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

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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.

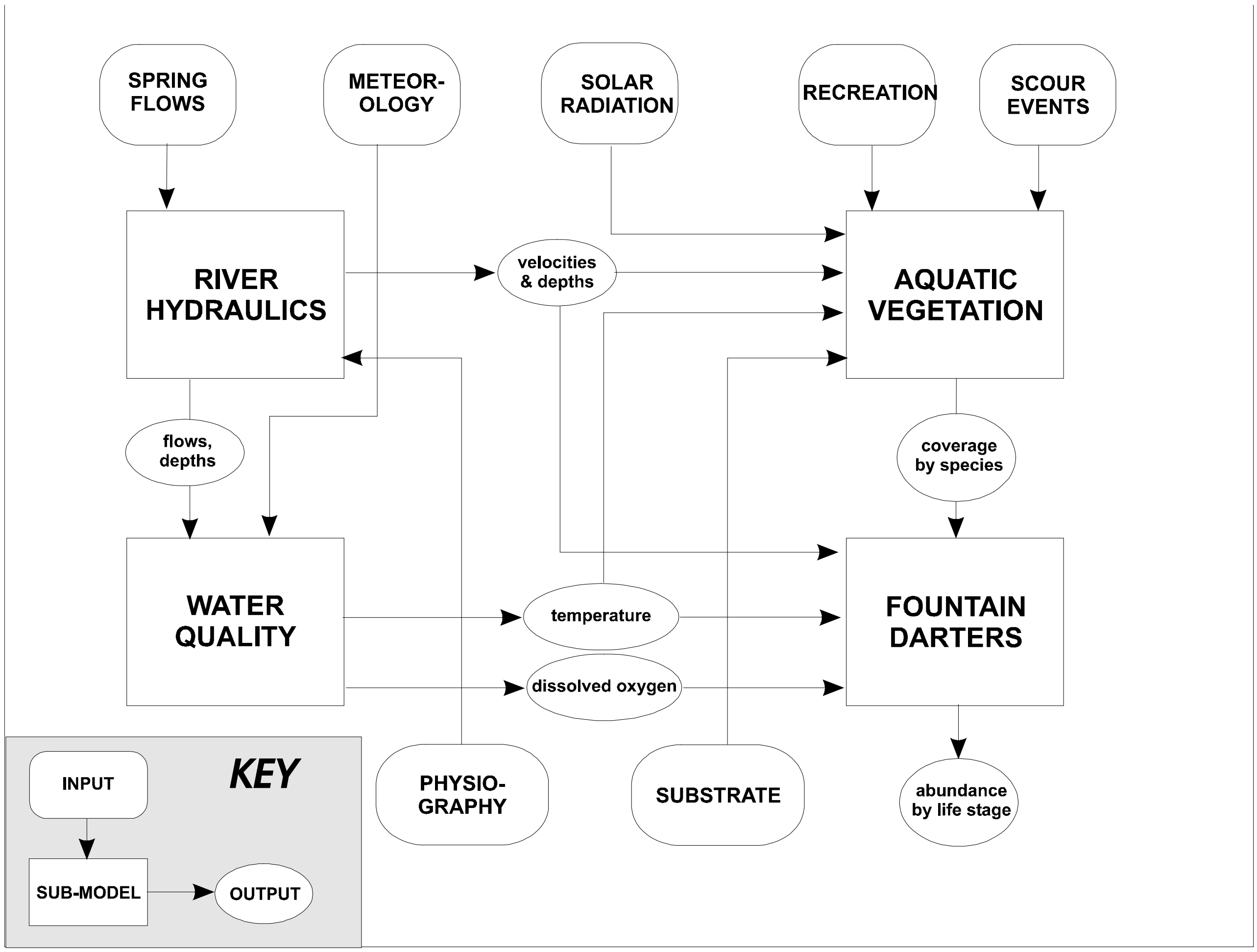

Figure ES1. Conceptual Model

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.

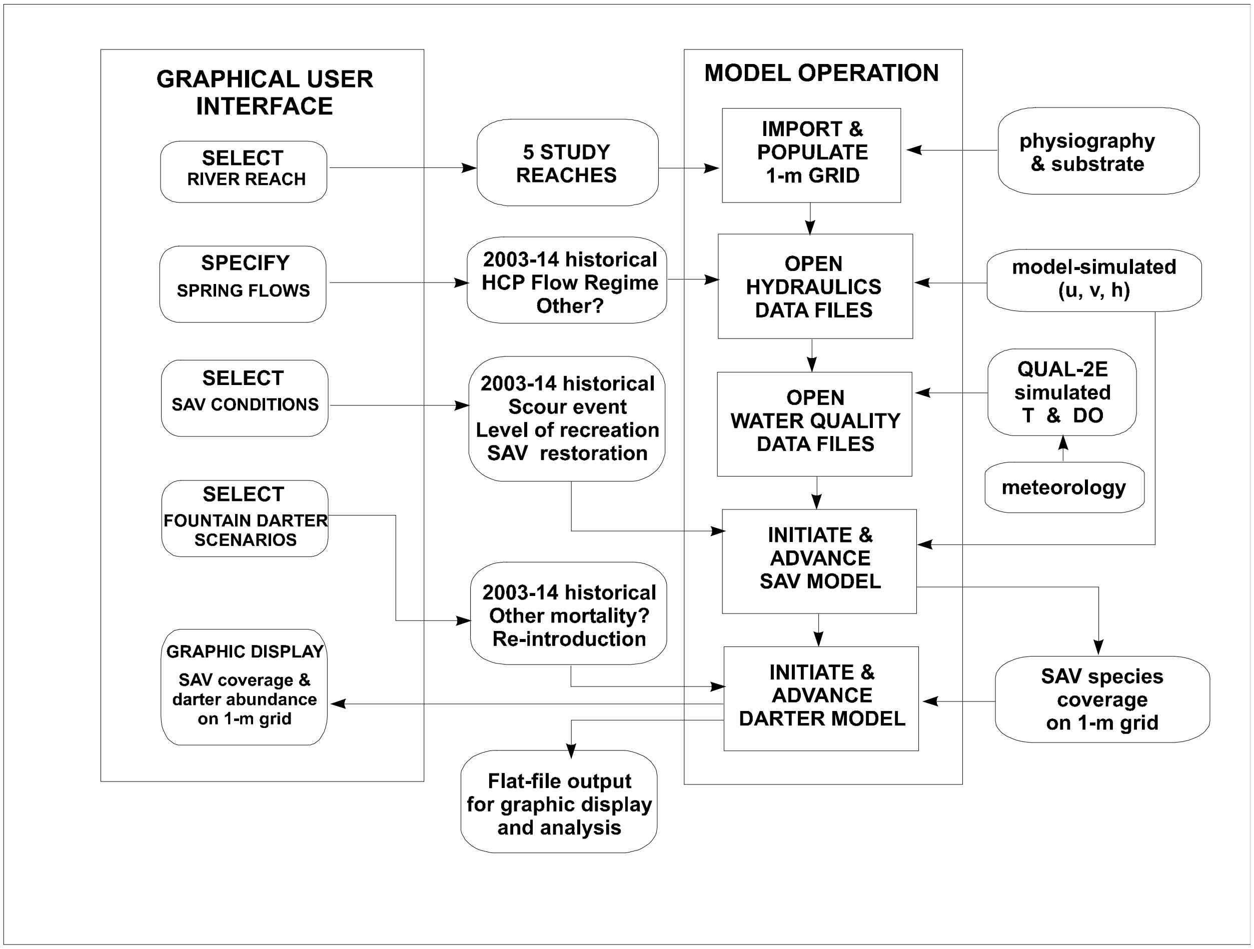

Figure ES2. Conceptual Operations Model

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.

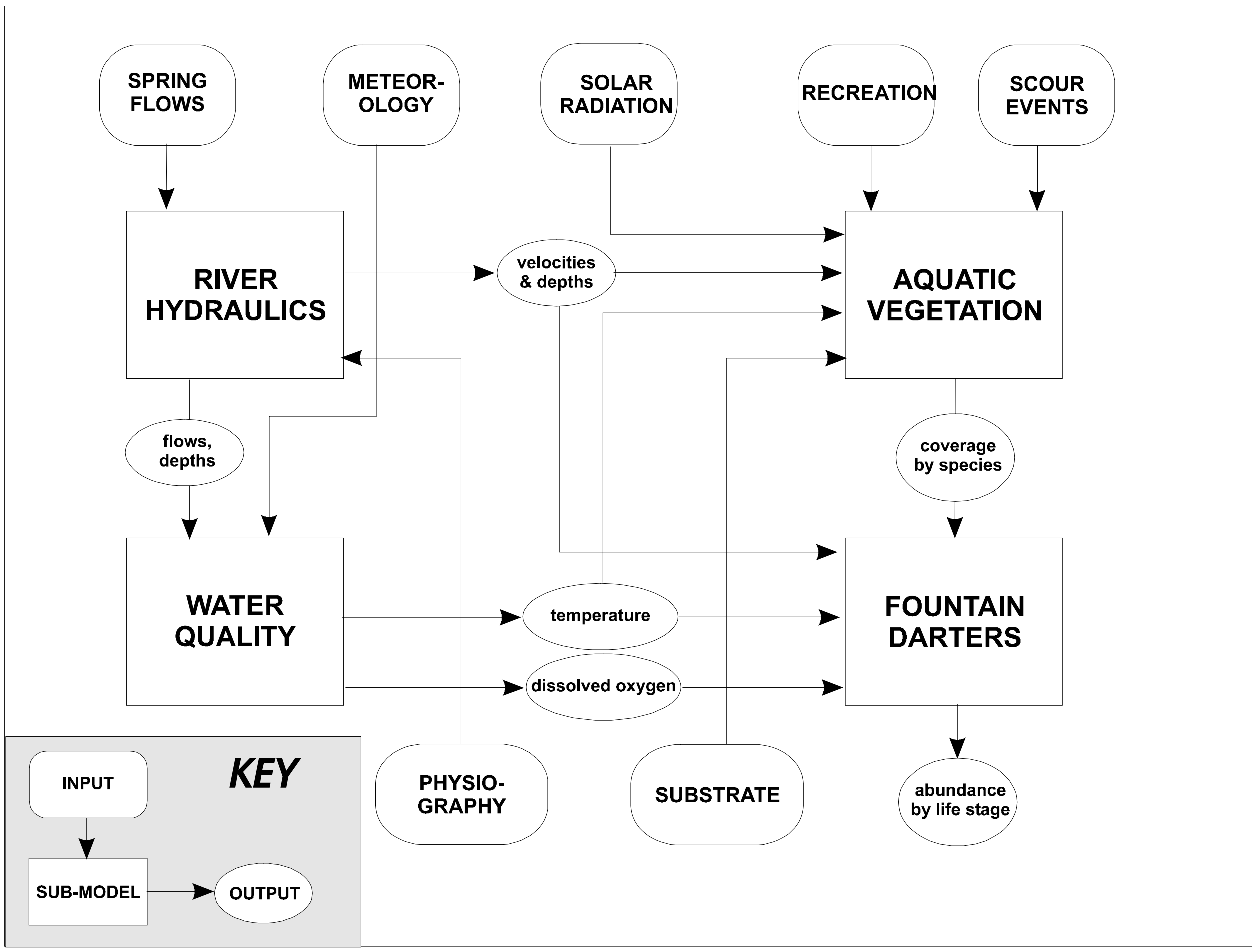

Figure 1. **Conceptual Model**

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 conceptual model (Figure 1) focuses on the fountain darter 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).

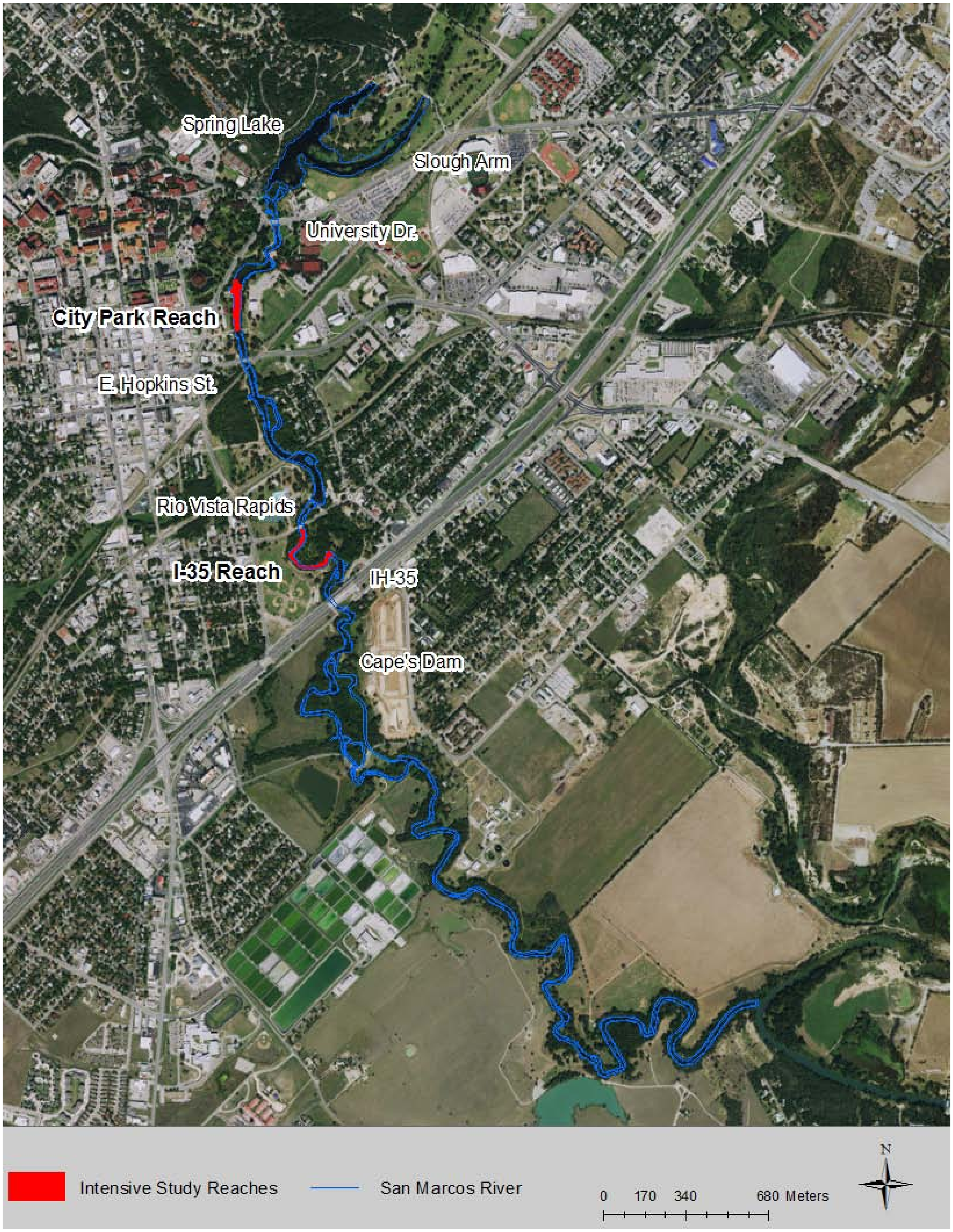

Figure 3. San Marcos River Ecomodel Simulation Reaches (City Park and I35).

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 (BIO-WEST 2015b). 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, 2015a). Although this reach is basically written off in the current

HCP flow-regime, it is a valuable model reach to provide a glimpse of 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 (BIO-WEST 2015a). 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 (BIO-WEST 2015b). 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, 2013b).

Water quality modeling

The suite of water-quality variables to be included in the model were limited to water temperature and dissolved oxygen (DO). 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 (BIO-WEST, 2013c), vegetation percent cover to biomass (Baylor, 2014) and Ludwigia competition (BIO-WEST, 2015c) study all provided direct input to the SAV submodel. The fountain darter fecundity (BIO-WEST, 2014c) and fountain darter movement study (BIO-WEST, 2014d) also provided direct input for the fountain darter submodel. Brief summaries of the 2014 and 2015 applied research studies mentioned 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.

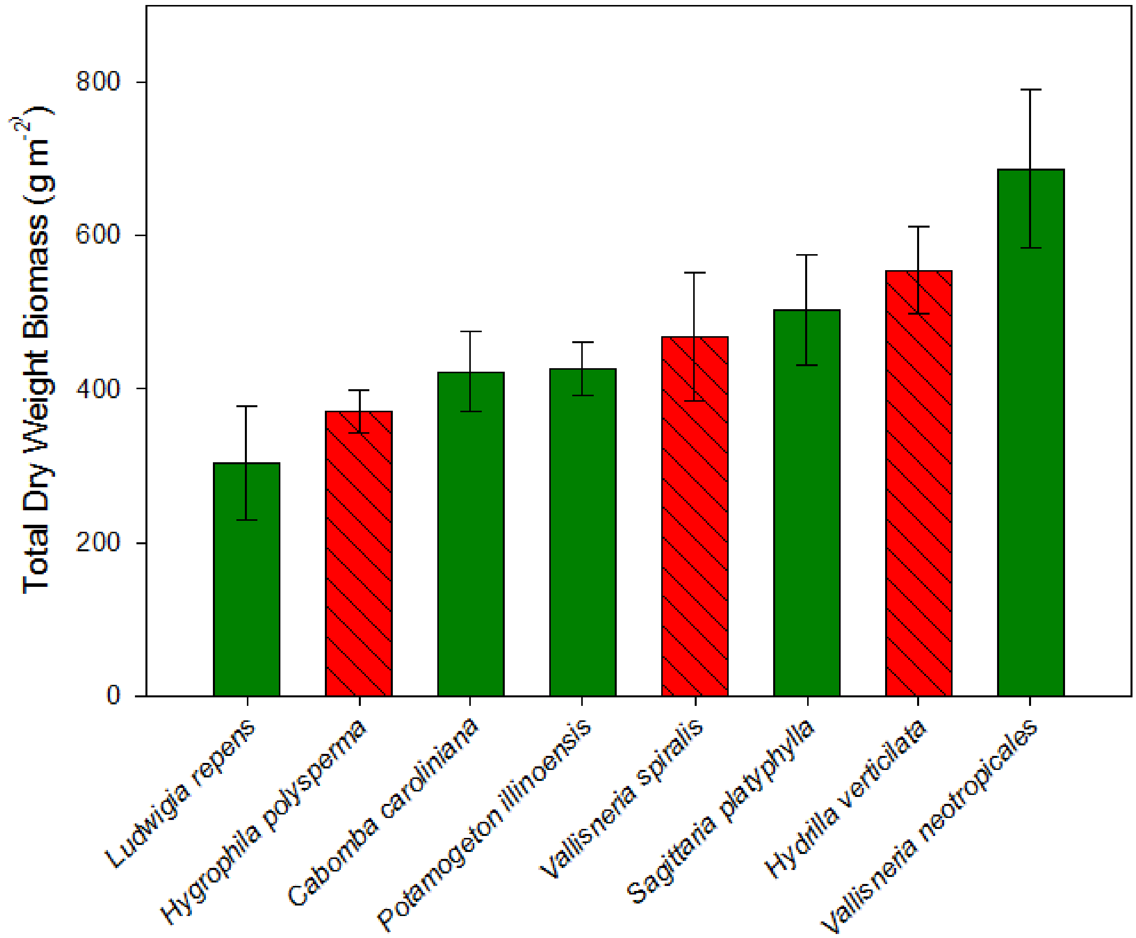

Figure 4. Total Dry Weight Biomass of Eight Aquatic Vegetation Species (Baylor 2014, Appendix B)

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).

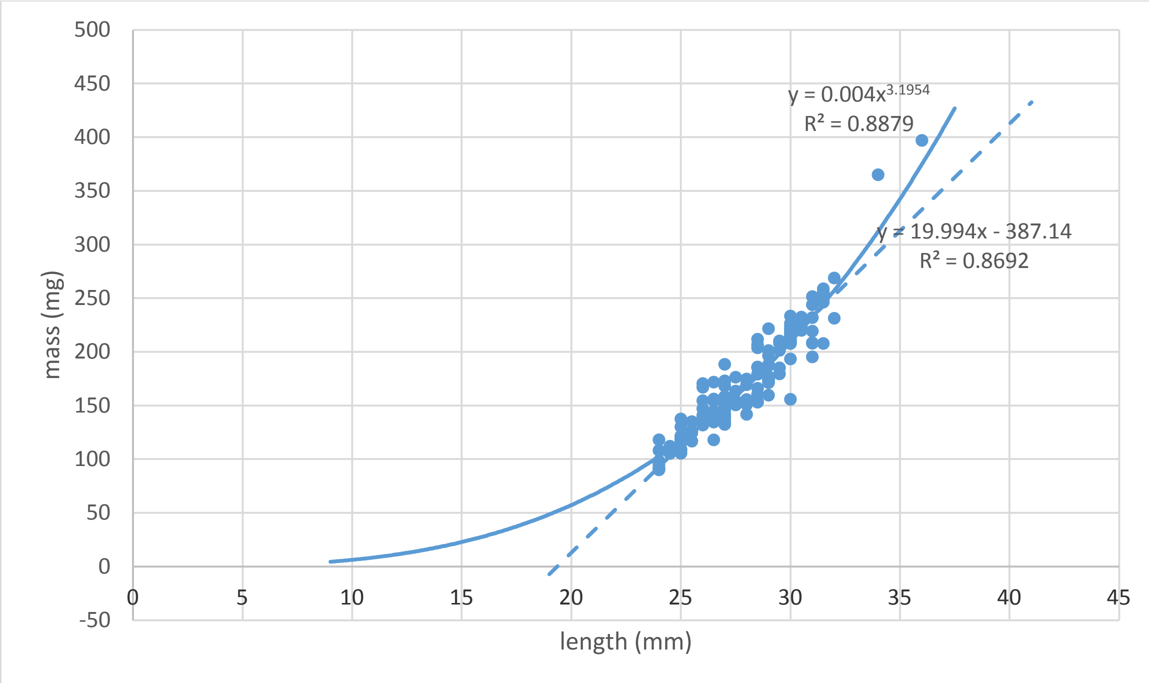

Figure 7. **Relationship of length to mass for the fountain darter.**

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.

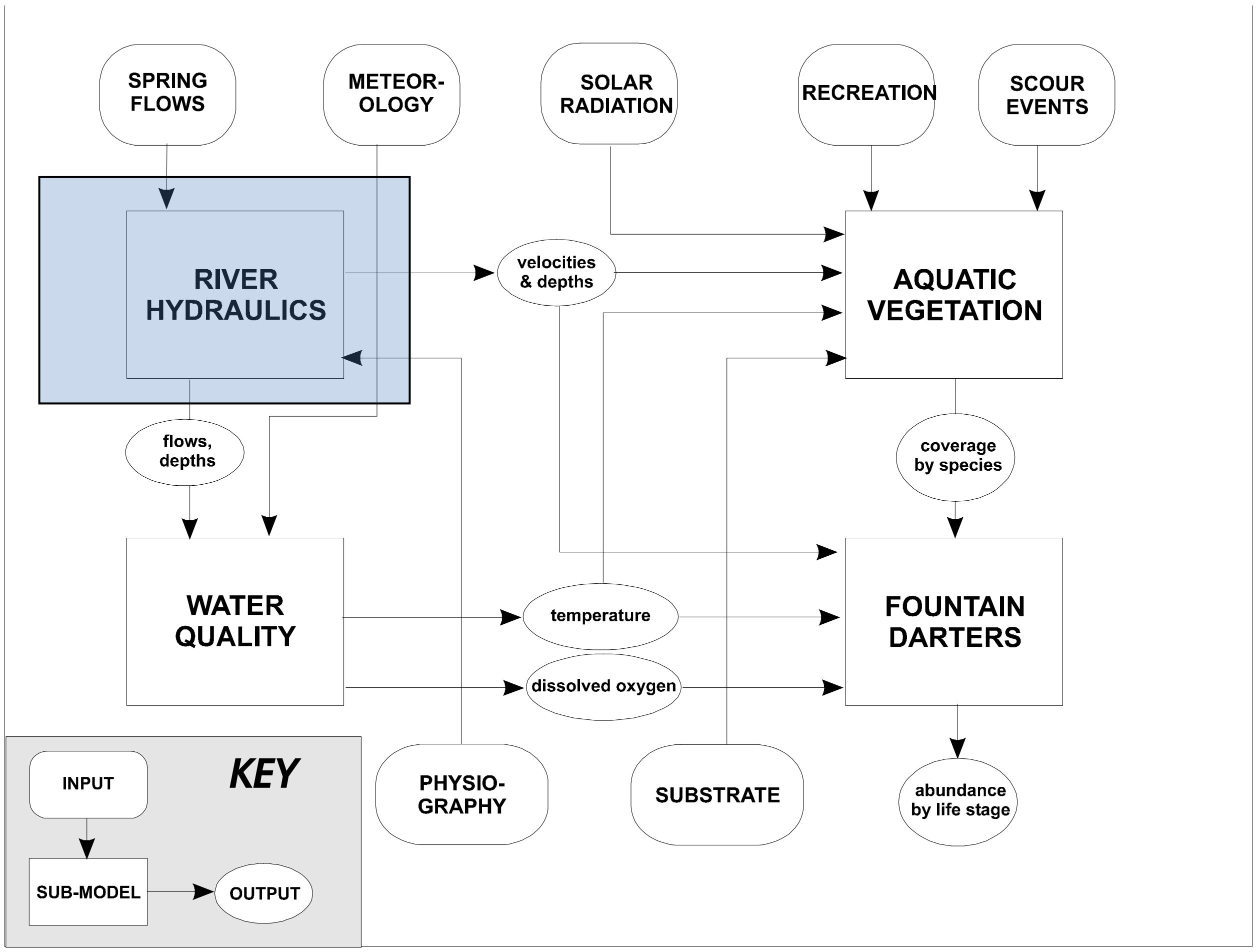

Figure 9. Conceptual model highlighting river hydraulics submodel.

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.

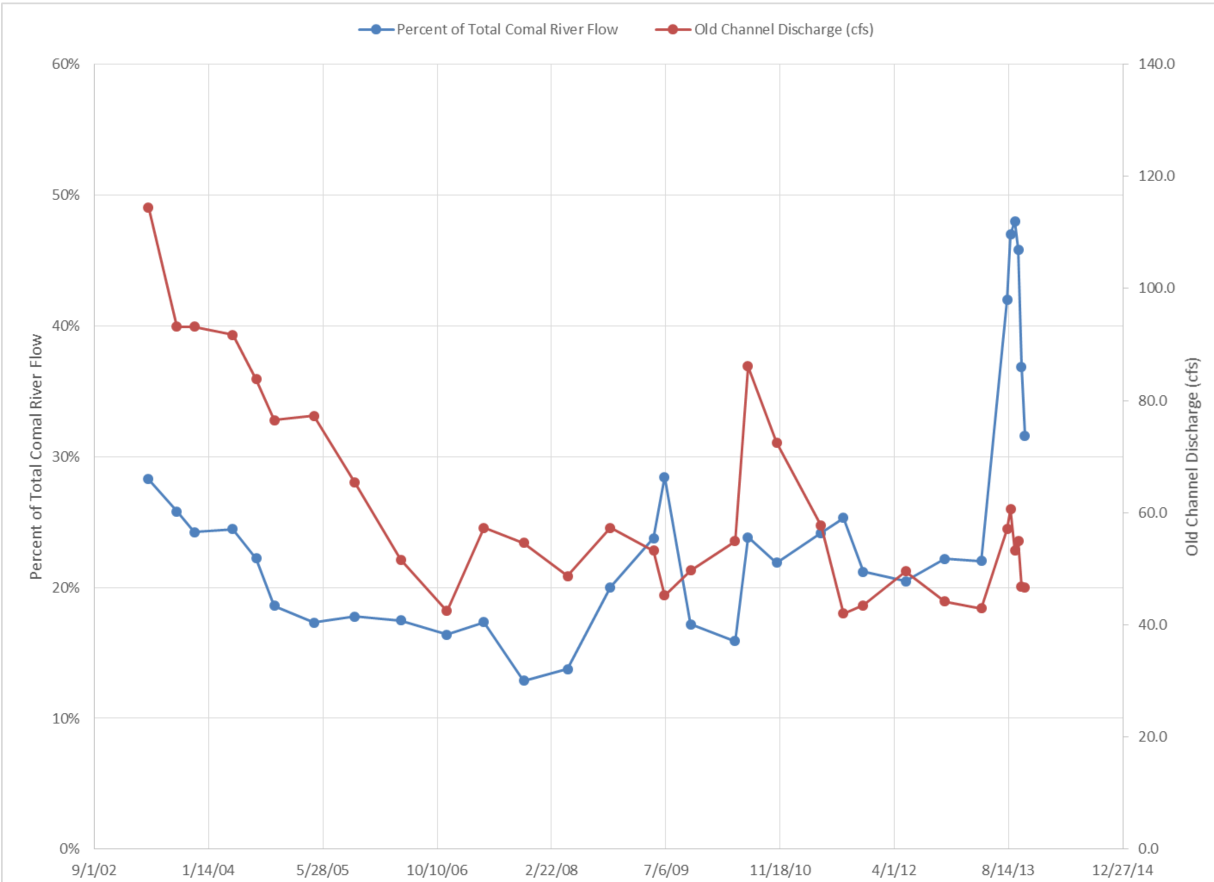

Figure 10. Empirical estimated discharges in the Old Channel of the Comal River and flows as a percent of total Comal River discharges.

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.

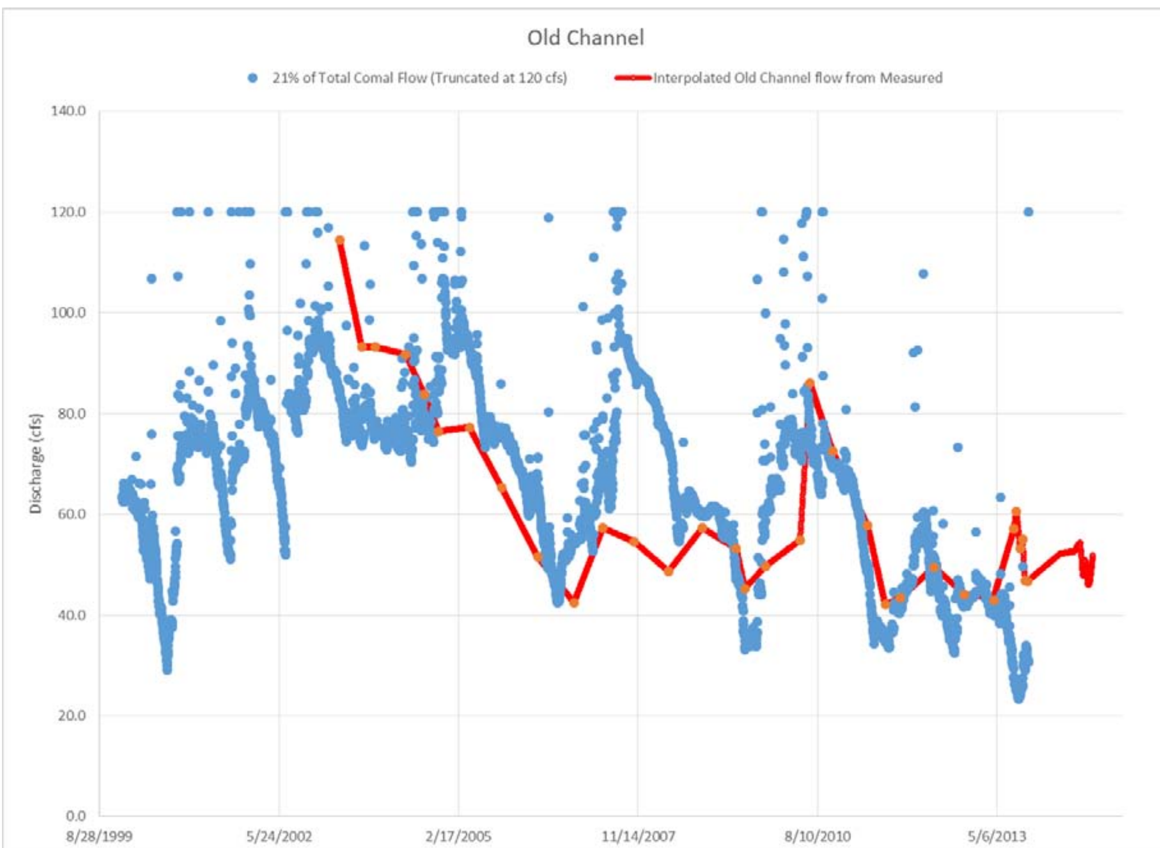

Figure 11. **Estimated daily discharges within the Old Channel modeling sites compared to the average of 21 percent of the total Comal River discharges (truncated to 120 cfs).**

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).

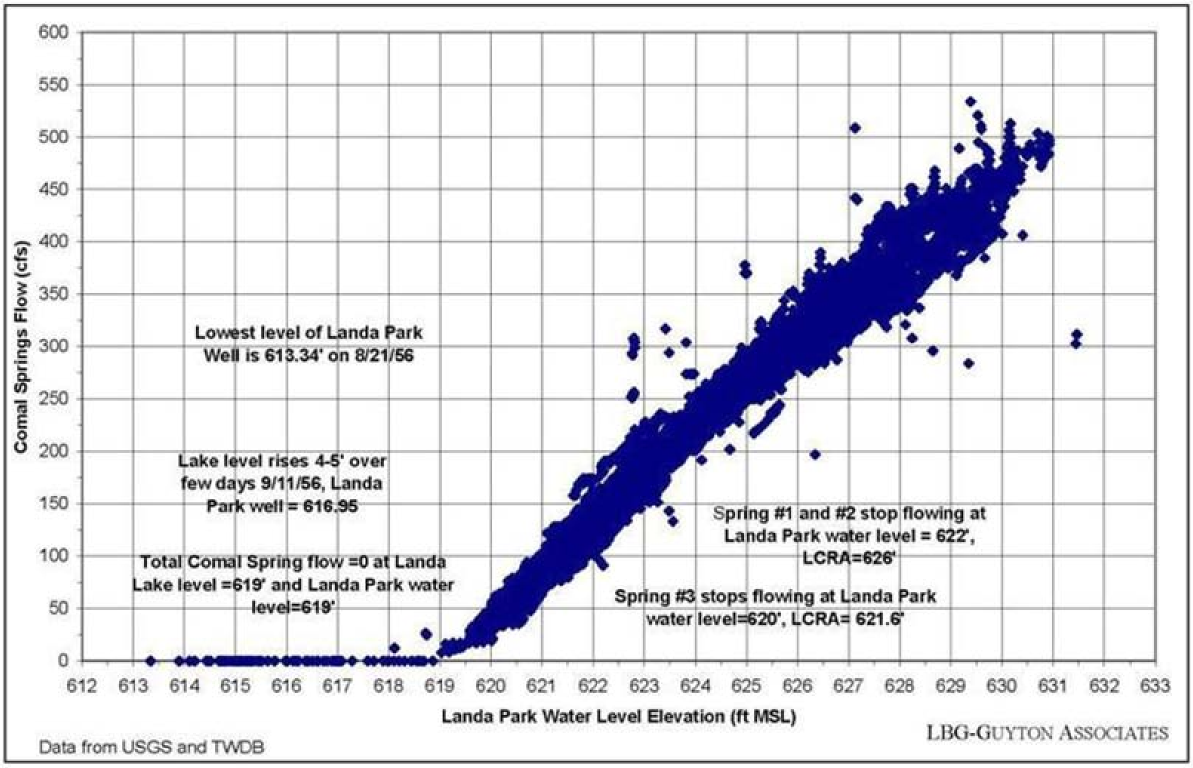

Figure 12. Relationship between the Landa Park well level versus Comal Springs flows for the 1948 to 2001 period (Guyton Associates, 2004).

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	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	0.01	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	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	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	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	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
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	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	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	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	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	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)	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
	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	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.

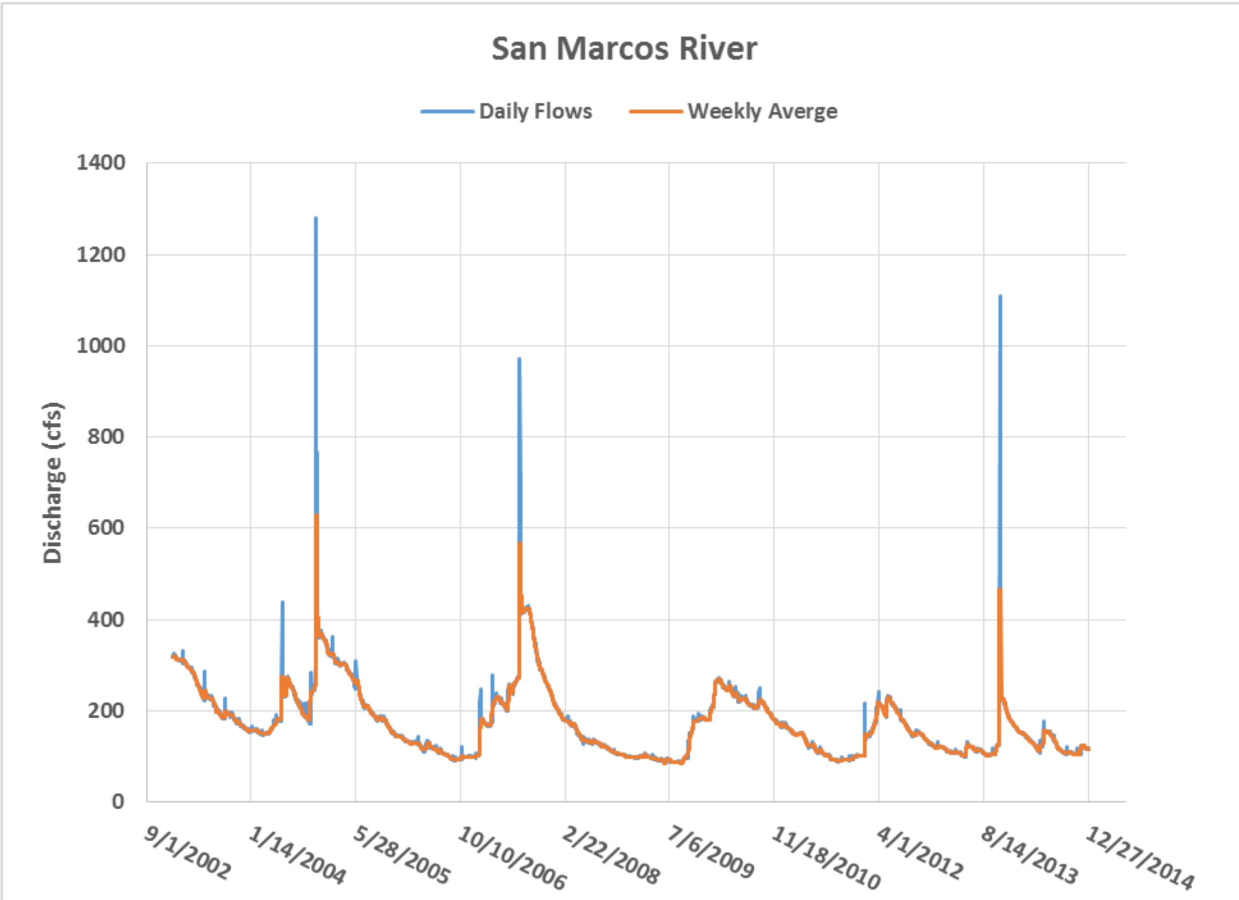

Figure 13. **Daily flows and Weekly Average Flows in the San Marcos River.**

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.

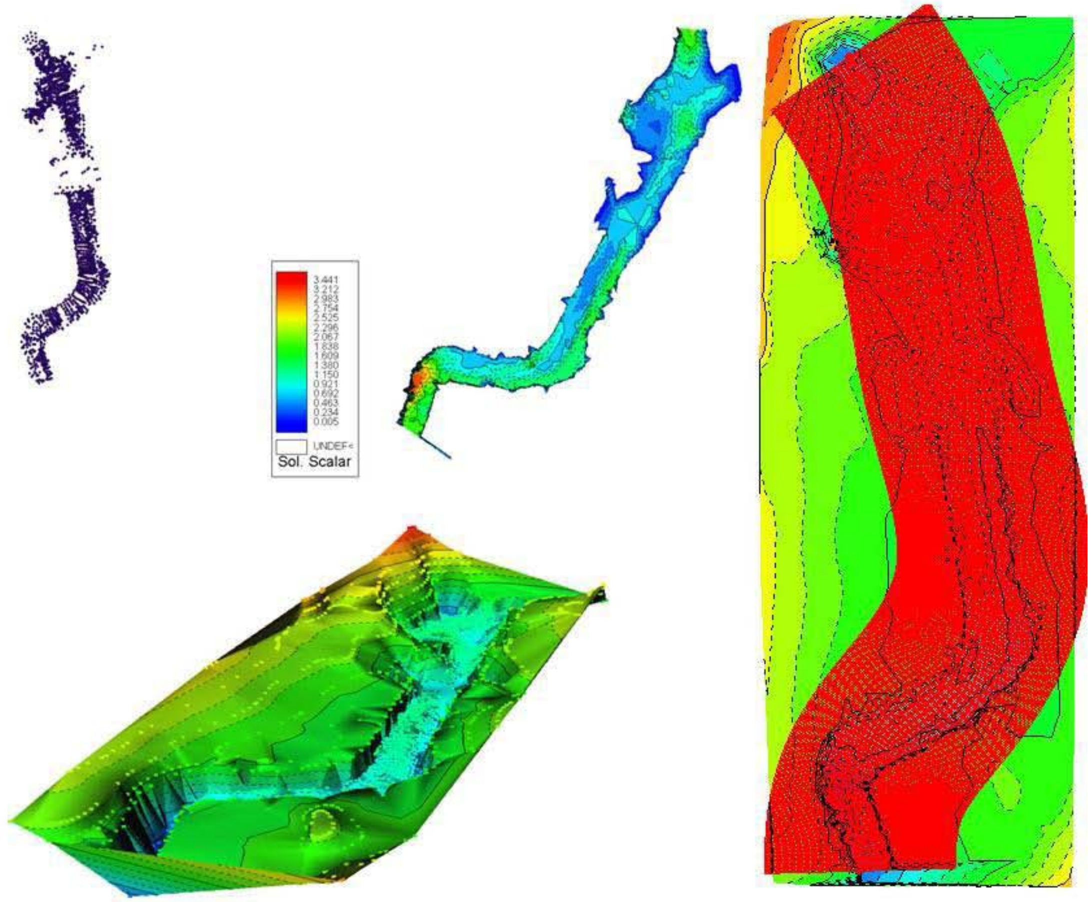

Figure 14. Example of field-measured topography points, depth contours, computational mesh overlay mapped onto topography, final 3-dimensional computation grid geometry used in MDSWMS hydraulic model (Hardy et al., 2010)

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.

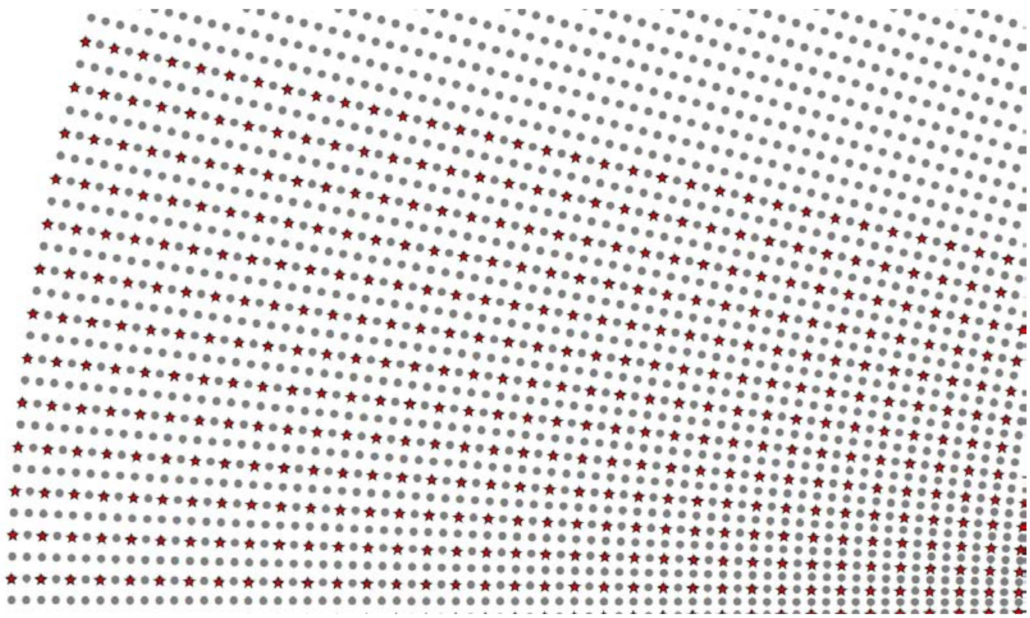

Figure 15. Example 0.5 meter grid extraction from 0.25 meter grid. Gray dots are original grid locations and red stars are the extracted grid locations. This extraction is then followed by a similar step to create a grid with 1-m resolution.

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.

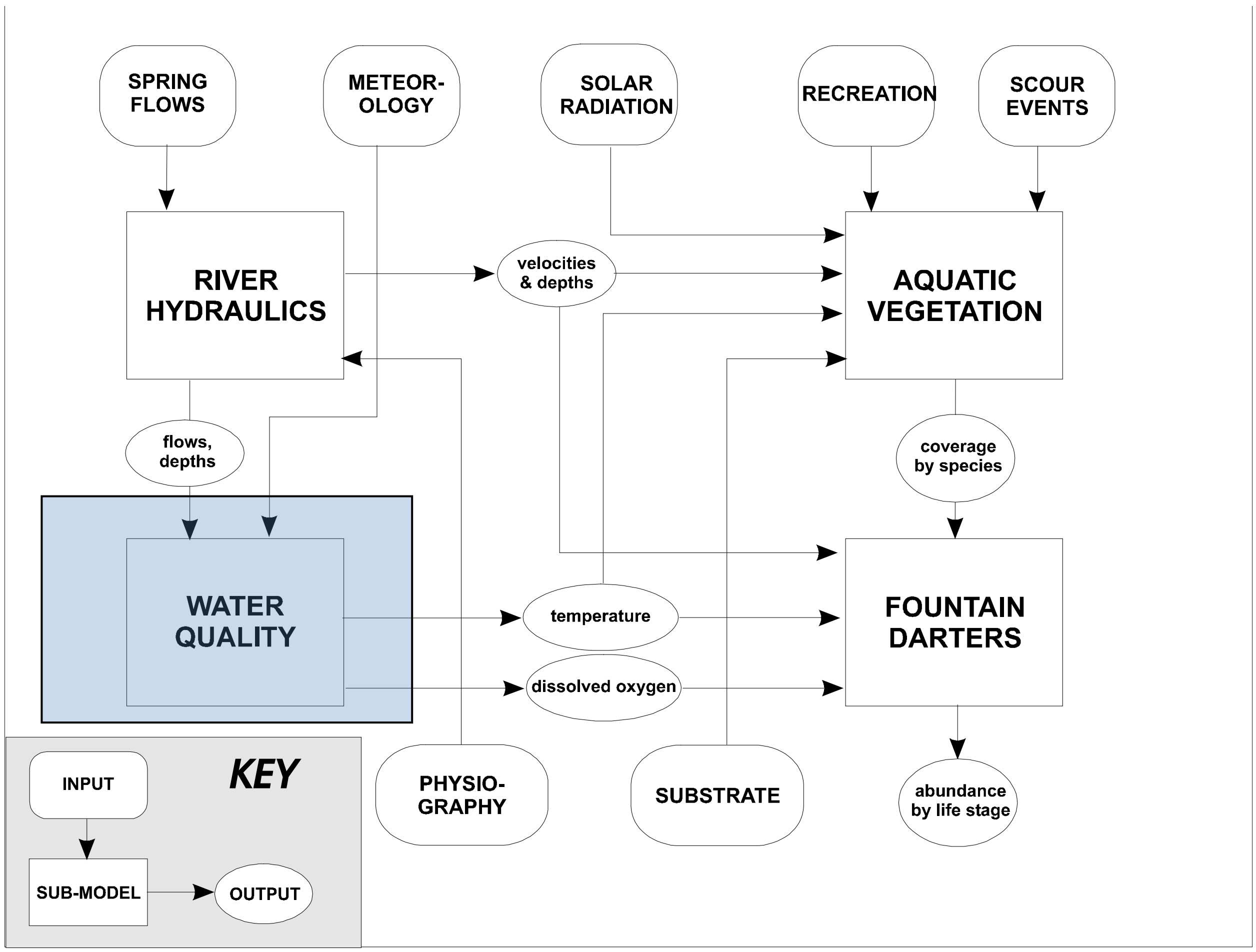

Figure 16. **Conceptual Model highlighting water quality submodel.**

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.

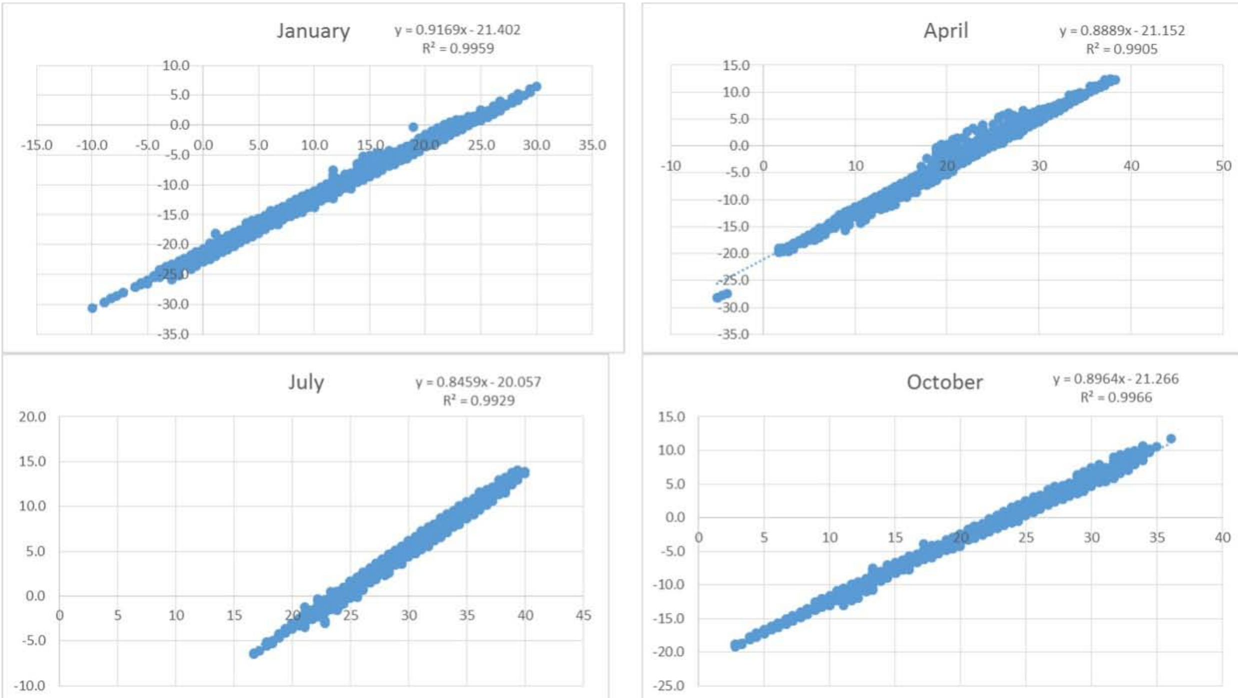

Figure 17. **Example regressions between air temperatures and the difference between air and water temperatures used to estimate hourly missing water temperatures at the Old Channel study site in the Comal River.**

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.

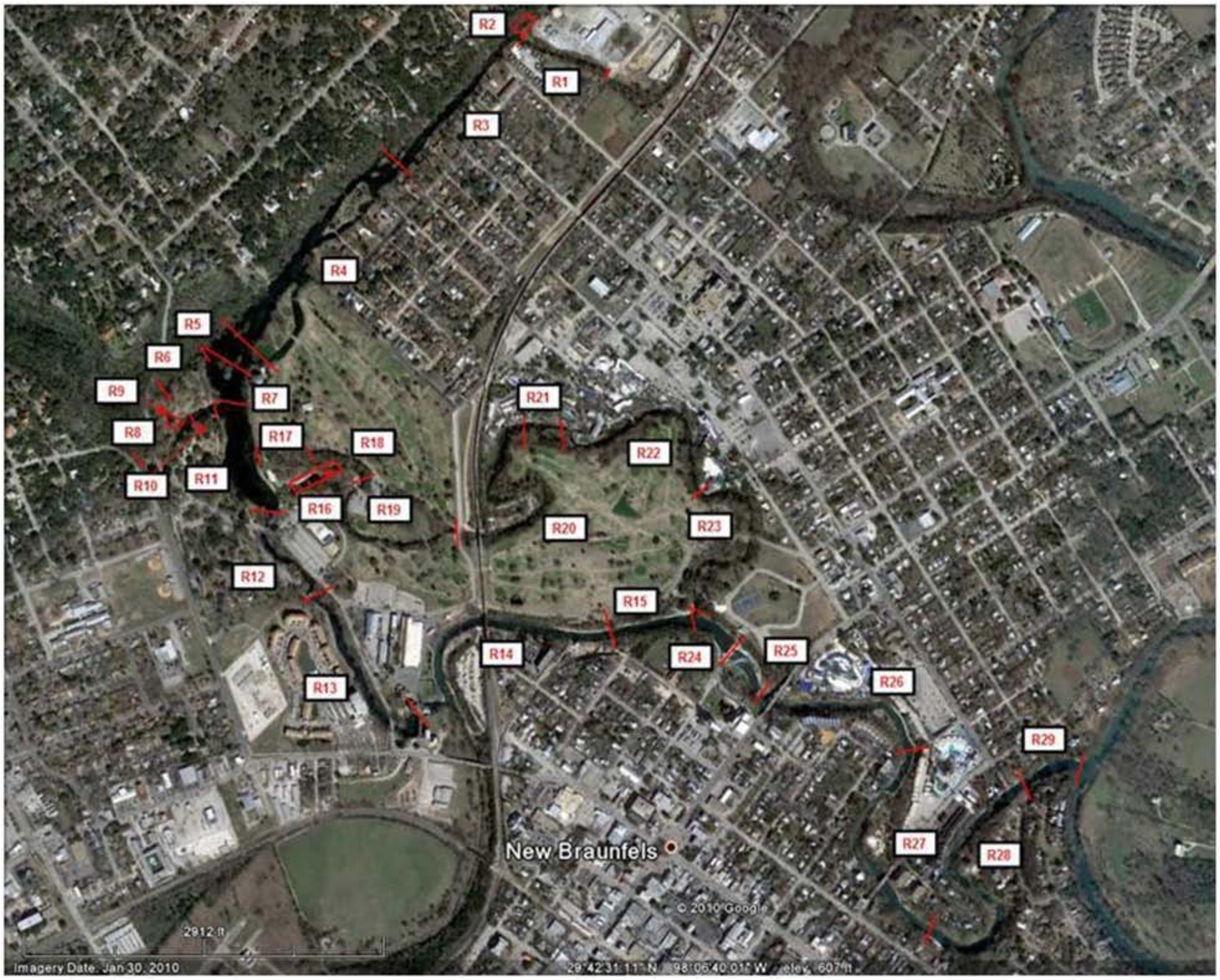

Figure 18. Qual2E computational river reaches used in modeling the Comal River system (after Hardy et al., 2010).

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.

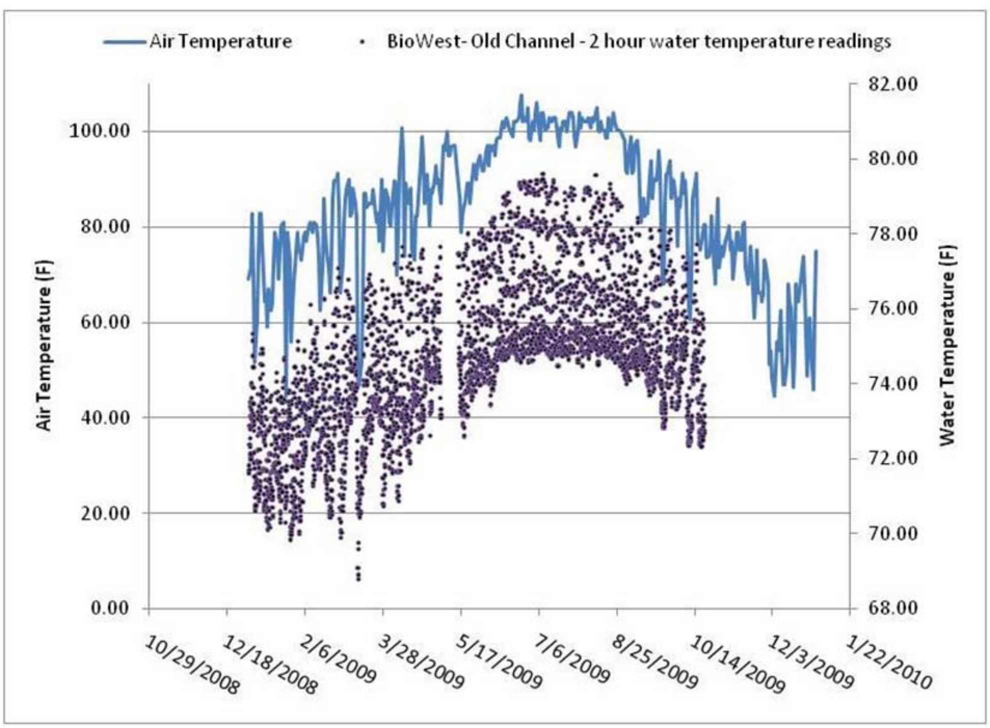

Figure 19. Maximum Daily Air Temperature and 2 hour interval recorded water temperatures from the Comal River in the Old Channel (BIO-WEST thermograph data).

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.

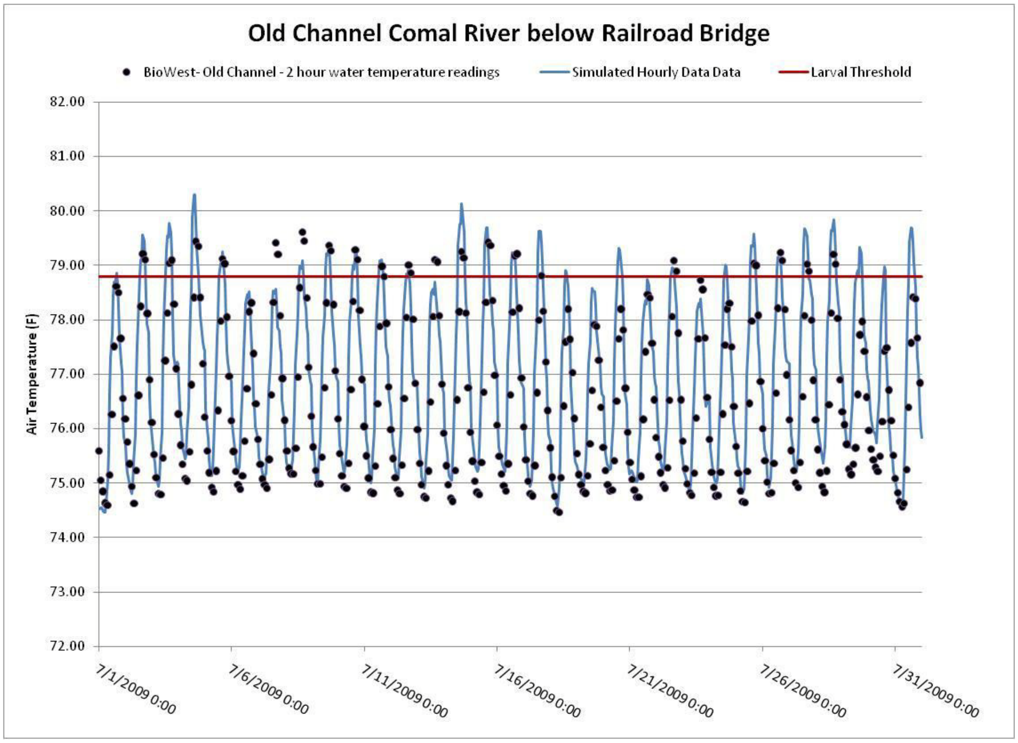

Figure 20. Simulated and observed water temperatures in the Old Channel of the Comal River during July 2009.

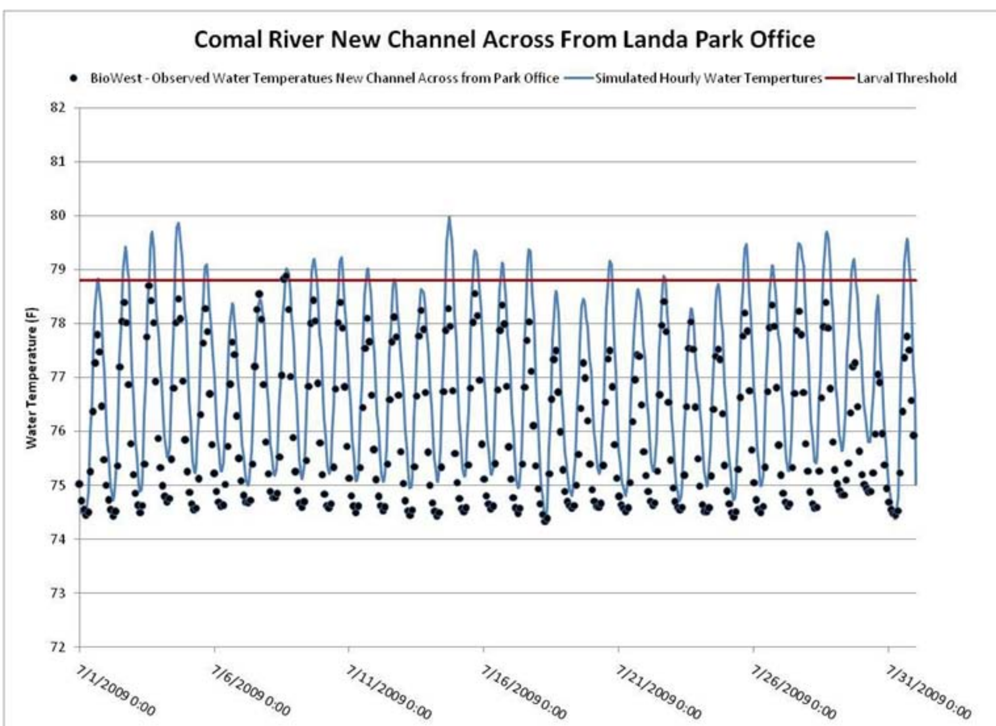

Figure 21. Simulated and Observed Water Temperatures in the New Channel of the Comal River during July 2009.

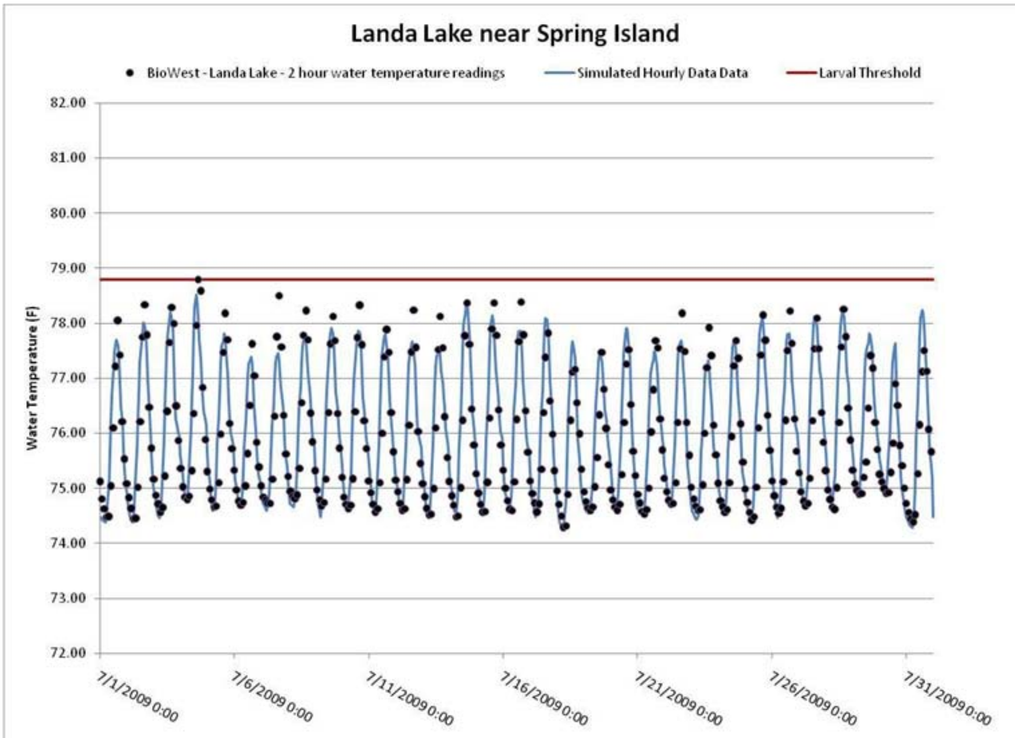

Figure 22. Simulated and observed water temperatures in Landa Lake near Spring Island during July 2009.

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 temperature.

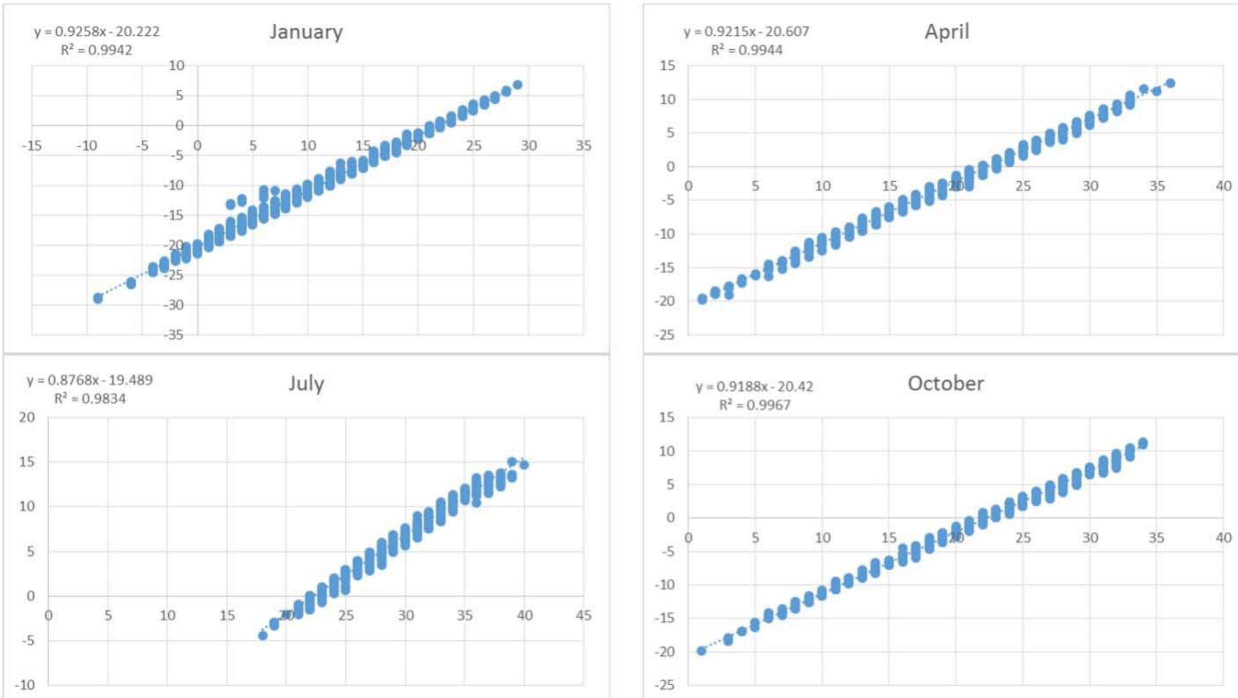

Figure 23. **Example regressions between air temperatures and the difference between air and water temperatures used to estimate hourly missing water temperatures at the City Park study site in the San Marcos River.**

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:

Headwaters

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),

Point Loads

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.

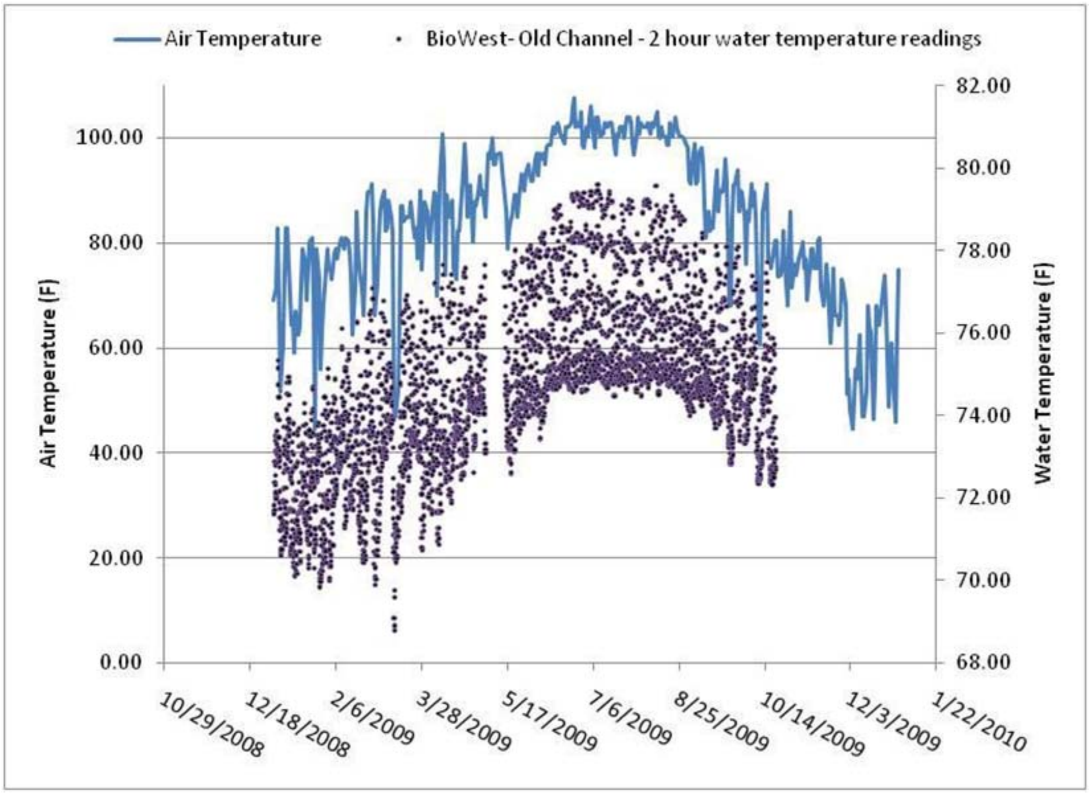

Figure 25. **Maximum Daily Air Temperature and 4 hour interval recorded water temperatures from the San Marcos River at City Park (BIO-WEST thermograph data).**

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.

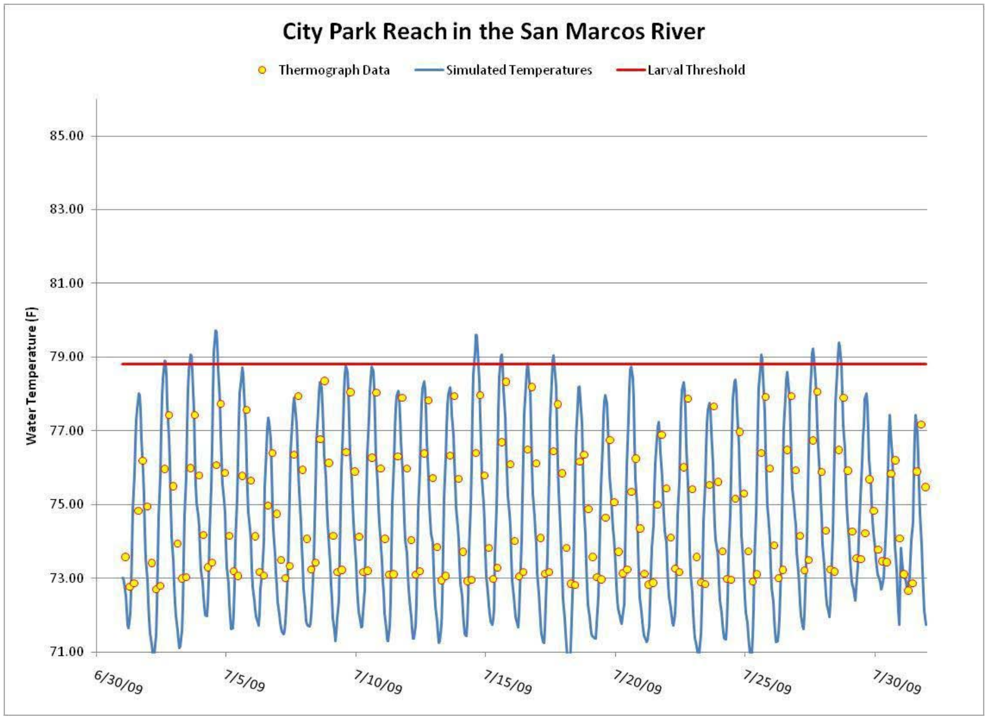

Figure 26. Simulated and observed water temperatures in City Park, San Marcos River during July 2009.

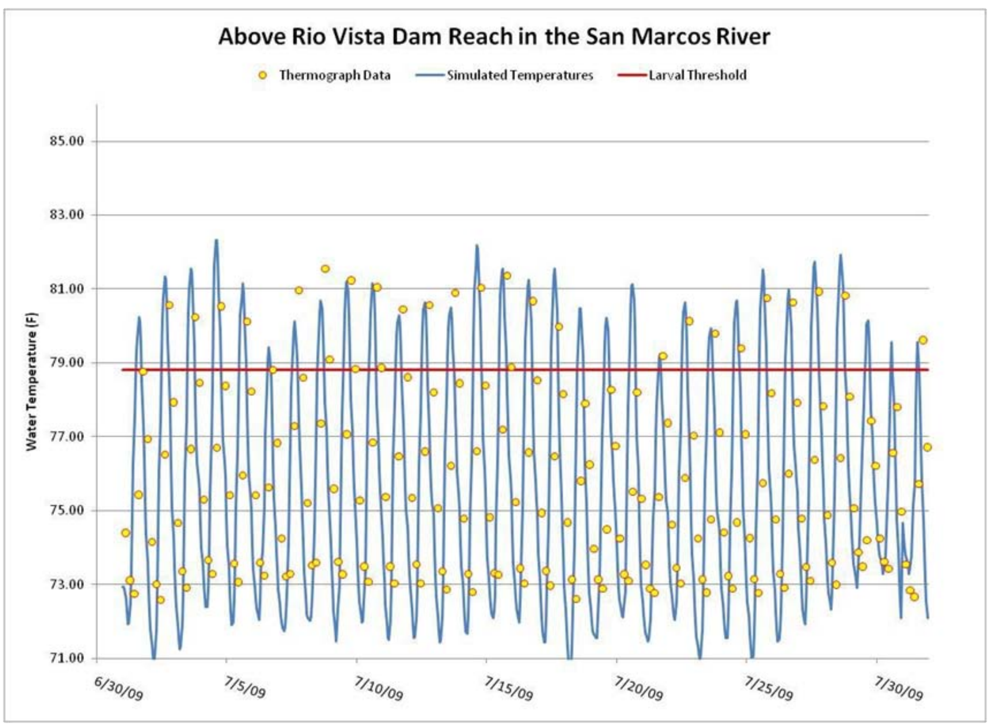

Figure 27. Simulated and observed water temperatures above Rio Vista, San Marcos River during July 2009.

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.

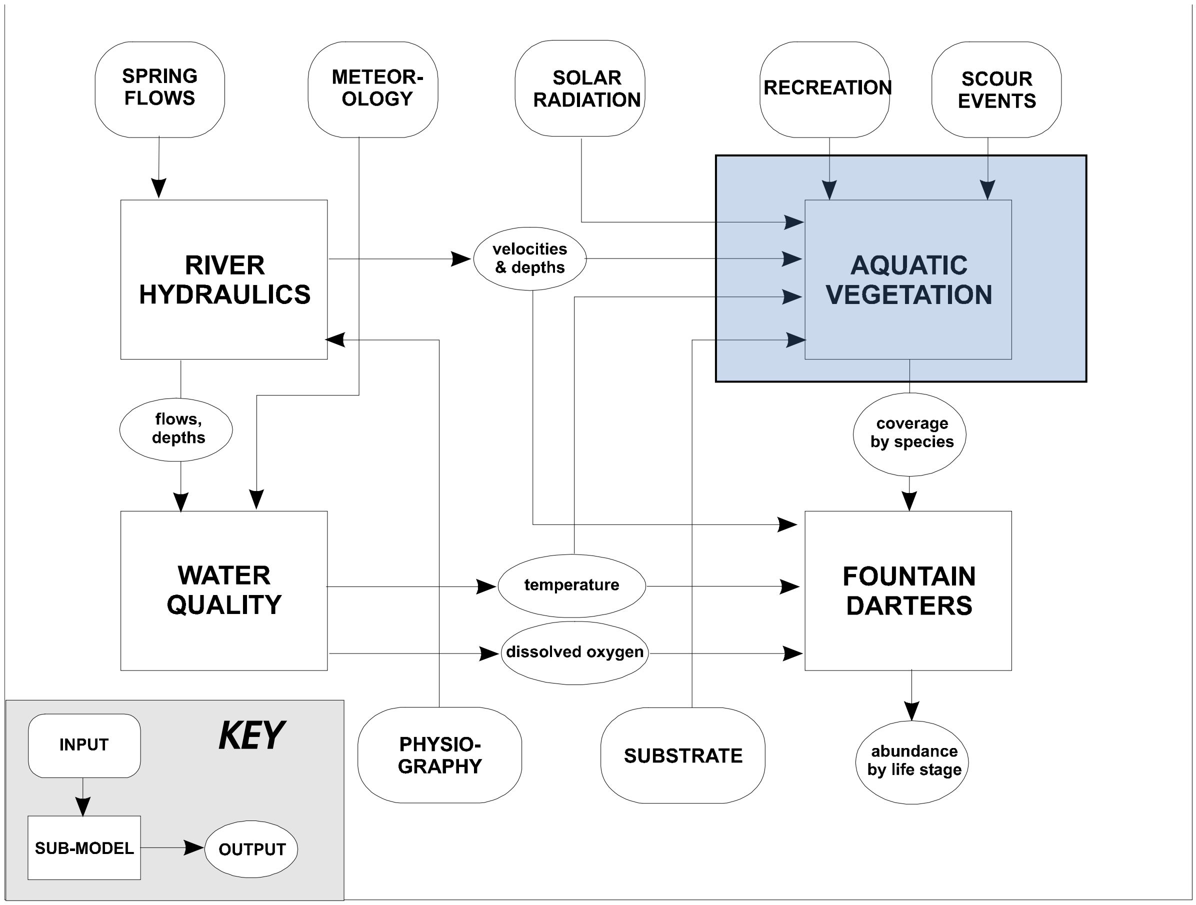

Figure 28. Conceptual Model highlighting Aquatic Vegetation

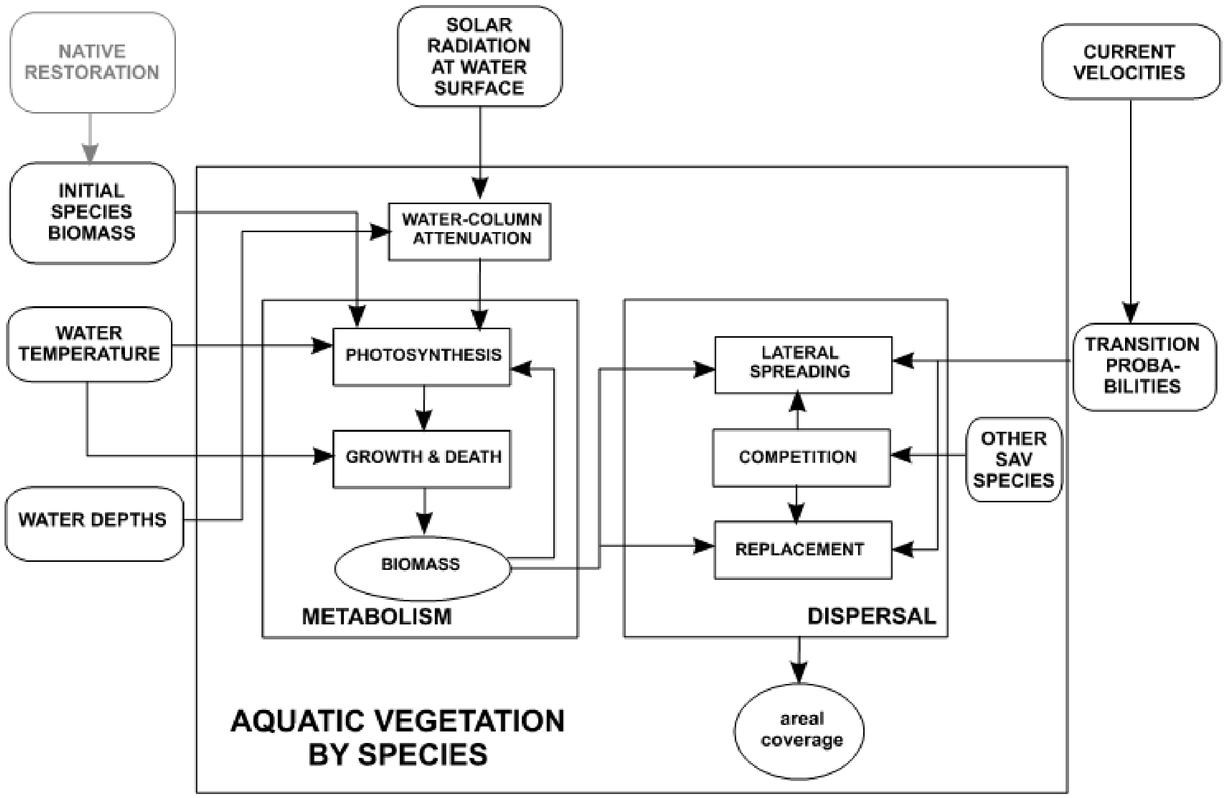

Figure 29. Aquatic Vegetation Conceptual submodel

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 species-specific 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) Existing SAV models have been calibrated for lake systems in Northern climates, which have distinct seasonal variations in temperature, turbidity, and nutrient concentrations. The San Marcos and Comal systems are spring-fed, relatively temperature-constant, clear water systems that do not experience the extreme nutrient fluctuations observed in the systems where the existing models were parametrized. As a result, the parameterization of the existing models is 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.

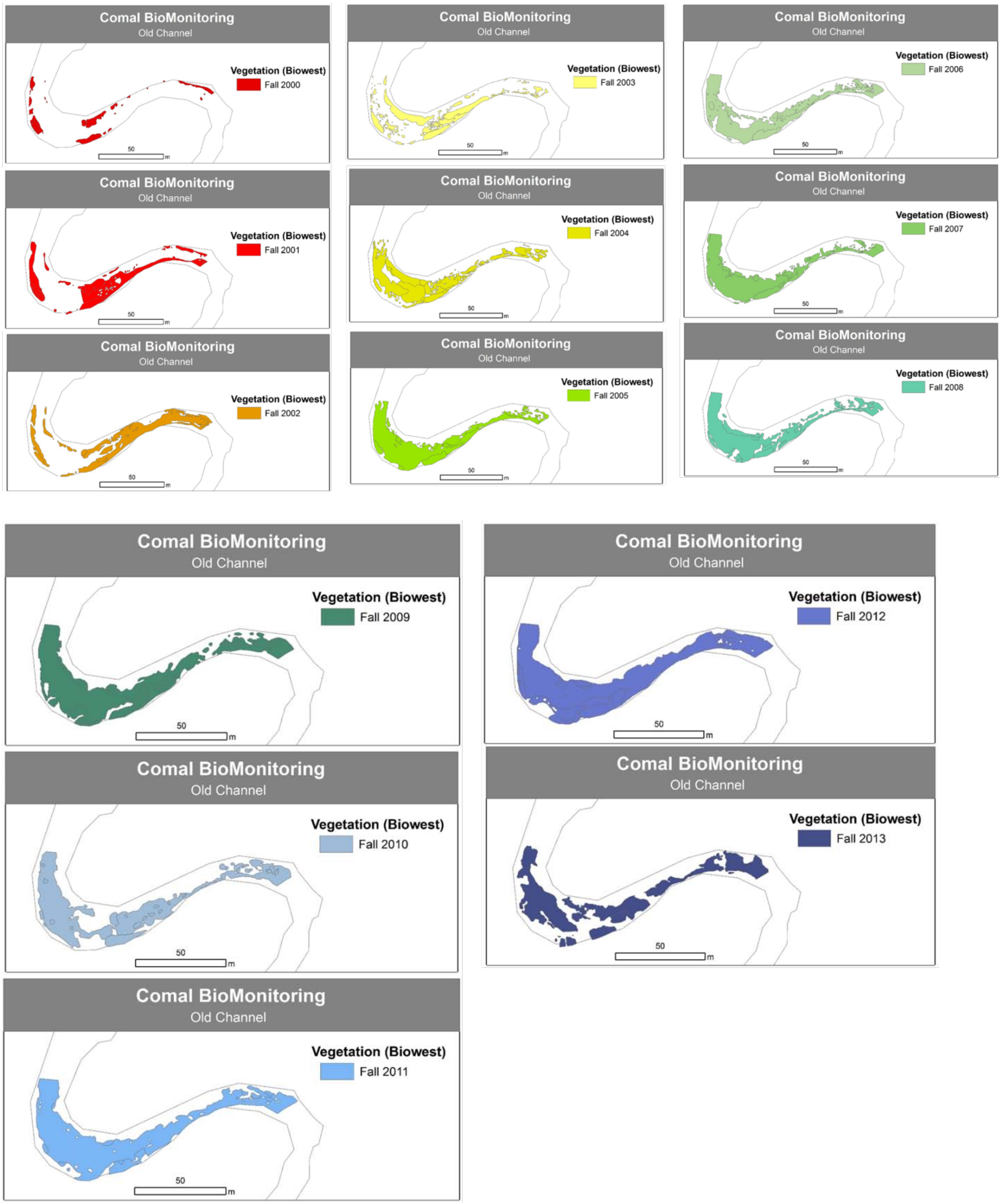

Figure 30. Shapefiles of vegetative coverage for the Old Channel in the Comal River System

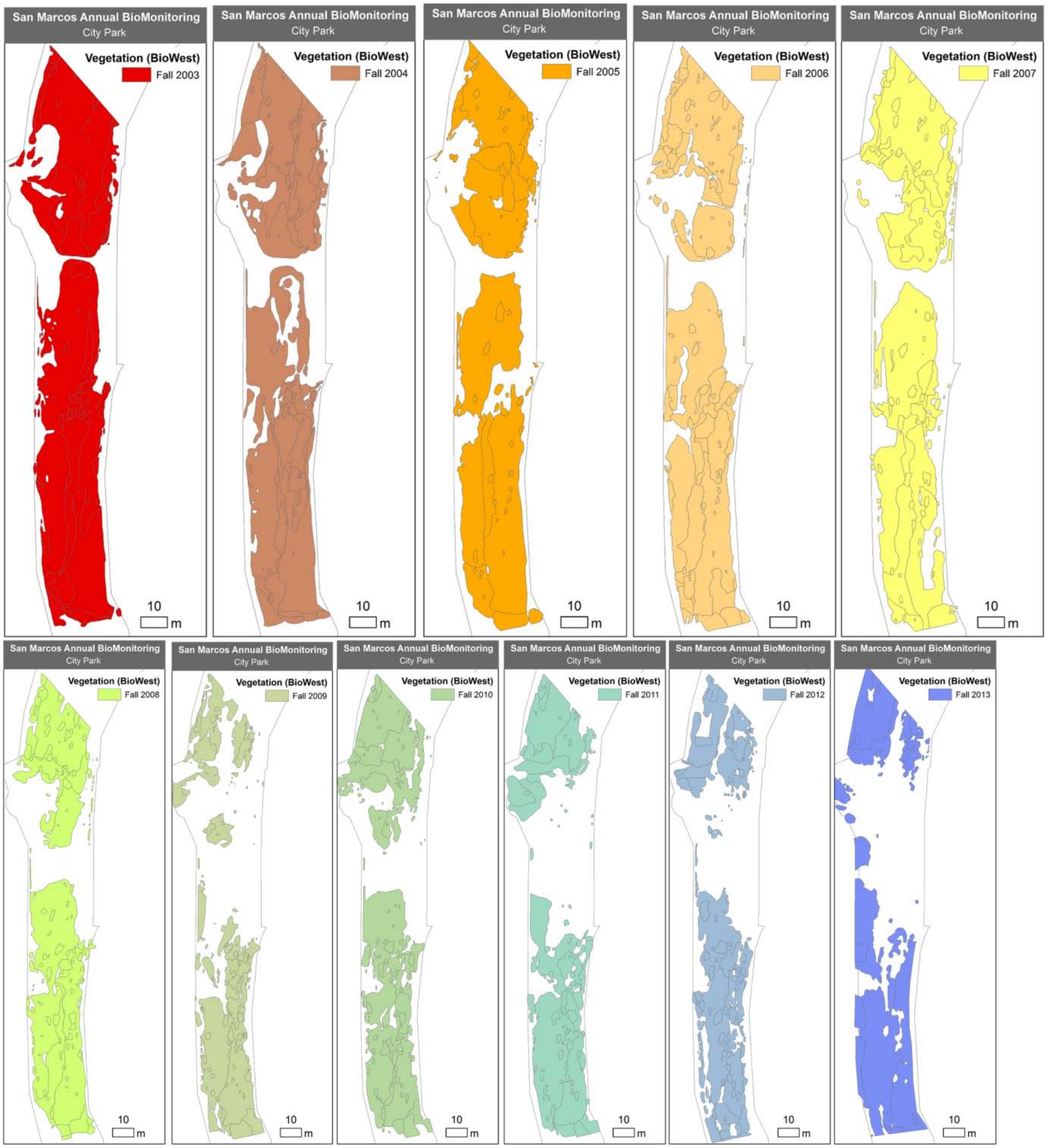

Figure 31. Shapefiles of vegetative coverage for the City Park Reach in the San Marcos River System.

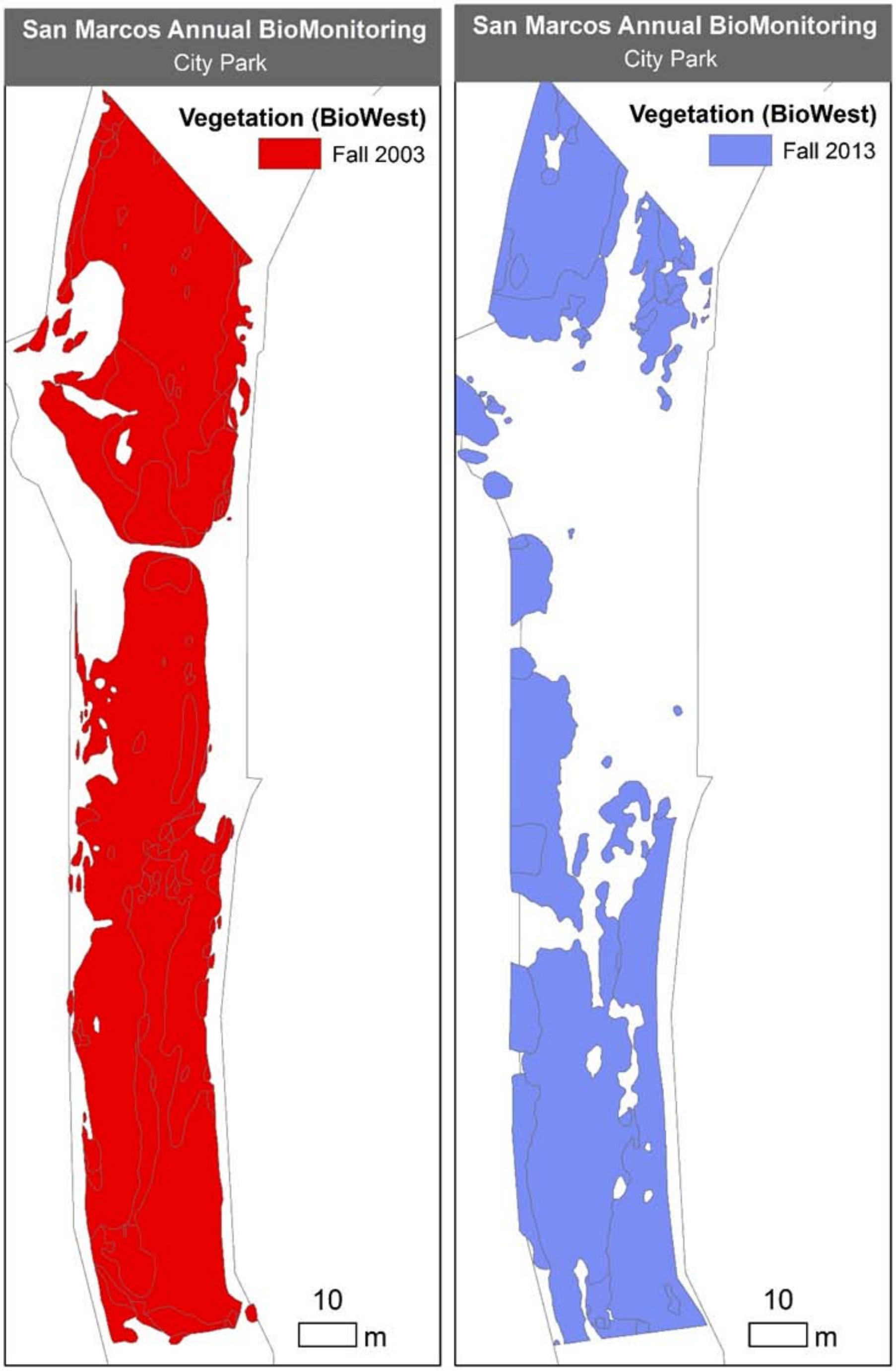

Figure 32. Shapefiles of vegetative coverage for the City Park Reach in the San Marcos River System (Fall 2003 and Fall 2013).

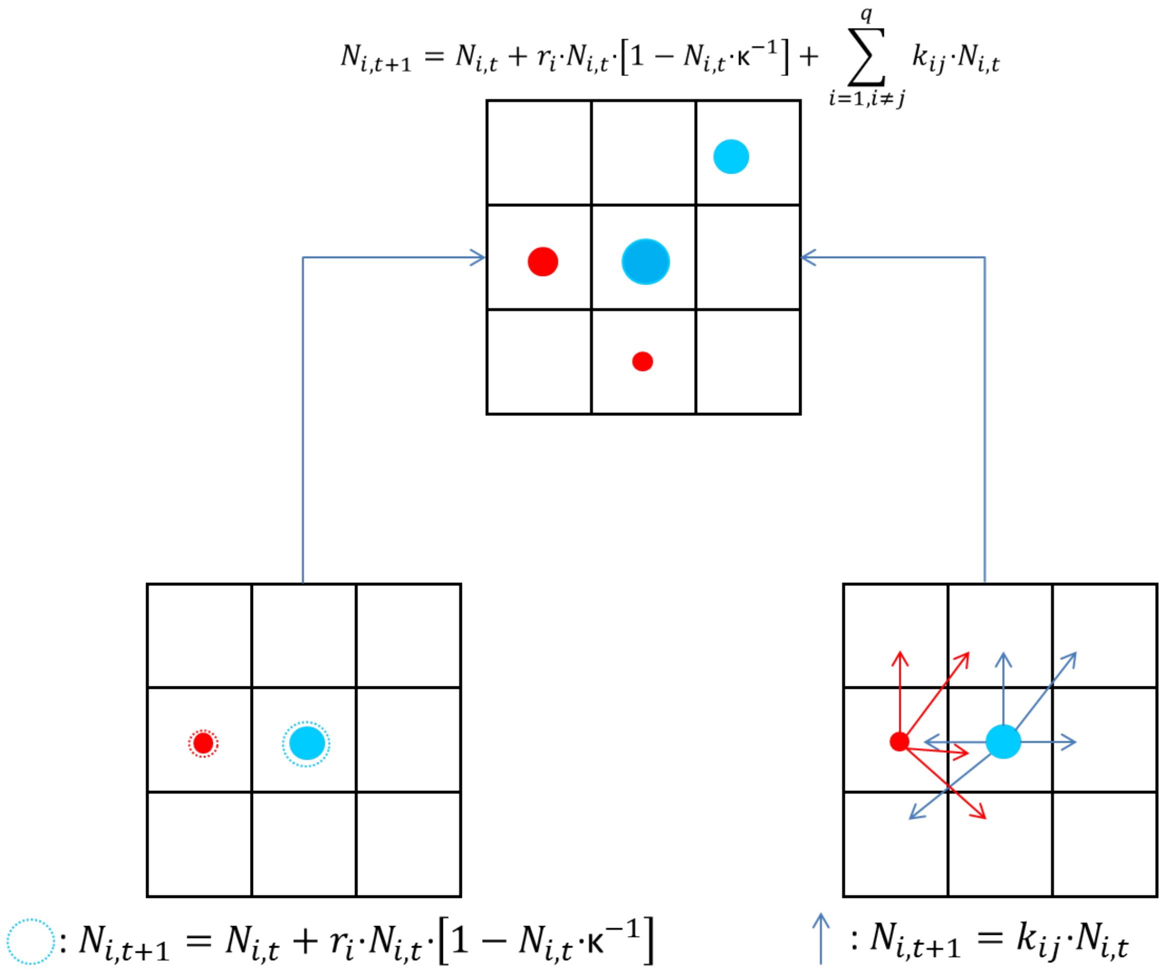

Figure 33. **Conceptual diagram of the mathematical framework that will be used to model growth and dispersal of different categories of vegetation (indicated by different colors). (A) Mathematical representation of local growth via a logistic equation where *ri* represents the spread rate of species *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 water temperature in the SAV submodel because these systems are spring-fed and have a relatively constant temperature (typically ranging from 21 - 26°C annually). Preliminary analysis indicated that aquatic macrophyte biomass accumulation was not sensitive to this relatively narrow band of temperature fluctuations. As a result, the effects of water temperature were not included in the photosynthesis equation. However, water temperature was included in the mortality estimates to account for potential extremes (cold or hot) that may occur during periods of extended low-flow.

We also elected not to include nutrients in the SAV submodel based on the general assumption that the aquatic macrophytes in the Comal and San Marcos systems are not nutrient limited. The overall idea is that aquatic macrophytes get most nutrients from sediments (via roots and stems) - not the water column (Barko and Smart, 1980) and that the sediments in these systems, which remain mostly undisturbed, provide the nutrients for the aquatic macrophyte communities. We are comfortable with this assumption based on the abundant and vibrant aquatic macrophyte communities present in both systems. The equation used for photosynthesis is adaptable, and can add a nutrient component if sediment nutrient data becomes available contrary to our present understanding.

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

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[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

$$\Delta W = W_s P - W(R_m + M) \qquad (1)$$

Where ΔW is the change in plant weight for a given day, W_s is the weight of the sprout, 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

$$P = P_{max} * \frac{I}{I + H_I} \qquad (2)$$

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).

$$\boldsymbol{Declination = -asin(sin(23.45) * \left(\cos\left(2 * \pi * \frac{day + 10}{365}\right)\right.} \tag{3}$$

$$sinld = \sin(lat) * \sin(declination) \qquad i_1$$

$$cosld = \cos(lat) * \cos(declination) \qquad i_2$$

$$aob = \frac{sinld}{cosld} \qquad i_3$$

$$\boldsymbol{daylength = 12 * \left(1 + 2 * \frac{\mathrm{asin}(aob)}{\pi}\right)} \tag{4}$$

$$\boldsymbol{hour_i = 12 + (daylength * 0.5 * gaussian\ weight_j)} \tag{5}$$

$$DsinB = 3600 * \left(daylength * sinld + 24 * cosld * \sqrt{(1 - aob^2) / \pi}\right) \qquad i_4$$

$$dsinBE = 3600 * \left(daylength * (sinld + 0.4 * (sinld^2 + cosld^2 * 0.5)) + 12 * cosld * (2 + 3 * 0.4 * sinld) * (1 - aob2) / \pi\right. \qquad i_5$$

$$sinb = max\left(0, \left(sinld + cosld * cos(2 * \pi * (hour_i + 12) / 24)\right)\right. \qquad i_6$$

$$\boldsymbol{PAR = 0.5 * dailyradiation * sinb * (1 + 0.4 * sinb) / dsinBE} \tag{6}$$

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

$$\boldsymbol{I_z = PAR * e^{(-0.12 * z) - (Kp * biomass_{>z})}} \tag{7}$$

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*.

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[3] http://www.lakeaccess.org/ecology/lakeecologyprim3.html

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

$$\boldsymbol{TDGA = daylength * \sum_{h=1}^{3}\left(GW_h * \sum_{z=1}^{3} P_{z_i}\right)} \qquad (8)$$

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 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.

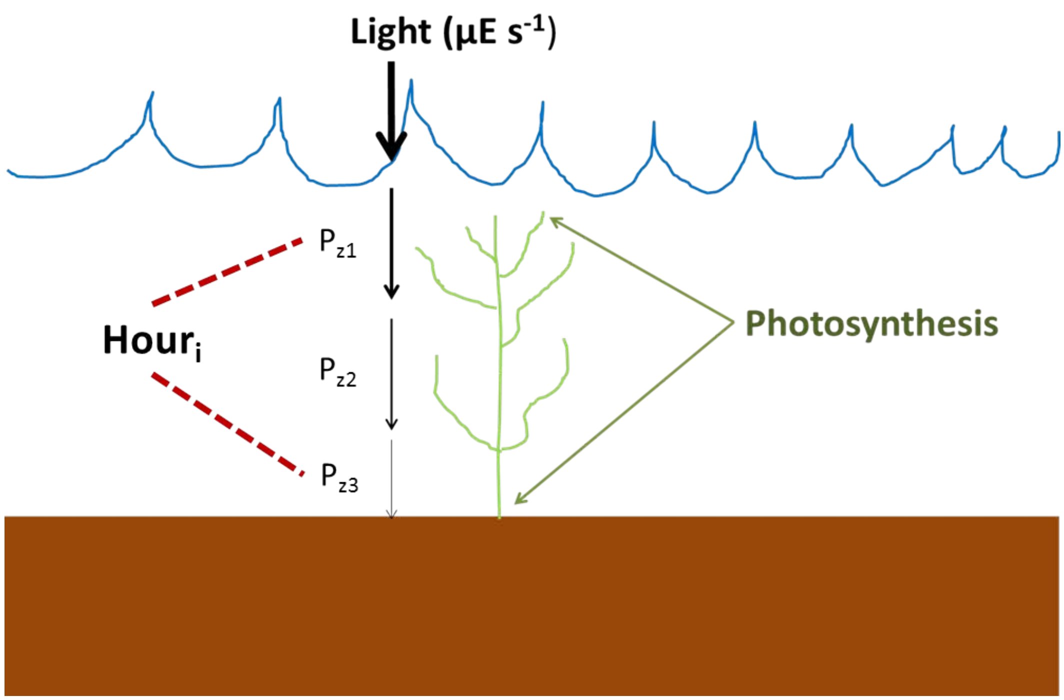


Figure 34. Conceptual model of Guassian integration of photosynthesis

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

$$\boldsymbol{R_{AG} = 0.0225 * \left(Q_{10}{}^{(temp-25)/10}\right)} \qquad \textbf{(9)}$$
$$\boldsymbol{R_{BG} = 0.015 * \left(Q_{10}{}^{(temp-25)/10}\right)} \qquad \textbf{(10)}$$

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):

$$\boldsymbol{G_{Growth} = F_{AG}G_{AG} + F_{BG}G_{BG}} \qquad \textbf{(12)}$$

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

$$\boldsymbol{BM_{t+1} = BM_t + F * \left(\frac{W_{glucose} - R}{G_{growth}}\right)} \qquad \textbf{(13)}$$

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.

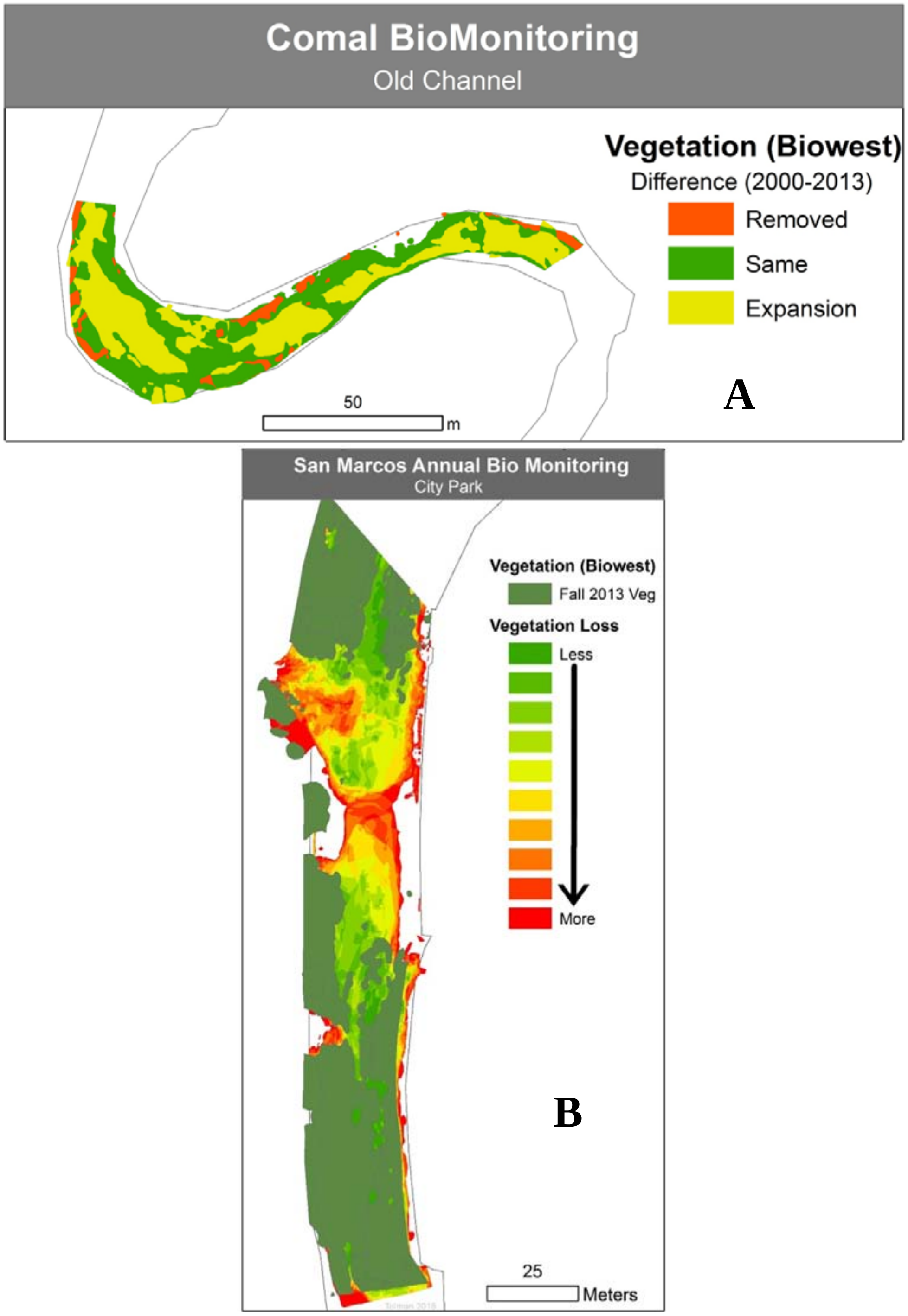


Figure 35. **Depiction of frequency of occupancy of a given cell over time in the Old Channel (A), and City Park (B) reaches. Red and orange colors indicate that locations oscillated between vegetated and unvegetated during the course of the 13 year study, while green colors indicate those locations 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.

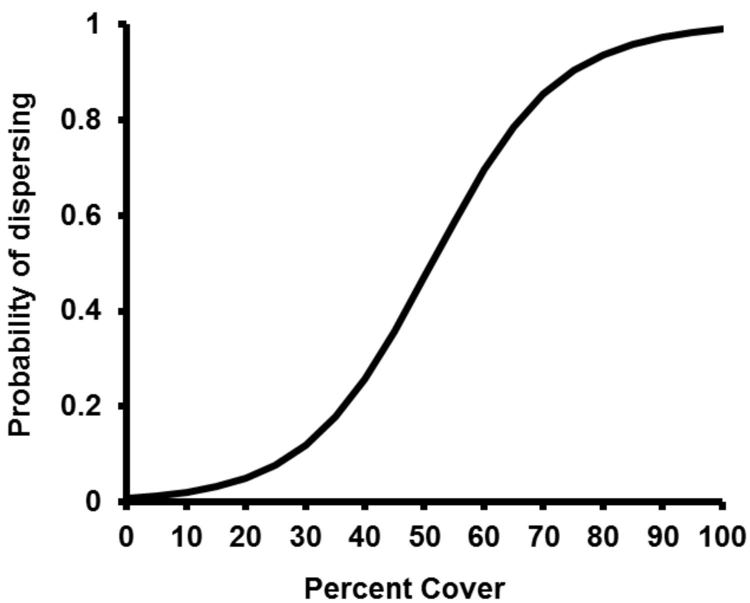


Figure 36. Example of logistic function used to calculate probability of dispersal based on percent cover of vegetation for each cell. Logistic curve was derived following Railsback and Grimm, 2014.

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.

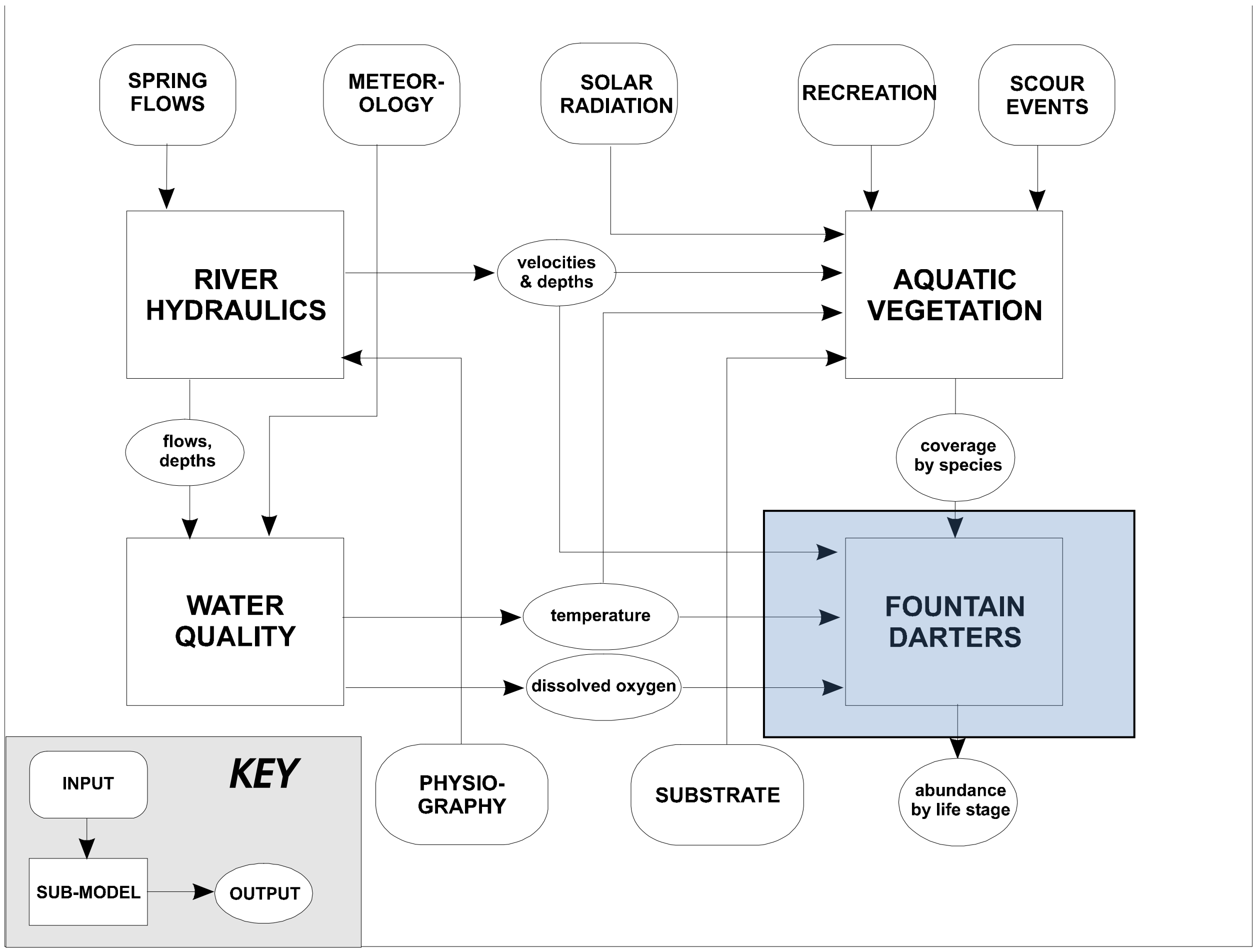


Figure 37. Conceptual model highlighting fountain darter submodel

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 Dr. Michael Longnecker (Professor and Associate Department Head, Department of Statistics) of 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.

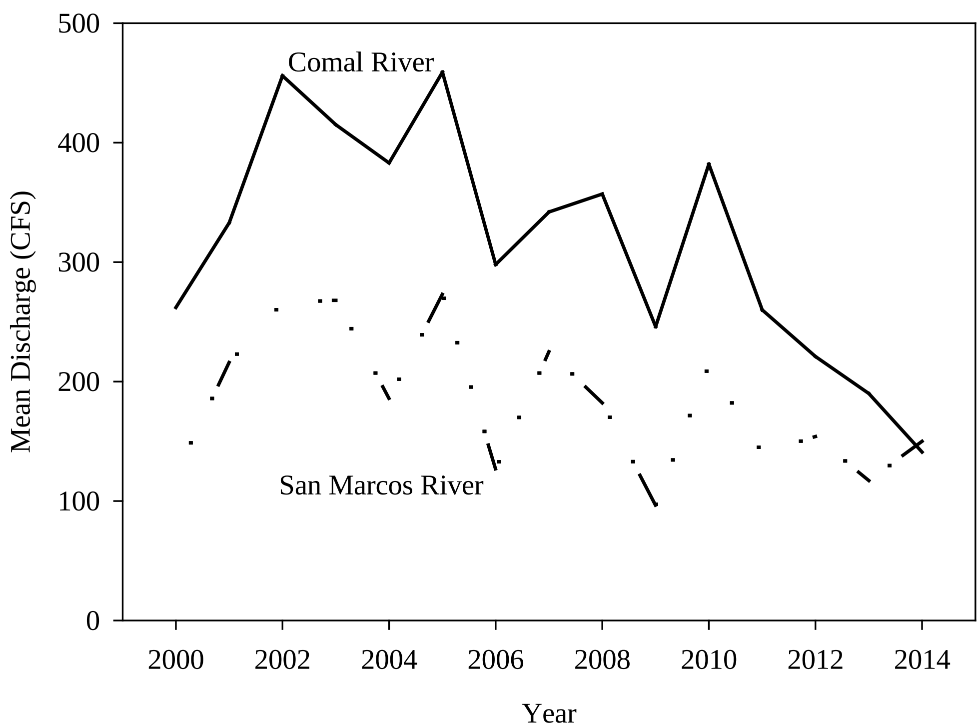


Figure 38. Mean annual discharge in cubic feet per second (CFS) during the period of observation (2000 - 2014).

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.

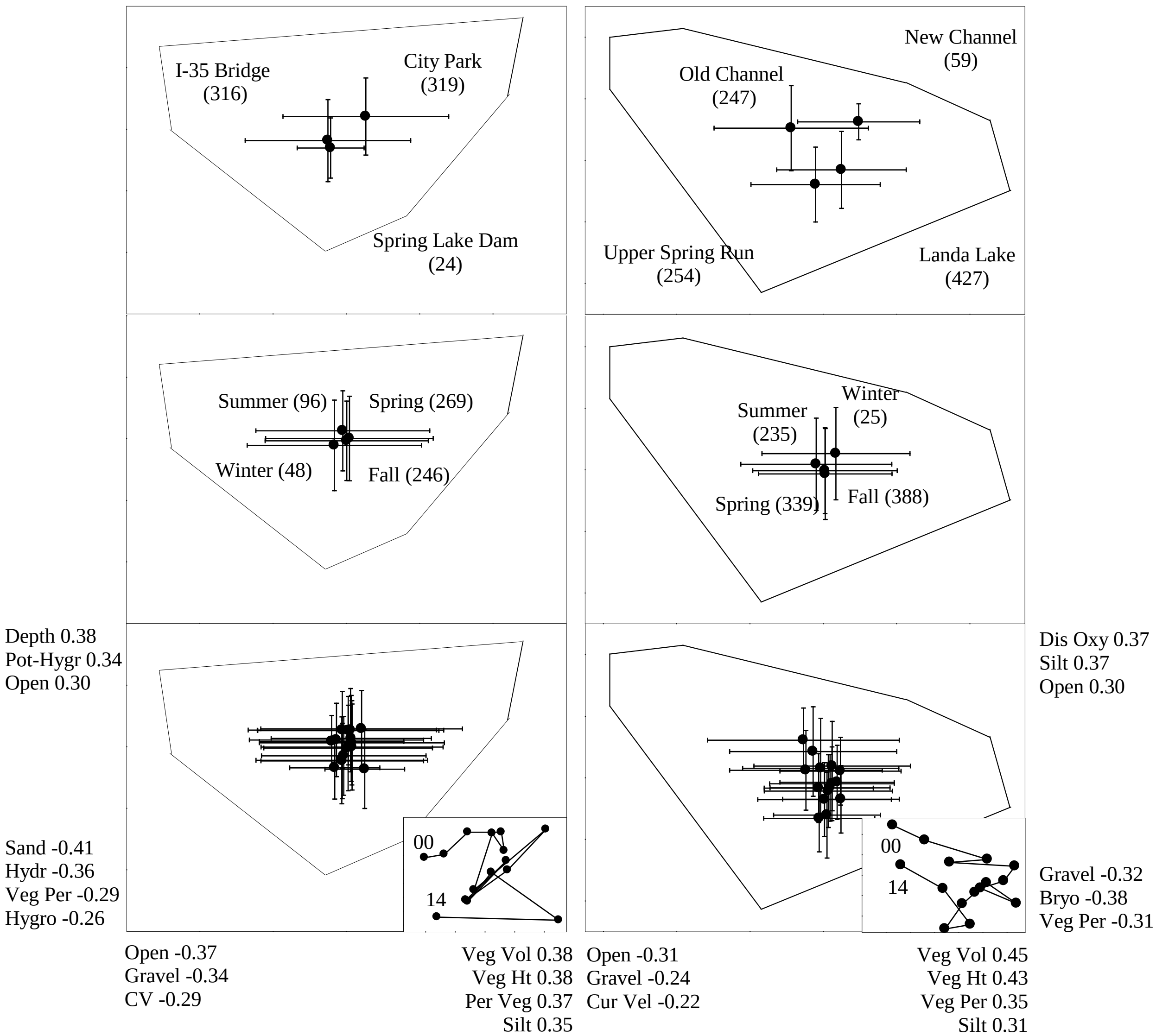


Figure 39. Mean and 1 SD for PC I and PC II scores by site (top graph), season (middle) and year (bottom). Number per site and season are in parentheses. For year, subset plot indicates trajectory of mean PC I and PC II scores starting in 2000 (00) and ending in 2014 (14). Abbreviated variables on axes: 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.

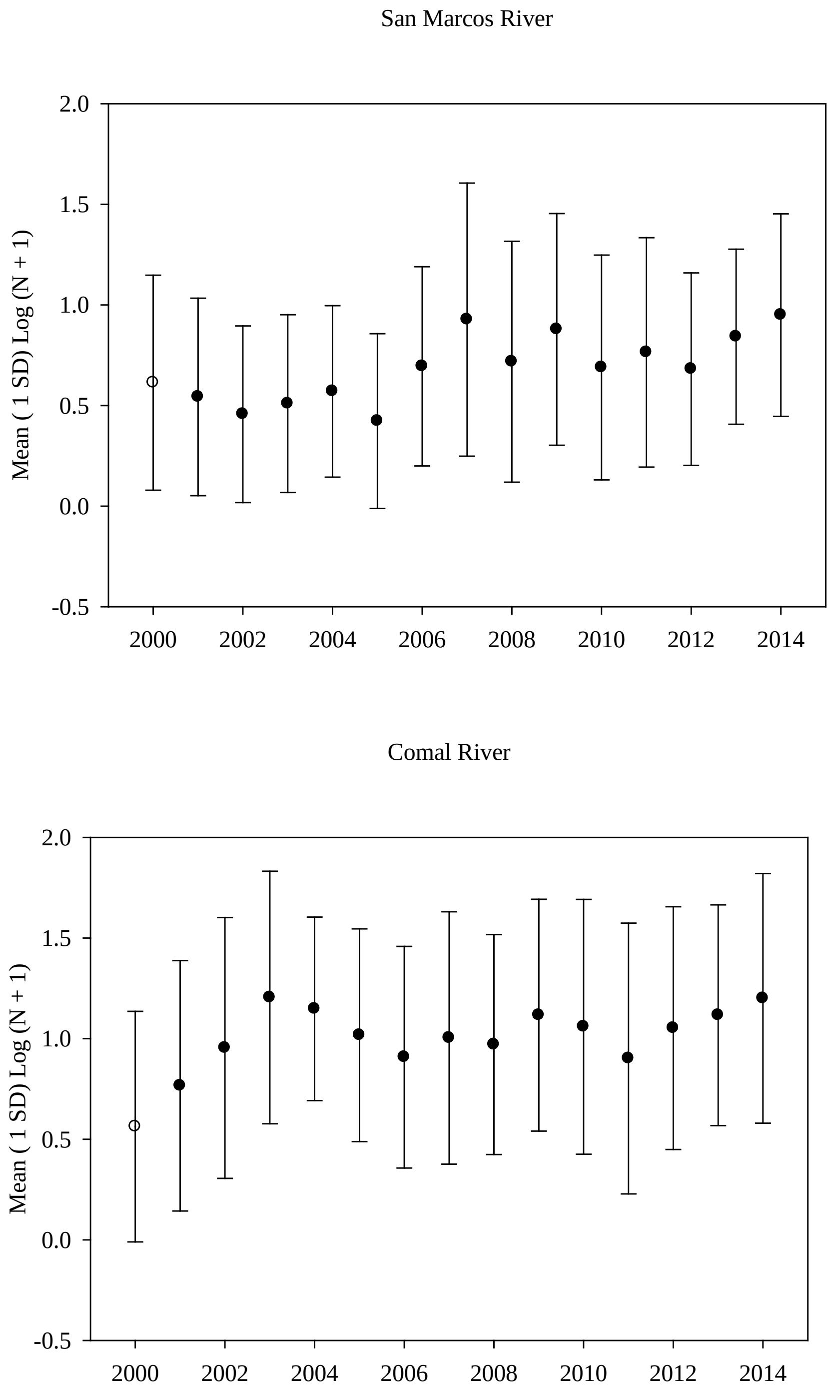


Figure 40. Mean ± 1 SD abundance (Log [N+1]) of fountain darters quantified in the San Marcos River (top panel) and Comal River (bottom panel) between 2000 and 2014.

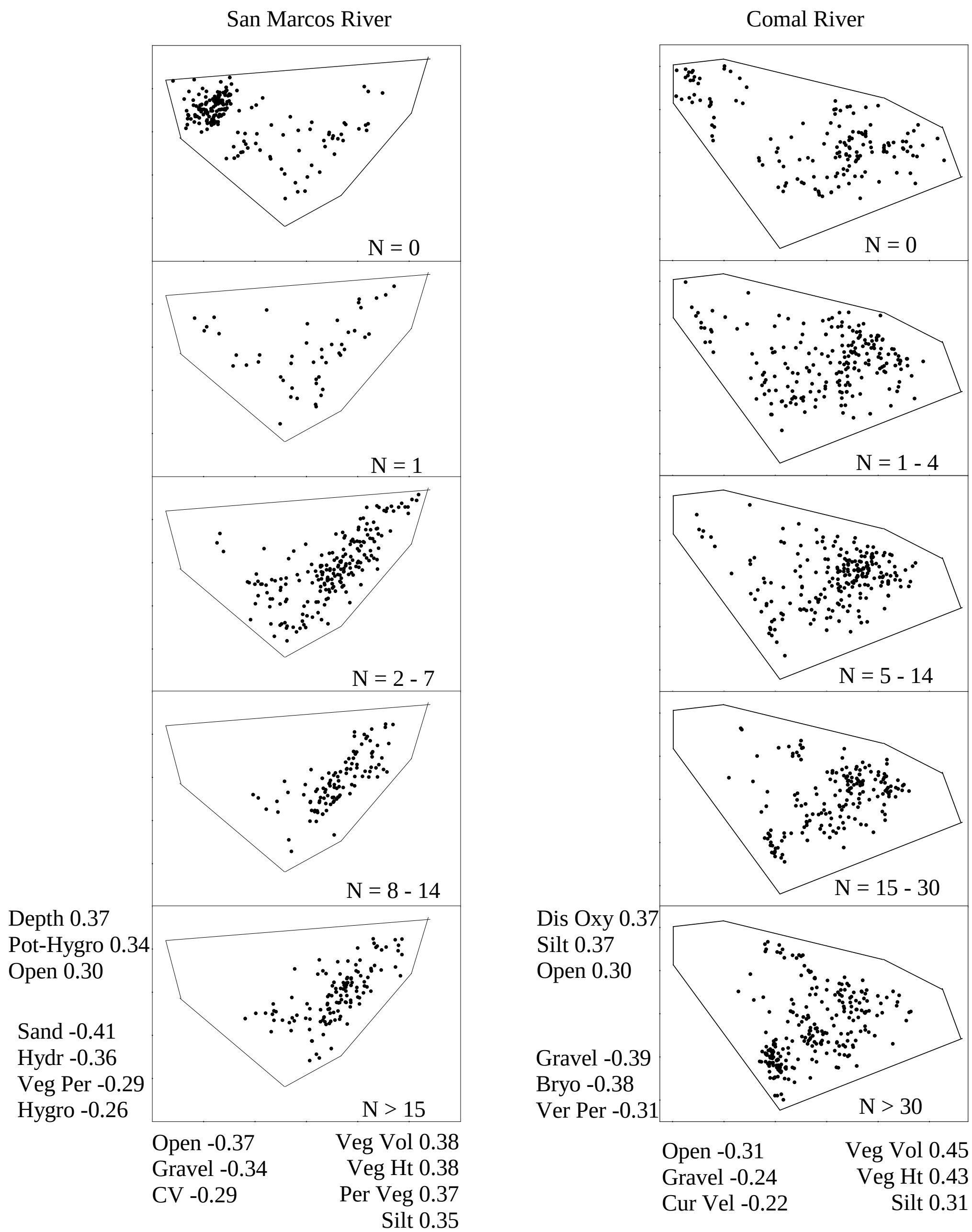


Figure 41. Bi-plots of principal components I and II factors for the San Marcos River (left panel) and Comal River (right panel). Principal component gradients (axes descriptions) listed on the two bottom graphs are the same for graphs within each panel. Solid line envelops all drop net data (not shown). 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.

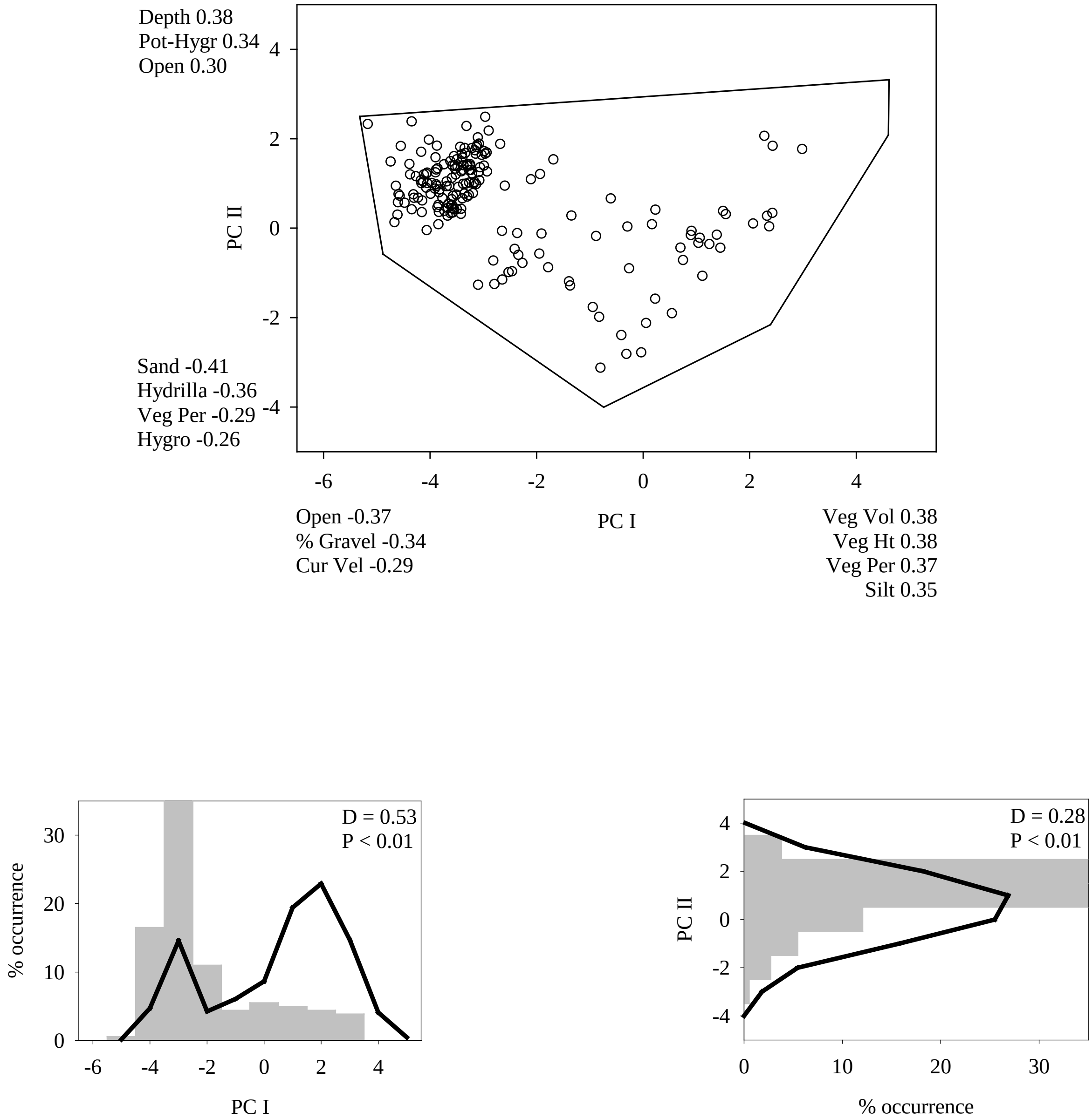


Figure 42. **Distribution of Category 0 (samples without fountain darters) along PC I and II (top panel) and by percent occurrences between observed (gray bars) and expected (black line) along PC 1 (bottom left) and PC 2 (bottom right) for San Marcos River. Significance between observed and expected 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.**

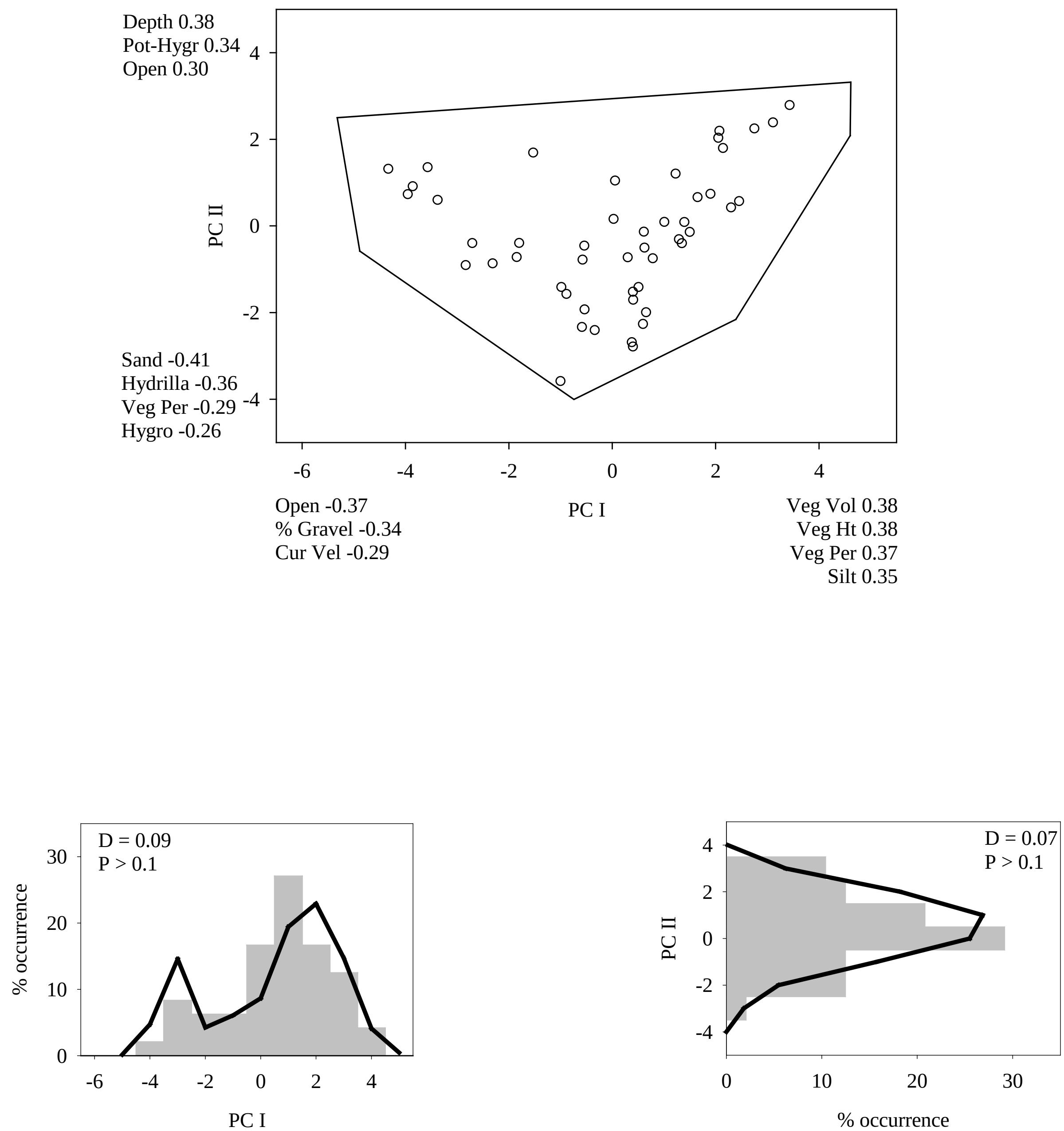


Figure 43. **Distribution of Category I (samples with one fountain darter) along PC I and II (top panel) and by percent occurrences between observed (gray bars) and expected (black line) along PC 1 (bottom left) and PC 2 (bottom right) for San Marcos River. Significance between observed and expected 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.**

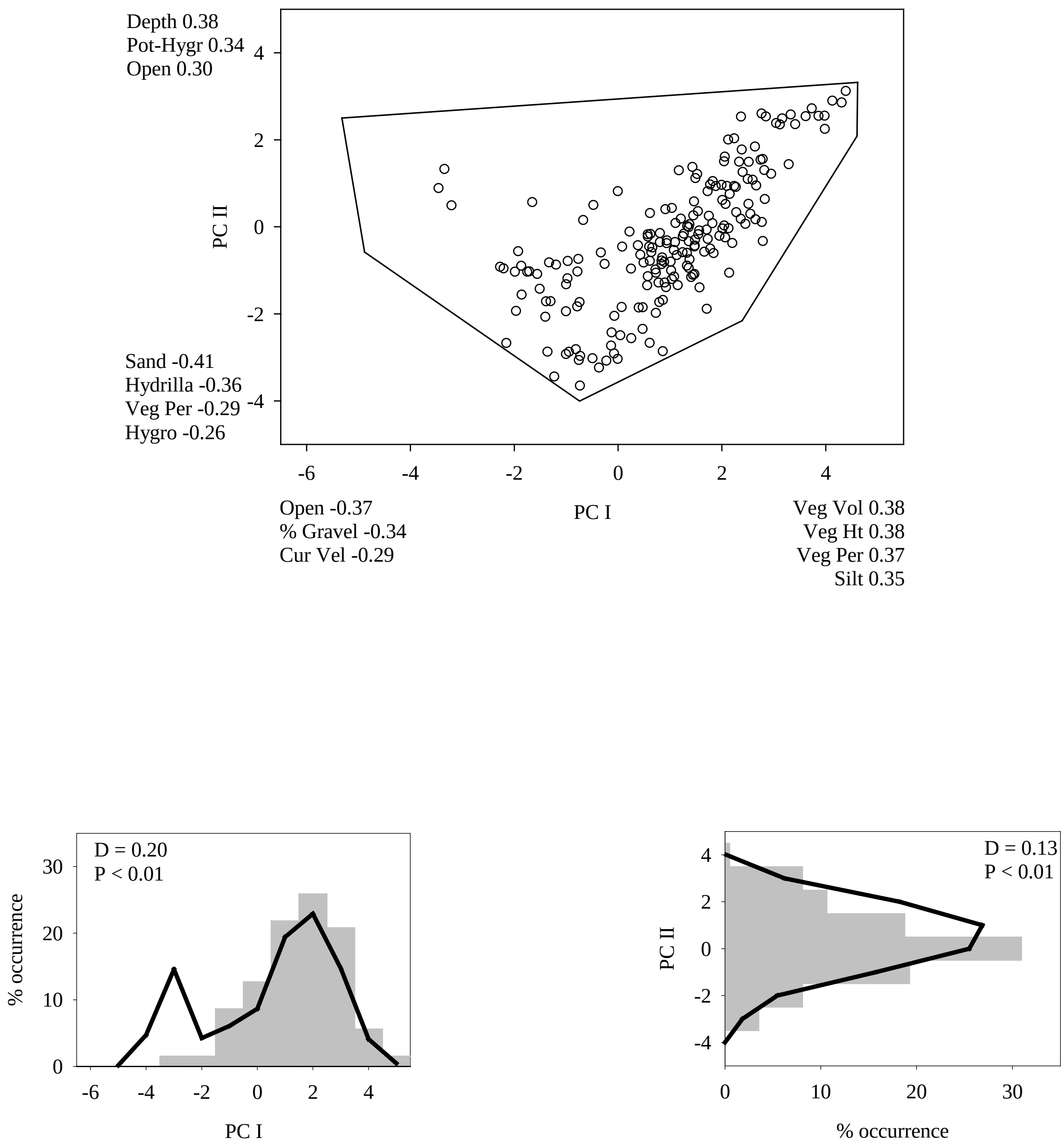


Figure 44. Distribution of Category II (samples with two to seven fountain darters) along PC I and II (top panel) and by percent occurrences between observed (gray bars) and expected (black line) along PC 1 (bottom left) and PC 2 (bottom right) for San Marcos River. Significance between observed and 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.

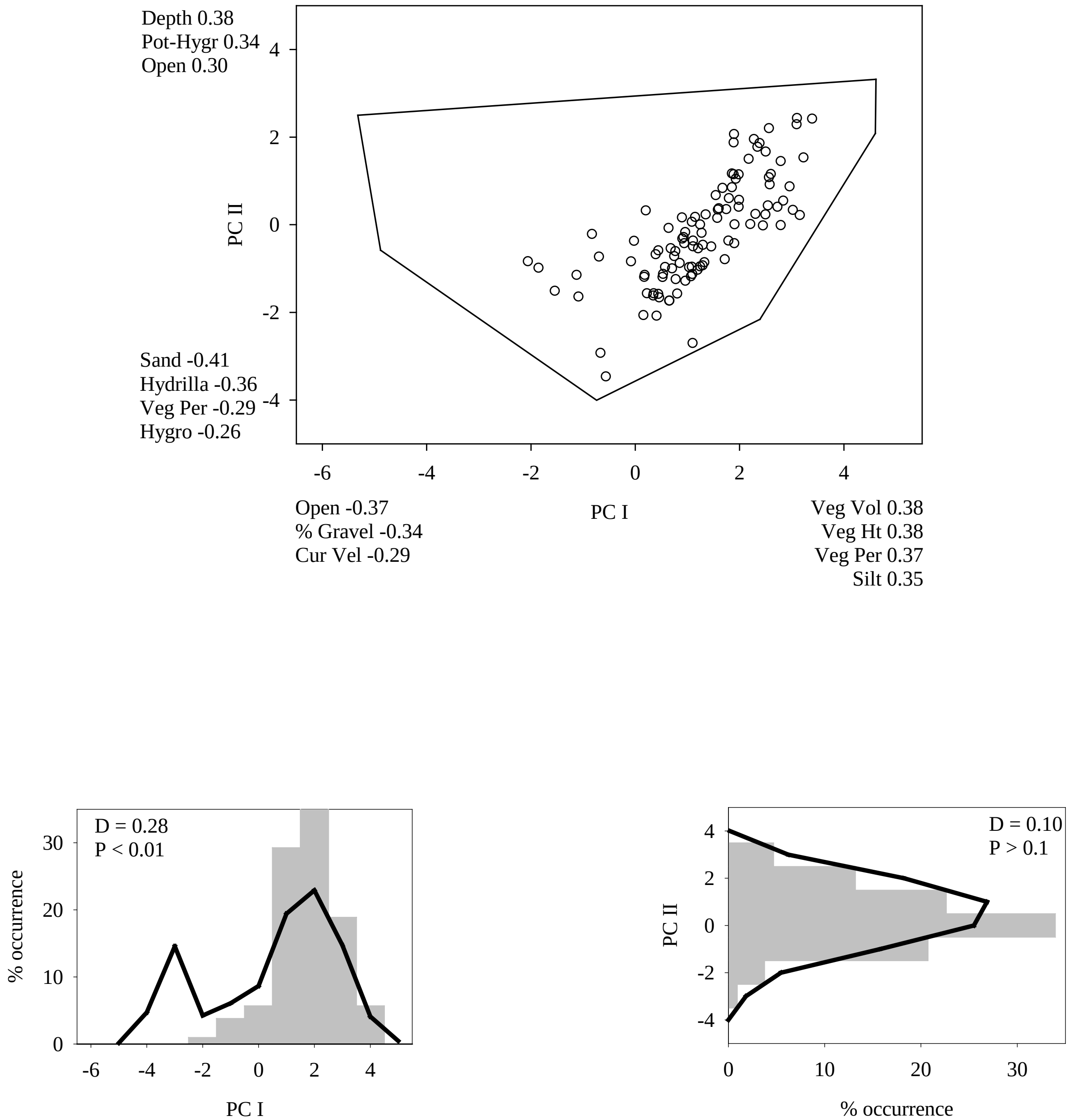


Figure 45. Distribution of Category III (samples with 8 to 14 fountain darters) along PC I and II (top panel) and by percent occurrences between observed (gray bars) and expected (black line) along PC 1 (bottom left) and PC 2 (bottom right) for San Marcos River. Significance between observed and expected 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.

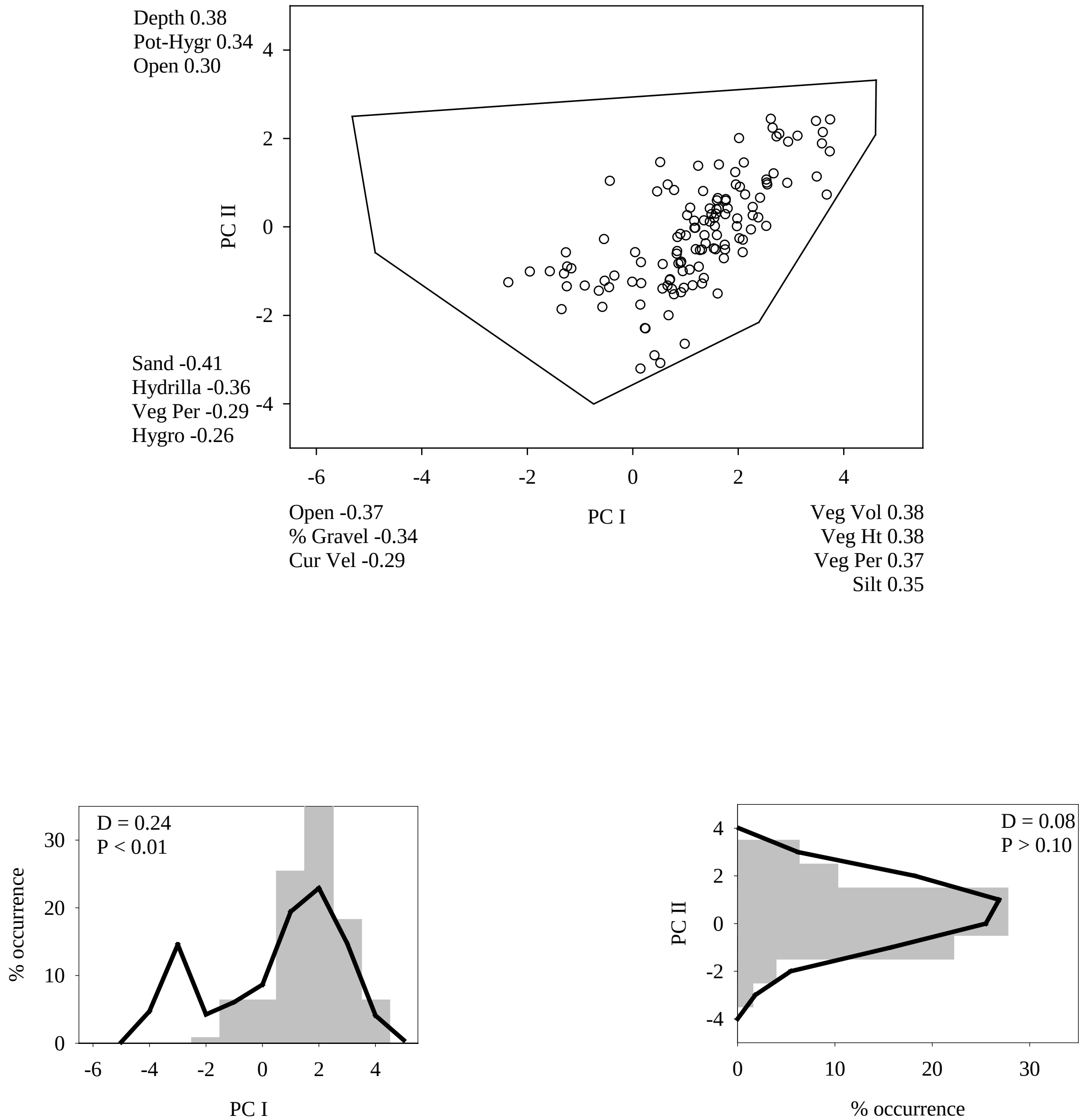


Figure 46. **Distribution of Category IV (samples with 15 to 242 fountain darters) along PC I and II (top panel) and by percent occurrences between observed (gray bars) and expected (black line) along PC 1 (bottom left) and PC 2 (bottom right) for San Marcos River. Significance between observed and expected 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.**

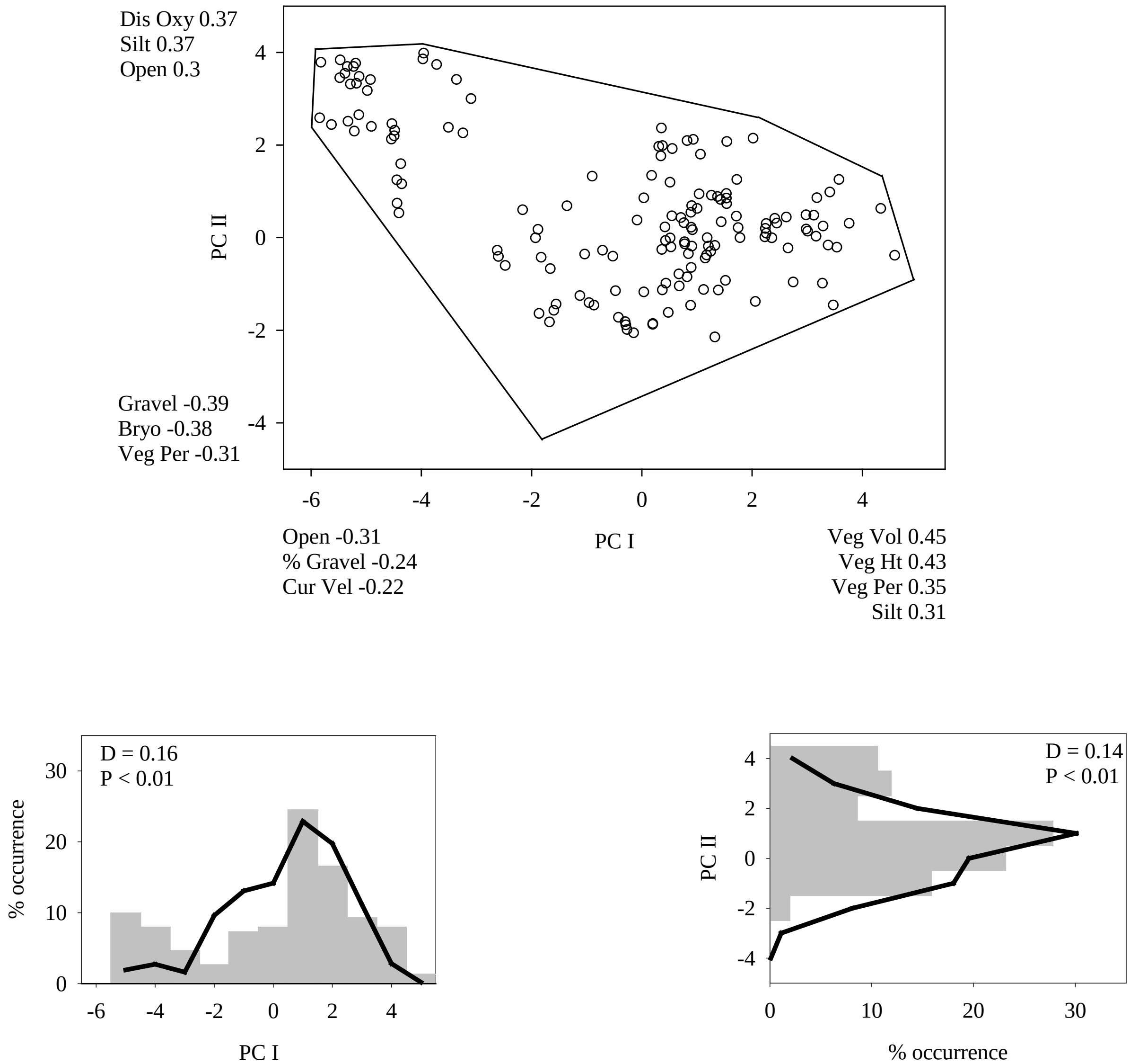


Figure 47. Distribution of Category 0 (samples without fountain darters) along PC I and II (top panel) and by percent occurrences between observed (gray bars) and expected (black line) along PC 1 (bottom left) and PC 2 (bottom right) for Comal River. Significance between observed and expected distributions 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.

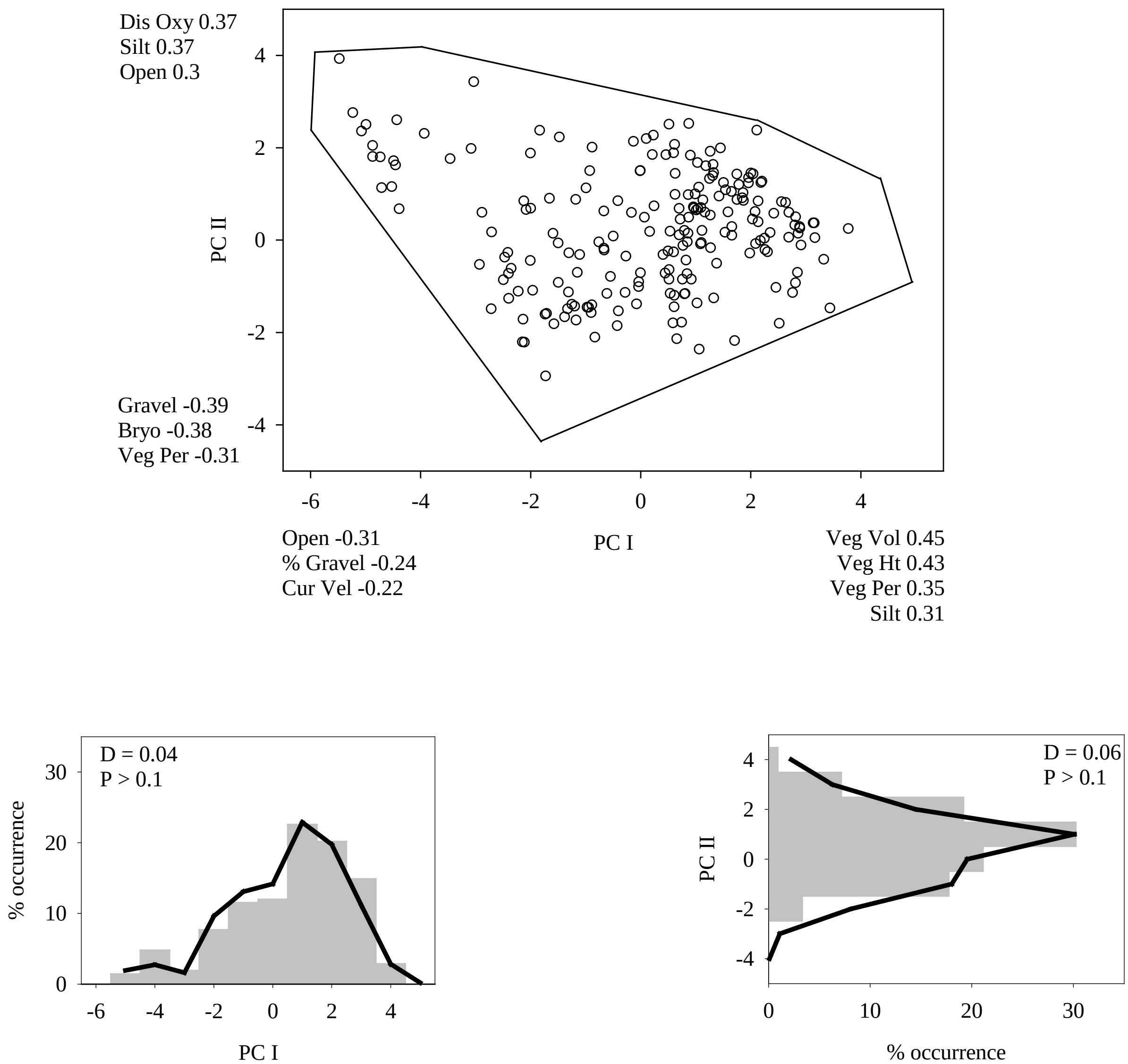


Figure 48. Distribution of Category I (samples with one to four fountain darters) along PC I and II (top panel) and by percent occurrences between observed (gray bars) and expected (black line) along PC 1 (bottom left) and PC 2 (bottom right) for Comal River. Significance between observed and expected 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.

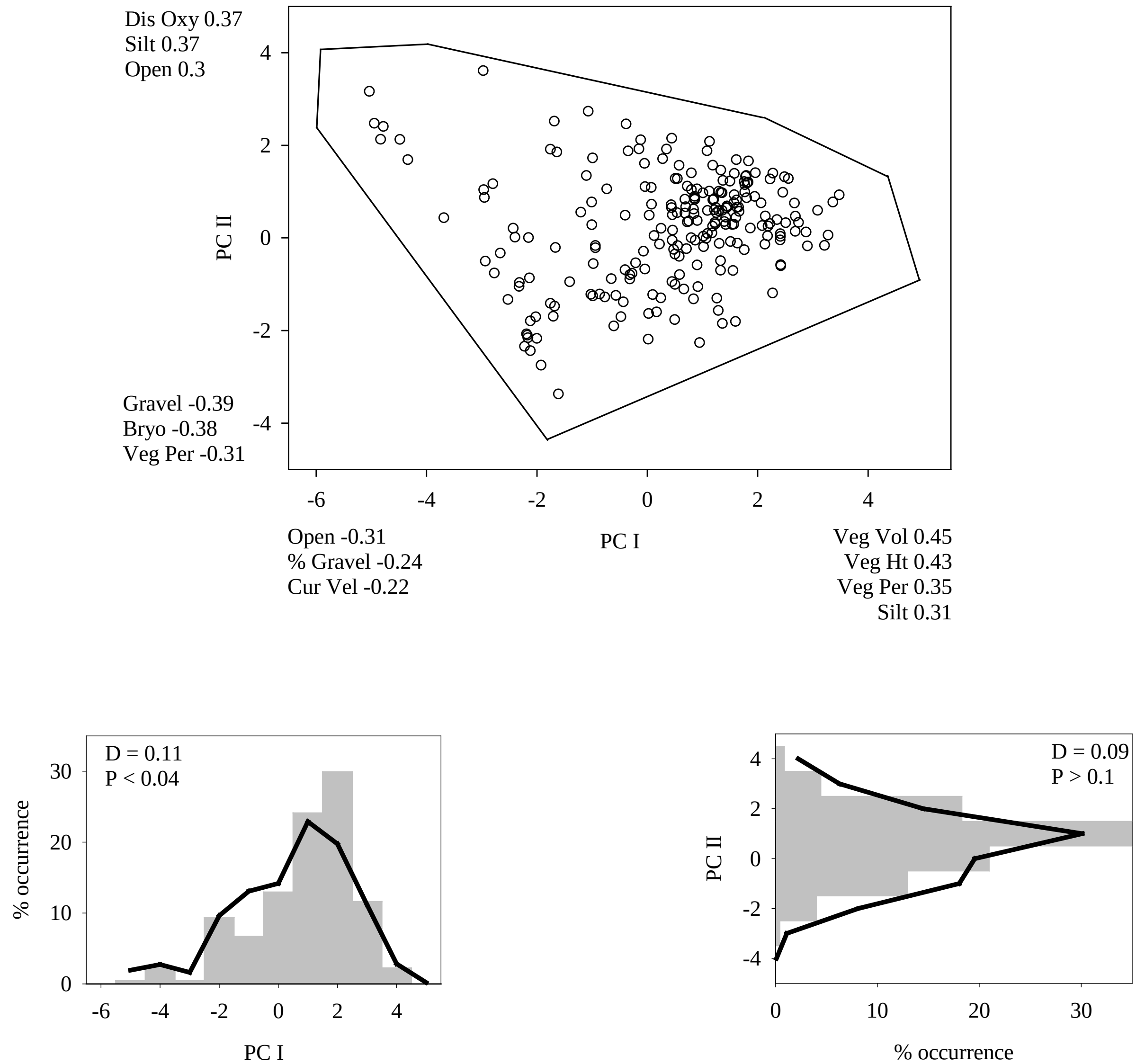


Figure 49. Distribution of Category II (samples with 5 to 14 fountain darters) along PC I and II (top panel) and by percent occurrences between observed (gray bars) and expected (black line) along PC 1 (bottom left) and PC 2 (bottom right) for Comal River. Significance between observed and expected distributions 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.

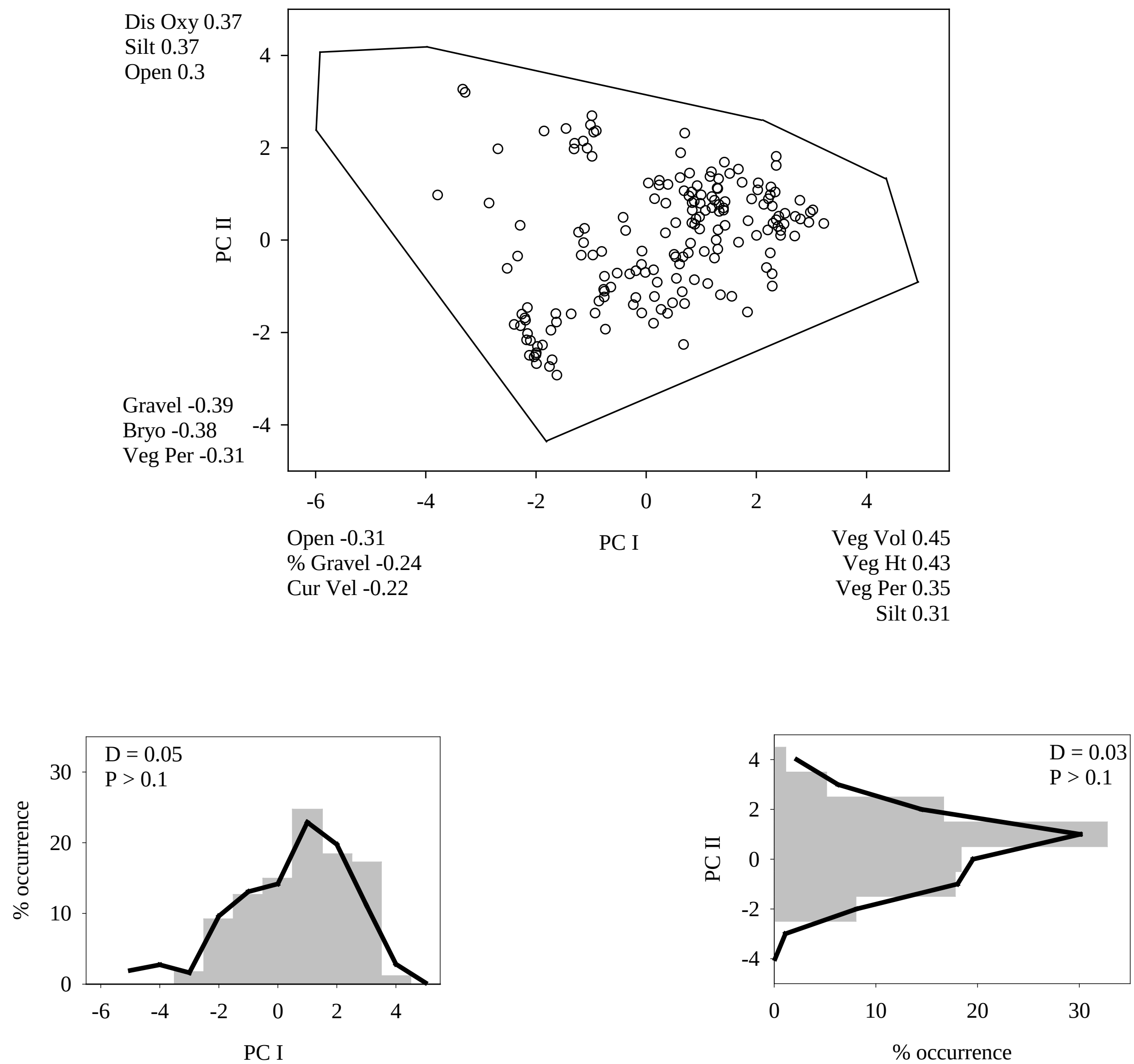
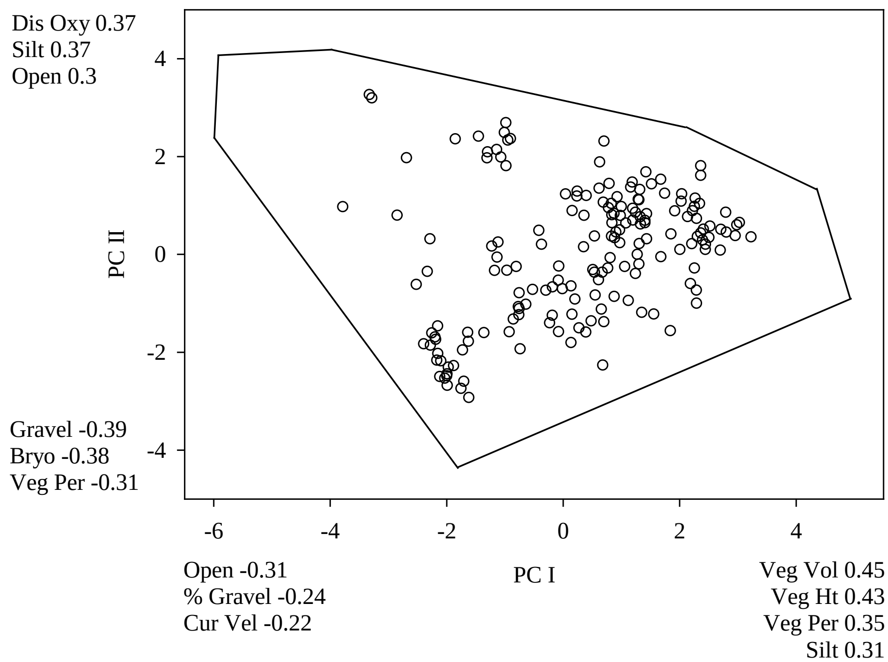
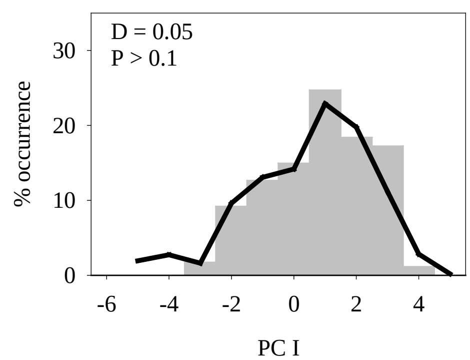


Figure 50. Distribution of Category III (samples with 15 to 30 fountain darters) along PC I and II (top panel) and by percent occurrences between observed (gray bars) and expected (black line) along PC 1 (bottom left) and PC 2 (bottom right) for Comal River. Significance between observed and expected 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.





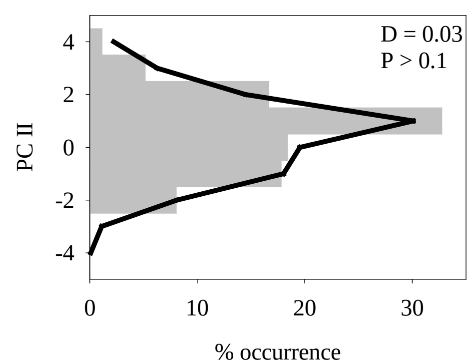


Figure 51. Distribution of Category IV (samples with 31 to 212 fountain darters) along PC I and II (top panel) and by percent occurrences between observed (gray bars) and expected (black line) along PC 1 (bottom left) and PC 2 (bottom right) for Comal River. Significance between observed and expected 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:

$$P(Y_i = K) = \frac{exp(\alpha_K + \beta_K X_i)}{c_i}, \text{ where } K = 2, 3, 4, \text{ or } 5, \quad (1)$$

$$P(Y_i = K) = \frac{1}{c_i}, \text{ where } K = 1, \quad (2)$$

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 ≤ 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.

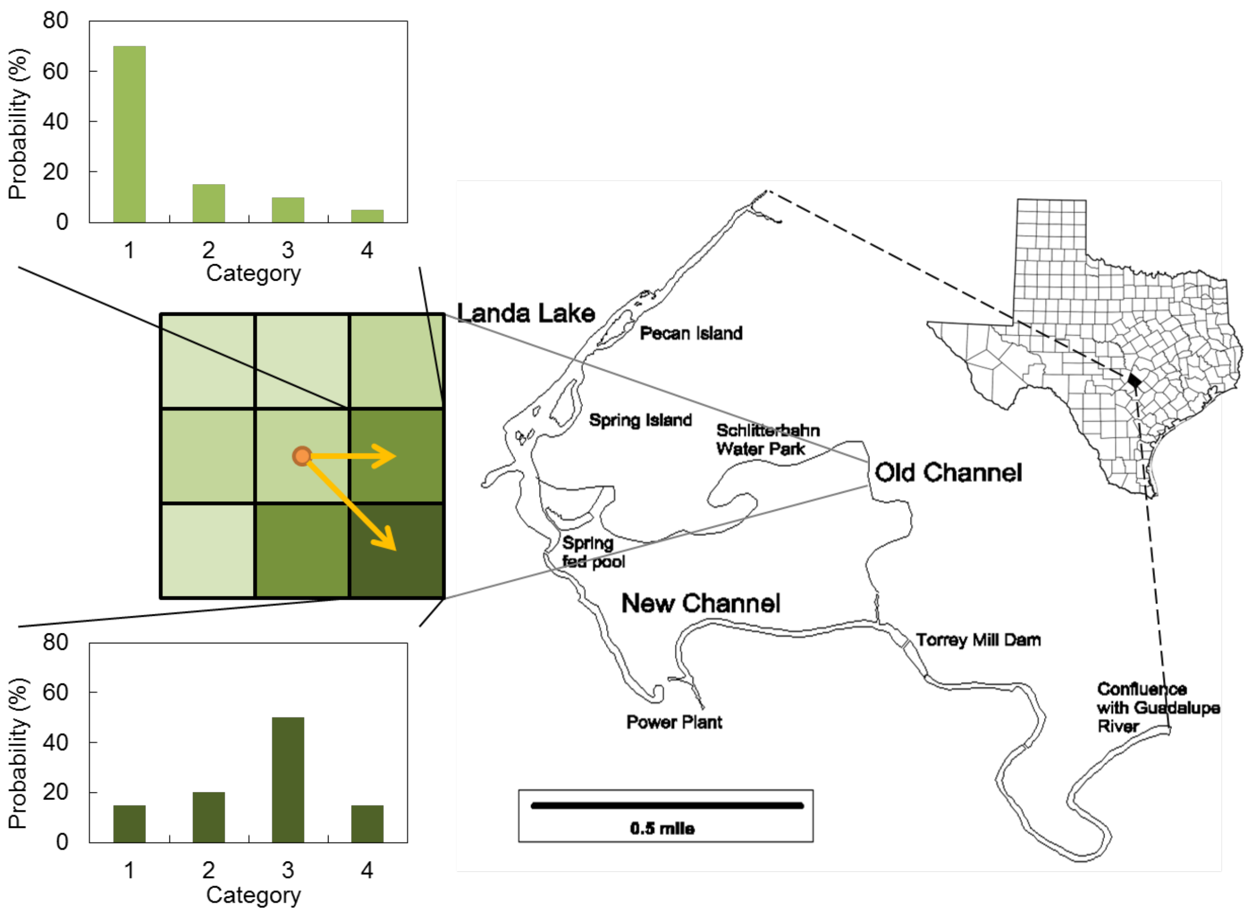


Figure 52. Conceptual diagram of multinomial logit regression model and movement

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.

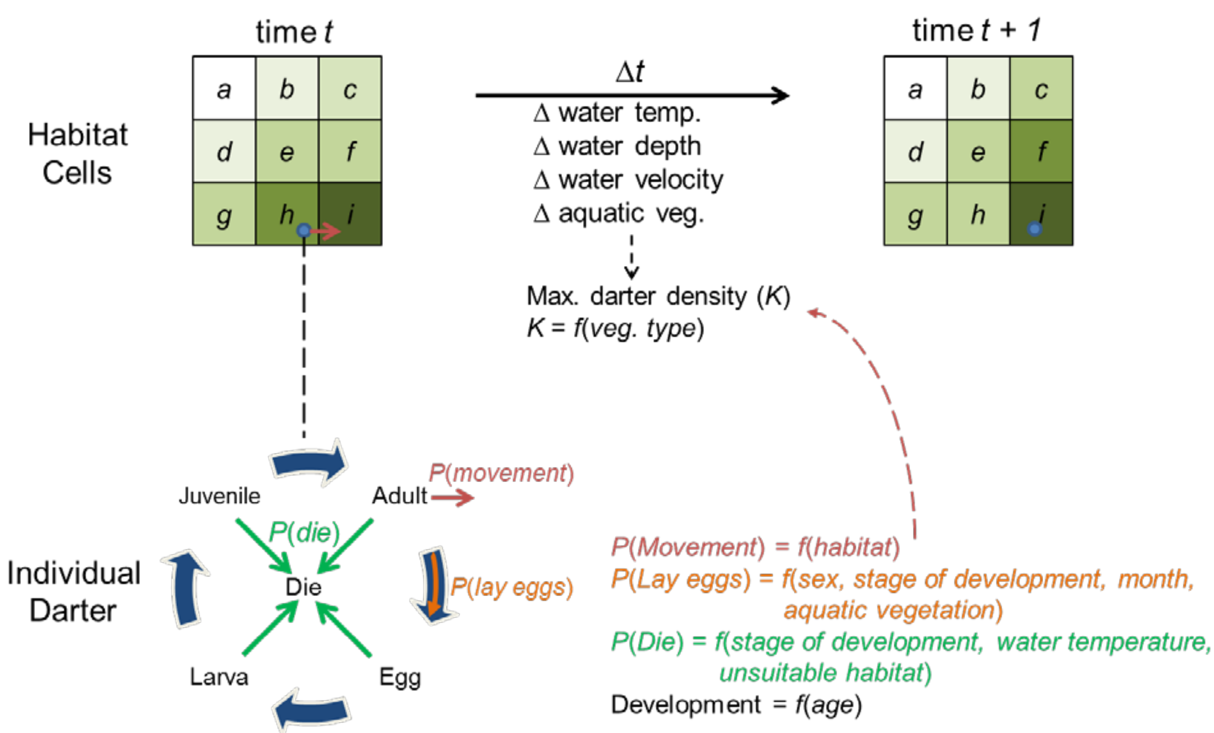


Figure 53. **Conceptual diagram of the spatially-explicit, individual-based, simulation model representing fountain darter population dynamics in response to changes in aquatic vegetation and hydrological conditions.**

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.

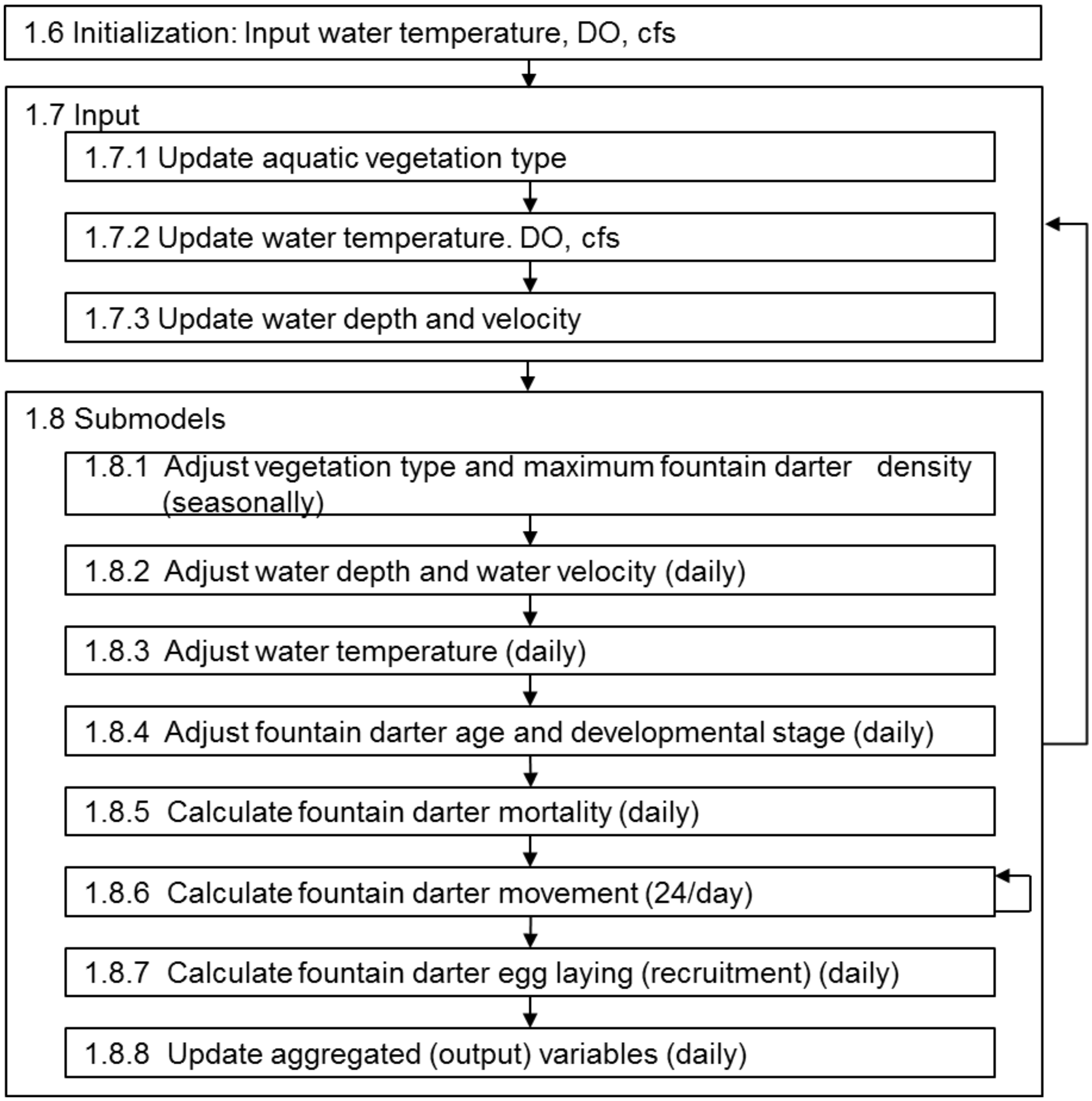


Figure 54. Overview of the sequence of events and processes involved in the execution of the fountain darter population dynamics model.

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.

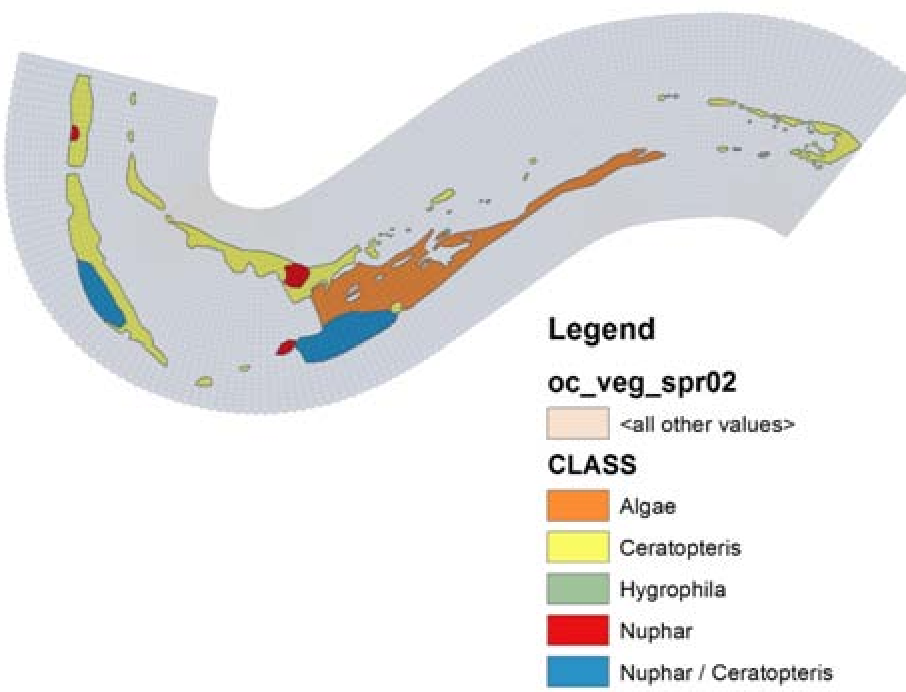


Figure 55. Example of a spatial join of vegetation mapping data polygons and the corresponding one meter hydraulic grid in the Old Channel of the Comal River.

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.

Low oxygen tolerance (point of equilibrium loss)
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.

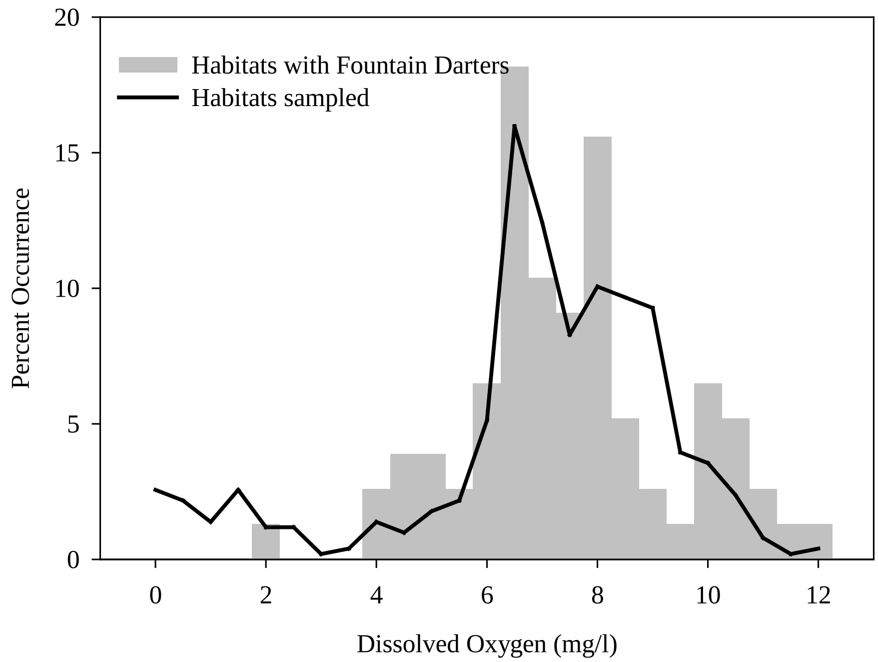


Figure 56. Dissolved oxygen comparison between habitats sampled with seines (N = 507; black line) and habitats (N = 77) with Fountain Darters (N = 203) taken from multiple sites seasonally for one year in the San Marcos River (Behen 2013). Dissolved oxygen was measured with a YSI multi-probe during daylight 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).

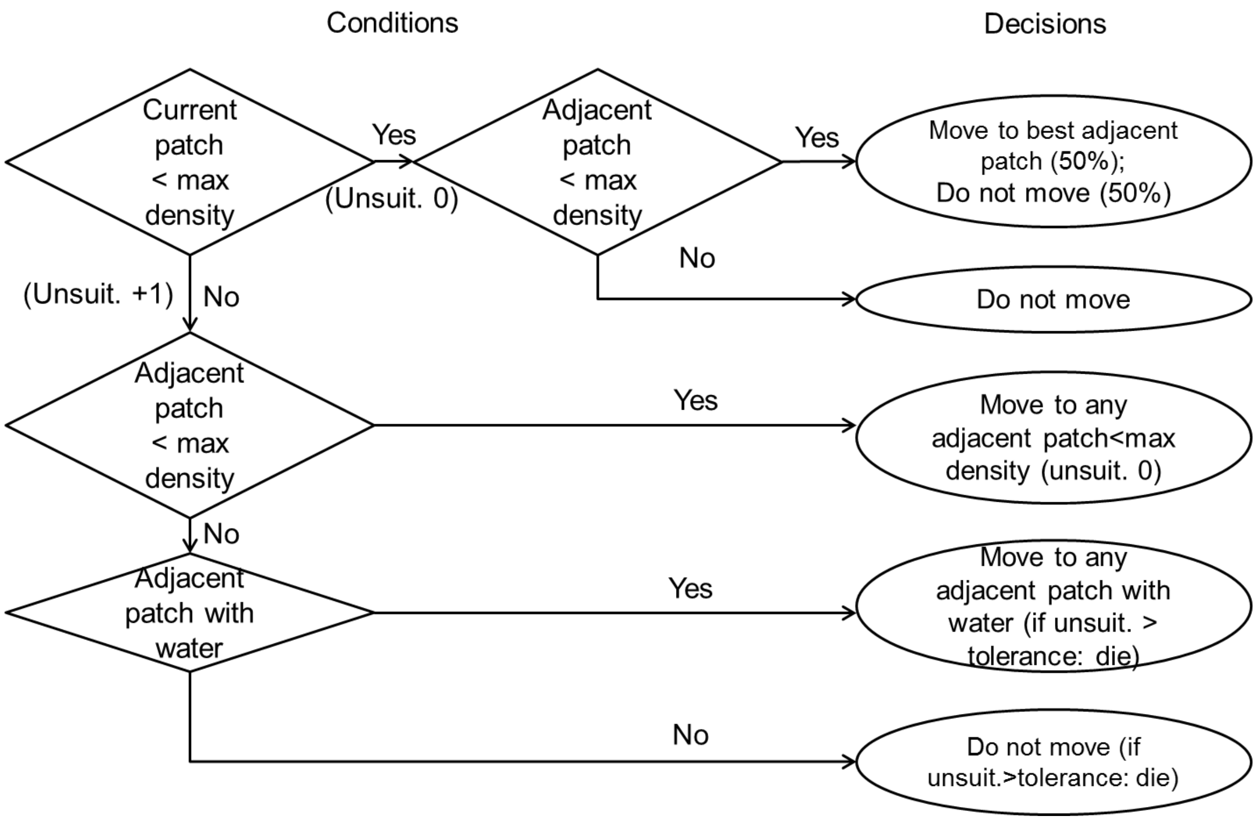


Figure 57. Summary of fountain darter movement rules. MD represents the number of juveniles plus adults that can be supported by the vegetation type in the habitat cell, ε represents the probability of moving to an adjacent habitat cell, ν' represents the number of consecutive moves during which the 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

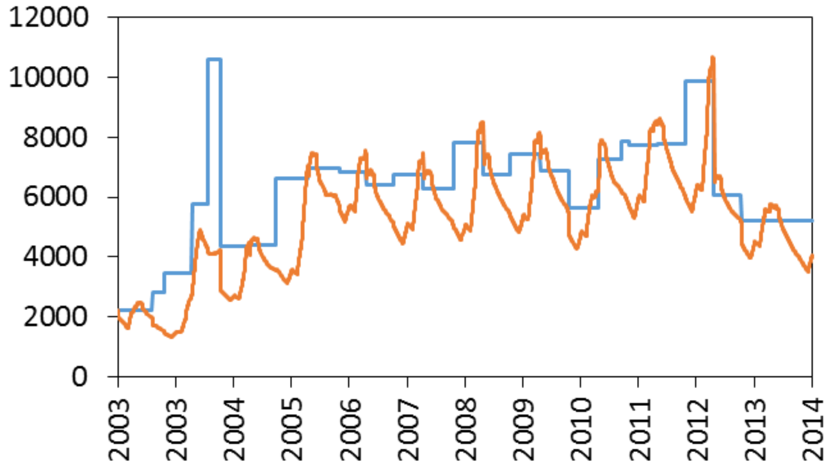
Verification

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).

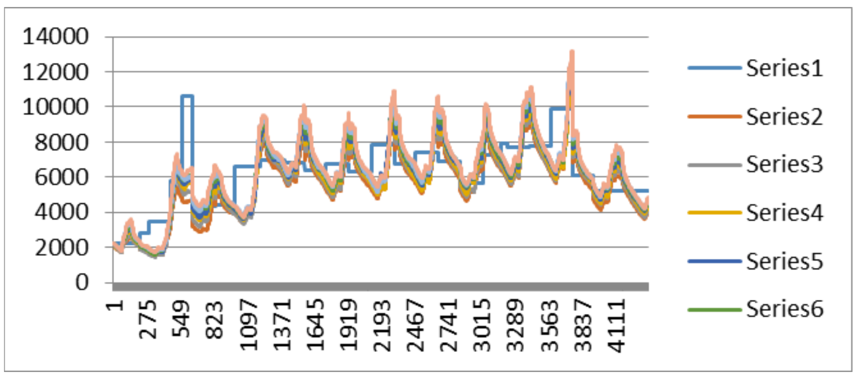
Calibration

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)

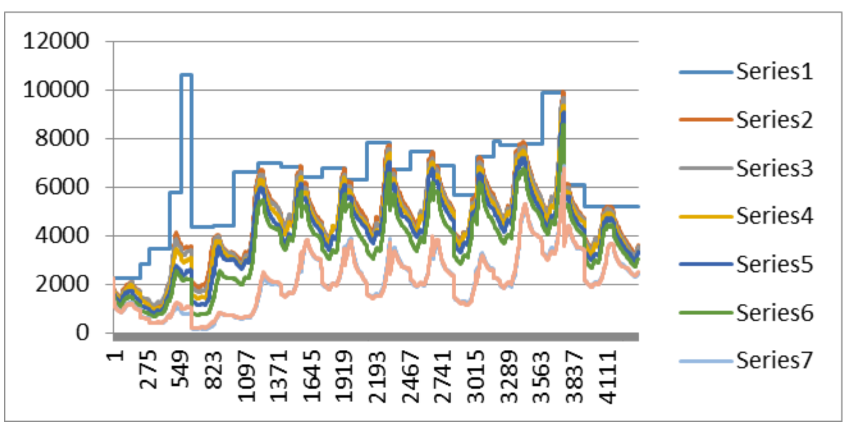
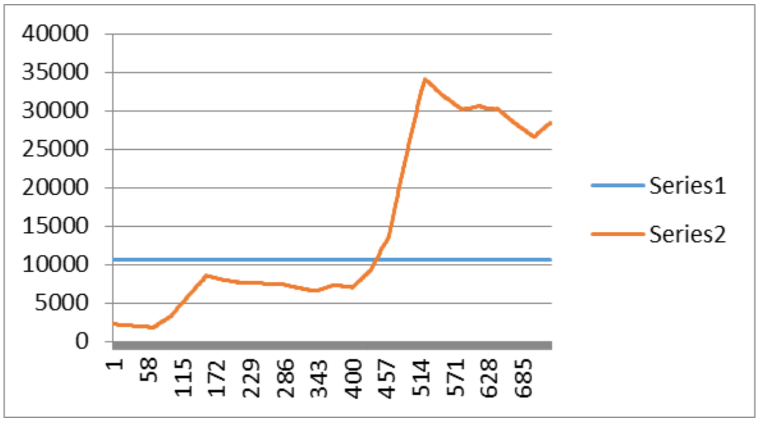


Figure 58. **Estimated maximum darter density and simulated number of juvenile plus adult fountain darters in the Old Channel of the Comal River using (a) the baseline value of v (12) and values of v that were (b) higher and (c) lower than baseline.**

(a)



(b)

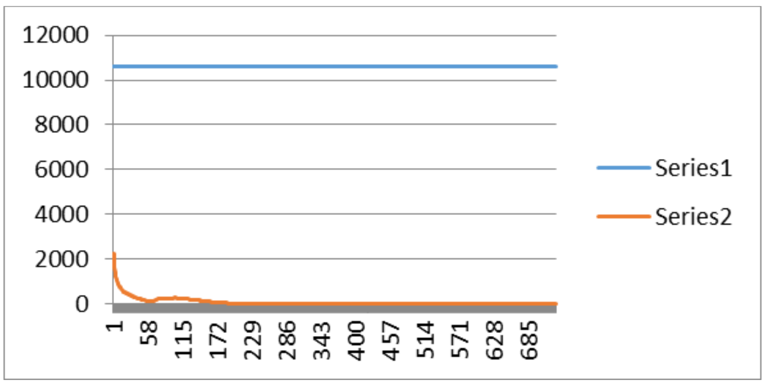


Figure 59. **Estimated maximum darter density and simulated number of juvenile plus adult fountain darters in the Old Channel of the Comal River (a) with no limit on the number of consecutive moves that a juvenile or adult fountain darter can survive without finding favorable habitat (v = 99999), and (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).

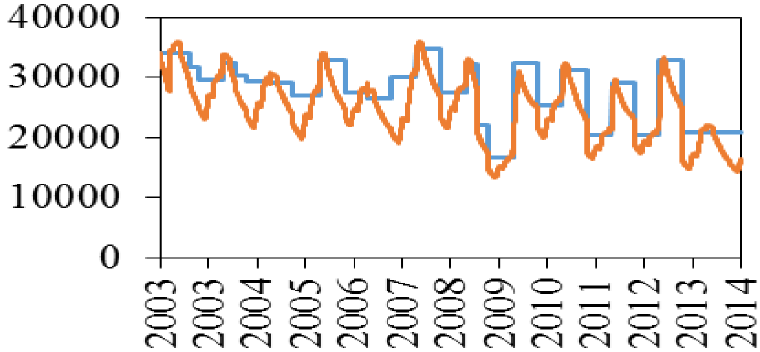


Figure 60. **Estimated maximum darter densities and simulated numbers of juvenile plus adult fountain darters in the City Park reach of the San Marcos River (with v =12).**

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.)

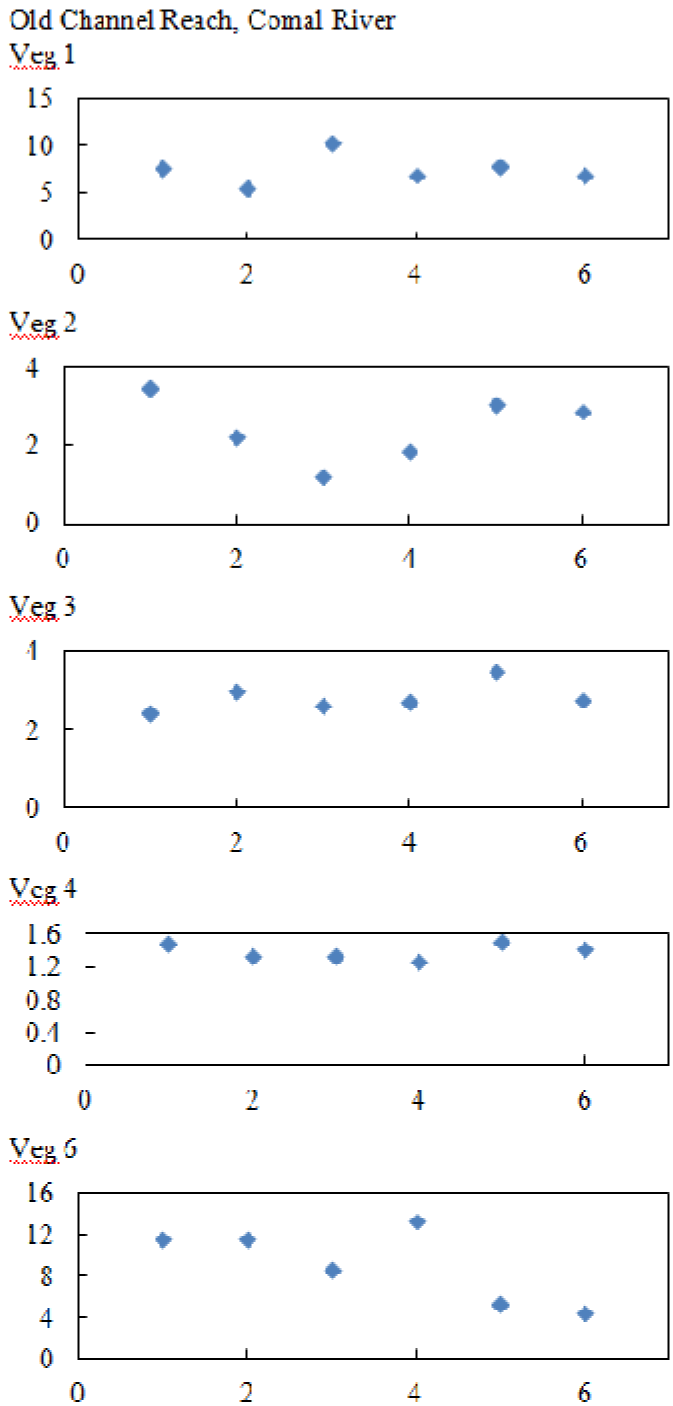


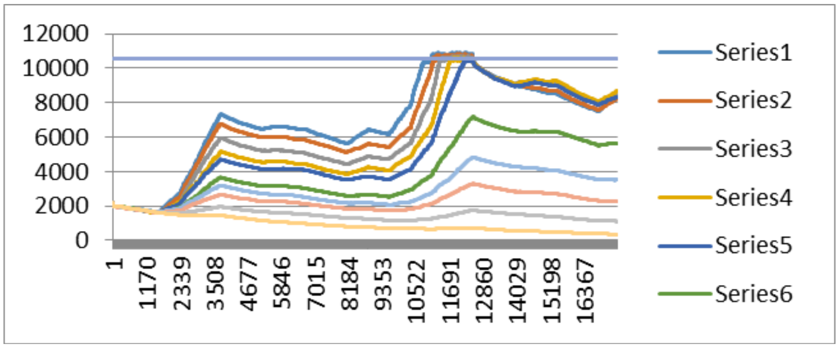
Figure 61. **Comparisons of the mean number of fountain darters per square meter captured in field versus simulated drop net samples in the indicated vegetation types in the Old Channel study reach of the Comal River. In each graph the first five dots, from left to right, represent means from five replicate stochastic (Monte Carlo) simulations, and the sixth dot represents the mean of field samples collected from 2003 through 2013.**

Sensitivity analysis

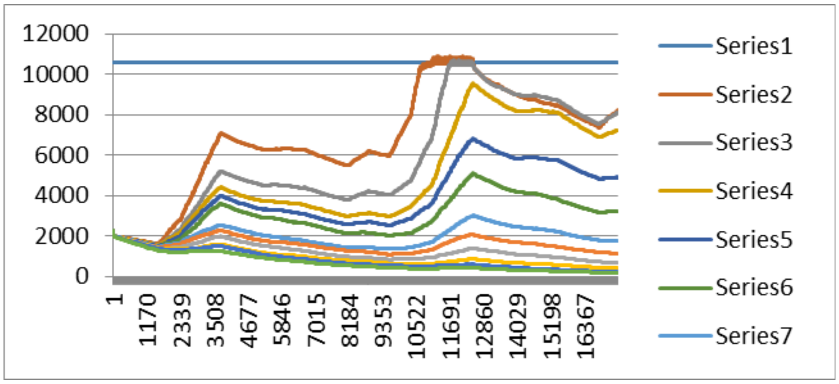
We focused sensitivity analysis on the model parameters that most directly affect fountain darter population growth/recovery: (1) recruitment (clutch size), (2) mortality (base mortality rates of life stages), and (3) movement (ε; probability of moving from a habitat cell that currently is below its 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)

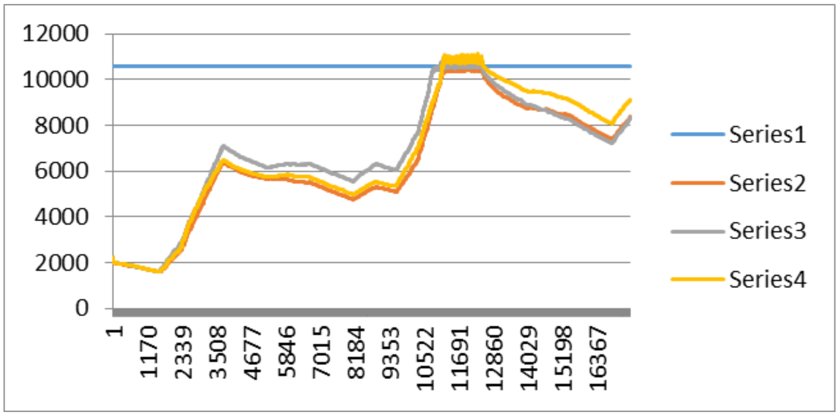


Figure 62. **Sensitivity of fountain darter population growth rate to changes in model parameters affecting (a) recruitment (clutch size), (b) mortality (base mortality rates of life stages), and (c) movement (ε; probability of moving from a habitat cell that currently is below its MD to an adjacent 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.

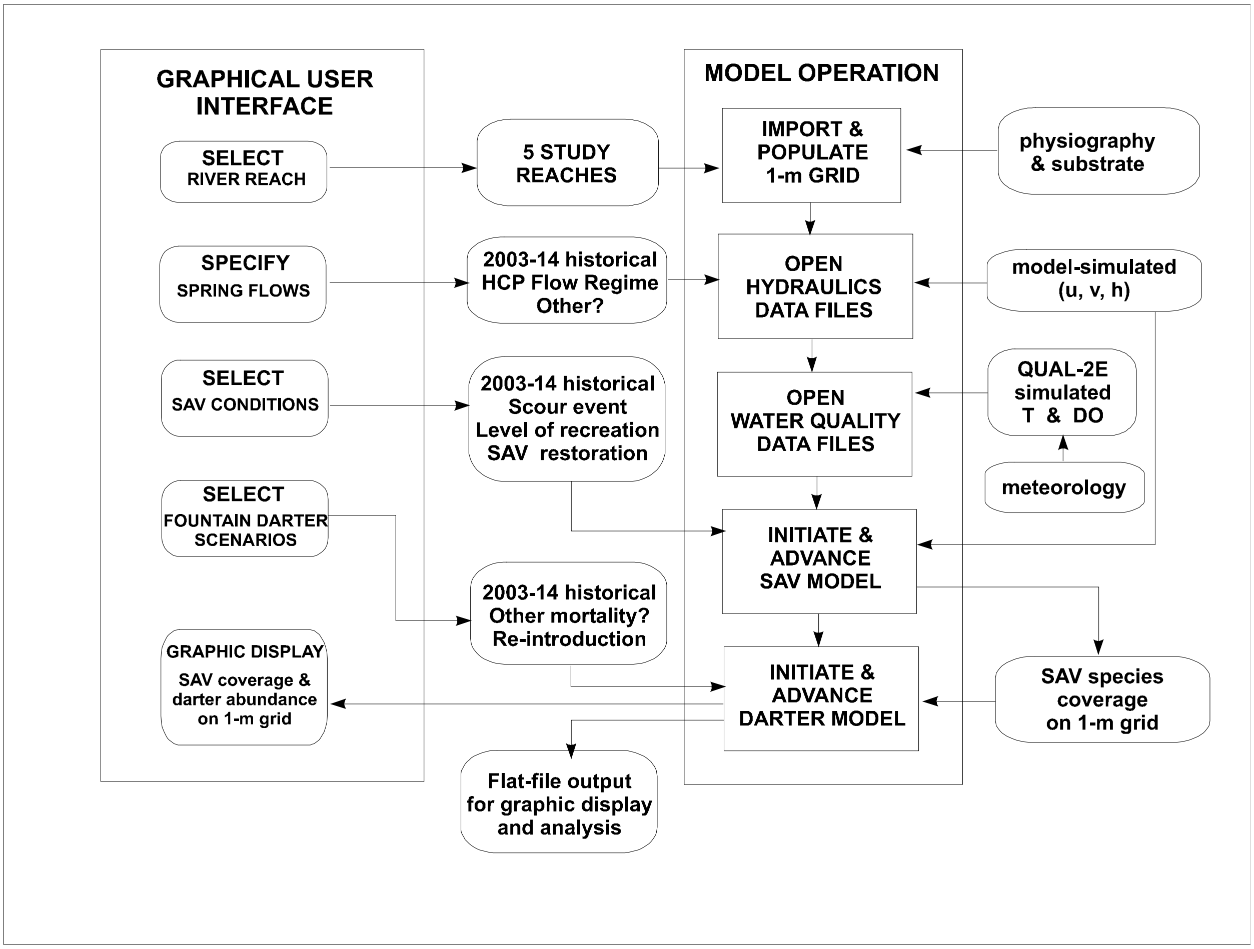


Figure 63. **Conceptual Operations Model**

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.

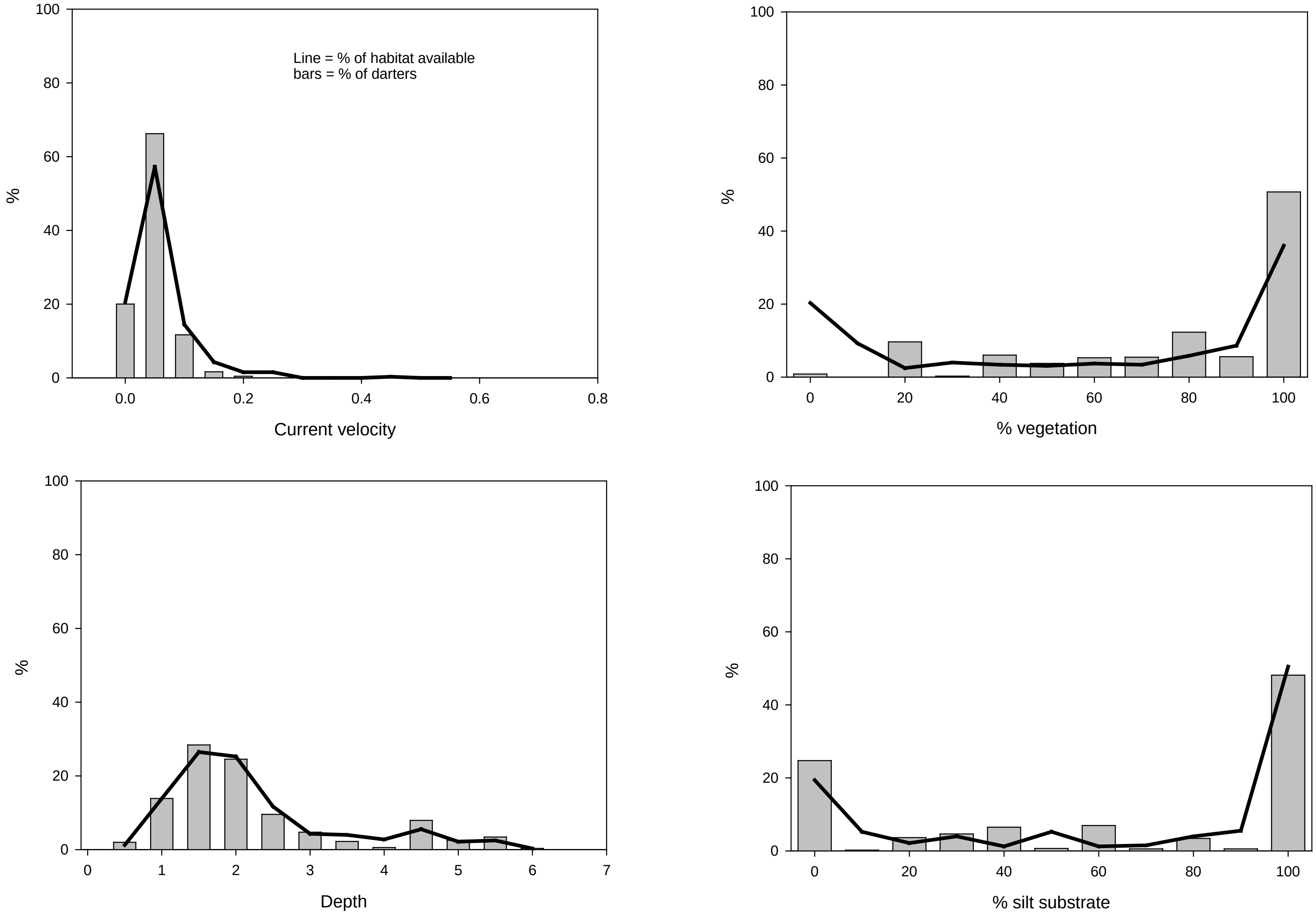
2. Watercourse conditions and fountain darter data

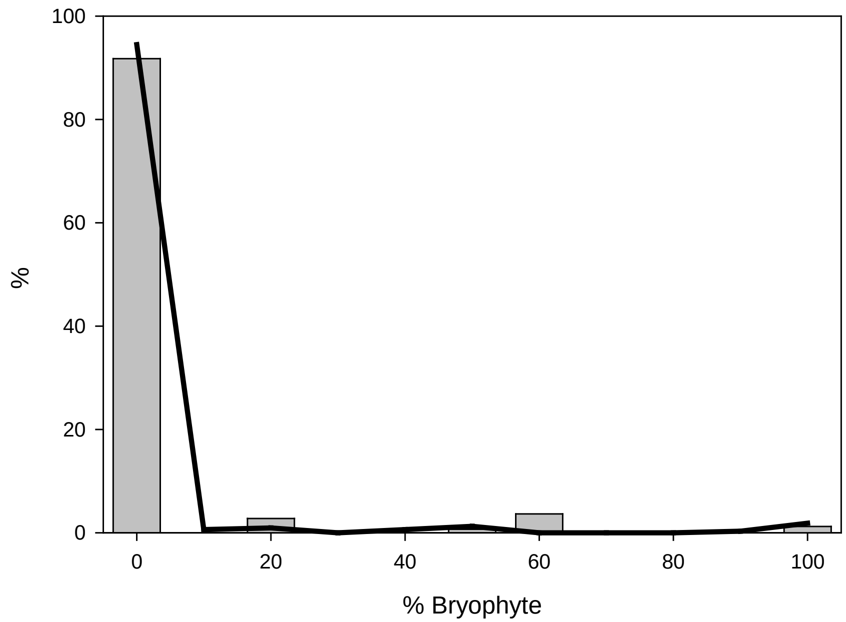
Tim reported that the October floods scoured out a fair amount of the submerged aquatic vegetation (SAV's) in the San Marcos in localized areas. Peak flow was around 800 cfs. The Blanco was up into the IH35 bridge, around 80,000 cfs.

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.

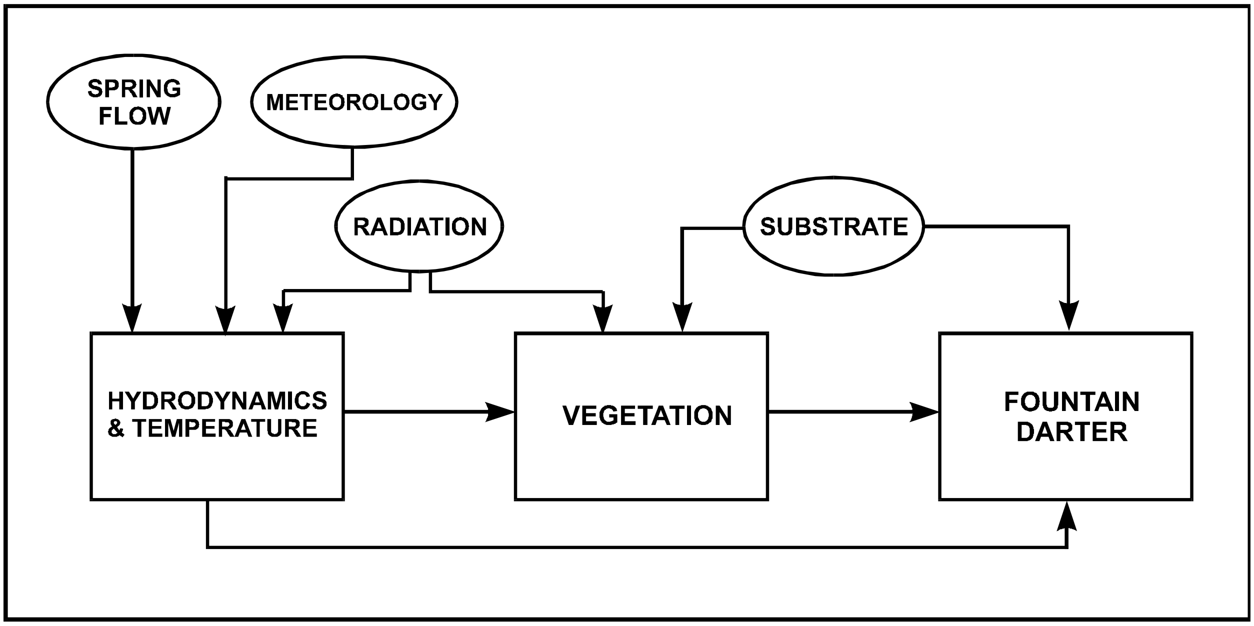


Figure 2 - Causal-link diagram for present version of fountain darter model with vegetation model operating

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

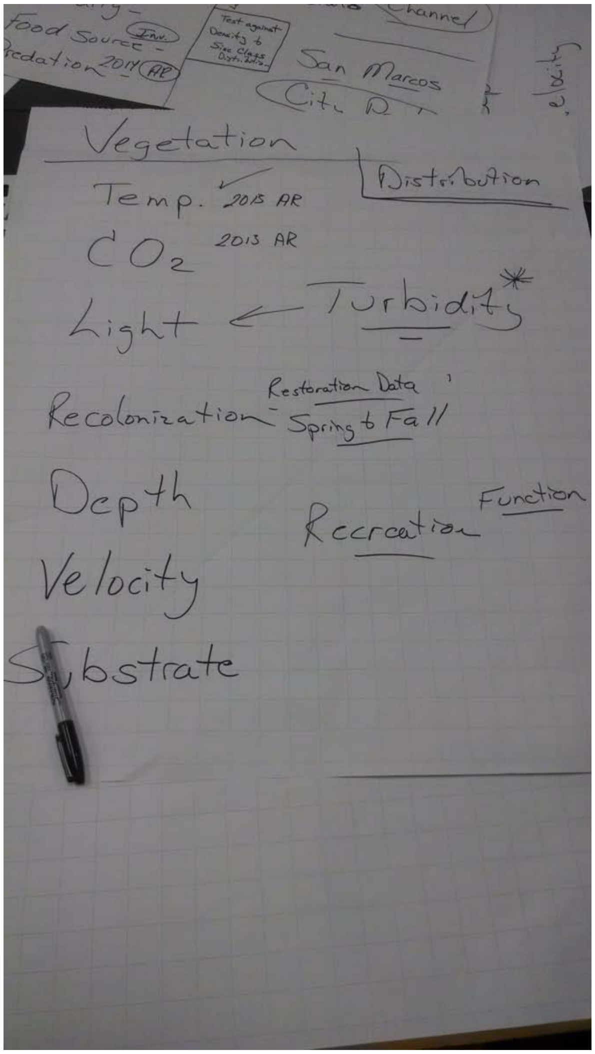
A description of each of these variables and their planned incorporation into the fountain darter model should be presented in the 2013 annual report.

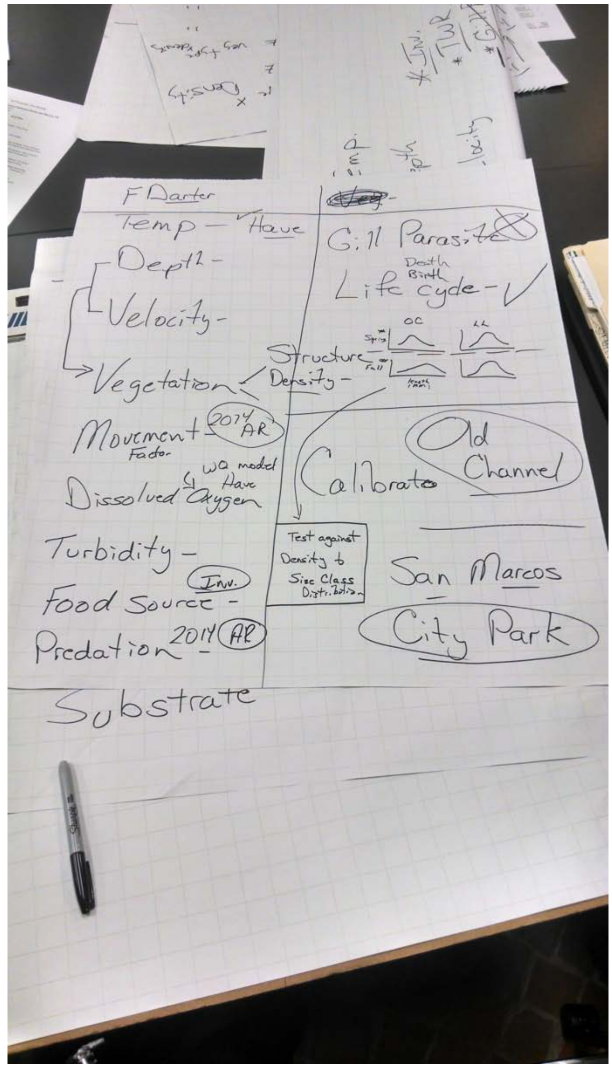
3. SAV modeling

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)

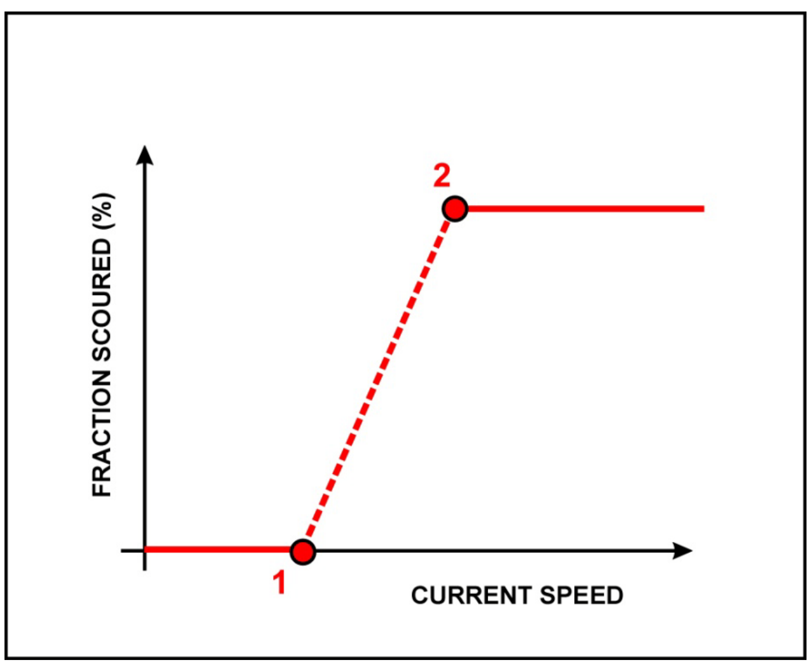
From: Ward, George H
Sent: Thursday, February 06, 2014 12:06 PM (e-mail)
To: 'Edmund Oborny'; Swannack, Todd M ERDC-EL-MS; Hardy, Thom; Doyle, Robert D.; Timothy Bonner; William Grant; hsuan006@neo.tamu.edu; Thomas Hardy; Todd S
Subject: Post script to yesterday re: veg modeling

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:

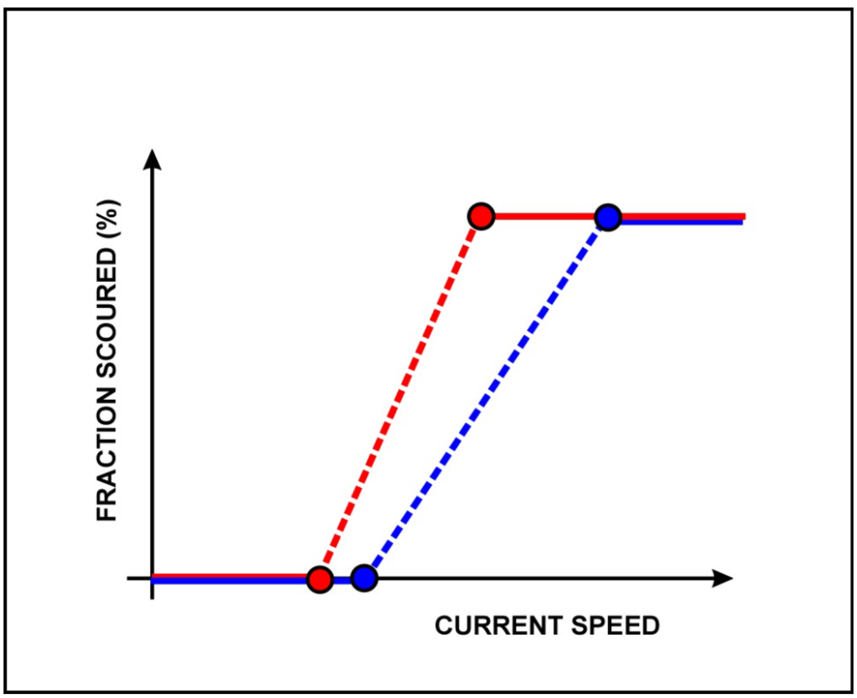


(NOTE to Thom. The real parameter would be shearing stress, which to first-order varies as the square of current speed. Because the relation is monotonic passing through the origin, we can scale the stress threshold to current speed, which is more desirable for management because it can be easily determined 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:

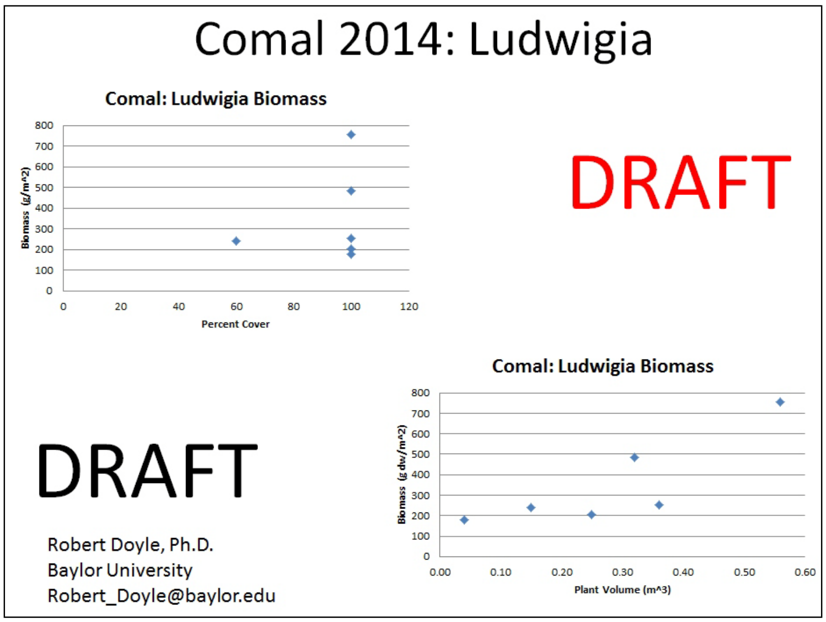
10:00	Meet and greet – Bill: South American stories and continued discussion of the spelling of Todd's last name.
10:15	Robert: update on the plant/biomass field study currently underway.
10:45	Todd: informal presentation on the status of the SAV
12:00	Repair to Schlotsky's for lunch (thank you, Bio-West)
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.

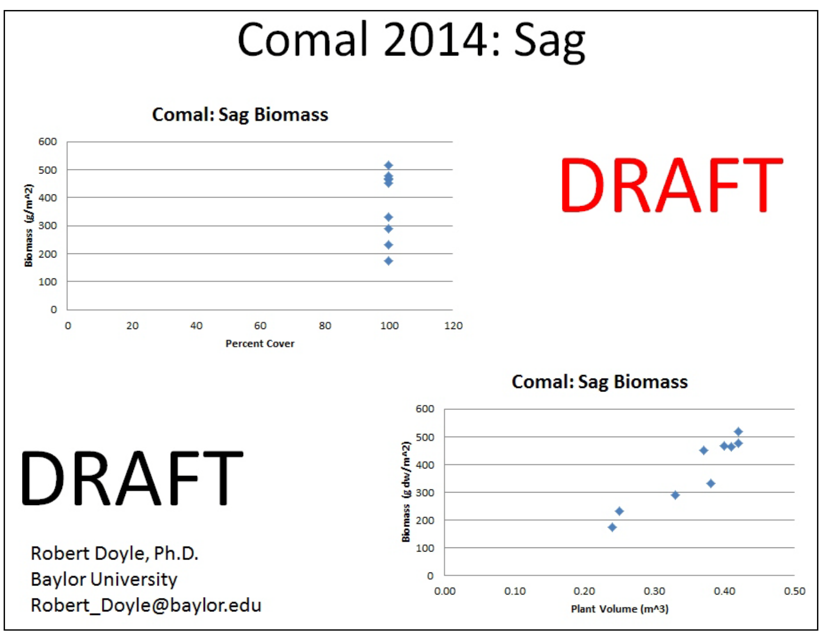
1. Field studies of biomass/coverage relations

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.

Madsen, J.D., 1997: Vegetative spread of Eurasian Watermilfoil colonies. *J. Aquat. Plant Mgmt. 35*, 63-68.

Madsen, J.D., 1999: *Predicting the invasion of Eurasian Watermilfoil into northern lakes*. Tech. Rep. A-99-2, Aquatic Plant Control Research Program, Waterways Experiment Station, U.S. Corps of Engineers, Vicksburg.

Scheffer, M., A. Bakema and F. Worelboer, 1993: MEGAPLANT: a simulation model of the dynamics of submerged plants. *Aquatic Biology 45*, 341-356.

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:

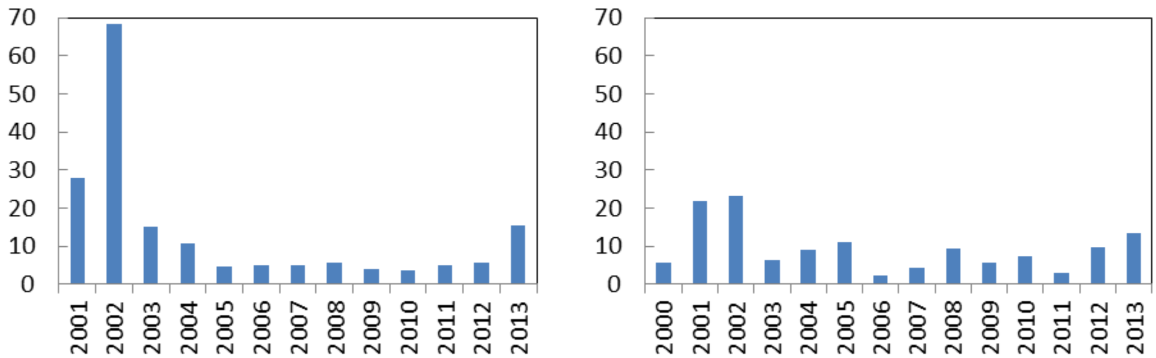
Year	Date	A1	A2	C1	C2	CT1	CT2	H1	…
2000	31-Aug	11	64	0	0	0	0	0	…
2000	13-Sep	53	85	0	0	3	0	0	…

- 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:

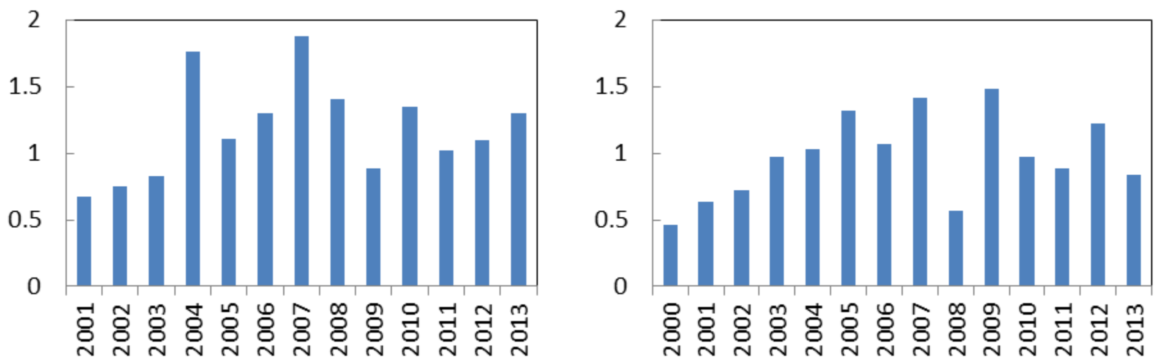
Year	Month	FD number	Veg Height	Algae	Bryophytes	Ceratopteris	Open	…
2000	8	11	0.32	0.2	0.8	0	0	…
2000	8	64	0.32	0.5	0	0	0.5	…

- We would like to know the precise definition of the variable in each column in the “water quality” table.

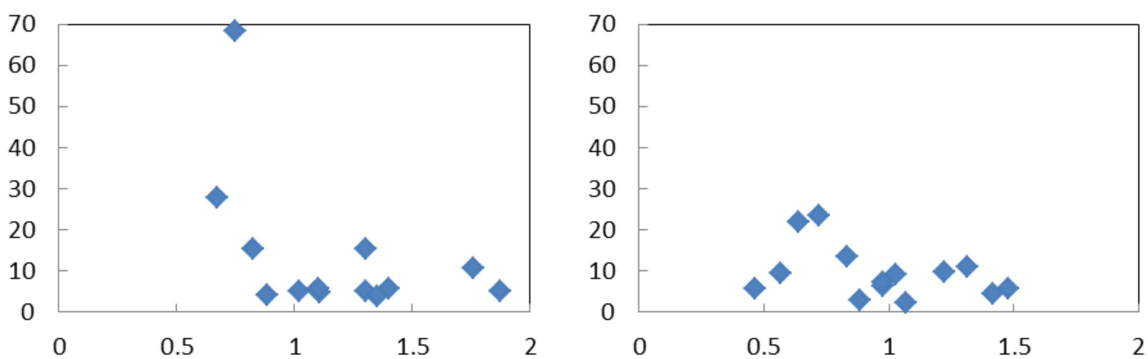
Average densities of fountain darters in a site during spring (on left) and fall (on right) from 2001/2000 to 2013:



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

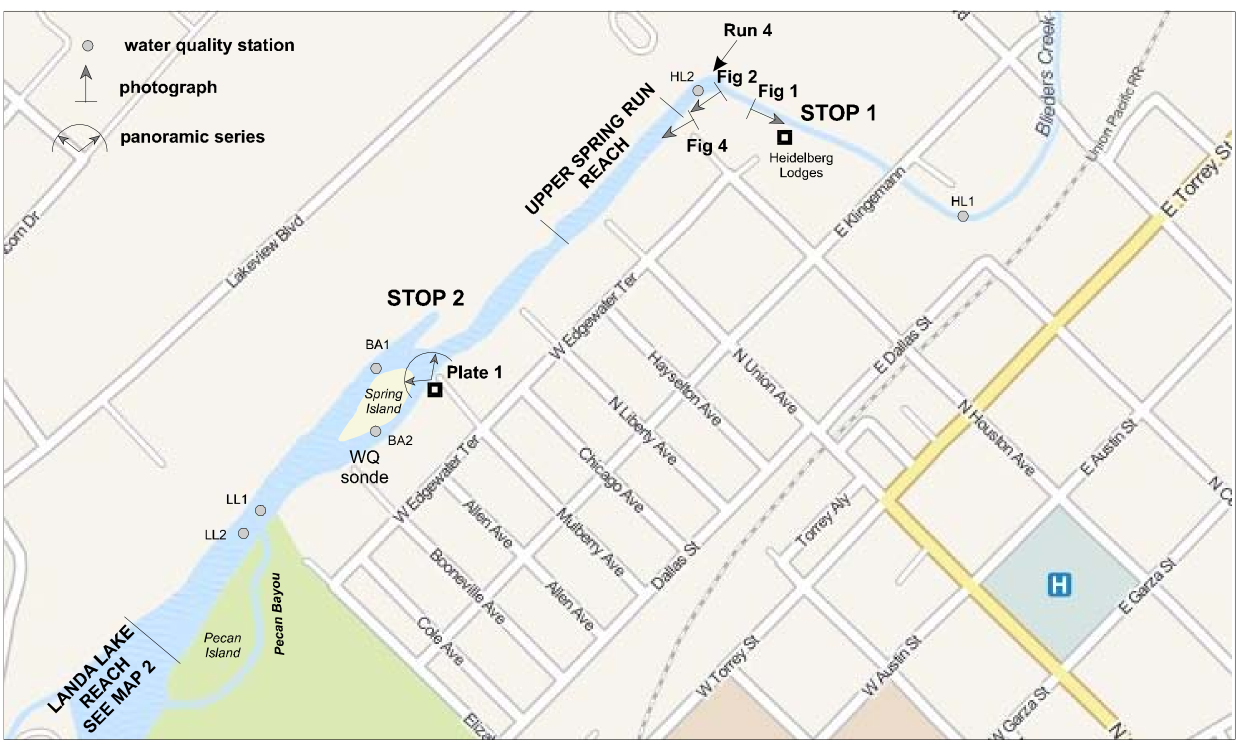
FROM: George H. Ward, scribe, with much input from Ed Oborny
DATE: 31 August 2014
SUBJECT: Notes on Darter Modeling Team field trip to Comal River and springs, 7 Aug 2014 CDT

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

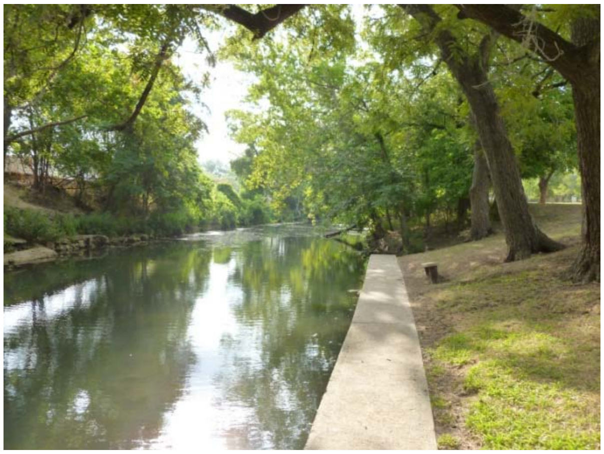


Fig 1 - Blieders Creek

Fig 2 - Spring Run 5

Fig 3 - Upstream from Upper Spring Run Reach

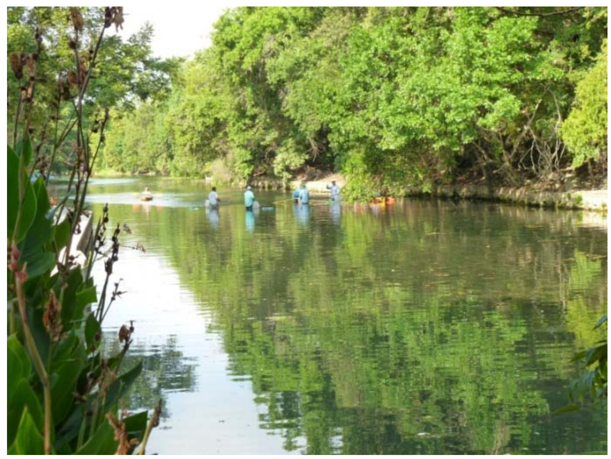


Fig 4 - Sampling underway in Upper Spring Run Reach

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

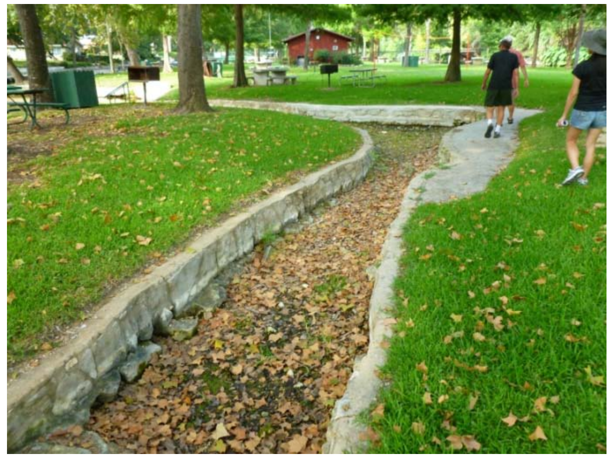


Fig 6 - Spring Run 6

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

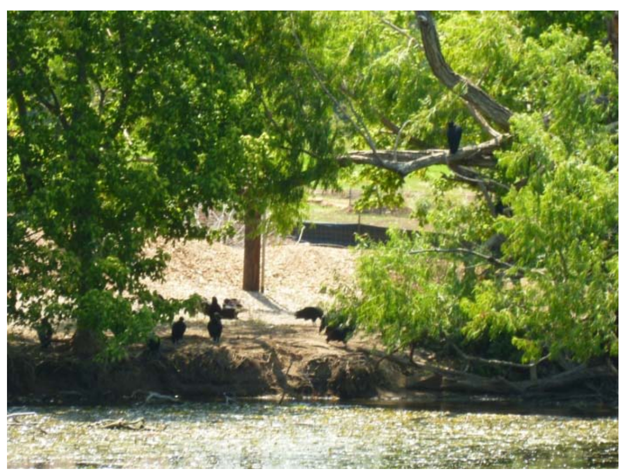
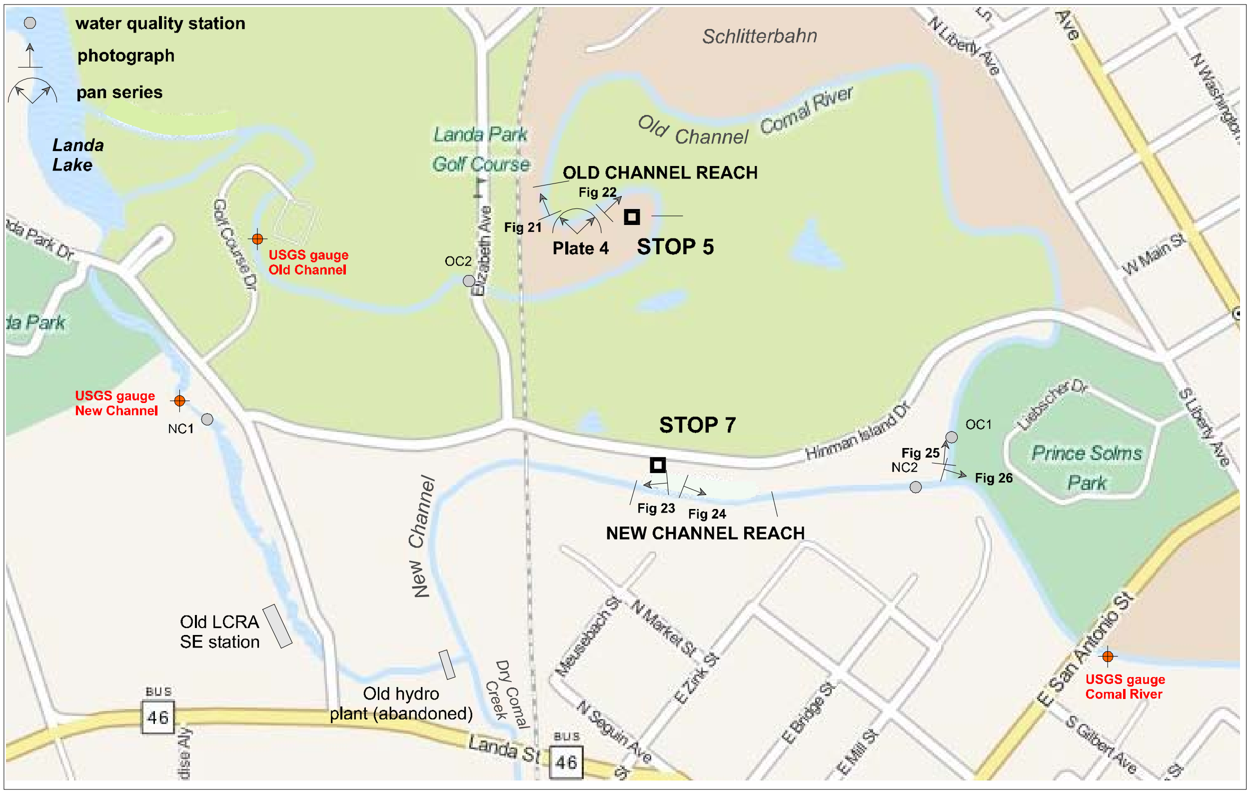


Fig 20 - Vultures hanging out around Landa Lake



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

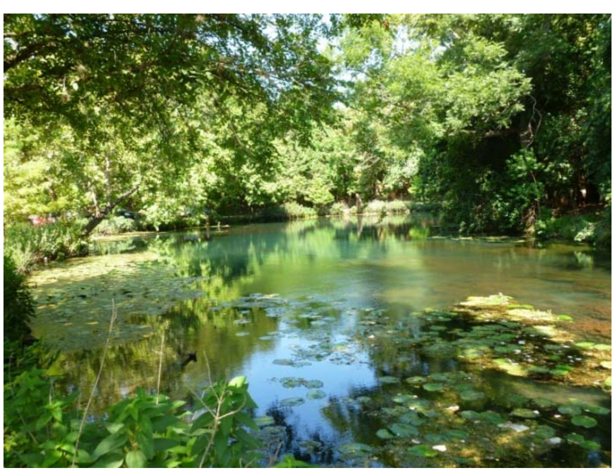


Fig 21 - Old Channel looking downstream

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

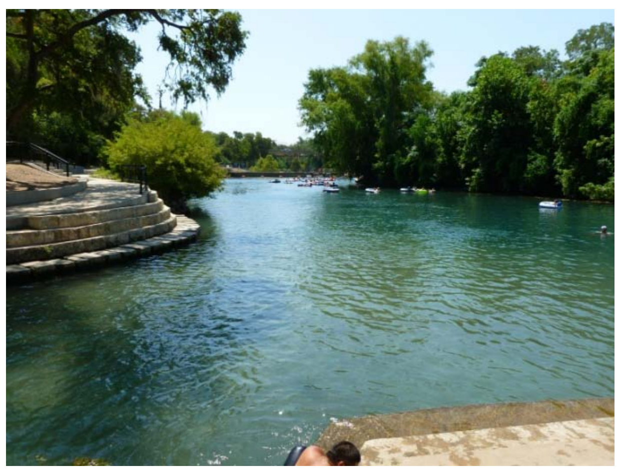


Fig 26 - New Channel, Cullen Dam in distance (Tube chute on right descending end)

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.

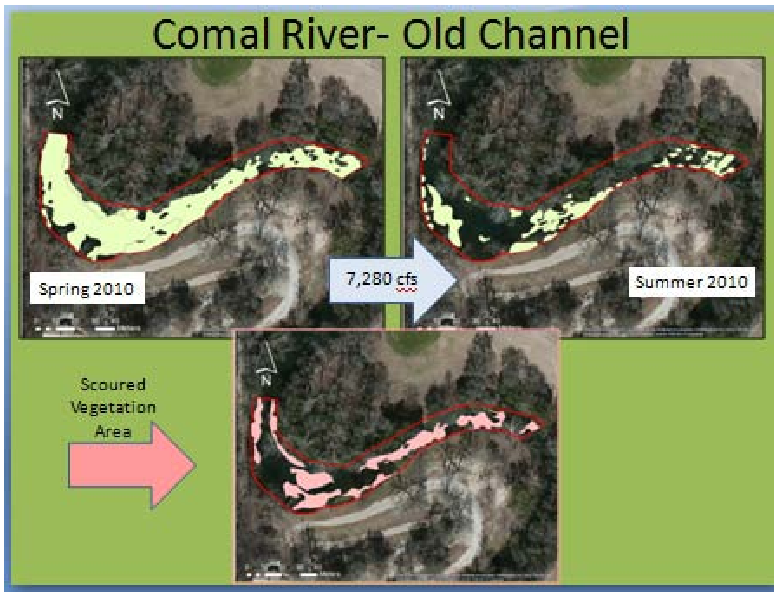
2. SAV studies

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.

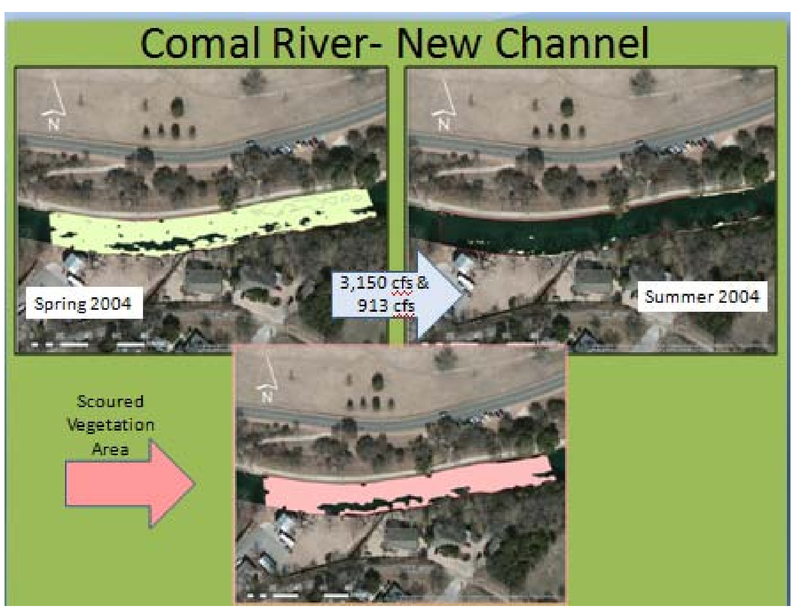
Todd reports continued good progress on the SAV modeling. He is evolving a combination of Charisma (the successor to Megaplant, see van Nes et al., 2003) and the metabolic functions developed in the ERDC models, see his handout summary attached. Some discussion of the use of shoot:root ratio as a measure 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

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, pt 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:

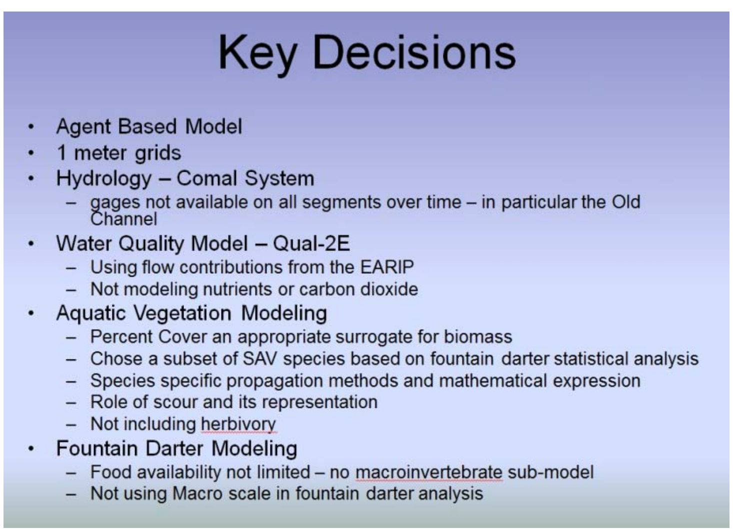
Ed Oborny	BIO-WEST	Bill Grant	TAMU	Thom Hardy	WSG
Robert Doyle	Baylor	Tim Bonner	TSU	Rose Wang	TAMU
Todd Swannack	ERDC	Jake Jackson	BIO-WEST	George Ward	UT

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.

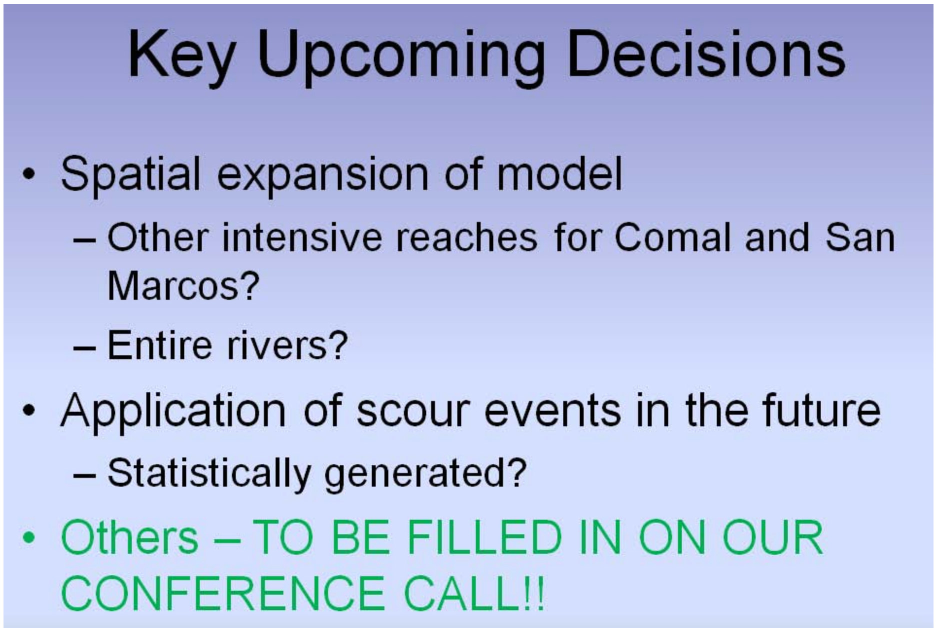
Robert may discuss carrying capacity in terms of percent cover, how the lab results enable us to link the observed vegetation densities to the modeled biomasses. He expressed concern at the use of numeric categories for persistence. These are qualitative classes, not quantitative, and the numeric designations 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

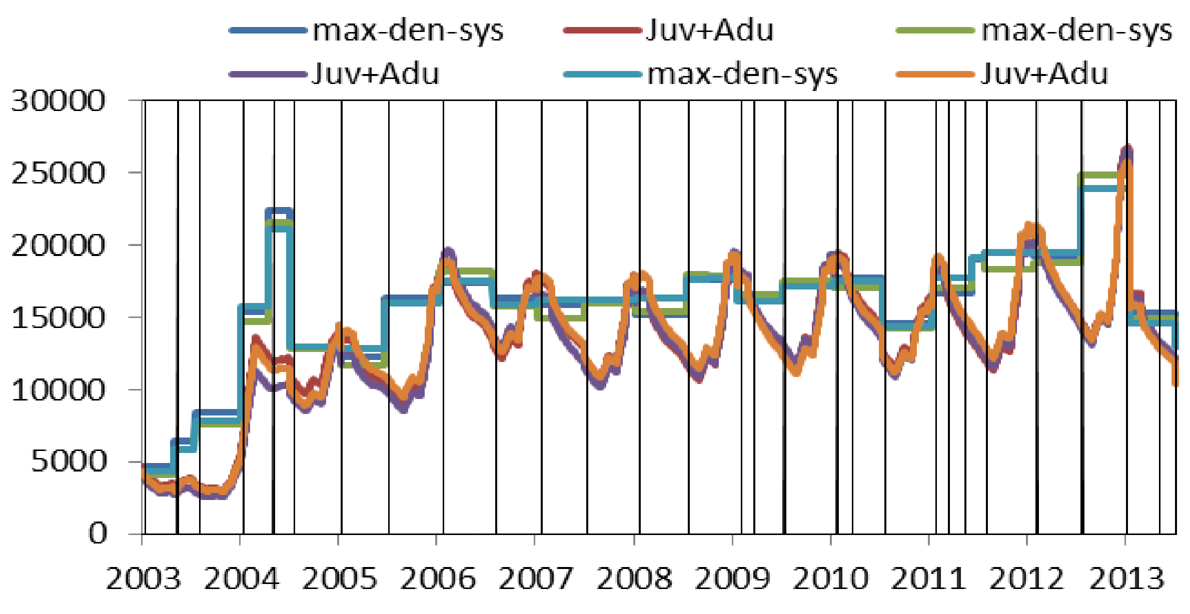
San Marcos Springs

AIC = 1436.515 (df = 24)

Variable	overall p-value	Category 2		Category 3		Category 4		Category 5	
		estimate	p-value	estimate	p-value	estimate	p-value	estimate	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.

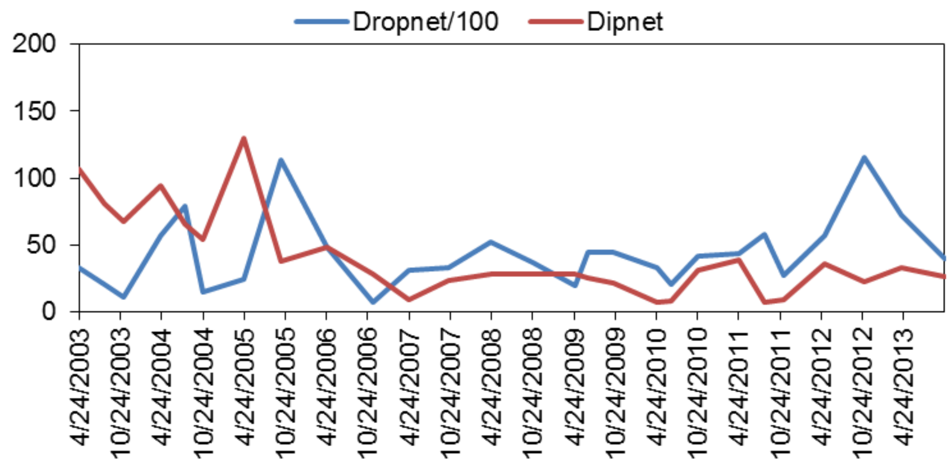
Given Todd's impending matrimonial endeavors, the following tasks should be completed before he takes the plunge:

- 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

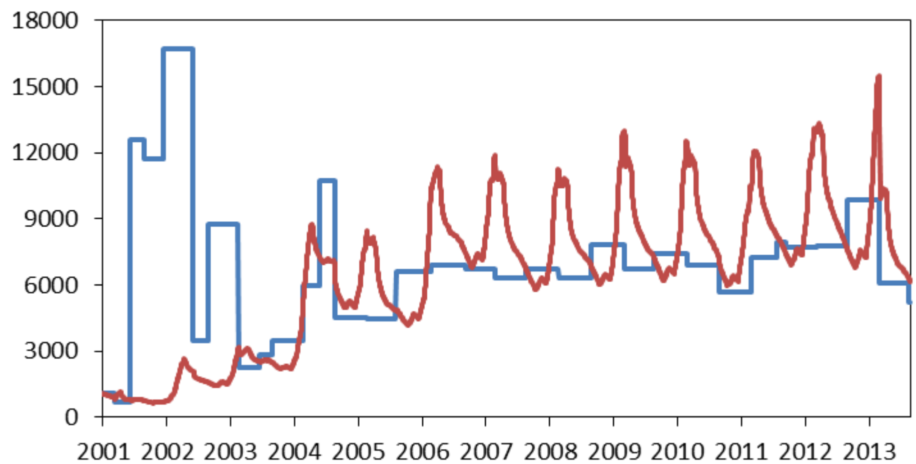
Vege	Average #/m2 of Dropnet	Average #/m2 of 96hr rule	Average #/m2 of 19hr rule	Average #/m2 of 12hr rule
1	20.17	11.34	9.89	8.49
2	3.48	2.04	1.21	1.29
3	3.09	4.64	3.67	3.48
4	1.78	1.91	1.17	0.96
6	12.83	11.33	11.33	10.33

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.)

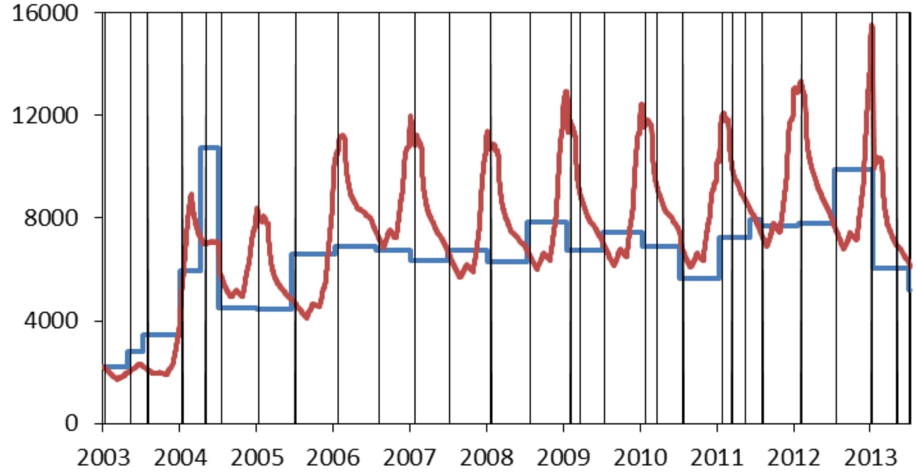
Specific model versus prediction results:

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.

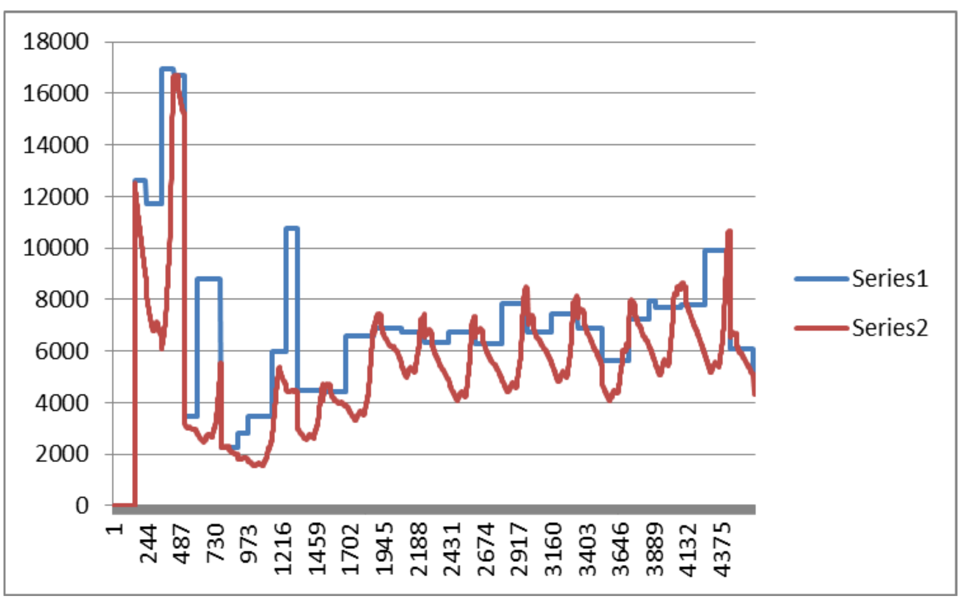
The next meeting was set for 9 October, and has been subsequently confirmed for those members of the Team that had to be absent on 3 September.

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

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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

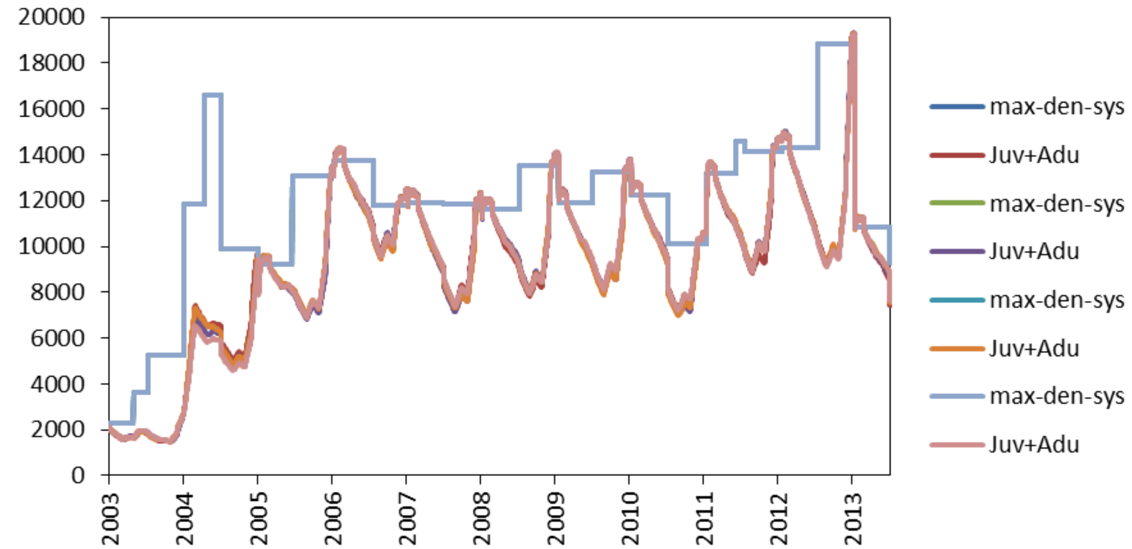
Vege Type	Average	StdDev	Minimum	Maximum	Sample size
0	1.32	2.15	0	8	34
1	21.96	22.73	0	105	42
2	3.12	3.88	0	17.5	33
3	4.13	3.87	0	21	87
4	1.78	1.39	0	5.5	47
6	11.58	4.82	6	20.5	6
10	8.00	N/A	8	8	1

Comal Springs

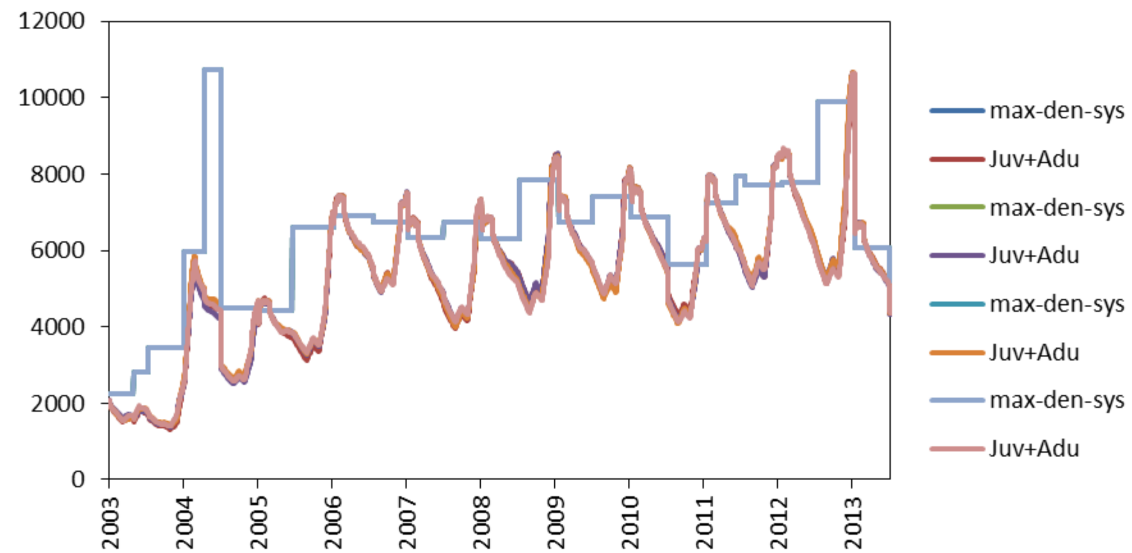
Vege Type	Average	StdDev	Minimum	Maximum	Sample size
0	1.29	2.59	0	15	62
1	19.34	22.36	0	105	48
2	3.12	3.88	0	17.5	33
3	7.37	8.35	0	38.5	297
4	12.15	14.41	0	85	138
6	24.91	20.28	0	96	152
7	5.27	14.00	0	106	95
8	5.58	9.95	0	58	86
10	9.58	9.09	0	42.5	91

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.

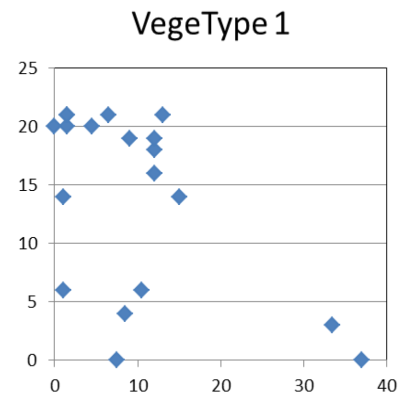
5. Additional matters

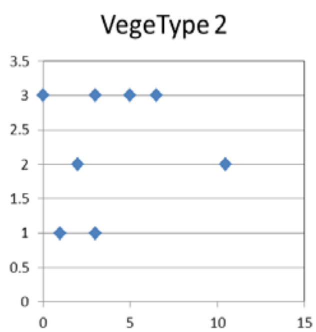
Slides to Ed by 27 Oct for the NAS presentation. Drafts of chapter texts by 9 November.

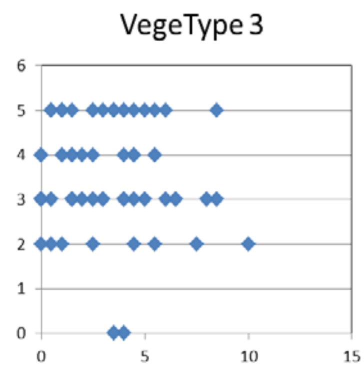
Next meeting: 10:00 CST 13 November at Bio-West offices.

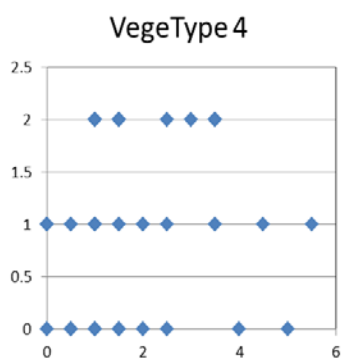
Survey (x) and simulated (y) darter density for each vegetation type (Rep 3) from Comal Old Channel data:

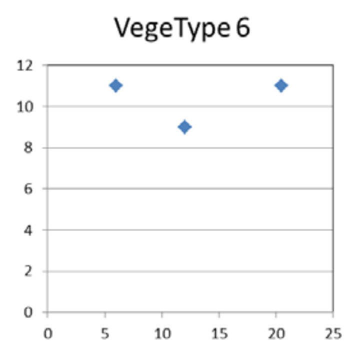
VegeType	E
1	-1.589
2	-0.263
3	-0.423
4	-0.456
6	-0.170
All	-0.5138







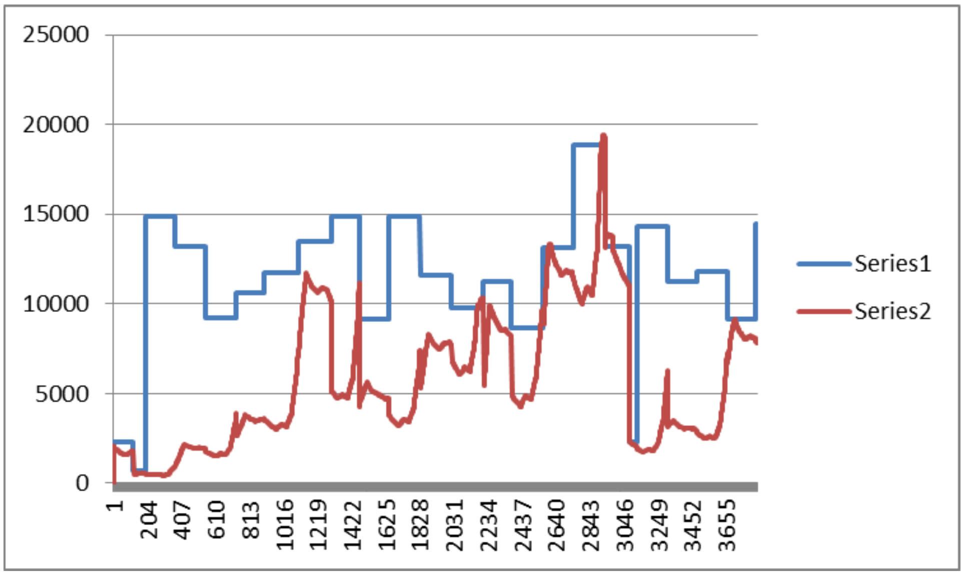




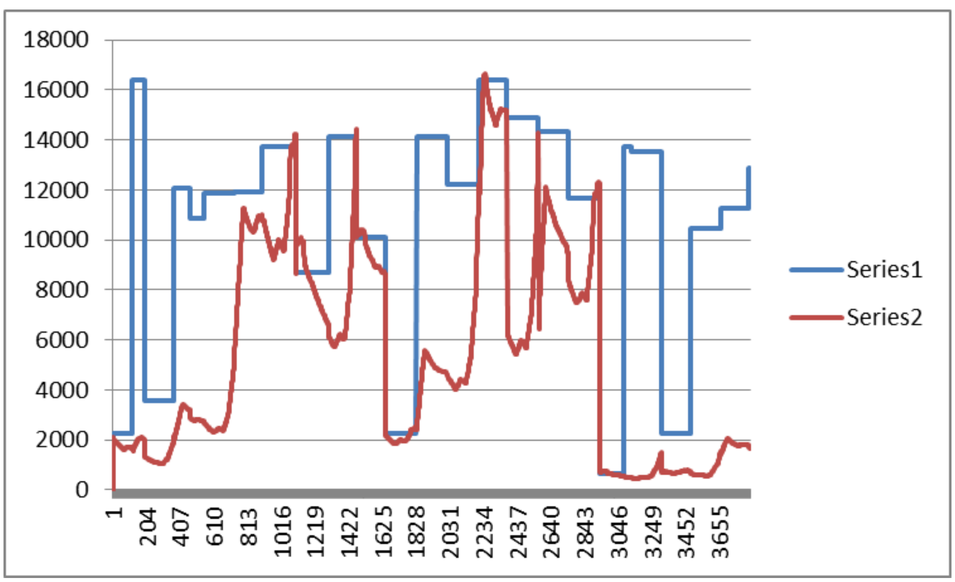
ADDENDUM 1
From Bill Grant, TAMU

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

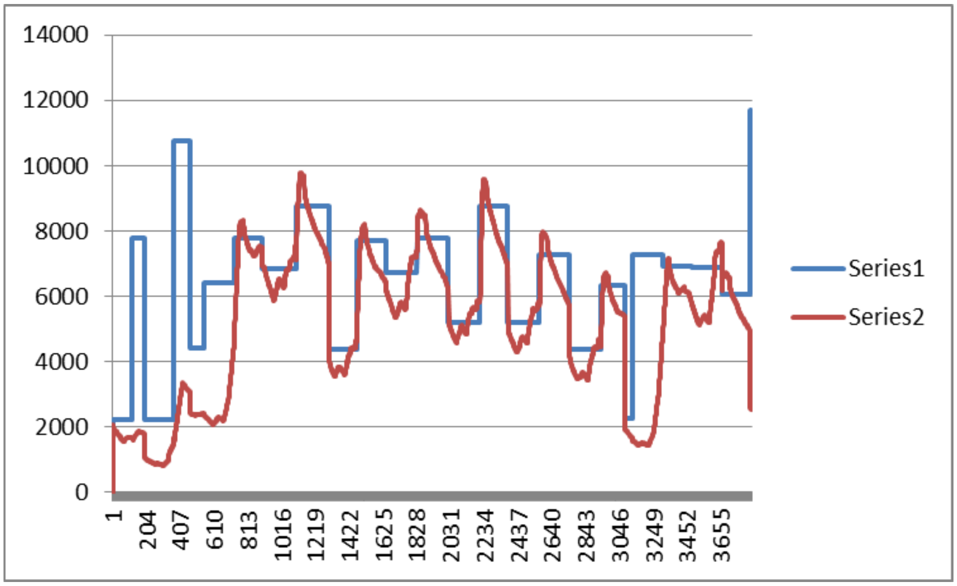


Rep #2

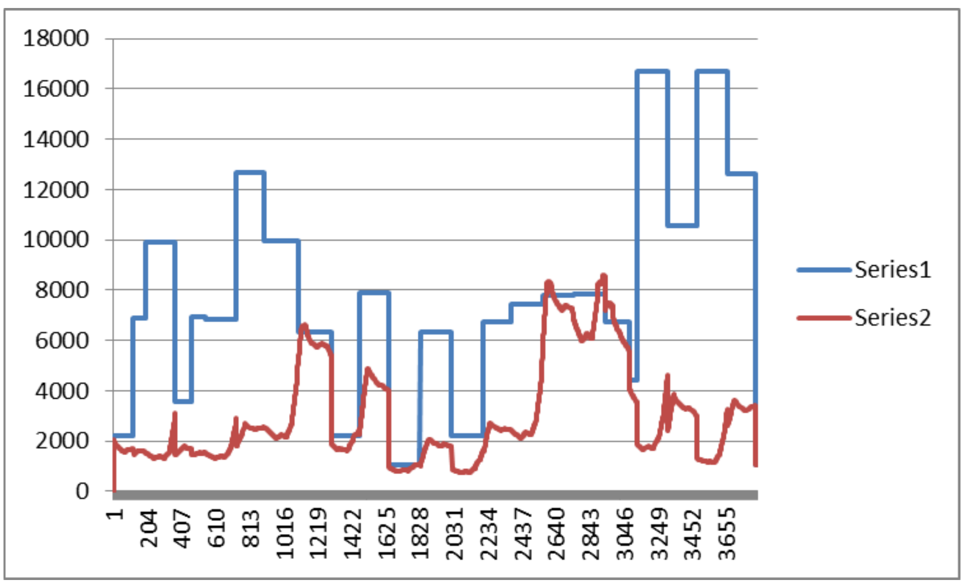


Based on Veg data from Old Channel Only (Random Sequence of Years)

Rep #1

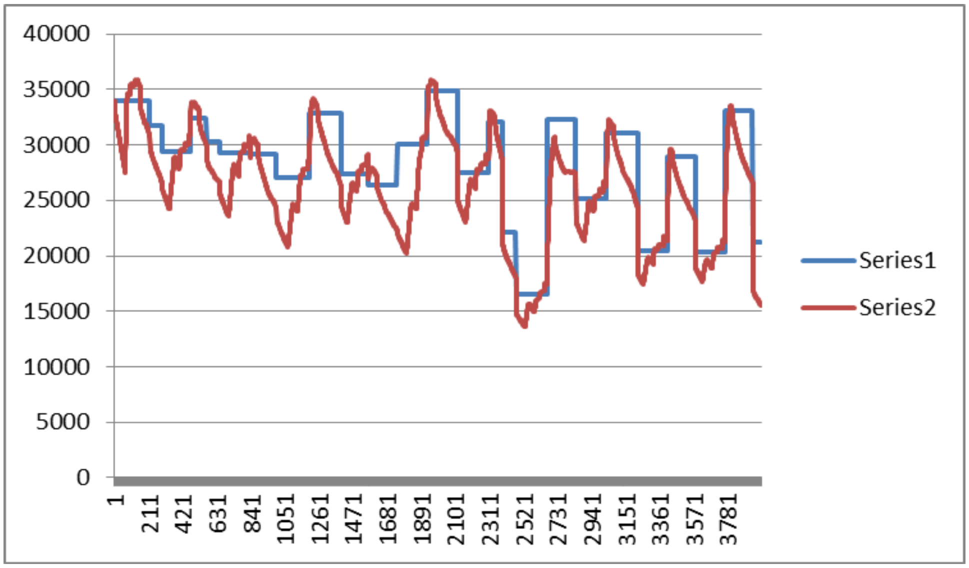


Rep #2



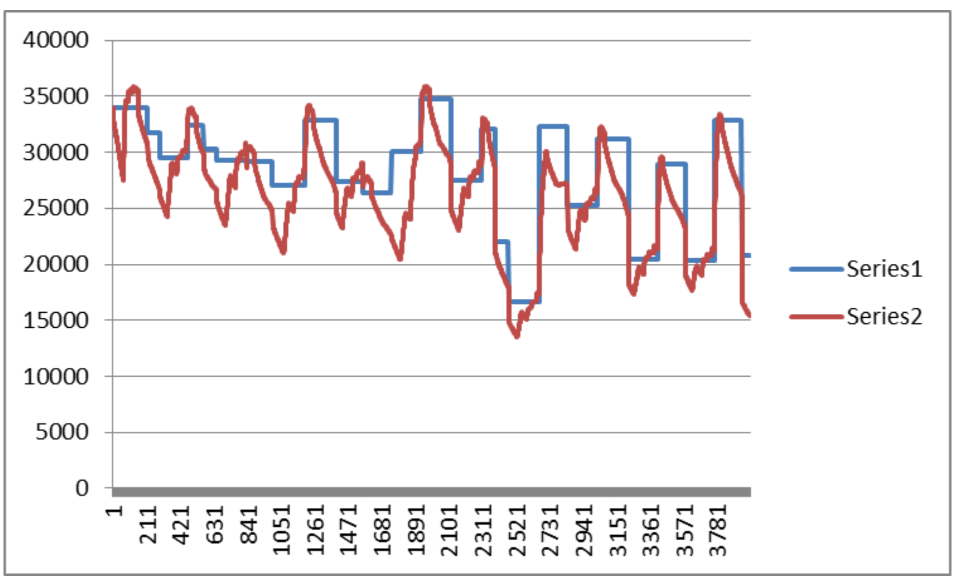
Based on Veg data from City Park Only (Appropriate Chronological Sequence of Years)

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!)

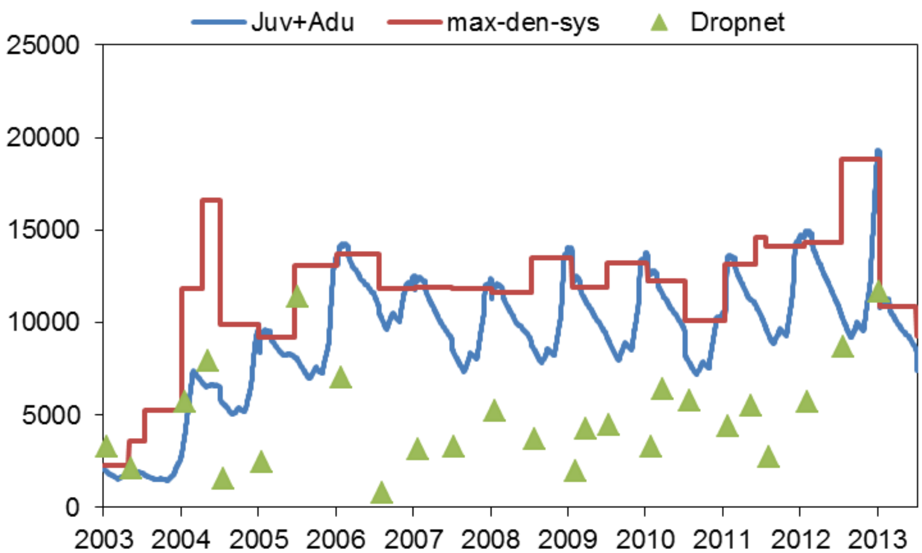


ADDENDUM 2

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*:

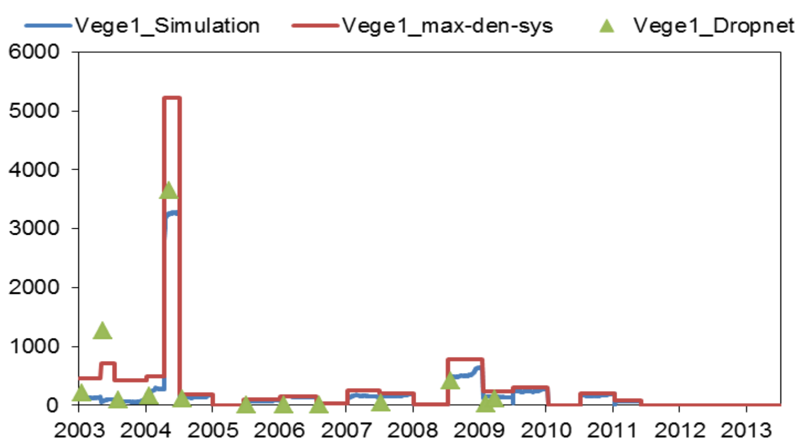


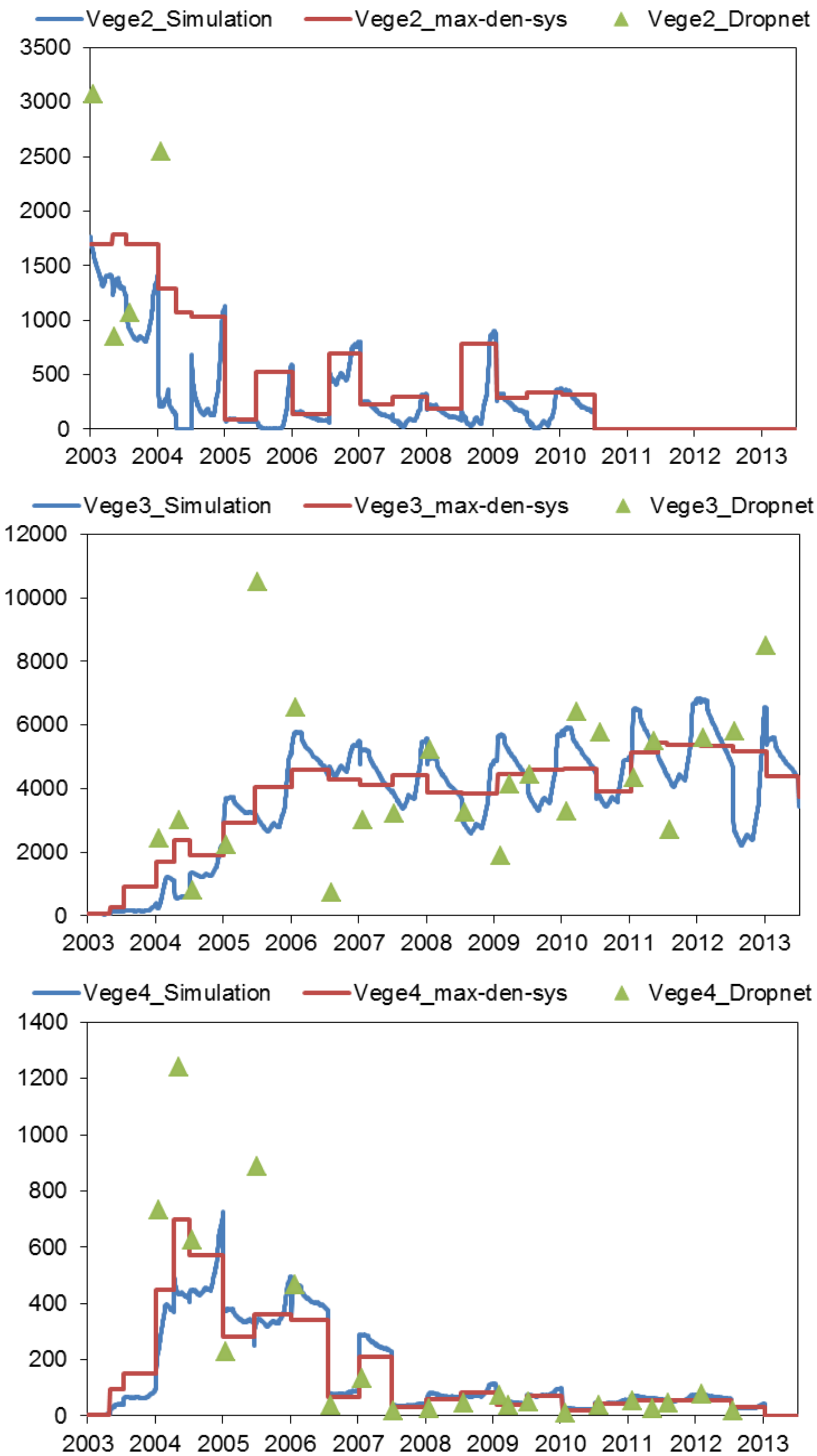
*During the dropnet sample days, BioWest only surveyed few vegetation types (4 to 7 dropnets) and hence the abundance calculated by using Σ(average darter density in vegetation type i) × (# of cells in vegetation type i) is always lower.

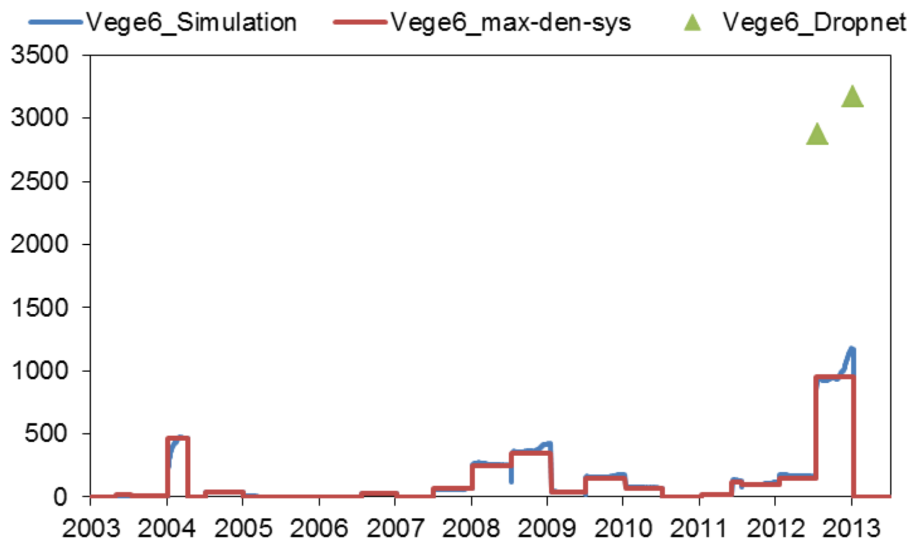
By vegetation type:

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
Vege4_Simulation
Vege4_max-den-sys
Vege4_Dropnet
1400
1200
1000
800
600
400
200
0



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:

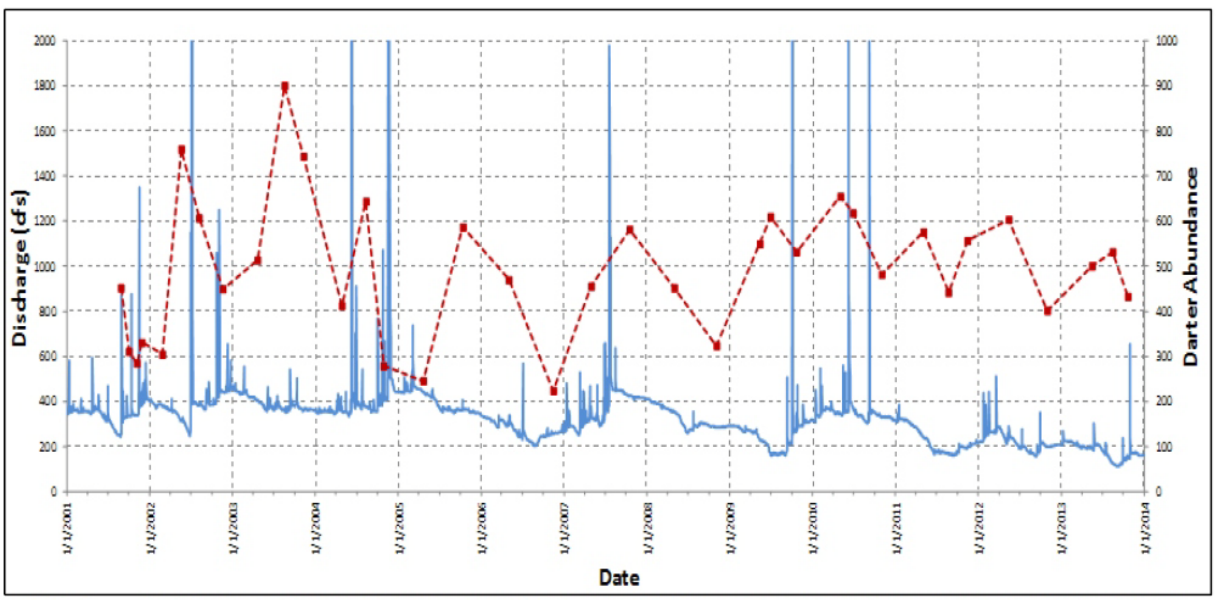


Figure 21. **Abundance of fountain darters from each drop net sampling event (red dashed line) plotted over a hydrograph of mean daily discharge from the USGS gauge on the Comal River at New Braunfels (blue line).**

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.

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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

<u>Sample Collection</u>

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).

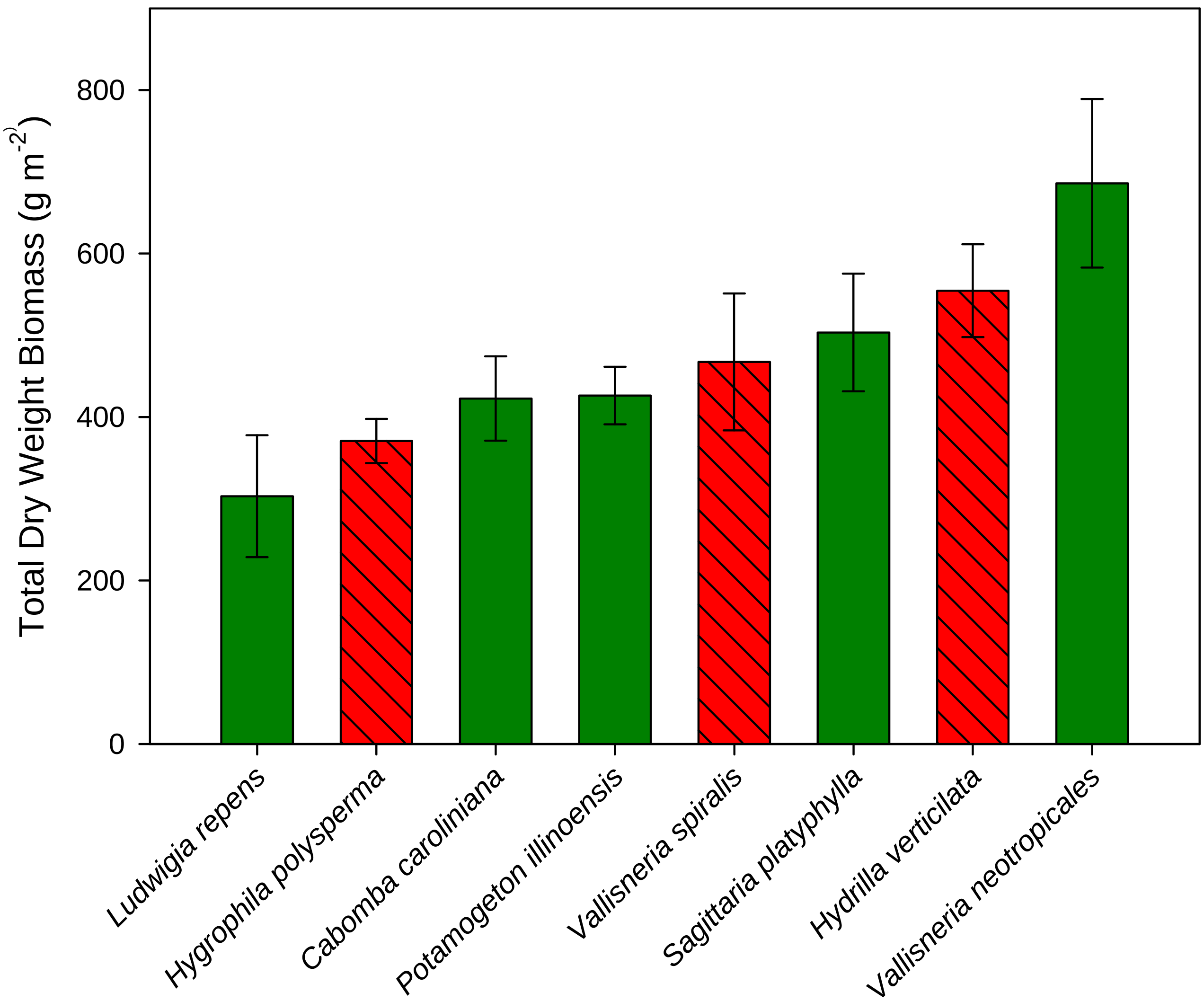

Figure 2. Average (± SE) of total plant biomass per meter squared for samples heavily dominated by a single plant species (90-100% cover reported). Native species are shown in solid green and non-native species in red hash.

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).

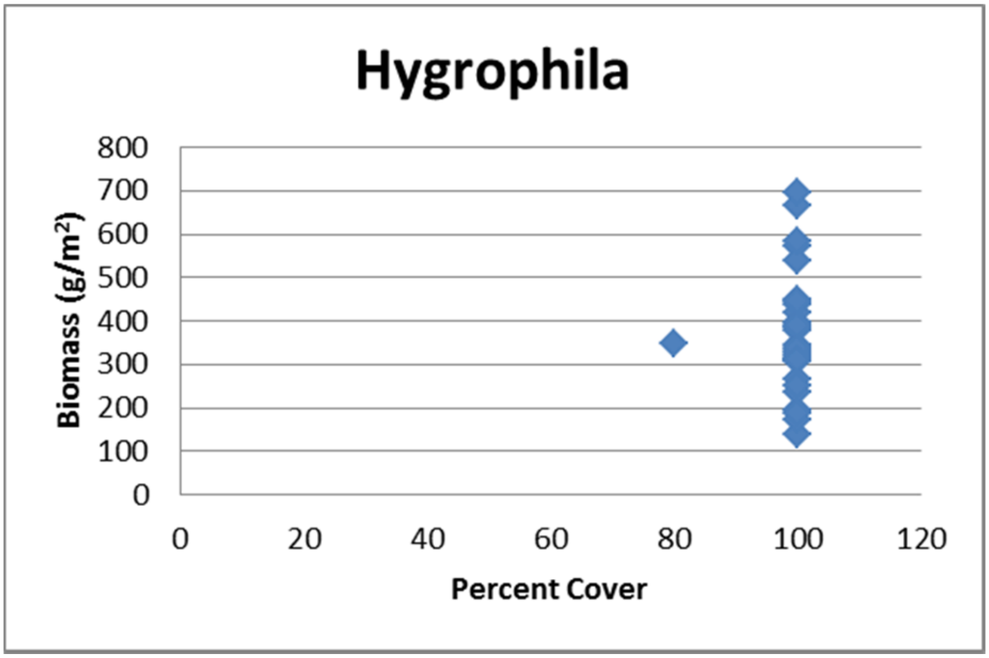

Figure 3. Total dry weight biomass vs. observed percent cover for 29 samples of Hygrophila collected on the Comal and San Marcos Rivers, TX.

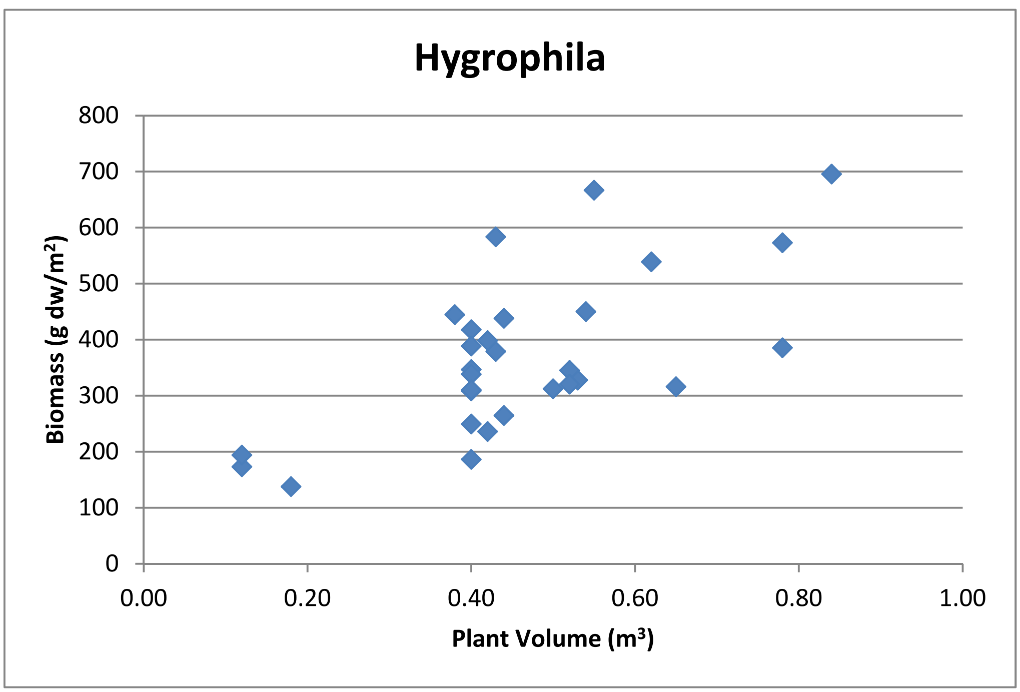

Figure 4. Total dry weight biomass vs. occupied plant volume for 29 samples of Hygrophila collected on the Comal and San Marcos Rivers, TX.

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 $0.33m^2$ 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

Field support by BIO-WEST is gratefully acknowledged. Additionally, Mr. Connor Costello provided assistance in processing the samples back in the Baylor Wetland Lab.

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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.

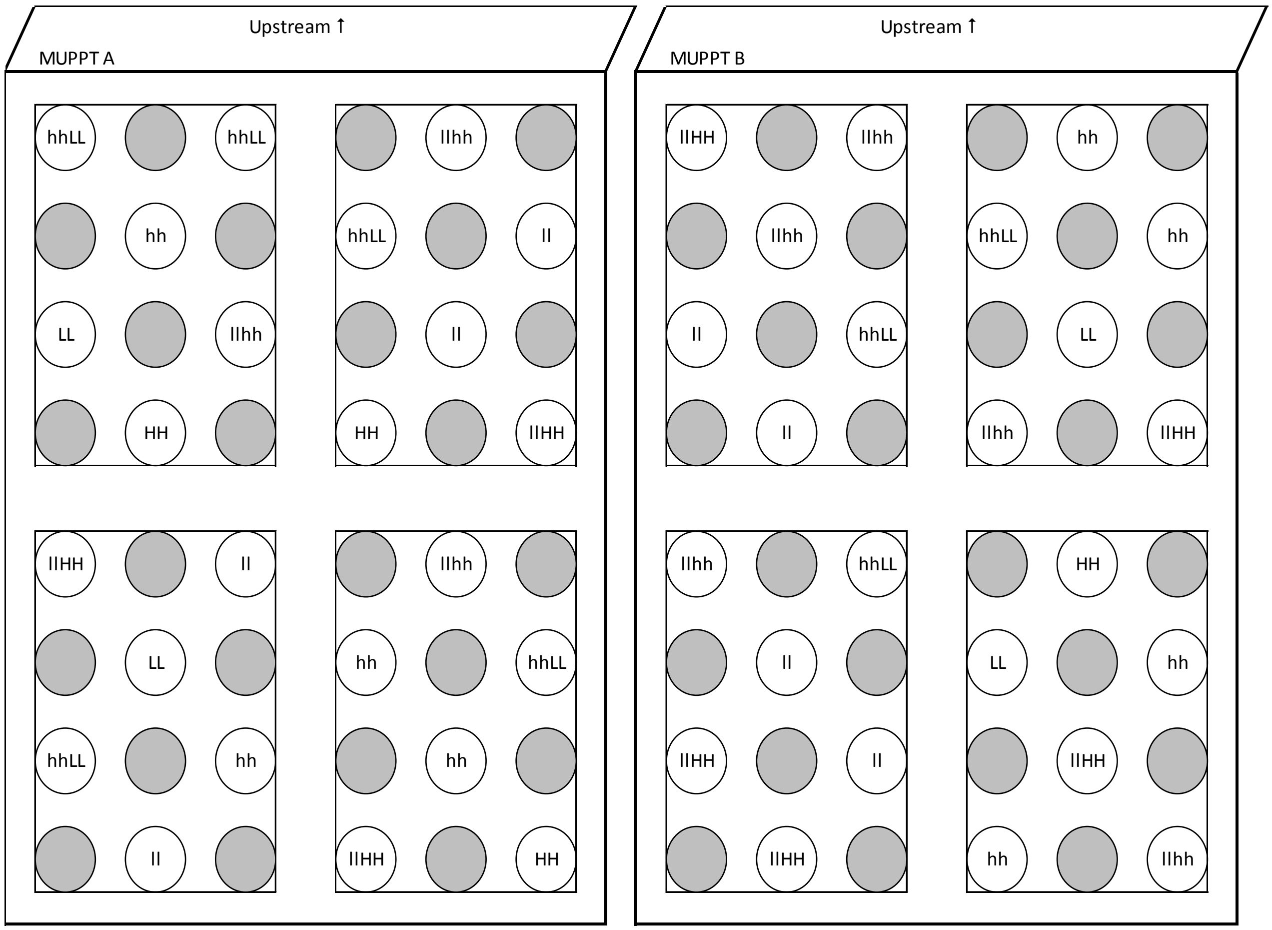

Figure 2. **Illustrated arrangement of alternating experimental pot placement within two MUPPTS anchored at each location. Open circles display the 7 possible experimental combinations (Table 3), and gray circles represent empty spaces.**

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.

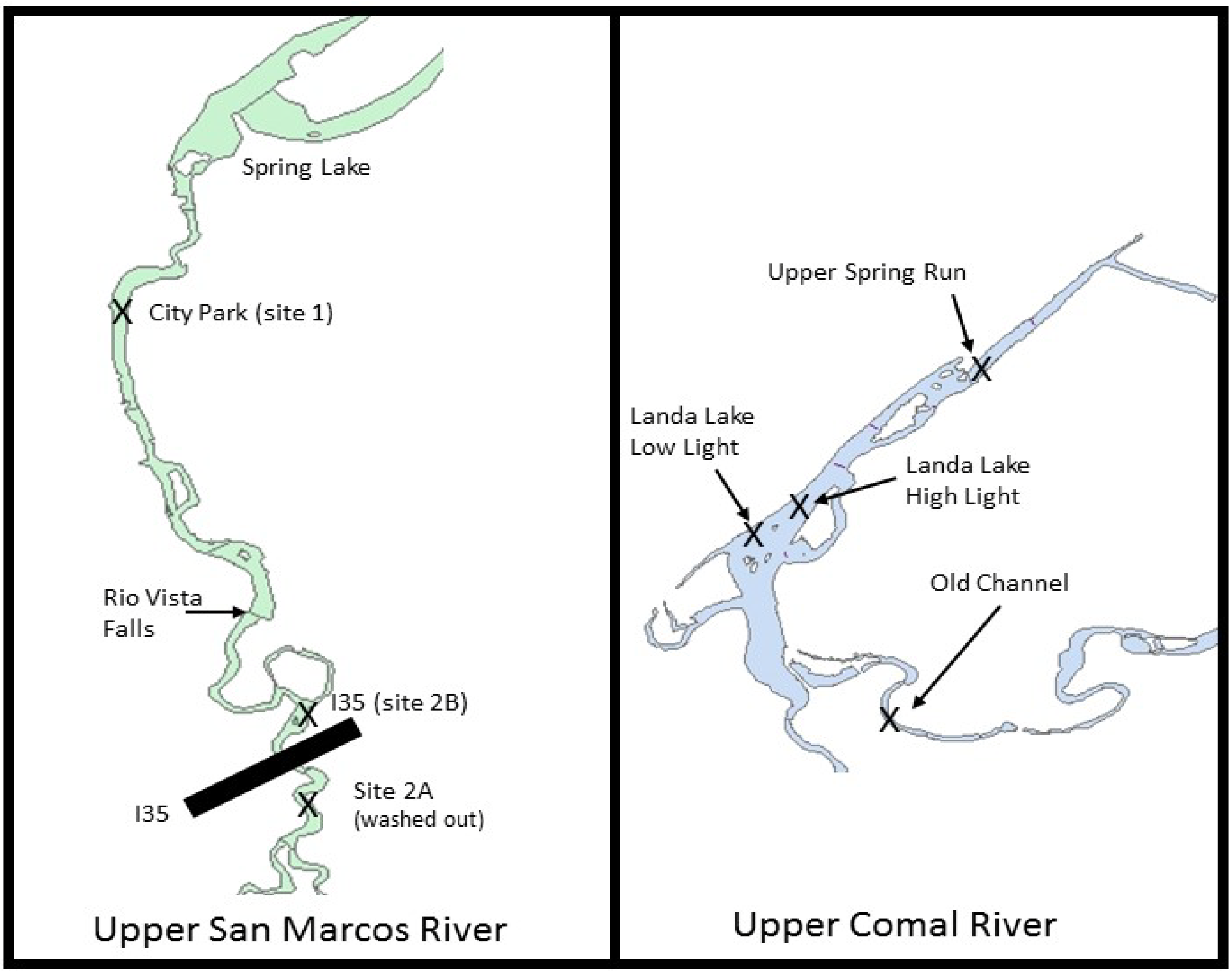

Figure 3. **Maps of the upper San Marcos and upper Comal Rivers showing locations of MUPPT deployment for competition experiments.**

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.

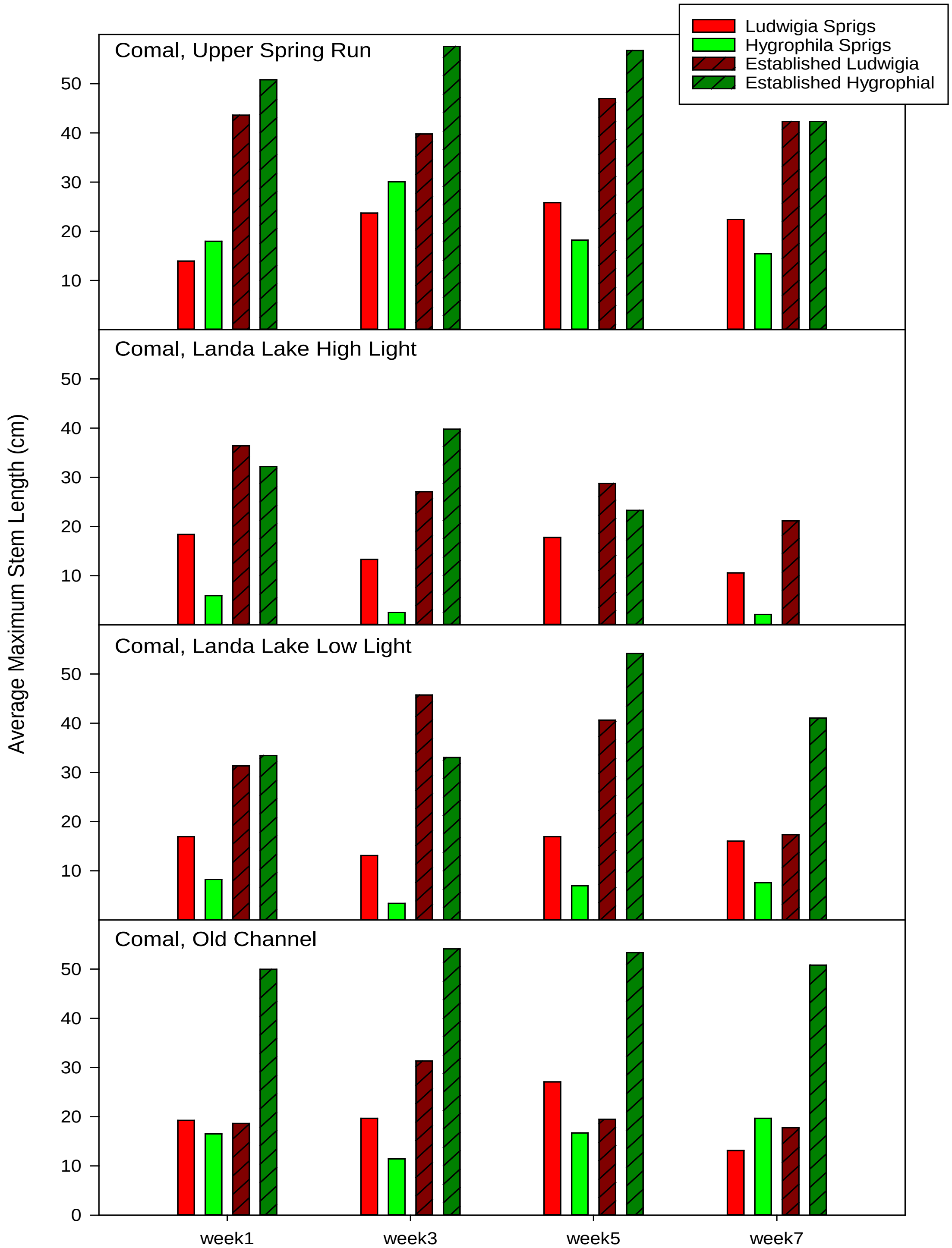

Figure 4. **Average maximum stem length of plants at each of the four experimental locations on the Comal River. Data is shown for sprigs of Ludwigia (red) and Hygrophila (green) as well as established Ludwigia (dark red, hatched) and Hygrophila (dark green, hatched).**

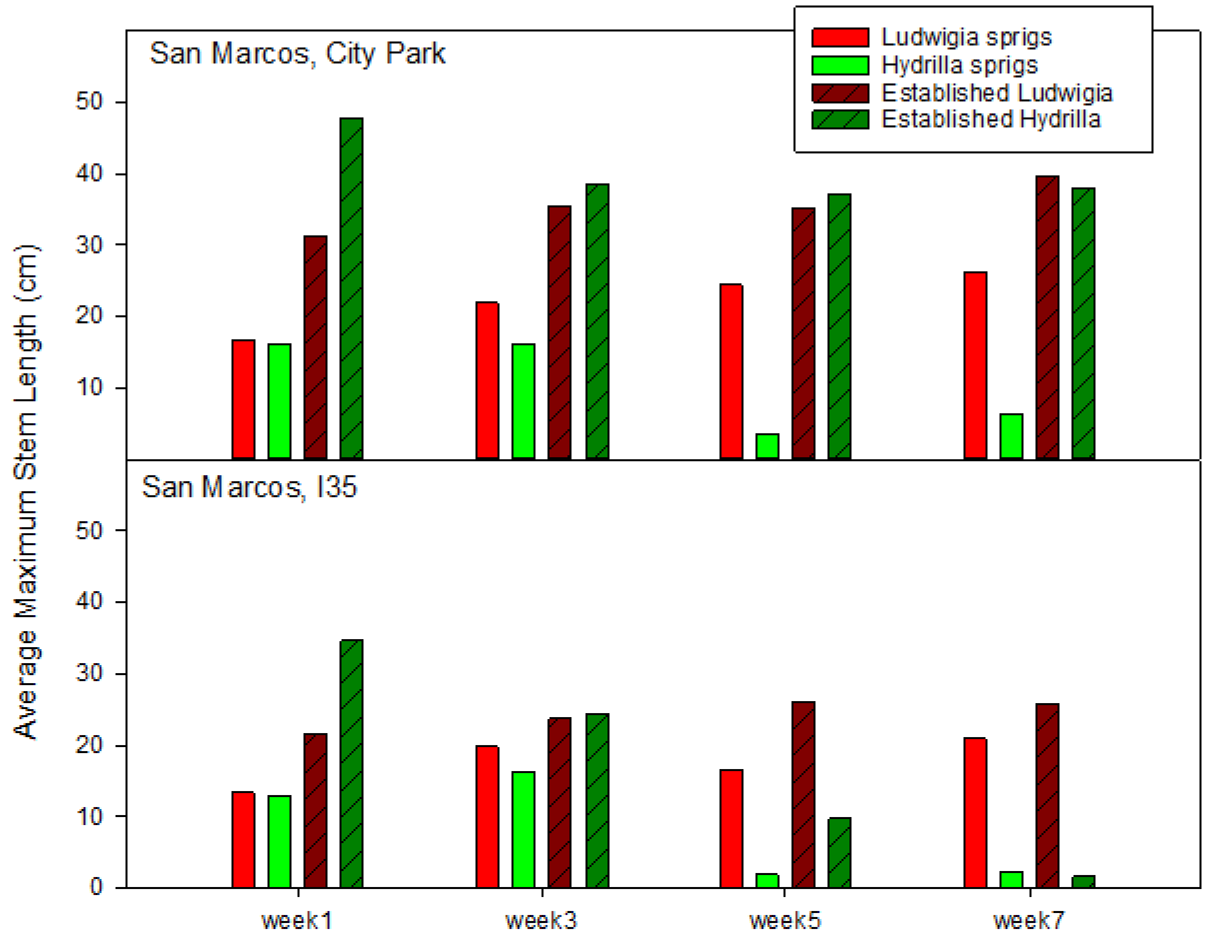

Figure 5. **Average maximum stem length of plants at each of the two experimental locations on the San Marcos River. Data is shown for sprigs of Ludwigia (red) and Hydrilla (green) as well as established Ludwigia (dark red, hatched) and Hydrilla (dark green, hatched).**

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)

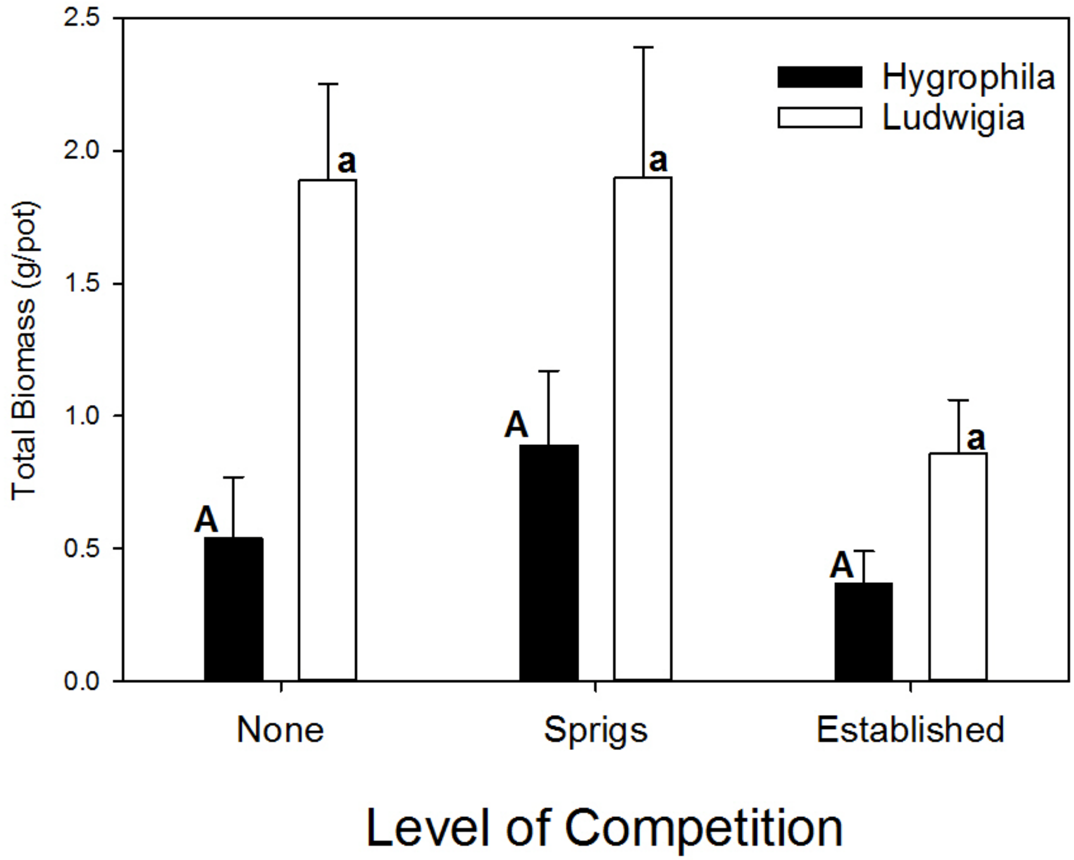

Figure 7. **Final total biomass of plants of Hygrophila (black bars) or Ludwigia (white bars) grown from two planted sprigs under three levels of competition (no competitor, two sprigs of competitor, established competitor). Shown are mean +/- SE. Two Way ANOVA (Location X Treatment) analysis showed 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)

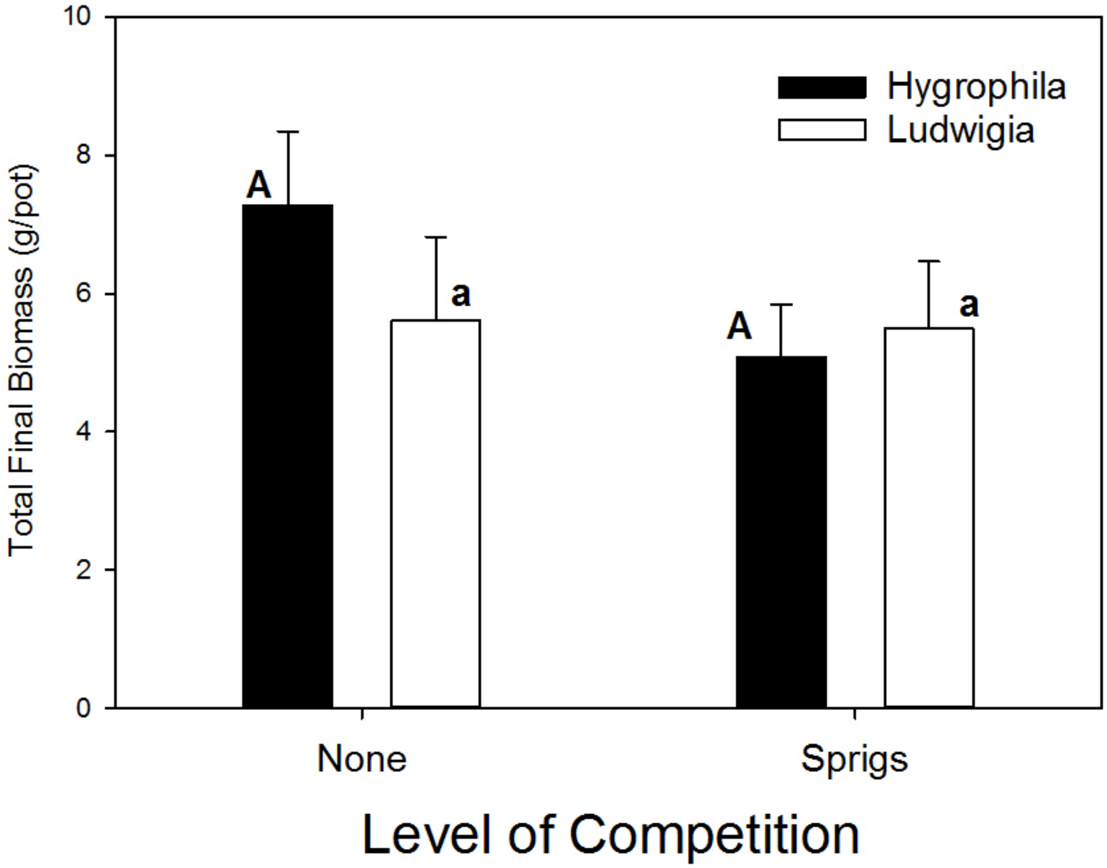

Figure 8. **Final total biomass of established plants of Hygrophila (black bars) or Ludwigia (white bars) grown with no competitive pressure (none) or invaded by two sprigs of the other species (sprigs). Shown are mean +/- SE. Two Way ANOVA (Location X Treatment) analysis showed no significant interaction 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)

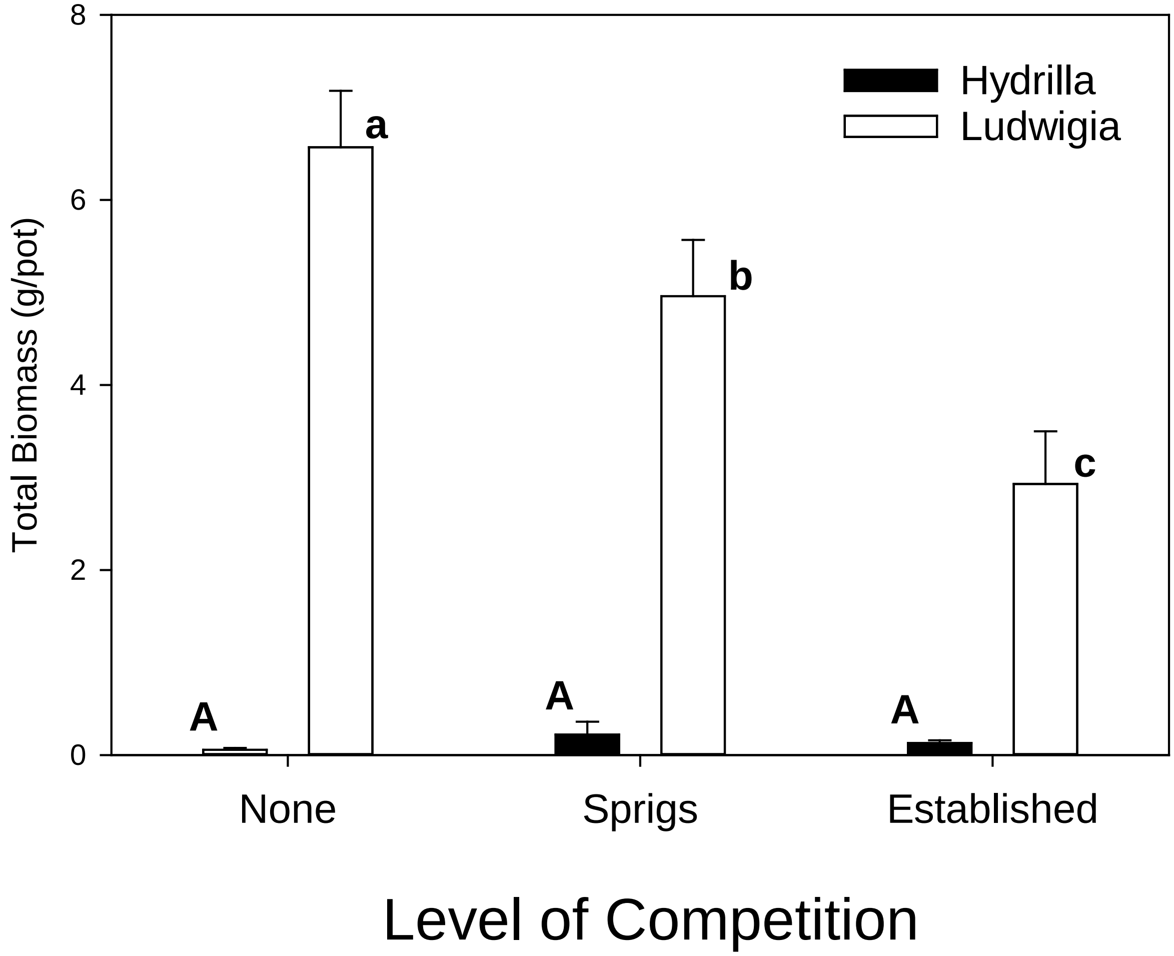

Figure 9. **Final total biomass of plants of Hydrilla (black bars) or Ludwigia (white bars) grown from two planted sprigs under three levels of competition (no competitor, two sprigs of competitor, established competitor). Shown are mean +/- SE. Two Way ANOVA (Location X Treatment) analysis showed no 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*

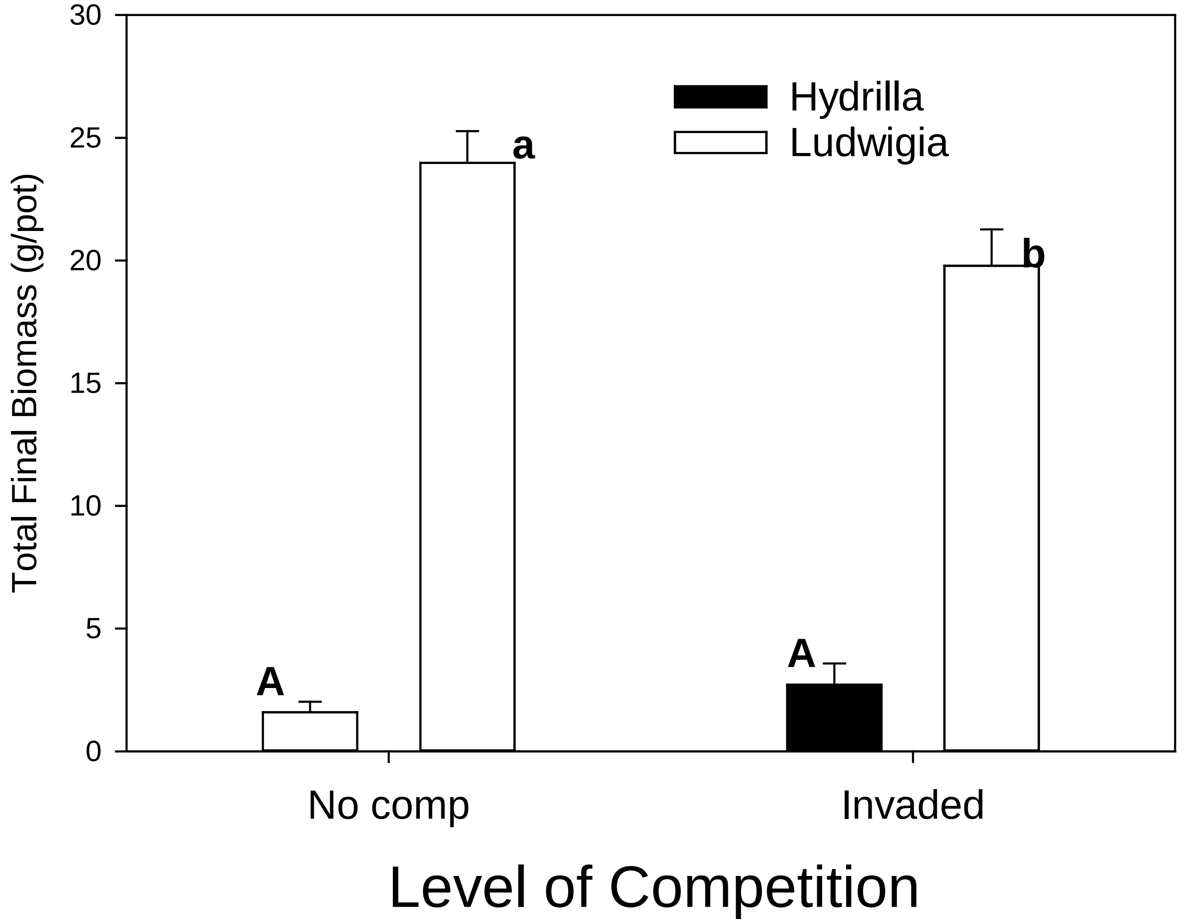

Figure 10. **Final total biomass of established plants of Hydrilla (black bars) or Ludwigia (white bars) grown with no competitive pressure (none) or invaded by two sprigs of the other species (sprigs). Shown are mean +/- SE. Two Way ANOVA (Location X Treatment) analysis showed no significant interaction 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

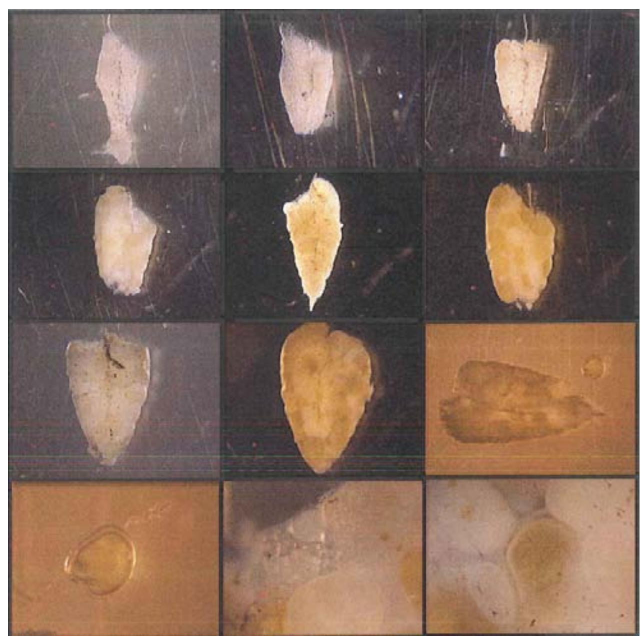
Effects of Low-Flow on Fountain Darter Reproductive Effort

EFFECTS OF LOW FLOW ON FOUNTAIN DARTER REPRODUCTIVE EFFORT

HABITAT CONSERVATION PLAN (HCP) 2014 APPLIED RESEARCH

Texas State University San Marcos Department of Biology

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.

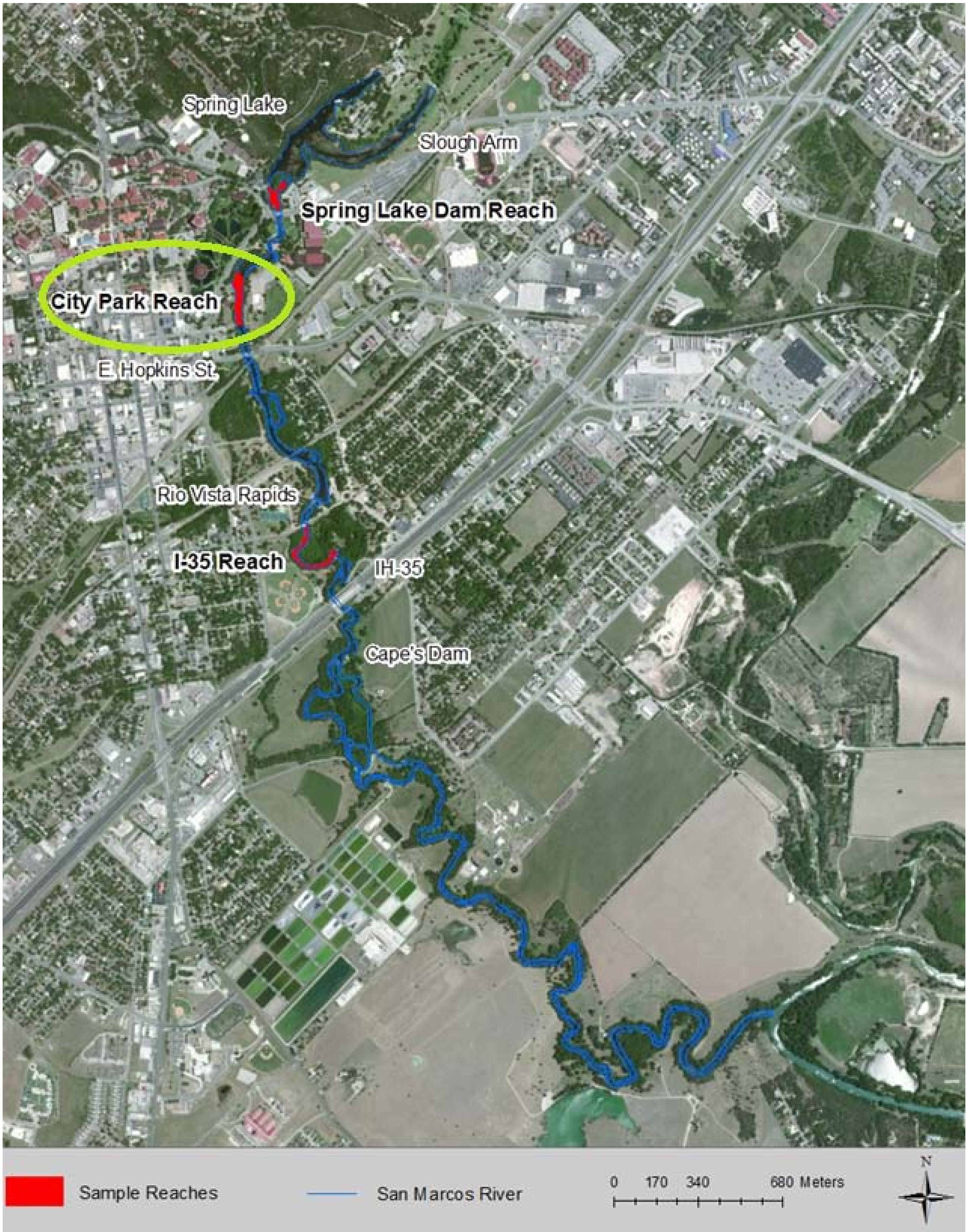

Figure 1. City Park sampling reach on the San Marcos River used for the Fecundity Study.

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).

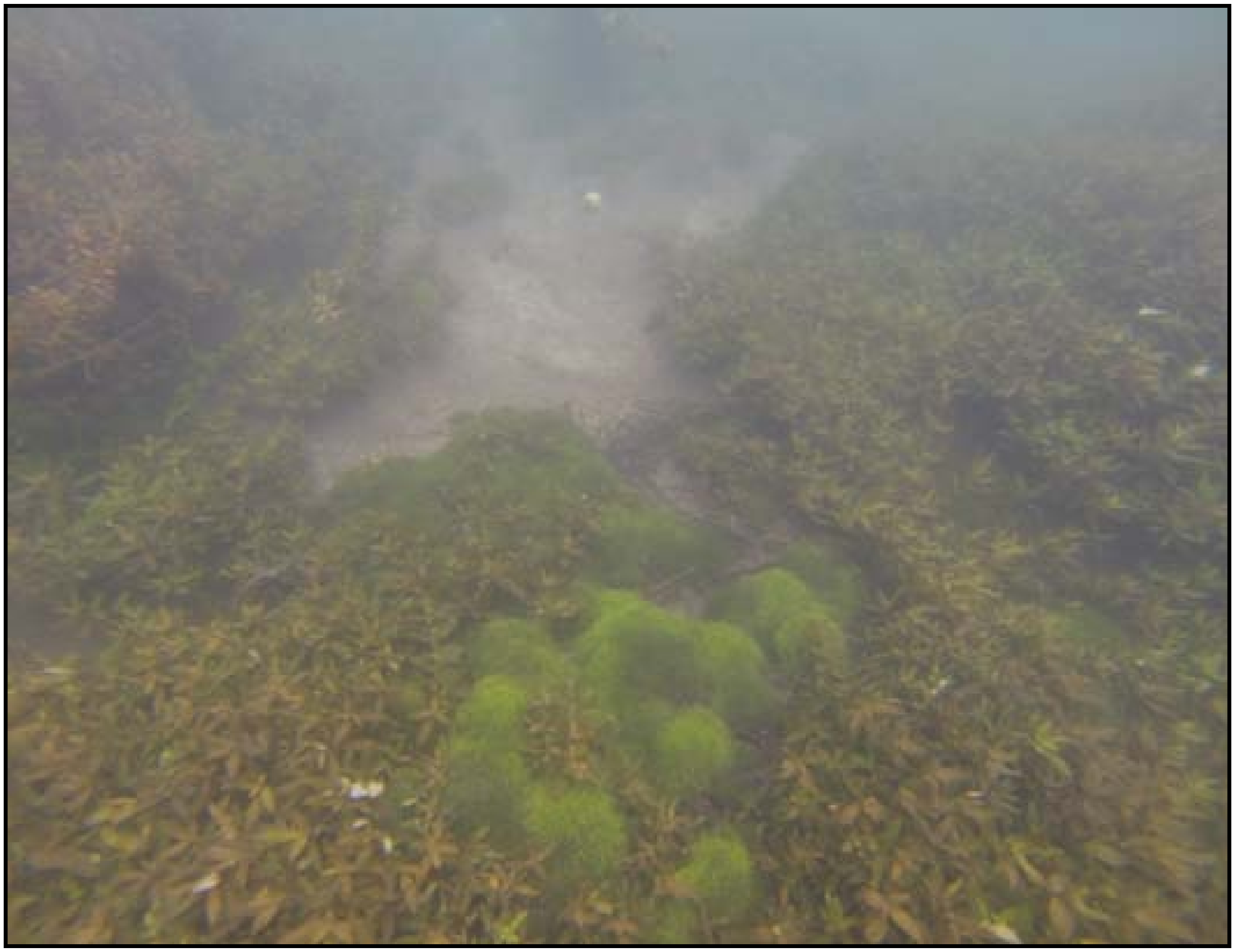

Figure 4. **Examples of "short" aquatic vegetation sampled in during monthly field collections.**

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).

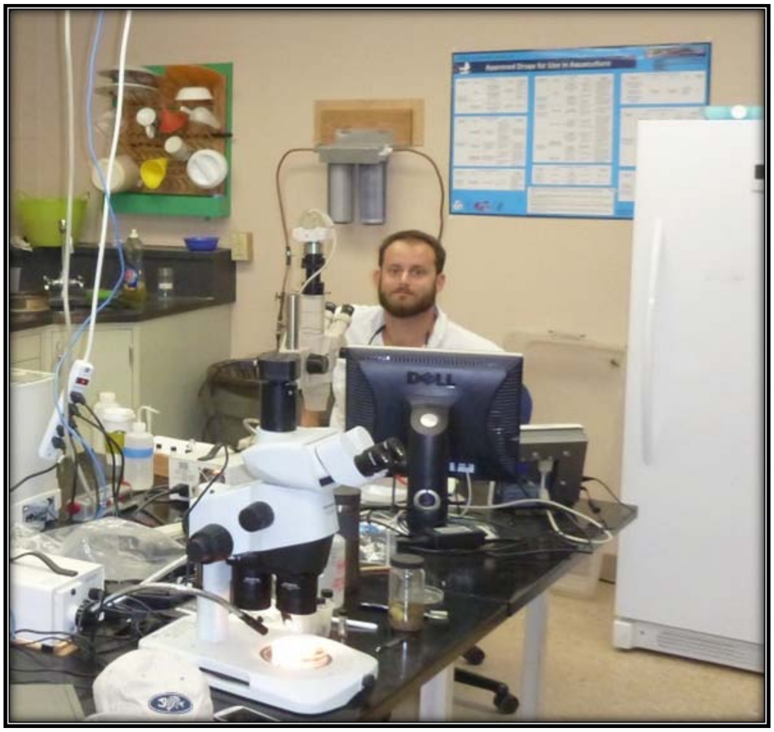

Figure 5. Laboratory analysis of ovary stage conducted at the SMARC.

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.

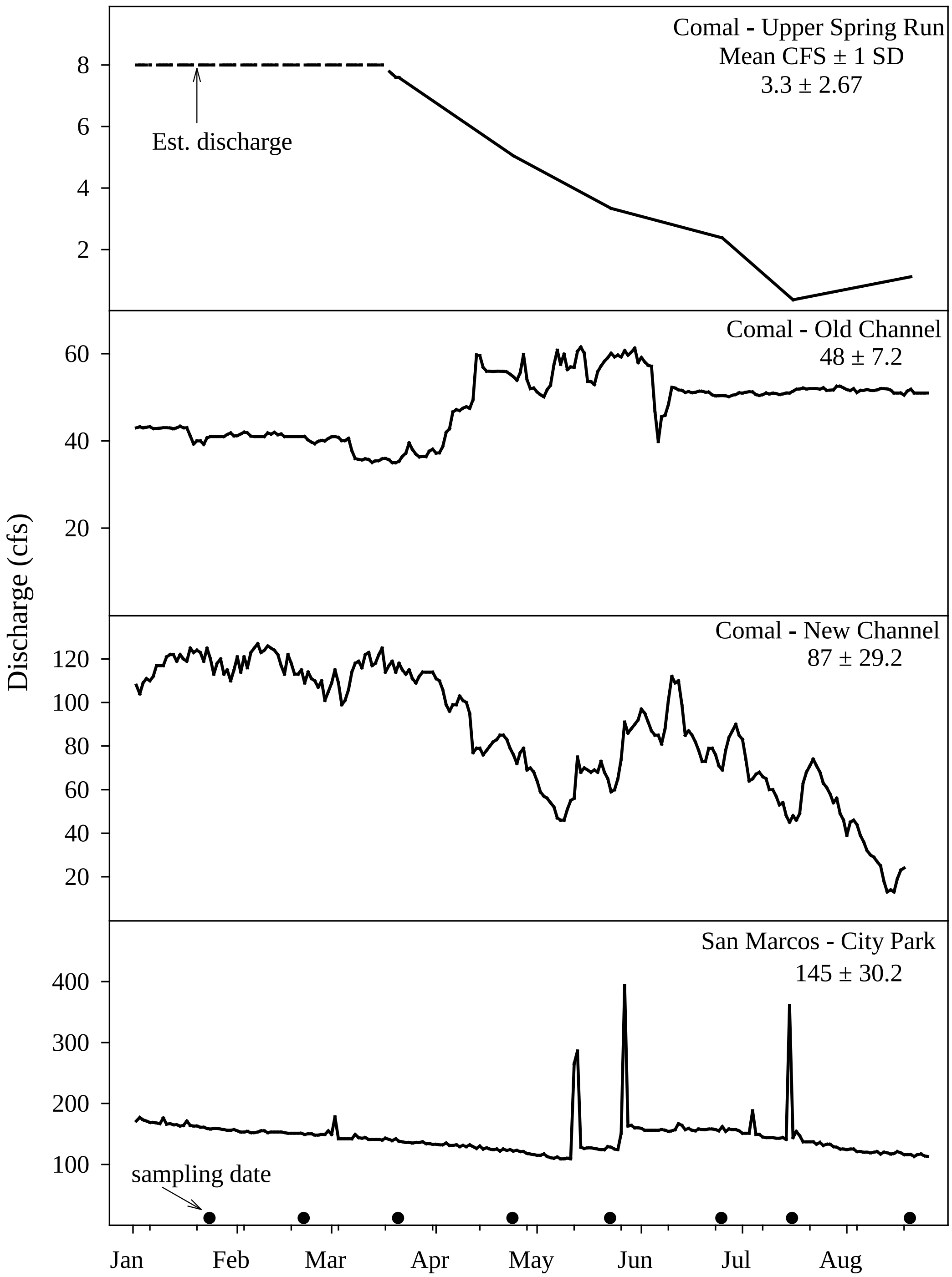

Figure 6. **Hydrograph (mean daily discharge) for each study site and collection dates of Fountain Darters taken. Comal River-Upper Spring Run hydrograph was calculated via transect method on the day of sampling.**

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 (µS*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

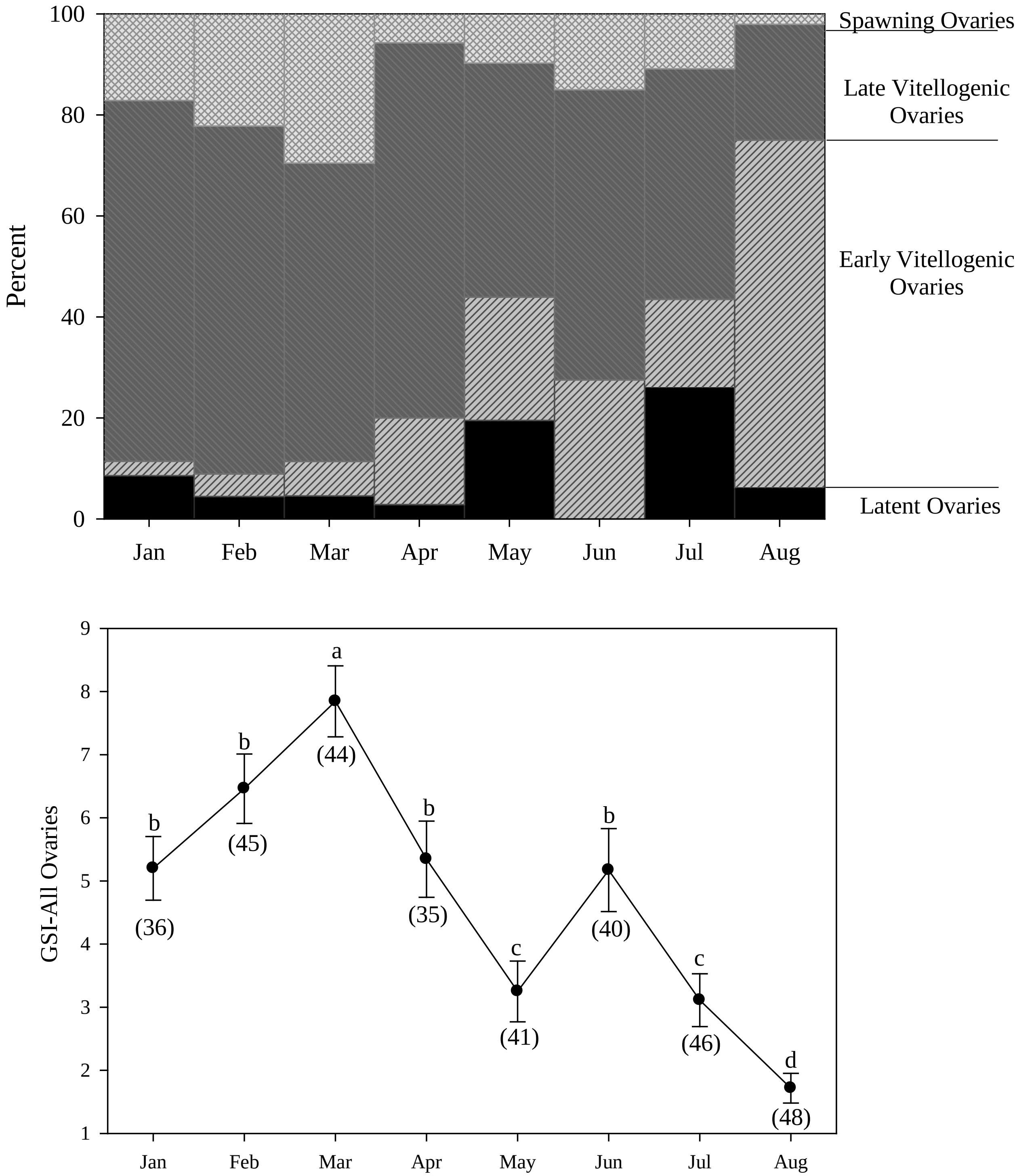

Figure 7. **Ovarian stages for all Fountain Darters (N = 355, upper panel) and gonadosomatic indices for all female Fountain Darters (lower panel) taken from four sites on San Marcos River and Comal River. Parenthetical numbers represent N of fishes per month. Same lower case letter represent no significant difference between months.**

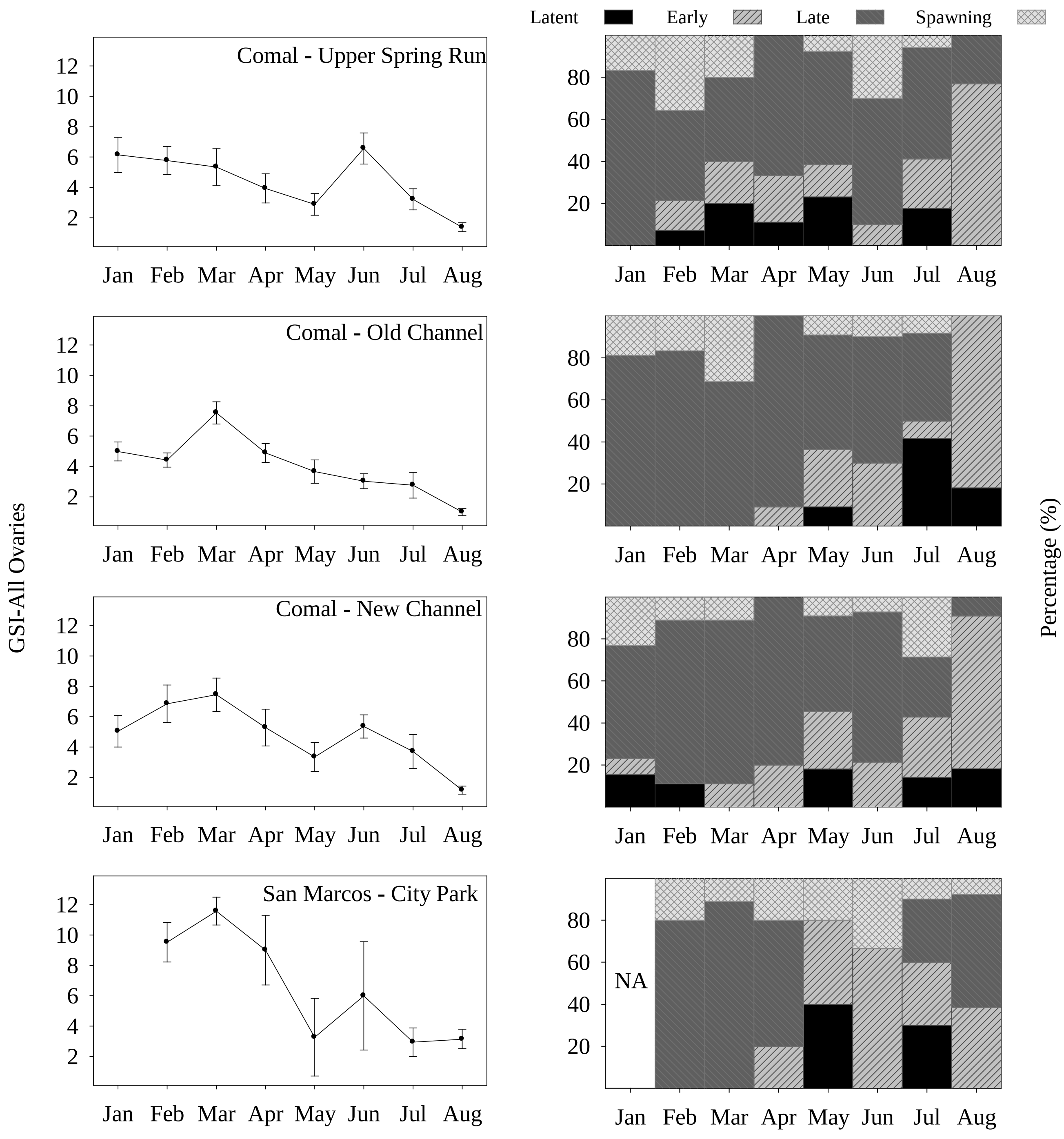

Figure 8. **Gonadosomatic indices for fishes >24 mm in TL (size of sexual maturity) among four sites (left panel) and associated ovarian stages (right panel).**

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).

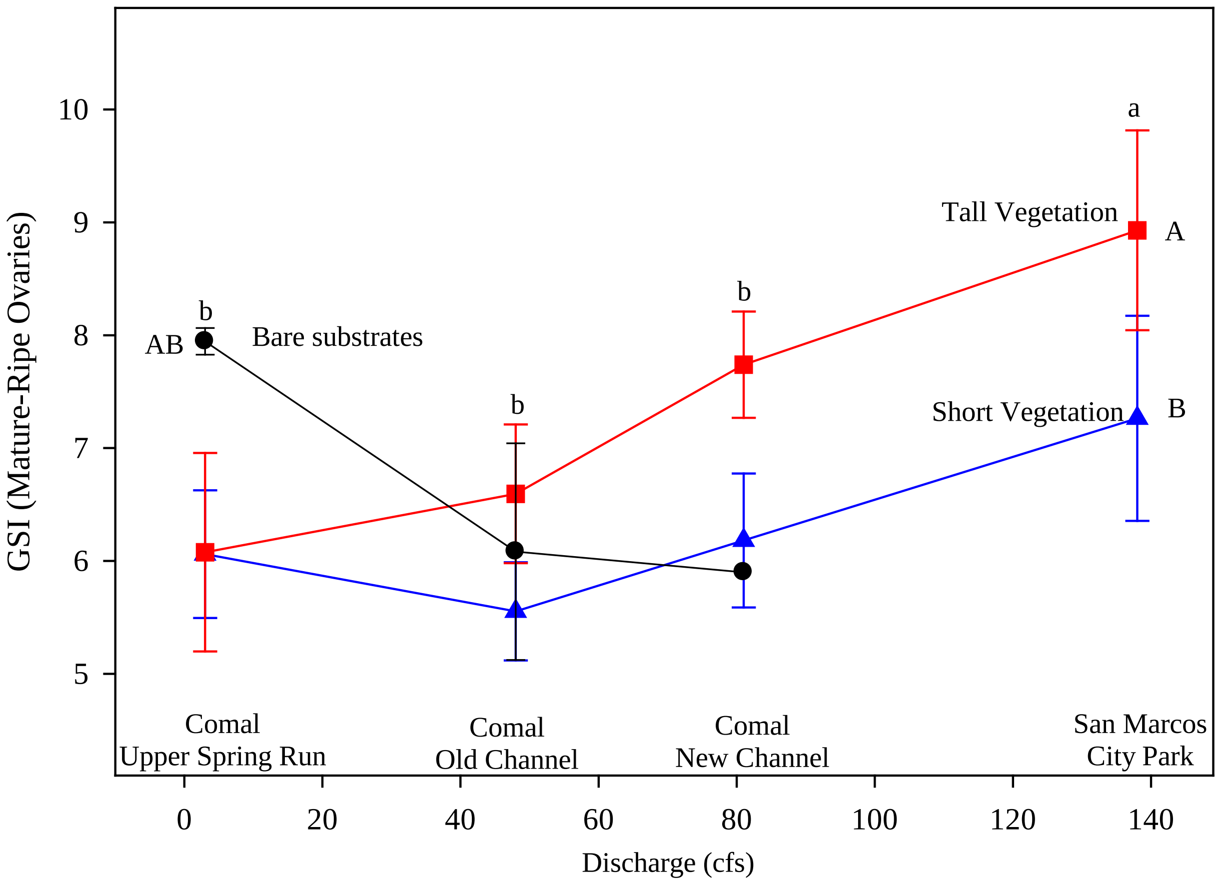

Figure 9. **Gonadosomatic indices for fishes with Mature-Ripe Ovaries by site and mean daily discharge and by vegetation type. Same upper case letters represent no significant vegetation type effect. Same lower case letters represent no significant site effect.**

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.

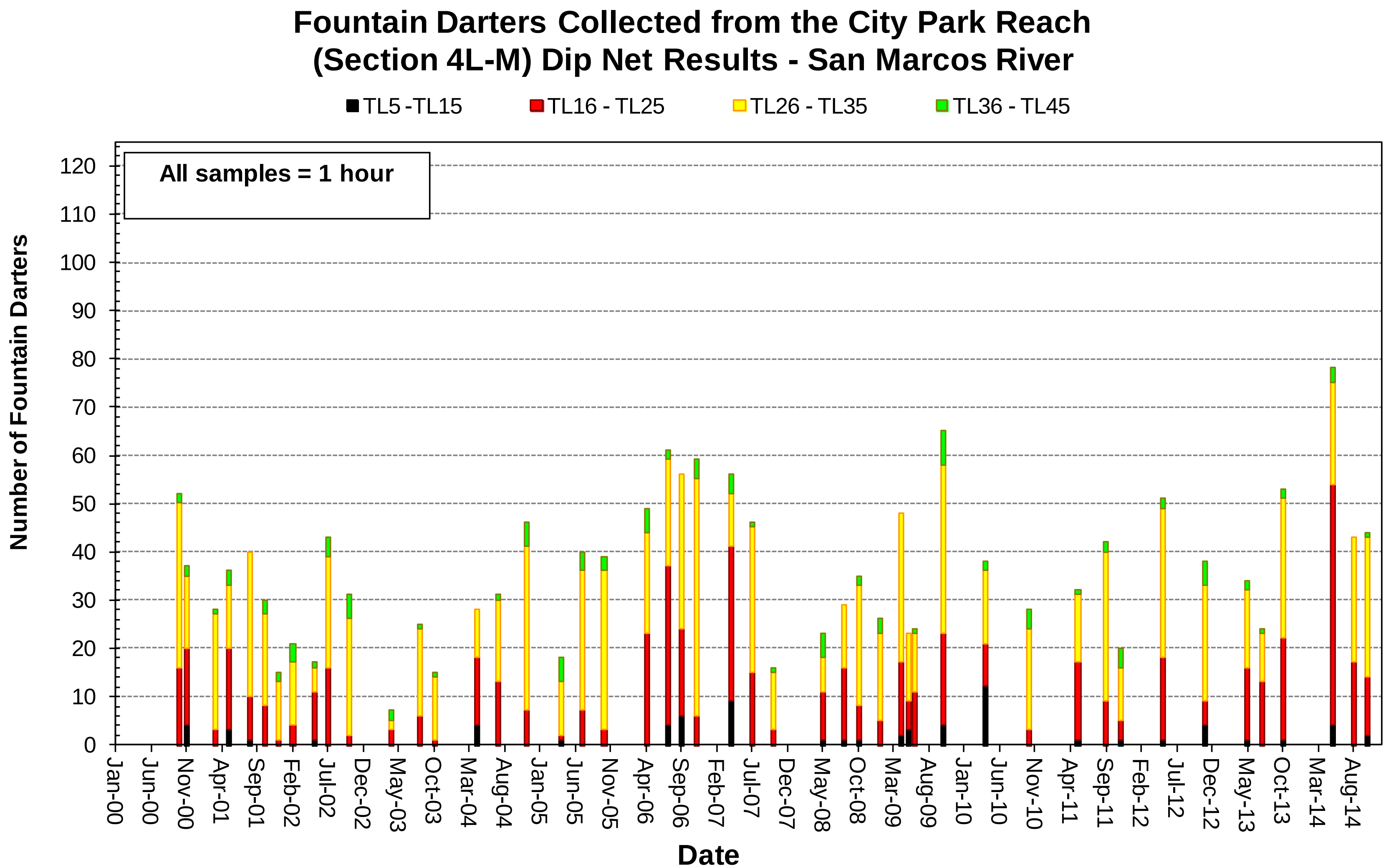

Figure 10. **Fountain Darter dip net results over time from City Park of the San Marcos River.**

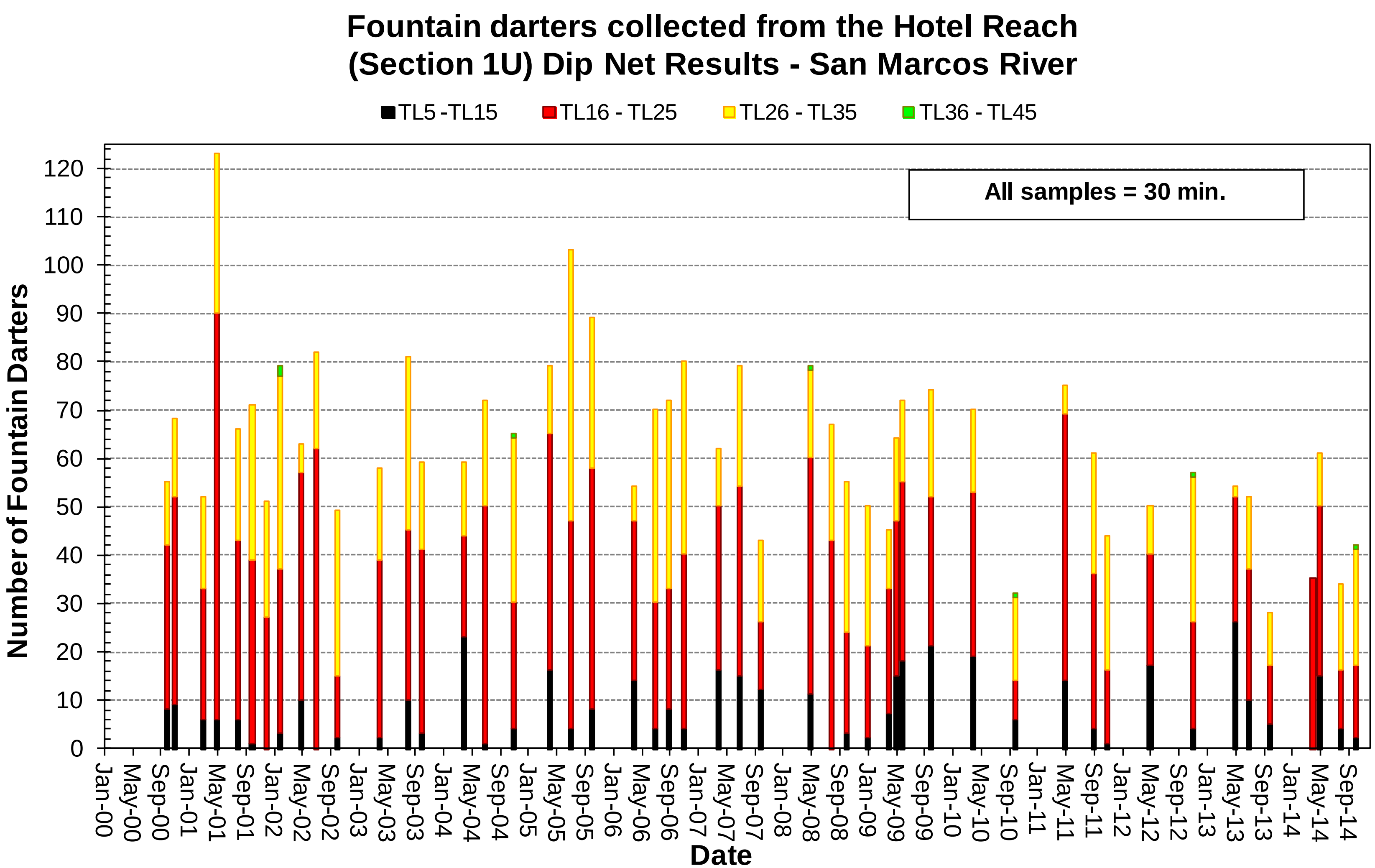

Figure 11. **Fountain Darter dip net results over time from Spring Lake of the San Marcos River.**

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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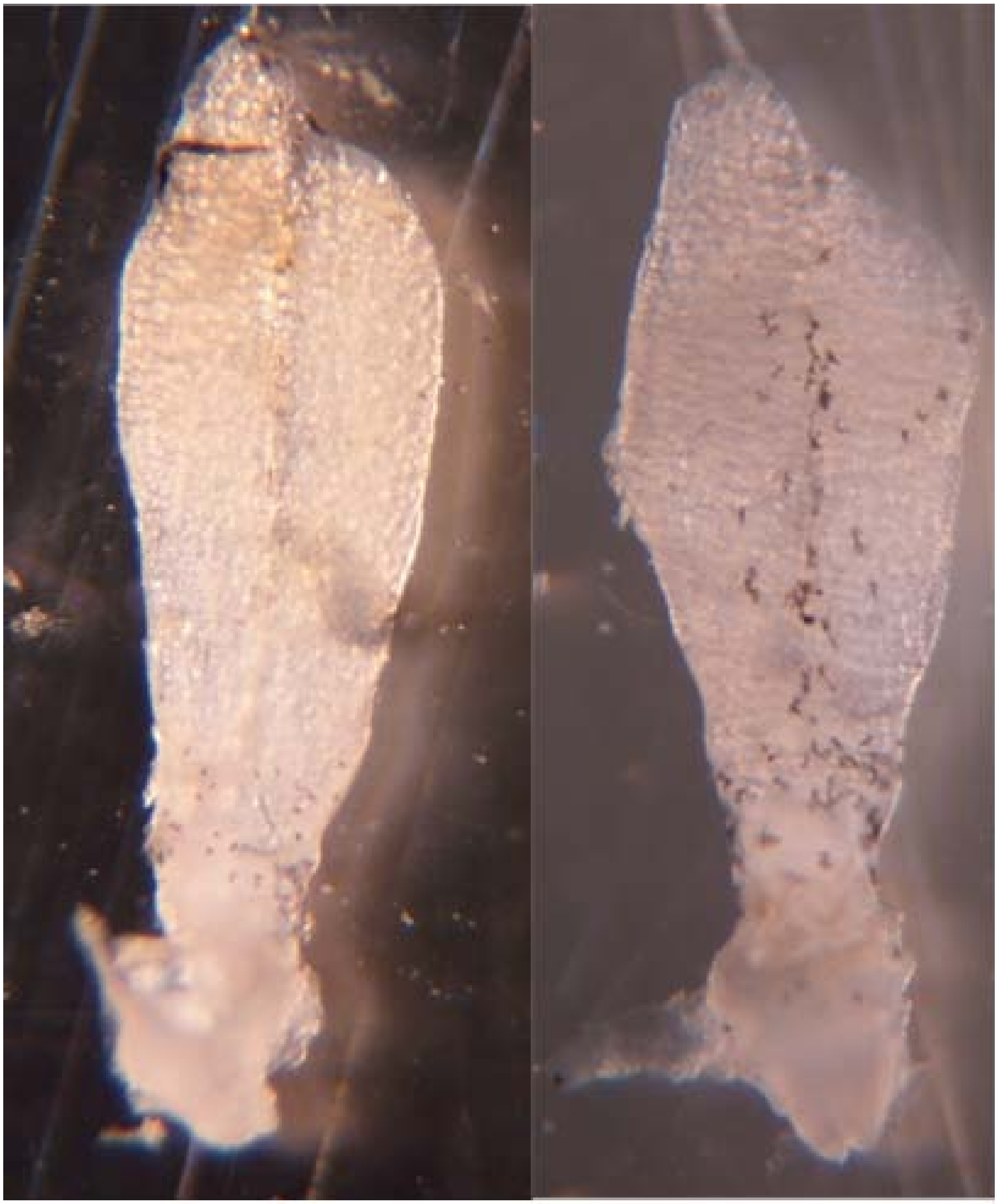
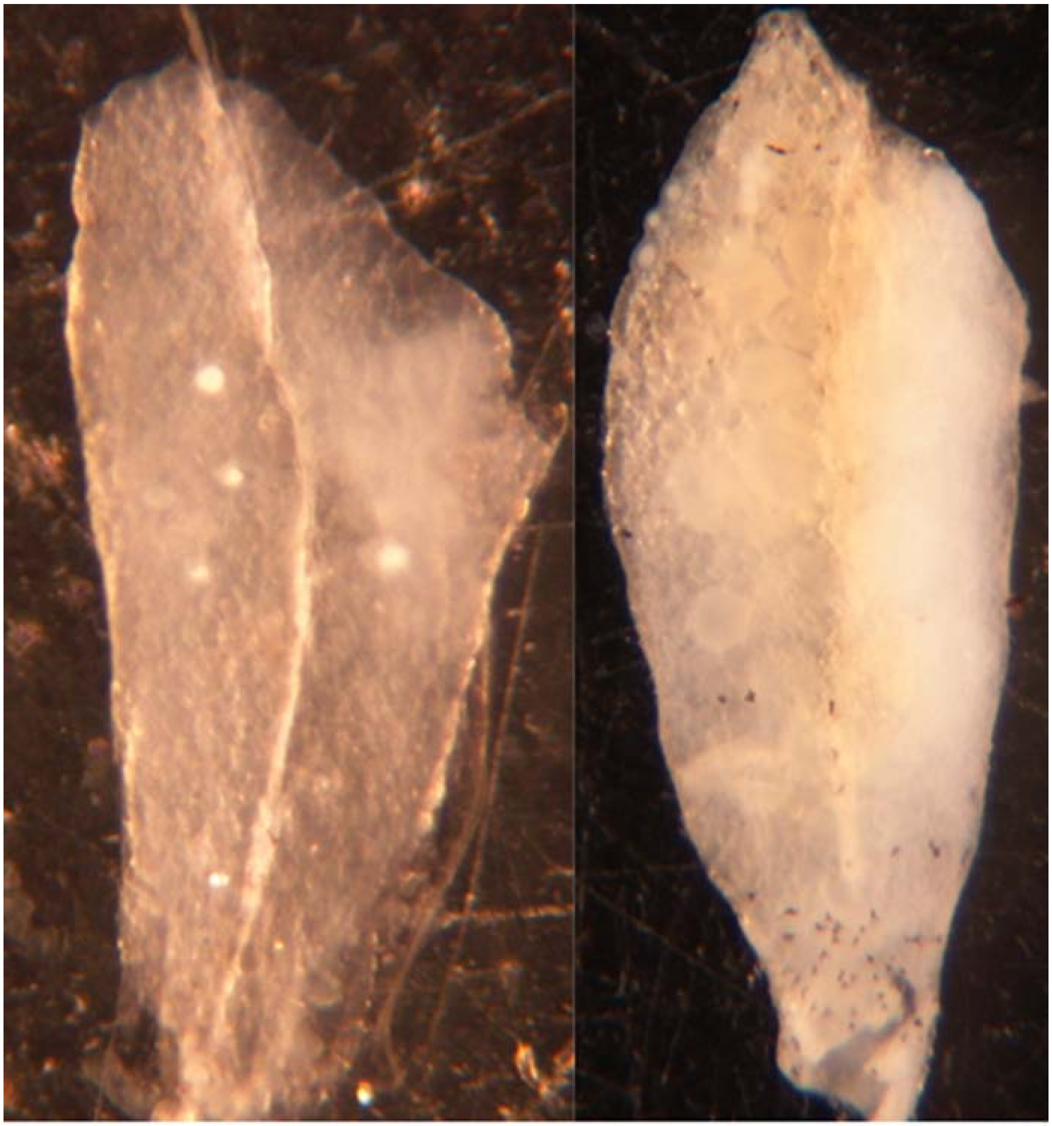
Appendix 1. Stages of ovarian and oocyte development in Fountain Darters taken from January through August 2014 among four sites on the San Marcos and Comal rivers.

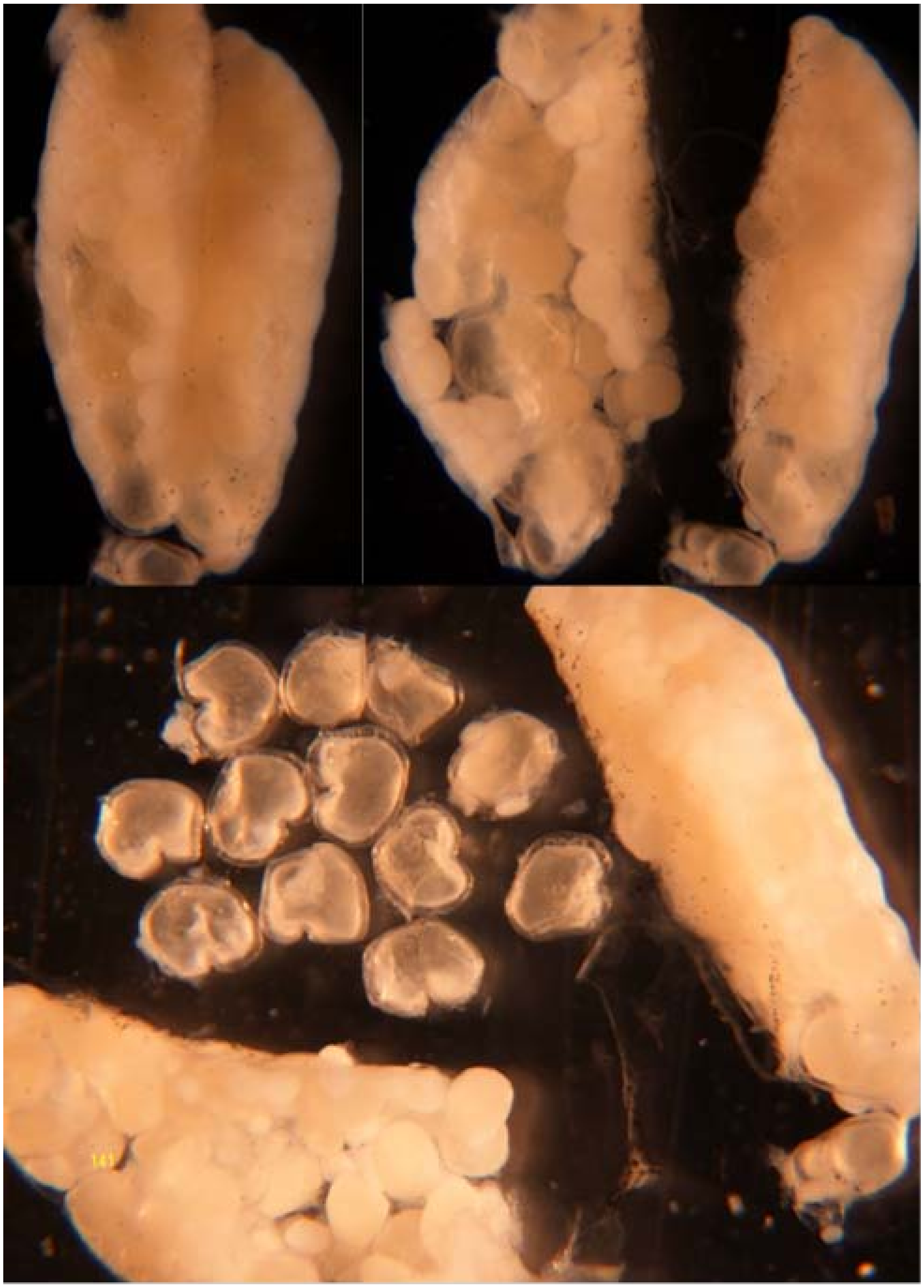


Pre-vitellogenic Ovaries; latent condition, semi translucent. Beginning stage of female Fountain Darter sexual maturity.



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

Prepared for:

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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.

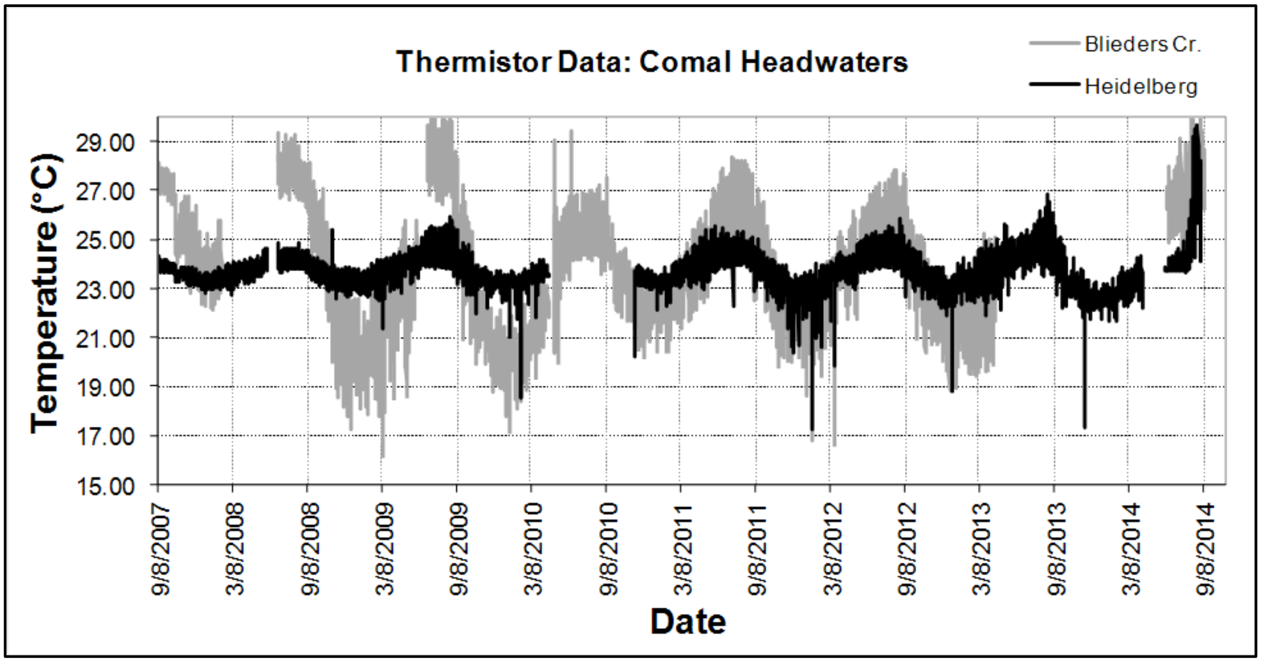

Figure 2. **Water temperature data from Blieders Creek and the Upper Spring Run reach (Heidelberg).**

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

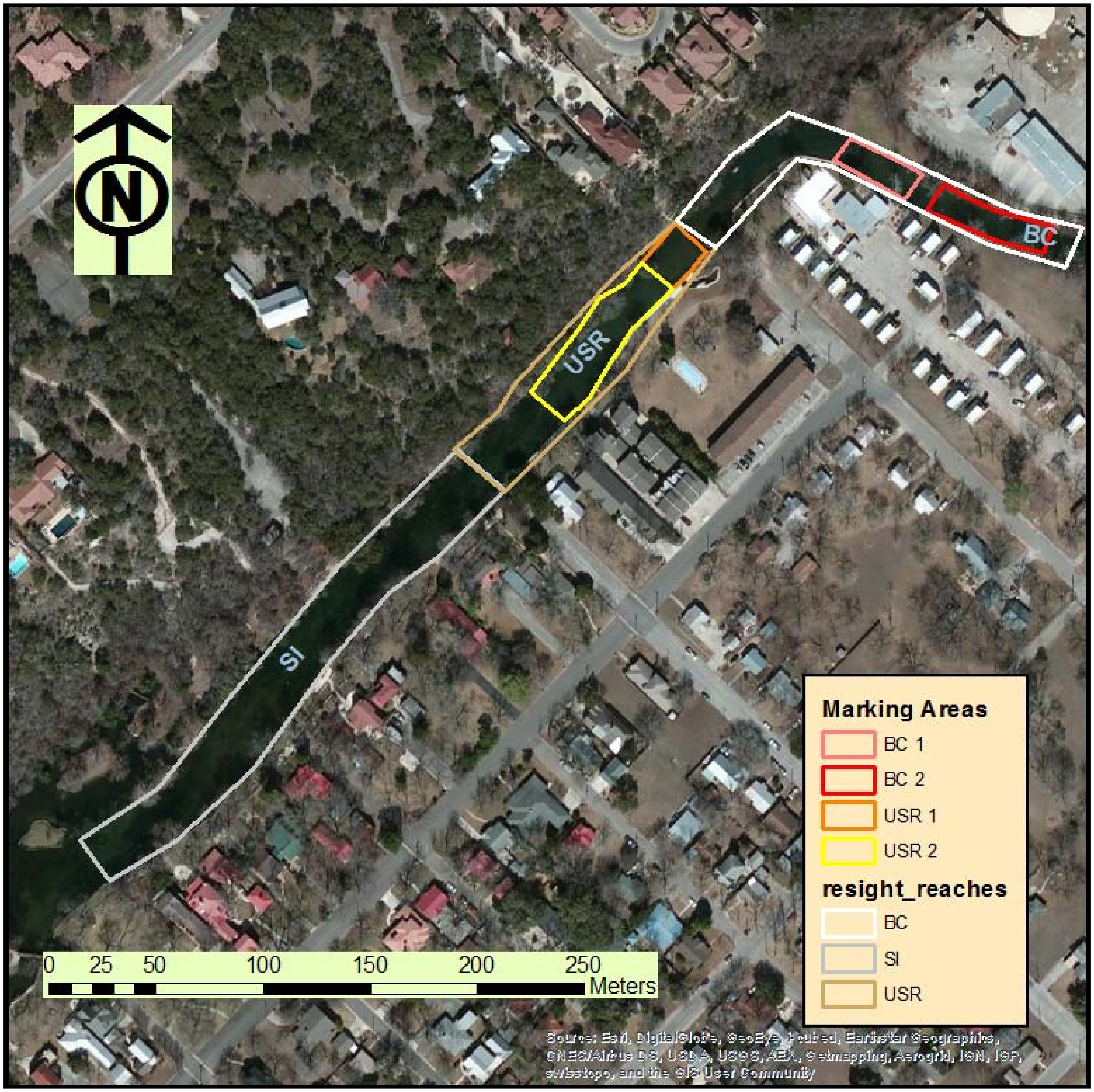

Figure 4. **Areas where fountain darters were collected and marked (Marking Areas) and areas where visual searches were conducted (resight_reaches).**

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.

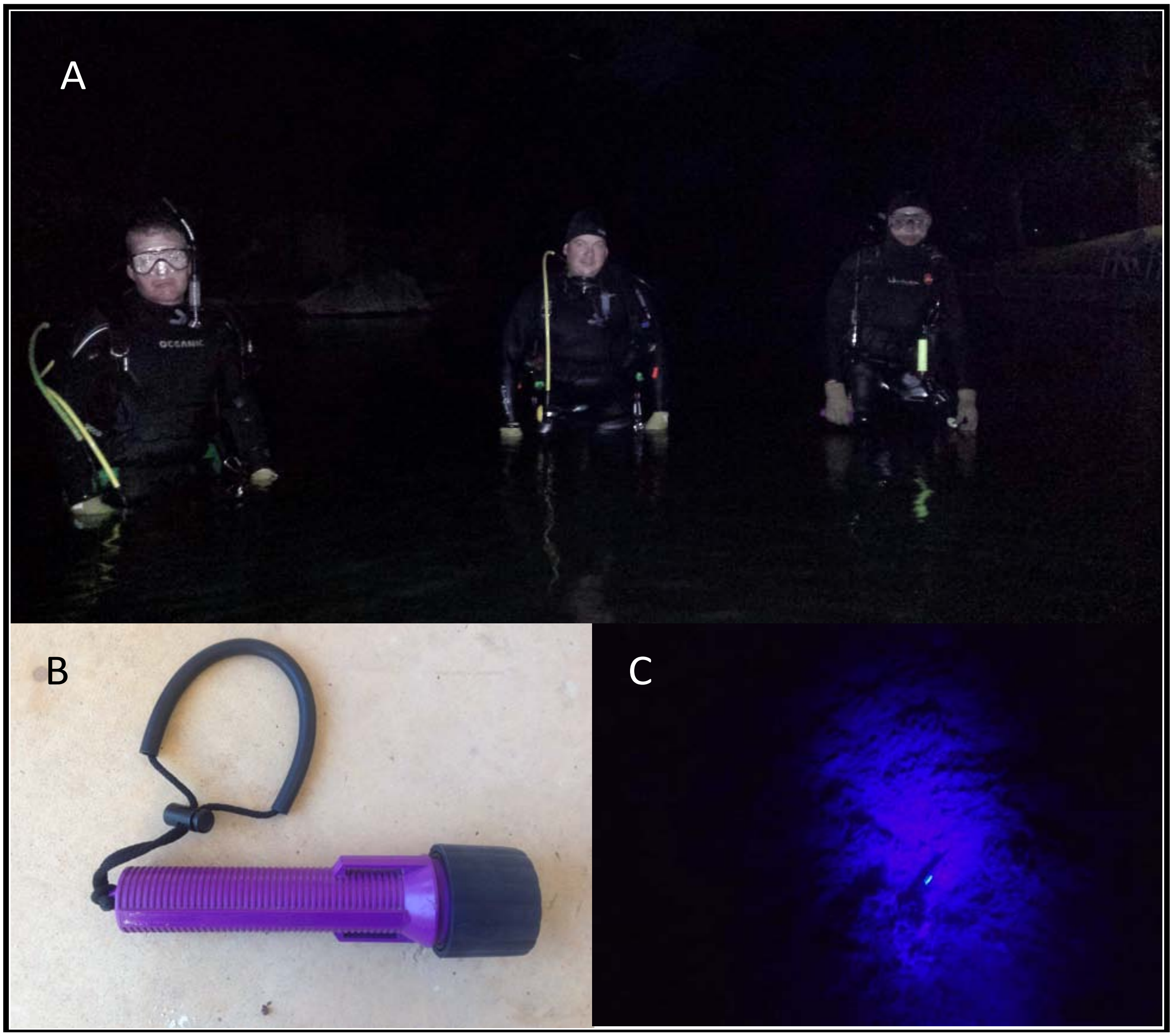

Figure 9. **BIO-WEST divers preparing for night relocation surveys (A) using VI lights (B) to relocate fountain darters with fluorescent visual implant elastomer (VIE) tags (C).**

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.**

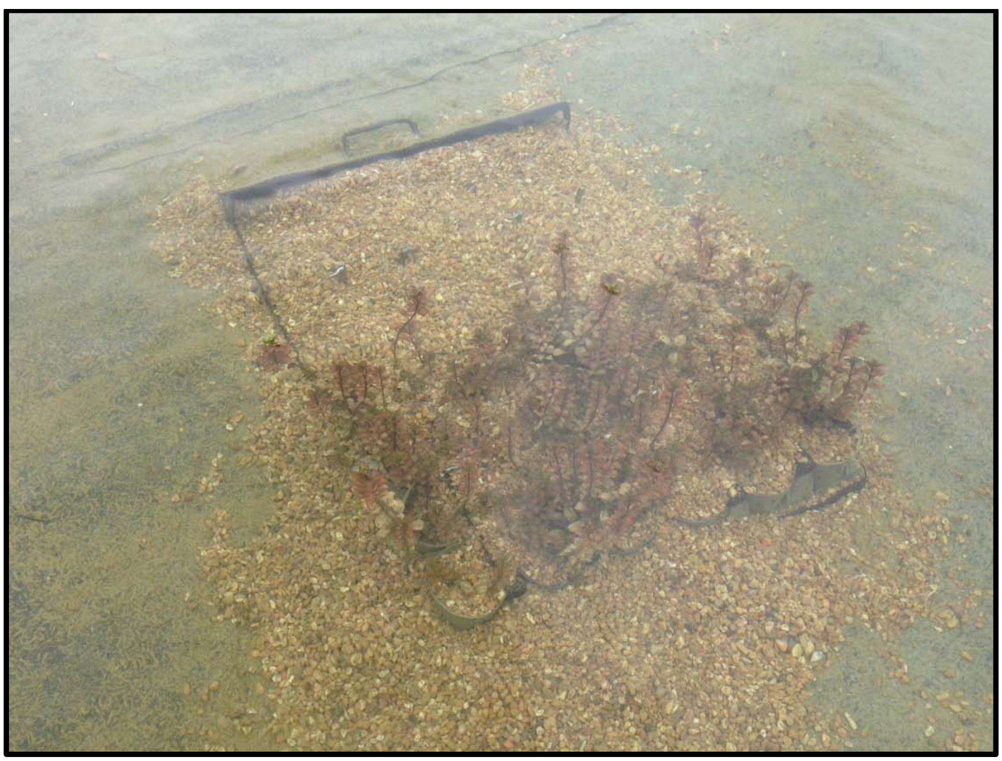

Figure 11. **Pea gravel being applied to mask MUPPT structure and homogenize available habitat patches for experimental trials.**

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.

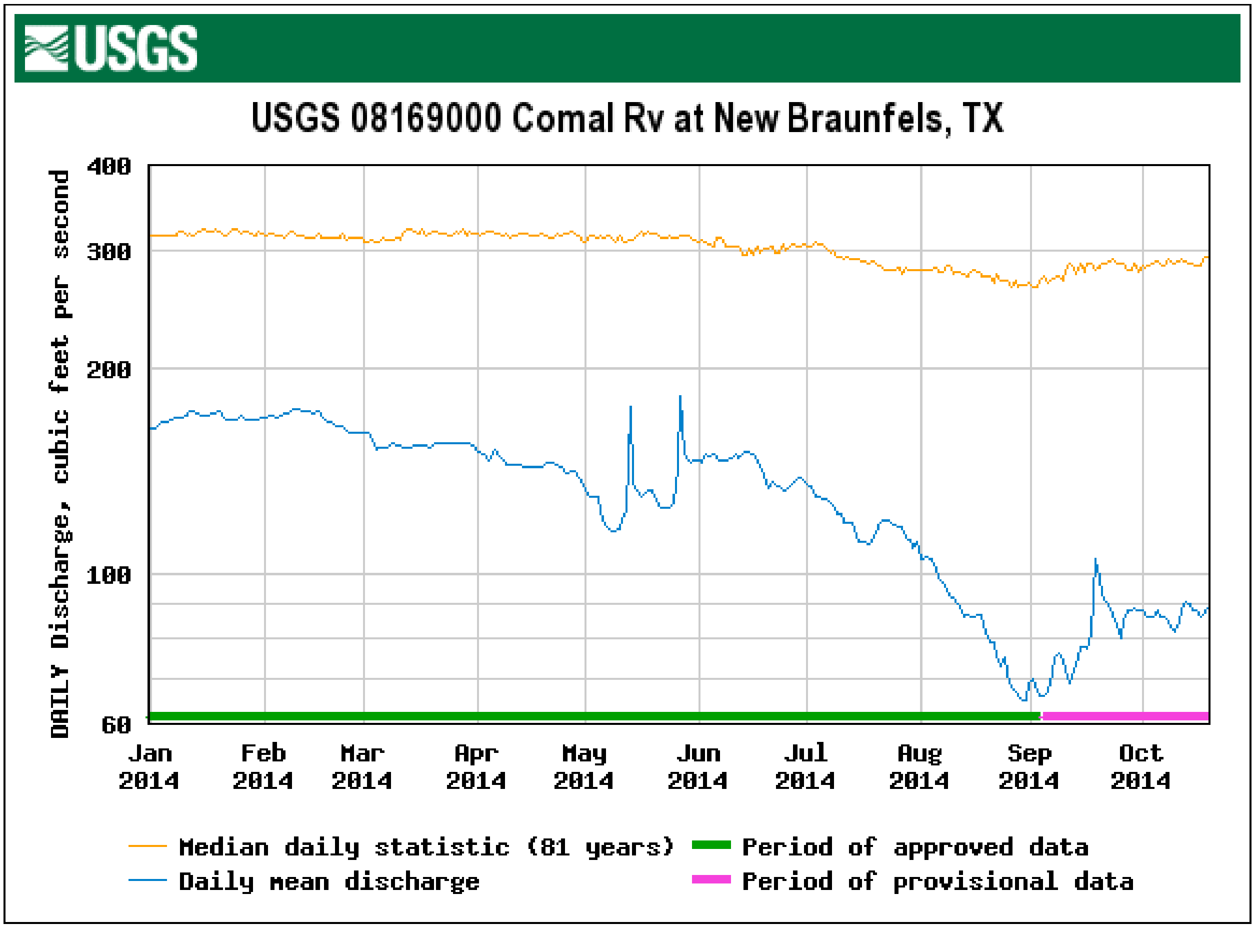

Figure 13. **Mean daily discharge (cfs) from the USGS gauge (#08169000) on the Comal River at New Braunfels, Texas from January 1–October 19, 2014.**

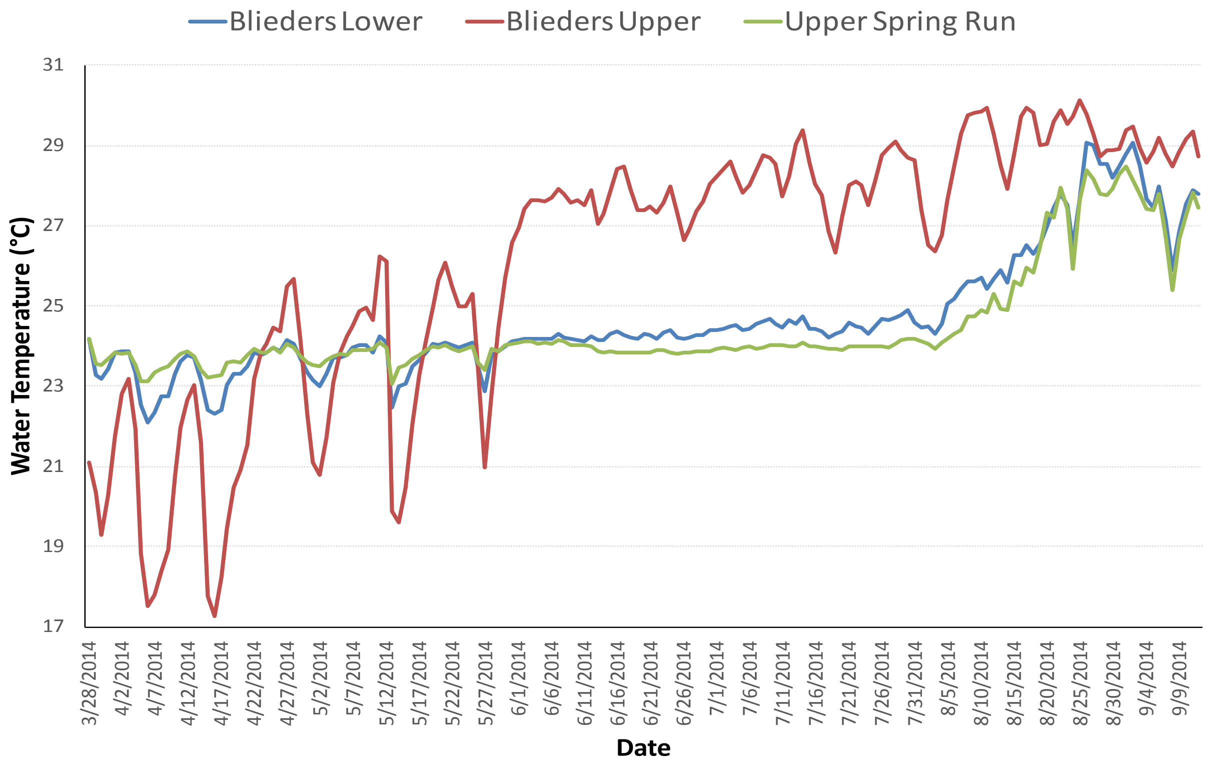

Figure 14. **Daily average water temperature taken from three locations within the study area.**

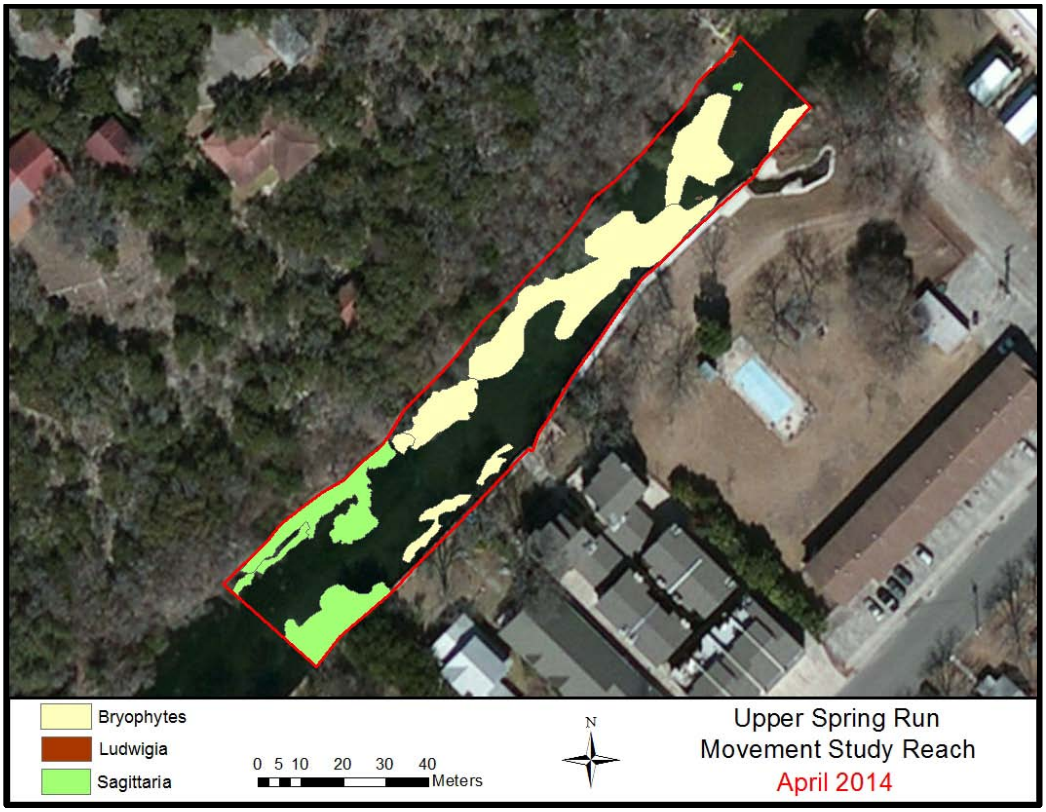

Figure 15. Aquatic vegetation present in the Upper Spring Run reach during April 2014.

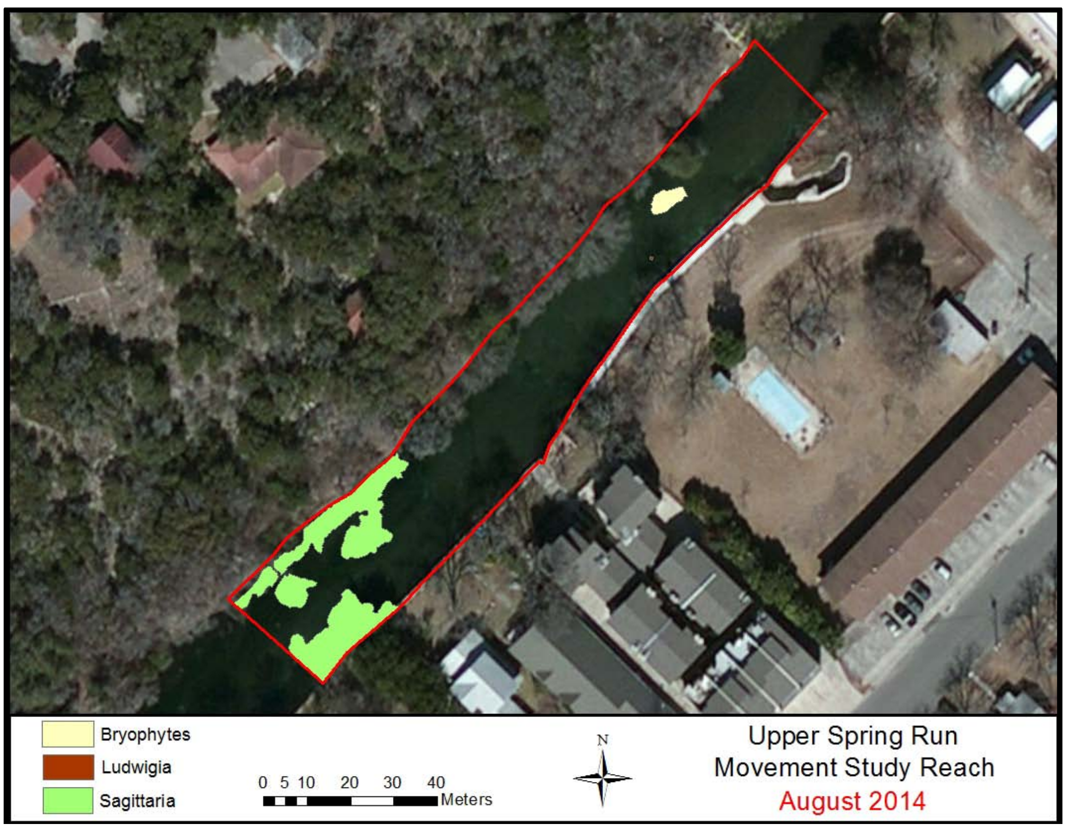

Figure 16. Aquatic vegetation present in the Upper Spring Run reach during August 2014.

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.

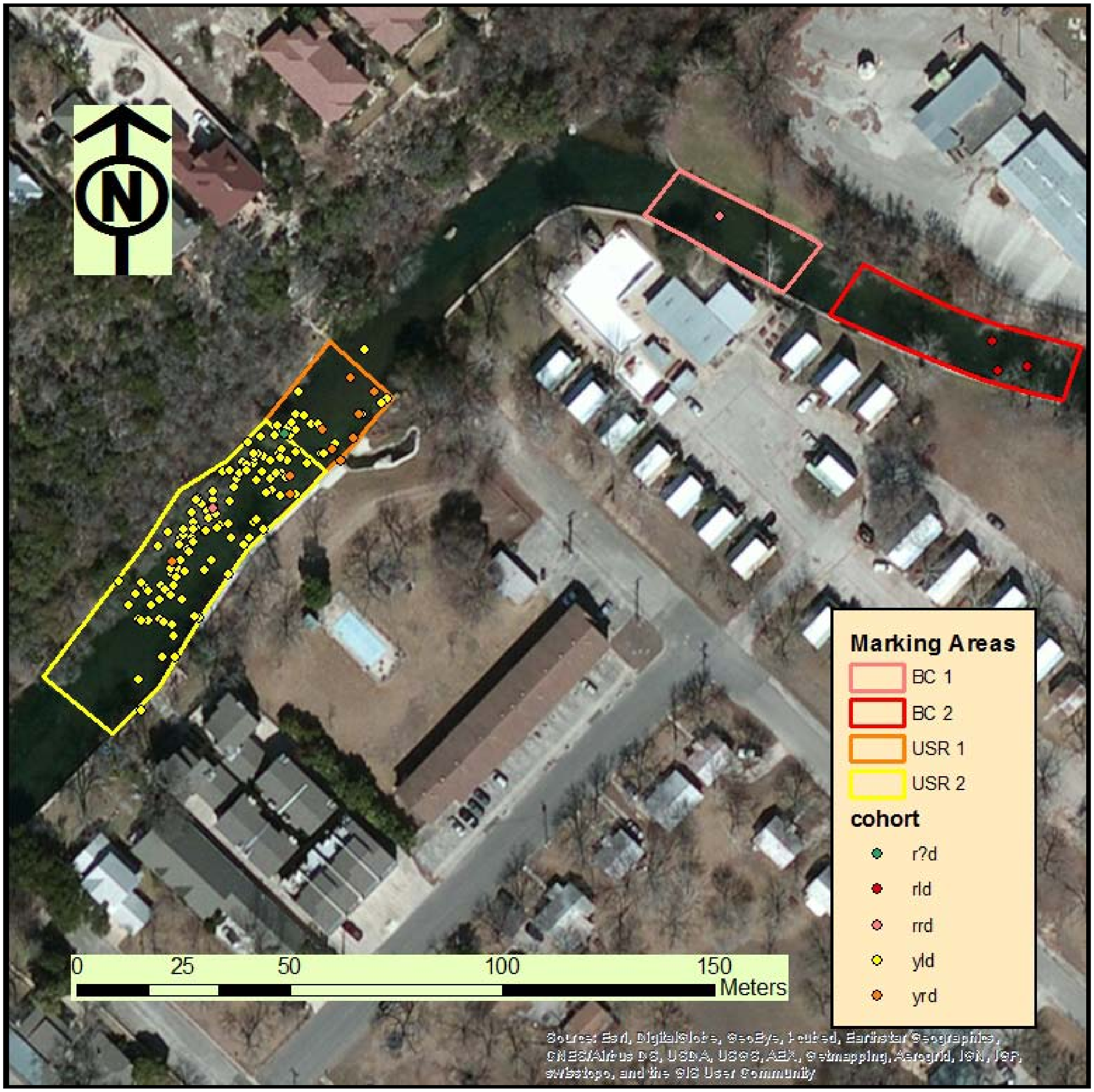

Figure 17. **Fountain darters relocated over the course of the study period. Relocation points are represented by the same color as the area in which fountain darters were originally marked.**

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:

$$E = 1 - \frac{\sum_{t=1}^{T}\left(Q_o^t - Q_m^t\right)^2}{\sum_{t=1}^{T}\left(Q_o^t - \overline{Q_o}\right)^2} \qquad (9)$$

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

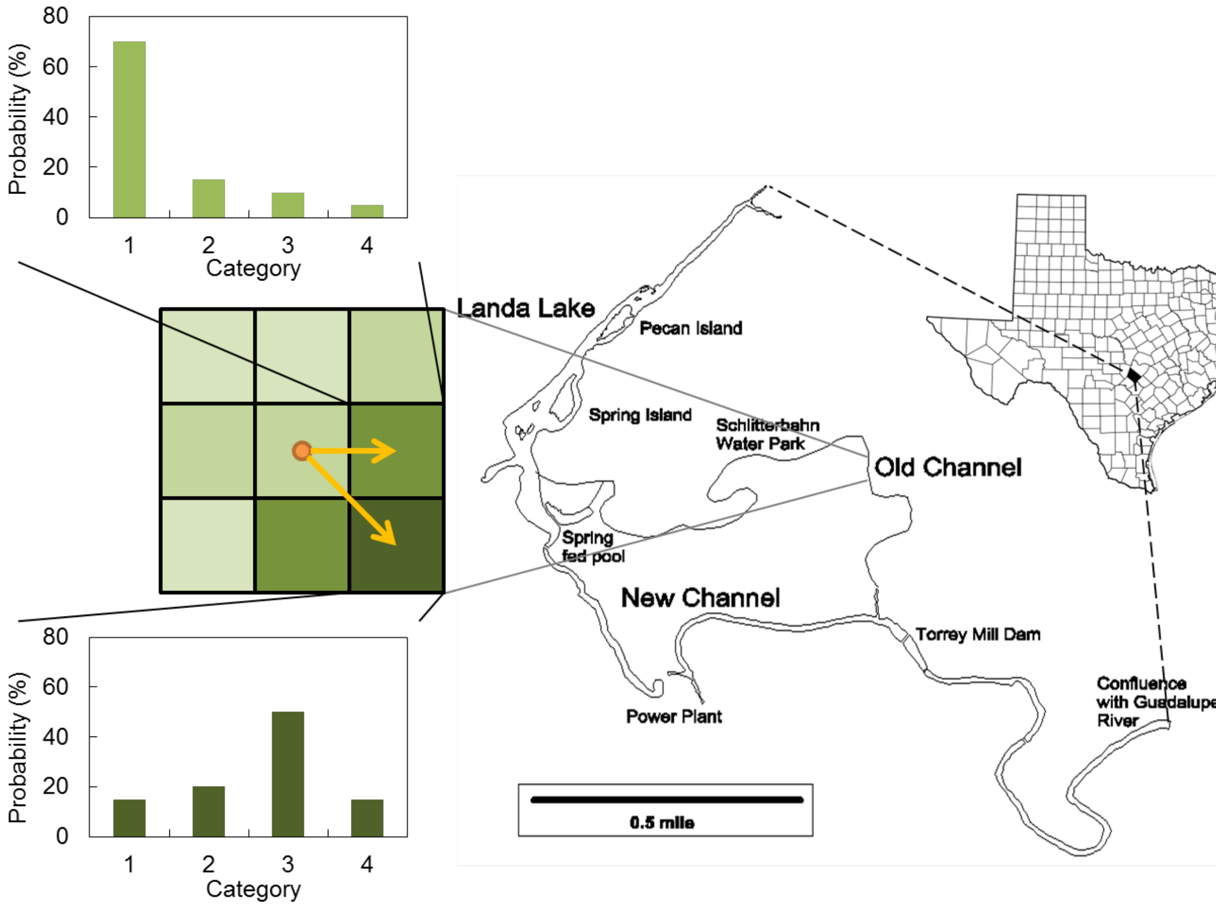

80
60
40
20
0
Probability (%)
1
2
3
4
Category
Landa Lake
Pecan Island
Spring Island
Schlitterbahn Water Park
Old Channel
Spring fed pool
New Channel
Torrey Mill Dam
Power Plant
Confluence with Guadalupe River
0.5 mile
80
60
40
20
0
Probability (%)
1
2
3
4
Category

Figure 1

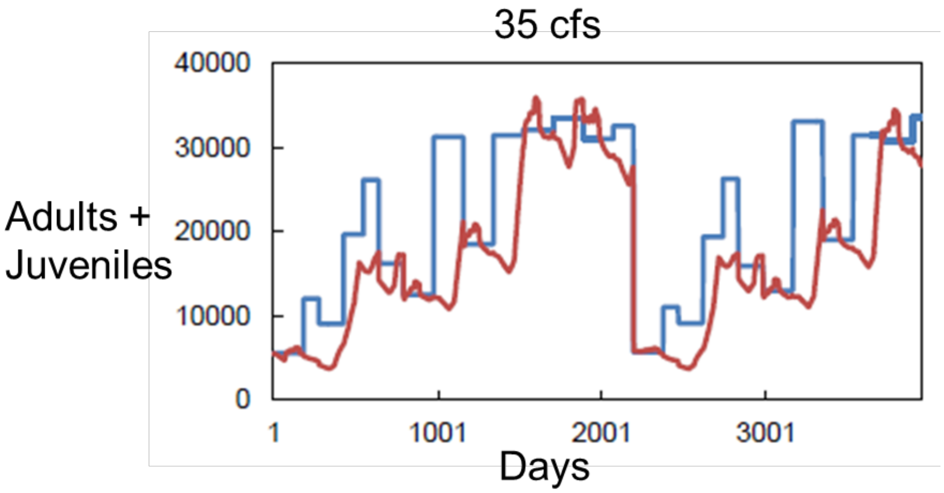
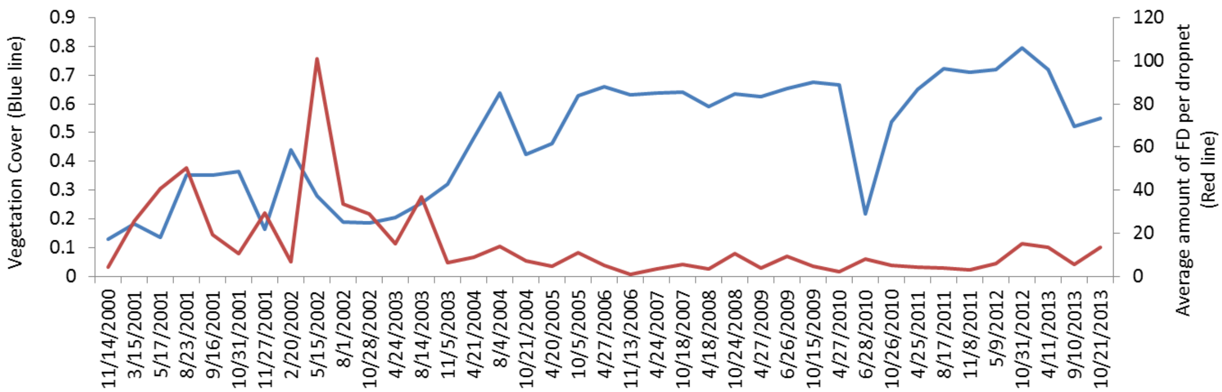
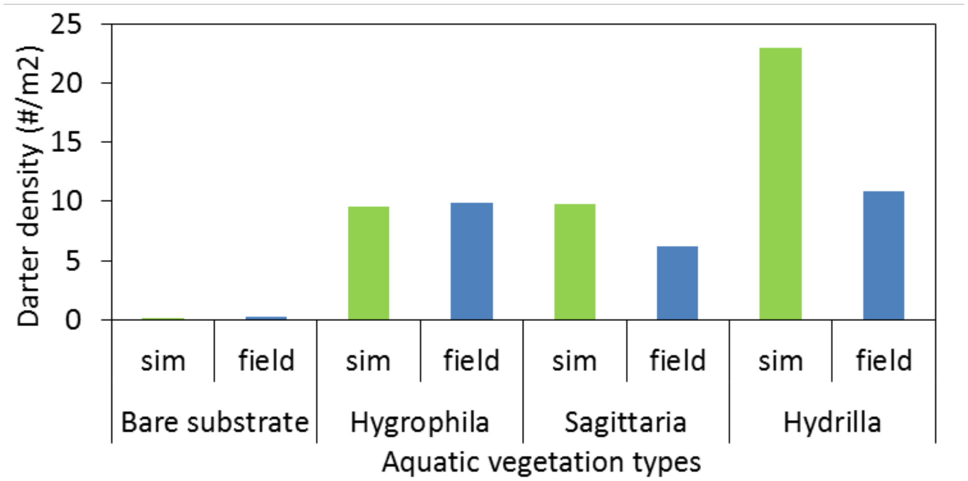

35 cfs
Adults +
Juveniles
40000
30000
20000
10000
0
1
1001
2001
3001
Days

Figure 2



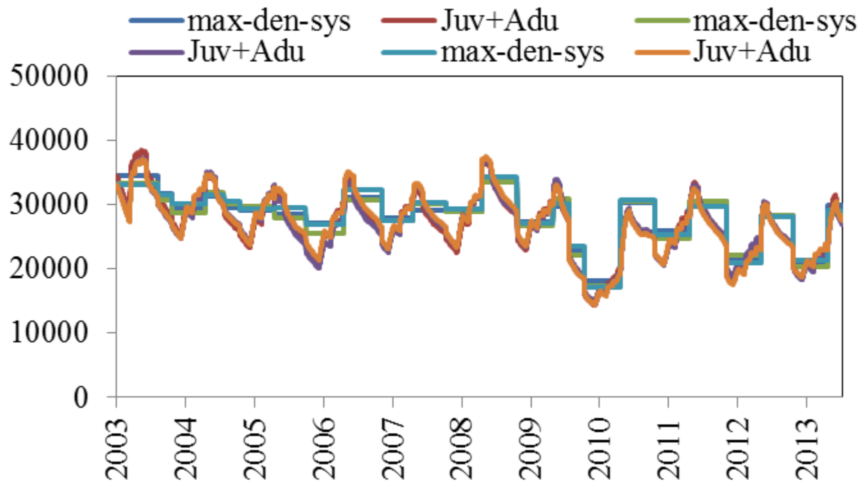
Vegetation Cover (Blue line)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
120
100
80
60
40
20
0
Average amount of FD per dropnet (Red line)
11/14/2000
3/15/2001
5/17/2001
8/23/2001
9/16/2001
10/31/2001
11/27/2001
2/20/2002
5/15/2002
8/1/2002
10/28/2002
4/24/2003
8/14/2003
11/5/2003
4/21/2004
8/4/2004
10/21/2004
4/20/2005
10/5/2005
4/27/2006
11/13/2006
4/24/2007
10/18/2007
4/18/2008
10/24/2008
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

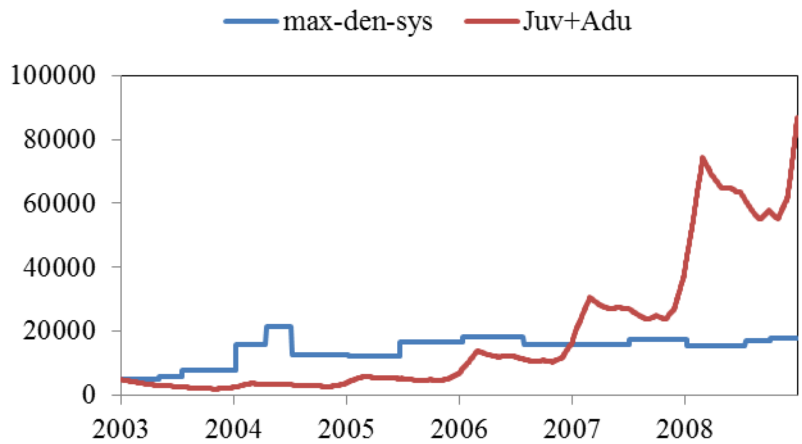
Figure 4



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)


max-den-sys
Juv+Adu
100000
80000
60000
40000
20000
0
2003
2004
2005
2006
2007
2008

(b)

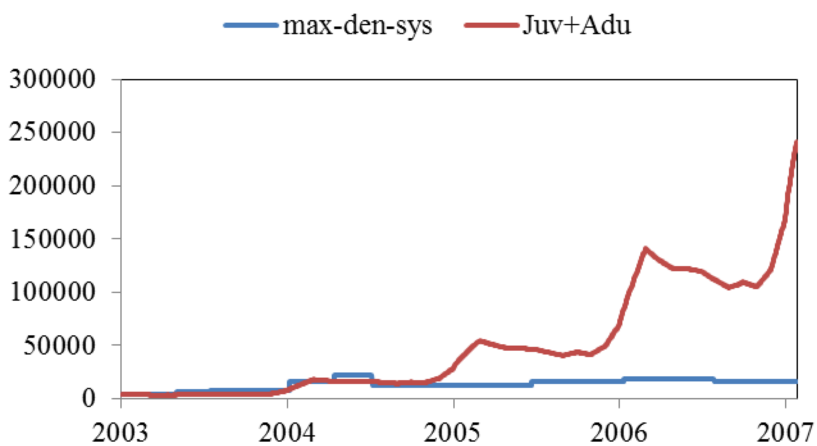
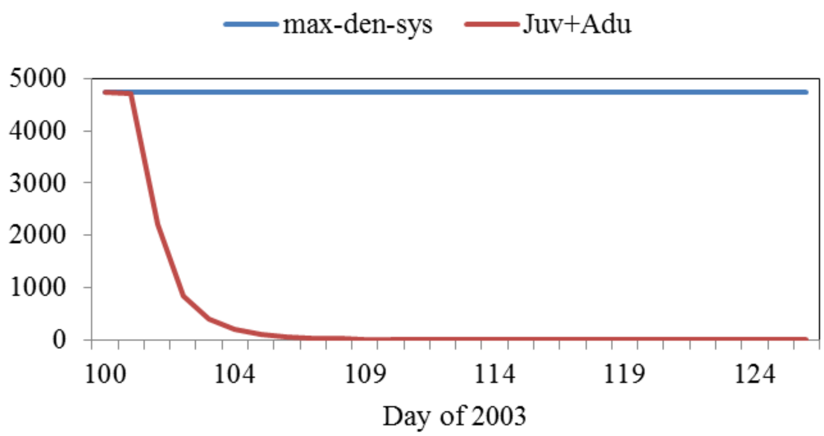
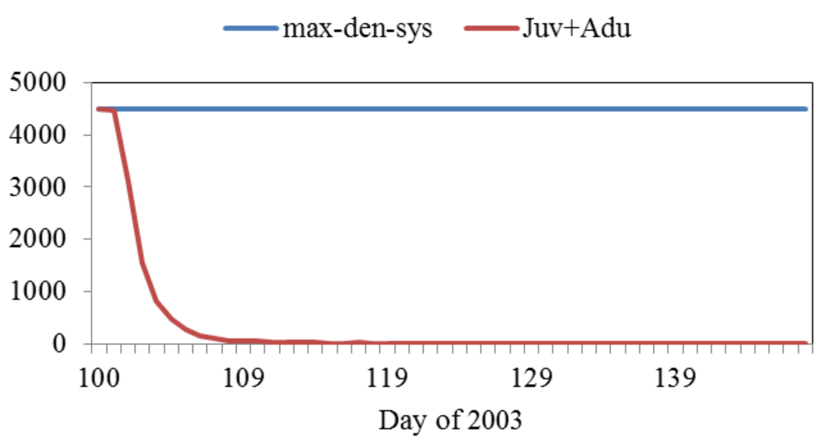

max-den-sys
Juv+Adu
300000
250000
200000
150000
100000
50000
0
2003
2004
2005
2006
2007

Figure 6

(c)



(d)



(e)

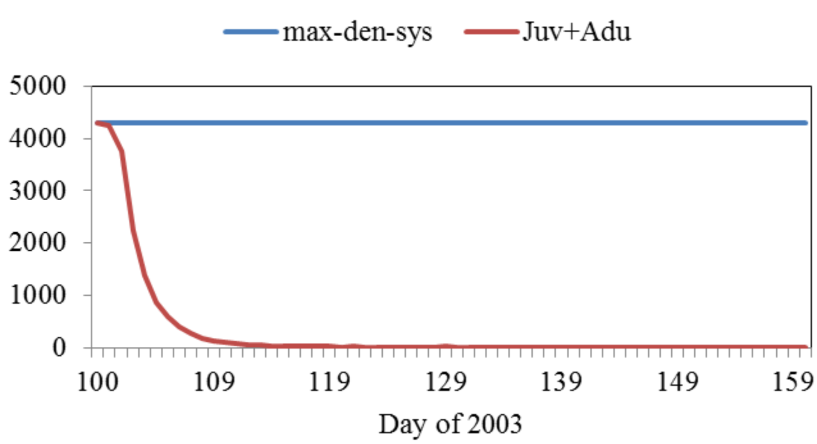
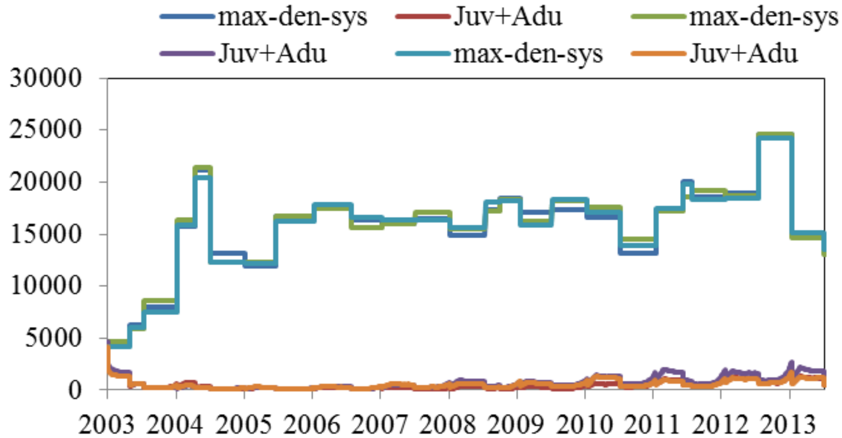
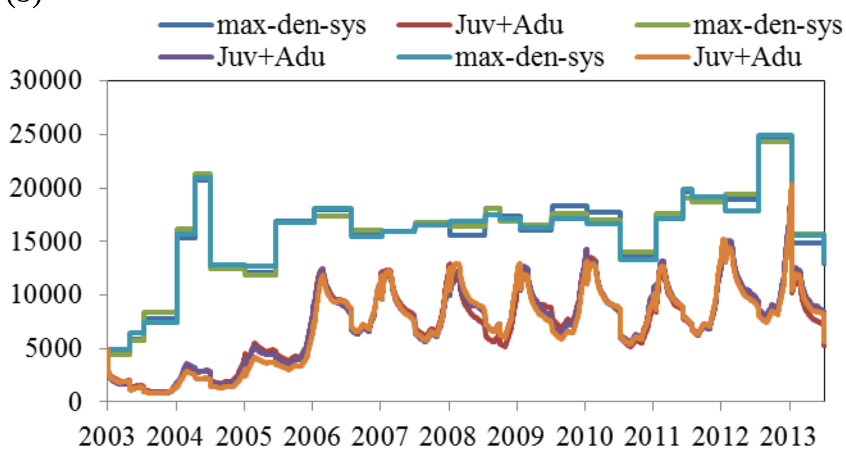


Figure 6 Cont.

(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)

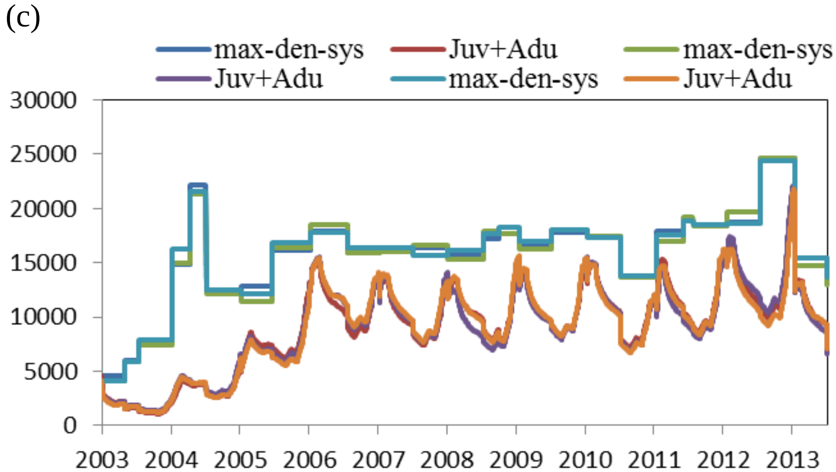
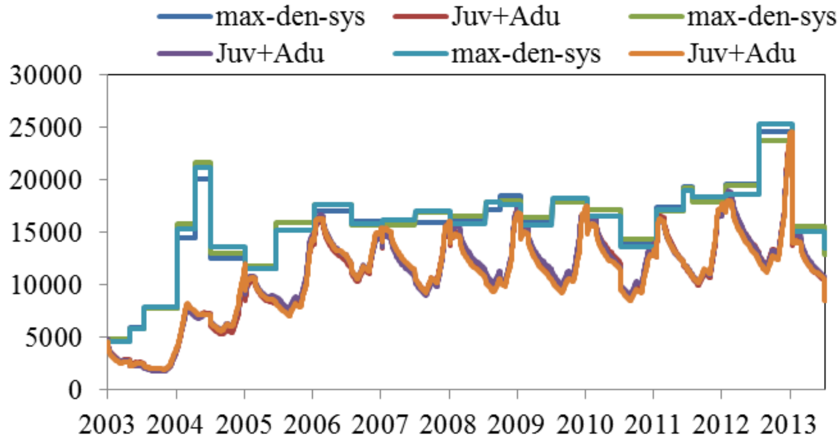

max-den-sys
Juv+Adu
max-den-sys
Juv+Adu
max-den-sys
Juv+Adu
30000
25000
20000
15000
10000
5000
0
2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013

Figure 7

(d)



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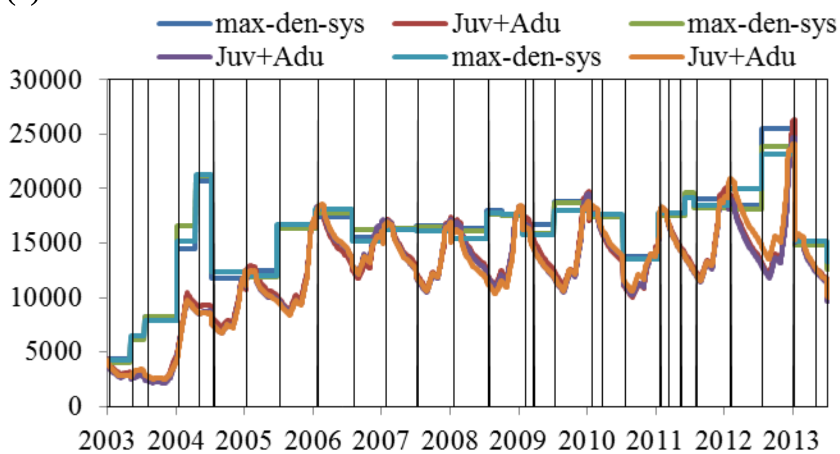


(f)

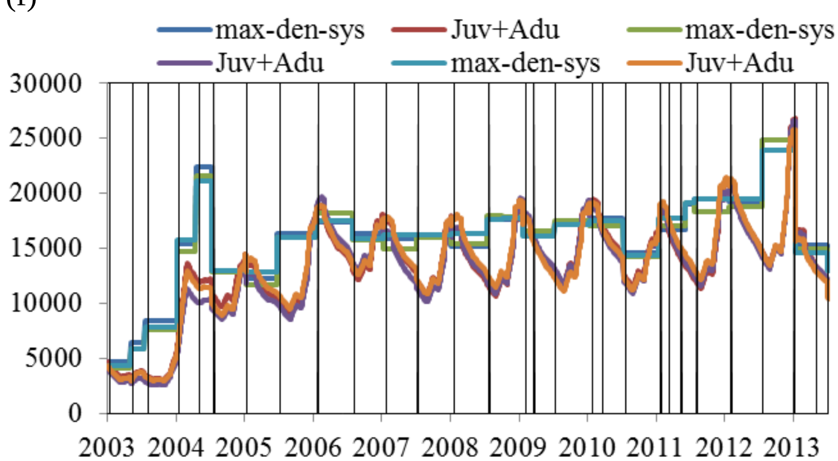
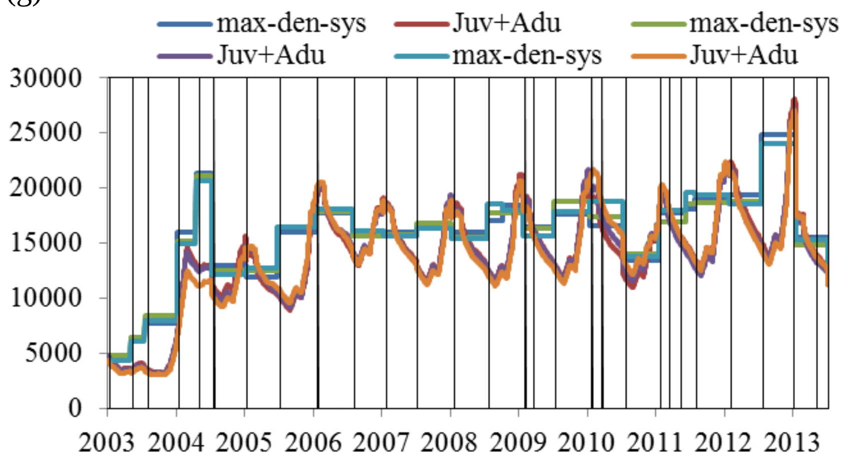
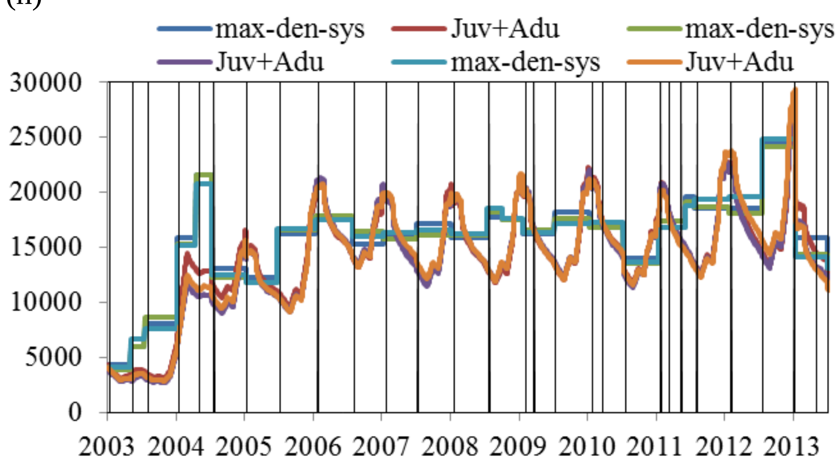


Figure 7 Cont.

(g)



(h)



(i)

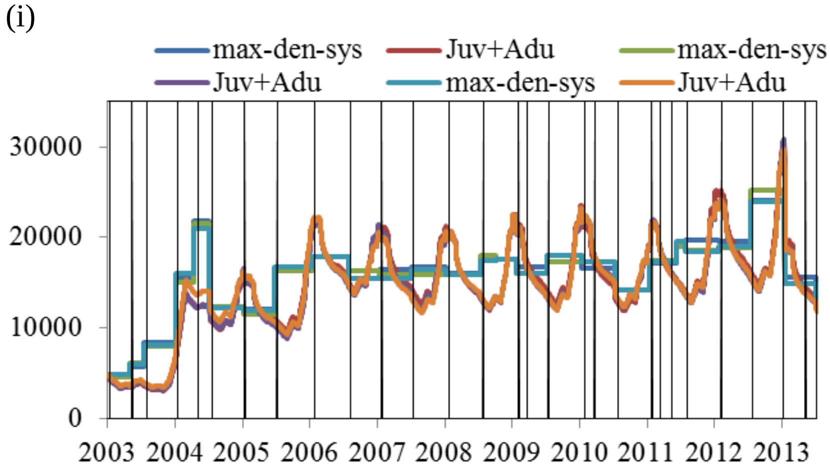
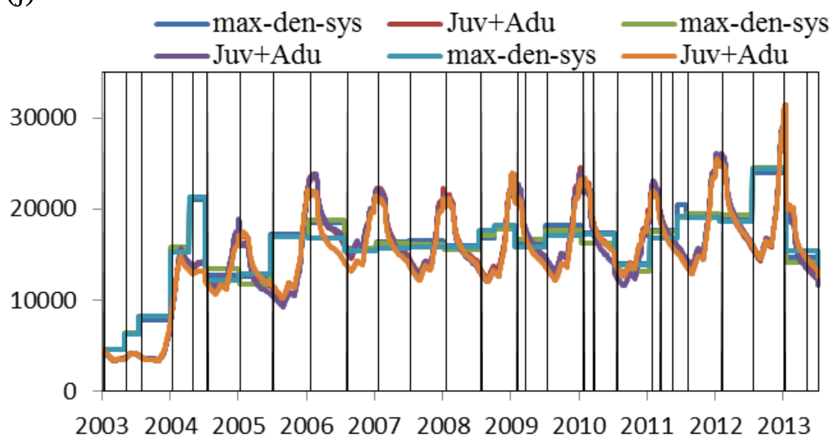


Figure 7 Cont.

(j)



(k)

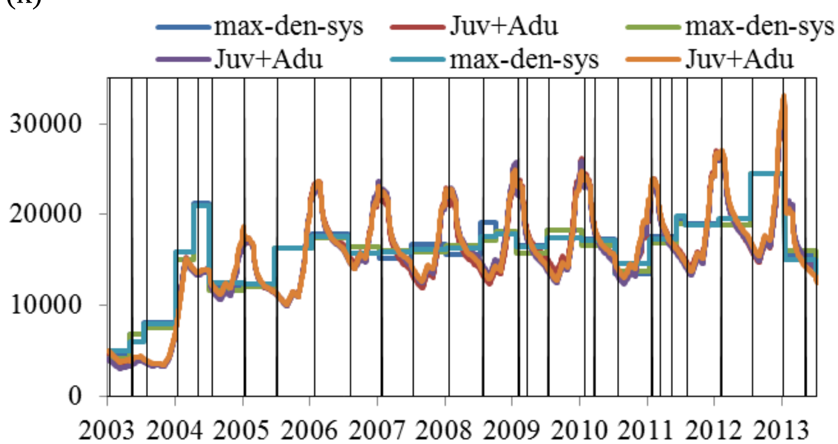
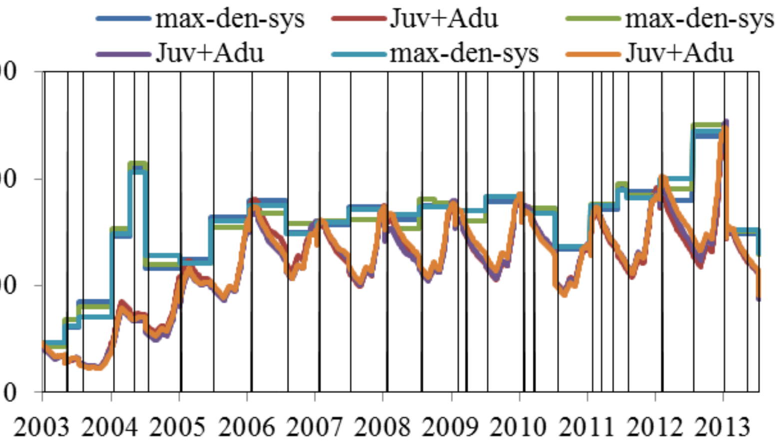
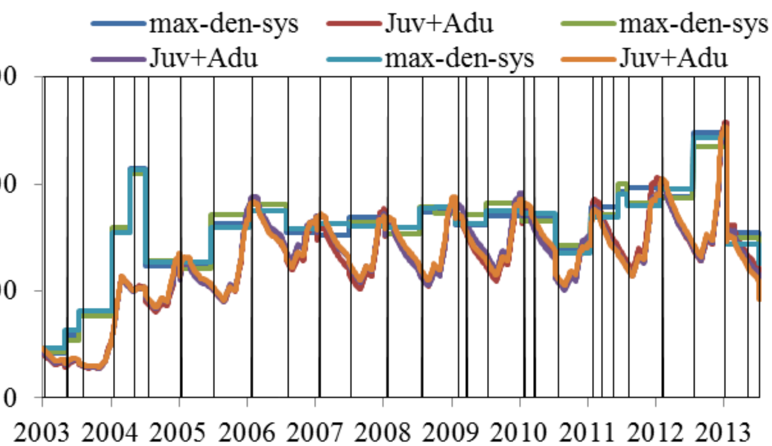


Figure 7 Cont.

(a)



(b)



(c)

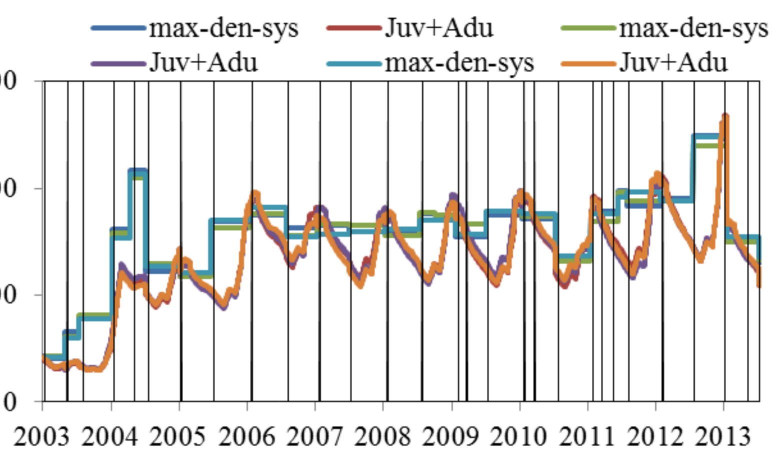
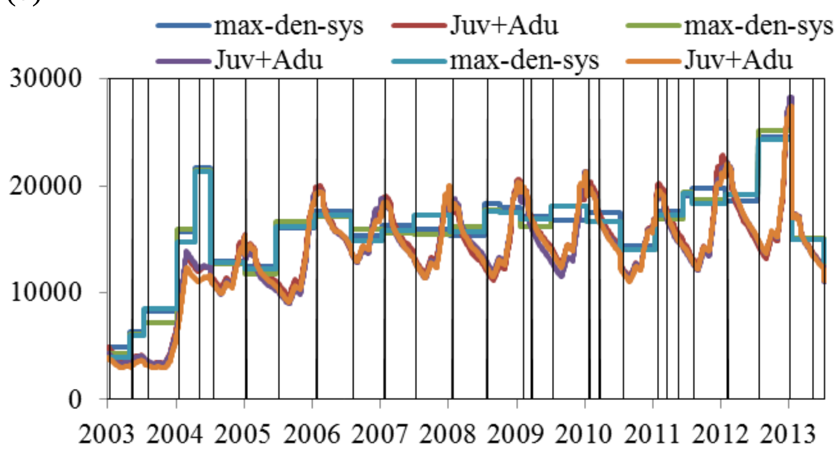


Figure 8 Cont.

(d)



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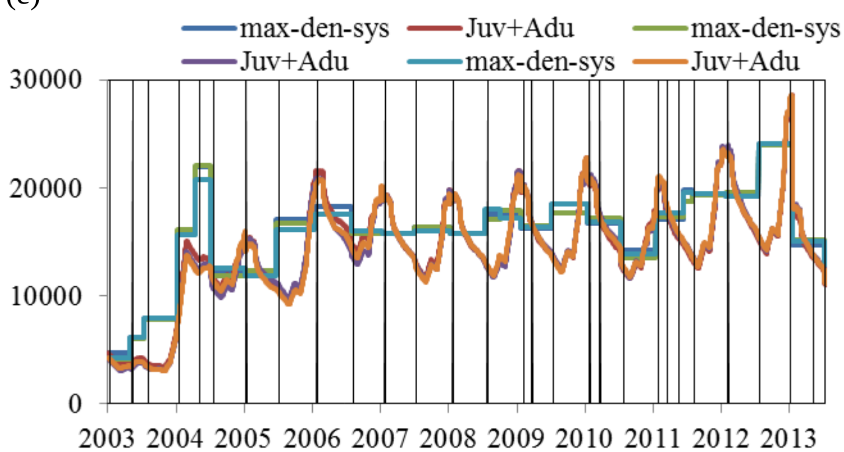
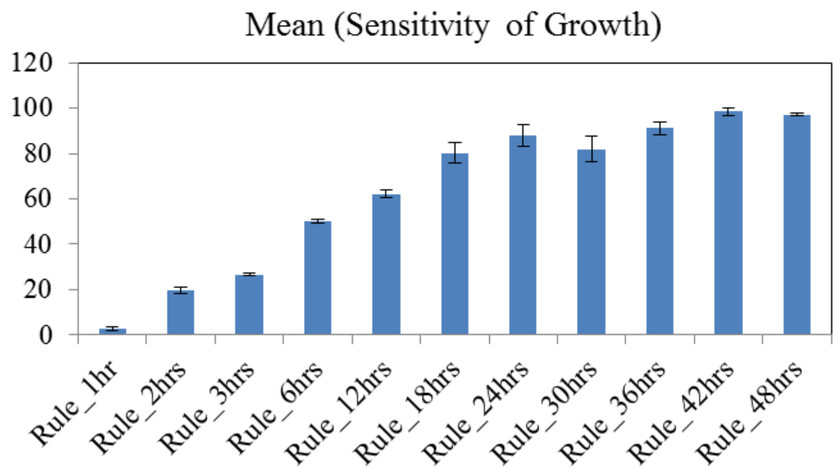


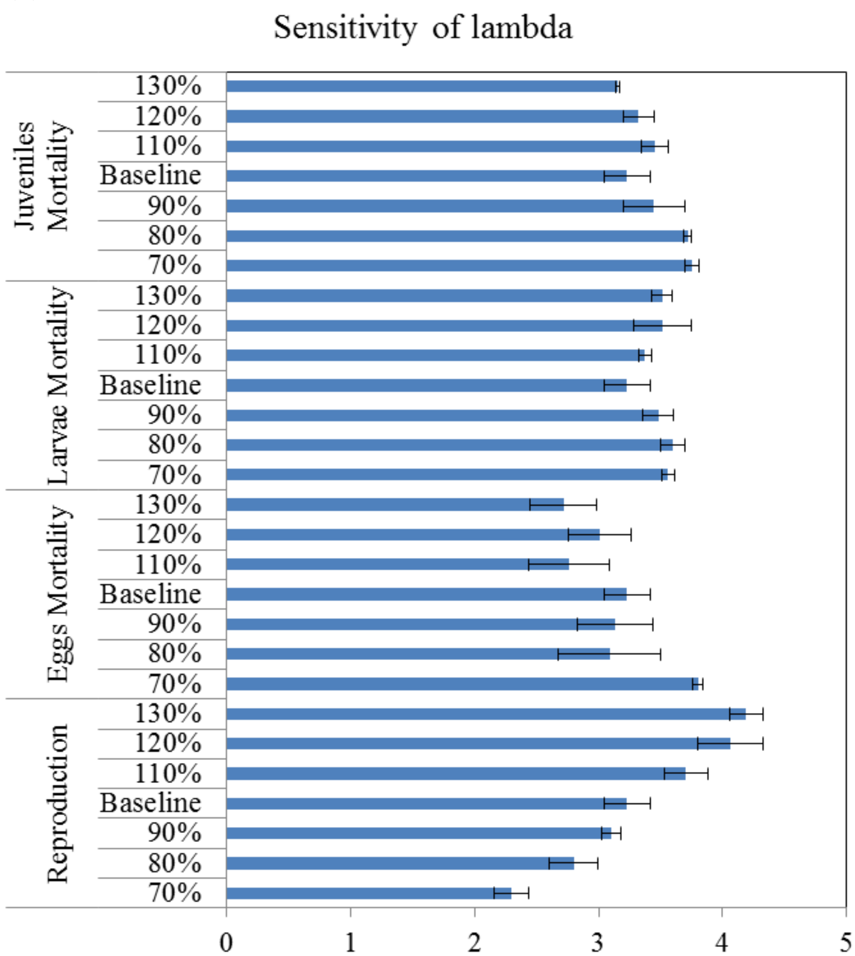
Figure 8 Cont.

(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

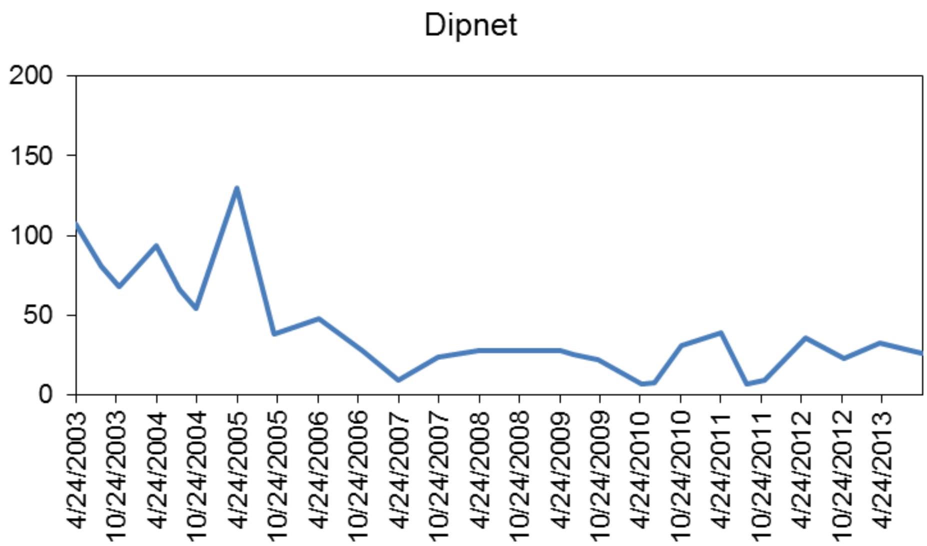
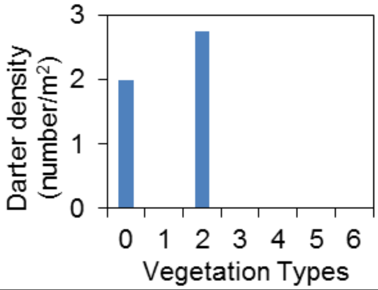

Dipnet
200
150
100
50
0
4/24/2003
10/24/2003
4/24/2004
10/24/2004
4/24/2005
10/24/2005
4/24/2006
10/24/2006
4/24/2007
10/24/2007
4/24/2008
10/24/2008
4/24/2009
10/24/2009
4/24/2010
10/24/2010
4/24/2011
10/24/2011
4/24/2012
10/24/2012
4/24/2013

Figure 10

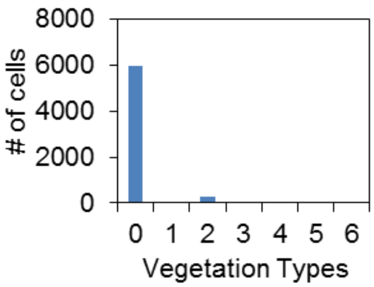
2000

Fall (Nov. 14th)

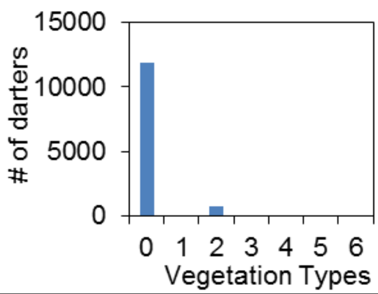
Darter density (#/m^2) in each veg type



# of cells of each veg type



Total # of darter in each veg cell



Total # of darters

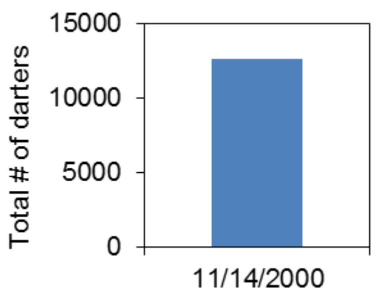


Figure 11

2001

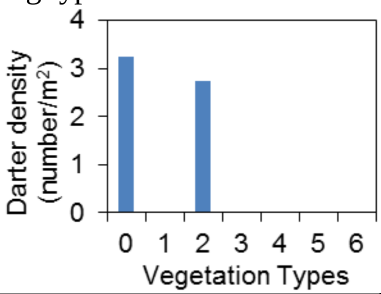
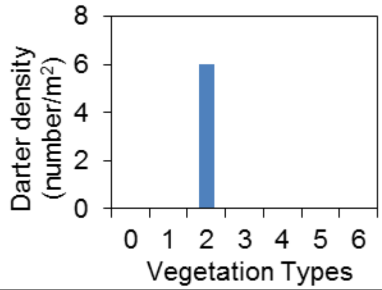
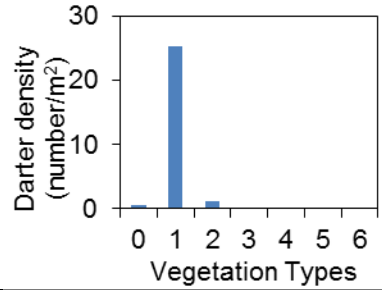
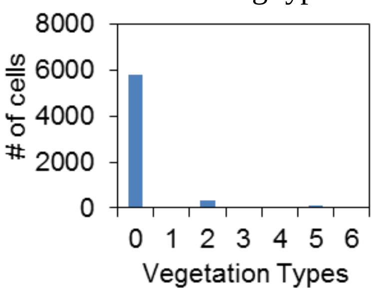
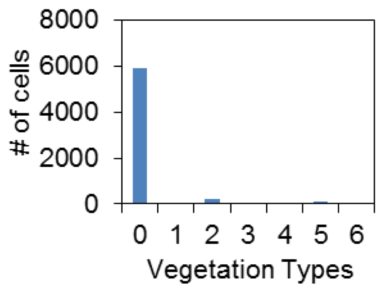
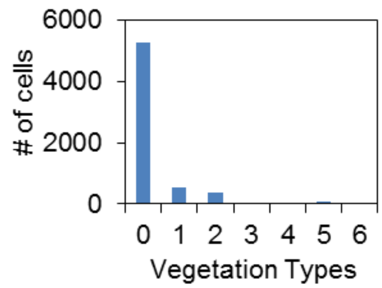
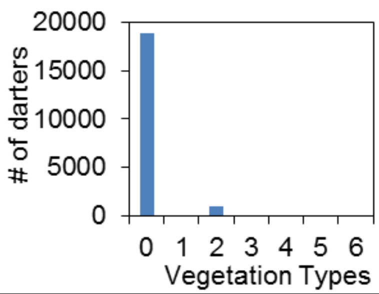
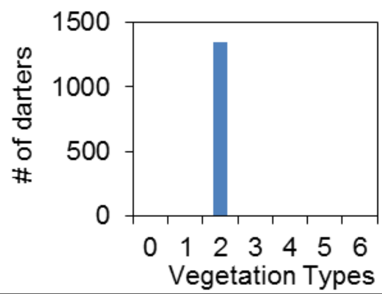
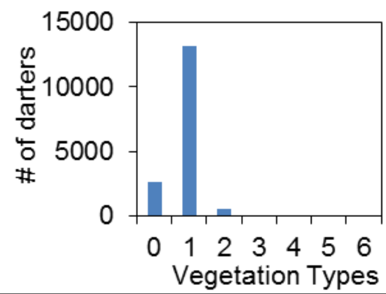
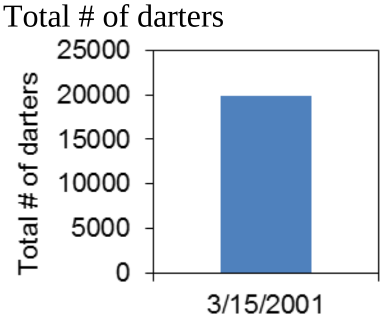
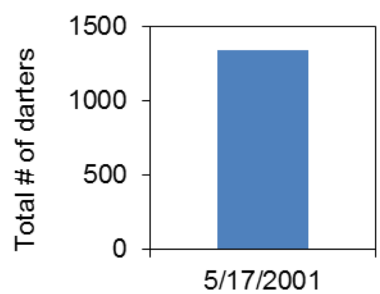
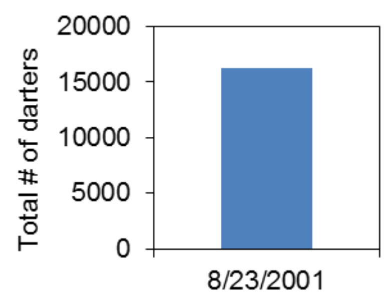
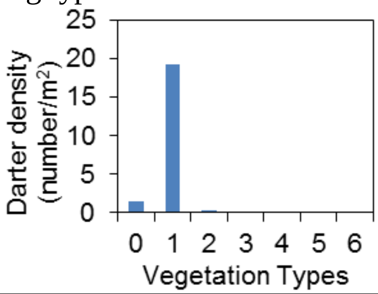
Winter (Mar.15th)	Spring (May 17th)	Summer (Aug. 23rd)
Darter density (#/m^2) in each veg type 		
# of cells of each veg type 		
Total # of darter in each veg cell 		
Total # of darters 		

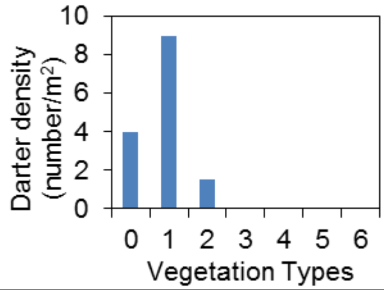
Figure 11 Cont.

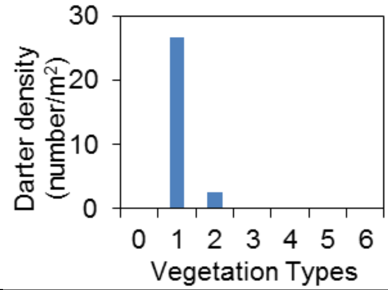
2001

Critical Period I (Sep. 16th)	Fall (Oct. 31st)	Critical Period II (Nov. 27th)

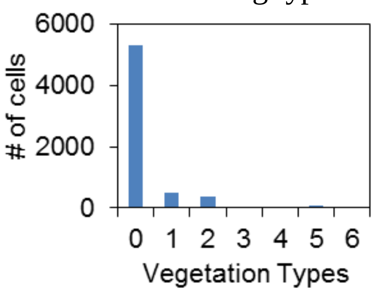
Darter density (#/m^2) in each veg type

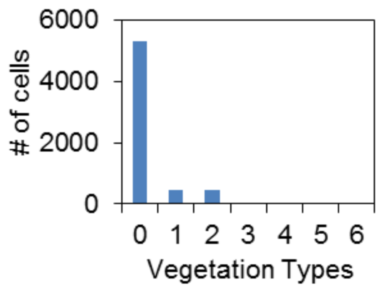


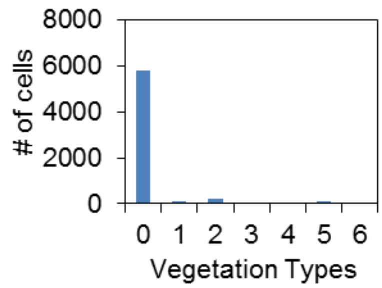




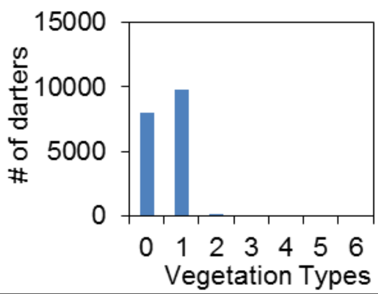
# of cells of each veg type

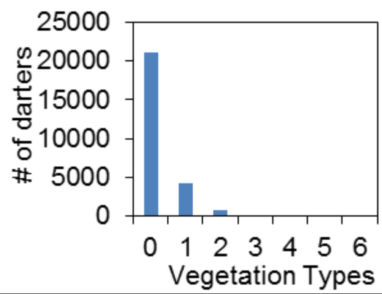


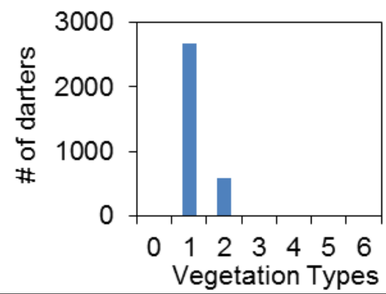




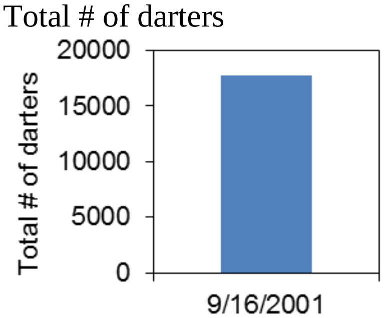
Total # of darter in each veg cell

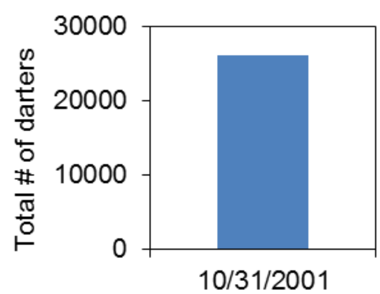






Total # of darters





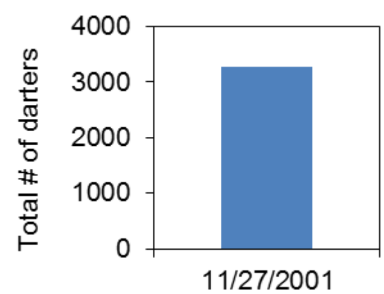
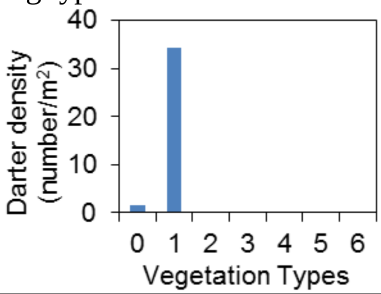


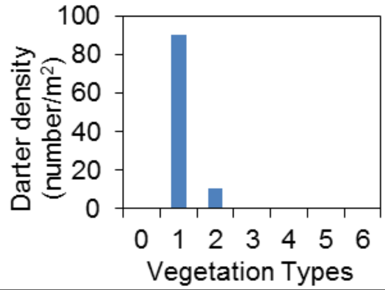
Figure 11 Cont.

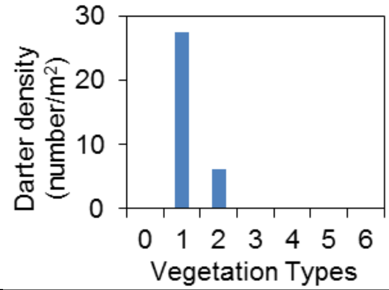
2002

Winter (Feb. 20th)	Spring (May 15th)	Summer (Aug. 1st)

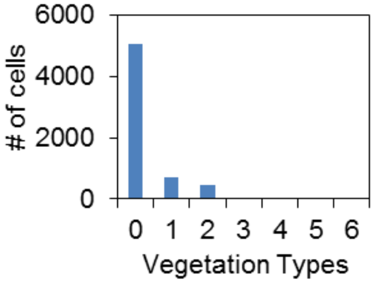
Darter density (#/m^2) in each veg type

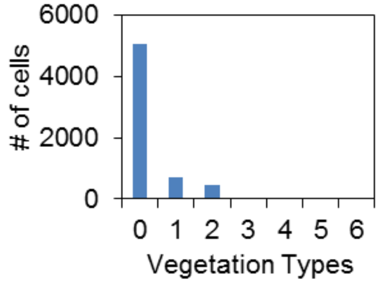


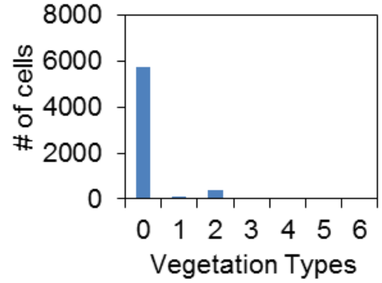




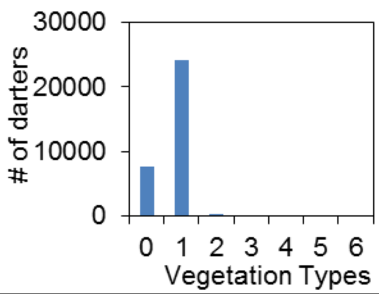
# of cells of each veg type

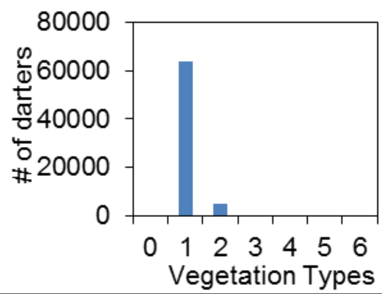


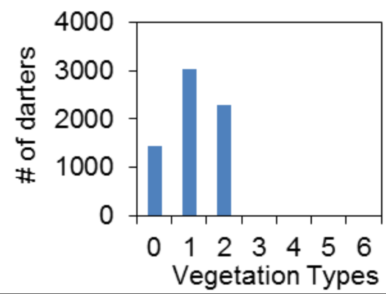




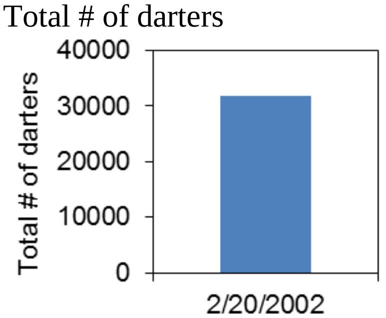
Total # of darter in each veg cell

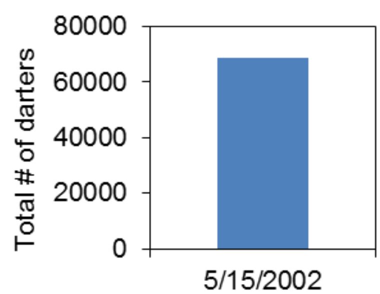






Total # of darters





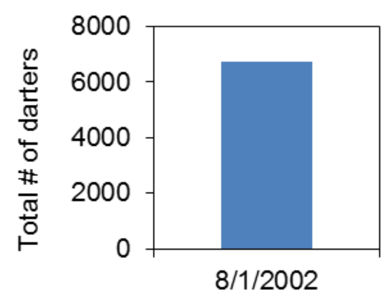
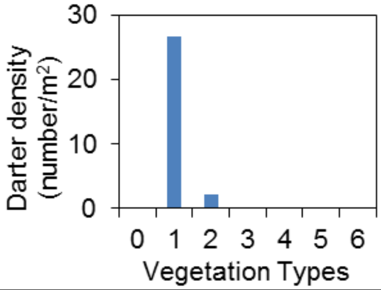


Figure 11 Cont.

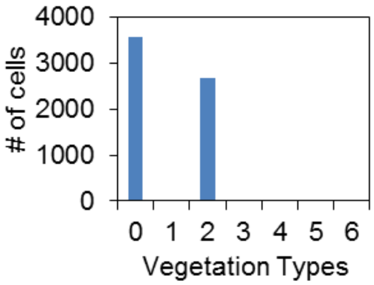
2002

Fall (Oct. 28th)

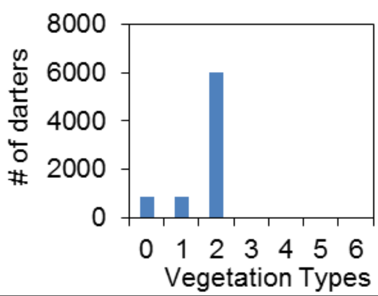
Darter density (#/m^2) in each vege type



# of cells of each veg type



Total # of darter in each veg cell



Total # of darters

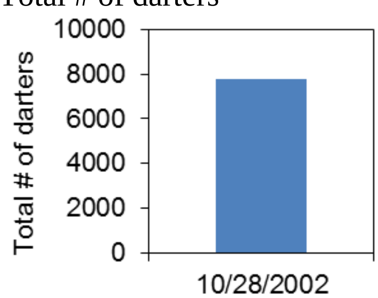


Figure 11 Cont.

2003

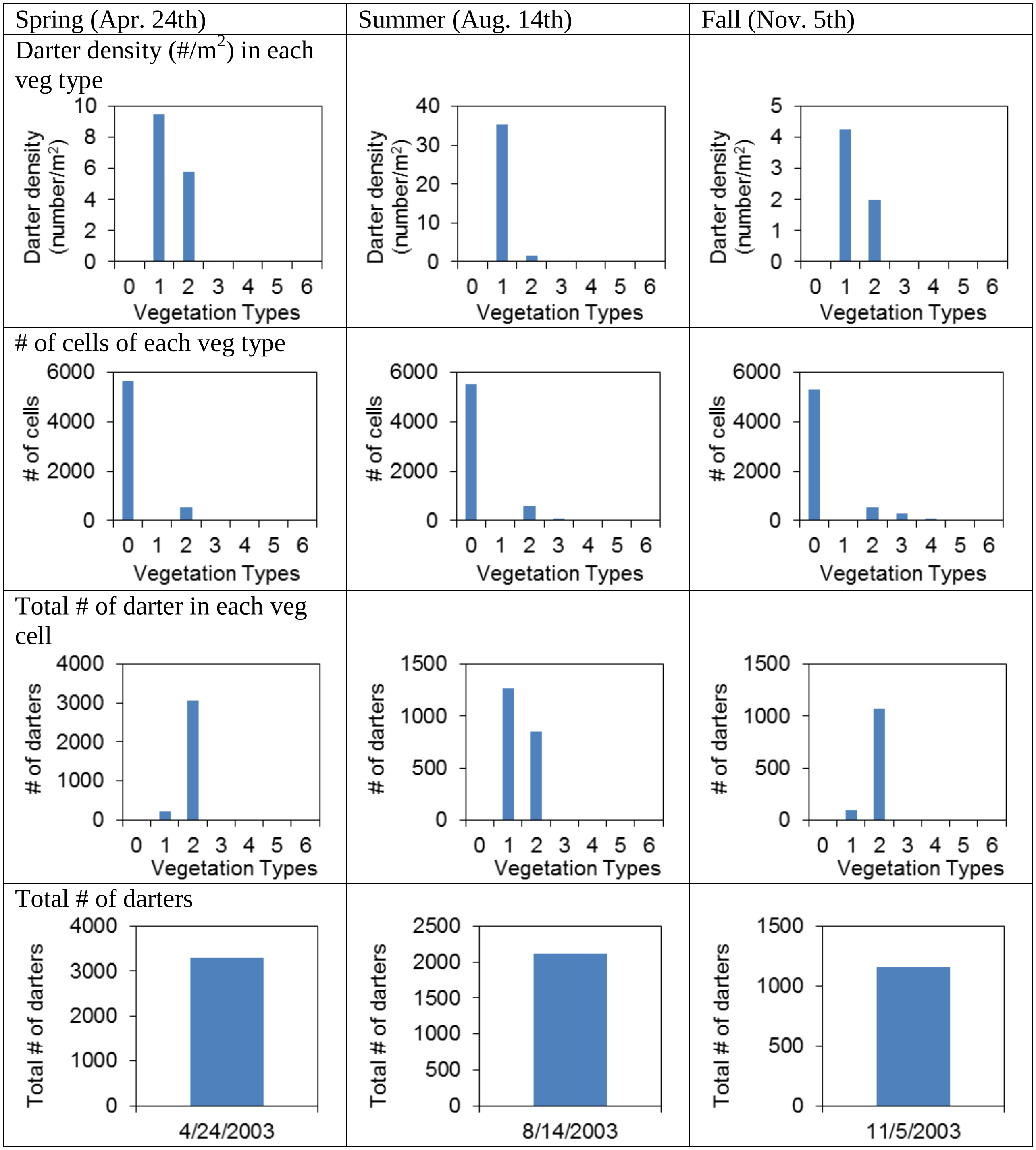
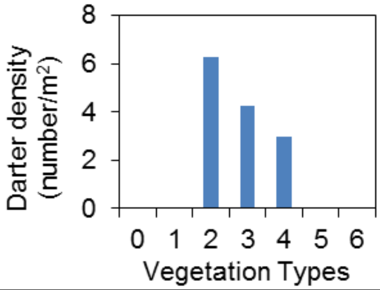


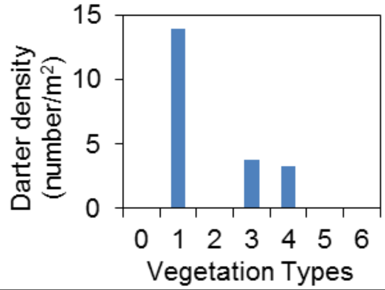
Figure 11 Cont.

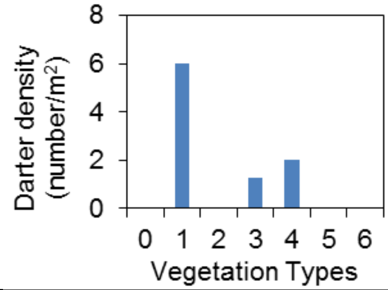
2004

Spring (Apr. 21st)	Summer (Aug. 4th)	Fall (Oct. 21st)

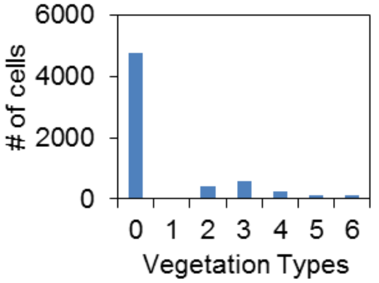
Darter density (#/m^2) in each veg type

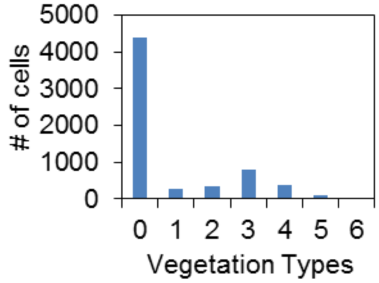


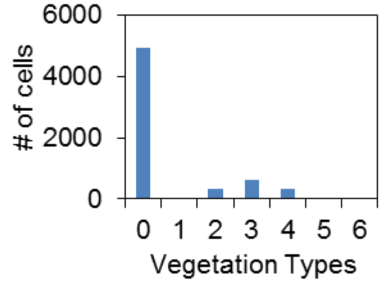




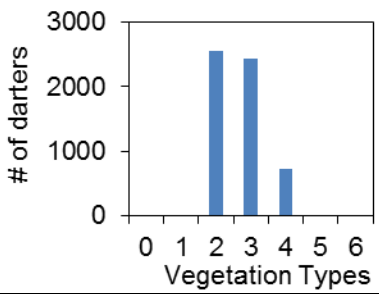
# of cells of each veg type

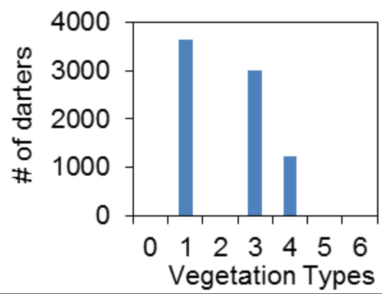


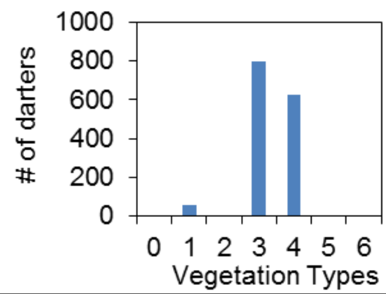




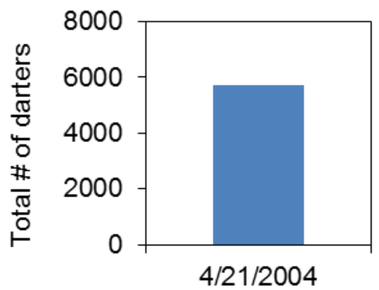
Total # of darter in each veg cell

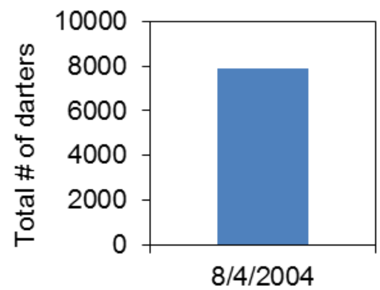






Total # of darters





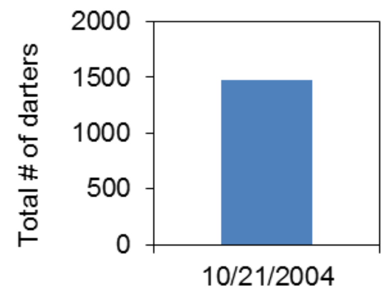
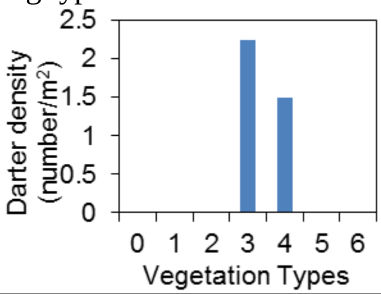


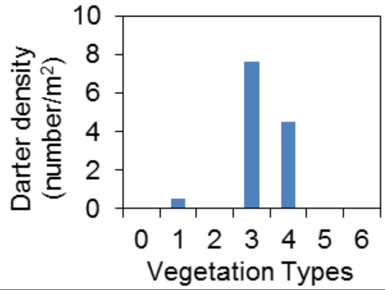
Figure 11 Cont.

2005

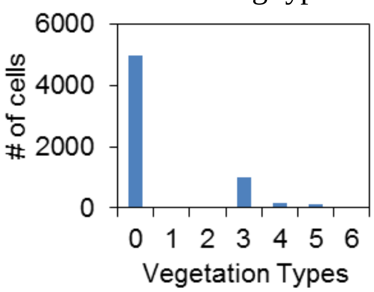
Spring (Apr. 20th) | **Fall (Oct. 5th)**

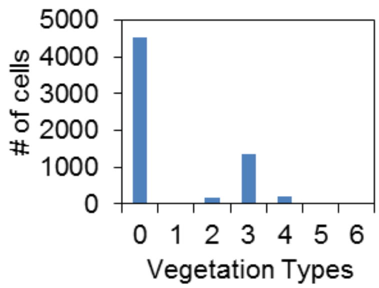
Darter density (#/m^2) in each veg type



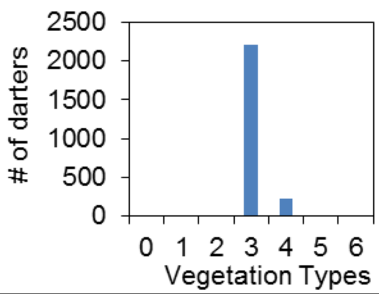


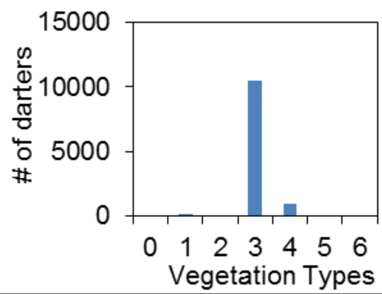
# of cells of each veg type



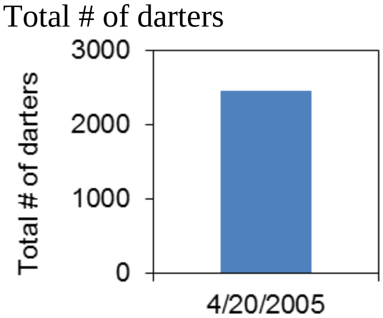


Total # of darter in each veg cell





Total # of darters



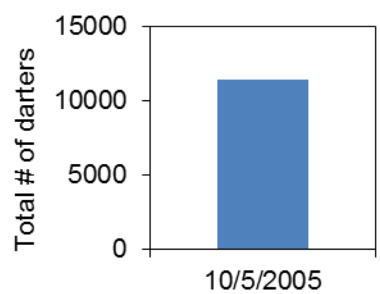


Figure 11 Cont.

2006

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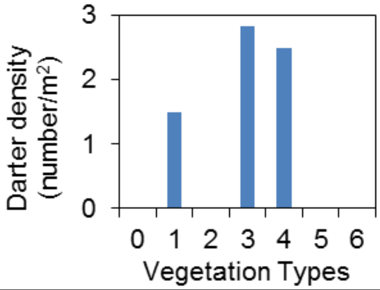

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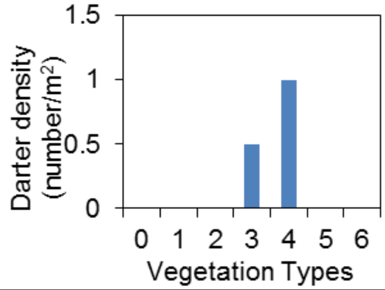

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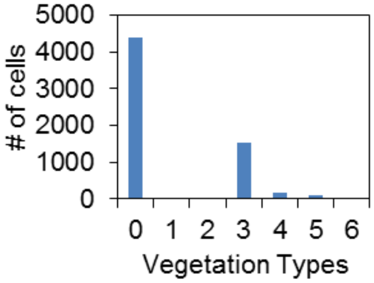
<tr><td># of cells of each veg type



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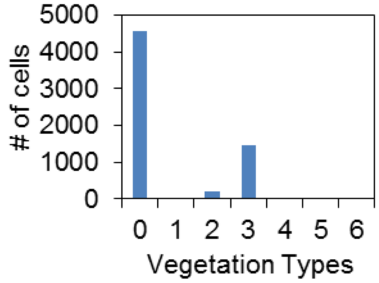

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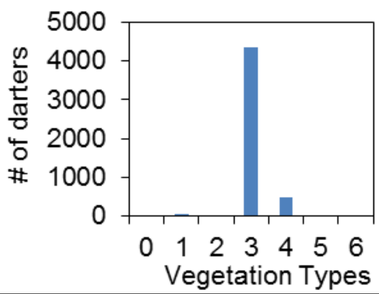

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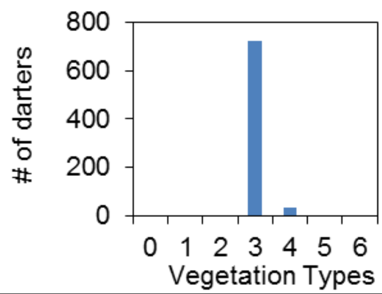

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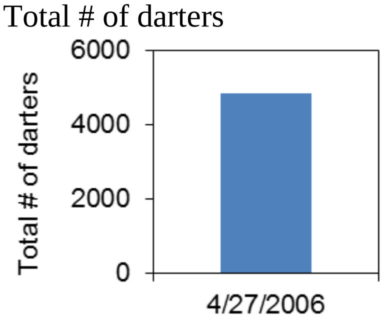
<tr><td>Total # of darters



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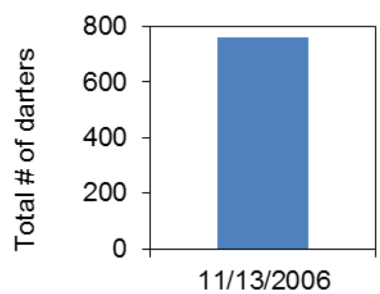

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Figure 11 Cont.

2007

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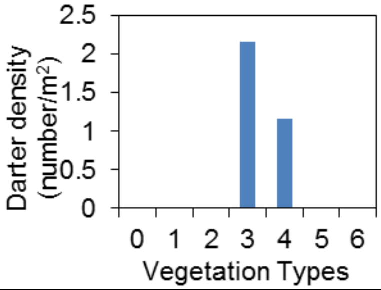
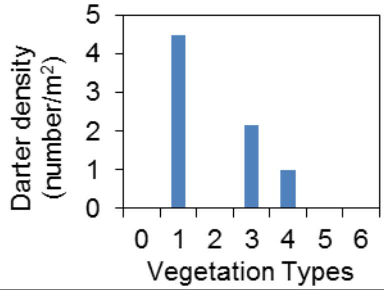
</td><td></td></tr>
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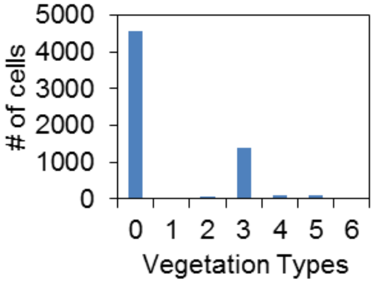
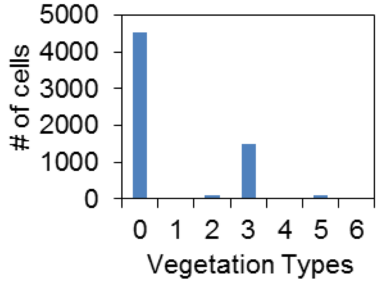
</td><td></td></tr>
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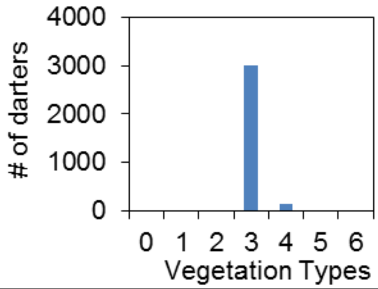
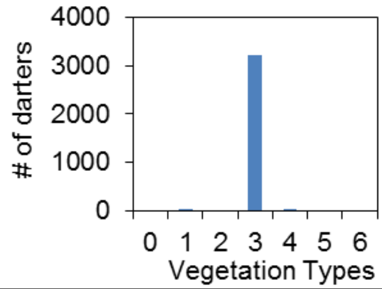
</td><td></td></tr>
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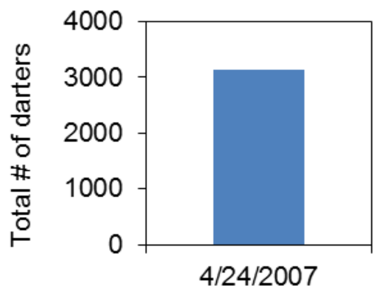
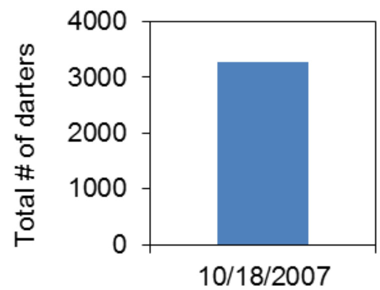
</td><td></td></tr>
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Figure 11 Cont.

2008

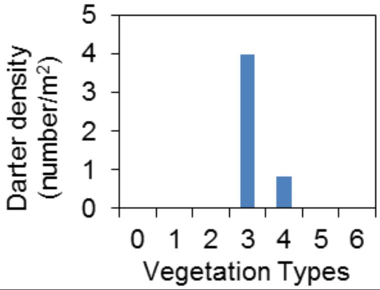
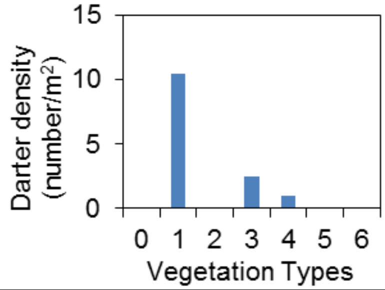
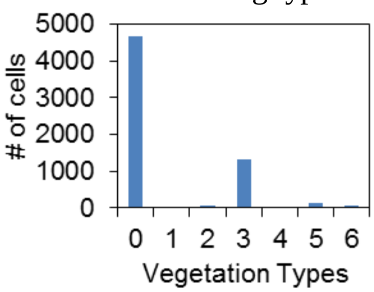
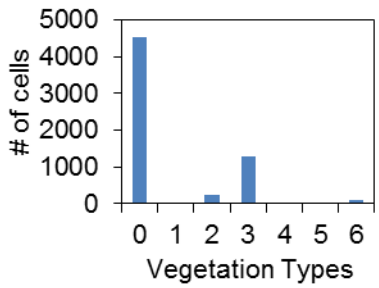
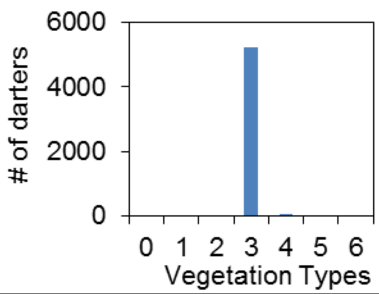
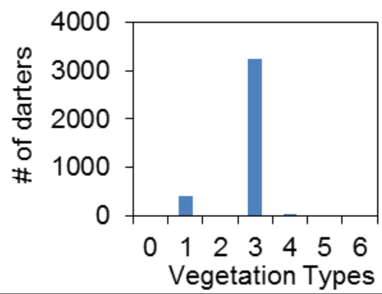
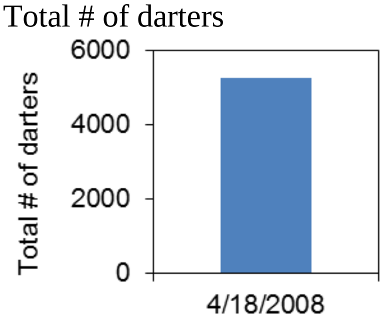
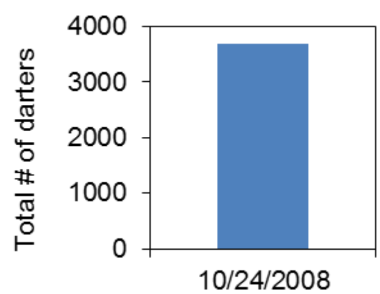
Spring (Apr. 18th)	Fall (Oct. 24th)
Darter density (#/m^2) in each veg type 	
# of cells of each veg type 	
Total # of darter in each veg cell 	
Total # of darters 	

Figure 11 Cont.

2009

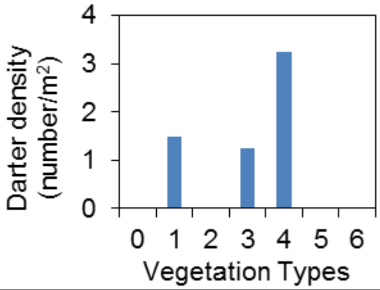
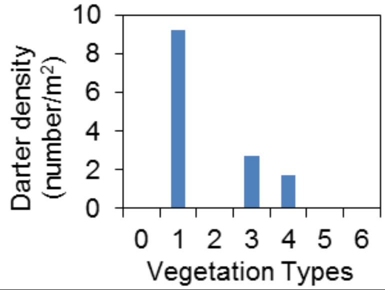
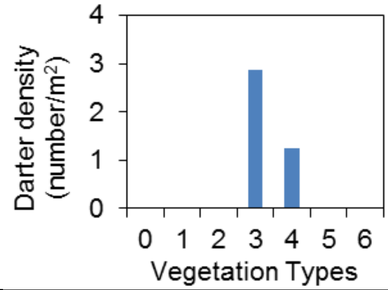
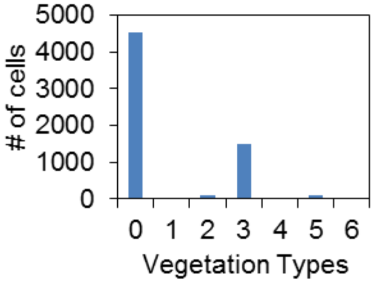
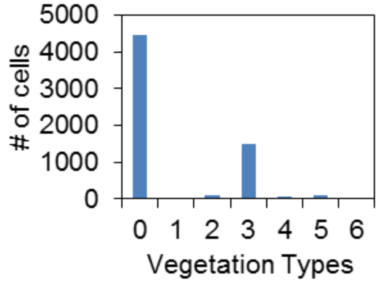
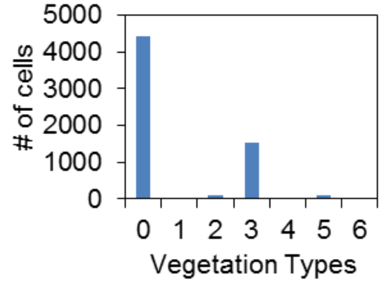
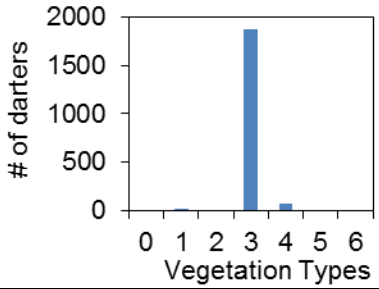
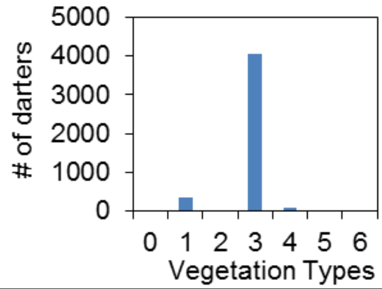
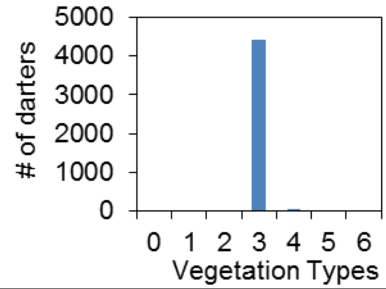
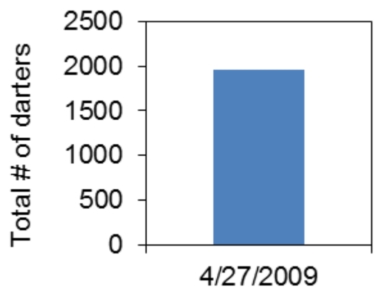
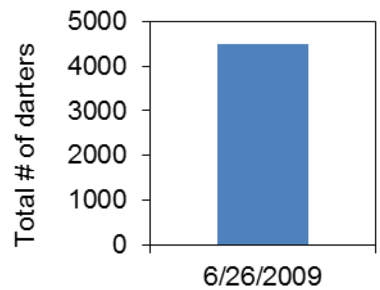
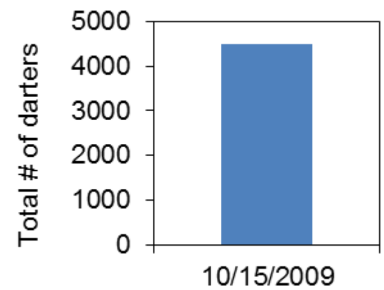
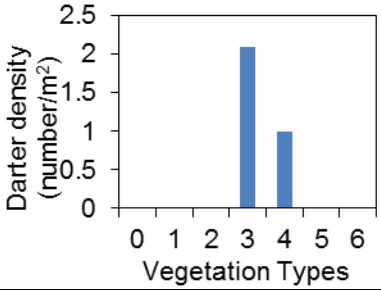
Spring (Apr. 27th)	Critical Period (Jun. 26th)	Fall (Oct. 15th)
Darter density (#/m^2) in each veg type 		
# of cells of each veg type 		
Total # of darter in each veg cell 		
Total # of darters 		

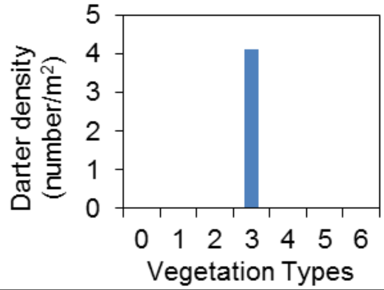
Figure 11 Cont.

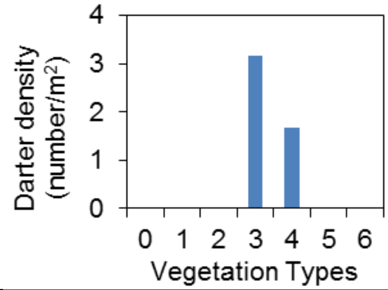
2010

Spring (Apr. 27th)	Critical Period (Jun. 28th)	Fall (Oct. 26th)

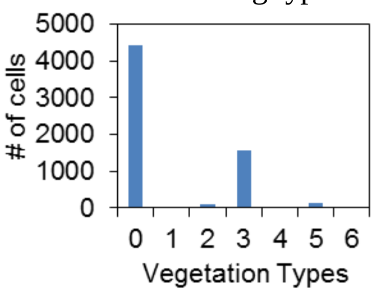
Darter density (#/m^2) in each veg type

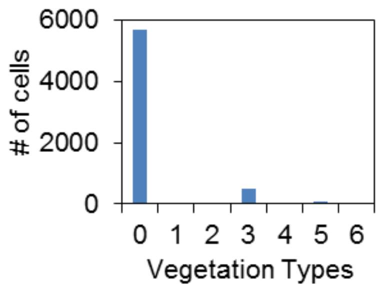


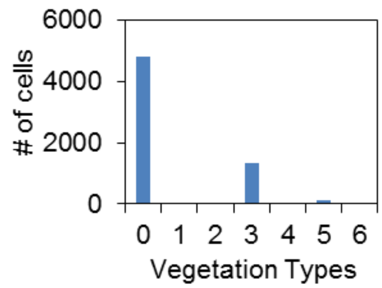




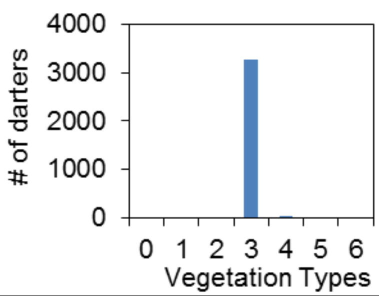
# of cells of each veg type

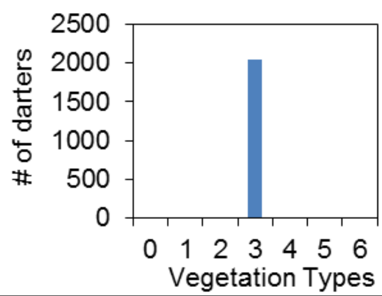


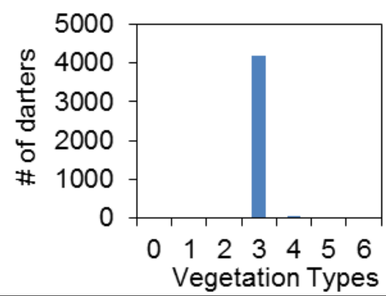




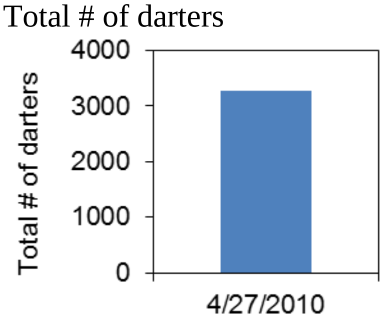
Total # of darter in each veg cell

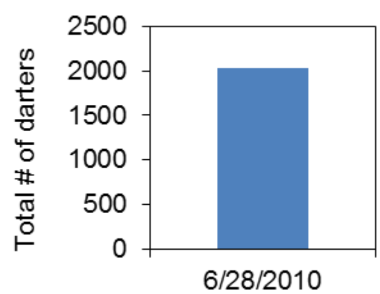






Total # of darters





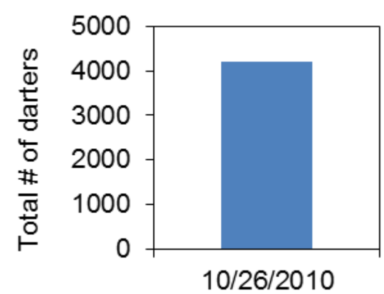
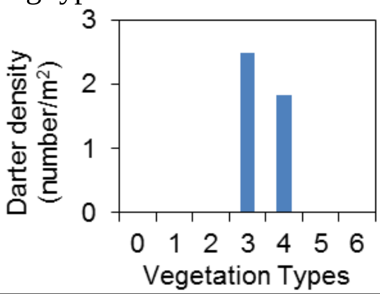


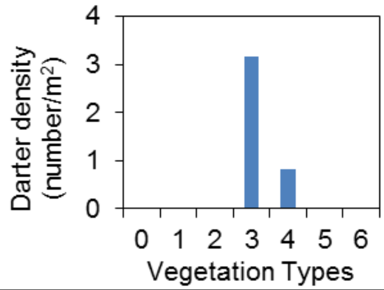
Figure 11 Cont.

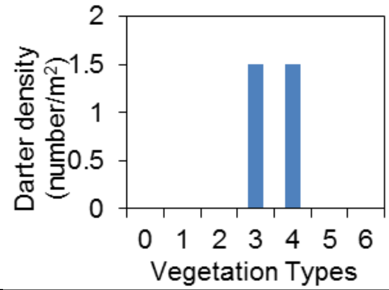
2011

Spring (Apr. 25th)	Critical Period (Aug. 17th)	Fall (Nov. 8th)

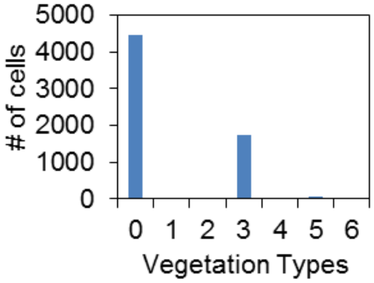
Darter density (#/m²) in each veg type

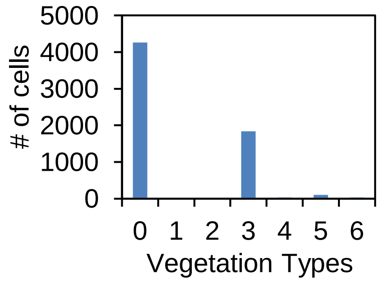


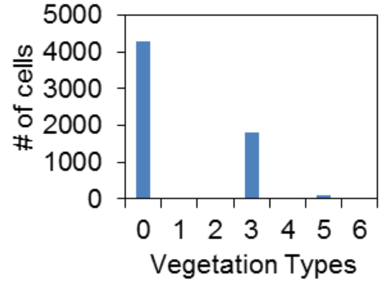




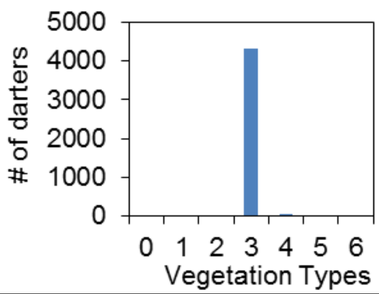
# of cells of each veg type

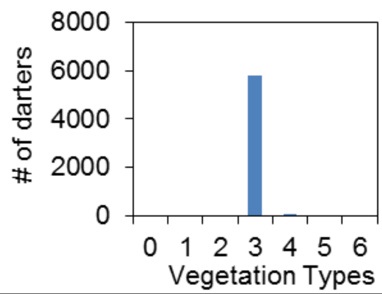


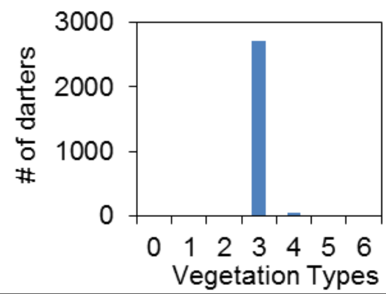




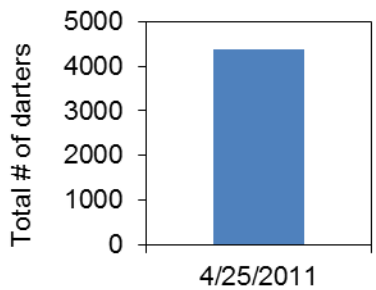
Total # of darter in each veg cell

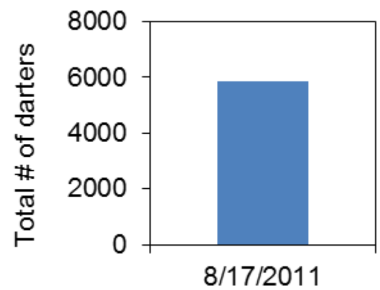






Total # of darters





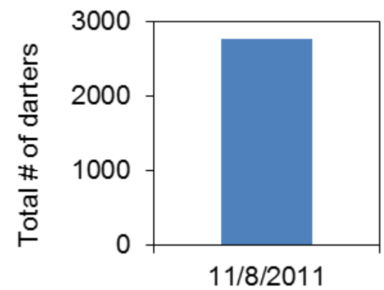


Figure 11 Cont.

2012

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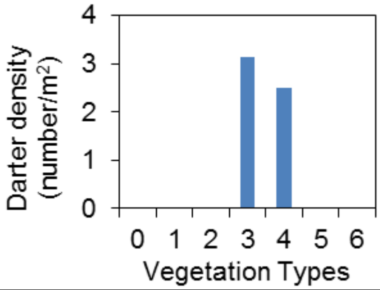
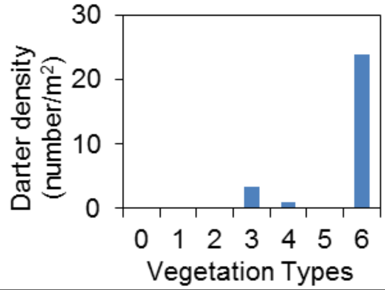
</td><td></td></tr>
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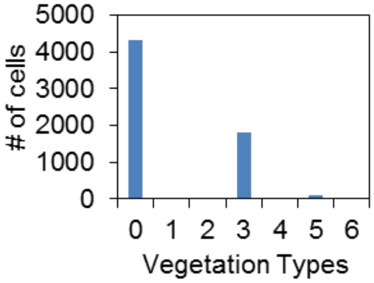
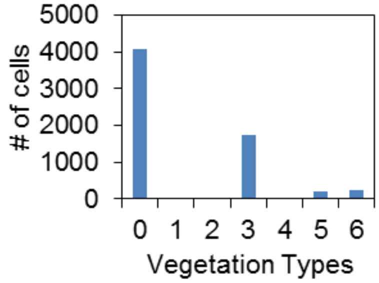
</td><td></td></tr>
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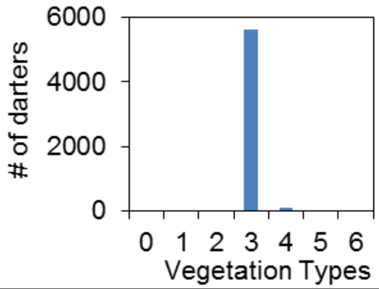
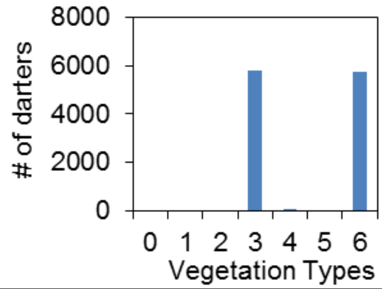
</td><td></td></tr>
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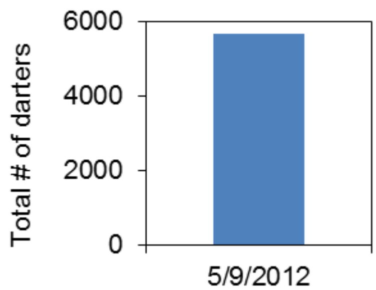
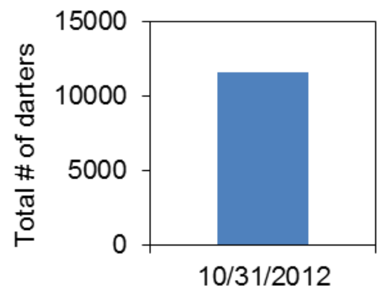
</td><td></td></tr>
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Figure 11 Cont.

2013

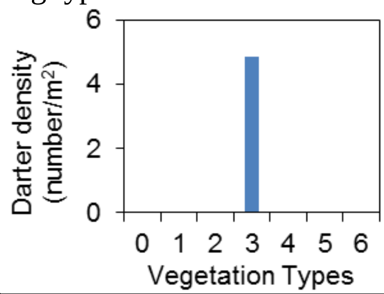
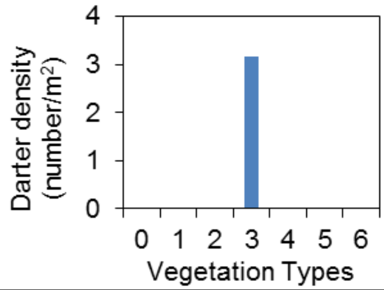
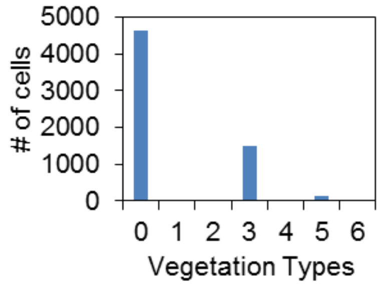
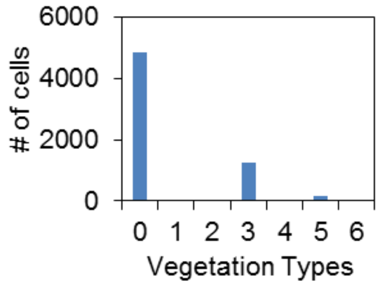
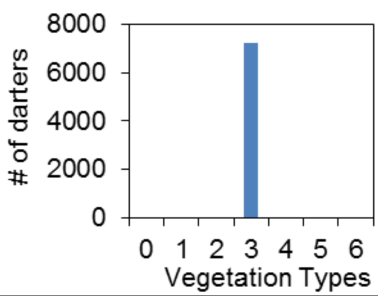
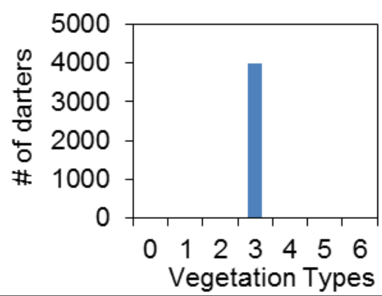
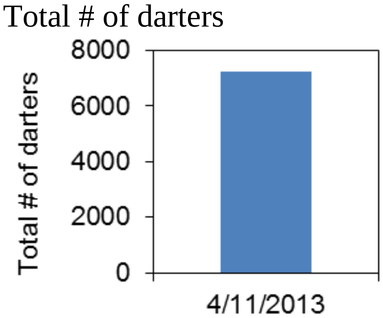
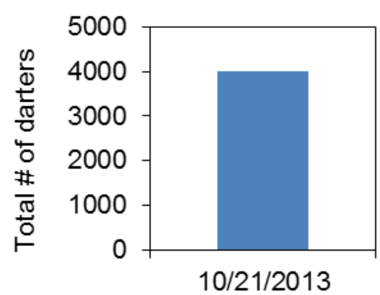
Spring (Apr. 11th)	Fall (Oct. 21st)
Darter density (#/m^2) in each veg type 	
# of cells of each veg type 	
Total # of darter in each veg cell 	
Total # of darters 	

Figure 11 Cont.

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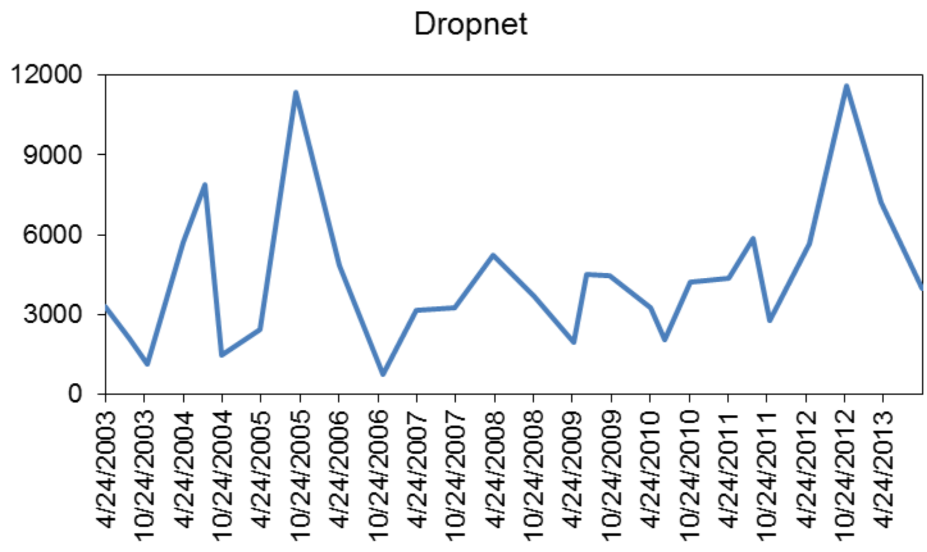
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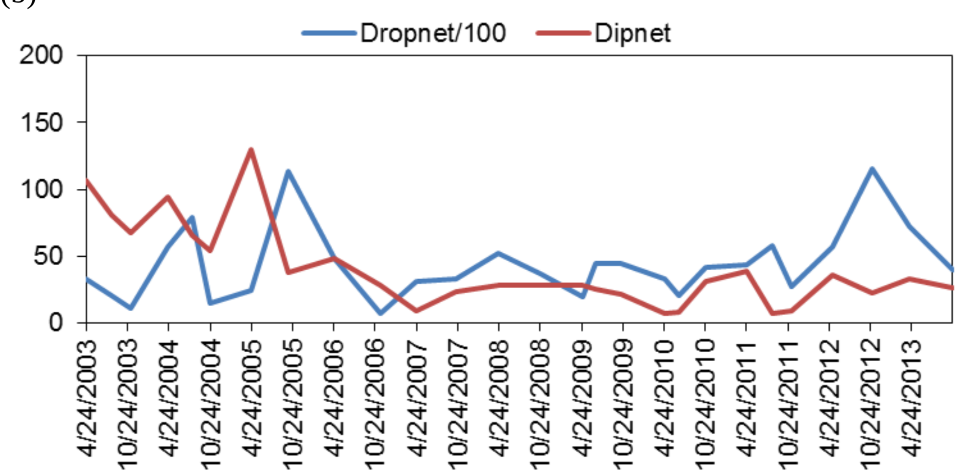
(b)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4/24/2013

Figure 12

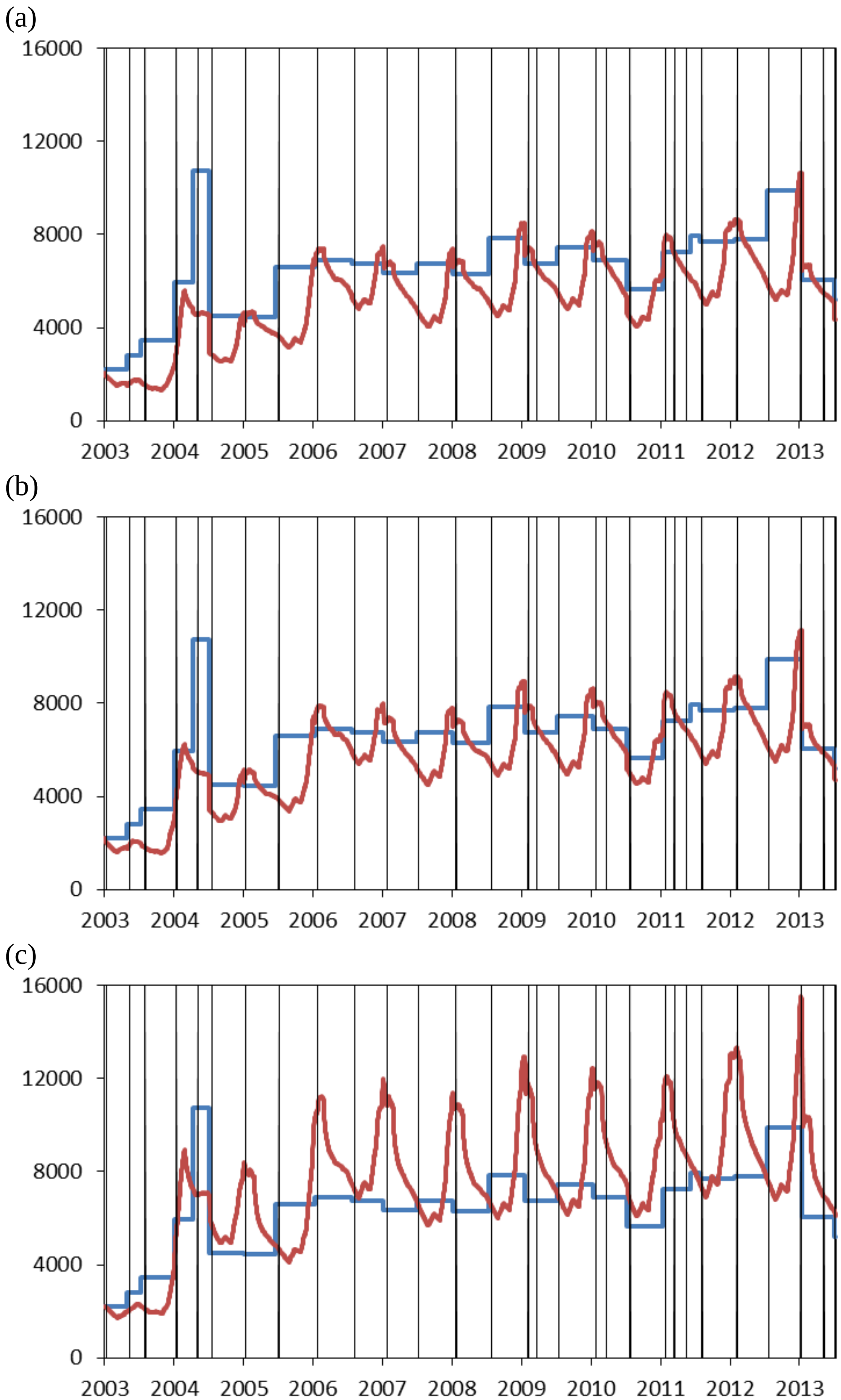

(a)
16000
12000
8000
4000
0
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
(b)
16000
12000
8000
4000
0
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
(c)
16000
12000
8000
4000
0
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013

Figure 13

(a)

(b)

(c)

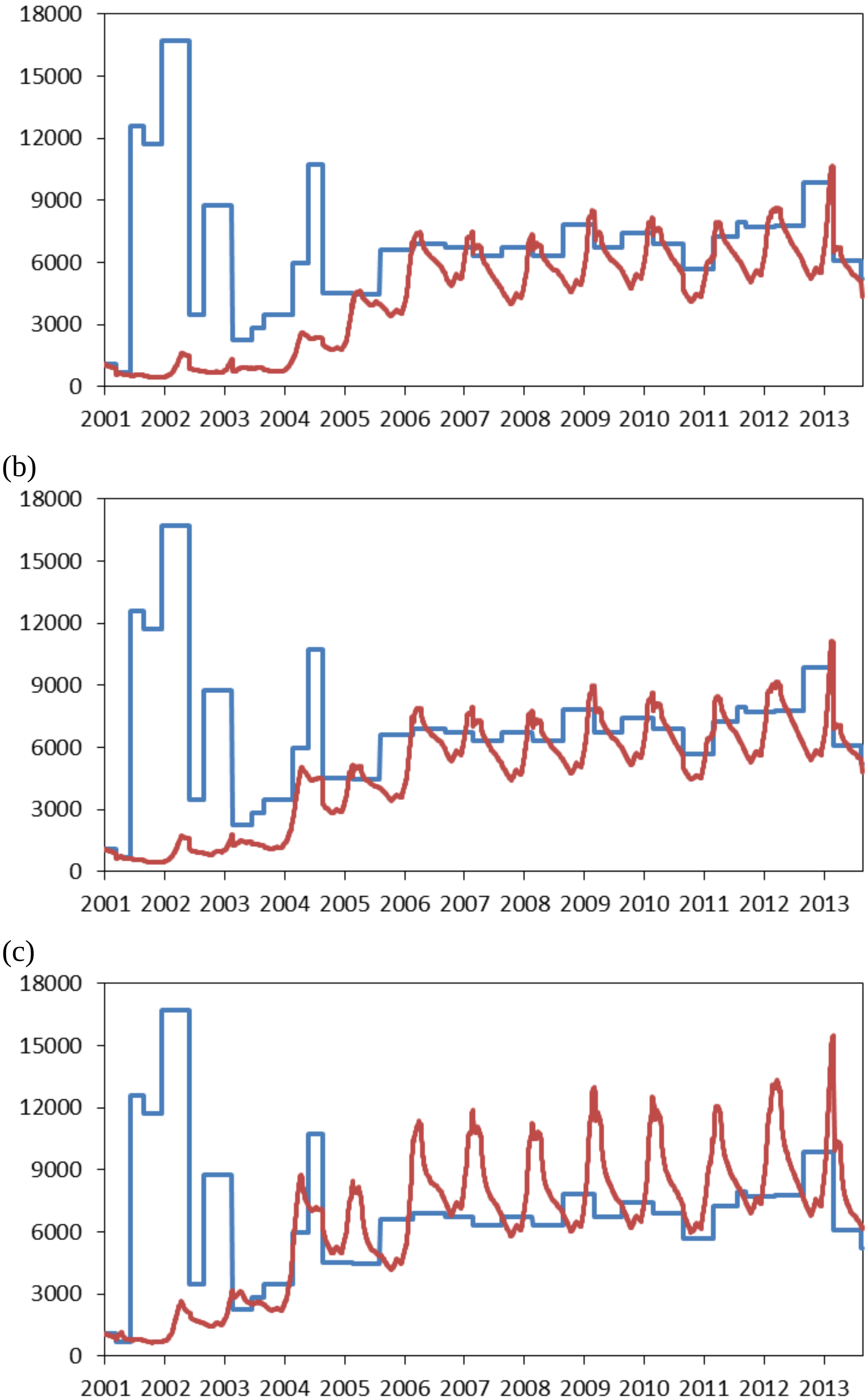
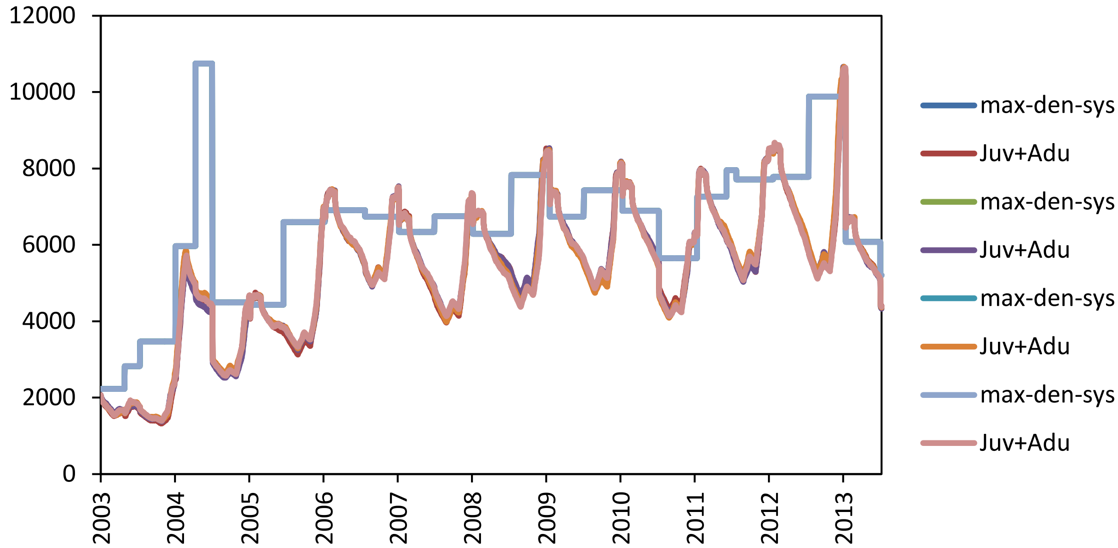


Figure 14



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

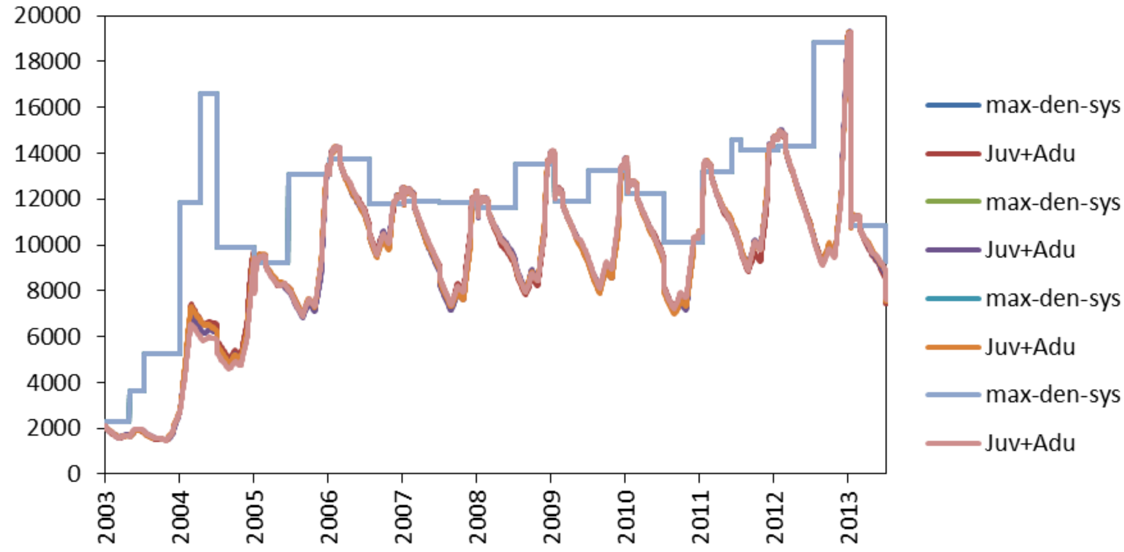

20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
max-den-sys
Juv+Adu
max-den-sys
Juv+Adu
max-den-sys
Juv+Adu
max-den-sys
Juv+Adu

Figure 16

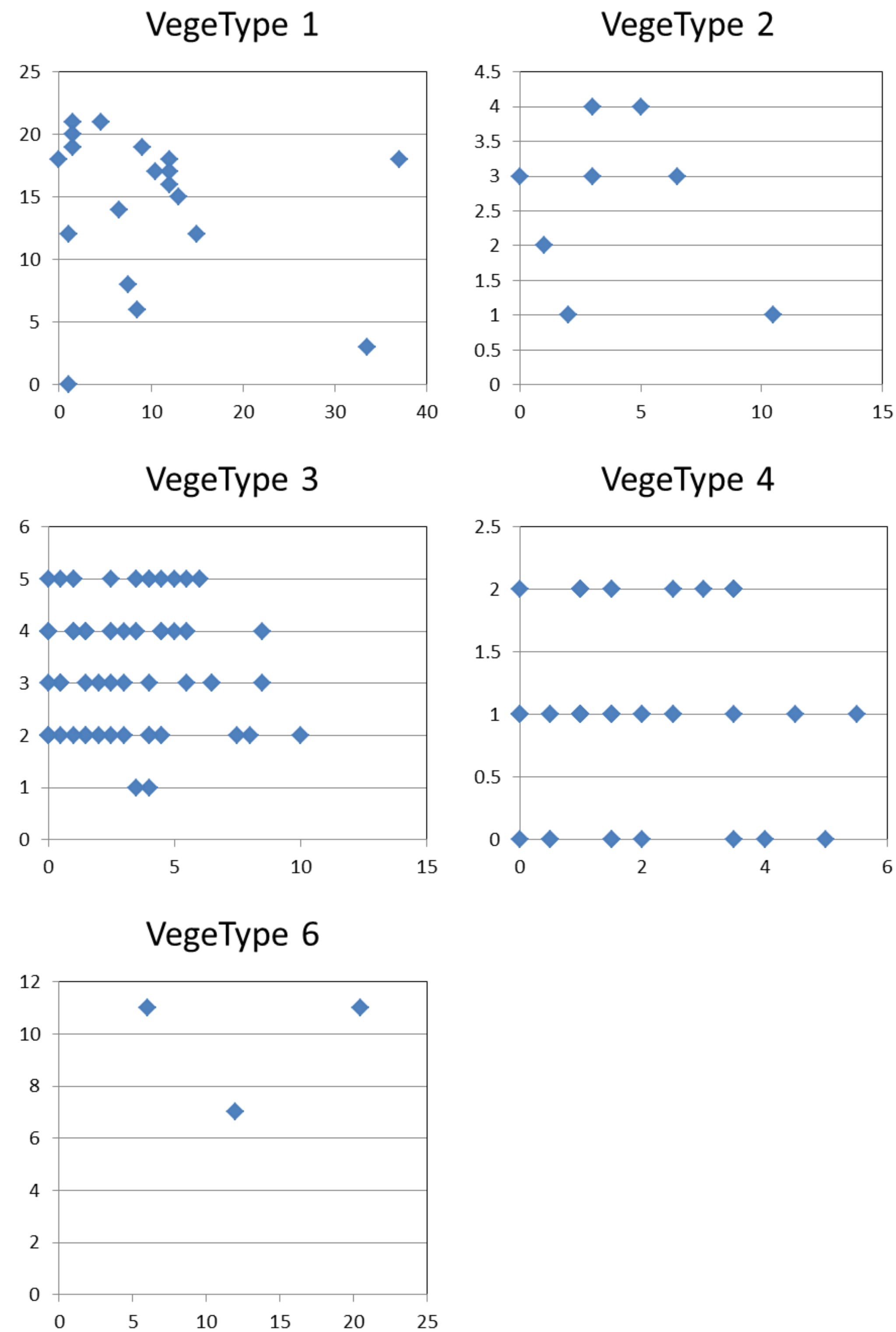


Figure 17 (a) Rep 1

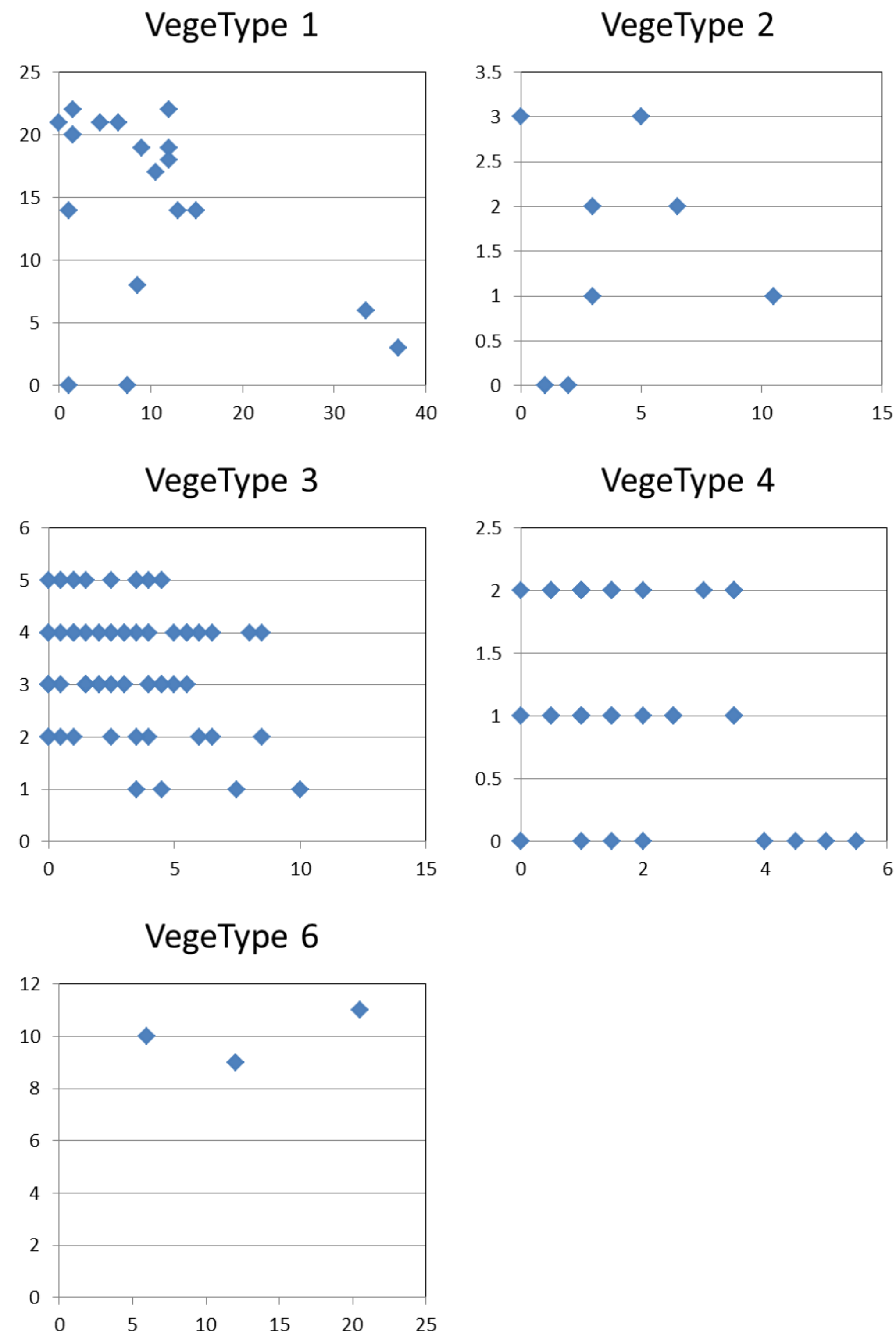


Figure 17 (a) Rep 2

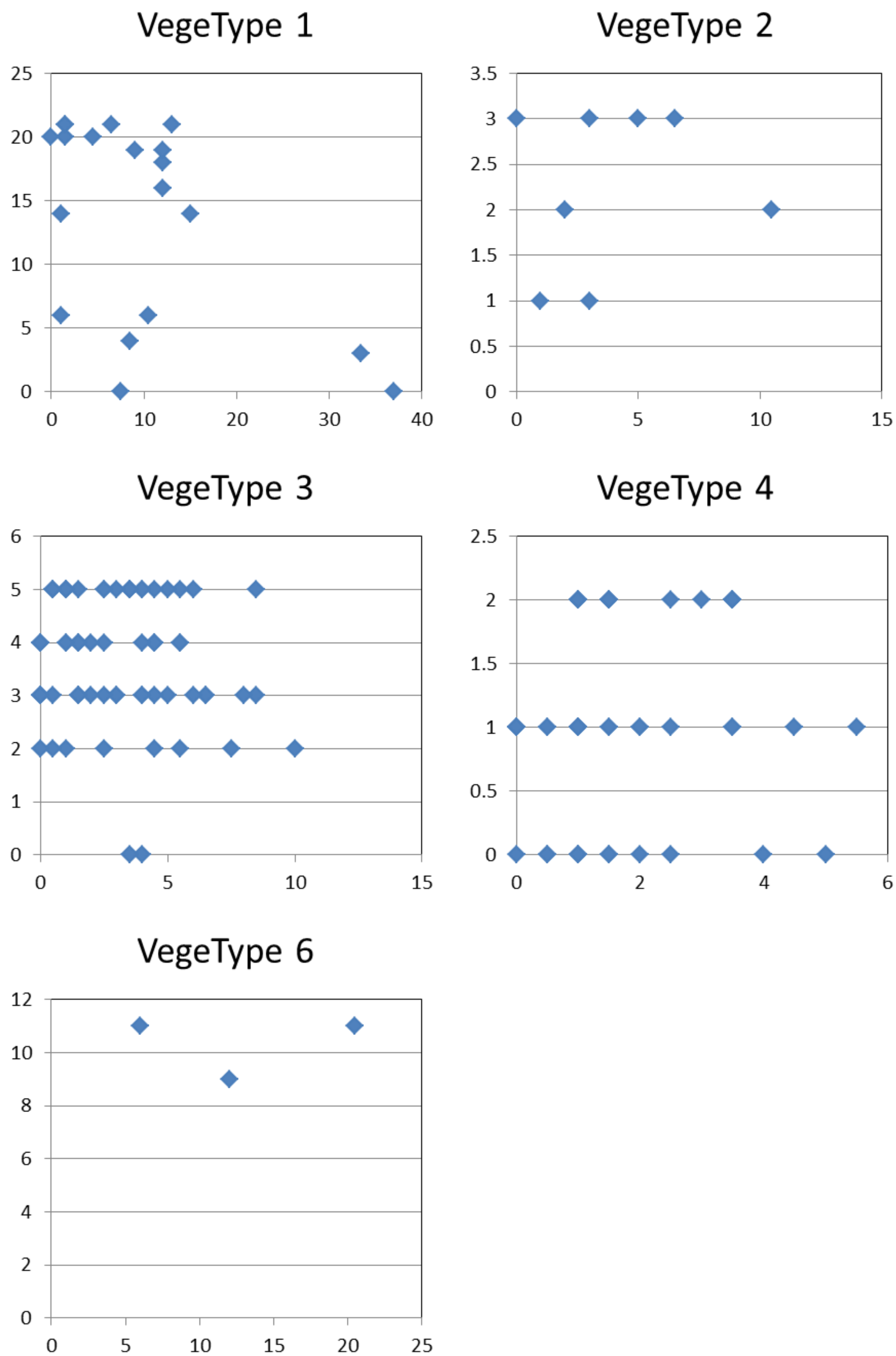


Figure 17 (a) Rep 3

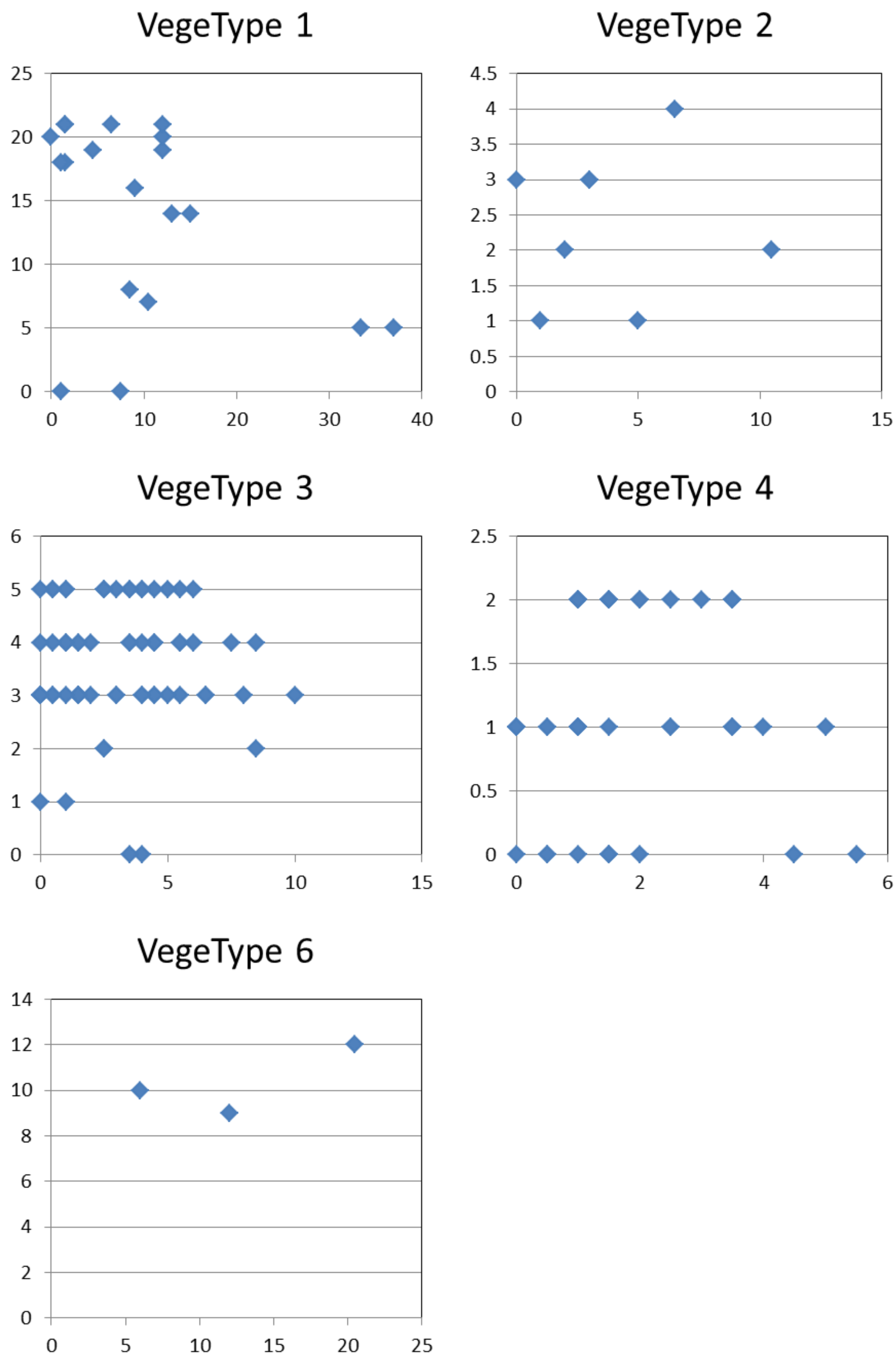


Figure 17 (a) Rep 4

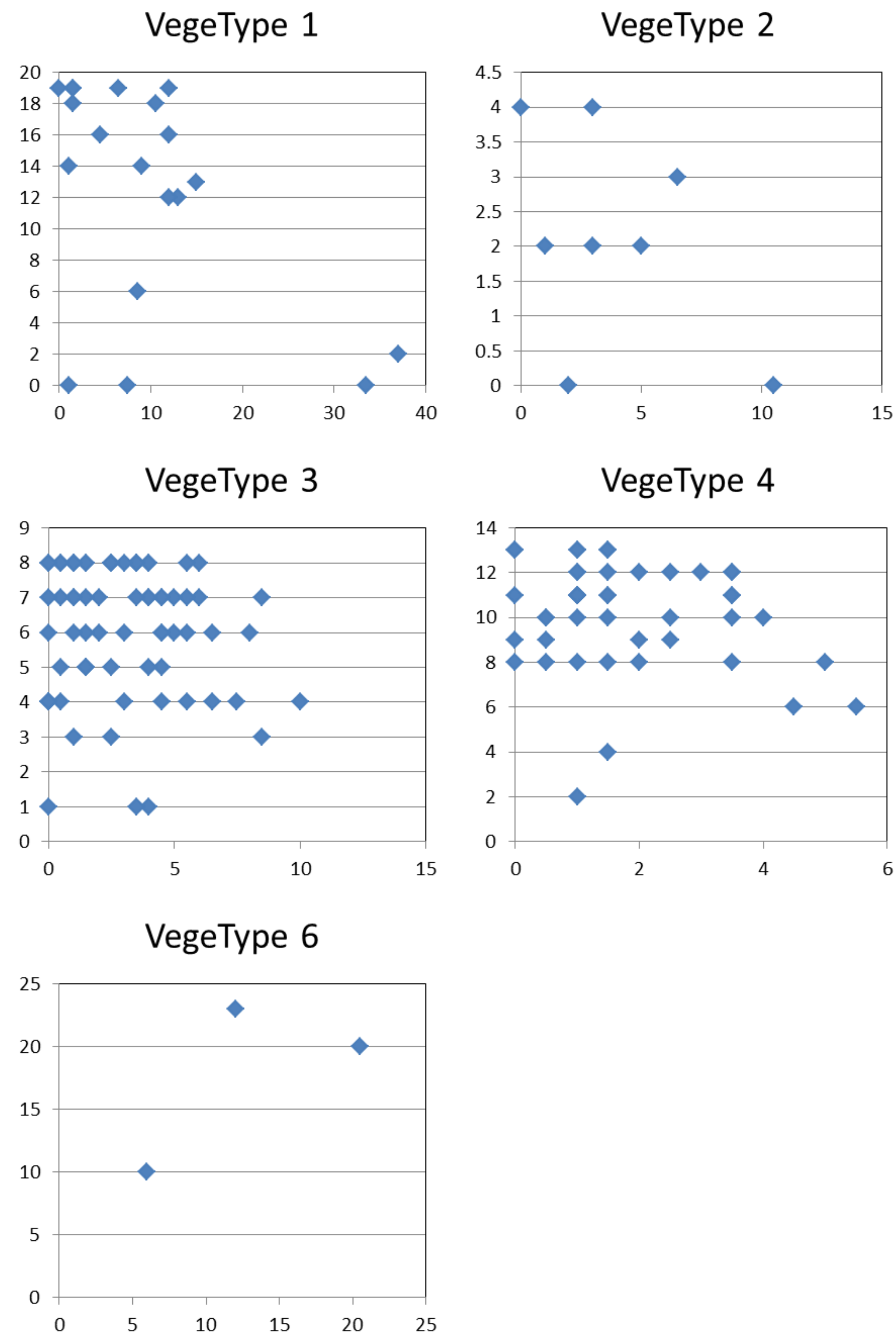


Figure 17 (b) Rep 1

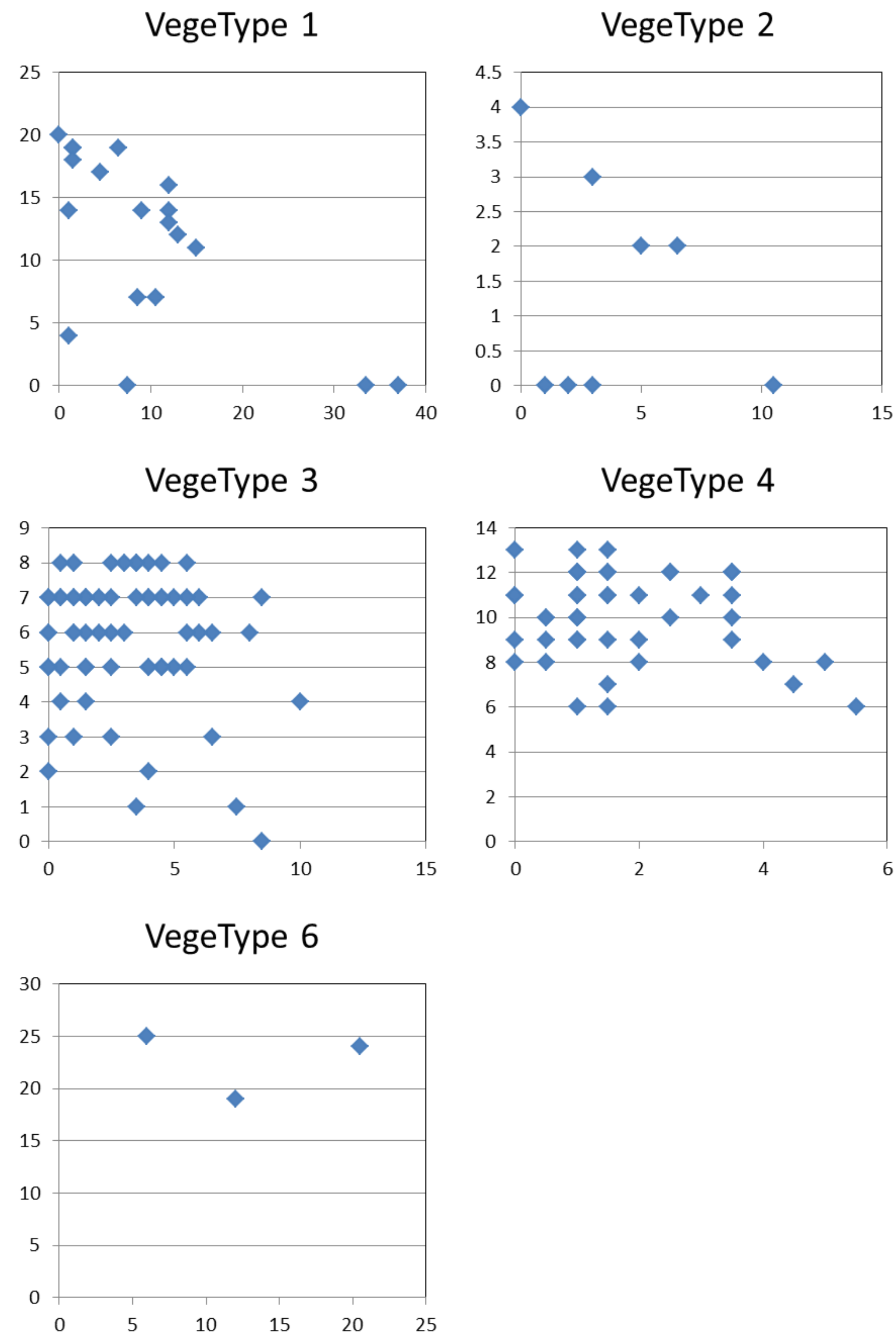


Figure 17 (b) Rep 2

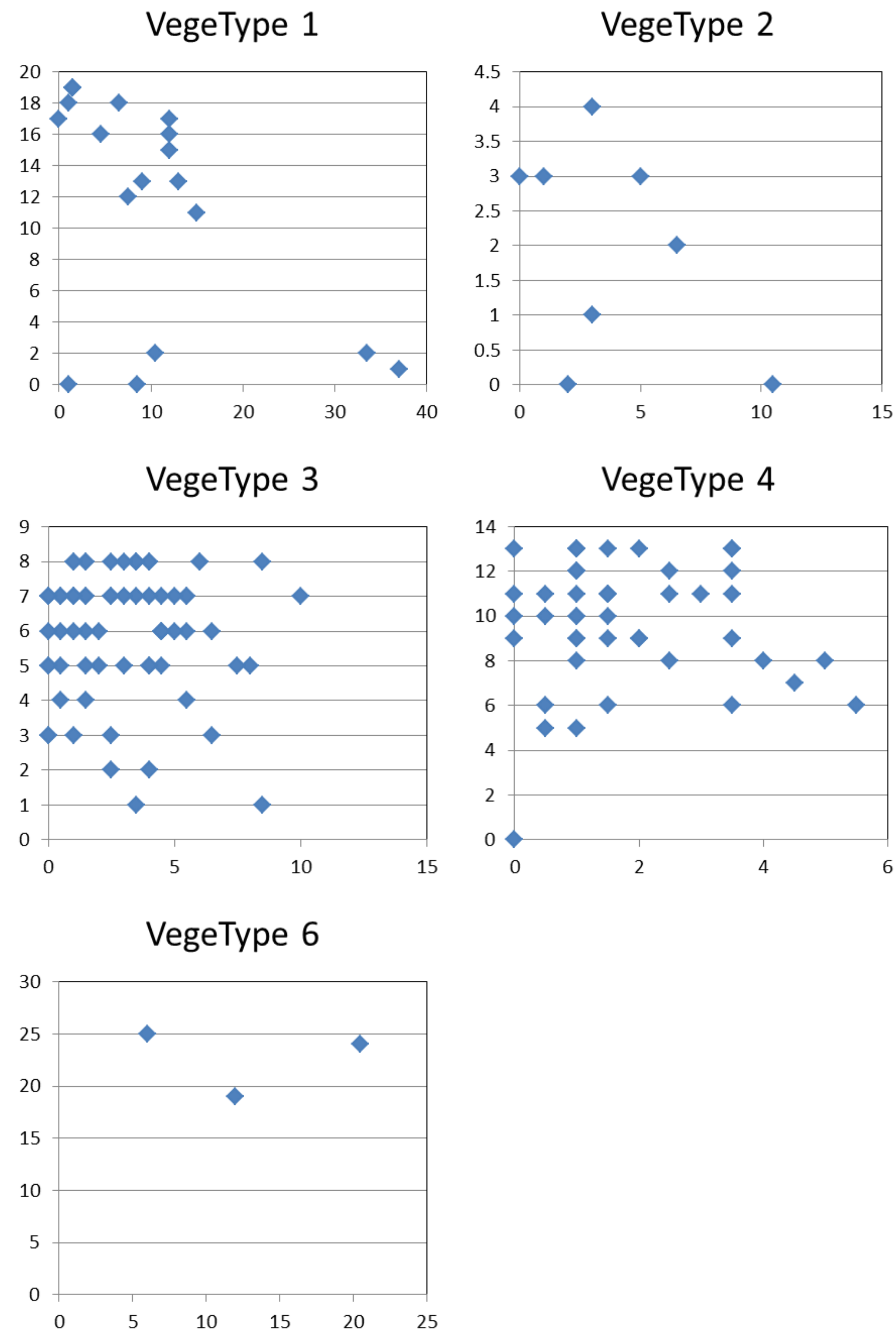


Figure 17 (b) Rep 3

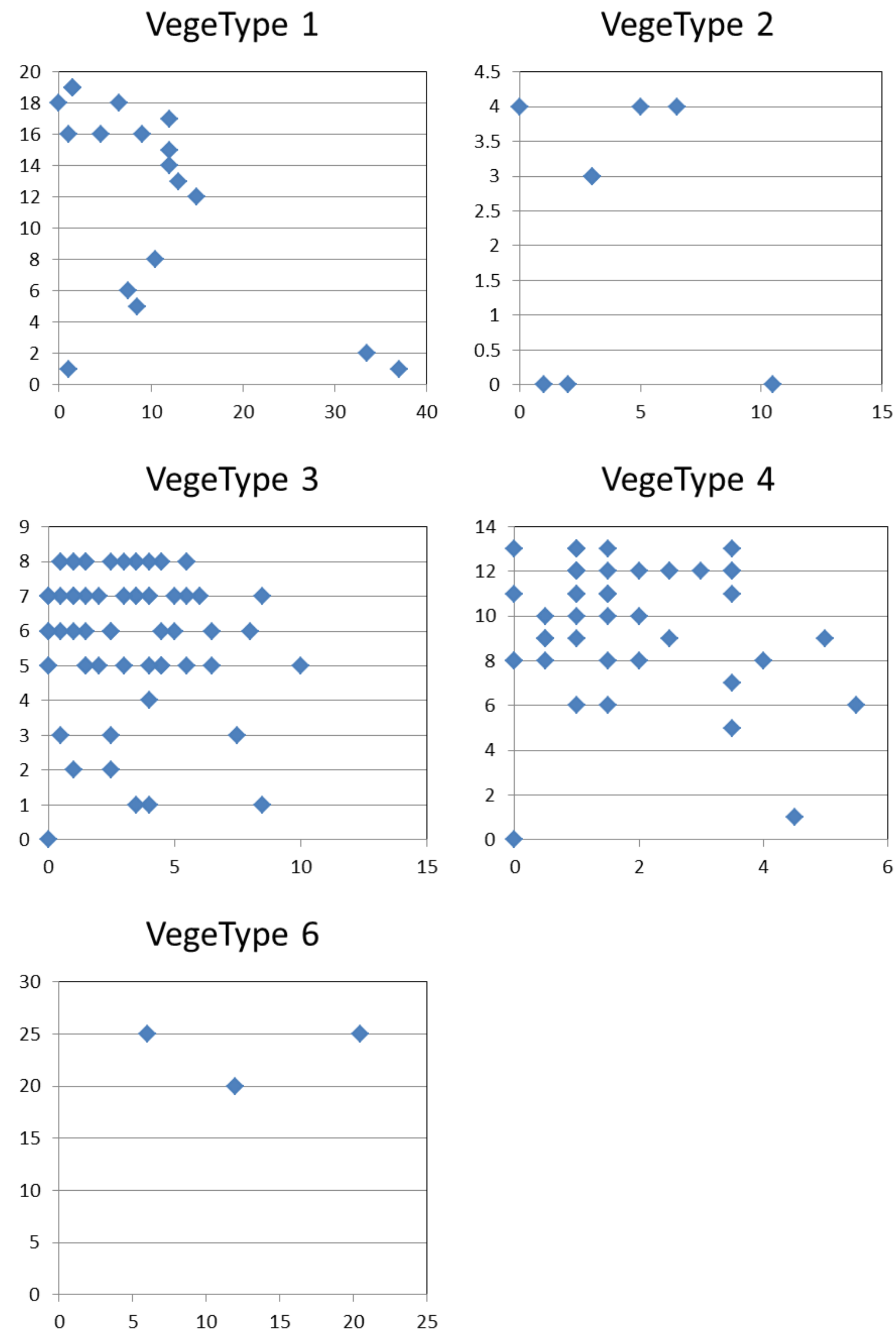


Figure 17 (b) Rep 4

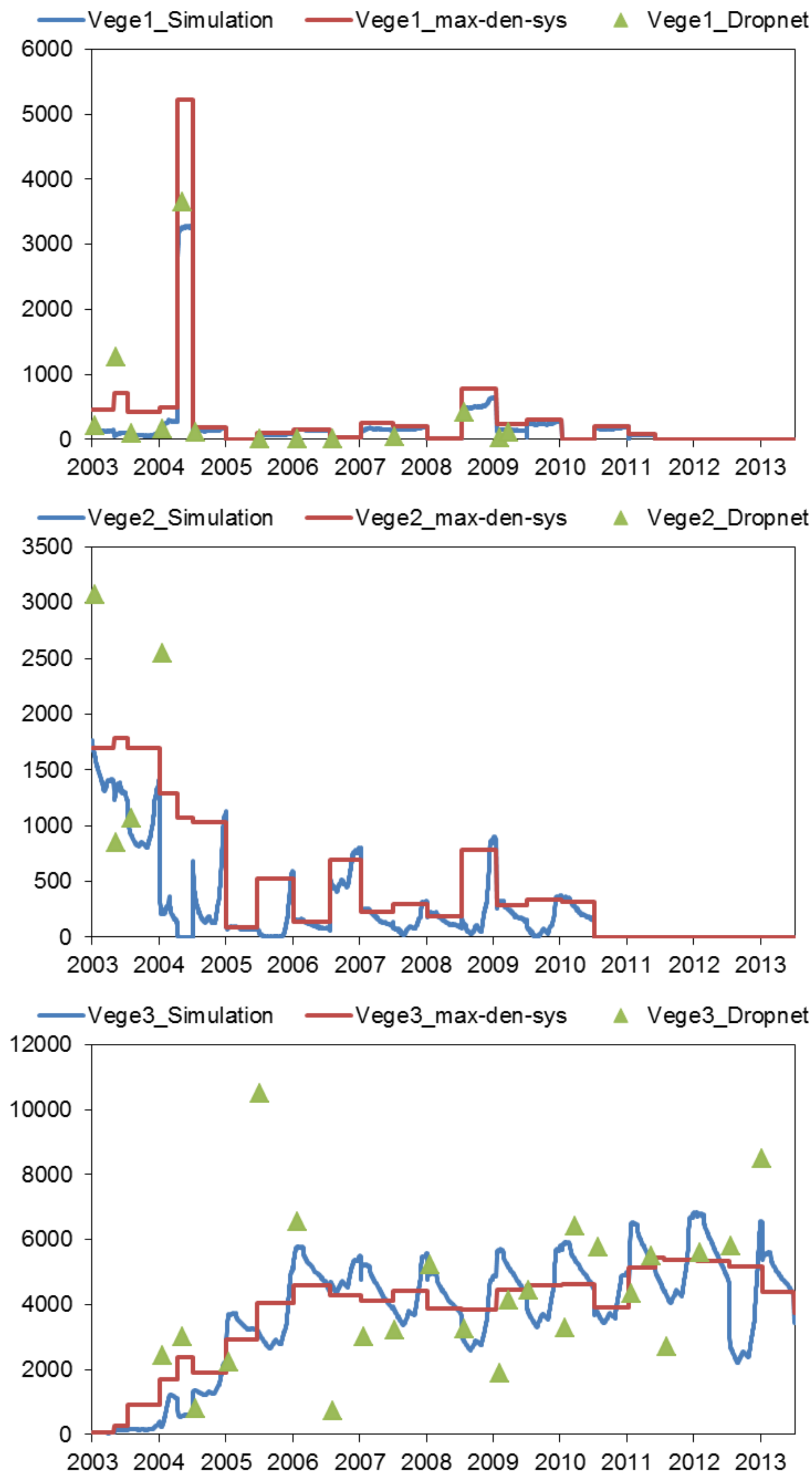
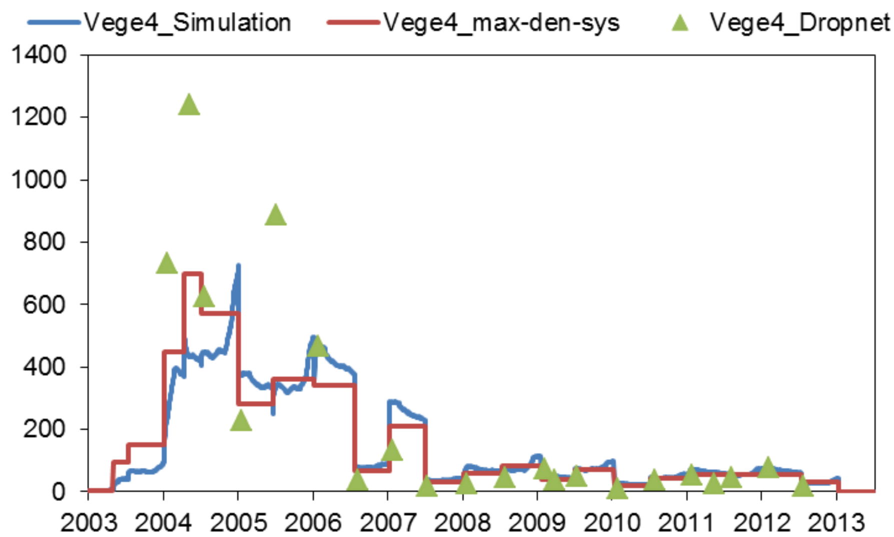

Vege1_Simulation
Vege1_max-den-sys
Vege1_Dropnet
6000
5000
4000
3000
2000
1000
0
2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013
Vege2_Simulation
Vege2_max-den-sys
Vege2_Dropnet
3500
3000
2500
2000
1500
1000
500
0
2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013
Vege3_Simulation
Vege3_max-den-sys
Vege3_Dropnet
12000
10000
8000
6000
4000
2000
0
2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013

Figure 18



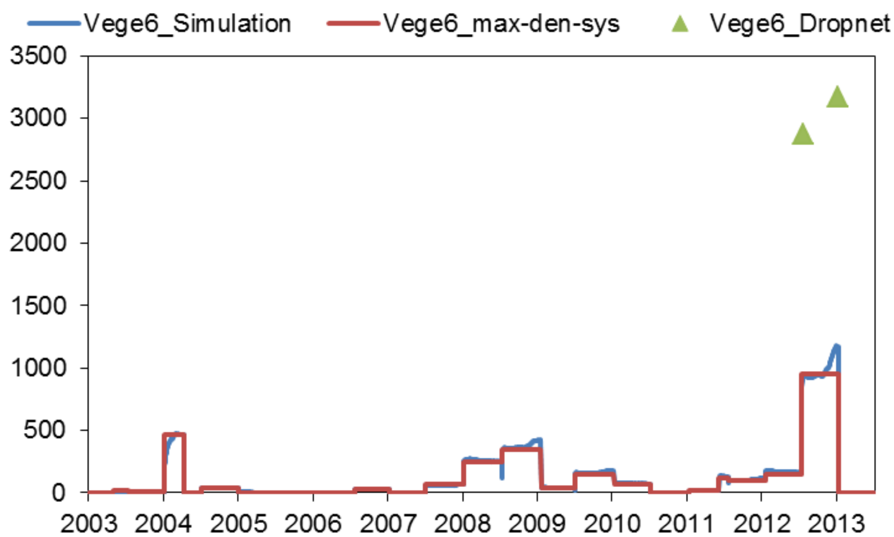


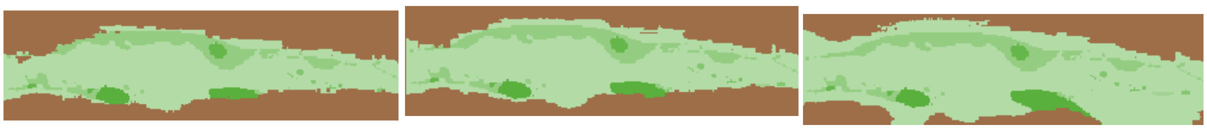
Figure 18 Cont.

APPENDIX G

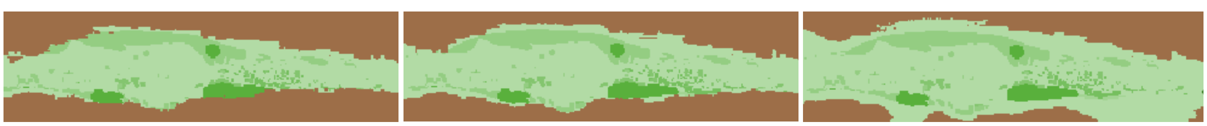
Preliminary Stage Verification Output

10 cfs 35 cfs 80 cfs

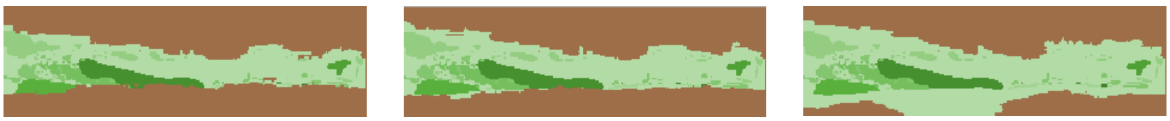
(a) Spring 2003



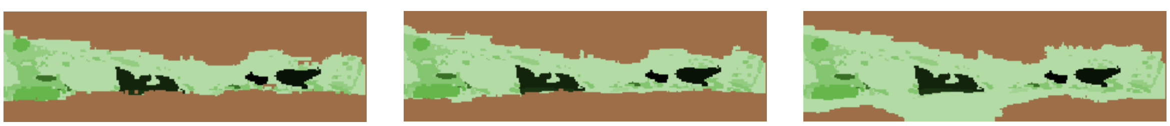
(b) Summer 2003



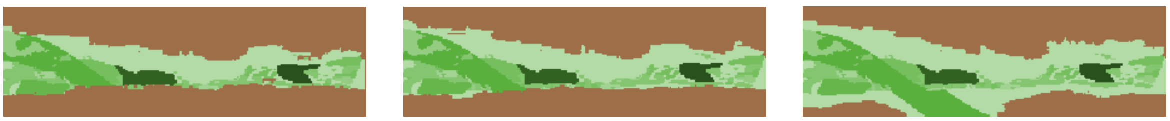
(c) Fall 2003



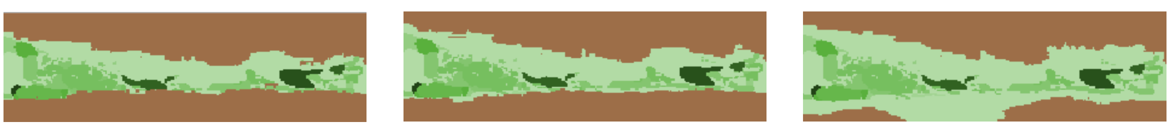
(d) Spring 2004



(e) Summer 2004

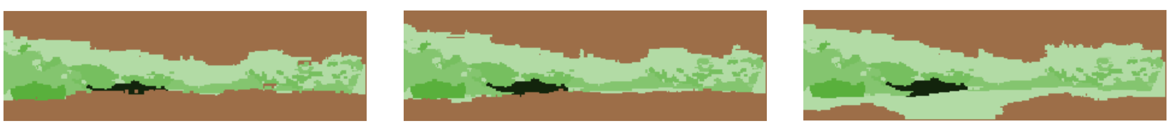


(f) Fall 2004

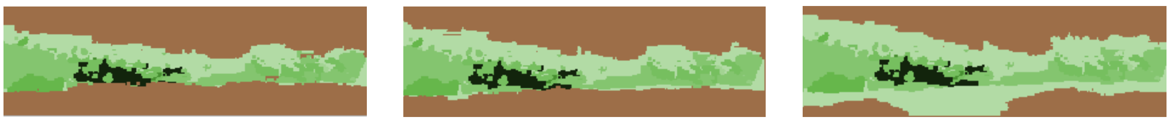


(g) Spring 2005

(h) Fall 2005

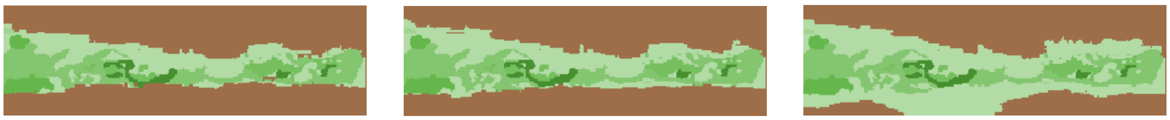


(i) Spring 2006



(j) Fall 2006

(k) Spring 2007



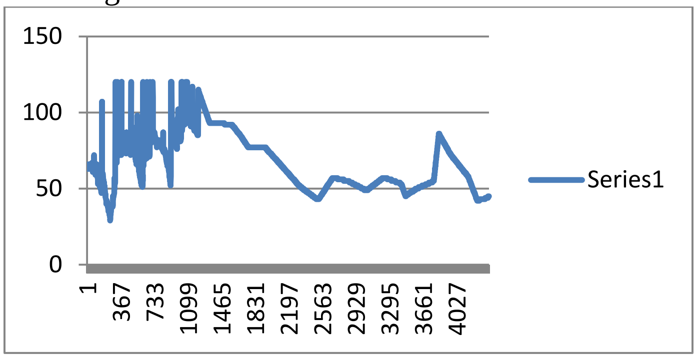
(l) Fall 2007

(m) Spring 2008

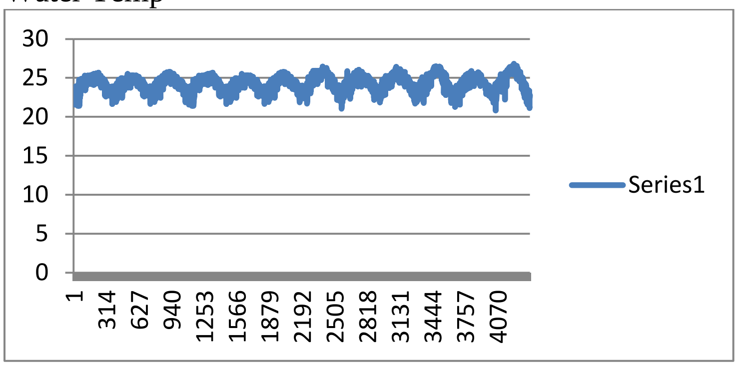
(n) Fall 2008

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.

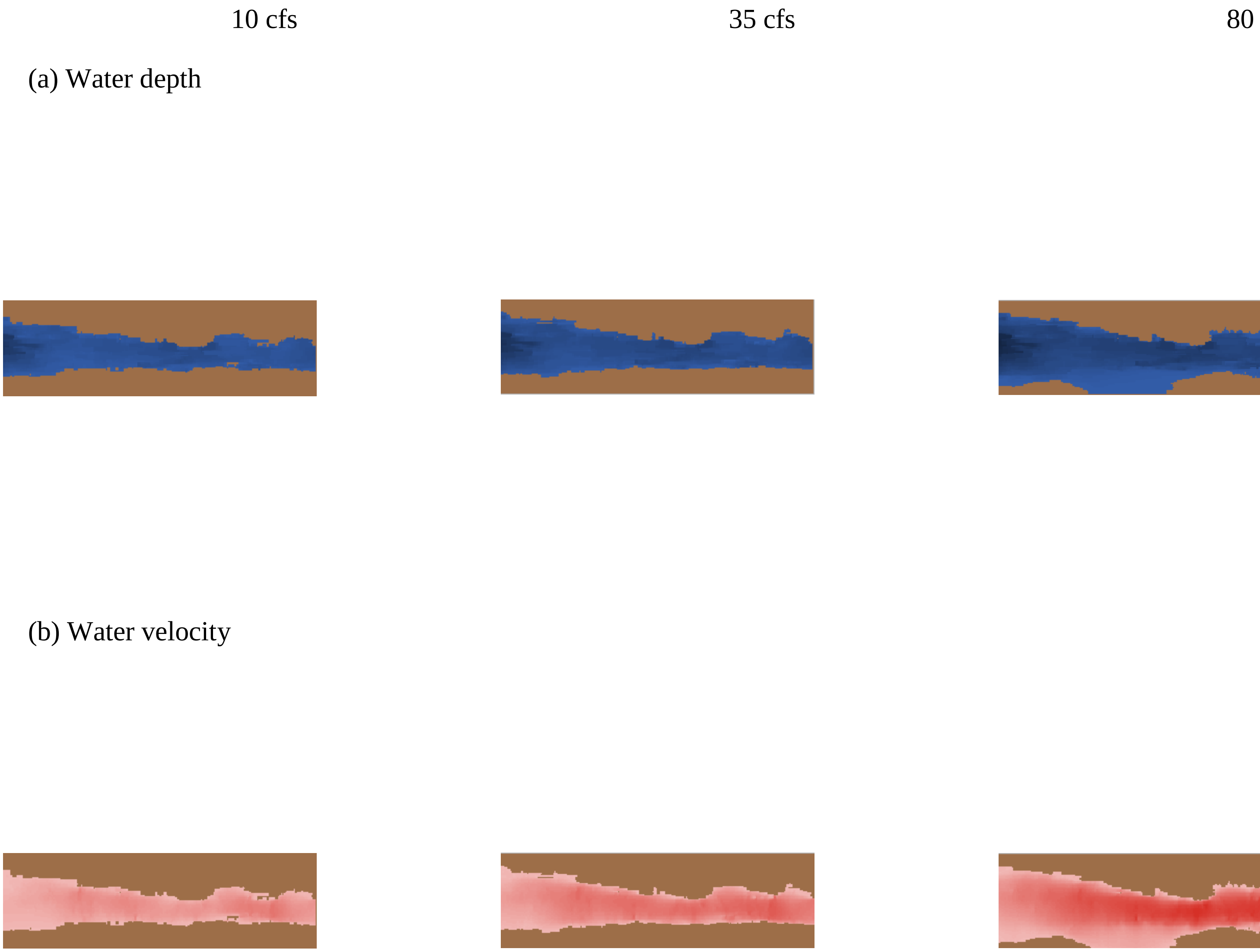
Discharge



Water Temp

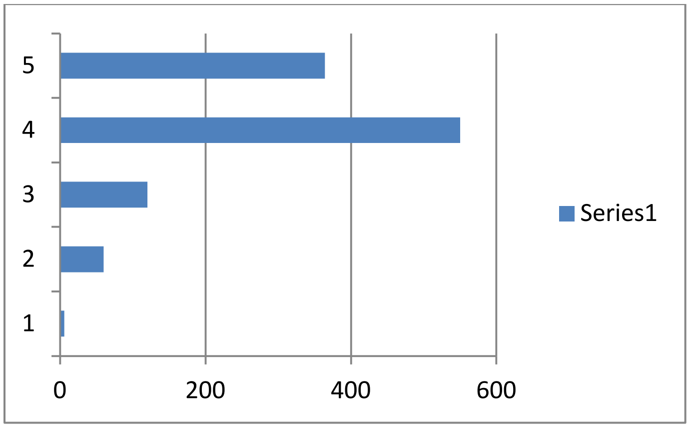


Appendix G. Figure 2. Simulated temporal dynamics of historical water discharge and temperature from 2003 through 2014 for the Old Channel of the Comal River.

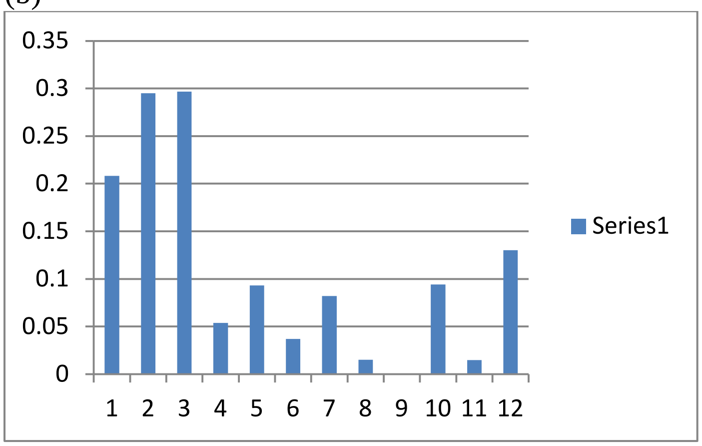


Appendix G. Figure 3. Simulated spatial-temporal dynamics of fountain darter habitat conditions as indicated by the simulated distribution of (a) water depth and (b) water velocity at 0.28, 0.99, and 2.26 m3 sec-1 (10, 35, and 80 cfs), respectively, in the Old Channel of the Comal River. Darker colors 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).