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Appendix K1

Comal Springs Riffle Beetle Habitat Connectivity Study
Final Report, December 14, 2015

Comal Springs Riffle Beetle Habitat Connectivity Study

FINAL REPORT **December 14, 2015**

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Executive summary

The Comal Springs riffle beetle *Heterelmis comalensis* (Coleoptera: Elmidae) is a federally-endangered aquatic beetle endemic to the Edwards Aquifer—specifically, areas of seeps and spring upwellings in the Comal and San Marcos systems. These beetles and their associated habitat are covered under a 2013 United States Fish and Wildlife Service (USFWS) Incidental Take Permit (ITP) maintained by the Edwards Aquifer Habitat Conservation Plan (EAHCP). The objective of the EAHCP applied research is to fill in critical data gaps for the covered species and to answer key questions posed in the EAHCP in order to inform future management decisions and possible adaptive management solutions. To this end, in this document we describe an applied research project performed in 2015 that examined water quality and survival of Comal Springs riffle beetles to establish a laboratory location for 2015 experimentation, a subsequent evaluation of potential surrogate beetle species, and a series of laboratory and field investigations examining key components of upwelling and lateral habitat connectivity.

Although both the USFWS San Marcos Aquatic Resource Center (SMARC) and Texas State University Freeman Aquatic Building (FAB) laboratories exhibited fairly similar water quality conditions with similar lack of temporal variability during our preliminary investigation, there were profound differences in the survival of riffle beetle adults with extensive mortality at FAB. As such, all subsequent 2015 riffle beetle laboratory studies associated with this project were conducted at SMARC. In response to the unknown causes for this mortality concern, Dr. Nowlin and Texas State University (both outside of this project) have been working closely with the Edwards Aquifer Authority to determine the cause for the adult mortality and how to manage the issue.

The surrogate evaluations suggest that *Microcylloepus pusillus* is likely not the best candidate to serve as a surrogate species for the Comal Springs riffle beetle in physiological stress studies associated with variation in environmental conditions, especially water temperature. This conclusion was further reinforced with stable isotope results that documented that the diet of *M. pusillus* was much more reliant upon periphyton and therefore feeds on an entirely different food chain than the Comal Springs riffle beetle.

Results from the upwelling habitat connectivity study conducted at SMARC using the Riffle Beetle Aquifer Simulation System revealed greater survival in treatments connected to organic matter via water flow than in treatments that were disconnected to organic matter over the course of the study. Additionally, qualitative observations of beetle movement over the course of the upwelling experiment suggest that beetles in Experimental Flow Chambers with flowing water connected via flow through organic material tended to be more active and often frequented the organic material provided near the surface of the upwelling. The laboratory observation regarding connectivity and organic matter was independently supported by field studies using stable isotopes to examine the Comal food chain.

Results from the field investigations indicate that inputs of terrestrial-derived materials are likely to be particularly important for the diet (and thus conservation) of the Comal Springs riffle beetle. In fact, model results indicate that Comal Springs riffle beetle adults and larvae derive 80% and 73% of their diet from terrestrial-derived materials (wood and leaves combined). The

results of this study suggest that a lateral connection to terrestrial matter sources may be particularly important for two of the invertebrate species listed under the EAHCP: Comal Springs riffle beetle and the Comal Springs dryopid beetle (*Stygoparnus comalensis*). Lower spring discharge and declining flows can lead to disconnection of the aquatic environment from the bank and to lower water surface areas of aquatic habitats, potentially leading to decreased inputs of terrestrial material that may support populations of these wood-associated taxa. In addition, decreased flow rates and water velocities could also lead to lower rates of downstream transport of terrestrial materials from more canopy-covered upstream areas (e.g., the more canopy-covered Spring Runs) to more open areas with lower rates of terrestrial inputs (e.g., Spring Island area).

Results from the stable isotope work indicate that the third EAHCP covered and federally listed Comal invertebrate, Peck's cave amphipod (*Stygobromus pecki*), another spring-associated consumer may have a more plastic feeding strategy than the Comal Springs riffle beetle and Comal Springs dryopid beetle and may be able to switch to an alternate basal resource food chain as environmental conditions vary spatially.

Relative to this investigation, it was clear that there are potentially a large number of additional studies that should be considered. Experiments for consideration could include examining the performance of riffle beetles to environmental stimuli (water temperature, organic matter connection or disconnection, levels of siltation, etc.) over extended periods of time (months) in order to establish "preferred" conditions for maintenance of both wild and refuge populations. It may also be extremely insightful to monitor physiological responses (instead of behavioral responses) to increasing temperature, such as respiration rates, immune function, and the concentration of various biomolecules.

Finally, based upon the two preliminary studies we conducted, it appears that passive sampling pits or wells and Bou-Rouch samples are not likely to be efficient methods to estimate beetles. However, the efficiency of these and other methods should be considered for more thorough investigation in a systematic study that explicitly examines and compares various collection methods in the field. In addition to these comparisons, other data should be considered for collection in the lab, including the dispersal or movement ability of beetles, the ability to mark and re-capture beetles in small-scale settings, and the ability of beetles to hold onto surfaces at different flow or suction rates.

Acknowledgments

The project team would like to acknowledge the USFWS SMARC scientists and staff for the generous use of their facilities and expertise.

1.0 INTRODUCTION

The Comal Springs riffle beetle *Heterelmis comalensis* (Coleoptera: Elmidae) is a federally endangered aquatic beetle endemic to the Comal and San Marcos springs systems in central Texas. Current species range is restricted to the headwaters of the Comal and San Marcos springs, as well as to areas of seeps and upwellings from the Edwards Aquifer within Landa Lake in the Comal system (Bowles et al. 2003, BIO-WEST 2002, Gibson et al. 2008, Norris and Gibson 2013). *Heterelmis comalensis* is a small, flightless riffle beetle that requires aquatic habitat throughout its life history (Bosse et al. 1988, Brown 1987). Like other elmid beetles, *H. comalensis* is understood to prefer habitats featuring high-quality water flowing over firm substrates with little-to-no silt cover and relatively uniform temperature, dissolved oxygen (DO), and pH levels (Brown 1987, USFWS 1997, Bosse et al. 1988, Crowe and Sharp 1997, Gibson et al. 2008, Bosse 1979).

Disappearance of surface flow in desert systems disconnects subsurface and surface habitats and has been observed to disrupt typical ecological processes in stream systems by changing species composition (Valett et al. 1992). However, though research on low-flow conditions and critical drought stages exist for surface taxa (e.g., Wright et al. 1994, Harrison 2000, Williams 1977), the same cannot be said for subsurface species: we were unable to locate any studies that determine a critical level of drought at which subterranean or hyporheic taxa are most at risk (Boulton 2003).

In this report we describe some initial testing of research facilities, an evaluation of surrogate beetle species, and a series of novel experiments examining key components of upwelling and lateral habitat connectivity of *H. comalensis*. Due to potential issues associated with differences in the survivability of *H. comalensis* at the Freeman Aquatic Building (FAB) at Texas State University and the United States Fish and Wildlife Service (USFWS) San Marcos Aquatic Resource Center (SMARC) observed in 2014, intensive water quality monitoring at both sites were conducted in 2015 (Section 1). Subsequently, we examined the suitability of several riffle beetle species as surrogates for *H. comalensis* for future studies (Section 2). However, the main focus of this applied research effort was testing the effects of water level decrease and surface habitat disconnection on *H. comalensis* under laboratory (Section 3) and field conditions (Section 4).

To test upwelling connectivity, we conducted laboratory experiments to assess the importance of organic matter availability on the spatial habitat preference and survival and/or ecological death of *H. comalensis* subjected to constant upwelling flow that was either connected or disconnected from organic material. This effect is of interest in light of the possibility that worsening drought and low-flow conditions may increase the disconnection of subsurface habitats from the surface edge habitats hypothesized to be important to *H. comalensis* in terms of protective habitat, food availability, or a combination of the two. Experiments were conducted in a wet laboratory at SMARC using a custom-built Riffle Beetle Aquifer Simulation System (RBASS) aquarium unit. The RBASS was engineered to create “spring upwelling” mesocosms that provide water quality and light conditions simulating those found in riffle beetle habitats in the Comal Springs/River system, and is valuable in that it allows controlled experimentation in an upwelling environment with the application of several replicates and/or several different treatments simultaneously.

The field portion of the study examined whether the abundance and distribution of *H. comalensis* is associated with spring openings that contain organic matter (i.e., root material, leaves), whether they utilize the organic matter and/or biofilms on the organic matter as a food source, and whether riffle beetles have the ability to respond to changing water levels over the course of the drawdown season. Conclusions and recommendations are presented in Section 5 with literature cited and reviewed presented in Section 6.

2.0 LITERATURE REVIEW

Prior to initiation of the study, an extensive literature review was conducted relating to 1) population distributions and habitat associations of *H. comalensis*; 2) dispersal ability, habitat connectivity, and life history of *H. comalensis*; 3) surface-subsurface interactions for flowing water invertebrates; 4) food habits and trophic ecology of *H. comalensis;* and 5) use of surrogate species for habitat connectivity studies. Since periods of extended drought greatly influence the amount and persistence of spring flows at Comal Springs, determining the likelihood of low flow periods in the future is critical for habitat management of the listed species dependent on these habitats for their continued presence in the system. The probabilities of a shorter-term (3-5 years) and longer-term (7-10 years; equivalent to the drought of record in the 1950's) drought periods are estimated to be approximately 0.2% and 0.1%, respectively (EARIP 2011). The Edwards Aquifer Recovery and Implementation Plan (EAHCP) set the long-term mean and minimum daily discharge objectives for Comal Springs at 225 ft^3/s (cubic feet/second) and 30 ft^3/s, respectively.

Historical data and modeling results indicate some of the potential loss of habitat and habitat degradation associated with reduction in spring flows. It has been observed that Spring Runs 1 and 2 generally cease to flow when total Comal Springs flow is ~130 ft^3/s, and Spring Run 3 generally ceases to flow when Comal Springs total flow is about 50 ft^3/s (LBG Guyton 2004). Modeling results suggest that discharge will be less than 120 ft^3/s for a total of 127 months and less than 45 ft^3/s for a total of seven months during a repeat of the drought of record (in the 1950's) with Phase 1 of the EAHCP implemented (EARIP 2011). Modeling efforts also indicate that a repeat of the drought of record (with Phase 1 of the EAHCP fully implemented) will lead to the total flows in the Comal Springs system to be < 30 ft^3/s for a two month period (EARIP 2011). If flows drop below 30 ft^3/s, it is expected that the main spring runs in the system (Spring Runs 1 through 6) will be dry for a considerable period of time, and that the remaining aquatic habitat for the Comal Springs riffle beetle within the system will be limited to portions of Landa Lake and the Spring Island area. Cumulatively, this information indicates that it is possible for several, if not most, of the spring runs in the Comal system to cease flowing for extended periods of time (i.e., months to years) and for a significant reduction of aquatic habitat to occur, should the Comal system again experience conditions similar to those of the drought of record.

Obviously, there is substantial uncertainty inherent with predictions about the duration and extent of low flow conditions at Comal Springs, but the effects of these predicted scenarios and droughts of lesser durations will likely affect the quality and quantity of habitat for listed species. In particular, the Comal springs riffle beetle has a fairly limited spatial distribution within the system, so changes in flow could lead to areas suitable for riffle beetle habitat in the system becoming reduced in area and fragmented, potentially leading to the spatial separation of beetles

from potential higher quality food resources they utilize. However, little is known about consequences of decreased flows and the potential effects on habitat connectivity for the Comal Springs riffle beetle. The purpose of this literature review is to provide information on the life history and surface-subsurface interactions and how these factors may affect the population and distribution of the Comal Springs riffle beetle. We also reviewed the literature for research that discusses the use of aquatic invertebrate surrogates.

Population Distributions and Habitat Associations of the Comal Springs Riffle Beetle

Riffle beetles (Order Coleoptera, Family Elmidae) typically live in swifter flowing areas in high water-quality streams and rivers. Adult riffle beetles generally crawl along benthic surfaces and respire through a plastron on the ventral side of their abdomen (Brown 1987, White and Roughley 2008, Elliott 2008a). *Heterelmis comalensis* is typically collected on cotton cloth lures in areas within a 80 cm from spring sources in the Comal system (Gibson et al. 2008, Cooke 2012). In hard-packed gravel habitats commonly found adjacent to spring openings, *H. comalensis* is found at the depths of 2-10 cm (Bosse 1979). The precise mechanisms for the apparent spatial restriction of *H. comalensis* within the Comal system are unknown, but may involve spatial variation in water quality parameters and habitat associations. Data indicate that riffle beetles prefer spring water characterized by high CO_2, low DO, and slightly lower pH in comparison to surface-water-dominated streams (Cooke 2012). Other species of more widespread elmids (i.e., *Stenelmis* spp.) also exhibit habitat preferences for stable gravel-cobble substrates and coarse woody debris, rather than unstable sand and mud substrates (Phillips 1995).

It is currently thought that a reduction in spring flow that leads to loss (desiccation) of habitat or reduces water quality of occupied riffle beetle habitat will likely impact their fitness and survival. Undoubtedly, water quantity will be the primary issue in the Spring Runs and along the western shoreline during substantial low-flow events, as springs within these areas will cease to flow and the habitat associated with the presence of the Comal Springs riffle beetle (i.e., areas around spring orifices) will be dry. However, as flows decline at Comal Springs and the remaining aquatic habitat is reduced to portions of Landa Lake along the western shoreline downstream of Spring Island (EARIP 2011), it is likely that the water temperature will increase and dissolved oxygen (DO) concentrations will drop. A recent study conducted for the EAHCP found that Comal Springs riffle beetles could tolerate rapid changes in temperature and DO concentrations (i.e., beetles could withstand up to 45°C before loss of function and tolerate 0 mg DO/L for several minutes without suffering obvious ill effects), but that their tolerance ranges to short-term temperature changes were substantially narrower than a closely-related elmid species (*Heterelmis glabra*; Nowlin et al. 2014). However, the sensitivity of riffle beetles to longer-term and more slowly-occurring changes in temperature and DO remain to be determined.

Dispersal Ability, Habitat Connectivity, and Life History of the Comal Springs Riffle Beetle

Although many adult aquatic coleopterans emerge from aquatic habitats for dispersal flights to other aquatic locations, many riffle beetle species typically cannot disperse great distances via flight (White and Roughley 2008). Indeed, some elmid species are thought to be flightless as adults (Elliott 2008a) and other species can fly relatively short distances after emergence but their flight muscles degenerate after they re-enter the water (Hinton 1976). The complete loss of adult flight (degenerated wings) or the rapid loss of wings after adult emergence suggests that retaining flight capability may not be compatible with utilization of plastron respiration because

of the maintenance of substantial sub-elytral air space required for functional wings (Thorpe and Crisp 1947). Thus, smaller bodied adult elmids like *H. comalensis* typically do not have the ability to disperse great distances and move relatively slowly via crawling in their aquatic habitats or through drifting downstream.

On benthic surfaces, smaller-bodied invertebrates like elmids are more resistant to dislodging during high flow events than larger-bodied invertebrates (Turcotte and Harper 1982), but the use of benthic surface habitats can vary substantially among species and within life stages of a single species. In general, elmid larvae are less sensitive to lower water velocities and hydraulic stagnation than adult stages (Walters and Post 2011) because larvae are less dependent on flowing water conditions (i.e., less rheophilous) than adults because they utilize gills for respiration (Elliott 2008a). Later larval elmid instars can develop tracheal air sacs that provide a Cartesian driver system for controlling the specific gravity of the body and allow drift towards preferred pupation sites along stream banks (Brown 1987). Drifting of individuals occurs mostly at night, most likely in response to gaining access to food resources or to escape sub-optimal environmental conditions (Elliott 2008b; Brown 1987; Reisen 1977). Higher mortality rates of newly hatched or overwintering larvae occurs during drift events, as they are then at high risk of being washed away by the current and/or dispersed to sub-optimal habitats (Reisen 1977; Elliott 2008a).

Variation in environmental conditions can alter the timing and magnitude of emergence of adult elmids, including *Microcylloepus pusillus* and *Heterelmis* spp. (Reisen 1977). In temperate systems with a high level of seasonal variation in environmental conditions (e.g., Quebec, North America), individual elmids can persist in the larval stage for 2-3 years and the adult stage lasts for approximately a year (Lesage and Harper 1976). In temperate systems, both adult and larval elmids exhibit a great deal of seasonal variation in life stage timing, and air/water temperatures greatly influences the duration of the pupation period (Lesage and Harper 1976). In contrast, *H. comalensis* and *M. pusillus* in the Comal Springs system exhibit non-seasonally influenced emergence patterns and have overlapping, asynchronous generations (Bowles et al. 2003). This lack of seasonality in emergence and life history patterns in *H. comalensis* is largely thought to be a consequence of environmental conditions at spring-influenced systems like Comal and San Marcos springs because they exhibit little seasonal variation. In other systems with limited variation in environmental conditions (e.g., the tropics), emergence of *Heterelmis* adults occurs during periods of low intensity, low current velocities, and high food availability, and oviposition occurs when temperature is highest and water level is lowest (Passos et al. 2003). It is important to note that although the USFWS can successfully house both adult and larval *H. comalensis* to the extent that adults mate and oviposit in aquaria, they have so far had only minimal success in getting adults to emerge after pupation. Currently, there is a clear need to better understand potential mechanisms and conditions leading to successful adult emergence of *H. comalensis*.

Surface-Subsurface Interactions for Flowing Water Invertebrates

The subsurface hyporheos, or hyporheic zone, of flowing water systems may act as a refuge for benthic invertebrates during periods of low flow or even in apparently dry stream beds (Williams and Hynes 1974). It provides refuge from drying and enables invertebrates to recolonize the surface once the disturbance has passed (Dole-Olivier et al. 1997). Drought-induced changes to in-stream environment such as hydraulic stagnation, increased/decreased water temperatures, increased fine sediment deposition, and altered macrophyte composition can affect the macroinvertebrate community by reducing habitat quantity and quality as well as access to preferred food resources. During low flow periods, the contraction of wetted stream width can cause in-stream organismal densities to increase and lead increased resource competition in the hyporheic zone (Dewson et.al 2007). Complete cessation of flow in the hyporheic zone can lead to loss of suitable habitat for invertebrates seeking refuge in the hyporheos through the eventual complete desiccation of hyporheic sediments (Boulton & Stanley 1995), anoxia in the hyporheos (Smock et al. 1994), and the lack of interstitial habitat due to clogging of interstices by fine sediments (Bo et al. 2006).

In addition to its limited geographic distribution, specificity in preferred habitat types, lack of mobility, and potential sensitivity to habitat degradation, the genetic variation of *H. comalensis* populations in the West Shoreline, Spring Island, and San Marcos springs populations suggests limited gene flow among these populations. Therefore, if springs at Comal or San Marcos springs cease to flow for extended periods of time, genetic variation across the remaining variable populations could be lost (Gonzales 2008). Although *H. comalensis* was described as a species after Comal Springs stopped flowing during the drought of record (Bosse et al. 1988), is has been assumed that *H. comalensis* populations were present in the Comal system prior to the drought of record and were able to persist for the 144-day no-flow period in 1956. It remains unknown precisely how beetles persisted in the Comal system during the drought of record, or how the drought of record affected riffle beetle populations, and if riffle beetles have the ability to rapidly recover from a large-magnitude drought events. It has been hypothesized that *H. comalensis* persisted through the drought of record through life cycle aestivation or by retreating into spring heads, the aquifer, or down into the hyporheos (Bowles et.al 2003).

Previous research has determined that for perennial species of riffle beetles (i.e., species that live for multiple years), pool habitats connected via some flowing surface water can serve as refugia for both larvae and adults during drought periods (Burk and Kennedy 2013). Elmid use of these refuge areas is thought to be associated with the relatively higher water quality and constancy of flow in these habitat patches during drought conditions (Burk 2012). In addition, during extreme drought events, elmids can take refuge in shaded disconnected pools where environmental temperature and evaporative losses are moderated by riparian shading (Burk and Kennedy 2013). However, elmids may also utilize subsurface (hyporheic) environments when flows are low; elmid body shape is such that they can tolerate small spaces (elmid adults are typically of small body size, while larvae have slender, flexible bodies), and elmids have been found to survive in the hyporheos for relatively long periods of time during periods of low flow (Boulton and Foster 1998; Marchant, 1988).

Food Habits and Trophic Ecology of Comal Springs Riffle Beetle

Potential food resources for *H. comalensis* have not been clearly identified in the Comal and San Marcos systems. Most literature sources state that riffle beetles are generally biofilm scrapers that can utilize detrital materials (Brown 1987). Currently, the standard capture method for *H. comalensis* in Comal is through the use of cloth lures (R. Gibson, USFWS; *pers. comm.*). Presumably, Comal Springs riffle beetles are attracted to the lures to gain access to the biofilms that grow there (Gibson et al. 2008). A more widely-distributed elmid species, *Heterelmis vulnerata*, is often associated with coarse woody debris with biofilm coverage and loose bark and/or interstitial spaces. The biofilm and interstitial spaces are thought to be used as concealment from the predators and biofilms may serve as algal and fungal food sources for the beetles (Phillips 1995). Seagle (1982) found that the gut contents of larvae and adults of three different riffle beetle species (*Stenelmis crenata, Stenelmis mera,* and *Optisoservus trivittatus*) were dominated by detritus-like materials, including wood xylem and unidentified organic matter and mineral particles, while algal material was consumed to a much lesser extent. Thus, it has been suggested that elmids should be reclassified as detritivores-herbivores rather than as strictly herbivores, with the exception of known xylophagus genera (i.e., *Lara*) (Seagle 1982). Cannibalistic foraging has been observed in some elmids (i.e., *M. pusillus*), but this behavior was attributed to nutritional deprivation and is probably not a common foraging strategy (Brown and Shoemake 1969). Currently, the precise food sources and trophic ecology of *H. comalensis* remains unknown. In addition, it is unknown whether a reduction in spring flow may lead to the disconnection of *H. comalensis* from potential or preferred food sources, such as terrestrial organic matter and detritus which may be most concentrated along the bank.

Use of Surrogate Species for Habitat Connectivity Studies

Given the sensitive nature and protected status of the *H. comalensis*, there is high potential value in determining whether other similar insect species may be used as test surrogates, especially as such could allow for improved understanding of tolerance to changes in habitat and quantity and quality while preventing the unnecessary take of protected organisms. Suitable surrogate organisms should share certain traits in common with the organism in question, such as life history, physiology, developmental patterns, food preferences, trophic ecology, habitat associations, sensitivity to environmental conditions, and dispersal ability.

Several species of elmids have been used as surrogate species to *H. comalensis* for a number of studies. The elmid *M. pusillus*, while in another genus, occurs in some of the same habitats as *H. comalensis* in the Comal system; the mechanisms facilitating the occurrence of *H. comalensis* and *M. pusillus* in similar habitats in Comal Springs are not well known, but their coexistence may be related to patterns in availability of preferred substrates, and competition with each other and with other species may influence their population distribution (Bowles et al. 2003). Both *M. pusillus* and *H. comalensis* occur in the same habitats at Comal Springs (spring runs 1, 2, and 3), indicating a possible preference for similar substrate composition (i.e., substrates dominated by gravel-sized particles), although the absence of *H. comalensis* and presence of *M. pusillus* at Spring Run 4, where substrate composition is dominated by silt, sand, and smaller gravels, suggests that *H. comalensis* may be more sensitive to changes in substrate size than *M. pusillus* (Bowles et al. 2003). In addition, adult *M. pusillus* are winged and are assumed to be capable of flight, whereas *H. comalensis* adults are generally thought to be incapable of flight. This suggests that *M. pusillus* may have greater potential dispersal ability, a wider population distribution in

Comal Springs, as well as a greater tolerance to changes in habitat than *H. comalensis* (Brown and Shoemaker 1969; Bowles et al. 2003). However, these differences are not well-elucidated and have yet to be examined in depth.

Heterelmis glabra and *H. vulnerata* may also serve as potential surrogates for *H. comalensis*. Data suggest that while *H. comalensis* and *H. glabra* are closely related, populations have been historically isolated from one another with little-to-no recent gene flow (Gonzales 2008). More recently, research associated with the EAHCP found that tolerance ranges to rapid and short-term temperature changes for *H. comalensis* were substantially narrower than for *H. glabra* (Nowlin et al. 2014), suggesting that *H. comalensis* is more sensitive to changes in habitat and water quality than *H. glabra*. Finally, *H. vulnerata* is typically found in surface-water-dominated sites and not with spring-dominated systems, suggesting that it has clearly different water quality and habitat requirements that *H. comalensis*. Cumulatively, this information indicates that there is clearly a need to determine whether effects of low flow on *H. comalensis* can be correctly assessed by using *M. pusillus*, *H. glabra,* and *H. vulnerata* as surrogate species.

3.0 METHODS AND MATERIALS

3.1 Potential Water Quality Differences between SMARC and FAB

Due to potential issues associated with differences in the survivability of *H. comalensis* and other riffle beetle species at FAB at Texas State University, we conducted high-frequency water quality monitoring at FAB and SMARC over a two-week period to determine whether either facility exhibited rapid changes in conditions that might affect the captive beetle populations. At each facility, an auto-logging datasonde (YSI multiparameter probe at SMARC and an In-Situ Inc. TROLL multiparameter probe at FAB) was placed on March 1, 2015 in the incoming water flow to a living stream in a plastic bucket (so that there were no influences of aeration) that logged data on temperature (°C), dissolved oxygen (DO; mg/L), conductivity (µS/cm), and pH at 10 minute intervals. Each sonde was calibrated to known standards on site. We did not cross-calibrate the sondes with each other because we were not interested in direct comparison of conditions across sites, but rather in the relative change in conditions within each site and how any potential changes would affect survival of beetles.

Concurrently with the installation of the sondes at each facility, we collected fifty individuals of both *H. comalensis* and *M. pusillus* on March 16, 2015 from Comal Springs by hand picking and transported individuals in high-quality coolers filled with site water to both facilities. Twenty-five individuals of each species were held in separate flow-through chambers on living streams at FAB (n = 25) and SMARC (n = 25). We additionally collected *H. glabra* (n = 50) from Finnegan Springs at the Devils River on March 21, 2015 by hand-picking from cloth lures. *Heterelmis glabra* individuals were transported to FAB and SMARC in high-quality coolers containing Finnegan Spring water and introduced into the flow-through chambers at FAB (n = 25) on March 21, 2015 and at SMARC (n = 25) on March 23, 2015. The plastic flow-through chambers that housed riffle beetle populations of each species at both the facilities contained pre-cleaned limestone river cobbles and well-conditioned sycamore (*Plantanus occidentalis*) leaves, a presumed food source. Data from the sondes were downloaded on a weekly or bi-weekly basis

and examined to determine if there were pronounced or rapid fluctuations in the conditions of the supply water quality (e.g., rapid drops in DO or abrupt changes in conductivity) at both the facilities. Survival of beetles at both facilities was checked every day and beetle mortality was recorded. The beetle survival at both the facilities was observed and recorded everyday until March 31, 2015 (Section 4.1).

On April 1, 2015, an additional flow-through chamber containing adults of *M. pusillus* (n = 22) was transferred from SMARC to FAB and connected to the water source in FAB wet lab. This transfer was done to verify the occurrence of 100% beetle mortality at FAB in a short period of time (Section 4.1) when all the conditions in the flow-through chambers were exactly similar at both the facilities and the only difference was the water source.

3.1.1 Data Analysis

Differences in riffle beetle survival and water quality at the two facilities were assessed by examining the number of beetles that were alive at each location during the study duration and by examining the variability in the various water quality parameters at each location over the study period. Variability in water quality parameters was assessed by determining the minimum, maximum, and standard deviation of values during the temporal span of the study.

3.2 Surrogate Testing for Effects of Water Temperature

Based on the results obtained by survival testing at SMARC and FAB (Section 4.1), all subsequent experiments examining riffle beetle responses to different environmental temperatures were performed in temperature-controlled living stream systems at SMARC. Adult beetles of each species (i.e., *H. comalensis*, *M. pusillus* and *H. glabra*) were collected in the wild using the previously described methods, transported to SMARC, and maintained in flow-through chambers with a constant addition of Edwards Aquifer well water for at least two weeks before being used in experiments.

Populations of adults were housed in flow-through chambers held within living stream systems and gradually acclimated to temperatures set at approximately spring outflow temperatures (23^{o}C) prior to the start of experiments. *Heterelmis glabra* was selected as a potential surrogate because it is the most closely related species to *H. comalensis* and is also considered to be spring-associated in terms of its habitat use. We also utilized *M. pusillus* in experiments because it is often found in the same habitats as *H. comalensis* and *H. glabra*, but its overall distribution within these habitats is thought to be more cosmopolitan and not as spring-restricted as the two *Heterelmis* species.

In this study, we focused on potential differences in survival among the three species during relatively long-term exposure to gradually-increasing temperatures. We elected to use this approach in our comparison for two main reasons. First, we decided to use temperature as the variable of interest because previous experiments with *H. comalensis* and *H. glabra* indicated that these species appeared to be sensitive to temperature when they increased to ~30^{o}C (Nowlin et al. 2014). Second, we elected to use a long-term and gradual increase design so that we could somewhat mimic the types of conditions that these beetles would experience in natural systems.

Previous studies (Nowlin et al. 2014) only had the opportunity to examine responses of *H. comalensis* to relatively short-term temperature increases (changes over a few hours); however, in spring systems, temperature increases are likely to be persistent and gradual as spring flows decline in the summer months. Thus, we elected to examine how gradual step-wise increases affected survival of beetles so that longer-term effects would have time to manifest themselves in animals.

For this portion of the study, the experimental setup included 10-L plastic tubs which lay within a living stream with continuous supply of Edwards Aquifer water (Figure 1). The plastic tub had a layer of pea-sized aquarium gravel in the bottom and a magnetic drive pump (~700 g/h or 44 L/min; Pentair Aquatic ecosystem, Model MD7). A temperature control unit (Frigid Units, Inc.) was placed in an initial reservoir and could be set to the desired temperature via digital interface. A temperature and optical DO probe was placed in the reservoir and DO and temperature were recorded continuously over the period of the experiment on a laptop computer with Loggers Pro software. The outlet of the magnetic pump was connected to flexible clear vinyl tubing which led to a manifold with multiple splitters in the line – each line led to an airtight high density polyethylene (HDPE) chamber that had inflow and outflow lines and would serve to house beetles during experiments. Openings to the inflow and outflow tubes to the chambers were covered with a fine mesh (100µm aperture) to prevent beetle escape. Individual beetles of each species were housed in individual HDPE chambers that were suspended in a living stream using a plastic screen mesh. Three plastic screens were suspended in the living stream and each plastic screen with 5 cups housed 5 individuals of the same species (one individual in each cup). Replacement of water into each beetle holding chamber was estimated at 120 mL/min, allowing for complete replacement of the chamber volume every 30 seconds.

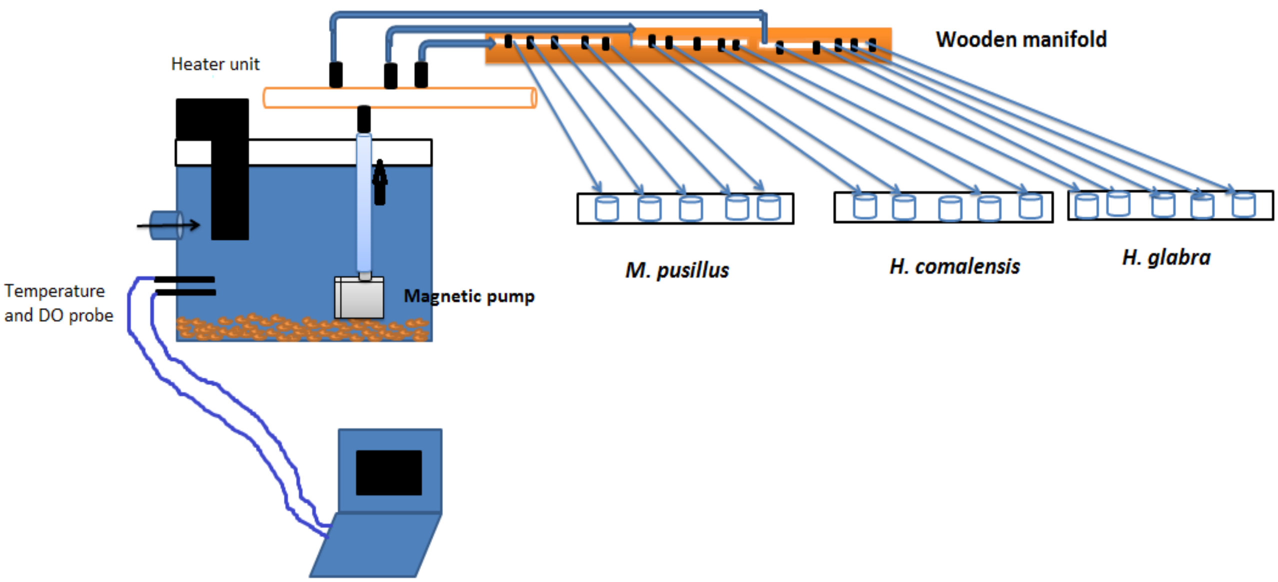

Figure 1. Schematic of the system used for the surrogate experiment. Incoming water temperature was ~23°C, and changes to water temperature were accomplished by setting the desired temperature on the heater unit.

The long-term experiments at different temperature treatments with *H. comalensis*, *H. glabra* and *M. pusillus* were conducted at SMARC from June 15 to July 20, 2015 (35 days). Five individuals of each species were subjected to gradually increasing target temperature treatments of 23°C, 26°C, 28°C, 29°C, 30°C, and 32°C or until all beetles died or exhibited a loss of response to a stimulus. The DO concentrations during experiments was always $\geq$5.9 mg/L. Beetles were initially kept at 23°C for one week (7 d) and then the temperature was slowly raised to 26°C over a period of ~2.5 days to allow for acclimation to experimental temperatures. Water temperatures in the reservoir varied from the programmed temperature by ± 1°C over the course of a given week, but mean temperatures were maintained at the desired temperature. After the period of acclimation to the new temperature, beetles in all the chambers were kept at each treatment temperature for 5 days and checked daily at 10:00 AM for signs of mortality or a loss of response (LOR) to an external stimulus (gentle agitation of the chamber; see Nowlin et al. 2014). If a beetle was observed to exhibit an LOR, it was immediately removed from its chamber and placed into an individual container at initial acclimation conditions (23°C, >4 mg/L) and repeatedly observed every few hours to note whether it recovered or died.

3.2.1 Data Analysis

In order to examine potential differences among the different riffle beetle species over the course of the progressively increasing temperatures, we examined the temperature at which beetles would die. Temperatures at which individuals became unresponsive/died were compared among species using one-way ANOVA. The temperature of death of each individual of each species was considered an independent observation. We assessed assumptions of normality and homogeneity of variances prior to analysis. Significance was inferred at $p \leq 0.05$.

3.3 Upwelling Habitat Connectivity

Based on the results obtained by survival testing at SMARC and FAB (Section 4.1), all subsequent 2015 laboratory experiments examining riffle beetle responses were conducted at SMARC. All water used in experimental apparatuses in the course of this laboratory investigation originated from a series of groundwater wells operated by SMARC

3.3.1 Refugium Tanks

Specific to the upwelling experiment, two refugia tanks were constructed based on the designs of tanks used to house *H. comalensis* at SMARC. Polyvinyl chloride (PVC) piping with nine holes drilled along the length was installed at the top of the refugium tanks and connected to the wet lab's water source to allow constant, low-level flow to enter the refugia from the top at several points. Four PVC drains capped with fine mesh were installed in the bottom of the refugia tanks to facilitate constant outflow. Tanks were stocked with boiled limestone rocks and oven-dried leaves from trees growing along the banks of Comal Springs (Figure 2). Refugia were covered with a non-light-penetrating polyethylene tarp to block light, mimicking the darkness typical of subterranean and/or interstitial spring habitats.

Figure 2. Refugium tank, showing water flowing in through the top and draining out through the bottom, with rock and leaf substrates.

3.3.2 Riffle Beetle Aquifer Simulation System

In order to conduct riffle beetle applied research for a wide variety of possible experiments, a self-contained hydraulic simulation system was designed and identified as the Riffle Beetle Aquifer Simulation System or "RBASS" (Figure 3). The primary objectives considered in the construction of the RBASS were to create a once-through flow hydraulic system that was both inert in composition and completely self-contained. The term "once-through flow" refers to the one-time use of source water that enters the system and exits without any reuse or recirculation. The term "self-contained" refers to the ability to host aquatic insects in multiple individual chambers and prevent escape. The RBASS can be divided into the following two main components: (1) the Hydraulic Distribution Arena (HDA) and, (2) the Experimental Flow Chambers (EFCs). The following narrative summarizes the construction and function of this system.

Figure 3. Photograph of RBASS system setup at SMARC wet laboratory showing six RBASS environmental flow chambers, upwelling flow spigots, black nylon cover, and inlet water line. The refugia tank can be seen at the far end of the green fiberglass living stream.

<u>Hydraulic Distribution Arena (HDA)</u>

The purpose of the HDA is to distribute aquifer source water to numerous self-contained flow chambers and to act as a containment arena and visual flow return valley. This objective was accomplished by constructing a complex, variably controlled plumbing system housed within a highly visible support structure.

Flow

The hydraulic or once-through flow plumbing system starts with a 0.75-inch female hose fitting that can receive a standard garden hose end for source water. A PVC ball valve regulates flow into the system and is followed by a food-grade 50-micron polypropylene sediment depth filter. This filter is fitted with a diverter valve that enables the system to either utilize or bypass the filter unit. In addition to a clear housing for visual filter inspection, the system includes a pre- and post-filter water pressure gauge to identify pressure differentiation, which might indicate a clogged filter. The 50-micron filter system also provides secondary containment and visual detection of an aquatic beetle that might escape the flow chamber and travel upstream. After the

filter system, 0.75-inch PVC pipe distributes the water to six PVC ball valves, each positioned between two flow chambers. From that point the water is distributed through a plastic manifold providing flow variations at three independent locations in each flow chamber. Water is expelled from the top of the flow chambers into a common return valley, which is drained through a 117-micron stainless steel wire mesh. The water level in the return valley can be regulated by the last PVC ball valve exiting the structure.

Construction
In addition to the aforementioned plumbing infrastructure, the HDA housing is constructed of white, 13-mm-thick expanded closed-cell PVC. The structure is held together with stainless steel screws and (where sealant is necessary) aquarium-grade silicone. The common return valley is removable from above to allow future plumbing access and to second as storage.

Experimental Flow Chambers (EFCs)
The EFCs are constructed of 0.24-inch clear acrylic measuring 24 inches high, 6 inches wide, and 0.75 inches deep (dimensions of open space between acrylic panel walls). Chamber seams are chemically fused (or “welded”) for a watertight seal using Weld-On 4, an acrylic adhesive commonly used in aquarium construction. Water inlet ports are bored through the acrylic material along one side of the flow chamber near the bottom, middle, and top. These ports allow 0.25-inch tubing to be inserted and capped with a tapered, threaded plastic diffuser end/spigot. The plastic tapered end allows for a compression seal between the tubing and acrylic, while the diffuser not only evenly distributes flow, but also prevents aquatic beetles from escaping from the EFC into the source water. A 1.50-inch by 4.0-inch opening is cut into the acrylic approximately 1 inch from the top to allow water to exit the chamber before overflowing. At this opening, a 117-micron T304 stainless steel mesh is secured by a removable compression plate to allow for cleaning and any necessary future replacement. Outside of the ECF, an acrylic shelf is affixed below and on the sides of this opening to divert outflow water into the HDA return valley. Lastly, an acrylic insert topped with closed-cell PVC was used as a sealed removable top.

A total of 12 EFCs were constructed to allow multiple replicate samples and flow variations. During experimentation, a total of six EFCs could be placed on each side of the HDA. For this study, six randomly chosen EFCs were used to support two treatments.

Ambient Light Enclosure
In order to mimic aphotic aquifer conditions, a cover was constructed to prevent ambient light exposure to the RBASS (Figure 4). This cover was constructed from a black, non-light-penetrating, vinyl-reinforced nylon, which is supported by a 0.75-inch PVC sub-frame. Four independent panels are sewn into each side of the cover to allow access to the RBASS at selected locations. Each panel is sewn together at the top and secured laterally by Velcro seams and extended to the facility floor.

Heat Exchange Process
Water destined for the RBASS and refugia tanks was sent through a chiller and hose assembly in order to regulate water temperature by protecting against periods of possible temperature variation caused by thermal convection between warm daytime soil and underground pipes (Figure 4). The chiller was maintained at approximately 68.3°F and contained 150 feet of 5/8’’ garden hose for the RBASS inlet and a length of 50 feet of ½’’ garden hose for the refugia inlet.

Figure 4. Photograph of RBASS cover and adjacent chiller and heat exchange system.

Surface Organic Layer

To supply organic matter for the experiment originating from known *H. comalensis* habitat, Anacua leaves from Spring Run 3 at Comal Springs were collected and transported to SMARC. Leaves were then dried in SMARC's drying oven and subsequently weighed to determine equal amounts for placement in each surface organic layer (SOL) (Figure 5). Once placed within the 2 –ply mesh SOL, the bundles were allowed to condition together in a bucket supplied by the RBASS inlet water for one week prior to use in the study.

Figure 5. Photograph of Anacua leaves used in the surface organic layer (SOL) packs.

3.3.3 Study design

On September 10, 2015, 60 *H. comalensis* were collected from Comal Springs using "hand-picking" collection techniques developed by researchers at SMARC to collect wild *H. comalensis* for stocking the refugia at that facility. Beetles were brought immediately to SMARC and placed in the refugia for a weeklong acclimation period.

The RBASS unit was set up to implement a design to test the effect of the organic matter connection or disconnection on beetle survival in upwelling flow in RBASS EFCs over a 30-day period. On 21 September 2015, nine *H. comalensis* were placed in each of 6 RBASS EFCs equipped with a substrate of two equal-sized vertical layers of 2,000-micron nylon mesh layered together. Following the acclimation period, SOL packets were added to the top of each EFC and secured by the addition of an extra layer of mesh substrate (Figure 6). After one additional acclimation week, outlet lines in the middle of the three disconnection-treatment tanks were opened at a rate which allowed the formation of a gap between the water line and the SOL layer over a period of three days. Beetles were then kept in the tanks until October 21, 2015.

Treatments were randomly assigned to 6 EFCs (3 EFCs with organic matter connection and 3 EFCs without organic matter connection). Visual counts were conducted and observed beetle spatial location was plotted within each EFC every other day. Water quality parameters were recorded once per week at two depths in each EFC (upper and lower). Water quality measurements included flow (L/s), water temperature (ºC), and dissolved oxygen (DO) (mg/L), and were taken on the side of each EFC opposite the flow spigots. Additional water quality measurements were logged by thermisters (HOBO® TidbiT v2 Water Temperature Data Loggers) every 10 minutes. Temperature and DO were recorded with a handheld portable meter (HACH® HQ40d multi-parameter meter) using a luminescent optical DO probe (HACH® IntelliCAL™ LDO101 probe). Flow was measured by recording the time required to fill a 100 mL beaker from an EFC inlet line identical to those providing water to experiment EFCs.

3.3.4 Data Analysis

Survival data (proportion of survivors pooled across replicates within each treatment due to small sample size) from the trial were assessed for greater survival of beetles in connection treatments than disconnection treatments by a one tailed test for the equality of two proportions (prop. test) with continuity correction for small sample size as implemented in R version 3.0.3 (R Development Core Team 2008).

Figure 6. Photograph of EFC with surface organic layer (SOL), EFC inlet (bottom diffuser cap), EFC outlet (middle diffuser cap), and grid overlay.

3.4 Lateral Habitat Connection and Diet Study

This field portion of the study examined whether the abundance and distribution of *H. comalensis* are associated with spring openings that contain organic matter (i.e., woody material, leaves), whether they utilize the organic matter and/or biofilms on the organic matter as a food source, and whether riffle beetles have the ability to respond to changing water levels over the course of the drawdown season. Thus, this portion of the study had three objectives: (1) to determine if beetles can be sampled with the use of passive sampling pits located within areas that are considered to be "preferred" or "associated" habitat; (2) to determine if beetles populations at spring sites have the ability to change patterns in their dispersion within spring run habitats both laterally and vertically as water levels decline over the course of a summer and fall; and (3) to determine if beetles are actively using terrestrial organic matter and/or the biofilms associated with organic matter as a food source.

3.4.1 Passive Sampling Pit Study

In order to meet the first two objectives, we utilized passive sampling "pits" or "wells" that were installed into benthic substrates around spring openings that are frequently associated with the presence of *H. comalensis*. We elected to use sampling pits because they would passively sample the beetles in their habitat and not act as a "lure" that would cause them to aggregate in specific area. Currently, the technique used to collect and to enumerate beetles relies upon the use of poly-cotton cloth lures, which beetles (adults and larvae) are attracted to and the resulting densities on the cloths may be higher than in the surrounding environment. The use of sampling pits is commonly done to passively collect hyporheic and sub-surface invertebrate fauna (e.g., Clinton et al. 1996), but passive sampling pits have not been previously used to sample for *H. comalensis*. Thus, we first performed a pilot study to determine if pits would effectively collect beetles. Sampling pits were constructed of 3/4" (1.905 cm) diameter PVC that was cut into 25 cm lengths and were perforated (5 mm diameter openings) to assess vertical distribution of beetles along the 5 cm depth intervals (Figure 7). PVC spacers were inserted into the pit which separated the 5 cm vertical intervals within sampling pits. Half of the sampling pits contained a small piece of poly-cotton cloth lure (used for the collection of *H. comalensis*) and the other half did not contain the cloth lure. Wells were capped at both ends.

Figure 7. Picture of the passive sampling pits utilized to examine the vertical and lateral distribution of riffle beetles in Comal SR3.

For the pilot study, sampling pits were installed at three sites in Comal Spring Run 3 (SR3; Figure 8) by hand digging up to a depth of 50 cm on April 8, 2015. Site 1 was a running seep surrounded with gravel and cobble located under a tree near the first bridge at SR3. The second site located downstream to the first site, was a seep covered with gravel and cobbles and was shaded by a tree. Site 3 was located downstream of Site 2 and was again within a spring outflow which was shaded by a tree. Site 3 had higher composition of silt in comparison to the other two sites. For the study, we installed a total of six pits, with 2 pits (one with cloth lures and one without) at each site. After installation, sampling pits were removed from sites after 3 weeks on April 30, 2015 and the organisms present in the pits were recorded.

Figure 8. Spring site along Spring Run 3 in the Comal system where *H. comalensis* commonly occurs. Sampling pits and Bou-Rouch collections were performed along this reach.

3.4.1.1 Data Analysis

For this portion of the project, we were initially interested if we could capture riffle beetles using passive pit samplers. If we received positive results, then we would have expanded the study, but because we did not capture any riffle beetles in the sampling pits (Section 4.4), we did not statistically analyze the data for this initial experiment.

3.4.2 Bou-Rouch Collection Study

In addition to the installation of passive sampling pits, we also utilized another passive sampling method to examine the lateral and vertical distribution of riffle beetles in the Comal system: a Bou-Rouch sampler. We extracted eight interstitial water (and presumably organisms) samples from three sites in Spring Run 3 using Bou-Rouch method (Bou and Rouch, 1967) on April 30, 2015. Bou-Rouch sampling involves the process of sampling hyporheic water from a

temporarily-installed well and a hand-pump (Figure 9). Bou-Rouch sampling is a fairly common practice to passively-sample hyporheic invertebrates (Hunt and Stanley 2000), but its effectiveness for sampling *H. comalensis* has not been explored.

Figure 9. Picture of a Bou-Rouch pump used for collections along Comal SR3.

For this portion of the study, we collected eight Bou-Rouch samples from four locations near spring openings in areas where *H. comalensis* have been previously collected using cloth lures. At each of the four locations, we collected two Bou-Rouch samples: one sample in which we installed the temporary well and pumped water for a fixed period of time and one sample in which we first bubbled CO_2 into the well for a known period before pumping water out of the well. Previous studies have expressed concern that some organisms have the ability to grasp onto larger substrates and prevent uptake during pumping (Fraser and Williams 1997), thus we elected to bubble CO_2 into ½ of the well samples in an attempt to anesthetize organisms prior to sampling.

The Bou-Rouch samples were collected by hammering a steel pipe (2.5 cm internal diameter, with 0.5 cm pores in the pipe) to a depth of 25-50 cm below the streambed. Interstitial water and fine sediment were extracted using a hand held pump attached to Bou-Rouch pipe (Bou & Rouch, 1967) and filling a 5 gallon plastic bucket (approximately 19 liters) and then filtered the contents through a 100-µm aperture mesh net. For half of the samples, we first bubbled CO_2 into the internal space of the Bou-Rouch pipe and below ground to anaesthetize organisms in the vicinity of the sampler before extracting the interstitial water. CO_2 was bubbled into the well at

a relatively low pressure for a 3 minute period from a commercial CO_2 canister with a flexible vinyl tube connected to an air stone; while directing CO_2 into wells, bubbles were clearly visible emanating from around the well at a radius extending up to approximately 10 cm from around the well. All hyporheic samples collected in the mesh net were preserved using 95% ethanol after we looked through the sample to remove any species of concern (e.g., *S. pecki*). Invertebrates in each sample were sorted and identified in the lab.

3.4.2.1 Data Analysis

For the Bou-Rouch study, we examined if there were differences in the number of captured invertebrates in standard Bou-Rouch collections and CO_2-amended Bou-Rouch collections using Students t-test. We assessed assumptions of normality and homogeneity of variances prior to analysis. Significance was inferred at $p \leq 0.05$.

3.4.3 Stable Isotope Study

In order to examine the potential diet of *H. comalensis* and to assess whether lateral connectivity is important for supplying resources to the riffle beetle, we conducted a study in which we assessed the diet of the Comal Springs riffle beetle and other invertebrate consumers in the Comal Springs food web using stable isotopes of carbon (δ13C) and nitrogen (δ15N). The published literature suggests that riffle beetles in general are biofilm scrapers, but there is a diversity of biofilm scraping strategies that can be utilized by stream organisms. Thus, to determine if riffle beetles are utilizing organic matter or biofilms associated with organic matter, we collected *H. comalensis* and other organisms from sites where they are present for analysis of stable isotopes. Stable isotopes are a commonly-used method in diet studies, and the ratio of stable isotopes of carbon (13C:12C or δ13C) can be used to determine which food items a consumer it utilizing (Fry 2006; Boecklen et al. 2011; Layman et al. 2012).

In late June 2015, we collected macroinvertebrates (including adult and larval *H. comalensis*) by hand picking along Spring Run 3 and along Spring Island. Invertebrates were generally sorted in the field while alive and active and kept in vials containing water from the site for 1-2 hours to allow them to void their gut contents. Organisms were then brought back to the lab, sorted and then dried at 60^{o}C for 48 h in plastic weigh boats.

In addition to organisms, we also collected several types of basal resources that would potentially support consumers in the Comal food web. We collected in-stream detritus of two types that are present in the system: well-conditioned leaves (typically sycamore and pecan, *Carya illinoinensis,* leaves) and well-rotted wood. We chose to collect wood because *H. comalensis* (and the Comal Springs dryopid beetle, *Stygoparnus comalensis*) is frequently found on pieces of porous rotted woody material (R. Gibson and W.H. Nowlin, *pers. obs.*). Because wood was relatively rotted, we did not attempt to identify wood to species. Both leaf and wood samples were collected in at least triplicate from each location (SR3 and Spring Island). Wood and leaf material was stored in plastic bags and brought back to the lab, where it was dried at 60^{o}C for 48 h in plastic weigh boats. We additionally collected at least 6 relatively flat rocks from each location for algal biofilms. Rocks were placed in plastic bags within a cooler and brought back to the lab to remove biofilms. Biofilms were removed by scrubbing the upper rock surface with a nylon bristled brush and washing the material with DI water into a clean HDPE beaker. A portion of this slurry was then filtered onto ashed and pre-weighed 25-mm diameter

Whatman GF/F filters; for each rock, we collected two filters. One filter (to be used for δ15N analyses) was immediately placed into an oven and at dried at 60^{o}C for 48 h. The other filter was placed into a fuming HCl chamber for at least 8 hours to remove inorganic precipitated carbonates from the algal biofilms, which affect bulk δ13C values (Pound et al. 2011); this filter would be used for δ13C values only. After fuming, filters were dried at 60^{o}C for 48 h.

After all samples were sorted, treated and dried, we prepared samples for analysis at the University of California – Davis Stable Isotope Facility. All organisms, leaves, and wood samples were homogenized and ground to a fine powder using a mill grinder or a mortar and pestle that was thoroughly cleaned between samples. In a few cases, individual organism were of great enough mass for individual analysis, but in most cases we were forced to create composite samples of 2-10 individuals so that there was enough mass to run ^{13}C and ^{15}N analyses. For all organisms, leaves, and wood samples, we analyzed at least 3 and up to 6 independent samples for stable isotopes of C and N after packing homogenized material into tin capsules. We folded and packed algal filters into tin capsules for analysis.

Stable isotopes of C and N were analyzed using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Samples were combusted at 1000°C in a reactor packed with chromium oxide and silvered copper oxide. Following combustion, oxides were removed in a reduction reactor (reduced copper at 650°C). The helium carrier then flowed through a water trap (magnesium perchlorate) and the resulting N_2 and CO_2 were separated on a Carbosieve GC column (65°C, 65 mL/min) before entering the IRMS. Glass fiber filters were analyzed for ^{13}C and ^{15}N isotopes using an Elementar Vario EL Cube or Micro Cube elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Samples were combusted at 1000°C in a reactor packed with copper oxide and lead chromate. Following combustion, oxides were removed in a reduction reactor (reduced copper at 650°C). The helium carrier then flowed through a water trap (magnesium perchlorate). N_2 and CO_2 were separated using a molecular sieve adsorption trap before entering the IRMS. For each isotope run, samples were interspersed with several replicates of at least two different laboratory standards. The laboratory standards, which are selected to be compositionally similar to the samples being analyzed, were previously calibrated against NIST Standard Reference Materials (IAEA-N1, IAEA-N2, IAEA-N3, USGS-40, and USGS-41). Each sample's preliminary isotope ratio is measured relative to reference gases analyzed with each sample. These preliminary values are finalized by correcting the values for the entire batch based on the known values of the included laboratory standards. The long-term standard deviation during analysis is ± 0.2‰ for ^{13}C and ± 0.3‰ for ^{15}N. Final delta values are expressed relative to international standards Vienna PeeDee Belemnite for C and Air for N.

3.4.3.1 Data Analysis

Stable isotope data for the various basal food resources (leaves, rotted wood, and algae) and for consumer species (including *H. comalensis*) were initially compared between sites (Spring Run 3 and Spring Island) to determine if there were any longitudinal changes in stable isotope values for δ^{13}C and δ^{15}N as water moved from Spring Run 3 to the Spring Island area. Food resources of the same type and the same species were compared between sites using separate one-way ANOVAs for δ^{13}C and δ^{15}N values. We also initially compared the various potential basal food

web resources (leaves, rotted wood, and algae) within a given site using separate one-way ANOVAs for $\delta^{13}C$ and $\delta^{15}N$ values. We assessed assumptions of normality and homogeneity of variances prior to analysis and significance was inferred at $p \leq 0.05$.

In order to determine the likely basal food resources for consumers in the food web, we utilized a Bayesian inference approach to food source determination using the stable isotope composition of the various food sources and consumers. Bayesian approaches to isotopic mixing models are becoming more widely used (Layman et al. 2012) because, unlike traditional linear mixing models, they allow us to incorporate sources of variability within the model, permit the analysis of multiple dietary sources for consumers, and generate potential dietary solutions as true probability distributions (Parnell et al. 2010). Although, there is some debate about the relative importance and kinds of assumptions that must be made with the use of isotopic mixing models, many ecologists are utilizing Bayesian approaches because of their utility when compared to traditional models.

In order to determine the relative importance of different basal food resources to different consumers in the Comal Springs foodweb, we followed the "best practices" guidelines as described in Phillips et al. (2014). We ran all isotope mixing models in the program Stable Isotope Analysis in R (SIAR) Version 4 (Inger et al. 2014). We first identified the potential basal food resources as leaves, rotted wood, and epilithic algae (periphyton) and used these as our resources in models (see Results below). Trophic enrichment factors (TEFs) for the models were taken from a meta-analysis of the literature (Caut et al. 2009) and used the mean (± 1SD) trophic enrichment factors of 1.33 ‰ ± 0.454 and 2.75 ‰ ± 1.637 for ^{13}C and ^{15}N, respectively. These values are the Δ13C for all "freshwater organisms" and the "overall" Δ15N for all organisms reported by Caut et al. (2009). We ran isotope mixing models to determine the proportional contribution of each basal food resource to each consumer species in the food web. We initially ran separate models for the Spring Run 3 and Spring Island food webs, but here we only report on the results of the mixing model that was run on the overall Comal Springs system, which includes consumers from both reaches. However, some consumer species appeared to exhibit differences in the basal food resources between the two sites (Section 4.4) and these differences were identified when we ran the initial ANOVAs examining spatial differences in $\delta^{13}C$ and $\delta^{15}N$ values in the same consumer species.

4.0 Results

4.1 Potential Water Quality Differences between SMARC and FAB

Water quality conditions (e.g., temperature, DO, and conductivity) within each site did not exhibit any large variation or display any values that would indicate that conditions were inappropriate for holding riffle beetles. At SMARC, we logged conditions from March 2, 2015 until March 8, 2015 when the sonde discontinued gathering data (Figure 10). However, during this time period there was minimal loss of riffle beetles at SMARC (in contrast to FAB; see below), so we discontinued collecting water quality data from SMARC. During the period of data collection at SMARC the mean temperature was 20.62°C and only varied from 20.01 – 21.28°C (Table 1). In general, temperature increased but only by approximately 1°C over the time period. Dissolved oxygen and conductivity also exhibited minimal changes over the same

time period, with mean DO and conductivity being 5.05 mg/L (range = 4.59 – 6.01 mg/L) and 554 µS/cm (range = 539 – 564 µS/cm), respectively (Figure 10; Table 1).

Table 1. Mean, median, minimum, maximum, and the coefficient of variation (CV; as a percent) of the water quality parameters measured at SMARC and FAB during the period of observation at each location.

	SMARC			FAB		
	Temperature (oC)	Conductivity (µS/cm)	DO (mg/L)	Temperature (oC)	Conductivity (µS/cm)	DO (mg/L)
Mean, Median	20.62, 20.51	553.94, 562.00	5.05, 5.08	22.21, 22.25	574.45, 574.83	6.82, 6.83
Min, Max	20.01, 21.28	539.00, 564.00	4.59, 6.01	21.86, 22.45	559.00, 583.34	6.56, 7.06
CV	1.98	1.98	5.47	0.71	1.07	1.07

At FAB, water quality conditions were logged from March 2, 2015 until April 14, 2015, well after the conclusion of the survival trials (Figure 11; see survival trial results below). FAB wet lab water was also of relatively high quality (as determined by the measured parameters) and did not exhibit any wide temporal changes over the recoding period. FAB mean temperature was 22.21^{o}C and varied from 21.86 – 22.45^{o}C (Table 1). Over the time period, temperature of FAB water appeared to oscillate slightly, but this change was <1^{o}C over the time period. Dissolved oxygen and conductivity also exhibited minimal changes over the same time period, with mean DO and conductivity being 6.82 mg/L (range = 6.56 – 7.06 mg/L) and 574 µS/cm (range = 559 – 583 µS/cm), respectively (Figure 11; Table 1). Over the time period, the conductivity at FAB increased slightly (Figure 11), but this increase was minimal (~30 µS/cm) and could be entirely due to drift associated with the extended deployment of the sonde.

Although both SMARC and FAB exhibited fairly similar water quality conditions with similar lack of temporal variability, there were profound and striking differences in the survival of riffle beetle adults at each location. Of the 25 individual *H. comalensis*, *H. glabra*, and *M. pusillus* that were initially placed at SMARC on March 16, 2015 (for *H. comalensis* and *M. pusillus*) and March 23, 2015 (for *H. glabra*), only 1 *M. pusillus* died and all other individuals were alive after several weeks. In contrast, mortality of individuals of all three species was observed within 3 days of introduction to FAB. By March 31, 2015, all individuals of all three species at FAB had died.

Examination of the water quality data for FAB during this period does not reveal any rapid fluctuations or exhibit any values that would be immediately distressing for riffle beetles (i.e., drop of DO near 0 mg/L, sudden increase in temperature to >30^{o}C; Figure 11). Thus, at that point, we elected to continue and perform all subsequent laboratory studies associated with this project at SMARC.

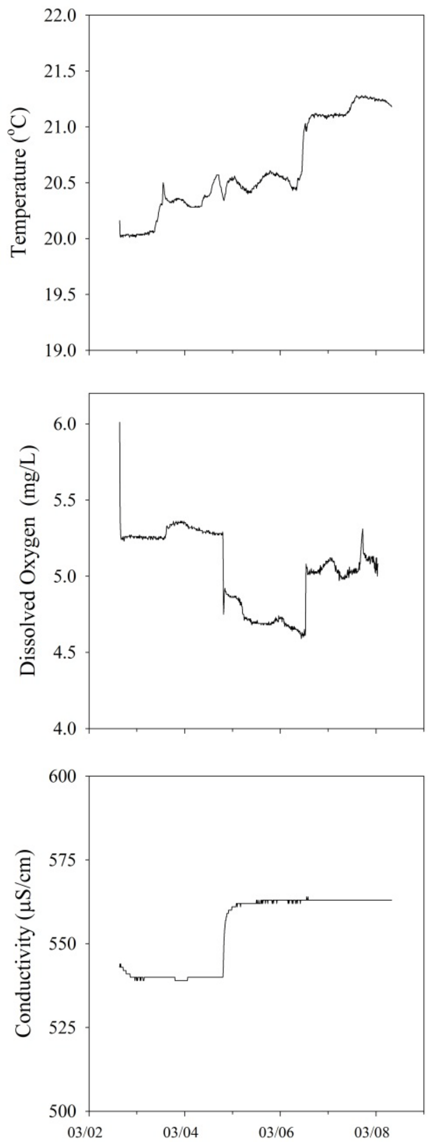

Figure 10. Temporal changes in the water quality parameters measured at SMARC during the experimental period. The data sonde terminated measurements on March 8, 2015; however, a majority of the beetles were still alive at SMARC facility more than 6 months after the period of observation ended.

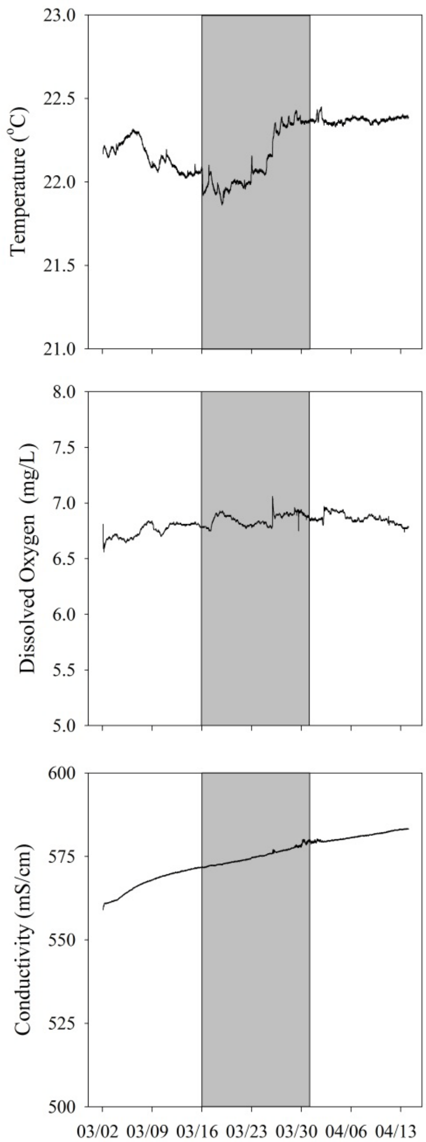

Figure 11. Temporal changes in the water quality parameters measured at FAB during the experimental period. The sonde measured water quality parameters throughout the period of beetle observation. The period of beetle observation is indicated by the shaded time period in each panel. All beetles were dead by the end of March.

4.2 Surrogate Testing for Effects of Water Temperature

During the experiment in which individuals of each species were exposed to gradually increasing water temperatures, the temperatures achieved in the experimental set-ups was relatively close to the desired set-points. In the beginning of the experiment and during the initial 7-day acclimation period, all individuals of all of species survived until the first increase in temperature (Figure 12). However, once the first temperature adjustment began and the temperature was increased to 26^{o}C, individuals of both *H. comalensis* and *H. glabra* died. As the temperature further increased over the 35-day experiment, both *H. comalensis* and *H. glabra* individuals died until all individuals were dead by 29^{o}C. In contrast, *M. pusillus* exhibited a different mortality pattern as temperatures increased (Figure 12). All *M. pusillus* individuals survived until 28^{o}C and then individuals began to quickly die after temperatures were increased beyond that threshold (all individuals were dead by 30^{o}C). These differences in temperature-dependent survival translated into significantly different temperatures among species for individual deaths ($F_{2, 12} = 4.84$; $p = 0.029$), with *M. pusillus* exhibiting significantly higher impairment temperatures than *H. glabra* (Tukey HSD; $p = 0.023$). However, results of the post-hoc analyses indicated that *H. comalensis* was not significantly different than *M. pusillus* (Tukey HSD; $p = 0.301$) or *H. glabra* (Tukey HSD; $p = 0.301$).

These relatively long-term temperature-dependent survival thresholds are consistent with previous work that examined upper temperature limits in *H. comalensis* and *H. glabra* (Nowlin et al. 2014). In relatively short-term temperature experiments (temperature change over a few hours) on these species, Nowlin et al. (2014) found that both *H. glabra* and *H. comalensis* exhibited evidence of stress (increased movement around the experimental chamber) when temperatures crossed a threshold of 28 – 31^{o}C. The same study conducted longer-term temperature experiments (change over a few days) with *H. glabra*, and found that LOR thresholds were 34 - 36^{o}C. Nowlin et al. (2014) concluded that *H. comalensis* and *H. glabra* likely experience physiological stress starting at temperatures ~28^{o}C and that the longer beetles spend at these temperatures, the effect is likely to be cumulative. Indeed, the “thresholds” we found for all three species in the present study are also in line with thresholds reported for other plastron utilizing beetle species (i.e., Harpster 1941, 1944).

In the present study, we found that *M. pusillus* exhibited a slightly higher mortality threshold temperature (onset of mortality at 29^{o}C) than the two *Heterelmis* species (mortality onset occurred at 26-27^{o}C). These results make sense in light of the types of habitats these species are associated with in natural systems. It has been hypothesized that organisms living in thermally stable environments, such as subterranean systems, the deep oceans, and spring-influenced ecosystems should be stenothermal (having a narrow thermal tolerance range) (Mermillod-Blondin et al. 2013). Both *H. glabra* used in this research and *H. comalensis* are associated with spring outflows, but *M. pusillus* is found in a variety of habitats often with less thermally-stable environments. Our results are consistent with this hypothesis and indicate that *M. pusillus* is less sensitive to longer-term temperature changes than *H. comalensis* and *H. glabra*. These results also suggest that *M. pusillus* is likely not the best candidate to serve as a surrogate species in physiological stress studies associated with variation in environmental conditions, especially water temperature.

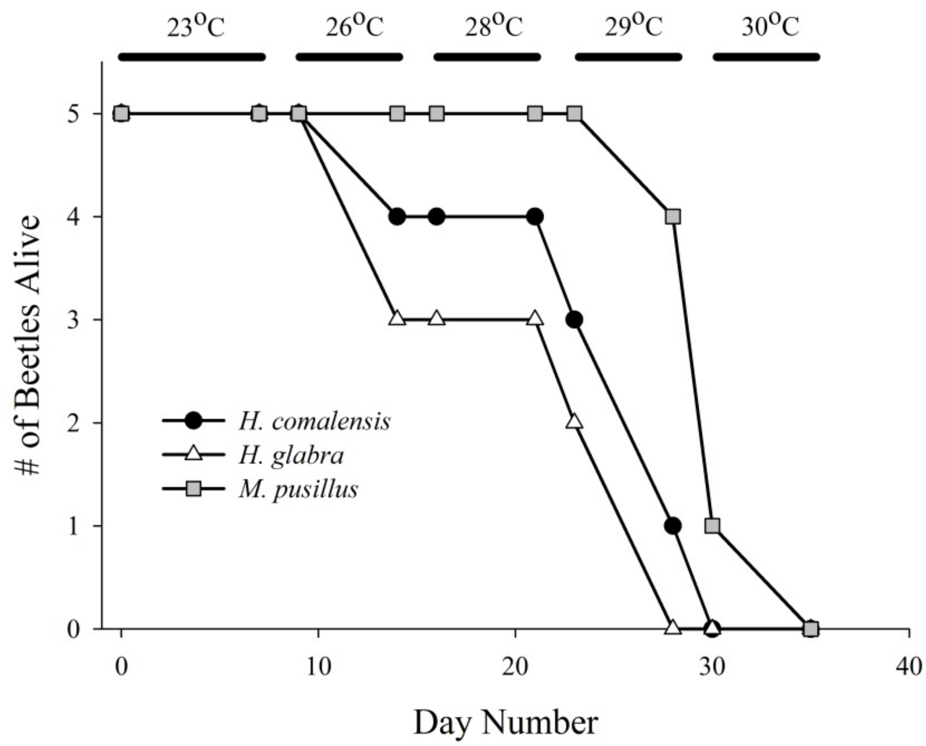

Figure 12. **Time series of the results of the longer-term gradual temperature increase surrogate testing study. The number of beetles alive (n = 5 initially) of each species is plotted as a function of time. The time period of consistent temperature maintenance is indicated by the dark lines across the top of the graph and the intervening short-duration periods of increasing to the next temperature are indicated by the gaps in the lines.**

4.3 Upwelling Habitat Connectivity

Discharge was represented as continuous water movement through each EFC at a constant rate that did not cause riffle beetle agitation. Several preliminary trials were conducted to determine levels at which beetles would become disoriented or dislodged from the mesh substrate and fall to the bottom of the EFC. Several trials were also conducted to make sure that the discharge rates would stay constant between treatments after water levels were dropped with flow exiting the EFC through the middle outlet versus the top of the EFC as for the connected treatments.

Overall, discharge within each EFC remained constant and similar to each other over the course of the experiment ranging from approximately 0.000084 to 0.000095 cfs. Discharge was measured in the EFCs as the time (approximately 37 to 42 seconds) it took to fill a 100 mL beaker. Measurements were based on an average of 5 reps conducted weekly over the course of the experiment in conjunction with the standard water quality parameter measurements.

Table 2 shows the standard water quality measurements (DO and temperature) that were taken weekly over the course of the experiment. As shown in Table 2, measurements across all treatments remained very consistent with DO ranging from 4.19 to 4.85 mg/L and water temperature from 21.4 to 22.2 °C over the course of the experiment. Figure 13 shows the continuous (10-minute intervals) thermister data from the RBASS inlet and outlet locations which further supports the water temperature conditions experienced within the EFCs.

Table 2. Standard parameter water quality results per treatment during the experiment.

Date	Time Range	EFC No. and Panel Section								
		EFC 1 Disconnected	EFC 2 Connected		5 Disconnected	8 Connected		9 Disconnected	12 Connected	
		lower	lower	upper	lower	lower	upper	lower	lower	upper
		Dissolved Oxygen (mg/L)								
10/6/2015	14:30 to 16:25	4.36	4.3	4.24	4.32	4.3	4.22	4.28	4.38	4.25
10/13/2015	13:47 to 14:04	4.81	4.75	4.69	4.85	4.72	4.66	4.71	4.78	4.67
10/20/2015	15:59 to 16:41	4.36	4.23	4.23	4.33	4.26	4.19	4.24	4.27	4.21
		Water temperature (°C)								
10/6/2015	14:30 to 16:25	21.6	21.6	21.6	21.9	21.6	21.7	21.5	21.8	21.8
10/13/2015	13:47 to 14:04	22.0	21.8	21.9	21.9	21.7	21.8	21.4	22.0	22.2
10/20/2015	15:59 to 16:41	21.7	21.5	21.5	21.6	21.6	21.7	21.5	21.8	21.8

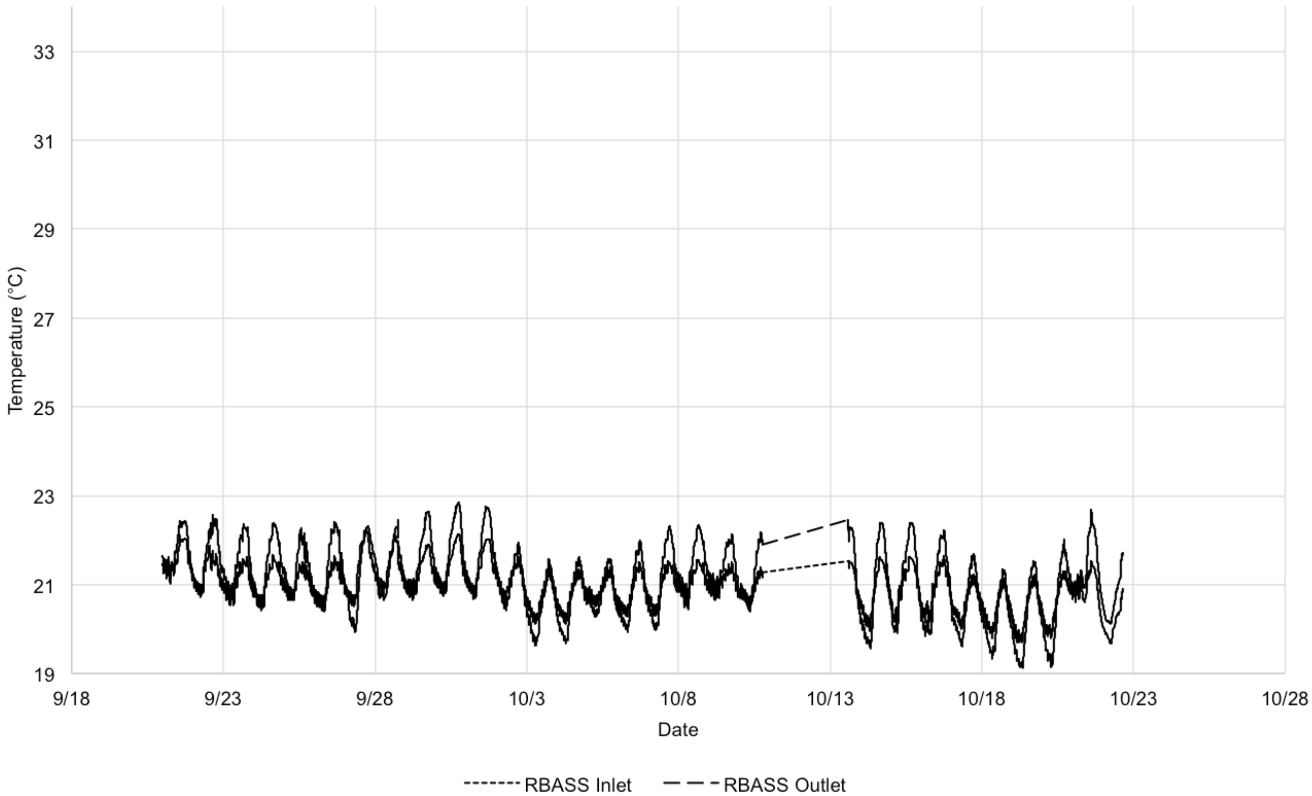

Figure 13. Time series plot of RBASS inlet and outlet temperature (°C) over course of experiment.

Following the termination of the study on October 21, 2015, the experiment tear down procedure was performed as follows. The beetle position within each SOL was noted. Each SOL was removed and cut with scissors and placed in a labeled cup. Each SOL was carefully taken apart and leaves examined for beetles, leaf by leaf. The EFC mesh (substrate) panel was then removed and placed on a labeled white tray. Beetle locations and activity of each beetle (i.e. "active" = crawling around; "inactive' = motionless) was logged to the extent practicable. Once retrieved all beetles were placed in cups with refugia water (Figure 14). Any beetles remaining in the EFC were removed via a long-handled acrylic fan-bristle brush (Figure 14). The number of beetles was counted and recorded, locations logged and all recovered beetles were placed in recovery bags inside the refugia tank. Recovery bags consisted of aquarium filter bags labeled for each EFC tank which were subsequently held in the refugia for 24 hours, before final determination of survival. Upon completion of the study, all live beetles were donated to SMARC.

Figure 14. Comal Springs riffle beetles during experiment tear down.

Table 3 presents the survival results from the experiment. Twenty-six of 27 beetles survived in EFCs that maintained flowing water connection through the SOL, whereas a total of 7 beetles died in the EFCs that were disconnected via water flow from the SOLs. Results of the one tailed test of proportions supported the conclusion that survival was higher for individuals in connected treatments ($p = 0.028$). Even when the data is subjected to a less powerful 2 tailed test it is still significant at the 90% level ($p=0.055$).

Table 3. Results of upwelling connectivity study in terms of survival per treatment.

EFC No.	Treatment	Comal Springs riffle beetles		
		Stocked	Survived	Percent survival
1	Disconnection	9	9	100
2	No disconnection	9	8	89
5	Disconnection	9	5	56
8	No disconnection	9	9	100
9	Disconnection	9	6	67
12	No disconnection	9	9	100
	Total	54	46	85

SUMMARY		
Treatment	Average Survival	STDEV
Disconnection	74.07	23.13
No disconnection	96.30	6.42

Qualitative beetle spatial location data was recorded by marking observed beetle positions within RBASS EFCs both in a logbook chart and on the clear acrylic EFC surface itself every other day. When practical, visual identification was conducted under the cover to minimize potential disturbance to the beetles from light exposure (Figure 15). Even so, limitations of this method include: (1) the impossibility of identifying individual beetles (and thus their movement) and (2) difficulty in ascertaining whether some beetles were dead or simply inactive when making observations through the EFC surface (this determination is best performed once beetles have been removed from EFCs and can be examined closely). Because of these limitations, we found only limited opportunity for analysis of beetle movement patterns, and have restricted the interpretation of this data to a brief qualitative discussion presentation of all movement data (Figure 16, Appendix A).

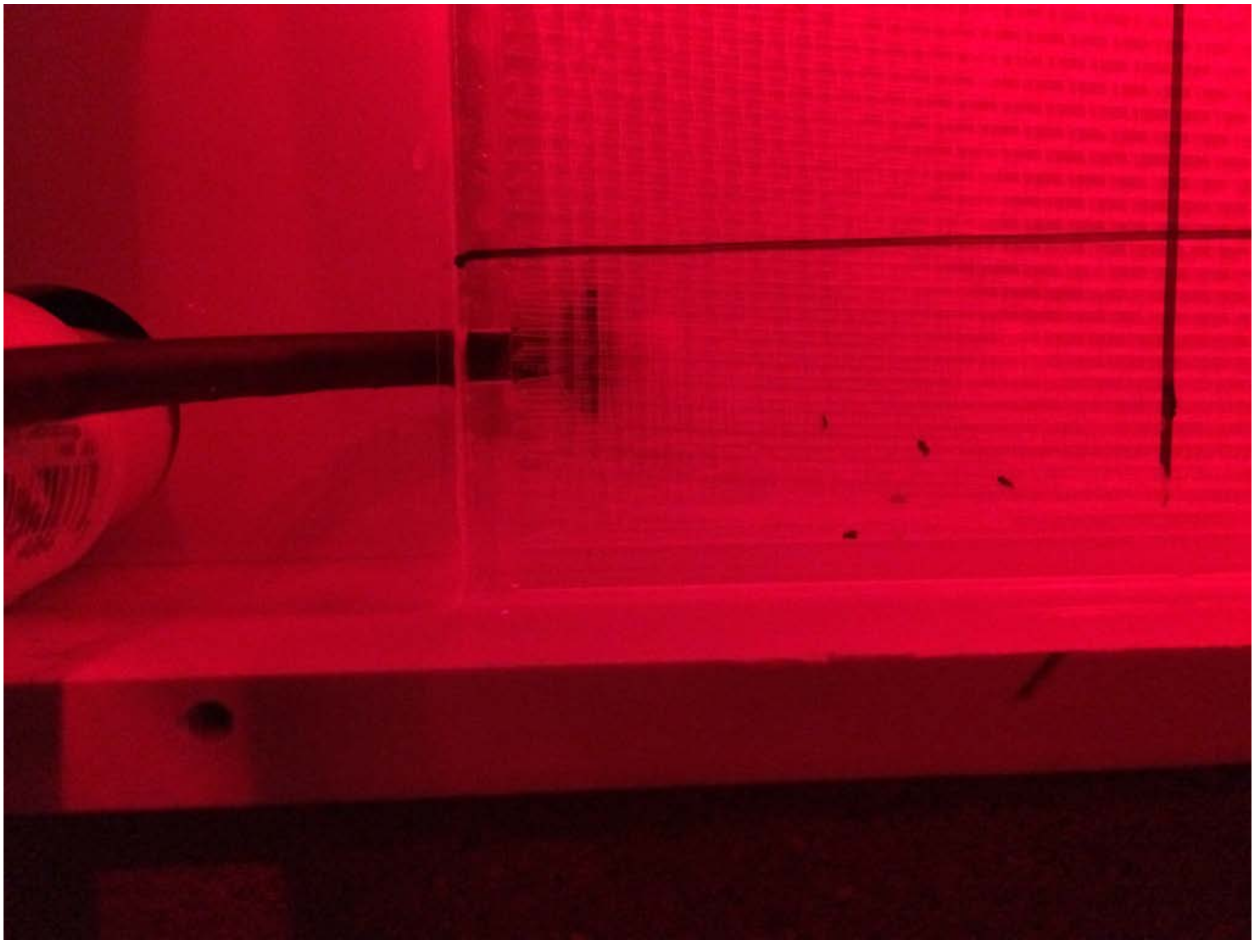

Figure 15. Location documentation conducted under the cover.

In general, qualitative observations and documentation over the course of the experiment suggest that beetles in EFCs with flowing water connected to the SOL tended to explore the entire tank, but often returned to the SOL. As such, a lot of the documented observations for these treatments show very few beetles outside of the SOL. In contrast, the beetles in the EFCs that were disconnected from the SOL spent the majority of time in the concentrated flow around the inlet and outlet in their respective tanks. We speculate that this might have occurred for a couple of reasons. The first is that the screen material blocking the outlet did build up a small amount of organic material over the course of the study. We mention this in that our 2014 riffle beetle work with *H. glabra* (BIO-WEST 2014) often had beetles attaching themselves above the water line to fine mesh that had organic material on it. Another possible explanation is that in the absence of organic matter, the beetles were simply attracted to areas of water flow / current.

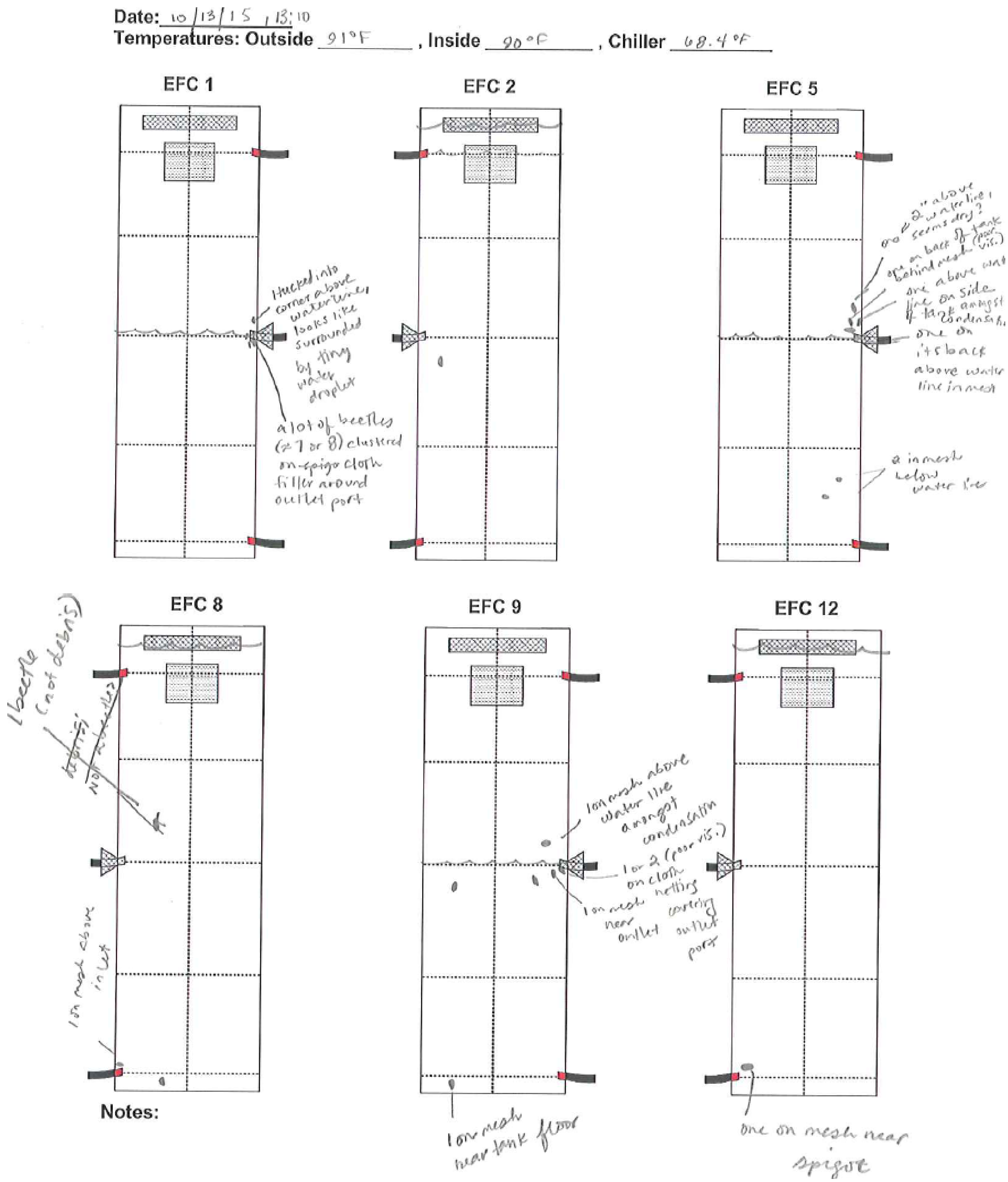

Figure 16. Beetle spatial location observations from October 13, 2015.

4.4 Lateral Habitat Connection and Diet Study

Passive Sampling Pit Study

In the passive sampling pit study, the installed wells collected relatively few organisms over the three-week period (Table 4). Although this time period is consistent with other studies (e.g., Clinton et al. 1996), the numbers of organisms was relatively low (1 – 4 individual taxa collected per pit). In total, pits only collected three taxonomic groups or species (*Stygobromus pecki*, *Psephenus* sp., and Lumbriculid worms) from depths of 20 – 25 cm. Although differences in the number of each species and the total number of invertebrates among pits with and without lures did not significantly differ (one-way ANOVA; $F_{1,5} \geq 3.00$, $p \geq 0.116$ for all analyses), organisms were only collected from pits without cotton-poly lures. Most importantly, however, we did not collect any adult or larval riffle beetles in sampling pits over the three-week period. Although all of these sites are commonly associated with the presence of riffle beetle adults and larvae, the low densities of individuals we collected in samplers and the lack of riffle beetles in this portion of the study (the initial pilot study), we elected to not continue with a more complex and labor intensive monitoring of riffle beetle lateral and vertical movement within the benthos.

Table 4. The mean number (min – max) of invertebrates collected over a 3-week period in passive sampling pits without cloth lures and with cloth lures in Spring Run 3.

	W/O Lure (n = 3)	W/ Lure (n = 3)
S. pecki	0.7 (0 - 1)	0 (0)
Psephenus	2.0 (0 - 4)	0 (0)
Lumbriculidae	0.7 (0 - 1)	0 (0)

Bou-Rouch Collection Study

In the portion of the study that preliminarily examined the efficacy of using a Bou-Rouch sampler to assess vertical and lateral movement of riffle beetles within substrates, we collected eight samples from locations in which adult and larval beetles are frequently encountered. Much like the collections made with the passive sampling pits, the numbers of organisms collected per sample was relatively low (Table 5). The specific taxa collected with the Bou-Rouch were similar to those collected with the sampling pits, but overall number of taxa per sample was in general greater in Bou-Rouch samples. Overall, we detected a total of 5 species/taxa within samples (*Stygobromus* sp., *Psephenus* sp., Lumbriculid worms, *Lircelolus* sp., and *Tarebia granifera*). There were no significant differences between the number of individual taxa and the total number of individuals across all taxa when the samples collected with CO_2 and those collected without CO_2 were compared (one-way ANOVA; $F_{1,7} \geq 0.18$, $p \geq 0.356$ for all analyses).

Table 5. The mean number and range (min – max) of invertebrates in Spring Run 3 collected with a Bou-Rouch sampling without the addition of CO_2 prior to pumping and with the addition of CO_2 prior to pumping.

Treatment	Taxon	Mean	Range
No CO_2	*Stygobromus* sp.	0.25	(0 - 1)
	Psephenus sp.	0.25	(0 - 1)
	Lumbriculidae	0.5	(0 - 1)
	Lirceolus spp.	0.25	(0 - 1)
	Tarebia granifera	0.25	(0 - 1)
	Total invertebrates		(0 - 3)
CO_2 added	*Stygobromus* sp.	0.25	(0 - 1)
	Psephenus sp.	0.5	(0 - 2)
	Lumbriculidae	0.25	(0 - 1)
	Total invertebrates	1	(0 - 4)

Although we took into account and standardized several of the critical variables that can influence the capture/collection rate of Bou-Rouch samples (e.g., volume collected, pore size of the sampler), we did not directly account for other variables that might affect collection efficiency, such as variation in sediment porosity and a direct estimate of the rate of sample collection (i.e., pumping rate) (Hunt and Stanley 2000). Our estimated pumping rates (approximately 4-6 L/min) were relatively high and should have produced higher invertebrate yields (Hunt and Stanley 2000). Most importantly, however, we did not collect any adult or larval riffle beetles in samples. Given the absence of riffle beetles in the samples we collected, we elected to not continue with a more complex and labor intensive monitoring of riffle beetle lateral and vertical movement within the benthos using the Bou-Rouch method. The lack of beetles in Bou-Rouch samples could be a consequence of several morphological or physiological adaptations of the beetles, including the ability of beetles to cling tightly to flowing surfaces and an ability to tolerate relatively high CO_2 levels in an already CO_2 saturated environment.

General Comments on Passive Collection Methods and Future Riffle Beetle Studies

Current techniques for collection of *H. comalensis* are limited to two main methods: the use of cotton-poly lure cloths and through hand-picking them from surfaces. Intensive searching efforts indicate that riffle beetles in the Comal system tend to be of relatively low abundance (a few individuals per square meter; R. Gibson, *pers. obs.*), so digging by hand through substrates and hand picking individuals is very time and labor intensive. Thus, this technique is likely not the most efficient when trying to collect riffle beetles for monitoring or population/density estimates. Indeed, it may take several hours of searching and disturbing the substrates around spring openings to collect a few individuals (W.H. Nowlin, *pers. obs.*). However, hand-picking may be the preferred method if there is a need to collect a few individuals or if an investigator is not concerned with disturbing substrates.

The use of cloth lures is now the "established" method for monitoring beetle numbers in the Comal system because beetles tend to congregate on the lures at densities higher than the

surrounding environment. However, the lure presumably serves as an attractant and therefore concentrates beetles in a given location, thereby affecting the ability of investigators to make reliable estimates of population density or size. Thus, there has been an increased interest in trying to find "passive" sampling methods for beetles that would allow researchers and management personnel to gain increased ability to make reliable density estimates. Based upon the two preliminary studies we conducted, it appears that passive sampling pits or wells and Bou-Rouch samples are not likely to be efficient methods to estimate beetles.

Stable Isotope Study

As a part of the stable isotope study, we collected individuals of 13 different taxa for analysis, including several species that are of concern in the EAHCP (i.e., *Stygobromus pecki*, *Stygoparnus comalensis*, and *H. comalensis*) (Table 6). The suite of taxa included a diversity of different feeding types, so that we could elucidate most of the major feeding groups within the invertebrates found in the Comal system. We initially assessed the food web at the two sampling locations separately (Spring Run 3 and Spring Island). We analyzed a total of 10 taxa in both Spring Run 3 and Spring Island.

The food web of Spring Run 3 included a diversity of consumers that utilized differing proportions of the three *a priori* identified basal food resources (i.e., periphyton, conditioned leaf litter, and partially rotted wood). In terms of the basal food resources that likely support the food web, in Spring Run 3, both the $\delta^{13}C$ and $\delta^{15}N$ values differed among the basal food resources ($\delta^{13}C$: one-way ANOVA, $F_{2,9} = 15.18$, $p = 0.001$; $\delta^{15}N$: one-way ANOVA, $F_{2,9} = 3.98$, $p = 0.047$) (Figure 17). Periphyton $\delta^{13}C$ in periphyton was relatively deplete (-31.5‰) when compared to wood (-27.31‰). However, well-conditioned leaf material exhibited $\delta^{13}C$ values (-30.62‰) that did not significantly differ from that of periphyton (Tukey HSD, $p > 0.05$), but was significantly different from that of wood (Tukey HSD, $p < 0.05$). In contrast, the $\delta^{15}N$ values of periphyton and wood were not different from one another (Tukey HSD, $p < 0.05$), while leaves had a significantly enriched $\delta^{15}N$ when compared to wood and periphyton (Tukey HSD, $p < 0.05$).

The consumers in Spring Run 3 appeared to be grouped into two general food chains within the overall food web. One group of consumers, which was composed of *Psephenus* sp., *M. pusillus* (adults and larvae grouped together), baetid mayfly nymphs, and helicopsychid caddis fly larvae, were likely feeding directly on periphyton. In contrast, the other main food chain was likely feeding on wood-based biofilms and was composed of *H. comalensis* (adults and larvae grouped together) and *Hyalella*. Elevated above these two groups (as indicated by relatively enriched $\delta^{15}N$ values were snail species (*Elimia*, *Tarebia*, and *Melanoides*). Snails in stream systems often exhibit somewhat elevated $\delta^{15}N$ values (e.g., Pound et al. 2011) because they may be less selective grazers than other algivorous invertebrate groups, such as psephenids (Anderson and Cabana 2007). *Stygobromus pecki* was the consumer exhibiting the highest trophic position in the SR 3 community, indicating that it is likely predatory on other invertebrates in the system.

Table 6. Mean and ± 1 SE values, and the number of samples analyzed to determine δ13C and δ15N of consumers and potential basal food resources collected at the two sites (Spring Run 3 and Spring Island) in the Comal system. Consumers with an * after their name had an *n* = 1 and were not analyzed within an individual site, but were combined into the same taxonomic group when the entire Comal system food web was analyzed. Species with (all) indicates the mean value of adults and larvae were pooled for the individual site, but adult and larvae were split out for the entire Comal system analysis.

Spring Run 3

Consumer	δ13C ‰	± 1 SE	*n*	δ15N ‰	± 1 SE	*n*
Baetidae	-34.814	0.223	4	4.682	0.153	4
Elimia sp.	-32.855	0.516	3	7.771	0.069	3
Helicopsychidae	-34.042	0.367	5	4.535	0.247	5
Heterelmis comalensis (all)	-26.674	1.618	3	4.965	0.430	3
Hyalella azteca	-27.997	0.971	4	5.330	0.094	4
Melanoides tuberculata	-28.066	0.683	3	7.682	0.275	3
Microcylloepus pusillus (all)	-35.067	0.152	5	4.471	0.241	5
Psephenus sp.	-35.142	0.202	4	3.277	0.303	4
Stygobromus pecki	-27.761	0.726	4	9.404	0.225	4
Tarebia granifera	-31.045	0.509	3	8.218	0.206	3
Source						
Leaves	-30.619	0.360	3	5.670	1.018	3
Perphyton	-31.591	0.722	4	3.729	0.198	4
Wood	-27.086	0.132	6	4.026	0.069	3

Spring Island

Consumer	δ13C ‰	± 1 SE	*n*	δ15N ‰	± 1 SE	*n*
Baetidae*	-31.053	--	1	6.994	--	1
Elimia sp.	-32.214	0.400	4	6.910	0.148	4
Helicopsychidae	-33.117	0.452	4	4.570	0.114	4
Heptageniidae	-30.574	0.972	4	6.036	0.172	4
Heterelmis comalensis (adult)	-26.559	0.441	3	4.571	0.089	3
Heterelmis comalensis (larvae)	-27.349	0.321	5	4.817	0.151	5
Hyalella azteca	-32.005	0.191	3	5.596	0.182	3
Microcylloepus pusillus (adult)	-33.711	0.504	5	4.434	0.065	5
Microcylloepus pusillus (larvae)*	-32.438	--	1	4.683	--	1
Psephenus sp.	-33.699	1.610	5	2.940	0.353	5
Stygobromus pecki	-30.339	0.462	4	9.275	0.293	4
Stygoparnus comalensis	-24.230	0.059	4	5.564	0.115	4
Tarebia granifera	-31.246	0.260	4	6.937	0.166	4
Source						
Leaves	-29.314	0.077	3	3.966	0.096	3
Perphyton	-31.435	1.192	4	4.138	0.144	4
Wood	-27.086	0.132	6	4.026	0.069	3

The food web in the Spring Island section of the Comal system was similar to the patterns found in Spring Run 3 (Figure 17). Again, the basal resources (periphyton, leaves, and wood) exhibited significantly different $\delta^{13}C$ values (one-way ANOVA, $F_{2, 12} = 13.04$, $p = 0.002$). Again, wood exhibited significantly enriched $\delta^{13}C$ values when compared to periphyton (Tukey HSD, $p < 0.05$), but was not significantly different from leaves (Tukey HSD, $p > 0.05$). In addition, leaf periphyton $\delta^{13}C$ values were intermediate to those of periphyton and wood and not significantly differ from those of periphyton $\delta^{13}C$ values (Tukey HSD, $p > 0.05$). In contrast, the $\delta^{15}N$ values did not differ among sources in the Spring Island site (one-way ANOVA, $F_{2, 9} = 0.570$, $p = 0.590$). The consumers in the Spring Island food web exhibited a similar configuration to SR3. Again, *Psephenus*, *M. pusillus*, and Helicopsychidae were associated with periphyton and larval and adult *H. comalensis* were more closely associated with wood. In addition, *Stygoparnus comalensis* was also closely associated with wood, as indicated by its enriched $\delta^{13}C$ value. Heptageniid mayfly nymphs and the snails *Tarebia* and *Elimia* were more associated with periphyton (as indicated by $\delta^{13}C$ values), but exhibited a higher estimated trophic position as estimated by $\delta^{15}N$ values. Again, less-selective grazers Heptageniidae and snails often exhibit higher $\delta^{15}N$ values than other grazers such as psephenids (Anderson and Cabana 2007).

However, in contrast to the SR3 site, the two amphipods (*Hyalella* and *Stygobromus pecki*) appeared to shift over more toward the periphyton-based food chain at the Spring Island site, with the $\delta^{13}C$ values of both of these consumers getting significantly more deplete (and therefore more reflective of an algal-based signature) in the downstream Spring Island site (*Hyalella*: one-way ANOVA: $F_{1, 7} = 16.40$, $p = 0.007$; *S. pecki*: one-way ANOVA: $F_{1, 7} = 8.97$, $p = 0.024$). The shift in basal resources for these two species may indicate some spatial variation and plasticity in their feeding strategy as the canopy cover opens up downstream at Spring Island.

We then combined the data from both sites to create the food web for the overall Comal system (Figure 18), but still reflected the shift in basal resource food chains associated with *S. pecki* and *Hyalella*. Again, in the overall Comal system food web, the two main food chains based upon two most differing basal food resources (periphyton versus wood) were apparent, with *H. comalensis* adults and larvae, *Stygoparnus comalensis*, *Hyalella* at SR3, *Melanoides*, and *Stygobromus pecki* at SR 3 associated with the wood biofilm based food chain. The remaining consumers in the food web and *Hyalella* and *S. pecki* at Spring Island were largely associated with the periphyton-based basal resource food chain.

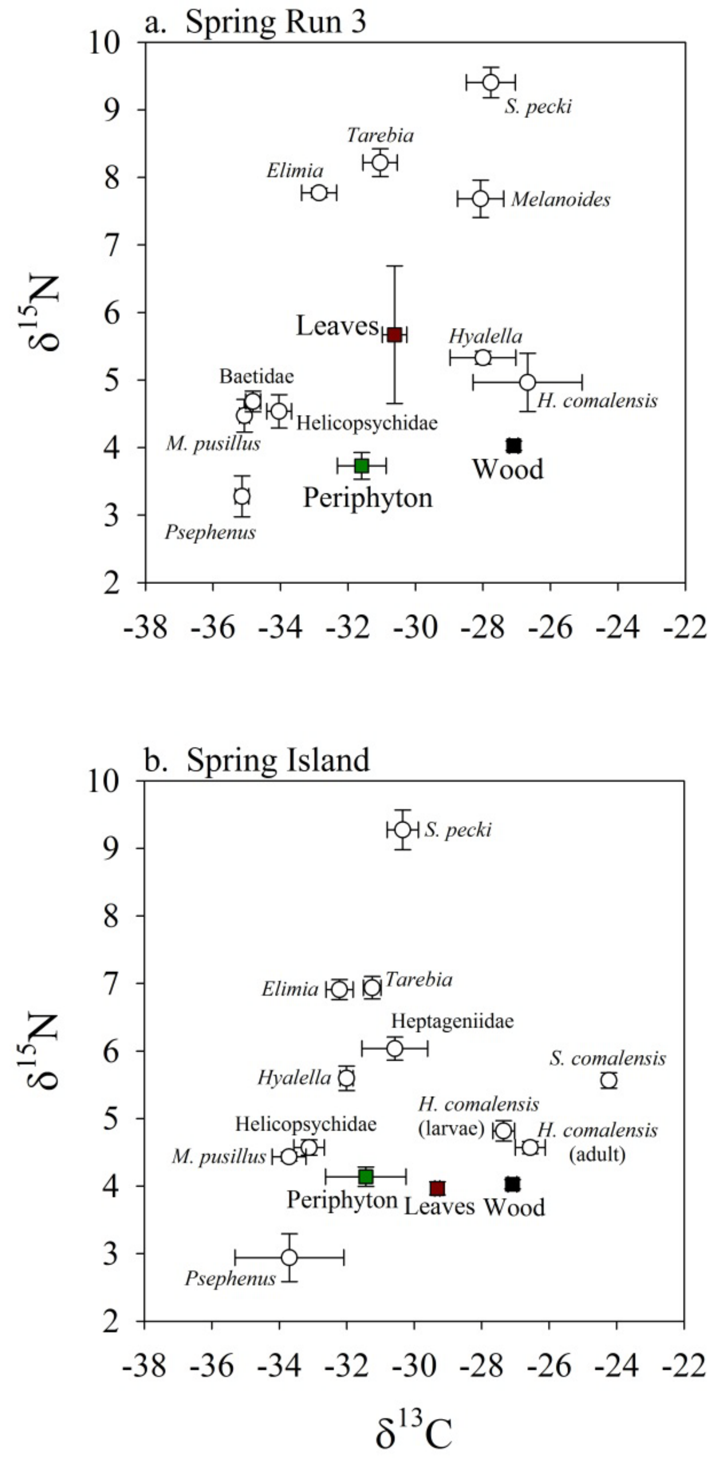

Figure 17. **Isotope bi-plots for consumers and basal food resources in (a) Spring Run 3 and (b) Spring Island. Each point represents the mean δ13C and δ15N values for each consumer or source (bars are ± 1 SE). See Table 6 for raw data.**

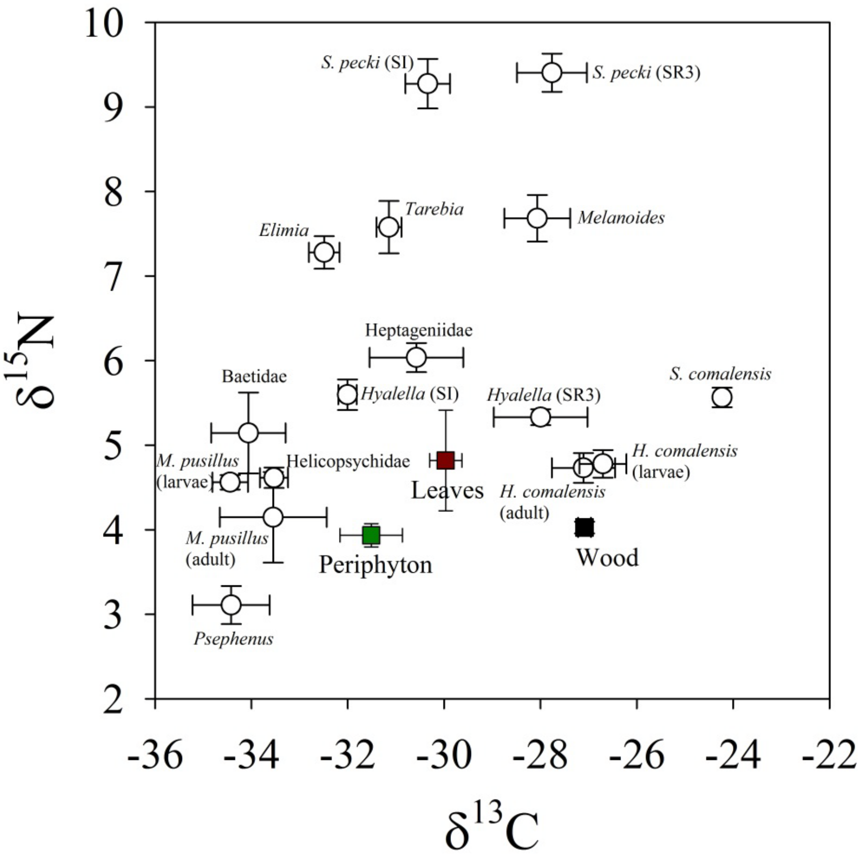

Figure 18. Isotope bi-plots for consumers and basal food resources in the entire Comal system. Each point represents the mean δ13C and δ15N values for each consumer or source (bars are ± 1 SE). Mean δ13C and δ15N values for *Hyalella* and *Stygobromus pecki* in Spring Island (SR3) and Spring Island (SI) have been plotted separately because δ13C for these two species (and presumably the basal food resource) differed significantly between SR3 and SI (see text for analysis).

When the combined data set was run in SIAR, the model output for each of these species generally reflected the differences that were apparent in the isotope bi-plot (Table 7). In this report, we provide the mean, mode and 95% credibility intervals for the model outputs for each consumer in the Comal food web; the actual posterior probability distributions and matrix plots are available upon request. The mean of the posterior probability distributions for *Psephenus*, helicopsychid caddis flies, *Tarebia*, and *M. pusillus* adults indicated that these consumers had diets that consisted of materials that was >50% of periphyton origin; this result clearly suggests that a majority of the diet of these consumers is periphyton-derived. In addition, there were several other consumers (*Hyalella* in Spring Island, *S. pecki* in Spring Island, *Elimia*, Heptageniidae, Baetidae, and *M. pusillus* larvae) who's posterior probability distributions indicated that periphyton-derived matter made up the largest single contributor of their diet (mean values ranging from 36 – 45% of the diet). In contrast, SIAR runs for several consumers indicated a large contribution of terrestrial-derived materials (wood and leaves combined) was

the resource base supporting their diets. Posterior probability distributions indicated that the majority of the diets of both *Hyalella* and *S. pecki* in SR3 were derived from terrestrial detritus (64 and 72%, respectively). *Stygoparnus comalensis* had a proportional diet contribution of 44% from only wood and 71% from both types of terrestrial materials. Finally, model results indicate that *H. comalensis* adults and larvae derive 80% and 73% of their diet from terrestrial-derived materials (wood and leaves combined). In particular, *H. comalensis* larvae were the consumer in the Comal system which exhibited the highest proportional contribution of wood-derived materials to its diet (mean contribution of 59%).

Table 7. Summary output data from SIAR model runs examining the percent contribution of various basal resources to the isotopic signatures of consumers in the Comal system food web. Summary results presented are the mean and mode percent contribution of each source to each consumer after the model run for each consumer (n of iterations = 30,000), as well as the upper and lower values of the 95% credibility intervals. Posterior probability distributions and matrix plots for each consumer are available upon request.

	Leaves		Periphyton		Wood	
Consumer	Mean, Mode	95% CI	Mean, Mode	95% CI	Mean, Mode	95% CI
Baetidae	28%, 35%	0 - 60%	45%, 42%	3 - 86%	25%, 5%	0 - 56%
Elimia sp.	32%, 36%	2 - 56%	47%, 44%	15 - 84%	21%, 4%	0 - 46%
Helicopsychidae	19%, 4%	0 - 49%	68%, 87%	32 - 99%	13%, 3%	0 - 40%
Heptageniidae	28%, 31%	0 - 56%	47%, 45%	11 - 87%	25%, 6%	0 - 52%
Heterelmis comalensis (adult)	25%, 5%	(0 - 53%)	27%, 25%	0 - 51%	48%, 46%	19 - 80%
Heterelmis comalensis (larvae)	21%, 5%	0 - 48%	20%, 16%	0 - 44%	59%, 64%	31 - 89%
Hyalella azteca (SR3)	28%, 32%	0 - 58%	36%, 38%	2 - 66%	36%, 38%	3 - 64%
Hyalella azteca (SI)	27%, 31%	0 - 58%	49%, 45%	11 - 91%	24%, 5%	0 - 53%
Microcylloepus pusillus (adult)	25%, 4%	0 - 55%	55%, 46%	18 - 96%	21%, 4%	0 - 49%
Microcylloepus pusillus (larvae)	33%, 35%	0 - 65%	36%, 39%	0 - 69%	31%, 34%	0 - 62%
Psephenus sp.	28%, 35%	0 - 58%	50%, 44%	12 - 93%	22%. 5%	0 - 50%
Stygobromus pecki (SR3)	38%, 39%	0.5 - 72%	28%, 32%	0 - 53%	35%, 37%	3 - 61%
Stygobromus pecki (SI)	39%, 42%	0.5 - 71%	47%, 47%	7 - 84%	15%, 3%	0 - 41%
Stygoparnus comalensis	27%, 7%	0 - 57%	29%, 36%	0 - 60%	44%, 40%	3 - 85%
Tarebia granifera	31%, 33%	0.8 - 58%	55%, 50%	21 - 92%	14%, 3%	0 - 38%

These results indicate that inputs of terrestrial-derived materials are likely to be particularly important for the diet (and thus conservation) of *H. comalensis* and *S. comalensis*. Both of these species are commonly found on decaying woody material in the field and individuals can be maintained in the lab by supplying well-conditioned leaf material (W.H. Nowlin and R. Gibson, *unpublished data*). Reliance on wood-associated biofilms is not a new observation in riffle beetles. Seagle (1982) found that the gut contents of larvae and adults of three riffle beetle species (*Stenelmis crenata*, *Stenelmis mera*, and *Optisoservus trivittatus*) were dominated by detritus-like materials, including wood xylem, unidentified organic matter, and mineral particles, while algal material was consumed to a much lesser extent. Thus, it has been suggested that elmids as a whole should be reclassified as detritivores-herbivores rather than as strictly herbivores, with the exception of known xylophagus genera (i.e., *Lara*) (Seagle 1982).

The results of this study suggest that a lateral connection to terrestrial matter sources may be particularly important for two of the species listed under the EAHCP: *Heterelmis comalensis* and *S. comalensis*. Lower spring discharge and declining flows can lead to disconnection of the

aquatic environment from the bank and to lower water surface areas of aquatic habitats, potentially leading to decreased inputs of terrestrial material that may support populations of these wood-associated taxa. These results also support the importance of a well developed riparian area essential for the conservation of the endangered Comal beetles stated as Primary Constituent Element (PCE) 4 in the critical habitat designation (USFWS 2007) and PCE(3) in critical habitat revision (USFWS 2013). In addition, decreased flow rates and water velocities could also lead to lower rates of downstream transport of terrestrial materials from more canopy-covered upstream areas (e.g., the more canopy-covered Spring Runs) to more open areas with lower rates of terrestrial inputs (e.g., Spring Island area).

Results from our study indicate that *Stygobromus pecki*, another spring-associated consumer covered under the EAHCP may have a more plastic feeding strategy than *H. comalensis* and *Stygoparnus comalensis* and may be able to switch to an alternate basal resource food chain as environmental conditions vary spatially. However, our results indicate that the feeding strategy of *H. comalensis* and *S. comalensis* appear to be largely dependent upon wood- and leaf-based biofilms, and this dependence doesn't change between sites. Therefore, the feeding strategy of these two species do not appear to respond to spatial changes in the canopy cover and presumably amount of terrestrial detritus inputs. Thus, downstream populations of *H. comalensis* and *S. comalensis* may be particularly sensitive to lower inputs of terrestrial detritus material. Our results also suggest that *M. pusillus* may not be an appropriate surrogate for *H. comalensis* in that the diet of *M. pusillus* was much more reliant upon periphyton and therefore feeds on an entirely different food chain.

5.0 Conclusions

In conclusion, this study conducted water quality evaluations and survival trials to establish a laboratory location for 2015 riffle beetle experimentation, a subsequent evaluation of potential surrogate beetle species, and a series of laboratory and field investigations examining key components of upwelling and lateral habitat connectivity of *H. comalensis*.

Although both SMARC and FAB exhibited fairly similar water quality conditions with similar lack of temporal variability, there were striking differences in the survival of riffle beetle adults with extensive mortality at FAB. As such, all subsequent 2015 riffle beetle laboratory studies associated with this project were conducted at SMARC. In response to the unknown causes for this mortality concern, Dr. Nowlin and Texas State University (both outside of this project) have been working closely with the Edwards Aquifer Authority to determine the cause for the adult mortality and how to manage the issue. It is currently established that the downstairs FAB Holding House water does not cause mortality in adult beetles and that the Wet Lab water does not cause mortality for adults when it is first passed through a flow-through activated charcoal filter. Assurances from Texas State University Facilities have been granted that they will work to determine the source of the problem, fix the problem, and that they will supply whatever charcoal filtration needs are required so that researchers can successfully conduct any future riffle beetle research at FAB.

The surrogate evaluations demonstrated that the relatively long-term temperature-dependent survival thresholds were consistent with previous work that examined upper temperature limits

in *H. comalensis* and *H. glabra* (Nowlin et al. 2014). Additionally, the "thresholds" found for all three species tested in the present study are also in line with thresholds reported for other plastron utilizing beetle species (i.e., Harpster 1941, 1944). In the present study, we found that *M. pusillus* exhibited a slightly higher mortality threshold temperature than the two *Heterelmis* species. These results make sense in light of the types of habitats these species are associated with in natural systems. It has been hypothesized that organisms living in thermally stable environments, such as subterranean systems and spring-influenced ecosystems should be stenothermal (having a narrow thermal tolerance range) (Mermillod-Blondin et al. 2013). Our results are consistent with this hypothesis and indicate that *M. pusillus* is less sensitive to longer-term temperature changes than *H. comalensis* and *H. glabra*. These results also suggest that *M. pusillus* is likely not the best candidate to serve as a surrogate species in physiological stress studies associated with variation in environmental conditions, especially water temperature.

The use of cloth lures is now the "established" method for monitoring beetle numbers in the Comal system because beetles tend to congregate on the lures at densities higher than the surrounding environment. However, the lure presumably serves as an attractant and therefore concentrates beetles in a given location, thereby affecting the ability of investigators to make reliable estimates of population density or size. Thus, there has been an increased interest in trying to find "passive" sampling methods for beetles that would allow researchers and management personnel to gain increased ability to make reliable density estimates. Based upon the two preliminary studies we conducted, it appears that passive sampling pits or wells and Bou-Rouch samples are not likely to be efficient methods for monitoring beetles.

Results from the upwelling habitat connectivity study conducted in SMARC laboratory using the RBASS revealed a statistically significant difference with greater survival in treatments connected to organic matter than in treatments that were disconnected via water flow to organic matter over the course of the study. Additionally, qualitative observations of beetle movement over the course of the upwelling experiment suggest that beetles in EFCs with flowing water connected via flow through organic material tended to more active and often frequented the organic material provided near the surface of the upwelling.

Results from the field investigations indicate that inputs of terrestrial-derived materials are likely to be particularly important for the diet (and thus conservation) of *H. comalensis* and *S. comalensis*. In fact, model results indicate that *H. comalensis* adults and larvae derive 80% and 73% of their diet from terrestrial-derived materials (wood and leaves combined). The results of this study suggest that a lateral connection to terrestrial matter sources may be particularly important for two of the species listed under the EAHCP: *H. comalensis* and *S. comalensis*. Lower spring discharge and declining flows can lead to disconnection of the aquatic environment from the bank and to lower water surface areas of aquatic habitats, potentially leading to decreased inputs of terrestrial material that may support populations of these wood-associated taxa. In addition, decreased flow rates and water velocities could also lead to lower rates of downstream transport of terrestrial materials from more canopy-covered upstream areas (e.g., the more canopy-covered Spring Runs) to more open areas with lower rates of terrestrial inputs (e.g., Spring Island area).

Results from our study indicate that *Stygobromus pecki*, another spring-associated consumer covered under the EAHCP may have a more plastic feeding strategy than *H. comalensis* and *S. comalensis* and may be able to switch to an alternate basal resource food chain as environmental conditions vary spatially. Backing up results from the water temperature surrogate testing study, our stable isotope results also suggest that *M. pusillus* may not be an appropriate surrogate for *H. comalensis* in that the diet of *M. pusillus* was much more reliant upon periphyton and therefore feeds on an entirely different food chain.

6.0 Recommendations for future applied research

The EAHCP Science Committee and National Academy of Science committees have both recommended additional life history and environmental stimulus work to be conducted with *H. comalensis.* Our study team concurs with that assessment and are actively engaged with 2016 EAHCP applied research directly assessing life history requirements from egg to adult. Additional research is being conducted on the endangered Comal invertebrates relative to rearing in captivity via on-going and upcoming refugia applied research.

Relative to this study, it was clear that the long-term cumulative stress experiments provide useful initial information on thermal thresholds for riffle beetles and their potential limits of acclimation for increasing environmental temperatures, but there are potentially a large number of additional studies that should be considered. Experiments for consideration could include examining the performance of beetles (both *H. glabra* and *H. comalensis*) to environmental stimuli (water temperature, organic matter connection or disconnection, levels of siltation, etc.) over extended periods of time (months) in order to establish "preferred" conditions for maintenance of both wild and refuge populations. It may also be extremely insightful to monitor physiological responses (instead of behavioral responses) to increasing temperature, such as respiration rates, immune function, and the concentration of various biomolecules (e.g., Mermillod-Blondin et al. 2013). The RBASS again proved to be a viable research tool to consistently control environmental variables in a simulated upwelling environment. To expand on the upwelling habitat connectivity assessment, it might prove valuable to conduct a study evaluating three scenarios of organic matter connectivity; the two tested in this study (connected and disconnected) with a third which would consist of a disconnected treatment that received infrequent rainfall events filtering over surface organic matter.

Finally, based upon the two preliminary studies we conducted, it appears that passive sampling pits or wells and Bou-Rouch samples are not likely to be efficient methods to estimate beetles. However, the efficiency of these and other methods should be considered for more thorough investigation in a systematic study that explicitly examines and compares various collection methods in the field. In addition to these comparisons, other data should be considered for collection in the lab, including the dispersal or movement ability of beetles, the ability to mark and re-capture beetles in small-scale settings, and the ability of beetles to hold onto surfaces at different flow or suction rates.

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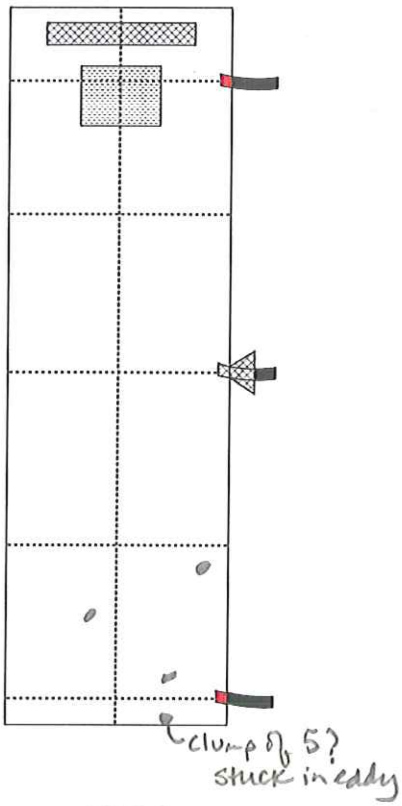
APPENDIX A

Comal Springs riffle beetle location data sheets

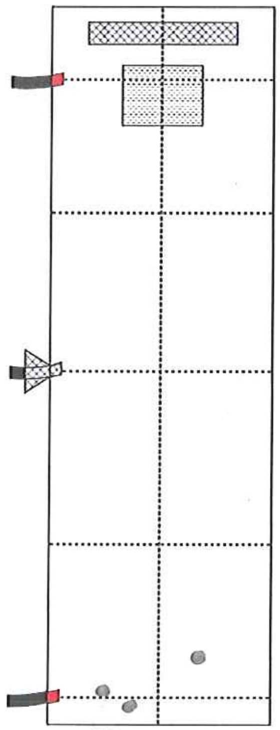
Date: 09/22/15, 12:20

Temperatures: Outside 90°F **, Inside** 90°F **, Chiller** 68.9°F

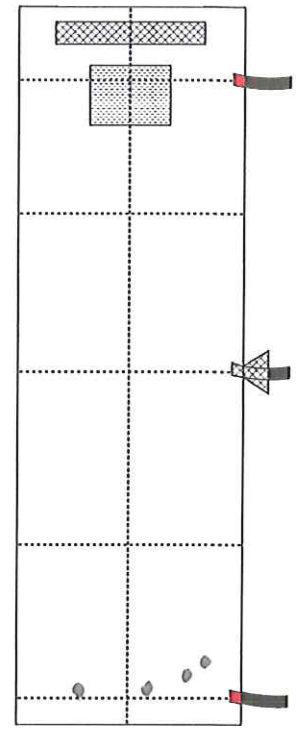
EFC 1



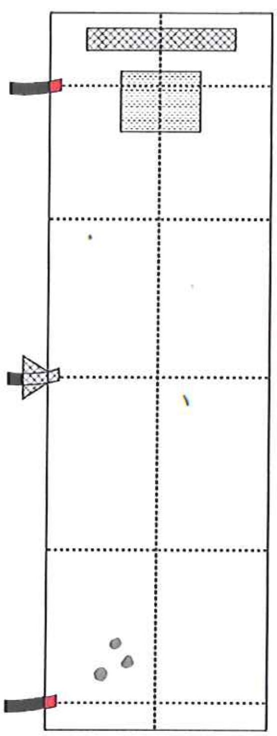
EFC 2



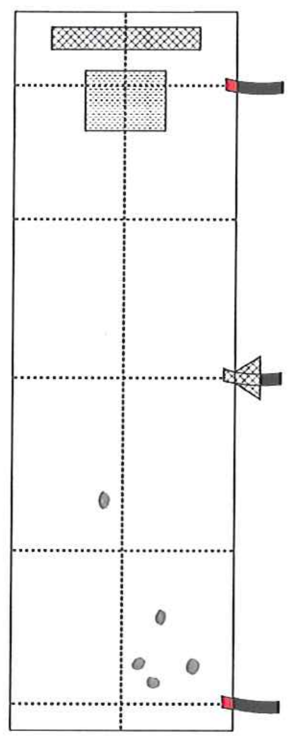
EFC 5



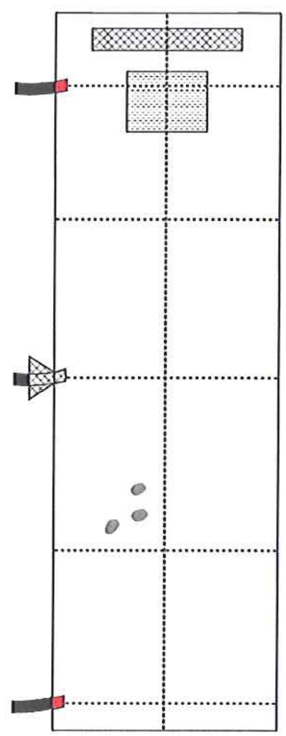
EFC 8



EFC 9



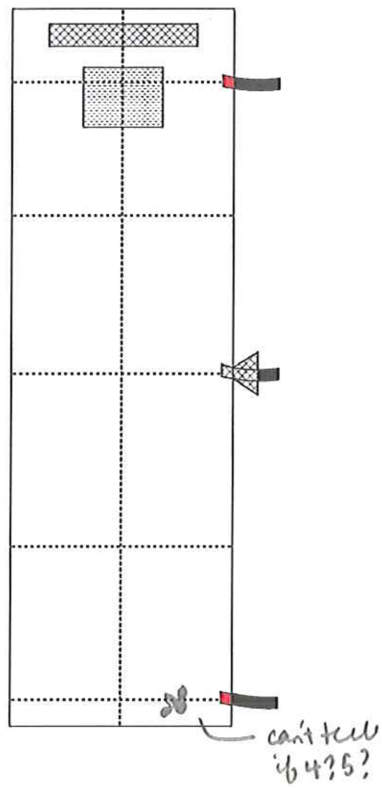
EFC 12



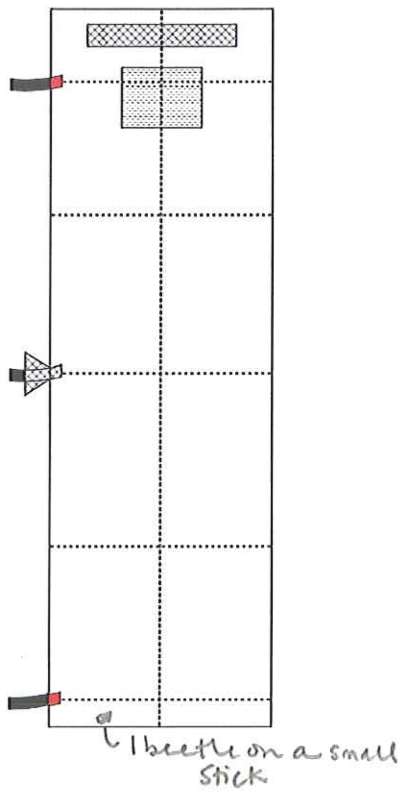
Notes:

Date: 09/26/15, 18:30
Temperatures: Outside 90°F **, Inside** 92°F **, Chiller** 68.1°F

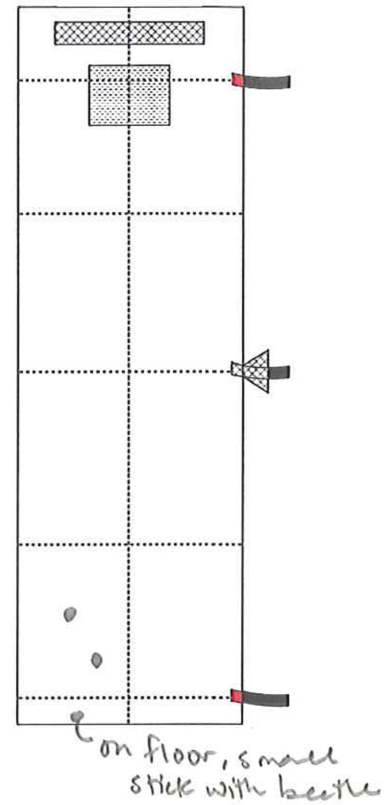
EFC 1



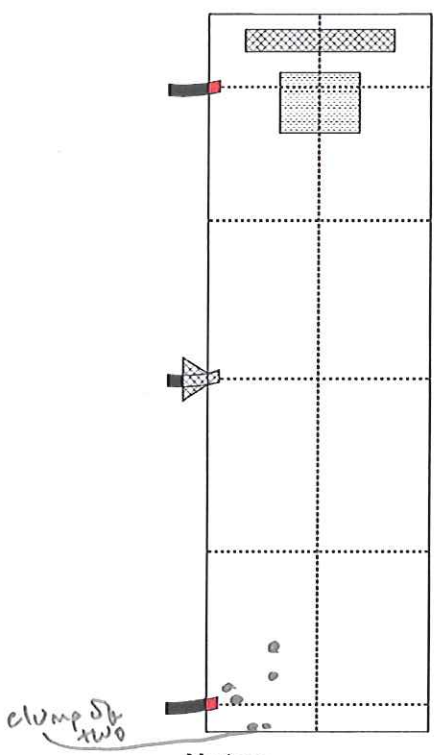
EFC 2



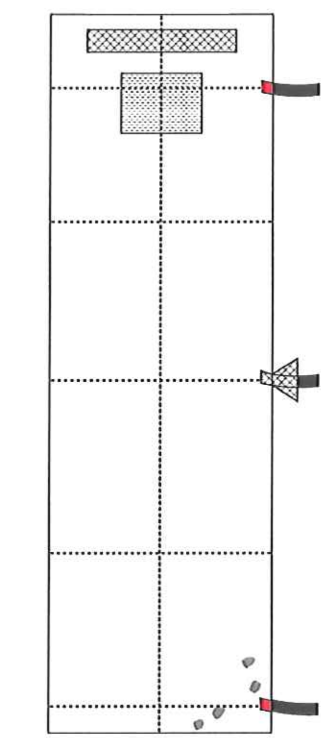
EFC 5



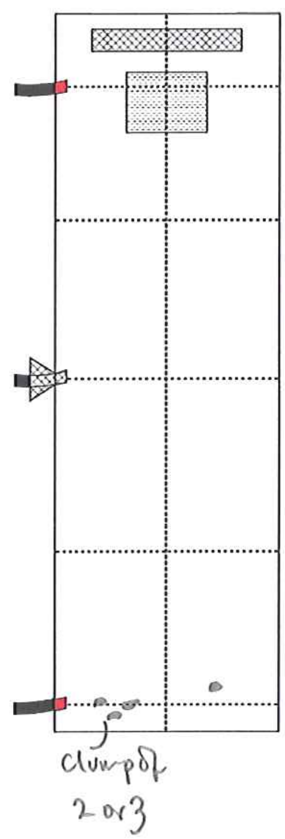
EFC 8



EFC 9



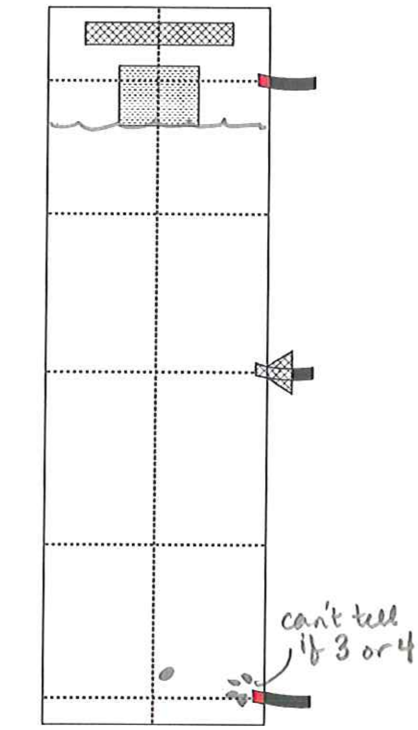
EFC 12



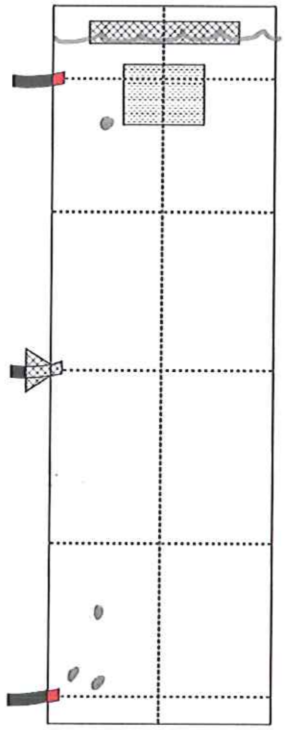
Notes:

Date: 09/28/15, 18:07
Temperatures: Outside 84°F , **Inside** 86°F , **Chiller** 68.3°F

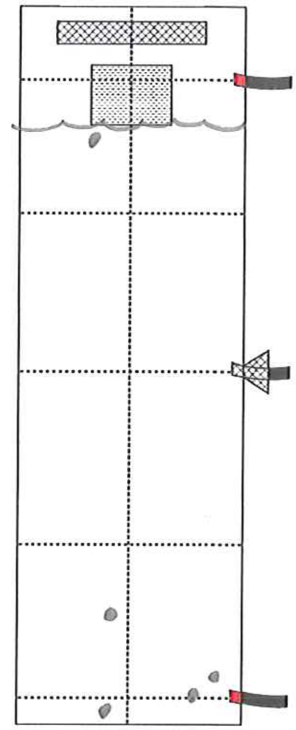
EFC 1



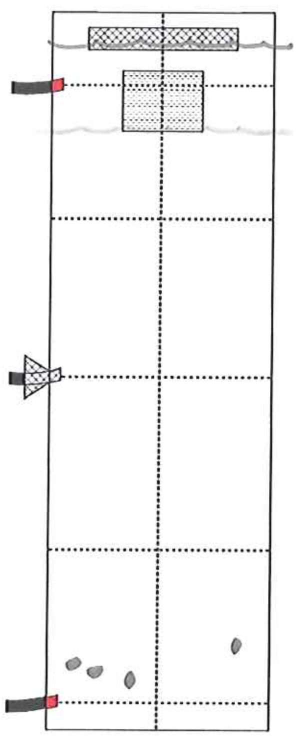
EFC 2



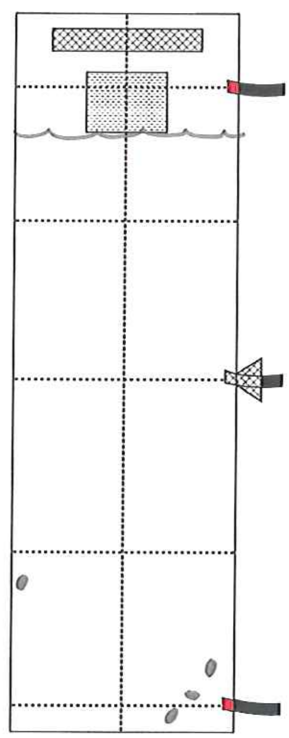
EFC 5



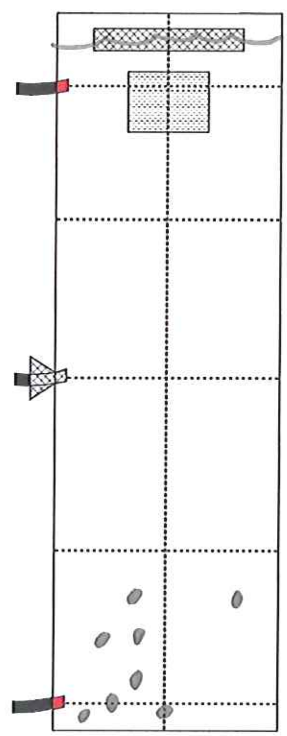
EFC 8



EFC 9



EFC 12

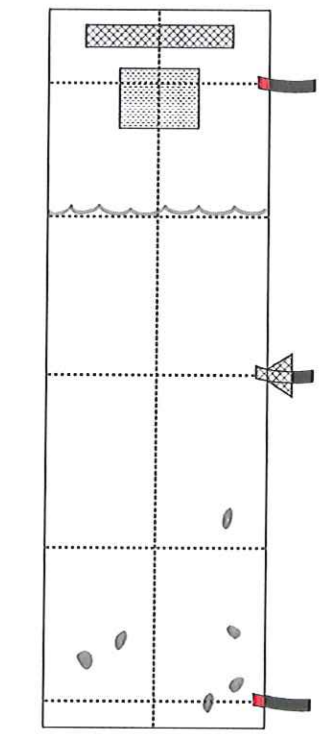


Notes:

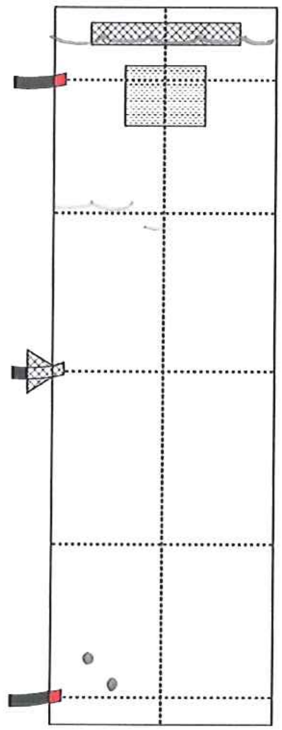
Date: 09/29/15, 14:15

Temperatures: Outside 91°F, Inside 88°F, Chiller 68.3°F

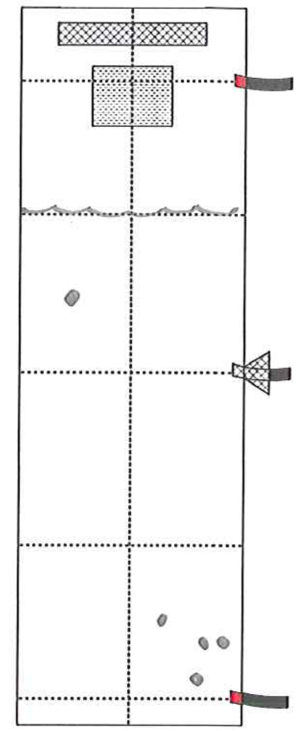
EFC 1



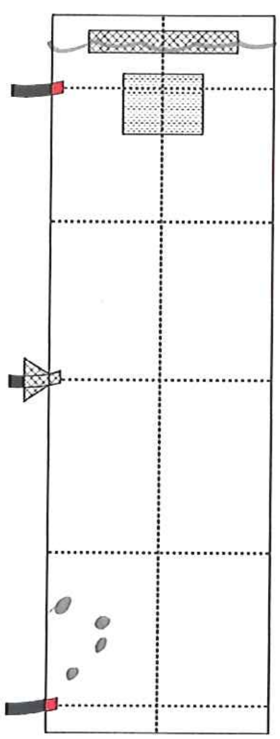
EFC 2



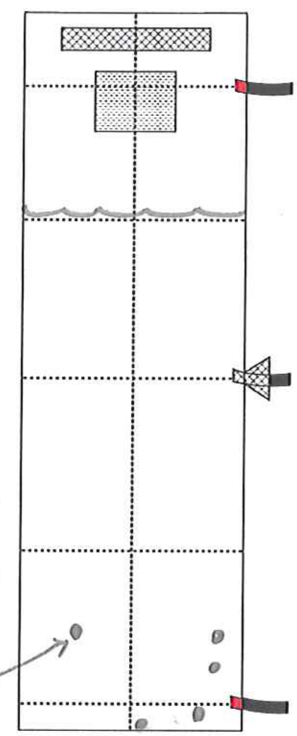
EFC 5



EFC 8

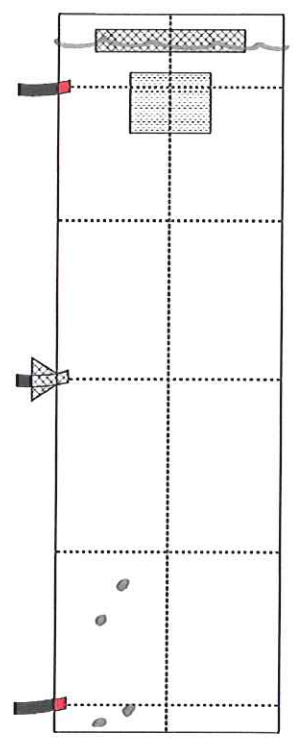


EFC 9



when this beetle saw my headlamp light he started crawling up on the mesh

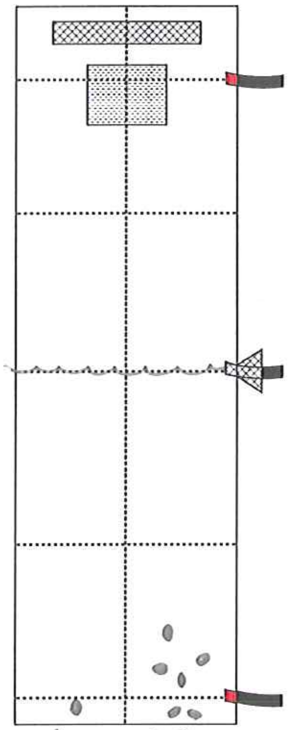
EFC 12



Notes:

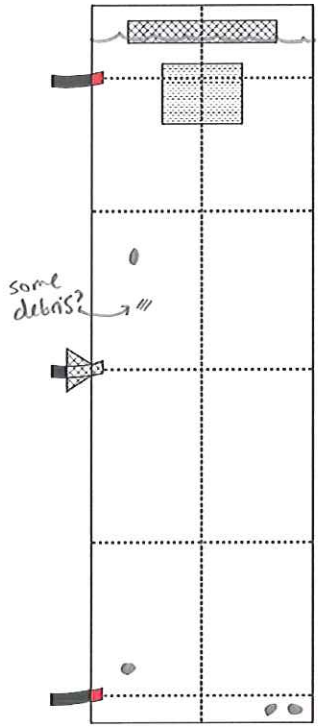
Date: 10/01/15 15:17
Temperatures: Outside 85°F **, Inside** 95°F **, Chiller** 68.6°F

EFC 1

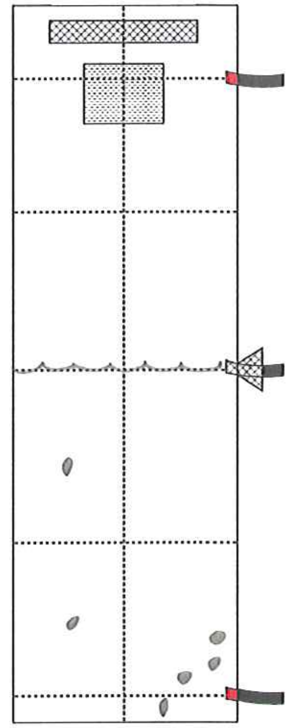


3 beetles hanging on mesh at bottom

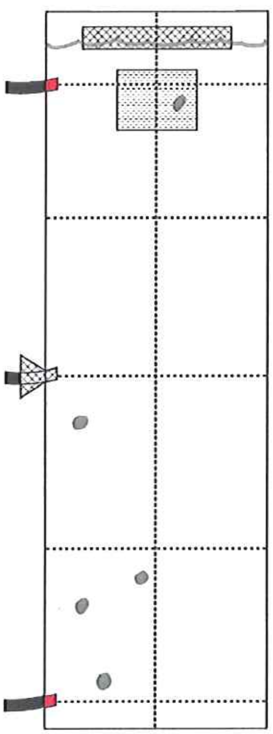
EFC 2



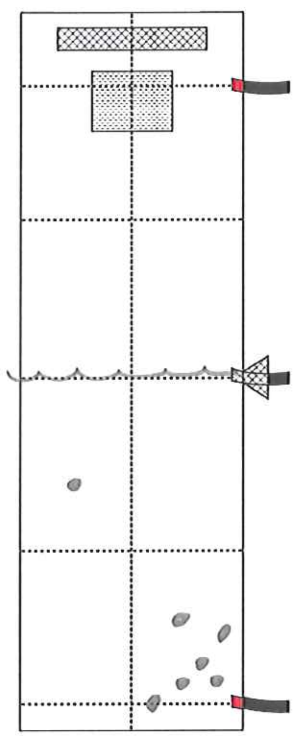
EFC 5



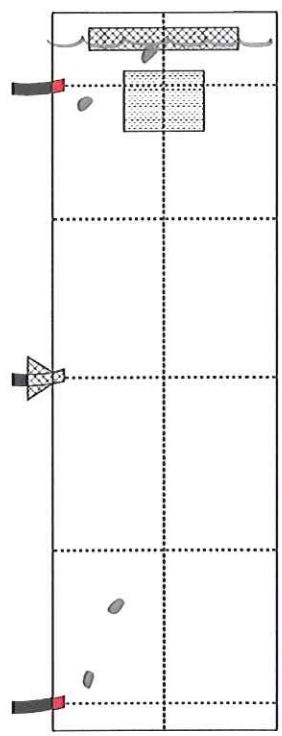
EFC 8



EFC 9

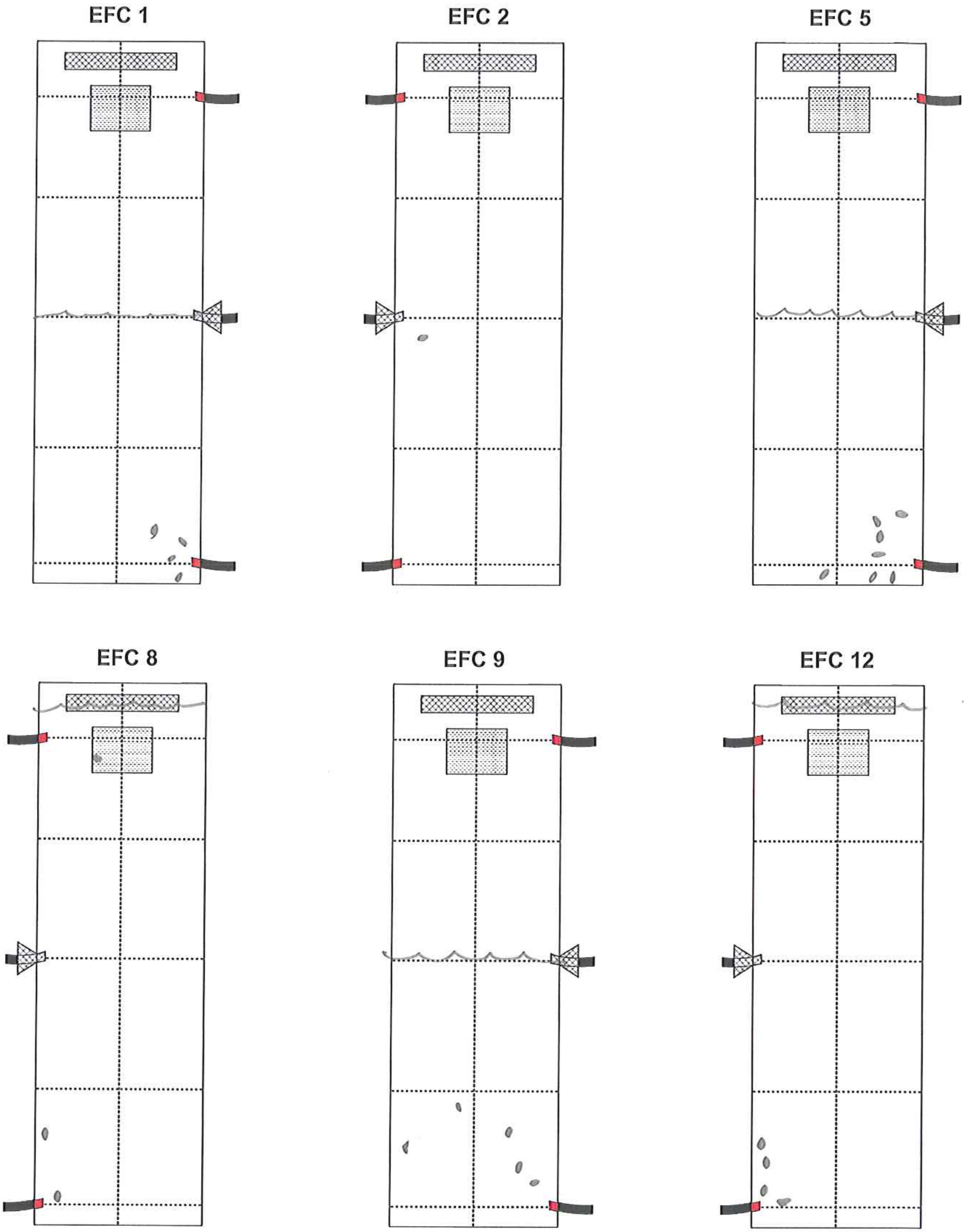


EFC 12



Notes: finished dropping water levels today at 15:37.

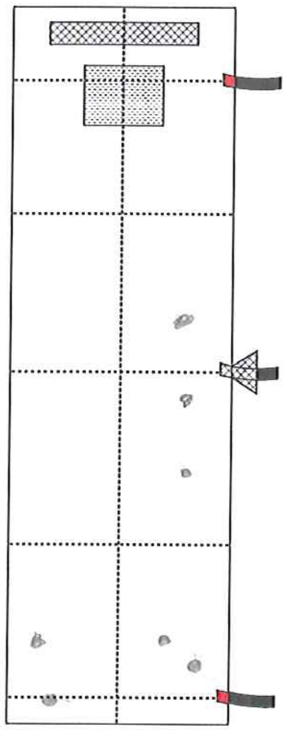
Date: 10/03/15 ,08:15
Temperatures: Outside 70 °F, Inside 64°F, Chiller 68.8°F



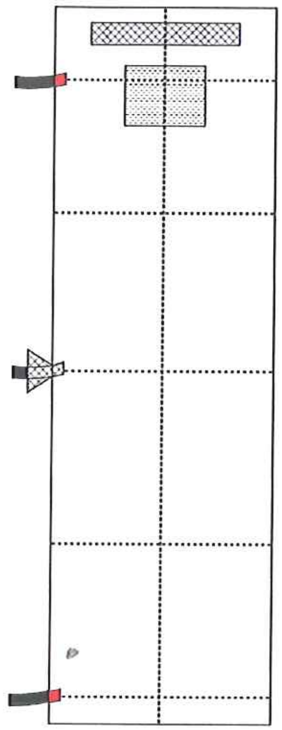
Notes:

Date: 10/5/2015 11:20 am
Temperatures: Outside 77°F , Inside 80 F , Chiller 68.2 F

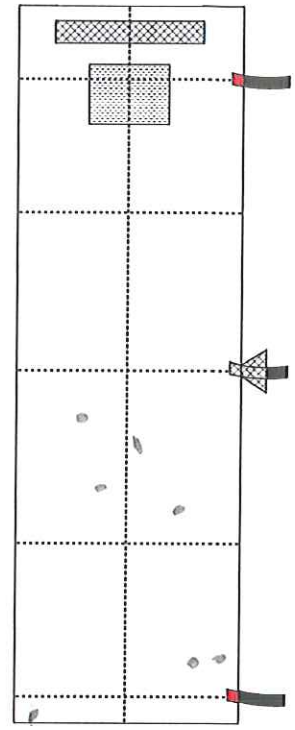
EFC 1



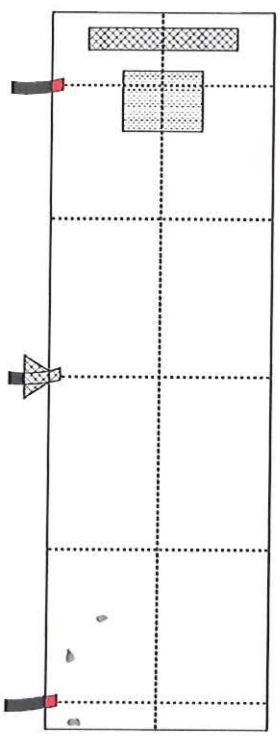
EFC 2



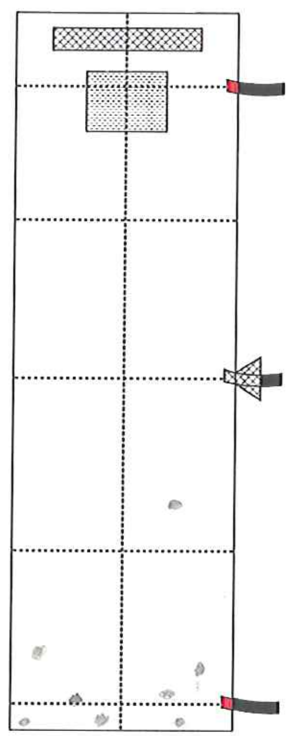
EFC 5



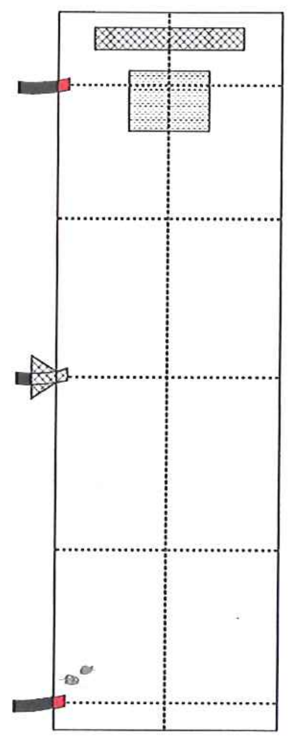
EFC 8



EFC 9



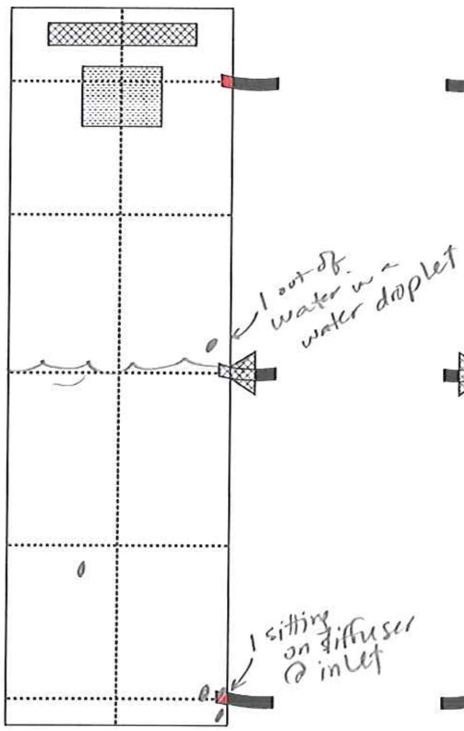
EFC 12



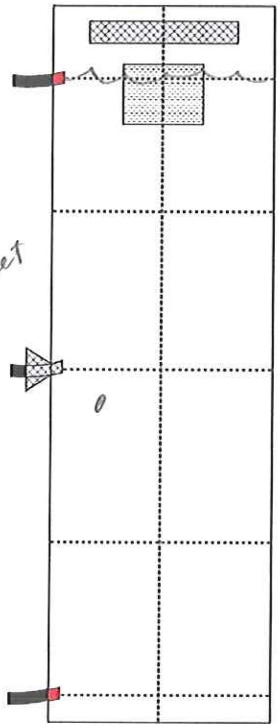
Notes:

Date: 10-07-15
Temperatures: Outside 90 °F, Inside 90 °F, Chiller 68.5 °F

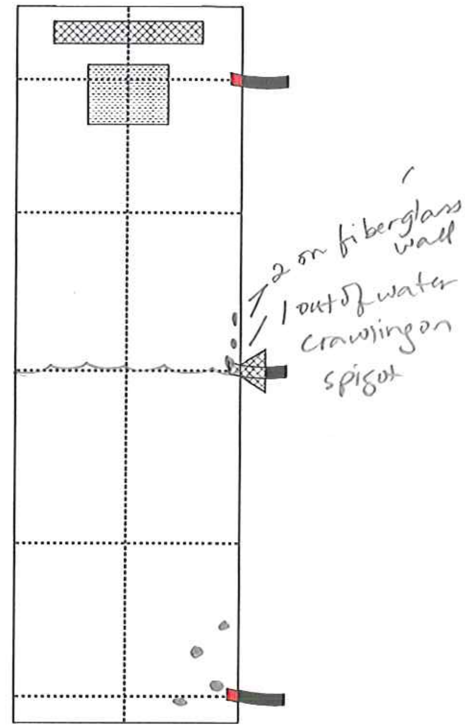
EFC 1



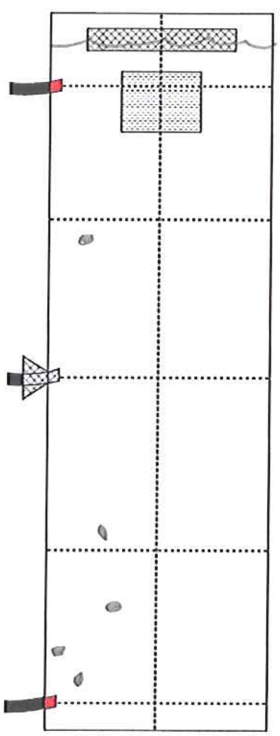
EFC 2



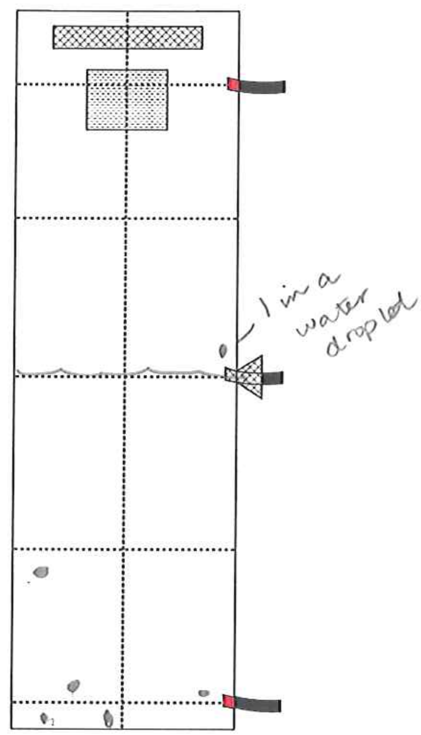
EFC 5



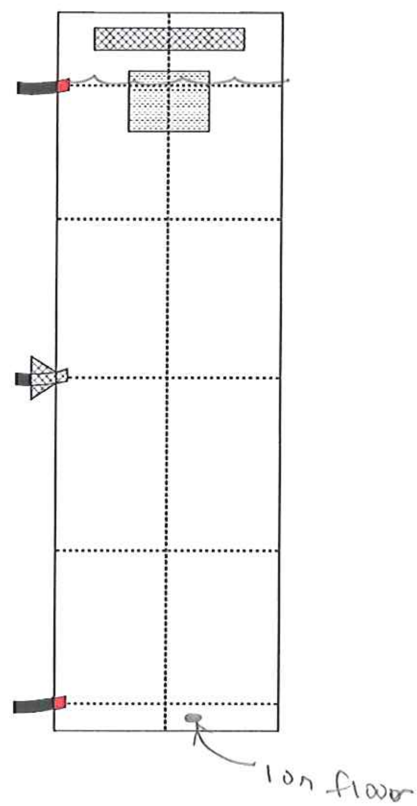
EFC 8



EFC 9



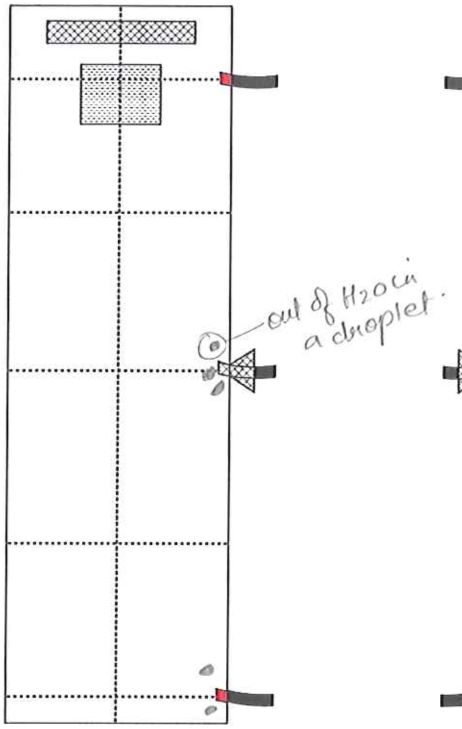
EFC 12



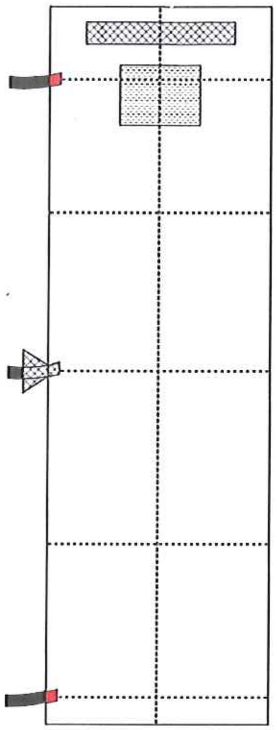
Notes:

Date: 10/08/05
Temperatures: Outside 90°F **, Inside** 85°F **, Chiller** 68.5F

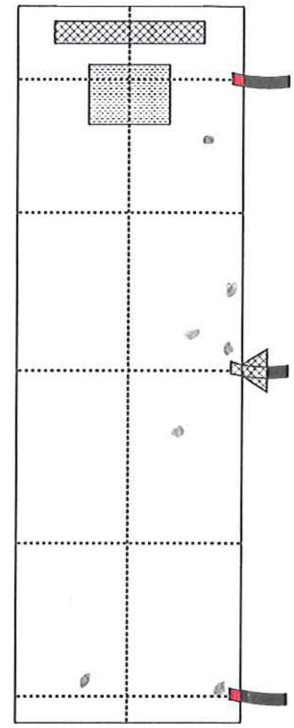
EFC 1



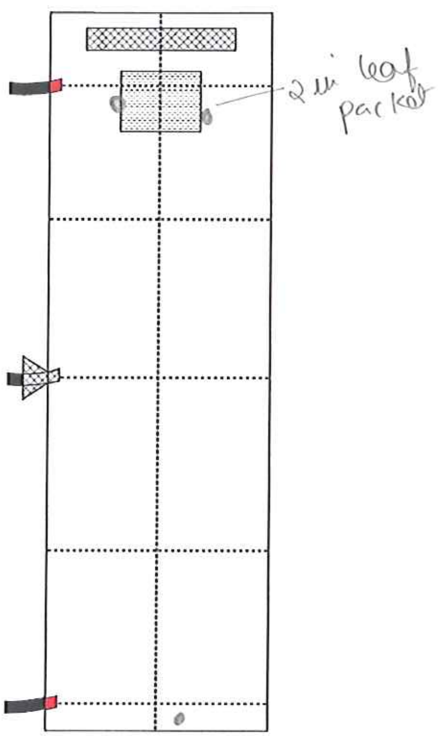
EFC 2 — None seen.



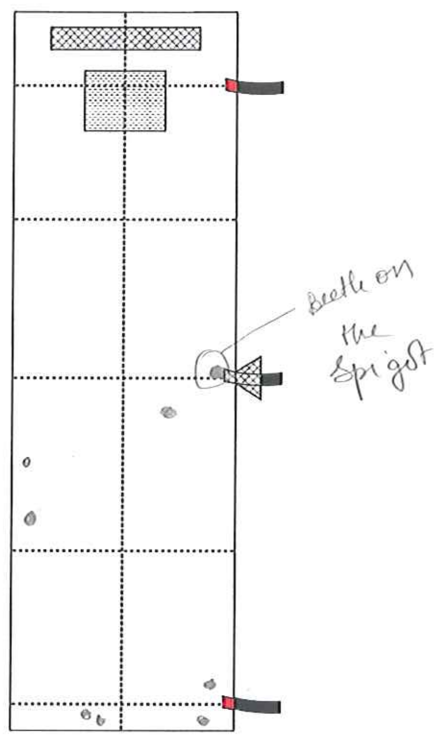
EFC 5



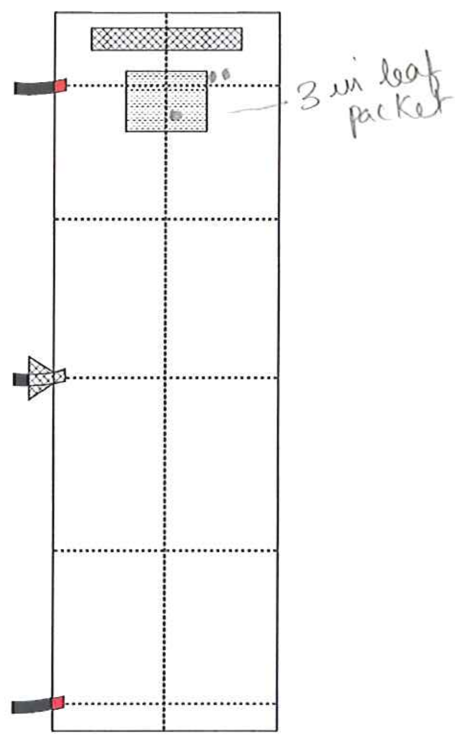
EFC 8



EFC 9



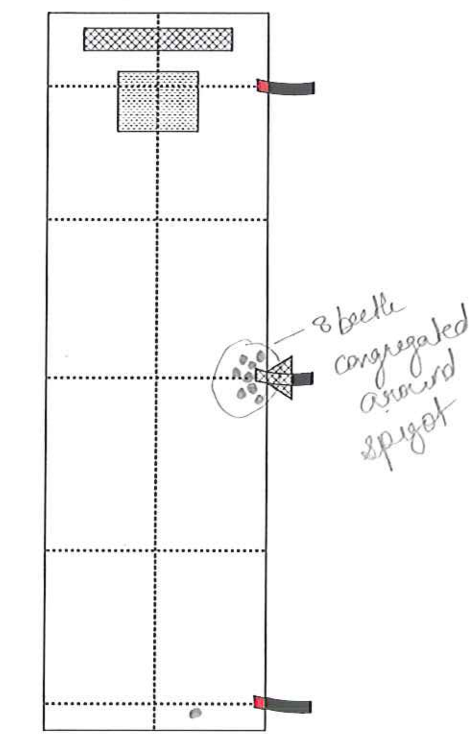
EFC 12



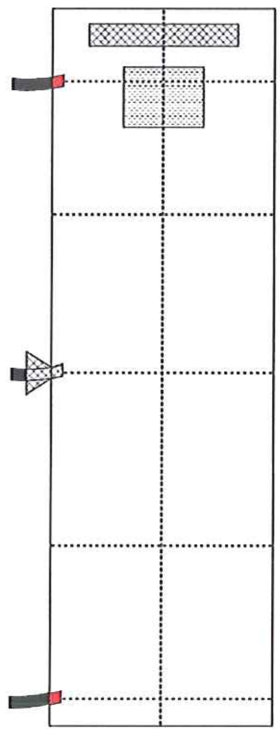
Notes:

Date: 10/10/2015
Temperatures: Outside 91°F, Inside 88°F, Chiller 68.5

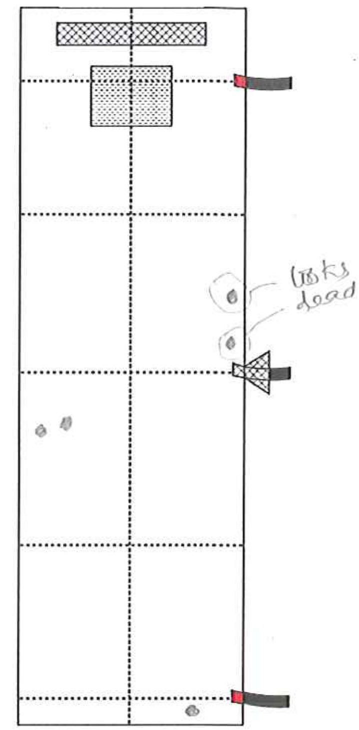
EFC 1



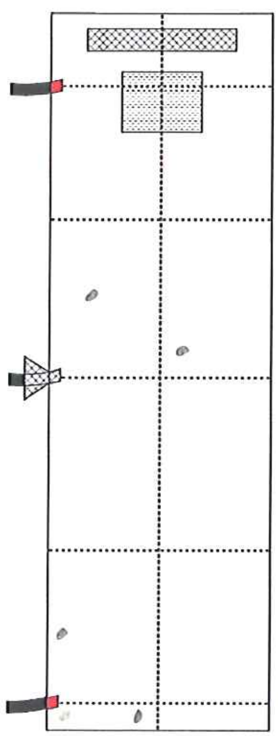
EFC 2



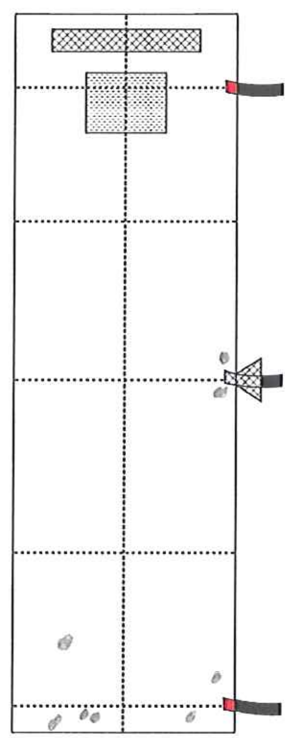
EFC 5



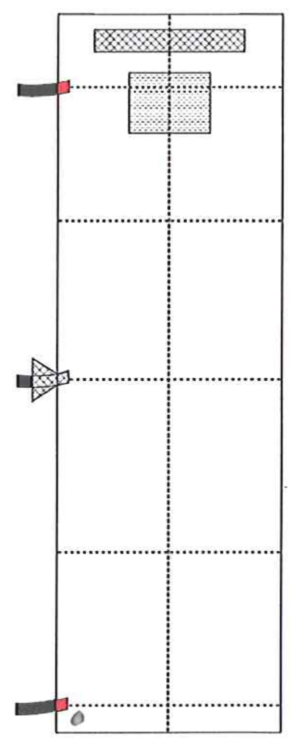
EFC 8



EFC 9

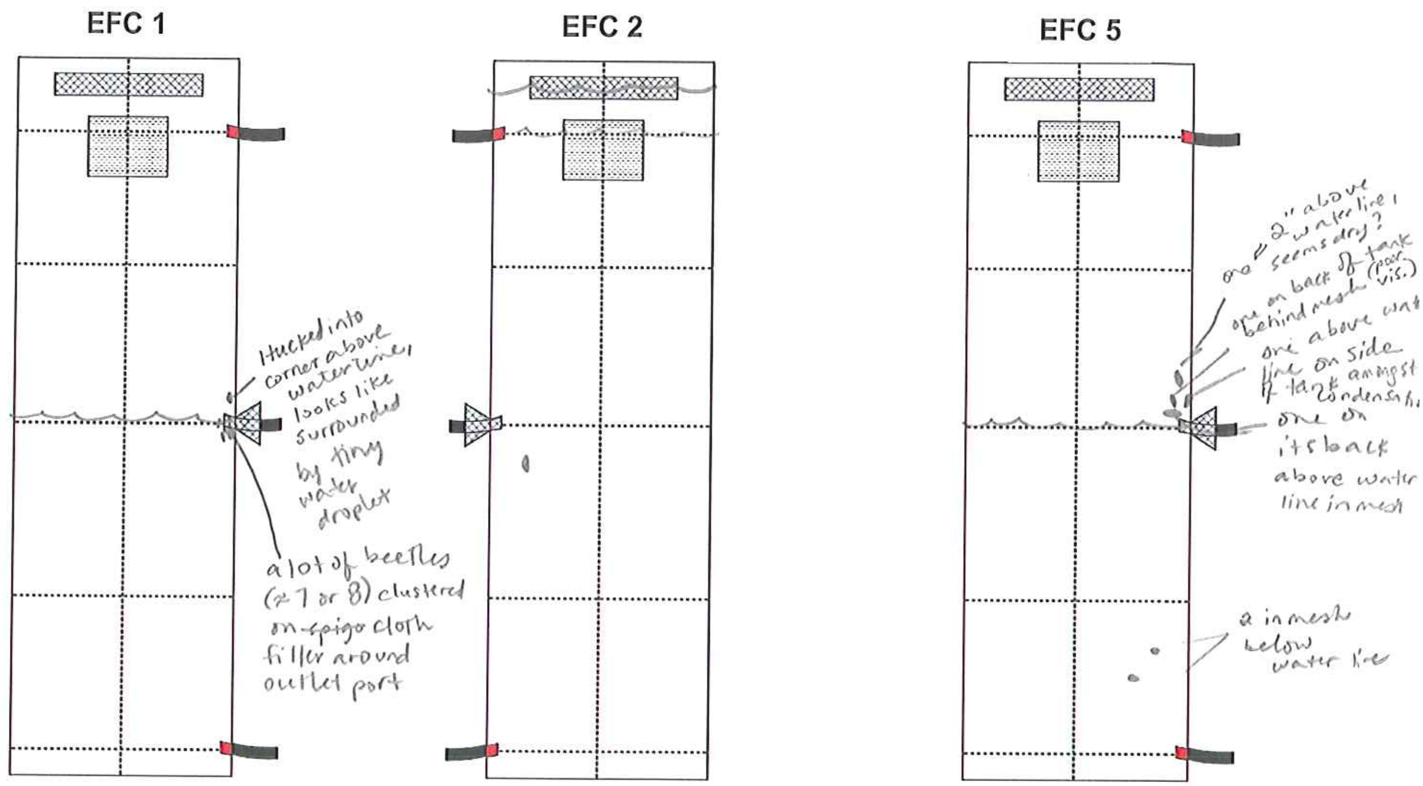


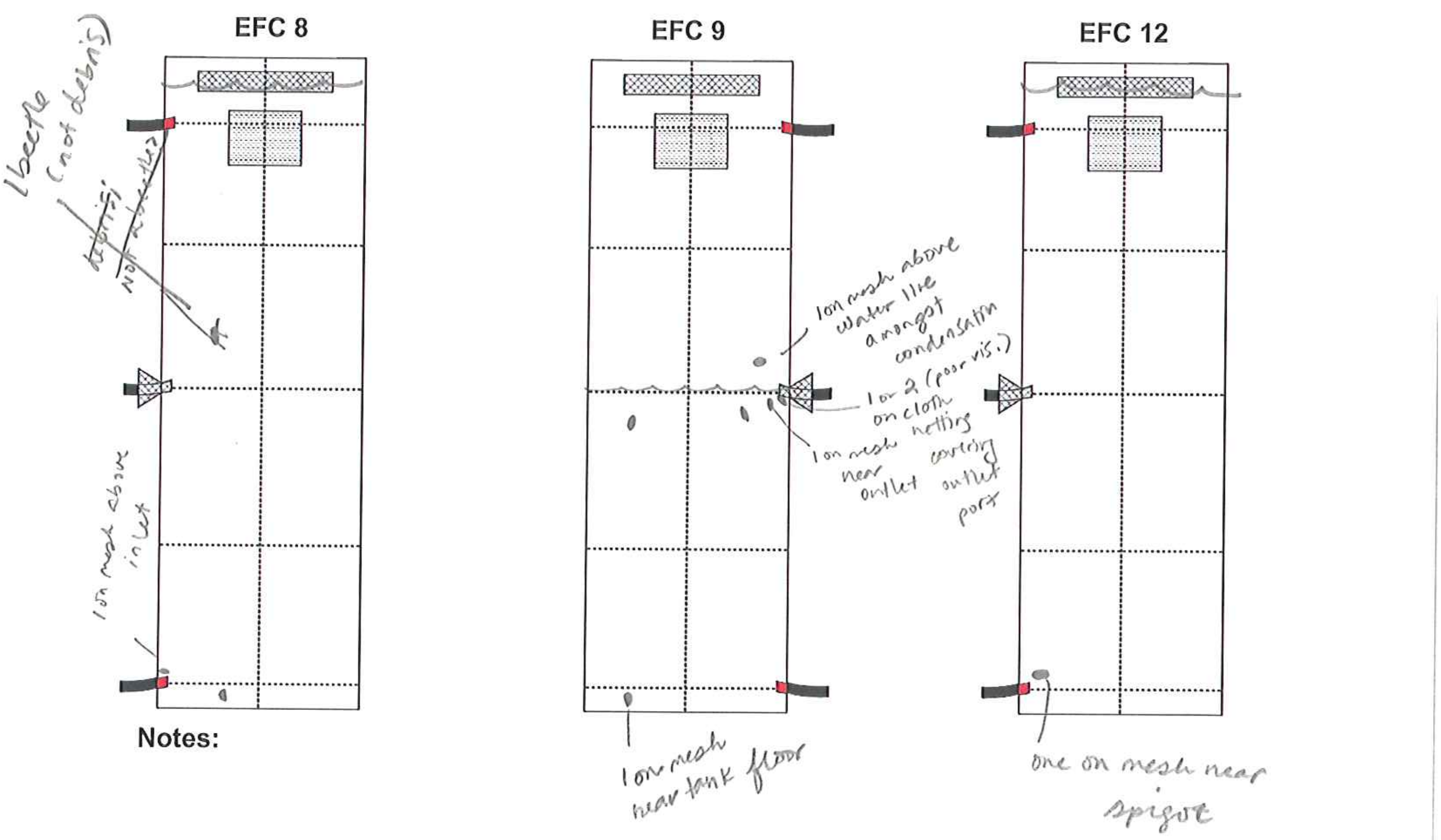
EFC 12



Notes:

Date: 10/13/15, 13:10
Temperatures: Outside 91°F , **Inside** 90°F , **Chiller** 68.4°F

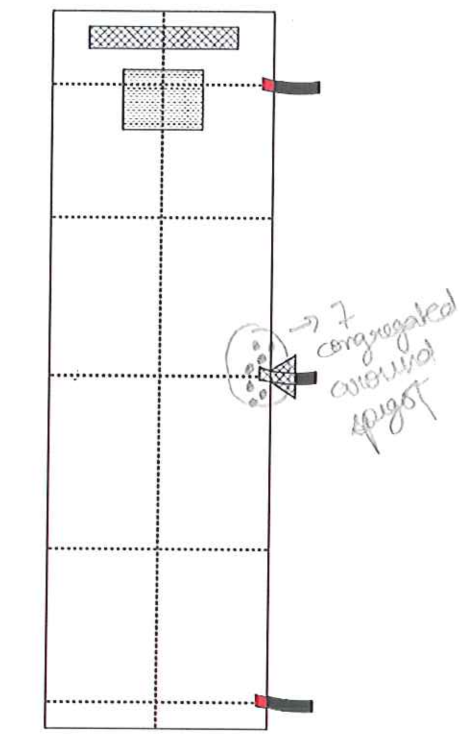




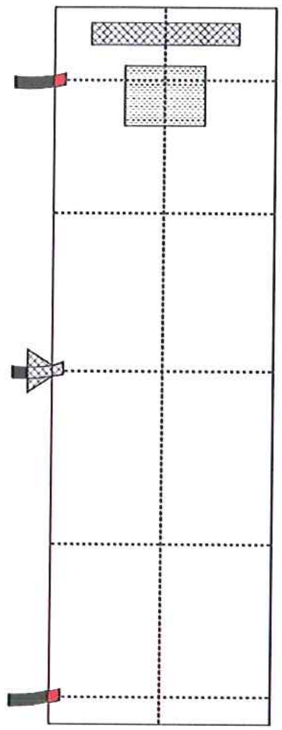
Notes:

Date: 10/15/2015
Temperatures: Outside 94°F, Inside 86°F, Chiller 68.4F

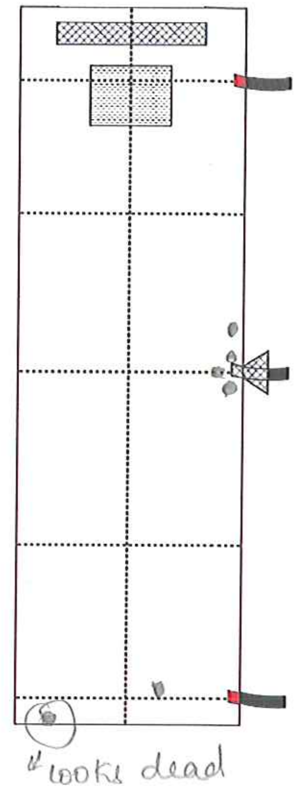
EFC 1



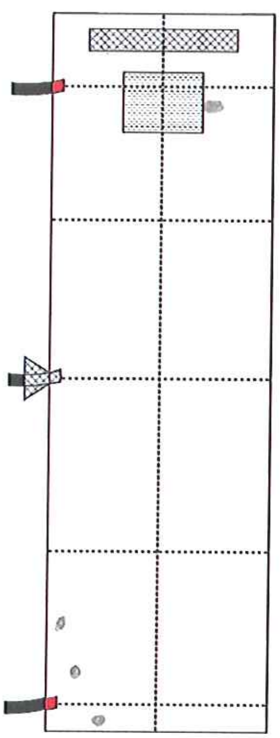
EFC 2



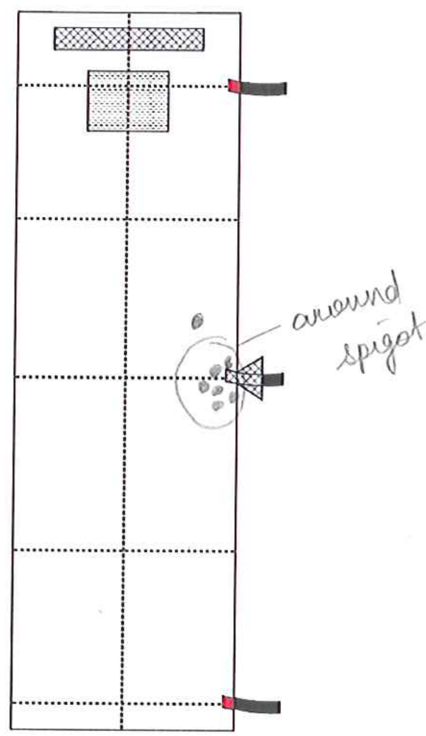
EFC 5



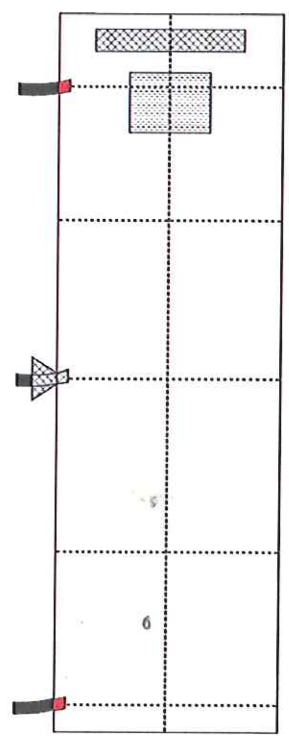
EFC 8



EFC 9



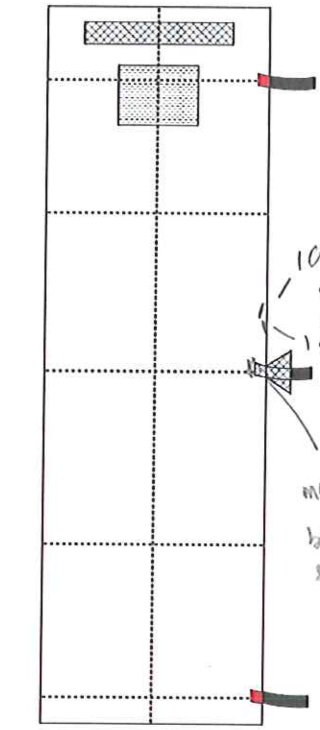
EFC 12



Notes:

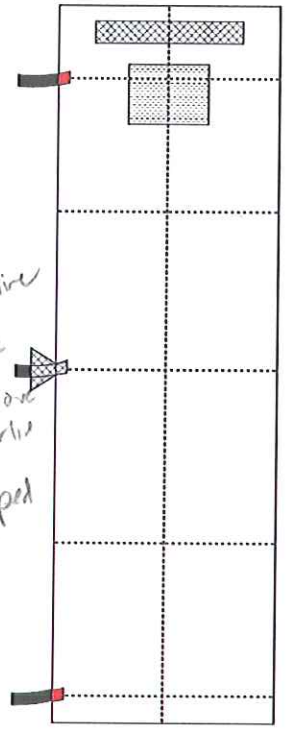
Date: 10/17/2015 10:44
Temperatures: Outside 75 °F, Inside 81 °F, Chiller 68.2 °F

EFC 1

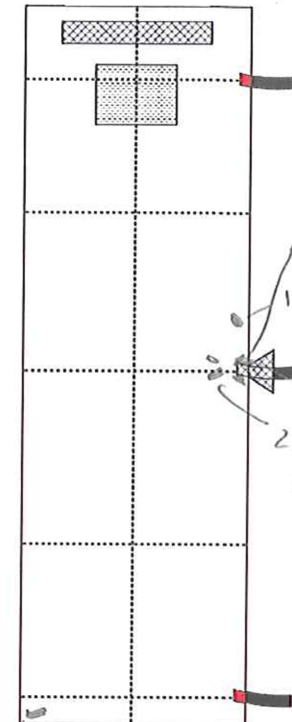


1 crawling on side of tank ≈2" above water line
1 sitting on side of tank ≈1.5" above waterline
mass of beetles clumped together on outlet

EFC 2 – NONE visible

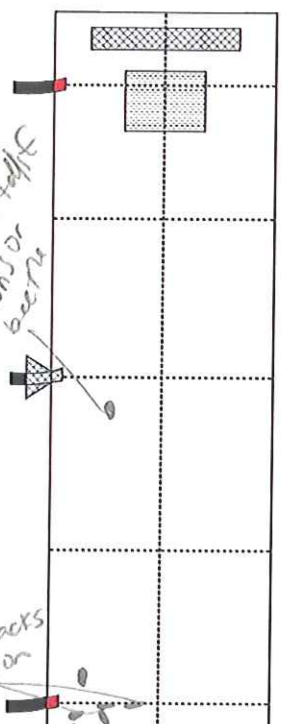


EFC 5



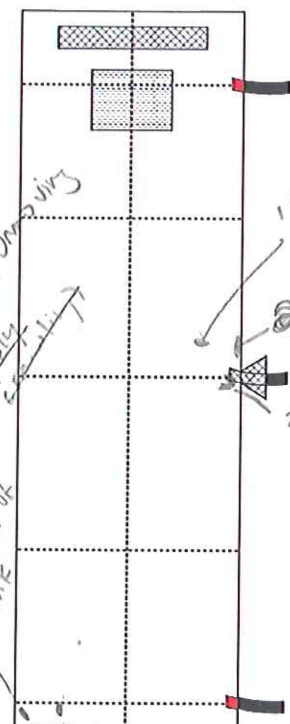
1 on tank wall very near outlet
1 unmoving ≈ 2" above water line
2 on outlet
2 on mesh near outlet
1 on its back on bottom of tank; unmoving

EFC 8



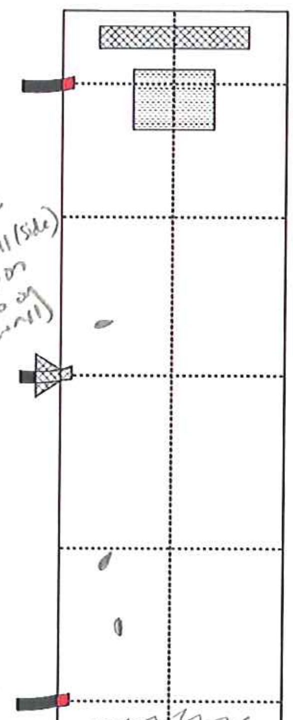
1 on 4 tuft debris or beetle
2 on backs supine on floor unmoving
2 on mesh near bottom
quite a bit of organic debris

EFC 9



inactive/unmoving (seems to be on tank bottom in corner of tank)
1 in mesh above water line
1 on tank wall (side) (condensation also on wall)
2 on outlet
1 on mesh on bottom of tank

EFC 12

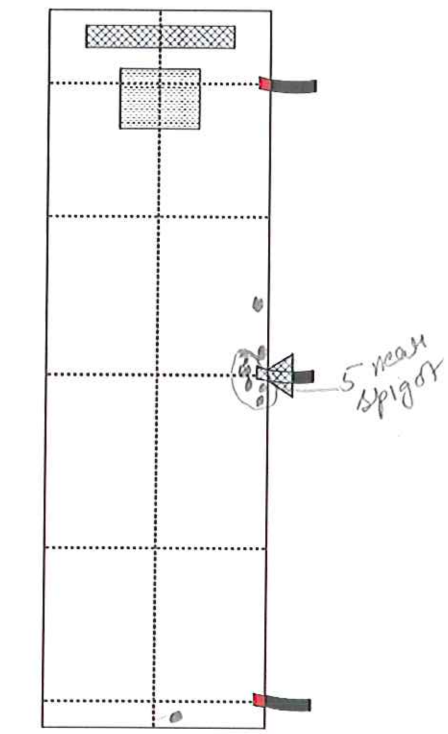


quite a bit of small diameter organic debris

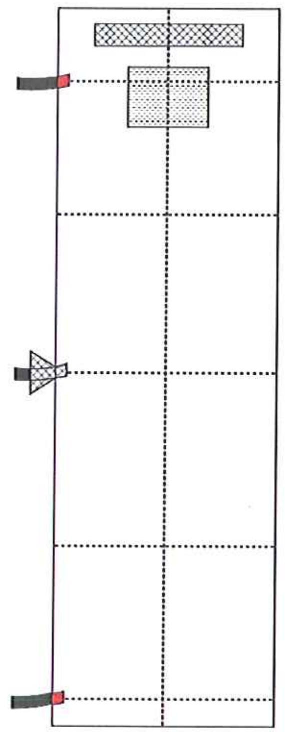
Notes:

Date: 10/19/2015
Temperatures: Outside 84°F **, Inside** 88°F **, Chiller** 68.5

EFC 1



EFC 2



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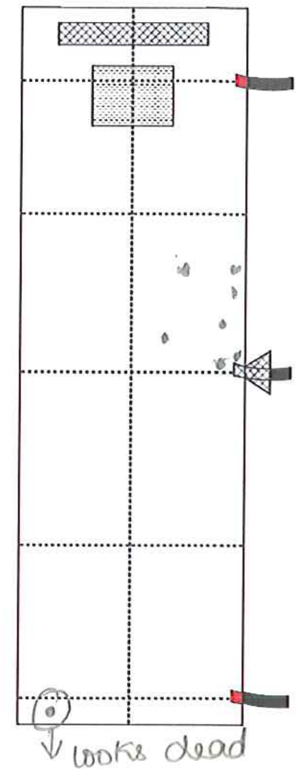
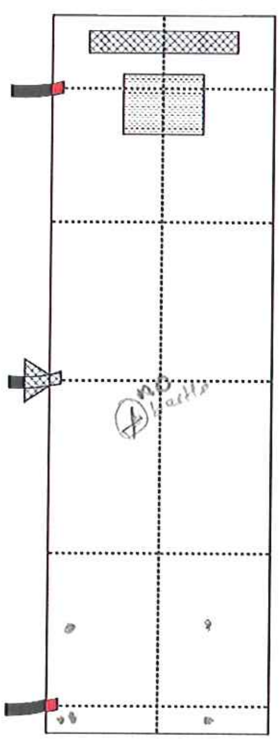
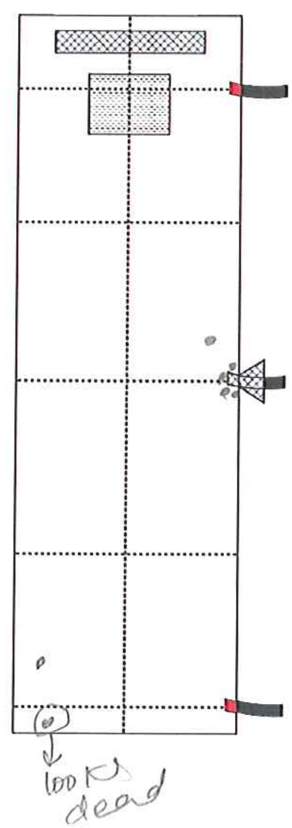


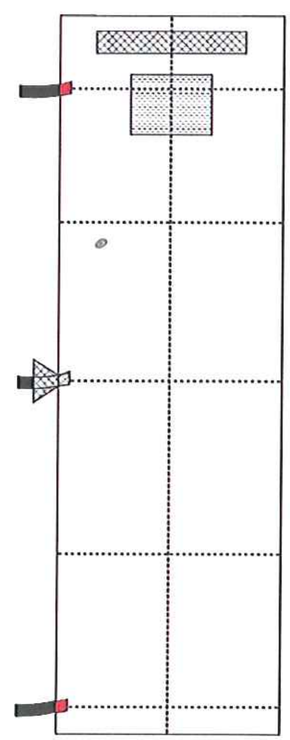
EFC 8



EFC 9



EFC 12



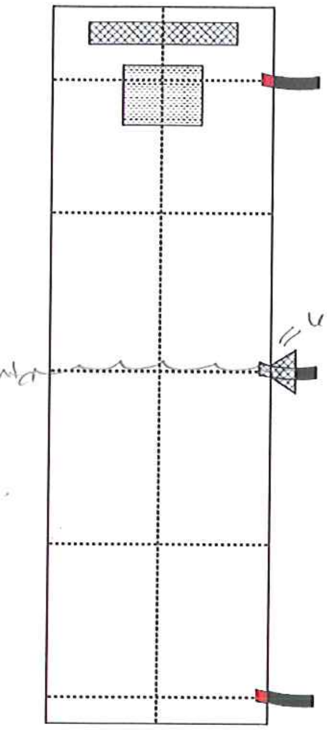
Notes:

Date: 10-21-15 10:30

Temperatures: Outside 79°F , **Inside** 81°F , **Chiller** 158°F??? Chiller seems broken. The water is still cool + running, but chiller is off

EFC 1

[1] Approx. 6-8 beetles visible; Approx 1-3 beetles unaccounted for



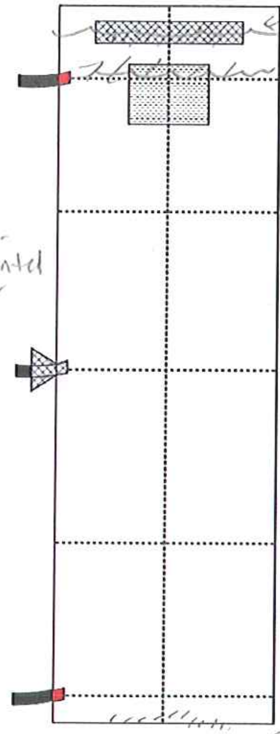
EFC 2

[2] Approx 0 beetles visible; Approx 9 unaccounted

≈ 6-8? beetles for sitting on outlet cover

debris also in mesh @ overflow

← No beetles visible



lots of debris

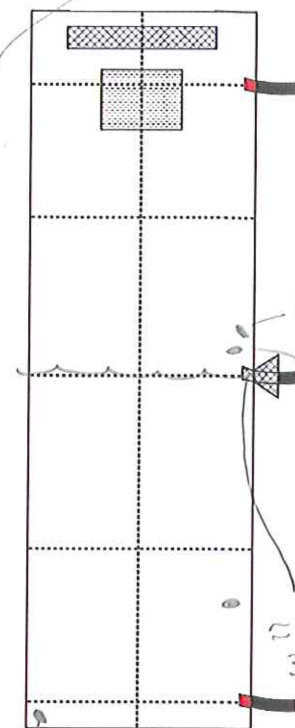
EFC 5

[5] ≈ Approx. 7 beetles visible, Approx 2 unaccounted for

1 beetle motionless on tank wall above water line

1 beetle inactive, clinging to mesh above water line

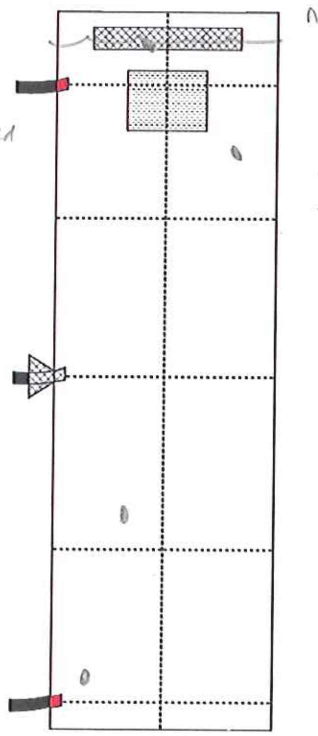
≈ 3 beetles sitting on outlet cover



1 beetle on back @ tank bottom

EFC 8

[8] 4 beetles visible, 5 unaccounted for

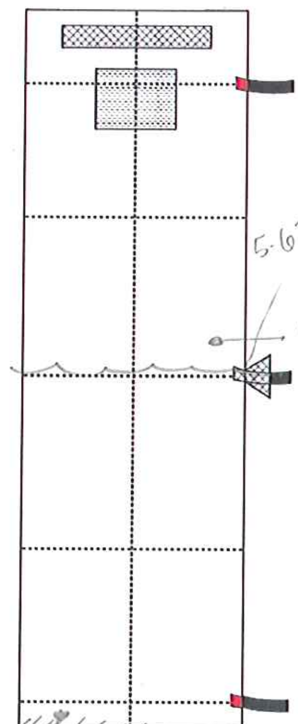


EFC 9

[9] Approx. 7 beetles visible, Approx 2 unaccounted for

5-6? or more beetles sitting on top of outlet cover

1 clinging to mesh above water line



looks like one beetle inactive, supine on tank floor, legs folded

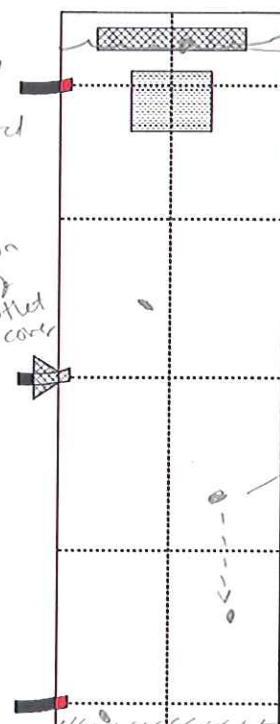
Some debris at tank bottom

EFC 12

[12] 4 beetles visible, 5 unaccounted for

1 on mesh of outflow just below water line

can't determine if beetle or debris — beetle! ** moved here in 5 minutes after check



lots of debris on tank floor

1 beetle clinging to mesh

Notes:

* all debris at tank bottoms appears to be small POM

** the beetles seem to move a lot when exposed to light

FINAL LOCATIONS

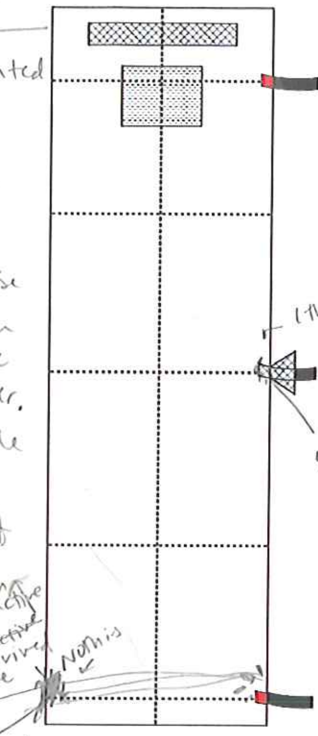
Date: 10-21-15
Temperatures: Outside ________ **, Inside** ________ **, Chiller** 68 °F

[1] FINAL

- 9 accounted for, none in SOL.
- 5 of those had been on fabric mesh filter.
- very little POM on bottom of tank
- 8 placed in net; 4 looked inactive but quickly revived; 1 inactive; 1 alive but died during extrication

These were alive but had been obscured by substrate

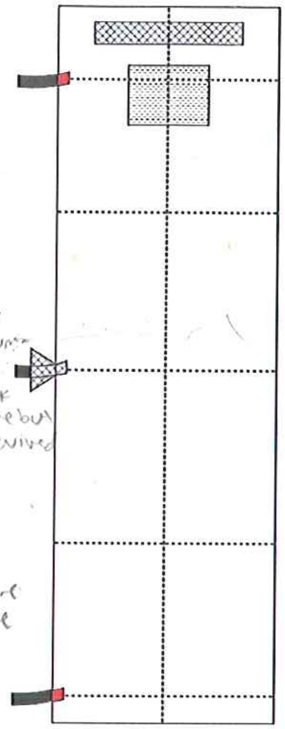
EFC 1



1 that had been above water column on side of tank was inactive but revived

5 beetles had been sitting on outlet; all were alive

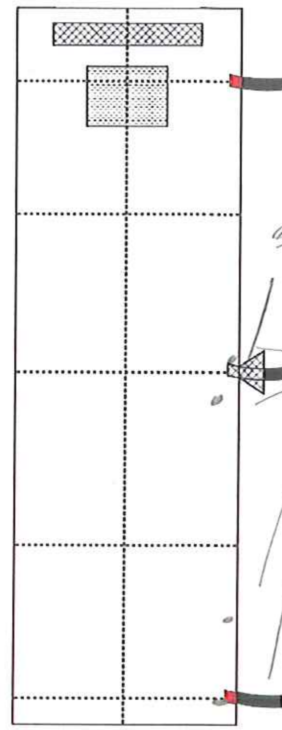
EFC 2



[2] FINAL

NONE VISIBLE ON MESH EVEN AFTER CLOSE INSPECTION

EFC 5

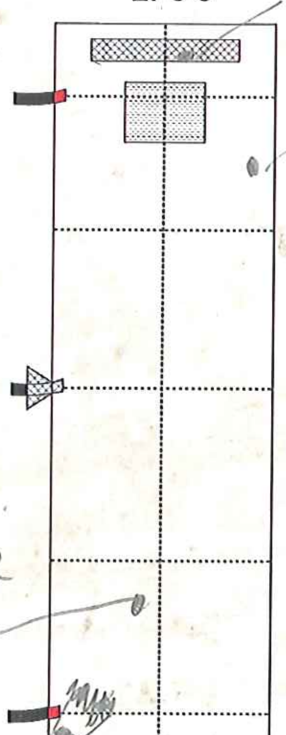


3 beetles on outlet seemed inactive

3 of the 4 beetles on mesh apparently alive; 1 inactive

Seems to be 1 stuck above water line

EFC 8



alive

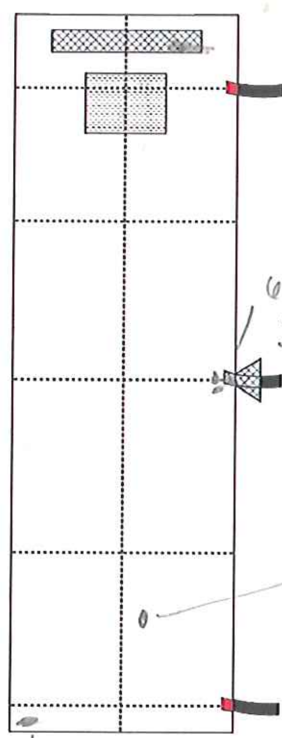
alive

alive

alive

the one on its back in the corner/bottom of the tank was alive

EFC 9

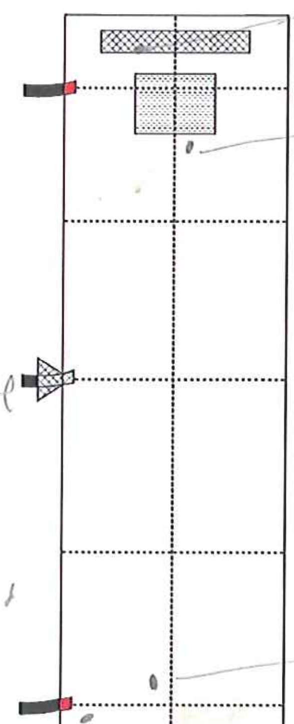


6 beetles 3 active in 3 inactive

1 alive, obscured by mesh

1 inactive

EFC 12



1 alive on mesh

seemed inactive

seemed inactive

alive

Notes:

Appendix K2

Final Report for Ludwigia repens Competition Study

Final Report for *Ludwigia repens* Competition Study

Edwards Aquifer Authority Contract #14-727L

PREPARED BY

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1.0 Introduction

The San Marcos and Comal Rivers have unique aquatic plant communities that support a wide variety of native and endemic wildlife including several listed species. In 2013 the Edwards Aquifer Habitat Conservation Plan (EAHCP) was enacted to enhance and expand habitat for covered species including the fountain darter (*Etheostoma fonticola*). Part of this long-term plan includes removal of the non-native aquatic plant species *Hydrilla verticillata* and *Hygrophila polysperma* and reintroduction of native aquatic plants such as *Ludwigia repens* – all of which will be referred to by their genus name throughout this report. Hydrilla and Hygrophila are becoming increasingly abundant in these systems (Lemke, 1989; Bowles and Bowles, 2001) and tend to support fewer numbers of fountain darters than certain species of native aquatic plants (BIO-WEST, 2015). The persistence and expansion of Hydrilla and Hygrophila pose a threat to efforts in re-establishing beneficial native aquatic vegetation for *E. fonticola* (Bormann, 2012). Predicting the long-term success of revegetation efforts and which species, native or non-native, dominate is vital in the development of a submerged aquatic vegetation module for the EAHCP Ecological model.

Interspecific competition, or the success of a particular plant species relative to another, is a potentially important factor in determining the complex structure of aquatic plant communities. Abiotic factors like substrate and water quality (Szosszkiewicz et al, 2014) as well as differences in species-specific characteristics such as growth rate, plant architecture, reproductive vigor and susceptibility to herbivory (Spencer and Bowes, 1985), phenological plasticity (Garbey et al, 2004; Thouvenot et al, 2013) and, in certain cases, chemical defenses (Gopal, 1993; Gross, 2003) all play a role in the distribution and abundance of species within the plant community. While competitive pressure among naturally co-existing species may appear to be low (Chambers and Prepas, 1990), various studies suggest that these communities do display spatiotemporal variability based on interactions between competitive ability and environmental gradients (McCreary, 1991; Barrat-Segretain, 1996). Non-native species may possess traits that confer a competitive advantage over native species, decreasing species richness, facilitating shifts in community composition and precipitating negative effects throughout the ecosystem (Santos et al, 2011).

Invasive aquatic plant species are well known for their ability to spread rapidly via fragmentation of stems, basal rooting structures, such as stolons, tubers or corms, or specialized structures, such as turions, which can detach and move downstream or float on currents into new locations colonizing in rapid fashion (Sculthorpe, 1967; Langeland and Sutton, 1980). Typically aquatic plants reproduce asexually (Arbor, 1920; Haynes, 1988) and vegetative structures are primed for growth upon settling into new habitat with root structures or leaves still attached (Sutton, 1996). As a consequence, in many cases, invasion of an aquatic species into new areas can take very little time (Santamaria, 2002). For example Eurasion watermilfoil, *Myriophyllum spicatum*, a

widespread problematic submersed aquatic plant has been documented to establish and dominate littoral zones of lakes within two to three years after introduction (Aiken et al., 1979; Newroth, 1985) and is known to suppress growth of a native species (Agami and Waisel, 1985). A North American native *Elodea nuttaalii* has spread rapidly in Japan's largest lake covering the lake bottom within a few years after introduction there (Kadono, 2004). Closer to home in the San Marcos system the exotic plant *Cryptocoryne becketti* was documented to quickly establish and spread within 2 years after initial discovery with a recorded expansion rate of 80% a year (Doyle, 2001) and annual mapping by BIO-WEST has shown the dramatic expansion of Hygrophila in the Old Channel Study Reach of the Comal River (BIO-WEST, 2015). Some invasive aquatic plants not only colonize rapidly but they can displace native aquatic plants by producing a dense canopy structure limiting light availability to other submersed species.

With recent documented expansions of invasive aquatic plants within the San Marcos and Comal systems data is needed to predict how native plants may respond. Few studies regarding native versus non-native aquatic plant competition have been conducted with regard to either of these systems. In one particular study, Doyle et al. (2003) conducted a study in a static container (35 gallon barrels) within an outdoor raceway to evaluate the competitive ability of *Ludwigia repens* against *Hygrophila polysperma*. Our experiment expanded upon that of Doyle et al. (2003) to help further understand the competitive outcome under more realistic environmental flow and ambient light conditions and to additionally investigate the competition between Ludwigia and Hydrilla.

Ludwigia repens (Forester), red ludwigia, is a perennial obligate aquatic plant native to the Comal and San Marcos rivers with common distribution throughout Texas. Ludwigia is an amphibious plant that produces both submersed and emergent growth and can grow terrestrially as well. The architecture of Ludwigia is characterized as caulescent and multi-branched. Submersed growth is typically upright within the water column and nodal rooting is common while terrestrial growth is typically low growing and prostrate. Ludwigia is considered prime habitat for the fountain darter (*Etheostoma fonticola*) and is being utilized in the restoration of darter habitat in both systems.

Hygrophila polysperma (Roxb.) T. Anderson is a non-native plant introduced from Asia. *Hygrophila polysperma* is morphologically similar to Ludwigia in many ways and has been confused with Ludwigia in some instances. Hygrophila is common within the Comal and San Marcos rivers but is not a common invasive plant in Texas as its known distribution is limited to Comal and San Marcos Rivers and San Felipe creek in Val Verde County (Williams, 2013). Like Ludwigia, Hygrophila is also amphibious exhibiting both completely submersed forms, emergent forms and terrestrial growth.

Hydrilla verticillata (L. f.) Royle is another non-native submersed plant introduced from Africa and Eurasia. Hydrilla is a widespread and common invasive aquatic plant with widespread

distribution in the United States. It too is an obligate aquatic plant, but does not produce emergent or terrestrial growth forms. Hydrilla only exists as a submersed aquatic plant typically producing dense growth in upright fashion towards the water surface producing a thick canopy. Absent in the Comal River, Hydrilla is common in the San Marcos River but has been successfully controlled in Spring Lake where it was once the dominant aquatic plant species (Williams, et al. 2011).

The data reported here provide information on the short-term (10 week) early establishment and growth period of viable sprigs of Ludwigia, Hygrophila, and Hydrilla under three levels of competition from the other species. Additionally, it evaluated the short term (10 week) impact(s) of sprig invasion from a competing species on the continued growth and development of established plants. These experiments were conducted at various locations within the Comal and San Marcos Rivers to provide more realistic environmental conditions than was possible with the static tank experiments previously conducted by Doyle et al. (2003).

2.0 Materials and Methods

Two separate studies were conducted to compare the competitive interactions of Ludwigia with Hygrophila and Hydrilla. The site of the Ludwigia X Hygrophila study took place within the Comal River. Since Hydrilla does not occur in the Comal system the Ludwigia X Hydrilla study was conducted separately in the San Marcos River located approximately 12 km north of the Comal River. Both rivers are spring-fed systems fed by the Edwards Aquifer and have similar water quality and general biological characteristics.

2.1 Study Design

Two separate but related two-factor factorial experiments for each species pair (Ludwigia X Hygrophila and Ludwigia X Hydrilla) comprised the studies (Tables 1 and 2). In each experiment the impact of competition (C) and location (L) was evaluated separately for each species.

The first experiment of each study (Table 1A, Table 2A) was designed to document initial establishment and growth of colonizing sprigs of each species in three competitive environments. Two sprigs of each species were planted into pots with no competition (empty pots without a competitor species) moderate competition (pots with 50:50 ratio Ludwigia: competitor sprigs) and high competition (pots with established plants of the competitor species). A second experiment evaluated the continued growth of established plants of Ludwigia or the non-native species without competition with those “invaded” by sprigs of the competing species (Table 1B, Table 2B). Experimental design and analysis followed that of Doyle et al., 2003. The combined experiments resulted in seven different treatments (Table 3).

Table 1. Comal River Ludwigia X Hygrophila competition study designs. A) Top. 3x4 Two-Factor Factorial Design (Competition X Location) for the Ludwigia X Hygrophila and Hygrophila X Ludwigia sprig competition experiments. Eight replicate plantings of sprigs of each species into three competitive environments were made at each of four locations. B) Bottom. 2X4 Two-Factor Factorial Design (Competition X Location) for established plants with and without invasion by sprigs of the other species. Invasion treatment was replicated eight times at each location, while the non-invaded treatment was replicated only four times at each location.

A. Sprig Experiments		3X Level of Competition		
		No Competition	Moderate Competition	High Competition
4X Locations	Landa Lake, High Light	8X	8X	8X
	Landa Lake, Low Light	8X	8X	8X
	Upper Spring Run	8X	8X	8X
	Old Channel	8X	8X	8X

B. Established Plant Experiments		2X Level of Competition	
		Not Invaded	Invaded by 2 sprigs
4X Locations	Landa Lake, High Light	4X	8X
	Landa Lake, Low Light	4X	8X
	Upper Spring Run	4X	8X
	Old Channel	4X	8X

Each of the two competition experiments were replicated at multiple locations: four locations on the Comal for the Hygrophila study (Table 1), and two locations on the San Marcos for the Hydrilla study (Table 2).

Table 2. **San Marcos River Ludwigia X Hydrilla competition study designs. A) Top. 3x2 Two-Factor Factorial Design (Competition X Location) for the Ludwigia X Hydrilla and Hydrilla X Ludwigia sprig competition experiments. Eight replicate plantings of sprigs of each species into three competitive environments were made at each of two locations. B) Bottom. 2X4 Two-Factor Factorial Design (Competition X Location) for established plants with and without invasion by sprigs of the other species. Invasion treatment was replicated eight times at each location, while the non-invaded treatment was replicated only four times at each location.**

A. Sprig Experiments		3X Level of Competition		
		No Competition	Moderate Competition	High Competition
2X Locations	City Park	8X	8X	8X
	I 35	8X	8X	8X

B. Established Plant Experiments		2X Level of Competition	
		Not Invaded	Invaded by 2 sprigs
	City Park	4X	8X
	I35	4X	8X

For the Ludwigia X Hygrophila or Ludwigia X Hydrilla experiments seven treatments were included (Table 3). The same treatments were used at all study locations. Our treatment nomenclature utilizes lower case letters to designate sprigs of a species and capital letters to designate established plants. The first three treatments utilize only plant sprigs planted into previously empty pots of sediment. These include freshly collected Ludwigia sprigs planted in monoculture into empty pots (ll), Hygrophila (or Hydrilla) sprigs planted in monoculture into empty pots (hh), a 50/50 mix of Ludwigia sprigs and Hygrophila (or Hydrilla) sprigs (llhh, 2 sprigs of each species). The use of newly sprigged fragments in empty pots provides information on the colonization potential of both species when free of competitive pressures (ll and hh). The 50:50 sprig mixture (llhh) provides information on the competitive outcome of “equal start” moderate-competition environments. The high-competition environment was obtained by planting sprigs of each species into pots of established plants of the other species (hhLL and llHH).

Table 3. Treatments for Ludwigia vs. Hygrophila (or Hydrilla) competition experiments.

Symbol	Treatment	Count
ll	Ludwigia sprigs into empty pot (No competition)	8
hh	Hygrophila (or Hydrilla) sprigs into empty pot (No competition)	8
ll hh	50 : 50 mix Ludwigia and Hygrophila (or Hydrilla) sprigs into empty pots (Moderate competition)	8
ll HH	Ludwigia sprigs planted into pots of established Hygrophila (or Hydrilla) (High competition for the sprigs; invasion scenario for established plant))	8
hh LL	Hygrophila (or Hydrilla) sprigs planted into pots of established Ludwigia (High competition for the sprigs; invasion scenario for established plant)	8
HH	Growth of established Hygrophila (or Hydrilla) plants (no competition from invading sprigs)	4
LL	Growth of established Ludwigia plants (no competition from invading sprigs)	4

Four treatments utilized established plants of the native or the competitor species (Figure 1). Sprigs of Ludwigia or the competitor species were planted into the pots containing established plants (llHH, hhLL) while other pots containing only established plants (HH, LL) were used to track the continued plant growth without any competitive pressure from invading fragments of the other species. All individual pots were secured within Mobile Underwater Plant Propagation Trays (MUPPT) developed and used for EAHCP restoration and applied research projects (Figure 1).

Note that the llHH and hhLL pots serve dual purpose. The sprig growth in these pots represents the growth of plant sprigs in high-competition environments (Experiment 1A or 2A). The continued growth of the established plant following invasion from the sprigs is the invaded scenario of the established plant experiments (Experiments 1B and 2B).

Figure 1. **Example of Experimental layout of treatments within a MUPPT (left) and MUPPT deployed in the San Marcos River (right). Examples of pots of several of the treatments are highlighted.**

2.2 Initial Setup and Sampling

Seven experimental treatments (Table 3) were randomly assigned and simultaneously placed into paired MUPPTs similar to the arrangement diagrammed in Figure 2. A total of 48 pots contained 8 replicates of 5 treatments – only Ludwigia sprigs (ll), only Hygrophila or Hydrilla sprigs (hh), a combination of sprigs (llhh), established plants with sprigs of the opposite species (LLhh and hhLL) – and 4 replicates of established plants for both species (LL and HH). Adjacent spaces were left empty to minimize interaction between pots, resulting in two MUPPTs being needed at each location.

Pre-established plants and sprigs were planted in 600mL quart-sized nursery pots filled with native silty/clay sediment collected from the respective rivers in which the study was carried out. Native sediment was collected in areas with no plant growth and further screened for plant propagules to prevent extraneous plant growth in treatments. Established plants were obtained by pre-culturing plants for three weeks in MUPPTs near the Landa Lake High Light location (Comal study) or at the experimental location used on the San Marcos (City Park) to allow robust

initial establishment and growth. Healthy plants of uniform size were selected for the experiment as well as to obtain initial biometric measurements. Stem cuttings were collected from healthy, established plants and inspected to ensure they had no visible signs of herbivory or disease. Sprigs 20cm in length were selected for experimental use and harvested for initial biomass.

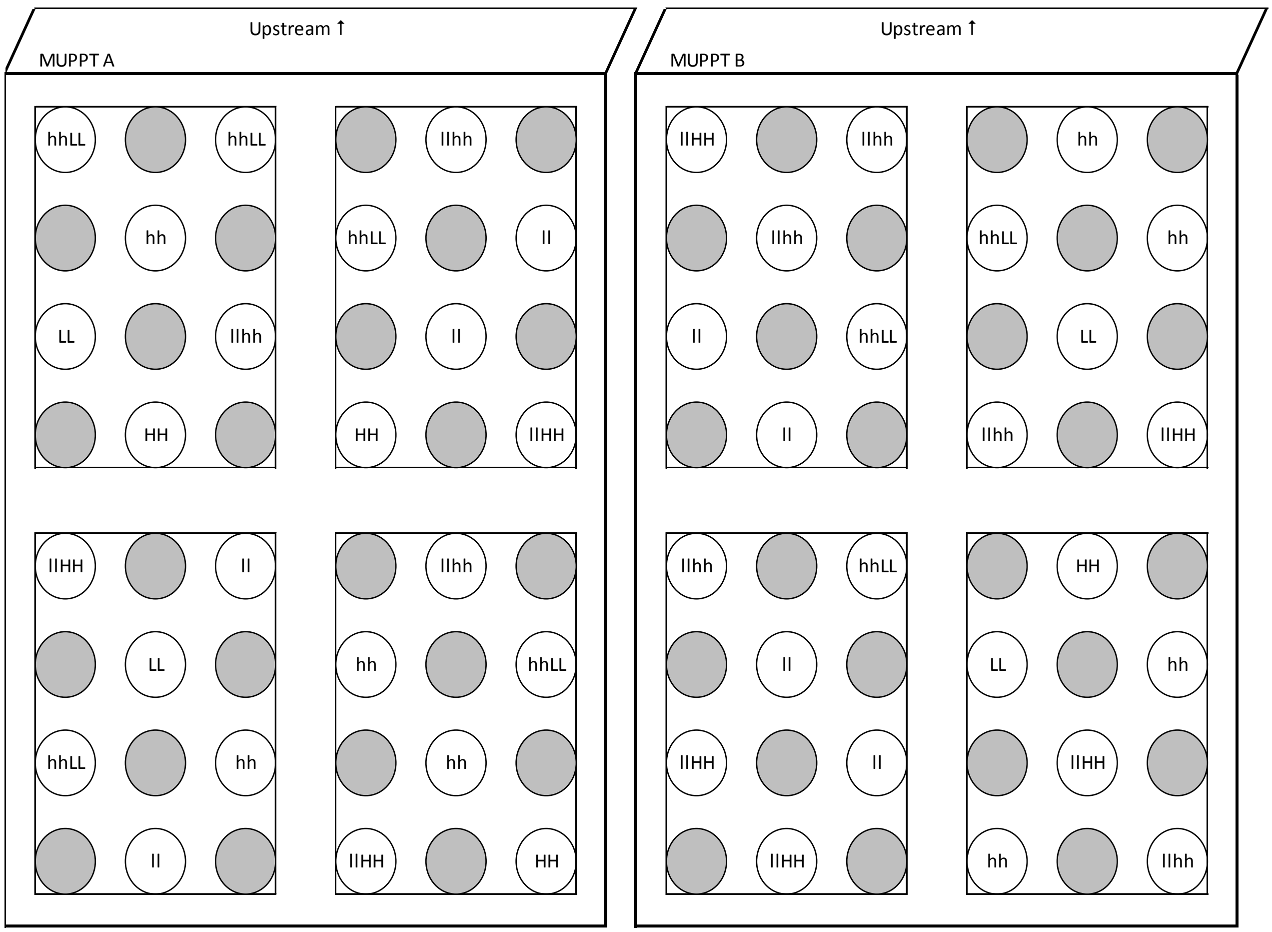

Figure 2. **Illustrated arrangement of alternating experimental pot placement within two MUPPTS anchored at each location. Open circles display the 7 possible experimental combinations (Table 3), and gray circles represent empty spaces.**

Four locations were selected on the Comal River to represent the variability of environmental conditions found within this system. Locations were selected within the Upper Spring Run (USR), Landa Lake in a shaded location (Landa Lake Low Light, LLLL), Landa Lake in a full sun exposure location (Landa Lake High Light, LLHL), and the Old Channel (OC; Figure 3). The Landa Lake High Light location was adjacent to the MUPPT culture station for restoration plantings while the Landa Lake Low Light location was along the western shoreline under the shade of an overhanging live oak tree. All four of these locations were initially planted on May 13, 2015 and harvested on July 27, 2015. In the San Marcos River two locations were chosen.

One location (1) above Rio Vista falls at City Park (CP) and another location (2A) below Rio Vista falls (Figure 3).

Rio Vista falls provides a distinctive dissection in the velocity characteristics of the San Marcos with river velocities below this point typically faster than velocities above the falls. The San Marcos study was initiated on April 23, 2015. Unfortunately, the significant flood event of May 2015 scoured out and destroyed the portion of the experiment at the downstream location (2A). The City Park location was minimally impacted, and continued until it was harvested on June 30, 2015. In order to provide information from the lower portion of the river, another site near the I35 crossing was selected (location 2B or I35, Figure 3) and plantings were initiated on July 6, 2015. The plants at this downstream location were harvested on September 11, 2015.

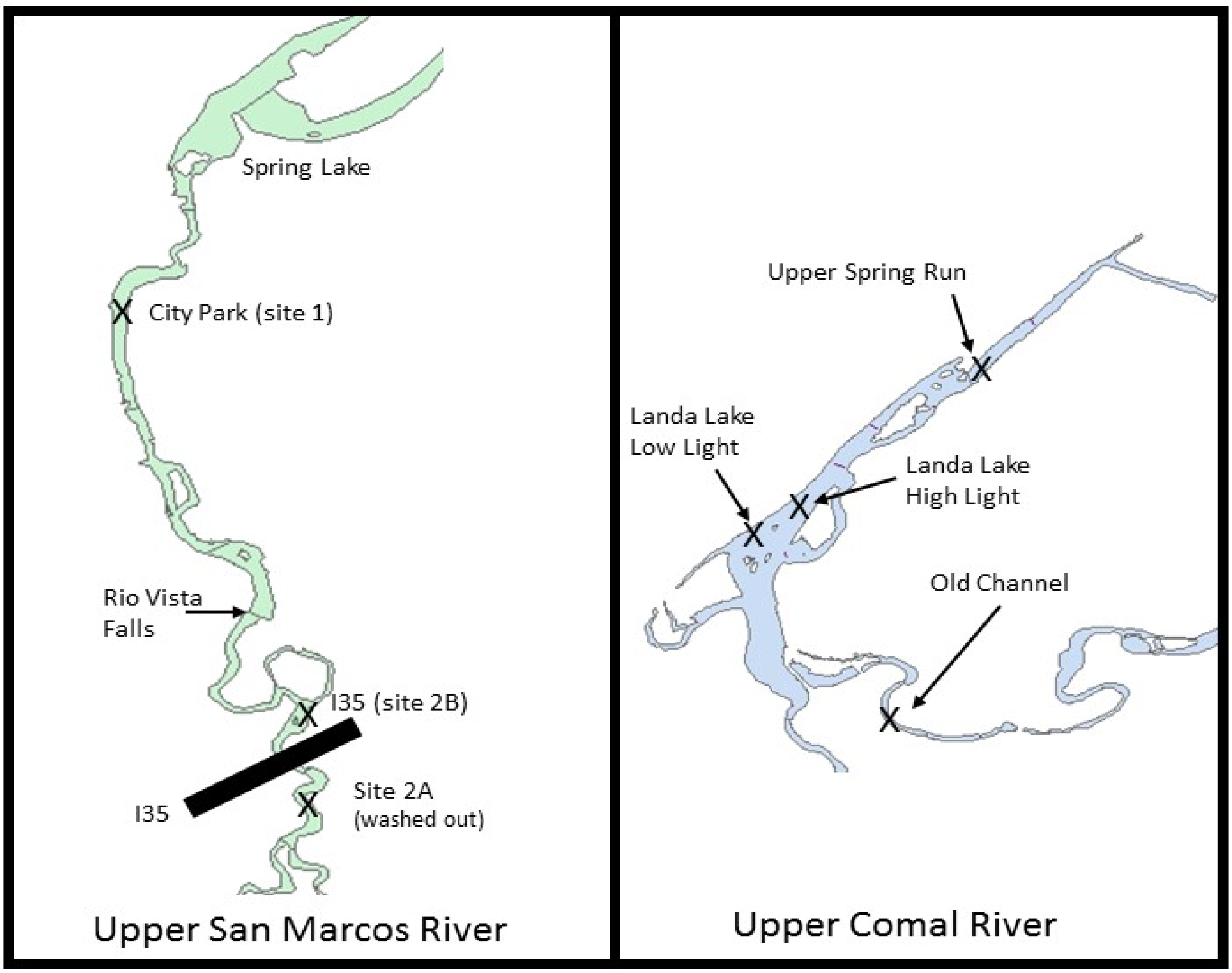

Figure 3. **Maps of the upper San Marcos and upper Comal Rivers showing locations of MUPPT deployment for competition experiments.**

After plantings were made, monitoring of growth and environmental characteristics (total depth, velocity at 80% and 20% of depth, temperature, DO and pH) occurred once per week. Photosynthetically active radiation or PAR was measured intermittently at each location over the course of several days using the Odyssey™ deployable waterproof sensor. Each experimental location, maximum stem length per species was recorded on two randomly selected individuals per treatment. Velocity and water depth were measured weekly with a Marsh-Mcbirney flo-mate while pH, temperature and dissolved oxygen (DO) were recorded at each location with a YSI™ multiparameter sonde.

Plants were harvested after 10 weeks of growth. Morphometric characteristics (stem counts and lengths) were recorded, then samples were separated into above-and-below ground tissues and dried at 60 °C for >72 hours then weighed to the nearest 0.1 mg at Baylor University.

3.0 Results

3.1 Initial measurements and Environmental conditions

Sprigs and established plants of both species were harvested to provide initial biomass estimates for each experiment. These average initial dry-weight biomass values (g/pot) ± SE, (n) were:

Comal River, Ludwigia pair of sprigs (g/pot), 0.47 ± 0.05 (16)

Comal River, Hygrophila pair of sprigs (g/pot), 0.27 ± 0.07 (16)

Comal River, established Ludwigia (g/pot), 4.15 ± 0.61 (6)

Comal River, established Hygrophila (g/pot), 2.17 ± 0.29 (6)

San Marcos (1) CP, Ludwigia pair of sprigs (g/pot), 0.48 ± 0.03 (25)

San Marcos (1) CP, Hydrilla pair of sprigs (g/pot), 0.23 ± 0.01 (30)

San Marcos (1) CP, established Ludwigia (g/pot), 4.79 ± 0.49 (6)

San Marcos (1) CP, established Hydrilla (g/pot), 2.65 ± 0.59 (6)

San Marcos (2B) I35, Ludwigia pair of sprigs (g/pot), 0.38 ± 0.03 (13)

San Marcos (2B) I35, Hydrilla pair of sprigs (g/pot), 0.54 ± 0.02 (16)

San Marcos (2B) I35, established Ludwigia (g/pot), 6.28 ± 0.30 (6)

San Marcos (2B) I35, established Hydrilla (g/pot), 2.63 ± 1.24 (6)

Environmental factors at each experimental location are summarized in Table 4. The recorded PAR maximums for each location were LLHL: 876 E/m² ; LLLL: 620 E/m² ; OC: 699 E/m² day. ; CP: 620 E/m² day. Average daily PAR at the LLHL location were 26% higher than average daily PAR measurements at the LLLL location. Data from USR and I35 were not recoverable.

Table 4. Summary of environmental parameters (± SE) for locations selected for the competition experiments. Depth and Velocity were measured in U.S. and converted to metric.

Location	Depth (cm)	Temp (°C)	DO (mgL^{-1})	pH	Vel. at 80% ($msec^{-1}$)	Vel. at 20% ($msec^{-1}$)
Comal River						
USR	98 ± 1	24.3 ± .2	4.67 ± .18	7.62 ± .04	0.08 ± .01	0.2 ± .01
LLHL	95 ± 2	24.1 ± .1	4.71 ± .18	7.46 ± .10	0.09 ± .02	0.23 ± .02
LLLL	120 ± 1	23.9 ± .1	4.61 ± .14	7.63 ± .07	0.09 ± .02	0.27 ± .02
OC	92 ± 1	23.9 ± .1	4.88 ± .08	7.62 ± .03	0.05 ± .03	0.56 ± .02
San Marcos River						
I35 (2B)	79 ± 1	22.2 ± .2	5.02 ± .20	7.55 ± .05	0.32 ± .07	1.03 ± .06
CP (1)	95 ± 4	22.1 ± .1	5.99 ± .29	7.42 ± .07	0.32 ± .12	0.6 ± .05

3.2 Plant growth over study period.

Figures 4 and 5 show the average growth of plant sprigs and established plants in the Comal (Ludwigia and Hygrophila) and the San Marcos (Ludwigia and Hydrilla). These data show that growth of Ludwigia was relatively robust at all locations. Growth of Hygrophila and Hydrilla was much more variable, and in general much less robust than the growth of Ludwigia.

Ludwigia sprigs (red bars, Figures 4 and 5) showed good establishment and growth in all experiments, although maximum stem length remained relatively modest as the plants appear to have mostly grown laterally. Established plants of Ludwigia showed very consistent data through time. Because the plants were in relatively high light environments, the plants tended to "bush out" rather than grow in length, a common adaptation for high-light growth environments. This effect is evident from the observation of the plants at San Marcos City Park (Site 1) at the end of the growth period (Figure 6). The MUPPT is very full of robust Ludwigia plants, although it is evident that the plants are "bushy" rather than elongated. Hygrophila sprigs in the Comal showed growth similar to that of Ludwigia sprigs at USR and OC, but lower growth in the two locations within Landa Lake. Hygrophila sprigs required repeated sprigging within the first week as many initial sprigs did not remain in their pots. In the San Marcos, Hydrilla sprigs tended to decline towards the end of the experimental growth periods. Established Hydrilla grew well at City Park (Site 1), but declined through time at the I35 (Site 2B) location.

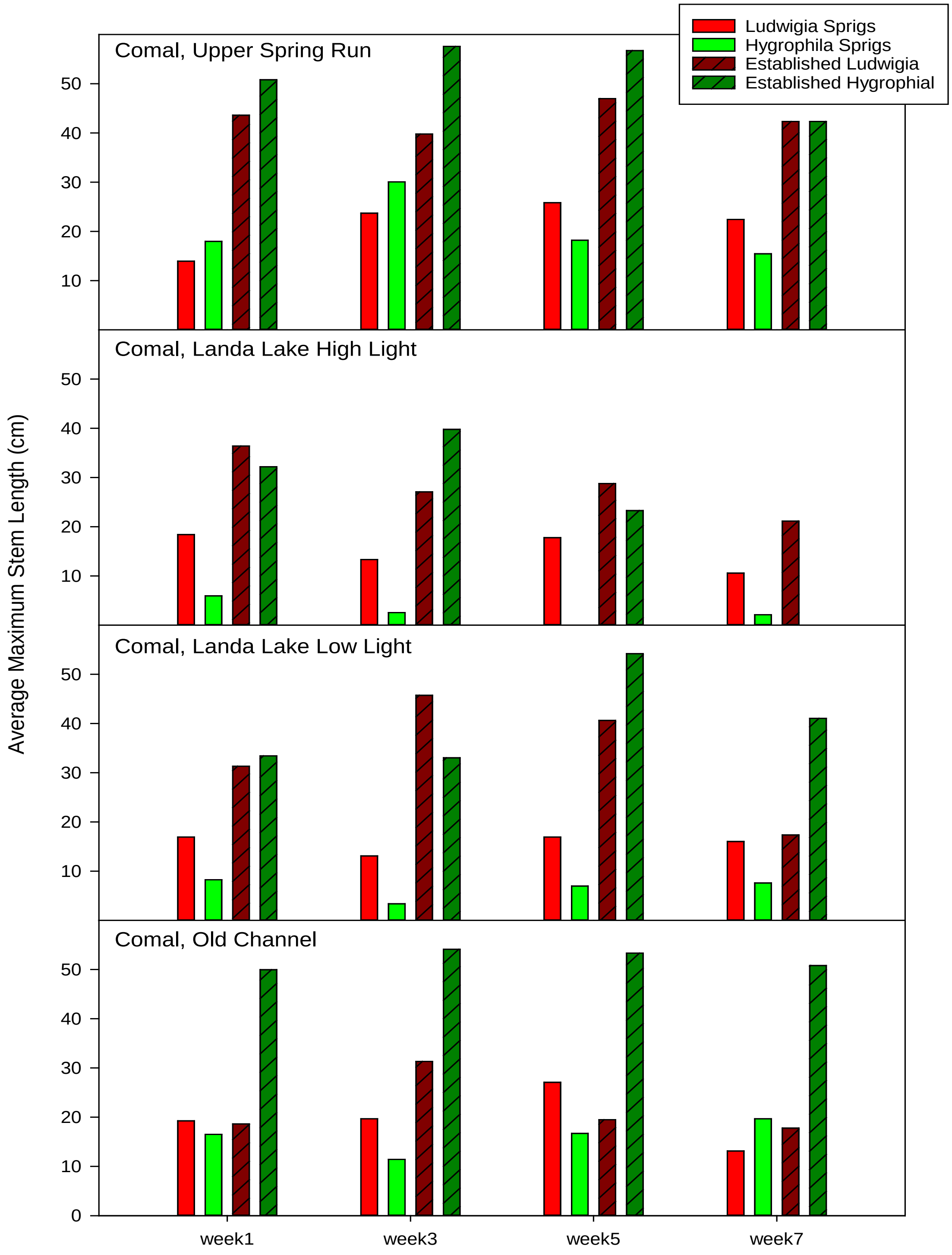

Figure 4. **Average maximum stem length of plants at each of the four experimental locations on the Comal River. Data is shown for sprigs of Ludwigia (red) and Hygrophila (green) as well as established Ludwigia (dark red, hatched) and Hygrophila (dark green, hatched).**

Figure 5. **Average maximum stem length of plants at each of the two experimental locations on the San Marcos River. Data is shown for sprigs of Ludwigia (red) and Hydrilla (green) as well as established Ludwigia (dark red, hatched) and Hydrilla (dark green, hatched).**

Figure 6. MUPPT at final harvest at San Marcos City Park (Site 1) location. Ludwigia plants (red) showed very robust growth. Hydrilla plants (green) showed variable success, although some plants were clearly very healthy.

3.3 Ludwigia X Hygrophila Sprig Competition Experiments.

Table 5 reports the outcome of the two-way ANOVA investigating the impact of competition (C) and location (L) on the growth of establishing sprigs of Ludwigia and Hygrophila. Notably, the lack of significant interaction between the two factors (C X L) allows evaluation of the C and L main effects. This lack of a significant interaction effect confirms that the pattern of competition impacts on the plant growth was consistent across all four planting locations and vice versa, the impacts of location were consistent regardless of level of competition.

Competition was not significant ($p>0.05$) for all growth parameters measured for both species. Even though the competition factor was not significant at the 0.05 level, Ludwigia total mass and total number of shoots showed a tendency toward lower values when the sprigs were planted into established Hygrophila ($P=0.07$, Table 5, Figure 7, white bars). However, there was no indication of lowered growth when the Ludwigia sprigs were planted with Hygrophila sprigs. The average maximum length at harvest and allocation of tissues to above ground versus below ground tissues of Ludwigia sprigs were not impacted by competition (Table 5, P=0.30, 0.62, respectively).

When planted in monoculture (two sprigs in empty pots), the biomass of Ludwigia at the end of the growth period exceeded that of Hygrophila by about 3.5x (Table 5, Figure 7). This result differs from that of Doyle et al. (2003) where the plants in monoculture had virtually identical growth.

Table 5 shows strong location effects on the growth of both species, indicating that the planting location had strong impacts on growth at all levels of competition. The location effect is significant for Ludwigia total mass and number of shoots. The biomass and number of shoots of Ludwigia was consistently 2-3x higher at the Landa Lake high light location (LLHL) than at the Landa Lake Low Light (LLLL) and the Old Channel (OC) locations. The impacts of location were much more severe for Hygrophila, where the plants were virtually eliminated at LLHL (possibly by herbivory) but was much higher at the OC location. Only in the OC was the growth of Hygrophila higher than the growth of Ludwigia.

Table 5. **Final mean and standard error (SE) for growth parameters of Ludwigia or Hygrophila sprigs grown under varying levels of competition (none, sprigs, established) at four locations in the Comal River. Also shown is the significance level of the two-way ANOVA testing effect of competition levels and location. Differences among competition levels or among locations determined by HSD-Tukey post hoc comparisons if interaction term was not significant and indicated by different letter superscripts.**

	Competition Treatments (C)			Locations (L)*				Two-way ANOVA		
	None	Sprigs	Est.	LLHL	LLLL	USR	OC	C X L	C	L
Ludwigia										
Total Mass (g)	1.89[a] (0.36)	1.90[a] (0.49)	0.86[a] (0.20)	2.47[b] (0.60)	0.96[a] (0.28)	1.75[ab] (0.45)	1.01[a] (0.25)	0.39	0.07	0.04
# shoots	2.59[a] (0.50)	2.25[a] (0.46)	1.28[a] (0.30)	3.21[b] (0.64)	1.04[a] (0.23)	2.25[ab] (0.56)	1.67[a] (0.41)	0.22	0.07	0.01
Max Lgth (cm)	20.4[a] (2.6)	20.7[a] (3.1)	15.1[a] (2.9)	13.0[a] (1.6)	16.6[a] (3.3)	23.4[a] (3.6)	21.8[a] (3.9)	0.94	0.30	0.11
AG:BG	4.09[a] (0.61)	4.31[a] (0.51)	3.28[a] (0.97)	3.23[a] (0.43)	4.60[a] (1.01)	3.74[a] (0.82)	4.25[a] (0.82)	0.47	0.62	0.73
Hygrophila										
Total Mass (g)	0.54[a] (0.23)	0.89[a] (0.28)	0.38[a] (0.12)	0.02[a] (0.01)	0.09[ab] (0.05)	0.95[bc] (0.21)	1.35[c] (0.42)	0.33	0.19	0.00
# shoots	0.94[a] (0.36)	1.18[a] (0.26)	0.72[a] (0.18)	0.13[a] (0.07)	0.21[a] (0.08)	1.63[b] (0.35)	1.83[b] (0.42)	0.48	0.40	0.00
Max Lgth (cm)	8.8[a] (3.17)	16.3[a] (4.0)	7.6[a] (2.6)	0.3[a] (0.2)	5.0[a] (2.8)	19.1[b] (4.4)	18.6[b] (4.6)	0.19	0.08	0.00
AG:BG	4.66[a] (1.63)	5.86[a] (1.20)	2.02[a] (0.58)	0.50[a] (1.41)	2.88[a] (1.38)	3.81[a] (0.81)	5.49[a] (1.35)	0.34	0.08	0.00

*Locations: Landa Lake High Light (LLHL), Landa Lake Low Light (LLLL), Upper Spring Run (USR), Old Channel (OC)

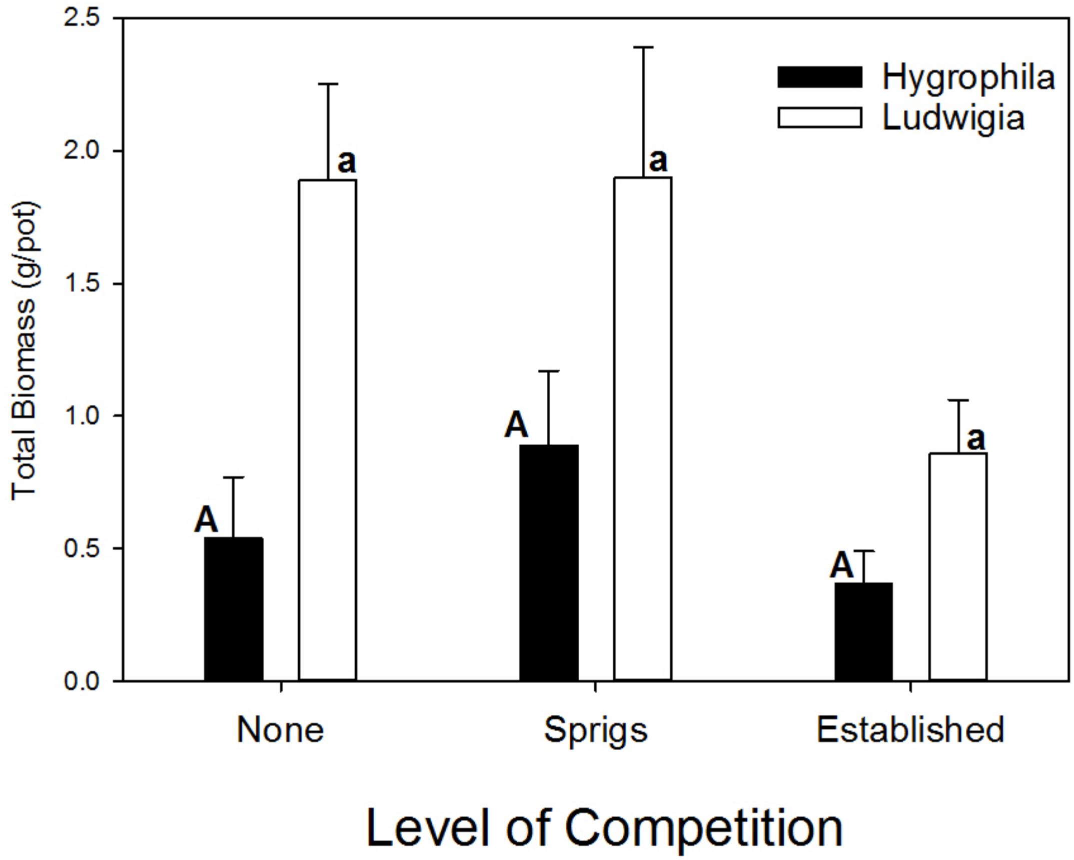

Figure 7. **Final total biomass of plants of Hygrophila (black bars) or Ludwigia (white bars) grown from two planted sprigs under three levels of competition (no competitor, two sprigs of competitor, established competitor). Shown are mean +/- SE. Two Way ANOVA (Location X Treatment) analysis showed no significant interaction between terms. The treatment factor was not significant for either species (P=0.19 Hygrophila, P=0.07 Ludwigia).**

3.4 Ludwigia X Hygrophila Continued Growth of Established Plants With and Without Invasion.

Table 6 shows the outcome of the two-way ANOVA investigating the impact of competition (C) and location (L) on the continued growth of established plants with and without invasion by sprigs of the other species. For both species, the lack of a significant interaction effect (C X L) allows the evaluation of the main effects (C and L) on the growth of the plants. Again, this fact confirms that the pattern of competition impact on the plant growth was consistent across all four planting locations and vice versa, the impact of location was consistent regardless of level of competition.

The continued growth of established Ludwigia plants was not impacted by invasion with Hygrophila sprigs. The averages of plants grown without competitive pressure and those invaded by sprigs of Hygrophila were virtually identical (Table 6, Figure 8). This result differs strongly from that of Doyle et al. (2003), where invasion of sprigs suppressed the continued growth of Ludwigia by 35%.

Surprisingly, the growth of Hygrophila was somewhat impacted by invasion by Ludwigia sprigs (Table 6). Hygrophila shoot number was significantly reduced ($P=0.03$) while total biomass showed a tendency to be reduced by about 30% ($P=0.10$, Figure 8) and plants tended to have lower proportional growth of above ground tissues ($P=0.06$). This comparison was not made by Doyle et al. 2003.

The continued growth of established Ludwigia and Hygrophila plants was also strongly impacted by planting location (Table 6, $P<0.00$ for all parameters measured). For example, the total biomass of Ludwigia at USR was 6.5X higher than that in the OC. The location impact was even larger for Hygrophila where total biomass at the OC site exceeded that at LLHL by more than 15X.

Table 6. **Final mean and standard error (SE) for growth parameters of established Ludwigia or Hygrophila grown without competitive pressure (none) or after invaded by two sprigs of the other species (invaded) at four locations in the Comal River. Also shown is the significance level of the two-way ANOVA testing effect of competition levels and location. Differences between competition levels or among locations determined by HSD-Tukey post hoc comparisons if interaction term was not significant and indicated by different letter superscripts.**

	Competition Treatments (C)		Locations (L)*				Two-way ANOVA		
	None	Invaded	LLHL	LLLL	USR	OC	C X L	C	L
Ludwigia									
Total Mass (g)	5.60^{a} (1.21)	5.49^{a} (0.97)	6.24^{b} (1.17)	2.42^{a} (0.75)	11.67^{c} (1.40)	1.78^{a} (0.40)	0.61	0.91	0.00
# shoots	7.94^{a} (1.42)	4.94^{a} (0.88)	5.08^{a} (0.80)	1.92^{a} (0.50)	11.75^{b} (1.63)	2.33^{a} (0.68)	0.88	0.37	0.00
Max Lgth (cm)	26.3^{a} (4.9)	24.7^{a} (2.8)	17.2^{a} (1.7)	20.7^{a} (5.1)	45.1^{b} (2.0)	18.0^{a} (4.9)	0.40	0.67	0.00
AG:BG	1.81^{a} (0.39)	1.84^{a} (0.38)	1.09^{a} (0.12)	1.19^{a} (0.44)	4.06^{b} (0.58)	0.72^{a} (0.18)	0.16	0.99	0.00
Hygrophila									
Total Mass (g)	7.27^{a} (1.87)	5.08^{a} (0.85)	0.74^{a} (0.24)	3.88^{ab} (0.95)	7.31^{bc} (0.93)	11.32^{c} (2.17)	0.29	0.10	0.00
# shoots	6.00^{b} (1.38)	4.04^{a} (0.60)	0.92^{a} (0.29)	3.08^{ab} (0.82)	5.67^{b} (0.86)	9.17^{c} (1.23)	0.06	0.03	0.00
Max Lgth (cm)	38.1^{a} (6.4)	31.9^{a} (4.2)	4.0^{a} (1.1)	34.5^{b} (7.0)	41.9^{bc} (4.4)	55.4^{c} (3.8)	0.63	0.21	0.00
AG:BG	4.32^{a} (1.18)	2.54^{a} (0.52)	0.46^{a} (1.07)	2.87^{a} (0.94)	3.95^{ab} (0.86)	5.71^{b} (0.72)	0.14	0.06	0.00

*Locations: Landa Lake High Light (LLHL), Landa Lake Low Light (LLLL), Upper Spring Run (USR), Old Channel (OC)

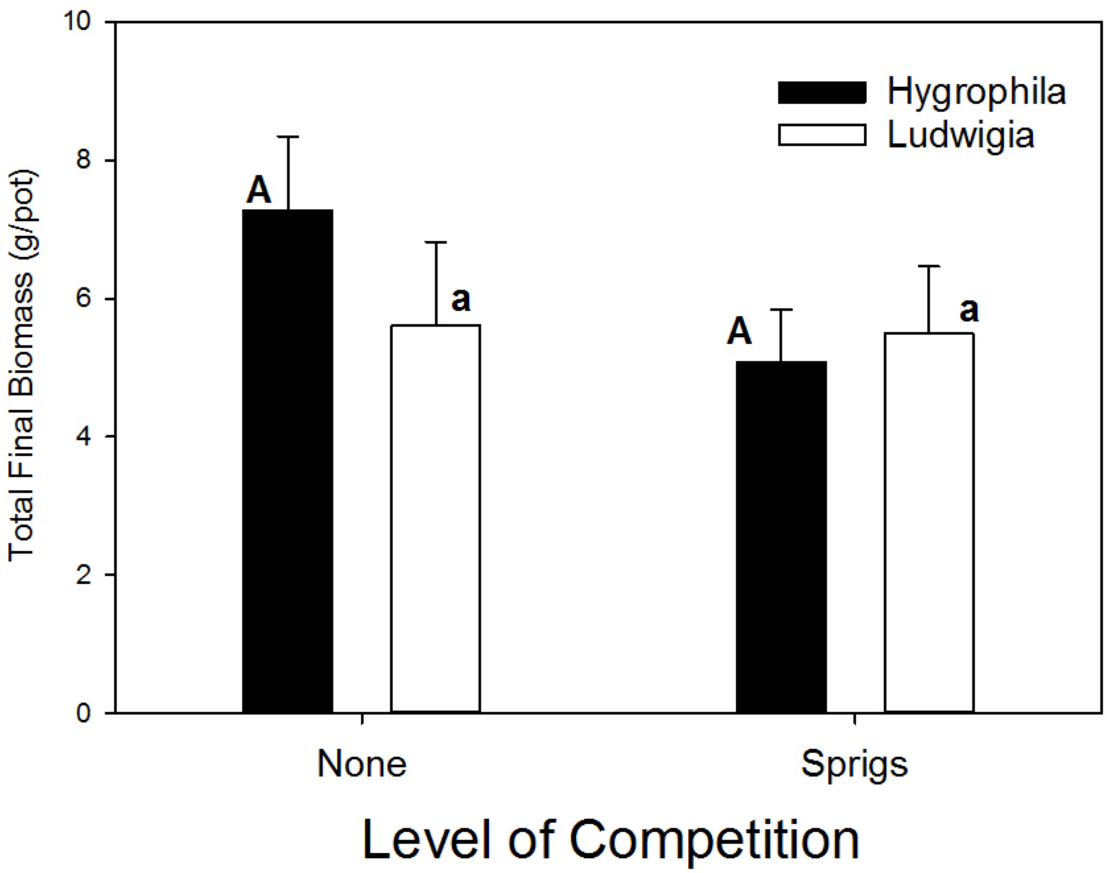

Figure 8. **Final total biomass of established plants of Hygrophila (black bars) or Ludwigia (white bars) grown with no competitive pressure (none) or invaded by two sprigs of the other species (sprigs). Shown are mean +/- SE. Two Way ANOVA (Location X Treatment) analysis showed no significant interaction between terms. The treatment factor (shown) was not significant for either species (P=0.10 Hygrophila, P=0.91 Ludwigia).**

3.5 Ludwigia X Hydrilla Sprig Experiments.

Table 7 reports the outcome of the two-way ANOVA investigating the impact of competition (C) and location (L) on the growth of establishing sprigs of Ludwigia and Hydrilla in the San Marcos River. Notably, the lack of significant interaction between the two factors (C X L) allows evaluation of the C and L main effects. This lack of a significant interaction effect confirms that the pattern of competition impacts on the plant growth was consistent across each planting location and vice versa, the impacts of location were consistent regardless of level of competition. This finding is particularly significant in light of the fact that the experiments at the two locations on the San Marcos did not occur simultaneously. As described earlier, the initial downstream location planted on April 23 was completely scoured by flooding prior to harvest. This downstream site was re-planted at I35 (Site 2B) in early July. Hence, the "location" factor for the San Marcos also contains a "season" factor imbedded in it.

The very poor survival and growth of Hydrilla sprigs when grown without competition was a very surprising outcome (Table 7, Figure 9). In fact, by the end of the experiment, most pots planted with Hydrilla sprigs failed to survive at all. Importantly, this identical same result was found for Hydrilla sprigs at both locations, which include the upstream planting made in April and the downstream planting in July. Ludwigia survival and growth when planted into empty pots was vigorous, and much higher than that of Hydrilla (Figure 9).

Ludwigia biomass accumulation over the experimental growth period was negatively impacted by Hydrilla competition, despite the poor growth of the Hydrilla sprigs. Ludwigia sprigs competing with Hydrilla sprigs or with established plants of Hydrilla showed significant declines of 25% and 64% respectively compared to Ludwigia sprigs grown alone (Figure 9). Additionally, all Ludwigia growth parameters measured showed a significant negative response to Hydrilla competition. In addition to biomass, shoot number, maximum length, and proportional investment in above ground tissues were all significantly lower for sprigs planted into pots with established Hydrilla (Table 7).

The level of Ludwigia competition was not a significant factor in Hydrilla growth. Hydrilla sprig growth was statistically similar at all levels of Ludwigia competition. However, the overall very poor growth of Hydrilla sprigs likely masks any possible competitive impact Ludwigia may have had.

The location factor was significant for Ludwigia total mass and number of shoots, with higher values for plants grown at the I35 location. In contrast, location was not a significant factor for Hydrilla biomass or stem number, likely due to the overall poor growth of Hydrilla sprigs at both locations.

Table 7. **Final mean and standard error (SE) for growth parameters of Ludwigia or Hydrilla sprigs grown under varying levels of competition (none, sprigs, established) at two locations in the San Marcos River. Also shown is the significance level of the two-way ANOVA testing effect of competition levels and location. Differences among competition levels or among locations determined by HSD-Tukey post hoc comparisons if interaction term was not significant and indicated by different letter superscripts.**

	Competition Treatments (C)			Locations (L)*		Two-way ANOVA		
	None	Sprig	Est.	CP	I35	C X L	C	L
Ludwigia								
Total Mass (g)	6.57^{c} (0.61)	4.96^{b} (0.61)	2.39^{a} (0.57)	3.78^{a} (0.41)	5.87^{b} (0.63)	0.32	*0.00*	*0.00*
# shoots	5.44^{b} (0.80)	4.19^{ab} (0.39)	3.19^{a} (0.52)	3.04^{a} (0.29)	5.50^{b} (0.56)	0.20	*0.01*	*0.00*
Max Lgth (cm)	34.5^{b} (1.4)	29.6^{ab} (1.5)	26.3^{a} (2.2)	30.8^{a} (1.2)	29.46^{a} (1.8)	0.56	*0.02*	0.50
AG:BG	7.01^{b} (0.74)	6.14^{ab} (0.83)	4.69^{a} (0.58)	7.54^{b} (0.66)	4.36^{a} (0.32)	0.24	*0.03*	*0.00*
Hydrilla								
Total Mass (g)	0.06^{a} (0.02)	0.22^{a} (0.14)	0.13^{a} (0.03)	0.17^{a} (0.10)	0.11^{a} (0.02)	0.26	0.42	0.55
# shoots	0.38^{a} (0.18)	0.94^{a} (0.27)	1.06^{a} (0.25)	0.88^{a} (0.21)	0.71^{a} (0.19)	0.12	0.09	0.53
Max Lgth (cm)	2.5^{a} (1.3)	6.2^{a} (3.7)	5.1^{a} (1.8)	7.2^{a} (2.7)	1.6^{a} (0.7)	0.37	0.54	0.07
AG:BG	1.00^{a} (0.58)	2.15^{a} (1.35)	2.07^{a} (1.60)	4.07^{b} (1.74)	0.23^{a} (0.08)	0.52	0.45	*0.02*

*Locations: City Park (CP), Interstate I35 crossing (I35)

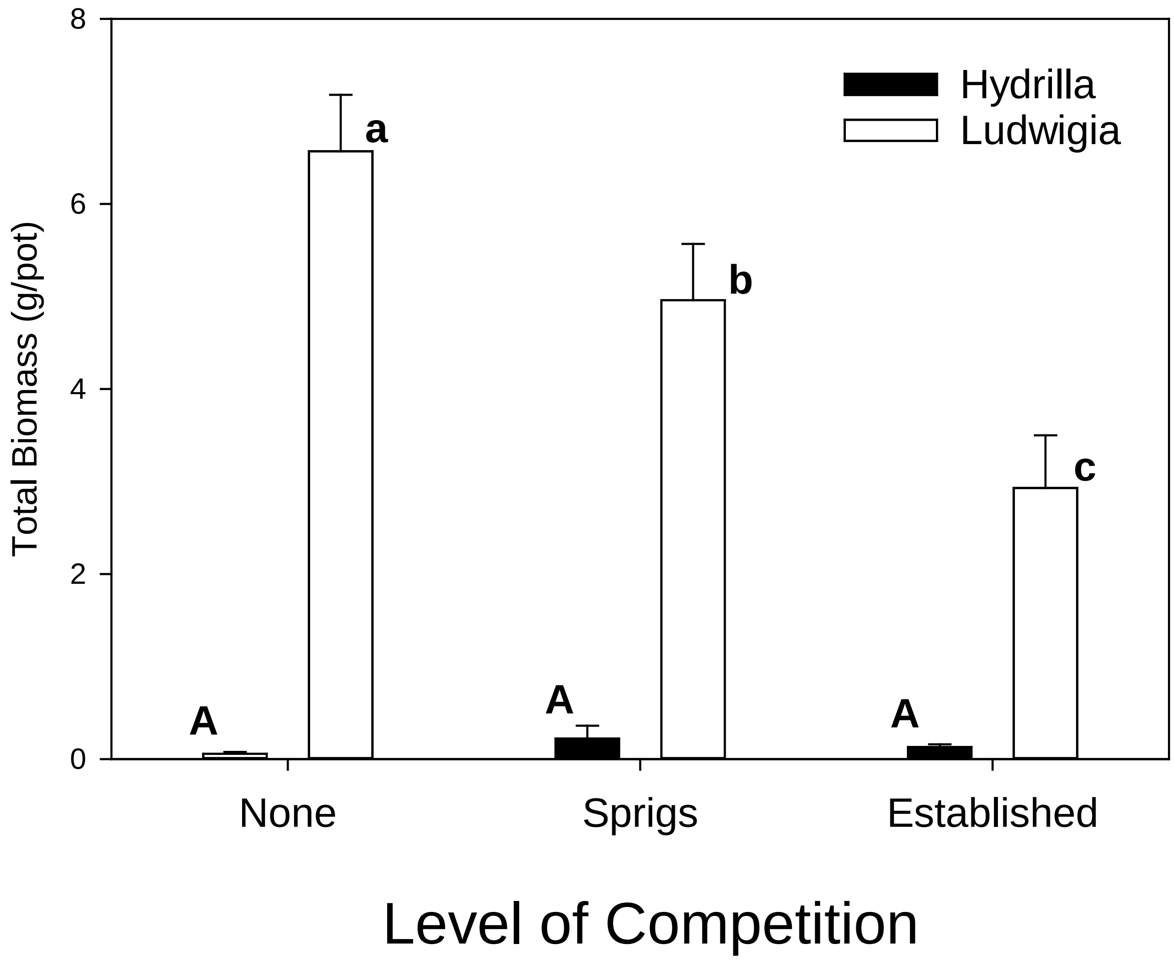

Figure 9. **Final total biomass of plants of Hydrilla (black bars) or Ludwigia (white bars) grown from two planted sprigs under three levels of competition (no competitor, two sprigs of competitor, established competitor). Shown are mean +/- SE. Two Way ANOVA (Location X Treatment) analysis showed no significant interaction between terms. The competition factor was significant for Ludwigia (P=0.00) with declining total biomass as level of competition increased. The competition factor was not significant for Hydrilla (P=0.42) although these results appear to be highly impacted by heavy herbivory and biomass loss.**

3.6 Ludwigia X Hydrilla Continued Growth of Established Plants With and Without Invasion. Table 8 shows the outcome of the two-way ANOVA investigating the impact of competition (C) and location (L) on the continued growth of established Ludwigia and Hydrilla plants with and without invasion by sprigs of the other species. For both species, the lack of a significant interaction effect (C X L) for most parameters allows the evaluation of the main effects (C and L) on the growth of the plants. Again, this fact confirms that the pattern of competition impact on the plant growth was consistent across both planting locations and vice versa, the impact of location was consistent regardless of level of competition.

The continued growth of established Ludwigia plants was impacted by invasion with Hydrilla sprigs (Figure 10). The biomass of established Ludwigia plants invaded by Hydrilla was significantly reduced by 17% relative to plants continuing to grow without invasion. This invasion impact is particularly notable given the overall poor growth of the Hydrilla sprigs. Possibly, under conditions with higher Hydrilla growth, the impact on the Ludwigia may be higher.

The continued growth of established Hydrilla plants was not impacted by Ludwigia competition (P=0.32). There was no statistically significant difference in any of the growth parameters measured for Hydrilla plants invaded by Ludwigia relative to uninvaded plants.

The continued growth of established Ludwigia and Hydrilla plants was significantly impacted by planting location. The total biomass and number of shoots of established Ludwigia plants at the end of the experimental growth period were significantly higher at I35 relative to that at City Park, while the opposite was true for Hydrilla (Table 8).

However, the overall growth of the two species was strikingly different. Overall, established Ludwigia plants growing without competitive pressure was more than 15X higher than that of established Hydrilla growing alone (Figure 10).

Table 8. **Final mean and standard error (SE) for growth parameters of established Ludwigia or Hydrilla grown without competition (none) or after invaded by two sprigs of the other species (invaded) at two locations in the San Marcos River. Also shown is the significance level of the two-way ANOVA testing effect of competition levels and location. Differences between competition levels or among locations determined by HSD-Tukey post hoc comparisons if interaction term was not significant and indicated by different letter superscripts.**

	Competition Treatments (C)		Locations (L)*		Two-way ANOVA		
	None	Invaded	CP	I35	C X L	C	L
Ludwigia							
Total Mass (g)	23.98[b] (1.29)	19.79[a] (1.48)	18.16[a] (1.43)	24.20[b] (1.32)	0.52	*0.04*	*0.01*
# shoots	11.88[a] (1.32)	11.69[a] (0.69)	9.75[a] (0.62)	13.75[a] (0.71)	0.22	0.86	*0.00*
Max Lgth (cm)	40.4 (2.7)	40.9 (1.3)	43.8 (1.2)	37.7 (1.8)	*0.01*	0.82	0.00
AG:BG	4.20[a] (0.33)	4.33[a] (0.27)	4.38[a] (0.31)	4.19[a] (0.29)	0.38	0.78	0.49
Hydrilla							
Total Mass (g)	1.59[a] (0.43)	2.71[a] (0.87)	3.86[b] (1.03)	0.81[a] (0.14)	0.26	0.32	*0.03*
# shoots	5.50[a] (1.02)	4.63[a] (0.68)	5.08[a] (0.75)	4.75[a] (0.86)	0.43	0.49	1.00
Max Lgth (cm)	25.7[a] (9.14)	24.7[a] (6.9)	46.0[b] (6.4)	4.2[a] (0.8)	0.99	0.89	*0.00*
AG:BG	1.31[a] (0.49)	2.00[a] (0.65)	3.29[b] (0.68)	0.26[a] (006)	0.34	0.35	*0.00*

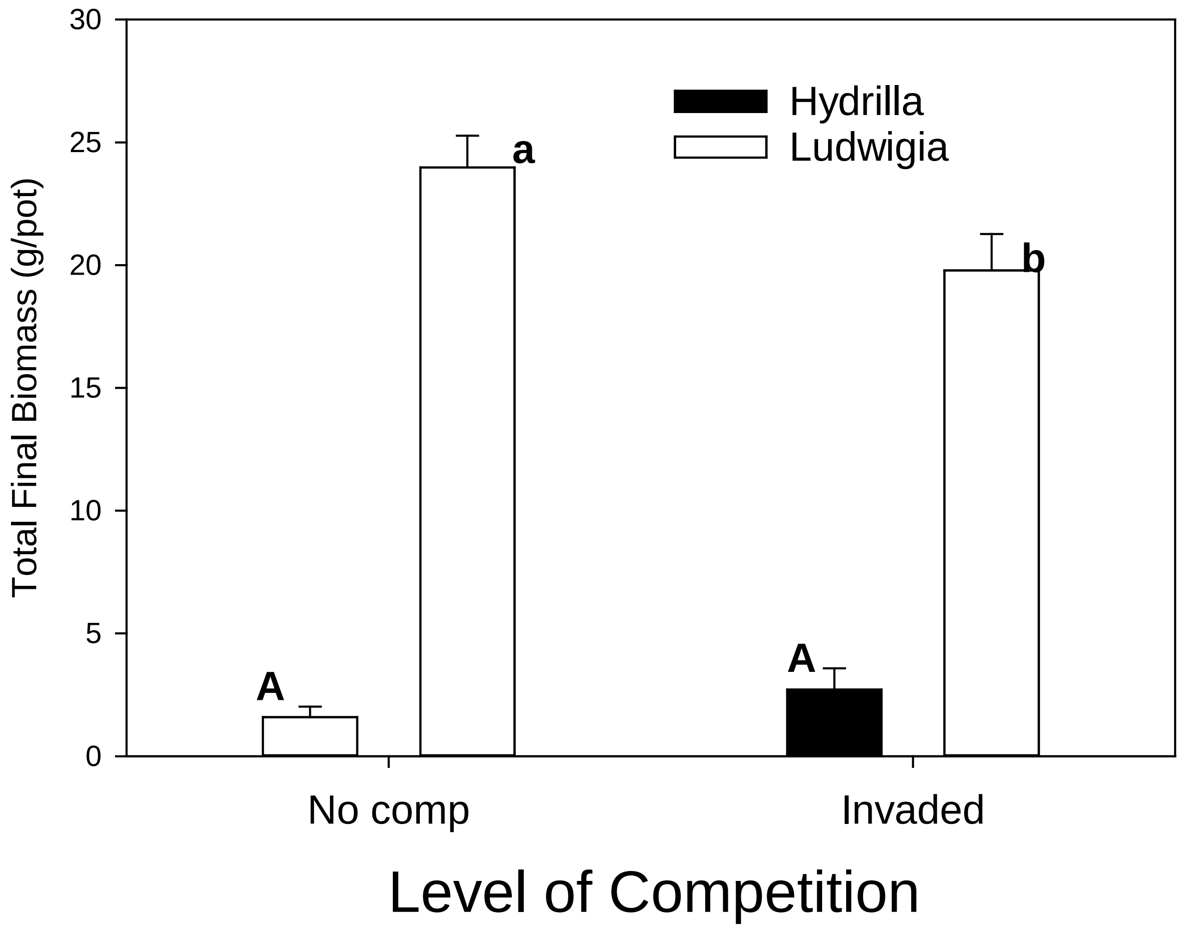

Figure 10. **Final total biomass of established plants of Hydrilla (black bars) or Ludwigia (white bars) grown with no competitive pressure (none) or invaded by two sprigs of the other species (sprigs). Shown are mean +/- SE. Two Way ANOVA (Location X Treatment) analysis showed no significant interaction between terms. The competition factor (shown) was significant for Ludwigia (P=0.04) with lower biomass levels in pots invaded by Hydrilla sprigs. The competition factor was not significant for Hydrilla (P=.32) although these results appear to be highly impacted by heavy herbivory and biomass loss.**

4.0 Discussion

Ludwigia is a native plant that appears to face competitive pressure from Hygrophila and Hydrilla, two widely distributed non-native species in the Comal (Hygrophila) and San Marcos (Hygrophila and Hydrilla) Rivers. All of these species share a similar branching growth form and are capable of asexual reproduction via establishments of viable sprigs. However, Ludwigia provides better habitat for the endangered fountain darters, and is currently being widely used in native plant restoration efforts in both rivers.

4.1 Growth of all species without competition

The results of the short-term competition experiments are generally good news for the continued use of Ludwigia in habitat restoration/enhancement efforts. The growth of Ludwigia sprigs (ll treatment) under no-competition conditions exceeded that of Hygrophila and Hydrilla (hh treatments). In fact, the establishment and growth of sprigs of the native species was more than 3X higher than Hygrophila (Table 5) and more that 10x higher than Hydrilla (Table 7) in our 10-week growth experiments. Both Hygrophila and Hydrilla sprigs appear to have suffered high mortality and poor growth under the experimental conditions tested. Likewise, the total biomass of established Ludwigia plants growing without competition (LL) was similar to that of Hygrophila (HH) (Table 6, Figure 8) and much higher than that observed for Hydrilla (HH) (Table 8, Figure 10).

These in-situ experiments include effects other than competitive interactions between the plants. Notably, we believe that herbivory negatively affected all experimental plants and proved particularly detrimental to the establishment of Hygrophila and Hydrilla sprigs. During routine monitoring we observed that the Hygrophila and Hydrilla sprigs often appeared damaged, and in some cases were entirely missing from the planted pots. In the Comal study red swamp crayfish (*Procambarus clarkii*) were observed burrowing into soil within pots and final harvest and clipped stems of some plants, especially those growing in the Landa Lake High Light location, were evident. For the established Hygrophila and Hydrilla plants, a potential explanation for the loss or zero net gain in biomass could be due to the brittle or easily fragmenting nature of the stems – a potential trade-off which might be advantageous for dispersal and colonizing new habitats.

The strong growth of Ludwigia under “no competition” conditions confirm the experience of restoration efforts in the Comal River that Ludwigia establishment and short-term growth is excellent.

4.2 Impacts of Hygrophila Competition and Location on Ludwigia Growth.

The growth of Ludwigia sprigs was not impacted by Hygrophila competition under the conditions tested in the Comal River. These results differ sharply from those of Doyle et al. (2003) that found that Ludwigia sprig relative growth rate was strongly impacted by competition

from Hygrophila sprigs (-40%) and profoundly suppressed by the presence of established Hygrophila plants (-80%).

The continued growth of established Ludwigia was likewise not impacted by competition from invading sprigs of Hygrophila (Table 6). These results also differ from those of Doyle et al. (2003) that found that total biomass of Ludwigia invaded by Hygrophila sprigs to be only 65% of that of uninvaded plants.

Ludwigia growth showed a strong location effect in the Comal River (Tables 5 and 6). The final biomass of the Ludwigia plants that developed from the sprigs varied significantly (2.4X) among the four locations, with higher values at Landa Lake High Light and lower values in the Old Channel and Landa Lake Low Light. Likewise, the final biomass of the established Ludwigia plants at the end of the experimental growth period varied by a factor of 6.5X with the highest values observed at the USR site and the lowest values seen in the OC. It is not surprising that Ludwigia showed strong location impacts, as we deliberately selected locations with variability in the factors known to impact plant growth, especially flow and light conditions. The overall growth of Ludwigia sprigs at Landa high light was more than 2.5 X higher than Landa low light (Table 5, 2.47 g versus 0.96 g) while overall growth of established Ludwigia was also greater than 2.5X at the high light location than at the low light location (Table 6, 6.24 g versus 2.42 g). While light did dramatically impact biomass accumulation, it did not necessarily impact the outcome of competition between Ludwigia and Hygrophila species. The mechanisms regulating the location effect, however, were not clear from these experiments and may warrant additional study to tease out impacts of light or velocity on the competitive interactions between plant species.

4.3 Impacts of Hydrilla Competition and Location on Ludwigia Growth.

In the San Marcos River, Ludwigia sprig growth was impacted by both Hydrilla competition and location. Ludwigia sprigs planted with Hydrilla sprigs or into pots of established Hydrilla showed significant suppression of 25% and 64%, respectively relative to pots growing without any Hydrilla competitor (Table 7). Likewise, established pots of Ludwigia showed significant (17%) suppression of growth when invaded by Hydrilla sprigs. These impacts are particularly notable given the overall poor growth of Hydrilla. For reasons we have not identified, the overall growth of Hydrilla at both locations was much lower than Ludwigia and much lower than expected based on previous experience with Hydrilla. Hydrilla is a widely distributed and successful invasive species that has been shown to be a very strong competitor, especially in “equal start” competition experiments (Smart et al., 1994, Van et al., 1999). However, the results of a New Zealand study which paired Hydrilla with various aquatic species indicate that its growth varies depending on the species with which it is planted and, subsequently, has variable impacts on the resultant biomass of that species (Hofstra et al., 1999).

Ludwigia also showed a significant location effect. Both sprigs and established Ludwigia plants showed significantly higher growth at the I35 site than at the City Park site.

4.4 Summary Evaluation

Overall, these data indicate positive short-term establishment and growth characteristics for Ludwigia, and supports the continued use of the species for restoration efforts. Ludwigia used in restoration efforts is likely to effectively establish and quickly colonize unvegetated areas of the rivers. In fact, the growth of Ludwigia sprigs was higher over the 10-week growth periods than either Hygrophila or Hydrilla. Although both non-native species appear to have suffered from herbivory impacts, there is no reason to believe that the experimental conditions used do not reflect actual levels of herbivory impacts in these systems. Therefore, Ludwigia planted into currently unvegetated areas or areas where the non-native plants have been removed are likely to grow very well.

Furthermore, Ludwigia may be less susceptible to competition impacts than previously documented. Under our experimental growth conditions, Ludwigia sprigs or established Ludwigia plants were not impacted by Hygrophila competition. Ludwigia sprigs and established plants were negatively impacted by Hydrilla, but even there all treatment levels showed significant positive growth.

While a common outcome of invasive versus native plant competition is that the invasive plant wins (hence the term "invasive") our data show that experiments conducted in situ may show a different outcome. While the biotic growth potential of a species is often linked to invasive species success, the outcome can depend on other factors too. Soil fertility, selective grazing pressures, propagule pre-emption and water velocity as well as other stressors are all factors which may promote the success of a native species and the depression of an introduced species or vice versa. Several studies have investigated the ability of *Vallisneria americana* to dominate over *Hydrilla verticillata* (Van et al., 1999, Smart et al., 1994) but soil fertility seems to determine the outcome. In our study Hydrilla continued to exert impacts upon Ludwigia despite a reduction in top growth biomass. *Hydrilla verticillata* is known to produce dense below ground biomass and propagules which may continue to compete with neighboring plant species despite its loss of stems and leaves. Also, although the Hydrilla plants were not present in some pots at the time of the final harvest, earlier growth in the season may have slowed the growth of the native plant.

The pre-emption of propagule establishment from mature native plant communities can play a preventative role in invasive plant success (Chadwell and Englehardt, 2008). In our study invasion of Hygrophila sprigs had virtually no impact upon established Ludwigia plants. As shown in studies with other invasive aquatic plants the establishment and dominance of the invasive may depend on the degree of intact native plant cover in the area of introduction. If a

well-developed native plant community exists at the site of introduction then the opportunity for invasion may substantially decrease (Bickel and Perrett, 2014).

Preferential grazing can heavily impact both native and introduced plants (Parker and Hay, 2005) and evidence suggests that this may be determined by the nutrient content, phenolic compounds or chemical or physical defenses of individual plant species (Lodge, 1990). We witnessed what was believed to be heavy herbivore grazing on Hygrophila and Ludwigia at both Landa Lake sites. While this factor probably does not fully explain our findings, we believe the effect of herbivory warrants further investigation (see below).

Finally, physical characteristics can greatly influence growth of aquatic macrophytes. As witnessed in our study, where location played a significant factor for all three species, exposure to gradients in velocity, depth and light can have significant impacts on plant growth and success. Stream velocities can provide positive conditions for plant growth yet aquatic plant biomass can be greatly reduced once a threshold is surpassed (French and Chambers, 1996) (Madson and Douglas, 2001). However certain species show phenotypic plasticity towards velocity and light gradients and can maintain vigorous growth compared to less adaptable species. A recent competition study conducted by Bilbo (2015) between *Hydrilla verticillata* and *Potamogeton illinoensis* also carried out in the San Marcos River bolsters our findings which indicate Hydrilla growth is not as vigorous when subjected to velocities above a certain threshold and several local studies have been conducted regarding occupancy of aquatic plant species along velocity gradients (Saunders et al., 2001) (Williams, 2013)

In conclusion, our study has shown that in-situ testing of competition between native and non-native aquatic vegetation species in the Comal and San Marcos systems provides differing results than when tested in a no-flow laboratory environment (Doyle et al., 2003). This updated information may be extremely valuable to the development of the EAHCP Ecological model and will be provided directly to that project team for consideration. The study also emphasizes that the successful establishment of aquatic plants is strongly location dependent and furthermore depends on a variety of factors and stressors and that the origin of the plant (native or non-native) does not automatically dictate the success of establishment or the competitive outcome.

5.0 Future Study Considerations

As is common with many studies the outcome of the data tends to ask more questions than provide answers. As such below are a few study questions instigated by the current study which may warrant further investigation.

1. What is the quantity and viability of aquatic plant propagules in the San Marcos and Comal Rivers?

The success of native and non-native aquatic plant establishment relies heavily on propagule production and distribution. In 2000 the distribution and dispersal of propagules of native and

nonnative species was investigated in the San Marcos River (Owens et al., 2001). One indication garnered from this study was that propagules of non-native species dominated across all study locations while propagules of native species were poorly represented and many not viable. Unfortunately, this study was not repeated in the Comal River. With on-going large scale removal of invasive plant species and re- introduction of native species a current understanding of propagule loading rates and viability would be important to help determine the future sustainability and outcome of the restoration projects in both systems.

2. What is the nutrient availability and how does nutrient partitioning influence growth of aquatic plants in the San Marcos and Comal Rivers?

As discussed previously several factors affect the recruitment, growth, persistence and expansion of aquatic plants in river systems. Nutrient stoichiometry—the ways in which aquatic plants use and partition nutrients—is an important process which either limits or drives the productivity of aquatic plants, but species respond to and use nutrients differently (Barko et al., 1991). Elevated levels of sediment nitrogen can limit the productivity of aquatic plant species or increase productivity in other species and uptake mechanisms of nutrients varies greatly by species (Fang et al., 2007). In essence, one factor which contributes to the growth and health of aquatic plants within these systems is sediment nutrients which have yet to be researched in-depth in either the Comal or San Marcos systems. A study to investigate the fertility of the sediment and how native and introduced plant species use or partition those nutrients would be an important step towards understanding and predicting the prolonged composition of the aquatic plant community in both systems.

3. What role does herbivory play in the establishment, growth and expansion of aquatic plants in the San Marcos and Comal Rivers?

Another observation often noted during active restoration and experimentation efforts is the impact of herbivory on plant establishment and continued growth. Defoliation pressures on the native and non-native species in this system are not well understood as they are imposed by a wide array of herbivorous vertebrate and invertebrate species. Many insect species are known to have specialized, co-evolved relationships with aquatic host plants, affecting not only floating or emergent leaf tissue but submerged anatomical features as well (Harms and Grodowitz, 2009). Recent documentation details the destructive impacts of a moth species' aquatic larvae on the native aquatic plant nurseries at the San Marcos Aquatic Resources Center (Hutchinson et al., 2015). Destruction of plant growth by aquatic caterpillars has been observed in the field as well. The invasive giant rams-horn snail (*Marisa cornuarietis*) - known to have a voracious appetite - and other herbivorous mollusks have been observed and documented feeding on the local vegetation (Grantham et al., 1995; Horne et al., 1992; Karatayev et al., 2009). Other common species with aquatic plant-dominated diets include crayfish, turtles, tilapia and water fowl. Observational and reported data suggest that the sustainability of restoration efforts could benefit from a deeper analysis of herbivore pressures.

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Appendix K3

Suspended Sediment Impacts on Texas Wild-Rice Study Scope of Work

SUSPENDED SEDIMENT IMPACTS ON TEXAS WILD RICE STUDY
SCOPE OF WORK

Task 1. Literature Review

TEXAS STATE will complete a comprehensive literature review of the effects of suspended sediment on aquatic plant communities and associated aquatic macroinvertebrates. This will include a focus on urban stream systems and in particular systems with high levels of recreational use.

Task 2. Methodology Development

TEXAS STATE will develop the project methodology and present the methodology and reasoning to the Science Committee for review and approval.

Subtask 2.1 Develop Experimental Design and Detailed Methodologies

TEXAS STATE. TEXAS STATE will develop a statistically valid study to evaluate the effect of suspended sediments on the aquatic plant community and associated macroinvertebrate community, with emphasis on heavily recreated and urban streams. TEXAS STATE will be required to provide justification for the selection of the environmental variables and the methodologies selected to conduct the study.

The study will include, at a minimum, the quantity of suspended sediments as well as the diel, weekly and seasonal suspended sediment patterns. It will include the suspended sediments effect on light attenuation, and the impacts on the aquatic vegetation and the macroinvertebrate community. TEXAS STATE will develop lists and comprehensive maps of the aquatic vegetation community and the macroinvertebrate community at each sample site.

Subtask 2.2 Present Methodologies to the Science Committee for Review

Upon completion of Subtask 2.1, TEXAS STATE will present the proposed design and methodologies to the EAHCP Science Committee, at a date to be determined by the HCP Director, for review before implementation of any activities in the field. TEXAS STATE will give a brief presentation and must be prepared to answer any questions from the Science Committee. In collaboration with HCP staff, recommendations provided by the Science Committee will be considered for inclusion in final research methodologies.

Task 3. Conduct Applied Research

TEXAS STATE will carry out experimentation consistent with the methodologies developed in Task 2 and approved by the Science Committee. TEXAS STATE will keep a project notebook

containing a description of the assumptions and methodologies used in the study analysis. The notebook shall be organized in such a way as to allow replication of the steps, calculations, and procedures used by the Consultant to reach conclusions, described in the draft final report. The project notebook shall include a CD of all raw data collected in the project and will be submitted with the draft final report. In addition, TEXAS STATE will take photographs of the experimentation (if applicable) throughout its various phases and make these photos available on the data CD and utilized in reports submitted to the EAA (where applicable).

Recreational Use of the River – TEXAS STATE will utilize all weather cameras at four locations to take pictures of specific reaches of the river. Images will be analyzed to divide recreational use into one of six categories including: swimming, tubing, fishing, kayaking, angling and dogs. Recreational use data will be linked to water quality data collected through the use of water quality sondes.

Water Quality Sampling – TEXAS STATE will maintain two water quality sondes at the Hopkins Street bridge and the IH-35 overpass. Data will be downloaded and sondes are calibrated on a bi-weekly basis. Data on turbidity (NTU), temperature (0C), and conductivity (~S/cm) will be collected at 15 minute intervals.

TEXAS STATE will collect water quality grab samples for the river during periods of high recreational activity; during periods of low recreational activity. Samples will be analyzed for total suspended solids, non-volatile suspended solids and turbidity.

TEXAS STATE will conduct mapping and classification of aquatic vegetation prior to water quality sampling. Sampling for vegetation and macroinvertebrates will be conducted approximately six (6) times per calendar year.

Drift Sampling – TEXAS STATE will collect drift samples at each study site prior to benthic or vegetation sampling in a given sampling period. Samples will be sorted in the laboratory and all invertebrates will be identified according to different functional feeding groups. All processed invertebrates for each sample will be preserved for later taxonomic identification.

Benthic Sampling – TEXAS STATE will sample the study sites using a proportional sampling design based on the percent aerial coverage of available 'habitat types' developed from the study site maps.

Benthic samples will be obtained by setting out quadrats at the selected location. A drift net will be placed at the downstream end of each quadrat. Macroinvertebrates

will then be identified and placed in different functional feeding groups. All invertebrate samples will be archived for potential future taxonomic analyses.

Vegetation Sampling – TEXAS STATE will collect vegetation samples from each site to estimate the mass of total solids, non-volatile solids, organic matter, carbonates, and epiphyton (algae attached to vegetation) biomass on different vegetation species and growth forms. The amount of total solids, non-volatile solids, carbonates, and epiphyton biomass will be expressed as the mass per unit surface area of the leaf (mg/cm2) and the mass per unit wet mass of the leaf (mg/g).

Texas Wild-Rice and Photosynthetically Active Radiation (PAR) – TEXAS STATE will test the threshold of a reduction in PAR on the vegetative growth of Texas wild-rice (TWR). TEXAS STATE will conduct an *in situ* study in Spring Lake. Initial values for flow rate, depth, and PAR will be recorded and then weekly observations of each treatment site thereafter. Weekly observations will involve recording the number of plants remaining in each treatment site, the estimated amount of average length and number of leaves for each plant, the amount of vegetative mat accumulation within the treatment site, accumulation of sediment deposit on any plants, and identifiable herbivory or vandalism of the site.

A single factor ANOVA will be used to determine the effect of light attenuation on biomass allocation. Post-hoc analyses will be conducted to determine differences between treatments.

Texas Wild-Rice *in situ* Sediment Responses - To assess impacts to TWR, TEXAS STATE will randomly select and plant approximately 75 Texas wild-rice plants at five treatment sites each with varying depth, velocity, light availability, and exposed turbidity values within the San Marcos River. Experiments will be conducted in late winter/early spring to represent minimal recreation impacts and also in mid-summer to represent maximum recreational impacts.

The sites chosen will represent conditions of: 1) ideal habitat (to act as the control site), 2) high recreation impact, 3) variable water velocities, and 4) variations in sunlight availability either by overhead canopy shading or water depth. Plants will be protected from herbivory and external damage by constructing exclosures. We propose to repeat this experiment twice based on a late winter early spring low recreation period versus a mid-summer high recreation period.

Task 4. Draft and Final Reports

TEXAS STATE will include in the Draft and Final Report a section describing the assumptions and methodology used by TEXAS STATE in generating the data, analysis and conclusions. The reports will summarize observations regarding analysis, trends, conclusions, and provide recommendations to the EAA for potential future research (if applicable).

The draft Final Report, will be submitted for review and comment no later than 30 days after the conclusion of the research. The Final Report will be due within 30 days after receipt of EAA review and comment.

TEXAS STATE will provide the Final Report, the project related data, and the project notebook in digital format. This digital report shall be contained appropriate electronic storage media, and shall be in Searchable Adobe Acrobat format, or other agreed upon format.

Task 5. Meetings and Presentations

At the direction of the HCP Director, TEXAS STATE will attend and be prepared to discuss the project at a minimum of two meetings with the Science Committee and two meetings with the Implementing Committee.

Appendix K4

Algae and Dissolved Oxygen Dynamics of Landa Lake and the Upper Spring Run

Algae and Dissolved Oxygen Dynamics of Landa Lake and the Upper Spring Run

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Executive Summary

The BIO-WEST, Inc. Project Team, including staff from Baylor University's Center for Reservoir and Aquatic Systems Research and Aqua Strategies Inc, developed and implemented specific studies to evaluate potential impacts to the habitat of the federally-listed endangered fountain darter (*Etheostoma fonticola*). The studies were divided to address two component topics during the 2015 field season:

A. determination of the potential impacts of benthic algal mats on underwater vegetation (macrophytes and bryophytes), and

B. characterize the daily variability in dissolved oxygen (DO) throughout the Upper Spring Run reach (USR) and Landa Lake; and evaluate the current and potential impacts of floating vegetation mats on DO diel dynamics.

These topics were prioritized for 2015 following back to back low-flow years when extensive benthic algal mats and floating vegetation mats were observed to develop and persist in the Comal River / Springs system (Comal system) through much of each summer. Anecdotal observations over the years suggest that the benthic algal mats may have significant negative impacts on the aquatic macrophytes and bryophytes that provide prime habitat for the fountain darter. Likewise, the widespread occurrence of floating vegetation mats suggest that the physical shading and possible in situ decomposition might negatively impact diel oxygen dynamics in a way detrimental to the fountain dater (i.e. periods of low DO at the sediment:water interface where the darter typically lives).

In 2013, the Comal system was subjected to lower than average flow conditions with the average monthly discharge ranging from 124 cubic feet per second (cfs) to 223 cfs. In 2014, monthly mean discharges declined considerably more with monthly discharge ranging from 80 cfs to 169 cfs. This resulted in one of the lowest discharge conditions observed in the Comal system in nearly 30 years. For a significant period of time during each summer cover of green filamentous algae was observed to increase and became dominant as flows decreased. As a result the USR and Landa Lake reaches saw a significant decrease in bryophyte cover and some reduction in cover of aquatic macrophytes. The increase in filamentous algae cover presumably resulted in an increase in floating vegetation mats produced as algae clumps senesced and floated to the surface. These large clumps of algae then become entangled with other organic debris and continued to accumulate as flows and water level decreased in Landa Lake.

In 2015 spring flow and discharge conditions improved substantially with mean monthly discharge in the first half of the year ranging from 154 to 386. Recent restoration measures implemented by the Edwards Aquifer Habitat Conservation Plan (EARIP 2011) in Landa Lake have played an important role in improving the overall robustness of this system in times of drought. However, the inevitable return of drought and resulting low-flow will no doubt cause a

recurrence of conditions witnessed from 2013 through 2014 when green filamentous algae was widespread and vegetation mats covered large areas of the lake's surface. While some of these impacts can be managed, controlled or mitigated for (like physical removal of vegetation mats) others are a bit harder to curb (such as filamentous algae blooms) without insight into factors that promote these conditions. It is our desire that the information provided within this report will provide some insight to the nature of green filamentous algae in the USR and Landa Lake. Also this report details studies regarding the occurrence and composition of floating vegetation mats and how these floating vegetation mats may impact the DO availability in Landa Lake.

Collections were made of representative filamentous algae present in April 2015 throughout the majority of the Comal system. These collections showed that two genera dominated the collections, *Spirogyra* and *Cladophora*. The spatial and temporal distribution of the more abundant, *Spirogyra* benthic mats was monitored during April-September 2015. Five permanent transects were established through the USR in areas where historically we had seen abundant benthic mats develop. Unlike in 2013 and 2014, benthic mats in 2015 ended up being poorly developed over the course of the monitoring period, likely a result of the higher than average flow conditions. An interesting and important observation from the mapping efforts was the observation that heavy benthic algal mats rarely co-occur with benthic bryophyte mats. It is possible that the development of thick algal mats quickly suppress the benthic bryophytes which, unlike macrophytes, have no way to grow taller and potentially alleviate shading from the algae.

Water quality was monitored at fixed points throughout the system to better understand possible nutrient limitation for the benthic mats. These data show very high levels of dissolved nitrogen (N) but vanishingly low levels of dissolved phosphorus (P). Although nutrient limitation studies per se were not conducted, the very high available N: available P ratios strongly suggest phosphorus limitation at least in the water column.

To address the fundamental question of algae impacts on macrophytes and bryophytes, we created an algae mat enclosure and compared growth within that enclosure to growth in an adjacent open plot without any benthic algae. The effort to artificially create a dense benthic mat was only moderately successful. However, despite the lack of persistence of a dense algal mat within the enclosure, we observed poorer growth within the enclosure for three of four vegetation planting types evaluated.

Over the course of 2015, up to 14 DO sensors were deployed simultaneously throughout USR and Landa Lake to characterize the daily variability of DO. We found that during 2015 the vast majority of the system showed no evidence of low-DO conditions that would be detrimental to the fountain darter. However, we did find that in stagnant backwater areas the nighttime DO did drop below 4.0 mg/L (a "bright line" established in the HCP for fountain darter habitat concerns). While these habitats currently account for only a small proportion of the system, it is likely that stagnant zones would increase under very low-flow conditions. We then compared daily DO variability at the sediment:water interface (where darters spend the majority of their

life) versus the water column at five locations and found little cause for concern under current flow conditions.

We also monitored the spatial and temporal distribution of floating vegetation mats. Not surprisingly, the areal extent of the mats in 2015 was significantly lower than that measured during 2014. We provide the first quantitative data on the composition and areal density of the floating vegetation mats in Landa Lake. During 2015 the mats were mostly composed of living bryophytes and algae, as well as broken fragments of macrophytes and terrestrial debris. These tissues accumulated through the year and formed mats with total dry weight biomass of up to 1.5 kg m-2 by the end of the summer. We subsequently conducted assays of biological oxygen demand (BOD) and found that the mats pose a potential demand of up to 700 g O2 m-2. Additionally, we determined the material decays rapidly, with decay half-lives of 12-35 days.

Finally, a conceptual analysis of the measurements made during 2015 along with literature values was conducted to evaluate the overall influence of floating vegetation mats on the DO dynamics in Landa Lake. This analysis suggests that the DO conditions within the lake are more greatly influenced by atmospheric reaeration, water column algal activity and macrophyte activity than by the decomposition of the floating vegetation mats under current conditions. However, if conditions were to change so that the floating vegetation mats covered 25% or greater of the lake surface, the vegetation mats assume a much larger role in determining daily DO fluctuations.

Overall a wealth of information was collected during 2015 related to the benthic algae mats and floating vegetation mats and their potential impacts on the ecosystem. The original intent of several of these studies were to be conducted during a low flow year as was predicted for 2015. However, record amounts of rainfall during Spring 2015 quickly changed the hydrological outlook for the year. With that change, those specific investigations morphed from attempting to characterize low-flow conditions to documenting baseline conditions on algae and DO patterns under average to above average springflow. While it was not the original intent, characterizing this baseline data will be invaluable moving forward. Several results provide evidence that under low-flow conditions there may be a higher cause for concern related to benthic or floating mats negatively impacting fountain darter habitat. However, to fully understand the impacts and stress that drought places on a system such as the Comal River it is our hope that certain components of this study would be repeated when drought conditions return. In particular, we recommend the continued use of MiniDOT sampling especially when concerns arise regarding DO measurements at specific locals, such as the lake bottom or other micro habitats, or when drought conditions return. Lastly, it is also recommended that a comprehensive Landa Lake Management Plan be considered that includes protocols and timelines for nutrient, algae and DO monitoring and management of floating vegetation mats.

CHAPTER 1

ALGAE IDENTIFICATION

Material and Methods

In order to provide a basic identification list of the common filamentous algae in the Comal River a survey of algae was conducted April 2015. Grab samples of algae were collected in locations from the Upper Spring Run (USR) reach to Hinman Island and GPS points were taken at sample collection locations with substrate or plant species noted. Samples were returned to the laboratory and identified to genus using Bellinger's "*Freshwater Algae Identification and Use as Bioindicators, 2015*" key. Additionally, digital photographs were taken of selected samples under the microscope and sent for expert determination by algae taxonomists. Vouchers of samples were fixed in 3% formalin and preserved in alcohol (EtOH) for further identification to species if necessary.

Results

Algae genera collected and identified include *Cladophora, Spirogyra, Calothrix, Chara, Lyngbya, Phormidium, Enteromorpha, Vouchera* and *Oedogonium.* The predominant algae genera identified from grab samples collected were *Spirogyra* and *Cladophora* while other algae genera were represented in only a few samples (Figure 1.1). Samples of *Spirogyra* were commonly associated with aquatic macrophytes most notably *Saggitaria platyphylla* and commonly formed continuous colonies across multiple bottom strata. We refer to these large benthic colonies as algae turf. *Cladophora* commonly occupied bare substrate or was loosely attached to *Vallisneria neotropcialis* (Vallisneria) or *Ludwigia repens* (Ludwigia).

Spirogyra can be easily identified in the field by its bright green color. Under the microscope the spiral arrangement of its chloroplasts are evident (Figure 1.2). It is slippery to the touch and is loosely arranged into mats. When picked up out of the water *Spirogyra* colonies dissociate into long slippery strands or fall apart all together. It is noticeably present in certain areas of the USR and Landa Lake throughout the year and was most likely the dominant turf forming algae in the USR and Landa Lake during the low flow period of 2014. Due to its widespread coverage in 2014 *Spirogyra* became the focus algae for all subsequent portions of the algae study.

Cladophora can be identified by its darker color. In many cases it is almost black in appearance and forms clumps or colonies around rocks or other hard substrate. It can be found loosely attached to aquatic plants and is noticeably present in the USR, Landa Lake and gravelly areas of the Old Channel. *Cladophora* tends to retain its long filamentous structure when removed from the water.

Overall algae cover was minimal in April 2015 with only small patches of *Spirogyra* and *Cladophora* algae located in the USR and Landa Lake. The macroalgae *Chara* sp. however was a dominate component in the USR, where it formed large stands.

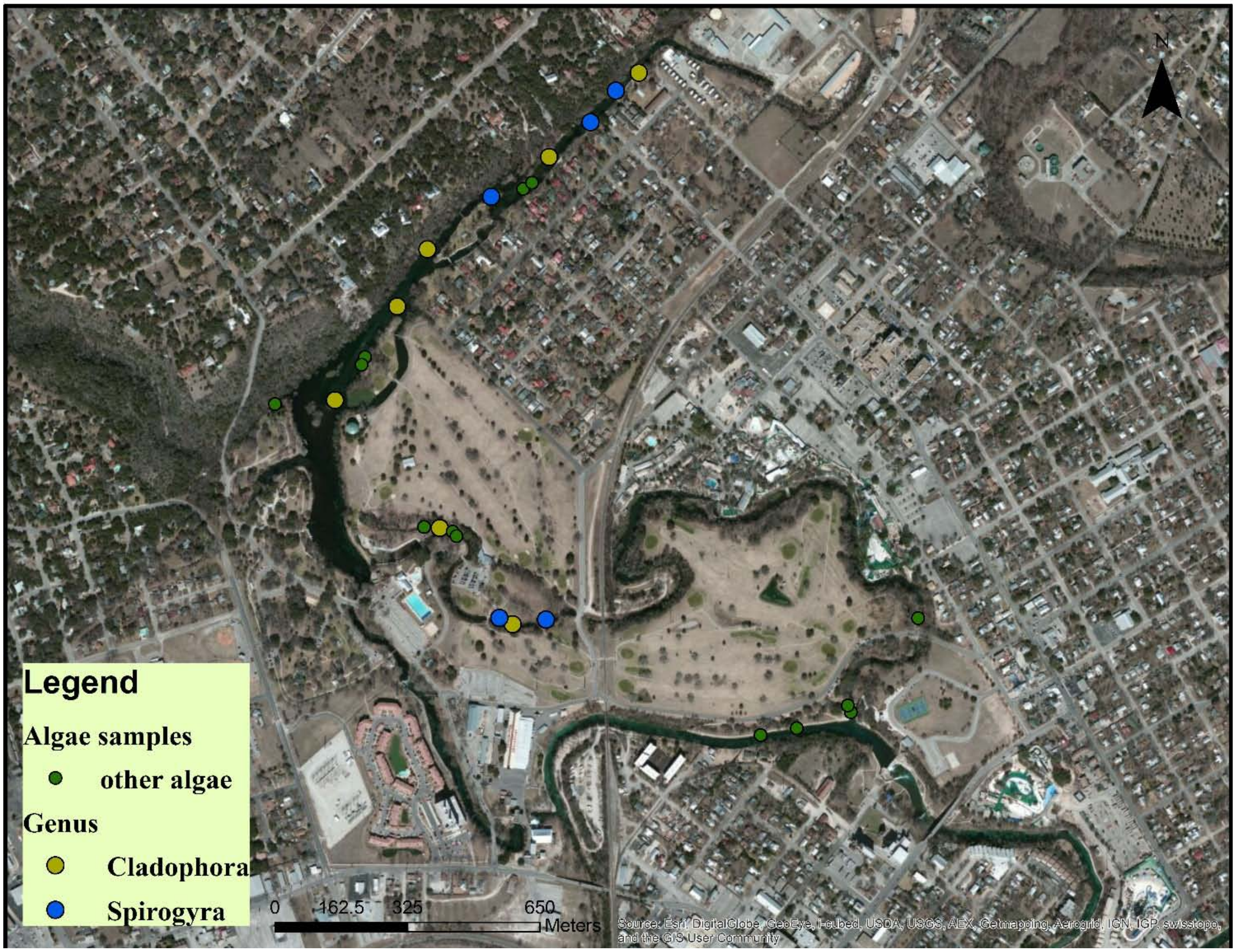

Figure 1.1 Map of algae grab samples collected from Upper Spring Run to the confluence of the Old and New Channels.

Table 1.1 Location attributes for each grab sample

Location	Genus	Flow	Position
1	*Cladophora*	Fast	on Substrate
2	*Spirogyra*	Fast riffle	in Bryophyte
3	*Lyngbya*	Medium	Mix w/ Riccia & Ludwigia
4	*Spirogyra*	Fast	on Substrate
5	*Cladophora*	Fast	on Substrate
6	*Spirogyra*	Low	on Substrate
7	*Spirogyra*	None	Floating
8	*Cladophora*	Low	on Substrate
9	*Spirogyra*	Low	on Substrate and Sagittaria
10	*Calothrix w/ Cladophora*	Low	on Substrate
11	*Chara*	Low	on Substrate and Bryophyte
12	*Cladophora*	Low	on Substrate
13	*Spirogyra*	Low	on Sagittaria
14	*Spirogyra*	Low	on Substrate .
15	*Cladophora*	Medium/High	Floating
16	*Cladophora*	Medium/Slow	Floating
17	*Cladophora*	Medium/Slow	on Substrate
18	*Lynghya*	Medium/Slow	on Substrate and Sagittaria
19	*Cladophora*	Medium/Slow	attached on Ludwigia
20	*Phormidium ?*	Fast	on cobble
21	*Cladophora*	Medium	on Cabomba
22	*Cladophora*	Medium	on concreteStairs
23	unknown	Medium	on wood
24	*Vouchera*	Medium	on concrete
25	*Oedogonium*	Fast	on substrate

Figure 1.2 Microscope and field images of *Spirogyra* (top) and *Cladophora* (bottom)

Discussion and Conclusions

The most common algae found in grab samples were *Spirogyra*. Primarily a shallow water algae this genus is very much a generalist commonly found in ditches, slow moving streams, backwaters and littoral zones across all climatic gradients. It can grow loosely attached to substrate, macrophytes or other underwater structures where its filaments over winter and expand (Sheath and Cole, 1992). As *Spirogyra* filaments expand oxygen bubbles trapped within filaments can bring *Spirogyra* clumps to the surface where it is quite capable of existing as floating mats (Hillebrand, 1983). *Spirogyra* is not considered an indicator for water quality integrity due to its very general growth needs and wide habitat ranges. The other common genera of algae collected in grab samples were *Cladophora*. This algae is typically found in streams and rivers but is common in lakes as well. *Cladophora* filaments directly anchor to substrate such as

rocks and gravel which allow it to prosper along shorelines of lakes and in faster flowing streams. However *Cladophora* filaments can detach from the anchoring holdfast during the growing season forming floating mats. Unlike *Spirogyra*, *Cladophora* tends to degrade once detached so that floating mats composed of *Cladophora* filaments can cause severe water quality issues such as elevated *E. coli* levels, odors and anoxic conditions (Vanden Heuvel et al., 2010). Excessive *Cladophora* growth can be indicative of degraded water quality and nutrient enrichment. Other filamentous algae genera were limited in distribution and warrant no further discussion in this report.

In the past any algae mapped as part of bio monitoring has been lumped in a general "filamentous algae" category without discerning individual genera. Although algae ID can be complicated we have provided further identification which can help better assess the spatial distribution of algae types for biological monitoring purposes. Further investigatory analysis for consideration would be to assess biological patterns such as seasonal variability between algae type, habitat suitability per algae type, and algal community dynamics over a broader time spectrum as well as spatial expansion of analysis to include the Old and New Channel.

Works Cited and Recommended Readings

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CHAPTER 2

COMPARISON OF ALGAE COVER TO OTHER AQUATIC VEGETATION TYPES IN FIELD TRANSECTS AND MAPPING

Materials and Methods

Field studies were used to further investigate how algae, specifically *Spirogyra*, expands within the system over the course of a growing season and how it may impact other types of vegetation. A field-based rapid algae survey (Barbour, 1999) was conducted in March, June, July and August 2015 along 5 transects located in the Upper Spring Run (USR) (Figure 2.1). At each transect and sampling date, a modified point-intercept method was used to sample randomly chosen points along each transect. Over the five sampling events, this resulted in a total of 26 sampling points per transect. At each point a plastic grid subdivided into 110 cells (1.5 cm x 1.5 cm in size) was placed onto the substrate, and the number of cells containing algae was tallied. To determine the dominant vegetation type, the number of cells containing bryophyte and substrate or macrophytes was quantified. Depth and velocity at 60% of depth was measured at each sample point. Standard water quality parameters including water temperature, pH, dissolved oxygen and conductivity were measured in three locations along the transect (river right, center and river left) using a YSI multiparameter sonde.

Additionally, mapping of macrophytes, algae and bryophytes was conducted within a 10 meter wide plot bisected by each transect (Figure 2.2). Plot parameters were mapped and the perimeter of algae, macrophyte and bryophyte patches within the plot was traced using a Trimble GPS unit to produce area cover estimates for each vegetation type. Algae turf was mapped in selected months across all of USR and Landa Lake to provide a broader picture of when and where algae turf develops.

Figure 2.1 (Left) Five survey transects were set up across the Upper Spring Run. (Right) plastic grids were placed at randomly selected points along the transect and the number of cells occupied by algae, bryophyte or other types counted.

Figure 2.2 Algae transects (blue lines) and corresponding algae plots (white rectangles) were used to survey the presence of *Spirogyra* algae turf in the Upper Spring Run.

To supplement the ongoing investigation into the impact of dissolved nutrients, routine water samples were collected from four locations within Landa Lake and USR (Figure 2.3). On some occasions additional grab samples were collected at individual transects. All grab samples were collected coincident with mapping and assessment efforts. Laboratory analysis was performed at Baylor's Center for Reservoir and Aquatic Ecosystem Research lab (CRASR) using a method to detect low TP and SRP concentrations, as low as 0.0002 to 0.0005 mg/L,

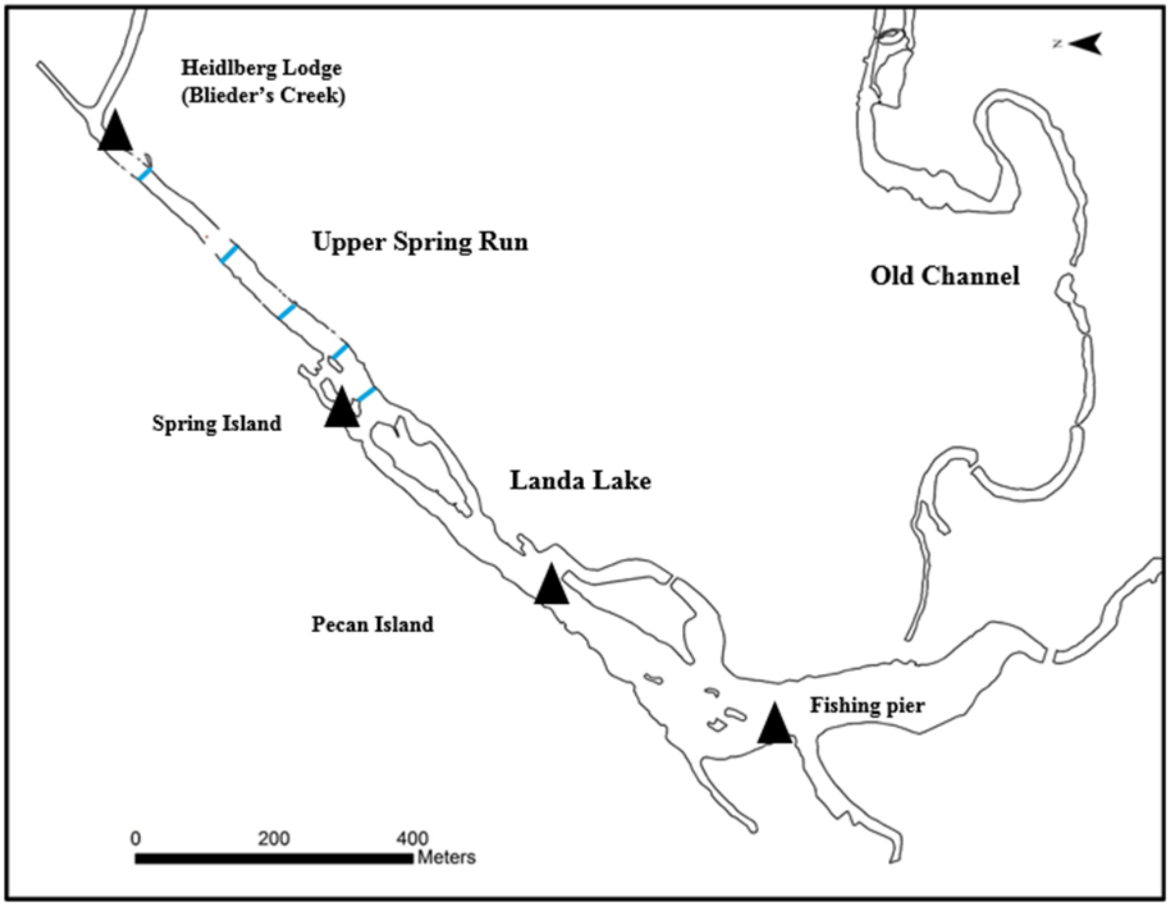

Figure 2.3 Map of Landa Lake and Upper Spring Run showing four routine sampling stations designated by triangles (Heidelberg, Spring Island, Upper Pecan Island, Landa Lake Fishing Pier). The location of the algae transects are indicated by blue lines.

Samples were collected as sub-surface (ca. 30 cm) grab samples into plastic bottles which had been acid washed (5% HCL) and triple rinsed with local water. All samples were immediately put on ice and returned to Baylor University for analysis. We analyzed these samples for total N (TN), total P (TP), NO_2-N + NO_3-N (NO3-N), and soluble reactive P (PO4-P) with colorimetric methods (APHA 1995) on a LachatH Quik-Chem 8500 flow-injection autoanalyzer (Hach Instruments, Loveland, Colorado). We measured TN and TP concentrations after persulfate digestion.

Results

Over the course of four sampling events transect data and mapping showed relatively low coverage of *Spirogyra* algae in the USR compared with other vegetation types (Figure 2.4). Transect number 1 was the only transect with *Spirogyra* observed during all sampling periods with algae occupying 73% of the total sampling points. *Spirogyra* was occasionally observed in transects 3 (35%) rarely observed in transects 4 (15%) and 5 (8%) never observed in transect 2. In contrast 73% of points in transect two were occupied by bryophyte followed by transect 3 (34%) and transect 5 (30%). Transects four and one both had 19% of sampling points occupied by bryophytes. Across all transects combined only 3% of points were occupied by both algae and bryophytes. A majority of sampled points in transect 4 and 5 were occupied by aquatic macrophytes, most commonly *Sagittaria platyphylla* and *Chara* sp. Although *Chara* sp is a macro algae its growth form is very similar to that of vascular macrophytes therefore it was combined with the macrophyte data.

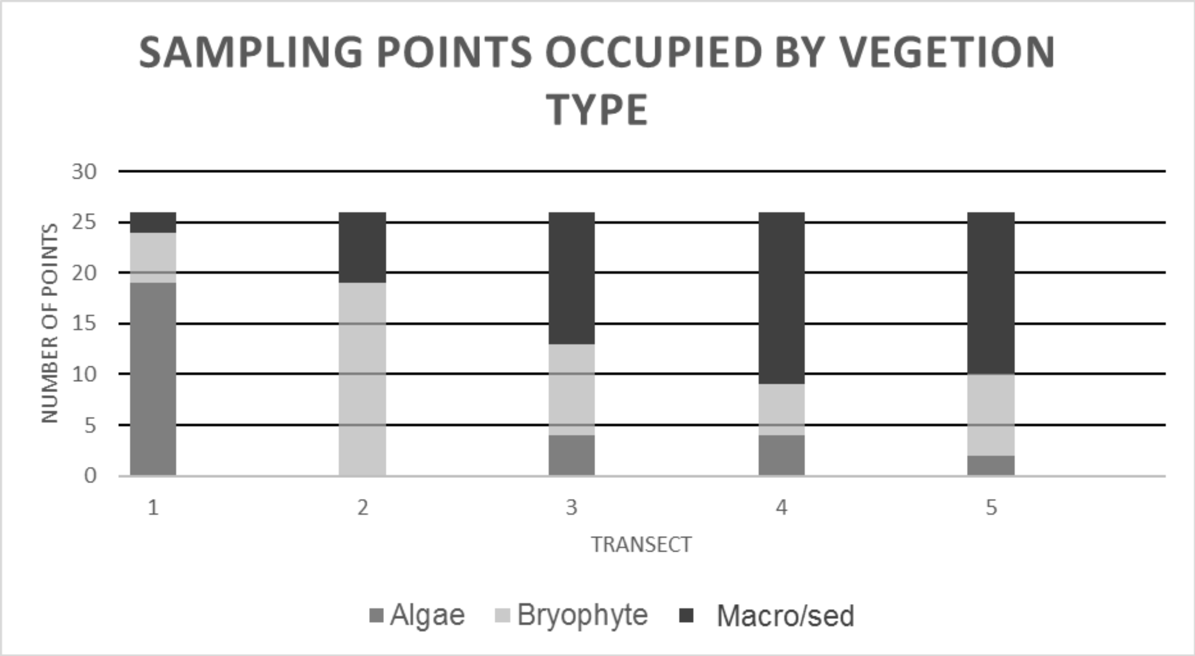

Figure 2.4 Total number of sampling points (n=26) occupied by vegetation type along each transect. Transect one had the highest number of points occupied by algae (grey) while all other transects were either dominated by bryophyte (light grey) or macrophyte and sediment (dark grey).

Mapping of plots showed relatively little coverage of *Spirogyra* turf in the USR compared to other vegetation types. Figure 2.5 shows the combined cover of each vegetation type per month. Though present in all plots at some point in time *Spirogyra* turf never dominated cover within

the plots as a whole but instead expanded and retreated, by more than 50% over time. Bryophyte cover doubled from March to June while macrophyte expansion remained somewhat stable. Plots were covered with the most vegetation in July at which time algae expansion peaked, along with bryophyte and macrophyte cover, within the plots. When looking at vegetation cover within each individual plot only Plot 1 maintained cover of all vegetation types across all months while other plots were dominated by a single vegetation type, either bryophyte or macrophyte, but never algae (Figure 2.6). Plot 1 was the only plot in which turf algae persisted across all months while in other plots algae was only present occasionally. Again macrophytes were exclusively composed of *Sagittaria platyphylla* and *Chara* sp. A map set of plots is available in Appendix I.

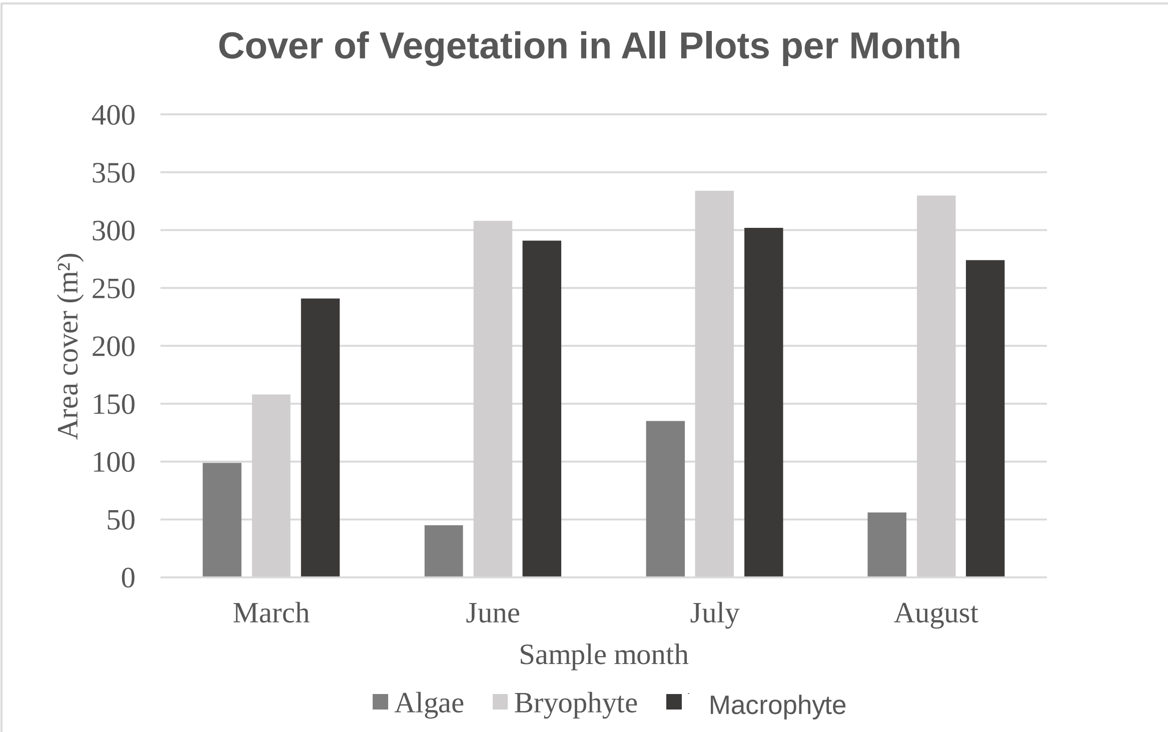

Figure 2.5 Cover of vegetation types by month when plots are combined.

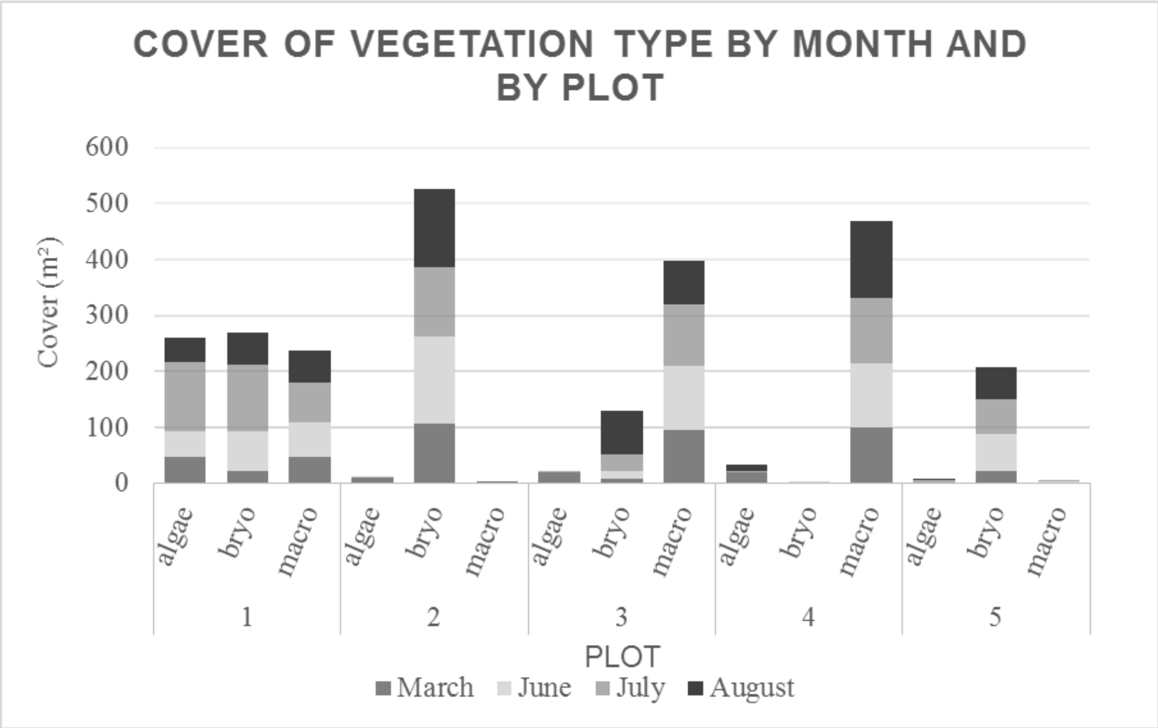

Figure 2.6 Cover of vegetation types by month and by plot. Total area of plots were plot 1: 304 m²; plot 2: 256m²; plot 3: 291m²; plot 4: 295m²; plot 5: 211m². Due to overlap of vegetation types total cover of vegetation can exceed the plot area.

Environmental parameters are summarized in Table 2.1. Overall water quality parameters were quite uniform due in part to the strong spring water influx into the USR. Water temperatures, pH and conductivity all remained stable while there was slight variability in dissolved oxygen. While turbidity was not quantified, water clarity throughout the monitoring period remained high even during periods of recreation. Although the USR does receive some moderate recreation these activities are limited throughout the day. Velocity is an important factor in algae dominance as persistent water flow can easily loosen algae fragments and transport them downstream. This action can quickly decrease algae density or eliminate algae turf altogether. During our study period mean daily discharge hovered around 300 cfs compared to 2014 when mean daily discharge across the same time frame ranged from 110 cfs to 80 cfs.

Mean monthly velocity measurements (Figure 2.7) were similar across our sampling dates, although the range of velocities was highest in the months of June and July - most likely a result of increased spring discharge. Velocity means were also relatively similar across all transects (Figure 2.8) but maximum velocities at transects 2 and 5 were well above all other transects. Transects 2 and 5 are notably dominated by bryophytes. The USR is a relatively uniform water body with very little heterogeneity in bathymetry or bank-to-bank width, and water flow originates entirely from spring upwellings and a few small spring runs eliminating most eddy flow and strong currents more prominent downstream. However the long straight layout of the

USR provides little shelter from velocity pulses allowing scour action during rain events or increased discharge to occur across a wide area.

Table 2.1 Average and Standard Error reported for parameters collected at each transect location.

Transect	pH	D.O. (mg/L)	Temp (°C)	Cond. (µs/cm).	Velocity (ft/sec.)
1	7.34 ± .02	6.61± .12	24.13± .18	565.48± 5	0.09± .01
2	7.34± .04	7.34± .32	24.48± .20	563.19± 4	0.19± .01
3	7.41± .04	7.11± .30	24.25± .14	562.47± 5	0.09± .007
4	7.34± .03	6.32± .19	24.73± .10	565.06± 5	0.10± .01
5	7.27± .03	5.23± .06	24.00± .06	562.92± 5	0.11±.01

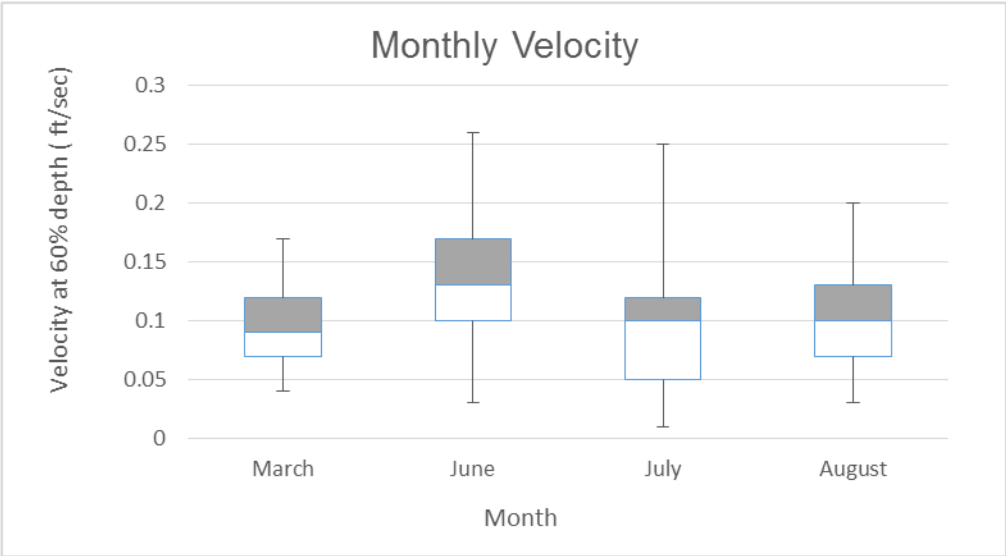

Figure 2.7 Average monthly water velocities measured at 60% of the total depth. Maximum velocity was highest in June and July.

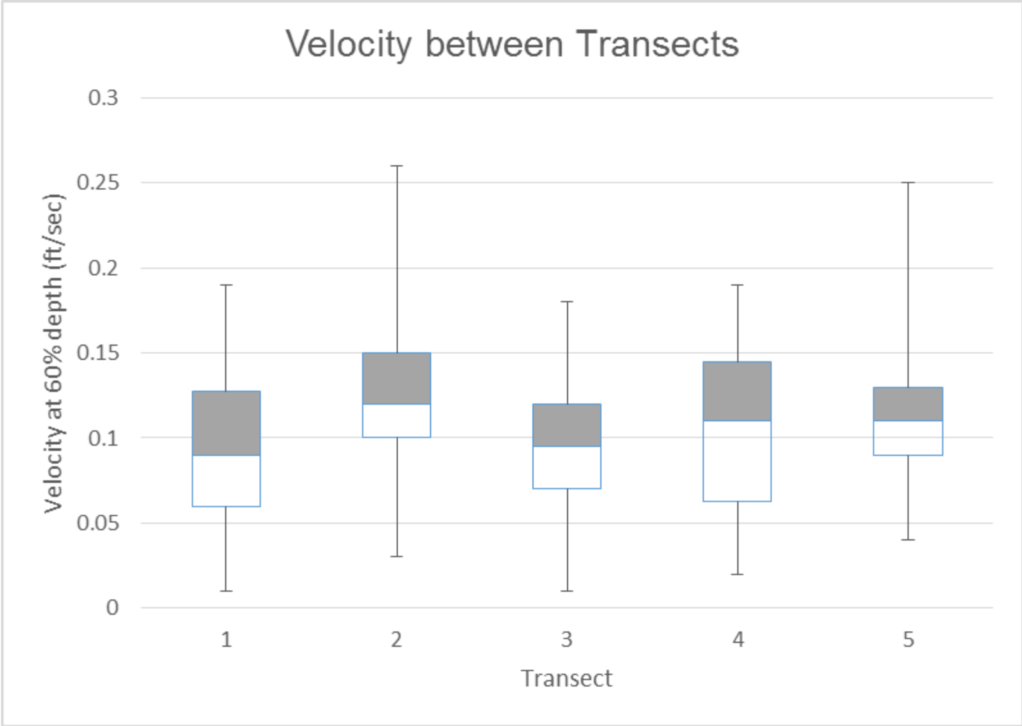

Figure 2.8 Average water velocities at each transect over the sampling period.

Nutrient availability is typically correlated to algae growth and algae blooms. In this study we collected grab samples to test for the availability of important nutrients such as Phosphorus (dissolved and particulate) and Nitrogen. On a seasonal scale Phosphorus levels remained notably low, at or just above detection limits in parts per billion (ppb) across all locations while available nitrogen was greater (Figure 2.9). This high N:P ratio indicates a Phosphorus limited system despite stormwater influx earlier in the season. Measured nutrients showed virtually no changes at temporal or spatial scales as Phosphorus remained just above detectable limits regardless of sampling location or sampling period. Small non-reoccurring spikes in total phosphorus (TP) were observed at specific locations known to harbor congregations of waterfowl. Although Nitrogen was measured at orders of magnitude greater than TP, Nitrogen levels remained consistent, within the 1 to 2 ppm range, regardless of location or sampling period (Figures 2.10, 2.11).

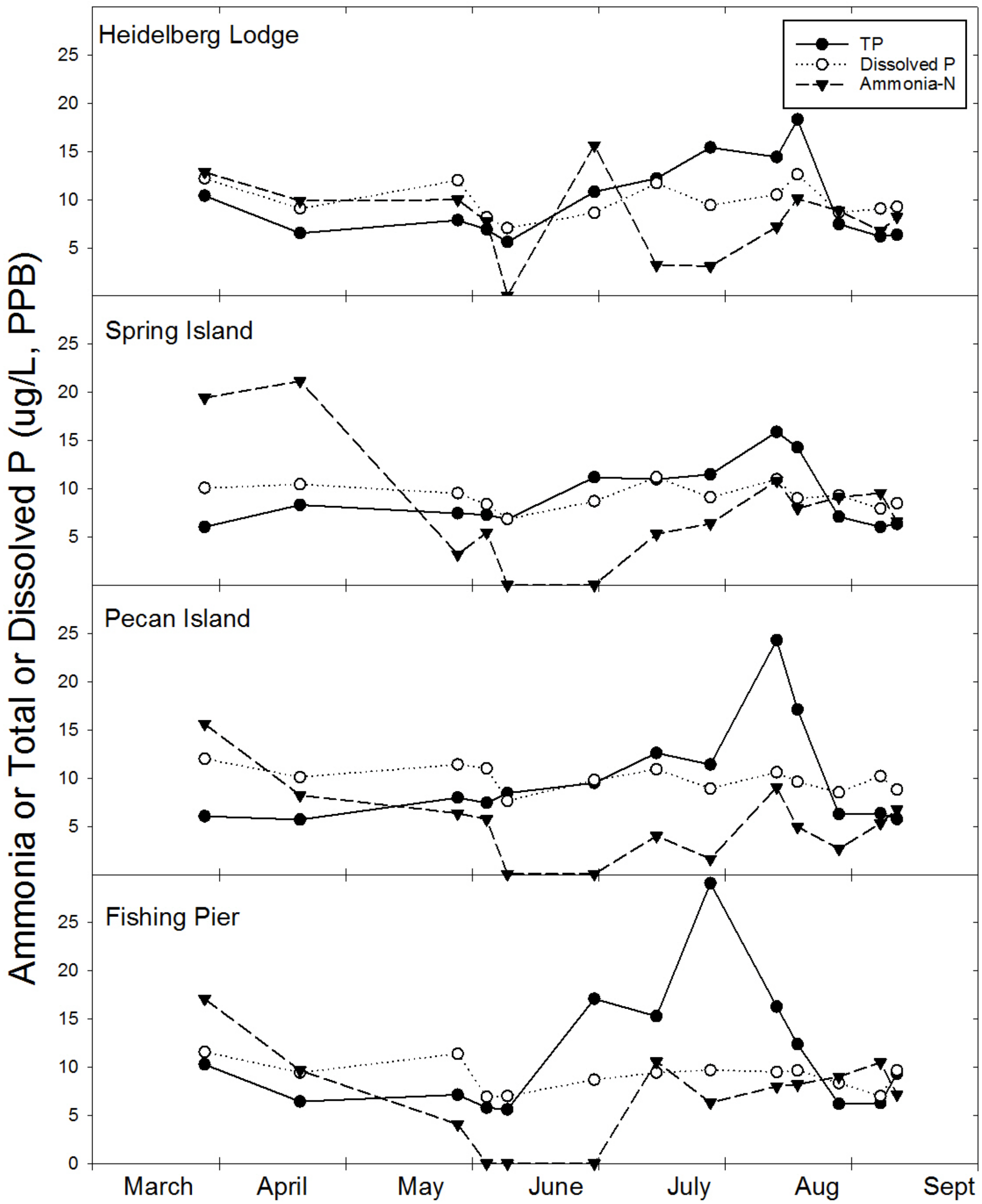

Figure 2.9 March-September 2015 NH3, Dissolved P and Total P at four locations on the Comal River, TX. The very low levels of TP and Dissolved P are notable, and are always very near the analytic detection limits. The occasional spikes in P observed are possibly related to recreation or large number of waterfowl sometimes observed.

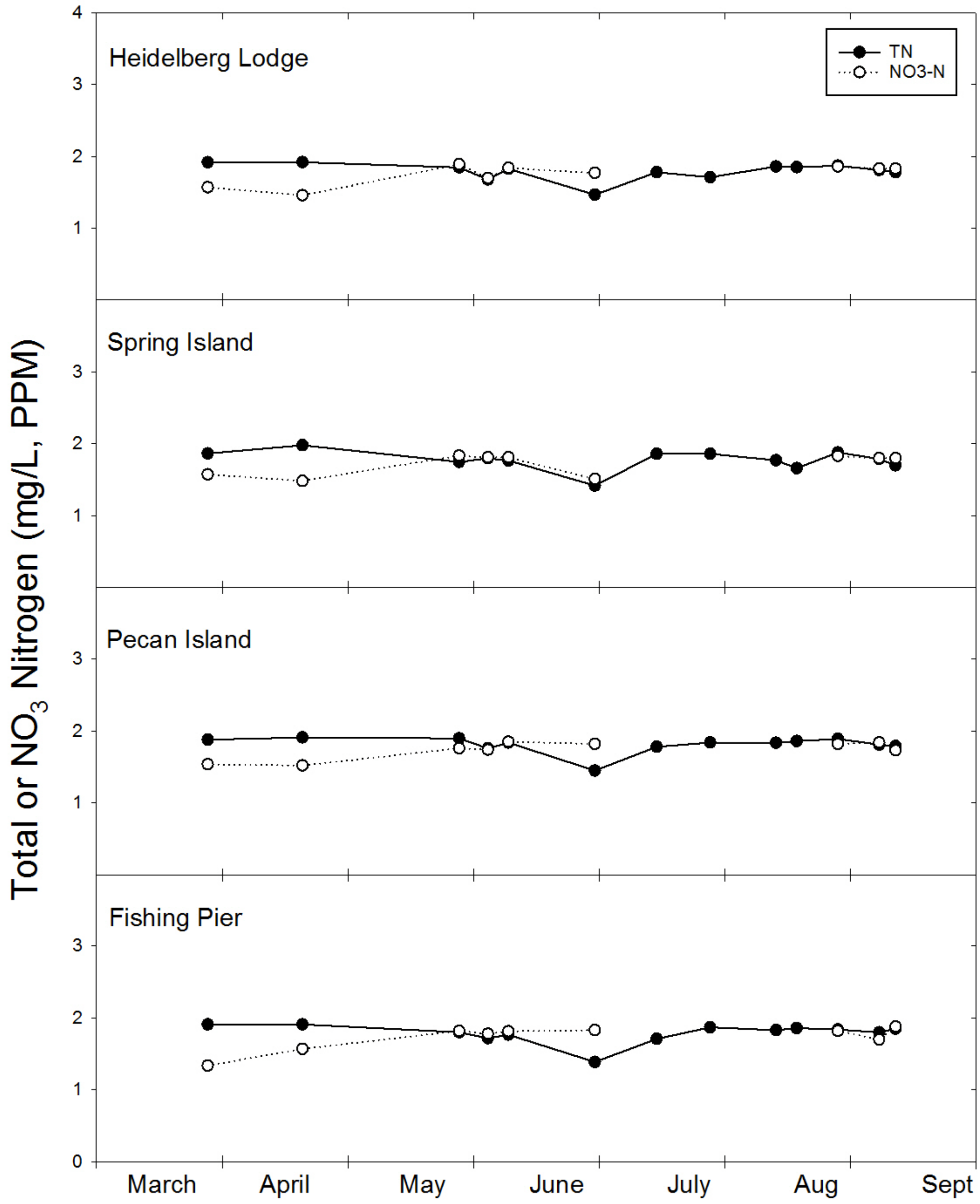

Figure 2.10 March-September 2015 NO_3 and Total N at four locations on the Comal River, TX. Total N is almost entirely composed on NO_3-N.

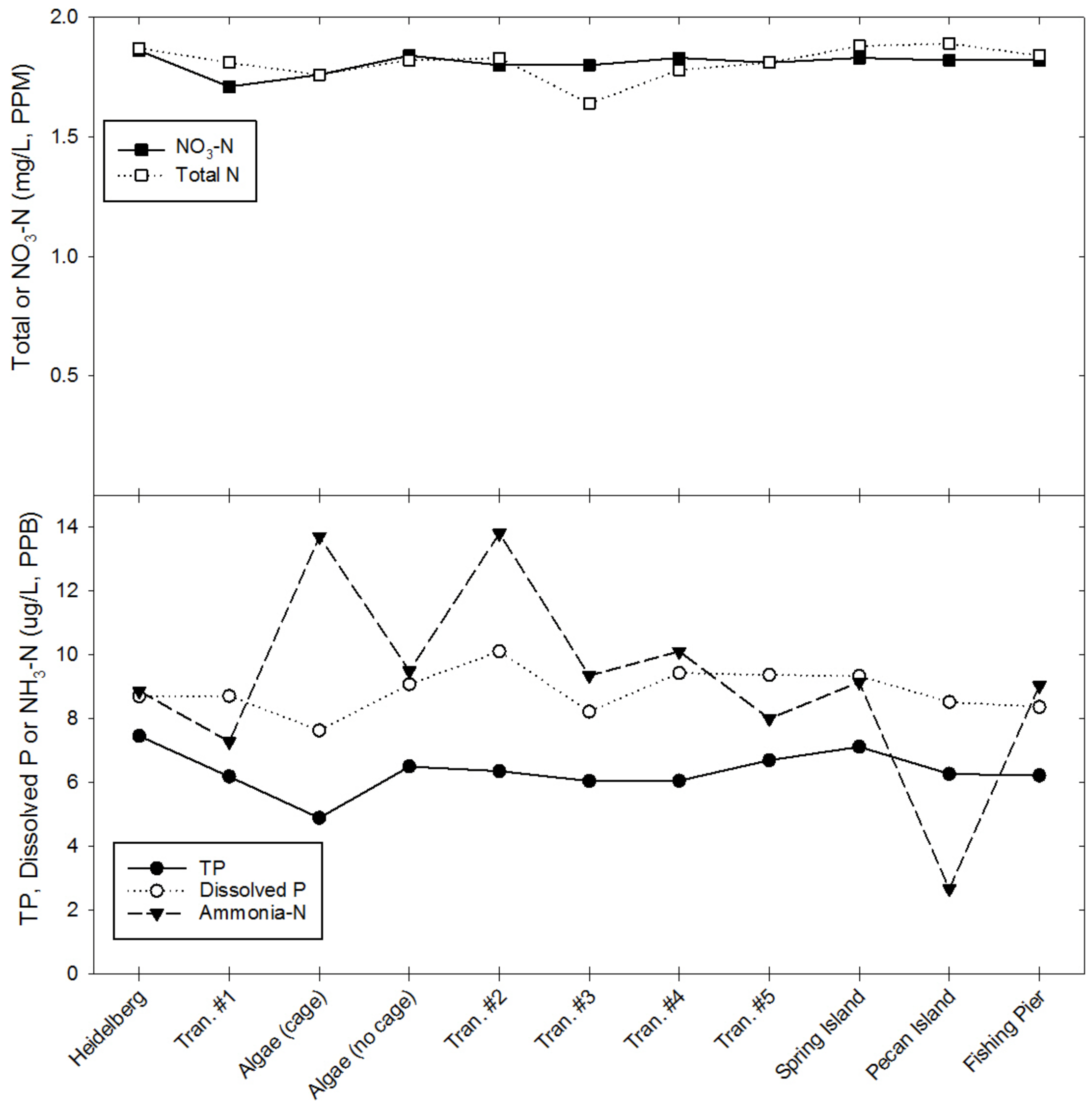

Figure 2.11 Spatial transect of water quality parameters from Heidelberg to the Landa Lake Fishing Pier made on August 28, 2015. The lack of variability among the locations is notable, indicating that in-lake processes are not happening quickly enough to impact the waters flowing through the system.

Mapping of algae in the USR and Landa Lake was conducted in selected months prompted by observed changes in algae cover or distribution (Figures 2.12 – 2-15). Table 2.3 shows the dates of mapping and corresponding total cover of algae turf mapped in Landa Lake and USR combined.

A baseline mapping event occurred in March to document where algae occurred before the growing season began. In March *Spirogyra* turf algae was prominent in isolated pockets located intermittently along the USR reach while no turf forming algae was present in Landa Lake (Figure 2.12). However, by July, a large expansion of turf forming algae occurred. By July 1, algae turf had decreased in the middle section of the USR while expanding in the area around Spring Islands where it began to cover *Sagittaria platyphylla*. Turf forming algae also expanded into Landa Lake at this time, forming large turf mats at the upper end of Pecan Island on bare substrate and along the Eastern shoreline. Turf algae were also present in spring run 1 as well where it formed on top of bare sediment. Through July, algae began to decrease in some areas but remained dominate in others, most notably Spring Island, where it continued to cover *Sagittaria platyphylla* and gravel substrate. However, by October, algae turf decreased in the Spring Island area yet expanded again in the middle portion of USR as well as the middle of Landa Lake where it began covering large areas of bryophyte (Figure 2.15).

Table 2.3 Mapping dates and cover of turf algae in the Upper Spring Run and Landa Lake

Mapping Month and Day	Cover in m^2
March 3	1,417
July 1	5,478
August 28	3,255
October 19	3,108

Figure 2.12 March baseline map of turf algae in Landa Lake and the Upper Spring Run.

Figure 2.13 Location of turf algae in Landa Lake and Upper Spring Run in July

Figure 2.14 Location of turf algae in Landa Lake and Upper Spring Run in August

Figure 2.15 Location of turf algae in Landa Lake and Upper Spring Run in October

Discussion

Transect sampling and plot mapping showed that algae never became a dominate vegetation type in the USR in 2015, contrary to observations made in 2014 when algae turf dominated this area. Transect sampling, plot mapping and large scale mapping of algae turf in Landa Lake and the USR all indicate strong variability in spatial coverage of turf algae, which expanded and retracted multiple times over the course of the sampling period. Even when turf algae were present in large areas density was still low and spotty. Interestingly, algae expansion and cover remained low through June a period in which algae expansion was anticipated to begin. However, mapping and transect sampling did show higher cover of algae in July. The cool rainy weather in May and first part of June 2015 possibly decreased optimal conditions for algal growth earlier in the season, but by July, long sunny days and no rain events produced better conditions for algal growth. However, the complete lack of rain in July and August could also have limited nutrient inputs into the USR and Landa Lake, causing algae to retreat or change distribution several times over the sampling periods. In some instances algae turf disappeared altogether only to form in other locations.

Despite the seemingly irregular distribution of algae during this study, some patterns can be seen from our survey. First, there seems to be an inverse relationship between algae dominance and bryophyte dominance. Algae and bryophytes rarely intermixed and areas dominated by algae, while few, tended not to be occupied by bryophytes and vice versa. Although algae turf has been observed growing over bryophytes where algae persists for periods of time bryophytes tend to disappear. In contrast, areas that are dominated by bryophyte were not largely occupied by turf algae (Figure 2.16). This pattern has been noted in past years, especially in 2014, when algae turf expanded throughout the USR as flow conditions declined concurrently with bryophytes decreasing and seemingly only persisting in shaded locations where algae growth was probably limited by light availability. In this study it was hard to determine if algae turf negatively impacted bryophytes because of the limited distribution of turf algae. All three vegetation types seemingly coexisted well in transect 1/ plot 1 in 2015. Strong spring upwellings located in this area possibly flushed algae filaments from the substrate while providing satisfactory conditions for bryophyte growth. In areas along this transect where spring upwellings were not present, algae and *Sagittaria platyphylla* dominated. Algae is often associated with *Sagittaria platyphylla* which did not seem to be negatively impacted by algae turf at least over the course of this study.

Second, turf algae are present regardless of the season. In March multiple pockets of turf algae were mapped throughout the USR and pockets of algae have been observed to persist through the winter months as well and this persistence is probably due to *Spirogyra*'s ability to withstand cool water temperatures and lower light levels (Graham et al., 1995). As the growing season progresses these “mother colonies” eventually give rise to algae turf in multiple locations. For this study, one interesting note is how often algae turf translocated to different areas over the course of the mapping periods. In the USR and Landa Lake algae turf commonly disappeared

only to colonize in another location. The Spring Island area of the USR was the only sampling location regularly occupied by turf algae and large scale mapping shows that algae in this location persisted, albeit fragmented, for the longest period of time.

Figure 2.16 Algae and bryophyte rarely intermixed.

Finally, turf algae dominance could be correlated with large scale nutrient availability. Phosphorus remained at very low detection levels in all sampling locations corroborating the limited historical nutrient data indicating that the Comal system may be Phosphorus limited. This lack of Phosphorus might limit algal growth, but raises the question to why algae turf is present at all? *Spirogyra* algae is considered a generalist compared to other algae genera. The highly variable distribution of algae turf could mean *Spirogyra* is either able to utilize Phosphorus at miniscule amounts or that turf algae is able to utilize Phosphorus at a microscale where Phosphorus is available at the sediment-water interface or around very localized nutrient inputs. Other factors that might impact the variable appearance and distribution of turf algae are grazing pressure from various invertebrates, birds, and human recreation in the USR.

Velocity could also dictate where algae turf settles and expands due to the unrooted and loosely organized growth structure of *Spirogyra*. Although Biggs et al. (1998) suggest that *Spirogyra* filaments can withstand stream velocities up to 0.98 ft/sec. before shearing away from substrates turf algae would not be expected to form in areas of strong current or could easily be displaced by scouring events from storm water pulses. In 2015 the USR did experience higher water flow and velocities compared to previous years and this could have played a part in algae distribution, but no correlation between algae location and velocities were observed during this study due to the overall lack of algae along transects.

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CHAPTER 3

IMPACTS OF BENTHIC ALGAE MATS ON AQUATIC VEGETATION

Materials and Methods

In order understand how filamentous benthic algal blooms impact the growth of aquatic plants and bryophytes a field study was conducted during the summer of 2015. A site with existing *Spirogyra* benthic algae mats, or turf algae, and another nearby without algae were identified in the Upper Spring Run (USR) to provide a treatment ("+Algae") and a reference ("no-Algae") plot (Figure 3.1). Since water flow conditions in the USR were at or above historical averages at the time of this study and algae growth was not dense, the team decided to devise a way to attempt to simulate conditions indicative of drought-induced low flow to promote the persistence of algae for the "algae" treatment. In order to accomplish this, a 3 x 3 meter enclosure was constructed around an area with gravel substrate and turf algae but no other vegetation present. Steel T-posts were erected around which silt fencing was secured so that it reached from the sediment to about 15cm above the surface of the water. The silt fencing enclosed the entire area, blocking water flow into the enclosure and producing lentic conditions (Figure 3.2). To promote heavy algal growth, the enclosure was periodically seeded with *Spirogyra* algae collected from nearby. To produce the reference, or no-Algae, plot another 3 x 3 meter area with gravel substrate and without vegetation or algae was designated approximately10 meters downstream of the +Algae treatment enclosure (Figure 3.2). Here, T-posts were erected and the area was demarcated with a rope and signage to prevent recreational traffic from entering the plot. Otherwise, the location was left open to water flow and regularly cleared of any accumulated algae or debris.

The goal of this study was to determine how the presence of benthic algae mats affects the growth of two aquatic vegetation types, vascular plants and bryophytes. Additionally, two growth stages of vegetation, "established" and "new growth", were included to help determine if algae impacted established plants differently than new-growth plants. Native species, discussed below, were chosen for this experiment.

Ludwigia repens, or Ludwigia, is a native aquatic plant that is easily propagated and used in ongoing aquatic habitat restoration in the Comal River. Due to these factors, Ludwigia was chosen to represent the vascular plant type for this study. Established Ludwigia was propagated by collecting 20cm long apical stem fragments from parent colonies located in Landa Lake. These stem fragments were planted into quart-sized nursery pots filled with native sediment also

collected from Landa Lake. Ten stem fragments were planted per pot, and potted plants were allowed to establish in an in situ nursery in Landa Lake for three weeks. This was sufficient time for the plants to establish and root development to fill the nursery pots. Twelve established plants of equivalent size were selected for use in the study and divided between two plastic nursery trays which were assigned to the +Algae treatment or no-Algae site. Twelve new-growth Ludwigia plants were planted in the same manner as the established plants (ten 20cm apical fragments per pot) but given no pre-culture establishment period. Then, they were divided between two nursery trays and placed in either the treatment (+Algae) or reference (no-Algae) plot. Additional established Ludwigia plants and Ludwigia stem fragments were harvested to obtain initial biomass and morphometric parameters.

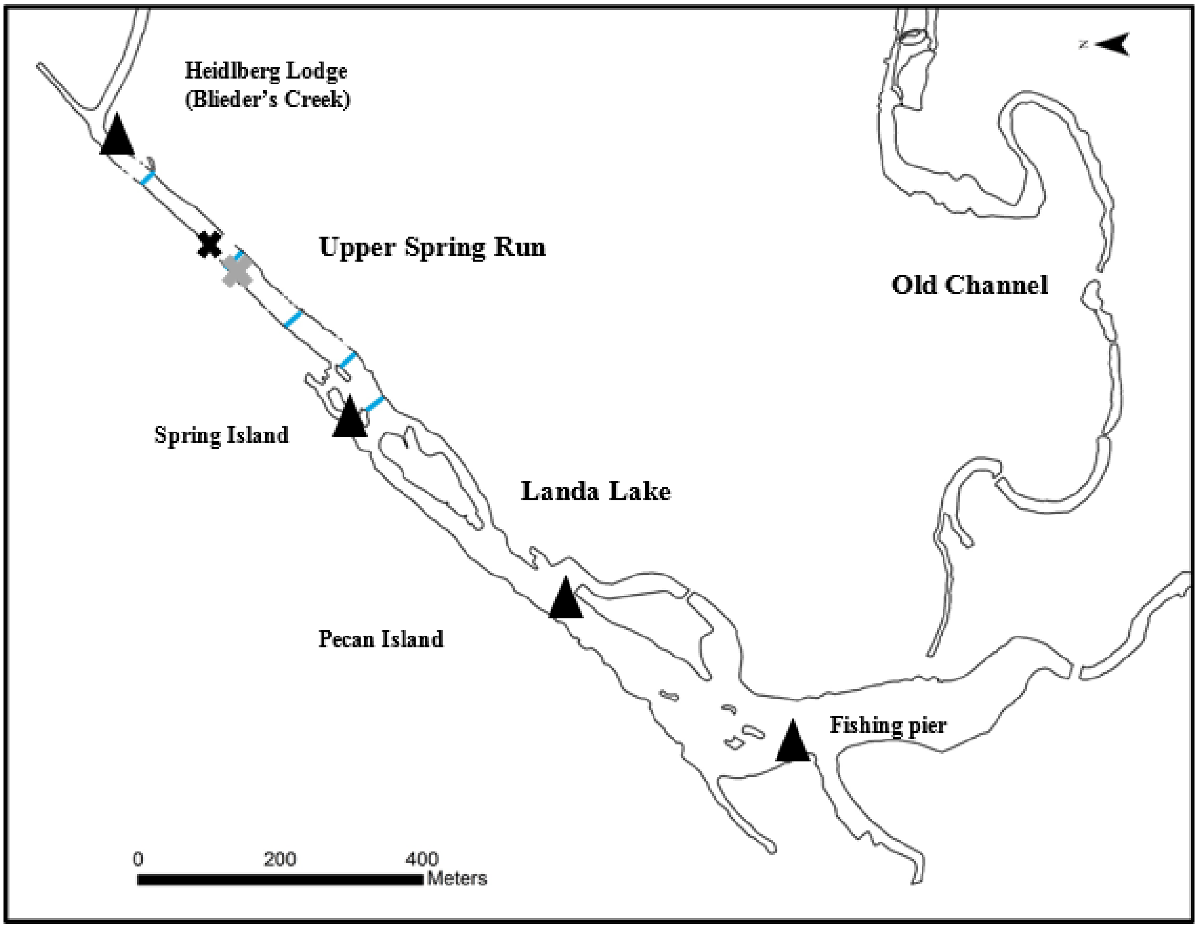

Figure 3.1 Location of the +Algae (black X) and no-Algae (grey X) plots in the Upper Spring Run in reference to algae transects (blue lines) and dissolved nutrients sampling stations (black triangles).

Figure 3.2 3 x 3 meter experimental enclosures - Contained (Top) and Open (bottom).

Bryophytes often occur naturally in the USR as a mixture of species. Therefore, no single species was isolated for use in this study, instead bryophyte clumps were collected from the USR. Established bryophytes were pre-cultured by sandwiching living bryophyte material between window screening inserted into 7 x 7 inch cells composed of egg crate grating. Individual cells were then combined into a 1m^2 bryophyte nursery tray. Bryophyte cells were allowed to grow in situ in Landa Lake until bryophyte covered or nearly covered the entire surface of the cell which took approximately 3 months (Figure 3.3). This method was preferred over previous bryophyte propagation methods which contained bryophyte within a mesh bag or cup. This method allows bryophyte to grow exposed to ambient conditions as though the plant were growing naturally on substrate or rock.

Figure 3.3 Established bryophytes after 3 months of culture in situ.

For the beginning of the study, twelve cells of approximately equivalent bryophyte cover were assigned to either the +Algae or no-Algae plot. The same methods were used to produce and distribute the twelve new-growth bryophyte cells, without the pre-culture growth period. Additional established and new bryophyte cells were harvested for initial biomass estimates.

Each plot (+Algae and no-Algae) contained two trays with a total of 12 Ludwigia plants (six established and six new-growth types) as well as 12 bryophyte cells (six established and six new-growth). The study duration was 63 days and was initiated on July 23, 2015. For each harvest event, three randomly selected replicates of all four experimental plantings (established Ludwigia and bryophytes, and new-growth Ludwigia and bryophytes) were harvested from the treatment (+Algae) and reference (no-Algae) plots on August 21 and at the end of the experimental growth period on September 24, 2015. Ludwigia plants were measured for stem count and maximum stem length, then separated for above- and below-ground biomass, dried at 60° C for 72 hours and weighed to the nearest 0.01g. Bryophytes were separated from their cell, cleaned, dried and weighed to the nearest 0.01g as well. At the end of the experimental growth period, the biomass of each vegetation type at day 60 was compared between the +Algae treatment and the no-Algae reference using a Student *t-test*.

During the experimental growth period, the experimental plots were monitored weekly over the duration of the study. Visual assessments were made weekly to estimate % cover and approximate thickness of algae in the +Algae treatment plot. If any algae were present in the no-Algae reference plot, it was manually removed.

Water quality was also monitored within the two experimental plots. A YSI multiparameter sonde was used to measure pH, dissolved oxygen and temperature weekly. Water velocity was measured in both plots with a Marsh McBirney 2000 flow meter. Water samples were also collected periodically at the two experimental locations as well as a location approximately 150 m upstream (Heidelberg Lodge) and approximately 400 m downstream (Spring Island). Samples were collected as sub-surface (ca. 30 cm) grab samples into plastic bottles which had been acid washed (5% HCL) and triple rinsed with local water. All samples were immediately put on ice and returned to Baylor University for analysis. We analyzed these samples for total N (TN), total P (TP), NO_2-N + NO_3-N (NO3-N), ammonia N (NH4-N) and soluble reactive P (PO4-P) with colorimetric methods (APHA 1995) on a LachatH Quik-Chem 8500 flow-injection autoanalyzer (Hach Instruments, Loveland, Colorado). We measured TN and TP concentrations after persulfate digestion.

Results

Measured water quality parameters showed little difference between the +Algae and no-Algae treatments despite the +Algae treatment being enclosed by silt fencing (Table 3.1). The only parameter notably different between the two treatments was the lowered velocity in the +Algae enclosure which ranged only between .01 and .03 ft/s throughout the duration of the study. As expected, the velocity in the no-Algae treatment was higher and variable (0.06 to 0.12 ft/s) since this location was open to water flow. Although we anticipated the possibility that the +Algae enclosure might differ in water quality values compared to the no-Algae treatment this was not observed.

Table 3.1 Mean and Standard Error of physiochemical parameters measured weekly from July 23 to September 24, 2015 at the +Algae and no- Algae treatments (n= 8).

		pH	D.O. (mg/L)	Temp (°C)	Cond. (µs/cm).	Velocity (ft/sec.)
Treatment	+Algae	7.50 ± .08	4.12 ± .0.29	23.9 ± 0.28	576 ± 0.72	0.02 ± .003
	no-Algae	7.43 ± .07	4.58 ± 0.17	24.0 ± 0.12	576 ± 0.78	0.09 ± .008

The nutrient concentrations in the +Algae and no-Algae reference plots were very similar to each other and to the sites immediately above and below the experimental site (Table 3.2). PO4-P, TP and NH4-N were typically below 10 ug/L and very near the analytical detection limits (≈ 5 ug/L). TP and PO4-P were virtually identical, indicating very little suspended material in the water column. In very sharp contrast, NO3-N and TN were very high (>1500 ug/L). Virtually all the TN was NO3-N, indicating very little suspended material in the water column. The ratio of available N to available P was very high and averaged 221. This indicates a very strongly P-limited system. Most algae need N and P at a ratio of about 7N:1P on a mass basis (15:1 on a molar basis). Available N:P ratios above 10 (mass basis) are considered P-limited.

Table 3.2 Nutrient parameters measured at benthic algae mat experiment location and at a location upstream (Heidelberg) and downstream (Spring Island). Concentrations shown in ug/L (PPB). Available N= NO3-N + NH4-N. Available P= PO4-P

Date	Location	PO4-P	NH4-N	NO3-N	TP	TN	TN:TP	Avail N: Avail:P
28-Aug	Heidelberg	9	9	1860	7	1870	251	215
	+Algae	8	14	1760	5	1760	361	233
	no-Algae	9	9	1835	6	1820	280	203
	Spring Island	9	9	1830	7	1880	264	197
7-Sep	Heidelberg	9	7	1830	6	1810	293	203
	+Algae	7	8	1740	5	1370	298	253
	no-Algae	8	7	1810	6	1780	276	215
	Spring Island	8	10	1800	6	1790	295	227
11-Sep	Heidelberg	9	8	1830	6	1780	280	199
	+Algae	6	3	1695	4	1680	423	265
	no-Algae	8	8	1800	7	1800	261	226
	Spring Island	9	7	1800	6	1700	267	212

Algae cover and thickness in the +Algae plot varied significantly through the study. Visual estimates of percent cover and average mat thickness are noted in Table 3.3. Significant amounts of algae in the +Algae treatment covered both Ludwigia growth types for a short time and loose algae often floated on the water surface. Algae was mostly absent in the +Algae plot at the conclusion of the study. Algae was rarely thick enough to cover a significant portion of upright growth exhibited by Ludwigia. However, being low growing and mat forming, the bryophyte frames were covered by algae turf mats for much of the experimental growth period. We anticipated algae turf mats forming and algae biomass remaining more consistent in the +Algae enclosure given that it was protected, but this did not occur. Even so, there was a considerably higher benthic algae turf presence in the treatment than in the control during the study. Comparison photos between the +Algae plot and the no- Algae plot are shown in Figure 3.4.

Table 3.3 Measured thickness of benthic algae turf mats in the +Algae plot during study period.

DATE	% COVER	THICKNESS
8/3	100%	0.8 cm
8/11	100%	1 cm
8/17	100%	10 cm
8/26	20%	1 cm
8/31	10%	.8 cm
9/9	40%	1.2 cm
9/14	100%	1.2 cm
9/21	20%	1.2 cm

Figure 3.4 A and B show the no-Algae plot where algae was mostly absent if it was observed. C and D show the +Algae treatment. While algae did become thick and entangled within Ludwigia (D) it did not remain attached for a prolonged period of time. Yet bryophytes, located near the substrate, were subjected to a longer period of algae cover even when algae was not dense.

Ludwigia stem lengths and the biomass of all vegetation classes are represented in Figures 3.5 and 3.6. The pattern of vegetation biomass over the experimental period shows that none of the vegetation types showed increases in biomass in either the treatment or the reference plot (Figure 3.6). Even so, differences existed between the +Algae and no-Algae plots by the end of the experiment for three of the four vegetation types. Established Ludwigia and established bryophytes maintained their initial biomass in the no-Algae plot, while their biomass in the +Algae treatment plot declined significantly by the end of the experiment. New-growth Ludwigia showed little variability through time as there was no significant difference in biomass between the +Algae and no-Algae plots. New-growth bryophytes declined in both plots, although they declined significantly more in the +Algae treatment plot than in the no-Algae plot. At the 32 day harvest established Ludwigia in the +Algae treatment exhibited more elongated stem growth

then established Ludwigia in the no-Algae plot which were bushier with multiple secondary branching (Figure 3.5). At the conclusion of the study it appeared that herbivory or some other physical damage occurred to Ludwigia plants in the +Algae plot as stems of Ludwigia were found floating in the enclosure. These were gathered to include with biomass estimates but it is unknown how much biomass was lost or potentially consumed by herbivore activity. Furthermore, it is unclear if any biomass was also lost from the reference plot, since any broken stems would have floated away.

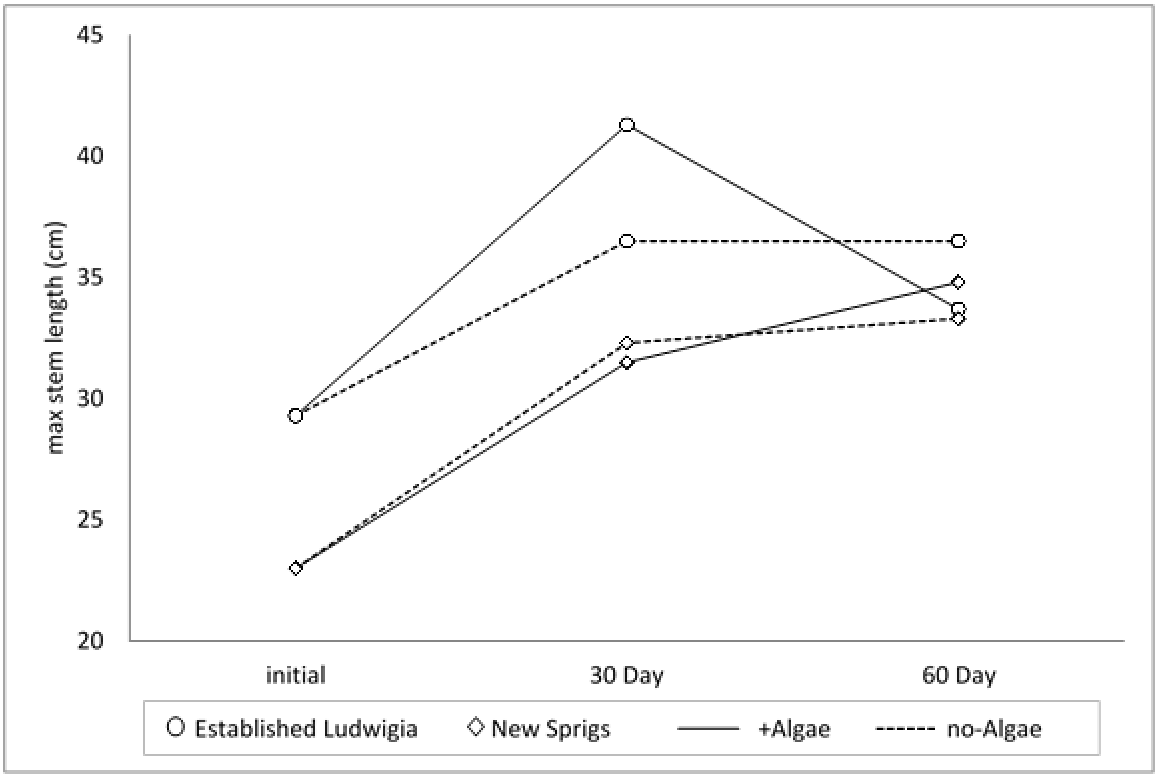

Figure 3.5 Stem lengths of established and new growth Ludwigia in the +Algae and reference plots. Established Ludwigia showed the greatest increase in stem length at the 32 day harvest but by the end harvest stem length was reduced by physical damage.

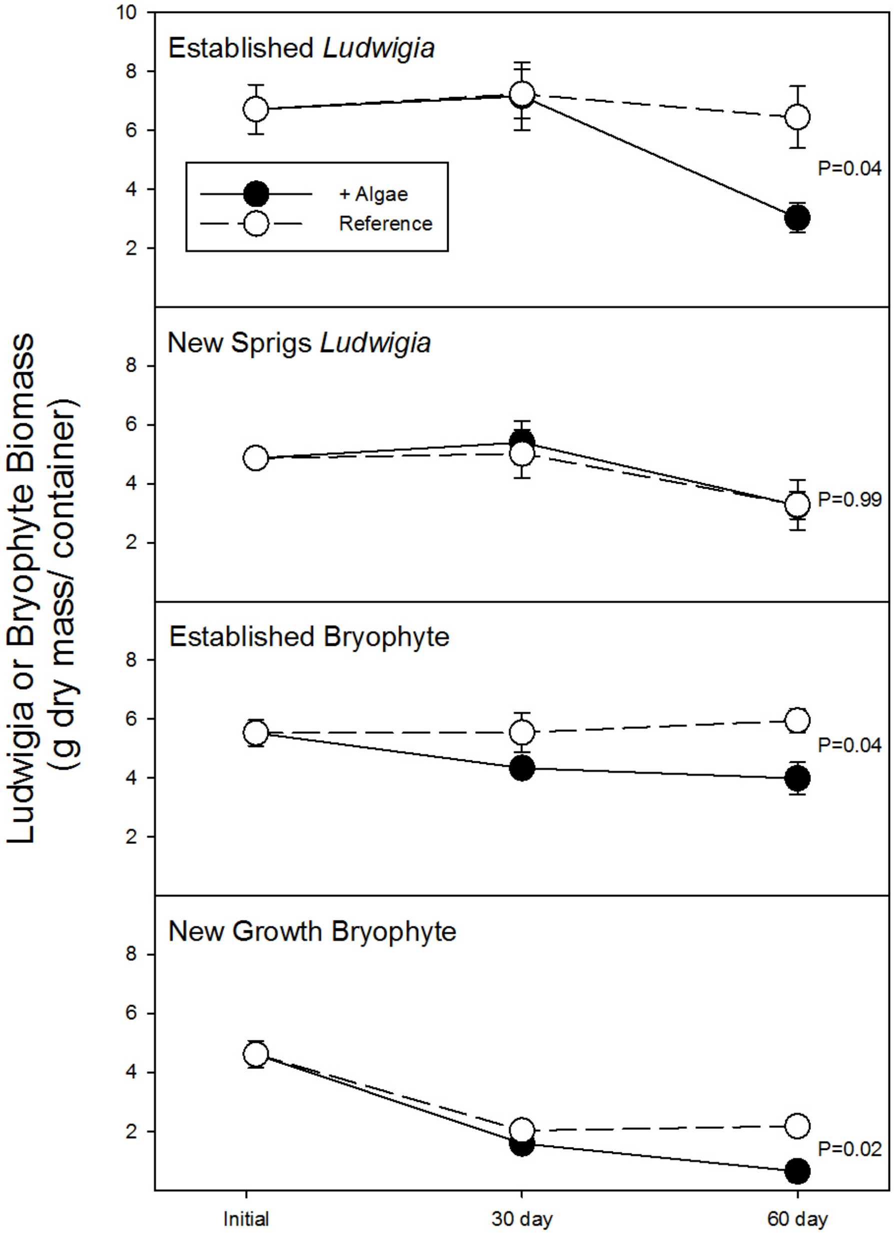

Figure 3.6 Vegetation biomass for each of the four vegetation types through the experimental growth period. The p-value of a Student *t-test* comparing the final biomass in the +Algae treatment and the Reference plot is shown.

Discussion

In interpreting the results of this study, it is difficult to decipher the critical level at which algae turf negatively impacts plant growth. Three of the four plant types (Established Ludwigia, Established bryophyte and new growth bryophyte) showed significant differences in biomass between the +Algae and no-Algae plots at the end of the study. Since growing conditions were similar between the two plots in all regards it is likely that algae played a significant role yet it is unclear if algae alone impacted plant types or if other factors did since new bryophyte and new Ludwigia decreased in biomass in both plots. Herbivory may have impacted the Ludwigia plants, but bryophytes are generally not preferred fodder for most aquatic herbivores, including crayfish, so this factor may be an unlikely contributor in bryophyte decline in the no-Algae plot (Parker et al., 2007). Established bryophyte showed the most immediate impact. Biomass of established bryophytes in the +Algae plot showed declines by the 32-day harvest and was significantly lower by the end of the experimental period. This result may indicate that established bryophytes are sensitive to the presence of algae even over a short period of time. Due to its growth form, bryophytes in the +Algae plot were undoubtedly more severely shaded by the algae turf than the upright growing Ludwigia, which could project stems and leaves above the algae turf even when algae turf was at its thickest. Literature does suggest that bryophytes are sensitive to competition from algae and other plant species and P uptake in bryophytes per unit biomass is less than algae (Steinman and Boston, 1993)(Stream Bryophyte Group, 1999). Unfortunately, algae turf biomass did not remain dense for prolonged periods so that we could fully evaluate the effects of Ludwigia exposure to algae turf.

Although the impact of benthic algal mats on freshwater vegetation does not appear common in the literature, seagrass meadows may provide a useful analog system for understanding potential impacts. Seagrass meadows are similar to freshwater macrophyte beds in that they are ecosystem engineer species that provide habitat and important ecosystem services. These benthic marine and estuarine communities are well-studied and may provide some insight into how algal mats influence benthic freshwater macrophyte and bryophyte communities, like those in Landa Lake. Shading caused by dense benthic algal mats is the primary mechanism of concern for seagrass communities, because of the short growth form of many seagrass plant species vegetation cannot grow above or out of the algal cover. Eelgrass (*Zostera* sp) seedlings appear to be particularly susceptible and were strongly impacted when shaded by dense algal mats (Rasmussen et al, 2011). However, mature seagrass vegetation is also affected by prolonged periods of algal shading (Gustafsson and Bostrom, 2014; Hauxwell et al, 2001; McGlathery, 2001). A secondary concern in marine systems is that of alterations in nutrient and dissolved gas concentrations. Multiple studies have noted that benthic macroalgal mats can create anoxic conditions well above the sediment interface and produce unfavorable biogeochemical conditions, such as potentially toxic concentrations of ammonium as tissues senesce and remineralize (Hauxwell et al, 2001; McGlathery, 2001; Qiuying and Dongyan, 2013). However, Rasmussen et al (2012) found that transient mats with more ephemeral drift-algae cover may not strongly impact mature plant communities. During 2015 the benthic mats observed in our experimental plot were possibly more like these transient ephemeral mats in growth pattern as well as duration.

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CHAPTER 4

DISSOLVED OXYGEN DYNAMICS IN LANDA LAKE AND THE UPPER SPRING RUN

4.1 SPATIAL DISTRIBUTION OF DISSOLVED OXYGEN

4.1.1 INITIAL INVESTIGATION OF SPATIAL DISTRIBUTION OF DISSOLVED OXYGEN IN LANDA LAKE

Materials and Methods

Information on the variation in dissolved oxygen (DO) spatially throughout Landa Lake has rarely been collected. Currently diel DO is only measured on a continuous basis at one location, about mid-lake 29°42'48.06"N; 98° 8'7.19"W, and these data are used to make management decisions for Landa Lake as a whole. To better understand the spatiotemporal distribution we measured DO and corresponding water temperature at 14 locations in Landa Lake and Upper Spring Run (USR) for one week from July 28 to August 5, 2015. DO measurements were collected with multiple MiniDOT DO sensors (Figure 4.1) available from Precision Measurement Engineering (PME Inc. Vista, CA). These sensors utilize the recently developed optical fluorescence technology and have been widely used by the United States Geological Survey in streams and rivers across the United States. Before deployment sensors were tested in the laboratory at Baylor University against the YSI brand data sonde (Xylem Inc.) by logging data of both instruments simultaneously while bubbling air then nitrogen to produce air-saturated followed by anoxic conditions. MiniDOT measurements were found to be equivalent to a YSI datasonde utilizing similar optical technology as MiniDOT values were within a few percent of the YSI values at all measurement periods.

Figure 4.1 MiniDOT dissolved oxygen sensor deployed in Landa Lake during summer 2015.

Biofouling of deployed sensors is a well-known problem for long-term biomonitoring. We initially deployed sensors in four locations of Landa Lake in the vertical "upright" (sensor facing upward) position recommended by PME Inc. However, we found that when the sensors were in high-light environments, heavy biofouling happened within 2-3 days and DO maxima of >12 mg/L and overnight minima of < 2 mg/L were recorded (Figure 4.2). We therefore modified to deploy the sensors in a horizontal position, and when possible facing north to minimize light exposure to the surface of the sensor. This resulted in much less observable biofouling at most locations over the deployment period of 8 days (Figure 4.2). However, at low-flow environments, notably site #14, rapid biofouling continued to be a significant issue. In these low-flow locations cleaning occurred as often as possible, but typically only every 5-7 days. As a consequence data collected after 2 days of deployment or cleaning at these sites were interpreted with caution.

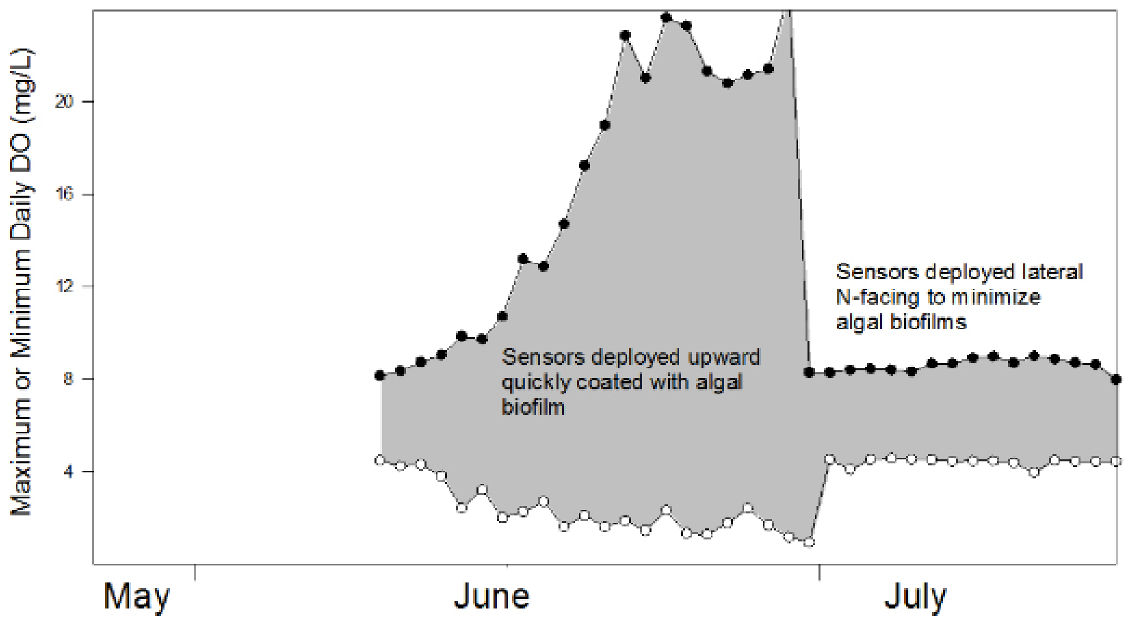

Figure 4.2 Upward versus horizontal deployment results of biofouling activity.

Measurement locations for the 8 day evaluation were selected during a reconnaissance investigation in late June conducted by team members. At this time, the HCP aeration project water quality probe was in place and operating. We chose measurement sites in a variety of locations throughout Landa Lake, excluding spring runs, to incorporate differing habitat types (deep, shallow, with current, stagnant, vegetated, non-vegetated, etc.) and capture the wide range of conditions that are experienced throughout Landa Lake and the USR reach (Figure 4.3). Table 4.1 provides a description of the chosen sites. The spatial evaluation study was initiated on July 28 and continued for one week with sensors being downloaded on August 5. During this time period, total system discharge in the Comal system ranged from approximately 300 to 340 cfs. From a historical perspective, this represents total system discharge conditions slightly above the long-term historical average which is considerably different from the 65 cfs total system discharge conditions experienced during summer 2014.

MiniDOTs were deployed at approximately 60 % of water depth. In locations where the depth was less than 2 meters, the MiniDOTs were cable tied to a t-post that was driven into the sediment. Where the depth was greater than 2 meters, a floatation device was wrapped around the sensor, which was then tied to an anchor with a length of rope that allowed it to float at appropriate depth. At each station total water depth was measured as well as water velocity at 20%, 60% and 80% of depth. Vegetation type around the station was also recorded as well as a gps waypoint.

During deployment MiniDOTs were cleaned after 3 days. At the end of the 8 day deployment data was downloaded. To download the data, the sensors were collected, wiped down, opened,

and turned off. Four of the MiniDOTs had SD cards that stored the data; these SD cards were removed, inserted into an SD card reader, downloaded onto a YUMA Tablet, then wiped clean and returned to the MiniDOT. The other 10 sensors were downloaded via usb cable that was plugged directly into the sensor from the YUMA.

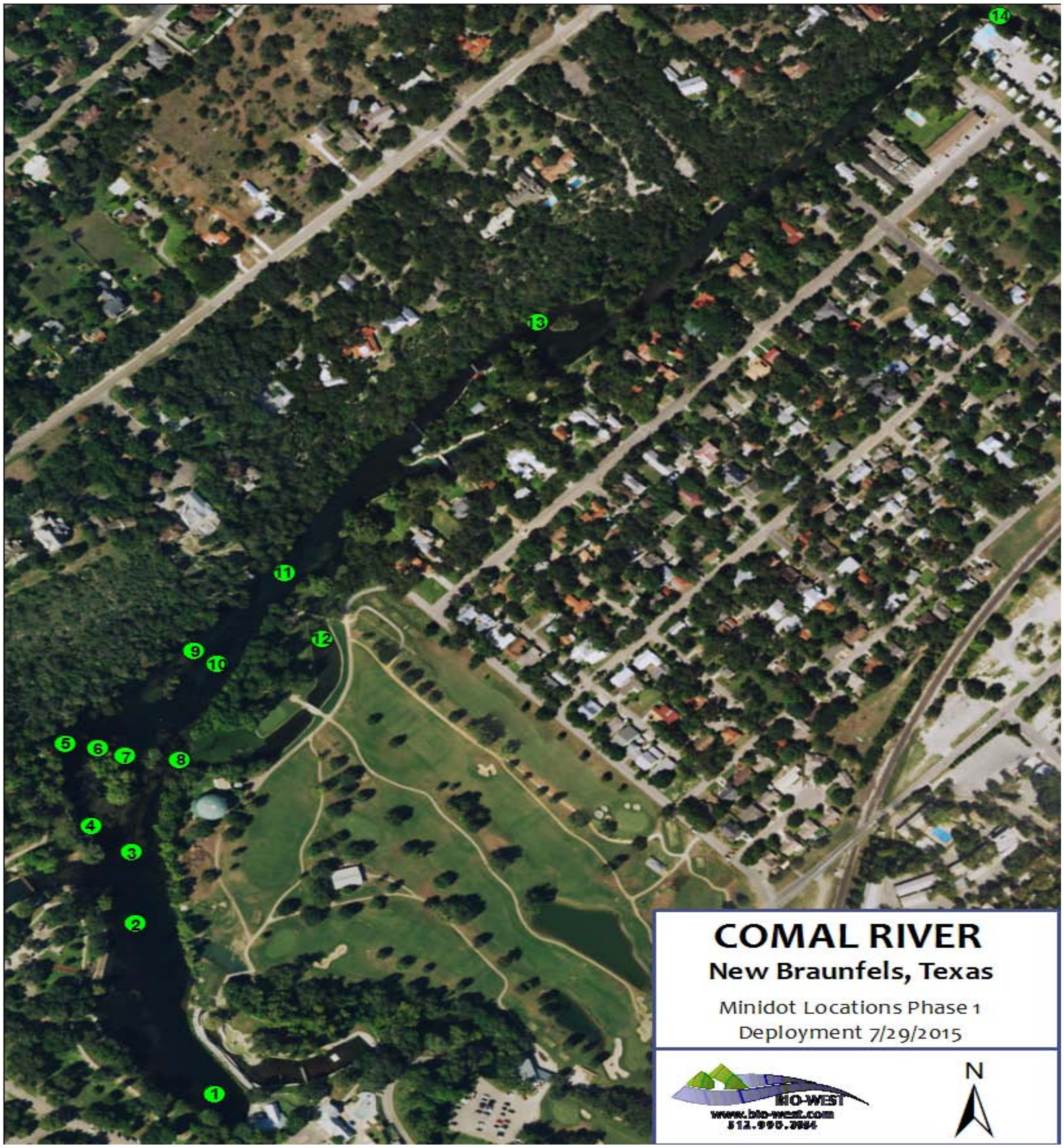

Figure 4.3 Location of 14 MiniDOT dissolved oxygen sensors during initial spatial evaluation study.

Table 4.1 Description of 14 MiniDOT dissolved oxygen sensor locations in Landa Lake. Sensors were deployed at approximately the 0.6V depth.

Station #	*DESCRIPTION*	*Total Depth (cm)*	*Vegetation*	*Approx Flow (0.6V m/sec)*
1	*Downstream Buoy in Landa Lake*	*152*	*Vallisneria*	*0.42*
2	*Adjacent to Paddleboat rental area*	*128*	*dense Vallisneria*	*0.06*
3	*At Existing DO probe for Aerator Project*	*143*	*dense Vallisneria*	*0.08*
4	*Adjacent to Fishing Pier in Vallisneria*	*155*	*Vallisneria*	*0.19*
5	*Adjacent to Gazebo at the outflow of Spring Run 3*	*180*	*edge of Vallisneria*	*0.29*
6	*Top of Island 1 in three islands area*	*125*	*dense Vallisneria*	*0.05*
7	*Upstream of Island 3 in three islands area*	*98*	*Sagittaria*	*0.34*
8	*Lower Pecan Island backwater area*	*70*	*Nuphar, Cabomba*	*0.04*
9	*Northwest shore across from Pecan Island*	*119*	*Bryophyte*	*0.22*
10	*Mid Channel location near MUPPT nursery*	*155*	*Bryophyte*	*0.21*
11	*Northwest shore near Cable*	*223*	*Bryophyte*	*0.12*
12	*Adjacent to Golf Course in Pecan Island backwater*	*85*	*Cabomba*	*0.08*
13	*Upstream of Spring Island*	*98*	*Sagittaria, Cabomba*	*0.09*
14	*Adjacent to Heidelberg Lodge*	*158*	*Nuphar, Cabomba*	*0.03*

Results

Results from the week-long spatial study are presented in Figure 4.4. A solid red line is place on each chart representing the HCP goal of 4.0 mg/L DO (EARIP 2011). In general, diel DO ranged between approximately 4.0 and 9.0 mg/L at most stations. The exception to this was in backwater areas, stations 8, 12 and 14, where very little water exchange takes place. Dissolved oxygen conditions at station 8 dipped down to approximately 3.0 mg/L on day 2 and continued to dip below 4.0 mg/L each subsequent morning of the study. Considering the location of this sonde, a shallow slow moving area surrounded by *Nuphar*, this was not unexpected. In fact, this likely provides a glimpse of what might be expected when total system discharge conditions are considerably lower causing pockets of considerably lower velocity fields.

Sites 12 and 14 were also located in areas with considerably lower velocities and would likely have experienced DO conditions less than 4.0 mg/L similar to station 8 on a daily basis. Although these conditions were experienced, biofouling of the probes at these locations occurred rapidly despite regular cleaning efforts causing both extremely high and low measurements of DO at these locations (Figure 4.4). Further investigations later in the summer and fall improved our confidence that bio fouling was the culprit to the high and low data at these locations during this study period. As such, we are not confident in the DO measurements reported in Figure 4.4 for these stagnant sites after more than two days of deployment.

Although we had anticipated the 2014 drought conditions to continue into 2015 low flow conditions did not continue through the summer of 2015. In fact one significant rain event occurred in May increasing discharge significantly. As such, this spatial data represents what is to be expected during average total system discharge conditions. It was also unfortunate that during this study period, the continuous monitoring sonde used for official determination of DO and other water quality parameters in Landa Lake was inoperable and removed from Landa Lake for repair so that no comparison could be made between our data and the data collected by that instrument.

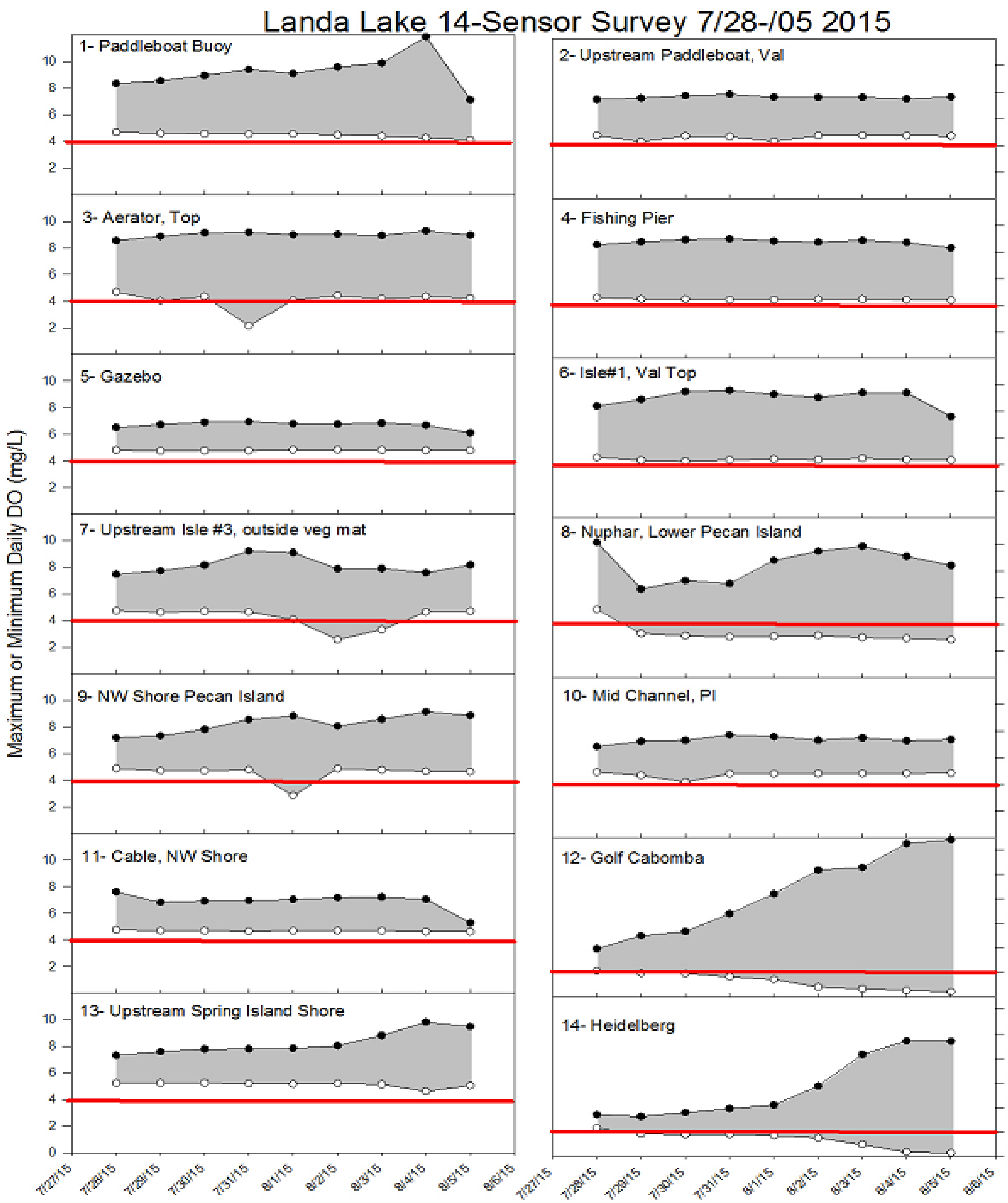

Figure 4.4 Maximum or Minimum dissolved oxygen results (mg/L) from 14 MiniDOT sensors during spatial evaluation study. Data at stations 8, 12 and 14 were heavily affected by biofouling.

4.1.2 ADDITIONAL INVESTIGATION OF THE SPATIO-TEMPORAL VARIABILITY OF DISSOLVED OXYGEN ALONG VERTICAL GRADIENTS AND MICROHABITATS IN LANDA LAKE

Materials and Methods

Building upon the results of the one-week spatial survey, we implemented a longer-term monitoring project (August 6 through October 6, 2015) at six locations in Landa Lake and the USR (Figure 4.5). We deployed sensors such that in addition to continuing to monitor large-scale horizontal spatial variability we could compare variability at a vertical scale and among different microhabitats. Comparative sampling included: a) DO at the bottom vs. DO at the top of the water column (where DO is often measured for monitoring purposes), and b) DO under a vegetation mat vs. DO outside a vegetation mat. Dissolved Oxygen measurements at the sediment-water interface is extremely important since fountain darters are mostly associated with this microhabitat. A variety of vegetation types were included across the locations as well. Dominant macrophyte species in Landa Lake include *Vallisneria neotropicalis* (Vallisneria) and *Sagiitaria platyphylla* (Sagittaria). Thick bryophyte turf, almost entirely composed of *Riccia fluitans*, was present along the sediment surface at some bottom locations while aquatic macrophytes were present in others. In some locations no vegetation was present. Vegetation mats accumulate regularly on the surface of Landa Lake.

Attributes of each location are provided in Table 4.2. Four MINIDOTs remained at previous sampling stations used for the initial one week study in order to continue long term data collection at these sites. Two new stations were selected in open water adjacent to large vegetation mats and two other stations were selected within the middle of vegetation mats for comparative sampling (Figure 4.6). Three stations received only one MINIDOT while five stations received two MINIDOTs for comparative sampling. At these locations a t-post was driven into the sediment and the sensors attached to the t-post. The top sensor was typically deployed about 20-30 cm below the water surface while the bottom sensor was deployed just above the sediment or any existing algae/bryophyte turf (Figure 4.6). Typically the bottom sensors were deployed 2-10 cm from the sediment surface, depending on presence or absence of photosynthetic benthic communities (most commonly, thick bryophyte turf). MiniDOTs were placed in a horizontal position to limit biofouling and debris build up. After deployment MINIDOTS were cleaned regularly every 3 to 5 days and data was downloaded once per week. Over the course of this study three MiniDOT sensors failed during the summer deployment period. Human tampering flooded the internal components of one MiniDOT and two others failed due to damage to the sensor membrane. At the end of the deployment period, the remaining MiniDOT sensors were returned to the laboratory at Baylor University and re-checked under saturation and anoxic conditions. All remaining sensors showed excellent performance.

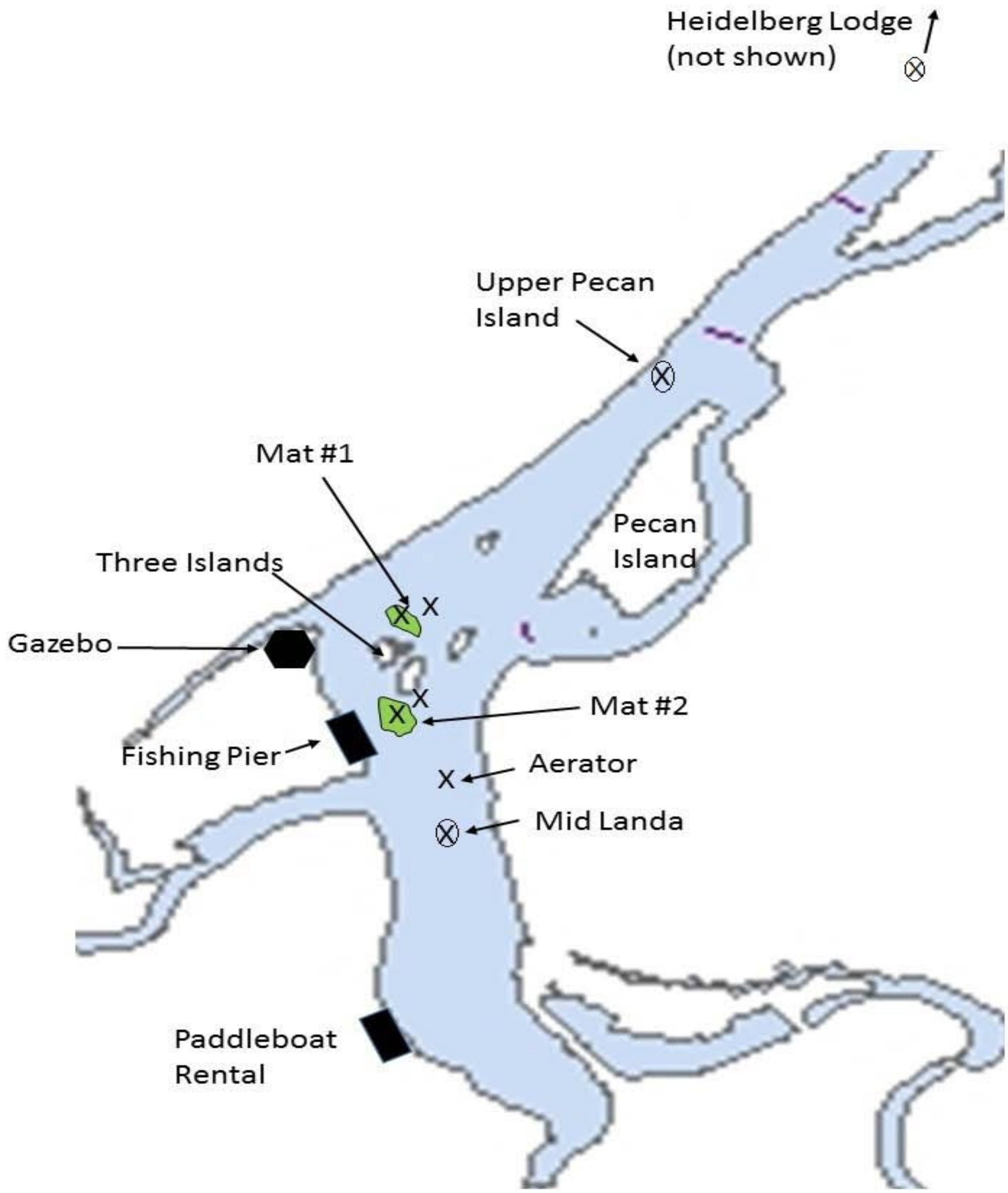

Figure 4.5 Location of eight MiniDOT dissolved oxygen stations selected for the August-October DO survey. Stations with X received one MINIDOT placed at mid-depth while locations with encircled X received two MINIDOTS placed at the sediment-water interface and just below the water surface. The Heidelberg Lodge location (not shown) received one MINIDOT.

Table 4.2 Attributes of locations selected for this prolonged study. Daily data was generated for up to 62 consecutive days (8/5/2015-10/6/2015). The total number of days for which data is available is shown for each location, along with the number of days where daily minima fell below 4.0 mg/L. Overall average minimum DO for all days for which data is available is also shown.

Location ID	Depth, Vegetation	Approx. flow (m/sec)	Total # days	# days < 4.0 mg/L	Avg. Min (mg/L)
Mid Landa Paddleboat (top, A)	115 cm. Dense Vallisneria. Sensor deployed just above Vallisneria canopy (30 cm depth)	0.2V= 0.44	62	3	4.72
Aerator (top, B)	139 cm. Adjacent to dense Vallisneria. Sensor 30 cm below surface.	0.2V= 0.22	62	16	4.14
Aerator (bottom, C)	139 cm. Moderate bryophytes at bottom. Sensor 20 cm above sediment	0.8V= 0.01	62	**62**	**2.73**
Mat #2 Interior (top, D)	73 cm. Dense Vallisneria. Thick mat. Sensor at 20-30 cm below surface	0.2V= 0.08	62	1	4.61
Mat #2 Interior (bottom E)	73 cm. Very little benthic community. Sensor 5-10 cm off bottom.	0.8V= 0.04	62	1	4.49
Mat #2 Outside (top, F)	98 cm. Moderate Vallisneria. Sensor 20-30 cm below water surface.	0.2V= 0.31	62	2	4.57
Mat #2 Outside (bottom, G)	98 cm. Moderate bryophytes. Sensor 5 cm above bryophytes (15-20 cm above sediment surface)	0.8V= 0.04	22	0	4.51
Mat #1 Interior (top, H)	90 cm. Moderate Vallisneria. Moderate mat of bryophyte and algae. Sensor 20-30 cm below surface	0.2V= 0.05	62	0	4.51
Mat #1 Interior (bottom, I)	90 cm. Very little benthic community. Sensor 5-10 cm off bottom.	0.8V= 0.02	62	0	4.38
Mat #1 Outside (top, J)	109 cm. Edge of dense Vallisneria, adjacent to dense Sagittaria and open area. Sensor 20-30 cm below surface	0.2V= 0.18	62	0	4.55
Mat #1 Outside (bottom, K)	109 cm. Dense bryophytes at bottom near sensor and adjacent in Sagittaria bed. Sensor 5 cm above bryophyte mat.	0.8V= 0.01	62	**29**	**3.99**
Top Pecan Island (top, L)	191 cm. 60 cm below water surface. Sparse bryophytes in the area.	0.6V= 0.20	26	6	4.41
Heidelberg Lodge (top, M)	151 cm. Moderate Nuphar and Cabomba. Sensor at 20-30 cm below surface. Very rapid biofouling!	0.2V= 0.03	62	**58**	**0.86**

Figure 4.6 Top. Sarah Hester of Baylor University places MiniDOT sensors within the middle of floating vegetation mats. Mats are composed of a variety of aquatic and terrestrial debris. Bottom. Placement of MiniDOT sensors at the top and bottom of the water column. Note the dense bryophyte turf, composed of *Riccia fluitans*, located along the bottom.

Results

The daily maximum and minimum DO recorded by each sensor at each of the locations is presented in Figure 4.7. One area of interest are differences in diel DO between the top and bottom of the water column. DO measurements are often taken at the top of the water column and water quality modeling usually integrates the entire water column based on this one measurement. However, fountain darters are more impacted by oxygen conditions at the bottom of the water column. Five locations had sensors deployed at the top and bottom of the water column (Aerator station, inside mat 1, outside mat 1, inside mat 2, outside mat 2).

The Aerator station is immediately adjacent to the solar powered aerator system installed in Landa Lake as part of the HCP (Figure. 4.5). This location is relatively deep (~1.4 m) and has dense Vallisneria immediately surrounding the area. The sensors were deployed in an opening in the Vallisneria with a moderate layer of bryophytes located along the bottom (Figure 4.7 B and C). At this station the daily maximum DO was usually a bit higher at the top than at the bottom (46 of 62 days), although the magnitude of this was relatively modest (usually <1.0 mg/L higher). The overnight minima were lower at the bottom sensor. At the top of the water column the overnight DO minima was never below 4.0 mg/L while the bottom DO measurements dropped below the 4.0 mg/L threshold on a nightly basis for the entire duration of deployment (Table 4.2). Average DO minima at the top of the water column was 4.14 mg/L while at the bottom sensor the average daily minimum value was 2.73 mg/L, below the HCP 4.0 mg/L goal. During five separate periods the overnight lows were very low (< 1 mg/L, Figure 4.7 C). These appear to be periods when the benthic bryophyte mat "fluffed" up to engulf the sensor. Several times when we serviced the sensors we noted that the bryophytes had accumulated around and on top of sensors. Perhaps the physical presence of the t-post and sensor facilitated this phenomena.

Stations located within the vegetation mats were set at the approximate center of two selected vegetation mats and sensors were deployed at the water surface and the sediment-water interface while two other sensor locations were set well outside and slightly upstream of these vegetation mats with MiniDOT sensors again deployed at the water surface and the sediment-water interface (Figure 4.5). The vegetation mats were primarily composed of bryophyte and algae, although some macrophyte pieces accumulated from time to time. The dominant vegetation beneath both of the vegetation mats was Vallisneria which filled the entire water column while the outside station was at the interface of the Vallisneria bed, and adjacent Sagittaria bed which was heavily colonized by bryophytes between the plants, and an unvegetated area covered by dense bryophytes.

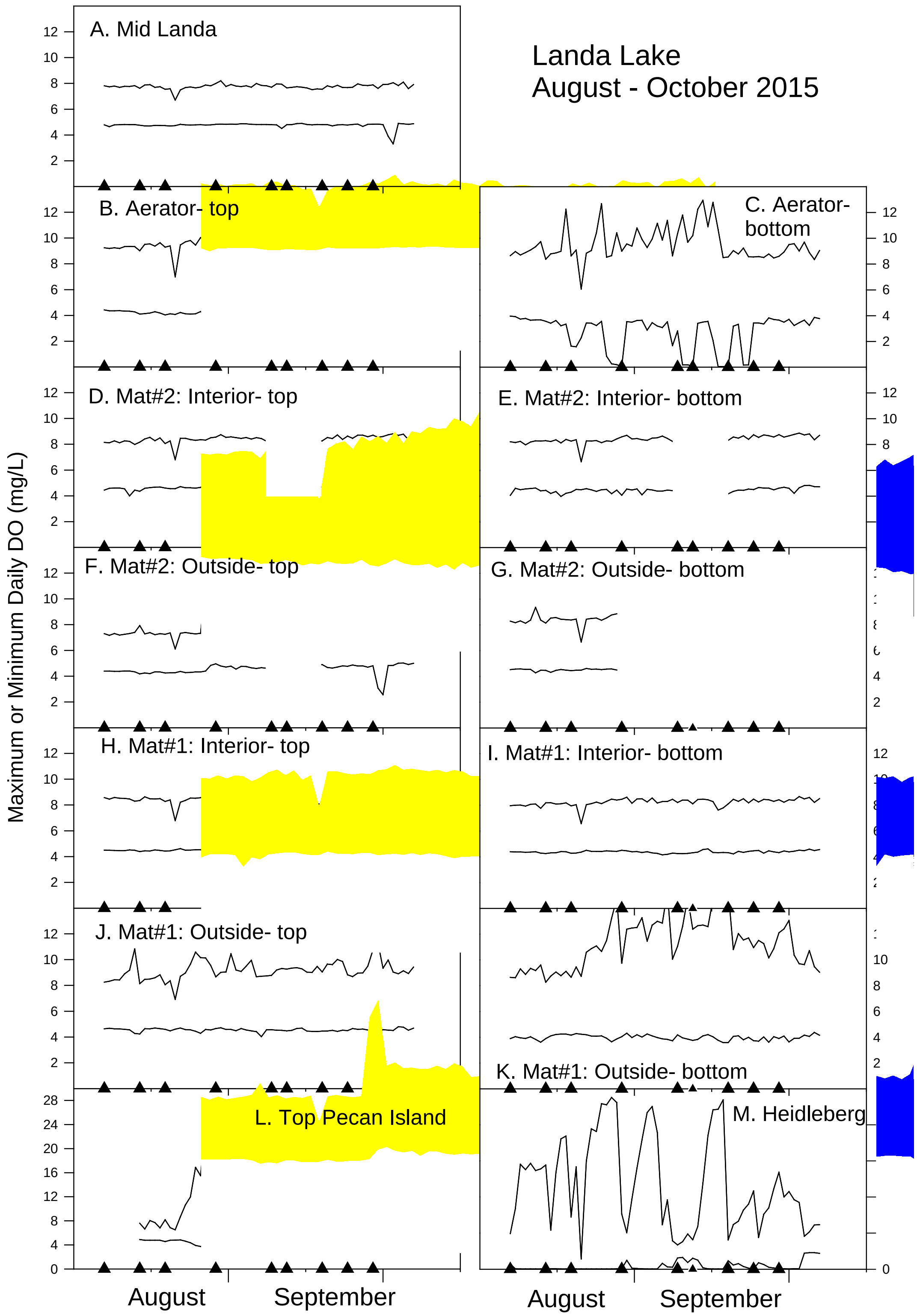

Figure 4.7 Maximum or Minimum dissolved oxygen results (mg/L) from MiniDOT sensors during deployment at the 13 locations. Dates when sensors were serviced are shown as triangles along the date timeline. Yellow= top sensors, Blue= near bottom.

At the station outside of vegetation mat #1 *(*Figure 4.7, J and K*)* overnight minima at the top of the water column (Figure 4.7, J) averaged 4.55 mg/L over the 62 nights sensors were deployed and never had an overnight minima below 4.0 mg/L DO (Table 4.2). The bottom sensor at this station recorded DO levels less than 4.0 mg/L on 29 of the 62 days of deployment, although the average overnight DO was 3.99 mg/L and the minimum overnight DO recorded was only 3.59 mg/L. Severe DO depletion below 2 mg/L was not observed at this location. Daily maximum DO at the top ranged from 7-10 mg/L (Figure 4.7 J) while DO maximum values at the bottom were often in the 10-13 mg/L range (Figure 4.7 K). DO maxima and minima were more extreme at this location at the bottom of the water column near the metabolically active bryophyte bed.

At the station within vegetation mat #1 maximum and minimum DO readings were muted through time at the top and bottom (Figure 4.7 D and E). Daily maxima were typically just above 8 mg/L at both the top and bottom of the water column. Overnight minima at the top and bottom of the water column was never below 4 mg/L and averaged 4.51 and 4.38 at the top and bottom of the water column, respectively.

The daily maximum and minimums of DO at the top and the near bottom MiniDOT sensors at Mat #1 are shown in Figure 4.8. These same data are shown in Figure 4.7 (H, I, J, K) but are re-plotted here to allow easier comparison of data inside versus outside the mats. At this station, daily maxima were consistently higher outside the mat at both the top and bottom of the water column (Figure 4.8, yellow circles compared to black circles). The difference in DO maxima were particularly pronounced near the bottom (Figure 4.8, bottom panel), likely due to the presence of that outside bottom sensor near the very metabolically active bryophyte mat. Daily minima at the top of the water column were virtually identical (Figure 4.8, Top Panel, triangles). Overnight minima at the bottom of the water column were actually slightly lower outside the mat rather than under the mat (Figure 4.8, Bottom Panel, triangles). This is likely also due to the very thick bryophyte mat present at this location.

The location of vegetation mat #2 was located just downstream of the three islands in Landa Lake and immediately in front of the fishing pier (Figure 4.5). This mat was composed of decomposing macrophytes, bryophytes, algae and terrestrial vegetation. Sensors here were also deployed at the surface and bottom both inside and immediately outside the mat on the upstream side. The dominant vegetation both inside and outside the mat was Vallisneria.

At the outside location of mat #2 overnight minima at the top of the water column (Figure 4.7 F) was usually well above 4.0 mg/L and averaged 4.57 mg/L over the 62 days. The two days with minimum DO's below 4 mg/L deviate sharply from most dates and may be related to floating vegetation fragments or algae wrapping around these sensors. Data was collected at the bottom of this location for the first 22 days of deployment. After this, the sensor was moved to allow continued monitoring at other locations after one of the sensors there was destroyed. Minimum DO were never below 4 mg/L and averaged 4.51 mg/L.

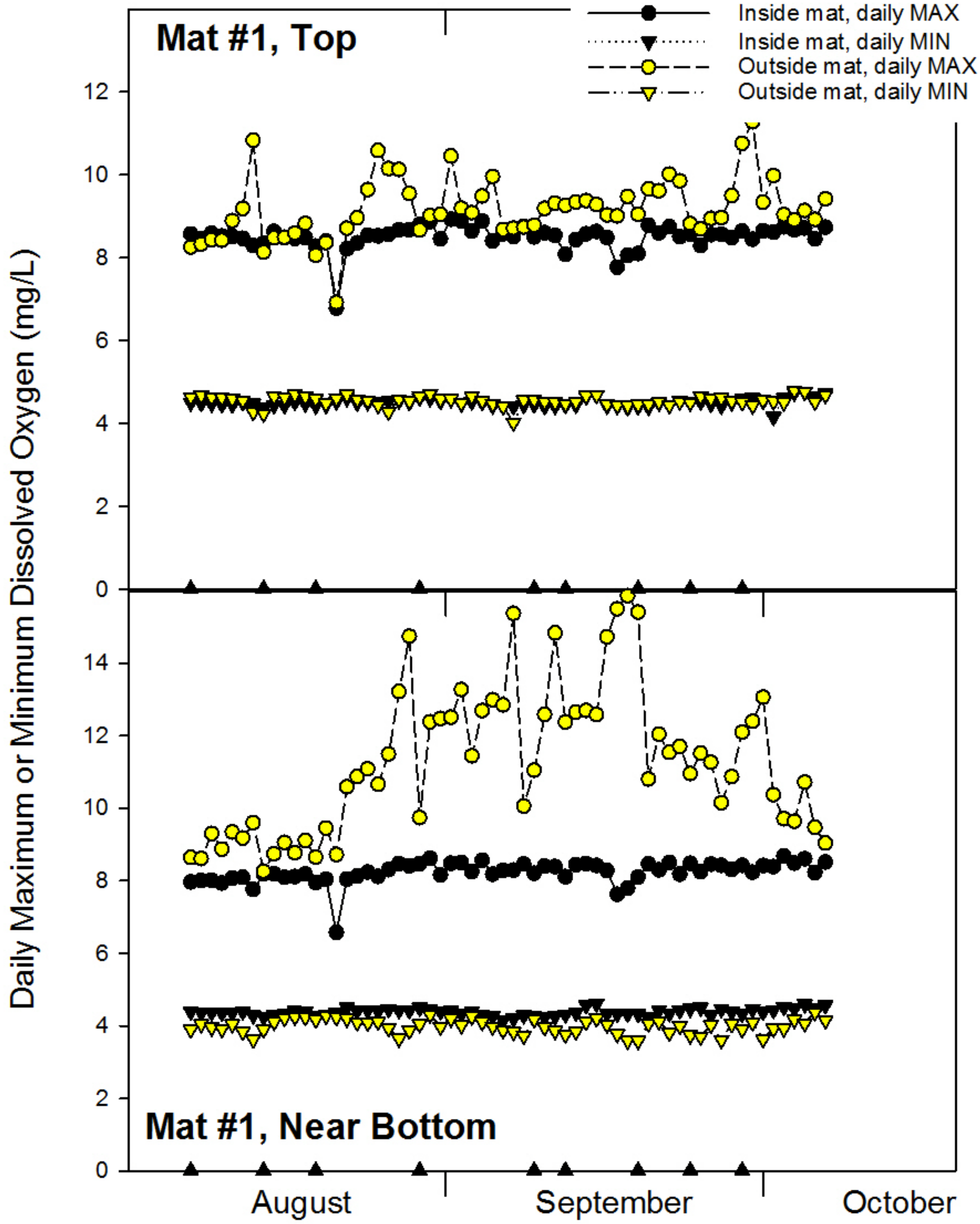

Figure 4.8 Daily maximum and minimum levels of dissolved oxygen at Mat #1. Top Panel shows data for the top of the water column, while the bottom panel shows data for the sensor near the sediment surface. Yellow symbols are used for sensors outside the mat and black symbols for sensors inside the mat. Circle are used for sensors at the top of the water column while down triangles are used for the near-bottom sensors.

The sensors deployed within mat #2 *(*Figure 4.7 D and E*)* showed a muted pattern similar to that seen at mat #1 for DO readings at the top and bottom of the water column. DO minima below 4.0 mg/L was recorded only once at each position, and both of these values were actually >3.9 mg/L, so just barely below the level of concern. Daily maximum values were typically just above 8.0 mg/L. The daily maximum and minimums of DO at the top and the near bottom at Mat #2 are shown in Figure 4.9. These same data are shown in Figure 4.7 (D, E, F, G) but are re-plotted here to allow easier comparison of data inside versus outside the mats.

During the first few weeks of deployment, the DO at the top of the water column was a bit higher inside the mat than outside the mat, although daily minima were virtually identical (Figure 4.9, top panel). Towards the end of August, we had to move the MiniDOT positioned at the bottom outside the mat due to equipment failure in another location. At that time we re-positioned the outside the mat sensor to a depth of 60 cm to represent a mid-water column reading. From that point on, the daily DO maxima outside the mat exceeded the maxima inside the mat for the top sensor. We believe that by re-positioning the sensor, we positioned it closer to the Vallisneria canopy, and therefore the observed daily maxima was higher. Daily maxima and minima at the bottom of the water column (Figure 4.9, Bottom Panel) were virtually identical throughout the sampling period.

Water temperatures remained remarkably constrained between 23 and 25 °C in all stations except Heidelburg which exceeded the 25 °C mark initially in August (Appendix II, Figure II-1). Also temperature data showed no significant differences between location (top versus bottom; inside mat versus outside mat) indicating waters were well mixed throughout the water column as well underneath floating vegetation mats (Figure 4.10).

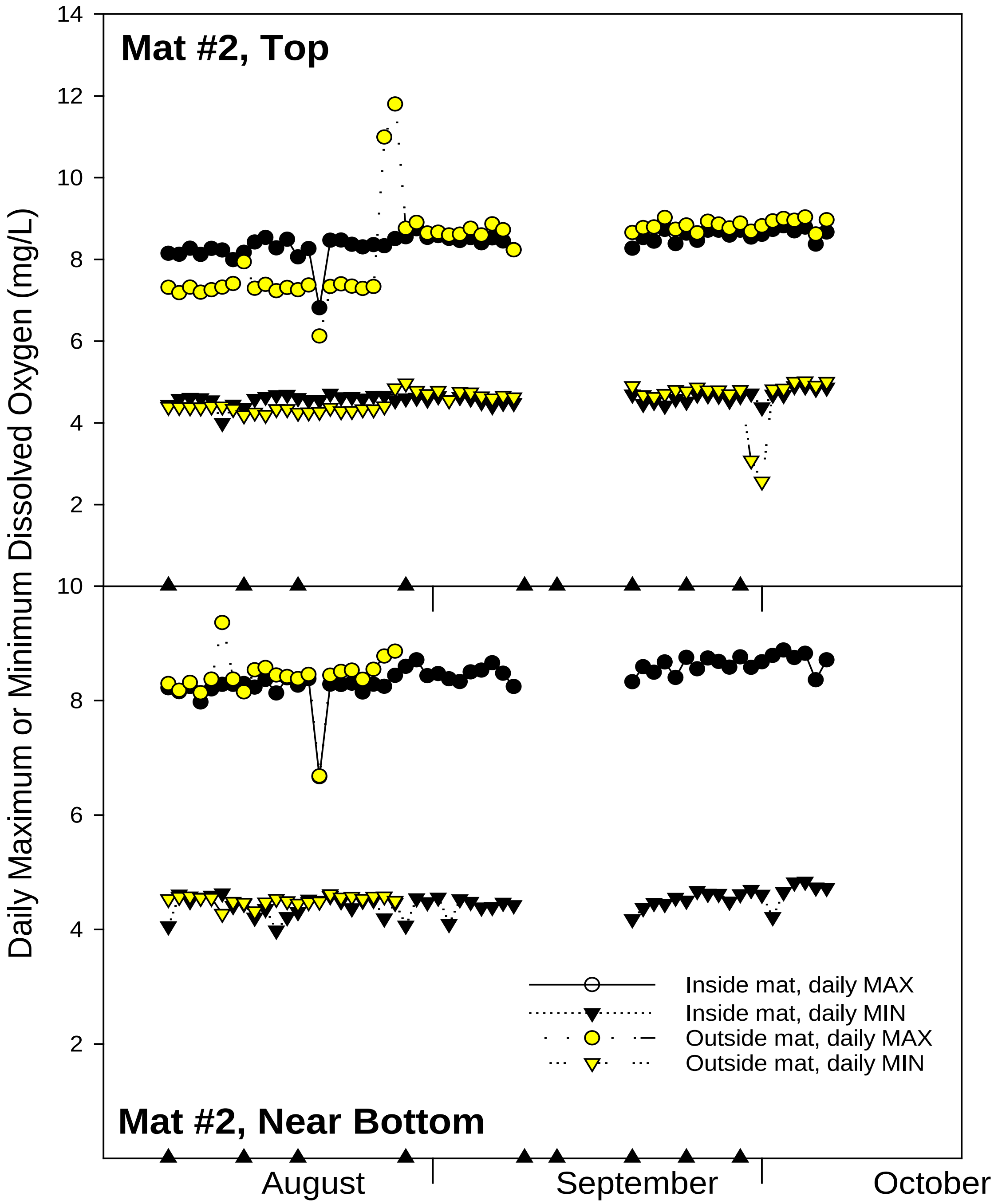

Figure 4.9 Daily maximum and minimum levels of dissolved oxygen at Mat #2. Top Panel shows data for the top of the water column, while the bottom panel shows data for the sensor near the sediment surface. Yellow symbols are used for sensors outside the mat and black symbols for sensors inside the mat. Circle are used for sensors at the top of the water column while down triangles are used for the near-bottom sensors.

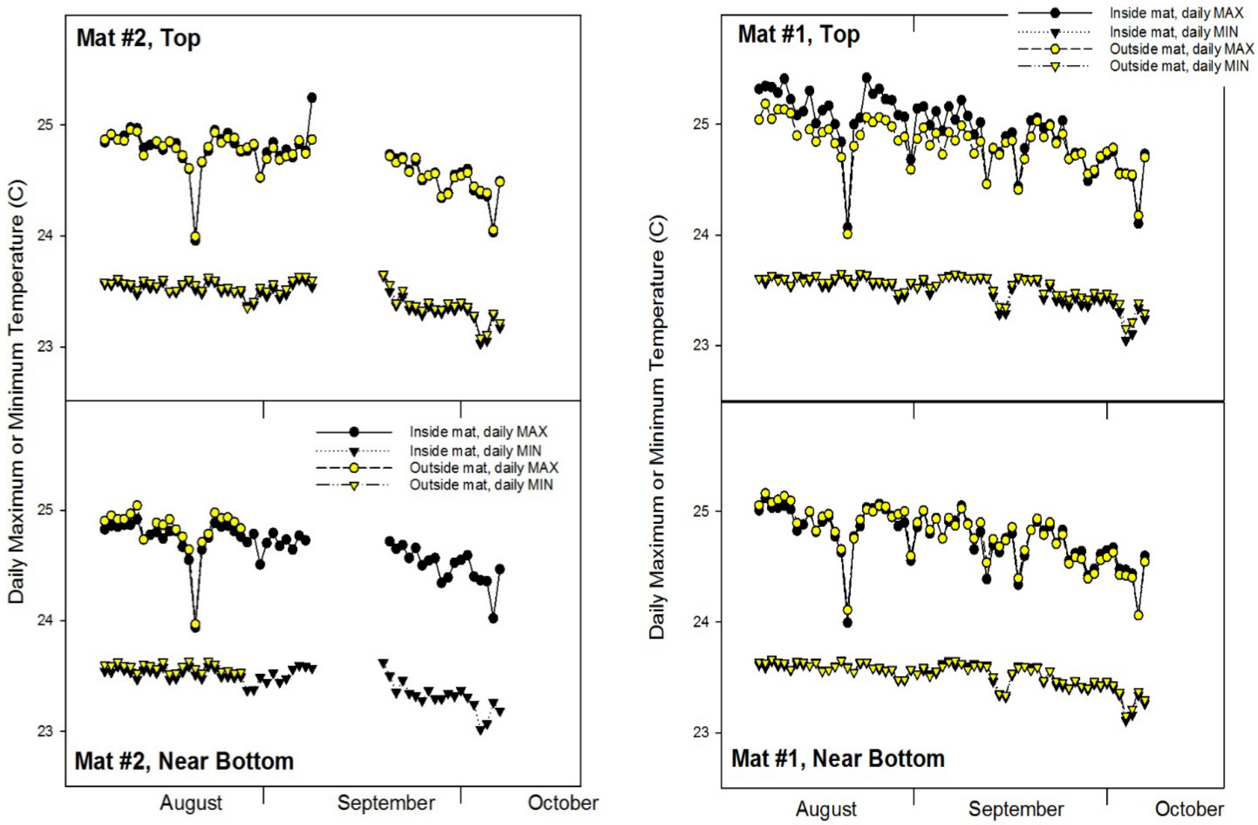

Figure 4.10 Maximum or Minimum water temperature results (° C) from MiniDOT sensors located within mats or outside of mats and at the top or bottom of the water column.

Discussion

The single area of concern indicated by the prolonged DO monitoring in 2015 relates to very low minimum values at low-flow locations with very dense vegetation such as the Nuphar bed at the bottom end of Pecan Island (Figure 4.4 Station #8) and the Cabomba in the channel behind Pecan Island near the golf course (Figure 4.4 Station #12). Although these locations also suffer from very rapid biofouling, we believe the data support concern for low DO conditions in these low-flow environments. Overnight DO minimums fell well below 4 mg/L even immediately following sensor cleaning. These findings are of potential concern since under very low flows during drought, these types of conditions may occupy a much larger portion of the Landa Lake and the USR than was observed in 2015. The location at Heidelberg Lodge (Figure 4.4 Station #14; Figure 4.7 M) experienced the highest degree of variability in DO with hypoxic conditions routinely being measured. This site has experienced severe and well documented hypoxic conditions in the past as a result of dinoflagellate blooms (Gilpin, 2012) but biofouling complicates the interpretation of our data after a few days of deployment. If hypoxic and even anoxic conditions are actually present this suggests these minima continue regardless of flow

conditions. Also, darters are commonly collected here so if hypoxic to anoxic oxygen conditions occur, the darters are apparently able to avoid them.

Comparison of DO dynamics inside and outside of vegetation mats showed few areas of concern. Overall, these data indicate that the vegetation mats had very little impact over diurnal DO patterns during the 2015 season. In particular, there is no evidence that minimum DO levels under the mats fall to levels that provide a concern for fountain darters. Perhaps the rather minimal vegetation mat formation during 2015 contributed to this finding. However, we believe that as long as measurable flow continues beneath the mats, there is likely to be relatively little problem related to localized DO minima.

Even so, two general trends emerge from evaluation of these patterns.

1) Dissolved oxygen appears more variable outside the mats than beneath the mats. The day-to-day variability of DO maxima and minima within the mats is remarkably constant at both the top and bottom of the water column (Figures 4.8 and 4.9, black symbols). We believe the pattern of more variable DO outside the mats is caused by two factors. A) Apparent fluctuations caused by higher biofouling at the outside locations. The sensors outside the mats were much more prone to develop significant biofouling by attached algae, no doubt because of the higher light climate (i.e. sensors not shaded by the mat). However, after discovering this issue, we made frequent trips to service and clean the sensors, so we believe the impact of biofouling is modest. B) Real DO fluctuations related to more metabolically active communities. The DO dynamics beneath the mats are generally free from localized swings caused by daytime photosynthesis (since the mats effectively shade the water column). Therefore, it is perhaps not surprising that the DO maxima tend to be much higher outside the mats. This is especially true at the bottom location of vegetation mat #1. This sensor was positioned just above a very metabolically active bryophyte bed, and we believe the large daily swings in DO reflect real variability at this site.
Although the low DO values occasionally recorded at the bottom sensors in this study are accurate, they likely do not reflect a concern for darter habitat (for example, see Figure 4.7 C). We believe these very low DO readings occur when the benthic bryophyte or algae layers expand to engulf our sensors (so that the sensors are within the mat matrix, not just above the mat surface as desired). Since the darters could likely easily avoid the low DO's within the matrix of the bryophyte layer, the low DO recorded may not indicate a concern under current flow conditions. With regards to the data at the aerator site (Figure 4.7 C) the minimum DOs excluding the five "low oxygen" periods was 3.34 mg/L. This value likely reflects the true DO minima at the bottom of the water column (but above the benthic bryophyte mat). While below the HCP 4.0 mg/L goal, these values do not likely represent a serious threat to fountain darters.

2) DO conditions beneath the mats do not appear to be unfavorable for fountain darters. The minimum DOs at the locations beneath the mats were almost identical to those outside the mats. In fact, the exception to that is the lower DOs outside of Mat #1, due to the high metabolic activity of the bryophyte bed, as explained above. Yet fountain darters are routinely observed, sampled and collected within this vegetation type. If DO within bryophyte beds routinely drops below the 4 mg/L then questions remain as to how fountain darters thrive in this habitat setting.

4.2 INFLUENCES OF VEGETATION MATS ON THE OXYGEN DEMAND IN LANDA LAKE

4.2.1 COMPOSITION AND BIOMASS OF VEGETATION MATS

Materials and Methods

Floating vegetation mats form each year on Landa Lake and are thought to potentially pose concerns for DO dynamics. While we generally know the mats are composed of uprooted (or senesced) macrophyte fragments, algae, bryophytes and terrestrial debris, we have no data on the proportion of these components within mats. Therefore, this study was carried out to quantify the vegetation mats in terms of mat composition (macrophyte, bryophyte, algae, terrestrial), total dry mass (DM) and ash-free dry mass (AFDM) on an area basis.

Each month between May and September 2015 two mat locations in Landa Lake were sampled to determine the composition and biomass of the mats (Figure 4.11). The location of mat #1 was constant through the study being located just upstream of the “three islands” in Landa Lake. The second mat sampled each month varied depending on where we felt the most extensive mats were developing. During 2015 the areal extent of mat formation appeared lower than experienced in recent years.

At each mat we collected three to four samples. The specific sampling location was distributed so that visual variability observed was represented in the samples. Typically two samples were collected from the apparent core of the mat and one or two additional samples collected from outlying areas of the mats. Samples were collected by isolating and cutting a known area of mat. We used a standard soil sieve with a total area of 0.033 m^2. At each location, the sieve was carefully positioned below the mat and slowly raised to the surface. The mat was compressed against the sieve edge and cut to isolate the mat sample (Figure 4.12). The mat sample was quickly transferred to extra-large zip lock bags and put on ice. The samples were transported back to the laboratory at Baylor University.

In the laboratory, mat samples were sorted by tissue type (macrophyte, bryophyte, algae, terrestrial). These samples were dried to constant weight at 60 °C and weighed to nearest 0.1 mg. Each sample was then homogenized using a heavy duty kitchen blender. This produced a coarse, but homogenous sample. Subsamples were taken and ashed at 550 °C for 2 hours to estimate Ash Free Dry Mass (AFDM).

Figure 4.11 Location of selected vegetation mats for study of composition and BOD.

Figure 4.12 Dr. Robert Doyle & Kelsey Biles of Baylor University collecting samples from vegetation mat #1 for composition and BOD analysis.

Results

The average mat composition and total dry mass and ash-free dry mass for the two mats sampled each month are shown in Figure 4.13. Overall, the mats in 2015 were primarily composed of floating bryophyte and algae tissues, although periodic accumulation of macrophyte and terrestrial plant debris was apparent. The algae and bryophyte tissues collected were always green and actively growing tissues instead of dead and decaying material. At the end of the summer, the mats were noticeably thicker and heavier with very little decaying tissue present. In August and September, mat dry mass was in the range of 550-1,550 g m^{-2}, whereas the dry mass in May was <400 g m^{-2}.

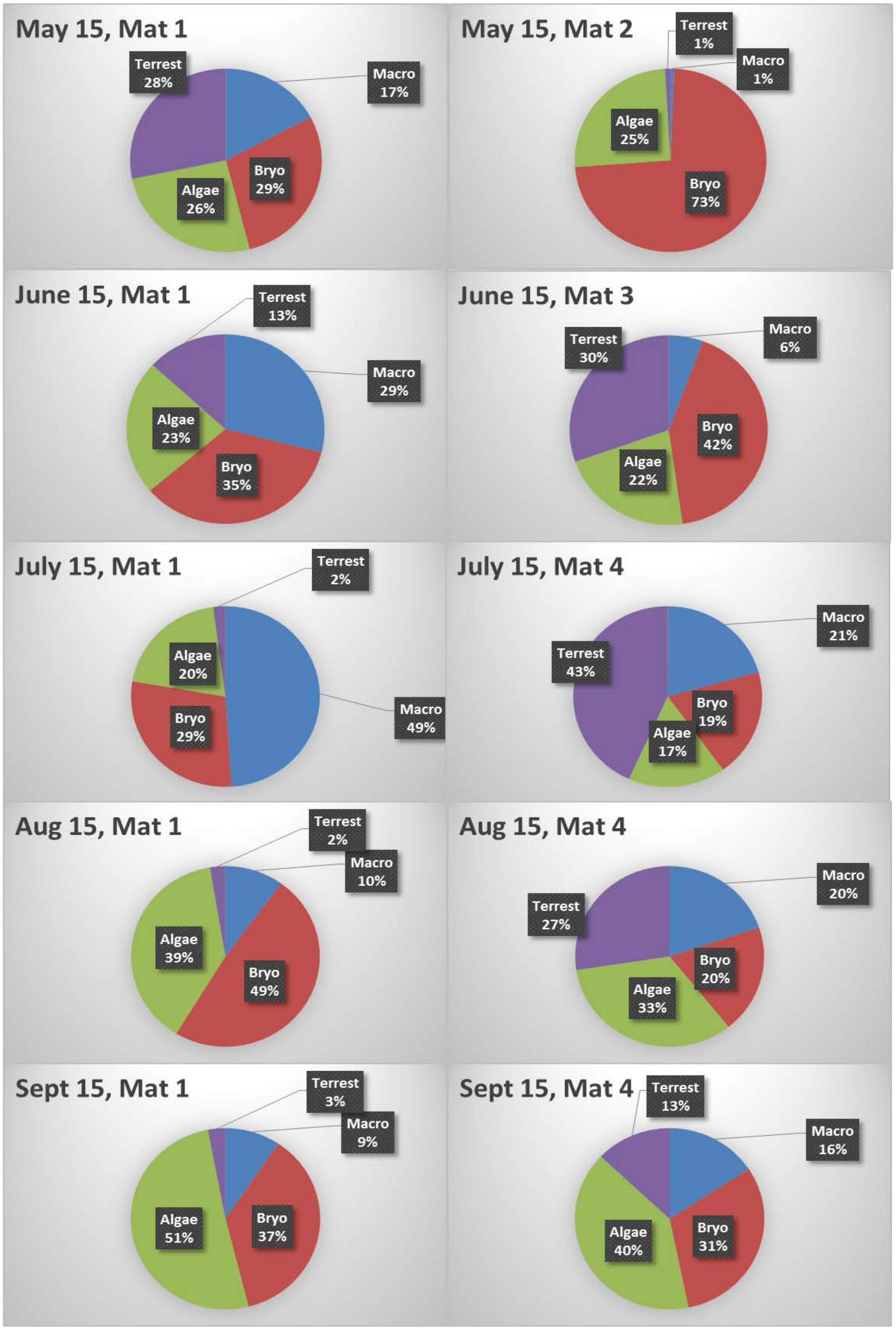

Figure 4.13 Composition of mats collected during each month. Shown are averages of dry mass samples for 3-4 quadrats collected at each mat site each month.

4.2.2 POTENTIAL BIOLOGICAL OXYGEN DEMAND OF VEGETATION MATS.

Materials and Methods.

The data reported in this section were measured primarily to provide information for a DO estimate/model that will be discussed in more detail in Chapter 5. The development of the vegetation mats poses a potential concern for DO dynamics because the mats are composed of organic material which may decompose within the lake, causing oxygen demand on the system. To address this question, we measured the *potential* biological oxygen demand of the mats by determination of the Ultimate Biological Oxygen Demand (BO_{ult}) of each tissue component of the mats. These were then combined based on the measured mat composition to estimate the total potential biological oxygen demand of the mats per m^2. Additionally vegetation mats were mapped to provide an estimated cover in square meters which could be used to further calculate the estimated total BOD of vegetation mats in situ. As mentioned, these data are of use primarily in assisting modeling efforts to predict DO dynamics under various scenarios (Chapter 5).

The Ultimate Biological Oxygen Demand (BO_{ult}) was determined from the dried tissues as an estimate of the maximum potential oxygen demand represented by the mats. At the beginning of the study we verified that dried and fresh samples of algae and bryophytes produced equivalent estimates of BO_{ult}. The spatial distribution of the vegetation mats were mapped each month using high-resolution GPS equipment (Trimble). Each mat was individually mapped by carefully circumnavigating the edges. To estimate potential BOD, we utilized an adaptation of the BOD_{ult} method of Ostapenia et al. 2009. For each mat sampled, three or four ground, homogenized subsamples of dominant tissue type were analyzed for BOD_{ult}. For each BOD estimate, approximately 20 mg was weighed out to nearest 0.1 mg and added to 330 ml BOD bottles filled with artificial lake water (deionized water to which key salts are added). The incubation water was aerated by bubbling with air so that initial DO was at 100% air-saturation. A 1 ml sample of unfiltered water from the Comal River was added to each bottle as a microbial inoculum. Samples were maintained in the dark at 20C in a BOD incubator. The DO in each bottle was measured every 3-7 days for 60-90 days. If DO in the bottle dropped below 15% saturation, the bottle was re-aerated. The decline in DO between samplings is a measure of the biological oxygen demand.

The cumulative BOD through time was plotted. From these data BOD-kinetic parameters [BOD_{ult} (mg O_2 L^{-1}) and the reaction constant k (day^{-1}) were calculated with the Microsoft Excel Solver tool that uses the Generalized Reduced Gradient (GRG) Nonlinear optimization code assuming first order kinetics (equation 1).

$$(1)\ BOD = BOD_{ult}\ (1-e^{-kt})$$

Results

Mapping of vegetation mats occurred in April, June, July, August, September and October. Vegetation mats tended to expand from one mapping event to the next with October resulting in the highest cover, 3,141 m^2, yet this was still below September 2014 cover which reached 3,877 m^2 (Figures 4.14 and 4.15) . Vegetation mats tend to persist around the three islands area and also tended to form around structures such as fallen trees, hanging limbs and man-made structures. Vegetation mats will also form where Vallisneria leaves reach the surface and debris becomes entangled in the leaves. Such was the case this year despite higher flows.

Cover of individual study mats studied varied by mat and by month. Mat #1 which was sampled repeatedly throughout the study ranged from 71 m^2 in April to 439 m^2. In May Mat #2 was 241 m^2. Mat #3 in June covered 63 m^2 and was sampled once as well and Mat #4, sampled multiple times, covered 112 m^2 in July, 381 m^2 August and 552 m^2 in September.

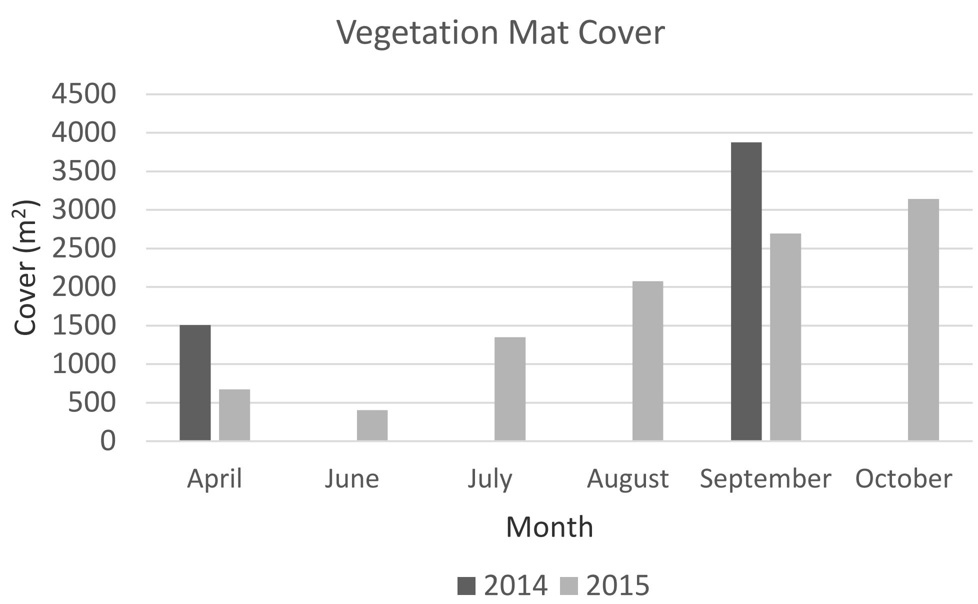

Figure 4.14 Total cover of vegetation mats during sampling months (2015) and compared to dates sampled in 2014.

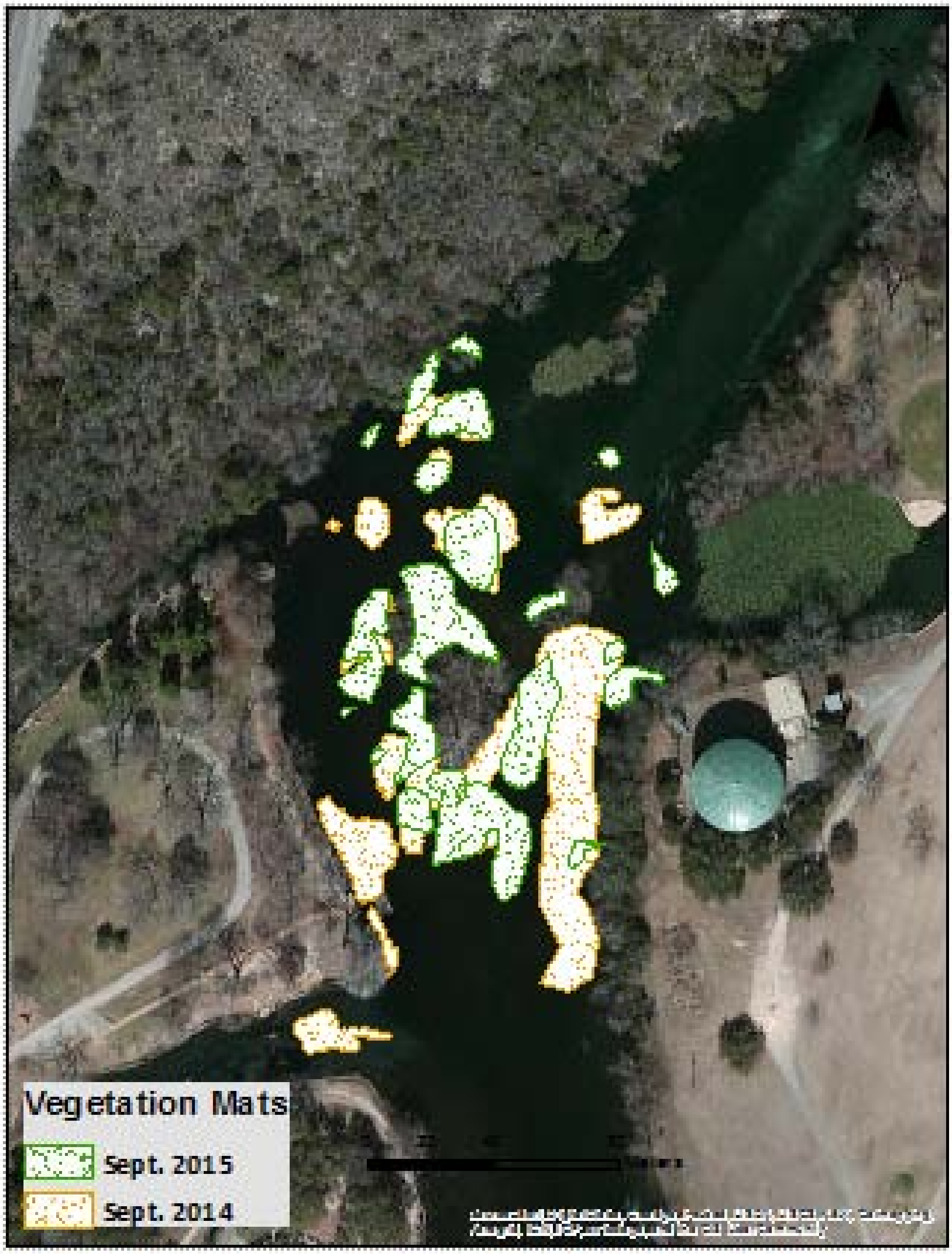

Figure 4.15 Map comparing cover of vegetation mat cover in September of 2014 and September of 2015.

The curve-fitting protocol yielded excellent fit to the experimental data (Figure 4.16). The BOD_{ult} values were then normalized to biomass by dividing by the used in each assay and corrected for the incubation volume.

Table II-1 in Appendix II shows the results of each assay conducted. The BOD_{ult} estimates are presented here in biomass-specific (gram dry mass, g dm) oxygen mass units (g O_2 g dm^{-1}). The values were surprisingly restricted and ranged from 0.28 to 0.83 g O_2 g^{-1} dry mass. Conversion to carbon mass units using a conversion factor of 0.375 (multiply by 0.375), assuming a theoretical molar respiration coefficient of 1.0 could be made if desired. The *k* parameter (decay coefficient) ranged from 0.02-0.06 d^{-1}, with equivalent half-lives of 35-12 days. Although this decay rate may not reflect *in situ* rates due to the drying and homogenization process, it provides some basis for estimating potential decay timelines.

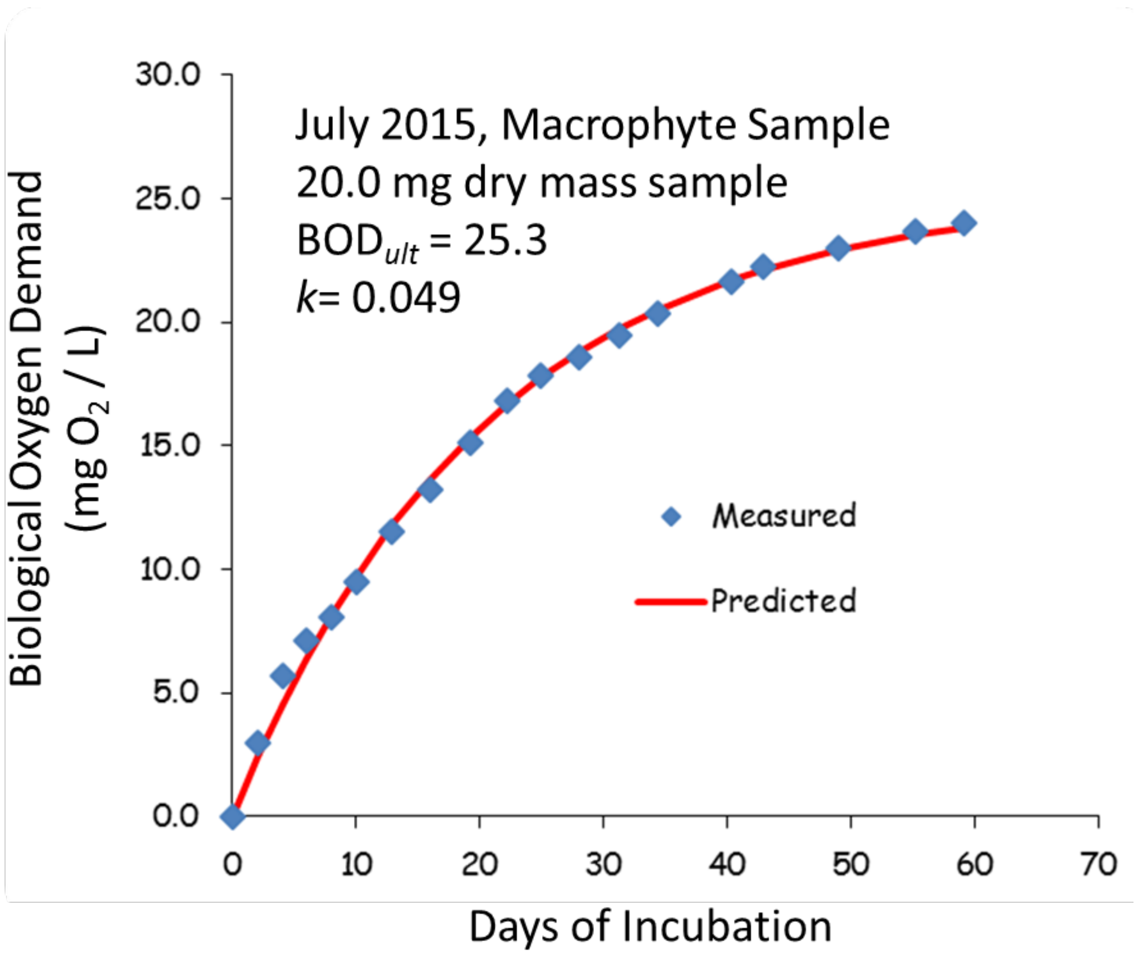

Figure 4.16 Measured Cumulative Biological Oxygen Demand through time (blue diamonds) and empirical curve fit to the data.

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The BOD data from Table 4.3 were extrapolated to an areal basis for each month based on rates measured each month for each tissue type and the measured composition of the mats during that time period. These estimates result in a potential ultimate oxygen demand per m^2 (Table 4.3). Total potential oxygen demand for the mats ranged from about 100 to 700 g O_2 m^{-2} for the data analyzed. The higher potential estimates come from mat#2 late in the summer. These data are used in Chapter 5 to provide a simple model to evaluate the potential impacts of the vegetation mats on the DO dynamics of Landa Lake.

Table 4.3 Total mat biomass, mat composition, BOD_{ult} for each tissue type and Potential Ultimate Oxygen Demand for the mat per m^2.

Date	Mat ID	Total Mat Biomass ($g\ m^{-2}$)	AFDM ($g\ m^{-2}$)	Mat composition ($g\ m^{-2}$) Macro	Bryo	Algae	Terrest	BOD_{ult} ($g\ O_2/g$ dry mass) Macro	Bryo	Algae	Terrest	Potential Ultimate Oxygen Demand ($g\ O_2\ m^{-2}$) Macro	Bryo	Algae	Terrest	TOTAL
15-May	1	360	264	62.0	103.5	92.6	101.6	0.62	0.69	0.46	0.34	38	71	43	35	187
	2	295	165	1.8	216.4	74.5	2.8	0.62	0.69	0.46	0.34	1	149	34	1	186
15-Jun	1	546	405	158.0	190.1	125.8	71.9	0.62	0.61	0.55	0.34	98	116	69	24	308
	2	625	477	35.3	263.4	136.3	190.4	0.62	0.61	0.55	0.34	22	161	75	65	322
15-Jul	1	204	142	100.5	58.9	41.4	4.3	0.55	0.50	0.42	0.34	55	29	17	1	104
	2	1008	743	208.1	195.7	168.2	436.4	0.55	0.50	0.42	0.34	114	98	71	148	431
15-Aug	1	562	331	56.4	273.7	217.3	14.8	0.70	0.61	0.49	0.34	39	167	106	5	318
	2	975	634	148.8	293.5	380.5	122.3	0.70	0.61	0.49	0.34	104	179	186	42	511
15-Sep	1	651	304	61.3	238.4	331.5	19.4	0.63	0.55	0.43	0.34	39	131	143	7	319
	2	1535	1147	303.6	298.8	511.4	420.8	0.63	0.55	0.43	0.34	191	164	220	143	719

Total Biomass: Total dry mass per meter squared
AFDM (Ash Free Dry Mass) = loss on ignition estimate of organic matter
Mat composition= we separated the mat into tissues, and weighted each separately
BOD_{ult} = ultimate oxygen demand per unit mass for each tissue type. Each sampling period value is an average of 5-7 replicate measures for that tissue type.
Potential Ultimate Oxygen Demand = Tissue biomass X BOD_{ult} ($g\ O_2\ m^{-2}$ of mat surface)
Note: if you make assumptions about how quickly decomp takes place (30 days?) you could get a daily demand per m^{-2}

Discussion

The development of vegetation mats during the summer of 2015 was substantially lower than observed in previous years. Compared to 2014, the maximum distribution of mats seen in September was 30% lower than that observed in 2014. We believe the more modest accumulation of vegetation mats are due at least in part to the higher flows experienced this past summer. Periods of low flow are more conducive to the accumulation of vegetation mats.

The vegetation mats showed substantial gains in total areal mass during the year. At the beginning of the sampling period (May) the mats were visually thinner than they were later in the year. These early season mats had total dry weights of < 400 g m^{-2} while later in the season the mats were much thicker and heavier (>1000 g m^{-2}).

We report here the first estimates of vegetation mat composition in the Comal system. The mats are composed of decaying macrophyte and terrestrial debris and (apparently) viable algae and bryophytes. During 2015 algae and bryophytes composed more than half the mat biomass in all but one mat sampled (mat 4, July 2015) when a very large amount of terrestrial biomass was present.

The BOD assays provide evidence that the bioavailable organic matter can be rapidly decomposed. The potential oxygen demand reported ranged from 0.28 to 0.83 g O_2 g^{-1} dry mass and appear quite narrowly constrained. The decay constants reported ranged from 0.02-0.06 d^{-1}, with equivalent half-lives of 35-12 days. While the BOD of terrestrial debris was measured only once, it showed among the lowest BOD_{ult} and *k* values. These lower values likely reflect less bioavailable organic matter per unit total biomass as well as tissues that are more difficult to decompose. The BOD parameters for algae, bryophytes and macrophytes were all similar and higher than that of terrestrial debris.

Previous studies have examined phosphorous (P) dynamics in shallow lakes, and Wang et al (2008) determined that various forms of P, including soluble reactive phosphorous (SRP), are released from lake sediments when DO concentrations fall below 1mgL^{-1}. Our water quality analyses have indicated that the system is phosphorous-limited, and future assessment of the soil nutrient profile would give us a better idea of what would happen under low-flow-induced hypoxia.

Aquatic vegetation governs the concentrations of dissolved gasses within their environment. Submerged vegetation utilizes dissolved inorganic carbon (DIC) and enriches the water with the oxygen produced as a byproduct of the photosynthetic process. Emergent or floating vegetation, however, has a physiological advantage in its capacity for atmospheric gas exchange. This atmospheric exchange often results in the depletion of oxygen within the surrounding aquatic environment as respiratory tissues are submerged and photosynthetically generated oxygen is released into the air rather than water (Caraco et al, 2006). The majority of the aquatic vegetation within Landa Lake is submerged and contributes to the stable daily DO profiles observed.

Due to the rare nature of this type of ecosystem, literature regarding the effects of floating mats of vegetation within a spring-fed shallow lake is scarce. However, we can glean insight from an assortment of studies that have investigated particular aspects and impacts of vegetation mats in other systems. The literature mirrors our observations in that it is difficult to draw definitive conclusions about the potential harm vegetation mats might pose.

It seems intuitive that the accumulation of floating vegetation constitutes a direct threat as they modify local conditions for the species around which they congregate and reduce light availability to submerged plants. One local experiment found that mats composed of drifting vegetation that became entangled and accumulated around artificial obstructions had negative

effects on various growth parameters of endangered *Zizania texana*, including obvious mechanical damage to plant tissues (Power, 1996). The consequences of shading as well as diel DO and pH changes can be more pronounced where mat canopies are dense and broad (Frodge et al, 1990; Caraco et al, 2006). Mats composed of bryophyte and algal material might indirectly affect the food web as they attract invertebrates to the surface and away from benthic habitats (Power, 1990). Observational studies of floating islands of vegetation in Florida lakes found that mats collect sediment and develop more complex root systems over time, which could permanently alter the areas in which they form (Mallison et al, 2001).

Although they are seen as having mostly negative impacts, ephemeral mats composed of an amalgam of species and vegetation types might benefit the system in a number of ways. First, they might have protective and regenerative properties for the associated plants and seeds under fluctuating hydrological conditions, lending to the propagation and diversity of plant assemblages within the system (Somodi and Zoltan, 2002; Cherry and Gough, 2005). Additionally, extensive shallow vegetation mats might insulate the underlying water column and prevent wind-induced turbidity during times of reduced springflow (Stronsnider and Nairn, 2010).

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CHAPTER 5

PREDICTING THE IMPACTS OF VEGETATION MATS ON THE BIOLOGICAL OXYGEN DEMAND IN LANDA LAKE

Materials and Methods

Two factors in Landa Lake are being investigated with this work: low dissolved oxygen (DO) concentration and floating vegetation mats. In the summers of 2013 and 2014 when total spring flow was much lower than average, DO in Landa Lake exhibited a diel trend reflective of algal activity in the water column: high DO during the day along with algal photosynthesis and low DO before dawn following algal respiration at night (Figure 5.1). While the exact influence of floating vegetation mats on lake conditions has not yet been quantified, a program has begun to periodically remove the floating mats from the lake to potentially improve the DO conditions as well as promote recreation and aesthetic value. For this study we aimed to address the relative influence of the floating vegetation mats on DO processes in the lake in order to provide input on how best to manage vegetation mats or if control and removal is deemed necessary.

Among many localized physical influences, the main factors affecting DO levels in a lake environment are temperature, water movements, atmospheric reaeration and internal biological processes (like algal photosynthesis and respiration) (Thomann and Mueller 1987, Caraco et al. 2006). The following are general considerations for each main factor:

- Temperature
  - Saturation concentration of DO
  - Kinetic rates of change
- Water movements
  - Inflows
  - Outflows
  - Circulation (internal mixing of lake waters, vertically and horizontally)
- Reaeration from atmospheric exchange
  - Wind patterns
  - Surface area
- Internal sources or sinks
  - Benthic (Sediment Oxygen Demand)
  - Water column (Biological Oxygen Demand)
    - Aquatic plants (macrophytes)
    - Algae
    - Aquatic organisms

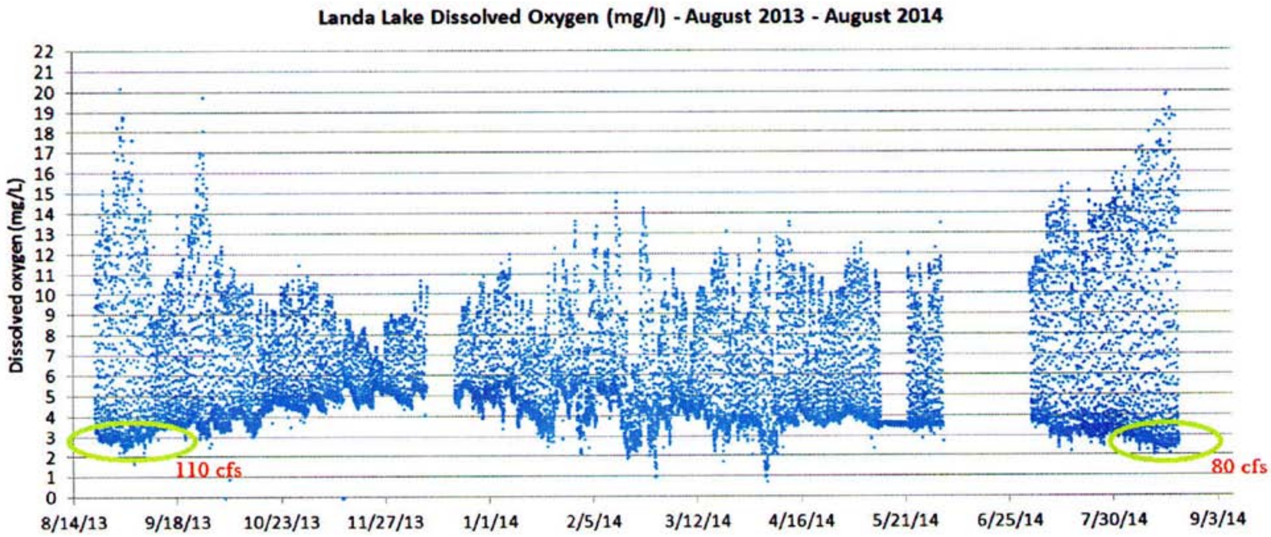

Figure 5.1 Dissolved oxygen time history in Landa Lake, 2013-2014 (SWCA 2013, 2014).

In many lake systems, the internal biological sources and sinks can at times impact DO levels more than water movements and reaeration. In Landa Lake this dominance of internal sources and sinks has been documented during periods where diurnal DO measurements exhibit patterns of increased DO during the day (macrophyte and algae photosynthesis with DO production) and decreased DO at the end of a night (algae respiration with DO consumption) (Figure 5.1). In Landa Lake the cycle is exacerbated since waters originating from an underground source are typically low in dissolved oxygen as a result of atmospheric isolation.

The impact of aquatic plants on DO is very complex. Beyond interaction of all of the broad category factors noted above, the growth, death and decomposition of aquatic algae and plants varies widely by species and depends upon light, nutrient levels (primarily phosphorus and nitrogen), and by access to a nutrient source (sediments or water column) (Thomann and Mueller 1987). Many simple modeling studies consider water movements and reaeration, and then approximate all of these internal factors by lumping them into a two parameters, biochemical oxygen demand (BOD) and sediment oxygen demand (SOD). Other studies go a little farther and augment BOD with additional time-varying parameters including light, nitrogen and phosphorus levels. The most complex studies may replace BOD entirely by attempting to track DO dynamics across multiple species of algae and macrophytes according to time-varying levels of light, ammonia, nitrates, organic nitrogen, ortho-phosphate, growth rates, decay rates and other parameters (Cole and Wells 2011).

As a first conceptual attempt to characterize the DO processes that most influence Landa Lake, this project takes a simple modeling approach: considering water movements, reaeration, SOD, BOD, algae and macrophyte presence. The measured BOD of the algal mats ("BOD_mat") is considered separately from typical instream BOD concentrations arising from storm runoff events ("BOD_runoff").

Our approach is to use time-varying DO data provided by the initial DO measurements collected using MiniDOTs reported in Chapter 4 in Landa Lake to investigate how each of the factors contributes to either an increase or decrease in DO. The following assumptions apply to this analysis:

- The lake is fully mixed and homogeneous
- No settling
- Simple un-calibrated kinetics using literature or regional default rates and coefficients
- Lake water is 25°C
- DO saturation concentration is 10.75 mg/L at 25°C
- Initial condition DO is 3 mg/L at 6am
- Predicted DO condition is at 6am on following day (last column, Table 5.1)
- Algal mats reduce atmospheric reaeration proportional to surface coverage
- Algal mat area is the maximum measured September 23, 2014 (Figure 5.2)
- Algal mat composition is maximum measured in 2015 (719 gO2/m2)
- Algal mat rate of consumption of DO is as measured in 2015 (0.05/day)
- Algal mat is composed of completely dead material contributing to BOD (this a is conservative assumption, considering approximately 20% of the mat was observed to be dead and the remaining 80% live algae)
- Algal mat "availability" is estimated based upon proportion of surface coverage (14%); mat material is not evenly distributed throughout entire volume but is only locally available at the mat for decay and oxygen consumption
- For Algae scenarios, water column algae chlorophyll-*a* concentration is at TCEQ screening level, 26.7 ug/L
- For Macrophyte scenarios, macrophyte chlorophyll-*a* concentration is twice the algae concentration (there is no basis for this broad assumption that likely underestimates macrophyte amounts)
- Lake surface area analyzed is the area of interest in Figure 5.3 (27,278 m2)

Table 5.1 Dissolved oxygen investigation scenarios.

Scenario	DO	Q	Wind	SOD	BOD_runoff	Algae_chla	Macro_chla	BOD_mat			DO
	initial							Area	Density	Availability	pred; 6am
	mg/L	cfs	mph	gO2/m2	mg/L	ug/L	ug/L	m2	g/m2	fraction	mg/L
1	3	212	-	0.6	-	-	-	-	-	-	5.67
2	3	212	-	0.6	5	-	-	-	-	-	5.2
3	3	212	-	0.6	-	-	-	3877	719	1	1.94
4	3	212	-	0.6	-	-	-	3877	719	0.14	4.83
5	3	212	-	0.6	-	26.7	-	3877	719	0.14	6.32
6	3	212	-	0.6	-	26.7	53.4	3877	719	0.14	3.97
7	3	80	-	0.6	-	26.7	-	3877	719	0.14	5.52
8	3	80	-	0.6	-	26.7	53.4	3877	719	0.14	3.01
9	3	30	-	0.6	-	26.7	-	3877	719	0.14	4.95
10	3	30	2	0.6	-	26.7	-	3877	719	0.14	5.55
11	3	30	2	0.6	-	26.7	53.4	3877	719	0.14	3.05
12	3	30	2	0.6	5	26.7	53.4	3877	719	0.14	2.53
13	3	30	2	0.6	-	26.7	53.4	9692	288	0.35	2.23

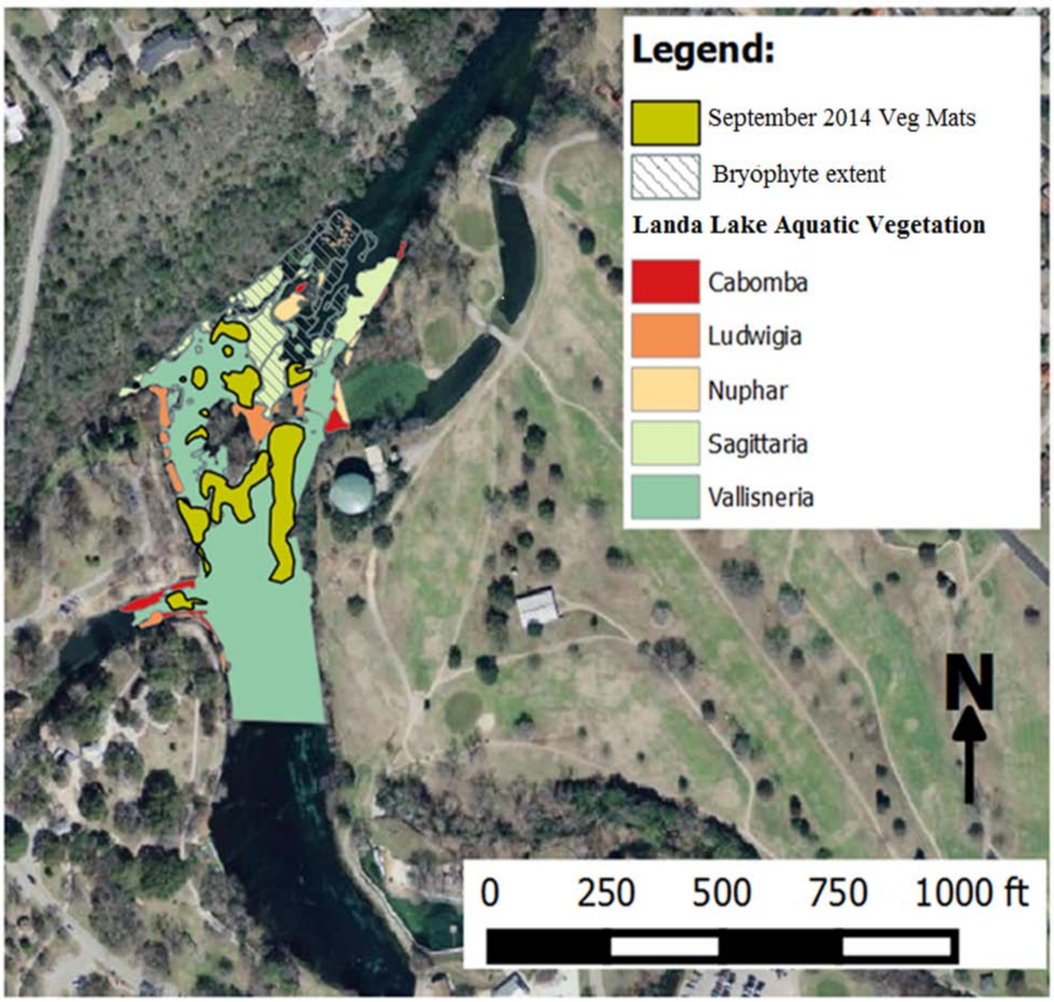

Figure 5.2 Landa Lake study area with vegetation mapping and maximum algal mat surface area in September 2014.

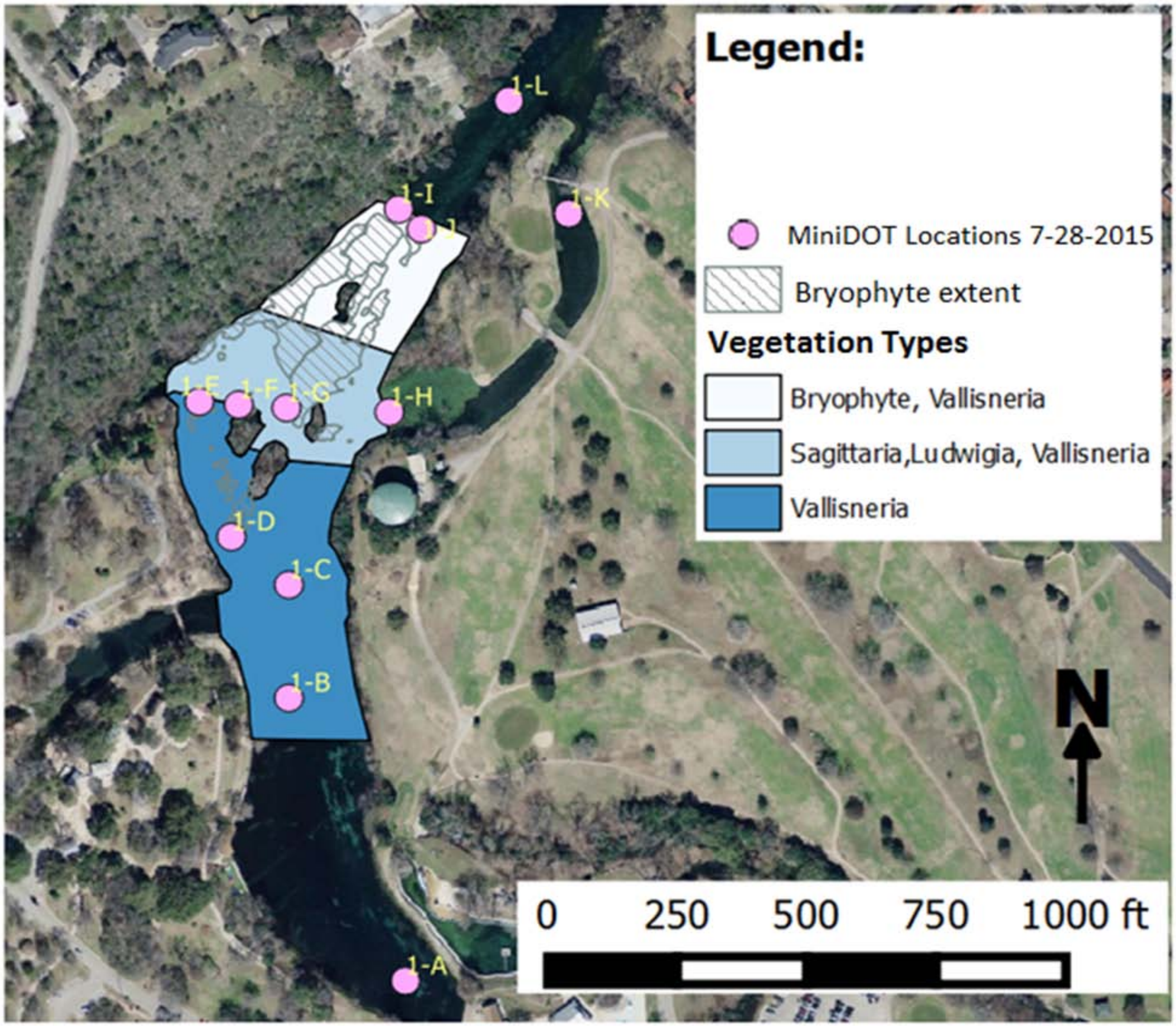

Figure 5.3 Landa Lake study area with generalized vegetation zones, initial MiniDOT locations and bryophyte areas.

Results

Scenarios investigated as part of this project are presented in Table 5.1. Three general flow scenarios are presented: 212 cfs (representing summer 2015 conditions), 80 cfs (summer 2014) and 30 cfs (minimum spring flow). Multiple sub-scenarios are presented for each flow scenario to investigate components having greatest influence on DO.

Scenario 1 represents a very simple condition where DO begins at 3 mg/L and is influenced only by atmospheric reaeration resulting from mixing caused by flow-induced water velocity. After 24 hours the DO concentration rises to 5.7 mg/L. After about 10 days the DO concentration

approaches saturation concentration (Figure 5.4); the hydraulic reaeration exhibits the largest magnitude influence on DO until approximately day 10 when the increase resulting from reaeration is offset by the decrease from the SOD. Scenario 2 includes BOD from, for example, a storm runoff event; the DO concentration after 24 hours is lower (5.2 mg/L) indicating that the runoff BOD exhibits influence, but the reaeration remains a greater influence since DO rises from initial condition of 3 mg/L.

Scenario 3 illustrates that the decaying algal mats have greater initial negative impact on DO than the positive atmospheric reaeration (Figure 5.5). This scenario, however, assumes that the entire mat is evenly distributed throughout the entire water body. A more reasonable assumption is that the mats remain aggregated and decompose locally at the mat location; Scenario 4 assumes that only the portion of the water body in direct contact with the mat (in this case, 14% surface area coverage) exerts influence on DO concentration. With this assumption, the relative influence of the decomposition of mats is small compared to reaeration (Figure 5.6).

The potential influence of water column algae and aquatic macrophytes was evaluated in Scenarios 5 and 6. As a starting place for this investigation, the TCEQ screening level for chlorophyll-*a* was used for the water column algae concentration. The chlorophyll-*a* level for macrophytes was assumed to be double the algae concentration. Both of these assumptions are in need of further data gathering to verify and determine more appropriate scenarios and to calibrate appropriate photosynthesis and respiration rates. The results indicate that algae and macrophytes can have a much more significant influence on DO than the decay of algal mats (Figure 5.7).

Scenario 7 and 8 evaluate the same inputs for a lower flow rate, 80 cfs (Figure 5.7). DO concentration after 24 hours (3.0 mg/L) is comparable to that measured during summer of 2014 (Figure 5.1).

Scenarios 9 through 13 evaluate 30 cfs, the minimum springflow contemplated for the Comal Springs system. At this low flow rate, hydraulic reaeration is negligible and wind-driven reaeration (Scenario 11, Figure 5.8) is not as great as the hydraulic reaeration from high flow conditions. As a result, the DO is lower after 24 hours (3.1 mg/L) compared to conditions at 80 cfs. Scenario 12 investigates the possibility of a small storm runoff event contributing BOD to the system at 30 cfs; the DO after 24 hours is lower, approximately 2.5 mg/L (Table 5.1).

Scenario 13 investigates the same total biomass as previous scenarios, but the algae mat is stretched to an increased surface area (double the size, 28% coverage). The mat density of 288 gO2/m2 is well within the range of measured mat density (minimum measured 104 gO2/m2). At this area of coverage, the combined influence to reduce surface reaeration and to increase contact of decaying mat across the lake area begins to cause a detrimental effect (Figure 5.9).

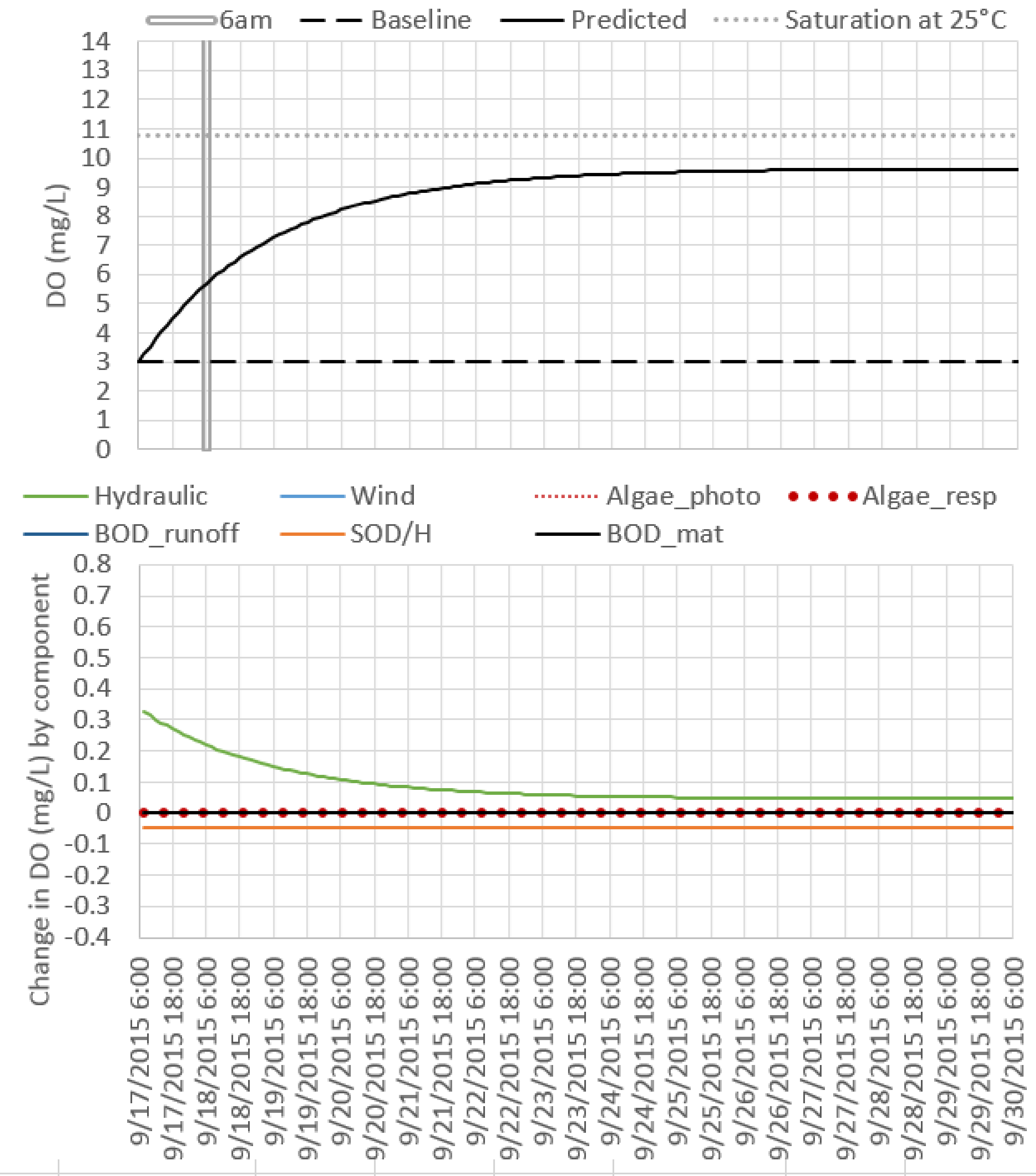

Figure 5.4 Scenario 1 – 212cfs with hydraulic reaeration and SOD only.

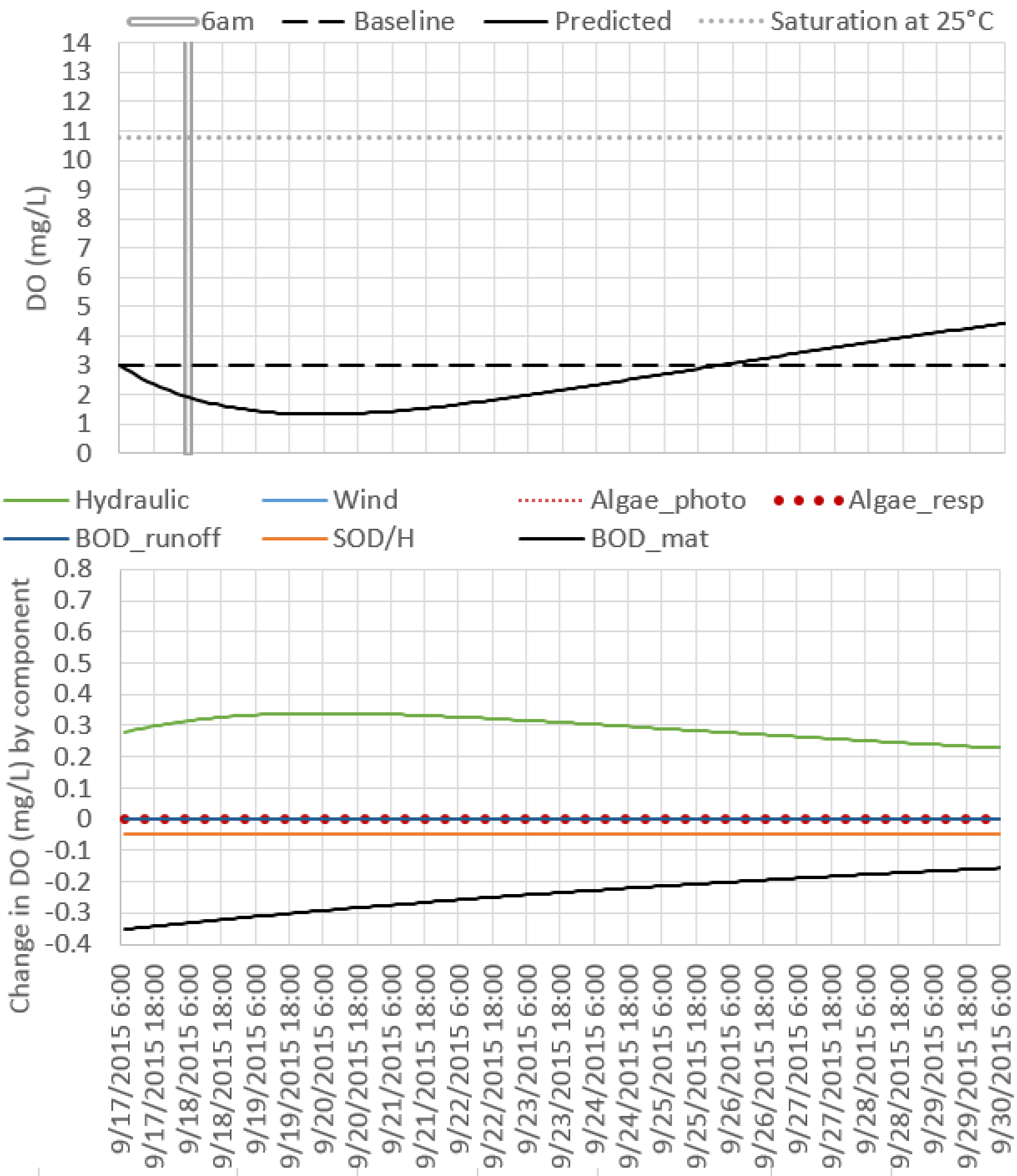

Figure 5.5 Scenario 3 - 212cfs with hydraulic reaeration, SOD and 100% of BOD_mat.

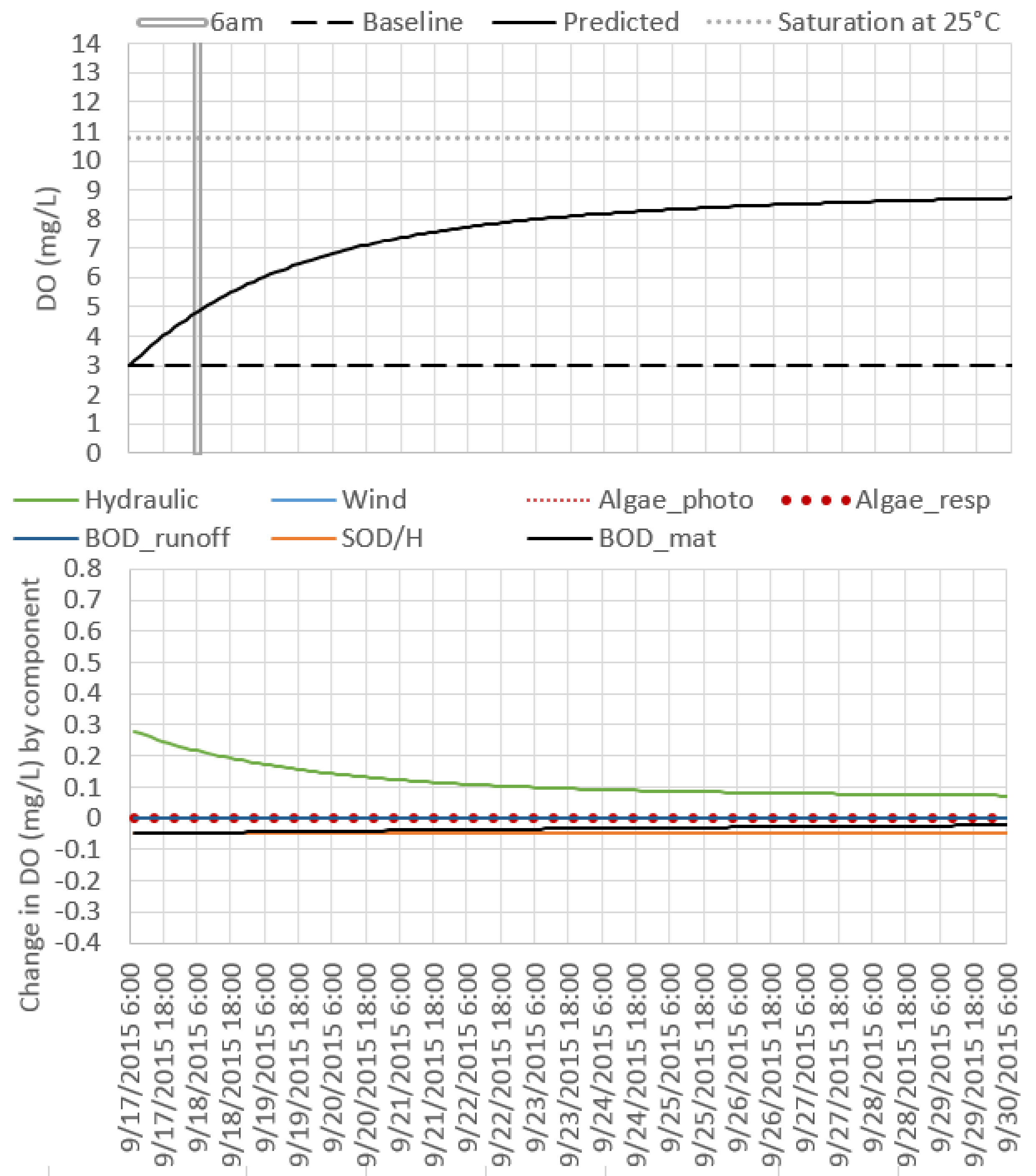

Figure 5.6 Scenario 4 – 212cfs with hydraulic reaeration, SOD and 14% of BOD_mat.

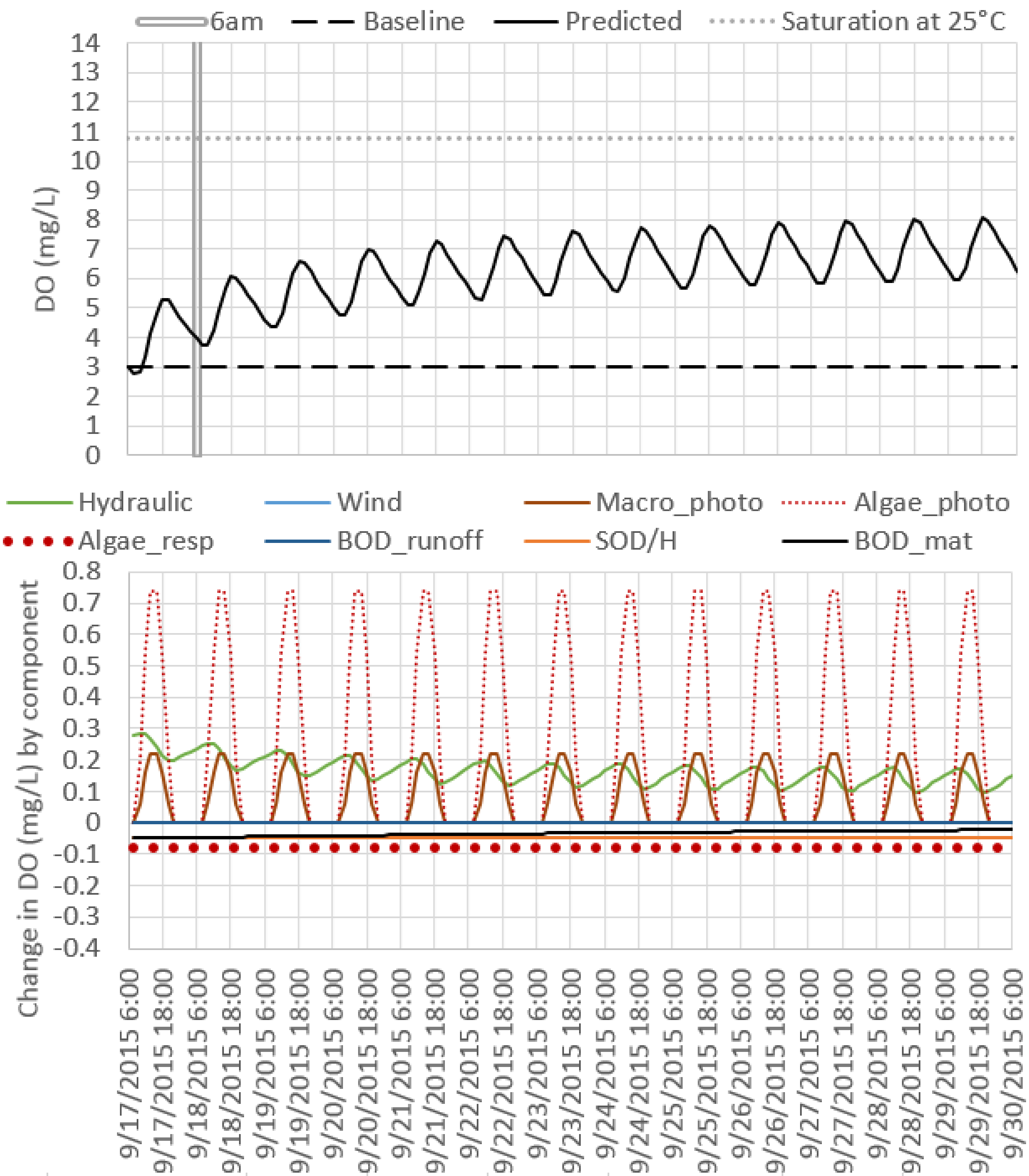

Figure 5.7 Scenario 6 - 212cfs with hydraulic reaeration, SOD, 14% of BOD_mat and assumed algae and macrophytes.

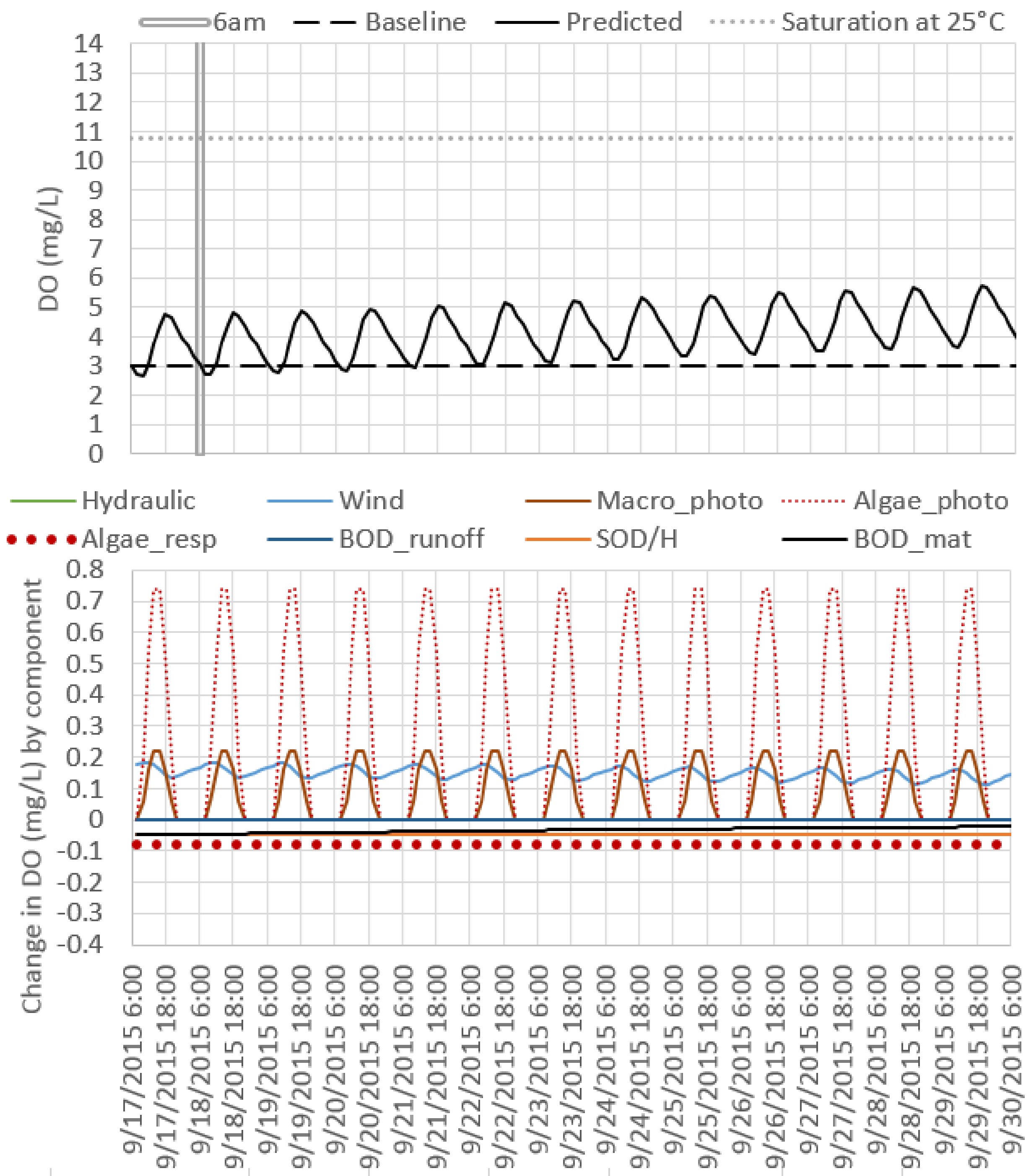

Figure 5.8 Scenario 11 - 30cfs with wind reaeration, SOD, 14% of BOD_mat and assumed algae and macrophytes.

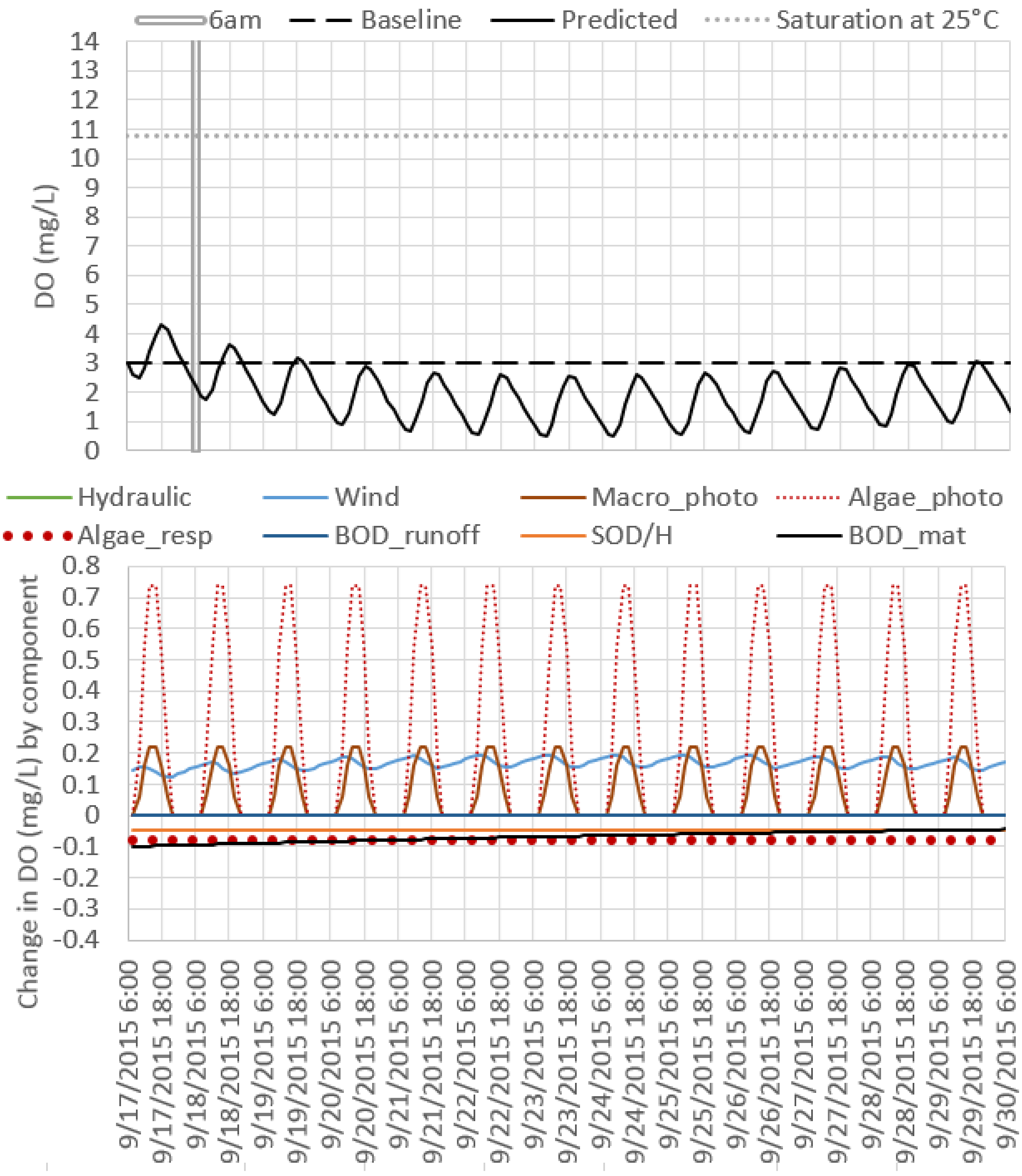

Figure 5.9 Scenario 13 - 30cfs with wind reaeration, SOD, 28% of BOD_mat (expanded area) and assumed algae and macrophytes.

Discussion

While the floating vegetation mats have been shown to have an influence on the DO dynamics within Landa Lake, the results of this very preliminary analysis indicate that the vegetation mats, at surface area coverage levels measured as part of this study, exhibit less influence to reduce DO than atmospheric reaeration influences increases in DO. As noted during sampling of vegetation mats, very little material is dead or decomposing within mats. Additionally, if assumed values are reasonable, algae mats exhibit much less influence than sediment oxygen demand, algae and macrophytes. Because of the limited influence, removal of algal mats at currently observed levels from the system is not anticipated to have significant positive influence on lake DO conditions.

However, if algal mat coverage exceeds 25% of the study area (the middle portion of the lake shown in Figure 5.3), which may occur during extended periods of extremely low springflow, this preliminary analysis suggests that removal of the mats would provide improvement to DO conditions.

This simple conceptual analysis predicts DO concentrations within a range comparable to observed DO concentrations. However, comparison of these preliminary uncalibrated model predictions to observations make clear the model is not yet accounting for the magnitude of DO production and consumption evident in observation data (assuming observation data is accurate and not significantly affected by biofouling). Additional complexity in the processes are evident, and both the kinetic rates and concentration scenarios need to be fine-tuned. The next phase of this work should focus on gathering field data supportive of calibrating a model:

- water-column algal concentrations including chlorophyll-*a* concentrations
- macrophyte biomass, spatial variability of biomass density
- water column nutrient concentrations
- spatial variability of sediment oxygen demand (considering dense macrophyte coverage in some areas and bare gravel in other areas).

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CHAPTER 6

SUMMARY CONCLUSIONS AND FUTURE STUDY RECOMMENDATIONS

This report deals with two well-known but poorly understood phenomena that occur each year on the Comal River, especially in Landa Lake and USR. These two phenomena are the development of benthic algae turf mats (benthic mats) and the development of floating vegetation mats (vegetation mats). While both have been thought to pose potential problems to endangered species, including the fountain darter, little quantitative information exists about either.

The development of benthic mats and their impacts on submersed vegetation is relatively well-studied in sea grass ecosystems, but appears extremely limited within the freshwater literature. The periodic presence of thick and widespread benthic mats of algae is a well know phenomena in the Landa Lake and USR reaches of the Comal River. Likewise, the development and persistence of floating vegetation mats have often been observed in Landa Lake. Several studies were conducted during 2015 to provide information related to the benthic algae mats and floating vegetation mats and their potential impacts on the ecosystem and we provide some conclusions and suggestions regarding future studies below. Originally, these studies were planned to occur during a low flow year as was predicted for 2015. However, record amounts of rainfall during late May and early June quickly changed the hydrological outlook for the year. With that sudden change, these studies morphed from attempting to characterize low-flow conditions to documenting baseline conditions on algae and dissolved oxygen patterns under average to above average springflow. While it was not our anticipation for this to be a baseline study, the information remains quite valuable. To fully understand the impacts and stress that drought places on a system such as the Comal River it is our hope that certain components of this study would be repeated when drought conditions return.

<u>Chapter 1</u> reports the collection and identification of the multiple genera of algae present in the Comal River. This is not an exhaustive list and additional information would be useful to better understand which algae species and varieties are present spatially throughout the Comal system. Furthermore a greater understanding of the habitat niches required by each algae type could provide some insight as to which algae genera may come to dominate the algal community when river conditions move into severe drought. *Spirogyra* dominated in 2015 while *Cladophora* or another type may dominate under different conditions.

Chapter 2 reports efforts to evaluate the spatial and temporal distribution of the benthic turf algae, composed of *Spirogyra*, as well as measurements of available nitrogen and phosphorus that may contribute to the development of algae turf. Towards this end, periodic maps of the system were made and permanent transects established to document the spatial/temporal variability of the mats. During 2015 the benthic mats were relatively poorly developed relative to previous years. The more limited development of benthic mats is hypothesized to be due to the relatively high flows which persisted throughout the 2015 year relative to the extremely low flows present in 2014 when algae dominated the USR. Literature suggests that *Spirogyra* can tolerate stream like conditions but its growth form and biomass allocation under such conditions is different compared to stagnant or low flow conditions.

The water quality monitoring showed very high levels of dissolved forms of nitrogen, especially nitrate (NO3-N). NO3-N is very high in the upwelling spring flows with concentrations typically above 1.5 mg/L (PPM). Ammonia nitrogen (NH4-N), while almost always measurable, was much lower and in the 5-20 ug/L (PPB) range. Dissolved phosphate (PO4-P) was always low and near the detection limits of 5 ug/L (PPB). These data show a ratio of available N:P that is very high, averaging 221. This indicates the available phosphorus is the limiting element for algae growth in the Comal system. Balanced growth (assuming common tissue levels of N and P) would suggest that the algae need N and P in a (mass) ratio of about 7N:1P (equivalent to about 15N:1P on a molar basis). N:P (mass) ratios greater than 10 are indicative of P-limitation. Hence we conclude that the benthic algae mats in Landa Lake and the USR are most likely P-limited.

One concerning observation from the 2015 monitoring effort is that benthic algae and benthic bryophyte mats rarely co-occur. Perhaps the development of benthic algae mats shades the benthic bryophytes which seemed to be the case in 2014. Future research regarding the ecology of *Spirogyra* would be beneficial to determine or predict how this algae would respond to changes in situ. Nutrient enrichment studies would conclusively demonstrate P- limitation and artificial substrate growth studies could help determine growth rate of *Spirogyra* which was seemingly fickle during 2015. Additionally, continued regular mapping of algae turf would provide the ability to document how algae turf changes over longer periods of time. In the future distinguishing between *Spirogyra* algae and *Cladophora* algae instead of lumping algae into one "filamentous algae" category would provide a beneficial data set towards any further HCP or biomonitoring measures especially for fountain darter sampling. Although not discussed in depth in Chapter 2 *Chara* has become a dominant component in the USR over the last two years. This macroalgae is mapped as part of the annual biomonitoring regime but the cause of its sudden resurgence in the USR and how it might compete with other vegetation types in the USR is worthy of additional study.

Chapter 3 reports efforts to conduct an experimental test of the impacts of benthic algae mats on Ludwigia and bryophytes. Originally we proposed to position actively growing pots of Ludwigia and racks of bryophytes in areas with and without benthic algal mats. However, due to the poor

development of algae mats during 2015 this approach was not possible. Instead we attempted to create a single "dense algae" enclosure and compare growth of Ludwigia and bryophytes in that enclosure to that of replicates growing in an adjacent plot free of benthic algae mats. We used both established and new-growth (viable but not pre-established) cultures of both Ludwigia and mixed bryophytes.

The +Algae enclosure resulted in a modest benthic algae mat for a substantial portion of the summer. Despite not having a thick mat, we observed negative impacts of the algae turf on Ludwigia and bryophytes. Established Ludwigia and Established bryophytes declined in +Algae treatment, but maintained original biomass in the reference area. New-growth bryophytes declined significantly more in +Algae treatment than the reference treatment. It is unclear why the new-growth bryophytes also declined in the reference site. New-growth Ludwigia showed no difference between the +Algae and reference treatments.

For future research we propose repeating this experiment when and if conditions become favorable for more expansive and persistent algae turf mats to develop. It is unknown how well other macrophyte species compete with algae turf for light and nutrients. Vegetation mapping shows no discernible impacts of algae turf upon Sagittaria but this has not been investigated on a smaller scale. A better understanding of how algae turf impacts bryophyte growth would be beneficial since fountain darters rely heavily on bryophytes as habitat. Little is known regarding bryophyte ecology, growth rate and nutrient requirements in general.

Chapter 4 reports several studies that sought to provide better understanding of DO dynamics and the floating vegetation mats that develop in the Landa Lake and USR region of the Comal River. Deployment of DO sensors throughout the Landa Lake and USR showed little cause for concern related to low DO under the 2015 flow conditions except for low-flow, stagnant portions of the system. Three more stagnant locations showed a pattern of low overnight DO measurements. While these low-flow locations covered relatively little of the system in 2015, they might represent a much larger portion of the available habitat under low-flow conditions.

Comparison of DO at the benthic surface versus the top of the water column show DO is more variable at the sediment surface than at the top of the water column. However, there was no evidence that DO above the benthic vegetation drops to extremely hypoxic or anoxic levels to threaten fountain darters. A few bottom sensors record very low DO measurements, but these were *within* benthic bryophyte mats which fountain darters likely could avoid.

Surprisingly, the DO dynamics do not appear to be strongly impacted by vegetation mats. Although DO is less variable under the mats than immediately outside the mats, there was no evidence of severe DO depletion beneath the mats. However, the lack of impact may be directly related to the modest mat formation in 2015. Additionally, good flow under the 2015 mats allowed the water column to mix thoroughly. During a low-flow year with more extensive mats,

the results might be different. Maximum areal cover of floating vegetation mats was lower in 2015 than in 2014 and mats did not persist as long in 2015 as they did in 2014.

The floating vegetation mats sampled in 2015 were composed mostly of bryophyte, macrophyte, and algae, with periodic inputs of terrestrial debris. The thickness and areal mass of the mats increased through the year. Early in the year the mats were relatively thin with dry mass of less than 0.4 kg m^{-2}. By the end of the summer the mats had increased in areal biomass by a factor of 3-4x with estimates of 1.0-1.5 kg dry mass per m^2 being commonly measured.

The floating vegetation mats pose a potential biological oxygen demand of up to 700 g O_2 m^{-2}. The organic material present showed relatively rapid decay rates. The observed decay rates ranged from 0.02 to 0.06 d^{-1} which are equivalent to half-lives of 12-35 days. That is, half of the bioavailable portion of these tissues decompose in 12-35 days. While mats may not pose a direct influence on the BOD in Landa Lake they can shade submerged plants reducing biomass and ultimately killing submersed aquatic plants. This was seen in 2014 when floating vegetation mats covered large areas of Ludwigia which had been planted as part of the HCP restoration effort.

Chapter 5 describes a conceptual analysis to determine the relative influence of the algal mats on overall DO conditions in the lake. The conceptual analysis incorporated the results of velocity measurements (Chapter 2), the algal mat mapping, DO conditions, and BOD data (Chapter 4) and literature values. The conceptual analysis shows that DO conditions in the lake are more greatly influenced by atmospheric reaeration, water column algal activity and macrophyte activity than by the decomposition of existing extents and composition of floating vegetation mats. However, if the vegetation mats expanded to cover more than 25% of the lake surface area, they could have a significant effect in reducing DO concentrations as a result of reduced surface reaeration. To better quantify the DO variability within the lake across a wider range of flow and nutrient inflow conditions, a calibrated model should be considered as a next step.

Finally, we recommend continuing the use of MiniDOT sampling especially when concerns arise regarding DO measurements at specific locals, such as the lake bottom or other micro habitats, or when drought conditions return. MiniDOTs have been shown to be extremely useful due to their portability and can be deployed quickly if necessary. It is also recommended that mapping of floating algae mats continue on a regular basis especially if low flows are anticipated to return. While algae and floating vegetation mats did not seem to pose major issues for Landa Lake in 2015 due to improved conditions, it is inevitable that the Comal River will return to drought conditions increasing the chances of damaging repercussions to the habitat quality from these phenomena. A comprehensive Landa Lake Management Plan including improved protocols and timelines for nutrient, algae and DO monitoring and management of floating vegetation mats would be a proactive approach to mitigate potential negative impacts that might occur during another sustained drought.

APPENDIX I

Figure I-1 March mapping of algae plots.

Figure I-2 June mapping of algae plots

Figure I-3 July mapping of algae plots

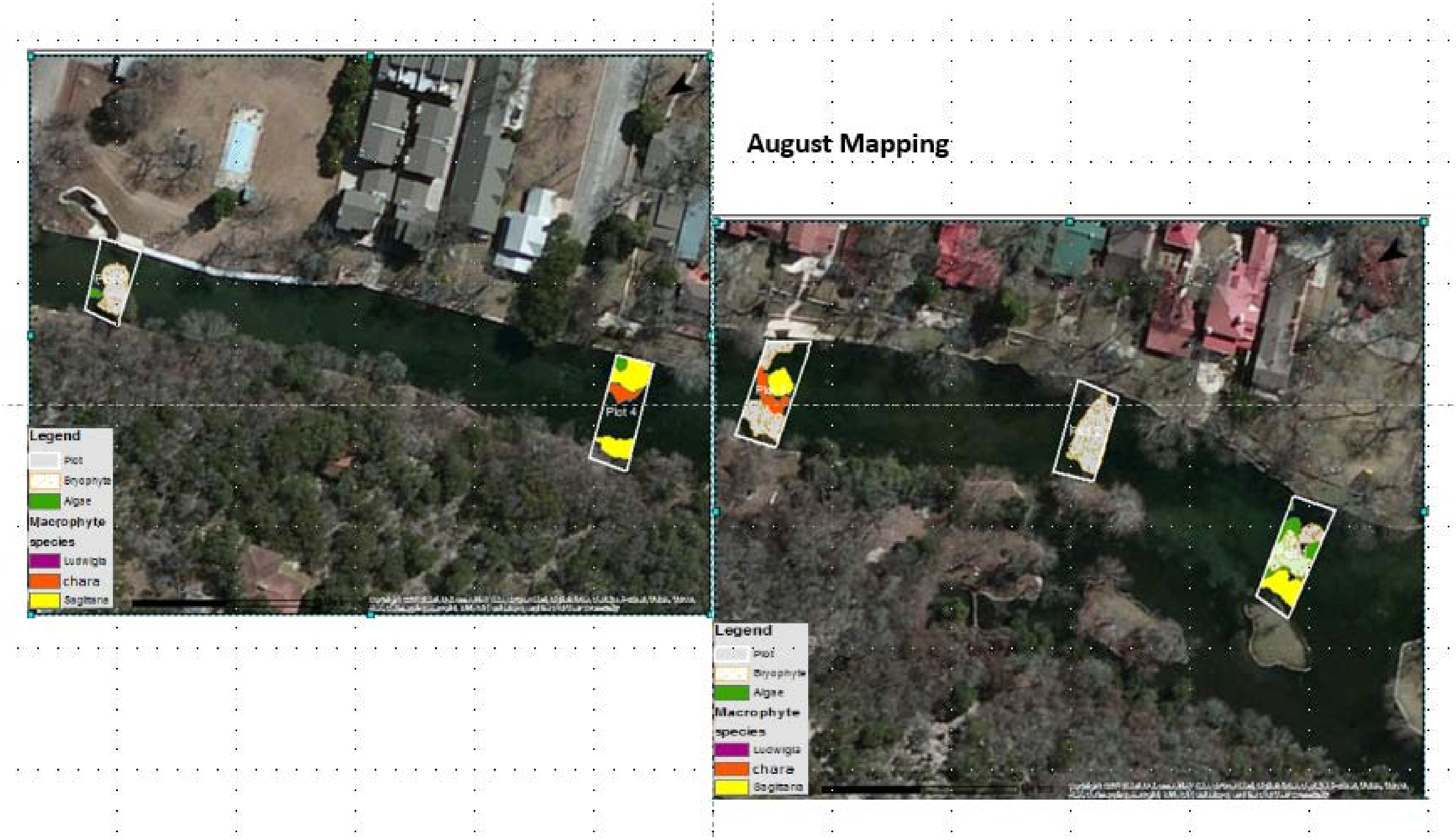

Figure I-4 August mapping of algae plots

APPENDIX II

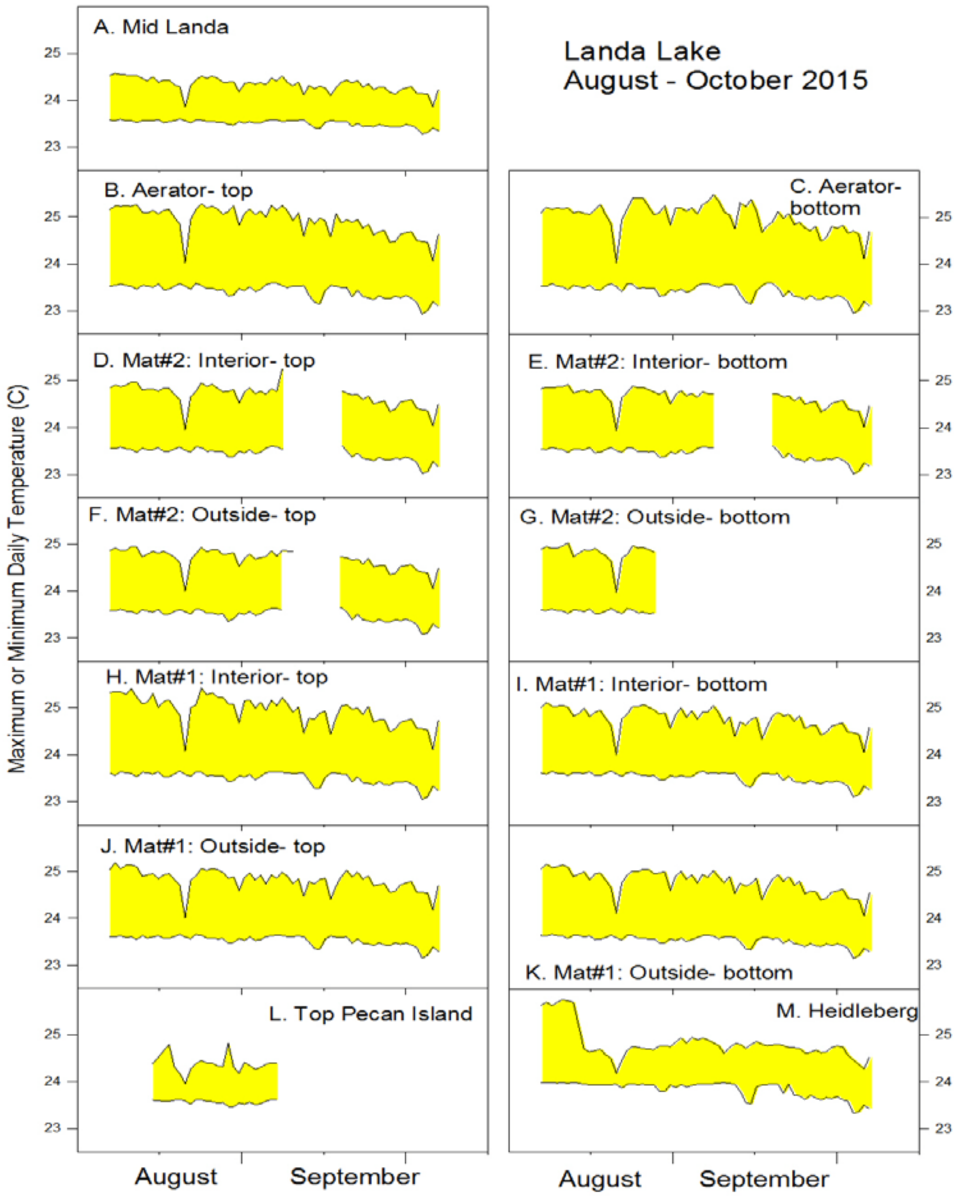

Figure II-1. Maximum or Minimum temperature results (°C) from MiniDOT sensors during deployment at the 13 stations.

Table II-1 Measured BOD_{ult} and k for each sample of each tissue type analyzed.

Date	Sample Bottle ID	k (day^{-1})	BOD_{ult} ($g\ O_2\ g^{-1}$)
May-15	1A_Bryo	0.032	0.73
	1B_Bryo	0.030	0.77
	1C_Bryo	0.031	0.58
	2A_Bryo	0.026	0.53
	2B_Bryo	0.025	0.75
	2C_Bryo	0.025	0.76
	1A_Algae	0.036	0.65
	1B_Algae	0.041	0.38
	1C_Algae	0.038	0.50
	2A_Algae	0.025	0.41
	2B_Algae	0.011	0.46
	2C_Algae	0.020	0.37
June-15	54: 1A_Macro	0.050	0.59
	55: 1B_Macro	0.053	0.60
	56: 1C_Macro	0.046	0.63
	77: 2A_Macro	0.046	0.69
	78: 2B_Macro	0.050	0.56
	79: 2C_Macro	0.038	0.67
	90: 1A_Bryo	0.062	0.55
	91: 1B_Bryo	0.058	0.56
	92: 1C_Bryo	0.050	0.68
	98: 2B_Bryo	0.054	0.61
	99: 2C_Bryo	0.052	0.66
	101: 1A_Algae	0.059	0.55
	102: 1B_Algae	0.054	0.46
	113: 2A_Algae	0.052	0.64
	114: 2B_Algae	0.053	0.52
	115: 2C_Algae	0.060	0.57
July-15	1A_Macro	0.044	0.64
	1B_Macro	0.028	0.53
	1C_Macro	0.037	0.59
	1D_Macro	0.040	0.48
	2A_Macro	0.047	0.52
	2B_Macro	0.049	0.54
	2C_Macro	0.049	0.53
	1A_Bryo	0.043	0.50
	1B_Bryo	0.036	0.51
	1C_Bryo	0.038	0.51
	1D_Bryo	0.028	0.41
	2A_Bryo	0.050	0.59
	2B_Bryo	0.060	0.53
	2C_Bryo	0.043	0.43
	1A_Algae	0.052	0.43
	1B_Algae	0.029	0.38
	1C_Algae	0.026	0.40
	1D_Algae	0.028	0.40
	2A_Algae	0.052	0.50
	2B_Algae	0.047	0.38
	2C_Algae	0.048	0.45
	1A_Terr	0.021	0.28
	1B_Terr	0.026	0.28
	2A_Terr	0.020	0.41
	2B_Terr	0.019	0.59
	2C_Terr	0.022	0.40

Date	Sample Bottle ID	k (day^{-1})	BOD_{ult} ($g\ O_2\ g^{-1}$)
August-15	1A_Macro	0.044	0.61
	1B_Macro	0.036	0.72
	1C_Macro	0.046	0.73
	1D_Macro	0.043	0.72
	2A_Macro	0.047	0.79
	2B_Macro	0.038	0.80
	2C_Macro	0.044	0.72
	2D_Macro	0.040	0.54
	1A_Bryo	0.050	0.59
	1B_Bryo	0.054	0.62
	1C_Bryo	0.046	0.50
	1D_Bryo	0.049	0.62
	2A_Bryo	0.046	0.65
	2B_Bryo	0.052	0.70
	2C_Bryo	0.051	0.51
	2D_Bryo	0.049	0.69
	1A_Algae	0.052	0.43
	1B_Algae	0.054	0.51
	1C_Algae	0.039	0.48
	1D_Algae	0.052	0.48
	2A_Algae	0.063	0.62
	2B_Algae	0.061	0.62
	2C_Algae	0.052	0.38
	2D_Algae	0.063	0.42
September-15	1A_Macro	0.019	0.83
	1B_Macro	0.021	0.70
	1C_Macro	0.023	0.69
	2A_Macro	0.048	0.54
	2B_Macro	0.038	0.56
	2C_Macro	0.046	0.52
	2D_Macro	0.050	0.58
	1A_Bryo	0.040	0.45
	1B_Bryo	0.035	0.47
	1C_Bryo	0.038	0.48
	2A_Bryo	0.048	0.66
	2B_Bryo	0.045	0.53
	2C_Bryo	0.050	0.60
	2D_Bryo	0.043	0.67
	1A_Algae	0.053	0.39
	1B_Algae	0.018	0.36
	1C_Algae	0.037	0.30
	2A_Algae	0.059	0.53
	2B_Algae	0.056	0.44
	2C_Algae	0.056	0.44
	2D_Algae	0.056	0.53

Figure II-2 Vegetation mats in Landa Lake often serve as foraging sights for birds and sunning sights for turtles.

Figure II-3 Very little algae turf was present in the +Algae plot at the time of final harvest.

Figure II-4 An example of all for vegetation types used as treatments in the Chapter 4 study. (Upper right) established-Ludwigia (Upper left) new growth-Ludwigia (Lower right) established-bryophyte and (Lower left) new growth-bryophyte.

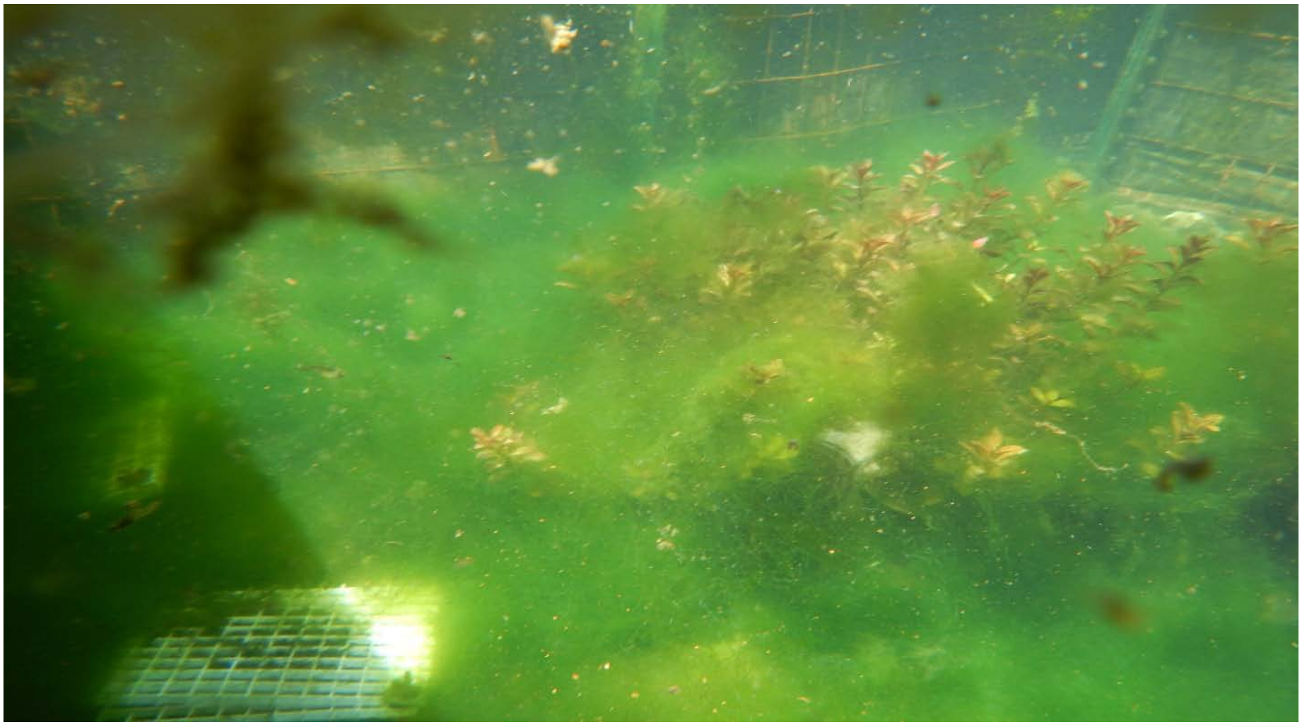

Figure II-5 Algae covering vegetation in the +Algae Plot.

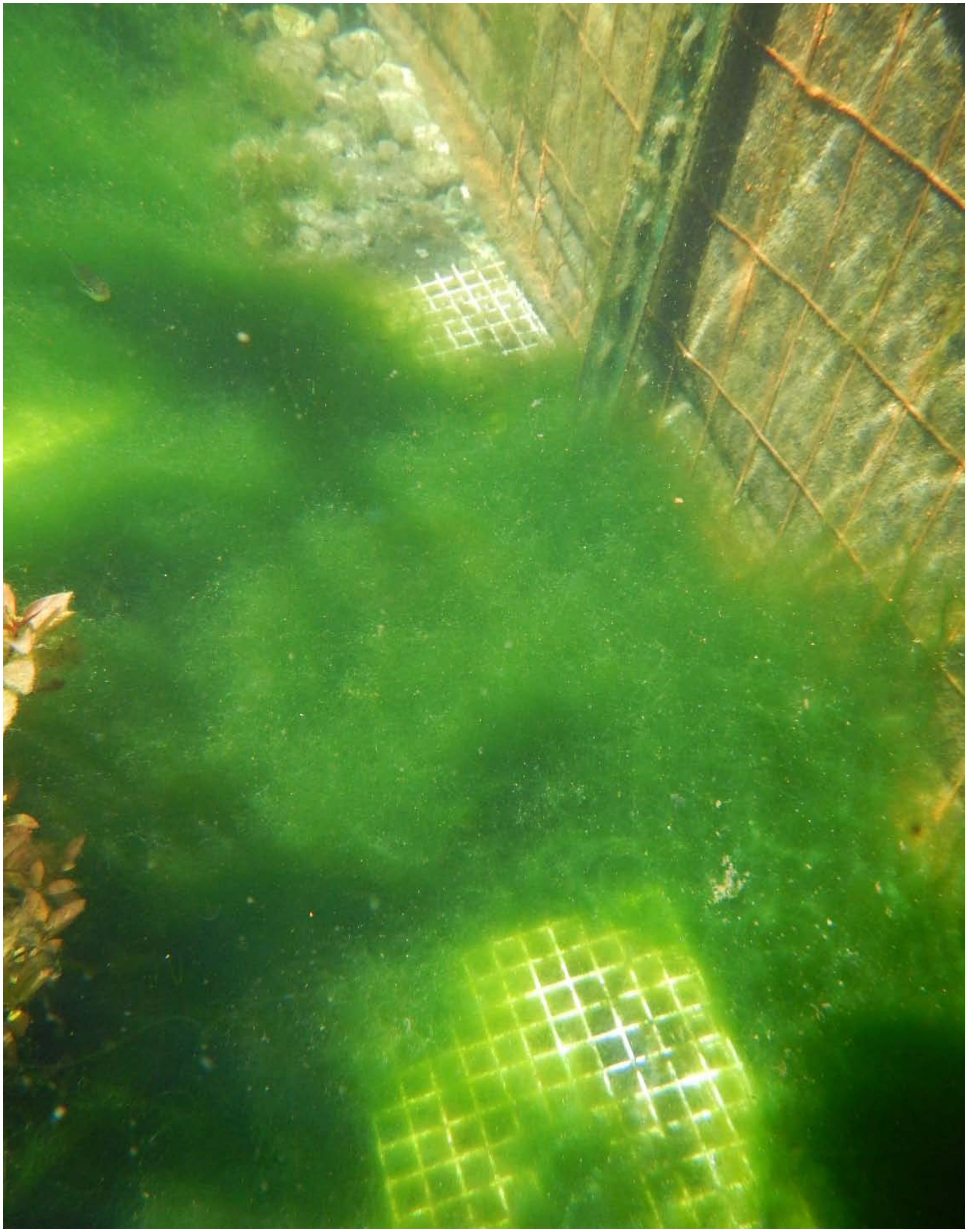

Figure II-6 Algae covering bryophyte cells in the +Algae Plot

Appendix K5

Refugia Research: Development of Husbandry and Captive Propagation Techniques for Invertebrates Covered Under the Edwards Aquifer Habitat Conservation Plan

REFUGIA RESEARCH: DEVELOPMENT OF HUSBANDRY AND CAPTIVE PROPAGATION TECHNIQUES FOR INVERTEBRATES COVERED UNDER THE EDWARDS AQUIFER HABITAT CONSERVATION PLAN

FINAL REPORT **February 17, 2016**

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EXECUTIVE SUMMARY

The primary objectives of this research were first to thoroughly review relevant literature, and secondly to assess and evaluate various methods and techniques that might successfully be used to study and establish refugium for the federally-listed invertebrates found in springs and wells of the Edwards Aquifer and in the Edwards Aquifer Habitat Conservation Plan. All experiments initially used surrogate species closely related to endangered species before actual testing on the endangered species. This minimized the chance of harm to the troglobitic amphipod *Stygobromus pecki* and the spring-adapted riffle beetle *Heterelmis comalensis* during testing.

Four sets of experiments were conducted. Each was designed to answer specific questions related to handling and detailed study, holding, basic reproductive traits, and culture of endangered organisms. All experiments were designed to address substantial gaps in current knowledge that might present obstacles to the successful holding and culture of refuge populations.

Anesthesia techniques that do not affect long- and short-term survival are required so that individual invertebrates can be identified, imaged, and studied at high magnification. This is essentially impossible while animals are moving. Our results showed that a dilute solution of ethanol (EtOH) was the most effective anesthetic and had almost no short- or long-term detrimental effects on any of the tested organisms. High concentrations of dissolved CO_2 were also effective for anesthesia of beetle larva, but logistics were more difficult that use of EtOH.

Responses of aquifer-adapted organisms to light exposure was evaluated to determine how it might alter behavior and/or metabolic rates (i.e., organism stress). Responses varied between five different amphipod species, but results indicate that successful culture of the amphipod *Stygobromus pecki* will probably require complete darkness and short observation periods under low-intensity long-wavelength (red) light in order to minimize changes to behavior, metabolic rates, and/or stress levels.

Amphipod reproduction experiments were performed on *Stygobromus pecki* to assess reproductive rates and sexual maturation rates. No changes in female reproductive development were observed during a two-month study period, other than release of young from a single female that was gravid at the beginning of the study. More work is needed to determine the time required for ovaries to develop and mature in female *Stygobromus* amphipods. This experiment did show that pairing equal- or larger-sized males with equal- or smaller-sized females resulted in the lowest incidence of cannibalism, and that pairing large females with smaller males should be avoided for this reason.

Experiments to assess the best methods for holding and growing groups or individuals of *Stygobromus pecki* proved that amphipods fed and grew measurably over a two-month period. Holding amphipods in groups was shown to be effective, provided there is habitat available for young and smaller amphipods to hide and avoid cannibalism.

1. INTRODUCTION

The Edwards Aquifer Habitat Conservation Plan (EAHCP) calls for the establishment of captive refuge populations for the species of concern associated with the Edwards Aquifer and the springs emerging from the aquifer. To develop successful captive propagation programs for the invertebrate species covered under the EAHCP, captive rearing, life history, and environmental requirements research needs to be conducted. Five aquatic invertebrates: the endangered Comal Springs riffle beetle (*Heterelmis comalensis*), Comal Springs dryopid beetle (*Stygoparnus comalensis*), and Peck's cave amphipod (*Stygobromus pecki*); and the petitioned Edwards Aquifer diving beetle (*Haideoporus texanus*) and Texas troglobitic water slater (*Lirceolus smithii*) are covered under the HCP. The San Marcos Aquatic Resources Center (SMARC) operated by the U.S. Fish and Wildlife Service maintains some of these species on site and has performed a variety of research projects on maintaining captive populations and propagating them for long-term refuge purposes. However, there are still several substantial questions and issues associated with many of these taxa which currently impede the ability of resource managers to maintain captive populations. For example, the USFWS can successfully hold Comal Springs riffle beetles and Peck's cave amphipods in captivity, but has experienced difficulties in refugium establishment with low numbers of beetles successfully pupating into adults and reduced survival of amphipods likely due to cannibalism. Additionally, very little is known about life history and the environmental requirements of any of the covered species.

In order to develop methods for captive propagation, four preliminary studies were executed during 2015. These studies looked at anesthesia, light response, mating behavior, and different holding systems for some of the protected species. The anesthesia of test organisms is considered crucial to be able to determine their sex, developmental stage, length, weight, and to individually mark or photograph organisms. Organisms' response to light could affect their stress level and health in captivity. Thus, understanding the mating behavior of Peck's cave amphipod and the role cannibalism plays in reproduction, if any, is essential to management of the refugia. The role of cannibalism and methods to limit it during all life stages also must be determined if populations are to be maintained in substantial numbers.

This document contains the results and methods we used to address the four main research projects: (1) methods for anesthesia for invertebrates held in captivity, (2) responses of epigean and/or hypogean invertebrates to light, (3) reproduction of amphipods (specifically the Peck's cave amphipod) held in captivity, and (4) methods for holding Peck's cave amphipods in captivity.

2. LITERATURE REVIEW

2.1. Anesthesia

Typically, invertebrate anesthetic agents rely on the respiratory system of organisms to gain entry into the nervous system of invertebrates where chemical disruption of synapses anesthetizes the organism (Cooper, 2011; Lewbart and Mosley, 2012). Amphipods and riffle beetle larvae respire through gills (Graham, 1990), thus water soluble chemicals are an effective

delivery method of anesthetics because of direct chemical exchange with water. However, adult riffle beetles respire through a plastron which indicates that aqueous solutes may not be an effective method for delivery of anesthetic to adult riffle beetles. This is because there is no direct chemical exchange with water but rather with the trapped gas bubble which is reliant upon atmospheric exchange of gasses with the surrounding water. Therefore, for adult riffle beetles, a gaseous anesthetic bubbled into solution is likely be a more effective method. Alternatively, use of a chemical anesthetic with a low vapor pressure, such as ethanol or ether, may be effective. Because water breathing organisms tend to have a larger exchange surface with water than air breathing organisms have with air (Graham, 1990), air breathing organisms may require higher concentrations of anesthetics because of smaller absorptive surfaces and therefore less chemical exchange with the environment when compared to water breathing organisms.

2.1.1. Specific information on effects of different anesthesia methods on Coleopterans

Very little work has been performed on the effects of different anesthesia agents and methods on aquatic beetles. However, a fair amount of work has been done on the anesthesia of terrestrial beetles, where CO_2 treatments are the widely utilized anesthetic of choice (Lewbart and Mosley, 2012). This may be an effective method for anesthesia of both adult and larval riffle beetles because CO_2 will be readily taken up by respiratory mechanisms, but CO_2 has been shown to cause permanent changes in behavior of anesthetized coleopterans (Lizé *et al.*, 2010) and affect survivability and fertility of other insects (Barron, 2000; Champion de Crespigny, F. E. and Wedell, 2008). Another method that has been used to anesthetize insects is cold induced anesthesia, but this has also been shown to also affect survivability (Barron, 2000; Champion de Crespigny and Wedell, 2008) and is likely inappropriate for spring- or aquifer-associated organisms which are likely to experience limited *in situ* environmental temperature variation and are thus likely adapted to these conditions (i.e., stenothermal). Volatile chemicals agents such as ethers or ethyl acetate have also been used for anesthesia and have been shown to have effects on survivability and behavior comparable to CO_2 (Loru *et al.*, 2010).

2.1.2. Specific information on different anesthesia methods on Amphipods

Both soluble chemical and gaseous chemicals bubbled into solution have been used as anesthetics for numerous species of aquatic crustaceans in a diversity of settings. The efficacy and survival rate varies appreciably between these two general anesthesia methods. For example, Cothran (2008) found that CO_2 treatments had 44% mortality when used on one species of *Hyalella* and almost complete mortality when used on another smaller species of *Hyalella*. In the same study, clove oil was used on the smaller *Hyalella* species and mortality was only 26% (Cothran, 2008); however, these mortality rates are likely much higher than what would be considered acceptable for refugium purposes. In contrast, Venarsky and Wilhelm (2006) found that that there was a positive relationship between CO_2 dose and mortality within different size classes of *Gammarus minus*. Cumulatively, these studies suggest that mortality associated with CO_2 anesthesia may be avoided at proper dosages.

Clove oil is a commonly used chemical anesthetic, but the duration of exposure to clove oil also had a positive relationship with increased mortality while time to anesthesia was shown to be negatively correlated with dose, and time to recovery was shown to be positively correlated with dose (Venarsky and Wilhelm, 2006). Ultimately, it appeared that a size appropriate dose and exposure duration could be elucidated that would effectively anesthetize the organisms while minimalizing mortality to an acceptably low rate (Venarsky and Wilhelm, 2006). However, no information has been collected on the effect of clove oil on the long term survival and fecundity of anesthetized organisms.

A more commonly used chemical anesthetic, which has also been tested on a diversity of vertebrates and invertebrates, is tricaine mesylate (a.k.a., tricaine methanesulfonate, TMS, and MS-222). MS-222 has been demonstrated to be effective at anesthetizing a diversity of invertebrates, including amphipods (Lewbart and Mosley, 2012), and if used properly can have low mortality rates (Ahmad, 1969). Like clove oil, dosage concentration is negatively correlated with time to anesthesia and positively correlated with time to recovery (Ahmad, 1969). Temperature was also shown to be negatively correlated with time to anesthesia (Ahmad, 1969), though as previously stated, we do not recommend that temperature be manipulated as it is unlikely that aquifer-adapted organisms can tolerate temperature fluctuations because they are likely to have evolved in physicochemically stable environments. The relationship between size of amphipod and concentration of dose has not been studied but it is likely that these two factors are positively correlated. Unfortunately, the long term effects of MS-222 on survivability and fecundity has not been studied.

Other anesthetics that have been used on crustaceans are dilute chlorobutanol and ethanol (EtOH) though these have not been tested on amphipods nor have their long term effects on survival or fecundity been studied (Ross *et al.*, 2009). However, the investigators in this project have previously used EtOH to short-term anesthetize hypogean amphipods and isopods from the Edwards Aquifer, but have not conducted a systematic study to determine tolerance limits and dosages.

2.2. Responses to light exposure by subterranean and subsurface invertebrates

Housing of organisms and observation of mating behaviors presents several substantial issues with regard to the conditions in which organisms are held and observed. In particular, subterranean organisms may have preferences or tolerances with regard to light levels (e.g., intensity) and the types of light (i.e., wavelengths) that are present. Though few studies have addressed light-dependent responses of stygobiontic invertebrates, a multitude of methods have been used that quantify responses of a diversity of organisms to light. In general, these responses are quantified based on the relative amount of movement in light versus dark areas, degree of avoidance of illuminated areas, and rate of respiration in light *versus* dark conditions. In most cases, it was determined that stygobiontic and troglodytic organisms have the ability to detect light (even eyeless forms) and put significant effort into avoiding light (Simcic and Brancelj, 2007; Borowsky, 2011). Considering that data indicate that many cave organisms tested to date

avoid light, it is likely that exposure to light may be stressful and disadvantageous to culturing organisms.

Information on the sensitivity of stygobiontic amphipods of the Edwards Aquifer to light has yet to be explored thoroughly and published. In contrast, subterranean amphipods from other geographic regions have been studied to a greater extent. The amphipod *Niphargus* is adapted to subterranean groundwater environments of Europe, is eyeless (and presumably blind), and has had aspects of their sensitivity to light studied including phototaxis (Borowsky 2011). Borowsky (2011) studied dorsal light reflex (a response of some organisms whereby they orient their dorsal surface towards the origin of light; Foxon, 1939) in *Niphargus* spp. and found no evidence that they exhibit this reflex. Borowsky (2011) also examined the relative amount of movement in illuminated and dark conditions and if *Niphargus* would move into areas protected from direct light and found that the intensity of the effect was positively related to the intensity of the light treatment. Simcic and Brancelj (2007) compared the sensitivity of subterranean aquatic amphipods and epigean aquatic amphipods, using *Niphargus stygius* and *Gammarus fossarum*, respectively. To compare the relative effect of light on these taxa, both *N. stygius* and *G. fossarum* were exposed to multiple intensities of light under identical conditions and the authors monitored the consumption of oxygen to infer rate of respiration (potentially indicating a stress response). *N. stygius*, had a significantly greater oxygen consumption rate when exposed to light than in the dark. In addition, the authors found that oxygen consumption rate was positively related to light intensity. In contrast, the authors observed no effect of light on respiration rates of the epigean species, *G. fossarum* (Simcic and Brancelj, 2007). Previous studies have indicated rate of oxygen consumption is correlated to stress levels in organisms (Kedwards *et al.*, 1996; Simcic and Brancelj, 2007). These data suggest that exposure to light is stressful to some species of subterranean amphipods. Therefore, it is likely that successful culture of stygobiontic amphipods in the refugium may require that organisms rarely if ever be exposed to light.

Avoiding exposure to light has been hypothesized to be advantageous to subterranean-adapted fauna, because a sightless condition in an epigean environment is almost certainly associated with a suite of disadvantages. This is likely because once a subterranean-adapted organism is exposed to surface conditions, a suite of disadvantages arise and mate finding and predator avoidance at the surface may be more difficult (Fišer et al. 2014). Indeed, some genetic data are consistent with this hypothesis in that it appears there is selection in cave invertebrates for the ability to detect light (Crandall and Hillis, 1997). Taking into consideration that most cave organisms tested to date (see references above) avoid light, it is likely that exposure to light may be stressful and disadvantageous when culturing organisms in a refuge setting.

2.3. Reproduction of subterranean amphipods

Like many aspects of the ecology and of subterranean amphipods, information on their life history is not abundant in the literature. In general, it appears that subterranean amphipods (like other subterranean species) have a much slower rate of reproduction than epigean species. Most epigean species of amphipods have multiple generations per year (i.e., multivoltine), while subterranean amphipods typically take at least a year to mature (i.e., univoltine) (Venarsky *et al.*, 2007; Crawford and Tarter, 1979). However, the subterranean hyporheic amphipod, *Niphargus aquilex aquilex*, has been shown to have the capacity to produce up to two generations per year

(Gledhill and Ladle*et al.*, 1969). This suggests that surface species may not be suitable surrogates for trying to study developmental rate, but may still be useful for studying developmental events at an accelerated rate.

The pairing of male and female amphipods and "optimal" sex ratios is not well understood. The sex ratio in *Crangonyx forbesi* was shown to fluctuate on an annual cycle (Crawford and Tarter, 1979). During winter months (*C. forbesi* breeding season), males outnumbered females, while during the summer no males were observed (Crawford and Tarter, 1979). It has been suggested that the greater abundance of males during the breeding season corresponds to females having synchronous pre-copulatory molts (Crawford and Tarter, 1979; Bollache and Cezilly, 2004). Sex ratios may also become distorted due to the mechanism of sex determination in amphipods. That is, amphipods do not have all sex determining alleles located on discrete sex chromosomes; rather, sex determining alleles are distributed across several chromosomes and sex is inherited much like a quantitative trait. Furthermore, it has been shown that certain pairings can lead to exclusively male or female offspring (Sutcliffe, 1992). Environmental factors have also been shown to affect or at least covariate with sex, as well (Sutcliffe, 1992; Watt and Adams, 1993; McCabe and Dunn, 1997) and infection with microsporidians (Bulnheim and Vávra, 1968) and chemical pollutants (Gross *et al.*, 2001) have also been shown to affect sex ratios or the development of sexual characteristics in amphipods suggesting that sex determination in amphipods may behave like a developmentally plastic and quantitative trait. Therefore, it is crucial to maintain proper pedigrees of amphipods and ideal culture conditions ratios in the refugium to ensure against improper development or heavily biased sex ratios which could lead to the collapse of specific or preferred culture lineages. Cumulatively, these data indicate that amphipods which are housed in a refuge context (i.e., *S. pecki*) require that reproduction is thoroughly understood.

Currently, there is no information in the literature on the mating behavior for any stygobiontic amphipod species in the world. However, there is a plethora of information on mate selection and timing of reproduction in epigean amphipods and there is some information on reproductive cycle of wild-caught stygobiontic amphipods. Amphipods are thought to only able to mate after the female molts, because only then is the cuticle of the females' exoskeleton flexible enough to allow the release of eggs through the genital pores into the marsupium (Bollache and Cezilly, 2004). Because females are only momentarily receptive to mating, males typically guard a female prior to her molting to insure that he fathers offspring. Molt cycles in males also appear to have a role in reproductive timing because males approaching a molt tend to not be willing to enter into amplexus because they will inevitably have to release the female upon molting thus never actually copulating with the female; a wasted investment in a mating (Bollache and Cezilly, 2004). Mate guarding comes at a cost to males by hindering their ability to forage, thereby reducing lipid stores and hindering growth (Robinson and Doyle, 1985). In response to this energetic cost, males tend to not enter into amplexus unless they have sufficient amounts of stored lipids and glycogen to wait out the females molt cycle and only if the female is expected to molt before the male (Plaistow *et al.*, 2003). Therefore, it is likely that proper nourishment is necessary to offset the nutritional costs of amplexus. In addition, there appears to be significant cannibalism in captive populations of *S. pecki* (R. Gibson, *pers. obs.*). However, keeping both males and females well-fed likely reduces the tendency of cannibalistic interactions to occur.

It has been proposed that because larger females are more fecund, that there is greater competition among males for access to larger females (Bollache and Cézilly, 2004). The resulting consequence is that larger males preferentially out-compete smaller males for larger females, thus there appears to be size assortative paring between males and females; at least in some species of amphipods (Bollache and Cézilly, 2004; Franceschi *et al.*, 2010). However, male-female pairs in amplexus with smaller females tend to have greater swimming efficiency than pairs in amplexus with larger females, suggesting that males tend to be larger than females in scenarios with predation (Adams and Greenwood, 1983). Selection for larger males is also compounded by female resistance to amplexus (Jormalainen and Merilaita, 1995). The ultimate consequence of these size-specific interactions is that females must have a large enough male suitor if mating is to be successful, but also that the female must be large enough (and thus fecund enough) to be worth the male investing in amplexus. However, under scenarios where selection has been relaxed, size selection will only be effected by choosing for the most fecund (thus largest) female that a male is large enough to restrain.

2.4. Amphipod holding and culturing techniques

In order to protect against loss of individuals held in a refugium setting, it is important that the range of conditions are survivable and mortality is minimized. Ideally, organisms should be housed within an "optimal" range of conditions and within this range, conditions should maximize survival and production of offspring for the refuge. In the context of establishing a refuge for the Edwards Aquifer, the Edwards biota live in environments with presumably little environmental variation and it is therefore expected that organisms will perform best under relatively stable conditions that mimic the physicochemistry of the Edwards Aquifer. In addition, this suggests that Edwards Aquifer organisms, regardless of taxonomy, are likely to have similar environmental requirements. However, this also implies that all Edwards Aquifer organisms have been afforded equal opportunities to become adapted to their environment; which is almost certainly not the case. Therefore, closely related groundwater fauna can be physiologically quite different, as with some *Niphargus* species (Issartela *et al.*, 2005) presumably because more recently distributed species have not been afforded the opportunity to become as "ideally" adapted.

In general, the metabolic rate of stygobionts appears to be low when compared to epigean relatives (Hervant *et al.*, 1997; Mezek *et al.*, 2010). Multiple distantly related taxa have been shown to have the ability to go without food for long durations without depleting energy reserves, while closely related surface taxa have been shown to deplete energy reserves during the same duration of starvation (Hervant *et al.*, 1997; Mezek *et al.*, 2010). Therefore, it is likely that refugium stygobionts will require relatively less feeding when compared to epigean species. However, in order to promote or maintain breeding in stygobionts, it is likely that organisms will need to be fed enough food to offset fitness costs (Plaistow *et al.*, 2003).

Among the amphipods in the hypogean genus *Stygobromus*, similarity in gross morphology alone does not appear to recapitulate phylogenetic relationships (Culver *et al.*, 2010). Within *Stygobromus*, a relationship seems to exist between the pore size of the habitat, the gross morphology, and the overall size of the species (Culver *et al.*, 2010). Other species of hypogean amphipods (i.e., *Niphargus* in Europe) also exhibit the same kind of patterns (Trontelj et al.

2012). The survival rate of *Gammarus pseudolimnaeus* (an epigean aquatic amphipod) offspring co-varies with pore size of habitat (Waters, 1984). Therefore, habitats in refugia will likely require species-specific pore spaces (such as mesh or other structure) in order to optimize refuge habitat suitability and survivability.

In addition to porosity, there is likely a relationship between survival/growth rates and physicochemical conditions. The survival rate of molting amphipods is closely related to the amount of dissolved Ca^{2+} in the water (Zehmer *et al.*, 2002). The same study found that low Ca^{2+} waters were shown to be deadly to most molting *Gammarus pseudolimnaeus*, which appears to be a large factor in determining the geographic range limits of this species. Edward Aquifer water is calcium-rich, therefore having sufficient Ca^{2+} for molting amphipods is not likely to be an issue. However, if refugium stock are moved to different locations, it is important that water at the new locations is rich in calcium; at least during molting.

Determining how to house individuals in order to track individual development and increase survival is critical. *Stygobromus pecki* has a tendency for cannibalism, thus knowing how to hold individuals (in group set ups or in individual containers) is critical to determine how to house individuals.

3. METHODS AND MATERIALS

3.1. Anesthesia

3.1.1. Test subjects and use of surrogates

Initial testing utilized surrogate species to avoid unnecessary mortalities of legally-protected species. When preliminary trials were completed with surrogates, protected species were then tested with relatively small numbers to refine understanding of proper dose of anesthetic. Fortunately, there is abundant locally distributed species in the same family as *S. pecki* (Crangonyctidae), both epigean and subterranean. *Stygobromus flagellatus* and *S. russelli* are the most locally abundant subterranean species and both were used as surrogates for anesthesia testing before *S. pecki* were tested. *Stygobromus flagellatus* and *S. russelli* were collected from drift nets placed over spring outflows at the headwaters of the San Marcos River (San Marcos Springs). The surrogate for the Comal Springs riffle beetle (*Heterelmis comalensis*) was *Heterelmis* cf. *glabra*., collected from Finnegan Springs along the upper Devils River, Val Verde County, Texas. All surrogates were acclimated to captivity for at least two weeks prior to testing to ensure that transport and handling did not confound results. Because it was unknown which anesthetics would be effective on which taxa and what concentration was best to use for each anesthetic, surrogate testing was used as an opportunity to determine the best prescribed doses for anesthesia trials on legally protected species. This was largely accomplished through insight from published literature and trial and error using surrogates species.

3.1.2. Source of legally protected experimental organisms

Adult *Heterelmis comalensis* were collected using poly cotton lures following the methods of Gibson *et al.* (2008) and Huston *et al.* (2015). *Heterelmis comalensis* larvae were produced in captivity by breeding of collected adults. *Stygobromus pecki* were collected by dip netting

sediments in spring openings of Comal Springs using aquarium nets. *Stygobromus pecki* were separated by hand from sediments immediately after collection. All specimens were acclimated to captivity for at least two weeks prior to testing to ensure that transport and handling did not confound results.

3.1.3. Initial test for susceptibility

Taxa were first tested at the highest prescribed dose (based on results from surrogate testing) of each anesthetic to test which anesthetics each taxon was susceptible to. If anesthesia or death occurred it was conclude that the taxon was susceptible to the anesthetic and therefore further testing using that anesthetic was warranted. Subsequent anesthesia trials at different concentrations were attempted to elucidate the most effective and practical anesthetic for each taxa and the most appropriate dose for that anesthetic. All anesthesia trials were maintained at 22^{o}C (approximately *in situ* conditions).

3.1.4. Anesthesia trials

3.1.4.1. Experimental groups

Each type of anesthetic was administered to the appropriate taxa at four different concentrations per anesthetic (Table 1 and Table 2), thus there were four treatment groups per anesthetic type. Within treatment groups we replicated (*n*=3) for each taxon within each treatment level; the number and type of test subjects used for each treatment group is presented in Table 3. None of the test subjects used for any of the anesthesia trials were used for any other treatments in order to avoid pseudoreplication.

Table 1. Concentration of anesthetics tested on *Stygobromus pecki* determined from preliminary surrogate testing.

	Treatment Concentrations			
Anesthetic	**1**	**2**	**3**	**4**
MS-222 (mg/L)	800	1200	1800	2700
(µL Clove Oil/L H_2O)	50	100	200	400
% concentration EtOH	2	6	14	22

Table 2. Concentration of anesthetics tested on *Heterelmis comalensis* determined from preliminary surrogate testing. Clove oil was only tested on larvae and CO2 was only tested on adults.

	Treatment Concentrations			
Anesthetic	**1**	**2**	**3**	**4**
(µL Clove Oil/L H_2O)	100	200	400	800
% concentration EtOH adults (larvae)	6 (2)	14 (6)	22 (14)	30 (22)
CO_2 (ppm)	130	-	-	-

Table 3. Number of individuals per treatment group by taxon

	Taxon		
Anesthetic	**Larval Beetles**	**Adult Beetles**	**Amphipods**
MS-222	0	0	12
CO_2	0	3	0
Clove Oil	12	0	12
EtOH	12	12	12
Sum=>	24	15	36

3.1.4.2. Preparation of treatment solutions

Clove oil (Eugenol)

A stock solution of 1 part clove oil to 3 parts 100% EtOH was prepared. After preparing the stock solution of clove oil, a 100 mL solution of Edwards artesian well water and clove oil was prepared at the prescribed concentrations (Table 1and2) for each replicate. The solution was

placed in a clean 125 mL Erlenmeyer flask and placed in a water bath of artesian water on a flow through system to insure thermal stability.

MS-222

A 100 mL solution of Edwards Aquifer artesian well water and MS-222 was prepared at the prescribed concentrations for each replicate (Table 1). The solution was placed in a clean 125 mL Erlenmeyer flask and placed in a water bath of artesian water on a flow through system to insure thermal stability.

EtOH

EtOH solutions of 100 mL were prepared at the prescribed concentrations (Table 1and 2) for each replicate by mixing 100% EtOH with artesian water. The solution was placed in a clean 125 mL Erlenmeyer flask and placed in a water bath of artesian water on a flow through system to insure thermal stability.

CO_2

CO_2 was bubbled into solution in a 2-L sealed container filled with 1 L of Edwards Aquifer artesian well water until reaching saturation. As CO_2 was bubbled into solution, concentration in solution was monitored using a CO_2 meter (OxyGuard CO_2 Analyzer). Despite using a sealed chamber, controlling the amount of CO_2 that went into solution was extremely difficult (see Coyle *et al.* 2004). Therefore, we only assessed one dose level of CO_2 in this study.

3.1.4.3. Experimental procedure

After preparing the experimental chamber with each individual anesthetic treatment, each individual test subject was placed into a separate 100 mL flask of the treatment solution. Upon placement into the solution, a stop watch was started and observation was made until the subject became anesthetized. If 30 min elapsed and the subject was still not anesthetized, this was recorded as not sensitive to treatment dose. If anesthesia occurred, the duration until anesthetized was recorded and subjects were removed from the anesthetic and washed with untreated Edwards Aquifer artesian well water. For *S. pecki*, if thirty minutes of exposure to anesthetic had elapsed and the test subject was still not anesthetized, this was recorded as "not sensitive to treatment dose". For *H. comalensis*, if sixty minutes of exposure to anesthetic had elapsed and the test subject was still not anesthetized, this was recorded as "not sensitive to treatment dose" (except for CO_2 which took appreciably longer for anesthesia to occur; see below). Afterwards, each test subject was photomicrographed at 10x magnification using Olympus Cellcens camera system and software at the standard shutter speed of 3.395 milliseconds. Length was then estimated for each individual using the measuring tool in the Cellcens software system. Following photography, test subjects were placed into individual holding chambers suspended in a flow through system of artesian water. Subjects were then monitored until they recovered and the time until recovery was recorded. If 3 hours elapsed and a test subject was still inactive, this was recorded as a mortality event for the individual.

Surviving test subjects were kept and monitored for an additional month to determine if there was any longer-term effects of each anesthetic on survivability. Anesthesia trials were maintained at 22ºC for the entire duration of the study.

3.1.4.4. Analysis

Multiple single factor ANOVAs were run to determine if size of test subject and/or treatment concentration could explain the variation observed in how long it took for test subjects to become anesthetized and how long it took for test subjects to become revived post anesthesia.

3.2. Light response

3.2.1. Test subjects

Stygobromus pecki Holsinger is federally listed as endangered and is known only from three localities: Comal Springs, Hueco Springs, and a monitoring well in Panther Canyon; all in Comal County, Texas (Krejca 2005; Gibson *et al.* 2008). This species is thought to be exclusively subterranean due to its lack of eyes and is therefore thought to only be encountered incidentally at spring openings. Despite numerous other species of subterranean *Stygobromus* reported or described from across the Edwards Aquifer, the diversity of this genus in the Edwards Aquifer is poorly understood. Therefore, the degree of endemism of this genus is not known, nor is the extent and distribution of most *Stygobromus* species understood.

Despite disparities in knowledge on *Stygobromus*, some species are better understood than others. In this study we tested and compared the relative sensitivity to light of five *Stygobromus* species; the species used depended on availability. All of the species used in this study had some aspects of their habitat preferences understood with the exception of an undescribed *Stygobromus* species that has only been collected from Garden Ridge well in Comal County, Texas. *Stygobromus pecki* is thought to be an exclusively subterranean species that is found in the waters beneath Comal Springs and is therefore expected to encounter epigean waters at an evolutionarily relevant frequency. In the context of this study, we define an evolutionarily relevant frequency as being frequently enough to act as a selection pressure on populations. *S. flagellatus* is thought to be a deep phreatic species that under natural conditions should not be expected to encounter epigean waters at an evolutionarily relevant frequency. *S. bifurcatus* is thought to be an interstitial exclusively subterranean species that is also not expected to encounter epigean waters at an evolutionarily relevant frequency. Finally, *S. russelli* is thought to be an interstitial hyporheic and subterranean species that is thought to incidentally encounter epigean waters at an evolutionarily relevant frequency. If some of these species retained or redeveloped the ability to detect light due to selection, it is hypothesized that this would only occur in the species which are expected to encounter epigean waters at an evolutionarily relevant frequency.

All *Stygobromus* used in this study were collected using drift nets placed over spring openings with the exception of *S. pecki*, which was collected by dip netting loose sediments of spring openings at Comal Springs, and *Stygobromus spp.*, which was collected by bottle trap form the Garden Ridge Well. Sampling localities and species collected at each is present in Table 4.

Sample size per species varied depending on availability. Prior to any experimentation, all test subjects were acclimated to captivity for at least two weeks prior to any experimentation.

Table 4. Collection localities for *Stygobromus* used in this study.

Locality	Coordinates	Taxa Collected
Big Boiling	30° 56.626'N 97° 32.199'W	*S. bifurcatus*; *S. russelli*
Comal Springs	29° 42.859'N 98° 8.156'W	*S. pecki*
Garden Ridge	29° 38.553'N 98° 18.442'W	*Stygobromus sp.*
Robertson	30° 56.679'N 97° 32.509'W	*S. russelli*
San Marcos Springs	29° 53.596'N 97° 55.864'W	*S. bifurcatus; S. russelli*
Sessom Springs	29° 53.427'N 97° 56.213'W	*S. russelli*

3.2.2. Experimental design

In the initial phase of this portion of the research we wished to determine which taxa, if any, respond to being exposed to any portion of the visible spectrum of light. To do this, all taxonomic groups were initially exposed to full spectrum light. This was done because organisms which only respond to a portion of the light spectrum should also respond to full spectrum light. Therefore, if organisms initially responded to full spectrum light, then further experimentation was performed to determine if there were threshold intensities below which they no longer respond and/or if organisms only responded to certain portion of the light spectrum (Figure 1).

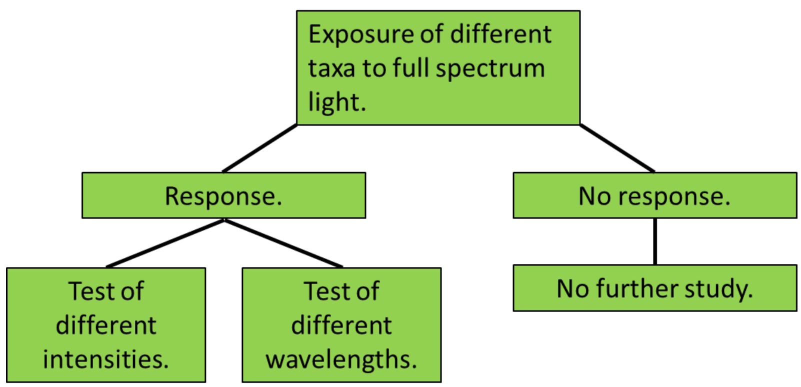

Figure 1. Flow chart of methodology used to test sensitivity of organisms to light.

3.2.3. Lighting apparatus

The lighting apparatus was made by cutting a 15-cm inch diameter hole in the center of the bottom of an inverted 20-L bucket 25-cm in diameter. The bucket was then painted on the

interior surface several times with a non-transparent black paint to insure that as little light escaped or penetrated as possible. A dome shaped metal lamp slightly larger in diameter than the hole in the bucket was affixed face down over the hole so that when the light was turned on the angle of incidence was nearly perpendicular to the horizontal surface. This apparatus was used for all light experiments.

3.2.4. Initial test for light response

A 5.5-W full-spectrum LED light bulb (equivalent to a 40-W halogen bulb) was used for all aspects of this phase of study. Light source was calibrated to 45 µmol m^{-2} s^{-1} of irradiance by adjusting the distance from the test subject and the amount of current to the bulb (via rheostat control). All organisms were submitted to two types of experiments, one that examined if there was a behavioral response to light exposure and another that assessed changes in rate of respiration (O_2 consumption) when exposed to light. Increases in respiration was assumed to be indicative of increased stress.

3.2.5. Behavioral response

A light avoidance experiment was used to determine which taxa preferred to not be exposed to direct light. Test subjects were placed in a 9-cm diameter petri dish with two quarters of its upper surface of the cover painted black with a nontransparent black paint (Figure 2). Light sources were oriented at a perpendicular angle of incidence from directly above. Petri dishes were filled with fresh Edwards Aquifer artesian well water at the beginning of each trial. All individual test subjects were placed in the petri dish and allowed ten minutes to acclimate in the dark prior to making any observations because preliminary trials suggested that ten minutes was an acceptable duration to allow organism to recover from the stress of being handled. After test subject acclimation, the light source was turned on and observations were taken for a 15 minutes period with one individual observation taken at 1 minute intervals. At each observation time, it was noted if the test subject was in one of the light or the dark quarters. At the end of each trial, each replicate had one response variable calculated: proportion of duration spent avoiding direct light. The difference of this proportion from 0.5 was then calculated as the true response variable because it is expected that an organism that shows no preference would be found in the dark and the light quarters half the time each due to randomness alone. Therefore, the null expectation was a value of 0.5 and departure from this value was considered to be a response. The magnitude of this response was tested for significance with a single factor ANOVA using the factor ‘species’ to predict the relative degree of response to light among the different species tested. A Tukey’s HSD was run to determine which species responded in a similar fashion and if there was any general response categories that taxa could be assigned to.

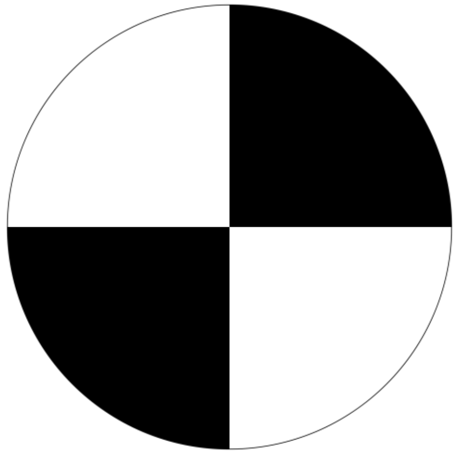

Figure 2. Schematic of a petri dish with two quarters of its upper surface covered in black paint.

3.2.6. Stress response

It is thought that exposure to light may induce stress in some subterranean species. To estimate stress, rate of dissolved oxygen (DO) consumption was compared between test subjects held in the dark *versus* in full spectrum light. We assumed that elevated respiration is related to elevated stress levels. Respiration rates were estimated using Qubit systems OX1LP-30 DO cuvettes with built-in Clark cell type polarographic oxygen sensors. After calibration of the cuvette systems, but prior to testing subjects for sensitivity to light, the DO consumption of the O_2 electrodes was estimated by running the cuvettes filled with 5 mL of water in the dark and full-spectrum light to insure that differences in lighting did not affect the rate of O_2 consumption by the electrodes themselves, and to gain an understanding of the base-line rate of O_2 consumption of the O_2 electrodes.

Using this system, an individual test subject was placed in a cuvette filled with 5 mL of artesian water and was allowed to acclimate to the chamber for 10 minutes to recover from the stress of handling. The test subject was then randomly assigned to an order in which they would receive each of the two treatments: dark or full-spectrum light. The subject was exposed to each treatment for 15 minutes while DO consumption was recorded at 30 second intervals with a 10 minute interval between the two treatments to allow organisms to recover and reacclimatize from any stress that may have been induced from the prior treatment. All experiments were carried out in a dark room. Light incidence on test subjects was perpendicular to the bottom of the cuvette. Cuvettes were jacketed with an artesian water flow through system in order to maintain thermal stability.

Changes in DO measurements over time were converted to a proportion of total DO consumed across all of the treatments for one individual in order to control for variation between test subjects due to size or other physiological differences. The combined data for all test subjects belonging to the same species, or same species from the same collection site, were regressed across elapsed time as the X axis. The slope between the dark and light treatments was then

compared using analysis of covariance (ANCOVA) within and across taxa to determine if species was a valid predictor of response to light and if the response varied within species across collection localities.

Net proportional DO consumption was also calculated for every test subject for both treatments. A two factor ANOVA with the factors 'species' and 'locality' as predictors was run to test if subjects belonging to the same species explained most of the variance or if response varied between populations within designated species.

3.2.7. Further study of taxa shown to respond to light

Species that responded behaviorally and physiologically to light were further studied and analyzed to determine what aspect(s) of full spectrum light they respond. Species that did not show a metabolic response to high intensity full spectrum light were not studied further. Likewise, species that did not respond behaviorally to high intensity full spectrum light were not studied further. Response of any type at lower intensities of full spectrum or at partial spectrum light would unlikely when high intensity full spectrum light failed to produce a response. *Stygobromus pecki* was the only species that showed both behavioral and stress responses to light and was the only species further studied.

3.2.7.1. Study of response to varying wavelengths

Full spectrum light is the combined irradiance of all the wavelengths of visible light, therefore full spectrum light will always have greater luminous flux than only a single wavelength if emitted from a device of the same wattage. In an effort to insure that differences in light intensity were not confounding results and to isolate the variable 'wavelength', it was necessary to calibrate each wavelength tested to the same amount of radiant flux. Therefore, prior to each treatment replicate the light source was recalibrated to 6 µmol m^{-2} s^{-1} of irradiance by adjusting the amount of current for each of the wavelengths tested using a FieldScout LightScout Quantum Light Meter. This was done in an effort to insure that any variation in intensity due to calibration error was spread as evenly as possible across treatment replicates.

Response to the different wavelengths of light was tested using the above described behavioral and respiratory methods. In both cases, the treatment replicates were given 10-mins acclimatization after handling before collecting any data. After the acclimatization period, test subjects were exposed to three wavelengths in a randomized order in an effort to insure that any residual response from the previous treatment would be randomly distributed across treatment replicates so that order of treatment was not a confounding factor. Three wavelengths were tested (red ≈ 700 nm; amber ≈ 600 nm; and blue ≈ 500 nm) on *S. pecki* (*n*=5 for each treatment).

3.2.7.2. Study of varying intensities of full spectrum

In order to determine if response to light is related to the intensity of light, different intensities of full spectrum light were tested. The intensities were 1.5 µmol m^{-2} s^{-1}; 3 µmol m^{-2} s^{-1}; 6 µmol m^{-2} s^{-1}, and 12 µmol m^{-2} s^{-1} for the behavioral study, and 6 µmol m^{-2} s^{-1}; 12 µmol m^{-2} s^{-1}, and 24 µmol m^{-2} s^{-1} for the respiratory study. Each treatment was calibrated to the appropriate intensity of light by adjusting the amount of current and the distance of the light source from the testing chamber. Response to the different intensities of light was tested using the above described behavioral and respiratory methods. In both cases, the treatment replicates (n=5) were given 10 min of acclimatization before collecting data. After this period, test subjects were exposed to thre intensities tested in a randomized order in an effort to insure that any residual response from the previous treatment would be randomly distributed across treatment replicates so order of treatments was not potentially confounding results.

3.3. Amphipod reproduction

Nine adult females with visibly gravid ovaries (indicating they would be receptive to copulation after their next molt) and nine adult males were selected at random from a stock culture of *S. pecki* (two females were also already brooding offspring in marsupiums). Each female was then paired with a male and the size of both individuals was measured in length by photomicrographing each individual at 10x magnification using Olympus Cellcens camera system and software at the standard shutter speed of 3.395 milliseconds. Length was then estimated for each individual from its respective photograph using the measuring tool in the Cellcens software system. These measurements were used to estimate the size ratio of the pair. Male-female pairings were adjusted so that there were 3 replicates of each of the following size ratio categories: male larger, male and female relatively equal in size, and female larger. Pairs were then placed in a transparent 1 L container with a mesh substrate set up on a flow through system that received a constant flow of artesian well water 0.5 L per minute.

After establishing mating pairs, initial observations on the reproductive condition of females. Observations were then taken weekly on the reproductive condition of females if amplexus or copulation was observed, and if cannibalism occurred within pairs. Female reproductive condition was categorized into the following categories: visible ova in ovaries, ovaries gravid with eggs, eggs laid in marsupium, linear embryos in eggs, and neonates in marsupium. This data was taken in an attempt to study the developmental rate of the reproduction in females. Observations were made across six weeks unless obvious cannibalism, death for other reasons, or successful reproduction occurred before six weeks elapsed; in which case the duration to one of these events was recorded. At the end of the six weeks each pair was categorized as either successfully reproducing or not. Throughout this experiment all amphipod pairs were fed commercially-available fish flakes *ad libitum*.

It was hypothesized that pairs with males larger than females would have reproductive success more frequently. To test if size ratio of pairs affected mating success, a single factor ANOVA was run using the factor “size ratio” to predict the response variable: reproductive success. A logistic regression was also used to test if size ratio and mating success have a predictable directional relationship.

3.4. Amphipod holding and culture

The current method employed at the SMARC to house and their cultures of *S. pecki* is a “group holding system”. These systems utilize plastic containers supplied with a constant supply of Edwards Aquifer well water through a simulated upwelling on the benthic surface which provides dispersed flow. Constant flow limits the development of anoxic areas, while large surface area filtered drainage reduces the likelihood of clogging and flooding. A nylon-net substrate with various pore sizes is provided to increase the three dimensional surface areas for the amphipods to utilize and to limit contact between individuals. Typically large numbers (*n* ranging from 30 – 60) of amphipods are placed together into these containers which has resulted in high mortality, presumably due to cannibalism. In order to reduce cannibalism, group held populations are well-fed.

Using this system, it is impossible to track the growth and development of individual amphipods. Therefore, two other types of holding systems are being proposed which will allow for holding amphipods individually. The first of these systems is the use of individual “flow chambers”. The second of these proposed systems is a “suspended static array”. Both of these chamber systems were designed to house amphipods individually to eliminate the possibility of cannibalism and also to allow for the study of growth and development of individual amphipods.

3.4.1. Individual flow chambers

Chambers were made by modifying commercially available single-cup reusable coffee filters (Keurig brand) that were 26 mL in total volume. Chambers were modified to have a water line feeding in while water was allowed to drain out freely through the filter mesh while submerged in a water bath of artesian water in an effort to insure that chambers remained filled to their maximum volume as well as maintained thermal stability. Each system consisted of 5 individual chambers fed from the same water supply and maintained in same water bath.

3.4.2. Suspended static array

The static array consisted of multiple commercially available single-cup reusable coffee filters arranged into an array with the chambers completely submerged. Each array consisted of 5 individual chambers maintained in the same water bath with a common supply of flow-through artesian water. In contrast to the “flow chambers”, this system only had water supplied to the water bath and no water was pumped through the individual chambers. Therefore, in this system, chambers only received water *via* passive diffusion through mesh.

3.4.3. Group holding system

Group holding systems held 5 organisms together in the same container. These containers were designed as described above and measured 25 by 15 cm on the benthic surface area and were filled to a volume of 2.5 L. Group holding systems received a constant supply of artesian water on a flow through system.

3.4.4. Experimental design

To compare the efficacy of these systems, an equal number of amphipods were placed into each replicate (5 individuals per replicate); there were three replicates for each type of holding system. Each replicate received the exact same rate of flow to insure that water supply was not a confounding variable; flow rate was calculated by timing the duration required to collect 1L of water from the outflow of each system and then calibrating each system receive 1 to 1.2 L of flow per minute. 36 *S. pecki* were collected by dip netting from Comal Springs in Landa Park on 20 July 2015. These 36 amphipods were randomly distributed into three groups of 12. Because it is only possible to identify *Stygobromus* spp. to species when they are adults only adult amphipods were used from the Landa Park collection. However, in order to properly execute this study, amphipods of all sizes needed to be included. Therefore, 9 immature amphipods that had been hatched and reared in captivity at SMARC (and therefore of known species identity) were selected and randomly distributed into three groups of 3. The three groups of immature amphipods were then randomly added to one of the group of adult amphipods to make for three groups of 15 amphipods that each had a broad range of sizes represented. Each group was then randomly assigned to one of the three treatments. From these three groups, 5 amphipods were selected one at a time and randomly assigned to a replicate within their treatment group until all three replicates had received 5 amphipods.

Amphipods placed into replicates had standard length estimated by photomicrographing each individual at 10x magnification using Olympus Cellcens camera system and software at the standard shutter speed of 3.395 milliseconds. Length was then estimated for each individual from its respective photograph using the measuring tool in the Cellcens software system. Using this length information, mean length and variance was estimated for each replicate. Holding trials were begun on 21 July, 2015 and ended on 11 November, 2015; if any amphipods died in the first week of the holding trials they were replaced. After the prescribed holding period (3 months) length was measured again using the same method for all remaining amphipods; % survival was also calculated. The hypothesis was that in the group holding system, % survival would decrease significantly compared to either of the individual holding systems and that in the group system, the mean size would increase on account of the larger individuals cannibalizing smaller individuals (thus eliminating the effect of smaller individuals on the mean) and the variance would also be reduce significantly in the group holding system when compared to the individual systems because the remaining amphipods in the group systems would all be of similar size (the largest amphipods). All study amphipods were fed commercially available fish flakes *ad libitum*.

4. RESULTS
4.1. Anesthesia
4.1.1. Clove Oil

During surrogate testing, none of the adult riffle beetles (*H. glabra*) survived anesthesia at any of the treatment doses of clove oil tested. Clove oil showed some promise for use on beetle larvae and *Stygobromus* spp. therefore, we further tested this anesthetic on legally protected *H. comalensis* larvae and *S. pecki*.

4.1.1.1. Stygobromus pecki

Individual length explained a significant amount of variation in time duration until anesthetized (F=7.008, p=0.024) but not in the duration until revived post anesthesia (F=0.015, p=0.904). Likewise, concentration was found to explain variation in duration until anesthetized (F=7.55, p=0.021) but not duration until revived (F=0.023, p=0.883). No discernable relationship was found between treatment dose and survival rate though there was appreciable mortality across the treatment groups one month post anesthesia.

4.1.1.2. *Heterelmis comalensis* larvae

Individual body length was not found to explain a significant amount of variation in duration until anesthetized (F=0.369, p=0.557) or variation in duration until revived (F=0.012, p=0.915). None of the variation in duration until anesthetized (F=0, p=0.99) or duration until revived (F=1.322, p=0.277) was explained by treatment concentrations. No discernable relationship was found between treatment dose and survival rate though there was appreciable mortality across the treatment groups one-month post anesthesia. The lack of explainable variation for any of the parameters for this anesthetic suggests that this is not a feasible choice of anesthetic for *H. comalensis* larvae.

4.1.2. MS-222

During surrogate testing, concentrations of MS-222 orders of magnitude higher than that necessary to euthanize vertebrates showed no effect on riffle beetles. Therefore, it was decided that this was not a practical anesthetic for use on riffle beetles as concentrations necessary to anesthetize beetles would be high enough to be potentially pose a health risk to laboratory workers.

Again, concentrations of MS-222 much higher than what was necessary for the euthanasia of vertebrates were shown to be effective at anesthetizing *Stygobromus* spp. Therefore this anesthetic was further tested in anesthesia trials on *S. pecki*. Length was not found to explain

variation in time until anesthetized (F=0.452, p= 0.516) or time until revived (F=0.269, p=0.615) across any of the treatment levels. Concentration of dose did explain some variation in duration until anesthetized (F=20.108, p<<0.001) but did not significantly explain variation in duration until revived (F=1.687, p=0.223). Differences in duration until anesthetized across the different treatment levels are depicted in Figure 3. Only the lowest concentration varied significantly from the other treatment levels (Figure 3; Table 5). Only one individual died across all of the treatment groups one month post anesthesia, therefore it does not appear that survival varied by treatment dose nor does it appear that MS-222 is lethal to *S. pecki* at any concentration tested.

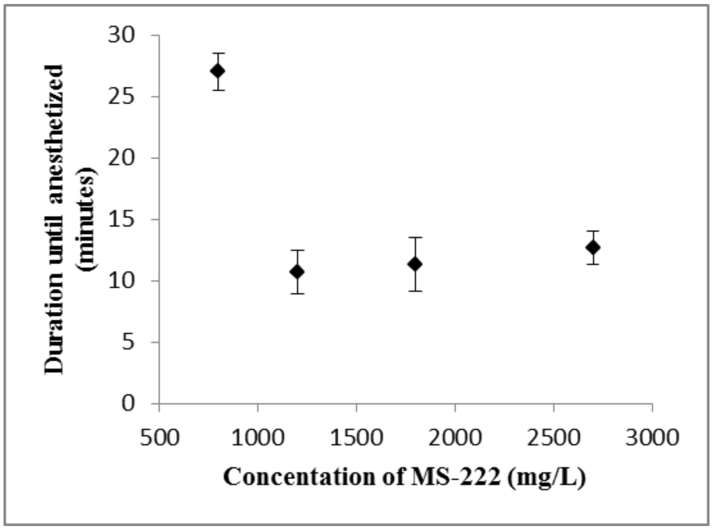

Figure 3. Duration until anesthetized for *Stygobromus pecki* exposed to four different concentrations of MS-222. The three higher concentrations showed no difference in duration until anesthetized. Error bars represent one standard error around the sample mean.

Table 5.) Tukey's HSD indicated that 800 mg/L MS-222 was the only concentration that differed from any of the other concentrations.

Comparison	diff	lwr	upr	p
800-1200	16.33	8.489	24.17	0.001
800-1800	15.67	7.823	23.51	0.001
800-2700	14.33	6.490	22.18	0.002
1200-1800	-0.667	-8.511	7.178	0.992
1200-2700	-2.000	-9.844	5.844	0.845
1800-2700	-1.333	-9.178	6.511	0.946

4.1.3. EtOH

4.1.3.1. Stygobromus pecki

Individual amphipod body length was not found to explain a significant amount of variation in duration until anesthetized (F=0.508, p=0.492) or variation in duration until revived (F=0.055, p=0.819). However, concentration was found to be a highly significant predictor of variation in duration until anesthetized (F=67.67, p<<0.001) and duration until revived (F=42.87, p<<0.001). Only one individual died across all treatment groups one month post anesthesia, thus it does not appear that long term survivability was affected by the EtOH treatment doses used in this study. Duration until anesthetized significantly predicted duration until revived (F=14.9, p<0.001). Interestingly, EtOH was the only anesthetic we tested as a part of this project that exhibited this relationship.

4.1.3.2. *Heterelmis comalensis* larvae

Boby length was not found to explain a significant amount of variation in duration until anesthetized (F=1.007, p=0.339) or variation in duration until revived (F=0.167, p=0.691). Concentration was not found to be a significant predictor of variation in duration until anesthetized (F=2.695, p=0.132); however, concentration was found to be a highly significant predictor of duration until revived (F=41.37, p<<0.001). One month post anesthesia, only two individuals had died and the deaths that occurred were correlated to treatment dose, suggesting that the range of treatment doses in this study did not affect long term survivability.

4.1.3.3. *Heterelmis comalensis* adults

Body length was not found to explain significant variation in duration until anesthetized (F=0.051, p=0.827) or variation in duration until revived (F=0.005, p=0.947). However, concentration was found to be a significant predictor of variation in duration until anesthetized (F=24.89, p<0.001) and duration until revived (F=14.0 p=0.004). Three individuals died

immediately following anesthesia; no additional individuals died after one month post anesthesia. Duration until anesthetized significantly predicted duration until revived (F=16.36, p=0.002). Again, as in the results for *S. pecki*, EtOH was the only anesthetic tested which exhibited this relationship.

4.1.4. CO_2

We only tested CO_2 on adult *Heterelmis* of both species. Despite using a presumably airtight sealed chamber, we experienced great difficulty establishing specific concentrations of CO_2 in solution. Therefore, we were only able to test the effect of CO_2 at ≈130 ppm on adults of both species. Our results showed that even at this high concentration, it took a mean duration of 96 minutes (range = 83 – 120 minutes) for individuals to become anesthetized. The duration until revived ranged from 22 to 30 minutes and there was no observed mortality during anesthesia or one month post anesthesia.

4.1.5. Discussion

Following the initial surrogate testing we concluded that clove oil should not be used at any concentration on adult riffle beetles. Even at the smallest concentrations used in this study, all of the surrogate individuals died. It is unclear why all adult riffle beetle surrogates died after exposure, but it is suspected that clove oil, being hydrophobic itself, interferes with the hydrophobic properties of the plastron (Brown, 1987; White and Roughley, 2008) preventing efficient respiration.

During initial surrogate testing of clove oil on *Stygobromus pecki*, it was observed that surrogates that died during exposure to clove oil turned black in color after death. This is in stark contrast to the opaque white color of live *Stygobromus* spp. It is suspected that at high dosages of clove oil, *Stygobromus* spp. essentially suffer chemical burns and die. Based on these initial findings, it was decided that clove oil should only be tested for efficacy on *Heterelmis comalensis* larvae and *Stygobromus pecki* and only at light dosages. Following experimental testing on *H. comalensis* larvae and *S. pecki*, it was concluded that clove oil is not the most feasible of anesthetics for either of these organisms. This is based on a lack of reliable predictors for duration until anesthetized and until revived, as well as relatively high mortality that was not predictable by treatment concentration. Therefore, it is our recommendation that clove oil not be used as an anesthetic for these species.

After surrogate testing with MS-222, it was determined that riffle beetles are not sensitive to this anesthetic at practically-applied dosages. Therefore, MS-222 was only tested for efficacy on *Stygobromus pecki*. Interestingly, our findings suggest that there is a threshold dosage that above which an increase in concentration of dosage does not increase the anesthesia efficacy of MS-222. This threshold was found to exist between 800 - 1200 µL/L suggesting it may be possible to achieve maximum efficacy with this anesthetic using the minimum dosage above the threshold concentration. The asymptote of efficacy in respect to concentration above 1000 µL/L

concentration of MS-222 in water has been observed in other taxa of amphipods (Ahmad, 1969). Testing of MS-222 on *S. pecki* during experimentation suggests that this anesthetic is not highly lethal to *S. pecki* at any of the concentrations tested. Furthermore, only one individual died across all of the treatment groups one month post exposure; therefore, these results are very encouraging as it does not appear to be possible to overdose *S. pecki* with MS-222 within the concentration range tested, thus reducing concerns of user error in future research.

During both surrogate testing and anesthesia trials on legally protected taxa, EtOH showed the greatest promise as an anesthetic in future studies. EtOH was the only anesthetic that duration until anesthetized significantly predicted duration until revived for any of the taxa tested. Indeed, this relationship was observed for both *S. pecki* and *H. comalensis* adults and likely would have been observed for *H. comalensis* larvae if we were able to obtain larger sample sizes. This relationship is very useful in being able to estimate how long anesthetized individuals will remain anesthetized and how long a window of time is available to work with individuals before they become active again. Recovery time post-anesthetization was significantly predicted by treatment concentration for both *S. pecki* and *H. comalensis* adults, and all other organisms tested. These results once again emphasize the utility of the use of EtOH as an anesthetic because of the level of control possible. Aside from *H. comalensis* adults, there was no appreciable mortality for any of the treatment groups thus suggesting that EtOH may be the ideal anesthetic for *S. pecki* and *H. comalensis* larvae. However, *H. comalensis* adults were relatively sensitive to higher concentrations of EtOH while not at all sensitive to lower concentrations, which failed to anesthetize any individuals in those treatment groups. Conversely, two of the three individuals in the highest concentration treatment group died immediately following anesthesia suggesting that this concentration is above or approaching a lethal threshold. Though there was a predictable relationship for all parameters measured using EtOH as an anesthetic on *H. comalensis* adults, the mortality results suggest that the window of anesthetic dose is quite narrow, suggesting that EtOH may not be the most practical for *H. comalensis* adults.

CO_2 was not used on *Heterelmis* surrogate larvae because other studies have shown CO_2 interferes with proper development of coleopteran larvae (Lizé *et al.*, 2010); nor was it tested on *Stygobromus* surrogates because other studies have shown appreciably high mortality of amphipods anesthetized with CO_2 (Cothran, 2008). Therefore, CO_2 was only tested on adult *Heterelmis*. Anesthesia of surrogates had no mortality, therefore it was determined it was safe to test CO_2 on *H. comalensis*. The difficulty establishing specific concentrations of CO_2 in solution was attributed to great solubility of CO_2 in water. The high solubility of CO_2 in water and difficulties of controlling concentration has also been reported in other studies (Coyle *et al.*, 2004). Because there was no mortality during anesthesia or one month post anesthesia, and there was low variation in anesthesia duration, CO_2 may be the best option for anesthesia of *H. comalensis* adults despite the length of time required to anesthetize individuals and the difficulties associated with controlling concentration.

None of the anesthetics tested showed a predictable relationship between size of organism and sensitivity to anesthetics for any of the taxa. This is likely due to the rate of uptake of anesthetic being directly proportional to the size (volume) of the organism. Therefore, size of organism should not be a consideration when deciding anesthetic concentration. It was observed during surrogate testing that anesthetics at any concentration were lethal to individuals that had recently

molted. This is likely due to the permeability of the post-molt soft exoskeleton. We recommend that great care be taken to ensure that organisms are not anesthetized post-molt until their exoskeletons have hardened.

Our study only examined the effect of concentration on the duration post exposure to anesthetic until anesthetized, the duration post anesthesia until revived, and the immediate and long-term survival of individuals post anesthesia. Although we have some insight into the survival rate of the various organisms at the various concentrations of the various anesthetics tested, we have virtually no information on the long term effects that the different anesthetics would have on the development and fecundity of anesthetized organisms. Therefore, our recommendations for best anesthetic are only based on the factors for which we have data. Thus, it may be determined in the future after further research that certain anesthetics should not be used because of the negative effects they have on the life history of anesthetized organisms. We also anesthetized each individual only once across the entire duration of our study; however, if one was to conduct a long term study on the growth and development study organisms would have to be anesthetized multiple times across their life span, thus cumulative effect may become a factor. Therefore, future research should address the long term effects of anesthetics on development and fecundity, and on the cumulative effect of repeated anesthesia.

4.2. Light response

4.2.1. Initial test for light response

4.2.1.1. Behavioral response

The study of the behavioral response of the various *Stygobromus* species tested suggests that species identity is a good predictor of whether or not a species will respond to light, and the magnitude of those responses (Table 6). Only two of the species were found to respond to light and differences in the magnitude of the response of those two species was indistinguishable (Table 7).

Table 6. ANOVA of behavioral response of the various *Stygobromus* species to light. The response variable was proportion of time spent avoiding exposure to direct light. 50% of time spent in light/dark is indistinguishable from random; therefore, magnitude of difference from 50% was the response variable analyzed. Taxon was found to significantly predict response to light.

SOV	df	F	p
Taxon	4	11.79	<<0.001
Locality	4	1.37	0.271
Taxon:Locality	1	0.066	0.799
Residuals	27		

Table 7. Tukey's HSD comparing all possible comparisons of behavioral response between the different *Stygobromus* species tested. *Stygobromus russelli* and *S. pecki* differed significantly from all other species but did not differ from each other.

Taxa Comparison	diff	lwr	upr	*p* adj
S. russelli-S. flagellatus	0.307	0.132	0.482	<<0.001
S. russelli-S. bifurcatus	0.36	0.145	0.576	<<0.001
S. pecki-S. bifurcatus	0.305	0.065	0.544	0.007
S. pecki-S. flagellatus	0.251	0.048	0.455	0.009
GR *S.* sp.-*S. russelli*	-0.227	-0.443	-0.011	0.035
GR *S.* sp.-*S. pecki*	-0.171	-0.411	0.068	0.259
GR *S.* sp.-*S. bifurcatus*	0.133	-0.15	0.417	0.658
S. russelli-S. pecki	0.056	-0.098	0.209	0.832
GR *S.* sp.-*S. flagellatus*	0.08	-0.174	0.334	0.890
S. flagellatus-S. bifurcatus	0.053	-0.2	0.307	0.973

4.2.1.2. Stress response

Table 8. Slopes and R^2s of the regressions of O^2 consumption for each species of *Stygobromus* tested. In the dark treatment none of the species had predictable variation in O^2 consumption except for *S. russelli* ($R^2=0.74$). However, in the light treatments, both *S. pecki* and Garden ridge *Stygobromus* sp. showed highly explainable variation which was also accompanied by a much steeper slope than what had been recorded for the dark treatments for both of these species. Interestingly, taxa was not found to be a significant predictor of variation in net O^2 consumed ($F=1.74$, $p=0.17$) but locality was ($F=3.99$, $p=0.009$) which suggest that niche adaptation may best explain variation in sensitivity to light. Another intriguing aspect of these results in that *S. russelli* did not show a respiratory response though they did respond behaviorally and Garden Ridge *S.* sp. did show a respiratory response despite not responding behaviorally.

Taxon	Locality	N	R^2 (dark)	R^2 (light)	Slope (dark)	Slope (light)
S. flagellatus	San Marcos	5	0.42	0.58	0.08	0.076
S. bifurcatus	Big Boiling, San Marcos	3	0.69	0.69	0.069	0.069
S. russelli	San Marcos, Sessom	10	0.74	0.82	0.074	0.068
Stygobromus sp.	Garden Ridge	3	0.37	0.85	0.03	0.086
S. pecki	Comal Springs	7	0.44	0.93	0.036	0.07

4.2.2. Further study of taxa shown to respond to light

4.2.2.1. Study of response to varying wavelengths

Behavioral

Table 9. Wave length was found to significantly predict the magnitude of behavioral response of *S. pecki* to light.

SOV	Df	Sum Sq	Mean Sq	F	p
Wavelength	1	0.235	0.235	25.38	<0.001
Residuals	13	0.12	0.009		

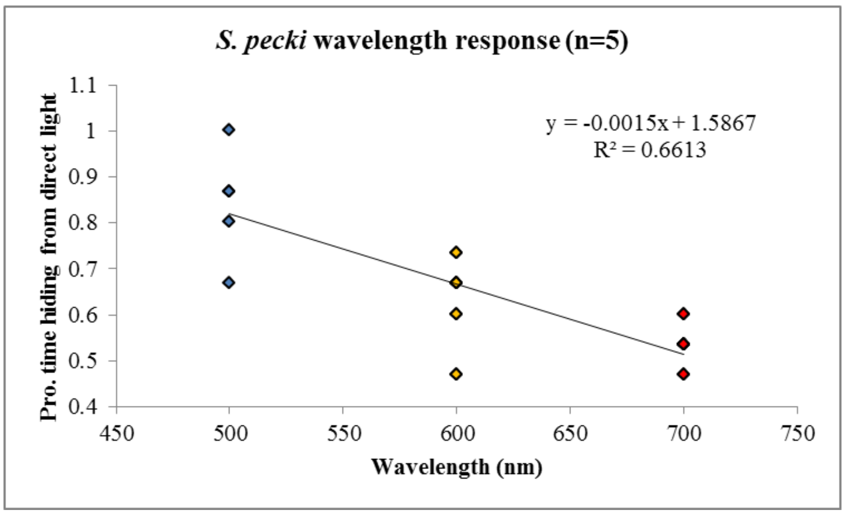

Figure 4. The magnitude of behavioral response of *S. pecki* to light was inversely related to wavelength.

Stress

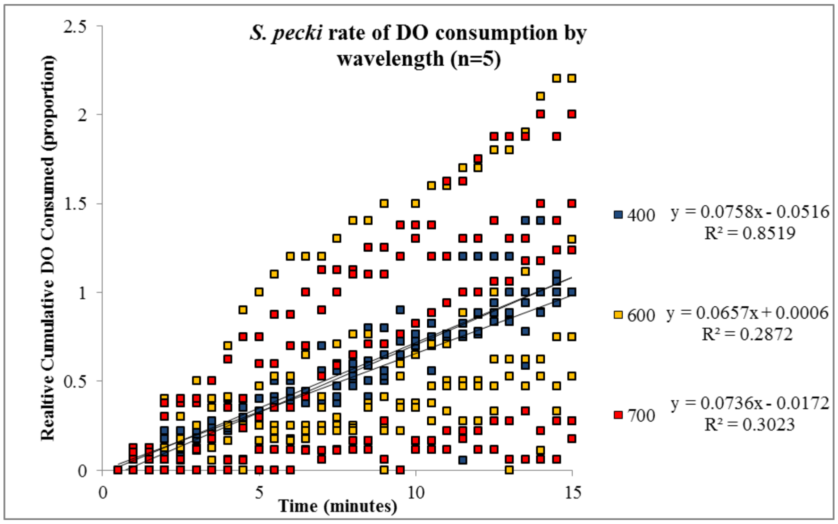

Figure 5. O^2 consumption of *S. pecki* was only found to be predictable when exposed to blue light (≈ 400 nm) despite all three treatments having similar slopes.

4.2.2.2. Study of varying intensities of full spectrum

Behavioral

Table 10. The magnitude of behavioral response of *S. pecki* to full spectrum light was found to be positively related to intensity.

SOV	Df	Sum Sq	Mean Sq	F value	P
Intensity	1	0.148	0.148	7.8	0.012
Residuals	18	0.341	0.0189		

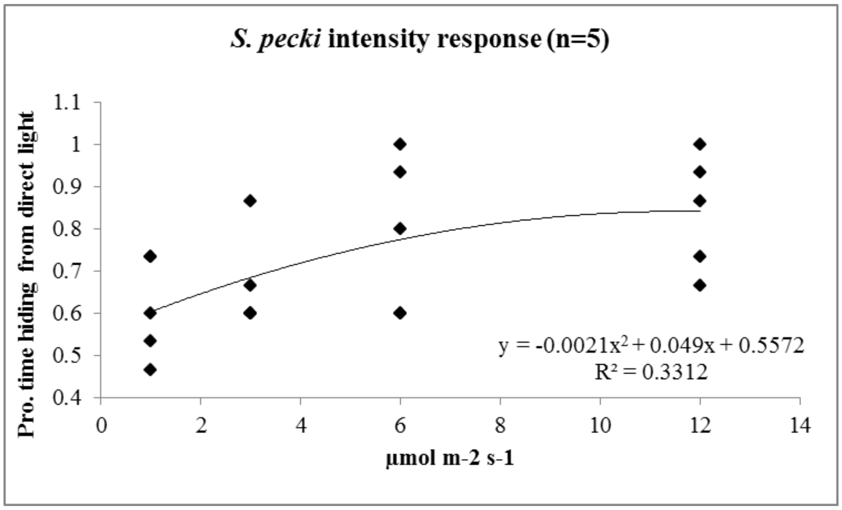

Figure 6. Despite an appreciable amount of variation between individuals, time spent avoiding light was positively related to the intensity of light.

Stress

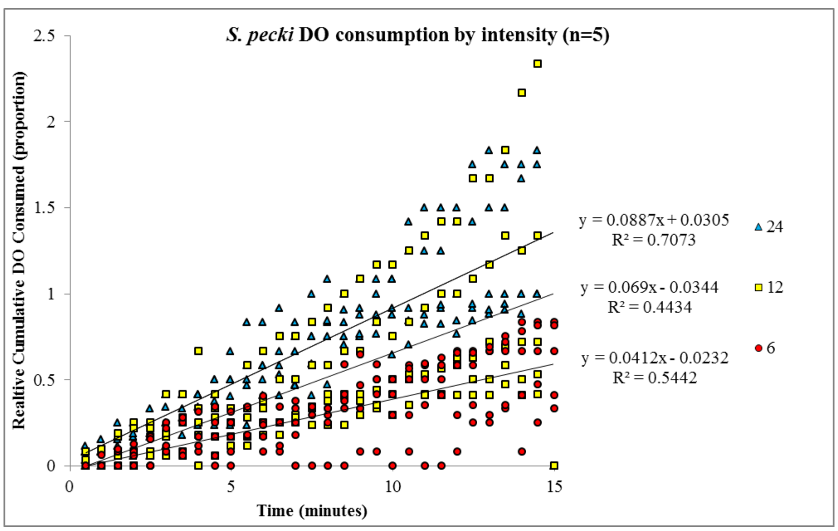

Figure 7. Once again, the greatest observable difference between treatments was differences in explainable variation which was positively related to the intensity of light. Only the highest intensities tested had an acceptable amount of explainable variation (R^2=0.71). Intensities tested were 6, 12 and 24 µmol m^2 s^{-1}.

4.2.3. Discussion

Darkness is one of the primary environmental factors that affect adaptations of subterranean species, leading to the reduction or disappearance of eyes, loss of pigmentation, and increased sensitivity of non-visual senses that presumably enhance fitness (Aden 2005). The reduction or loss of eyes in most troglomorphic organisms has been attributed to a fitness gain associated with

elimination of these structures (Romero and Green, 2005). Eyes are presumed to be functionally useless in the dark and therefore the relatively high energetic cost of developing and maintaining eyes affords no advantages that could offset the cost of investment and justify maintaining eyes. However, selection also appears to discriminate against non-synonymous nucleotide substitutions in the rhodopsin molecule (a presumably “useless” gene if sight is not important) in cave invertebrates as much as it does against surface invertebrates (Crandall and Hillis 1997). Furthermore, subterranean organisms that are presumed to be blind have been shown to be able to detect light and prefer to avoid exposure to direct light (Schlagel and Breder, 1947; Borowsky, 2011; Friedrich *et al.*, 2011, and this study). These findings are curious and difficult to explain, but it is possible that selection to remain in a cave environment outweighs the fitness disadvantages associated with maintaining light sensitive organs. If this is the case, it is no doubt because it is almost certainly disadvantageous for a sightless organism to find itself in an epigean environment.

Previous studies have shown that increased rates of oxygen consumption are indicative of environmental stress in an organism (Kedwards *et al.*,1996; Simcic and Brancelj, 2007). The stress induced by being exposed to light was demonstrated by Simcic and Brancelj (2007), who found that *Niphargus stygius* had up to a 130% increase in O_2 consumption rate when exposed to light; suggesting that avoiding light is most advantageous despite the metabolic cost involved because exposure at the surface likely represents an evolutionary dead end as finding a mate on the surface is extremely improbable and falling victim to predation is more probable.

In our study, three of the five species of *Stygobromus* responded to light, but there is no evidence from any study at present that would indicate the presence of photoreceptor nerve cells in the body of any *Stygobromus* species. Even though three of the species tested responded to light, only *S. pecki* had detectable responses in terms of behavior and DO consumption. *S. russelli* only responded behaviorally, and the Garden Ridge *Stygobromus* sp. only responded in DO consumption study. With other subterranean taxa responding to and avoiding light (e.g., Simcic and Brancelj, 2007; Borowsky, 2011), it is not surprising that many of the taxa in this study did, as well. Prior to this study it was not known if the species of concern, *S. pecki*, was sensitive to light or preferred to avoid direct exposure to light. Our findings indicate that *S. pecki* is the most sensitive of the *Stygobromus* species we examined. This is not surprising given the spring associated nature of *S. pecki*. It is difficult to explain why *S. russelli* and Garden Ridge *Stygobromus* sp. responded behaviorally to light, but not in terms of increased DO consumption. However, differences in niche specialization and common ancestry may be able to explain this after further research.

S. pecki response (avoidance of light) was shown in this study to be positively related to the intensity of full spectrum light, and inversely related to wavelength of light at a fixed intensity. Our results show that both light intensity and wavelength significantly predicted the magnitude of the response of *S. pecki* to light. However, wavelength was a better predictor, explaining more variation in the response variable in both the behavioral and stress response studies. This result is not surprising, as water is known to filter out longer wave length light first, therefore only allowing shorter wavelength light to reach an appreciable depth in water. However, there was no wavelength or intensity of light that *S. pecki* did not respond to. Because of these findings we recommend that the best culture technique for *S. pecki* in refugia would be in the

complete absence of light in order to reduce inducing increased stress. However, this condition simply is not continuously feasible if refuge workers are to have interaction with culture stock. Therefore, in order to ensure that organisms are not unnecessarily stressed while still allowing workers to maintain cultures, it may be possible to use illumination with only long wavelength portions of the visible light spectrum at low intensities. Cumulatively, these results lead us to conclude that the best measure would be to use only red light and at very low intensities.

4.3. Amphipod reproduction

Across the two-month study duration, no noticeable development was noticed in the ovaries of any of the females. In fact, the only change observed was the release of young from the marsupium of one of the females that was gravid at the beginning of the study. This showed that the duration of the experiment was not long enough to observe any measurab le development through the reproductive cycle. However, insight into the potential pairing combinations was possible given the disequilibrium of cannibalism by the two sexes (Table 11). This data suggests that males and females should be of roughly equal size to insure that neither are cannibalized and that females should never be larger than males in any of the pairings. Table 11.) Females accounted for a disproportionately greater amount of observed cannibalism, especially when females were larger. However, no instance of cannibalism was observed by the smaller of the two in any of the pairings.

Treatment	*n*	Proportion of males cannibalized	Proportion of females cannibalized
male larger	2	0	0.5
female larger	3	0.667	0
same	4	0.25	0
total	10	0.333	0.111

4.4. Amphipod holding and culture

Survivorship across the three treatment groups (Table 12) was found to vary significantly between treatments [$F_{2,6}$=16.75, p=0.0035, df=2]. However, all that variation could be accounted for in the significantly poorer survivorship of the ‘individual’ treatment group (Table 13).

Table 12.) Survivorship per replicate of each treatment across three months.

Treatment	**Replicate**	**Surviving proportion (month 1)**	**Surviving proportion (month 2)**	**Surviving proportion (month 3)**
Group	1	0.8	0.8	0.8
Group	2	1	1	1
Group	3	1	0.8	0.8
Static	1	1	1	1
Static	2	1	1	1
Static	3	1	1	1
Individual	1	0.6	0.4	0.4
Individual	2	0.6	0.2	0.2
Individual	3	0.6	0.6	0.6

Table 13.) Tukey's HSD indicated that survivorship did not vary significantly between the 'static' and 'group' treatments but did vary significantly for all other comparisons.

Comparison	diff	lwr	upr	*p*
Indi-Group	-0.467	-0.801	-0.133	0.012
Static-Indi	0.600	0.266	0.934	0.004
Static-Group	0.133	-0.201	0.467	0.483

Net growth did not vary significantly between treatment groups regardless of survivorship [$F_{2,}$)= 0.083, p=0.922]

Figure 8).

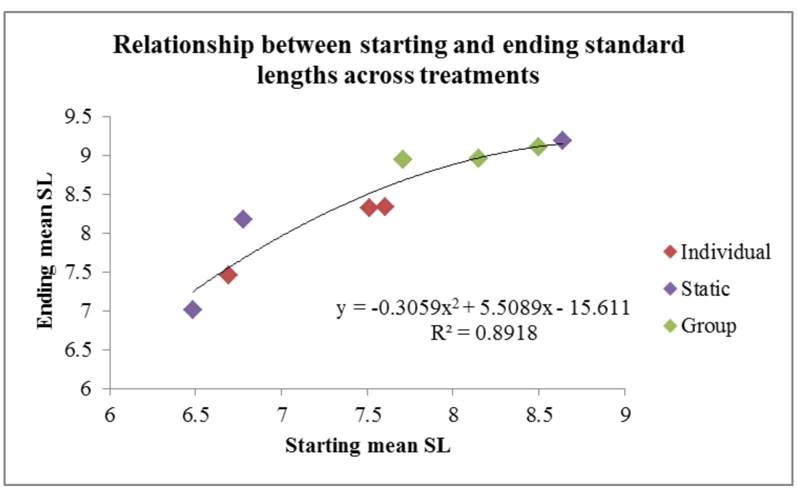

Figure 8. The ending standard length (mm) was predicted by starting standard length with a similar relationship between all replicates of all treatment groups suggesting that treatment had no effect on net growth.

4.4.1. Discussion

Results from this study suggest that the static array and group holding systems do not differ in survivorship. Although this is well supported by our data, we did not use neonates in this study. Observations made prior to this study suggested that *S. pecki* only cannibalize appreciably smaller individuals. The smallest individual used in one of the group holding treatment groups was 4.28 mm in length, which is more than twice as large as freshly hatched *S. pecki* neonates in length. Therefore, it is believed that if neonates had been incorporated in this study (which were not available at the time of this study) that the static array holding system would have had appreciably higher survivorship.

The individual flow through systems had the lowest survivorship of all treatments. The reason for this is because of the mechanics of the flow through system used at SMARC. The water supply used for all aspects of this study is prone to getting air bubbles in the lines. In the group and static array holding systems, any air fed into these systems through water lines escaped at the surface of the water. However, in the individual flow through chambers, none of the chambers were open to the surface and water that flowed in had to flow out through the mesh side panels. Because mesh size had to be small enough to prevent the escape of organisms, it also prevented the escape of air bubbles due to surface tension, thus gradually filling the individual holding chambers with air. Though effort was taken to routinely check chambers and remove any accumulated air, it was difficult to stay ahead of the rate at which chambers filled with air, thus making this holding system too tedious to be feasible for holding large numbers of organisms. However, flow-through systems could be used if they were re-designed to eliminate the issues of gas bubble accumulation that were encountered in our experimental system.

Therefore, based on prior observations and findings presented here, the recommendations for culturing are to use static array systems for monitored growth and development of neonates and to protect them from cannibalism, and then use group systems for larger organisms to allow them to encounter each other and mate. This would require frequently checking group systems for brooding females and moving any brooding females to isolation. Isolation systems currently used at SMARC for brooding females have proven to be very successful in separating mother from freshly hatched neonates. This system has two chambers stacked on top of each other separated with mesh large enough for young to pass through but too small for adults to pass through. The brooding female is placed in the upper chamber and when she releases neonates from here marsupium they are able to escape through the mesh where she cannot follow. With periodic checks of this system, neonates are able to be removed unharmed and placed in a static array system where they can be grown out to a large enough size to be introduced into group holding systems. Recommendations for use of this proposed method are based on present research and therefore future research is necessary to test the efficacy of the implementation of this proposed methodology.

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Appendix K6

Letter to the Texas State Attorney General's Office requesting an opinion

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OPINION COMMITTEE

FILE # ML-47636-14
I.D. # 47636

RQ-1220-GA

Edwards Aquifer Legislative Oversight Committee

September 3, 2014

VIA EMAIL opinion.committee@texasattorneygeneral.gov

The Honorable Greg Abbott
Attorney General of Texas
Attn: Opinion Committee
P.O. Box 12548
Austin, Texas 78711

RE: Request for opinion concerning whether a special district, or political subdivision of the state, may provide funding in the form of a grant to the federal government for the implementation of a program with public benefit under certain terms and conditions proposed by the federal government.

Dear Attorney General Abbott:

I respectfully request an Attorney General Opinion concerning the ability of the Edwards Aquifer Authority ("EAA") to provide funding to the U.S. Fish & Wildlife Service for the implementation of a refugia program under certain terms and conditions proposed by the Service. As you know, the Texas Constitution generally prohibits political subdivisions such as the EAA from providing grants of public funds to other entities, with certain exceptions. Upholding the requirements of the constitution is of course our first duty in all matters. The Edwards Aquifer Habitat Conservation Plan Program ("EAHCP"), including the refugia program, is a critical part of ensuring continued regional management of the Edwards Aquifer. I believe the EAA, other parties to the EAHCP and the public would benefit from clarification of the application of the Texas Constitution, statutes, and case law to the proposed refugia program to ensure that all legal requirements have been met.

The EAA is required to implement a refugia program under the EAHCP which was developed at the direction of the Texas Legislature in the Edwards Aquifer Authority Act. The EAHCP became effective in 2013 when the U.S. Fish and Wildlife Service ("Service") issued to the EAA, the City of New Braunfels, the City of San Marcos, the City of San Antonio, acting by and through its San Antonio Water System Board of Trustees, and Texas State University, an Incidental Take Permit ("ITP") under the federal Endangered Species Act of 1973 ("ESA").[1]

[1] As described more thoroughly in the attached brief, the refugia program is designed to temporarily house endangered species outside of their native habitats to protect their survival during times of stress, with the goal of reintroduction to the habitat at a later time.

This ITP provides users of the Southern Segment of the Edwards Aquifer ("Aquifer"), and the EAA in regulating the Aquifer, protection from Section 9(a) take liability under the ESA. The EAA is responsible for the general management and oversight of the EAHCP, including the establishment of a refugia program, subject to the duties and responsibilities held solely or jointly by the other permittees.

The EAA does not currently have refugia facilities nor is there the staff to implement a refugia, therefore, the EAHCP requires the EAA to contract with the Service to support, coordinate with, and provide funding to the Service for the implementation of the EAA's refugia program. However, the Service also does not currently have refugia facilities or necessary staff.

It is estimated that implementation of the refugia program will cost approximately $25,178,955 over the fifteen (15)-year term of the ITP. This amount includes costs to construct, equip, and staff the additional facilities necessary for the Service to have the capacity to be able to operate and maintain the refugia. The EAA will fund the refugia through its normal funding mechanism – aquifer management fees assessed against permitted users of the Aquifer.

In order for the Service to create a refugia program for the EAHCP, the Service has indicated that all refugia facilities must be located on its federally-owned land, that the improvements and equipment must be owned by the Service, and that additional staff necessary to operate the refugia must be Service employees.

Finally, and importantly, the Service is requiring that all of the costs for the additional construction, equipment, supplies, and staff necessary for the Service to operate the refugia must be funded solely by the EAA with no federal contributions. The Service advises that unless and until the EAA fully funds the refugia, and the facilities are constructed and staff hired, the Service is unable to provide any refugia services for the EAHCP.

These terms and conditions proposed by the Service appear to implicate the prohibition against the gratuitous grant of public credit and funds under Article III, Section 52(a), Texas Constitution, by political subdivisions, such as the EAA. Moreover, it is unclear whether the EAA has the statutory authority to enter into such an arrangement with the Service. Specifically, the following issues are presented:

1. May the EAA provide funding to the Service for new additional buildings and associated works and facilities, and equipment and supplies, necessary to implement the EAA's refugia program if such buildings, works, facilities, and equipment must be owned solely by the Service and be physically located on real property owned by the Service?

2. If so, does the EAA have the statutory authority to enter into a non-joint refugia project contract with the Service and make advance payments to the Service for the costs of the refugia program?

3. Are the EAA-funded refugia facilities to be owned and operated by the Service to be considered "district facilities" for purposes of Chapter 49, Subchapter I, such

that the proposed refugia contract must meet the requirements of the subchapter, or may the EAA waive such requirements in the face of Service objections?

Under the authority to issue advisory opinions granted to the Attorney General pursuant to Article IV, Section 22 of the Texas Constitution, and Subchapter C of Chapter 402 of the Texas Government Code, please accept this letter as a request for a written opinion responding to the questions raised herein. Attached you will find a brief providing you with additional background facts, the questions presented, and a discussion of the law relative to the issues raised.

In light of the critical role that the refugia program plays in the implementation of the EAHCP and the resolution of the endangered species issues relative to the management of the Aquifer, the resolution of the legal issues raised herein is necessary and in the public's interest. Furthermore, although I am fully supportive of the protection of this region's natural resources and acknowledge the importance of this particular refugia program, I want to be certain that the state of Texas is equally protected in the continued appropriate use and oversight of these funds. It is a top priority for me to ensure that ratepayer dollars supporting this program are spent in compliance with the law and sufficiently safeguarded for the purpose for which they were intended, well into the future. Accordingly, your opinion on the foregoing issues is respectfully requested.

Your attention to this matter is greatly appreciated. If you have any questions regarding this request, please do not hesitate to contact me at (512) 463-0325 in my Capitol Office.

Sincerely,

Doug Miller

Representative Doug Miller, Chair
Edwards Aquifer Legislative Oversight Committee

Cc: The Honorable Rick Perry, Governor
The Honorable David Dewhurst, Lieutenant Governor
The Honorable Joe Straus, Speaker of the House of Representatives
The Honorable Troy Fraser, Chairman of Senate Natural Resources
The Honorable Allan Ritter, House of Representatives

Attachment

RE: Request for opinion concerning whether the Edwards Aquifer Authority ("EAA") may provide funding to the U.S. Fish & Wildlife Service ("Service") for the implementation of a refugia program under certain terms and conditions proposed by the Service

1. QUESTIONS PRESENTED

1. May the EAA provide funding to the Service for new additional buildings and associated works and facilities, and equipment and supplies, necessary to implement the EAA's refugia program if such buildings, works, facilities, and equipment must be owned solely by the Service and be physically located on real property owned by the Service?

2. If so, does the EAA have the statutory authority to enter into a non-joint refugia project contract with the Service and make advance payments to the Service for the costs of the refugia program?

3. Are the EAA-funded refugia facilities to be owned and operated by the Service to be considered "district facilities" for purposes of Chapter 49, Subchapter I, such that the proposed refugia contract must meet the requirements of the subchapter, or may the EAA waive such requirements in the face of Service objections?

2. BACKGROUND FACTS

2.1 Edwards Aquifer Recovery Implementation Program

From 2006 through 2011, the EAA, along with many other stakeholders, worked to cooperatively develop a "recovery implementation program" for the benefit of the federally-listed threatened and endangered species[2] associated with the Edwards Aquifer ("Aquifer").[3] This program came to be known as the "Edwards Aquifer Recovery Implementation Program" ("EARIP").

The EAA, the Service, the Texas Commission on Environmental Quality, the Texas Parks and Wildlife Department, the Texas Department of Agriculture, the Texas Water Development Board and other stakeholders were required to prepare a "program document," which could be in the form of a "habitat conservation plan" ("HCP")[4] to support the issuance of

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[2] These species are known as the "Covered Species." For a list of the eleven Covered Species associated with the Aquifer, *see* Edwards Aquifer Habitat Conservation Plan at 1-10, Table 1-3 (Nov. 2012). This list also includes three "petitioned" species which have not yet been officially declared to be either threatened or endangered. A copy of the EAHCP is available from the EAHCP's website at http://www.eahcp.org/files/uploads/Final%20HCP%20November%202012.pdf.

[3] *See* Act of May 28, 2007, 80th Leg., R.S., ch. 1430, § 12.06, 2007 Tex. Gen. Laws 5848, 5904-5908 ("S.B. 3") (amending the Edwards Aquifer Authority Act, Act of May 30, 1993, 73rd Leg., R.S., ch. 626, 1993 Tex. Gen. Laws 2350, as amended, to add new Section 1.26A requiring the EAA to develop a "recovery implementation program").

[4] The federal Endangered Species Act of 1973 ("ESA") is codified at 16 U.S.C.A. §§ 1531-1544 (West 2014). An HCP is a document supporting an application for an Incidental Take Permit under Section 10(a)(1)(B) of the ESA (16 U.S.C.A. § 1539(a)(1)(B)) that, among other things, contains measures that the applicant will take to minimize and mitigate to the maximum extent practicable the impacts of an otherwise lawful activity on the threatened and

an "incidental take permit" ("ITP")[5] by the Service to the EAA and other permittees.[6] As finally developed by the EARIP participants, the program document included several other documents. Among these documents was an interlocal contract under Chapter 791, Texas Government Code, referred to as the "funding and management agreement" ("FMA").[7] The purpose of the FMA is to provide the terms and conditions for the management and funding of the implementation of the EAHCP.[8] Another document included was an "implementing agreement" ("IA").[9] The purpose of the IA is to define the roles and responsibilities of those parties interested in the EAHCP to ensure a common understanding of actions to be undertaken by ITP permittees to minimize and mitigate the effects of the use and regulation of the Aquifer on the threatened and endangered species associated with the Aquifer.[10]

In January 2012, the EAA, in conjunction with the City of New Braunfels, the City of San Marcos, the City of San Antonio acting by and through its San Antonio Water System Board of Trustees, and Texas State University (the "Other Permittees"), filed a joint application for an ITP with the Service. The application included the HCP, the FMA and the IA. After the ITP application was filed, the work of the EARIP participants was essentially complete and the EARIP began to wind down its activities and was replaced by the EAHCP administered by the EAA.

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endangered species that may be affected by the activity. *Id.* § 1539(a)(2)(A)(ii), (B)(ii)); *see also* 50 C.F.R §§ 17.22(b)(1)(iii)(B), (b)(2)(i)(B); 17.32(b)(1)(iii)(C)(2), (b)(2)(i)(B) (2013).

[5] An ITP issued under Section 10(a)(1)(B) of the ESA (16 U.S.C.A. § 1539(a)(1)(B)) acts as an exception to "take" liability under Section 9(a)(1)(B) of the ESA (16 U.S.C.A. § 1538(a)(1)(B)) by authorizing activities that may result in the "take" of protected species if the "taking is incidental to, and not the purpose of, the carrying out of an otherwise lawful activity." *Id.* § 1539(a)(1)(B). For example, the withdrawal of groundwater from the Aquifer by well owners, and the regulation of such withdrawals by the EAA, are lawful activities under Texas law. *See generally* Edwards Aquifer Authority Act, Act of May 30, 1993, 73rd Leg., R.S., ch. 626, 1993 Tex. Gen. Laws 2350, as amended ("EAA Act"), §§ 1.08; 1.14-1.20. The EAA Act is uncodified. The EAA Act, with all amendments incorporated therein, is available from the EAA's website at http://edwardsaquifer.org/legislation-and-rules/the-eaa-act.

[6] *Id.* § 1.26A(b), (c), and (d).

[7] *See* Funding and Management Agreement by and among the Edwards Aquifer Authority, the City of New Braunfels, the City of San Marcos, the City of San Antonio, acting by and through its San Antonio Water System Board of Trustees, and Texas State University – San Marcos to fund and manage the Habitat Conservation Plan for the Edwards Aquifer Recovery Implementation Program (eff. Jan. 1, 2012). A copy of the FMA is available from the EAHCP's website at http://www.eahcp.org/files/uploads/Funding_and_Management_Agreement_(Appendix_R).pdf.

[8] *Id.*, Recital B at 2.

[9] *See* Implementing Agreement by and among the Edwards Aquifer Authority, the City of New Braunfels, the City of San Marcos, the City of San Antonio acting by and through its San Antonio Water System Board of Trustees, Texas State University – San Marcos, and the Texas Parks and Wildlife Department and United States Fish and Wildlife Service to implement the Habitat Conservation Plan for the Edwards Aquifer Recovery Implementation Program (eff. Mar. 4, 2013). A copy of the IA is available from the EAHCP's website at http://www.eahcp.org/files/admin-records/NEPA-and-HCP/Imp_Agr_Doc_with_TCEQ_sig.pdf.

[10] *Id.* at 1.

2.2 Edwards Aquifer Habitat Conservation Plan Program

In February 2013, the Service approved the application and issued ITP TE63663A-O to the EAA and the Other Permittees to be effective March 2013.[11] By issuing the ITP, the Service also approved the HCP, the FMA, and the IA, and the approved HCP has come to be known as the Edwards Aquifer Habitat Conservation Plan Program ("EAHCP").[12] Since issuance, the EAA and the Other Permittees have been implementing the EAHCP, however, the EAA is responsible for the general management and oversight of the EAHCP, including the establishment of the refugia program, subject to the duties and responsibilities held solely or jointly by the Other Permittees.[13]

The approved EAHCP includes "minimization and mitigation measures" (known as "Conservation Measures")[14] that "are designed to ensure that incidental take resulting from the Covered Activities will be minimized and mitigated to the maximum extent practicable and will not appreciably reduce the likelihood of the survival and recovery of covered species associated with the Aquifer and Comal and San Marcos Springs and Rivers ecosystems."[15]

2.3 The EAA's Refugia Program

Among the Conservation Measures to be implemented by the EAA, and most relevant to this request, is a refugia program.[16] Paragraph K of the ITP provides as follows:

> The EAA will support and coordinate with the USFWS (Service) on the work relating to the San Marcos Aquatic Resource Center's operation and maintenance of a series of off-site refugia at the Service's San Marcos, Uvalde, and Inks Dam facilities. (Section 6.4 of the HCP). The support of the refugia will augment the existing financial and physical resources of these facilities, and provide supplementary resources for appropriate research activities, as necessary, to house and protect adequate populations of Covered Species and expanded knowledge of their biology, life histories, and effective reintroduction techniques. The use of this support will be limited to the Covered Species in the EARIP HCP.

The above language is nearly identical to that set out in Section 5.1.1 of the EAHCP.

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[11] A copy of ITP TE63663A-O is available from the EAHCP's website at http://www.eahcp.org/files/admin-records/NEPA-and-HCP/USFWS_Permit_03-18-2013_rcvd_1030_a.m._Final.pdf.

[12] *See supra* note 1 for the EAHCP.

[13] FMA § 2.2.

[14] *See* EAHCP, ch. 5 for the complete catalog of the Conservation Measures.

[15] EAHCP § 1.1.1. "Covered Activities" means those activities described in Chapter 2 of the EAHCP for which incidental take authorization of Covered Species is authorized pursuant to the ITP. Examples of Covered Activities include the withdrawal of groundwater from the Aquifer, the regulation of the Aquifer by the EAA, the management of public recreation on the Comal and San Marcos Rivers by New Braunfels and San Marcos, respectively, diving classes at Texas State University, the operation by SAWS of its Aquifer Storage and Recovery facility, and the development of a "state scientific area" by the Texas Parks and Wildlife Department.

[16] EAHCP § 5.1.1.

As used in the EAHCP, a refugium means an off-site facility designed and dedicated to the care, housing, and maintenance of individuals or populations of Covered Species in an artificial habitat to protect them from and to avoid the negative effects of drought disturbance, disease outbreaks, and water quality impairment in the Comal and San Marcos Springs and Rivers ecosystems.

The primary purpose of the EAHCP refugia program is to provide a location where viable source populations of Covered Species can survive these disturbances, and be reintroduced and repopulate in the Comal and San Marcos Springs and Rivers ecosystems following a disturbance in non-refugia areas.

To accomplish this, three categories of population stocks of Covered Species will be collected and managed at the refugia: (1) "standing stocks" will always be in the refugia as backup in the event an emergency occurs in the Comal and/or San Marcos Springs and Rivers ecosystems; (2) "refugium stocks" will be collected when conditions in the wild for breeding are poor and held in the refugia for breeding and possible later reintroduction of offspring back into the wild; and (3) "salvage stocks" will be collected to prevent death when conditions in the wild have deteriorated and they will be held in the refugia for possible later reintroduction when conditions in the wild improve.

In addition, it is planned that research will be conducted at the refugia that would be directed at husbandry, propagation, and reintroduction of Covered Species maintained at the refugia.

The proposed locations for the three refugia facilities under the EAHCP are the San Marcos Aquatic Resource Center ("SMARC") near San Marcos, Texas, the Inks Dam National Fish Hatchery ("Inks Dam") near Burnet, Texas, and the Uvalde National Fish Hatchery ("Uvalde") near Uvalde, Texas. These three facilities are owned and operated by the Service.

2.4 Terms and Conditions Proposed by the Service for the Refugia Contract

Neither the EAA nor the Service currently has the facilities or staff to implement the ITP- and EAHCP-mandated refugia program. Nonetheless, Paragraph K of the ITP and Section 5.1.1 of the EAHCP require the EAA to support, coordinate with, and provide funding to the Service for the refugia program. This will necessarily be accomplished under a contract between the EAA and the Service.

The cost to construct, operate, maintain, and staff refugia at the SMARC, Inks Dam, and Uvalde facilities is estimated to be $25,178,955 over the fifteen (15)-year term of the ITP.[17] The approximate cost to construct new buildings and associated works, facilities, equipment, and

[17] EAHCP Table 7.1. The ITP became effective on March 18, 2013, and expires on March 21, 2028. ITP at 1. Since the ITP is in year two (2) of its term, and the refugia program is not yet under implementation, the estimated total implementation costs for the refugia would need to be downwardly adjusted by the amounts in Table 7.1 for each calendar year during the ITP that the refugia program has not been implemented.

utilities is estimated to be $10,854,000. The estimate for staff support and services is $14,324,955.

Conceptually, the Service proposes a management contract that would contain the terms and conditions under which it would be willing to contract with the EAA to implement the refugia program.[18] This contract would encompass the construction of the additional buildings and associated works and facilities by the Service, the acquisition of necessary equipment and supplies, and staffing as necessary to provide the Service with sufficient capacity to perform the refugia services. The Service has indicated that deviations from the proposed terms and conditions will not be accepted. Because the Service does not currently have the necessary capacity to provide refugia services to the EAA, the Service has indicated that its offer is conditioned upon the EAA providing full funding in advance of the Service performing any refugia-related activities. Additionally, the Service advises that until the necessary capacity is in place, the Service is unable to and will not provide any refugia services to the EAA, including even reduced level refugia activities pending the construction of the necessary facilities.

Relative to the additional buildings, works, facilities, equipment, and supplies, the Service has indicted that they must be owned by the Service and located on federally-owned land, with no ownership, title or interest of any kind in the EAA. Nor does the Service propose any reversionary interest in the EAA after the expiration of the ITP term in March 2028. Thus, the Service would continue to own all of the improvements funded by the EAA even though the improvements on federal land may continue to have a useful life that will not accrue to the benefit of the EAA. Control over the design, construction and equipment plans and specifications, cost schedules, procurement, construction, and construction management and oversight, and operation and maintenance would be vested exclusively in the Service.

Relative to the additional staff, they would be employees of the Service. Control over these employees would be with the Service.

Regarding providing refugia-related professional services (as opposed to construction activities), the Service proposes a contract in the form of a "reimbursable agreement" in which the Service would provide staffing and services and other products. The agreement would provide for: (1) a scope of work; (2) the cost of the work; (3) a transfer of funds from the EAA to the Service in advance of the Service performing any work; (4) annual work plans to include the specific work to be done in a particular calendar year and the cost of such services; and (5) after performance of the services, the Service would bill against the advanced funds provided by the EAA with a detailed documented accounting of the funds spent, billed, and balances, all through a federal accounting system.

In short, the Service proposes for the EAA to engage and fund the Service to perform all refugia-related construction and services as long as the EAA provides full funding in advance. The Service has indicated that as long as the EAA provides the agreed-to funding, it will

[18] *See* Letter from L. Stewart Jacks, Assistant Regional Director, Fish and Aquatic Conservation, Service to Roland Ruiz, General Manager, EAA (July 16, 2014) (on file with the EAA).

consider the EAA to be in full compliance with Paragraph K of the ITP, and the EAA will have met its legal duty to implement a refugia program under Section 5.1.1 of the EAHCP.[19]

2.5 Funding of the EAHCP

The ITP was issued subject to "full and complete compliance with, and implementation of, the EARIP HCP and all specific conditions contained herein."[20] The IA provides that: "Permittees will provide such funds as may be necessary to carry out their respective obligations under the HCP, as set out in Section 7 of the HCP, and amplified in Article 5 of the FMA."[21] The EAHCP provides that:

> To issue the ITP, USFWS must find that the Applicants 'will ensure that adequate funding for the [HCP] will be provided.' (16 U.S.C. § 1539(a)(2)(B)(iii)). To satisfy this requirement, the costs of implementing the HCP are set out below [in chapter 7 of the EAHCP] along with the assurance that funding will be available to implement the HCP. Specifics regarding the funding arrangements for the HCP are found in Articles Three and Five of the FMA, (Appendix R) and that are generally described briefly in Sections 7.1.1 and 9.1.1 below.[22]

Under the FMA, the EAA, beginning January 1, 2013, has the obligation to fully fund the implementation costs of the EAHCP.[23] The FMA provides that the implementation costs for the EAHCP will be funded primarily through "program aquifer management fees" ("PAMFs") assessed by the EAA against holders of EAA-issued groundwater withdrawal permits.[24] The EAA is authorized to assess its regular aquifer management fees ("AMFs") to finance the administrative expenses and programs of the EAA authorized under the EAA Act.[25] A PAMF is a category of AMF collected by the EAA under Section 1.29 of the EAA Act to fund the costs of the implementation of the EAHCP.[26] The EAHCP is one of the expenses and programs under the EAA Act and, therefore, the EAA may assess AMFs to fund this program.[27] The EAA must

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[19] Letter from Adam Zerrenner, Field Office Supervisor, Austin Ecological Services Field Office, Service, to Nathan Pence, Program Manager, EAA (Nov. 14, 2013) (on file with the EAA). Essentially, this acknowledgment shifts the burden of actual day-to-day compliance for the operation of the refugia from the EAA to the Service in exchange for the EAA's commitment to fund the refugia program.

[20] ITP ¶ E.

[21] IA § 10.0.

[22] EAHCP § 7.0. *See also* 50 C.F.R §§ 17.22(b)(1)(iii)(B), (b)(2)(i)(C); 17.32(b)(1)(iii)(C)(2), (b)(2)(i)(C) (2013).

[23] FMA §§ 3.2, 5.2.1; and EAHCP § 7.1.2.

[24] FMA §§ 5.1, 5.2.2; and EAHCP § 7.1.2. Contributions are also anticipated from other governmental, corporate, and associational entities. FMA §§ 5.1, 5.3, 5.5.2; and EAHCP § 7.1.2.

[25] *See* EAA Act § 1.29(b).

[26] *See* FMA § 1.1.41; and *see also* EDWARDS AQUIFER AUTHORITY RULES § 709.18(a)(2) (2013). A copy of the EAA's rules are available from the EAA's website at http://edwardsaquifer.org/legislation-and-rules/rules-and-regulations.

[27] *See* EAA Act §§ 1.11(d)(9), 1.14(h), and 1.26A.

annually assess PAMFs at levels sufficient to fully fund the implementation of the EAHCP.[28] The amount of funding that is required to meet the full funding obligations is provided for in Section 5.2.1 of the FMA and Table 7.1 of the EAHCP, and includes a line item for the refugia program.[29]

3. DISCUSSION

In order to evaluate the issues raised in this request, it is important to evaluate the legal nature of the EAA and its legal authority generally and specifically, with respect to threatened and endangered species protection, the expenditure of its funds and contracting.

3.1 Legal Nature of the EAA

The EAA is a conservation and reclamation district created by the EAA Act pursuant to Article XVI, Section 59 of the Texas Constitution.[30] The Legislature characterizes the EAA as a "special regional management district"[31] The EAA is also a groundwater conservation district ("GCD") under Chapter 36 of the Texas Water Code.[32] As such, the EAA is a political subdivision of the state and stands on the same footing as counties and other political subdivisions.[33]

3.2 Legal Authority of the EAA in General

The legal authority of the EAA is initially found in its organic act – the EAA Act. Section 1.08(a) of the Act also provides that the EAA may look to Chapters 36,[34] 49,[35] and 51 of the

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[28] FMA §§ 5.2.1, 5.2.2.

[29] FMA § 5.2.1; EAHCP § 7.1.2, and Table 7.1. The actual level of estimated funding initially required by the EAA for calendar year 2013 was $20,416,847. *See* FMA §§ 3.2; 5.2.1; and EAHCP §§ 7.1.1, 7.1.2, and Table 7.1. Beginning in 2014, the EAA's funding obligation may be adjusted up or down based on actual experience. FMA § 5.2.1. However, the EAA's funding obligation for any year may never be increased beyond the level of the funding obligation for 2013, adjusted for a 2% increase, compounded annually for the years that have elapsed since 2013. *See id.* §§ 3.2, 5.2.1; and EAHCP § 7.1.2.

[30] EAA Act §§ 1.02(a); 1.06(b).

[31] *Id.* § 1.01.

[32] *See Edwards Aquifer Auth. v. Chem. Lime, Ltd.*, 291 S.W.3d 392, 399 at n. 37 (Tex. 2009) (finding that the EAA is a "district" as defined in Section 36.001(1), Texas Water Code); *see also In re Edwards Aquifer Auth.*, 217 S.W.3d 581, 587 (Tex. App.—San Antonio 2006, orig. proceeding).

[33] *See* TEX. CONST. art. XVI, § 59(b); *Bennett v. Brown Cnty. Water Improvement Dist. No. 1*, 272 S.W.2d 498, 500 (Tex. 1954), *accord Willacy Cnty. Water Control and Improvement Dist. No. 1 v. Abendroth*, 177 S.W.2d 936, 937 (Tex. 1944).

[34] Section 1.08(a) of the Act does not specifically reference Chapter 36, Texas Water Code, but, instead, refers to Chapter 52, Texas Water Code: "The authority has all of the rights, powers, privileges, authority, functions, and duties provided by the general law of this state, including Chapters 50, 51, and 52, Water Code . . ." In 1995, Chapter 52 was repealed and recodified at Chapter 36. *See* Act of May 29, 1995, 74th Leg., R.S., ch. 933, §§ 2, 6, 1995 Tex. Gen. Laws 4673, 4679, 4701. Texas courts have held that due to this recodification, Chapter 36 applies to the EAA. *See e.g., In re Edwards Aquifer Auth.,* 217 S.W. 3d at 588 ("[b]ased on the plain language of the statute, we hold that Chapter 36 of the Water Code applies to the Authority"); *see also Edwards Aquifer Auth. v. Chem. Lime, Ltd.*, 291 S.W.3d 392; and *Edwards Aquifer Auth. v. Horton*, No. 04-09-00375-CV, 2010 WL 374551 (Tex.

Texas Water Code, for additional legal authority, as well as other general laws applicable to authorities created under Article XVI, Section 59, of the Texas Constitution.[36]

The Texas Constitution specifically provides that special districts created under Article XVI, Section 59, have only such powers and authorities as "may be conferred by law."[37] Therefore, unlike home rule cities, a groundwater conservation district, such as the EAA, has only those powers expressly granted to it by the Legislature,[38] and those powers that are necessarily implied in order to carry out its express powers.[39] If a statute does not grant a power expressly, or by reasonable implication, then a GCD has no legal authority to act.[40] If a GCD exceeds its powers, such conduct is *ultra vires* and is void.[41]

App.—San Antonio 2010, pet. denied) (mem. op., not designated for publication) (applying certain sections of Chapter 36 to the EAA); and *see also* Tex. Att'y Gen. LO-97-012 (1997).

[35] Section 1.08(a) of the Act does not specifically reference Chapter 49 of the Water Code, but, instead, refers to Chapter 50 of the Water Code. Chapter 50 was repealed and recodified at Chapter 49. *See* Act of May 25, 1995, 74th Leg., R.S., ch. 715, §§ 2, 39, ch. 49, 1995 Tex. Gen. Laws 3755, 3802. Based on the same logic as discussed above, Chapter 49 also applies to the EAA. *See e.g.* Tex. Att'y Gen. Op. No. JC-0006, 2 (1999).

[36] EAA Act § 1.08(a).

[37] TEX. CONST. art. XVI, § 59(b).

[38] *See, e.g., Mobil Oil Corp. v. Matagorda Cnty. Drainage Dist. No. 3*, 597 S.W.2d 910, 913 (Tex. 1980) ("The distinction between the broad powers of such municipalities and the limited powers of special districts such as drainage districts has been previously recognized by this court."); *Franklin Cnty. Water Dist. v. Majors*, 476 S.W.2d 371, 373 (Tex. Civ. App.—Texarkana 1972, writ ref'd n.r.e.) ("A water district . . . can only do that which is authorized by the statute creating it."); and *Tri-City Fresh Water Supply Dist. No. 2 of Harris Cnty. v. Mann*, 142 S.W.2d 945, 948 (Tex. 1940) ("The powers of such districts are measured by the terms of the statutes which authorized their creation, and they can exercise no authority that has not been clearly granted by the legislature.")

[39] *See* Tex. Att'y Gen. Op. No. JC-11 (1999) (citing *Tex. Roofing Co. v. Whiteside,* 385 S.W.2d 699, 701 (Tex. Civ. App.—Amarillo 1964, writ ref'd n.r.e) as standing for the proposition that political subdivisions have "only those powers expressly conferred on them by the constitution or by statute or those necessarily implied from the powers conferred") *and Benavides Ind. Sch. Dist. v. Guerra*, 681 S.W.2d 246, 249 (Tex. App.—San Antonio 1984, writ ref'd n.r.e) (indicating that a political subdivision cannot act in a manner contrary to express or implied statutory authority or else its action is void).

[40] *See Franklin Cnty. Water Dist*, 476 S.W.2d at 373; *Benavides*, 681 S.W.2d at 249 (explaining that a political subdivision's action is void if it is executed in a manner contrary to express or implied statutory authority). Relatively recent examples of the need for GCDs to demonstrate their express authority to support their actions include *Guitar Holding Company, L.P. v. Hudspeth County Underground Water Conservation District No. 1*, 263 S.W.3d 910, 917 (Tex. 2008) (GCD's actions to link transfer permits to existing permits exceeded its statutory authority under Chapter 36, Texas Water Code); *South Plains Lamesa R.R., Ltd. v. High Plains Underground Water Conservation Dist. No. 1*, 52 S.W.3d 770, 779-80 (Tex. App.—Amarillo 2001, no pet.) (GCD not authorized to limit groundwater withdrawals based on tract size because Chapter 36, Texas Code, did not "clearly authorize" the regulation of groundwater withdrawals by that method); and Tex. Att'y Gen. Op. No. GA-498, 11 (2007) (EAA not authorized to issue permits comprised of "senior" and "junior" withdrawal rights, with junior rights being interrupted when the Aquifer was below a certain level, as to do so exceeded its authority under EAA Act).

[41] *Houston Natural Gas Corp. v. Nueces Cnty. Water Improvement Dist. No. 1,* 157 S.W.2d 170, 171 (Tex. Civ. App.—San Antonio 1941) (holding the distribution of natural gas to be *ultra vires*).

3.3 Legal Authority of the EAA Relative to Species Protection

The EAA was created to manage and regulate withdrawals from the Aquifer.[42] Section 1.08(a) of the EAA Act provides that the EAA has "all of the powers, rights, and privileges necessary to manage, conserve, preserve, and protect the aquifer and to increase the recharge of, and prevent the waste or pollution of water in, the aquifer."[43] The EAA, however, has the additional important function of developing and implementing a program to protect the federally-listed threatened or endangered species associated with the Aquifer. In this regard, the EAA was created in 1993 in response to, and to provide a mechanism to prevent, a federal takeover of the Aquifer under the ESA.[44] Section 1.01 of the EAA Act acknowledges that a legislative policy in creating the EAA was to, among other things, "effective[ly] control . . . the [Aquifer] . . . to protect terrestrial and aquatic life . . ."[45]

To provide for the EAA's implementation of its species protection program, the Legislature provided the EAA with the following express authority:

1. The EAA may hold permits under federal law pertaining to the ESA.[46]

2. The EAA is required to manage withdrawals from the Aquifer to protect aquatic and wildlife habitat, and protect species that are designated as threatened or endangered under the ESA.[47]

3. The EAA is required to implement and enforce water management practices,

[42] The portion of the Aquifer over which the EAA exercises its jurisdiction is the segment of the Aquifer within the boundaries of the EAA lying between Brackettville, Texas, in Kinney County and Kyle, Texas, in Hays County. EAA Act §§ 1.03(1); 1.08(a), (b). This segment is referred to as the Southern Segment of the Aquifer (as opposed to the Barton Springs or Northern Segments of the Aquifer, over which the EAA has no jurisdiction). The Southern Segment is also sometimes referred to as the "San Antonio Segment."

[43] *Id.* § 1.08(a).

[44] *See Sierra Club v. Lujan*, No. MO-91-CA-069, 1993 WL 151353 (W.D. Tex. 1993) (opinion issued); *Sierra Club v. Babbitt*, 995 F.2d 571 (5th Cir. 1993); *Sierra Club v. City of San Antonio*, 112 F.3d 789 (5th Cir. 1997), *cert. denied*, 522 U.S. 1089 (1998). In creating the EAA, a *Burford* abstention defense became available to the State to successfully defend the Aquifer from management by the federal courts under the ESA. *Id.* Indeed, the 5th Circuit in *City of San Antonio* stated its views on the passage of the EAA Act as follows:

> [T]he Edwards Aquifer Act can fairly be characterized as a comprehensive regulatory scheme. It represents a sweeping effort by the Texas Legislature to regulate the aquifer, with due regard for all competing demands for the aquifer's water. The Act vests the . . . Authority with 'all the powers and privileges necessary to manage, conserve, preserve, and protect the aquifer . . .' The Authority controls withdrawals from the aquifer through a permit system.. . . The Act also specifically addresses the preservation of endangered species. Under § 1.14 of the Act the Authority must 'protect aquatic and wildlife habitat' and 'protect species that are designated as threatened or endangered under applicable federal or state law.'

Id. at 794.

[45] EAA Act § 1.01.

[46] *Id.* § 1.11(d)(9). This subsection also authorizes the EAA to hold permits under state law.

[47] *Id.* § 1.14(a) (6), and (7).

procedures, and methods to ensure that, not later than December 31, 2012, the continuous minimum springflows at Comal Springs and San Marcos Springs are maintained to protect endangered and threatened species to the extent required by federal law.[48]

4. In 2007, the Legislature required the EAA to cooperatively develop a "recovery implementation program" for the benefit of the federally-listed threatened and endangered species associated with the Aquifer.[49]

5. The EAA is also required to adopt a final "critical period plan" in light of the recovery implementation program.[50]

3.4 Extension of Public Credit or Public Funds by Political Subdivisions

As discussed above in Section 2.4, the EAHCP requires the EAA to support, coordinate with, and provide funding to the Service for the implementation of the refugia program under Section 5.1.1 of the EAHCP. This will necessitate the EAA entering into a contract with the Service for this purpose. The terms and conditions proposed by the Service are set out in Section 2.4 above and appear to implicate the prohibition against gratuitous grants of public credit and funds under Article III, Section 52(a), Texas Constitution, by political subdivisions, such as the EAA.

Article III, Section 52(a) of Article III provides as follows:

> [T]he Legislature shall have no power to authorize any county, city, town, or other political corporation or subdivision of the State to lend its credit or to grant public money or thing of value in aid of, or to any individual, association or corporation whatsoever, or to become a stockholder in such corporation, association or company. . . .

This section prohibits the state from authorizing political subdivisions to gratuitously lend their public credit or public moneys to any individual, association, or corporation. Similar prohibitions for state agencies are found at Article III, Sections 50 and 51, Texas Constitution.

As discussed above in Section 2.4, the Service is requiring that the EAA expend its public funds to construct permanent improvements to be owned by the Service on Service-owned land. The Attorney General has had multiple occasions to consider whether a state agency or a political subdivision may expend public funds to construct permanent improvements on land not owned by the state agency or political subdivision in light of the prohibition against gratuitous grants of public credit or funds. The general rule appears to be that, if certain criteria are met,

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[48] *Id.* § 1.14(h). The "federal law" referred to in Section 1.14(h) is undoubtedly the ESA. *See id.* § 1.11(d)(9) (authorizing the EAA to seek permits under the ESA).

[49] *Id.* § 1.26A.

[50] *Id.* § 1.26(a).

public funds may be expended without contravening Article III, Sections 50, 51, and 52(a) if the improvements are made on land in which the funding agency holds a sufficiently long-term leasehold interest.[51] The criteria that need to be satisfied to authorize such an arrangement are that: (1) the expenditure of funds is supported by statutory authority;[52] (2) a valid public purpose will be served; (3) adequate consideration will be received by the funding agency; (4) adequate controls by the funding agency are maintained to ensure that the public purpose will be achieved; and (5) the benefit to the land owner is merely incidental to and not the primary purpose of the expenditure.[53]

The Attorney General recognized the appropriateness of public expenditures in several circumstances not involving a lease. First, the Attorney General found that state funds may be expended to construct university-owned improvements on property owned by a trust dedicated to the support and benefit of the university.[54] Second, the Attorney General has approved the expenditure of public funds to construct permanent improvements on land owned by a university, notwithstanding the existence of reversionary interests held by the grantor.[55] Finally, county expenditures to help construct a school swimming pool on school-owned property were found to be permissible if the county has the statutory authority to do so and retains a sufficient level of joint responsibility and continued participation in the project.[56]

The Service's proposed terms and conditions discussed in Section 2.4 in essence provide for a political subdivision (i.e., the EAA) to serve as a funding agency to a federal agency (i.e., the Service) to construct federally-owned buildings on federally-owned land so that the federal agency will have the capacity to perform an activity (i.e., implement a refugia program) that the political subdivision desires to have the federal agency perform on its behalf. This scenario does not appear to have been previously considered by the Attorney General.[57]

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[51] *See e.g.,* Tex. Att'y Gen. Op. No. LO-97-078 (1997) (state expenditures to construct airport on land leased to the state); Tex. Att'y Gen. Op. No. MW-290 (1981) (county expenditures to improve hospital leased from another district); Tex. Att'y Gen. Op. No. H-416 (1974) (state expenditures to assist in the construction of a city airport on school-owned property leased to a city); Tex. Att'y Gen. Op. No. H-403 (1974) (state expenditures to construct state livestock export station on privately-owned property leased to the state); and Tex. Att'y Gen. Op. No. H-257 (1974) (state expenditures to construct state recreational facilities on federally-owned property leased to the state). Similarly, public expenditures to acquire and rehabilitate a privately-owned building with the intent to lease the building back to the previous owner have been found not to violate the constitutional limitation on gratuitous grants of public funds. Tex. Att'y Gen. Op. No. H-445 (1974).

[52] *See e.g.* Tex. Att'y Gen. Op. No. JM-65 (1983) (noting that "a primary issue is whether the county has authority to perform the specific service [for which it is seeking to contract]. Without that authority, an expenditure by the county constitutes a donation of county's funds in violation of the constitution."); Tex. Att'y Gen. Op. No. MW-532 (1982) (state expenditures to private landowners to reforest their privately-owned lands without statutory authority violates Article III, Section 51); and Tex. Att'y Gen. Op. No. MW-329 (1981) (county expenditures to non-profit corporation to construct building for job skill training without statutory authority violates Article III, Section 52).

[53] *See supra* notes 50 and 51.

[54] Tex. Att'y Gen. Op. JM-551 (1986).

[55] Tex. Att'y Gen. Op. MW-514 (1982); and Tex. Att'y Gen. Op. No. H-655 (1975).

[56] Tex. Att'y Gen. Op. No. H-413 (1974).

[57] *See supra* notes 50 and 51 and the authorities discussed therein.

As discussed below in Section 3.5, the EAA has the statutory authority to implement a refugia program and to engage the Service to assist in this program and such an expenditure would have the valid public purpose of protecting the Covered Species associated with the Aquifer. However, it is not clear, based on prior Attorney General opinions, especially because the title to the buildings, works, facilities, and equipment necessary to implement the EAA's refugia program would be solely in the Service, and these improvements and equipment would be required to be physically located on Service-owned land, whether the EAA would receive adequate consideration, whether the EAA would be able to maintain adequate control to ensure that the public purpose was being achieved, or whether the benefit to the federal government would be merely incidental. Accordingly, guidance is sought from the Attorney General on whether the EAA may provide funding to the Service under the terms and conditions proposed by the Service, or whether such funding would contravene the gratuitous grant prohibition of Article III, Section 52(a).

Additional guidance is sought on whether certain contractual provisions would be legally adequate for the EAA to ensure that: (1) adequate consideration will be received by the EAA for the funding of improvements and equipment to be owned by the Service on Service-owned land; (2) adequate control will be maintained by the EAA to ensure that the EAA's public purpose will be achieved; and (3) any benefit to the federal governmental will be only "incidental." Specifically, guidance is sought from the Attorney General on whether a contractual requirement that the Service use the EAA-funded improvements and equipment solely to implement the EAA's refugia program for the benefit of the Covered Species and/or the shifting of operational compliance to the Service[58] would be sufficient to satisfy the adequate consideration requirement, notwithstanding the fact that the EAA has no ownership, leasehold, or reversionary interest of any kind in such improvements and equipment. As for the control requirement, the Attorney General's guidance is sought on whether the right to inspect, monitor, and audit the refugia facilities and activities would be a sufficient control to ensure that the public purpose of the EAA's funding was being achieved. This issue is of particular importance in light of the fact that the EAA will have no ownership, leasehold, or reversionary interest of any kind in the improvements and equipment, and the staffing of the facilities will be solely by federal employees not subject to EAA control. Finally, in light of the federal ownership of all land, improvements, and equipment, and the federal staffing of the refugia, coupled with the duty of the EAA to solely and fully fund the refugia program, the Attorney General's guidance is sought on whether a contractual requirement that the Service use the EAA-funded improvements and equipment solely to implement the EAA's refugia program for the benefit of the Covered Species would be sufficient to ensure that the EAA's funding under these circumstances will result in only an "incidental" benefit to the Service, and avoid a result that the primary purpose of the funding is to benefit the Service.[59]

[58] *See supra* note 18.

[59] It would also seem that in order for the EAA to protect its investment in the refugia facilities, the provision to use the improvements funded by the EAA solely for the purpose of the EAHCP's refugia program would need to be accompanied by appropriate default provisions in the event the Service does not or is not able to use the facilities solely for this purpose, including, for example, a reversionary interest in the EAA, with other remedies such as a right of access and the ability to assume control of the facility. It should be noted that the term of the refugia contract would be for the unexpired term of the ITP. Such term may be less than the useful life of any improvements and equipment funded by the EAA. Moreover, there is always a possibility that the federal government could defund or otherwise close the SMARC, Inks Dam, and/or Uvalde facilities. It is not understood by the EAA that the Service

3.5 Statutory Authority Relative to Advance Payments and Non-Joint Projects

As discussed above in Section 2.4, the Service is requiring that the EAA provide refugia funding to the Service before the Service constructs any improvements, acquires any equipment or supplies, or performs any refugia-related services. Guidance is sought on whether any constitutional or statutory provisions prohibit such advance payments and whether the EAA has the statutory authority to enter into a non-joint refugia project with the Service.

The Attorney General has had occasion to consider whether a state agency or political subdivision may make such "advance payments," in light of the prohibition against gratuitous grants of public credit or money. As discussed below, the general rule appears to be that, if certain criteria are met, advance payments of public funds may be made by a political subdivision without violating Article III, Section 52(a).

The Texas Attorney General has opined that the constitutional prohibitions against gratuitous grants are not intended to prevent the direct accomplishment of a legitimate public purpose by the mere fact that a private entity may be otherwise benefited.[60] Indeed, the Attorney General has indicated that he "has issued a number of opinions approving the advance payment of public funds to private parties for the achievement of a public purpose."[61]

Similar to the discussion above in Section 3.4 relative to the construction of improvements, the Attorney General has stated that the fundamental principles relative to Sections 50, 51, and 52, of Article III regarding gratuitous grants are that "the constitutional provisions are not violated when public funds are expended for the achievement of a public purpose, when the public receives adequate consideration in return, and when the governmental body retains control over the use of the funds to ensure that the public purpose is achieved."[62] The Attorney General has also opined that whether a particular expenditure of public funds meets constitutional muster "is left, in the first instance, within the sound discretion of the governing body that proposes to pay public funds to a private entity."[63]

As for arrangements between two governmental entities, the First Court of Appeals in *State ex rel. Grimes County Taxpayers Association v. Texas Municipal Power Agency* has stated as follows:

> Many cases could be cited which involve an arrangement between two governmental entities in which one rendered agreed services to the other in exchange for money paid at a different time than when

will agree to any reversionary interest or other appropriate default remedies in the EAA after the expiration or termination of the ITP, or in the event of closure or defunding of the refugia sites. Although the ITP is renewable (*see* ITP Block 4), the nature of such renewal cannot be entirely known at this time. *See* 50 C.F.R. § 13.22 (2013).

[60] *See e.g.* Tex. Att'y Gen. Op. No. JM-1030 (1989).

[61] *Id.*

[62] *Id.*

[63] *Id.*

> services were rendered . . . Two requirements must be met in such a transaction. (1) The purpose for which the obligation or payment or transfer was made must be within the power of the entity incurring the obligation or making the payment or transfer of funds [citations omitted]. (2) The political entity that receives the funds has to be obligated (by statute or contract) to use the funds for the public purpose.[64]

In *Grimes*, the court held that early payments by four cities to the Texas Municipal Power Agency were neither lacking in consideration, nor were they grants, donations, or gratuities, but instead "were payments made for services rendered and *to be rendered*."[65] As a result, these payments, despite being prepayments, were not in violation of Section 52 of Article III of the Texas Constitution. The Attorney General, relying on *Grimes*, has stated that Section 50 or Article III "does not prohibit advance payment by one governmental entity for services which another governmental entity is obligated to render in the future."[66]

While the Attorney General has appeared to generally approve of advance payments if certain criteria are met, guidance from the Attorney General is sought as to whether the EAA may legally provide advance funding to the Service for both construction, equipment and supplies, and services under the terms and conditions proposed by the Service without contravening the gratuitous grant prohibition of Article III, Section 52(a).

Assuming that advance payments are permissible under Article III, Section 52(a), Texas Constitution as discussed above, it is necessary to determine whether the EAA has statutory authority to enter into the contract proposed by the Service.

Section 1.11(d)(2) of the EAA Act[67] generally authorizes the EAA to enter into contracts. This section does not prescribe any limitations or qualifiers. This would seem to indicate that the EAA has broad authority to enter into contracts necessary to implement its responsibilities under the Act, including to contract for services, research, construction, ownership, operation, and maintenance of the refugia program.

Sections 49.213(b) and Section 49.213(c)(5) and (7), Texas Water Code, similarly appear to provide broad contracting authority to the EAA respectively as follows:

> A district may enter into contracts with any person or any public or private entity in the performance of any purpose or function permitted by a district.[68]

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[64] *State ex rel. Grimes Cnty. Taxpayers Ass'n v. Texas Muni. Power Agency*, 565 S.W.2d 258, 265 (Tex. App. — Houston [1st Dist.] 1978, no writ) (internal citations omitted).

[65] *Id* at 265 (emphasis added).

[66] *See* Tex. Atty. Gen. Op. No. MW-55 (1979).

[67] EAA Act § 1.11(d)(2). The Act also specifically authorizes the EAA to contract for services to conduct research. *Id.* § 1.27(d).

[68] TEX. WATER CODE ANN. § 49.213(b) (West 2008).

> A district may enter into contracts . . . with persons[,] or any public or private entities[,] on the terms and conditions the board may consider desirable, fair, and advantageous for: . . .
> (5) the maintenance and operation of any works, improvements, facilities, plants, equipment, and appliances of the district or of another person or public or private entity;[69] . . .
> (7) the exercise of any other rights, powers, and duties granted to a district.[70]

As discussed in Sections 1.1, 1.2, 1.3 and 3.3, the refugia program is necessary to accomplish a purpose for which the EAA was created – the protection of the Covered Species associated with the Aquifer, and would be for a purpose or function permitted by the EAA, as well as the exercise of a right, power, and duty granted to the EAA. Similar to Section 1.11(d)(2) of the EAA Act, these sections do not prescribe any limitations or qualifiers on the contracting authority of a district. Indeed, Section 49.213(c) provides that districts may contract on the terms and conditions the district's board may consider desirable, fair, and advantageous. Therefore, Sections 49.213(b) and Section 49.213(c)(7) would appear to support the proposition that the EAA may broadly contract with the Service for services, research, construction, ownership, and operation, and maintenance of the refugia program on the terms and conditions the EAA's Board of Directors ("Board") may consider desirable, fair, and advantageous, including non-joint projects and the making of advance payments.

Supporting this broad contracting authority is Section 49.211(b), Texas Water Code, which gives districts broad authority to own, construct, operate, and maintain facilities necessary to accomplish its purposes as follows:

> A district is authorized to purchase, construct, acquire, own, operate, maintain, repair, improve, or extend inside and outside its boundaries any and all land, works, improvements, facilities, plants, equipment, and appliances necessary to accomplish the purposes of its creation or the purposes authorized by this code or any other law.[71]

Relative to services contract, Section 49.067(b), Texas Water Code, likewise appears to provide districts broad authority to set the terms and conditions for services contracts, including the terms for payment, as follows:

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[69] Note that Section 49.213(c)(5) authorizes a district to enter into a contract for the maintenance and operation of works and improvements owned by another person or entity, whether public or private. Although this subsection does not so expressly provide, it can be observed here that for facilities owned by another entity, the operation and maintenance would have to benefit the district by relating to a right, power, duty, function, or purpose that the district is authorized by law to perform.

[70] *Id.* § 49.213(c)(5), (7) (West 2008).

[71] *Id.* § 49.211(b) (West 2008).

> Notwithstanding any other law, a contract for technical, scientific, legal, fiscal, or other professional services must be approved by the board unless specifically delegated by board action. The terms and conditions of such a contract, including the terms for payment, are subject to the decision of the board unless specifically delegated by board action. The board through such action cannot abrogate its fiscal responsibility.[72]

Therefore, based on the sections of the EAA Act and Chapter 49 discussed above, the EAA would appear to have broad authority to contract with the Service to construct, own, operate, and maintain all land, facilities, and equipment, as well as for services and research necessary to implement the refugia program, and the determination of the terms of payments under the proposed refugia contract would rest generally with the EAA's Board, and that the project may be non-joint and the payments under the refugia contract may be made in advance of the Service performing the services. However, these sections, because of their breadth, offer no guidance on those terms and conditions that would be necessary for the contract to contain in order to satisfy the requirements of Article III, Section 52(b) of the Texas Constitution. Additionally, clarification is needed with respect to these broad grants of contracting authority in light of other sections in Chapter 49 which appear to provide for a more limited contracting authority under certain circumstances that are relevant to the Services's proposed terms and conditions for the refugia contract.

Several provisions of Chapter 49 of the Texas Water Code specifically authorize districts, including the EAA, to contract for *joint* projects. Section 49.213(a) addresses joint contracting projects as follows:

> A district may contract with a person or any public or private entity for the joint (constructing, financing, ownership, and operation of any works, improvements, facilities, plants, equipment, and appliances necessary to accomplish any purpose or function permitted by a district, or a district may purchase an interest in any project used for any purpose or function permitted by a district.[73]

Section 49.227, Texas Water Code, further provides:

> A district or water supply corporation may act jointly with any other person or entity, private or public, whether within the State of Texas or the United States, in the performance of any of the powers and duties permitted by this code or any other laws.[74]

As discussed above, the refugia program is necessary for the EAA to protect the Covered Species associated with the Aquifer. Therefore, under Sections 49.213(a) and 49.227, the EAA may

[72] *Id.* § 49.067(b) (West 2008).

[73] *Id.* § 49.213(a) (West 2008).

[74] *Id.* § 49.227 (West 2008).

contract with the Service for the *joint* construction, financing, ownership, and operation of the refugia program. However, these sections, standing alone, are silent as to whether the EAA is authorized to contract with the Service for the refugia project unless and only if the project is, in fact, *jointly* constructed, financed, owned, and operated. The Services's proposed terms and conditions proposed do not to appear to meet these criteria because the financing for the refugia is solely by the EAA, and the EAA has no ownership interest, nor any other role in the refugia construction or operation.

Accordingly, the Attorney General's guidance is sought as to whether the EAA has the statutory authority to enter into a refugia contract with the Service in which the construction, financing, ownership, and operations will not be joint in that the EAA will be providing all of the funding to the Service, the EAA will have no ownership interest in the improvements funded by the EAA, and the EAA will have no participation in the construction or operations of the refugia, although the refugia will be for a permissible purpose and function of the EAA.

Furthermore, guidance is also sought from the Attorney General as to whether Section 49.276 applies to the EAA and prohibits the EAA from making advance payments to the Service for the construction of the additional improvements necessary for the Service to perform the refugia services, and, if not, whether the EAA otherwise has the statutory authority to make advance payments. Section 49.276(a) provides that "district[s] shall pay the contract price of construction contracts *only* as provided in [Section 49.276]."[75] Subsection (b) requires that districts "make progress payments under construction contracts monthly as the work proceeds, or at more frequent intervals as determined by the board or its designee, on estimates approved by the board or its designee."[76] Subsection (d) provides for retainages and that districts are not obligated to pay interest on amounts retained except as so provided.[77] In light of Section 49.213(c)'s apparent broad grant of discretion to district boards in structuring their contracts "on the terms and conditions the board may consider desirable, fair, and advantageous," may the EAA deem advance payment to the Service to be "desirable, fair, and advantageous" and, therefore, within the authority of the EAA to make advance payments?

Finally, based on the above discussion, while the EAA may have general authority to contract with the Service for services related to the refugia program, the issue remains whether the EAA may make advance payments for such *services* (as opposed to construction) as required by the Service. None of the sections discussed above authorizing the EAA to contract with the Service relative to the EAA's refugia program specifically authorize the EAA to make advance payments for services.

Section 49.067(b), Texas Water Code, notes that the terms and conditions of service contracts, including the terms of payment, are generally subject to the discretion of a district.[78] However, this section seems to be nothing more than a restatement of the basic principle that the

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[75] *Id.* § 49.276(a) (West 2008).

[76] *Id.* § 49.276(b).

[77] *Id.* § 49.276(d).

[78] *Id.* § 49.067(b).

governing body of a district has primary jurisdiction over contracting, and that such authority may be delegated within limits. Therefore, this section does not seem to amount to an authorization for the EAA Board to approve advance payment contracts for services.

Section 49.213(c), Texas Water Code, authorizes a board to enter into service contracts with persons or other entities "on the terms and conditions the board may consider *desirable, fair, and advantageous*." Thus, it appears that as long as a contractual term or condition is considered by the EAA Board to be "desirable, fair, or advantageous," then it would be in the discretion of the Board to approve its inclusion as a term or condition of a contract. Accordingly, guidance is sought from the Attorney General on whether the EAA has statutory authority under Section 49.213(c) to find that the advance payment to the Service for refugia-related services is permissible as being "desirable, fair, and advantageous" and may be incorporated into the refugia contract.

3.6 Applicability of Chapter 49, Subchapter I

Another issue relative to the construction of the refugia facilities for which the Attorney General's guidance is sought is whether Subchapter I of Chapter 49, Texas Water Code,[79] applies to the proposed refugia contract and thus, would require that the refugia contract comply with the procurement requirements in that subchapter. Section 49.271(a) provides that "*[a]ny* contract made by the board for *construction work* shall conform to the provisions of [chapter 49]."[80] This section appears to place no qualifiers on the nature of the construction contract to which Subchapter I might apply. Similarly, Section 49.276(a) provides that the "district shall pay the contract price of construction contracts only as provided in this section."[81] On the other hand, Section 49.273(a) provides that "board[s] shall contract for construction . . . of *district facilities* and for the purchase of equipment, materials, machinery, and all things that constitute or will constitute the plant, works, facilities, or improvements *of the district* in accordance with [Section 49.273, Texas Water Code.][82] It is not clear whether Subchapter I of Chapter 49 applies to the construction contemplated by the refugia contract: (1) as the EAA will not own any of the improvements for which the EAA is proposed to provide all of the funding; (2) the Service is proposed to be solely responsible for the design, construction and equipment plans and specifications, cost schedules, procurement, construction, and construction management and oversight, and operation and maintenance; and (3) the contract will not be a traditional construction contract in which the EAA will be contracting to construct facilities that it will own, but instead will be funding the construction of facilities to be owned exclusively by the federal government, notwithstanding the fact that the contract may contain a provision requiring the Service to use the facilities solely for the benefit of the EAA's refugia program. Guidance is sought as to whether the EAA-funded refugia facilities to be owned and operated by the Service are to be considered "district facilities" for purposes of Chapter 49, Subchapter I, such that the proposed refugia contract must meet the requirements of the subchapter, or whether the EAA may waive such requirements in the face of Service objections.

[79] *Id.* §§ 49.271- .279 (West 2008 & Supp. 2014).

[80] *Id.* § 49.271(a) (West 2008), (West Supp. 2014).

[81] *Id.* § 49.276(a) (West 2008).

[82] *Id.* § 49.273(a) (West Supp. 2014).

4. CONCLUSION

An Attorney General opinion on the legal issues raised herein concerning whether the EAA may provide funding to the Service for the implementation of a refugia program under certain terms and conditions proposed by the Service is necessary and in the public's interest and is respectfully requested.

Appendix K7

Letter opinion from Texas State Attorney General's Office

KEN PAXTON
ATTORNEY GENERAL OF TEXAS

March 9, 2015

The Honorable Doug Miller
Chair, Special Purpose Districts Committee
Texas House of Representatives
Post Office Box 2910
Austin, Texas 78768-2910

Opinion No. KP-0008

Re: Whether the Edwards Aquifer Authority may provide funding to the U.S. Fish & Wildlife Service for the implementation of a refugia program (RQ-1220-GA)

Dear Representative Miller:

You ask three questions related to whether the Edwards Aquifer Authority ("EAA") may provide funding to the U.S. Fish & Wildlife Service ("USFWS") for the implementation of a refugia program under certain terms and conditions proposed by USFWS.[1]

EAA is "a special regional management district" created by the Legislature through the Edwards Aquifer Authority Act ("EAA Act").[2] The stated purposes of EAA are "to protect terrestrial and aquatic life, domestic and municipal water supplies, the operation of existing industries, and the economic development of the state." EAA Act § 1.01. Among other duties, EAA is responsible for the regulation of withdrawals of groundwater from the aquifer by well owners. *Id.* § 1.15. EAA has recognized that performing its statutory duties could result in the "taking" of endangered species, which is prohibited by federal law under the Endangered Species

[1]Letter from Honorable Doug Miller, Co-Chair, Edwards Aquifer Legislative Oversight Comm. to Honorable Greg Abbott, Tex. Att'y Gen. at 2–3 (Sept. 3, 2014), https://www.texasattorneygeneral.gov/opinion/requests-for-opinion-rqs ("Request Letter").

[2]EAA Act § 1.01, http://www.edwardsaquifer.org (Legislation and Rules). The EAA Act remains uncodified. Citations are to the EAA Act's current sections, without separate reference to amending sections. Official citations are included in the session laws. *See* Act of May 30, 1993, 73d Leg., R.S., ch. 626, 1993 Tex. Gen. Laws 2350, *amended by* Act of May 16, 1995, 74th Leg., R.S., ch. 524, 1995 Tex. Gen Laws 3280; Act of May 29, 1995, 74th Leg., R.S., ch. 261, 1995 Tex. Gen. Laws 2505; Act of May 6, 1999, 76th Leg., R.S., ch. 163, 1999 Tex. Gen. Laws 634; Act of May 25, 2001, 77th Leg., R.S., ch. 1192, 2001 Tex. Gen. Laws 2696; Act of May 27, 2001, 77th Leg., R.S., ch. 966, §§ 2.60–.62, 6.01–.05, 2001 Tex. Gen. Laws 1991, 2021–22, 2075–76; Act of May 25, 2001, 77th Leg., R.S., ch. 1192, 2001 Tex. Gen. Laws 2696; Act of June 1, 2003, 78th Leg., R.S., ch. 1112, § 6.01(4), 2003 Tex. Gen. Laws 3188, 3193; Act of May 23, 2007, 80th Leg., R.S., ch. 510, 2007 Tex. Gen. Laws 900; Act of May 28, 2007, 80th Leg., R.S., ch. 1351, §§ 2.01–2.12, 2007 Tex. Gen. Laws 4612, 4627–34; Act of May 28, 2007, 80th Leg., R.S., ch. 1430, §§ 12.01–12.12, 2007 Tex. Gen. Laws 5848, 5901–09; Act of May 21, 2009, 81st Leg., R.S., ch. 1080, 2009 Tex. Gen. Laws 2818.

Act ("ESA"). 16 U.S.C.A. § 1538(a)(1)(B) (West 2010).[3] Therefore, EAA, joined by several other entities, sought and obtained from USFWS an incidental take permit.[4] The Permit precludes liability under the ESA for any taking that is "incidental to, and not the purpose of, the carrying out of an otherwise lawful activity." *Id.* § 1539(a)(1)(B).

To obtain the Permit, the ESA required EAA and other stakeholders to submit a plan that specified the steps they would take to "minimize and mitigate" the impact of any taking. *Id.* § 1539(a)(2)(A)(i–ii). Accordingly, EAA and a number of other entities prepared a habitat conservation plan outlining specific measures that the applicants would undertake to protect the covered species.[5] USFWS approved the Plan and issued the Permit in February 2013. Permit at 1. "The authorization granted by [the Permit is] subject to full and complete compliance with, and implementation of, the" Plan. *Id.*

As part of the Plan, EAA is required to "support and coordinate with the USFWS on the work relating to the . . . operation and maintenance of a series of off-site refugia at USFWS's San Marcos, Uvalde, and Inks Dam facilities." *See* Plan, § 5.1.1. You describe a refugia in this context as "an off-site facility designed and dedicated to the care, housing, and maintenance of individuals or populations of Covered Species in an artificial habitat to protect them from and to avoid the negative effects of drought disturbance, disease outbreaks, and water quality impairment in the Comal and San Marcos Springs and Rivers ecosystems." Request Letter at 7. You explain that USFWS has proposed "a management contract that would contain the terms and conditions under which it would be willing to contract with the EAA to implement the refugia program." *Id.* at 8. Under the terms of that proposed contract, USFWS would acquire or construct buildings, facilities, equipment and supplies, which would be owned by USFWS and located on federal land. *Id.*

You first ask whether EAA may provide funding to USFWS to implement the refugia program if the "buildings, works, facilities, and equipment must be owned solely by [USFWS] and be physically located on real property owned by" USFWS. *Id.* at 4. You are specifically concerned that EAA's provision of funding to USFWS may implicate the prohibition against gratuitous grants of public credit and funds under article III, section 52(a) of the Texas Constitution. *Id.* at 13.

Article III, section 52 prohibits the Legislature from authorizing a political subdivision "to lend its credit or to grant public money or thing of value in aid of, or to any individual, association

[3]Under the ESA, to "take" is defined as "to harass, harm, pursue, hunt, shoot, wound, kill, trap, capture, or collect, or to attempt to engage in any such conduct." 16 U.S.C.A. § 1532(19) (West 2010). Section 1538(a)(1)(B) provides, with certain exceptions, that "with respect to any endangered species of fish or wildlife listed pursuant to section 1533 of this title, it is unlawful for any person subject to the jurisdiction of the United States to . . . take any such species within the United States or the territorial sea of the United States." *Id.* § 1538(a)(1)(B).

[4]*See* EAA Act § 1.11(d)(9) (expressly authorizing the EAA to hold permits under the ESA); USFWS Permit TE63663A-0,http://www.eahcp.org/files/admin-records/NEPA-and-HCP/USFWS_Permit_03-18-2013_rcvd_1030_ a.m._Final.pdf ("Permit").

[5]*See* Edwards Aquifer Authority Recovery Implementation Program, Habitat Conservation Plan (Nov. 2012) ("Plan"), http://www.eahcp.org/ ("Documents and Publications," "Habitat Conservation Plans and Appendices").

or corporation." TEX. CONST. art. III, § 52(a). Answering your question first requires a determination as to whether a court would consider USFWS, a federal agency, an "individual, association or corporation" for purposes of article III, section 52. *Id.* Courts have considered some local governmental entities, such as school districts, to be corporations for purposes of article III, section 52. *See, e.g., San Antonio Indep. Sch. Dist. v. Bd. of Trs. of San Antonio Elec. & Gas Sys.*, 204 S.W.2d 22, 25 (Tex. Civ. App.—El Paso 1947, writ ref'd n.r.e.) (concluding that article III, section 52 prohibits a city from donating "its funds to an independent municipal corporation such as an independent school district"). The Texas Supreme Court, however, has concluded that a state agency does not "qualify as an individual, association or corporation under section 52(a)" and that the provision therefore "does not prohibit transfers to a state agency." *Tex. Mun. League Intergov'l Risk Pool v. Tex. Workers' Comp. Comm'n*, 74 S.W.3d 377, 384 (Tex. 2002); *see also Harris Cnty. Flood Control Dist. v. Mann*, 140 S.W.2d 1098, 1103 (Tex. 1940) (orig. proceeding) (concluding that statutes authorizing a district to cooperate with the federal government in carrying out the purposes of the district did not violate article III, section 52). Although we find no case expressly stating that a federal agency is not an "individual, association or corporation" for purposes of section 52, the same rationale that applies to a state agency would likely apply to a federal agency. Thus, a court is likely to conclude that a federal agency like USFWS is not an "individual, association or corporation" under article III, section 52 and that the constitutional provision would not prohibit EAA from providing funds to USFWS to implement the refugia program consistent with EAA's purposes.

In your second question, you ask whether EAA has the statutory authority to enter into a non-joint refugia project contract with USFWS. Request Letter at 2. The Legislature has granted EAA broad authority to "enter into contracts." EAA Act § 1.11(d)(2); Tex. Att'y Gen. Op. No. GA-0708 (2009) at 3. Furthermore, the EAA has "all of the powers, rights, and privileges necessary to manage, conserve, preserve, and protect the aquifer," expressly including the authority granted by chapters 36, 49, and 51 of the Water Code.[6] EAA Act § 1.08. Thus, if EAA determines that entering into the refugia project contract with USFWS under the terms USFWS proposes is necessary to manage, conserve, preserve and protect the aquifer, a court would likely conclude that the EAA has statutory authority to do so.[7]

You suggest that two provisions in chapter 49 of the Water Code may prohibit the EAA from contracting with USFWS "unless and only if the project is, in fact, *jointly* constructed, financed, owned, and operated." Request Letter at 20. The two provisions you raise are sections

[6]Subsection 1.08(a) of the EAA Act provides that the EAA's authority includes that granted by chapters 50, 51 and 52 of the Water Code. Chapters 50 and 52 were repealed by the Legislature in 1995 and replaced with chapters 49 and 36, respectively. Courts have since held that the recodified chapters apply to the EAA. *See, e.g., In re Edwards Aquifer Auth.*, 217 S.W.3d 581, 587–88 (Tex. App.—San Antonio 2006, orig. proceeding).

[7]You also ask in relation to this question whether EAA may provide advance funding to USFWS for construction, equipment and supplies "without contravening the gratuitous grant prohibition of Article III, Section 52(a)." Request Letter at 17. Because we have already concluded that a court is unlikely to consider USFWS an "individual, association or corporation" under article III, section 52, we do not address this question further.

49.213(a) and 49.227, which authorize special law districts to contract for joint projects with other entities. Subsection 49.213(a) provides:

> A district may contract with a person or any private or public entity for the joint construction, financing, ownership, and operation of any works, improvements, facilities, plants, equipment, and appliances necessary to accomplish any purpose or function permitted by a district, or a district may purchase an interest in any project used for any purpose or function permitted by a district.

TEX. WATER CODE ANN. § 49.213(a) (West 2008). Section 49.227 states:

> A district or water supply corporation may act jointly with any other person or entity, private or public, whether within the State of Texas or the United States in the performance of any of the powers and duties permitted by this code or any other laws.

Id. § 49.227. The language of sections 49.213(a) and 49.227 provides affirmative grants of authority for districts to act jointly with other entities; however, nothing in either provision suggests that those statutes are intended to limit the EAA's broad contracting authority granted in the EAA Act.

Your final question asks whether chapter 49, subchapter I of the Water Code will apply to the refugia contract between EAA and USFWS. Request Letter at 2–3. Chapter 49, subchapter I is titled "Construction, Equipment, Materials and Machinery Contracts," and it outlines a number of procurement requirements for "[a]ny contract made by the board for construction work." TEX. WATER CODE ANN. § 49.271(a) (West Supp. 2014); *see generally id.* §§ 49.271–.279 (West 2008 & Supp. 2014). In particular, you question whether section 49.276 of the Water Code prohibits EAA from making advance payments to USFWS for the construction of the additional improvements necessary to operate the refugia program.[8] As you describe it, although the proposed management contract between EAA and USFWS will require USFWS to either acquire or construct facilities and improvements, EAA will be contracting for USFWS's services to operate the refugia program. You explain that USFWS will have "[c]ontrol over the design, construction and equipment plans and specifications," which suggests that the contract between EAA and USFWS itself may not be a contract for construction work, making subchapter I inapplicable. Request Letter at 8. Even if a court were to conclude that the contract between EAA and USFWS were a contract for construction work, section 49.278 excepts certain contracts from the application of subchapter I, including contracts for "professional services" and "contracts for

[8]Subsection 49.276(b) of the Water Code provides that "[t]he district will make progress payments under construction projects monthly as the work proceeds, or at more frequent intervals as determined by the board or its designee, on estimates approved by the board or its designee." TEX. WATER CODE ANN. § 49.276(b) (West 2008).

services or property for which there is only one source or for which it is otherwise impracticable to obtain competition." TEX. WATER CODE ANN. § 49.278(a)(2), (4) (West 2008). Under the terms of the contract, USFWS is the only source that may provide and operate a refugia program. For these reasons, a court would likely conclude that chapter 49, subchapter I does not apply to a contract between EAA and USFWS for the operation of a refugia program.[9] You do not ask, and we do not address, the propriety of USFWS requiring as a condition of an incidental take permit that the applicant fund structures on USFWS property or fund USFWS employees.

[9]You do not ask, and we do not address, the constitutional authority of the federal government to regulate the taking of the endangered species that are part of the Plan. *See GDF Realty Invs., Ltd. v. Norton*, 326 F.3d 622, 640–41 (5th Cir. 2003) (holding that application of the ESA's take provision to intrastate species is a constitutional exercise of the Commerce Clause power); *but see People for the Ethical Treatment of Prop. Owners v. U.S. Fish & Wildlife Serv.*, No. 2:13–cv–00278–DB, 2014 WL 5743294, at *8 (D. Utah Nov. 5, 2014) (holding that the Commerce Clause "does not authorize Congress to regulate takes of a purely intrastate species that has no substantial effect on interstate commerce").

SUMMARY

Article III, section 52 of the Texas Constitution prohibits the Legislature from authorizing a political subdivision "to lend its credit or to grant public money or thing of value in aid of, or to any individual, association or corporation." Based on Texas Supreme Court precedent, a court is likely to conclude that the United States Fish and Wildlife Service is not an "individual, association or corporation" under article III, section 52, and that the constitutional provision therefore would not prohibit the Edwards Aquifer Authority from providing funds to the United States Fish and Wildlife Service to implement a refugia program.

If the Edwards Aquifer Authority determines that entering into the refugia project contract with the United States Fish and Wildlife Service under the terms the Service proposes is necessary to manage, conserve, preserve and protect the aquifer, a court would likely conclude that the Edwards Aquifer Authority Act provides statutory authority to do so.

A court would likely conclude that chapter 49, subchapter I of the Water Code does not apply to a contract between the Edwards Aquifer Authority and the United States Fish and Wildlife Service for the operation of a refugia program.

Very truly yours,

KEN PAXTON
Attorney General of Texas

CHARLES E. ROY
First Assistant Attorney General

BRANTLEY STARR
Deputy Attorney General for Legal Counsel

VIRGINIA K. HOELSCHER
Chair, Opinion Committee
Assistant Attorney General, Opinion Committee

Appendix K8

RFP No. 142-15-HCP to solicit proposals from qualified vendors for the purpose of providing salvage refugia, practicing husbandry for the care and propagation of the eleven (11) covered species as required by the Edwards Aquifer Habitat Conservation Plan (Salvage Refugia Operations)

EDWARDS AQUIFER
AUTHORITY

4-A\1.25

March 30, 2015

Dear Interested Offeror:

The Edwards Aquifer Authority (the "EAA") is requesting proposals from qualified vendors for the purpose of providing salvage refugia, practicing husbandry for the care and propagation of the eleven (11) covered species as required by the Edwards Aquifer Habitat Conservation Plan (proposal #142-15-HCP).

Attached is the proposal package. Please complete the attached sheets with one (1) unbound original, three (3) copies and one (1) electronic copy Proposal Summary (Attachment A1), Cost Proposal Summary (Attachment A2), and Client Reference forms (Attachments B1-B3) and submit to:

Ms. Cyndi Holman
Procurement Specialist
Edwards Aquifer Authority
900 E. Quincy Street
San Antonio, TX 78215

Proposals must be submitted only on the attached proposal summary forms and are to be sealed with "SALVAGE REFUGIA OPERATIONS PROPOSAL" indicated on the top of the envelope. Proposals are due in the EAA offices no later than 10:00 a.m., Wednesday, April 22, 2015, at which time the proposals will be opened. **PROPOSALS RECEIVED AFTER THE DEADLINE WILL NOT BE ACCEPTED AND WILL BE RETURNED IMMEDIATELY UNOPENED.**

Proposals offering less than 90 calendar days for acceptance by the EAA from the date set for opening will be considered nonresponsive and will be rejected.

The EAA reserves the right to reject any and all proposals.

A pre-proposal meeting is scheduled for Wednesday, April 15, 2015 at 10:00 a.m., at the EAA office.

If you have any questions, please call Ms. Holman at (210) 222-2204.

Sincerely,

Roland Ruiz
General Manager

REQUEST FOR PROPOSALS FOR
SALVAGE REFUGIA OPERATIONS PROPOSAL
FOR THE
EDWARDS AQUIFER AUTHORITY
PROPOSAL NO. 142-15-HCP

Issued by:
Edwards Aquifer Authority
900 E. Quincy Street
San Antonio, TX 78215
(210) 222-2204

Issue Date:	Monday, March 30, 2015
Proposals Close:	Wednesday, April 22, 2015
Time:	10:00 a.m., Central Time

SECTION 1
PROJECT INTRODUCTION AND BACKGROUND

1.1 PURPOSE OF REQUEST FOR PROPOSALS (RFP)

The Edwards Aquifer Authority (the "EAA") is requesting proposals from qualified vendors for the purpose of providing salvage refugia, practicing husbandry for the care and propagation of the eleven (11) covered species as required by the Edwards Aquifer Habitat Conservation Plan. This project is expected to continue through December 2016.

1.2 DESCRIPTION OF PROPOSED PROJECT

The primary purpose of this project is to conduct salvage refugia operations, for the eleven (11) covered species identified in the EAHCP and listed for coverage under the ITP.

This project is intended to provide for the collection, husbandry, captive propagation, and re-introduction of covered species individuals collected from their native habitat when it is determined by the EAA and the U.S. Fish and Wildlife Service (USFWS) that significant covered species kills in the wild are imminent or on-going.

1.3 PROJECT SCHEDULE

The following is the tentative schedule for selection and award of the EAA's procurement:

Description	Date
RFP Advertisements	Sunday, March 29, 2015 Sunday, April 5, 2015 Sunday, April 12, 2015
Pre-Proposal Meeting	Wednesday, April 15, 2015 10:00 a.m., Central Time
Last Date for Questions	Wednesday, April 15, 2015 12:00 p.m., Central Time
Proposals Close	Wednesday, April 22, 2015 10:00 a.m., Central Time

SECTION 2
INSTRUCTION TO OFFERORS

2.1 REQUESTS FOR INFORMATION

This RFP is being issued by the EAA, San Antonio, Texas, which is the sole point of contact for purposes of information concerning this RFP. The EAA reserves the right to issue addenda if required. All questions and inquiries regarding this request for proposal may be submitted in writing to Ms. Cyndi Holman, Procurement Specialist, by 12:00 p.m., Central Time, Wednesday, April 15, 2015. Requests for information received prior to the above stated deadline are to be responded to in writing by the EAA in the form of an addendum addressed to all proposal specification recipients.

The EAA will conduct a pre-proposal meeting with prospective Offerors on Wednesday, April 15, 2015 at 10:00 a.m. in the Artesian Room of the EAA's office located at 900 E. Quincy Street.

Submission of a proposal shall be considered *prima facie* evidence that the Offeror has familiarized himself/herself with, and understands, the solicitation, its terms and general conditions, etc., under which the contract is to be awarded, administered, and performed. The EAA will not be responsible for any interpretations or misinterpretations of any oral instructions.

2.2 SUBMISSION REQUIREMENTS

Offerors are required to submit their proposals on the attached Proposal Summary forms (see Attachment A1). Proposal envelopes are to be plainly marked, "SALVAGE REFUGIA OPERATIONS PROPOSAL".

Offerors are required to submit their proposals no later than 10:00 a.m., Central Time, on Wednesday, April 22, 2015, to:

Ms. Cyndi Holman
Procurement Specialist
Edwards Aquifer Authority
900 E. Quincy Street
San Antonio, TX 78215
cholman@edwardsaquifer.org
(210) 222-2204

NO FACSIMILE PROPOSALS WILL BE ACCEPTED.

Upon receipt by the EAA, each proposal will be stamped with the date and time received and stored unopened in a secure place until the proposal opening. All proposals become the property of the EAA, which will hold the contents of all proposals confidential until an award is made.

PROPOSALS RECEIVED AFTER THE TIME SET FOR THE OPENING WILL BE DECLARED LATE AND NOT ELIGIBLE FOR OPENING AND CONSIDERATION. THE EAA IS NOT RESPONSIBLE FOR MAIL, COURIER OR OTHER DELIVERY METHODS, IN-TRANSIT TIME OR NON-DELIVERY. LATE DELIVERIES WILL BE HELD UNOPENED. OFFEROR WILL BE ADVISED BY MAIL THAT HIS/HER PROPOSAL WAS LATE AND NOT ACCEPTED AND WILL BE ALLOWED TO PICK UP HIS/HER PROPOSAL PACKAGE OR FURNISH A "CALL TAG" AND HAVE THE PACKAGE PICKED UP BY A COURIER.

2.3 PROPOSAL FORMAT

The EAA requires that submitted proposals adhere to the following general format to simplify the review process. Failure to follow the required format or to respond to each specification may result in rejection of the proposal. All proposals must be submitted on the enclosed forms in duplicate, or photocopies of the forms and electronically on CD or flash drive format. Failure to do so may result in rejection of the proposal.

2.3.1 General Requirements

Submit the names of three client references for whom similar refugia operations and or research has been provided within the past five (5) years on the form contained in Attachments B1-B3, Client Reference. Do not use the EAA as one of the three references. The description must provide the following minimum information:

- o Client name;
- o Client contact name;
- o Client address and telephone number(s);
- o Date contract began; and
- o Description of services.

The Offeror agrees EAA staff may contact the references given.

2.3.2 Response to Commercial Questions and Statements

Please answer the questions indicated in Attachment A1, Proposal Summary, directly and specifically. All pricing is to be included in Attachment A2, Cost Proposal Summary. Any exceptions to any of the requirements and specifications contained in this RFP must be noted in the allotted space in Attachment A1. Attachments A1 and A2 must be returned with the Offeror's response.

2.3.3 Cost Proposal

Provide, under separate sealed envelope, an itemized list of projected costs necessary to complete this project. Use the forms provided in Attachment A2, Cost Proposal Summary, to quote a price for the salvage refugia operations project described in this RFP. The EAA is exempt from sales tax, but could be subject to other types of taxes. If any other type of tax is added, please specify.

2.4 MINORITY-OWNED AND WOMEN-OWNED BUSINESSES

The EAA strongly encourages minority and women-owned businesses to submit proposals. The EAA also encourages applicants, in those instances when joint venturing and/or subcontracting is appropriate, to form joint ventures and/or provide subcontract opportunities to minority and women owned firms.

2.5 PROPOSALS BINDING

Proposals must set forth accurate and complete information as required by this RFP (including attachments). Negligence upon the part of the Offeror in preparing the proposal confers no right of withdrawal after the time fixed for the submission of proposals.

2.6 LATE PROPOSALS, MODIFICATIONS, OR WITHDRAWALS

Proposals received after the date and the time indicated will not be considered and will be returned unopened if the Offeror is identified on the envelope.

Proposals may be withdrawn or modified in writing prior to the proposal opening. Responses that are resubmitted or modified shall be sealed and resubmitted to the Procurement Specialist prior to the proposal opening.

2.7 PROPOSAL COSTS

All costs for preparing the proposals are to be borne by the Offeror and may not be included in the cost proposal.

2.8 PROPOSAL SIGNATURE

The EAA will prepare a contract for the successful Offeror using the name exactly as it appears on the proposal. Therefore, it is imperative the Offeror sign the proposal using correct and complete legal names and titles.

2.9 CONTRACT AWARD

The EAA reserves the right to accept or reject any and all proposals. Unless all proposals are rejected or the solicitation is cancelled, the contract is to be awarded to the Offeror whose proposal best meets the requirements and criteria set forth in these specifications. No proposal is to be considered binding upon the EAA until a contract has been awarded. The EAA reserves the right to award the proposal and/or contract to more than one Offeror.

Contract award is to be issued to the successful Offeror by letter. The vendor shall not begin any work on this contract until such time as a Notice to Proceed has been issued by the General Manager.

2.10 CONTRACT

It is expressly understood by the Offerors that written notice of award by the EAA will constitute acceptance of the proposal.

2.11 CONTRACTOR SELECTION

2.11.1 Selection Process

The selection process will include the following steps:

1. Receipt of proposals.
2. Review of proposals submitted.
3. Evaluation of proposals and ranking of Offerors-EAA staff shall review all information available from the selection process and rank the Offerors.
4. Contract Award-The EAA will award the contract to the Offeror whose proposal is the most advantageous to the EAA and which will result in the most economical provision of these required services to the EAA.

2.11.2 Selection Criteria

Selection will be based on the following criteria which are listed in order of importance:

1. Commercial Quality (65 Points)
   - o Proven scientific understanding of endangered species issues
   - o Experience with similar programs
   - o Adequate consideration of scientific constraints
   - o Possession of or plan to obtain appropriate permits from United States Fish and Wildlife Services (USFWS) for activities including endangered species, or any other requisite permits
   - o Ability to start immediately
   - o Ability to house all the covered species
   - o Ability to house those species whose triggers will occur first
   - o Knowledge of the project area
   - o Demonstration of qualified project personnel
   - o Customer references and satisfaction of existing customers
   - o Proposal quality

   *If permit(s) are not currently held by firm, proposal must include a date by which such permit(s) will be granted and describe the process by which the permit(s) will be obtained.

2. Cost Factors (30 points)
   - o Total cost
   - o Additional costs

3. Financial Stability (5 points)
   - o Current financial condition

2.12 CONFIDENTIAL MATERIAL

Proposals will remain confidential until an award is made, except for the information that is public during a proposal opening. At that time, all information is public unless considered confidential by the Public Information Act, such as trade secrets and financial information. Offeror must indicate if any of the information provided constitutes an exception to the Public Information Act. All information not labeled as confidential will be presumed to be public information.

SECTION 3
SPECIFICATIONS

3.1 BACKGROUND INFORMATION

The EAA is a political subdivision of the State of Texas. Governed by an elected board of directors, the EAA is empowered to manage, preserve, and protect the Edwards Aquifer.

This project is intended to provide for the collection, husbandry, captive propagation, and re-introduction of covered species individuals collected from their native habitat when it is determined by pre-established triggers outlined in the HCP that signify that losses of covered species in the wild are imminent or on-going. This project is expected to continue through December 2016.

Section K of EAA's ITP requires that the EAA provide for refugia to house and protect the covered species in the event that a drought or other disaster may threaten the species' continued existence without intervention.

Offerors will be required to demonstrate extensive knowledge of refugia operations, care and propagation, and research of the eleven covered species listed in the Habitat Conservation Plan.

Offerors may be required to conduct research to assist in successfully implementing refugia for the covered species and may include: physiology, life histories, effective reintroduction techniques, collection techniques, husbandry, and propagation.

The use of EAA funds will be limited to the operation and maintenance of refugia for the covered species, other operations and research activities will not be funded.

The primary purpose of this project is to conduct salvage refugia operations, for the eleven (11) covered species listed below and identified in the EAHCP and listed for coverage under the ITP.

Common Name	Scientific Name	ESA Status
Fountain Darter	*Etheostoma fonticola*	Endangered
Comal Springs Riffle Beetle	*Heterelmis comalensis*	Endangered
San Marcos Gambusia	*Gambusia georgei*	Endangered*
Comal Springs Dryopid Beetle	*Stygoparnus comalensis*	Endangered
Peck's Cave Amphipod	*Stygobromus pecki*	Endangered
Texas Wild Rice	*Zizania texana*	Endangered
Texas Blind Salamander	*Eurycea rathbuni*	Endangered
San Marcos Salamander	*Eurycea nana*	Threatened
Edwards Aquifer Diving Beetle	*Haideoporus texanus*	Petitioned
Comal Springs Salamander	*Eurycea* sp.	Petitioned
Texas Troglobitic Water Slater	*Lirceolus smithii*	Petitioned

*The San Marcos gambusia was last collected in the wild in 1983 and may already be extinct.

3.2 MINIMUM SPECIFICATIONS

3.2.1 HCP Salvage Refugia Operations

PROPOSED SCOPE OF WORK

In responding to this RFP, the Offeror shall discuss the general approach to tasks, specific methodologies, infrastructure, project management and team organization, strategies to accomplish work in a timely manner, and budget requirements to complete each of the tasks listed below. After the negotiation of a contract and for the following scope of work, the successful Offeror will be a Contractor with the EAA

Task 1. Collection, establishment, and maintenance of salvage stocks of refugium populations of covered species when triggered based on the following table;

The Contractor will collect, house, and care for the appropriate number of covered species as required based on the following table:

Common Name	HCP Trigger	No. Species Collected
Fountain Darter	1. Comal - <50% mean aquatic vegetation (Landa Lake and Old Channel) and <20% darter presence system wide; or <25% mean aquatic vegetation (Landa Lake and Old Channel) and <30% darter presence system wide 2. San Marcos - 75 cfs or less for 4 consecutive days at University Drive gauge	San Marcos - 2,500 Comal - 2,000
Comal Springs Riffle Beetle	Comal - >30 cfs when only one of the three monitored sites continues to have 6 or more adult beetles collected in a 24 hr sample period using cotton lures.	500
Comal Springs dryopid beetle	Comal - When any standard or conventional water quality parameter exceeds the historical range of water quality parameter for the Edwards Aquifer by 10% or more.	500
Peck's cave amphipod	When any standard or conventional water quality parameter exceeds the historical range of water quality parameter for the Edwards Aquifer by 10% or more.	500
Texas blind salamander	When any standard or conventional water quality parameter exceeds the historical range of water quality parameter for the Edwards Aquifer by 10% or more.	500
San Marcos gambusia	Not considered	Not considered
Texas wild-rice	<3,500 m^2 total coverage in the San Marcos River; or TWR stands exist at <3 distinct sections as described by the Biological Monitoring seven sections.	1500

San Marcos salamander	<50% suitable habitat (Biological Monitoring locations) and <20% salamander density; or <25% suitable habitat (Biological Monitoring locations) and <30% salamander density	500
Comal Springs salamander	<50% suitable habitat (Biological Monitoring locations) and <20% salamander density; or <25% suitable habitat (Biological Monitoring locations) and <30% salamander density	500
Texas Troglobitic Water Slater	Comal - When any standard or conventional water quality parameter exceeds the historical range of water quality parameter for the Edwards Aquifer by 10% or more.	500
Edwards Aquifer Diving Beetle	Not considered	500

Task 2. Conduct research as necessary to expand knowledge of the covered species' physiology, environmental requirements, health and disease issues, life histories, and effective reintroduction techniques. The Contractor will propose, with the confirmation of EAA staff, specific research needed to accomplish Task 1.

Task 3. Develop and refine animal rearing methods and captive propagation techniques for the Covered Species. The Contractor will document the successes and failures of each rearing methods and captive propagation techniques.

Task 4. Reintroduction of species and monitoring of recovery in the event of a loss of species in their native environment in coordination with USFWS.

Task 5. Final report describing all activities completed under this project including "lessons learned". This task also requires a "stand alone" procedural/ operations manual.

Task 6. Attend meetings and give presentations to the Science Committee, Implementing Committee, and EAA Board of Directors as requested by the EAHCP Program Manager. Offeror should budget for a minimum of three meetings each year.

ADDITIONAL INFORMATION TO OFFERORS

1. Proposals shall discuss the process and methodology for completing the tasks described in this RFP. It should identify all subcontractors that will be used on the project and the task on which each subcontractor will be used.

2. The Contract will be a time and material contract with a specified "not to exceed" amount. The budget will be itemized by tasks. Cost estimates should take personnel, infrastructure, supplies, equipment, and travel into account for each task. Project personnel should be identified by position title and the hourly rate. The budget should clearly indicate the indirect costs or overhead charged for the work.

3. This project requires the use of Edwards Aquifer groundwater. The proposal should include discussion of the water source for the proposed project including purveyor, access point, etc.

4. The proposal should discuss infrastructure and facilities (existing or proposed), and location of refugia.

5. The proposal should discuss health issues including disease control and disinfection of effluent discharge and quarantine of the covered species brought in.

6. The Offeror may propose to provide refugia for all or some of the eleven covered species. The proposal should clearly indicate which species the proposal addresses.

7. Scope alterations are encouraged and should contain detailed discussions of task alternatives, alternative task budgets, and alternative timelines. If scope alternatives are provided, the Offeror will provide a separate budget table as described in section 3.2.2 of this RFP.

8. The EAA reserves the right to select any combination of tasks and subtasks for the final scope of work.

3.2.2 Pricing Information

Respondents are required to submit itemized cost proposals for items as described in this RFP in a separate sealed envelope. The form shown in Attachment A2 shall be used for all costs. All charges are to be included in the cost proposal. All price quotations shall be valid for at least 90 days from the proposal opening.

SECTION 4
TERMS AND CONDITIONS

4.1 STANDARD FORM OF CONTRACT

Attached is a copy of the EAA's Standard Form of Contract stating the general terms and conditions which will be contained in any contract resulting from this RFP (see Attachment C). Exhibit A, Scope of Work, of the contract form is attached for informational purposes only. Exhibit A is not to be completed at this time. It will be completed upon award of contract. Offerors, by virtue of submitting a proposal, acknowledge, understand, and agree to these terms and conditions, unless such is indicated on the Proposal Summary.

4.2 LAWS AND REGULATIONS

The EAA requires that all responses to this RFP, and any contracts that may result, be in accordance with the laws and regulations of the State of Texas. Furthermore, the awarded Contractor must adhere to all Occupational Safety and Health Administration standards as applicable to the contracted work.

4.3 PROPOSAL ACCEPTANCE PERIOD

All prices and conditions of the proposal shall remain in effect for 90 days after the date set for the proposal opening. Proposals offering less than 90 calendar days for acceptance by the EAA from the date set for opening will be considered nonresponsive and will be rejected.

The EAA's Code of Ethics is attached for your reference only (see Attachment D). No action is necessary.

4.4 TIME OF COMPLETION

The Contractor shall take all necessary and appropriate actions to complete the project in accordance with the ongoing schedule incorporated in the resultant contract.

4.5 INSURANCE REQUIREMENTS

4.5.1 Worker's Compensation Insurance

The Contractor shall procure and shall maintain during the life of the contract, Worker's Compensation and Employer's Liability Insurance as required by applicable State law for all of his/her employees to be engaged in work at the site of the project under the contract and, in case of any such work sublet, the Contractor shall require the subcontractor(s) similarly to provide

Worker's Compensation Insurance for all of the latter's employees to be engaged in such work unless such employees are covered by the protection afforded by the Contractor's Workers Compensation and Employer's Liability Insurance. In case any class of employees engaged in hazardous work on the project under the contract is not protected under the worker's compensation statutes, the Contractor shall provide and shall cause each subcontractor to provide adequate Employer's Liability Insurance in the amount of $500,000 for the protection of such of his/her employees as are not otherwise protected.

4.5.2 Contractor's (Commercial General) Public Liability and Property Damage Insurance and Vehicle Liability Insurance

The Contractor shall procure and shall maintain during the life of the contract Commercial General Liability (Bodily Injury and Property Damage) Insurance and Automobile (Vehicle) Liability Insurance coverage as specified below. The General Liability Policy shall also include products and Completed Operations Insurance in the same limits as the General Liability coverage as well as an endorsement providing Broad Form Contractual Liability coverage:

INSURANCE COVERAGE

a. Worker's Compensation Statutory

and

b. Employer's Liability $100,000/500,000/100,000

Commercial General (Public) Liability insurance including coverages for the following:

a. Premises operations
b. Independent contractors
c. Products/completed operations
d. Personal injury
e. Contractual liability
f. Medical payments
g. Underground hazard
h. Explosion and collapse hazard
i. EAA's property in Contractor's care, custody, or control

a. Each Occurrence: $1,000,000

and

b. General Aggregate: $2,000,000

Comprehensive Automobile Liability Insurance, including coverage for loading and unloading hazards, for:

a. Owned/Leased vehicles
b. Non-owned vehicles and;
c. Hired vehicles

Combined single limit for bodily injury and property damage of $500,000 per occurrence or its equivalent.

4.5.3 Subcontractor's Public Liability and Property Damage Insurance and Vehicle Liability Insurance

The Contractor shall either (1) require each subcontractor to procure and to maintain during the life of his/her subcontract, Subcontractor's Public Liability and Property Damage Insurance, and Vehicle Liability Insurance of the type and in the amounts specified or, (2) insure the activities in his/her policy as specified.

4.5.4 Additional Insureds

The Contractor shall provide in the Liability Policies of Insurance the Edwards Aquifer Authority as additional insured as it relates to this contract.

4.5.5 Scope of Insurance and Special Hazards

Insurance required under the above sections shall provide adequate protection for the Contractor and his/her subcontractors, respectively, against damage claims which may arise from operations under the contract, whether such operations be by the insured or by anyone directly or indirectly employed by him/her, and also against any of the special hazards which may be encountered in the performance of the contract, including explosions, collapse and underground hazards.

4.5.6 Proof of Insurance Coverage

The Contractor shall furnish the EAA with insurance certificates for him/her and his/her subcontractors showing the type, coverage, limits of liability, class of operations covered, effective dates, date of expiration of policies and name of insurance companies prior to beginning any work. The attached Insurance Requirement Affidavit is required to be submitted along with the Proposal. The Contractor must furnish the EAA with 30 days written notice by either certified mail or personal delivery should any of the above described policies change that materially affect the coverage or be cancelled prior to the expiration date. All notices shall be mailed to:

Ms. Cyndi Holman
Procurement Specialist
Edwards Aquifer Authority
900 E. Quincy Street
San Antonio, TX 78215

Strike the following language as indicated on the cancellation section of the certificate of insurance: "SHOULD ANY OF THE ABOVE DESCRIBED POLICIES BE CANCELLED BEFORE THE EXPIRATION DATE THEREOF, THE ISSUING COMPANY WILL ~~ENDEAVOR TO~~ MAIL 30 DAYS WRITTEN NOTICE TO THE CERTIFICATE HOLDER NAMED TO THE LEFT. ~~BUT FAILURE TO MAIL SUCH NOTICE SHALL IMPOSE NO OBLIGATION OR LIABILITY OF ANY KIND UPON THE COMPANY, ITS AGENTS OR REPRESENTATIVES~~."

EDWARDS AQUIFER AUTHORITY
INSURANCE REQUIREMENT AFFIDAVIT

To Be Completed By Appropriate Insurance Agent/Broker

I, the undersigned Agent/Broker, certify that the insurance requirements contained in this proposal document have been reviewed by me with the below identified Contractor. If the below identified Contractor is awarded this contract by the Edwards Aquifer Authority, I will be able to, within fifteen (15) days after being notified of such award, furnish a valid insurance certificate to the EAA meeting all of the requirements defined in this proposal.

____________________________ ____________________________
Agent (Signature) Agent (Print)

Name of Agent/Broker: ____

Address of Agent/Broker: ______________________________________

City/State/Zip: ____

Agent/Broker Telephone #: (   ) __________________________________

Date: ____

CONTRACTOR'S NAME: ______________________________________
(Print or Type)

NOTE TO AGENT/BROKER
If this time requirement is not met, the EAA has the right to reject this proposal and award the contract to another. If you have any questions concerning these requirements, please contact Ms. Cyndi Holman at (210) 222-2204.

ATTACHMENT A1
EDWARDS AQUIFER AUTHORITY
PROPOSAL SUMMARY
FOR HABITAT CONSERVATION PROGRAM
SALVAGE REFUGIA OPERATIONS
PROPOSAL #142-15-HCP

Vendor Name: ____

Submit to: Ms. Cyndi Holman
Procurement Specialist
Edwards Aquifer Authority
900 E. Quincy Street
San Antonio, TX 78215

Attach your proposal for (state project) ______________________________

____

to this document.

The following exceptions to the Specifications are noted:

____

____

Response to Commercial Questions and Statements

What is the current financial status and condition of the proposing entity?

____

____

The EAA reserves the right to request financial statements as needed.

Please mark one of the following:

_________ I agree with the terms and conditions of the contract form as contained in Attachment C to the proposal specifications.

_________ I do not agree with the terms and conditions of the attached contract form as contained in Attachment C to the proposal specifications.

I would like to take exception to the following contract provisions and am proposing alternative contract language as follows:

____

____

____

The undersigned certifies that the information contained in this proposal has been carefully checked and is submitted as correct and that he/she is authorized to submit this proposal on behalf of the Offeror named below.

Signed: ____
Name

____
Printed Name

____
Title

____
Company

____
Address

____

____
Telephone No.

ATTACHMENT A2
EDWARDS AQUIFER AUTHORITY
COST PROPOSAL SUMMARY
FOR HABITAT CONSERVATION PROGRAM
SALVAGE REFUGIA OPERATIONS
PROPOSAL #142-15-HCP

Vendor Name: ____

Submit to: Ms. Cyndi Holman
Procurement Specialist
Edwards Aquifer Authority
900 E. Quincy Street
San Antonio, TX 78215

The undersigned proposes to furnish the prices shown below in accordance with the specifications and the response attached hereto. It is expressly agreed that the EAA has the right to reject any or all proposals submitted if such action is deemed in its interest. The total cost is $_______________, the breakdown of which is:

Task 1 __________________________	$____________________________
Task 2 __________________________	$____________________________
Task 3 __________________________	$____________________________
Task 4 __________________________	$____________________________
Task 5 __________________________	$____________________________
Task 6 __________________________	$____________________________
Other ( )	$____________________________
*TOTAL	$____________________________

*Do not include sales tax in your cost proposal. If any other type of tax is added, please give full description of tax type.

The undersigned certifies that the prices contained in this proposal have been carefully checked and are submitted as correct and that he/she is authorized to submit this proposal on behalf of the Offeror named below.

Proposals offering less than 90 calendar days for acceptance by the EAA from the date set for opening will be considered nonresponsive and will be rejected.

Signed: ______________________________

Name

____

Printed Name

____

Title

____

Company

____

Address

____

____

Telephone No.

ATTACHMENT B1

CLIENT REFERENCE

For each reference, complete the following information:

Client Name: ____

Client Contact Name: ____

Position: ____

Client Address: ____

____

____

Client Telephone Number(s): __________________________________

Date Contract Began: ____

Description of Services: ______________________________________

____

____

Cost: ____

ATTACHMENT B2

CLIENT REFERENCE

For each reference, complete the following information:

Client Name: ____

Client Contact Name: ____

Position: ____

Client Address: ____

____

____

Client Telephone Number(s): __________________________________

Date Contract Began: ____

Description of Services: ______________________________________

____

____

Cost: ____

ATTACHMENT B3

CLIENT REFERENCE

For each reference, complete the following information:

Client Name: ____

Client Contact Name: ______________________________________

Position: ____

Client Address: ____

____

____

Client Telephone Number(s): ________________________________

Date Contract Began: ______________________________________

Description of Services: ____________________________________

____

____

Cost: ____

ATTACHMENT C
FOR INFORMATIONAL PURPOSES ONLY

CONTRACT No. XXX-XX-XXX
BETWEEN THE
EDWARDS AQUIFER AUTHORITY
AND
[Insert the Contractor's name]
FOR [Insert the name of the Project]

This Contract is made and entered into by and between the EDWARDS AQUIFER AUTHORITY, ("EAA"), a political subdivision of the State of Texas, with its principal place of business located at 900 E. Quincy Street, San Antonio, Texas 78215, and [Insert the Contractor's name] ("Contractor"), a [Insert the nature of the Contractor] with its principal place of business located at [Insert Contractor's address]. Each of these entities is, at times, referred to in this Contract individually as a "Party," and both are referred to collectively as "Parties."

RECITALS

WHEREAS, the Edwards Aquifer Authority ("EAA") was created in 1993 by the Edwards Aquifer Authority Act of May 30, 1993, 73rd Leg., R.S., ch. 626, 1993 Tex. Gen. Laws 2350; as amended ("Act"); and

WHEREAS, under Section 1.02 of the Act, the EAA is a conservation and reclamation district created by virtue of Article XVI, Section 59 of the Texas Constitution, and is a governmental agency and body politic and corporate vested with the full authority to exercise the powers and to perform the functions specified in the Act; and

WHEREAS, under Section 1.08(a) of the Act, the EAA has all the powers, rights and privileges necessary to manage, conserve, preserve, and protect the Edwards Aquifer ("Aquifer") and to increase the recharge of, and prevent the waste or pollution of water in, the Aquifer; and

WHEREAS, under Section 1.11(d)(2) of the Act, the EAA may enter into contracts; and

WHEREAS, the EAA Board of Directors approved this Contract on [Insert date], and authorized the EAA General Manager to execute the Contract; and

WHEREAS, [Insert description of the other Party]; and

WHEREAS, [Insert basic description of the transaction]; and

WHEREAS, it is in the public interest that the EAA enter into this Contract.

AGREEMENT

NOW THEREFORE, for and in consideration of the mutual promises and agreements set forth in this Contract, the EAA and the Contractor agree as follows:

ARTICLE I – TERM; DESCRIPTION OF WORK

Section 1.1. Term. This Contract is effective and commences on [Insert date] ("Effective Date"), and terminates on [Insert date] ("Expiration Date").

Section 1.2. Services. Subject to the terms and conditions of this Contract, the EAA engages the Contractor to perform, for the benefit of the EAA, the work set forth and described in this Contract and in the following documents ("Services") which are attached hereto: (1) the Scope of Work which is attached hereto as Exhibit A ("Scope of Work"); (2) the Budget Estimate which is attached hereto as Exhibit B ("Budget Estimate"); (3) the Labor Categories Rates & Personnel Chart which is attached hereto as Exhibit C ("Labor Categories Rates & Personnel Chart"); and (4) [Add other Exhibits as needed]. The Contractor accepts such engagement and agrees to devote its best efforts and abilities, and furnish all necessary labor, machinery, equipment, tools, and transportation necessary in furtherance of its engagement under this Contract.

Section 1.3. Commencement and Completion of Services. The Contractor will commence performing the Services immediately upon the date of receipt of the written notice to proceed issued by the EAA's General Manager. All Services will be completed and delivered to the EAA by the Expiration Date and shall be completed in compliance with the schedules, budgets, descriptions and specifications contained herein and in the Exhibits attached hereto. It shall be the Contractor's responsibility to ensure that the completion times for the tasks required under this Contract are met. [[Add if applicable: "At the sole option of the EAA, this Contract may be renewed and extended for up to [Insert number] periods of one additional year each, with such election made by the EAA giving the Contractor written notice to renew and extend this Contract prior to the end of the then Contract period."]] Time is of the essence in the performance of this Contract.

ARTICLE II – AMENDMENTS

Section 2.1. This Contract may be amended only by written agreement of the Parties.

Section 2.2. Amendments by the General Manager. The Board of Directors of the EAA delegates the authority to the General Manager to enter into amendments to this Contract without further authorization by the Board consistent with the General Manager's authority to enter into contracts under Section 4.01 of the EAA's Bylaws.

ARTICLE III – COMPENSATION

Section 3.1. Fees and Expenses. The EAA agrees to pay the Contractor for the Services rendered under this Contract in accordance with the Scope of Work, Budget Estimate, and Labor Categories, Rates & Personnel Chart, but in no event shall payments to the Contractor exceed

$______________. The Contractor may not exceed this amount and will be responsible for the payment of all of its other and additional costs and expenses. The Contractor is not authorized to expend any additional funds in excess of this amount without the prior written approval from the EAA. The EAA will not be held accountable for any unauthorized work performed or funds expended by the Contractor in providing the Services under this Contract.

Section 3.2. Payment. All invoices from the Contractor to the EAA for the Services performed under this Contract shall be sent monthly and shall provide an itemization of the Services rendered, costs and expenses incurred. The terms of each invoice shall be net thirty (30) days upon EAA receipt and approval of that invoice.

ARTICLE IV – INDEPENDENT CONTRACTOR

Section 4.1. No Employment Relationship. The Parties understand and agree that this Contract does not create a fiduciary relationship between them, that they are separate entities, that the Contractor is an independent contractor with respect to the performance of the Services and is not subject to the direct or continuous control and supervision of the EAA, and that nothing in this Contract is intended to make either Party a subsidiary, joint venturer, partner, employee, agent, servant or representative of the other Party for any purpose whatsoever. The Contractor shall provide any and all equipment and materials necessary for the performance of the Services under this Contract. The EAA shall have no right of direction or control of the Contractor, or its employees and agents, except in the results to be obtained, and in a general right to order the performance of the Services to start or stop as agreed to herein, to inspect the progress of the Services, and to receive reports. The Contractor shall accommodate reasonable requests from the EAA to allow EAA employees, agents or representatives to accompany and observe Contractor personnel in carrying out the Services under this Contract.

ARTICLE V – CONTRACTOR PERSONNEL AND SUBCONTRACTORS

Section 5.1. Personnel. The Contractor will provide any and all personnel necessary for its performance of the Services. The Contractor will be responsible for its employees and agents in all respects, including, without limitation, their compliance with applicable laws and their safety, including without limitation, all Occupational Safety and Health Administration (OSHA) standards, requirements, and regulations. The Contractor indemnifies and holds harmless the EAA, its officers, employees and directors, from and against any claims bought by any employee, subcontractor or other agent of the Contractor relating in any way to the Services performed under this Contract.

Section 5.2. Subcontractors. In performing the Services under this Contract, the Contractor may retain and utilize as its subcontractors, to the extent that they are not already employees of the Contractor, those individuals identified to and approved in writing by the EAA, in advance. The EAA, in consultation with the Contractor, shall have the right to terminate, limit, or alter, at any time, the participation of any subcontractor utilized by the Contractor. No additional subcontractors may be retained by the Contractor to perform any Services under this Contract without the prior written consent of the EAA, provided that no such consent shall be necessary for the retention of any subcontractor previously approved by the EAA and identified by the

Contractor on the Effective Date of this Contract. The Contractor will be responsible for its subcontractors in all respects including their compliance with applicable laws and their safety, including without limitation, all OSHA standards, requirements, and regulations.

[Insert this section if necessary]
Subcontracting services under this contract may be provided by:

-

ARTICLE VI – TERMINATION

Section 6.1. Termination. The EAA may terminate this Contract at any time, including at the expiration of each budget or payment period during the term of this Contract, with or without cause, upon ten (10) days prior written notice to the Contractor. Upon receipt of such termination notice, the Contractor shall immediately stop all work in progress, including all work performed by subcontractors. Insofar as possible, all work in progress will be brought to a logical termination point. Within thirty (30) days of the final invoice following termination, the EAA shall pay the Contractor all moneys then due and owing for the Services rendered, costs and expenses reasonably incurred up to the time of termination.

ARTICLE VII – OWNERSHIP OF MATERIALS

Section 7.1. Ownership. All information, documents, property, or materials produced, created, or supplied under this Contract by the Contractor, its employees, agents or subcontractors or anyone else, and whether finished or unfinished or in draft or final form, will be the property of the EAA. The EAA shall have unlimited rights to technical and other data resulting directly from the performance of the Contractor's Services under this Contract.

Section 7.2. Delivery of Documents upon Termination. Upon termination of this Contract under Sections 1.3 or 6.1, all such information, property and materials not already in the possession of the EAA will be promptly delivered to the EAA.

Section 7.3. Nondisclosure of Documents. The information, documents, property, or materials produced, created or supplied under this Contract by the Contractor, including preliminary technical reports and studies, shall not be disclosed by the Contractor to any third-party without the prior written consent of the EAA. The Contractor shall immediately advise the EAA of any requests for any such information, document, property, or materials by a third-party. The unauthorized disclosure of such information, documents, property, or materials in violation of this section shall, in the sole judgment of the EAA, constitute a breach of this Contract and shall be subject to all applicable remedies at law or equity.

Section 7.4. Record Copies. The Contractor shall retain a record copy of all information, documents, property, or materials developed in the course of performing the Services. Upon request of the EAA, such information, documents, property, or materials will be promptly supplied to the EAA, including after the Expiration Date or the termination of this Contract under Section 6.1. The EAA will reimburse the Contractor for actual cost of time and expenses of reproduction of such materials when requested.

ARTICLE VIII – NON-PERFORMANCE

Section 8.1. The Contractor warrants that it will perform all Services in a good and workmanlike manner, strictly in accordance with the standards of the Contractor's profession, the Scope of Work, and as otherwise provided in this Contract and the Exhibits hereto. The Contractor's failure to timely perform the Services as warranted and agreed shall constitute a breach of this Contract and shall be subject to all applicable remedies at law or equity. Judgment of nonperformance shall rest solely with the EAA.

ARTICLE IX – LIQUIDATED DAMAGES

Section 9.1. If the Contractor fails to complete the Services required to be provided under this Contract by the Expiration Date, then the Contractor shall pay to the EAA as liquidated damages, the sum of $________ per day, beginning on the day first following the Completion Date and continuing for each day until actual completion of the Scope of Work, provided that any such delay in completion is due to the Contractor and is not attributable to a failure of the EAA, its employees or agents, or any third parties who are not subcontractors of the Contractor, to perform its responsibilities in coordinating, reviewing or otherwise performing the Services under this Contract. The EAA, in its sole discretion, reserves the right to waive this provision in whole or in part. These liquidated damage amounts are in addition to, and not in lieu of, any other damages due to the EAA, or any other remedies available to the EAA as a result of a default by the Contractor under this Contract.

[or THIS ARTICLE LEFT BLANK]

ARTICLE X – BOND COVERAGE

Section 10.1. If requested by the EAA as security for the performance of the terms of this Contract, the Contractor will provide the EAA, for its benefit, with a performance bond in the sum of 100% of the anticipated costs to perform the Services under this Contract. Such bond shall be in form and substance in all respects satisfactory to the EAA and shall be issued by a surety company authorized to do business in Texas and in all respects satisfactory to the EAA. The Contractor, through its agent of record, shall notify the EAA in writing of any changes in bonding coverage within thirty (30) days prior to any effective date of the change.

[or THIS ARTICLE LEFT BLANK]

ARTICLE XI – INSURANCE

Section 11.1. Insurance Coverages. During the term of this Contract, the Contractor shall obtain and maintain in effect, at Contractor's expense, appropriate insurance policies protecting the Contractor and the EAA, and their respective officers, directors and employees, against any loss, liability, personal injury, death, property damage or any expense arising out of the performance of the Services under this Contract, including, without limitation: (1) worker's compensation insurance in compliance with applicable state law; (2) comprehensive general

liability insurance, insuring against property damage, personal injury and death, in an amount of no less than $1,000,000.00 per occurrence; (3) automobile liability insurance in an amount no less than $1,000,000.00; (4) umbrella liability insurance in an amount of no less than $1,000,000.00. Said insurance policies shall be with insurance carriers licensed to do business in Texas. The Contractor shall be responsible for requiring that its subcontractors carry and maintain adequate insurance coverage.

Section 11.2. Additional Insureds. The Contractor shall name the EAA and its officers, directors and employees as "additional insureds" on all of the insurance policies specified in Subsection 11.1 above, or with respect to the worker's compensation insurance, contain waivers of subrogation by Contractor and the insurance carrier in favor of the EAA. Not later than the date of receipt of the written notice to proceed under Section 1.3, the Contractor must provide the EAA with certificates of insurance to be issued directly to the EAA by the Contractor's insurance agent, identifying the specified coverage. The Contractor, through its agent of record, shall notify the EAA of any changes in coverages within thirty (30) days prior to any effective date of change.

Section 11.3. No limitations. Contractor's obligation to obtain and maintain the foregoing policy or policies in the amounts specified shall not be limited in any way by reason of any insurance which may be maintained by the EAA, nor shall Contractor's performance of this obligation relieve it of liability under the indemnity provisions set forth in Section 12.2.

ARTICLE XII – ASSUMPTION OF RISK AND INDEMNIFICATION

Section 12.1. Risk. The Contractor shall assume all risks associated with the Contractor's or its subcontractors' performance under this Contract and shall waive any claim against the EAA and other participants for damages arising out of the performance of the Services under this Contract.

Section 12.2. Indemnification. The Contractor shall defend, indemnify and hold harmless the EAA, its directors, employees and agents from any and all damages, loss, or liability of any kind whatsoever, including the costs of litigation and attorneys' fees arising from (a) contracts or arrangements between the Contractor and any third parties entered into in performing this Contract, (b) any claims brought by any person relating to this Contract or the Services provided hereunder, or (c) the quality of the Services or the performance of the Services covered by this Contract.

ARTICLE XIII – NOTICES

Section 13.1. Notices to the EAA. All notices or communications under this Contract to be mailed or delivered to the EAA shall be in writing and shall be sent to the EAA's principal place of business as follows, unless and until the Contractor is otherwise notified:

EDWARDS AQUIFER AUTHORITY
900 E. Quincy Street
San Antonio, Texas 78215
ATTENTION: ROLAND RUIZ, GENERAL MANAGER

Section 13.2. Notices to the Contractor. All notices or communications under this Contract to be mailed or delivered to the Contractor shall be in writing and shall be sent to the address of the Contractor as follows, unless and until the EAA is otherwise notified:

____

____

____

ATTENTION: ____________________________

Section 13.3. Effective Date of Notice. Any notices or communications required to be given in writing by one Party to the other shall be considered as having been given to the addressee on the date the notice of communication is posted by the sending Party.

ARTICLE XIV – MISCELLANEOUS

Section 14.1. Entire Agreement. This Contract and the attached Exhibits constitute the entire agreement between the Parties regarding the Services to be performed by the Contractor and there are no representations, warranties, agreements or commitments between the Parties except as set forth herein. Unless otherwise authorized herein, no amendments or additions to this Contract shall be binding on the Parties unless in writing and signed by the Parties.

Section 14.2. Non-Waiver. No delay or failure by either Party to exercise any right under this Contract, nor any partial or single exercise of that right, shall constitute a waiver of that or any other right, unless otherwise expressly provided herein.

Section 14.3. Headings. Headings in this Contract are for convenience only and shall not be used to interpret or construe its provisions.

Section 14.4. Governing Law. This Contract shall be deemed to have been executed and performed in the State of Texas and shall be construed in accordance with and governed by the laws of the State of Texas. Venue for any disputes or claims arising from this Contract shall be exclusively in the proper courts in Bexar County, Texas.

Section 14.5. Counterparts. This Contract may be executed in two or more counterparts, each of which shall be deemed an original but all of which together shall constitute one and the same instrument.

Section 14.6. Binding Effect. The provisions of this Contract shall be binding upon and inure to the benefit of the Parties and their respective successors and assigns; provided, however, that the Contractor may not assign any of its rights nor delegate any of its duties hereunder without the EAA's prior written consent.

Section 14.7. Validity. The invalidity of any provision or provisions of this Contract shall not affect any other provision of this Contract, which shall remain in full force and effect, nor shall the invalidity of a portion of any provision of this Contract affect the balance of such provision.

Section 14.8. Non-Waiver of Immunity. Nothing in this Contract is intended as any waiver by the EAA of any immunity from suit to which it is entitled under Texas law.

Section 14.9. Survival. Termination of this Contract for breach shall not constitute a waiver of any rights or remedies available at law or in equity to a Party to redress such breach. All remedies, either under this Contract or at law or in equity or otherwise available to a Party, are cumulative and not alternative and may be exercised or pursued separately or collectively in any order, sequence or combination. In addition, to these provisions, applicable provisions of this Contract shall survive any termination of this Contract.

Section 14.10. Attachments. The Exhibits, schedules and/or other documents attached hereto or referred to herein are incorporated herein and made a part hereof for all purposes. As used herein, the expression "Contract" means the body of this Contract and such attachments, Exhibits, schedules and/or other documents, and the expressions "herein," "hereof," and "hereunder" and other words of similar import refer to this Contract and such attachments, exhibits, schedules and/or other documents as a whole and not to any particular part or subdivision thereof.

Section 14.11. Costs. If any legal action, arbitration, or other proceeding is brought by a Party for the enforcement of this Contract or because of an alleged breach or default of this Contract, the prevailing Party shall be entitled to recover reasonable costs incurred, including but not limited to attorney's fees, in such action or proceeding in addition to any other relief to which it or they may be entitled.

Section 14.12. Authority to Contract. Each Party represents and warrants for the benefit of the other Party that: (1) it has the legal authority to enter into this Contract; (2) this Contract has been duly approved and executed; (3) no other authorizations or approvals are or will be necessary in order to approve this Contract and to enable that Party to enter into and comply with the terms and conditions of this Contract; (4) the person executing this Contract on behalf of each Party has the authority to bind that Party; and (5) the Party is empowered by law to execute any other agreement or documents and to give such other approvals, in writing or otherwise, as are or may hereafter be required to implement and comply with this Contract.

Section 14.13. Officers or Agents. No officer or agent of the Parties is authorized to waive or modify any provision of this Contract. No amendment to or rescission of this Contract may be made except by a written document signed by the Parties' authorized representatives.

IN WITNESS WHEREOF, this Contract is executed as of the day and date first written above.

EDWARDS AQUIFER AUTHORITY	(INSERT NAME OF CONTRACTOR)
By:________________________ Roland Ruiz General Manager	By:______________________________ (Name) (Title)
ATTEST:	ATTEST:
By:________________________ Jennifer Wong-Esparza Assistant to the Board Secretary	By:______________________________ (Name)__________________________ (Title)___________________________

APPROVED AS TO FORM:

____
Darcy Alan Frownfelter
General Counsel
Edwards Aquifer Authority

EXHIBIT A
SCOPE OF WORK

EXHIBIT B
BUDGET ESTIMATE

EXHIBIT C
LABOR CATEGORIES AND PERSONNEL CHART

ATTACHMENT D

<u>FOR INFORMATIONAL PURPOSES ONLY</u>

EDWARDS AQUIFER AUTHORITY

CODE OF ETHICS FOR EAA EMPLOYEES

Every employee is expected to perform his or her job duties satisfactorily, to maintain a high level of personal conduct on the job, to render courteous and efficient service to the public, to be mindful of safety practices and to exercise care in the use of EAA property.

Each employee of the EAA is a public servant and, as such, is held to the highest standard of ethical conduct. Consistent with this public trust, employees may not:

- use their official positions to secure special privileges or exemptions for themselves or others;
- grant any special consideration, treatment or advantage to any citizen, individual or group beyond any available to every other citizen, individual or group;
- disclose, without proper authorization, confidential information that could adversely affect the property, management or affairs of the EAA;
- directly or indirectly use any confidential information for their own personal gain or benefit, or for the private interest of others;
- engage in any outside activities that will conflict with, or will be incompatible with, the duties assigned to them in the course of their employment with the EAA; or that would reflect discredit upon the EAA; or in which their employment with the EAA would give them an advantage over others engaged in competition with the employee's personal business or vocational pursuits. This policy will not prohibit employees from performing any services for another organization if the General Manager determines there is no conflict with EAA duties and responsibilities;
- represent, directly or indirectly, or appear on behalf of private interests before the EAA Board of Directors; nor will they represent any private interest in any action or proceeding involving the EAA; nor will they accept a retainer or compensation that is contingent upon a specific action taken by the EAA;
- use EAA funds, supplies, equipment, vehicles or facilities for any purpose other than conducting official business; or
- have a financial interest, direct or indirect, in any contract with the EAA, or in the sale to the EAA of any land, materials, supplies or services, except on behalf of the EAA as an employee.

The previous list of prohibited activities is not all-inclusive. Violation of the public trust is prohibited and may result in dismissal.

Employees are permitted, as private citizens, to support political candidates for public office. An employee may not, however, participate actively in any candidate's campaign for membership in the EAA board of directors. Employees may not hold or run for a political office, the responsibilities of which may affect, directly or indirectly, the EAA. Likewise, employees are not permitted to use their working time or EAA resources to participate in a political campaign or any other political activity. The term "participate" includes, without limitation, making political speeches, telephone solicitation, distributing political literature, or writing or handling letters related to a political campaign or activity. Political posters may not be displayed on EAA property. Employees are not required to contribute to any political fund or render any political service to any person or party whatsoever.

Employees are prohibited from soliciting or accepting any gifts, gratuities, favors, loans or other objects of monetary worth that might reasonably tend to influence the employee in the discharge of official duties or that the employee knows or should know is being offered with the intent to influence the employee's official conduct.

Appendix K9

2015–2016 Salvage Refugia Research Plan

EDWARDS AQUIFER AUTHORITY

2015–2016 SALVAGE REFUGIA RESEARCH PLAN

SWCA ENVIRONMENTAL CONSULTANTS
Sound Science. Creative Solutions.

SUBMITTED BY

SWCA Environmental Consultants
6200 UTSA Boulevard, Suite 102
San Antonio, Texas 78249

IN ASSOCIATION WITH

The San Antonio Zoo
and SeaWorld San Antonio

San Antonio Zoo

SeaWorld

INTRODUCTION

The goal of salvage refugia is to collect and maintain captive stocks of listed Edwards Aquifer species when certain environmental triggers are met so that the species are available for reintroduction following a low-flow or other catastrophic event. The salvage refugia program has been established as part of compliance with the Edwards Aquifer Recovery Implementation Program Incidental Take Permit (ITP) and the associated Edwards Aquifer Habitat Conservation Plan (EAHCP). As mitigation, the EAHCP requires the establishment of off-site refugia to maintain captive populations of the Covered Species when it is determined that significant loss has occurred as a result of a catastrophic event, such as prolonged drought or chemical spill. This proposed research plan will improve our understanding of collection in a way that will provide a benefit to the collective refugia program, but is particularly focused on effective collection methods during a salvage collection event. Salvage collection is triggered when Covered Species die-offs in the wild are imminent or ongoing as outlined in the EAHCP (described below). The target collection numbers for salvage collection is not expressly identified in the EAHCP; however, in developing the *Refugia Review: Edwards Aquifer Habitat Conservation Plan,* HDR considered known captive propagation methodologies and the U.S. Fish and Wildlife Service (USFWS) *San Marcos/Comal/Edwards Aquifer Rare, Threatened, and Endangered Species Contingency Plan* to identify target collection amounts (HDR 2015). This research plan has been developed to describe and guide the currently anticipated research for the salvage refugia program in late 2015 and calendar year 2016.

In general, the proposed research will be focused on improving collection methods for the Comal Springs dryopid beetle (*Stygoparnus comalensis*) and the Texas blind salamander (*Eurycea rathbuni*). Since individuals collected as part of this research will be provided to the refugia facility, additional research on captive husbandry is proposed to support the eventual long-term refugia operations and ensure effective maintenance of captive populations. The research activities identified in this research plan will commence as soon as facilities are operational and USFWS and Texas Parks and Wildlife Department permits are in place. In the interim we have engaged with current research that is being conducted at the San Antonio Zoo and the San Marcos Aquatic Resources Center (SMARC).

COLLECTION METHODS

COMAL SPRINGS DRYOPID BEETLE

The Comal Springs dryopid beetle (CSDB) is one of 11 Covered Species included in the EAHCP. Salvage triggers for the CSDB occur when springflow at the Comal Springs falls below 30 cubic feet per second *and* when any standard or conventional water quality parameter within one of three Edwards Aquifer wells exceeds the historical range of water quality parameters by 10% or more (Recon et al. 2012). When triggers are met, it is expected that up to 500 individuals will be collected from the Comal Springs system for placement in the salvage refugia facility. In a salvage situation, it is likely that conditions will continue to decline and an efficient collection technique is essential to ensure that the maximum numbers of individuals are safely and efficiently collected. Unfortunately, none of the current collection methods effectively collect large quantities of CSDBs in a short period of time. Thus, the purpose of this proposed research is to investigate alternative collection methods to identify techniques that will increase the amount of CSDBs that can be collected during a salvage collection event.

The CSDB is considered a stygobiont (an obligate subterranean aquatic organism) and is the only known stygobiont within the beetle family dryopidae (Barr and Spangler 1992). The CSDB occurs in the Edwards Aquifer and has only been collected in two general locations, Comal Springs in Comal County, Texas, and Fern Bank Springs in Hays County, Texas. This species is only known to occur underground (or near spring orifices) within the Edwards Aquifer, resulting in inherent challenges when attempting to collect individuals.

Adult CSDBs are aquatic, have vestigial eyes, and are apparently flightless (Barr and Spangler 1992). Adults can absorb dissolved oxygen with the use of a plastron (a thin sheet of air) (Arsuffi 1993) and have been observed rising above the water surface in captivity (Barr and Spangle 1992). CSDB larvae, and all other known dryopid larvae, do not possess gills and are considered terrestrial or semi-terrestrial (Brown 1987). Thus, it is presumed that CSDB larvae inhabit air-filled voids spaces within the aquifer. These void spaces may contain food sources such as organic detritus and tree roots (Barr and Spangle 1992) and possibly serve as oviposition and pupation and sites.

PREVIOUS COLLECTION METHODS AND RESULTS

It is our understanding that to-date, less than 300 CSDB beetles have been historically collected from the wild (Arsuffi 1993; Barr 1993; Barr and Spangler 1992; Gibson et al. 2008; R. Gibson, USFWS, pers. comm. to B. Hall on 22 Sept 2015). Between 1987 and 1992, 58 adults and 27 larvae were collected and from 2002 to 2013, 71 adults and 51 larvae were collected; for a total of 207 individuals (R. Gibson, USFWS, pers. comm. to B. Hall on 22 Sept 2015). BIO-WEST has also incidentally collected a small number (exact number unknown) of CSDBs during annual monitoring of the Comal Springs riffle beetle (*Heterelmis comalensis*) (BIO-WEST 2005, 2006, 2007, 2008, 2014, 2015). It is likely that the paucity of historic collections of CSDB is likely attributable to current methodologies which do not directly sample what we understand to be the beetle's habitat.

Currently utilized methods collect CSDBs that have been ejected from the aquifer and/or individuals that occur at the extreme fringe of their presumed habitat, such as near spring orifices and upwellings. Previous collection methods have included kick nets, Hess samplers, hand sampling, bottle traps, drift nets, and cotton-cloth lures (Arsuffi 1993; Barr 1993; Barr and Spangler 1992; BIO-WEST 2005, 2006, 2007, 2008, 2014, 2015; Gibson et al. 2008; R. Gibson, USFWS, pers. comm. to C. Collins on 22 Sept 2015). Kick netting and Hess sampling are likely destructive to CSDBs and their habitats which, coupled with the preexisting vulnerability present during a salvage collection event, make this a less than ideal collection technique. Hand sampling has a historically low success rate, is labor intensive, and can also be potentially destructive to habitat. Bottle traps have been used to sample wells with limited success (R. Gibson pers. comm. to C. Collins 22 Sept 2015). Setting drift nets over spring orifices and upwellings is relatively non-invasive and has successfully resulted in the collection of living specimens. However, as shown in Table 1 below, this method has a relatively low capture rate that appears to favor larvae over adults.

Table 1. A summary of drift rate success of previous CSDB collections utilizing drift nets in Comal Springs, Comal County, and Fern Bank Springs, Hays County, Texas (as summarized in Gibson et al. 2008; BIO-WEST 2004–2015).

Location	Date	Drift time (hrs)	No. CSDB*	Drift Rate (No./24 hrs)
Comal Springs	August 1992	397.5	5 (1A, 4L)	0.3
Comal Springs	May/August 2003	451.6	5 (2A, 3L)	0.3
Comal Springs	2003–2014	1871.6	47 (4A, 41L, 1P)	0.6
Fern Bank Springs	August 1992	131.5	13 (1A, 12L)	2.4
Fern Bank Springs	May/August 2003	304.8	1L	0.1

* CSDB = Comal Springs dryopid beetle; A = Adult, L = Larvae, P = Probable pupae

Cotton-cloth lures are comprised of 60% cotton and 40% polyester and have been utilized to monitor Comal Springs riffle beetles in the Comal Springs system and San Marcos Springs with success (BIO-WEST 2005, 2006, 2007, 2008, 2014, 2015; Gibson et al. 2008). Cotton-cloth lures have also occasionally attracted CSDBs (BIO-WEST 2005, 2006, 2007, 2008, 2014, 2015; Gibson et al. 2008). The cotton-cloth lure method is the currently preferred non-lethal method for collecting Comal Springs riffle beetles, CSDBs, and other invertebrates (Gibson et al. 2008). Cotton-cloth lures are left out at least three to five weeks to foster biofilms (primarily bacteria and fungi) that are considered potential food sources that attract invertebrates. Cotton-cloth lures seem to catch low numbers of CSDB but, unlike drift netting, tend to favor adults (Gibson et al. 2008; R. Gibson pers. comm. to C. Collins 22 Sept 2015).

Using cotton-cloth lures, BIO-WEST has incidentally collected a few CSDBs during biological monitoring of Comal Springs riffle beetles in spring runs and upwellings in Comal Springs. BIO-WEST captured three CSDBs in 2007, six in 2013, and one in 2014 (BIO-WEST 2008, 2014, 2015). Gibson et al. (2008) also collected four CSDBs using cotton-cloth lures during invertebrate surveys of Comal Springs and Fern Bank Springs. Thus, if improved upon, a lure of some type and configuration may be suitable for collecting multiple numbers of CSDBs.

PROPOSED COLLECTION METHODS

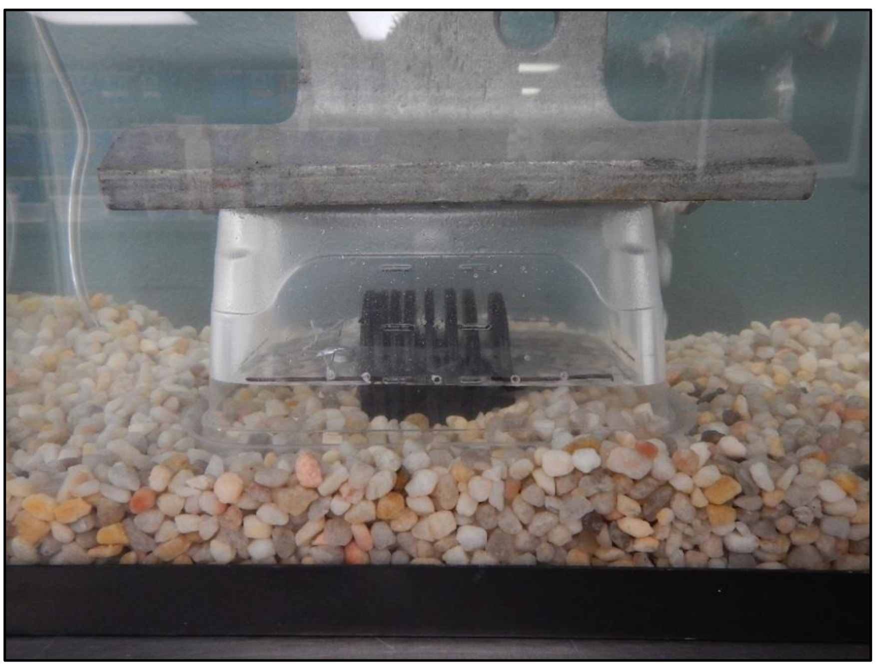
We are not proposing further research on existing methods that have proved ineffective in the past; rather we are proposing new research using cotton-cloth lures with modifications to methods previously intended to target species other than the CSDB. For this research, potential collection locations will be selected based on previous locations where CSDB have been historically collected in the Comal Springs system (Arsuffi 1993; Barr 1993; Barr and Spangler 1992; BIO-WEST 2005, 2006, 2007, 2008, 2014, 2015; Gibson et al. 2008; R. Gibson, USFWS, pers. comm. to B. Hall on 22 Sept 2015). These locations may include Spring Runs 1, 2, and 3 and upwellings in and around the Spring Island area. From 2002 to 2014, Spring Run 1 has yielded four adults and 29 larvae; Spring Run 2: 23 adults and one larva; Spring Run 3: one adult and 20 larvae; and Spring Island upwellings: 29 adults and no larvae (R. Gibson pers. comm. to B. Hall on 22 Sept 2015). These areas have historically produced the most CSDBs in Comal Springs.

In addition to cotton-cloth lures, we will test the common invertebrate sampler, Hester-Dendy (HD) (Hester and Dendy 1962). The HD sampler is a multi-plate substrate sampler that is typically used for biological monitoring to sample macroinvertebrate communities. Like cotton-cloth lures, HDs likely foster the growth of biofilms that provide food sources for invertebrates. Adult CSDBs have been found on pieces of wood lying on top of upwellings (Gibson et al. 2008). Thus, HDs made of native materials such as live oak (*Quercus fusiformis*), bald cypress (*Taxodium distichum*), or pecan (*Carya illinoensis*) tree species found in and around Comal Springs (C. Collins pers. obs., others), for example, may foster the appropriate type and amount of biofilms or habitat to attract CSDB individuals. HDs made of three different materials identified as HD-Type A, HD-Type B, and HD-Type C will be tested.

Gas bubbles are readily observed arising from upwellings in Comal Springs (LBG-Guyton Associates 2004). The gas bubbles are an apparent indication of ground water discharge and its content has been found to be largely atmospheric (LBG-Guyton Associates 2004). Gas goes into solution when atmospheric air is entrained and/or dissolved during surface water recharge. The gas comes out of solution with a reduction in pressure when aquifer water rises in elevation. The gas gets trapped within gravel and cobbles during the discharge of water into the spring system. This trapped gas may serve as potential habitat (air-filled void spaces) for the CSDB. Interestingly, no gas bubbles are readily observed in San Marcos Springs (LBG-Guyton Associates 2004). This *may* explain why CSDBs have not been found in the San Marcos Spring system. However, we do not know the status of gas bubbles in Fern Bank Springs, the only other known location for CSDBs.

Four upwelling areas (four replicates) will be sampled. Each upwelling area will have eight lure types that will be placed over, and partially buried within, eight randomly selected boils (areas where gas bubbles are observed rising from floor) of the upwelling. Four of the lure types (cotton-cloth lure, HD-Type A, HD-Type B, and HD-Type C) will be placed over four boils in the upwelling. Four additional lures, composed of the same materials as the previous four lures, will also be placed over and partially buried within boils. However, these lures will be placed under "bell traps." The larvae of CSDB are thought to be terrestrial or semi-terrestrial, relying on void spaces within the aquifer. It is also possible that adults favor void spaces, particularly dark spaces, as they are troglobitic. The lures placed under bell traps (consisting of black plastic containers, size to be determined) will contain an air/water interface. Each bell trap will be placed over a boil with noticeably rising gas bubbles. The released gas, largely atmospheric (LBG-Guyton Associates 2004), will be trapped inside the container (Photograph 1). The water level within the bell trap will be regulated with small holes punched into the container at the appropriate water level height, so that the lures will be partially underwater and partially above water (Photograph 2). Thus, inside the bell trap, the environment will be dark and contain aquatic and terrestrial environments. Lures, over time, will potentially grow biofilms that may attract CSDBs should they occur nearby within the upwelling (see Photograph 2.). Each upwelling (replicate) will have eight lures (variables). Sampling will start preferably in April or May, a time of the year when CSDBs have historically been collected (USFWS 1997). Lures will be checked every week for at least 12 weeks.

Photograph 1. Gas escaping from an upwelling. The released gas is largely atmospheric (LBG-Guyton Associates 2004).



Photograph 2. Gas released from the upwelling will be trapped within a "bell trap." The water level will be regulated with holes punched at the appropriate level. Portions of the lure will be both underwater and above water. In the field, the container will be black or opaque to simulate a dark, void space.

STATISTICAL ANALYSIS

The proposed experiment will include six HD treatments and two cloth lure control treatments—all crossed with bell trap covers and no covers—placed at four spring upwells. Upwelling sites are considered independent and will serve as replicates for a total of 32 experimental units. Total numbers of CSDBs will be recorded at each sampling trip and evaluated using analysis of variance (ANOVA). Due to the possible low prevalence of CSDBs, additional analyses may be performed using a nonparametric equivalent of an ANOVA, such as a Kruskal-Wallis test, using a ranked system. Additional water quality measurements may also be analyzed using regression analysis, where appropriate.

EXPECTED OUTCOMES

Capture success will be expressed as captured numbers of CSDB/time. Trapping methods will be compared to determine if the new sampling methods increase CSDB capture rates. It is our expectation that capture rates will increase with new sampling methods as compared to previous methods. Additionally, we also expect that trapping methods that most closely mimic the natural habitat of CSDBs (bell traps), will result in a higher capture rate.

COORDINATION

Our team will coordinate all activities with the City of New Braunfels Parks Department, BIO-WEST, Texas Parks and Wildlife Department, and the USFWS San Marcos Aquatic Research Center.

PERMITTING

The following permits are required to conduct the collection methods study for the Comal Springs dryopid beetle: Texas Parks and Wildlife Scientific Research Permit and USFWS Native Endangered and Threatened Species-Scientific Purposes, Enhancement of Propagation or Survival Permit.

Our team has applied for both of these permits and is coordinating with appropriate agency staff to obtain these permits.

TEXAS BLIND SALAMANDER

The Texas blind salamander (TBS) is a neotenic (retains juvenile characteristics in adult form) plethodontid salamander. The TBS is considered a stygobiont (an obligate subterranean aquatic organism) that exhibits troglomorphic traits. Its body is unpigmented and its limbs are long and slender. It lacks external eyes and its head is broad with a flattened snout. There are no definite external characteristics that can be used to determine sex. Juvenile TBS have been observed throughout the year, this is likely due to the thermally constant waters of the aquifer environment. The TBS has only been collected from wells and springs located in the San Marcos pool of the Edwards Aquifer, Hays County, Texas (Longely 1978). As this species is only known to occur underground within the Aquifer, collecting this species is inherently challenging.

Salvage triggers for the TBS occur when springflow at San Marcos Springs falls below 50 cubic feet per second *and* when any standard or conventional water quality parameter within one of three Edwards Aquifer wells exceeds the historical range of water quality parameters by 10% or more (Recon et al. 2012). When triggers are met, it is expected that as many living, undamaged individual TBSs as possible will be collected from the spring systems for placement in the salvage refugia facility. In a salvage situation, it is likely that conditions will continue to decline and an efficient collection technique is essential to ensure that the maximum numbers of individuals are collected. Unfortunately, none of the current collection methods effectively collect large quantities of TBSs in a short period of time. Thus, the purpose of this proposed research is to improve upon current collection methods that may increase the amount of TBSs that can be collected during a salvage collection event.

PREVIOUS COLLECTION METHODS

TBS have been collected from eight sites in Hays County including Rattlesnake Well, Ezell's Cave, San Marcos Springs, Artesian Well, Federal Fish Hatchery Well, Primer's Well, Wonder Cave, and Johnson's Well (Krejca 2007; Longely 1978). For this research, potential collection locations will be selected based on previous known locations and available access to wells and springs. Our current access includes Primer Well, Johnson's Well, and Rattlesnake Well. We hope to gain access to other locations as they become available, such as Diversion Spring-San Marcos Springs and Sessom's Spring.

Previous methods for collecting salamanders have included dip netting, hand collecting, drift netting, and various trapping methods (Krejca 2007; Mitchell and Reddell 1965; Uhlenhuth 1921; USFWS 2014). Bottle traps have been successfully used to capture groundwater organisms (Hutchins et al. 2010; Hutchins and Orndorff 2009; Malard et al. 2002; Purvisa and Opsahl 2005) including blind salamanders (D. Fenolio per. obs.). However, the capture rate (numbers per unit time) of TBSs with the use of bottle traps and various bottle trap configurations, has not been compared. Drift nets placed over springs and spring diversions have also been successful at capturing blind salamanders.

PROPOSED COLLECTION METHODS

We propose to compare capture rate (numbers of individual per unit time) of TBSs between two bottle trap designs within at least three well sites. We will also determine capture rate of TBSs at springs/spring diversions utilizing drift nets. Bottle trap designs will consists of plastic bottles with either a single inverted lid (single-opening) (Photograph 3)or two inverted lids (double-opening). Each bottle trap design will be deployed for six weeks within each season. Drift nets will be deployed at springs/spring diversions at the same time as bottle traps. All bottle traps and drift nets will be checked weekly during four seasons: winter, spring, summer, and fall. Seasons are defined as follows: January / February / March = Winter; April / May / June = Spring; July / August / September = Summer; and October / November / December = Fall.

STATISTICAL ANALYSIS

We proposed to use an ANOVA to compare capture rates of TBSs between single-opening and double-opening bottle traps within sites, between sites, and between seasons. Due to the possible low prevalence of TBSs, additional analyses may be performed using a nonparametric equivalent of an ANOVA using a ranked system. We will use a T-Test to compare capture rates between well sites and springs/spring diversions. Additional water quality measurements may also be analyzed using regression analysis, where appropriate.

Photograph 3. Single-opening bottle trap design.

EXPECTED OUTCOMES

Capture success will be expressed as captured numbers of TBSs/time. The bottle trap design that captures the most TBSs over time will be the preferred trapping method during salvage collections. With this study, we will likely not determine if one well site or spring site has more TBSs than the others. We will however, determine which trap design captures more TBSs than others at a particular well site. We might find that one trap design captures more TBSs than the other designs at one well site. Conversely, another design may be more successful at a different well site. A successful trap design may be site specific. It is anticipated that during salvage collection, drift nets will also be deployed over springs and spring diversions. Drift net capture rates will be compared to bottle trapping rates. It should be noted that TBSs collected in drift nets are individuals that happen to be expelled from their habitat (the aquifer) as opposed to bottle trapping that traps TBSs within their habitat. These two collection methods are not comparable; however, estimates on capture rates on these two capture techniques would be useful in determining the amount of time needed to collect specimens during salvage operations.

COORDINATION

Our team will coordinate all activities with Texas State University – Meadows Center, BIO-WEST, Texas Parks and Wildlife Department, and the USFWS San Marcos Aquatic Research Center.

PERMITTING

The following permits are required to conduct the collection methods study for the Texas blind salamander: Texas Parks and Wildlife Scientific Research Permit and USFWS Native Endangered and Threatened Species-Scientific Purposes, Enhancement of Propagation or Survival Permit.

Our team has applied for both of these permits and is coordinating with appropriate agency staff to obtain these permits.

HUSBANDRY RESEARCH

COORDINATION WITH ONGOING SMARC RESEARCH

SMARC has offered to make F1 salamanders (Texas blind salamander and San Marcos salamander [*Eurycea nana*]) available for husbandry research. Our project team is currently investigating this option, but has some concerns about the appropriateness of introducing a known pathogen, *Microsporidium* spp., into the new salvage refugia facilities at the zoo. Members of our project staff are currently receiving hands-on training at SMARC from SMARC employees in basic husbandry of the salamanders by assisting in the care of the F1 salamanders.

HUSBANDRY RESEARCH AT THE SAN ANTONIO ZOO

Since research during the salvage refugia term is focused on refining collection methods for the CSDB and the TBS, it is expected that individuals will be collected and transported to the facility for use as standing stock for the long-term refugia operation. Since the long-term refugia facility may not be operational by the time individuals are collected, it is assumed that collected individuals will be transported to the San Antonio Zoo. For this reason, some captive husbandry protocols need to be refined to support healthy individuals in the captive environment.

Our project team has identified a series of questions that will be applied to the species held in captivity. It is expected that many of these questions will be answered through routine observations and diligent record keeping.

Data collected will also include:

1. Physical baseline of research specimens (length, weight, health observations) with follow-up measurements (up to twice per month).
2. Tank water quality (temperature, dissolved oxygen, hardness, pH, nitrates/nitrites, ammonia).
3. Video surveillance (when and where appropriate).

4. Observations and documentation of behavior (e.g., feeding, use of cover/shelter in containers, breeding, cannibalism) via visual data collection, photographic, night-vision video recording equipment, and video surveillance. Lighting used will be based on lessons learned from current SMARC research.
5. Breeding success rate (number of eggs, percent hatched, survival rates, cannibalism of offspring)

RESEARCH QUESTIONS

Question 1:

Can Target Species/surrogate species be collected in a manner that does not damage them?

Question 2:

Can Target Species/surrogate species be transported to the lab in a manner that does not damage them?

Progress Metric:

Target Species/surrogate species are collected and delivered to the lab and survive for an excess of two weeks.

Question 3:

Will the Target Species/surrogate species eat in captivity?

Progress Metric:

Target Species/surrogate species are collected and delivered to the lab and eat in captivity.

Question 4:

What foods will the Target Species/surrogate species accept in captivity?

Progress Metric:

The Target Species/surrogate species eats in captivity and food preferences, if any, are noted.

Question 5:

Is there a food delivery method that increases consumption in Target Species/surrogate species?

Progress Metric:

A food delivery pathway is identified that increases food consumption in Target Species/surrogate species.

Question 6:

Will the Target Species accept and absorb multiple food types?

Progress Metric:

The Target Species/surrogate species accepts multiple food types; fecal sample testing indicates nutrients are being absorbed. *****

***** Some subterranean species have specific dietary parameters and this question may not apply to all species.

Question 7:

What does a growth curve from the juvenile condition to the adult condition look like in Target Species?

Progress Metric:

A growth curve for a Target Species/surrogate species is produced via laboratory collected data. Use feasible techniques for marking and measuring specimens including anesthetics currently being evaluated by SMARC.

Question 8:

Can reproductive behavior be induced with changes in captive conditions (e.g., water conductivity shifts, temperature changes, changing water flow rates)?

Progress Metric:

Some manipulation of environmental conditions is identified that can induce reproductive behavior.

Question 9:

Is there an ideal group size in a laboratory enclosure?

Progress Metric:

Differently numbered groups are maintained in otherwise identical laboratory conditions and a subsequent analysis demonstrates that either better growth rates and/or reproductive behavior are achieved by one group size over others (statistically significant difference).

REPORTING

Detailed methodologies and results of all research conducted for the salvage refugia program will be summarized and documented in the salvage refugia program annual report submitted annually to the Edwards Aquifer Authority.

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Appendix K10

RFP No. 149-15-HCP to solicit proposals from qualified vendors for the providing refugia operations and practicing husbandry for the care and propagation of the eleven (11) covered species as required by the Edwards Aquifer Habitat Conservation Plan (Long Term Refugia Operations)

EDWARDS AQUIFER
AUTHORITY

September 21, 2015

Dear Interested Offeror:

The Edwards Aquifer Authority (the "EAA") is requesting proposals from qualified vendors for providing refugia operations and practicing husbandry for the care and propagation of the eleven (11) covered species as required by the Edwards Aquifer Habitat Conservation Plan (Proposal #149-15-HCP).

Attached is the proposal package. Please complete the attached sheets with one (1) unbound original, three (3) bound copies and one (1) electronic copy (Attachment A1), Cost Proposal Summary (Attachment A2), and Client Reference forms (Attachments B1-B3) and submit to:

Ms. Cyndi Holman
Procurement Specialist
Edwards Aquifer Authority
900 E. Quincy Street
San Antonio, TX 78215

Proposals must be submitted only on the attached proposal summary forms and are to be sealed with "EDWARDS AQUIFER HABITAT CONSERVATION PLAN LONG TERM REFUGIA OPERATIONS PROPOSAL" indicated on the top of the envelope. Proposals are due in the EAA offices no later than 3:00 p.m., Thursday, October 29, 2015, at which time the proposals will be opened. **PROPOSALS RECEIVED AFTER THE DEADLINE WILL NOT BE ACCEPTED AND WILL BE RETURNED IMMEDIATELY UNOPENED.**

Proposals offering less than 90 calendar days for acceptance by the EAA from the date set for opening will be considered nonresponsive and will be rejected.

The EAA reserves the right to reject any and all proposals.

A mandatory pre-proposal meeting is scheduled for Wednesday, October 7, 2015 at 9:00 a.m., at the EAA office.

If you have any questions, please call Ms. Holman at (210) 222-2204.

Sincerely,

Roland Ruiz
General Manager

REQUEST FOR PROPOSALS
FOR EDWARDS AQUIFER HABITAT CONSERVATION PLAN
LONG TERM REFUGIA OPERATIONS
FOR THE
EDWARDS AQUIFER AUTHORITY
PROPOSAL NO. 149-15-HCP

Issued by:
Edwards Aquifer Authority
900 E. Quincy Street
San Antonio, TX 78215
(210) 222-2204

Issue Date:	Monday, September 21, 2015
Proposals Close:	Thursday, October 29, 2015
Time:	3:00 p.m., Central Time

SECTION 1
PROJECT INTRODUCTION AND BACKGROUND

1.1 PURPOSE OF REQUEST FOR PROPOSALS (RFP)

The Edwards Aquifer Authority (EAA), located in San Antonio, Texas, is soliciting statements of team qualifications and technical proposals from qualified vendors to provide a "turn-key" Refugia Operation for the Covered Species under the Edwards Aquifer Habitat Conservation Plan Program (EAHCP). The primary purpose of the EAHCP Long Term Refugia Operations is to provide for the development and operations of Captive Propagation Facilities which will provide artificial habitats where viable source populations of Covered Species can be protected and survive during episodes of compromised habitat conditions (i.e. drought, disease outbreaks, water quality impairment, etc.) in the Comal and San Marcos Springs and River systems with the intent to reintroduce and repopulate, following an extinction, back into the wild (i.e., non-refugia springs and river ecosystems).

1.2 DESCRIPTION OF PROPOSED PROJECT

The Awarded Team shall provide a "turn-key" Refugia Operation, for the eleven (11) Covered Species of the EAHCP. The refugia operations team, in coordination with EAA, will manage all aspects of Refugia Operations for a period equal to the duration of the Incidental Take Permit (valid through March 31, 2028).

Qualified teams shall demonstrate expertise in fisheries science; fisheries facility planning, engineering, and operations; hatchery/refugia program development and management; invertebrate and aquatic research; and environmental compliance.

A record of developing programs in a manner that demonstrates the ability to meet the requirements of the Edwards Aquifer Authority Act, Edwards Aquifer Habitat Conservation Plan and associated Incidental Take Permit (ITP), will be a required criteria for vendor qualification. Prospective vendor teams shall describe their approach to developing a conceptual construction design and management plan in coordination with EAA and USFWS to maintain compliance with the Edwards Aquifer Habitat Conservation Plan Program (EAHCP).

1.3 PROJECT SCHEDULE

The following is the tentative schedule for selection and award of the EAA's procurement:

Description	Date
RFP Advertisements	Sunday, September 20, 2015 Sunday, September 27, 2015 Sunday, October 4, 2015
Mandatory Pre-Proposal Meeting	Wednesday, October 7, 2015 9:00 a.m., Central Time
Last Date for Questions	Wednesday, October 7, 2015 12:00 p.m., Central Time
Proposals Close	Thursday, October 29, 2015 3:00 p.m., Central Time

SECTION 2
INSTRUCTION TO OFFERORS

2.1 REQUESTS FOR INFORMATION

This RFP is being issued by the EAA, San Antonio, Texas, which is the sole point of contact for purposes of information concerning this RFP. The EAA reserves the right to issue addenda, if required. All questions and inquiries regarding this request for proposal must be submitted in writing to Ms. Cyndi Holman, Procurement Specialist, by 12:00 p.m., Central Time, Wednesday, October 7, 2015. Requests for information received prior to the above stated deadline are to be responded to in writing by the EAA in the form of an addendum addressed to all proposal specification recipients.

The EAA will conduct a mandatory pre-proposal meeting with prospective Offerors on Wednesday, October 7, 2015 at 9:00 a.m. 900 E. Quincy Street, San Antonio TX 78215. The EAA will not accept proposals from Offerors who did not have a representative in attendance of the pre-proposal meeting.

Submission of a proposal shall be considered *prima facie* evidence that the Offeror is familiar with, and understands, the solicitation, its terms and general conditions, etc., under which the contract is to be awarded, administered, and performed. The EAA will not be responsible for any interpretations or misinterpretations of any oral instructions.

2.2 SUBMISSION REQUIREMENTS

Offerors are required to submit their proposals on the attached Proposal Summary forms (see Attachment A1). Proposal envelopes are to be plainly marked, "EDWARDS AQUIFER HABITAT CONSERVATION PLAN LONG TERM REFUGIA OPERATIONS PROPOSAL".

Offerors are required to submit their proposals no later than 3:00 p.m., Central Time, on Thursday, October 29, 2015 to:

Ms. Cyndi Holman
Procurement Specialist
Edwards Aquifer Authority
900 E. Quincy Street
San Antonio, TX 78215
cholman@edwardsaquifer.org
(210) 222-2204

NO FACSIMILE PROPOSALS WILL BE ACCEPTED.

Upon receipt by the EAA, each proposal will be stamped with the date and time received and stored unopened in a secure place until the proposal opening. All proposals become the property of the EAA, which will hold the contents of all proposals confidential until an award is made.

PROPOSALS RECEIVED AFTER THE TIME SET FOR THE OPENING WILL BE DECLARED LATE AND NOT ELIGIBLE FOR OPENING AND CONSIDERATION. THE EAA IS NOT RESPONSIBLE FOR MAIL, COURIER OR OTHER DELIVERY METHODS, IN-TRANSIT TIME OR NON-DELIVERY. LATE DELIVERIES WILL BE HELD UNOPENED. OFFEROR WILL BE ADVISED BY MAIL THAT HIS/HER PROPOSAL WAS LATE AND NOT ACCEPTED AND WILL BE ALLOWED TO PICK UP HIS/HER PROPOSAL PACKAGE OR FURNISH A "CALL TAG" AND HAVE THE PACKAGE PICKED UP BY A COURIER.

2.3 PROPOSAL FORMAT

The EAA requires that submitted proposals adhere to the following general format to simplify the review process. Failure to follow the required format or to respond to each specification may result in rejection of the proposal. All proposals must be submitted on the enclosed forms in triplicate, or photocopies of the forms and electronically on CD or flash drive format. Failure to do so may result in rejection of the proposal.

2.3.1 General Requirements

To be considered responsive, responsible, reliable, qualified, and possessing the ability to acquire or possess an adequate location (which meets the definition for redundancy) design, construction, and management and operation of the entire project for a period of up to the year 2028 (ITP authorization period). More specifically to be considered qualified, vendor teams, must provide their approach and demonstrate their technical ability to fulfill the criteria for development and management of refugia facilities as described in Section 3.2.

Submit the names of three client references for whom similar captive propagation and refugia operations and services have been provided within the past five (5) years on the form contained in Attachments B1-B3, Client Reference. Do not use the EAA as one of the three references. The description must provide the following minimum information:

- Client name;
- Client contact name;
- Client address and telephone number(s);
- Date conservation activity began; and
- Description of services/ conservation activities.

The Offeror agrees EAA staff may contact the references given.

2.3.2 Response to Commercial Questions and Statements

Please answer the questions indicated in Attachment A1, Proposal Summary, directly and specifically. All pricing is to be included in Attachment A2, Cost Proposal Summary. Any exceptions to any of the requirements and specifications contained in this RFP must be noted in the allotted space in Attachment A1. Attachments A1 and A2 must be returned with the Offeror's response.

2.3.3 Cost Proposal

Provide, under separate sealed envelope, an itemized list of projected costs necessary to complete this project. Use the forms provided in Attachment A2, Cost Proposal Summary, to quote a price for the EAHCP Long Term Refugia Operations described in this RFP. The EAA is exempt from sales tax, but could be subject to other types of taxes. If any other type of tax is added, please specify.

2.4 MINORITY-OWNED AND WOMEN-OWNED BUSINESSES

The EAA strongly encourages minority and women-owned businesses to submit proposals. The EAA also encourages applicants, in those instances when joint venturing and/or subcontracting is appropriate, to form joint ventures and/or provide subcontract opportunities to minority and women owned firms.

2.5 PROPOSALS BINDING

Proposals must set forth accurate and complete information as required by this RFP (including attachments). Negligence upon the part of the Offeror in preparing the proposal confers no right of withdrawal after the time fixed for the submission of proposals.

2.6 LATE PROPOSALS, MODIFICATIONS, OR WITHDRAWALS

Proposals received after the date and the time indicated will not be considered and will be returned unopened, if the Offeror is identified on the envelope.

Proposals may be withdrawn or modified in writing prior to the proposal opening. Responses that are resubmitted or modified shall be sealed and resubmitted to the Procurement Specialist prior to the proposal opening.

2.7 PROPOSAL COSTS

All costs for preparing the proposals are to be borne by the Offeror and may not be included in the cost proposal.

2.8 PROPOSAL SIGNATURE

The EAA will prepare a contract for the Successful Offeror using the name exactly as it appears on the proposal. Therefore, it is imperative the Offeror sign the proposal using correct and complete legal names and titles.

2.9 CONTRACT AWARD

The EAA reserves the right to accept or reject any and all proposals. Unless all proposals are rejected or the solicitation is cancelled, the contract is to be awarded to the Offeror whose proposal best meets the requirements and criteria set forth in these specifications. No proposal is to be considered binding upon the EAA until a contract has been awarded. The EAA reserves the right to award the proposal and/or contract to more than one Offeror.

Contract award is to be issued to the Successful Offeror by letter. The vendor shall not begin any work on this contract until such time as a Notice to Proceed has been issued by the General Manager.

2.10 CONTRACT

It is expressly understood by the Offerors that written notice of award by the EAA will constitute acceptance of the proposal.

2.11 CONTRACTOR SELECTION

2.11.1 Selection Process

The selection process will include the following steps:

1. Receipt of proposals.
2. Review of proposals submitted.
3. Evaluation of proposals and ranking of Offerors - EAA staff shall review all information available from the selection process and rank the Offerors.
4. To assist in the selection process, the EAA may choose to interview the highest ranking Offerors.
5. The EAA will award the contract to the Offeror whose proposal is the most advantageous to the EAA and which will result in the most economical provision of these required services to the EAA.

2.11.2 Selection Criteria

Selection will be based on the following criteria which are listed in order of importance:

1. Commercial Quality (55 points)
    - Proven scientific understanding of endangered species issues
    - Experience with similar programs
    - Adequate consideration of scientific constraints
    - Possession of, or plan to obtain, appropriate permits from United States Fish and Wildlife Services (USFWS), Texas Parks and Wildlife Department (TPWD) or any other requisite permit, as needed for Refugia Operations.
    - Description of technical approach for the development of a Refugia Master Plan
    - Ability and approach to house (i.e., property, facilities, water, and utilities) standing, refugia and salvage stock of the covered species in a primary and redundant facility through March 31, 2028
    - Ability to safely collect and transport individuals to and from natural habitats and refugia facilities
    - Knowledge of the environmental conditions in the project areas and species' habitats
    - Documented methods to insure ethical treatment of animals
    - Ability to handle all aspects of operations, including: budgeting and invoicing

2. Cost Factors (30 points)
    - Total cost
    - Contingency Costs (estimated costs for services not included or specified)

3. Timing (10 points)
    - Offerors will be scored on their ability to have fully functioning Refugia Operations in place by January 1, 2017(earlier preferred), or as close to that date as possible

4. Financial Stability (5 points)
    - Current financial condition

2.12 CONFIDENTIAL MATERIAL

Proposals will remain confidential until an award is made, except for the information that is public during a proposal opening. At that time, all information is public unless considered confidential by the Public Information Act, such as trade secrets and financial information. Offeror must indicate if any of the information provided constitutes an exception to the Public Information Act. All information not labeled as confidential will be presumed to be public information.

SECTION 3
SPECIFICATIONS

3.1 BACKGROUND INFORMATION

The EAA is a political subdivision of the State of Texas. Governed by an elected board of directors, the EAA is empowered to manage, preserve, and protect the Edwards Aquifer.

This project is intended to provide for the collection, husbandry, captive propagation, and reintroduction of covered species individuals collected from their native habitat when it is determined by pre-established triggers outlined in the HCP that signify that losses of covered species in the wild are imminent or on-going. This project is expected to continue through March 2028.

Section K of EAA's ITP[1] requires that the EAA provide for refugia to house and protect the covered species in the event that a drought or other disaster may threaten the species' continued existence without intervention.

Offerors will be required to demonstrate extensive knowledge of refugia operations, care and propagation, and research of the eleven covered species listed in the Habitat Conservation Plan.

Offerors will be required to conduct research to assist in successfully implementing refugia for the covered species[2] and may include: physiology, life histories, effective reintroduction techniques, collection techniques, husbandry, genetics and propagation.

The use of EAA funds will be limited to the operation and maintenance of refugia for the covered species or related surrogates used for research purposes; other operations and research activities will not be funded.

The primary purpose of this project is to conduct refugia operations, for the eleven (11) covered species listed below and identified in the EAHCP and listed for coverage under the ITP.

[1] As amended on January 20, 2015
[2] See the "Additinoal Information for Offerors, # 13" in this RFP

Common Name	Scientific Name	ESA Status
Fountain Darter	*Etheostoma fonticola*	Endangered
Comal Springs Riffle Beetle	*Heterelmis comalensis*	Endangered
San Marcos Gambusia	*Gambusia georgei*	Endangered[3]
Comal Springs Dryopid Beetle	*Stygoparnus comalensis*	Endangered
Peck's Cave Amphipod	*Stygobromus pecki*	Endangered
Texas Wild Rice	*Zizania texana*	Endangered
Texas Blind Salamander	*Eurycea rathbuni*	Endangered
San Marcos Salamander	*Eurycea nana*	Threatened
Edwards Aquifer Diving Beetle	*Haideoporus texanus*	Petitioned[4]
Comal Springs Salamander	*Eurycea* sp.	Petitioned[5]
Texas Troglobitic Water Slater	*Lirceolus smithii*	Petitioned[6]

*The San Marcos gambusia was last collected in the wild in 1983 and may already be extinct.

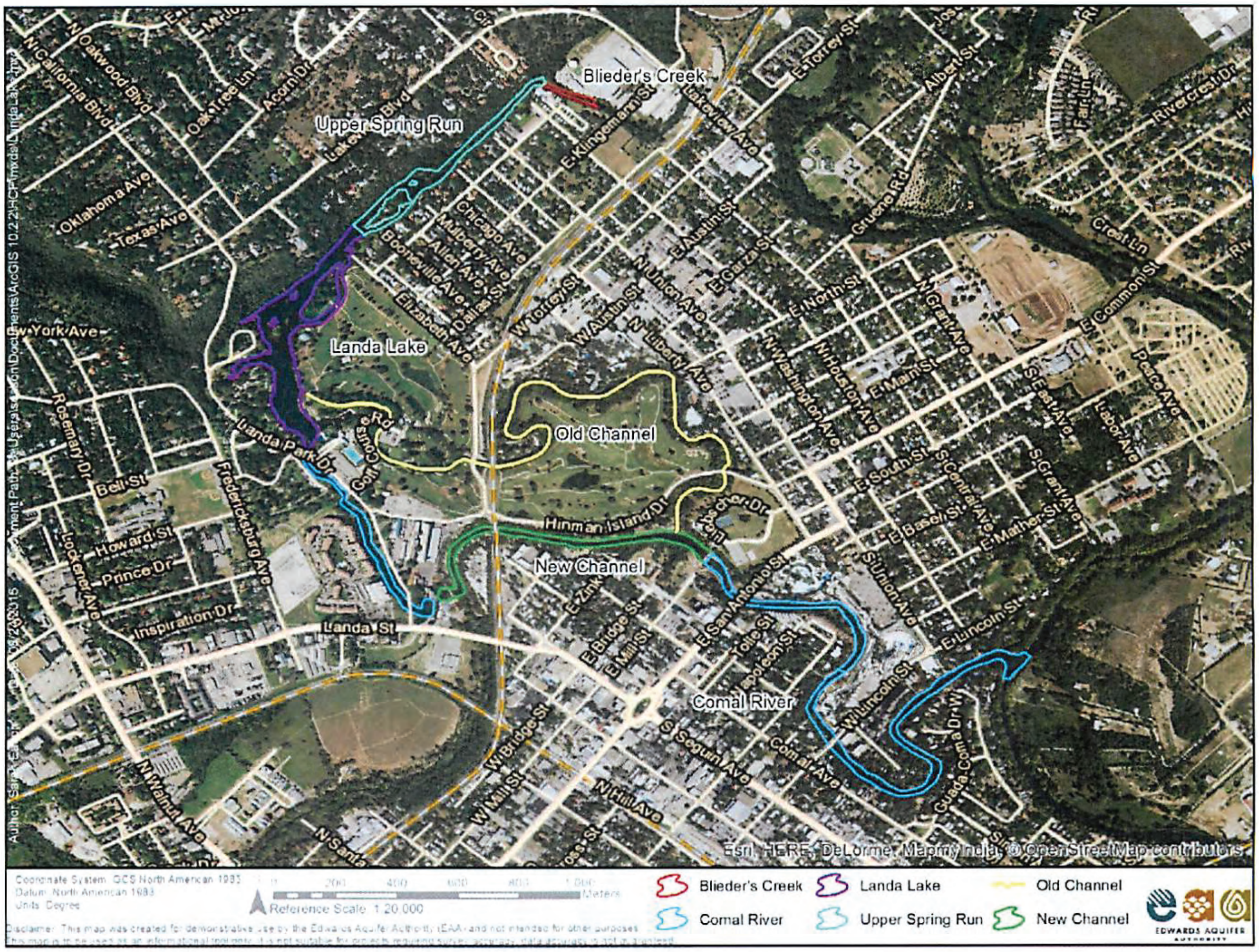
PROJECT AREA

This project is focused on the species that are dependent on the ecosystems that surround Spring Lake in San Marcos and the San Marcos River downstream to Interstate 35 Bridge (Map 1); and Landa Lake and the Comal River (Map 2) maps below:

[3] Presumed extinct; See the "Additional Information for Offerors, # 13" in this RFP
[4] See the "Additional Information for Offerors, # 13" in this RFP
[5] See the "Additional Information for Offerors, # 13" in this RFP
[6] See the "Additional Information for Offerors, # 13" in this RFP

San Marcos/Spring Lake Project Area Map (Map 1)

New Braunfels/Landa Lake Project Area Map (Map 2)



3.2 MINIMUM SPECIFICATIONS

3.2.1 Edwards Aquifer Habitat Conservation Plan Long Term Refugia Operations

Proposed Scope of Work
The Awarded Contractor will be expected to provide fully operational refugia capable of meeting the requirements as described in the proposed Scope of Work.

The refugia will be operated in accordance with guidelines identified in the EAHCP, Section K of the ITP[7], and the USFWS "Policy Regarding Controlled Propagation of Species Listed under the Endangered Species Act." The Awarded Contractor, in coordination with EAA, shall manage all aspects of Refugia Operations through March 2028.

Refugia Operations are defined herein to include the following:

- One main off-site[8] refugia facility and one redundant off-site refugia facility. Refugia facilities should provide for, at a minimum, the space requirements and infrastructure specifications presented in the conceptual layout (see Attachment C), as well as, all requirements in the "Additional Information for Proposers" section of this RFP.
- Refugia program staffed with qualified and permitted individuals for all EAHCP Covered Species
- Collection, establishment, and maintenance of standing stocks, refugia stocks, and salvage stocks for the EAHCP covered species[9]
- Development and refinement of animal rearing methods and captive propagation techniques
- Preparation of a Research Plan to be used for the duration of the ITP, with specific goals and milestones
- Conduct research, as defined in the Research Plan, to further refine refugia operations
- Preparation of a Reintroduction Plan for each Covered Species[10]
- Reintroduction of affected species, as defined in the Reintroduction Plan, if triggered
- Refugia program permitting and reporting, as required by: the ITP, USFWS, TPWD, the EAHCP Annual Report, HCP Annual Workplans and compliance meetings with Regulators.

In responding to this RFP, the Offeror shall discuss the general approach to tasks, specific methodologies, infrastructure, project management and team organization, strategies to accomplish work in a timely manner, and budget requirements to complete each of the

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[7] As amended on January 20, 2015

[8] Offsite is defined as "away from the principle area of activity". For our Refugia program, it simply refers to a location far enough away from the springs ecosystems (Comal and San Marcos), so that if the springs' ecosystem is compromised by some action (flood, fire, natural or man caused disaster, etc.), the offsite facility is not compromised by the same action

[9] See the "Additional Information for Offerors, # 13" in this RFP

[10] See the "Additional Information for Offerors, # 13" in this RFP

tasks listed below. After the negotiation of a contract and for the following scope of work, the Successful Offeror will be a Contractor with the EAA.

Task 1. Provide fully functioning Refugia for the EAHCP Covered Species[11]. Facilities: services will include facilities use and maintenance, utilities, water source, daily operations, and staffing.

Refugia Stocks: collect, establish, and maintain standing stocks, refugia stocks, and salvage stocks of Covered Species (when triggered). Standing Stock, Refugia Stock, and Salvage Stock target numbers and captive populations[12] should be divided between the primary facility and redundant facility. The division of these numbers may remain flexible and should be managed in consultation with the EAA. Refer to Table 2 for species numbers and other requirements.

Permits: the Contractor will be responsible for identification and acquisition of all required Federal, State and local permits. The Contractor shall provide EAA with drafts, for review, of all permit application before submittal. The Contractor will prepare, in cooperation with and approval from the EAA, a detailed Disposition Plan to be included in the Federal Recovery Permit.

Additionally, at a minimum of once a quarter and up to monthly if needed, the Contractor shall be required to meet at the Refugia facility with EAHCP staff. Together, the Contractor and EAHCP staff will review the facility, current research, and discuss any contractual compliance issues or budgeting concerns. The EAA, with reasonable notice to the Contractor, may visit/access the Refugia facilities at any time.

Task 2. Conduct research as necessary to expand knowledge of the EAHCP Covered Species[13] for refugia operations. At the direction of the EAA, the Contractor will conduct research including but not limited to, species' physiology, environmental requirements, health and disease issues, life histories, genetics and effective reintroduction techniques. In cooperation with and with approval from the Edwards Aquifer Authority, the Contractor will prepare, a specific Research Plan for the duration of the ITP focusing on these items:

- Specifying necessary research topics for the species
- Listing appropriate possible research questions for the species
- Discussion of previous and current genetic studies
- Researching federally listed species only
  - Identifying the listed species with the greatest data needs (for refugia purposes)
  - Identifying the order that the listed species are most likely to be affected
  - Identifying listed species most difficult to re-introduce
- Reintroduction methods and techniques for each listed species
- Timelines and milestones

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[11] See the "Additional Information for Offerors, # 13" in this RFP
[12] The collection of species should comprise a 50:50 male/female sex ratio to the greatest extent possible
[13] See the "Additional Information for Offerors, # 13" in this RFP

The draft Research Plan is due to EAA within 180 days of notice to proceed and will be presented by the Contractor to the EAHCP Science Committee for input. Research will begin no later than 365 days from Notice to Proceed.

Task 3. Develop and refine animal rearing methods and captive propagation techniques for the Covered Species. The Contractor will document the success and failures of each rearing method and captive technique.

Task 4. Reintroduction of species and monitoring of recovery in the event of a loss of species in their native environment. The Contractor will prepare, in cooperation with and approval from the EAA, a specific Reintroduction Plan for each Covered Species[14]. See the flow diagram in Attachment D for a general concept of the steps in reintroduction.

Task 5. Submit annual reports describing all activities completed under this project including "lessons learned." This task also requires the development (and annual updates) of a stand-alone procedural/operations manual.

Additionally, the Contractor shall prepare the EAHCP Annual Work Plan for Refugia, as required by Section 4.4 of the Funding and Management Agreement of the EAHCP and the Annual Report as required by Section U of the ITP.

Task 6: Attend meetings and give presentations to the USFWS regulatory division, EAHCP Science Committee, EAHCP Implementing Committee, and EAA Board of Directors as requested by the EAHCP Program Manager. Offeror should budget for a minimum of five professional presentations/meetings in the first two years and three each year thereafter.

ADDITIONAL INFORMATION TO OFFERORS

1. Proposals should discuss the process and methodology for completing the tasks described in this RFP, and should identify all subcontractors that will be used on the project and the task on which each subcontractor will be used.
2. The Contract will be a time and material contract with a specified "not to exceed" amount.
   The budget will be itemized by tasks. Cost estimates should take personnel, infrastructure, supplies, equipment, and travel into account for each task. Project personnel should be identified by position title and the hourly rate. The budget should clearly indicate the indirect costs or overhead charged for the work.
3. This project requires the use of untreated Edwards Aquifer groundwater. The proposal should include discussion of the water source for the proposed project, access point, etc.
4. The proposal should discuss infrastructure and facilities, and location of refugia, including:
   - Square Footage
   - Power/Generator/Power Backup
   - Lab facilities (onsite or access to)
   - Salvage Pods
   - Facilities for Texas Wild Rice (presumably outside or in a greenhouse)

[14] See the "Additional Information for Offerors, # 13" in this RFP

- o Bio-security/Quarantine methods
- o Edwards Groundwater
- o Appropriate effluent capabilities

The EAA has an existing contract to provide salvage refugia operations through December 2016. This contract includes the use of 2-4 "pods"[15] to care and house protected species. The Successful Offeror will be expected to use the pods for at least some portion of the Long Term Refugia Operations.

5. The proposal should discuss all aspects of disease control, biosecurity and health issues including:
   - Quarantine of all wild caught individuals
   - Certified disease free stocks for reintroduction
   - Disinfection of the refugia facility and equipment
   - Isolation plan for appropriate species, stocks of species, and species from each spring system
   - Appropriate treatment of effluent waste water
   - Prevention of disease introduction to the refugia from:
     - i. Non Refugia species
     - ii. Water Source
     - iii. Human introduction (staff and public)
     - iv. Food source
     - v. Etc
   - Biosecurity of facilities
   - Prevention of escapement of all life stages of the Covered Species.

6. Scope alterations should contain detailed discussions of task alternatives, alternative task budgets, and alternative timelines. If scope alternatives are provided, the Offeror will provide a separate budget table as described in section 3.2.2 of this RFP.

7. The EAA reserves the right to select any combination of tasks and subtasks for the final scope of work.

8. The Successful Offeror will include EAA staff in all discussions with USFWS.

9. A record of developing programs in a manner that demonstrates the ability to meet the requirements of the Edwards Aquifer Authority Act, will be a required criteria for vendor qualification. Prospective vendor teams will describe their approach to developing a Refugia in coordination with EAA and USFWS to maintain compliance with the EAHCP.

10. Offerors will be required to demonstrate extensive knowledge of refugia operations, care and propagation, and research of the eleven (11) covered species listed in the EAHCP. Offerors may be required to conduct research to assist in successfully implementing refugia for the covered species and may include: physiology, life

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[15] A pod is a retrofitted storage/shipping container. The use of pods, while a relatively new concept, has been implemented at several facilities across the globe including the Australian Amphibian Research Center, the Atlanta Botanical Gardens, the Central Florida Zoo, the Chilean Amphibian Research Center at National Zoo in Santiago, Chile, and others.

histories, effective reintroduction techniques, collection techniques, husbandry, and propagation.

11. There are 11 Covered Species in the EAHCP. Of the 11, 8 have federal listing status and 3 are proposed for federal listing.

    One of the listed species is the San Marcos gambusia, presumed extinct. No space (sq ft) shall be required in the facilities for the gambusia and no resources shall be expended on collection or research of the San Marcos gambusia. In the event that the San Marcos gambusia is inadvertently collected or identified, the Contractor shall work with EAA to draft an action plan to address refugia for the San Marcos gambusia.

    Priority shall be given to listed species, rather than proposed species. i.e. The research and reintroduction plans should address all aspects/issues associated with listed species prior to work on proposed species.

12. The refugia program will be developed to maintain standing stock[16], refugia stock[17], and salvage stock[18] (if necessary). Standing stock and refugia stock will be collected and maintained in a manner that preserves genetic integrity of covered species. The refugia program will also be developed in a manner that allows for the adaptation of target numbers and management units as new information about the species becomes available. Current standing stock, refugia stock, and salvage stock target numbers for each species are as follows:

[16] Standing Stocks - continuously maintained; to account for unforeseen event

[17] Refugium Stocks - original and primary brood stock for preservation; breeding stock; collected after concern is identified.

[18] Salvage Stocks – harvested when significant Covered Species kills in the wild are imminent or on-going; occurs when triggers are met.

Table 2.
Species Triggers and Numbers

Species	Standing Stock		Refugia Stock		Salvage Stock	
	Trigger	Target #	Trigger	Target #	Trigger	Target #
Fountain Darter (Comal)	ASAP	1,000	< 150 cfs	1,000 minus standing stock	< 50% mean aquatic vegetation (Landa lake and Old Channel) and darter presence system wide; or <20% mean aquatic vegetation (Landa Lake and Old Channel) and <30% darter presence system wide	2000
Fountain Darter (San Marcos)	ASAP	1,000	< 80 cfs	1,000 minus standing stock	≤ 75 cfs for four (4) consecutive days	2500
Texas Wild-Rice	ASAP	430	< 120 cfs	430 minus standing stock	< 3,500 m^2 total coverage in the San Marcos River; or Texas wild-rice exists at < three (3) of the seven (7) distinct sections (Biological Monitoring sections)	1500
Texas Blind Salamander	ASAP	500	< 105 cfs	500 minus standing stock	When any standard or conventional water quality parameter exceeds the historical range of water quality parameters for the Edwards Aquifer by ≥ 10%	500
San Marcos Salamander	ASAP	500	< 120 cfs	500 minus standing stock	< 50% suitable habitat (Biological Monitoring locations) and < 20% salamander density; or < 25 % suitable habitat (Biological Monitoring locations) and < 30% salamander density	500
Comal Springs Salamander	ASAP	500	< 120 cfs	500 minus standing stock	< 50% suitable habitat (Biological Monitoring locations) and < 20% salamander density; or < 25 % suitable habitat (Biological Monitoring locations) and < 30% salamander density	500
Peck's Cave Amphipod	ASAP	500	< 30 cfs	500 minus standing stock	When any standard or conventional water quality parameter exceeds the historical range of water quality parameters for the Edwards Aquifer by ≥ 10%	500
Comal Springs Riffle Beetle	ASAP	500	< 120 cfs	500 minus standing stock	< 30 cfs when only one (1) of three (3) monitored sites continues to have six (6) or more adult Comal Springs riffle beetles collected in a 24 hr sample period using cotton lures	500

Species	Standing Stock Trigger	Standing Stock Target #	Refugia Stock Trigger	Refugia Stock Target #	Salvage Stock Trigger	Salvage Stock Target #
Comal Springs Dryopid Beetle	ASAP	500	< 30 cfs	500 minus standing stock	When any standard or conventional water quality parameter exceeds the historical range of water quality parameters for the Edwards Aquifer by ≥ 10%	500
Edward's Aquifer Diving Beetle	ASAP	500	< 30 cfs	500 minus standing stock	When any standard or conventional water quality parameter exceeds the historical range of water quality parameters for the Edwards Aquifer by ≥ 10%	500
Texas Troglobitic Water Slater	ASAP	500	< 50 cfs	500 minus standing stock	When any standard or conventional water quality parameter exceeds the historical range of water quality parameters for the Edwards Aquifer by ≥ 10%	500

*Since the gambusia is considered extinct, they are not included in this list.

3.2.2 Pricing Information

Respondents are required to submit itemized cost proposals for items as described in this RFP in a separate sealed envelope. The form shown in Attachment A2 will be used for all costs. All charges are to be included in the cost proposal. All price quotations will be valid for at least 90 days from the proposal opening.

SECTION 4
TERMS AND CONDITIONS

4.1 STANDARD FORM OF CONTRACT

Due to the nature of this RFP, the resulting contract with the Successful Offeror will be different from the EAA's standard professional services contract. Terms of the contract will be negotiated based on the specifics of the proposal selected.

4.2 LAWS AND REGULATIONS

The EAA requires that all responses to this RFP, and any contracts that may result, be in accordance with the laws and regulations of the State of Texas. Furthermore, the awarded Contractor must adhere to all Occupational Safety and Health Administration standards as applicable to the contracted work.

4.3 PROPOSAL ACCEPTANCE PERIOD

All prices and conditions of the proposal shall remain in effect for 90 days after the date set for the proposal opening. Proposals offering less than 90 calendar days for acceptance by the EAA from the date set for opening will be considered nonresponsive and will be rejected.

The EAA's Code of Ethics is attached for your <u>reference</u> <u>only</u> (see Attachment E). No action is necessary.

4.4 TIME OF COMPLETION

The Contractor shall take all necessary and appropriate actions to complete the project in accordance with the ongoing schedule incorporated in the resultant contract.

4.5 INSURANCE REQUIREMENTS

4.5.1 Worker's Compensation Insurance

The Contractor shall procure and shall maintain during the life of the contract, Worker's Compensation and Employer's Liability Insurance as required by applicable State law for all of his/her employees to be engaged in work at the site of the project under the contract and, in case of any such work sublet, the Contractor shall require the subcontractor(s) similarly to provide Worker's Compensation Insurance for all of the latter's employees to be engaged in such work unless such employees are covered by the protection afforded by the Contractor's Workers Compensation and Employer's Liability Insurance. In case any class of employees engaged in hazardous work on the project under the contract is not protected under the worker's compensation statutes, the Contractor shall provide and shall cause each subcontractor to provide adequate Employer's Liability Insurance in the amount of $500,000 for the protection of such of his/her employees as are not otherwise protected.

4.5.2 Contractor's (Commercial General) Public Liability and Property Damage Insurance and Vehicle Liability Insurance

The Contractor shall procure and shall maintain during the life of the contract Commercial General Liability (Bodily Injury and Property Damage) Insurance and Automobile (Vehicle) Liability Insurance coverage as specified below. The General Liability Policy shall also include products and Completed Operations Insurance in the same limits as the General Liability coverage as well as an endorsement providing Broad Form Contractual Liability coverage:

INSURANCE COVERAGE

a. Worker's Compensation — Statutory

and

b. Employer's Liability — $100,000/500,000/100,000

Commercial General (Public) Liability insurance including coverages for the following:

a. Premises operations
b. Independent contractors
c. Products/completed operations
d. Personal injury
e. Advertising injury
f. Contractual liability
g. Medical payments
h. Underground hazard
i. Explosion and collapse hazard
j. EAA's property in Contractor's care, custody, or control

a. Each Occurrence: $1,000,000

and

b. General Aggregate: $2,000,000

Comprehensive Automobile Liability Insurance, including coverage for loading and unloading hazards, for:

a. Owned/Leased vehicles
b. Non-owned vehicles
c. Hired vehicles

Combined single limit for bodily injury and property damage of $500,000 per occurrence or its equivalent.

4.5.3 Subcontractor's Public Liability and Property Damage Insurance and Vehicle Liability Insurance

The Contractor shall either (1) require each subcontractor to procure and to maintain during the life of his/her subcontract, Subcontractor's Public Liability and Property Damage Insurance, and Vehicle Liability Insurance of the type and in the amounts specified or, (2) insure the activities in his/her policy as specified.

4.5.4 Additional Insureds

The Contractor shall provide in the Liability Policies of Insurance the Edwards Aquifer Authority as additional insured as it relates to this contract.

4.5.5 Scope of Insurance and Special Hazards

Insurance required under the above sections shall provide adequate protection for the Contractor and his/her subcontractors, respectively, against damage claims which may arise from operations under the contract, whether such operations be by the insured or by anyone directly or indirectly employed by him/her, and also against any of the special hazards which may be encountered in the performance of the contract, including explosions, collapse and underground hazards.

4.5.6 Proof of Insurance Coverage

The Contractor shall furnish the EAA with insurance certificates for him/her and his/her subcontractors showing the type, coverage, limits of liability, class of operations covered, effective dates, date of expiration of policies and name of insurance companies prior to beginning any work. The attached Insurance Requirement Affidavit is required to be submitted along with the Proposal. The Contractor must furnish the EAA with 30 days written notice by either certified mail or personal delivery should any of the above described policies change that materially affect the coverage or be cancelled prior to the expiration date. All notices shall be mailed to:

Ms. Cyndi Holman
Procurement Specialist
Edwards Aquifer Authority
900 E. Quincy Street
San Antonio, TX 78215

Strike the following language as indicated on the cancellation section of the certificate of insurance: "SHOULD ANY OF THE ABOVE DESCRIBED POLICIES BE CANCELLED BEFORE THE EXPIRATION DATE THEREOF, THE ISSUING COMPANY WILL ~~ENDEAVOR TO~~ MAIL 30 DAYS WRITTEN NOTICE TO THE CERTIFICATE HOLDER NAMED TO THE LEFT. ~~BUT FAILURE TO MAIL SUCH NOTICE SHALL IMPOSE NO OBLIGATION OR LIABILITY OF ANY KIND UPON THE COMPANY, ITS AGENTS OR REPRESENTATIVES~~."

EDWARDS AQUIFER AUTHORITY
INSURANCE REQUIREMENT AFFIDAVIT

To Be Completed By Appropriate Insurance Agent/Broker

I, the undersigned Agent/Broker, certify that the insurance requirements contained in this proposal document have been reviewed by me with the below identified Contractor. If the below identified Contractor is awarded this contract by the Edwards Aquifer Authority (EAA), I will be able to, within fifteen (15) days after being notified of such award, furnish a valid insurance certificate to the EAA meeting all of the requirements defined in this proposal.

________________________ ________________________
Agent (Signature) Agent (Print)

Name of Agent/Broker: ________________________

Address of Agent/Broker: ________________________

City/State/Zip: ________________________

Agent/Broker Telephone #: ( ) ________________________

Date: ________________________

CONTRACTOR'S NAME: ________________________
(Print or Type)

NOTE TO AGENT/BROKER
If this time requirement is not met, the EAA has the right to reject this proposal and award the contract to another. If you have any questions concerning these requirements, please contact Ms. Cyndi Holman at (210) 222-2204.

ATTACHMENT A1
EDWARDS AQUIFER AUTHORITY
PROPOSAL SUMMARY
EDWARDS AQUIFER HABITAT CONSERVATION PLAN
LONG TERM REFUGIA OPERATIONS
PROPOSAL #149-15-HCP

Vendor Name: ____

Submit to: Ms. Cyndi Holman
Procurement Specialist
Edwards Aquifer Authority
900 E. Quincy Street
San Antonio, TX 78215

Attach your proposal for Edwards Aquifer Habitat Conservation Plan Long Term Refugia Operations to this document.

The following exceptions to the Specifications are noted:

____

____

____

Response to Commercial Questions and Statements

What is the current financial status and condition of the proposing entity?

____

____

____

The EAA reserves the right to request financial statements as needed.

The undersigned certifies that the information contained in this proposal has been carefully checked and is submitted as correct and that he/she is authorized to submit this proposal on behalf of the Offeror named below.

Signed: ____
Name

____
Printed Name

____
Title

____
Company

____
Address

____

____
Telephone No.

ATTACHMENT A2
EDWARDS AQUIFER AUTHORITY
COST PROPOSAL SUMMARY
EDWARDS AQUIFER HABITAT CONSERVATION PLAN
LONG TERM REFUGIA OPERATIONS
PROPOSAL #149-15-HCP

Vendor Name: ____

Submit to: Ms. Cyndi Holman
Procurement Specialist
Edwards Aquifer Authority
900 E. Quincy Street
San Antonio, TX 78215

The undersigned proposes to furnish the prices shown below in accordance with the specifications and the response attached hereto. It is expressly agreed that the EAA has the right to reject any or all proposals submitted if such action is deemed in its interest. The total cost is $______________, the breakdown of which is:

Task 1	$________________________
Task 2	$________________________
Task 3	$________________________
Task 4	$________________________
Task 5	$________________________
Task 6	$________________________
Shipping/Installation	$________________________
Other	$________________________
*TOTAL	$________________________

*Do not include sales tax in your cost proposal. If any other type of tax is added, please give full description of tax type.

Proposals offering less than 90 calendar days for acceptance by the EAA from the date set for opening will be considered nonresponsive and will be rejected.

The undersigned certifies that the prices contained in this proposal have been carefully checked and are submitted as correct and that he/she is authorized to submit this proposal on behalf of the Offeror named below.

Signed: ______________________________________
Name

____
Printed Name

____
Title

____
Company

____
Address

____

____
Telephone No.

ATTACHMENT B1

CLIENT REFERENCE

For each reference, complete the following information:

Client Name: ____

Client Contact Name: ____

Position: ____

Client Address: ____

____

____

Client Telephone Number(s): _________________________________

Date Contract Began: _______________________________________

Description of Services: _____________________________________

____

____

Cost: ____

ATTACHMENT B2

CLIENT REFERENCE

For each reference, complete the following information:

Client Name: ____

Client Contact Name: ____

Position: ____

Client Address: ____

____

____

Client Telephone Number(s): __________________________________

Date Contract Began: ____

Description of Services: ______________________________________

____

____

Cost: ____

ATTACHMENT B3

CLIENT REFERENCE

For each reference, complete the following information:

Client Name: ____

Client Contact Name: ______________________________________

Position: ____

Client Address: ____

____

____

Client Telephone Number(s): _________________________________

Date Contract Began: ______________________________________

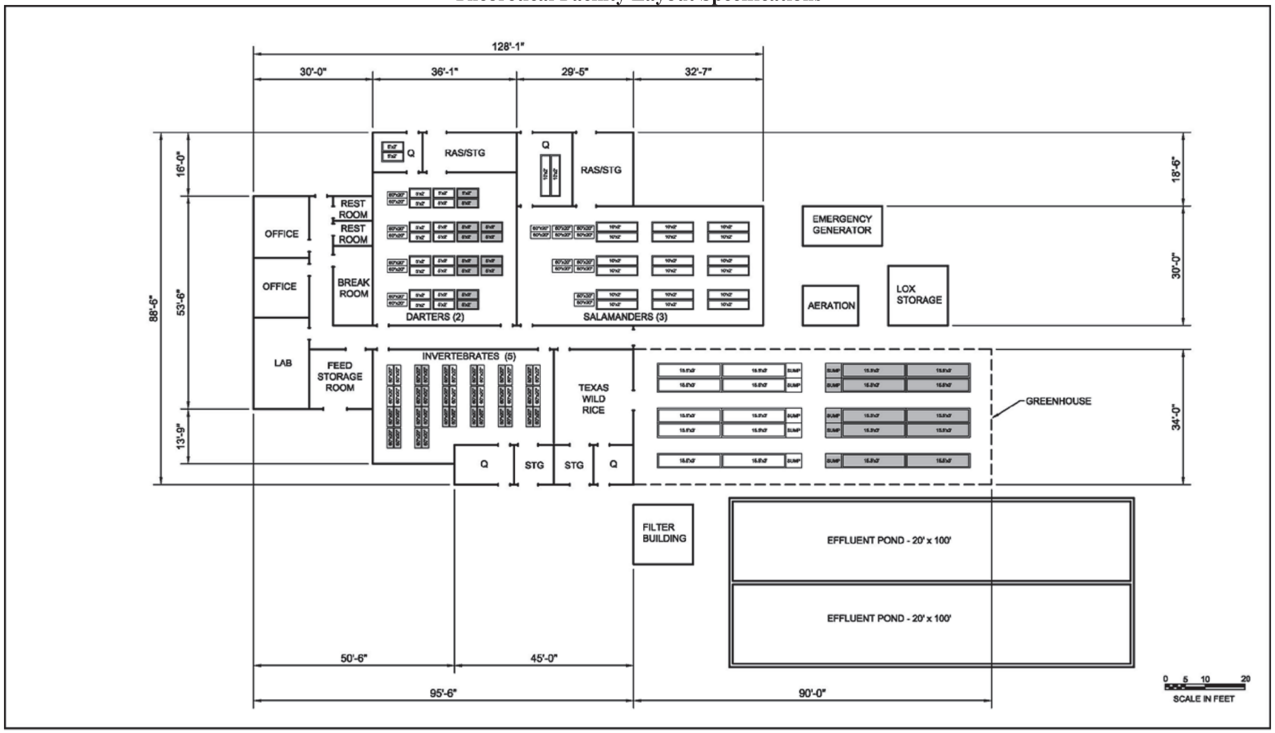
Description of Services: ____________________________________

____

____

Cost: ____

ATTACHMENT C
Theoretical Facility Layout Specifications



CONCEPT DRAWINGS FOR PLANNING PURPOSES ONLY
NOT FOR CONSTRUCTION

NOT DEVELOPED FOR A DESIGNATED FACILITY OR SPECIFIC LOCATION

LEGEND:

RAS - RECIRCULATING AQUICULTURE SYSTEM
Q - QUARANTINE
STG - STORAGE
LOX - LIQUID OXYGEN
- SALVAGE STOCK

HDR

Edwards Aquifer Authority

Refugia Layout

DATE
April 17, 2015

FIGURE
Figure B-1

EAA Refugium Rearing Specifications - Standing Stock/Refugia

Species	Mgmt Units (MUs)	Species per MU	Survival	Species/ MU plus Mortality	Lifestage	Rearing Vessel	Density	Vessels per MU	Vessels per System	System Dimensions	Total No. of Systems	Salvage Number	Salvage Addt'l Space	New Water Flow (gpm) Note f	Reuse Flow(gpm) Note g	Salvage New Water Flow(gpm) Note f	Salvage Reuse Flow (gpm) Note g
Fountain Darter (Comal)	4	250	76%	310	Egg & Larval	21 L tank (5.5 gal)	1.58 fish/l (6 fish/gal)	9.39 = 10	10	60"W x 20"D x 58"T	4 (1 per MU)			2.4	24		
					Adult	140 L trough (37 gal)	1.58 fish/L (6 fish/gal)	1.39 = 2	1	2'W x 5'L x 0.5' H	8 (2 per MU)	2000	12 troughs Note c and d	4.8	48	7.2	72
Fountain Darter (San Marcos)	4	250	76%	310	Egg & Larval	21 L tank (5.5 gal)	1.58 fish/l (6 fish/gal)	9.39 = 10	10	60"W x 20"D x 58"T	4 (1 per MU)			2.4	24		
					Adult	140 L trough (37 gal)	1.58 fish/L (6 fish/gal)	1.39 = 2	1	2'W x 5'L x 0.5' H	8 (2 per MU)	2500	Included in Fountain Darter (Comal)	4.8	48		
Texas Wild-Rice	10	Varies, total plants = 430	80%	538	Seedling												
			90%	478	Adult	Fiberglass raceway (870 gal)	1 plant / 8" dia. pot	MUs separated by grating	48	3'W x 15.5'L x 2.5'D	10	1500	10 rcwys Note a and b Flow: see Note h	29.5	2950	29.5	2950
Texas Blind Salamander	13	500 for all MUs	77%	650 for all MUs	Egg	10 gal env. chamber	6 adults/tank (4 F, 2 M)	2	8	60"W x 20"D x 58"T	3.25 = 4			1.2	12		
					Larval	114 L tank	1 / L	1	6	60"W x 20"Dx 58"T (4 units / system)	2.16 = 2			0.6	6		
					Adult	Fiberglass trough (1,136 L)	1 per 10 L	2	6	2'W x 10'L x 2'D	6	500	Note e	6	60		
San Marcos Salamander	6	500 for all MUs	77%	650 for all MUs	Egg	10 gal env. chamber	6 adults/tank (4 F, 2 M)	2	8	60"W x 20"D x 58"T	2			0.6	6		
					Larval	114 L tank	1 / L	1	6	60"W x 20"Dx 58"T (4 units / system)	1			0.3	3		
					Adult	Fiberglass trough (1,136 L)	1 per 10 L	1	6	2'W x 10'L x 2'D	6	500	Note e	6	60		
Comal Springs Salamander	4	500 for all MUs	77%	650 for all MUs	Egg	10 gal env. chamber	6 adults/tank (4 F, 2 M)	2	8	60"W x 20"D x 58"T	1			0.3	3		
					Larval	114 L tank	1 / L	1	6	60"W x 20"Dx 58"T (4 units / system)	1			0.3	3		
					Adult	Fiberglass trough (1,136 L)	1 per 10 L	1	6	2'W x 10'L x 2'D	6	500	Note e	6	60		
Peck's CaveAmphipod	3	500 for allMUs	80% (assumed)	625 for allMUs	All life stages	50 L tank	9 per 50 L	69	9	60"W x 20"D x 82"T	8	500	Add 8 systems	9.6	96	9.6	96
Comal SpringsRiffle Beetle	2	500 for allMUs	80% (assumed)	625 for allMUs	All life stages	50 L tank	22 per 50 L	28	9	60"W x 20"D x 82"T	3	500	Add 3 systems	3.6	36	3.6	36
Comal SpringsDryopid Beetle	1	500 for allMUs	80% (assumed)	625 for allMUs	All life stages	50 L tank	22 per 50 L	28	9	60"W x 20"D x 82"T	3	500	Add 3 systems	3.6	36	3.6	36
Edward's AquiferDiving Beetle	2	500 for allMUs	80% (assumed)	625 for allMUs	All life stages	50 L tank	22 per 50 L	28	9	60"W x 20"D x 82"T	3	500	Add 3 systems	3.6	36	3.6	36
Texas TroglobiticWater Slater	1	500 for allMUs	80% (assumed)	625 for allMUs	All life stages	50 L tank	22 per 50 L	28	9	60"W x 20"D x 82"T	3	500	Add 3 systems	3.6	36	3.6	36
Total														**89.2**	**3547**	**60.7**	**3262**

ATTACHMENT D

Protocol for Reintroduction of EAHCP Covered Species

LABORATORY PREPARATION FOR EFFECTIVE REINTRODUCTION

- Develop a Genetics Management Plan for Refugia Stocks
- Exposure to Environmental Conditions to Maximize Survival through Physiological Adaptations
- Predator Avoidance during introduction
- Determination of prefered introduction life stage
- Utilized Dispersal Methods/Mechanisms

PRE-RELEASE PROTOCOL DEVELOPMENT

(consultation w/ USFWS required)

- Determine Optimal Number, Size, and Condition to Release
- Determine Optimal Time and Location for Release
- Develop a Model to Define and Gauge Reintroduction Success
- Develop a Recovery Plan for Each Species?

COMPROMISED HABITAT

DETERMINATION OF NEED FOR REINTRODUCTION

- Conduct species population and detection survey for species of concern
- Determine extinction has occured
- Consult with U.S. Fish and Wildlife Service
- Consult with Texas Parks and Wildlife

PREPERATION OF REFUGIA STOCKS FOR REINTRODUCTION

- Disease Screening
- Genetic-Based Defect Screening
- Additinoal breeding to produce stocking numbers

POST-RELEASE MONITORING AND EVALUATION

- Initiate Intense Monitoring for Reintroduction Evaluation and Modification
- Reintroduction Approaches will be Evaluated and Revised Based on Survival/Mortality Estimates

CONTINGENCY PLAN

- Modify stocking plan (location, timing, lifestage, etc.) if necessary
- Additional Stockings

ADAPTIVE MANAGEMENT

ATTACHMENT E

FOR INFORMATIONAL PURPOSES ONLY

EDWARDS AQUIFER AUTHORITY

CODE OF ETHICS FOR EAA EMPLOYEES

Every employee is expected to perform his or her job duties satisfactorily, to maintain a high level of personal conduct on the job, to render courteous and efficient service to the public, to be mindful of safety practices and to exercise care in the use of EAA property.

Each employee of the EAA is a public servant and, as such, is held to the highest standard of ethical conduct. Consistent with this public trust, employees may not:

- use their official positions to secure special privileges or exemptions for themselves or others;
- grant any special consideration, treatment or advantage to any citizen, individual or group beyond any available to every other citizen, individual or group;
- disclose, without proper authorization, confidential information that could adversely affect the property, management or affairs of the EAA;
- directly or indirectly use any confidential information for their own personal gain or benefit, or for the private interest of others;
- engage in any outside activities that will conflict with, or will be incompatible with, the duties assigned to them in the course of their employment with the EAA; or that would reflect discredit upon the EAA; or in which their employment with the EAA would give them an advantage over others engaged in competition with the employee's personal business or vocational pursuits. This policy will not prohibit employees from performing any services for another organization if the General Manager determines there is no conflict with EAA duties and responsibilities;
- represent, directly or indirectly, or appear on behalf of private interests before the EAA Board of Directors; nor will they represent any private interest in any action or proceeding involving the EAA; nor will they accept a retainer or compensation that is contingent upon a specific action taken by the EAA;
- use EAA funds, supplies, equipment, vehicles or facilities for any purpose other than conducting official business; or
- have a financial interest, direct or indirect, in any contract with the EAA, or in the sale to the EAA of any land, materials, supplies or services, except on behalf of the EAA as an employee.

The previous list of prohibited activities is not all-inclusive. Violation of the public trust is prohibited and may result in dismissal.

Employees are permitted, as private citizens, to support political candidates for public office. An employee may not, however, participate actively in any candidate's campaign for membership in the EAA board of directors. Employees may not hold or run for a political office, the responsibilities of which may affect, directly or indirectly, the EAA. Likewise, employees are not permitted to use their working time or EAA resources to participate in a political campaign or any other political activity. The term "participate" includes, without limitation, making political speeches, telephone solicitation, distributing political literature, or writing or handling letters related to a political campaign or activity. Political posters may not be displayed on EAA property. Employees are not required to contribute to any political fund or render any political service to any person or party whatsoever.

Employees are prohibited from soliciting or accepting any gifts, gratuities, favors, loans or other objects of monetary worth that might reasonably tend to influence the employee in the discharge of official duties or that the employee knows or should know is being offered with the intent to influence the employee's official conduct.

Appendix K11

2014 Water Audit and Infrastructure Analysis Report for the City of Natalia, Texas

2014 WATER AUDIT AND INFRASTRUCTURE ANALYSIS REPORT

FOR
CITY OF NATALIA, TEXAS

EDWARDS AQUIFER
AUTHORITY

Queen City of the Southwest
Land of the Bluebonnets
THE CITY OF NATALIA

Prepared by:

LNV

TBPE FIRM NO. F-366

8918 Tesoro Drive, Suite 401
San Antonio, Texas 78217
Ph: (210) 822-2232 Fax: (210) 822-4032

STATE OF TEXAS
BYRON G. SANDERFER
99241
LICENSED
PROFESSIONAL ENGINEER

03/08/16

Table of Contents

Introduction

LNV, Inc. has been retained by the Edwards Aquifer Authority (EAA) for a Water Conservation Audit to identify areas where water losses are occurring and provide recommendations to reduce this amount within the City of Natalia Water System. This analysis is in general accordance with the Texas Water Development Board (TWDB), the American Water Works Association (AWWA), and the best practices for water audits of similar water systems.

This report was completed by using the top-down “desktop” water audit approach. This approach utilizes available existing records provided by the City of Natalia to review the quantity of water pumped into the system, where the water is being used, and determine areas where the water is being lost. Some estimation was required to provide values for main breaks and flushing of lines.

Background

The City of Natalia serves approximately 1,663 residents and maintains approximately 14 miles of water distribution mains with 573 service connections (based on 2012 Water Audit Report). The city relies solely on the Edwards Aquifer for its potable water source through the use of two active wells. There are currently three wells owned by the city but only two are being used on a daily basis and connected to the system. The two active wells are located approximately 4.5 miles north of Natalia along FM 463 and supply the city through an 8-inch PVC transmission main that enters the distribution system at the intersection of FM 471 and Cresson St., Appendix C shows the current wells and distribution system based on City records and working knowledge of the system. The city does not import or export water to their system.

The distribution system is comprised of 2-inch, 6-inch, and 8-inch water distribution mains. These mains are comprised of various material types; such as PVC, Asbestos Cement, and Steel. Services to both residential and commercial properties consist of mostly Blue Poly Pipe and PVC with a few being made of Copper. Both the Blue Poly Pipe and PVC services are known to leak due to their inability to flex with ground movements. The city is looking into means of replacing the service lines with Copper as water mains are being replaced throughout

the city.

Average known water losses range between 30 percent and 35 percent which have been consistent over the past several years. The Edwards Aquifer Authority (EAA) has tasked LNV, Inc. to review the past data for the year 2014 and provide recommendations on how to minimize the water loss for the City of Natalia.

The City of Natalia owns an EAA permit for 266.67 acre-feet per calendar year. The city is allowed to withdraw this amount of water per year from the Edwards Aquifer if water restrictions are not in place. In 2014 the city pumped approximately 200 acre-feet. The amount of water used by the City of Natalia is directly related to the demand and Critical Period Management Plan reductions.

During times of drought reductions, the EAA imposes required withdraw reductions on all EAA groundwater permit holders. The EAA is tasked with managing the Edwards Aquifer by ensuring the volume of water the city pumps depends on the Critical Period Management Plan imposed on by the EAA during periods of drought. **Table No. 1, Edwards Aquifer Authority Drought Restrictions** shows the percent reduction required by all groundwater permit holders using more than 3 acre-feet per year. These reductions are enforced based on a rolling 10 day average. **Table No. 1** is a representation of what the City of Natalia is allowed to withdraw during the enforcement of the Critical Period Management Plan on an annualized basis.

Edwards Aquifer Authority Drought Restrictions			
Restriction	Aquifer Depth	Percent Reduction	Permit Amount (acft)
No Restrictions	>660	0%	266.67
Stage 1	<660	20%	213.33
Stage 2	<650	30%	186.67
Stage 3	<640	35%	173.33
Stage 4	<630	40%	160.00
Stage 5	<625	44%	149.33

Note: Permit amount is related to The City of Natalia's allowed withdraw

Table No. 1, Edwards Aquifer Authority Drought Restrictions

These reductions are imposed on the groundwater permit holders and watering restrictions are then passed on to the customers by way of restricted days allowed for irrigation purposes to help conserve water.

In the 2014 calendar year the city was required to reduce usage by 35 percent (annualized) limiting their EAA permit to withdraw at 173.33 acre-feet. The city exceeded their permit and had to go to the water market and purchase an additional 40 acre-feet (also required to meet the 35 percent reduction) allowing them to ultimately withdraw approximately 200 acre-feet. As history has shown, more droughts will come and go causing the city to keep a watchful eye on the amount of water readily available for their consumption.

Water Audit

A Water Audit can be completed by using two methods; a top-down approach and a bottom-up approach. Both methods are trying to achieve the same goal; account for all water introduced into the water distribution system and develop a plan of action to reduce losses within the water distribution system.

This report will follow the top-down approach by using data collected by the city throughout the 2014 calendar year. **Table No. 2, 2014 Water Analysis** lists all collected data for the 2014 calendar year. This table was populated using information from the 2014 Monthly Field Reports (Appendix F). These Monthly Field Reports are completed each month by city staff and provided to city council each month as progress report regarding the usage of water per each month. Based on the values provided by the city, the average loss of water for the 2014 year was 27.08 percent. This was determined by summing up the value of Approximate Volume lost resulting from Leaks and Water Not Billed and divided by the total Volume of Water Pumped into the water distribution system. According to TWDB, the state average for water loss of all reporting water systems for the year 2014 was around 12 percent. For water systems providing service below a population of 10,000 the average water loss was 18 percent.

2014 Water Analysis							
MONTH	WATER PUMPED (GAL)	WATER BILLED (GAL)	CITY USE (GAL)	APPROX. LEAKS (GAL)	APPROX. FLUSHING (GAL)	WATER NOT BILLED (GAL)	WATER LOSS (%)
JANUARY	5,460,080	3,554,651	33,285	750,000	450,000	672,144	26.05%
FEBRUARY	5,703,810	4,215,541	37,650	600,000	150,000	700,619	22.80%
MARCH	5,014,530	3,319,439	16,600	700,000	350,000	628,491	26.49%
APRIL	5,403,730	3,437,253	39,450	800,000	450,000	677,027	27.33%
*MAY	5,447,323	3,723,712	25,603	700,000	300,000	698,008	25.66%
JUNE	5,107,490	3,635,008	26,011	600,000	200,000	646,471	24.40%
JULY	5,562,860	3,540,725	21,084	800,000	500,000	701,051	26.98%
AUGUST	5,979,290	4,359,766	24,400	600,000	250,000	745,124	22.50%
SEPTEMBER	5,979,290	4,359,766	31,000	600,000	250,000	738,524	22.39%
OCTOBER	5,925,370	4,212,722	25,751	500,000	200,000	986,897	25.09%
NOVEMBER	5,278,060	3,636,026	16,400	500,000	200,000	925,634	27.01%
DECEMBER	4,506,040	2,689,937	10,000	625,000	350,000	831,103	32.31%
TOTAL	65,367,873	44,684,546	307,234	7,775,000	3,650,000	8,951,093	
**ADJ. TOTAL (98%)	66,701,911	44,684,546	307,234	7,775,000	3,650,000	10,285,131	27.08%

*Note: Data not provided for month of May (except for leaks and flushes), Values averaged from other months

**Note: 98% Accuracy adjustment for pumps at wells

Note: Water Loss (%) includes Approx. Leaks and Water not Billed divided by Water Pumped

Note: Data used to populate this table was taken from the 2014 Monthly Field Reports

Table No. 2, 2014 Water Analysis

The current distribution system is comprised of approximately 14 miles of 2-inch, 6-inch, and 8-inch water mains with material types consisting of PVC, Asbestos Cement, and Steel. The total length was determined by using existing distribution maps provided by the city and importing the information into ArcGIS 10.2.2 over an aerial image of the city and summing up each length. **Table No. 3, Length of Distribution Water Main Sizes** shows a tally of each water main size with the approximate length. Appendix C is provided to show the distribution network within the boundaries of the City of Natalia.

Length of Distribution Water Main Sizes		
Distribution Water Main Size	Approx. Length (LF)	Approx. Length (MI)
8"	46,920	8.89
6"	22,551	4.27
2"	5,248	0.99
Total	74,719	14.15

*Note: Numbers were approximated using existing distribution maps and ArcGIS 10.2.2 to map the system

Table No. 3, Length of Distribution Water Main Sizes

In 2012, the City of Natalia completed a Water Audit Report by using the form provided within the "Water Loss Audit Manual for Texas Utilities" developed by the Texas Water Development Board (TWDB), see Appendix D. The current 2014 Water Audit Report form completed by LNV, Inc. is shown in Appendix E. This form takes information regarding the distribution system such as length of pipes, number of services, and volume of water pumped into the system and helps the city identify how much water is actually being lost within the system. At the end of the water audit form, it asks for the unit cost of water and allows you to calculate the approximate amount of revenue lost due to the volume of water not leased within the system. In 2012 the City of Natalia lost $99,381.92 in water revenues due to Apparent and Real Losses (See Appendix D). Based on the data collected from 2014, the City of Natalia lost approximately $70,972.11 from Apparent and Real Losses (See Appendix E).

The City of Natalia has made improvements since the last Water Audit was performed. In 2012, the city reported a loss of approximately 34 percent as compared to 27.08 percent as indicated in the 2014 Water Audit form. The city credits this to identifying areas prone to breaks and leaks and making repairs before an issue arises. They are also keeping an eye on customer meters that show signs of pending failure or inaccuracies. These meters are replaced at the first sign of failure or inaccuracy so the proper volume of water used can be properly billed.

There are two volumes every water supplier should be aware of: Authorized Consumption and Water Losses. Authorized Consumption is comprised of Billed and Unbilled Authorized Consumption. These are classified as revenue waters. Water Losses are comprised of Apparent and Real Losses. Apparent Losses include Unauthorized Consumption, Customer

meter under-registering, and Billing adjustments/waivers. Real Losses are comprised of physical losses to the system.

According to the Field Reports provided by the city for the calendar year of 2014, a total of 66,701,911 gallons (204.7 acre-feet) were pumped into the system based on an adjusted pumping rate of 98%. Authorized Consumption totaled 48,641,780 gallons (149.3 acre-feet) and Water Loss totaled 18,060,131 gallons (55.4 acre-feet). This equates to approximately 27.08 percent of the water pumped into the system is lost through documented leaks (approximate value) and unmetered non-billed water loss.

Infrastructure Improvements Analysis

The City of Natalia currently owns and maintains approximately 14 miles of water distribution mains comprised of 2-inch, 6-inch, and 8-inch diameter pipes which consist of PVC, Asbestos Cement, and Steel pipe materials. Services are comprised of Blue Poly Pipe, PVC, and Copper. LNV met with Mr. Art Smith, Director of Public Works for the City of Natalia, on two occasions to collect data and listen to his concerns regarding the distribution system. Mr. Smith provided insight regarding areas of concern and what his thoughts were regarding the loss of water.

Three concerns the city expressed to LNV were Residential/Commercial Meter inaccuracies, Production Well Meters, and Billing adjustments.

Replacing faulty or inaccurate meters requires the least amount of excavation when it comes to addressing water losses. Water meters are typically enclosed in a box/vault that is visible from the surface and replacing one takes minimal staff and time to complete. Replacing meters requires minimal excavation and minimal service impact for residents in effected areas. It also allows the city to identify where the Blue Poly Pipe and PVC services are located and plan for replacement when the distribution main supplying those services is funded. The 2012 Water Audit (copied for 2014 Water Audit) stated the Customer Water Meters were averaging 95 percent accuracy throughout the distribution system. This appears to be high and should be verified with a certified company. Since the city is actively replacing the meters on an as needed basis, the average accuracy for the customer meters is estimated to be between 85 and 95 percent. This means the city is losing money from faulty meters and the actual water loss within the system is reduced because of the inaccuracy of the meters is increased. The customer meter

accuracy loss would then reduce the amount of water lost within the system because some of it would be accounted for within the customer meter accuracy loss, Item No. 25 of the 2014 Water Audit Form (Appendix E).

The City of Natalia is currently replacing between 50-75 meters each year and with 573 service connections within the distribution system it will take between 8-12 years before all the meters have been replaced. Testing water meters for their accuracies will also help save costs because if the meter is still holding its accuracy then replacing the meter is not required. Keeping records of where meters were replaced and where ones have held their accuracy will also assist the city in determining where to spend its resources. Not every meter will be required to be replaced.

Billing adjustments will also need to be further evaluated. The automatic billing software used by the City of Natalia takes the meter readings (field readings, written by hand) and provides a bill to the customer. The issue is there is a flat rate for the first 1,000 gallons used and every 100 gallons used after the initial amount is charged by a unit fee based on the total amount of water used.

The billing records provided to LNV by the city indicate the city only charges customers by increments of 1,000 gallons. Whether these numbers are rounded up or down is unknown. Reviewing the data provided for the period of August 2014 - August 2015 suggest that the city is not billing the full amount. Several customers use 1,000 gallons for one month and then 0 gallons the next several months followed by 1,000 gallon consumption. Either the property was vacant or the gallons were not rolled over to the next month until the 1,000 gallon mark was met. The actual meter readings need to be reviewed and compared to see if each gallon is being charged and accounted for. This will show if each month is being carried over or if the billing software is starting over each month.

The city believes the billing software is starting each month over and the city is losing revenue because of rounding issues and not accounting for water being used. The city estimates approximately 200,000 to 300,000 gallons per month are being lost through the billing software not rolling over the gallons from the previous months. If this is the case, the customer billing contributes to approximately 2.4-3.6 million gallons (7.3-11 acre-feet) of revenue water not accounted for each year.

Some additional concerns expressed by the city were a few streets located on the north

side of the distribution system. Third, Fourth, Fifth, and Sixth St. between FM 471 and Pearson St. are more prone to leaks and water main breaks than the rest of the distribution system. The pipe material within this area is comprised of 6-inch Asbestos Cement pipe and 2-inch Steel pipe. That being said, these pipe need to be replaced by upsizing them to 8-inch PVC. This will allow for better pressure within the immediate area, the existing Blue Poly Pipe and PVC services should also be replaced with Copper, and allow for better fire flow because of the larger volume of water being stored directly within the system.

The city currently implements several methods for managing the distribution system. **Table No. 4, Continued Management of System** lists several actions that can be implemented with the corresponding benefits.

Continued Management of System		
Item	Description	Benefits
1	Annually Check Accuracy of Production Well Meters	Accurately withdraw correct amounts
2	Manage water within the distribution system to reduce the amount of water used for flushing purposes	Reduce amount of water required to flush system
3	Perform Leak Detection Surveys regularly to locate areas of concern	This will identify areas that need to be replaced
4	Perform Inferred Surveys using Unmanned Aerial Vehicle to detect leaks along the transmission main from the two production wells	This will identify areas of leaks/breaks along the transmission main
5	Request residents to report concerns or suspicious activates regarding unauthorized use of water	Greater ability to catch leaks sooner with the additional eyes watching over the community
6	Request residents to report any areas that show signs of leaks (green areas/increased vegetation, soft spots, ponding)	Greater ability to catch leaks sooner with the additional eyes watching over the community
7	Verify billing software is correctly charging customers for water usage based on amount used each month and bill based on increments of 100 instead of 1,000 gallons	Identify if customers are being billed correctly and the volume of water charged is correctly being accounted for

Table No. 4, Continued Management of System

By implementing any number of the items listed in **Table No. 4** will help the city move towards lowering the volume of water loss each year.

Some action items to consider are listed in **Table No. 5, Improvements to Distribution System.** The items listed within this table are improvements that can be developed with a replacement schedule based on identifying areas prone to leaks and breaks.

Improvements to Distribution System							
Item	Description	Unit	Unit Price	Quantity	Total Cost	Approx. Volume Saved (gal/year)	Benefits
1	Perform a study to detect leaks throughout the distribution system and develop a Capital Improvements Project list with a yearly goal for construction projects	EA	Varies	N/A	N/A	N/A	This will identify areas and prioritize areas that need to be addressed
2	Replace Blue Poly Pipe and PVC services with Copper	EA	$2,000	573	$1,146,000	2,536,818	Reduce leaks and breaks
3	Upsize existing 2-inch and 6-inch water mains with 8-inch PVC water mains	LF	$80	27,799	$2,223,920	Varies	Reduce leaks and breaks; Increase available daily flow rate to customers; Increase available flow rate for fire flow; Increased capacity
4	Replace existing Asbestos and Steel water mains with 8-inch PVC water mains	LF	$80	12,700	$1,016,000	Varies	Reduce leaks and breaks
5	Replace Residential/Commercial Meters	EA	$350	573	$200,550	Varies	Improves accuracy; Correctly bill customers for usage
6	Reduce the number of "dead ends" within the distribution system by looping the ends of mains	LF	$80	2,000	$160,000	Varies	Reduces amount of water required to flush system; Provides loops within the system; Increased capacity for all types of demands; Provides continuous service

Note: Costs are from SAWS June 2014 Average Unit Bid Pricing

Unit Prices are approximate based on 2014 SAWS numbers and should be verified prior to any budgeting takes place

Unit Prices only include the cost for construction and do not include permit fees, engineering design, etc.…

Table No. 5, Improvements to Distribution System

The items listed within **Table No. 5** are a building block for the city to further investigate and make a plan on how to start investing into the distribution system by replacing the more prone to leak/break areas and upsize them from the existing 2-inch or 6-inch to 8-inch PVC mains. It is key to understand and know where to best allocate your resources to receive the best investment from the use of available funds. Having a study performed identifying areas and establishing a Capital Improvements Project list is a road map the city can use to make progress in reducing the amount of water lost within their system.

Estimated Water Rate Impact

Several measures were discussed within the Infrastructure Improvements Analysis on ways to reduce water losses. Each of these measures tackles a specific way of addressing a water loss. In order to compete these tasks, it takes additional revenue. Having studies conducted to specifically identify problem areas and establishing a list with a time line to complete will help the city make progress in managing their system.

Something the City of Natalia should consider is to complete a Development Impact Fee Study. This study allows the city to recover costs by providing public facilities for new development. Since Love's has taken residence within the city limits, other large retailers may decide to establish their presence within the city limits and collecting fees from these new developments will help offset the costs of new infrastructure to meet their demands. In addition to the Development Impact Fee Study a Rate Study needs to be completed to find ways to generate more revenue. Looking at cities of similar size and adopting their practices and billing structure would be a start.

Conclusion

The City of Natalia has shown improvement from their Water Audit of 2012 to their 2014 Water Audit in which the water lost was reduced. A reduction from 34 percent to 27.08 percent shows the city is making progress toward reducing water lost within their system. They are using the resources they have to best manage the system they are responsible for. The City of Natalia is actively pursuing all means and methods to improving the existing infrastructure including actively pursuing grants through various agencies and organizations.

Based on the findings within this report, the city has the ability to recover approximately

$71,000.00 worth of revenue water per year which equates to approximately 18 million gallons (55 acre-feet) of water for the year 2014.

References

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Water Management International (www.wmi-water.com). n.d.

Appendix:

A. Figure No. 1 - Location Map

B. Figure No. 2 - City of Natalia Overview Map

C. Figure No. 3 - Existing Water Distribution System Map

D. 2012 TWDB Water Audit for Natalia

E. 2014 TWDB Water Audit for Natalia

F. 2014 Monthly Field Reports

A. Figure No. 1 – Location Map

0 15,000 30,000 Feet

LNV engineers | architects | surveyors

TBPE FIRM NO. F-366

Location Map

Natalia, TX

LNV Proj. No.	150349
Date	10/8/2015
Scale	1 inch = 30,000 feet
File	Natalia Location Map
Drafted	JS

N W E S

Figure 1

S:\Projects\Edwards Aquifer Authority\150349 City of Natalia Water Loss Assessment\000\Design\GIS\Natalia Location Map.mxd

B. Figure No. 2 – City of Natalia Overview Map

LNV
engineers | architects | surveyors
TBPE FIRM NO. F-366

Overview Map

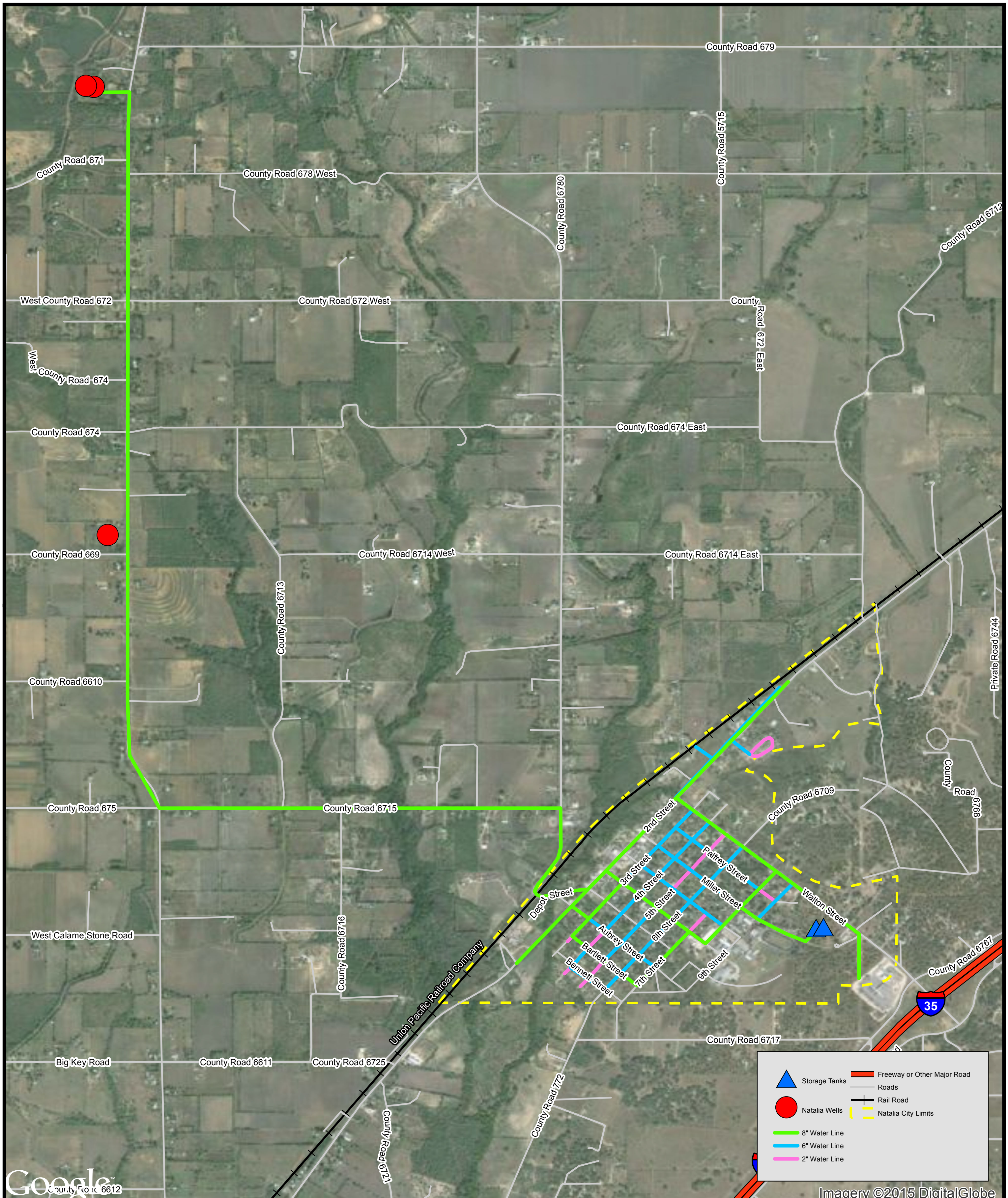
Natalia, TX

LNV Proj. No.	150349
Date	10/8/2015
Scale	1 inch = 1,500 feet
File	Natalia
Drafted	JS

Figure 2

S:\Projects\Edwards Aquifer Authority\150349 City of Natalia Water Loss Assessment\000\Design\GIS\Natalia.mxd

C. Figure No. 3 – Existing Water Distribution System Map



LNV
engineers | architects | surveyors
TBPE FIRM NO. F-366

Existing Water Distribution System Map

Natalia, TX

LNV Proj. No.	150349
Date	10/30/2015
Scale	1 inch = 2,500 feet
File	Natalia water lines
Drafted	JS

Figure 3

S:\Projects\Edwards Aquifer Authority\150349 City of Natalia Water Loss Assessment\000\Design\GIS\Natalia water lines.mxd

D. 2012 TWDB Water Audit for Natalia

TEXAS WATER DEVELOPMENT BOARD

P.O. BOX 13231, CAPITOL STATION

AUSTIN, TX 78711-3231

2012 Water Audit Report

A. Water Utility General Information

1. Water Utility Name: City of Natalia
2. Contact:
   - 2a. Name: Art Smith
   - 2b. Telephone #: 830-663-2926
   - 2c. Email Address: asmith@cityofnatalia.com
3. Reporting Period: From 1/1/2012 To 12/31/2012
4. Source Water Utilization, percentage: Surface Water 0.00 % Ground Water 100.00 %
5. Population Served:

		Assessment Scale
5a. Retail Population Served	1,663	
5b. Wholesale Population Served	0	
6. Utility's Length of Main Lines, miles	17.00	0
7. Number of Wholesale Connections Served	0	
8. Total Retail Metered Connections	573	
9. Service Connection Density (Number of retail service connections / miles of main lines)	33.71	
10. Average Yearly System Operating Pressure (psi)	53.00	0
11. Volume Units of Measure:	Gallons	

B. System Input Volume

			Assessment Scale
12. Produced Water	71,366,000	gallons	0
13. Production Meter Accuracy (enter percentage)	98.00	%	0
14. Corrected Input Volume	72,822,449	gallons	
15. Total Water Purchased	0	gallons	0
16. Total Wholesale Water Sales	0	gallons	0
17. Total System Input Volume (Corrected input volume, plus imported water, minus exported water)	72,822,449	gallons	

C. Authorized Consumption

			Assessment Scale
18. Billed Metered	43,339,175	gallons	0
19. Billed Unmetered	0	gallons	0
20. Unbilled Metered	337,176	gallons	0
21. Unbilled Unmetered	3,925,000	gallons	0
22. Total Authorized Consumption	47,601,351		

TEXAS WATER DEVELOPMENT BOARD

P.O. BOX 13231, CAPITOL STATION

AUSTIN, TX 78711-3231

2012 Water Audit Report

gallons

D. Water Losses

23. Water Losses (Line 17 minus Line 22)	25,221,098	gallons	

E. Apparent Losses

24. Average Customer Meter Accuracy (Enter percentage)	95.00	%	0
25. Customer Meter Accuracy Loss	2,281,009	gallons	
26. Systematic Data Handling Discrepancy	0	gallons	0
27. Unauthorized Consumption	182,056	gallons	0
28. Total Apparent Losses	2,463,065	gallons	

F. Real Losses

29. Reported Breaks and Leaks (Estimated volume of leaks & breaks repaired during the audit period)	10,225,000	gallons	0
30. Unreported Loss (Includes all unknown water loss)	12,533,033	gallons	0
31. Total Real Losses (Line 29, plus Line 30)	22,758,033	gallons	
32. Water Losses (Apparent + Real) (Line 28 plus Line 31) = Line 23	25,221,098	gallons	
33. Non-revenue Water (Water Losses + Unbilled Authorized Consumption) (Line 32, plus Line 20, plus Line 21)	29,483,274	gallons	

G. Technical Performance Indicator for Apparent Loss

34. Apparent Losses Normalized (Apparent Loss Volume / # of Retail Service Connections/365)	12	gallons

H. Technical Performance Indicators for Real Loss

35. Real Loss Volume (Line 31)	22,758,033	gallons
36. Unavoidable Annual Real Losses, volume (calculated)	0	gallons
37. Infrastructure Leakage Index (calculated) (Equals real loss volume divided by unavoidable annual real losses)	0.00000	
38. Real Losses Normalized (Real Loss Volume / # of Service Connections / 365)	109	gallons

(This indicator applies if service connection density is greater than or equal to 32 / mile)

39. Real Losses Normalized — 0 gallons
(Real Loss Volume/Miles of Main Lines/365)
(This indicator applies if service connection density is less than 32/mile)

I. Financial Performance Indicators

	Value		Assessment Scale
40. Total Apparent Losses (Line 28)	2,463,065	gallons	
41. Retail Price of Water	$0.00339		0
42. Cost of Apparent Losses (Apparent loss volume multiplied by retail cost of water, Line 40 x Line 41)	$8,349.79		
43. Total Real Losses (Line 31)	22,758,032.65		
44. Variable Production Cost of Water* (*Note: in case of water shortage, real losses might be valued at the retail price of water instead of the variable production cost.)	$0.00400		0
45. Cost of Real Losses (Real Loss multiplied by variable production cost of water, Line 43 x Line 44)	$91,032.13		
46. Total Assessment Scale			0
47. Total Cost Impact of Apparent and Real Losses	$99,381.92		
48. Comments			
49. Total Water Loss %	34.63	%	
50. GPCD (Gallons Per Capita Per Day) Input	119.97		
51. GPCD (Gallons Per Capita Per Day) Loss	41.55		

E. 2014 TWDB Water Audit for Natalia

Texas Water Development Board
Water Audit Worksheet

A. Water Utility General Information

1. Water Utility Name: City of Natalia
2. Contact: Name Art Smith

   Telephone# 830-663-2926 Email Address asmith@cityofnatalia.com
3. Reporting Period: From 01 / 01 / 2014 to 12 / 01 / 2014
4. Source Water Utilization, percentage: Surface Water 0 % Groundwater 100 %
5. Population Served:

   a. Retail Population Served 1,663

   b. Wholesale Population Served 0

		Assessment Scale
6. Utility's Length of Main Lines, miles	14	______
7. Number of Wholesale Connections Served	0	
8. Number of Retail Service Connections Served	573	
9. Service Connection Density *(Number of retail service connections/Miles of main lines)*	40.93	
10. Average Yearly System Operating Pressure (psi)	53	______

11. Volume Units of Measure (check one):

    _____ acre-ft _____ million gallons ______ thousand gallons X gallons

B. System Input Volume

12. Water Volume from own Sources	65,367,873	______
13. Production Meter Accuracy (enter percentage)	98 %	______
14. Corrected Input Volume	66,701,911	
15. Wholesale Water Imported	0	______
16. Wholesale Water Exported	0	______
17. **System Input Volume** *(Corrected input volume, plus imported water, minus exported water)*	66,701,911	

		Assessment Scale
C. Authorized Consumption		
18. Billed Metered	44,684,546	____
19. Billed Unmetered	0	____
20. Unbilled Metered	307,234	____
21. Unbilled Unmetered	3,650,000	____
22. Total Authorized Consumption	48,641,780	
D. Water Losses		
23. Water Losses *(Line 17 minus Line 22)*	18,060,131	
E. Apparent Losses		
24. Average Customer Meter Accuracy *(Enter percentage)*	95 %	____
25. Customer Meter Accuracy Loss	2,351,818	
26. Systematic Data Handling Discrepancy	0	____
27. Unauthorized Consumption	185,000	____
28. Total Apparent Losses	2,536,818	
F. Real Losses		
29. Reported Breaks and Leaks *(Estimated volume of leaks and breaks repaired during the audit period)*	7,775,000	____
30. Unreported Loss *(Includes all unknown water loss)*	7,748,313	____
31. Total Real Losses *(Line 29, plus Line 30)*	15,523,313	
32. Water Losses (Apparent + Real) *(Line 28 plus Line 31) = Line 23*	18,060,131	
33. Non-revenue Water (Water Losses + Unbilled Authorized Consumption) *(Line 32, plus Line 20, plus Line 21)*	22,017,365	

Assessment Scale

G. Technical Performance Indicator for Apparent Loss

34. Apparent Losses Normalized (Apparent Loss Volume/# of Retail Service Connections/365) — 12.13

H. Technical Performance Indicators for Real Loss

35. Real Loss Volume *(Line 31)* — 15,523,313

36. Unavoidable Annual Real Losses, volume (calculated) — 3,127,893

37. Infrastructure Leakage Index (calculated) — 0
(Equals real loss volume divided by unavoidable annual real losses)

38. Real Losses Normalized (Real Loss Volume/# of Service Connections/365) — 74.22
(This indicator applies if service connection density is greater than 32/mile)

39. Real Losses Normalized (Real Loss Volume/Miles of Main Lines/365) — N/A
(This indicator applies if service connection density is less than 32/mile)

I. Financial Performance Indicators

40. Total Apparent Losses *(Line 28)* — 2,536,818

41. Retail Price of Water — $0.0035 — ____

42. Cost of Apparent Losses — $8,878.86
(Apparent loss volume multiplied by retail cost of water, Line 40 x Line 41)

43. Total Real Losses *(Line 31)* — 15,523,313

44. Variable Production Cost of Water* — $0.0040 — ____
*(*Note: In case of water shortage, real losses might be valued at the retail price of water instead of the variable production cost.)*

45. Cost of Real Losses — $62,093.25
(Real loss multiplied by variable production cost of water, Line 43 x Line 44)

46. Total Assessment Score — ____

47. Total Cost Impact of Apparent and Real Losses — $70,972.11

Appendix 1.2

Water Audit Worksheet Instructions

(All numbers used in this worksheet are for example purposes only)

The following instructions can be used in completing the Water Audit Worksheet. The instructions are labeled by line number shown on the worksheet. The Water Audit Worksheet requests that the water utility enter general information and water supply, consumption, and loss quantities. It also requests assessment scores representing the degree of validation of individual components. For those components that include an assessment line, enter a number between 1 and 5. (See Appendix 1.3 for more information.) If a component does not apply, then enter 0 (for example, if the water utility does not import any water, enter 0 for wholesale water imported). You may visit the TWDB Web site for the online version of the water audit:

http://www.twdb.state.tx.us/conservation/municipal/waterloss/

A. Water Utility Information

1. **Water Utility Name:** List the formal name of the water utility for which the water audit exists.
2. **Contact:** List the name of the primary contact person responsible for completing the water audit for the water utility, the telephone number, and email address.
3 **Reporting Period:** Enter calendar year or fiscal year dates for the reporting period.
4. **Source Water Utilization:** Enter percentages to represent the proportions of surface water and groundwater withdrawn for source water supply. Remember that the total of the two percentages must equal 100%.
5. **Population Served:** List separately the retail and wholesale populations served. You may multiply the number of connections by three if needed to estimate the retail population.
6. **Utility's Length of Main Lines, miles:** List the total length of pipeline in the water distribution system in miles.
7. **Number of Wholesale Connections Served:** List the number of wholesale interconnections supplying water to other water utilities.
8. **Number of Retail Service Connections Served:** List the number of retail customer service connections served by the utility's water distribution system.
9. **Service Connection Density:** Calculate the service connection density by dividing the number of retail customer service connections by the length of miles of pipeline in the water distribution system.
10. **Average Yearly System Operating Pressure:** List the average pressure across the entire water distribution systems for the audit period. If a hydraulic model of the network exists, the average pressure can be calculated by the model; otherwise, an estimate can be used.
11. **Volume Units of Measure:** Select the volume units of measure for the water audit. The units must be consistent throughout the entire water audit. If choosing million gallons for system input (from production meters), then authorized consumption (billed and unbilled) and all other entries must also be entered in million gallons. This typically requires a conversion for billed metered consumption.

B. System Input Volume: The total water supplied to the infrastructure. It is the total of all production meter readings for the entire year. List the volume or percentage requested in each item, along with the scores from Appendix 1.3 that in your judgment best represent the degree of validation of the data.

12. **Water Volume from own Sources:** Includes all water taken as source water from permitted sources, such as rivers, lakes, streams, and wells.
13. **Production Meter Accuracy (enter a percentage):** Achieved by calibrating or verifying the accuracy level (expressed as a percentage) of production meters. For example purposes, if the meter over-registered by 4 percent, enter 1.04; if it under-registered by 4 percent, enter .96.
14. **Corrected Input Volume (calculated automatically online):** The sum obtained when the production meter adjustment is either added to or subtracted from the system input volume. Divide "water volume from own sources" by the production meter accuracy. You must add the decimal point when the calculation is done manually (for example, to .96).

    Example: If "water volume from own sources" registered 1.8 MG/year through two production meters, which were found to be collectively under-registering flow by 4 percent, then the corrected input volume (CIV) is:

Corrected Input Volume = (1,800,000)/(0.96) = 1,875,000

15. **Wholesale Water Imported:** Amount of purchased wholesale water transferred into the utility's water distribution system from other water suppliers.
16. **Wholesale Water Exported:** Amount of wholesale water transferred out of the utility's distribution system. It may be put into the system initially but is only in the system for a brief time for conveyance reasons.
17. **System Input Volume:** Calculated as the corrected input volume plus water imported minus water exported (Line 14, plus Line 15, minus Line 16).

C. Authorized Consumption: All water that has been authorized for use or consumption by the utility or its customers. Remember to convert these volumes into the same units as the water delivery volume. Note: Any type of legitimate consumption should be classified in one of the four components of authorized consumption.

18. **Billed Metered:** All retail water sold and metered.
19. **Billed Unmetered:** All water sold but not metered.
20. **Unbilled Metered:** All water metered but not billed, such as back flushing water, parks, golf courses, and municipal government offices.
21. **Unbilled Unmetered:** All water not billed or metered, such as flushing fire hydrants.
22. **Total Authorized Consumption:** The total of the above four components, automatically calculated in the online worksheet.

D. Water Losses: Water delivered to the distribution system that does not appear as authorized consumption.

23. Calculated as the difference of the system input volume and total authorized consumption (Line 17 minus Line 22).

E. Apparent Losses: Water that has been consumed but not properly measured or billed. These losses represent under-registered or under-billed water that occurs via customer meter inaccuracy, systematic data handling error in the customer billing system, and unauthorized consumption:

24. **Average Customer Meter Accuracy:** List the composite accuracy percentage for your customer's meters. This percentage is typically derived from meter testing results. A representative assessment of customer meter accuracy can be obtained by testing as few as 50 meters.

25. **Customer Meter Accuracy Loss:** Obtained by dividing the billed metered water volume by the degree of average customer meter accuracy (Line 18 ÷ Line 24).

    Example: If billed metered (line 18) consumption registered 1.5 MG/year and random meter testing found customer meters to be collectively under-registering flow by 8 percent (so they are 92 percent accurate), then the customer meter accuracy loss is:

    Custom Meter Accuracy = [(1,500,000)/(0.92) – 1,500,000] = 130,434.78 gallons

26. **Systematic Data Handling Discrepancy:** List the estimated volume of water recorded by customer meters but distorted by meter reading or billing system error.

27. **Unauthorized Consumption (theft):** Estimate amount of water loss due to theft. Include an estimate of water taken illegally from fire hydrants, as well as water loss at the customer service connection. Theft at the customer connection can include tampering with meters or meter reading equipment, in addition to illegal taps and other similar occurrences.

28. **Total Apparent Losses:** This value is calculated automatically online as the sum of customer meter accuracy loss, systematic data handling error, and unauthorized consumption.

F. Real Losses: These are physical losses from the pressurized water distribution system, including water mains and all appurtenances (for example, valves and hydrants) and customer service connection piping. Real losses represent water that is lost from the distribution system prior to reaching the customer destination.

29. **Reported Breaks and Leaks:** Reported breaks and leaks are brought to the attention of the water utility by customers, public safety officials, other utilities, or other members of the general public. Usually these visible water main breaks are very disruptive and water utilities respond quickly to these events, so the run duration of the break or leak is relatively short. Estimate the total volume of water loss during the water audit period from reported breaks and leaks that were repaired during the year. Leakage flow rates must be estimated for various types of breaks and leaks, as well as the approximate duration of the breaks or leaks prior to repair.

30. **Unreported Loss:** This is a "catch-all" volume, meaning that this volume of real losses is the quantity that remains after authorized consumption, apparent losses, and reported leakage have been subtracted from the system input volume. In every water distribution system, even those employing effective active leakage control programs, there exists some amount of undetected leakage. Some of this loss is comprised of unreported leakage that has not yet been detected in leak surveys. It also includes a subcomponent known as background leakage, which is the collective weeps and seeps at pipe joints and on customer service connections that cannot be detected with acoustic sounding devices. Any degree of error in quantifying metered and estimated volumes in the water audit results in error in this component. As the validation of the water audit improves over time, so will the level of validation of the unreported loss volume.

31. **Total Real Losses:** This value is calculated automatically online as the sum of reported breaks and leaks and unreported loss.
32. **Water Losses:** Calculated as the sum of apparent losses and real losses. This value should equal the value of Line 23. This line is included as a balancing check.
33. **Non-revenue Water:** Calculated as the sum of apparent losses, plus real losses, plus unbilled metered consumption and unbilled unmetered consumption. This is the water that does not contribute to the water utility billings.

G. Technical Performance Indicator for Apparent Loss: Performance indicators are quantitative measures of key aspects within the utility. Using these indicators, the utility will have a history to track its performance from year to year. One performance indicator exists for apparent loss.

34. **Apparent Losses Normalized:** Calculated as the volume of apparent loss, divided by the number of retail customer service connections, divided by 365 days. This performance indicator allows for reliable performance tracking in the water utility's efforts to reduce apparent losses.

H. Technical Performance Indicator for Real Loss: Several performance indicators exist for real loss.

35. **Real Loss Volume:** This is the quantity from Line 31.
36. **Unavoidable Annual Real Losses:** Calculated reference value using the equation shown in Table 3-2. This is a theoretical value of the technical low level of leakage that might be attained in a given water utility, based upon several system specific parameters.
37. **Infrastructure Leakage Index:** This performance indicator is calculated as the ratio of real losses over the unavoidable annual real losses. The index measures the water utility's leakage management effectiveness and is an excellent performance indicator for comparing performance among water utilities. The lower the value of the infrastructure leakage index, the closer the utility is operating to the theoretical low level of the unavoidable annual real loss. Appendix 1.4 gives general guidance on setting preliminary leakage reduction targets using the infrastructure leakage index without changing water pressure.
38. **Real Losses Normalized:** Calculated as the real loss volume, divided by the number of retail service connections, divided by 365. Use this calculation if the service connection density is greater than, or equal to, 32 per mile. This indicator allows for reliable performance tracking in the water utility's efforts to reduce real losses.
39. **Real Losses Normalized:** Calculated as the real loss volume, divided by the number of miles of pipeline, divided by 365. Use this calculation if the service connection density is less than 32 per mile. This indicator allows for reliable performance tracking in the water utility's efforts to reduce real losses.

I. Financial Performance Indicators

40. **Total Apparent Losses:** List the volume from line 28.
41. **Retail Price of Water:** Water utility rate structures usually feature multiple tiers of pricing based upon volume consumed. For the water audit, it is best to use a single composite price rate to represent the retail cost of water, which is used to place a value on the apparent losses. The largest number of accounts in most utilities is residential accounts; therefore, the residential pricing tier may be used in place of weighted calculations to determine a composite rate.
42. **Cost of Apparent Losses:** Calculated by multiplying the apparent loss volume by the retail price of water. This represents the potential amount of missed revenue due to apparent losses.
43. **Total Real Losses:** List the volume from line 31.
44. **Variable Production Cost of Water:** Marginal production cost including variable costs, which are typically the costs of raw water, energy, and chemicals. If applicable, the cost of raw water should include the price of take or pay contracts. These costs are applied to determine the cost impact of real losses. In cases of water shortage, real losses might be valued at the retail price of water instead of the variable production cost.
45. **Cost of Real Losses:** Calculated by multiplying the real loss volume by the variable production cost of water. These costs represent the additional operating costs incurred by the water utility due to the real losses (in other words, leakage).
46. **Total Assessment Score:** Add the individual assessment scores to obtain a total.
47. **Total Cost Impact of Apparent and Real Losses:** Calculated by adding lines 42 and 45. This amount indicates the cost inefficiency encountered by the water utility for losses. This cost value can be objectively weighed against potential loss control programs to determine the cost effectiveness of such programs.

If you or the utility has any software application questions, please call Juan Moran-Lopez at 512-463-0987 or email: Juan.Moran-Lopez@twdb.texas.gov

For more information on water audits, visit the American Water Works Association Web site: http://www.awwa.org/Resources/topicspecific.cfm?ItemNumber=3653&navItemNumber=1583

F. 2014 Monthly Field Reports

To Governing Body
From: Art Smith
Subject: Field Report for January 2014

Sent sewer samples to Pollution Control	2, 9, 16, 23, 30
Sent water site samples to Pollution Control	16

City vehicles for the month.

	Amount	Miles	Gallons	MPG
Ford F-150 #1	$402.21	2,141	132.54	16.20
Ford F-150 #2	$418.95	1,406	137.22	11.00
Chevy	$163.02	776	53.45	14.52
GMC 1ton	Not in Daily Use			

City Water Use

	Gallons	Cost
City office	1,000	$19.00
Natalia PD	2,000	$21.00
Sewer Plant	9,000	$35.00
Library	5,000	$27.00
Little League	0	$0.00
Veterans Memorial	0	$0.00
Fire Department	2,000	$21.00
Fire Trucks	11,285	$40.00
Scotts	3,000	$23.00

	Gallons	Last Month
Leaks	750,000	500,000
Flushing	450,000	300,000

Cost for Leaks & Flushing
$173.09

City Water Use	1,233,285	$359.09
Total Gallons Pumped	5,460,080	
Gallons Sold	3,554,651	
Well #7	2,000	$0.29
Total Usage	4,787,936	
Gallons Lost	672,144	$96.95
Loss Percentage	14.04%	
Total City's Cost		$456.32

Services turned off.	18
Survices turned on.	17

One service remains turned off due to non-payment. They have not contacted the office.

16 water meters were replaced.

To Governing Body
From: Art Smith
Subject: Field Report for February 2014

Sent sewer samples to Pollution Control	6, 13, 20, 27
Sent water site samples to Pollution Control	13

City vehicles for the month.

	Amount	Miles	Gallons	MPG
Ford F-150 #1	$374.08	1,894	118.44	16.20
Ford F-150 #2	$289.81	813	93.11	12.00
Chevy	$161.03	632	52.45	12.05
GMC 1ton	Not in Daily Use			

City Water Use

	Gallons	Cost
City office	1,000	$19.00
Natalia PD	1,000	$19.00
Sewer Plant	20,000	$62.00
Library	10,000	$37.00
Little League	0	$0.00
Veterans Memorial	0	$0.00
Fire Department	1,000	$19.00
Fire Trucks	1,650	$20.30
Scotts	3,000	$23.00

	Gallons	Last Month
Leaks	600,000	750,000
Flushing	150,000	450,000

Cost for Leaks & Flushing
$108.18

City Water Use	787,650	$307.48
Total Gallons Pumped	5,703,810	
Gallons Sold	4,215,541	
Well #7	0	$0.00
Total Usage	5,003,191	
Gallons Lost	700,619	$101.06
Loss Percentage	14.00%	
Total City's Cost		$408.53

Services turned off.	12
Survices turned on.	12

To Governing Body
From: Art Smith
Subject: Field Report for March 2014

Sent sewer samples to Pollution Control 6, 13, 20, 27
Sent water site samples to Pollution Control 13

City vehicles for the month.

	Amount	Miles	Gallons	MPG
Ford F-150 #1	$245.88	1,318	75.92	16.20
Ford F-150 #2	$312.63	1,187	97.16	11.00
Chevy	$274.53	759	84.55	8.98
GMC 1ton	Not in Daily Use			

City Water Use

	Gallons	Cost
City office	1,000	$19.00
Natalia PD	2,000	$21.00
Sewer Plant	2,000	$21.00
Library	3,000	$23.00
Little League	2,000	$21.00
Veterans Memorial	0	$0.00
Fire Department	1,000	$19.00
Fire Trucks	2,600	$21.00
Scotts	3,000	$23.00

	Gallons	Last Month
Leaks	700,000	600,000
Flushing	350,000	150,000

Cost for Leaks & Flushing
$151.45

City Water Use	1,066,600	$319.45
Total Gallons Pumped	5,014,530	
Gallons Sold	3,319,439	
Well #7	0	$0.00
Total Usage	4,386,039	
Gallons Lost	628,491	$90.65
Loss Percentage	14.33%	
Total City's Cost		$410.10

Services turned off. 23
Survices turned on. 21

Two services remain turned off due to non-payment.

To Governing Body
From: Art Smith
Subject: Field Report for April 2014

Sent sewer samples to Pollution Control	3, 10, 17, 24
Sent water site samples to Pollution Control	24

City vehicles for the month.

	Amount	Miles	Gallons	MPG
Ford F-150 #1	$435.52	1,774	127.93	16.50
Ford F-150 #2	$337.86	902	98.82	13.00
Chevy	$192.01	937	55.99	16.74
GMC 1ton	Not in Daily Use			

City Water Use

	Gallons	Cost
City office	2,000	$21.00
Natalia PD	1,000	$19.00
Sewer Plant	12,000	$42.00
Library	6,000	$29.00
Little League	8,000	$33.00
Veterans Memorial	0	$0.00
Community Garden	1,000	$19.00
Fire Department	0	$0.00
Fire Trucks	6,450	$29.00
Scotts	3,000	$23.00

	Gallons	Last Month
Leaks	800,000	700,000
Flushing	450,000	350,000

Cost for Leaks & Flushing
$180.30

City Water Use	1,289,450	$395.30
Total Gallons Pumped	5,403,730	
Gallons Sold	3,437,253	
Well #7	2,000	$0.29
Total Usage	4,726,703	
Gallons Lost	677,027	$97.65
Loss Percentage	14.32%	
Total City's Cost		$493.24

Services turned off.	18
Survices turned on.	16

Two services remain turned off due to non payment.

To Governing Body
From: Art Smith
Subject: Field Report for June 2014

Sent sewer samples to Pollution Control	5, 12, 19, 26
Sent water site samples to Pollution Control	19

City vehicles for the month.

	Amount	Miles	Gallons	MPG
Ford F-150 #1	$412.50	1,720	121.938	15.60
Ford F-150 #2	$345.95	1,282	102.39	11.60
Chevy	$377.31	1,328	111.93	11.86
GMC 1ton	Not in Daily Use			

City Water Use

	Gallons	Cost
City office	2,000	$21.00
Natalia PD	1,000	$19.00
Sewer Plant	2,000	$21.00
Library	1,000	$19.00
Little League	12,000	$42.00
Veterans Memorial	0	$0.00
Community Garden	2,000	$21.00
Fire Department	1,000	$19.00
Fire Trucks	2,011	$21.00
Scotts	3,000	$23.00

	Gallons	Last Month
Leaks	600,000	700,000
Flushing	200,000	300,000

Cost for Leaks & Flushing
$115.39

City Water Use	826,011	$206.00
Total Gallons Pumped	5,107,490	
Gallons Sold	3,635,008	
Well #7	0	$0.00
Total Usage	4,461,019	
Gallons Lost	646,471	$93.25
Loss Percentage	14.49%	
Total City's Cost		$299.25

Services turned off.	25
Survices turned on.	25

To Governing Body
From: Art Smith
Subject: Field Report for July 2014

Sent sewer samples to Pollution Control 3, 10, 17, 24, 31
Sent water site samples to Pollution Control 10

City vehicles for the month.

	Amount	Miles	Gallons	MPG
Ford F-150 #1	$415.43	2,096	122.441	16.70
Ford F-150 #2	$422.21	1,554	124.02	11.30
Chevy	$282.23	882	83.81	10.52
GMC 1ton	$408.65	1,004	106.35	9.44

City Water Use

	Gallons	Cost
City office	1,000	$19.00
Natalia PD	1,000	$19.00
Sewer Plant	13,000	$44.50
Library	0	$0.00
Little League	1,000	$19.00
Veterans Memorial	0	$0.00
Fire Department	0	$0.00
Fire Trucks	2,084	$21.00
Scotts	3,000	$23.00

	Gallons	Last Month
Leaks	800,000	600,000
Flushing	500,000	200,000

Cost for Leaks & Flushing
$187.51

City Water Use	1,321,084	$333.01
Total Gallons Pumped	5,562,860	
Gallons Sold	3,540,725	
Well #7	2,000	$0.29
Total Usage	4,861,809	
Gallons Lost	701,051	$101.12
Loss Percentage	14.42%	
Total City's Cost		$434.42

Services turned off. 15
Survices turned on. 12

2 services remain locked due to non-payment, and the other service is still off due to the death of the resid

To Governing Body
From: Art Smith
Subject: Field Report for August 2014

Sent sewer samples to Pollution Control	7, 14, 21, 28
Sent water site samples to Pollution Control	14

City vehicles for the month.

	Amount	Miles	Gallons	MPG
Ford F-150 #1	$294.81	1,374	89.21	16.10
Ford F-150 #2	$406.39	1,415	122.72	12.00
Chevy	$204.85	796	61.70	12.90
GMC 1ton	$517.25	1,118	135.94	8.20

City Water Use

	Gallons	Cost
City office	2,000	$21.00
Natalia PD	1,000	$19.00
Community Garden	1,000	$19.00
Sewer Plant	5,000	$27.00
Library	0	$0.00
Little League	0	$0.00
Veterans Memorial	0	$0.00
Fire Department	1,000	$19.00
Fire Trucks	11,400	$39.50
Scotts	3,000	$23.00

	Gallons	Last Month
Leaks	600,000	800,000
Flushing	250,000	500,000

Cost for Leaks & Flushing
$122.60

City Water Use	874,400	$146.50
Total Gallons Pumped	5,979,290	
Gallons Sold	4,359,766	
Well #7	0	$0.00
Total Usage	5,234,166	
Gallons Lost	745,124	$107.47
Loss Percentage	14.24%	
Total City's Cost		$253.97

Services turned off.	18
Survices turned on.	17

One service remains shut off because the resident moved out.

To Governing Body
From: Art Smith
Subject: Field Report for September 2014

Sent sewer samples to Pollution Control	4, 11, 18, 25
Sent water site samples to Pollution Control	11

City vehicles for the month.

	Amount	Miles	Gallons	MPG
Ford F-150 #1	$360.47	1,743	112.405	16.50
Ford F-150 #2	$413.05	1,569	128.52	13.00
Chevy	$242.01	960	75.74	12.68
GMC 1ton	Not in Daily Use			

City Water Use

	Gallons	Cost
City office	1,000	$19.00
Natalia PD	0	$0.00
Sewer Plant	21,000	$64.50
Library	1,000	$19.00
Little League	4,000	$25.00
Veterans Memorial	0	$0.00
Fire Department	0	$0.00
Fire Trucks	1,000	$19.00
Scotts	3,000	$23.00

	Gallons	Last Month
Leaks	600,000	600,000
Flushing	250,000	250,000

Cost for Leaks & Flushing
$122.60

City Water Use	881,000	$292.10
Total Gallons Pumped	5,979,290	
Gallons Sold	4,359,766	
Well #7	0	$0.00
Total Usage	5,240,766	
Gallons Lost	738,524	$106.52
Loss Percentage	14.09%	
Total City's Cost		$403.10

Services turned off.	6
Survices turned on.	6

To Governing Body
From: Art Smith
Subject: Field Report for October 2014

Sent sewer samples to Pollution Control 2, 9, 16, 23, 31
Sent water site samples to Pollution Control 9

City vehicles for the month.

	Amount	Miles	Gallons	MPG
Ford F-150 #1	$378.77	1,948	125.898	16.60
Ford F-150 #2	$377.33	1,669	124.74	12.00
Chevy	$249.99	1,024	84.37	12.14
GMC 1ton	Not in Daily Use			

City Water Use

	Gallons	Cost
City office	1,000	$19.00
Natalia PD	1,000	$19.00
Sewer Plant	1,000	$19.00
Library	0	$0.00
Little League	2,000	$21.00
Veterans Memorial	0	$0.00
Fire Department	1,000	$19.00
Fire Trucks	16,751	$52.00
Scotts	3,000	$23.00

	Gallons	Last Month
Leaks	500,000	600,000
Flushing	200,000	250,000

Cost for Leaks & Flushing
$100.97

Estimated Water Loss from billing method
(included in City Water Use)
250,000

City Water Use	975,854	$272.97
Total Gallons Pumped	5,925,370	
Gallons Sold	4,212,722	
Well #7	2,000	$0.29
Total Usage	5,188,576	
Gallons Lost	736,794	$106.27
Loss Percentage	14.20%	
Total City's Cost		$383.24

Services turned off. 17
Survices turned on. 16

1 service remains turned off for nonpayment.

To Governing Body
From: Art Smith
Subject: Field Report for November 2014

Sent sewer samples to Pollution Control	6, 13, 20, 25
Sent water site samples to Pollution Control	13

City vehicles for the month.

	Amount	Miles	Gallons	MPG
Ford F-150 #1	$241.90	1,476	90.32	15.80
Ford F-150 #2	$312.47	1,248	115.12	12.50
Chevy	$154.23	838	56.21	14.90
GMC 1ton	Not in Daily Use			

City Water Use

	Gallons	Cost
City office	2,000	$22.00
Natalia PD	1,000	$20.00
Sewer Plant	1,000	$20.00
Library	0	$0.00
Little League	8,000	$34.00
Veterans Memorial	0	$0.00
Fire Department	1,000	$20.00
Fire Trucks	400	$20.00
Scotts	3,000	$23.00

	Gallons	Last Month
Leaks	500,000	500,000
Flushing	200,000	200,000

Cost for Leaks & Flushing
$100.97

Estimated Water Loss from billing method
(included in City Water Use)
250,000

City Water Use	966,503	$259.97
Total Gallons Pumped	5,278,060	
Gallons Sold	3,636,026	
Well #7	2,000	$0.29
Total Usage	4,602,529	
Gallons Lost	675,531	$97.44
Loss Percentage	14.68%	
Total City's Cost		$360.06

Services turned off.	8
Survices turned on.	8

To Governing Body
From: Art Smith
Subject: Field Report for December 2014

Sent sewer samples to Pollution Control	4, 11, 18, 23, 30
Sent water site samples to Pollution Control	18

City vehicles for the month.

	Amount	Miles	Gallons	MPG
Ford F-150 #1	$99.44	1,014	63.537	16.00
Ford F-150 #2	$266.12	1,402	115.80	12.50
Ford F-150 #3	$204.94	1,054	95.54	14.50
Chevy	$138.11	658	55.81	11.80
GMC 1ton	Not in Daily Use			

City Water Use

	Gallons	Cost
City office	1,000	$20.00
Natalia PD	1,000	$20.00
Sewer Plant	3,000	$24.00
Library	0	$0.00
Little League	2,000	$22.00
Veterans Memorial	0	$0.00
Fire Department	0	$0.00
Fire Trucks		
Scotts	3,000	$23.00

	Gallons	Last Month
Leaks	625,000	500,000
Flushing	350,000	200,000

Cost for Leaks & Flushing
$140.63

Estimated Water Loss from billing method
(included in City Water Use)
250000

City Water Use	1,235,144	$249.63
Total Gallons Pumped	4,506,040	
Gallons Sold	2,689,937	
Well #7	0	$0.00
Total Usage	3,925,081	
Gallons Lost	580,959	$83.80
Loss Percentage	14.80%	
	Total City's Cost	$334.87

Services turned off.
Survices turned on.

Appendix K12

Predictive Ecological Models for the Comal and San Marcos Ecosystems
Project Presentation

PREDICTIVE ECOLOGICAL MODEL(S) FOR THE COMAL AND SAN MARCOS ECOSYSTEMS

(Comal and San Marcos Springs)

HABITAT CONSERVATION PLAN
EDWARDS AQUIFER

EDWARDS AQUIFER AUTHORITY

February 11, 2015

MODELING TEAM

Bill Grant, Texas A&M University

Hsiao-Hsuan (Rose) Wang , Texas A&M University

Robert Doyle, Baylor

Todd Swannack, Environmental Research & Development Center/ Waterways Experiment Station

Thom Hardy, Watershed Systems Group

Timothy Bonner, Texas State University

Ed Oborny, Jake Jackson, BIO-WEST

George Ward, University of Texas

Overview

- Agent Based Model (Swannack)
- Hydraulic modeling (Hardy)
- Water quality modeling (Hardy)
- Percent Cover to Biomass study (Doyle)
- Aquatic Vegetation Modeling (Swannack)
- Food Source Analysis (Jackson)
- Fountain Darter Modeling (Wang and Grant)
- Overview of Key Decisions (Ward)
- Schedule and Next Steps (Oborny)

Key Decisions

- Agent Based Model
- 1 meter grids
- Hydrology – Comal System
  - gages not available on all segments over time – in particular the Old Channel
- Water Quality Model – Qual-2E
  - Using flow contributions from the EARIP
  - Not modeling nutrients or carbon dioxide
  - Using maximum temperature and minimum dissolved oxygen
- Aquatic Vegetation Modeling
  - Percent Cover an appropriate surrogate for biomass
  - Chose a subset of SAV species based on fountain darter statistical analysis
  - Species specific propagation methods and mathematical expression
  - Not including herbivory
- Fountain Darter Modeling
  - Food availability not limited – no macroinvertebrate sub-model
  - Not using Macro scale in fountain darter analysis

Overview

- Agent Based Model (Swannack)
- Hydraulic modeling (Hardy)
- Water quality modeling (Hardy)
- Percent Cover to Biomass study (Doyle)
- Aquatic Vegetation Modeling (Swannack)
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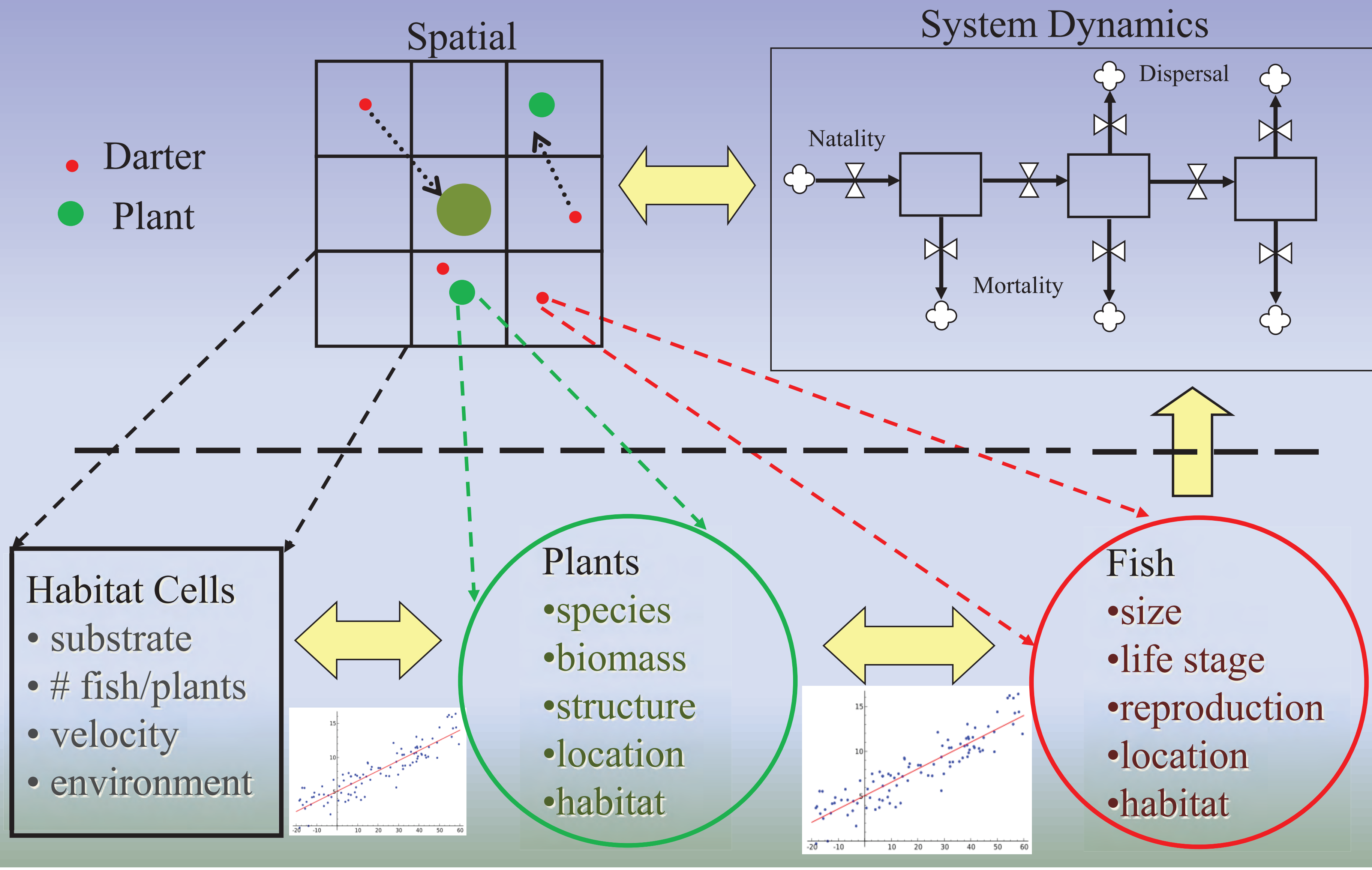
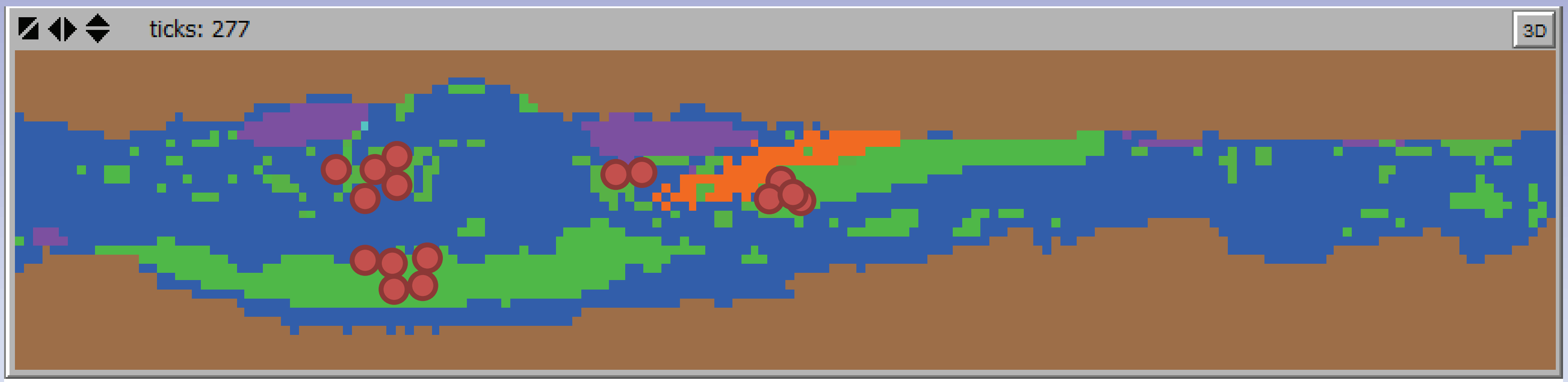
Conceptual overview of typical ecological models



Conceptual overview of spatially-explicit, agent-based model



- Captures individual variability, ecological and physical processes in a dynamic, spatial framework which can be used to test scenarios/inform management decisions

Agent-based References

- Grimm V. & Railsback S.F. (2005) *Individual-based Modeling and Ecology*. Princeton University Press, Princeton.
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- Mazaris A.D., Broder B. & Matsinos Y.G. (2006) An individual based model of a sea turtle population to analyze effects of age dependent mortality. *Ecological Modelling* **198**, 174-82.
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- Rossmanith E., Blaum N., Grimm V. & Jeltsch F. (2007) Pattern-oriented modeling for estimating unknown pre-breeding survival rates: The case of the Lesser Spotted Woodpecker. *Biological Conservation* **135**, 555-64.
- Swannack T.M., Grant W.E. & Forstner M.R.J. (2009) Projecting population trends of endangered amphibian species in the face of uncertainty: A pattern-oriented approach. *Ecological Modelling* **220**, 148-59.
- Uchmanski J. & Grimm V. (1996) Individual-based modelling in ecology: what makes the difference? *Trends in Ecology & Evolution* **11**, 437-41.
- Wiegand T., Jeltsch F., Hanski I. & Grimm V. (2003) Using pattern-oriented modeling for revealing hidden information: a key for reconciling ecological theory and application. *Oikos* **100**, 209-22.

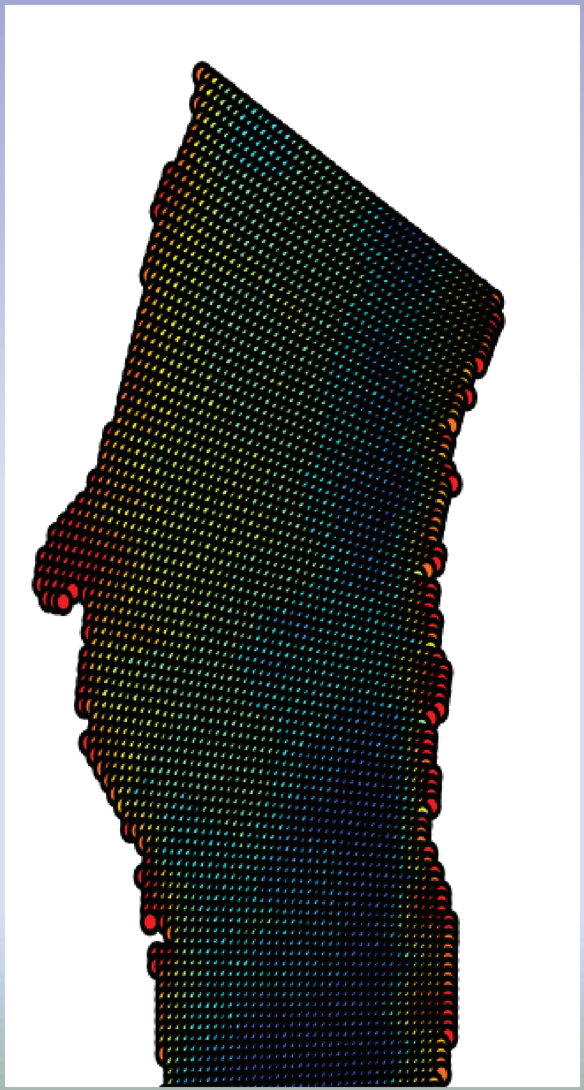
Overview

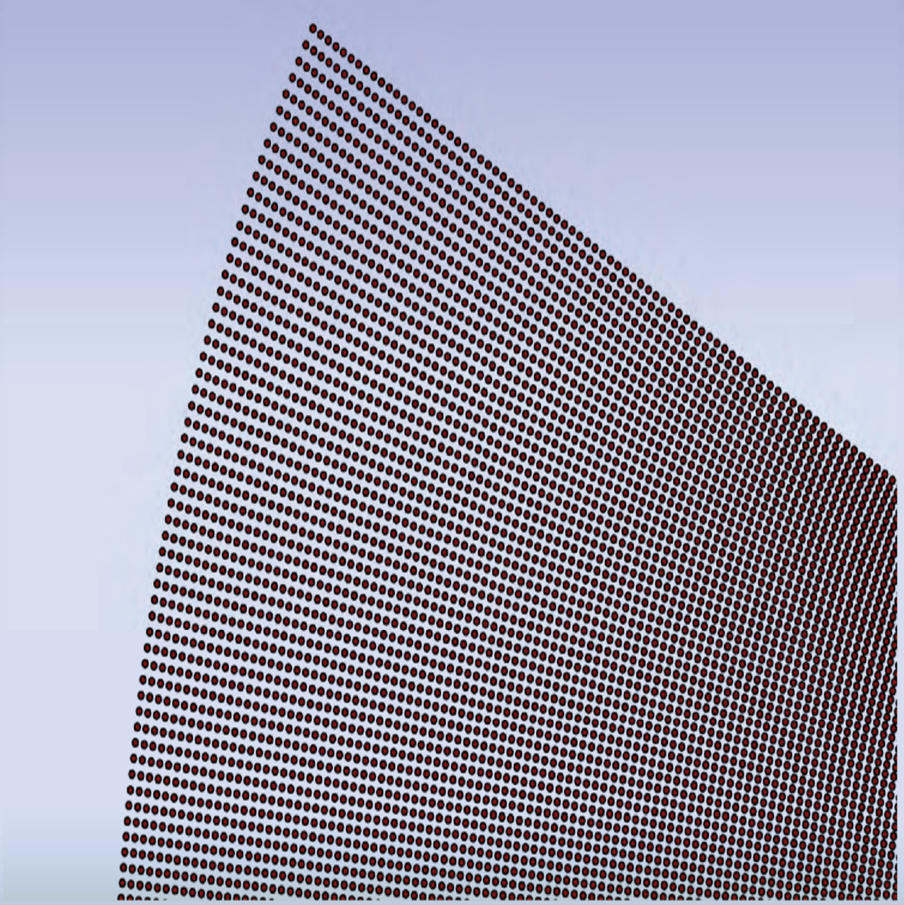
- Agent Based Model (Swannack)
- Hydraulic and Water Quality modeling (Hardy)
- Percent Cover to Biomass study (Doyle)
- Aquatic Vegetation Modeling (Swannack)
- Food Source Analysis (Jackson)
- Fountain Darter Modeling (Wang and Grant)
- Overview of Key Decisions (Ward)
- Schedule and Next Steps (Oborny)

Computational Grids

- Original 0.25 meter computational grids were resampled to generate 1.0 meter grids.
  - No interpolations
  - Corresponding nodes were simply extracted

City Park Study Reach – Hydraulic Grid: 1 Meter Resolution

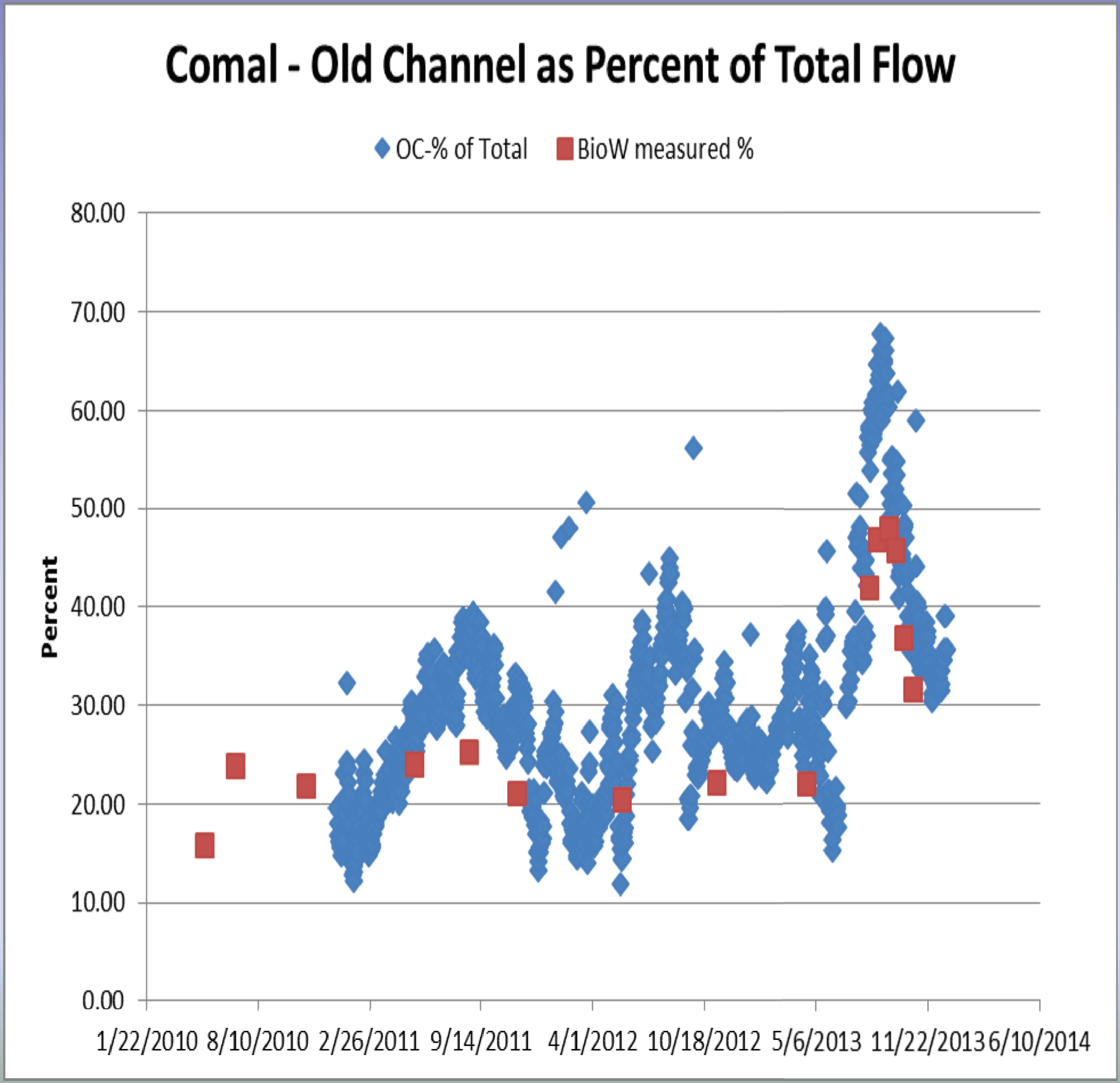


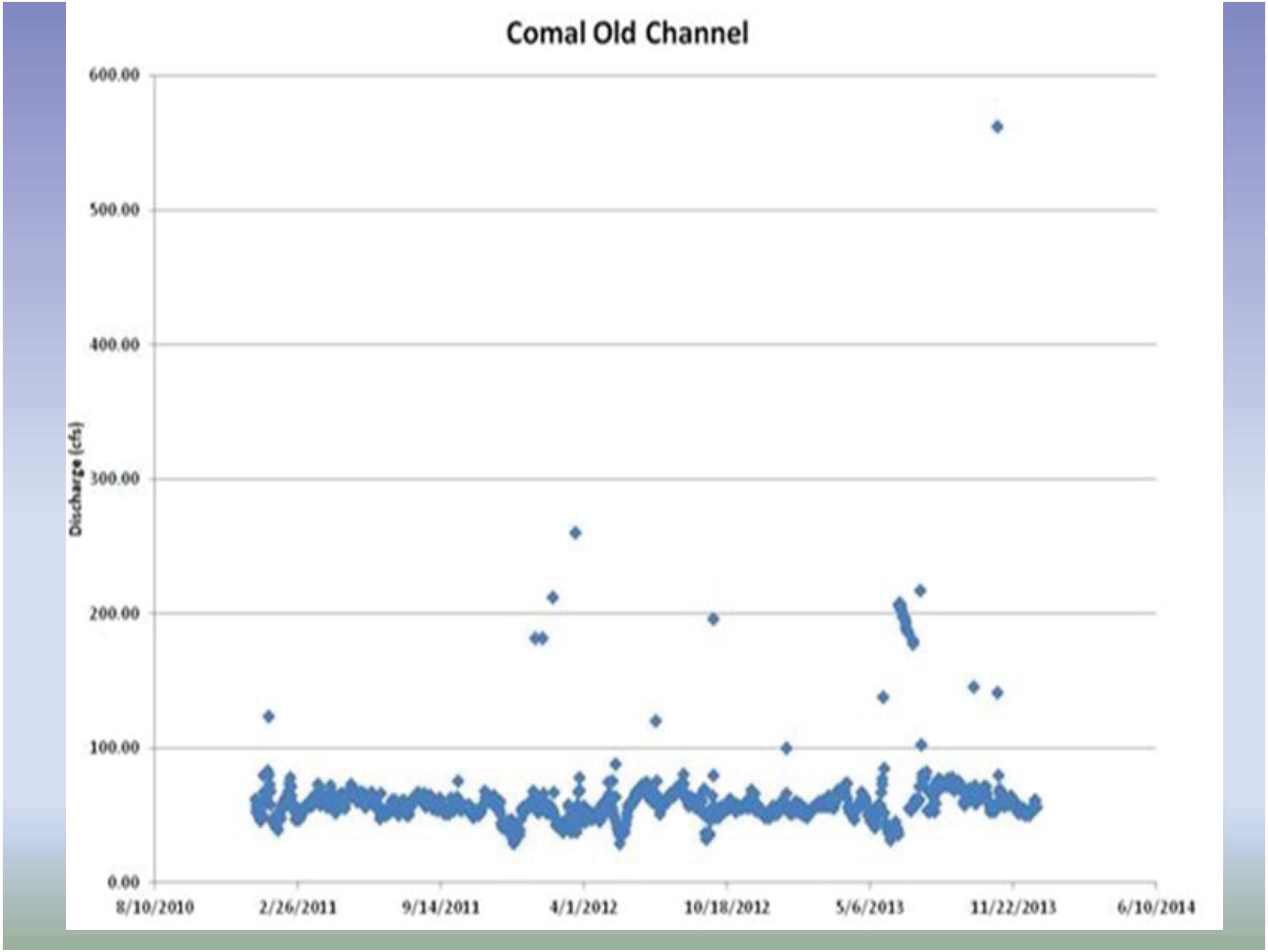


City Park Study Reach with Spatially Joined Vegetation Overlay

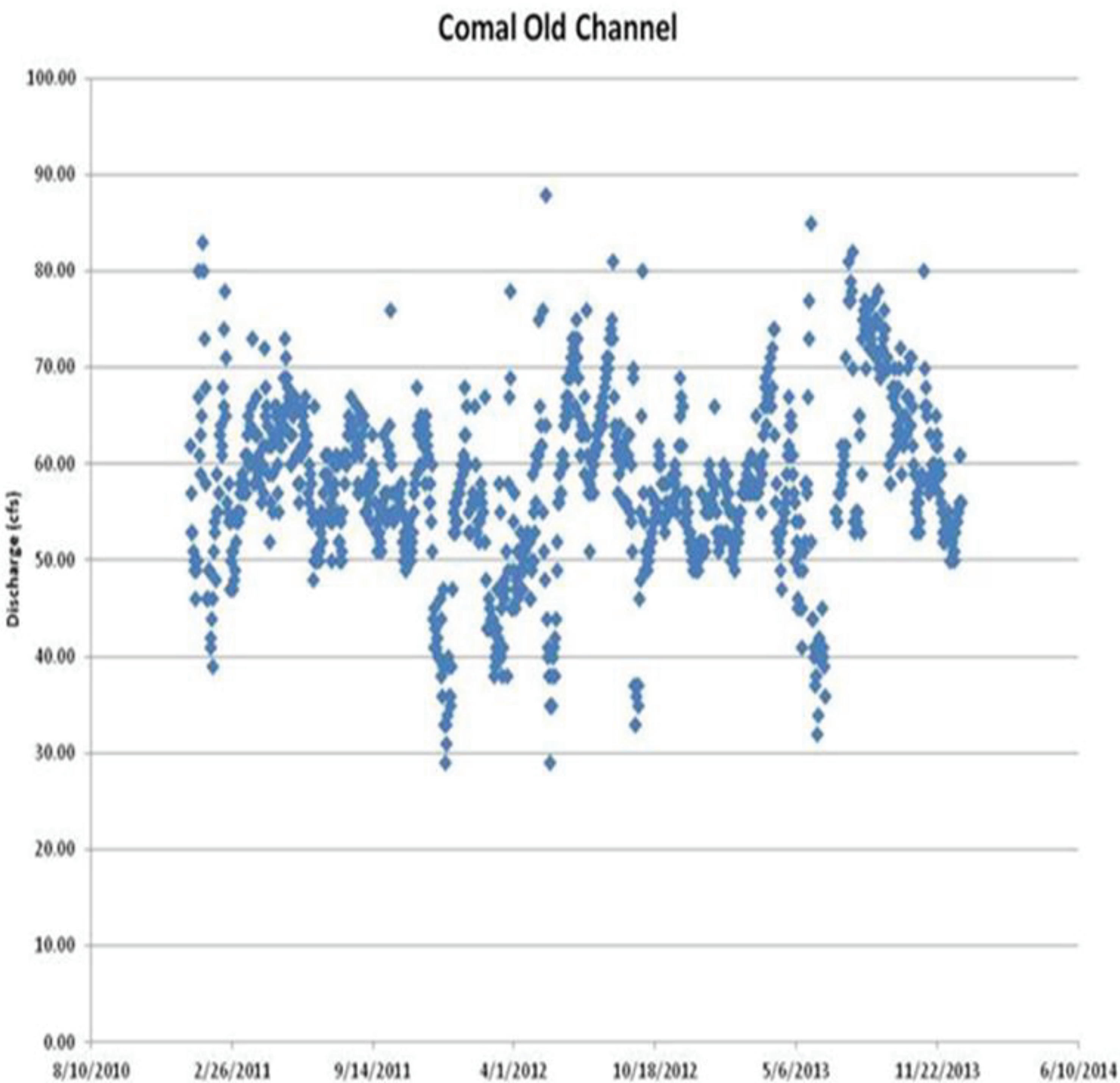
Interpolation of Hydraulic Grids for Flow Rates – Old Channel

- The hydraulic solutions in the Old Channel were used to linearly interpolate the hydraulic properties at 1 cfs increments between 10 and 80 cfs
- Cross checking of interpolated values to simulated values resulted in less than a 3 percent MSE between simulated and interpolated values

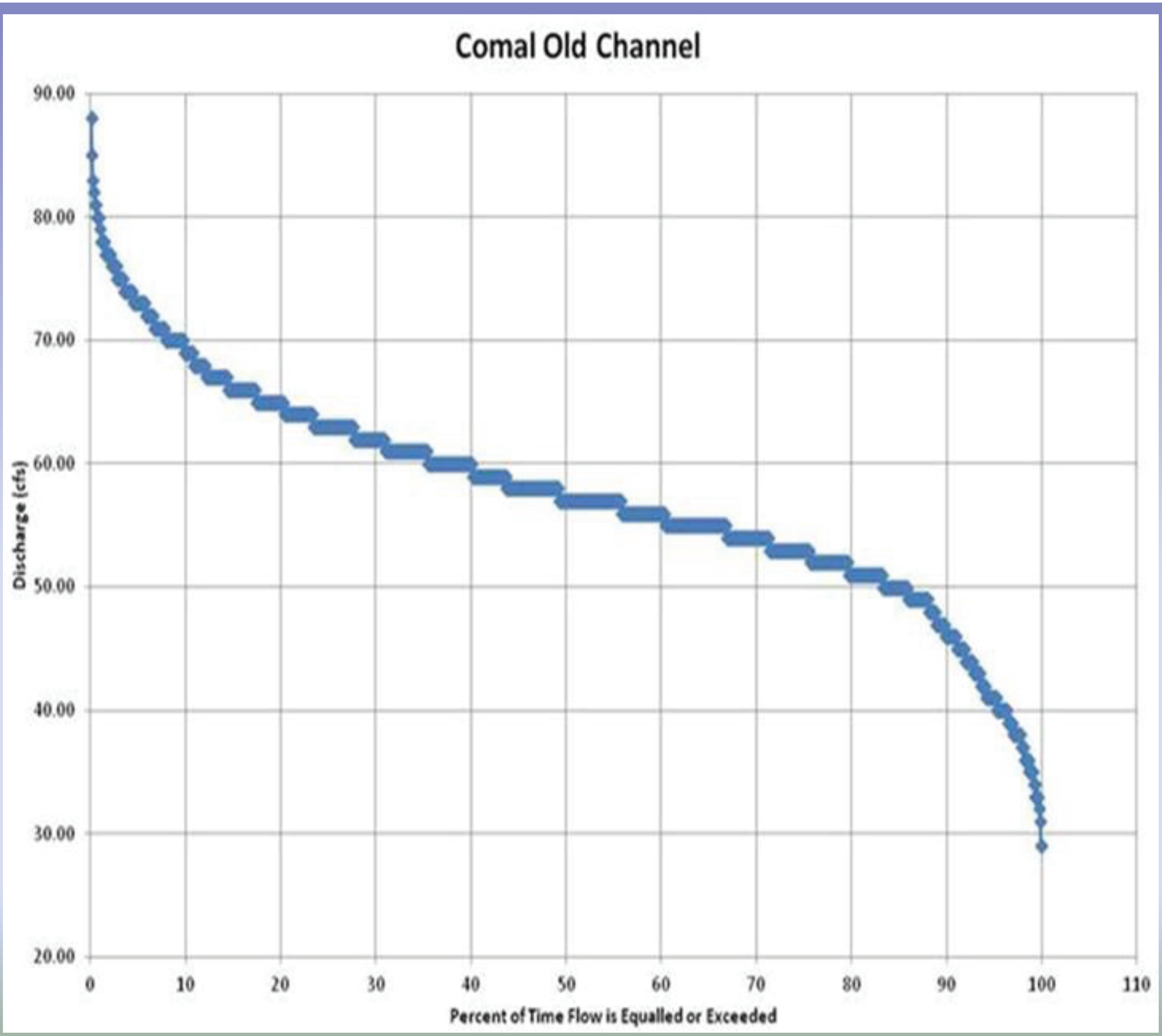

Comal - Old Channel as Percent of Total Flow
OC-% of Total
BioW measured %
Percent
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
1/22/2010
8/10/2010
2/26/2011
9/14/2011
4/1/2012
10/18/2012
5/6/2013
11/22/2013
6/10/2014



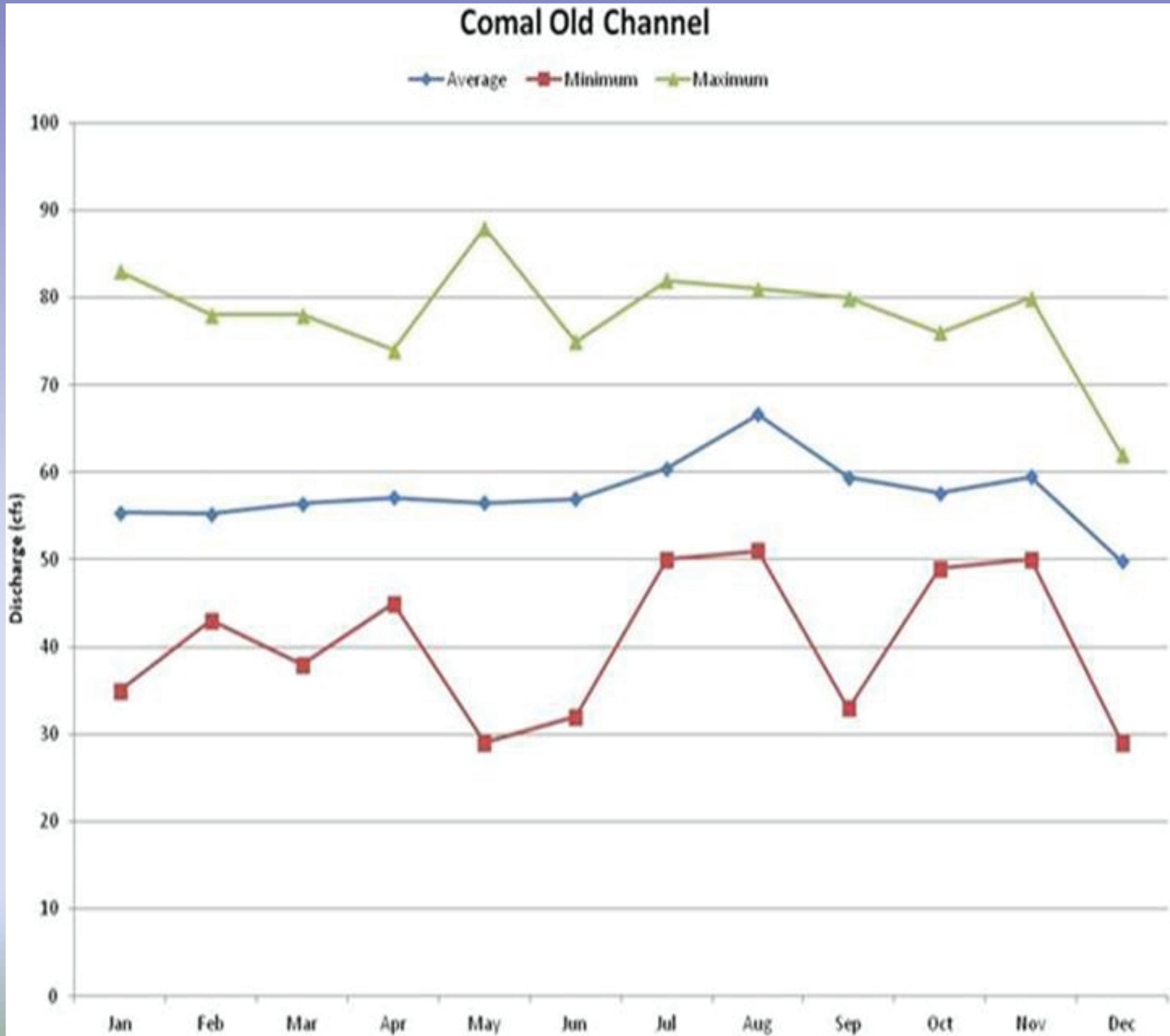
Comal Old Channel
Discharge (cfs)
600.00
500.00
400.00
300.00
200.00
100.00
0.00
8/10/2010
2/26/2011
9/14/2011
4/1/2012
10/18/2012
5/6/2013
11/22/2013
6/10/2014



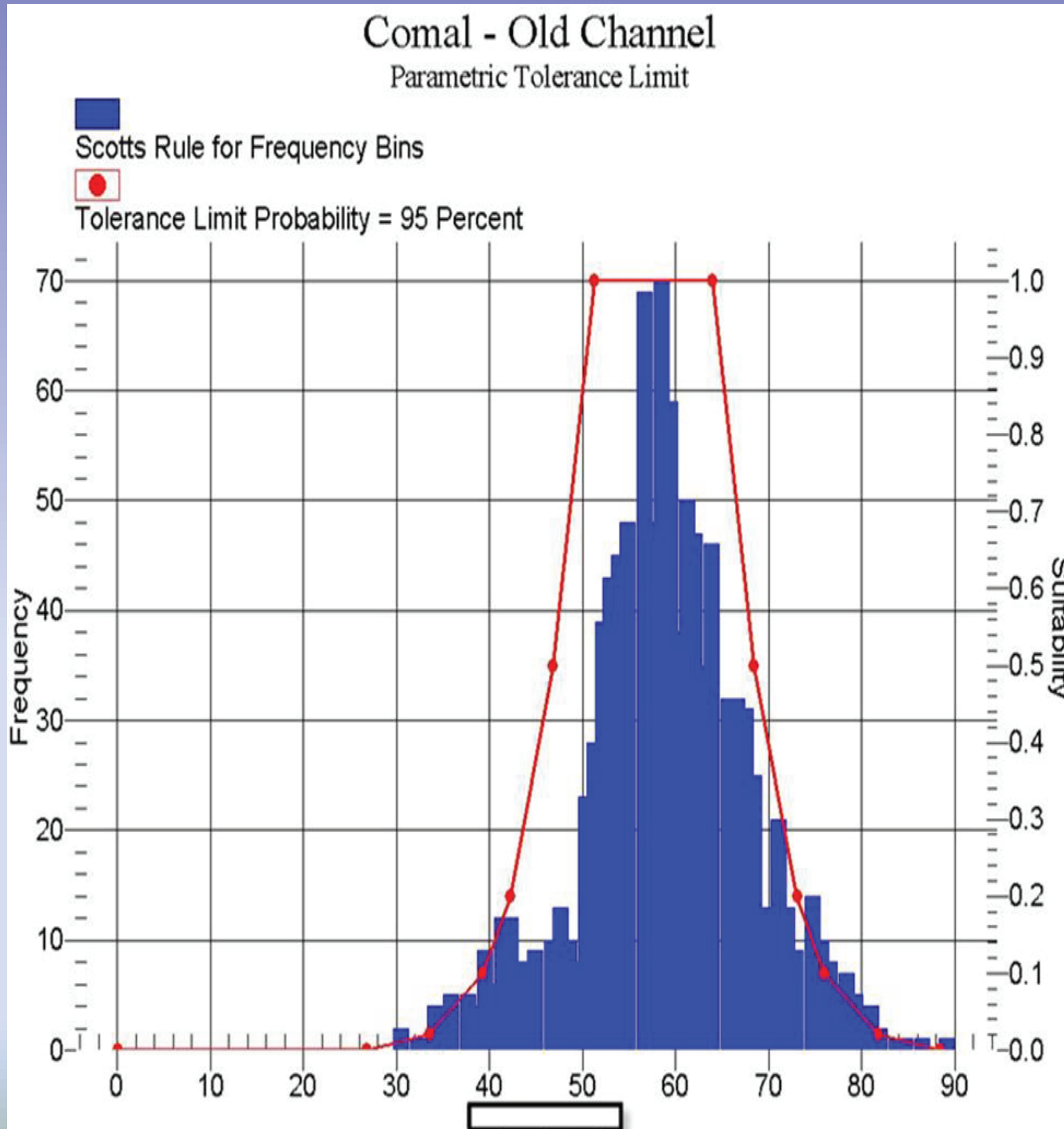
Comal Old Channel
Discharge (cfs)
100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
8/10/2010
2/26/2011
9/14/2011
4/1/2012
10/18/2012
5/6/2013
11/22/2013
6/10/2014



Comal Old Channel
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
Discharge (cfs)
0
10
20
30
40
50
60
70
80
90
100
110
Percent of Time Flow is Equalled or Exceeded

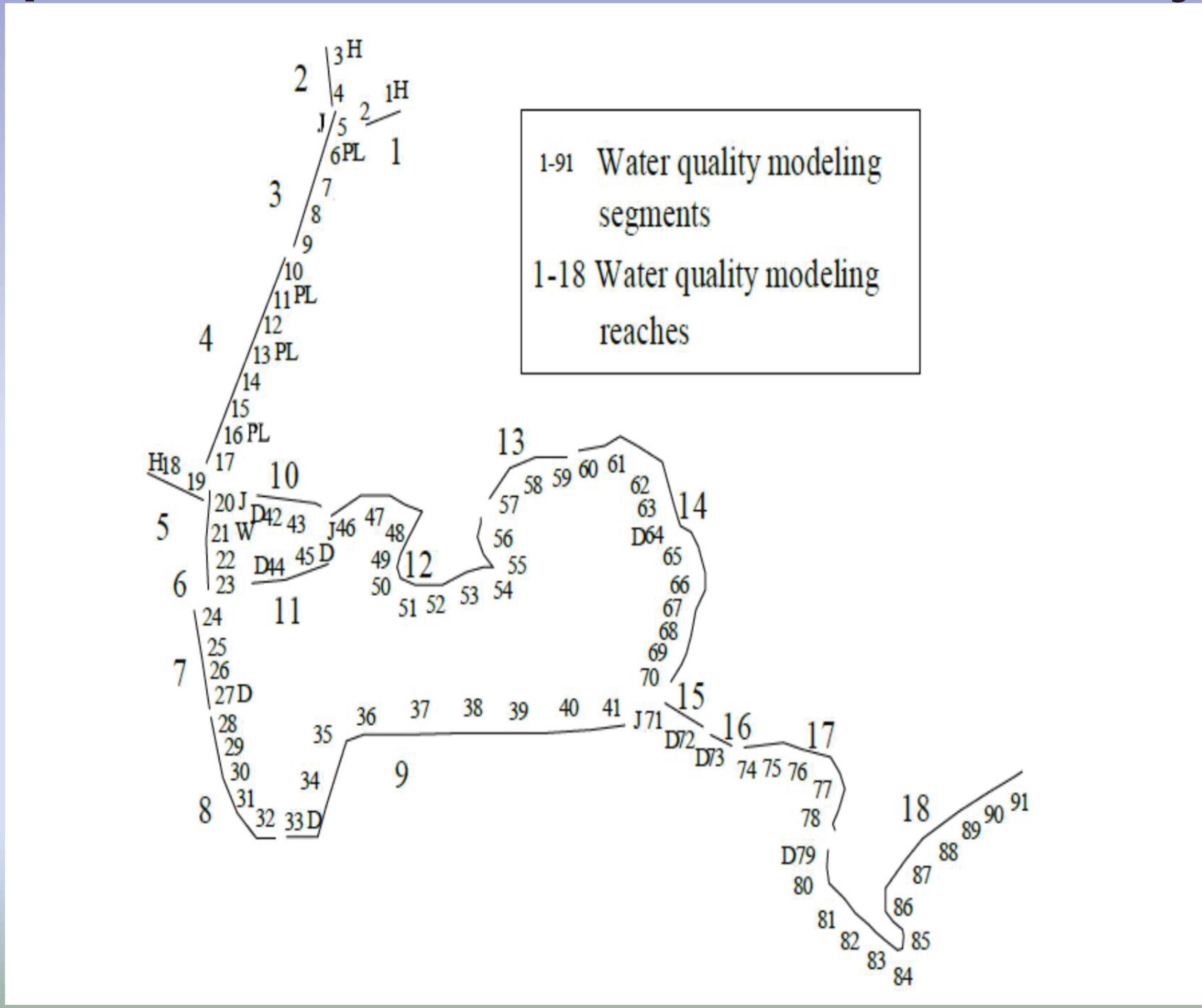


Comal Old Channel
Average
Minimum
Maximum
Discharge (cfs)
100
90
80
70
60
50
40
30
20
10
0
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec



Comal - Old Channel
Parametric Tolerance Limit
Scotts Rule for Frequency Bins
Tolerance Limit Probability = 95 Percent
Frequency
Suitability
0
10
20
30
40
50
60
70
80
90
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0

Qual2e – Calibration and Simulation Temperature and Dissolved Oxygen

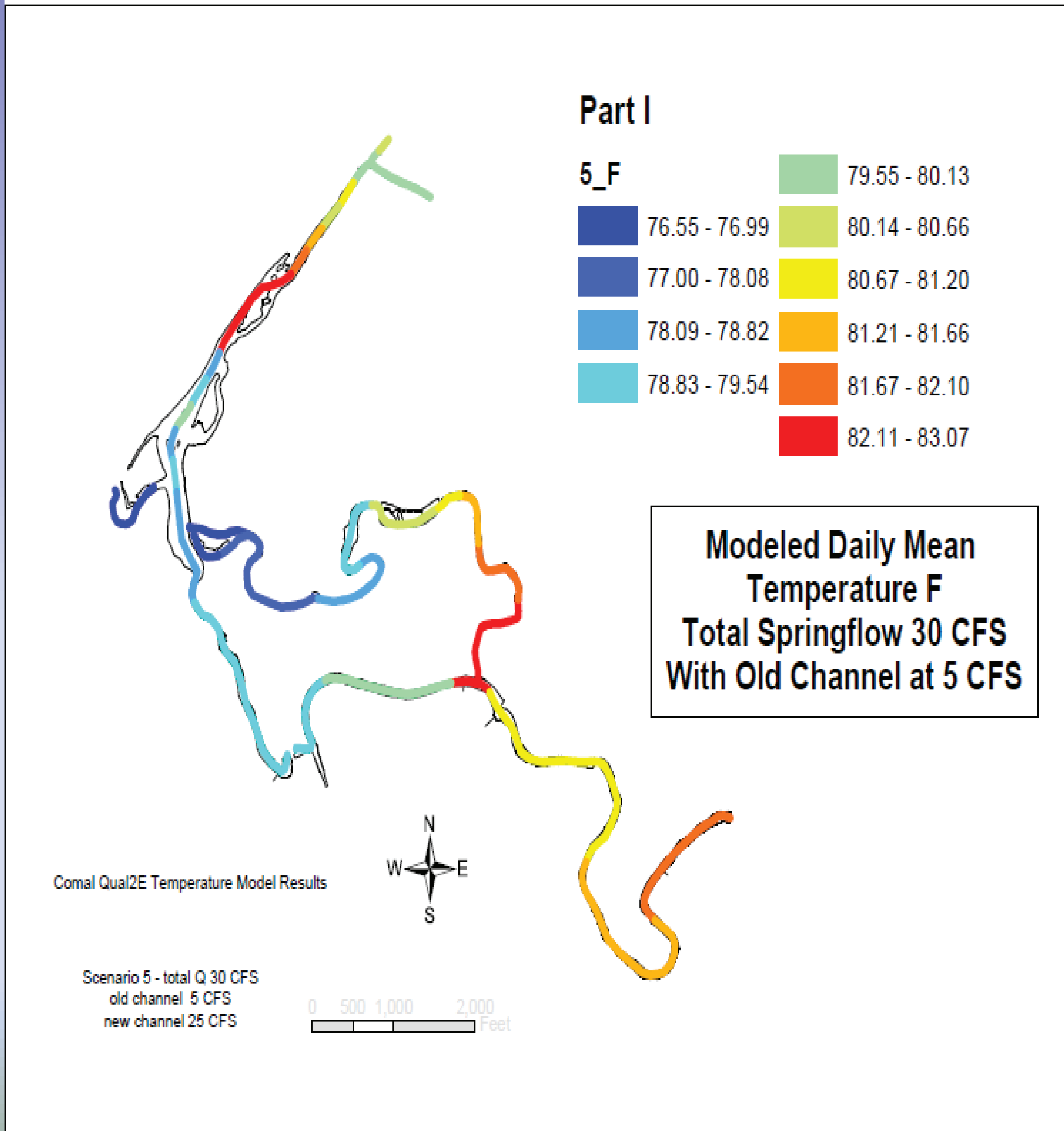


Calibrations

- Seasonal for Selected Years over the Simulation Period
  - Temperature
  - Dissolved Oxygen
- Mixed Data Temporal density available
  - Temperature
  - Dissolved Oxygen

Simulations

- Hourly Temperature
- Hourly Dissolved Oxygen
- Extract Average and Maximum Daily Temperature
- Extract Average and Minimum Dissolved Oxygen
- Associate Temperature and Dissolved Oxygen onto Computational Grid Locations
- Develop Simple Scenario Builder Interface for Management and Simulation of Qual2E for future management scenarios


Part I
5_F
76.55 - 76.99
77.00 - 78.08
78.09 - 78.82
78.83 - 79.54
79.55 - 80.13
80.14 - 80.66
80.67 - 81.20
81.21 - 81.66
81.67 - 82.10
82.11 - 83.07
Modeled Daily Mean Temperature F Total Springflow 30 CFS With Old Channel at 5 CFS
Comal Qual2E Temperature Model Results
N
W
E
S
Scenario 5 - total Q 30 CFS
old channel 5 CFS
new channel 25 CFS
0 500 1,000 2,000
Feet

Overview

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Study Overview

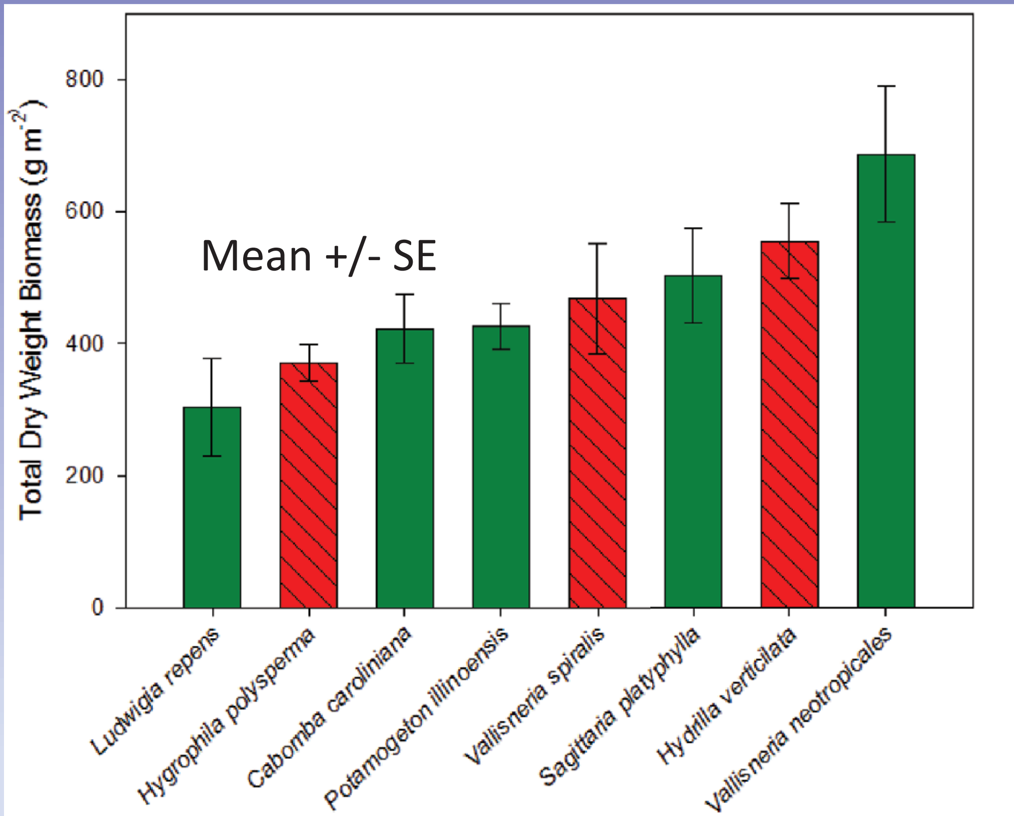
- 13 years of "cover" maps for both rivers- but no biomass data
- Most plant growth models have plant biomass as output parameter.

- Study Plan
  – Characterize % cover (experienced mappers)
  – Collect quantitative above and below ground dry biomass samples
  – Determine relationship between these variables
  – Determine "carrying capacity" for each species

Plants Selected

Table 1. Aquatic plant species studied with information about species origin (native/exotic) and DAFOR scale estimate of abundance.

Species	Native/Exotic	Abundance
Cabomba caroliniana	Native	occasional
Hygrophila polysperma	Exotic	abundant - dominant
Ludwigia repens	Native	occasional
Sagittaria platyphylla	Native	frequent
Vallisneria spiralis	Exotic	rare (SM), absent (Comal)
Hydrilla verticilata	Exotic	abundant (SM), rare (Comal)
Potamogeton illinoensis	Native	occasional (locally abundant)
Vallisneria neotropicalis	Native	absent (SM), abundant - dominant (Comal)



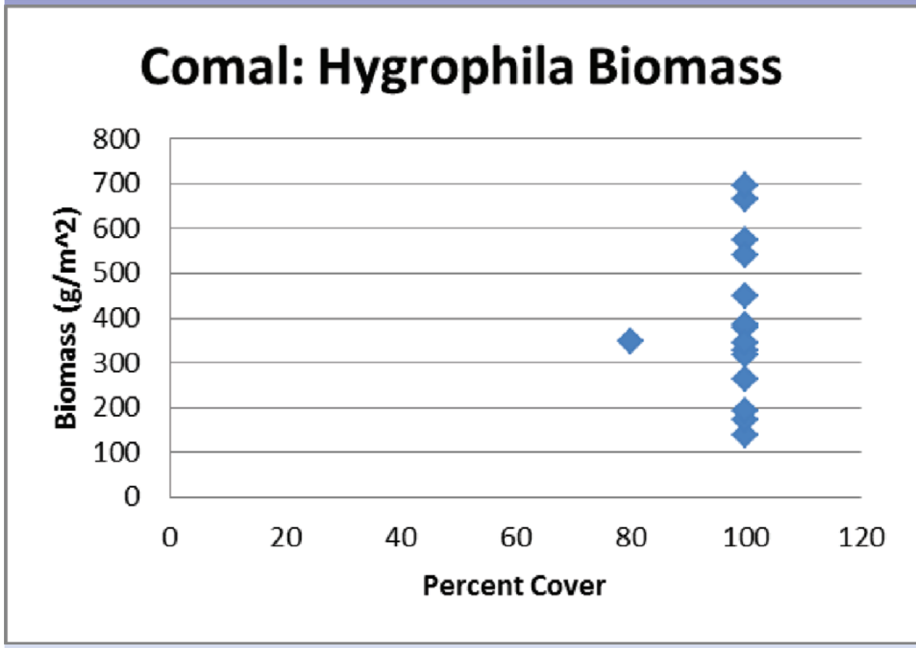
Carrying Capacity Samples

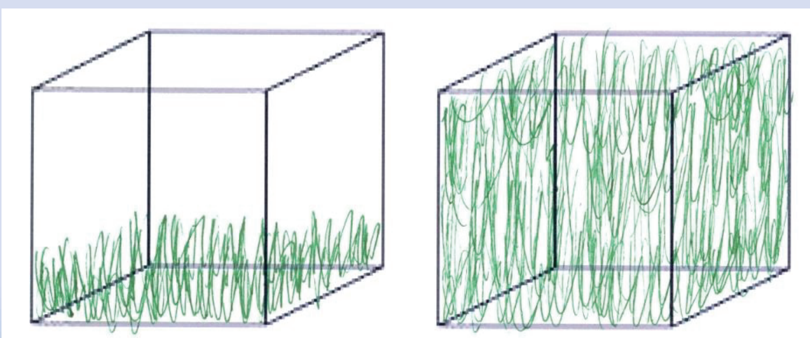
- Data for 3 exotic and 5 native species
- >2x variability in biomass for full cover samples

Table 2. Summary statistics for all samples of each species with 90-100% cover designation

		100% Cover Total Biomass (g/m^2)					Below Ground	
Species	Count	Mean	SE	Median	Min	Max	% BG	SE
Cabomba	7	422.6	51.7	499.2	207.9	558.1	17.3%	2.1%
Hygrophila	28	370.6	27.0	341.7	137.7	695.4	11.6%	1.2%
Ludwigia	8	303.0	74.6	201.8	157.8	757.1	27.6%	3.3%
Sagittaria	12	503.3	72.1	465.8	173.6	888.2	24.8%	1.3%
Comal Val	15	685.9	103.1	657.3	249.2	1939.2	23.7%	1.6%
Hydrilla	11	554.4	56.8	515.1	307.4	861.1	9.9%	1.3%
Potamogeton	11	426.2	35.2	458.1	236.7	575.4	33.2%	2.1%
SM Val	3	467.3	83.8	441.3	336.8	623.7	55.1%	2.5%

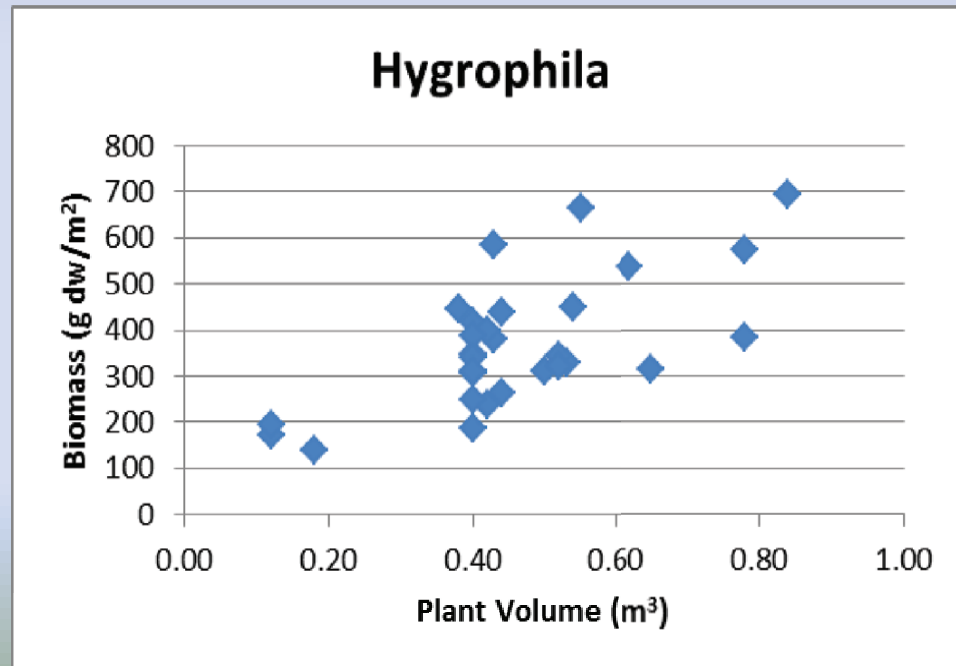
Comal 2014: Hygrophila





Same COVER

Different VOLUME



Biomass vs. Plant Volume

Table 3. Results of linear regression of total biomass vs plant volume

Species	p	r^2	n	slope	SE
Cabomba	<0.001	90.3	15	1228.1	107.8
Hygrophila	<0.001	92.4	29	772.7	41.8
Ludwigia	0.005	67.8	9	726.0	176.8
Sagittaria	<0.001	93.2	15	1327.3	96.3
Comal Val	<0.001	65.9	15	1432.3	275.3
Hydrilla	<0.001	84.5	11	825.2	111.9
Potamogeton	<0.001	93.3	11	1277.3	106.7
SM Val	Insufficient samples				

Robert Doyle, Ph.D.
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Ecomodel utility

- Provides way to estimate historic biomass levels for aquatic vegetation maps we have
- Provides data to allow model to know when a cell is at **Carrying Capacity** for a given species

Overview

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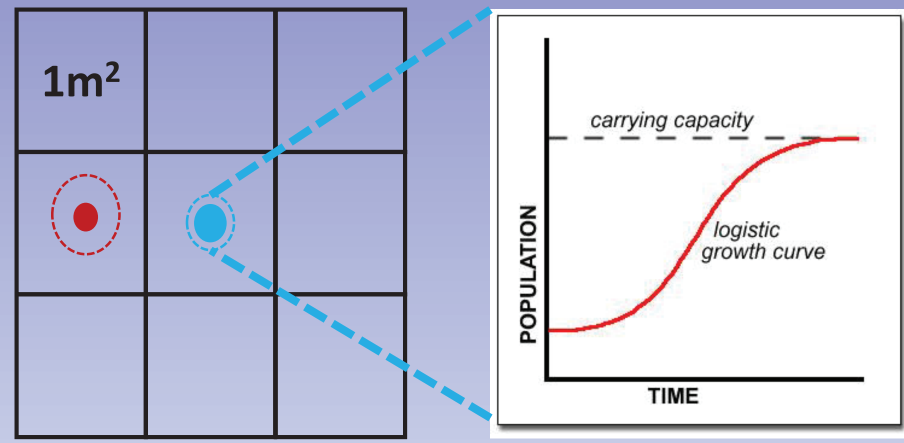
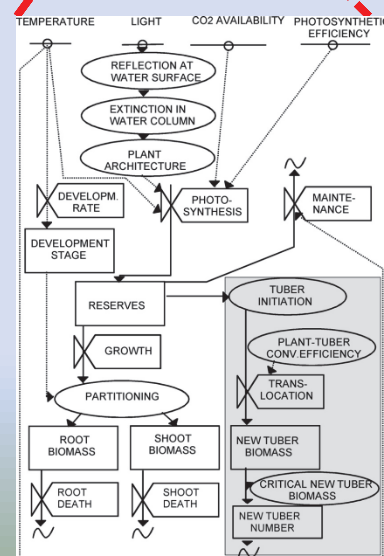
Growth Modeling

- Simplify growth models to capture critical components
  - Modeling on 1m^2 area. Growth calculated daily.
  - Add characteristics for structure, native/non-native to agent-class
  - Current data indicate darters are more often found in native species (e.g., *Ludwigia* vs *Hygrophila*, which are structurally similar)
  - Simulate intracell growth
    - *N* = biomass in cell *i*
    - *r* = intrinsic growth rate
    - κ = carrying capacity for each cell (going to try to link this to percent cover)



$$N_{i,t+1} = N_{i,t} + r_i \cdot N_{i,t} \cdot \left[1 - N_{i,t} \cdot \kappa^{-1}\right]$$



Growth modeling con't.

- The plant growth model is based on three existing approaches: MEGAPLANT (Scheffer et al 1993), Charisma, a spatially explicit update of MEGAPLANT (van Nes et al 2003), ERDC Models (Best and Boyd), Teh (2006)

$$\Delta W = W_s P - W(R_m + M) \quad (1)$$

$$R_m = r_{20} * Q_{10}^{((T-20)/10)} \quad (2)$$

$$P = P_{max} * \frac{I}{I+H_I} * \frac{S*T^{pt}}{T^{pt}+H_T^{pt}} * \frac{H_D}{D+H_D} \quad (3)$$

$$I_{z,t} = I_0 * e^{-K_d - K_p * b_z} \quad (4)$$

$$I_{z,t} = I_0 * e^{-K_d} \quad (5)$$

$$(T) = \frac{1.35 * T^3}{T^3 + 14^3} \quad (6)$$

$$N_j = \frac{a_s * B}{b_s} \quad (7)$$

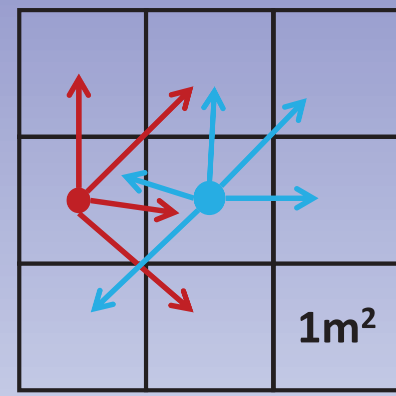
$$A'_L(t) = \frac{A_m \varepsilon k I_0 \exp\left\{-k\left[0.5(1+t)LAI\right]\right\}}{A_m + \varepsilon k I_0 \exp\left\{-k\left[0.5(1+t)LAI\right]\right\}} \quad (8)$$

Plant Growth Inputs

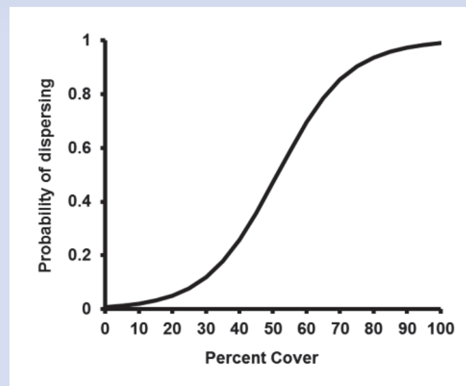
- Temperature, depth, velocity (hydraulic model)
- Solar Radiation (NASA database)
- Light attenuation coefficients (scientific literature)
- Species specific parameters (from scientific literature, and Baylor percent-cover study)
  - Plant structure, growth-related parameters, etc…
- Historic geographic distributions
- Turbidity may be included based on 2015 Applied Research Study

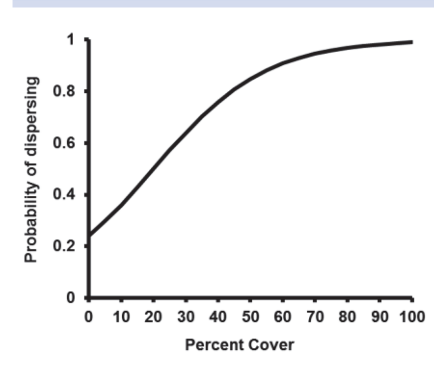
Dispersal Modeling

- What's the probability that a plant in cell *j* is colonized by plants from cell *i?*
- Dispersal calculated monthly.
- Following Wang et al 2010, 2012
  - Currently exploring different formulations for dispersal kernals for aquatic plants.
  - Process differs per species
  - Dispersal probabilities change with changes in percent cover, and species type (particularly invasive/native)
- Competition will be updated with results of 2015 Applied Research



: $N_{j,t+1} = k_{ji} \cdot N_{j,t}$





Dispersal Processes

Species	Seed	Fragments	Persistence	Other
Cabomba	N	Y	Medium	
Hydrilla	N	Y (high)	Medium	Tuber
Hygrophila	Y (maybe not viable)	Y (high)	Low	
Ludwigia	Y (mabye not viable)	Y (med)	Low	
Potamogeton	Y	Y (med)	High	
Sagittaria	Y	N	Very High	Tuber
Vallisneria	N	N	Very high	
Texas Wild Rice	Y	N	High	Tillers

Bryophytes are being modeled as probability of occurrence (in each cell)

Plant Growth Model Outputs

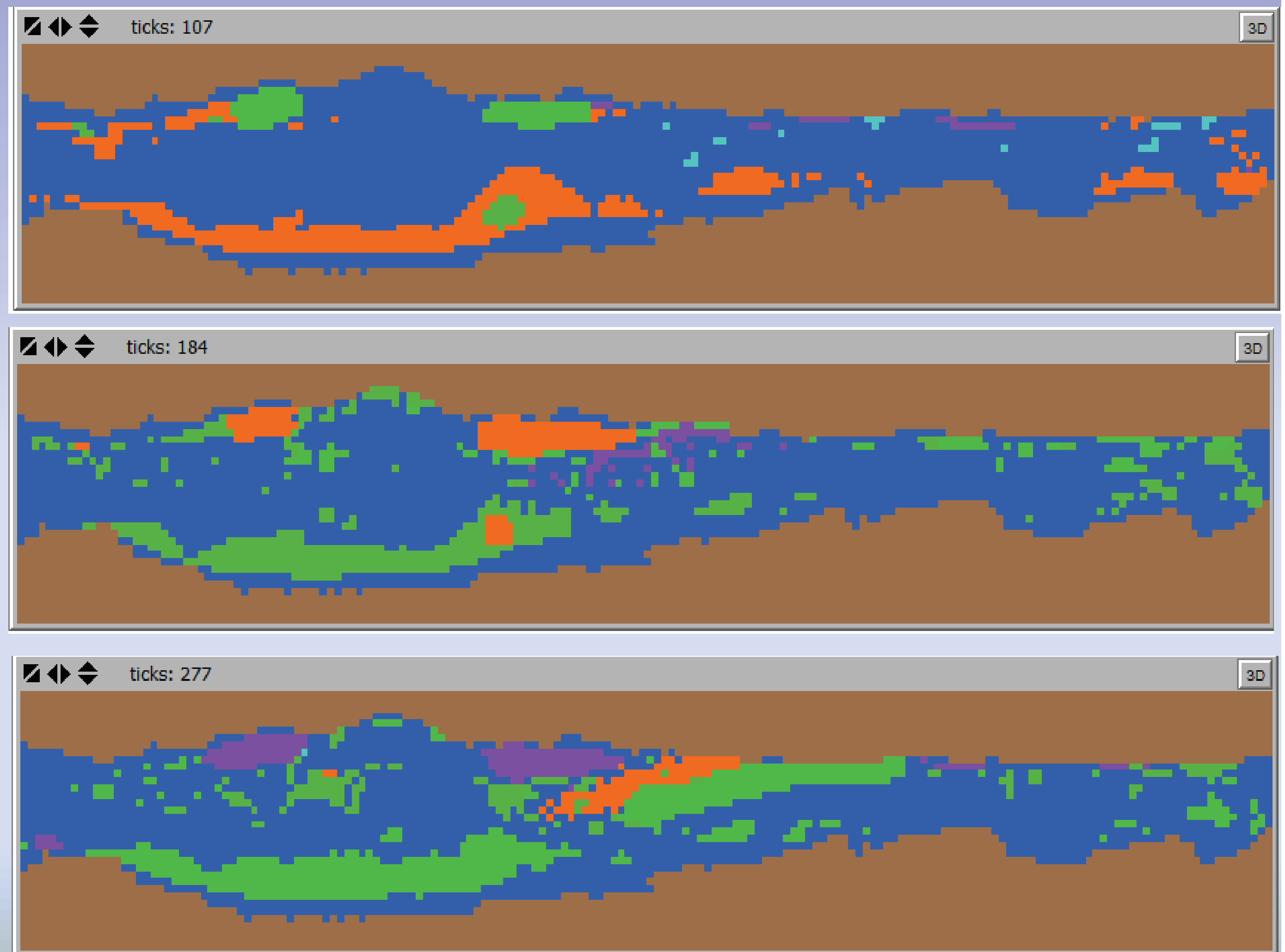
- Biomass (per species)
  - Above ground/Belowground(g)
- Percent cover
- Plant Height

Spatial & Dispersal Model Outputs

- Presence/Absence of Bryophytes (Algal dynamics might be included pending results of 2015 Applied Research Study)
- Probabilities of new cell being colonized
- Landscape coverages

Model Visualizations

Green: Algae
Orange: Ceratopteris
Lime: Hygrophila
Cyan: Ludwigia
Violet: Nuphar
Magenta: Bryophytes



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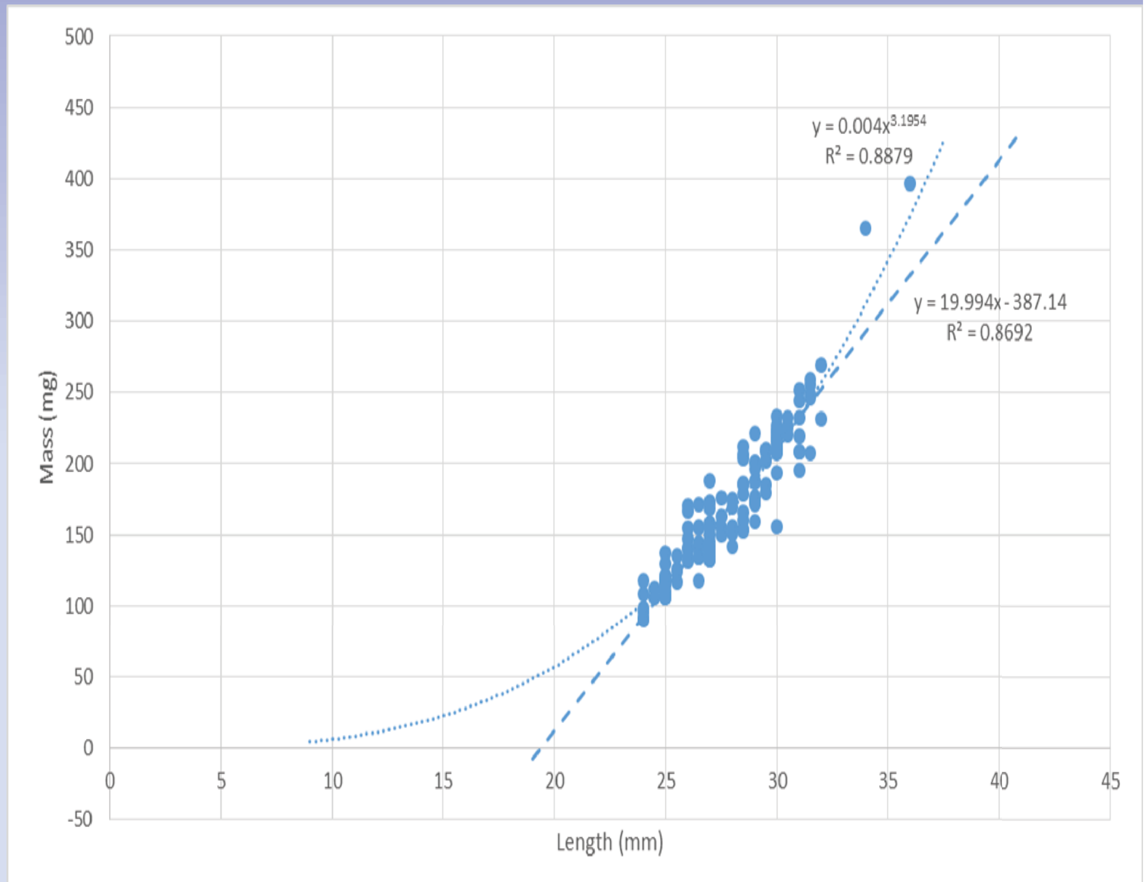
Fountain Darter Prey Consumption

- Estimated as 5% of fountain darter mass/day
- Fountain darter length and mass data from Fecundity Study (n = 417) examined for length by mass relationship
- Average length of fountain darters captured in bio-monitoring drop net samples (2001-2014) from each major vegetation type converted to average mass
- This value multiplied by 2014 density estimates for each habitat type/system to estimate biomass requirement from prey
- Maximum density observed substituted in previous equation for 2014 density to estimate maximum requirement



Fountain darter average length data (**Mean Length** (sd), N, *n*)

	Comal	San Marcos
Bryophytes	**23.53** (6.46), 150, *7929*	-
Cabomba	**25.24** (5.32), 87, *1760*	**24.47** (6.43), 79, *1247*
Hydrilla	-	**24.14** (7.16), 145, *2048*
Hygrophila	**23.80** (6.35), 267, *4403*	**24.99** (7.44), 149, *1534*
Ludwigia	**24.88** (5.94), 132, *3547*	**23.44** (6.88), 2, *9*
Sagittaria	**24.59** (5.35), 49, *1001*	**23.86** (6.41), 12, *100*
Vallisneria	**24.55** (6.13), *986* , 62	**26.93** (6.66), 8, *215*

Hyalella azteca

- Most abundant invertebrate in all samples
- Previous studies have demonstrated importance in fountain darter diets (Shenck and Whiteside 1977, Bergin 1996)
- Collected length data from Comal (n = 69) and SM (n = 77)
- Used average length and length-mass relationship of Bencke et al. 1999 to estimate average mass
- Average mass * average density = estimate of amphipod prey available

Results

		BRY (150,-)	CAB (87, 79)	HYD (-, 145)	HYG (267,149)	LUD (132, 2)	SAG (49, 12)	VAL (62, 8)
Comal	Amphipod biomass	5384.5 (13)	1817.05 (12)		1392.04 (18)	5812.9 (14)	1312.59 (14)	284.068 (4)
	Biomass required	131.845 (**2.44%**)	57.425 (**3.16%**)		38.06 (**2.73%**)	80.82 (**1.39%**)	30.025 (**2.28%**)	30.99 (**10.9%**)
	Max required	463.63 (8.6%)	235.75 (13%)		180.28 (13%)	424.35 (7.3%)	75.06 (5.7 %)	171.56 (60%)
San Marcos	Amphipod biomass		4770.96 (8)	6922.0 (12)	1957.76 (12)		4842.7 (8)	4173.87 (4)
	Biomass required		43.76 (**0.92%**)	34.07 (**0.49%**)	29.27 (**1.5%**)		19.19 (**0.4%**)	103.33 (**2.47%**)
	Max required		144.95 (3%)	631.58 (9.1%)	181.48 (9.35)		60.6 (1.25%)	271.34 (6.5%)

Food Availability Conclusion

- Overall, abundance *H. azteca* (one of many potential prey species present) far exceeds needs of fountain darters
- Data collected from within natural systems, so predation etc. are likely captured in the data
- Turnover rates for invertebrates range from approximately 2.5-5 with a mode of 3.5 (Waters 1969)
- *H. azteca* have been shown to have a turnover rate of 3.3% per day (in a different environment) and a potential rate in laboratory experiments of 4% per day (Cooper 1965)
- Invertebrate food availability is not likely to be limiting for fountain darters

Food Source References

- Benke, A., Huryn, A., Smock, L., & Wallace, J. (1999). Length-mass relationships for freshwater macroinvertebrates in North America with particular reference to the Southeastern United States. Journal of the North American Benthological Society, 18(3), 308-343.
- Bergin, S. J. (1996). Diet of the fountain darter *Etheostoma fonticola* in the Comal River, Texas. Thesis. San Marcos, Texas: Texas State University.
- Cooper, W. E. (1965). Dynamics and production of a natural population of a fresh-water amphipod, *Hyalella azteca*. Ecological Monographs, 35(4), 377-394.
- Waters, T. F. (1969). The turnover ratio in production ecology of freshwater invertebrates. The American Naturalist, 103(930), 172-185.

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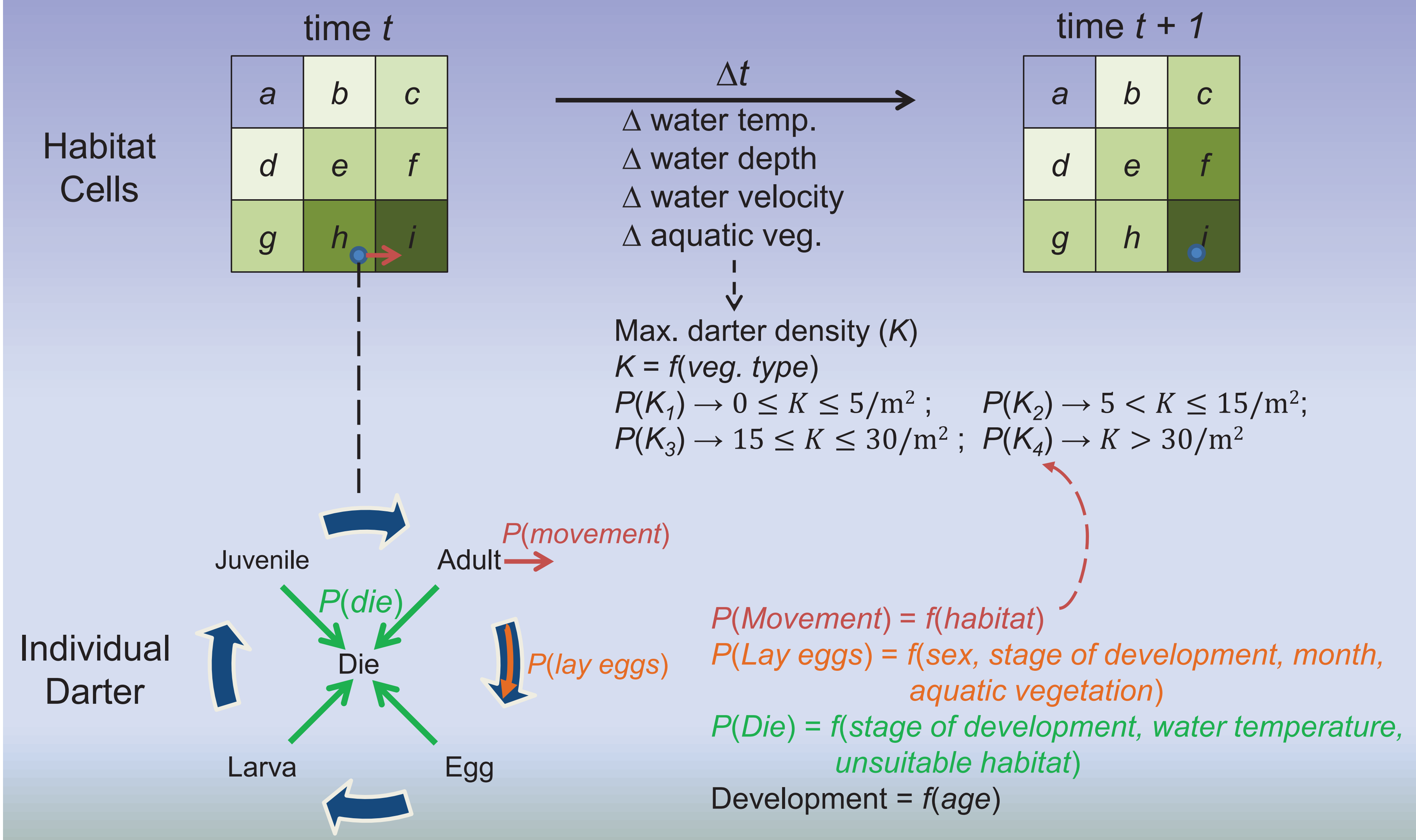
Overview of model



Sequence of model calculations

1. Initialization of habitat cells and fountain darters

2. Input
   - 2.1 Input time series of vegetation types
   - 2.2 Input time series of water temperatures
   - 2.3 Input time series of water depths and water velocities

3. Submodels
   - 3.1 Adjust vegetation type and calculate maximum fountain darter density (seasonally)
   - 3.2 Adjust water depth and water velocity (daily)
   - 3.3 Adjust water temperature (daily)
   - 3.4 Adjust fountain darter age and developmental stage (daily)
   - 3.5 Calculate fountain darter mortality (daily)
   - 3.6 Calculate fountain darter movement (hourly)
   - 3.7 Calculate fountain darter egg laying (recruitment) (daily)
   - 3.8 Update aggregated (output) variables (daily)

Fountain darter reproduction and mortality

Probability of having eggs (this month)	Probability of egg laying (daily)
Jan.: 0.1714	0.1855
Feb.: 0.2220	0.2405
Mar.: 0.2955	0.3198
Apr.: 0..0571	0.0618
May: 0.0976	0.1056
Jun.:0.1500	0.1624
Jul.: 0.1087	0.1176
Aug.: 0.0208	0.0225
Sep.: 0.0000	0.0000
Oct.: 0.0732	0.0792
Nov.: 0.1500*	0.1624*
Dec. 0.1500*	0.1624*

♀ Stage ♂ (age-days)	Probability of dying (daily)
Egg (0-5)	$0.03 + f(T)$
Larva (6-65)	$0.031 + f(T)$
Juvenile (66-185)	$0.00149 \times f(T)$
♀ Young adult ♂ 42% (186-735) 58%	$0.00149 \times f(T)$
♀ Old adult ♂ (736-1100)	$0.00545 \times f(T)$
♀ Death old age ♂ (1101)	1.0

Egg:

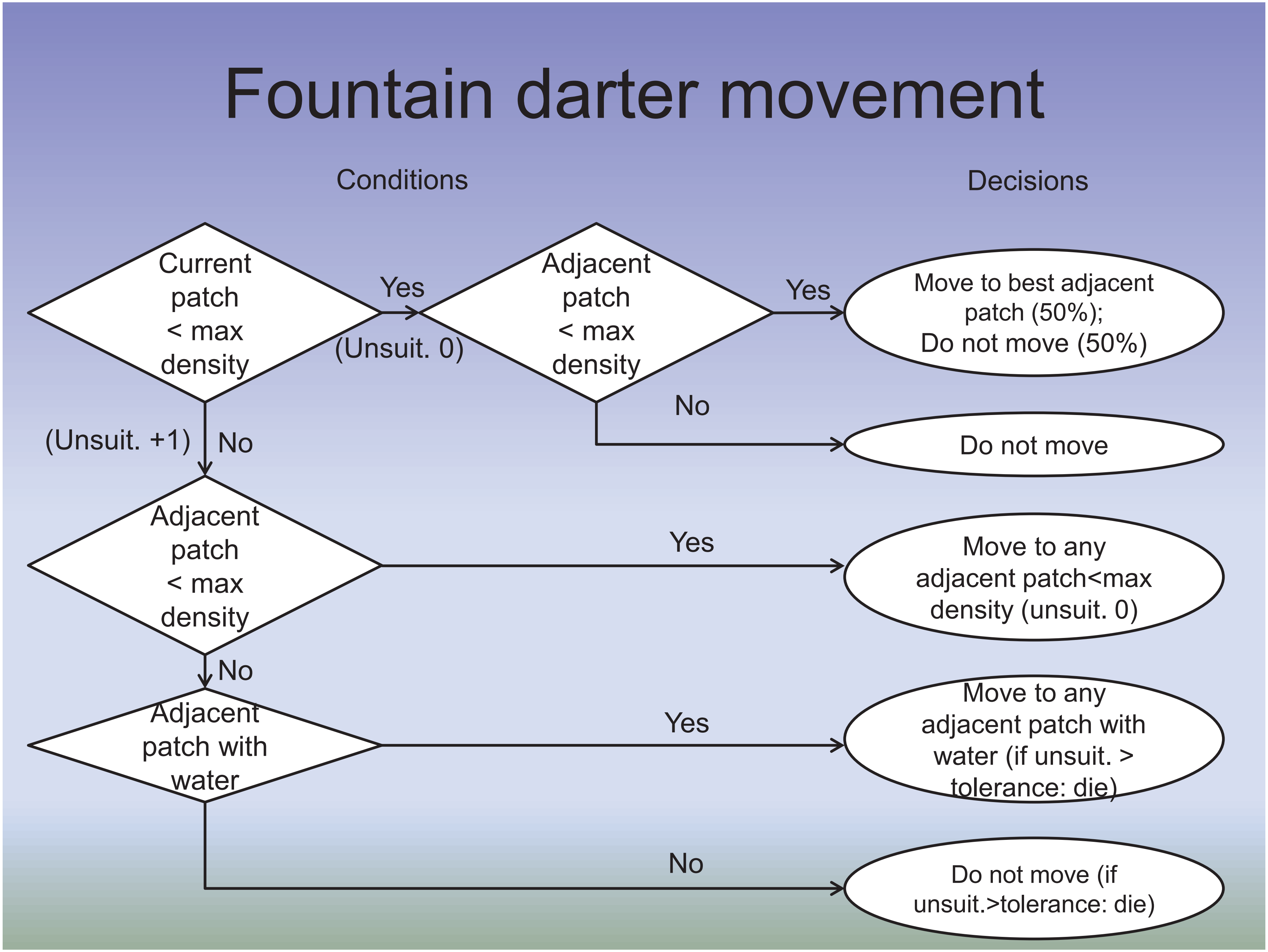
$T \leq 23C$: $f(T) = 0.025$
$23C < T \leq 27C$: $f(T) = -0.6075+0.0275^{*}T$
$T > 27C$: $f(T) = 0.135$

Larva:

$T \leq 22C$: $f(T) = 1/[1+\exp(-7.31+5.43\times\ln(T))]$
$T > 22C$: $f(T) = 1/[1+\exp(310.96-89.83\times\ln(T))]$

Juvenile and young adult:

$T \leq 0C$: $f(T) = 3$
$0C < T \leq 8C$: $f(T) = 3-0.025\times T$
$8C < T \leq 22C$: $f(T) = 1$
$22C < T \leq 30C$: $f(T) = -4.5+0.25\times T$
$T > 30C$: $f(T) = 3$



Fountain darter movement
Conditions
Decisions
Current patch < max density
Yes
(Unsuit. 0)
Adjacent patch < max density
Yes
Move to best adjacent patch (50%); Do not move (50%)
No
Do not move
(Unsuit. +1)
No
Adjacent patch < max density
Yes
Move to any adjacent patch<max density (unsuit. 0)
No
Adjacent patch with water
Yes
Move to any adjacent patch with water (if unsuit. > tolerance: die)
No
Do not move (if unsuit.>tolerance: die)

Data

- EAA Biological Monitoring: Dropnet data.
- Time frame: 2000-2014.
- Spatial coverage: 4 reaches in Comal Spring and 3 reaches in San Marcos Spring.

Objective

- Understand and estimate the relationship between the density of fountain darters and available environmental variables.
- Quantify the above relationship to assist with the development of fountain darter dynamics model (e.g., estimation of carrying capacity and development of movement rules)

Data organization

- We have merged and organized the EAA biological monitoring data.
- Density of fountain darter (#/m^2).
- We have selected several micro-environmental variables which are believed to affect the density of fountain darters: coverage of Cabomba (%), Hydrilla (%), Hygrophila (%), POT_HYG (%), Potamogeton (%), Sagittaria (%), Vallisneria (%), MainVegPer (%), MainVegHeight (ft), WithBryo (Yes/No), WaterDepthFt, Velocity, Temp, DO, SpCond, and pH.
- We have selected and/or calculated several reach-based macro-environmental variables: Flow, T_GreenAlgae (%), T_Cabomba (%), T_Hydrilla (%), T_Hygrophila (%), T_Ludwigia (%), T_Potamogeton (%), T_Sagittaria (%), T_Vallisneria (%), T_Zizania (%), T_Open (%).

Progress: Linear regression

- Dependent variable: density of fountain darters
- Independent variables: all environmental variables.
- $y_i = \beta_1 x_{i1} + \cdots + \beta_p x_{ip} + \epsilon_i$
- Results: $R^2 < 0.4$
- What next?
  - ➢ Exploring Generalized Linear Models (GLMs)

Progress: Two-Level Hierarchical Logit Model

- Purpose: accounting for the influence of micro- and macro-environments on fountain darter density

Micro-environmental factors **Macro-environmental factors**

$$p(j) = \frac{e^{V_j/\theta_k} \cdot \left(\sum_{i \in C_k} e^{V_i/\theta_k}\right)^{\delta_k - 1}}{\sum_{k'} \left(\sum_{i \in C_{k'}} e^{V_i/\theta_{k'}}\right)^{\delta_{k'}}}$$

- Dependent variable: Density category - 1 (no fountain darters found; 343 observations), 2 (low; from 1 fountain darter to 0.5 SD below the mean; 542 obs.), 3 (fair; 0.5 SD either side of the mean; 563 obs.), 4 (high; 0.5 to 1.5 SD above the mean; 132 obs.), and 5 (very high; greater than 1.5 SD above the mean; 92 obs.)

Progress: Two-Level Hierarchical Logit Model

- Flaw: all macro-environmental factors in the same reach have the same values which results in under- or overestimation of the interpretative power of each significant macro-environmental factor.
- What next?
  - ➢ Exclude macro-environmental factors
  - ➢ Either combine data from the two springs to run “a” model or separate data to run a separate model for each spring? Defining categories?
    - ✓ After consulting with Dr. Michael Longnecker (Dept. of Statistics, Texas A&M University), we decided to run a separate model for each spring, with density categories for each based on expert opinion.

Multinomial Logit Model

- $P(Y_i = K) = \frac{exp(\alpha_K + \beta_K X_i)}{c_i}$, where *K* = 2, 3, or 4 (1)

  $P(Y_i = K) = \frac{1}{c_i}$, where *K* = 1 (2)

  and where

  $c_i = 1 + \sum_{K=2}^{4}[exp(\alpha_K + \beta_K X_i)]$ (3)
- Check multicollinearity: VIF (variance inflation factor) and correlation matrix
- Model selection criteria: AIC (Akaike information criterion)
- Model evaluation: AUC (area under the ROC curve; ROC: receiver operating characteristic)

Multinomial Logit Model

- Dependent variable for Comal Spring: Density (*D*) category - 1 ($0 \leq D \leq 5$; 393 observations), 2 ($5 < D \leq 15$; 204 obs.), 3 ($15 < D \leq 30$; 124 obs.), and 4 ($D > 30$; 74 obs.).
- Dependent variable for San Marcos Spring: Density (*D*) category - 1 ($0 \leq D \leq 2$; 131 observations), 2 ($2 < D \leq 8$; 151 obs.), and 3 ($8 < D \leq 15$; 58 obs.).

Multinomial Logit Model

	Comal Spring	San Marcos Spring
VIF	< 10 (no multicollinearity)	< 10 (no multicollinearity)
Correlation matrix	< 0.5 (no correlation)	< 0.5 (no correlation)
AIC	1583.038 (smallest value)	863.538 (smallest value)
AUC*	0.7972 (model reliability and validity: good)	0.7510 (model reliability and validity: fair)

* We evaluated the reliability and validity of our models as fair (0.50 < AUC = 0.75), good (0.75 < AUC = 0.92), very good (0.92 < AUC = 0.97), or excellent (0.97 < AUC = 1.00) based on the value of AUC .

Multinomial Logit Model

Comal Spring	Overall p-value	2	3	4
Constant	-	7.7584	9.5793	2.1008
Bryophytes	<.0001	4.3455	4.2244	9.8051
Cabomba	<.0001	4.4947	3.3249	8.6736
Ceratopteris	0.0329	3.4015	1.8596	-5.4335
FilamentousAlgae	<.0001	6.0201	4.7125	12.1659
Hygrophila	<.0001	3.5061	2.8677	6.6600
Ludwigia	<.0001	3.9065	3.8657	8.1887
Sagittaria	0.0126	2.2736	1.2025	5.7577
Vallisneria	0.0005	3.0407	1.2385	6.8683
WithBryo	<.0001	1.8536	1.935	2.8385
WaterDepthFt	0.0326	-0.3647	-0.3881	-0.0018
SpCond	0.0483	-0.0018	-0.0009	0.00022
pH	<.0001	-1.4116	-1.7139	-1.6568

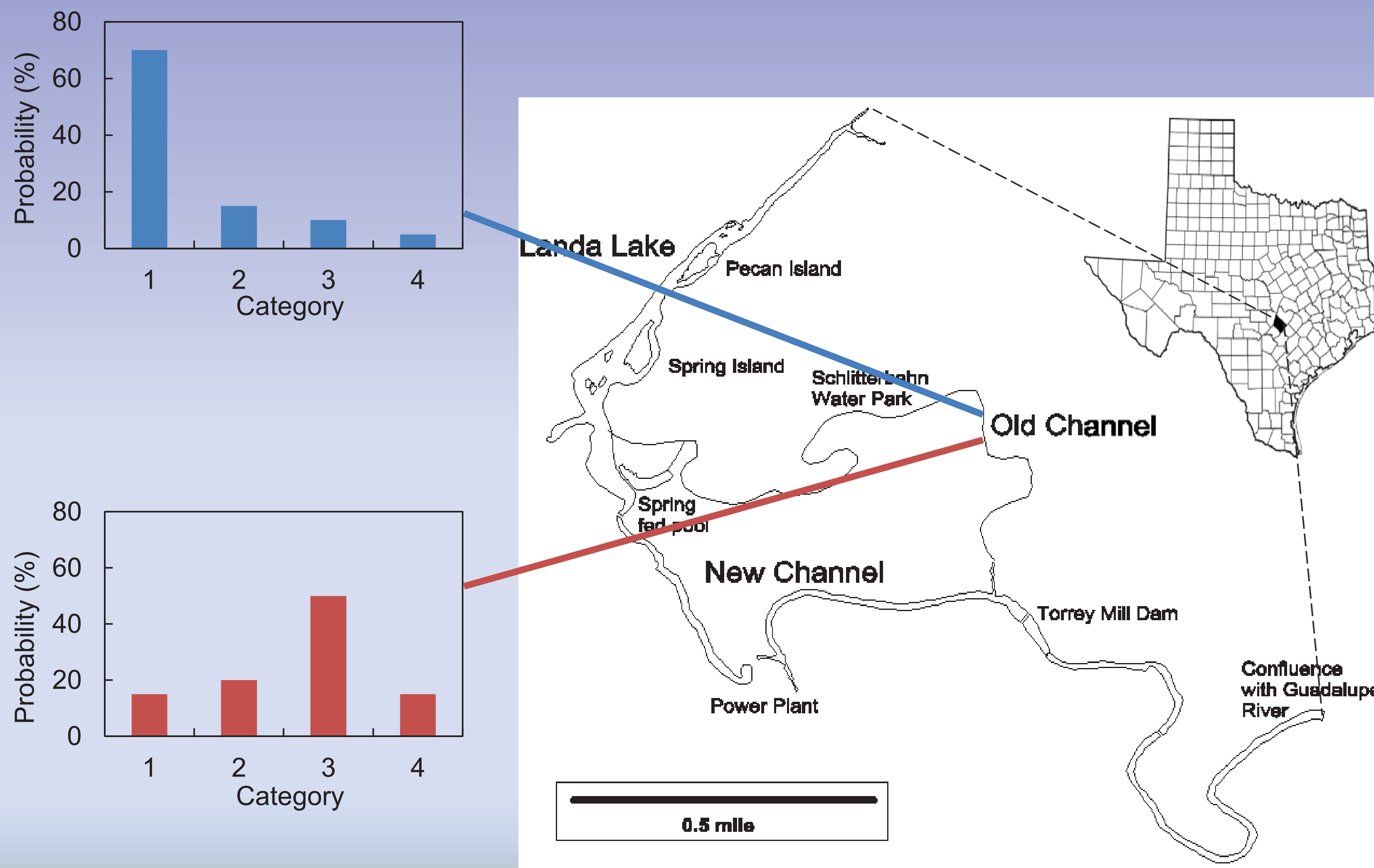
San Marcos Spring	Overall p-value	2	3
Constant	-	-10.171	-26.605
Cabomba	0.0048	3.3529	3.4414
Hydrilla	0.0881	2.2244	1.8748
Hygrophila	0.0069	2.9458	1.6812
POT_HYG	0.0094	2.7433	2.0642
MainVegPer	0.0827	-0.0058	0.0559
WaterDepthFt	0.1403	-0.1848	0.2231
Velocity	0.041	-3.0728	-4.7604
Temp	0.0244	0.3914	0.5201
SpCond	0.0219	-0.0038	-0.0032
pH	0.0586	0.3487	1.1541

Multinomial Logit Model

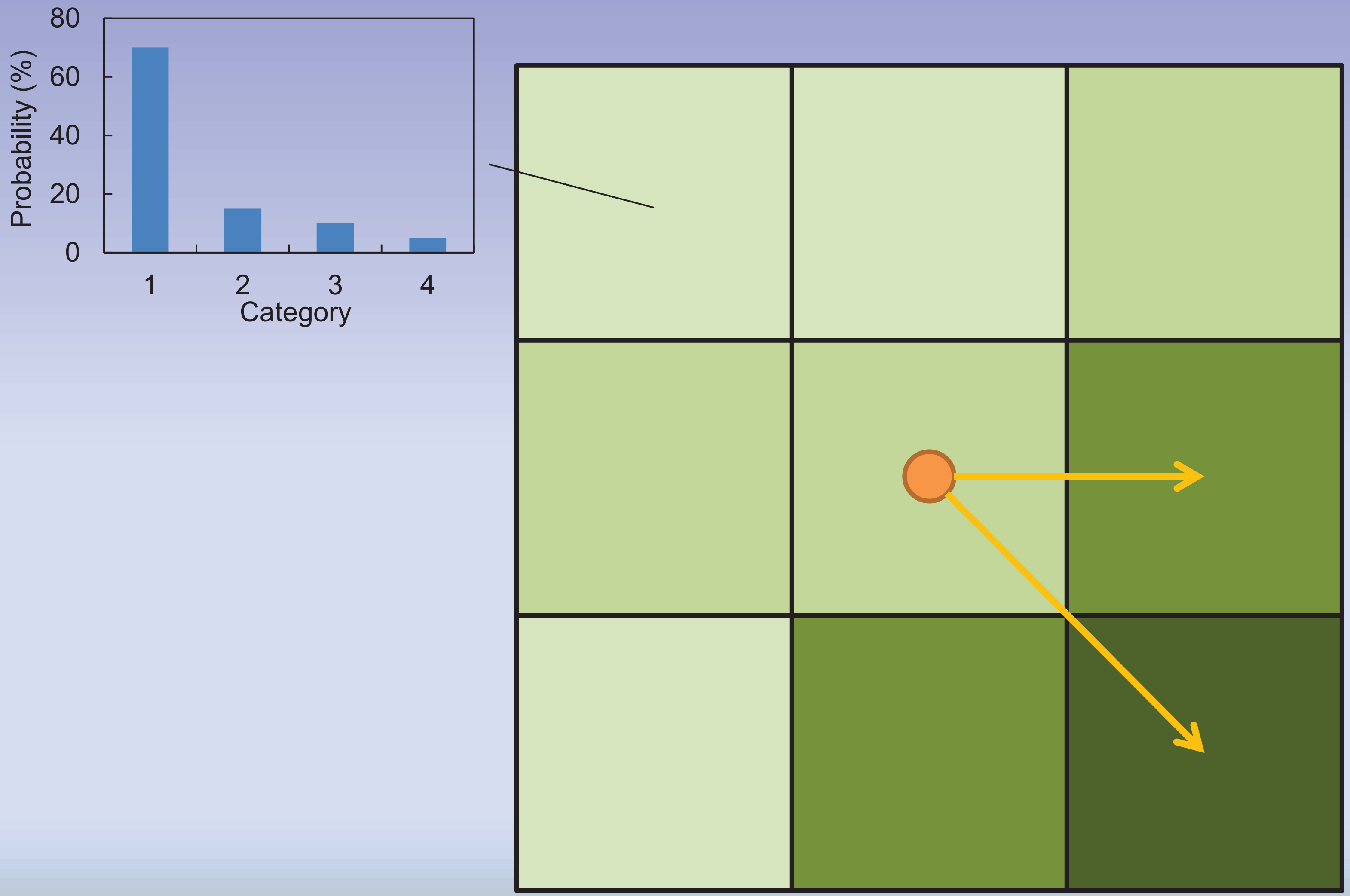
- Comal Spring (model with only vegetation variables)

	Overall p-value	2	3	4
Constant	-	-2.527	-3.273	-8.534
Bryophytes	<.0001	4.094	4.8522	10.562
Cabomba	<.0001	2.3687	1.9795	6.5969
FilamentousAlgae	<.0001	3.5954	3.1458	9.6475
Hygrophila	0.0002	1.7462	1.9114	5.3002
Ludwigia	<.0001	2.216	3.0779	7.2021
Sagittaria	0.0202	1.0847	0.8749	5.2995
Vallisneria	0.0032	1.4683	0.4913	5.7802

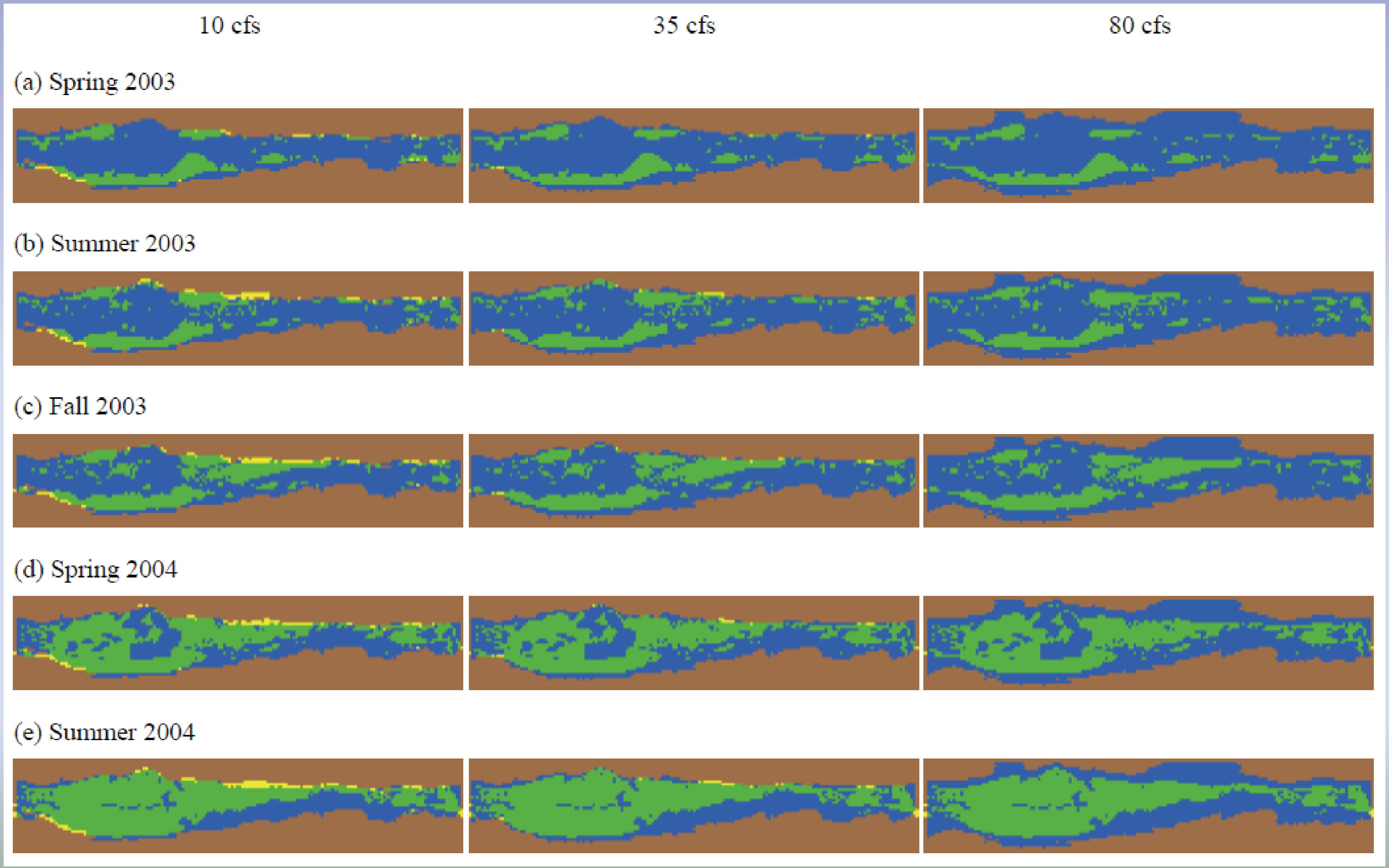
Application



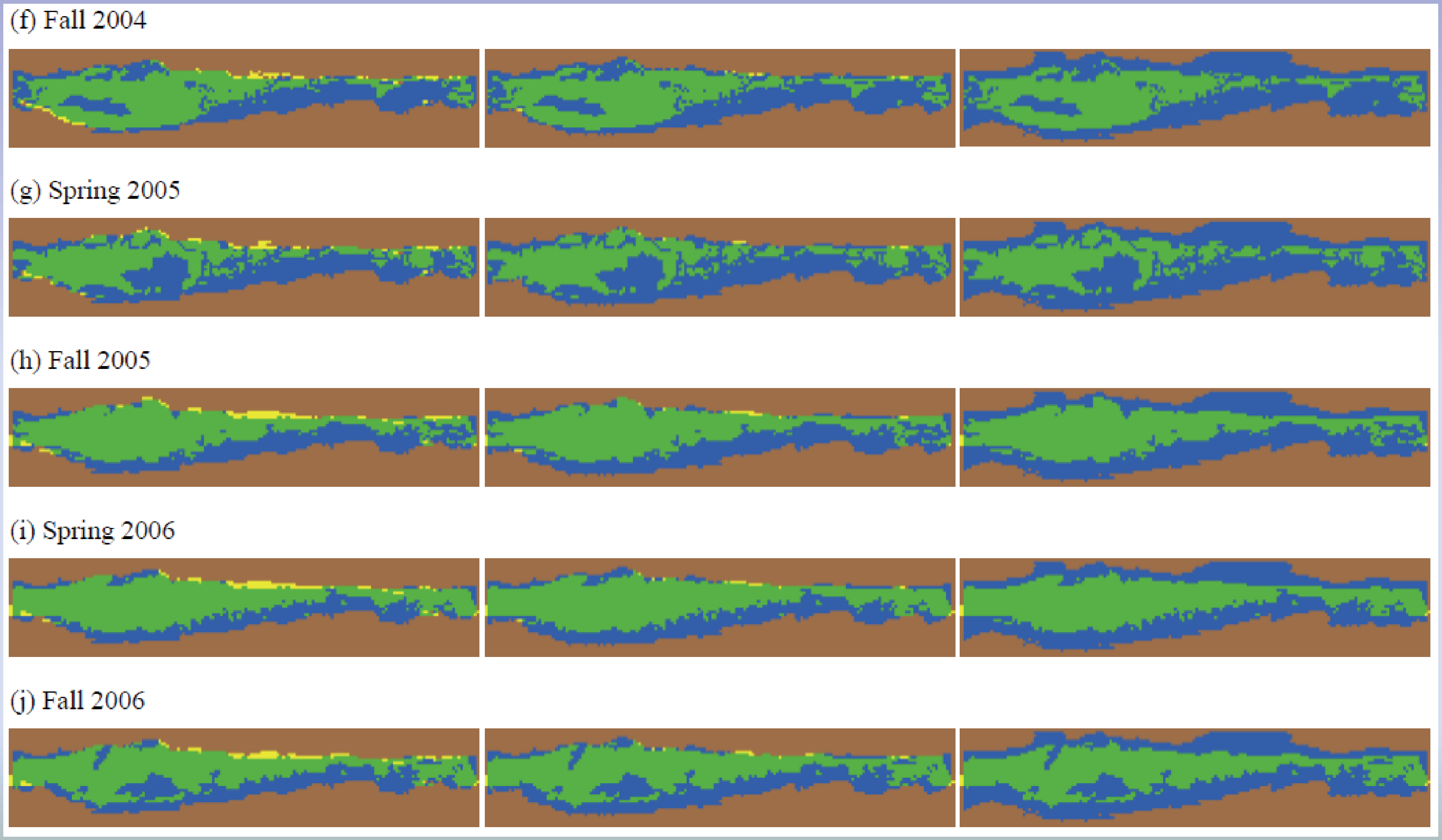
Application



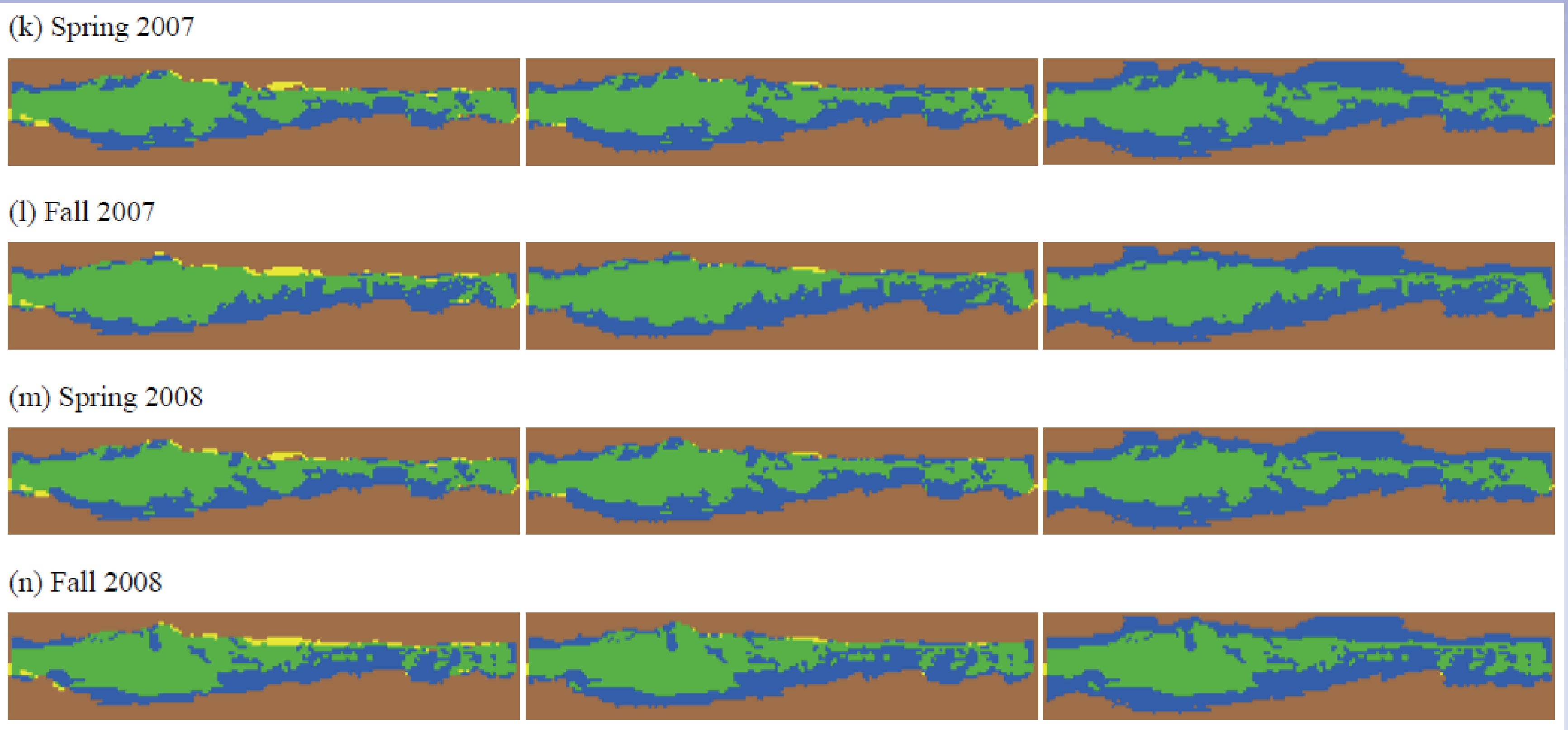
Simulated spatial-temporal distribution of aquatic vegetation



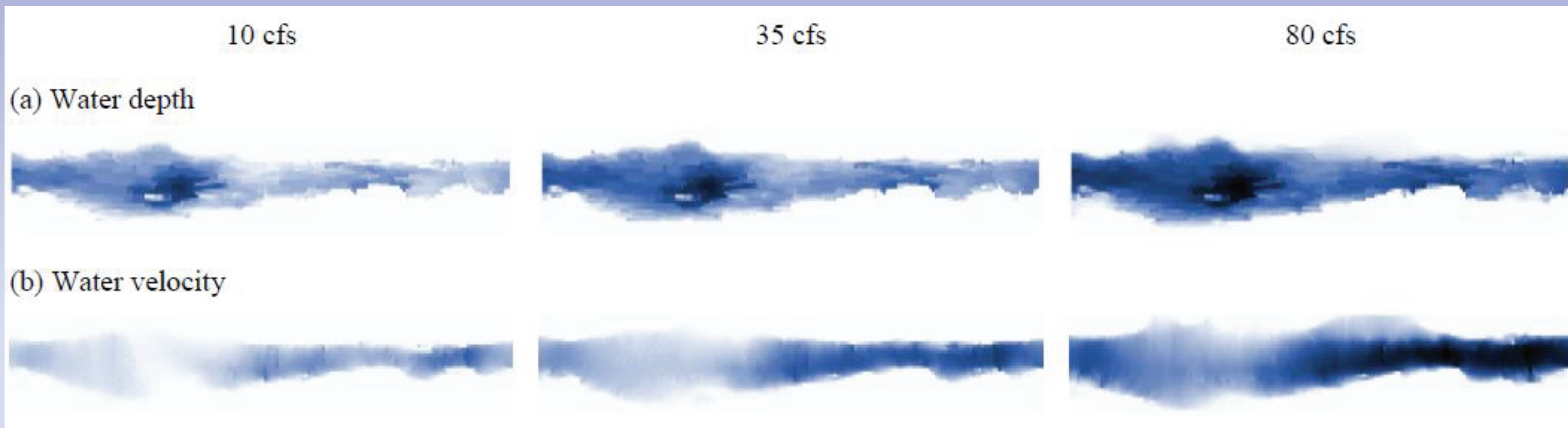
Simulated spatial-temporal distribution of aquatic vegetation



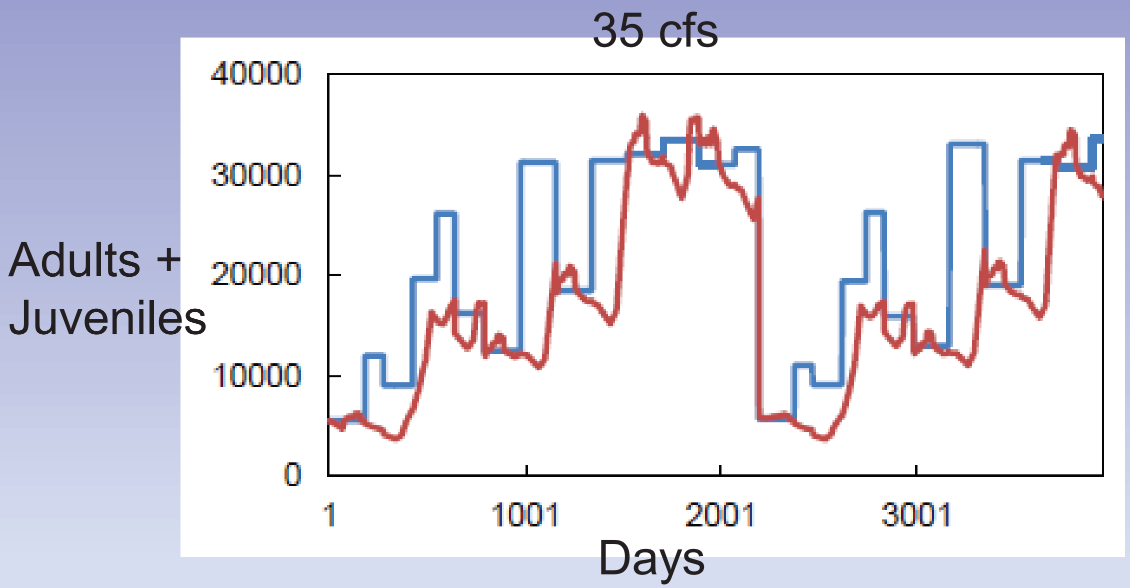
Simulated spatial-temporal distribution of aquatic vegetation



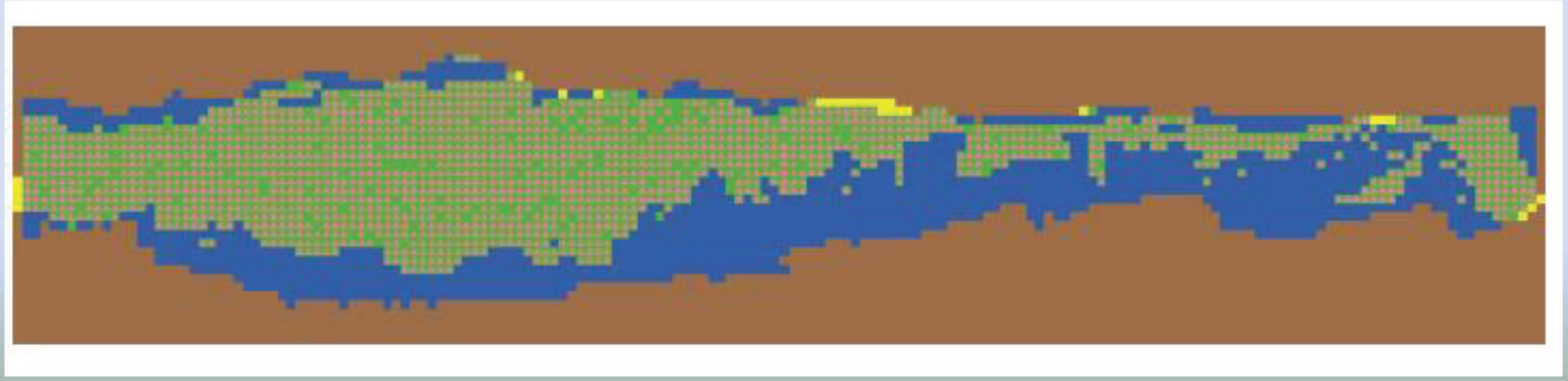
Simulated spatial-temporal distribution of water depth and water velocity



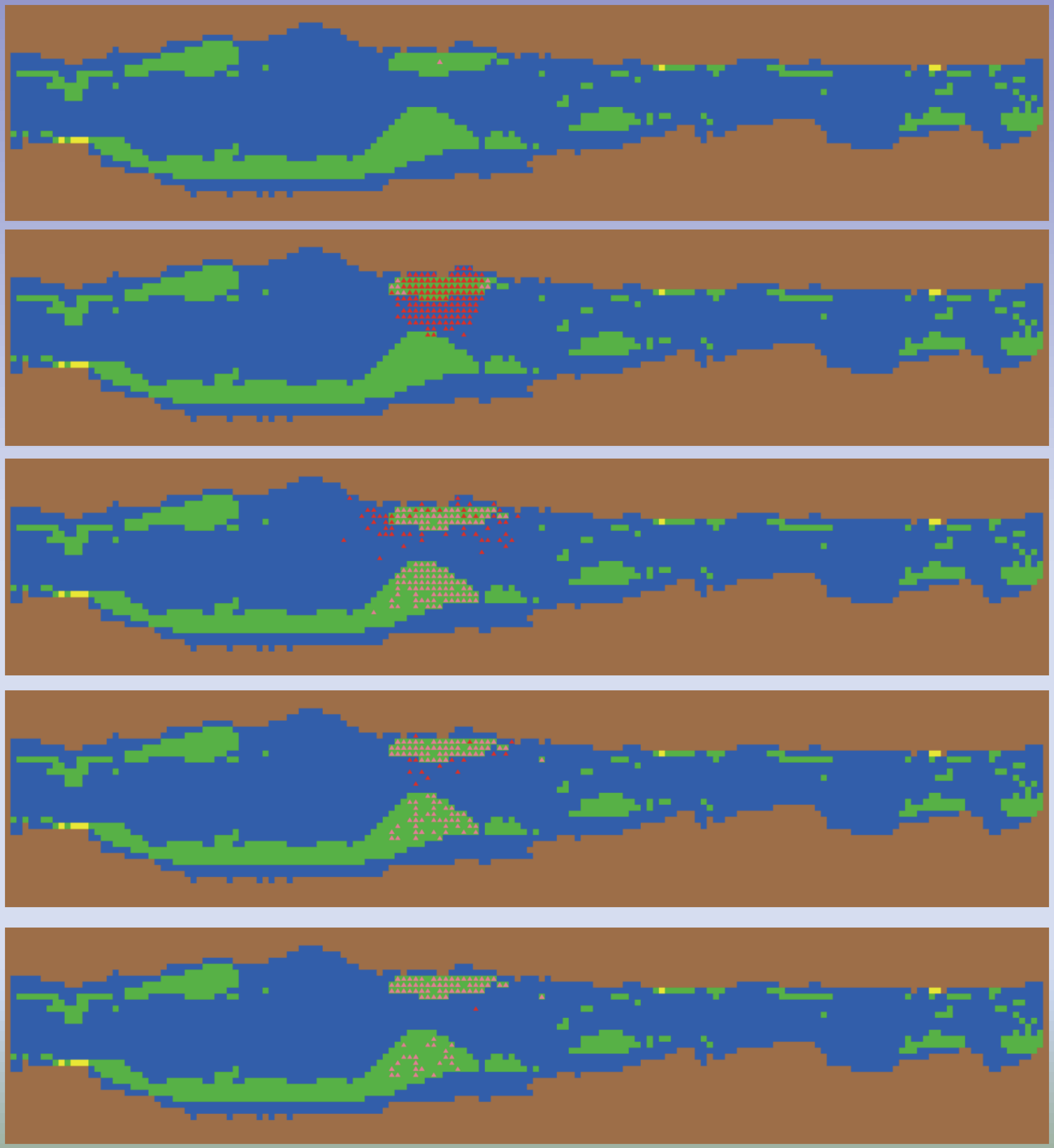
Simulated fountain darter population trends



Simulated fountain darter distribution



Simulated fountain darter movements



Overview

- Agent Based Model (Swannack)
- Hydraulic and Water Quality modeling (Hardy)
- Percent Cover to Biomass study (Doyle)
- Aquatic Vegetation Modeling (Swannack)
- Food Source Analysis (Jackson)
- Fountain Darter Modeling (Wang and Grant)
- Overview of Key Decisions (Ward)
- Schedule and Next Steps (Oborny)

Key Decisions

- Agent Based Model
- 1 meter grids
- Hydrology – Comal System
  - gages not available on all segments over time – in particular the Old Channel
- Water Quality Model – Qual-2E
  - Using flow contributions from the EARIP
  - Not modeling nutrients or carbon dioxide
  - Using maximum temperature and minimum dissolved oxygen
- Aquatic Vegetation Modeling
  - Percent Cover an appropriate surrogate for biomass
  - Chose a subset of SAV species based on fountain darter statistical analysis
  - Species specific propagation methods and mathematical expression
  - Not including herbivory
- Fountain Darter Modeling
  - Food availability not limited – no macroinvertebrate sub-model
  - Not using Macro scale in fountain darter analysis

Key Upcoming Decisions

- Spatial expansion of model
  - Other intensive reaches for Comal and San Marcos?
  - Entire rivers?
- Period of simulation
- Application of scour / recolonization
  - Statistically generated?
- Uncertainty Analysis
- Scenario Development
  - In the future

Schedule and Next Steps

- March 2015
  - Submit Year 3 scope of work for approval
- Summer 2015
  - Complete integration / linkage of water quality, aquatic vegetation and fountain darter models.
  - Complete – calibrated models
    - Old Channel reach of the Comal River
    - City Park reach of the San Marcos River
- December 2015
  - Submit annual model status report
- Summer 2016
  - Complete spatial expansion modeling in both systems
  - Final Model validation
- December 2016
  - Complete users manual and submit final working models to EARIP

Questions / Comments

- FEEDBACK WELCOME
  - March 11th

Appendix K13

Predictive Ecological Model for the Comal and San Marcos Ecosystems
Project Interim Report

PREDICTIVE ECOLOGICAL MODEL FOR THE COMAL AND SAN MARCOS ECOSYSTEMS PROJECT

Edwards Aquifer Habitat Conservation Plan

Contract No. 13-637-HCP

INTERIM REPORT

Prepared for

Edwards Aquifer Authority

Prepared by

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Dr. Timothy Bonner (Texas State University)
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Dr. William Grant (Texas A&M University)
Dr. Thom Hardy (Watershed Systems Group)
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Dr. George Ward (University of Texas Austin)

December 31, 2015

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EXECUTIVE SUMMARY

Since 2013, a team of scientists and engineers have been engaged in developing a predictive ecological model for use in management decisions regarding factors affecting the San Marcos and Comal Rivers, notably the magnitudes and time variations in spring flows to these systems as specified objectives of the Edwards Aquifer Habitat Conservation Plan (HCP) Phase 1 implementation. This document reports progress and presents status of the model development project.

In the real world, the target species are embedded within a larger ecosystem that includes the physical environment, especially water flows and water quality, and biological organisms that interact with the population of imperiled species in the rivers. One attribute of a model is that it depicts only those components and processes that are important for its intended purpose. In this project, the fundamental question demanding the use of a model is:

What will happen to the Covered Species and their habitats at HCP (Phase 1) allowed flow levels and durations?

This has guided the decisions of which components and processes are included in the model, and their priorities of development. This project represents Stage 1 of model development in which the fountain darter (*Etheostoma fonticola*) is the principal target species for management.

The model formulation was founded on the principle of determinism, that is, the model is intrinsically mechanistic. This means that the key causal relations are explicitly depicted in the model. A conceptual model was constructed and revised throughout the modeling effort to depict the overall model structure, the present version of which is shown in Figure ES1.

Substantial data resources are available and have been used in the present study. Over the years, a wealth of information has been collected on the Comal and San Marcos springs and river systems, their physical behavior and the unique species that inhabit them. This includes various scientific studies by academics, consultants, state and federal workers. The longest continuous and on-going comprehensive biological data collection effort for these systems is the HCP biological monitoring program, an outgrowth of the Edwards Aquifer Authority (EAA) Variable

Flow Study. This program includes a plethora of sampling components. Several sampling strategies and locations are employed that are designed to cover the entire extent of endangered species habitats in both systems, and to allow for holistic ecological assessments. Over the past 15 years, species-specific habitat and community data have been collected via this robust and multi-faceted sampling program. In this project, the focus is on the fountain darter drop net data, submerged aquatic vegetation mapping data, and water quality data collected via that program.

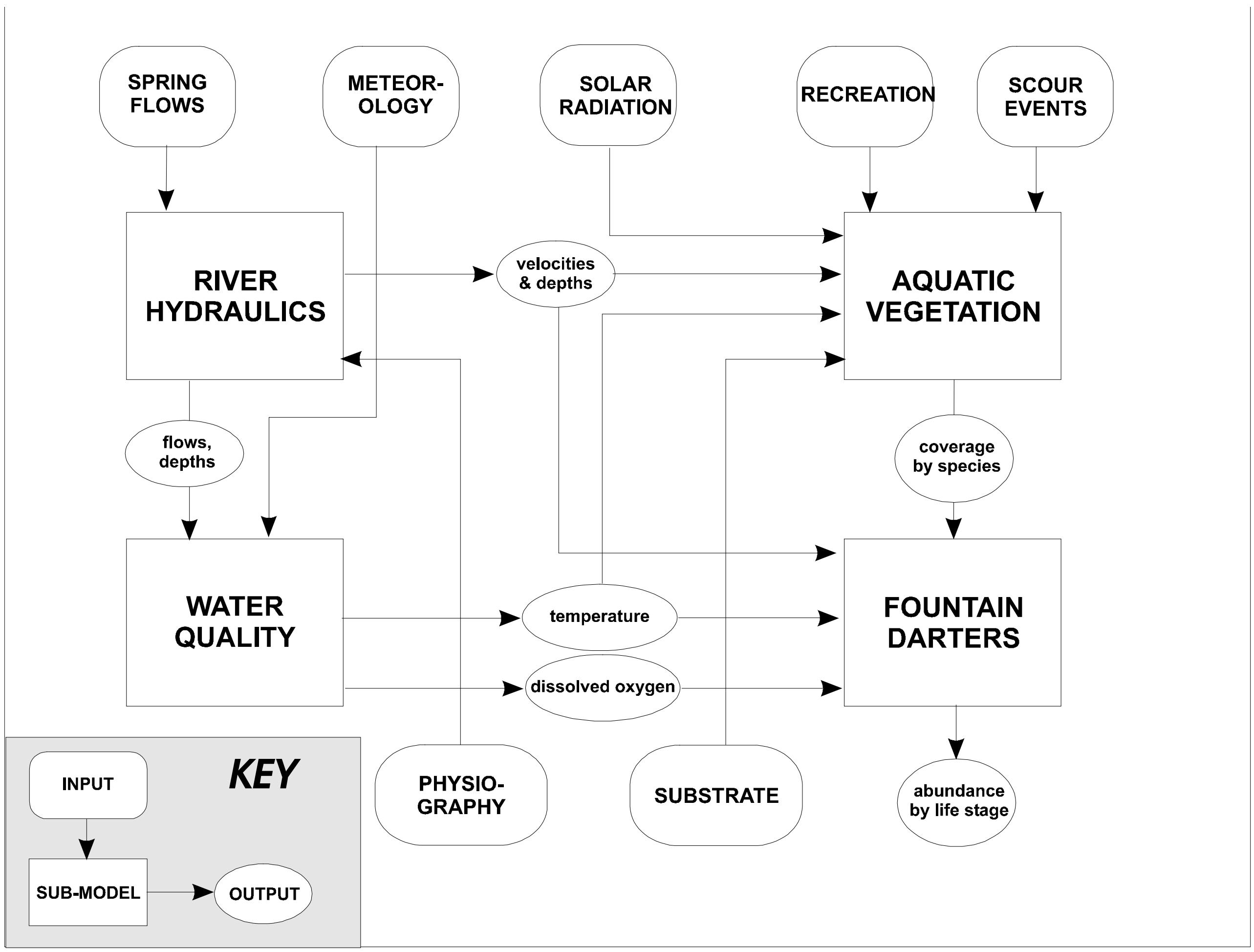

Figure ES1. Conceptual Model

Over the development of the project, five study reaches were selected based upon available data resources, a variety of external forcings, diverse habitats, and existing populations of fountain darters. During this initial phase of model formulation and development, the project was

confined to two study reaches, the Old Channel reach of the Comal River and the City Park reach of the San Marcos River. In 2016, the project team will expand the simulation model to include two additional reaches in the Comal system (Upper Spring Run reach and Landa Lake) and one additional reach on the San Marcos system (I35 reach). All ecomodel reaches selected have intensive biological data collected since 2000, and collectively provide a diversity of habitat conditions as well as natural and anthropogenic influences.

In addition to these data resources, the project has employed the results of historical research as well as special studies conducted as HCP applied research projects. Historical research and modeling included efforts from the San Marcos Observing System and the Edwards Aquifer Recovery Implementation Program (2009-2012), while HCP applied research included studies on aquatic vegetation tolerance (2013), percent cover to biomass of target aquatic vegetation (2014), fountain darter fecundity (2014), fountain darter movement (2014), and native versus non-native aquatic vegetation competition (2015). Results from these focused applied research efforts were used to the degree practical to parameterize the ecological submodels where appropriate.

Hydrology addresses the larger scale transfers of water between terrestrial and aquatic systems and is typically based on empirical measurements of the total flow rate associated with gage locations, employing principles of mass (or volume) balance. Hydraulics addresses the dynamics of water motion within river channels and is utilized to derive the estimates of the spatial distribution of depth and velocity within the channel at target flow rates. In this project, calibrated two-dimensional hydrodynamic models are used to estimate the spatial distribution of depth and velocity for simulated discharges in both the Comal and San Marcos systems.

The hydrology utilized in the modeling was derived from empirical measurements of flow collected at USGS gages within both systems over the 2003-2014 period and spot measurements of the discharge collected at specific locations as part of monitoring efforts. Daily flows within the San Marcos River were taken directly from the USGS gage below Spring Lake Dam (USGS 08170500) while gage data within the Comal River were taken from the gage in the New Channel (USGS 08168932) and the total Comal River above the confluence with the Guadalupe

River (USGS 08169000). Published data on spring flows in conjunction with synoptic flow measurements for both river systems were utilized to partition the spatial contribution of flows as noted below for the purpose of water quality modeling. Flow partitioning within the Old Channel of the Comal River was derived from empirical spot measurements over the simulation period for calibration and validation purposes while simulated scenario flows were based on assumed flow partitioning outlined in the HCP.

Past work on the two rivers has included application of the U.S. Geological Survey Multi-dimensional Surface Water Modeling System (MDSWMS), which models the horizontal components of current velocity and the water-surface elevation at a fine computational resolution (0.25-m grid spacing) within the river channel. These models for each river were adapted for use in the ecological model. The models were utilized to simulate the spatial distributions of depth and velocities for target flow rates at each study site and the corresponding results used to extract data on a coarser 1-m grid for input into the vegetation and fountain darter models.

Two water quality parameters are considered vital to fountain darter health, *viz*. temperature and dissolved oxygen (DO). Each of these is potentially impacted by low spring flows. The model employed for water quality in both river systems is the Environmental Protection Agency (EPA) model QUAL-2E, a one-dimensional (longitudinal) model of mass balance in the watercourse, i.e., the model predicts the average substance concentration across the cross section of the river channel. The existing Qual2E models previously developed for the Comal and San Marcos River systems were utilized as the platform for simulation of hourly water temperature and DO values. From these model runs, daily values of average and maximum water temperature and minimum DO in each study reach are then provided as inputs to the vegetation and/or fountain darter models. Because these water quality parameters are fairly homogenous in the rivers, the spatial resolution of the model is much coarser than that of the hydraulic model, each QUAL-2E segment ranging about 60-600 m in length.

One of the fundamental ecological attributes affecting fountain darter populations is habitat, for which the submerged aquatic vegetation (SAV) is essential. A comprehensive review of existing software products for SAV modeling was carried out. While some features of these models were

incorporated into the present SAV model, it proved necessary to develop a custom model that captures the critical processes of the vegetation communities within the San Marcos and Comal ecosystems. This has required a considerable effort within this project because in explicitly treating multispecies SAV communities with dispersal and competition as well as the traditional processes of growth and senescence; the model is advancing the underlying science. Both the SAV and the fountain darter models are implemented within the NetLogo agent-based modeling (ABM) framework, a time- and space-dependent numerical simulation. The spatial increment is 1 m, which is a compromise between the detail of habitat variation in the river, and what is sufficient for management decisions as well as computationally efficiency. The model simulates vegetation growth, density, and colonization of several SAV species found in the Comal and San Marcos rivers. This is a hybrid model: while some of the physical processes are based upon deterministic processes, others, notably dispersal, rely upon statistical models based upon the observational data base for the two rivers.

The modeling approach for the fountain darter component was to develop a time-advancing, spatially-explicit, individual-based model implemented in NetLogo, representing fountain darter population dynamics using HCP biological monitoring data collected since 2000 as the foundation. The underlying relations between habitat characteristics and darter populations (as monitored by the drop net program) were characterized statistically. Inputs to the fountain darter model include hydrology/hydraulic data, daily mean and max water temperature and daily minimum DO, and SAV distribution and densities (Figure ES1). For initial model calibration work, a de-coupled version of the fountain darter model was created, in which the output from the SAV model into the fountain darter model is disabled, and the SAV distributions and densities are taken directly from field observations. This allowed parameterization of the fountain darter model to proceed without the complexity of simultaneously calibrating the SAV model. Once the SAV model is fully operational and calibrated, it will be coupled with the fountain darter model for the final calibrations. All fountain darter calibration results shown in this report are with the de-coupled fountain darter model.

The approach to model calibration was to select two of the study reaches for initial model implementation and calibration, namely the Old Channel reach in the Comal River, and City

Park reach in the San Marcos River. The hydraulic model (MDSWMS) and water-quality model (QUAL-2E) have been calibrated and tested in previous studies of the two rivers. In the present model structure, these models are driven by hydrology inputs and operated to generate water velocity and water surface elevations at their respective spatial resolutions of 0.25 m and 60-600 m, respectively. Input data from the former are extracted for the 1-m NetLogo grid.

Among the tasks remaining to be completed in the ongoing work in this project are:

(1) completion of the calibration work on the SAV model for both primary study reaches;
(2) sensitivity studies of the SAV model to hydrological inputs;
(3) completion of calibration work on the de-coupled fountain darter model and additional sensitivity studies;
(4) calibration and verification studies on the fountain darter model coupled with the SAV model;
(5) sensitivity studies on the coupled SAV-fountain darter model;
(6) extension of model development work (formulation, calibration and sensitivity) to the other three ecomodel reaches;
(7) completion of a user-oriented operational version of the model; and
(8) documentation of the model.

With respect to (7) and (8), as work on the overall model structure, its formulation, and implementation for the two primary reaches in the Comal and San Marcos systems has proceeded, the team has been mindful that the ultimate product is an operational computer code capable of being set up and run by EARIP Signatory staff. The design of the model has therefore reflected the intention to cast it in a format amenable to such use.

The general sequence of model operation, as presently conceived, is shown in Figure ES2. In most respects this figure parallels the conceptual model of Figure ES1 (as it should) but emphasizes the strategy of data transfer between the major model components.

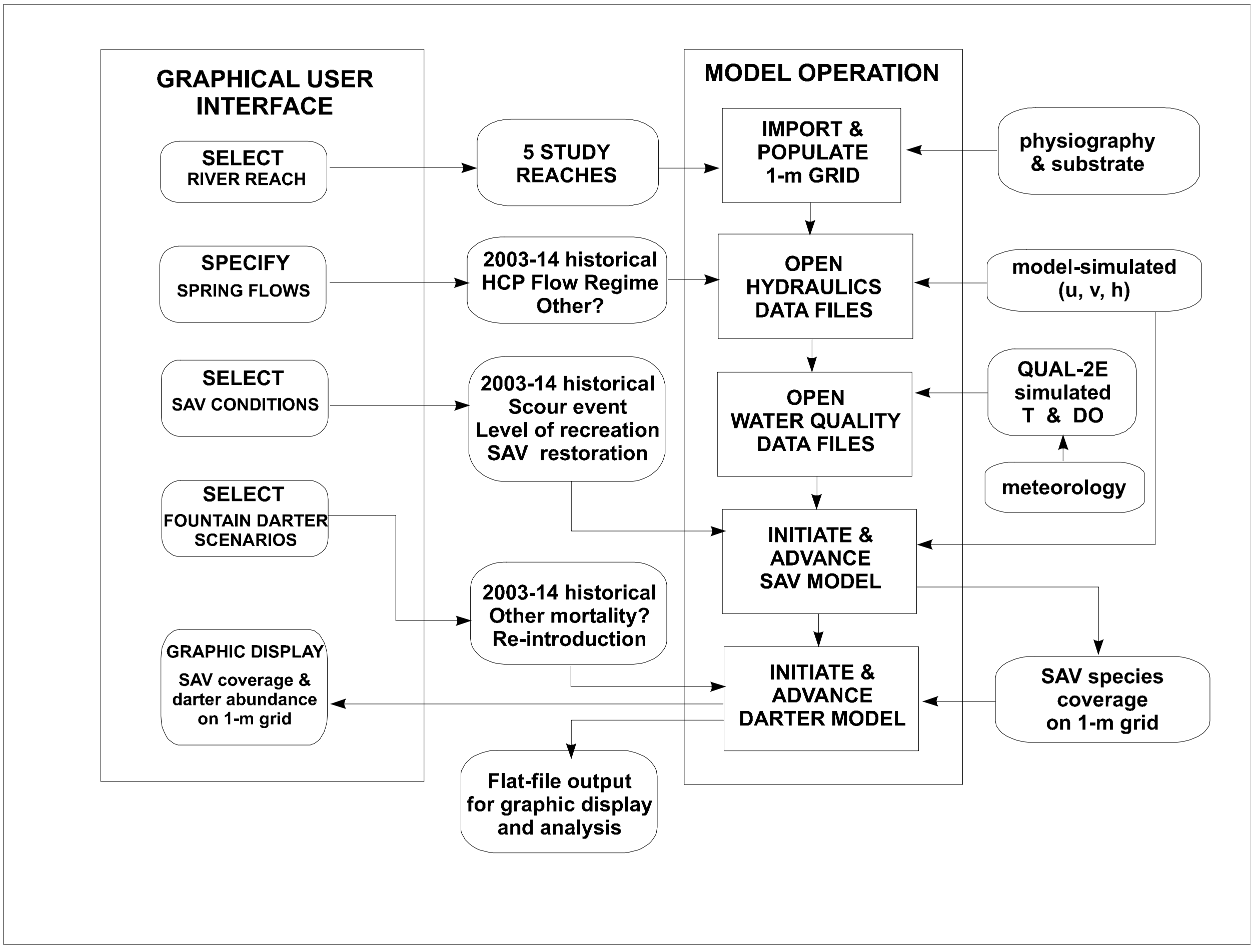

Figure ES2. Conceptual Operations Model

Four attributes of the operational version of the model have been delineated:

(1) Model set-up and operation through a graphical user interface (GUI)
(2) Standardized initiation to ensure comparability of time-series model runs
(3) Limited input options specifically tailored to management questions to simplify operation of model
(4) Range of output formats to facilitate post-run analysis and displays

Programming on the GUI has been underway for the past year, and will include a stepwise selection process for model set-up as well as spatial display of simulated SAV and darter evolution in time (using the NetLogo visual display). Model inputs will employ standardized initiation options to minimize the effect of starting transients, and will be based upon the concept of hydrological scenarios, notably the 2003-2014 historical flows and the HCP (Phase 1) flow

management objectives, as well as other potentially useful scenarios. A variety of output formats will be available to support display and analysis of the model results.

Though the completed, validated and operational fountain darter simulation model will complete this contracted effort at the end of 2016, this likely will not be the end of ecomodel development for the Comal and San Marcos springs ecosystems. Other management scenarios will likely present themselves as being desirable for inclusion in the model operation. Extensions of the scope of the model will require re-examination of the simplifications employed in this work, and possibly entail additional parameterization and validation.

As highlighted in the HCP, there is uncertainty inherent with predictions about the duration and extent of low flow conditions at Comal Springs, but the effects of these predicted scenarios and droughts of lesser durations will likely affect the quality and quantity of habitat for other HCP covered species. In particular, the Comal springs riffle beetle (*Heterelmis comalensis*) has a fairly limited spatial distribution within the system, so changes in flow could lead to areas suitable for riffle beetle habitat in the system becoming reduced in area and fragmented. It was the judgment of this team that the information base for the riffle beetle is presently inadequate to construct an ecosystem model focused upon this species. The project team concurs with the National Academy of Science's recommendations for EARIP to consider detailed monitoring studies and applied research to define the life history, habitat, water-quality, and food sources for the riffle beetle, and the future development of a population model.

1 Introduction

As described in the Edwards Aquifer Habitat Conservation Plan (HCP), flow-regimes for both the Comal and San Marcos systems have been prescribed as management objectives linked to the biological goals for the Covered Species (EARIP 2012). Although those objectives were approved by the U.S. Fish and Wildlife Service (USFWS) for Phase 1 implementation, there is still uncertainty inherent with 1) predictions about the duration and extent of low flow conditions at Comal and San Marcos Springs, and 2) the effects of these predicted scenarios and droughts of lesser durations on the quality and quantity of habitat for listed species. Thus, the HCP specified two primary *purposes* for including a predictive ecological model in the Adaptive Management Plan, and three "objectives" for each:

(1) Identify and describe specific ecological responses —

- (i) to assist in identifying and quantifying the effects of various environmental factors, including groundwater withdrawal, recreation, parasitism, restoration, etc. on ecological changes in these ecosystems and associated species;
- (ii) to assist in establishing potential threshold levels for these ecosystems and associated species relative to potential environmental stressors;
- (iii) to assist the overall scientific effort to better understand the interrelationships among the various ecological factors affecting the dynamics of these ecosystems and associated species.

(2) Quantify, predict and project impacts —

- (i) to predict specific ecological responses of the Comal and San Marcos Springs/River ecosystems and associated Covered Species to various environmental factors, both natural and anthropogenic;
- (ii) to project long-term effects of the Covered Activities on these ecosystems and associated species to facilitate designation of Phase II biological goals and strategies for achievement;
- (iii) to assist in mitigation design, implementation, and monitoring, as well as permitting, where applicable.

These are not the usual types of objectives specified for a model research and development project (e.g., Grant et al., 1997; Grimm and Railsback, 2005; Turchin, 2003), but have more the character of how and why the model is to be used. This illustrates that this project, though titularly research, has a practical and utilitarian goal, to produce a model capable of depicting

responses of the ecosystem to various external factors (including scenarios of Covered Activities), which can be used to *assist* the management enterprise. Despite the multiplicity of uses that such a model will afford, the fundamental question demanding the use of a model is:

What will happen to the Covered Species and their habitats at HCP (Phase 1) allowed flow levels and durations?

As communicated throughout this effort, the first stage of model formulation posits that the fountain darter (*Etheostoma fonticola*) is the principal species whose response must be determined, and that the set of controls governing the response is the characteristics of stream habitat (primarily water temperature and aquatic vegetation) available.

In order to best serve the requirements of the Edwards Aquifer Recovery Implementation Program (EARIP), and consonant with the specifications of the HCP, the model formulation was founded on the principle of determinism, that is, the model is intrinsically mechanistic. This means that the key causal relations are explicitly depicted in the model. The substantial field data resources of the HCP are exploited in statistical submodels that parameterize these relations and in testing the predictive capability of the model. The modeling philosophy of representing the principal controlling factors and processes tempered with a results-oriented pragmatism follows that articulated by Grant and Swannack (2008).

As a refresher, the Ecosystem Modeling Team (Team) consists of professionals from the University of Texas, Texas State University, Texas A&M University, Baylor University, US Army Engineer Research and Development Center (ERDC), Watershed Systems Group, Inc., and BIO-WEST, Inc. The team is comprised of modelers, statisticians, engineers, and scientists, several of which have spent the majority of their careers working with the threatened and endangered species in the Comal and San Marcos River systems.

The Team engaged in multiple technical coordination meetings initially aimed at outlining the modeling strategies based on the assessment of available data, literature reviews, feasible modeling approaches, and integration of required modeling component linkages. As the project progressed, the meetings (typically monthly in person) provided an opportunity to provide

updates on modeling activities in the various disciplines, identify potential problems, address concerns, and brainstorm on solutions for each modeling component as well as overall model integration. It also provided the team the opportunity to discuss comments and voiced concerns of the HCP Science Committee and National Academy of Science, as well as stay up to speed with and incorporate ongoing HCP applied research, biological monitoring, or restoration activity data as appropriate. Meeting minutes comprising the project notebook are provided in Appendix A.

Since submitting the first interim report, the project team has had the opportunity to directly provide updates to and solicit input from the HCP Science Committee, HCP Implementing Committee and National Academy of Sciences (NAS) on several occasions as shown below.

- May 12, 2014 NAS Committee update at EAA
- February 11 , 2015 HCP Science Committee update in San Marcos
- March 11, 2015 HCP Science Committee update in San Marcos
- March 19, 2015 HCP Implementing Committee at EAA
- March 24, 2015 EAA Research and Technology – Board Subcommittee
- October 27, 2015 NAS Committee update at EAA
- November 10, 2015 HCP Science Committee update in San Marcos
- December 17, 2015 HCP Implementing and Science Committees at EAA

Throughout the course of this project, the project team through Edwards Aquifer Authority's (EAA) facilitation has also held open dialogue with both NAS and the HCP Science Committee members to provide additional clarification, solicit input, and address questions or comments.

The focus of this interim status report is on model component development and evaluation. This included conceptual model development for the overall fountain darter simulation model which consists of a series of linked submodels. The submodels include hydraulics, water quality, submerged aquatic vegetation (SAV), and fountain darter life cycle and movement. In addition, special studies were performed directly for the ecomodel effort or indirectly through the HCP applied research program. These studies are briefly summarized within this interim report to provide additional context for model development. Additionally, a key component of the project extends beyond just how the model functions, but how can it be used. Therefore, a preliminary operations conceptual model was developed and is discussed towards the close of the document.

Finally, this report is titled “Interim” as this project is still very much in progress. A discussion on next steps, key upcoming decision points, and schedule are presented throughout the document with a closing observation on future considerations.

1.1 Data Resources

Over the years, a wealth of information has been collected on the unique species that inhabit Comal and San Marcos springs. To fulfill their Senate Bill 3 responsibilities, an Edwards Aquifer Area Expert Science Subcommittee for the Edwards Aquifer Recovery Implementation Program was formed and subsequently generated two reports (commonly known as K-charge and J-charge reports that evaluated the best available science collected up until the time of the reports. Both report titles bulleted below provide excellent summaries and descriptions of the available data resources and existing modeling tools prior to 2010.

- Evaluation of Designating a San Marcos Pool, Maintaining Minimum Springs Flows at Comal and San Marcos Springs, and Adjusting the Critical Period Management Triggers for San Marcos Springs (EAAESS 2008
- Analysis of Species Requirements in Relation to Spring Discharge Rates and Associated Withdrawal Reductions and Stages for Critical Period Management of the Edwards Aquifer (EAAESS 2009)

The longest continuous and on-going comprehensive data collection effort for these systems is the HCP biological monitoring program. Section 6.3.1 of the HCP describes the path forward that was implemented in 2013 for the continuation of Biological Monitoring that was initiated in 2000. A good overview and description of the original development and scoping of the biological monitoring program is provided in BIO-WEST (2007c). Originally, the biological monitoring program (formerly known as the Edwards Aquifer Authority Variable Flow Study) included Comprehensive sampling during “normal” set temporal periods, as well as specific triggered sampling for low-flow events (Critical Period sampling). Since the implementation of the HCP those initial goals and objectives have been expanded and refined through the EARIP process.

It is important to recognize that many different sampling components are included in the HCP biological monitoring program and several sampling location strategies are employed. The

sampling locations selected are designed to cover the entire extent of endangered species habitats in both systems, but also allow for holistic ecological interpretation, while maximizing resources where practical and when applicable. Consequently, the current design employs five basic sampling location strategies for the Comal and San Marcos systems as follows with associated sampling components:

1. System-wide sampling
   - Full system Aquatic Vegetation Mapping—once every 5 years

2. Select longitudinal locations
   - Temperature monitoring—thermistors
   - Water quality sampling—during low-flow sampling
   - Fixed station photography
   - Discharge measurements

3. Intensive Study Reach Sampling (4 reaches-Comal, 3 reaches – San Marcos)
   - Aquatic vegetation mapping
   - Fountain darter drop netting
   - Fountain darter presence/absence dip netting

4. Intensive Springs Sampling
   - Endangered Comal invertebrate sampling
   - Comal and San Marcos salamander sampling

5. River Section/Segment Sampling
   - Fountain darter timed dip net surveys
   - Macroinvertebrate community sampling
   - Fish community sampling

Over the past 15 years, a wealth of species-specific, habitat, and community data have been collected via this robust and multi-faceted sampling program. Germane to this project, the focus will be on the fountain darter drop net data (Section 2.5), submerged aquatic vegetation data (Section 2.4), and water quality data (Section 2.3) collected by this program over the years.

In addition to long-term biological monitoring, EAA, USGS, TCEQ and others have been collecting water quality data from these spring systems over many years. Additionally, the HCP

implemented a more intensive water and sediment quality monitoring program in 2013 (SWCA 2014).

With the implementation of the HCP came the establishment of an applied research program focused on addressing key data gaps relative to the covered species and habitats in the Comal and San Marcos systems. Over the first three years of the HCP Applied Research Program, several applied research projects have been conducted by researchers at Baylor University, Texas State University, BIO-WEST, and the Meadows Center that provided direct or indirect input to the ecomodel project. A list of these is presented below with summaries of key projects presented in Section 2.1.

- 2013 Applied Research
  - Aquatic vegetation tolerance study
  - pH drift study
  - Food source tolerance study

- 2014 Applied Research
  - Fountain darter movement under low-flow conditions in the Comal Springs / River ecosystem
  - Effects of low flow on fountain darter reproductive effort
  - Effects of predation on fountain darters

- 2015 Applied Research
  - Ludwigia interference and competition study
  - Algae dynamics and dissolved oxygen depletion study
  - Effects of turbidity on submerged aquatic plants

Finally, during the development of the ecomodel project, specific studies have been implemented by the Team to directly answer key questions and provide guidance in model development. These studies have included a vegetation percent cover to biomass study performed by Baylor University as well as a food source desktop evaluation conducted by BIO-WEST and Texas State University. Additionally, two additional efforts are underway by the Team including a fountain darter mortality study in the wild as well as random drop net sampling throughout each system. Both efforts were designed to inform model components or test model output and will be incorporated into the final report.

1.2 Conceptual Model

The uses of models permeate science and engineering. The last half century has seen an explosion of literature about types, formulations and applications of models. Presentation of models in science has even found its way into elementary school curriculum. The essence of a science or engineering model is contained in this statement:

> Model – a simplified depiction of a natural entity that exhibits its important features while eliminating or suppressing matters of irrelevant detail.

Strictly, this is only a quasi-definition, because it fails to specify what exactly is meant by "important" and "irrelevant," but it expresses the spirit of a model in the phrase "simplified depiction". A model is a representation of the essential behavior of the "entity," i.e., only those aspects that are considered "important." Much stock is placed in a parsimonious model that still succeeds in replicating natural behavior, and the terms "mimic" and "simulate" are often used to describe successful model behavior (e.g., Bender, 1978; Sober, 2015).

A model can be a physical depiction. Many laboratory experiments are models of reality in which external factors are controlled. Physical models of watercourses (a.k.a. "hydraulic models") have been an engineering staple for centuries (Ivicsics, 1975; Levi, 1995; Fatherree, 2004). A model can also be a set of mathematical relations whose variables measure features of the natural entity, i.e. a mathematical model. This may be the most important type of model because of its rigor and versatility, and has acquired even greater importance in the latter half of the twentieth century with the availability of high-speed computers enabling the numerical solution of even horrendously complicated equations.

Both science and engineering models are quantitative. Both models seek to express relations between external or "forcing" variables and the resultant variables that characterize the natural entity. That is, both models are causal, connecting the natural entity to external factors by cause-and-effect processes. The distinction between a science model and an engineering model is less one of formulation and more one of purpose. The scientist employs a model to clarify concepts, to appraise the relative importance of processes and/or variables, and to explore the behavior of

the modeled entity. The engineer uses a model for estimation of the effect of some configuration of external factors on the natural entity, as a guide to designing means of controlling the response or ameliorating its impacts. We note that while development of a model of the spring's ecosystems requires traditional scientific analyses, its intended application is much closer in principle to an engineering model.

Underlying the details of how a model is constructed or formulated is the assessment of the modeler of which variables adequately represent the natural entity, what other variables control the system, and what processes operate to relate the external variables to the response of the entity. This assessment is drawn from experience, intuition, and insight, and is itself a model of reality — a conceptual model. It may be communicated by pictures, or diagrams, or gestures and grunts. (Odum, 1994, famously devised a symbolic language for diagramming a conceptual model of an ecosystem.)

The HCP modeling project reported here began with the formulation of a conceptual model by the members of the team, and this model has been repeatedly revised during the course of the work. The present version of this conceptual model is shown in Figure 1. Oblong boxes indicate external controls ("inputs"), which may originate from data (perhaps involving a separate model) or by direct specification. The boxes identify the key submodels, whose development proceeded separately at the outset of the project. (Two of these, the hydraulic and water quality submodels, were developed by previous HCP projects, and adapted for use in the present work.) Ovals indicate variables predicted by the submodels ("output"). The arrows show the direction of causality, and can also be regarded as the flow of information. It is apparent that the "natural entity" referenced above (by this rather clumsy phrase) as the subject of a model is in fact a system (Checkland, 1993; Odum, 1994; Meadows, 2008). Each of the main submodels will be addressed separately in this report.

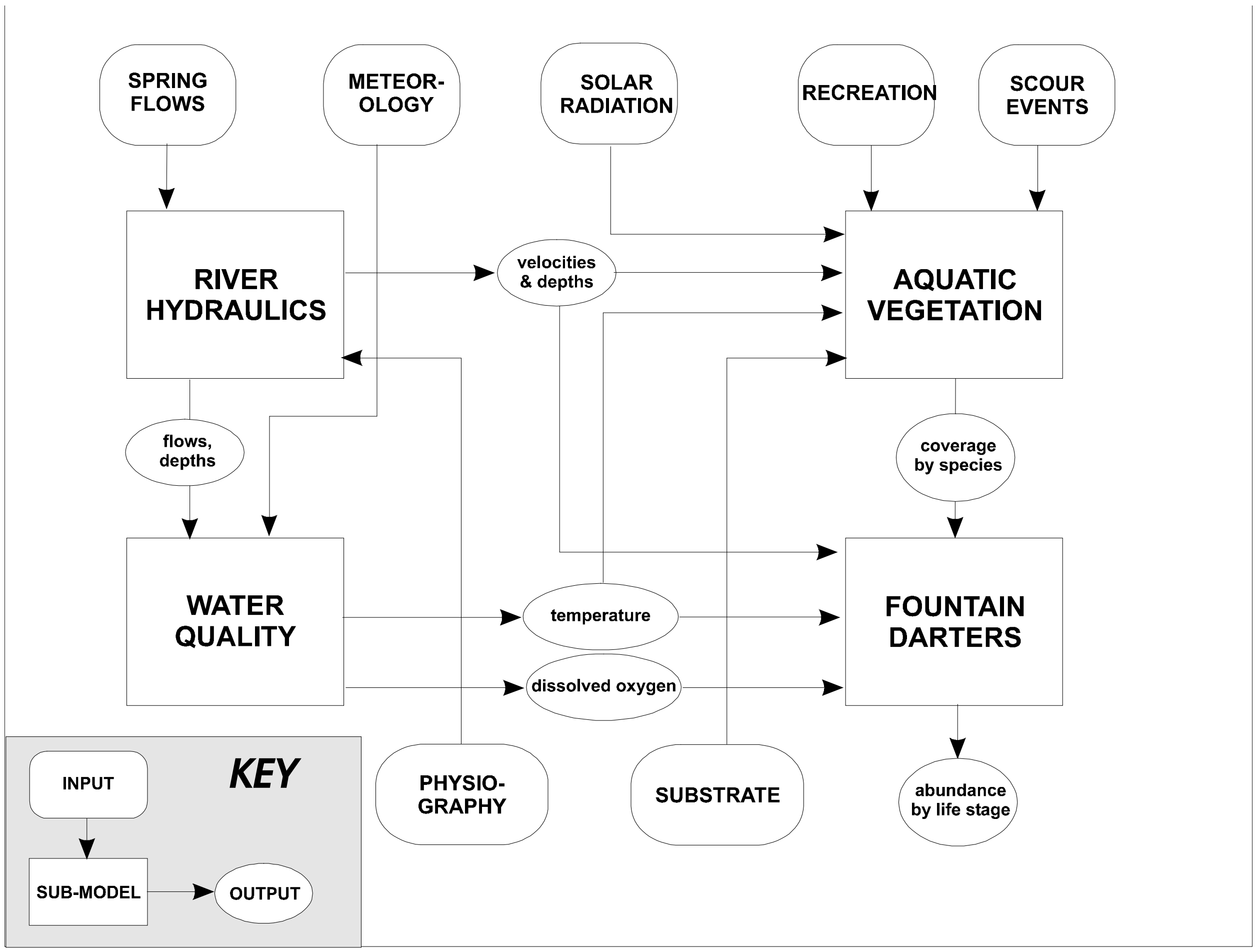

Figure 1. **Conceptual Model**

1.3 Key Decision Points

During the course of model development, the team was confronted with a series of decisions on model formulation and implementation. Many of these decisions were matters of technical detail or did not have a crucial impact on model development. Some were key decisions that represented forks in the road of model development. Pursuant to EARIP desires, decisions were documented (mainly in the meeting notes, collected here in Appendix A). The major decisions are presented in detail in the following chapters. Here these key decisions are listed and briefly described.

Agent-based Modeling

The main submodel is the population model of the fountain darter (see Figure 1), because the sustainability of the present population of this species is the central motivation for the HCP (Phase 1) flow prescriptions. While traditional instream flow modeling approaches have been used in the past, it was the consensus of the team that a fresh modeling approach would take better advantage of the considerable data resources available to the project. The Agent-based Model (ABM, a.k.a. Individual-based Model, IBM) was selected. This was considered to afford a means of simulating the time evolution of darter distributions in space, subject to time-varying external factors, and also enable the injection of random variables into the model.

NetLogo model platform

One of the principal software products presently available for implementing ABM's is NetLogo (Wilensky 1999). Again, some of the team members had previous experience with the software. Besides the low cost of the software, adoption of a widely tested and highly regarded software is a better option for the EARIP than having the team author its own ABM software.

Fountain darter model grid

The spatial resolution of each of the submodels is different, determined by the intrinsic variability of the physical relationships underlying the model, the resolution of field data, and the demands on computing capacity. Selection of a grid resolution for the fountain darter model was postponed until sufficient experience had been obtained with the early versions of the darter model. After this experimentation, a grid resolution of 1 meter was selected as being a satisfactory compromise between the incremental steps of darter movement and computational demands and execution times.

Study reaches: Original selection and additional reaches

The Old Channel study reach of the Comal River and the City Park study reach of the San Marcos River were selected as the primary reaches within each system for model development and testing (Figures 2 and 3). Each reach selected has intensive biological data collected since 2000. These primary reaches provided a nice diversity of habitat conditions as well as anthropogenic influences, as summarized below.

Figure 2. Comal River Ecomodel Simulation Reaches (Old Channel, Landa Lake, and Upper Spring Run).

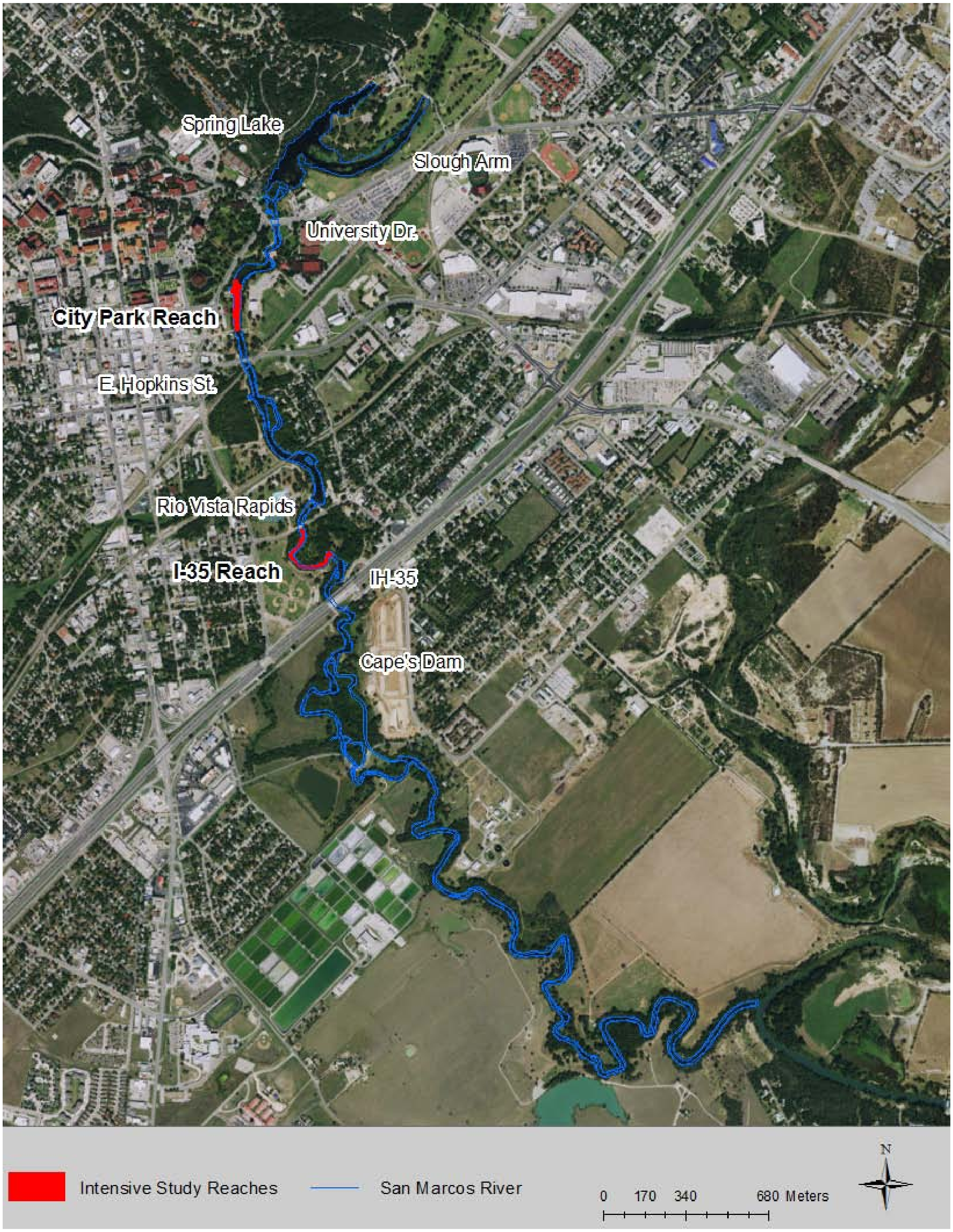

Figure 3. **San Marcos River Ecomodel Simulation Reaches (City Park and I35).**

In general, the Old Channel reach of the Comal River maintains a fairly constant flow controlled by a series of culverts and/or pass through the spring-fed swimming pool. Over time this reach has maintained high quality fountain darter habitat with limited to no recreational pressure. An installation of a USFWS sponsored culvert in the early 2000s resulted in altered flow conditions during the wet period of late 2003 and 2004. This higher flow condition caused drastic changes to aquatic vegetation (i.e. fountain darter habitat) (BIO-WEST, 2007c). Subsequent recovery of aquatic vegetation in this reach when flow returned to more typical conditions were represented by a near complete change from native to non-native aquatic vegetation. This change in aquatic vegetation resulted in an overall decline of the quality of fountain darter habitat in this reach (BIO-WEST, 2007c). The team felt that these changes over time directly related to changing flow conditions made this site a logical choice to initiate modeling activities. Additionally, extensive HCP habitat restoration is presently being conducted in this important stretch of the Comal River.

The City Park reach of the San Marcos River was selected as the primary reach in this system because of vastly differing characteristics from the Old Channel. The City Park reach is heavily recreated, and thus experiences considerable changes in aquatic vegetation from spring to fall and then back to spring each year. Unlike the Old Channel reach, the City Park reach has maintained relatively low quality fountain darter habitat over time. The lower quality characterization is resultant of higher flows and resulting deeper and faster hydraulic properties along with large quantities of non-native vegetation.

In 2016, the Team will expand the simulation model to include two additional reaches in the Comal system (Upper Spring Run reach and Landa Lake) and one additional reach on the San Marcos system (I35 reach).

The Upper Spring Run reach of the Comal system was selected because it is the first reach in either system to experience impacts from low flow conditions. In 2014, this reach experienced nearly 5 consecutive months of less than 2 cfs total discharge. During this period, impacts to aquatic vegetation were documented as were reduced densities of fountain darters in drop net sampling data (BIO-WEST, 2015b). Although this reach is basically written off in the current

HCP flow-regime, it is a valuable model reach to provide a glimpse and what might occur in other study reaches when they get to lower discharge conditions.

In contrast to the Upper Spring Run reach, the Landa Lake reach of the Comal system was selected because it has supported very static high quality fountain darter habitat conditions over the past 15 years. This reach also maintains some of the deepest areas of the system which are anticipated to support wetted area at very low discharges. Similar to the Old Channel, only limited recreational activity is present in this reach. Finally, this reach supports an immense amount of aquatic vegetation biomass which has raised some water quality concerns should a die-off occur during low-flow conditions.

The I-35 reach of the San Marcos system was selected because it supports higher quality habitat than City Park, with less overall recreation. This reach also supports a more natural channel with riparian coverage and channel meanders as opposed to straight channel with concrete bulkheads and limited tree coverage as represented by the City Park reach. In addition, this reach supports a more diverse community of aquatic vegetation. Similar to the Old Channel reach of the Comal River, the I-35 reach has experienced flow related changes to fountain darter habitat over time since the reconstruction of Rio Vista Dam in the mid 2000s (BIO-WEST, 2013a).

Water quality modeling

The suite of water-quality variables to be included in the model were limited to water temperature and dissolved oxygen (DO). There was little evidence for significant nutrient limitation in the study rivers, so nitrogen and phosphorus species were not considered. Similarly, the concentrations of carbon dioxide ($CO2$) are high and stable. The darters and, to a lesser extent, aquatic vegetation are primarily sensitive to water temperature, especially its stability during extreme low flows, so this is a necessary water quality variable. Although there has been limited oxygen depletion in the study rivers (in Landa Lake due to artificial reaeration), under drought conditions it is conceivable that DO may drop below the limits of toleration for darters. Therefore this variable was also included in the model. Moreover, to simplify the linkage between the water quality submodel and the darter submodel, the key variables selected were daily maximum temperature and daily minimum DO.

Aquatic vegetation model

Almost all computational vegetation models employ biomass as the basic dependent variable. The formulation of the present model is no exception. Because the field data is measured as fraction of areal coverage by species, the need to explore whether such observations could be converted to equivalent biomass was recognized early in the project and led to recommendation for a special study. The results of that study were affirmative, that conversions could be formulated relating the two measures of vegetation density.

The need to explicitly model plant propagation meant developing new vegetation model components based upon current literature and analysis of field data. This represents a major advance over present SAV models, and entailed several subordinate decisions, which are documented in detail in the report text.

Prey component in darter model

Darters eat a variety of invertebrates, and inclusion of these food sources would necessitate separate submodels for each prey species. Based upon estimates of standing crop of categories of invertebrates and the daily requirements for darters, it was determined that availability of food was not a limiting factor for the darter populations. The decision was made to disregard food availability in the current version of the darter model.

2 Model Component Development and Evaluation

2.1 Special Studies

As noted in the data resources section, HCP applied research projects have been influential in the development of the HCP Ecological model. In particular, the aquatic vegetation tolerance studies (2013), vegetation percent cover to biomass (2014) and Ludwigia competition (2015) study all provided direct input to the SAV submodel. The fountain darter fecundity (2014) and fountain darter movement study (2014) also provided direct input for the fountain darter submodel. Brief summaries of the 2014 and 2015 applied research studies are provided in this section with full reports included in Appendices B-E. In addition, a brief overview of the

macroinvertebrate food source investigation is included to highlight an example of the Team's decision making process on model components during the development phase.

Finally, it is important to note that two additional studies (fountain darter mortality in the wild and random drop net sampling for validation exercises) are scheduled for 2016, both of which are anticipated to provide direct inputs or testing of the fountain darter submodel.

Vegetation Percent Cover to Biomass

The aquatic vegetation models being adapted and validated include the USACE ERDC vegetation growth models (Best et al., 2001) as well as the "MEGAPLANT" (Scheffer et. al., 1993) model, both of which utilize vegetation biomass as the primary response variables in the models. However, we have virtually no aquatic vegetation biomass data for the plant species in the Comal and San Marcos Rivers. Instead, we have over 15 years of detailed maps of both rivers that show species distribution and estimates of percent cover for each mapped species. These vegetation cover maps all exist in ESRI ArcGIS® format and provide a robust record of the vegetation dynamics on the Comal and San Marcos Rivers during the past 15 years.

The objective of the Baylor (2014) study (full study report provided in Appendix B) was to determine the relationship between observed vegetation percent cover and dry weight biomass. These data establish the range of total dry weight biomass and proportion of above ground to below ground tissues for eight species of interest on the Comal and San Marcos Rivers (Figure 4). In addition, we provide regression relationships of total dry weight biomass versus occupied plant volume that can be used when those data are available. Those data are available for all maps generated by Dr. Doyle (1998-2001), and can be estimated with reasonable accuracy for all of the BIO-WEST mapping efforts if needed.

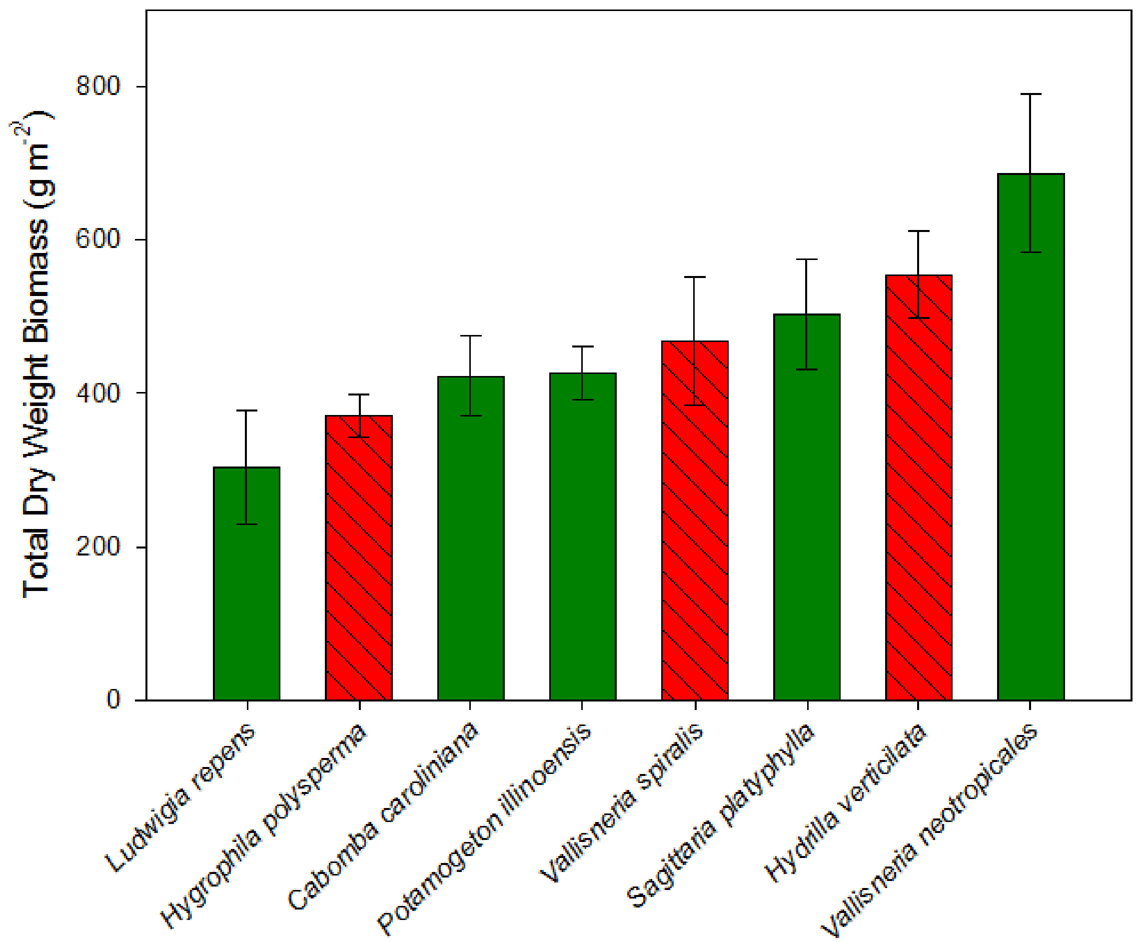

Figure 4. Total Dry Weight Biomass of Eight Aquatic Vegetation Species (Baylor 2014, Appendix B)

Three outcomes of this study show encouraging promise for providing reasonable biomass data to inform the vegetation model development efforts for the HCP. First, the variability around the “100%” cover samples appears to be reasonably constrained. Evidence comes in that the standard error (SE) around the mean for all species ranged only between 7.3-24.6% (Appendix B, Table 2 and Figure 2). The average SE variability around the mean for all species sampled was 13.7%. Second, the species to species variability is well defined (Appendix B, Table 2 and Figure 2). This provides realistic and constrained targets for maximum biomass per m^2 for each of the key species. Finally, the biomass to plant volume relationship described (Appendix B, Table 3) is also well bounded so that even more accurate estimates of biomass can be made provided vegetation height data is available. The average SE of the slope is only 12.4%, indicating that plant volume explains the vast majority of the variability in vegetation biomass.

Since the vegetation height data are directly available for some maps and reasonable estimates can be made for most of the maps, we now have robust data to inform the SAV modeling efforts.

Aquatic Vegetation Competition

The data from BIO-WEST (2015c) (full study provided in Appendix C) provide information on the early establishment and growth period of viable sprigs of Ludwigia, Hygrophila, and Hydrilla under three levels of competition from the other species. Additionally, it evaluated the short term impact(s) of sprig invasion from a competing species on the continued growth and development of established plants. These experiments were conducted at various locations within the Comal and San Marcos Rivers to provide more realistic environmental conditions than was possible with the static tank experiments previously conducted by Doyle et al. (2003) (Figure 5).

Figure 5. Doyle et al. 2003 and BIO-WEST 2015c (Appendix C)

Overall, these data indicate positive short-term establishment and growth characteristics for Ludwigia, and supports the continued use of the species for restoration efforts. Ludwigia used in restoration efforts is likely to effectively establish and quickly colonize unvegetated areas of the rivers. In fact, the growth of Ludwigia sprigs was higher over the 10-week growth periods than either Hygrophila or Hydrilla (Appendix C). Although both non-native species appear to have suffered from herbivory impacts, there is no reason to believe that the experimental conditions

used do not reflect actual levels of herbivory impacts in these systems. Therefore, Ludwigia planted into currently unvegetated areas or areas where the non-native plants have been removed are likely to grow very well.

Furthermore, Ludwigia may be less susceptible to competition impacts than previously documented. Under our experimental growth conditions, Ludwigia sprigs or established Ludwigia plants were not impacted by Hygrophila competition. Ludwigia sprigs and established plants were negatively impacted by Hydrilla, yet all treatment levels showed significant positive growth (Figure 6).

Figure 6. MUPPT at final harvest at San Marcos City Park (Site 1) location. Ludwigia plants (red) showed very robust growth. Hydrilla plants (green) showed variable success, although some plants were clearly very healthy (Appendix C).

In conclusion, this study has shown that in-situ testing of competition between native and non-native aquatic vegetation species in the Comal and San Marcos systems provides differing results than when tested in a no-flow laboratory environment (Doyle et al., 2003). This updated information was used to aid in the development of the SAV submodel. The study also emphasizes that the successful establishment of aquatic plants is strongly location dependent and

furthermore depends on a variety of factors and stressors and that the origin of the plant (native or non-native) does not automatically dictate the success of establishment or the competitive outcome.

In addition, Bilbo (2015) recently investigated Hydrilla in the San Marcos River as it relates to competition with native species. Similar to the aforementioned competition investigation, this thesis involved a competition study to determine if native species can out-compete non-native species under a set of environmental conditions. The experiment was conducted within Spring Lake at the headwaters of the San Marcos River in 2014. A three-factor replacement design: (water velocity, substrate type, and competitive pressure) was employed to assess competitive interaction between a native species (Potamogeton) and non-native species (Hydrilla). Illinois pondweed (*Potamogeton illinoensis*) and Hydrilla were potted in monoculture (intraspecific competition) and mixtures (interspecific competition) using sand or silt sediment, and high or low velocity for a period of seven weeks. Above- and belowground dry biomass, total stem length, and number of stems were measured.

Across all treatments, pondweed demonstrated significantly ($P<0.05$) higher growth rates than Hydrilla (Bilbo 2015). Substrate type and monocultures were not statistically significant factors in plant growth, however growth indices indicated that total dry biomass of both plants was slightly higher in sand substrate and high velocity. Intraspecific competition was determined to be greater than interspecific competition for both species, and both species produced more biomass when in monoculture and at lower ratios in mixtures. Therefore, data from this thesis suggests optimal growing conditions for Illinois pondweed to out-compete Hydrilla are in sand substrate and higher velocity conditions. As with results from the Ludwigia competition study, results from this thesis were used to update parameters in the current SAV submodel.

Macroinvertebrate Food Source

Conservation concerns for the fountain darter often involve hypotheses about factors that might limit darter populations, especially in the event of large environmental changes. One of these factors is the invertebrate community that comprises the fountain darter food source. Past studies have contributed to our knowledge of which of the innumerable invertebrate species may be used

by fountain darter populations in their extant range (Bergin, 1996; Schenk and Whiteside, 1977). These studies have illustrated that the amphipod *Hyalella azteca* is an important component of the fountain darter's diet, and they are also raised as the primary food source for the darter in captive assurance and research collections. This species is very abundant in both the San Marcos and Comal springs systems, representing by far the most abundant taxa present in all invertebrate samples collected by BIO-WEST during HCP biological monitoring efforts in 2013 and 2014 (BIO-WEST, 2015a and 2015b).

For this analysis, average biomass (mg) of amphipods (*Hyalella azteca*) contained in 1 m^2 of major vegetated habitat types in the Comal and San Marcos rivers was estimated using the length – mass relationships of Bencke et al. (1999) along with length data from *H. azteca* collected from each system (n = 77 [San Marcos] and 69 [Comal]) and invertebrate density data collected by BIO-WEST researchers in 2013 and 2014. Average mass of fountain darters in mg was estimated from the average length of darters captured in corresponding HCP biological monitoring drop net samples from 2001 – 2014 using a power curve (Figure 7) constructed with length and mass data collected from fountain darters (n = 417) in both systems as part of another ongoing study (BIO-WEST, 2014c) to predict length / weight relationships. For comparison, the daily biomass required by the estimated population of fountain darters in each habitat type was calculated as 5 % of darter mass (Dr. Tim Bonner, personal communication), using average mass in mg multiplied by 2014 fountain darter density estimates for each habitat. To provide a highly conservative estimate of food source consumption relative to abundance, fountain darter requirements at "carrying capacity" were estimated similarly but substituting the maximum density (# darters m^{-1}) observed for that habitat type since 2001 for the 2014 observed density (Table 1).

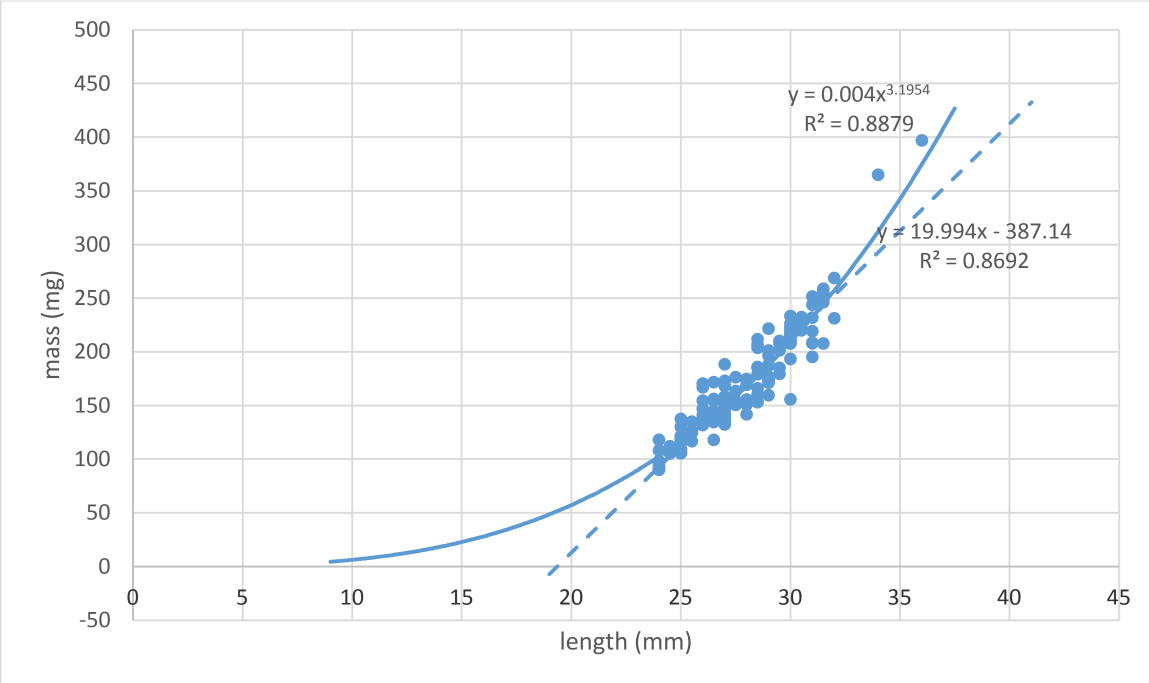

Figure 7. **Relationship of length to mass for the fountain darter.**

Table 1. **Estimates of total available food source (amphipod) biomass (mg) compared to estimated intake requirements of the darter population (mg) in 1 square meter of major vegetated habitat types in the Comal and San Marcos rivers. Parenthetical values in "Biomass required" cells represent the percentage of the standing crop biomass consumed by daily fountain darter needs at population densities. (BRY = bryophytes, CAB = Cabomba, HYD = Hydrilla, HYG = Hygrophila, LUD = Ludwigia, SAG = Sagittaria, VAL = Vallisneria)**

		BRY	CAB	HYD	HYG	LUD	SAG	VAL
Comal	Amphipod biomass (mg)	5384.5	1817.05		1392.04	5812.9	1312.59	284.068
	Biomass required (mg)	131.845 (**2.44%**)	57.425 (**3.16%**)		38.06 (**2.73%**)	80.82 (**1.39%**)	30.025 (**2.28%**)	30.99 (**10.9%**)
	Max required (mg)	463.63	256.9		192.81	490.74	589.37	171.56
San Marcos	Amphipod biomass (mg)		4770.96	6922.0	1957.76		4842.7	4173.87
	Biomass required (mg)		43.76 (**0.92%**)	34.07 (**0.49%**)	29.27 (**1.5%**)		19.19 (**0.4%**)	103.33 (**2.47%**)
	Max required (mg)		144.95	631.58	181.48		60.6	271.34

Examination of these data show that fountain darter needs represent a small proportion of the average standing crop of *H. azteca*, even at very high densities. In fact, aquatic invertebrates are generally accepted as having turnover rates of 2.5 – 5 % daily with a mode of 3% (Waters, 1969) and *H. azteca* specifically have been shown to exhibit a turnover rate of 3.3% daily (Cooper, 1965). Thus, in most cases estimates of use by fountain darters are under the daily replacement rate of the prey. These prey abundance data were collected from fully ecologically functional habitats and replicated, and therefore any influence of predation, competition, or other processes as they occur in these systems are inherently included in the estimates. We should note that while this may not represent an ideal, intensive academic study of fountain darter metabolic ecology, the intent is to determine the relative importance of invertebrate prey taxa abundance in the context of the long list of potential model parameters that could be used to simulate or predict darter abundance. It is also important to note that *H. azteca* represent but one of many taxa present in these habitats that are used as prey by fountain darters. Given the conclusions of this simple analysis of existing data, there is nothing in the data to suggest that availability or abundance of invertebrate prey is likely to be limiting for the fountain darter. As we briefly examined estimates based only on a single taxon from the breadth of taxa known to be consumed by the species, it seems it could be safely assumed that availability of prey could only increase with consideration of other taxa and the productivity of the systems in question. The Team therefore made the determination not to pursue a macroinvertebrate food source component in the Ecological model at this time.

Fountain Darter Fecundity

The fecundity study (full report provided in Appendix D) directly assessed the influence of flow and aquatic vegetation on fountain darter reproduction. Type and/or structure of aquatic vegetation are key components of fountain darter habitat in the HCP Ecological model. Information generated from this work provided direct measurements of reproductive success and expenditure for fountain darters throughout the year, which were subsequently incorporated into the fountain darter life cycle submodel.

Study results from this 2014 HCP applied research effort differ slightly than the results reported by Schenck and Whiteside (1977). Schenck and Whiteside (1977) reported peaks in

reproductive effort as greater proportions of females containing mature ova in February and March and again in July and August. Conversely, we found a general decrease in reproductive effort from Spring through Summer. Our study results, however, are consistent with reproductive efforts reported spawning patterns in other spring-associated minnows (McMillan, 2011) and spring-associated darters (Folb 2010). In addition, our results are consistent with field observations within the San Marcos and Comal Rivers (BIO-WEST, 2014a, 2014b). Small fountain darters (5 – 15mm, <60 days old; Brandt et al., 1993) were captured in the San Marcos River-City Park during dip netting events 23 of the 47 events (49%) since 2000 with most occurrences noted during the Spring (Appendix D, Figure 10). Small fountain darters were taken more often (44 of 47 events; 94%) in Spring Lake (Appendix D, Figure 11) than in San Marcos River-City Park, but higher proportions were again found in the Spring. In the Comal River, similar patterns are evident: New Channel (46% of samples contained small fountain darters), Old Channel (79%), Upper Spring Run (71%), and Landa Lake (90%) which again documents differences among sites. However, as with the San Marcos River, peaks in the Comal system were most evident in the Spring at all stations.

Collectively, fountain darters reproduce for at least eight months (January – August) but reproductive effort is not equal among months or among sites (discharge). Mechanisms underlying reduced reproductive energy at discharges <145 cfs and in tall vegetation are unknown at this time. Density-dependent mechanisms, such as prey availability and fountain darter densities, are potential factors regulating reproductive investment. Density dependent mechanisms influencing reproductive effort (investment and seasonality) have potentially interesting links to quality of habitats via field observations. As noted above, occurrences of small fountain darters are more frequent in Landa Lake and Spring Lake (>90% occurrence among samples) than in Old Channel and Upper Spring Run (71 – 79%) or San Marcos-City Park and New Channel (<50%). Though reproductive investment appears to be higher in San Marcos, City Park reach, the greater frequency of small fountain darters year round at Upper Spring Run and Old Channel suggest extended spawning.

Fountain Darter Movement

This study (full report provided in Appendix E) was conducted to examine fountain darter movement under deteriorating habitat conditions caused by low-flow scenarios. Previous research conducted in the Old Channel of the Comal River has shown that fountain darters move little in quality habitat under a stable flow regime (Dammeyer et al., 2013). Specifically, fountain darters moved an average of 10 meters (m) over the course of the year, with a maximum movement of 95 m in 26 days. However, should habitat conditions begin to deteriorate; movement could potentially increase as fountain darters search for more suitable conditions. To investigate this, over 2,000 individual fountain darters were captured from the headwaters of the Comal River, injected with fluorescent visual implant elastomer (VIE) marks under their skin, and released during a low-flow period in spring and summer 2014 (Figure 8). A variety of methods were used to relocate the tagged fountain darters and thus monitor movement and habitat utilization.

Figure 8. BIO-WEST and USFWS personnel marking fountain darters in the Comal system.

Over the course of the study, total system discharge at Comal Springs declined drastically, reaching levels that had not been experienced in over 20 years. During late August and early September 2014, spring flow within the study area was essentially zero (<1 cfs), although some groundwater infiltration was noted in certain areas along the river bottom. Aquatic vegetation, which is the key fountain darter habitat component within the study reach, became covered in filamentous algae and eventually disappeared completely. Water temperatures, which typically fluctuate between 23°C and 26°C over the course of a year peaked at over 30°C, with two straight weeks over 26°C. Extremely low discharge conditions, coupled with extensive habitat decline, provided the study team with a very favorable situation to observe movement of wild fountain darters in a stressed environment.

A total of 149 fountain darters were relocated during the study. In general, despite the low-flow conditions observed, fountain darters were relatively sedentary, moving an average of 20.9 m (median = 17.9 m) from their release point over the course of the study. However, two fountain darters, which were tagged in Blieders Creek, made relatively long movements of approximately 130 m toward areas of increased spring influence in the Upper Spring Run. These represent the longest recorded movements ever documented for wild fountain darters. Despite these two relatively long movements from Blieders Creek to the Upper Spring Run, no fountain darters were documented moving downstream of the Upper Spring Run into the spring-influenced habitat that was available near Spring Island. The distance to this habitat (>250 meters), along with observations made by divers suggesting that much of the wetted area between became comparatively warm and stagnant, may have presented a barrier to fountain darter movement.

Average distance moved (20.9 m) and maximum distance moved (131 m) in this study was slightly greater than that documented under stable habitat conditions by Dammeyer et al. (2013) (10 m and 95 m, respectively). This may suggest slightly increased movement as fountain darters searched for more suitable habitat. However, this may also be an artifact of a more expansive study area. This study provided interesting insight into fountain darter movement, habitat selection, and potential population dynamics under low-flow, no-vegetation conditions. When aquatic vegetation disappeared in July and early August, and water temperatures increased, rather than moving, fountain darters adjusted their habitat utilization to that available within the

local area. They were observed using interstitial spaces in gravel and cobble substrates as concealment, and were occasionally seen occupying open silt flats during this time period.

Results of this study show that even under extreme low-flow conditions, long-distance movement of fountain darters was rare. This has direct implications to ecological model parameterization. At the initiation of the modeling effort, a decline in habitat within the ecological model resulted in a concomitant decline in the number of fountain darters occupying that habitat, with no movement factor incorporated. This study suggests that movement/emigration of fountain darters from disappearing vegetation/habitat does occur but is not likely to completely counteract a projected population decline, particularly if additional habitat is more than approximately 20 m away. At maximum, fountain darters were observed moving over 100 m. However, this is based on the maximum distance moved by only a few individuals. For the current fountain darter movement submodel, the median distance moved during extreme low-flow conditions (17.9 meters) is currently incorporated.

2.2 Hydraulics

Characterization of the aquatic environment in terms of water quantity and quality requires two different levels of characterization. The first is the estimation of the total flow volume at specific locations over time (hydrology), where differences between spatial locations are obtained via a mass balance of the flow. The second level of characterization refers to the physical attributes of the flow volume in terms of the spatial distribution of depths and velocities (hydraulics) (Figure 9). Hydrology is typically estimated from empirical measurements of the total flow rate associated with gage locations while the hydraulics are estimated over spatial domains based on calibration and application of hydraulic simulation models. Its importance in this work is that it is the fundamental input specification for an operational model.

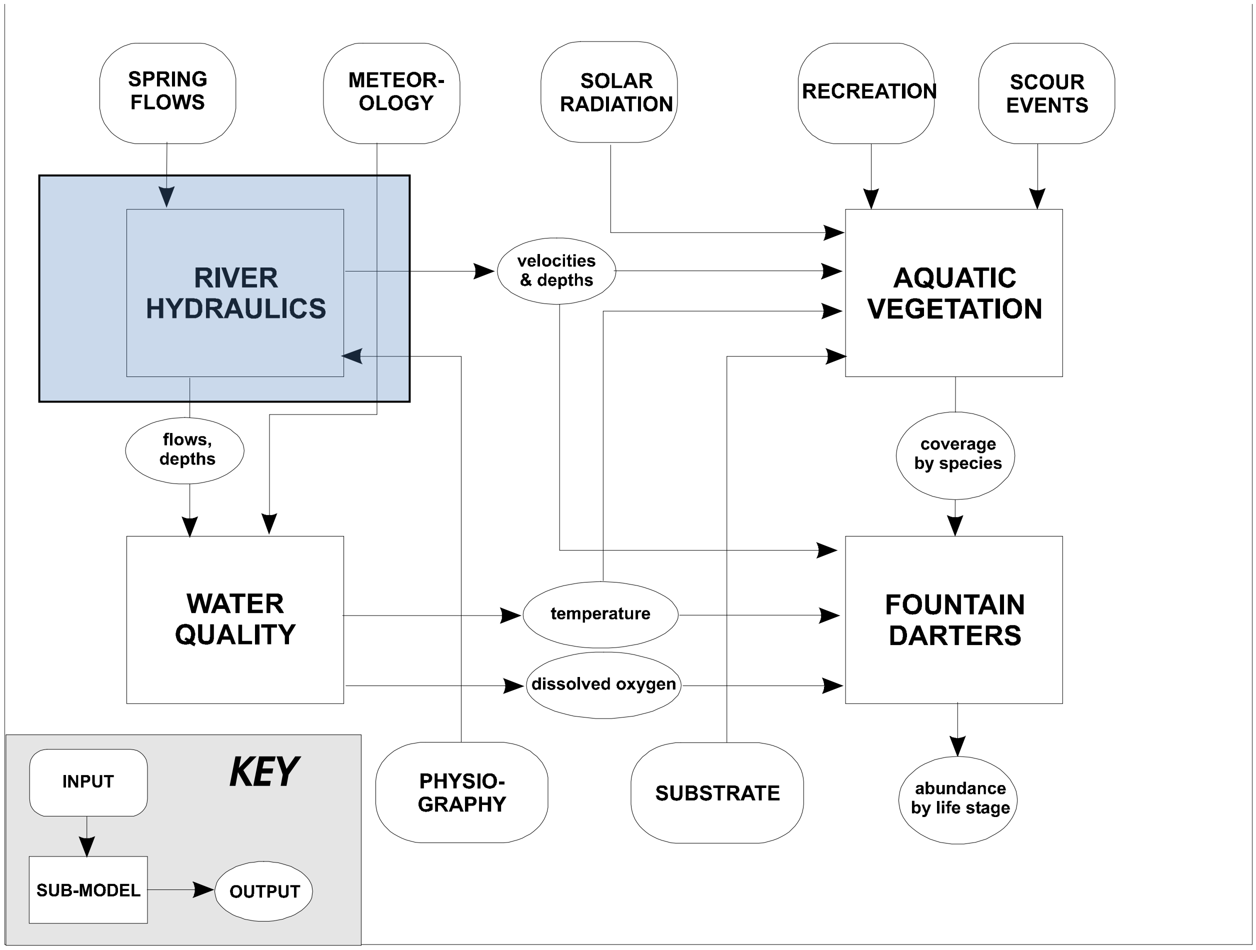

Figure 9. Conceptual model highlighting river hydraulics submodel.

Comal River Hydrology

Although the total Comal River discharge is recorded at a gage upstream from its confluence with the Guadalupe River near New Braunfels, Texas, estimating the flows between the new channel and old channel reaches are hampered by several factors. There is a lack of continuous (daily) gage data within the Old Channel over the desired simulation period and the measured flows in the Old Channel do not represent a consistent proportion of the total Comal River flows (Figure 10). Flows in the Old Channel ranged between 13 and 48 percent of total Comal River flows with a median of approximately 21 percent. In general, at total Comal River flows less than about 200 cfs, the Old Channel flows were approximately 45 percent. However, flows into the Old Channel historically (and now) were controlled by manipulation of culverts from Landa Lake and flows passing through the spring fed pool in Landa Park. At higher discharges (e.g.,

floods) flows from Landa Lake overtop the culverts into the Old Channel and total Comal River flows are often influenced by the contribution of ungaged flows (e.g., Dry Comal drainage) such that flows in the Old Channel cannot be directly derived by the difference between total Comal River and New Channel gaged flows.

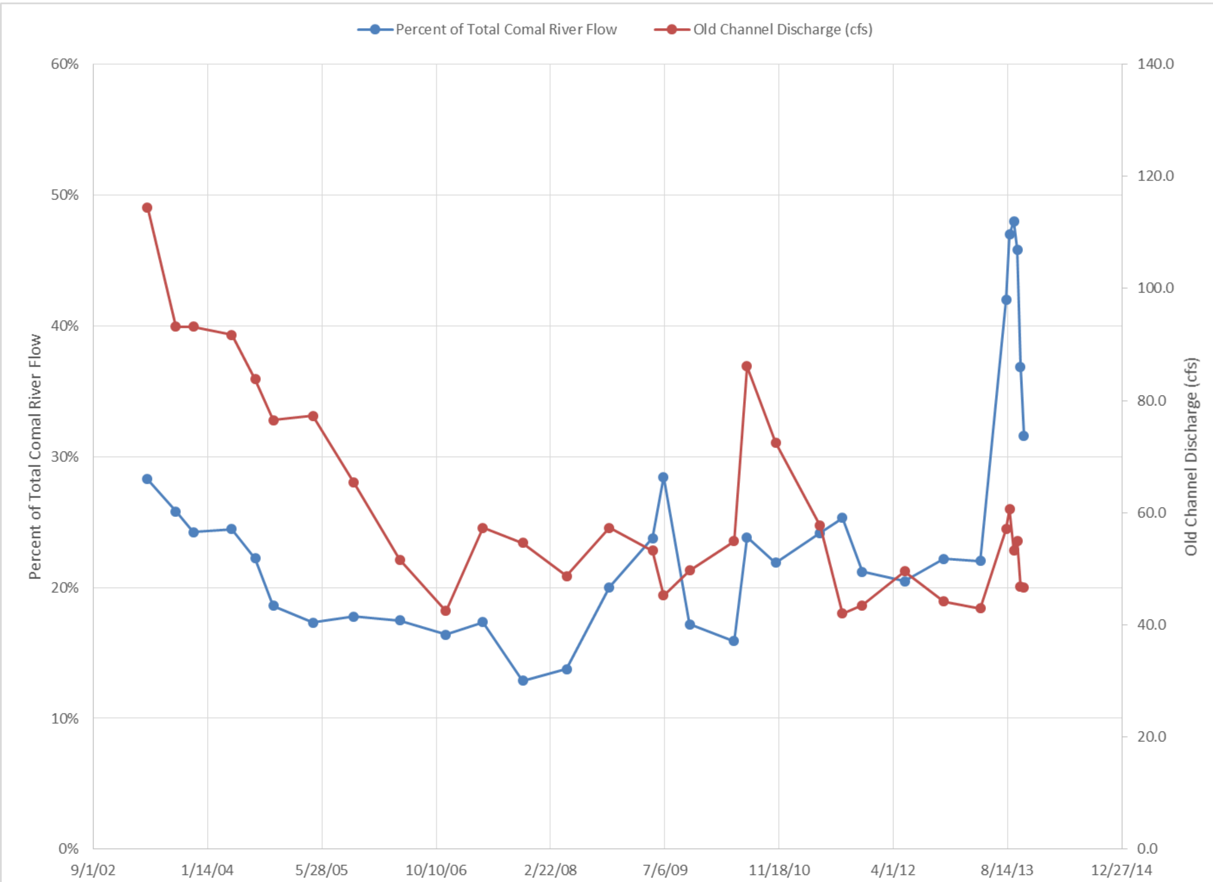

Figure 10. **Empirical estimated discharges in the Old Channel of the Comal River and flows as a percent of total Comal River discharges.**

Use of the estimated Old Channel flows within the ecomodel is also constrained by the available hydraulic simulation grids in the Old Channel which only simulate a maximum flow of 80 cfs. New culverts were added to the Old Channel bypass from Landa Lake as part of the HCP restoration efforts and the current recommended flow splits between the Old and New channels of the Comal River restrict controlled flow rates within the Old Channel to a maximum of 80 cfs.

Based on these factors, the estimated daily flows within the Old Channel were derived from a simple linear interpolation of the measured flows. We felt this was the most parsimonious

approach to support calibration and validation of the fountain darter model within the Old Channel (Figure 11). The flows were truncated at 120 cfs which represents the highest empirical measured flow within the Old Channel. Old Channel flows for arbitrary future flow scenarios will be constrained by the maximum culvert flow capacity in conjunction with flow through the Spring Fed Pool at Landa Park (i.e., approximately 80 cfs). As noted previously, in order to accommodate computational efficiency within the ecomodel, the estimated daily flows were aggregated to 7 day averages. We believe this 'smoothing' is justified given the relatively constant daily flow rates where variations in daily flows are not expected to result in demonstrable responses in either fountain darters or vegetation based on empirical monitoring over the past 10+ years. This weekly averaged flow value was used to set the corresponding headwater and point load contributions and corresponding flows within the Old Channel for use in Qual2E.

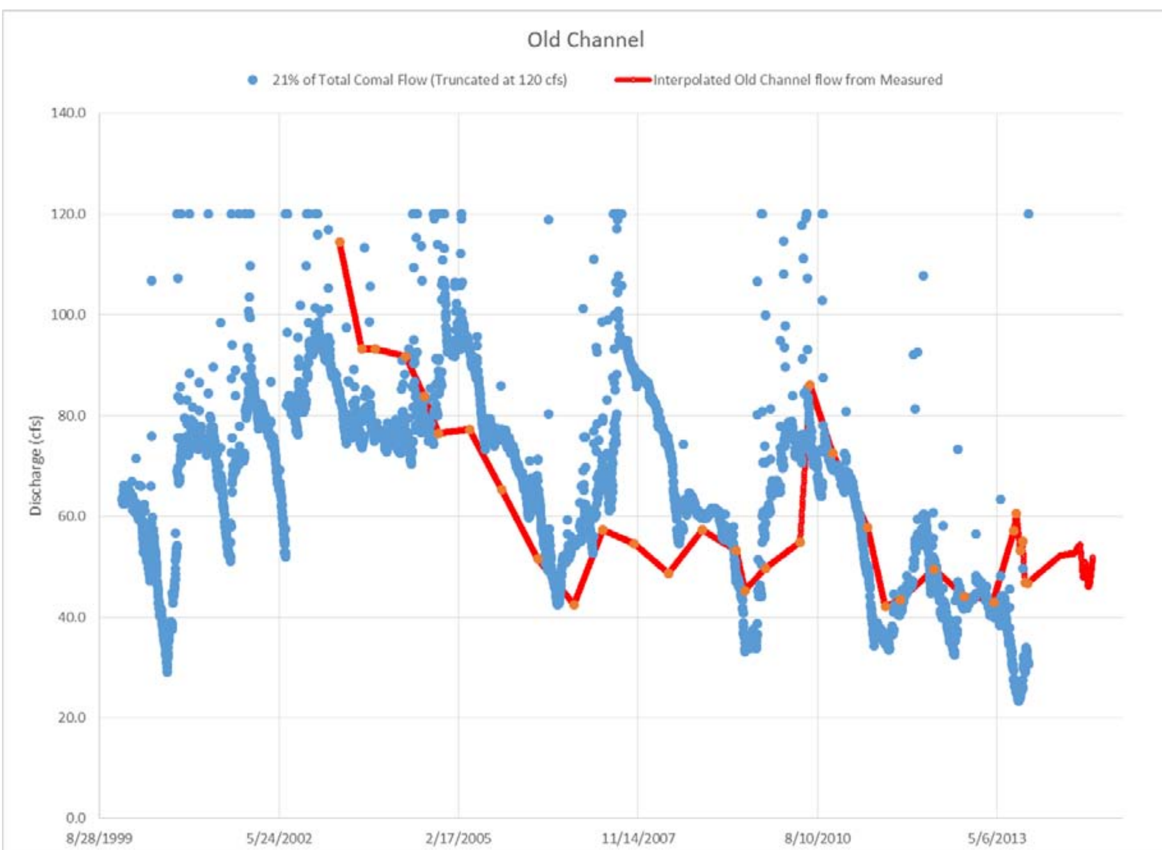

Figure 11. **Estimated daily discharges within the Old Channel modeling sites compared to the average of 21 percent of the total Comal River discharges (truncated to 120 cfs).**

Table 2 shows the percent contribution to the total Comal River flow for specific springs based on field measurements for a range of observed total Comal River discharges (BIO-WEST, 2010a). These data indicate that on average for these flow ranges the total contribution of the main spring runs to the total Comal River discharge is on the order of 25 percent. The data also suggest that as the total Comal River discharge decreases, the total contribution of the main spring runs begins to decrease and that there is a differential reduction between the specific spring runs.

Table 2. Total Comal River discharge and the percent contribution of main spring runs based on empirical measurements.

Total Comal River Flow (cfs)	Spring #1 (%) of Total Flow	Spring #2 (%) of Total Flow	Spring #3 upper (%) of Total Flow	Spring #3 lower (%) of Total Flow	Spring Flow as Percent of Total Comal Flow
159	4.80	3.50	4.70	13.60	21.90
224	6.90	1.50	5.10	11.50	19.90
259	9.50	1.30	5.60	13.00	23.80
286	7.90	2.10	4.20	13.00	23.00
295	9.30	1.30	9.60	12.30	22.90
330	9.70	1.50	4.80	12.10	23.30
351	7.10	1.10	2.50	9.10	17.30
361	11.80	1.70	10.40	13.70	27.20
368	10.20	1.40	9.20	11.90	23.50
375	11.50	1.70	9.80	13.20	26.40
377	13.30	2.30	11.10	13.90	29.50
385	11.20	1.50	9.70	12.30	25.00
405	12.10	1.80	9.90	13.20	27.10
411	12.20	1.80	10.30	13.30	27.30
424	10.00	1.50	3.50	12.30	23.80
446	14.40	2.40	10.20	13.20	30.00
Averages					
341	10.12	1.78	7.54	12.60	24.49

The analysis by Guyton Associates (2004) of historical water levels and spring flows was used as a basis for estimating main spring run discharges under lower flow conditions. Figure 12 shows the relationship between the Landa Park well levels versus total Comal Springs flow for the 1948 to 2001 period (Guyton Associates, 2004).

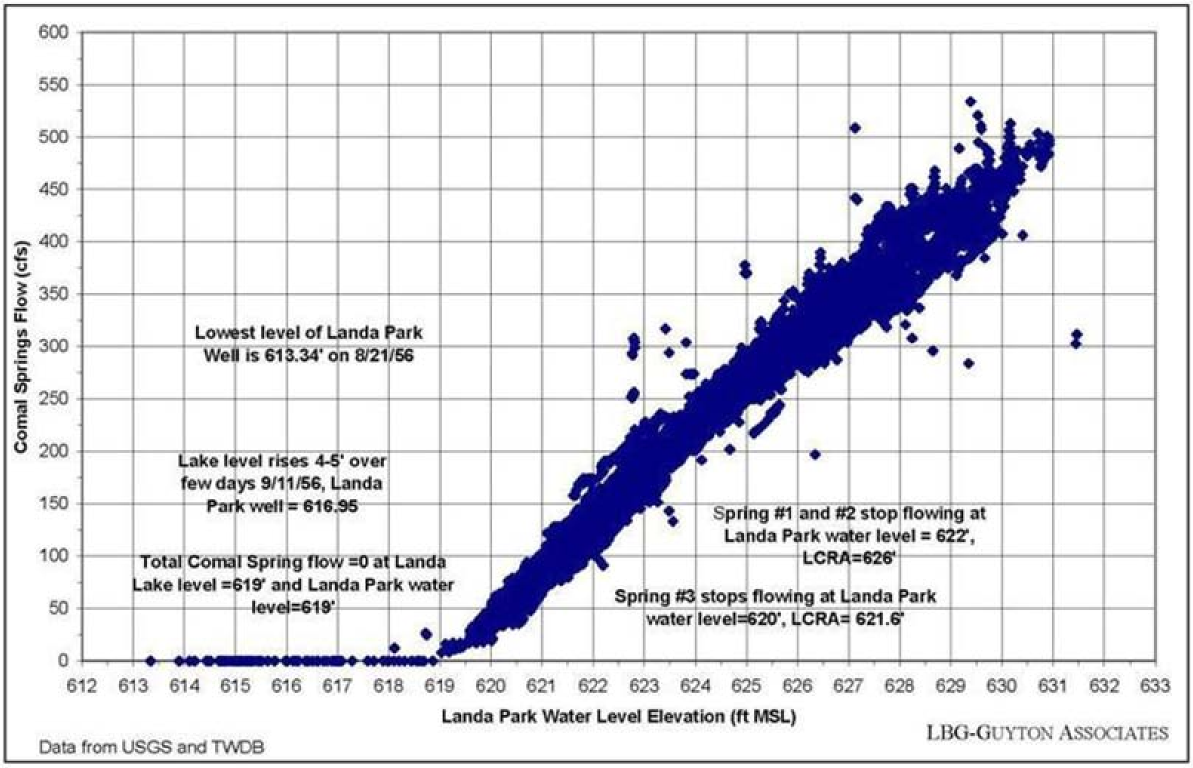

Figure 12. **Relationship between the Landa Park well level versus Comal Springs flows for the 1948 to 2001 period (Guyton Associates, 2004).**

These results show that historically Spring Run 1 and 2 stop flowing at a discharge that ranges between approximately 150 to 100 cfs and that Spring 3 stops flowing at a discharge range between approximately 60 and 20 cfs based on the measured water surface elevations. It was therefore assumed that as flows drop below the observed flow ranges reported in Table 2, flow contributions from the main spring runs will diminish to a point that all flow will be provided by the springs within Landa Lake proper and primarily along the western shore margin near the main spring runs and from various spring locations in the vicinity of Spring Island and Pecan Islands (Brune, 1981; Guyton Associates, 2004).

For the purposes of modeling, it was assumed that Spring Runs 1 and 2 would stop flowing at a total Comal River discharge of 130 cfs and that Spring Run 3 would stop flowing at a total Comal River discharge of 50 cfs. The percent contributions for each main spring run were initially set to the values associated with a total Comal River flow of 160 cfs, which is equivalent to the lowest observed discharge listed in Table 2. The percent contributions were assumed to linearly decrease to zero at the flow rates where springs were assumed to stop flowing.

However, due to analytical constraints on headwater elements within Qual2E, a nominal spring flow of 0.01 cfs was assigned to each main spring run (and headwater) for all simulated flow rates where springs or headwaters were assumed to have ceased flowing. Headwater inflows as a function of total Comal River discharge are provided in Table 3.

Table 3. Assumed headwater inflows (cfs) for each headwater as a function of total Comal River discharge.

HeadWaters Discharge (cfs)															
NW_Branch	0.01	0.01	0.01	0.01	0.01	0.01	2.20	2.40	2.60	2.80	3.00	3.20	3.40	3.60	3.80
NE_Branch	0.01	0.01	0.01	0.01	0.01	0.01	0.06	0.06	0.07	0.07	0.08	0.08	0.09	0.09	0.10
Spring_Run_3	0.01	0.01	0.01	0.01	0.01	2.35	2.59	2.82	3.06	3.29	3.53	3.76	4.00	4.23	4.47
SR1_Head	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Spring_Run_2	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
OC-Woods	10.50	14.00	17.50	21.00	24.50	28.00	31.50	35.00	38.50	42.00	42.00	42.00	42.00	42.00	42.00
OC-SPring_fed_p	4.50	6.00	7.50	9.00	10.50	12.00	13.50	15.00	16.50	18.00	18.00	18.00	18.00	18.00	18.00
Total Comal Discharge (cfs)	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95
	15.00	20.00	25.00	30.00	35.00	40.00	45.00	50.00	55.00	60.00	60.00	60.00	60.00	60.00	60.00

HeadWaters Discharge (cfs)															
NW_Branch	4.00	4.20	4.40	4.60	4.80	5.00	5.20	5.40	5.60	5.80	6.00	6.20	6.40	6.60	6.80
NE_Branch	0.10	0.11	0.11	0.12	0.12	0.13	0.13	0.14	0.14	0.15	0.15	0.16	0.16	0.17	0.17
Spring_Run_3	4.70	4.94	5.17	5.41	5.64	5.88	6.11	6.35	6.58	6.82	7.05	7.29	7.52	7.76	7.99
SR1_Head	0.01	0.01	0.01	0.01	0.01	0.01	1.25	1.30	1.34	1.39	1.44	1.49	1.54	1.58	1.63
Spring_Run_2	0.01	0.01	0.01	0.01	0.01	0.01	2.28	2.36	2.45	2.54	2.63	2.71	2.80	2.89	2.98
OC-Woods	42.00	42.00	42.00	42.00	42.00	42.00	42.00	42.00	42.00	42.00	42.00	42.00	42.00	42.00	42.00
OC-SPring_fed_p	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00
Total Comal Discharge (cfs)	100	105	110	115	120	125	130	135	140	145	150	155	160	165	170
	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00

Assumed Flow Splits for Old and New Channel

Flow partitioning between the Old Channel and New Channel are shown in Table 4. For all simulated flows above 70 cfs, the flow in the old channel was assumed to be maintained at 60 cfs. This maximum value was selected to avoid vegetation scour that has been observed at higher flow rates that can reduce both the quantity and quality of darter habitat in this section of the Comal River. We note that the culvert capacities can in fact accommodate flow of at least 80 cfs and therefore this upper limit may be modified in future scenario evaluations. For all other simulations, 70 percent of the flow into the old channel was assumed to be through the culverts (Reach 17) and the remaining 30 percent through the spring fed pool (Reach 16).

Table 4. Assumed Flow Splits in the Old Channel for Total Flow Rates in the Comal River. (Note: 60 cfs is assumed to be in the old channel at all total Comal River discharges above 70 cfs).

Total Comal River Discharge (cfs)	Old Channel Flow (cfs)
25	15
30	20
35	25
40	30
45	35
50	40
55	45
60	50
65	55
70	60

San Marcos Hydrology

Daily flows for the San Marcos River were taken from the USGS gage (08170500 San Marcos River at San Marcos, TX) for the period January 1, 2003 through December 31, 2014 (Figure 13). Individual spring flows within Spring Lake were treated as a single incremental flow as the study reaches are located below all spring flow inputs in Spring Lake. This approach assumes that the total discharge is distributed along the entire reach length of Spring Lake which closely approximates the spatial distribution of springs (Hardy et al., 2010). This is considered a pragmatic assumption given the available data on spring flows (Guyton Associates, 2004) and

lack of quantitative data on individual spring flow discharges with changes in total San Marcos River discharge.

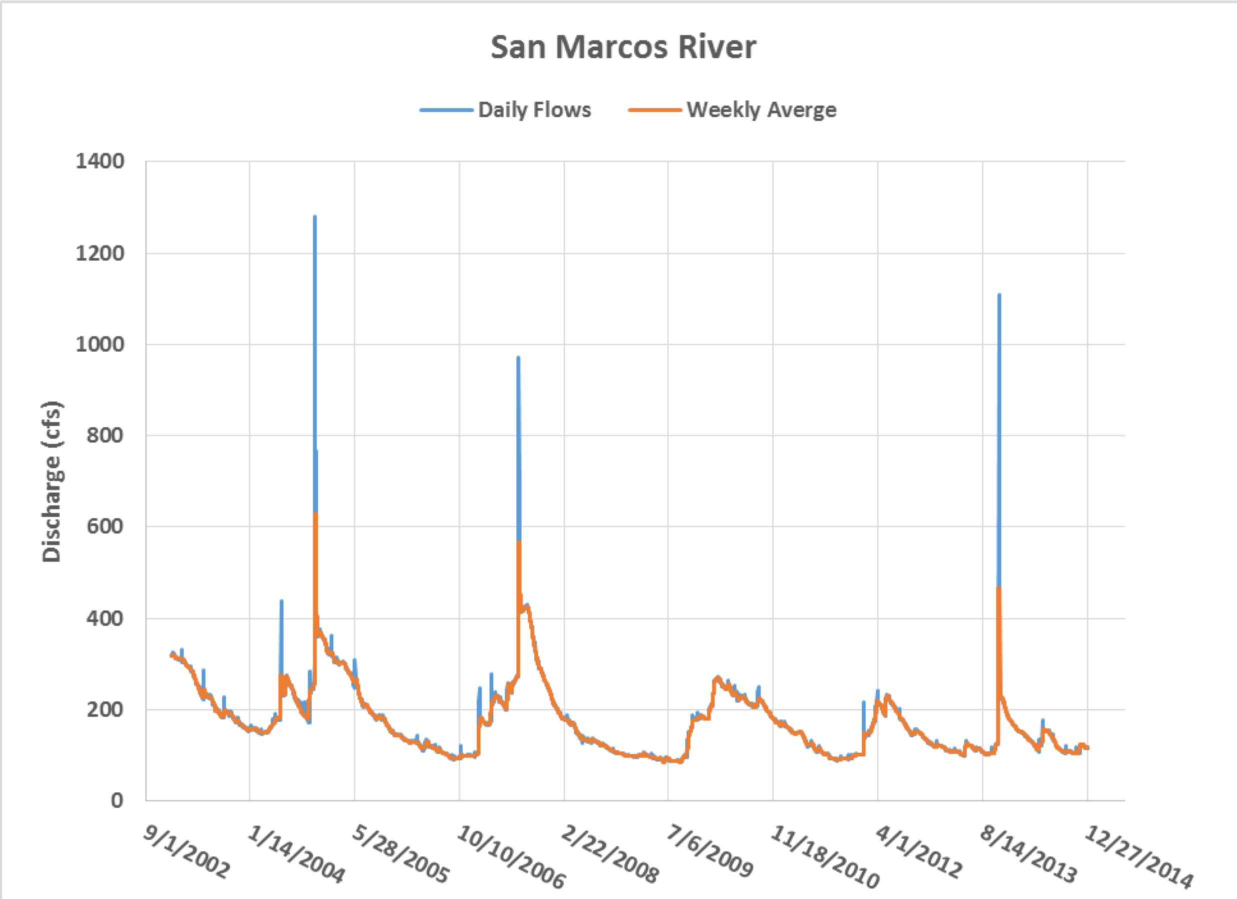

Figure 13. Daily flows and Weekly Average Flows in the San Marcos River.

The physical attributes or spatial characteristics of the flow within study reaches are modeled by a version of the so-called shallow-water equations *sans* rotation terms (e.g., Kundu, 1990). These are vertically averaged equations that describe the horizontal components of current velocity and the water-surface elevation. The equations are solved numerically using a boundary-following curvilinear two-dimensional grid (Figure 14). This is the native hydrodynamic model contained within the U.S. Geological Survey (USGS) Multidimensional Surface Water Modeling System (MDSWMS), which is a versatile GUI-based modeling software that has had extensive application to prediction of transverse circulations in rivers (e.g., Conoway and Moran, 2004; McDonald et al., 2006; Hardy et al., 2010). The model solutions as

presently implemented for the Comal and San Marcos Rivers are steady state, in that the model is run to equilibrium for a prescribed magnitude of flow within the old channel.

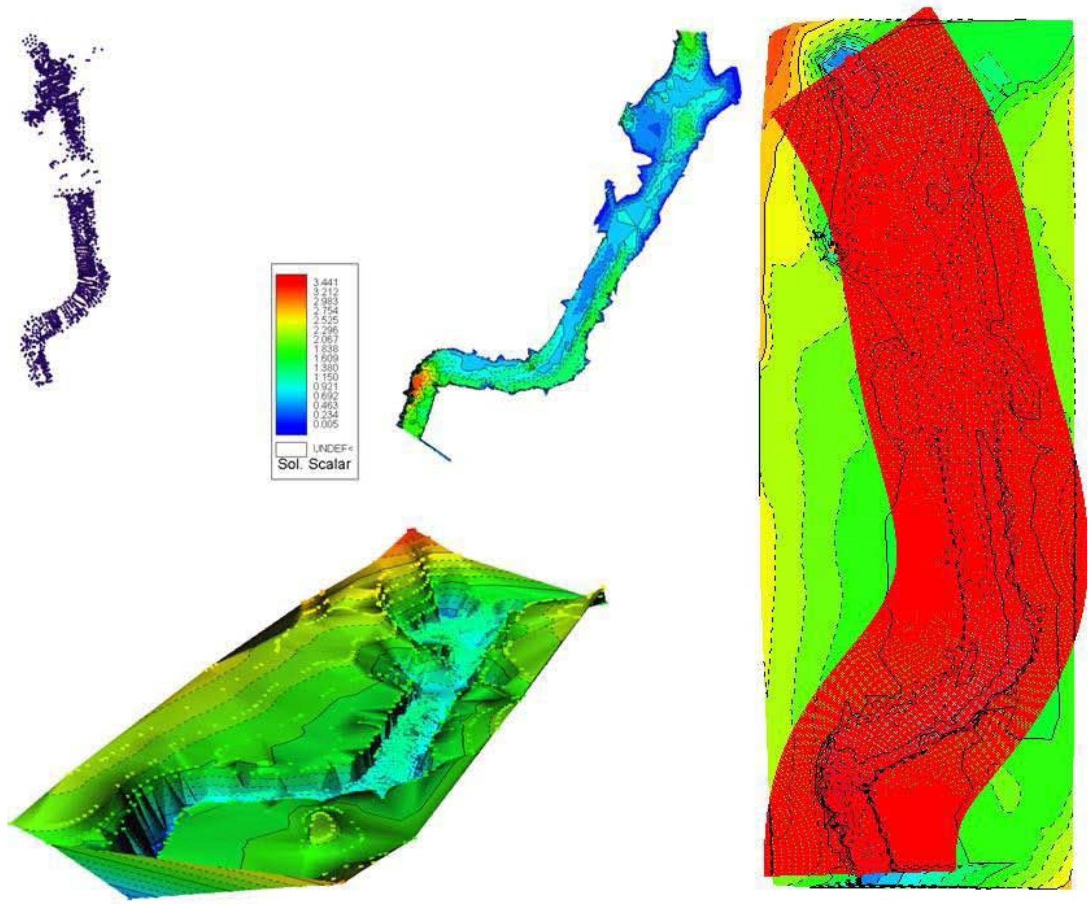

Figure 14. **Example of field-measured topography points, depth contours, computational mesh overlay mapped onto topography, final 3-dimensional computation grid geometry used in MDSWMS hydraulic model (Hardy et al., 2010)**

Running the hydraulic models in dynamic mode required an unacceptable cost in computational time for direct linkage with the fountain darter submodel. Alternatively, the hydraulic model(s) were run at a number of steady state solutions for target discharges for each river system and these pre-computed solutions were formatted for use within the fountain darter model.

The 2-dimensional hydraulic simulations for study sites in the Comal and San Marcos Rivers were adapted from Hardy et al. (2010). After much deliberation, the consensus of the team was

that representing the environmental data at 1-m spacing would be a satisfactory compromise between depiction of darter abundance and computational overhead of the darter model. Therefore, the original 0.25 meter computational grids for the hydrodynamic model (including substrate properties) were subsampled to derive 1.0 meter resolution grids for output files to the fountain darter model. This was accomplished by extracting the corresponding grid points at 1 meter increments from the orthonormal rectilinear grid structure (Figure 15). At each extracted grid point, the corresponding water depth and velocity were retained for use. The spatial extent of each simulation reach (e.g. Old Channel study reach of the Comal River) was utilized to clip the corresponding hydraulic grids to the same spatial domain as the long term aquatic vegetation monitoring data.

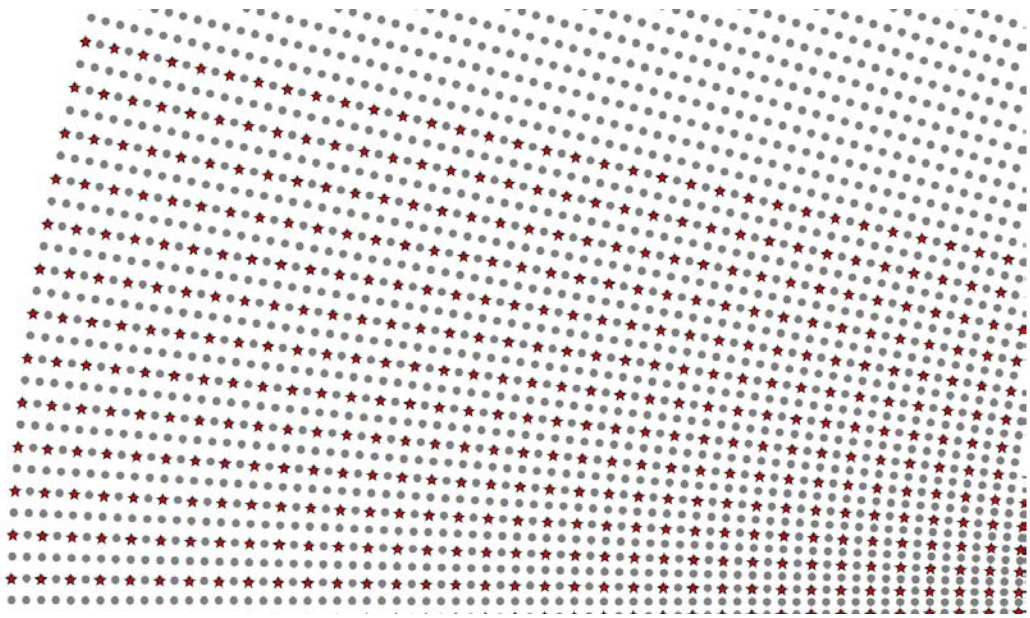

Figure 15. Example 0.5 meter grid extraction from 0.25 meter grid. Gray dots are original grid locations and red stars are the extracted grid locations. This extraction is then followed by a similar step to create a grid with 1-m resolution.

Table 5 lists the hydraulic model simulation flows for the Comal and San Marcos study sites. The Old Channel hydraulic grids were used to linearly interpolate the depth and velocity at each grid point between adjacent simulation discharges to derive one cfs incremental solutions between 10 and 80 cfs. Pair-wise comparisons of interpolated values between three known discharges (e.g., simulated results at 10 and 30 were used to interpolate results at 20 and compared to the simulated results at 15) showed less than an average of 3 percent differences

over the spatial domain of the model. This flow increment in the resolution of the hydraulic model simulations was initially thought necessary to specify flows for calibration and verification purposes within the Old Channel. However, no interpolations of hydraulic properties at un-simulated discharges were undertaken for any other study reaches in either the Comal or San Marcos River systems.

Table 5. Hydraulic Simulation Flows in the Comal and San Marcos River Systems.

Hydraulic Model Simulation Flows (cfs)		
Comal - Old Channel	San Marcos - City Park	
10	30	120
20	45	140
30	50	160
35	55	180
40	60	200
45	70	220
50	80	240
55	90	260
60	100	
70		
80		

2.3 Water Quality

For the purposes of determining the sufficiency of the HCP (Phase 1) flow levels for maintaining the population of fountain darters, two water quality parameters were considered to be crucial, *viz*. temperature and dissolved oxygen (DO). Each of these is potentially impacted by low spring flows. Under these conditions, we do not anticipate effects arising from, for example, altered nutrient concentrations or various toxics. (We note that both rivers lie in urban areas and may eventually be exposed to excessive loads, but this is currently beyond the scope of this initial modeling effort.) The role played by water quality in the overall fountain darter model is shown in the conceptual model of Figure 16. As with the hydraulic model, it was judged more efficient to de-couple the actual operation of the water quality model from the vegetation and darter models by pre-computing temperature and DO values over the entire simulation period. For the initial calibration and validation period of the fountain darter model, the observed (or estimated) daily flows and measured meteorological

data were utilized to compute the corresponding minimum, average and maximum daily water temperature and minimum daily DO at each study reach. These associated daily values were then provided to the vegetation and/or fountain darter model as inputs.

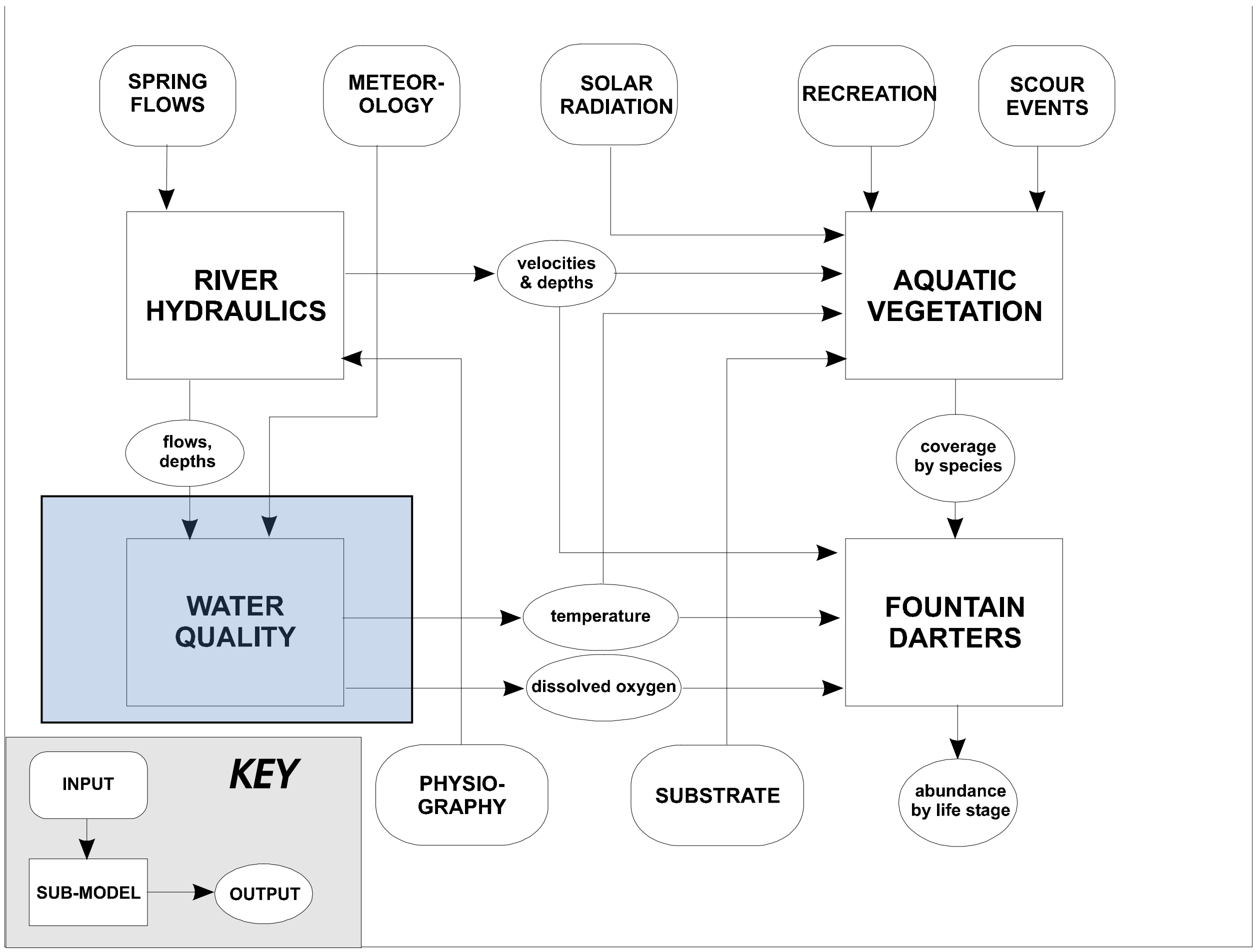

Figure 16. **Conceptual Model highlighting water quality submodel.**

Thermograph data for limited time periods were available from locations within the Old Channel of the Comal River and the City Park reach of the San Marcos River. These data records were utilized to derive hourly historic time series of water temperature at these locations for use by the Team, in particular to validate the temperature function of the water quality model. Historic DO data were more limited, only available for spot measurements associated with seasonal drop net sampling, and therefore could not be utilized to reconstruct hourly values.

The model employed for water quality in both river systems is QUAL-2E, disseminated and supported by the Environmental Protection Agency (EPA). This model was originally adapted from QUAL, a stream and river model first developed by the Texas Water Development Board in the early 1970's. This model has been widely used worldwide for addressing water quality in streams and rivers (Ward and Benaman, 1999). This is a one-dimensional (longitudinal) model of mass balance in the watercourse, i.e., the model predicts the average substance concentration across the cross section of the river channel. The model itself is steady state, but among the alterations to the coding, components of the model address diurnal variation in temperature and DO. Details of QUAL-2E formulations are given by Chapra (1997). The existing Qual2E models for the Comal and San Marcos River systems developed by Hardy et al. (2010) were utilized as the platform for simulation of hourly water temperature and dissolved oxygen values. The original models were calibrated to hourly data for a typical summer low flow condition and then used to simulate the 2009 calendar year for use in the evaluation of HCP flow regimes for both systems. In the present project, the models were extended to cover the entire January 1, 2003 through December 31, 2014 simulation period.

Comal River

Historic Water Temperatures

Thermograph data from the Old Channel was utilized to construct the hourly time series of water temperatures for the simulation period. Missing values were generated using two approaches. In those instances where less than three hours were missing, the missing values were linearly interpolated from adjacent values. For longer periods of missing values, regression equations based on the difference between hourly air and water temperatures were developed on a monthly basis (e.g., Figure 17; Table 6). Over 96 percent of the predicted hourly water temperatures based on the regression equations were within 1 degree of measured data. The hourly water temperature data was then reduced to the minimum, average and maximum daily water temperatures.

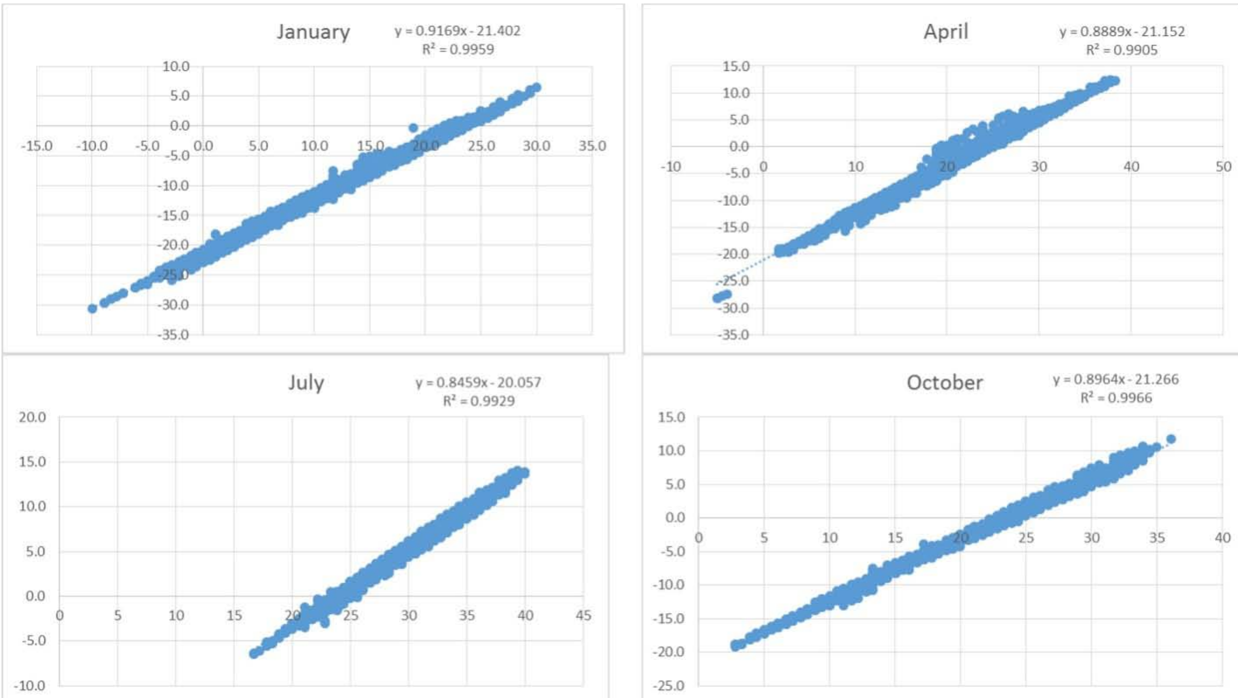

Figure 17. **Example regressions between air temperatures and the difference between air and water temperatures used to estimate hourly missing water temperatures at the Old Channel study site in the Comal River.**

Table 6. **Regression equations and r2 for prediction equations of the difference between air and water temperatures on a monthly basis for the Old Channel in the Comal River.**

	Equation	R^2
Jan	y = 0.9169x - 21.402	0.996
Feb	y = 0.9045x - 21.326	0.996
Mar	y = 0.8957x - 21.271	0.994
Apr	y = 0.8889x - 21.152	0.991
May	y = 0.8772x - 20.900	0.993
Jun	y = 0.8486x - 20.118	0.994
Jul	y = 0.8459x - 20.057	0.993
Aug	y = 0.8592x - 20.301	0.994
Sep	y = 0.8814x - 20.923	0.994
Oct	y = 0.8964x - 21.266	0.997
Nov	y = 0.9138x - 21.529	0.997
Dec	y = 0.9194x - 21.472	0.996

Modeled Water Temperatures and Dissolved Oxygen

The physical reach structure of the Comal Qual2E model is shown in Figure 18. The specific reaches are summarized in Table 7. The three Ecomodel simulation reaches for the Comal are

represented by the Upper Spring Run (Reach 3), Landa Lake (Reaches 5 and 7 combined) and the Old Channel (Reach 20). This interim report only provides simulation results for the Old Channel Reach. The other two Ecomodel reaches will be incorporated into the final report.

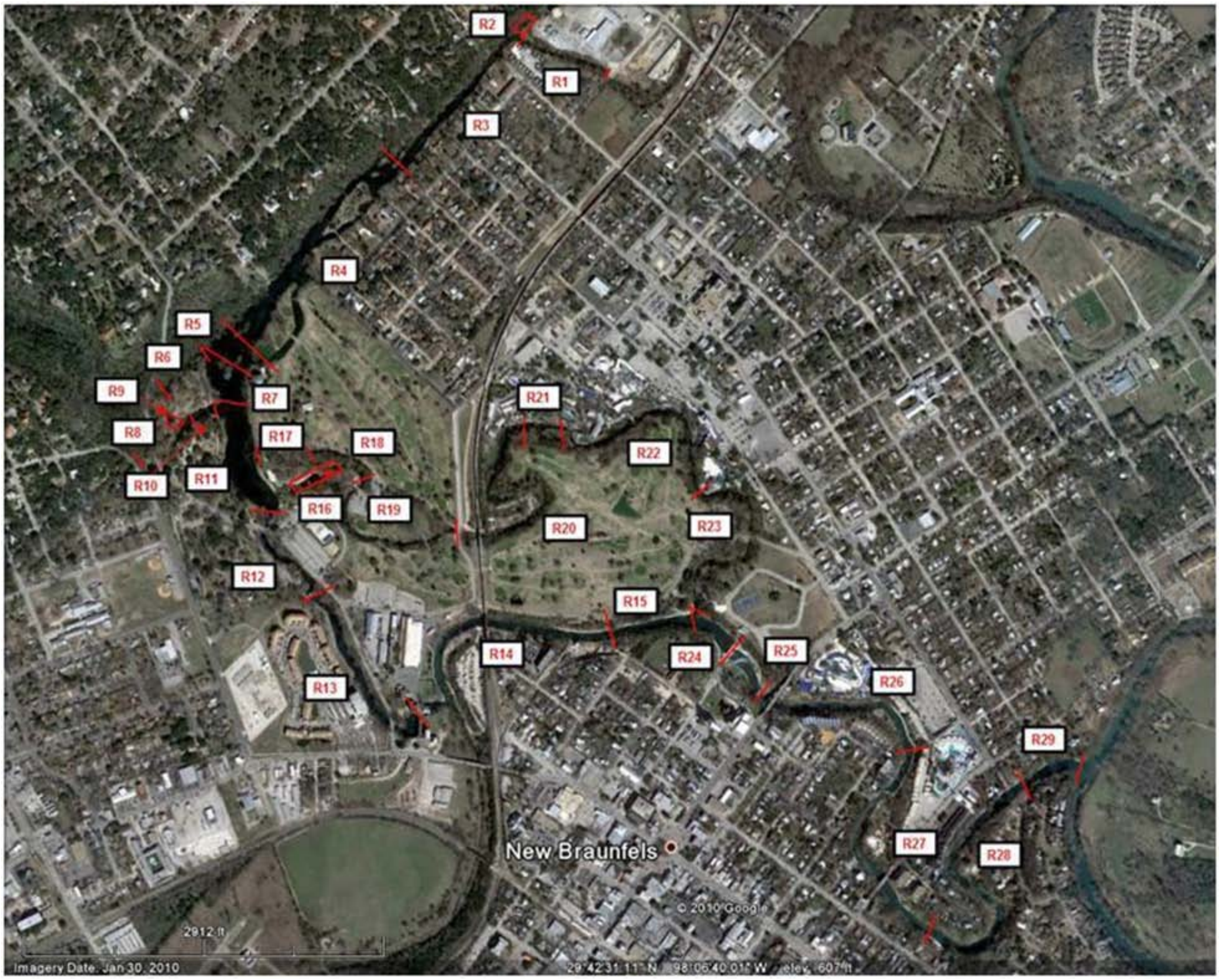

Figure 18. **Qual2E computational river reaches used in modeling the Comal River system (after Hardy et al., 2010).**

Table 7. Comal River QUAL-2E segmentation

Segment	*Name*	*Length (m)*	*Segment*	*Name*	*Length (m)*
1	NW Branch	244	16	OC-Woods	213
2	NE Branch	91	17	OC-Spring fed pool	152
3	Upper Landa	427	18	OC-Below SF pool	61
4	Mid Landa 1	610	19	OC golf course	518
5	Mid Landa 2	61	20	OC Middle 1	610
6	Spring Run 3	152	21	OC Middle 2	152
7	Below SR 3	91	22	OC Lower 1	610
8	SR1 Head	335	23	OC Lower 2	427
9	Spring Run 2	91	24	Above Clemens	122
10	SR1 b/l SR2	152	25	Above USGS weir	122
11	Lower Landa	335	26	Above Vnotched	579
12	Lake to weir	396	27	Above Guadalupe	610
13	Weir to power p	518	28	Above Guadalupe	610
14	Lower new chann	610	29	Above Guadalupe	61
15	Lower new chann	274			

The Comal system is represented by seven headwater inputs and 44 point loads, the later representing various spring sources identified within Landa Lake (Hardy et al., 2010):

Headwaters

1. The NE Branch (Reach 1 – Bleeders Creek),
2. NW Branch (Reach 2),
3. Spring Run 1 (Reach 6),
4. Spring Run 2 (Reach 9),
5. Spring Run 3 (Reach 8),
6. Old Channel outlet (Reach 17) and,
7. The Spring Fed Pool outlet (Reach 16).

Point load locations for Landa Lake springs were taken from the spatial mapping provided in Brune (1981) and assigned to the nearest computational element within each Qual2E reach (Hardy et al., 1998).

Assumed Headwater and Point Loads within the Comal River System

Flow contribution of the 44 point loads associated with various spring sources were estimated according to relative spring size (discharge) as identified in Brune (1981), reported or assumed

spring elevations based on measured bathymetry of Landa Lake, and as a factor of total Comal discharge. Guyton Associates (2004) estimated that Spring Run 3 stops flowing at a total Comal River discharge of approximately 50 cfs, which corresponds to an elevation of 620 feet. Based on Landa Lake bathymetry, headwaters in Reach 1 and 2 were set to 0.01 cfs for simulated flows below a total Comal River discharge of 50 cfs while spring sources in Reach 2 (i.e., point loads) and the first three point loads in Reach 3 were assigned a value of zero, since they are at an elevation above 620 feet. It is also assumed that at flows below 50 cfs, Spring Run 5 (Nolte Apartments) stops flowing since it is approximately six inches above the lake elevation. At total Comal River discharges above 50 cfs, point loads were proportionally increased based on their assumed size (Table 8).

For all simulations, a constant water temperature of 74.5 (F) was assumed for headwater and point load sources with the exception of Reach 1 (Bleeders Creek) headwater inflows, which was assigned an initial value of 80.0 (F) based on temperature monitoring data for summer months. It is recognized that this introduces some bias during the colder months in the current simulations and will be modified for the final simulations based on the ongoing analysis of air and water temperature data at this location.

Table 8. Assumed point load discharges for Landa Lake utilized in the Qual2E modeling runs.

Total Comal Flow (cfs)	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100	105	110	115	120	125	130	135	140	145	150	155	160	165	170
Point Load Flow (cfs)																														
1	0.00	0.00	0.00	0.00	0.00	0.00	1.79	1.95	2.12	2.28	2.44	2.61	2.77	2.94	3.10	3.26	3.43	3.59	3.75	3.92	4.08	3.96	4.11	4.26	4.41	4.57	4.72	4.87	5.02	5.18
2	0.00	0.00	0.00	0.00	0.00	0.00	1.24	1.35	1.47	1.58	1.70	1.81	1.92	2.04	2.15	2.27	2.38	2.49	2.61	2.72	2.83	2.66	2.76	2.86	2.97	3.07	3.17	3.28	3.38	3.48
3	0.00	0.00	0.00	0.00	0.00	0.00	1.24	1.35	1.47	1.58	1.70	1.81	1.92	2.04	2.15	2.27	2.38	2.49	2.61	2.72	2.83	2.66	2.76	2.86	2.97	3.07	3.17	3.28	3.38	3.48
4	0.00	0.00	0.00	0.00	0.00	0.00	0.63	0.69	0.75	0.81	0.87	0.93	0.98	1.04	1.10	1.16	1.22	1.28	1.34	1.40	1.45	1.21	1.25	1.30	1.35	1.40	1.44	1.49	1.54	1.58
5	0.23	0.28	0.33	0.37	0.42	0.26	1.24	1.35	1.47	1.58	1.70	1.81	1.92	2.04	2.15	2.27	2.38	2.49	2.61	2.72	2.83	2.66	2.76	2.86	2.97	3.07	3.17	3.28	3.38	3.48
6	0.23	0.28	0.33	0.37	0.42	0.26	1.24	1.35	1.47	1.58	1.70	1.81	1.92	2.04	2.15	2.27	2.38	2.49	2.61	2.72	2.83	2.66	2.76	2.86	2.97	3.07	3.17	3.28	3.38	3.48
7	0.23	0.28	0.33	0.37	0.42	0.26	1.24	1.35	1.47	1.58	1.70	1.81	1.92	2.04	2.15	2.27	2.38	2.49	2.61	2.72	2.83	2.66	2.76	2.86	2.97	3.07	3.17	3.28	3.38	3.48
8	0.23	0.28	0.33	0.37	0.42	0.26	1.24	1.35	1.47	1.58	1.70	1.81	1.92	2.04	2.15	2.27	2.38	2.49	2.61	2.72	2.83	2.66	2.76	2.86	2.97	3.07	3.17	3.28	3.38	3.48
9	0.23	0.28	0.33	0.37	0.42	0.26	1.24	1.35	1.47	1.58	1.70	1.81	1.92	2.04	2.15	2.27	2.38	2.49	2.61	2.72	2.83	2.66	2.76	2.86	2.97	3.07	3.17	3.28	3.38	3.48
10	0.23	0.28	0.33	0.37	0.42	0.26	1.24	1.35	1.47	1.58	1.70	1.81	1.92	2.04	2.15	2.27	2.38	2.49	2.61	2.72	2.83	2.66	2.76	2.86	2.97	3.07	3.17	3.28	3.38	3.48
11	0.23	0.28	0.33	0.37	0.42	0.26	1.24	1.35	1.47	1.58	1.70	1.81	1.92	2.04	2.15	2.27	2.38	2.49	2.61	2.72	2.83	2.66	2.76	2.86	2.97	3.07	3.17	3.28	3.38	3.48
12	0.23	0.28	0.33	0.37	0.42	0.26	1.24	1.35	1.47	1.58	1.70	1.81	1.92	2.04	2.15	2.27	2.38	2.49	2.61	2.72	2.83	2.66	2.76	2.86	2.97	3.07	3.17	3.28	3.38	3.48
13	0.23	0.28	0.33	0.37	0.42	0.26	1.24	1.35	1.47	1.58	1.70	1.81	1.92	2.04	2.15	2.27	2.38	2.49	2.61	2.72	2.83	2.66	2.76	2.86	2.97	3.07	3.17	3.28	3.38	3.48
14	0.23	0.28	0.33	0.37	0.42	0.26	1.24	1.35	1.47	1.58	1.70	1.81	1.92	2.04	2.15	2.27	2.38	2.49	2.61	2.72	2.83	2.66	2.76	2.86	2.97	3.07	3.17	3.28	3.38	3.48
15	0.23	0.28	0.33	0.37	0.42	0.26	1.24	1.35	1.47	1.58	1.70	1.81	1.92	2.04	2.15	2.27	2.38	2.49	2.61	2.72	2.83	2.66	2.76	2.86	2.97	3.07	3.17	3.28	3.38	3.48
16	0.23	0.28	0.33	0.37	0.42	0.26	1.24	1.35	1.47	1.58	1.70	1.81	1.92	2.04	2.15	2.27	2.38	2.49	2.61	2.72	2.83	2.66	2.76	2.86	2.97	3.07	3.17	3.28	3.38	3.48
17	0.23	0.28	0.33	0.37	0.42	0.26	1.24	1.35	1.47	1.58	1.70	1.81	1.92	2.04	2.15	2.27	2.38	2.49	2.61	2.72	2.83	2.66	2.76	2.86	2.97	3.07	3.17	3.28	3.38	3.48
18	1.48	1.77	2.07	2.36	2.66	2.75	0.47	0.51	0.56	0.60	0.64	0.69	0.73	0.78	0.82	0.86	0.91	0.95	0.99	1.04	1.08	0.84	0.87	0.90	0.93	0.97	1.00	1.03	1.06	1.10
19	1.48	1.77	2.07	2.36	2.66	2.75	0.47	0.51	0.56	0.60	0.64	0.69	0.73	0.78	0.82	0.86	0.91	0.95	0.99	1.04	1.08	0.84	0.87	0.90	0.93	0.97	1.00	1.03	1.06	1.10
20	0.35	0.42	0.49	0.56	0.64	0.50	0.81	0.85	0.89	0.93	0.97	1.01	1.05	1.09	1.12	1.16	1.20	1.24	1.28	1.32	1.36	1.11	1.13	1.16	1.19	1.22	1.24	1.27	1.30	1.33
21	1.48	1.77	2.07	2.36	2.66	2.75	0.47	0.51	0.56	0.60	0.64	0.69	0.73	0.78	0.82	0.86	0.91	0.95	0.99	1.04	1.08	0.84	0.87	0.90	0.93	0.97	1.00	1.03	1.06	1.10
22	1.48	1.77	2.07	2.36	2.66	2.75	0.47	0.51	0.56	0.60	0.64	0.69	0.73	0.78	0.82	0.86	0.91	0.95	0.99	1.04	1.08	0.84	0.87	0.90	0.93	0.97	1.00	1.03	1.06	1.10
23	1.48	1.77	2.07	2.36	2.66	2.75	0.47	0.51	0.56	0.60	0.64	0.69	0.73	0.78	0.82	0.86	0.91	0.95	0.99	1.04	1.08	0.84	0.87	0.90	0.93	0.97	1.00	1.03	1.06	1.10
24	1.48	1.77	2.07	2.36	2.66	2.75	1.33	1.45	1.57	1.70	1.82	1.94	2.06	2.19	2.31	2.43	2.55	2.67	2.80	2.92	3.04	2.87	2.98	3.10	3.21	3.32	3.43	3.54	3.65	3.76
25	1.48	1.77	2.07	2.36	2.66	2.75	1.33	1.45	1.57	1.70	1.82	1.94	2.06	2.19	2.31	2.43	2.55	2.67	2.80	2.92	3.04	2.87	2.98	3.10	3.21	3.32	3.43	3.54	3.65	3.76
26	0.91	1.09	1.27	1.46	1.64	1.62	1.33	1.45	1.57	1.70	1.82	1.94	2.06	2.19	2.31	2.43	2.55	2.67	2.80	2.92	3.04	2.87	2.98	3.10	3.21	3.32	3.43	3.54	3.65	3.76
27	0.91	1.09	1.27	1.46	1.64	1.62	1.33	1.45	1.57	1.70	1.82	1.94	2.06	2.19	2.31	2.43	2.55	2.67	2.80	2.92	3.04	2.87	2.98	3.10	3.21	3.32	3.43	3.54	3.65	3.76
28	0.91	1.09	1.27	1.46	1.64	1.62	1.33	1.45	1.57	1.70	1.82	1.94	2.06	2.19	2.31	2.43	2.55	2.67	2.80	2.92	3.04	2.87	2.98	3.10	3.21	3.32	3.43	3.54	3.65	3.76
29	0.91	1.09	1.27	1.46	1.64	1.62	1.33	1.45	1.57	1.70	1.82	1.94	2.06	2.19	2.31	2.43	2.55	2.67	2.80	2.92	3.04	2.87	2.98	3.10	3.21	3.32	3.43	3.54	3.65	3.76
30	0.91	1.09	1.27	1.46	1.64	1.62	1.33	1.45	1.57	1.70	1.82	1.94	2.06	2.19	2.31	2.43	2.55	2.67	2.80	2.92	3.04	2.87	2.98	3.10	3.21	3.32	3.43	3.54	3.65	3.76
31	0.91	1.09	1.27	1.46	1.64	1.62	1.33	1.45	1.57	1.70	1.82	1.94	2.06	2.19	2.31	2.43	2.55	2.67	2.80	2.92	3.04	2.87	2.98	3.10	3.21	3.32	3.43	3.54	3.65	3.76
32	0.91	1.09	1.27	1.46	1.64	1.62	1.33	1.45	1.57	1.70	1.82	1.94	2.06	2.19	2.31	2.43	2.55	2.67	2.80	2.92	3.04	2.87	2.98	3.10	3.21	3.32	3.43	3.54	3.65	3.76
33	0.91	1.09	1.27	1.46	1.64	1.62	1.33	1.45	1.57	1.70	1.82	1.94	2.06	2.19	2.31	2.43	2.55	2.67	2.80	2.92	3.04	2.87	2.98	3.10	3.21	3.32	3.43	3.54	3.65	3.76
34	0.91	1.09	1.27	1.46	1.64	1.62	1.33	1.45	1.57	1.70	1.82	1.94	2.06	2.19	2.31	2.43	2.55	2.67	2.80	2.92	3.04	2.87	2.98	3.10	3.21	3.32	3.43	3.54	3.65	3.76
35	0.91	1.09	1.27	1.46	1.64	1.62	1.33	1.45	1.57	1.70	1.82	1.94	2.06	2.19	2.31	2.43	2.55	2.67	2.80	2.92	3.04	2.87	2.98	3.10	3.21	3.32	3.43	3.54	3.65	3.76
36	0.91	1.09	1.27	1.46	1.64	1.62	1.33	1.45	1.57	1.70	1.82	1.94	2.06	2.19	2.31	2.43	2.55	2.67	2.80	2.92	3.04	2.87	2.98	3.10	3.21	3.32	3.43	3.54	3.65	3.76
37	0.01	0.01	0.01	0.01	0.01	2.23	2.45	2.67	2.89	3.12	3.34	3.56	3.78	4.01	4.23	4.45	4.67	4.90	5.12	5.34	5.56	5.79	6.01	6.23	6.45	6.68	6.90	7.12	7.34	7.57
38	0.01	0.01	0.01	0.01	0.01	2.23	2.45	2.67	2.89	3.12	3.34	3.56	3.78	4.01	4.23	4.45	4.67	4.90	5.12	5.34	5.56	5.79	6.01	6.23	6.45	6.68	6.90	7.12	7.34	7.57
39	0.93	1.12	1.31	1.49	1.68	1.87	3.30	3.60	3.90	4.20	4.50	4.80	5.10	5.40	5.70	6.00	6.30	6.60	6.90	7.20	7.50	7.80	8.10	8.40	8.70	9.00	9.30	9.60	9.90	10.20
40	0.23	0.28	0.33	0.37	0.42	0.26	0.42	0.46	0.50	0.54	0.58	0.62	0.66	0.70	0.73	0.77	0.81	0.85	0.89	0.93	0.97	2.98	3.10	3.21	3.33	3.44	3.56	3.67	3.79	3.90
41	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	1.25	1.30	1.34	1.39	1.44	1.49	1.54	1.58	1.63
42	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	1.25	1.30	1.34	1.39	1.44	1.49	1.54	1.58	1.63
43	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	1.25	1.30	1.34	1.39	1.44	1.49	1.54	1.58	1.63
44	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	1.25	1.30	1.34	1.39	1.44	1.49	1.54	1.58	1.63

Meteorological Data

Hourly meteorological data (net solar radiation, cloudiness, dry and wet bulb temperature, barometric pressure, and wind speed) from the New Braunfels Airport was utilized for calibration and simulation for the period January 1, 2003 through December 31, 2014. Missing hourly values were interpolated from adjacent time steps for short periods or substituted from similar overall metrological periods based on antecedent or post daily values when more than 2 days long. The hourly data was reduced to every 3 hours for use in the Qual2E simulations.

Calibration

The 2009 water year was retained for calibration because it represented an extended hot and dry condition during the low flow summer period and empirical water temperature data was available for key locations within the Comal River for the purpose of model calibration (e.g., Figure 19). Calibration of the water temperature model focused on July as this coincided with both low flows and highest observed water and air temperatures that are anticipated to represent the most limiting conditions for fountain darters.

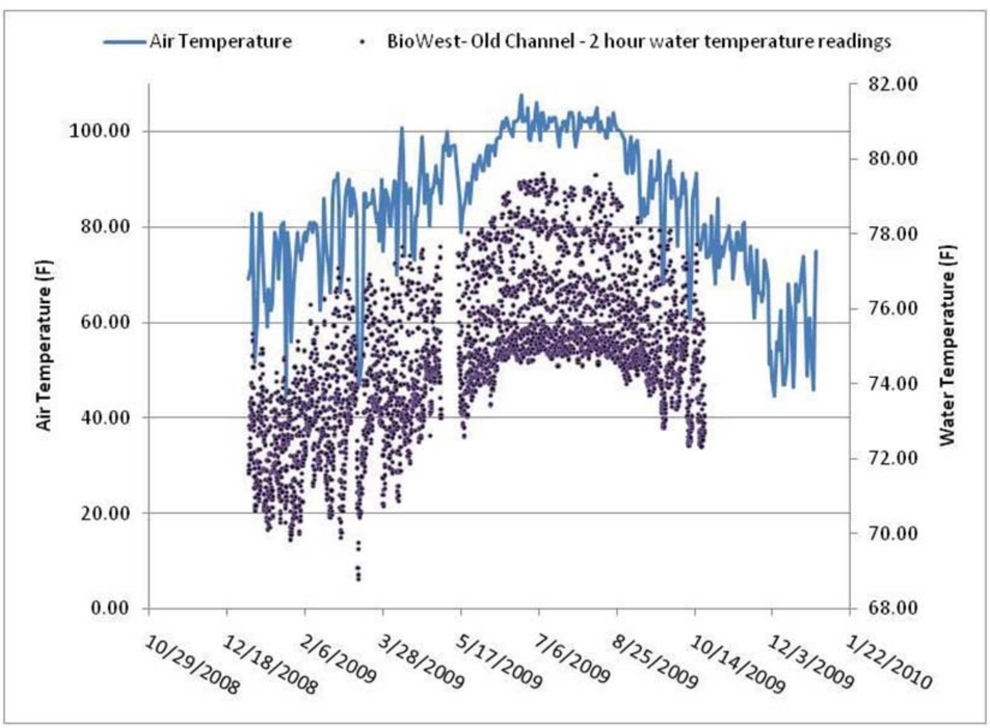

Figure 19. **Maximum Daily Air Temperature and 2 hour interval recorded water temperatures from the Comal River in the Old Channel (BIO-WEST thermograph data).**

Qual2E was run in dynamic simulation mode to estimate the hourly water temperatures and compared to the available thermograph data at key locations within the Comal River system. Initial calibration runs were made at a total Comal River discharge of 165 cfs as this was the July 2009 average discharge and flows in the old channel were set to 45 cfs based on measurements by BIO-WEST on July 2, 2009. Examples of simulated and observed hourly water temperatures at three key locations are provided in Figures 20 through 22.

The results demonstrate that the simulated water temperatures at the calibration flows (old and new channel) are within approximately 1.0 to 0.5 degrees (F) over the entire 31 day simulation period. The calibrated Qual2E model was used to simulate the hourly temperatures and dissolved oxygen throughout the Comal River from January 1, 2003 through December 31, 2014 using the assumed flow splits and flow contributions as noted below. In order to accommodate computational efficiency within the ecomodel, the estimated daily flows were aggregated to 7 day averages as described below. This flow value was used to set the corresponding daily headwater and point load contributions and corresponding flows within the Old Channel for use in Qual2E. The model was then used to simulate the hourly temperature and DO using this weekly constant flow rate, while the 3 hourly meteorological data was allowed to vary day to day.

Simulation results were post processed to extract the daily minimum, average, and maximum water temperatures and minimum DO within the Qual2E reaches that corresponded to the ecomodel reaches. It was assumed that given the relatively short ecomodel reaches that the corresponding Qual2E simulation results could be applied uniformly to all computational cells within the ecomodel reach.

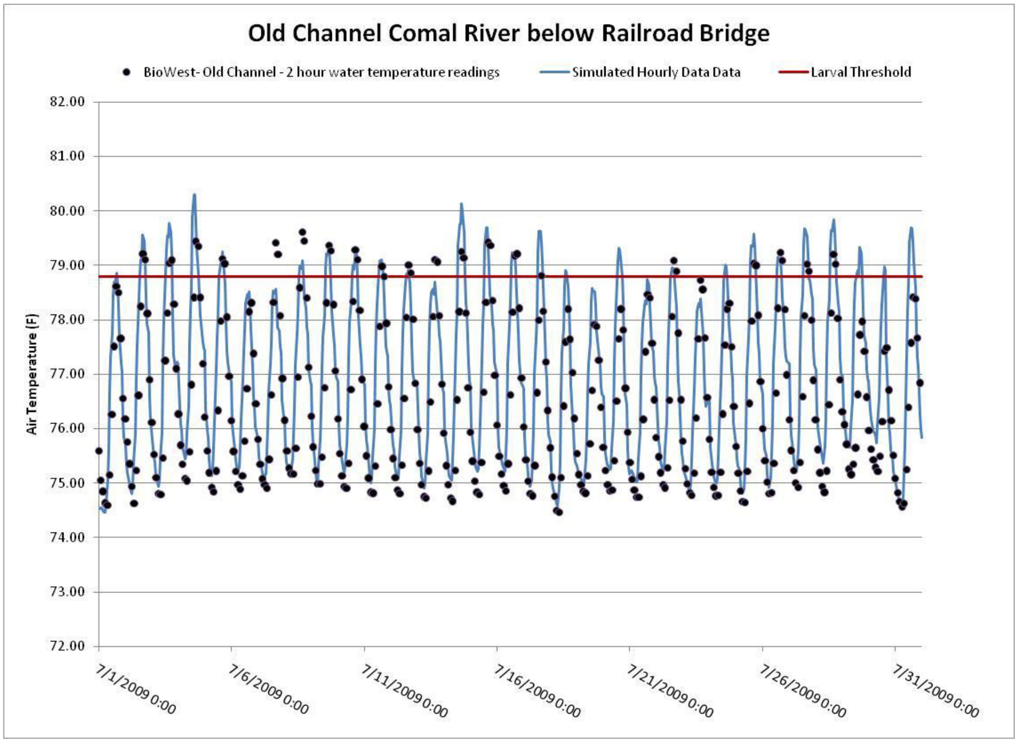

Figure 20. **Simulated and observed water temperatures in the Old Channel of the Comal River during July 2009.**

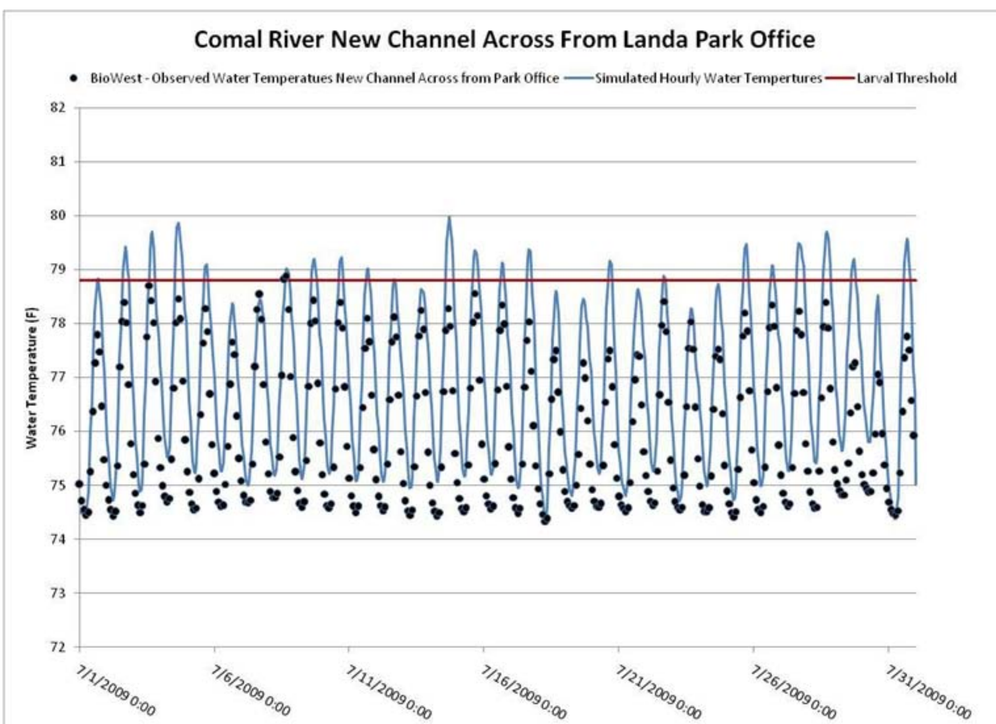

Figure 21. **Simulated and Observed Water Temperatures in the New Channel of the Comal River during July 2009.**

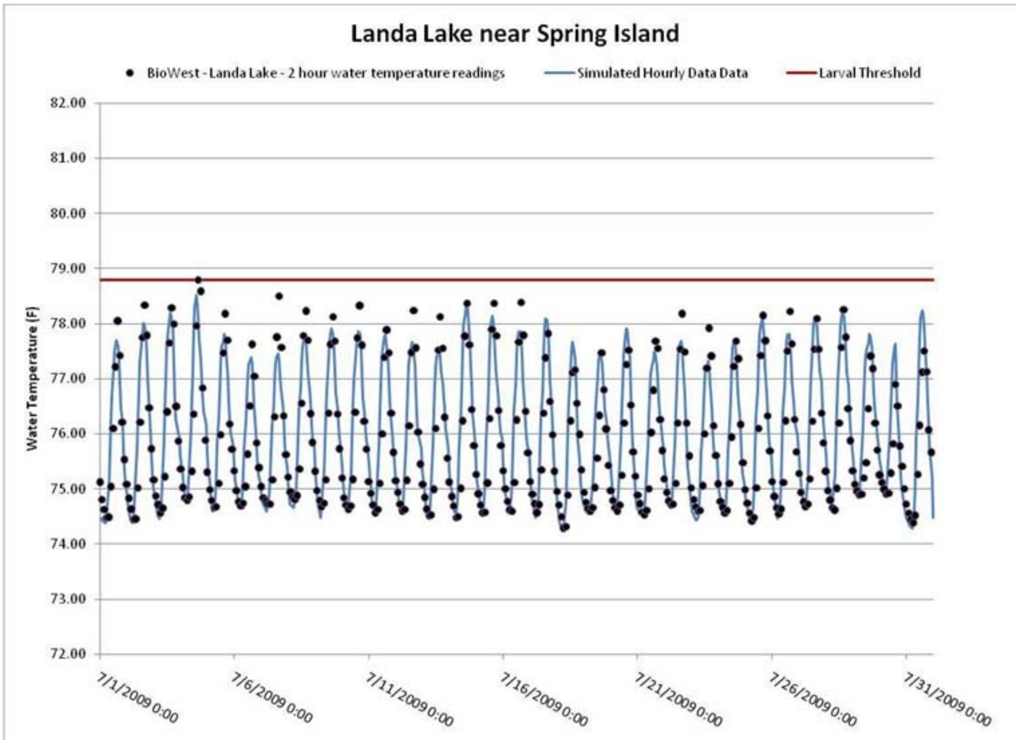

Figure 22. **Simulated and observed water temperatures in Landa Lake near Spring Island during July 2009.**

San Marcos River

Historic Water Temperatures

Thermograph data from City Park was utilized to construct the hourly time series of water temperatures for the simulation period. Missing values were generated using two approaches. In those instances where less than three hours were missing, the missing values were linearly interpolated from adjacent values. For longer periods of missing values, regression equations based on the difference between hourly air and water temperatures were developed on a monthly basis (e.g., Figure 23; Table 9). Over 96 percent of the predicted hourly water temperatures based on the regression equations were within 1 degree of measured data. The hourly water temperature data was then reduced to the minimum, average and maximum daily water.

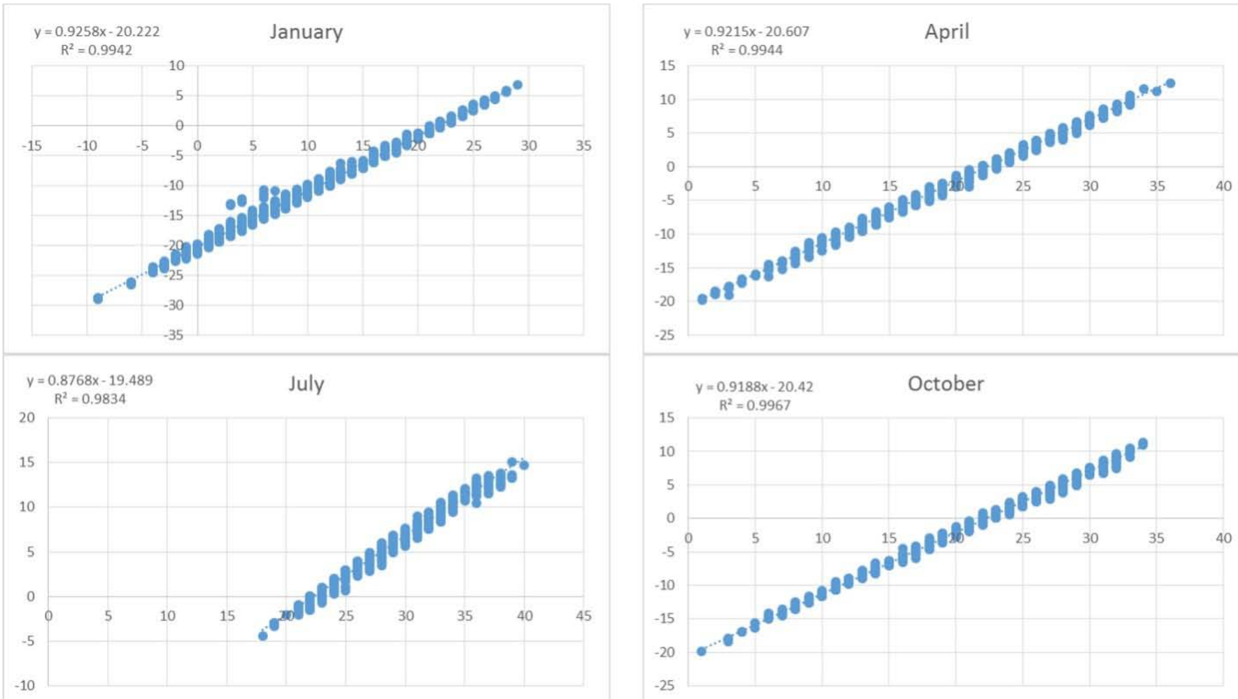

Figure 23. **Example regressions between air temperatures and the difference between air and water temperatures used to estimate hourly missing water temperatures at the City Park study site in the San Marcos River.**

Table 9. **Regression equations and r2 for prediction equations of the difference between air and water temperatures on a monthly basis for City Park in the San Marcos River.**

	Equation	R^2
Jan	y = 0.9258x - 20.222	0.994
Feb	y = 0.9269x - 20.423	0.996
Mar	y = 0.9224x - 20.514	0.995
Apr	y = 0.9215x - 20.607	0.994
May	y = 0.9164x - 20.523	0.992
Jun	y = 0.8906x - 19.837	0.998
Jul	y = 0.8768x - 19.489	0.983
Aug	y = 0.8855x - 19.632	0.984
Sep	y = 0.9059x - 20.188	0.990
Oct	y = 0.9188x - 20.420	0.997
Nov	y = 0.9272x - 20.378	0.996
Dec	y = 0.9288x - 20.262	0.996

Modeled Water Temperatures and Dissolved Oxygen

The physical reach structure of the San Marcos Qual2E model is shown in Figure 24.

The individual reaches are summarized in Table 10. The two ecomodel simulation

reaches for the San Marcos are represented by City Park (Reach 7) and I35 (Reach 9). This interim report only provides simulation results for the City Park Reach. The I35 ecomodel reach will be incorporated into the final report.

Figure 24. Qual2E computational river reaches used in modeling the San Marcos River system (after Hardy et al., 2010).

Table 10. San Marcos River QUAL-2E segmentation

Segment	*Name*	*Length (m)*	*Segment*	*Name*	*Length (m)*
1	Upper Mn Spr Lk	396	12	Below Cape's Dam	610
2	Upper Spr Lk Sl	213	13	State Hatchery	518
3	Mid Spr Lk Slou	518	14	Mill Race	579
4	Lower Spr Lk Sl	244	15	Lower SM A	183
5	Lower Spring Lk	244	16	Lower SM B	610
6	University Drive	305	17	Lower SM C	610
7	City Park	610	18	Lower SM D	610
8	Above Rio Vista	610	19	Lower SM E	610
9	Below Rio Vista	549	20	Lower SM F	610
10	Glover's Ditch	335	21	Lower SM G	610
11	Above Capes Dam	549			

The San Marcos system is represented by four headwater reaches and four point loads as follows:

<u>Headwaters</u>

1. Spring Lake Headwater (Reach 1),
2. Spring Lake Slough Headwater (Reach 2),
3. Glover's Ditch Headwater (Reach 10),
4. Mill Race Diversion Headwater (Reach 14),

<u>Point Loads</u>

1. Sessoms Creek Point load,
2. Mill Race Discharge Point load,
3. State Fish Hatchery Point load,
4. San Marcos Wastewater Treatment Plant Point load

Assumed Spring Flows for San Marcos Headwater and Point Loads

Individual spring flows within Spring Lake were treated as a single incremental inflow within Reach 1. This approach within Qual2E assumes that the total discharge is distributed along the entire reach length which closely approximates the spatial distribution of springs within Spring Lake (Hardy et al., 2010). This is considered a pragmatic assumption given the available data on spring flows (Guyton Associates, 2004) and lack of quantitative data on individual spring flow discharges with changes in total San Marcos River discharge. Changes in total San Marcos discharge were modeled by

changes to the headwaters and incremental inflow values within Reach 1 as shown in Table 11.

Table 11. Assumed headwater and point load discharges for the San Marcos River.

San Marcos Discharge (cfs)	45	50	55	60	65	70	75	80	85	90	100	110	120	130
Spring Lake Headwater	3.1	3.4	3.8	4.1	4.4	4.8	5.1	5.5	5.8	6.1	6.8	7.5	8.2	8.9
Incremental Inflow Reach 1	41.9	46.6	51.3	55.9	60.6	65.2	69.9	74.5	79.2	83.9	93.2	102.5	111.8	121.1
Spring Lake Slough	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Sessoms Creek	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
State Fish Hatchery	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0
Wastewater Plant	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6

Meteorological Data

Hourly meteorological data (net solar radiation, cloudiness, dry and wet bulb temperature, barometric pressure, and wind speed) from the San Marcos Airport was utilized for calibration and simulation for the period January 1, 2003 through December 31, 2014. Missing hourly values were interpolated from adjacent time steps for short periods or substituted from similar periods based on antecedent or post daily values when more than 2 days long. The hourly data was reduced to every 3 hours for use in the Qual2E simulations.

Calibration

The 2009 water year was retained for calibration because it represented an extended hot and dry condition during the low flow summer period and empirical water temperature data was available for key locations within the San Marcos River for the purpose of model calibration (e.g., Figure 25). Calibration of the water temperature model focused on July as this coincided with both low flows and highest observed water and air temperatures that are anticipated to represent the most limiting conditions for fountain darters.

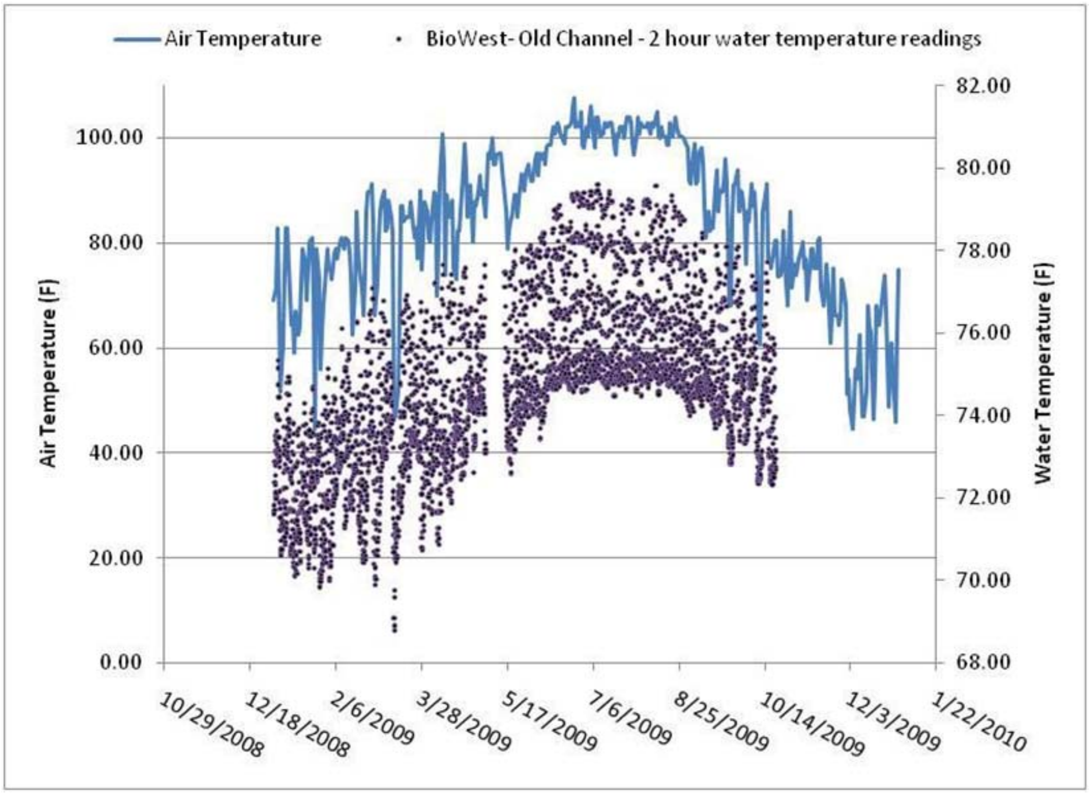

Figure 25. **Maximum Daily Air Temperature and 4 hour interval recorded water temperatures from the San Marcos River at City Park (BIO-WEST thermograph data).**

Qual2E was run in dynamic simulation mode to estimate the hourly water temperatures and compared to the available thermograph data at key locations within San Marcos River system. Initial calibration runs were made at a total San Marcos River discharge of 89 cfs as this was the July 2009 average discharge. Examples of simulated and observed hourly water temperatures at three key locations are provided in Figure 26 and Figure 27.

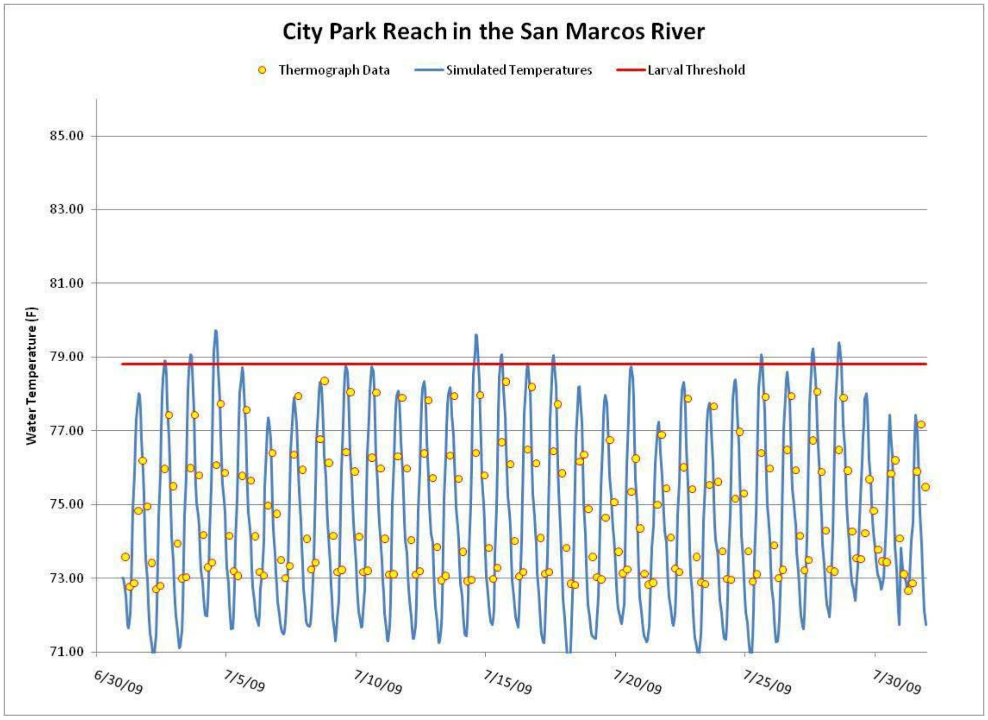

Figure 26. **Simulated and observed water temperatures in City Park, San Marcos River during July 2009.**

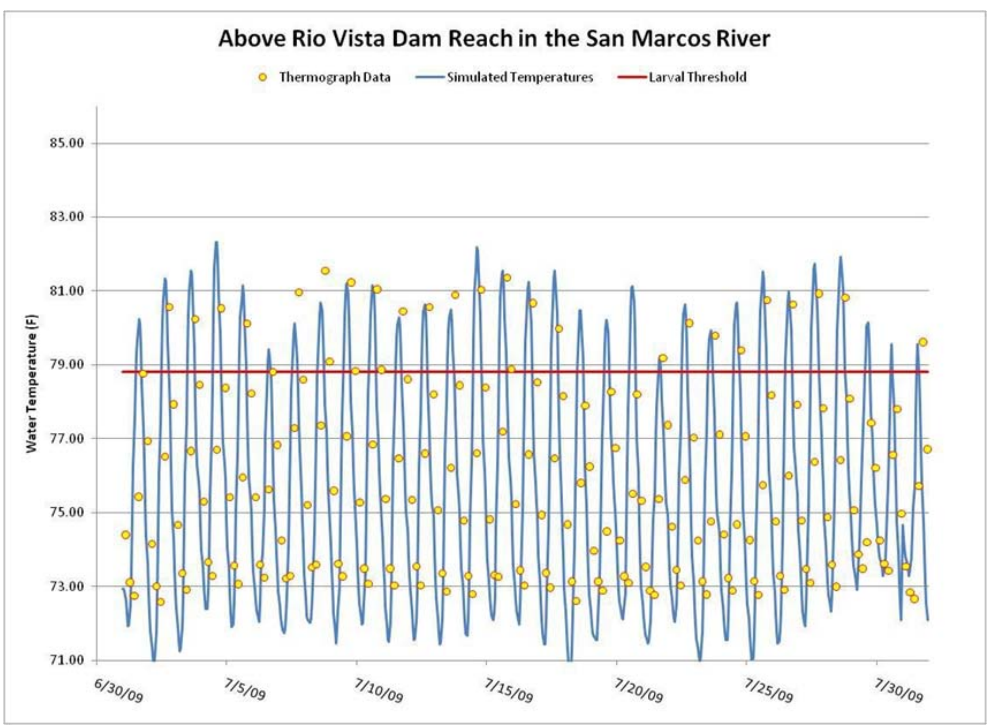

Figure 27. **Simulated and observed water temperatures above Rio Vista, San Marcos River during July 2009.**

The results demonstrate that the simulated water temperatures at the calibration flow are within approximately 1.0 to 0.5 degrees (F) over the entire 31 day simulation period. The calibrated Qual2E model was used to simulate the hourly temperatures and dissolved oxygen throughout the San Marcos River from January 1, 2003 through December 31, 2014 using the assumed headwater and point load flow contributions as noted above. In order to accommodate computational efficiency within the ecomodel, the daily flow values were used to compute 7 day averages for use in the simulations as noted below.

Simulation results were post processed to extract the daily minimum, average, and maximum water temperatures and minimum DO within the Qual2E reach that corresponded to the ecomodel reach. It was assumed that given the relatively short ecomodel reach (City Park) that the corresponding Qual2E simulation results could be applied uniformly to all computational cells within the ecomodel reach.

2.4 Submerged Aquatic Vegetation

Submerged aquatic vegetation (SAV) is considered one of the major drivers of fountain darter population dynamics by serving as shelter and by providing habitat for aquatic invertebrate prey items. Given the importance of SAV in the fountain darter life cycle, understanding the factors that affect SAV persistence is paramount for successful aquatic ecosystem management in the Comal and San Marcos Rivers. Its role in the overall conceptual model of fountain darters is shown in Figure 28. A detailed conceptual model of the aquatic vegetation component alone is displayed in Figure 29. We re-emphasize that this model has full time-space depiction, but these dimensions of the model are suppressed in these causal-flow diagrams for clarity.

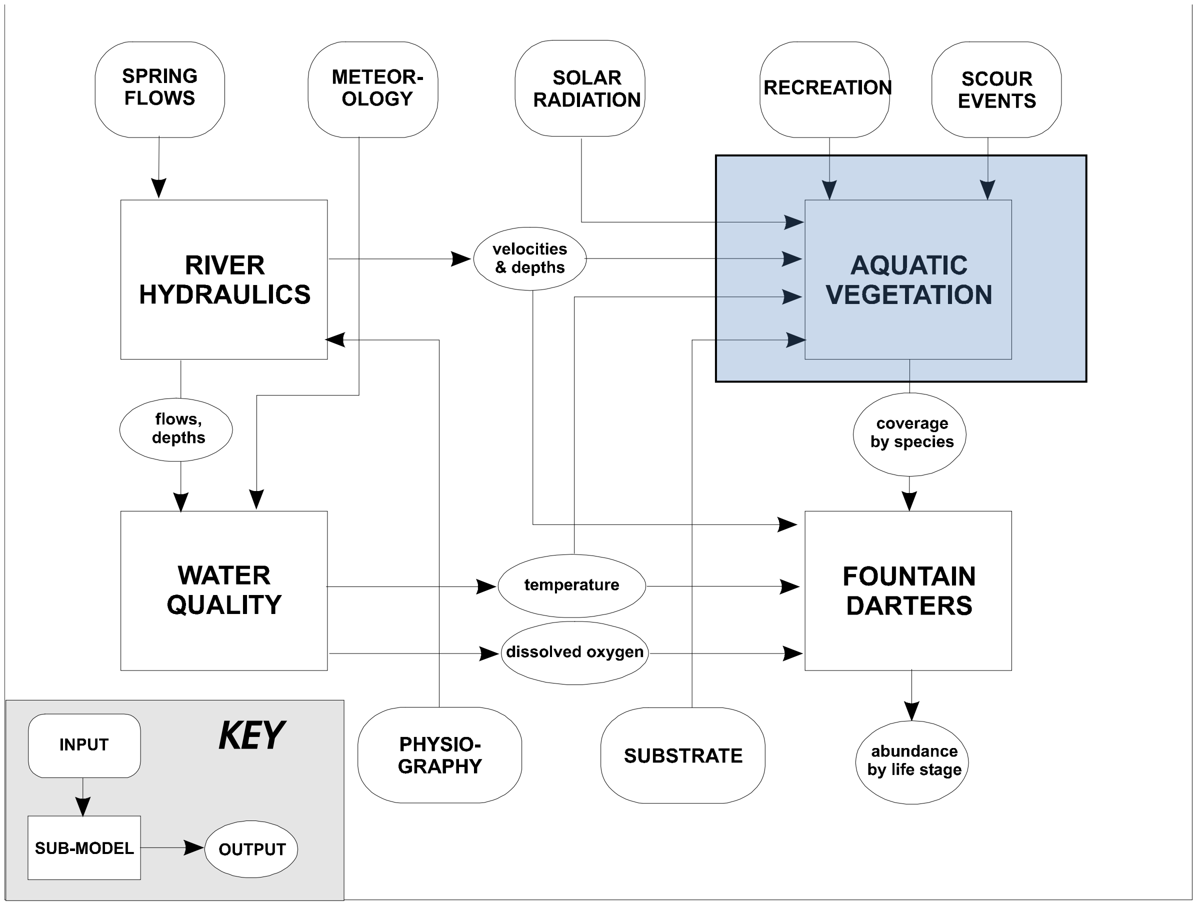

Figure 28. Conceptual Model highlighting Aquatic Vegetation

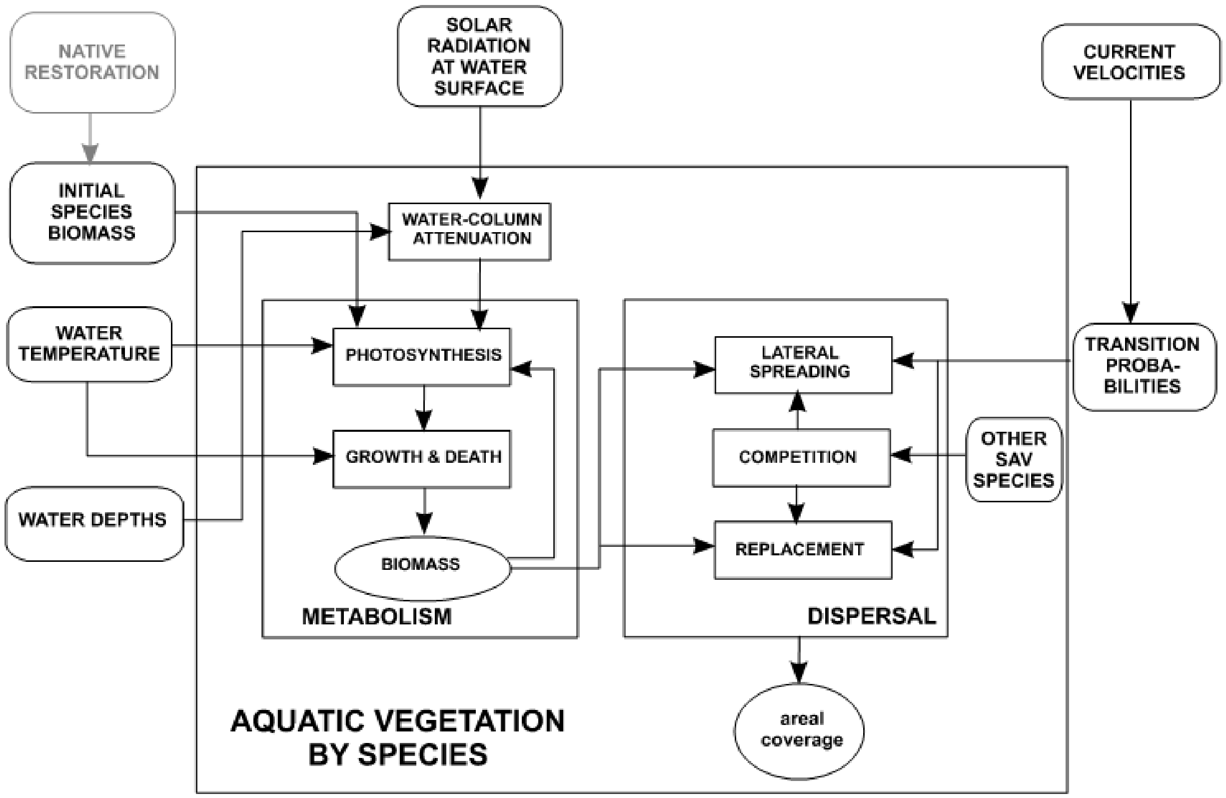

Figure 29. Aquatic Vegetation Conceptual submodel

The SAV modeling effort first explored whether existing models could be integrated into the fountain darter model. Six existing SAV models were evaluated for their use as components (i.e., submodels) in the San Marcos and Comal ecosystem models. Four models were developed by the US Army Engineer Research and Development Center (henceforth ERDC) – *Hydrilla sp*. (Best and Boyd, 1996), *Mirophyllum sp*. (Best and Boyd, 1999), *Vallisneria americana* (Best and Boyd, 2001, 2007, 2008), and *Stuckenia pectinata* (syn. *Potamogeton pectinatus*) (Best and Boyd, 2003). For detailed summaries of the ERDC models see specific model descriptions cited above and Best et al. (2011) for the generalized approach. The other two (MEGAPLANT (Scheffer et al., 1993), and Charisma (van Nes et al., 2003) were developed to model growth of plants in northern Europe. The models were evaluated for their overall ability to meet the objectives of this study, and specific consideration was given to how it could be integrated with the hydraulic and fountain darter submodels.

The aquatic vegetation models have the same general structure: they are spatially-implicit, bioenergetic-based, carbon-growth, mechanistic models that simulate biomass dynamics under various environmental conditions. The ERDC models focused on single species, but the other two were more generalized and could be parameterized for multiple taxa. For each model, biomass accumulation (measured as dry matter accumulation, including subterranean tuber formation) is a function of irradiance, temperature, carbon dioxide (CO_2) availability, and taxa-specific plant characteristics. Growth is assumed to occur in a pest-, disease-, and competitor- free environment. The collective focus of these models was to develop a suite of quantitative tools to understand environmental impact that management has on biomass accumulation of the respective species.

The questions being asked of the San Marcos and Comal ecosystem models are inherently spatial (e.g., how do fountain darter populations redistribute themselves with changes in vegetative cover?; how does the vegetative community redistribute itself over time naturally or as a result of disturbance?). Therefore, spatial processes such as dispersal and recolonization should be included within the SAV models in order to address these questions. Both the hydraulic and fountain darter models are spatially-explicit, and operate at the same spatial scale. Model integration would be relatively seamless if vegetation was modeled at the same spatial scale as well. However, the existing models were spatially implicit and did not explicitly consider space. Two different strategies were considered for implementing and integrating an existing model into a spatial framework compatible with the hydrodynamic and fountain darter models:

(1) Utilizing a model integration framework to link the fountain darter, hydraulic, and vegetation models.
(2) Reprogramming the vegetation models in the same platform as the fountain darter model (NetLogo, an object oriented language used for spatially-explicit modeling)

Model integration frameworks, such as Open Modeling Interface (OPEN MI), FRAMES, or the Object Modeling System (OMS), are designed to integrate models without changing existing model structure or code. Briefly, models are encapsulated within the integration framework, and user-designed input-output structures pass relevant information to-and-from

models as needed at the appropriate time steps and spatial scales. For the ecomodel, this approach would involve choosing the appropriate integration platform, determining how the models should communicate amongst each other (e.g., what information, and how often, is output from the hydraulic model passed to the fountain darter and vegetation models), and determining how the feedbacks among the models work. The strength of model integration lies in taking advantage of existing models. However, since both the fountain darter and vegetation models needed to be converted to spatially-explicit versions, utilizing this approach was not feasible.

Given the familiarity of the Team with object oriented programming, and that the fountain darter model was being reprogrammed in NetLogo (a language common among the modeling team) the Team chose to evaluate the feasibility of reprogramming one of the models into this language. Given the similarities in the code structure among all the aquatic vegetation models, we chose to recode the Vallisneria model (henceforth VALLA) as a test case, for two reasons: (1) Vallisneria is a common species in the system, and (2) the most recent version of the model (Best and Boyd, 2007, 2008) contains the impact of flow on biomass accumulation. In order to facilitate future modeling efforts, VALLA was reprogrammed using the spatial domain and input parameters of the fountain darter model. Input parameters include time series of hydrodynamic variables and aquatic vegetation maps from 2003 to 2008.

There were several issues with a direct conversion of VALLA to a spatially-explicit version.

(1) Originally, there was not a method to quantitatively represent the relationship between biomass and spatial coverage. For example, a 15 g increase in biomass cannot be directly correlated with a concurrent change in spatial coverage. Without understanding this relationship, spatial coverage cannot be projected with any degree of accuracy from the VALLA model, which is crucial since spatial coverage is currently thought to be a major driver in fountain darter population dynamics.

(2) VALLA does not model dispersal or species-species interactions. The vegetative communities of San Marcos and Comal systems are incredibly dynamic and the community composition can change over the course of a single year (Figures 30

through 32). These processes must be included in the model in order to capture the dynamics of these systems.

(3) VALLA was parameterized from data from the northern phenotype of Vallisneria, which produces overwintering buds, whereas the southern phenotype does not. Within the existing model, the formation of winter buds controls spring biomass. This process is hard coded into the model and removing it, which would be necessary to represent the southern phenotype, would fundamentally change the structure of the model. Likewise, the other SAV models were not parameterized in southern climes.

(4) All of the existing models were calibrated for lentic systems with variable temperatures, turbidity, and nutrient concentrations. The San Marcos and Comal systems are spring-fed, relatively temperature-constant, clear water systems without extreme nutrient limitations. As a result, the parameterization of the existing models are inappropriate for the San Marcos and Comal systems. Further, the existing models contain other processes which may be superfluous given the objectives of the San Marcos and Comal ecosystem models. An example includes computing below-ground biomass on daily time step, which likely doesn't impact fountain darter dynamics.

(5) Implementing VALLA on the fine spatial scale of the fountain darter model increased computational time to the point of computational intractability (the model cannot execute within a reasonable time). The dynamism of the plant communities of the San Marcos and Comal rivers (see Figures 30 through 32) requires the model contain components that account for stochastic disturbance events, such as scouring, and recolonization after such events.

(6) In order to meet the overall objectives of the ecomodel, the vegetation model must be able to address how vegetation interacts with other ecosystem components, including the fountain darter. Since the presence of aquatic vegetation and structure of the vegetative community are important drivers in fish distributions (Rossier et al., 1996), these components must be included within the vegetation submodels.

Based on this analysis, the team decided to develop a custom model that captures the critical processes of the vegetation communities within the San Marcos and Comal ecosystems. The aquatic vegetation model is a spatially-explicit, agent-based model, programmed in NetLogo (the same language as the fountain darter model). The model is driven by environmental and physical parameters including:

- Temperature
- Light (including the effect of turbidity)
- Velocity
- Depth
- Substrate

Growth and senescence are based on the relevant functions from the ERDC, MEGAPLANT, and Charisma models, but simplified using threshold-based equations when appropriate (see below). Generalized functions for partitioning biomass were modified from Teh (2006).

Plant dispersal is a poorly understood process, and will be modeled using empirical data. We attempted to follow the mathematical framework established by Wang et al. (2011, 2012) (Figure 33), which quantifies both local (intra-cell) and regional (inter-cell) changes in spatial coverage of terrestrial vegetation, including representing (A) local growth via a logistic equation where r_i represents the spread rate of species i, and κ is the percentage of land cover), (B) intercellular dispersal, where k_{ij} is the dispersal from cell i to cell j, (C) synthesis of the two processes into a spatially-explicit agent-based framework, but these processes did not adequately capture the dynamics of the aquatic vegetation in the San Marcos and Comal systems. Thus, recolonization is parameterized based on an analysis from vegetation colonization/recolonization data accumulated from 2003 to 2008.

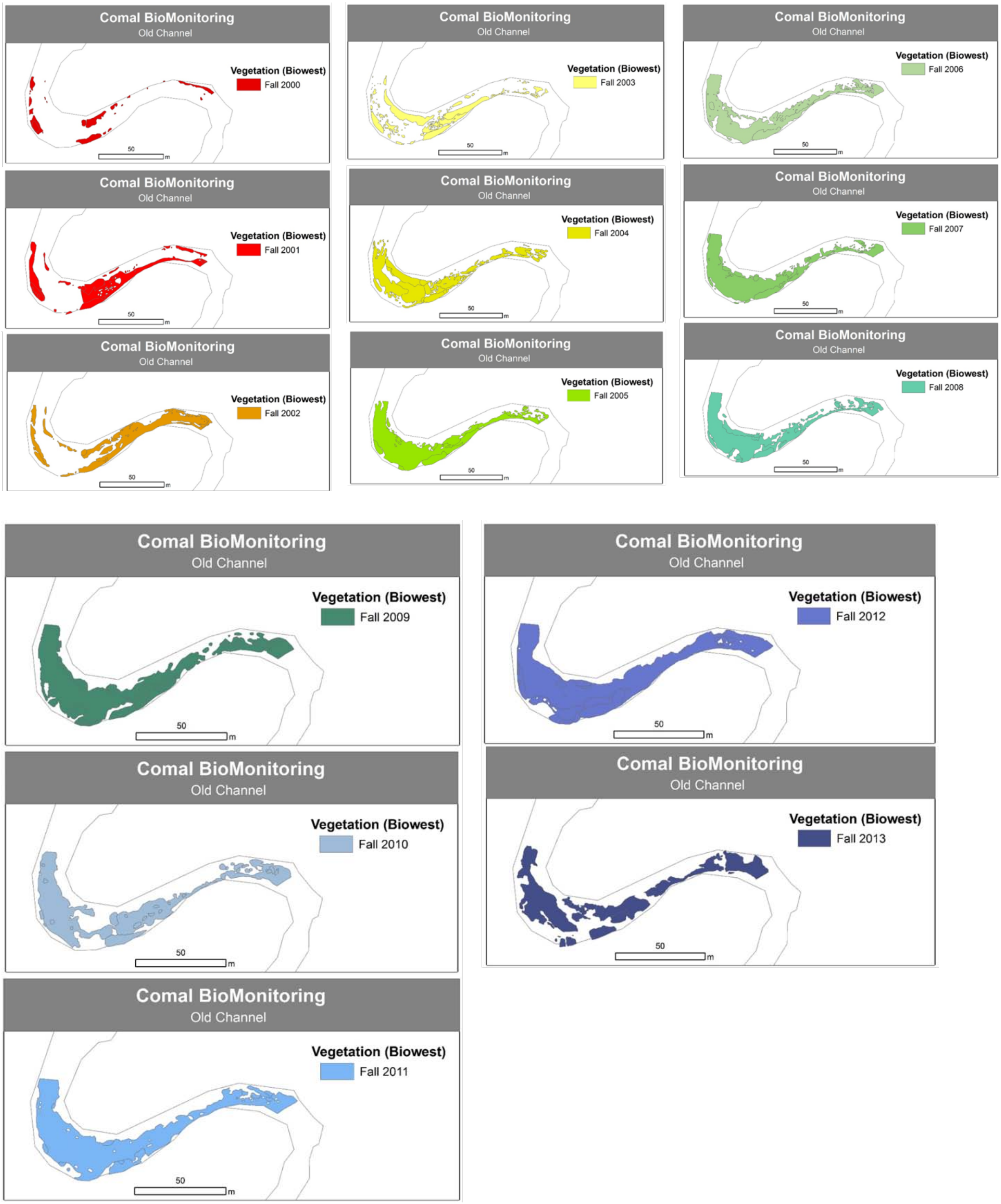

Figure 30. Shapefiles of vegetative coverage for the Old Channel in the Comal River System

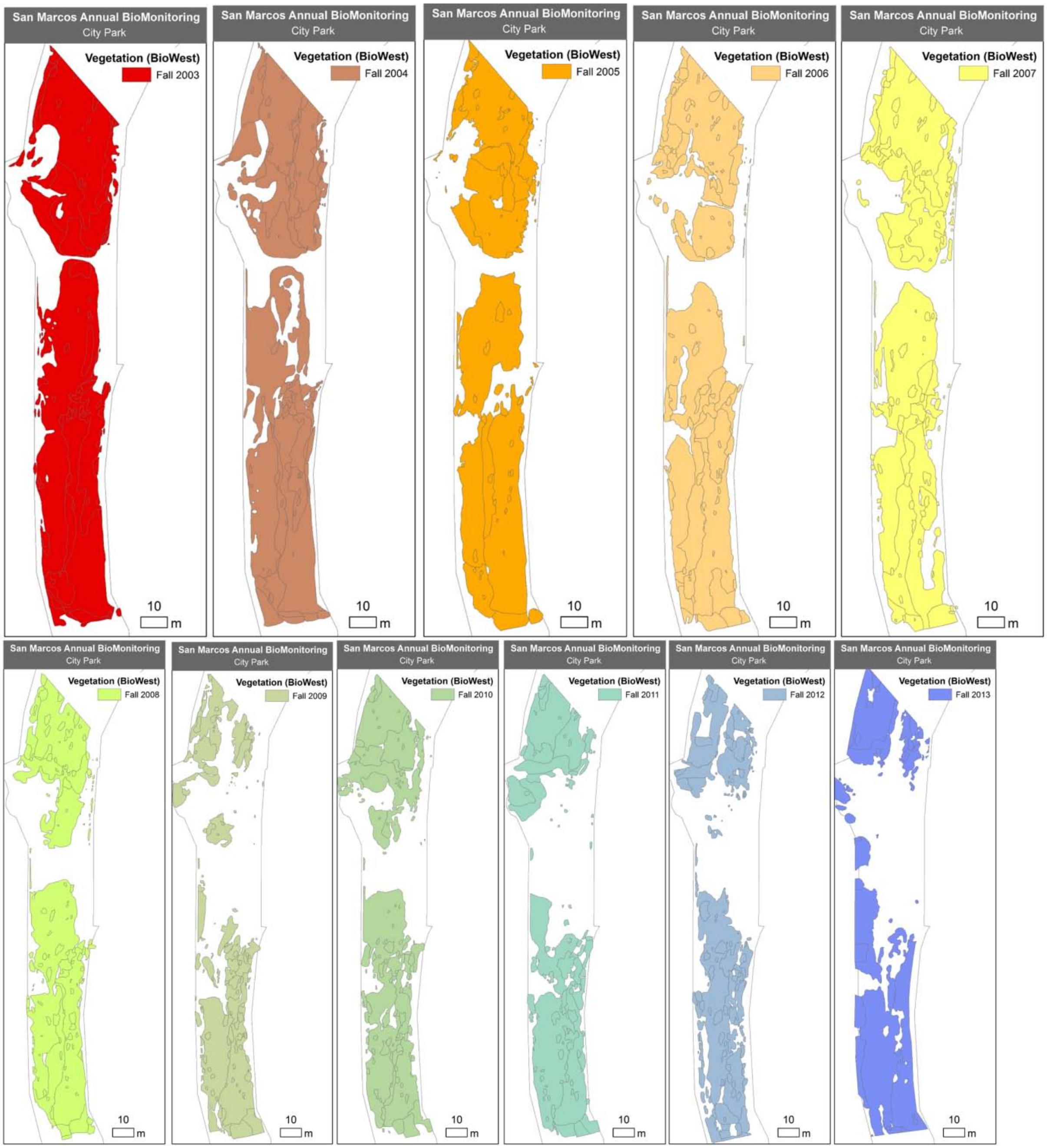

Figure 31. Shapefiles of vegetative coverage for the City Park Reach in the San Marcos River System.

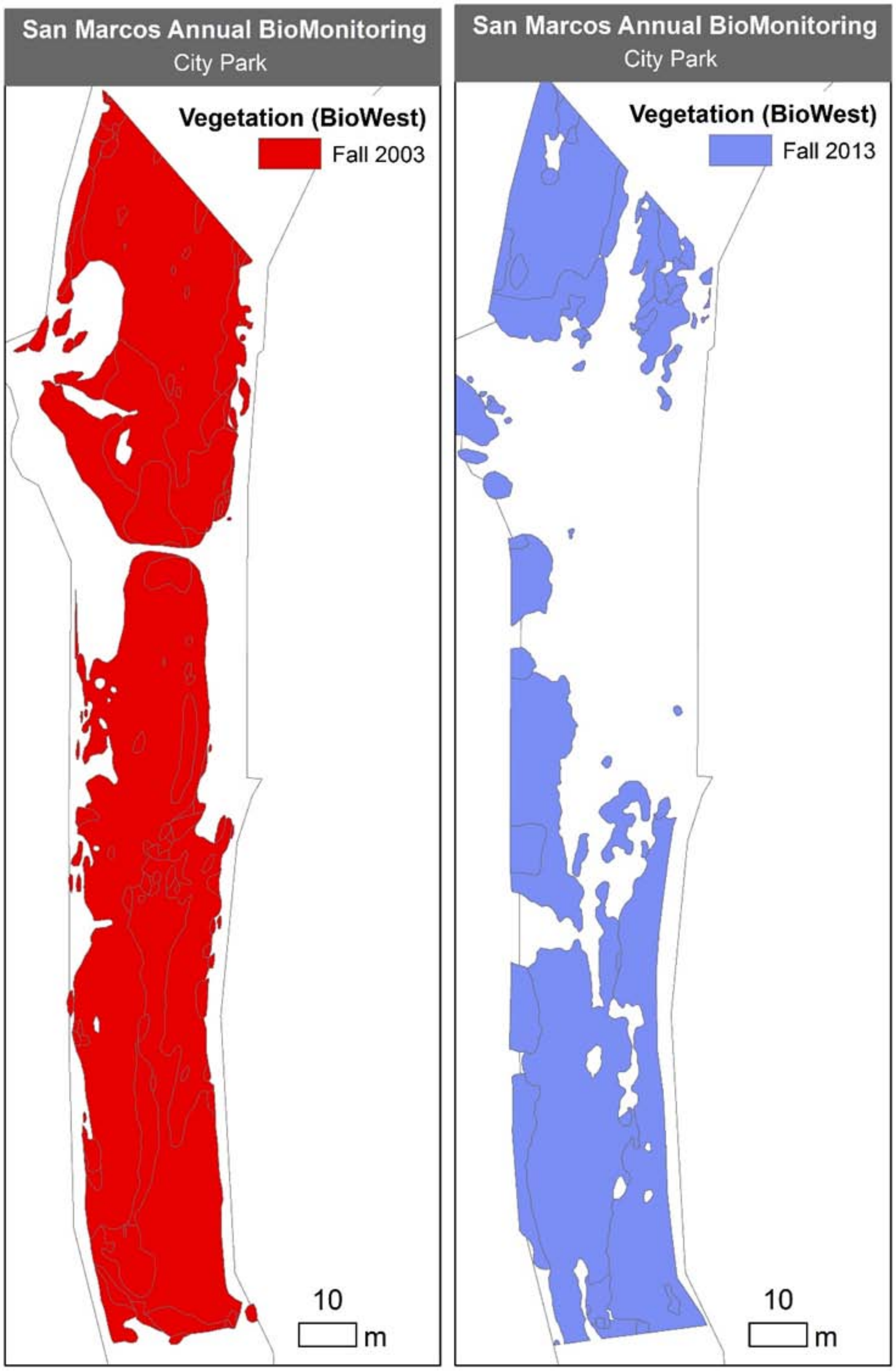

Figure 32. **Shapefiles of vegetative coverage for the City Park Reach in the San Marcos River System (Fall 2003 and Fall 2013).**

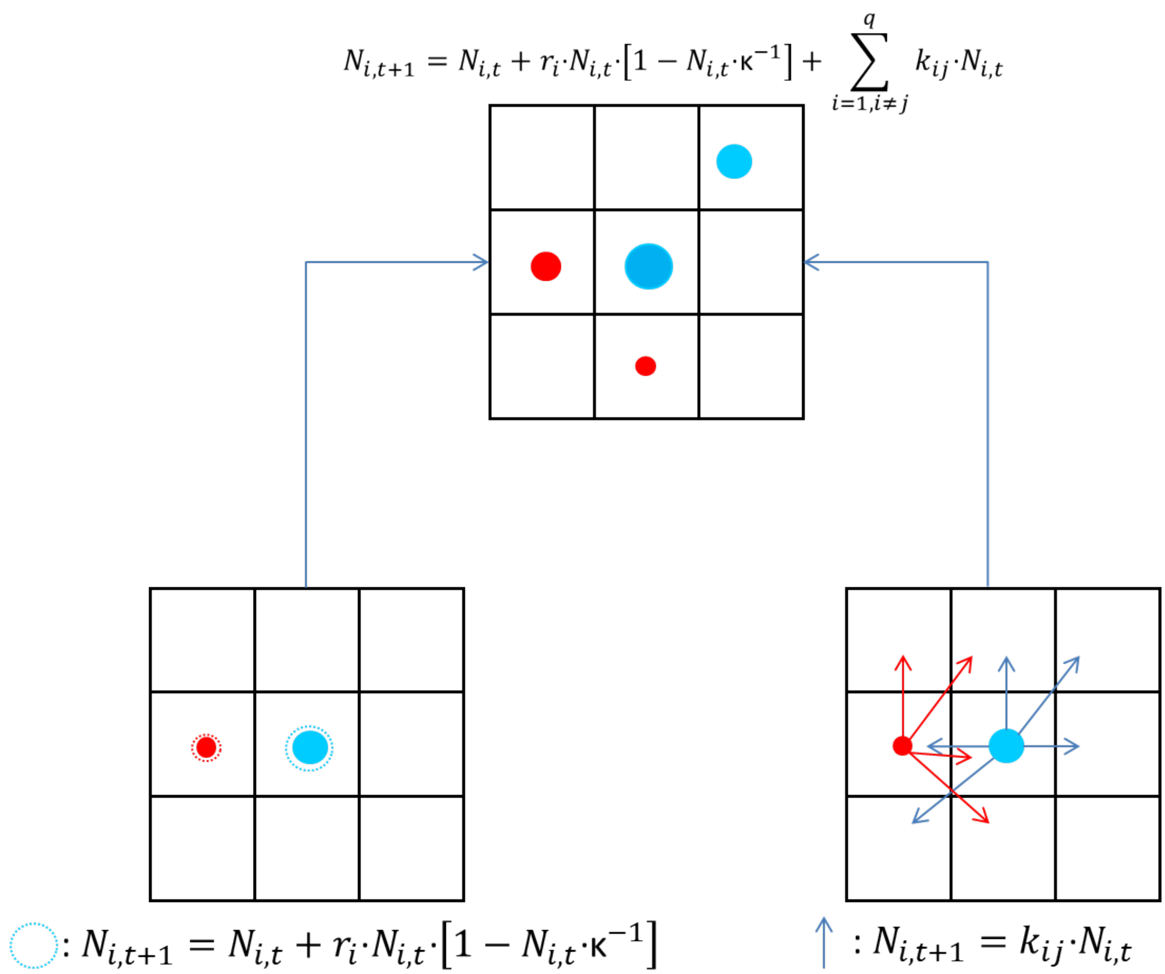

Figure 33. **Conceptual diagram of the mathematical framework that will be used to model growth and dispersal of different categories of vegetation (indicated by different colors). (A) Mathematical representation of local growth via a logistic equation where *ri* represents the spread rate of species *i*, and κ is the percentage of land cover), (B) Mathematical representation of intercellular dispersal, where *kij* is the dispersal from cell i to cell *j*, (C) mathematical representation of the synthesis of the two approaches into a spatially-explicit agent-based framework.**

Model Overview and Description

Model Overview

The model simulates vegetation growth, density, and colonization of eight SAV species found in the spring-fed Comal and San Marcos rivers (for a list of species see Table 12). The formulations for the SAV submodel are based on earlier models (Best and Boyd, 2001; Scheffer et al., 1993; van Nes et al., 2003), but have been modified for clear water, spring-fed, temperature-constant systems.

Table 12. List of species being modeled in the Comal and San Marcos systems.

Species
Cabomba
Hydrilla
Hygrophila
Ludwigia
Potamogeton
Sagittaria
Vallisneria
Texas Wild Rice

The model is spatially-explicit (i.e., geo-referenced and grid-based with a cell size of $1m^2$), stochastic, process-based, and programmed in NetLogo v5.2. The model simulates daily accumulation of biomass through photosynthesis, which is controlled by photosynthetically-active solar radiation and water depth. The model has a daily time step, but biomass accumulation is calculated using three-point Gaussian integration over both time and the depth profile for photosynthetic accumulation of biomass to be estimated in more detail (Best and Boyd, 2001). We did not include the effects of temperature or nutrients because these systems are spring-fed and have a relatively constant temperature (ranging from 21 - 24°C annually), and do not appear to be water column nutrient limited.

Colonization of unvegetated cells, or conversion from one species type to another occurs once a month and is based on a series of conditions, including the historical records of particular cells being vegetated, the type of species in a cell, the relative resilience of a species to disturbance, and a matrix of transition probabilities that quantify the probability of a cell transitioning from one species to another. The transition matrix was calculated from

thirteen years of field mapping efforts. For computational efficiency the model allows one species type to occur per cell.

Model Initialization

In addition to the physical and water quality data from the hydrodynamic submodel (velocity, depth, temperature, and DO), the SAV submodel is initialized with geo-referenced shapefiles of vegetation maps collected during field mapping in 2000 (Figures 30 and 31), monthly extraterrestrial radiation[1], and a user-defined latitude in degrees[2].

Model Description

Plant growth, in terms of biomass gained or lost (in grams/day) is modeled on a daily timestep and is calculated as

$$\Delta \boldsymbol{W} = \boldsymbol{W_s P} - \boldsymbol{W}(\boldsymbol{R_m} + \boldsymbol{M}) \qquad (1)$$

Where ΔW is the change in plant weight for a given day, W_s is the weight of the sprout, P is the amount of biomass gained through photosynthesis per unit weight of the plant, W is the weight of individual plant, R_m is respiration, and M is mortality.

Photosynthesis

Photosynthesis is affected by in-situ light (I), and distance from the top of the plant (D) using Michaelis-Menten saturation functions and a maximum value of photosynthetic accumulation (P_{max}), which can be calibrated for different species. The Michaelis-Menten function for light assimilation provides a good approximation of photosythentic response to light (Carr *et al.* 1997). Since light intensity follows a daily cycle, and varies with depth, photosynthesis is calculated at multiple times per day and at multiple depths in the vegetation, and is then integrated into a total daily value using Gaussian integration (Goudriaan and van Laar (1994), explained in section 2.2.2). Photosynthesis is calculated as

[1] Monthly radiation can be found at http://w2.weather.gov/climate/ or http://www.fao.org/docrep/x0490e/x0490e0j.htm

[2] For the Comal and San Marcos Rivers, 29.7° N latitude was used

$$P = P_{max} * \frac{I}{I + H_I} \quad (2)$$

Where P_{max} represents the daily production of the plant top at 20°C (which assumes no resource limitation). The defaults for P_{max} is 0.01 g g^{-1} d^{-1}, but is calibrated to match growth rates of different species. I is the daily value photosynthetically available radiation (PAR), H_I is the half-saturation coefficient of light (100 μE m^{-2} s^{-1}), D is the distance from the top of the plant, and H_D is the half-saturation coefficient of depth (1m). Since these rivers are not nutrient or temperature limited, we did not model their effects on growth.

In situ light

In aquatic systems, the availability of light is the driving factor controlling photosynthesis (Carr *et al.*, 1997). Irradiance follows daily and seasonal cycles, resulting in spatio-temporal patterns of light availability and growth patterns. These patterns are captured by including solar declination (eq. 3) and day length (eq. 4) to calculate PAR. This method uses the terminology and follows the ASTRO and TOTASSIM procedures of Goudriaan and van Laar (1994). Briefly, day of year (*day*) is used as an input to calculate solar declination (eq. 3), which is then combined with latitude (*lat*) in intermediate equations (i_1 through i_3) to calculate day length (eq. 4). *Daylength* is then used to calculate a specific hour when photosynthesis occurs (eq. 5). Finally, PAR (μE m^{-2} s^{-1}) at the water surface is estimated as 50% of the total irradiation given the day of year, hour, declination, and latitude (intermediate calculations i_4 through i_6).

$$\boldsymbol{Declination = -asin(sin(23.45) * \left(\cos\left(2 * \pi * \frac{day + 10}{365}\right)\right.} \quad (3)$$

$$sinld = \sin(lat) * \sin(declination) \quad i_1$$

$$cosld = \cos(lat) * \cos(declination) \quad i_2$$

$$aob = \frac{sinld}{cosld} \quad i_3$$

$$\boldsymbol{daylength = 12 * \left(1 + 2 * \frac{\mathrm{asin}(aob)}{\pi}\right)} \quad (4)$$

$$\boldsymbol{hour_i = 12 + (daylength * 0.5 * gaussian\ weight_j)} \quad (5)$$

$$DsinB = 3600 * \left(daylength * sinld + 24 * cosld * \sqrt{(1 - aob^2) / \pi}\right) \quad i_4$$

$$dsinBE = 3600 * \left(daylength * (sinld + 0.4 * (sinld^2 + cosld^2 * 0.5)) + 12 * cosld * (2 + 3 * 0.4 * sinld) * (1 - aob2) / \pi\right) \quad i_5$$

$$sinb = max\,(0, \left(sinld + cosld * cos(2 * \pi * (hour_i + 12) / 24)\right) \quad i_6$$

$$\boldsymbol{PAR = 0.5 * dailyradiation * sinb * (1 + 0.4 * sinb) / dsinBE} \quad (6)$$

Light attenuation in the water column follows the Lambert-Beer law (following van Nes et al., 2003). Self-shading is included, and is based on species -specific light attenuation coefficients (K_p), which provides a negative feedback for growth (i.e., the more biomass that accumulates the less light reaches the lower layers of the plants. Irradiance at a given depth (*z*) is calculated as

$$\boldsymbol{I_z = PAR * e^{(-0.12 * z) - (Kp * biomass_{>z})}} \quad (7)$$

Where *PAR* represents the photosynthetically available radiation at the surface, – 0.12 is the light attenuation coefficient of the water[3], *z* is the depth of the water at which photosynthesis is occurring, and $biomass_{>z}$ is the biomass above depth *z*.

Gaussian Integration

Since photosynthesis occurs throughout daylight hours, and irradiance changes throughout the day, PAR is calculated three times at three different depths per plant (Figure 34), and then integrated using three point Guassian integration, which has been shown to provide accurate estimates of daily accumulation of biomass (Goudriaan and van Laar, 1994). Total daily gross assimilation (*TDGA*) in grams (*g*) is calculated as

$$\boldsymbol{TDGA = daylength * \sum_{h=1}^{3}\left(GW_h * \sum_{z=1}^{3} P_{z_i}\right)} \quad (8)$$

Where *daylength* is the length of a given day, in hours (*h*), *GW* is the Gausssian weight used to weight the hourly photosynthesis (*P*) that was accumulated at depth *z* with irradiance (*i*). Gross assimilation is needed for growth and maintenance of the plant, which are based on their glucose requirement. Therefore, the *TDGA* was converted into the weight of glucose

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[3] http://www.lakeaccess.org/ecology/lakeecologyprim3.html

for potential plant growth ($W_{glucose}$) by multiplying it by the aboveground biomass of the plant and $\frac{30}{44}$ (Teh, 2006). Once biomass is converted to glucose it is partitioned to above-ground and below-ground parts of the plant.

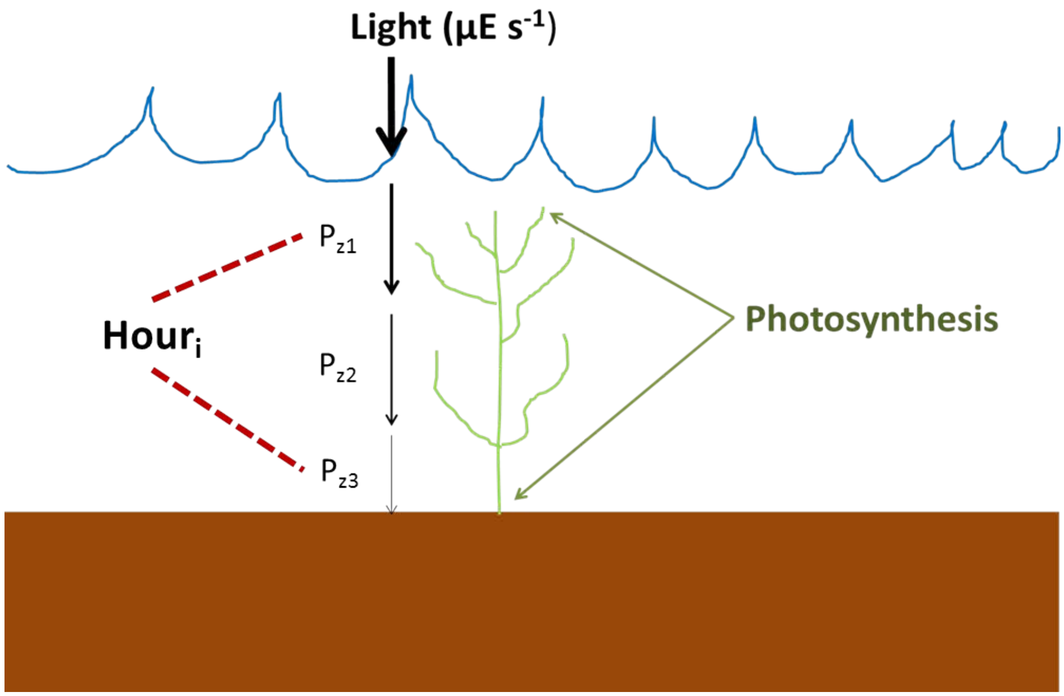


Figure 34. Conceptual model of Guassian integration of photosynthesis

Respiration

Maintenance respiration is needed for plants to continue to live. The model estimates maintenance respiration based on daily temperature and the biomass in the above and below ground sections of the plants. Maintenance respiration rates (*R*) for above-ground (*AG*) and below-ground (*BG*) biomass were based on a Q_{10} formulation (i.e., the measure of the rate of change of a by increasing the temperature by 10°C), and are calculated as

$$\boldsymbol{R_{AG} = 0.0225 * \left(Q_{10}{}^{(temp-25)/10}\right)} \quad \textbf{(9)}$$

$$\boldsymbol{R_{BG} = 0.015 * \left(Q_{10}{}^{(temp-25)/10}\right)} \quad \textbf{(10)}$$

where Q_{10} is a constant and set at 2, temp is daily temperature, and 0.0225 and 0.015 are the maintenance respiration coefficients for *AG* and *BG* biomass, respectively (based on values in Teh, 2006, Table 7.1).

Plant growth

The difference between gross photosynthesis and maintenance respiration is the amount of assimilate available for growth. The glucose requirement for growth (G_{Growth}) is calculated using the following equation from Teh (2006):

$$G_{Growth} = F_{AG}G_{AG} + F_{BG}G_{BG} \qquad (12)$$

where F is the fraction of dry matter allocated to each plant part and G is the glucose requirement for growth of each plant part. The G estimates used for each plant part are from Teh (2006, Table 7.4), with aboveground biomass being the sum of the above ground plant sections. The incremental plant part biomass gain per day is then estimated as

$$BM_{t+1} = BM_t + F * \left(\frac{W_{glucose} - R}{G_{growth}}\right) \qquad (13)$$

If R is greater than the weight of glucose for potential plant growth, no growth occurs.

Morphological maximums are input parameters based on the literature or field data collected during this study (Table 13), and are set in place to ensure plants sizes do not exceed biological limits. If after growth is simulated the species specific aboveground biomass exceeds the user-defined maximum aboveground biomass ($BM_{AG\text{-}Max}$), the aboveground biomass is truncated to the maximum value. If after growth is simulated the species specific root mass exceeds the user-defined maximum root mass ($R_{M\text{-}Max}$), the root mass is truncated to the maximum value.

In some cases, the aboveground biomass is less than the user-defined minimum requirement for photosynthesis to occur. This is particularly true for some plants after colonization of new cells. When this happens the model simulates plant growth by translocating 1% of the root biomass to the aboveground biomass, following methods used by Best and Boyd (2001).

Table 13. Parameter table for growth model for two species Potamogeton and Vallisneria

Parameter	Description	Unit	Vegetation Species	
			Potamogeton	*Vallisneria*
S_D	Average stem density per plant	count	3[1]	35[1]
H_{Max}	Maximum stem height	cm	80[1,2,3]	34.7[2]
S_M	Maximum mass of each stem	g	6[1]	0.09[3]
RL_{Max}	Maximum root length	cm	60[4]	30[4]
$P_{D\text{-}Max}$	Maximum plant density per 0.5 m^2	count	11.23[5]	3.15[3]
$CSA_{Average}$	Average cross-sectional area of a stem	cm^2	0.231[6]	0.155[5]
R_{RAB}	Root-to-aboveground biomass ratio	ratio	0.429[7]	1.128[4]
R_{RS}	Root-to-shoot ratio	ratio	0.95[8]	1.10[4]
MinRoot	Minimum root size	g	0.001 [b]	0.001 [b]
MinSize	Minimum size for photosynthesis	g	0.5 [b]	0.5 [b]
Dispersal	# of 0.5 m increments traversed per year	count	8[9]	1[6]
$Season_{Begin}$	First day of growing season	Julian day	107[10]	121[4]
$Season_{End}$	Last day of growing season	Julian day	226[11]	274[3]
LeafDO	First day of leaf die off	Julian day	163[11]	244[4]
k	Plant tissue light extinction coefficient	$m^{-2}g^{-1}$	0.0235 [a]	0.0235 [a]
H_I	Half-saturation constant for light	$\mu Em^{-2}s^{-1}$	14 [a]	14 [a]
P_{max}	Maximum daily production	$g^{-1}hr^{-1}$	0.01 [a]	0.01 [a]
WintStor	Winter storage of biomass	proportion	0.33 [b]	0.33 [b]
WintDie	Additional winter die off	proportion	0.05 [b]	0.05 [b]
$F_{greenleaves}$	Biomass allocation to leaves	proportion	0.50[7]	0.27 [b]
F_{stem}	Biomass allocation to stem	proportion	0.20[7]	0.20 [b]
F_{roots}	Biomass allocation to roots	proportion	0.30[7]	0.53[4]

Conversion and dispersal

Currently, there are few models that explicitly quantify the relationship between environmental conditions, and the ability of a plant to colonize new areas or be replaced by another species. We have developed an approach that simulates changes in vegetative cover over time based ecological dispersal theory and on empirical estimates gathered from 13 years of vegetation mapping, and are currently implementing it into the model. We model two distinct processes: dispersal of vegetation into adjacent, unvegetated cells, and the conversion of a cell from one vegetation type to another.

Vegetation coverage for each reach was mapped at least twice a year for thirteen years (e.g, Figures 30 and 31). Spatial analysis indicated that both vegetation coverage and species composition were dynamic. Within each reach, there were specific areas that were never vegetated, others that remained vegetated, and other locations that oscillated between

vegetated and unvegetated (Figure 35A and B). For each cell, we determined the probability of being vegetated by developing a frequency distribution of vegetation history for that specific location.

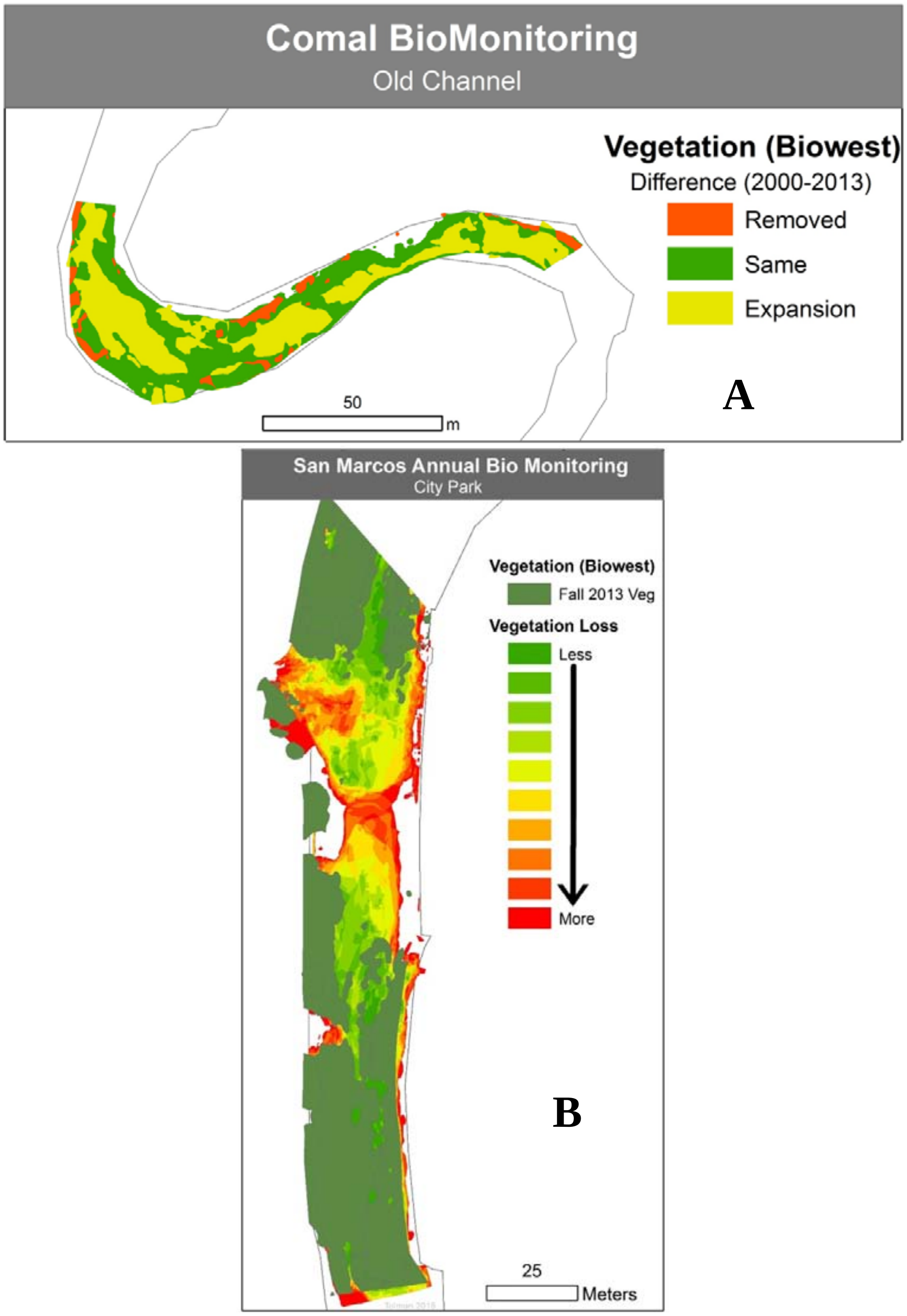


Figure 35. **Depiction of frequency of occupancy of a given cell over time in the Old Channel (A), and City Park (B) reaches. Red and orange colors indicate that locations oscillated between vegetated and unvegetated during the course of the 13 year study, while green colors indicate those locations remained mostly vegetated.**

Dispersal by aquatic vegetation can take place through seed deposits, clonal growth, and/or fragments settling and rooting downstream. We did not model specific dispersal processes, rather, colonization of unvegetated cells is based on a series of conditional probabilities that were calculated for each cell as follows

1) First, we query the unvegetated cells and determine the likelihood of a given cell to be vegetated. If a cell has a high likelihood of being vegetated, then
2) we determine the likelihood of the specific species within a given cell dispersing into new areas. If the species is likely to disperse we then
3) calculate a probability of dispersal based on the percent cover of the plant species in the occupied cell. The model creates shape parameters for a logistic distribution based on the percent cover of vegetation for each of vegetated cell. This function then generates a probability of dispersal, which is lowest at low values for percent cover, and highest as the cover approaches 100% (Figure 36). Dispersal into unvegetated cells is calculated by comparing the probability of dispersal to a random number drawn from a uniform distribution between 0 and 1; dispersal occurs if the random number is less than the probability of dispersal.

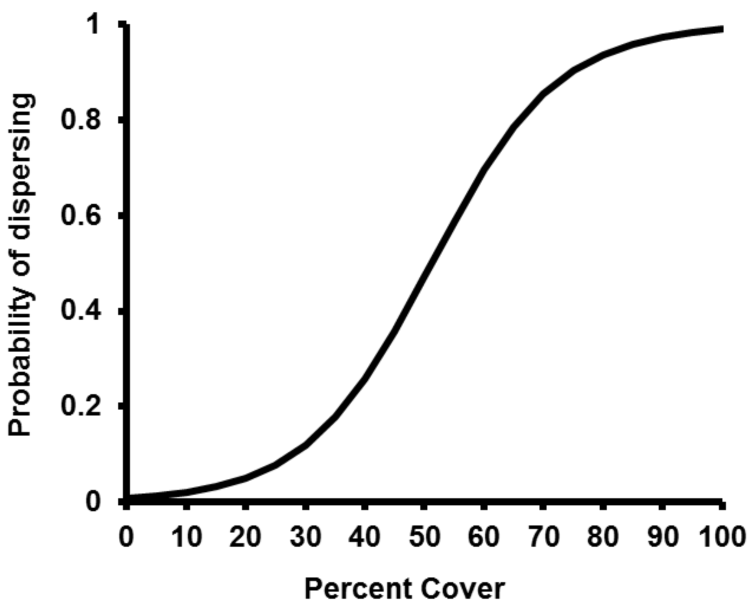


Figure 36. **Example of logistic function used to calculate probability of dispersal based on percent cover of vegetation for each cell. Logistic curve was derived following Railsback and Grimm, 2014.**

The newly colonized neighboring cell will receive 10% of the parent plant's leaf, stem, and root biomass, but the parent plant is not taxed for dispersing (i.e., the parent plant does not

lose biomass). Further, a parent plant is only allowed to disperse once per year and collectively a plant will only disperse as far as the user-defined input for dispersal distance (in this case, to a neighboring cell).

Species composition within the study areas was dynamic, and would often change within a given year or across years. To capture this phenomenon, we determined the probability of a cell converting from one species to another over a given time period by incorporating reach-specific vegetation history into the model. Using the mapped vegetation data, we developed a series of transition matrices that estimated the probability of conversion from a given species to a given species (e.g., converting from Vallisneria to Hygrophila) for both a short (2 consecutive samples) or long (3 consecutive samples) time periods. Cell conversions are calculated once every three months, and are determined by the specific combination of vegetative species within that cell. The model stores the history of every cell's vegetation types, and then the probability of converting to another type is determined by comparing a random number drawn from a uniform distribution to the cumulative frequency distribution of becoming another species.

Mortality

Senescence is based on overall growth patterns and based on temperature. It is lowest in the summer, and highest in the winter. Death rates and their corresponding temperatures were based on Best and Boyd (2001). Senescence was integrated into the equation for incremental plant part biomass gain per day (see equation 13) such that

$$BM_{t+1} = BM_t + F * \left(\frac{W_{glucose} - R}{G_{growth}}\right) - d * BM_t \qquad (14)$$

Mortality is also associated with disturbance events, including scour and restoration. Currently, neither restoration nor scour are implemented in the model. Conceptually, the model assumes disturbance associated with a recreation or scour causes direct mortality to plants through excess flow, or human-mediated disturbance. Therefore, any plants within cells that are impacted by these events die.

The depletion in plant parts or in some cases the mortality of a plant occurred other plant parts were depleted. For example, if at any point the below-ground biomass was depleted, or if the above ground biomass falls below a user-defined threshold then entire plant died. This might occur if the annual senescence for a plant part consistently exceeded the incremental plant part biomass gain.

Plant attributes

There are several measureable plant attributes that are important to the growth of aquatic vegetation. For simplicity and computational efficiency we categorized all stems, shoots, and leaves as aboveground biomass, and roots and other below-ground matter as belowground biomass.

Aboveground height (*H*) is calculated based on biomass:root ratio, following Best and Boyd (2001), and it cannot exceed the water depth of its cell. Root length (RL) is calculated as

$$\boldsymbol{RL = R_{RS} * H} \qquad \textbf{(15)}$$

where R_{RS} is a user-defined root-to-shoot ratio. If the root length overshoots a user-defined, maximum root length, the root length is truncated to the user-defined maximum root length. Maximum root biomass ($R_{M\text{-}Max}$) was then calculated as a portion of BM_{max}, such that

$$\boldsymbol{R_{M-Max} = R_{RAB} * BM_{max}} \qquad \textbf{(16)}$$

where R_{RAB} is the root to aboveground biomass ratio.

Analysis of Vegetation Patterns

Review of vegetation data set and statistics overview

The composition and distribution of submerged aquatic vegetation was mapped in the spring and fall at Old Channel, Comal River between 2000-2013 and at City Park Reach, San Marcos River between 2003 -2013. Additional seasons were observed at Old Channel from 2001-2002 and at City Park in 2003-2004, as summarized in Table 14. Observations were also made following various water flow events at each site.

Table 14. **SAV mapping schedule.**

Site	Year	Season: spring	summer	fall	winter	Flow
City Park Reach, San Marcos River System	2003	x	x	x		
	2004	x	x	x		
	2005	x		x		
	2006	x		x		Low
	2007	x		x		
	2008	x		x		
	2009	x	x	x		
	2010	x		x		
	2011	x		x		
	2012	x		x		
	2013	x		x		
Old Channel, Comas River System	2000			x		
	2001	x	x	x	x	High
	2002	x	x	x	x	
	2003	x	x	x		
	2004	x	x	x		
	2005	x		x		
	2006	x		x		
	2007	x		x		
	2008	x		x		
	2009	x		x		Low
	2010	x		x		High
	2011	x		x		
	2012	x		x		
	2013	x		x		

To analyze the effects of site, season and flow on presence/absence of specific vegetation species, we ran generalized linear models (GLMs) using binomial distributions for each species of interest. A random model effect was incorporated to account for repeated measures through time and the Bound Optimization by Quadratic Approximation (BOBYQA) algorithm was employed. All regression analyses were run using R v3.2.1, using the glmer routine. Species specific analyses were limited to species with counts of >200 individuals. In addition to species specific analyses, we included a general analysis of vegetated/unvegetated cells within each mapped area. Data were grouped to determine

differences among 2 seasons (spring, fall), 3 seasons (summer, spring, fall), and 4 seasons, divided by availability. The influence of high and low flow was also explored for years in which these data were available.

Regressions analyses

Two season analysis across sites

To determine the effect of seasonal changes from spring to fall on vegetation, we ran logistic regressions (as described above) using spring and fall data from both study sites throughout the entire study period (2001-2013). The following plants were included in species-specific analyses: Hygrophila, Ceratopteris, Nuphar, Vallisneria, Zizania, Sagittaria, Potamogeton, Ludwigia and Riccia. Algae and bare space were also considered in separate analyses. Results revealed significant differences in vegetation due to site for Hygrophila, Nuphar, Riccia, and Ludwigia (Table 15). Patterns of Ceratopteris distribution showed significant difference by site and year (Table 15). In all cases in which site differences were found, relative abundance was significantly higher at the Old Channel, Comal river site. The remaining species did not show any significant changes in distribution with respect to year, site or fall/spring season and there were no significant differences seen in bare space occupation. Seasonal differences between spring and fall were not significant.

Table 15. **Results of logistic regression models incorporating two season data (fall, spring) from sites in the San Marcos and Comal rivers. Sites were mapped from 2000-2013. Significant effects are shown in bold. Random effects of repeated sampling were included in all analyses.**

Species	Fixed Effects	Estimate	Std Error	z	p
Ceratopteris	Intercept	1172.63	274.97	4.26	**<0.001**
	Season	-0.39	0.94	-0.42	0.66
	Year	-0.59	0.14	-4.30	**<0.001**
	Site	5.39	1.00	5.40	**<0.001**
Hygrophila	Intercept	-662.93	138.85	-4.77	**<0.001**
	Season	-0.02	0.48	-0.04	0.97
	Year	0.33	0.07	4.75	**<0.001**
	Site	0.68	0.48	1.42	0.16
Ludwigia	Intercept	-139.02	268.64	-0.52	0.61
	Season	-0.55	0.94	-0.57	0.56
	Year	0.06	0.13	0.48	0.64
	Site	5.85	1.04	5.64	**<0.001**
Nuphar	Intercept	-176.11	291.56	-0.60	0.55
	Season	0.49	1.04	0.47	0.64
	Year	0.08	0.15	0.57	0.57
	Site	5.90	1.10	5.37	**<0.001**
Riccia	Intercept	-325.97	343.21	-0.95	0.34
	Season	-0.86	1.20	-0.72	0.47
	Year	0.16	0.17	0.92	0.36
	Site	4.11	1.27	3.24	**0.001**

Three season analysis across sites

Including the effect of seasonal vegetation changes across three seasons - summer, spring, and fall –reduced the number of years of available data to four (2002-2004; 2009). Individual analyses for bare space, algae and all species with counts > 200 allowed for the inclusion of the following: Hygrophila, Hydrilla, Ceratopteris, Nuphar, Vallisneria, Zizania, Sagittaria, Potamogeton, Ludwigia, and Riccia. Most species show no significant differences in site, season, or year. Exceptions included significant site differences in the abundance of Ceratopteris, Ludwigia, and bare space in which there was both higher abundance and more empty space at the Old Channel, Comal River site (Table 16). In addition, we found a significant yearly difference in the abundance of Hygrophila, due to very low abundance in 2002 (Table 16).

Table 16. Results of logistic regression models incorporating three season data (fall, spring, summer) from sites in the San Marcos and Comal rivers. Data was included from 2002-2004, and 2009. Significant effects are shown in bold. Random effects of repeated sampling were included in all analyses.

Species	Fixed Effects	Estimate	Std Error	z	p
Ceratopteris	Intercept	902.11	477.45	1.89	0.06
	Season Spring:Fall	-0.44	1.34	-0.33	0.74
	Season Summer:Fall	-0.58	1.42	-0.41	0.68
	Year	-0.45	0.24	-1.91	0.06
	Site	5.48	1.17	4.67	**<0.001**
Hygrophila	Intercept	-967.88	333.33	-2.9	**<0.01**
	Season Spring:Fall	-0.24	0.99	-0.24	0.81
	Season Summer:Fall	-0.09	1.05	-0.09	0.93
	Year	0.48	0.17	2.89	**<0.01**
	Site	-0.16	0.86	-0.19	0.85
Ludwigia	Intercept	-280.64	706.3	-0.4	0.69
	Season Spring:Fall	-0.7	2.06	-0.34	0.73
	Season Summer:Fall	1.2	2.14	0.56	0.58
	Year	0.13	0.35	0.38	0.7
	Site	4.57	1.8	2.54	**0.01**
Bare Space	Intercept	-715.72	730.48	-0.98	0.33
	Season Spring:Fall	0.27	2.18	0.12	0.9
	Season Summer:Fall	0.63	2.3	0.28	0.78
	Year	0.36	0.36	0.98	0.33
	Site	4.55	1.91	2.38	**0.02**

Four season analysis at Old Channel, Comal River

Two years, 2001 and 2002, were sampled in all four seasons at the Old Channel site. Regression analyses of all species present (Hygrophila, Algae, Ceratopteris, Nuphar) as well as algae and bare space showed some seasonal patterns at this site. Specifically, Ceratopteris abundance differed by year and season such that every seasonal contrast except for summer and winter differed (Table 17). Nuphar abundance also differed by year and season, with all seasonal contrasts distinct except spring and winter (Table 17). Both algae and bare space showed significant yearly and seasonal differences, in which each year and season was distinct (Table 17). Abundance of Hygrophila did not change seasonally or between the two years analyzed.

Table 17. Results of logistic regression models incorporating four season data Old Channel, Comal River system. Data was included from 2001-2002. Significant effects are shown in bold. Random effects of repeated sampling were included in all analyses. (TS 6)

Species	Fixed Effects	Estimate	Std Error	z	p	Linear Contrast (LC)	LC Estimate	LC Std Error	LC z	LC p
Ceratopteris	Intercept	-356.10	85.46	-41.67	**<0.0001**	spring, fall	-3.00	0.06	-48.71	**0.00**
	Season Spring:Fall	-3.00	0.06	-48.71	**<0.0001**	summer, fall	-2.61	0.06	-46.31	**0.00**
	Season Summer:Fall	-2.61	0.06	-46.31	**<0.0001**	winter, fall	-2.49	0.05	-45.43	**0.00**
	Season Winter:Fall	-2.49	0.05	-45.43	**<0.0001**	summer, spring	0.39	0.07	5.70	**0.00**
	Year	1.78	0.04	41.66	**<0.0001**	winter, spring	0.50	0.07	7.40	**0.00**
						winter, summer	0.11	0.06	1.73	0.30
Nuphar	Intercept	3236.47	278.06	11.64	**<0.0001**	spring, fall	2.37	0.24	9.79	**<0.001**
	Season Spring:Fall	2.37	0.24	9.79	**<0.0001**	summer, fall	1.67	0.25	6.71	**<0.001**
	Season Summer:Fall	1.67	0.25	6.71	**<0.0001**	winter, fall	2.37	0.24	9.79	**<0.001**
	Season Winter:Fall	2.37	0.24	9.79	**<0.0001**	summer, spring	-0.70	0.16	-4.31	**<0.001**
	Year	-1.62	0.14	-11.67	**<0.0001**	winter, spring	0.00	0.15	0.00	1.00
						winter, summer	0.70	0.16	4.31	**<0.001**
Algae	Intercept	90.45	103.93	0.87	0.38	spring, fall	-1.79	0.11	-15.93	**<0.001**
	Season Spring:Fall	-1.79	0.11	-15.93	**<0.0001**	summer, fall	0.29	0.07	4.21	**<0.001**
	Season Summer:Fall	0.29	0.07	4.21	**<0.0001**	winter, fall	0.44	0.07	6.62	**<0.001**
	Season Winter:Fall	0.44	0.07	6.62	**<0.0001**	summer, spring	2.07	0.11	18.77	**<0.001**
	Year	-0.05	0.05	-0.91	0.36	winter, spring	2.23	0.11	20.31	**<0.001**
						winter, summer	0.16	0.06	2.45	0.07
Bare	Intercept	2540.20	71.90	35.33	**<0.0001**	spring, fall	3.06	0.06	53.88	**<0.0001**
	Season Spring:Fall	3.06	0.06	53.88	**<0.0001**	summer, fall	2.05	0.05	43.02	**<0.0001**
	Season Summer:Fall	2.05	0.05	43.02	**<0.0001**	winter, fall	1.82	0.05	39.51	**<0.0001**
	Season Winter:Fall	1.82	0.05	39.51	**<0.0001**	summer, spring	-1.00	0.06	-17.31	**<0.0001**
	Year	-1.27	0.04	-35.31	**<0.0001**	winter, spring	-1.23	0.06	-21.51	**<0.0001**
						winter, summer	-0.23	0.05	-4.60	**<0.0001**

Flow

We examined the influence of high and low flow on vegetation on a site specific basis. At the Comal river site, analyses were run on years 2001, 2009 and 2010, due to occurrence of sampled high and low flow events. Separate, repeated-measures logistic regressions were run for each species present as well as bare space. Our analyses indicate that the abundance of Ceratopteris, Hygrophila, and Nuphar and bare space was significantly different in high flow than in low and average flow conditions (Table 18). These species also had significant changes in abundance depending on the year examined (Table 18). Ludwigia abundance was impacted by flow as well, and changed significantly under each flow regime, but showed no yearly differences (Table 18). Algae abundance was impacted by every flow type and every year sampled, showing significant differences for both factors (Table 18). In contrast Riccia abundance showed no differences due to flow or year.

The San Marcos site had only one year (2006) in which communities were sampled following a flow event. As a result, our analyses are limited to comparisons of low flow and normal flow at this site. Bare space, as well as five species that had counts >100 were examined separately. A significant effect of flow was found for the species Hydrilla, Potamogeton, Sagittaria and Zizania (Table 18). There was no effect of low flow on Hygrophila or bare space abundance.

Community composition

To identify the factors responsible for differences in vegetation at the community level (i.e., community composition and abundance of all species identified), we used the ANOSIM routine in Primer-e v.6. This multivariate approach uses a Bray-Curtis distance matrix describing community distance for each sampling time. Sites were analyzed separately to determine the effects of season, year and flow on changes in vegetation.

Table 18. Results of logistic regression models examining the effect of flow on vegetation abundance at Old Channel, Comal River system. Data was included from 2001, 2009, 2010. Significant effects are shown in bold. Random effects of repeated sampling were included in all analyses. (TS 7)

Species	Fixed Effects	Estimate	Std Error	z	p
Ceratopteris	Intercept	-4.08	0.07	-61.20	**<0.0001**
	Flow Low:High	0.04	0.15	0.26	0.79
	Flow Average:High	0.12	0.06	2.12	**0.03**
	Year 2009:2001	-1.76	0.08	-20.72	**<0.0001**
	Year 2010:2001	-2.87	0.11	-27.02	**<0.0001**
Hygrophila	Intercept	-16.39	0.72	-22.67	**<0.0001**
	Flow Low:High	2.84	0.11	26.85	**<0.0001**
	Flow Average:High	8.96	0.08	36.49	**<0.0001**
	Year 2009:2001	11.14	0.71	15.61	**<0.0001**
	Year 2010:2001	10.88	0.71	15.25	**<0.0001**
Ludwigia	Intercept	-26.46	212.33	-0.13	0.90
	Flow Low:High	3.27	0.79	4.12	**<0.0001**
	Flow Average:High	2.46	0.75	3.27	**<0.0001**
	Year 2009:2001	17.17	212.33	0.08	0.94
	Year 2010:2001	16.34	212.33	0.08	0.94
Nuphar	Intercept	-7.36	0.15	-49.24	**<0.0001**
	Flow Low:High	0.55	0.20	2.80	**0.01**
	Flow Average:High	0.49	0.11	4.61	**<0.0001**
	Year 2009:2001	0.52	0.12	4.32	**<0.0001**
	Year 2010:2001	0.90	0.10	8.96	**<0.0001**
Riccia	Intercept	-38.65	451.75	-0.09	0.93
	Flow Low:High	15.42	348.14	0.04	0.97
	Flow Average:High	12.89	348.14	0.04	0.97
	Year 2009:2001	17.12	287.89	0.06	0.95
	Year 2010:2001	15.89	287.89	0.06	0.96
Algae	Intercept	-4.12	0.07	-59.38	**<0.0001**
	Flow Low:High	0.83	0.27	3.11	**<0.01**
	Flow Average:High	-0.19	0.06	-3.02	**<0.01**
	Year 2009:2001	-3.28	0.20	-16.53	**<0.0001**
	Year 2010:2001	-4.76	0.32	-14.94	**<0.0001**
Bare	Intercept	4.47	0.06	74.21	**<0.0001**
	Flow Low:High	-0.82	0.06	-12.88	**<0.0001**
	Flow Average:High	-0.80	0.04	-22.49	**<0.0001**
	Year 2009:2001	-1.95	0.04	-49.30	**<0.0001**
	Year 2010:2001	-1.29	0.03	-38.64	**<0.0001**

Results indicate that on a site-specific level, differences in community composition are due to a yearly effect rather than a seasonal or flow induced effect (Tables 19 and 20). Significant p values were only found associated with year. These results are consistent at both sites. The infrequency of low flow events sampled at City Park did not allow for a 2-way ANOSIM of season and flow.

Table 19. **Results of logistic regression models examining the effect of flow on vegetation abundance at City Park, San Marcos River system. Data was included from 2006. Significant effects are shown in bold. Random effects of repeated sampling were included in all analyses.**

Species	Fixed Effects	Estimate	Std Error	z	p
Hydrilla	Intercept	-10.17	0.11	-94.37	**<0.0001**
	Flow Average:Low	-0.37	0.07	-5.38	**<0.0001**
Hygrophila	Intercept	-11.58	0.14	-79.98	**<0.0001**
	Flow Average:Low	0.07	0.09	0.73	0.47
Potamogeton	Intercept	-13.74	0.44	-31.24	**<0.0001**
	Flow Average:Low	1.73	0.27	6.46	**<0.0001**
Sagittaria	Intercept	-14.51	0.50	-28.93	**<0.0001**
	Flow Average:Low	1.24	0.30	4.21	**<0.0001**
Zizania	Intercept	-13.30	0.35	-38.31	**<0.0001**
	Flow Average:Low	0.63	0.21	3.00	0.00
Bare	Intercept	10.75	0.10	108.59	**<0.0001**
	Flow Average:Low	0.05	0.07	0.75	0.45

Table 20. **Changes in community composition at Old Channel, Comal River System.**

Primer-e Routine		R	p
Nested ANOSIM	Season (nested)	0.69	0.11
	Year	0.63	**0.01**
ANOSIM	Year	0.62	**0.01**
	Flow	-0.1	0.59
ANOSIM	Season	0.1	0.076
	Flow	0.21	0.29

Transition probabilities

Community change can also be examined by determining the likelihood that a vegetated point within each mapped site remains occupied with the same species, changes to another species or becomes bare. To identify the chance of these possibilities, we calculated mean relative transition probabilities for each species using data from all available years and the spring and fall seasons. We calculated the probability of transition through two seasons (spring-fall as well as fall-spring) as well as three seasons (spring-fall-spring). The results of these calculations are presented in Tables 21 through 25. Generally, highest transition probabilities were found when points became unoccupied and returned to bare space, and when points did not transition to a new vegetated state (i.e., the same species occupied a point through multiple seasons).

Table 21. Changes in community composition at City Park reach, San Marcos River System.

Primer-e Routine		R	p
Nested ANOSIM	Season (nested)	0	0.67
	Year	0.3	**0.003**
ANOSIM	Year	0.19	**0.03**
	Flow	-0.1	0.1

Table 22. **Mean and standard deviation of transition probability from fall to spring for each species and bare space across 12 years of sampling at Old Channel, Comal River site.**

Mean Transition Probability		**Spring**						
		Algae	Bare	*Ceratopteris*	*Hygrophila*	*Ludwigia*	*Nuphar*	*Riccia*
Previous Fall	Algae	0.117	0.790	0.044	0.026	0.000	0.019	0.004
	Bare	0.001	0.946	0.007	0.037	0.005	0.003	0.002
	Ceratopteris	0.002	0.596	0.264	0.047	0.001	0.089	0.002
	Hygrophila	0.000	0.117	0.005	0.851	0.014	0.009	0.004
	Ludwigia	0.004	0.267	0.000	0.280	0.438	0.005	0.006
	Nuphar	0.000	0.248	0.024	0.079	0.000	0.645	0.004
	Riccia	0.005	0.603	0.000	0.240	0.005	0.048	0.100

StdDev of Transition Probability		**Spring**						
		Algae	Bare	*Ceratopteris*	*Hygrophila*	*Ludwigia*	*Nuphar*	*Riccia*
Previous Fall	Algae	0.028	0.014	0.030	0.030		0.030	0.030
	Bare	0.004	0.001	0.004	0.004	0.004	0.004	0.004
	Ceratopteris	0.014	0.009	0.012	0.013	0.014	0.013	0.014
	Hygrophila	0.009	0.008	0.009	0.003	0.009	0.009	0.009
	Ludwigia	0.035	0.030		0.030	0.026	0.035	0.035
	Nuphar		0.031	0.036	0.035		0.022	0.036
	Riccia	0.049	0.031		0.042	0.049	0.048	0.046

Table 23. Mean and standard deviation of transition probability from spring to fall for each species and bare space across 12 years of sampling at Old Channel, Comal River site.

Mean Transition Probability		Fall						
		Algae	Bare	*Ceratopteris*	*Hygrophila*	*Ludwigia*	*Nuphar*	*Riccia*
Previous Spring	Algae	0.154	0.214	0.433	0.184	0.000	0.000	0.015
	Bare	0.008	0.901	0.046	0.035	0.004	0.004	0.002
	Ceratopteris	0.008	0.250	0.606	0.083	0.005	0.044	0.004
	Hygrophila	0.002	0.175	0.012	0.774	0.011	0.011	0.015
	Ludwigia	0.001	0.350	0.000	0.263	0.385	0.000	0.001
	Nuphar	0.014	0.155	0.260	0.086	0.000	0.450	0.035
	Riccia	0.029	0.535	0.000	0.309	0.016	0.037	0.074

Std Dev of Transition Probability		Fall						
		Algae	Bare	*Ceratopteris*	*Hygrophila*	*Ludwigia*	*Nuphar*	*Riccia*
Previous Spring	Algae	0.0649	0.0625	0.0531	0.0637			0.0700
	Bare	0.0040	0.0013	0.0039	0.0039	0.0040	0.0040	0.0040
	Ceratopteris	0.0219	0.0191	0.0138	0.0211	0.0220	0.0215	0.0220
	Hygrophila	0.0084	0.0076	0.0083	0.0040	0.0083	0.0083	0.0083
	Ludwigia	0.0338	0.0273		0.0291	0.0266		0.0338
	Nuphar	0.0289	0.0268	0.0251	0.0279		0.0216	0.0286
	Riccia	0.0632	0.0437		0.0533	0.0636	0.0630	0.0617

Table 24. **Three season mean transition probability from spring to fall to spring for each species and bare space across 12 years of sampling at Old Channel, Comal River site.**

Mean transition probability		Spring (current)							
Spring (previous)	**Fall(previous)**	**Algae**	**Bare**	***Ceratopteris***	***Hygrophila***	***Ludwigia***	***Nuphar***	***Riccia***	**sum**
Algae	**Algae**	0.484	0.516	0.000	0.000	0.000	0.000	0.000	1.000
	Bare	0.026	0.795	0.000	0.128	0.051	0.000	0.000	1.000
	Ceratopteris	0.023	0.862	0.023	0.057	0.034	0.000	0.000	1.000
	Hygrophila	0.000	0.222	0.000	0.667	0.056	0.000	0.056	1.000
	Ludwigia	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Nuphar	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Riccia	0.000	0.667	0.000	0.000	0.333	0.000	0.000	1.000
Bare	**Algae**	0.213	0.713	0.036	0.027	0.000	0.004	0.008	1.000
	Bare	0.001	0.965	0.004	0.021	0.004	0.002	0.002	1.000
	Ceratopteris	0.002	0.790	0.173	0.017	0.001	0.014	0.003	1.000
	Hygrophila	0.000	0.212	0.007	0.716	0.042	0.016	0.007	1.000
	Ludwigia	0.010	0.203	0.000	0.285	0.475	0.013	0.013	1.000
	Nuphar	0.000	0.275	0.036	0.108	0.000	0.575	0.006	1.000
	Riccia	0.008	0.687	0.000	0.122	0.000	0.046	0.137	1.000
Ceratopteris	**Algae**	0.000	0.938	0.063	0.000	0.000	0.000	0.000	1.000
	Bare	0.004	0.594	0.140	0.247	0.006	0.008	0.000	1.000
	Ceratopteris	0.002	0.327	0.510	0.076	0.000	0.084	0.001	1.000
	Hygrophila	0.000	0.037	0.029	0.632	0.294	0.007	0.000	1.000
	Ludwigia	0.000	0.000	0.100	0.000	0.000	0.900	0.000	1.000
	Nuphar	0.000	0.500	0.000	0.000	0.000	0.500	0.000	1.000
	Riccia	0.111	0.000	0.000	0.889	0.000	0.000	0.000	1.000
Hygrophila	**Algae**	0.000	0.565	0.000	0.435	0.000	0.000	0.000	1.000
	Bare	0.001	0.471	0.011	0.487	0.014	0.009	0.008	1.000
	Ceratopteris	0.000	0.265	0.190	0.450	0.000	0.095	0.000	1.000
	Hygrophila	0.000	0.093	0.005	0.890	0.004	0.005	0.003	1.000

	Ludwigia	0.000	0.342	0.000	0.404	0.247	0.000	0.007	1.000
	Nuphar	0.000	0.267	0.000	0.218	0.000	0.505	0.010	1.000
	Riccia	0.000	0.638	0.000	0.289	0.000	0.032	0.041	1.000
Ludwigia	**Algae**	0.000	0.000	0.000	1.000	0.000	0.000	0.000	1.000
	Bare	0.000	0.508	0.004	0.276	0.211	0.000	0.000	1.000
	Ceratopteris	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Hygrophila	0.000	0.205	0.000	0.635	0.160	0.000	0.000	1.000
	Ludwigia	0.000	0.295	0.000	0.226	0.479	0.000	0.000	1.000
	Nuphar	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Riccia	0.000	0.000	0.000	0.000	1.000	0.000	0.000	1.000
Nuphar	**Algae**	0.000	0.118	0.765	0.000	0.000	0.118	0.000	1.000
	Bare	0.000	0.550	0.053	0.206	0.000	0.183	0.008	1.000
	Ceratopteris	0.000	0.163	0.207	0.021	0.000	0.609	0.000	1.000
	Hygrophila	0.000	0.181	0.039	0.394	0.157	0.220	0.008	1.000
	Ludwigia	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Nuphar	0.000	0.228	0.020	0.045	0.000	0.705	0.002	1.000
	Riccia	0.000	0.366	0.000	0.171	0.000	0.171	0.293	1.000
Riccia	**Algae**	0.286	0.286	0.000	0.429	0.000	0.000	0.000	1.000
	Bare	0.000	0.313	0.000	0.679	0.000	0.000	0.008	1.000
	Ceratopteris	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Hygrophila	0.013	0.160	0.000	0.773	0.000	0.027	0.027	1.000
	Ludwigia	0.000	0.500	0.000	0.500	0.000	0.000	0.000	1.000
	Nuphar	0.000	0.222	0.000	0.000	0.000	0.778	0.000	1.000
	Riccia	0.000	0.444	0.000	0.389	0.000	0.000	0.167	1.000

Table 25. Standard deviation of three season mean transition probability from spring to fall to spring for each species and bare space across 12 years of sampling at Old Channel, Comal River site.

Stdev of transition probability		Spring (current)						
Spring (previous)	**Fall(previous)**	**Algae**	**Bare**	***Ceratopteris***	***Hygrophila***	***Ludwigia***	***Nuphar***	***Riccia***
Algae	**Algae**	0.090	0.090	0.000	0.000	0.000	0.000	0.000
	Bare	0.025	0.065	0.000	0.054	0.035	0.000	0.000
	Ceratopteris	0.016	0.037	0.016	0.025	0.020	0.000	0.000
	Hygrophila	0.000	0.069	0.000	0.079	0.038	0.000	0.038
	Ludwigia							
	Nuphar							
	Riccia	0.000	0.272	0.000	0.000	0.272	0.000	0.000
Bare	**Algae**	0.018	0.020	0.008	0.007	0.000	0.003	0.004
	Bare	0.000	0.001	0.000	0.001	0.000	0.000	0.000
	Ceratopteris	0.001	0.008	0.007	0.002	0.000	0.002	0.001
	Hygrophila	0.000	0.009	0.002	0.010	0.004	0.003	0.002
	Ludwigia	0.006	0.023	0.000	0.026	0.029	0.007	0.007
	Nuphar	0.000	0.035	0.014	0.024	0.000	0.038	0.006
	Riccia	0.008	0.041	0.000	0.029	0.000	0.018	0.030
Ceratopteris	**Algae**	0.000	0.061	0.061	0.000	0.000	0.000	0.000
	Bare	0.003	0.022	0.016	0.019	0.004	0.004	0.000
	Ceratopteris	0.001	0.013	0.014	0.007	0.000	0.008	0.001
	Hygrophila	0.000	0.016	0.014	0.041	0.039	0.007	0.000
	Ludwigia	0.000	0.000	0.095	0.000	0.000	0.095	0.000
	Nuphar	0.000	0.158	0.000	0.000	0.000	0.158	0.000
	Riccia	0.105	0.000	0.000	0.105	0.000	0.000	0.000
Hygrophila	**Algae**	0.000	0.103	0.000	0.103	0.000	0.000	0.000
	Bare	0.001	0.012	0.003	0.012	0.003	0.002	0.002
	Ceratopteris	0.000	0.030	0.027	0.034	0.000	0.020	0.000
	Hygrophila	0.000	0.003	0.001	0.003	0.001	0.001	0.001
	Ludwigia	0.000	0.039	0.000	0.041	0.036	0.000	0.007

	Nuphar	0.000	0.044	0.000	0.041	0.000	0.050	0.010
	Riccia	0.000	0.033	0.000	0.031	0.000	0.012	0.013
Ludwigia	**Algae**	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Bare	0.000	0.032	0.004	0.029	0.026	0.000	0.000
	Ceratopteris							
	Hygrophila	0.000	0.026	0.000	0.031	0.023	0.000	0.000
	Ludwigia	0.000	0.025	0.000	0.023	0.027	0.000	0.000
	Nuphar							
	Riccia	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nuphar	**Algae**	0.000	0.078	0.103	0.000	0.000	0.078	0.000
	Bare	0.000	0.043	0.020	0.035	0.000	0.034	0.008
	Ceratopteris	0.000	0.019	0.021	0.007	0.000	0.025	0.000
	Hygrophila	0.000	0.034	0.017	0.043	0.032	0.037	0.008
	Ludwigia							
	Nuphar	0.000	0.020	0.007	0.010	0.000	0.022	0.002
	Riccia	0.000	0.075	0.000	0.059	0.000	0.059	0.071
Riccia	**Algae**	0.171	0.171	0.000	0.187	0.000	0.000	0.000
	Bare	0.000	0.041	0.000	0.041	0.000	0.000	0.008
	Ceratopteris							
	Hygrophila	0.013	0.042	0.000	0.048	0.000	0.019	0.019
	Ludwigia	0.000	0.250	0.000	0.250	0.000	0.000	0.000
	Nuphar	0.000	0.139	0.000	0.000	0.000	0.139	0.000
	Riccia	0.000	0.117	0.000	0.115	0.000	0.000	0.088

SAV Modeling summary and continued effort

The SAV in the San Marcos and Comal Rivers is exposed to a different suite of environmental and physical parameters than the SAV that have been traditionally modeled. SAV models have historically been developed in lentic systems, particularly at northern climes where growth patterns are seasonal, and are often subject to nutrient limitations. These models cannot be applied to vegetation in spring-fed, clear-water lotic systems where the temperature is relatively constant. Further, these models have all been spatially-implicit single species models and do not contain functions for dispersal or multiple species of vegetation, nor have they been integrated with hydrodynamic or models of other taxa. In order to meet the objectives of the modeling project, we needed a SAV model that could incorporate the unique environmental and physical characteristics of the San Marcos and Comal systems, could capture the dynamics of multiple species, and that could be integrated with a hydrodynamic and fountain darter model. Since an existing model could not sufficiently address the objectives of the modeling study, we developed a novel model for the questions being asked of the HCP. Once we decided to develop a new model, we dedicated significant effort to capturing critical processes that affect plant growth in spring-fed rivers without making the model overly complex. We were not only concerned with accurately capturing the dynamics of vegetation growth and dispersal, but also had to consider how the SAV model would integrate with the hydrodynamic and fountain darter models so the model would run in a reasonable amount of time.

The SAV model plant growth model is process-driven and calculates daily biomass accumulation through photosynthesis. The equations used in the model are translations of equations used in well-established SAV models. The growth component of the SAV model is complete, and has been parameterized and calibrated for all but 3 species. The calibration for the remaining species will not involve much effort and values for the parameters will be gathered from the applied research studies or scientific literature. Since the growth module is process-driven, it is easily portable to all the ecomodel reaches in the system.

The dispersal component is where the majority of the modeling remains for the SAV model. Dispersal is poorly understood in aquatic plants, particularly in river systems. We first attempted to model SAV dispersal using a process-based approach (as described above), but we were

unable to accurately simulate the dynamics of the system. Currently, we are statistically analyzing the 13 years of vegetation mapping data in order to quantify the changes in vegetation over time (approach is described above). We will use the results of the statistical analysis as a basis for our approach for modeling SAV dispersal. Using this approach, we will be able to quantify system-level attributes such as diversity, persistence, species composition, patch shape, among others. This, along with the results from the applied research studies on SAV, will provide us with a quantitative foundation upon which to build the dispersal functions for the SAV. Dispersal will be modeled probabilistically by considering the likelihood of

(a) a species successfully colonizing a given geo-referenced cell, given its historical ability to maintain vegetation
(b) species X occupying a new cell, given that it has to outcompete other species in neighboring cells,

The dispersal model will be evaluated based on its ability to recreate the system-level patterns that were revealed during the statistical analyses of the mapping data.

2.5 Fountain Darter

The fountain darter model is the final component of the conceptual model of how darters respond to external conditions, see Figure 37. Unlike the more physical components of the overall model, *viz*. the hydraulics and water quality models, we do not have sound deterministic physical principles for darter populations upon which a numerical model may be based. For hydraulics and water quality, we have the equations of momentum (derived from Newton's laws of motion) and continuity, coupled with physically or chemically-based process equations, such as frictional loss at the streambed, evaporation from the water surface, reaeration at the surface, and kinetics within the water column. In contrast, for fountain darters, we have only the principles of accounting, and external forcing must be specified based upon empirical relations inferred from observation. Following a detailed literature search and evaluation of existing data, our goal was to develop a spatially-explicit, individual-based model representing fountain darter population dynamics using HCP biological monitoring data collected since 2000 as the foundation.

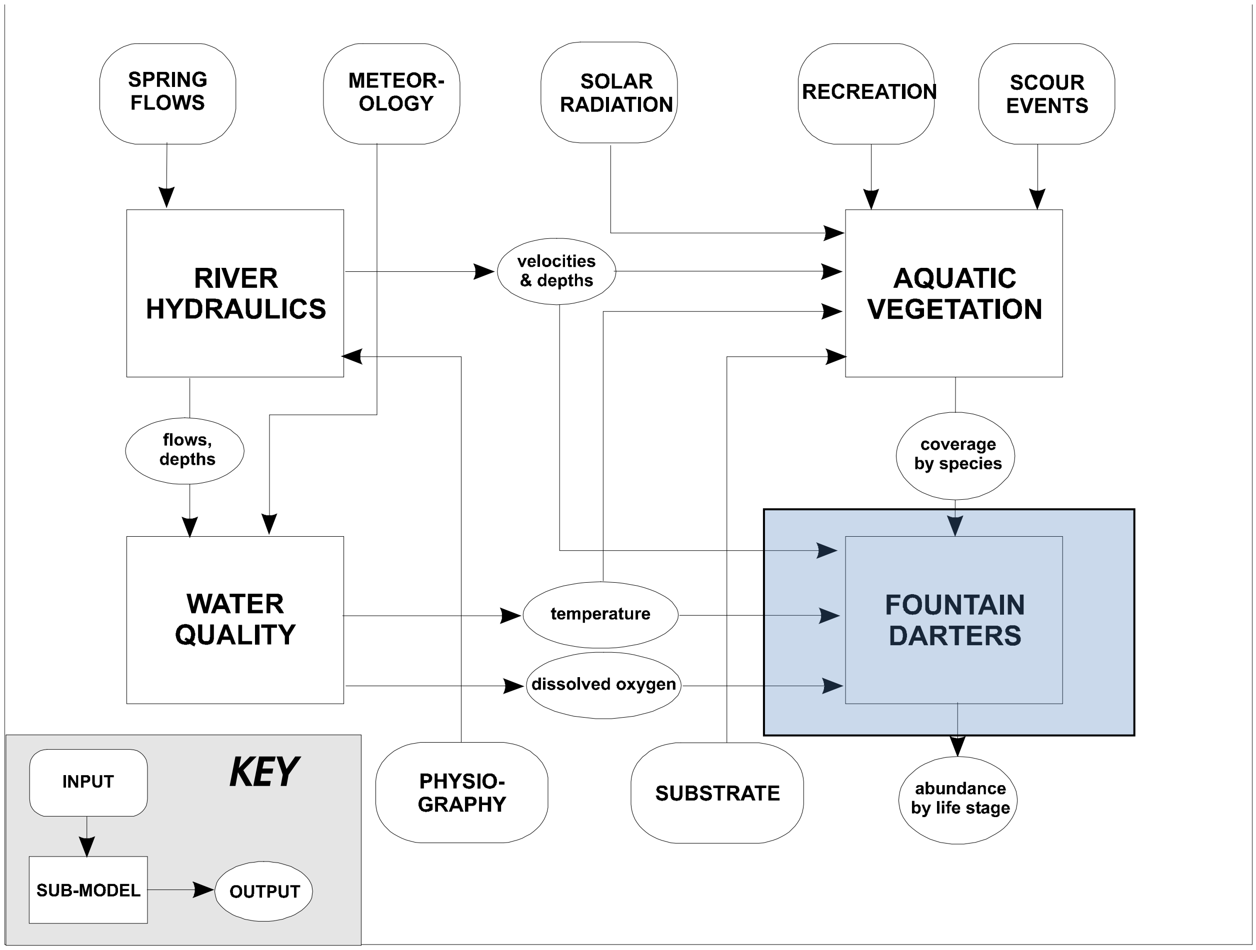


Figure 37. Conceptual model highlighting fountain darter submodel

Fountain Darter Historical Drop Net Data

A drop net is a sampling device originally designed by the USFWS to sample fountain darters and other benthic fish species. The net encloses a known area (2 square meters [m^2]) and allows a thorough sample by preventing escape of fishes occupying that area. A large dip net (1 m^2) is used within the drop net and is swept along the length of the river substrate 15 times to ensure complete enumeration of all fishes trapped within the drop net. Four drop net study reaches are sampled in the Comal system, and three drop net study reaches in the San Marcos River. Prior to a drop net sampling event, all aquatic vegetation is mapped within the drop net sample reach per respective system. Using the dominant vegetation types in the current HCP biological monitoring contract, drop net sites are generated following a stratified design based on the present aquatic vegetation coverage.

At each drop net location the substrate type (Table 26), water depth (m), vegetation type (species, Table 27), height of vegetation (VEGHT, m), presence of bryophytes within other vegetation (WBRYO, Table 27) and percent coverage of dominant vegetation are recorded (MAINPER), along with velocity at 15 centimeters above the bottom (CV), water temperature (TEMP), conductivity (COND), pH, and dissolved oxygen (DO) (Table 28). Fountain darters are identified, enumerated, measured for total length (mm), and returned to the river (outside the drop net) at the point of collection. More explicit details regarding the experimental design and procedures of biological monitoring are available in BIO-WEST (2015a,b)

In total, the fountain darter drop net data available for this analysis consist of 1,661 total observations, 1,002 from the Comal Springs system and 659 from the San Marcos Springs system. A concise summary of the fountain darter drop net data is provided below.

Table 26. Number of observations in which different substrate types were observed in each river.

	SILT	**SAND**	**GRAVEL**	**COBBLE**	**BEDROCK**
COMAL	551	45	333	72	1
SAN MARCOS	369	64	212	13	1

Table 27. Number of observations in each river within each dominant vegetation species strata. All strata are dominant vegetation types, though an additional variable (With Bryophytes) is included which represents observations which were observed to contain bryophytes within the dominant vegetation as this is hypothesized to provide a different habitat class.

	COMAL	SAN MARCOS
Bryophytes	152	0
Cabomba	91	78
Ceratophylum	33	0
Filamentous algae	42	0
Green algae	6	0
Hydrilla	0	167
Hygrophila	297	167
Ludwigia	138	2
Open	62	148
Potamogeton/hygrophila	0	68
Potamogeton	0	13
Sagittaria	95	10
Vallisneria	86	6
With Bryophytes	161	0

Table 28. Summary of continuous variables contained in the dropnet data.

	COMAL			SAN MARCOS		
	mean	***median***	***stdev***	***mean***	***median***	***stdev***
DARTER ABUNDANCE	21.19	9.00	29.67	8.61	4.00	14.60
MAINPER	88.25	100.00	31.63	71.51	95.00	40.02
VEGHT	0.39	0.37	0.27	0.32	0.30	0.27
DEPTH	0.85	0.85	0.24	0.64	0.64	0.26
CV	0.03	0.02	0.05	0.12	0.03	0.18
TEMP	23.71	23.68	0.75	22.08	22.09	0.92
DO	6.44	6.34	1.37	7.87	7.85	1.36
COND	561.24	553.00	35.84	601.07	601.00	44.31
PH	7.35	7.32	0.33	7.48	7.50	0.35

A statistical analysis of the historical data was an important first step in the parameterization of the simulation model. There are several modeling methods (including Poisson regression, negative binomial regression and zero-inflated count models) commonly used as analytical techniques for dealing with single species count data. In this study, we preliminarily investigated several methods on the front end including the aforementioned techniques along with a more detailed principal component analysis described in the following section. Based on conversations with professors in the Statistics Department at Texas A&M University we also explored many different aspects of a multinomial logit regression model summarized following the principal component analysis section and further documented in Appendix F.

Fountain Darter Historical Drop Net Data Principal Components Analysis

To evaluate the fountain darter habitat associations, Dr. Tim Bonner conducted a multivariate approach to assess spatial (i.e., among sites) and temporal (among seasons and through time)

patterns in San Marcos and Comal rivers sampled habitats and the association between habitats and fountain darter abundance.

Methods

Principal component analysis (PCA) implemented by PROC PRINCOMP in the SAS ver. 9.2 (SAS Institute Inc., 2008) was used to assess linear combinations of habitat characteristics among sampled habitats taken by BIO-WEST during a fifteen year period (2000 through 2014) within the San Marcos River and Comal River. A separate PCA was developed for each river system. Dominant substrate types were denoted as dummy variables. Other variables were ratio data (e.g., percent vegetation) or continuous data (e.g., water temperature) and z-transformed before analyses. PC scores I and II were averaged by site, season, and year to visually assess spatial and temporal trends in habitat parameters. Likewise, temporal patterns in Fountain darter abundance were plotted through time to visually assess if Fountain darter abundance trends among years. Numbers of fountain darters per sampled habitat were $\log_{10}$ (N +1) transformed to improve assumption of linearity and averaged among habitats by year. Numbers of fountain darters per sampled habitat were converted to categorical variables. In the San Marcos River, abundance categories were 0 (N = 0 fish), I (1), II (2 – 7), III (8 – 14, and IV (15 – 242). In the Comal River, abundance categories were 0 (N = 0), I (1 - 4), II (5 – 14), III (15-30), and IV (31 – 212). Rationale in using categories rather than count data include: to improve linear relationship between darter abundance and independent variables because of positive skews in the number of sampled habitats with no to few darters (50% of all sampled habitats had <4 darters in the San Marcos River and <10 darters in the Comal River), and rarity of sampled habitats with large counts (N = 2 of habitats with >100 darters in the San Marcos River and N = 31 in the Comal River). Ranges of darters within each category differed by river because of differences in fountain darter abundances by river. Nevertheless, qualitative values of each category are the same between both rivers: Category 0 (no darters), Category I (occurs but in low abundance), Categories II - IV (increasing levels of abundance). Relationships between habitat gradients (PC axes I and II) and abundance categories of Fountain darter were assessed with linear regression (PROC GLM; SAS). Relationships between habitats gradients (PC axes I and II) and each abundance category were assessed with Kolmogorov-Smirnov test (K-S test). Observed percent

frequency of each abundance category and expected percent frequent of all PC scores (I and II) were calculated for each PC axes using a histogram with a bin frequency of 1.

Results

Habitat Assessment

Mean annual discharge ranged from 97 to 273 cfs in the San Marcos River and 141 to 456 cfs in the Comal River between 2000 and 2014 (Figure 38). Among 659 samples from the San Marcos River and 987 samples from the Comal River, silt substrate (>50%) with vegetation (>70%) was the most common type of habitat sampled (Table 29). Most abundant dominant vegetation sampled was Hydrilla and Hygrophila (23%) in the San Marcos River and Hygrophila (28%) and Bryophytes (14%) in the Comal River. Sampled habitats on average were in sluggish current velocities (<0.12 m/s) and shallow depths (<0.84 m). Water quality parameters were consistent through time and not considered limiting, although water temperature exceeded optimum spawning temperatures <1% of the time.

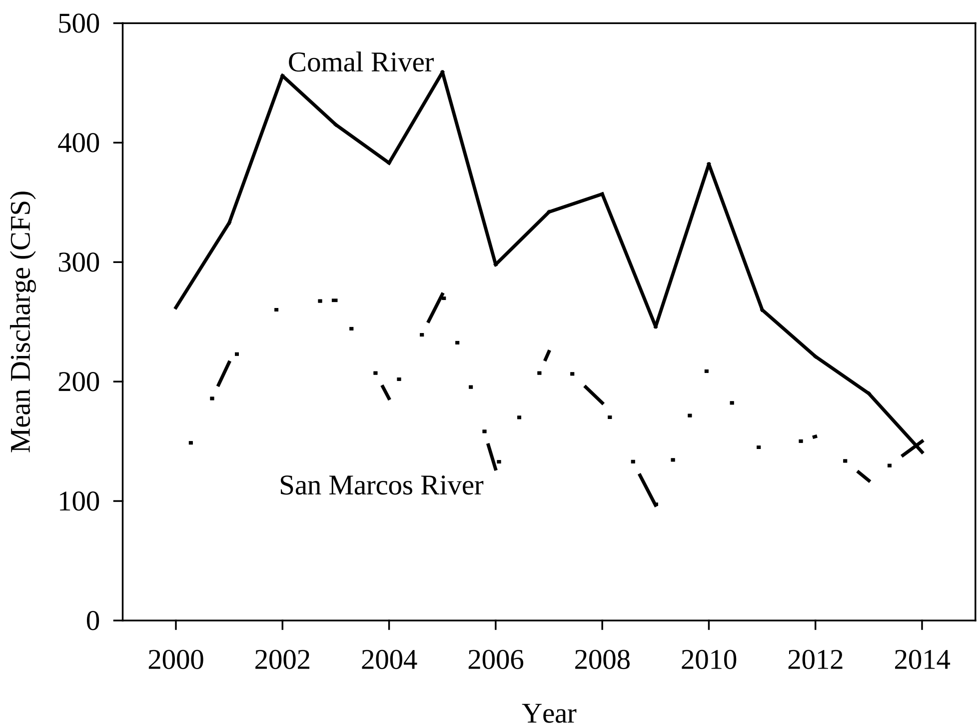


Figure 38. Mean annual discharge in cubic feet per second (CFS) during the period of observation (2000 - 2014).

Table 29. Statistics for water and habitat parameters of drop nets taken from the San Marcos (N = 659) and Comal (N = 987) rivers. Dominant substrates represent the relative abundance of each substrate among all drop nets.

	San Marcos River				Comal River			
	Mean	1 SD	Minimum	Maximum	Mean	1 SD	Minimum	Maximum
Water temperature (°C)	22.1	0.92	18.6	27.7	23.7	0.75	21.1	28.7
pH	7.5	0.35	6.0	8.4	7.3	0.33	6.5	9.6
Conductivity (uS/cm)	601	44	489	710	561	36	443	755
Dissolved oxygen (mg/l)	7.9	1.35	3.2	12.9	6.4	1.37	3.3	10.7
Current velocity (m/s)	0.12	0.182	0.00	1.28	0.03	0.046	0.00	0.40
Water depth (m)	0.64	0.257	0.09	1.37	0.84	0.243	0.01	1.43
Vegetation								
% Vegetation (all)	71	40	0	100	87	26	0	100
With bryophytes	< 0.1	0.039	0	1	0.16	0.361	0	1
Veg Height (m)	0.33	0.275	0	1.3	0.38	0.268	0	1.2
Veg Volume (m^3)	0.60	0.519	0	2.3	0.73	0.523	0	2.5
Dominance (%)								
Open	22	42	0	100	6	23	0	100
Bryophytes					14	34	0	100
Cabomba	11	31	0	100	9	27	0	100
Ceratopteris					3	16	0	100
Filamentous Algae					4	17	0	100
Green Algae					0.38	6	0	100
Hydrilla	23	41	0	100				
Hygrophila	23	40	0	100	28	44	0	100
Ludwigia	0.3	5	0	100	12	30	0	100
Mixed (Pot & Hygro)	10	29	0	100				
Potamogeton	1.7	11	0	100				
Sagittaria	1.3	11	0	100	9.0	28	0	100
Vallisneria	0.9	9	0	100	8.6	28	0	100
Dominant Substrate	Relative %				Relative %			
% Silt	56				55			
% Sand	10				4			
% Gravel	32				33			
% Cobble	2				7			
% Bedrock	0.2				0.1			

Principal component axes I and II explained 29% of the total variation in habitat variables within the San Marcos River (Table 30). Axis I explained 22% of the total variation and described vegetation, substrate, and current velocity gradients. Habitats with negative PC I scores along axis I were swifter current velocities with gravel substrates and lesser amounts of vegetation. Habitats with positive PC I scores were densely vegetated with silt substrates and slow current velocities. Axis II explained 7% of the total variation and described a depth and vegetation gradient. Habitats with negative PC II scores were shallow with predominantly Hygrophila

vegetation and sand substrates. Habitats with positive PC II scores were from deeper depths with mixed stands of Potamogeton-Hygrophila or lesser amounts of vegetation.

Table 30. Principal components I and II loadings for each parameter used in models. Bold represents parameters with strongest loadings per gradient.

	San Marcos River		Comal River	
Parameter:	PC 1	PC II	PC 1	PC II
Water temperature	-0.024	0.099	-0.040	0.055
pH	-0.070	-0.248	-0.043	0.121
Conductivity	0.004	0.007	-0.003	-0.269
Dissolved oxygen	-0.076	-0.016	-0.065	**0.369**
Current velocity	**-0.294**	0.086	**-0.229**	0.165
Water depth	0.189	**0.378**	0.047	-0.093
Vegetation				
% Vegetation (all)	**0.373**	**-0.289**	**0.345**	**-0.312**
With bryophytes	0.007	-0.062	0.079	0.005
Veg Height	**0.377**	0.164	**0.439**	0.002
Veg Volume	**0.381**	0.141	**0.447**	-0.011
Dominance				
Open	**-0.366**	**0.296**	**-0.307**	**0.301**
Bryophytes			-0.197	**-0.376**
Cabomba	0.132	0.115	0.102	0.127
Ceratopteris			0.028	0.158
Filamentous Algae			-0.084	0.201
Green Algae			-0.025	-0.067
Hydrilla	0.035	**-0.364**		
Hygrophila	0.109	**-0.259**	0.182	0.030
Ludwigia	-0.006	-0.062	-0.038	-0.120
Mixed (Pot & Hygro)	0.171	**0.342**		
Potamogeton	0.018	0.096		
Sagittaria	0.020	-0.013	0.049	-0.049
Vallisneria	-0.017	-0.050	0.205	-0.020
Dominant Substrate				
Silt	**0.353**	0.114	**0.310**	**0.373**
Sand	-0.021	**-0.418**	0.032	-0.096
Gravel	**-0.335**	0.112	**-0.243**	**-0.389**
Cobble	-0.084	0.103	-0.178	0.063
Bedrock	-0.017	-0.017	0.014	0.016

Principal component axes I and II explained 24% of the total variation in habitat variables within the Comal River. Axis I explained 15% of the total variation and described vegetation and substrate gradient. Sampled habitats with negative PC I scores along axis I were gravel substrates with swifter current velocities and lesser amounts of vegetation. Sampled habitats with positive PC I scores were densely vegetated with silt substrates. Axis II explained 9% of the total variation and described substrate, water quality, and vegetation gradient. Sampled habitats with negative PC II scores were with gravel substrates and bryophytes. Sampled habitats with positive PC II scores were with silt substrates, lesser amounts of vegetation, and higher dissolved oxygen concentrations.

Sampled habitats varied little among sites, season, and years (Figure 39). In the San Marcos River, sampled habitats in City Park consisted of more vegetation (positive on PC 1) than I-35 Bridge and Spring Lake Dam. In the Comal River, sampled habitats in Landa Lake and New Channel consisted of more vegetation than Upper Spring Run and Old Channel. Sampled habitats in Landa Lake and Upper Spring Run consisted of more bryophytes than Old Channel and New Channel. Seasonal shifts in vegetation amounts were not discernible in either system. Among years, shifts towards more vegetation were observed in both systems, followed by shifts to less vegetation by 2014.

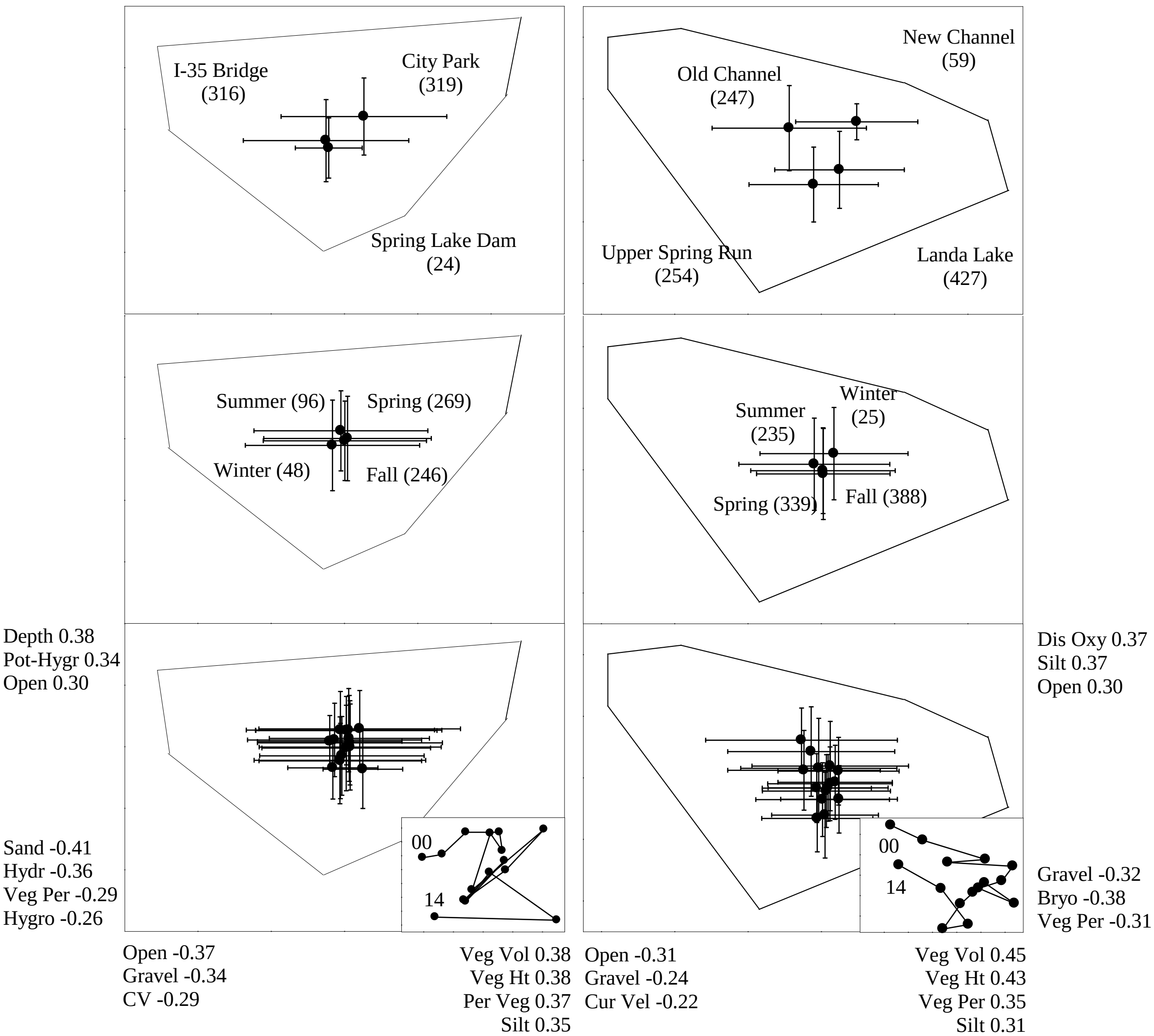


Figure 39. **Mean and 1 SD for PC I and PC II scores by site (top graph), season (middle) and year (bottom). Number per site and season are in parentheses. For year, subset plot indicates trajectory of mean PC I and PC II scores starting in 2000 (00) and ending in 2014 (14). Abbreviated variables on axes: Bryo = Bryophytes; Cur Vel = current velocity; CV = current velocity; Dis Oxy = dissolved oxygen; Hydr = Hydrilla; Hygro = Hygrophila; Pot-Hygr = mixed Potamogeton and Hygrophila; Veg Ht = Vegetation Height; Veg Per = percent coverage of main vegetation in dropnet samples Veg Vol = volume of vegetation in dropnet samples.**

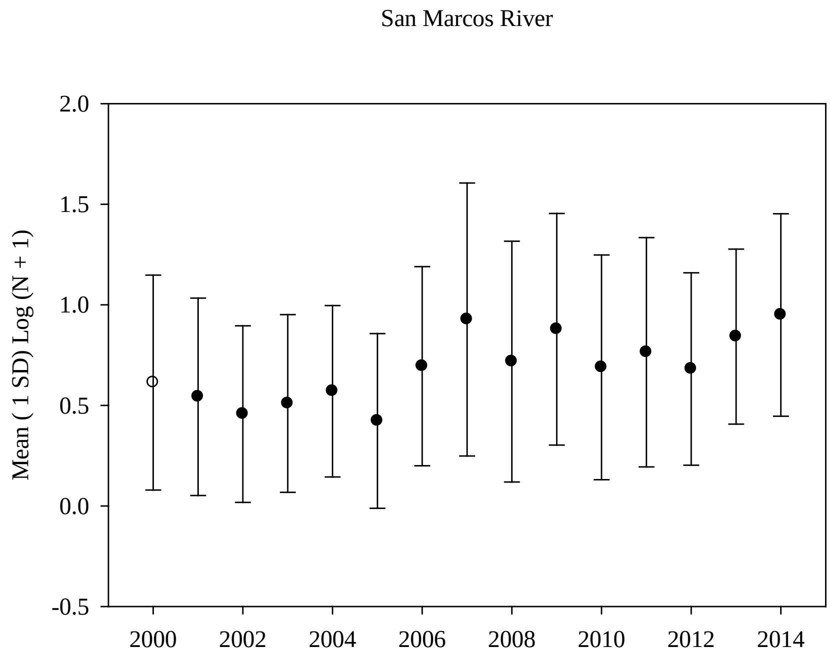
Fountain Darter abundance and habitat associations

A total of 5,704 fountain darters was captured from the San Marcos River and 20,929 fountain darters were captured from the Comal River. Mean (± 1 SD) was 8.7 ± 14.64 fish per sample in the San Marcos River and 21.2 ± 29.71 fish per sample in the Comal River. Maximum numbers of fountain darters captured per sample was 241 from the San Marcos River and 212 from the Comal River. Mean (± 1 SD) abundance of fountain darters by year ranged from 3.3 ± 4.23 to 23.9 ± 46.32 in the San Marcos River and 8.6 ± 16.72 to 33.6 ± 41.97 in the Comal River. In both rivers, plots of numbers of darters among sampled habitats by year suggested increasing trends in abundance (Figure 40).

Fountain darter abundance categories (0 – IV) were positively related to PC axis I (regression analyses: slope = 0.411; $P < 0.01$; $r^2 = 0.42$) and negatively related to PC axis II (slope = -0.233; $P < 0.01$; $r^2 = 0.05$) in the San Marcos River. By abundance categories, observed distributions differed ($P < 0.01$) from expected along PC I for Category 0 (K-S test statistic: D = 0.53), Category II (D = 0.20), Category III (D = 0.28), and Category IV (D = 0.24) (Figures 41 through 51). Observed distributions differed ($P < 0.01$) from expected along PC II for Category 0 (D = 0.28), and Category II (D = 0.13). Sampled habitats among Category 0 were associated with less vegetation, gravel substrates, and swifter current velocities. Sampled habitats among Category I were ubiquitously distributed among the available habitats. Sampled habitats among Categories II – IV were associated with greater amounts of vegetation, silt substrate, and sluggish current velocities.

Fountain darter abundance categories (0 – IV) were not related to PC axis I (P = 0.35) and negatively related to PC axis II (slope = -0.272; $P < 0.01$; $r^2 = 0.08$) in the Comal River. By abundance categories, observed distributions differed ($P < 0.04$) from expected along PC I for Category 0 (K-S test statistic: D = 0.16), Category II (D = 0.11), and Category IV (D = 0.19). Observed distributions differed ($P < 0.01$) from expected along PC II for Category 0 (D = 0.14), and Category IV (D = 0.23). Sampled habitats among Category 0 were associated with less vegetation, gravel substrates, and swifter current velocities. Sampled habitats among Category II were associated with more vegetation. Sampled habitats among Category IV were associated

with less vegetation volume and height along PC 1 and with more bryophytes over gravel substrates along PC 2.



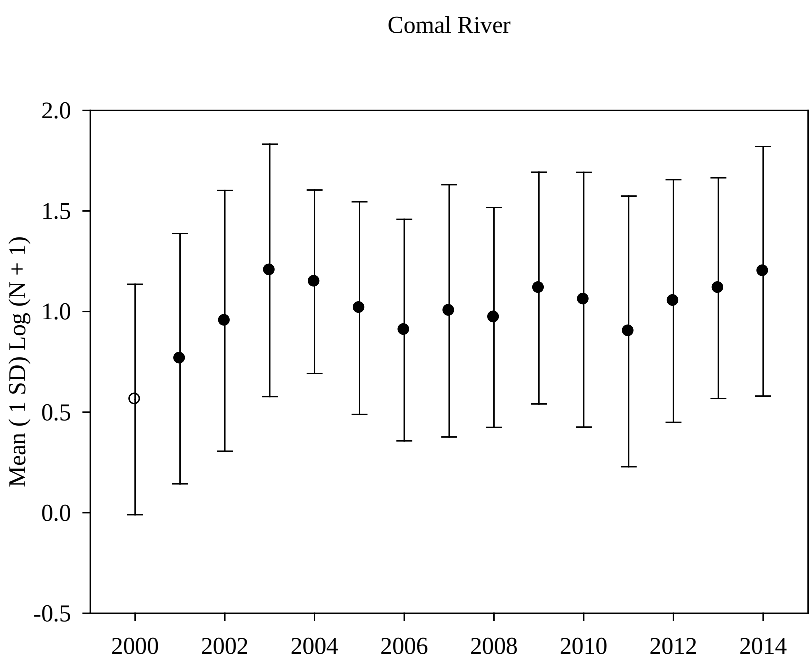


Figure 40. Mean ± 1 SD abundance (Log [N+1]) of fountain darters quantified in the San Marcos River (top panel) and Comal River (bottom panel) between 2000 and 2014.

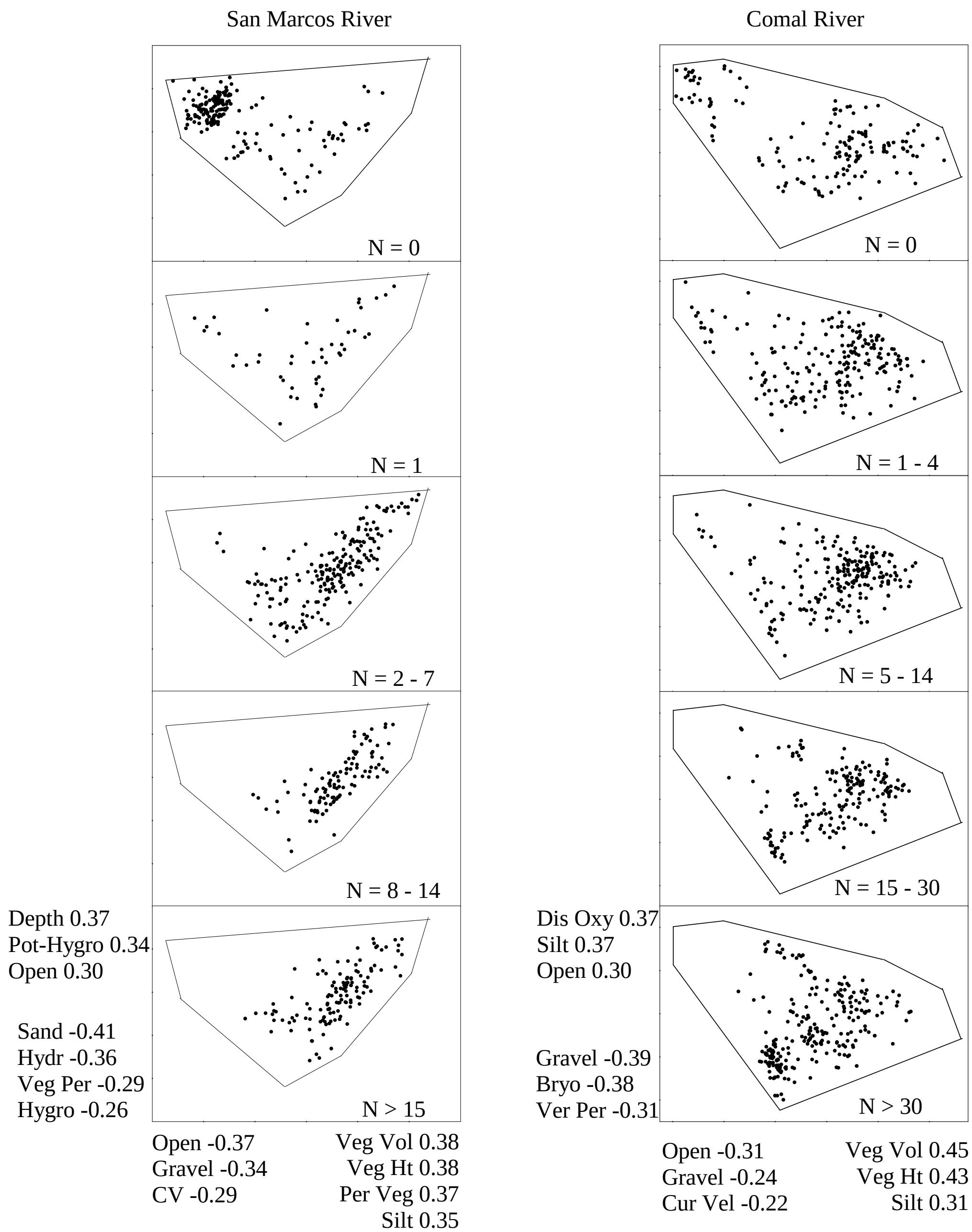


Figure 41. Bi-plots of principal components I and II factors for the San Marcos River (left panel) and Comal River (right panel). Principal component gradients (axes descriptions) listed on the two bottom graphs are the same for graphs within each panel. Solid line envelops all drop net data (not shown). Black circles represent drop nets by abundance category. Abundance category descriptions (0 – IV) are listed on each graph with N representing the number of darters in each sample. Abbreviated variables on axes: Bryo = Bryophytes; Cur Vel = current velocity; CV = current velocity; Dis Oxy = dissolved oxygen; Hydr = Hydrilla; Hygro = Hygrophila; Pot-Hygro = mixed Potamogeton and Hygrophila; Veg Ht = Vegetation Height; Per Veg = percent coverage of main vegetation in dropnet samples Veg Vol = volume of vegetation in dropnet samples.

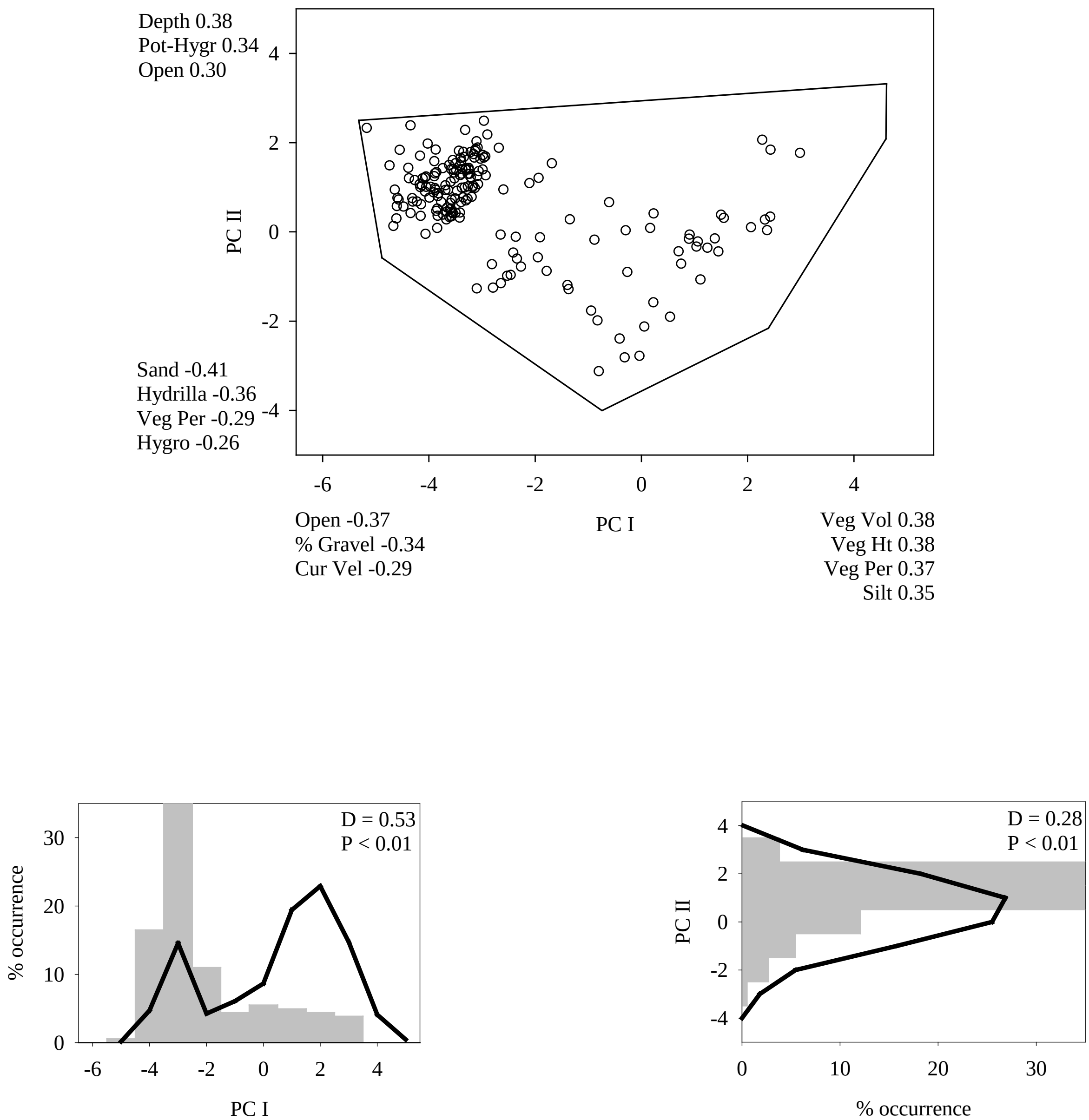


Figure 42. **Distribution of Category 0 (samples without fountain darters) along PC I and II (top panel) and by percent occurrences between observed (gray bars) and expected (black line) along PC 1 (bottom left) and PC 2 (bottom right) for San Marcos River. Significance between observed and expected distributions were assessed with Kolmogorov-Smirnov test (D). Abbreviated variables on axes: Cur Vel = current velocity; Hygro = Hygrophila; Pot-Hygr = mixed Potamogeton and Hygrophila; Veg Ht = Vegetation Height; Veg Per = percent coverage of main vegetation in dropnet samples Veg Vol = volume of vegetation in dropnet samples.**

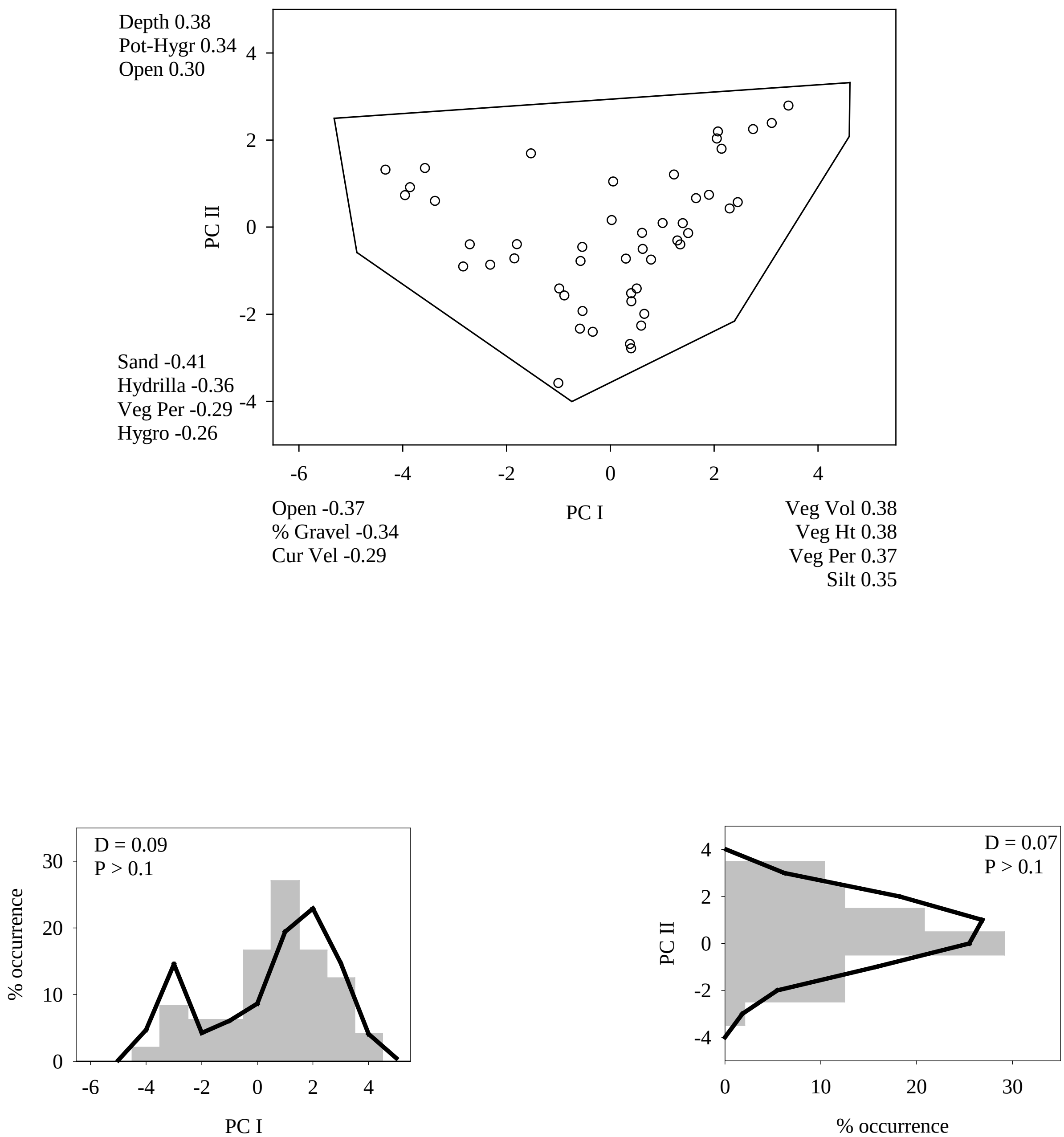


Figure 43. Distribution of Category I (samples with one fountain darter) along PC I and II (top panel) and by percent occurrences between observed (gray bars) and expected (black line) along PC 1 (bottom left) and PC 2 (bottom right) for San Marcos River. Significance between observed and expected distributions were assessed with Kolmogorov-Smirnov test (D). Abbreviated variables on axes: Cur Vel = current velocity; Hygro = Hygrophila; Pot-Hygr = mixed Potamogeton and Hygrophila; Veg Ht = Vegetation Height; Veg Per = percent coverage of main vegetation in dropnet samples Veg Vol = volume of vegetation in dropnet samples.

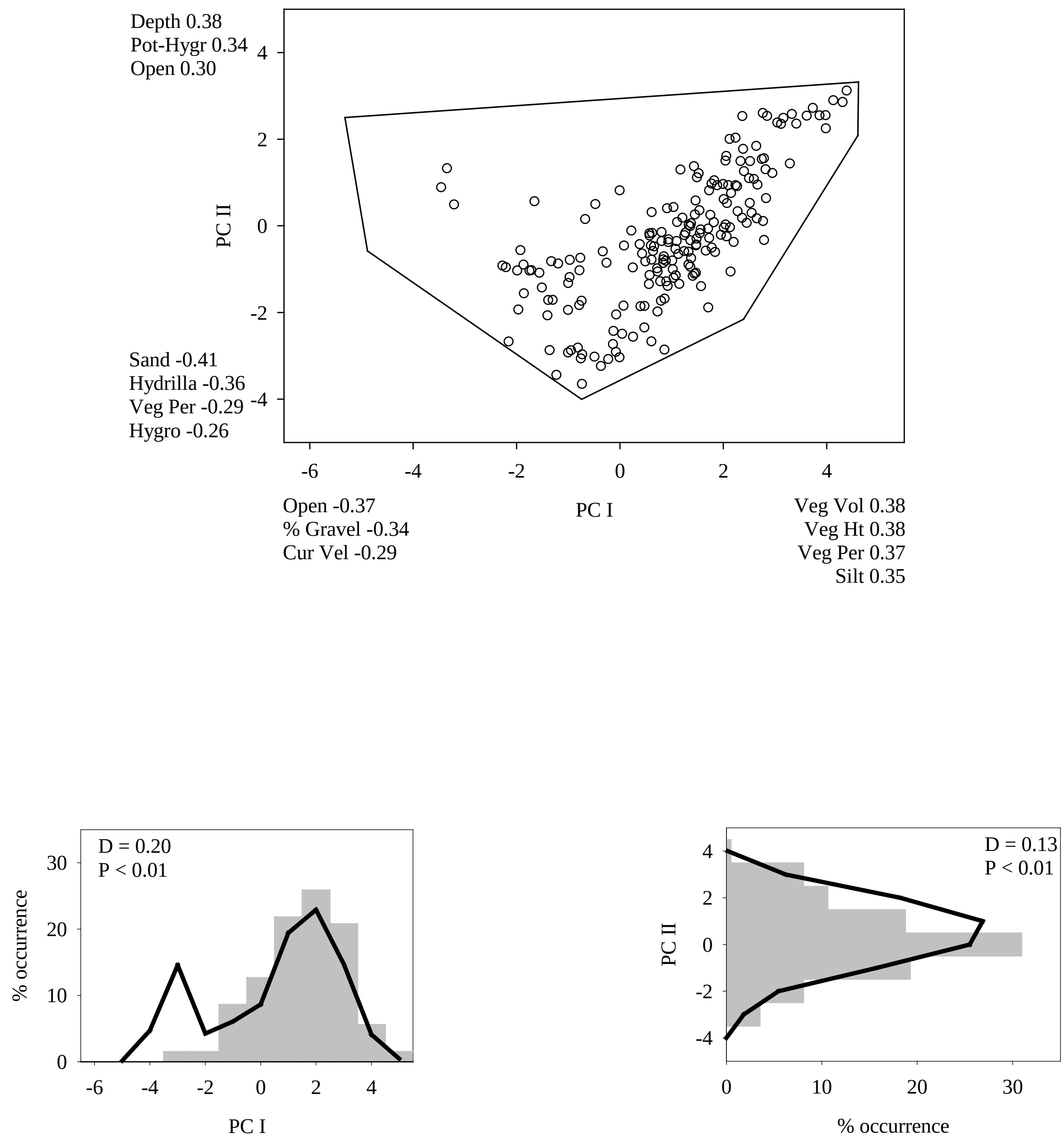


Figure 44. **Distribution of Category II (samples with two to seven fountain darters) along PC I and II (top panel) and by percent occurrences between observed (gray bars) and expected (black line) along PC 1 (bottom left) and PC 2 (bottom right) for San Marcos River. Significance between observed and expected distributions were assessed with Kolmogorov-Smirnov test (D). Abbreviated variables on axes: Cur Vel = current velocity; Hygro = Hygrophila; Pot-Hygr = mixed Potamogeton and Hygrophila; Veg Ht = Vegetation Height; Veg Per = percent coverage of main vegetation in dropnet samples Veg Vol = volume of vegetation in dropnet samples.**

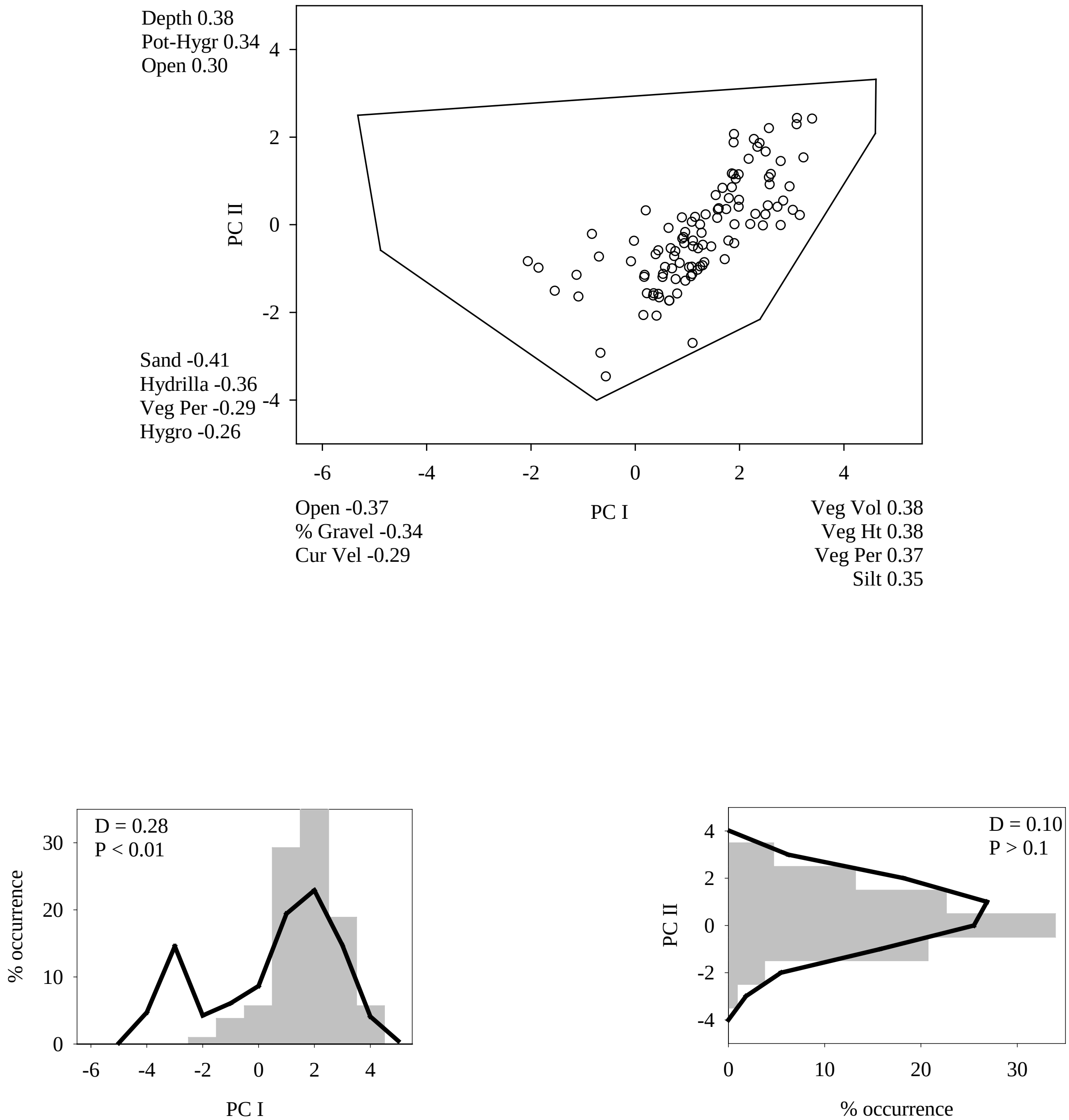


Figure 45. **Distribution of Category III (samples with 8 to 14 fountain darters) along PC I and II (top panel) and by percent occurrences between observed (gray bars) and expected (black line) along PC 1 (bottom left) and PC 2 (bottom right) for San Marcos River. Significance between observed and expected distributions were assessed with Kolmogorov-Smirnov test (D). Abbreviated variables on axes: Cur Vel = current velocity; Hygro = Hygrophila; Pot-Hygr = mixed Potamogeton and Hygrophila; Veg Ht = Vegetation Height; Veg Per = percent coverage of main vegetation in dropnet samples Veg Vol = volume of vegetation in dropnet samples.**

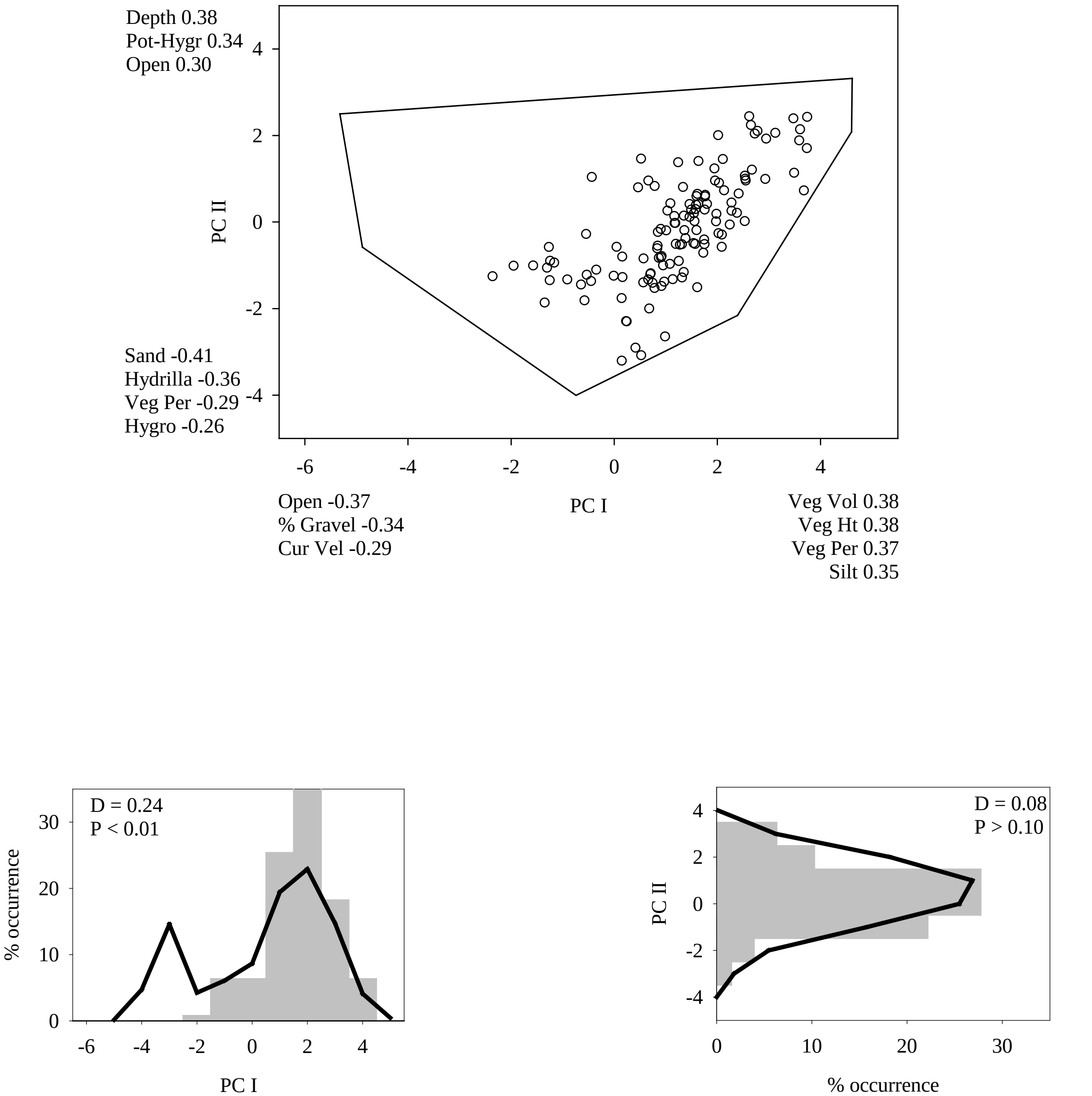


Figure 46. **Distribution of Category IV (samples with 15 to 242 fountain darters) along PC I and II (top panel) and by percent occurrences between observed (gray bars) and expected (black line) along PC 1 (bottom left) and PC 2 (bottom right) for San Marcos River. Significance between observed and expected distributions was assessed with Kolmogorov-Smirnov test (D). Abbreviated variables on axes: Cur Vel = current velocity; Hygro = Hygrophila; Pot-Hygr = mixed Potamogeton and Hygrophila; Veg Ht = Vegetation Height; Veg Per = percent coverage of main vegetation in dropnet samples Veg Vol = volume of vegetation in dropnet samples.**

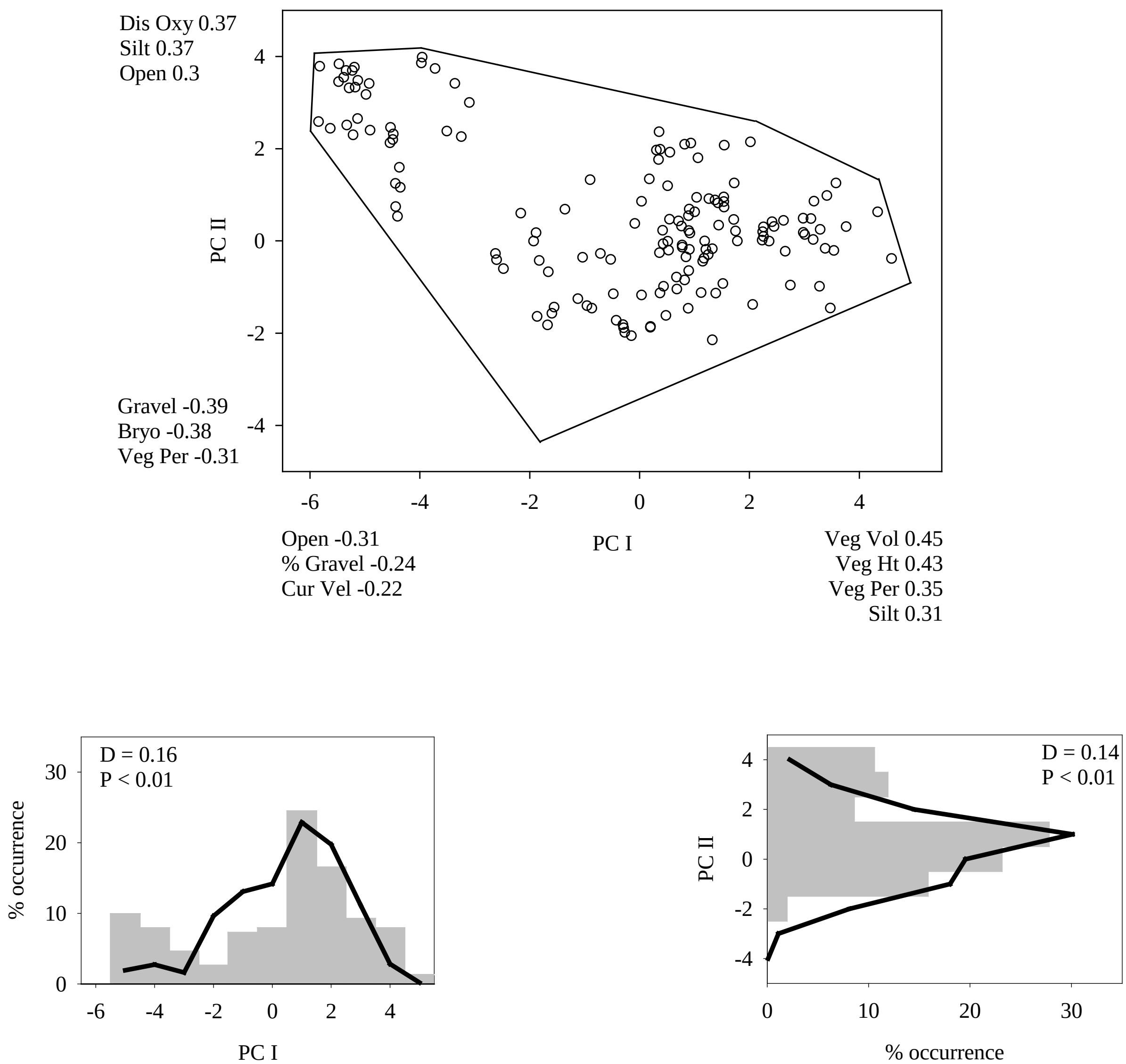


Figure 47. Distribution of Category 0 (samples without fountain darters) along PC I and II (top panel) and by percent occurrences between observed (gray bars) and expected (black line) along PC 1 (bottom left) and PC 2 (bottom right) for Comal River. Significance between observed and expected distributions was assessed with Kolmogorov-Smirnov test (D). Abbreviated variables on axes: Bryo = Bryophytes; Cur Vel = current velocity; Dis Oxy = dissolved oxygen; Veg Ht = Vegetation Height; Per Veg = percent coverage of main vegetation in dropnet samples Veg Vol = volume of vegetation in dropnet samples.

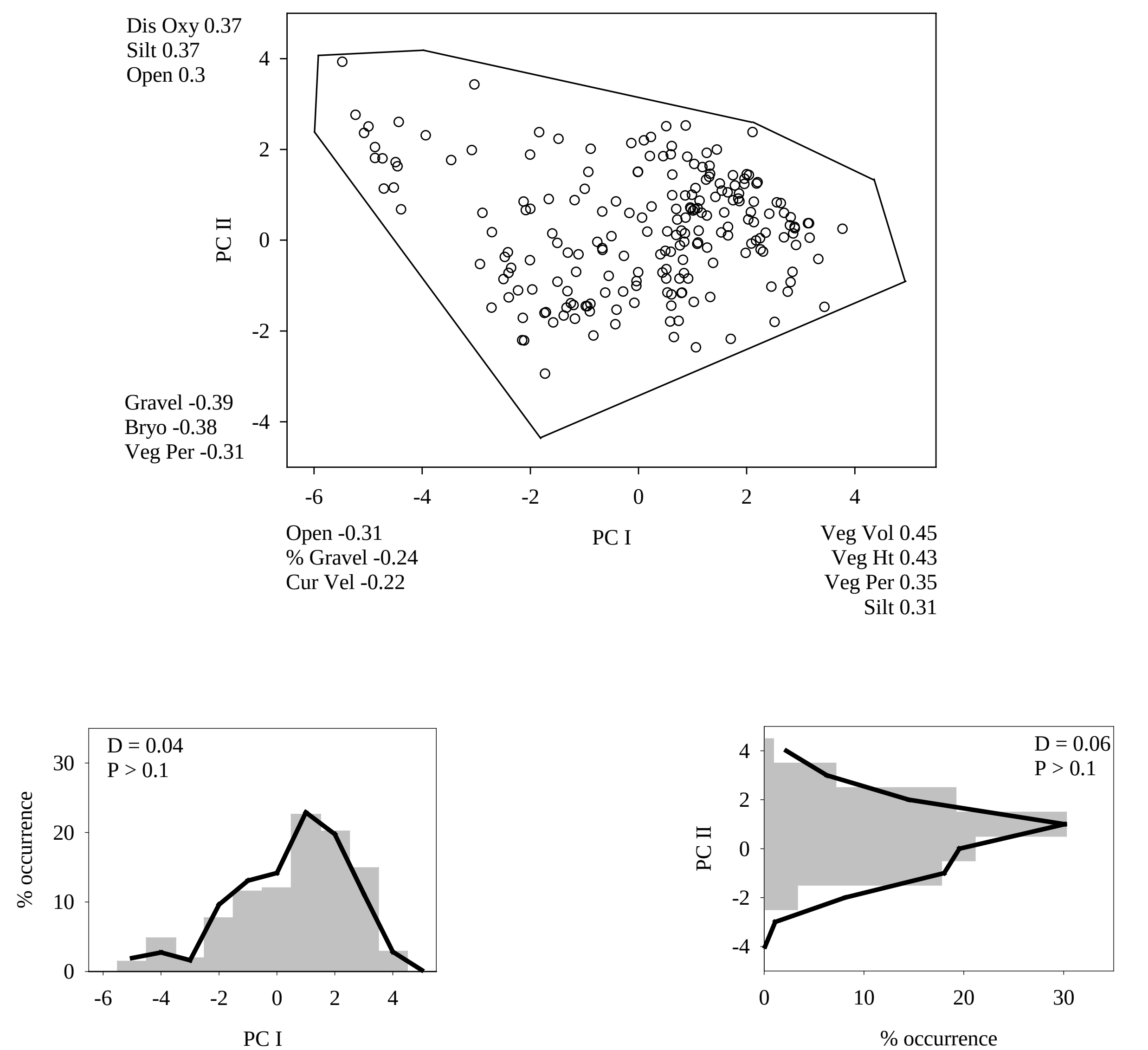


Figure 48. **Distribution of Category I (samples with one to four fountain darters) along PC I and II (top panel) and by percent occurrences between observed (gray bars) and expected (black line) along PC 1 (bottom left) and PC 2 (bottom right) for Comal River. Significance between observed and expected distributions was assessed with Kolmogorov-Smirnov test (D). Abbreviated variables on axes: Bryo = Bryophytes; Cur Vel = current velocity; Dis Oxy = dissolved oxygen; Veg Ht = Vegetation Height; Per Veg = percent coverage of main vegetation in dropnet samples Veg Vol = volume of vegetation in dropnet samples.**

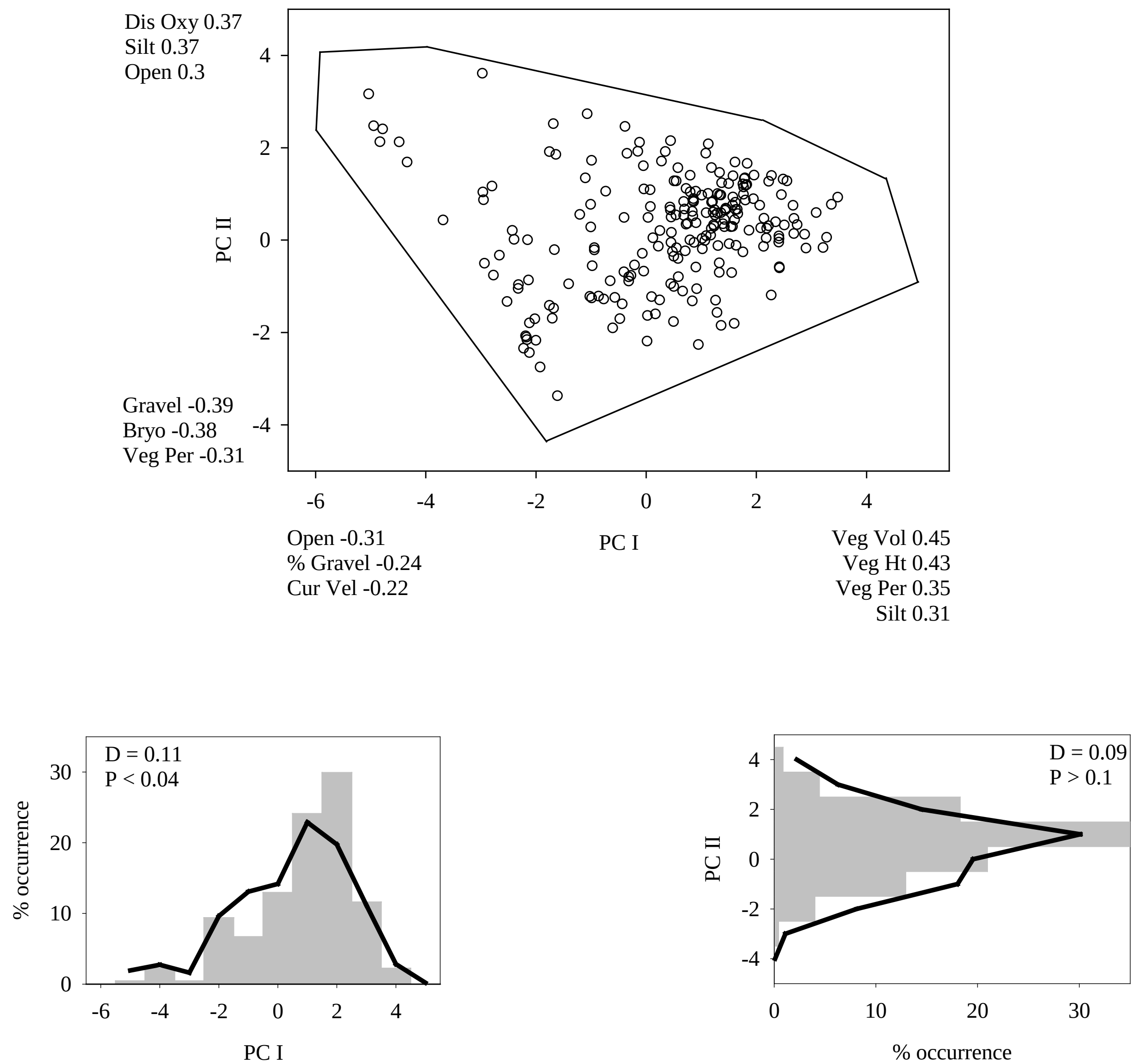


Figure 49. **Distribution of Category II (samples with 5 to 14 fountain darters) along PC I and II (top panel) and by percent occurrences between observed (gray bars) and expected (black line) along PC 1 (bottom left) and PC 2 (bottom right) for Comal River. Significance between observed and expected distributions was assessed with Kolmogorov-Smirnov test (D). Abbreviated variables on axes: Bryo = Bryophytes; Cur Vel = current velocity; Dis Oxy = dissolved oxygen; Veg Ht = Vegetation Height; Per Veg = percent coverage of main vegetation in dropnet samples Veg Vol = volume of vegetation in dropnet samples.**

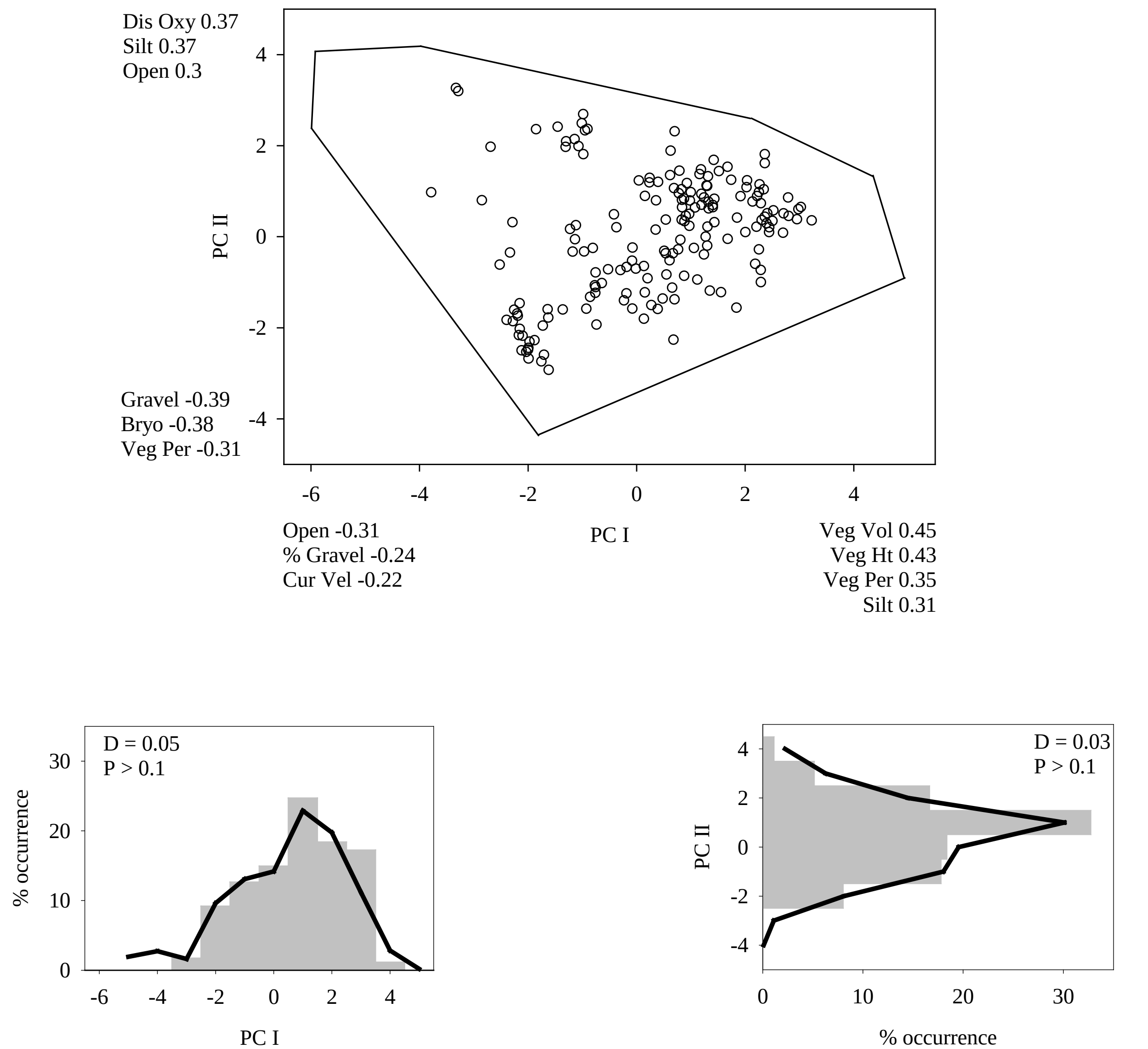


Figure 50. Distribution of Category III (samples with 15 to 30 fountain darters) along PC I and II (top panel) and by percent occurrences between observed (gray bars) and expected (black line) along PC 1 (bottom left) and PC 2 (bottom right) for Comal River. Significance between observed and expected distributions was assessed with Kolmogorov-Smirnov test (D). Abbreviated variables on axes: Bryo = Bryophytes; Cur Vel = current velocity; Dis Oxy = dissolved oxygen; Veg Ht = Vegetation Height; Per Veg = percent coverage of main vegetation in dropnet samples Veg Vol = volume of vegetation in dropnet samples.

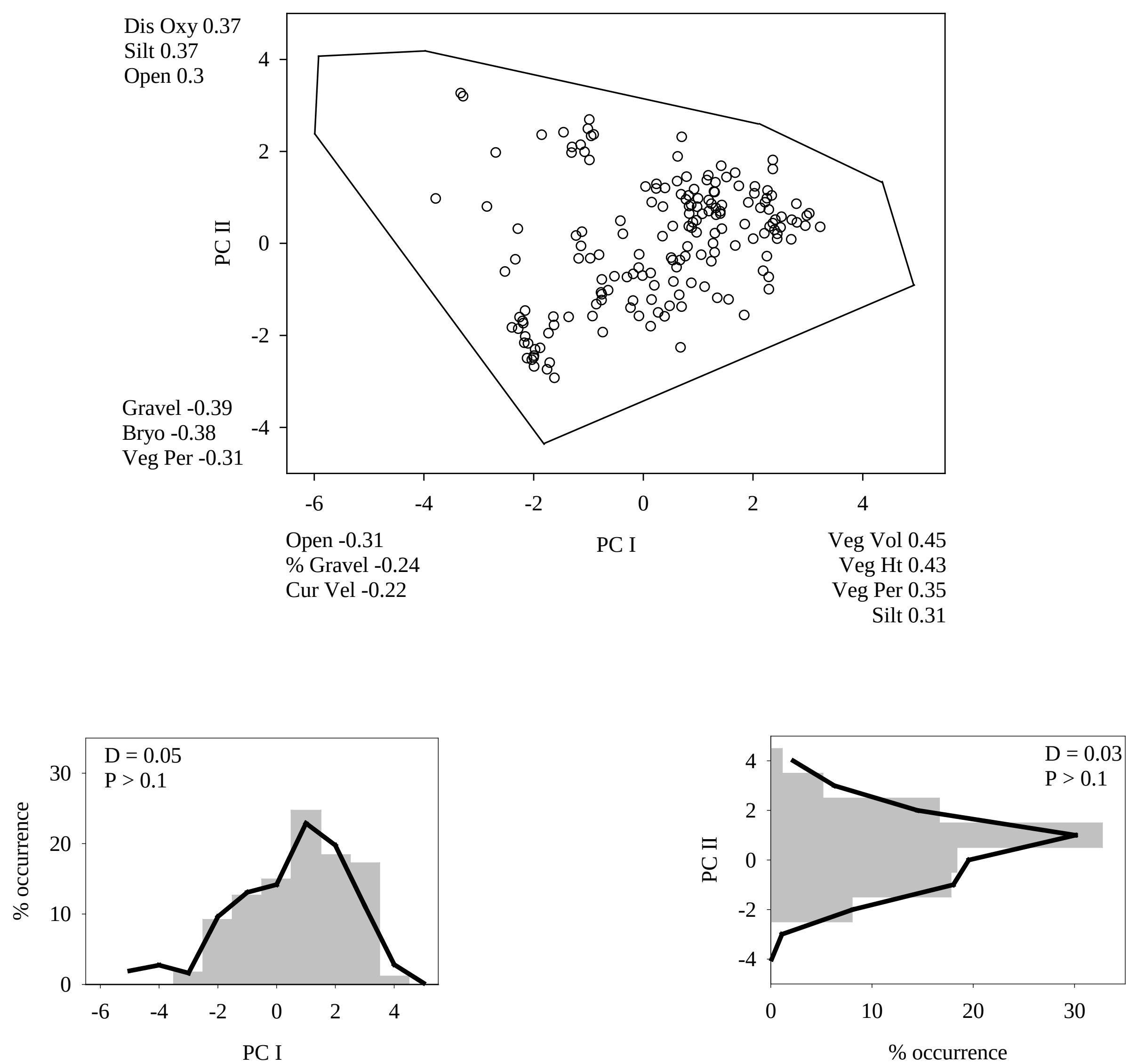


Figure 51. **Distribution of Category IV (samples with 31 to 212 fountain darters) along PC I and II (top panel) and by percent occurrences between observed (gray bars) and expected (black line) along PC 1 (bottom left) and PC 2 (bottom right) for Comal River. Significance between observed and expected distributions was assessed with Kolmogorov-Smirnov test (D). Abbreviated variables on axes: Bryo = Bryophytes; Cur Vel = current velocity; Dis Oxy = dissolved oxygen; Veg Ht = Vegetation Height; Per Veg = percent coverage of main vegetation in dropnet samples Veg Vol = volume of vegetation in dropnet samples.**

In summary, sampled habitats without darters were characterized as less vegetation, gravel substrates, and swifter current velocities in both rivers. Overall, some level of darters (1 to 4) can be found in any of the sampled habitats, whereas a greater number of darters were associated with greater amounts of vegetation, silt substrates, and sluggish current velocities in the San Marcos River. There are, in general, greater number of darters found throughout the available habitats in the Comal River with the highest numbers typically associated with bryophytes. In conclusion, although these rivers are dynamic (i.e. aquatic vegetation comes and goes, substrates change, with current velocities that are spatially highly variable for a given discharge, we have seen floods and drought) fountain darters appear to be well adapted to the conditions observed over the past 15 years in each system.

Fountain Darter Historical Drop Net Data Analysis

To further examine the extensive fountain darter drop net data set for model parameterization, we developed a statistical model representing the relationship between potential maximum darter densities and their environmental variables. We first re-organized drop net data including aquatic vegetation, water depth, velocity, and dissolved oxygen concentration in the Comal River and San Marcos River from 2003 through 2014. We then used different approaches including statistical methods and empirical analyses such that the most appropriate method could explain the relationship between potential maximum darter densities and the environmental variables well (significantly) in both views of statistic and ecology. A brief description of these trials in chronological order is presented below with a detailed documentation in Appendix F. The statistical analysis in our study, i.e. multinomial logit regression, provides a location (a cell) with a probability of darter density being in any of five categories and hence provides stochasticity in our simulation model. In the real world, two (almost) identical habitats seldom will have the same abundance of darters. An advantage of the multinomial logit model is to reflect this variation at the local level. In each cell, the probability of observing darter abundance values within each of the five density categories is calculated by the multinomial logit model. These probabilities could be viewed as the possible habitat qualities for each cell.

The number of, as well as the ranges of the response categories for raw fountain darter counts were delineated using a combination of expert opinion and review of the distribution of darter

abundance values in the data. The original goal of this process was to define categories that were considered representative of ecologically meaningful levels of abundance. These units could then be more easily applied in a management context than explicit abundance estimates. The final decision was to employ five categories of darter abundance in each system (Table 31).

Table 31. Range of fountain darter density values composing darter abundance probability categories developed for the multinomial logit model.

category	***1***	***2***	***3***	***4***	***5***
COMAL	0	1-5	6-15	16-30	>30
SAN MARCOS	0	1-2	3-8	9-15	>15

Finally, we applied the model to estimate the potential maximum darter densities for each habitat cell in the fountain darter individual-based, spatially-explicit simulation model.

Potential predictors of fountain darter abundance

Previous research including our PCA has identified several potential predictors of occurrence or abundance of fountain darters or similar species, including coverage and height of aquatic vegetation, presence of particular plant species, and water depth, velocity, temperature, conductivity, pH, and dissolved oxygen. Drawing on this literature and preliminary analysis, as well as extensive personal field observations, we selected a set of variables to include in our analysis (Table 32).

Table 32. Descriptions, values or units of measure, and means or frequencies of vegetation characteristics and water features as potential determinants of fountain darter density in (a) Comal and (b) San Marcos Springs, Texas.

(a)

Variable	Variable description	values or units of measure	Mean (range)[a] or frequency
Substrate types			
Gravel	Gravel	0: no	0: 540
		1: yes	1: 256
Sand	Sand	0: no	0: 755
		1: yes	1: 41
Silt	Silt	0: no	0: 418
		1: yes	1: 378
Silt_Gravel	Silt over gravel	0: no	0: 725
		1: yes	1: 71
Vegetation characteristics			
Open	Bare	%	5.92 (0 – 100)
Bryophytes	Bryophytes coverage	%	15.97 (0 – 100)
Cabomba	Cabomba coverage	%	8.83 (0 – 100)
Ceratopteris	Ceratopteris coverage	%	2.99 (0 – 100)
Fil_Algae	Filamentous Algae coverage	%	4.15 (0 – 100)
Green_Algae	Green Algae coverage	%	0.25 (0 – 100)
Hygrophila	Hygrophila coverage	%	29.95 (0 – 100)
Ludwigia	Ludwigia coverage	%	12.54 (0 – 100)
Sagittaria	Sagittaria coverage	%	9.38 (0 – 100)
Vallisneria	Vallisneria coverage	%	8.97 (0 – 100)
With_Bryo	With bryophytes overlap with main vegetation	0: no 1: yes	0: 526 1: 269
VegCover	Main vegetation coverage	%	93.04 (10 – 100)
VegHeight	Main vegetation height	Ft	1.35 (0.10 – 3.8)
Water features			
Depth	Water depth	Ft	2.80 (0.7 – 4.7)
Velocity	Water velocity		0.03 (0.02 – 0.40)
Temperature	Water temperature	C	23.64 (21.05 – 34.80)
DO	Dissolved oxygen		6.29 (3.26 – 10.70)
Cond	Conductivity		532.40 (0.55 – 755.00)
pH	pH value		7.33 (6.50 – 9.59)

[a]Numbers inside the parentheses are the range of the variable.

Table 32 Continued. Descriptions, values or units of measure, and means or frequencies of vegetation characteristics and water features as potential determinants of fountain darter density in (a) Comal and (b) San Marcos Springs, Texas.

(b)

Variable	Variable description	values or units of measure	Mean (range)[a] or frequency
Substrate types			
Cobble	Cobble	0: no	0: 369
		1: yes	1: 1
Gravel	Gravel	0: no	0: 319
		1: yes	1: 51
Sand	Sand	0: no	0: 323
		1: yes	1: 47
Silt	Silt	0: no	0: 121
		1: yes	1: 249
Silt_Gravel	Silt over grave	0: no	0: 348
		1: yes	1: 22
Vegetation characteristics			
Open	Bare	%	22.37 (0 – 100)
Cabomba	Cabomba coverage	%	11.05 (0 – 100)
Hydrilla	Hydrilla coverage	%	23.29 (0 – 100)
Hygrophila	Hygrophila coverage	%	22.94 (0 – 100)
POT_HYG	Potamogeton and Hygrophila coverage	%	9.67 (0 – 100)
Potamogeton	Potamogeton coverage	%	1.67 (0 – 100)
Sagittaria	Sagittaria coverage	%	1.29 (0 – 100)
Vallisneria	Vallisneria coverage	%	1.29 (0 – 100)
VegCover	Main vegetation coverage	%	71.10 (0 – 100)
VegHeight	Main vegetation height	Ft	1.07 (0 – 4.3)
Water features			
Depth	Water depth	Ft	2.25 (0.3 – 100)
Velocity	Water velocity		0.11 (-0.03 – 1.28)
Temperature	Water temperature	C	22.08 (18.59 – 27.70)
DO	Dissolved oxygen		7.87 (3.20 – 12.85)
Cond	Conductivity		578.25 (0.59 – 710.00)
pH	pH value		7.48 (6.00 – 8.44)

[a]Numbers inside the parentheses are the range of the variable.

We tried to estimate the relationship between potential maximum darter densities and their environmental variables using data collected over a five-year period. Two potential criticisms of any approach are that our estimates of the relationship are unique to our methods of analyses (Elith and Graham, 2009) and to our specification of the variables included in that analysis (Agresti, 2007). These criticisms are generic problems related to structural uncertainty in the

mathematical representation of natural systems (Walters, 1986). Hence, we used a range of different designs (from dependent variables settings to independent variables settings to different statistical analyses methods) to understand how to appropriately present the relationship. The possibility remains that there might be a more powerful method (Elith and Graham, 2009) and/or a more useful variables design (Wang et al., 2011). Evaluation of the relative merits of the different methodological approaches to estimate the relationship between endangered species abundance and their environmental variables currently is a topic of much debate. Hence, it remains a fruitful area of further investigation. In the sections that follow, we present details of the statistical analyses chronologically.

Statistical methods (October 2014 – July 2015)

Applying multinomial logit regression model in a combined dataset (data from both Comal and San Marcos Springs (October 2014)

We used multinomial logit regression model and all samples in Comal and San Marcos springs from 2000 to 2013 to understand the effects of environmental variables on the potential maximum darter densities in both springs. The distribution of fountain darter density was assumed normal, and categories (*K*) were assigned using the following rule: 1 (no fountain darter found; 343 observations), 2 (low; from 1 fountain darter to 0.5 SD below the mean; 542 obs.), 3 (fair; 0.5 SD either side of the mean; 563 obs.), 4 (high; 0.5 to 1.5 SD above the mean; 132 obs.), and 5 (very high; greater than 1.5 SD above the mean; 92 obs.), where mean = 20.23 and SD (standard deviation) = 27.08.

Multinomial logit regression model, a generalized linear model (GLM), was used to analyze the relationship between fountain darter density and environmental variables. GLMs are a generalization of linear regression models which allow various distributions for the response and error terms in the model (Agresti, 2007). The multinomial logit regression is used to calculate the probability of category membership of a dependent variable, in this case fountain darter density, based on multiple independent variables in an arbitrary number of categories. The independent variables can be either dichotomous (i.e., binary) or continuous (i.e., interval or ratio in scale). Multinomial logit regression is an extension of binary logistic regression that allows for more than two categories of the dependent or outcome variable. Like binary logistic

regression, multinomial logistic regression uses maximum likelihood estimation to evaluate the probability of categorical membership (Starkweather and Moske, 2011).

Each measurement in our dataset could have fallen into any of the five density categories *K*, where *K* = 1, 2, 3, 4, or 5. Therefore, we assumed that density category placement did not tend to happen in any particular order, and that the categories were strictly nominal. For a given sample *i*, we defined the density category as a response Y_i, where $Y_i = K$. We assumed a multinomial distribution for the response Y_i with class probabilities $P(Y_i = K)$. The model has the form:

$$P(Y_i = K) = \frac{exp(\alpha_K + \beta_K X_i)}{c_i}, \text{ where } K = 2, 3, 4, \text{ or } 5, \qquad (1)$$

$$P(Y_i = K) = \frac{1}{c_i}, \text{ where } K = 1, \qquad (2)$$

and where

$$c_i = 1 + \sum_{K=2}^{5} [exp(\alpha_K + \beta_K X_i)]. \qquad (3)$$

The parameter vectors α_K and β_K relate to category *K*, and the vector X_i is a row of the design matrix containing independent environmental variables for a sample *i*. Note that:

$$\sum_{K=1}^{5} P(Y_i = K) = 1. \qquad (4)$$

SAS ver. 9.2 (SAS Institute Inc., 2008) was used to fit the models. Variable selection and parameter estimation process continued until the selection criteria, as described below, were optimized. The models that optimized the criteria, subject to the constraint of equations for each *K* (eqs. (1) and (2)), were then selected. Having fitted the models, the probabilities that density falls into a given category in the sample *i* can be calculated.

The best model was identified by removing non-significant terms one at time and re-estimating the model (Agresti, 2007) until the Akaike Information Criterion score (AIC; Akaike, 1973) could not be lowered further. The reliability and validity of the models were evaluated based on the area under Receiver Operating Characteristic (ROC) curve (Area Under Curve; AUC) as fair ($0.50 < AUC \leq 0.75$), good ($0.75 < AUC \leq 0.92$), very good ($0.92 < AUC \leq 0.97$), or excellent

(0.97 < AUC ≤ 1.00) (Hosmer and Lemeshow, 2000). The AUC was computed for all ten comparison pairs (e.g. Y_i = 1 vs. Y_i = 2) and the results averaged (Hand and Till, 2001). Model selection was conducted using SAS ver. 9.2 (SAS Institute Inc., 2008) and model evaluation using the pROC package (Robin et al., 2011) in R ver. 2.14.1 (R Development Core Team, 2006).

Applying the two levels hierarchical logit model in a combined dataset (data from both Comal and San Marcos Springs (November 2014)

We used the two levels hierarchical logit model for a combined dataset (Comal Springs and San Marcos springs) to account for the influences of micro-(sample scale) and macro-(reach scale) environments on potential maximum darter densities. The choice probability of the generic alternative *j*, *p*(*j*), of the two levels hierarchical logit model is obtained as:

$$p(j) = p(k) \cdot p(j/k) \tag{5}$$

where *p*(*k*) is the choice probability of group *k* including alternative *j*, and *p*(*j*/*k*) represents the conditional choice probability of *j* given *k*. The analytical expression of *p*(*k*) and *p*(*j*/*k*) are the following:

$$p(k) = \frac{\left(\sum_{i \in C_k} e^{V_i/\theta_k}\right)^{\delta_k}}{\sum_{k'}\left(\sum_{i \epsilon C_{k'}} e^{V_i/\theta_{k'}}\right)^{\delta_{k'}}} \tag{6}$$

$$p(j/k) = \frac{e^{V_j/\theta_k}}{\sum_{i \epsilon C_k} e^{V_i/\theta_k}} \tag{7}$$

Hence, combining the above two equations:

$$p(j) = \frac{e^{V_j/\theta_k} . \left(\sum_{i \epsilon C_k} e^{V_i/\theta_k}\right)^{\delta_k - 1}}{\sum_{k'}\left(\sum_{i \epsilon C_{k'}} e^{V_i/\theta_{k'}}\right)^{\delta_{k'}}} \tag{8}$$

The micro-environmental variables included those listed in Table 32. However, we added variables at the macro-environmental scale: representing reach, season and areal coverage of vegetation at this scale.

In addition, we also used multiple methods to check for multicollinearity: (1) The VIF (variance inflation factor) of model is < 10, then it is taken as an indicator that no multicollinearity is present in our model. (2) Multicollinearity arises when the predictor variables are strong correlated among themselves. In such a case, multicollinearity inflates the errors. Hence, we examine the correlation matrix of predictor variables if they are measured in continuous scales and see whether their correlation coefficients are higher than should be expected.

Applying the two levels hierarchical logit models in each spring system (December 2014)

We used the two levels hierarchical logit model for each spring system individually because the results (Appendix F, Table 4) did not capture the specific effects of each spring. Hence, we re-defined the categories: Fountain darter mean abundance: 19.24, standard deviation (SD): 26.99. Category 1 (no fountain darter found; 90 observations in Comal spring and 23 obs. in San Marcos spring); category 2 (low; from 1 fountain darter to 0.5 SD below the mean; 209 obs. in Comal spring and 148 obs. in San Marcos spring); category 3 (fair; 0.5 SD either side of the mean; 315 obs. in Comal spring and 174 obs. in San Marcos spring); category 4 (high; 0.5 to 1.5 SD above the mean; 107 obs. in Comal spring and 21 obs. in San Marcos spring); and category 5(very high; greater than 1.5 SD above the mean; 74 obs. in Comal spring and 4 obs. in San Marcos spring). There were total 795 obs. and 370 obs. in Comal and San Marcos springs, respectively.

Accordingly, micro-environmental variables included those listed in Table 32a and macro-environmental variables included season, Flow, and areal coverage of each vegetation type at the reach scale in Comal Springs. Micro-environmental variables for San Marcos springs included those in Table 32b with the following exception: Cobble was removed due to low sample size. Macro-environmental variables again included season and areal coverage of vegetation types at the reach scale in San Marcos Springs.

Finally, we modified some independent variables: (1) Replaced CP (critical period) with real season, and (2) Deleted some macro-level vegetation types which only exist in very small areas.

Applying the two levels hierarchical logit model and multinomial logit regression model in each springs (January 2015)

Because we found that the macro-environmental variables could possibly dilute the effects of the micro-environmental variables in each reach, we ran two models in each spring system. The first model is two levels hierarchical logit model which uses both macro- and micro-environmental variables and the second model is multinomial logit regression model which only use micro-environmental variables.

Application of the multinomial logit regression model (February 2015)

We used the probabilities calculated from the multinomial logit regression model to set up the potential maximum darter densities in each cell and then used this rule to drive the movement of fountain darter. We represented the conceptual model in Figure 52.

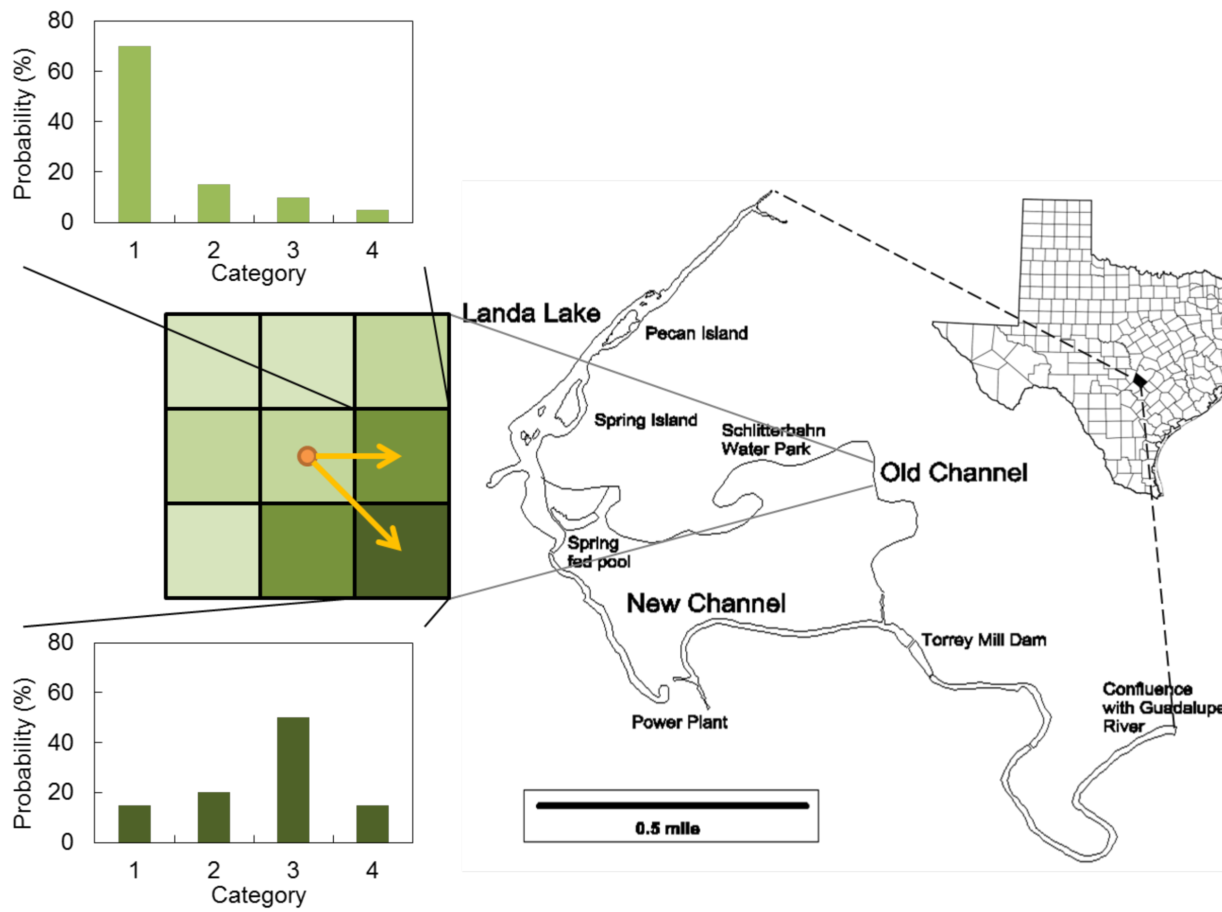


Figure 52. **Conceptual diagram of multinomial logit regression model and movement**

Refine the drop net data and rerun the multinomial logit regression model (March 2015)

We re-ran the multinomial logit regression model in Comal Springs after Team members edited some missing information of the drop net data.

Rerun the multinomial logit regression model excluding the variables of pH and Conductivity (May and June 2015)

We re-ran the multinomial logit regression model in both springs because we will not have values of pH (they are not being modeled) and conductivity as independent variables in the future. After having the best multinomial logit regression model incorporated in the fountain darter spatially-explicit, individual-based model samples in San Marcos Springs, we then compared the indicated vegetation types based on drop net sampling to simulated drop net using paired t-test.

Incorporating the results of multinomial logit regression model (estimated maximum darter density, MD), movement rules and consecutive moves (v) in the fountain darter spatially-explicit, individual-based model (July 2015)

We incorporated the results of multinomial logit regression model (estimated maximum darter density, MD), movement rules and consecutive moves (v) in the fountain darter spatially-explicit, individual-based model. We only ran 3 reps of baseline simulation (with movement rule and 18 hours limitation) for the darters in City Park in San Marcos Springs. However, we designed a range of different settings of movement rules and consecutive moves (v) in Old Channel in Comal Springs. We ran a range of different settings of movement rules and consecutive moves (v) in Old Channel in Comal Springs.

The null models included with (1) random movement and no hour limitation for darters to stay in unfavorable habitats without dying, (2) movement rule and no hour limitation for darters to stay in unfavorable habitats without dying, (3) random movement and 12hours limitation for darters to stay in unfavorable habitats without dying, (4) random movement and 18hours limitation for darters to stay in unfavorable habitats without dying, or (5) random movement and 24hours limitation for darters to stay in unfavorable habitats without dying.

We then ran a set of models to determine the consecutive moves (*v*) included with movement rule and (1) 1 hour, (2) 2 hours, (3) 3 hours, (4) 6 hours, (5) 12 hours, (6) 18 hours, (7) 24 hours, (8) 30 hours, (9) 36 hours, (10) 42 hours, or (11) 48 hours limitation for darters to stay in unfavorable habitats without dying. In addition, we ran a set of models to determine the consecutive moves (*v*) included with stay rule and (1) 6 hours, (2) 12 hours, (3) 18 hours, (4) 24 hours, or (5) 30 hours limitation for darters to stay in unfavorable habitats without dying.

Finally, we evaluated the fountain darter spatially-explicit, individual-based model based on (1) system level results including (i) comparison of estimated maximum darter density and simulated number of juvenile plus adult fountain darters, and (ii) sensitivity analyses, and (2) the comparison of the indicated vegetation types based on drop net sampling to different designs of simulated drop net using paired t-test. Sensitivity analyses included (1) comparison of models with movement rule and different consecutive moves (*v*) and (2) comparison of the effects of different demographic parameters on lambda of fountain darter.

Empirical analyses (April 2015 – November 2015)

Understand the relationship between fountain darter density and aquatic vegetation types based on drop net data and aquatic vegetation maps (April 2015)

Based on the preliminary results in February 2015, we found the potential maximum darter densities did not meet the general trends of observation. Hence, we drew upon the drop net data and aquatic vegetation maps to understand the relationship between fountain darter density and aquatic vegetation types empirically.

Revisit the drop net data and apply the new information in the fountain darter spatially-explicit, individual-based model (August 2015)

Based on the different versions of statistical analyses, our team found that the maximum density generated from the statistical analyses (e.g. max-den-sys in Appendix F, Figure 7 and 8) did not match the general observation (dip net data, Appendix F, Figure 10) of fountain darter in Comal Springs. Hence, we revisited the drop net data in Comal Springs. We used the drop net data in each aquatic vegetation type in each sampling period to multiply the cells of the aquatic vegetation. We then summarized these values from all aquatic vegetation types to represent the

estimated overall fountain darter abundance in each sampling period in Comal Springs. The detailed calculation could be found in Appendix F, Figure 11. Finally, we overlapped the estimated overall fountain darter abundance and dip net data.

After revisiting the drop net data, we thought that it could be an option for us to use the estimated overall fountain darter abundance in each sampling period in Comal Springs as the potential maximum fountain darter density. Hence, we integrated the new information in the fountain darter spatially-explicit, individual-based model which started running from 2003 and examined the performance of the new version of model based on (1) comparison of estimated maximum darter density and simulated number of juvenile plus adult fountain darters in the system level, (2) comparison of the indicated vegetation types based on drop net sampling to different designs of simulated drop net using paired t-test and (3) comparison of the specific vegetation type based on drop net sampling to different designs of simulated drop net using paired t-test.

In addition, we tested the initial effects on the fountain darter spatially-explicit, individual-based model. We integrated the new information in the fountain darter spatially-explicit, individual-based model which started running from 2001 and examined the performance of the new version of model following the same procedure which was described in the previous paragraph.

Apply the reach specific information in the fountain darter spatially-explicit, individual-based model (September and October 2015)

After integrating the empirical approach of analyzing drop net data in Comal Springs to the fountain darter spatially-explicit, individual-based model, we applied the approach but used only reach specific drop net data (Old Channel) to the simulation model. We then compared the indicated vegetation types based on drop net sampling to different designs of simulated drop net using Nash-Sutcliffe model efficiency coefficient. The equation of Nash-Sutcliffe model efficiency coefficient is:

$$E = 1 - \frac{\sum_{t=1}^{T}\left(Q_o^t - Q_m^t\right)^2}{\sum_{t=1}^{T}\left(Q_o^t - \overline{Q_o}\right)^2} \quad (9)$$

where $Q_o{}^t$ is (observed) sampled density of fountain darter at time t, Q_m^t is simulated density of fountain darter at time t, $\overline{Qo}$ is the mean of (observed) sampled density of fountain darter.

Nash–Sutcliffe efficiency can range from $-\infty$ to 1. An efficiency of 1 ($E = 1$) corresponds to a perfect match of simulated density to the sample density. An efficiency of 0 ($E = 0$) indicates that the model predictions are as accurate as the mean of the sample density. An efficiency less than zero ($E < 0$) occurs when the observed mean is a better predictor than the model. Essentially, the closer the model efficiency is to 1, the more accurate the model is.

Analyses of estimated maximum, simulated, and drop net data of darter densities based on each aquatic vegetation type (November 2015)

We analyzed the estimated maximum, simulated, and drop net data of darter densities based on each aquatic vegetation type. The estimated maximum darter densities were calculated as (average darter density from 2003 to 2013 in vegetation type *i*) × (# of cells in vegetation type *i*) and the drop net-based darter densities were calculated as (average darter density at survey time in vegetation type *i*) × (# of cells in vegetation type *i*).

In summary, although the identification of environmental factors and habitat characteristics that potentially determine maximum darter densities is relatively simple, the establishment of quantitative relationships with a solid empirical basis remains a challenge. Factors affecting densities operate at different spatial and temporal scales, resulting in data limitations and modeling challenges. Two potential criticisms of any quantitative approach are that the parameter estimates upon which the resulting relationship is based are unique to the particular method of analysis used and to the particular specification of the variables included in that analysis. These criticisms are generic problems related to structural uncertainty in the mathematical representation of natural systems. To date, we have used a wide variety of different approaches ranging from more sophisticated statistical methods to simpler empirical analyses (as previously noted, the timeline and results - including all tables and figures from these analyses are presented in detail in Appendix F). For use in the fountain darter population dynamics simulation model, we favor a simple, empirically-based approach that assigns a maximum darter density to each simulated habitat cell probabilistically based on the cumulative frequency distribution of darter densities in drop net samples collected from the vegetation type corresponding to the vegetation type of the simulated habitat cell. Simulation model runs

(described below) using this approach to assign maximum darter densities to habitat cells generate simulated darter densities that are comparable to darter densities observed in field.

Model description

Concurrent with the statistical evaluation, we developed a spatially-explicit, individual-based, model representing fountain darter population dynamics in response to changes in aquatic vegetation and hydrological conditions (Figure 53). We first verified that the model generated spatial-temporal dynamics of aquatic vegetation, water depth, velocity, and DO concentration similar to those observed in each of several reaches in the Comal River and the San Marcos River from 2003 through 2014. We then calibrated the model such that the simulated abundance of fountain darters in each reach responded appropriately to historical changes in these habitat conditions. Finally, we evaluated the model by comparing simulated drop net samples to those observed in the field. In the sections that follow, we present details of the model following the protocol suggested by Grimm et al. (2006) for describing individual-based models.

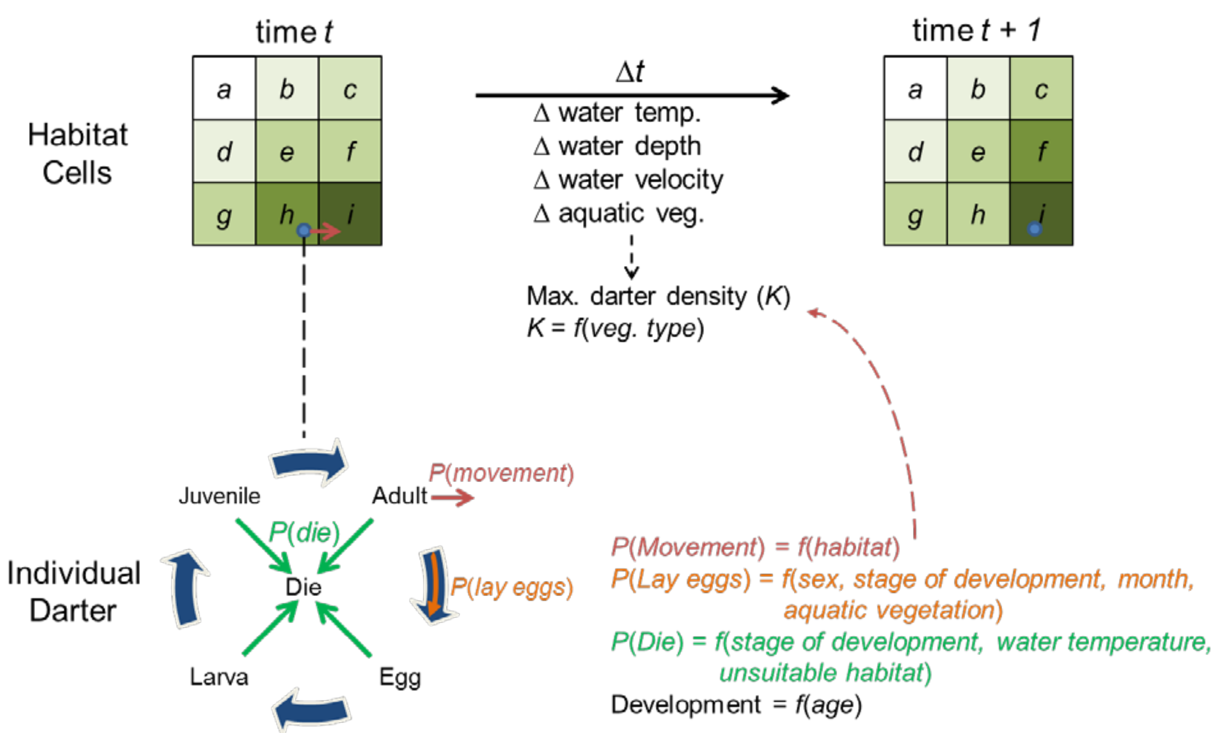


Figure 53. **Conceptual diagram of the spatially-explicit, individual-based, simulation model representing fountain darter population dynamics in response to changes in aquatic vegetation and hydrological conditions.**

The purpose of the model is to simulate the population dynamics of fountain darters in response to changes in habitat conditions that might result directly or indirectly from changes in water flow within the Comal River and the San Marcos River. The ability to simulate fountain darter responses to spatial-temporal changes in the distribution and species composition of aquatic vegetation, as well as water temperature, DO concentration, depth, and velocity, as they pass through egg, larval, juvenile, and adult life stages is of particular interest.

State variables include (1) a reach-specific number (tens of thousands) of $1m^2$ habitat patches arrayed in a rectangular grid representing the area of, and immediately adjacent to the given reach, derived from the MDSWMS (USGS 2013) 2-dimensional hydrodynamic model calibrated for the reach (Hardy et al., 2010), and (2) a variable number (up to several tens of thousands) of individual fountain darters. Attributes of habitat patches include location (latitude, longitude), vegetation type (Table 33), water temperature (C), DO concentration (mg/L), depth (m), and velocity (m^3 sec^{-1}). Attributes of fountain darters include sex, age (days), life stage (egg, larva, juvenile, young adult, old adult), location (habitat patch currently occupied), and, for adult females, reproductive state (whether or not they are reproductively active, and whether or not they have laid eggs within the last month). Attributes of habitat patches that can change over time include vegetation type, water temperature, DO concentration, depth, and velocity. Attributes of fountain darters that can change over time include age, life stage, and location.

Table 33. Aquatic vegetation types represented as attributes of simulated habitat patches, including code used in vegetation mapping in the field, and associated cover type, species, numeric code used in simulation model, and abbreviations found in data files.

Mapped Code	Cover type	Species	Numeric Code	Abbreviation
0	Bare substrate/too deep		0	Bare
1	Algae	Filamentous algae	1	Alg
2	Ceratopteris	Ceratopteris	2	Cera
3	Hygrophila	Hygrophila	3	Hygro
4	Ludwigia	Ludwigia	4	Lud
5	Nuphar	Nuphar	5	Nuph
6	Nuphar/Ceratopteris	Nuphar	5	Nuph
7	HYG40	Hygrophila	3	Hygro
8	HYG-LUD50	Hygrophila	3	Hygro
9	Riccia	Bryophytes	6	Bryo
10	Bryophytes	Bryophytes	6	Bryo
11	LUDW50	Ludwigia	4	Lud
12	LUDW70	Ludwigia	4	Lud
13	Veg mat		0	Bare
14	Hyg30/Ludw40	Hygrophila	3	Hygro
15	Hyg40/Ludw10	Hygrophila	3	Hygro
16	LUDW40	Ludwigia	4	Lud
17	LUDW60	Ludwigia	4	Lud
N/M (Not Mapped)	Sagittaria	Sagittaria	7	Sag
N/M	Vallisneria	Vallisneria	8	Vall
N/M	Hydrilla	Hydrilla	9	Hydr
N/M	Cabomba	Cabomba	10	Cab
N/M	Potamogeton	Potamogeton	11	Pot
N/M	Texas wild rice	Texas wild rice	12	Rice

We programmed the model and executed simulations in NetLogo (Wilensky, 1999), exported simulation results to Excel© (Microsoft, 2003) for archiving and temporal graphics. During each simulation, the system is initialized by assigning each habitat cell a vegetation type, as well as a water temperature, DO concentration, depth, and velocity, and by assigning each individual fountain darter a sex, age, life stage, and location (Figure 54). Simulations are driven by daily time series of values representing estimated historical water discharge (cfs), and water temperatures and DO concentrations from the 1 January 2003 to 31 December 2014.

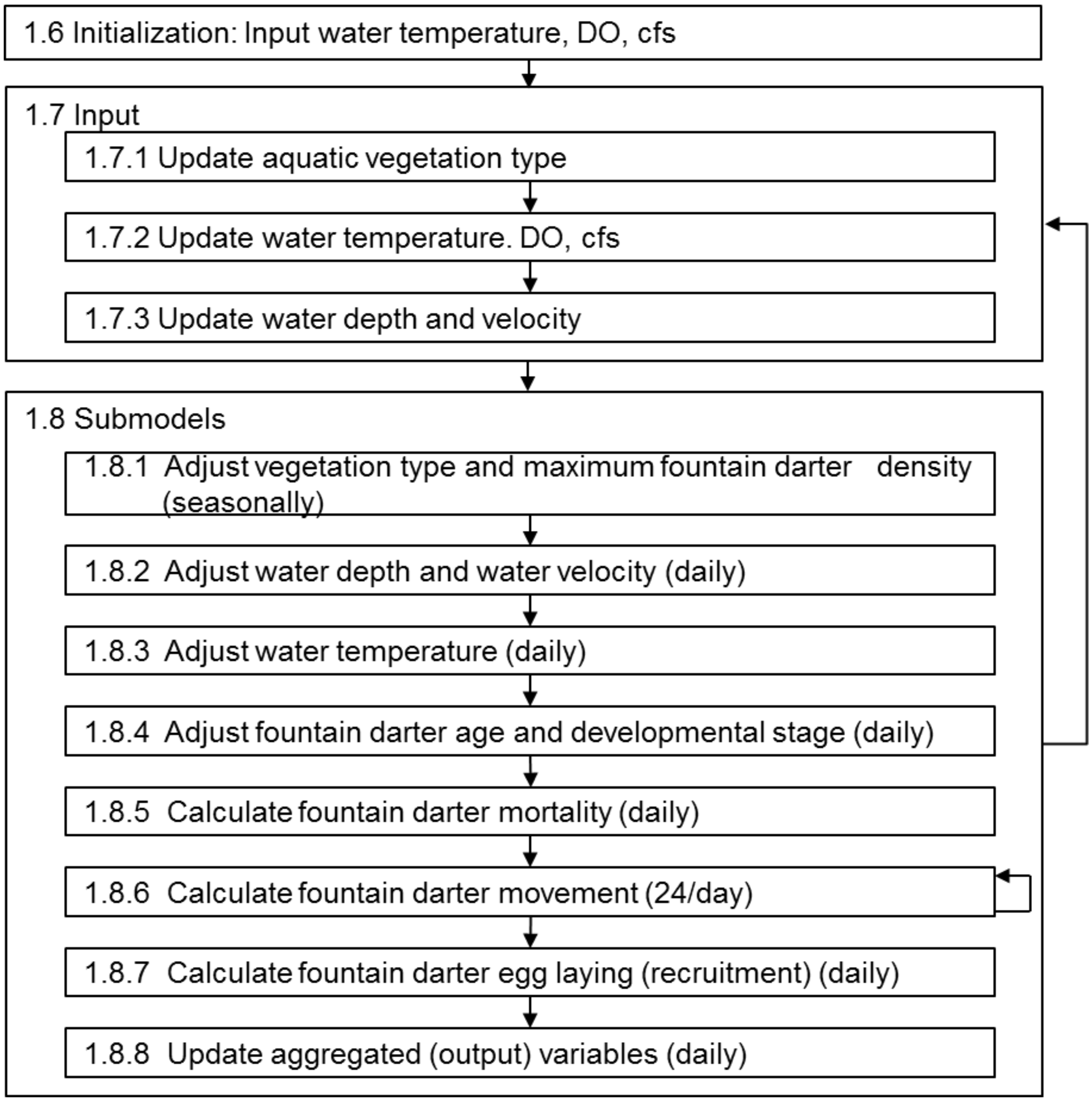


Figure 54. Overview of the sequence of events and processes involved in the execution of the fountain darter population dynamics model.

Historical daily water discharges for the given reach are used to estimate the associated water depths and water velocities for each habitat cell within the reach for that day. Next, iteratively during the simulation, (1) values representing estimated daily water discharge, and water temperature and DO concentration are adjusted according to their respective input time series, (2) water depth and water velocity in each habitat cell are adjusted based on the estimated daily water discharge, and (3) effects of these changes on the mortality, movement, and egg-laying (recruitment of new individuals) of fountain darters are calculated. Estimated historical

vegetation changes occur seasonally (spring, summer, and fall of 2003 and 2004; spring and fall of 2005 to 2014). Fountain darters may make up to 24 movements each day, but aging, development (from egg to larva to juvenile to adult), mortality, and egg laying are calculated on a daily basis. During the simulation of each fountain darter activity (move, age, develop, die, lay eggs), individuals are selected in random order, that is, the first randomly selected individual is given the opportunity to perform the given activity, then the second randomly selected individual, then the third, and so on. The aggregated variables that describe the state of the system include the number of habitat patches with each type of aquatic vegetation, and the numbers and proportions of eggs, larvae, juveniles, young adults, old adults, males, and females in the fountain darter population. All of these aggregated variables are updated daily.

Basic principles

Motivation for development of such a model came from the perceived need to refine the representation, both functionally and spatially, of the response of fountain darters to changes in spring flow and/or changes in the amount of habitat provided by aquatic vegetation potentially resulting from future water demands of an increasing human population (Mora et al., 2013). Although hydrological models of the Edwards Aquifer (Schulman et al., 1995; Lindgren et al., 2004) are available, as is a framework for assessing levels of spring flow needed to maintain fountain darter habitat (INSE, 2004, Hardy et al., 2010), to our knowledge the only population dynamics model for the fountain darter was developed quite recently by Mora et al. (2013). Their model is a compartment model based on difference equations representing the effect of spring flow and water temperature on fountain darter recruitment and survival, which they used to project fountain darter population sizes under various scenarios of reduced spring flows. In the present study, we describe development of a spatially-explicit, individual-based, population dynamics model for the fountain darter emphasizing more mechanistic connections among spring flow, the distribution of aquatic vegetation, and fountain darter recruitment, survival, and development.

Emergence

Spatial and temporal patterns of abundance of fountain darters in the various life stages (egg, larvae, juvenile, young adult, old adult) emerge as system-level properties as a result of

empirically-based spatial and temporal patterns of habitat characteristics (vegetation type, water temperature, water depth, water velocity), empirically-based rates of fountain darter egg-laying, development, and survival, and hypothesized rules governing fountain darter movement.

Sensing

Fountain darters are “aware” of their age and life stage, the characteristics of the habitat cell in which they currently are located, and the number of consecutive time steps that they have been in habitat cells without aquatic vegetation.

Interaction

Habitat cells and fountain darters interact implicitly in that movement, survival, and egg-laying of fountain darters is affected by the characteristics of the habitat cell in which they currently are located.

Stochasticity

During initialization of the model, age and life stage of fountain darters are assigned randomly based on empirical probabilities that result in age- and stage-class distributions approximating those observed in the field. During simulations, movement, survival, and egg-laying of fountain darters are determined probabilistically.

Observation

Output from the model includes time series of daily values of water discharge, the numbers and proportions of habitat patches containing each type of aquatic vegetation, the vegetation-based, estimated carrying capacity of the reach for fountain darters (juveniles and adults only), and the numbers and proportions of eggs, larvae, juveniles, and adults in the fountain darter population.

Model initialization

The system is initialized by assigning each habitat cell an aquatic vegetation type, and a water temperature, DO concentration, depth, and velocity such that the resulting simulated habitat patterns resemble those observed during the spring of 2003 in the particular reach of the Comal River or the San Marcos River being simulated, and by assigning each individual fountain darter an age, life stage, and location such that the resulting age- and stage-class distributions and sex ratio of the simulated population approximate those observed in the field during 2003 (BIO-WEST, 2004a), and such that all simulated darters are located in habitat cells with aquatic

vegetation (Figure 55). The initial number of juvenile plus adult darters is calculated based on the estimated maximum darter density that can be supported by the aquatic vegetation within the reach. The maximum darter density associated with each type of aquatic vegetation is based on analyses of drop net data collected in the Old Channel Reach of the Comal River or the City Park Reach of the San Marcos River, whichever river contains the reach being simulated, from 2003 through 2013 (BIO-WEST, 2004a – 2014a, BIO-WEST, 2004b – 2014b). The maximum darter density of each habitat cell (MD_i; the number of juveniles plus adults that can be supported by the vegetation type in habitat cell *i*) is assigned probabilistically based on the cumulative frequency distribution of the density of darters (individuals / m^2) collected in drop nets placed in that vegetation type in the field.

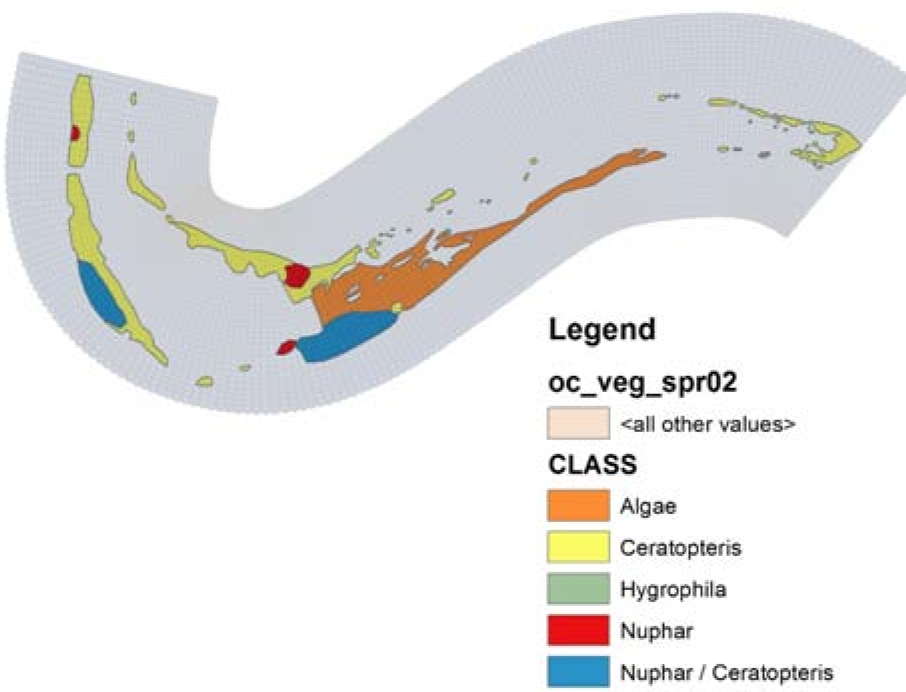


Figure 55. Example of a spatial join of vegetation mapping data polygons and the corresponding one meter hydraulic grid in the Old Channel of the Comal River.

Input to the model includes time series of values representing, for the particular reach of the Comal River or the San Marcos River being simulated, (1) the aquatic vegetation type within each habitat cell, (2) the water discharge, temperature, and DO concentration for the entire reach,

and (3) the water depth and velocity in each habitat cell associated with the specific water discharge rates.

Aquatic vegetation type

Aquatic vegetation maps were developed by physically delineating the vegetation polygons in the field using GPS (BIO-WEST, 2004a – 2014a, BIO-WEST, 2004b – 2014b). The corresponding vegetation polygons were spatially mapped to the hydrodynamic computational grid using ArcMap 9.3 (ESRI, 2014).

Water discharge, temperature, and DO concentration

Mean daily water discharge was estimated based on data from a gauge as described in Section 2.2. In the preliminary stage, mean daily water temperatures were derived from temperatures recorded at 15-minute intervals near that gauge. In some instances, short intervals of missing data were interpolated using simple linear interpolation. For the final model, all temperature results will be estimated based on hydrodynamic simulations using Qual-2E (see Section 2.3). Mean daily DO concentrations were estimated based on hydrodynamic simulations using Qual-2E (see Section 2.3).

Water depth and velocity

Results of hydraulic simulations of water depth and velocity for various water discharge rates within and beyond historical ranges (0.28 to 2.26 m^3 sec^{-1} ; 10 to 80 cfs) using MDSWMS were used to interpolate the depth and velocity each habitat cell . Water depths and velocities associated with discharge rates not simulated using MDSWMS were estimated by linear interpolation. Interpolated values at known water discharges showed less than a 3.0 percent variation in interpolated depth and velocities when compared to the simulated hydraulic attributes (see Section 2.2).

Submodels

Adjust vegetation type and maximum darter density

For the preliminary assessment, the corresponding one meter hydraulic grids in each simulation reach (i.e., Old Channel in the Comal River and City Park in the San Marcos River) were used to

conduct a spatial join of the available vegetation monitoring data from 2003 through 2014 (e.g., Figure 55). In this version of the fountain darter model, the input from the SAV submodel is disabled, and instead replaced by the observed SAV distributions. This allows parameterization of the fountain darter to proceed without the complexity of simultaneously calibrating the SAV model. This incarnation of the fountain darter model is referred to hereafter as the de-coupled version to distinguish from the final version in which SAV is fully coupled into the fountain darter submodel.

Vegetation coding for specific vegetation types varied over the course of field studies between 2003 and 2014. A standardized coding scheme for vegetation and substrates was developed and used to standardize all the spatially joined data sets for both river systems.

In this de-coupled version, since continuous vegetation time histories are not available from the SAV model, vegetation types are adjusted during the spring (1 March, day-of-year 60), summer (1 July, day-of-year 182), and fall (1 October, day-of-year 274) of 2003 and 2004, and during the spring (1 March, day-of-year 60) and fall (1 September, day-of-year 244) of 2005 to 2014, with the vegetation type assigned to each habitat cell based on the input time series of vegetation data. Immediately following the adjustment of the vegetation type within any given habitat cell *i*, the maximum darter density of that cell (MD_i) is adjusted accordingly. As noted above, upon completed calibration of the SAV submodel, this de-coupled version will be replaced with the direct linkage to the SAV submodel results.

Adjust water discharge, temperature, and DO concentration

Mean water discharges, and mean water temperatures and DO concentrations are adjusted daily, with a single discharge, water temperature, and DO concentration assigned to the entire reach (global variables) based the input time series of discharge, temperature, and DO concentration data.

Water temperature impacts to fountain darter life stages and reproductive success are based on existing literature (Brandt et al., 1993, Bonner et al., 1998, McDonald et al., 2007). Although spawning success and larval growth show declines in a laboratory setting at temperatures over 27

°C, it is a conservative temperature trigger; the lethal limit (50% mortality) for larval fountain darters is 31.9° C and approximately 3.0° C higher for adults (Brandt et al., 1993, Bonner et al., 1998, McDonald et al., 2007).

Relative to dissolved oxygen (DO) tolerances for model parameterization, TCEQ standards for mean (minimum, 24-h period) DO concentrations are 5.0 (3.0) for high to 6.0 (4.0) for exceptional Aquatic Life Use (TCEQ 2010 Standards https://www.tceq.texas.gov/assets/public/waterquality/standards/TSWQS2010/TSWQS2010_rule.pdf).

Among darters, DO critical concentrations range from 1.09 to 3.39 mg/l (Hlohowskyj and Wissing, 1987; Hartline, 2013). Based on available information, DO concentrations are similar among habitat generalists and swiftwater/riffle specialists.

<u>Low oxygen tolerance (point of equilibrium loss)</u>
Greenside Darter (riffle specialist): 3.39 mg/l
Fantail Darter (habitat generalist): 2.03 mg/l
Rainbow Darter (riffle specialist): 1.64 mg/l
Bronze Darter (swift water specialist): 1.09 – 3.39 mg/l; temperature: 20 – 24°C
Greenbreast Darter (swift water specialist): 1.99 – 2.59 mg/l; temperature: 20 – 28°C
Blackband Darter (habitat generalist): 2.63 – 3.05 mg/l; temperature: 20 – 24°C

Critical concentration of DO (i.e., loss of equilbrium) and concentration of DO for reduced reproduction are unknown for the fountain darter. Based on field collections, fountain darters were collected in habitats ranging from 2.47 to 12.3 mg/l DO (Behen, 2013). Fountain darter habitat use versus habitat available were proportional similar between 4.0 to 12.0 mg/l (Figure 56). Fountain darter habitat use was less than expected (i.e., available) at DO concentrations < 4.0 mg/l (excluding the observation at 2.47 mg/l), which typically occur in the Slough Arm reach of Spring Lake. Other factors might exclude fountain darters from Slough Arm; therefore, habitat available for fountain darters might overestimate availablity and, consequently, underestimate use of the low DO habitats.

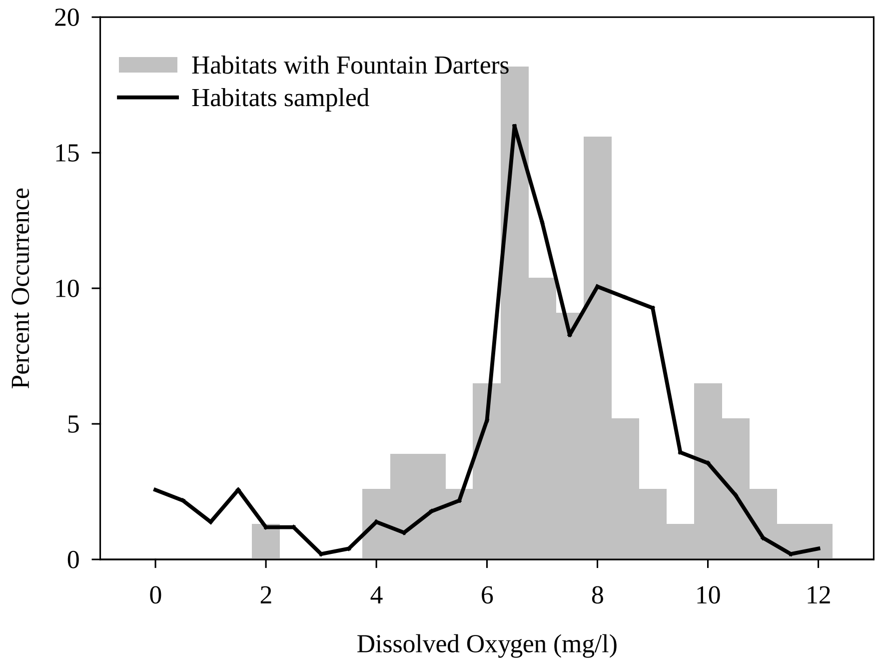


Figure 56. Dissolved oxygen comparison between habitats sampled with seines (N = 507; black line) and habitats (N = 77) with Fountain Darters (N = 203) taken from multiple sites seasonally for one year in the San Marcos River (Behen 2013). Dissolved oxygen was measured with a YSI multi-probe during daylight hours (07:00 to 17:00 hours).

The Team determined the following DO conditions for model parameterization as described in the fountain darter mortality description below. At conditions greater than 4.0 mg/l DO, no impacts are projected to any fountain darter life stage. The justification is existing data and staying above the minimum standards for exeptional quality habitat per TCEQ Aquatic Life use classification. Above 3.0 mg/l DO, adults darters are not impacted in the model, but larval and egg life stages are. This is based on adults being found in these areas, the existing literature regarding low oxygen tolerances of habitat generalists darters and staying above the minimum standards for high quality habitat per TCEQ Aquatic Life use classification. At present, 2 mg/l is the threshold for death of all fountain darter life stages in the model. This is based on the literature on lower end tolerances from laboratory studies: 2 of 3 darter species measured by Hlohowskyj and Wissing (1987) were near 2.00 mg/l (1.64 and 2.03 mg/l), 2 of 3 darter species measured by Hartline (2013) had lower range near 2.00 mg/l (1.09 and 1.99 mg/l).

Adjust water depth and water velocity

Water depths and velocities are adjusted daily, with the water depth and velocity assigned to each habitat cell based on the water depth and velocity data input file associated with the mean water discharge being simulated for that day.

Adjust fountain darter age and developmental stage

Fountain darter ages are updated daily, with developmental stages updated from egg to larva at 6 days of age (Simon et al., 1995), from larva to juvenile at 66 days of age, from juvenile to young adult at 186 days of age, and from young adult to old adult at 736 days of age (Brandt et al., 1993).

Calculate fountain darter mortality

Fountain darter mortality related to water temperature is calculated on a daily basis, with the probability of dying (*pd*) of each individual calculated as a function of its stage of development and the water temperature in the habitat cell in which the individual is located. For eggs, larvae, juveniles, young adults, and old adults, respectively:

pd_{eggs} = (base-mort-egg + egg-mort-temp)

where egg-mort-temp = 0.025 if temp <= 23C

= -0.6075 + 0.0275 * temp if 23C < temp <= 27

= 0.135 if temp > 27C

pd_{larvae} = (base-mort-lar + lar-mort-temp)

where lar-mort-temp = 1 / (1 + exp (-7.31 + 5.43 * ln temp)) if temp <= 22C

= 1 / (1 + exp (310.96 – 89.83 * ln temp)) if temp > 22C

$pd_{juv\text{-}yng\ adu}$ = (base-mort-juv-yngadu * juv-adu-mort-temp)

where juv-adu-mort-temp = 3 if temp <= 0C

= 3 - 0.025 * temp if 0C < temp <=8C

$= 1$ if $8C < temp <= 22C$

$= -4.5 + 0.25 * temp$ if $22C < temp <= 30C$

$= 3$ if $temp > 30C$

$pd_{old\ adults}$ = (base-mort-juv-oldadu * juv-adu-mort-temp)

The base mortality rates for eggs, larvae, juveniles/young adults, and old adults, were 0.03, 0.031, 0.00149, and 0.00545, respectively, were based on information in Pitcher and Hart (1982) and Brandt et al. (1993), and the water temperature effects on mortality were based on information in Bonner et al. (1998).

Fountain darter mortality related to DO concentration (mg/l) is calculated on a daily basis, with the probability of dying (*pd*) of each individual calculated as a function of its stage of development and the current DO concentration in the reach. For larvae, and juveniles/adults, respectively:

$pd_{larvaeDO} = 1 - (1 / (1 + \exp(-5.3 * (DO - 3))))$

$pd_{juv\text{-}aduDO} = 1 - (1 / (1 + \exp(-10.6 * (DO - 2.5))))$

Eggs and larvae also die if the habitat cell in which they are located losses its aquatic vegetation, juveniles and adults also die if they fail to find suitable habitat (see next section on darter movements), and old adults also die when they reach 1100 days of age (about 3 years old).

Calculate fountain darter movement

Juvenile and adult fountain darters may make up to 24 movements per day, whereas eggs and larvae are immobile. Movement rules, which are hypothetical, but which result in movement patterns generally consistent with those based on field data collected from marked individuals (BIO-WEST 2014c, Appendix E), are summarized in Figure 57. (1) If an individual is located in a habitat cell that currently is below its estimated maximum darter density (MD; the number of juveniles plus adults that can be supported by that vegetation type), and there are no adjacent habitat cells below their MD, then the individual will not move from the cell it currently occupies. (2) If an individual is located in a habitat cell that currently is below its MD, and one

or more of the adjacent habitat cells is below their MD, then the individual has a probability (ε = 0.50) of moving to one of those habitat cells (randomly chosen), and a probability ($1 - \varepsilon$) of remaining in the cell it currently occupies. This rule allows individuals to move about larger aggregates of suitable habitat cells and prevents situations in which suitable habitat cells near the center of large patches become inaccessible due to "barriers" formed by suitable, fully-occupied habitat cells. (3) If an individual is located in a habitat cell that currently is at or above its MD, and one or more of the adjacent habitat cells is below their MD, then the individual moves to one of those habitat cells (randomly chosen). (4) If an individual is located in a habitat cell that currently is at or above its MD, and none of the adjacent habitat cells is below their MD, but one or more of the adjacent habitat cells has water, then the individual moves to one of those habitat cells (randomly chosen). (5) If an individual is located in a habitat cell that currently is at or above its MD, and none of the adjacent habitat cells is below their MD, and none of the adjacent habitat cells has water, then the individual will not move from the cell it currently occupies. If an individual has not occupied a habitat cell that was below its MD (has not found favorable habitat) within an arbitrarily specified number of consecutive moves (ν), it dies (ν = 12; see model calibration section below).

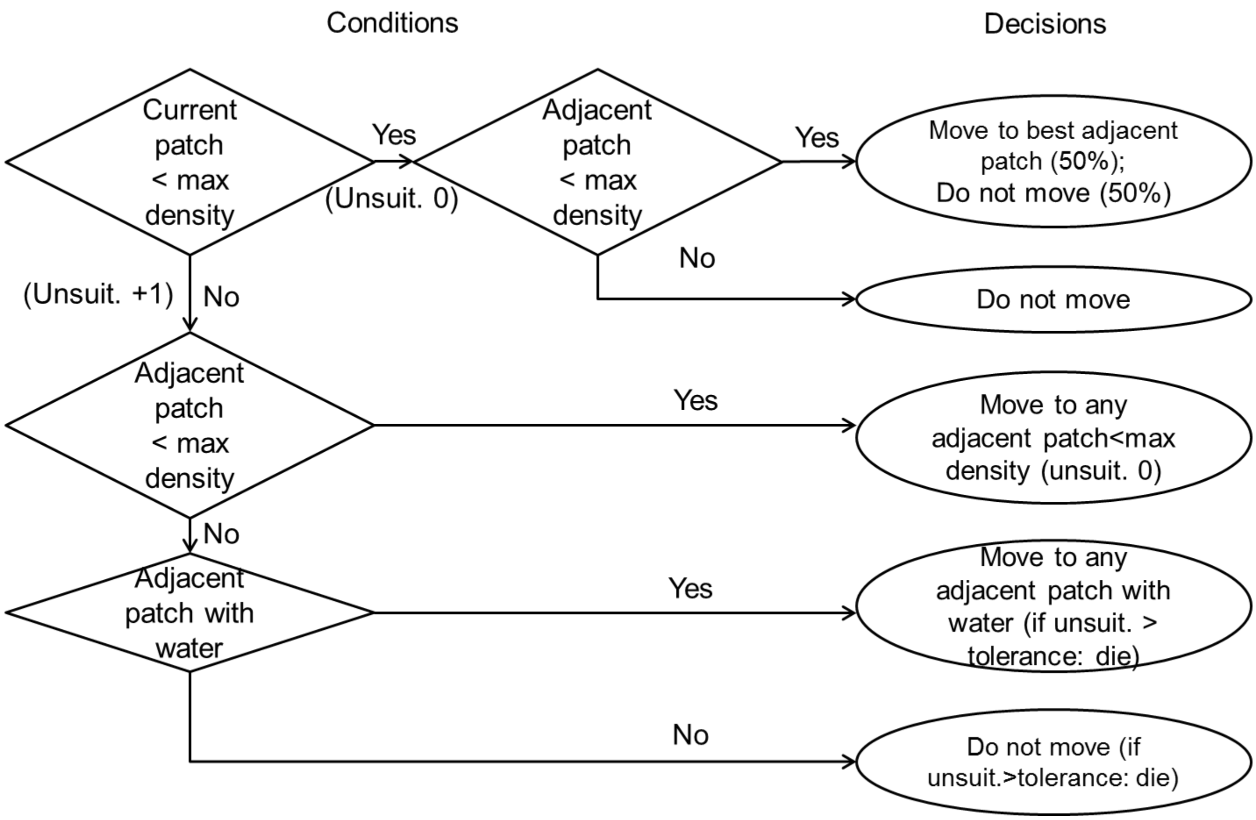


Figure 57. Summary of fountain darter movement rules. MD represents the number of juveniles plus adults that can be supported by the vegetation type in the habitat cell, ε represents the probability of moving to an adjacent habitat cell, ν' represents the number of consecutive moves during which the individual has not occupied a habitat cell that was below its MD (has not found favorable habitat), and ν represents the maximum number of consecutive moves that the individual can survive in unfavorable habitat. Parenthetical numbers refer to decision steps described in the text.

Calculate fountain darter egg laying (recruitment)

Fountain darter egg laying is calculated on a daily basis, with the probability that an adult female lays eggs calculated as a function of month-of-year, the presence of aquatic vegetation in the habitat cell in which the individual is located, and whether or not the individual has laid eggs within the last month. The proportion of adult females that are reproductively active during the months of January through December are 0.1944, 0.2889, 0.3182, 0.0571, 0.0976, 0.1750, 0.1304, 0.0208, 0.0, 0.0976, 0.0328, and 0.1296, respectively, (BIO-WEST 2014d, Appendix D). For those reproductively active females that are located in a habitat cell with aquatic vegetation and that have not laid eggs within the last month, the daily probabilities of laying eggs during the months of January through December, are 0.014, 0.033, 0.027, 0.020, 0.013, 0.006, 0.033, 0.061,

0.008, 0.006, 0.004, and 0.002, respectively, based on McDonald et al. (2007) and BIO-WEST (2014d, Appendix D). If eggs are laid, the clutch size is 19 (Schenck and Whiteside, 1977).

Conduct drop net sampling

On each day of simulated time that corresponds to the first day of a historical drop net sampling period in the reach being simulated, the model "samples" fountain darters in each of the same vegetation types that were sampled in the field, with the relative sampling effort distributed across the different vegetation types as it was in the field. That is, for each drop net field sample in a given vegetation type, the model randomly selects a habitat cell with that vegetation type and records the number of juvenile plus adult darters in that habitat cell. If the vegetation type sampled in the field is not present in the model (based on the historical vegetation maps), the model records a "99999" and that simulated sample is not used in subsequent analyses. (Before comparing simulated and field samples, the number of darters in each field samples is divided by 2; drop nets sampled an area of 2 m^2 in the field, whereas the size of the habitat cells in the model is 1 m^2.)

Update aggregated (output) variables

Aggregated variables describing the state of the system that are calculated daily and written to output files include: (1) the total number of habitat patches with aquatic vegetation, (2) the numbers of habitat patches with each type of aquatic vegetation, (3) the maximum fountain darter density, (4) the total number of juvenile plus adult fountain darters, and (5) the proportions of eggs, larvae, juveniles, young adults, old adults, males, and females in the fountain darter population. At the end of each simulation, the number of fountain darters (juveniles plus adults) caught in each vegetation type during each simulated drop net sample are written to a file which also contains the number of fountain darters caught in each vegetation type during each the drop net sampling conducted in the field.

Preliminary stage of simulation modeling

Verification

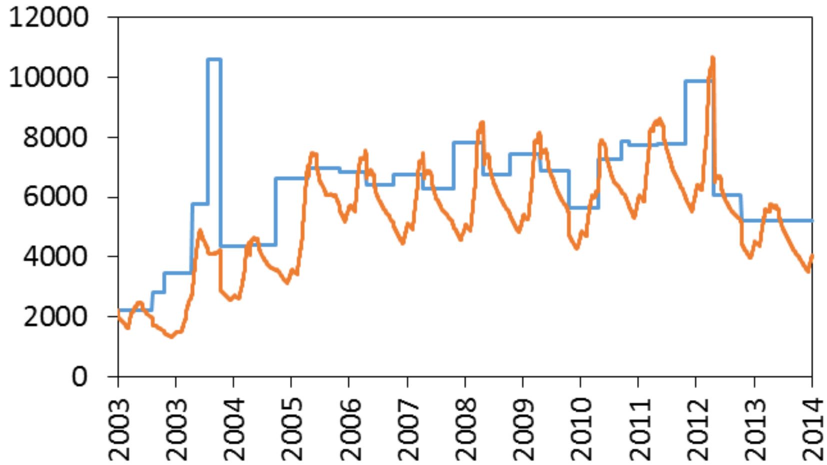
We first verified that the model code appropriately generated historical habitat conditions for each of the reaches of the Comal River and the San Marcos River by simulating spatial-temporal

dynamics of aquatic vegetation (Appendix G, Figure 1), as well as the temporal dynamics of water discharge and temperature concentration from 2003 through 2014 for each reach and comparing simulation outputs to the corresponding time series of input data (Appendix G, Figure 2). We then verified that the model code generated appropriate spatial distributions of water depth and velocity over a range of different water discharges for each of the reaches by comparing simulated depth and velocity patterns with those generated by MDSWMS at the corresponding discharges (Appendix G, Figure 3). Finally, we verified that the model code represented the development of fountain darters through egg, larva, juvenile, young adult, and old adult life stages, as well as the seasonality of reproduction, in accordance with the empirically-based life history parameters used in the model (Appendix G, Figure 4).

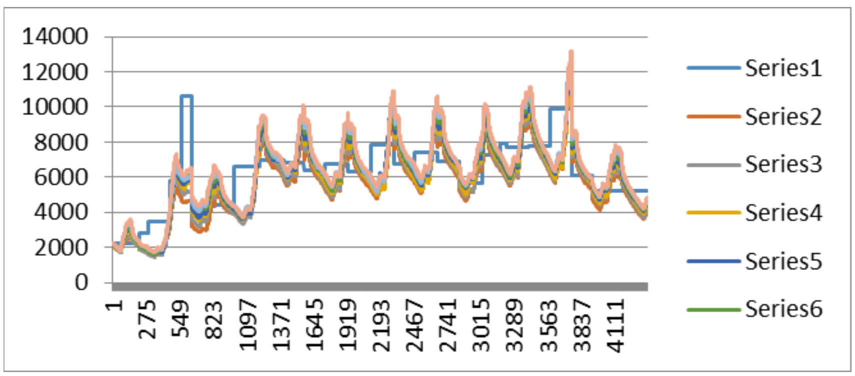
Calibration

For the de-coupled fountain darter model calibration, we used the version of the model that was parameterized to represent the Old Channel reach of the Comal River. We calibrated this version of the model by adjusting v (the number of consecutive moves that a juvenile or adult fountain darter can survive without finding favorable habitat) such that the simulated number of juveniles plus adults increased toward, but did not markedly exceed, the estimated maximum darter densities that could be supported by the aquatic vegetation ($\sum MD_i$; where MD_i is the number of juveniles plus adults that can be supported by the vegetation type in habitat cell *i*; see Section 1.6. and Section 1.8.1) within the Old Channel reach from 2003 to 2014. These two criteria were met with $v = 12$ (Figure 58a), whereas with higher and lower values of v, the number of juveniles plus adults increased beyond (Figure 58b), and failed to reach (Figure 58c), the estimated maximum darter density, respectively. When we removed the limit on the number of consecutive moves that a juvenile or adult fountain darter can survive without finding favorable habitat ($v = 99999$), the number of juveniles plus adults increased exponentially (Figure 59a), and when we replaced the movement rules with random movement the population could not sustain itself (Figure 59b).

(a)



(b)



(c)

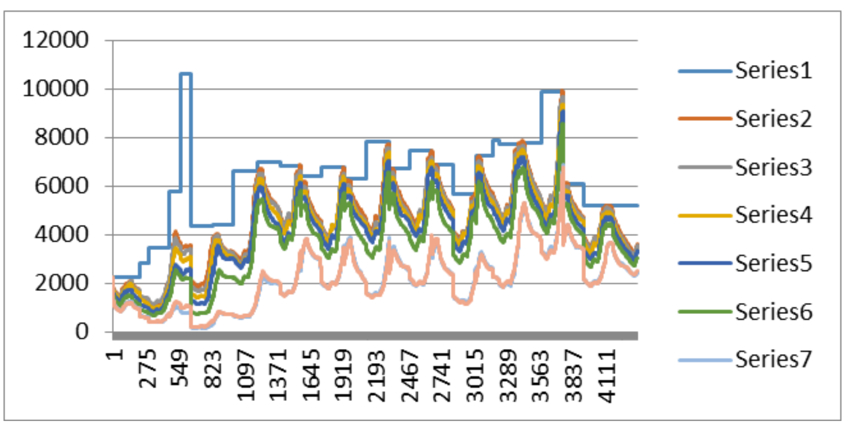
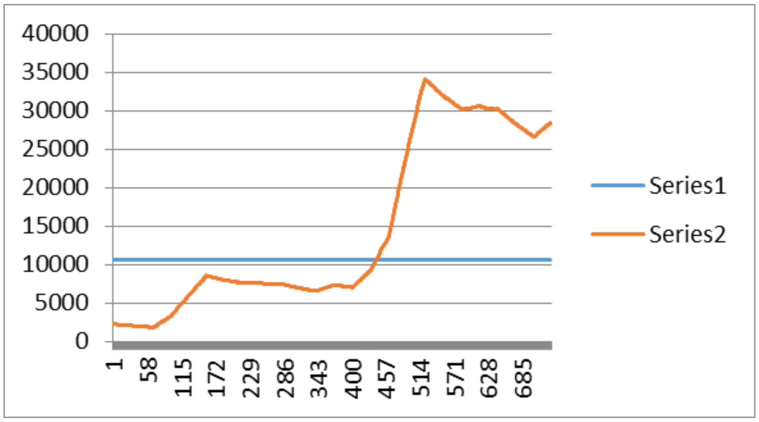


Figure 58. **Estimated maximum darter density and simulated number of juvenile plus adult fountain darters in the Old Channel of the Comal River using (a) the baseline value of v (12) and values of v that were (b) higher and (c) lower than baseline.**

(a)



(b)

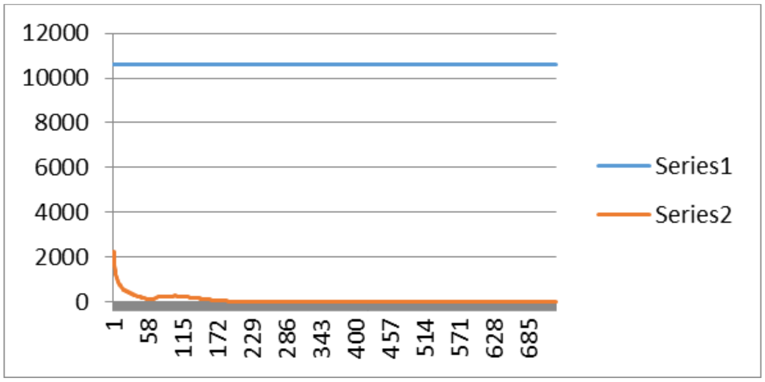


Figure 59. **Estimated maximum darter density and simulated number of juvenile plus adult fountain darters in the Old Channel of the Comal River (a) with no limit on the number of consecutive moves that a juvenile or adult fountain darter can survive without finding favorable habitat (v = 99999), and (b) with the movement rules replaced with random movement.**

Validation

For the de-coupled model validation, we used the version of the model that was parameterized to represent the City Park reach of the San Marcos River. We evaluated model performance by comparing the simulated trends in the numbers of juveniles plus adults to the estimated maximum darter densities that could be supported by the aquatic vegetation within this reach from 2003 to 2014 (with $v = 12$). The relationship of the simulated numbers of juveniles plus adults to the estimated maximum darter densities generated by this version of the model, without further calibration, was essentially the same as that generated by the calibrated (Old Channel reach) version of the model (Figure 60).

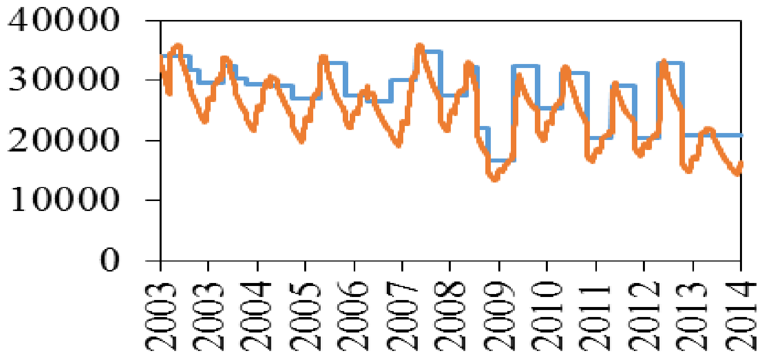


Figure 60. **Estimated maximum darter densities and simulated numbers of juvenile plus adult fountain darters in the City Park reach of the San Marcos River (with v =12).**

For the second phase of model validation, we will use each of five versions of the model (parameterized to represent the Old Channel, Landa Lake, and Upper Spring Run reaches of the Comal River, and the City Park and I35 reaches of the San Marcos River) to simulate historic conditions from 2003 to 2014, and compare the number of darters captured in simulated drop net samples to the corresponding drop net samples collected in field. We will run 5 replicate stochastic (Monte Carlo) simulations representing each reach. To date, we have completed this second phase of model validation for the Old Channel reach of the Comal River. Ranges in the mean number of fountain darters per square meter captured in simulated drop net samples in each of the various vegetation types in the Old Channel reach, with one exception (vegetation type 6, which was sampled only 3 times in the field), encompassed the numbers of fountain darters per square meter captured in the corresponding field drop net samples (Figure 61). (Note that, due to the abrupt changes in the historical vegetation maps (the SAV inputs to the de-coupled version of the fountain darter model) which result in increases in the simulated darter population lagging behind abrupt increases in estimated maximum darter densities, we have adjusted the number of darters in each of the field drop net samples by the proportion of the estimated maximum darter density represented by the simulated darter population on the date of the sample. For example, if the simulated darter population divided by the estimated maximum darter density is 0.75, we multiply the number of darters in the field drop net sample by 0.75.)

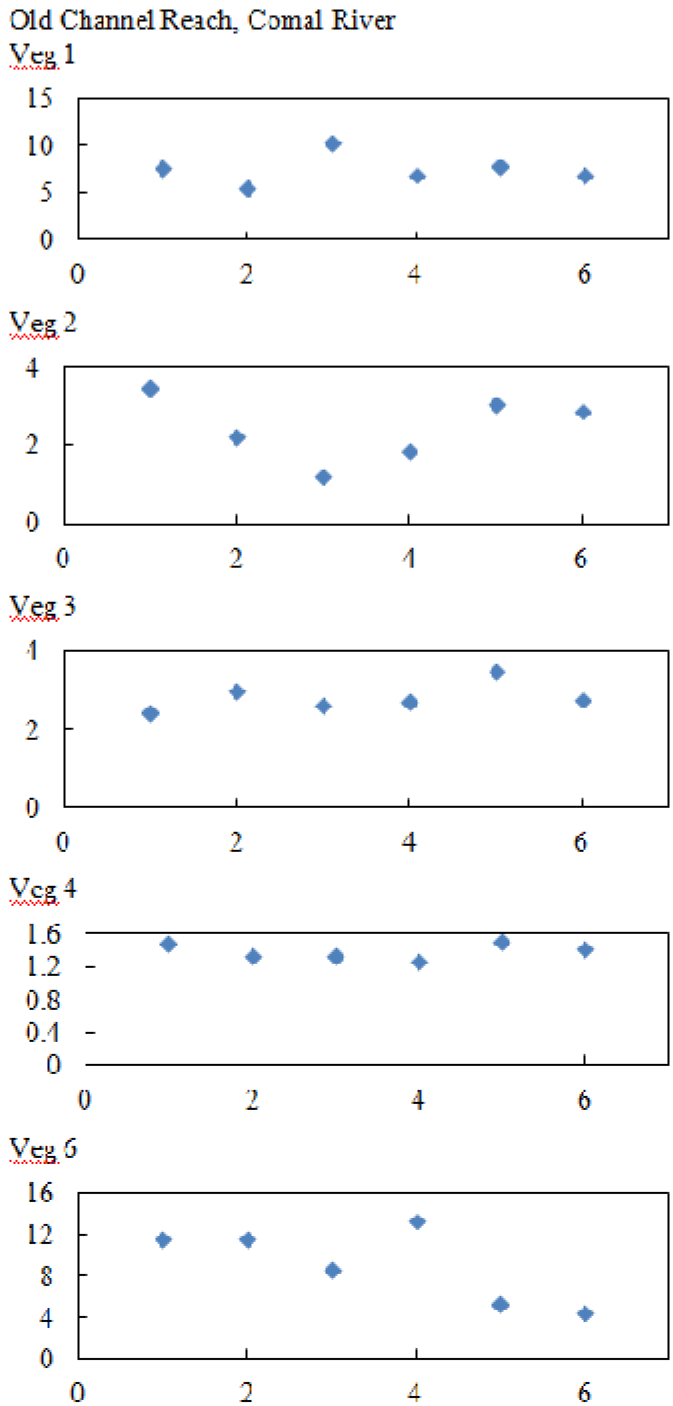


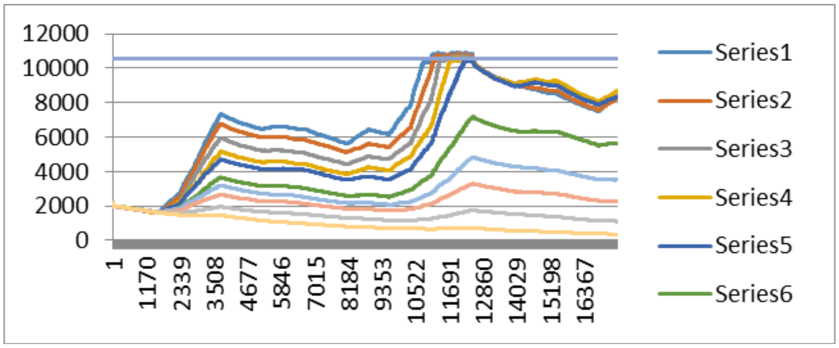
Figure 61. **Comparisons of the mean number of fountain darters per square meter captured in field versus simulated drop net samples in the indicated vegetation types in the Old Channel study reach of the Comal River. In each graph the first five dots, from left to right, represent means from five replicate stochastic (Monte Carlo) simulations, and the sixth dot represents the mean of field samples collected from 2003 through 2013.**

Sensitivity analysis

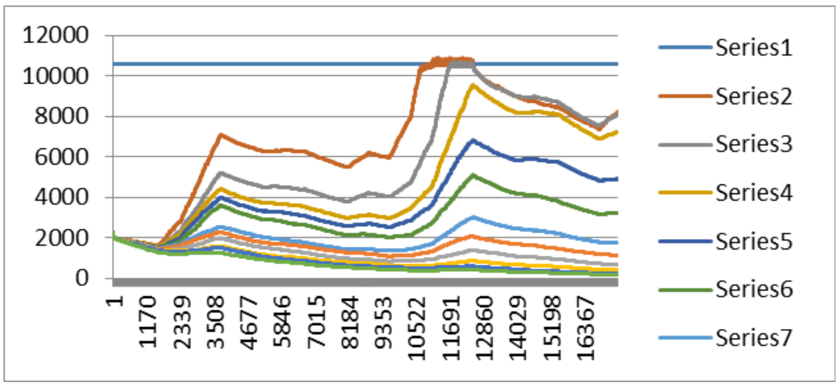
We focused sensitivity analysis on the model parameters that most directly affect fountain darter population growth/recovery: (1) recruitment (clutch size), (2) mortality (base mortality rates of life stages), and (3) movement (ε; probability of moving from a habitat cell that currently is below its MD to an adjacent habitat cell that is below its MD, which also affects mortality). For these simulations, we used the version of the model that was parameterized to represent the Old Channel reach of the Comal River. However, after model initialization was complete, we changed the distribution of aquatic vegetation types from that representing the spring of 2003 to that representing the summer of 2004, and maintained this distribution throughout the simulation. Thus, the relatively small initial fountain darter population of ≈2,000 juveniles plus adults associated with the aquatic vegetation of the spring of 2003 immediately found itself in a habitat that could support ≈10,600 juveniles plus adults, and thereby could express its maximum growth potential. We ran three sets of simulations in which we sequentially (1) reduced clutch size to 90, 80, … , 20, and 10% of its baseline value, (2) increased the base mortality rates of stages (eggs, larvae, juveniles/young adults, and old adults) by 10, 20, …, 90, and 100% of their baseline values, and (3) increased ε by 100% of its baseline value and reduced ε to 0.

With clutch size reduced to 60% of its baseline value, the simulated population still could increase from ≈2,000 to > 10,000 juveniles plus adults by early June of 2004 (peak population levels occur in June) and sustained a net annual growth rate (λ) of 3.74, but with reductions > 60% population increases were noticeable less (Figure 62a). With base mortality rates increased by 10% relative to its baseline value, the simulated population still could increase from ≈2,000 to > 10,000 juveniles plus adults by early June of 2004 (with $\lambda = 3.63$), but with increases > 10% population increases were noticeable less (Figure 62b). Whether ε was increased to 100% or decreased to zero, the simulated population still could increase from ≈2,000 to > 10,000 juveniles plus adults by early June of 2004 (with $\lambda \geq 3.74$) (62c).

(A)



(B)



(C)

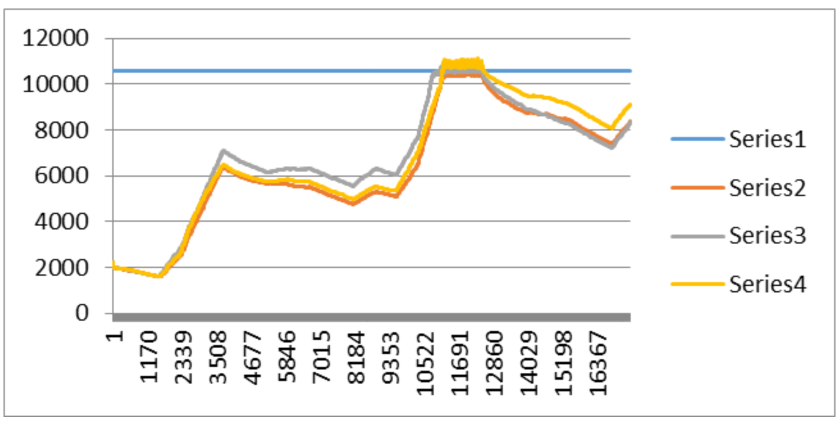


Figure 62. **Sensitivity of fountain darter population growth rate to changes in model parameters affecting (a) recruitment (clutch size), (b) mortality (base mortality rates of life stages), and (c) movement (ε; probability of moving from a habitat cell that currently is below its MD to an adjacent habitat cell that is below its MD, which also affects mortality). See text for details of experimental design.**

Summary of fountain darter simulation modeling completed to date and on-going

In summary, we have developed a spatially-explicit, individual-based, model representing fountain darter population dynamics in response to changes in aquatic vegetation and hydrological conditions. We have verified that the model generates spatial-temporal dynamics of water depth, velocity, and DO concentrations similar to those observed in the Old Channel reach of the Comal River and the City Park reach of the San Marcos River from 2003 through 2014. To date, we have used historical vegetation data as input to the model, referred to as the de-coupled version. These input data will be replaced by simulated vegetation responses to hydrological conditions when the fountain darter population dynamics submodel is coupled with the SAV submodel. We have calibrated the de-coupled version of the model representing the Old Channel reach of the Comal River such that the simulated abundance of fountain darters in this reach responds appropriately to historical changes in habitat conditions. We evaluated the model using the version of the model representing the City Park reach of the San Marcos River by comparing the simulated trends darter densities to the estimated maximum darter densities that could be supported by the aquatic vegetation within this reach from 2003 to 2014.

The relationship of the simulated darter densities to the estimated maximum darter densities generated by this version of the model, without further calibration, was essentially the same as that generated by the calibrated (Old Channel reach) version of the model, that is, simulated trends paralleled observed trends. We have further evaluated the model using the version of the model representing the Old Channel reach of the Comal River by comparing simulated drop net samples to those observed in the field. Ranges in the numbers of fountain darters per square meter captured in simulated drop net samples in all but one of the vegetation types (one for which there were few field samples) encompassed the numbers of fountain darters per square meter captured in the corresponding field drop net samples. Although this comparison does not constitute a validation in the strict sense of the term, since some of the field drop net data were used to quantify the model, it does lend confidence to the functioning of the processes represented in the model. Finally, using the version of the model representing the Old Channel reach of the Comal River, we have examined the sensitivity darter population growth/recovery rates to changes in the values of parameters representing recruitment, mortality, and movement.

Work currently underway includes the development of an additional three versions of the model parameterized to represent the Landa Lake and Upper Spring Run reaches of the Comal River, and the I35 reach of the San Marcos River. As soon as the final adjustments to the SAV submodel have been completed, we will couple that submodel to each of the five versions of the darter submodel. Technical (programming) aspects of this coupling have been completed using the Old Channel version of the model. We then will use the coupled model to simulate fountain darter population response to various environmental scenarios, which are described in the following section.

3 Fountain Darter Simulation Model Application

3.1 Final Simulation Model

The work on the ecosystem modeling has been directed toward completing each of the components of the overall conceptual model diagrammed in Figure 1. The technical approach was based upon a sharply focused appreciation of the application of this model to evaluating the HCP (Phase 1) flow regime, specifically whether the fountain darter populations can be sustained under this particular set of spring flows. The model requirements to answer this question guided suitable approximations and simplifications, which were incorporated into the model formulation.

A key decision collectively made near the outset was to focus the effort on "study reaches" rather than the entire systems. Over the development of the project, five ecomodel reaches were selected based upon available data resources, a variety of external forcings, diverse habitats, and existing populations of fountain darters. During this initial phase of model formulation and development, the project was confined to two primary reaches, the Old Channel of the Comal River and the City Park reach of the San Marcos River. At present time, the Team believes that working models in these five ecomodel reaches will answer the foundational question of whether fountain darter populations can be sustained per the HCP (Phase 1) flow regime. By concentrating on carefully selected reaches for model application, a satisfactory answer can be achieved without the expense and complexity of a complete river-system model, which could require years for model calibration and verification.

The opportunity of the project to take advantage of the previous efforts of the San Marcos Observing System, EARIP, historical research and targeted work supported by the HCP in developing and applying numerical models of velocity and water depth (the hydraulic model), and of temperature and dissolved oxygen (the water quality model) meant that these activities are the furthest advanced at this point in time. So much so that these components are considered to be complete, there remaining only the tasks of streamlining the transfer of output from these models into the ecosystem models. This same opportunity was not available for either the SAV or the fountain darter model. Instead, these had to be developed from first principles, relying upon extensive analyses of the data resources. Both models are advancing the state of the art, and at this point are not complete, though the preliminary results are encouraging. The final step in model integration is linking the output of the SAV model as an input to the fountain darter simulation model.

3.2 Model Operation

The conceptual model of Figure 1 in some respects suggests how the actual computational model might be structured. There are, however, practical aspects of implementing the indicated model executions to simplify the set-up and application of the model to a specific problem. To guide this aspect of model development—by which is meant the construction of an operating computer program to carry out the numerical operations underlying the ecosystem model—a companion conceptual operations model was formulated, presented in Figure 63. In this diagram the arrows indicate the actual transfer of information from one subunit of the program to another, which in some cases corresponds to the conceptual model, but in other circumstances is specific to the functioning of the computer.

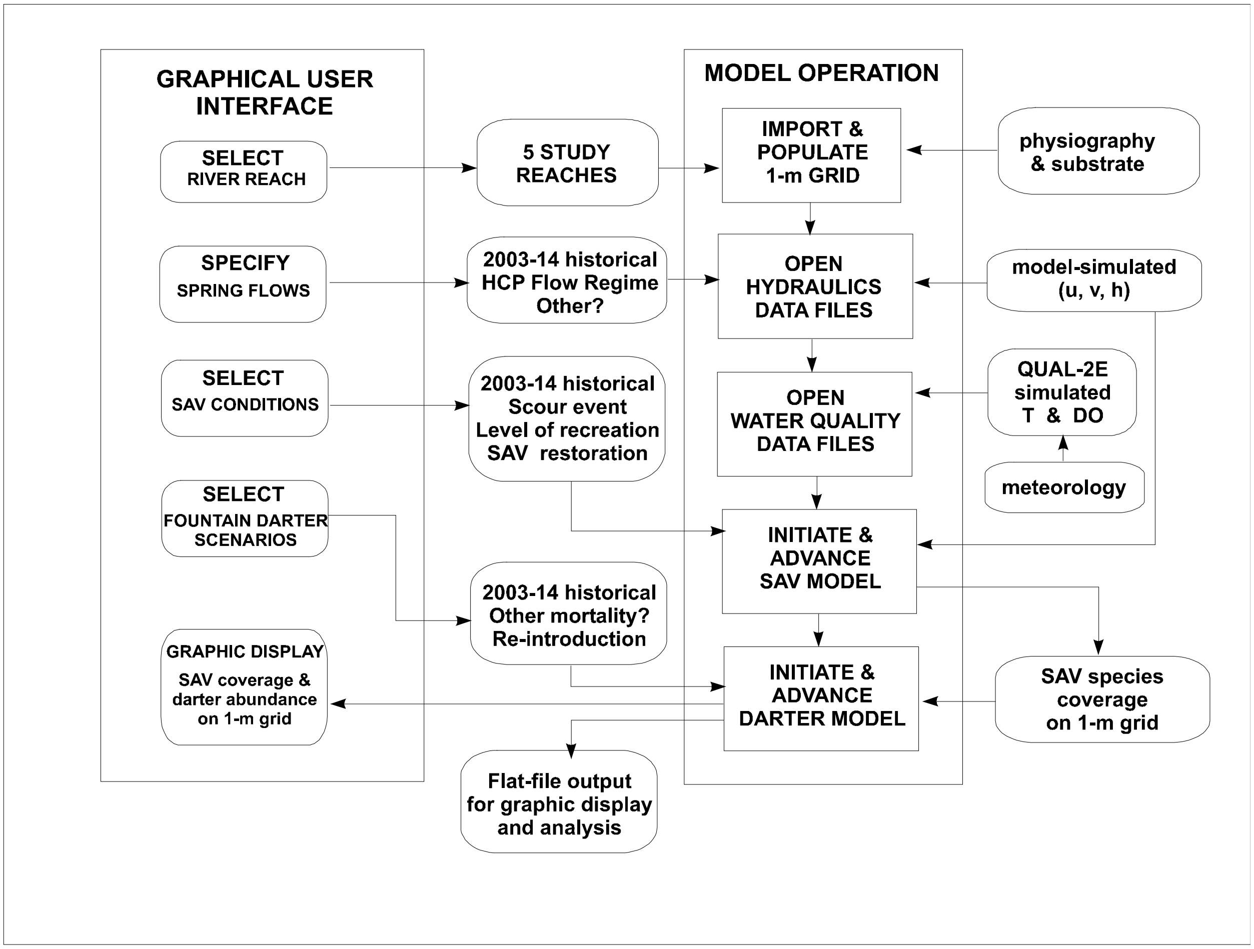


Figure 63. **Conceptual Operations Model**

From the standpoint of operating a computer program, several desired features of the program may be delineated:

(1) Model set-up and operation through a graphical user interface (GUI)
(2) Standardized initiation to ensure comparability of time-series model runs
(3) Limited input options specifically tailored to management questions to simplify operation of model
(4) Range of output formats to facilitate post-run analysis and displays

Desideratum (1) recognizes the ubiquity of GUI's in modern microcomputer operations, and the intuitive value of such an interface in working through the tedious process for setting up and executing complex numerical models. Work has been underway for some time by the project team on the series of GUI options to be used to set up and execute the ecosystem model. The prominent role of the GUI in directing various inputs to the model is shown in Figure 63.

Unlike the simplified conceptual model (Figure 1), the actual model operation must contend with the complexity of spatial distribution of variables being advanced in time. Every element of the conceptual model, including the inputs and processes, changes from point to point in space and from one time step to the next. This imposes a problem from the outset (literally) because the model must be initiated with values at all positions in the spatial domain for each of the dependent and independent variables. Practically, these "initial conditions" are unknown. Even when field data are available, this will never comprise measurements of each variable at every grid point in space (and even if it did, these measurements would include random errors). Therefore any arbitrary initial condition will contain inconsistencies between the variable values and the complex of equations relating one variable to another. These inconsistencies are referred to as a "starting transient", and as the model advances in time and the model equations are repeatedly applied over the spatial domain, these starting transients will decay in time from the model system. (This decay time is sometimes called "flushing time" in riverine modeling, or "spin-up" time in coastal modeling, a term borrowed from large-scale dynamical models in which the rotation of the earth is included.)

The user should not be expected to deal with such technicalities, so the intent of Desideratum (2) above is that a small library of spatial-domain populations of model variables will be created by running the model forward in time with steady inputs until the model values equilibrate. Such a field of model variables values will then be internally consistent and suitable for serving as initial conditions for operational runs. It is intended that this initiation step be largely automated and implemented by the computer without significant intervention by the user, so it is implicit in the opening of hydraulic and water-quality files and the initiation operations of the SAV and fountain darter models, shown in Figure 63.

The practical application of the finished model program will be in addressing specific management problems that may confront the EARIP. A model with unlimited capabilities for set-up and input would place unrealistic demands on the Signatory's staffs, members of the Science Committee, stakeholders, etc in learning the modeling system and components. Instead, it is the team's conviction that the model should present a small number of likely management

cases for which the model can be activated with prepared inputs, subject to manipulation in magnitude. This is the philosophy underlying Desideratum (3) above. Specific examples will be described below.

Finally, one important use of the model will be communication of model results to members of the EARIP and to the public at large. Additional processing and graphic depiction of model results will be useful in facilitating this communication. It is important, therefore, that a model simulation provide sufficient output to support this communication function in versatile, robust formats for importation into spreadsheet programs, statistical packages, and graphic image applications. This is the intent of Desideratum (4).

Several examples may clarify the envisioned model applications. Of course, the single most important model scenario is a low-flow summer condition with substantially diminished spring flows. The user will first select the river reach of concern (or perhaps address each reach in sequence), as indicated in Figure 63. Next spring flows are specified, perhaps together with season. (This specification is still under evaluation, and may take the form of selection from several scenarios, may involve the direct user input of spring flow magnitudes or may use seasonal HCP flows embedded within the 2003-14 standard time period.) From this input, the model will import the necessary spatial-domain grid with physiography, and populate the fields of velocity and water levels, followed by daily minimum DO and daily maximum temperatures, see Figure 63. Finally, the user selects the SAV and fountain darter scenarios. For most comparative evaluations, especially of sustainability of the fountain darter populations, the 2003-14 standard time history will be used. However, the model will accommodate some special management problems, as described further below. At this point, the model is run and provides a display of the evolution of the SAV and fountain darter spatial distributions with time within the GUI (Figure 63). In addition, ASCII (text) files will be output at a specified frequency capable of being imported into additional special-purpose programs, such as spreadsheets.

One of the principal concerns of the team is the impact of loss of aquatic vegetation on the fountain darter population. After a loss event, the concern is the length of time required for vegetation to recover, and the species that will probably be dominant. The effects of recreation

on vegetation will be treated by reducing or eliminating (i.e., zeroing the coverage of) all species in specific areas known to be subjected to heavy recreational use, for time periods every summer corresponding to the tourist season. This at present will require the use of GIS to modify the appropriate vegetation coverage polygon(s). Upon the termination of the recreation season, the SAV model will re-vegetate these impacted areas by regrowth through rooting of seeds, plant fragments and rhizomes. An even more catastrophic process is the occasional scour event associated with floods in the river. In the present model, a scour event is assumed to remove all SAV's, and the modeling problem is to simulate the re-establishment of vegetation in the affected areas.

The SAV component of the model can also be used to simulate the effect of plant-community restoration, by initializing the SAV distribution with the desired native plants. Again, this will be handled by re-initializing the area in which a hypothetical restoration project is to take place, determining the model response of vegetation growth, then simulating the effects of the new habitats on fountain darters. This at present will require the use of GIS to modify the appropriate vegetation coverage polygon(s).

Similarly, several impact events and/or management strategies for fountain darters can be capable of simulation by proper specification of initial populations and/or process parameters, e.g., increases in mortality due to disease or parasites, total loss due to catastrophic spills, and rates of population growth after re-introduction of the species. This at present will require the use of GIS to modify the appropriate vegetation coverage polygon(s).

4 Next Steps and Future Considerations

Year 3

It should be neither overreach nor palliation to observe that as an interim progress report, this reports work in progress, and there remains work to be done. Among the tasks remaining, the SAV model will be brought to completion and implemented as an input to the fountain darter simulation model. Additional validation work will be necessary for the combined models. Once the model performance is judged satisfactory for the two primary study reaches (Old Channel on

the Comal River and the City Park reach of the San Marcos River), model operation for the remaining three ecomodel reaches will be undertaken. This will also entail more extensive validation, with cross comparison of the key parameterizations over the five ecomodel reaches. This validation will also include an assessment and quantification of uncertainty in both data and model, and its use in interpreting model results.

Additional development of the complete computer code is necessary, with integrated GUI and a range of scenarios at the disposal of the user. Preparation of these scenarios will be a major undertaking, with the initial focus on the HCP flow regime and the standard time period operation (i.e., 2003-2014). A brief user's guide will be prepared and training sessions offered to the Signatory's staff in late 2016 as per contractual requirements.

Future Considerations

Though the completed, validated and operational fountain darter simulation model will complete this contracted effort in late 2016, this likely will not be the end of model development for the Comal and San Marcos springs ecosystems. Other management scenarios may present themselves as being desirable for inclusion in the model operation. Extensions of the scope of the model will require re-examination of the simplifications employed in this work, and possibly entail additional parameterization and validation. In particular, the EARIP may consider extending the model to address the impacts of storm runoff on nutrient loads and loads of toxic compounds. With respect to nutrients, under extreme low-flow conditions, and/or with increasing urbanization of the river watersheds, reaches may become eutrophic. This could be prejudicial for the fountain darter population, as well as other species in the rivers.

As stated in the HCP, there is uncertainty inherent with predictions about the duration and extent of low flow conditions at Comal Springs, but the effects of these predicted scenarios and droughts of lesser durations will likely affect the quality and quantity of habitat for other HCP Covered species. In particular, the Comal springs riffle beetle has a fairly limited spatial distribution within the system, so changes in flow could lead to areas suitable for riffle beetle habitat in the system becoming reduced in area and fragmented, potentially leading to the spatial separation of beetles from potential higher quality food resources they utilize. It was the

judgment of this team that the information base for the riffle beetle is presently inadequate to construct an ecosystem model focused upon this species. The project team concurs with the National Academy of Science's recommendations for the EARIP to consider focused monitoring studies and/or applied research to define the habitat, water-quality, and food sources for the riffle beetle, and the future development of a population model.

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APPENDIX A

HCP Ecomodel Team Meeting Minutes

June 2013 - October 2015

MEMORANDUM

FROM: George H. Ward
DATE: 20 June 2013
SUBJECT: Notes on kickoff teleconference, 20 Jun 2013

Participants:

Ed Oborny	BIO-WEST
Robert Doyle	Baylor
Tim Lewis	ERDC
Thom Hardy	WSG
Todd Swannack	ERDC
Bill Grant	TAMU
George Ward	UT

1. Contracting status

Baylor, WSG, Ward complete and active.
TSU, TAMU, ERDC underway.
More info is needed for TAMU and ERDC. Ed will discuss offline.

There was some discussion of the time reporting requirements for invoicing. EAA will ultimately need to see documentation of individual's hours on specific project subtasks. This may present a problem for the universities, as the level of detail is typically hours on the project account, but does not extend to subtask delineation nor does it include hours spent but not formally charged.

Also, all non-labor expenses (travel, meal, etc.) will need to be itemized and accompanied by a receipt.

Ed noted that the BIO-WEST invoice will be sent to EAA o/a the 4th of each month, so if it is important that a subcontractor have an invoice included in that mailing, BIO-WEST needs to receive it no later than the 3rd.

2. "Project notebook" deliverable

This is apparently motivated by the contracting staff at EAA who has found this practice useful. The prime requirement is a documentation of the process by which key decisions were arrived at.

It will be important to document our work as it proceeds by brief technical memoranda, internal memoranda and "notes to self", to expedite team communication and to facilitate report preparation, so this practice should also assist in creating "notebooks". No particular format is suggested, as it is our impression that the notebooks will be informal compilations.

3. Task statements, subcontractor scope and schedules

Ed reviewed the organization of the first year's effort into various subtasks and the assignments of responsibility. Principal initial effort will be the lit surveys for aquatic macroinvertebrates and Comal Springs riffle-beetle modeling. Reviews should be organized as an overview of the present status of modeling with a fairly comprehensive list of primary citations. The lit review should summarize the modeling approaches (which we expect will be only a few broad categories) and provide a brief summary of suitability for EAA.

Three data acquisition internal memos (WSG – Utah State work / ERDC – SAV models / TAMU – fountain darter model) will need to be completed by early August (o/a 1 August) for sharing the present status of existing work among team members. In addition, two data mining memos (SAV and fountain darter) will need to be completed by the end of August to facilitate internal scope preparation.

Despite the fact that a lot of work will be carried out by October, this will not end the task effort, especially for 3.1 and 3.2. Though the remaining budget will be limited, it will be important that some modeling work be carried out in the remainder of the year.

It is suggested that we plan to have two meetings to fulfill contractual obligations: one in the Oct-Nov timeframe with the science committee/ implementing committee/EAA) to review our progress and discuss calibration data sets; and one in the spring (o/a early March) with the same groups to go over recommendations and future work.

Thom noted the importance of skull sessions to identify specific capabilities that each model will need to have to satisfy the requirements of EAA. It may be helpful that some of these be in person, at least among the members located in the Central Texas area.

In summary, Ed reiterated that several deadlines and deliverables (most internal but one external) will be coming up in the next quarter as follows:

- August 1 – Three data acquisition internal memos
- August 30 – Two internal data mining/coordination memos
- August 30 – Literature review from WSG to PI
- Sept 16 – Internal Scopes for SAV and fountain darter
- Sept 30 – Submittal of literature review to EAA

4. Communication

Communication among the project members is an important dimension of this work. It is suggested that we try to have a brief teleconference about every two weeks starting in the latter half of July. Suggestions for suitable days/times are solicited. In addition, we anticipate frequent communication via e-mails as the work progresses.

MEMORANDUM

FROM: George H. Ward
DATE: 27 September 2013
SUBJECT: Notes on Ecosystem Team meeting, 20 Sep 2013
Meadows Institute Offices, San Marcos

Attendance:

Thom Hardy	WSG	Bill Grant	TAMU
Ed Oborny	BIO-WEST	Rose Wang	TAMU
Robert Doyle	Baylor	Tim Bonner	TSU
Todd Swannack	ERDC	George Ward	UT
Tim Lewis	ERDC (remote via call-in)		

1. Contracting status

All contracts in place and underway. Todd is now resident in office at Texas State.

Discussion of the time reporting requirements for invoicing. EAA will probably require documentation of individual's hours on specific project subtasks, though it is not clear how this will be handled for subcontractors. It will be important for the academic team members, who are paid by appointment typically on a semester basis, to keep independent records of time spent on the EAA project, in case documentation is required later. Also, all non-labor expenses (travel, meal, etc.) will need to be itemized and accompanied by a receipt. Tips are not honored.

Robert Gulley has retired. Nathan Pence is the new HCP program manager.

2. Review of Scope

The scope items for the present contract were briefly reviewed. Ed's reading of the EAA intent is, while flow is a management objective for the HCP, the overall objective is biological condition. Therefore what is ultimately needed is a model that can address flow regimes, e.g., for Comal Springs:

> a drop to one month at 30 cfs
> followed by six months at 60 cfs

while maintaining a long-term average of 195 cfs, cf. Section 1.7.1.2 of the HCP, and relate these to ecosystem health. That is, the model needs to be capable of accepting a time signal of flow as input. Basically, the project needs to refine the conditions stated in the HCP.

Controlling and other external factors were discussed for possible inclusion in the model. Gill parasite component may be useful in management, but data show a decline of the parasite (reasons unknown). The macroinvertebrate model may be useful as a model food source to the darters. The darter can tolerate up to 34.5°C, but amphipods may not be able to, so a lower threshold may be the more effective temperature constraint. Hyalella may serve as proxy for amphipods. Mayflies are also a prime food source, maybe even more than amphipods. Canopy cover should be considered. Thom noted that his temperature model includes shading. Functional ecogroups might be better than individual species.

Geographical distributions of fountain darters were discussed in context of the extent of spatial depiction necessary. Fountain darters typically don't use the spring runs, and are mainly in the lakes and river reaches, particularly where temperatures are stable. Tim B.: darters are inactive under good conditions, but when conditions are changing no one really knows how far they will move. He is presently studying this in an ongoing project. Is a detailed computational grid really necessary? Why not identify those regions in which certain species are known to occur (or not) and model as a single spatial region connected to others?

3. Review of data availability

Data holding in the various categories of vegetation, inverts, fish, external conditions were briefly reviewed. Marcus Geary (EAA) noted as source for hydrological data.

4. Draft report on riffle beetle & invertebrates

Task 1 requires preparation of a literature review addressing two topics: (1) possible modeling strategies for the riffle beetle & (2) a modeling approach for aquatic macroinverts. Dr Hardy has prepared a draft addressing both. (He included a literature review on Hyalella, to serve as a proxy for inverts.) The team agreed that these are in fact independent topics, because the riffle beetle is addressed solely because of its endangered status, not because of its role in the ecosystem, while the macroinvert modeling is necessitated by its function as a food source. The possibility of separating these into two separate reports was discussed. On the one hand, these are independent subjects, but on the other hand, there is some overlap.

Some discussion was devoted to identifying the target readership for the report. George expressed discomfort that the draft report seems to be addressed to the Science Committee, instead of a reader at the level of, say, Dr. Gulley, noting that after all it is the EAA, not the Science Committee, that cuts our checks. The consensus of the group, however, was that the Science Committee is the appropriate audience, because it is exclusively their opinion that dictates the EAA's acceptance of the report.

A re-organization of the draft report was proposed, and Dr Hardy will undertake this revision, which will then be reviewed by the team. The decision of whether to submit independent reports was deferred until the report is re-drafted.

5. Key questions or requirements, and next steps

Several key questions/requirements were identified in the course of discussion and summarized as follows:

Macroinverts need to be explicitly addressed and incorporated into the model(s).

Bonner: The Team needs to have a brief but directed consideration that will limit the theoretical models to what are needed & applicable to Comal and San Marcos Springs.

While it is the consensus that spatial dependency is important, we need to think through exactly how we will depict this in the modeling. It was noted that different vegetation species react differently to hydrology, particularly to flooding.

We need to formulate conceptual model(s) of the springs systems and rivers. In particular, an expanded conceptual model for each of the fountain darter and SAV's is needed. Bill and Todd, respectively, will prepare first-cuts at these.

Todd will begin setting up a one-species model for vegetation to facilitate our consideration of this aspect of the modeling and to identify needed parameters.

How will we be able to validate the model for extreme low-flow events until these actually occur? The suggestion was made to seek data from other systems where such low flows are more common. This needs to be looked into.

First draft of conceptual models to be prepared by TAMU and submitted within a few days. Upon project team review, a conference call will be conducted Friday, October 4th at 2pm to provide feedback on the conceptual models.

Next face to face meeting tentatively scheduled for October 25th with location to be determined.

MEMORANDUM

FROM: George H. Ward, scribe
DATE: 7 October 2013
SUBJECT: Notes on teleconference, 4 October 2013

Participants:

Ed Oborny	BIO-WEST
Thom Hardy	WSG
Todd Swannack	ERDC
Rose Wang	TAMU
Bill Grant	TAMU
Tim Bonner	TSU
George Ward	UT

1. Conceptual model of fountain darter

The draft conceptual models sketched by the TAMU team members was discussed. Key discussion points summarized as follows:

As formulated, the model appears comprehensive, including land-use, presumably runoff from the watershed, etc. Are we still mainly concerned with spring flows in the range 0-125 cfs, say, or are going to model the world? Emphasis remains on the low flows, though we need the capability to input a "regime", or a time variation of these flows. But for the conceptual model it's a good idea to include every potential control just to remind us that these operate at some scale, though we may choose to neglect them for our specific scenarios.

There isn't a food source for the darter. Do we need one? Yes, we need to include a food source, maybe one or two more boxes with generic categories, e.g. amphipods.

The gill parasite needs to be brought into the model. We can't lose sight of its potential impact on the darter population.

Some discussion of movement rules for the darter. Thom remembered a similar individual-based model developed by Railsback and will try to distribute a copy. He followed up with a copy of Railsback et al. (1999, *Ecological Modeling 123*), attached. (Subsequently, this scribe attempted to download USFS Report PSW-GTR-182 but the Forest Service site is down due to the government shutdown. However, Railsback et al. 2012, *Natural Resource Modeling 15*, may be useful, also attached.)

To move from the conceptual model to the quantitative model, Bill and Rose need input from the experts in two categories: (1) a list of what needs to be in the model, (2) rough, even qualitative functional depictions of the key processes, e.g. temperature response. Bill and Rose will prepare

graphical blank forms for each key process and distribute these to the team by 9 October, and the team will sketch the functional responses as they perceive them and return to TAMU by 15 October, whereupon Bill and Rose will incorporate these into a first draft of the darter model.

2. Literature search draft report

This scribe has re-formatted the draft report prepared by Thom and distributed it to the team.

Consensus is that the bullets summarizing the recommendations from the EAA expert modeling panel of last summer should be deleted, since we have moved beyond this level of modeling. It would be good to have an initial paragraph describing the literature search, e.g., the number of documents located and reviewed, though not explicitly cited in the text.

The suggestion was made that we back off stating a recommendation for a model for either invertebrates in general or the riffle beetle, because we are not sufficiently along in the review to commit. This is, after all, a literature review, but not necessarily a decision point.

There may be a BBN model diagram specific to the riffle beetle analogous to Fig. 11 from Jean Cochrane. Thom and a few members of the team have a vague memory that this has been presented in the past.

A revised version will be forthcoming shortly with mainly edits, which will be distributed at once to the team. Any substantive changes or additions from the team need to be supplied quickly, as we are already behind the delivery date for this report.

3. Next meetings

We will have a telephone conference call Monday 21 October (2013) at 2:00 PM.

The next meeting will be at the Meadows Institute, 13 November 2013 at 1:00.

FROM: George H. Ward, scribe
DATE: 21 October 2013
SUBJECT: Notes on teleconference, 21 October 2013

Participants:

Ed Oborny	BIO-WEST
Thom Hardy	WSG
Todd Swannack	ERDC
Rose Wang	TAMU
Bill Grant	TAMU
Tim Bonner	TSU
George Ward	UT

Objective: Discuss the "judgments" of darter dependency on external parameters requested by TAMU team.

At this point, only Ed has responded to the request of Bill and Rose for inputs to their conceptual model for darters.

Bill clarified the desired format of the exercise. The *increase* of darter population ("recruitment," which will in fact include several mechanisms that increase the population) or *decrease* ("mortality", likewise, incorporating several mechanisms that decrease the population) should be thought of as a factor that will multiply the respective "base" rate, hence its depiction as a dimensionless variable. How this variable changes with an external parameter, such as temperature or plant cover, as "professional judgment," is what TAMU is seeking for the first version of the darter model. For now, the effect of several external parameters will be determined by simply multiplying the scaling factors. Later, more complicated formulations can be accommodated.

Bill further described the three categories or uses of data from the perspective of developing a systems simulation model:

(1) driving variable data,
(2) evaluation data,
(3) data that are analyzed to quantify functional relationships within the model

In the last case, the results of the analyses, perhaps a regression equation, actually become part of the model. Driving (or external) variables (1) affect the system but are not affected by the system. For example, we might use a time series of rainfall data to generate primary production, thus changing the state of the system, but future rainfall is

unaffected by these changes to the system. Variables such as rainfall are not *inherently* driving variables, it depends on the system of interest. For example, massive deforestation would affect rainfall patterns. Evaluation* data (2) often are time series of population sizes or standing crop biomasses, which represent real-world observations on the things we are trying to simulate. We don't use these data to construct the model, but rather we compare them to their simulated counterparts to see how well the model is performing. The third category of data (3) may come in many forms, but the distinguishing feature is that they are used to quantify the functional relationships in the model, often taking the form of rate equations, or expressing the likelihood that some process will occur.

Ed has supplied an older STELLA model from an earlier HCP report. The relations depicted are correlative, that is, regressions of the population versus the external parameter. Bill pointed out that what he needs is a *rate* of increase or decrease associated with the external parameter, which does not necessarily follow from a correlative relation.

Some questions about the scale (i.e., units) for certain external parameters. Sediment texture (grain-size), for example. The Wentworth scale is fine. Current could be in units of speed, or represented by flow.

Ed expressed concern that attempting to depict multiple variables may result in "double-dipping", for example, representing the effect of current on darter population explicitly may duplicate the relation already implicit in another variable that current affects, e.g., vegetation cover of a species that is scoured by high currents, or substrate texture that is governed by statistics of current speed.

This emphasizes the importance of a sound conceptual model, because there may indeed be several different relations on the same variable, depending upon the intermediate mechanisms operating. Bill observed that the time scale of response is important here. If a plant species is scoured out by high currents, does it simply grow back, or does some sort of successional development take place? Though this question was offered as an example of what other considerations are invoked by looking at longer time scales, Thom noted that this question has been addressed for the San Marcos by Hannan and Dorris (1970).

The main purpose of the model is to test the flow regime specified in the HCP, then to determine, on the one hand, whether the specified flows can be reduced without appreciable impact on the ecosystem, and on the other whether higher flows are needed to preserve the ecosystem. But we made it clear to EAA that a fully operational model cannot be completed within the first year of this project. We should, however, be striving to have an operational framework that we can demonstrate on a PC. Such a model will

* Bill doesn't like the term "validation."

also be useful to the team in formulating future research studies to be undertaken in the following years, by better identifying critical information deficiencies.

Thom recommended that we start with fundamentally simple relations, and use the data to test these relations. He would also like us to start thinking about how we are going to incorporate spatial variability into the model(s).

Bill and Rose want to use NETLOGO instead of STELLA, part because it is freely available, while STELLA requires purchase of a license. They have already tested the older demographic model (from Mora et al., 2013) in NETLOGO and found it to give equivalent answers.

Tim is sitting on a lot of data from the San Marcos that requires some number-crunching to get the inputs that TAMU is looking for. He believes he can carry out the necessary calculations and get something to TAMU by the end of the week.

George remarked that "equifinality" is a concern, particularly with uses of data to evaluate a model, i.e., Bill's category (3), and will need to be discussed later.

Todd is working on the single-species model. He is also working on extracting functional forms for SAV responses to external parameters and will have something for the team by the end of the week.

Over the next three weeks, leading up to the 13 November team meeting, the team should be looking at the preliminary model results and interacting among ourselves in modifying the inputs to achieve a realistic (sort-of) behavior.

References

Hannan, H., and T. Dorris, 1970: Succession of a macrophyte community in a constant temperature river. *Limn. Oceanogr. 15*, 442-453.

Mora, M., W. Grant, L. Wilkins, H.-H. Wang, 2013: Simulated effects of reduced spring flow from the Edwards Aquifer on population size of the fountain darter (Etheostoma fonticola). *Ecol. Model. 250*, 235-243.

MEMORANDUM

FROM: George H. Ward, scribe
DATE: 19 November 2013
SUBJECT: Notes on Ecosystem Team meeting, 13 November 2013
Meadows Institute Offices, San Marcos

Attendance:

Thom Hardy	WSG	Bill Grant	TAMU
Tim Bonner	TSU	Rose Wang	TAMU
Todd Swannack	ERDC	George Ward	UT

1. Deliverables status

Invertebrate modeling report (EA HCP Ecosystem Modeling Team, 2013) transmitted to EAA. No response. The silence is deafening.

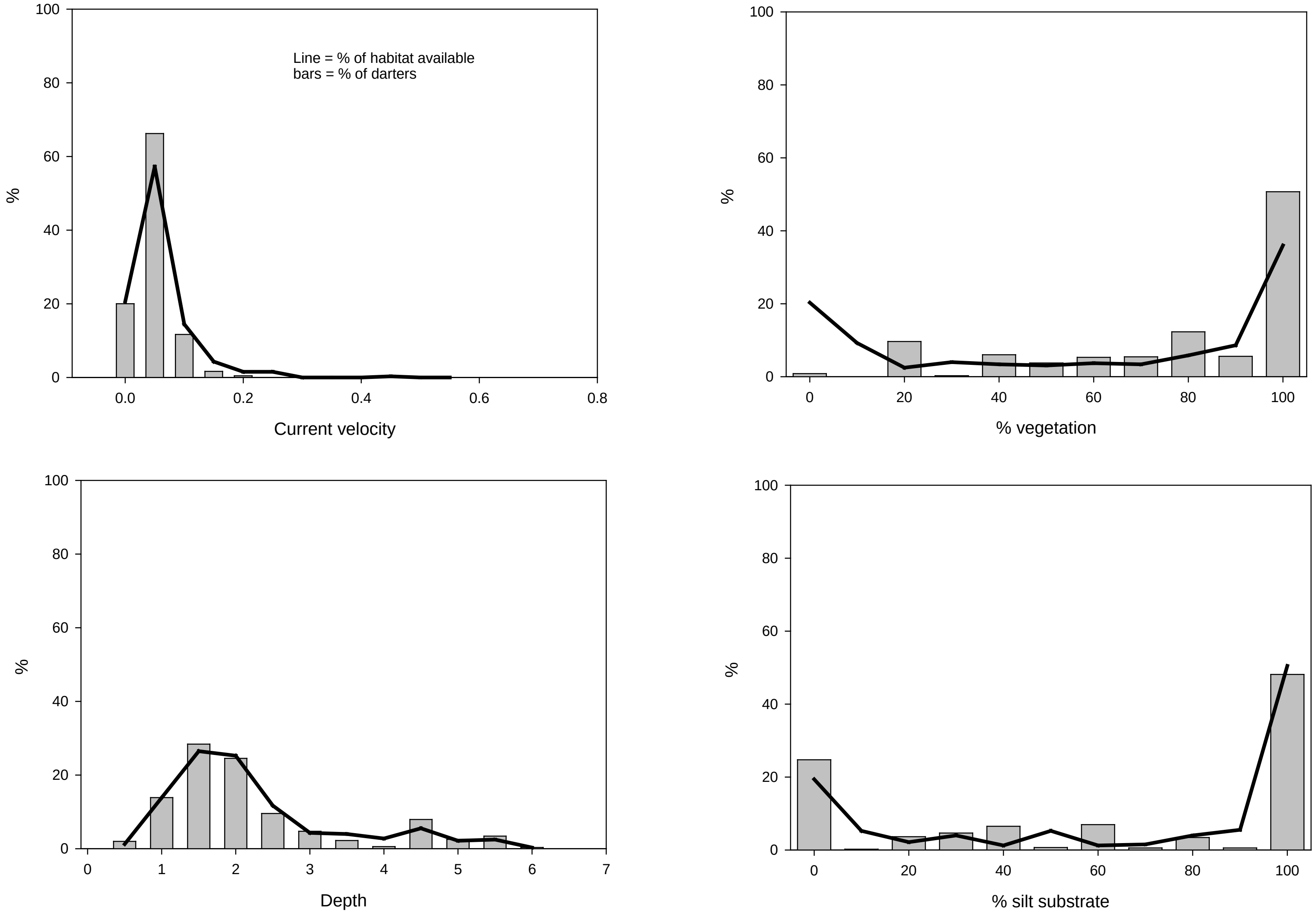
2. Watercourse conditions and fountain darter data

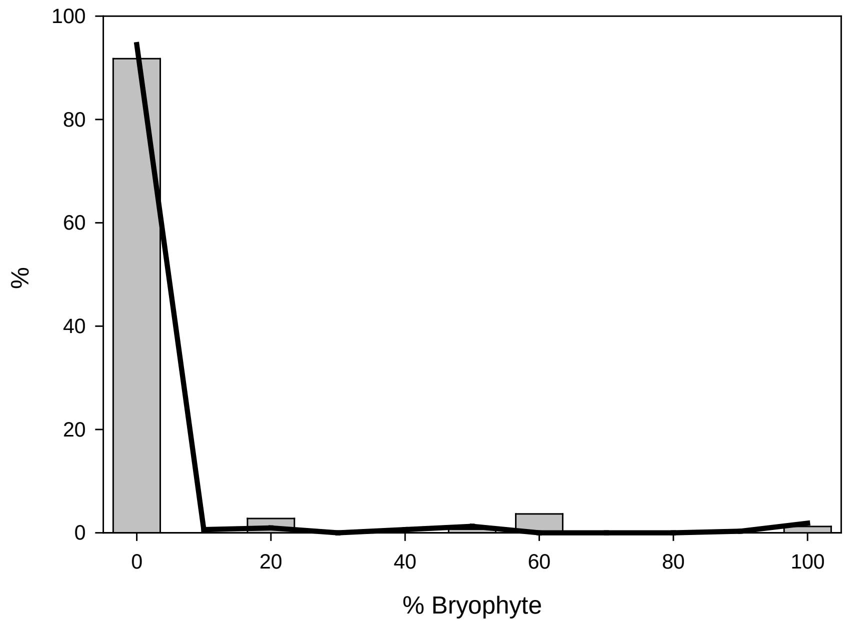
Tim reported that the October floods scoured out a fair amount of the submerged aquatic vegetation (SAV's) in the San Marcos in localized areas. Peak flow was around 800 cfs. The Blanco was up into the IH35 bridge, around 80,000 cfs.

Tim then reviewed analyses he has carried out for his SCUBA diving fountain darter surveys from the San Marcos. For each cross section at which he sampled, the section was subdivided into distinct combinations of depth, substrate and vegetation ("habitats") and the proportion of habitats available was computed. Then the numbers of darters found in each "habitat" were weighted based on abundance to compare habitat available versus habitat used by fountain darters (Attachment 1). Similarities in habitat available and habitat used suggest (statistically analyses forthcoming) that fountain darters are positively associated with low current velocities, independent of depth, amount of silt substrate, availability of bryophytes (a contrast to findings in the Comal River) and amount of vegetation as long as some vegetation is present within a habitat patch. Quantified habitat associations are available to assist in the development of the ecological model.

Results from the San Marcos do not fully track the results of monitoring in the Comal, specifically in that fountain darters are found over bare substrate in the San Marcos and there is not a strong association with Bryophytes. It was suggested by Tim and Thom that darters are simply using boundary layers as velocity shelters and may be reflecting plant/substrate morphology effects on current velocity (i.e., structure rather than species). Tim requested suggestions for other analyses to which his data might be subjected.

Figure 1
Provisional plots of Bonner data on habitat and darter versus habitat parameters




100
80
60
40
20
0
%
0
20
40
60
80
100
% Bryophyte

3. Fountain darter modeling

Bill and Rose have incorporated the external-factor relations provided by the team into a first-cut NETLOGO model of darter populations with four geographical cells. Demonstrations of the model were presented. They began with a "demographics" only model, i.e. no external forcing, then demonstrated how each relation (on flow, depth, etc.) is incorporated into the model, and the resulting effect on the population. The population responds to population density as a randomized down-gradient movement. More sophisticated rules can be formulated, and there was a discussion of the forms these rules might take. Now the model has a loop embedded in each time step, so a fish can move several times.

Thom and the TAMU team had previously experimented with NETLOGO and determined that it could easily accommodate on the order of 10^4 computational cells or grid locations. This led to the suggestion that the model be implemented on one of the 100-m reaches that Bio-West has been sampling over the past 12 years, because the 0.25-m grid system that Thom employs in his hydrodynamic-temperature model could be ingested by NETLOGO for a reach this size (100 m x 10 m x 4 x4 nodes per sq meter = 16,000).

There was some discussion of how best to input this hydrodynamic information. "Coupling" the models would present a programming challenge that is probably unnecessary. Rather, the hydrodynamic model output can be generated as a time-series text file, which is simply read in, timestep-by-timestep, into NETLOGO. Bill and Rose are to consider what input format would work best for them and advise Thom. Then Thom will produce an input "driver" file of variables

$$\varphi, \lambda, h, T, u, veg$$

i.e., latitude, longitude, water surface elevation, temperature, current speed at each node, and vegetation type at each node, resp. For this formulation, vegetation will be treated as a categorical variable, e.g. values = 1, 2, 3, etc., corresponding to each of eight or so dominant species, determined from the vegetation polygons the Bio-West has mapped on the 100-m reaches. The TAMU team will then set up a NETLOGO grid over the 100-m reach to model darter behavior. There was some discussion of superposing the model output on a raster-image map or aerial of that section of the river.

4. Vegetation modeling

Todd reported on the ERDC modeling. Per the team's decision (Ward, 2013), ERDC is proceeding with a stripped-down version of its model to address a single species. This is requiring some re-programming, in which Todd is presently addressing coding computational conundrums.

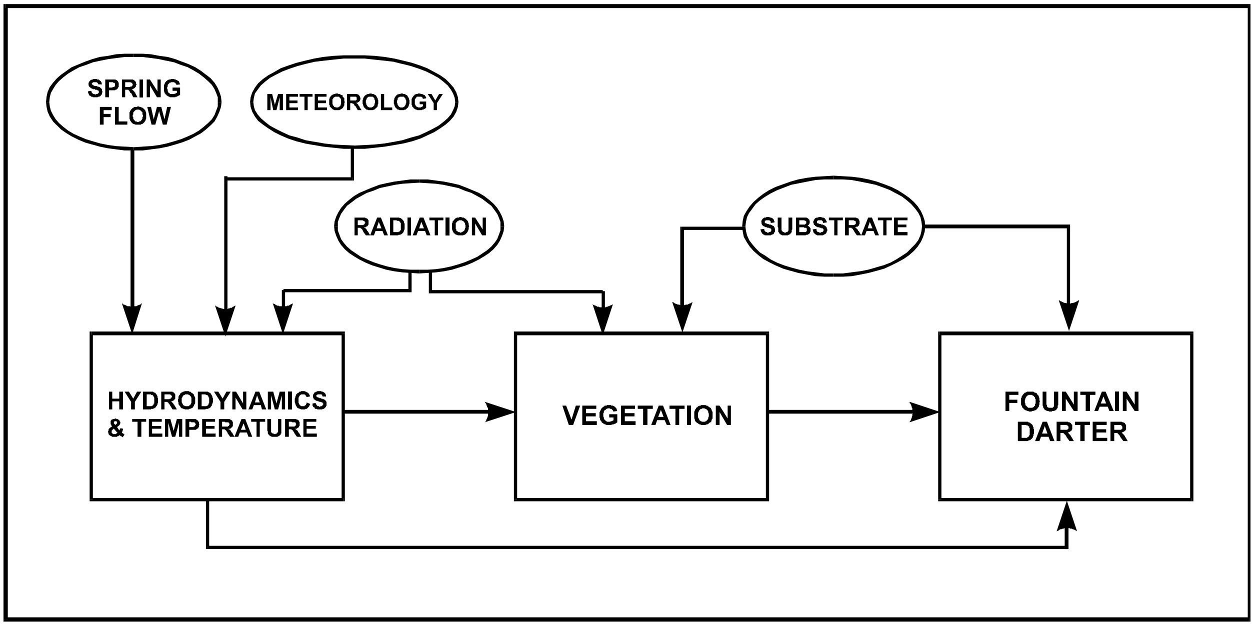


Figure 2 - Causal-link diagram for present version of fountain darter model with vegetation model operating

Ultimately, the output from the vegetation model will be linked into the darter model in a similar manner to the external drivers of Section 3 above. This model formulation is essentially feed-forward, see the causal-link diagram of Fig. 2, so model linkage can be accomplished through input/output file transfers.

5. Model parameterization and testing

Once a test reach is identified, it will be necessary to quantify undetermined parameters in the darter model formulation. The team agreed on an approach in which six consecutive years of data will be used to delineate the model parameters (i.e., “parameterization” or “calibration”) and the remaining six years will be used to test the model’s predictive capability. Ideally, the first six years would be used for parameterization and the other for testing. However, it is important that the six years used for parameterization exhibit as high a range as possible in the values of the external variables. It is possible that the best such six-year period may occur in the center of the 12-year data period. In this case, the model test in the remaining six years will be carried out in a 12-year simulation.

This is not a calibration-verification exercise, but more of a testing exercise to assess the ability of the simplified model framework to predict abundance of darters. There are many factors potentially remaining to be incorporated into the model formulation, such as nutrient inputs, darter prey, and vegetation dynamics.

One additional benefit that NETLOGO may provide was discussed by the team, *viz*., the potential for creating a scaled-down version suitable for dissemination to the stakeholder community to explore on their own.

6. Next steps

Any suggestions to Tim for further analyses should be forwarded to him.

Thom will examine the Bio-West data to (1) provisionally select the most favorable 100-km station for the modeling exercise, and (2) provisionally select the best six-year period for model parameterization. These selections will be discussed in a teleconference with Ed & George. Tim will participate also if he has no other conflicts at the scheduled time.

The date for this telephone conference needs to be set, ideally in the first week of December if not before.

Bill and Rose will delineate the optimum format for a text file containing the hydrodynamic and vegetation information for input into the present NETLOGO model, and transmit to Thom.

It was the consensus of the team that the next meeting should occur in late January or February. A telephone conference at some intermediate point, e.g. early January, would be desirable to assess progress on the modeling.

References

EA HCP Ecosystem Modeling Team, 2013: *Literature review: Invertebrate modeling framework and modeling approaches for the Comal riffle beetle*. Task 1 report, Edwards Aquifer Recovery Implementation Program, Habitat Conservation Plan, Edwards Aquifer Authority, San Antonio.

Ward, G., 2013: Notes on Ecosystem Team meeting, 20 Sep 2013. Modeling Team, Edwards Aquifer Recovery Implementation Program, Habitat Conservation Plan, Edwards Aquifer Authority, San Antonio.

MEMORANDUM

FROM: George H. Ward, scribe
DATE: 8 January 2014
SUBJECT: Notes on teleconference, 8 January 2013

Participants:

Ed Oborny	BIO-WEST	Bill Grant	TAMU
Thom Hardy	WSG	Tim Lewis	ERDC
Todd Swannack	ERDC	Gary Dick	ERDC
Rose Wang	TAMU	George Ward	UT

Objectives: Review status of darter and vegetation modeling. Start work on deliverables.

***Modeling*:**

There has been a substantial activity in extending the darter model to address a spatially complex river segment (for which the Old Channel reach was selected) with time-dynamic inputs, in which Bill, Rose, Thom and Ed have participated. There have been a few glitches manifested as data have been prepared in suitable formats and transmitted to Bill and Rose, but these have been worked out. The latest issue, that NETLOGO accepts only a rectilinear grid, while Thom's hydraulics are based upon a curvilinear channel-following grid, can be dealt with by simply treating the hydraulic model grid as a matrix whose entries correspond to the NETLOGO cells. Thom determined that the geometric error entailed is negligible. Two types of input files will be produced for the modelers: (1) variables that are spatially homogeneous but temporally dynamic, (2) variables that are spatially heterogeneous but temporally static except for a small number of quantum changes. The principal representative of the former is water temperature. The latter includes vegetation and substrate categories, and hydrodynamic variables (water depth, current velocity). The input structures for each were discussed.

The present status of the darter model is that the refined grid has been successfully implemented, and the model executes satisfactorily with 60,000 model darters. Bill describes these as "dummy darters" because they have not yet received specification of how they respond to population density, physicochemical variables and habitat variables. The single-species aquatic vegetation model is not as far along as the darter model but progress is being made. Todd has been sustaining programming bugs with the *Vallisneria* model, that have driven him to re-code a substantial part of the program. This is still underway.

There was considerable discussion of the model framework and procedures, where we are now and where we are going. These are small steps in a sustained process to ultimately arrive at a fully operational, coupled model capable of exploring alternative future scenarios. In the present year, our goal is to get models "up and running," by which is meant models with partial capabilities that yield reasonable-looking answers. Emphasis is on achieving bug-free operation

of computer programs with sufficient generality to support the EAA modeling objectives. The next step for the darter model will be the detailed specification of darter behavior, including reaction to aquatic conditions. The next step for the vegetation model will be specification of appropriate growth and kinetic factors for the species modeled, including reactions to water and substrate chemistry. Our selected test domain is the Old Channel reach of the Comal River. Ultimately, the models will be calibrated for one six-year period and verified against the other (as discussed in the 19 November meeting). For this initial development phase, the darter and aquatic vegetation models will be developed independently, with a "place-holder" array in the darter model for vegetation parameters. Temporarily, this array will be filled with observed vegetation distributions from the field work. Once the separate models have been carried forward to a satisfactory level of validation*, they will be coupled, so that the predicted vegetation distribution will populate the input array in the darter model.

Thom raised the question of whether the heterogeneous arrays, which are updated only about 4 times a year, should be interpolated to the intervening times or simply subjected to a quantum change. For present purposes, the latter was judged to be the more efficient procedure. Also, it was decided that temperature inputs every hour would be sufficient time resolution for the darter and aquatic vegetation models. Ed will be extracting hourly temperature from the BIO-WEST data bases and sending to Thom, who will fill the data gaps with a sinusoid diurnal variation. Ed will also examine the dates of aquatic vegetation mapping data and specify input-update dates one-two months before each data collection event, and provide these to Thom, Rose and Bill. Thom will integrate the aquatic vegetation distribution and substrate data with the hydraulic output to create multi-variable input arrays for the model domain at each date specified.

It was noted that the current velocity data is within the Old Channel, and does not directly relate to total system discharge. For the present model implementation work, this is not an issue, but at some point in the future, the relationship between the two will need to be incorporated.

Gary raised the question of why *Vallisneria* was selected as the model species, given the USFWS determination that *Vallisneria* is not native. We had decided that our initial model set-up would be a single-species model, to be later expanded to include other species. *Vallisneria* was a handy choice because some of the important growth and kinetic parameters were already on hand. Also, the FWS determination notwithstanding, *Vallisneria* is an important species in these systems, being dominant in some areas. There was a discussion of the value of identifying the plants, not by species, but by structural (or architectural) attributes, since this would better reflect their importance to the darter community. Robert observed that the Corps *Vallisneria* parameters are appropriate for Northern ecotypes, but not for Southern ecotypes, which are evergreen.

* Bill doesn't like the term "validation."

Deliverables:

Ed enumerated the deliverables for this contract. Each of these, with discussion, is summarized below:

1) Any identified *2015 applied research* needed for any species including Texas wild-rice. We need to discuss and generate ASAP if we feel any specific parameters could really benefit from a more direct look in 2015.

Robert observed that it will be difficult to implement any kind of rigorous vegetation study and have results within only one year. Perhaps some studies validating growth/uptake kinetics for key species.

We need to supply a bullet list of potential topics. The team should be thinking about this. Jot down some ideas and provide to Ed or George by 17 January.

2) Subtask 3.3 (TWR) and Subtask 3.4 (Gill parasite) have internal team memos due by WSG (Thom Hardy) to the team on Feb. 3rd. Topics of the memos are thoughts on how to move forward with these 2 additional model components, or should we. Input from all to WSG prior to then should occur. Memos will go in the project notebook and then be summarized in the Draft Report.

Ed commented that recent work at the FWS Aquatic Resources Center (ARC) is indicating that the gill parasite may not be as important as first thought. Tim Bonner later clarified that the results indicate no relationship between fountain darter swimming ability and *C. formosanus* infection levels.

Thom will take responsibility for compiling first drafts of these memos. The team needs to provide rough notes to Thom by 24 January.

3) Task 4 - Recommendations for future work and 2014 contract scope of work. Input from all, compiled by Ward. Due to EAA no later than March 3rd.

These can be brief, in the format of contract statements of work. These will have to be vetted by the Science Committee before EAA can begin the task of formulating next year’s scope of work, so the deadline of delivery to EAA is firm. More discussion of this will take place in our team meeting of 5 February.

4) Task 5 - Draft 2013 report - Lead authors - TAMU fountain darter, ERDC aquatic vegetation, WSG will compile. Will include all 2013 activities (lit review overview, Subtask 3.3 and 3.4 memos summarized, etc.) Due to Ward by March 14th, Due to EAA by April 1st.

Ed envisions a relatively concise report of no more than about 25 pages. It is now time for the principal modelers to begin drafting their reports, along with prosecuting the technical work. Again, more discussion of this at the upcoming team meeting.

MEMORANDUM

FROM: George H. Ward, scribe
DATE: 17 February 2014
SUBJECT: Notes on Ecosystem Team meeting, 5 February 2014
Meadows Institute Offices, San Marcos

Attendance:

Thom Hardy	WSG	Bill Grant	TAMU
Tim Bonner	TSU	Rose Wang	TAMU
Todd Swannack	ERDC	Robert Doyle	Baylor
Ed Oborny	BIO-WEST	George Ward	UT

1. Scope and budget for 2014

The project team needs to submit a 2014 scope of work to EAA in early March in order to meet EAA review deadlines for Board approval in April. This is a separate document from the Annual Report and will need to include our projected plan moving forward in 2014 including Scope and Budget. There are two strategies of scoping that should be considered: (1) same budget as this year, i.e., a total of $170K, (2) what we'd really need to do the work. **Feedback on your individual tasks should include both scenarios and are due to George by 21 February.** Once compiled, Ed will get some additional information from EAA about which of these is more realistic.

If all goes perfectly, the EAA Board will approve our 2014 scope at their April meeting.

2. Fountain darter modeling

Bill and Rose reported on the present status of this effort. The test application for the Old River Channel of Comal is currently in the model. Depth & velocity at each cell have been implemented as a table look-up function, flow ranging 2 – 80 cfs with $\Delta = 1$. Can stop model and change flow rate and veg distribution. Now veg changes seasonally on annual cycle. Programming for fountain darters incorporated into code, so they are hardwired part of model. Temperatures are homogeneous and change hourly.

There was some discussion of whether to generate time series using gaussian model. Not critical path, can implement it easily now (one line of code with a function call) and re-examine later in the project.

Present darter model has only temperature as an external variable, though the framework is there for life cycle simulation. Need to add coding for the response of darters to vegetation.

The report on darter (and SAV) modeling should be concise but sufficient to show the large amount of work conducted to get to this point Summarize progress on the model. Copious bullets. Include a run of the darter model with (1) normal temperatures & flow, (2) drought temperatures & flows, as demonstration of capability, i.e., proof of concept.

Variables that should be included in the darter modeling:

Temperature
Movement (include in 2014 work)
Dissolved oxygen (from QUAL-2E model)
Turbidity
Food source (invertebrates)
Predation (include in 2014 work)
Vegetation (need to include structure and density)

Depth and velocity are needed but their effects are only indirect, through SAV

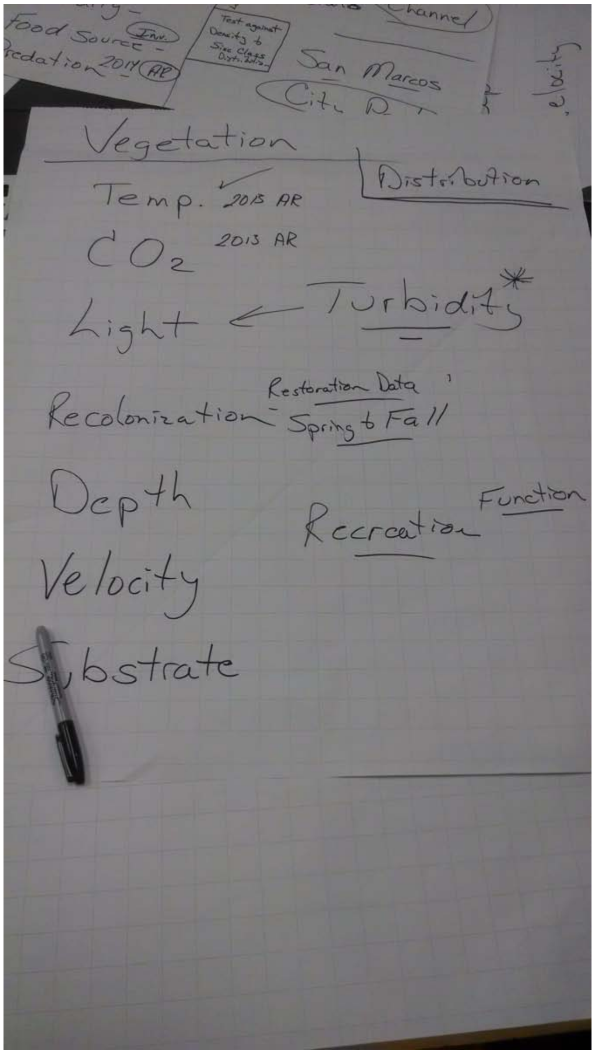
A description of each of these variables and their planned incorporation into the fountain darter model should be presented in the 2013 annual report.

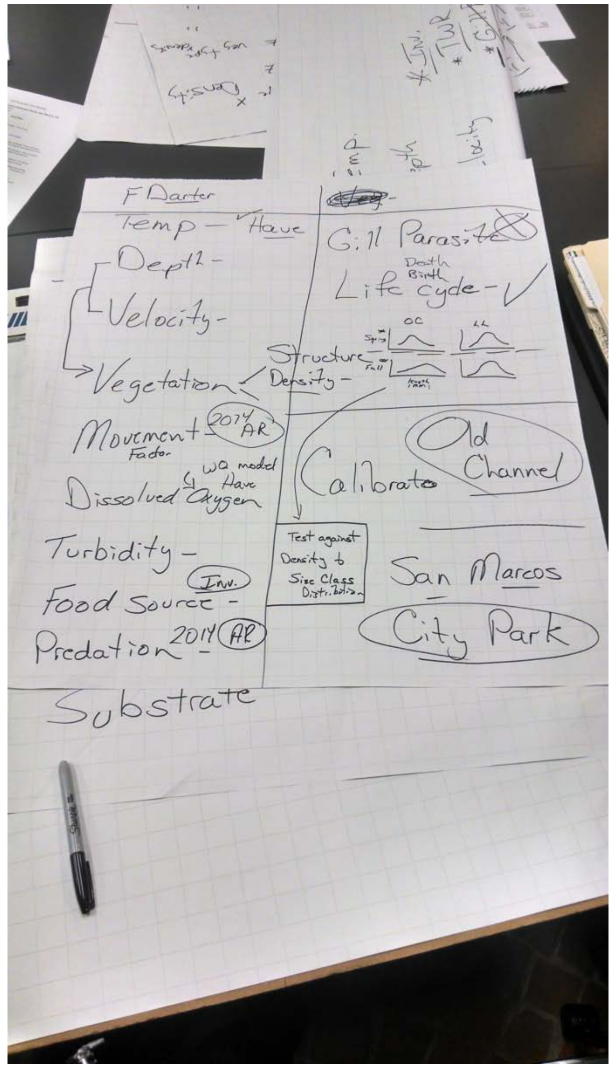
3. SAV modeling

Todd reported on the present status of this work. The Corps models are plant-specific but not spatially dependent. However, in both project streams, there are complex changes in vegetational distribution. Thinking now about developing an areal-coverage model. Maybe two separate models, one for areal coverage, the other for physiological responses. The later would employ some of the same functions of the Corps models, but scaled down and simplified.

Discussion about how to proceed. Ed suggests a model for each structural category, since structure may be the factor that dictates the response of darters to vegetation. Robert observed that the spatial variation within structural categories will vary with species. Some will blink out then reappear. Others, like *Sagittaria* and *Vallisneria*, are more or less permanent and coverage will expand and retract. Thom noted that storm or extreme temperature events, which are essentially stochastic, can knock vegetation back. Also recreation could be an annual function applied to reduction of coverage.

The decision was make to develop a simplified vegetation model w/ thresholds and recolonization functions, but incorporating stripped down versions of the Corps biomass growth & senescence. Three classes of vegetation are to be addressed at first, though the decision of what these classes would mean (structural categories, dominant species, etc.) is postponed for later consideration.





Variables that need to be included in vegetation model:

- Temperature
- CO2
- Light (including the effect of turbidity)
- Depth
- Velocity
- Substrate
- Recolonization after events (including a recreation function)

As for the fountain darter model, a description of each of these variables and their planned incorporation into the SAV model(s) should be presented in the 2013 annual report.

4. Additional modeling

Need to extend the darter model to include food sources. Gill parasite no longer appears that important, so consider removing it from consideration. Additionally, Thom mentioned a parallel effort on a parasite model in the Pacific northwest, that might be applicable should we determine to explore this variable down the road.

If we are going to address food for the darter, then we will need some kind of macroinvertebrate model. This should yield seasonal density of macroinverts by vegetation type.

Darters eat many things. Should we be spending resources to model a specific food component? Are there any data on maintenance levels of food requirement, e.g., energy units? Tim B. is working on this now. As an off-the-top estimate, he judges that darters need about 1/10 of the invert biomass available. A small project to refine this estimate would be useful.

Thom is studying physical impacts on sediment (human activities, recreation, dogs, etc.) and their effects on vegetation & inverts.

Riffle beetles pose a problem. We really don't know enough about these & their interaction with the substrate to effectively model. Ed thinks this is the organism likely to be impacted for flows less than 30 cfs. General consensus is to take riffle beetles off the table for the present.

In addition to the SAV modeling already underway, maybe we should consider a Texas Wild Rice model for next year. More interest was expressed in the processes that would have to be depicted in such a model. For example, the effects of suspended sediments in the water column on PAR.

5. Future work

The team agreed that it will be better to focus on two reaches in 2014, e.g. Old River in the Comal and City Park in the San Marcos, instead of seven, to better focus on testing and validation.

Potential separate research items for 2014:

(1) PAR as function of depth, turbidity & Canopy shading. Thom has been working with lidar-based ray tracing to estimate light impingent upon the City Park reach of San Marcos, so has some quantification of canopy effects.

(2) Feeding/caloric intake, mantenance energy (intake) requirements:
- (a) Back-of-envelope estimate based on literature on evacuation rates.
- (b) determine density of inverts
- (c) estimate fountain darter caloric intake requirement

(d) estimate community consumption of inverts by other fish
(e) estimate food availability of fountain darer compared to requirement

Potential 2015 topics for inclusion in HCP Applied Research:

(1) Relation between biomass (predicted by SAV model) and measurable parameters (e.g., % cover).

(2) Turbidity effect on other vegs (contingent upon 2014 Texas wild rice studies)

6. 2013 Annual Report

2013 Report - Lead authors - TAMU fountain darter, ERDC aquatic vegetation, WSG will compile. Will include all 2013 activities (lit review overview, Subtask 3.3 and 3.4 memos summarized, etc.)

Deadlines:
All sections to WSG – 3 March or sooner
Compiled draft to George – 14 March.
Draft to team for review – 21 March.
Comments to Ward – 28 March.
Final to EAA – 1 April.

7. Next Conference calls /Team meeting

2/26: Conference Call to discuss 2014 Scope and Budget and Annual Report progress: Wednesday 26 February at 2 pm. Ed will send out call information.

3/19: Conference Call to discuss Annual Report edits: Wednesday 19 March at 2 pm. Ed will send out call information.

3/26: Conference Call to discuss Annual Report final edits: Wednesday 26 March at 2 pm. Ed will send out call information.

4/23: Team Meeting at the Meadows Center work on model interaction and variable incorporation Wednesday 23 April (10am to 5pm)

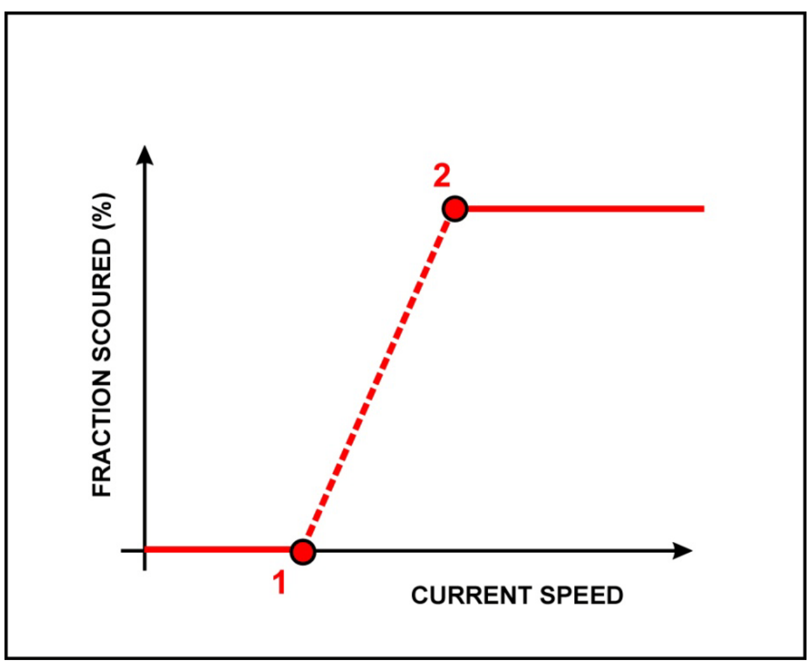
From: Ward, George H
Sent: Thursday, February 06, 2014 12:06 PM (e-mail)
To: 'Edmund Oborny'; Swannack, Todd M ERDC-EL-MS; Hardy, Thom; Doyle, Robert D.; Timothy Bonner; William Grant; hsuan006@neo.tamu.edu; Thomas Hardy; Todd S
Subject: Post script to yesterday re: veg modeling

Team –

During our discussion of SAV (a.k.a. veg) modeling yesterday I was quietly suffering a bout of indigestion, wondering if we were delicately avoiding the elephant in the room. I didn't want to interrupt our organized and productive discussions with my amateurish nattering, so now that it's relatively safe, would like to add this postscript.

Do we need to give more attention to the mechanics of scouring of SAV? It's sounding like these are the major events that completely alter plant distributions and dominance. Ed mentioned one instance in which the recovery after such an event entailed complete replacement of the previous veg by hydrophila. While we obviously cannot hope to predict such events in advance, if we could quantify the threshold of scour, we should be able to use our hydrological record to make some statements about frequency (return periods) of such major events, and mean times between events, which could be useful from a planning perspective.

Taking a simple-minded approach, at minimum for a given species we need to quantify (1) the current speed below which the plant is completely immune to being scoured and (2) the current speed at which it is gone, goodbye, schematically something like this:

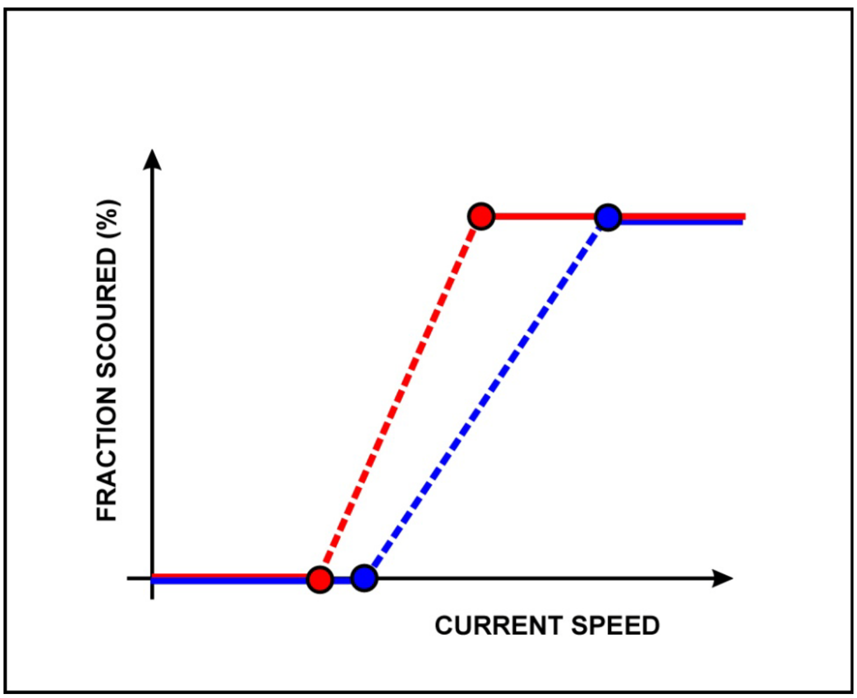


(NOTE to Thom. The real parameter would be shearing stress, which to first-order varies as the square of current speed. Because the relation is monotonic passing through the origin, we can scale the stress threshold to current speed, which is more desirable for management because it can be easily determined from flow.)

The transition from threshold 1 to threshold 2 is probably pretty complicated. For EAA purposes, however, we can probably get by with an approximate linear relation as shown by the broken line.

(NOTE to Thom. We probably expect this to behave analogously to the momentum impulse response in mechanics, in which the increment in momentum is the integral of the applied force over a short but finite time duration δt.   "Short" here is relative to the time scale of growth of theveg. Thus the scour response would be proportion to the mean current speed V times δt. A flood pulse over a short time would produce the same scour as a moderate flow sustained over a longer time. Ed noted one example when a moderate spring flow was maintained over several months due to high infiltration into the aquifer, achieving a scour of vegetation. We see the same phenomenon in the Colorado below Austin whenever releases are made to supply the rice farmers downstream. The resulting current is not hugely swift, as might be experienced during a flood event, but sustained over the 2-3 months of the irrigation season, the SAV's are cleaned out of the river channel.)

Several hypotheses might explain the re-growth post-event, e.g. from sheltered stocks upstream, from seed banks, or perhaps differential responses to the scour event, such as sketched here:



in which the blue species would have a re-growth advantage if the current is high enough to remove the red but leave at least a few percent of the blue intact.

My questions to you are:

(1) Is this something we should consider for additional study?
(2) We of course are not the only researchers to have encountered this, and there is a literature out there about scour of vegetation. From a quick Google a few minutes ago, a lot of it appears to address development after removal by scour, but there is probably some that addresses the scour resistance of species. Should we at least carry out a cursory literature review? Maybe there is some data out there for the same or similar species that we could exploit.
(3) Would it be of value to consider proposing some lab studies (in flumes, for instance) to better quantify this?
(4) From the mountain of data that has been accumulated from the two rivers, could we sort and stack by current speed, then analyze preceding flow data to draw some rough inferences of the threshold values?

--- george

MEMORANDUM

FROM:	George H. Ward, scribe
DATE:	27 February 2014
SUBJECT:	Notes on teleconference, 26 February 2013

Participants:

Ed Oborny	BIO-WEST	Bill Grant	TAMU
Thom Hardy*	WSG	Tim Lewis	ERDC
Rose Wang	TAMU	George Ward	UT
Tim Bonner	TSU		

*Thom had to sign off early because of a previous commitment

Objectives: Review status of reporting

1. 2014 Ecomodeling Scopes

The PI has received next-year scopes and cost estimates from TAMU, WSG and ERDC. Ed will be compiling scopes for Baylor, TSU, BIO-WEST, and administrative efforts. We are advised that we are not bound to the 1-year cost previously scheduled, due to the compression of the modeling time frame, but should determine exactly what each investigator needs to accomplish in the forthcoming year and estimate costs accordingly. Ward will use the scopes already submitted to prepare a skeleton for the scope/budget, which will then be modified as the revised texts become available.

Ed mentioned that EAA sent out an email soliciting 2015 applied research projects from the ecomodel team. The one study we previously agreed on was an aquatic vegetation biomass to percent cover field study. Ed mentioned that a one-page scope following EAA guidance is required for that proposal and due by March 17th.

Is there need for a scope item addressing amphipod modeling? The consensus was such a task is premature until the fountain darter modeling is further advanced.

Is there need for addressing scouring of vegetation, per Ward's e-mail of 6 February? Thom suggested some flume studies using planted vegetation, to measure the critical shearing stress. Tim Lewis indicated that the lab facilities are available at ERDC. Tim and Thom will draft a one-page proposal to be added to the scope.

The estimation of energetic requirements for the fountain darter and the available food will be undertaken by Tim Bonner, but will be handled separately through TSU's contract. Ed and Tim B. will work on language to incorporate into the 2014 scope.

2. Report drafts

TAMU has submitted a draft final report. ERDC has not yet. These need to be submitted to Thom by 3 March.

3. Report to NRC/NAS Review Committee

Ward briefly reported on his presentation to the NRC review committee. There are several modelers on the Committee that were hungry for details. He noted the need to allow some time in the cost estimates for responding to questions and/or comments from the NRC. At the same time, it will be important to shield the project team as much as possible from a diversion of time and effort.

4. Science Committee presentation

Today (26 Feb) Ed made a presentation to the Science Committee, essentially the same one made to the NRC and Implementing Committee earlier this month. Once of the members (Miguel Acevedo) had a lot of questions about the modeling: how are we addressing uncertainty, how will the models be calibrated, etc.

Actions:

ERDC report due to Thom by Monday, March 3. Please send to George and Ed as well.

Tim L. needs to prepare a first-draft of an experimental task to measure critical stress and send on to Thom. Then back to George by March 5.

Tim B. needs to prepare a paragraph description of a fountain darter food source evaluation and provide to Ed. Then back to George by March 5.

George needs to pull together the scopes received thusfar as a straw-man document and send to Ed by March 7. Ed will incorporate TSU, Baylor, BW and get back to George by March 10. George will distribute to the team soon thereafter for review. Submittal to EAA asap at that point.

Invoices due to Ed by Monday (March 3) noon. There are only 2 more (March and April) opportunities for submitting 2013 invoices.

MEMORANDUM

FROM: George H. Ward, scribe
DATE: 15 April 2014
SUBJECT: Notes on teleconference, 7 April 2013

Participants:

Ed Oborny	BIO-WEST	Robert Doyle	Baylor
Thom Hardy	WSG	Tim Lewis	ERDC
Bill Grant	TAMU	Todd Swannack	ERDC
Rose Wang	TAMU	Tim Bonner	TSU
Gary Dick	ERDC	George Ward	UT

Objectives: Status of project

1. Status of 2013 Interim Status Report, schedule, etc.

A draft of the subject report should now be in the hands of the participants. Your review is requested. Please provide comments to George and/or Ed by 16 April. We will incorporate comments and prepare a revised draft immediately.

2. 2014-2016 Ecomodel Scope & Budget

The original projected budget for this work was $175K/year. The sum of the estimated budgets for the next year of work was over twice this. For contractual purposes, EAA management hoped we might be able to compress the work to be completed by the end of 2016 (which would be commensurate with the anticipated budget estimates the team has already provided), so that a single contract extension could be issued.

Although the budget estimates were considered sound, there was discomfort among the team about accelerating the work to complete everything by the end of 2016. The goal would be to have an operating model with a user-friendly frontend whose operation could be easily communicated to EAA staff to make actual model runs. It has been noted (by EAA) that the groundwater modeling project was on a faster timeline and lesser budget.

There was concern whether two-and-a-half years was sufficient calendar time (as opposed to human time) to bring the model to that level of performance, including validation for all study reaches. It was observed that several research projects to provide input to the modeling would be reaching completion in the 3rd project year, which would be when the model would already have been validated.

The team discussed the many unknowns that would have to be resolved within such a period. It was also remarked that the groundwater modeling had not started from practically zero, as was

the case for ecomodeling, so the time frames for the two should not be comparable. It was agreed that at the end of the next year, the team should be in a much better position to project required modeling tasks and budgets to complete the work.

Ed will report back to EAA, and suggest that funding and contracting be handled year-to-year.

3. NAS/NRC Review and Meetings

The members should feel free to meet with the NRC committee as much as desired. However, if such meetings begin to represent an undue burden on team time, then George or Ed should be advised, and EAA will be contacted to determine the course of action.

While we are free to interact with the NRC committee, we are instructed not to provide any materials in writing to the committee without first clearing such transmittals with EAA.

The NRC committee has requested additional presentations from the Ecosystem Team on 12-13 May 2014, despite being advised that the team is just starting its work and the work has not advanced to the point that we much to communicate. Apparently, the committee is very interested in the present direction of the vegetation modeling and the fountain darter modeling, so it will be necessary that Bill Grant and Todd Swannack take the lead on these presentations. (It is likely that Thom Hardy will be needed as well, but he will be participating in these meetings for other topics so will be on hand. Similarly, Ed Oborny will already be at these meetings for other topics and will be available to assist if needed.)

4. Next Ecomodeling Team meeting

The next internal project team meeting is scheduled at 1000 on 23 April. It has been moved to the BIO-WEST offices in Round Rock.

5. Other business

Bill is preparing a paper on fountain darter modeling (water quality / land use interactions) but based upon material available from the open literature. No material from the HCP projects is used nor will be cited. He asked the team's opinion as to whether such a paper from a member of the modeling team would be an issue with EAA. It was the judgment of the Team that this is clearly external to the present HCP work, and that he should feel free to proceed.

MEMORANDUM

FROM: George H. Ward, scribe
DATE: 28 April 2014
SUBJECT: Notes on Ecosystem Team meeting, 23 April 2014
BIO-WEST offices, Round Rock

Attendance:

Bill Grant	TAMU	Robert Doyle	Baylor
Rose Wang	TAMU	Todd Swannack	ERDC
Ed Oborny	BIO-WEST	George Ward	UT

Agenda:

(1) Discuss NRC interactions (Oborny)
(2) Go over the Year 2 scope of work and assignments - Who is doing what, when, etc. (Oborny)
(3) Finalize the 2013 Ecomodel Report (Ward)
(4) Discuss available biological data for model parameterization - both fountain darter and aquatic veg (Oborny / Hardy)
(5) Discuss biomass to percent cover study (Doyle)
(6) Discuss ecological relationships (All)

1. NRC Interactions

Earlier Bill, Rose and Ed had an extended conversation with Dr. Kenneth Rose (NRC) concerning the background and procedure for the fountain darter modeling. This conversation made it clear that at some point the team needs to address the background for the project, distinction between Phase I and Phase II of the HCP, data collection over the years, and the information that is on hand. Ultimately this will constitute the early chapters in the formal project report, but given the NRC review, an early production of a background document seems appropriate. Ed was given the task of producing a first draft.

Ed reiterated the EAA desire that the team interact as much as necessary (and desired) with the NRC, but not transmit anything in writing without the prior approval of the Authority.

2. Year 2 Scope

Ed went over the Year 2 Scope and assignments. At this point, three of the four approvals necessary have been secured. The fourth step is for the EAA board to formally approve at their May 13th board meeting. Ed also provided an update of the desire of EAA to compress the work into a single contract ending in 2016 at which time the team will produce a model "product"

capable of operation by EAA personnel. It was acknowledged that the team believes that such a short delivery date may result in a model that is incomplete in some respects, but Ed believes that EAA will be willing to work with the team to further advance the model formulation and performance after this period.

Ed is in the process of compiling the Team Notebook from e-mails and memos on meetings and teleconferences (such as the present missive). There is no specific delivery date for this document, but it will be maintained for inspection by EAA at any time, and for transmittal at the conclusion of the contract.

3. Finalize Ecomodel Report

The team went through the draft Interim Status Report page-by-page and identified additional text and figures, particularly in model description, model platform, etc., that will be supplied by Bill Grant and Todd Swannack. Todd will provide specific citations from *Ecological Modelling* and a revised conceptual diagram. Bill will add the Grimm et al. citation and text explaining the IBM protocols (which arose during the conversation with Dr. Rose). These pieces will be provided to George and Ed by Monday, and the final draft assembled for delivery to EAA on 30 April.

Ward reiterated his suggestion made at the outset of the project that we generate the report as we work, in the form of rough internal documents (like Appendix A and Appendix B for the fountain darter work) and technical memorandums. This will greatly ease the end of project panic to throw together a report, and will produce a generally higher quality document.

4. Biological data for modeling

It has become apparent that many of the team is not familiar with the biological collections that have been made over the years. In order to better exploit this reservoir of data, Ed undertook an overview of the programs and the data that they have produced. This led to an extended discussion of the differences between the two rivers, the character of the sampled reaches, representativeness of the reaches vis-à-vis vegetation communities. Some of the information Ed showed has not yet made its way into reports to EAA, so Ed is going to put this in an internal memo for distribution to the team.

5. Vegetation modeling

An extended discussion took place among the team, notably Robert and Todd, about the proposed research on incorporating the upcoming studies of the relation of plant structure and percent cover, which is observed in the field, to biomass, which is the product of the model.

6. Fountain darter modeling

There was a wide-ranging discussion among the participants on this topic, and the integration with the SAV model. One suggestion that Dr. Rose had made was to code up our own model, using FORTRAN as the language, to improve on the running time of NETLOGO. The team remarked that once we start coding our own models, this project could take years. Furthermore, there is no indication at this time that NETLOGO running time is a limitation on the modeling. If it becomes so, then we can aggregate the spatial grid to compensate. (The present 0.25 m x 0.25 m grid is far more resolved than is likely warranted.) A user interface with sliding-bar inputs could be easily created as a NETLOG front-end.

7. Other business

Since this is the last month under the present authorization, it is important that all invoices be submitted to Ed ASAP.

No date was set for the next team meeting. It may be desirable, however, for a meeting among the fish modelers to ensure that all the necessary data has been transmitted to these members of the team.

MEMORANDUM

FROM: George H. Ward, scribe
DATE: 11 July 2014
SUBJECT: Notes on Ecosystem Team meeting, 1000 2 July 2014 CDT
BIO-WEST offices, Round Rock

Attendance:

Bill Grant	TAMU	Robert Doyle	Baylor
Rose Wang	TAMU	Todd Swannack	ERDC
Ed Oborny	BIO-WEST	George Ward	UT

Tim Bonner in afternoon

Agenda:

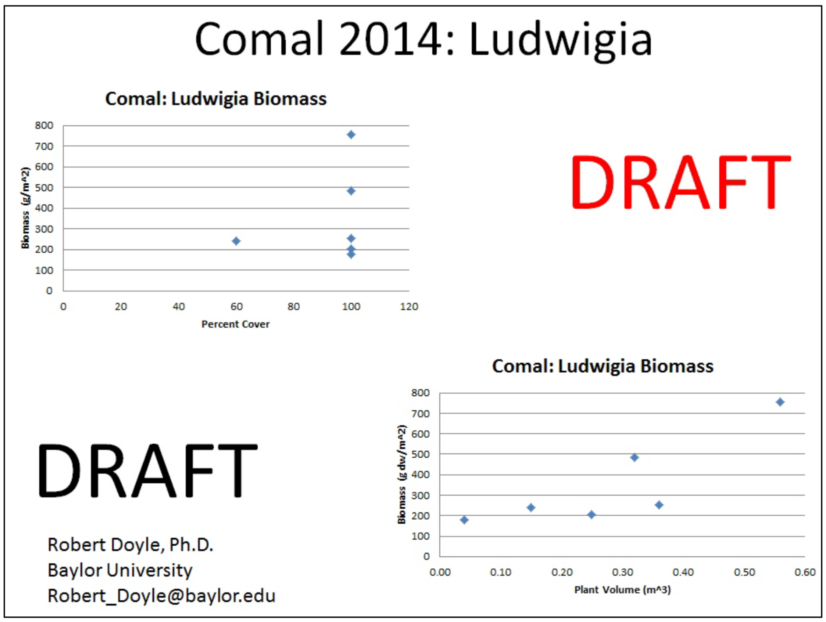
10:00	Meet and greet – Bill: South American stories and continued discussion of the spelling of Todd's last name.
10:15	Robert: update on the plant/biomass field study currently underway.
10:45	Todd: informal presentation on the status of the SAV
12:00	Repair to Schlotsky's for lunch (thank you, Bio-West)
1:00	Rose and Bill: informal presentation on the fountain darter data mining and the current status of the fountain darter model in the Old Channel.
2:30	Discussions on parameterization, relationships, interaction between SAV and fountain darters, etc. Steps forward, assignments, next meeting.

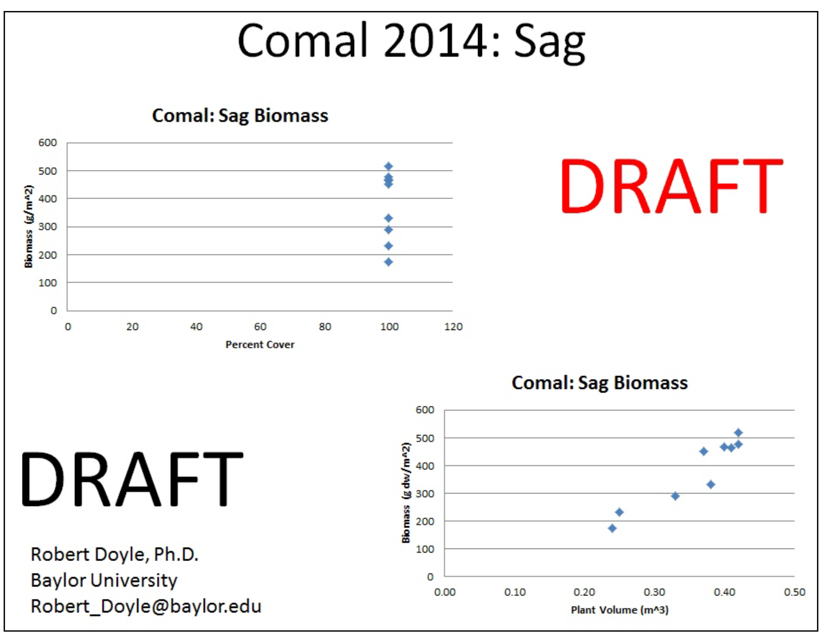
1. Field studies of biomass/coverage relations

Two field trips to Comal & San Marcos Rivers during June. Several protocols for SAV sampling were tried, e.g. cataloging rosettes, tangles, etc. Ultimately a method for sampling plant biomass, above and below ground, was devised using a large porous frame to compress the vegetation to the bed, followed by extraction of a central core as deep into the substrate as necessary to encompass the root zone.

This method yields a direct measure of biomass per unit area. The samples so obtained are still in the drying ovens, but Robert is optimistic that this is the way to go.

Preliminary measures of biomass evidence a large scatter as a function of fractional coverage (as a percent). Robert has discovered that for some of these plant species, there is an additional dependency on average plant height (estimated as total depth – depth to canopy, both of which are routinely measured in drop net surveys). In the figures below, plant height is multiplied by area sampled to get plant volume.





Robert will be departing for the Amazon in the following week, but his students have their instructions on how to proceed in his absence.

As part of this project, Sarah, one of Robert's grad students, is carrying out a literature review on response of Comal/ San Marcos SAVs to bed scour. This is the initial step of one of our research tasks to examine bed stress and critical stress for incipient scour. Based on this initial lit review, a more extensive literature evaluation will be conducted by the team this fall, so we can await the student's work and supplement it, if necessary. The team is starting the planned statistical analysis of field data coupling observations of large-sale SAV scour and re-growth with hydrology and other forcing factors (including recreation).

2. SAV Modeling

Todd reports good progress now that he has backed off to a monospecific code. He is combining a "megaplant" conceptual model (Scheffer, 1993) with some of the USCE kinetic/ growth relations. This is presently coded in NETLOGO with NLS files. Todd is also doing some preliminary work on dispersal and how to code that into the model. He is experimenting with assigning an attribute "native" versus "invasive" with an associated probability distribution. Robert suggested consulting the work of Madsen at USCE WES on plant dispersal (e.g., Madsen, 1997, 1999).

3. Darter data mining and modeling

Bill and Rose provided a written summary of their work thus far (attached as Appendix A). This is summarized as follows:

- We spent most of our time re-organizing the June data. We would like to suggest a new format for recording the field data.
- We only tested the information based on the spring data set, the fall data set, and the combined data set. We did not take into account some potentially important events that occurred during the 14-year period covered by these data (e.g., scouring or drought). There were some similar correlations in our three analyses that could prove useful during model evaluation (assuming these preliminary results do not change as a result of more thorough analyses).
- There also were some inconsistences in our preliminary results (all correlations were positive based on the spring data set, whereas some correlations were positive but others were negative based on the fall data set), perhaps due to the failure to incorporate the important events such as scouring and drought.
- We plan to pursue using time series analysis to understand the data well.
- We plan to incorporate the water quality data to understand the relationship between fountain dater density and biotic (vegetation types) and abiotic (water quality) variables.
- We are currently (very early) in the process of reprogramming the fountain darter model in C++ to allow faster execution of the model which will be needed to perform the

relatively many simulations during sensitivity analyses (as suggested by Dr. Kenneth Rose of the National Academy of Science review team).

Much of Rose's efforts in June have been directed toward re-formatting of the massive data holdings from Comal/San Marcos Rivers to facilitate various kinds of statistical analyses as well as forming inputs to the model. She posed several questions to the team that have grown out of this work. Notably, the velocity measurements are made at a depth of 15 cm. There was some discussion of the current speeds immediately off the bed surface. Though these are sensibly nil, some of the team argue that there would be a small current here (driven by the water-column current speed). This would of course be incapable of sensing by the equipment used in the field measurement program.

She also inquired about where in the water column the darters are found. Tim observed that darters do not have a swim bladder so they tend to be benthic, being concentrated at or just above the bottom, though occasional individuals are found resting on plant structures within the water column. Robert suggested looking at reports done by Dibble at WES addressing plant architecture and its parameterization (Dibble et al., 1997a, 1997b). This might be useful in quantifying darter dependence on plant structure. Ed remarked that the high 2001-02 fountain darter densities in the Old Channel study site that Rose was describing in the memo were in association with very high filamentous algae (species uncertain), which have coarse thick green streamers packed with amphipods (they crawl up your arm!). The food and cover (protection) provided by this "good" algae resulted in large densities of darters being found within. It was emphasized that this filamentous algae should not be confused with the slimy bright green algae that covers other parts of the system (most notably the Upper Spring Run reach) during hot, summertime periods. Tim opines that darter use of vegetation is highly associated with structure. Ed noted that when St Augustine is inundated by higher flows extending into people's lawns, it's full of amphipods and darters. The fish hatchery even uses artificial turf for habitat. Ward: There must be some other factor at work, evidenced by the different darter densities in Ludwigia (native) versus Hygrophila (nonnative), though their structures are practically identical.

To address some of Rose's concerns, Ed summarized briefly the protocols in data collection and field data entry. First the reach is mapped. Then based on veg density, random sites are picked within each dominant veg category. The meaningful distinction in field sites is therefore veg type. The protocol is to always perform six (6) drop net samples in the Old Channel study reach, regardless of veg types, even when there are not three dominant veg categories. However, "algae" in the field notes always refers to the bright-green periphyton that covers plant structure during summer, not the massive filamentous algae that was present and sampled in the early 2000's. It was decided that a memo from Ed summarizing the field data collection protocols, especially for drop-net sampling, would be useful.

4. Action items

1) Todd – send Mega Plant paper to all
2) Sarah (BU) – SAV scour lit review
3) Sarah (BU) – work up 2nd set of cover/biomass samples
4) BW & WSG – Flood vs. veg coverage analysis
5) Robert – send 98 flood info to BW
6) BU Start Ludwigia study (later in August/September)
7) BW Drop net sampling memo to team
8) BW presence/absence data to Rose (TAMU)
9) TSU / BW – Food availability analysis
10) Ed forward to George – email from Kenny Rose summary

5. Next meetings

Two limited meetings with TSU/BW/TAMU in College Station between now and August 15th. Primary attendance will be those involved in darter data mining (though anyone is welcome). Dates and times to be determined.

The next full team meeting is set for Wednesday 27 August at BIO-WEST - Round Rock. Tentative agenda:

BU – update on field studies and lit review
TSU – update on food availability
BW/ WSG – update on scour/veg analysis
TAMU – statistics update and FD model presentation
ERDC – Veg Model demo – show and tell

References

Dibble, E., K. Killgore, and S. Harrel, 1997a: *Assessment of fish-plant interactions*. Misc. Pap. A-97-6, Aquatic Plant Control Research Program, Waterways Experiment Station, U.S. Corps of Engineers, Vicksburg.

Dibble, E., K. Killgore, and G. Dick, 1997b: *Measurement of plant architecture in seven aquatic plants*. Misc. Pap. A-97-7, Aquatic Plant Control Research Program, Waterways Experiment Station, U.S. Corps of Engineers, Vicksburg.

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APPENDIX FROM BILL GRANT AND ROSE WANG

Brief outline of what we have done in June, 2014

- We examined all of the files from Ed and decided to re-organize them so that we could use the information for our present purposes more efficiently. That is, we reformatted the data to facilitate informal searches for trends of interest and, particularly, to facilitate data management associated with statistical analyses.

- After reformatting, we first focused our attention on the drop net data with the goal of relating fountain darter density to aquatic vegetation at each site (which usually has a major aquatic vegetation type identified) in each reach (CP, I35, LL, NCR, OCR, SLD, and USR) in each season (spring, summer, and fall) in each year (2000-2014). (Depending on the results of these analyses, these data almost surely will provide valuable information for model evaluation and, perhaps, also for parameterizing portions of the model, most likely, the darter movement sub-model.

- We queried the information from "dartersup2date_May2014.mdb." Specifically, we merged four tables (Darters, SiteCodes, Site, and WaterQuality) into one table (named "QueryDarter_SiteCodes_Site_WaterQ") based on site codes.

- We narrowed our initial analyses to the relationship between darters and aquatic vegetation in June, hence we used only part of the above queried table, including information from tables of "Darters," "SiteCodes," and "Site". We will analyze other months, and also the water quality portion of these data, in the near future.

- We extracted information from "QueryDarter_SiteCodes_Site_WaterQ.xlsx" and created "2014JuneAnalyses.xlsx." There are several sheets in "2014JuneAnalyses.xlsx." – The first one includes "year," "survey date," "reach," and "the numbers of fountain darters in each survey site." (We have some questions regarding the presence and absence of the different vegetation types in different samples.) We have not yet completed this sheet, although we have completed the old channel part for June. The format is as follows:

Year	Date	Reach	A1	A2	C1	C2	CT1	CT2	H1	…
2000	31-Oct	CP	0	0	0	0	0	0	7	…
2000	1-Nov	I-35	0	0	3	7	0	0	0	…

- We also are separating the above data table into specific reaches to analyze the potential effect of the different biotic and/or abiotic factors in each reach on the relationship of fountain darter and aquatic vegetation. Once again, we have not yet completed this sheet, but we have completed the old channel part for June. The format is as follows:

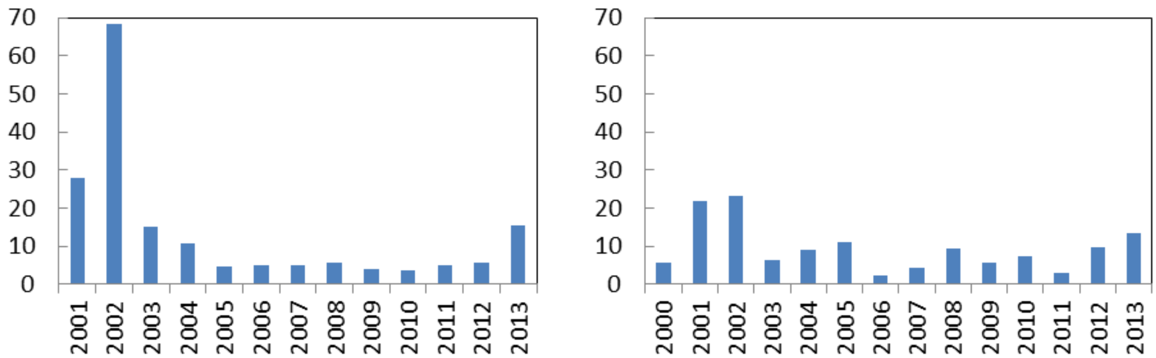
Year	Date	A1	A2	C1	C2	CT1	CT2	H1	…
2000	31-Aug	11	64	0	0	0	0	0	…
2000	13-Sep	53	85	0	0	3	0	0	…

- As we were organizing the above information, we noticed that even though some site codes are the same (e.g. A1 or H1), sites with the same code could have noticeably different proportions of the different aquatic vegetation types. Hence, we created seven sheets, one based on each of the seven reaches, containing more detailed information including "year," "month," ""FD number," "Veg Height," "Algae," "Bryophytes," "Ceratopteris," and so forth. As above, we have not yet completed this sheet, but we have completed the old channel part for June. The format is as follows:

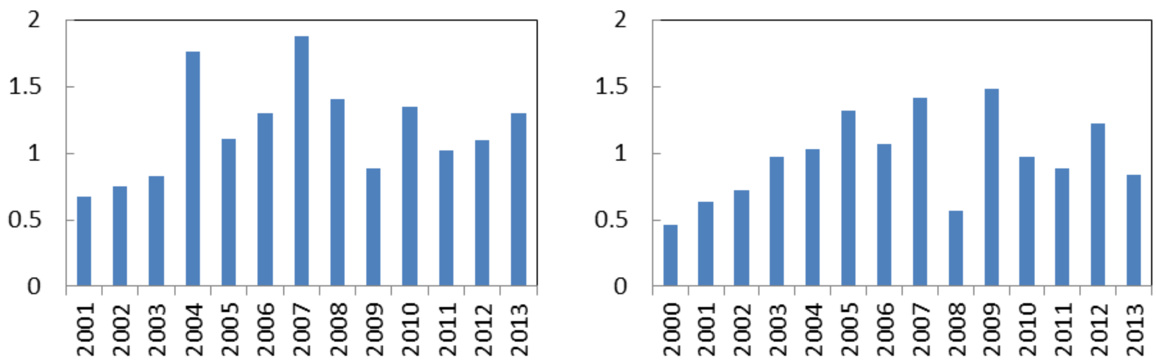
Year	Month	FD number	Veg Height	Algae	Bryophytes	Ceratopteris	Open	…
2000	8	11	0.32	0.2	0.8	0	0	…
2000	8	64	0.32	0.5	0	0	0.5	…

- We would like to know the precise definition of the variable in each column in the "water quality" table.

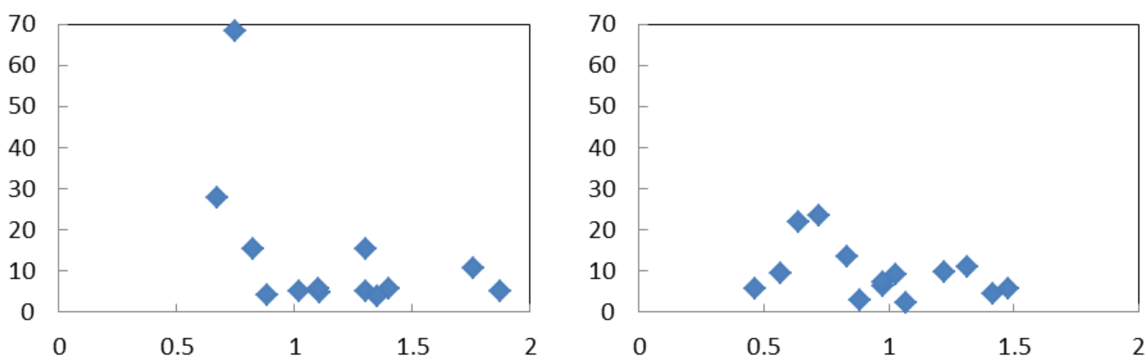
Average densities of fountain darters in a site during spring (on left) and fall (on right) from 2001/2000 to 2013:



Average vegetation height in the same site during spring (on left) and fall (on right) from 2001/2000 to 2013:



Based on the above information, we tried to develop the relationship between fountain darter density (y axis) and vegetation height (x axis). We found the relationship is negative but weak (R^2<0.4).



We tried to find the relationship between average density of fountain darter (1) during spring, (2) during fall, and (3) throughout the year (spring, summer, and fall) and the proportional coverage of several aquatic vegetation types via stepwise linear regression:

Spring data set

(Ludwigia and Raccia omitted in the regression model because of collinearity.)

The first step:

Source	SS	df	MS
Model	38557.3099	7	5508.18713
Residual	52609.3654	69	762.454571
Total	91166.6753	76	1199.56152

Number of obs	=	77
F(7, 69)	=	7.22
Prob > F	=	0.0000
R-squared	=	0.4229
Adj R-squared	=	0.3644
Root MSE	=	27.613

FDnumber	Coef.	Std. Err.	t	P>|t|	Beta
Algae	166.8552	285.8301	0.58	0.561	1.652798
Bryophytes	122.3565	286.1299	0.43	0.670	.5675198
Ceratopteris	110.1488	288.2202	0.38	0.704	.9509384
Open	86.17313	285.9443	0.30	0.764	.561669
Filamentousalgae	143.5708	329.6494	0.44	0.665	.0788057
Hygrophila	101.9979	285.5187	0.36	0.722	1.365846
Ludwigia	100.9891	285.5685	0.35	0.725	1.029014
_cons	-95.40701	285.4728	-0.33	0.739	.

The last step:

note: Raccia omitted because of collinearity

Source	SS	df	MS
Model	36241.0837	1	36241.0837
Residual	54925.5916	75	732.341222
Total	91166.6753	76	1199.56152

Number of obs	=	77
F(1, 75)	=	49.49
Prob > F	=	0.0000
R-squared	=	0.3975
Adj R-squared	=	0.3895
Root MSE	=	27.062

FDnumber	Coef.	Std. Err.	t	P>|t|	Beta
Algae	63.6506	9.048127	7.03	0.000	.6304963
_cons	6.164223	3.398429	1.81	0.074	.

These preliminary results showed that (1) coverage of most of the aquatic vegetation types contribute positively to fountain darter density (1st one: algae, 2nd: Filamentous algae, 3rd: Bryophytes) even though these variables were not statically significant and (2) the final model consisted of only one statistically significant variable "algae," however, this variable had relatively high explanatory power. Thus these results indicated the importance of the proportional cover of algae in explaining fountain darter density.

Fall data set:

(Bryophytes, Filamentous algae, Nuphar and Raccia omitted in the regression model because of collinearity.)
The first step:

Source	SS	df	MS
Model	3773.65111	6	628.941852
Residual	9307.69136	66	141.025627
Total	13081.3425	72	181.685312

Number of obs =	73
F(6, 66) =	4.46
Prob > F =	0.0008
R-squared =	0.2885
Adj R-squared =	0.2238
Root MSE =	11.875

FDnumber	Coef.	Std. Err.	t	P>|t|	[95% Conf.	Interval]
VegHeight	-1.132109	3.360313	-0.34	0.737	-7.84119	5.576973
Algae	.6388232	7.941462	0.08	0.936	-15.21682	16.49446
Bryophytes	0	(omitted)				
Ceratopteris	-18.03116	10.00027	-1.80	0.076	-37.99734	1.935018
Open	-12.94066	9.198177	-1.41	0.164	-31.30542	5.424088
Filamentousalgae	0	(omitted)				
Nuphar	0	(omitted)				
Hygrophila	-15.49941	8.528307	-1.82	0.074	-32.52673	1.527901
Ludwigia	-20.08414	8.013712	-2.51	0.015	-36.08403	-4.084247
Raccia	0	(omitted)				
_cons	23.95916	6.903635	3.47	0.001	10.17561	37.74271

The last step:

Source	SS	df	MS
Model	3353.99818	3	1117.99939
Residual	9727.34429	69	140.976004
Total	13081.3425	72	181.685312

Number of obs =	73
F(3, 69) =	7.93
Prob > F =	0.0001
R-squared =	0.2564
Adj R-squared =	0.2241
Root MSE =	11.873

FDnumber	Coef.	Std. Err.	t	P>|t|	[95% Conf.	Interval]
Ceratopteris	-17.00458	5.840746	-2.91	0.005	-28.65655	-5.352611
Filamentousalgae	0	(omitted)				
Nuphar	0	(omitted)				
Hygrophila	-13.27928	3.567018	-3.72	0.000	-20.39529	-6.163278
Ludwigia	-18.00692	4.201995	-4.29	0.000	-26.38967	-9.624168
Raccia	0	(omitted)				
_cons	19.94263	2.475601	8.06	0.000	15.00395	24.88132

The R^2 was relatively low. However, algae was the only factor positively correlated (not statistically significant) with fountain darter density based on the results of the 1^{st} step. Some aquatic vegetation types (Ceratopteris, Hygrophila and Ludwigia) were negatively correlated (statistically significant) with fountain darter density. This finding was different from the previous one which showed all vegetation types were positively correlated with fountain darter density.

Combined data set (spring, summer, and fall data sets)
The first step:

Source	SS	df	MS
Model	51040.9768	10	5104.09768
Residual	85129.0875	191	445.702029
Total	136170.064	201	677.463007

Number of obs =	202
F(10, 191) =	11.45
Prob > F =	0.0000
R-squared =	0.3748
Adj R-squared =	0.3421
Root MSE =	21.112

FDnumber	Coef.	Std. Err.	t	P>|t|	[95% Conf.	Interval]
VegHeight	.1026602	3.465649	0.03	0.976	-6.733202	6.938522
Algae	24.75734	42.66542	0.58	0.562	-59.39857	108.9133
Bryophytes	-3.882688	43.53501	-0.09	0.929	-89.75384	81.98847
Ceratopteris	-17.07819	43.10098	-0.40	0.692	-102.0932	67.93686
Open	-28.55226	43.83459	-0.65	0.516	-115.0143	57.9098
Filamentousalgae	3.763401	134.755	0.03	0.978	-262.0358	269.5626
Nuphar	-70.03505	218.8681	-0.32	0.749	-501.7441	361.674
Hygrophila	-19.17428	42.88323	-0.45	0.655	-103.7598	65.41126
Ludwigia	-21.98806	42.78482	-0.51	0.608	-106.3795	62.40336
Raccia	116.5712	427.514	0.27	0.785	-726.684	959.8263
_cons	26.25355	42.64877	0.62	0.539	-57.86953	110.3766

The last step:

Source	SS	df	MS
Model	48160.639	1	48160.639
Residual	88009.4254	200	440.047127
Total	136170.064	201	677.463007

Number of obs =	202
F(1, 200) =	109.44
Prob > F =	0.0000
R-squared =	0.3537
Adj R-squared =	0.3504
Root MSE =	20.977

FDnumber	Coef.	Std. Err.	t	P>|t|	[95% Conf.	Interval]
Algae	43.47057	4.155266	10.46	0.000	35.27682	51.66433
_cons	6.266893	1.684836	3.72	0.000	2.944571	9.589214

These preliminary results showed that (1) most aquatic vegetation types were correlated (either positively or negatively) with fountain darter density (this result was different from the one based on the spring data set) and (2) the final model consisted of only one statistically significant variable “algae,” however, this variable had relatively high explanatory power. Thus these results indicated the importance of the proportional cover of algae in explaining fountain darter density (this result is the same as the one based on the spring data set).

MEMORANDUM

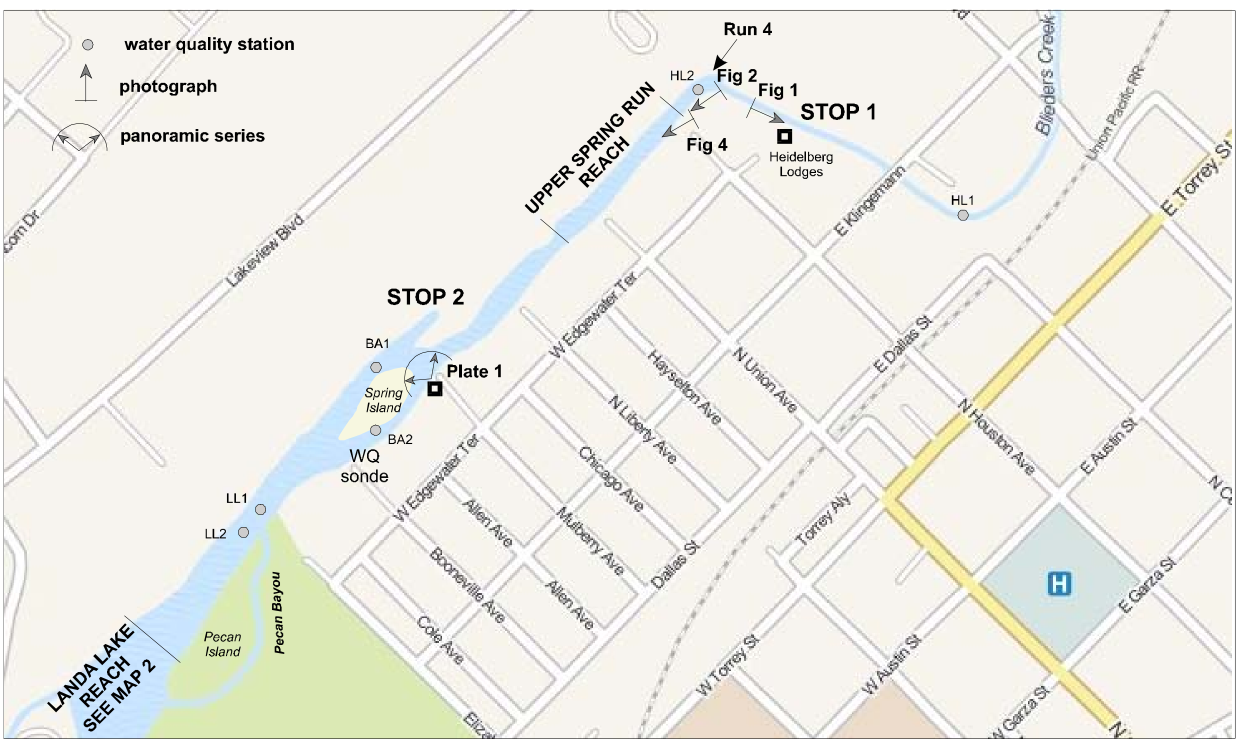
FROM: George H. Ward, scribe, with much input from Ed Oborny
DATE: 31 August 2014
SUBJECT: Notes on Darter Modeling Team field trip to Comal River and springs, 7 Aug 2014 CDT

Attendance:

Bill Grant	TAMU	Ed Oborny	BIO-WEST
Rose Wang	TAMU	George Ward	UT

Ed Oborny presented a guided tour of the Comal spring system with special emphasis on those aspects immediately pertinent to the fountain darter modeling effort.

The field trip began at Blieders Creek, the upstreammost point of the Comal study area, see Map 1, Figs. 1-2. No perceptible streamflow here (Fig 1). Ed pointed out *Cabomba* and other aquatic macrophytes occupying areas of Blieders Creek. The first upwellings begin at the confluence of the Upper Spring Run and Blieders Creek, visible as intermittent streams of bubbles. There was no flow in Upper Spring Run 5 (Fig. 2). The Upper Spring Run reach (Fig. 3) was relatively clear of vegetation due to summer swimmers, but revegetates during the winter. We came upon the BIO-WEST field crew engaged in collection of fountain darters (Fig. 4), and observed the



Map 1 - Blieders Creek and upper reach of Landa Lake

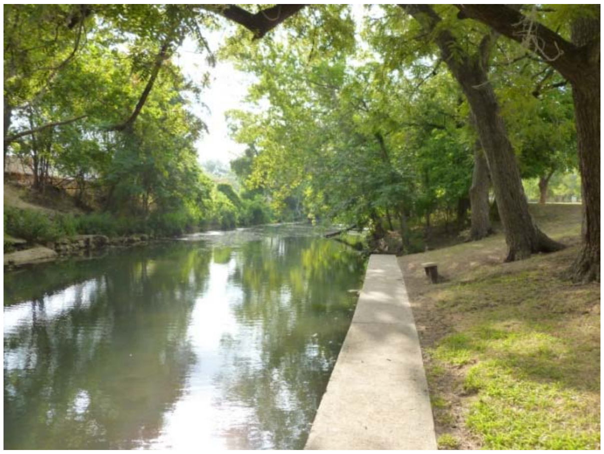


Fig 1 - Blieders Creek

Fig 2 - Spring Run 5

Fig 3 - Upstream from Upper Spring Run Reach

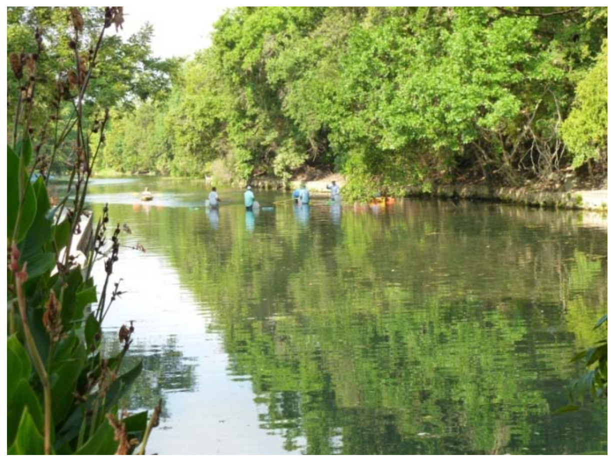


Fig 4 - Sampling underway in Upper Spring Run Reach

fluorescent tagging of darters. The modeling team inspected the Upper Spring Run Reach, one of the reaches ultimately to be modeled.

The next stop (Stop 2) was at the upstream end of Spring Island, see Map 1 and Plate 1, where we examined both reaches around the island. The team was shown the locations of the recording thermistors. There is much upwelling spring flow in this reach, and darters and endangered inverts are plentiful. *Sagittaria* is prevalent on the north side of the island. There is a high density of bryophytes interspersed among the aquatic vegetation (Fig. 5). There were also dense growths of the algae that coats the aquatics during the summer. Spring Run 6 was not flowing, though there was subsurface seepage (Fig. 6). This reach is productive. Dissolved oxygen (DO) rises to around 13 ppm at midday, though the concentration in spring water is only around 4.

Fig 5 - Bryophytes

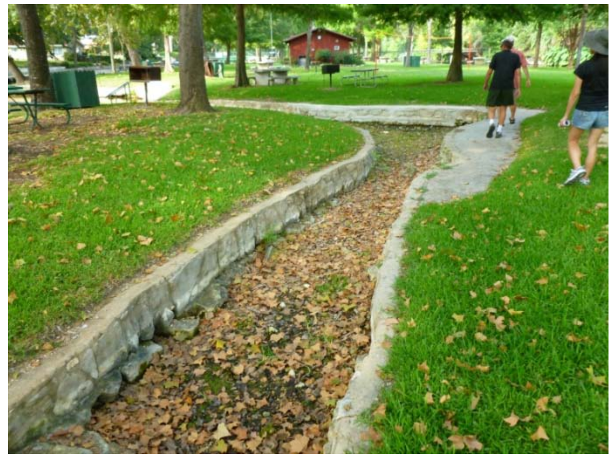


Fig 6 - Spring Run 6

Map 2 – Landa Lake Reach and Landa Park

Stop 3 (Map 2) was on the golf course overlooking the Landa Lake Reach. There was a lot of *Cabomba* evident in Pecan Bayou with most of it flowering at the time of our tour. Ed summarized the drainage changes underway at the golf course and pointed out the old and new outlets from Landa Lake into the old river channel. The old outlet structure at Landa Lake and at the headwater of the Old Channel are shown in Figs. 7 and 8, resp. The new outlets, “Large Culvert” (east), and the small culvert into the springfed pool (west), separated by about 50 ft, are shown in Figs. 9 and 10, resp. These outlets supplemented the old outlet starting in 2004. (The old outlet was capped earlier this year.) The discharge from Landa Lake into the headwater of the old channel, Figs. 11 and 12, was estimated by Ed to be about 45 cfs. This is controlled by the east (Main) outlet (Fig. 9), a manual gate, which is set by adjusting the gate height while discharge measurements are made downstream in the old channel, the target flow being 50 cfs. The west outlet supplies water to the springfed pool, and has a capacity of about 5 cfs. The discharge from the pool conflows with the old channel downstream.

Fig 7 - Old (closed) outlet from Landa Lake

Fig 8 - Old outlet in headwater of old channel

Fig 9 - New east (Main) outlet from Landa Lake

Fig 10 - New west (springfed pool) outlet from Lake

Fig 11 - Discharge into old channel

Fig 12 - Headwater of old channel

We walked downstream to the first bend, where sedimentation had built an island (subsequently removed in April 2013). This reach is a fast-flowing stream with natural tree canopy. Here there is a cut bank on the convex side of the bend (left descending bank), slated for stabilization. Figs 13 and 14 show some of the vegetation in this reach. Both *Hygrophila* and *Ludwigia* are present here, with the former nearly completely restored with the latter at this time. Much restoration work has been carried out through this reach, and it represents excellent darter habitat.

Fig 13 - *Cabomba* in old channel

Fig 14 - Restored *Potamogeton* (green) and *Ludwigia* (red) in old channel

Later in the day, we worked our way around to the opposite side of Landa Lake, Stop 6 (Map 2), where we were able to examine several of the major springs. Clearly, the drought continues, as there was practically no flow from these major orifices and spring runs, apart from some lateral seeps (Figs 15-18). From here we observed the Landa Lake study reach from the north shore of the lake (Figs. 19-20, Plate 3).

Fig 15 - Spring Run 1

Fig 16 - Spring Run 1 headwaters

Fig 17 - Panther Canyon

Fig 18 - Major orifice in Spring Run 1

Fig 19 - Vallisneria in Landa Lake

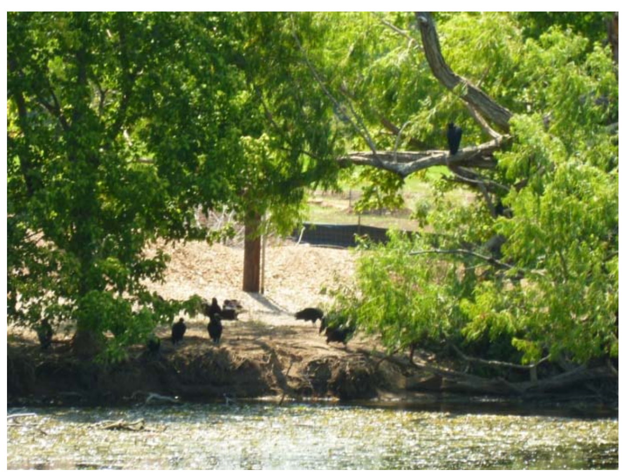
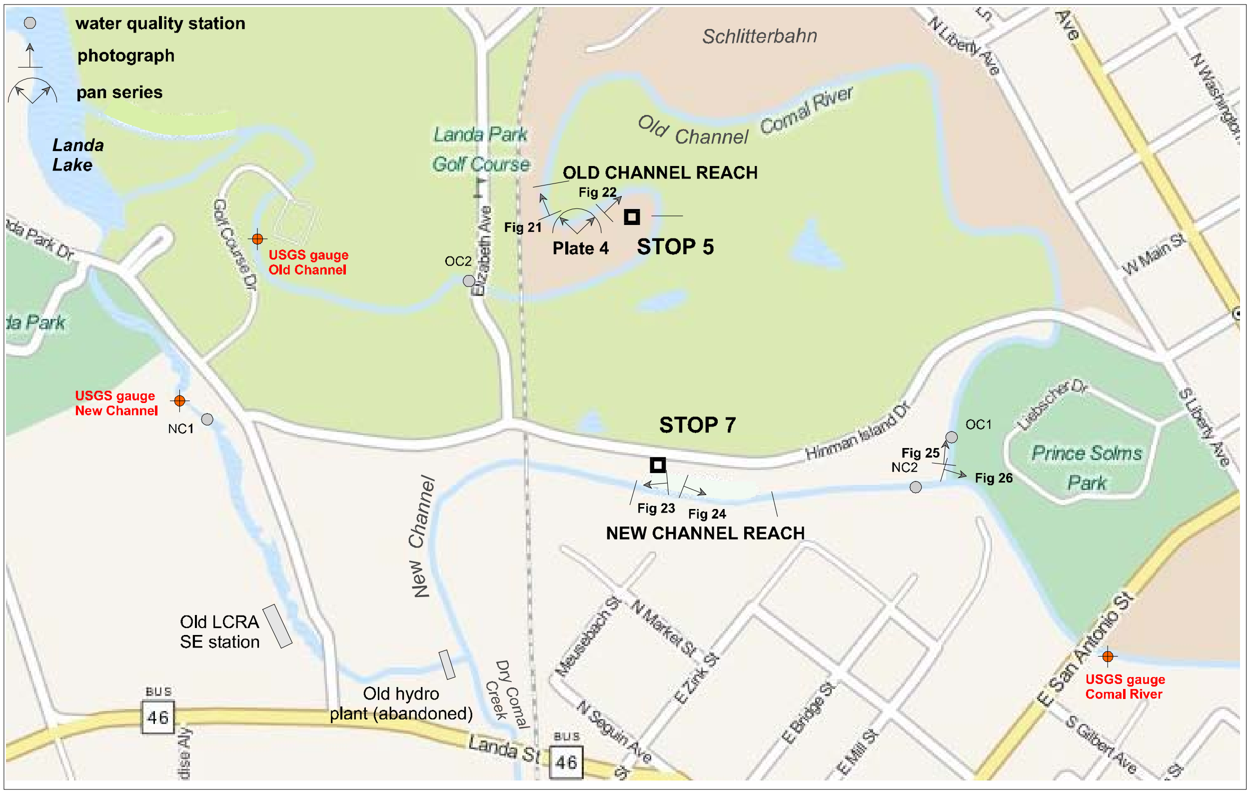


Fig 20 - Vultures hanging out around Landa Lake



Map 3 - Old Channel and New Channel of Comal River

We now moved down river to the Schlitterbahn parking area on the old channel, Stop 5 (Map 3). Here the river flow is that from Landa Lake, with no additional contributions. Some time was spent in studying the old river channel reach, which is the primary validation site for the modeling work presently underway. This section of the old river was excellent habitat in the early 2000’s, but is now vegetated primarily by *Hygrophila* with some bryophytes (Figs. 21-22). At one time, the filamentous algae with long streamers was prevalent here, but after the

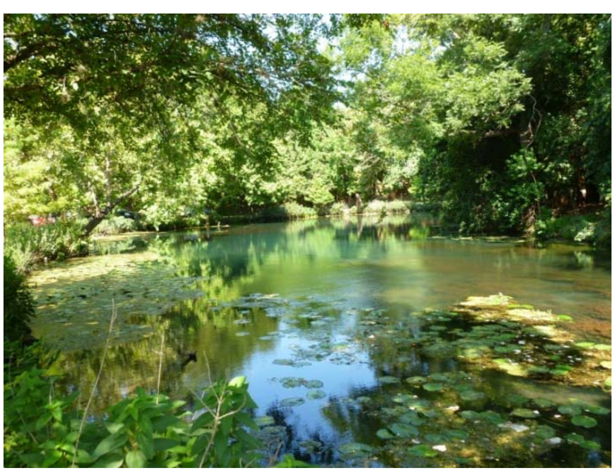


Fig 21 - Old Channel looking downstream

Fig 22 - Old Channel looking upstream

installation and operation of the New Culvert in 2003-04 most all native aquatic vegetation has been replaced with non-native *Hygrophila*.

After visiting the springs on the west side of Landa Lake (see above), we toured the new channel from the outlet from Landa Lake downstream. Ed estimated the flow in the new channel on this day to be approximately 65 cfs. Stop 7 was at the New Channel Reach. The channel here is urbanized and subject to heavy recreation, Figs. 23-26.

Fig 23 - New Channel looking upstream

Fig 24 - New Channel looking downstream

Fig 25 - New Channel at bridge crossing

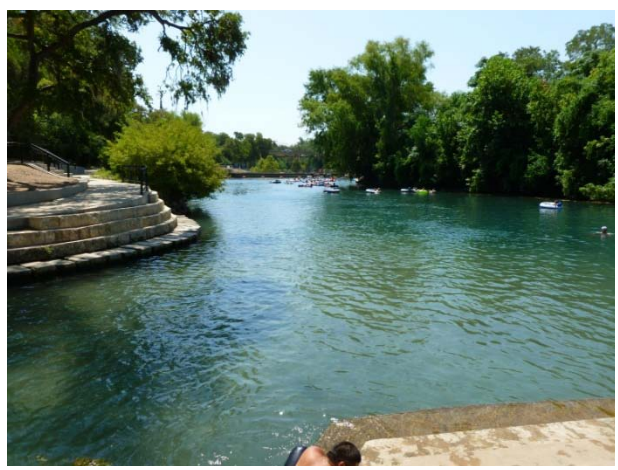


Fig 26 - New Channel, Cullen Dam in distance (Tube chute on right descending end)

Plate 1 – Panoramic series, upstream end of Spring Island

Plate 2 – Panoramic series, bend and cut bank of old river channel

Plate 3 – Panoramic series, Landa Lake reach, from north shore

Plate 4 – Panoramic series, Old Channel Reach

MEMORANDUM

FROM: George H. Ward, scribe
DATE: 13 August 2014
SUBJECT: Fountain darter modeling meeting, 7 Aug 2014 CDT

Attendance:

Bill Grant	TAMU	Ed Oborny	BIO-WEST
Rose Wang	TAMU	Tim Bonner	TSU
George Ward	UT		

Discussions:

Following the morning field trip to the Comal River, the fountain darter modeling team plus interlopers met with Dr Bonner at the Freeman Aquatic Building at Texas State University to review progress on the darter model. This evolved into a wide-ranging discussion on the behavior of the darters and the analysis of data.

Rose and Bill have been exploring various statistical approaches to the darter data collected over the years, which might reveal functional dependencies on environmental factors, especially vegetation. Rose presented some preliminary results of multivariate analyses using a suite of community metrics, e.g. diversity, as independent variables. It was observed that evaluation of these metrics would be impaired because the method of quantifying and reporting SAV characteristics was founded on the premise that darter abundance will be dependent upon the species of vegetation present, so reporting focused on the dominant one or two species. Therefore, it might be better to treat vegetation as a categorical variable and apply analysis of covariance to address both categorical and continuous measures. It was suggested that the emphasis be on partial correlation (or partial covariance) in reporting results.

The discussion returned to the problem that fraction of areal vegetation coverage, as reported in the field data (as percent), does not account for the density of the vegetation (see memo on meeting of 2 July 2014). Ed reviewed the convention for reporting bryophytes intermixed with another SAV, and the interpretation of bryophyte presence. To test the hypothesis that it is the bryophytes that provide structural attraction to the darters, can these observations be used to estimate the bryophyte areal abundance alone? That is, may observations of 60% *Hygrophila* with 50% bryophytes (say) allow an estimate of bryophyte abundance of 30% ((0.60 x 0.50)? This question could not be resolved within the discussion, and it was tabled for later consideration.

Tim presented some preliminary results of darter stomach-content analyses carried out by him and his students. The motivation for requesting this analysis was to determine the daily food requirement of a darter, and therefore provide guidance to the team as to whether a separate compartment in the darter model for food availability would be necessary. If available food

exceeds the darter requirement by, say, several orders of magnitude, it could be safely assumed that food is not a limiting variable for darter abundance. Conversely, if the daily food requirement is on the same order as food available, then the food source would need to be explicitly considered. This would then necessitate a submodel for amphipod abundance.

Although there was considerable variance in the data, the food requirement appears to be around 1% of body weight. (This agrees with Dr Bonner's preliminary back-of-the-envelope estimates before this work began.) This can be combined with the estimated abundance of darters and compared to the population of amphipods to complete the analysis. However, one interesting aspect of Dr Bonner's data is that the differentiation of stomach contents according to *types* of food indicates that, contrary to expectations, amphipods comprise a small proportion of the darter's diet. This is based upon separating the food into identifiable units, and reporting the number of said units. These need to be converted to biomass to completely quantify the relative importance of amphipods.

MEMORANDUM

FROM: George H. Ward, scribe
DATE: 15 September 2014
SUBJECT: Fountain darter modeling meeting, 27 Aug 2014 CDT

Attendance:

Robert Doyle	Baylor	Thom Hardy	WSG	Bill Grant	TAMU
Ed Oborny	BIO-WEST	Todd Swannack	ERDC	Rose Wang	TAMU
Tim Bonner	TSU	Jeremy Webster	BIO-WEST	George Ward	UT

1. Current status of Comal system

Ed reviewed the Comal springs and river system, re-capping the field trip of 7 August. The Comal system is at its lowest level since 1990. The major spring runs are nearly dry, but water may be found under rocks. Tim has seen a decrease in numbers of darters in the Upper Spring run area, but this is true of all fish in that area. Some darters are hiding under rocks and surviving. Ed is seeing a reduction at his stations in the Upper Spring Run reach and New Channel. But there is no evidence of the "skinny fish" syndrome (cf. court case). Tim notes that darters are consuming foods. A brief discussion of crayfish predation followed. Tim is catching darters in upper spring run evidencing reproduction. At the time of the meeting, water temps are well below 27°C, but if the drought holds, he will be able to test hypothesis of reproductive failure at around 27°. Tim has seen stratification in temperature between the upper foot and near-bottom. Small cool upwellings continue, and are probably sustaining this stratification.

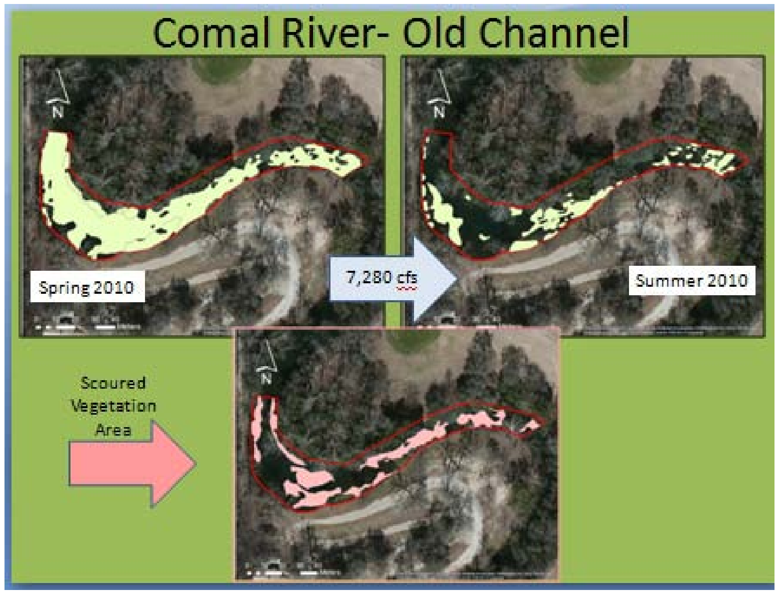
2. SAV studies

Robert reviewed some preliminary findings on sensitivity of key veg species to low CO_2, and ability to switch to bicarbonates. *Ludwigia* can switch, but *Cabomba* is obligate-CO_2. *Vallisneria* readily uses bicarb if CO_2 is unavailable. Sarah continues to work on literature review of current scour of SAV's.

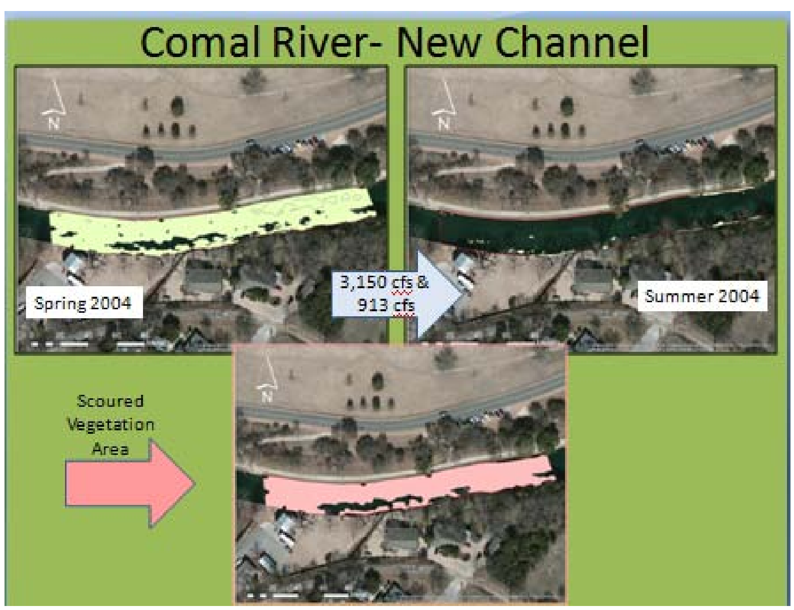
Todd reports continued good progress on the SAV modeling. He is evolving a combination of Charisma (the successor to Megaplant, see van Nes et al., 2003) and the metabolic functions developed in the ERDC models, see his handout summary attached. Some discussion of the use of shoot:root ratio as a measure of below-ground biomass. Robert has this data for the principal species. Todd can also modify the light equations, (4) and (5) in the handout, to include attenuation due to suspended sediment. Thom has data on turbidity. He notes that this typically exhibits a diel pattern as well as a daily (low mid-week, high weekends) and seasonal progression, tracking tourism activity. Todd needs a height to length parameterization (see "plant growth" in handout). Robert observed that the biomass:volume plot is essentially biomass:plant height. In future, Todd plans to improve Charisma's modeling of plant mortality.

3. Analysis of scour in historical data

Jeremy and Ed presented an overview of the analyses BIO-WEST is carrying out of the historical vegetation scour in these systems. They are using the GIS displays of vegetation distribution to create a pre-flood/post-flood difference display of the areas scoured by past flood events.



In the 2010 event (total system flow 7,280 cfs), for example (see above), extensive scour occurred in the Old Channel: *Sagittaria* survived but everything else scoured. The 2004 event scoured the new channel (see below).



This stimulated discussion of sedimentary processes. During low flows sediment accumulates in the new channel, which then supports SAVs, but then the next flood event takes it out. The question BIO-WEST would like to explore is whether this sort of analysis will be sufficient to estimate thresholds of scour for a given combination of SAV's and sediment texture. Since these results are in GIS, the areas of individual veg species may be quantified. The preliminary results in spreadsheet format were presented:

	A	V	W	X	Y	Z	AA	AB	AC	AD	AE	AF
1	Pulse Date	6/9/2004, 6/30/04				10/25/2004, 11/17/04, 11/22/04				7/20/2007		
2	Pulse (cfs)	3150, 913				1070, 2600, 6860				1980		
3	Dry Comal									6/29/07, 7/3/07, 7/20/07		
4	Dry Comal									649, 520, 1750		
5		Spring_04	Summer_04	Veg Difference		Fall_04	Spring_05	Veg Difference		Spring_07	Fall_07	Veg Difference
6	Date	4/21/2004	8/3/2004	Days Between		10/19/2004	4/21/2005	Days Between		4/27/2007	10/18/2007	Days Between
7	Flow	363	382	105		385	443	185		343	425	175
8	Layer											
9	Algae	0.0	47.4	47.4		0.0	70.2	70.2		0.0	0.0	0.0
10	Bryophytes	0.0	0.0	0.0		0.0	0.0	0.0		49.9	0.1	-49.8
11	Cabomba	272.1	0.9	-271.2		3.1	0.0	-3.1		106.9	0.3	-106.5
12	Ceratopteris	0.0	0.0	0.0		0.0	0.0	0.0		0.0	0.0	0.0
13	Hygrophila	3300.3	77.2	-3223.0		619.6	18.1	-601.5		1107.6	0.8	-1106.8
14	Limnophila	0.0	0.0	0.0		0.0	0.0	0.0		0.0	0.0	0.0
15	Ludwigia	3.9	0.4	-3.5		0.5	0.0	-0.5		8.4	0.0	-8.4
16	Nuphar	0.0	0.0	0.0		0.0	0.0	0.0		0.0	0.0	0.0
17	Sagittaria	0.0	0.0	0.0		0.0	0.0	0.0		0.0	0.0	0.0
18	Vallisneria	0.0	0.0	0.0		0.0	0.0	0.0		0.0	0.0	0.0
19				0.0				0.0				0.0
20	Total	3576.3	125.9	-3450.4		623.2	88.2	-535.0		1272.7	1.3	-1271.5

USR / USR Pulse / LL / LL Pulse / OCR / OCR Pulse / NCR / NCR Pulse

Robert's data from before and after the 1998 flood is also being examined.

Should succession be of concern in interpreting replacement of one veg type with another over time? Robert's opinion is that aquatic plant communities are rarely stable enough for the conventional paradigms of succession to be applicable. He referenced a past Corps position paper addressing this question.

Another major problem with the present analyses is accurately defining the flow rate actually experienced in the monitored reaches. The flows given above are for the total system as measured downstream at the USGS gauges. The flows manifested in different subreaches are clearly determined by the local distribution of rainfall and runoff creating the flood events.

4. Darter analyses

Rose and Bill summarized their statistical analyses of the historical data. Focusing on the Old Channel only, they ran a 15 single-factor ANOVA to correlate darter numbers with major vegetation types. This confirms the qualitative judgment that higher darter numbers are associated with bryophytes. However, dividing the samples into with and without bryophytes did not produce a statistically significant difference, with the exception of *Hygrophila*, due to the

small sample sizes of the with-bryophyte categories. There was discussion on whether the analysis should break subreaches out or encompass the whole system. They will be evaluating both moving forward. They propose to use a multinomial logit regession model. There was discussion of the possible value in constraining or weighting the analyses by the amount of various vegetation types.

5. Action

Ed proposed the following action items:

BW:	Get San Marcos veg data to Thom for scour review
WSG:	San Marcos set-up for City Park reach
	QUAL-2E conversion
BU:	Continue lit search
	Start San Marcos *Ludwigia* study
	Continue biomass-to-cover study
TAMU:	Work with BW on database updates
	Look at Comal and San Marcos systems, and individual reaches
	Evaluate drop net density data relative to available aquatic vegetation
	Multinomial logit regression
ERDC:	Get with Robert & populate equations with real data or Robert's opinion
	Get with Rose on math
TSU	Continue food source analysis with benthic macroinvertebrate data

The next team meeting is scheduled for 1000 9 October at BIO-WEST offices (Round Rock)

References

Carr, G., H. Duthie, and W. Taylor, 1997: Models of aquatic plant productivity: a review of the factors that influence growth. *Aquatic Botany 59*, 195-215.

van Nes, E., M. Scheffer, M. van den Berg, and H. Coops, 2003: Charisma: a spatial explicit simulation model of submerged macrophytes. *Ecological Modelling 159*, 103-116.

APPENDIX
Model Description
Todd Swannack, ERDC

The plant growth model is based on three existing approaches

1) MEGAPLANT (Scheffer et al 1993, Aquatic Botany)
2) Charisma, a spatially explicit update of Charisma (van Nes et al 2003, Ecological Modelling)
3) ERDC Models (Best and Boyd)

General description

Plant growth, in terms of biomass gained or lost (in grams/day) is modeled on a daily timestep. Plant growth is calculated as

$$\Delta W = W_s P - W(R_m + M) \qquad (1)$$

Where ΔW is the change in plant weight for a given day, W_s is the weight of the sprout, W is the weight of individual plant, R_m is respiration, and M is mortality.

Photosynthesis

Plant growth is calculated by estimating daily photosynthetic growth. Photosynthesis is calculated using a suite of Monod (i.e., Michaelis-Menten) equations (Carr *et al.* 1997). These equations have 1 parameter, the half-saturation coefficient (H_x) which indicates the concentration where growth is reduced by 50%. Photosynthesis is affected by in situ light (I), temperature (T, °C), and distance to the top of the plant (D). Photosynthesis is calculated as

$$P = P_{max} * \frac{I}{I+H_I} * \frac{S*T^{pt}}{T^{pt}+H_T^{pt}} * \frac{H_D}{D+H_D} \qquad (3)$$

Where represents the daily production of the plant top at 20°C (assuming no light limitation). The defaults for P_{max} is 0.01 g g^{-1} d^{-1}, H_I is the half-saturation coefficient of light (100 μE m^{-2} s^{-1}), H_T is the half-saturation coefficient of temperature, S is the temperature factor, *p*t is the power of a Hill function, and H_D is the half-saturation coefficient for distance (set at 1 m).

In-situ light (*I*)

In-situ light is the primary driver of plant growth. Irradiation values are needed at any time of day to integrate total daily photosynthesis (i.e., photosynthesis will need to be calculated at several depths at several times to get total daily *P*)[1]. Irradiation is represented as effective irradiation and is attenuated based on depth. Irradiation at depth *z* can modeled two different ways. One considers self-shading (4) and the other does not (5).

$$I_{z,t} = I_0 * e^{-K_d - K_p * b_z} \qquad (4)$$

$$I_{z,t} = I_0 * e^{-K_d} \qquad (5)$$

where I_z is the irradiation at depth z at time t (μE m^{-2} s^{-1}), I_0 is the photosynthetically available radiation (PAR) at the surface (μE m^{-2} s^{-1}), K_d is the light attenuation coefficient of the water (m^{-1}), Kp is the attenuation coefficient of the plant material (0.02 m^2 g^{-1}) and b_z is the biomass of the

[1] I'm not sure of the best way to do this efficiently

plant material above depth *z* (g m^{-2}). It is assumed that PAR is 50% of total irradiation, and that 10% of light is reflected from the water's surface.

Plant growth

Plants only grow during the growing season (15 March – 31 October, days 75 – 304). At the beginning of the growing season. At the beginning of the growing season, sprouts take biomass from the roots until that source is exhausted.

Plants grow by accumulating biomass via photosynthesis. Plant length is calculated from a fixed length to width ratio (A 1 m sprout weighs 0.1 g m^{-1})[2]. Plants have a maximum length, which is species specific (currently set at 4 m). When a plant reaches its maximum length, it adds biomass evenly

Root biomass is accumulated as plants grow at 6% of aboveground biomass.

Temperature

Temperature is modeled as a Hill function (similar to Monod functions, but with an added exponent (*pt*), that can better describe transitions from one state to another). Within the model, *pt* is set to 3, *S* is set to 1.35, and H_T is set to 14 (following both Charisma and MEGAPLANT)

$$f(T) = \frac{1.35 * T^3}{T^3 + 14^3} \quad (6)$$

Respiration

Respiration depends on temperature and is based on a Q_{10} formulation (i.e., the measure of the rate of change of a by increasing the temperature by 10°C). The default value of Q_{10} is 2.

$$R_m = r_{20} * Q_{10}^{((T-20)/10)} \quad (2)$$

Where R_m is the maintenance respiration (g g^{-1} d^{-1}), r20 is the respiration at 20°C, and T is temperature (°C)

Mortality

Mortality (M) is currently represented as a constant percentage of biomass lost per day (following ERDC models). This was the cleanest way to model mortality. Mortality in Charisma and MEGAPLANT focused on mortality caused by wave-damage, herbivory, and competition at high densities (using a thinning law).

Grazing and recreation can be added to the model.

Seasonal die offs (represented by maximum age of plants) can be added easily as well.[3]

Reproduction and Dispersal[4]

From 15 April to 15 May, plants allocate a percentage of their biomass towards tubers/seeds accumulating between 13 – 20% of their biomass for reproductive output. At the end of the

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[2] Does this seem right? This number is an estimate for dry weight to length for *Potamogeton pectinatus*.

[3] What is the maximum age of an aquatic plant?

[4] Formulated, but not implemented. Pending discussion with Robert and remaining crew

growing season, that biomass is transformed to seeds/tubers. The total number of seeds/tubers that is produced is calculated as

$$N_j = \frac{a_s * B}{b_s} \quad (7)$$

Where N_j is the total number of seeds/tubers dispersed by plants (# yr^{-1}), a_s is the fraction of the plant biomass allocated to seeds (g yr^{-1}), B is the total plant biomass (g), and b_s is the biomass of a seed (g)

NEXT STEPS

1) Need to import time-series of irradiation[5], temperature, and depth, and make sure math works across time (more than 1 day).
2) Dispersal is not currently in the model. I should probably do that.
    a. Seeds?
    b. Adventitious roots?
3) Should I include a thinning law?
4) Other comments?

References

Carr G.M., Duthie H.C. & Taylor W.D. (1997) Models of aquatic plant productivity: a review of the factors that influence growth. *Aquatic Botany* **59**, 195-215.

[5] Does anyone know where to get irradiation data?

MEMORANDUM

FROM:	George H. Ward, scribe
DATE:	31 October 2014
SUBJECT:	Ecomodeling team meeting, 1000 9 October 2014 CDT

Attendance:

Bill Grant	TAMU	Thom Hardy	WSG	Rose Wang	TAMU
Ed Oborny	BIO-WEST	Todd Swannack	ERDC	Tim Bonner	TSU
Jake Jackson	BIO-WEST	George Ward	UT		

1. Current status of Comal system and HCP projects

Ed reviewed the present, drought status of Comal and San Marcos springs and river systems. Comal had some rains and has come back to 85 cfs total system flow (60 cfs old channel, 25 cfs new channel). San Marcos is still flowing about 103 cfs and looks good.

Some "gardening" projects shut down in summer due to low flows. EAA working with USFWS to get these reinstated. Five research proposals selected for forthcoming year. ERDC was the only proposer on the vegetation scour study, so has been selected (by default).

2. SAV studies

Todd reports continuing progress. Still working on the integration problem (see 27 Aug notes). Has coded four separate "strategies" of SAV dispersal. More are in preparation. As more information is needed on exactly how our plants disperse, he has also been reviewing the literature on these species. Todd displayed model operation for the dispersal functions presently operative. Validating will require constructing confidence isopleths. Thom will share with Todd the results from empirical studies of plant removal in the San Marcos system.

3. Fecundity & predation of fountain darters

Tim's project is drawing to a close. The report is due in November, but the data and results are available to the team now. Tim summarized these. He is using the gonadal somatic index (GSI, the fraction of body weight represented in the gonads) to quantify energy invested in spawning. Most warm water fish spawn multiple times through the year, including fountain darters. Year-round spawning is rare in fish; out of about 640 species, four (4) are reported to be year-round spawners. Darters are year-round species. The GSI does show a variation in reproductive effort. He observed a decrease in summer, which may be related to recreational activity. This pattern is starting to look like that of other spring-water fish. Also, there is different reproductive effort dependent upon relative vegetation height. Todd noted that the SAV model will predict vegetation height.

Bass and crayfish are prominent predators of fountain darters. Bass is also a predator on crayfish. Tim's experiments are indicating that predation appears to be additive, i.e., the sum of bass and crayfish. This suggests that fountain darters are the preferred prey of bass even when crayfish are present.

Tim also observed that the underlying concept of a lot of this work is "patchiness" in time and space.

3. Darter modeling

The fountain darter model is now being carried forward on two "platforms", *viz*. Netlogo and C++. The program in C++ was suggested by the National Academy reviewers because the Netlogo version was judged to be too slow for execution of a large number of scenarios. Indeed, the C++ version is about an order of magnitude faster in execution. On the other hand, it lacks the graphical output of Netlogo. The SAV files are being generated by Thom in both formats. These are based on the GIS veg maps produced by BIO-WEST. When the SAV model becomes operational, these "data file" inputs will be replaced by output from the SAV model. Todd: "All of this stuff will work."

Thom gave an overview of the water quality model. The Old Channel w/ constant flow rate is relatively simple. The San Marcos City Park reach is more complex. This has been modeled with flows ranging 40 – 280 cfs at intervals of 5 cfs for low flows, increasing to 20 cfs for higher flows. These then serve as "look up" tables for the darter model. To incorporate water quality, the QUAL2E water quality model will have to simulate the entirety of each river system with hourly met data inputted (and constant flow rates). Temperature does not vary a lot spatially, so can be handled by assigning one T value at 100 m intervals. Thom needs specifications for the time/space resolution needed in the SAV and fountain darter models for growth terms, etc. If threshold behavior is manifested, e.g., if DO falls below 2.0 the fish croak, then the WQ model will need to simulated PR, whereupon DO's < 2 could be extracted and applied as appropriate. To operate the darter model for predictive scenarios, it will be necessary to specify met conditions, from which the temperature model will be run at specified flow, to produce a file of temps at 100 m nodes.

Ed: we'll need to address DO for "political reasons". Generally, DO has not been a problem. But recently in the low flows, Landa Lake patches of vegetation are driving diel variation in DO, with a range of 2 – 17 mg/L. Could we run a range of flows, for separate seasons, to archive a look-up library of temperatures? This needs to be given some thought. It may prove to be more efficient to actually re-run QUAL-2E as part of the fountain darter model execution. We also have to distinguish between the model runs that the ecosystem team will want to make versus those that will be carried out by EAA staff. With respect to the latter, maybe we should consider defining some simplified scenarios to facilitate model runs for management purposes.

4. Drop-net darter analyses

In August, Rose and Bill summarized their statistical analyses of the historical data, based upon a 15 single-factor ANOVA to correlate darter numbers with major vegetation types. Rose has reorganized the dropnet data weighting by the dominant vegetation areal coverage. Upon reflection, they believe a better strategy might be to use logit analysis. This is a categorical version of the multivariate logistic regression method. This has a history of application to biological data, primarily in the past 20 years (e.g., Hosmer and Lemeshow, 1989; Cramer, 2002). A preliminary application of this method was presented, as follows. Categories 2 – 5 are ranges of numbers of darters and is the dependent variable. The individual independent variables are retained according to their small p values. The final retained variables in the model are:

Variable	Overall *P*-value	Category 2		Category 3		Category 4		Category 5	
		Estimated coefficient	*P*-value	Estimated coefficient	*P*-value	Estimated coefficient	*P*-value	Estimated coefficient	*P*-value
Constant	–	0.6196	0.7452	-7.9018	0.0004	-13.2625	<0.0001	-18.3240	<0.0001
Bryophytes	0.0006	2.3115	0.0566	3.7323	0.0021	4.5881	0.0006	5.2690	0.0003
Cabomba	<0.0001	3.5416	0.0009	5.2066	<0.0001	5.3816	<0.0001	5.3257	<0.0001
Ceratopteris	0.0310	1.9023	0.0078	1.4136	0.0181	-10.5104	0.0854	-9.5265	0.0862
FAlgae	<0.0001	14.4100	0.0018	19.1906	0.0025	19.4989	0.0019	22.5046	0.0060
Hydrilla	0.0490	1.0638	0.0118	1.0090	0.0052	0.4889	0.6103	0.7305	0.1142
Hygrophila	<0.0001	1.5035	<0.0001	2.2374	<0.0001	2.0593	0.0007	1.6246	0.0574
Ludwigia	<0.0001	2.6431	<0.0001	3.7054	<0.0001	4.2030	<0.0001	3.9002	<0.0001
POT_HYG	0.0059	3.1083	0.0054	3.3643	0.0029	2.5123	0.1088	-8.9735	0.9847
VegPer	<0.0001	0.0114	0.4134	0.0794	<0.0001	0.0939	<0.0001	0.1372	<0.0001
VegHeight	<0.0001	-0.9266	0.2333	1.7744	0.0371	3.0889	0.0097	4.4971	0.0033
VegVol	0.0002	0.0124	0.1446	-0.0139	0.1329	-0.0289	0.0264	-0.0443	0.0078
WithBryo	<0.0001	1.3088	0.0995	2.8370	0.0002	3.3302	<0.0001	3.8677	<0.0001
WaterDepthFt	<0.0001	-0.6532	0.0002	-0.9541	<0.0001	-0.9467	<0.0001	-0.7526	0.0001
Gravel	0.0048	0.1786	0.7300	0.7942	0.1770	1.7812	0.0188	3.9518	0.0010
Sand	0.0410	0.6767	0.3130	1.7995	0.0143	1.9821	0.0518	3.6941	0.0254
Silt	0.0022	0.9754	0.0573	1.8355	0.0017	2.0219	0.0113	4.4331	0.0004
Silt_Gravel	0.0496	0.1198	0.1429	1.0638	0.1130	0.8677	0.1637	2.8651	0.0398
Speed	0.0458	2.6844	0.0973	2.2843	0.0720	-1.3615	0.0110	-4.7245	0.0377
SpCond	0.0479	-0.00217	0.0212	-0.00031	0.0925	0.00319	0.0779	-0.00146	0.0469

This stimulated considerable discussion, including the roles of abiotic and seasonal variation in the darter data, and the potential effects of stratification. It was noted that some of these independent variables may, in fact, be correlated. This would undermine the use of p values to reduce the number of variables and needs to be examined.

5. Darter movement as indicated in tagging studies

Jake and Ed made a brief presentation on the drop net and tagging experiments, particularly in the upper spring runs of Comal under this summer's low flow conditions. Low densities were found in Blieders, where they tagged maybe 200 darter of which they recovered 6, of which 3 were still in Blieders. Further down toward and in Landa Lake, densities were higher, but movement was limited, apparently independent of the vegetation cover. Jake noted a zone below Union Street where emergent *Sagittaria* had sealed off the creek. Just upstream there was a zone of higher turbidity, higher temperatures, and generally "crappy" water.

It's difficult to assert that there is a continuing reduction in population. They may simply be diving into the substrate. Tim believes some, even many, are in fact dying. There followed an extended discussion of darter movement, what we know and how do we know it.

6. Action

Ed summarized the following action items:

1) Thom – WQ model each system – pre-compute response matrix (Max, Min, Avg Temp) Min DO. Level of effort – get with Ed. Send memo to team.
2) TAMU – incorporate DO thresholds in the existing model (get with Bonner on values)
3) TAMU and Todd – list of inputs and formats of needed data – from each other and Thom – Circulate to team.
4) Todd – Habitat Quality – visit with Thom and Robert – work on.
5) Todd – plug dispersal approach into larger model and test 3 types of dispersal as well as growth
6) Continued discussion on fecundity incorporation into darter model. (Tim and TAMU)
7) Jake and Rose discuss movement study and dispersal ranges for incorporation
8) TAMU – look at systems – season or month, flow
9) George's statistics request to Rose regarding collinearity of the independent variables

References

Cramer, J.S., 2002: The origins of logistic regression. Discussion paper 119/4, Tinbergen Institute, and University of Amsterdam.

Hosmer, D., and S. Lemeshow, 1989: *Applied logistic regression*. New York: John Wiley & Sons.

MEMORANDUM

SUBJECT:	Ecomodeling team meeting, BIO-WEST offices, 1000 18 November 2014 CDT
FROM:	George H. Ward, scribe
DATE:	24 November 2014

Attendance:

Bill Grant	TAMU	Thom Hardy	WSG	Rose Wang	TAMU
Ed Oborny	BIO-WEST	Sarah Hester	Baylor	Jake Jackson	BIO-WEST
George Ward	UT				

Todd Swannack, ERDC, was ill but participated via telephone.

1. Current status of Comal system and HCP projects

Ed reviewed the present, continuing-drought status of Comal and San Marcos springs and river systems. The hill country got some rain in October and the J-17 index well (San Antonio) rose about 8 ft. Total flow in the Comal river system rose from 90 to about 120 cfs, and is still holding at 120 cfs. In the San Marcos, there was no significant response.

EAA has now received clarification from USFWS about Provision M of the Incidental Take Permit, so most of the unobtrusive project work that had been put on hold is now reinstated.

Fall monitoring was completed in late October – early November, though the data will not be immediately used in this study because some weeks will be required to process the data. The three darter studies from the present year's research projects are now complete and the reports finalized. Ed will send out copies of all three to the team. With respect to next year's research, ERDC was the only proposer on the vegetation scour study and was therefore selected for the work. Unfortunately, EAA and the Army could not agree on the payment process for the project, so it has been cancelled.

2. SAV modeling

An ailing Todd reported via telephone in a monotone wheeze. Progress on programming the SAV model continues. He is now inserting the code for dispersal into the "big" model (in contrast to the prototype "minimodels" he was using to test the scripts and do preliminary evaluations of the dispersal mathematical functions). As can be expected in such a complicated model, this introduced some bugs in the program, but in general is looking good. He made a presentation to the science committee, which has raised some questions about details of the model formulation that he will need to address, particularly in the mechanisms for dispersal. He also had extended conversations with two members of the National Academy review team. This has led to some alternative growth functions that he wants to study and evaluate for possible incorporation into the SAV model.

3. SAV field studies

Sarah Hester represented the Baylor team. About 90% of the veg cover samples have been analyzed (above and below-ground biomass) for the seven dominant SAV species. She showed some preliminary results from these data relating cover and plant volume to biomass. The San Marcos and Comal *Vallisneria* are different species, which accounts for the difference in biomass for this plant.

She also reported on the distributed planting experiments with *Ludwigia*, in which MUPPT-grown plants were transplanted over larger reaches in both the San Marcos and Comal rivers. The survival is variable, and tends to decline with several environmental variables (substrate, flow velocity), but these may be proxies for location in the river and hence may reflect human impacts.

She is completing the literature review on scour of SAV, which now consists of an annotated bibliography of 28 articles. Thom asked for an advance copy so he can compare to his document collection. He may have some gray-literature reports that she has not seen, e.g., studies of stalk vulnerability to current speed.

4. Darter modeling

Thom is still working on the generation of the flow and weather response matrix for DO and temperature to be generated from his QUAL-2E water quality model of the river systems (see memo for 9 October meeting). By Thanksgiving, he is planning to complete a memo to the team considering the two alternatives: (1) look-up tables from pre-computed scenarios, and (2) embedding calls to the QUAL-2E executable from within the darter model code. The pros and cons of each approach were briefly discussed by the team.

Thom also raised the question of whether the present grid system of 0.25 m, which is employed by the hydraulic model, is unnecessarily small, and suggested that the SAV and substrate data (and the hydraulic model output) could be aggregated at, say, 1 meter resolution. This would vastly improve the running time of the Netlogo darter model with minor sacrifice in accuracy. (Selection of an appropriate spatial resolution has been discussed since the September 2013 team meeting, but a provisional operational darter/SAV model was needed to quantify the running time.)

These and a few other issues about the details of model structure were raised. It was decided that the modelers (i.e., Thom, Todd, Rose and Bill) should hammer these matters out in a conference call, which was set for 25 November.

5. Drop-net darter analyses

Rose and Bill presented updated results from their multivariate logit analysis of the drop-net data (see memo of 9 October 2014 meeting). There are now two classes of external (independent) variables considered. The first is micro-variables, applicable to the specific location of the drop-net sample, which include:

> Cobble, Gravel, Sand, Silt, Silt_over_gravel, Bryophytes, Cabomba, Ceratopteris, Fil_algae, Green_algae ,Hydrilla, Hygrophila, Ludwigia, POT_HYG, Potamogeton, Sagittaria, Vallisneria, MainVegHeight, MainVegVol, WithBryo, WaterDepthFt, Velocity, Temp, DO, SpCond, pH

(These are the same variable set considered in the first version of this analysis, see notes for 9 October meeting.) The second is macro-variables, which apply to the entirety of the reach in which the drop-net sample point is located, and include:

> CP (critical period), Fall, Spring, Summer, Winter, T_Green_algae, T_Bryophytes, T_Cabomba, T_Ceratophyllum, T_Ceratopteris, T_Eichhornia, T_Heteranthera, T_Hydrilla, T_Hydrocotle, T_Hygrophila, T_Justicia, T_Ludwigia ,T_Nuphar, T_Potamogeton, T_Rorippa, T_Sagittaria, T_Vallisneria, T_Zizania, T_Open, T_Fil_algae, T_Chara, T_Limnophila (where "T_" designates reach total or reach average)

The procedure has been coded so that additional analyses can now be performed efficiently. Several variations in the analysis were suggested by the team: (1) separate the data for the two river systems; (2) separate the Old Channel data into pre- and post-2005.

Also, collinearity was examined. High values were found for those variables related by definition, such as a SAV species at the sample point and the total reach value (T_ …) for that same species. The team believed that 0.8 as a criterion for excess collinearity was too high, as it excludes only these types of related variables, but that a value of 0.5 was more appropriate, given the noise in this type of data.

It was recommended that Rose and Bill document their analyses and preliminary results in a technical memorandum internal to the modeling team. Bill noted that what they are ultimately trying to extract from these analyses are rates of fecundity and mortality, backing into these from the dynamics of darter density. The environmental controls are all part of Habitat Quality, which is assumed to drive the behavior and net fecundity (over mortality) in the model.

This spurred a discussion of the darter model formulation. Central is the specification of movement and carrying capacity, both of which are driven by environmental factors. In the present formulation, the model should initially use temperature effects on reproduction as

indicated by literature, ditto temperature effects on mortality, and ditto minimum required DO. Seasonality for reproduction to be modified by Bonner et al. 2014 (in press), possibly affected by Habitat Quality (maybe just be on the reach level). Assume predation, competition, etc. captured in Habitat Quality. The consensus is that the darters do not really move that much. They tend to stay in one place, and if disturbed move a short distance away. If we want other factors (e. g. parasites) we would need to develop professional judgment relationships.

6. Action

Ed summarized the following action items:

(1) Send EAA Applied Research fountain darter (movement, fecundity, and predation) to project team – Ed
(2) Bonner / BW subgroup meeting - Bonner – food source
(3) BW – WSG – shear stress subgroup meeting
(4) WQ – response matrix call out or compute– Final memo by Thanksgiving – Thom
(5) Conference call – Thom, Bill, Rose, Todd – 10am next Tuesday.
(6) Todd/Ed with Robert/Sarah to talk about studies and incorporation into SAV model. Also talk about habitat quality – December
(7) Bill/Rose – write up of logit method – include discussion of collinearity
(8) Ed/Jake/Tim – TAMU December – get Rose's update analysis – Dec.
(9) TAMU – incorporate DO thresholds in the existing model (get with Bonner on values) – above meeting.
(10) Continued discussion on fecundity incorporation into darter model. (Tim and TAMU)
(11) Jake and Rose discuss movement study and dispersal ranges for incorporation
(12) Ed send out outline for Feb 11 presentation.
(13) Sarah send Thom & George Annotated bibliography to Thom for review and response.

Next full team meeting is scheduled for January 6th and 7th (if needed) at the Meadows Center of TSU in San Marcos.

Prepared by Todd Swannack 25 November 2014

Notes for Modeling Team Meeting (Bill, Rose, Thom, and Todd)

- Benefits of changing resolution to $1m^2$
    - Matches scale of processes we're trying to model
        - Field data (drop net, etc)
        - Plant growth/dispersal
    - Faster computation time
    - Less input data
    - Thom has correct data format
    - Netlogo should run faster
        - Use read to end of file
        - Assign attributes to cells with those coordinates
    - Unanimous decision to move forward with $1m^2$
    - Action items
        - Thom will update grids for hydro & veg and send to Bill
        - Todd will get grids/input commands from Bill
        - Todd will finish the script to transform veg coding from Ed's coding scheme to modeled vegetation
        - Thom and Todd will send Rose input data for her stat models

- Will start to explore R-Netlogo linkages
        - Bill/Rose: Tomek will explore
        - Todd: Follow up with colleague at CERL

Do we need scour study?

- Represent it as a probability?
    - Pick group of cells at random and have those scales be scoured?
- Represent it empirically?
- It is a spatially-explicit process, so needs spatial component
- Do we force recreation of past, or exploration of how scour events affect darters?
- What level of detail do we need/is important for scour?
- Ecological modeling approach vs hydrologic approach
    - Levels of uncertainty vary, but overall system should be
- System experts could be used for scour from recreation events
- Then scour from floods could be incorporated at once per long term flood event (e.g., once every 10 years)
- Decision point not needed now, but need to be forward-thinking about how scour will be included in model
    - See what Sarah comes up with for scour lit review, then make a decision as to how to model it

MEMORANDUM

SUBJECT: Ecomodeling team meeting, Meadows Center, Texas State University, 0900 CST 6 January 2015
FROM: George H. Ward, scribe
DATE: 25 February 2015

Attendance:

Bill Grant	TAMU	Thom Hardy	WSG	Rose Wang	TAMU
Ed Oborny	BIO-WEST	Todd Swannack	ERDC	Jake Jackson	BIO-WEST
Tim Bonner	TSU	Robert Doyle	Baylor	George Ward	UT

1. Current status of Comal and San Marcos systems

Ed: The drought continues. Nine straight months of total Comal flow < 130 cfs, Upper spring run <3 cfs, though old river channel continues at about 60 cfs. San Marcos flowing 110-115 cfs steady, conditions good.

2. Darter modeling

Bill summarized the darter model status. All information received is being incorporated in the model(s):

	NETLOGO	C++
City Park	√	√
Comal Old Channel	√	√

The stats indicated aquatic vegetation (veg) to be important, but depth and flow not significant. The relations have been coded into the model mainly as probabilistic expressions. Four veg categories proved to be significant, but specification of the darter movement is still incomplete.

Rose led an extensive discussion about the conflicting roles of point veg versus reach-scale (denoted T_) factors (micro- versus macro-) in the drop-net data. In the San Marcos, the T_-variables are generally positive in the relation to darter abundance, while in the Comal, these variables are generally negative. Consensus for the reason is that the T_ variables are confounding the analysis. Do we even need to consider this scale of response? This led to a discussion of the "ovoid" of response of a darter, judged to be < 5 m. Conclusion: Reach evaluation is a different analysis, not to be combined with (or into) the "micro" or point data. Some of the participants, however, would like to see "universal field" equations that would be applicable to darters in *both* the San Marcos and Comal systems. Water depth is also a confounding factor, because it is gear-based, thus carries with it an intrinsic bias. It was decided that we need to re-run the stat analyses using only the micro-scale variables. Rose worked on it.

This led to a discussion of the carrying capacity for fountain darters of a given habitat. Ed opined that this is essentially a statement of the probability that a darter will be found in a model cell of a particular combination of environmental factors. For a square (cell) of given vegetation make-up and water quality, there were three ways discussed to estimate carrying capacity: (1) development of cumulative frequency for four categories of vegetation; (2) the historical observed maximum darter density; (3) probabilities of darter density with a cutoff based upon vegetation. In addition there should be an upper boundary on total movement of individuals.

3. Food source

Jake outlined the results of the BIO-WEST experiments and calculations of invertebrate food availability for the fountain darters. Invert samples were collected in 2013 & 2014. This analysis started with *Hyallela* because of its abundance. Results are:

		BRY (150,0)	CAB (87, 79)	HYD (0, 145)	HYG (267,149)	LUD (132, 2)	SAG (49, 12)	VAL (62, 8)
Comal	Amphipod biomass	5384.5 (7)	1817.05 (6)		1392.04 (10)	5812.9 (6)	1312.59 (8)	284.068 (2)
	Biomass required	131.845 **(2.44%)**	57.425 **(3.16%)**		38.06 **(2.73%)**	80.82 **(1.39%)**	30.025 **(2.28%)**	30.99 **(10.9%)**
	Max required	463.63	256.9		192.81	490.74	589.37	171.56
San Marcos	Amphipod biomass		4770.96 (4)	6922.0 (6)	1957.76 (5)		4842.7 (4)	4173.87 (2)
	Biomass required		43.76 **(0.92%)**	34.07 **(0.49%)**	29.27 **(1.5%)**		19.19 **(0.4%)**	103.33 **(2.47%)**
	Max required		144.95	631.58	181.48		60.6	271.34

According to Jake, the parenthetical numbers under the veg codes are the number of samples from Comal, San Marcos for that veg type. In the row for each system, they are the number of invert samples in that veg type. The percentages represent the proportion of the estimated mean standing crop that would be taken by the estimated darter needs. Using 5% of darter mass as an estimate of the daily food intake requirement, even with the maximum observed darter density, the food supply far exceeds this daily requirement.

The conclusion is that food availability is not a limiting factor and does not need to be explicitly considered. As a corollary, there is no need for an amphipod population model, at least at this stage of model development. In the write-up for this work, it will be necessary to address some of the qualifications of this conclusion, e.g., there may be a minimum and/or maximum temperature within the range of the darter that affects the density of amphipods.

4. Other environmental limits

Thom noted a paper by William E. Cooper on *H. azteca* in *Ecological monographs*. He will be circulating copies to the team.

Dissolved oxygen is not clear-cut. There is confusion in the literature between DO stress and DO lethality. Tim did find a paper that seems to support a threshold of about 2 mg/L for spring-fed rivers, based upon a level at which taxa richness begins to decline. In lab setting, lethality is < 2, looks like 0.5-1.0, the uncertainty arising from physiological response time. There ensued discussion of whether 2 mg/L is a reasonable number given the other approximations involved in the modeling. Instantaneous or durational? What exactly do we assume to happen at DO < 2, reproduction ceases? death? Maybe we need to apply the model diagnostically to pursue answers. There was a reference to Dr. Al Groger at TSU and the EAA data. Tim will look into the effects of DO on reproduction in the darter.

5. Water quality modeling

Thom announced that after much study it is now decided that QUAL-2E will *not* be embedded in the veg-darter model as a dynamic simulation. This would make model operation much too complex. Instead, QUAL-2E will be operated “off-line” and arrays of model output will be generated for various combinations of climate and hydrology. That is, these arrays of time-space distribution of hydraulic and water quality variables will be input into the darter model then interpolated as necessary.

The 2003-2010 water quality data preparation is now done for Old Channel, and Thom is working on the San Marcos.

Tim will send out a paper on turbidity effects on darters.

6. SAV modeling

Todd reported that the model is still in the prototype stage, just received updated 1-meter grid data, and is now incorporating scour events as probabilistic responses. Factors affecting growth/death of a species, say *Ludwigia*: (1) light at surface (from sun), and attenuation with depth & turbidity (water clarity), (2) temperature. Species growth characteristics are different. Nutrients aren’t explicitly considered, as they don’t appear to change with flow. Similar growth seems to be exhibited in silts and muds.

Todd -- Formulation of persistence scoring per December meeting with Robert was discussed. Needs to program these also as probabilities. Now coding different vegetation species. Still fussing with light attenuation. Has improved depictions of dispersal including mechanism.

These are being incorporated into the model based upon each 1 m2 square containing exactly one species. Bryophytes will be handled as "overlay", i.e., a second (presence/absence) attribute. Native versus invasive is an attribute in the model because invasives exhibit much faster regrowth after scour events.

Ed – This summer in the upper spring run in Comal, we lost 30-40% of plants under low flows. He thinks this is some kind of stagnant water phenomenon. Robert opines this is related to the carbon balance. In stagnant water, the plant loses C. For example, wild rice will either die or become emergent.

This led to a discussion of "future" flood/scour scenarios, and how to implement these in the model. Do we input difference levels of flood damage as function of flood intensity? Bill noted that we will have to run many replicates for each scenario, as a monte-carlo exercise.

Have we satisfactorily delineated flood-scour effects or do we need to repeat the scour-study RFP? General consensus is that it is now too late in the modeling schedule. By the time the work would be completed, the modeling effort will be over. For now, between the analysis of Thom and BIO-WEST, and the present SAV model formulation, we've wired around it. The effect of recreation is represented as a scour function of people pressure, i.e., an absence/presence variable. As we get into model applications, we may need to re-visit the scour issue.

What about CO_2? Not really a problem, except perhaps for the few species that are CO_2-obligates. Otherwise there is ample CO_2/CO_3 in the river systems.

It was suggested that Todd use his model to evaluate the potential effects of shading on plant growth. This may be species differentiated.

7. Additional topics

There was an extensive discussion of how we will go about measuring the validation of the model. The plan has been to calibrate on the 03-08 period then verify against 09-13. Do they need to be chronological? Perhaps it would be better to select appropriate years from the data-collection history.

Tim will provide new fecundity information to Bill and Rose, including new results on seasonality.

Rose completed the separate San Marcos and combined systems stat analyses. Comal was recomputed omitting the T_-variables. These results look good except for the high degree of "noise" in the San Marcos system. Apparently the results are sensitive to how the categories of darter density are defined. Rose will experiment with these and report back to the team.

The team then turned its attention to the upcoming presentation to the HCP Science Committee. Two important slides will be (1) a summary of the key decisions made thus far in the modeling work, and (2) identification of the upcoming decisions that the team will face. We need to solicit the input of the Science Committee in the latter.

Ed & George will put together a “draft” of the presentation, incorporating slides from Thom, Rose & Bill, Robert and Todd by 2 February, and the team will discuss and edit this presentation in a conference call 1200-1400 5 Feb. The next ecoteam meeting was scheduled for 26 February at TAMU.

MEMORANDUM

FROM: George H. Ward, scribe
DATE: 17 February 2015
SUBJECT: Notes on teleconference, 1200 CST 5 February 2015

Participants:

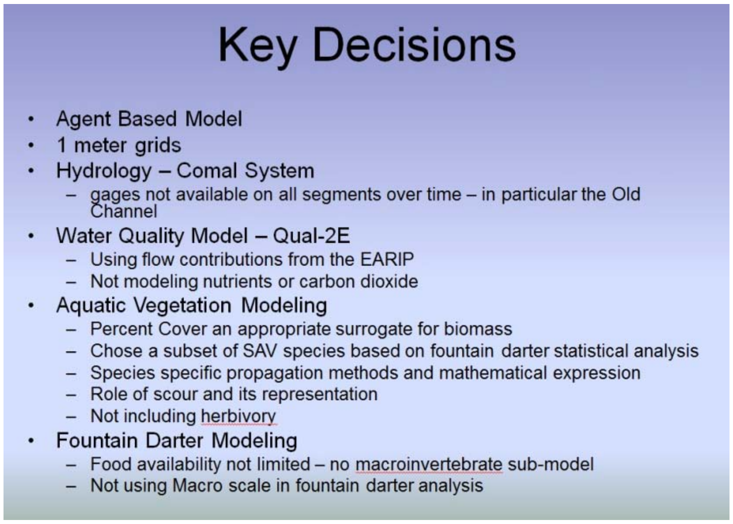
Ed Oborny	BIO-WEST	Bill Grant	TAMU	Thom Hardy	WSG
Robert Doyle	Baylor	Tim Bonner	TSU	Rose Wang	TAMU
Todd Swannack	ERDC	Jake Jackson	BIO-WEST	George Ward	UT

Objectives: Review Power Point for presentation to HCP Science Committee
Briefly review progress on model development (in the course of the above)
Respond to questions from Dr. Conrad Lamon, new member of Science Committee

Discussions:

Ed remarked that it is important to elicit information from the HCP Science Committee at this meeting, because we will need to submit a draft scope of work for next year's efforts to EAA in early March and need to have the Committee's buy-in on what we propose.

Ed has synthesized a (draft) power point from the slides & graphics contributed by the team, and led the discussion of its contents. He will open the presentation with introductions of the team members, and a quick overview of the project. He suggests that the slide showing key decisions made thus far be presented next to set up the individual presentations of the team.



Thom noted that decisions were made to use the modeled max temperature and min DO as the water-quality parameters, though these might not be regarded as “key” decisions. Also, pH was represented as important in Rose’s analysis, but we are not modeling pH.

Todd remarked that he and Thom are still working on how exactly to formulate scour and doesn’t believe that it has yet risen to the point of being identified as a “key” decision. This item was removed from the “key decisions” slide and added to “Key questions”.

Some discussion of how to present agent-based modeling. It was suggested that a slide enumerating the advantages and disadvantages of the method would be useful. Bill cautioned that we’re not using a compartment model framework.

Discussion on how much detail Thom should go into on his slides. A depiction of the 1-m grid for the old channel reach would be useful. Also some information about the utility of changing to the 1-m grid versus the 0.25 m-grid intrinsic to the hydraulic model.

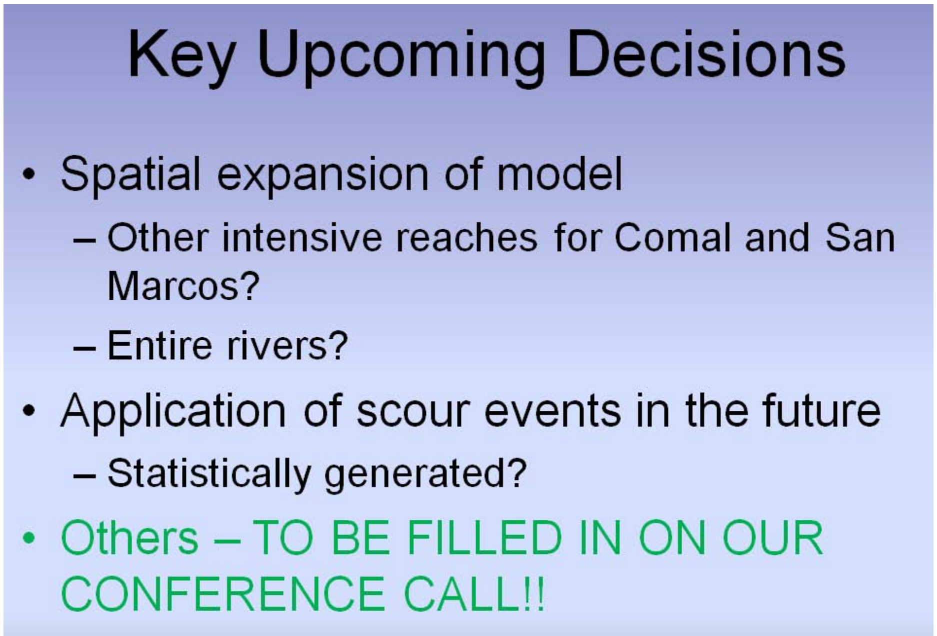
Robert may discuss carrying capacity in terms of percent cover, how the lab results enable us to link the observed vegetation densities to the modeled biomasses. He expressed concern at the use of numeric categories for persistence. These are qualitative classes, not quantitative, and the numeric designations are easily misinterpreted. He suggested “high”, “moderate”, “low” etc., instead.

On the SAV slides, Todd suggested adding bullets to identify those factors we are awaiting that will be determined by 2015 research studies. Todd has created animations of the SAV model, but at present it is literally like watching grass grow, because the development of vegetation in response to external controls takes place slowly.

The food source evaluations will be handled by Jake. His slides were considered fine by the team, and no further alterations were proposed.

The fountain darter work (along with the supporting habitat quality analyses) is considered to be the meat of the presentation. Rose and Bill went over their slides. While it is possible to prepare some movies showing the simulations, it’s Bill’s opinion that the movies aren’t terribly interesting because to the untrained eye there is not a lot happening.

The final slides will review the key decisions again, as a summary, followed by the key questions (or upcoming decisions), which is where we need the comments of the Science Committee.



These key questions include how to incorporate scour, how to handle recolonization after scour events. Thom suggested scenario development as another upcoming decision, which led to a discussion of different kinds of model scenarios serving different objectives, e.g. model validation, long-term simulations, critical hydrological conditions (drought), testing of the HCP flow goals, etc.

The presentation will then conclude with the overall schedule for the rest of the project.

At this point the conference call was joined by Alicia Reinmund, Bob Hall and Rick Ilgner of the EAA staff, and Dr. Conrad Lamon, and the remainder of the conference addressed questions of Dr. Lamon. His principal concerns were:

(1) discontinuation of fountain-darter sampling in bare areas
(2) details of the multicategory (logit) analyses by TAMU
(3) lack of attention given uncertainty

After much discussion, Dr Lamon indicated that (1) he still has reservations about not sampling bare areas, but will discuss further at some point; (2) he needs to study the details of the logit model and its application in this project. The fact that it is not THE model for fountain darters but is merely one component, viz. the basis for habitat quality definition, seemed to mitigate his concerns. With respect to (3), the team has been concerned about quantifying and expressing uncertainty since the outset of the project. The fact that it is not yet explicitly addressed does not mean that we are neglecting it, but rather that the fundamental deterministic models (for SAV and darters) need to be developed as a first priority.

MEMORANDUM

SUBJECT: Ecomodeling team meeting, Nagle Hall, Texas A&M University, 1200 CST 26 February2015
FROM: Ed Oborny and George Ward, scribes
DATE: 5 March 2015

Attendance:

Bill Grant	TAMU	Thom Hardy	WSG
Rose Wang	TAMU	Ed Oborny	BIO-WEST
Jake Jackson	BIO-WEST	Tim Bonner	TSU
George Ward	UT		

Objectives

The principal objectives of this meeting were (1) review the comments of the HCP Science Committee following the presentations made last month, (2) to review status of the modeling efforts, especially the goals to be met by May, and (3) identify the work elements that need to be addressed within the forthcoming contractual period, for incorporation into the draft scope of work due to EAA on 1 March.

1. HCP Science Committee comments

Apart from Dr. Lamon, the Science Committee's comments were brief, to-the-point and will not entail a substantial effort for response. We need a short (two-sentence, say) response on the use of agent-based (IBM) models, attach the list of references from Todd's presentation, plus Ken Rose's latest publication (K. Rose et al., 2015: Best modeling practices, etc. *Ecol. Mod. 300*, 12-29). This should satisfy this concern of the HCP Science Committee (HCPSC).

QUAL-2E is the way to go with water quality at this point. With this many other aspects of the model, we don't know the relative importance under critical conditions. Once we start running the entire model, we'll know better what factors prove to be controlling and require further study and/or alternative models.

Data range of model simulations is 1 April 2003 through 30 November 2013 (which captures the vegetation surveys performed in spring and fall). We can run any subperiod, according to model development and testing needs. The apparent paradox that we are not modeling pH and conductivity even though these emerged as statistically significant in the analyses is due to this significance arising from anomalous events, e.g. storm hydrographs, probe failures, etc.

During the HCPSC meeting, Dr. Arsuffi pressed for bioenergetics studies to confirm that food supply is not presently limiting for the fountain darter. It's hard to justify the expense of this, since our back-of-the-envelope calculation looked only at amphipods (finding these to be at least

an order of magnitude greater than the estimated food requirements of the darter) without considering the variety of alternative food sources available.

Thom will call Dr. Jacqulyn Duke directly regarding her comment “that the model functions for flow pulses include two separate functions: one that inclues total discharge for each daily step”. Thom is convinced a phone conversation will alleviate any concerns on capturing flow pulses in the model.

Lamon’s comments may take more effort, but need to be limited as there is no point in diverting time and resources into alternative statistical analyses. One-on-one communication with Conrad may be the best response format. Basically, there are three strategies of response: (1) perform suggested stat analyses and report to the HCPSC by their 11 March meeting; (2) simply comment that we’ve studied the data and considered (and tried) various statistical models, consulted with experts on the TAMU statistics faculty, and are happy with the performance of the darter model with the present stat-based HQI; (3) ignore the comments. After some discussion, the team believed that (2) best represents our position and confidence in the results. Bill and Rose will draft a response by next Tuesday for review by the team.

2. Modeling

The team engaged in a lengthy discussion of the present status of the modeling. Unfortunately, Todd was dealing with a family health crisis and could not make the meeting, but he and Bill have had numerous exchanges in the past several weeks, so Bill summarized the status of the SAV modeling, and is comfortable that the model will be calibrated for both the Old Channel and City Park reaches by the end of May. The status of the darter model was described. Bill has run simulations on various points in parameter space, i.e. changing only one parameter leaving the others fixed, to determine sensitivity of the model. He showed numerous plots of darter density under these various parameter configurations.

Bill also noted that the new version of NetLogo is now available, which, among other things, was supposed to be much faster in execution than the previous version. However, the TAMU researchers are finding that this version is actually slower. Since they’ve had the model framework for only a few days, they have unable to determine the reason, and will be communicating with the program developers.

Rose led a discussion on the preparation of a manuscript to describe the multinomial logit data analysis and application in more detail. Rose will send a draft to the team in the near future. The goal for the team is to review and fill in the sections assigned to each member by the end of March. Suggestions for journals to submit this manuscript are also welcomed by Rose.

Bill and Rose need to verify that they have all of the input data for the San Marcos test reach. This is not clear in their records. Thom and the TAMU researchers will work this out.

3. Scope of Work for June 2015 – December 2016

Need paragraphs putting forth work statements and costs estimates for the next 18 months starting 1 June by next Monday, when Ed is required to submit the Year 3 proposal.

Spatial expansion of the model. Upon consideration and contemplation of HCPSC comments, the project team made the decision to expand with two additional study reaches in the Comal system and one in the San Marcos supported by the following rationale.

Comal System: Expand to include the Upper Spring Run study reach and Landa Lake study reach. The Upper Spring Run reach is the most likely reach to first experience impacts related to low-flow conditions and has already experienced flow-related impacts during recent drought conditions. The Landa Lake reach has been the most stable habitat over the last 15 years and is presumed to remain the same as it will likely be the last water body protected under extremely low-flow conditions. As the Ecomodel objective is to test the applicability and protection of the HCP flow regime, both ends of the spectrum appear most appropriate.

San Marcos System: Expand to include the I35 study reach. This allows for one study reach upstream of Rio Vista Dam (City Park reach- in progress) and one below. The upstream reach provides an index for conditions experienced more near Spring Lake dam including more consistent water quality yet a high level of recreational activity. The downstream reach (I35) provides conditions further away from the source including increased water temperatures, increased turbidity, etc. with somewhat lower recreational pressure.

There will be two reports emerging from this work: a final quasi-technical report documenting the model development and scientific bases, and a brief users manual that will enable EAA or its contractor to set up the model and run it for a specific scenario. Thom observed that we will need one complete scenario set-up and execution for the user's manual to serve as a demonstration case. Which such scenario needs to be given some thought in the next scope of work. It is important that the final report for this project include a section on model deficiencies and recommendations for additional work.

There was much discussion of the fact that relative to other HCP activities, this project is underfunded and on an ultra-aggressive time scale. Ed reviewed the political issues surrounding the present budget and schedule. As such, the team carefully considered the Year 3 scope, schedule, and budget in order to meet the goals of the HCP ecomodel as well as the time and budget constraints. Should the Year 3 Scope proposed by the Ecomodel team be accepted, all should be good. However, if additional activities are requested by the HCPSC, NAS reviewers, Implementing Committee, or Stakeholders, the project team will be required to adjust the Year 3 budget and likely schedule request.

Other business
The next team meeting will be 24 March at BIO-WEST offices in Round Rock. Due to schedule conflicts in the morning, the meeting will start a 1pm. Ed will send a notice and draft agenda approximately one week prior to the meeting.

Postscript

In the days following the above meeting, additional exchanges took place via e-mail concerning the role of SAV data and the SAV model in calibration of the darter model. These discussions are transcribed below:

From: Edmund Oborny [mailto:eoborny@bio-west.com]
Sent: Friday, February 27, 2015 10:23 AM
To: William Grant; hsuan006@neo.tamu.edu
Cc: Swannack, Todd M ERDC-EL-MS; Ward, George H; Doyle, Robert D.; Edmund Oborny
Subject: Ecomodel conversation with Todd

Hi Bill and Rose,

I had a great talk with Todd this morning who assures me he will have a working SAV model for the Old Channel of the Comal by the end of March, and subsequently one for the City Park reach of the San Marcos by end of May.

I encourage both of you and Todd to have frequent communication over the next several weeks and months to hammer all this out including the ultimate linkage from the SAV model to the FD model that is scheduled to be in place by the end of May as well.

As such, my recommendation is to increase communication and incorporate Todd's Old Channel SAV model when it gets to you in late March, rather than embarking on the interpolation/ smoothing vegetation exercise that we discussed yesterday.

Are you cool with this George?

Cheers!

Ed

From: George Ward, UT (gward@utexas.edu)
Sent: Friday, February 27, 2015
To: Edmund Oborny; William Grant; hsuan006@neo.tamu.edu
Cc: Swannack, Todd M ERDC-EL-MS; Doyle, Robert D.; 'TH31@TxState.edu'
Subject: RE: Ecomodel conversation with Todd

Hi Ed and Bill and Rose and Todd –

I'm not sure I'm cool with it or not. (How's that for equivocation?)

Ultimately THE model is the coupled SAV-Fountain Darter model, and it is the performance of that model that we are ultimately concerned about.

But as an intermediate step in model development, we need to validate* the SAV Model against vegetation-survey observations, and validate* the Fountain Darter Model against darter abundance (and maybe size-class) data.

We don't expect the SAV Model to nail the observed veg distributions exactly, and we don't expect the Darter Model to nail the observed abundances exactly. However, when we validate the coupled SAV-Darter Model, any prediction errors in the simulated SAV will be passed on to the simulated darters, in addition to whatever errors are introduced by the parameters of the Darter Model itself. If we try to wire around the intermediate step of separately validating the SAV and Darter Models, moving directly to the coupled model, then Bill and Rose could potentially be ripping their collective hair out trying to match the darter observations by manipulating Darter Model parameters, when a substantial error is arising from the SAV model that they have no means of adjusting.

The advantage of validating the Darter Model using the observed SAV data is that this source of error is eliminated, and Rose and Bill can quantify the model parameters based entirely upon the processes of metabolism and movement. I think this advantage is huge, and to skip over this step in the interest of saving time will simply be making the validation task harder.** But there are issues.

* I know Dr Grant does not like this term, but for the short duration of this e-mail let me use it to describe the general processes that we are calling "calibration" and "verification", to avoid getting into the minutiae of matching data, versus assessing model error, versus diagnosing said error, versus investigating means of revision. If this still causes indigestion in some members of the team, simply replace the word "validation" with the word "calibration", and take a healthy dose of Pepto-Bismol.

** Indeed, one of the first diagnostic tests that Bill and Rose will almost probably make is to run the darter model with observed veg data, somehow rendered as a continuous input, in order to isolate the errors in the Darter Model from the errors contributed by the SAV Model. So we really can't avoid this intermediate step though it is debatable when in the validation process it should be done.

One issue that confronts Bill and Rose is that we have veg observations only every six months or so (i.e., they are "sparse" in time), and have no knowledge of exactly what those veg distributions look like in the intervening periods. To assume that they are constant for months then abruptly shift to another value is patently unrealistic, and moreover the sudden shifts in veg will induce numerical transients in the behavior of the simulated darters that may dominate the errors in darter behavior. The problem is how to render the observed data that are sparse in time as a more realistic time signal input, which will minimize corruption of the simulated darter behavior. There are two strategies on the table:

(1) Use the "calibrated" SAV Model of simulated veg as inputs to the Darter Model.

(2) Use some kind of artificial smoothing of the quantum jumps in the observed veg time signal to make it more "realistic".

Strategy (2) could be done simply by using, say, a multi-point sliding average prior to and following each quantum jump. I understand that Rose and Bill also have a more sophisticated smoothing scheme they have used before, but it will take more time to implement and test. Strategy (2), if successful, will eliminate buggy darter behavior by removing the quantum jumps in veg distribution, but will not necessarily create a "realistic" time distribution in the intervening period.

Strategy (1) is in fact operation and validation* of the Coupled Model, which I'm concerned about for the reasons given above.

I'm wondering if there is a way to create (3) an interpolative time series that responds correctly to seasonal forcing, as a hybrid of (1) and (2), which we should consider. Todd, what about making stepwise simulations with your SAV model, or maybe some stripped-down version of it, to act in effect as an interpolator between two successive surveys of vegetation? For each pair of data you might proceed as follows. Use the earlier survey data to initialize the model, then integrate forward to the date of the later survey. Force the model so that it predicts the later survey results exactly (or close to). Each pair of successive surveys are treated independently of the others, so how the model time signal is forced to pass through the two data points applies only to that pair. The results from applying this to all of the surveys is a smoothly varying time signal that passes through each veg survey data point. This would then serve as the input to Bill and Rose's model. The nice thing about this approach is that your SAV model should respond to seasonal changes in insolation and turbidity, thereby creating a more realistic transition from one survey to the next.

I'm sweeping a lot of detail under the carpet. The "data points" above are in fact % coverage of each species for each 1-m square model grid. Does this mean that you have to manually adjust

* See previous footnote.

the parameters for each grid cell and each veg species? Geez, I hope not. I'm hoping that a quick algorithm could be written that extracts the time signal for each species at a grid cell (as a vector of values for each time step between the surveys) then algebraically scales this time signal to pass through the first and second survey values for each pair of surveys.

I'm also aware that each of the smoothing approach (2) and the hybrid approach (3) is making two grand assumptions:

(A) The observed veg data is without error

(B) The range of veg cover is limited to what we actually observed

The first (A) is not a big problem. Typically, we go into the validation* task making this assumption, then after the model is calibrated do an *a posteriori* uncertainty analysis that includes estimating the standard error in the data. But (B) is a different matter. The surveys probably do not capture the entire range of the % cover. By limiting the synthesized time signal to this range may be introducing a substantial error. The SAV Model on the other hand, when fully validated, will track each veg species as it responds to seasonal changes in sunlight, turbidity, water quality, etc., and the simulated % cover may exceed the surveyed values (say, in summer) or be less than the surveyed values (say, in dead of winter).

I guess I'm wondering whether there is something to recommend this intermediate step and the resulting provisional validation* of the Darter Model as a useful exercise that will ultimately move us closer to a validated Coupled Model, rather than attempting to validate the Darter Model using simulated veg distributions which may themselves have prediction errors. Should Bill and Rose be carrying out this provisional validation* in the interim, by either strategy (2) or strategy (3)? Or should we be cool with waiting for Todd to validate his SAV Model then use its simulated veg distributions to drive the Darter Model for validation?

I'm not sure I'm cool with it or not.

--- George

From: William Grant <William.Grant@agnet.tamu.edu>
Sent: Sunday, March 01, 2015 12:55 PM
To: Ward, George H
Cc: Edmund Oborny; hsuan006@neo.tamu.edu; Swannack, Todd M ERDC-EL-MS; Doyle, Robert D.; TH31@TxState.edu
Subject: RE: Darter Model Validation versus SAV surveys

Hi George:

Thanks so much for your, as always, most thoughtful comments (so, I'm being absolutely serious about that). Also, if I may continue in an atypically (for me) serious mode for just a moment, two thoughts occur to me.

First, the veg signal that we currently are using to drive the model has served a useful purpose in that it demonstrates the simulated population's ability to respond to abrupt improvement and deterioration of their habitat, which is superimposed on their "normal" seasonal fluctuations in density and stage structure, which is more clearly seen during times when the veg is not changing. So this has been a good exercise.

Second, now that we have the quantitative link between the veg types and darter densities (the results of Rose's statistical analyses) in good shape, we are in the process of adding code to the model that will allow us to sample the simulated darter population with drop nets at the times and places (i.e., in the veg types) that correspond to the field samples collected in the Old Channel.

We also are re-organizing the drop net data files to facilitate the comparison of simulated and field data, and are double-checking that we have the completely updated time series of water depths, velocities, temperatures, and flow rates read into both the Comal and San Marcos versions of the model (thanks Thom for your most recent contribution in this regard!). The testing of this new code for drop net sampling the simulated darter population is independent of the manner in which we generate the veg signal (although, of course, the results of the comparisons of simulated and observed darter densities will depend on the trajectories of the simulated darter population as it passes through these sampling times and, thus, the results of the comparisons will remain tentative until we have coupled to the "real" veg model).

My point with regard to this second thought is simply that, given the new code will not generate and check itself spontaneously nor instantaneously, regardless of the team's decision on how to proceed, Rose and I probably should not focus our main efforts on developing an interim veg model for at least a little bit longer anyway. So, while I agree completely with the points you make, perhaps the team has at least a bit of time to ponder the decision (and for Todd to work!).

Well, with that, I'll slip back into my normal mode of communication – and leave you with the image of the rest of us pondering (in appropriate surroundings, sipping (or chugging, as the case may be) appropriately mind-freeing, creative-thought-provoking beverages) while we watch Todd slaving away (trying to find devilishly hidden bugs) with the veg model. Hope you are having a nice weekend (are you in The Cave? Someone told me that's the name of a pub just across the street from the UT campus, is that right?)

– Bill

MEMORANDUM

SUBJECT: Ecomodeling team meeting, BIO-WEST offices, Round Rock
1300 CDT 24 March 2015
FROM: George Ward, scribe
DATE: 1 April 2015

Attendance:

Bill Grant	TAMU	Rose Wang	TAMU
Ed Oborny	BIO-WEST	Jake Jackson	BIO-WEST
Tim Bonner	TSU	George Ward	UT

Thom was committed to budgetary meetings at Texas State. Due to a mix-up in scheduling, Todd had a conflict with today's meeting. Since SAV modeling would not be discussed, Robert opted out. The emphasis of today's meeting was therefore on the fountain darter modeling and related statistical analyses.

Agenda

1. Review the responses to the Year 3 scope from the Science Committee and the Implementing Committee.
2. Discuss the draft report of the National Academy of Sciences (NAS) review report, which has recently been made available to the team.
3. Review the status of the major components of the team effort, *viz*. the fountain darter model, the SAV model and the water quality (WQ) model and data transfer.

1. Presentations and meetings

During the past two weeks, Ed has given three presentations on the project, to the HCP Science Committee, the HCP Implementing Committee (IC), and EAA's Research and Technology Committee (R&TC). The IC and R&TC went fine. The Science Committee presentation generally went well, particularly given the absence of country-western dancing in the next room, except for some concerns with the uncertainty analyses. Dr Lamon did not appear satisfied with the Ecomodeling Team responses to his questions, and his dissatisfaction mainly focused on the Team's not exploring more multivariate analyses before settling on the logit approach. Some of his concerns may be due to an apparent misunderstanding that the statistical model is the only model for fountain darters, rather than a method for formulating the habitat quality component of the much more involved Individual-Based Model (IBM) of the darter population. Bill and Rose should have some one-on-one discussions with Dr Lamon to try to resolve these differences. Jake has done some experimental runs of some of the alternative analyses suggested by Dr. Lamon, finding essentially the same suite of external variables as emerged from the logit analyses. Also, there were some rumblings over the conclusion that invertebrate density (as food) is not limiting for the darter.

Danny Reible (NAS Committee Chair) also made a presentation on the results of the National Academy of Sciences (NAS) review at the IC meeting, which is discussed in the following section.

2. NAS Draft Report

The section of the NAS draft addressing the state of the ecosystem modeling was fair and helpful. Of course, the NAS suffered from the disadvantage of carrying out its review early in the modeling process, so there were few concrete results to be reviewed.

During the IC meeting, Dr Reible presented a summary of the findings of the NAS, in which he emphasized and/or fleshed out some of the recommendations on the ecosystem modeling. One of these was to express concern of the NAS that the fountain darter modeling will be completed within the specified 18 months, and recommended that the habitat suitability modeling be updated as back-up in case the darter model is not ready. The team had a rather negative reaction to this, not the least because the habitat suitability approach does not yield robust results for the HCP-specified flow regime. The NAS (according to Dr Reible) recommends that the ecomodeling team convene a “workshop” of experts to input to the process. One or two members of the SC (at their subsequent meeting) embraced this and amplified it to suggesting such workshops on a regular (1-2 month) basis.

3. Status of darter modeling

Although Thom Hardy could not attend today’s meeting, he sent the following summary of his status via e-mail to the team:

> Basically, I have given Bill/Rose/Todd all the daily flow values for the Old Channel and City Park. The daily minimum, average, and maximum water temperatures for the simulation period of record and all the vegetation maps spatially joined with the underlying hydraulic grids in NetLogo format. I am working on the technical memos for the revision in the Old Channel hydrology and the hydrology for City Park for the project notebooks. There is still a small technical issue on generation of flow rates (+/- for new scenarios) in the Old Channel I need to think through for 'future conditions' that may by different that just the estimated flows for the calibration and simulation period we are currently using. I am also working on a technical memo on how I estimated the daily minimum, average, and maximum daily temperatures at both City Park and the Old Channel while that is still fresh.
>
> I am now focused on the continued recalibration of the Qual2E model for both systems to permit simulation of the period of record. At this point there are no DO excursions that I found in reviewing the available WQ data provided by Ed (or my data from the San Marcos) that impact any of the vegetation or darter limiting factors in the models for the calibration or validation work at this point so the simulations from Qual2E are not needed in the 'short term' (read next few week). I suspect I will finish my re-calibration for temperature and DO in the next couple of weeks and then work with Bill/Rose/Todd on

passing the simulation information to NetLogo as needed. This will be basically a flat file format that Bill/Todd can read into NetLogo that assigns the temperature(s) and DO at each computational node on a daily basis for whatever period is simulated in the water quality model. The setup, simulation, and then parsing of the data will all be handled via the WQ utility tool that I am working on once I am happy with the calibration runs for each system.

The remainder of the meeting was devoted to review and discussion of the fountain darter modeling. Bill and Rose have successfully run the model for the old channel (with placeholder vegetation data) for the period 2004-2014. They displayed the plotted results from this exercise together with the field data. Only one darter variable was used as a calibration parameter, namely the time assigned for a darter to be in poor habitat before expiring (either to predation or stress). Several suggestions were offered by the team: (1) incorporate the old channel data for 2000-03 into the input files and re-run. (2) Plot each vegetation type separately. (3) Superpose the measured darter densities. (4) Though time out of suitable habitat is an available variable, a similar exercise should be made with the other darter movement parameters, independently. This could lead into a sensitivity analysis for each one. (5) Are we seeing a density response to the distribution of veg types? (6) Select a shorter run period, e.g., 2 years, and make repeated replicate runs to quantify the variability latent in all of the various probability values (now generated by random number). Todd reported to Ed that work is progressing well on the SAV model for the old channel, and it is expected that by the next meeting, the SAV should be incorporated into the fountain darter model.

Rose & Bill plan to consult, again, with the statisticians at TAMU with regard to the questions raised by Dr Lamon. As noted above, Jake will try some of the alternative stat analyses. Tim will run a Q&D principal components analysis (a.k.a., empirical orthogonal function depiction). All of this needs to be written up in a format suitable for incorporation into the final report for more detailed review by the team.

Other business:

The next team meeting will be 12 May at BIO-WEST offices in Round Rock starting at 10 AM.

MEMORANDUM

FROM: George H. Ward, scribe
DATE: 31 May 2015
SUBJECT: Notes on teleconference, 1000 CDT 5 May 2015

Participants:

Ed Oborny	BIO-WEST	Bill Grant	TAMU	Tim Bonner	TSU
Rose Wang	TAMU	Jake Jackson	BIO-WEST	George Ward	UT

Objectives: Respond to questions from Dr. Conrad Lamon
Achieve statistical enlightenment

Discussions:

Alicia Reinmund-Martinez (EAA) has had conversations with Dr Lamon and boiled his concerns down to three questions:

1. What was the rationale behind aggregating the data into the categories 0-5, 5-15, 15-30, >30? Was it to address the zero FD density numbers?

2. Where has the multinominal logit model been used with single species count (or density) data? Examples?

3. Please provide a description of the data used for the FD model development.

She requests that the team formulate responses to these questions for transmittal to Dr Lamon.

With respect to (1), the consensus was that these categories were matters of judgment, based on examination of the count data. Speculations were offered that a sensitivity to the specific categories might be of use, testing whether the same forcing variables emerged with different categories. Tim expressed particular interest in the 0 category from a presence/absence viewpoint.

Rose needs to tighten up the description of the data in response to (3). Perhaps her Table 1 with additions from Tim would suffice. Thom Hardy was unable to participate in the teleconference but sent the following comment concerning question (3) via e-mail:

> One meter hydraulic computational grids with predictions of depth and velocity at simulated discharges were derived from previously calibrated and reviewed hydrodynamic models for each system clipped to the spatial extant of each study site.

Hydraulic grids were spatially joined to available vegetation/substrate polygon maps on a seasonal/yearly basis for each study reach.

Daily flows in the Old Channel were estimated from a combination of total Comal Spring flows and spot measurements within the old channel. Daily flow values in the San Marcos River were derived from measured data at the USGS gage at the University Bridge.

Minimum, average and maximum daily water temperatures for each study site were derived from thermograph data and missing data either interpolated from adjacent hourly data and/or from relationships with hourly air temperatures from the San Marcos and New Braunfels airport weather stations.

Ward will provide some literature citations in responses to (2).

The discussions then turned to statistical models and finishing up the statistical foundations for specifying habitat quality and darter behavior. Rose/Bill and Jake will be exploring alternative analyses. There is no expectation that this will change any of the identifications of primary external variables, because we've already been down that road.
We're really doing these alternative analyses as a matter of documentation of our earlier decisions. It was noted by one cynical voice, probably your scribe, that having to document everything in this manner is not consistent with the need to work in the most efficient and speedy manner in order to stay on schedule.

It was noted that we need to start all of these alternative analyses with the same variable list. And we should include substrate. Jake suggested that we could use the newer (2015) data to test predictive power of some of these models. Some discussion followed about specifically how to treat the "with bryophyte" category.

The teleconference concluded with the reminder that we will be having a team meeting in one week, when some of these matters can have additional airing.

APPENDIX

The discussion begun in this teleconference continued through an exchange of e-mails. For completeness, they are archived below:

From: Jacob Jackson [mailto:jjackson@bio-west.com]
Sent: Tuesday, May 05, 2015 1:48 PM
To: Bonner, Timothy H; Hsiaohsuan Wang; Ward, George H
Cc: Edmund Oborny
Subject: Revised data

Howdy everyone,
Here are the re-re-refined dropnet data. It is the same as the previous version, with the removal of the bryophytes within bryophytes. If you detect any additional issues, let me know and I'll fix them. Tim, I left the missing data missing, as I do not intend to impute missing values for my analysis. See y'all next week,

Jake Jackson
Bio-West, Inc.

From: Bonner, Timothy H <TBonner@txstate.edu>
Sent: Tuesday, May 05, 2015 3:09 PM
To: Jacob Jackson; Hsiaohsuan Wang; Ward, George H
Cc: Edmund Oborny
Subject: My thoughts on missing data

Summary of missing data (Comal River) is below. Note: One missing column point kicks out the entire row (a drop net).

In the Comal River, 56 rows (5% of the data) are missing non-essential data: two without depths, the rest missing dissolved oxygen, specific conductance, or pH, and a few are missing substrate. If deleting, all of 2012 Fall collection will be lost because of 1 wq measurement missing. Information on 1,279 darters will be deleted (6% of total darters).

Cost per drop net is fairly expensive. Forgetting to take a dissolved oxygen measurement negates the entire sample? Maybe if I want a quick way to work through the data. Absolutely not if my crew and I busted our butts to collect the data and one field hand forgot to record pH.

Folks conducting field research and measuring a lot of data are very much aware, under the best of circumstances, that missing data happens. Of course, we would kick out a row if we didn’t count the darters. Or, didn’t record veg type, veg amount, depth, substrate…etc. But one missing point?

There are simple ways to handle this (which are established in the literature). For one, add in the average of the column. This datum point will be a no effect, while allowing the other points within the row to matter. However, we can do even a better job of estimating the missing point. Add in the average but just for the one site. Substrate is pretty easy to estimate as well.

It's all about credible estimations. I already filled in the missing data and didn't have any issues (made the changes in red for transparency purposes). If I did feel uncomfortable about an estimate, then I could always delete the row.

Inserting missing data does open ourselves up for criticism ("you can't make up data") but we can provide a decent estimation and justification in most (all) cases. After all, even our depth measures are an estimation. 0.83 meters is not the true depth but an estimation. True depth would have a bunch more decimal places.

Loss of 5% of the data and 6% of the fish is troubling to me. I recommend taking the extra time and fill in the missing data. I can do this, with justifications (and a set of rules) in about 30 minutes.

Thoughts from others?

Timothy H. Bonner
Texas State University

From: Hsiaohsuan Wang [mailto:hsuan006@tamu.edu]
Sent: Tuesday, May 05, 2015 3:50 PM

Hi Jake & Tim,

Thanks for the data and detailed explanation, respectively. We would like to have Tim's updated data to run the analyses. Hence, we are looking forward to it.

Best,
Rose

From: Bonner, Timothy H <TBonner@txstate.edu>
Sent: Tuesday, May 05, 2015 4:05 PM
To: Hsiaohsuan Wang

Working on it now. I've got to work back through the spreadsheet and convert to prose to numbers. Once done, I'll work on missing data. Maybe later this evening or early AM.

Timothy H. Bonner

From: Bonner, Timothy H <TBonner@txstate.edu>
Sent: Wednesday, May 06, 2015 7:00 AM
To: Hsiaohsuan Wang
Cc: Jacob Jackson; Ward, George H; Edmund Oborny
Attachments: Ecomodel Revised 5 6 15.xlsx; Notes on Missing data.docx

All:

Revised data attached.

Column titles in Blue: Substrate as dummy variables. I inserted Substrate into the main data set. Other qualifiers (year, site, season) also converted to dummy variables but to the far left. These will not be used in the models, but I'll use them later to assess annual, site, and season differences among PCA scores.

I converted "silt of gravel" to silt (dummy variables, not the prose). Any problems with this?

About 100 rows were salvaged. I've attached notes, documenting the changes along of a description of rules used to guide changes. Also, I highlighted each change in the spreadsheet with red.

In all cases, I believe missing data were easily replaced with a suitable estimate. Nothing controversial in my opinion.

Tim

From: Bonner, Timothy H <TBonner@txstate.edu>
Sent: Wednesday, May 06, 2015 12:48 PM
To: Hsiaohsuan Wang
Cc: Jacob Jackson; Ward, George H; Edmund Oborny
Subject: RE: Revised data

Jake:

I'm finally comprehending what you were saying about % veg. It isn't percent of Veg X but percent of the dominant veg X. Hence, we can have open with bryophyte. Therefore open = >50% without veg. By no means does open = bare. This comprehension is important subsequent the interpretation.

We could have turn % Veg X into dummy variables. However, they are pretty much dummy variables any ways but with a little more information. As such, I say keep them as is.

Tim

From: Jacob Jackson <jjackson@bio-west.com>
Date: Wed, May 6, 2015 at 3:41 PM
Subject: Re: Revised data

That's cool, but I still can't help but think we should then ditch MainVegPer, since it is the exact same value within a dom veg type and I think it is confounding the result. I don't think recoding the VegX variables as P/A will result in any loss of information because the value (percentage) is still present and in a linear combination results in:

1 * beta *Percentage for presence, or
0 * beta * Percentage for absence

(depending on variable selection of course). I think this would allow for a better opportunity to discriminate among effects of specific veg species vs. simply percent cover. This is also more reflective of the sampling design, which is stratified where X number of samples are taken in each veg type each occasion with the goal of sampling as homogenous an area as possible. I think that this intentional stratification means that we are trying to coerce the percent cover values into a continuous variable for each species when it is not appropriate. That being said, y'all should be able to instruct me that I am off the reservation if that is the case. I know we need to gain traction so you guys let me know the consensus view so I can produce models comparable to yours.

From: Hsiaohsuan Wang <hsuan006@tamu.edu>
Sent: Wednesday, May 06, 2015 10:48 PM
To: Jacob Jackson; Timothy Bonner
Cc: Ward, George H; Edmund Oborny; William Grant
Subject: Re: Revised data

Hi Jake and Tim,

Thank you so much for explaining your points. On our end, we feel the opinions from both sides make sense. Hence, we will run both and check the performances of two models.

Best,
Rose (& Bill)

From: Bonner, Timothy H <TBonner@txstate.edu>
Sent: Thursday, May 07, 2015 8:45 AM
To: Hsiaohsuan Wang; Jacob Jackson
Cc: Ward, George H; Edmund Oborny; William Grant
Subject: RE: Revised data
Attachments: Correlation matrix.xlsx; Presentation.pptx

Jake et al.:

Attached is a covariance and correlation matrix for the Comal River. Since MainVegPer (VegPer) is summed across all plant types, it is not highly correlated with any particular veg type. It varies little with any Veg X. Does this addresses your concern?

Note that MainVegPer is highly correlated with Open. Open might be a candidate for dropping, since we can estimate Open with high accuracy if we know MainVegPer. PCA handles high redundancy among a few variables very well. Hence no real benefit in dropping (% variation explain could go from 20% to 22%).

With these thoughts in mind, I'm viewing PCA has an exploratory tool to understand gradients among our data (site, season, year, things not necessary to address in the GLM but useful for the biology of the system) and therefore complements (sets up) the GLM. Each model does something a little different with the data. As such, we should not force all of the parameters into each model. Symmetry of models is not important because we are not comparing which model is "better".

PCA can handle all parameters. I'm using all. GLM? Develop the most logical model possible, which could include dropping a few parameters. My suggestion is let the GLM dictate direction. Develop the most parsimonious model as possible, for the benefit of predicting Fountain Darter abundance to be used in the simulation model.

Attached (Presentation) is the revised set of tables/figures for the report (first six slides) and a step by step PCA analysis (for our meeting next week or for the Science Committee). Between now and our next meeting, I'll work on the report/publication.

One crazy thought (to further elucidate benefits of complementary stats or demonstrates my lack of understanding for the simulation model):

> Step 1. Use only the parameters with high loadings (Bold in Slide 2) on PC I and II in your GLM (9 parameters in Comal and 12 parameters in San Marcos River). Therefore, PCA was used as a parameter reduction technique (one of its purposes).
>
> Step 2 (alternative a): Add additional parameters deemed useful for the simulation model, such as DO and water temperature.
>
> Or,Step 2 (alternative b): Ignore additional parameters deemed useful for the simulation model. Do they really belong in the GLM? Can't the simulation model run with GLM plus additional rules, such as water temperature (min, max, optimum), Dissolved oxygen (min, max, optimum), others?

Always available for a phone call, if we need to think through this in real time.

Tim

From: Bonner, Timothy H <TBonner@txstate.edu>
Sent: Thursday, May 07, 2015 2:06 PM
To: Hsiaohsuan Wang; Jacob Jackson
Cc: Ward, George H; Edmund Oborny; William Grant
Subject: RE: Revised data
Attachments: Habitat and Abundance Report THB 5 7 15.docx

My revised report is attached. Feel free to use it as the start of our report or I can make it a self-contained chapter.

If viewing the attached as our report, all of my writing should be considered rudimentary (or concepts) at this point. Once more of the machine is assembled, more rounds of grooming will be necessary. I've included my Methods and Results, leaving in notes about Report (and ms) Intro and Discussion. I've included figures and tables for the publication. I started an appendix to add other tables, figures, smaller scale "chapters" (e.g., I started a "why use abundance categories instead of raw counts), which will be useful for the report but not necessarily for the publication. As such, we are not limited in what we include. In fact, I think we should include everything except the kitchen sink, but keep it organized: publication level material in the main report/ms, side stories, sub-analyses/plots/tables, and kitchen sinks in appendices.

I'll be in the field all day tomorrow but will be available over the weekend or next Monday to visit about any of the linear models.

Tim

From: Hsiaohsuan Wang <hsuan006@tamu.edu>
Sent: Thursday, May 07, 2015 4:08 PM
To: Bonner, Timothy H
Cc: Jacob Jackson; Ward, George H; Edmund Oborny; William Grant

Hi Tim,

We found a minor bug in your description. We designed the categories based on density (D) not abundance, so the categories are:

Comal:
1: D= 0
2: 0 < D <= 5
3: 5 <D <= 15
4: 15 < D <= 30
5. D > 30

San Marcos:
1: D= 0
2: 0 < D <= 2
3: 2 <D <= 8
4: 8 < D <= 15
5. D > 15

Rose & Bill

From: Bonner, Timothy H <TBonner@txstate.edu>
Sent: Thursday, May 07, 2015 5:23 PM
Subject: RE: Revised data

Thanks. I didn't catch it.

Do you recall why we decided on density (0.5 of abundance) instead of abundance? I vaguely recall to convert to square meter, so the drop nets are 2 sq meters?

From: William Grant <William.Grant@agnet.tamu.edu>
Sent: Friday, May 08, 2015 10:20 AM
Subject: RE: Revised data

Hi Tim (and All) – Yes, each drop net sample covered 2 square meters. I don't remember the date or the details of our discussion when we decided to use individuals per square meter rather than individuals per the 2 square meters encompassed by the drop net. But I am quite sure it was a group decision – perhaps someone can reconstruct the reasoning from their notes at that meeting.

Hope all is well – take care – and we'll see you next Tues. in Round Rock

– Bill & Rose

From: Hsiaohsuan Wang <hsuan006@tamu.edu>
Sent: Sunday, May 10, 2015 4:01 PM
Subject: Re: Revised data
Attachments: Results_based on updated data.docx

Dear All,

Attached is the updated results using the most current(modified/edited) data. If Tim and Jake would, please take a look. We can compare this with our previous results and Jake's results when we meet this Tuesday.

Best,
Rose & Bill

From: Jacob Jackson <jjackson@bio-west.com>
Sent: Monday, May 11, 2015 9:19 AM
Attachments: Darte_OverdispersionModels_revised0507.docx

Howdy everyone,

The results of negbin and zip analyses of the updated data are attached. Let me know if you have questions.

From: Bonner, Timothy H <TBonner@txstate.edu>
Sent: Monday, May 11, 2015 10:45 AM
Subject: RE: Revised data

Jake:

Follow up question: The multinomial logit model used by Rose is the negative binomial model?

Minor comment: "The response variable considered was counts of fountain darters in drop net samples collected from 2001-2014". Did you delete 2000 data before running your analysis or should the statement be revised to "net samples collected from 2000-2014"?

Tim

From: Bonner, Timothy H <TBonner@txstate.edu>
Sent: Monday, May 11, 2015 9:17 AM
To: Hsiaohsuan Wang; William Grant
Cc: Jacob Jackson; Ward, George H; Edmund Oborny
Subject: RE: Revised data
Attachments: Comal River all data and Cat 5 data.xlsx

Rose:

Thanks for sending.

Questions (perhaps for tomorrow's discussion):

1. Estimates listed in Table 2 by Category shows "strength" or "loading" of the variable. It is the slope with (+) or (-) indicating correlation? For example, Cat 5 silt estimate is 4.97. P-value <0.001. Interpretation is that silt has the second most "strength" or "power" in predicting Cat 5 densities of darters (Sand is first, with bryophytes third)? All three being positive means direct relationships with Cat 5 abundance?

Alternatively, maybe we can't interpret estimate as variable strength because variables differ in scale (range of cv: 0 – 0.4; range of conductivity: 443 – 755). If true, then magnitude of each estimate needs to be adjusted by the scale of the variable in order to interpret strength.

2. Loadings/variable strength/slopes were determined by regression using all of the data. Therefore, habitats with Cat 5 densities had relatively more sand, silt, and with Bryo than those without Cat 5 densities (Cat 1 – 4), correct? Same interpretation for all of the variables correct (e.g., habitats with lower pH [-2.231] had fewer Cat 5 densities than habitats with Cat 1 – 4)?

I'm attempting to clearly understand the meaning of each estimate, so I can compare back to the data (see attached) and understand how the model is performing.

For example, I calculated means of variables (and relative abundances for substrate) for sampled habitats (N = 90) with Cat 5 densities and Cat 1 – 4 densities. I inserted your model estimates and P-values (I only show the estimates with P < 0.05). I expected that positive model estimates (e.g., water temperature) would associate with parameters that had greater means for Cat 5 habitats than Cat 1 - 4 habitats. For Water Temperature (estimate = 0.53, P-value = 0.048), mean of Cat 5 habitats was 24.0; mean of Cat 1 – 4 habitats was 23.7. Not much difference hence estimate is fairly low compared to other
variable parameters. Makes sense to me. Most of the other comparisons (represented in green squares makes sense to me).

However, silt estimate was positive 4.97 (and if my interpretation is correct is positively related to darter abundance) and therefore should have a higher mean % silt than Cat 1-4 habitats. However, it does not. % silt of Cat 5 habitats was 39%; % silt of Cat 1-4 was 56%. There were others that didn't make sense to me (with red squares).

I would like to repeat comparisons for all categories (Cat 1 vs. the rest, Cat 2 vs. the rest, etc.) but wanted to make sure that I understand model estimates first so I have context for my exercise. This is part of using PCA and linear models to compare findings. I did not find much in associations between habitats and lower categories of fountain darters, so I'm trying to think through what the linear model is detecting.

Tim

From: Jacob Jackson <jjackson@bio-west.com>
Sent: Monday, May 11, 2015 9:19 AM
Subject: Re: Revised data
Attachments: Darte_OverdispersionModels_revised0507.docx

Howdy everyone,

The results of negbin and zip analyses of the updated data are attached. Let me know if you have questions.

MEMORANDUM

SUBJECT: Ecomodeling team meeting, BIO-WEST offices, Round Rock
1000 CDT 12 May 2015
FROM: George Ward, scribe
DATE: 2 June 2015

Attendance:

Bill Grant	TAMU	Rose Wang	TAMU
Ed Oborny	BIO-WEST	Jake Jackson	BIO-WEST
Tim Bonner	TSU	Todd Swannack	ERDC/USCE
George Ward	UT		

Agenda

1. NAS, Science Committee, springs condition
2. QUAL-2E model for DO, linkage to FD simulation model for Old Channel & City Park
3. SAV growth/dispersion model, calibration for Old Channel & City Park reaches, linkage with FD model
4. FD dropnet data statistics
5. Progress on FD simulation model for Old Channel & City Park
6. Responses to Conrad Lamon's questions, transmitted through EAA
7. Schedule, deadlines, next steps

1. NAS, Science committee, springs condition

Ed reported. EAA presently reviewing NAS report and preparing response/reaction. Not really relevant to the Ecosystem modeling work. Science Committee is still active.

San Marcos now flowing above average due to recent rains. Comal about 210-215 cfs, highest in three years but still well below the long-term average.

2. QUAL-2E status

Thom was unable to attend. He reported the following via e-mail (12 May):

> The visual basic .NET interface is perhaps half done. The user has to select either the San Marcos or the Comal and then selects which study site(s) they want to 'modify'. There then are several options available. Change the underlying hydrology by changing the input flows and/or change an existing sequence of flows by a factor (constant). They can also change/edit/input different meteorological data for the selected period of simulation.

The program will then 'run Qual2E' and parse the outputs to the appropriate input files needed by Bill/Rose and Todd.

Technical Issues. I am making code changes to the underlying Qual2E source code to recompile it to run under either a Windows 32 or 64 bit environment. The existing spawn and wait function within .Net works but Windows 8.1 onward won't allow the existing executable to run while Windows 7 will since it retained the WOW (Windows over Windows) for backward compatibility that was dropped in subsequent versions of windows. Dropped Bill Gates from the Christmas Card list. Anyway, recoding is going fine and from previous efforts like this, there will not be any compatibility issues with various Windows operating systems. The interface is already Windows version neutral.

3. SAV growth/dispersion model

Todd reported that he and Robert have had several fruitful meetings on the formulation of the SAV model. *Potamogeton* & *Vallisneria* biomass conversion now implemented. The model is operating on daily time step inputs, though the model calculations address diurnal solar inputs, takes data inputs of light (through date & latitude), temperature, depth, and outputs above- and below-ground biomass. The model is basically modular.

Turbidity is not an issue except after floods or around recreation areas. This is independent of flow and velocity so will have to be handled by some input procedure. An empirical relation of some sort will be needed.

Todd is still working on a relation between flow and production, which will affect some species (e.g., Texas wild rice). ERDC has done work in the past on velocity effects (boundary layer fluxes around stems and leaves) that might be adapted.

The opinion is that vegetation is always absent in certain areas due to either high velocities or tree shading. Substrate may also be involved (Todd is looking into this).

The next priority in model development is extending the model formulation to *Hygrophila* and other plants. Need to start exploring variation of plant coverage as a function of depth, velocity & temperature. Todd will put one of his stat staff on it.

4. Fountain darter dropnet statistics

There have been issues about the meaning of percent dominance in the data. Selection of a sample site is based on reach dominance of a given plant (or open/bare). The sampling design attempts to find a homogeneous region for the drop sample but in some instances this failed.

There were 19 cases identified in which the reach dominants did not exhibit sufficient abundance in the dropnet sample to be representative. The consensus was that these 19 data points (out of hundreds) should be excluded from the stat analyses.

The "w/bryophyte" category is valid only in the past five or so years. Earlier data have been "converted" to "w/bryophytes" whenever bryophytes were recorded. There was discussion about how this was done and whether it accurately reflects the field sheets. A few field sheets were pulled and found to be consistent with the entry in the data base, but it was decided that additional spot checking would be needed. Tim volunteered to undertake this, and consult with Jake who would supervise the retrieval of the field sheets & their interpretation.

Decisions:

(1) Main veg %
(2) 0,1 categorical variable
(3) veg code, 1 categorical variable
for vegetation (e.g., *Cabomb*a, algae, etc.)

5. Progress on fountain darter simulation model for Old Channel

The dropnet stat analyses were a natural segue into the fountain darter IBM work. Bill & Rose reported on its status. Modifications in the data base will entail some re-runs that may affect the habitat quality specifications for the FD model.

The categories of FD abundance have now been changed to separate a zero category. There was discussion about how "real" the zeroes are, i.e. do these mean absolute absence of FD's or merely sparse FD's. The consensus is that the sampling is carried in such a way that a zero recorded very likely means there are no darters in the sample.

- Rose – rerun – 3 way statistics
  - Main veg percentage and veg percentage
  - Main veg percentage and change veg to presence / absence
  - Main veg percentage – veg clumped into single variable
- Jake – based on Rose's rerun – rerun whatever he needs to

See Appendix below.

6. Responses to Dr. Lamon's three questions

1. What was the rationale behind aggregating the data into the categories 0-5, 5-15, 15-30, >30? Was it to address the zero FD density numbers?

> Jake will respond to this. (Note that we have recently changed the categories to separate the zero class.)

2. Where has the multinominal logit model been used with single species count (or density) data? Examples?

Ward's literature citations are considered adequate.

3. Please provide a description of the data used for the FD model development.

This stimulated much discussion. What exactly is he looking for? How much detail should we go into? Is a simple description of the data collection protocols and the variables measured sufficient? Should we provide a copy of the data base in spreadsheet form? Do we need to go into any of our stat analyses?

Perhaps because of the lateness of the day, we converged on (1) provide a description of the data, and (2) provide a preliminary (and brief) summary of stat analyses. Tim and Rose are to provide stat analyses & discussions.

Ward was tasked with pulling together the letter from these contributions.

7. Scheduled items

The next meeting will be 1000 CDT Tuesday 9 June at BIO-WEST in Round Rock.

Ed left for the South Pacific.

APPENDIX

Following the meeting some of the discussions were continued via e-mail. For archival purposes, these are included here.

From: **Bonner, Timothy H <TBonner@txstate.edu>**
Sent: Wednesday, May 13, 2015 7:37 AM
To: Jacob Jackson; Hsiaohsuan Wang; William Grant; Edmund Oborny; Ward, George H
Subject: Revised data 5 13 2015
Attachments: Ecomodel Revised 5 13 15.xlsx
Notes on data grooming Stats Model.docx

Attached is the revised data set (Tab "Revised 5 13 15"). I did not delete the two tabs with previous versions (for reference purposes), but I colored the cells red (hurts the eyes). Do not use.

On Tab "Revised 5 13 15", I converted percent veg as 0 and 1 (cells highlighted in color). I kept dominant veg type with percentages in place (different color). Rose wanted to run both scenarios. Column "Vegvolume" was deleted. Also 18 rows were deleted because they did not have a dominant veg type listed. Deleted rows are provided in a separate tab for viewing. Under Sample column, I reassigned sample numbers (sequential 1 – 1628; necessary because of row deletions).

Next steps (from notes of our discussion yesterday):

0. Verify that the revisions were done correctly and per our discussion yesterday. Did I forget something?

1. Jake verifies that wbryo is properly noted for all rows by spot checking a subset of the data (until confident that the column is accurate).

2. If ok or changes made, Jake will send back "revised 5 13 15" by only changing (or not) the wbry column.

3. Rose will run the stats model and let us know which model works best for her (veg as %, veg as dummy, dominant veg as a nominal variable). We will call this the full model. The reduced model (only the parameters needed by the Simulation Model) will be forthcoming and not part of the report due on June 3. Is this correct?

4. Jake reruns his analyses to address Q1 (Science Committee inquiry).

5. I will rerun PCA

6. Within the next couple of weeks, Rose and I will integrate our findings into a single report (Q3-Science Committee inquiry). Rose will provide Stats Methods, Results, Tables; Tim will Provide PCA Methods, Results, Tables/Figures. Can BioWest provide a paragraph or two (pulled from previous reports) on how sampling approach? I will then add a paragraph on “data grooming”, which will precede Stats methodologies.

George: For the record, I updated my “Missing data” file with additional notes on the latest data grooming (now called “Notes on data grooming Stats Model”).

Comments?

Tim

From: Bonner, Timothy H <TBonner@txstate.edu>
Sent: Thursday, May 14, 2015 8:33 AM
To: Jacob Jackson; Hsiaohsuan Wang; William Grant; Edmund Oborny; Ward, George H
Subject: wbryo update and revisions
Attachments: Ecomodel Revised 5 14 15.xlsx

Friends:

Jake ran a query on “wbryo” within the secondary veg type. He produced 156 rows of data. Compared to our existing data set, I found three rows contained “wbry =1” that shouldn’t be (SiteCodes: 389, 429, 739) and one row (Site Code 1645) that was “wbry=0” but should be “wbry=1”. I corrected these entries accordingly. The revised data sheet is attached.

Ed and Jake: Please check with original data sheets and confirm Sitecodes 389, 429, and 739 (more info is below about these collections) do not have “wbry”. This will be a double check of Jake’s query.

Another question: Jake also produced sitecodes 1741 and 1755 with “wbryo”. I assume that these are 2015 samples? Please confirm. If not, we are missing rows for some unknown reason.

View more checks and then the data should be good for analysis.

389	429	739
5/20/2002	8/6/2002	4/20/2005
USR	USR	LL
cl	cl	cl
H2	H1	L1
gravel	silt over gravel	gravel
Hygrophila	Hygrophila	Ludwigia

From: Edmund Oborny <eoborny@bio-west.com>
Sent: Thursday, May 14, 2015 9:01 AM
To: Bonner, Timothy H
Cc: Jacob Jackson; Hsiaohsuan Wang; William Grant; Ward, George H; J Hull
Subject: Re: wbryo update and revisions

Tim,

Data books show that your 3 site codes (389, 429, and 739) all have bryophytes.

Site code 1645 also has bryophytes.

In each case they were coded as Riccia which is a type of bryophyte.

Site codes 1741 and 1755 are October 2014 samples from Landa Lake. In Rose's original analysis, she only had data through Spring 2014, since we started this analysis process over a year ago. I don't know if this caused the discrepancy with those points.

I have cc:d Jeremy Hull as well as he will be the best person to double check numbers while Jake is out the next several days.

Cheers!

Ed

From: Bonner, Timothy H <TBonner@txstate.edu>
Sent: Friday, May 15, 2015 4:49 PM
To: William Grant; Hsiaohsuan Wang
Cc: Jacob Jackson; Ward, George H; Edmund Oborny; J Hull (jhull@bio-west.com)
Subject: Revised data
Attachments: Ecomodel Revised 5 15 15 430P.xlsx
Notes on data changes 5 15 2015.docx

Attached is the revised dataset. Bryo column was doubled checked. Some dropnets were missing. We added them back, but not the ones we agreed to remove. Missing cell data were estimated as before.

We randomly checked 3% of the spreadsheet data with the BioWest database. We had 100% concordance. No need to check further, in my opinion.

Specifics on data changes, additions, and double checks are listed in the word document.

Rose: The revised spreadsheet (the only one not in “All Red”) is available for your analyses.

Tim

From: Hsiaohsuan Wang <hsuan006@tamu.edu>
Sent: Friday, May 22, 2015 10:33 PM
To: Bonner, Timothy H
Cc: William Grant; Jacob Jackson; Ward, George H; Edmund Oborny; J Hull
Subject: Re: Revised data
Attachments: Results.docx

Dear All,

Attached is our results. In general, they all look good (models 1 and 2 and/or 3 in both springs). We have hard time to determine which one would be the best. Of course, we can simply run the AUC and check the performance. However, before we do so, we would like to have Tim's and Jake's opinions about which model makes better sense in fountain darter's ecology. Many thanks in advance. :)

Best, Rose

From: Bonner, Timothy H <TBonner@txstate.edu>
Sent: Sunday, May 24, 2015 7:06 PM
To: Hsiaohsuan Wang
Cc: William Grant; Jacob Jackson; Ward, George H; Edmund Oborny; J Hull
Subject: RE: Revised data
Attachments: Some observations and questions THB.docx

Rose:

Thanks for sending! I’ve attached some thoughts and questions to this email. Please review when you get a chance. Also, feel free to call and discuss. Some of the questions might be easier to answer over the phone rather than writing.

Thanks,
Tim

MEMORANDUM

SUBJECT:	Ecomodeling team meeting, BIO-WEST offices, Round Rock 1000 CDT 9 June 2015
FROM:	George Ward, scribe
DATE:	18 August 2015

Attendance:

Jake Jackson	BIO-WEST	Ed Oborny	BIO-WEST
George Ward	UT	Tim Bonner	TSU (in afternoon)

Todd Swannack (ERDC) and Rose Wang (TAMU) called in. Thom Hardy (WSG) was conflicted but sent in comments. Robert Doyle (Baylor) was sampling the rivers for effects of flood. Bill Grant (TAMU) had appointment with jury.

1. Springs and rivers conditions

San Marcos significantly flooded in May-June, in particular due to backwater from Blanco. Comal at 250, aquifer up 40 ft compared to last year at this time.

2. QUAL-2E status

Thom e-mailed the following status report:

> I have a working version of Qual2E executable that will run on Windows 7 or 8 versions of the operating system. I am working through a small technical glitch to allow installation without having to have Administrator privileges and then installing it to a directory structure NOT under Program Files since Windows 8 won't allow scratch files to be written to a sub-directory 'for security reasons', even if you have administrator privileges. Go figure, Billy G does not trust us mere mortals.
>
> The preliminary runs for calibration in both Comal and San Marcos are almost complete. At least as far as the lack of real calibration data we have to work with. I have started some simple test scenario runs to convince myself the results are at least what I think is reasonable for conditions we have no data for. For example, I turned off the sun and held the air temperature constant and the water temperatures did not change in the river. That is at least what one might expect although it could be argued that with no sun, we would likely freeze.
>
> The small interface is probably about a week from being completed now that I have an executable that can be used. I have not decided yet to use a spawn and wait versus a dynamic link library to call Qual2E from the interface. There are pros and cons to both and I am leaning toward the spawn and wait since I can trap for an execution error from the FORTRAN program and not blow the interface back to the desktop which happens with a DLL ling instead. I don't trust folks to not

abuse the interface and pass non-license data to Qual2E and I have no mood to error trap all the possible garbage combinations. Integration with Bill/Rose/Todd is trivial as I will just pass the DO to the same grid file input format they are using for temperature.

3. SAV growth/dispersion model

Todd spoke with Thom about velocity data and plans to have this factor incorporated into the SAV model soon. The literature shows velocity to be important to dispersal. The model is working for invasive species now (i.e.., Hygrophila, Hydrilla, etc.) but the parameter set can be further refined. Dispersal model is now process-based. However, the literature is inadequate on processes of dispersal, so we need to revert to empirical formulae. His staff at ERDC are working on this now.

Turbidity/recreation not yet in the model. Substrate plays a role and discussions with Thom about this factor are underway, particularly with respect to how to incorporate this into the model framework.

The SAV model is shifting more and more from a process-based approach to statistical/data-based approach. Dispersal is proving far more complicated than Todd thought it would be. By our July meeting we may need to be thinking about Plan B. Todd is putting statistical equations in the model now and the preliminary results are looking good. Also there is little change in the fountain darter model with the different stat relations.

4. Fountain darter model and dropnet statistics

Rose reported on the re-analysis of darter dropnet data (see memo for meeting in May). She and Bill tend to favor Model 1 because it retains more of the vegetation species. Jake noted that BIO-WEST didn’t actually sample all of these veg species other than opportunistically in sites dominated by other vegetation. The zeroes in the data, e.g., are not really measured as 0. Model 2 seems a better depiction of the actual sampling strategy. There followed an extensive discussion of Model 1 versus Model 2. In the San Marcos, Model 2 drops out Hydrilla and Potamogeton. Jake opined that this is because we are forcing the model to do this. Todd offered that Model 2 is easily defensible from model-selection theory. The consensus was that Model 2 is the favored depiction, and the Team made the executive decision to adopt that model.

5. Additional business: response to comments of Dr Lamon

The Team turned to a discussion of responding to the questions submitted by Dr Lamon of the HCP Science Committee. For Question 3 (see previous memoranda), it was decided to base the description of the data on the 24 July 2014 memo from BIO-WEST to Bill and Rose. A sidebar discussion was motivated by the question about the categories of darter density. It was noted

that the number of darters is not a proportionate measure of habitat. It is more meaningful to differentiate “a few” and “a lot”. Moreover, the precision of the count is not essential to the characterization. This needs to be communicated in our response.

Ed, Jake and George then undertook the formulation of a Memorandum response from the Team to Dr Lamon addressing the three questions he raised. The plan was for Jake to hand-deliver the memo at the EAA meeting later in the week.

MEMORANDUM

SUBJECT: Ecomodeling team meeting, BIO-WEST offices, Round Rock
1000 CDT 21 July 2015
FROM: George Ward, scribe
DATE: 13 August 2015

Attendance:

Bill Grant	TAMU	Thom Hardy	WSG	Rose Wang	TAMU
Ed Oborny	BIO-WEST	Todd Swannack	ERDC	Jake Jackson	BIO-WEST
George Ward	UT				

Tim Lewis, Gary Dick and Lynde Dodd of ERDC participated by conference call.

Robert Doyle (Baylor) was in the wilds of Brazil. Tim Bonner (TSU) was in the wilds of Port Mansfield.

1. Current status of Comal and San Marcos systems

Ed: Flow levels remain up due to the extensive rains in May and June. BIO-WEST went out to survey the San Marcos River the week after the Blanco flood. The San Marcos is flowing at > 300 cfs, about twice its long-term average. Comal River discharge did not get as high. New channel of the Comal River scoured some aquatic vegetation due to flows from Dry Comal. Flow now is about 320 cfs, above average and about five times last summer's flow. Because of the controlled release, the flow in Old Channel continues at around 60 cfs.

Thom: The gauge on San Marcos was up to 800 but this was all backwater from the flood on the Blanco, even took out Thom's experiment at Rio Vista. Some discussion about the role of backwater in creating "high water" events in the San Marcos.

2. General status of project work

Robert Doyle's work this summer is showing that *Ludwigia* will hold its own over non-natives such as *Hygrophila* in a flowing stream. Thom is finding similar results for *Potamogeton*.

Robert's team is installing 14 minisondes in the Comal system (including areas of dense algal mats) to monitor the detailed time and space variation of dissolved oxygen (DO). Just like last summer, Landa Lake is not maintaining a DO > 4 ppm on occasion in the early morning hours (as measured in a *Vallisneria* bed with a probe just off the lake bottom). The City of New Braunfels is using its Landa Lake aerators every night.

Is water temperature a problem? Thus far, no. Last summer, neither river exhibited an area where temperatures reached lethal limits for juvenile or adult fountain darters. Apparently the groundwater seeps appear to moderate temperatures. However, under the lowest HCP flow condition spossible, temperatures may rise, so it is a model variable.

Thom reviewed the QUAL-2E model status. For both systems the model can be off 0.5-1.0 ppm DO. The team regards this as good, since this on the order of the accuracy of the DO probes. There are a few spots needing additional work. The model DO in the flow from Spring Lake is lower than the measurements due to poor representation of in the spillway from the lake. Thom is completing the GUI interface. The user will be limited to changing flows in the input specification. Also, the relative contributions of the different springs will be hardwired and not available for change. At present these relative contributions are based upon earlier work carried out by Thom. The Team agreed that this relative contributions need to remain fixed in the model.

Now there is talk about removing Capes Dam. The team agrees that to include this in the model goes beyond our present scope. This will have to be considered in future work. (Further this dam location is downstream of the two model reaches.)

Thom expects to have the code finished and testing completed by the end of August. Can start sending template files to Bill now for incorporation into the fountain darter simulation model.

An interim progress report draft needs to be complete by 15 November. The Team decided to show a model run of the 13-year history.

3. SAV Modeling

Todd reviewed the status of the SAV model. Growth in the model is now modular. There are three different versions of the photosynthesis equations (mainly differing in which variables are included). Dispersal is improved but still needs work. ERDC is looking at additional attributes that would control the increase in biomass, e.g. limiting the number of runners per season. Code has been added to keep track of dispersing species.

Much of the past month's effort has been spent on stat analyses, to understand what is going on in the system. Aggregated over the entire system, relative coverage (i.e., relative composition) is the basic variable. The analysis needs to be extended to find total (absolute, not relative) coverage. ERDC carried out a number of probabilistic analyses to quantify differences among sites. GLM's have been applied to all sites combined. Now work is underway on the Old Channel separately. (Thom noted that one of his students has already done this for the City Park reach.) The model boils down to habitat quality, dispersal and growth (including senescence)

with external forcing (management, scour). Thom suggests assigning a risk of scour, recreation-based from 13 years of data.

Todd notes that the incorporation of statistical results means that we are working toward a probabilistic model. This is going to require multiple reps in execution. The darter model is already probabilistic, but additional reps will drive up run time considerably.

4. Darter modeling

Rose and Bill presented the new statistical results, in which pH and conductivity are omitted. From the AIC criterion, the model would be judged to be still satisfactory. The results are more “interesting” (larger number of contributing variables) due to the greater number of vegetation species. (“Mainper” = dominant species; “CV” = current velocity.)

Comal Springs

AIC = 2424.197 (df = 60)

Variable	overall	Category 1		Category 2		Category 3		Category 4	
	p-value	estimate	p-value	estimate	p-value	estimate	p-value	estimate	p-value
Silt	<.0001	1.2956	0.0022	2.3575	<.0001	1.6247	0.0062	4.6367	<.0001
Sand	<.0001	2.0764	0.02	4.1702	<.0001	4.5564	<.0001	6.97	<.0001
gravel	0.0001	1.1181	0.0071	1.873	0.0005	1.9984	0.0005	4.7627	<.0001
Bryo	<.0001	2.6678	0.0016	5.6075	<.0001	6.8753	<.0001	7.4034	<.0001
Cabom	<.0001	2.6997	0.0004	3.9653	<.0001	5.1362	<.0001	3.8497	0.0002
FilAlg	<.0001	1.5288	0.1685	3.6152	0.0012	4.6041	0.0003	5.2499	<.0001
Hygro	<.0001	1.1027	0.0003	1.5058	<.0001	2.5287	<.0001	0.4149	0.544
Lud	0.0002	1.6565	0.0011	2.0672	0.0003	3.392	<.0001	1.6122	0.0417
Open	0.0721	-2.3788	0.0035	-0.8837	0.2413	-13.4965	0.9702	-14.2634	0.9675
Sag	0.01	-1.1103	0.001	-1.2197	0.0098	-0.3636	0.6324	-1.1506	0.1435
Mainper	0.0736	-0.0208	0.0062	-0.00206	0.6346	-0.00248	0.6613	0.00182	0.6432
WBryo	<.0001	1.8466	0.0013	3.7791	<.0001	4.0361	<.0001	4.7138	<.0001
Depth	0.0037	-1.1185	0.0307	-2.0738	0.0004	-2.0432	0.0016	-1.2259	0.0995
CV	0.0478	-1.497	0.5515	-2.2842	0.4634	0.3249	0.9206	6.8015	0.0586
Temp	<.0001	-0.2052	0.1266	0.0851	0.6001	0.3427	0.0701	0.7407	0.0011
Intercept	--	6.977	0.0364	-3.2767	0.404	-10.8421	0.019	-24.5001	<.0001

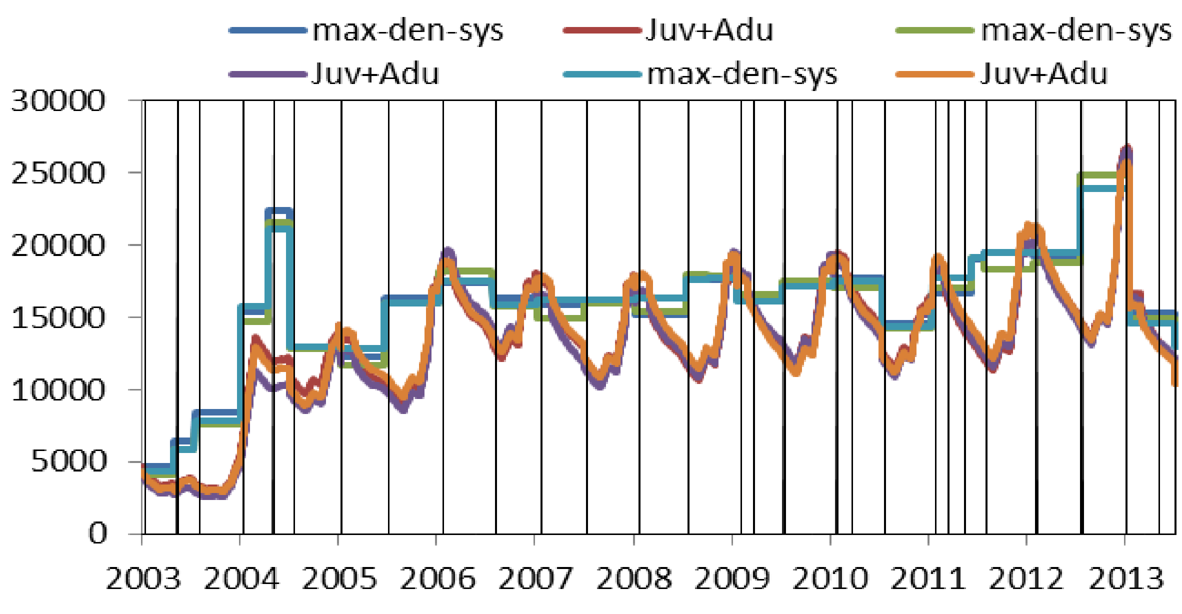
San Marcos Springs

AIC = 1436.515 (df = 24)

Variable	overall p-value	Category 2 estimate	Category 2 p-value	Category 3 estimate	Category 3 p-value	Category 4 estimate	Category 4 p-value	Category 5 estimate	Category 5 p-value
Cabom	0.0417	1.5857	0.1378	2.1504	0.0419	2.5568	0.0172	2.205	0.0493
Hygro	0.015	0.4114	0.3086	0.8813	0.0286	0.0158	0.9745	-0.2255	0.7137
Mainper	<.0001	0.0332	<.0001	0.0475	<.0001	0.0916	<.0001	0.092	0.0014
Veght	0.0028	2.7798	0.0031	3.5367	0.0003	2.638	0.0202	1.1137	0.4174
Depth	0.0001	-2.7642	0.0003	-3.6957	<.0001	-2.6556	0.0062	-1.7924	0.1227
CV	0.0247	-1.4832	0.112	-3.0727	0.0065	-2.5076	0.091	-13.5556	0.0267
Intercept	--	-0.8899	0.1134	-1.6866	0.0196	-7.1035	0.0001	-7.4306	0.0098

Rose and Bill then displayed a series of 11-year simulations (2003-2013) in which darter numbers were initialized at the estimated carrying capacity (assumed equal to the maximum observed density) and various movement strategies are used in the model to respond to habitat quality. One example follows:

Rules: including movement rules and 18 hours limitation for darters to stay in unfavorable habitats without dying (3 reps)



The statistics of comparison between dropnet survey data and the simulated darter abundance (numbers per sq m) were tabulated as follows:

Comparison between dropnet survey and simulated results with movement rules and hours limitation

	Dropnet	*1hr*	*2hrs*	*3hrs*	*6hrs*	*12hrs*	*18hrs*
Mean	20.3254	0.8203	5.5552	7.2740	9.6358	10.5516	10.4470
t Stat		2.6662	2.6529	2.4364	2.1705	1.8611	1.9843
P(T<=t)		0.0223	0.0226	0.0295	0.0410	**0.0609**	**0.0520**
t Critical one-tail		2.0150	2.0150	2.0150	2.0150	2.0150	2.0150

	Dropnet	*24hrs*	*30hrs*	*36hrs*	*42hrs*	*48hrs*
Mean	20.3254	10.4508	10.7727	10.3785	11.1751	11.0177
t Stat		1.9905	1.9721	2.1698	1.9016	1.9560
P(T<=t)		**0.0516**	**0.0528**	0.0411	**0.0578**	**0.0539**
t Critical one-tail		2.0150	2.0150	2.0150	2.0150	2.0150

Comparison between dropnet survey and simulated results with stay rules and hours limitation

	Dropnet	*6hrs_Stay*	*12hrs_Stay*	*18hrs_Stay*	*24hrs_Stay*	*30hrs_Stay*
Mean	20.3254	8.244633	8.806788	9.894979	9.807317	9.811449
t Stat		2.139721	2.229409	2.006383	1.890742	2.059683
P(T<=t)		0.042674	0.038111	**0.050556**	**0.058624**	0.047236
t Critical one-tail		2.015048	2.015048	2.015048	2.015048	2.015048

There followed much handwringing by the Team concerning an apparent lack of agreement of these model results with independent fountain darter dipnet data. Bill noted that model parameterization is still underway, for example there is a limit to migratory distance per unit time in the model, but there have been no studies thusfar of the effect of varying this parameter.

The Team decided to explicitly include FltAlg as a habitat variable to potentially capture the observed drop in darter population post-2003. In addition, the upper bound for category 4 will be changed, to the maximum darter density recorded per the empirical drop net database. Additionally the time to death will be increased, and a probability will be assigned to the "death" operation, so that some small fraction survives the season.

Next meeting

The next team meeting will be 1000 3 September at BIO-WEST offices in Round Rock.

MEMORANDUM

SUBJECT: Ecomodeling team meeting, BIO-WEST offices, Round Rock
1000 CDT 3 September 2015
FROM: George Ward, scribe
DATE: 18 September 2015

Attendance:

Robert Doyle	Baylor	Bill Grant	TAMU	Rose Wang	TAMU
Ed Oborny	BIO-WEST	Todd Swannack	ERDC	Jake Jackson	BIO-WEST
George Ward	UT	Tim Lewis	ERDC (telephone)		

1. Current status of Comal and San Marcos systems and of the project

Ed: After the heavy rains in May and June, the spigot is now off and we are sliding back into drought conditions. The Comal is starting to drop, from 400 to 230, while the San Marcos is continuing to hold a bit better.

The HCP Science Committee has been off for the summer but is scheduled to meet next Wednesday, and then again on 10 November.

2. Status of hydraulic model

Thom is continuing to develop the graphic user interface for the model. The Team agreed with Thom's decision to hard-wire the distribution of spring flows among the orifices. This will greatly simplify the GUI, and relieve the user of having to "make up" inputs. With respect to the flow split on the Comal (new versus old channel), Thom recommends limiting the input flow for the old channel to 80 cfs. BIO-WEST has learned that this is even too high to operate, because this high a flow starts to scour out restored areas on the old channel. The Team concurs with assigning this upper limit to the model inputs.

Tasks for Thom include:

- Finish up the QUAL-2E model for DO and temp and get package to Bill/Rose when done.

3. SAV Modeling

Todd is continuing the work of specifying SAV dispersion by an empirical model developed from statistics of observations. He has produced maps of the City Park (San Marcos) and Comal

reaches showing total vegetation (not individual species) over time, displaying GIS layers of annual presence/absence and changes (increase, decrease, no change) distributions. Todd has worked out transition probabilities of one species being replaced by another for a season and a year, and proposes that these transition probabilities can be used to quantify dispersal. Robert noted that the growth rate of a patch of vegetation from lab and field studies can be estimated geometrically. Concern was expressed by the Team that such an approach eliminates depiction of causal responses to external factors, notably river flow. It was suggested that a modification of this approach be pursued in which the veg data will be first stratified. It's not clear yet which categories of stratification should be used. Probably post-scour periods versus stable periods, and various categories of steady flow. Then transition probabilities can be computed for each stratum.

Todd remarked that it is important to realize that these statistical depictions are an alternative to the model component of "dispersal", which he is pursuing because it is becoming apparent that to model dispersal deterministically is too much of an advance over the present state of the science on this process.

The Team engaged in a discussion of how the SAV data might be usefully stratified, particularly whether we have adequate data to stratify by flow. Ed noted that a "take analysis" has been prepared by BIO-WEST and submitted to USFWS for several years. These "take analyses" document changes in veg related to flood, average or drought years, and suggest there is a veg versus Q relation. Ed will send copies of these to Robert and Todd. An additional possibility is to stratify by recreation.

What about upstream veg as a source for dispersal? For example, important upstream sources of *Hygrophila* to Landa Lake was the swimming pool, Landa Lake and upper spring run, but these sources have been removed since BIO-WEST and Baylor have replaced these colonies primarily with native aquatic vegetation. However, Blieders Creek still has *Hygrophila*, but it's not clear that Blieders represents the same magnitude of source that the swimming pool etc. had on dispersal in the Lake. The Team agreed to postpone further work on this dispersal process pending new evaluations of transition probabilities based upon the above considerations.

We need to exploit all of the growth research that's come out of our special projects. The discussion moved into details of incorporating these into the SAV model: whether to calibrate or validate, how to incorporate river flow and/or spring flow, etc. It was noted that water depth may be the more important variable, rather than flow, because of its spatial variability across the section. Should velocity be incorporated into the growth model? This has been done in some models in the literature. Todd is looking into this. It may be sufficient to represent it as a threshold phenomenon.

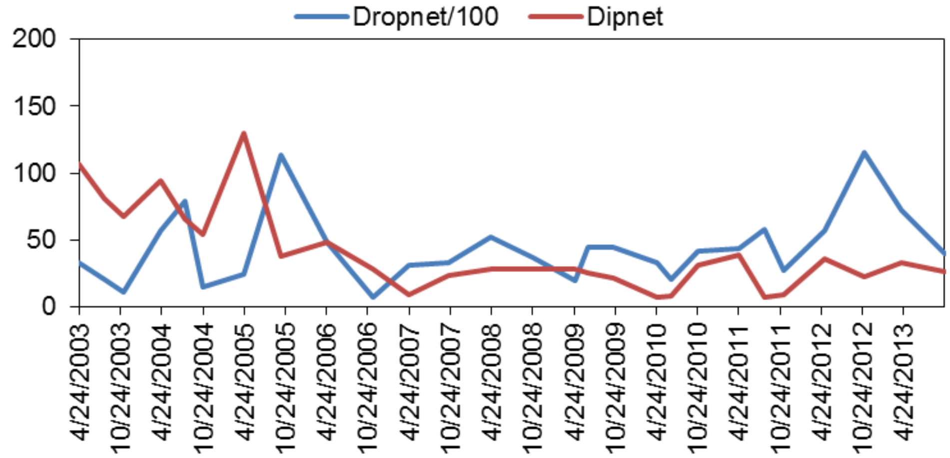
Given Todd's impending matrimonial endeavors, the following tasks should be completed before he takes the plunge:

- Stratified evaluation
  - Flow – Ed send take analysis breakdown for flow years to Todd, Robert, team.
  - Recreation – sort of incorporated already by season – look at available summer data??
  - Depth, velocity, substrate
- Get with Robert on Lud competition study
  - Flow – study reach specific
- Get with Robert and Casey on restoration growth data
  - Inform the equations?
  - Validate the results?
- Current velocity for growth model. Is this continuous or is this just a threshold value.
- Provide Bill/Rose with an updated version (simplified) of the existing Net Logo veg model (growth and dispersal) so Rose can check the behavior of the darter population.

4. Darter modeling

Bill and Rose have been experimenting with model simulations trying to find some means of reproducing the downward trend of darter abundance shown in the dipnet data (see figure following), and they are now wondering how applicable that early dipnet data may be relative to this modeling effort. Ed is fine with letting the dipnet data go, if the Team agrees that it may not adequately represent darter abundance. The Team agreed.

Once again, the specification of carrying capacity became a discussion topic. We should not be basing this specification on max darter density in the Old Channel alone. Need to include, say, all *Ludwigia* darter densities throughout the Comal, i.e. set carrying capacity = max darter density (for each SAV) in the entire system.



Rose and Bill presented summaries of comparisons of model to observed (dropnet) fountain darter abundance for the period 2001-13:

- 96 hours rule: p-values = 0.1742 (There is a statistically significant difference between the outcome of this scenario and the dropnet data.)
- 19 hours rule: p-values = 0.0200 (There is no statistically significant difference between the outcome of this scenario and the dropnet data.)
- 12 hours rule: p-values = 0.0063 (There is no statistically significant difference between the outcome of this scenario and the dropnet data.)

Specific comparison based on each aquatic vegetation

- 96 hours rule: p-values = 0.4306 (There is no statistically significant difference between the outcome of this scenario and the dropnet data.)
- 19 hours rule: p-values = 0.8227 (There is no statistically significant difference between the outcome of this scenario and the dropnet data.)
- 12 hours rule: p-values = 0.9477 (There is no statistically significant difference between the outcome of this scenario and the dropnet data.)

Descriptive statistics

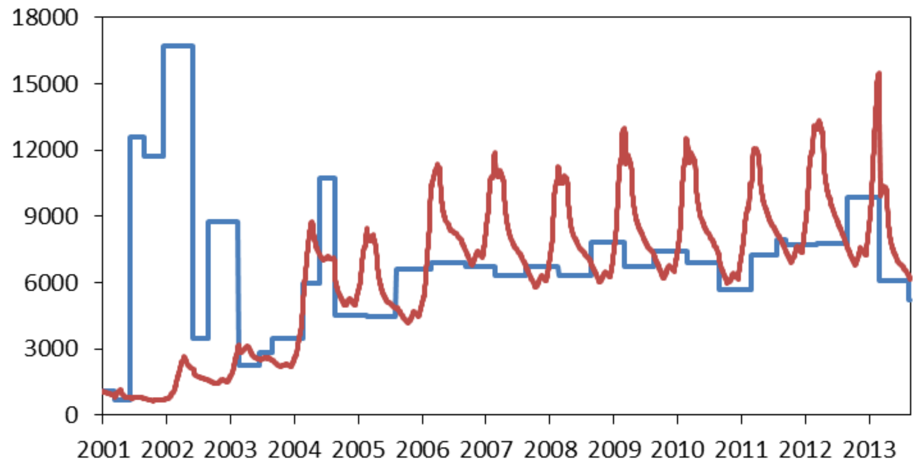
Veg e	Average #/m2 of Dropnet	Average #/m2 of 96hr rule	Average #/m2 of 19hr rule	Average #/m2 of 12hr rule
1	20.17	11.34	9.89	8.49
2	3.48	2.04	1.21	1.29
3	3.09	4.64	3.67	3.48
4	1.78	1.91	1.17	0.96
6	12.83	11.33	11.33	10.33

Some concern was expressed about the use of the p-value as a measure of model performance. As an alternative, the Nash-Sutcliffe index was suggested.*

There transpired much discussion about the simulation versus data. There was concern about the 2001-02 data points in the old channel dragging down the simulation for 2-3 years due to the anomalously small values. Ed also observed that these data points were from the first years of the program during which field protocols were still being worked out. The likely presence of a “starting transient” in the initial period of simulation was also noted, of an unknown duration but perhaps extending for several years. A starting transient is an artifact of any complex time-simulating model, which arises from an imbalance or inconsistency among the various terms in the model equations due imprecision in initial conditions. In this case, the input initial abundance of fountain darters specified throughout the model domain (most computational cells of which lack data and must be estimated) imposed on the corresponding observed distributions of substrate, vegetation cover, etc., is the probable source of the starting transient.

The Team considered that the starting transient due to inconsistencies in initial values may be further compounded by the extreme variations in observed darter abundance in the first three years of the simulation period (2001-03, see below).

96 hours rule



* The ratio of data variance about the model prediction, to variance about the mean of the data is given by:

$$V = \sum (x - x_{mod})^2 / \sum (x - \bar{x})^2$$

where x is the measured values of the modeled variable with mean value $\bar{x}$, x_{mod} is the model predicted value corresponding to the measured value, and the sums range over all measurements. Therefore, the analogy to explained variance of a regression is the variance in the data explained by the model, i.e. $1 - V$. This has lately been accorded the elevated title of Nash-Sutcliffe efficiency index, though it did not originate with Nash and Sutcliffe (1970), and is only vaguely related to efficiency.

Bill and Rose had anticipated this concern and presented a second simulation beginning in 2003 (thereby not attempting to mimic the extreme variations in the first two years), as follows:

Comparison: Simulation from 2003

General comparison

- 96 hours rule: p-values = 0.0008 (There is a statistically significant difference between the outcome of this scenario and the dropnet data.)
- 19 hours rule: p-values = 0.2174 (There is no statistically significant difference between the outcome of this scenario and the dropnet data.)
- 12 hours rule: p-values = 0.4272 (There is no statistically significant difference between the outcome of this scenario and the dropnet data.)

Specific comparison based on each aquatic vegetation

- 96 hours rule: p-values = 0.4421 (There is no statistically significant difference between the outcome of this scenario and the dropnet data.)
- 19 hours rule: p-values = 0.8282 (There is no statistically significant difference between the outcome of this scenario and the dropnet data.)
- 12 hours rule: p-values = 0.9494 (There is no statistically significant difference between the outcome of this scenario and the dropnet data.)

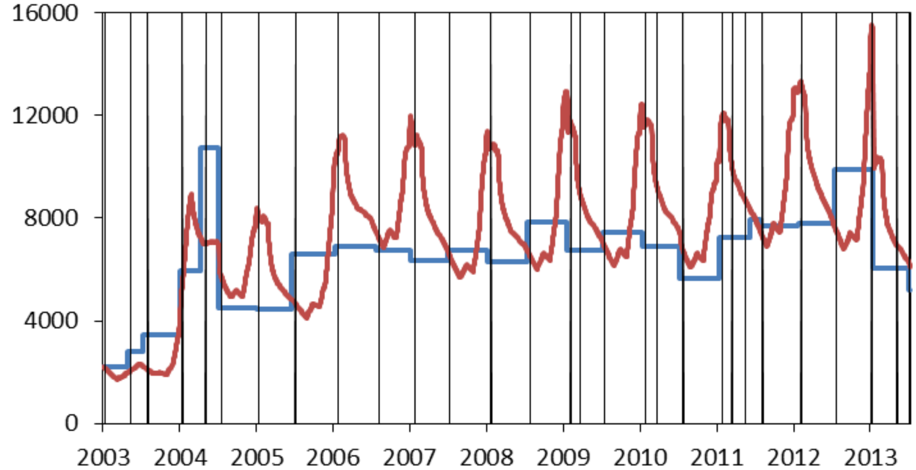
Specific model versus prediction results:

Vege	Average #/m2 of Dropnet	Average #/m2 of 96hr rule	Average #/m2 of 19hr rule	Average #/m2 of 12hr rule
1	9.87	16.58	14.26	13.95
2	3.88	3.25	2.75	2.13
3	3.09	4.72	3.83	3.61
4	1.78	2.19	1.26	1.04
6	12.83	11.00	10.67	10.33

Some members of the Team would like to see further comparisons for the first two years, see following figure, since there is a possibility that the large excursions in darter abundance are in fact real (a response to the high concentrations of beneficial algae in those years). They requested that TAMU try another simulation in which the model is initiated at around the 2012 peak in abundance, so see how the model depicts the recession in abundance following the major shift in aquatic vegetation in 2003.

It was also suggested that other initial values be tried developed from an extended simulation run of the model after starting transients have been flushed from the model domain, say the mean values for 2013. As an alternative for examining model response, it was suggested that model runs with the order of model-year inputs (veg, river flow etc.) being randomly shuffled.

96 hours rule



For Bill and Rose, neither of whom is involved in Todd's matrimonial exercise, the do-list includes:

- Another parameter to quantify the performance of the model, viz. Sutcliffe – Nash
- Average of number of darters in each vegetation type – using whole Comal system vs. just Old Channel
- Scatterplot graphs for comparison of model vs. drop net data with std dev bars on the data
- Initiate model starting in Fall 2001
- Initiate model using the 2013 values
- Randomly shuffle the years, to sample with replacement – permute the veg maps. To test how the model responds to a different time series.
- Figure out a good way to represent the variability in the number of samples per veg type, and estimates of darter density.

5. Additional matters

Ed will make the presentation to the HCP Science Committee in November. (George has a conflict on this date so will not be able to participate.) He requests that Todd, Bill, Robert and Thom provide three or four slides (or *.avi movie file, as appropriate) that would exemplify the status of the work for inclusion in his Power Point, no later than 27 October.

The time for authoring an interim report is approaching quickly. All PI's should outline their sections, prior to the next meeting. The final outline will be formulated at that meeting.

The Team agreed that a flow chart of the complete model system showing inputs, outputs, and general model structure should be prepared as part of the interim report. Discuss and come up with our thoughts of what the user can make the model do. George will develop a flow chart based on those discussions.

Tim – send DO mortality thresholds to Bill/Rose as soon as possible.

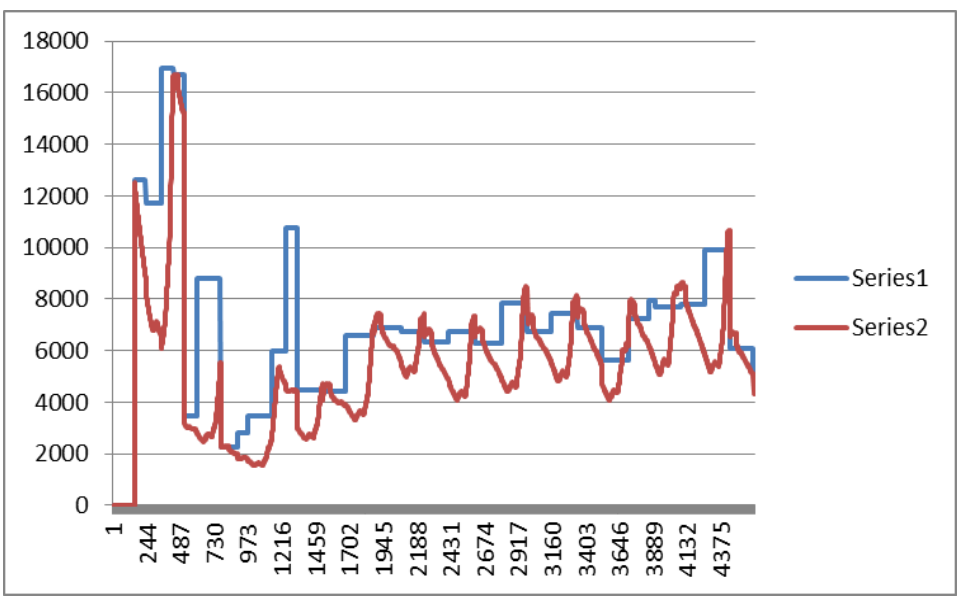
The next meeting was set for 9 October, and has been subsequently confirmed for those members of the Team that had to be absent on 3 September.

References

Nash, J., and J. Sutcliffe, 1970: River flow forecasting through conceptual models: Part I, A discussion of principles. *J. Hydrol. 10*, 282-290.

ADDENDUM (from Bill Grant, 7 September 2015):

FYAA (for your amazement and amusement) – results of a simulation in which darters were initialized at the estimated (based on historical vegetation) maximum density in Aug. 2001. Note the essentially density-independent rate of population growth when new habitat suddenly becomes available during the period of net population growth.



MEMORANDUM

SUBJECT: Ecomodeling team meeting, BIO-WEST offices, Round Rock
1000 CDT 9 October 2015
FROM: George Ward, scribe
DATE: 5 November 2015

Attendance:

Robert Doyle	Baylor	Bill Grant	TAMU	Rose Wang	TAMU
Ed Oborny	BIO-WEST	Todd Swannack	ERDC	Jake Jackson	BIO-WEST
George Ward	UT	Thom Hardy	WSG (telephone)		

1. Current status of Comal and San Marcos systems and of the project

Ed: Comal holding in the low 200's, San Marcos holding. Both systems looking good.

The National Academy panel will have a field trip on 27 October. In association with this, on 28 October, a presentation on the status of the modeling effort has been requested. Ed will make this presentation & solicited input from the Team for his Power Point. George suggested a series of flow charts laying out the underlying conceptual model, plus a flow chart depicting the envisioned operation of the model. Ed requested candidate slides from the Team members by 27 October.

Ed will also present a model overview and status report to the HCP Science Committee on 10 November. (George begged off due to a teaching commitment at the Marine Science Institute.) Hopefully, the NAS materials can be adapted for presentation to the Science Committee.

The Interim Report is coming due. The Team reviewed the outline, see Table 1. Special studies will require a synopsis. We need to identify last summer's work and anything else that has been used in the model formulation. Writing assignments were made in previous meetings and by arbitrary decree by Ed. Draft texts of the various chapters are due 9 November.

2. Status of SAV model

Robert reported that the experiments on *Ludwigia* competition versus *Hydrilla* and *Hygrophila* are now complete and data analysis is underway. Casey (BIO-WEST) has mapped the restoration data, which can now be used to estimate expansion rates if we decide we need them.

Todd reports that the statistical work (outlined in the previous Team meeting) is now completed. Water depth is one of the model input variables and this provides a direct link to flow. He has

TABLE 1 – Draft outline of Interim Report with author assignments

Chapter 1 – Model Concept

- Introduction
  - Overview *(Ed)*
  - Lay out the question
- Conceptual Model – Model Description
  - Conceptual Model *(George and Rose & Team)*
- Data Resources
  - List of information used and resources *(Ed / George following input from team)*
- Activities to Date
  - Timeline *(Ed)*
  - Summary of Meetings *(Ed)*
  - Key Decision points *(George following input from Team)*
    - Hydrology *(Thom)*
      - Old Channel flow gage
    - Water quality *(Thom)*
    - Study reaches (description of each reach) *(BW)*
    - SAV decisions *(Todd)*
    - FD decisions *(Bill and Rose)*

Chapter 2 – Model Component Development and Evaluation

- Special Studies *(BW)*
  - Fecundity *(Tim B.)*
  - Movement *(BW)*
  - Distributed plantings *(Robert)*
  - Competition / Interference *(Robert)*
- Hydraulics and Hydrology *(Thom)*
  - Historical conditions
  - Hydraulic evaluation (reference previous reports)
- Water Quality *(Thom)*
  - Historical characterization of WQ (DO and Temp)
    - Thermisters
    - QUAL-2E evaluation / Sensitivity Analysis / Uncertainty Analysis
- Submerged Aquatic Vegetation *(Todd)*
  - Existing Data and characterization of aquatic vegetation in two study reaches. (BW)
  - Growth
  - Dispersion
  - Evaluation / Sensitivity Analysis / Uncertainty Analysis

(continued)

TABLE 1
(continued)

Fountain Darter Life Cycle and Movement *(Bill and Rose)*
- Existing conditions – data resources (*BW*)
- Drop net statistics
- Carrying capacity
- Movement
- Dissolved oxygen tolerances *(Tim B.)*
- Evaluation / Sensitivity Analysis / Uncertainty Analysis

Chapter 3 Fountain Darter Simulation Model Preliminary Evaluation

Preliminary Stage *(Bill and Rose)*
- Predicted vs Observed
- Sensitivity Analysis – Uncertainty Analysis

Final Stage *(George)*
- Steps still needed to be incorporated

Chapter 4 - Fountain Darter Simulation Model Application

What is the question *(Ed and George)*
- Can the fountain darter population sustain itself under various scenarios of flow (including the HCP flow regime)?
- Answer presented as
- Relative comparison of fountain darters numbers per sample reach
- How does this relate to the whole system
  - Old Channel spill example – reintroduction to Landa Lake

What can the user manipulated and evaluate with the FD Simulation Model *(Ed and George)*
- Flow and associated DO and temp changes
- Vegetation Changes
  - Recreation
  - Major Scour Events
  - Restoration projects
    - Types of vegetation
    - Native to non native slider
- Fountain darter changes
  - Reintroduction
    - Rates of recovery
  - Increase in mortality
    - Disease
    - Parasites
    - Toxic spills

(continued)

TABLE 1
(continued)

Chapter 5 – Next Steps and Future Considerations (Ed and George)
- Year 3
    - Additional 3 study reaches, but the same methodology
- Future Considerations
    - Scenario Testing
    - More parameters to slide and smile (Water temp, DO?)
    - Storm runoff and impacts (toxic chemicals)
    - Eutrophication (nutrient input and manifestation)
    - Riffle Beetle Model?

References *(All)*
Appendices – to be determined

not yet finished the analysis of "take" data, but hopes to finish this while on his honeymoon. Still having problems getting the SAV model to agree with field observations when one veg type totally replaces another. A discussion followed of how to validate a monte-carlo simulation against only one realization (i.e., only one set of field data). Todd needs to implement a negative feedback on growth.

Todd has found and repaired the negative biomass bug in the SAV model and will be sending an update to the linked model at TAMU. We now have a completely linked SAV + FD model, though work is still ongoing on the dispersal mechanisms.

3. Hydraulics and water quality

Thom summarized the status of the model coupling and user interface. The model reads Y-M-D (year+month+day) and the associated flow rate, 8 daily readings of met data, then runs QUAL then moves on the next day. This is the basic time series process for both hydraulic output and water quality, to be fed to the linked SAV+FD model. There are still a few bugs to be worked out in the interface. He has concern about allowing the user complete freedom to specifiy start/end time/dates since this could lead to unrealistic runs, e.g. a 2-week period when darters take months to grow out. The Team decided that this was too much flexibility, and instead the user should be presented with a set of scenarios to choose from. E.g., the model might run only the 13-year period starting in 2001. The user can modify Q, etc. in subperiods & can extract a limited time period from the output, but will have to run out the full simulation period.

Thom needs SAV maps and associated data for the three new reaches in the San Marcos & Comal. Ed will send these today or Monday.

4. Fountain darter modeling

Bill: The FD team is still struggling with long run times of the San Marcos (City Park) model. May be too many land cells or too many darters. (George suggested an experimental model run with swaths of cells removed, to see how much model acceleration would result.) But the highest priority are:

(1) input gridded data for the other reaches (Landa Lake, Upper Spring Run, IH35)
(2) DO and temperature data for the two primary reaches (Old Channel and City Park)

Rose displayed some recent results from the FD model. FD densities by veg type (summed over all grids with the same veg type in the model reach) are shown in Table 2.

Table 2 -Darter density in each vegetation type:

Old Channel

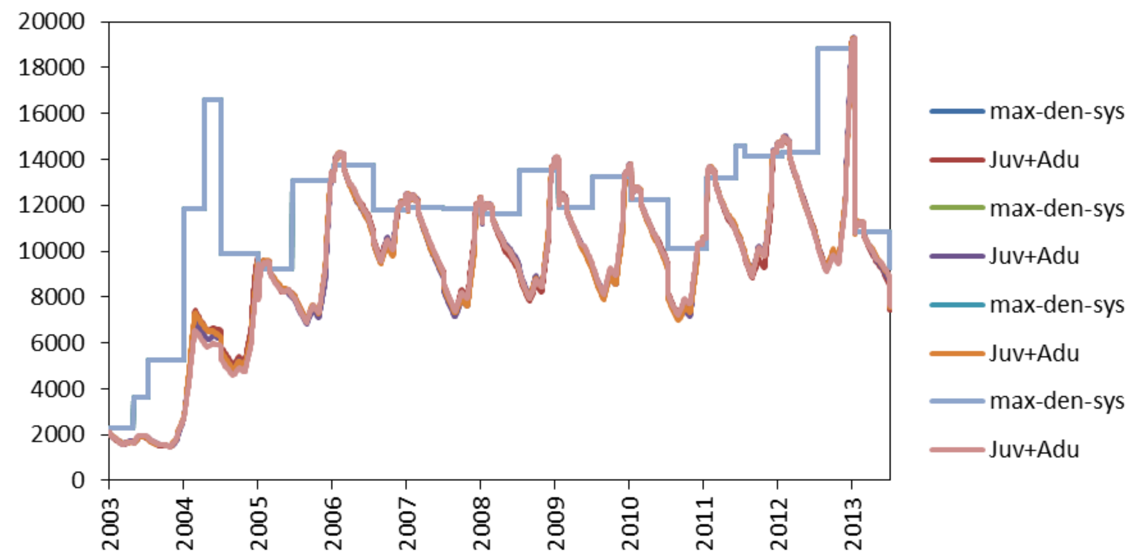
Vege Type	Average	StdDev	Minimum	Maximum	Sample size
0	1.32	2.15	0	8	34
1	21.96	22.73	0	105	42
2	3.12	3.88	0	17.5	33
3	4.13	3.87	0	21	87
4	1.78	1.39	0	5.5	47
6	11.58	4.82	6	20.5	6
10	8.00	N/A	8	8	1

Comal Springs

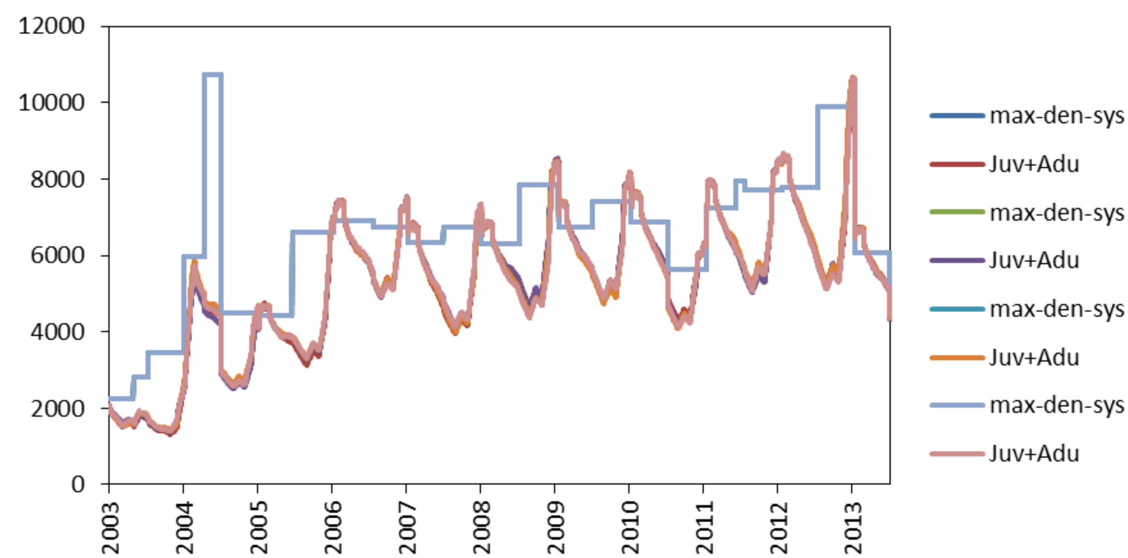
Vege Type	Average	StdDev	Minimum	Maximum	Sample size
0	1.29	2.59	0	15	62
1	19.34	22.36	0	105	48
2	3.12	3.88	0	17.5	33
3	7.37	8.35	0	38.5	297
4	12.15	14.41	0	85	138
6	24.91	20.28	0	96	152
7	5.27	14.00	0	106	95
8	5.58	9.95	0	58	86
10	9.58	9.09	0	42.5	91

Model simulations starting in 2003 for total darters in the two primary reaches were shown. The following graphs show max darters in system (blue line) and simulated number of darters (juveniles + adults, red line), in which max limit for survival in unsuitable habitat = 12 consecutive hours (original value) and the upper limit on darters in best habitat category = 36 (original value)

Based on Comal data



Based on old channel data



Some members of the team asked whether field data could be superposed on this time-series simulation. Rose provided this several days later via e-mail, which stimulated an exchange among the team members, summarized in Addendum 2.

Plots of field observations of darter abundance (abscissa) in the Comal Old Channel versus simulated abundance (ordinate), for each veg type were then shown, along with the calculated Nash-Sutcliffe efficiency *E*:

$$E = 1 - \frac{\sum_{t=1}^{T}(Q_o^t - Q_m^t)^2}{\sum_{t=1}^{T}(Q_o^t - \overline{Q_o})^2}$$

where $Q_o{}^t$ is (observed) sampled density of fountain darter at time *t*, Q_m^t is simulated density of fountain darter at time *t*, $\overline{Qo}$ is the mean of (observed) sampled density of fountain darter.
Properties of *E*:

- Nash–Sutcliffe efficiency can range from $-\infty$ to 1.
- An efficiency of 1 (*E* = 1) corresponds to a perfect match of simulated density to the sample density.
- An efficiency of 0 (*E* = 0) indicates that the model predictions are as accurate as the mean of the sample density.
- An efficiency less than zero (*E* < 0) occurs when the observed mean is a better predictor than the model.
- Essentially, the closer the model efficiency is to 1, the more accurate the model is. However, the efficiency coefficient is sensitive to extreme values and might yield sub-optimal results when the dataset contains large outliers.

The results (an example of which follows) would suggest that the model still needs some adjustment. There was also a discussion on how best to compare the simulated data to the field data.

A set of time-series plots were accidently left behind at College Station. Bill provided these by e-mail after the meeting. They are included here as Addendum 1.

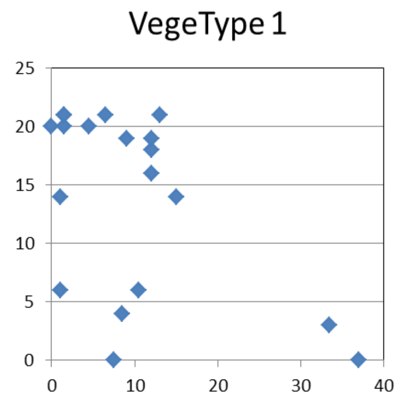
5. Additional matters

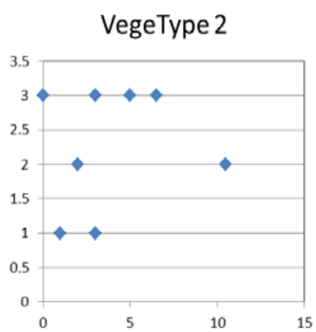
Slides to Ed by 27 Oct for the NAS presentation. Drafts of chapter texts by 9 November.

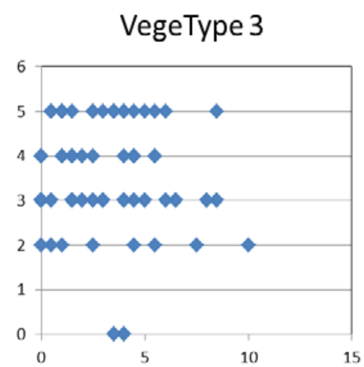
Next meeting: 10:00 CST 13 November at Bio-West offices.

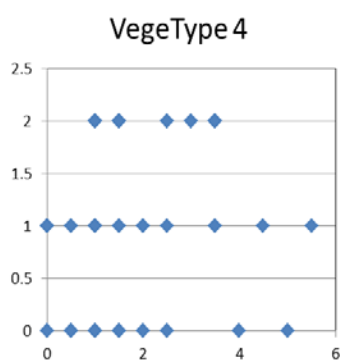
Survey (x) and simulated (y) darter density for each vegetation type (Rep 3) from Comal Old Channel data:

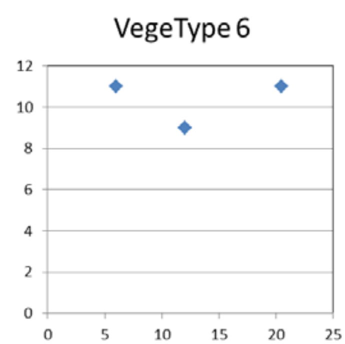
VegeType	E
1	-1.589
2	-0.263
3	-0.423
4	-0.456
6	-0.170
All	-0.5138









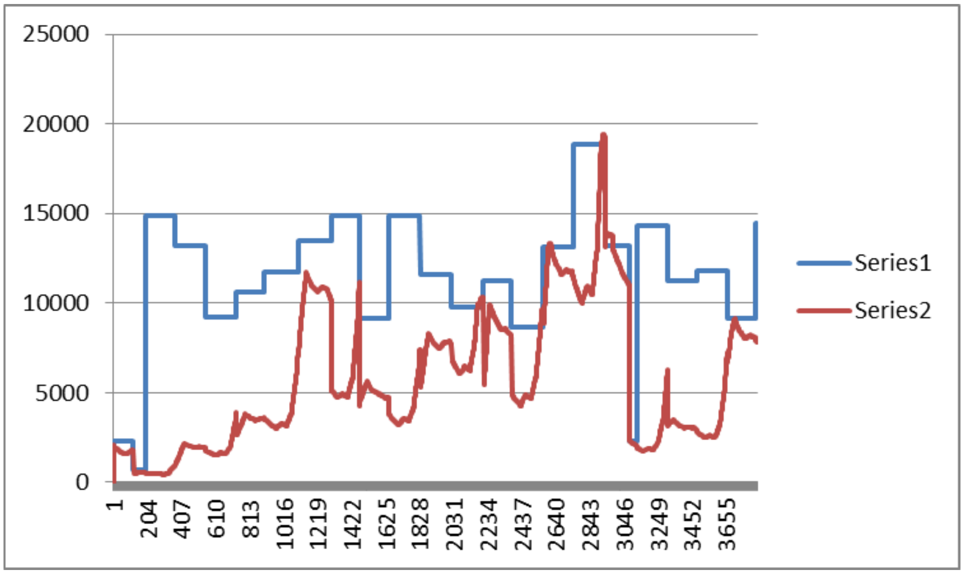


ADDENDUM 1

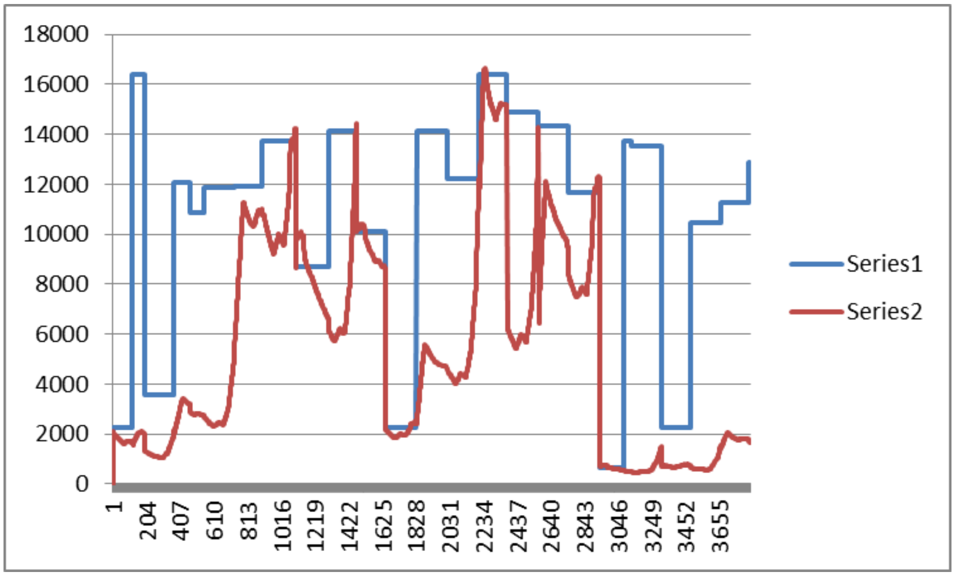
From Bill Grant, TAMU

Here are some simulation results that we forgot to bring to the 9 Oct. 2015 meeting in Round Rock. (Folks with the appropriate security clearance will understand the color coding)
Based on Veg data from Entire Comal (Random Sequence of Years)

Rep #1

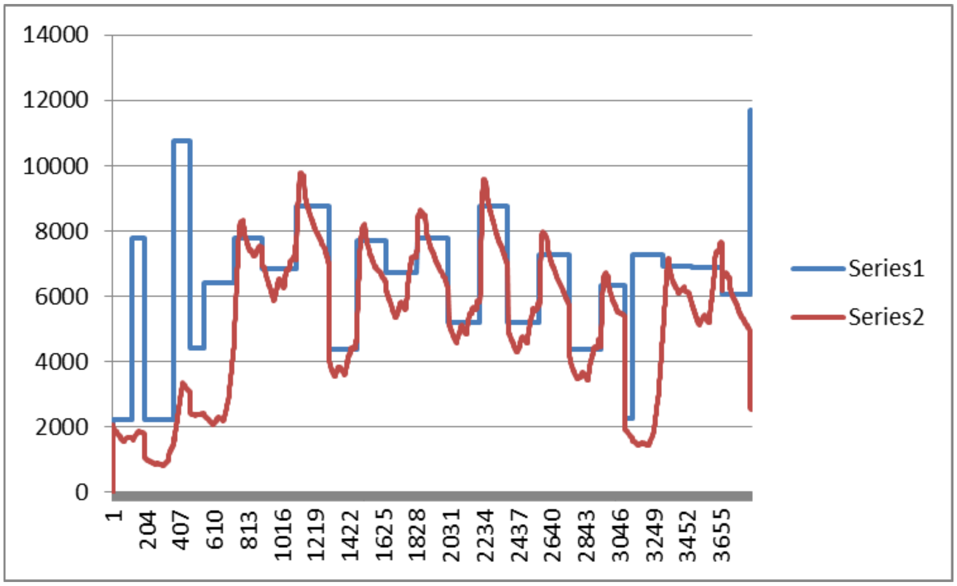


Rep #2

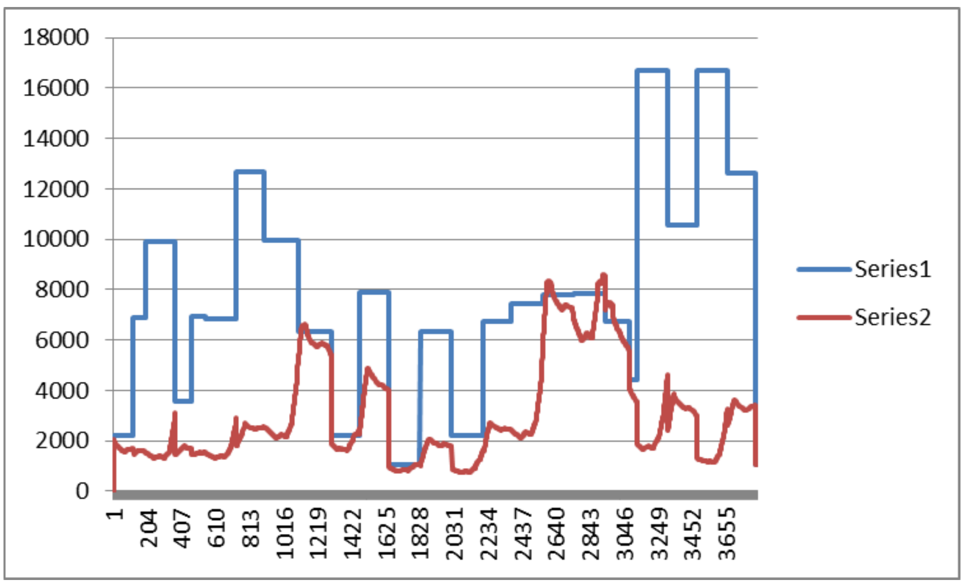


Based on Veg data from Old Channel Only (Random Sequence of Years)

Rep #1

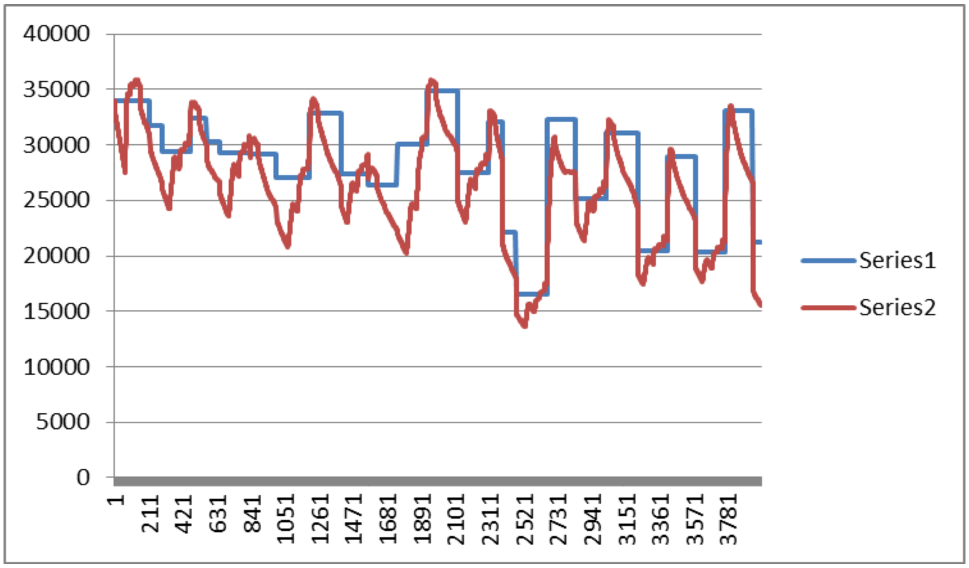


Rep #2

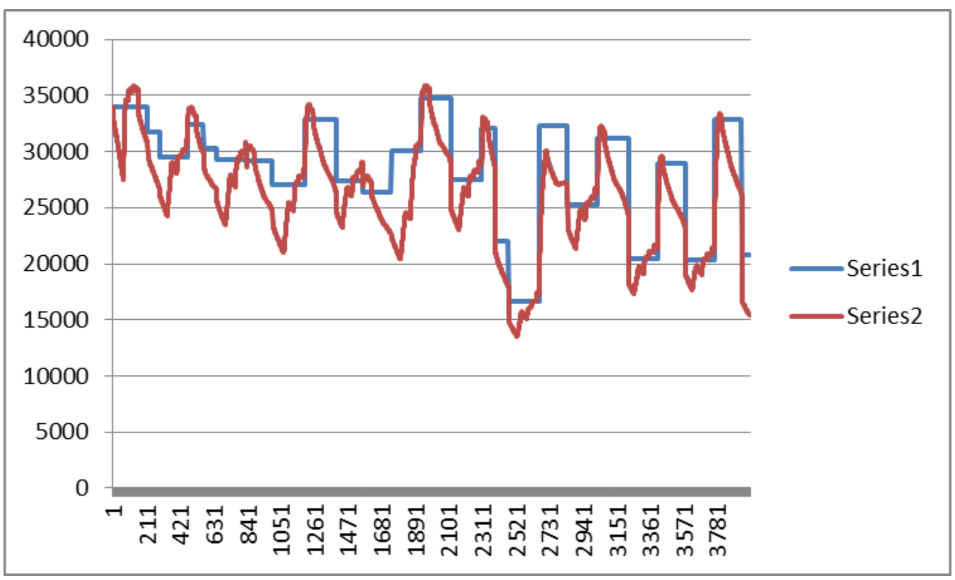


Based on Veg data from City Park Only (Appropriate Chronological Sequence of Years)

Rep #1



Rep #2
(This should have been based on entire San Marcos Veg, but, evidently, I did not adjust the "slider" appropriately – a sign of the extreme pressure under which we are operating!)

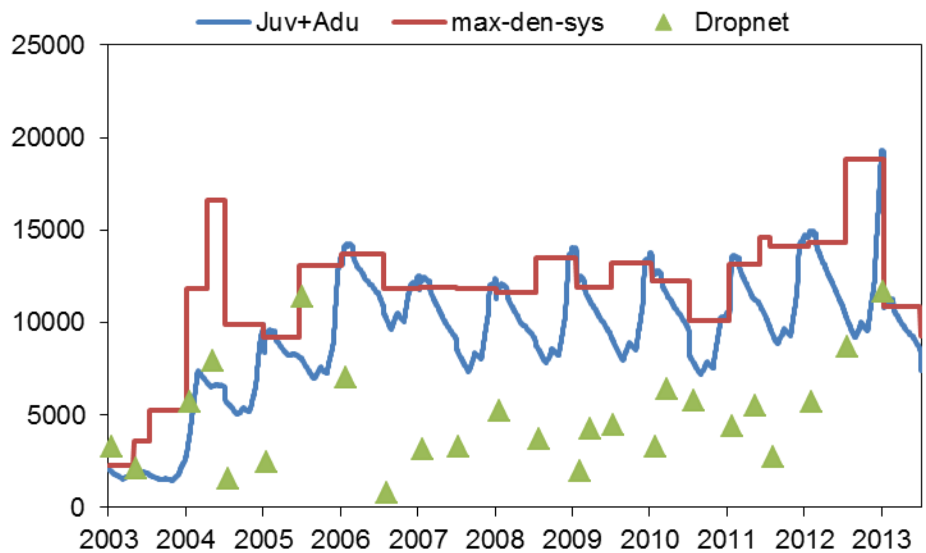


ADDENDUM 2

In response to the request during the meeting to add the dropnet field data to the FD 11-year simulations, Rose provided the following results via e-mail on 4 November. The resulting e-mail exchange among Team members is appended.

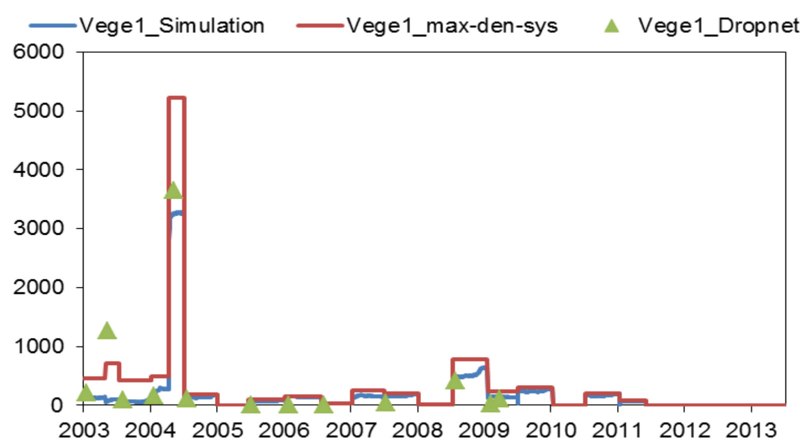
Fountain darter simulation in Comal Springs

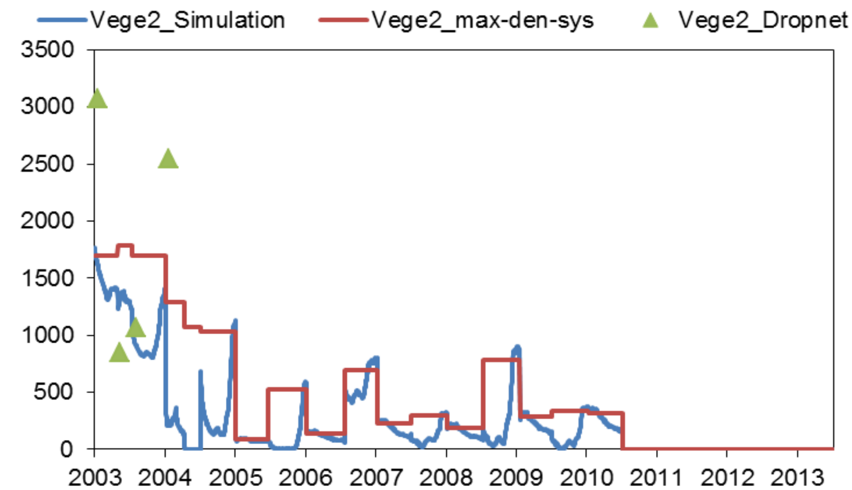
Overall*:

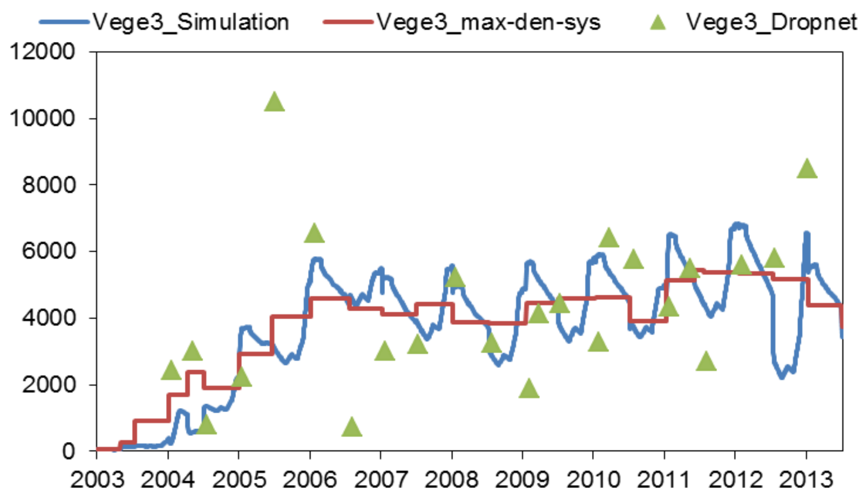


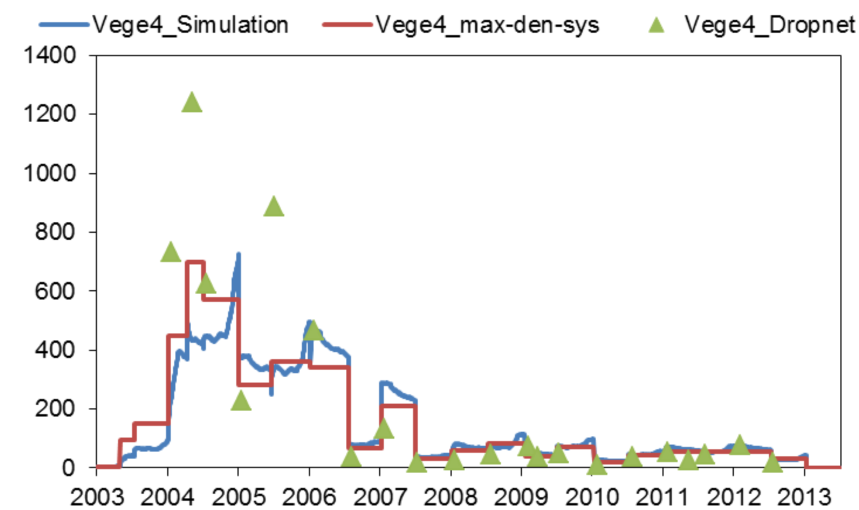
*During the dropnet sample days, BioWest only surveyed few vegetation types (4 to 7 dropnets) and hence the abundance calculated by using Σ(average darter density in vegetation type i) × (# of cells in vegetation type i) is always lower.

By vegetation type:
Max-den-sys: (average darter density from 2003 to 2013 in vegetation type i) × (# of cells in vegetation type i)
Dropnet: (average darter density at survey time in vegetation type i) × (# of cells in vegetation type i)




Vege2_Simulation
Vege2_max-den-sys
Vege2_Dropnet
3500
3000
2500
2000
1500
1000
500
0
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013


Vege3_Simulation
Vege3_max-den-sys
Vege3_Dropnet
12000
10000
8000
6000
4000
2000
0
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013


Vege4_Simulation
Vege4_max-den-sys
Vege4_Dropnet
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800
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400
200
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2003
2004
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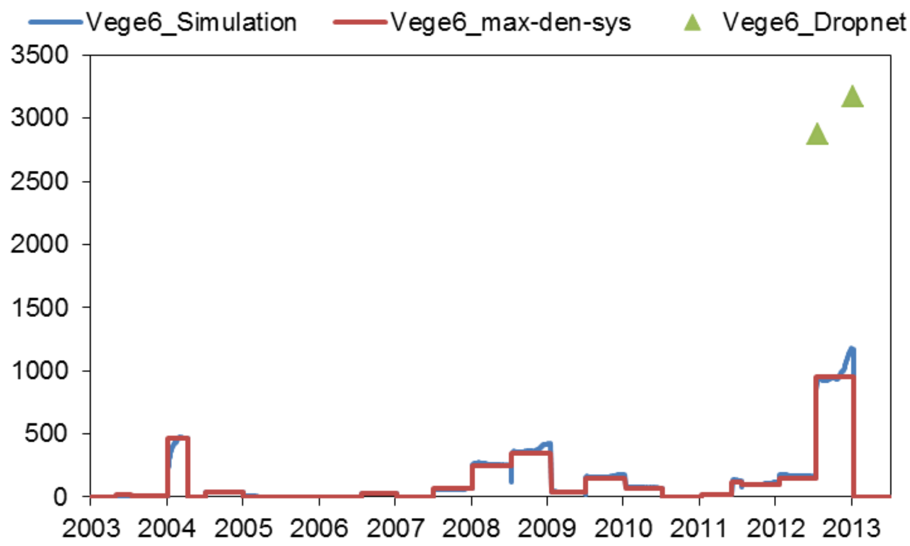
2009
2010
2011
2012
2013



From: Ward, George H
Sent: Wednesday, November 04, 2015 4:45 PM
To: 'Hsiaohsuan Wang'
Cc: wegrant; Edmund Oborny

Hi Rose –

Thanks very much for this. The model results for the individual veg types look much better than the stat index would suggest.

The first figure is a head scratcher, however. This appears to me to be a model simulation for something that we have no field data for, i.e. no field data for darters totaled over all veg types for a specific dropnet survey date, and is therefore irrelevant. However, what about field data for the total darter abundance summed over the veg types actually surveyed?

-- George

From: Hsiaohsuan Wang <hsuan006@tamu.edu>
Sent: Thursday, November 05, 2015 11:26 AM
To: Ward, George H
Cc: wegrant; Edmund Oborny
Attachments: Fountain darter simulation in Comal Springs_v2.docx

Dear George,

Your point about the problem of comparing the darters totaled over all veg types is absolutely right. We don't think there is a good way to make such a comparison. The veg types in which darters were sampled in the field do not correspond exactly with the veg types represented in the model. So, I have deleted the field data points from the first graph. Actually, the red line (max-den-sys) represents the synthesis of all of the field data correlating darter density with veg type. Hopefully, it makes sense.

Best, Rose

From: Ward, George H
Sent: Thursday, November 05, 2015 1:40 PM
To: 'Hsiaohsuan Wang'
Cc: wegrant; Edmund Oborny; 'Jacob Jackson'

Hi Rose –

The revised figure is nice, BUT it removes the essential thing we need to display, which is the extent of agreement of model and data. (No data = no information.)

The following figure is taken from the 2013 Biomonitoring report:

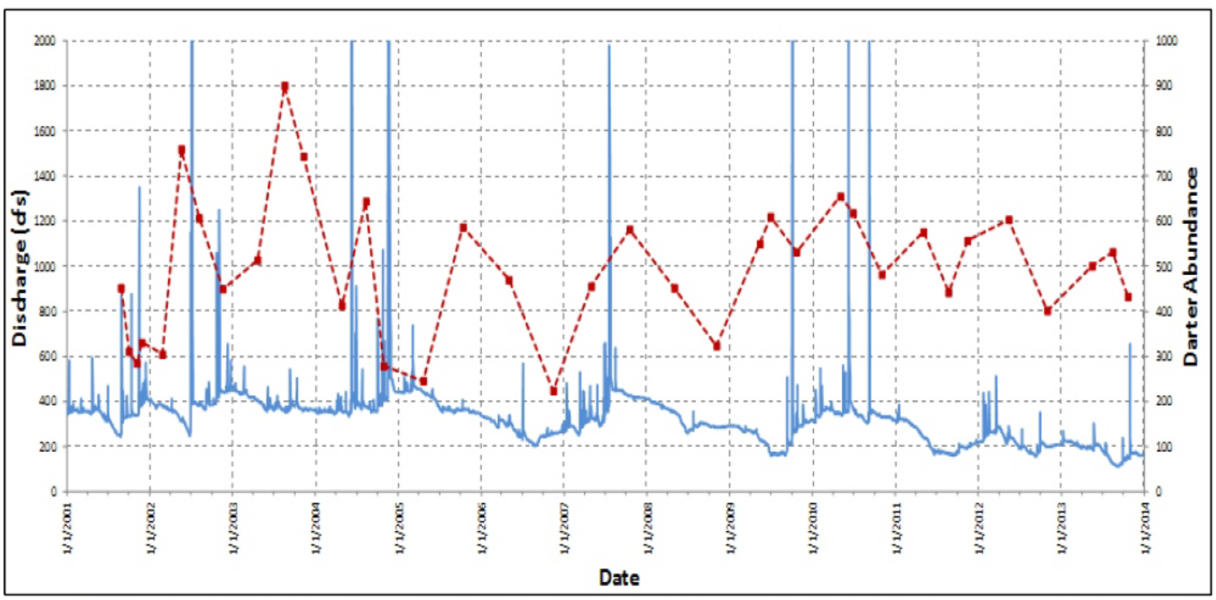


Figure 21. **Abundance of fountain darters from each drop net sampling event (red dashed line) plotted over a hydrograph of mean daily discharge from the USGS gauge on the Comal River at New Braunfels (blue line).**

This and similar figures appear in the BIO-WEST reports. It's logical for someone to ask: how do the model predictions compare to the actual field data as shown in this figure (the red data points)? We need to figure out how to extract the information from the modeled darter abundances so that the model results are directly comparable to the field data. Clearly, simply averaging model abundances over all of the grid points in the model domain is not comparable, first because the dropnets don't sample each grid point, and second because a lot of the modelled grid points will have fewer or no darters, and, as you've already noted, will reduce the averaged value considerably.

One thing we might do is identify the grid cells that correspond to the dropnet sample locations, and extract only those model values for each date/time of the sample event, then average these model values over the modeled reach. I think this may be how the BIO-WEST data were obtained. We need Ed's and Jake's confirmation of how exactly the above numbers of darter abundance were computed. Then we need to see if we can replicate this using model output.

-- George

From: Grant, William E <wegrant@tamu.edu>
Sent: Friday, November 06, 2015 6:02 AM
To: Jacob Jackson
Cc: Ward, George H; hsuan006@tamu.edu; Edmund Oborny

Jake – The model "samples" fountain darters on (or about – as they say in court) each date that the field samples were taken by selecting the same number of cells in the same vegetation types as were sampled in the field and recording the number of darters in each of these cells.

For example, in the Old Channel, on day-of-year 113 in the year 2003, the model sampled 5 cells. I can't tell from the model code, which I'm looking at now, which veg. types were sampled (I'd have to look at the drop net sample input data file – and I'm too lazy to do that right now), but I'm confident the veg. types are paired up correctly with the field data. If the veg. type sampled in the field is not present in the model (based on the historical veg. maps) the model records a "99999" and that simulated sample is not used in the analysis.

Before Dr. Rose compares the simulated and actual samples, she divides the field samples by 2 to convert from a 2 sq.m. to a 1 sq.m. basis, which is the size of the cells in the model. On my list of things to do as I'm updating our model description for the draft report is to formalize the description of the simulated drop net sampling. But, actually, what I just wrote might not be too far from what a more polished version might look like (that is, I already have sufficient coffee on board to be more or less lucid – well, I'll let you be the judge of that!). Hope this helps – take care - Bill

W. E. Grant

From: Ward, George H
Sent: Friday, November 06, 2015 8:53 AM
To: 'Grant, William E'; Jacob Jackson
Cc: hsuan006@tamu.edu; Edmund Oborny

Right. But how does BIO-WEST accumulate the field data to get FD density for the entire channel reach, such as shown in Fig. 21? We need to accumulate the model data the same way in order to compare them. Jake?

-- George

From: Jacob Jackson <jjackson@bio-west.com>
Sent: Friday, November 06, 2015 2:50 PM
To: Ward, George H
Cc: Grant, William E; hsuan006@tamu.edu; Edmund Oborny

The data used to derive the figure in question is a summation of the counts from each drop net sample (regardless of veg strata) in that event. In this chart, this was summed over 22 samples/event (6 Upper Spring Run, 10 Landa Lake, and 6 Old Channel). These data did not appear to be presented the same way in the 2014 report, possibly due to an additional sampling reach being added which made the comparison less straight forward. I hope this answers your question. If I understood Bill's model sampling description correctly, it is sampling the same # of "drop nets" in the same veg types (if available), so merely summing those abundance (count) values should be appropriate to compare to this. The larger question in my opinion is if that would really tell us much, maybe so if it is within the estimate of error in the field data? Might it be more useful to bootstrap samples from the model cells and use a BUP with a CI to compare to the field data and its CI?

APPENDIX B

Vegetation Percent Cover to Biomass

Edwards Aquifer Authority: 2014 Ecomodeling:

Vegetation Percent Cover to Biomass

Report of Research Activities

Prepared for:
BIO-WEST, Inc.

Prepared by:
Robert Doyle, Sarah Hester
Baylor University, Center For Reservoir and Aquatic Systems Research
&
Casey Williams, BIO-WEST

11/25/2014

CRASR

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Background and Objective

The Edwards Aquifer Habitat Conservation Plan (HCP) for the Comal and San Marcos Springs/River ecosystems specifies the development of predictive ecological models to guide development of and support future decisions within the Adaptive Management Plan (AMP). One effort focuses on modeling aquatic vegetation responses to various ecological factors so that the foundational habitat for endangered species can be better understood and predicted. The aquatic vegetation models being adapted and validated include the US Army Engineer Research and Development Center (ERDC) vegetation growth models (Best et. al. 2001) as well as the "MEGAPLANT" (Scheffer et. al. 1993) model, both of which utilize vegetation biomass as the primary response variables in the models.

Unfortunately, we have virtually no aquatic vegetation biomass data for the plant species in the Comal and San Marcos Rivers. Instead, we have over 15 years of detailed maps of both rivers that show species distribution and estimates of percent cover for each mapped species. These data are the results of GPS mapping efforts conducted by Robert Doyle (1998-2001), BIO-WEST (2001-2014), and Chetta Owens (LAERF, 2009). These vegetation cover maps all exist in ArcView and provide a robust record of the vegetation dynamics on the Comal and San Marcos Rivers during the past 15 years.

The objective of this study is to determine the relationship between observed vegetation percent cover and dry weight biomass. If this relationship can be reliably established it will allow the rich historic data with maps showing percent cover to provide realistic estimates of biomass. In this way past biomass changes in response to know environmental factors (low flow periods, floods, etc) can be used for model development and validation. Furthermore, the model outputs (biomass) can be used to estimate vegetation percent cover, which may have more direct application to fountain darter *Etheostoma fonticola* use of the habitat.

Review on Quantification of Aquatic Macrophytes

Quantification of plants is among the oldest of ecological endeavors and has a rich and diverse history. Quantification of aquatic plant communities has likewise been of interest, especially with regards to managing nuisance aquatic vegetation (Madsen et. al. 1991), impacts on ecosystem processes (Carpenter and Lodge 1986), quality of macroinvertebrate habitat (e.g. Theel et. al. 2008), or importance to fisheries or fish communities (e.g. Ferrer and Dibble 2005).

The need for information related to aquatic plant quantification methods led to the 1993 Aquatic Vegetation Quantification Symposium held at the 10th annual meeting of the North American Lake Management Society. The results of that symposium were published in Lake and Reservoir Management (volume 7, 1993). These papers included an overview of options for aquatic plant quantification (Madsen and Bloomfield 1993), use of line transect methods (Titus 1993), identification and mapping (Newroth 1993), experimental design considerations in field studies (Spencer and Whitehand 1993) and the much-cited guidance on biomass

techniques for quantification (Madsen 1993). No details are given in that symposium to use of GIS/GPS technologies because of the prohibitive cost and low availability of that technology.

However, early use of GIS was already being made among some users (Remillard and Welch 1993). In subsequent years the rapid development of GIS/GPS technologies and the lower cost and higher availability of the methods resulted in widespread use of these techniques (Caloz and Collet 1997, Muller 1997, Lehmann and Lachavanne 1997). Use of GIS and high-resolution GPS units for mapping the distribution of aquatic plant communities is now firmly established as standard method (Madsen 1999, Sawyer and Keeler-Wolf 2009). The Comal and San Marcos River maps all utilized high-resolution hand held GPS units to map the boundaries of the plants in the rivers. For each polygon generated the species and apparent percent cover of each species in the polygon were recorded. Percent cover was estimated visually by experienced mappers. For the 1998-2001 maps generated by R. Doyle, the water depth and vegetation height was also recorded. While vegetation height was not recorded as part of the BIO-WEST mapping efforts, vegetation height was recorded for other sampling efforts at the same time and in the same locations (e.g. drop nets) so that vegetation height can be estimated for most sampling events should that be needed.

Materials and Methods

Sample Collection

Above and below ground biomass samples were collected for eight different aquatic macrophyte species of the Comal and/or San Marcos Rivers during the summer and fall 2014. General information about each species including native/exotic status and qualitative abundance on a DAFOR scale (dominant >90%, abundant 51-90%, frequent 21-50%, occasional 6-20%, or rare <5%) is provided in Table 1.

Table 1. Aquatic plant species studied with information about species origin (native/exotic) and DAFOR scale estimate of abundance.

Species	Native/Exotic	Abundance
Cabomba caroliniana	Native	occasional
Hygrophila polysperma	Exotic	abundant - dominant
Ludwigia repens	Native	occasional
Sagittaria platyphylla	Native	frequent
Vallisneria spiralis	Exotic	rare (SM), absent (Comal)
Hydrilla verticilata	Exotic	abundant (SM), rare (Comal)
Potamogeton illinoensis	Native	occasional (locally abundant)
Vallisneria neotropicalis	Native	absent (SM), abundant - dominant (Comal)

We selected naturally occurring plant stands showing no evidence of recent disturbances. For most samples we selected plant stands with near 100% vegetation cover to allow species-specific relationships to be determined. In addition, the majority of all plants in the Comal River occur in near monospecific stands. For each sample we recorded the apparent percent cover of the stand and collected biomass samples. Percent cover was estimate by one or both of two scientists (Robert Doyle; Casey Williams) who were involved with some of the original

mapping efforts so that we could relate the biomass samples collected to the vegetation maps as accurately as possible. In addition we recorded water depth and vegetation height.

Samples were collected on four separate trips during the summer/fall of 2014 (6/2-3, 9/17, 10/1, & 10/29). Biomass samples were collected in one of two ways: a) quadrat or b) compressed canopy. The two samples were tested side by side in a Hygrophila stand and produced samples within 10% of each other.

Quadrat Method. This method generally followed that of Madsen (1993) for destructive sampling of above and below ground tissues. A weighted 3-sided quadrat of know size (0.100-0.125 m^2) was carefully positioned around the basal stems or rosettes of the vegetation to be sampled. The above ground portion of any plants within the quadrat were then carefully clipped at the sediment surface and removed. This above ground vegetation was field rinsed to remove any sticks or loose debris and collected into labeled bags. The below ground (roots & rhizomes) were then harvested by carefully excavating the sediment to a depth of at least 20 cm or until further excavation failed to contain roots. These sediments were field washed through a 0.1 mm sieve and stored in labeled plastic bags.

Compressed Canopy Method. In some cases dense canopies made separation of above ground biomass difficult (especially for dense Hygrophila and Hydrilla samples). In these cases we compressed the vegetation canopy against the sediments by lowering a chain-link fence gate (112 cm x 122cm) into which we had created a 20cm X 20 cm opening (Figure 1).

Figure 1. A) Chain-link fence gate used to compress vegetation canopy for subsequent sample. B) Compressed canopy with sampling cylinder inserted through opening to isolate vegetation sample.

This modified gate was then carefully lowered over the vegetation canopy effectively compressing the above ground vegetation against the sediment. We then inserted a metal coring cylinder (20.6 cm diameter, 0.033m^2 area) into the sediment to a depth of 30 cm isolating an above ground and below ground sample Figure 1.B.

The above ground vegetation within the cylinder was then carefully collected, field rinsed and bagged. The sediments within the cylinder were then collected by hand and sieved through a 1 mm sieve to collect the below ground roots and rhizomes.

The samples were kept on ice and returned to the labs at Baylor University. There each sample was carefully rinsed. If a sample contained multiple species, the above ground and below ground biomass of each species was separated and dried separately. Separation of the below ground roots was actually quite easily accomplished based on the quite distinct nature of each species roots. Each sample was dried for at least 72 hours to constant weight at 60 °C and then weighed. Data are expressed on a per m^2 basis (Appendix 1).

In addition to percent cover, we estimated the above ground plant volume for each sample. This "plant volume" was determined by multiplying the height of each plant times the percent cover of the sample. In addition, for each sample we computed the percent of the total plant biomass contributed by the below ground tissues (% BG).

Results

For each species we computed the mean, SE, median, range of the total biomass as well as the mean and SE of % BG tissues for all samples with 90-100 % cover (Table 2).

Table 2. Summary statistics for all samples of each species with 90-100% cover designation

		100% Cover Total Biomass (g/m^2)					Below Ground	
Species	Count	Mean	SE	Median	Min	Max	% BG	SE
Cabomba	7	422.6	51.7	499.2	207.9	558.1	17.3%	2.1%
Hygrophila	28	370.6	27.0	341.7	137.7	695.4	11.6%	1.2%
Ludwigia	8	303.0	74.6	201.8	157.8	757.1	27.6%	3.3%
Sagittaria	12	503.3	72.1	465.8	173.6	888.2	24.8%	1.3%
Comal Val	15	685.9	103.1	657.3	249.2	1939.2	23.7%	1.6%
Hydrilla	11	554.4	56.8	515.1	307.4	861.1	9.9%	1.3%
Potamogeton	11	426.2	35.2	458.1	236.7	575.4	33.2%	2.1%
SM Val	3	467.3	83.8	441.3	336.8	623.7	55.1%	2.5%

The average total plant biomass for these species varied from near 300 g m^{-2} (*Ludwigia repens*) to almost 700 g m^{-2} (*Vallisneria neotropicalis*) (Figure 2).

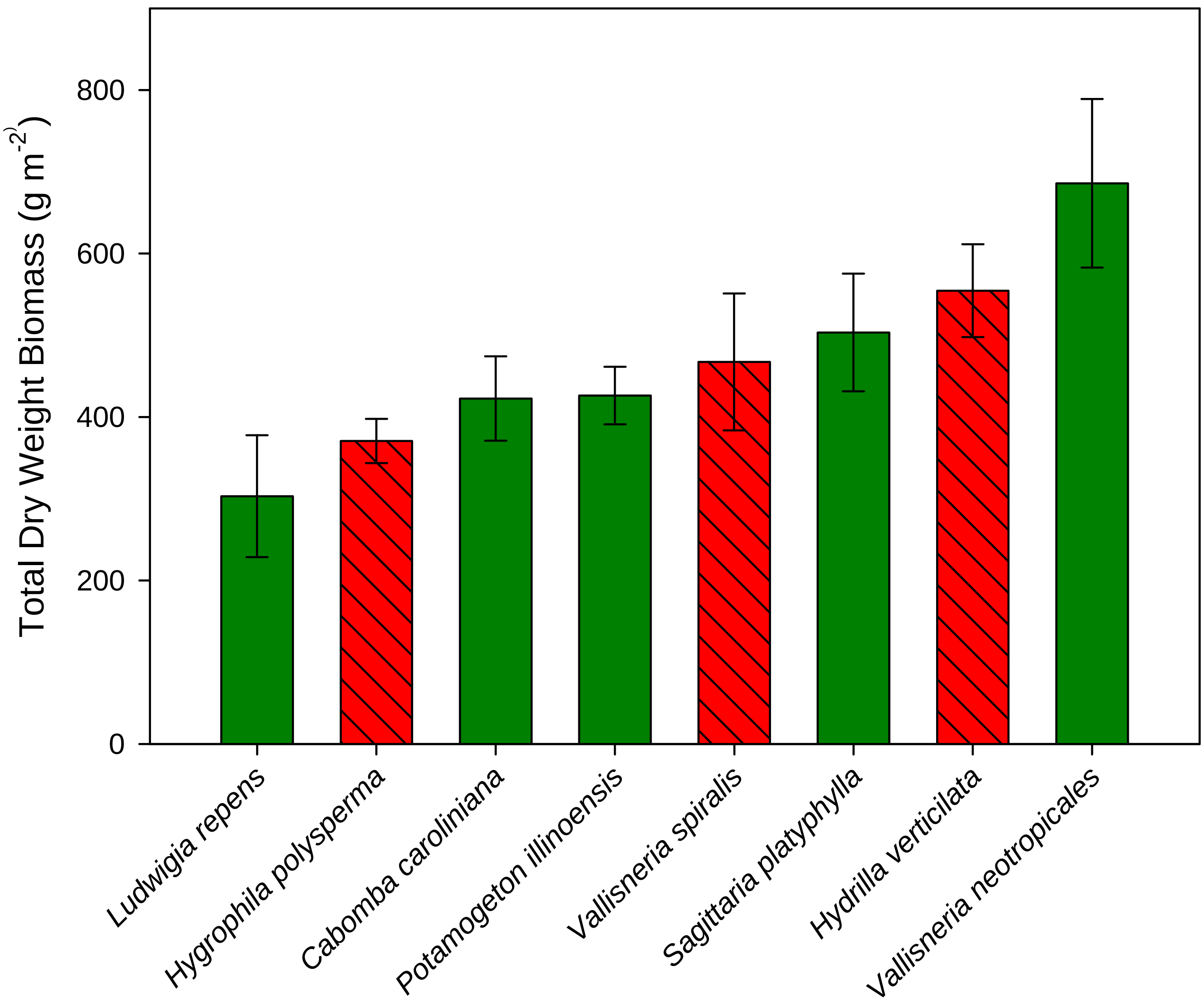

Figure 2. Average (± SE) of total plant biomass per meter squared for samples heavily dominated by a single plant species (90-100% cover reported). Native species are shown in solid green and non-native species in red hash.

Linear regression of biomass vs. percent cover has limited utility. As an example, Figure 3 shows the biomass vs. percent cover for the 29 samples of Hygrophila collected. Because the vast majority of plant samples found and sampled within these river systems had very high observed percent cover (80-100%), there is simply insufficient spread of the data to allow effective linear regression. However, a much more useful relationship emerges when total plant biomass is plotted against plant volume (Figure 4).

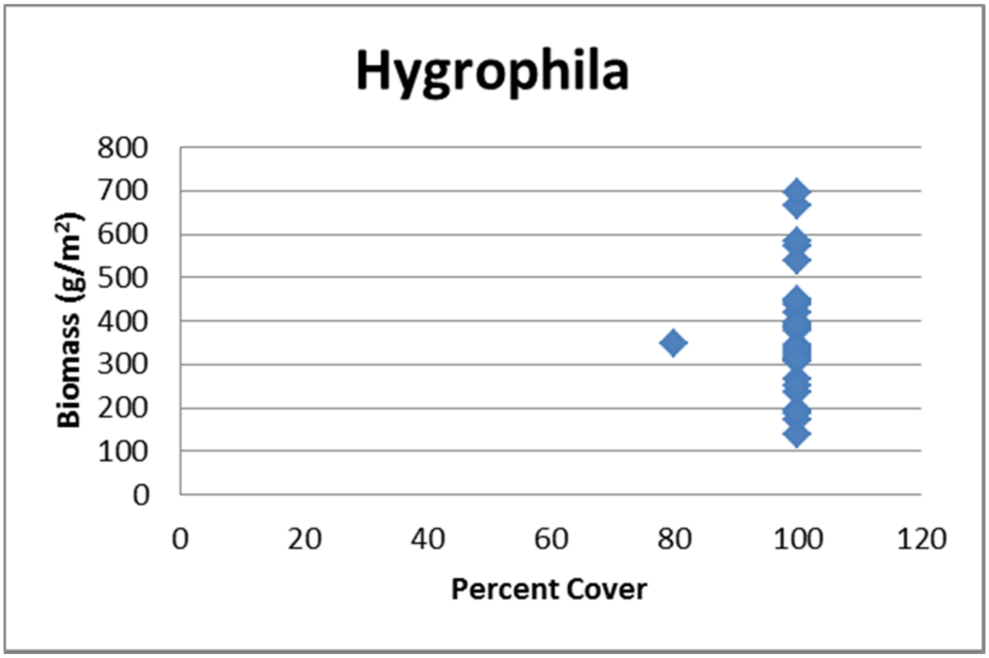

Figure 3. Total dry weight biomass vs. observed percent cover for 29 samples of Hygrophila collected on the Comal and San Marcos Rivers, TX.

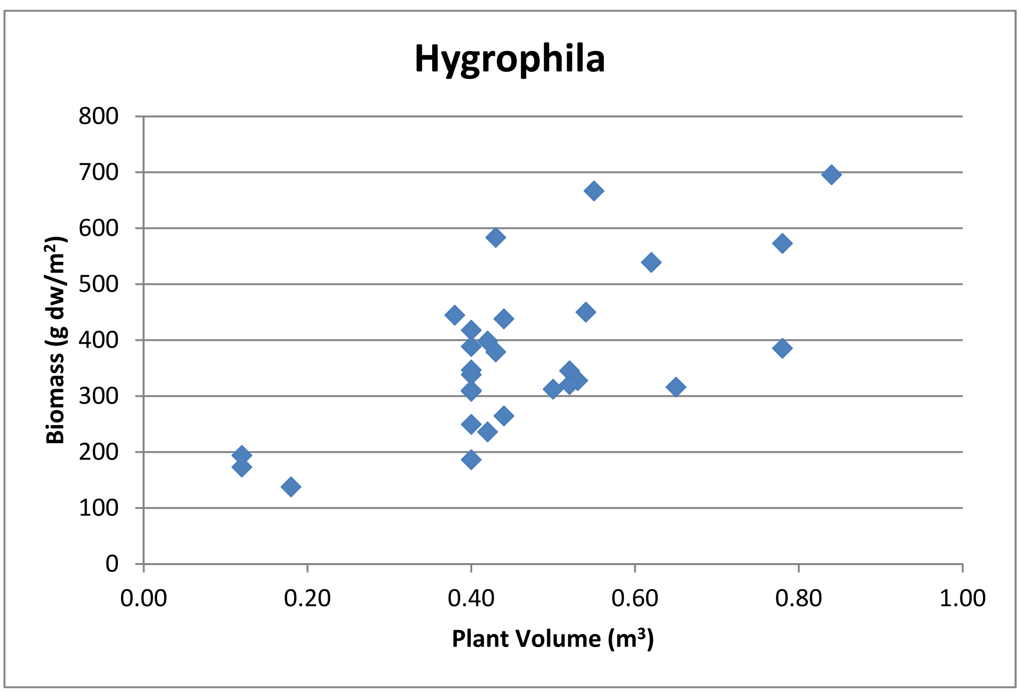

Figure 4. Total dry weight biomass vs. occupied plant volume for 29 samples of Hygrophila collected on the Comal and San Marcos Rivers, TX.

The results of the linear regression of total plant biomass vs. plant volume for each of the species are shown in Table 3. A highly significant relationship explaining much of the variability emerged for each species. For this regression analysis we required the regression to pass through the zero origin.

Table 3. Results of linear regression of total biomass vs plant volume

Species	p	r^2	n	slope	SE
Cabomba	<0.001	90.3	15	1228.1	107.8
Hygrophila	<0.001	92.4	29	772.7	41.8
Ludwigia	0.005	67.8	9	726.0	176.8
Sagittaria	<0.001	93.2	15	1327.3	96.3
Comal Val	<0.001	65.9	15	1432.3	275.3
Hydrilla	<0.001	84.5	11	825.2	111.9
Potamogeton	<0.001	93.3	11	1277.3	106.7
SM Val	Insufficient samples				

Discussion

These data establish the range of total dry weight biomass and proportion of above ground to below ground tissues for eight species of interest on the Comal and San Marcos Rivers, TX. In addition, we provide regression relationships of total dry weight biomass vs. occupied plant volume that can be used when those data are available. Those data are available for all maps generated by R Doyle (1998-2001), and can be estimated with reasonable accuracy for all of the BIO-WEST mapping efforts if needed.

The percent below ground tissues for the various species vary widely. They appear to fall into two general groups with low and high investment of biomass into below ground tissues.

Hydrilla, Cabomba and Hygrophila both invest relatively little into roots and rhizomes with only 9.9% ± 1.3%, 11.6% ± 1.2% and 17.3 ± 2.1% (mean ± SE) respectively. This low level of investment in below ground tissues may be related to the anecdotal observation that these species seem to "move around" the system, appearing and later disappearing from an area. However, three of the Hydrilla samples collected in late October 2014 had subterranean turions (tubers). These asexual reproductive structures are produced during short days and can be viable in the sediments for many years. Two of the five samples collected 10/29/2014 each had three tuber in the $0.33 m^2$ sample collected (= 91 tubers m^{-2}). The presence of Hydrilla tubers within the sediments dramatically increases the likelihood of persistence of this species in an area since the tubers can remain viable in the sediments for many years.

Ludwigia, Sagittaria, Potamogeton and Comal Vallisneria (likely *V. neotropicalis*) had more significant investment of biomass into root tissues (range = 24-33%) consistent with the more permanent nature of colonies of these species within the river systems. Sagittaria and Vallisneria appear to be particularly stable in time and space within these systems. The Vallisneria in the San Marcos (likely the exotic *V. spiralis*) has very high % biomass below ground (55%), although it is currently not widely distributed and occurs only in discrete patches.

Three outcomes of this study show encouraging promise for providing reasonable biomass data to inform the vegetation model development efforts for the HCP AMP. First, the variability around the "100%" cover samples appears to be reasonably constrained. Evidence comes in that the standard error around the mean for all species ranged only between 7.3-24.6% (Table 2 and Figure 2). The average SE variability around the mean for all species sampled was 13.7%. Second, the species to species variability is well defined (Table 2 and Figure 2). This provides realistic and constrained targets for maximum biomass per m^2 for each of the key species. Finally, the biomass to plant volume relationship described (Table 3) is also well bounded so that even more accurate estimates of biomass can be made provided vegetation height data is available. The average SE of the slope is only 12.4%, indicating that plant volume explains the vast majority of the variability in vegetation biomass. Since the vegetation height data are directly available for some maps and reasonable estimates can be made for most of the maps, we should have robust data to inform the modeling efforts.

Recommendations for Future Studies

No additional data appears to be needed for most of the species reported on here. However, two species investigated showed less robust data than the other species. The variability in Ludwigia data was much higher than that of other species. The reason for this higher variability is not known. Also, we have limited data for the relatively rare non-native Vallisneria species on the San Marcos River (*V. spiralis*). If tighter data is needed for either of these species, additional samples can be collected in 2015.

Additionally, we did not collect biomass data for the endangered Texas Wildrice (*Zizania texana*). Biomass sampling is by nature destructive. However, if actual biomass data are required by the modelers for this important and increasingly widespread species (on the San Marcos River), we can generate these data in 2015.

Finally, if vegetation height is deemed necessary by the modelers, some level of effort will be needed to organize those data from the maps for which it exists or estimate the data for the BIO-WEST maps.

Acknowledgements

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Appendix 1

Data collected for determination of biomass to percent cover analysis.

River	Species	% Cover	Depth (cm)	Plant Height (cm)	Plant Vol (m^3)	Sediment	Total Biomass (g/m^2)	Below Ground (% of total)
Comal	Cabomba	100	68.0	27.0	0.27	Soft Silt	499.2	20.9%
Comal	Cabomba	100	70.0	24.0	0.24	Soft Silt	365.8	10.8%
Comal	Cabomba	100	60.0	28.0	0.28	Soft Silt	517.3	26.0%
Comal	Cabomba	80	53.0	23.0	0.18	Soft Silt	268.1	15.5%
Comal	Cabomba	80	63.0	33.0	0.26	Soft Silt	241.3	13.6%
Comal	Cabomba	70	53.0	30.0	0.21	Soft Silt	130.8	16.5%
Comal	Cabomba	100	56.0	30.0	0.30	Soft Silt	284.6	13.5%
Comal	Cabomba	100	53.0	53.0	0.53	Soft Silt	525.2	21.3%
Comal	Cabomba	100	40.0	35.0	0.35	Soft Silt	558.1	16.7%
Comal	Cabomba	30	64.0	24.0	0.07	Silt	98.4	15.0%
Comal	Cabomba	30	78.0	18.0	0.05	Silt	89.9	8.6%
Comal	Cabomba	30	60.0	12.0	0.04	Silt	157.2	16.9%
Comal	Cabomba	80	60.0	22.0	0.18	Soft Silt	111.5	25.1%
Comal	Cabomba	80	58.0	18.0	0.14	Soft Silt	137.1	16.1%
Comal	Cabomba	100	56.0	20.0	0.20	Soft Silt	207.9	12.0%
Comal	Ludwigia	100	58.0	32.0	0.32	Gravel	484.7	23.1%
Comal	Ludwigia	100	56.0	56.0	0.56	Gravel	757.1	17.6%
Comal	Ludwigia	100	56.0	36.0	0.36	Gravel	254.1	30.6%
Comal	Ludwigia	100	40.0	25.0	0.25	Gravel	205.1	18.2%
Comal	Ludwigia	60	43.0	15.0	0.09	Gravel	239.5	50.2%
Comal	Ludwigia	100	50.0	4.0	0.04	Gravel	177.5	42.8%
Comal	Ludwigia	100	60.0	55.0	0.55	Soft Silt	157.8	20.8%
Comal	Ludwigia	100	60.0	50.0	0.50	Soft Silt	198.6	28.6%
Comal	Ludwigia	100	60.0	50.0	0.50	Soft Silt	189.3	38.8%

Appendix 1 Continued.

River	Species	% Cover	Depth (cm)	Plant Height (cm)	Plant Vol (m^3)	Sediment	Total Biomass (g/m^2)	Below Ground (% of total)
Comal	Sagittaria	100	42.0	24.0	0.24	Gravel	173.6	24.5%
Comal	Sagittaria	100	45.0	25.0	0.25	Soft Silt	232.3	28.7%
Comal	Sagittaria	100	51.0	33.0	0.33	Soft Silt	289.4	34.0%
Comal	Sagittaria	100	52.0	42.0	0.42	Soft Silt	477.6	19.9%
Comal	Sagittaria	100	48.0	41.0	0.41	Soft Silt	463.6	19.6%
Comal	Sagittaria	100	46.0	38.0	0.38	Soft Silt	331.8	19.3%
Comal	Sagittaria	100	72.0	42.0	0.42	Soft Silt	517.3	24.2%
Comal	Sagittaria	100	70.0	40.0	0.40	Soft Silt	468.0	27.6%
Comal	Sagittaria	100	82.0	37.0	0.37	Silt	451.5	30.7%
Comal	Sagittaria	30	38.0	14.0	0.04	Gravel	165.5	31.7%
Comal	Sagittaria	30	50.0	22.0	0.07	Gravel	327.9	52.4%
Comal	Sagittaria	30	48.0	20.0	0.06	Gravel	154.8	32.2%
San Marcos	Sagittaria	100	74.0	54.0	0.54	Soft Silt	861.6	22.6%
San Marcos	Sagittaria	100	74.0	54.0	0.54	Soft Silt	888.2	23.7%
San Marcos	Sagittaria	100	72.0	52.0	0.52	Soft Silt	885.1	22.5%
Comal	Vallisneria	100	72.0	66.0	0.66	Soft Silt	742.6	19.0%
Comal	Vallisneria	100	81.0	69.0	0.69	Soft Silt	671.4	16.7%
Comal	Vallisneria	100	78.0	64.0	0.64	Soft Silt	521.2	19.7%
Comal	Vallisneria	100	62.0	54.0	0.54	Gravel	657.3	21.4%
Comal	Vallisneria	100	58.0	36.0	0.36	Gravel	664.4	19.7%
Comal	Vallisneria	100	51.0	33.0	0.33	Gravel	420.2	29.4%
Comal	Vallisneria	100	34.0	34.0	0.34	Gravel	901.5	29.9%
Comal	Vallisneria	100	46.0	46.0	0.46	Gravel	586.9	27.3%
Comal	Vallisneria	100	36.0	36.0	0.36	Gravel	361.5	32.5%
Comal	Vallisneria	100	32.0	24.0	0.24	Gravel	686.3	36.9%
Comal	Vallisneria	100	42.0	34.0	0.34	Gravel	1939.2	15.5%
Comal	Vallisneria	100	38.0	30.0	0.30	Gravel	989.9	20.1%
Comal	Vallisneria	100	58.0	40.0	0.40	Soft Silt	446.0	23.3%
Comal	Vallisneria	100	50.0	36.0	0.36	Soft Silt	249.2	19.3%
Comal	Vallisneria	100	50.0	35.0	0.35	Soft Silt	451.0	24.8%

Appendix 1 Continued.

River	Species	% Cover	Depth (cm)	Plant Height (cm)	Plant Vol (m^3)	Sediment	Total Biomass (g/m^2)	Below Ground (% of total)
Comal	Hygrophila	100	48.0	43.0	0.43	Silt	378.8	15.7%
Comal	Hygrophila	100	53.0	53.0	0.53	Soft Silt	327.7	13.5%
Comal	Hygrophila	100	63.0	55.0	0.55	Soft Silt	666.7	4.4%
Comal	Hygrophila	100	36.0	18.0	0.18	Gravel	137.7	14.7%
Comal	Hygrophila	100	28.0	12.0	0.12	Gravel	173.0	17.4%
Comal	Hygrophila	100	24.0	12.0	0.12	Gravel	193.9	12.8%
Comal	Hygrophila	100	78.0	78.0	0.78	Soft Silt	572.8	2.8%
Comal	Hygrophila	100	78.0	78.0	0.78	Soft Silt	385.2	5.0%
Comal	Hygrophila	100	84.0	84.0	0.84	Soft Silt	695.4	3.5%
Comal	Hygrophila	100	60.0	44.0	0.44	Soft Silt	264.5	8.5%
Comal	Hygrophila	80	83.0	50.0	0.40	Soft Silt	346.4	4.4%
Comal	Hygrophila	100	78.0	65.0	0.65	Soft Silt	316.0	7.3%
Comal	Hygrophila	100	72.0	52.0	0.52	Soft Silt	345.1	3.8%
Comal	Hygrophila	100	70.0	54.0	0.54	Soft Silt	449.9	4.9%
Comal	Hygrophila	100	72.0	62.0	0.62	Soft Silt	538.9	5.7%
San Marcos	Hygrophila	100	58.0	50.0	0.50	Soft Silt	312.3	11.8%
San Marcos	Hygrophila	100	52.0	52.0	0.52	Soft Silt	320.6	16.8%
San Marcos	Hygrophila	100	58.0	43.0	0.43	Soft Silt	583.4	9.9%
San Marcos	Hygrophila	100	55.0	40.0	0.40	Soft Silt	417.6	12.4%
San Marcos	Hygrophila	100	58.0	42.0	0.42	Soft Silt	236.0	30.7%
San Marcos	Hygrophila	100	58.0	42.0	0.42	Soft Silt	398.2	16.7%
San Marcos	Hygrophila	100	52.0	44.0	0.44	Soft Silt	437.9	9.2%
San Marcos	Hygrophila	100	52.0	38.0	0.38	Soft Silt	444.6	12.9%
San Marcos	Hygrophila	100	52.0	40.0	0.40	Soft Silt	308.5	18.6%
San Marcos	Hygrophila	100	52.0	40.0	0.40	Soft Silt	388.6	21.7%
San Marcos	Hygrophila	100	52.0	40.0	0.40	Soft Silt	338.3	18.0%
San Marcos	Hygrophila	100	64.0	40.0	0.40	Soft Silt	186.3	8.4%
San Marcos	Hygrophila	100	64.0	40.0	0.40	Soft Silt	249.3	7.4%
San Marcos	Hygrophila	100	64.0	40.0	0.40	Soft Silt	310.2	9.4%

Appendix 1 Continued.

River	Species	% Cover	Depth (cm)	Plant Height (cm)	Plant Vol (m^3)	Sediment	Total Biomass (g/m^2)	Below Ground (% of total)
San Marcos	Hydrilla	100	72.0	52.0	0.52	Soft Silt	329.6	7.2%
San Marcos	Hydrilla	100	72.0	56.0	0.56	Soft Silt	492.6	7.7%
San Marcos	Hydrilla	100	72.0	56.0	0.56	Soft Silt	307.4	7.1%
San Marcos	Hydrilla	100	100.0	100.0	1.00	Soft Silt	808.9	12.8%
San Marcos	Hydrilla	100	100.0	100.0	1.00	Soft Silt	696.8	6.8%
San Marcos	Hydrilla	100	100.0	100.0	1.00	Soft Silt	623.1	5.6%
San Marcos	Hydrilla	100	75.0	55.0	0.55	Silt	515.1	4.3%
San Marcos	Hydrilla	100	75.0	40.0	0.40	Silt	861.1	11.5%
San Marcos	Hydrilla	100	40.0	25.0	0.25	Sand	451.9	15.6%
San Marcos	Hydrilla	100	72.0	30.0	0.30	Sand	365.4	14.7%
San Marcos	Hydrilla	100	80.0	35.0	0.35	Sand	647.0	16.0%
San Marcos	Potamogeton	100	40.0	22.0	0.22	Gravel	437.1	34.6%
San Marcos	Potamogeton	100	40.0	22.0	0.22	Gravel	410.7	41.1%
San Marcos	Potamogeton	100	40.0	22.0	0.22	Gravel	458.1	45.8%
San Marcos	Potamogeton	100	64.0	24.0	0.24	Silt	265.3	36.0%
San Marcos	Potamogeton	100	62.0	22.0	0.22	Silt	283.3	33.2%
San Marcos	Potamogeton	100	56.0	16.0	0.16	Silt	236.7	25.9%
San Marcos	Potamogeton	90	75.0	55.0	0.50	Silt	470.1	22.9%
San Marcos	Potamogeton	90	75.0	55.0	0.50	Silt	480.6	38.9%
San Marcos	Potamogeton	90	68.0	48.0	0.43	Silt	575.4	31.6%
San Marcos	Potamogeton	90	70.0	40.0	0.36	Silt	507.6	27.7%
San Marcos	Potamogeton	90	70.0	40.0	0.36	Silt	563.4	27.9%
San Marcos	Vallisneria	100	50.0	12.0	0.12	Gravel	623.7	57.0%
San Marcos	Vallisneria	100	50.0	12.0	0.12	Gravel	441.3	58.2%
San Marcos	Vallisneria	100	50.0	12.0	0.12	Gravel	336.8	50.0%

APPENDIX C

Ludwigia Competition Study

Final Report for *Ludwigia repens* Competition Study

Edwards Aquifer Authority Contract #14-727L

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1.0 Introduction

The San Marcos and Comal Rivers have unique aquatic plant communities that support a wide variety of native and endemic wildlife including several listed species. In 2013 the Edwards Aquifer Habitat Conservation Plan (EAHCP) was enacted to enhance and expand habitat for covered species including the fountain darter (*Etheostoma fonticola*). Part of this long-term plan includes removal of the non-native aquatic plant species *Hydrilla verticillata* and *Hygrophila polysperma* and reintroduction of native aquatic plants such as *Ludwigia repens* – all of which will be referred to by their genus name throughout this report. Hydrilla and Hygrophila are becoming increasingly abundant in these systems (Lemke, 1989; Bowles and Bowles, 2001) and tend to support fewer numbers of fountain darters than certain species of native aquatic plants (BIO-WEST, 2015). The persistence and expansion of Hydrilla and Hygrophila pose a threat to efforts in re-establishing beneficial native aquatic vegetation for *E. fonticola* (Bormann, 2012). Predicting the long-term success of revegetation efforts and which species, native or non-native, dominate is vital in the development of a submerged aquatic vegetation module for the EAHCP Ecological model.

Interspecific competition, or the success of a particular plant species relative to another, is a potentially important factor in determining the complex structure of aquatic plant communities. Abiotic factors like substrate and water quality (Szosszkiewicz et al, 2014) as well as differences in species-specific characteristics such as growth rate, plant architecture, reproductive vigor and susceptibility to herbivory (Spencer and Bowes, 1985), phenological plasticity (Garbey et al, 2004; Thouvenot et al, 2013) and, in certain cases, chemical defenses (Gopal, 1993; Gross, 2003) all play a role in the distribution and abundance of species within the plant community. While competitive pressure among naturally co-existing species may appear to be low (Chambers and Prepas, 1990), various studies suggest that these communities do display spatiotemporal variability based on interactions between competitive ability and environmental gradients (McCreary, 1991; Barrat-Segretain, 1996). Non-native species may possess traits that confer a competitive advantage over native species, decreasing species richness, facilitating shifts in community composition and precipitating negative effects throughout the ecosystem (Santos et al, 2011).

Invasive aquatic plant species are well known for their ability to spread rapidly via fragmentation of stems, basal rooting structures, such as stolons, tubers or corms, or specialized structures, such as turions, which can detach and move downstream or float on currents into new locations colonizing in rapid fashion (Sculthorpe, 1967; Langeland and Sutton, 1980). Typically aquatic plants reproduce asexually (Arbor, 1920; Haynes, 1988) and vegetative structures are primed for growth upon settling into new habitat with root structures or leaves still attached (Sutton, 1996). As a consequence, in many cases, invasion of an aquatic species into new areas can take very little time (Santamaria, 2002). For example Eurasion watermilfoil, *Myriophyllum spicatum*, a

widespread problematic submersed aquatic plant has been documented to establish and dominate littoral zones of lakes within two to three years after introduction (Aiken et al., 1979; Newroth, 1985) and is known to suppress growth of a native species (Agami and Waisel, 1985). A North American native *Elodea nuttaalii* has spread rapidly in Japan's largest lake covering the lake bottom within a few years after introduction there (Kadono, 2004). Closer to home in the San Marcos system the exotic plant *Cryptocoryne becketti* was documented to quickly establish and spread within 2 years after initial discovery with a recorded expansion rate of 80% a year (Doyle, 2001) and annual mapping by BIO-WEST has shown the dramatic expansion of Hygrophila in the Old Channel Study Reach of the Comal River (BIO-WEST, 2015). Some invasive aquatic plants not only colonize rapidly but they can displace native aquatic plants by producing a dense canopy structure limiting light availability to other submersed species.

With recent documented expansions of invasive aquatic plants within the San Marcos and Comal systems data is needed to predict how native plants may respond. Few studies regarding native versus non-native aquatic plant competition have been conducted with regard to either of these systems. In one particular study, Doyle et al. (2003) conducted a study in a static container (35 gallon barrels) within an outdoor raceway to evaluate the competitive ability of *Ludwigia repens* against *Hygrophila polysperma*. Our experiment expanded upon that of Doyle et al. (2003) to help further understand the competitive outcome under more realistic environmental flow and ambient light conditions and to additionally investigate the competition between Ludwigia and Hydrilla.

Ludwigia repens (Forester), red ludwigia, is a perennial obligate aquatic plant native to the Comal and San Marcos rivers with common distribution throughout Texas. Ludwigia is an amphibious plant that produces both submersed and emergent growth and can grow terrestrially as well. The architecture of Ludwigia is characterized as caulescent and multi-branched. Submersed growth is typically upright within the water column and nodal rooting is common while terrestrial growth is typically low growing and prostrate. Ludwigia is considered prime habitat for the fountain darter (*Etheostoma fonticola*) and is being utilized in the restoration of darter habitat in both systems.

Hygrophila polysperma (Roxb.) T. Anderson is a non-native plant introduced from Asia. *Hygrophila polysperma* is morphologically similar to Ludwigia in many ways and has been confused with Ludwigia in some instances. Hygrophila is common within the Comal and San Marcos rivers but is not a common invasive plant in Texas as its known distribution is limited to Comal and San Marcos Rivers and San Felipe creek in Val Verde County (Williams, 2013). Like Ludwigia, Hygrophila is also amphibious exhibiting both completely submersed forms, emergent forms and terrestrial growth.

Hydrilla verticillata (L. f.) Royle is another non-native submersed plant introduced from Africa and Eurasia. Hydrilla is a widespread and common invasive aquatic plant with widespread

distribution in the United States. It too is an obligate aquatic plant, but does not produce emergent or terrestrial growth forms. Hydrilla only exists as a submersed aquatic plant typically producing dense growth in upright fashion towards the water surface producing a thick canopy. Absent in the Comal River, Hydrilla is common in the San Marcos River but has been successfully controlled in Spring Lake where it was once the dominant aquatic plant species (Williams, et al. 2011).

The data reported here provide information on the short-term (10 week) early establishment and growth period of viable sprigs of Ludwigia, Hygrophila, and Hydrilla under three levels of competition from the other species. Additionally, it evaluated the short term (10 week) impact(s) of sprig invasion from a competing species on the continued growth and development of established plants. These experiments were conducted at various locations within the Comal and San Marcos Rivers to provide more realistic environmental conditions than was possible with the static tank experiments previously conducted by Doyle et al. (2003).

2.0 Materials and Methods

Two separate studies were conducted to compare the competitive interactions of Ludwigia with Hygrophila and Hydrilla. The site of the Ludwigia X Hygrophila study took place within the Comal River. Since Hydrilla does not occur in the Comal system the Ludwigia X Hydrilla study was conducted separately in the San Marcos River located approximately 12 km north of the Comal River. Both rivers are spring-fed systems fed by the Edwards Aquifer and have similar water quality and general biological characteristics.

2.1 Study Design

Two separate but related two-factor factorial experiments for each species pair (Ludwigia X Hygrophila and Ludwigia X Hydrilla) comprised the studies (Tables 1 and 2). In each experiment the impact of competition (C) and location (L) was evaluated separately for each species.

The first experiment of each study (Table 1A, Table 2A) was designed to document initial establishment and growth of colonizing sprigs of each species in three competitive environments. Two sprigs of each species were planted into pots with no competition (empty pots without a competitor species) moderate competition (pots with 50:50 ratio Ludwigia: competitor sprigs) and high competition (pots with established plants of the competitor species). A second experiment evaluated the continued growth of established plants of Ludwigia or the non-native species without competition with those “invaded” by sprigs of the competing species (Table 1B, Table 2B). Experimental design and analysis followed that of Doyle et al., 2003. The combined experiments resulted in seven different treatments (Table 3).

Table 1. Comal River Ludwigia X Hygrophila competition study designs. A) Top. 3x4 Two-Factor Factorial Design (Competition X Location) for the Ludwigia X Hygrophila and Hygrophila X Ludwigia sprig competition experiments. Eight replicate plantings of sprigs of each species into three competitive environments were made at each of four locations. B) Bottom. 2X4 Two-Factor Factorial Design (Competition X Location) for established plants with and without invasion by sprigs of the other species. Invasion treatment was replicated eight times at each location, while the non-invaded treatment was replicated only four times at each location.

A. Sprig Experiments		3X Level of Competition		
		No Competition	Moderate Competition	High Competition
4X Locations	Landa Lake, High Light	8X	8X	8X
	Landa Lake, Low Light	8X	8X	8X
	Upper Spring Run	8X	8X	8X
	Old Channel	8X	8X	8X

B. Established Plant Experiments		2X Level of Competition	
		Not Invaded	Invaded by 2 sprigs
4X Locations	Landa Lake, High Light	4X	8X
	Landa Lake, Low Light	4X	8X
	Upper Spring Run	4X	8X
	Old Channel	4X	8X

Each of the two competition experiments were replicated at multiple locations: four locations on the Comal for the Hygrophila study (Table 1), and two locations on the San Marcos for the Hydrilla study (Table 2).

Table 2. San Marcos River Ludwigia X Hydrilla competition study designs. A) Top. 3x2 Two-Factor Factorial Design (Competition X Location) for the Ludwigia X Hydrilla and Hydrilla X Ludwigia sprig competition experiments. Eight replicate plantings of sprigs of each species into three competitive environments were made at each of two locations. B) Bottom. 2X4 Two-Factor Factorial Design (Competition X Location) for established plants with and without invasion by sprigs of the other species. Invasion treatment was replicated eight times at each location, while the non-invaded treatment was replicated only four times at each location.

A. Sprig Experiments		3X Level of Competition		
		No Competition	Moderate Competition	High Competition
2X Locations	City Park	8X	8X	8X
	I 35	8X	8X	8X

B. Established Plant Experiments		2X Level of Competition	
		Not Invaded	Invaded by 2 sprigs
	City Park	4X	8X
	I35	4X	8X

For the Ludwigia X Hygrophila or Ludwigia X Hydrilla experiments seven treatments were included (Table 3). The same treatments were used at all study locations. Our treatment nomenclature utilizes lower case letters to designate sprigs of a species and capital letters to designate established plants. The first three treatments utilize only plant sprigs planted into previously empty pots of sediment. These include freshly collected Ludwigia sprigs planted in monoculture into empty pots (ll), Hygrophila (or Hydrilla) sprigs planted in monoculture into empty pots (hh), a 50/50 mix of Ludwigia sprigs and Hygrophila (or Hydrilla) sprigs (llhh, 2 sprigs of each species). The use of newly sprigged fragments in empty pots provides information on the colonization potential of both species when free of competitive pressures (ll and hh). The 50:50 sprig mixture (llhh) provides information on the competitive outcome of “equal start” moderate-competition environments. The high-competition environment was obtained by planting sprigs of each species into pots of established plants of the other species (hhLL and llHH).

Table 3. Treatments for Ludwigia vs. Hygrophila (or Hydrilla) competition experiments.

Symbol	Treatment	Count
ll	Ludwigia sprigs into empty pot (No competition)	8
hh	Hygrophila (or Hydrilla) sprigs into empty pot (No competition)	8
ll hh	50 : 50 mix Ludwigia and Hygrophila (or Hydrilla) sprigs into empty pots (Moderate competition)	8
ll HH	Ludwigia sprigs planted into pots of established Hygrophila (or Hydrilla) (High competition for the sprigs; invasion scenario for established plant))	8
hh LL	Hygrophila (or Hydrilla) sprigs planted into pots of established Ludwigia (High competition for the sprigs; invasion scenario for established plant)	8
HH	Growth of established Hygrophila (or Hydrilla) plants (no competition from invading sprigs)	4
LL	Growth of established Ludwigia plants (no competition from invading sprigs)	4

Four treatments utilized established plants of the native or the competitor species (Figure 1). Sprigs of Ludwigia or the competitor species were planted into the pots containing established plants (llHH, hhLL) while other pots containing only established plants (HH, LL) were used to track the continued plant growth without any competitive pressure from invading fragments of the other species. All individual pots were secured within Mobile Underwater Plant Propagation Trays (MUPPT) developed and used for EAHCP restoration and applied research projects (Figure 1).

Note that the llHH and hhLL pots serve dual purpose. The sprig growth in these pots represents the growth of plant sprigs in high-competition environments (Experiment 1A or 2A). The continued growth of the established plant following invasion from the sprigs is the invaded scenario of the established plant experiments (Experiments 1B and 2B).

Figure 1. **Example of Experimental layout of treatments within a MUPPT (left) and MUPPT deployed in the San Marcos River (right). Examples of pots of several of the treatments are highlighted.**

2.2 Initial Setup and Sampling

Seven experimental treatments (Table 3) were randomly assigned and simultaneously placed into paired MUPPTs similar to the arrangement diagrammed in Figure 2. A total of 48 pots contained 8 replicates of 5 treatments – only Ludwigia sprigs (ll), only Hygrophila or Hydrilla sprigs (hh), a combination of sprigs (llhh), established plants with sprigs of the opposite species (LLhh and hhLL) – and 4 replicates of established plants for both species (LL and HH). Adjacent spaces were left empty to minimize interaction between pots, resulting in two MUPPTs being needed at each location.

Pre-established plants and sprigs were planted in 600mL quart-sized nursery pots filled with native silty/clay sediment collected from the respective rivers in which the study was carried out. Native sediment was collected in areas with no plant growth and further screened for plant propagules to prevent extraneous plant growth in treatments. Established plants were obtained by pre-culturing plants for three weeks in MUPPTs near the Landa Lake High Light location (Comal study) or at the experimental location used on the San Marcos (City Park) to allow robust

initial establishment and growth. Healthy plants of uniform size were selected for the experiment as well as to obtain initial biometric measurements. Stem cuttings were collected from healthy, established plants and inspected to ensure they had no visible signs of herbivory or disease. Sprigs 20cm in length were selected for experimental use and harvested for initial biomass.

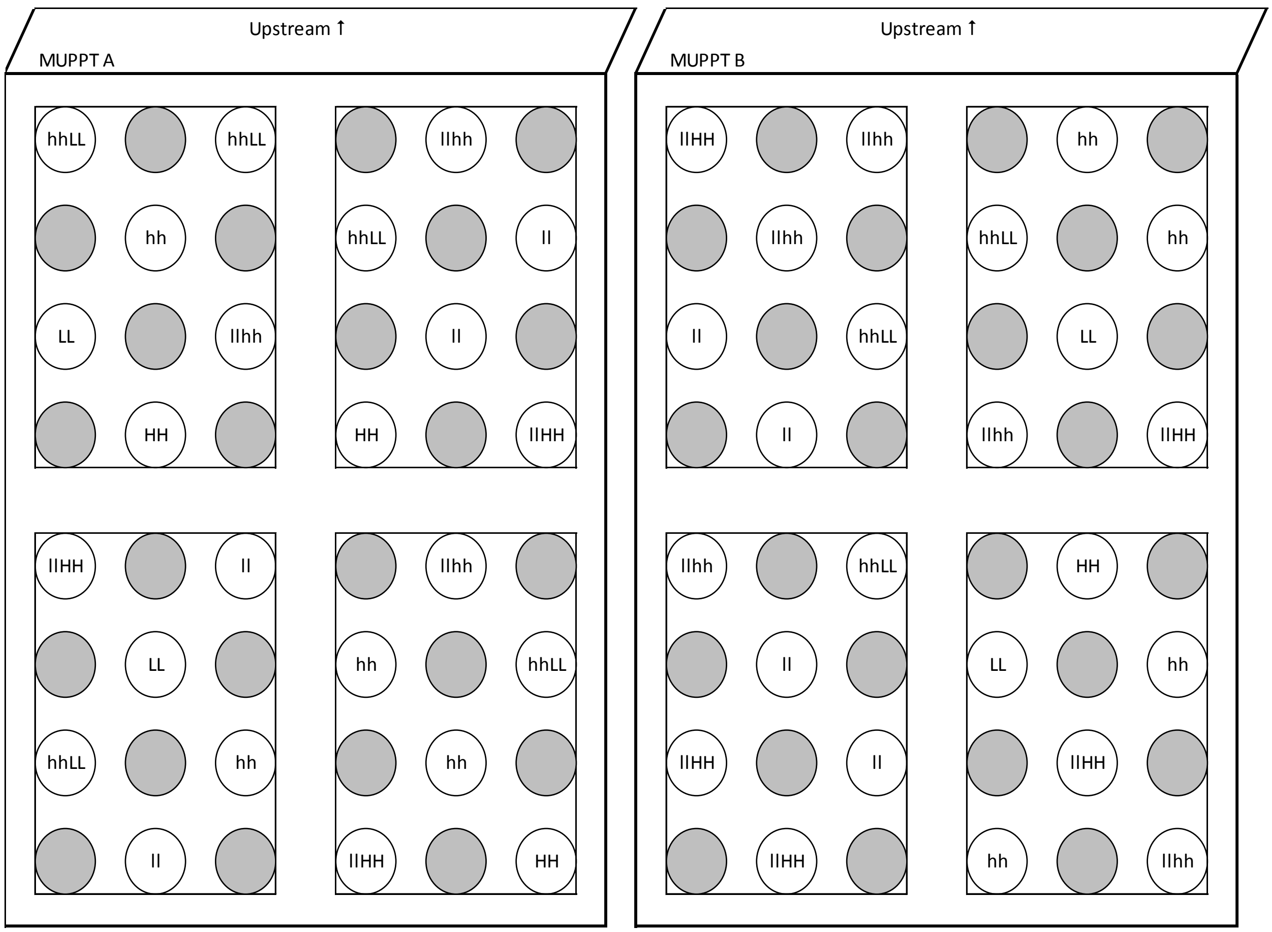

Figure 2. **Illustrated arrangement of alternating experimental pot placement within two MUPPTS anchored at each location. Open circles display the 7 possible experimental combinations (Table 3), and gray circles represent empty spaces.**

Four locations were selected on the Comal River to represent the variability of environmental conditions found within this system. Locations were selected within the Upper Spring Run (USR), Landa Lake in a shaded location (Landa Lake Low Light, LLLL), Landa Lake in a full sun exposure location (Landa Lake High Light, LLHL), and the Old Channel (OC; Figure 3). The Landa Lake High Light location was adjacent to the MUPPT culture station for restoration plantings while the Landa Lake Low Light location was along the western shoreline under the shade of an overhanging live oak tree. All four of these locations were initially planted on May 13, 2015 and harvested on July 27, 2015. In the San Marcos River two locations were chosen.

One location (1) above Rio Vista falls at City Park (CP) and another location (2A) below Rio Vista falls (Figure 3).

Rio Vista falls provides a distinctive dissection in the velocity characteristics of the San Marcos with river velocities below this point typically faster than velocities above the falls. The San Marcos study was initiated on April 23, 2015. Unfortunately, the significant flood event of May 2015 scoured out and destroyed the portion of the experiment at the downstream location (2A). The City Park location was minimally impacted, and continued until it was harvested on June 30, 2015. In order to provide information from the lower portion of the river, another site near the I35 crossing was selected (location 2B or I35, Figure 3) and plantings were initiated on July 6, 2015. The plants at this downstream location were harvested on September 11, 2015.

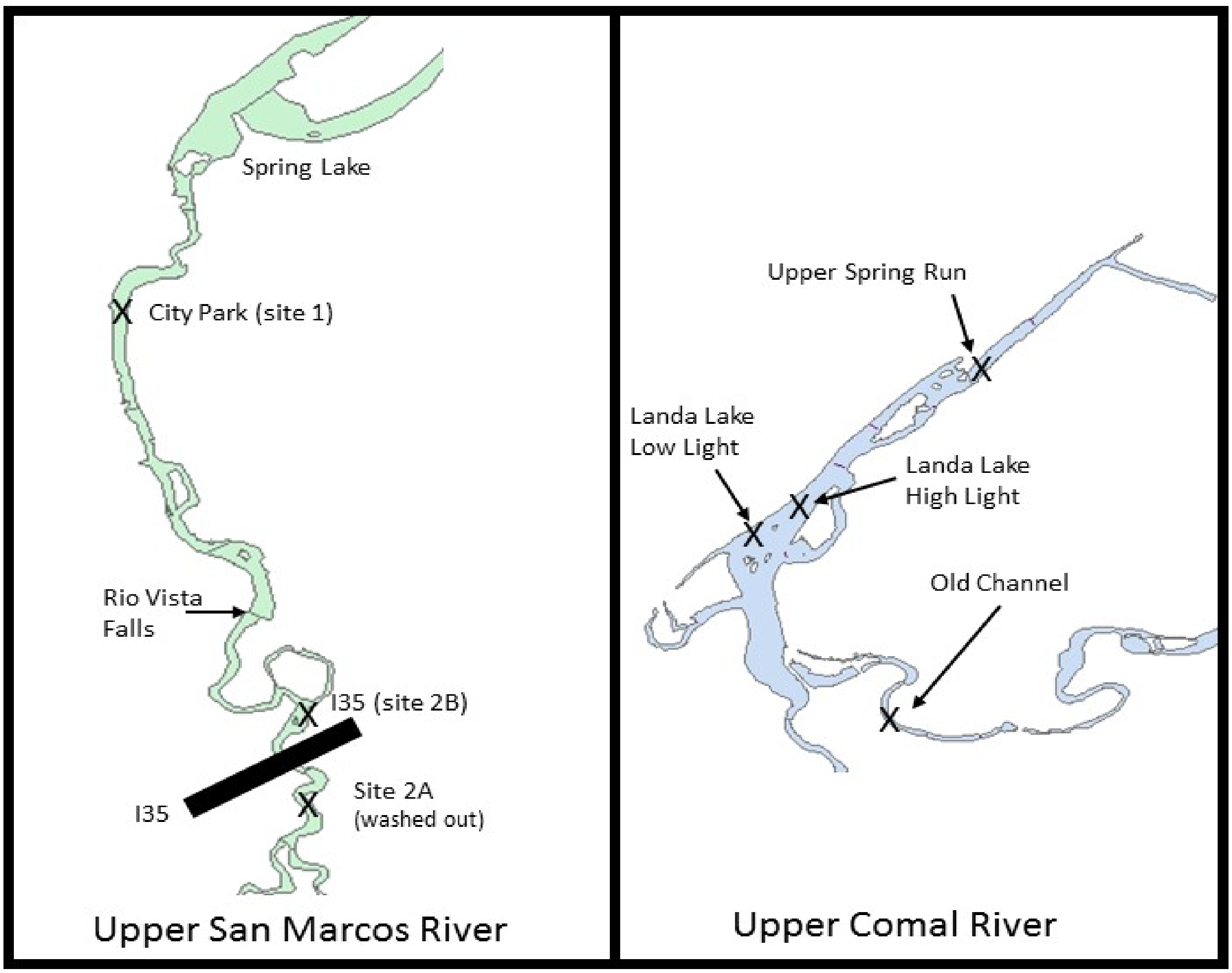

Figure 3. **Maps of the upper San Marcos and upper Comal Rivers showing locations of MUPPT deployment for competition experiments.**

After plantings were made, monitoring of growth and environmental characteristics (total depth, velocity at 80% and 20% of depth, temperature, DO and pH) occurred once per week. Photosynthetically active radiation or PAR was measured intermittently at each location over the course of several days using the Odyssey™ deployable waterproof sensor. Each experimental location, maximum stem length per species was recorded on two randomly selected individuals per treatment. Velocity and water depth were measured weekly with a Marsh-Mcbirney flo-mate while pH, temperature and dissolved oxygen (DO) were recorded at each location with a YSI™ multiparameter sonde.

Plants were harvested after 10 weeks of growth. Morphometric characteristics (stem counts and lengths) were recorded, then samples were separated into above-and-below ground tissues and dried at 60 °C for >72 hours then weighed to the nearest 0.1 mg at Baylor University.

3.0 Results

3.1 Initial measurements and Environmental conditions

Sprigs and established plants of both species were harvested to provide initial biomass estimates for each experiment. These average initial dry-weight biomass values (g/pot) ± SE, (n) were:

Comal River, Ludwigia pair of sprigs (g/pot), 0.47 ± 0.05 (16)

Comal River, Hygrophila pair of sprigs (g/pot), 0.27 ± 0.07 (16)

Comal River, established Ludwigia (g/pot), 4.15 ± 0.61 (6)

Comal River, established Hygrophila (g/pot), 2.17 ± 0.29 (6)

San Marcos (1) CP, Ludwigia pair of sprigs (g/pot), 0.48 ± 0.03 (25)

San Marcos (1) CP, Hydrilla pair of sprigs (g/pot), 0.23 ± 0.01 (30)

San Marcos (1) CP, established Ludwigia (g/pot), 4.79 ± 0.49 (6)

San Marcos (1) CP, established Hydrilla (g/pot), 2.65 ± 0.59 (6)

San Marcos (2B) I35, Ludwigia pair of sprigs (g/pot), 0.38 ± 0.03 (13)

San Marcos (2B) I35, Hydrilla pair of sprigs (g/pot), 0.54 ± 0.02 (16)

San Marcos (2B) I35, established Ludwigia (g/pot), 6.28 ± 0.30 (6)

San Marcos (2B) I35, established Hydrilla (g/pot), 2.63 ± 1.24 (6)

Environmental factors at each experimental location are summarized in Table 4. The recorded PAR maximums for each location were LLHL: 876 E/m² ; LLLL: 620 E/m² ; OC: 699 E/m² day. ; CP: 620 E/m² day. Average daily PAR at the LLHL location were 26% higher than average daily PAR measurements at the LLLL location. Data from USR and I35 were not recoverable.

Table 4. Summary of environmental parameters (± SE) for locations selected for the competition experiments. Depth and Velocity were measured in U.S. and converted to metric.

Location	Depth (cm)	Temp (°C)	DO (mgL^{-1})	pH	Vel. at 80% ($msec^{-1}$)	Vel. at 20% ($msec^{-1}$)
Comal River						
USR	98 ± 1	24.3 ± .2	4.67 ± .18	7.62 ± .04	0.08 ± .01	0.2 ± .01
LLHL	95 ± 2	24.1 ± .1	4.71 ± .18	7.46 ± .10	0.09 ± .02	0.23 ± .02
LLLL	120 ± 1	23.9 ± .1	4.61 ± .14	7.63 ± .07	0.09 ± .02	0.27 ± .02
OC	92 ± 1	23.9 ± .1	4.88 ± .08	7.62 ± .03	0.05 ± .03	0.56 ± .02
San Marcos River						
I35 (2B)	79 ± 1	22.2 ± .2	5.02 ± .20	7.55 ± .05	0.32 ± .07	1.03 ± .06
CP (1)	95 ± 4	22.1 ± .1	5.99 ± .29	7.42 ± .07	0.32 ± .12	0.6 ± .05

3.2 Plant growth over study period.

Figures 4 and 5 show the average growth of plant sprigs and established plants in the Comal (Ludwigia and Hygrophila) and the San Marcos (Ludwigia and Hydrilla). These data show that growth of Ludwigia was relatively robust at all locations. Growth of Hygrophila and Hydrilla was much more variable, and in general much less robust than the growth of Ludwigia.

Ludwigia sprigs (red bars, Figures 4 and 5) showed good establishment and growth in all experiments, although maximum stem length remained relatively modest as the plants appear to have mostly grown laterally. Established plants of Ludwigia showed very consistent data through time. Because the plants were in relatively high light environments, the plants tended to “bush out” rather than grow in length, a common adaptation for high-light growth environments. This effect is evident from the observation of the plants at San Marcos City Park (Site 1) at the end of the growth period (Figure 6). The MUPPT is very full of robust Ludwigia plants, although it is evident that the plants are “bushy” rather than elongated. Hygrophila sprigs in the Comal showed growth similar to that of Ludwigia sprigs at USR and OC, but lower growth in the two locations within Landa Lake. Hygrophila sprigs required repeated sprigging within the first week as many initial sprigs did not remain in their pots. In the San Marcos, Hydrilla sprigs tended to decline towards the end of the experimental growth periods. Established Hydrilla grew well at City Park (Site 1), but declined through time at the I35 (Site 2B) location.

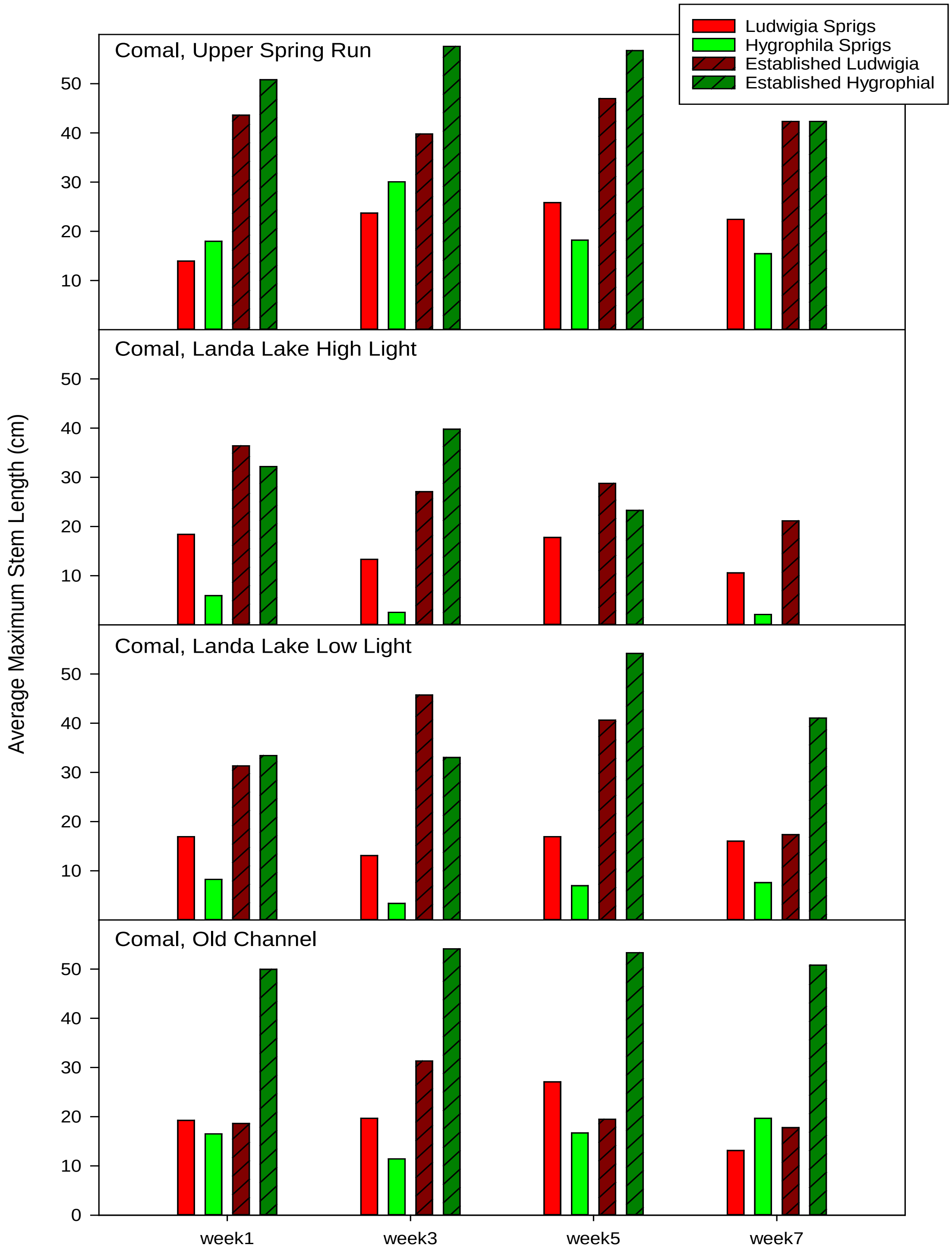

Figure 4. **Average maximum stem length of plants at each of the four experimental locations on the Comal River. Data is shown for sprigs of Ludwigia (red) and Hygrophila (green) as well as established Ludwigia (dark red, hatched) and Hygrophila (dark green, hatched).**

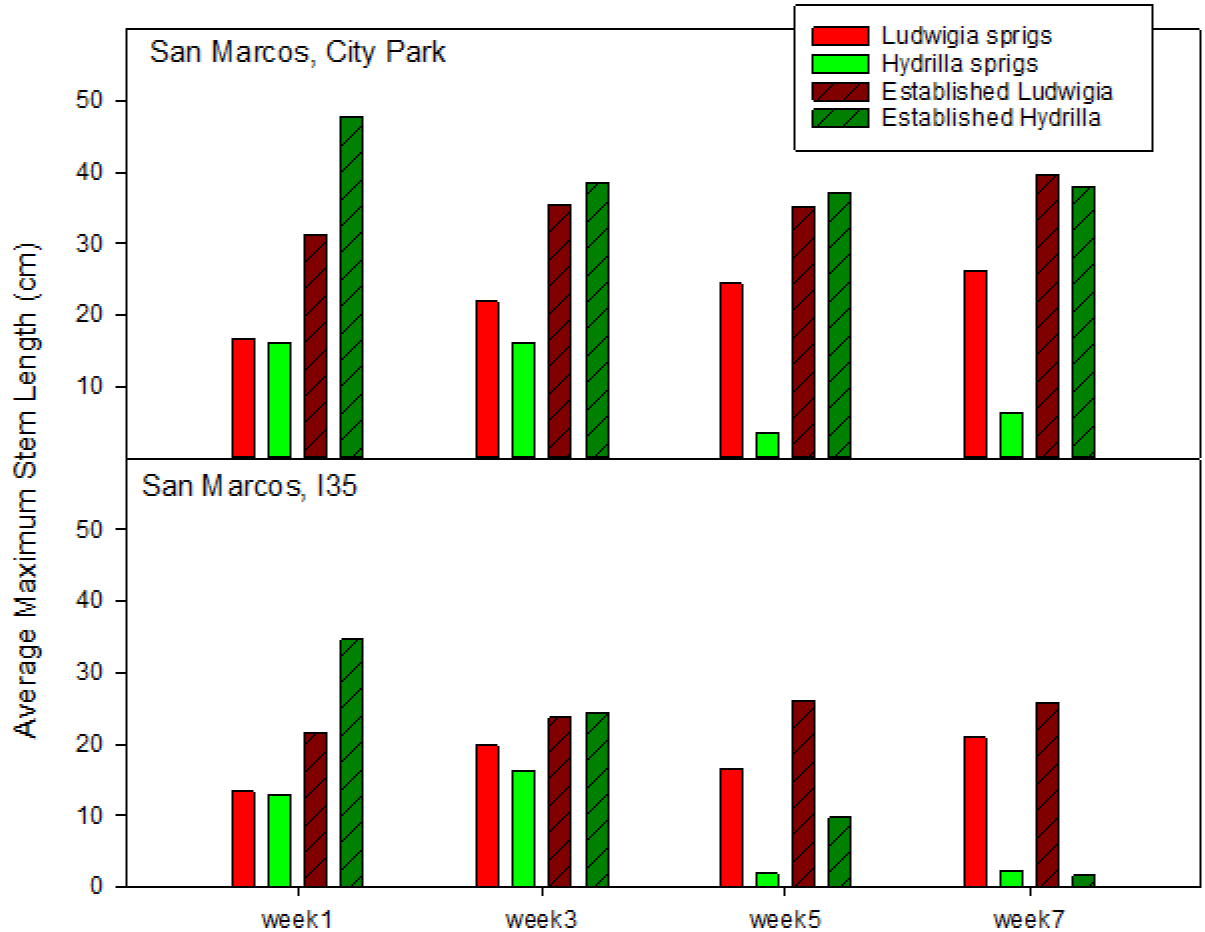

Figure 5. **Average maximum stem length of plants at each of the two experimental locations on the San Marcos River. Data is shown for sprigs of Ludwigia (red) and Hydrilla (green) as well as established Ludwigia (dark red, hatched) and Hydrilla (dark green, hatched).**

Figure 6. MUPPT at final harvest at San Marcos City Park (Site 1) location. Ludwigia plants (red) showed very robust growth. Hydrilla plants (green) showed variable success, although some plants were clearly very healthy.

3.3 Ludwigia X Hygrophila Sprig Competition Experiments.

Table 5 reports the outcome of the two-way ANOVA investigating the impact of competition (C) and location (L) on the growth of establishing sprigs of Ludwigia and Hygrophila. Notably, the lack of significant interaction between the two factors (C X L) allows evaluation of the C and L main effects. This lack of a significant interaction effect confirms that the pattern of competition impacts on the plant growth was consistent across all four planting locations and vice versa, the impacts of location were consistent regardless of level of competition.

Competition was not significant ($p>0.05$) for all growth parameters measured for both species. Even though the competition factor was not significant at the 0.05 level, Ludwigia total mass and total number of shoots showed a tendency toward lower values when the sprigs were planted into established Hygrophila ($P=0.07$, Table 5, Figure 7, white bars). However, there was no indication of lowered growth when the Ludwigia sprigs were planted with Hygrophila sprigs. The average maximum length at harvest and allocation of tissues to above ground versus below ground tissues of Ludwigia sprigs were not impacted by competition (Table 5, $P=0.30$, 0.62, respectively).

When planted in monoculture (two sprigs in empty pots), the biomass of Ludwigia at the end of the growth period exceeded that of Hygrophila by about 3.5x (Table 5, Figure 7). This result differs from that of Doyle et al. (2003) where the plants in monoculture had virtually identical growth.

Table 5 shows strong location effects on the growth of both species, indicating that the planting location had strong impacts on growth at all levels of competition. The location effect is significant for Ludwigia total mass and number of shoots. The biomass and number of shoots of Ludwigia was consistently 2-3x higher at the Landa Lake high light location (LLHL) than at the Landa Lake Low Light (LLLL) and the Old Channel (OC) locations. The impacts of location were much more severe for Hygrophila, where the plants were virtually eliminated at LLHL (possibly by herbivory) but was much higher at the OC location. Only in the OC was the growth of Hygrophila higher than the growth of Ludwigia.

Table 5. Final mean and standard error (SE) for growth parameters of Ludwigia or Hygrophila sprigs grown under varying levels of competition (none, sprigs, established) at four locations in the Comal River. Also shown is the significance level of the two-way ANOVA testing effect of competition levels and location. Differences among competition levels or among locations determined by HSD-Tukey post hoc comparisons if interaction term was not significant and indicated by different letter superscripts.

	Competition Treatments (C)			Locations (L)*				Two-way ANOVA		
	None	Sprigs	Est.	LLHL	LLLL	USR	OC	C X L	C	L
Ludwigia										
Total Mass (g)	1.89[a] (0.36)	1.90[a] (0.49)	0.86[a] (0.20)	2.47[b] (0.60)	0.96[a] (0.28)	1.75[ab] (0.45)	1.01[a] (0.25)	0.39	0.07	0.04
# shoots	2.59[a] (0.50)	2.25[a] (0.46)	1.28[a] (0.30)	3.21[b] (0.64)	1.04[a] (0.23)	2.25[ab] (0.56)	1.67[a] (0.41)	0.22	0.07	0.01
Max Lgth (cm)	20.4[a] (2.6)	20.7[a] (3.1)	15.1[a] (2.9)	13.0[a] (1.6)	16.6[a] (3.3)	23.4[a] (3.6)	21.8[a] (3.9)	0.94	0.30	0.11
AG:BG	4.09[a] (0.61)	4.31[a] (0.51)	3.28[a] (0.97)	3.23[a] (0.43)	4.60[a] (1.01)	3.74[a] (0.82)	4.25[a] (0.82)	0.47	0.62	0.73
Hygrophila										
Total Mass (g)	0.54[a] (0.23)	0.89[a] (0.28)	0.38[a] (0.12)	0.02[a] (0.01)	0.09[ab] (0.05)	0.95[bc] (0.21)	1.35[c] (0.42)	0.33	0.19	0.00
# shoots	0.94[a] (0.36)	1.18[a] (0.26)	0.72[a] (0.18)	0.13[a] (0.07)	0.21[a] (0.08)	1.63[b] (0.35)	1.83[b] (0.42)	0.48	0.40	0.00
Max Lgth (cm)	8.8[a] (3.17)	16.3[a] (4.0)	7.6[a] (2.6)	0.3[a] (0.2)	5.0[a] (2.8)	19.1[b] (4.4)	18.6[b] (4.6)	0.19	0.08	0.00
AG:BG	4.66[a] (1.63)	5.86[a] (1.20)	2.02[a] (0.58)	0.50[a] (1.41)	2.88[a] (1.38)	3.81[a] (0.81)	5.49[a] (1.35)	0.34	0.08	0.00

*Locations: Landa Lake High Light (LLHL), Landa Lake Low Light (LLLL), Upper Spring Run (USR), Old Channel (OC)

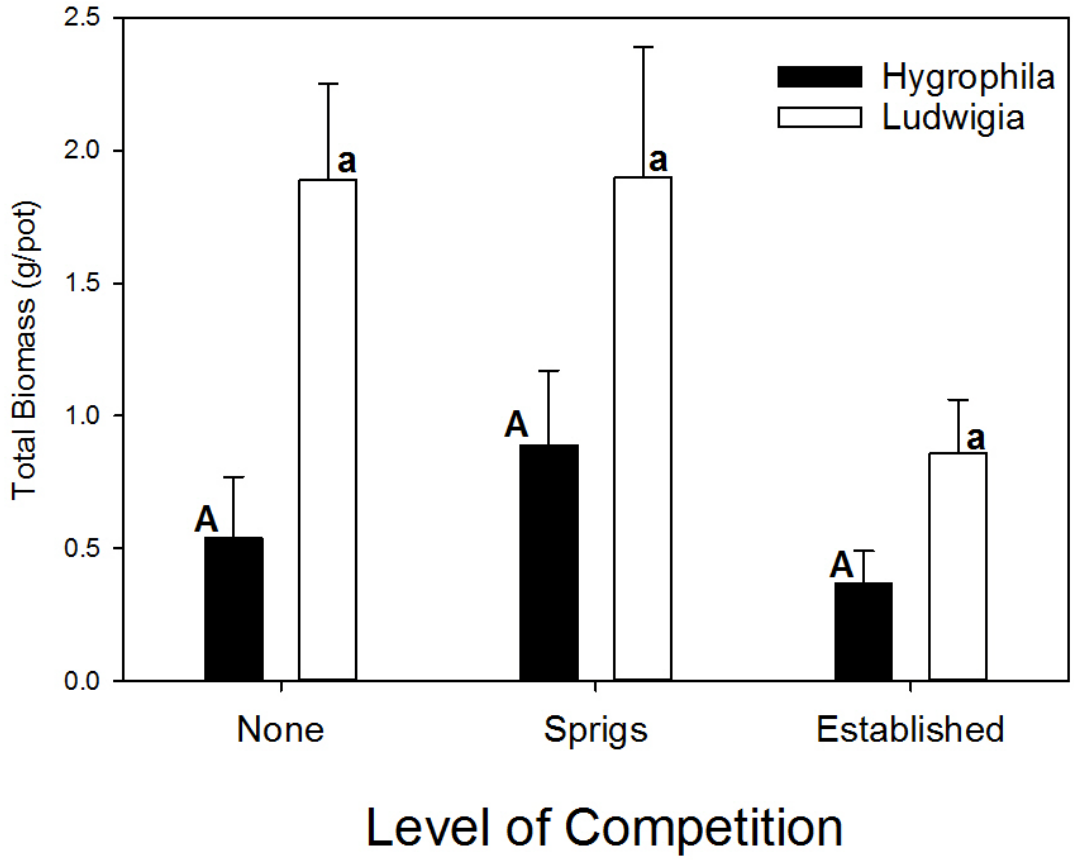

Figure 7. **Final total biomass of plants of Hygrophila (black bars) or Ludwigia (white bars) grown from two planted sprigs under three levels of competition (no competitor, two sprigs of competitor, established competitor). Shown are mean +/- SE. Two Way ANOVA (Location X Treatment) analysis showed no significant interaction between terms. The treatment factor was not significant for either species (P=0.19 Hygrophila, P=0.07 Ludwigia).**

3.4 Ludwigia X Hygrophila Continued Growth of Established Plants With and Without Invasion.

Table 6 shows the outcome of the two-way ANOVA investigating the impact of competition (C) and location (L) on the continued growth of established plants with and without invasion by sprigs of the other species. For both species, the lack of a significant interaction effect (C X L) allows the evaluation of the main effects (C and L) on the growth of the plants. Again, this fact confirms that the pattern of competition impact on the plant growth was consistent across all four planting locations and vice versa, the impact of location was consistent regardless of level of competition.

The continued growth of established Ludwigia plants was not impacted by invasion with Hygrophila sprigs. The averages of plants grown without competitive pressure and those invaded by sprigs of Hygrophila were virtually identical (Table 6, Figure 8). This result differs strongly from that of Doyle et al. (2003), where invasion of sprigs suppressed the continued growth of Ludwigia by 35%.

Surprisingly, the growth of Hygrophila was somewhat impacted by invasion by Ludwigia sprigs (Table 6). Hygrophila shoot number was significantly reduced ($P=0.03$) while total biomass showed a tendency to be reduced by about 30% ($P=0.10$, Figure 8) and plants tended to have lower proportional growth of above ground tissues ($P=0.06$). This comparison was not made by Doyle et al. 2003.

The continued growth of established Ludwigia and Hygrophila plants was also strongly impacted by planting location (Table 6, $P<0.00$ for all parameters measured). For example, the total biomass of Ludwigia at USR was 6.5X higher than that in the OC. The location impact was even larger for Hygrophila where total biomass at the OC site exceeded that at LLHL by more than 15X.

Table 6. **Final mean and standard error (SE) for growth parameters of established Ludwigia or Hygrophila grown without competitive pressure (none) or after invaded by two sprigs of the other species (invaded) at four locations in the Comal River. Also shown is the significance level of the two-way ANOVA testing effect of competition levels and location. Differences between competition levels or among locations determined by HSD-Tukey post hoc comparisons if interaction term was not significant and indicated by different letter superscripts.**

	Competition Treatments (C)		Locations (L)*				Two-way ANOVA		
	None	Invaded	LLHL	LLLL	USR	OC	C X L	C	L
Ludwigia									
Total Mass (g)	5.60^{a} (1.21)	5.49^{a} (0.97)	6.24^{b} (1.17)	2.42^{a} (0.75)	11.67^{c} (1.40)	1.78^{a} (0.40)	0.61	0.91	0.00
# shoots	7.94^{a} (1.42)	4.94^{a} (0.88)	5.08^{a} (0.80)	1.92^{a} (0.50)	11.75^{b} (1.63)	2.33^{a} (0.68)	0.88	0.37	0.00
Max Lgth (cm)	26.3^{a} (4.9)	24.7^{a} (2.8)	17.2^{a} (1.7)	20.7^{a} (5.1)	45.1^{b} (2.0)	18.0^{a} (4.9)	0.40	0.67	0.00
AG:BG	1.81^{a} (0.39)	1.84^{a} (0.38)	1.09^{a} (0.12)	1.19^{a} (0.44)	4.06^{b} (0.58)	0.72^{a} (0.18)	0.16	0.99	0.00
Hygrophila									
Total Mass (g)	7.27^{a} (1.87)	5.08^{a} (0.85)	0.74^{a} (0.24)	3.88^{ab} (0.95)	7.31^{bc} (0.93)	11.32^{c} (2.17)	0.29	0.10	0.00
# shoots	6.00^{b} (1.38)	4.04^{a} (0.60)	0.92^{a} (0.29)	3.08^{ab} (0.82)	5.67^{b} (0.86)	9.17^{c} (1.23)	0.06	0.03	0.00
Max Lgth (cm)	38.1^{a} (6.4)	31.9^{a} (4.2)	4.0^{a} (1.1)	34.5^{b} (7.0)	41.9^{bc} (4.4)	55.4^{c} (3.8)	0.63	0.21	0.00
AG:BG	4.32^{a} (1.18)	2.54^{a} (0.52)	0.46^{a} (1.07)	2.87^{a} (0.94)	3.95^{ab} (0.86)	5.71^{b} (0.72)	0.14	0.06	0.00

*Locations: Landa Lake High Light (LLHL), Landa Lake Low Light (LLLL), Upper Spring Run (USR), Old Channel (OC)

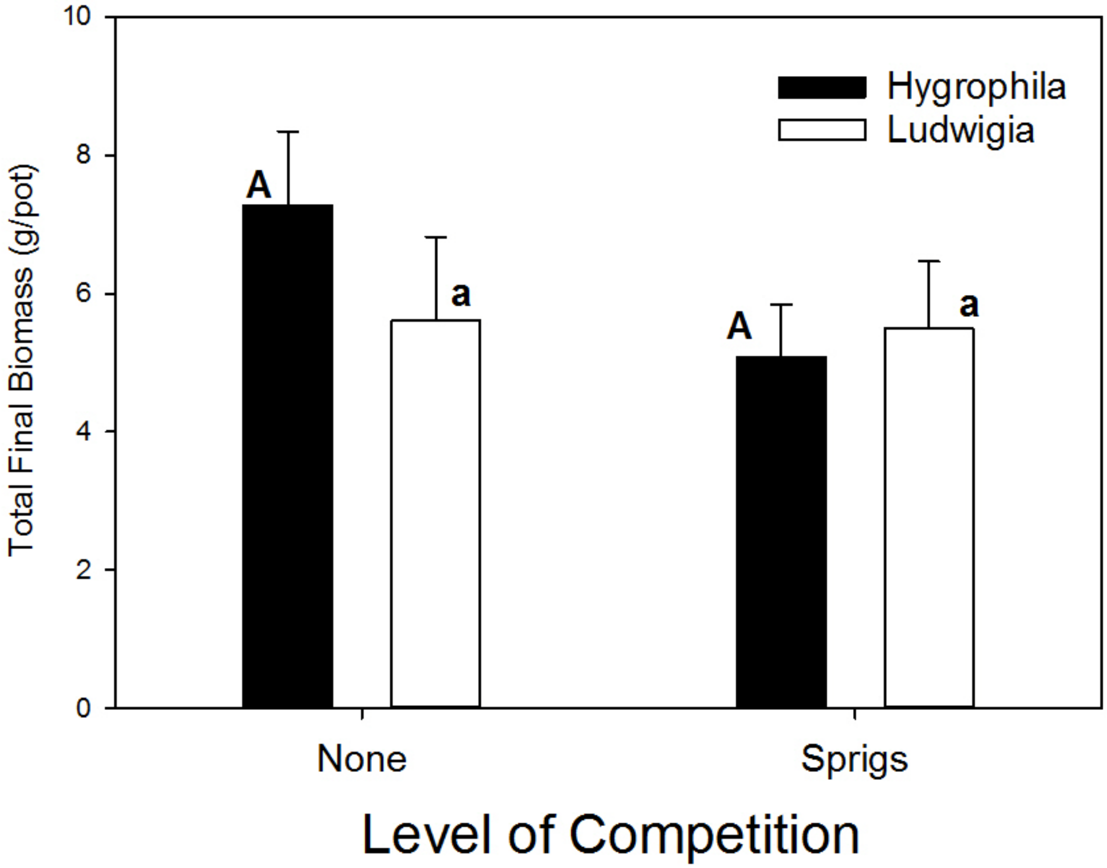

Figure 8. **Final total biomass of established plants of Hygrophila (black bars) or Ludwigia (white bars) grown with no competitive pressure (none) or invaded by two sprigs of the other species (sprigs). Shown are mean +/- SE. Two Way ANOVA (Location X Treatment) analysis showed no significant interaction between terms. The treatment factor (shown) was not significant for either species (P=0.10 Hygrophila, P=0.91 Ludwigia).**

3.5 Ludwigia X Hydrilla Sprig Experiments.

Table 7 reports the outcome of the two-way ANOVA investigating the impact of competition (C) and location (L) on the growth of establishing sprigs of Ludwigia and Hydrilla in the San Marcos River. Notably, the lack of significant interaction between the two factors (C X L) allows evaluation of the C and L main effects. This lack of a significant interaction effect confirms that the pattern of competition impacts on the plant growth was consistent across each planting location and vice versa, the impacts of location were consistent regardless of level of competition. This finding is particularly significant in light of the fact that the experiments at the two locations on the San Marcos did not occur simultaneously. As described earlier, the initial downstream location planted on April 23 was completely scoured by flooding prior to harvest. This downstream site was re-planted at I35 (Site 2B) in early July. Hence, the "location" factor for the San Marcos also contains a "season" factor imbedded in it.

The very poor survival and growth of Hydrilla sprigs when grown without competition was a very surprising outcome (Table 7, Figure 9). In fact, by the end of the experiment, most pots planted with Hydrilla sprigs failed to survive at all. Importantly, this identical same result was found for Hydrilla sprigs at both locations, which include the upstream planting made in April and the downstream planting in July. Ludwigia survival and growth when planted into empty pots was vigorous, and much higher than that of Hydrilla (Figure 9).

Ludwigia biomass accumulation over the experimental growth period was negatively impacted by Hydrilla competition, despite the poor growth of the Hydrilla sprigs. Ludwigia sprigs competing with Hydrilla sprigs or with established plants of Hydrilla showed significant declines of 25% and 64% respectively compared to Ludwigia sprigs grown alone (Figure 9). Additionally, all Ludwigia growth parameters measured showed a significant negative response to Hydrilla competition. In addition to biomass, shoot number, maximum length, and proportional investment in above ground tissues were all significantly lower for sprigs planted into pots with established Hydrilla (Table 7).

The level of Ludwigia competition was not a significant factor in Hydrilla growth. Hydrilla sprig growth was statistically similar at all levels of Ludwigia competition. However, the overall very poor growth of Hydrilla sprigs likely masks any possible competitive impact Ludwigia may have had.

The location factor was significant for Ludwigia total mass and number of shoots, with higher values for plants grown at the I35 location. In contrast, location was not a significant factor for Hydrilla biomass or stem number, likely due to the overall poor growth of Hydrilla sprigs at both locations.

Table 7. **Final mean and standard error (SE) for growth parameters of Ludwigia or Hydrilla sprigs grown under varying levels of competition (none, sprigs, established) at two locations in the San Marcos River. Also shown is the significance level of the two-way ANOVA testing effect of competition levels and location. Differences among competition levels or among locations determined by HSD-Tukey post hoc comparisons if interaction term was not significant and indicated by different letter superscripts.**

	Competition Treatments (C)			Locations (L)*		Two-way ANOVA		
	None	Sprig	Est.	CP	I35	C X L	C	L
Ludwigia								
Total Mass (g)	6.57[c] (0.61)	4.96[b] (0.61)	2.39[a] (0.57)	3.78[a] (0.41)	5.87[b] (0.63)	0.32	*0.00*	*0.00*
# shoots	5.44[b] (0.80)	4.19[ab] (0.39)	3.19[a] (0.52)	3.04[a] (0.29)	5.50[b] (0.56)	0.20	*0.01*	*0.00*
Max Lgth (cm)	34.5[b] (1.4)	29.6[ab] (1.5)	26.3[a] (2.2)	30.8[a] (1.2)	29.46[a] (1.8)	0.56	*0.02*	0.50
AG:BG	7.01[b] (0.74)	6.14[ab] (0.83)	4.69[a] (0.58)	7.54[b] (0.66)	4.36[a] (0.32)	0.24	*0.03*	*0.00*
Hydrilla								
Total Mass (g)	0.06[a] (0.02)	0.22[a] (0.14)	0.13[a] (0.03)	0.17[a] (0.10)	0.11[a] (0.02)	0.26	0.42	0.55
# shoots	0.38[a] (0.18)	0.94[a] (0.27)	1.06[a] (0.25)	0.88[a] (0.21)	0.71[a] (0.19)	0.12	0.09	0.53
Max Lgth (cm)	2.5[a] (1.3)	6.2[a] (3.7)	5.1[a] (1.8)	7.2[a] (2.7)	1.6[a] (0.7)	0.37	0.54	0.07
AG:BG	1.00[a] (0.58)	2.15[a] (1.35)	2.07[a] (1.60)	4.07[b] (1.74)	0.23[a] (0.08)	0.52	0.45	*0.02*

*Locations: City Park (CP), Interstate I35 crossing (I35)

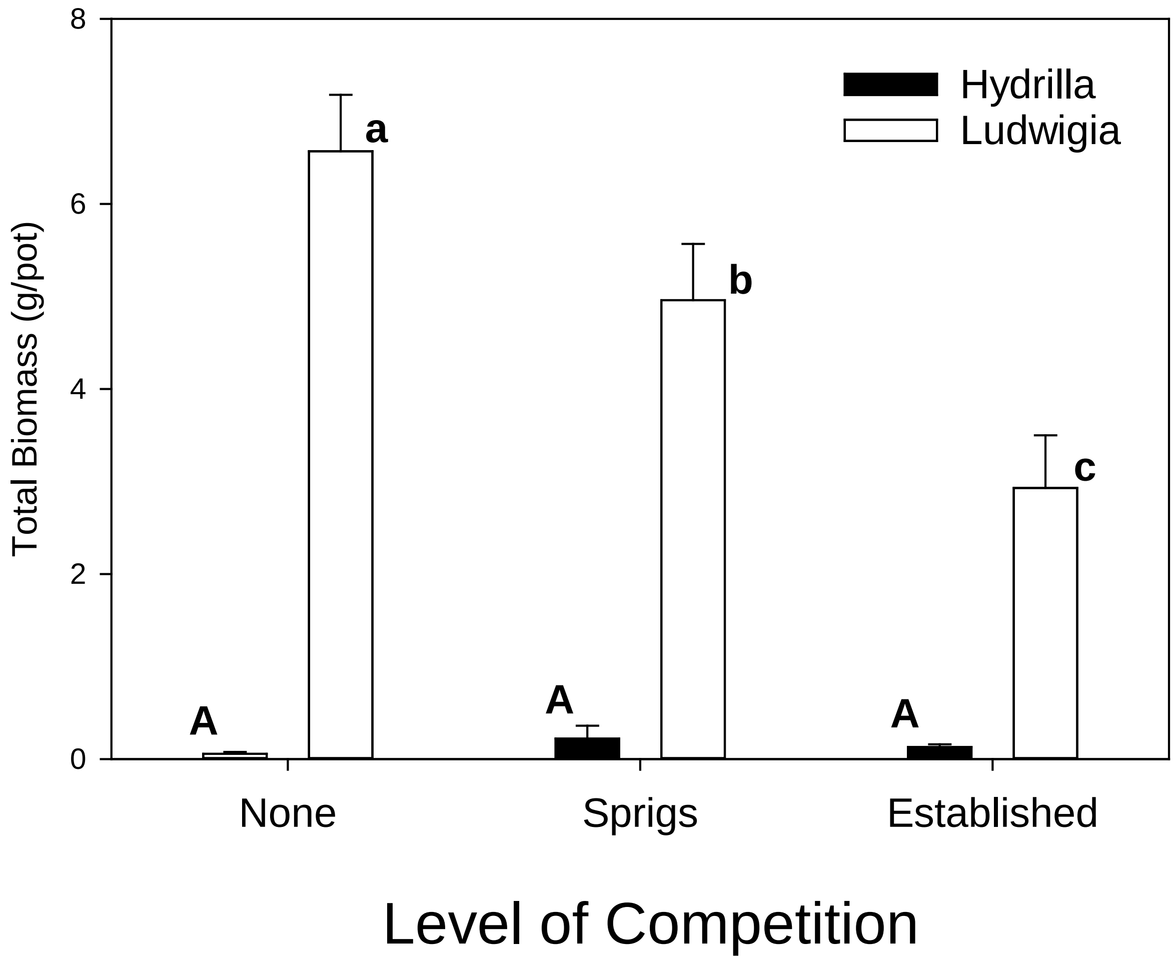

Figure 9. **Final total biomass of plants of Hydrilla (black bars) or Ludwigia (white bars) grown from two planted sprigs under three levels of competition (no competitor, two sprigs of competitor, established competitor). Shown are mean +/- SE. Two Way ANOVA (Location X Treatment) analysis showed no significant interaction between terms. The competition factor was significant for Ludwigia (P=0.00) with declining total biomass as level of competition increased. The competition factor was not significant for Hydrilla (P=0.42) although these results appear to be highly impacted by heavy herbivory and biomass loss.**

3.6 Ludwigia X Hydrilla Continued Growth of Established Plants With and Without Invasion. Table 8 shows the outcome of the two-way ANOVA investigating the impact of competition (C) and location (L) on the continued growth of established Ludwigia and Hydrilla plants with and without invasion by sprigs of the other species. For both species, the lack of a significant interaction effect (C X L) for most parameters allows the evaluation of the main effects (C and L) on the growth of the plants. Again, this fact confirms that the pattern of competition impact on the plant growth was consistent across both planting locations and vice versa, the impact of location was consistent regardless of level of competition.

The continued growth of established Ludwigia plants was impacted by invasion with Hydrilla sprigs (Figure 10). The biomass of established Ludwigia plants invaded by Hydrilla was significantly reduced by 17% relative to plants continuing to grow without invasion. This invasion impact is particularly notable given the overall poor growth of the Hydrilla sprigs. Possibly, under conditions with higher Hydrilla growth, the impact on the Ludwigia may be higher.

The continued growth of established Hydrilla plants was not impacted by Ludwigia competition (P=0.32). There was no statistically significant difference in any of the growth parameters measured for Hydrilla plants invaded by Ludwigia relative to uninvaded plants.

The continued growth of established Ludwigia and Hydrilla plants was significantly impacted by planting location. The total biomass and number of shoots of established Ludwigia plants at the end of the experimental growth period were significantly higher at I35 relative to that at City Park, while the opposite was true for Hydrilla (Table 8).

However, the overall growth of the two species was strikingly different. Overall, established Ludwigia plants growing without competitive pressure was more than 15X higher than that of established Hydrilla growing alone (Figure 10).

Table 8. **Final mean and standard error (SE) for growth parameters of established Ludwigia or Hydrilla grown without competition (none) or after invaded by two sprigs of the other species (invaded) at two locations in the San Marcos River. Also shown is the significance level of the two-way ANOVA testing effect of competition levels and location. Differences between competition levels or among locations determined by HSD-Tukey post hoc comparisons if interaction term was not significant and indicated by different letter superscripts.**

	Competition Treatments (C)		Locations (L)*		Two-way ANOVA		
	None	Invaded	CP	I35	C X L	C	L
Ludwigia							
Total Mass (g)	23.98[b] (1.29)	19.79[a] (1.48)	18.16[a] (1.43)	24.20[b] (1.32)	0.52	*0.04*	*0.01*
# shoots	11.88[a] (1.32)	11.69[a] (0.69)	9.75[a] (0.62)	13.75[a] (0.71)	0.22	0.86	*0.00*
Max Lgth (cm)	40.4 (2.7)	40.9 (1.3)	43.8 (1.2)	37.7 (1.8)	*0.01*	0.82	0.00
AG:BG	4.20[a] (0.33)	4.33[a] (0.27)	4.38[a] (0.31)	4.19[a] (0.29)	0.38	0.78	0.49
Hydrilla							
Total Mass (g)	1.59[a] (0.43)	2.71[a] (0.87)	3.86[b] (1.03)	0.81[a] (0.14)	0.26	0.32	*0.03*
# shoots	5.50[a] (1.02)	4.63[a] (0.68)	5.08[a] (0.75)	4.75[a] (0.86)	0.43	0.49	1.00
Max Lgth (cm)	25.7[a] (9.14)	24.7[a] (6.9)	46.0[b] (6.4)	4.2[a] (0.8)	0.99	0.89	*0.00*
AG:BG	1.31[a] (0.49)	2.00[a] (0.65)	3.29[b] (0.68)	0.26[a] (006)	0.34	0.35	*0.00*

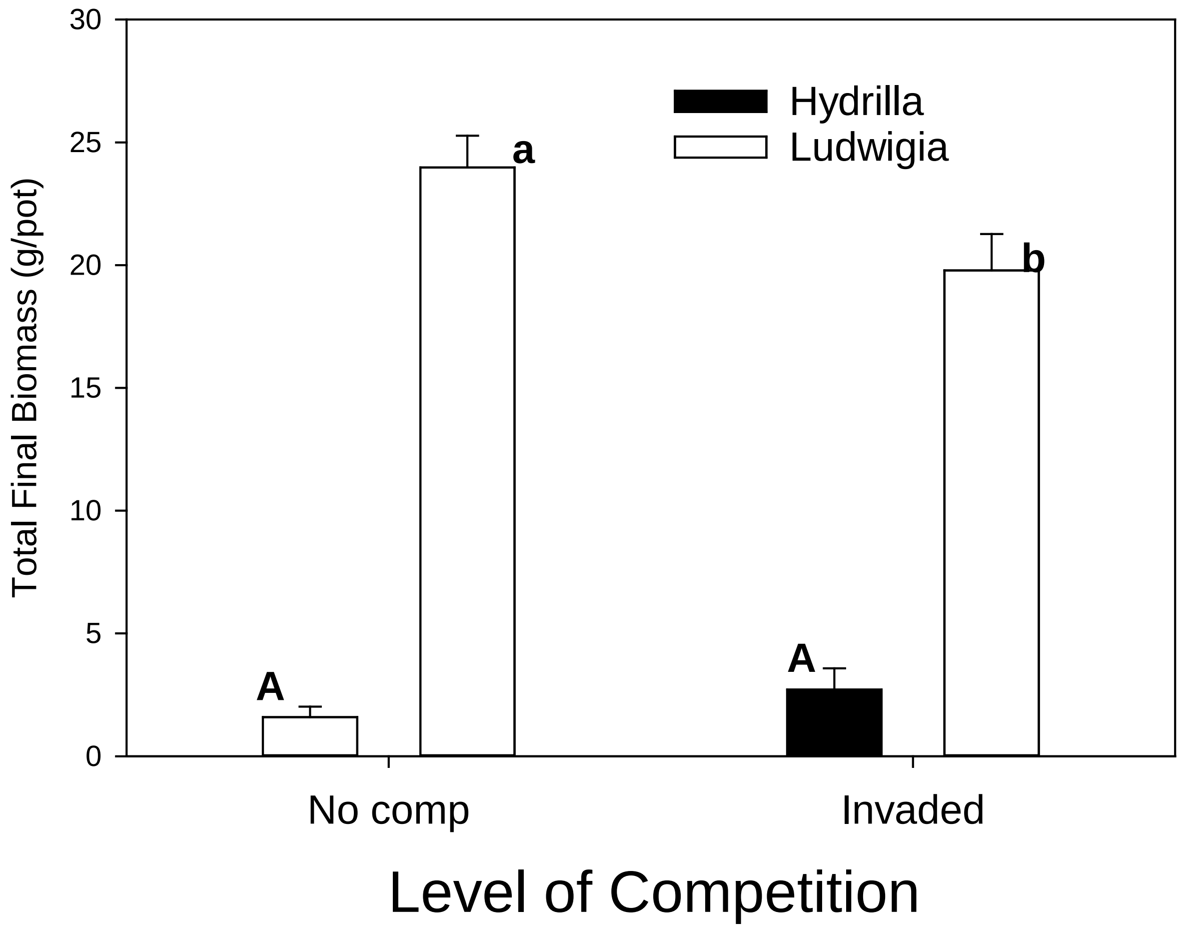

Figure 10. **Final total biomass of established plants of Hydrilla (black bars) or Ludwigia (white bars) grown with no competitive pressure (none) or invaded by two sprigs of the other species (sprigs). Shown are mean +/- SE. Two Way ANOVA (Location X Treatment) analysis showed no significant interaction between terms. The competition factor (shown) was significant for Ludwigia (P=0.04) with lower biomass levels in pots invaded by Hydrilla sprigs. The competition factor was not significant for Hydrilla (P=.32) although these results appear to be highly impacted by heavy herbivory and biomass loss.**

4.0 Discussion

Ludwigia is a native plant that appears to face competitive pressure from Hygrophila and Hydrilla, two widely distributed non-native species in the Comal (Hygrophila) and San Marcos (Hygrophila and Hydrilla) Rivers. All of these species share a similar branching growth form and are capable of asexual reproduction via establishments of viable sprigs. However, Ludwigia provides better habitat for the endangered fountain darters, and is currently being widely used in native plant restoration efforts in both rivers.

4.1 Growth of all species without competition

The results of the short-term competition experiments are generally good news for the continued use of Ludwigia in habitat restoration/enhancement efforts. The growth of Ludwigia sprigs (ll treatment) under no-competition conditions exceeded that of Hygrophila and Hydrilla (hh treatments). In fact, the establishment and growth of sprigs of the native species was more than 3X higher than Hygrophila (Table 5) and more that 10x higher than Hydrilla (Table 7) in our 10-week growth experiments. Both Hygrophila and Hydrilla sprigs appear to have suffered high mortality and poor growth under the experimental conditions tested. Likewise, the total biomass of established Ludwigia plants growing without competition (LL) was similar to that of Hygrophila (HH) (Table 6, Figure 8) and much higher than that observed for Hydrilla (HH) (Table 8, Figure 10).

These in-situ experiments include effects other than competitive interactions between the plants. Notably, we believe that herbivory negatively affected all experimental plants and proved particularly detrimental to the establishment of Hygrophila and Hydrilla sprigs. During routine monitoring we observed that the Hygrophila and Hydrilla sprigs often appeared damaged, and in some cases were entirely missing from the planted pots. In the Comal study red swamp crayfish (*Procambarus clarkii*) were observed burrowing into soil within pots and final harvest and clipped stems of some plants, especially those growing in the Landa Lake High Light location, were evident. For the established Hygrophila and Hydrilla plants, a potential explanation for the loss or zero net gain in biomass could be due to the brittle or easily fragmenting nature of the stems – a potential trade-off which might be advantageous for dispersal and colonizing new habitats.

The strong growth of Ludwigia under "no competition" conditions confirm the experience of restoration efforts in the Comal River that Ludwigia establishment and short-term growth is excellent.

4.2 Impacts of Hygrophila Competition and Location on Ludwigia Growth.

The growth of Ludwigia sprigs was not impacted by Hygrophila competition under the conditions tested in the Comal River. These results differ sharply from those of Doyle et al. (2003) that found that Ludwigia sprig relative growth rate was strongly impacted by competition

from Hygrophila sprigs (-40%) and profoundly suppressed by the presence of established Hygrophila plants (-80%).

The continued growth of established Ludwigia was likewise not impacted by competition from invading sprigs of Hygrophila (Table 6). These results also differ from those of Doyle et al. (2003) that found that total biomass of Ludwigia invaded by Hygrophila sprigs to be only 65% of that of uninvaded plants.

Ludwigia growth showed a strong location effect in the Comal River (Tables 5 and 6). The final biomass of the Ludwigia plants that developed from the sprigs varied significantly (2.4X) among the four locations, with higher values at Landa Lake High Light and lower values in the Old Channel and Landa Lake Low Light. Likewise, the final biomass of the established Ludwigia plants at the end of the experimental growth period varied by a factor of 6.5X with the highest values observed at the USR site and the lowest values seen in the OC. It is not surprising that Ludwigia showed strong location impacts, as we deliberately selected locations with variability in the factors known to impact plant growth, especially flow and light conditions. The overall growth of Ludwigia sprigs at Landa high light was more than 2.5 X higher than Landa low light (Table 5, 2.47 g versus 0.96 g) while overall growth of established Ludwigia was also greater than 2.5X at the high light location than at the low light location (Table 6, 6.24 g versus 2.42 g). While light did dramatically impact biomass accumulation, it did not necessarily impact the outcome of competition between Ludwigia and Hygrophila species. The mechanisms regulating the location effect, however, were not clear from these experiments and may warrant additional study to tease out impacts of light or velocity on the competitive interactions between plant species.

4.3 Impacts of Hydrilla Competition and Location on Ludwigia Growth.

In the San Marcos River, Ludwigia sprig growth was impacted by both Hydrilla competition and location. Ludwigia sprigs planted with Hydrilla sprigs or into pots of established Hydrilla showed significant suppression of 25% and 64%, respectively relative to pots growing without any Hydrilla competitor (Table 7). Likewise, established pots of Ludwigia showed significant (17%) suppression of growth when invaded by Hydrilla sprigs. These impacts are particularly notable given the overall poor growth of Hydrilla. For reasons we have not identified, the overall growth of Hydrilla at both locations was much lower than Ludwigia and much lower than expected based on previous experience with Hydrilla. Hydrilla is a widely distributed and successful invasive species that has been shown to be a very strong competitor, especially in “equal start” competition experiments (Smart et al., 1994, Van et al., 1999). However, the results of a New Zealand study which paired Hydrilla with various aquatic species indicate that its growth varies depending on the species with which it is planted and, subsequently, has variable impacts on the resultant biomass of that species (Hofstra et al., 1999).

Ludwigia also showed a significant location effect. Both sprigs and established Ludwigia plants showed significantly higher growth at the I35 site than at the City Park site.

4.4 Summary Evaluation

Overall, these data indicate positive short-term establishment and growth characteristics for Ludwigia, and supports the continued use of the species for restoration efforts. Ludwigia used in restoration efforts is likely to effectively establish and quickly colonize unvegetated areas of the rivers. In fact, the growth of Ludwigia sprigs was higher over the 10-week growth periods than either Hygrophila or Hydrilla. Although both non-native species appear to have suffered from herbivory impacts, there is no reason to believe that the experimental conditions used do not reflect actual levels of herbivory impacts in these systems. Therefore, Ludwigia planted into currently unvegetated areas or areas where the non-native plants have been removed are likely to grow very well.

Furthermore, Ludwigia may be less susceptible to competition impacts than previously documented. Under our experimental growth conditions, Ludwigia sprigs or established Ludwigia plants were not impacted by Hygrophila competition. Ludwigia sprigs and established plants were negatively impacted by Hydrilla, but even there all treatment levels showed significant positive growth.

While a common outcome of invasive versus native plant competition is that the invasive plant wins (hence the term “invasive”) our data show that experiments conducted in situ may show a different outcome. While the biotic growth potential of a species is often linked to invasive species success, the outcome can depend on other factors too. Soil fertility, selective grazing pressures, propagule pre-emption and water velocity as well as other stressors are all factors which may promote the success of a native species and the depression of an introduced species or vice versa. Several studies have investigated the ability of *Vallisneria americana* to dominate over *Hydrilla verticillata* (Van et al., 1999, Smart et al., 1994) but soil fertility seems to determine the outcome. In our study Hydrilla continued to exert impacts upon Ludwigia despite a reduction in top growth biomass. *Hydrilla verticillata* is known to produce dense below ground biomass and propagules which may continue to compete with neighboring plant species despite its loss of stems and leaves. Also, although the Hydrilla plants were not present in some pots at the time of the final harvest, earlier growth in the season may have slowed the growth of the native plant.

The pre-emption of propagule establishment from mature native plant communities can play a preventative role in invasive plant success (Chadwell and Englehardt, 2008). In our study invasion of Hygrophila sprigs had virtually no impact upon established Ludwigia plants. As shown in studies with other invasive aquatic plants the establishment and dominance of the invasive may depend on the degree of intact native plant cover in the area of introduction. If a

well-developed native plant community exists at the site of introduction then the opportunity for invasion may substantially decrease (Bickel and Perrett, 2014).

Preferential grazing can heavily impact both native and introduced plants (Parker and Hay, 2005) and evidence suggests that this may be determined by the nutrient content, phenolic compounds or chemical or physical defenses of individual plant species (Lodge, 1990). We witnessed what was believed to be heavy herbivore grazing on Hygrophila and Ludwigia at both Landa Lake sites. While this factor probably does not fully explain our findings, we believe the effect of herbivory warrants further investigation (see below).

Finally, physical characteristics can greatly influence growth of aquatic macrophytes. As witnessed in our study, where location played a significant factor for all three species, exposure to gradients in velocity, depth and light can have significant impacts on plant growth and success. Stream velocities can provide positive conditions for plant growth yet aquatic plant biomass can be greatly reduced once a threshold is surpassed (French and Chambers, 1996) (Madson and Douglas, 2001). However certain species show phenotypic plasticity towards velocity and light gradients and can maintain vigorous growth compared to less adaptable species. A recent competition study conducted by Bilbo (2015) between *Hydrilla verticillata* and *Potamogeton illinoensis* also carried out in the San Marcos River bolsters our findings which indicate Hydrilla growth is not as vigorous when subjected to velocities above a certain threshold and several local studies have been conducted regarding occupancy of aquatic plant species along velocity gradients (Saunders et al., 2001) (Williams, 2013)

In conclusion, our study has shown that in-situ testing of competition between native and non-native aquatic vegetation species in the Comal and San Marcos systems provides differing results than when tested in a no-flow laboratory environment (Doyle et al., 2003). This updated information may be extremely valuable to the development of the EAHCP Ecological model and will be provided directly to that project team for consideration. The study also emphasizes that the successful establishment of aquatic plants is strongly location dependent and furthermore depends on a variety of factors and stressors and that the origin of the plant (native or non-native) does not automatically dictate the success of establishment or the competitive outcome.

5.0 Future Study Considerations

As is common with many studies the outcome of the data tends to ask more questions than provide answers. As such below are a few study questions instigated by the current study which may warrant further investigation.

1. What is the quantity and viability of aquatic plant propagules in the San Marcos and Comal Rivers?

The success of native and non-native aquatic plant establishment relies heavily on propagule production and distribution. In 2000 the distribution and dispersal of propagules of native and

nonnative species was investigated in the San Marcos River (Owens et al., 2001). One indication garnered from this study was that propagules of non-native species dominated across all study locations while propagules of native species were poorly represented and many not viable. Unfortunately, this study was not repeated in the Comal River. With on-going large scale removal of invasive plant species and re- introduction of native species a current understanding of propagule loading rates and viability would be important to help determine the future sustainability and outcome of the restoration projects in both systems.

2. What is the nutrient availability and how does nutrient partitioning influence growth of aquatic plants in the San Marcos and Comal Rivers?

As discussed previously several factors affect the recruitment, growth, persistence and expansion of aquatic plants in river systems. Nutrient stoichiometry—the ways in which aquatic plants use and partition nutrients—is an important process which either limits or drives the productivity of aquatic plants, but species respond to and use nutrients differently (Barko et al., 1991). Elevated levels of sediment nitrogen can limit the productivity of aquatic plant species or increase productivity in other species and uptake mechanisms of nutrients varies greatly by species (Fang et al., 2007). In essence, one factor which contributes to the growth and health of aquatic plants within these systems is sediment nutrients which have yet to be researched in-depth in either the Comal or San Marcos systems. A study to investigate the fertility of the sediment and how native and introduced plant species use or partition those nutrients would be an important step towards understanding and predicting the prolonged composition of the aquatic plant community in both systems.

3. What role does herbivory play in the establishment, growth and expansion of aquatic plants in the San Marcos and Comal Rivers?

Another observation often noted during active restoration and experimentation efforts is the impact of herbivory on plant establishment and continued growth. Defoliation pressures on the native and non-native species in this system are not well understood as they are imposed by a wide array of herbivorous vertebrate and invertebrate species. Many insect species are known to have specialized, co-evolved relationships with aquatic host plants, affecting not only floating or emergent leaf tissue but submerged anatomical features as well (Harms and Grodowitz, 2009). Recent documentation details the destructive impacts of a moth species' aquatic larvae on the native aquatic plant nurseries at the San Marcos Aquatic Resources Center (Hutchinson et al., 2015). Destruction of plant growth by aquatic caterpillars has been observed in the field as well. The invasive giant rams-horn snail (*Marisa cornuarietis*) - known to have a voracious appetite - and other herbivorous mollusks have been observed and documented feeding on the local vegetation (Grantham et al., 1995; Horne et al., 1992; Karatayev et al., 2009). Other common species with aquatic plant-dominated diets include crayfish, turtles, tilapia and water fowl. Observational and reported data suggest that the sustainability of restoration efforts could benefit from a deeper analysis of herbivore pressures.

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APPENDIX D

Effects of Low-Flow on Fountain Darter Reproductive Effort

EFFECTS OF LOW FLOW ON FOUNTAIN DARTER REPRODUCTIVE EFFORT

HABITAT CONSERVATION PLAN (HCP) 2014 APPLIED RESEARCH

TEXAS STATE UNIVERSITY
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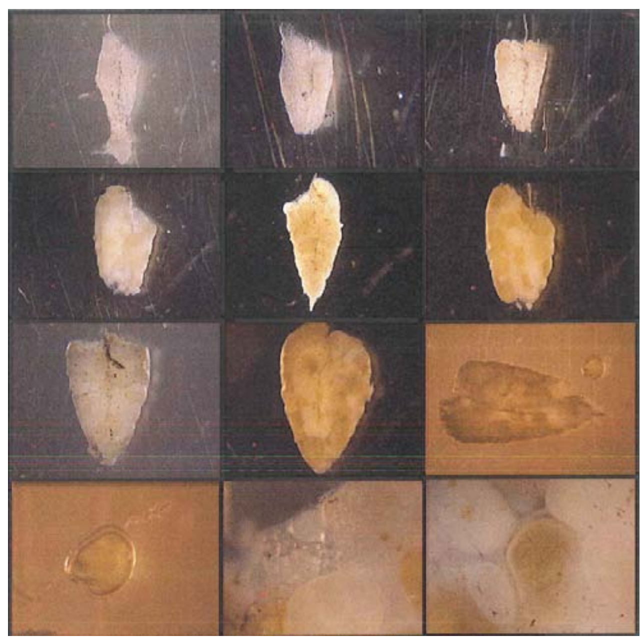
BIO-WEST



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FINAL REPORT

October 2014

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1.0 INTRODUCTION

The Edwards Aquifer Habitat Conservation Plan (HCP) is founded on long-term biological goals for the covered species that inhabit the Comal and San Marcos springs/river ecosystems (EARIP 2011). To support the long-term biological goals, flow management objectives (flow regimes) were established that are presumed to be protective of the threatened and endangered species in these systems. The low-flow conditions (discharge and extended durations) incorporated in the HCP flow regime and projected to occur during severe drought have occurred very infrequently (or not at all) during the historical record. Consequently, complete testing of ecological response(s) to these conditions in the wild is unlikely. Therefore, testing of simulated conditions in laboratory and/or field environments is mandatory to address HCP unknowns.

Section 6.3.4 of the HCP lays out the path forward for answering key questions and filling in data gaps to test assumptions and ultimately assist with management decisions. The focus in 2013 was on addressing several key questions surrounding physical habitat and food source responses both related to the federally-listed endangered Fountain Darter *Etheostoma fonticola*. In 2014, three additional applied research projects focused on the Fountain Darter were conducted. This report focuses on the effects of low flow on Fountain Darter fecundity.

Reproductive success of slackwater and benthic fishes is reduced under low flow conditions, attributed to greater variability in physical habitats and to increases in organic substrates (Schlosser 1982, Falke et al. 2010). As flows decrease, aquatic vegetation (e.g., physical habitat) proliferates but not homogeneously among plant taxa (Bunn and Arthington 2002, Riis and Hawes 2002). Physical habitat alteration, such as changes in the plant community, can reduce foraging efficiency and alter spatial distributions and habitat quality (Dibble et al. 1997, Dibble 2010). Modified vegetative structural complexity (i.e., low-growing vs. tall-growing, sparse vs. dense macrophytes) in conjunction with accumulation of organic sediments under a declining hydrograph, can limit spawning and nursery habitats of stream fishes, especially those that attach eggs to plants or substrates (Dibble et al. 1997). As such, studies documenting the effects of the alteration of flow regime within associated habitats of the Fountain Darter are of extreme importance to the HCP.

Reduction in base flow restricts the amount of available habitat for spring-associated fishes (Hubbs 1995), likely fragments habitats and impedes movement (Dammeyer et al. 2013), decreases Fountain Darter reproductive success (Brandt et al. 1993, Bonner et al. 1998), and increases intraspecific competition (Araujo 2012) and gill-parasite mortality (McDonald et al. 2006, Tolley-Jordan and Owen 2008). Modeling suggests that reducing the 19-year mean base flow conditions (184 cfs) to 58 cfs (32% of current base flow) would noticeably reduce Fountain Darter populations in the San Marcos River (Mora et al. 2013). Empirical evidence supports this prediction, given that Fountain Darters were considered extirpated from the Comal River in 1973, attributed to cessation of spring flows though possibly affected by rotenone treatment to remove non-native fishes in the 1950s, and a catastrophic flood in 1972, prior to documenting the extirpation in the mid-1970s (Schenck and Whiteside 1976).

Given that low flow conditions will alter the habitats of the Fountain Darter, we predict that reproductive effort within levels of vegetated to non-vegetated habitat types and within high to low discharge environments will be reduced, respectively. To test this prediction, we first established a

baseline in Fountain Darter reproductive readiness among a gradient of flow regimes and among vegetation types. Objectives of this study were to quantify elements of Fountain Darter reproduction (gonadal recrudescence, ovarian development) among available flow gradients ranging from 5 to 120 cfs in the wild and among physical habitat types and substrates (open substrates, low-growing and tall-growing aquatic vegetation).

HCP Ecological Model Parameterization

The Fountain Darter fecundity study directly assessed the influence of flow and aquatic vegetation on Fountain Darter reproduction. Type and/or structure of aquatic vegetation are key components of Fountain Darter habitat in the HCP Ecological Model while discharge is a driving variable. Information generated from this work could provide direct measurements of reproductive success and expenditure for Fountain Darters throughout the year. Although the report puts forward parameters (reproductive effort by month, flow, and vegetation type) for consideration in model parameterization, it is emphasized that specific use of any of the 2014 applied research will be determined by the HCP ecosystem modeling team with guidance from the HCP Science Committee.

Recommendations for Future Applied Research

The Fountain Darter fecundity study was successful in evaluating the relationship in reproductive effort between discharge and vegetation type. However, it is acknowledged that this study only represents one partial year of data collection and did occur during an extended drought under total system discharge conditions not observed at Comal Springs since 1990. It is also described in the discussion section that unique habitat areas such as Spring Lake in the San Marcos system and Landa Lake in the Comal System may provoke different reproductive responses. As such, monthly sampling for an additional year with the collection of female Fountain Darters from existing sites and additional habitat areas is recommended.

Acknowledgments

The project team would like to acknowledge the U.S. Fish and Wildlife Service San Marcos Aquatic Resource Center (SMARC) scientists and staff. In particular, we thank Dr. Ken Ostrand and Dr. Tom Brandt for their guidance, assistance, patience and cooperation. As described throughout this report, Dr. Ostrand was integral in monthly field collection efforts and access was provided to SMARC laboratory facilities for all histology evaluations. We would also like to thank the HCP Science Committee for their timely input regarding approaches and methods for research activities.

2.0 DATA REVIEW AND AVAILABLE LITERATURE

For this reproductive assessment, the data review and literature compilation were performed for two major categories including baseline reproductive rates of Fountain Darters and habitats used by Fountain Darters for egg deposition. Each topic is addressed below.

Baseline reproductive rates of Fountain Darters: Fountain Darters are sexually dimorphic with males having distinct coloration in dorsal fins and short, pointed genital papillae and females having less intense pigmentation in dorsal fins and long, forked genital papillae (Schenck and Whiteside 1977). Sex ratios are slightly skewed toward males (1.39:1:00). Minimum length of reproduction

is 24 mm in total length (Schenck and Whiteside 1977) at age 3.5 months (Linam et al. 1993) to 6 months (Brandt et al. 1993). Numbers of ova (ovulated oocytes within the ovary) are related to female length in darters with larger females producing more ova, though size of ova is independent of female length (Schenck and Whiteside 1977, Marsh 1986). Ova occur in female Fountain Darters year round, suggesting a protracted spawning season (12-months) but with reproductive peaks in late winter and late summer (Schenck and Whiteside 1977). Fountain Darters are batch spawners, producing a mean of 9 to 14.5 eggs per day during a 33 d period in a hatchery setting (Bonner et al. 1998) with 5 to 27 days, on average, between batches (Brandt et al. 1993). Eggs are released at water temperatures ranging from 3 to 30°C (Brandt et al. 1993) with optimum egg production ranging between 14 and 26° (Bonner et al. 1998, McDonald et al. 2007).

Habitats used by Fountain Darters for egg deposition: Fountain Darters are facultative phytophilic spawners (Simon 1999) depositing adhesive eggs on macrophytes (Strawn 1956, Phillips et al. 2011) but also on hard substrates lacking vegetation (Brandt et al. 1993). To date, Fountain Darters deposit eggs have been observed on *Rhizoclonium*, *Ludwigia*, *Sagittaria*, and *Zizania* (Phillips et al. 2011), but this is likely an incomplete list. Fountain Darters associate with a wide variety of vegetation, including *Riccia*, *Rhyzoclonium*, *Hydrilla*, *Ludwigia*, *Potamogeton*, *Sagittaria*, *Vallisneria*, *Hygrophila*, and *Cabomba* (Schenck and Whiteside 1976, Linam et al. 1993, Phillips et al. 2011, Alexander and Phillips 2012, Araujo 2012, Dammeyer et al. 2013) and areas without vegetation (Crowe and Sharp 1997, Araujo 2012, Behen 2013). Fountain Darters, in general, associate with slackwater and low velocity habitats, ranging in depths from < 0.5 m to 5 m with silt to cobble substrates (Behen 2013). Sister species within Subgenus Microperca (*E. microperca* and *E. proeliare*; Near et al. 2011) also are associated with slackwater to run habitats consisting of detrital terrestrial leaves, woody debris, and dense vegetation (Burr and Page 1978; Paine et al. 1981; Johnson and Hatch 1991).

3.0 MATERIALS AND METHODS

3.1 Field Collections

Sampling occurred monthly starting in January proceeding into August, 2014. Sample sites included City Park reach of the San Marcos River (Hays County, Texas) (Figure 1) and New channel, Old channel, and upper spring run reaches of the Comal River (Comal County, Texas) (Figure 2). Within each site, dip nets (16.5" hoop x 1/16" mesh) (Figure 3) and seines (2m x 1m x 1/16'' mesh) were used to capture female Fountain Darters > 24 mm in total length (TL). Immature (< 24 mm TL) and male Fountain Darters were promptly returned to the immediate area of capture. Females selected for laboratory analysis were placed in a lethal dose of MS-222 and preserved in a 10% solution of buffered formalin.

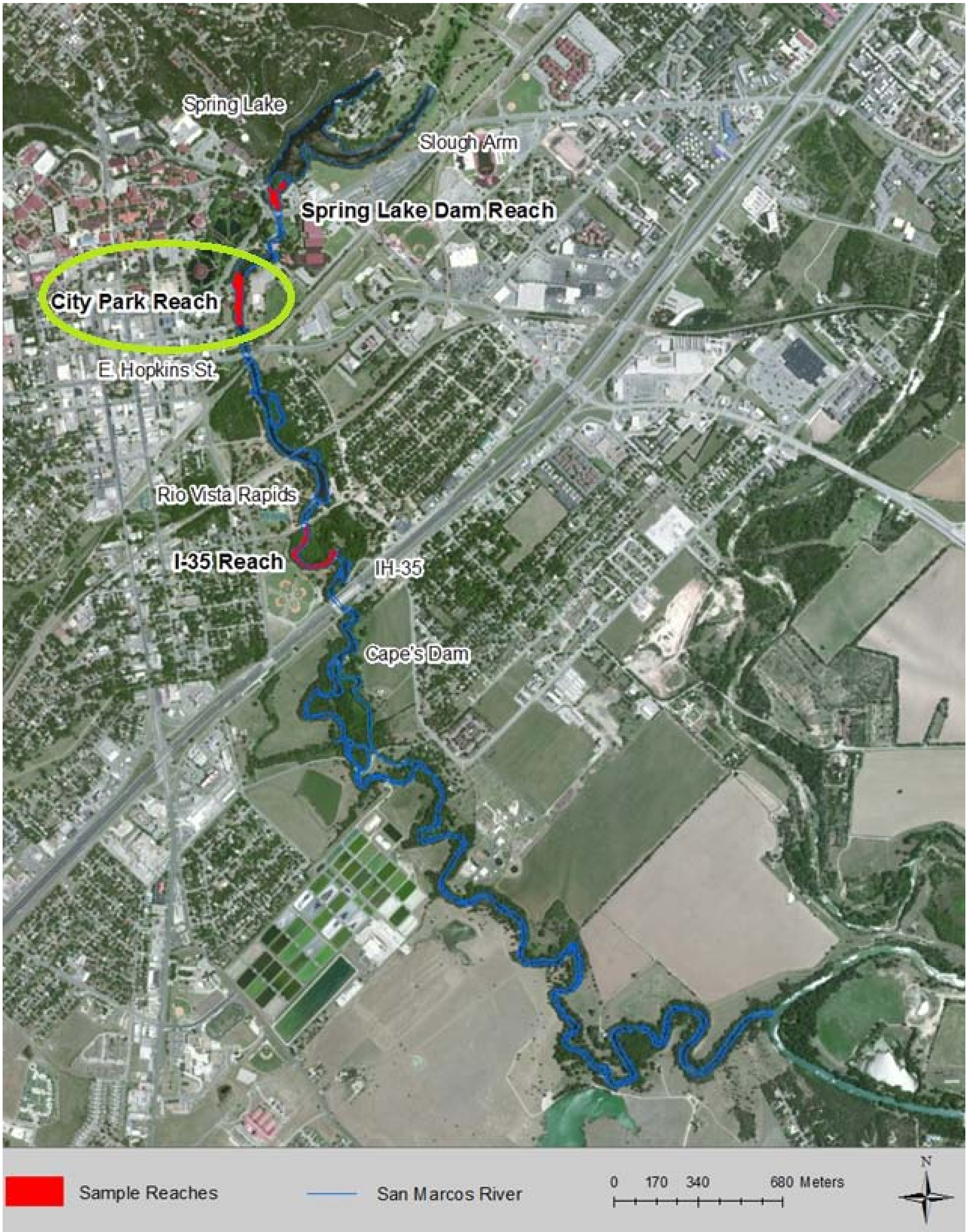


Figure 1. City Park sampling reach on the San Marcos River used for the Fecundity Study.

Figure 2. Upper Spring Run, Old Channel, and New Channel study reaches on the Comal River.

Figure 3. Monthly field collections for mature female Fountain Darters in the Old Channel of the Comal River.

Three vegetation types per site were sampled. Vegetation types were bare substrates (no vegetation), short vegetation (macrophytes < ½ of water depth) (Figure 4), and tall vegetation (macrophytes stands > ½ of water depth). Sample depth in meters (m) and vegetation height (m) were measured, along with visual estimation of the dominant vegetation. Current velocity was measured using a Marsh-McBirney Flo-mate™ portable velocity flow meter within vegetation and above (where applicable), and at 60% of the water depth for habitats without vegetation. Water quality was measured using a YSI 556 Multi-parameter System and included dissolved oxygen (mg/l), pH, temperature (˚C), and specific conductance (µS/cm). Percent substrate composition was visually estimated using a modified Wentworth scale (silt: <0.06 mm, sand: 0.06–1.99 mm, gravel: 2–63 mm, cobble: 64–255 mm, boulder: >256 mm, and bedrock).

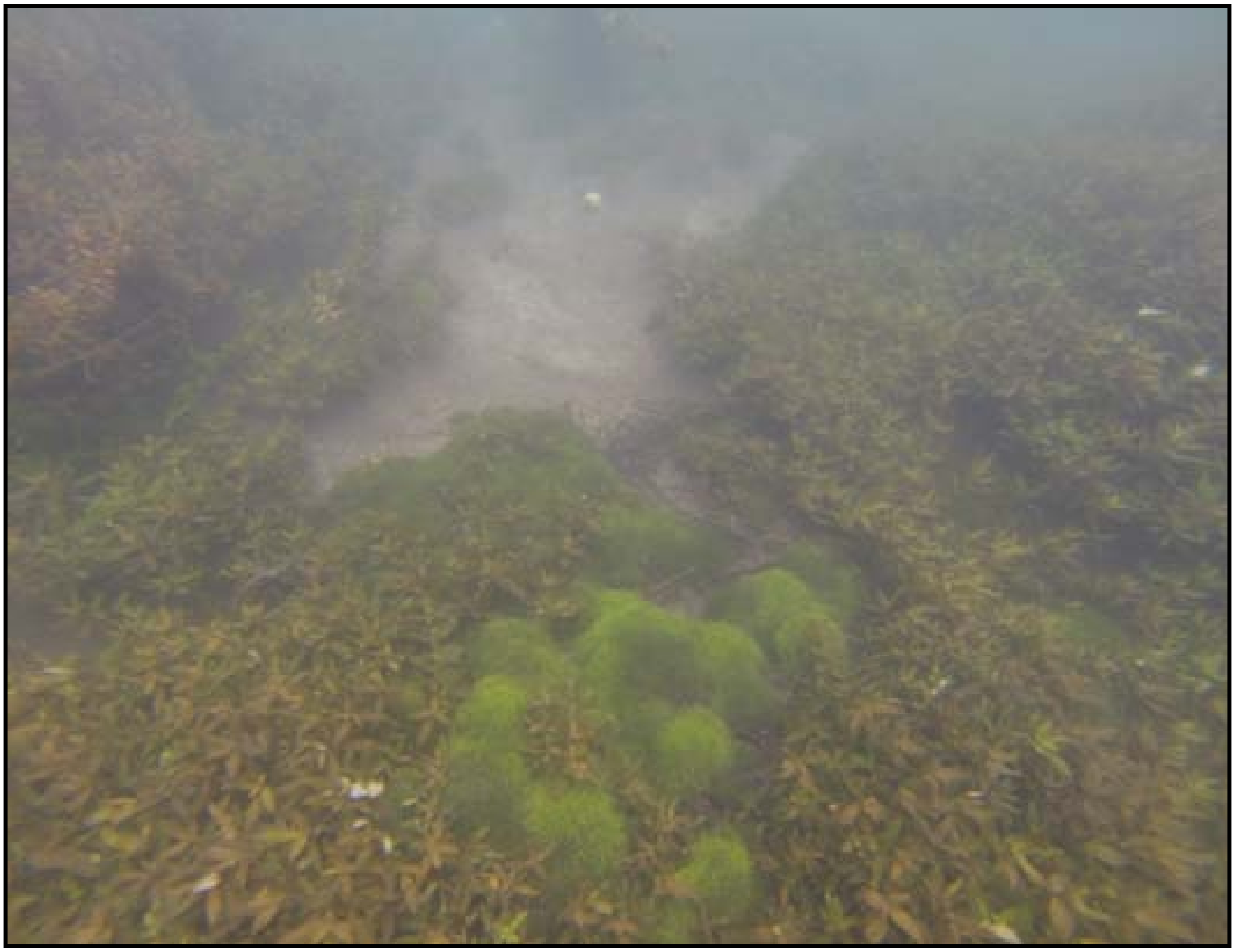


Figure 4. **Examples of "short" aquatic vegetation sampled in during monthly field collections.**

3.2 Laboratory Analysis

Samples were allowed to fix in solution for two weeks. Fish were transferred from formalin to 70% ethanol, total length was measured, and ovaries were excised. Gonadosomatic index (GSI) was estimated as the percent ratio of ovary to eviscerated body weight (liver, intestinal tract, and other viscera removed). Oocyte to ova maturation and ovarian stage was estimated using methodologies specific to darters modified from Heins and Baker (1989), Heins et al. (1992), and Heins (1995). Following recommendations provided by Brewer et al. (2008), a small sample of ovaries were selected from hatchery stock and conditioned to specific stages of development. These individuals were used for histology to confirm classification of ovarian stage. All laboratory analyses were conducted at the U.S. Fish and Wildlife Service San Marcos Aquatic Resources Center (SMARC), in San Marcos, Texas (Figure 5).

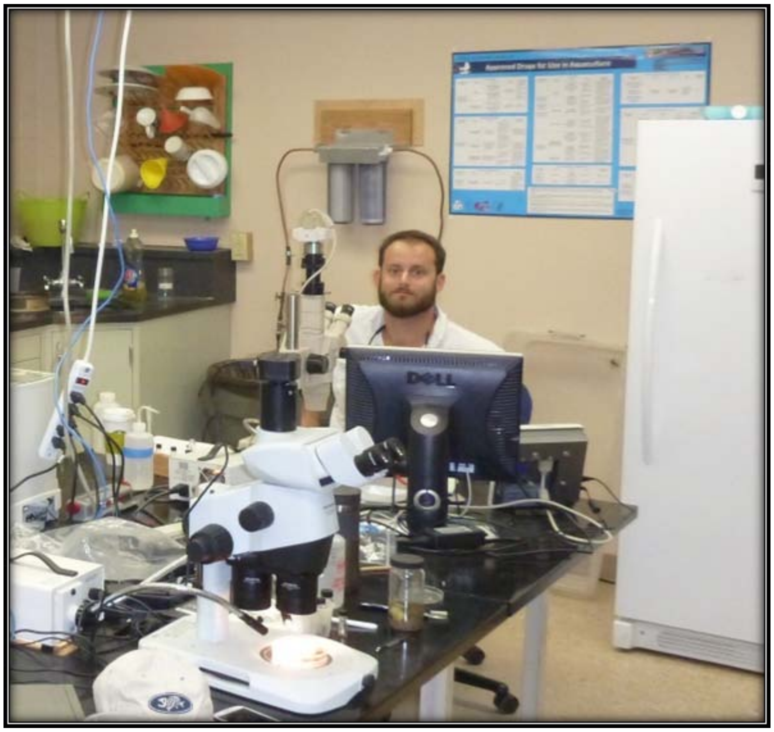


Figure 5. Laboratory analysis of ovary stage conducted at the SMARC.

Ovarian stage was separated into four distinct categories: pre-vitellogenic, early vitellogenic, late vitellogenic, and spawning (Appendix 1). Pre-vitellogenic ovaries appeared small, clear or translucent and were classified as latent (Heins 1995). Early vitellogenic ovaries were larger than latent, and opaque in appearance with a moderate amount of oocytes enlarged (Heins 1995). Late vitellogenic ovaries were greatly enlarged, and contained larger oocytes in the last stages of vitellogenesis or pre-ovulation (Heins et al. 1992; Heins 1995). Spawning ovaries contained ova distinguished by a small infold and enlarged chorion similar to descriptions provided by Schenck and Whiteside (1977) and Heins (1995).

3.3 Experimental Design and Data Analysis

The experimental design for this study involved the following response variables: Gonadosomatic index and percent of mature ovaries. The experimental unit was an individual female Fountain Darter and the following three treatments were tested:

Treatment 1: Flow regime (four levels)
Treatment 2: Vegetation type (three levels)
Treatment 3: Month (eight levels)

This resulted in an 8 x 4 x 3 = 96 design. Five replications per treatment were selected to control variability relating to batch spawning fishes (5 x 96 = 480 female Fountain Darters). Availability by site, month, and substrate type dictated the total number of female darters, so total catch was less than the anticipated 480.

Mean daily discharge (cubic feet per second; cfs) was obtained from USGS Stations (Comal River-Old Channel: 08168913; Comal River-New Channel: 08168932; San Marcos River: 08170500). Mean discharge was calculated via a transect method at Comal River-Upper Spring Run.

Gonadosomatic indices were calculated for all fishes by site and month and for Mature-Ripe ovaries only. Monthly differences in GSI-All Ovaries were assessed across all sites with one-factor ANOVA ($\alpha = 0.05$), followed by post-hoc Fisher's Least Significance Difference, to determine if reproductive effort is homogenous across months as expected for a year-round spawning fish. Mature-Ripe ovaries were selected as the most sensitive indicator of reproductive effort, because this stage of late vitellogenic ovary is the most advanced without ovulation and egg release (Heins and Baker 1989). Hence, weights of Mature-Ripe ovaries are not influenced by prior release (minutes to hours) of ovum. Differences in GSI-Mature-Ripe Ovaries (dependent variable) by site (categorical independent variable; represents differences in discharge) and vegetation type (categorical independent variable) were assessed with a two-factor ANOVA, followed by post-hoc Fisher's Least Significance Difference test. Site*Vegetation interaction term was not significant ($P = 0.67$) and dropped from the linear model.

4.0 RESULTS AND DISCUSSION

Mean discharge (± 1 SD) ranged from 3.3 (2.7) cfs in the Comal River-Upper Spring Run to 145 (30.2) cfs in the San Marcos River-City Park (Figure 6). Discharge during the period of observation decreased in the Comal River-Upper Spring Run, stayed fairly constant in the Comal River-Old Channel, decreased in the Comal River- New Channel, and slightly decreased in San Marcos River-City Park, though supplemented with multiple pulse flow events. Mean water temperature (calculated at location of each fish taken) ranged between 21.5°C at San Marcos River-City Park to 24.2°C at Comal River-Upper Spring Run (Table 1). Minimum water temperatures ranged between 18.6°C at San Marcos River-City Park to 20.3°C at Comal River-Old Channel, and maximum water temperature ranged between 22.7°C at San Marcos River-City Park to 29.6°C at Comal River-Upper Spring Run. The lowest dissolved oxygen level (4.5 mg/l) was observed at Comal River-Upper Spring Run. Fish were taken from a variety of substrate types, through predominantly from gravel at Comal River-Upper Spring Run or from silt at the other three sites. Mean relative vegetation heights ranged from 15 to 31% of water depth for short vegetation and 57 to 71% of water depth for tall vegetation.

Among all sites and for 335 Fountain Darters, four stages of ovarian development were found from January through August 2014, except latent ovaries were absent in June 2014 (Figure 7). Occurrences of spawning ovaries and late vitellogenic ovaries from January through August indicate egg release throughout the study period. However, proportions of spawning ovaries and late vitellogenic ovaries decreased through time. Fish with spawning ovaries and late vitellogenic ovaries comprised >50% of the breeding population from January through July and <25% in August. Reproductive effort, as measured by GSI-All Ovaries, differed ($P < 0.01$) among months with a peak in March, elevated in January, February, April, and June, and decreased in May, July, and August. Hence, reproductive effort was not constant through time.

Among individual sites, GSI-All Ovaries generally decreased from January through August (Figure 8). Occurrence of spawning ovaries indicated egg release during each month and site, except in April and August at Comal-Upper Spring Run, Comal-Old Channel, and Comal-New Channel.

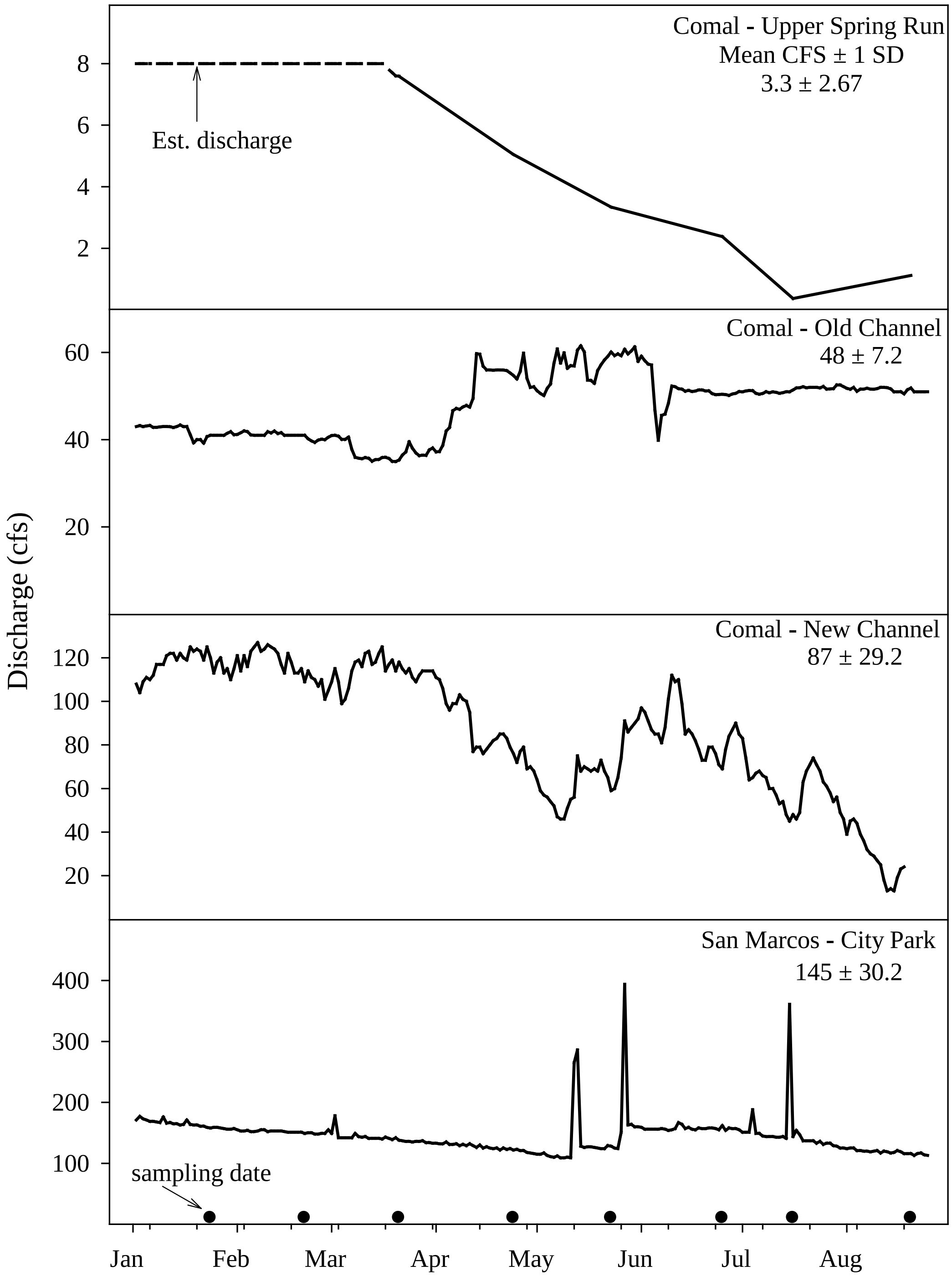


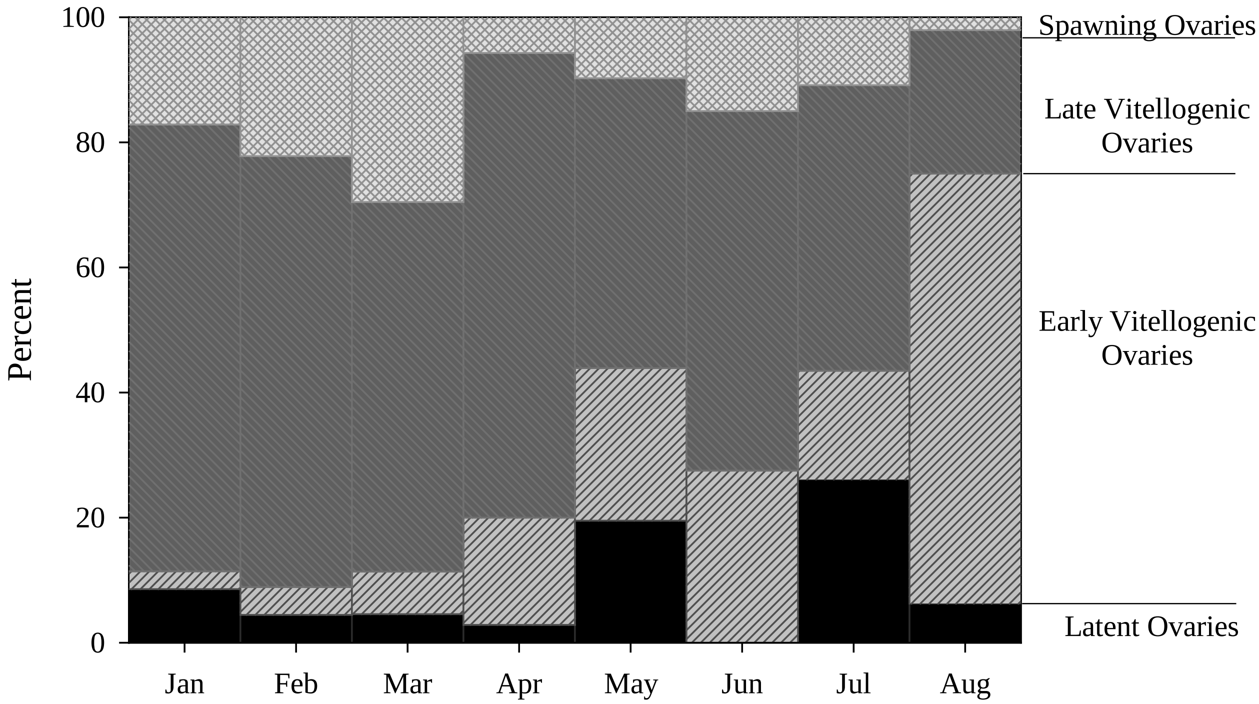
Figure 6. **Hydrograph (mean daily discharge) for each study site and collection dates of Fountain Darters taken. Comal River-Upper Spring Run hydrograph was calculated via transect method on the day of sampling.**

Table 1. Water quality measurements among four sites and across months.

River	Comal River			Comal River		
Site	Upper Spring Run			Old Channel		
Mean Discharge (cfs^{-1})	3.3			48		
	Mean (SE)	Min	Max	Mean (SE)	Min	Max
Temperature (˚C)	24.18 (0.2)	19.5	29.6	22.7 (0.2)	20.3	24.3
pH	7.33 (0.04)	7	7.8	7.49 (0.02)	7.28	8.19
Dissolved Oxygen ($mg*l^{-1}$)	7.97 (0.2)	4.54	11.86	8.33 (0.14)	6.4	12.8
Specific Conductance ($\mu S*cm^{-1}$)	558 (2.2)	491	590	551 (2.8)	516	595
Habitat Type	Bare	Short	Tall	Bare	Short	Tall
	mean (SE)	mean (SE)	mean (SE)	mean (SE)	mean (SE)	mean (SE)
Depth (m)	1 (0.05)	0.68 (0.03)	0.77 (0.03)	1.0 (0.06)	0.68 (0.04)	0.71 (0.03)
Current Velocity ($m*s^{-1}$)	0 (0.0)	0.02 (0.00)	0.01 (0.01)	0.24 (6.7)	0.06 (0.00)	0.09 (0.01)
Aquatic Vegetation Height (m)	-	0.09 (0.01)	0.49 (0.03)	-	0.19 (0.02)	0.37 (0.02)
Relative Height Index	-	0.15 (0.02)	0.67 (0.04)	-	0.29 (0.03)	0.57 (.04)
% Woody Debris	11 (7.62)	0	0	28.4 (10.1)	0	0
% Substrate						
Silt	13 (5.4)	6.7 (1.9)	12.9 (4.8)	12.5 (7.2)	96.9 (2.6)	98.2 (0.58)
Sand	1 (0.6)	1.7 (0.77)	2.3 (2.3)	20.6 (7.9)	0.13 (0.13)	0
Gravel	58 (10.3)	65.6 (5.5)	55.9 (6.5)	18.4 (7.1)	2.56 (2.1)	0
Cobble	24 (8.8)	26.0 (5.9)	29.0 (5.6)	40.6 (9.6)	0.38 (0.38)	1.178 (.57)
Boulder	2 (1.7)	0	0	7.8 (3.0)	0	0

Table 1 (continued). Water quality measurements among four sites and across months.

River	Comal River			San Marcos River		
Site	New Channel			City Park		
Mean Discharge (cfs^{-1})	81			138		
	Mean ± (SE)	Min	Max	Mean ± (SE)	Min	Max
Temperature (˚C)	22.8 (0.22)	18.8	24.9	21.5 (0.20)	18.6	22.7
pH	7.69 (0.02)	7.48	7.99	7.43 (0.04)	7.14	7.68
Dissolved Oxygen ($mg*l^{-1}$)	8.53 (0.14)	7.12	10.83	8.58 (0.16)	6.77	10.95
Specific Conductance ($\mu S*cm^{-1}$)	558 (2.8)	517	600	584 (3.9)	533	620
Habitat Type	Bare	Short	Tall	Bare	Short	Tall
	mean (SE)	mean (SE)	mean (SE)	mean (SE)	mean (SE)	mean (SE)
Depth (m)	0.44 (0.06)	0.84 (0.05)	0.72 (0.02)	0.47 (0.09)	0.85 (0.04)	0.74 (0.04)
Current Velocity ($m*s^{-1}$)	0.07 (0.02)	0.06 (0.01)	0.06 (0.01)	0.09 (0.03)	0.08 (0.02)	0.06 (0.01)
Aquatic Vegetation Height (m)	-	0.26 (0.03)	0.47 (0.02)	-	0.23 (0.02)	0.56 (0.06)
Relative Height Index	-	0.31 (0.03)	0.66 (.02)	-	0.29 (0.03)	0.71 (0.05)
% Woody Debris	0	0	0	0	0	0
% Substrate						
Silt	13.6 (7.0)	90.4 (4.5)	92.0 (3.4)	50 (29.0)	88.4 (5.5)	78.4 (7.2)
Sand	25 (6.7)	0	0	43.3 (29.6)	11.5 (5.5)	19.8 (6.6)
Gravel	56.8 (8.9)	6.0 (3.3)	7.3 (3.3)	6.7 (6.7)	0	1.8 (0.7)
Cobble	4.6 (1.3)	0	0.71 (0.3)	0	0	0
Boulder	0	0	0	0	0	0



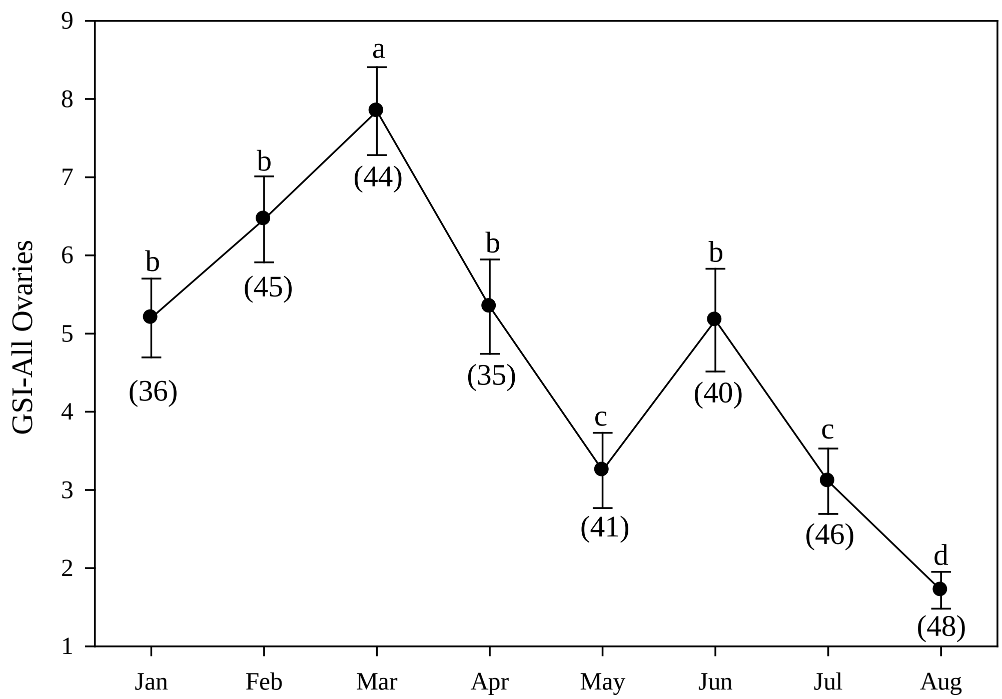


Figure 7. **Ovarian stages for all Fountain Darters (N = 355, upper panel) and gonadosomatic indices for all female Fountain Darters (lower panel) taken from four sites on San Marcos River and Comal River. Parenthetical numbers represent N of fishes per month. Same lower case letter represent no significant difference between months.**

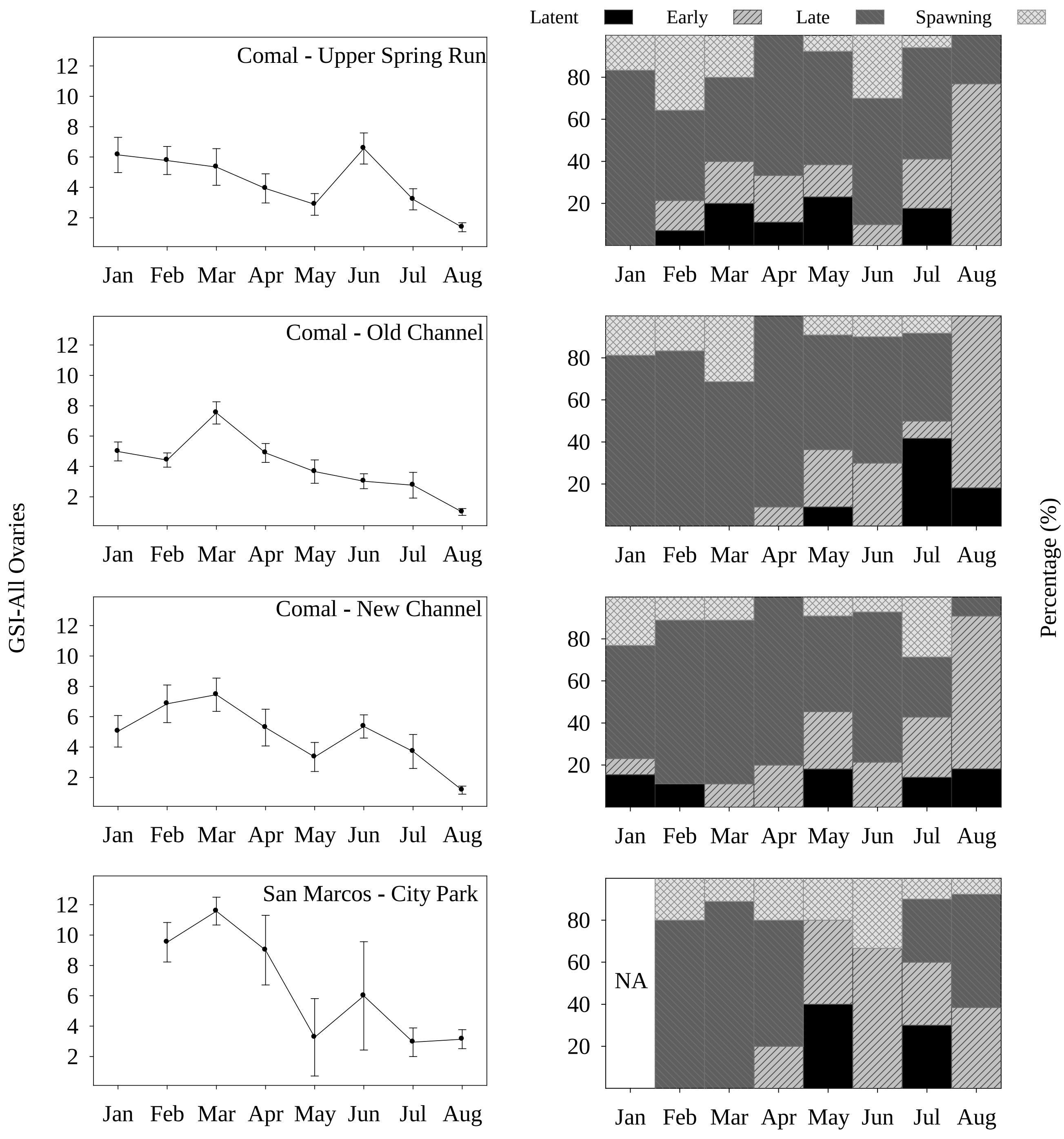


Figure 8. **Gonadosomatic indices for fishes >24 mm in TL (size of sexual maturity) among four sites (left panel) and associated ovarian stages (right panel).**

Gonadosomatic indices-Mature-Ripe Ovaries differed among sites and vegetation type (P<0.01) (Figure 9). Mean (± 1 SE, N) of GSI (Ripe-Mature Ovary) taken from San Marcos River-City Park was 8.4 (0.67, 21) and was greater than those taken from Comal River-New Channel (7.0 ± 0.38, 41), Comal River-Old Channel (6.1 ± 0.34, 39), and Comal River-Upper Spring Run (6.5 ± 0.39, 24). Mean of GSI (Ripe-Mature Ovary) taken from tall vegetation was 7.5 (0.35, 60), did not differ from those taken from bare substrate (7.0 ± 0.47, 12), and was greater than those taken from short vegetation (6.1 ± 0.29, 53).

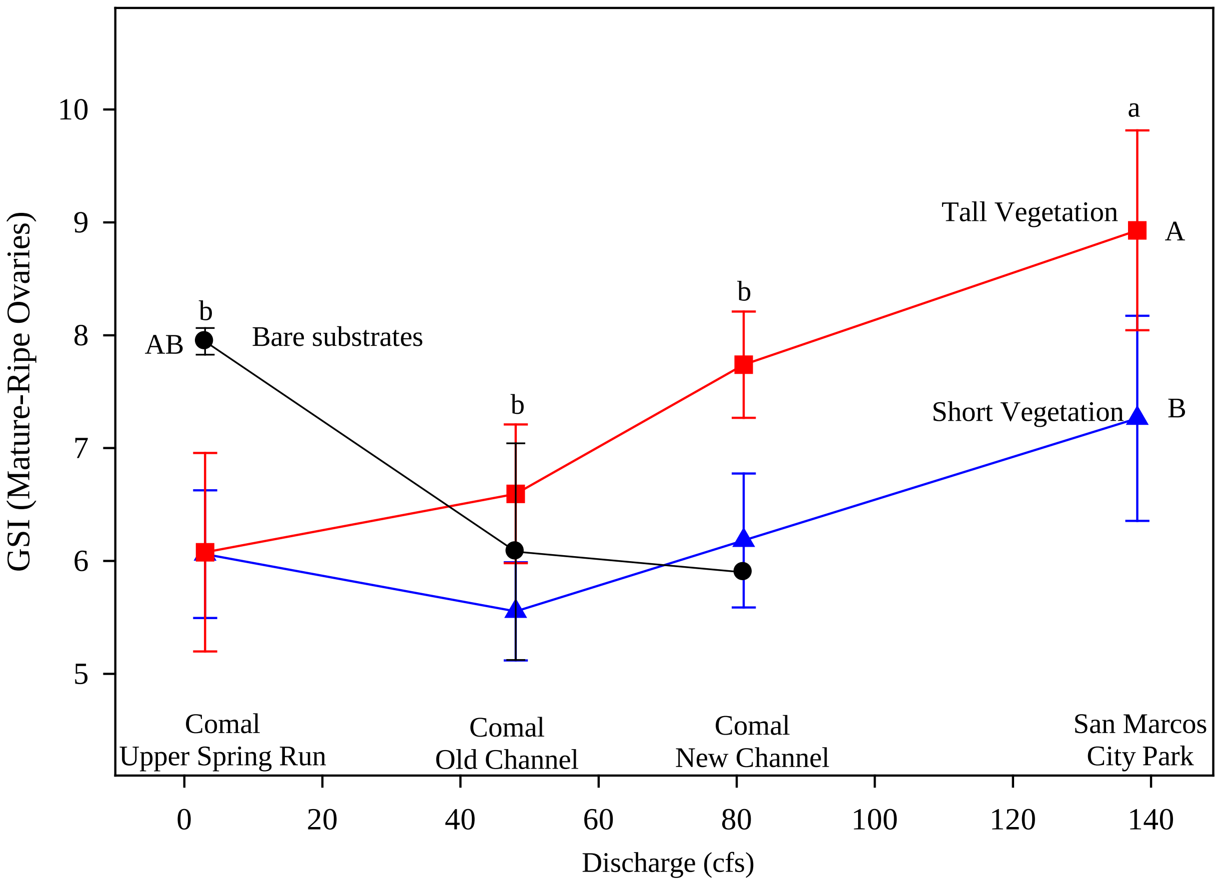


Figure 9. **Gonadosomatic indices for fishes with Mature-Ripe Ovaries by site and mean daily discharge and by vegetation type. Same upper case letters represent no significant vegetation type effect. Same lower case letters represent no significant site effect.**

Initial predictions on the relationship between reproductive effort and discharge were partially supported. Reproductive effort, as measured by GSI-Mature-Ripe Ovaries, was greater within greater discharge environments of the San Marcos River (mean discharge = 145 cfs). However, differences in reproductive effort were not detectable among discharges ranging from 3.3 to 87 cfs in the Comal River. Furthermore, spawning, as measured by occurrence of Spawning Ovaries, occurred at <1 cfs in Comal River-Upper Spring Run in July 2014. Therefore, differences in spawning among flow gradients were not detected under conditions encountered in 2014, whereas amount of energy invested into reproduction is dependent on discharge at levels >87 cfs.

Study results herein differ slightly than the results reported by Schenck and Whiteside (1977). Schenck and Whiteside (1977) reported peaks in reproductive effort as greater proportions of females containing mature ova in February and March and again in July and August. Conversely, we found a general decrease in reproductive effort from Spring through Summer. Our study results, however, are consistent with reproductive efforts reported spawning patterns in other spring-associated minnows (McMillan 2011) and spring-associated darters (Folb 2010). In addition, our results are consistent with field observations within the San Marcos and Comal Rivers (BIO-WEST 2014a, 2014b). Small Fountain Darters (5 – 15mm, <60 days old; Brandt et al. 1993) were captured in the San Marcos River-City Park during dip netting events 23 of the 47 events (49%) since 2000 with most occurrences noted during the Spring (Figure 10). Small Fountain Darters were taken more often (44 of 47 events; 94%) in Spring Lake (Figure 11) than in San Marcos River-City Park, but higher proportions were again found in the Spring. In the Comal River, similar patterns are evident: New Channel (46% of samples contained small Fountain Darters), Old Channel (79%), Upper Spring Run (71%), and Landa Lake (90%) which again documents differences among sites. However, as with the San Marcos River, peaks in the Comal system were most evident in the Spring at all stations.

Initial predictions on the relationship between reproductive effort and vegetation type were largely unsupported. Reproductive effort was greater in tall vegetation at Comal River-Old Channel, Comal River-New Channel, and San Marcos-City Park. Reproductive effort was greatest on bare substrates in Comal River-Upper Spring Run, likely attributed to limited amounts of vegetation within the site.

Collectively, Fountain Darters reproduce for at least eight months (January – August) but reproductive effort is not equal among months or among sites (discharge). Mechanisms underlying reduced reproductive energy at discharges <145 cfs and in tall vegetation are unknown at this time. Density-dependent mechanisms, such as prey availability and Fountain Darter densities, are potential factors regulating reproductive investment. Information on food items consumed is available for Fountain Darters collected during this study and could yield insight into potential diet differences among sites and vegetation types, but the information has yet to be quantified. Additionally, measures of Fountain Darter densities are available and will be evaluated against reproductive investment at a later date.

Density dependent mechanisms influencing reproductive effort (investment and seasonality) have potentially interesting links to quality of habitats via field observations. As noted above, occurrences of small Fountain Darters are more frequent in Landa Lake and Spring Lake (>90%

occurrence among samples) than in Old Channel and Upper Spring Run (71 – 79%) or San Marcos-City Park and New Channel (<50%). Though reproductive investment appears to be higher in San Marcos-City Park, the greater frequency of small Fountain Darters year round at Upper Spring Run and Old Channel suggest extended spawning. Comparisons between reproductive investment and spawning are potentially useful as an indicator of habitat quality.

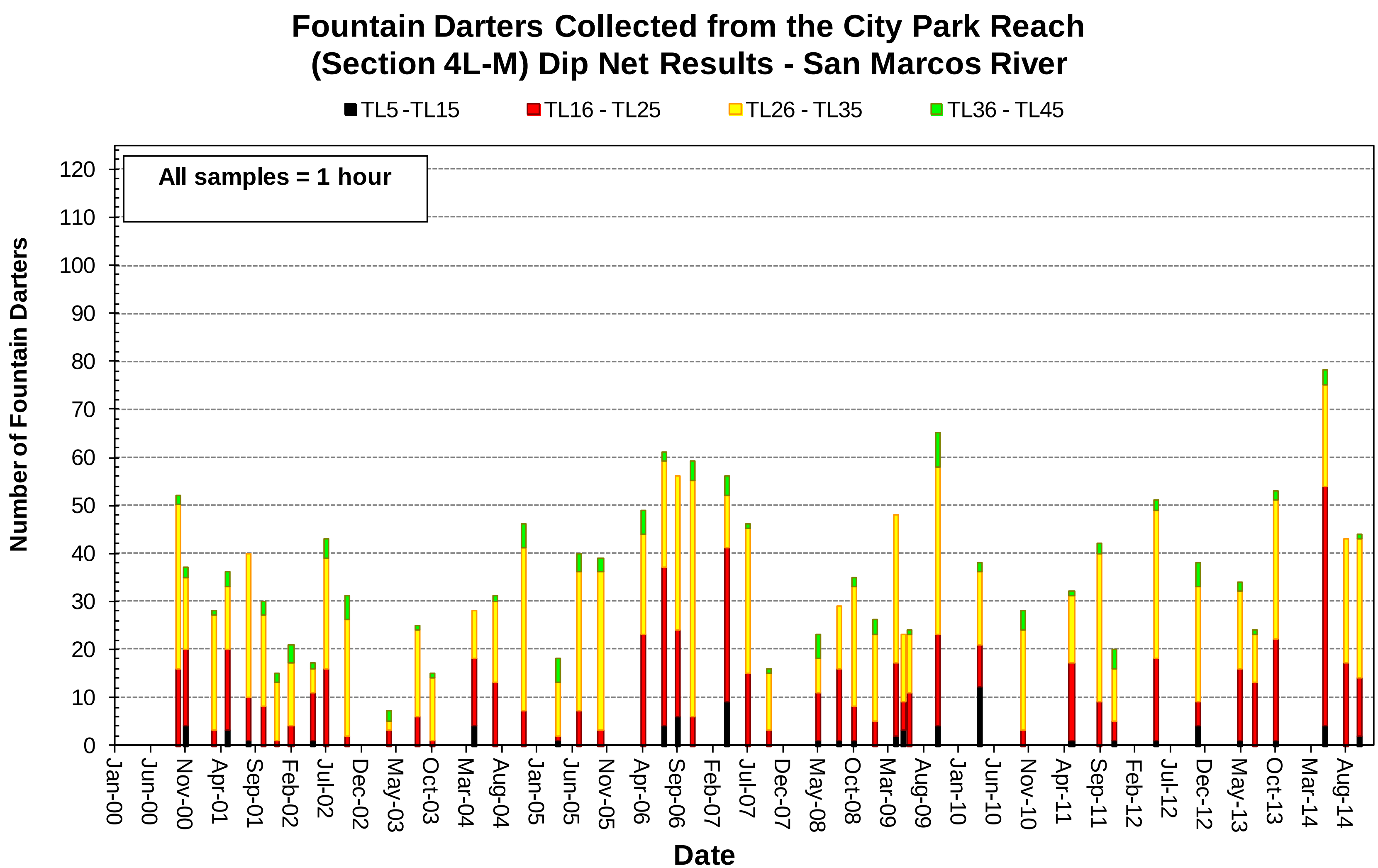


Figure 10. **Fountain Darter dip net results over time from City Park of the San Marcos River.**

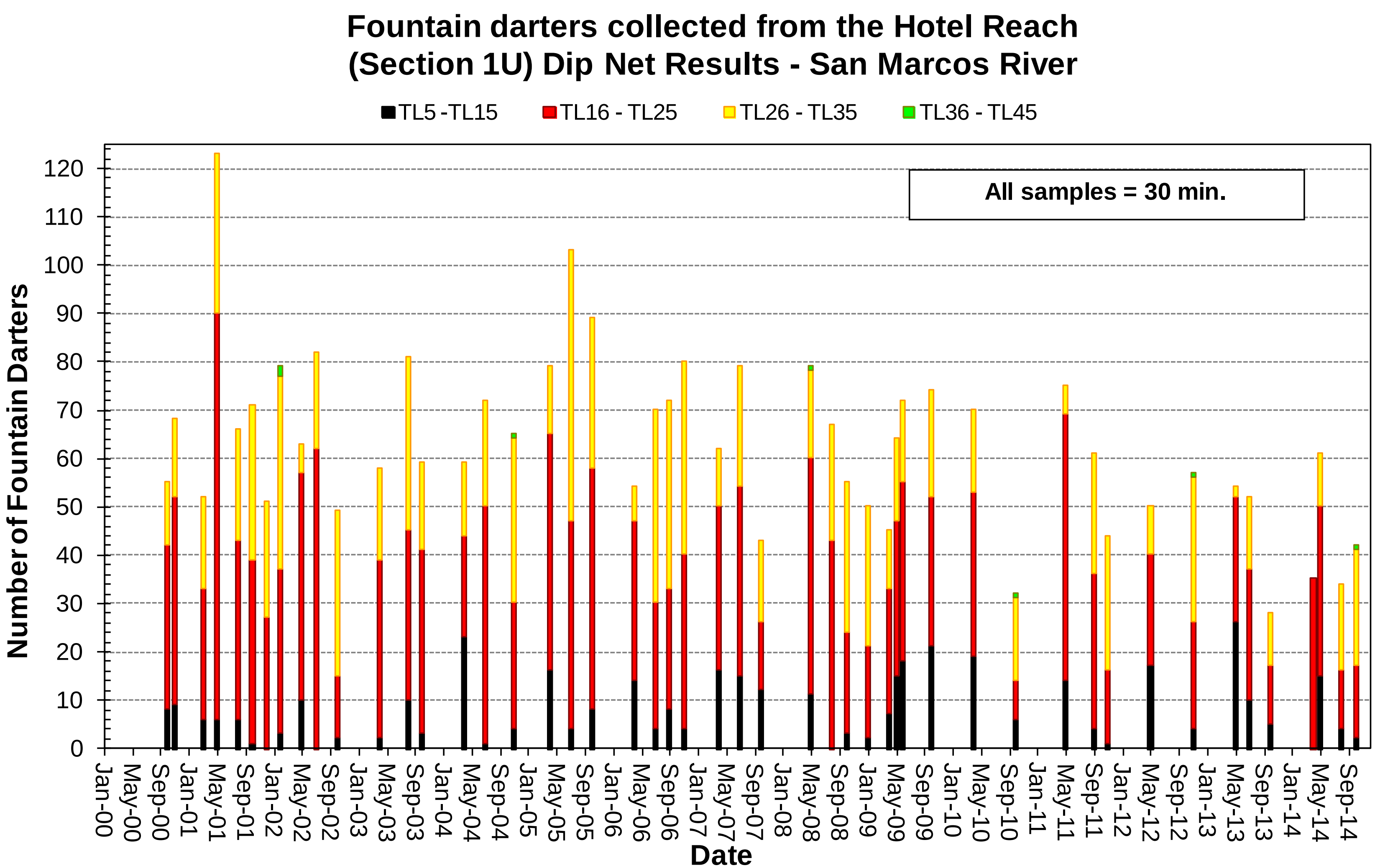


Figure 11. **Fountain Darter dip net results over time from Spring Lake of the San Marcos River.**

5.0 CONCLUSIONS

Fountain Darters are reported to be year round spawners. Evidence to date supports this but spawning effort is not equal among all months. Decreasing effort occurs during the summer months. Given the abiotic conditions recorded at each site, we hesitate to attribute this decrease to water year (below average flow this year) conclusively until comparable data are collected during an average or above average flow year. Reproductive effort differed among a flow gradient ranging between 3.3 and 145 cfs but only with marginal differences in GSI. Spawning did not cease across the flow gradient. In addition, vegetation type was associated with reproductive effort.

Mechanisms to explain the observed pattern are still being explored but likely include results of physical (structural components of vegetation) or biological (density-dependent) processes, such as amount of food available or number of conspecifics in the area. The relationships among physical and biological mechanisms and flow could offer insight on how flow indirectly affects Fountain Darter reproduction.

For the HCP Ecological parameterization, estimates of reproductive effort by month, flow, and vegetation type can be used to improve reproduction estimates in the model. Currently, the model is using water temperature as the primary determinant of reproduction. Information provided herein offers additional options to refine reproductive parameters in the final model.

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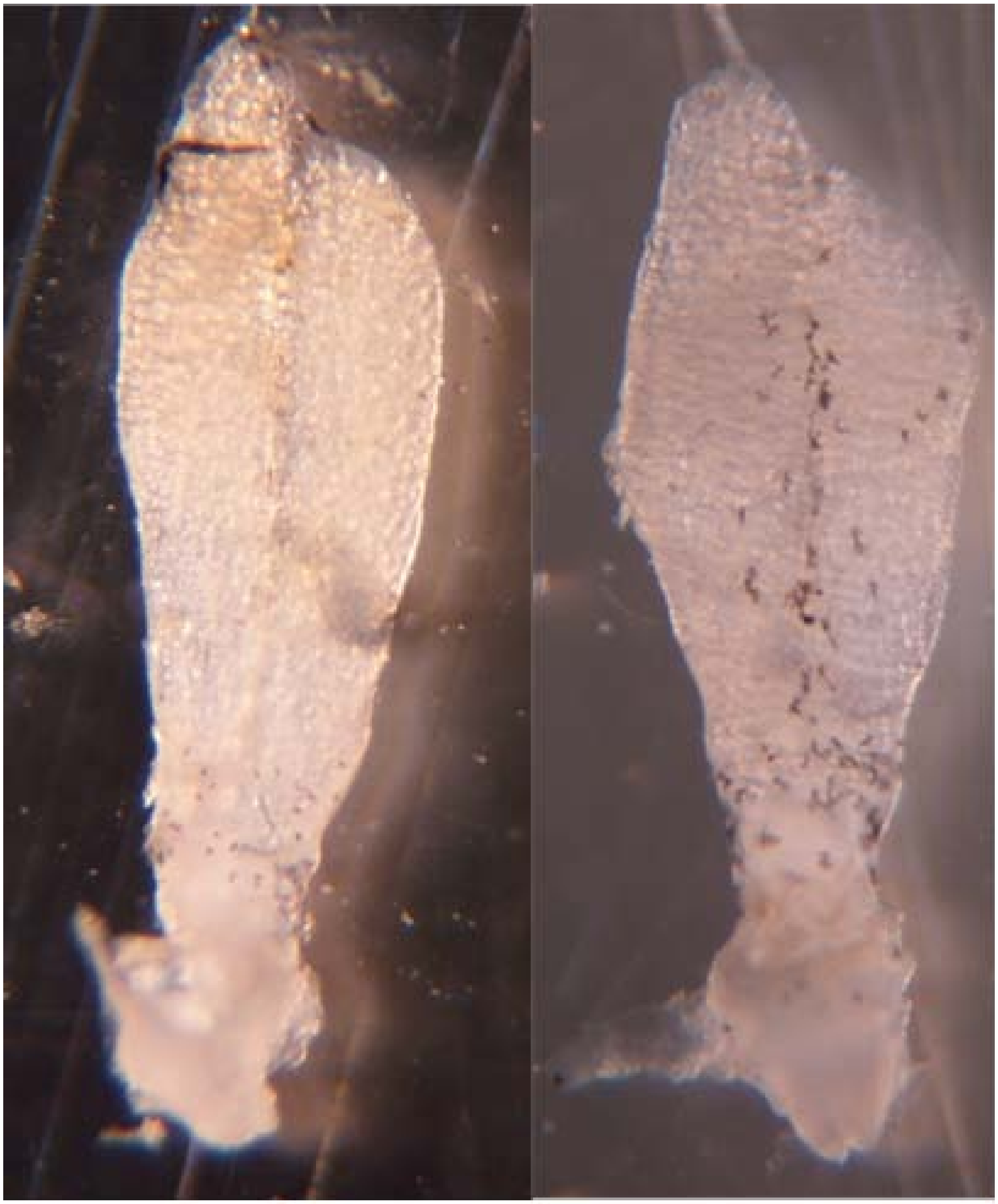
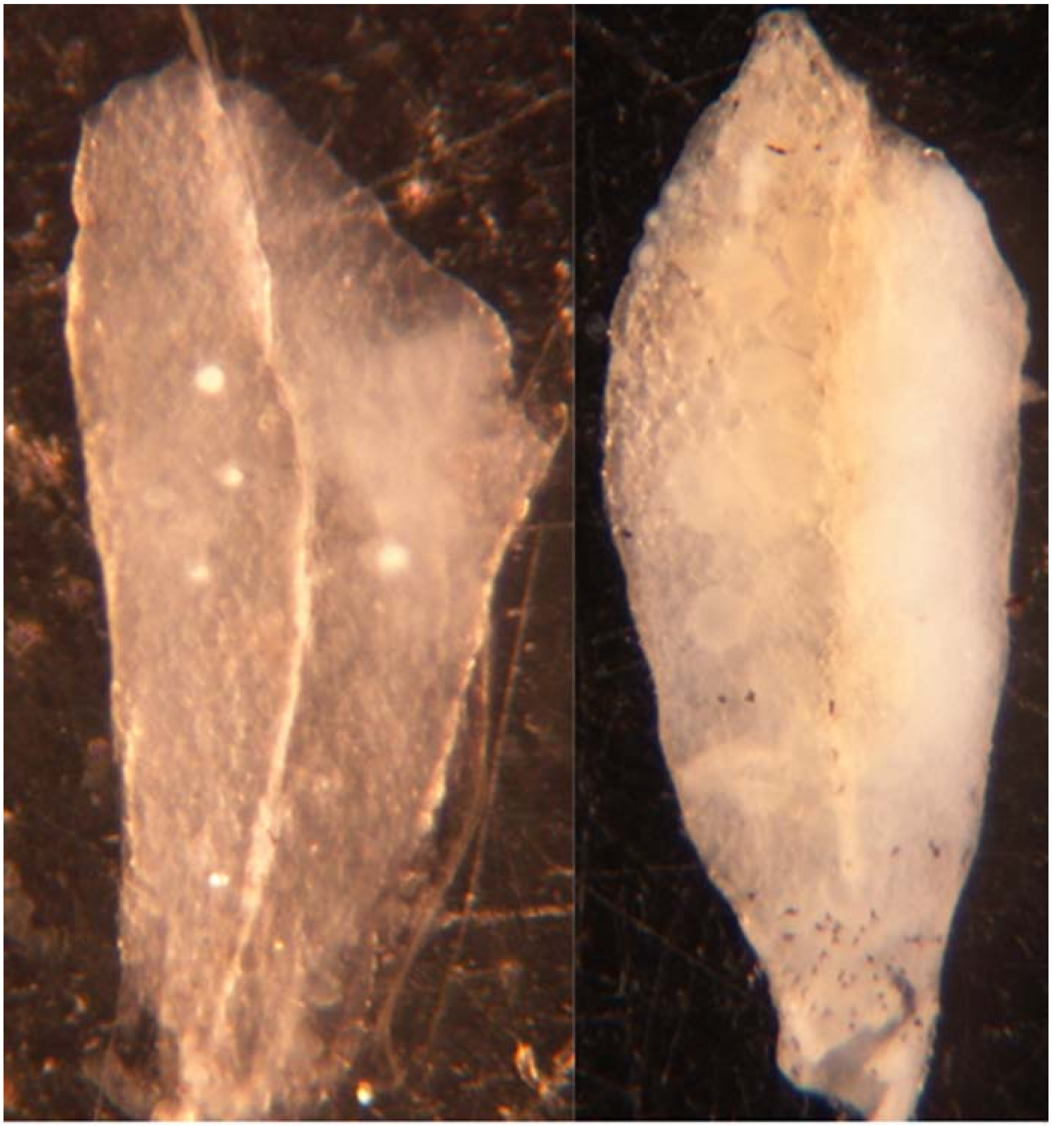
Appendix 1. Stages of ovarian and oocyte development in Fountain Darters taken from January through August 2014 among four sites on the San Marcos and Comal rivers.

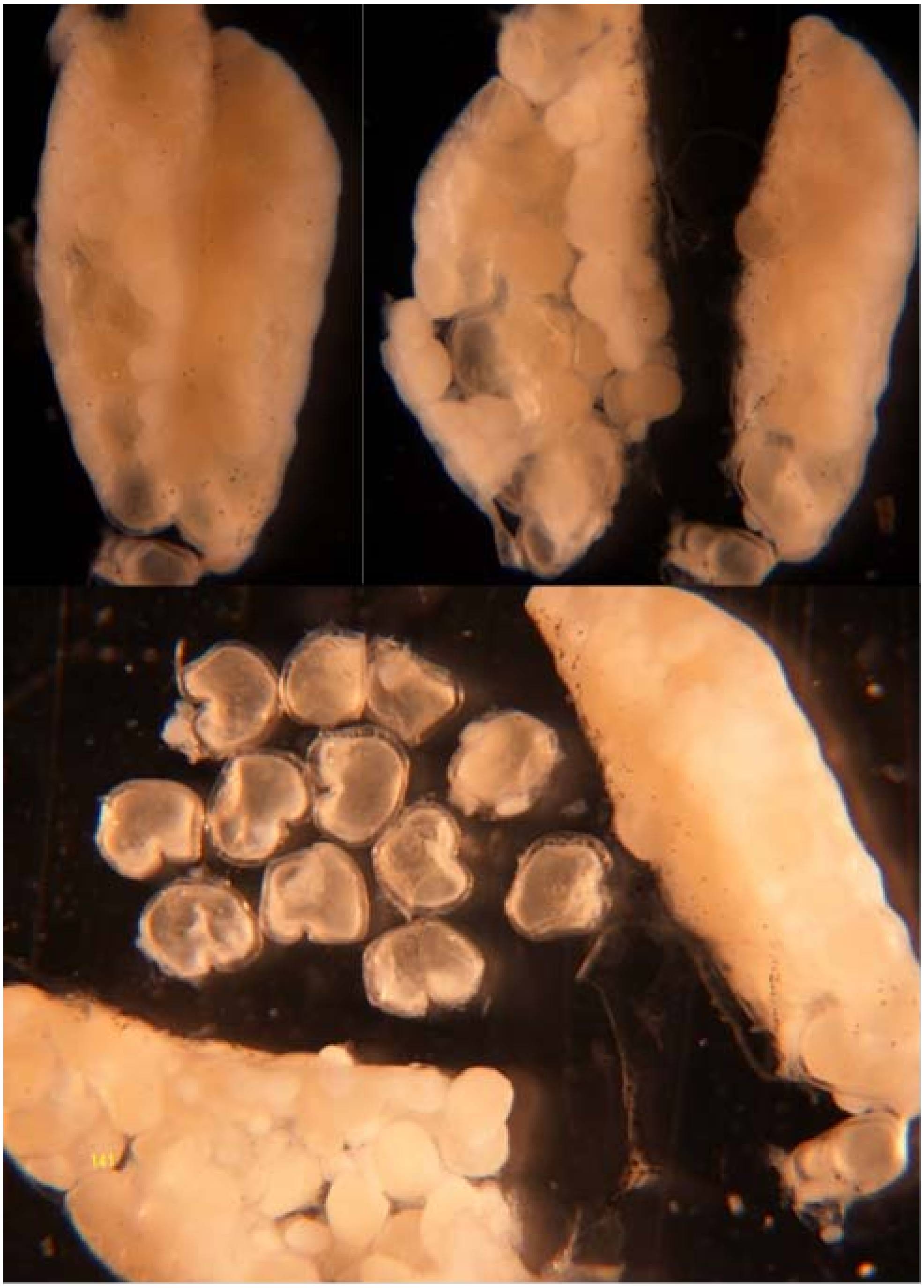


Pre-vitellogenic Ovaries; latent condition, semi translucent. Beginning stage of female Fountain Darter sexual maturity.



Early Vitellogenic Ovaries; beginning of vitellogenesis (yolk loading). Ovaries opaque, moderate amount of oocytes enlarged.

Late Vitellogenic Ovaries; towards end stage of vitellogenesis (yolk loading). Ovaries mostly opaque and enlarged. Oocytes noticeably engorged with yolk, chorion separation or thickening present but not overtly pronounced in non-ovulated clutch.



Spawning ovary; ovulated ovum present, ovaries enlarged. Ovum noticeably larger with yolk and chorion separation more distinct. Ovum with distinct infold on one side. Clutch size dependent upon relative time of capture from ovulation.

Ova; ovulated. Yolk appears as oil form; infolding present on one side. Chorion thickening greatly pronounced and viewed as translucent membrane surrounding the yolk (oil-like substance, opaque and off-white to yellow in color).

APPENDIX E

Fountain Darter Movement Under Low-low Conditions in the Comal Springs / River Ecosystem

FOUNTAIN DARTER MOVEMENT UNDER LOW-FLOW CONDITIONS IN THE COMAL SPRINGS / RIVER ECOSYSTEM

FINAL REPORT

October 30, 2014

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EXECUTIVE SUMMARY

A vital component of the Edwards Aquifer Habitat Conservation Plan (HCP) is the development of an ecological model to predict responses of the covered species to various flow regimes. Although development of the model is well underway, additional ecological data on the covered species is necessary to parameterize this model. This report describes studies conducted to examine fountain darter *Etheostoma fonticola* movement under deteriorating habitat conditions caused by low-flow scenarios.

Initial study plans included a field component examining movement of wild fountain darters in the Comal Springs / River ecosystem as well as a manipulative pond investigation in the event that low-flow conditions were not encountered in the wild. However, since extended low-flow conditions presented themselves in the Comal system during 2014 and complications were encountered in the experimental pond; all resources were diverted to the field study to maximize the information gained from the project.

Previous research conducted in the Old Channel of the Comal River has shown that fountain darters move little in quality habitat under a stable flow regime (Dammeyer et al. 2013). Specifically, fountain darters moved an average of 10 meters (m) over the course of the year, with a maximum movement of 95 m in 26 days. However, should habitat conditions begin to deteriorate; movement could potentially increase as fountain darters search for more suitable conditions. To investigate this, over 2,000 individual fountain darters were captured from the headwaters of the Comal River, injected with fluorescent visual implant elastomer (VIE) marks under their skin, and released during a low-flow period in spring and summer 2014. A variety of methods were used to relocate the tagged fountain darters and thus monitor movement and habitat utilization.

Over the course of the study, total system discharge at Comal Springs declined drastically, reaching levels that had not been experienced in over 20 years. During late August and early September, spring flow within the study area was essentially zero (<1 cfs), although some groundwater infiltration was noted in certain areas along the river bottom. Aquatic vegetation, which is the key fountain darter habitat component within the study reach, became covered in filamentous algae and eventually disappeared completely. Water temperatures, which typically fluctuate between 23°C and 26°C over the course of a year peaked at over 30°C, with two straight weeks over 26°C. Extremely low discharge conditions, coupled with extensive habitat decline, provided the study team with a very favorable situation to observe movement of wild fountain darters in a stressed environment.

A total of 149 fountain darters were relocated during the study. In general, despite the low-flow conditions observed, fountain darters were relatively sedentary, moving an average of 20.9 m (median = 17.9 m) from their release point over the course of the study. However, two fountain darters, which were tagged in Blieders Creek, made relatively long movements of approximately 130 m toward areas of increased spring influence in the Upper Spring Run. These represent the longest recorded movements ever documented for wild fountain darters. Despite these two relatively long movements from Blieders Creek to the Upper Spring Run, no fountain darters were documented moving downstream of the Upper Spring Run into the spring-influenced

habitat that was available near Spring Island. The distance to this habitat (>250 meters), along with observations made by divers suggesting that much of the wetted area between became comparatively warm and stagnant, may have presented a barrier to fountain darter movement.

Average distance moved (20.9 m) and maximum distance moved (131 m) in this study was slightly greater than that documented under stable habitat conditions by Dammeyer et al. (2013) (10 m and 95 m, respectively). This may suggest slightly increased movement as fountain darters searched for more suitable habitat. However, this may also be an artifact of a more expansive study area.

This study provided interesting insight into fountain darter movement, habitat selection, and potential population dynamics under low-flow, no-vegetation conditions. When aquatic vegetation disappeared in July and early August, and water temperatures increased, rather than moving, fountain darters adjusted their habitat utilization to that available within the local area. They were observed using interstitial spaces in gravel and cobble substrates as concealment, and were occasionally seen occupying open silt flats during this time period. These changes in habitat utilization could result in decreased prey availability and increased susceptibility to predation. As a result, an eventual decline in fountain darters would be anticipated should these conditions persist. It will be important to closely examine the HCP biological monitoring program data at the conclusion of this year's sampling to evaluate if a concurrent decline in fountain darter abundance occurred in the Upper Spring Run in late summer 2014.

HCP Ecological Model Parameterization

Results of this study show that even under extreme low-flow conditions, long-distance movement of fountain darters was rare. This has direct implications to ecological model parameterization. Currently, a decline in habitat within the ecological model results in a concomitant decline in the number of fountain darters occupying that habitat. At present, there is no movement factor incorporated. This study suggests that movement/emigration of fountain darters from disappearing vegetation/habitat is not likely to completely counteract a projected population decline, particularly if additional habitat is more than approximately 20 m away. At maximum, fountain darters were observed moving over 100 m. However, this is based on the maximum distance moved by only a few fountain darters. Perhaps a more appropriate cutoff to represent movement potential in the HCP ecological model would be the median distance moved during extreme low-flow conditions (17.9 meters).

In addition to providing input to the ecological model on movement potential under low-flow scenarios, this study also provided data on fountain darter population size within the study reach. These estimates may be useful in HCP ecological model calibration or validation within this reach. Finally, changes in habitat utilization that could result in decreased prey availability and increased susceptibility to predation should be considered during ecological model parameterization. Although the report puts forward parameters for consideration in model parameterization, it is emphasized that specific use of any of the 2014 applied research will be determined by the HCP ecosystem modeling team with guidance from the HCP Science Committee.

Recommendations for Future Applied Research

Should low-flow conditions continue or rebound in the first 6 months of 2015, it is recommended that follow-up relocation surveys in the Upper Spring Run reach be conducted. These surveys would test the following two hypotheses: (1) a complete loss of marked individuals would occur during extended drought, or (2) higher relocation percentages would accompany a rebound in total system discharge and subsequent anticipated habitat improvements in the spring. Additionally, determining population sizes in other study reaches in the Comal and San Marcos rivers would likely be beneficial in ecological model calibration or validation in those reaches.

Acknowledgments

The project team would like to acknowledge the U.S. Fish and Wildlife Service (USFWS) San Marcos Aquatic Resource Center (SMARC) scientists and staff. In particular, we thank Dr. Ken Ostrand and Dr. Tom Brandt for their guidance, assistance and cooperation. Dr. Ostrand was integral in all marking activities and his dedication to this project is very much appreciated.

1.0 INTRODUCTION

Section 6.3.4 of the Edwards Aquifer Habitat Conservation Plan (HCP) outlines applied research activities focusing heavily on the fountain darter *Etheostoma fonticola* and the Comal Springs riffle beetle *Heterelmis comalensis* (EARIP 2011). Additional ecological data on these species is needed to populate an ecological model, which is under development and serves as a crucial component to meet HCP goals and inform management decisions in coming years. To provide input to the ecological model, the initial round of applied research activities in 2013 focused on addressing several key questions regarding physical habitat and food source responses relative to the fountain darter under low-flow conditions. Specifically, applied research studies conducted in 2013 were aimed at determining the low-flow-induced abiotic conditions, which would result in impacts to aquatic vegetation (fountain darter habitat) and amphipod populations (fountain darter food source) (BIO-WEST 2013). Such habitat deterioration parameters can be incorporated into the ecological model, thus resulting in impacts to the fountain darter population as habitat conditions deteriorate. However, this assumes that degradation of habitat results in a similar degradation of the fountain darter population and does not account for the ability of fountain darters to move away from deteriorating habitat to more suitable areas. Therefore, to build upon the 2013 studies, a key question for 2014 applied research related to how fountain darter movement may be influenced by changes in habitat under low-flow-induced conditions. This report describes applied research conducted in 2014 relating to movement of fountain darters under such conditions.

One previous study, Dammeyer et al. (2013), has examined movement of wild fountain darters within the Old Channel of the Comal River. Their results show that fountain darters are not highly mobile, moving an average of 10 meters (m) within a year, and up to a maximum of 95 m. Fountain darters move among habitats more frequently than other darters, most often towards low-growing vegetation such as bryophytes or filamentous algae and most often in an upstream direction. However, the Dammeyer et al. (2013) study was conducted in the Old Channel of the Comal River, which typically exhibits a stable hydrograph and consistent habitat conditions. By contrast, the goal of this study was to further investigate movement relative to changes in habitat and temperature caused by low-flow regimes.

To accomplish this, a two-part study design was developed involving a field movement study in the Upper Spring Run reach of the Comal River as well as a manipulative pond study in an experimental pond at the U.S. Fish and Wildlife Service (USFWS) San Marcos Aquatic Resource Center (SMARC). Prior to initiation of the study, an extensive literature review was conducted relating to movement of freshwater fishes under varying hydrologic regimes, particularly fountain darters and other similar species. This literature review is summarized in Section 2. Section 3 provides information on the study design, describes the methods used, and presents challenges observed during implementation of these studies. Results are provided in Section 4, followed by conclusions and recommendations in Section 5. Finally, Section 6 lists the references cited throughout the document.

2.0 LITERATURE REVIEW

Movement of freshwater stream fish depends on an array of physical and environmental factors (Jackson et al. 2001). The restricted-movement paradigm predicts that small-bodied, resident fishes are relatively sedentary, moving <50 m under normal hydrological conditions (Gerking 1959, Gowan et al. 1994). *Etheostoma*, a very speciose genus composed of the smallest species of darters, conform to the predictions of the restricted-movement paradigm, being highly sedentary with 80 to 97% of individuals remaining within habitat patch of initial observation (Boschung and Nieland 1986, Labbe and Fausch 2000, Mundahl and Ingersoll 1983, Roberts and Angermeier 2007b). Among a few mobile individuals, mean distance moved is <200 m (Mundahl and Ingersoll 1983, Roberts and Angermeier 2007a). Movement among highly sedentary darters coincides with non-reproductive seasons (Mundahl and Ingersoll 1983, Scalet 1973), shifting habitat preferences as the darters grow (Labbe and Fausch 2000), and declining habitat quality (Mundahl and Ingersoll 1983, Roberts and Angermeier 2007b). Among swift-water darters, a 5% area loss of riffle habitats (i.e., shallow water habitats) because of summertime dewatering prompted fantail darters (*E. flabellare*) to move away from riffles (Roberts and Angermeier 2007b). Movement is also associated with density dependent mechanisms. Darter movement from patches has been shown to increase as resources became limited (Mundahl and Ingersoll 1983).

Fountain darters, like other darters, appear highly sedentary, moving on average 10 m within a year and up to 95 m within 26 days under a stable hydrograph (Dammeyer et al. 2013). When movement occurs, fountain darters move among habitats more frequently (51%) than other darters (3 to 20%; Mundahl and Ingersoll 1983; Labbe and Fausch 2000), most often towards low-growing vegetation, upstream, and during the winter and spring-summer seasons. Determining how and why fountain darters disperse throughout the Comal River system could be vital to the conservation of this species. Dammeyer et al. (2013) have offered insight into this fundamental question under a stable hydrograph; however, the goal of this study is to further investigate movement relative to changes in habitat and temperature caused by low-flow regimes. A wealth of information on aquatic vegetation preference by the fountain darter is available through long-term biological monitoring on both the Comal and San Marcos systems (BIO-WEST 2014a, BIO-WEST 2014b).

A mark-and-relocate study was conducted to determine how movement of fountain darters is affected by habitat and temperature changes under low-flow conditions. Fountain darter mark-and-relocate techniques utilized methods previously developed for darters and other small-bodied fishes, with visual implant elastomer (VIE) as the marking material. Although recapture success rate varies among movement studies (9–37%) (Belica and Rahel 2008, Dammeyer et al. 2013, Labbe and Fausch 2000, Roberts and Angermeier 2007b, Schaefer et al. 2003, Skyfield and Grossman 2008), VIE marking has been thoroughly tested (Belica and Rahel 2008, Holt et al. 2013, Labbe and Fausch 2000, Phillips and Fries 2009, Roberts and Angermeier 2004, Weston and Johnson 2008) and shows a high rate of retention (79–100%) accompanied with high survivorship (85–100%). Additionally, laboratory studies using darters (Phillips and Fries 2009, Roberts and Angermeier 2004) found VIE advantageous compared with other marking mediums, such as acrylic paint or photonic dye. Both visual (re-sight) and physical (dip net, recapture) methods were used for relocating fountain darters due to their habitat affinity (i.e., benthic fish

occupying areas of dense vegetation) (Alexander and Phillips 2012, Linam et al. 1993), characteristics of the study reach, and successes/suggestions of previous studies (Belica and Rahel 2008, Dammeyer et al. 2013, Holt et al. 2013, Jordan et al. 2008, Labbe and Fausch 2000, Skyfield and Grossman 2008).

3.0 DESIGN, METHODS, AND IMPLEMENTATION

3.1 Field Movement Study

3.1.1 Study Area

The Upper Spring Run reach of the Comal River near the Blieders Creek confluence (Figure 1) provided a well-suited area for the field movement study for two primary reasons. First, due to the elevation of springs in this reach, this area is the first to be impacted by springflow reductions as overall system discharge declines. Previous monitoring conducted in this reach as part of the HCP biological monitoring program has documented deterioration of habitat within this reach under low-flow conditions, as well as a corresponding decline in fountain darter abundance. Second, Blieders Creek merges with the river near the head of this reach. This intermittent creek is dependent upon local runoff and has a different temperature regime than the Upper Spring Run reach (Figure 2). Water temperatures in the middle and upper portions of Blieders Creek get much higher during the summer and much lower during the winter when compared to the spring-fed Comal River. Fountain darters are known to use the lower portions of the creek, although use of the area is expected to be seasonal, as water temperatures allow. Therefore, even if high-quality habitat conditions persisted in the Upper Spring Run reach over the course of the field movement study, habitat conditions in Blieders Creek were known to deteriorate each summer. This provided the study team an opportunity to observe movement of fountain darters in low springflow conditions. To keep track of water temperature conditions throughout the project area over the course of the study, three stationary water temperature monitors (HOBO tidbit V2) were placed at key locations and set to collect water temperature hourly (Figure 1).

3.1.2 Marking

Fountain darters were marked with fluorescent VIE tags using products and materials commercially available from Northwest Marine Technology, Inc. (www.nmt.us). Visual implant elastomer tags consist of a two-part silicone based material that is mixed immediately before use, injected under the skin as a liquid, and soon cures to a pliable solid. Visual implant elastomer marking has been thoroughly tested and has shown a high rate of retention (79–100%) accompanied with high survivorship (85–100%) on small fishes and darters, including the fountain darter (Belica and Rahel 2008, Holt et al. 2013, Labbe and Fausch 2000, Phillips and Fries 2009, Roberts and Angermeier 2004, Weston and Johnson 2008). Laboratory studies using darters found VIE advantageous compared with other marking mediums such as acrylic paint or photonic dye (Phillips and Fries 2009, Roberts and Angermeier 2004).

Figure 1. Map of Upper Spring Run and Blieders Creek study area.

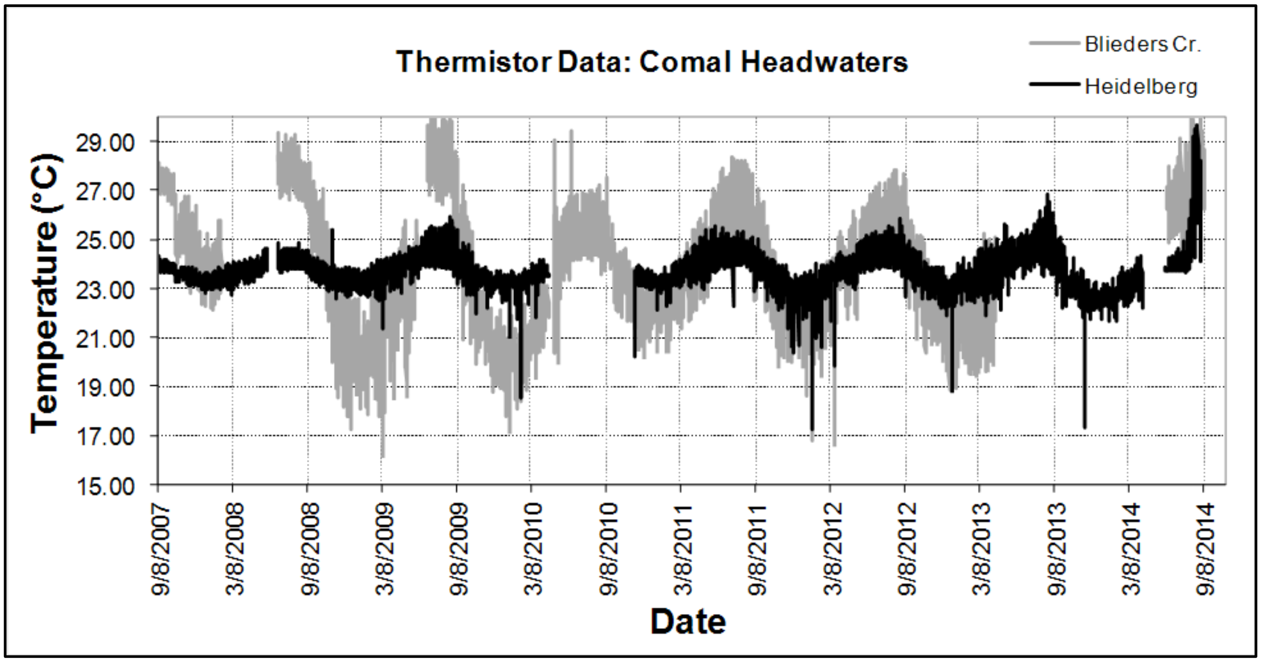

Figure 2. **Water temperature data from Blieders Creek and the Upper Spring Run reach (Heidelberg).**

To test mark retention and survivorship and provide marking practice prior to initiation of the field study, 35 adult fountain darters were marked at the SMARC on March 19, 2014. These fish were “extra” F1 hatchery stock scheduled to be euthanized if not used for another purpose. They were captured from their tank with a small aquarium net, injected with fluorescent blue VIE marks approximately 2–3 millimeters (mm) in length along the left side of the dorsal fin (Figure 3), immediately placed back into their tank of origin, and monitored for several weeks after tagging. These fish exhibited 100% survivorship and tag retention 1 month after marking, and were kept alive to be used later in the manipulative pond experiment.

From March 24 to August 7, 2,212 individual fountain darters were marked within four study locations (Table 1). Fountain darters were captured from two Upper Spring Run and two Blieders Creek (BC) sampling areas using dip nets and cohort marked according to area of initial capture (Figure 4). Darters from the upstream Upper Spring Run site (USR1) were marked with yellow fluorescent VIE on the right side of and adjacent to their dorsal fin, while fountain darters from the downstream Upper Spring Run site were marked with yellow fluorescent VIE on the left side of their dorsal fin. Upstream BC (BC2) were marked with red VIE on their left side, and downstream BC (BC1) fountain darters were marked with red on their right side (Figure 5).

Captured fountain darters were held in floating containers in the river that allowed water exchange and had shade covers until marking (Figure 6). During marking, fountain darters were removed from the floating container using an aquarium net, injected with a VIE mark, and quickly placed back into a separate container containing fresh river water. Insulated and aerated bait buckets were used as post-marking containers to reduce stress and mortality on hot days (Figure 7). To reduce handling stress, fountain darters were not individually measured. However, fountain darters less than approximately 20 mm total length were released without marking.

Figure 3. Visual implant elastomer -marked fountain darters from the initial trial run at the SMARC on March 19, 2014.

Table 1. Marking dates, marks used, and number of darters marked in each sampling area.

Location	Marking Dates	Mark	Total Number
USR1	Mar. 24, Apr. 18	Yellow / Right Dorsal	185
USR2	Mar. 24, Apr. 18, May 30, Aug. 7	Yellow / Left Dorsal	1,810
BC1	Mar. 25, Apr. 18	Red / Right Dorsal	154
BC2	Mar. 28, Apr. 18	Red / Left Dorsal	63
Total			2,212

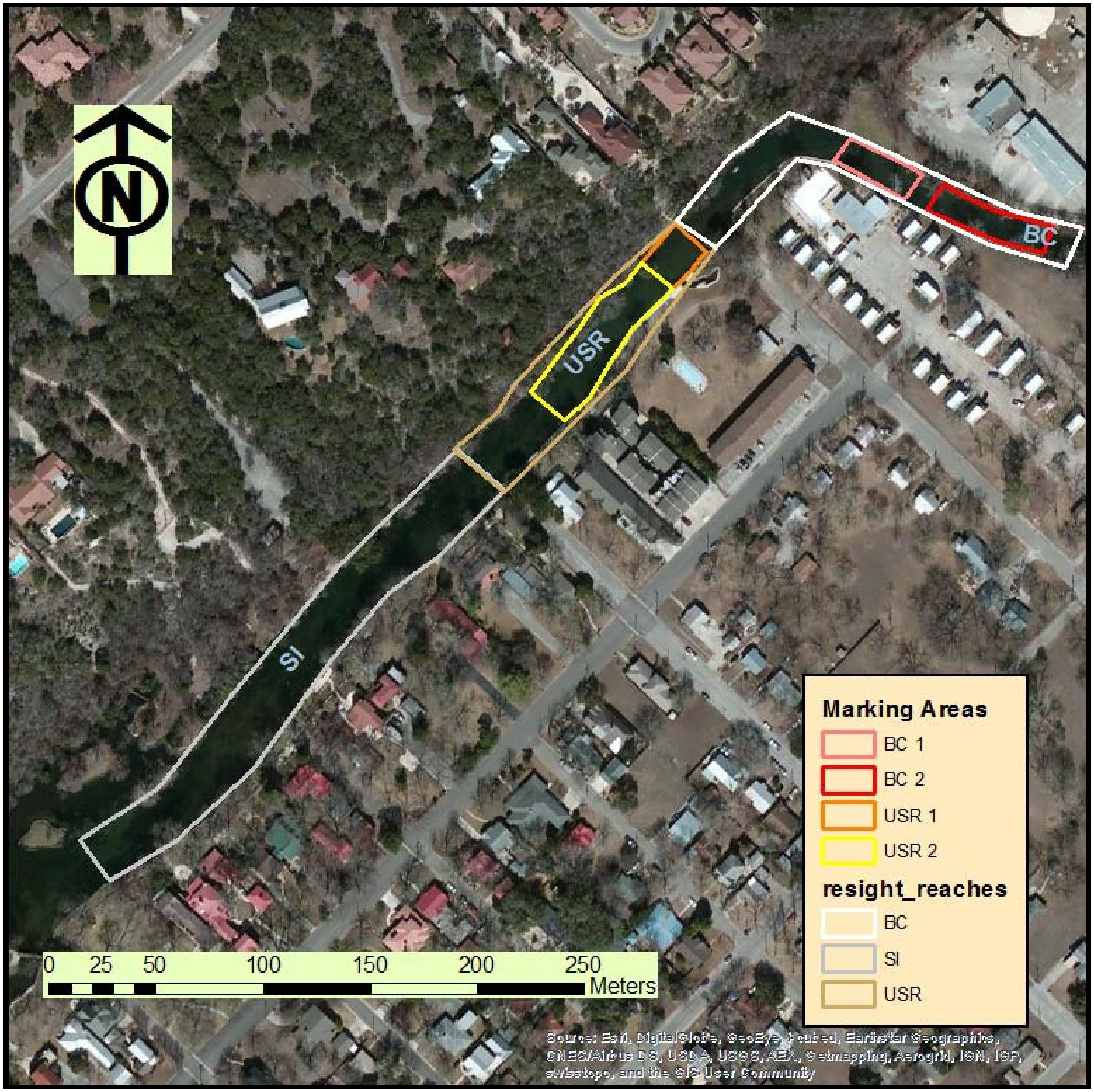

Figure 4. Areas where fountain darters were collected and marked (Marking Areas) and areas where visual searches were conducted (resight_reaches).

Figure 5. Examples of visual implant elastomer (VIE) marked fountain darters.

Figure 6. **Floating container constructed to retain darters in river water during marking events.**

Figure 7. BIO-WEST and USFWS personnel marking fountain darters at USR1.

After marking, fountain darters were held briefly to observe for mortality, they were released at designated release points within each marking area. Observed mortality rates prior to release averaged 2.5% (range: 0–14%) and consisted mainly of fountain darters in the smaller size classes. The number of fountain darters marked during an event was determined either by the maximal number that could be captured (Blieders Creek) or the number that could be marked by the end of day (Upper Spring Run).

Fountain darters that were recaptured from previous marking events during a new marking event were held in the aforementioned containers *in situ* for the duration of the event to prevent re-counting. For these relocations, the same data were recorded as during relocation surveys described below.

3.1.3 Relocating

Relocating marked fountain darters was conducted using three separate methods: recapturing them with dip nets (Figure 8), daytime SCUBA/snorkel visual surveys, and nighttime SCUBA/snorkel visual surveys with the aid of specially designed ultra-violet underwater flashlights (VI light; Northwest Marine Technology, Inc.) (Figure 9). The VI lights radiate a deep purple light that causes the tags to fluoresce, increasing visibility substantially when used in the dark or shade. Initially (April–June), daytime dip net and visual surveys were used to collect

Figure 8. **Dip netting for marked fountain darters in the Upper Spring Run reach.**

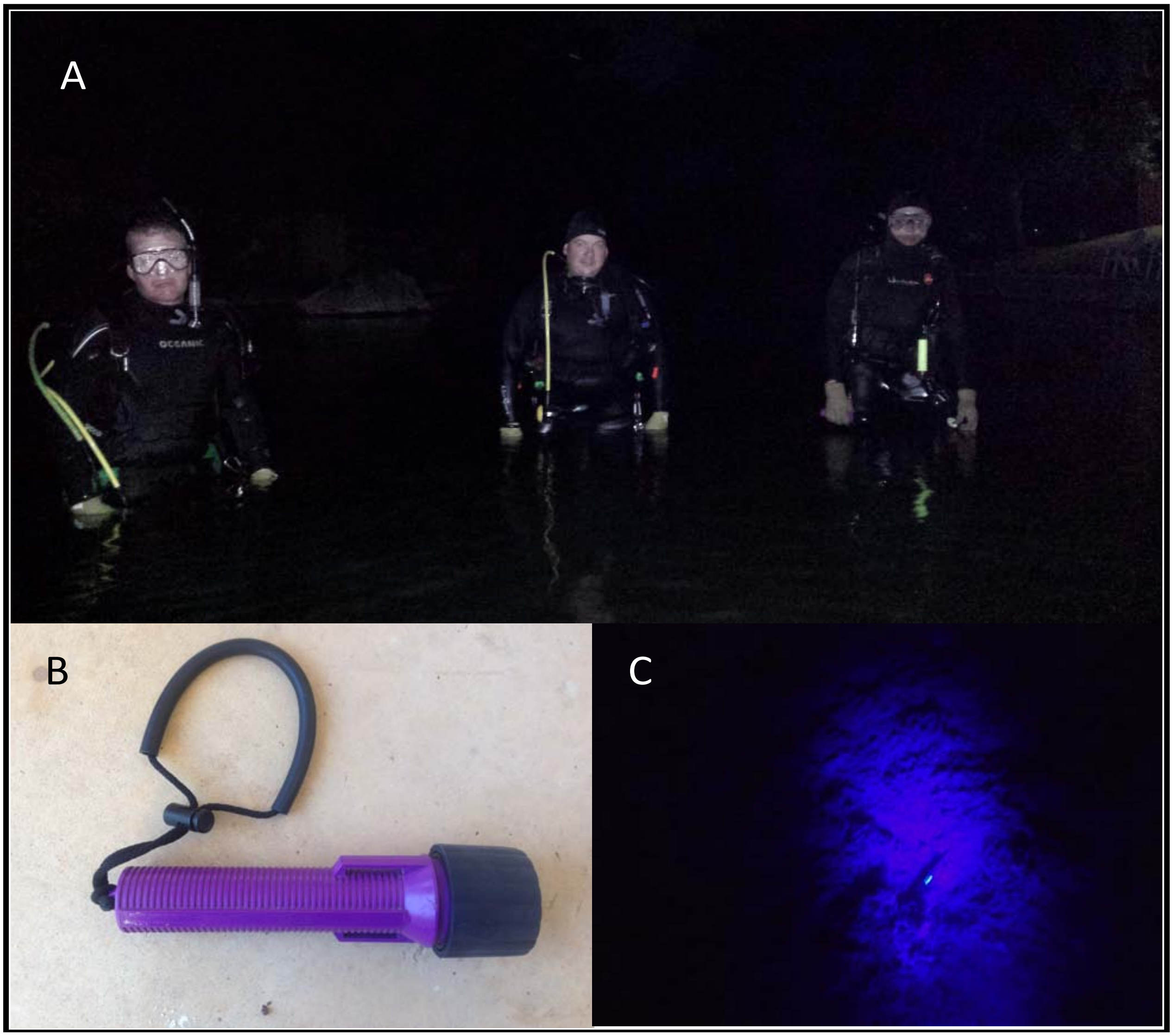

Figure 9. **BIO-WEST divers preparing for night relocation surveys (A) using VI lights (B) to relocate fountain darters with fluorescent visual implant elastomer (VIE) tags (C).**

most relocation data. However, preliminary data from relocation events in the Upper Spring Run using each technique showed that catch-per-unit-effort (CPUE) was highest using night visual surveys due to the substantial increase in visibility of the fluorescent tags (Table 2). Therefore, night SCUBA/snorkel visual surveys were used to relocate darters for the remainder of the study period (July–September).

Night visual surveys were conducted using either SCUBA or snorkel equipment according to the reach being surveyed and water level conditions at the time of the event. The study area was split into three different survey areas delineated by feasible access points (Figure 4). Each survey area could be covered thoroughly in a single night dive which typically lasted approximately 2–3 hours. Two to five observers swam through the chosen survey area parallel to one another in an upstream direction using VI lights to scan the substrate for fluorescent tags. Large rocks, aquatic

Table 2. Catch-per-unit-effort (CPUE) from initial relocation surveys using three different methods.

Date	Relocation Method	Total Person-Hours Effort	Number of Relocations	CPUE (darters/person-hr)
5/30/2014	Day Dipnet	25	4	0.16
6/24/2014	Day SCUBA/snorkel	10	4	0.40
7/1/2014	Night SCUBA/snorkel	9.75	18	1.85

vegetation, and algal mats were often moved to search for fountain darters hiding underneath or around these structures. In addition to VI lights, which provide little illumination, standard dive lights were also carried by each diver to help orient themselves in the dark underwater environment. Waterproof tank lights were strapped to each diver's back so that divers could keep track of each other's positions. An attempt was made by the divers to move through the study area at approximately the same rate, thus reducing potential overlap and ensuring that unique portions of the survey area were observed by each diver. An additional biologist accompanied divers in a kayak to record data and assist. Each time a marked fountain darter was relocated, a GPS waypoint was collected using a Garmin eTrex 30 handheld GPS. Additionally, time, mark description (color, location on fish), and notes on habitat (vegetation and/or substrate) were also recorded. During visual surveys, the number of unmarked fountain darters observed by each diver was also recorded. Standard water quality parameters (temperature, pH, conductivity, and dissolved oxygen [DO]) were recorded at the onset of each marking event using a HydroTech water-quality sonde. The number of observers and the time spent searching was recorded for each relocation event and CPUE was calculated as darters/person-hour.

3.1.4 Habitat Analysis

A variety of statistical analyses were used to explore relationships between fountain darter movement data and various habitat and discharge variables. Due to low sample size in other populations, only data from fountain darters marked in the USR1 and USR2 were subjected to statistical analysis. Analysis of Variance (ANOVA) was used to investigate association of recaptures (scaled by total number marked at the time of recapture) with habitat type. Linear regression was used to investigate relationships between the same response variable and weekly average temperature (from HOBO logger), DO (empirical from sampling event), and system discharge (based on U.S. Geological Survey [USGS] gauge #08169000). System discharge and weekly average temperature values were used in analyses as they were found to have significant ($p<0.001$), near-perfect (>0.90) Pearson's correlation with empirical data, but were more complete. For the same set of observations, the estimated distance of each relocation from the release point was calculated using ArcMap 10.2.2 (ESRI, Redlands, CA). These data were analyzed using linear regression to incorporate the variables study days (days since beginning of study), weekly average temperature, and discharge. Data in all statistical analyses were visually examined for departures from model assumptions in R version 3.0.3 (R Development Core Team 2008) using residual and quantile plots.

3.2 Manipulative Pond Study

For the manipulative pond study, it was proposed to use an experimental pond at the USFWS San Marcos Aquatic Resource Center (SMARC) to conduct a series of experiments investigating

movement of fountain darters. The initial experiments were designed to investigate the use of vegetated vs. non-vegetated habitat patches by fountain darters. Follow-up experiments would then examine movement of fountain darters once vegetated habitat patches were removed and pond levels were altered. One hundred thirteen experimental fountain darters were given fluorescent VIE (Northwest Marine Technology, Shaw Island, WA) marks adjacent to the dorsal fin to enhance observation during experiments and housed in a holding tank at SMARC.

Vegetated patches consisted of specially designed Mobile Underwater Plant Propagation Trays (MUPPTS) planted with *Ludwigia repens* (Figure 10). Vegetation was established in the pond in mid-April, approximately one month prior to the planned beginning of experiments. This was done to allow for colonization of the vegetated patches by invertebrates providing a food source for the experimental fountain darters representative of a natural system. To provide an initial population of invertebrates, amphipods were stocked from a nearby SMARC raceway on several occasions in April and May. Vegetated patches were arranged interspersed with equally-sized non-vegetated patches. The MUPPTS and pots as well as non-vegetated areas were covered with pea gravel to prevent fountain darters from using the structure of the MUPPTS as habitat (Figure 11). A system of dam boards was constructed at the pond drain to allow manipulation of water levels to facilitate experiments involving manipulation of the draw down rate of the pond and its effect on movement of the fountain darters among habitats. The inflow plumbing to the pond was modified to allow for manipulation of inflow rates. Finally, two HOBO tidbit V2 temperature loggers were placed in the pond to record water temperature approximately 2 inches above the pond bottom spaced equally from the shallow (inflow) end to deep (outflow) end of the pond.

In late May, as the experimental pond setup neared completion, temperature loggers documented large diel swings in water temperature with afternoon water temperatures exceeding 29°C. With hot summer conditions expected in the coming months, it was determined that water temperatures similar to the natural environment of the fountain darter could not be maintained at the current inflow rate and pond level. Therefore, extensive experimentation was conducted to examine the effect of various flow rates and pond water levels on water temperature within the pond. Over a series of preliminary experiments in May and June, pond inflow rates were adjusted from approximately 9 gallons/minute (gpm) to over 80 gpm, and pond levels were lowered by removing dam boards to reduce overall retention time in the pond. After each successive change in flow rate and/or water level, temperature dataloggers were used to monitor water temperatures in the pond. Even at the lowest possible water level amenable to experiments and the highest flow rate tested, water temperatures in the pond during mid-June pushed or exceeded 30°C with diel swings of 8–10 degrees. Therefore, in an attempt to further reduce retention time in the pond, a dividing wall was built with sandbags on June 25 (Figure 12). This wall cut the surface area of the pond approximately in half. However, even under this reduced surface area and a high flow rate, water temperatures in the pond still exhibited a large diel swing with maximum temperatures exceeding 30°C (Table 3). Therefore, in early July the decision was made to abandon the pond study and focus efforts on the field movement study. This decision was aided by the fact that discharge conditions in the Upper Spring Run reach and for the Comal System as a whole were approaching levels not observed in over 20 years. As such, the natural system was providing the perfect laboratory and efforts were thus expanded in the field to take advantage of these rarely seen conditions.

Figure 10. **MUPPTS being planted with *Ludwigia repens* in an experimental pond at the SMARC.**

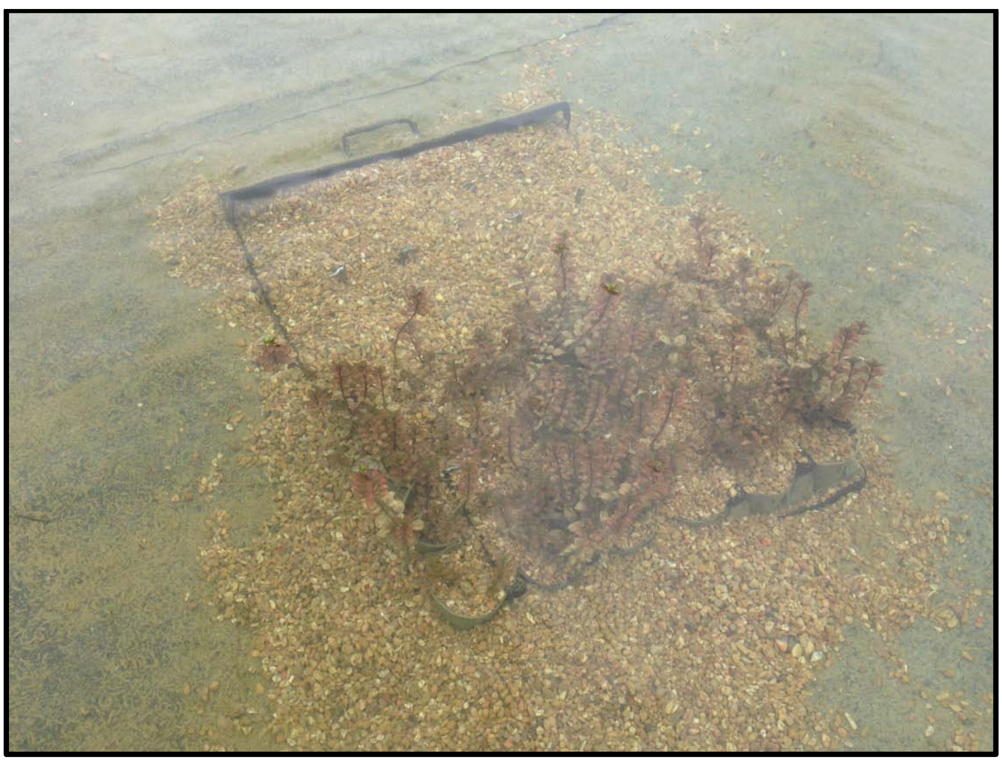

Figure 11. **Pea gravel being applied to mask MUPPT structure and homogenize available habitat patches for experimental trials.**

Figure 12. **Sandbag wall being constructed to reduce the area of the experimental pond, thereby increasing water exchange rates to facilitate improved water temperature control.**

Table 3. **Summary statistics of water temperature recorded from two different locations in the experimental pond during various temperature manipulation trials.**

Date	Shallow End Water Temperature (°C)			Deep End Water Temperature (°C)		
	Max	**Min**	**Avg.**	**Max**	**Min**	**Avg.**
May 09 – May 23	29.27	20.24	24.46	28.94	20.22	24.46
May 23 – May 30	29.27	20.25	24.46	28.94	20.22	24.46
May 30 – Jun 09	30.98	23.57	26.43	31.18	23.62	26.51
June 09 – June 13	31.89	22.94	26.74	32.30	22.92	26.66
June 13 – June 16	31.43	23.71	26.53	31.08	23.67	26.23
June 16 – June 19	29.67	23.30	25.55	29.46	23.30	25.50
June 19 – June 25	32.67	22.68	26.57	32.87	22.30	26.47
June 25 – June 30	31.38	20.08	25.60	31.48	20.08	25.25
June 30 – July 03	28.94	23.11	25.00	30.17	23.06	25.41

4.0 Results

4.1 Field Movement Study

4.1.1 Habitat Conditions Observed

Extreme low-flow conditions occurred at Comal Springs over the course of the study period (Figure 13). In fact, daily mean discharge dipped as low as 65 cubic feet per second (cfs) at the end of August. This represented the lowest daily mean discharge observed in over 20 years. Additionally, total system discharge remained below 100 cfs for 43 straight days from early August through mid-September. These conditions resulted in cessation of spring flow from many spring areas in the Upper Spring Run. As a result, Upper Spring Run discharge approached zero (<1 cfs) during late-August. During this time, water temperatures within the Upper Spring Run and Blieders Creek climbed considerably with daily average temperature approaching 30°C (Figure 14). However, even under these conditions, some spring flow/groundwater influence was still evident along the bottom in certain areas.

Aquatic vegetation represents the main fountain darter habitat component within the Upper Spring Run study reach and consists mainly of bryophytes with occasional summer blooms of filamentous algae. Blieders Creek typically contains large mats of muskgrass (*Chara* sp.), along with *Hygrophila polysperma*, filamentous algae, and *Nuphar* sp. Although coverage of aquatic vegetation within Blieders Creek remained relatively stable, coverage of aquatic vegetation within the Upper Spring Run reach varied considerably over the course of the year due to low springflow conditions. Data collected by BIO-WEST as part of the ongoing HCP biological monitoring shows that in early April, bryophytes covered approximately 34% of the Upper Spring Run study area (Figure 15). This had dropped to less than 1% coverage of bryophytes by August (Figure 16).

Low-flows and resulting deterioration of aquatic vegetation and water quality conditions in the study area provided the study team with a favorable situation for observing fountain darter movement patterns under extreme low-flow and unstable habitat conditions. Data on the number of fountain darters relocated, and their movement patterns, are described in the following sections.

4.1.2 Relocation Efficiency

In total, 149 fountain darters were relocated over the course of the study. The majority of these (136) were located during 22 separate relocation events between April 18 and September 10, 2014 (Table 4). Thirteen additional fountain darters were relocated incidentally during sampling for other applied research studies or during HCP biological monitoring activities during the study period. Given the total number of fountain darters marked (2,212), if each relocation is considered an independent observation, this results in an overall relocation rate of 6.7%. Although slightly lower, this is comparable to the recapture percentage (8.7%) observed in the previous fountain darter marking study conducted in the Old Channel Reach of the Comal River (Dammeyer et al. 2013). It is not surprising that the relocation rate was slightly lower in this study, given that the Upper Spring Run is a much more expansive aquatic environment than the Old Channel.

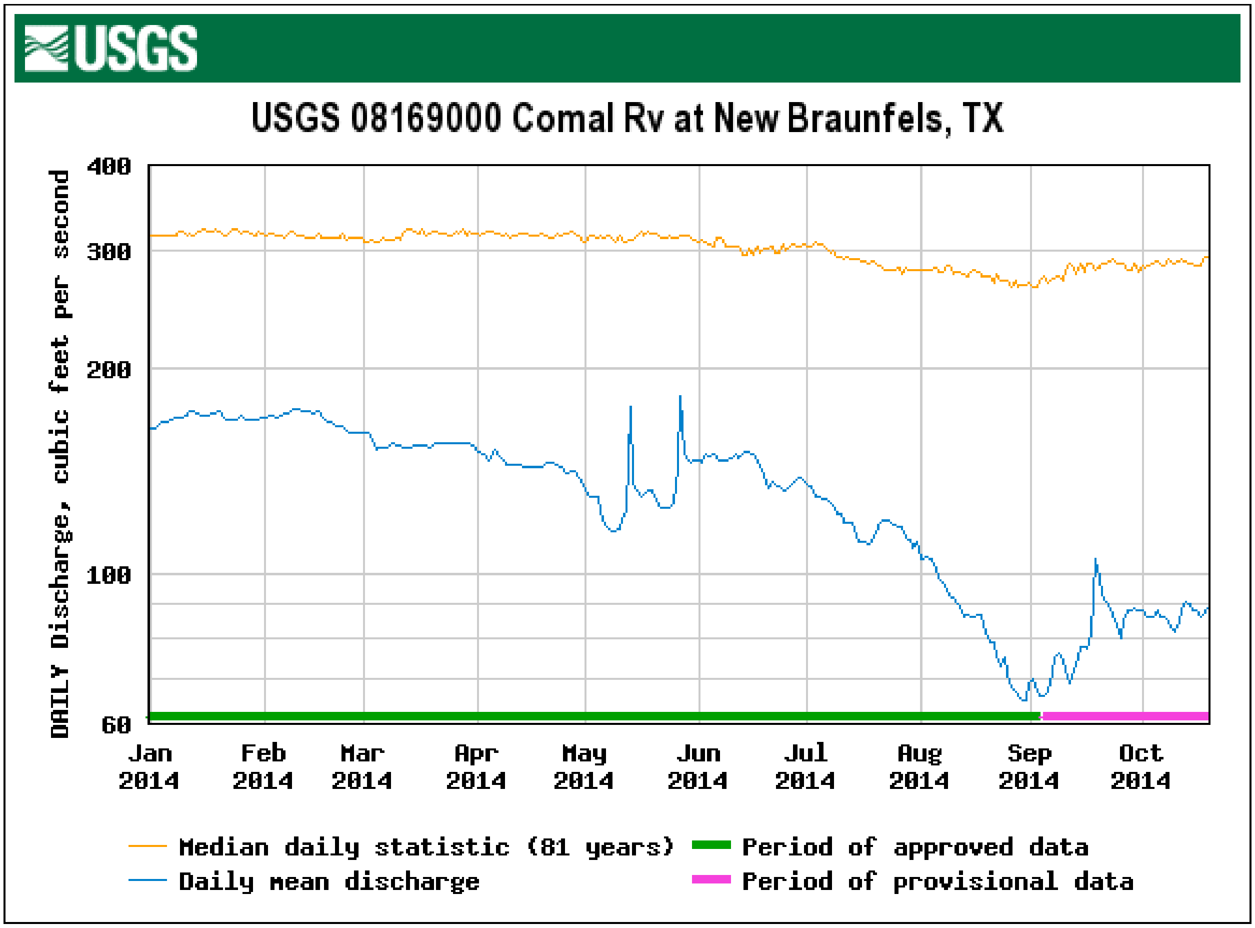

Figure 13. **Mean daily discharge (cfs) from the USGS gauge (#08169000) on the Comal River at New Braunfels, Texas from January 1–October 19, 2014.**

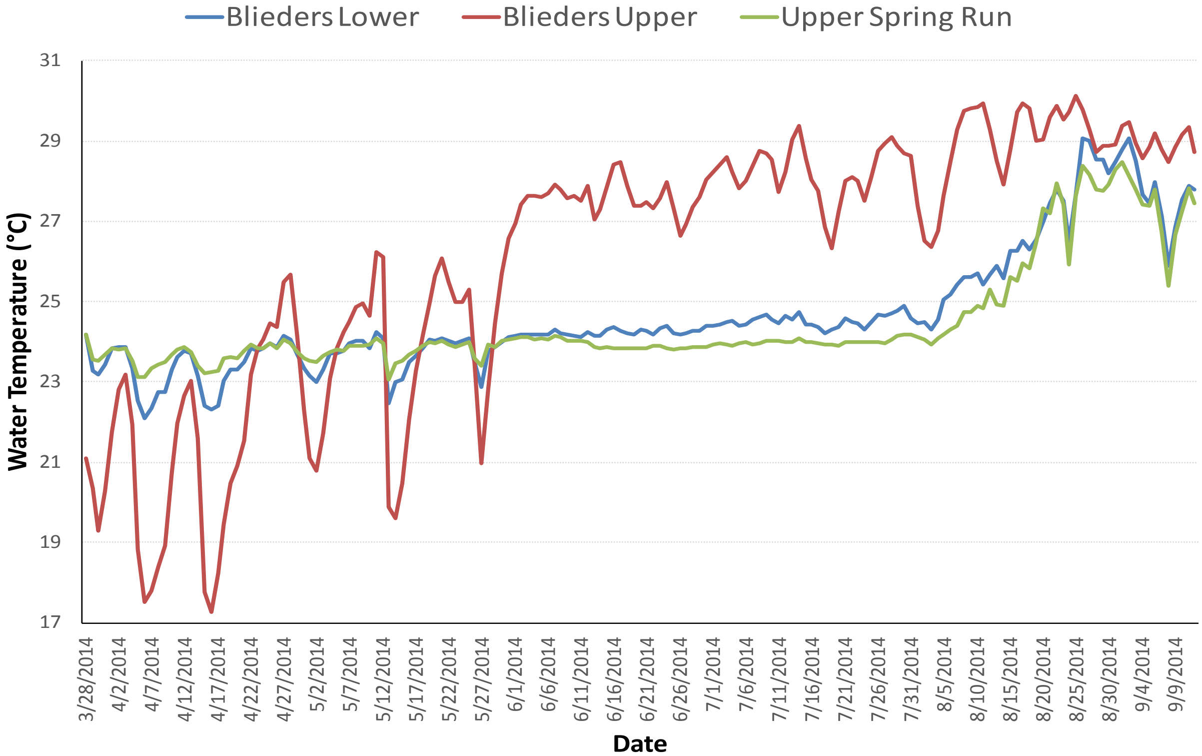

Figure 14. Daily average water temperature taken from three locations within the study area.

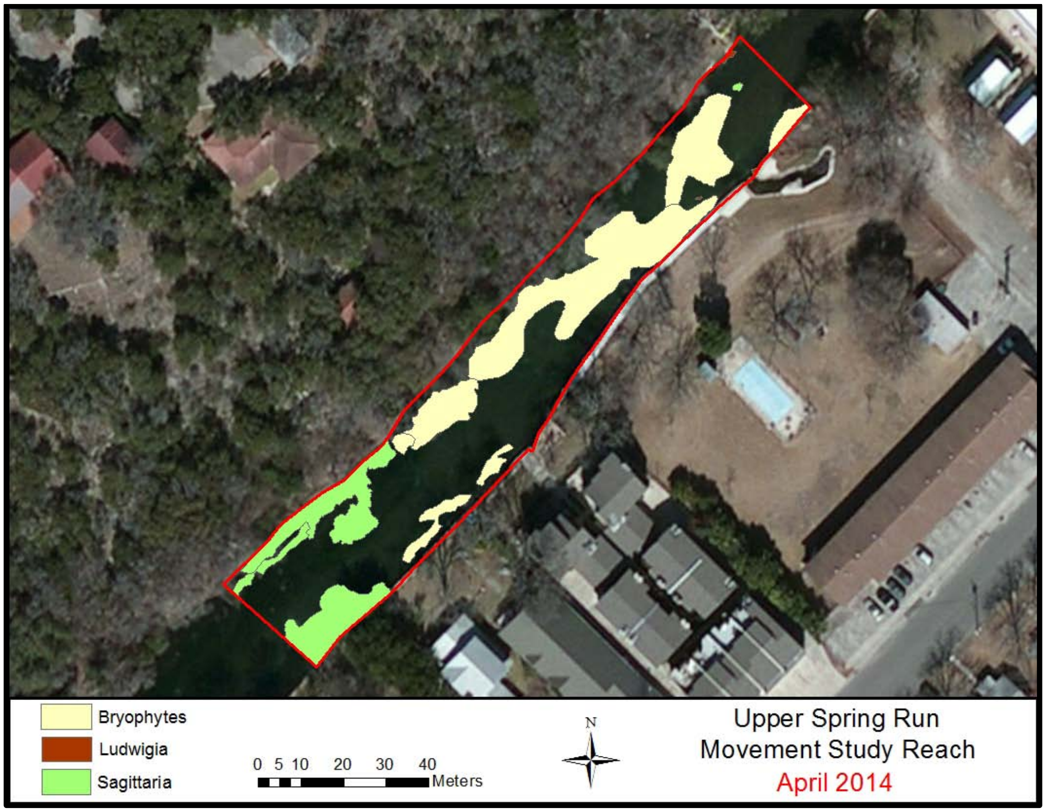

Figure 15. **Aquatic vegetation present in the Upper Spring Run reach during April 2014.**

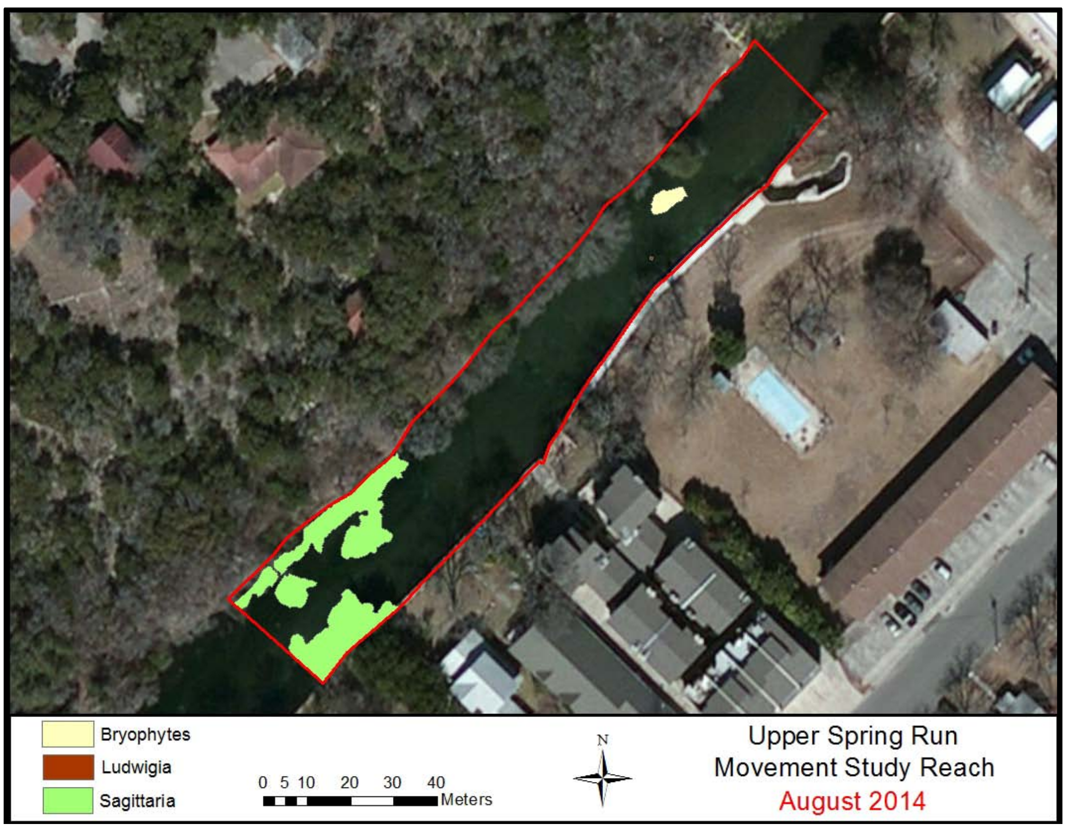

Figure 16. **Aquatic vegetation present in the Upper Spring Run reach during August 2014.**

Table 4. Date and details of fountain darter relocation events conducted from April through September 2014.

Date	Survey Area	Method	Number of Observers	Time Spent Searching (Hours)	Effort (Person-Hours)	Number of Relocations	Total Number Marked on Date of Survey	CPUE (Darters/person-hr)
4/18/2014	BC	Day Dipnet	4	1.00	4.00	2	386	0.50
4/18/2014	USR	Day Dipnet	4	3.00	12.00	2	386	0.17
4/24/2014	USR	Day Dipnet	4	1.25	5.00	4	757	0.80
5/16/2014	USR	Day Dipnet	4	4.50	18.00	8	757	0.44
5/30/2014	USR	Day Dipnet	5	5.00	25.00	4	757	0.16
6/23/2014	SI	Day Visual	4	2.00	8.00	0	1,348	0.00
6/24/2014	USR	Day Visual	4	2.50	10.00	4	1,348	0.40
6/26/2014	BC	Day Visual	3	2.00	6.00	0	1,348	0.00
7/1/2014	USR	Night Visual	3	3.25	9.75	18	1,348	1.85
7/9/2014	SI	Night Visual	3	2.75	8.25	0	1,348	0.00
7/10/2014	BC	Night Visual	3	2.50	7.50	1	1,348	0.13
7/23/2014	BC	Night Visual	3	2.25	6.75	0	1,348	0.00
7/24/2014	USR	Night Visual	3	2.25	6.75	17	1,348	2.52
7/29/2014	SI	Night Visual	4	1.50	6.00	0	1,348	0.00
7/30/2014	USR	Night Visual	3	1.75	5.25	9	1,348	1.71
8/12/2014	SI	Night Visual	4	1.50	6.00	0	2,212	0.00
8/13/2014	USR	Night Visual	4	1.75	7.00	42	2,212	6.00
8/26/2014	USR	Night Visual	4	2.00	8.00	17	2,212	2.13
8/28/2014	SI	Night Visual	4	1.75	7.00	0	2,212	0.00
9/9/2014	USR	Night Visual	4	1.50	6.00	8	2,212	1.33
9/10/2014	BC	Night Visual	2	1.50	3.00	0	2,212	0.00
9/10/2014	SI	Night Visual	3	1.75	5.25	0	2,212	0.00
Total				**49.25**	**180.50**	**136**	**2,212**	
Average CPUE								**0.82**

Also, it should be noted that this 6.7% estimate is slightly misleading since fountain darters were marked repeatedly through the course of the study, and many relocations took place with fewer total fountain darters marked. Regardless, low relocation rates are to be expected given the small size of the fountain darter (maximum length <2 inches) and its preference for complex benthic habitats.

Overall average CPUE was 0.8 fountain darters per person-hour (range: 0.0–6.0) and was highly dependent on the area surveyed, the total number of fountain darters marked, and the relocation technique used (Table 4). If only data from night visual surveys is analyzed since it was the most effective relocation technique, then CPUE within the Upper Spring Run survey area averaged 2.6 fountain darters/person-hour (range: 1.3–6.0). Data from night visual surveys show an average CPUE of 0.04 and 0.00 in the Blieders Creek and Spring Island survey areas, respectively. Although no fountain darters were marked in the Spring Island survey area, it was repeatedly surveyed to document any potential emigration from the Upper Spring Run Survey area as discharge declined. Two-hundred and seventeen fountain darters were marked within Blieders Creek (both sample areas combined). However, dense macrophyte beds within the creek made visual relocation of fountain darters difficult in this area, perhaps leading to the reduced CPUE.

4.1.3 Movement Patterns

In general, relocation data showed that fountain darters moved little from their initial area of capture (Figure 17). In fact, 84% of darter relocations were within the initial area of capture. The overall average distance moved was 20.9 m (median = 17.9 m) and ranged from a minimum of less than one meter to over 130 m (Table 5). This 130 m movement was a fountain darter that was tagged in BC1 during March or April and moved to the middle of USR2 by August 7. This exceeds the maximum movement found by Dammeyer et al. (2013) and, therefore, represents the longest movement ever recorded for a wild fountain darter. Additionally, one other fountain darter marked in Blieders Creek moved over 128 m. This red-marked darter was spotted in the upper portion of USR2 on April 22, 2014, during fish community sampling as part of the HCP biological monitoring. However, it was not determined whether this fish was tagged on the left or right side. Therefore, it was assumed this fish moved from BC1, which would be the closest site in Blieders Creek.

If the two long movements out of Blieders Creek are removed from analysis, then average distance moved at a given marking location varied between 14.0 and 21.0 m, with maximum movements ranging from 18.8 to 63.3 m. This represents slightly higher average movement than that reported by Dammeyer et al. (2013), who reported an average movement of 10 m in the Old Channel. Larger average movement in the Upper Spring Run during low-flow periods may represent fountain darters moving more in search of better physical habitat and/or feeding opportunities. Fountain darters in stable habitats within the Old Channel may have to move less to obtain the necessary resources. However, more movement within the Upper Spring Run may just be an artifact of a less confined study area.

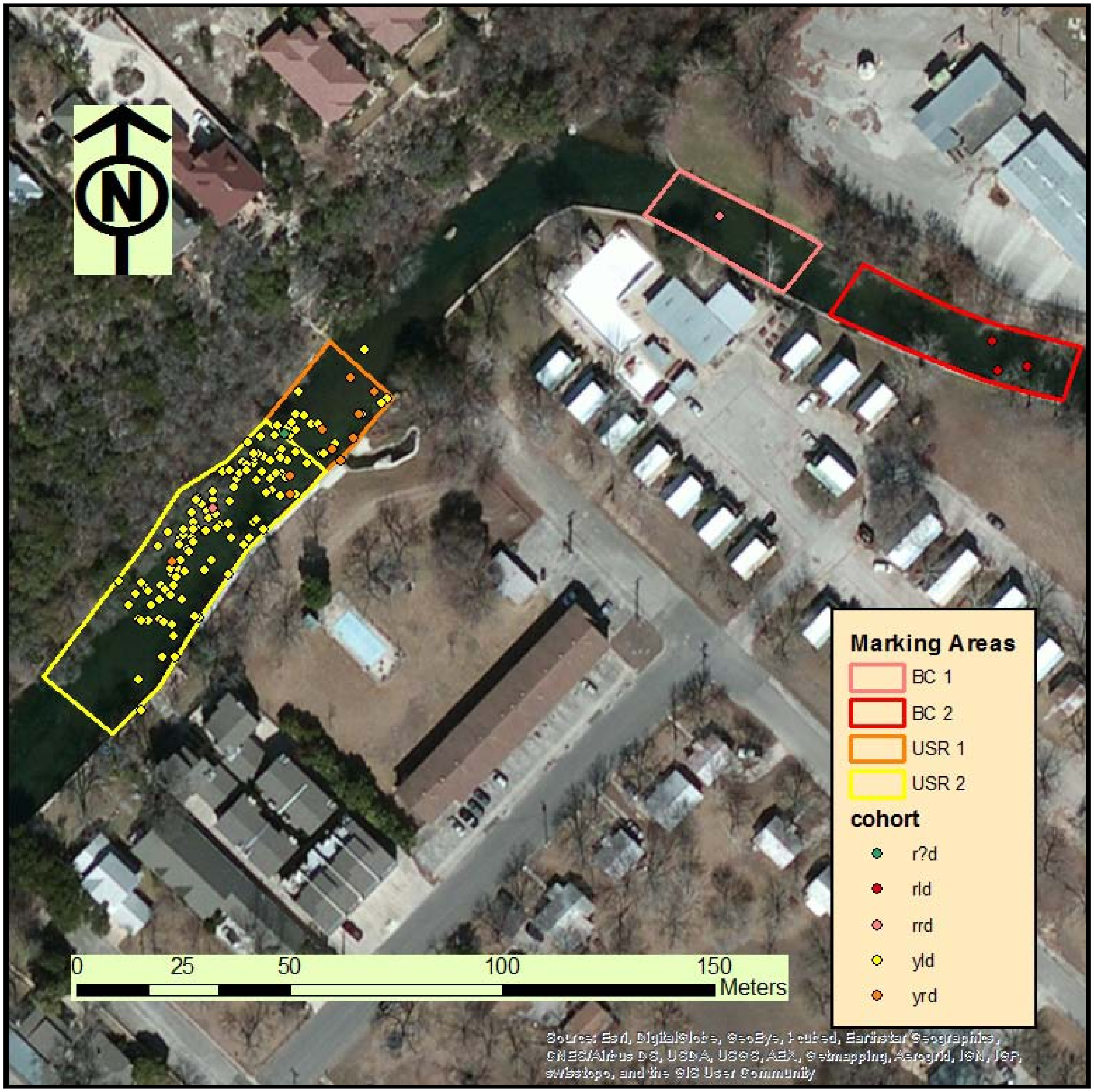

Figure 17. **Fountain darters relocated over the course of the study period. Relocation points are represented by the same color as the area in which fountain darters were originally marked.**

Table 5. Number of relocations, number of emigrations, and summary statistics for distance moved for each marking area.

Marking Area	Number of Relocations	Number of Emigrations	Distance Moved (meters)		
			Avg	Max	Min
BC1	2	1	74.5	131.1	17.9
BC2	3	0	14.0	18.8	11.3
BC?	1	-		≥128.6	
USR1	10	3	21.0	63.3	3.7
USR2	133	20	19.4	52.8	0.5
Overall	149	24	20.9	131.1	0.5

Despite over 27 person-hours of effort, no movements were observed from USR2 downstream into the Spring Island survey area. During extreme low-flow conditions in late August, more-thermally-stable vegetated habitat still occurred downstream at Spring Island, yet no fountain darters were observed moving in that direction. This may have been a result of lower quality habitat in the upstream portion of the Spring Island survey area. During late August surveys, divers noticed cooler spring-fed water in the downstream portion of the Spring Island survey area, followed by warmer more-stagnant conditions in the upstream portion of the Spring Island survey area. This middle area of warmer water may have prevented fountain darters from traveling in a downstream direction toward Spring Island. Instead, fountain darters remained within the Upper Spring Run survey area.

No temporal patterns in movement or location were observed. With discharge declining in early August, and relocations declining, a large marking event was conducted to boost relocation numbers and assess if movement under such conditions was different than during better conditions in spring. However, despite the conditions observed, relocations were continually found near the area of capture.

4.1.4 Habitat Analysis

No clear patterns were evident between relocation data and the various habitat variables recorded. ANOVA results did not show any relationship ($p=0.201$) between habitat type (bryophyte, algae, leaf litter/detritus, open substrate or under substrate) and the relative percentage of marked fountain darters detected (detections/# marked x 100). Linear regression of discharge, weekly mean temperature and DO on relative percentage of marked fountain darters detected showed no significant relationships or interactions among variables (adjusted r-squared -0.044, $F=0.8799$ on 7 and 13 df, $p=0.5476$). Using distance from release point as a response variable rather than percent capture, linear regression was conducted against the following variables: study days (days since inception of study), weekly average temperature, DO, and USGS discharge values. This analysis also found no significant relationships (adjusted r-squared 0.0177, $F=1.555$ on 4 and 119 df, $p=0.1908$) to exist in the data.

4.1.5 Population Estimates

Although this study was not explicitly designed for such purpose, the pooled relocation data from USR1 and USR2 was used to generate an abundance estimate for the Upper Spring Run study reach. Estimates of abundance and accompanying confidence intervals were produced by both Schnabel and Schmumacher-Eschmeyer methods using methods implemented in the R package “fishmethods” in R version 3.0.3 (R Development Core Team, 2008). As both estimation methods make some assumptions that the population is “closed” to immigration and emigration during the period data were collected, estimates were made using only the last three sampling occasions (August 13 and 26, and September 9) to generate a data set where the assumptions are most likely met.

The Schnabel method estimated the fountain darter population of the Upper Spring Run to be 21,692 with a 95% confidence interval of 18,099 to 27,064 individuals. Estimates from the Shumacher-Eschmeyer method were 16,138 individuals with a 95% confidence interval of 9,083–72,269.

5.0 Summary and Conclusions

In summary, habitat conditions observed in the study area during summer 2014 provided a very favorable scenario to observe fountain darter movement under low-flow-induced unstable habitat conditions. Bryophytes, which provide high-quality fountain darter habitat based on drop net density estimates from biomonitoring data (BIO-WEST 2014a) and are common in the study area during normal flow conditions, completely deteriorated from the study reach by mid-summer. Initially, bryophytes were overtaken by filamentous algae, which had bloomed during the intense sunlight and low-flow conditions. Such algal blooms are common in the Upper Spring Run reach under low-flow summertime conditions. However, by late August, as flows continued to drop, even the filamentous algae had disappeared and the reach contained essentially no aquatic vegetation. Drop net density data from the biomonitoring program suggest that areas containing no vegetation harbor few fountain darters. Additionally, the fairly stable water temperature typical in this reach increased substantially beginning in early August. Water temperatures, which typically fluctuate between 23 and 26°C in a normal year, peaked at over 30°C and remained above 26°C for over two straight weeks. Despite these conditions, relocation data showed that fountain darters remained in the Upper Spring Run area. No fountain darters were observed moving downstream toward vegetated spring-influenced habitats near Spring Island. Instead, fountain darters seemed to congregate around the few areas in the study reach that still had some groundwater influence. Although total discharge in this area was approaching zero, divers conducting visual surveys noted that some springflow was still trickling from a few areas along the river bottom. Noticeable stratification had developed in many areas with cooler spring-water along the river bottom and much warmer water in the upper two-thirds of the water column. These were the areas in which most relocations occurred during late August and early September when discharge was the lowest.

Although fountain darters marked in the Upper Spring Run were not observed to make large movements, two longer movements were observed from fountain darters that were marked in Blieders Creek. Blieders Creek is not spring-fed, and temperatures in the creek fluctuate much more than in the Upper Spring Run. Fountain darters had been previously documented in the lower and middle portion of the creek on occasion, although use of the area was considered to be

seasonal since water temperatures often exceed 30°C in the middle portion of the creek during summer months. Water temperature data collected as part of this study showed that temperatures in the upper portion of the creek were consistently above 27°C in early June. However, temperatures in the lower portion of the creek mirrored those in the Upper Spring Run and remained below 26°C until early August. Although only 6 relocations were made based on 217 fountain darters marked in Blieders Creek, two of those relocations represented fountain darters making large movements (> 125 m) from the creek downstream into the Upper Spring Run. These two movements represent the longest documented movements of wild fountain darters and document seasonal use of Blieders Creek.

When combined, relocation data from the Upper Spring Run and Blieders Creek show that even under extreme low-flow conditions (< 1 cfs), fountain darters are rather sedentary, moving on average 20.9 m (median = 17.9 m) from their release location. At maximum, fountain darters moved up to 131 m, although movements of this magnitude were rare (≈1% of all movements). This has direct implications to ecological model development. Currently, a decline in habitat within the ecological model results in a concomitant decline in fountain darter populations occupying that habitat, with no movement factor currently incorporated. This study suggests that movement/emigration of fountain darters from the area is not likely to counteract this projected population decline, even under extreme low-flow conditions, and particularly if additional habitat is more than approximately 20 m away.

In addition to providing input to the ecological model on movement potential under low-flow scenarios, this study also provided data on fountain darter population size within the study reach. Population size estimations calculated from mark recapture data from two different techniques ranged from approximately 16,000 to approximately 22,000 individuals within the Upper Spring Run study area. These estimates and associated error seem reasonable based on previous experience and HCP biological monitoring abundance estimates. In particular, population estimate data may be useful in HCP ecological model calibration or validation within this reach. Similar studies may be useful in the future for determining population size and model evaluation within other reaches.

Finally, this study provided interesting insight into fountain darter habitat selection and potential population dynamics under extreme low-flow, no-vegetation conditions. When aquatic vegetation disappeared from the Upper Spring Run study area in July and early August and water temperatures increased, fountain darters did not move out of the area looking for more suitable habitat. Instead, they were often observed hiding under gravel and cobble substrate in areas where springflow maintained adequate interstitial spaces for concealment. They were also observed using open silt substrate at times. These changes in habitat utilization could result in decreased prey availability and increased susceptibility to predation. In this case, an eventual decline in fountain darters would be anticipated should these conditions persist. An alternative interpretation is that the lack of movement from the study area may suggest that habitat within the area was still adequate for maintaining the fountain darter population. It will be important to closely examine the HCP biological monitoring program data at the conclusion of this year's sampling to evaluate if a concurrent decline in fountain darter abundance occurred in the Upper Spring Run in late summer 2014.

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APPENDIX F

Fountain Darter Drop Net Statistical Analysis

INTERIM FINAL PROGRESS REPORT: DROP NET STATISTICS FOR ESTIMATING MAXIMUM DARTER DENSITIES

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1. Statistical analysis overview

We developed a statistical model representing the relationship between potential maximum darter densities and their environmental variables. We first re-organized drop net data including aquatic vegetation, water depth, velocity, and dissolved oxygen concentration in the Comal River and San Marcos River from 2003 through 2014. We then used different approaches including statistical methods and empirical analyses such that the most appropriate method could explain the relationship between potential maximum darter densities and the environmental variables well (significantly) in both views of statistic and ecology. Finally, we applied the model to estimate the potential maximum darter densities for each habitat cell in the fountain darter individual-based, spatially-explicit simulation model.

2. Data

2.1 Source of data

EAA initiated fountain darter sampling in Comal and San Marcos Springs, Texas, USA during the summer of 2000 and have been sampling at least twice per year since that time. The 15-year (2000-2014) data set used in this study came from 795 and 659 drop net samples taken from four reaches of the Comal (Upper Spring Run, Landa Lake, Old Channel, and New Channel)

and three reaches of the San Marcos (City Park, I-35, SLD), respectively. A drop net is a sampling device originally designed by the U.S. Fish and Wildlife Service (USFWS) and subsequently modified by EAA to sample fountain darters and other benthic fish species. The net encloses a known area (2 m^2) and allows a thorough sample by preventing escape of fishes occupying the enclosed area.

Prior to each drop net sampling period, EAA mapped all aquatic vegetation within the reaches where the drop net samples were to be taken and identified the dominant types of vegetation and/or bare substrate within each reach. During sampling, EAA placed a drop net in a fixed number of sampling sites randomly selected within specific aquatic vegetation types within each sampling reach. The number of sampling sites per reach has not changed since initiation of the sampling program with the exception of the New Channel Reach, in which EAA discontinued sampling from 2004 to 2014 due to repeatedly poor habitat conditions and limited sampling areas. For each event, a minimum of two drop net samples per vegetation type was desired but not always possible. Additionally, if there were not enough dominant vegetation types to permit sampling different types, additional samples of the dominant aquatic vegetation present were taken.

For each drop net sample, EAA recorded the number of fountain darters within the net (EAA removed individuals from the net and returned them to the river just outside the net), water depth, vegetation type, height of vegetation, and areal coverage of vegetation within the net, as well as water temperature, velocity (15 centimeters above the stream bottom), conductivity, pH, and dissolved oxygen just outside the upstream edge of the net.

2.2 Potential predictors of fountain darter abundance

Previous work has identified several potential predictors of occurrence or abundance of fountain darters or similar species, including coverage and height of aquatic vegetation, presence of particular plant species, and water depth, velocity, temperature, conductivity, pH, and dissolved oxygen. Drawing on this literature, as well as extensive personal field observations, we selected a set of variables to include in our analysis (Table 1).

3. Methods

We tried to estimate the relationship between potential maximum darter densities and their environmental variables using data collected over a five-year period. Two potential criticisms of any approach are that our estimates of the relationship are unique to our methods of analyses (Elith and Graham, 2009) and to our specification of the variables included in that analysis (Agresti, 2007). These criticisms are generic problems related to structural uncertainty in the mathematical representation of natural systems (Walters, 1986). Hence, we used a range of different designs (from dependent variables settings to independent variables settings to different statistical analyses methods) to understand how to appropriately present the relationship. The possibility remains that there might be a more powerful method (Elith and Graham, 2009) and/or a more useful variables design (Wang et al., 2011). Evaluation of the relative merits of the different methodological approaches to estimate the relationship between endangered species abundance and their environmental variables currently is a topic of much debate. Hence, it remains a fruitful area of investigation further. In the sections that follow, we present details of the statistical analyses chronologically.

3.1 Statistical methods (October 2014 – July 2015)

3.1.1 Applying multinomial logit regression model in a combined dataset (data from both Comal and San Marcos Springs (October 2014)

We used multinomial logit regression model and all samples in Comal and San Marcos springs from 2000 to 2013 to understand the effects of environmental variables on the potential maximum darter densities in both springs. The distribution of fountain darter density was assumed normal, and categories (*K*) were assigned using the following rule: 1 (no fountain darter found; 343 observations), 2 (low; from 1 fountain darter to 0.5 SD below the mean; 542 obs.), 3 (fair; 0.5 SD either side of the mean; 563 obs.), 4 (high; 0.5 to 1.5 SD above the mean; 132 obs.), and 5 (very high; greater than 1.5 SD above the mean; 92 obs.), where mean = 20.23 and SD (standard deviation) = 27.08.

Multinomial logit regression model, a generalized linear model (GLM), was used to analyze the relationship between fountain darter density and environmental variables. GLMs are a generalization of linear regression models which allow various distributions for the response and error terms in the model (Agresti, 2007). The multinomial logit regression is used to calculate the probability of category membership of a dependent variable, in this case fountain darter density, based on multiple independent variables in an arbitrary number of categories. The independent variables can be either dichotomous (i.e., binary) or continuous (i.e., interval or ratio in scale). Multinomial logit regression is an extension of binary logistic regression that allows for more than two categories of the dependent or outcome variable. Like binary logistic regression, multinomial logistic regression uses maximum likelihood estimation to evaluate the probability of categorical membership (Starkweather and Moske, 2011).

Each measurement in our dataset could have fallen into any of the five density categories *K*, where *K* = 1, 2, 3, 4, or 5. Therefore, we assumed that density category placement did not tend

to happen in any particular order, and that the categories were strictly nominal. For a given sample i, we defined the density category as a response Y_i, where $Y_i = K$. We assumed a multinomial distribution for the response Y_i with class probabilities $P(Y_i = K)$. The model has the form:

$$P(Y_i = K) = \frac{exp(\alpha_K + \beta_K X_i)}{c_i}, \text{ where } K = 2, 3, 4, \text{ or } 5, \quad (1)$$

$$P(Y_i = K) = \frac{1}{c_i}, \text{ where } K = 1, \quad (2)$$

and where

$$c_i = 1 + \sum_{K=2}^{5} [exp(\alpha_K + \beta_K X_i)]. \quad (3)$$

The parameter vectors α_K and β_K relate to category K, and the vector X_i is a row of the design matrix containing independent environmental variables for a sample i. Note that:

$$\sum_{K=1}^{5} P(Y_i = K) = 1. \quad (4)$$

SAS ver. 9.2 (SAS Institute Inc., 2008) was used to fit the models. Variable selection and parameter estimation process continued until the selection criteria, as described below, were optimized. The models that optimized the criteria, subject to the constraint of equations for each K (eqs. (1) and (2)), were then selected. Having fitted the models, the probabilities that density falls into a given category in the sample i can be calculated.

The best model was identified by removing non-significant terms one at time and re-estimating the model (Agresti, 2007) until the Akaike Information Criterion score (AIC; Akaike, 1973) could not be lowered further. The reliability and validity of the models were evaluated based on the area under Receiver Operating Characteristic (ROC) curve (Area Under Curve; AUC) as fair ($0.50 < AUC \leq 0.75$), good ($0.75 < AUC \leq 0.92$), very good ($0.92 < AUC \leq 0.97$), or excellent ($0.97 < AUC \leq 1.00$) (Hosmer and Lemeshow, 2000). The AUC was computed for all ten comparison pairs (e.g. $Y_i = 1$ vs. $Y_i = 2$) and the results averaged (Hand and Till, 2001).

Model selection was conducted using SAS ver. 9.2 (SAS Institute Inc., 2008) and model evaluation using the pROC package (Robin et al., 2011) in R ver. 2.14.1 (R Development Core Team, 2006).

3.1.2 Applying the two levels hierarchical logit model in a combined dataset (data from both Comal and San Marcos Springs (November 2014)

We used the two levels hierarchical logit model for a combine dataset (Comal Springs and San Marcos Springs) to account for the influences of micro- and macro-environments on potential maximum darter densities.

The choice probability of the generic alternative *j*, *p*(*j*), of the two levels hierarchical logit model is obtained as:

$$p(j) = p(k) \cdot p(j/k) \tag{5}$$

where *p*(*k*) is the choice probability of group *k* including alternative *j*, and *p*(*j*/*k*) represents the conditional choice probability of *j* given *k*. The analytical expression of *p*(*k*) and *p*(*j*/*k*) are the following:

$$p(k) = \frac{\left(\sum_{i \in C_k} e^{V_i/\theta_k}\right)^{\delta_k}}{\sum_{k'}\left(\sum_{i \in C_{k'}} e^{V_i/\theta_{k'}}\right)^{\delta_{k'}}} \tag{6}$$

$$p(j/k) = \frac{e^{V_j/\theta_k}}{\sum_{i \in C_k} e^{V_i/\theta_k}} \tag{7}$$

Hence, combining the above two equations:

$$p(j) = \frac{e^{V_j/\theta_k} . \left(\sum_{i \in C_k} e^{V_i/\theta_k}\right)^{\delta_k - 1}}{\sum_{k'}\left(\sum_{i \in C_{k'}} e^{V_i/\theta_{k'}}\right)^{\delta_{k'}}} \tag{8}$$

The micro-environmental variables still included Cobble, Gravel, Sand, Silt, Silt_over_gravel, Bryophytes, Cabomba, Ceratopteris, Fil_algae, Green_algae ,Hydrilla, Hygrophila, Ludwigia,

POT_HYG, Potamogeton, Sagittaria, Vallisneria, MainVegHeight, MainVegVol, WithBryo, WaterDepthFt, Velocity, Temp, DO, SpCond, and pH. However, we added the macro-envirmental variables: CP, Fall, Spring, Summer, Winter, T_Green_algae, T_Bryophytes, T_Cabomba, T_Ceratophyllum, T_Ceratopteris, T_Eichhornia, T_Heteranthera, T_Hydrilla, T_Hydrocotle, T_Hygrophila, T_Justicia, T_Ludwigia ,T_Nuphar, T_Potamogeton, T_Rorippa, T_Sagittaria, T_Vallisneria, T_Zizania, T_Open, T_Fil_algae, T_Chara, and T_Limnophila.

In addition, we also used couple methods to check the multicollinearity: (1) The VIF (variance inflation factor) of model is < 10. It means no multicollinearity in our model. (2) Multicollinearity arises when the predictor variables are strong correlated among themselves. In such a case, multicollniearity inflates the errors. Hence, we examine the correlation matrix of predictor variables if they are measured in continuous scales and see whether their correlation coefficients are way too high.

3.1.3 Applying the two levels hierarchical logit models in each springs (December 2014)

We used the two levels hierarchical logit model for each springs because the results (Table 4) did not capture the specific effects of each spring. Hence, we re-defined the categories: Fountain darter mean abundance: 19.24, standard deviation (SD): 26.99. Category 1 (no fountain darter found; 90 observations in Comal spring and 23 obs. in San Marcos spring); category 2 (low; from 1 fountain darter to 0.5 SD below the mean; 209 obs. in Comal spring and 148 obs. in San Marcos spring); category 3 (fair; 0.5 SD either side of the mean; 315 obs. in Comal spring and 174 obs. in San Marcos spring); category 4 (high; 0.5 to 1.5 SD above the mean; 107 obs. in Comal spring and 21 obs. in San Marcos spring); and category 5(very high; greater than 1.5 SD

above the mean; 74 obs. in Comal spring and 4 obs. in San Marcos spring). There were total 795 obs. and 370 obs. in Comal and San Marcos springs, respectively.

Accordingly, micro-environmental variables included "Gravel, Sand, Silt, Silt over gravel, Bryophytes, Cabomba, Ceratopteris, Filamentous Algae, Green Algae, Hygrophila, Ludwigia, Sagittaria, Vallisneria, MainVegPer, MainVegHeight, WaterDepthFt, Velocity, Temp, DO, SpCond, and pH" and macro-environmental variables include "Spring, Summer, Fall, Winter, Flow, T_Green algae, T_Bryophytes, T_Cabomba, T_Ceratopteris, T_Hygrophila , T_Ludwigia, T_Nupha, T_Sagittaria, T_Vallisneria, T_Open, and T_Fil algae" in Comal Springs. Micro-environmental variables include "Gravel, Sand, Silt, Cabomba, Hydrilla, Hygrophila, POT/HYG, Sagittaria, Vallisneria, MainVegPer, MainVegHeight, WaterDepthFt, Velocity, Temp, DO, SpCond, and pH" and macro-environmental variables include "Spring, Summer, Fall, Winter, Flow, T_Green algae, T_Cabomba, T_Hydrilla, T_Hygrophila, T_Ludwigia, T_Potamogeton, T_Sagittaria, T_Vallisneria, T_Zizania, and T_Open" in San Marcos Springs.

Finally, we modified some independent variables: (1) Replace CP (critical period) with real season, and (2) Delete some macro-level vegetation types which only exist in very small areas.

3.1.4 Applying the two levels hierarchical logit model and multinomial logit regression model in each springs (January 2015)

Because we found that the macro-environmental variables could possibly dilute the effects of the micro-environmental variables in each reach, we ran two models in each spring. The first model is two levels hierarchical logit model which uses both macro- and micro-environmental variables and the second model is multinomial logit regression model which only use micro-environmental variables.

3.1.5 Application of the multinomial logit regression model (February 2015)

We used the probabilities calculated from the multinomial logit regression model to set up the potential maximum darter densities in each cell and then used this rule to drive the movement of fountain darter. We represented the conceptual model in Figure 1.

3.1.6 Refine the drop net data and rerun the multinomial logit regression model (March 2015)

We re-ran the multinomial logit regression model in Comal Springs after our teammates (Jake and Tim) edited some missing information of the drop net data.

3.1.7 Rerun the multinomial logit regression model excluding the variables of pH and Cond (May and June 2015)

We re-ran the multinomial logit regression model in both springs because we will not have values of pH and Cond as independent variables in the future. After having the best multinomial logit regression model incorporated in the fountain darter spatially-explicit, individual-based model samples in San Marcos Springs, we then compared the indicated vegetation types based on drop net sampling to simulated drop net using paired t-test.

3.1.8 Incorporating the results of multinomial logit regression model (estimated maximum darter density, MD), movement rules and consecutive moves (v) in the fountain darter spatially-explicit, individual-based model (July 2015)

We incorporated the results of multinomial logit regression model (estimated maximum darter density, MD), movement rules and consecutive moves (v) in the fountain darter spatially-

explicit, individual-based model. We only ran 3 reps of baseline simulation (with movement rule and 18 hours limitation) for the darters in City Park in San Marcos Springs. However, we designed a range of different settings of movement rules and consecutive moves (*v*) in Old Channel in Comal Springs. We ran a range of different settings of movement rules and consecutive moves (*v*) in Old Channel in Comal Springs.

The null models included with (1) random movement and no hour limitation for darters to stay in unfavorable habitats without dying, (2) movement rule and no hour limitation for darters to stay in unfavorable habitats without dying, (3) random movement and 12hours limitation for darters to stay in unfavorable habitats without dying, (4) random movement and 18hours limitation for darters to stay in unfavorable habitats without dying, or (5) random movement and 24hours limitation for darters to stay in unfavorable habitats without dying.

We then ran a set of models to determine the consecutive moves (*v*) included with movement rule and (1) 1 hour, (2) 2 hours, (3) 3 hours, (4) 6 hours, (5) 12 hours, (6) 18 hours, (7) 24 hours, (8) 30 hours, (9) 36 hours, (10) 42 hours, or (11) 48 hours limitation for darters to stay in unfavorable habitats without dying. In addition, we ran a set of models to determine the consecutive moves (*v*) included with stay rule and (1) 6 hours, (2) 12 hours, (3) 18 hours, (4) 24 hours, or (5) 30 hours limitation for darters to stay in unfavorable habitats without dying.

Finally, we evaluated the fountain darter spatially-explicit, individual-based model based on (1) system level results including (i) comparison of estimated maximum darter density and simulated number of juvenile plus adult fountain darters, and (ii) sensitivity analyses, and (2) the comparison of the indicated vegetation types based on drop net sampling to different designs of simulated drop net using paired t-test. Sensitivity analyses included (1) comparison of models

with movement rule and different consecutive moves (*v*) and (2) comparison of the effects of different demographic parameters on lambda of fountain darter.

3.2 Empirical analyses (April 2015 – November 2015)

3.2.1 Understand the relationship between fountain darter density and aquatic vegetation types based on drop net data and aquatic vegetation maps (April 2015)

Based on the preliminary results in February 2015, we found the potential maximum darter densities did not meet the general trend s of observation. Hence, we drew upon the drop net data and aquatic vegetation maps to understand the relationship between fountain darter density and aquatic vegetation types empirically.

3.2.2 Revisit the drop net data and apply the new information in the fountain darter spatially-explicit, individual-based model (August 2015)

Based on the different versions of statistical analyses, our team found that the maximum density generated from the statistical analyses (e.g. max-den-sys in Figure 7 or 8) did not match the general observation (dip net data, Figure 10) of fountain darter in Comal Springs. Hence, we revisited the drop net data in Comal Springs. We used the drop net data in each aquatic vegetation type in each sampling period to multiply the cells of the aquatic vegetation. We then summarized these values from all aquatic vegetation types to represent the estimated overall fountain darter abundance in each sampling period in Comal Springs. The detailed calculation could be found in Figure 11. Finally, we overlapped the estimated overall fountain darter abundance and dip net data.

After revisiting the drop net data, we thought that it could be an option for us to use the estimated overall fountain darter abundance in each sampling period in Comal Springs as the potential maximum fountain darter density. Hence, we integrated the new information in the fountain darter spatially-explicit, individual-based model which started running from 2003 and examined the performance of the new version of model based on (1) comparison of estimated maximum darter density and simulated number of juvenile plus adult fountain darters in the system level, (2) comparison of the indicated vegetation types based on drop net sampling to different designs of simulated drop net using paired t-test and (3) comparison of the specific vegetation type based on drop net sampling to different designs of simulated drop net using paired t-test.

In addition, we tested the initial effects on the fountain darter spatially-explicit, individual-based model. We integrated the new information in the fountain darter spatially-explicit, individual-based model which started running from 2001 and examined the performance of the new version of model following the same procedure which was described in the previous paragraph.

3.2.3 Apply the reach specific information in the fountain darter spatially-explicit, individual-based model (September and October 2015)

After integrating the empirical approach of analyzing drop net data in Comal Springs to the fountain darter spatially-explicit, individual-based model, we applied the approach but used only reach specific drop net data (Old Channel) to the simulation model. We then compared the indicated vegetation types based on drop net sampling to different designs of simulated drop net

using Nash-Sutcliffe model efficiency coefficient. The equation of Nash-Sutcliffe model efficiency coefficient is:

$$E = 1 - \frac{\sum_{t=1}^{T}\left(Q_o^t - Q_m^t\right)^2}{\sum_{t=1}^{T}\left(Q_o^t - \overline{Q_o}\right)^2} \tag{9}$$

where $Q_o{}^t$ is (observed) sampled density of fountain darter at time t, Q_m^t is simulated density of fountain darter at time t, $\overline{Qo}$ is the mean of (observed) sampled density of fountain darter. Nash–Sutcliffe efficiency can range from $-\infty$ to 1. An efficiency of 1 (E = 1) corresponds to a perfect match of simulated density to the sample density. An efficiency of 0 (E = 0) indicates that the model predictions are as accurate as the mean of the sample density. An efficiency less than zero (E < 0) occurs when the observed mean is a better predictor than the model. Essentially, the closer the model efficiency is to 1, the more accurate the model is.

3.2.4 Analyses of estimated maximum, simulated, and drop net data of darter densities based on each aquatic vegetation type (November 2015)

We analyzed the estimated maximum, simulated, and drop net data of darter densities based on each aquatic vegetation type. The estimated maximum darter densities was calculated by using (average darter density from 2003 to 2013 in vegetation type *i*) × (# of cells in vegetation type *i*) and the drop net based darter densities was calculated by using (average darter density at survey time in vegetation type *i*) × (# of cells in vegetation type *i*).

4. Results

4.1 Results of statistical methods (October 2014 – July 2015)

4.1.1 Results of multinomial logit regression model in a combined dataset (data from both Comal and San Marcos Springs (October 2014)

Results indicated that AIC reached its minimums (AIC = 2,060.442; model j; Table 2) once nine variables were removed: Open, Sagittaria, Cobble, Potamogeton, Green algae, DO, Vallisneria, pH, and Temp. The final model included the constant and 19 variables (Table 3). Although some variables were not significant for certain categories ($P > 0.05$), all variables included in the final model were significant overall (Table 3).

4.1.2 Results of the two levels hierarchical logit model in a combined dataset (data from both Comal and San Marcos Springs (November 2014)

Based on the minimum value of AUC, the final model included the constant and 24 variables (Table 4). As for the problem of multicollinearity, it might arises when r is greater than 0.80 (this is a rule of thumb). We found that there were some strong correlations between parameters: (1) MainVegHeight vs. MainVegVol ($r = 0.9785$), (2) T_Heteranthera vs. T_Justicia ($r = 0.8092$), and (3) T_Hydrilla vs. T_Potamogeton ($r = 0.8604$). After running our model, these variables are all excluded in the final model (Table 4).

4.1.3 Results of the two levels hierarchical logit models in each springs (December 2014)

We found that the spring specific models perform better than the model combining data from both springs. In addition, the final models of each spring are very different from each other and the variables selected in each spring make better ecological sense than the combined one (Table 5).

4.1.4 Results of the two levels hierarchical logit model and multinomial logit regression model in each springs (January 2015)

Even though the value of AIC is lower and the AUC is higher in the two levels hierarchical logit models in both springs (Tables 6 – 9), we decided to use multinomial logit regression model for estimating the potential maximum darter densities (Tables 10 – 11). The reason was that the design of the macro-environmental variables was inappropriate – we assigned the specific information of each reach to the drop net data which were sampled in that reach. Hence, it inflated the macro-environmental impacts and diluted the influences of micro-environmental variables.

4.1.5 Evaluation of the application of the multinomial logit regression model (February 2015)

Estimated maximum darter density using the application of the multinomial logit regression model and simulated number of juvenile plus adult fountain in the Old Channel of the Comal River using the baseline value of ν (12) looked matching well (Figure 2). The simulated number of juvenile plus adult fountain seems following the seasonal fluctuation appropriately.

4.1.6 Results of the multinomial logit regression model after refining the drop net data (March 2015)

We represented the results of the model including variable selection process, pairwise AUC scores, and variables included in the best model in Tables 12 – 14. The model with the mean AUC of 0.805 (Table 13) was considered good.

4.1.7 Results of the multinomial logit regression model excluding the variables of pH and Cond and the evaluation of the application of the multinomial logit regression model in the fountain darter spatially-explicit, individual-based model (May and June 2015)

We represented the results of the model including variable selection process and variables included in the best model in Tables 15 – 16. In addition, field and simulated drop net samples in San Marcos Springs were not statistically different (t = 3.18, p = 0.28, paired t-test) (Figure 4).

4.1.8 Results of incorporating the results of multinomial logit regression model (estimated maximum darter density, MD), movement rules and consecutive moves (*v*) in the fountain darter spatially-explicit, individual-based model (July 2015)

We only represent 3 reps of baseline simulation (with movement rule and 18 hours limitation) for the darters in City Park in San Marcos Springs in Figure 5. The system level results indicated that the fountain darter spatially-explicit, individual-based model responded to the estimated maximum darter density well.

The results of the null models in Old Channel in Comal Springs which the fountain darter population either declined or grew exponentially(Figure 6) did not represent the field observation.

The results of the models with movement or stay rule and consecutive moves (*v*) seemed more reasonable in certain range of consecutive moves (Figures 7 – 8).

The results of sensitivity analyses indicated that the models with movement rules and above 12 consecutive moves seemed not different from each other (Figure 9a). Among all demographic parameters, reproduction was the most sensitive parameter to affect lambda (Figure 9b).

Based on the results of comparison of the indicated vegetation types based on drop net sampling to simulated drop net using paired t-test, it seemed like the models with (1) movement rule and equal or greater than 12 consecutive moves (Table 17a), and (2) movement rule and

equal or greater than 18 consecutive moves (Table 17b) were not insignificant from the drop net sampling.

4.2 Results of empirical analyses (April 2015 – November 2015)

4.2.1 Trends of drop net data and aquatic vegetation maps from 2000 to 2013

We found that the vegetation coverage is about or less than 20% in OC occurred in 11/2000, 3/2001, 5/2001, 11/2001, 8/2002, 10/2002, 4/2003, and 6/2010 (Figure 3). When we just take a closer look, darter density in these periods is a little bit higher than average density cross the past 10 years. The reason for it is because the darters utilize the areas with Ceratopteris and Filamentous Algae in 11/2000, 3/2001, 5/2001, 11/2001, 8/2002, 10/2002, and 4/2003, and in the areas with Hygrophila in 6/2010. We then specifically ran the statistics for OC and OC_20%veg. The results showed that FD utilized the habitat with Hygrophila or Ceratopteris more than usual when the total vegetation coverage decreases to about 20% and most of the vegetation was Hygrophila or Ceratopteris.

4.2.2 Results of the estimated overall fountain darter abundance and the application to the fountain darter spatially-explicit, individual-based model (August 2015)

We overlapped the dip net data in Comal Springs and the estimated overall fountain darter abundance which was calculated from both drop net data and the aquatic vegetation maps (Figure 12). We found that the trends were not very similar. Hence, we thought it would not be appropriate to use the trend of dip net data to judge the potential maximum fountain darter density which was estimated based on statistical analyses.

The results of the fountain darter spatially-explicit, individual-based models starting from 2003 with movement or stay rule and consecutive moves (*v*) seemed more reasonable with 12 or 19 consecutive moves (Figure 13). Based on the results of comparison of the indicated vegetation types based on drop net sampling to simulated drop net using paired t-test, it seemed like the models with movement rule and 12 or 19 consecutive moves performed well with p-value = 0.4272 or 0.2174, respectively, which indicated that there were no statistically significant difference between the outcome of this scenario and the drop net data for these two models. The models with 96 consecutive moves were with p-value = 0.0008 which indicated that there was a statistically significant difference between the outcome of this scenario and the drop net data. However, based on the results of comparison of the specific vegetation type based on drop net sampling to simulated drop net using paired t-test, it seemed like the models with movement rule and 12 or 19 or 96 consecutive moves all performed well with p-value = 0.9494 or 0.8282 or 0.4421, respectively, which indicated that there were no statistically significant difference between the outcome of this scenario and the drop net data for these two models. We represent the descriptive statistics in Table 18.

The results of the fountain darter spatially-explicit, individual-based models starting from 2001 with movement or stay rule and consecutive moves (*v*) seemed more reasonable with 96 consecutive moves (Figure 14). Based on the results of comparison of the indicated vegetation types based on drop net sampling to simulated drop net using paired t-test, it seemed like the model with movement rule and 96 consecutive moves performed well with p-value = 0.1742 which indicated that there were no statistically significant difference between the outcome of this scenario and the drop net data. The models with 12 or 19 consecutive moves were with p-value = 0.0043 or 0.0200 which indicated that there was a statistically significant difference between

the outcome of this scenario and the drop net data. However, based on the results of comparison of the specific vegetation type based on drop net sampling to simulated drop net using paired t-test, it seemed like the models with movement rule and 12 or 19 or 96 consecutive moves all performed well with p-value = 0.9477 or 0.8227 or 0.4306, respectively, which indicated that there were no statistically significant difference between the outcome of this scenario and the drop net data for these two models. We represent the descriptive statistics in Table 19.

4.2.3 Results of the using the reach specific information (Old Channel) in the fountain darter spatially-explicit, individual-based model (September and October 2015)

The descriptive statistics of Old Channel and Comal Springs were very different from each other (Table 20). The system results of the fountain darter spatially-explicit, individual-based model based on Old Channel data (Figure 15) looked better than the one based on Comal Springs data (Figure 16). The Nash-Sutcliffe model efficiency coefficients of both models were < 0 (Table 21), but the model based on Old Channel data was better (Figures 17). Moreover, even though the efficiency coefficients are negative in both models, the efficiency coefficient is sensitive to extreme values and might yield sub-optimal results when the dataset contains large outliers which are our case.

4.2.4 Results of estimated maximum, simulated, and drop net data of darter densities based on each aquatic vegetation type (November 2015)

We overlapped estimated maximum, simulated, and drop net data of darter densities based on each aquatic vegetation type in Figure 18. The results seemed promising. We will investigate

the current approach further and find a most appropriate way to design the potential maximum darter densities.

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TABLES AND FIGURES

Table 1 Descriptions, values or units of measure, and means or frequencies of vegetation characteristics and water features as potential determinants of fountain darter density in (a) Comal and (b) San Marcos Springs, Texas, USA

(a)

Variable	Variable description	values or units of measure	Mean (range)[a] or frequency
Substrate types			
Gravel	Gravel	0: no	0: 540
		1: yes	1: 256
Sand	Sand	0: no	0: 755
		1: yes	1: 41
Silt	Silt	0: no	0: 418
		1: yes	1: 378
Silt_Grave	Silt over grave	0: no	0: 725
		1: yes	1: 71
Vegetation characteristics			
Open	Bare	%	5.92 (0 – 100)
Bryophytes	Bryophytes coverage	%	15.97 (0 – 100)
Cabomba	Cabomba coverage	%	8.83 (0 – 100)
Ceratopteris	Ceratopteris coverage	%	2.99 (0 – 100)
Fil_Algae	Filamentous Algae coverage	%	4.15 (0 – 100)
Green_Algae	Green Algae coverage	%	0.25 (0 – 100)
Hygrophila	Hygrophila coverage	%	29.95 (0 – 100)
Ludwigia	Ludwigia coverage	%	12.54 (0 – 100)
Sagittaria	Sagittaria coverage	%	9.38 (0 – 100)
Vallisneria	Vallisneria coverage	%	8.97 (0 – 100)
With_Bryo	With bryophytes overlap with main vegetation	0: no 1: yes	0: 526 1: 269
VegCover	Main vegetation coverage	%	93.04 (10 – 100)
VegHeight	Main vegetation height	Ft	1.35 (0.10 – 3.8)
Water features			
Depth	Water depth	Ft	2.80 (0.7 – 4.7)
Velocity	Water velocity		0.03 (0.02 – 0.40)
Temperature	Water temperature	C	23.64 (21.05 – 34.80)
DO	Dissolved oxygen		6.29 (3.26 – 10.70)
Cond	Conductivity		532.40 (0.55 – 755.00)
pH	pH value		7.33 (6.50 – 9.59)

[a]Numbers inside the parentheses are the range of the variable.

Table 1 Cont.
(b)

Variable	Variable description	values or units of measure	Mean (range)[a] or frequency
Substrate types			
Cobble	Cobblle	0: no 1: yes	0: 369 1: 1
Gravel	Gravel	0: no 1: yes	0: 319 1: 51
Sand	Sand	0: no 1: yes	0: 323 1: 47
Silt	Silt	0: no 1: yes	0: 121 1: 249
Silt_Grave	Silt over grave	0: no 1: yes	0: 348 1: 22
Vegetation characteristics			
Open	Bare	%	22.37 (0 – 100)
Cabomba	Cabomba coverage	%	11.05 (0 – 100)
Hydrilla	Hydrilla coverage	%	23.29 (0 – 100)
Hygrophila	Hygrophila coverage	%	22.94 (0 – 100)
POT_HYG	Potamogeton and Hygrophila coverage	%	9.67 (0 – 100)
Potamogeton	Potamogeton coverage	%	1.67 (0 – 100)
Sagittaria	Sagittaria coverage	%	1.29 (0 – 100)
Vallisneria	Vallisneria coverage	%	1.29 (0 – 100)
VegCover	Main vegetation coverage	%	71.10 (0 – 100)
VegHeight	Main vegetation height	Ft	1.07 (0 – 4.3)
Water features			
Depth	Water depth	Ft	2.25 (0.3 – 100)
Velocity	Water velocity		0.11 (-0.03 – 1.28)
Temperature	Water temperature	C	22.08 (18.59 – 27.70)
DO	Dissolved oxygen		7.87 (3.20 – 12.85)
Cond	Conductivity		578.25 (0.59 – 710.00)
pH	pH value		7.48 (6.00 – 8.44)

[a]Numbers inside the parentheses are the range of the variable.

Table 2 Variable selection process. Variables once removed were not returned to the model. The minimum value of AIC is in bold

Model ID	Variable removed	AIC
a	None	2087.182
b	Open	2080.263
c	Sagittaria	2073.303
d	Cobble	2070.058
e	Potamogeton	2082.773
f	Green algae	2075.917
g	DO	2073.684
h	Vallisneria	2068.423
i	pH	2064.734
j	Temp	**2060.442**
k	SpCond	3434.112
l	WaterDepthFt	3441.113

Table 3 Variables included in the best model according to the AIC criteria (model j)

Variable	Overall *P*-value	Category 2		Category 3		Category 4		Category 5	
		Estimated coefficient	*P*-value	Estimated coefficient	*P*-value	Estimated coefficient	*P*-value	Estimated coefficient	*P*-value
Constant	–	0.6196	0.7452	-7.9018	0.0004	-13.2625	<0.0001	-18.3240	<0.0001
Bryophytes	0.0006	2.3115	0.0566	3.7323	0.0021	4.5881	0.0006	5.2690	0.0003
Cabomba	<0.0001	3.5416	0.0009	5.2066	<0.0001	5.3816	<0.0001	5.3257	<0.0001
Ceratopteris	0.0310	1.9023	0.0078	1.4136	0.0181	-10.5104	0.0854	-9.5265	0.0862
FAlgae	<0.0001	14.4100	0.0018	19.1906	0.0025	19.4989	0.0019	22.5046	0.0060
Hydrilla	0.0490	1.0638	0.0118	1.0090	0.0052	0.4889	0.6103	0.7305	0.1142
Hygrophila	<0.0001	1.5035	<0.0001	2.2374	<0.0001	2.0593	0.0007	1.6246	0.0574
Ludwigia	<0.0001	2.6431	<0.0001	3.7054	<0.0001	4.2030	<0.0001	3.9002	<0.0001
POT_HYG	0.0059	3.1083	0.0054	3.3643	0.0029	2.5123	0.1088	-8.9735	0.9847
VegPer	<0.0001	0.0114	0.4134	0.0794	<0.0001	0.0939	<0.0001	0.1372	<0.0001
VegHeight	<0.0001	-0.9266	0.2333	1.7744	0.0371	3.0889	0.0097	4.4971	0.0033
VegVol	0.0002	0.0124	0.1446	-0.0139	0.1329	-0.0289	0.0264	-0.0443	0.0078
WithBryo	<0.0001	1.3088	0.0995	2.8370	0.0002	3.3302	<0.0001	3.8677	<0.0001
WaterDepthFt	<0.0001	-0.6532	0.0002	-0.9541	<0.0001	-0.9467	<0.0001	-0.7526	0.0001
Gravel	0.0048	0.1786	0.7300	0.7942	0.1770	1.7812	0.0188	3.9518	0.0010
Sand	0.0410	0.6767	0.3130	1.7995	0.0143	1.9821	0.0518	3.6941	0.0254
Silt	0.0022	0.9754	0.0573	1.8355	0.0017	2.0219	0.0113	4.4331	0.0004
Silt_Gravel	0.0496	0.1198	0.1429	1.0638	0.1130	0.8677	0.1637	2.8651	0.0398
Speed	0.0458	2.6844	0.0973	2.2843	0.0720	-1.3615	0.0110	-4.7245	0.0377
SpCond	0.0479	-0.00217	0.0212	-0.00031	0.0925	0.00319	0.0779	-0.00146	0.0469

Table 4 Variables included in the best model according to the AIC criteria

Variable	Overall P-value	Category 2		Category 3		Category 4		Category 5	
		Estimated coefficient	P-value	Estimated coefficient	P-value	Estimated coefficient	P-value	Estimated coefficient	P-value
Constant		5.6776	0.0493	0.4217	0.8897	-0.0714	0.9862	-0.3766	0.9445
Cobble	0.0007	-1.0254	0.0738	-2.206	0.0009	-1.4936	0.0566	-4.7085	0.0002
Gravel	0.0323	-0.5157	0.0981	-0.6858	0.0369	0.0592	0.8841	-0.5802	0.2536
Sand	0.1013	-0.12	0.8032	-0.5767	0.2483	0.4121	0.4803	-0.5361	0.562
Bryophytes	<.0001	0.0281	0.9838	1.0608	0.4415	1.6604	0.242	3.7192	0.0106
Fil_algae	<.0001	1.4635	0.4309	4.2819	0.0202	6.5658	0.0011	10.8217	<.0001
Potamogeton	0.0812	-4.4477	0.0587	-7.2215	0.0047	-17.325	0.9114	-15.481	0.9407
Sagittaria	<.0001	-1.916	<.0001	-2.5647	<.0001	-1.895	0.0118	-1.2266	0.2181
Vallisneria	<.0001	-2.133	0.0016	-4.1193	<.0001	-5.5023	<.0001	-4.3989	<.0001
WithBryo	<.0001	1.9027	0.0078	2.9577	<.0001	3.6147	<.0001	4.1649	<.0001
Velocity	0.0023	-2.6131	0.0586	-6.5596	0.0002	-8.7657	0.0063	-8.8623	0.0657
DO	0.068	0.0809	0.4549	0.2194	0.0457	0.0589	0.663	0.1619	0.3299
CP	0.004	0.00633	0.9858	0.221	0.5579	1.4327	0.0029	0.9643	0.1035
pH	0.1641	-0.7323	0.034	-0.6064	0.0954	-0.8819	0.0788	-1.3613	0.033
Spring	0.0021	-0.1553	0.6017	0.0826	0.7888	1.1435	0.0048	0.4958	0.3035
Summer	0.0003	-0.4237	0.4277	0.0459	0.9317	1.5881	0.0123	1.1146	0.1168
T_Bryophytes	0.0019	1.8094	0.2232	4.9281	0.0013	5.2275	0.0101	4.3212	0.0927
T_Hydrocotle	0.0831	7.022	0.9468	-161.7	0.1753	-259	0.3263	-112.1	0.6043
T_Nuphar	0.0697	19.0214	0.1164	30.1795	0.016	22.6813	0.2524	-21.173	0.5774
T_Chara	0.2409	-286.3	0.2477	-1717.7	0.0248	-10088	0.953	-8106.3	0.9779
T_Ludwigia	0.1238	-5.6922	0.5265	-3.1063	0.7366	-53.499	0.1026	-118.1	0.0169
T_Vallisneria	<.0001	0.1073	0.9305	3.9877	0.001	7.8917	<.0001	9.5374	<.0001
T_Zizania	<.0001	35.7371	0.0133	65.4157	<.0001	82.0468	<.0001	74.3518	0.0007
T_Open	0.0356	-0.5288	0.4609	0.4668	0.5241	1.6583	0.1294	0.9007	0.5912
T_Limnophila	0.0197	-351.6	0.0017	-308	0.0079	-415.9	0.0151	-425.2	0.0341

Table 5 Variables included in the best model in (a) Comal and (b) San Marcos Springs according to the AIC criteria

(a)

Variable	Overall P-value	Category 2		Category 3		Category 4		Category 5	
		Estimated coefficient	P-value	Estimated coefficient	P-value	Estimated coefficient	P-value	Estimated coefficient	P-value
Constant		-4.9335	0.0804	-9.8708	0.0015	-7.9093	0.0312	-14.078	0.0055
Gravel	0.0192	0.7424	0.2259	1.611	0.0195	1.6156	0.0419	4.1581	0.0012
Sand	0.0016	2.8285	0.0316	4.6251	0.0007	5.019	0.0006	6.9541	0.0003
Silt	<.0001	1.6805	0.0091	2.8407	<.0001	2.03	0.0169	5.4686	<.0001
Silt_gravel	0.0178	1.4284	0.0607	2.1989	0.0089	1.6653	0.1001	4.6558	0.002
Bryophytes	0.0014	-1.7951	0.2389	0.3093	0.839	1.0532	0.5099	2.4261	0.1454
Fil_algae	<.0001	1.3573	0.4964	5.2104	0.0089	7.0138	0.0019	13.5754	<.0001
Hygrophila	0.0147	-1.0307	0.1079	0.1966	0.7657	0.5514	0.451	-0.1126	0.9004
Sagittaria	0.0003	-3.8046	<.0001	-3.2289	0.0007	-2.0801	0.0705	-2.0819	0.1247
Vallisneria	<.0001	-2.2328	0.0035	-3.8595	<.0001	-5.3697	<.0001	-3.9702	0.0002
MainVegPer	0.0506	0.0104	0.6143	0.0474	0.0439	0.0358	0.2049	0.1006	0.014
MainVegHeight	0.0518	-1.8549	0.1215	0.9781	0.4392	0.7065	0.6831	3.8928	0.113
MainVegVol	0.0458	0.0161	0.2105	-0.0146	0.2872	-0.0122	0.5147	-0.0474	0.0735
WithBryo	<.0001	1.5665	0.0201	2.8531	<.0001	3.5557	<.0001	4.388	<.0001
DO	0.0069	0.217	0.1529	0.5119	0.0012	0.4356	0.0195	0.3559	0.0992
Spring	0.009	0.1681	0.6452	0.5689	0.1402	1.4761	0.0021	0.5571	0.3286
Summer	<.0001	0.2399	0.5864	0.9008	0.0483	2.1291	<.0001	2.0914	0.0006
Flow	0.0666	0.00658	0.018	0.0085	0.0031	0.00823	0.0193	0.00741	0.0785
T_Bryophytes	0.0159	0.5101	0.8086	-1.8589	0.3746	-4.7307	0.0541	-7.6181	0.0261
T_Hygrophila	<.0001	2.2284	0.1412	-2.3769	0.1163	-8.1883	0.0002	-15.672	0.0068
T_Ludwigia	0.0889	-13.429	0.1308	-15.203	0.0935	-53.798	0.0932	-134.1	0.0187
T_Nuphar	0.0069	36.9473	0.0243	57.5275	0.0008	49.3686	0.0581	-25.077	0.673
T_Sagittaria	0.0306	22.8202	0.0031	25.2145	0.0021	18.9196	0.0686	23.1914	0.0559
T_Open	<.0001	-0.1622	0.9114	-4.9666	0.0006	-7.4019	<.0001	-11.788	<.0001
T_FilAlgae	0.0668	-6.549	0.2356	-11.505	0.0575	-15.734	0.0723	-34.187	0.0042

Table 5 Cont.
(b)

Variable	Overall P-value	Category 2		Category 3		Category 4		Category 5	
		Estimated coefficient	P-value	Estimated coefficient	P-value	Estimated coefficient	P-value	Estimated coefficient	P-value
Constant		25.033	0.3582	-36.7333	0.2207	-187.2	0.0061	935.4	0.1203
Cabomba	0.0083	9.0209	0.7807	10.1315	0.7546	12.0459	0.7102	0.2886	0.9954
Velocity	0.0031	-3.0389	0.0638	-7.6019	0.0003	-9.8446	0.063	-12.6969	0.0975
Temp	0.015	0.1581	0.6172	0.606	0.0597	0.5548	0.1508	2.5903	0.1272
Flow	0.0267	-0.00678	0.0828	-0.00767	0.0578	0.00244	0.6874	0.1521	0.076
T_GreenAlgae	0.022	5.3051	0.788	15.6028	0.4184	47.0943	0.0416	1481.5	0.0506
T_Cabomba	0.0046	-24.4169	0.5575	43.548	0.3276	171.3	0.041	4157.5	0.0598
T_Hydrilla	0.0008	-16.0848	0.5111	34.8212	0.1999	187.2	0.0047	-648	0.1832
T_Hygrophila	0.0015	-23.9078	0.3916	26.7313	0.3765	144.7	0.0251	-1560.7	0.0754
T_Potamogeton	0.0012	-34.533	0.2236	16.5632	0.5929	156.3	0.0231	-1443	0.0891
T_Sagittaria	0.0031	-44.7291	0.4196	32.2797	0.5758	271.9	0.0099	-414.7	0.547
T_Zizania	0.0017	-32.5267	0.353	38.6973	0.3153	243.8	0.0071	68.5332	0.8759
T_Open	0.0007	-25.9126	0.3026	25.7946	0.3555	171.8	0.0105	-1331.4	0.0888

Table 6 Variable selection processes in (a) the two levels hierarchical logit model and (b) multinomial logit regression model in Comal Springs. Variables once removed were not returned to the model. The minimum value of AIC is in bold

(a)

Variable removed	AIC
None	1441.800
GreenAlgae	1441.802
Vallisneria	1435.803
Velocity	1429.840
MainVegHeight	1425.057
T_GreenAlgae	1420.592
Temp	1416.811
T_Cabomba	1413.386
WaterDepthFt	1411.989
Flow	1409.540
SpCond	1408.078
T_Ceratopteris	1408.847
T_Sagittaria	**1404.421**
T_Vallisneria	1405.268
T_Ludwigia	1409.042
T_FilamentousAlgae	1410.223

(b)

Variable removed	AIC
None	1602.649
GreenAlgae	1602.651
MainVegPer	1596.703
MainVegHeight	1591.225
Velocity	1586.141
DO	**1583.038**
Temp	1583.471

Table 7 Variable selection processes in (a) the two levels hierarchical logit model and (b) multinomial logit regression model in San Marcos Springs. Variables once removed were not returned to the model. The minimum value of AIC is in bold

(a)

Variable removed	AIC
None	842.875
WithBryo	837.03
Potamogeton	837.031
Vallisneria	839.139
Sagittaria	842.209
DO	837.158
pH	832.041
T_Vallisneria	828.098
MainVegHeight	824.02
WaterDepthFt	822.493
Flow	820.533
Hydrilla	**819.827**
POT_HYG	823.104
Velocity	823.636

(b)

Variable removed	AIC
None	872.907
WithBryo	867.298
Potamogeton	867.298
Vallisneria	869.777
Sagittaria	869.154
MainVegHeight	866.423
DO	**863.538**
WaterDepthFt	864.257
Hydrilla	866.035
POT_HYG	867.334

Table 8 Pairwise AUC scores for all combinations of darter density categories and mean AUC for (a) the two levels hierarchical logit model and (b) multinomial logit regression model in Comal Springs

(a)

AUC	1 vs 2	1 vs 3	1 vs 4	2 vs 3	2 vs 4	3 vs 4	Mean AUC
	0.7970	0.9466	0.9805	0. 7315	0. 8643	0. 7970	0. 853

(b)

AUC	1 vs 2	1 vs 3	1 vs 4	2 vs 3	2 vs 4	3 vs 4	Mean AUC
	0.7728	0.8596	0.9370	0.6360	0.8050	0.7728	0.797

Table 9 Pairwise AUC scores for all combinations of darter density categories and mean AUC for (a) the two levels hierarchical logit model and (b) multinomial logit regression model in San Marcos Springs

(a)

AUC	1 vs 2	1 vs 3	1 vs 4	2 vs 3	2 vs 4	3 vs 4	Mean AUC
	0.7413	0.9009	0.9557	0.7749	0.8583	0.8034	0.839

(b)

AUC	1 vs 2	1 vs 3	1 vs 4	2 vs 3	2 vs 4	3 vs 4	Mean AUC
	0.698	0.8114	0.8545	0.7167	0.8035	0.6218	0.751

Table 10 Variables included in the best model of (a) two levels hierarchical logit model and (b) multinomial logit regression model in Comal Springs according to the AIC criteria

(a)

Variable	Overall p-value	Category 2	Category 3	Category 4
Constant	-	12.7629	17.7838	28.6073
Bryophytes	<.0001	3.9417	6.4208	6.3332
Cabomba	0.0135	1.1052	1.6958	1.6018
Ceratopteris	0.0039	1.6018	4.8123	-8.6461
FilamentousAlgae	<.0001	6.0158	8.751	10.5942
Hygrophila	<.0001	2.4483	4.1791	2.4481
Ludwigia	<.0001	1.6947	3.5262	2.18
Sagittaria	<.0001	1.9578	4.0795	3.8731
MainVegPer	0.0486	0.00664	-0.025	0.0261
WithBryo	<.0001	1.4929	1.543	2.93
DO	0.0244	0.2792	0.1788	0.2641
pH	0.0009	-1.2701	-1.6356	-2.1946
T_Bryophytes	0.0105	-9.066	-10.5119	-26.2994
T_Hygrophila	0.0003	-10.3513	-12.9186	-31.4859
T_Ludwigia	0.0433	-10.339	-62.5925	-115.3
T_Vallisneria	0.0825	-5.8783	-4.9509	-18.3049
T_Open	0.0013	-11.761	-13.8727	-28.8402
T_FilamentousAlgae	0.0033	-16.7799	-17.8574	-41.4284

(b)

Variable	Overall p-value	Category 2	Category 3	Category 4
Constant	-	7.7584	9.5793	2.1008
Bryophytes	<.0001	4.3455	4.2244	9.8051
Cabomba	<.0001	4.4947	3.3249	8.6736
Ceratopteris	0.0329	3.4015	1.8596	-5.4335
FilamentousAlgae	<.0001	6.0201	4.7125	12.1659
Hygrophila	<.0001	3.5061	2.8677	6.66
Ludwigia	<.0001	3.9065	3.8657	8.1887
Sagittaria	0.0126	2.2736	1.2025	5.7577
Vallisneria	0.0005	3.0407	1.2385	6.8683
WithBryo	<.0001	1.8536	1.935	2.8385
WaterDepthFt	0.0326	-0.3647	-0.3881	-0.0018
SpCond	0.0483	-0.0018	-0.00094	0.000215
pH	<.0001	-1.4116	-1.7139	-1.6568

Table 11 Variables included in the best model of (a) two levels hierarchical logit model and (b) multinomial logit regression model in San Marcos Springs according to the AIC criteria

(a)

Variable	Overall p-value	Category 2	Category 3	Category 4
Constant	-	-70.8978	-231.7	-212.7
Cabomba	0.0011	1.6269	2.2446	2.9217
Hygrophila	0.0021	0.8609	-0.3504	-0.8761
POT_HYG	0.123	0.1495	-0.1969	-2.638
MainVegPer	0.0872	0.0094	0.0683	0.0714
Velocity	0.1163	-2.2086	-4.9639	-5.8
Temp	0.0012	0.5689	0.7875	0.7238
SpCond	0.0066	-0.00405	-0.00503	-0.00388
T_GreenAlgae	0.0045	8.9807	11.1086	49.0672
T_Cabomba	<.0001	71.0293	265.6	213.1
T_Hydrilla	<.0001	61.3801	202	194.5
T_Hygrophila	<.0001	55.8122	216.7	174.9
T_Ludwigia	0.0014	67.1282	410.2	248.2
T_Potamogeton	<.0001	56.6594	212.8	182
T_Sagittaria	<.0001	101.2	236.1	238.5
T_Zizania	<.0001	66.2649	255.4	260.5
T_Open	<.0001	59.2093	208	188

(b)

Variable	Overall p-value	Category 2	Category 3	Category 4
Constant	-	-10.171	-26.6053	-26.7749
Cabomba	0.0048	3.3529	3.4414	1.8017
Hydrilla	0.0881	2.2244	1.8748	0.5355
Hygrophila	0.0069	2.9458	1.6812	-0.1177
POT_HYG	0.0094	2.7433	2.0642	-1.1731
MainVegPer	0.0827	-0.00581	0.0559	0.0717
WaterDepthFt	0.1403	-0.1848	0.2231	0.1977
Velocity	0.041	-3.0728	-4.7604	-9.6547
Temp	0.0244	0.3914	0.5201	0.4473
SpCond	0.0219	-0.00381	-0.00317	-0.0037
pH	0.0586	0.3487	1.1541	1.3725

Table 12 Variable selection process. Variables once removed were not returned to the model. The minimum value of AIC is in bold

Model ID	Variable removed	AIC
a	None	1889.89
b	MainVegPer	1889.89
c	Open	1885.12
d	Velocity	1882.31
e	GreenAlgae	1879.07
f	MainVegHeight	1876.52
g	Temp	1871.23
h	Ceratopteris	**1871.03**
i	WaterDepthFt	1872.95

Table 13 Pairwise AUC scores for all combinations of darter density categories and mean AUC for the multinomial logit regression models in Comal Springs

AUC	1 vs 2	1 vs 3	1 vs 4	2 vs 3	2 vs 4	3 vs 4	Mean AUC
	0.7928	0.8763	0.9272	0.6502	0.8135	0.7684	0.805

Table 14 Variables included in the best model of the multinomial logit regression model in Comal Springs according to the AIC criteria

Variable	Overall P-value	Category 2		Category 3		Category 4	
		Estimated coefficient	P-value	Estimated coefficient	P-value	Estimated coefficient	P-value
Bryophytes	<.0001	0.0163	0.0023	0.0339	<.0001	0.0734	<.0001
Cabomba	<.0001	0.0245	<.0001	0.0324	<.0001	0.0575	0.0002
FilamentousAlgae	<.0001	0.0344	<.0001	0.0445	<.0001	0.0907	<.0001
Hygrophila	<.0001	0.0147	<.0001	0.0239	<.0001	0.0452	0.0022
Ludwigia	<.0001	0.0154	0.0006	0.0329	<.0001	0.056	0.0003
Sagittaria	0.0412	0.00146	0.7561	0.00594	0.4347	0.0427	0.0046
Vallisneria	0.0064	0.00811	0.0621	0.00569	0.4919	0.0474	0.0014
WithBryo	<.0001	1.8507	<.0001	2.0044	<.0001	2.4682	<.0001
WaterDepthFt	0.0495	-0.2574	0.0295	-0.3613	0.0119	-0.2361	0.2089
DO	0.0065	0.0712	0.3105	0.026	0.7714	0.4008	0.0007
SpCond	0.0474	-0.00131	0.0654	-0.00082	0.356	0.00186	0.1887
pH	<.0001	-1.2384	0.0001	-1.563	<.0001	-1.8534	0.0003

Table 15 Variable selection process in (a) Comal and (b) San Marcos Springs. Variables once removed were not returned to the model. The minimum value of AIC is in bold

(a)

Model ID	Variable removed	AIC
a	None	2441
b	bedrck	2441
c	Vall	2441
d	cobble	2434.97
e	Cerato	2430.84
f	GrAlg	2429.58
g	Veght	2425.61
h	DO	**2424.2**
i	Mainper	2426.62
j	Open	2431.01
k	CV	2430.07

(b)

Model ID	Variable removed	AIC
a	None	1501.45
b	bedrck	1501.45
c	cobble	1493.91
d	WBryo	1486.88
e	Pot	1479.34
f	Vall	1478.14
g	gravel	1473.24
h	Sand	1466.95
i	Open	1461.35
j	Sag	1458.09
k	Hydrilla	1452.2
l	Poghygr	1446.62
m	DO	1441.46
n	Silt	1437.29
o	Temp	**1436.52**
p	Cabom	1441.61

Table 16 Variables included in the best model of the multinomial logit regression model in (a) Comal and (b) San Marcos Springs according to the AIC criteria

(a)

Variable	Overall P-value	Category 1		Category 2		Category 3		Category 4	
		Estimated coefficient	P-value	Estimated coefficient	P-value	Estimated coefficient	P-value	Estimated coefficient	P-value
Silt	<.0001	1.2956	0.0022	2.3575	<.0001	1.6247	0.0062	4.6367	<.0001
Sand	<.0001	2.0764	0.02	4.1702	<.0001	4.5564	<.0001	6.97	<.0001
gravel	0.0001	1.1181	0.0071	1.873	0.0005	1.9984	0.0005	4.7627	<.0001
Bryo	<.0001	2.6678	0.0016	5.6075	<.0001	6.8753	<.0001	7.4034	<.0001
Cabom	<.0001	2.6997	0.0004	3.9653	<.0001	5.1362	<.0001	3.8497	0.0002
FilAlg	<.0001	1.5288	0.1685	3.6152	0.0012	4.6041	0.0003	5.2499	<.0001
Hygro	<.0001	1.1027	0.0003	1.5058	<.0001	2.5287	<.0001	0.4149	0.544
Lud	0.0002	1.6565	0.0011	2.0672	0.0003	3.392	<.0001	1.6122	0.0417
Open	0.0721	-2.3788	0.0035	-0.8837	0.2413	-13.4965	0.9702	-14.2634	0.9675
Sag	0.01	-1.1103	0.001	-1.2197	0.0098	-0.3636	0.6324	-1.1506	0.1435
Mainper	0.0736	-0.0208	0.0062	-0.00206	0.6346	-0.00248	0.6613	0.00182	0.6432
WBryo	<.0001	1.8466	0.0013	3.7791	<.0001	4.0361	<.0001	4.7138	<.0001
Depth	0.0037	-1.1185	0.0307	-2.0738	0.0004	-2.0432	0.0016	-1.2259	0.0995
CV	0.0478	-1.497	0.5515	-2.2842	0.4634	0.3249	0.9206	6.8015	0.0586
Temp	<.0001	-0.2052	0.1266	0.0851	0.6001	0.3427	0.0701	0.7407	0.0011
Intercept	--	6.977	0.0364	-3.2767	0.404	-10.8421	0.019	-24.5001	<.0001

(b)

Variable	Overall P-value	Category 1		Category 2		Category 3		Category 4	
		Estimated coefficient	P-value	Estimated coefficient	P-value	Estimated coefficient	P-value	Estimated coefficient	P-value
Cabom	0.0417	1.5857	0.1378	2.1504	0.0419	2.5568	0.0172	2.205	0.0493
Hygro	0.015	0.4114	0.3086	0.8813	0.0286	0.0158	0.9745	-0.2255	0.7137
Mainper	<.0001	0.0332	<.0001	0.0475	<.0001	0.0916	<.0001	0.092	0.0014
Veght	0.0028	2.7798	0.0031	3.5367	0.0003	2.638	0.0202	1.1137	0.4174
Depth	0.0001	-2.7642	0.0003	-3.6957	<.0001	-2.6556	0.0062	-1.7924	0.1227
CV	0.0247	-1.4832	0.112	-3.0727	0.0065	-2.5076	0.091	-13.5556	0.0267
Intercept	--	-0.8899	0.1134	-1.6866	0.0196	-7.1035	0.0001	-7.4306	0.0098

Table 17 The results of comparison of the indicated vegetation types based on drop net sampling to different designs of simulated drop net including models with (a) movement or (b) stay rule and different number of consecutive moves using paired t-test

(a)

	Dropnet	1hr	2hrs	3hrs	6hrs	12hrs	18hrs	24hrs	30hrs	36hrs	42hrs	48hrs
Mean	20.3254	0.8203	5.5552	7.274	9.6358	10.5516	10.447	10.4508	10.7727	10.3785	11.1751	11.0177
t Stat		2.6662	2.6529	2.4364	2.1705	1.8611	1.9843	1.9905	1.9721	2.1698	1.9016	1.956
P(T<=t)		0.0223	0.0226	0.0295	0.041	0.0609	0.052	0.0516	0.0528	0.0411	0.0578	0.0539

(b)

	Dropnet	6hrs	12hrs	18hrs	24hrs	30hrs
Mean	20.3254	8.24463	8.80679	9.89498	9.80732	9.81145
t Stat		2.13972	2.22941	2.00638	1.89074	2.05968
P(T<=t)		0.04267	0.03811	0.05056	0.05862	0.04724

Table 18 Descriptive statistics of mean (SD) fountain darter density (#/m^2) from drop net data and simulated from 2003 drop net data

Veg type	Drop net	12-hr rule	19-hr rule	96-hr rule	# of samples
1	9.87 (10.16)	13.95 (6.75)	14.26 (7.99)	16.58 (6.48)	19
2	3.88 (3.39)	2.13 (1.36)	2.75 (1.58)	3.25 (0.71)	8
3	3.09 (2.43)	3.61 (1.22)	3.83 (0.95)	4.72 (1.22)	69
4	1.78 (1.39)	1.04 (0.86)	1.26 (0.74)	2.19 (1.31)	47
6	12.83 (7.29)	10.33 (1.15)	10.67 (1.53)	11 (1.73)	3

Table 19 Descriptive statistics of mean (SD) fountain darter density (#/m^2) from drop net data and simulated from 2001 drop net data

Veg type	Drop net	12-hr rule	19-hr rule	96-hr rule	# of samples
1	20.17 (23.04)	8.49 (8.48)	9.89 (9.26)	11.34 (9.46)	35
2	3.48 (4.09)	1.29 (1.18)	1.21 (1.34)	2.04 (1.32)	28
3	3.09 (2.43)	3.48 (1.27)	3.67 (1.23)	4.64 (1.16)	69
4	1.78 (1.39)	0.96 (0.83)	1.17 (0.82)	1.91 (0.97)	47
6	12.83 (7.29)	10.33 (2.08)	11.33 (1.15)	11.33 (1.15)	3

Table 20 Descriptive statistics of fountain darter density (#/m^2) from drop net data in (a) Old Channel and (b) Comal Springs

(a)

Vege Type	Mean	SD	Minimum	Maximum	Sample size
0	1.323529	2.145758	0	8	34
1	21.96429	22.72862	0	105	42
2	3.121212	3.877077	0	17.5	33
3	4.126437	3.873898	0	21	87
4	1.776596	1.386301	0	5.5	47
6	11.58333	4.820961	6	20.5	6
10	8	N/A	8	8	1

(b)

Vege Type	Mean	SD	Minimum	Maximum	Sample size
0	1.290323	2.591835	0	15	62
1	19.34375	22.36428	0	105	48
2	3.121212	3.877077	0	17.5	33
3	7.37037	8.35089	0	38.5	297
4	12.15217	14.41925	0	85	138
6	24.90789	20.27652	0	96	152
7	5.268421	14.00452	0	106	95
8	5.575581	9.949141	0	58	86
10	9.576923	9.09332	0	42.5	91

Table 21 The Nash-Sutcliffe model efficiency coefficients for the model (4 reps) based on (a) Old Channel data or (b) Comal Springs data

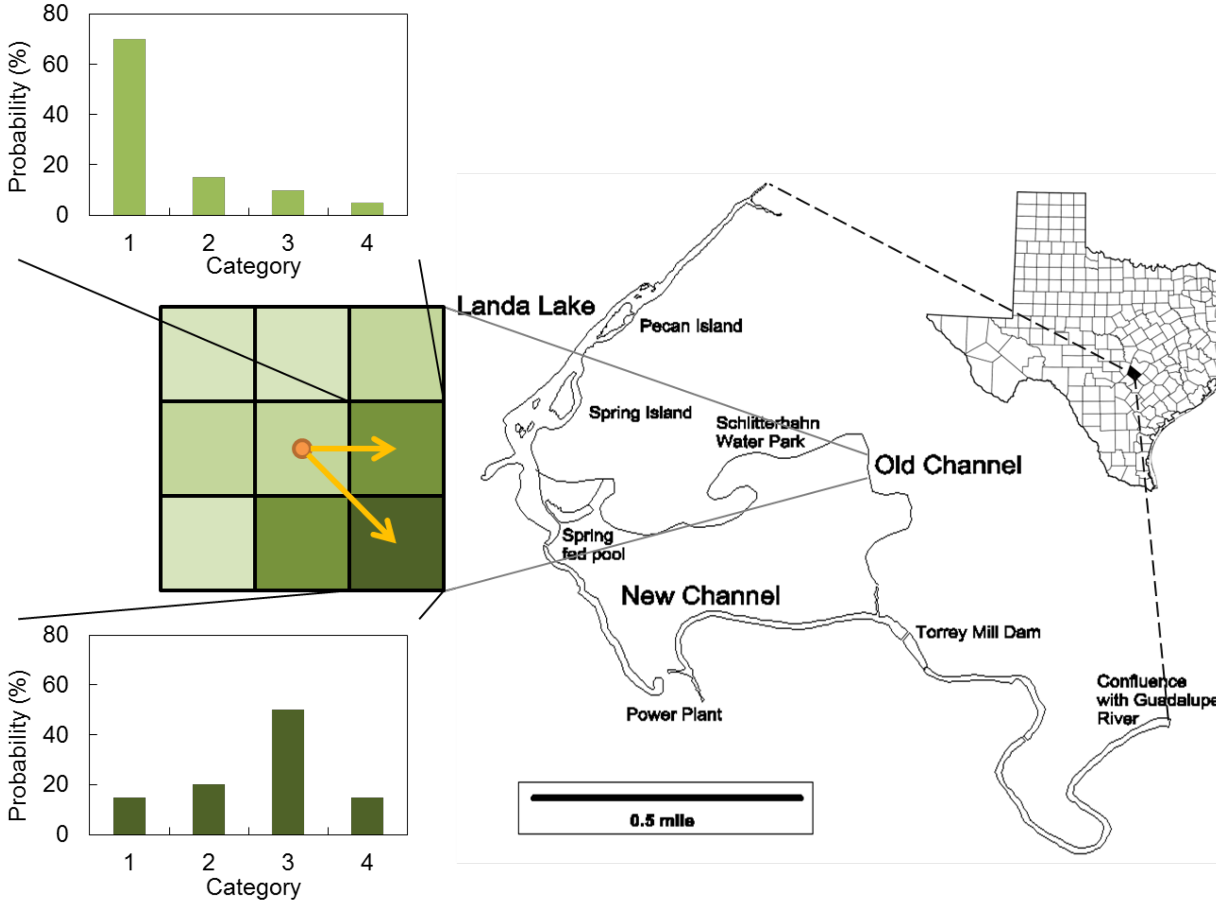
(a)

Vege Type	Rep 1	Rep 2	Rep 3	Rep 4
1	-0.794	-1.420	-1.589	-1.311
2	-0.437	-0.661	-0.263	-0.288
3	-0.274	-0.438	-0.423	-0.256
4	-0.490	-0.728	-0.456	-0.468
6	-0.321	-0.086	-0.170	0.084
All	-0.117	-0.445	-0.514	-0.354

(b)

Vege Type	Rep 1	Rep 2	Rep 3	Rep 4
1	-1.296	-1.368	-1.312	-1.227
2	-0.922	-1.109	-0.947	-0.723
3	-2.273	-2.168	-2.028	-2.253
4	-40.534	-39.413	-38.215	-39.923
6	-2.516	-2.977	-2.977	-3.194
All	-1.581	-1.600	-1.523	-1.545

FIGURES LEGEND


80
60
40
20
0
Probability (%)
1
2
3
4
Category
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Pecan Island
Spring Island
Schlitterbahn
Water Park
Old Channel
Spring
fed pool
New Channel
Torrey Mill Dam
Power Plant
Confluence
with Guadalupe
River
0.5 mile
80
60
40
20
0
Probability (%)
1
2
3
4
Category

Figure 1

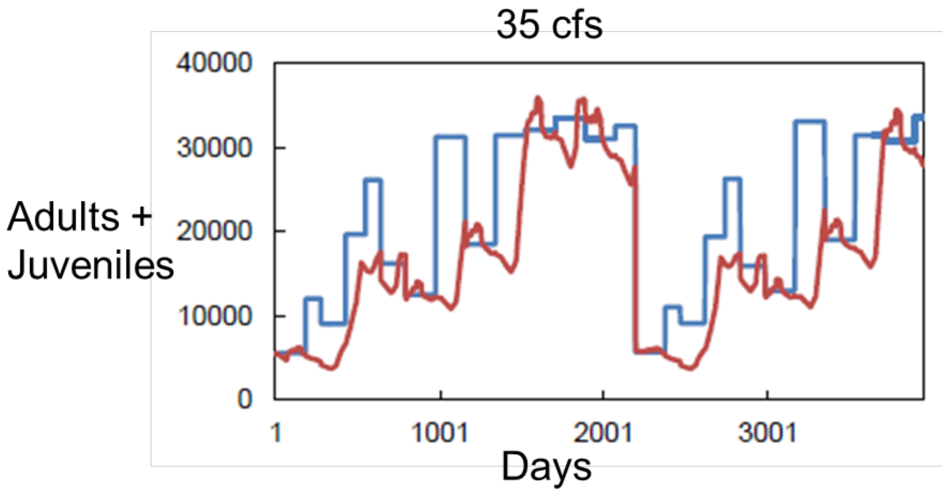
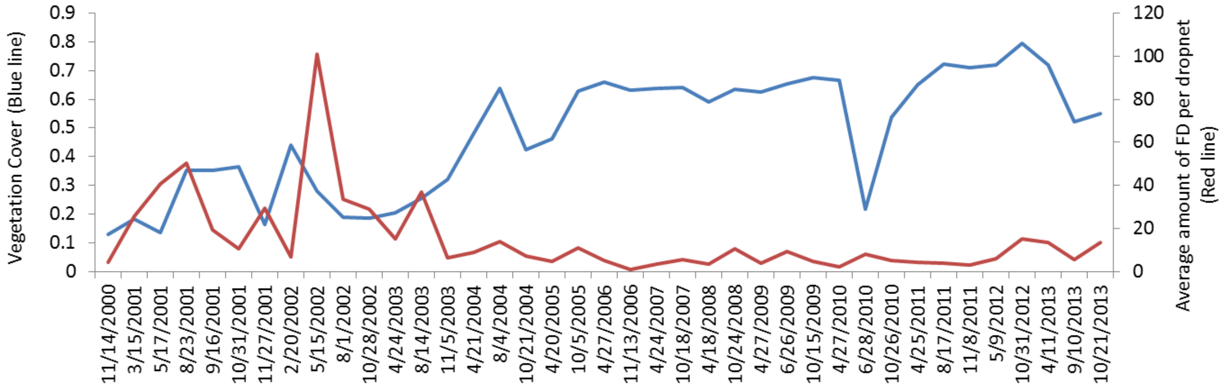

35 cfs
40000
30000
20000
10000
0
Adults +
Juveniles
1
1001
2001
3001
Days

Figure 2



Vegetation Cover (Blue line)
Average amount of FD per dropnet (Red line)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
0
20
40
60
80
100
120
11/14/2000
3/15/2001
5/17/2001
8/23/2001
9/16/2001
10/31/2001
11/27/2001
2/20/2002
5/15/2002
8/1/2002
10/28/2002
4/24/2003
8/14/2003
11/5/2003
4/21/2004
8/4/2004
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11/13/2006
4/24/2007
10/18/2007
4/18/2008
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4/27/2009
6/26/2009
10/15/2009
4/27/2010
6/28/2010
10/26/2010
4/25/2011
8/17/2011
11/8/2011
5/9/2012
10/31/2012
4/11/2013
9/10/2013
10/21/2013

Figure 3

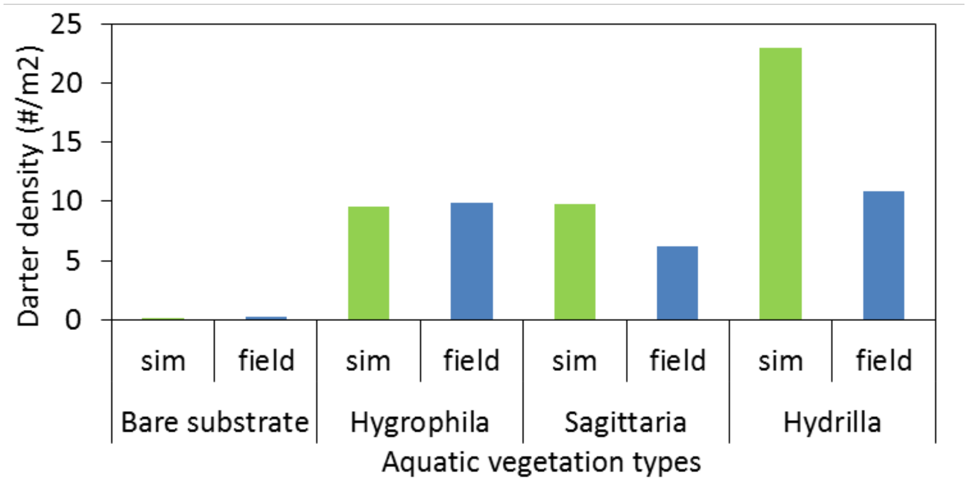
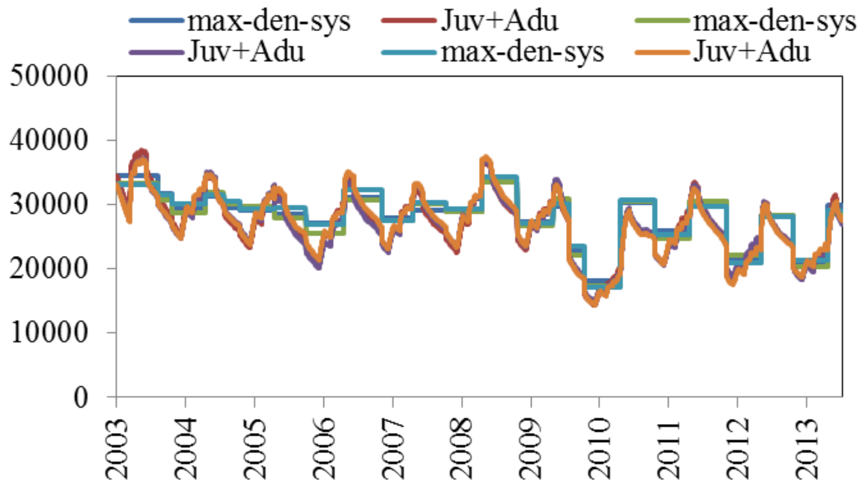

Darter density (#/m2)
25
20
15
10
5
0
sim
field
sim
field
sim
field
sim
field
Bare substrate
Hygrophila
Sagittaria
Hydrilla
Aquatic vegetation types

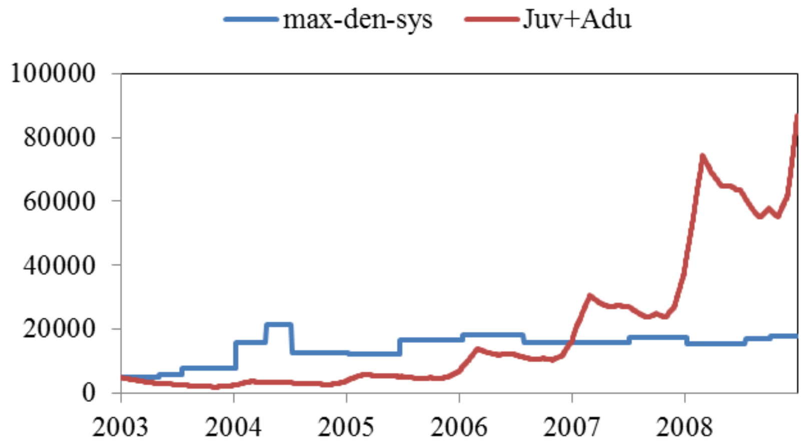
Figure 4



max-den-sys
Juv+Adu
max-den-sys
Juv+Adu
max-den-sys
Juv+Adu
50000
40000
30000
20000
10000
0
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013

Figure 5

(a)



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Juv+Adu
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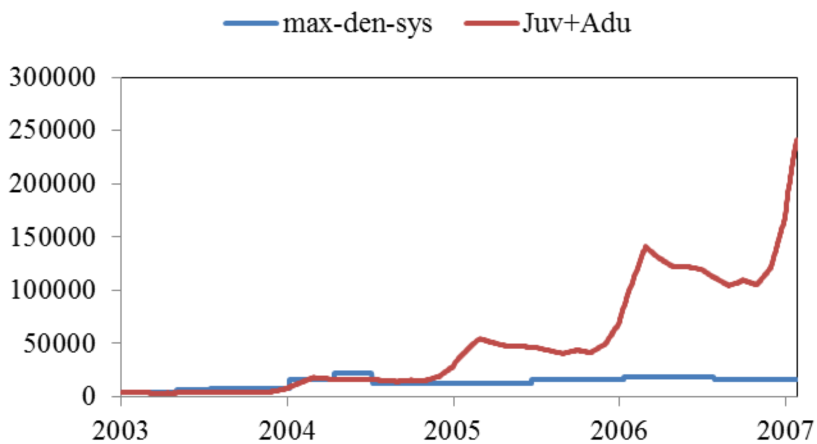
80000
60000
40000
20000
0
2003
2004
2005
2006
2007
2008

(b)



max-den-sys
Juv+Adu
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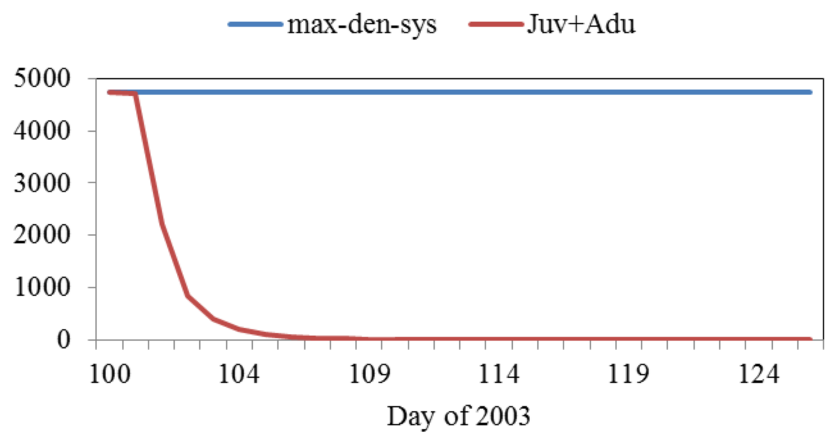
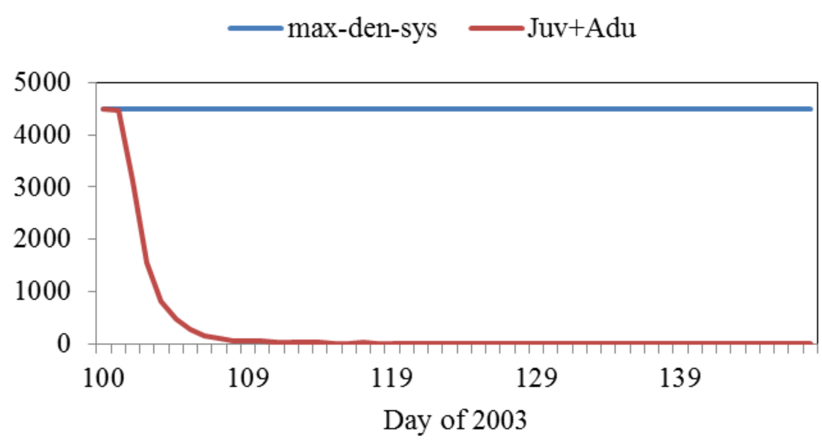
250000
200000
150000
100000
50000
0
2003
2004
2005
2006
2007

Figure 6

(c)



(d)



(e)

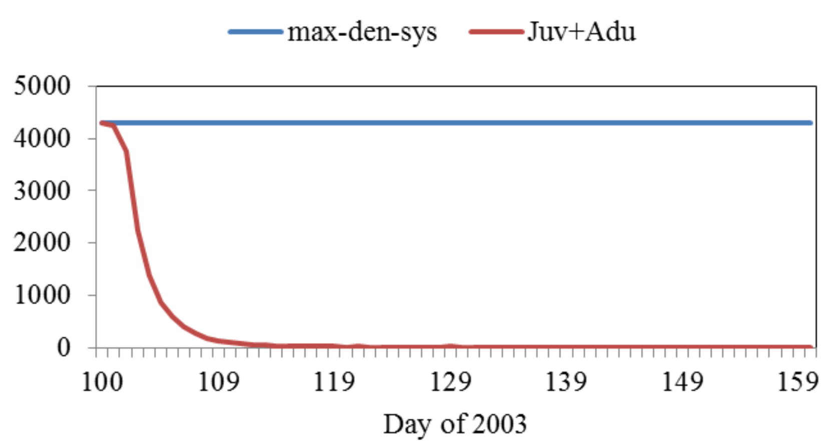


Figure 6 Cont.

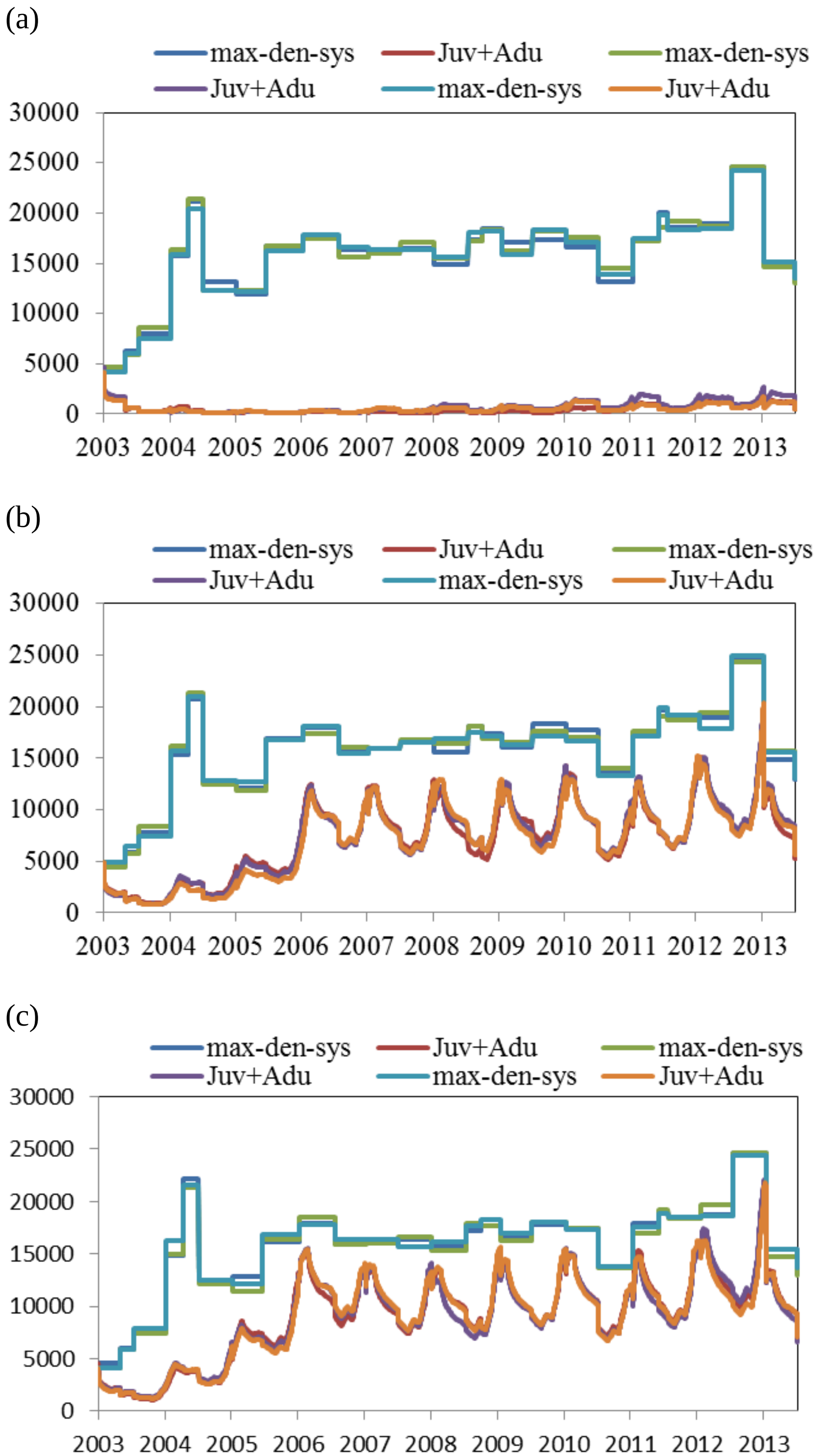
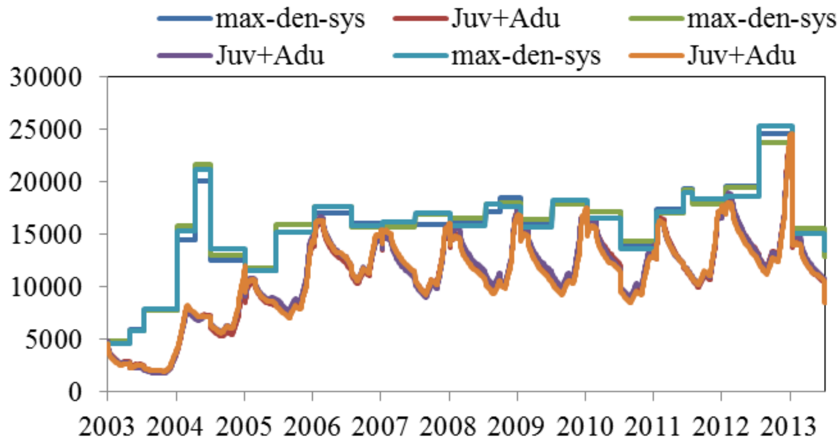
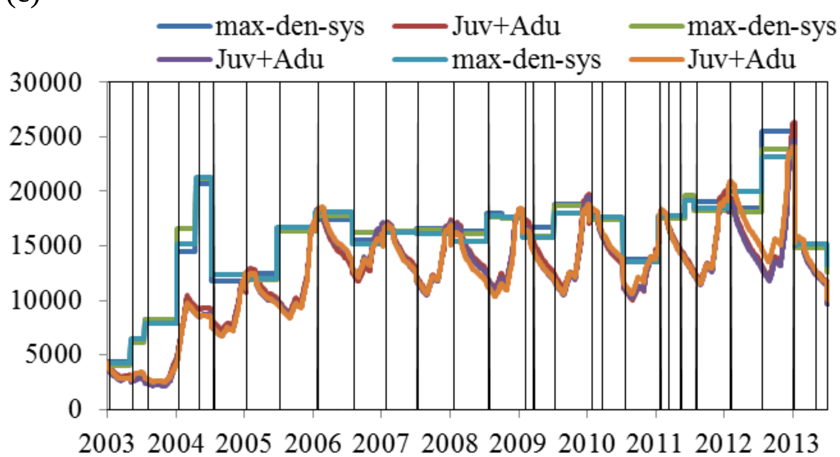

(a)
max-den-sys
Juv+Adu
max-den-sys
Juv+Adu
max-den-sys
Juv+Adu
30000
25000
20000
15000
10000
5000
0
2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013
(b)
max-den-sys
Juv+Adu
max-den-sys
Juv+Adu
max-den-sys
Juv+Adu
30000
25000
20000
15000
10000
5000
0
2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013
(c)
max-den-sys
Juv+Adu
max-den-sys
Juv+Adu
max-den-sys
Juv+Adu
30000
25000
20000
15000
10000
5000
0
2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013

Figure 7

(d)



(e)



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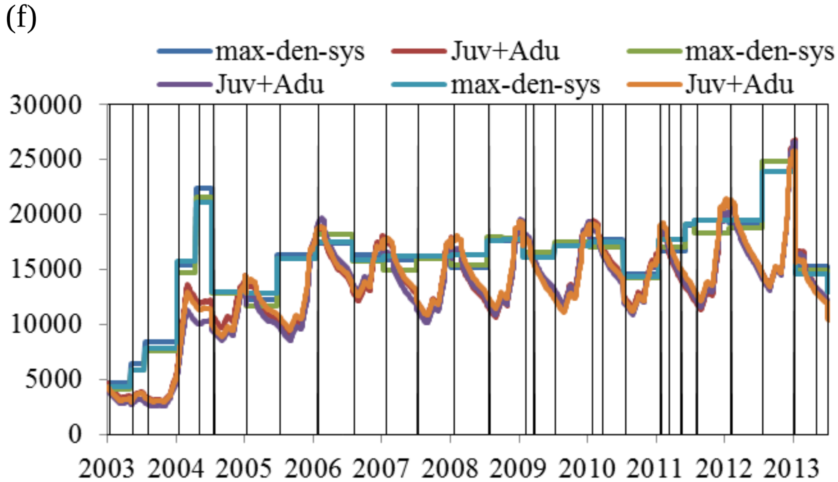
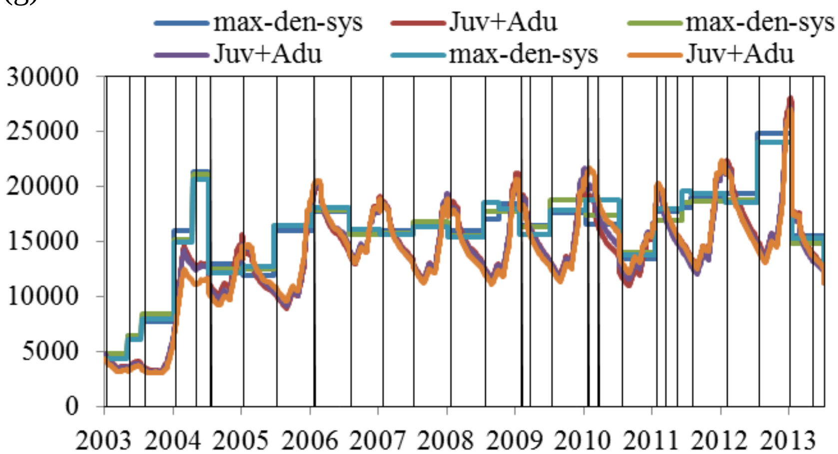


Figure 7 Cont.

(g)



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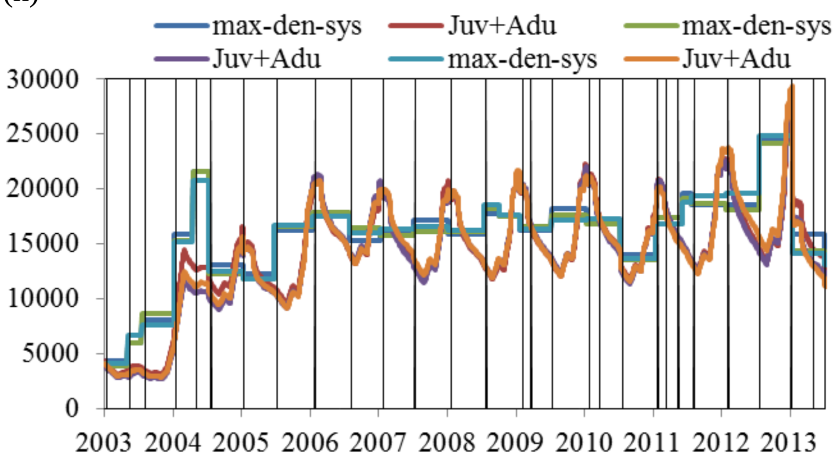

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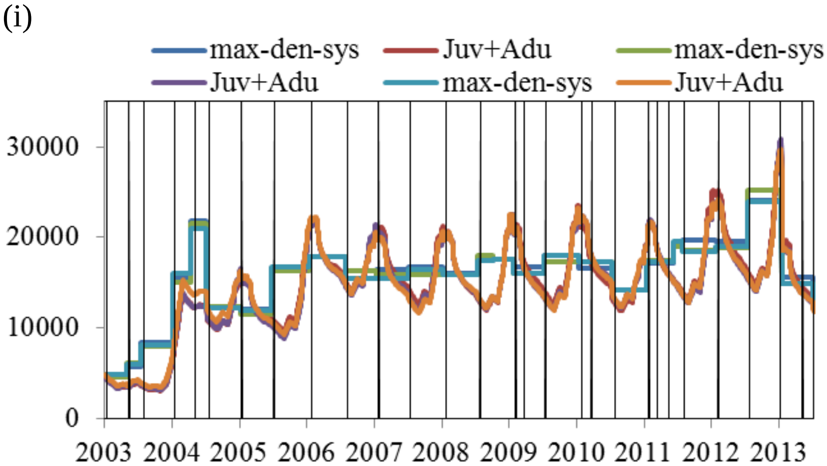
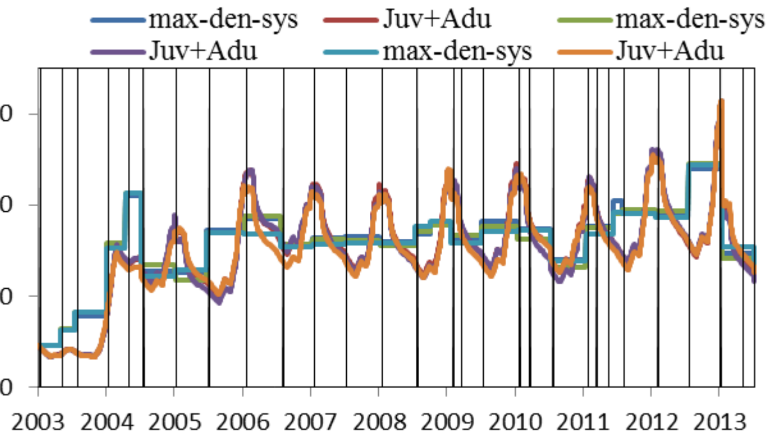


Figure 7 Cont.

(j)



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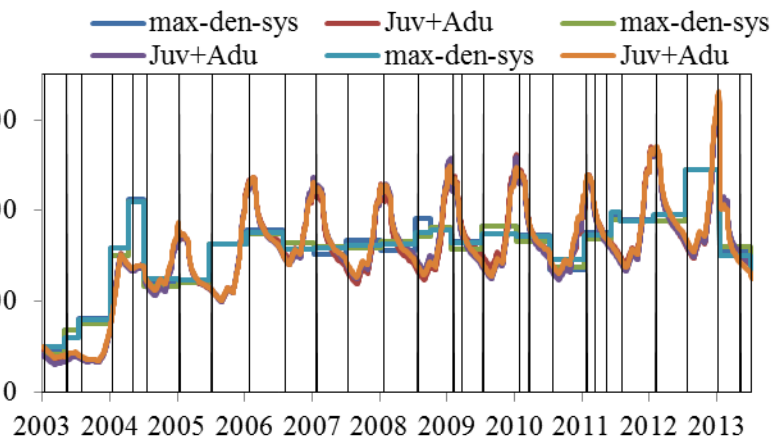
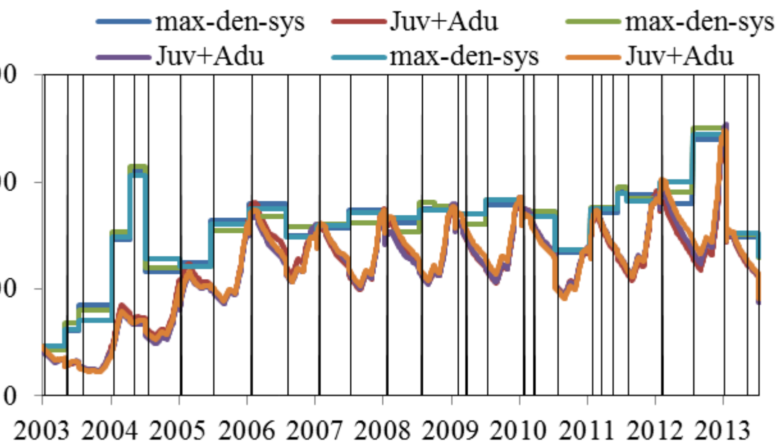


Figure 7 Cont.

(a)



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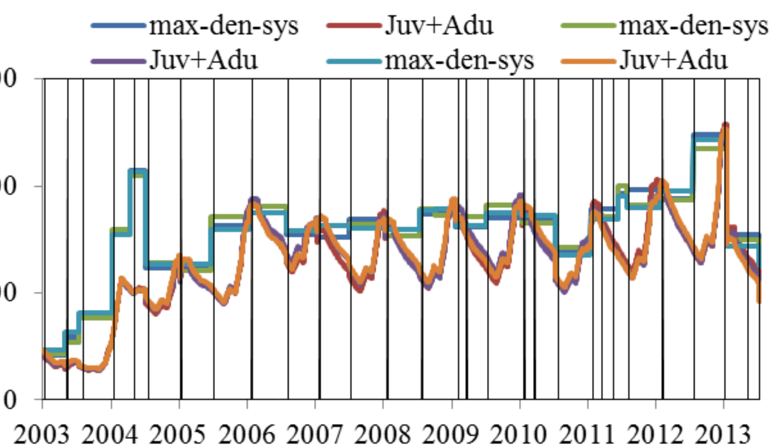

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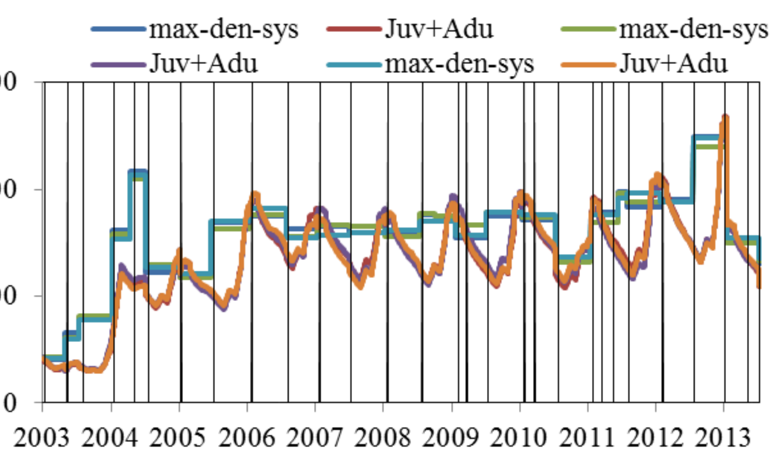
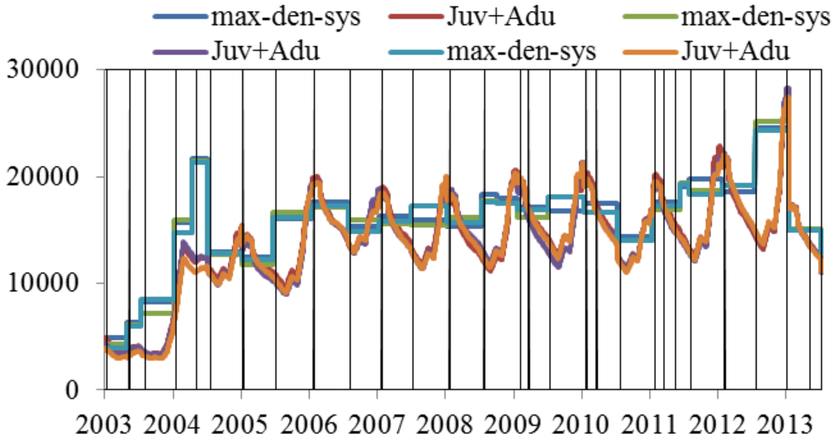


Figure 8 Cont.

(d)



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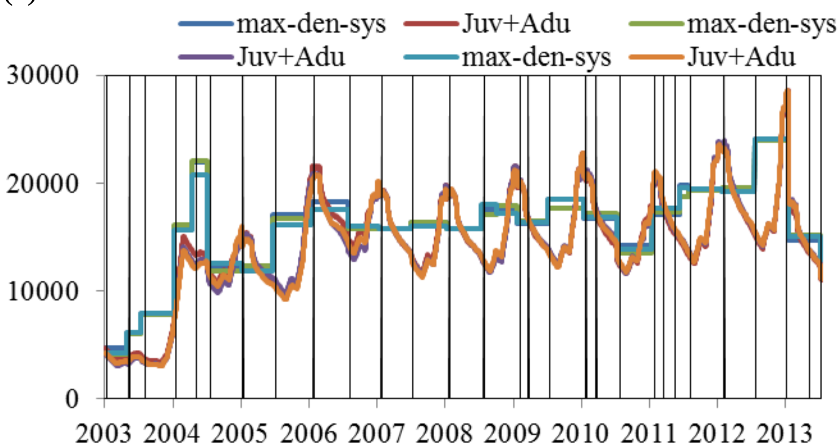
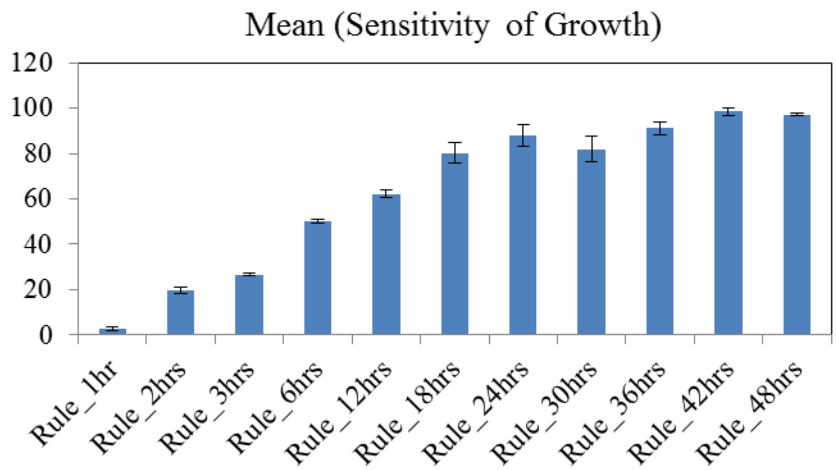


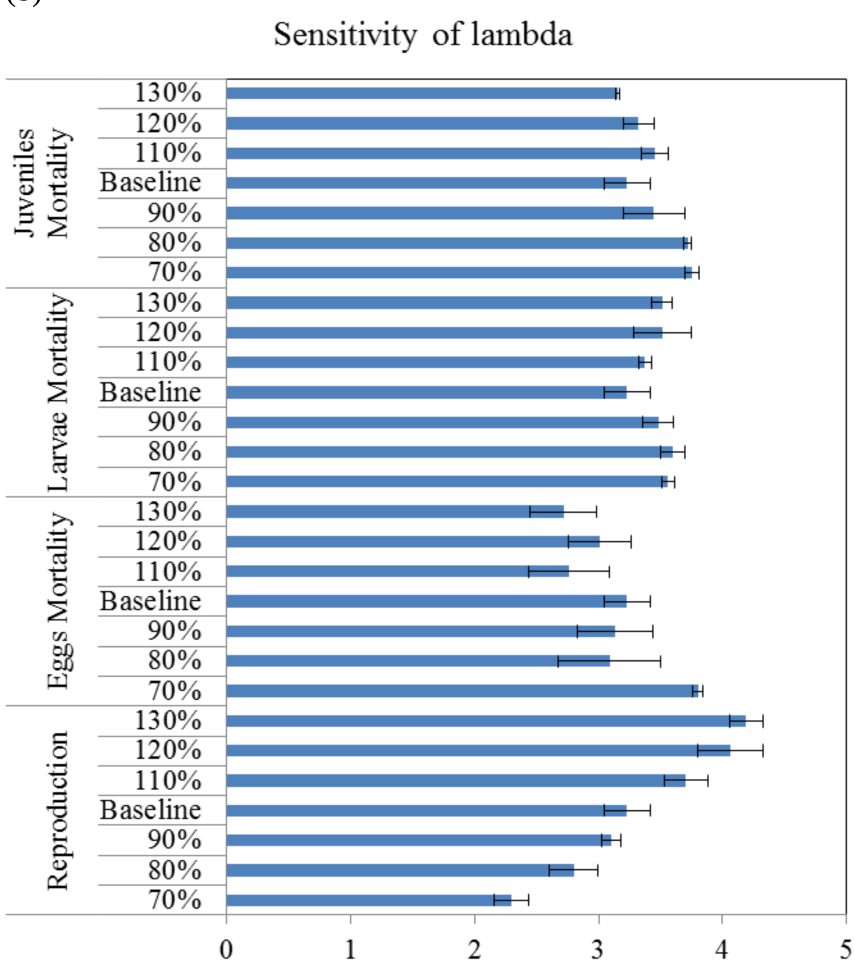
Figure 8 Cont.

(a)



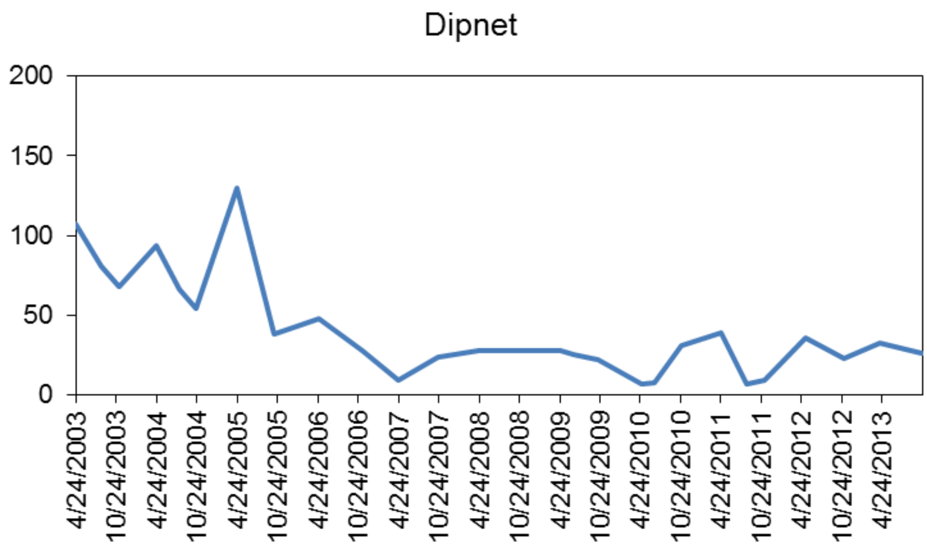
Mean (Sensitivity of Growth)
120
100
80
60
40
20
0
Rule_1hr
Rule_2hrs
Rule_3hrs
Rule_6hrs
Rule_12hrs
Rule_18hrs
Rule_24hrs
Rule_30hrs
Rule_36hrs
Rule_42hrs
Rule_48hrs

(b)



Sensitivity of lambda
Juveniles Mortality
Larvae Mortality
Eggs Mortality
Reproduction
130%
120%
110%
Baseline
90%
80%
70%
0
1
2
3
4
5

Figure 9



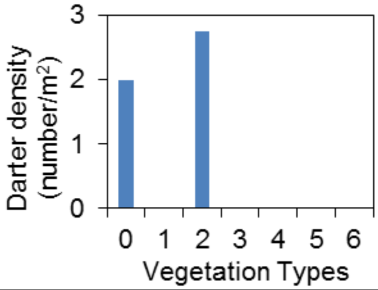
Dipnet
200
150
100
50
0
4/24/2003
10/24/2003
4/24/2004
10/24/2004
4/24/2005
10/24/2005
4/24/2006
10/24/2006
4/24/2007
10/24/2007
4/24/2008
10/24/2008
4/24/2009
10/24/2009
4/24/2010
10/24/2010
4/24/2011
10/24/2011
4/24/2012
10/24/2012
4/24/2013

Figure 10

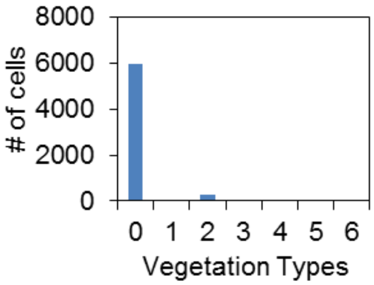
2000

Fall (Nov. 14th)

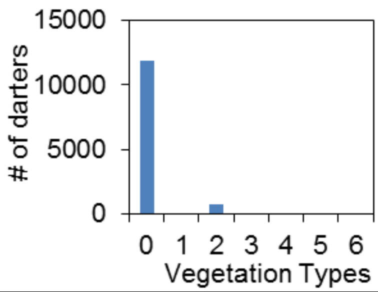
Darter density (#/m^2) in each veg type



# of cells of each veg type



Total # of darter in each veg cell



Total # of darters

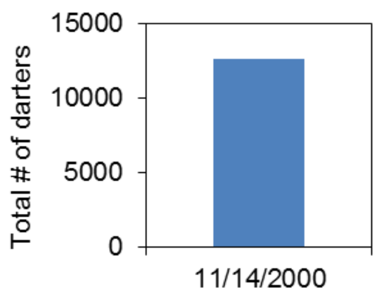
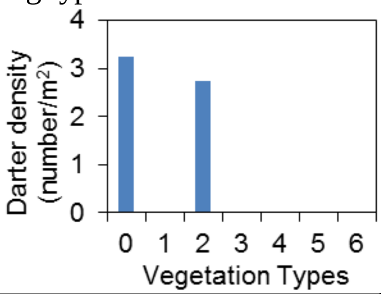


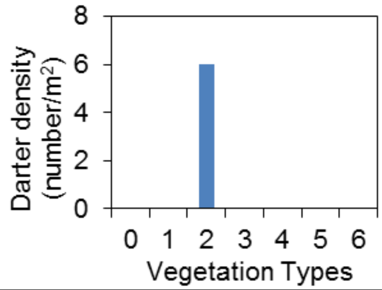
Figure 11

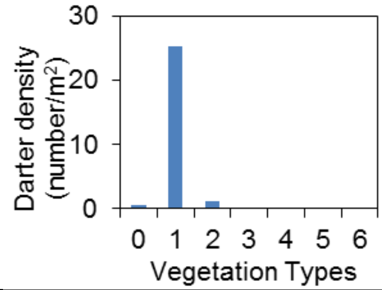
2001

Winter (Mar.15th)	Spring (May 17th)	Summer (Aug. 23rd)

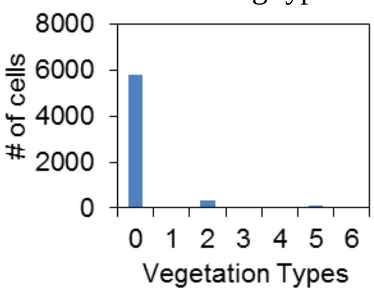
Darter density (#/m²) in each veg type

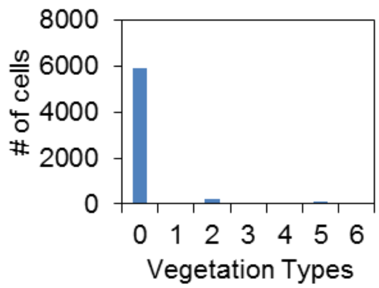


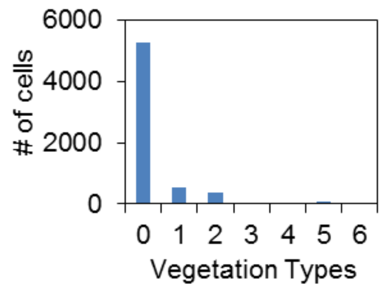




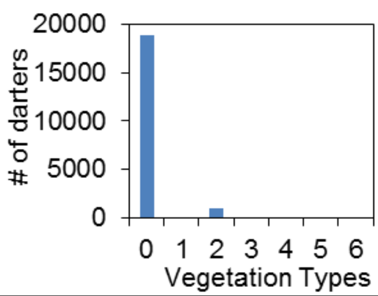
# of cells of each veg type

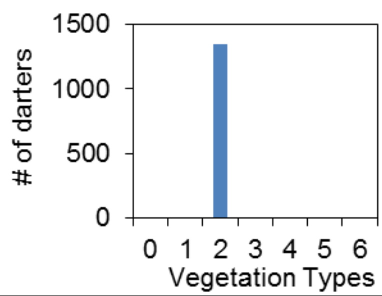


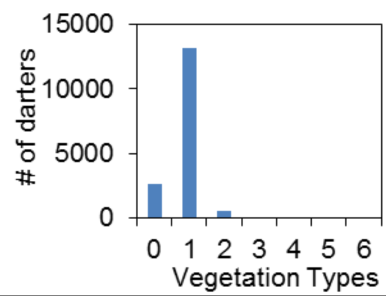




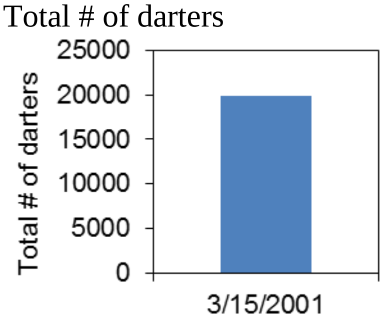
Total # of darter in each veg cell

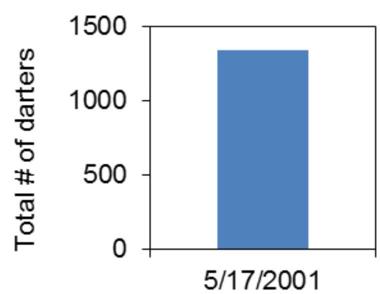






Total # of darters





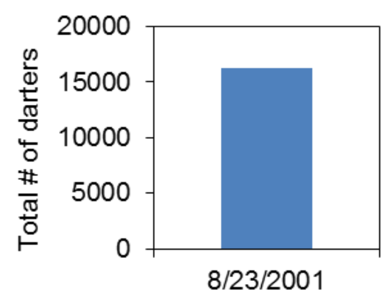


Figure 11 Cont.

2001

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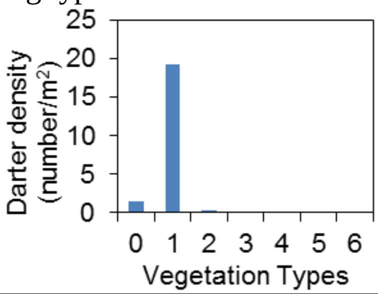
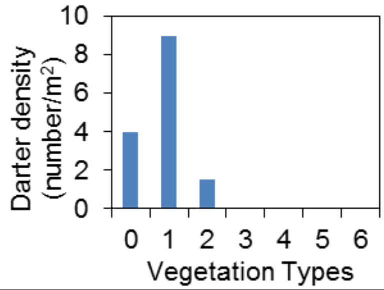
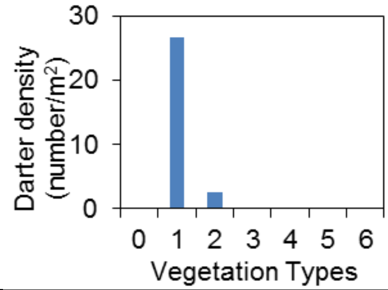

</td><td>
</td><td>
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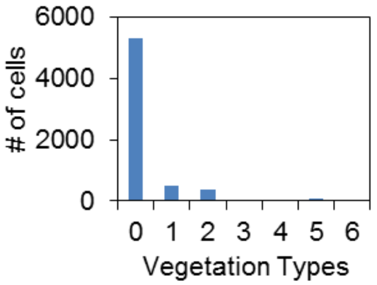
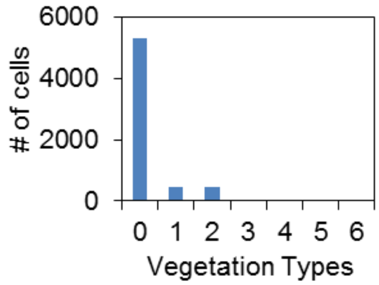
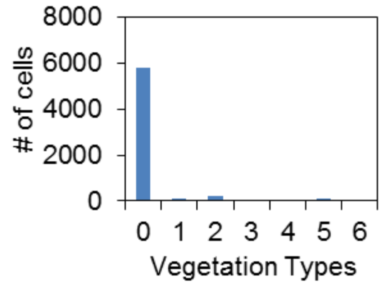

</td><td>
</td><td>
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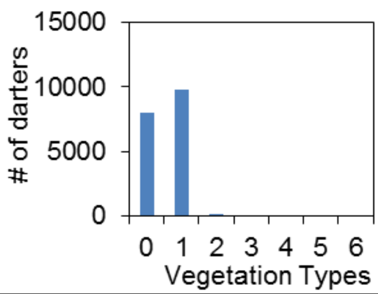
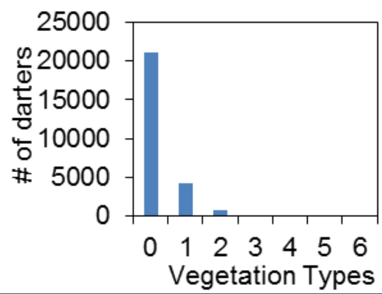
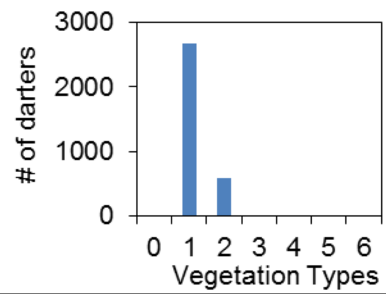
<tr><td>Total # of darter in each veg cell

</td><td>
</td><td>
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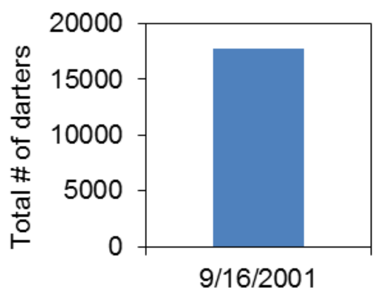
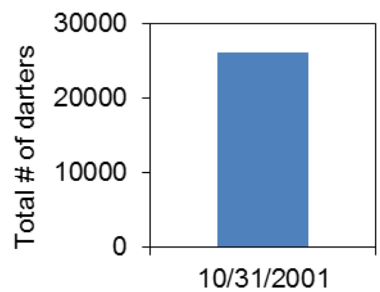
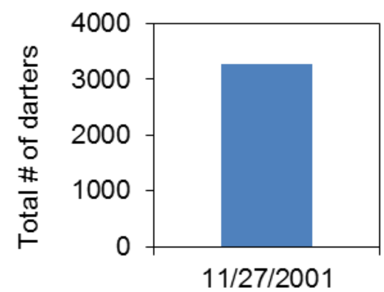

</td><td>
</td><td>
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Figure 11 Cont.

2002

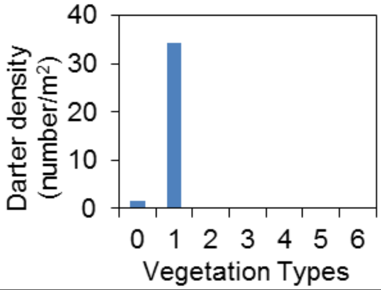
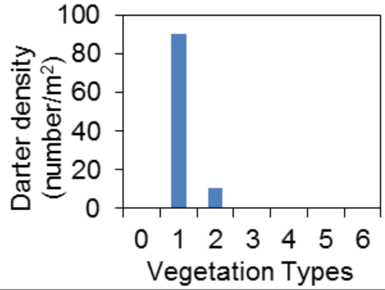
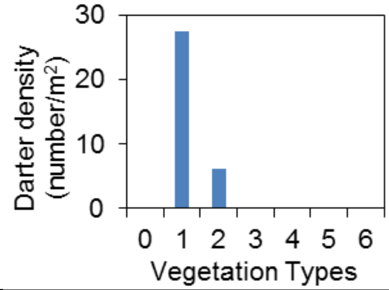
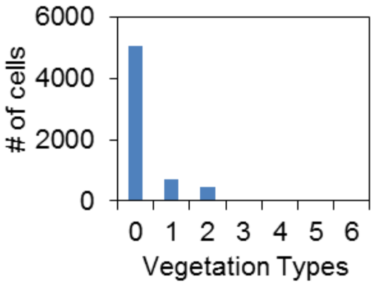
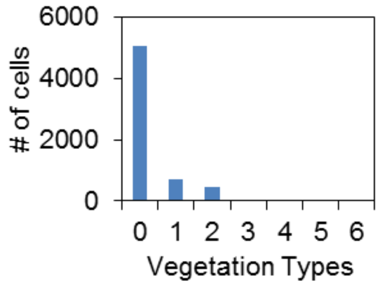
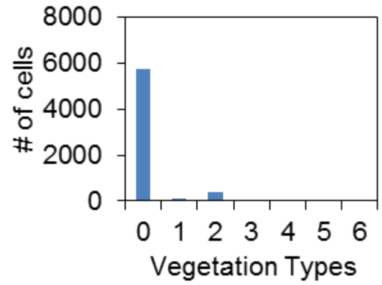
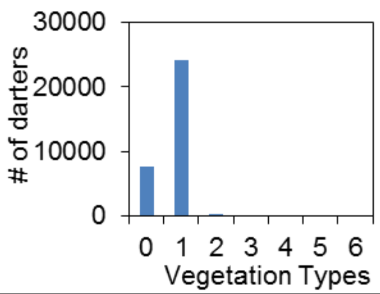
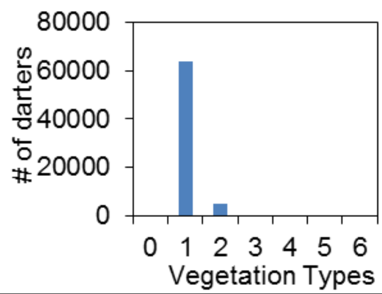
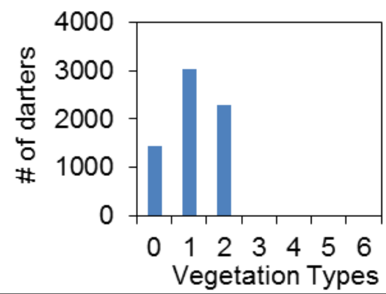
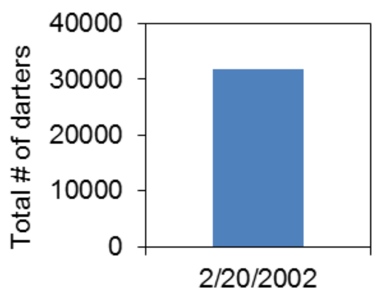
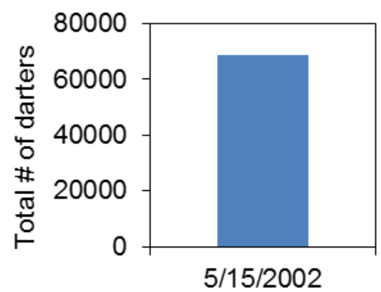
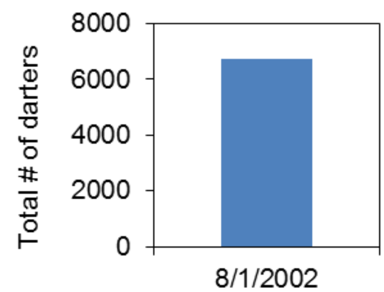
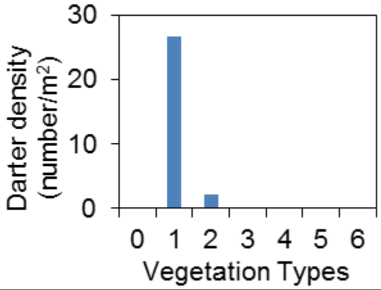
Winter (Feb. 20th)	Spring (May 15th)	Summer (Aug. 1st)
Darter density (#/m^2) in each veg type 		
# of cells of each veg type 		
Total # of darter in each veg cell 		
Total # of darters 		

Figure 11 Cont.

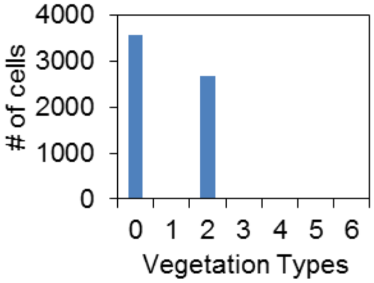
2002

Fall (Oct. 28th)

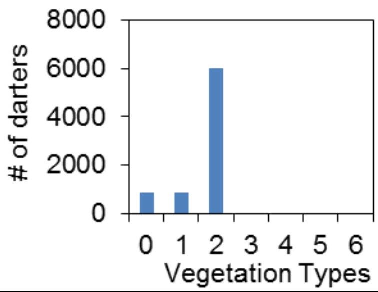
Darter density (#/m^2) in each vege type



# of cells of each veg type



Total # of darter in each veg cell



Total # of darters

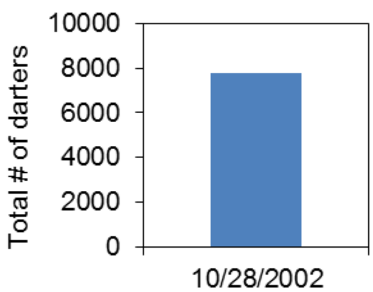


Figure 11 Cont.

2003

<table>
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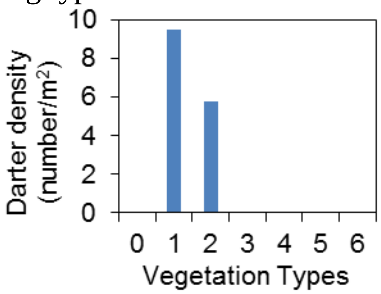
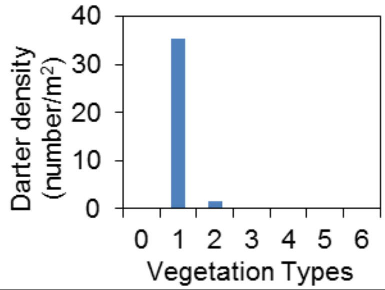
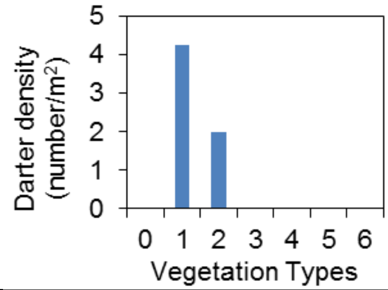
</td><td></td><td></td></tr>
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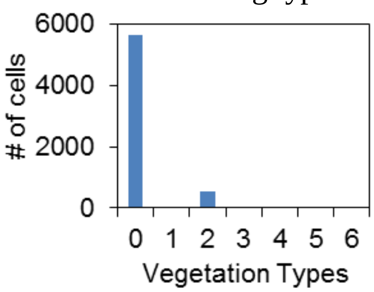
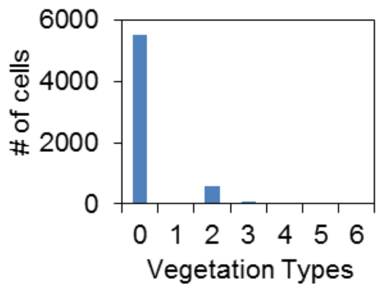
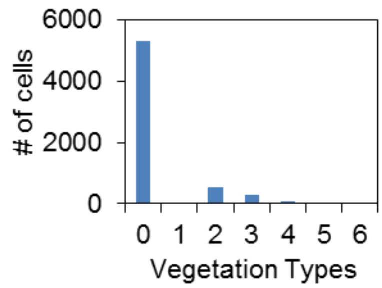
</td><td></td><td></td></tr>
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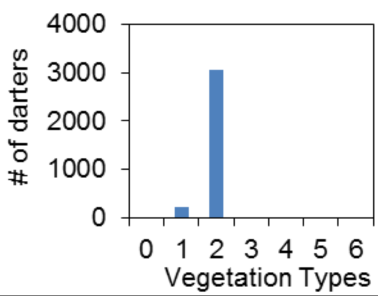
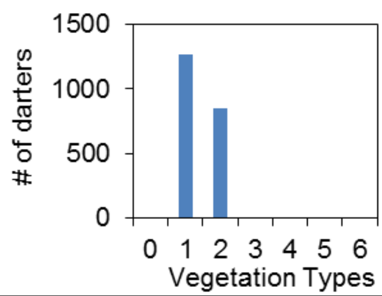
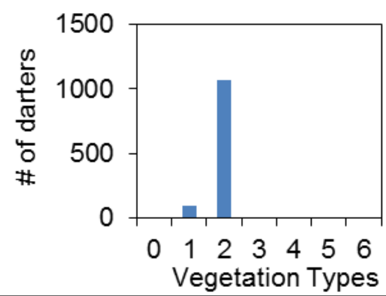
</td><td></td><td></td></tr>
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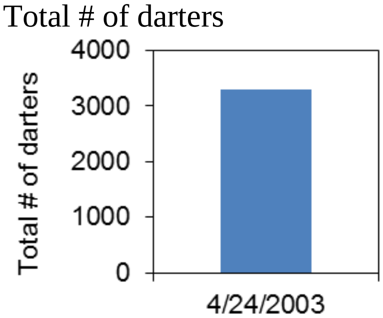
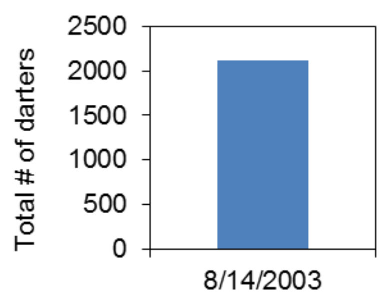
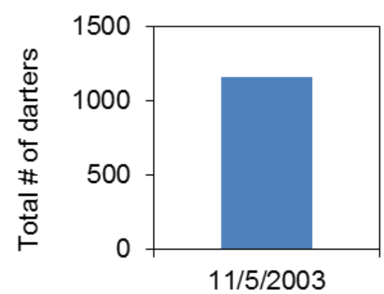
</td><td></td><td></td></tr>
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Figure 11 Cont.

2004

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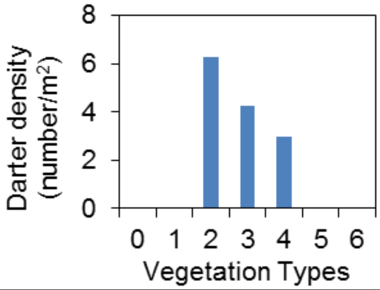
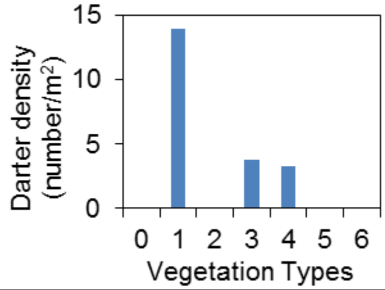
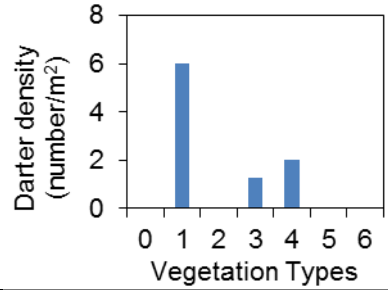

</td><td>
</td><td>
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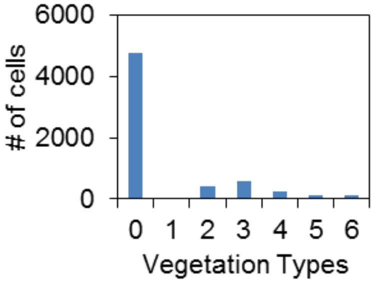
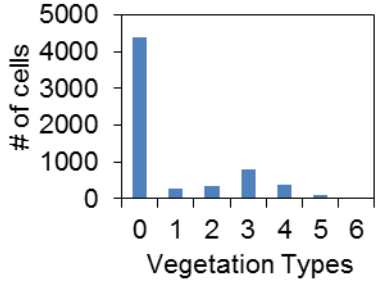
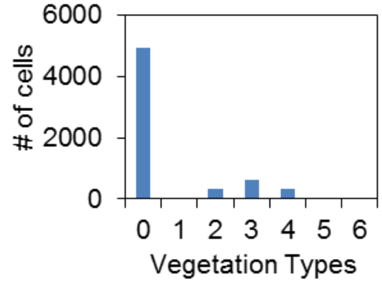

</td><td>
</td><td>
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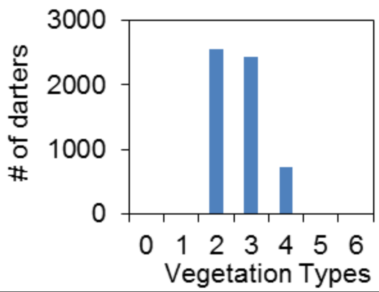
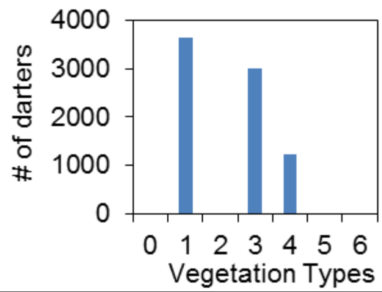
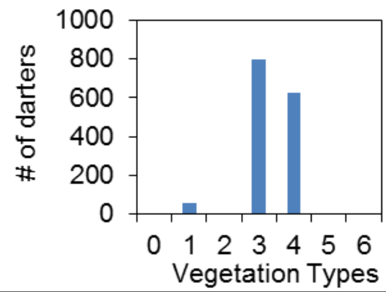

</td><td>
</td><td>
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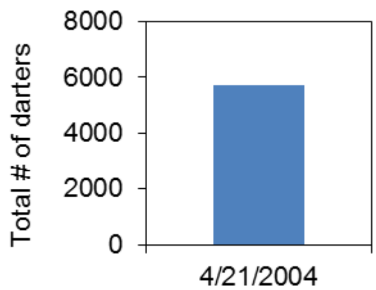
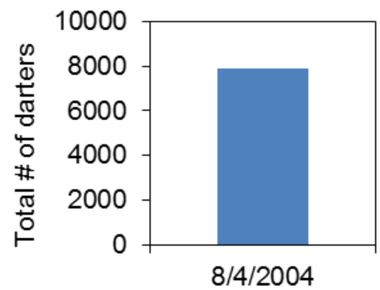
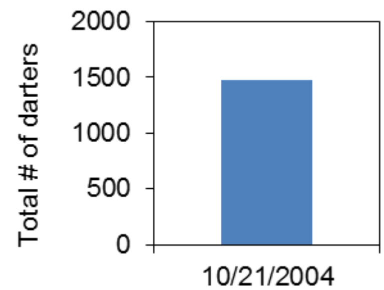

</td><td>
</td><td>
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Figure 11 Cont.

2005

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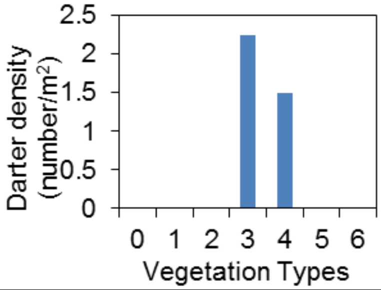
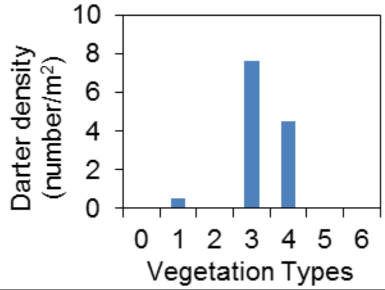

</td><td>
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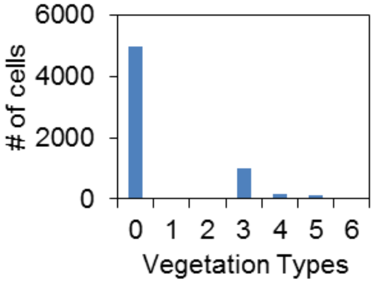
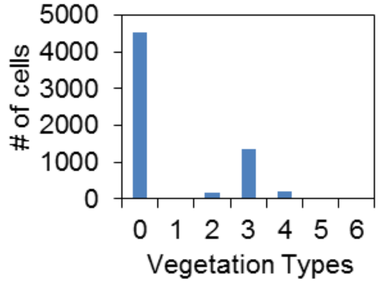

</td><td>
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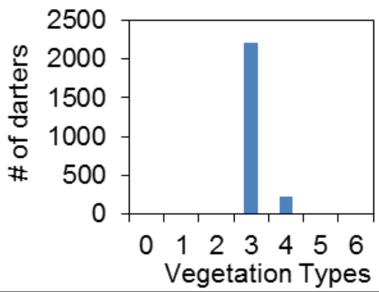
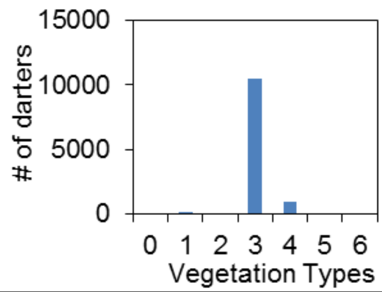

</td><td>
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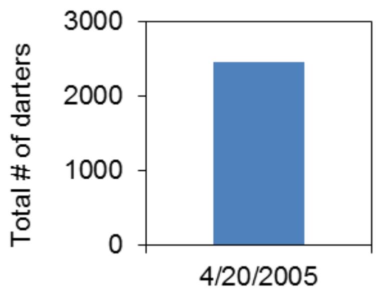
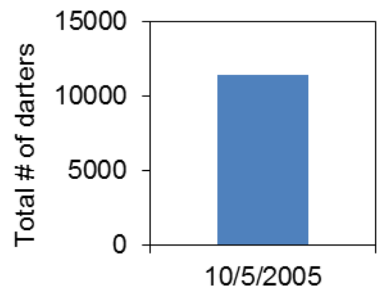

</td><td>
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Figure 11 Cont.

2006

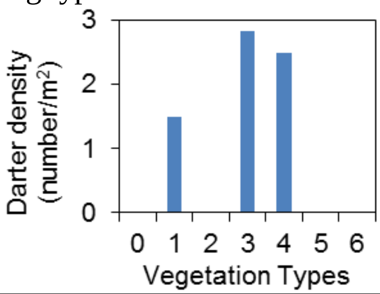
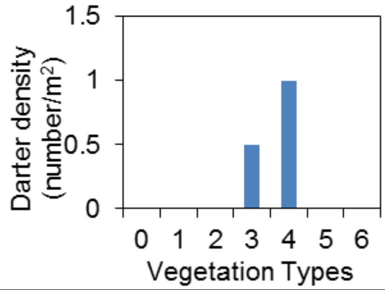
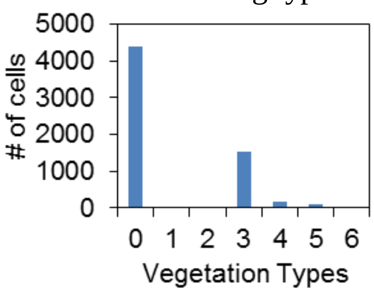
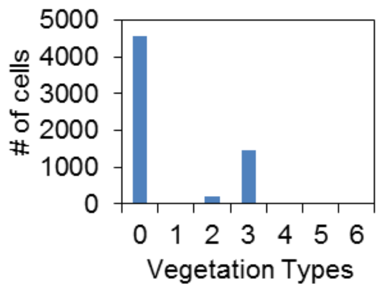
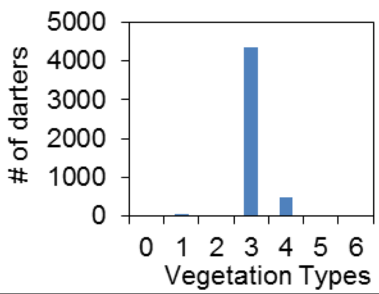
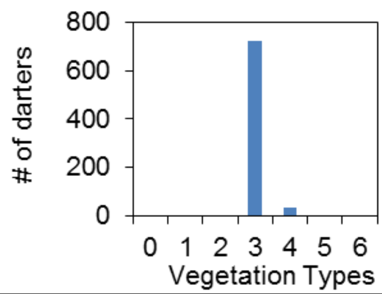
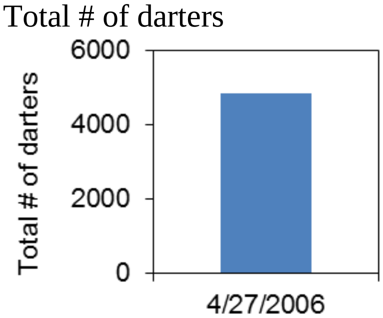
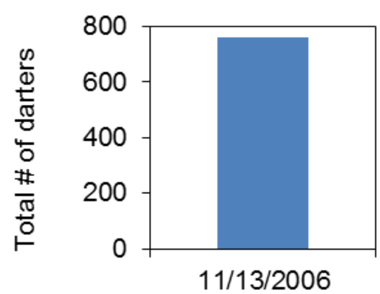
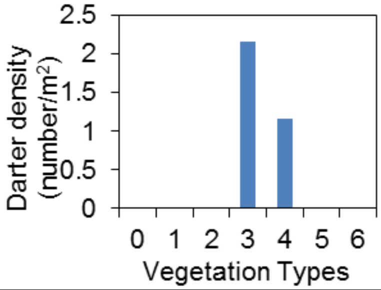
Spring (Apr. 27th)	Fall (Nov. 13th)
Darter density (#/m^2) in each veg type 	
# of cells of each veg type 	
Total # of darter in each veg cell 	
Total # of darters 	

Figure 11 Cont.

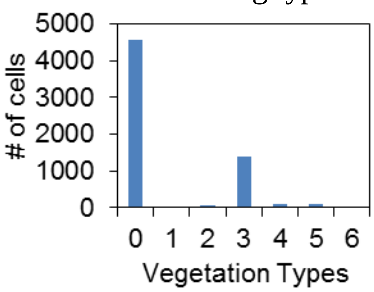
2007

Spring (Apr. 24th)

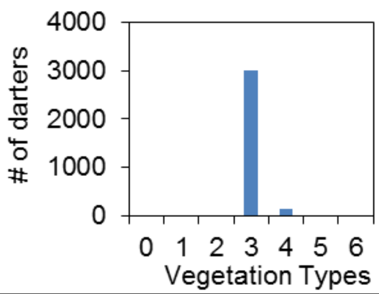
Darter density (#/m^2) in each veg type



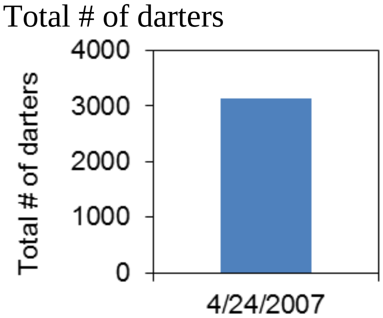
# of cells of each veg type



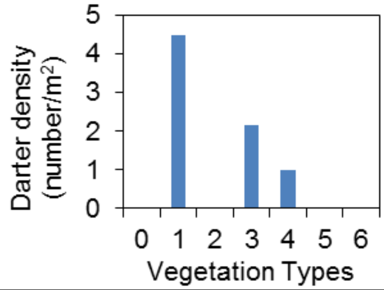
Total # of darter in each veg cell

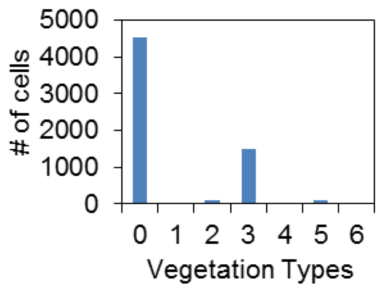


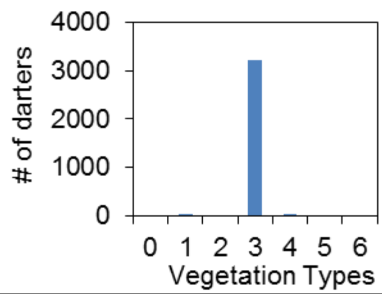
Total # of darters



Fall (Oct. 18th)







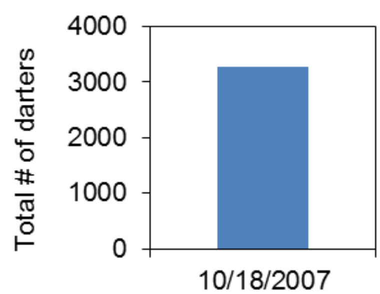


Figure 11 Cont.

2008

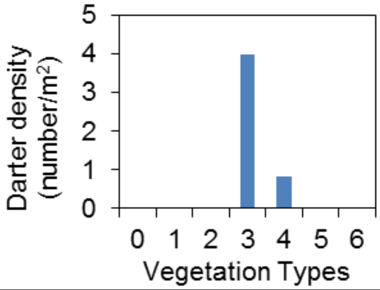
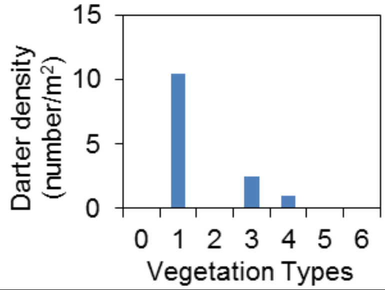
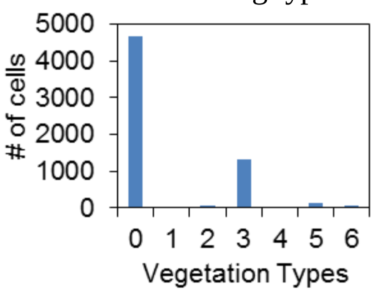
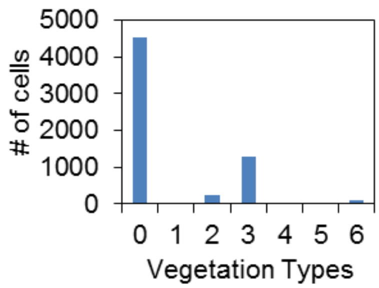
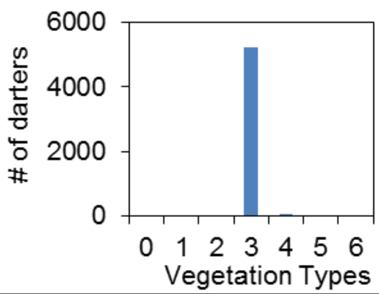
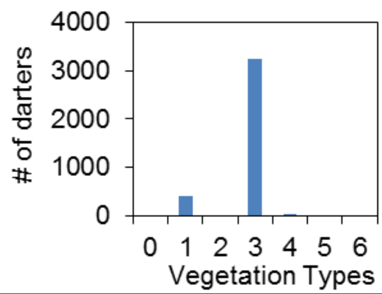
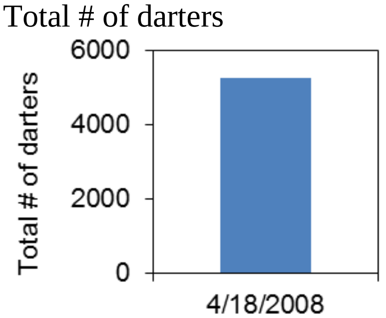
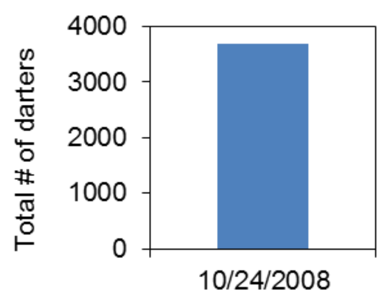
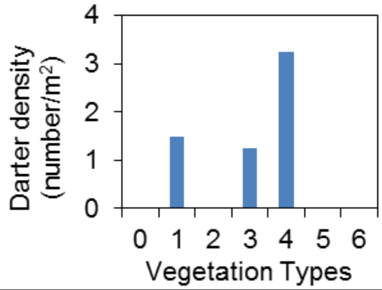
Spring (Apr. 18th)	Fall (Oct. 24th)
Darter density (#/m^2) in each veg type 	
# of cells of each veg type 	
Total # of darter in each veg cell 	
Total # of darters 	

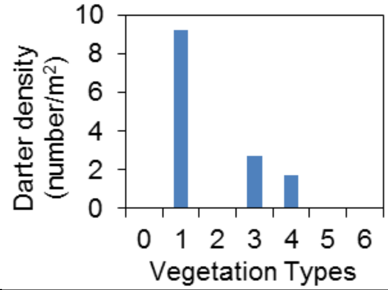
Figure 11 Cont.

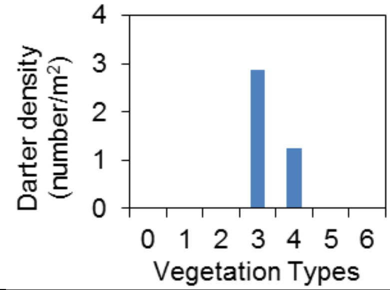
2009

Spring (Apr. 27th)	Critical Period (Jun. 26th)	Fall (Oct. 15th)

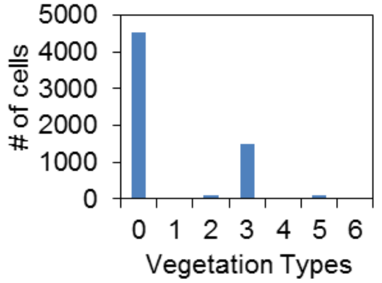
Darter density (#/m^2) in each veg type

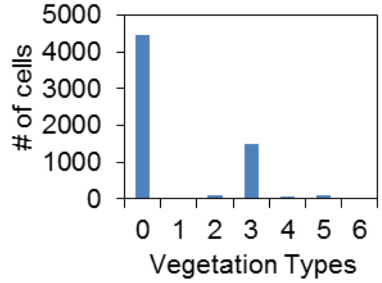


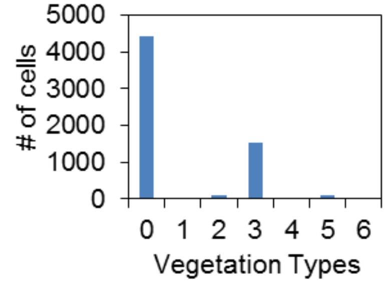




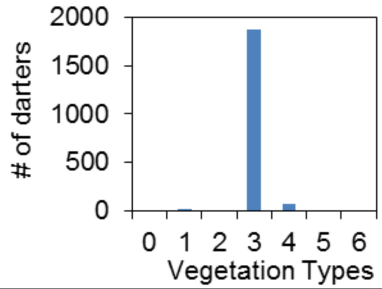
# of cells of each veg type

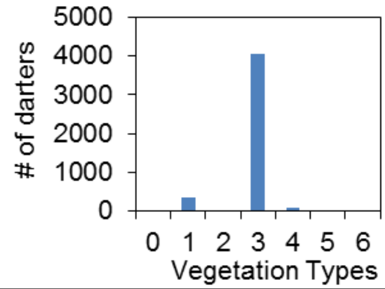


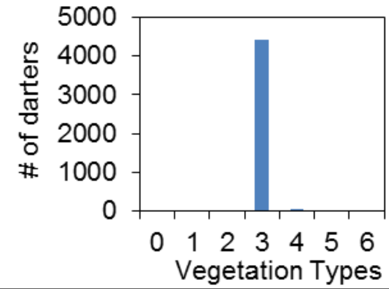




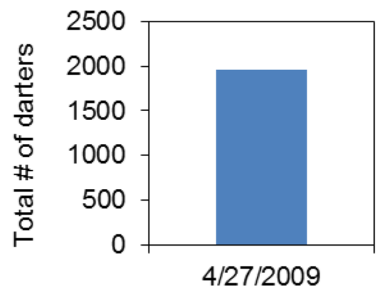
Total # of darter in each veg cell

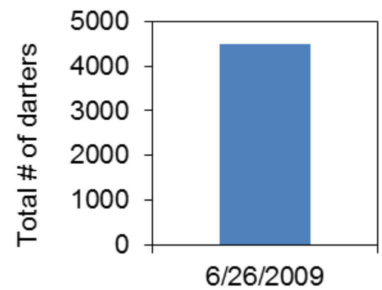






Total # of darters





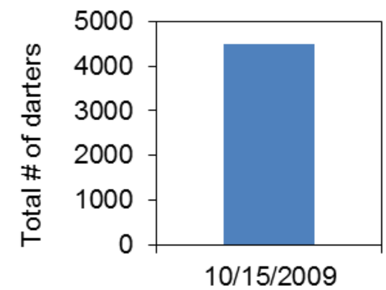


Figure 11 Cont.

2010

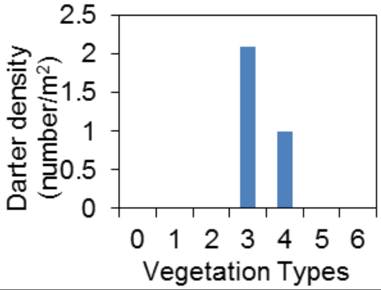
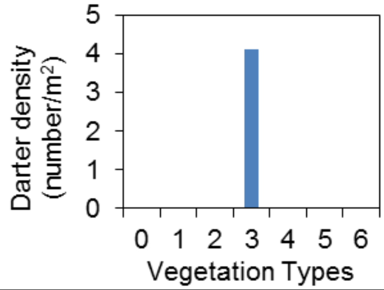
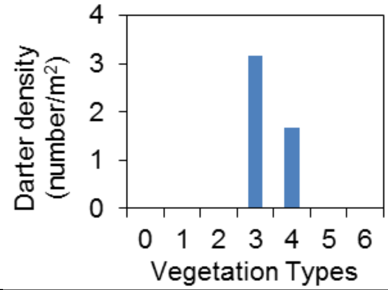
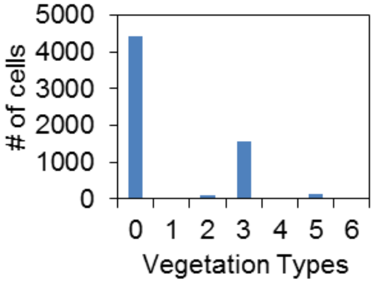
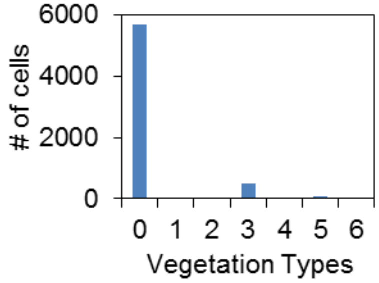
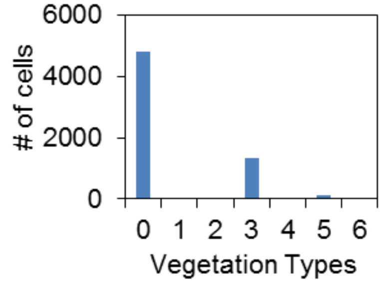
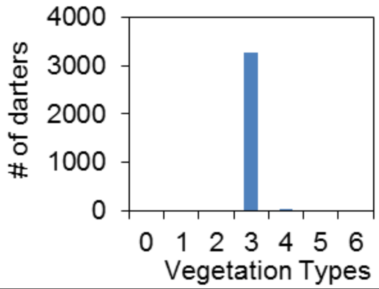
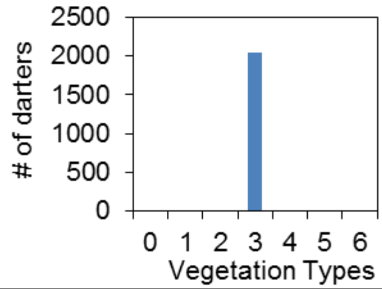
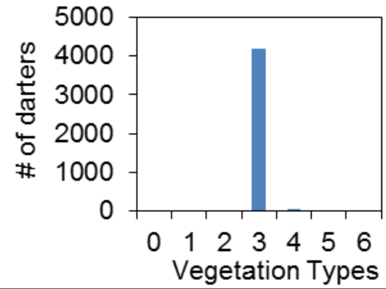
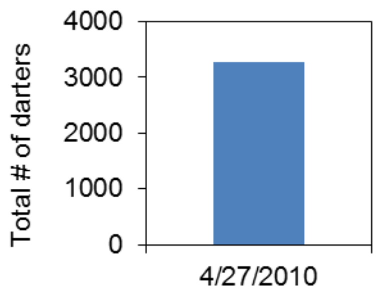
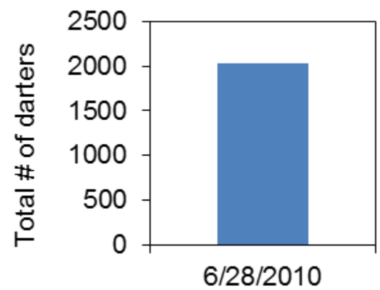
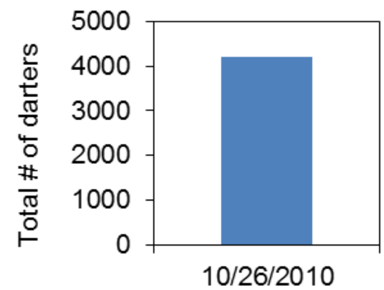
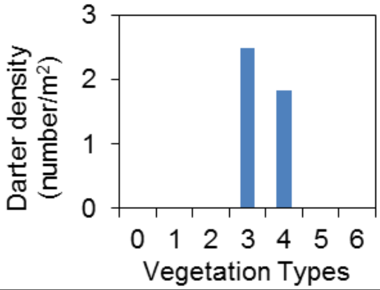
Spring (Apr. 27th)	Critical Period (Jun. 28th)	Fall (Oct. 26th)
Darter density (#/m^2) in each veg type  	 	
# of cells of each veg type  	 	
Total # of darter in each veg cell  	 	
Total # of darters  	 	

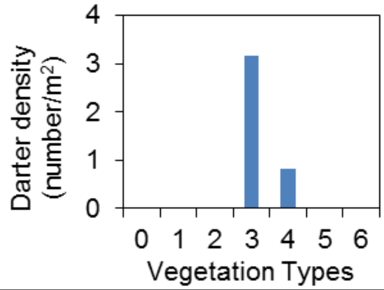
Figure 11 Cont.

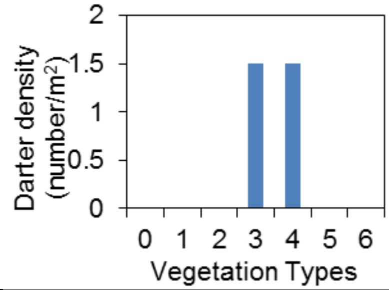
2011

Spring (Apr. 25th) | **Critical Period (Aug. 17th)** | **Fall (Nov. 8th)**

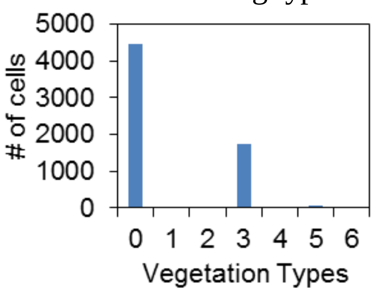
Darter density (#/m^2) in each veg type

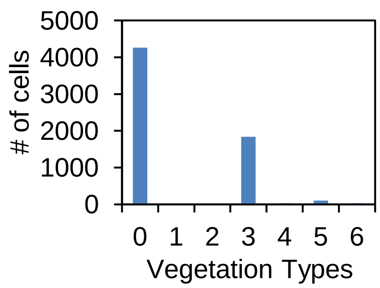


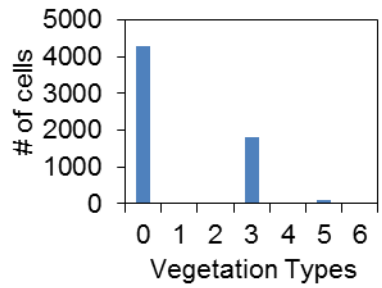




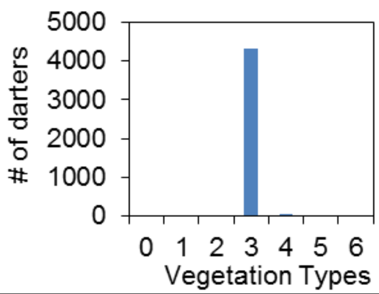
# of cells of each veg type

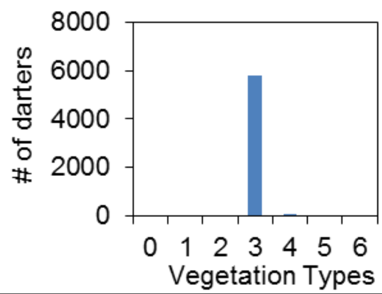


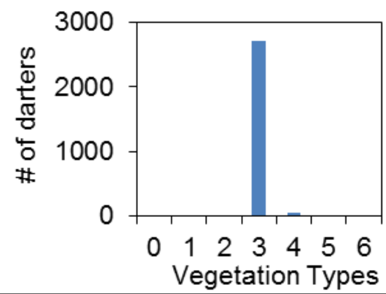




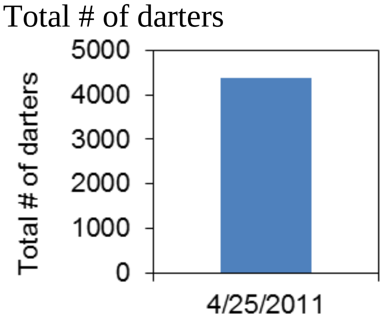
Total # of darter in each veg cell

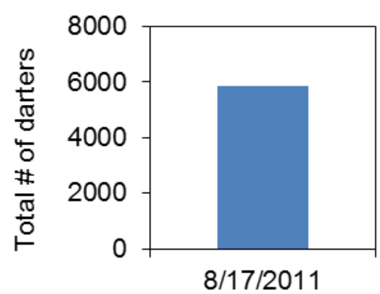






Total # of darters





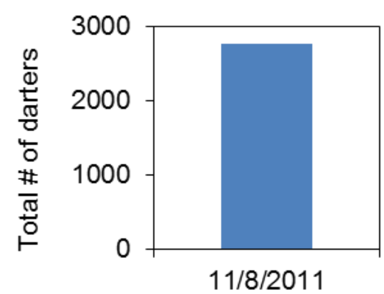


Figure 11 Cont.

2012

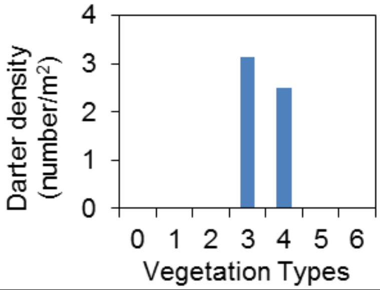
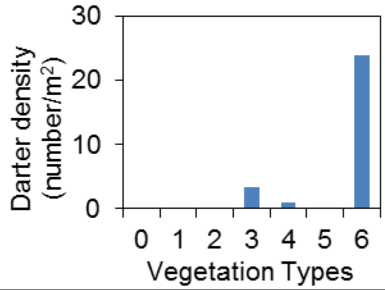
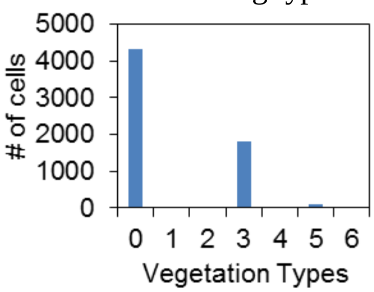
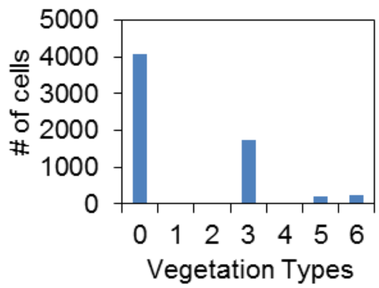
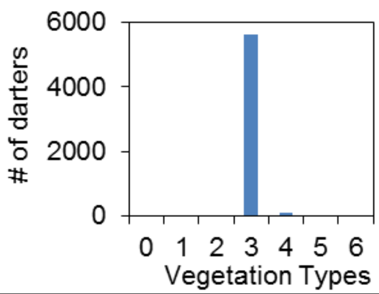
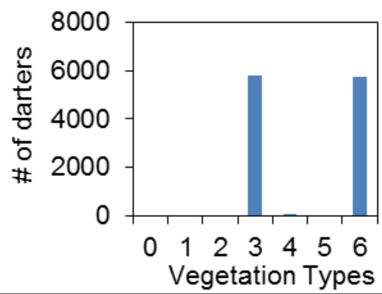
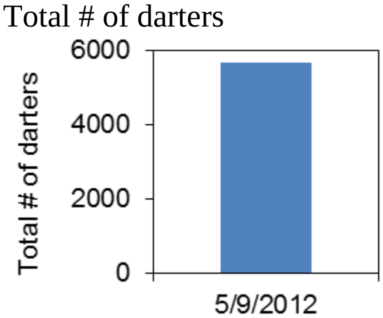
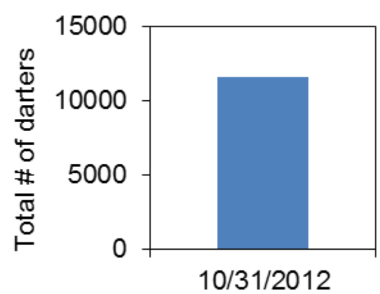
Spring (May 9th)	Fall (Oct. 31st)
Darter density (#/m²) in each veg type 	
# of cells of each veg type 	
Total # of darter in each veg cell 	
Total # of darters 	

Figure 11 Cont.

2013

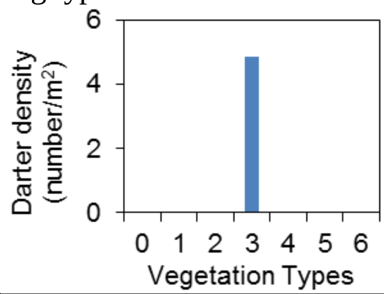
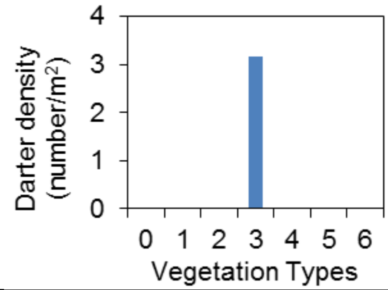
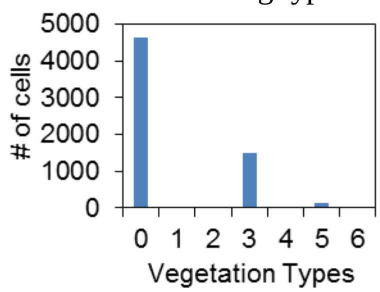
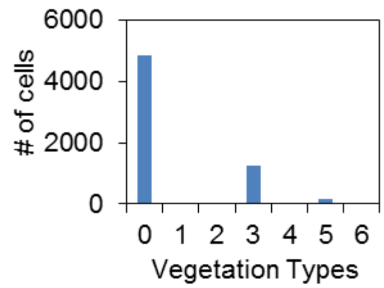
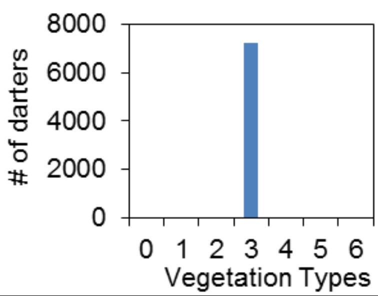
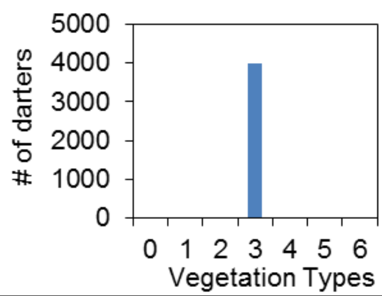
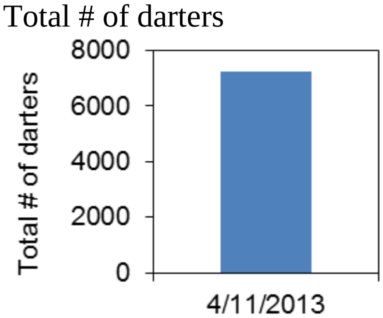
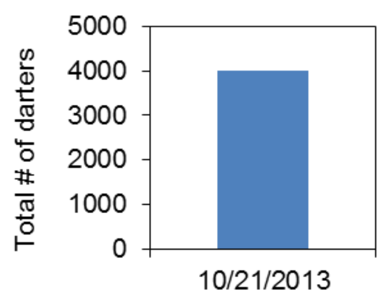
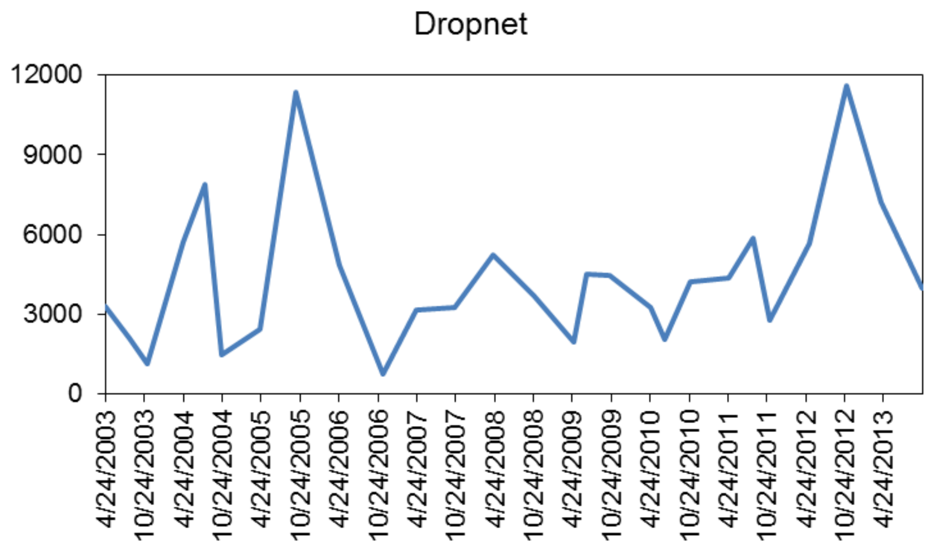
Spring (Apr. 11th)	Fall (Oct. 21st)
Darter density (#/m^2) in each veg type 	
# of cells of each veg type 	
Total # of darter in each veg cell 	
Total # of darters 	

Figure 11 Cont.

(a)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9000
6000
3000
0
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10/24/2003
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10/24/2004
4/24/2005
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10/24/2006
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4/24/2009
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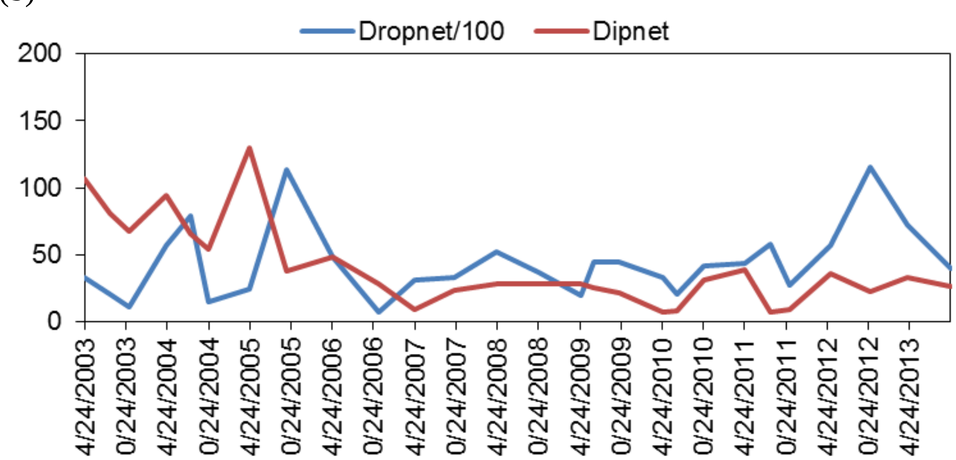
4/24/2013

(b)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Dipnet
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150
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50
0
4/24/2003
10/24/2003
4/24/2004
10/24/2004
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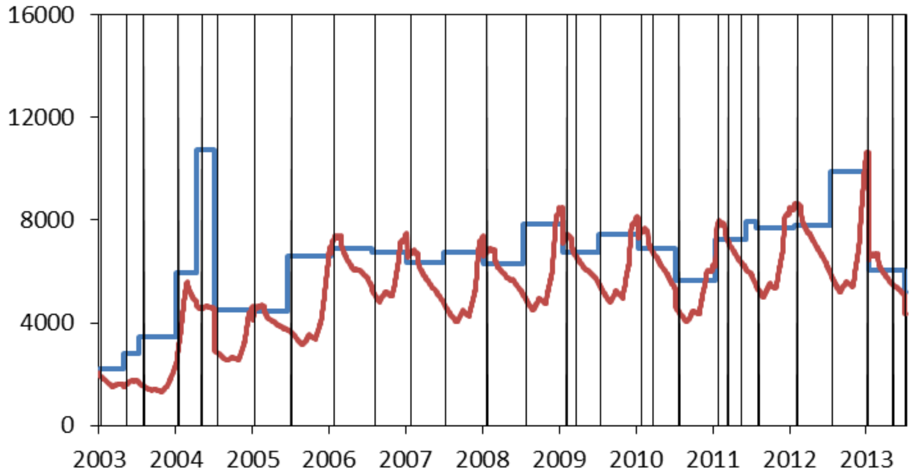
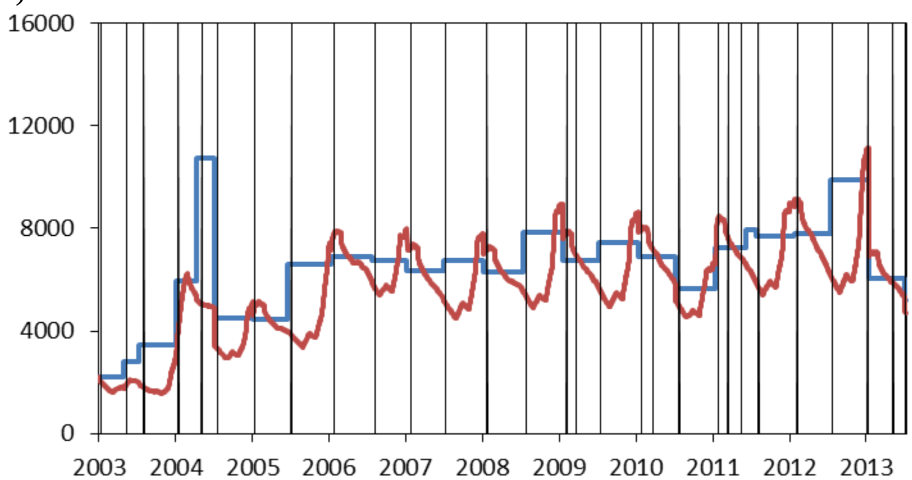
10/24/2008
4/24/2009
10/24/2009
4/24/2010
10/24/2010
4/24/2011
10/24/2011
4/24/2012
10/24/2012
4/24/2013

Figure 12

(a)



(b)



(c)

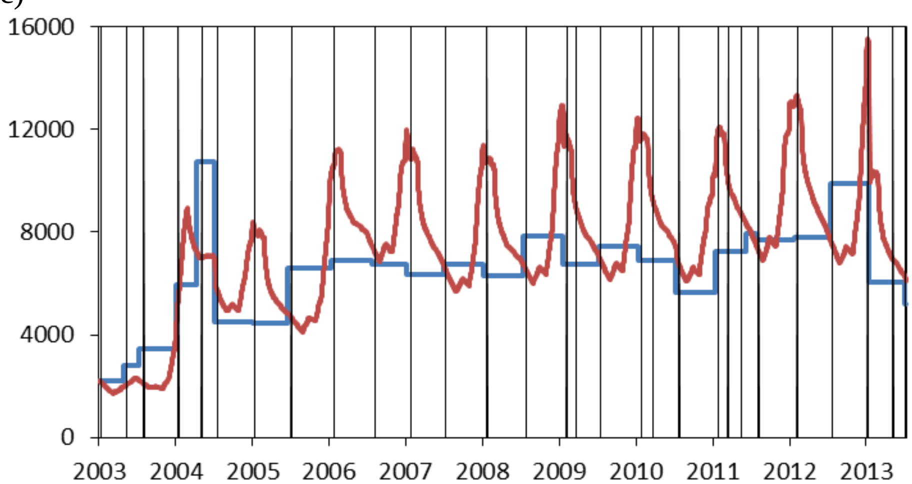
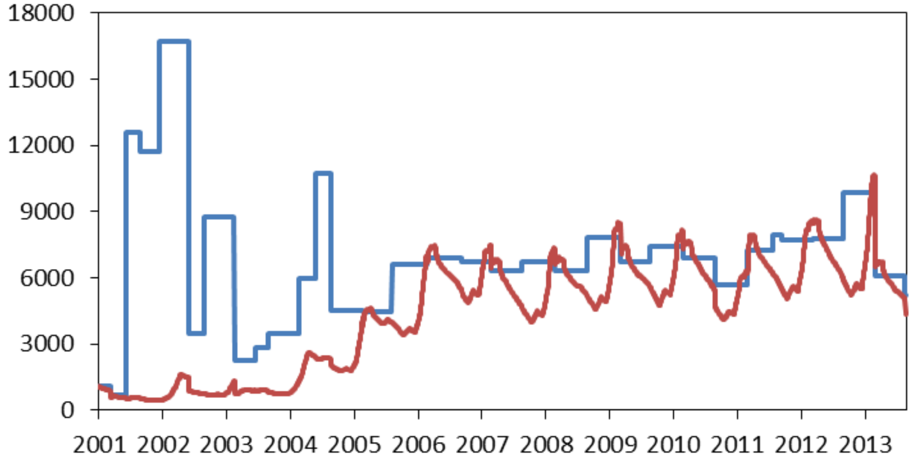
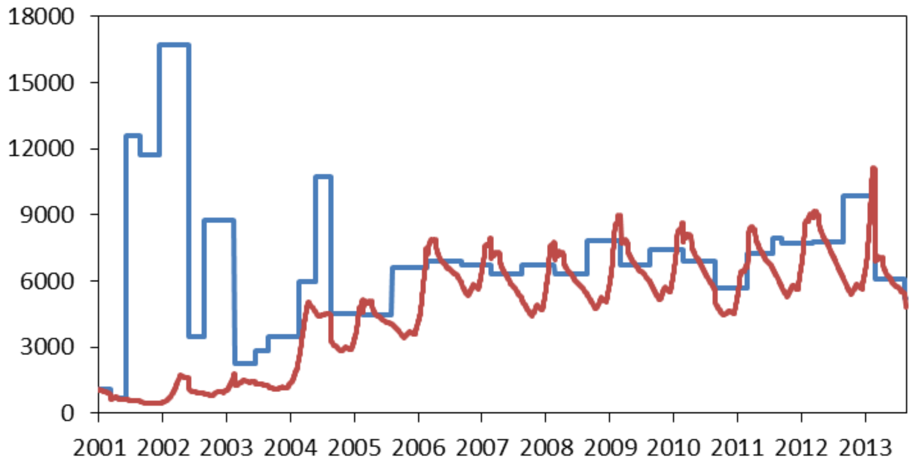


Figure 13

(a)



(b)



(c)

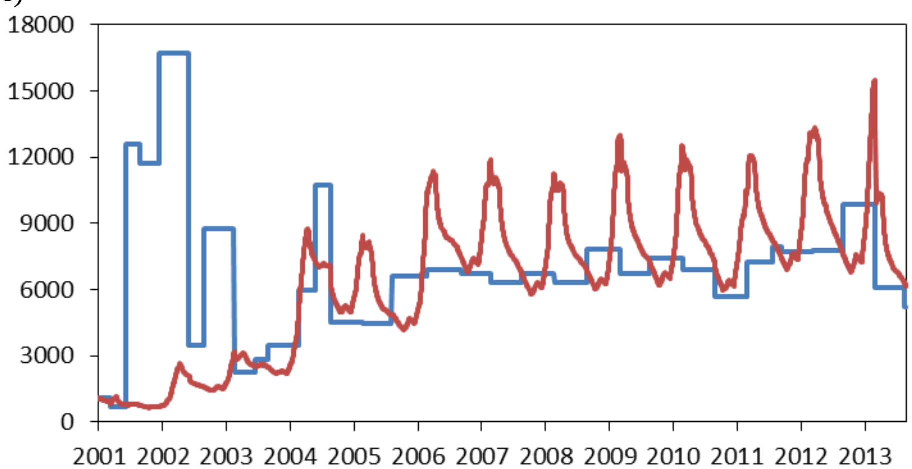
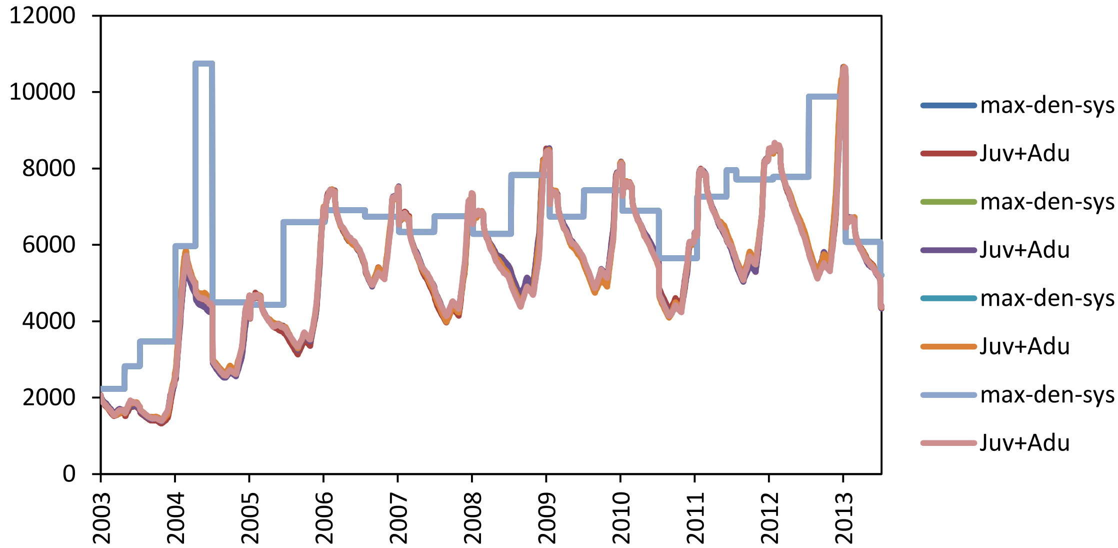
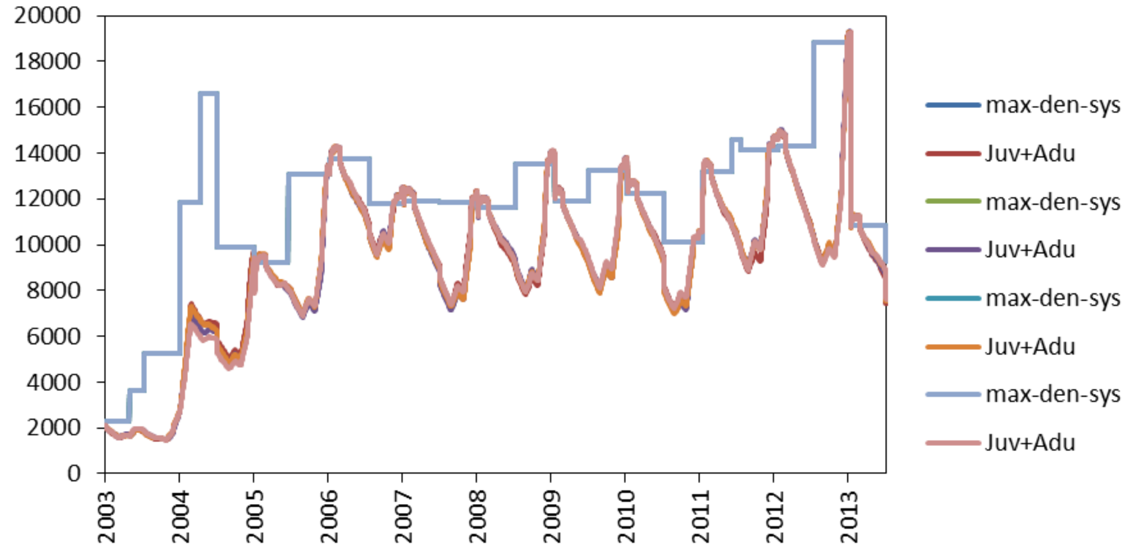


Figure 14



12000
10000
8000
6000
4000
2000
0
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
max-den-sys
Juv+Adu
max-den-sys
Juv+Adu
max-den-sys
Juv+Adu
max-den-sys
Juv+Adu

Figure 15



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14000
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0
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2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
max-den-sys
Juv+Adu
max-den-sys
Juv+Adu
max-den-sys
Juv+Adu
max-den-sys
Juv+Adu

Figure 16

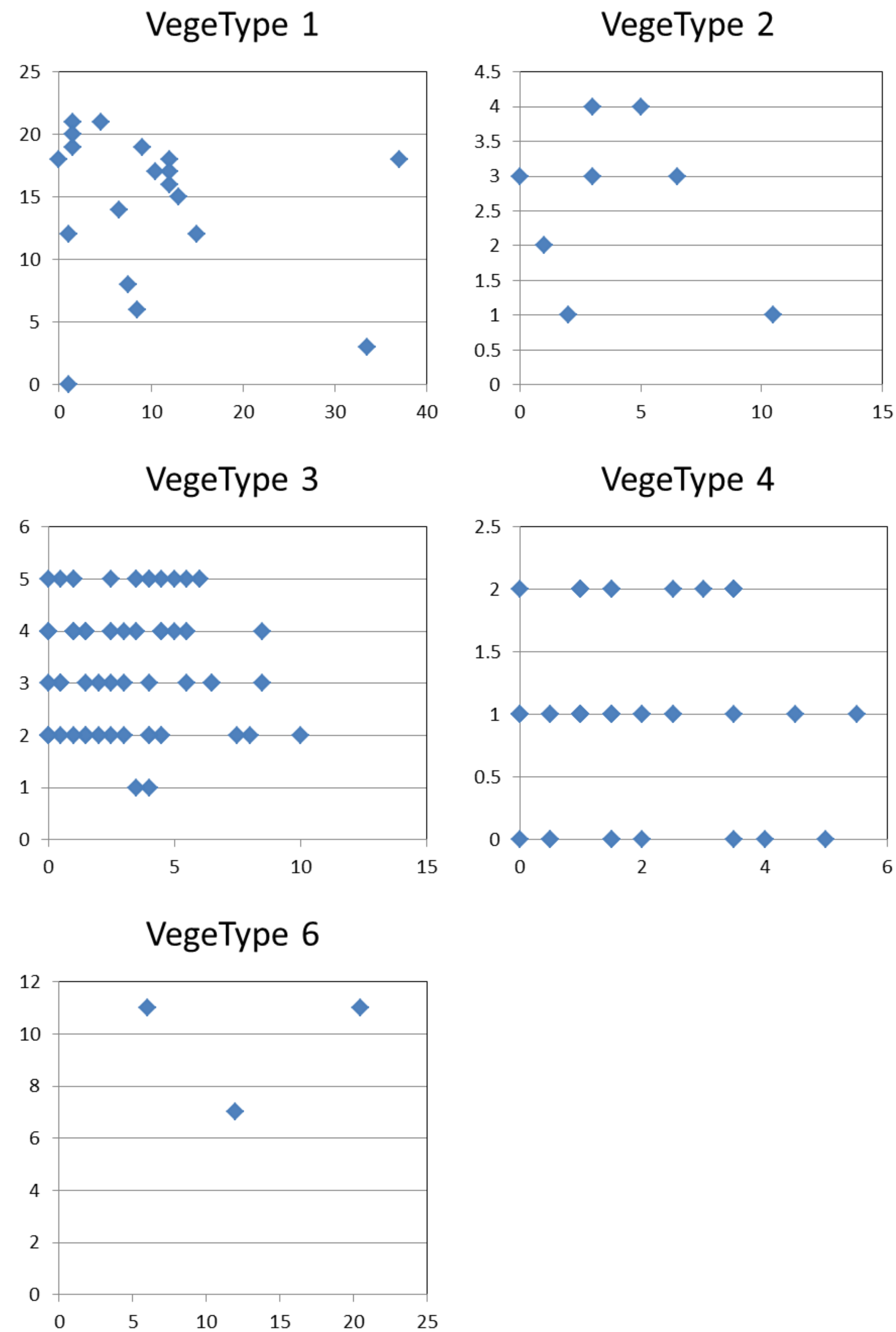


Figure 17 (a) Rep 1

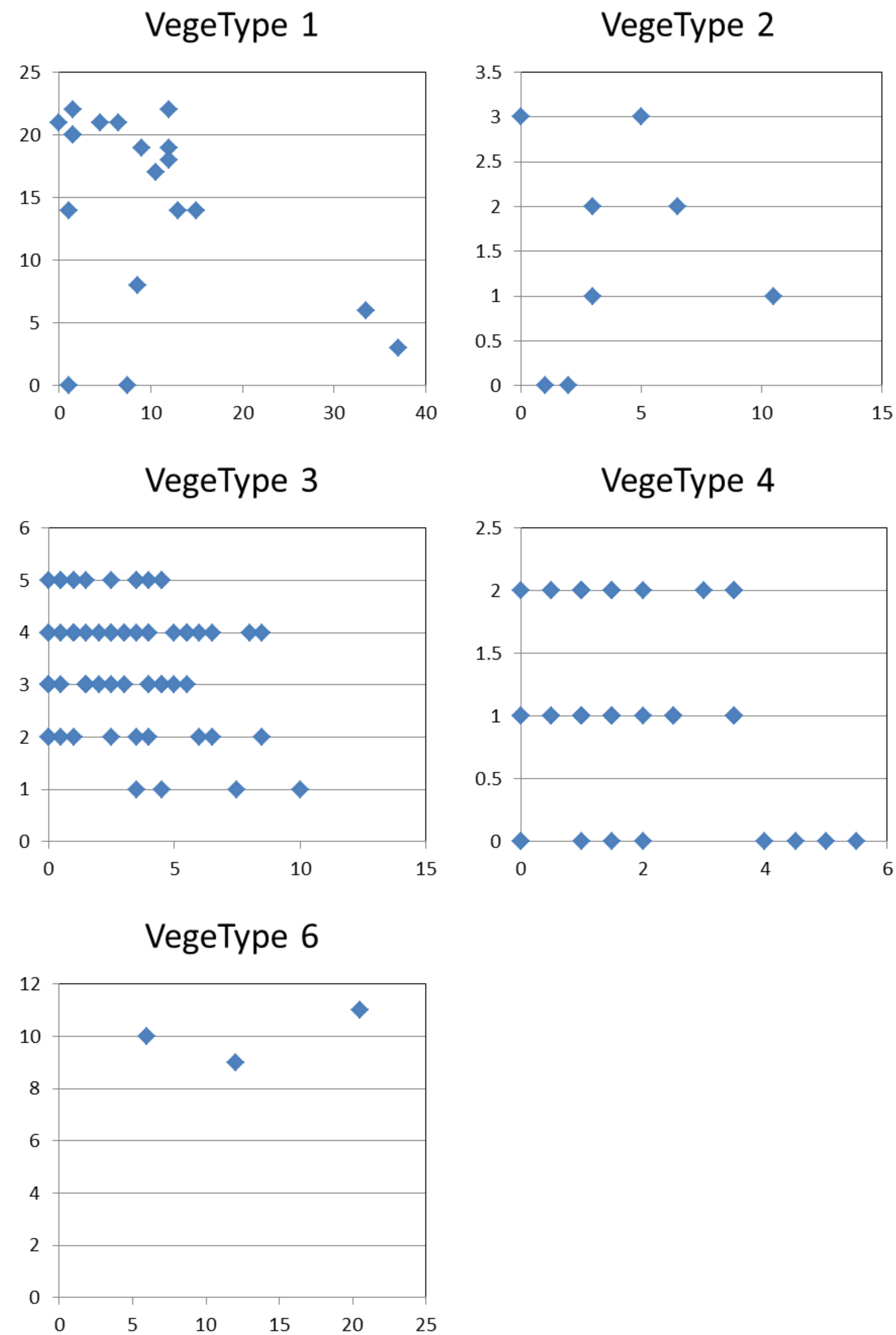


Figure 17 (a) Rep 2

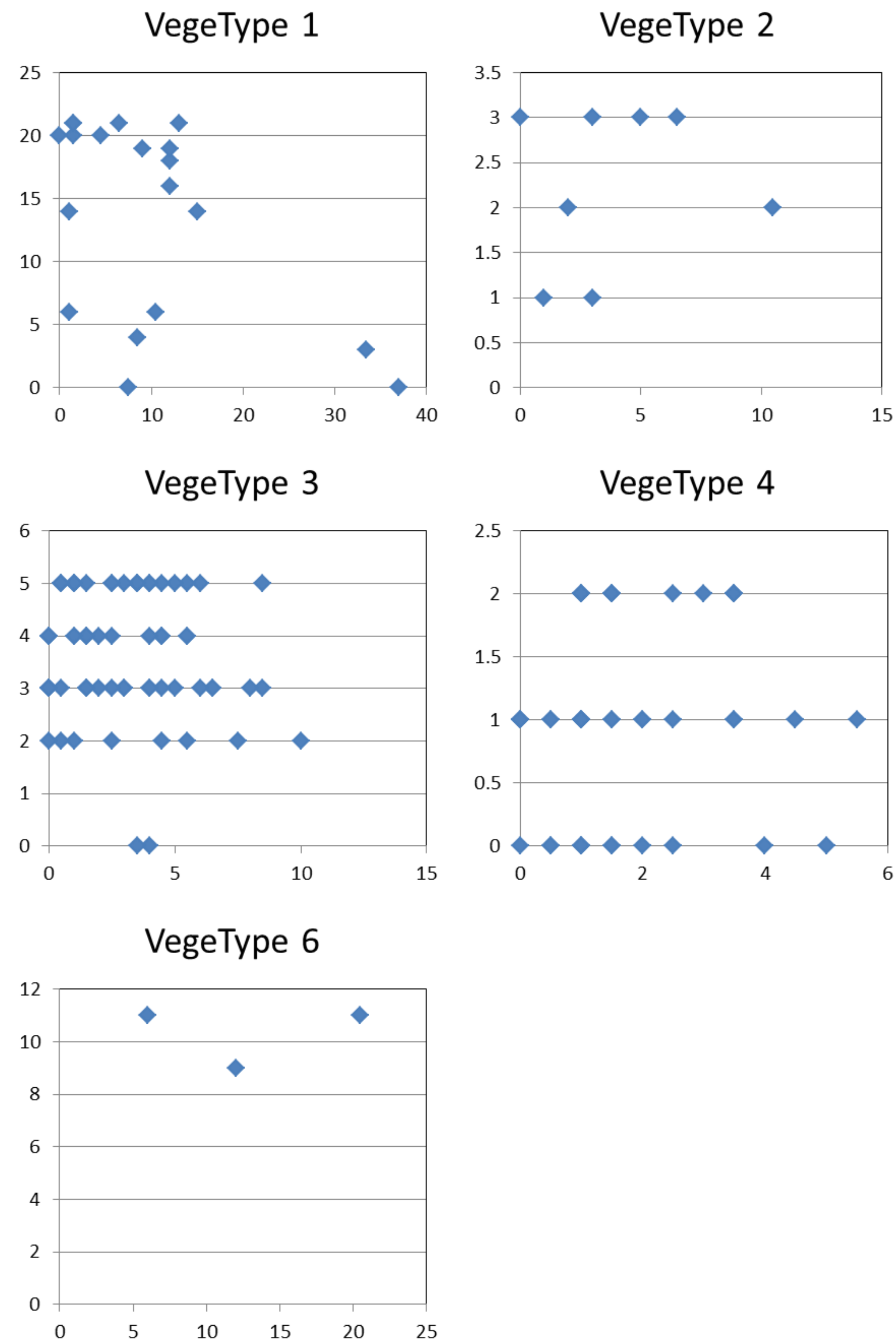


Figure 17 (a) Rep 3

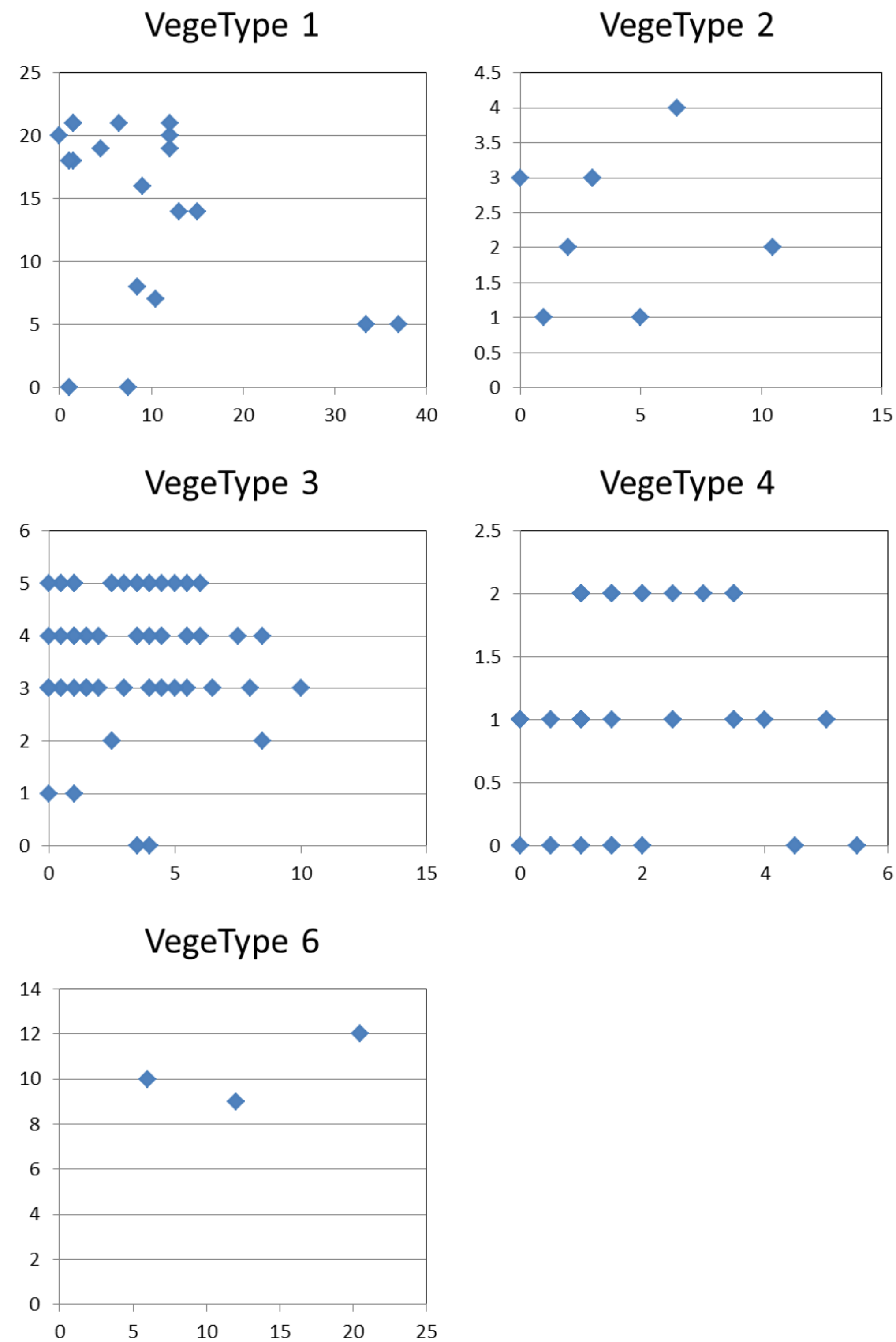


Figure 17 (a) Rep 4

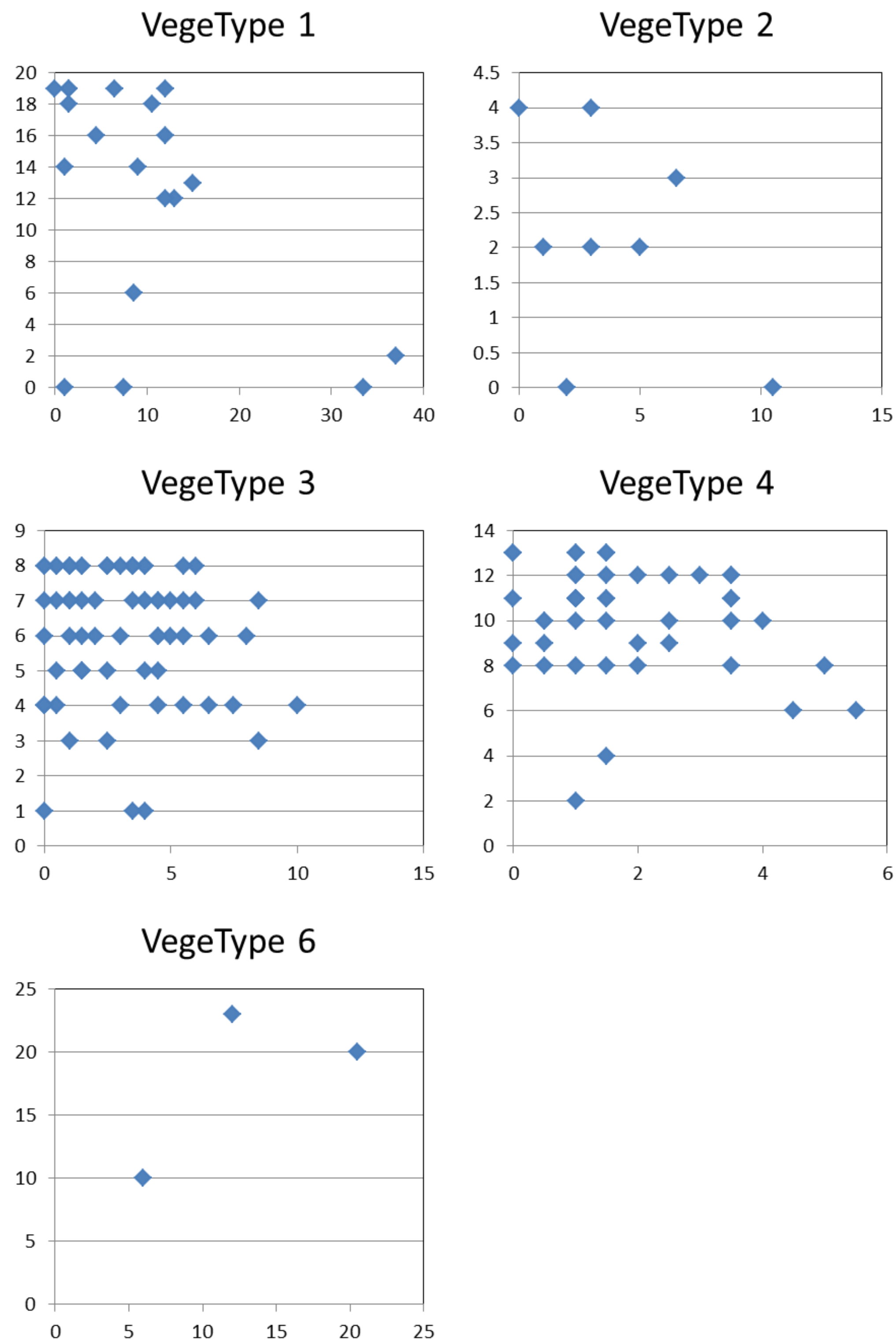


Figure 17 (b) Rep 1

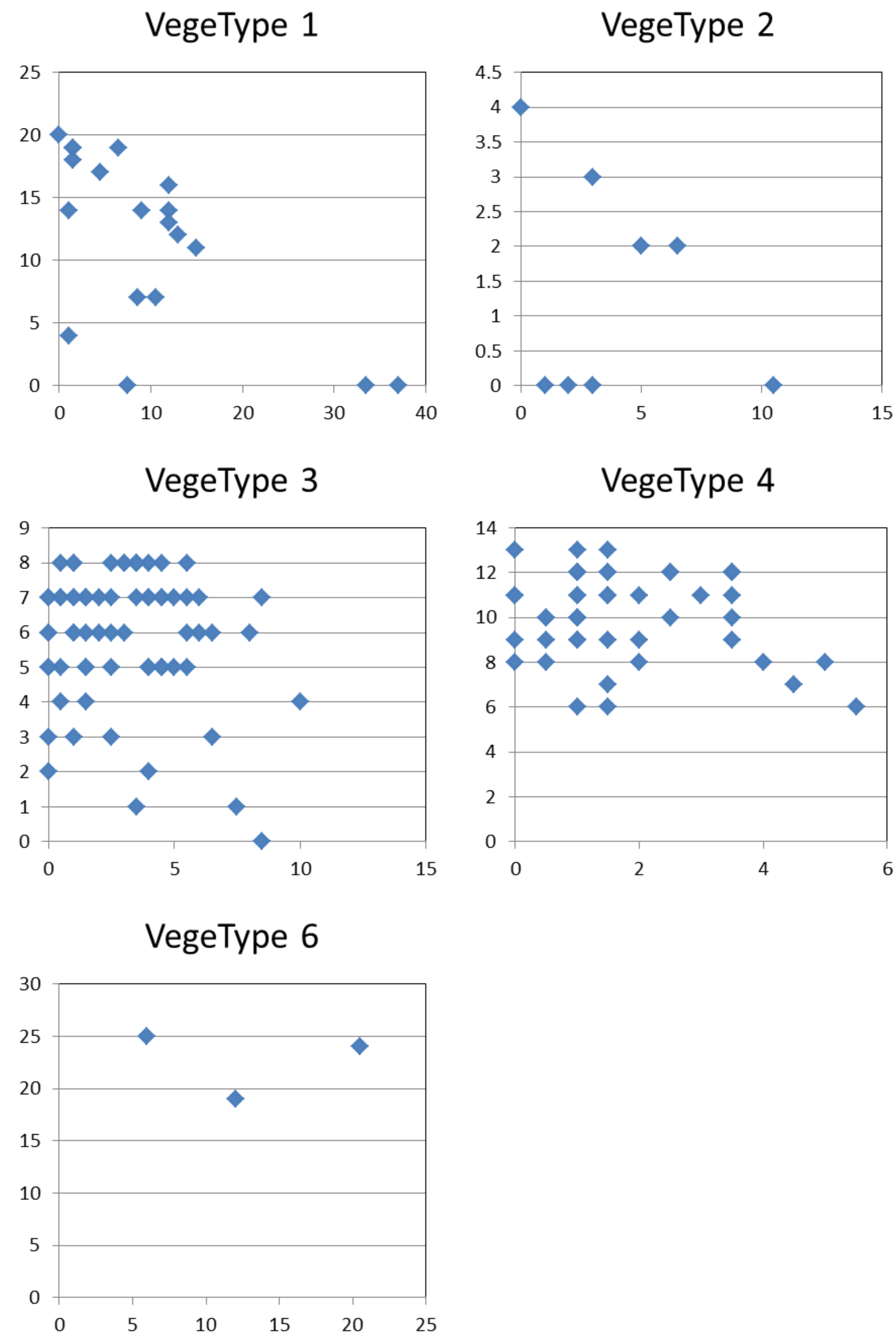


Figure 17 (b) Rep 2

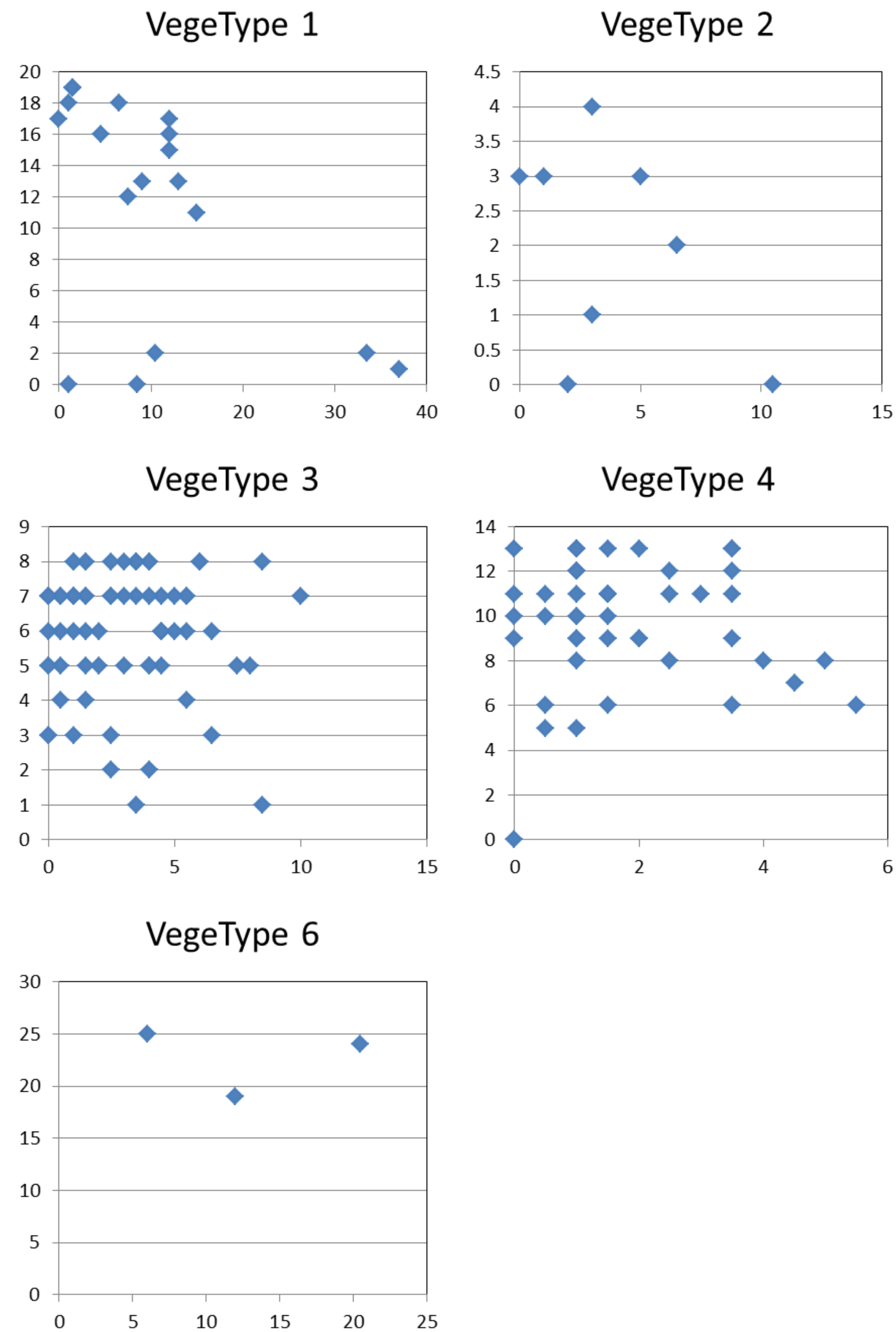


Figure 17 (b) Rep 3

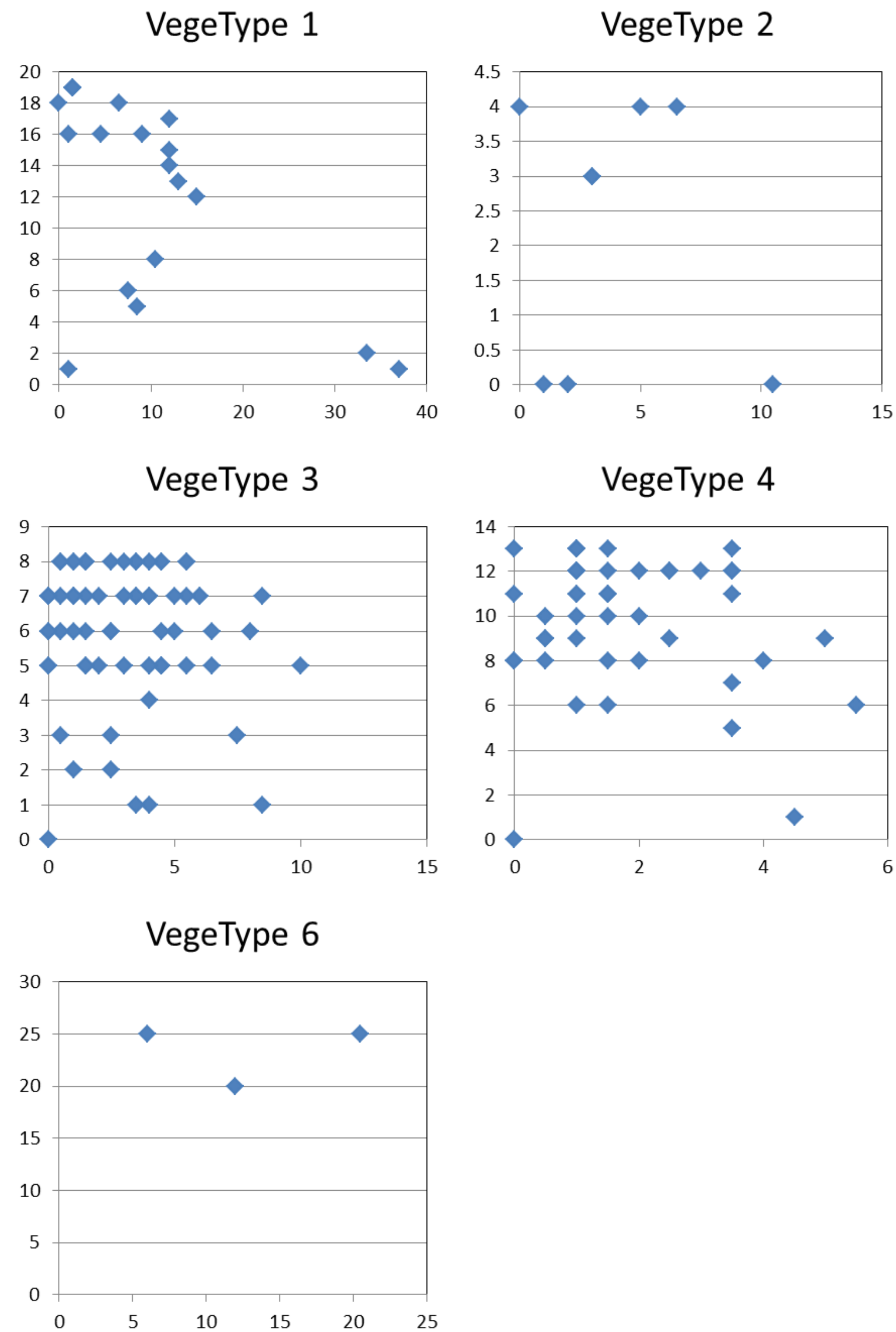


Figure 17 (b) Rep 4

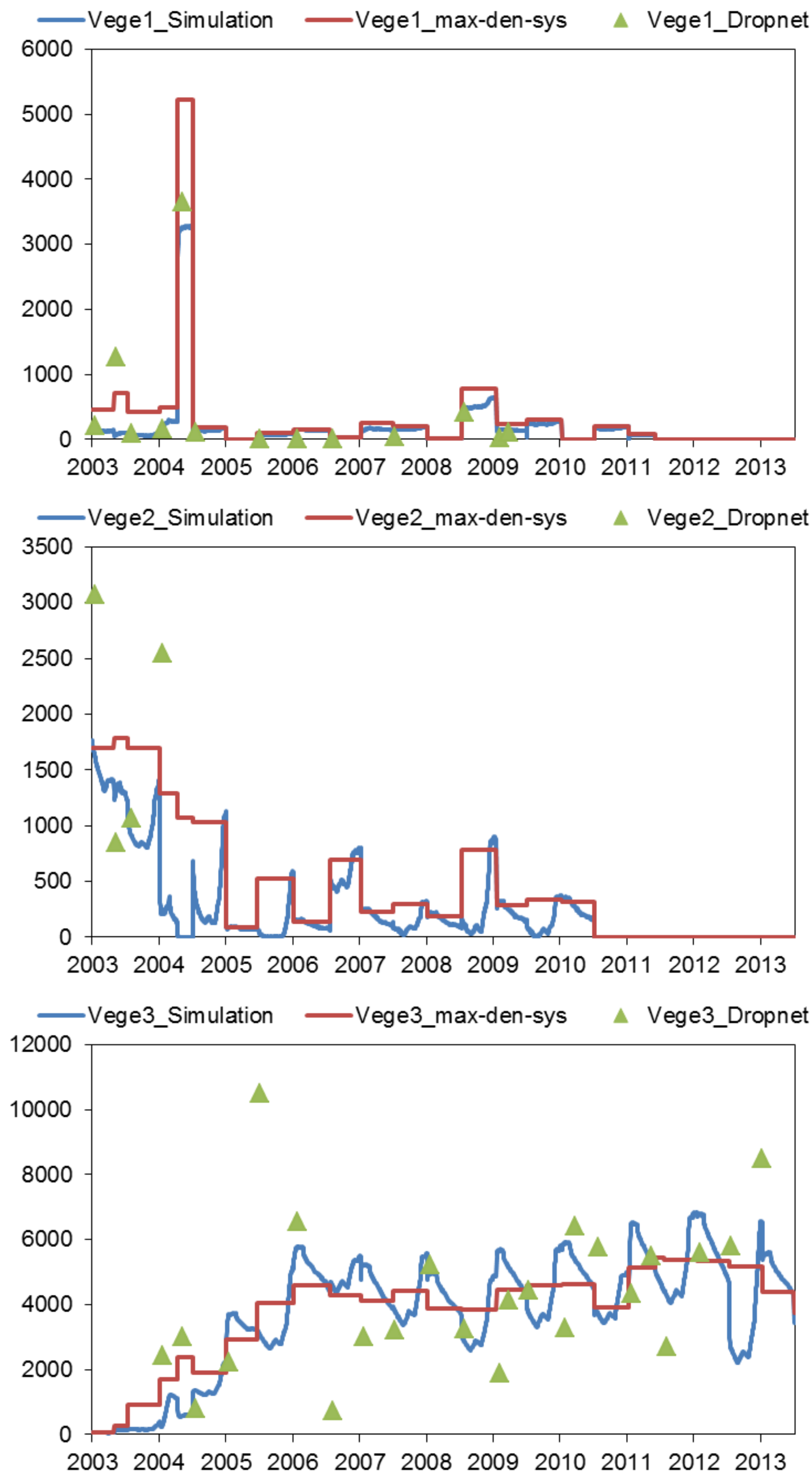

Vege1_Simulation
Vege1_max-den-sys
Vege1_Dropnet
6000
5000
4000
3000
2000
1000
0
2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013
Vege2_Simulation
Vege2_max-den-sys
Vege2_Dropnet
3500
3000
2500
2000
1500
1000
500
0
2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013
Vege3_Simulation
Vege3_max-den-sys
Vege3_Dropnet
12000
10000
8000
6000
4000
2000
0
2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013

Figure 18

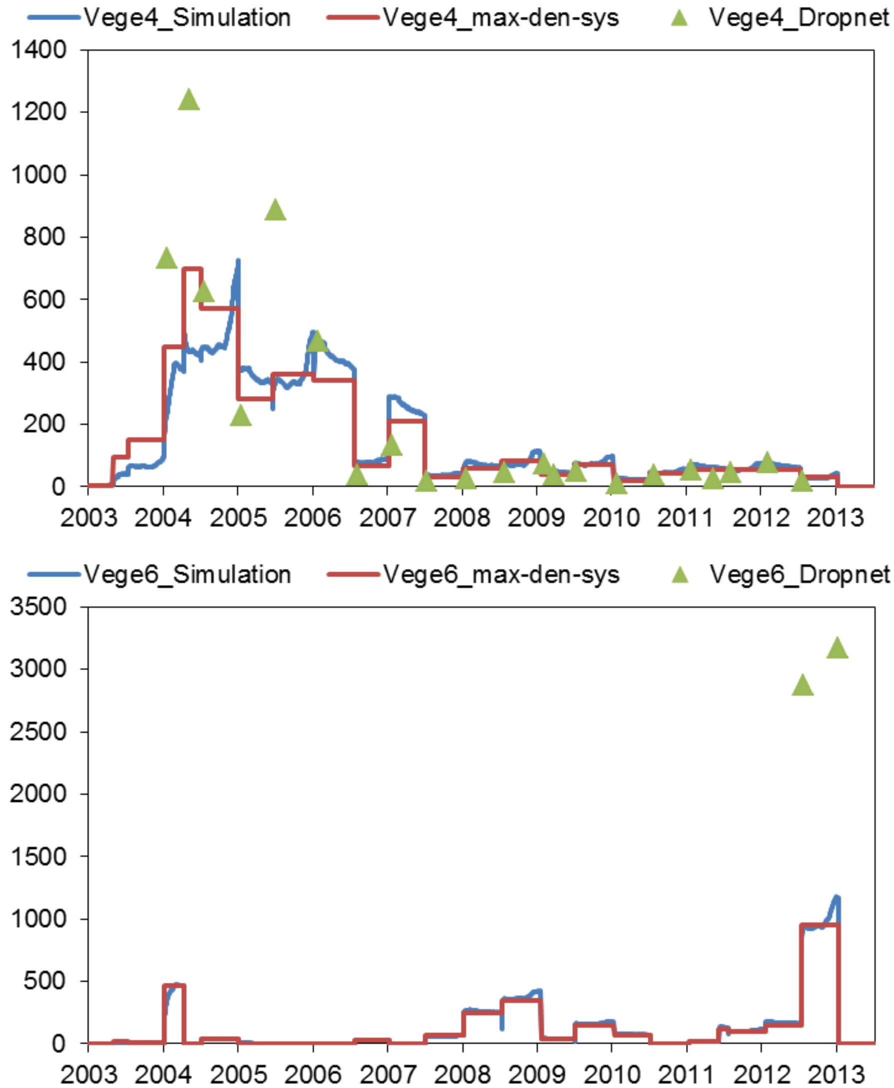


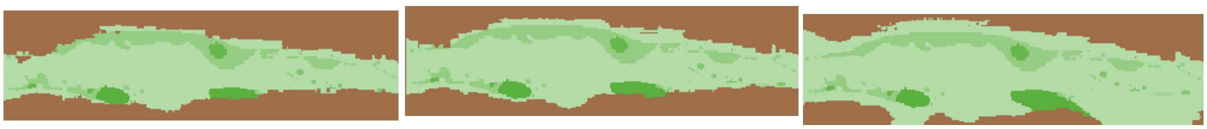
Figure 18 Cont.

APPENDIX G

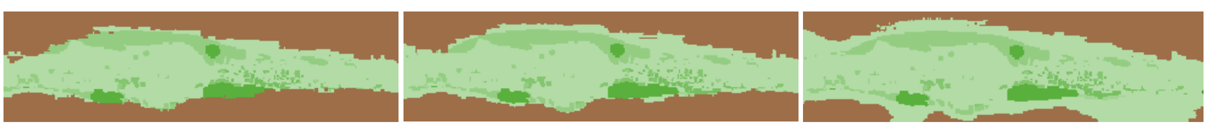
Preliminary Stage Verification Output

10 cfs 35 cfs 80 cfs

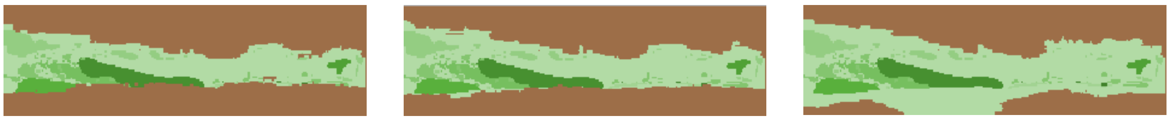
(a) Spring 2003



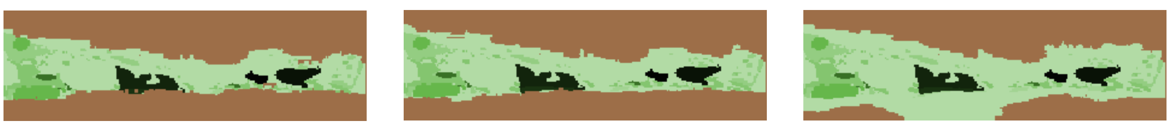
(b) Summer 2003



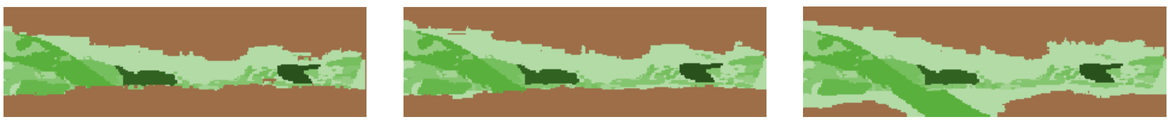
(c) Fall 2003



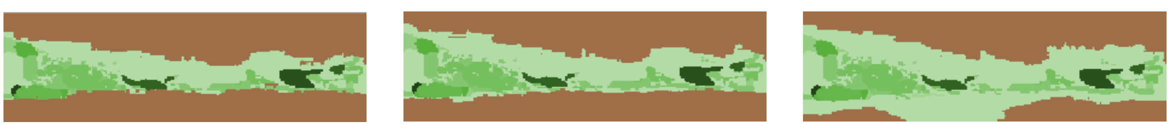
(d) Spring 2004



(e) Summer 2004

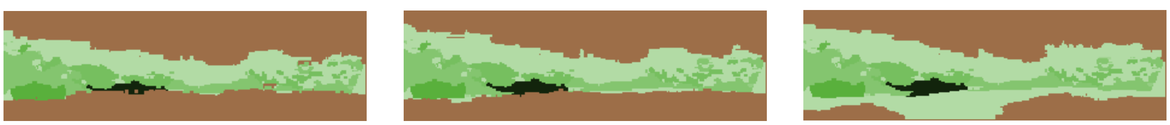


(f) Fall 2004

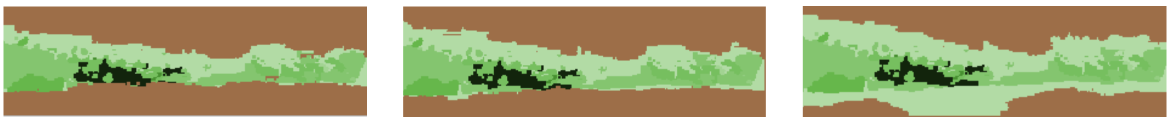


(g) Spring 2005

(h) Fall 2005

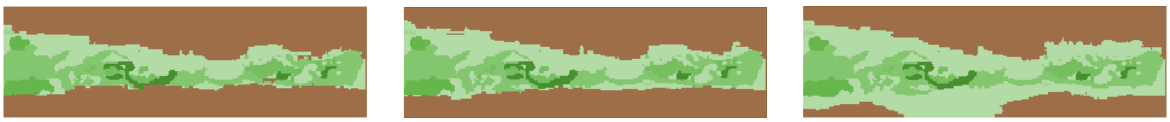


(i) Spring 2006



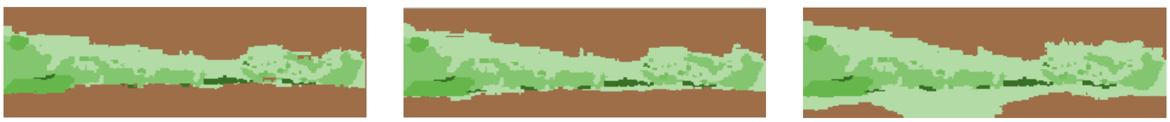
(j) Fall 2006

(k) Spring 2007



(l) Fall 2007

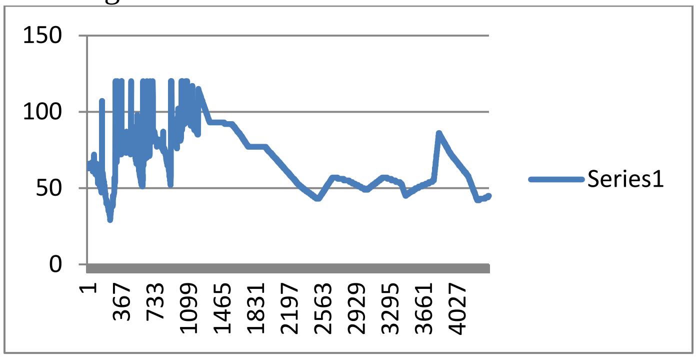
(m) Spring 2008



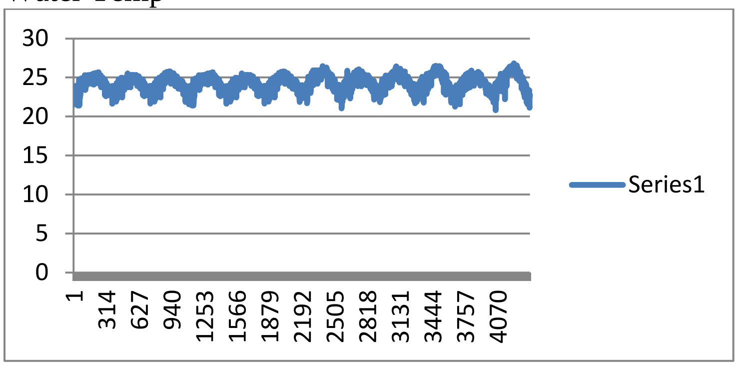
(n) Fall 2008

Appendix G. Figure 1. Simulated spatial-temporal dynamics of fountain darter habitat conditions as indicated by the simulated distribution of different aquatic vegetation types (different shades of green) in the Old Channel of the Comal River during (a) spring of 2003, (b) summer of 2003, (c) fall of 2003, (d) spring of 2004, (e) summer of 2004, (f) fall of 2004, (g) spring of 2005, (h) fall of 2005, (i) spring of 2006, (j) fall of 2006, (k) spring of 2007, (l) fall of 2007, (m) spring of 2008, and (n) fall of 2008 at 10, 35, and 80 cfs, respectively.

Discharge



Water Temp



Appendix G. Figure 2. Simulated temporal dynamics of historical water discharge and temperature from 2003 through 2014 for the Old Channel of the Comal River.

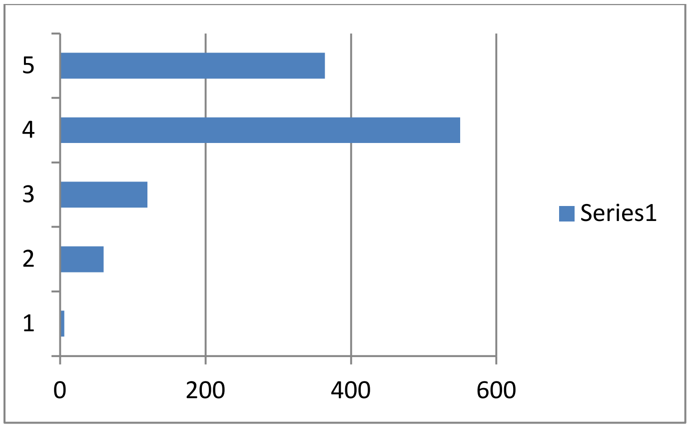
10 cfs 35 cfs 80

(a) Water depth

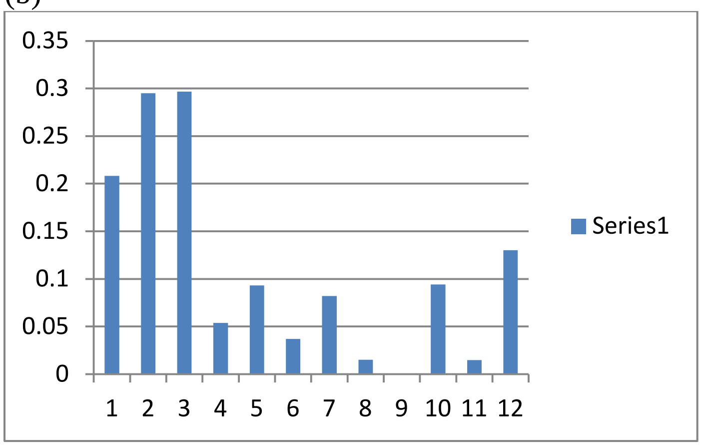
(b) Water velocity

Appendix G. Figure 3. Simulated spatial-temporal dynamics of fountain darter habitat conditions as indicated by the simulated distribution of (a) water depth and (b) water velocity at 0.28, 0.99, and 2.26 m3 sec-1 (10, 35, and 80 cfs), respectively, in the Old Channel of the Comal River. Darker colors represent deeper depths and faster velocities.

(a)



(b)



Appendix G. Figure 4. Simulated (a) development of fountain darters through egg, larva, juvenile, young adult, and old adult life stages (number of days spent in each life stage), and (b) seasonality of reproduction (proportion of adult females that are reproductively active each month).

Appendix K14

Annotated Bibliography of Publications Concerning Coal-Tar-Based Pavement Sealant

Annotated Bibliography of Publications Concerning Coal-Tar-Based Pavement Sealant

Prepared by Barbara J. Mahler, U.S. Geological Survey

July 16, 2012

Many of these publications are available at: http://tx.usgs.gov/coring/allthingssealcoat.html

General Information and Overviews

Parking Lot Sealcoat: A Major Source of Polycyclic Aromatic Hydrocarbons (PAHs) in Urban and Suburban Environments. Van Metre, P.C.; Mahler, B.J.; Scoggins, M.; and Hamilton, P.A., 2005. U.S. Geological Survey Fact Sheet 2005-3147, 4 p. http://pubs.usgs.gov/fs/2005/3147/

This early (2005) factsheet describes PAH contamination associated with runoff from coal-tar-sealed lots and associated deleterious effects to aquatic life and biological communities.

Coal-tar-based pavement sealcoat, polycyclic aromatic hydrocarbons (PAHs), and environmental health. Mahler, B.J., and Van Metre, P.C., 2011, U.S. Geological Survey Fact Sheet 2011–3010, 6 p. http://pubs.usgs.gov/fs/2011/3010/

This USGS factsheet provides an overview of the ways in which coal-tar-based sealcoat contaminates pavement dust, lake sediment, and house dust.

Coal-tar-based pavement sealcoat and PAHs: Implications for the environment, human health, and stormwater management. Mahler, B.J.; Van Metre, P.C.; Crane, J.L.; Watts, A.W.; Scoggins, M.; Williams, E.S., Environ. Sci. Technol., 2012. http://pubs.acs.org/doi/abs/10.1021/es203699x

This Feature article in *Environmental Science and Technology* summarizes the ways in which coal-tar-based sealcoat contaminates stormwater runoff, lake sediment, soil, house dust, and air, and implications for human and biological health and stormwater management.

Environmental Studies

Parking lot sealcoat: An unrecognized source of urban PAHs. Mahler, B. J.; Van Metre, P. C.; Bashara, T. J.; Wilson, J. T.; Johns, D. A., Environ. Sci. Technol. 2005, 39, (15), 5560-5566.

This article was the first to report the potential for coal-tar-based pavement sealcoat to be an important source of PAH contamination. The study of runoff from 13 parking lots found that concentrations of PAHs in particles in runoff from pavement with coal-tar-based sealcoat was, on average, 65 times higher than concentrations in particles in runoff from unsealed asphalt parking lots.

Concentrations of Polycyclic Aromatic Hydrocarbons (PAHs) and Major and Trace Elements in Simulated Rainfall Runoff from Parking Lots, Austin, Texas, 2003. Mahler, Barbara J.; Van Metre, Peter C.; Wilson, Jennifer T. 2004. USGS OFR 2004-1208. http://pubs.usgs.gov/of/2004/1208/ This report was subject to an "Information Quality Act" challenge from the sealcoat industry, to which the USGS responded. A press release summarized the USGS response. http://www.usgs.gov/newsroom/article.asp?ID=1642

This USGS report provides the data used in Mahler et al., 2005.

Trends in Hydrophobic Organic Contaminants in Lake Sediments Across the United States, 1970-2001. Van Metre, P.C. and Mahler, B.J., 2005. Environ. Sci. Technol., v. 39, no. 15, p. 5567-5574.

This scientific journal article documents upwards trends in PAH contamination in sediment in urban lakes across the United States.

PAHs underfoot: Contaminated dust from coal-tar sealcoated pavement is widespread in the United States. Van Metre, P. C.; Mahler, B. J.; Wilson, J. Environ. Sci. Technol. 2009, 43, (1), 20-25. Environ. Sci. Technol. 2009, 43, (1), 20-25.

This scientific journal article reports that concentrations of PAHs in dust swept from parking lots across the central, southern, and eastern U.S.—where coal-tar-based sealcoat use is most common—are in the 1000s of mg/kg, concentrations similar to those in contaminated soils of USEPA Superfund Sites.

Collection and Analysis of Samples for Polycyclic Aromatic Hydrocarbons in Dust and Other Solids Related to Sealed and Unsealed Pavement From 10 Cities Across the United States, 2005-07. Van Metre, P. C.; Mahler, B. J.; Wilson, J. T.; Burbank, T. L. 2008. USGS Data Series 361.

This USGS report provides data used in Van Metre et al., 2009.

Influence of coal-tar sealcoat and other carbonaceous materials on polycyclic aromatic hydrocarbon loading in an urban watershed. Yang, Y., Van Metre, P.C., Mahler, B.J., Wilson, J.T., Ligouis, B., Razzaque, M.M., Schaeffer, D.J., and Werth, C.J., 2010,: Environ. Sci. Technol., v. 44, p. 1217-1223.

This scientific journal article reports research using organic petrography to quantitatively determine the proportion of PAHs in dust and soil samples originating as coal-tar pitch. The study found that coal-tar pitch, used in coal-tar-based sealcoat, was a dominant

source of PAHs in the watershed, contributing as much as 99% of the PAHs in sealed parking lot dust, 92% in unsealed parking lot dust, 88% in commercial area soil, 71% in streambed sediment, and 84% in surficial lake sediment.

Particle-associated contaminants in street dust, parking lot dust, soil, lake-bottom sediment, and suspended and streambed sediment, Lake Como and Fosdic Lake watersheds, Fort Worth, Texas, 2004. Wilson, J.T., Van Metre, P.C., Werth, C.J., and Yang, Yaning, 2006: U.S. Geological Survey Data Series 211, 24 p.—ONLINE ONLY
http://pubs.usgs.gov/ds/2006/211/

This USGS report provides the data used in Yang et al.

Coal-tar-based parking lot sealcoat: An unrecognized source of PAH to settled house dust. Mahler, B. J.; Van Metre, P. C.; Wilson, J. T.; Musgrove, M.; Burbank, T. L.; Ennis, T.; Bashara, T. J., Environ. Sci. Technol. 2010, 44, 894-900.

This scientific journal article reports that concentrations of PAHs in house dust in residences adjacent to parking lots with coal-tar-sealcoated pavement were 25 times higher than those in house dust in residences adjacent to parking lots with unsealed pavement or pavement with asphalt-based sealcoat.

Contribution of PAHs from Coal-Tar Pavement Sealcoat and Other Sources to 40 U.S. Lakes. Van Metre, P. C.; Mahler, B. J. Sci. of the Total Environ., 2010, v.409, 334-344.

This scientific journal article reports that coal-tar-based sealcoat was, on average, the largest source of PAHs to sediment in 40 U.S. lakes, on the basis of a statistical source-apportionment approach. The article also reported that coal-tar-based sealcoat was the source of upward trends in PAH concentrations in seven of eight urban lakes investigated.

Polycyclic aromatic hydrocarbons in stormwater runoff from sealcoated pavements. Watts, A.W., Ballestero, T.P., Roseen, R.M., and House, J.P., Environ. Sci. Technol. 2010, v. 44(23), 8849-8854.

This scientific journal article reports that even partial coverage of a drainage area by coal-tar-based sealant resulted in increased PAH concentrations in sediment. A stormwater swale receiving runoff from both sealed and unsealed lots had PAH concentrations 25 times higher after sealant was applied than prior to sealant application.

Polycyclic aromatic hydrocarbons released from sealcoated parking lots -- A controlled field experiment to determine if sealcoat is a significant source of PAHs in the environment. University of New Hampshire Stormwater Center, 18 p.

This report, prepared for the USEPA, demonstrates that use of coal-tar-based sealcoat resulted in increases in PAH concentrations in adjacent soil, land-surface dust, air, and stormwater sediment.

Volatilization of polycyclic aromatic hydrocarbons from coal-tar-sealed pavement. Van Metre, P. C.; Majewski, M. S.; Mahler, B. J.; Foreman, W. T.; Braun, C. L.; Wilson, J. T.; Burbank, T. Chemosphere, 2012.

This scientific journal article reports PAH releases to air from in-use parking lots with and without coal-tar-based sealcoat. The mass of PAHs released to air per unit area of coal-tar-sealed pavement was 60 times greater than that released from unsealed asphalt pavement, even though in all but one case the sealant had been applied from 3 to 8 years prior to sampling.

PAH volatilization following application of coal-tar-based pavement sealant. Van Metre, P. C.; Majewski, M. S.; Mahler, B. J.; Foreman, W. T.; Braun, C. L.; Wilson, J. T.; Burbank, T. Atmos. Environ. 2012.

This scientific journal article reports enormous releases of PAHs to the atmosphere (one-quarter to one-half of the PAHs contained in the product) during the 15 days following application of coal-tar-based sealant. The authors estimate that PAH emissions from new coal-tar-based sealcoat applications each year (~1000 Mg) are larger than annual vehicle emissions of PAHs for the United States.

Biological and Ecological Health

The effects of coal tar based pavement sealer on amphibian development and metamorphosis. 2006. Bryer, P.J., Elliott, J.N., and Willingham, E.J. , Ecotoxicology, vol. 15(3), 241-247.

This scientific journal article reports that exposure to sediment contaminated with coal-tar-based pavement sealer resulted in stunted growth and slower development of the frog *Xenopus laevis*.

Coal-tar based pavement sealant toxicity to freshwater macroinvertebrates. Bryer, P.J., Scoggins, M., and McClintock, N.L., 2009. Environmental Pollution, v. 158, no. 5, p. 1932-1937.

This scientific journal article reports that exposure to sediment contaminated with coal-tar-based sealcoat resulted in decreased abundance and richness of freshwater macroinvertebrates, an important element in the aquatic food chain.

Occurrence of polycyclic aromatic hydrocarbons below coal-tar-sealed parking lots and effects on stream benthic macroinvertebrate communities. Scoggins, M., McClintock, N., Gosselink, L., and Bryer, P., 2007. Journal of the North American Benthological Society, v. 26, no. 4, p. 694-707.

This scientific journal article reports a significant decrease in the health of the ecological community downstream from points of discharge of runoff from coal-tar-sealcoated parking lots relative to ecological communities upstream.

Toxicity of coal–tar and asphalt sealants to eastern newts, *Notophthalmus viridescens*. 2010. Bommarito, T., Sparling, D.W., and Halbrook, R.S.

This scientific journal article reports that exposure of eastern newts to sediment contaminated with coal-tar-based sealcoat resulted in deleterious effects, including difficulty right themselves, impaired ability to swim, and diminished liver enzyme activities.

Toxicity of coal-tar pavement sealants and ultraviolet radiation to *Ambystoma Maculatum.* 2010. Bommarito, T., Sparling, D.W., and Halbrook, R.W.

This scientific journal articles reports that spotted salamanders exposed to sediment contaminated with coal-tar-based sealcoat in sediment had slower rates of growth and diminished ability to swim. Subsequent exposure to ultra-violet radiation resulted in genetic damage.

Human Health

Coal-tars and Derived products. 1985 International Agency for Research on Cancer (IARC) –, vol 35, 83 p. http://www.inchem.org/documents/iarc/vol35/coaltars.html

This landmark document describes the carcinogenic properties of coal tars and coal-tar pitches, and finds that there is sufficient evidence that coal-tar pitches are carcinogenic in humans.

Williams, E. S.; Mahler, B. J.; Van Metre, P. C. Coal-tar pavement sealants might substantially increase children's PAH exposures. Environ. Pollut. 2012.

This "New Initiatives" article in *Environmental Pollution* estimates that, although dietary ingestion has long been thought to be the primary route of human exposure to polycyclic aromatic hydrocarbons (PAHs), for children 3–5 years of age living in residences adjacent to parking lots with coal-tar-based sealcoat, non-dietary ingestion of PAHs (i.e., ingestion of house dust) is about 2.5 times that of dietary ingestion.

Public Policy

Contamination of Stormwater Pond Sediments by Polycyclic Aromatic Hydrocarbons (PAHs) in Minnesota: The Role of Coal Tar-based Sealcoat Products as a Source of PAHs. Crane, J.L., Grosenheider, K., and Wilson, C.B., 2010, Minnesota Pollution Control Agency, 64 p. http://www.pca.state.mn.us/index.php/view-document.html?gid=12960

This white paper by the Minnesota Pollution Control Agency describes the filling of stormwater ponds with PAH-contaminated sediments, the expense of deposing of the sediments, and the likelihood that coal-tar-based pavement sealants are a substantial contributor to the problem.

Research Funded by the PCTC (Pavement Coatings Technology Council)

Polycyclic aromatic hydrocarbons (PAHs) in Austin sediments after a ban on pavement sealers. 2010. Demott, R.P., Gauthier, T.D., Wiersema, J.M., and Crenson, G. Environmental Forensics, vol. 11, 372-382.

This scientific journal article reports no statistically significant different in PAHs in streambed sediment in Austin 2 years after a ban on use of coal-tar-based sealcoat.

Forensic assessment of refined tar-based sealers as a source of polycyclic aromatic hydrocarbons (PAHs) in urban sediments. 2012. O'Reilly, K., Pietari, J., and Boehm, P. Environmental Forensics, vol. 13, 185-196.

This scientific journal articles reports that, using statistical (forensic) analysis, the authors were not able to differentiate between the contribution of coal-tar-based sealants to PAHs and that of other sources.

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(REV. FEBRUARY 2012)

Manuscripts in Preparation

O'Reilly, K., Pietari, J. and Boehm, P. (2012). PAH Fingerprinting: A Cautionary Tale.

Publications in Refereed Journals:

O'Reilly, K., Pietari, J. and Boehm, P. (2012). A Forensic Assessment of Coal Tar Sealants as a Source of Polycyclic Aromatic Hydrocarbons in Urban Sediments. *Environmental Forensics,* in press.

O'Reilly, K., Pietari, J. and Boehm, P. (2011). Comment on "PAHs Underfoot: Contaminated Dust from Coal-Tar Sealcoated Pavement is Widespread in the U.S." *Environ. Sci. Technol.*, **2011**, *45* (7), pp 3185–3186

DeMott, R.P., Gauthier, T.D., Wiersema, J.M. and Crenson, G. (2010). PAHs in Austin Sediments after a Ban on Pavement Sealers. *Environmental Forensics*, 11:4, 372-382.

DeMott, R.P.; Gauthier,T.D. (2006) Comment on "Parking lot sealcoat: An unrecognized source of urban polycyclic aromatic hydrocarbons." *Environ. Sci. Technol.* **2006,** *40*(11), 3657-3658

Articles Published in Magazines for Professionals:

O'Reilly, K., Pietari, J. and Boehm, P. (2010). Polycyclic Aromatic Hydrocarbons in Stormwater and Urban Sediments: A Review. *Stormwater* Magazine. September 2010.

Presentations at Scientific Meetings:

O'Reilly, K., Pietari, J. and Boehm, P. (2011). Managing Risks: Will banning pavement sealers have the desired effect? Abstract and Poster Presented at the *32nd Annual Meeting of the Society of Environment Toxicology and Chemistry (SETAC)*, Boston, Nov. 2011.

DeMott, R.P. and Gauthier, T.D. (2011). Use of Mass Balance Bounding Estimates and Sensitivity Analysis to Prioritize PAH Inputs in Urban Systems. Abstract and Poster Presented at the *32nd Annual Meeting of the Society of Environment Toxicology and Chemistry (SETAC)*, Boston, Nov. 2011.

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Pietari, J., O'Reilly, K. and Boehm, P. (2011). Environmental Forensics for PAH Source Management: Pavement Sealants and Sediments. Abstract and Poster Presented at the *Sixth International Conference on Remediation of Contaminated Sediments*, New Orleans, LA Feb. 2011.

O'Reilly, K., Pietari, J. and Boehm, P. (2010). PAHs in Urban Sediments: Forensics Approaches for Assessing the Relative Contribution of Atmospheric Deposition. Abstract and Poster Presented at the *31st Annual Meeting of the Society of Environment Toxicology and Chemistry (SETAC),* Seattle, Nov. 2010.

Gauthier, T.D. and DeMott, R.P. (2008). Analysis of PAH Concentrations Detected in Austin Texas Stream Sediments Following a Ban on the Use of Coal Tar Sealers. Abstract of Presentation Made at the *29th Annual Meeting of the Society of Environmental Toxicology and Chemistry (SETAC),* Tampa, Nov. 2008.

Appendix K15

The *EAHCP Steward* Newsletter

HABITAT CONSERVATION PLAN EDWARDS AQUIFER

Steward Newsletter

The Edwards Aquifer Habitat Conservation Plan e-newsletter, "Steward," is published to highlight the efforts underway to protect the Comal and San Marcos Springs and ensure a healthy habitat for the threatened and endangered species.

EAHCP Looking Forward to 2015

The 2014 end-of-year joint meeting gave the EAHCP stakeholders a preview of the work to be expected and completed by the permittees in 2015.

Texas Wild Rice

As part of its 2015 plan, the City of New Braunfels will enhance its continual water quality monitoring with its water testing stations in Landa Lake. This equipment collects data every 30 minutes and provides critical information about oxygen levels in the lake at various times of the day. The City will also continue its sediment removal, river bank stabilization, nonnative plant/animal reductions, installation of additional aerators, and other measures in their effort to ensure excellent water quality in the Comal ecosystems and to benefit the endangered species. Additionally, the City of New Braunfels will be stepping up its "low impact development" program to teach the community how human activities can affect water quality, and the best practices for preventing pollution in the Comal River.

With the success in increasing Texas wild-rice coverage this past year, the City of San Marcos will be upgrading its recreation management programs and silt removal efforts for the plant's continued growth in 2015. In addition, the City will boost education programs for river users about sustainable river use and the listed species. Texas State University will gather information on recreational use of the river and the potential impacts of those activities on the ecosystem. Furthermore, additional litter-collection boats will be added to efficiently remove any trash and floating vegetation. In 2015, the City of San Marcos will remove approximately 1,000 square meters of fine sediment from the river bottom.

Finally, the Edwards Aquifer Authority continues to develop a predictive ecological model to evaluate the impacts of various water quality protection measures. This model is constructed from data collected through years of research and monitoring inside the Comal and San Marcos Springs systems. The 2015 goal is further development and calibration of a working model that will predict specific ecological responses and will help scientists fine tune strategies that will protect the species in both systems.

You can read more about the EAHCP work plans at this link.