Appendix L

City of New Braunfels Reports

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Appendix L1

City of New Braunfels Standard Operating Procedures for Managing Flow Partitioning between the Old and New Channels of the Comal River

City of New Braunfels Standard Operating Procedures for Managing Flow Partitioning between the Old and New Channels of the Comal River

August 31, 2015

Purpose: To manipulate flow from Landa Lake into the Old Channel of the Comal River in order to maintain flow partitioning between the Old and New Channels of the Comal River according to Table 5-3 of the Edwards Aquifer Habitat Conservation Plan (EAHCP).

Background: Installation of a Waterman C-10 gate structure was completed in June 2014. The control gate allows City of New Braunfels staff to increase or decrease flow rates from Landa Lake into the Old Channel of the Comal River, via a 48” culvert pipe, by adjusting the control gate.

The United States Geological Survey (USGS) operates three streamflow gages within the Comal River. A map indicating the locations of the USGS streamflow gages is shown in Figure 1. Gage 08168913 is located in the Old Channel of the Comal River at a golf course cart crossing situated between Golf Course Road and Elizabeth Avenue (Latitude 29°42'36.28", Longitude 98°07'53.98" NAD83). Gage 08168932 is located at a weir structure within the New Channel of the Comal River at the Mill Race (Latitude 29°42'32.26", Longitude 98°08'00.39" NAD83). Gage 08169000 is located within the Comal River at San Antonio Street (Latitude 29°42'21", Longitude 98°07'20" NAD27) and measures total Comal River flow. All three USGS streamflow gages are instrumented to provide near-continuous streamflow data (at 15 minute intervals) which is accessible via the USGS National Water System Web Interface. The USGS gage for the Old Channel of the Comal River (Gage 08168913) can be accessed at http://waterdata.usgs.gov/tx/nwis/uv/?site_no=08168913&PARAmeter_cd=00065,00060. The USGS gage for the New Channel of the Comal River (Gage 08168932) can be accessed at http://waterdata.usgs.gov/tx/nwis/uv/?site_no=08168932&PARAmeter_cd=00065,00060. The USGS gage for the Comal River (Gage 08169000), which measures total Comal River flow, can be accessed at http://waterdata.usgs.gov/tx/nwis/uv/?site_no=08169000&PARAmeter_cd=00065,00060.

Figure 1. Locations of Flow-Split Culvert Gate and USGS streamflow gages

Table 5-3 of the HCP (Figure 2) provides flow partitioning guidelines for the Old and New Channels of the Comal River at varied total Comal River springflows. City of New Braunfels staff will manipulate the culvert gate valve in order to maintain flow-split ratios provided in Table 5-3. Observations made by City of New Braunfels staff indicate that the discharge rating curve established for the flow control gate and culvert (Appendix 1) is consistent with measured streamflow at the USGS Old Channel gage. A Flow-Split Management Worksheet (Appendix 2) has been developed to record gate adjustments and streamflow rates prior to and following control gate adjustments.

Flow Split Management for Old and New Channels (Table 5-3 in EARIP HCP)						
Total Comal Springflow (cfs)	**Old Channel (Cfs)**			**New Channel (Cfs)**		
	Fall/ Winter		*Spring/ Summer*	*Fall/ Winter*		*Spring/ Summer*
350+	80		60	270+		290+
300	80		60	220		240
250	80		60	170		190
200	70		60	130		140
150		60			90	
100		60			40	
80		50			30	
70		50			20	
60		40			20	
50		40			10	
40		30			10	
30		20			10	

Figure 2. HCP Flow-Split Table

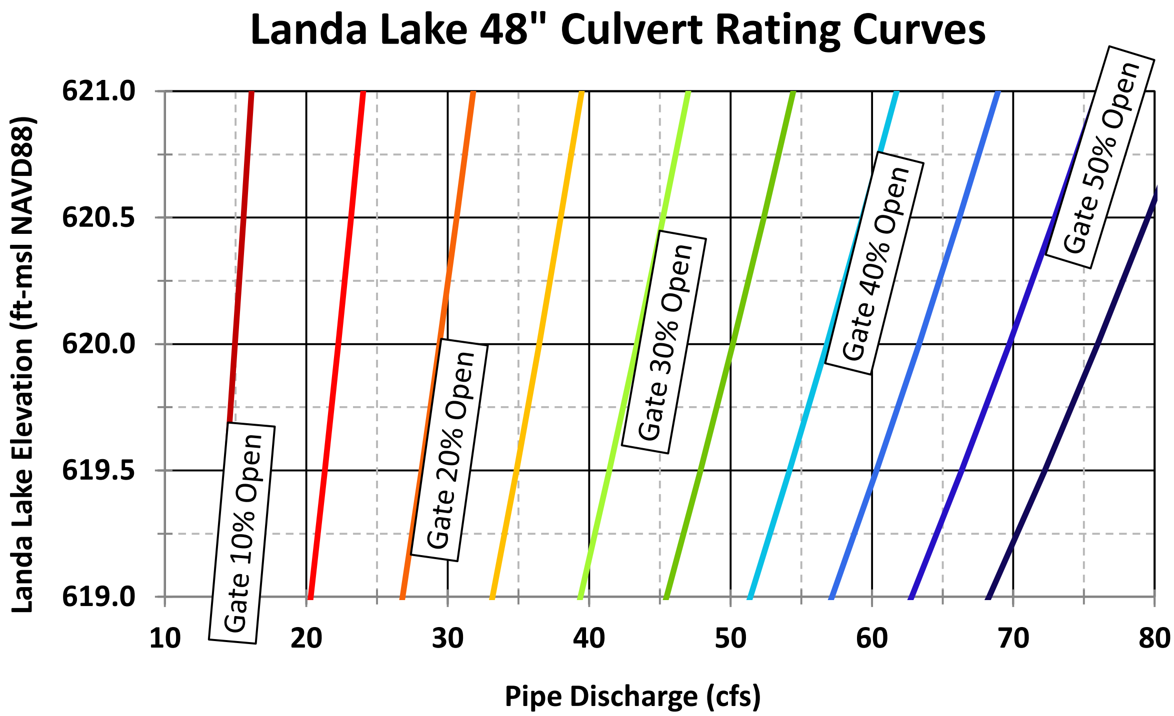
Procedures for Evaluating Comal River Streamflow Conditions and Making Adjustments to the 48" Culvert Control Gate

The following procedures will be implemented by City of New Braunfels staff when making adjustments to the 48" control gate system governing flow from Landa Lake into the Old Channel of the Comal River. Each of the three USGS streamflow gages within the Comal system are monitored by City of New Braunfels staff on a regular, on-going basis to evaluate the need for flow-split adjustments based on Table 5-3.

1. When streamflow conditions warrant the need for flow adjustments, existing streamflow conditions, according to USGS Comal River gages, are documented prior to making adjustments to the flow control gate.
2. Ultimately, adjustments to the control gate are made based on the USGS gages at the Old and New Channels of the Comal River (Gages 08168913 and 08168932, respectively). Achieving the target flow rate in the Old Channel is given priority over flow rates in the New Channel. The control gate discharge rating curve is used only as a reference when making gate adjustments. All data is recorded on the Flow-Split Management Worksheet.
3. A chain and lock has been installed on the gate wheel to prevent unauthorized adjustments to the gate system. Prior to making adjustments to the gate, remove the lock and chain using the key retained by City of New Braunfels staff.

4. Prior to making adjustments to the culvert gate, a measurement is taken from the top of the gate frame to the top of the gate wheel stem (Photo 1). This measurement is documented on the Flow-Split Management Worksheet to reflect the gate setting prior to adjustments.
5. The gate is opened/ closed by turning the gate control wheel one turn at a time. Each full turn of the gate wheel opens/ closes the control gate approximately 1/4". Control gate adjustments of 1/4" (or one full wheel turn) have previously resulted in approximately 1 cfs increase/ decrease in flow. Following control gate adjustments, an additional measurement is taken from the top of the gate frame to the top of the gate wheel stem. This measurement is also documented on the Flow-Split Management Worksheet.
6. Following any gate adjustment, at least two hours is allowed to pass to allow for two streamflow readings to be updated on the USGS streamflow web interface (the USGS gages log measurements on a 15 minute intervals but may take up to an hour to post to the web interface per discussion with Michael Nyman w/ USGS). City of New Braunfels staff documents USGS streamflow gage readings for each of the gages on the Flow-Split Management Worksheet.
7. Steps 4, 5, and 6 are repeated until Old Channel streamflow is observed to be consistent with the flow rates for the Old Channel as indicated on the flow-split table. If needed, adjustments of more than 10 cfs shall be made over 2-3 days, if possible, in order to avoid large flow pulses into the Old Channel.
8. The chain and lock is re-installed at any time that City of New Braunfels staff is not present at the gate structure.
9. The USGS streamflow gages are consulted again on the day following the initial adjustments to ensure adjustments were properly and target flow rates, per Table 5-3, were achieved.
10. Additional adjustments are made, as-needed, according to the above procedures until the proper flow-split ratios are met for the Old and New Channels of the Comal River according to Table 5-3 of the EAHCP.
11. All gate adjustments, and corresponding streamflow rates, are documented by City of New Braunfels staff on the Flow-Split Management Worksheet and are referred to when making subsequent flow adjustments. The standard operating procedures provided will be revised (and dated) as ongoing observations are made regarding the operation and functionality of the control gate and the management of flow partitioning.
12. The City of New Braunfels will coordinate with Edwards Aquifer Authority Habitat Conservation Program staff to notify the United States Fish and Wildlife staff of any planned flow adjustments that significantly deviate from Table 5-3 of the EAHCP.
13. The control gate will be exercised and maintained on a routine, ongoing basis to ensure continued operability and functionality. Any observed issues will be corrected immediately. The City of New Braunfels will perform an inspection of the flow-control gate and controls on a quarterly basis. The condition of the flow-control gate, required maintenance, and any other pertinent issues that may impact operation of the gate will be noted on the Flow-Split Control Gate Maintenance log (Appendix 3).
14. Appropriate training will be provided to any City of New Braunfels staff deemed responsible for making adjustments to the control gate.

Appendix 1. Rating Curve for 48” Culvert and Waterman C-10 Gate



A discharge rating curve has been developed for the 48” culvert and gate system. The rating curve provides a correlation of discharge to the percentage of gate open at varying Landa Lake elevations. The rating curve is used as a guideline when establishing gate position and flows, however, actual flow-split adjustments are made based on the USGS streamflow gages.

Appendix 2. Sample Flow Split Management Worksheet

Date	Time	Individual Performing Flow Observation/ Gate Adjustment	Streamflow Prior to Gate Adjustment				Gate Wheel Stem Height Prior to Adjustment (inches above gate frame)	Streamflow Following Gate Adjustment				Gate Wheel Stem Height After Adjustment (inches above gate frame)	# of wheel turns made	Observations
		Initials	Total Comal (cfs)*	Total Comal-Sum of OC&NC (cfs) **	Old Channel (cfs)***	New Channel (cfs)****		Total Comal (cfs)*	Total Comal-Sum of OC&NC (cfs) **	Old Channel (cfs) ***	New Channel (cfs)****			

*Total Comal streamflow as measured at USGS Gage 08169000

**Total Comal streamflow as a sum of streamflow measured at the USGS Old Channel and New Channel Gages

***Old Channel streamflow as measured at USGS Gage 08168913

****New Channel streamflow as measured at USGS Gage 0816893

Appendix 3. Sample Flow-Split Control Gate Maintenance Log

Flow-Split Control Gate Maintenance Log			
Date of Inspection	**Person Performing Inspection**	**Observed Issues**	**Summary of Maintenance Performed**

Reference Photo

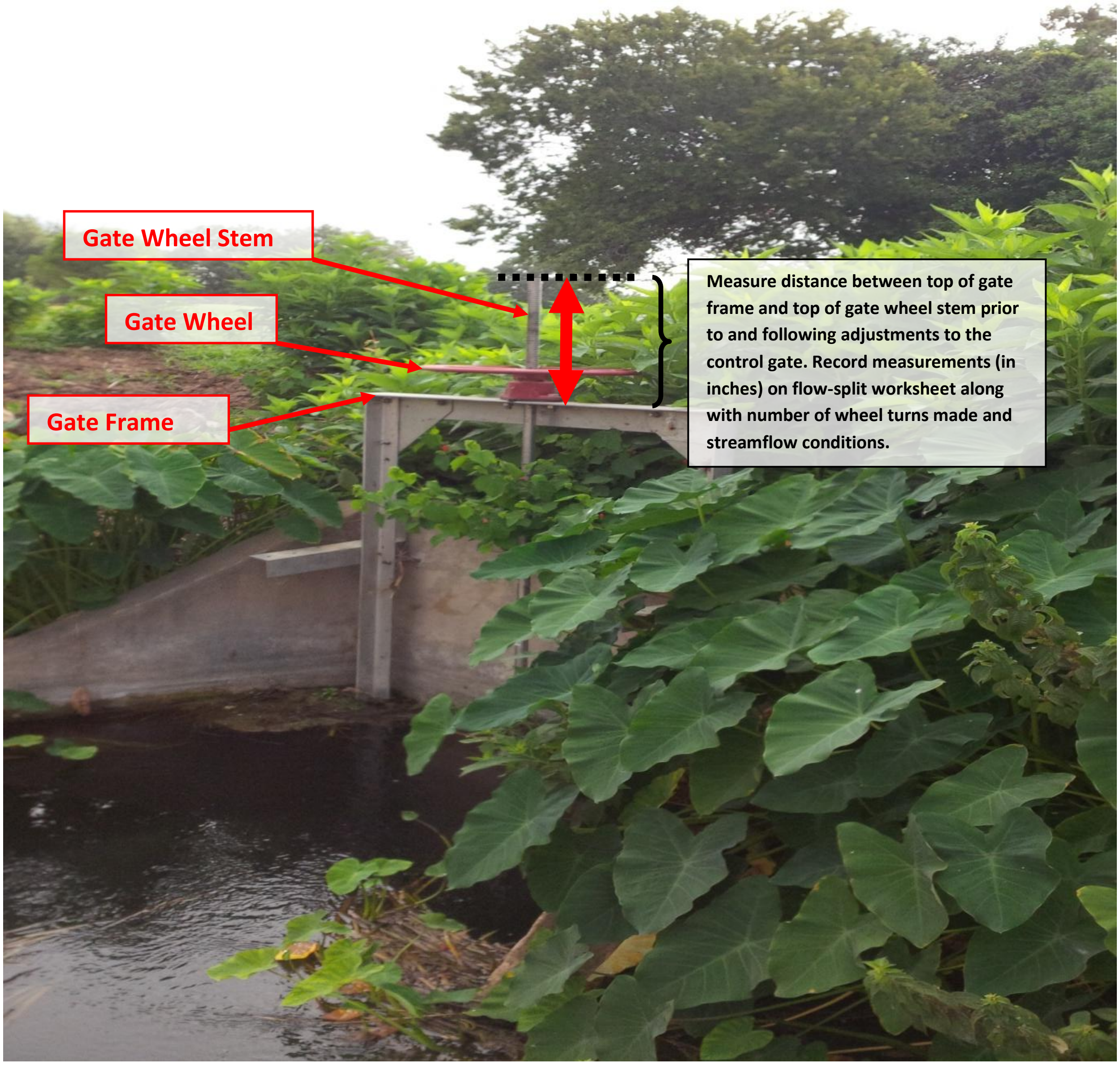


Photo 1. Waterman C-10 gate structure as viewed from the Landa Lake side of culvert.

Appendix L2

2015 Native Aquatic Vegetation Restoration in Landa Lake and Old Channel of the Comal River

2015 Native Aquatic Vegetation Restoration in Landa Lake and Old Channel of the Comal River

PREPARED FOR

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November 6, 2015

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1.0 INTRODUCTION

The Habitat Conservation Plan (HCP) developed as part of the Edwards Aquifer Recovery Implementation Plan (EARIP) provides for ecological monitoring and research with the aim of fostering high-quality habitat for the many threatened and endangered species found in the Comal system. As part of this program, scientists with BIO-WEST, Inc. teamed with researchers from Baylor University's Center for Reservoir and Aquatic Systems Research ("Baylor") to develop and implement a native aquatic vegetation restoration plan for Landa Lake and the Old Channel within the City of New Braunfels, Texas (BIO-WEST 2013). The intent of this HCP measure is to maintain high-quality habitat for the endangered fountain darter (*Etheostoma fonticola*) by both increasing the amount of available habitat and by improving the quality of the existing habitat. This report summarizes the restoration activities conducted from January 2015 through October 2015, including a summary of the restoration progress and recommendations for future work.

1.1 Project areas

The main project areas for 2015 vegetation restoration work include the general extent of the Landa Lake HCP biological monitoring study reach and a stretch of the Old Channel of the Comal River bounded on the upstream side by the Landa Lake outflow and bounded on the downstream side below Elizabeth Street at the downstream-most extent of the Old Channel HCP biological monitoring study reach (Figure 1). Landa Lake is a small impoundment covering approximately 18 acres. It is located within the city of New Braunfels, Texas and is a relatively shallow water body, reaching a maximum depth of approximately 10 feet. Landa Lake receives spring flow from several sources, including (1) spring runs and peripheral springs that issue from fissures along the western bank of the lake, (2) low-pressure upwellings on the bottom of the lake, and (3) from the "Upper Spring Run" area upstream of the lake. Landa Lake discharges into two channels: the Old Channel, which is the original water course of the upper Comal River, and the New Channel, which is a man-made canal. These two water courses meet downstream of Landa Lake, where they form the main stem of the Comal River.

Figure 1. Location of the Landa Lake and Old Channel restoration areas (Red). Green indicates the extent of 2013 and 2014 restoration activities in the Old Channel, yellow represents extent for 2015 activities, and dark blue indicates areas of future invasive vegetation removal and habitat restoration.

1.2 Comal River aquatic plant community

In 2013 the project team determined a path forward for the project by conducting site assessments to gage habitat quality within the project areas and by analyzing historical accounts in order to determine previous expanses of native vegetation. The historical analysis supported the theory that many pockets of native vegetation in Landa Lake and the Old Channel had been replaced by non-native aquatic plant species, and that the areas slated for restoration could, following restoration activities, once again support native plant communities.

1.2.1 Historical plant communities

Historically, the Comal Spring system has had a diverse native plant community. Approximately 10 species of obligate aquatic vascular plants are considered dominant in the vegetative community of the Comal system (Williams 2011). During surveys in 1993, Crowe (1997) identified and mapped six dominate aquatic plants. Later studies (Cauble 1998, Ourso 1998) found similar results. The Comal system also supports a robust community of aquatic mosses and liverworts, generally referred to as bryophytes. In addition to these native plants, several species of aquatic plants have been introduced into the Comal system. These include the floating fern *Ceratopteris thalictroides*, *Limnophila sessiliflora*, *Hydrilla verticillata* and *Hygrophila polysperma (*called *Hygrophila*

throughout this report*)*. While these plants are often considered extremely invasive species, so far *Hygrophila* is the only one with a distribution that encompasses a large area of the Comal system.

Hygrophila is an invasive aquatic plant species native to India, Sri Lanka, and Malaysia. It is an amphibious aquatic, able to survive well in submersed flowing conditions and also to grow terrestrially in muddy locations along the water's edge. *Hygrophila* is listed as a Federal Noxious weed by the USDA (USDA 2006). While its amphibious growth characteristics and reported tolerance to a wide range of light and water quality conditions contribute to its success as an invasive weed (Spencer and Bowes 1985), a recent study of *Hygrophila* in the San Marcos River shows the plant is mostly found in conditions of moderate water flow and silty substrates (Williams 2013).

1.2.2 Hygrophila invasion in the Old Channel

In Texas, *Hygrophila* was first identified in the San Marcos River and later the Comal, where it had probably been present for decades before being positively identified (Angerstein and Lemke 1994). *Hygrophila* is thought to have begun to dominate the Comal system at some point shortly before the year 2000, possibly due to scouring events that cleared habitat previously occupied by other species (Doyle 2002). In 2010, the total system coverage of *Hygrophila* in the Comal River was 25,752 m², making it the second most common aquatic plant after *Vallisneria* sp. (Williams 2011).

The last 200 river meters of the Old Channel restoration area, below Elizabeth Street, is designated as the Old Channel study reach for the Biological Monitoring Program of the HCP. Vegetation in this section has been mapped by BIO-WEST at least twice each year since 2001. This historical data is a valuable source regarding what the aquatic plant community looked like before invasion of *Hygrophila*, and, accordingly, how the community has changed after *Hygrophila*'s takeover (Figure 2). Maps made prior to 2002 showed that the Old Channel Study reach was dominated by algae and *Ceratopteris thalictroides*, with very little other submersed aquatic vegetation present. However, by 2012, this section was almost entirely dominated by *Hygrophila*, interspersed with *Ludwigia* and *Nupahar lutea*, a waterlily species.

As part of the HCP biological monitoring activities, in February 2013 the project team made up-to-date baseline maps of the Landa Lake (Figure 3) and Old Channel (Figure 4) restoration areas. These 2013 maps showed that *Ludwigia* was absent from the Old Channel biological monitoring reach below Elizabeth street. *Ludwigia*'s range was also very limited in the Landa Lake and Old Channel restoration areas (Table 1). In 2013, *Hygrophila* was the dominant submersed aquatic plant species in the Old Channel restoration stretch of the Comal River covering approximately 27 % of the total area (Table 1).

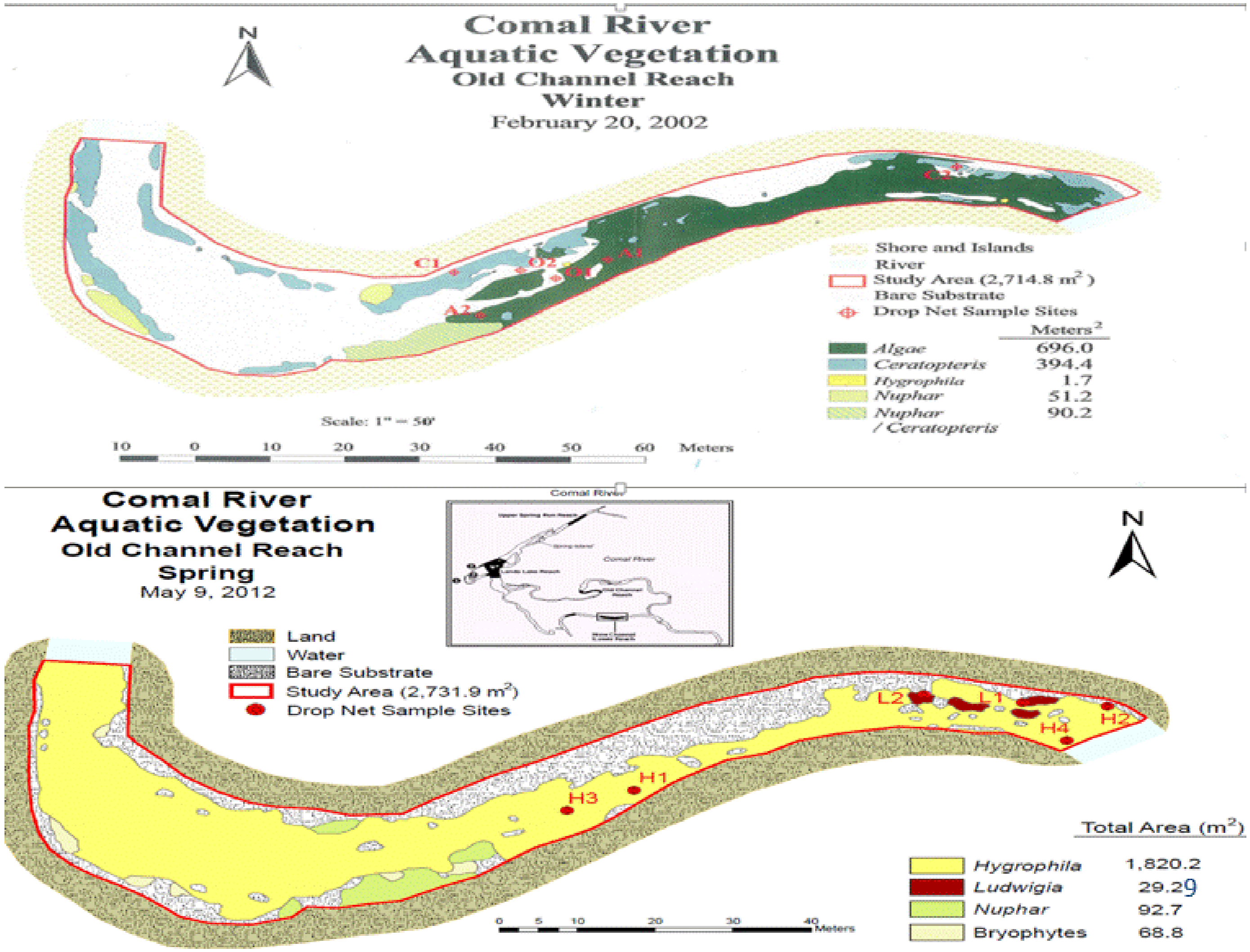

Figure 2. **Past vegetation mapping in a section of the Old Channel shows the composition of the aquatic plant community before (top) and after (bottom) *Hygrophila* invasion.**

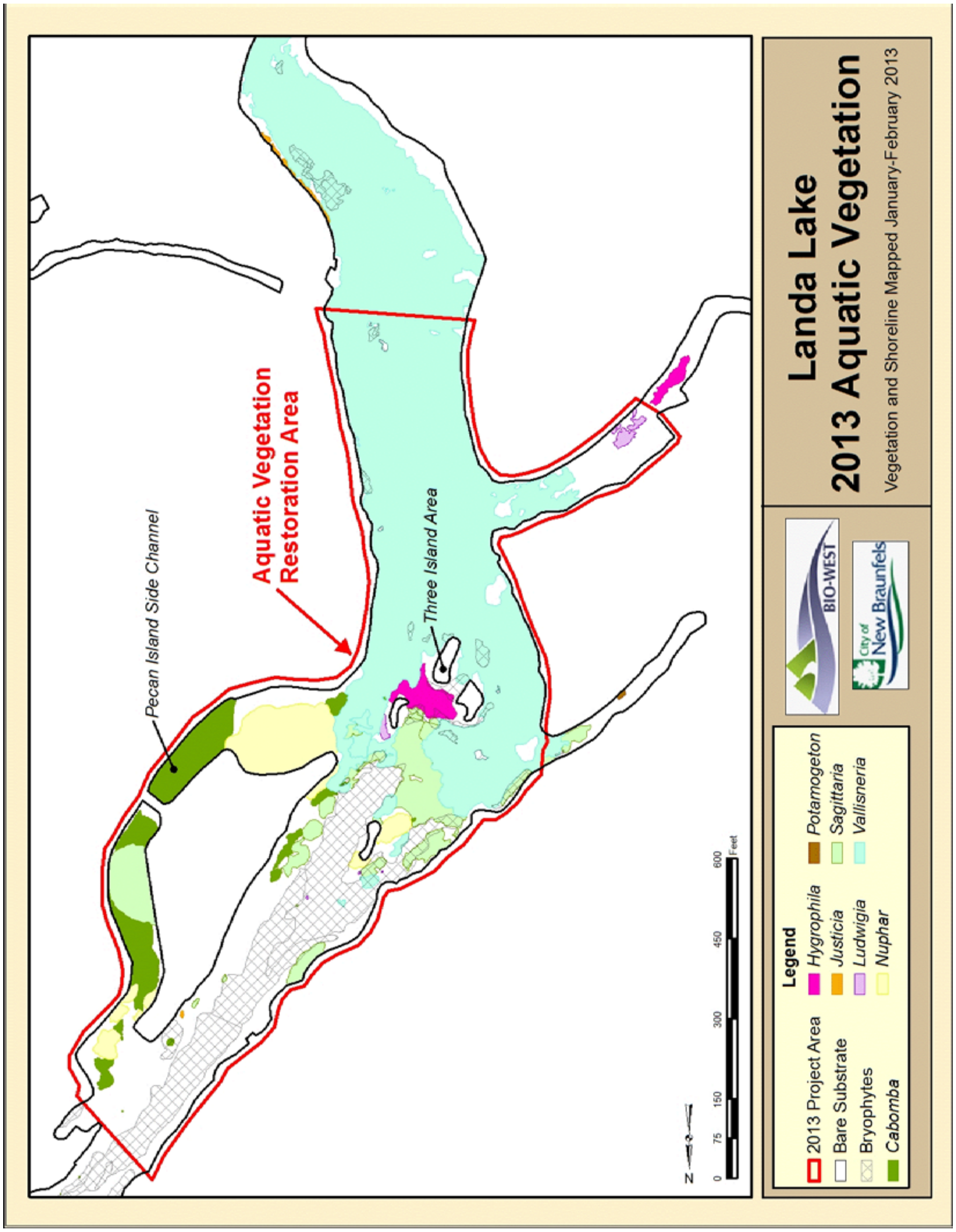

Figure 3. Pre-restoration mapped aquatic vegetation in Landa Lake (early 2013).

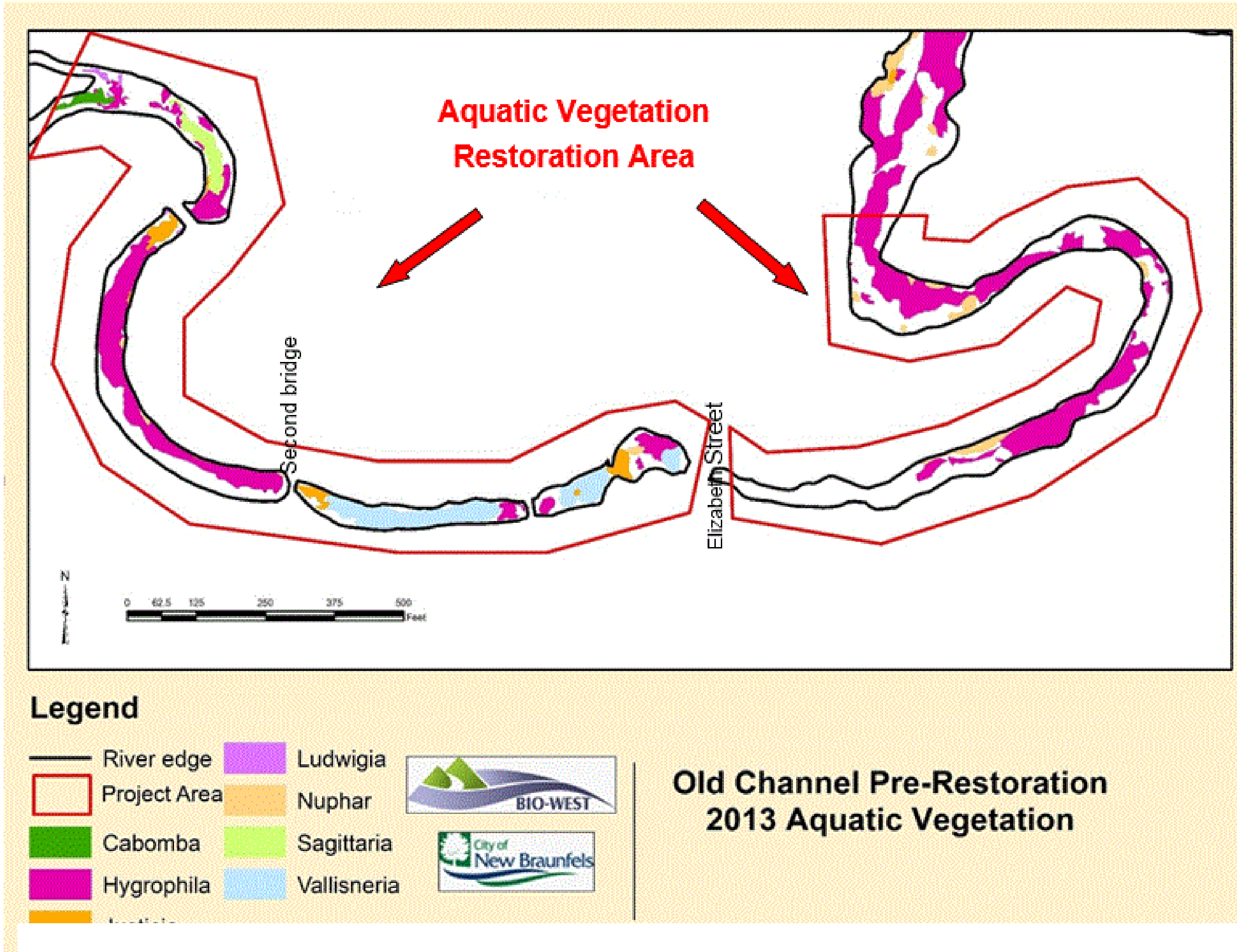

Figure 4. **Pre-restoration aquatic vegetation in Old Channel (early 2013).**

Table 1. Aquatic vegetation species and area coverage present in 2013 within the two Project Areas prior to HCP restoration activities.

Aquatic Plant Species	Area (m^2)	% of Total Area within Project Area
Landa Lake Project Area (47,889 m^2)		
Bryophytes*	7,019.9	14.7%
Cabomba Ɨ	2,713.1 (344.1)	5.7%
Hygrophila	522.9	1.1%
Justicia	6.4	0.01%
Ludwigia	191.7	0.4%
Nuphar	2,715.9	5.7%
Sagittaria	3,461.6	7.2%
Vallisneria	21,192.1	44.2%
Bare substrate	10,065.4	21%
Old Channel Project Area (8,050 m^2)		
Cabomba	117.3	1.6%
Colocasia	164.1	2%
Hydrocotyle	2.2	0.02%
Hygrophila	2,177.4	27%
Justicia	372.2	4.6%
Ludwigia	123.7	1.5%
Nuphar	70.3	0.9%
Sagittaria	281.6	3.5%
Vallisneria	1,132.7	14.1%
Zizaniopsis	130.7	1.6%
Bare substrate	3,477.8	43.2%

*Presented as bryophytes mapped as the dominant vegetation. An additional 1,026.8 m^2 of bryophytes is overlying other mapped vegetation types.

Ɨ This number has since been modified from previous reports. Number in parentheses removes the large amount of *Cabomba* located within Pecan Island side channel. This number (344.1) should be considered the baseline number for *Cabomba* in the Landa Lake restoration area.

1.2.3 Target aquatic plant species for restoration

In order to identify target native species for restoration, the project team looked at several factors, including the quality of habitat the species provides for the fountain darter, historical and current distribution of the species in the system, and the suitability of the species for propagation. Based on these factors the project team, in 2013, identified three native species—*Ludwigia, Cabomba* and *Sagittaria*—and their corresponding habitat types for use in restoration activities:

In addition to the three native aquatic species used in 2013, and two additional native aquatic plants used in 2014 (*Potamogeton* and nonvascular bryophytes/aquatic mosses), restoration efforts in 2015 included the addition of the vascular *Vallesneria*. A brief description of each vascular species is provided below.

Cabomba caroliniana

Cabomba is a perennial branching species which typically grows in large stands, in silty substrates, in low velocity areas, and downstream of velocity shelters. *Cabomba* is considered to be quality fountain darter habitat (BIO-WEST 2014a).

Current distribution in Landa Lake is limited but historically had a much wider distribution. Propagation difficulty for restoration purposes is under investigation.

Ludwigia repens

Ludwigia is a perennial branching species which typically grows in small to large areas as individual specimens or in large stands. *Ludwigia* grows in sand and gravel substrates, generally in areas with moderate velocities. *Ludwigia* provides excellent fountain darter habitat.

Ludwigia has very small distribution within the Comal system at present, but was historically widespread. It is easy to propagate (Doyle 2002).

Sagittaria platyphylla

Sagittaria is a perennial upright rosette species commonly found in silt and sand substrates, generally in areas with low to moderate velocities. Although *Sagittaria* offers low suitability for fountain darter habitat, it does provide good substrate for darter egg deposition (Phillips et al. 2011). *Sagittaria* also provides velocity shelters for *Cabomba* and bryophytes.

Historically, *Sagittaria* had a restricted distribution in the Comal system but is now widespread. It is also easy to propagate (Doyle 2002).

Potamogeton illinoensis (Illinois pondweed)

Potamogeton has had a historically expansive distribution in the Comal River, but total system mapping in 2013 showed it only occupied 3 m^2 within Spring Run 3 and a few small stands in the New Channel. It is a submersed aquatic plant that grows equally well in rapidly flowing or in still water. *Potamogeton* grows well in areas with cobble substrate where other native aquatic plant species grow poorly. It makes up a large portion of the aquatic plant community in the San Marcos River, where it is able to compete with *Hygrophila*. In the San Marcos River, density studies have shown that *Potamogeton* is fountain darter habitat, having an approximate density of about 6 darters per m^2 (BIO-WEST 2014b). *Potamogeton* is easy to propagate and is tolerant both of water drawdowns and deep water. *Potamogeton* is suited for restoration work in that it is robust, capable of thriving in very fast-moving water and rocky substrate, which *Ludwigia*, *Cabomba* and *Sagittaria* do not tolerate well. This allows *Potamogeton* to fill a niche that the other restoration plant species cannot. Secondly, *Potamogeton* produces an extensive rhizomatous root system in the sediment, which helps stabilize the streambed and provides good velocity shelter for other aquatic plant species to establish downstream. *Potamogeton* can help protect the system from scour events by (1) stabilizing the streambed, (2) providing velocity shelters for more scour-sensitive plants, such as *Cabomba* and *Ludwigia*, and (3) provide velocity shelters for

fountain darters.

Vallisneria neotropicalis (Eel grass)

Vallisneria is a dominant member of the aquatic plant community in Landa Lake. It is also found in a few isolated locations in the Old Channel. *Vallisneria* was first used in the restoration plan this year. Due to its affinity for flowing water, we utilized *Vallisneria* in selected locations as yet another velocity shelter for restored species. Similar to *Potamogeton*, *Vallisneria* disperses water velocity and reduces turbulent flow within the area downstream of its colony, providing optimal growing conditions for native velocity-sensitive species. It also provides an important ecosystem service by holding streambed sediment in place. However, *Vallisneria's* growth form is very different from *Potamogeton*. Its architecture of long, overarching leaves allows pockets of bryophytes to develop underneath the canopy, where turbulent flow is reduced. Usually these bryophyte pockets persist through high flow events. This characteristic is not seen in the densely growing *Potamogeton*. Utilizing *Vallisneria* provides another tool to help restore a sustainable and bio diverse plant community that has been absent in the Comal River for decades.

2.0 SPRING AND RIVER CONDITIONS IN 2015

Unlike 2014, daily discharge in 2015 remained near or above historical averages for the majority of the year, and well above the 130 cfs daily threshold of HCP Provision M, which requires work cessation if flows fall below that mark (Table 2, Figure 5).

Table 2. Average monthly streamflow in the Comal River 2013-2015

Month	Average Monthly Streamflow (cfs)		
	2013	2014	2015
January	222.5	169.3	146.6
February	219.5	169.0	186.6
March	204.5	154.9	194.5
April	196.0	144.9	198.8
May	201.0	132.5	247.5
June	202.4	143.0	323.9
July	170.3	119.5	334.6
August	127.5	84.8	268.3
September	124.4	79.9	214.2
October	164.1	89.8	210.7
November	172.7	142.5	[a]
December	161.6	135.8	[a]

[a] Data not available

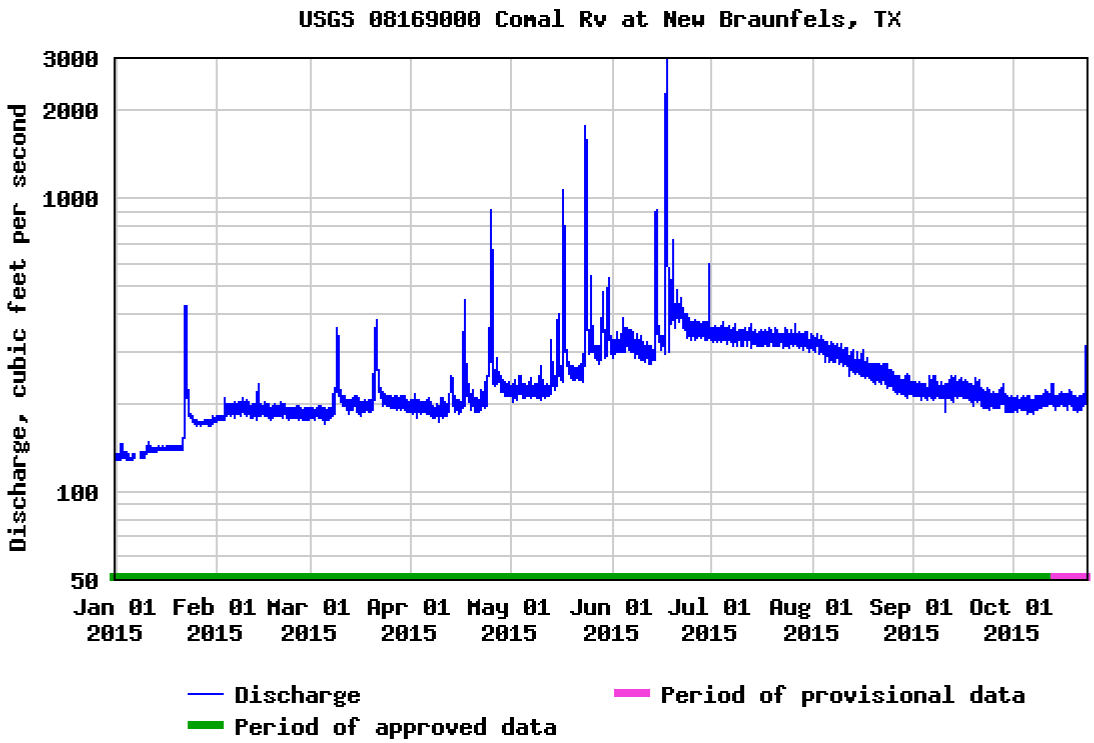

Figure 5. Discharge in the Comal River from January-October 2015 (USGS 2015).

3.0 PLANT PROPOGATION METHODS

Plants were propagated using the same methods as prior years. Mobile Underwater Plant Propagation Trays (MUPPTs) were utilized to propagate *Ludwigia* in the Landa Lake field nursery. *Ludwigia* was propagated in quart size nursery pots filled with clay based soil collected from Landa Lake. Pots were fitted into MUPPTs and plants were allowed to grow until they became rootbound within the pot, a period of about three weeks during peak growing season. Other target species were either transplanted or sprigged into the planting site from mother colonies within the Comal River. No off-site nursery-grown plants were introduced into the system in 2015.

4.0 AQUATIC VEGETATION RESTORATION PROGRAM

Successful restoration of aquatic plants can be negatively impacted by both abiotic and biotic factors. Due to the lack of an established root system, newly-planted transplants and sprigs are especially vulnerable to disturbance by changes in current velocity, and their growth can be decreased or inhibited by increased sedimentation rates, cover from bryophytes and algae, and increased turbidity. Herbivory by turtles, nutria, carp and insect larvae can also be detrimental to the establishment of native aquatic plants (Smart, Dick et al., 1998) (Hutchison et al., 2015). Herbivory has indeed occurred in several instances within the field nursery. However, the extent of herbivory is harder to determine once plants are planted. Since the Comal River is a unique ecosystem, and

ecological restoration is not an exact science, the project team continues to use an adaptive approach and system-specific planning. Prior to planting it was understood that not every restored site would produce similar results, even when the methods of *Hygrophila* removal and native plantings were similar.

Aquatic habitat restoration field efforts in the project areas consisted of three main activities, including (1) removal of invasive *Hygrophila*, (2) planting of native aquatic plants, and (3) monitoring and gardening of restored areas. Restoration occurred under the following permits obtained from the Texas Parks and Wildlife Department (TPWD):

a. Exotic species removal permit AVR 01 15-016 (for removal of *Hygrophila* and *Arundo*)

b. A permit to introduce fish, shellfish or aquatic plants into public waters (introduction of nursery grown plants) was obtained but not used in 2015

c. Plant species planted in 2015 included *Ludwigia*, *Cabomba*, *Sagittaria* and *Vallisneria*. No *Potamogeton* was planted in 2015. No permit was necessary for plants collected and replanted within the same waterbody.

4.1 *Hygrophila* removal

In 2015, significant effort was put into removing and eliminating *Hygrophila* in the restoration areas, as well as in upstream locales such as the Upper Spring Run (USR) and the spring-fed swimming pool. This upstream removal worked to prevent *Hygrophila* fragments drifting into restoration sites downstream.

In early 2014, most *Hygrophila* was removed from the Three Island area of Landa Lake, with one small remnant patch remaining. Mapping in early January of 2015 showed that this patch covered approximately 20 m^2. In March of 2015, this patch was removed, virtually eliminating *Hygrophila* from the Landa Lake restoration area. However, *Hygrophila* existed in several areas upstream in the USR reach. As these patches provided a continual supply of fragments to the downstream project area, it was decided that removal was necessary to prevent re-colonization of *Hygrophila* into Landa Lake. Initial removal of *Hygrophila* in USR began in May, and aquatic gardening of the area via the BIO-WEST SCUBA team continued intermittently until September (Figure 6 and Figure 7). *Hygrophila* in the USR was well rooted in gravel and cobble, and several passes were needed to thoroughly remove *Hygrophila* roots. In one particularly difficult location, a rubber pond liner held in place with sandbags was used to cover *Hygrophila* root mass. This barrier was left in place for three weeks, and when it was removed, no subsequent growth of *Hygrophila* in that location was observed. In total, 539 m^2 of *Hygrophila* were removed from the USR area. Another 12 m^2 of *Hygrophila* was removed from Landa Lake in the Pecan Island slough, outside of the Landa Lake restoration area. Small clumps of *Hygrophila* found in spring run 1 and along the Landa Lake dam were removed as well, but these were well under 1 m^2 combined. Although small, isolated clumps continue to exist in USR and elsewhere, these clumps are unlikely to provide substantial fragment material for *Hygrophila* spread in the near term, especially given the continued implementation of the aquatic gardening program in 2016.

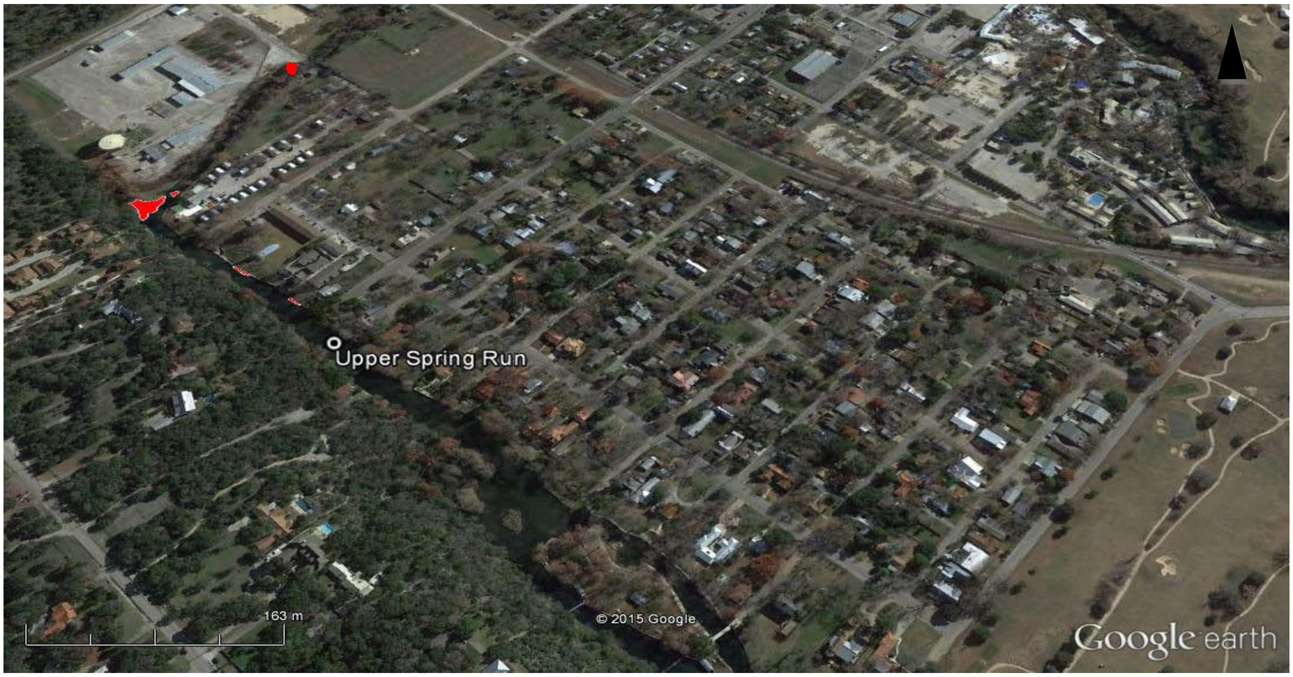

Figure 6. Locations of *Hygrophila* removed (red) from the Upper Spring Run in 2015.

Figure 7. *Hygrophila* removal along the east shoreline of Upper Spring Run (left) and at Heidelberg Lodges (right).

Hygrophila removal in the Old Channel restoration area was expansive. Starting in February, *Hygrophila* removal was concentrated in the area upstream of the second golf course bridge, as *Hygrophila* had regrown in that location due to moratorium on gardening and restoration work which occurred between July 2014 and January of 2015 while HCP Provision M was in effect.

Baseline mapping of the Old Channel section between the Landa Lake dam and the second golf course bridge in January 2015 showed 537 m² of *Hygrophila* cover (Figure 8). Most of this cover was considered regrowth, as approximately 435 m² of *Hygrophila* had been removed in this stretch in 2014. Removal of *Hygrophila* in this section was completed by April. Removal afterwards was then concentrated below Elizabeth Street bridge, the spring-fed swimming pool, and Upper Spring Run.

Removal of *Hygrophila* below Elizabeth Street began in May 2015. Due to the large extent of *Hygrophila* coverage and the wide river channel in this area, it was determined that methods for *Hygrophila* removal needed to be adjusted. A longer, more durable net and floating boom system was devised; this reached the entire width of the river channel so that larger sections of *Hygrophila* could be removed. Due to deeper water (i.e., 4 to 5 feet), the BIO-WEST dive team was essential for removing a majority of the biomass. Following previous methods, top growth was removed via hand or rake and allowed to float downstream into the net, where it was collected and removed from the water. In total, 1,122 m² of *Hygrophila* was removed from the Old Channel below Elizabeth Street to the stopping point for 2015 (Figure 9). This area was characterized as a monoculture of almost entirely *Hygrophila* 3 to 4 feet high extending bank to bank in many places. Additionally, *Hygrophila* was removed from three locations in the Old Channel upstream of Elizabeth Street, accounting for another 235 m² of *Hygrophila* removal.

During removal efforts in June 2014, it was observed that a large amount *Hygrophila* fragments originated from the spring-fed swimming pool and moved downstream through the Old Channel restoration area. Upon further inspection, the gravel bottom of the spring fed swimming pool was noted to be approximately 80% covered with *Hygrophila*. A large amount of fragmentation was occurring from this growth as swimmers tore *Hygrophila* stems and discharge washed these propagules downstream. As a result, the restoration team recommended that removal of *Hygrophila* from the swimming pool was necessary in order to eliminate this source of fragments and prevent re-colonization of *Hygrophila* downstream in the Old Channel restoration area. Due to the large amount of *Hygrophila* in the pool and the extent of its establishment, it was determined that removal of gravelly bottom material by excavator machine would quickly and most efficiently eliminate *Hygrophila* from the pool. Removal of *Hygrophila* from the swimming pool occurred from April 6 to April 8, 2015, and consisted of removal of 3 to 4 inches of pool bottom gravel across two thirds of the pool bottom. The material was hauled to a secure location contained within silt fencing and allowed to dry. After removal of bottom material the pool was revisited occasionally throughout the summer, whereupon any remaining *Hygrophila* fragments were removed. This additional effort removed an estimated 970 m² of *Hygrophila*.

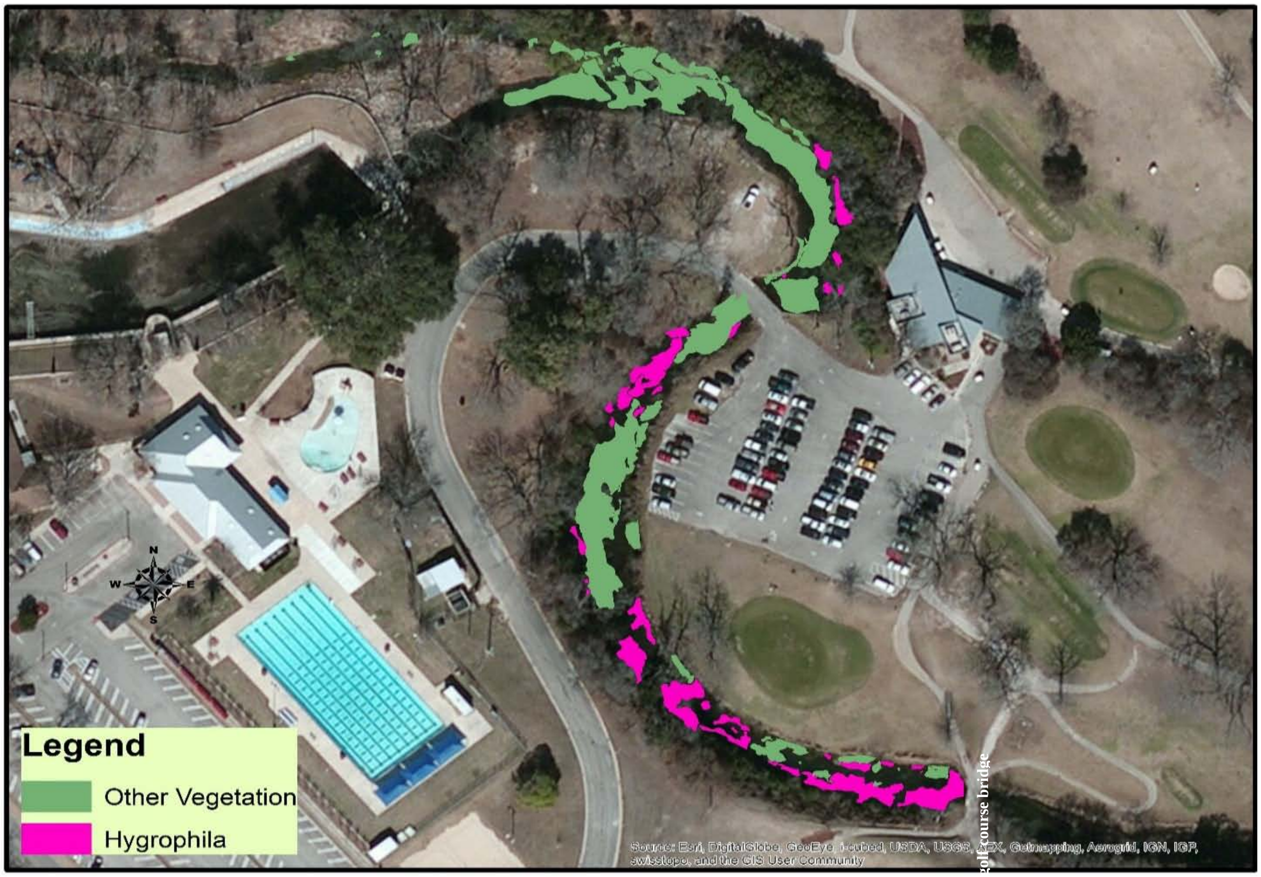

Figure 8. ***Hygrophila*** **distribution in Old Channel above the second golf course bridge, January 2015.**

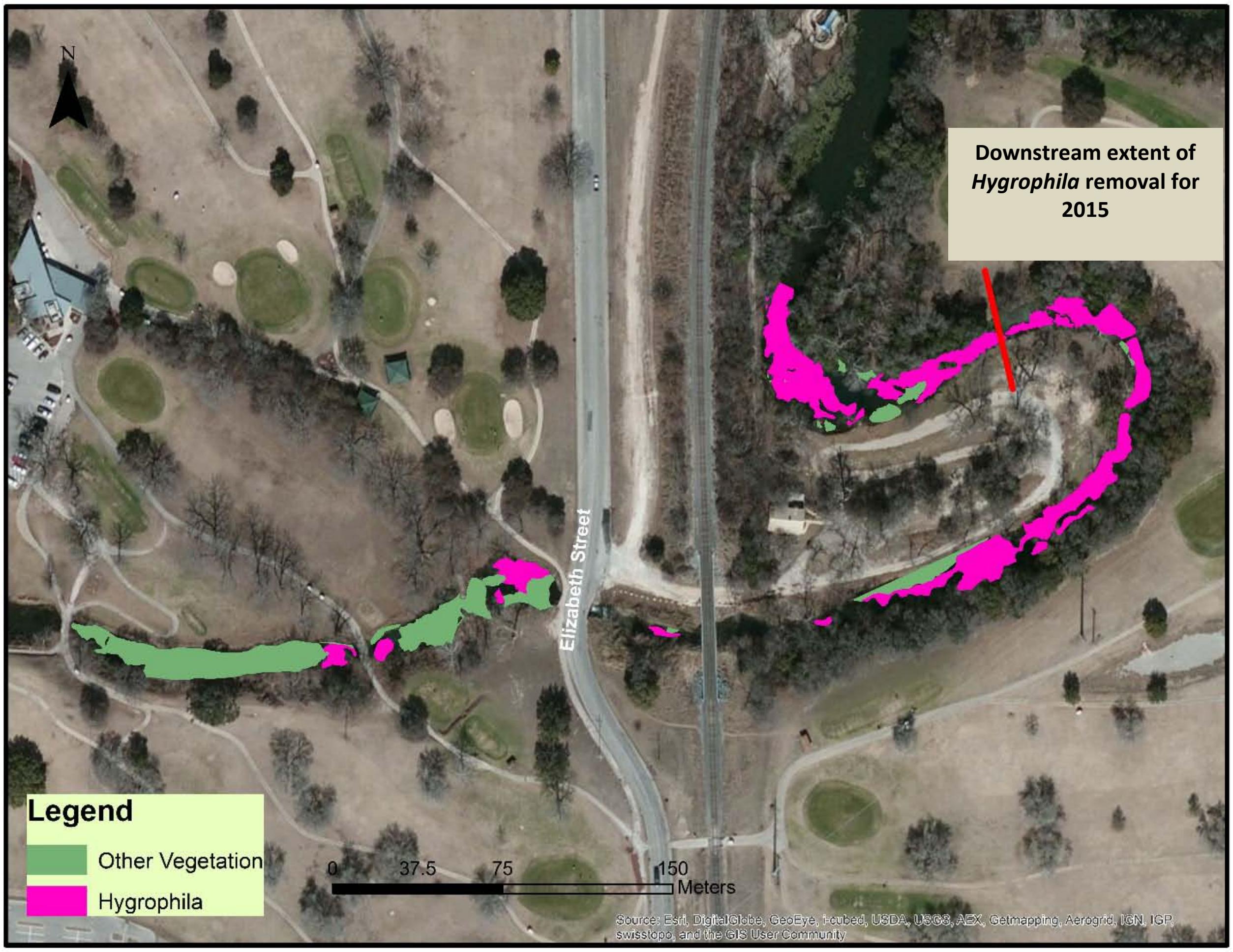

Figure 9. ***Hygrophila*** **distribution in Old Channel below the second golf course bridge, January 2015.**

Table 3. **Total area of** ***Hygrophila*** **removed per work section in 2015.**

Location/Section	Area Removed (m²)	Period of Work (2015)
Landa Lake Restoration Area	20	March
Old Channel Restoration Area	1,894	January to October
Swimming Pool	970	April
Upper Spring Run	539	April to August
Total for 2015	**3,423**	January to October

4.2 Native aquatic plant restoration

As discussed in the restoration plan, the Comal system offers a variety of habitat niches due to differences in sediment type, water velocities, water depths and habitat structure. The Landa Lake restoration sites are quite different from the Old Channel restoration site in these characteristics, and it was determined early on that several planting techniques and planting densities would be utilized, depending on the conditions of the area being planted, to improve success (Siliman et al. 2015).

The following planting patterns were proven to be effective in 2013 and 2014 and consequently were carried forward in 2015 activities:

1. Grouped plantings – Plants are placed close together forming a stand, clump, or mass.

2. Grid plantings – Plants are placed evenly on-center 1 to 2 feet apart.

Depending on restoration site substrate conditions, holes were excavated either using hand tools or by hand. Potted plants were removed from their propagation pots, placed into excavated holes, and surrounding soil was then tamped by hand to ensure good root-to-soil contact. Sprigs were also utilized for plantings in certain areas. Sprigs can be planted simply by making a deep divot in the sediment, inserting a bundle of stems, and then tamping down. Sprigs are planted in the same pattern as potted plants to improve cover and success.

4.3 Monitoring and aquatic gardening

Aquatic gardening is a necessary phase of the restoration process, since not all *Hygrophila* fragments can be entirely removed, and because fragments from upstream are constantly dislodging and floating downstream. In general, after an area has been restored, an aquatic gardening schedule of once per month during the growing season (April to September) seems appropriate for identifying and removing *Hygrophila* sprigs. In some cases, such as in the Landa Lake restoration area, no *Hygrophila* sprigs have been observed after removal was completed, while in other areas, such as the Old Channel, *Hygrophila* persists after restoration activities and so a gardening regime is continuous. In September, a total system gardening event took place to remove any *Hygrophila* sprigs which remained from summer removal activities.

Aquatic gardening entails more than just removal of *Hygrophila* sprigs. When moving into the Old Channel below Elizabeth Street, the restoration team removed long swaths of *Arundo donax*, log jams, and low-hanging limbs along the edges of the stream bank in order to prevent floating litter buildup. These litter mats capture debris including plant fragments and can allow *Hygrophila* fragments to root and grow. In some instances, these debris mats also block light from reaching planted native plants. Other aquatic gardening activities carried out in 2015 included supplemental planting of native plants in a few locations, removing dense bryophyte growth in newly-planted areas, and trimming *Ludwigia* beds to prevent topped-out growth.

Monitoring of restoration is an important step towards providing information on the success of the project and the efficacy of the methods used. Our monitoring program consists of vegetation mapping and quadrat sampling. In 2015, four mapping events were conducted in order to evaluate

the restoration project. The first was a baseline mapping event conducted in January 2015 before any restoration work was begun. Subsequent mapping occurred in April, August and October. Vegetation mapping is conducted by encircling the perimeter of plant patches with a kayak while GPS coordinates are collected via a Trimble GPS unit. Once a patch has been mapped, it is identified to species, and a cover or density estimate of the patch is entered. These methods allow us not only to evaluate the spatial expansion of plant species, but to qualitatively evaluate the density and health of restored stands. Quadrat sampling is conducted by estimating the percent cover within a 1m by 1m quadrat set within specific Landa Lake and Old Channel plots. For 2015, quadrat estimates were taken three times.

4.4 Vegetation mats

Vegetation mats form annually in Landa Lake and can typically be observed from spring into the fall season. Mats typically form and expand as excessive algae, bryophyte and plant growth senesces and floats to the surface. This material accumulates around branches, in *Vallisneria* leaves, and around shallow water areas. In 2014, these mats expanded greatly due to the low flows experienced that year. These mats were mapped twice in 2014 to get a sense of how much surface area they cover and where they form. In 2015, a more thorough study of the mats is ongoing, being conducted by Baylor University as part of a 2015 HCP applied research study (Figure10). During this study, mats were mapped 5 times: one baseline survey in April and then monthly from June to September. In 2014, concerns were raised regarding how the mats impact the aquatic habitat near and underneath them as they decompose. Vegetation mats are known to block out light to the aquatic vegetation beneath them, while also restricting water flow, thus limiting light and carbon dioxide availability and causing a die-off of native plants.

As noted, vegetation mats were mapped twice in 2014 and 5 times in 2015 (Figure 11). In April 2014, vegetation mats covered 1,505 m^2, while in April 2015 they covered only 671 m^2. By September 2014, the area covered by vegetation mats expanded to 3,877 m^2, compared to only 2,693 m^2 observed in September 2015. While vegetation mats cover a relatively small area of Landa Lake, they tend to form around obstructions, such as along the perimeter of and below the Three Island area (Figure 3). In our observations over the four month maintenance hiatus in 2014, vegetation mats had a negative impact on restored areas planted with *Ludwigia*. However, this did not appear to be the case in 2015, as vegetation mats did not tend to cover restoration plots. However, since vegetation mats form on Landa Lake even during average or above-average flows, it is important to understand that they provide a physical barrier to light, which in time can kill aquatic plants despite the fact that other water quality conditions remain optimal.

Figure 10. Dr. Robert Doyle (Baylor) collects vegetation mat samples of Landa Lake as part of a 2015 HCP applied research project.

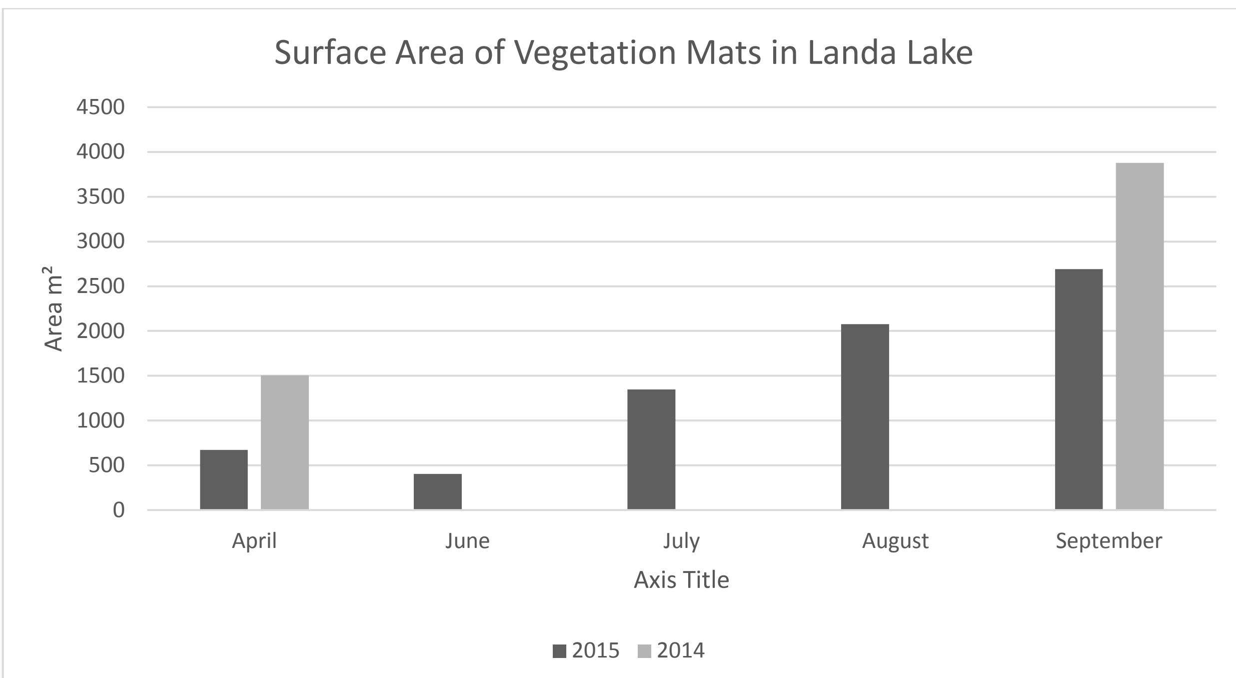

Figure 11. Surface area of vegetation mats in Landa Lake.

4.5 Pre- and post-restoration monitoring

For the purpose of monitoring the effects of habitat changes resulting from aquatic vegetation restoration, the BIO-WEST team incorporated pre- and post-restoration biological assessments in conjunction with the 2015 aquatic vegetation restoration on the Comal River. The aquatic restoration assessment study area was limited to the Old Channel restoration area between Sediment Island and the second golf cart bridge, and then resuming once again downstream of Elizabeth Street for approximately 400 river meters. Sampling sites were randomly selected within monospecific *Hygrophila* patches to evaluate fish and macroinvertebrate assemblages before *Hygrophila* removal. After removal, fish and macroinvertebrate surveys were used to assess community structure before aquatic vegetation had been introduced—this was essentially bare substrate—and then again after native plants had been planted and established. Post-restoration surveys were conducted within randomly selected patches of *Ludwigia* or other native vegetation which corresponded with the areas previously sampled pre-restoration. At each sampling site, teams recorded water column depth, velocity, vegetation type, percent coverage, height, and substrate.

Macroinvertebrate collections were made by placing a D-frame kick net downstream of a vegetation patch while agitating or kicking the vegetation, causing macroinvertebrates to be captured by the net. Kick net samples were collected for one minute, after which the contents were placed in plastic sampling bottles and preserved with 70% isopropyl alcohol. Macroinvertebrate samples were then identified to the family-level and used to compare differences in macroinvertebrate community abundances between pre- and post-*Hygrophila* removal and/or vegetation types occurring as a result of restoration efforts.

Fish communities were evaluated using drop net sampling. A drop net frame is thrown into the water and the buoyant surface frame is allowed to float on the surface effectively capturing all fish species with a given area, in this case 1 m^2. A large dip net [½m^2] that spans the width of the drop net was swept along the bottom substrate until fish numbers reached 0 to ensure complete collection of all fish trapped within the net. All fish were identified to species, enumerated, measured for total length, and returned to the river at the point of collection.

Results from the pre-, during, and post-restoration biological assessment will be reported in the 2016 Annual Restoration report. This monitoring regime should provide valuable information regarding how the aquatic community has changed throughout the process of restoration and whether the perceived benefits of aquatic vegetation restoration can be documented.

4.6 2015 restoration results

The methods utilized in 2013 and 2014 for native aquatic plant restoration were continued in 2015. Between January and October, a total of 21,427 native aquatic plants were planted. Landa Lake received 9,589 plants, while the Old Channel received 11,438 plants. The MUPPT nursery provided 4,138 plants, with the remaining plant material being transplanted or sprigged into restoration areas from mother colonies within the Comal System. Total planted area (plot area) in 2015 was 1,964 m^2. In total, since 2013 4,448 m^2 of area have been planted as part of the aquatic plant restoration program.

4.6.1 Old Channel restoration results

In 2015 1,130 m² were planted in eight restoration plots (R, S, T, U, V1, V3, V3, V4 and W) in the Old Channel (Figures 12-14), bringing the three year total of area planted in the Old Channel to 2,673 m². A total of 11,438 plants were installed into the Old Channel restoration area in 2015, with a majority of these planted in new plots, while some plants were installed as supplemental plantings into existing plots (Table 4). As in previous years, planting proceeded piecemeal downstream, with planting areas delineated by plots based on date of planting. Dense riparian shading prevented planting in several locations along the Old Channel restoration area, forcing some areas to be skipped. No planting occurred between the second golf course bridge and Elizabeth Street, as this area is already covered with native aquatic vegetation. However, removal of *Hygrophila* opened up some areas for future planting in this stretch. Extremely dense riparian canopy cover and large sections of exposed bedrock downstream of Elizabeth Street limit the area which can be planted at this location. Typically, native plants were planted in grouped plantings where sunlight was sufficient, and shady areas were left bare. However, bare substrate rarely stays entirely devoid of plant life, as bryophyte and algae turf have been observed colonizing on the sediment and bedrock.

Regular mapping of the Old Channel restoration area (Figures 15, 16, 17 and 18) shows an overall increase in cover of native aquatic vegetation between the baseline mapping in January and the October mapping event. Table 5 shows seasonal cover, in square meters, of the target species for restoration activities. Between January and April, cover of *Ludwigia* decreased sharply by 31 percent, but then regained some cover by August. Other target native aquatic plants species showed a steady increase in cover (i.e. Cabomba) or maintained their existing cover (i.e. *Sagittaria*). Due to the variability of stream habitat and the multitude of factors which can cause decline in plant cover it is hard to ascertain why *Ludwigia* experienced a decrease in overall cover through August 2015. Regardless of the decrease experienced through summer 2015, *Ludwigia* cover through August 2015 (505 m^2) is still over four times the pre restoration cover (123 m²) for this native species in the Old Channel restoration area. By October, cover of *Ludwigia* increased to 651 m², the highest amount observed all year.

Quadrat monitoring of the sediment island plot (yellow triangle) showed an average of 100 percent cover of *Ludwigia* in April, which was reduced to 20 percent cover by August. Between January and August GIS mapping showed *Sagittaria* increased 12 percent, while *Cabomba* showed an increase of 70 percent. Bryophyte cover in the Old Channel showed a remarkable increase of 164 percent (Figure 13). This is most likely due to the extensive removal of *Hygrophila*, which tends to deepen the water column and slow down water velocities enough for bryophytes to settle and establish. In the area above the second golf course bridge, banks of bryophyte several feet thick formed along the edge of the river channel. This could be detrimental to the native plants planted in this area, but for the most part these bryophyte colonies were pushed away from planted areas by the river current.

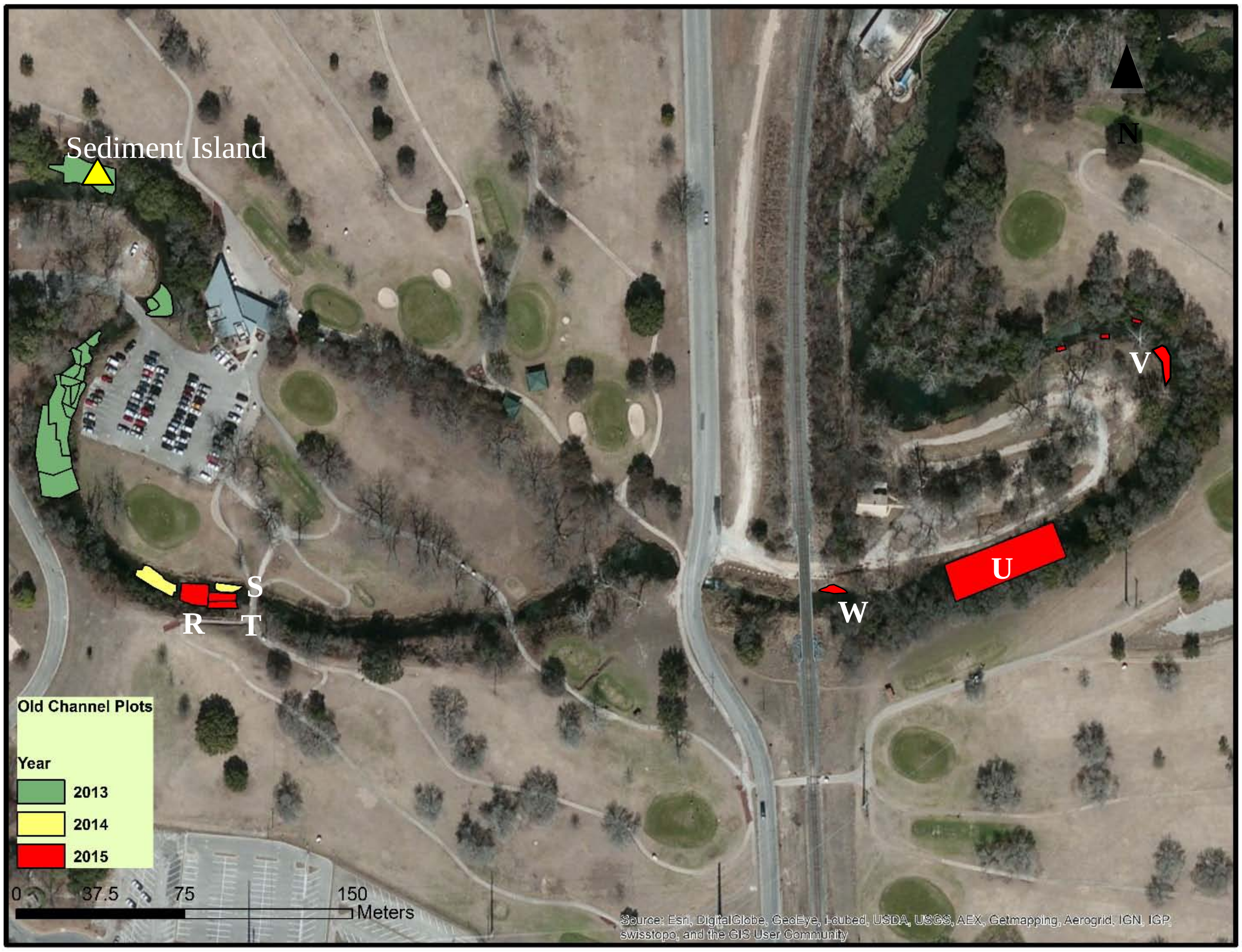

Figure 12. Restoration plots in the Old Channel project area. Yellow triangle indicates location of quadrat monitoring.

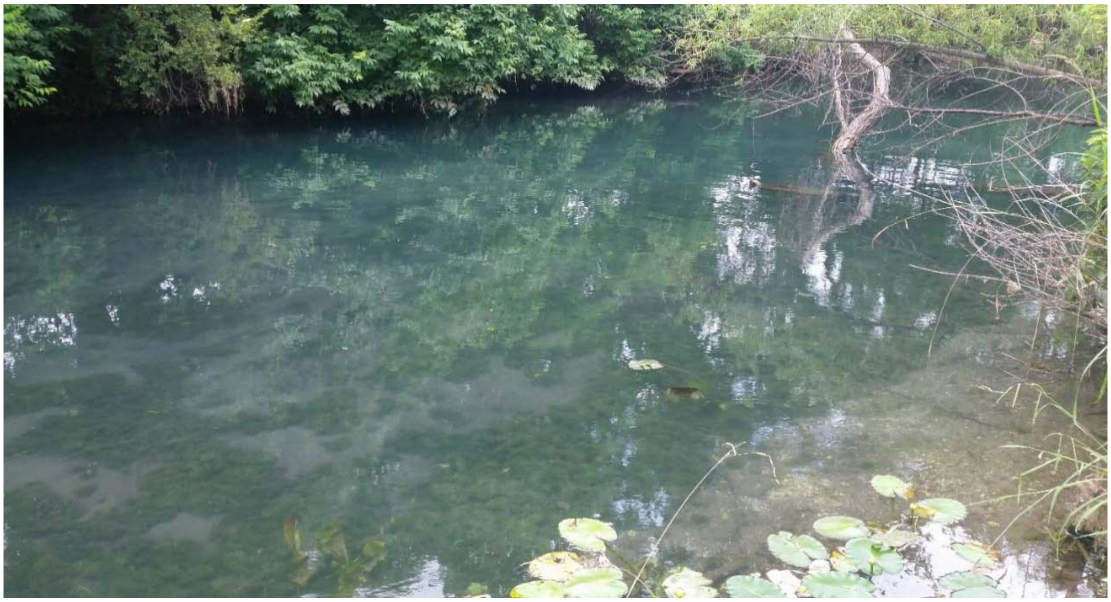

Figure 13. The Old Channel below Elizabeth Street after *Hygrophila* removal. Bryophytes quickly colonized the bare areas forming dense bryophyte turf along the river bed.

Figure 14. Old Channel plot V-4 after planting.

Table 4. Planting dates and number of native specimens planted within each Old Channel reach restoration plot

2015 Old Channel Restoration Plantings					
Date Planted	Plot	*Ludwigia*	*Sagittaria*	*Cabomba*	*Vallisneria*
3/23/2015	R		575		
3/23/2015	S		562	617	
3/25/2015	Q*	370			
5/11/2015	S, R	376			
5/11/2015	N,O,Q*	200			
6/16-17/2015	R		2,030	240	
6/23/2015	T			200	
7/7/2015	Sed. Isld*	192			
7/14-15/2015	U	624	1,800	90	650
8/24/2015	V 1-4	912			
9/30/2015	U			600	
9/30/2015	V1-4	1,000			
10/1/2015	V1-4	400			
Total		**4,074**	**4,967**	**1,747**	**650**

* Asterisk indicates supplemental plantings in pre-existing plots.

Table 5. Seasonal cover (m²) of target restoration species in the Old Channel restoration area indicated by GIS mapping

Species	January	April	August	October
*Ludwigia**	649	448	505	651
*Sagittaria**	340	386	386	504
*Cabomba**	104	139	177	170
Bryophyte	353	665	934	1,051
Hygrophila	2,602	2,526	1,105	944

* Approximately 281 m² of *Sagittaria* in the Old Channel restoration area was pre-existing and so was not planted in 2015.

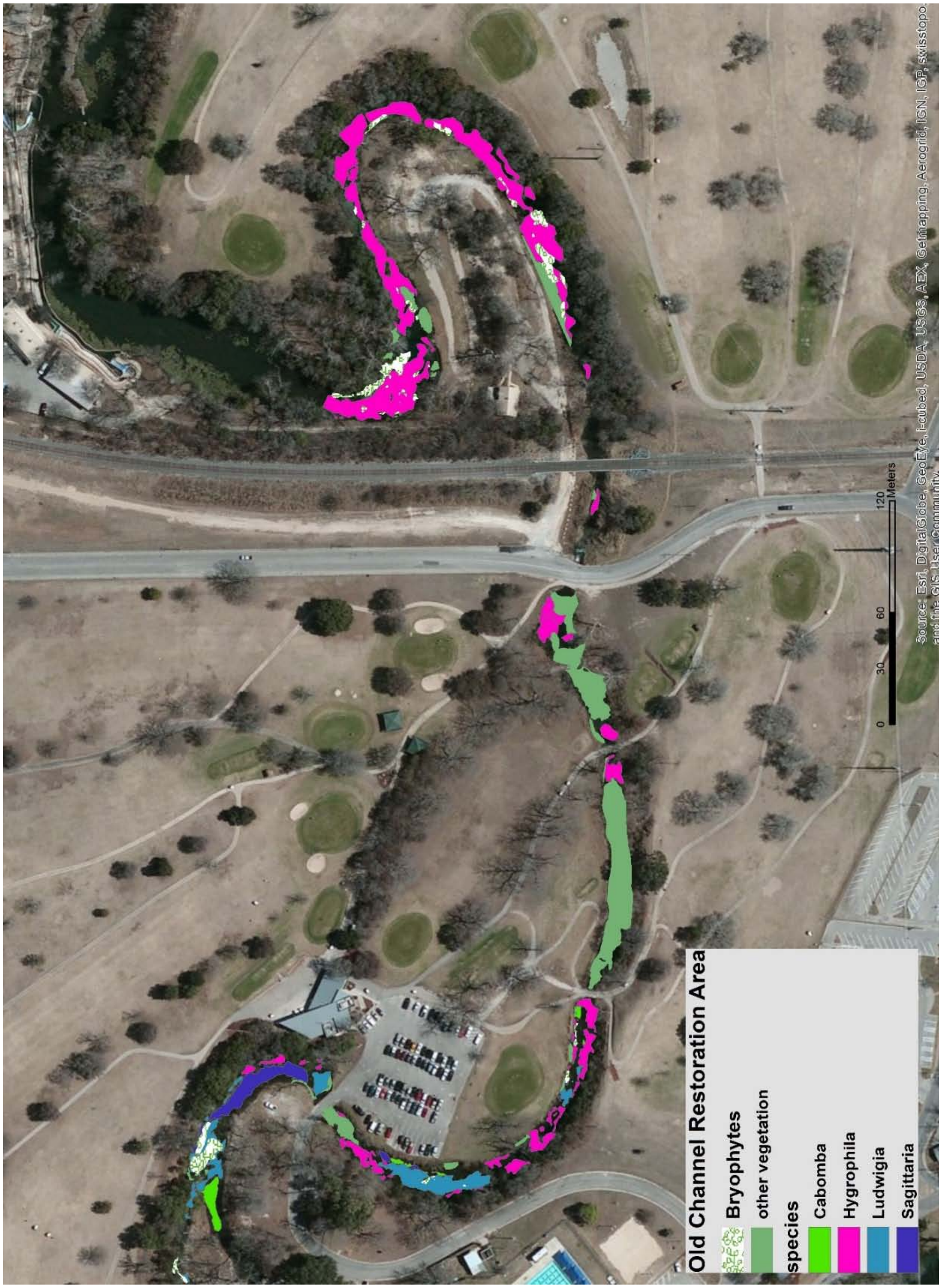

Figure 15. **Restored aquatic vegetation in the Old Channel in January 2015.**

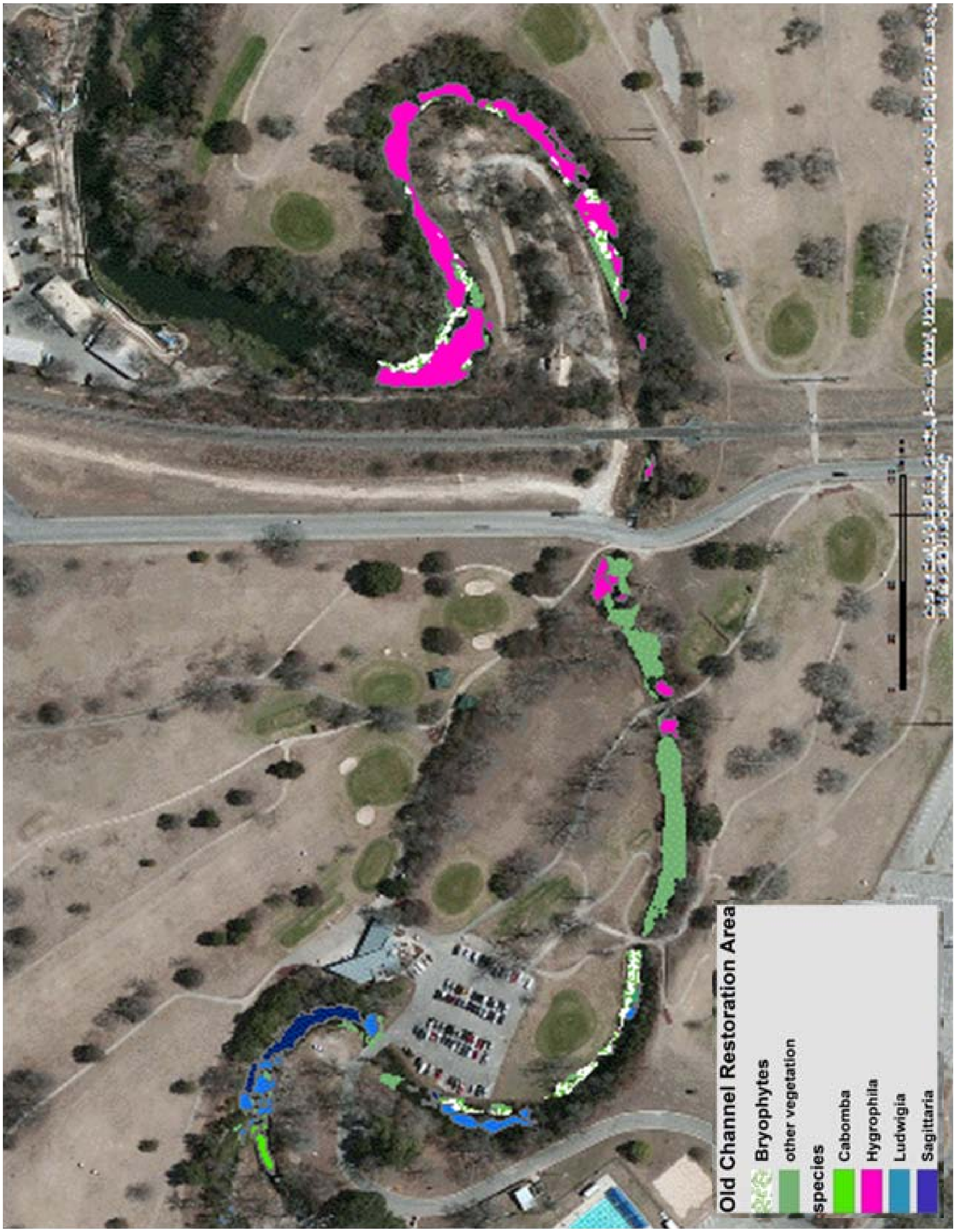

Figure 16. Restored aquatic vegetation in the Old Channel in April 2015.

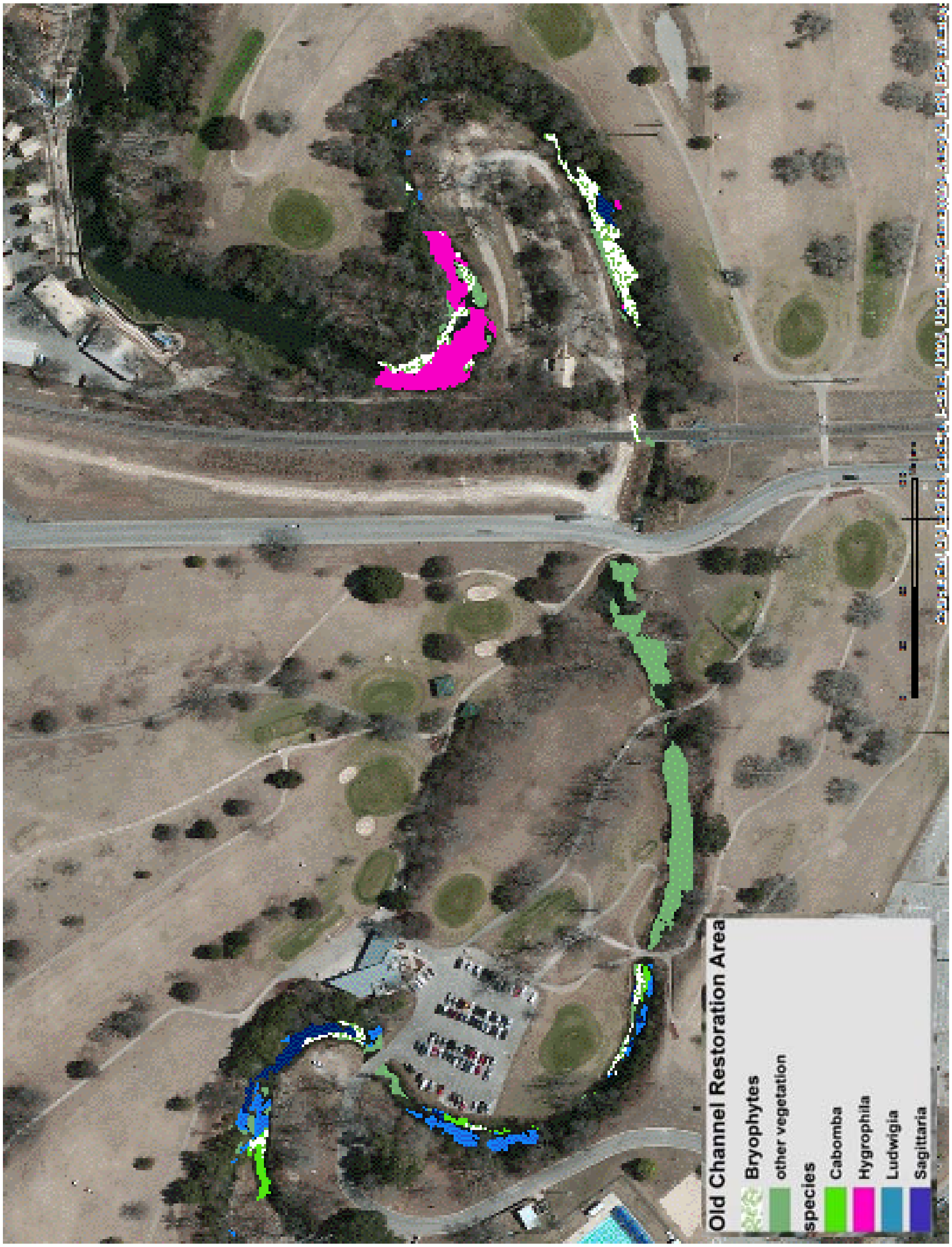

Figure 17. Restored aquatic vegetation in the Old Channel in August 2015.

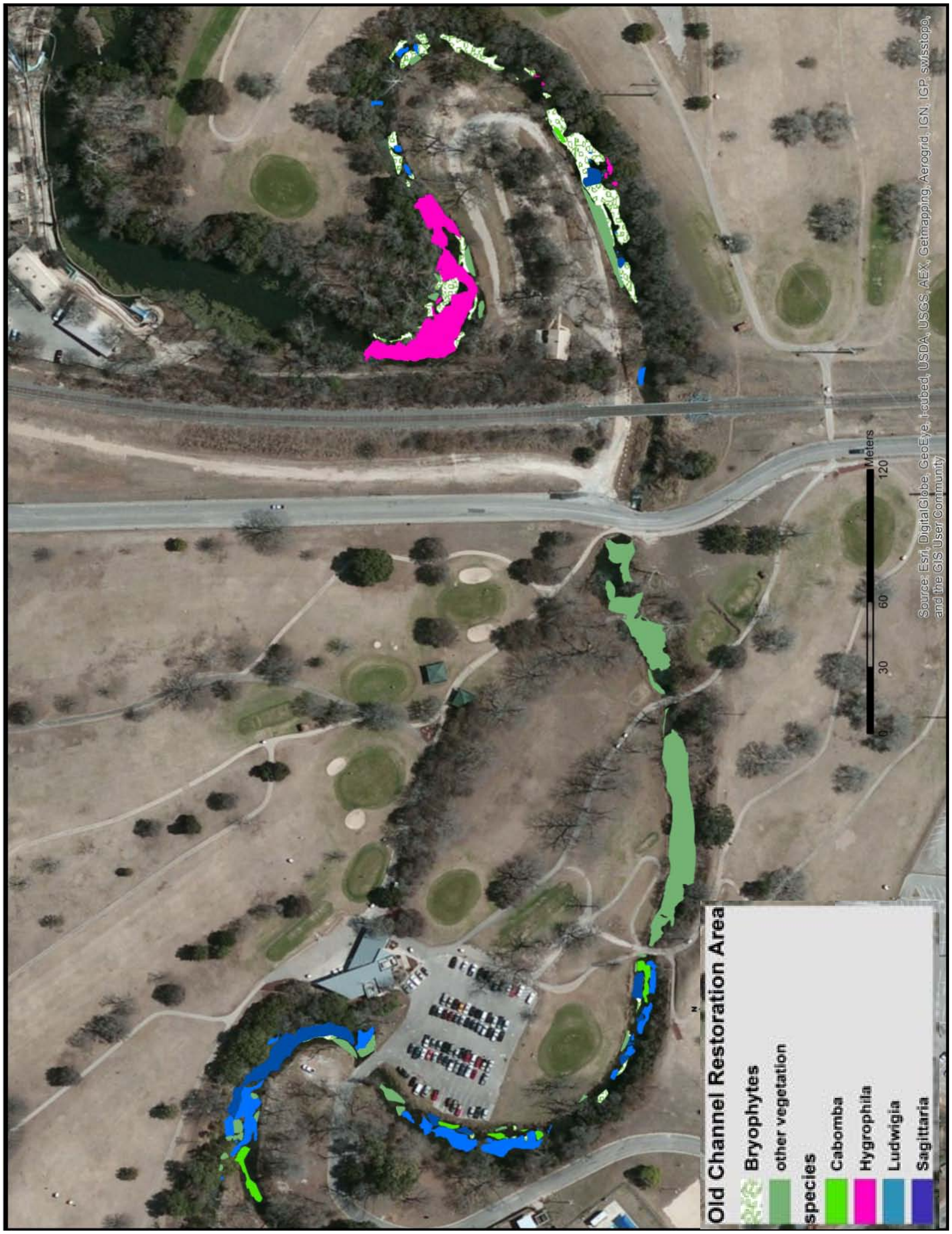

Figure 18. Restored aquatic vegetation in the Old Channel in October 2015.

4.6.2 Landa Lake Restoration Results

In 2015, 926 m² was planted in six restoration plots at Landa Lake (S2, T, U1, U2, U3, U4 and V) (Figure 19) bringing the three year total of area planted in Landa Lake to 2,694 m². A total of 9,989 plants were installed into the Landa Lake restoration area in 2015 (Table 6). A majority of these plants were installed into new plots for 2015 however some were planted as supplemental plantings into pre-existing plots. All new area planted in 2015 was area left bare from the Landa Lake wall construction project carried out in 2013 to 2014. These areas were previously covered in *Vallisneria,* which was smothered during wall construction. After wall construction, deep open areas were left near the Gazeebo south shoreline, which made perfect locations to try deep water (>4') restoration plantings, consisting mostly of *Ludwigia* and *Sagittaria*. Additionally, *Vallisneria* was planted to help provide a velocity buffer from the strong water flow exiting spring run 3. Ample flow and strong sunlight allowed plantings in this area to grow rapidly, with most plots reaching near 100% cover within weeks. These deep water areas are the most important restored area of Landa Lake, as they should provide import refuge habitat when Landa Lake depths decrease during drought conditions (Figure 20 and 21).

Regular mapping of the Landa Lake restoration area (Figures 22, 23, 24 and 25) show the cover of native aquatic vegetation between the baseline mapping in January and the October mapping event. Table 7 provides seasonal cover of target aquatic plant species in the Landa Lake restoration area in 2015. In general, cover of target species in the Landa Lake restoration area was highest in April. *Ludwigia* and *Cabomba* both saw large increases in cover between January and April mapping. During this time, *Ludwigia* cover increased 34 percent and *Cabomba* increased nearly 50 percent. By August, cover of these two species had reduced substantially, although August cover still remained above January cover. Coverage in October also showed a slight decline for these two native species. Quadrat sampling within selected plots of Landa Lake showed decreases in cover, as well. The largest loss of restored plant biomass occurred within the C and T plots. Plant cover in these plots showed winter cover near 100 percent, but this decreased to or below 20 percent cover by August.

Due to the variability in aquatic habitat, it is hard to ascertain why aquatic plant cover decreases during the growing period. While vegetation mats typically form in Landa Lake, and have been observed to smother native plants, as they did in 2014, these mats did not seem to develop over restoration plots in 2015, and did not directly contribute to decline in native aquatic plant cover. Other issues such as turbidity, human interference, and herbivory were directly observed to occur in or around restoration plots and these disturbances likely contributed to decreases in biomass.

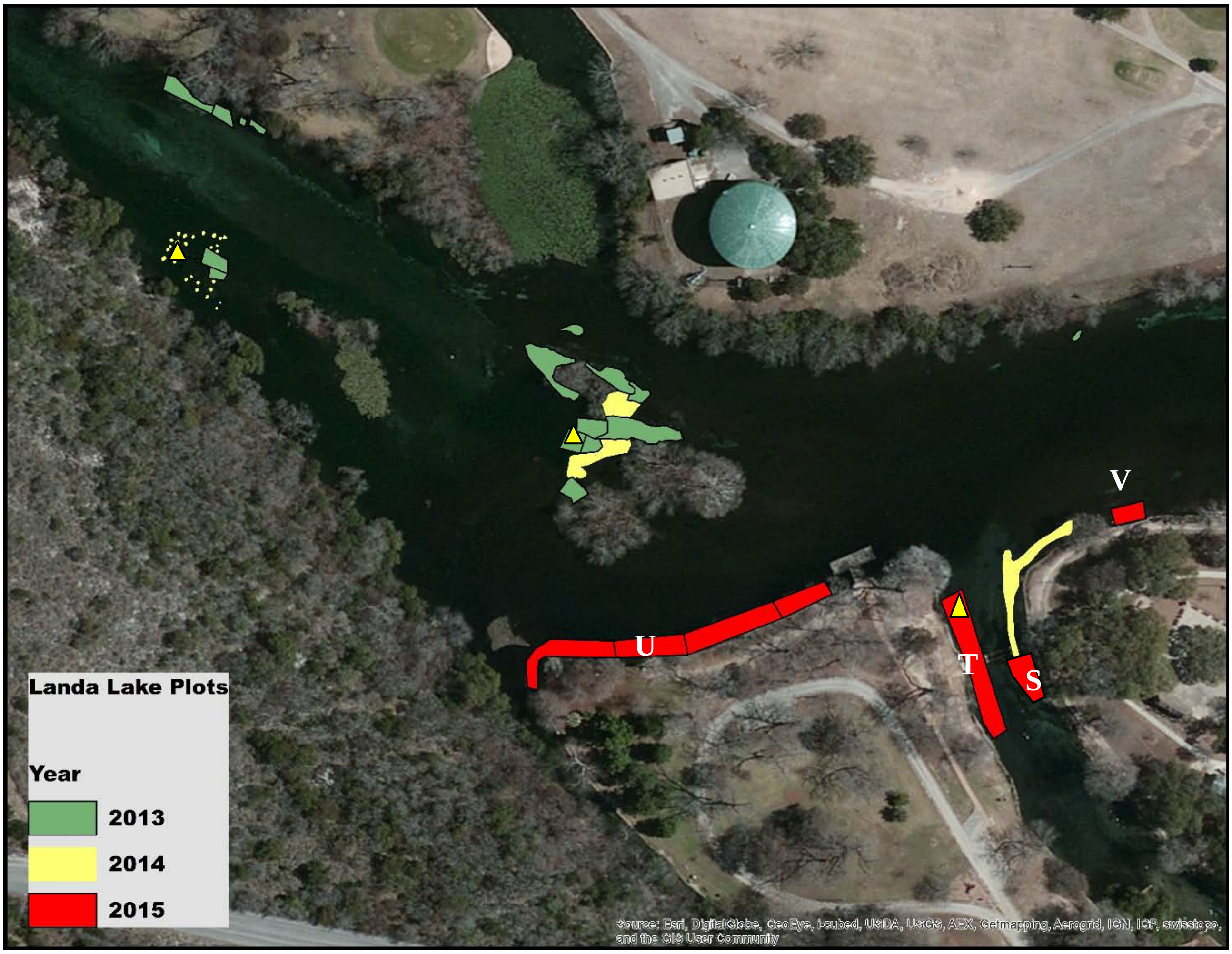

Figure 19. Restoration plots in the Landa Lake restoration area. Yellow triangle indicates location of quadrat monitoring.

Table 6. Planting dates and number of native specimens planted within each Landa Lake restoration plot. Asterisk indicates supplemental plantings in pre-existing plots.

2015 Landa Lake Restoration Plantings					
Date	Plot	*Ludwigia*	*Cabomba*	*Sagittaria*	*Vallisneria*
12/8/2014	M*	280			
12/9/2014	S2	145			
1/26/2015	T		50		
2/4-6/2015	T		950		
2/18/2015	T		600		
2/25/2015	T		400		
3/11/2015	S2	800			
3/12/2015	U1	200			
3/30/2015	U2	600		125	25
4/6-8/2015	U3	1,400		1,750	1,200
5/12/2015	U4	384			
6/10/2015	U4	480			
6/15/2015	V	200			
10/1/2015	U2	200			
10/1/2015	U3	200			
Total		**4,889**	**2,000**	**1,875**	**1,225**

Table 7. Seasonal cover (m²) of target restoration species in the Landa Lake restoration area indicated by GIS mapping.

Species	January	April	August	October
*Ludwigia**	460	701	486	476
*Sagittaria**	2,423	2,854	2,346	2,644
*Cabomba**	260	511	392	306
Bryophyte	1,723	2,412	N/A	2,109
Hygrophila	20	0	0	0
*Vallisneria**	15,524	14,991	14,911	13,556

* These numbers combine naturally occurring and planted *Sagittaria*, *Cabomba* and *Vallisneria* in Landa Lake

Figure 20. Landa Lake restoration plot U2 immediately after planting.

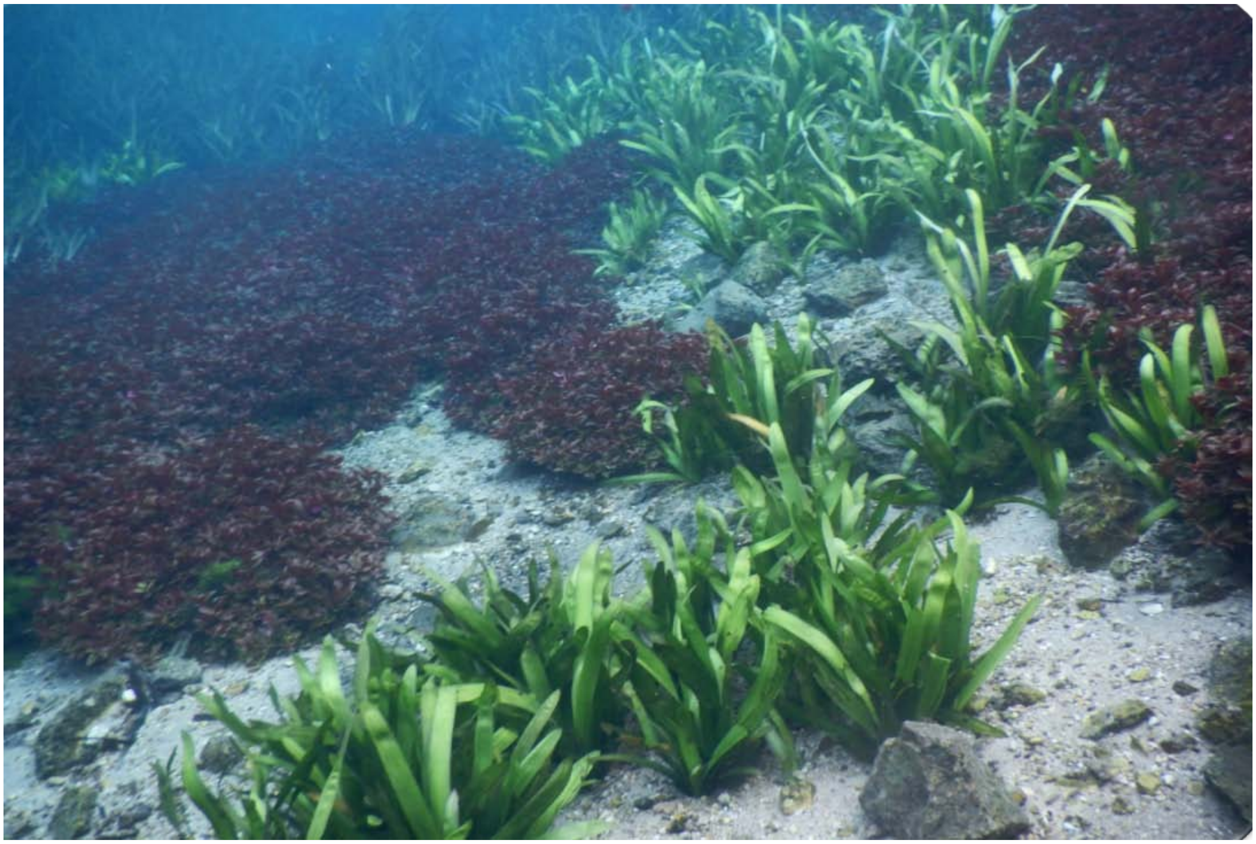

Figure 21. Landa Lake restoration plot U2 three weeks after planting.

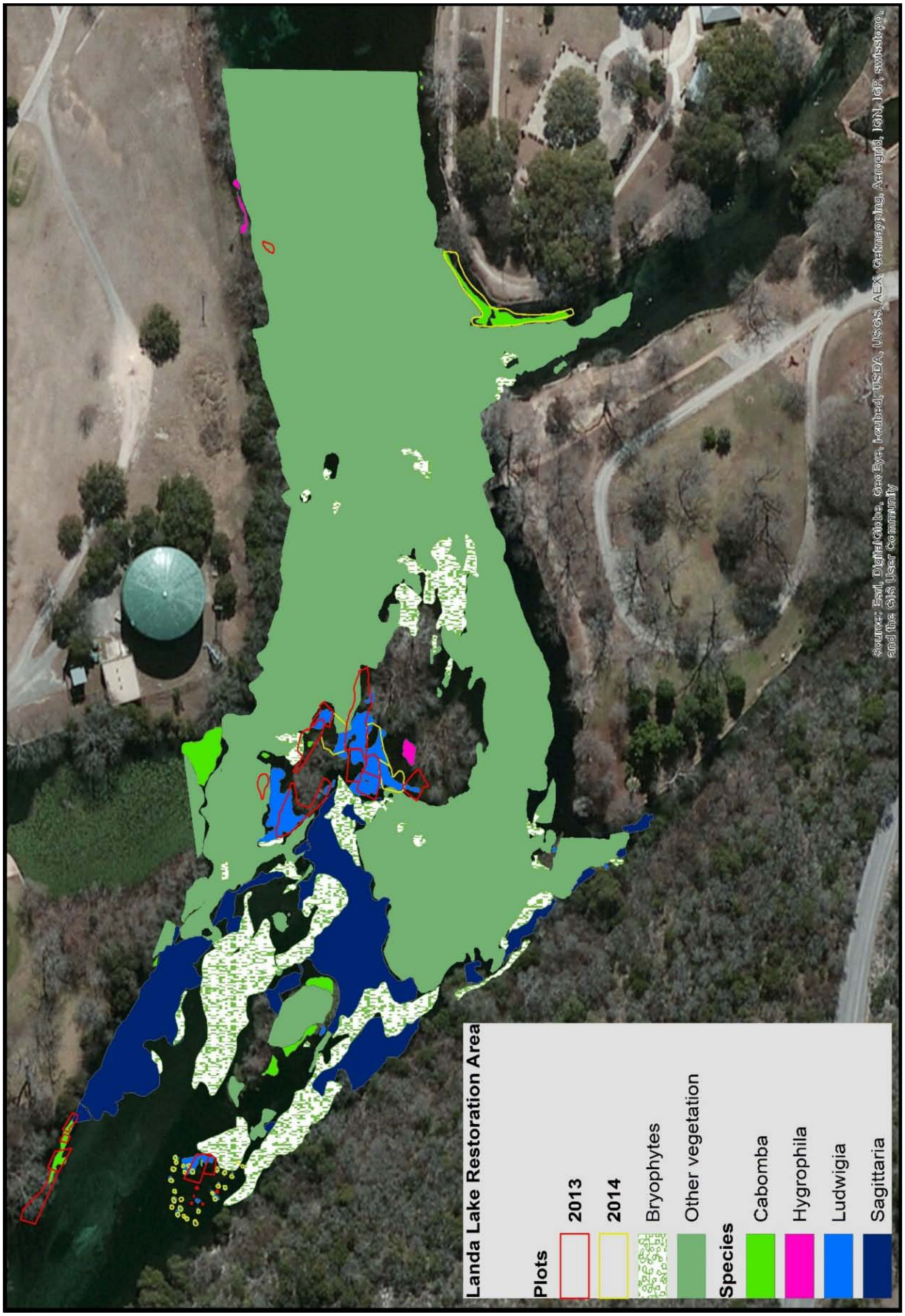

Figure 22. Restored aquatic vegetation in Landa Lake January 2015.

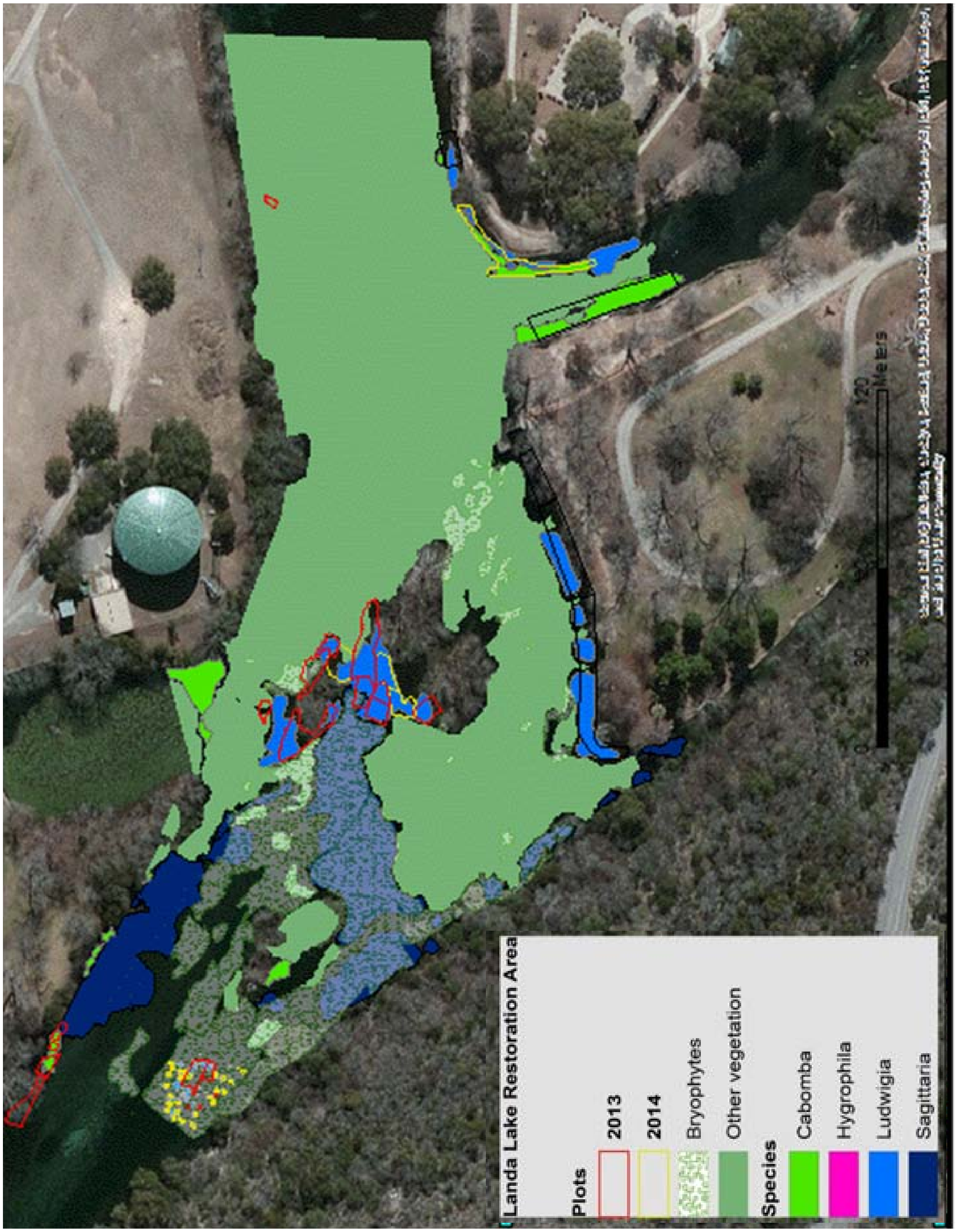

Figure 23. Restored aquatic vegetation in Landa Lake April 2015.

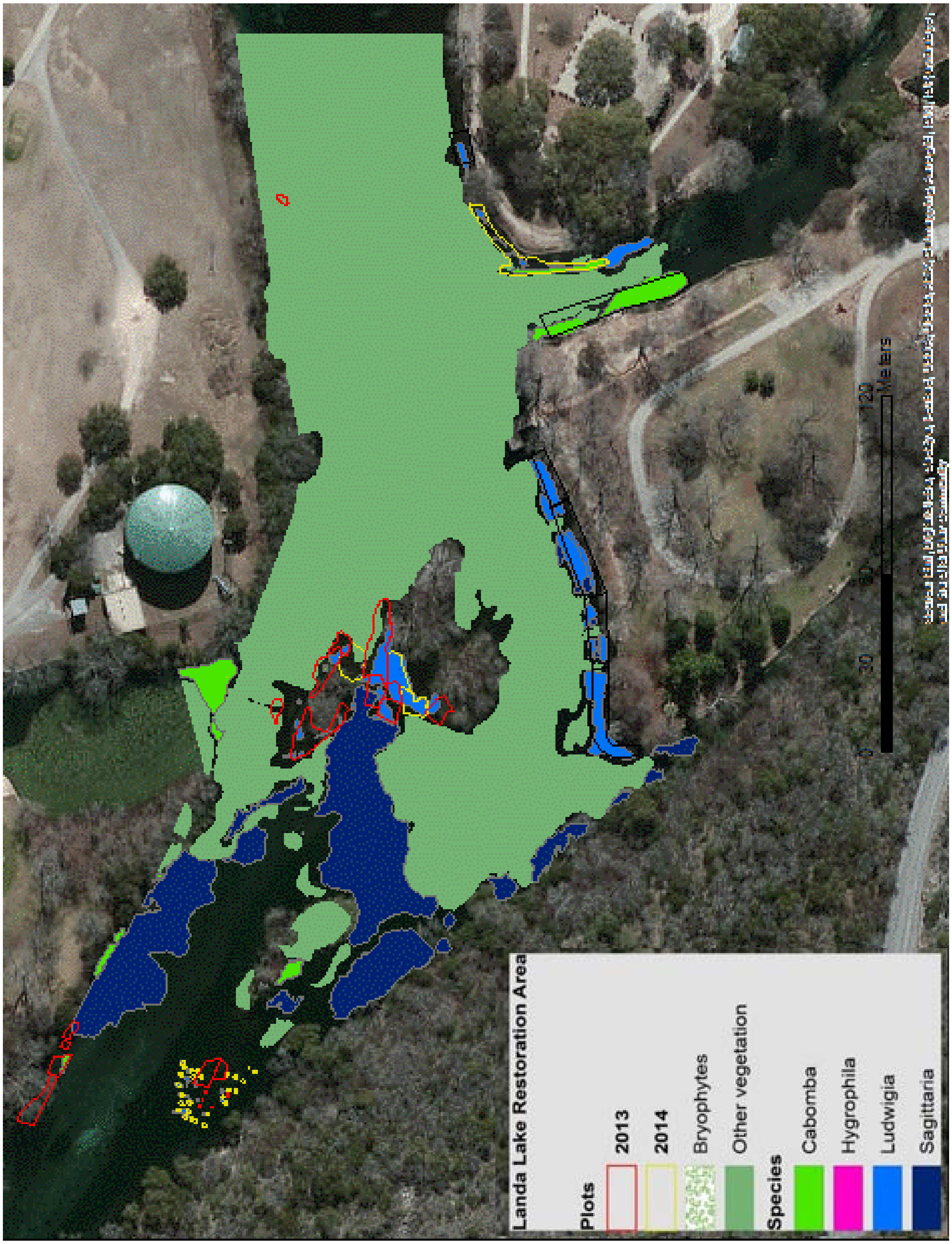

Figure 24. Restored aquatic vegetation in Landa Lake August 2015.

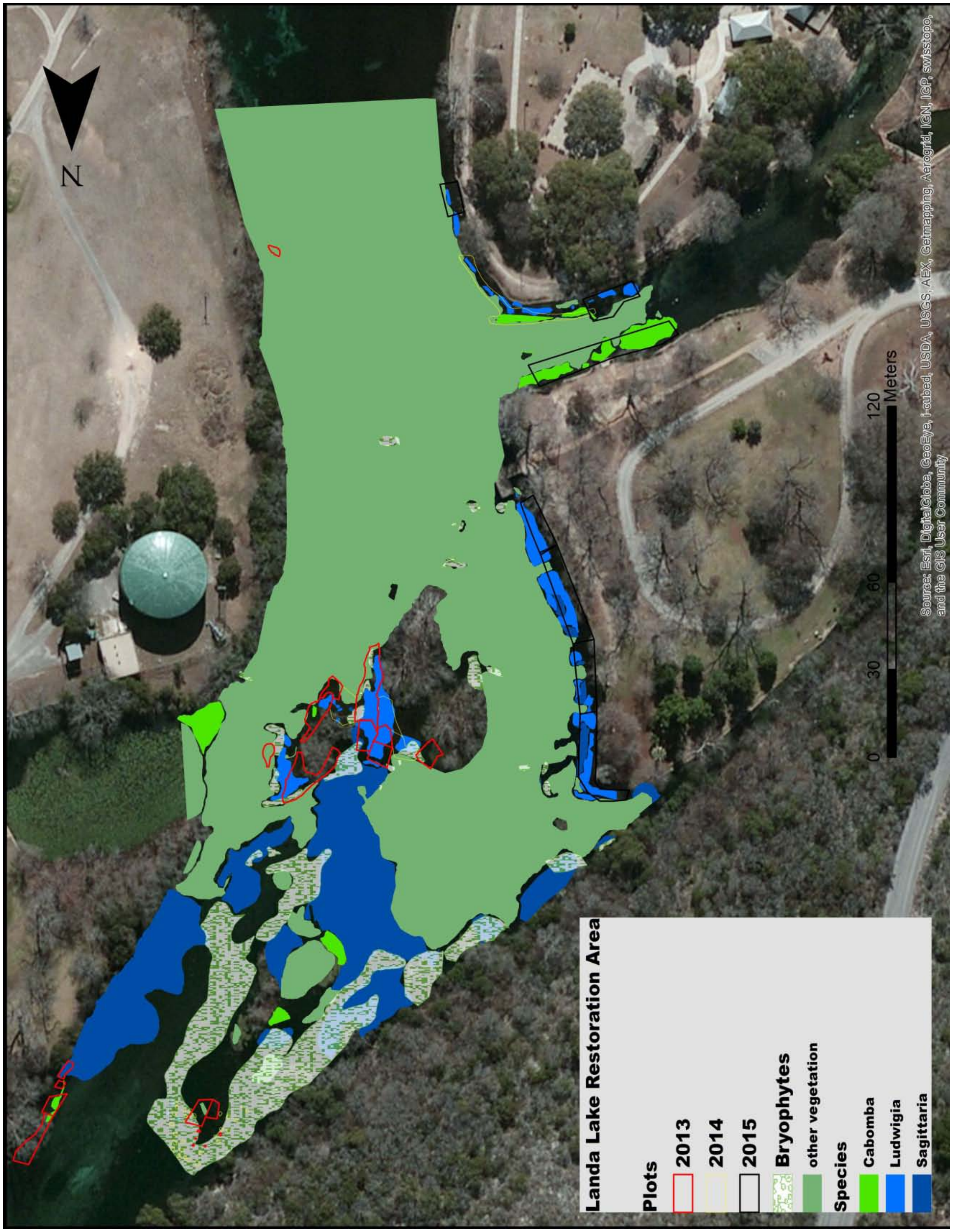

Figure 25. Restored aquatic vegetation in Landa Lake October 2015.

4.7 Restoration Summary

Results from vegetation mapping events show that target native vegetation is increasing in both Landa Lake and the Old Channel restoration areas, and that cover of native target species, especially *Ludwigia,* which was considerably limited in distribution within both restoration areas, have increased considerably compared to the pre-restoration period. Table 8 tracks *Ludwigia* and *Cabomba* cover from before restoration began to present. In the Old Channel, which was dominated by *Hygrophila* before removal, biodiversity of the plant community has increased. Species such as *Cabomba, Sagittaria, Ludwigia* and *Potamogeton* have been introduced back into areas of the Old Channel where they previously existed as indicated by historical mapping.

Table 8. Comparison of coverage of *Ludwigia* and *Cabomba* across all restoration mapping events to date.

	Landa Lake		Old Channel	
Mapping Period	***Ludwigia* Coverage (m^2)**	***Cabomba* Coverage (m^2)**	***Ludwigia* Coverage (m^2)**	***Cabomba* Coverage (m^2)**
Spring 2013	191	344	123	117
Fall 2013	318	356	940	56
Spring 2014	262	15	585	89
Fall 2014	225	71	479	72
Winter 2015	460	260	649	107
Spring 2015	701	511	448	139
Summer 2015	486	392	505	177
Fall 2015	**476**	**306**	**651**	**170**

The removal of *Hygrophila*, increase in biodiversity, and the expansion in the distribution of native plants provides habitat highly suited to the Comal system, which can hopefully provide refuge for fountain darters and other aquatic species in both times of plenty and in times of drought. Removal of *Hygrophila* from the Old Channel below Elizabeth Street opened up river channel, which was previously choked by the weed. Bottom sediment, which was composed of fine material when *Hygrophila* dominated, changed to more coarse sand, gravel and cobble after *Hygrophila* removal. The expanded water column allowed bryophytes to settle and attach to these coarse sediments, forming large colonies along the bottom. In some areas along the river edge, algae communities have begun covering gravel and cobble substrate. In Landa Lake, over 500 m^2 of native plants have been planted in water deeper than 4 feet. These deep-water plantings will provide refuge to fountain darters even during times of extreme drought when Landa Lake depths decrease and shallow patches of vegetation are exposed.

5.0 FUTURE STUDY CONSIDERATIONS

Many environmental factors affect the recruitment, growth and persistence of aquatic plants in river systems, including water clarity, water quality, nutrient availability, flow conditions and light conditions. 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, but the process of denitrification within dense stands of aquatic plants can produce self-limiting factors as well (Cufrey and Kemp 1992). In essence, one factor which drives the growth and health of aquatic plants within the Comal system is sediment nutrients which have yet to be researched in-depth in the Comal system. A study to investigate the makeup of the sediment and how native plant species use or partition those nutrients would be an important step towards understanding and predicting the future makeup of the Comal system aquatic plant community.

Over the past several years of restoration monitoring it has been noted that *Ludwigia* initially grows rapidly, forming thick, dense stands which then eventually thin and retreat into much smaller patches. Studies of patch dynamics in other stream macrophytes indicates that large patches produce self-limiting factors such as shading, nutrient competition, and physical drag or resistance, which in turn can decrease the rate of growth (Sand-Jensen, Madsen, 1992). Patch shape and size is also a factor of velocity, with the widest area of patches typically greater in the upstream end, and narrower patch widths oriented downstream. The dynamics of aquatic plant patchiness is not well understood for species living in the Comal River. Understanding why some restored species expand and retreat could improve restoration planting techniques.

A challenge that is continually present along the Old Channel restoration area is the amount of riparian canopy cover. Many large sections of the Old Channel restoration area are currently too shady for native aquatic plant establishment and expansion. Although these large areas may not be suitable for planting they do not necessarily remain bare. Bryophyte species, which tend to be more shade tolerant, have been observed to colonize bare areas producing thick turf. This habitat is quite suitable for the fountain darter and bryophyte colonization will be promoted by installing natural velocity shelters such as logs and boulders.

Aquatic gardening, monitoring and restoration activities will continue in 2016 in compliance with the HCP. *Hygrophila* removal will continue below Elizabeth Street and restoration planting will continue in Landa Lake as well as the Old Channel. Proposed activities include expanding restoration plantings into the Upper Spring Run from Bleiders Creek to Spring Island and in select locations (to be determined) of the New Channel of the Comal River. Riparian improvements are also proposed along the Old Channel project area in order to increase light availability necessary to enhance native aquatic vegetation survival and expansion.

6.0 REFERENCES

Angerstein, A.B., Lemke, D.E., 1994. First records of the aquatic weed *Hygrophila polysperma* (Acanthaceae) from Texas. Sida 16, 365–371.

Barko, J. W., G. Douglas, and S. R. Carpenter. 1991. Sediment interactions with submersed macrophyte growth and community dynamics. Aquatic Botany 41, 41-65.

BIO-WEST 2013. 2013 Restoration Plan for Aquatic Vegetation in Landa Lake and Old Channel Reach. May 13, 2013. Prepared for City of New Braunfels, TX, 72pp.

BIO-WEST 2014a. Habitat Conservation Plan Biological Monitoring Program. San Marcos Springs/River Ecosystem. 2013 Annual Report. Edwards Aquifer Authority. 80 p. plus Appendices.

BIO-WEST 2014b. Habitat Conservation Plan Biological Monitoring Program. Comal Springs/River Ecosystem. 2013 Annual Report. Edwards Aquifer Authority. 94 p. plus Appendices.

Cauble, Kathryn. 1998. Abundance and distribution of the freshwater snail fauna in Comal Springs-Landa Lake, New Braunfels, Comal Co., Texas. MS Thesis, Southwest Texas State University, San Marcos, Texas.

Crowe, J.C. and J.M. Sharp. 1997. Hydrologic delineation of habitats for endangered species: the Comal springs/river system. Environmental Geology. 30, 17-28.

Cufrey, J. M., and W. M. Kemp. 1991. Influence of the submersed plant, *Potamogeton perfoliatus*, on nitrogen cycling in estuarine sediments. Limnology and Oceanography 37, 1483-1495.

Doyle, R. 2002. Development of techniques for establishment of native aquatic vegetation in the Comal and San Marcos Rivers, Texas. Baylor University, Waco, Texas.

Madsen, J.D., P.A. Chambers, W.F. James, E.W. Koch and D.F. Westlake. 2001. The interaction between water movement, sediment dynamics and submersed macrophytes. Hydrobiologia. 444, 71-84.

Hutchinson, J. T., D. C., Huston, and J. R. Gibson. 2015. Defoliation of cultured creeping primrose willow (Ludwigia repens) and other aquatic plants by *Parapoynx obscuralis* (Lepidoptera: Crambidae). Southwestern Entomologist, 40, 227-232.

Ourso, Robert. 1998. Effects of current velocity on habitat suitability of exotic giant ramshorn snails (*Marisa cornuarietis*) in Comal Springs, Texas. MS Thesis, Southwest Texas State University, San Marcos, Texas.

Sand-Jensen, K. A. J., and T. O. M. VindbÆK Madsen. 1992. Patch dynamics of the stream macrophyte, *Callitriche cophocarpa*. Freshwater Biology 27, 277-282.

Siliman, B.R., Elizabeth Schrack, O., He, R. Cope et ali. 2015. Facilitation shifts paradigms and can amplify coastal restoration efforts. Proceedings of the National Academy of Sciences Early Edition. www.pnas.org/cgi/doi/10.1073/pnas.1515297112. Accessed October 29, 2015

Smart, R. M., Dick, G. O., and Doyle, R. D. 1998. Techniques for establishing native aquatic plants. J Aquat. Plant Manage. 36, 44-49.

Spencer, W. and G. Bowes., 1985. Limnophila and Hygrophila: A review and physiological assessment of their weed potential in Florida. J. Aquat. Plant Manage. 23,7-16.

United States Department of Agriculture, 2006**.** Federal noxious weed list. http://www.aphis.usda.gov/plant_health/plant_pest_info/weeds/downloads/weedlist.pdf. Accessed July, 2013

United States Fish and Wildlife Service, 2010, Biological and Conference Opinions of the Edwards Aquifer Recovery Implementation Program Habitat Conservation Plan Permit TE-63663A-O[Memorandum]. Albuquerque, NM: Department of the Interior 145-146.

(USGS) United States Geological Survey. 2015. National Water Information System: Web Interface for USGS Gage No. 08169000 for the period January 1, 2014 to October 1, 2015. [http://waterdata.usgs.gov]

Watson, G.W. and F.L. Rose. 1982. The effects of aquatic macrophytes on the stream microenvironment. Aquatic Botany 14, 309 -324.

Williams, C. R. 2013. Distribution and habitat requirements of *Hygrophila polysperma* on the San Marcos River, Hays County, Texas. MS Thesis, Texas State University, San Marcos, Texas.

Williams, C.R. 2011. Aquatic vegetation survey of the Comal River. River Systems Institute. San Marcos, Texas.

APPENDIX A
FIELDWORK PHOTOGRAPHS

Figure A1 Removing *Hygrophila* from above the first golf course bridge in the Old Channel in February of 2015. Due to implementation of HCP Provision M *Hygrophila* had not been removed from this area in 2014.

Figure A2 *Sagittaria* is harvested from Landa Lake for transplanting into restored areas. Previous to 2015 only small amounts of Saggitaria have been planted, but in 2015 large amounts were collected as bare rooted plants and transplanted in the Old Channel where the plant is useful in capturing bryophytes and decreasing river bed scour.

Figure A3 Approximately 970 m² of *Hygrophila* was removed from the spring fed swimming pool by excavator in April of 2015. This subsequently eliminated the discharge of *Hygrophila* fragments from the pool into downstream restoration areas in the Old Channel.

Figure A4 *Hygrophila* in the Upper Spring Run was removed to eliminate the source of *Hygrophila* to downstream restoration areas in Landa Lake. A floating barge helps contain *Hygrophila* during the removal process and provides a means of transporting it safely to shore for disposal.

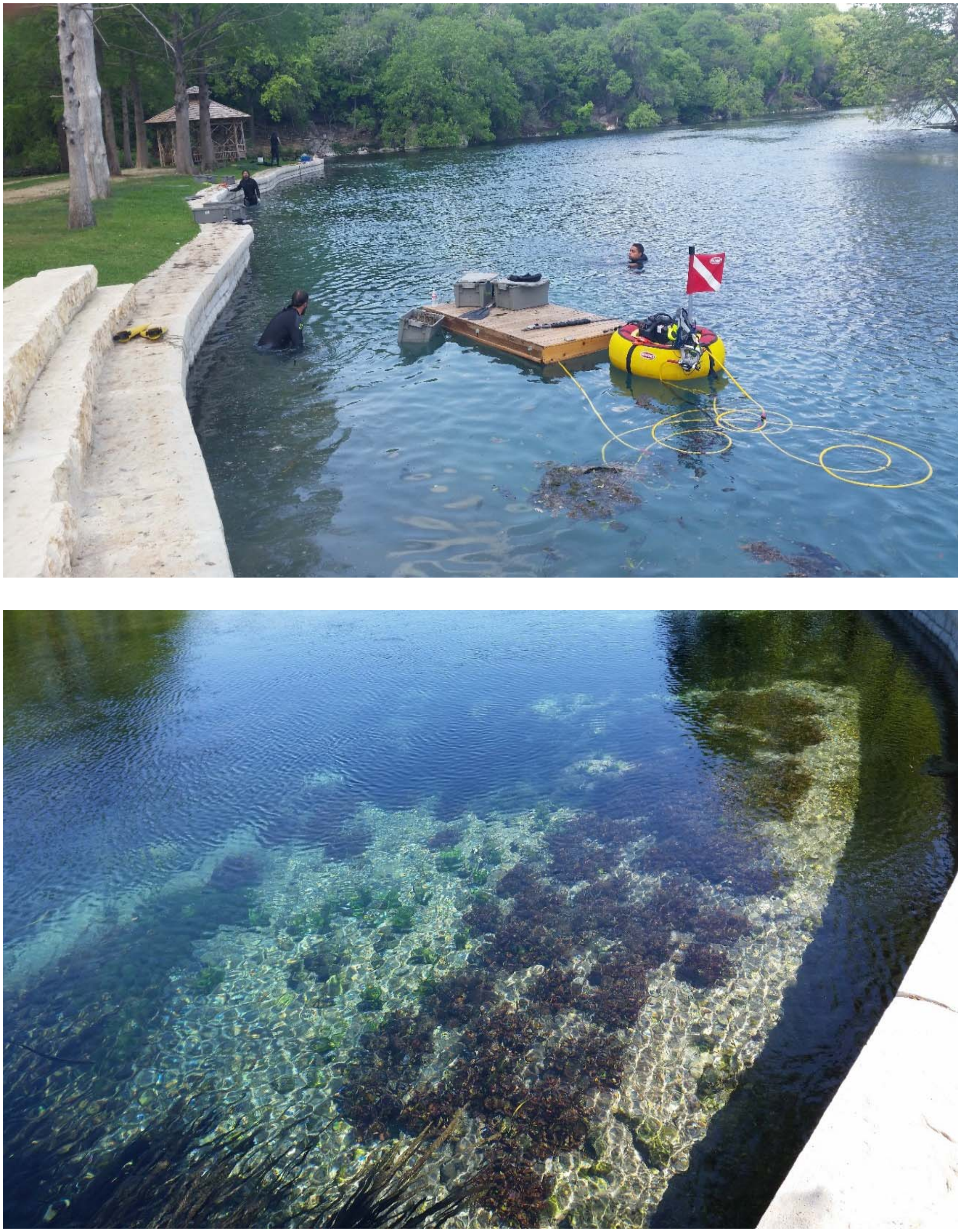

Figure A5 After the Landa Park rehabilitation project sections along the new retaining wall in Landa Park, which had previously been occupied by *Vallisneria*, were left bare of submersed vegetation. These areas provided perfect planting locations for native aquatic plants in deep water habitat. *Vallisneria* was also replanted in sections to provide velocity shelter for *Ludwigia* and *Sagittaria.*.

Figure A6 The Old Channel below Elizabeth Street is much wider and deeper than upstream sections and *Hygrophila* is much more dense (top). As a result a floating boom with a weighted net was used so that *Hygrophila* could be cleared across the entire width of the channel. The net was rigged at an angle so water velocity would concentrate *Hygrophila* biomass into one end for easy collection (bottom).

Figure A7 After *Hygrophila* was removed from the Old Channel below Elizabeth Street the substrate changed from dark colored silt to sand or gravel (top). Large areas of the Old Channel below Elizabeth Street are currently too shady to plant native aquatic plants so native plants, such as *Ludwigia,* are clumped together in prime growing locations (bottom).

Figure A8 Biological sampling in restored areas are ongoing as part of a biological monitoring regime. Sampling of the fish community via drop net (top) and the invertebrate community by dip net (bottom) takes place before *Hygrophil*a removal, after *Hygrophila* removal and after native plants are installed and established.

Appendix L3

Landa Lake Dissolved Oxygen Mitigation: 2015 Report

LANDA LAKE DISSOLVED OXYGEN MITIGATION:

2015 REPORT

Prepared for:

City of New Braunfels
300 Laurel Lane
New Braunfels, Texas 78130

Prepared by:

SWCA Environmental Consultants
10245 West Little York Drive, Suite 600
Houston, Texas 77040

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SWCA Project No. 24678

November 2015

CONTENTS

ATTACHMENTS

1.0 INTRODUCTION

This report provides a summary of the work performed by SWCA Environmental Consultants (SWCA) to monitor water chemistry, particularly dissolved oxygen (DO), within the Landa Lake portion of the Comal River in New Braunfels, Texas. This report summarizes work performed throughout 2015 under contract #NB13-006 with the City of New Braunfels (New Braunfels), including water quality measurement and operation of aerators. This report will receive supplemental data for the months of November and December before becoming a final annual report.

1.1 BACKGROUND

The Comal River is a sub-basin of the larger Middle Guadalupe River watershed, which is classified by the U.S. Geological Survey (USGS) as Hydrologic Unit #12100202. Measuring approximately 3.25 miles from the confluence of the most upstream spring runs to its mouth on the Guadalupe River, the Comal River is a spring-fed waterway that provides habitat for several endemic species. The river system consists of several spring runs that act as tributaries to Landa Lake, a broad, shallow impoundment that flows consistently based on upstream spring discharges (Map 1).

The hydrogeology and anthropogenic alterations to the Comal River have effectively isolated the biota of Landa Lake from other nearby waterways. At least five federally listed endangered species, namely fountain darter (*Etheostoma fonticola*), Texas blind salamander (*Typhlomolge rathbuni*), Comal Springs riffle beetle (*Heterelmis comalensis*), Peck's Cave amphipod (*Stygobromus pecki*), and Comal Springs dryopid beetle (*Stygoparnus comalensis*), are found within the Comal River system. An additional species of concern is the Comal Springs population of Texas salamander (*Eurycea neotenes*), which represents a distinct genetic history from other conspecifics. These species are dependent on springs that discharge from the Edwards Aquifer and the continued high water quality of the river for their survival. However, the re-routing of the Comal River and impoundment of Landa Lake for hydroelectric use has created a habitat that is geographically isolated from the rest of the Guadalupe River basin. The isolation of these communities causes the populations to diverge from one another but also reduces their genetic plasticity. As such, native species endemic to Landa Lake and the upstream spring runs are likely to be susceptible to alterations in the physical, chemical, and biological structure of the lake.

Spring-fed streams in arid regions, such as the Texas Hill Country, naturally vary in discharge quantity based on rainfall, aquifer recharge, and other abiotic factors. Decreased recharge, which is usually associated with drought conditions, generally leads to decreased spring discharges. In the case of the Comal Springs system, the southern portion of the Edwards Aquifer is recharged from an approximately 1,480-square-mile area that receives water from rainfall and losing streams flowing through the recharge zone as they percolate through unconfined limestone strata (Texas Commission on Environmental Quality [TCEQ] 2005). Recharge water in the Edwards Aquifer flows generally eastward through the confined strata of the aquifer until it discharges in the springs associated with the artesian zone (Map 2). Discharge quantity influences flow rates which, in turn, affects water chemistry and habitat suitability for species in these springs, their receiving streams, and Landa Lake. As such, changes in flow regimes, water chemistry, and introduction of exotic species can further endanger these already vulnerable species.

One of the more pressing concerns for the biota of Landa Lake is the dissolved oxygen content of the water column. Periods of high die-off by algae and aquatic vegetation as well as inputs of allochthonous organic material may cause depressed dissolved oxygen levels, especially during high temperature and high sunlight periods. Although the artesian springs maintain a relatively constant temperature, it should be noted that decreased discharge volumes and high water temperatures, both of which are typically

associated with high air temperature periods, are often associated with decreased dissolved oxygen content in the water column.

1.2 PURPOSE

New Braunfels extended their contract with SWCA Environmental Consultants (SWCA) to maintain water quality monitoring equipment within Landa Lake to track dissolved oxygen and other water quality parameters and, if necessary, take practicable steps to mitigate for depressed dissolved oxygen. Since installing a data sonde and telemetry system in 2013, this equipment has provided continuous monitoring of dissolved oxygen, temperature, pH, conductivity, and turbidity. These data provide guidance for management decisions related to maintaining adequate dissolved oxygen concentrations to support endangered species populations under periods of stress such as droughts, vegetation die-off, or pollution discharge.

This report summarizes the water quality data gathered during the 2015 field year (November 2014 – October 2015) for dissolved oxygen and other related constituents as measured in the main body of the lake near the water-sediment interface. These data aid in assessing the oxygen dynamics and provide needed feedback to guide the operation of aerators to maintain dissolved oxygen necessary to support wildlife within Landa Lake.

2.0 EQUIPMENT INSTALLATION AND IMPLEMENTATION

2.1 SONDE DEPLOYMENT AND OPERATION

No additional equipment was installed during the 2015 field season; however, the monitoring station was harried by operational errors throughout the year. Early in the field season (December 2014), sonde errors were noted through unacceptably low temperature readings. The sonde was kept deployed for several weeks until the sonde could be evaluated by Measurement Specialties. After an extensive investigation, the sonde was found to have a leak near the port, a reoccurring problem from 2014. The leak was addressed; however, shortly after being re-deployed the telemetry system failed. A site investigation revealed that the connection between the sonde cable and the telemetry system had been damaged causing the serial port to break from the wiring. As a result of the damage to the telemetry system, sonde data failed to be transmitted. The sonde once again experienced another error leading to the loss of data for much of the summer. Although a loaner probe was eventually provided, there were significant data gaps and a great deal of data that were unusable in the 2015 field season.

Continuous data collection for dissolved oxygen, conductivity, and temperature were only available from November 1, 2014, to December 6, 2014. However the temperature data were erroneous measurements (negative values), and no data on pH were collected. After December 6, 2014, data was intermittently recorded between January and September 2015 as the sonde continued to have difficulties. From September 18, 2015, to October 30, 2015 data was continuously recorded.

2.2 WATER QUALITY

During the 2013 and 2014 field seasons, SWCA established diel baseline dissolved oxygen patterns using data coinciding with increased temperatures and limited vegetation conditions (SWCA 2013, 2014). These data were used to determine the target dissolved oxygen concentration in the lake. Because of the relatively stable water temperatures, it was expected that dissolved oxygen would demonstrate a fluctuating pattern based on the influence of photosynthesis and respiration, resulting in depressed measurements at dawn and peak concentrations near midday. SWCA used these baseline patterns to detect deviations and trends toward low dissolved oxygen (≤2.0 milligrams per liter [mg/L]).

During all months the sonde was fielded, SWCA performed monthly on-site calibration for the monitored parameters to ensure the continued accuracy of the measured parameters. During each calibration event, the sonde probes for dissolved oxygen, temperature, pH, conductivity, and turbidity were inspected for signs of damage and maintained as needed (e.g., wipers replaced, electrolyte solutions replenished). As in previous years, discrete measurements continued to be collected every 30 minutes. To ensure prompt response to emergency conditions, SWCA maintained e-mail and text message alarms on the telemetry system; however, these were interrupted by telemetry failures. The precise locations of the monitoring and telemetry stations are provided in Map 3.

3.0 WATER QUALITY RESULTS AND DISCUSSION

Water quality data are collected every 30 minutes, with calibration, maintenance, telemetry errors, and necessary repairs being the interruptions in data collection. The data were regularly reviewed on ienvironet.com by SWCA staff to evaluate water quality trends. Additionally, ienvironet.com allows the user to set pre-defined alert criteria based on measured values. Based on initially collected dissolved oxygen values, SWCA elected to receive email alerts if dissolved oxygen concentrations were below 2.0 mg/L. Throughout the 2015 field season, this alert was sporadically triggered. The equipment communications during 2015 led to a total of 4,216 triggered events. The vast majority of these likely resulted from communications and electronics errors. It is likely that several of these incidents are associated with overnight anoxia; however, the efficacy of these alerts is dubious based on the general lack of trustworthy data.

All monitoring data were downloaded and interpreted to make inferences regarding water quality in Landa Lake. SWCA removed values that were known to be aberrant (e.g., values recorded during the calibration and sonde cleaning events or values otherwise known to be false). Furthermore, SWCA used an outlier analysis based on the distribution of the data to exclude data that were beyond two standard deviations of the mean. Again, the general lack of data for the year makes the usable data quite restricted.

It is important to note that long-term (i.e., interannual) inferences regarding water chemistry patterns were not possible based on the brevity of the monitoring period (i.e., May, 2013 through present) and the data errors during the 2015 season.

3.1 Daily Water Quality Patterns

3.1.1 Water Temperature

As in the 2014 dataset, the water temperature remained relatively constant throughout the monitoring period in which reliable continuous data were collected (i.e., September 18 – October 30, 2015). Water temperature gradually declined by approximately 1.5 degrees Celsius (°C) during September and October. The mean water temperature was 23.56°C with a standard deviation of 0.30. Thus 95% of the measurements were between 23.26 and 23.85°C. Similar to previously collected data, the 2015 data set was not sufficiently long to provide an idea of expected annual water temperature dynamics in Landa Lake. However the winter data is consistent with other temperate water bodies in showing a decline in water temperatures as the winter progresses (Figure 1).

Among the data that are available, the daily difference between low and high water temperatures varied narrowly throughout 2015. In most days, minimum temperatures were recorded in the overnight and early morning hours and maximum temperatures were recorded during early afternoon hours. Impingent solar radiation only caused an average difference of 1.42°C between the daily high and low water temperature during 2015. The daily temperature difference was similar to those seen in previous years.

3.1.2 Dissolved Oxygen

Dissolved oxygen in a water body is typically driven by many factors, chiefly temperature at the air-water interface, epilimnetic photosynthesis and respiration, and hypolimnetic sediment oxygen demand (Staehr et al. 2010). Temperature, the most important physical variable, has a well-established negative correlation with dissolved oxygen content in a water body. However, increased air and water temperature are typically driven by increases in ambient solar radiation. Therefore, although increased temperature should be associated with decreased dissolved oxygen, it is also strongly related to photosynthesis and its products (i.e., oxygen and carbohydrates). In a highly productive, photosynthesis-dominated system this would lead to increased dissolved oxygen during bright, mid-day hours as oxygen production vastly overwhelms respiration. However, sunset reverses this pattern as plants respire the glucose produced during daylight hours which, in turn, causes dissolved oxygen levels to decline through the night. These patterns were observed in previous years and appear to continue to be the driving dynamics for dissolved oxygen.

As was previously observed, diel oxygen fluctuation patterns observed in Landa Lake were as expected for an aquatic system rich with photosynthetic organisms. Dissolved oxygen typically oscillated between daily minima and maxima during the overnight (i.e., 10:00 P.M. to 8:00 A.M.) and early afternoon (i.e., 1:00 to 5:00 P.M.) hours, respectively (Figure 2).

Dissolved oxygen measurements at the probe ranged from 0.02 to 10.58 mg/L with percent saturation ranging from 1.56% to 121.4%; however, instantaneous high dissolved oxygen levels frequently exceeded 9.5 mg/L (Figure 3). Even through periods of high dissolved oxygen, daily mean dissolved oxygen remained relatively stable (generally between 5 and 7 mg/L). The stability of the mean dissolved oxygen suggests that days during which extremely high dissolved oxygen is observed also experience marked or prolonged periods of lower dissolved oxygen.

Throughout 2015, instantaneous measures of low dissolved oxygen value (<2.0 mg/L) were collected 4,216 times out of the 12,599 possible measurements collected. Many of these were likely associated with communications errors, however, this is difficult to determine in consideration of the paucity of data.

3.1.3 pH

As expected for a limestone-bed stream system, pH values were generally basic. Over the monitoring period, pH ranged from 6.90 to 8.22 standard pH units (SU) and averaged 7.25 (Figure 4), a range similar to that observed in previous years. No pH data are available for the spring months; however there was a slight increase in pH readings during the fall.

Similarly to dissolved oxygen, pH measurements demonstrate a fairly consistent daily cycle driven by photosynthesis and respiration (Wurts and Durborow 1992). As photosynthesis increases throughout the morning hours, dissolved carbon dioxide (CO_2) is taken up by plants and algae to produce carbohydrates and oxygen. The removal of CO_2 causes water pH to increase; however, as the temperature and dissolved oxygen increase, respiration by non-photosynthetic organisms also increases until CO_2 production exceeds CO_2 uptake by photosynthetic organisms. The transition of photosynthetic organisms to respiration during the night further increases dissolved CO_2, thereby depressing pH. In Landa Lake, this pattern is borne out by maximum daily pH readings recorded in the early afternoon (e.g., 1:00 P.M.) and minimum daily pH throughout the evening and early morning hours (Figure 5). Daily mean pH ranged between 6.99 and 7.37 SU with little variation from day to day.

3.1.4 Turbidity

As noted in previous years, there were no distinctive diel or monthly patterns associated with turbidity. Rather, variations in turbidity appear to be predicated by conditions beyond the spring runs or Landa Lake. Specifically, reliably measured turbidity in the lake varied between from 0 to 6,890 nephelometric turbidity units (NTU). However, it was assumed that values of over 2,000 NTU were outliers possibly related to biota on or near the sonde rather than accurate representations of the water quality in Landa Lake. However, there were also several rain events that may have caused higher turbidity values. With outliers (i.e., measurements outside of 2 standard deviations) removed, turbidity ranged between 0 and 1,310 NTU, with an average of 624.70 NTU throughout the monitoring period (Figure 6). As previously observed, during 2015 there were several days during which the measured turbidity did not exceed 1 NTU, which is consistent with the routinely transparent water within Landa Lake and the spring runs.

3.1.5 Specific Conductivity

During 2015, no daily cycle was evident for specific conductivity, which remained relatively consistent throughout the monitoring period. Toward the end of the monitoring period there was a slight decrease in specific conductivity from September to October (e.g., 581.24 to 537.38 microsiemens per centimeter [μS/cm]). However, in general, specific conductivity remained close to 569.35 μS/cm, with values typically ranging between 304.3 and 617 μS/cm (Figure 7). The relative constant specific conductivity in the lake across multiple years (2013–2015) is indicative of an aquatic system that is not heavily influenced by surface water. Likewise, the occasional decreases in specific conductivity are possibly related to storm events.

3.2 Relationships Among Variables

To better model the core relationships among the measured variables to a principal controlling physical variable, SWCA plotted all dissolved oxygen, pH, turbidity, and specific conductivity values against water temperature. As with previous data analyses, pH, turbidity, and specific conductivity values lacked any discernable relationship to water temperature (see Figure 8, 9, and 10 for pH, turbidity, and conductivity, respectively). The correlation coefficient for daily mean values of each of these variables was low ($r^2<0.15$). However, dissolved oxygen once again showed a stronger correlation with water temperature than all other variables (Figure 11), though this relationship is generally weak ($r^2=0.41$). This relationship is likely based on positive relationships of both water temperature and primary production with ambient solar radiation. In a highly productive, eutrophic system, increased water temperature and photosynthetic output (oxygen) are generally related to peak solar radiation and, therefore, correlated. In the case of Landa Lake, this relationship is likely limited by the relatively narrow range of water temperatures. Further data collection is necessary to better elucidate these relationships.

3.3 Aerators

Although oxygen depletion never reached 0 mg/L, aerators were activated with the timers set to run continuously during the overnight hours starting during the summer months. This was principally done at the request of New Braunfels based on the continued problems with the sonde and telemetry system. After some initial struggles with the aerators, the actuators were reconfigured to provide more consistent operation. There is insufficient data to indicate that the aerators substantially increased dissolved oxygen throughout Landa Lake.

Through a collaborative effort, Bio-West, Aqua Strategies, and SWCA carried out a study of aerator efficacy near the confluence of Blieder's Creek and the Upper Spring Run during mid-September, 2015. As part of this study, SWCA purchased as small, pilot-scale aeration system that was operated during

overnight hours. Based on the anticipation of overnight anoxia, the aerators were operated and the zone of effect in the water column was measured in situ. Preliminary results indicate that the efficacy of pad aerators is localized and that the configuration in Landa Lake is optimum for maintaining target dissolved oxygen values. Results of this study are provided in a separate report (Bio-West 2015).

4.0 RECOMMENDATIONS

Data from the fall have supported the tentatively identified relationships observed in previous water monitoring activities. The water quality parameters, specifically dissolved oxygen and pH, fluctuated daily and seasonally, as would be expected. Water temperature and specific conductivity decreased very slightly as the months progressed toward winter. Turbidity did not demonstrate any discernible patterns over the same period. To draw more substantial conclusions, SWCA recommends the continued monitoring of this station to identify long-term (e.g., seasonal, annual) water quality patterns and to better define the preliminary relationships identified in this report. Additionally, SWCA recommends examining the data in relation to climatological information (e.g., storm events, impingent light) to refine the patterns observed among the water quality parameters, especially dissolved oxygen.

Water quality studies are highly data-driven with monitoring activities and management decisions typically based on a rich, accurate data set drawn from multiple inputs. The continued sonde and telemetry errors encountered on multiple occasions during 2015 as well as the requisite downtime to address these problems left little information of value to aid in management decisions. Considering the importance of the data gathered for the, such persistent problems may require the placement of additional monitoring stations, the purchase of a spare sonde, replacement of probes, or replacement of the existing sonde with a differing model. The lack of data resulting from the sonde and telemetry errors creates an untenable situation for evaluating and managing Landa Lake.

Based on preliminary results of the study in Upper Spring Run, the efficacy of the aerators in their current configuration appears to be less than optimal. The limited findings of that study imply that the aerators may provide greater benefit in other locations, as was suggested when the project was originally scoped, and in additional locations. The supplemental study implies that several additional aerators may be needed to maintain target dissolved oxygen levels. Additionally, the current placement of the aerators likely provides little benefit to fountain darters. The currently deployed aerators are not highly amenable to relocation, as they are operated through solar panels.

SWCA agrees with the recommendations of the Supplemental Dissolved Oxygen Evaluation study (Bio-West 2015) that further study of the oxygen dynamics in Landa Lake and the reliability of the methods by which we evaluate these conditions are needed. Dissolved oxygen in this system is likely to depend on several variables including temperature, photosynthesis, oxygen demand (both from the decay of autochthonous material and respiration) and, therefore, additional studies are needed to improve oxygen management. Although the 2015 data is limited, dissolved oxygen measurements were bimodal and skewed heavily toward lower dissolved oxygen concentrations (Figure 12), indicating low oxygen conditions are typical for the system. The degree to which these overnight lows are caused by biological oxygen demand or chemical oxygen demand remain unknown. This uncertainty will continue to make management of the system a challenge.

5.0 REFERENCES

Bio-West. 2015. Supplemental Dissolved Oxygen Evaluation in Landa Lake – 2015. Memorandum to City of New Braunfels.

Horne, F.R., T.L. Arsuffi, and R.W. Neck. 1992. Recent Introduction and Potential Botanical Impact of the Giant Rams-Horn Snail, *Marisa cornuarietis* (Pilidae), in the Comal Springs Ecosystem of Central Texas. *The Southwestern Naturalist*. 27:2, pp 194-196.

Staehr, P.A., D. Bade, M.C. Van de Bogert, G.R. Koch, C. Williamson, P. Hanson, J.J. Cole and T. Katz. 2010. Lake metabolism and the diel oxygen technique: state of the science. *Limnology and Oceanography: Methods*. 8, pp 628-644.

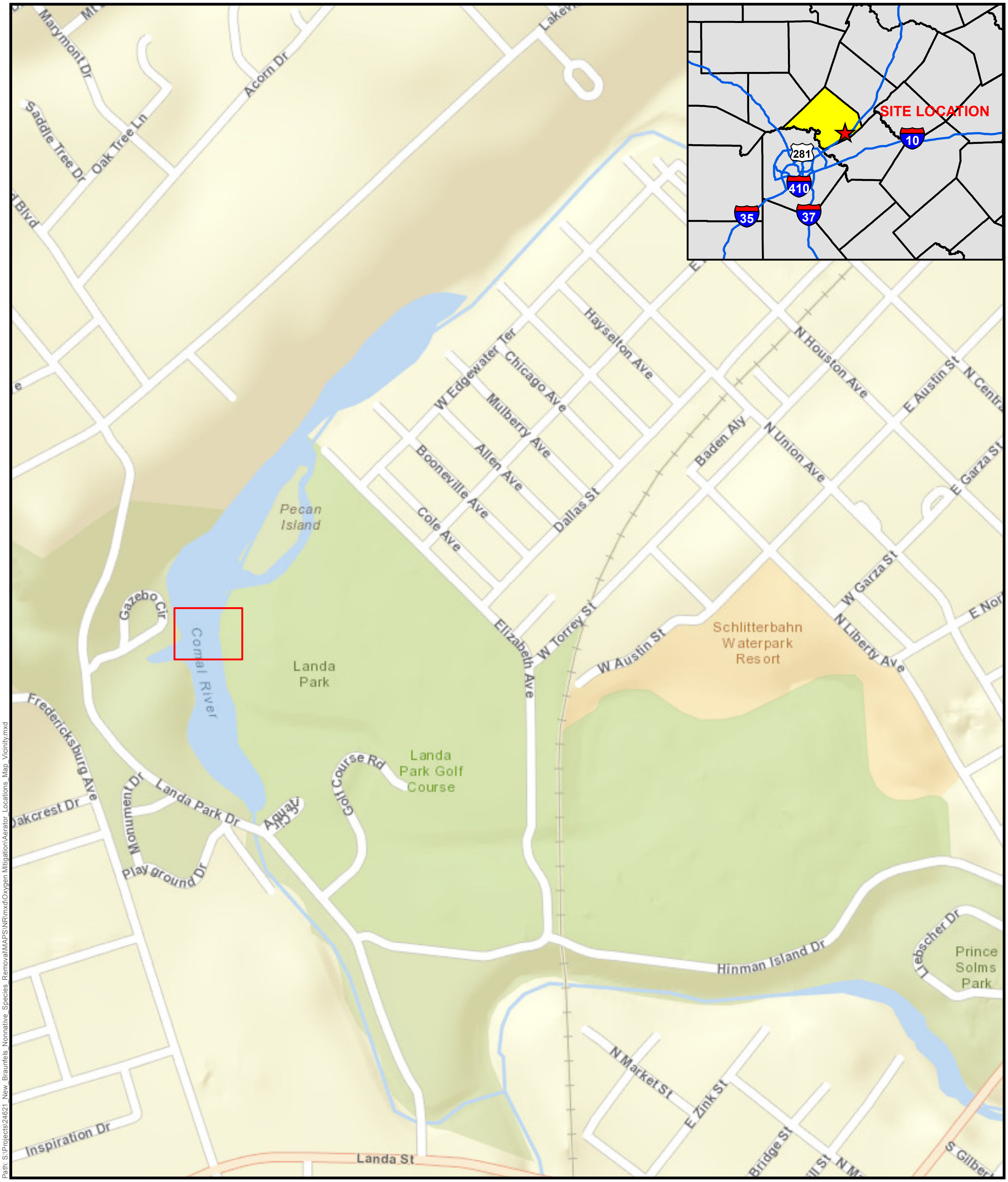
SWCA Environmental Consultants (SWCA). 2013. *Decaying Vegetation Removal and Dissolved Oxygen Mitigation: Interim Final Report – 2013*.

SWCA. 2014. Landa Lake Dissolved Oxygen Mitigation: 2014 Report.

Texas Commission on Environmental Quality (TCEQ). 2005. Edwards Aquifer Recharge Zone - Chapter 213 Rules. Available at: http://www.tceq.texas.gov/assets/public/gis/metadata/edw_tsms.pdf. Accessed December 2013.

Wurts, W.A. and R.M. Durborow. 1992. Interactions of pH, carbon dioxide, alkalinity and hardness in fish ponds. Southern Regional Aquaculture Center. Publication No. 464.

ATTACHMENT A
MAPS



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CITY OF NEW BRAUNFELS

STUDY AREA MAP
COMAL COUNTY, TEXAS

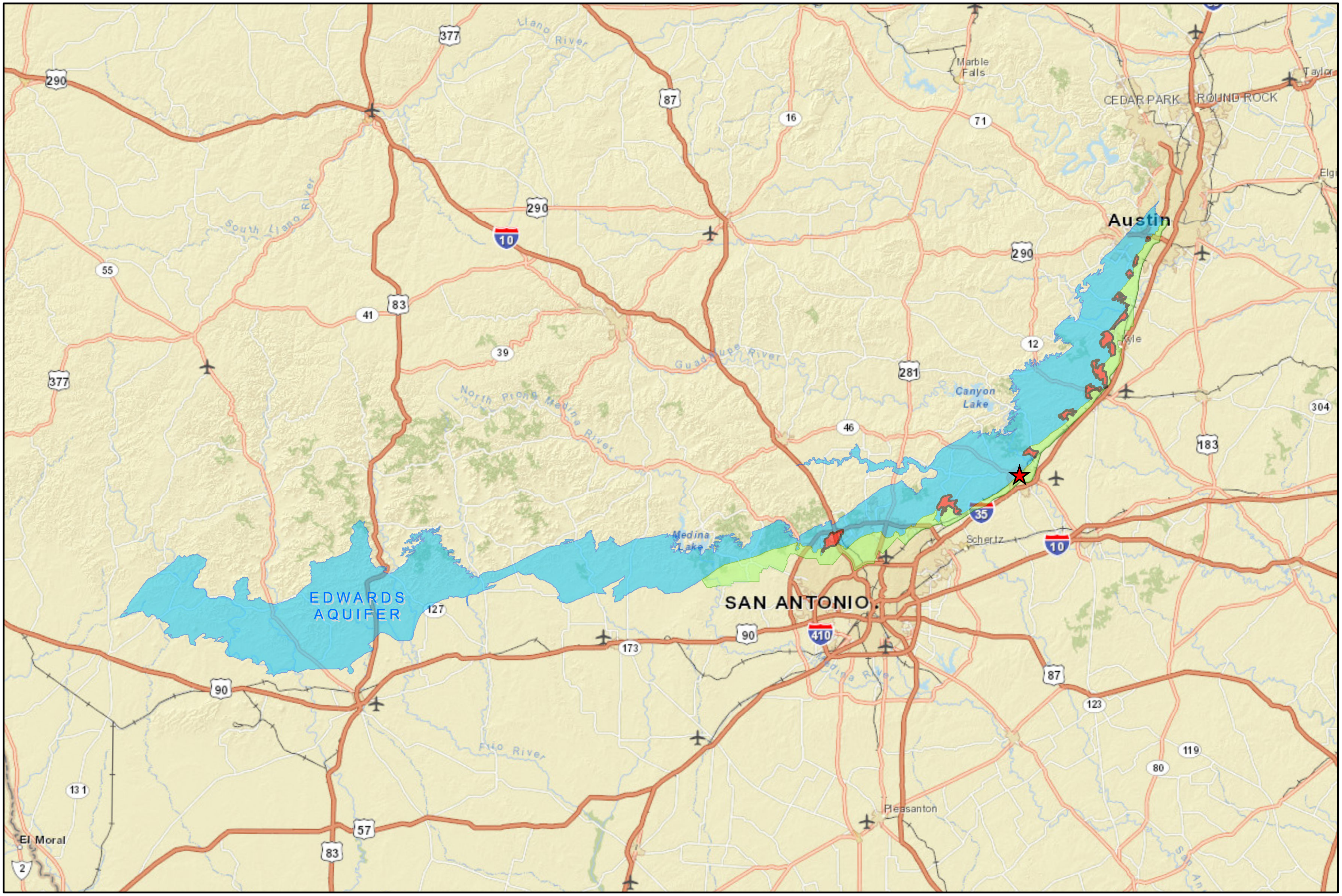
SHEET 1 OF 1 MAP 1

Study Area

Background:	ESRI Street Map
Scale:	1:10000
Created By:	AB
Approved By (or Quad)	RH
SWCA Project No.:	24678
Date Produced:	December 6, 2013

NAD 1983 State Plane Texas South Central Zone 4204

Feet: 0, 500, 1,000
Meters: 0, 100, 200, 300



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CITY OF NEW BRAUNFELS
EDWARDS AQUIFER RECHARGE AND TRANSITION ZONES - COMAL AND SAN MARCOS SPRINGS
COMAL COUNTY, TEXAS

SHEET 1 OF 1 MAP 2

★ Site Location
Recharge Zone
Contributing Zone within Transition Zone
Transition Zone

Background:	ESRI Street Map
Scale:	1:1,250,000
Created By:	AB
Approved By (or Quad):	RH
SWCA Project No.:	24678
Date Produced:	December 10, 2013

NAD 1983 StatePlane Texas South Central FIPS 4204 Feet

0 10 20 30 Miles
0 25 50 Kilometers

Path: S:\Projects\24621_New_Braunfels_Nonnative_Species_Removal\MAPS\NR\mxd\Oxygen Mitigation\Aerator_Locations_Aquifer_Map.mxd

Path: S:\Projects\24621_New_Braunfels_Nonnative_Species_Removal\MAPS\NR\mxd\Oxygen Mitigation\Aerator_Locations_Map.mxd

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CITY OF NEW BRAUNFELS

AERATOR & SONDE LOCATIONS MAP
COMAL COUNTY, TEXAS

SHEET 1 OF 1 MAP 3

Aerator Pads
Sonde
Compressor Station
Telemetry Station

N
S

Background:	ESRI World Imagery
Scale:	1:1500
Created By:	AB
Approved By (or Quad)	RH
SWCA Project No.:	24678
Date Produced:	December 6, 2013

NAD 1983 State Plane Texas South Central Zone 4204

0 100 200 Feet
0 20 40 60 Meters

APPENDIX B
FIGURES

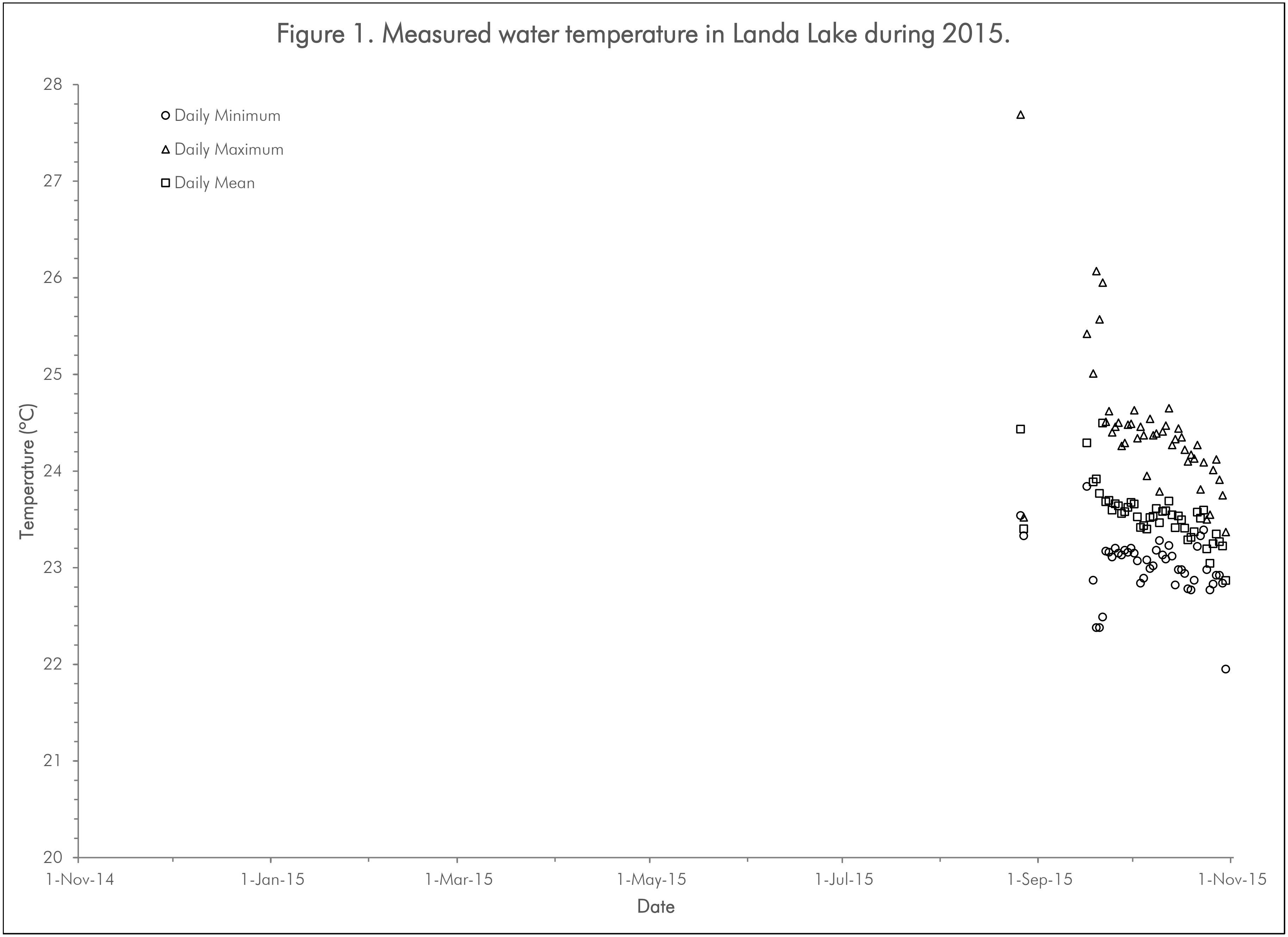


Figure 1. Measured water temperature in Landa Lake during 2015.
Daily Minimum
Daily Maximum
Daily Mean
Temperature (°C)
28
27
26
25
24
23
22
21
20
1-Nov-14
1-Jan-15
1-Mar-15
1-May-15
1-Jul-15
1-Sep-15
1-Nov-15
Date

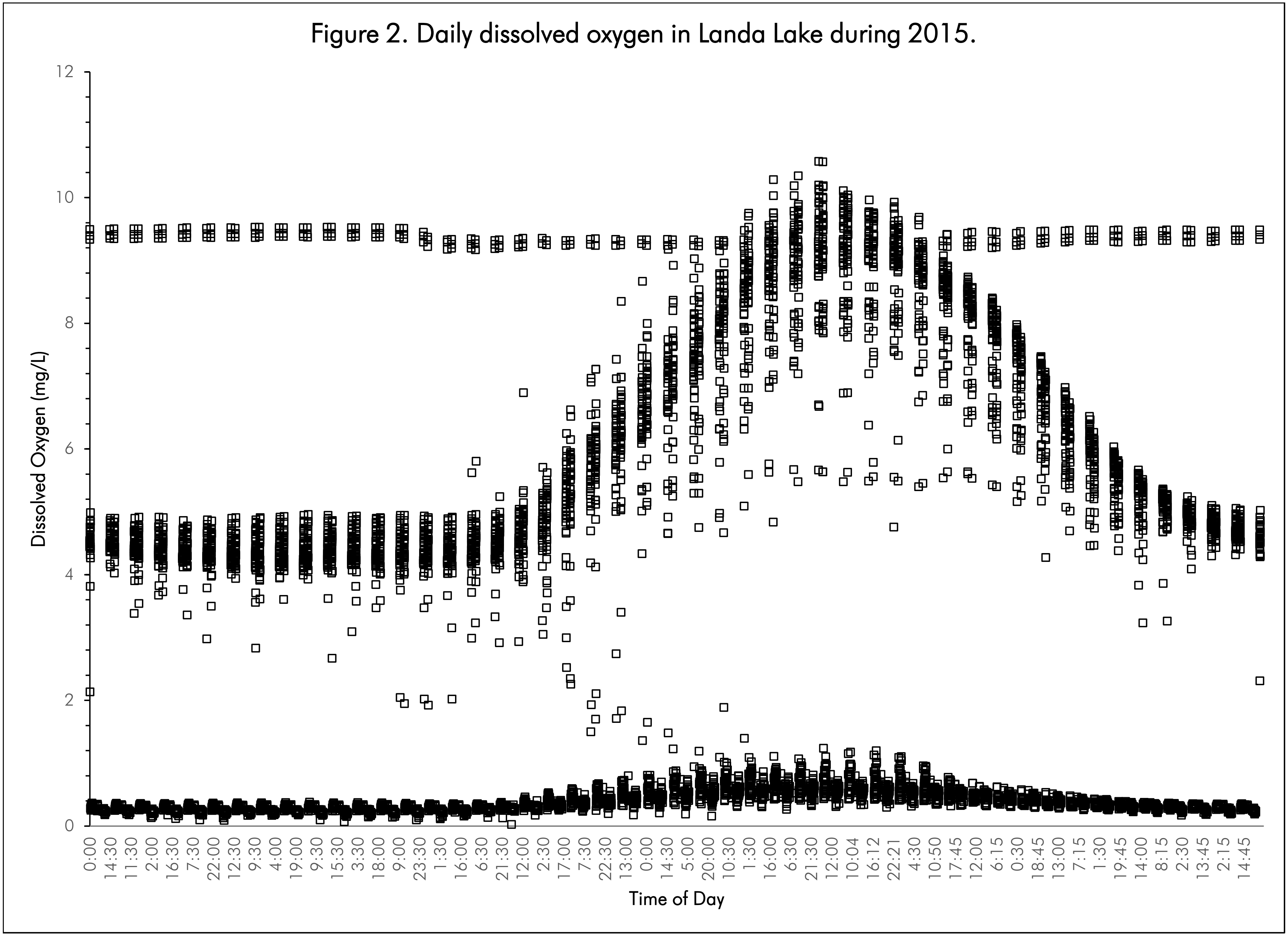


Figure 2. Daily dissolved oxygen in Landa Lake during 2015.
Dissolved Oxygen (mg/L)
Time of Day
0
2
4
6
8
10
12

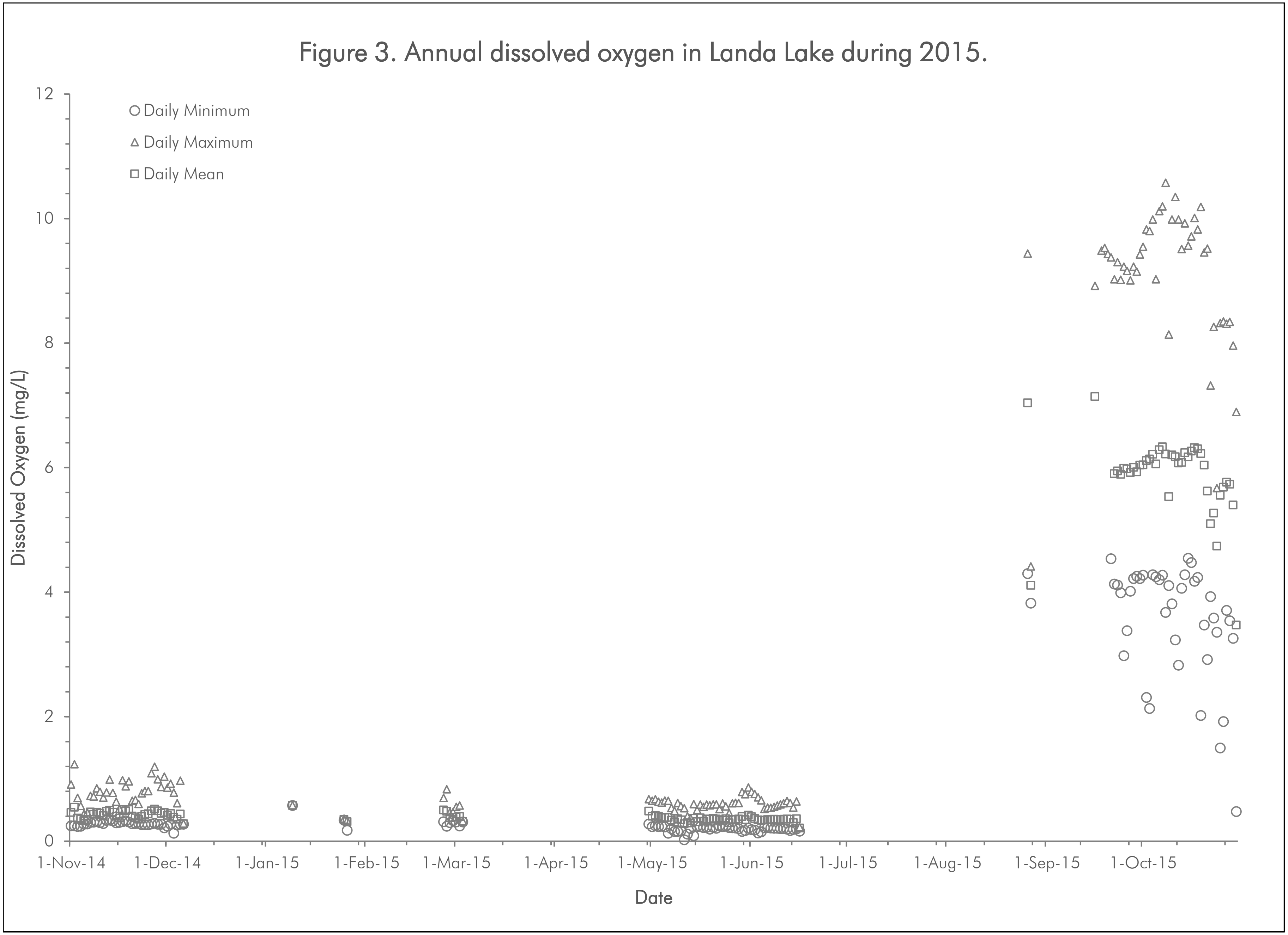


Figure 3. Annual dissolved oxygen in Landa Lake during 2015.
Daily Minimum
Daily Maximum
Daily Mean
Dissolved Oxygen (mg/L)
0
2
4
6
8
10
12
1-Nov-14
1-Dec-14
1-Jan-15
1-Feb-15
1-Mar-15
1-Apr-15
1-May-15
1-Jun-15
1-Jul-15
1-Aug-15
1-Sep-15
1-Oct-15
Date

Figure 4. pH measurements for Landa Lake during 2015.

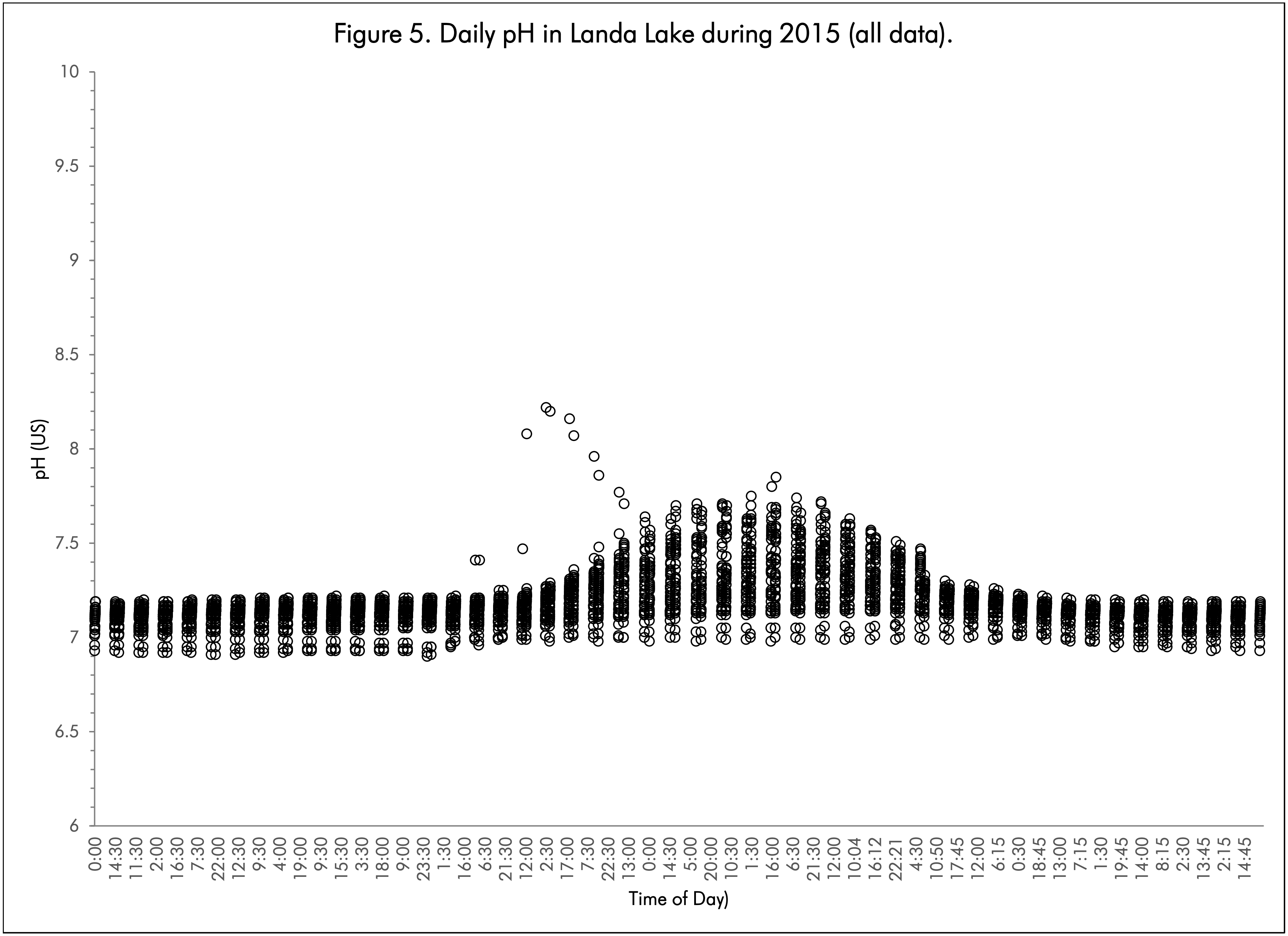


Figure 5. Daily pH in Landa Lake during 2015 (all data).
10
9.5
9
8.5
8
7.5
7
6.5
6
pH (US)
0:00
14:30
11:30
2:00
16:30
7:30
22:00
12:30
9:30
4:00
19:00
9:30
15:30
3:30
18:00
9:00
23:30
1:30
16:00
6:30
21:30
12:00
2:30
17:00
7:30
22:30
13:00
0:00
14:30
5:00
20:00
10:30
1:30
16:00
6:30
21:30
12:00
10:04
16:12
22:21
4:30
10:50
17:45
12:00
6:15
0:30
18:45
13:00
7:15
1:30
19:45
14:00
8:15
2:30
13:45
2:15
14:45
Time of Day)

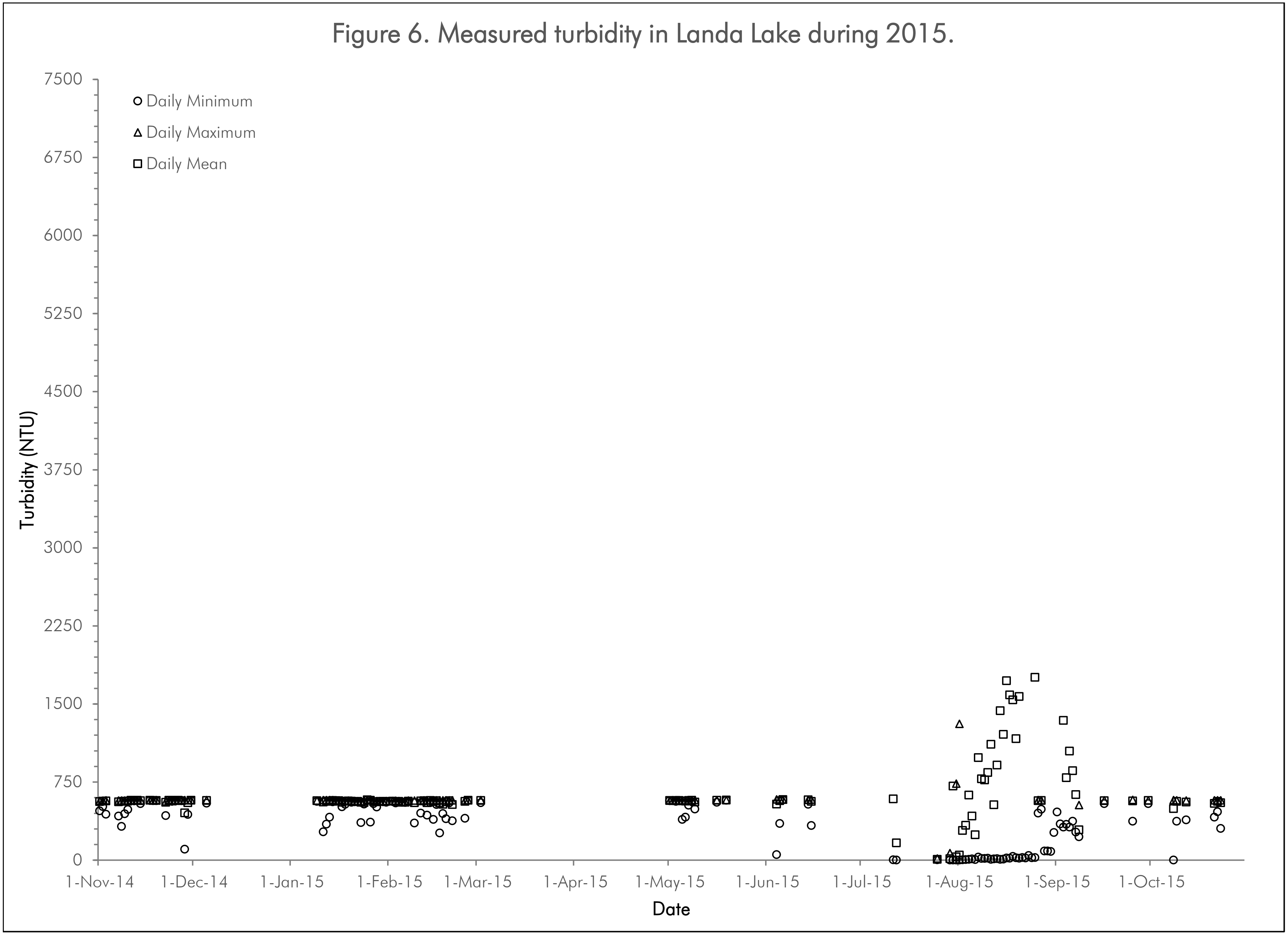


Figure 6. Measured turbidity in Landa Lake during 2015.
Daily Minimum
Daily Maximum
Daily Mean
Turbidity (NTU)
7500
6750
6000
5250
4500
3750
3000
2250
1500
750
0
1-Nov-14
1-Dec-14
1-Jan-15
1-Feb-15
1-Mar-15
1-Apr-15
1-May-15
1-Jun-15
1-Jul-15
1-Aug-15
1-Sep-15
1-Oct-15
Date

Figure 7. Measured specific conductivity in Landa Lake during 2015.

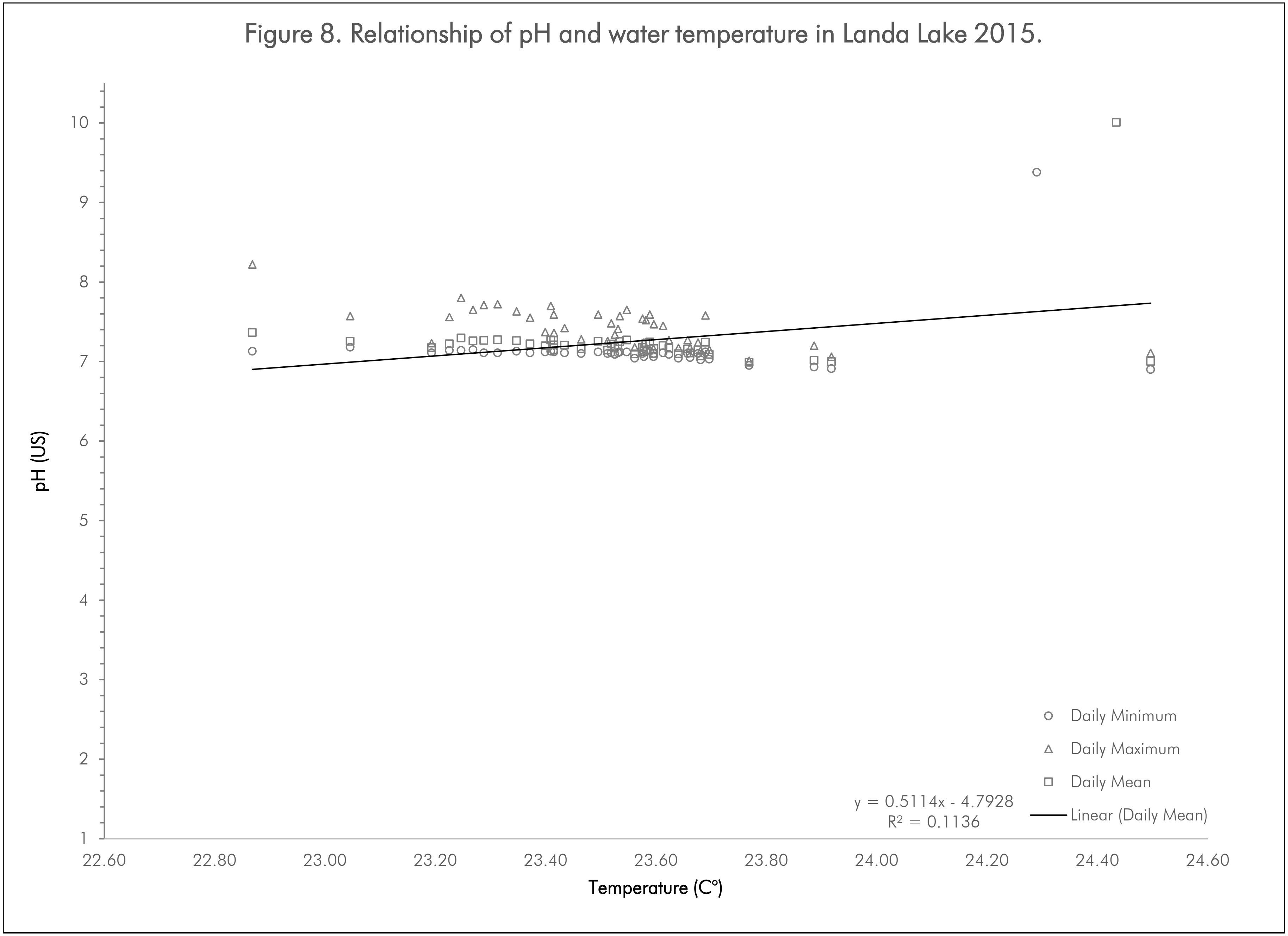


Figure 8. Relationship of pH and water temperature in Landa Lake 2015.
pH (US)
10
9
8
7
6
5
4
3
2
1
22.60
22.80
23.00
23.20
23.40
23.60
23.80
24.00
24.20
24.40
24.60
Temperature (C°)
Daily Minimum
Daily Maximum
Daily Mean
Linear (Daily Mean)
y = 0.5114x - 4.7928
R² = 0.1136

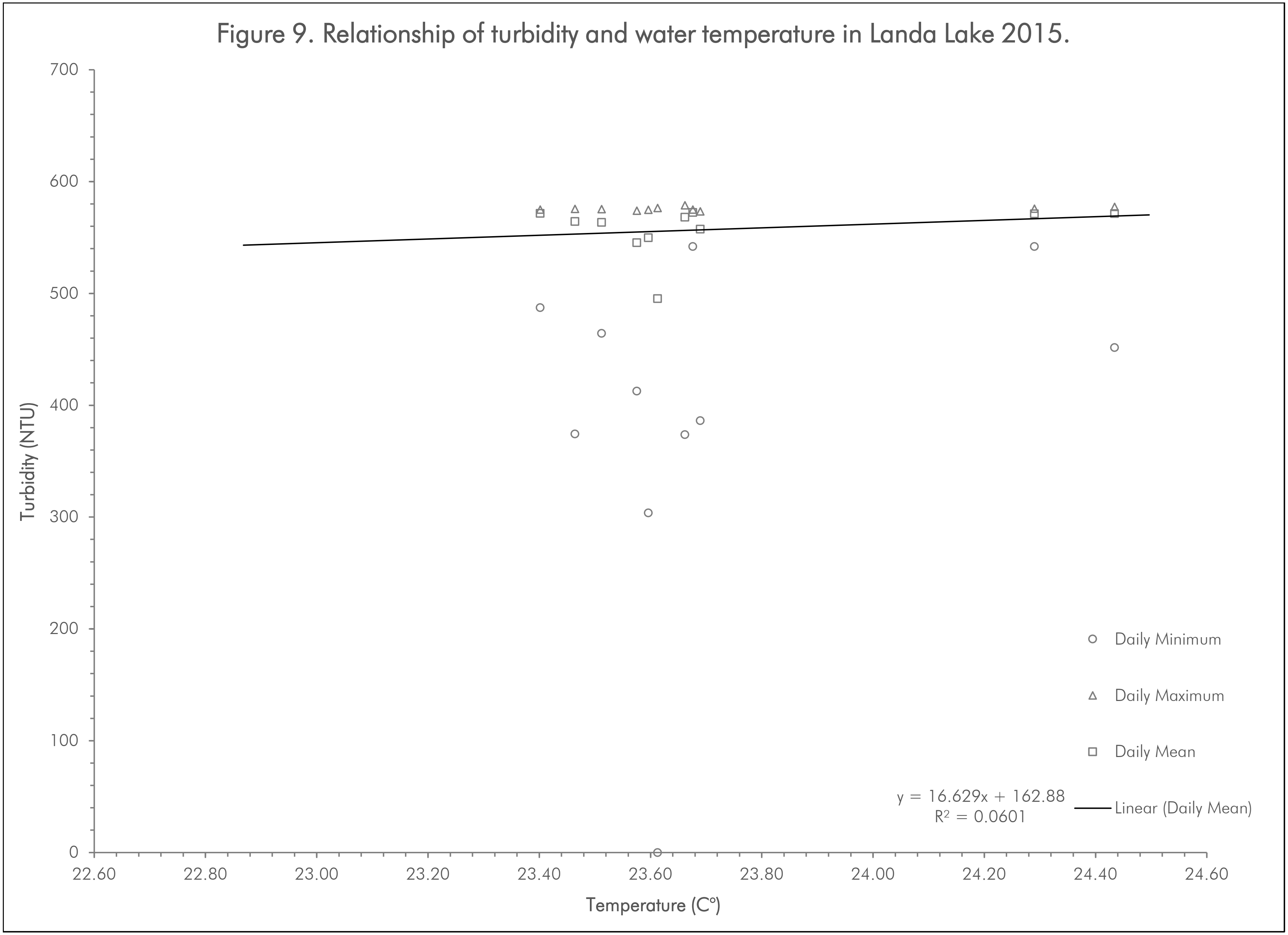


Figure 9. Relationship of turbidity and water temperature in Landa Lake 2015.
Turbidity (NTU)
700
600
500
400
300
200
100
0
22.60
22.80
23.00
23.20
23.40
23.60
23.80
24.00
24.20
24.40
24.60
Temperature (C°)
Daily Minimum
Daily Maximum
Daily Mean
y = 16.629x + 162.88
R² = 0.0601
Linear (Daily Mean)

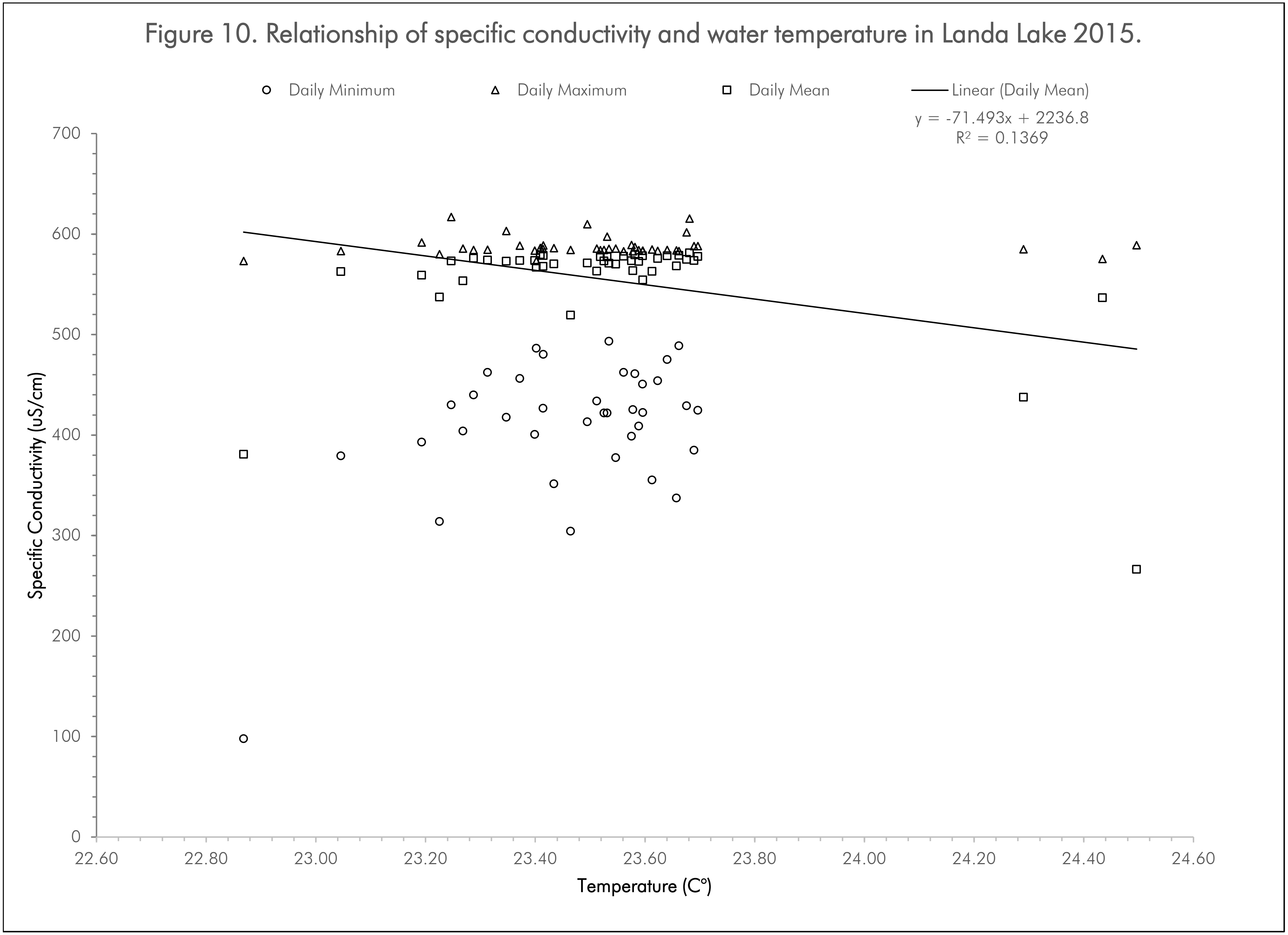
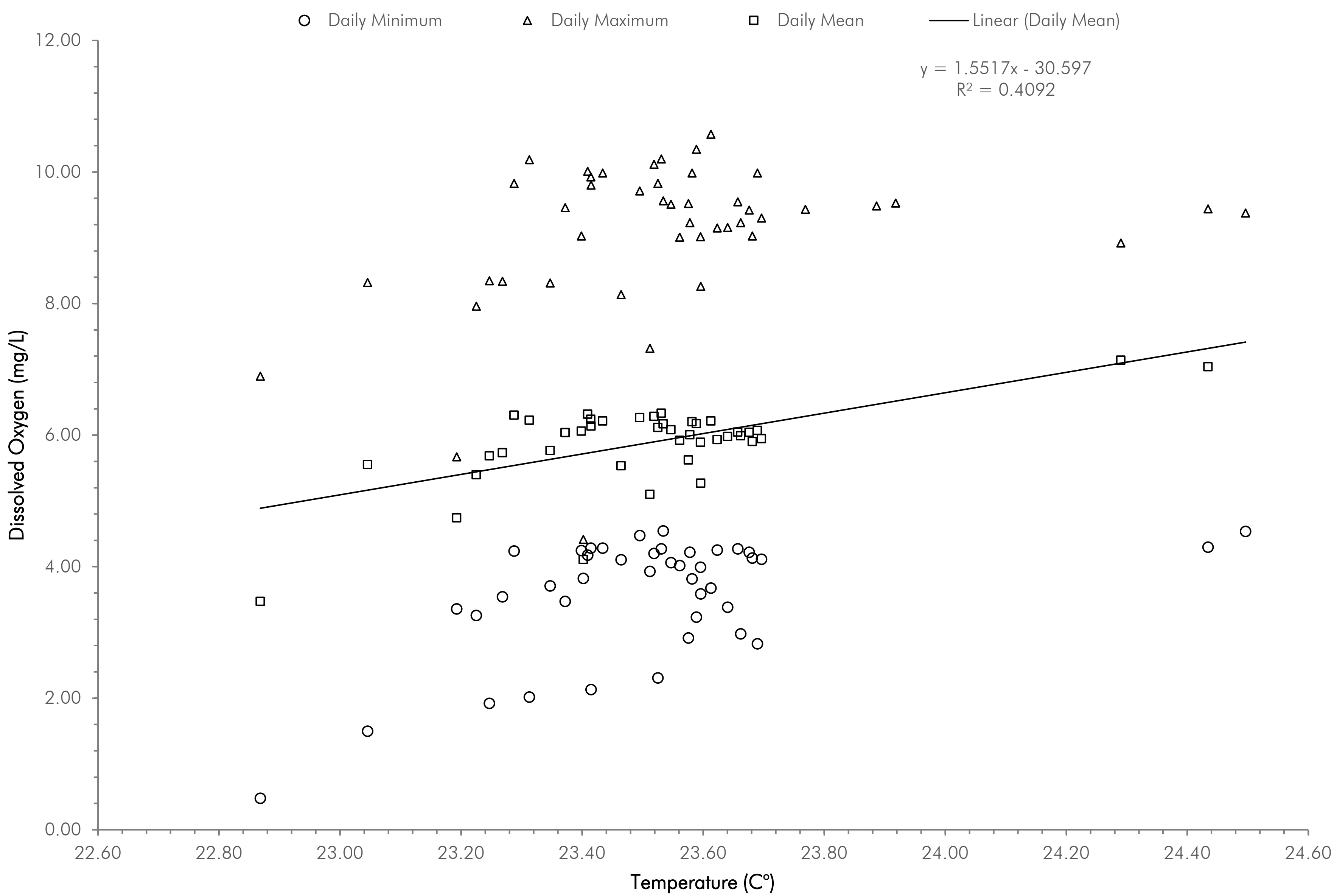


Figure 10. Relationship of specific conductivity and water temperature in Landa Lake 2015.
Daily Minimum
Daily Maximum
Daily Mean
Linear (Daily Mean)
y = -71.493x + 2236.8
R² = 0.1369
Specific Conductivity (uS/cm)
700
600
500
400
300
200
100
0
22.60
22.80
23.00
23.20
23.40
23.60
23.80
24.00
24.20
24.40
24.60
Temperature (C°)

Figure 11. Relationship of dissolved oxygen and water temperature in Landa Lake 2015



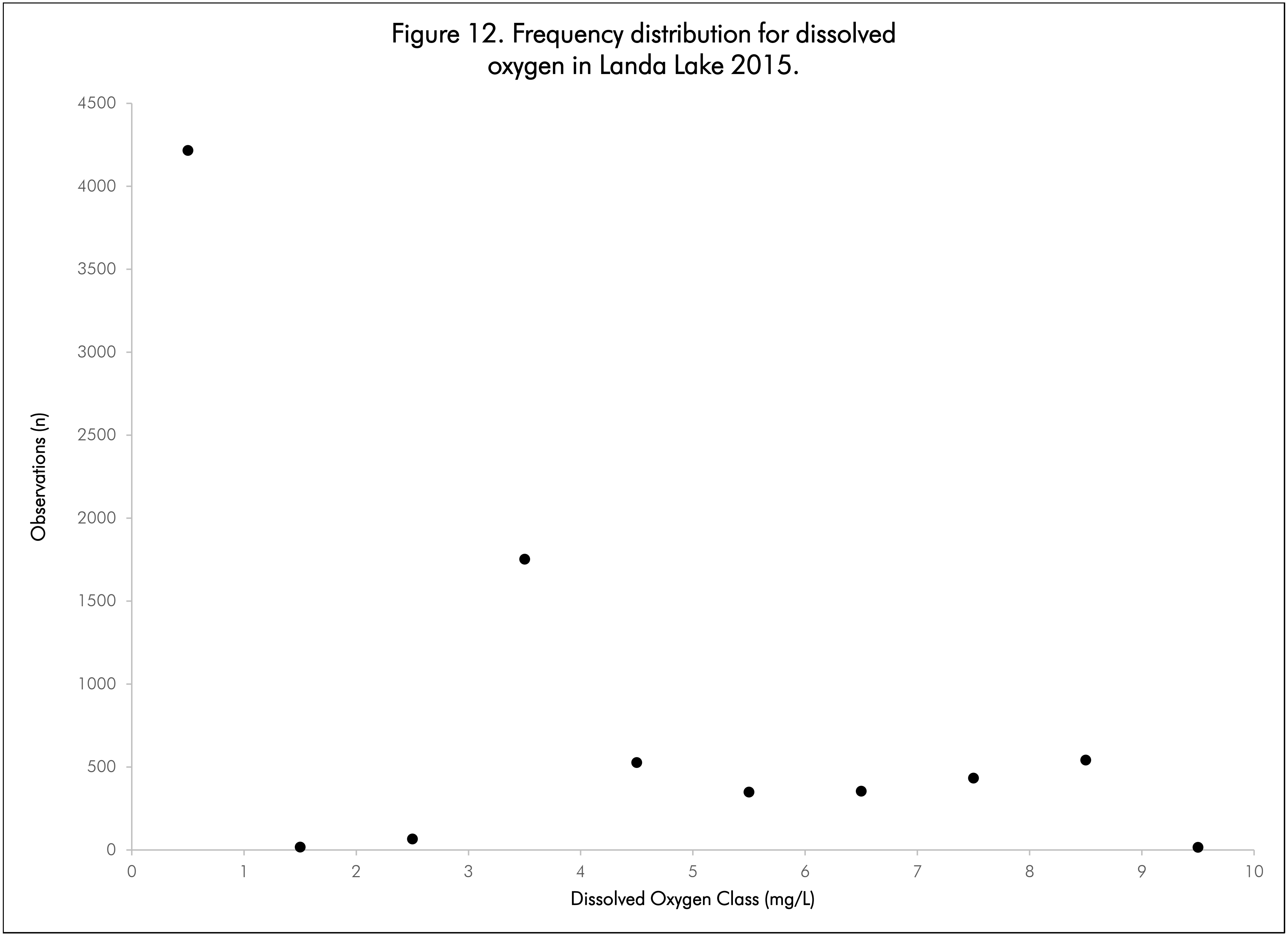


Figure 12. Frequency distribution for dissolved oxygen in Landa Lake 2015.
Observations (n)
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0
1
2
3
4
5
6
7
8
9
10
Dissolved Oxygen Class (mg/L)

Appendix L4

BIO-WEST Memorandum - Supplemental Dissolved Oxygen Evaluation in Landa Lake - 2015

BIO-WEST, Inc.
1812 Central Commerce Court
Round Rock, Texas 78664

MEMORANDUM

TO:	Zackary Martin, Mark Enders
FROM:	Ed Oborny, Tim Osting, Robert Doyle
DATE:	November 5, 2015
SUBJECT:	**SUPPLEMENTAL DISSOLVED OXYGEN EVALUATION IN LANDA LAKE - 2015**

EXECUTIVE SUMMARY

During summer 2015, the BIO-WEST project team conducted a supplemental dissolved oxygen evaluation in the Comal system focused on 1) evaluating the spatial distribution of dissolved oxygen (DO) in the Landa Lake relative to the established DO monitoring location for the HCP Aeration project, and 2) testing the efficacy of the current aerator setup in Landa Lake. Results from this investigation suggest that DO concentrations in flowing waters throughout Landa Lake in 2015 were generally > 4.0 mg/L and fairly consistent under the total system discharge conditions observed during the study period. From a historical perspective, this represents total system discharge conditions slightly above the long-term historical average which is considerably different from the 65 cfs total system discharge conditions experienced during summer 2014. Results from this study and additional work being conducted for a concurrent 2015 HCP applied research project focused on floating vegetation mat oxygen consumption showed that biofouling of sensors was a major factor in elevated and very low measurements of DO in the Comal system during 2015. Finally, calculations based on a trial diffuser study conducted and subsequent spreadsheet modeling with inherent assumptions suggest that the current aerator system employed in Landa Lake is insufficient to maintain HCP specified DO conditions (4.0 mg/L) during low-flow conditions in Landa Lake.

Based on these results, the project team recommends several activities for consideration in 2016 and beyond. Spatial examinations of DO should be conducted under a range of total system discharge conditions, primarily lower flow conditions than observed in 2015. A detailed evaluation of the HCP Aerator project DO database and calibration/probe cleaning notes may shed light on potential biofouling influenced results for that program. Additional work should be conducted to more narrowly focus diffuser deployments towards specific objectives and to determine the most efficient mechanical technology capable of accomplishing objectives. In order to accomplish or strive towards HCP directives, a better definition for DO management goals and objectives will need to be formulated moving forward. A goal of maintaining 4.0 mg/L DO throughout all fountain darter habitat in the Comal system is likely not possible from a practical or cost perspective. It is also likely not necessary for the survival and recovery of endangered species during and following low-flow events. To test this hypothesis, a series of diffuser studies (testing the number of and placement of diffusers) is recommended for 2016. These studies would focus on evaluating and potentially establishing a “protection zone” for fountain darters in key habitat locations from the Spring Island to Three Island area of Landa Lake should low-DO conditions result from future low-flow conditions in the system.

Finally, aside from membrane disc diffusers tested as part of this project and future recommended activities, there are other means for increasing DO in the Comal system. Alternate methods that may meet management objectives that have not been evaluated are those that can improve circulation (pumps, Solar Bees fans, etc.), and are recommended for further investigation. Because of the lake's large surface area, the natural reaeration processes combined with increased exchange of shallow and deep waters may be an effective method to increase overall DO conditions.

INTRODUCTION

To mitigate low dissolved oxygen (DO) concentrations caused by natural processes, engineered approaches are commonly designed and employed in waste water treatment plants, reservoirs and ponds. A typical mechanism to increase dissolved oxygen content of water is through use of a diffuser, a device that allows air (or pure oxygen in some applications) to bubble up through the water column. Other effective methods include those that promote circulation and mixing of waters. Circulation methods typically employ pump systems, fountains or large fan blades to pull deep water toward the surface to increase DO content by increasing contact with the atmosphere.

Considerations in design of aeration systems include oxygen demand of organisms in the water column and sediments, movement/circulation of water, shape of water body (e.g., surface area and depth) and water body volume, including whether there are inflows or outflows. These universal factors are anticipated to affect DO concentration in Landa Lake during low spring flow conditions, and this is a preliminary investigation into use of diffuser devices to counterbalance and increase DO concentrations. This preliminary comparative analysis considers two areas within Landa Lake: the main open water part of the lake near the observation pier, and the lake headwaters near Blieders Creek.

In 2013 a set of diffusers was installed in the lake near the observation pier in conjunction with a continuous DO monitor (Figure 1, SWCA 2014). The effectiveness of the main lake diffusers was unclear in response to natural low-flow and low-DO conditions occurring during summer 2014. The DO content of lake waters near the monitor did not appear to increase while the diffusers were in operation. This could have been an artifact of the location of the DO monitor being placed on the lake bottom in non-flowing waters (shielded by vegetation) (Figure 2), or lack of proximity to diffusers, or insufficient capacity of the diffusers to impart enough DO considering the characteristics (volume, velocity, depth) of this area of the lake.

The DO probe records data on a routine time step and is reported in real-time to the City of New Braunfels. The DO data is monitored to aid in decisions regarding triggering the series of diffusers that have been placed in Landa Lake (Figure 1) as part of HCP Aeration project. Questions that have been raised over the first few years of the HCP Aeration project are 1) whether this single monitor probe accurately represents DO conditions throughout larger portions of the lake, 2) whether biofouling might play a role in some of the extremely high and low DO values reported at times since initial placement, and 3) whether the diffuser arrangement currently implemented is sufficient to support DO conditions (> 4.0 mg/L) as specified in the HCP (EARIP 2011). As such, additional studies were conducted by BIO-WEST, Baylor University, and AquaStrategies (BIO-WEST project team) during 2015 to investigate these questions. This memorandum presents the results and recommendations stemming from the 2015 DO investigations.

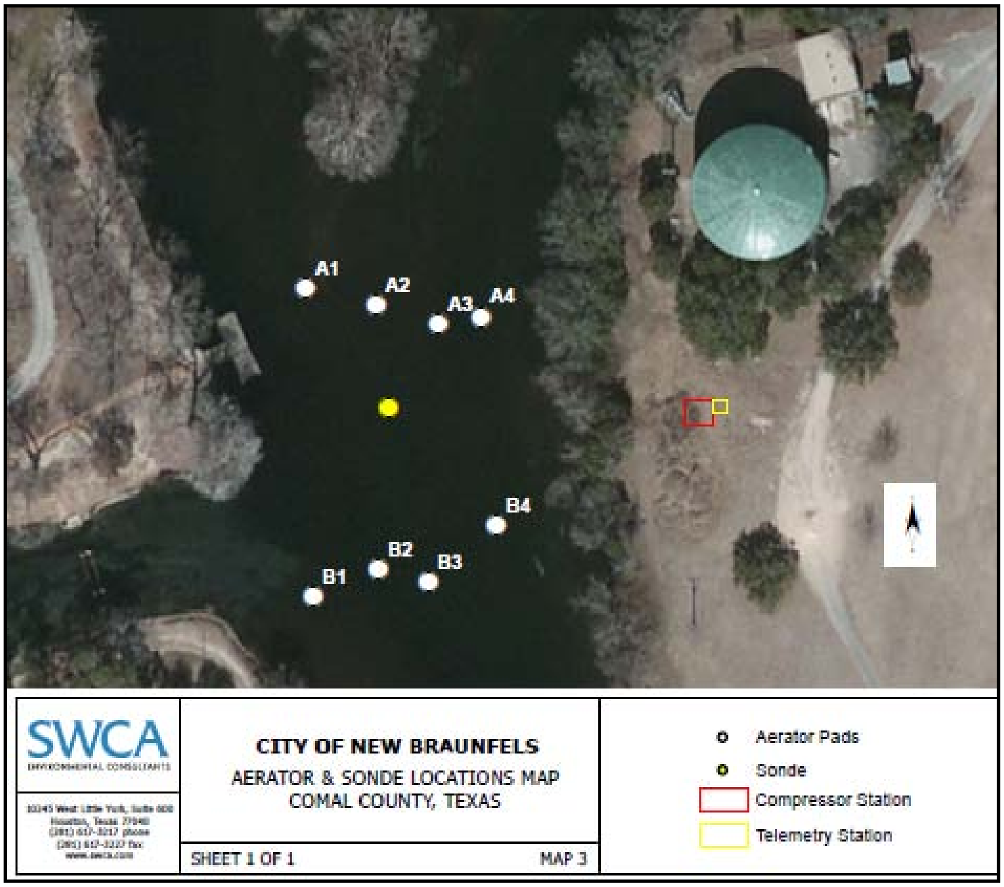


Figure 1: HCP Aeration Project. Water Quality Sonde and Aerator Pads (SWCA 2014).

Figure 2: Water quality multi-probe installed in Landa Lake for Aeration Project.

SPATIAL DISSOLVED OXYGEN EVALUATION

To investigate how representative existing DO measurements in the center of Landa Lake is to surrounding areas, a one-week spatial evaluation of DO was conducted within Landa Lake and the Upper Spring Run reach. For this investigation, 14 MiniDOT DO sensors were installed in the Comal system in late July and set to record measurements on 10 minute intervals. The MiniDOT logger is a completely submersible instrument that logs DO and water temperature measurements. MiniDOT DO sensors are made by Precision Measurement Engineering (PME, Vista CA) and utilize the newer optical fluorescence technology to measure DO concentrations. The sensors have been widely used by the USGS and were tested in the labs at Baylor University prior to deployment. Our tests show that the MiniDOTS are equivalent to a YSI datasonde utilizing similar optical technology. We logged data while bubbling sequentially with air and nitrogen to produce air-saturated and anoxic conditions. The MiniDOT values were within a few percent of the YSI at all measurement periods. The oxygen sensor is an optode that measures DO concentration in water through a fluorescence method. Figure 3 shows a MiniDOT DO sensor deployed at a mid-column location in Landa Lake.

Figure 3: MiniDOT dissolved oxygen sensor deployed during spatial expansion study

Biofouling of deployed sensors is a well-known problem for long-term biomonitoring. We initially deployed sensors in four locations of Landa Lake in the vertical "upright" (sensor facing upward) position recommended by PME. However, we found that when the sensors were in high-light environments, heavy biofouling happened within 2-3 days and DO maxima of >12 mg/L DO and overnight minima of < 2 mg/L were recorded. We therefore modified to deploy the sensors in

a horizontal position, and when possible facing north to minimize light exposure to the surface of the sensor. This resulted in much less observable biofouling at most locations for 5-7 days (Figure 4). However, at low-flow environments rapid biofouling continued to be a significant issue and at these sites data more than 48 hours after deployment or cleaning should be viewed with caution. Table 1 provides a description of each MiniDOT sensor location corresponding with Figure 5.

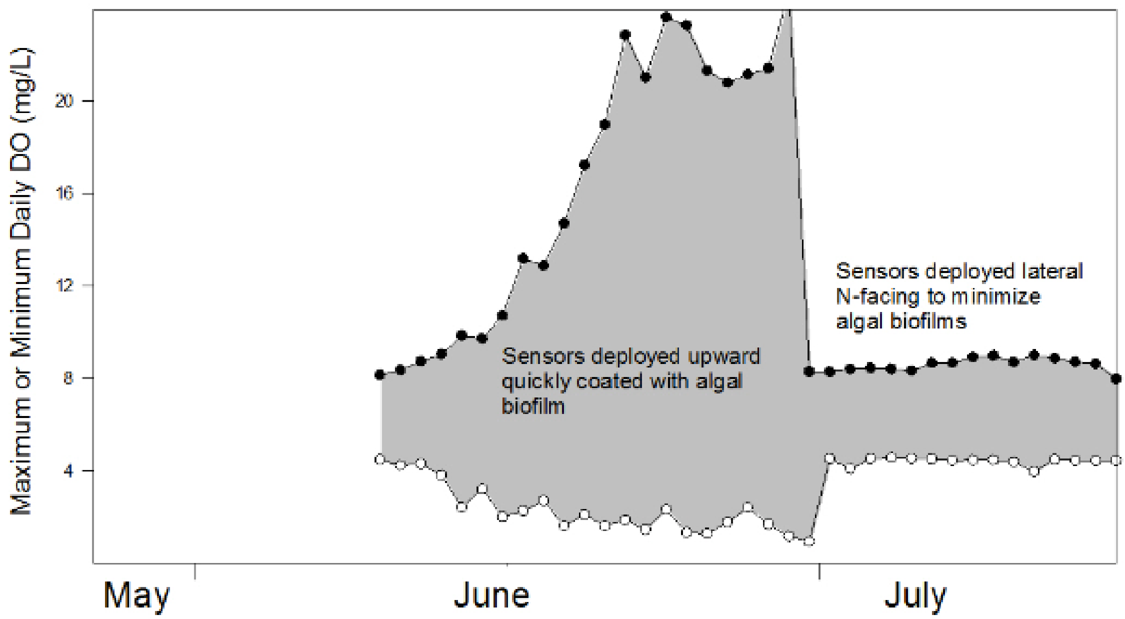


Figure 4: Upward versus lateral deployment results of biofouling activity.

TABLE 1 – Description of 14 MiniDOT dissolved oxygen sensor locations in Landa Lake

SITE #	***DESCRIPTION***
1	***Downstream Buoy in Landa Lake***
2	***Adjacent to Paddleboat rental area***
3	***At Existing DO probe for Aerator Project***
4	***Adjacent to Fishing Pier in Vallisneria***
5	***Adjacent to Gazebo at the outflow of Spring Run 3***
6	***Top of Island 1 in three islands area***
7	***Upstream of Island 3 in three islands area***
8	***Lower Pecan Island backwater area***
9	***Northwest shore across from Pecan Island***
10	***Mid Channel location near MUPPT nursery***
11	***Northwest shore near Cable***
12	***Adjacent to Golf Course in Pecan Island backwater***
13	***Upstream of Spring Island***
14	***Adjacent to Heidelberg Lodge***

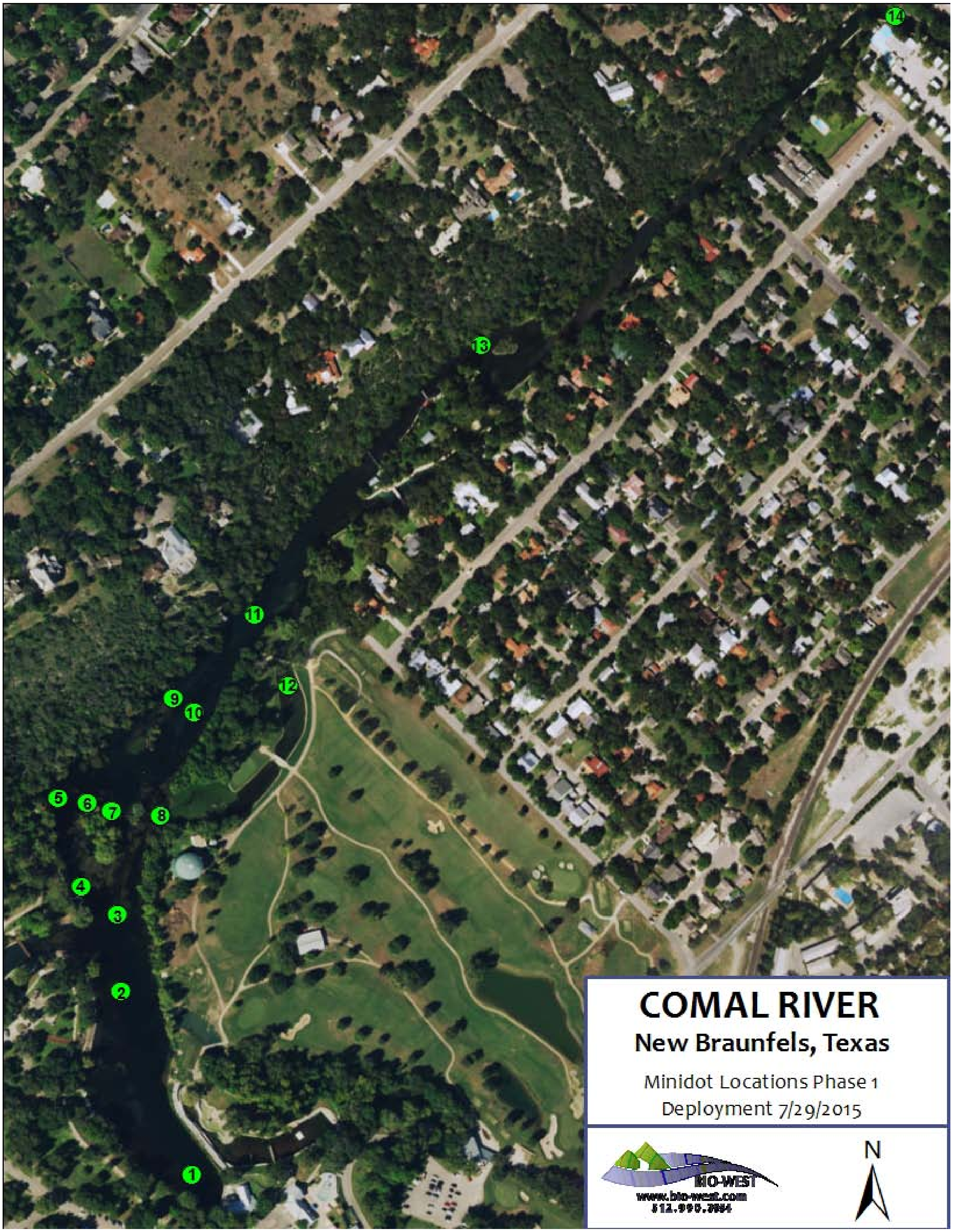


Figure 5: Location of 14 MiniDOT dissolved oxygen sensors during spatial evaluation study

Measurement locations were selected during a reconnaissance investigation in late June conducted by Ed Oborny and Casey Williams (BIO-WEST) and Dr. Robert Doyle (Baylor). At this time, the HCP Aeration project water quality probe was in place and operating. Measurement sites were located throughout the main portion of Landa Lake in differing habitat types (deep, shallow, with current, stagnant, vegetated, non-vegetated, etc.) to capture a wide range of conditions that are experienced throughout Landa Lake and the upper spring run reach. The spatial evaluation study was initiated on July 28th and continued for one week with sensors being downloaded on August 5th. During this time period, total system discharge in the Comal system ranged from approximately 300 to 340 cfs. From a historical perspective, this represents total system discharge conditions slightly above the long-term historical average which is considerably different from the 65 cfs total system discharge conditions experienced during summer 2014. Although unfortunate that lower flow conditions were not available in summer 2015 to test, the data does represent what is to be expected during average total system discharge conditions. It was also unfortunate that during this study period, the HCP Aeration project water quality probe was inoperable and not in Landa Lake for comparison.

Results from the week-long spatial study are presented in Figure 6. A solid red line is place on each chart representing the HCP goal of 4.0 mg/L DO. In general, DO conditions ranged between approximately 4.0 and 9.0 mg/L on a daily cycle at most stations (Figure 6). The exception to this was at the more stagnant areas (Sites 8, 12, and 14). Dissolved oxygen conditions at Site 8 dipped down to approximately 3.0 mg/L on day 2 and continued to dip below 4.0 mg/L each subsequent morning of the study. Considering the location of this sonde within a slow moving area surrounding by *Nuphar*, this was not unexpected. In fact, this likely provides a glimpse of what might be expected when total system discharge conditions are considerably lower causing pockets of considerably lower velocity fields.

Sites 12 and 14 were also located in areas with considerably lower velocities and would likely have experienced DO conditions less than 4.0 mg/L similar to Site 8 on a daily basis. Although these conditions were experienced, biofouling of the probes at these locations rapidly took place causing both extremely high and low measurements of DO at these locations (Figure 6). Upon further investigations this summer and fall with bio-fouling, we are confident that biofouling was occurring at these locations during this study period. As such, we are not confident in the DO measurements reported in Figure 5 for these 2 sites during the one-week study. It was surprising how quickly the biofouling would occur on the sensors upon installation and following sensor cleaning. During continued investigations, it was not uncommon for biofouling to occur within a day of installation, requiring frequent cleaning to obtain accurate measurements.

From an application standpoint, this is extremely important to note relative to the HCP Aeration project and associated water quality probe. It was quite evident during our studies that when the sensors started to record over 12-15 mg/L DO during the late afternoon those sensors were starting to accumulate biofilms that were directly influencing the DO measurements. During these times, extremely low DO conditions were reported the subsequent morning as expected. We point this out because during preliminary evaluations of the existing DO database for the HCP Aeration project we noted considerable DO measurements in excess of 15 mg/L in the late afternoon followed by extreme declines the next morning. Those subsequent declines triggered the activation of the aerators in Landa Lake. At this time, we are unsure how many of the low DO measurements that have triggered the aerators over the implementation period might be an artifact of biofouling. As such, we recommend that the City of New Braunfels investigate the HCP Aerator project DO database and calibration/probe cleaning notes in detail to address this uncertainty. In addition, the City of New Braunfels should inquire whether an automated wiper is being employed on the existing DO probe and, if not, investigate retrofitting the data sonde with a self-cleaning optical DO sensor.

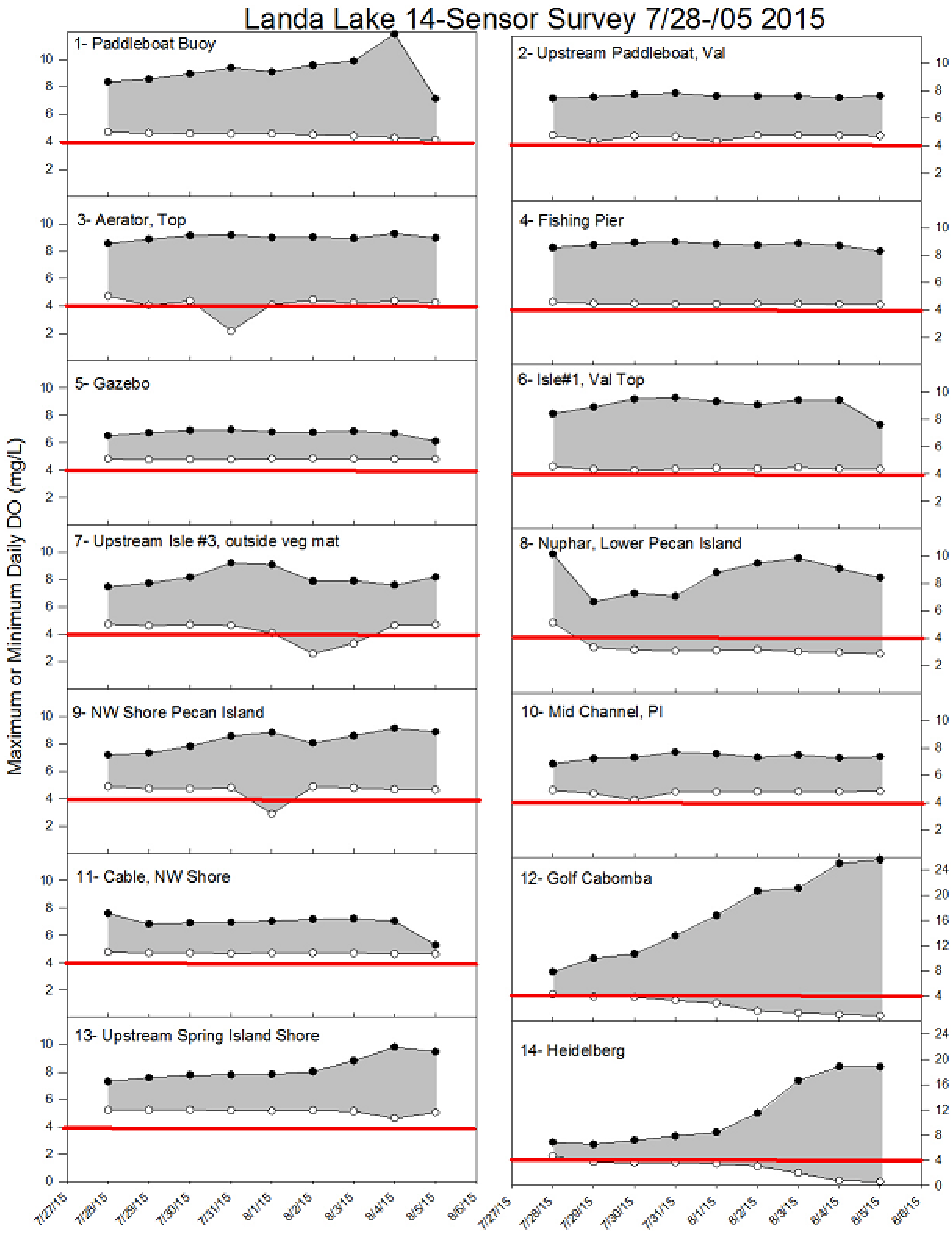


Figure 6: Maximum or Minimum dissolved oxygen results (mg/L) from 14 MiniDOT sensors during spatial evaluation study

DIFFUSER TRIAL STUDY

In mid-August, the BIO-WEST project team coordinated with the City of New Braunfels contractor (SWCA) in charge of the HCP Aeration project to set up a diffuser trial in Landa Lake aimed at evaluating the efficacy of the current aeration system. However, following an additional month and a half (August/September) of data collection to assess oxygen consumption of algal mats within the lake for a concurrent HCP applied research study, it became obvious that DO conditions within the main portion of Landa Lake were not declining low enough to conduct a meaningful test of the current aeration system this year. Dissolved oxygen conditions at Site 14 (Heidelberg Lodge) were experiencing nightly declines in DO that warranted further investigation. The BIO-WEST project team conducted a preliminary investigation of DO conditions at this location during the late afternoon (September 9th) and early morning (September 10th) hours to assess whether a diffuser study in this area might be applicable. Dissolved oxygen measurements during this investigation ranged from approximately 2.9 to 9.5 mg/L with a noticeable decline from late afternoon to the following morning. With this knowledge in hand, the BIO-WEST project team worked closely with SWCA to set up and conducted a diffuser trial study in the upper spring run reach / Blieders creek area.

Summary of Dissolved Oxygen Trial – September 17-18, 2015

Tim Osting (AquaStrategies), Ed Oborny and Michael Edwards (BIO-WEST), and Rick Howard and Taylor Guest (SWCA) met at the City of New Braunfels Landa Lake Park Office at 11:00am on September 17th. The study team conducted a pre-test experiment of the aeration system in Blieders Creek toward the upstream edge of the concrete barrier adjacent to Heidelberg Lodge (Figure 7).

Figure 7: Photographs of pre-test aeration experiment conducted by BIO-WEST and SWCA on September 17th.

SEPTEMBER 17, 2015

9/17/15 Formal Experiment

Following the pre-test experimentation, BIO-WEST proceeded to establish transects through the main study area (Figure 8). Each transect had a minimum of three locations with each diffuser transect (A and C) having five locations along each transect. Transect D had one additional location D13 out in the center of Spring Run 4. During this time, SWCA set up and installed the diffusers at sites C8 and A2 (Figure 8). Diffusers were tested and then turned off. BIO-WEST then retrieved the 5th MiniDOT sensor from the open algae enclosure experiment. Temperature and DO were recorded with a handheld portable meter (HACH® HQ40d multi-parameter meter) using a luminescent optical DO probe (HACH® IntelliCAL™ LDO101 probe).

9/17/15 - Baseline measurements – 5:00 to 6:32pm. Top and bottom measurements of DO and water temperature were taken at each transect location except D13 where only a mid-depth measurement was recorded. In addition to the transect location measurements, five additional measurements for DO and water temperature were taken directly at the five MiniDOT sensors. Note that MiniDOT sensor 5 at G21 was not installed until 7pm to ensure that Dr. Robert Doyle was fine with the location. As such, only Probes N (C9), DSN (D12), USN1, and USN2 had measurements during the 1st baseline measurement. Upon Dr. Doyle's arrival, the 5th MiniDOT sensor was installed at G21 (Figure 9).

Diffusers were turned ON at 7:30 pm. An underwater view of the diffusers in operation is shown in Figure 10.

9/17/15 – 1st Post-Diffuser measurements – 9:00 to 9:48pm. Top and bottom measurements of DO and water temperature were taken at each transect location except D13 where only a mid-depth measurement was recorded. In addition to the transect location measurements, five additional measurements for DO and water temperature were taken directly at the five MiniDOT sensors. All data was collected by BIO-WEST.

9/17/15 – 2nd Post-Diffuser measurements – 11:00 to 11:45pm. Top and bottom measurements of DO and water temperature were taken at each transect location except D13 where only a mid-depth measurement was recorded. In addition to the transect location measurements, five additional measurements for DO and water temperature were taken directly at the five MiniDOT sensors. All data was collected by BIO-WEST.

Diffusers were turned OFF at 11:52 pm.

SEPTEMBER 18, 2015

9/18/15 - Baseline measurements – 3:57 to 4:59am. Top and bottom measurements of DO and water temperature were taken at each transect location except D13 where only a mid-depth measurement was recorded. In addition to the transect location measurements, five additional measurements for DO and water temperature were taken directly at the five MiniDOT sensors. All data was collected by BIO-WEST and Baylor.

Diffusers were turned ON at 5:10 am.

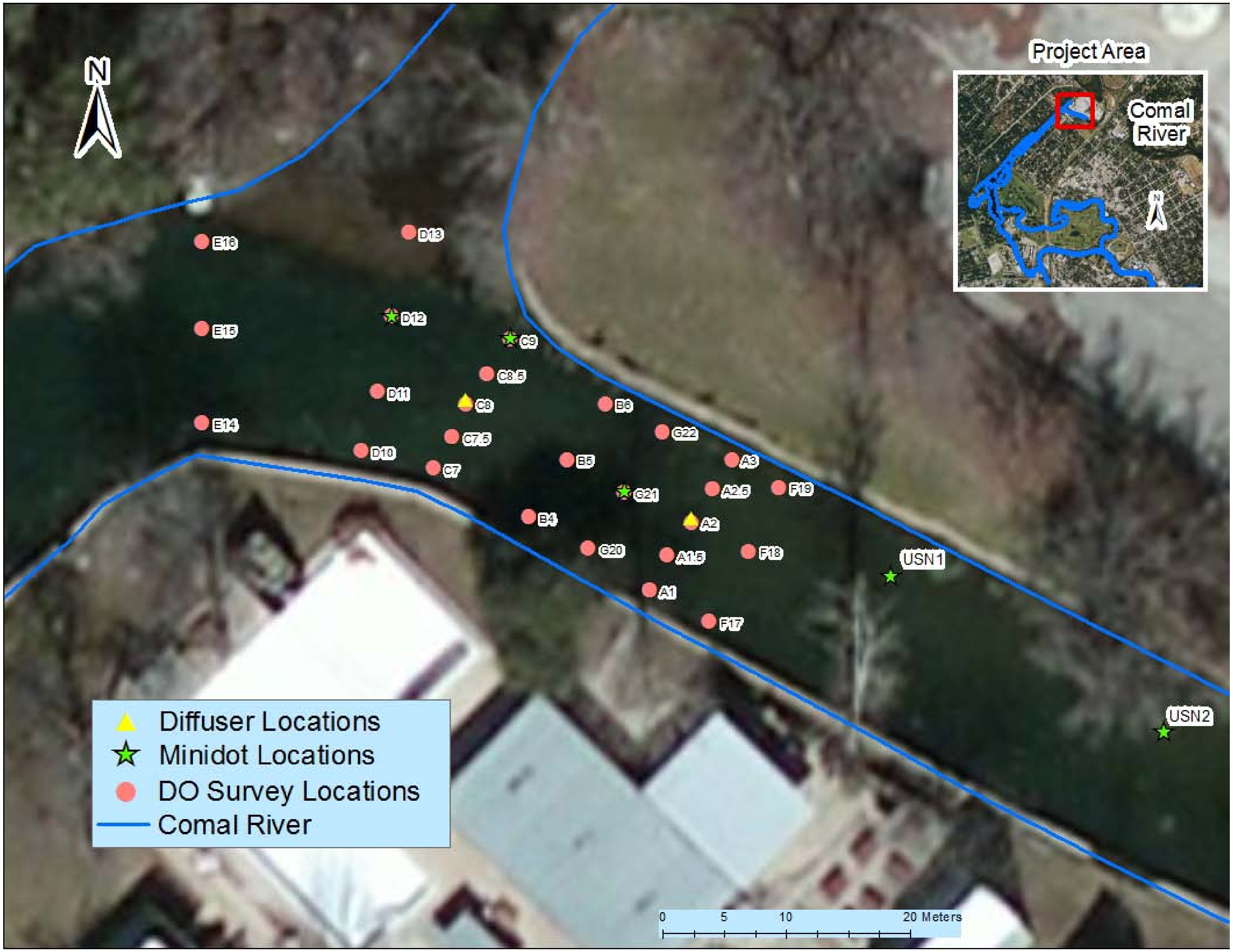


Figure 8: Sampling transects, diffuser and MiniDOT sensor locations for formal diffuser trial on September 17-18.

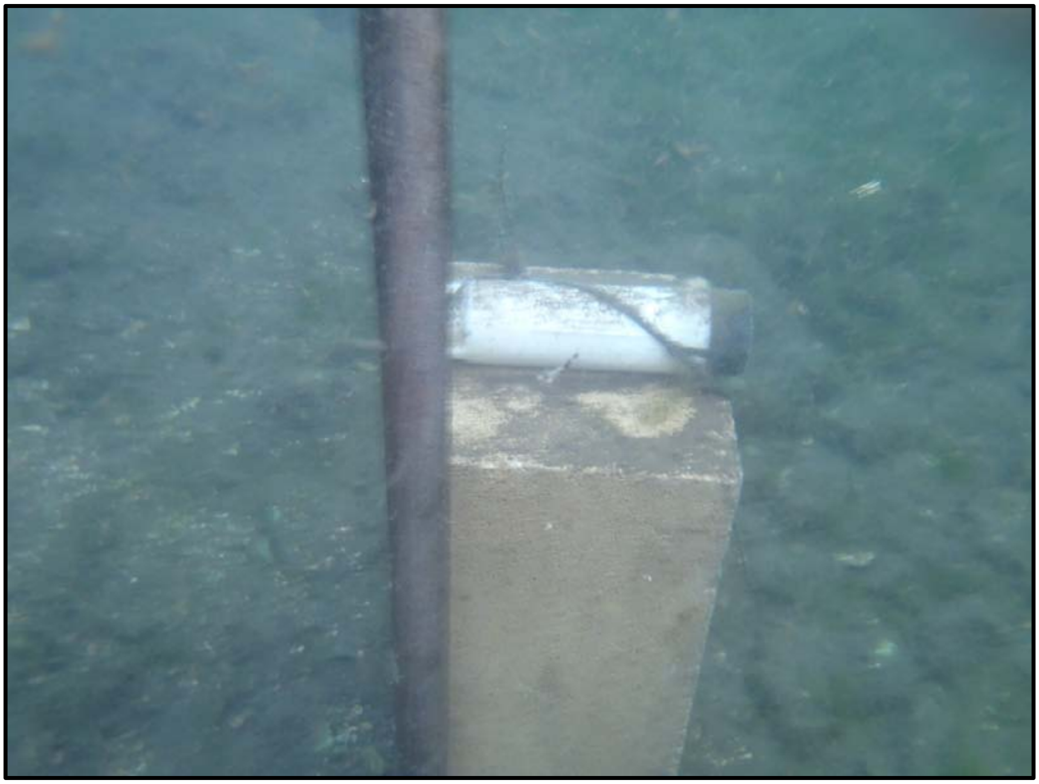


Figure 9: MiniDOT sensor placed at Site G21.

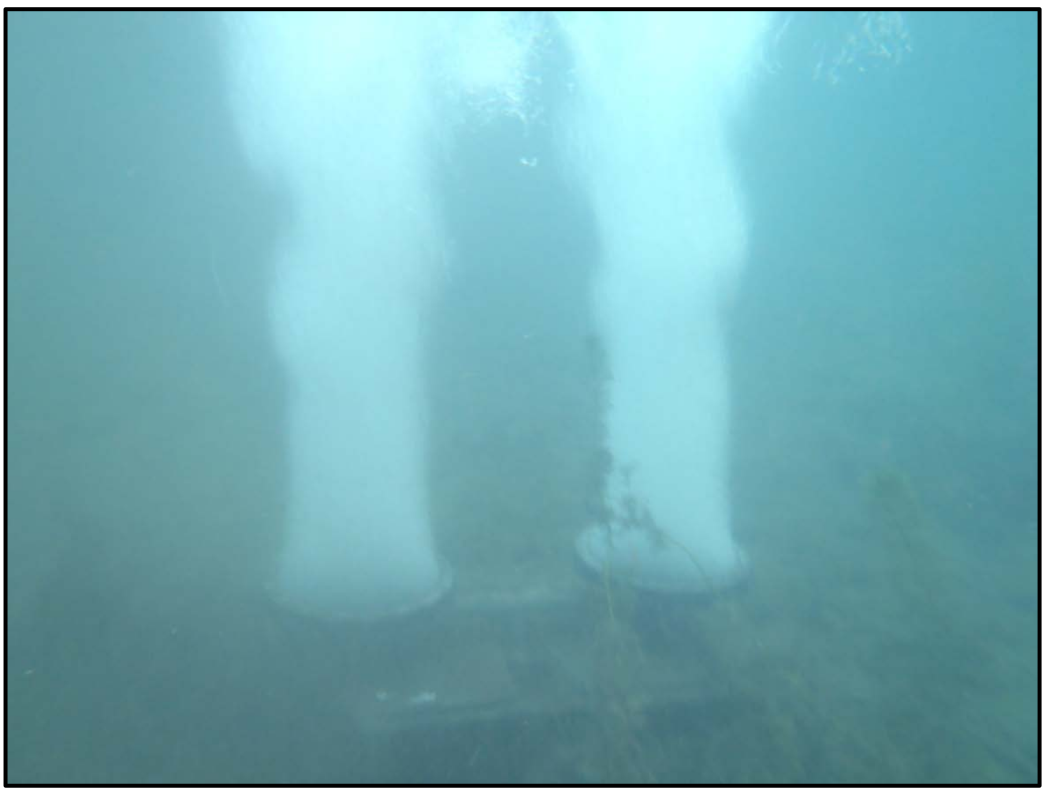


Figure 10: Underwater view of diffusers in operation.

9/18/15 – Upstream diffuser measurements – 5:09 to 5:41am. Dissolved oxygen measurements (top and bottom) were taken at Sites B5 and F18 at time 0. Measurements were initiated immediately upon turning on the diffusers. Both locations are approximately 15 feet upstream of their respective diffuser. An additional four sets of measurements were taken at each location at approximately 5, 10, 20, and 30 minutes post-startup. All data was collected by BIO-WEST and Baylor.

9/18/15 – Transect diffuser measurements – 5:43 to 6:38am. Following the individual site measurements, DO measurements (top and bottom) were taken at each of the five points along transects A and C. Measurements were started with the five points along transect A, then 5 feet upstream on centerline, 5 feet downstream on centerline, 10 feet downstream on centerline, and 15 feet downstream on centerline. The same set of nine measurement locations (top and bottom) were then conducted along transect C. The exact set of measurements were then repeated, first for transect A, then transect C. All data was collected by BIO-WEST and Baylor.

9/18/15 – Post-Diffuser full set of measurements – 6:41 to 7:30 am. Top and bottom measurements of DO and water temperature were taken at each transect location except D13 where only a mid-depth measurement was recorded. In addition to the transect location measurements, five additional measurements for DO and water temperature were taken directly at the five MiniDOT sensors. All data was collected by BIO-WEST and Baylor.

Following the last set of measurements and updating City of New Braunfels staff at approximately 7:45am (Figure 11), the project team dismantled the equipment and removed all transects.

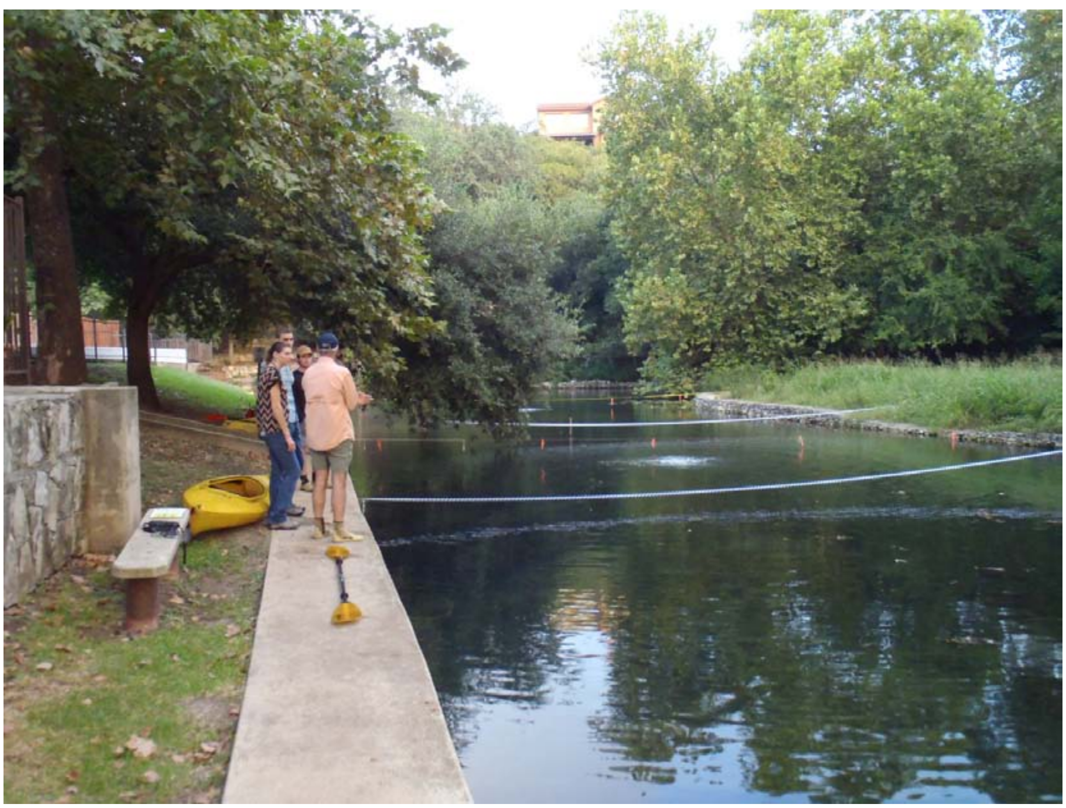


Figure 11: Project team providing an update to City of New Braunfels staff. View of transects and operating diffusers in the background.

CONCEPTUAL ANALYSIS OF DISSOLVED OXYGEN AUGMENTATION

Diffuser experiment in Blieders Creek

To estimate the effectiveness of diffusers for adding oxygen to waters, a limited experiment was conducted in Blieders Creek as described above. This area was considered a good control area since flow and velocity through this area at the time of the experiment was negligible, and the area was experiencing a bloom of algae. DO measurements leading up to the experiment showed an increase in DO during the day corresponding to algal photosynthesis and a decrease in DO during the early morning hours corresponding to algal respiration. This pattern allowed for testing the hypothesis that the diffusers could increase the DO content of waters during the early-morning hours.

In advance of the test, preliminary estimates indicated that injection of approximately 2 lbs of oxygen (O2) would be necessary to increase DO concentration in the experiment area from 2 mg/L to 5 mg/L if there is no continued biological demand for oxygen. This is based upon an assumed water control volume of approximately 10,500 cubic feet (87 feet of river, 40 feet wide, approximately 3 feet deep) with no exchange at the boundaries (no flow). Assuming an O2 content in one cubic foot of air is 0.017 lb, and that the air compressor and diffuser used in the experiment can deliver approximately 5 cubic feet of air per minute, and assuming perfect efficiency, the diffuser system could theoretically deliver the 2 lbs of O2 into the water column in less than 30 minutes. However, many factors prevent the full 2 lbs of O2 from being assimilated efficiently into the water column:

- Oxygen transfer efficiency - Typical efficiency of a disc membrane diffuser varies with air flow rate and height of water column above the diffuser (i.e., water depth). The standard oxygen transfer efficiency for disc membrane diffusers is on the order of 1.8% per foot of submergence, when measured in close proximity to the diffuser. At 3 feet submergence with efficiency of 5.4%, transferring 2 lbs of oxygen into the water would take closer to 10 hours than to the theoretical 30 minutes noted above (0.5 hr / 5.4%).
- Site depth – Depths at the Blieders Creek site range from 2 feet at the edges to 4 feet at the thalweg, with a typical average depth of approximately 3 feet. The diffusers were placed in an area of approximately 3.5 feet, and the 1 foot height of the diffuser equipment allows for interaction of air bubbles over approximately 2.5 feet of water column. This is not sufficient submergence to allow for maximum exchange of oxygen in the bubbles into the water.
- Site circulation – Velocity at the Blieders Creek site were very low, less than 0.05 feet per second on average when measured under comparable conditions the week preceeding the experiment. Observed velocity varies across any given cross-section, and ranged from 0.004 feet per second to 0.1 feet per second (Figure 12); this is effectively zero velocity since the action of taking measurements likely imparts local velocity on par with the observed velocity values. The low velocity and lack of water movement hampers redistribution of oxygenated water throughout the entire control volume. Additionally, upward movement of air bubbles from the diffusers was observed to impart localized circulation. However, the relative degree of circulation improvement across the entire control volume resulting from diffuser action was difficult to determine and likely on par with the degree of circulation imparted by the kayak used to move from measurement point to measurement point.

- Oxygen absorption – The rate of oxygen absorption from the air bubbles into the water column can be predicted as a function of temperature, pressure, density and antecedent oxygen content in the water. The rate is faster when the oxygen concentration in water is lower, then the rate slows as concentration increases. That is, it takes longer to increase from 4 mg/L to 5 mg/L than from 2 mg/L to 3 mg/L. Any diffuser-type device will generally be more effective when DO conditions are lower.

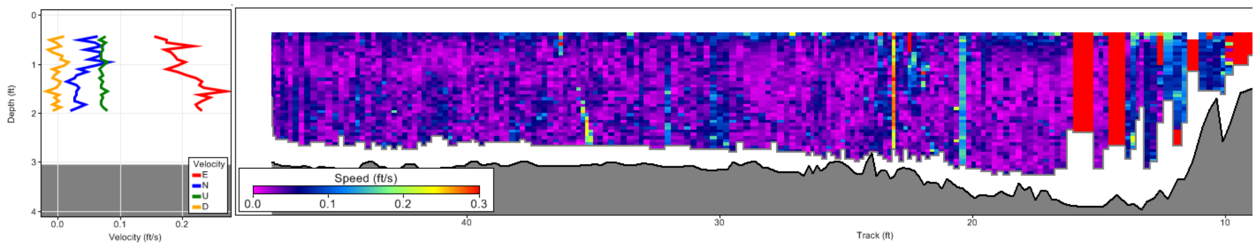


Figure 12: Velocity transect in Blieders Creek, near site D12 at confluence with Landa Lake headwaters (bad data shown red)

The immediate project area during the experiment exhibited DO content ranging from approximately 8.6 to 4.6 mg/L on average in the near-vicinity of the diffusers (Figure 13- 14). The overall trend was for DO to steadily decrease from the high in the late afternoon (5:30pm) to a minimum during the next early morning (4:30am) and remain constant at the minimum until the last measurement after daylight (7:00am). This ambient control DO condition can be considered to be based upon observations at the location marked "L-DSN" in Figure 13, located at the downstream edge of the test zone ("D-12" in Figure 13). The trend in DO content in the vicinity of the diffusers (sonde observation data at "I-Cinderblock" and "N-C9") was slightly higher compared to the DO trend in downstream areas ("L-DSN") over that timeframe (Figure 13). The upstream areas ("M-USN1" and "H-USN2") exhibited a larger DO swing as a result of increased algae activity.

Point measurements surrounding the diffuser were less conclusive than the MiniDOT sensor data time-series (Figure 15). DO concentration did not appear to significantly vary whether diffusers were on or off between the initial morning measurement (4:30am with diffusers off, average 4.6 mg/L) and the second morning measurement (7:00am with diffusers on); however, measurements at most observation locations resulted in an up-tick in the DO while diffusers were on, compared to DO reductions or random DO changes while diffusers were off.

The ambient oxygen concentration of 4.5 to 5.0 mg/L reduced the effectiveness of the diffusers; the moderate oxygen content hampers absorption of additional oxygen into the water column from the diffusers. Greater impact resulting from the diffusers would be anticipated and may have been measureable if ambient oxygen concentrations had been lower (e.g., closer to 2 mg/L).

A time-series spreadsheet model of the Blieders Creek site was developed to estimate the effectiveness of the diffusers, and compare theoretical calculations to observations. The model is based upon typical approaches to dissolved oxygen analysis in natural surface water bodies, along with empirical properties of oxygen transfer into water using membrane diffusers like the units used in the experiment and like those currently installed in the main body of Landa Lake.

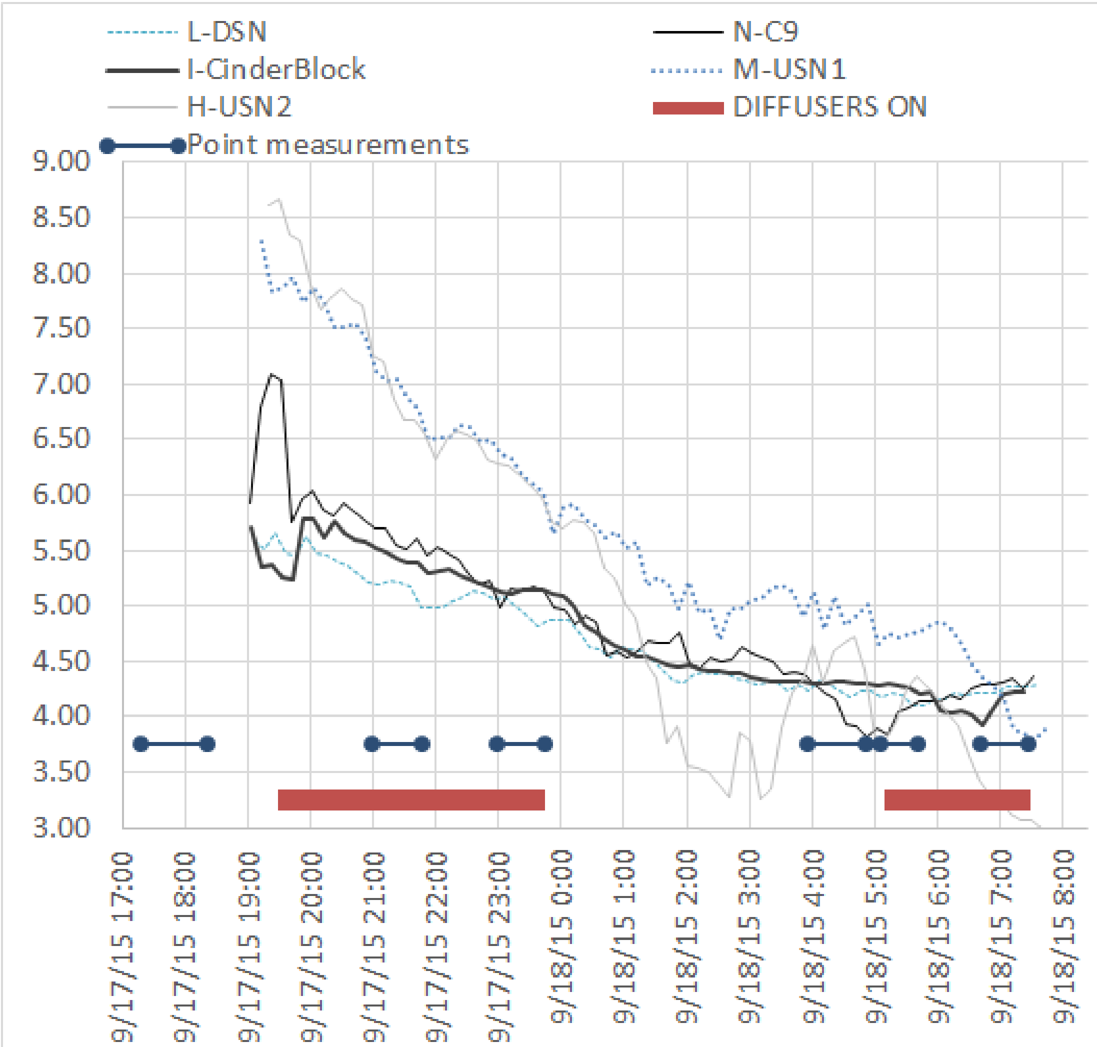


Figure 13: Blieders Creek dissolved oxygen Mini Dot sonde data (DO, mg/L), and time of diffuser experiment activities.

This type of membrane disc diffuser is commonly used in wastewater treatment applications, and the majority of research and design information is based upon that application. For biological treatment of waste streams, diffusers are used in tanks to increase oxygen content and promote aerobic respiration of biological organisms to increase breakdown of organic materials. Waste streams have a very high oxygen demand (much higher than Landa Lake waters) requiring a high oxygenation rate; therefore, in waste water applications disc diffusers are tightly spaced (1 to 6 disc diffusers per square meter) at the bottom of deep (12 feet to 20 feet) tanks.

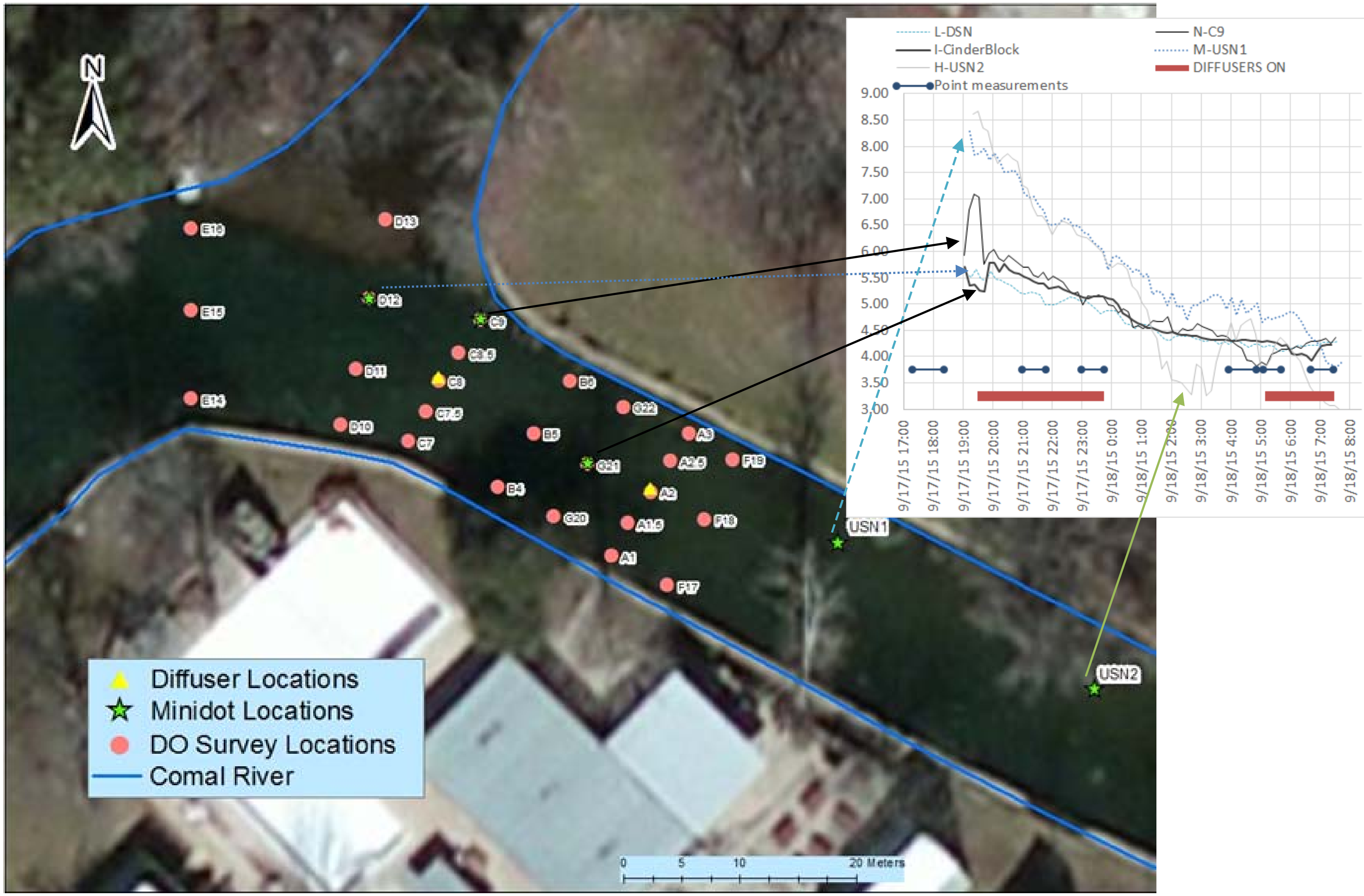


Figure 14: Blieders Creek dissolved oxygen measurement points during diffuser experiment (September 17-18, 2015).

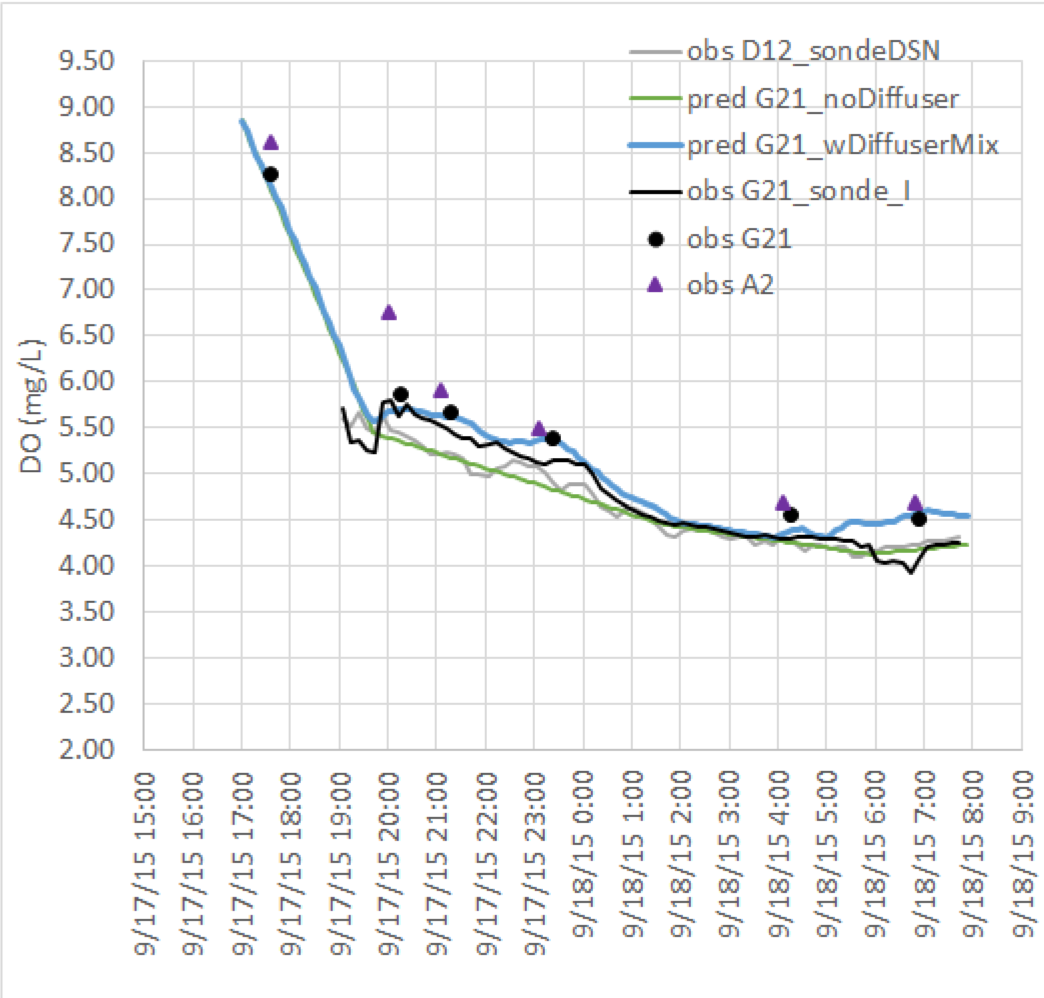


Figure 15: Blieders Creek – comparison of DO time series at MiniDOT sensor location G12, near diffuser A2.

For the Blieders Creek experiment, two sets of two-disc diffuser units were deployed (Figure 10). The spacing between each unit was approximately 60 feet, or one and a half channel widths (Figure 14). The green line labeled "pred G21_noDiffuser" represents an approximated average trend of DO at the G21 MiniDOT sensor location assuming no diffuser and based upon the general trend of reduced DO at the downstream observation location ("obs D12_sondeDSN") (Figure 15). The blue line labeled "pred G21_wDiffuserMix" represents the addition of the diffusers to the site area, as well as addition of increased mixing and circulation resulting from movement of the kayak within sites during measurement and diffuser periods (see Figure 13 for the periods). The predicted blue line (with diffuser) generally represents the trend in DO exhibited in this location's sonde data ("obs G21_sonde_I") and point observations ("obs G21" black dots and "obs A2" purple triangles). At these DO concentration levels, i.e., concentration between 4 and 5 mg/L, the combination of the diffuser and the increased circulation appeared to cause an increase in DO of approximately 0.5 mg/L.

Pertinent site characteristics influencing DO concentration at this site are the shallow depths (2.5 feet to 4 feet deep), lack of flow velocity and mixing with upstream/downstream areas. The zone

of influence of each double-disc diffuser unit was estimated to be reduced after 20 feet (distance from diffuser to "I_Cinderblock" sonde).

Diffusers in Landa Lake

When compared to the Blieders Creek/headwaters experiment site, the focus area (HCP Aeration project) for increasing DO during low-flow times in Landa Lake exhibits much larger volume, deeper depths, greater footprint area, increased flow-through and significant submerged aquatic vegetation.

Based on the work developed using Blieders Creek data, the effectiveness of diffusers in Landa Lake (Figure 16) is estimated. A one-night period of observed DO concentrations measured in Landa Lake for this project in summer 2015 was used to characterize a baseline test condition (yellow "DO_sonde_H" in Figure 17). Since the existing data observations are being used as the baseline, the diel trend of DO concentration caused by biological activity (algae) already accounts for circulation, wind reaeration and chemical, biological and sediment oxygen demand.

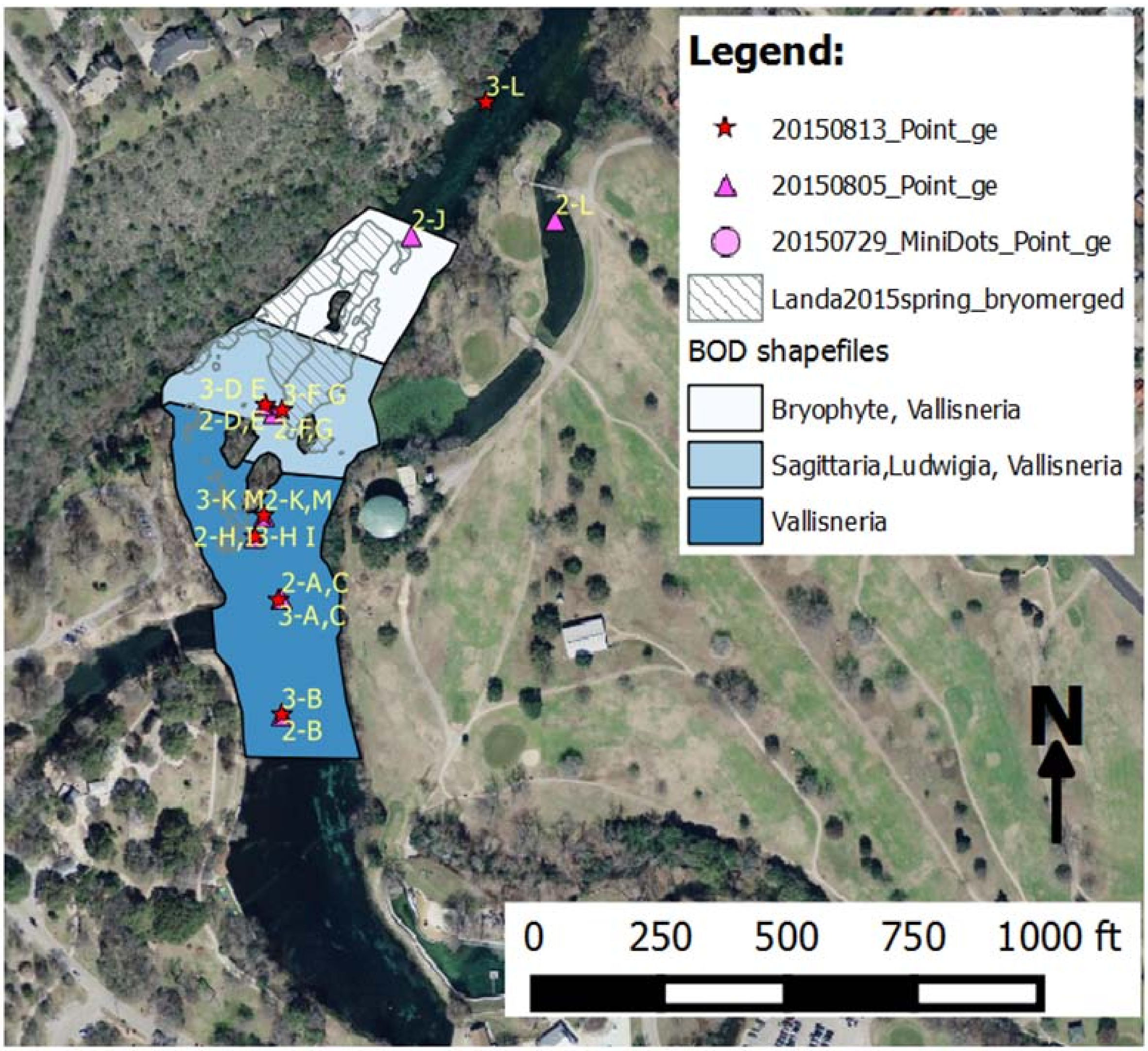


Figure 16: Map of Landa Lake vegetation zones and 2015 vegetation mat MiniDOT sensor locations.

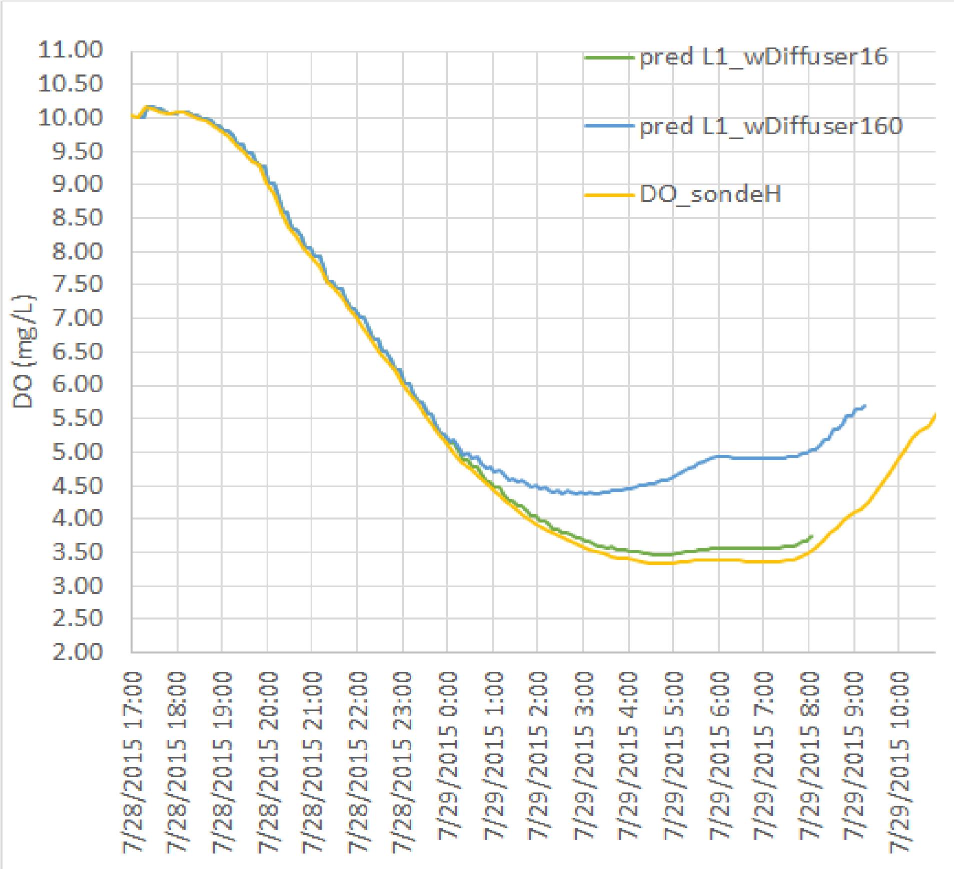


Figure 17: Landa Lake DO measurements, with predicted effect of 16 and 160 diffusers.

To estimate the effect diffusers could have had, the following assumptions are incorporated into this preliminary analysis:

- The area of interest consists of the main body of Landa Lake adjacent to the fishing pier (darkest blue "*Vallisneria*" area in Figure 16). This area is approximately 3.4 surface acres. The average depth is approximately 5 feet. The water temperature is assumed to be 25 °C.
- The area exhibits homogeneous DO conditions, no stratification, no lateral variability.
- No flow is occurring in the lake. NOTE: Even at the lowest flow levels, some velocity and water exchange will occur in the lake. This can have a beneficial effect to increase surface mixing and reaeration; alternately, this can have a detrimental effect to decrease DO concentration if adjacent areas are exhibiting low DO.
- Wind is negligible therefore surface reaeration is negligible.
- Disturbance resulting from recreation or measurement activities is negligible.
- Submerged vegetation does not negatively impact circulation and mixing.
- Air flow supplied to the diffusers is approximately 1.3 standard cubic feet per minute (scfm).
- The diffusers are turned on as soon as ambient DO reaches 5.0 mg/L, and they are turned off when ambient DO reaches 5.0 mg/L.
- Disc membrane diffusers are used with an approximate oxygen transfer efficiency of 1.8% per foot of submergence (i.e., 9% at 5 feet).

With noted assumptions, the impact of installing 160 diffusers at approximately 30 foot spacing is to increase the ambient DO concentration by 1 mg/L. For the conditions tested, this maintains a minimum DO concentration of 4.5 mg/L throughout the entire area and a return to 5.0 mg/L within 6 hours (blue line "pred L1_wDiffuser160", Figure 17). This is an improvement over the minimum DO concentration of 3.3 mg/L in the ambient waters. Use of 160 diffusers requires a considerable compressor with capacity of supplying 208 scfm.

Placing 16 diffusers throughout the same area at approximately 100 foot spacing does little to increase the DO concentrations (green line "pred L1_wDiffuser16", Figure 17) in comparison to ambient waters.

As an alternate, the 16 diffusers could be placed at 30 foot spacing. This would generally maintain a higher DO concentration consistent with the blue line (i.e., 160 diffusers), although the "protection zone" of higher DO would be approximately 8,100 square feet (0.19 acre), much smaller than the overall 3.4 acre area.

Based on the preliminary calculations and observations made for this project, additional work should be conducted to more narrowly focus future diffuser deployments towards specific objectives and to determine the most efficient mechanical technology capable of accomplishing objectives. Additional resolution on the footprint of protection area can help reduce the number of disc diffusers. In particular, if a different protection zone is more relevant to the target species (such as the white area in Figure 16), then the depths and surface area of that area may exhibit different DO characteristics. The range of protective ambient DO concentrations necessary for target species also plays a major role. If the minimum protective DO concentration is on the order of 3.0 mg/L, then this generally entails lower costs (less compressor power and fewer diffusers), compared to a minimum protective DO concentration of 4.0 to 5.0 mg/L.

Aside from membrane disc diffusers tested as part of this project, there are other means for increasing DO. Alternate methods that may meet management objectives that have not been evaluated are those that can improve circulation (pumps, Solar Bees fans, external air columns, etc.), and are recommended for further investigation. Because of the lake's large surface area, the natural reaeration processes combined with increased exchange of shallow and deep waters may be an effective method to increase overall DO conditions.

References

[EARIP] Edwards Aquifer Recovery Implementation Program. 2011. Habitat Conservation Plan and Appendices. December 2011.

SWCA. 2014. Landa Lake Dissolved Oxygen Mitigation: 2014 Report. Prepared for the City of New Braunfels. December 2014. 7 p. plus Attachments.

Appendix L5

Final 2015 Report for the Invasive Species Removal Project for the City of New Braunfels, Comal County, Texas

FINAL 2015 REPORT FOR THE INVASIVE SPECIES REMOVAL PROJECT FOR THE CITY OF NEW BRAUNFELS

COMAL COUNTY, TEXAS

Prepared for:

City Of New Braunfels

Prepared by:

SWCA Environmental Consultants

SWCA Project No. 24621

September 2015

FINAL 2015 REPORT FOR THE INVASIVE SPECIES REMOVAL PROJECT FOR THE CITY OF NEW BRAUNFELS

COMAL COUNTY, TEXAS

Prepared for:

City of New Braunfels
300 Laurel Lane
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SWCA Project No. 24621

September 29, 2015

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EXECUTIVE SUMMARY

At the request of the City of New Braunfels, SWCA Environmental Consultants (SWCA) has prepared a comprehensive invasive species removal report for the non-native species removal project in Landa Lake, Comal County, Texas (project area). The City of New Braunfels, in accordance with the Edwards Aquifer Habitat Conservation Plan (HCP), desires to eliminate or reduce the density of non-native animal species to minimize their impact to the Comal River ecosystem. The target non-native species include the vermiculated sailfin armored catfish (*Pterygoplichthys disjunctivus*), tilapia (*Oreochromis aureus*), nutria (*Myocastor coypus*), and giant ramshorn snail (*Marisa cornuarietis*). These non-native species are thought to compete for resources (e.g., habitat and food) with the native species of HCP concern. Due to their life history traits, burrowing into the sides of rivers and lake banks when they nest which causes soil destabilization and subsequent erosion, both the armored catfish and the nutria also are potentially responsible for a substantial amount of the damage observed along Landa Lake's embankments. Tilapia also excavate nests in the substrate, destroying vegetation. Recent research regarding the predatory nature of the giant ramshorn snail preying upon fountain darter eggs has indicated that this invasive species may have significant impacts to the endangered species as well.

SWCA conducted six 3-day-long removal efforts throughout the 2015 removal season, which spanned from February to July. Removal efforts were conducted in a similar manner as previous years. Both types of invasive fish were targeted with multiple removal methods. Areas of possible nutria habitation were found and trapped. Cumulative removal efforts for 2015 resulted in over 1,300 pounds of biomass removal. When combined with 2014's 3,845 pounds and 2013's 6,000 pounds of biomass removal, SWCA has removed over 11,000 pounds of invasive biomass—or approximately 4,300 tilapia, 800 armored catfish, 58 nutria, and several thousand snails in the three years' time.

Within the limitations of schedule, budget, and scope of work, SWCA warrants that this study was conducted in accordance with accepted environmental science practices, including the technical guidelines, evaluation criteria, and documented parameters in effect at the time this evaluation was performed.

The results and conclusions of this report represent the best professional judgment of SWCA scientists. No other warranty, expressed or implied, is made.

ACKNOWLEDGEMENTS

We would like to thank the City of New Braunfels for the opportunity to work in and help out this amazing ecosystem. We would also like to express our sincere gratitude to the employees at Heidelberg Lodge, specifically Chris Mock for the amazing hospitality over the past three years. Lastly we would like to thank Luci Cook-Hildreth at the Texas Parks and Wildlife for partnering with us on some very interesting research on the armored catfish.

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1.0 INTRODUCTION

The City of New Braunfels, in accordance with the Edwards Aquifer Habitat Conservation Plan (HCP), desires to eliminate or reduce the density of non-native animal species to minimize their impact to the Comal River ecosystem. The target non-native species include the vermiculated sailfin armored catfish (*Pterygoplichthys disjunctivus*), tilapia (*Oreochromis aureus*), nutria (*Myocastor coypus*), and the giant ramshorn snail (*Marisa cornuarietis*). These non-native species are believed to compete for resources (e.g., habitat and food) with the native species of HCP concern. Additionally, the life history traits of armored catfish and nutria are potentially responsible for a substantial amount of the damage observed along Landa Lake's embankments. Nutria and armored catfish both burrow into the sides of river and lake banks when they nest. This constant burrowing action causes soil destabilization and subsequent erosion. Tilapia also dig into substrate for their nests, destroying vegetation.

SWCA Environmental Consultants (SWCA) was hired to conduct a formal on-site invasive species investigation and removal. Prior to these efforts, a desktop review of these invasive species was conducted in 2013. SWCA's field efforts involved six sessions, each three days in length in February, March, April, May, June, and July of 2015.

2.0 METHODS

2.1 Desktop Review

SWCA's desktop review involved a review of literature and data published by the Texas Parks and Wildlife (TPWD), federal agencies including the U.S. Fish and Wildlife Service (USFWS), the Texas Natural Resources Information Systems (TNRIS), and other relevant academic peer reviewed publications.

2.1.1 Vermiculated Sailfin Armored Catfish (Pterygoplichthys disjunctivus)

The loricariid genus *Pterygoplicthys* consists of approximately 15 valid species native to South America (Brazil and Bolivia) along the Amazon River Basin (Armbruster and Page 2006; Nico et al. 2012). Species of this genus have become both introduced and established in numerous states across the southern United States such as Florida and Texas, and all over the world (Jones et al. 2013; Nico et al. 2012). In habitats where these species become established they tend to become abundant and have the ability to disperse rapidly (Capps et al. 2011; Nico et al. 2009a). *Pterygoplicthys disjunctivus* (Weber 1992), the species in question at Comal Springs is believed to have been introduced into Florida back in the late 1950s, and since then has become established in many major waterways throughout the state. It is believed that this species has approximately the same timeline of introduction in Texas. Barron (1964) reported finding this species in the San Antonio River in the early 1960s.

The species' unique morphology allows them to have a varied diet consisting of algae, small invertebrates, organic sediments, and even wood (Nico et al. 2009a). The genus has another morphological adaptation that allows them to become a "nightmare" invasive species: the ability to gulp, metabolize, and breathe air outside of water (Nico et al. 2012). These adaptations enable them to invade ecosystems with constant temperatures, low oxygen levels, and fast flowing (generally hypoxic), water that other fish are unable to colonize (Nico et al. 2009a). *P. disjunctivus* has been shown to utilize warm freshwater springs as thermal refuges from cold winter river temperatures in Florida (Gibbs et al. 2010). The constant temperature of Comal Springs makes the habitat a perfect refuge and breeding ground for this species.

Despite the abundance of this species throughout the southeast, very little research has been conducted on the life history or ecosystem impacts of this species (Gibbs et al. 2013). Known potential ecological impacts to native species include dietary resource competition with native fish, direct predation of eggs in regard to native bottom-nesting fish species, and erosion and bank destabilization due to tunneling during spawning season (male builds and guards nest and spawning sites) (Gibbs et al. 2013; Howells 1999; Nico et al. 2009a), and lastly females are known to be prolific iteroparous (multiple reproductive cycles over a lifetime) reproducers (Gibbs et al. 2013). It has been well documented that this species can cause economic loss to local fisheries due to aforementioned competition and predation (Nico et al. 2009a, 2009b, and 2012). All of these effects and the inherent ease of which the species can invade an environment make life history and removal programs extremely important.

2.1.2 Blue Tilapia (Oreochromis aureus)

The term "tilapia" refers to a group of African cichlids consisting of three genera: *Oreochromis*, *Sarotherodon*, and *Tilapia*. These genera are unique in that they are more tolerant of high salinity, high water temperature, low dissolved oxygen, and high ammonia concentrations than most common freshwater fish (Popma and Masser 1999; Trewavas 1983). Their ability to withstand such poor water quality has been instrumental in their increasing popularity as a cultured food fish (aqua culture), with carp being the only group of fish species more intensively farmed around the globe (Peterson et al. 2004). In conjunction with these unique physiological adaptations, tilapia are trophic generalists that have an extremely fast reproductive biology which is characterized by very short generation times, multiple clutches per annum, and extended breeding seasons (Dempster et al. 1993; Peterson et al. 2004). Consequently, this has led to the inadvertent, and occasionally intentional, introduction of several tilapia species to naturally occurring waterways around the United States (Howells 1999; Popma and Masser 1999). Common examples of these types of introduction include individuals escaping nearby fish farming operations during periods of flooding and pet trade releases. Nightmare examples of tilapine fish invasions can be found throughout the southeast. For example, Crutchfield (1995) documented the redbelly tilapia (*Tilapia zilli* Gervais) becoming the fourth most abundant fish species in North Carolina power plant reservoir, three years after accidental introduction. This population explosion resulted in the elimination of aquatic macrophytes in the reservoir, which in turn caused significant declines in native fish population (Crutchfield 1995; Peterson et al. 2004). Fortunately, tilapia's intolerance of sustained low water temperatures prevents individuals from overwintering in areas where water reaches the lower lethal temperature of approximately 50°F–52°F (Popma and Masser 1999). This is problematic in the case of Comal Springs and Landa Lake because its spring-fed headwaters maintain a year round temperature near 72°F which has allowed the tilapia population to continually increase per annum.

Species from *Sarotherodon* and *Oreochromis* are mouth brooders, which immediately pick up and store fertilized eggs in their buccal cavity up to several days after hatching (Popma and Masser 1999; Linde et al. 2008). This method of reproduction affords fry the chance to further develop until they become more capable of avoiding predation. Furthermore, tilapia species typically reach sexual maturity at a much smaller size which means a larger proportion of the population are actively spawning and can quickly replace individuals lost to predation or disease (Martin et al. 2010). Yet another potential issue arising from reproduction habits is due to tilapia's nest-building behavior, which might alter or destroy spawning grounds that could potentially be used by native fish species. In reference to tilapia's feeding habits, though they are often thought of as filter feeders, in reality their diet consists of a variety of items including algae, plankton, some aquatic macrophytes, planktonic and benthic aquatic invertebrates, larval fish, detritus, and decomposing organic matter (Linde et al. 2008; Martin et al. 2010; Popma and Masser 1999). Such a varied diet can prove problematic for nearby native fish populations as tilapia may either outcompete juveniles and adults or even directly consume newly hatched fry. By reducing the amount of available nutrients in the ecosystem, any native fish that require a more specialized diet are likely to be displaced as they are outcompeted by the generalist tilapia invaders.

2.1.3 Nutria (Myocastor coypus)

The nutria (*Myocastor coypus*) is a large semi-aquatic mammal in the family Rodentia that is native to much of South America. This species has been introduced to numerous countries around the world (including much of the United States) primarily for fur farming (Sheffels and Sytsma 2007). Nutria are generally thought to have been first released into the marshes surrounding New Orleans in the 1930s as an alternative to native species fur trade. In addition to escapees from fur farms in Louisiana, populations have been introduced in most of the Gulf States as well as Maryland, Washington, and Oregon as a method in controlling nonnative vegetation in bays, lakes, and ponds (LeBlanc 1994; Sheffels and Sytsma 2007).

Most of the extensive damage caused by nutria is a direct result of feeding and burrowing (Sheffels and Sytsma 2007), for example, the most significant categories of nutria damage that have been listed from Maryland and Louisiana is herbivory damage. In Oregon the most significant source of damage is the destruction of water control structures and associated erosion caused by nutria burrowing. Nutria are also capable of transporting parasites and pathogens transmittable to humans, livestock, and pets (Sheffels and Sytsma 2007). Expansion into public areas, environments heavily used by people (such as Landa Park), increases the potential for conflicts between nutria and humans (Sheffels and Sytsma 2007). Nutria attacks have been reported in isolated cases and nutria are rodents that carry a variety of transmittable parasites and pathogens.

2.1.4 Giant Ramshorn Snail (Marisa cornuarietis)

Marisa cornuarietis is commonly called the giant or Columbian ramshorn snail. This species of snail is a relative of the Apple snail, another invasive species. The giant ramshorn is typically yellow or brown in coloration and can grow to over 2 inches in diameter. The giant ramshorn is native to northern South America and southern Central America, but has been introduced to many environments in order to control aquatic vegetation (Horne et al. 1992). They have established themselves in several places within the United States including Florida and Texas (Comal Springs) (Horne et al. 1992). These snails have been found to wreak havoc on adopted environments due to their voracious appetite and ease of reproduction; they have a great appetite for plants both live and dead (Phillips et al. 2010).

This species has been documented in the San Marcos and Guadalupe River system since 1981 (Horne et al. 1992). The giant ramshorn snail was first identified in the Comal River system in June of 1984 when four empty shells were collected from Landa Lake (Horne et al. 1992). Method of introduction into the San Marcos and Comal Springs ecosystems is unknown but speculated to be aquarium releases due to the presence of the species in multiple pet stores in the area (Horne et al. 1992).

Possible impacts from this species have been identified over the years. Horne et al. (1992) acknowledged observing plants in many areas of Landa Lake that had been denuded of leaves or even grazed to the bottom. This species, when feeding, clip the basal stem of the plant before feeding on the cuttings. A common occurrence is that the snail will frequently lose their grip, allowing the aerenchymous cuttings to float away in the current to form large floating masses (Horne et al. 1992). Even today, SWCA biologists have observed this scenario on numerous occasions throughout the removal effort. One of the main causes for concern for this species could be its impact on the endangered species *Etheostoma fonticola*, the fountain darter. Phillips et al. (2010) has indicated that this species of invasive snail is a significant predator of fountain darter eggs.

2.1.5 Statistical Methods

Descriptive statistics including median, mean, and standard error of the weight and length of each fish species were performed for all three years—2013, 2014, and 2015—separately and comparatively. Each

fish species was then broken down and placed into frequency histograms by weight and length, with bin sizes of 0.1 kilogram and 1.0 centimeter, respectively. Independent t tests with heterostochasticity were run on ln transformed weight and length data. Sex ratios were also calculated for catfish, tilapia and nutria.

2.2 Invasive Species Removal

2.2.1 Fish

The project goal was to remove all non-native fish species observed, particularly the large breeding females. Areas of high invasive species density were located with the knowledge gained of the species biology. Areas containing loose substrate with abundant surrounding vegetation were targeted for tilapia. Areas that had significant erosion and holes in the lake substrate indicated possible nesting areas for the armored catfish (Appendix B, Photograph 3). Both species of invasive fish were removed with the use of four fyke nets during each trapping session. Fyke nets are passive traps that have 50-foot leads that guide fish into a 12-foot-long by 3-foot-wide hoop net. Tilapia were also targeted with the use of three gill nets of varying mesh size ranging from 2-inch to 3-inch mesh. Targeted fish are caught live and non-target fish are released alive. In addition, SWCA biologists snorkeled early in the morning and late in the afternoon (times of high fish activity) in areas of high fish density and speared all non-native fish possible.

Once removed from the water, all invasive fish were eviscerated, in accordance with state laws. The carcasses were measured, weighed to the nearest kilogram, and a total biomass removed during the sample was calculated. Total length was also taken to determine if, over time, the removal of adults affects target population demographics. As removal pressure increases, over time, the average size of fish captured should increase due to available food resources. SWCA biologists also attempted to determine sex of each individual to develop a sex ratio of what is being removed during the project's timeline.

Specimens of the vermiculated sailfin armored catfish were given to TPWD biologist Luci Cook-Hildreth for necropsy studies including gender, reproductive, and age analysis. The target is to eventually publish this material in a per-reviewed periodic journal.

2.2.2 Nutria

Between 8 and 13 Havahart® live traps baited with carrots, sweet potatoes, and apples were set during each trip in an effort to capture the invasive nutria. Traps were placed in areas frequented by nutria (evident by slides, scat, chewed vegetation, lake-wall erosion and damage, and other observations). This increased efficiency of target animal capture and reduced the likelihood of non-target animals entering the traps. All traps were checked around park closing time and again the next morning at approximately 7:30 a.m. All nutria captured were shot at the base of the skull with a high powered air rifle. This method has proven remarkably efficient in dispatching the animal quickly and humanely. All species removed were measured to the nearest centimeter and whole body weighed to the nearest gram.

2.3 Mapping

During the field survey, SWCA geographically referenced features using a Trimble GeoExplorer 6000 Global Positioning System (GPS) unit capable of sub-meter accuracy.

ESRI's ArcView 10 geographic information system (GIS) software was used to generate the attached vicinity and site layout maps (Appendix B, Photograph 3).

3.0 RESULTS

3.1 Invasive Removal

Over the 20 field days that SWCA performed invasive species removal, 113 vermiculated sailfin catfish, 516 tilapia, eight nutria, and 411 ramshorn snails were lethally removed from Landa Lake. Tables 1–6 are the results of each sampling session completed from February 2015 to July 2015. The total biomass, average length, and sex ratios are reported for each species.

Table 1. Non-Native Species Removal Biometrics for Session 1, February 2015

Species	Number Removed	Biomass (kg)	Biomass (pounds)	Avg. Length (cm)	Sex Ratio
Armored Catfish	9	11.89	26.21	45.7	0.33:1 Female bias
Tilapia	93	75.86	16.24	35	0.40:1 female bias
Nutria	2	5.36	11.81	NA	Both male
Giant Ramshorn Snail	103	NA	NA	3.41	NA
Totals	**207**	**93.11**	**205.27**	**NA**	**NA**

Table 2. Non-Native Species Removal Biometrics for Session 2, March 2015

Species	Number Removed	Biomass (kg)	Biomass (pounds)	Avg. Length (cm)	Sex Ratio
Armored Catfish	22	23.85	52.58	44.3	1.10:1
Tilapia	93	81.88	180.51	36.2	0.45:1 Female Bias
Nutria	1	2.18	4.8	NA	Female
Giant Ramshorn Snail	98	NA	NA	3.29	NA
Totals	**214**	**107.91**	**237.9**	**NA**	**NA**

Table 3. Non-Native Species Removal Biometrics for Session 3, April 2015

Species	Number Removed	Biomass (kg)	Biomass (pounds)	Avg. Length (cm)	Sex Ratio
Armored Catfish	6	6.86	15.12	46.1	0.33:1: Female Bias
Tilapia	109	101.4	223.54	36.6	Aprox: 1:1
Nutria	2	6	13.2	NA	1:1
Giant Ramshorn Snail	0	0	0	NA	NA
Totals	**117**	**114.26**	**251.9**	**NA**	**NA**

Table 4. Non-Native Species Removal Biometrics for Session 4, May 2015

Species	Number Removed	Biomass (kg)	Biomass (pounds)	Avg. Length (cm)	Sex Ratio
Armored Catfish	19	20.56	45.32	45.8	1.10:1
Tilapia	106	88.12	194.27	39.3	0.15:1 Ext- Male Bias
Nutria	2	7.3	17	NA	1:1
Giant Ramshorn Snail	0	NA	NA	NA	NA
Totals	**127**	**115.98**	**255.69**	**NA**	**NA**

Table 5. Non-Native Species Removal Biometrics for Session 5, June 2015

Species	Number Removed	Biomass (kg)	Biomass (pounds)	Avg. Length (cm)	Sex Ratio
Armored Catfish	45	44.73	98.61	45.4	0.25:1 Male Bias
Tilapia	84	77.98	171.9	37	0.25 :1 Male bias
Nutria	1	2.29	25.4	NA	Male
Giant Ramshorn Snail	156	0.92	4.6	3.26	NA
Totals	**286**	**125.92**	**277.6**	**NA**	**NA**

Table 6. Non-Native Species Removal Biometrics for Session 6, July 2015

Species	Number Removed	Biomass (kg)	Biomass (pounds)	Avg. Length (cm)	Sex Ratio
Armored Catfish	12	11.79	26	44.9	1:1
Tilapia	30	24.76	54.58	35.9	0.25 :1 Male bias
Nutria	0	NA	NA	NA	NA
Giant Ramshorn Snail	0	NA	NA	NA	NA
Totals	**42**	**36.55**	**80.57**	**NA**	**NA**

Figures 1A and 1B show the comparison between the removal years for mean weight for both fish species (SE error bars, Catfish p-value= <0.001, Tilapia p-value=<0.001). Figures 1C and 1D show the comparison of mean length between the removal years (SE error bars, Catfish p-value= 0.007, Tilapia p-value= <0.001).

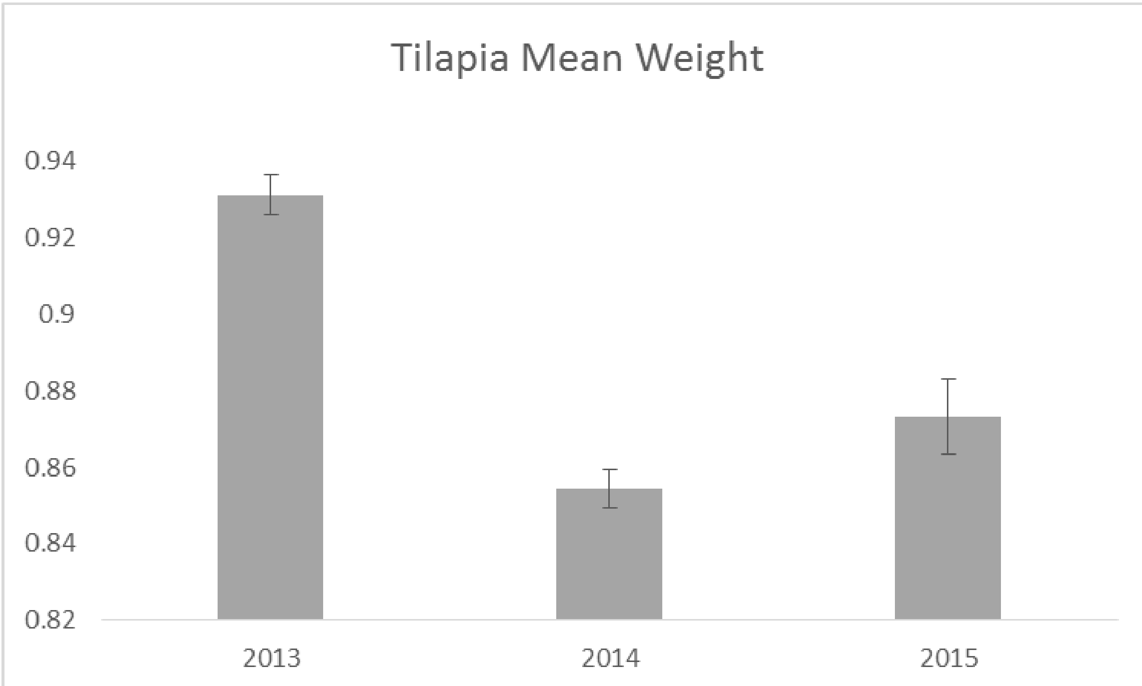


Figure 1A. Tilapia mean weight.

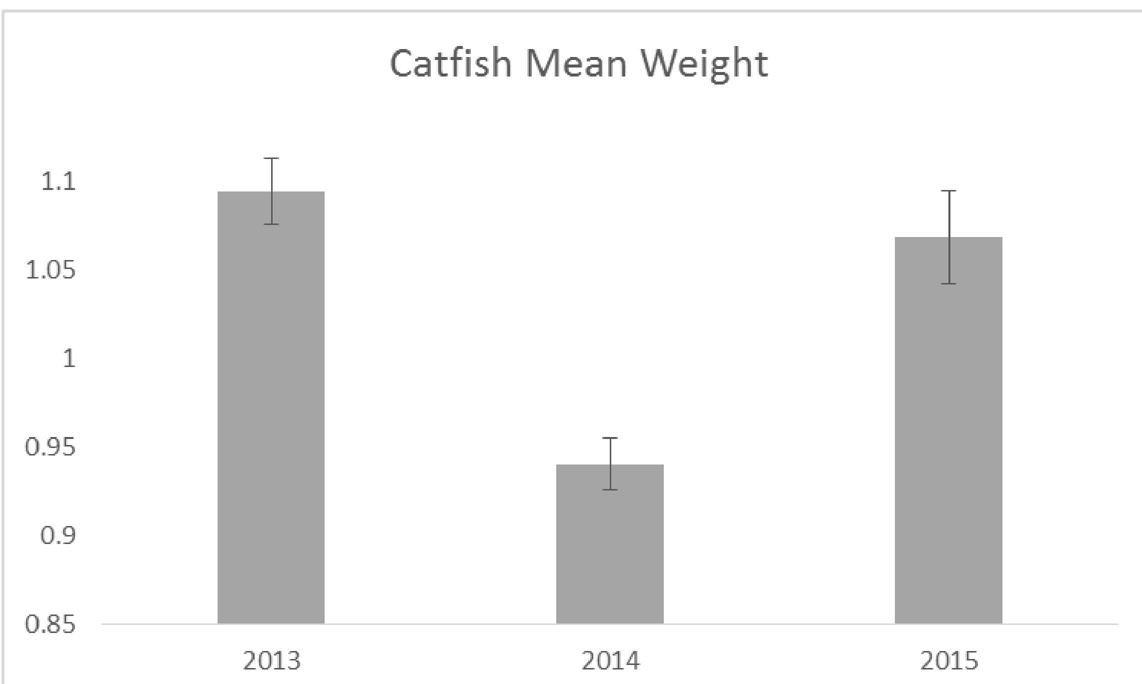


Figure 1B. Catfish mean weight.

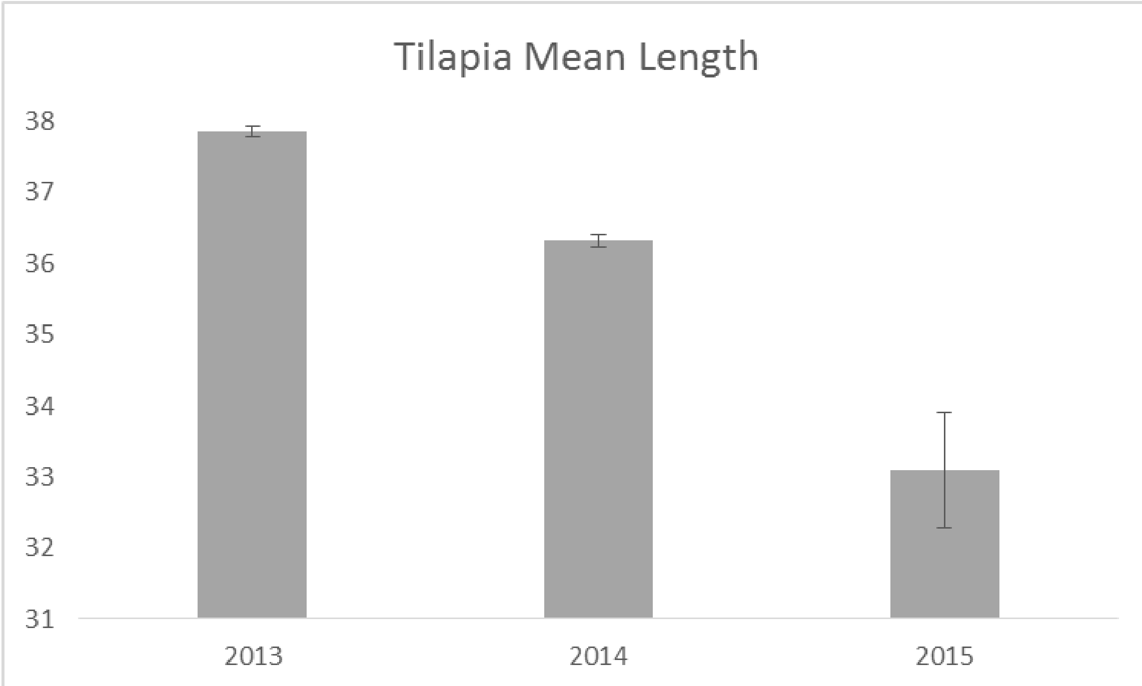


Figure 1C. Tilapia mean length.

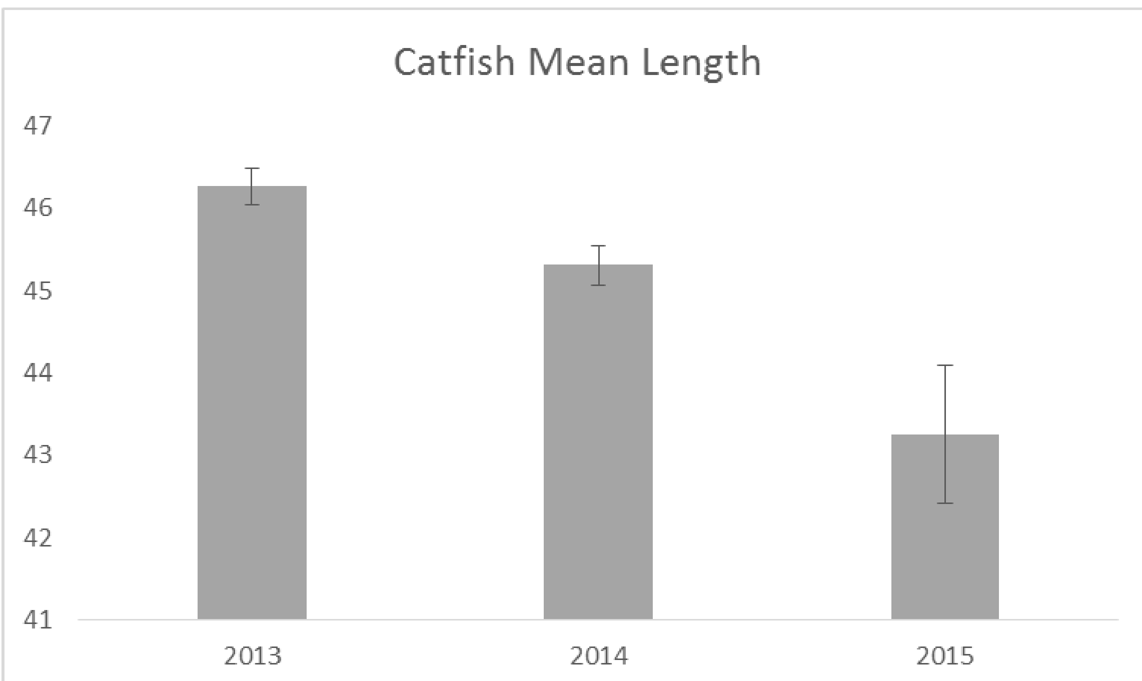


Figure 1D. Catfish mean length.

Figures 1E–1H show the comparison between the removal years for mean weight and length for both fish species by sex.

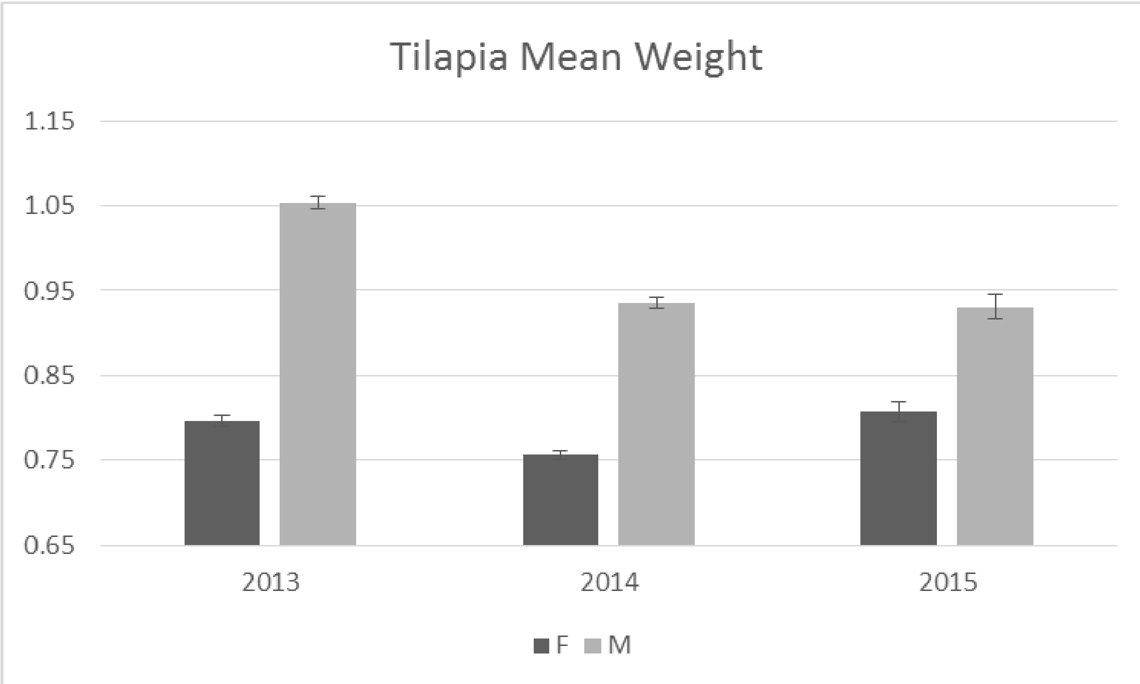


Figure 1E. Tilapia mean weight by sex.

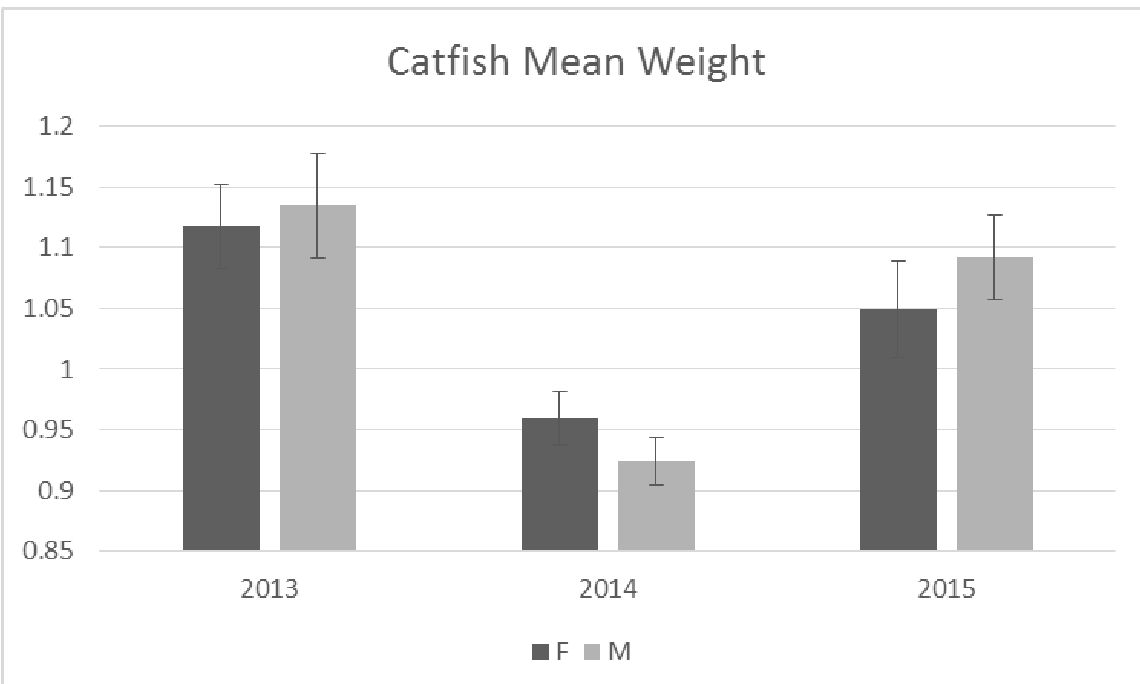


Figure 1F. Catfish mean weight by sex

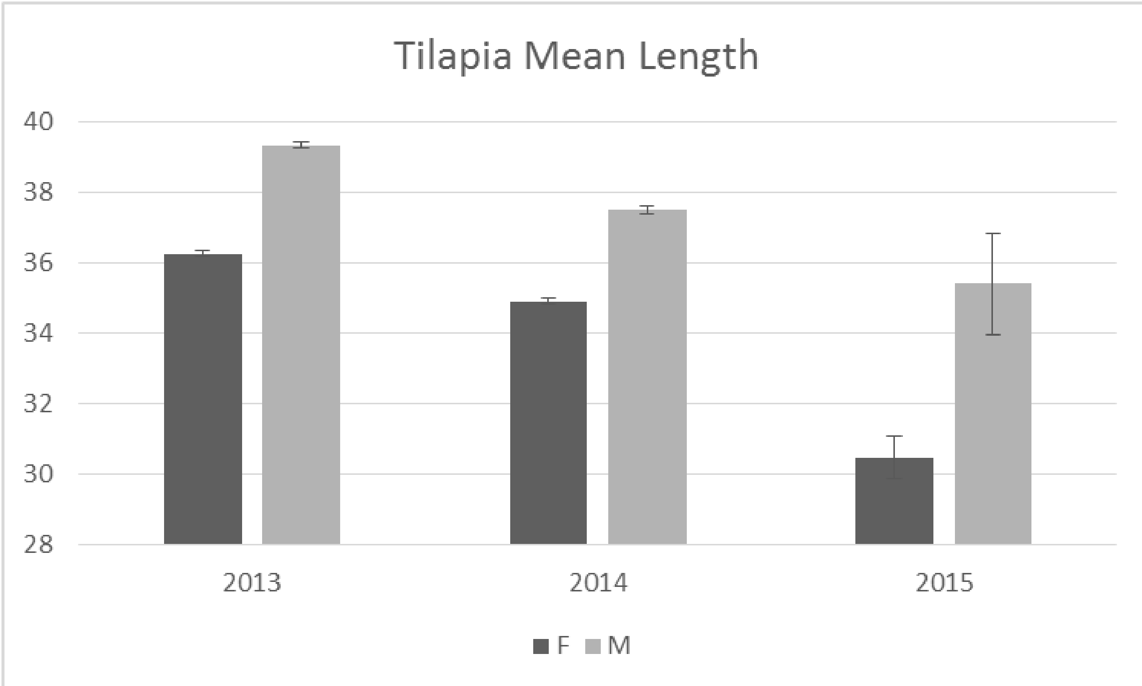


Figure 1G. Tilapia mean length by sex.

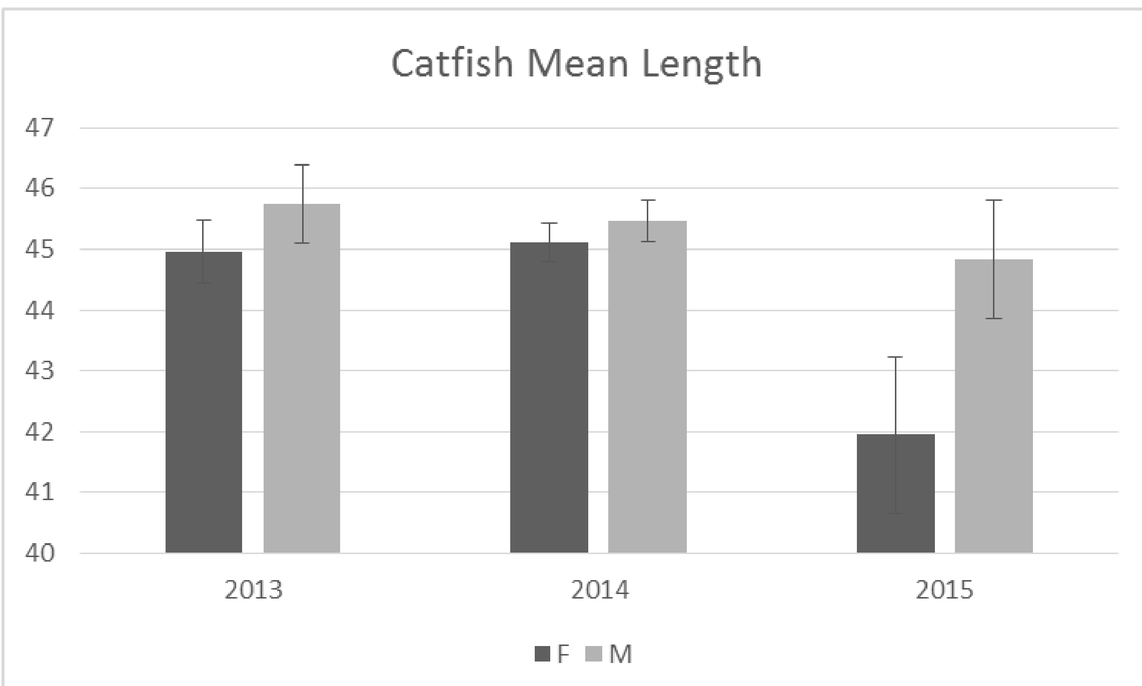


Figure 1H. Catfish mean length by sex.

Figures 2A–2C show the range of weights of catfish removed in 2013, 2014, and 2015

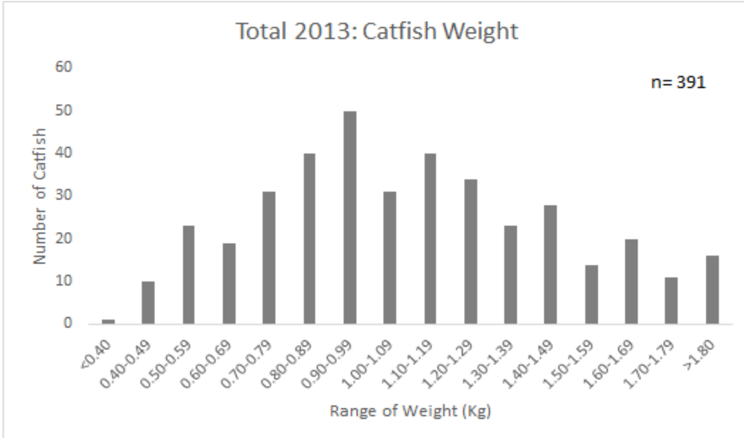


Figure 2A. 2013 total catfish weight.

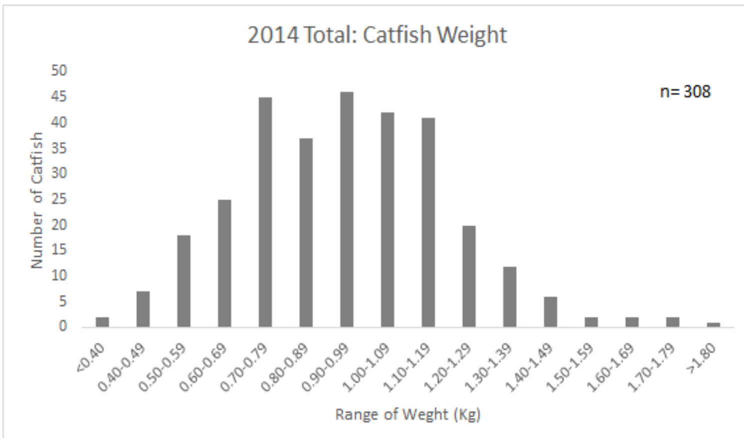


Figure 2B. 2014 total catfish weight.

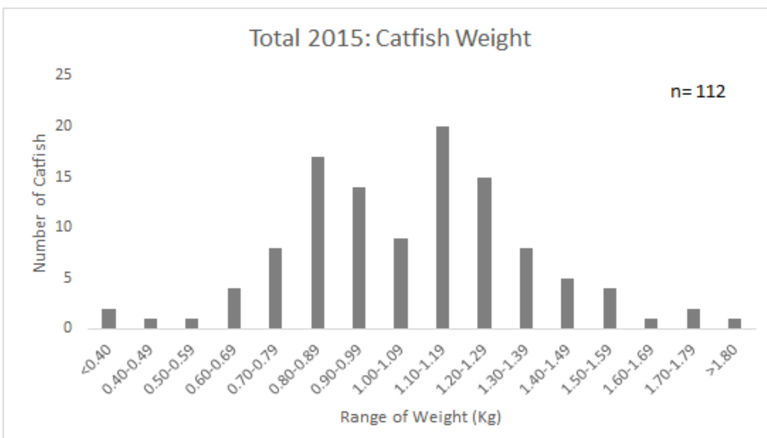


Figure 2C. 2015 total catfish weight.

Figures 3A–3C show the range of lengths of catfish removed in 2013, 2014, and 2015.

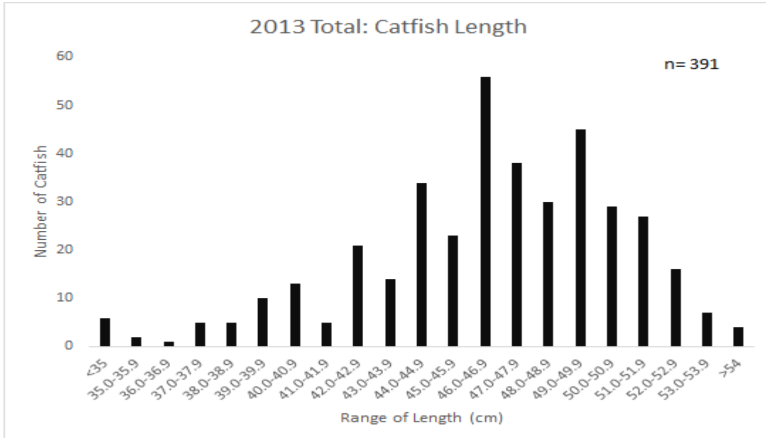


Figure 3A. 2013 total catfish length.

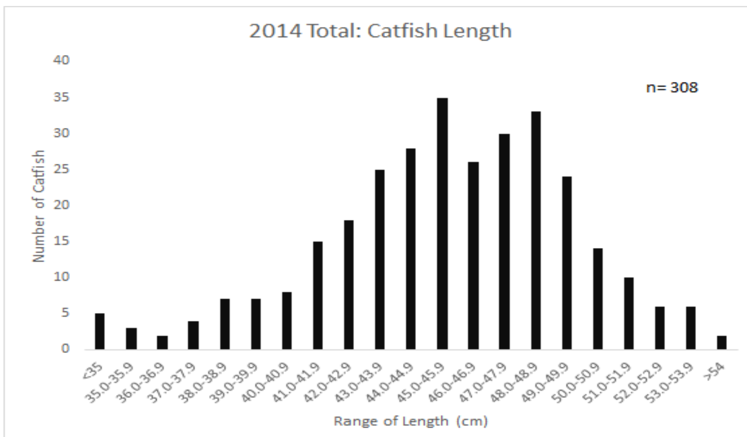


Figure 3B. 2014 total catfish length.

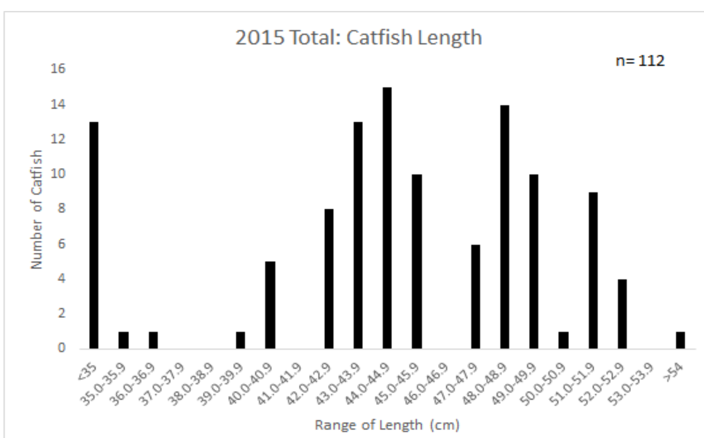


Figure 3C. 2015 total catfish length.

Figures 4A–4C show the range of weights of tilapia removed in 2013, 2014, and 2015.

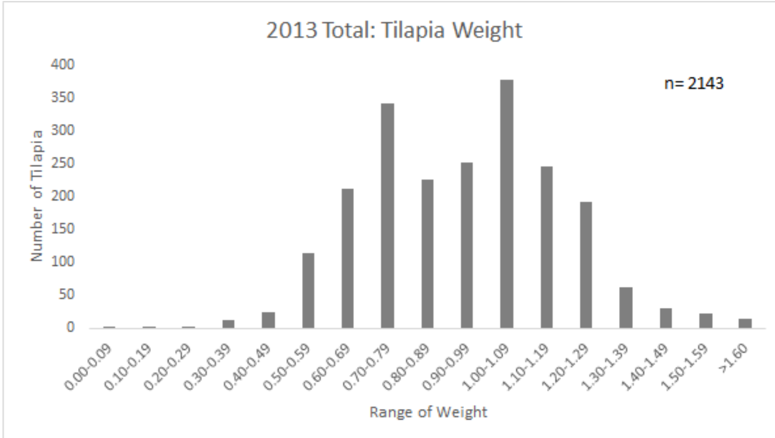


Figure 4A. 2013 total tilapia weight.

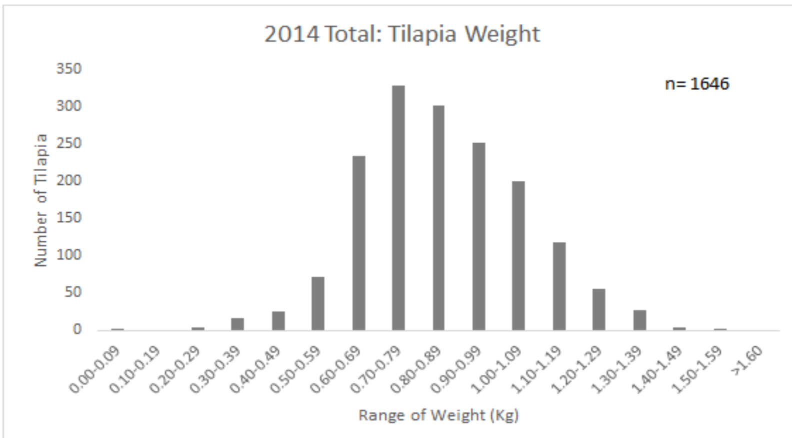


Figure 4B. 2014 total tilapia weight.

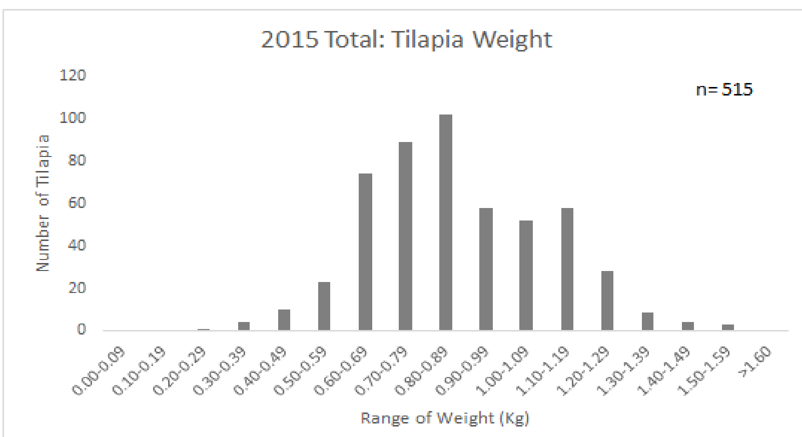


Figure 4C. 2015 total tilapia weight.

Figure 5A–5C: Range of lengths of tilapia removed in 2013, 2014, and 2015.

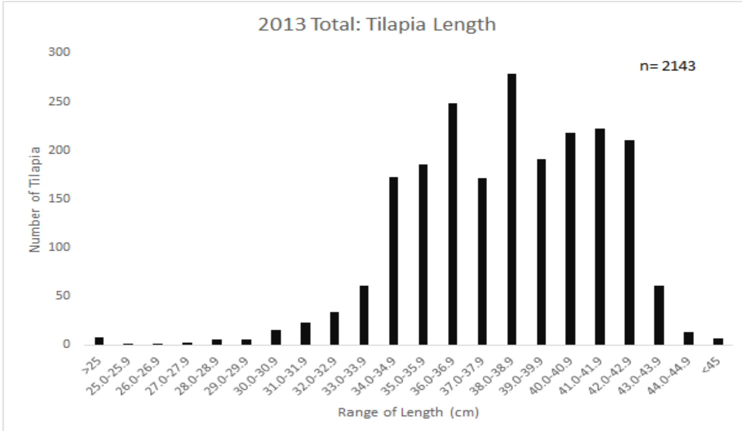


Figure 5A. 2013 total tilapia length.

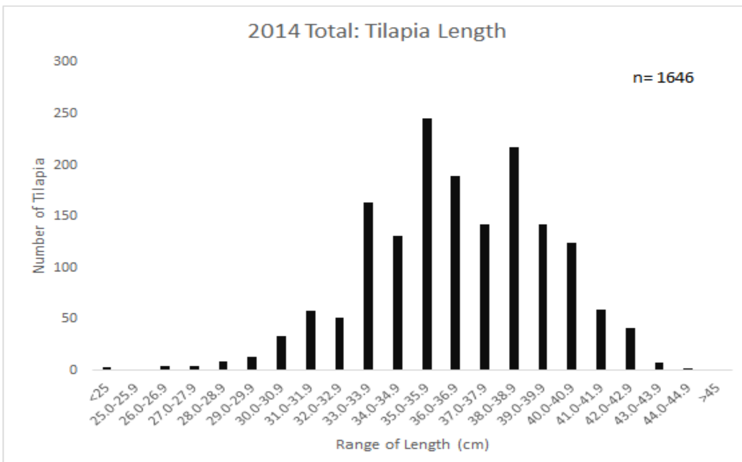


Figure 5B. 2014 total tilapia length.

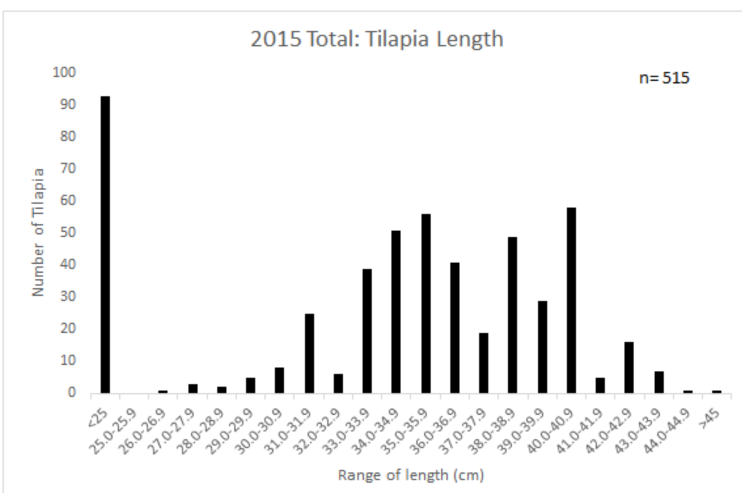


Figure 5C. 2015 total tilapia length.

Figures 6A and 6B show the catfish and tilapia sex ratios for the 2015 sampling year.

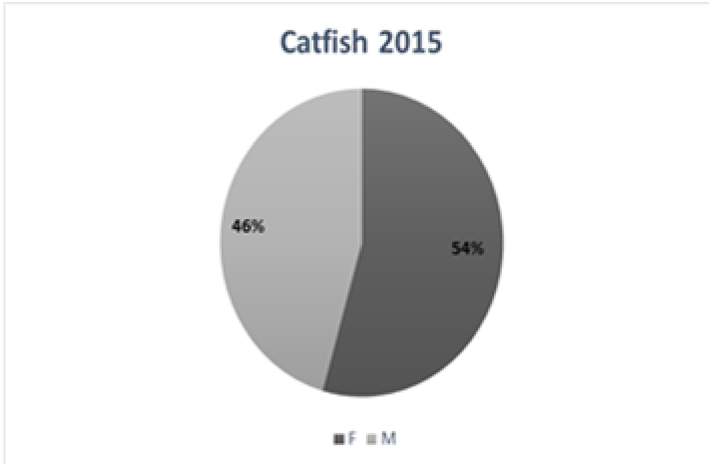


Figure 6A. Catfish sex ratios for 2015 sampling year.

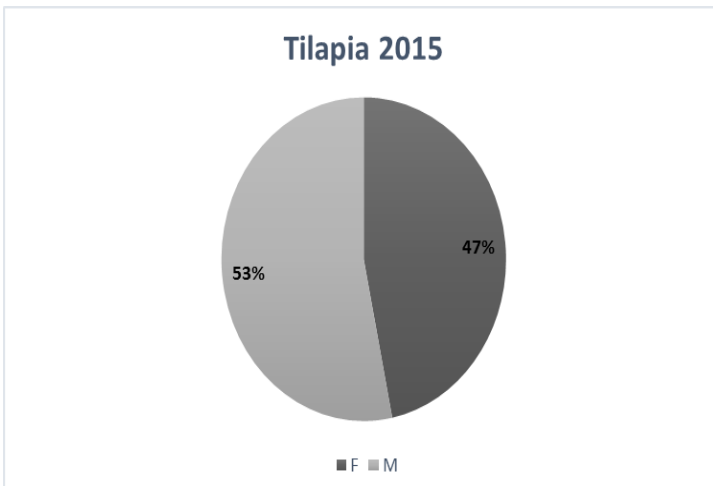


Figure 6B. Tilapia sex ratios for 2015 sampling year.

3.1.3 Hydrology

Central West Texas has gone through a long-term drought the past 3+ years. This drought has caused many local waterways to decrease in volume and size. Comal Springs, having a constant water supply, has dried out only once in recorded history, in the 1950s. Due to its propensity for continued flow, it is an extremely important ecosystem within the region; therefore, with the continued drought conditions, water levels should be monitored. Lower water levels could lead to lower oxygen levels within the water column, which could lead to subsequent algal blooms. These types of conditions should be monitored in conjunction with the invasive species removal since the majority of the removed biomass is from tilapia, which primarily consume algae (Dempster et al. 1993).

SWCA used the weather station in San Antonio, Texas (Location No. 417945) to determine regional rainfall measurements during the time of the field survey. This location was selected due to its proximity

to the project area, approximately 28 miles to the southwest. According to National Weather Service preliminary climatological data, over 25 inches of rain was recorded at the San Antonio weather station from February to July 2015. This is approximately 5 inches higher than average. There were several intense rain events that impacted our removal sessions in May and June.

The Natural Resources Conservation Service (NRCS) Wetlands Determination (WETS) weather station used to determine the normality of rainfall using The Direct Antecedent Rainfall Evaluation Method (DAREM) calculations data was the San Antonio Airport (NRCS 2014). The quarterly DAREM calculations for 2015 were calculated using observed rainfall data and comparative WETS data (Table 7). Based upon these calculations, this area of Texas was found to have wetter than normal to normal precipitation patterns, although two major rainfall events in May and June influenced the trend.

Table 7. National Oceanic and Atmospheric Administration (NOAA) Online Weather Data for San Antonio, Texas (Station No. 417945).

Month	30th Percentile	70th Percentile	Measured Rainfall (in)	Condition	Month Weight[b]	Score[c]	Quarterly Description[d]
January	0.62	2.03	3.67	3	1	3	
February	0.62	2.11	0.53	1	2	2	
March	0.79	2.30	2.97	3	3	9	14
April	1.05	3.15	7.54	3	1	3	
May	2.25	5.77	8.57	3	2	6	
June	2.17	5.25	6.42	3	3	9	18
July	0.38	2.35	0.07	1	2	2	
August	0.79	3.05	0.29	1	3	3	5
Total			**30.06**				

[a] Condition values are 1 for <30th percentile, 2 for between 30th and 70th percentile, 3 for >70th percentile
[b] Month Weight is 3 for the most recent month, 2 to the previous month, and so on.
[c] Score is the product of the condition and month weight.
[d] Drier than normal (sum is 6-9), normal (sum is 10-14), wetter than normal (sum is 15-18)

Source: NRCS 2012

3.3.3 Limitations and Warranty

Within the limitations of schedule, budget, and scope of work, SWCA warrants that this study was conducted in accordance with accepted environmental science practices, including the technical guidelines, evaluation criteria, and species' listing status in effect at the time this evaluation was performed.

The results and conclusions of this report represent the best professional judgment of SWCA scientists. No other warranty, expressed or implied, is made.

Please be aware that only the USFWS and/or lead federal agency can determine compliance with the Endangered Species Act.

4.0 DISCUSSION AND RECOMMENDATIONS

In 2015, the third year of SWCA's invasive species removal project, biologists removed a total of 1,308.83 pounds (approximately 65% of a ton) of biomass from Landa Lake. During the first two removal efforts, SWCA removed 6,010 pounds of biomass in 2013 and 3,823 pounds of biomass in 2014. The number of nutria, armored catfish, and tilapia that were removed from the system all significantly decreased, and a single individual goldfish was removed, a new non-native species. Also, both invasive fish species, tilapia, and armored catfish showed a shift toward smaller individuals.

Comparing the three years of removal efforts, there were several key shifts in the data. Most strikingly is the lack of nutria in the system during year 3. During the entire 2015 removal year, only eight total nutria were caught in the system, as compared to 2013 and 2014, when biologists captured 50 individuals. Importantly, of the eight individual nutria lethally removed from Comal Springs, five were male and three were female (Appendix B, Photograph 5). This strongly suggests that the breeding population of the nutria in the area has declined below the amount to sustain a population. We stress that subsequent trapping should occur during the next removal effort in 2016 to ensure that this is the case. Long-term monitoring for this species will also be important to deter new individuals from neighboring regions to re-establish and begin damaging the landscape again. As of right now we are aware of only two individuals left in the lake. These two will be intensely trapped next year.

SWCA biologists were able to capture and remove both tilapia and armored catfish in far less numbers as compared to the previous two removal years. In 2013, SWCA removed 391 individual armored catfish and 2,143 tilapia from Comal Springs which is comparable to the 308 and 1,646 respective captures in 2014. We were able to remove 112 armored catfish and 515 tilapia from the lake in 2015. A noticeable shift occurred when looking at the sizes of the removed fish. Each species showed a significant decrease in the average length and weight when compared between the first two capture years (see Figures 1A and 1B). This difference in size strongly implies that removal efforts are suppressing the population's ability to breed and to gain adult mass. Also, it must be stressed that a huge shift in smaller body size was noticed this year (See Figure 5C).

Successful invasive species removal projects that target fish over a long period of time strive for the population to show deceases in overall length and weight. What the results show is that the population is being stressed by the loss of adult (breeding) individuals. Catching smaller and smaller individuals each year displays, an impact to the species' breeding ability through direct loss of breeding individuals, as well as the species capacity to gain adult size over time. This significant decrease in both size and weight remains when the species are broken up by sexes, with the exception of catfish length. When broken down into size classes, you can see that the larger individuals are noticeably missing when comparing the 2015 sampling year to 2013 and 2014 respectively (See Figures 2A–5C). All of these trends in conjunction point toward the conclusion that, with continued removal efforts, the populations may hit a point at which the females are no longer large enough to breed.

Additionally, throughout the duration of the project SWCA paired with Luci Cook-Hildreth, from the TPWD, on a breeding and age study for the vermiculated sailfin catfish. SWCA biologists have captured and donated several hundred catfish for analysis. In 2013, all of the female armored catfish removed during the breeding season (late spring / early summer) had viable egg sacs. During the same time period in 2014, she did not find a single female that contained egg sacs. This trend continued with the 2015 removal effort. This shows definitively that we have effectively broken the ability of the catfish to breed within Landa Lake.

A significant population of tilapia remains within the lake system. In the 2013 and 2014 seasons, the golf course channel was the primary breeding habitat for tilapia. However, this area was not used this year; the

tilapia shifted their nesting grounds into southern part of Landa Lake. Although the golf course channel was not used for nesting to the same degree as 2013, we do believe it is being used as nursery habitat for the tilapia fry (young immature individuals).

SWCA did not prioritize the capture and removal of giant ramshorn snails over the other invasive species; the amount of invasive fish and nutria being removed did not allow the time needed to address this issue. We have tried various methods over the three years to try and remove this species, all with too little affect to warrant the time, effort, and money needed to invest in them.

While it is an improvement to be able to remove several hundred snails in a day, the best possible long-term removal effort would be through the use of biological removal means. The use of a population of native predators to decrease the population size of the snails would make the most meaningful difference. In the instance of this particular habitat, the common musk turtle (*Sternotherus odoratus*), has been shown to primarily prey on invasive species (Paterson and Lindeman 2009; Wilhelm and Plummer 2012). Paterson and Lindeman (2009) conducted a dietary study on a population of *S. odoratus* in the Lake Erie area and found that the native turtle species seemed to prefer invasive Eurasian mussel species. They found that the Erie population had increased head widths, presumably to adjust to this new and energy-dense food source (Paterson and Lindeman 2009). We have observed similar trophic phenomena at Comal Springs in relation to the resident *S. odoratus* population. In three years' time we have captured and marked > 1,500 common musk turtles. The majority of these turtles have larger head widths than what has been reported in other populations throughout the southeast (Munscher unpublished data). We hypothesized that the reason for this change in head width was that the musk turtles were actively preying on a larger than normal food source, the giant ramshorn snail. During one of the North American Freshwater Turtle Research Group's (NAFTRG's) quarterly turtle population monitoring in Landa Lake, the group kept 72 common musk turtles overnight to determine what they were feeding on within the lake. Of the 72 turtles, 64 had parts of giant ramshorn snails in their feces. This turtle species also preys heavily on melanoides snail species and the NAFTRG recorded 60 turtles with the possible remains of melanoides snails in their feces. In SWCA's professional opinion the only way to promote an increase in turtle population is through nesting habitat creation. Much of the Landa Lake area has been modified. Whether it be through private residences, the city golf course, or the city park. All of these areas have high human traffic and lack adequate nesting habitat needed for a large turtle population. Through the creation of nesting habitat, we would expect more young turtles to enter the ecosystem and in turn increase predation on the ample invasive snail population present.

Invasive species removal efforts typically take years to accomplish a goal of severe population depletion or entire eradication. Similar removal efforts in Florida targeting the same species have been ongoing for over 10 years with great success. One particular study at Volusia Blue Spring in Volusia County, Florida, has removed over 8,000 vermiculated sailfin catfish from the spring (Gibbs et al. 2013). To this day a few individuals are observed but the population's breeding capacity has been reduced enough that they no longer see the hundreds they have previously observed in a single day. Typically, invasive species eradication is not feasible due to the open nature of the target system (areas of immigration and emigration are available for the species to come and go from the ecosystem). Landa Lake does not fit an open system model. There is no direct overland flow into the lake nor is there likelihood of the various spillways within the lake flooding enough on a regular basis to allow for invasive species within the river to repopulate the lake area. It is our professional opinion that the three main invasive species present (nutria, tilapia, and armored catfish) within the lake ecosystem can be pushed to suffer breeding population crashes with continued high-level removal efforts over the next several years.

It is important to note that successful invasive species eradication efforts typically benefit biological diversity in their host ecosystem. However, there is also evidence that successful eradications can have unexpected impacts on native species and their host ecosystems. For example long-term invaders can

become a replacement source of nutrients. No one knows the over-encompassing impacts these invasive species have placed upon the Comal Springs ecosystem. After removing so much biomass of invasive fish, it is our professional opinion that, in time, a native fish reintroduction should be considered.

The 2015 removal season was a success. It has been shown through statistical testing that we are catching smaller individuals than the two previous years for both fish species. Additionally, over 80 of the 112 armored catfish removed came from two trips out of the new channel; since that point finding and removing armored catfish has been more difficult. We believe maybe one or two nutria are left around the lake that will be removed with more trapping effort during the next removal year.

5.0 SUMMARY

In 2015, SWCA performed an aggressive and successful third invasive species removal effort at Comal Springs, Landa Lake, New Braunfels, Texas. During the 2013 to 2015 removal efforts, a total of 63 field days, SWCA biologists removed approximately 11,300 pounds (5 tons) of invasive biomass. In the three years of removal efforts, noticeable impacts have already been observed on both the nutria and armored catfish populations. Subsequent removal efforts must be made every year for the foreseeable future in order to fully remove or to significantly impact their breeding populations. In regards to the tilapia population within the lake, we believe that our statistical analysis detailed above shows that we are seeing the desired trend of the reduction in overall body size. SWCA has removed approximately 4,260 tilapia and suspect that there could still be several hundred left in the lake. Giant ramshorn snail removal, while ongoing, may only see a lasting decrease through an increase in natural predation. This can be achieved through increasing turtle nesting ability or nest survival.

Extensive monitoring should continue for years to come to observe the impacts that this biomass removal has on the system. Native fish species should have less competition pressures and populations should be able to rebound because of it. It is worthy to note that certain native species typically present in a waterbody like Landa Lake have not been observed during the entire removal effort. For instance, no *Ictalurus* catfish species have been sighted in the 3-year project. This could be a result of competition due to the invasive species. Species such as catfish help regulate nutrient flow in lake systems. With the large removal of tilapia and armored catfish (addressing this nutrient issue) problems revolving around nutrient loading could occur. Algal blooms could become more abundant due to the loss of these invasive species without a native species to take up the opening niche. We suggest a possible native species reintroduction at a later date. However, more studies should be conducted before this can be done.

6.0 LITERATURE CITED

Armbruster, J.W., and L.M. Page. 2006. Redescription of *Pterygoplicthys punctatus* and description of a new species. *Neotropical Ichthyology* 4:401-409.

Barron, J. C. 1964. Reproduction and apparent over-winter survival of the suckermouth armored catfish, *Plecostomus* sp., in the headwaters of the San Antonio River. Texas Journal of Science 16:449–450.

Capps, K.A. 2011. Changes in community structure and ecosystem processes in response to armored catfish (Siluriformes: Loricariidae) invasion. Ph.D. dissertation, Cornell University. Ithaca, New York.

Crutchfield, J.U., Jr. 1995. Establishment and expansion of redbelly tilapia and blue tilapia in power plant cooling reservoir. *American Fisheries Society Symposium* 15:452-461.

Dempster, P.W., M.C.M. Beveridge, and D.J. Baird. 1993. Herbivory in the tilapia *Oreochromis niloticus*: a comparison of feeding rates on phytoplankton and periphyton. *Journal of Fish Biology* 43(3):385-392.

Gibbs MA, Mallinger M, Martin D, Ross M. 2010. Disturbance of the Florida Manatee *Trichechus manatus latirostris*) by an invasive catfish (*Pterygoplichthys disjunctivus*). *Southeastern Naturalist* 9(4): 635–648.

Gibbs, MA, B.N. Kurth, and C.D. Bridges. 2013. Age and growth of the loricariid catfish *Pterygoplichthys disjunctivus* in Volusia Blue Spring, Florida. Aquatic Invasions 8(2):207-2018.

Horne, F.R., T.L. Arsuffi, and R.W. Neck. 1992. Recent introduction and potential botanical impact of the giant rams-horn snail, Marisa cornarietis (Pilidae), in the Comal Springs ecosystem of Central Texas. *The Southwestern Naturalist* 37(2):194-196

Howells, R.G. 1999. Guide to identification of harmful and potentially harmful fishes, shellfishes, and aquatic plants prohibited in Texas. Revised edition. Texas Parks and Wildlife Department, Special Publication, Austin. 370 pp.

Jones, R.W., Weyl, OLF, E.R. Swartz, M.P. Hill. 2013. Using a unified invasion framework to characterize Africa's first loricariid catfish invasion. *Biological Invasions* 15:2139-2145.

LeBlanc, D.J. 1994. Nutria. In: *Prevention and Control of Wildlife Damage*. Cooperative Extension Division Institute of Agriculture and Natural Resources and USDA APHIS Animal Damage Control, B-71–B-80

Linde, R.A., J.I. Izquierdo, J.C. Moreira, and E. Garcia-Vazquez. 2008. Invasive tilapia juveniles associated with degraded river habitats. *Aquatic Conservation* 18(6) 891-895.

Martin, C.W., M.M. Valentine, and J.F. Valentine. 2010. Competitive interactions between invasive Nile tilapia and native fish: the potential for altered trophic exchange and modification of food webs. *PLoS ONE* 5(12):e14395.

Nico, L.G., H.L. Jelks, and T. Tuten. 2009a. Non-Native Suckermouth Armored Catfishes in Florida: Description of Nest Burrows and Burrow Colonies with Assessment of Shoreline Conditions. *Aquatic Nuisance Species Research Program Bulletin* 9(1): 1-30.

Nico, L.G., W.F. Loftus, and J.P. Ried. 2009b. Interactions between nonnative armored catfish (Loricaridae:*Pterygoplicthys*) and native Florida manatee (*Trichechus manatus latirostris*) in artesian springs. *Aquatic Invasions* 4:511-519.

Nico, L.G., P.L. Butt, G.R. Johnston, H.L. Jelks, M. Kail, and S.J. Walsh. 2012. Discovery of South American suckermouth armored catfishes (Loricariidae, Pterygoplichthys spp.) in the Santa Fe River drainage, Suwannee River basin, USA. *BioInvasion Records* 1(3)179-200.

National Oceanic and Atmospheric Administration (NOAA) Online Weather Data (NOWData). 2012. Climatology data for San Antonio, Texas (417945), Available online at http://www.nws.noaa.gov/climate/. Accessed August 2012.

Paterson, J.C., and J.V. Lindeman. 2009. Effects of Zebra and Quagga Museel (*Dreissena* spp.) invasion on the feeding habits of *Sternotherus odoratus* (Stinkpot) on Preque Islo, Northwestern Pennsylvania. *Northeastern Naturalist* 16(3):365-374.

Peterson, M.S., W.T. Slack, N.J. Brown-Peterson, and J.L. McDonald. 2004. Reproduction in nonnative environments: establishment of Nile tilapia *Oreochromis niloticus*, in coastal Mississippi watersheds. *Copeia* 2004: 842-849.

Phillips, C.T., M.L. Alexander, and R. Howard. 2010. Consumption of eggs of the endangered fountain darter (Etheostoma fonticola) by native and nonnative snails. *The Southeastern Naturalist* 55(1): 115-117.

Popma, T. and M. Masser. 1999. Tilapia: Life History and Biology. Southern Regional Aquaculture Center. Available online at: http://www.aquanic.org/publicat/usda_rac/efs/srac/283fs.pdf. Accessed March 24, 2005.

Sheffels, T.R. and M.D. Sytsma. 2007. Report on nutria management and research in the Pacific Northwest. Unpublished report. Portland, Oregon: Portland State University.

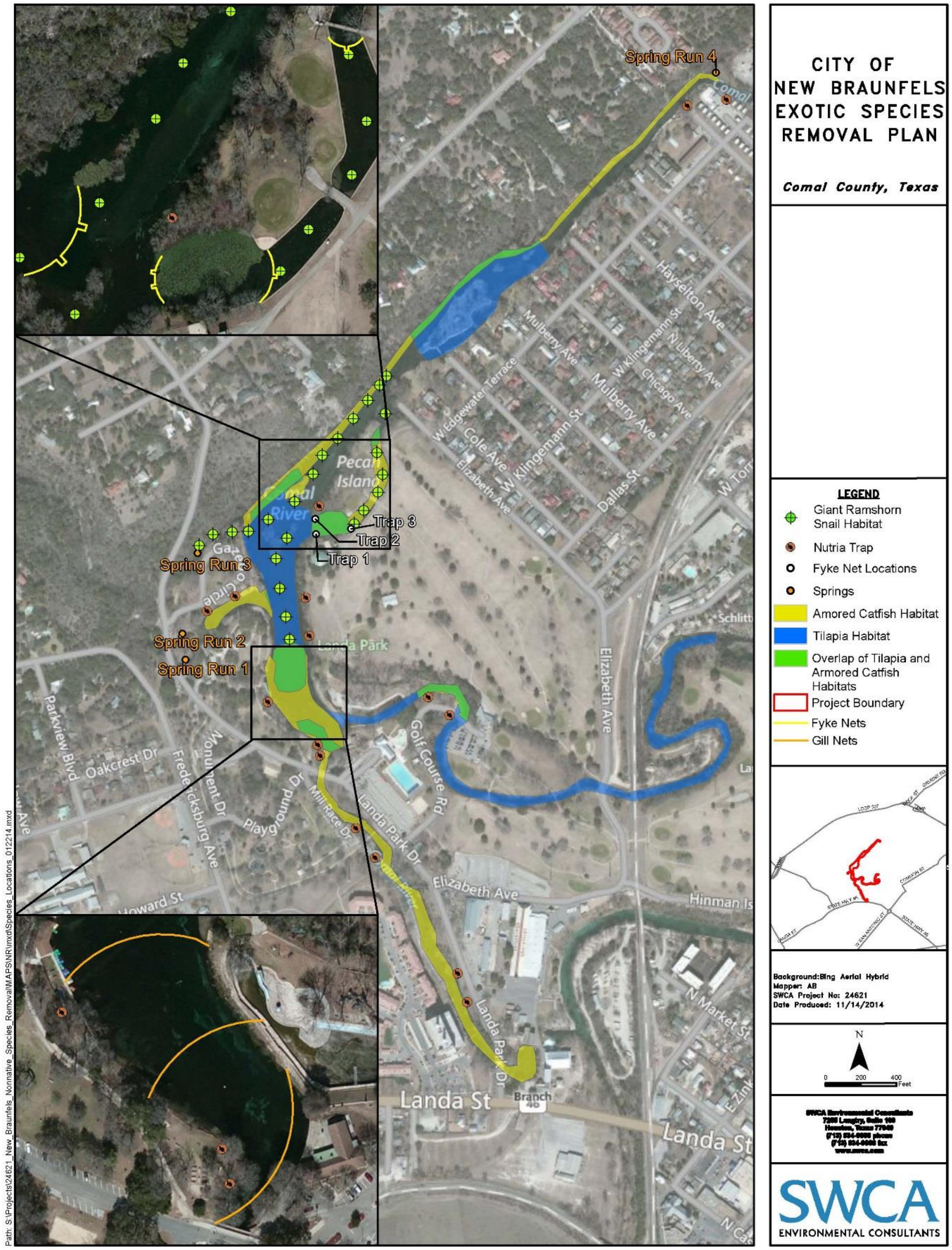
Trewavas, E. 1983. Tialpine fishes of the genera *Sarotherodon*, *Oreochromis* and *Danakilia*. Cornell University Press, Ithaca, NY.

Weber, C. 1992. Révision du genre *Pterygoplichthys* sensu lato (Pisces, Siluriformes, Loricariidae). *Revue Francaise d'Aquariologie* 19:1-36.

Wilhelm, C.E., and M.V. Plummer. 2012. Diet of radio tracked musk turtles, *Sternotherus odoratus*, in a small urban stream. *Herpetological Conservation and Biology* 7(2): 258-264.

Appendix A

Vicinity and Site Layout Map


CITY OF NEW BRAUNFELS EXOTIC SPECIES REMOVAL PLAN
Comal County, Texas
LEGEND
Giant Ramshorn Snail Habitat
Nutria Trap
Fyke Net Locations
Springs
Amored Catfish Habitat
Tilapia Habitat
Overlap of Tilapia and Armored Catfish Habitats
Project Boundary
Fyke Nets
Gill Nets
Background:Bing Aerial Hybrid
Mapper: AB
SWCA Project No: 24621
Date Produced: 11/14/2014
N
0 200 400 Feet
SWCA
ENVIRONMENTAL CONSULTANTS
Spring Run 4
Spring Run 3
Spring Run 2
Spring Run 1
Trap 1
Trap 2
Trap 3
Pecan Island
Landa Park
Landa St
Landa Park Dr
Elizabeth Ave
Golf Course Rd
Howard St
Hinman Is
Path: S:\Projects\24621_New_Braunfels_Nonnative_Species_Removal\MAPS\NR\mxd\Species_Locations_012214.mxd

Appendix B

Photographic Log

PHOTOGRAPHIC LOG

New Braunfels Invasive Species Removal Project

Photo 1 Overhead view of Landa Lake. This area is highly used by tilapia for nesting.

Photo 2 SWCA uses Jon Boats to check fyke nets and gill nets.

Photo 3 Underwater visual of tilapia captured in a fyke net.

Photo 4 Underwater visual of tilapia captured in a fyke net.

Photo 5 Turtles are captured in the fyke nets as well. Nets are checked twice a day release by catch.

Photo 6 SWCA biologist Lonnie Wolfe spearing a breeding male tilapia (mouth brooders).

Photo 7 A large bin filled with removed tilapia.

Photo 8 Processed armored catfish

Photo 9 Close up of an armored catfish.

Photo 10 A bin of over 30 armored catfish donated to TPWD for research.

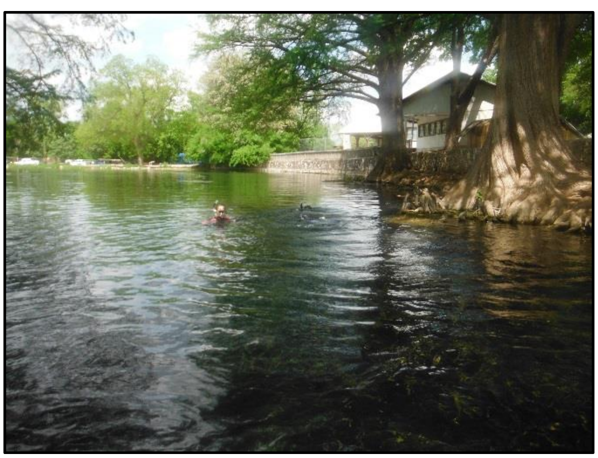


Photo 11 SWCA biologists Laura Rudolf and Eric Munscher chasing tilapia into a gill net.

Photo 12 Vermiculated armored catfish being speared by a Hawaiian sling.

PHOTOGRAPHIC LOG

New Braunfels Invasive Species Removal Project

Photo 13 Vermiculated armored sailfin catfish being processed by Hunter Thompson.

Photo 14 A small common goldfish removed from Landa Lake.

Photo 15 A common goldfish that was removed from Landa Lake.

Photo 16 Armored catfish being necropsied. In 2013–2014 75% females had eggs; in 2015 0 had eggs.

Photo 17 Armored catfish depicting the typical spinal deformity observed in Landa Lake specimens. Possible Inbreeding depression.

Photo 18 Nutria captured in live traps.

PHOTOGRAPHIC LOG

New Braunfels Invasive Species Removal Project

Photo 19 Side profile of invasive nutria that has been dispatched humanely.

Photo 20 Giant ramshorn snail on eelgrass in Landa Lake.

Photo 21 SWCA biologist Chris Collins measuring the width of several giant ramshorn snails.

Photo 22 Processing the giant ramshorn Snail.

Photo 23 Chris Collins and Laura Rudolf checking a gill net.

Photo 24 Laura Rudolf and Chris Collins checking gill nets.

Appendix L6

2015 Comprehensive Final Report for Gill Parasite (Centrocestus formosarus) Activities in the Comal River

2015 Comprehensive Final Report for Gill Parasite (*Centrocestus formosanus*) Activities in the Comal River

PREPARED FOR:

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November 3, 2015

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1.0 INTRODUCTION

The BIO-WEST, Inc. (BIO-WEST) Project Team (Project Team), including staff from Texas State University-San Marcos and the U.S. Fish and Wildlife Service San Marcos Aquatic Resources Center (SMARC), is pleased to submit this 2015 Report for Gill Parasite Activities in the Comal Springs/River (Comal system) to the City of New Braunfels. This document represents 2015 Gill Parasite studies as they build upon or relate to previous years activities.

The recent Edwards Aquifer Recovery Implementation Plan process led to development of the approved Edwards Aquifer Habitat Conservation Plan (HCP) (EARIP, 2011). In relation to the Comal system, the Edwards Aquifer Recovery Implementation Plan process and HCP identify a variety of options to improve and increase habitat for threatened and endangered species, including the fountain darter (*Etheostoma fonticola*), Comal Springs salamander (*Eurycea* sp.), Comal Springs riffle beetle (*Heterelmis comalensis*), Peck's cave amphipod (*Stygobromus pecki*), and the Comal Springs dryopid beetle (*Stygoparnus comalensis*).

Specific to *E. fonticola*, one of the proposed HCP minimization and mitigation measures is to conduct studies aimed at monitoring and reducing concentrations of the nonnative gill parasite *Centrocestus formosanus* in the Comal River study area (Figure 1). This parasite has a complex life cycle that includes the nonnative snail (*Melanoides tuberculatus*) as its first intermediate host, any of a wide variety of fishes as its secondary intermediate host, and a piscivorous wading bird as the definitive host. This parasite has been shown to negatively impact *E. fonticola*, and there are concerns that these impacts could worsen during low-flow conditions as parasite concentrations may increase due to a lower volume of water passing through the system.

Applied research and monitoring conducted as part of this study in 2013 established baseline data on distribution, abundance, and density of both the host snail (*M. tuberculatus*) and the free- swimming cercariae of the parasite *C. formosanus*. However, at the few sites that had been previously sampled, cercariae densities had changed considerably when compared to previous collections. Therefore, in 2014 additional snail and cercariae monitoring was implemented to gain insight into temporal patterns in abundance and density of snails and cercariae. In 2015, these protocols were modified for maximal efficiency based on 2014 results to monitor hosts (Section 2) and gill parasites (Section 3) for trends.

Research conducted in 2014 to better understand snail infection prevalence throughout the system not only yielded surprisingly high rates of snail infection in some areas compared to estimates of previous studies, but resulted in a refined and very efficient method for assessing snail infection. This method, which is presented in Section 4, was again applied in 2015, albeit on a smaller scale, to look for patterns in these rates.

The prevalence of the parasite in *E. fonticola* was investigated initially in 2014. Although collections from the late 1990s demonstrated that the parasite was prevalent and abundant in wild *E. fonticola* individuals from the Comal River (Mitchell, et al., 2000), little recent data were available. Parasites in gills of wild *E. fonticola* individuals collected from two sites on the Comal River were quantified in 2014, and additional individuals from another site were examined in 2015. The results of this examination are presented in Section 5.

Although great progress is being made via HCP investigations, there are still many instances where knowledge of the life history and population characteristics of the hosts and parasites, and their interactions, is lacking in the Comal system. In 2015, BIO-WEST researchers collaborated with Texas State University parasitologists in the development of a larger, scientific approach to furthering the understanding of host/parasite dynamics in the Comal system. The objective of this approach is to uncover new opportunities for informed management through refinement of knowledge of the intricacies of these ecological relationships. The initial testing and refinement of new methodologies was begun in 2015. This included the use of cutting edge genetic and morphometric analysis technologies to investigate subdivisions in snail host populations and their relationship to parasite infection as explained in Section 6. Finally, all of the above data are summarized and discussed in Section 7, which is followed by suggestions for 2016 monitoring and research in Section 8. References are listed in Section 9.

Figure 1. Comal River study area.

2.0 SNAIL DISTRIBUTION AND DENSITY MONITORING IN 2015

2.1 Methods

The goal of annual snail distribution and density monitoring conducted is to assess any changes in snail spatial distribution, density, or size-class structure within the Comal River study area (study area). With this goal in mind, the system-wide snail distribution survey conducted in 2013 was repeated in 2014 and 2015. Surveys were conducted within the same four sampling reaches (Figure 1):

- Upper Spring Run (USR)–from Spring Run 4 and Blieders Creek to the northern tip of Pecan Island,
- Landa Lake (LL)–from the northern tip of Pecan Island downstream to the Landa Park Drive Bridge near the dam,
- New Channel Reach (NCR)–from the Landa Park Drive Bridge downstream past the old power plant to Clemens Dam, and
- Old Channel Reach (OCR)–from Landa Lake culverts downstream to the confluence with the New Channel just upstream of Clemens Dam.

During March 12-17, 2015, a survey team comprised of one senior-level biologist and one technician surveyed for snails with dip nets. Although much of the river was sampled by wading, kayaks and specialized telescoping-handled dip nets were used for sampling deeper areas. In 2013 this survey was conducted independently by three different crews to generate accurate baseline data and assess effort requirements. However, the resulting data made it clear that each crew had similar findings. Thus, in 2014 and 2015, only one survey was conducted by a single crew. Each reach was covered as thoroughly as possible in an attempt to replicate the average effort expended per crew in 2013. Locations with high densities of *M. tuberculatus* were noted. A GPS point was taken to mark the location of every individual sample site. At each recorded sample site, 2–4 dip net sweeps were taken with specially designed snail dip nets. To most efficiently cover all available habitats, a staggered sampling approach was used. If no *M. tuberculatus* were collected during the first two dips, the team moved on to a new location. Similarly, if >50 *M. tuberculatus* were collected in the first two dips, the area was considered “high density” and the team moved to a new location. A four-dip maximum was conducted at any individual sampling site. Every *M. tuberculatus* over approximately 17 mm (the smallest size thought to be infected with the parasite) was counted, as were any giant ramshorn snails (*Marisa cornuarietis*). All captured *M. tuberculatus* and *M. cornuarietis* collected during the course of sampling were retained and destroyed. The survey team continued survey efforts until they had covered all four sampling reaches (Figure 2). In 2015, presence or absence of another exotic snail and trematode host, *Tarebia granifera*, was also recorded at each site.

Figure 2. Sampling coverage for comprehensive snail surveys in 2015.

To quantify the density of *M. tuberculatus* in "hot spot" areas of the system, density sampling was conducted both in areas sampled previously in 2013 (providing for detection of trends) and in new hot spot areas identified during the 2014 survey. Density sampling was conducted using a custom-designed snail density quadrat developed for this study. The device consisted of a 0.25 square-meter (m^2) quadrat constructed of 0.5-inch PVC pipe with sheet metal attached, which created a 4-inch fence that could be pushed into the substrate to prevent the quadrat from moving (Figure 3). Snails within the quadrat were removed with a snail dip net until no more snails were found. All *M. tuberculatus* were enumerated and measured to the nearest millimeter for comparison of length-frequency data among years. Three quadrat samples were conducted near each of 15 high-density areas spread among the USR, LL, and NCR. The OCR was excluded in 2013 as no high-density areas were found there during the system-wide survey, but this reach was added to 2014 and 2015 density sampling as hot spots were located during each of these system-wide surveys.

2.2 Results and Discussion

Overall, 197 sites were sampled, with 1,198 *M. tuberculatus* collected during the system-wide survey in 2015 (Table 1). Only 6 *M. cornuarietis* were captured, and *Tarebia granifera* were found present at 179 sites (90.8%). *M. tuberculatus* were present at 41.6% of sites sampled in 2015, slightly higher than 2014 or 2013 (36%). Similar to the results of the 2013 and 2014 surveys, the NCR had the highest captures of *M. tuberculatus* (397) while the OCR had the least (156).

Table 1. Overall capture results for *Melanoides tuberculatus* (MT) and *Marisa cornuarietis* (MC) from all sites sampled during the 2013, 2014 and 2015 system-wide surveys of the Comal River study area.

YEAR	NUMBER OF SITES	NUMBER OF MT	NUMBER OF SITES W/ MT	NUMBER OF SITES W/ >15 MT/DIP	NUMBER OF MC
2013	245	1,480	88	11	37
2014	222	1,628	79	12	16
2015	197	1,198	82	4	6

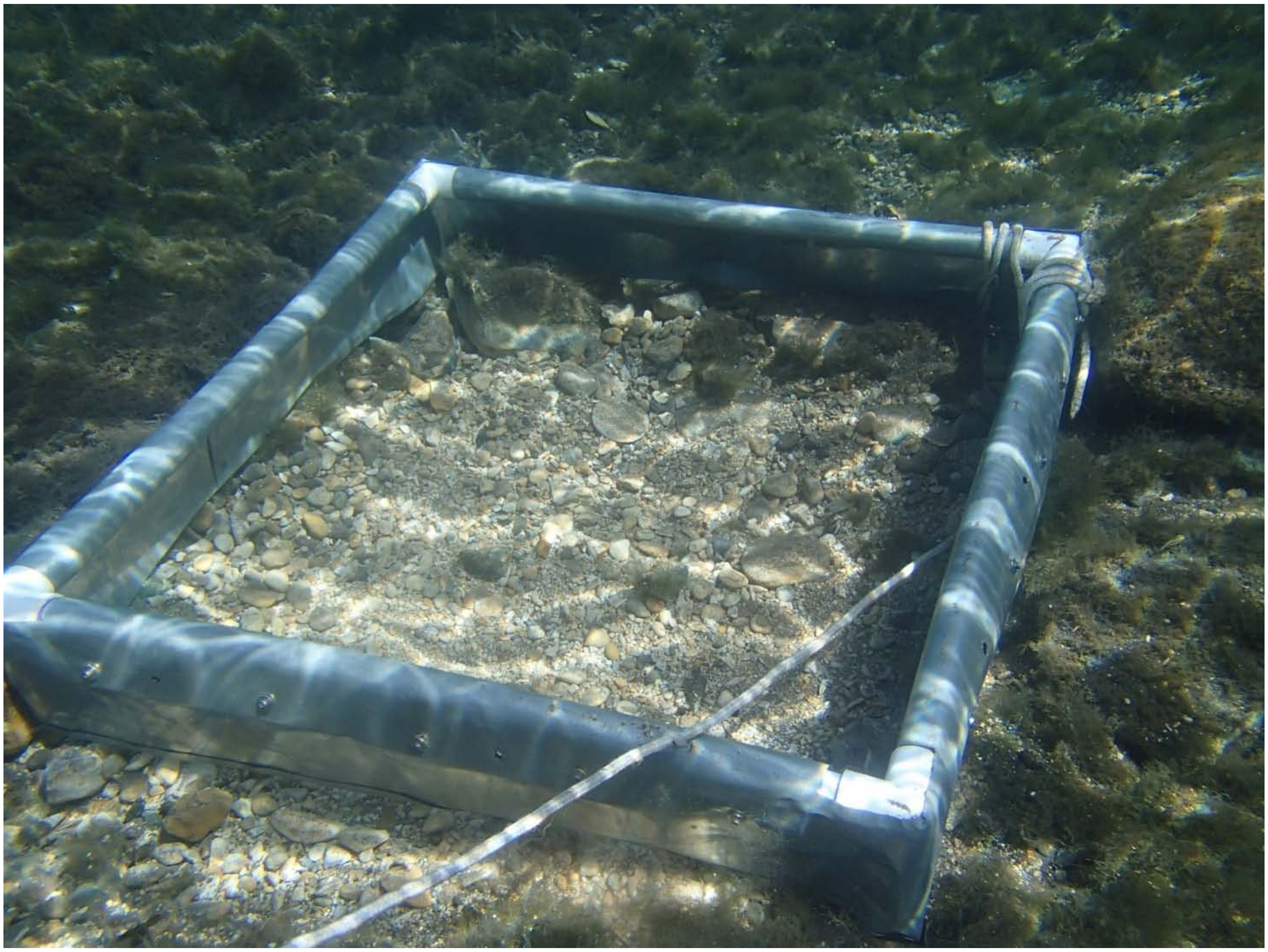


Figure 3. Snail density quadrats were used to estimate snail densities throughout the Comal system.

Average 2013 densities of *M. tuberculatus* in hot spots ranged from 179/m^2 to over 1,000/m^2, 2014 densities observed ranged from 50/m^2 to 850/m^2, and in 2015 the observations ranged from 33/m^2 to 936/m^2. Similar to 2014, the highest 2015 observed densities were in USR. While it does appear at a glance that densities in 2015 are reduced significantly in 2015 for LL, NCR, and OCR (Table 2), there are several factors that may have influenced this outcome. One such factor was the impact of construction activities in LL and NCR. Construction of new walls removed or altered some sampling sites that had been previously used, either preventing resampling or significantly altering the result. The new construction also often produced areas that are not conducive to our density sampling methods, as large cobbles and exposed wire snag nets and create interstitial spaces that cannot be sampled. However, when reach average densities are compared among years and their estimates of standard error are considered, density estimates are relatively static across years (Table 2).

Table 2. Mean yearly snail density estimates (± SE) per square meter averaged over samples within each reach.

YEAR	SAMPLING REACH[a]			
	USR	LL	NCR	OCR
2013	371.7 (±115.6)	399.3 (±70.9)	607.1 (±221.2)	---
2014	426.9 (±114)	350 (±103.3)	343.7 (±37.8)	146.2 (±32.6)
2015	480.2(±127.7)	185.3(±55.8)	147.1(±55.9)	62(±6)

[a] USR=Upper Spring Run, LL=Landa Lake, NCR=New Channel Reach, OCR=Old Channel Reach

Mean lengths of snails captured within each reach from 2013 – 2015 do not appear to differ greatly among sample years, however mean length of snails collected during density sampling in the OCR was consistently much lower than in the other reaches (Figure 4). In 2015, the USR showed fluctuation in some size categories, but given the similar mean and overall distribution this is most likely simple sampling error and possibly affected by the increased sample size in 2015 (Figure 4).

The interesting bimodal distribution in snail size structure present in the USR data all three years is reflected in the NCR data in 2015, illustrating a change in size structure within this population. This possibly represents increased reproduction or recruitment in this reach, but the exact mechanisms behind this unique distribution are unknown at this time. Further study of this population will be important in determining what causes this pattern of size distribution, and if it really is different. Analysis of genetic and morphometric data from snails in this reach, which has begun this year, will help inform as to the possible causes of these distributions. Data from LL distinctly showed an increase in the frequency of larger snails. It can be observed that the mean is nearly the same again as in 2013, and the range of means over the 3 year period is only 4mm, suggesting that this again may only be on the scale of sampling error and this distribution, like that found for the USR and OCR, has remained stable through time.

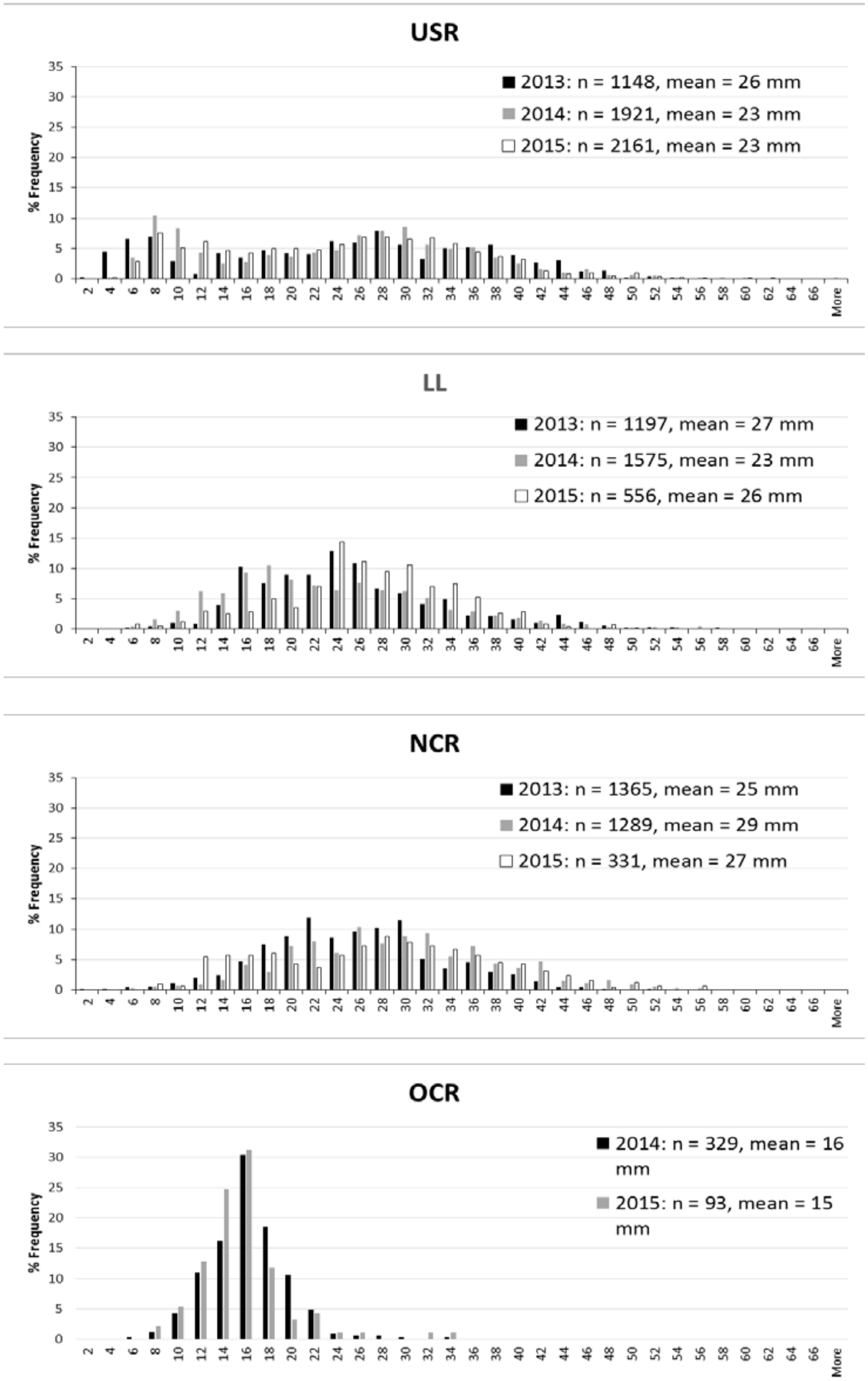


Figure 4. **Length frequency distribution of *Melanoides tuberculatus* collected from four reaches of the upper Comal River (Upper Spring Run [USR], Landa Lake [LL], New Channel Reach [NCR], and Old Channel Reach [OCR]) from 2013-2015.**

3.0 CERCARIAE MONITORING IN 2015

3.1 Methods

To quantify density of drifting gill parasite (*C. formosanus*) cercariae in the Comal River study area, four transects were selected for sampling in 2014 from the six transects sampled in 2013: SI, LL, OCR, and New Channel at Landa RV Park (RVP) (Figure 5). Based on 2013 and 2014 data, this was reduced to 3 sites (LL, OCR, RVP) thought to best represent the system as a whole and allow for the most efficient long-term monitoring of drifting cercariae. SI was dropped in 2015 due to the apparent scarcity of cercariae detected, additional effort required to sample that site, its upstream position in a system that appears to act in an additive fashion, and low observed host infection rates in 2014 studies (7%). The selected transects also allow for comparisons to additional types of data, such as encysted parasites in fish gills, due to concurrent sampling by other researchers.

At each of the selected transect locations, 5-L water samples were collected from six points that were evenly distributed throughout the water column both horizontally and vertically. For each transect, three sampling stations were established that were equally spaced across the stream channel perpendicular to flow. At each of these stations, two 5-L samples were collected, one approximately 5 cm from the surface and one at 60% of the depth at that location. Samples were collected using a modified livewell pump attached to a standard flow/depth measurement rod and buckets marked at the 5-L volume. At the time of collection, each water sample was immediately treated with 5 milliliters (ml) of formaldehyde to kill parasite cercariae, thus facilitating their capture (live cercariae can wiggle through the filter device). Filtration involved passing the sample through a specialized filter apparatus (Figure 6) containing three progressively finer nylon filters, the final filter having pores of 30 microns. After filtration of each sample, the 30-micron filter containing cercariae was removed from the filtration apparatus and placed in a Petri dish. Each sample was then stained with Rose Bengal solution and fixed with 10% formalin, at which point the Petri dish was closed and sealed with Parafilm for storage. Cercariae on each filter were later counted using high-power microscopy at the BIO-WEST laboratory.

Previous data on cercarial drift have noted large diel shifts in drifting cercariae densities. Therefore, to standardize for time of day, all samples were collected within a 2-hour window from 9:00 to 11:00 am. To accomplish this, only one site was sampled per day, and sampling was done on consecutive days whenever possible (some sampling periods were interrupted by weather/high water in 2014 and 2015) attempting to collect observations capturing minimal natural variation within a sample period. In 2015, each site was sampled 3 times within each primary sampling period to allow for assessment of variation within and among primary sampling periods and consequent evaluation of the error of this method. Sampling events in 2015 occurred February 25-March 17 (winter), June 1-11 (spring), and July13-23 (summer) and compared with 2014 collections occurring February 10–14, June 2–5, and August 11–14.

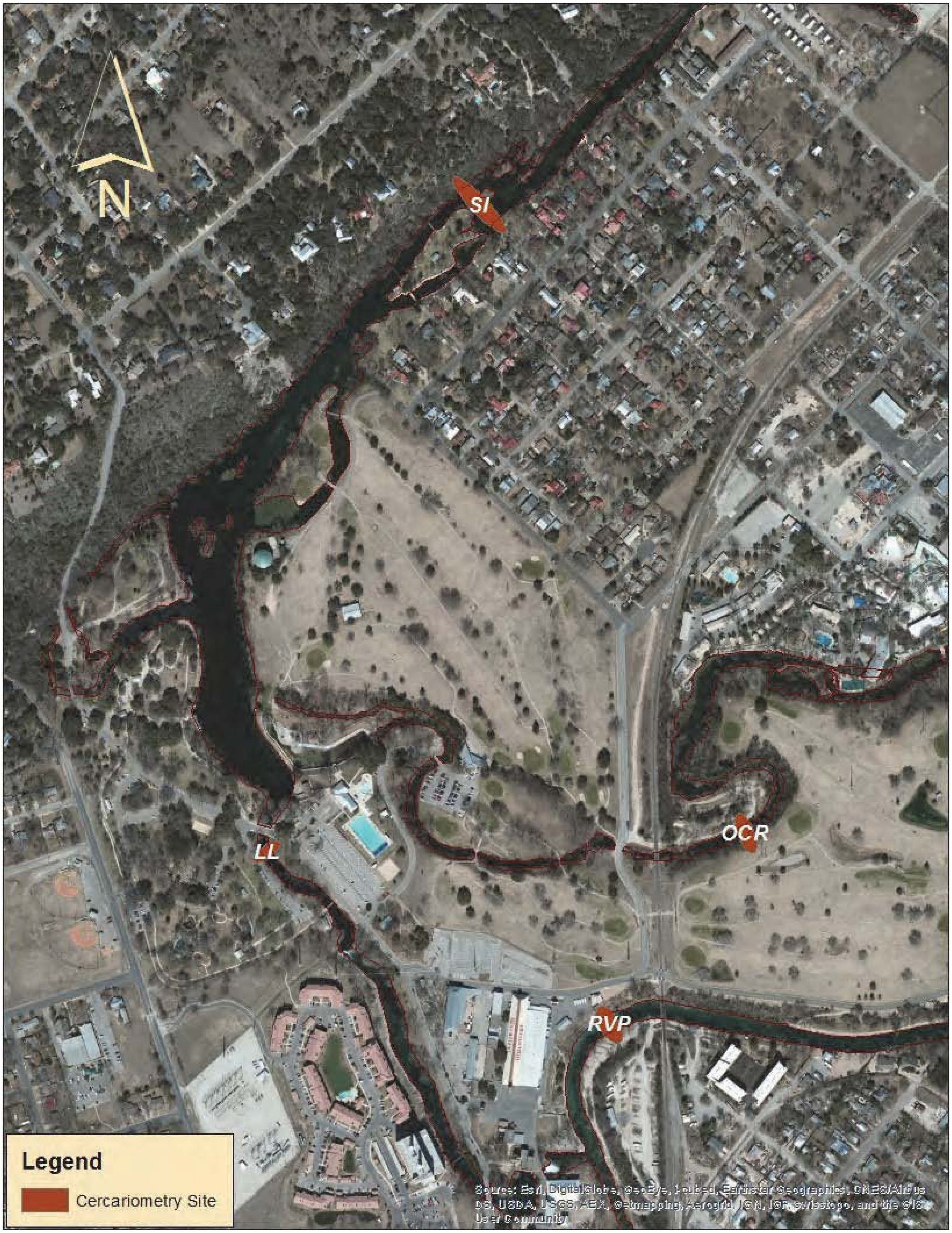


Figure 5. Location of cross sections used for monitoring drifting cercariae in the water column (cercariometry) are shown in red. SI not sampled in 2015.

Figure 6. **Filtration of water samples to collect gill parasite cercariae from the water column.**

3.2 Results and Discussion

In 162 individual 5-L samples, 2,311 total *C. formosanus* cercariae were detected in 2015, resulting in an overall annual, system wide mean of 2.8 (SE ±0.01) cercariae per liter. The corresponding mean from comparable 2014 samples was 4.9 (SE ±0.5) cercariae per liter. Thus, density of cercariae overall was lower in 2015, quite possibly due to shorter residence time of cercariae through the system due to higher flows in 2015 (Figure 7). This reduction is most apparent in the data from LL, where the greatest differences among yearly samples were observed (Table 3, Figure 7, Figure 8). Cercariae density results from OCR were essentially the same both years, with observed densities remaining lower than those at the other sites. Less seasonal fluctuation was observed among samples from RVP collected in 2015, though the annual means for this site are relatively close (Table 3, Figure 8).

Cercariae abundance in 2015 was much less variable among sites and more stable in relation to season at each site (Figure 8). Observed densities at LL and OCR sites in 2015 followed a similar pattern to 2014, increasing over the year, though this increase was of much less magnitude at LL (Figure 8). This is further evidence of a concentration effect of low flows such as those observed in 2014 (Figure 7). Even if cercarial production remained similar throughout the year, cercariae would be expected to become more concentrated as spring flows decrease, producing a corresponding increase in residence time thus greater accumulation/higher density. Observed 2014 concentrations at RVP had shown a peak in spring and decline in summer, thought to have resulted from settling out of cercariae in the water column in the New Channel above the power plant (between LL and RVP) due to reduced flow. This peak was not observed in 2015, when the observed cercariae densities were more or less stable over the year. These data suggest that *E. fonticola* in lower Landa Lake and the upper New Channel will be exposed

to increased parasite concentrations under low flows, but moderate base flows as observed in 2015 mediate cercariae accumulation to some degree.

Table 3. Mean cercariae/liter (±SE) collected during parasite monitoring events in 2014 (annual mean 4.9 (±0.5)) and 2015 (annual mean 2.8 (±0.01)).

Transect[a]	Year	Month			
		Winter	Spring	Summer	OVERALL
LL					
	2014	4.4 (±0.4)	6.1 (±0.5)	13.3 (±0.6)	**7.9 (±1.0)**
	2015	2.6 (±0.3)	2.6 (±0.3)	3.4 (±0.3)	**2.9 (±0.2)**
OCR					
	2014	0.4 (±0.1)	1.0 (±0.2)	2.0 (±0.3)	**1.1 (±0.2)**
	2015	1.4 (±0.2)	1.9 (±0.2)	2.4 (±0.2)	**1.9 (±0.1)**
RVP					
	2014	3.8 (±0.3)	7.8 (±0.9)	4.8 (±0.4)	**5.6 (±0.2)**
	2015	4.5 (±0.7)	3.1 (±0.3)	3.6 (±0.3)	**3.7 (±0.2)**

[a] LL=Landa Lake outflow, OCR=Old Channel at Elizabeth Avenue, RVP=New Channel at Landa RV Park

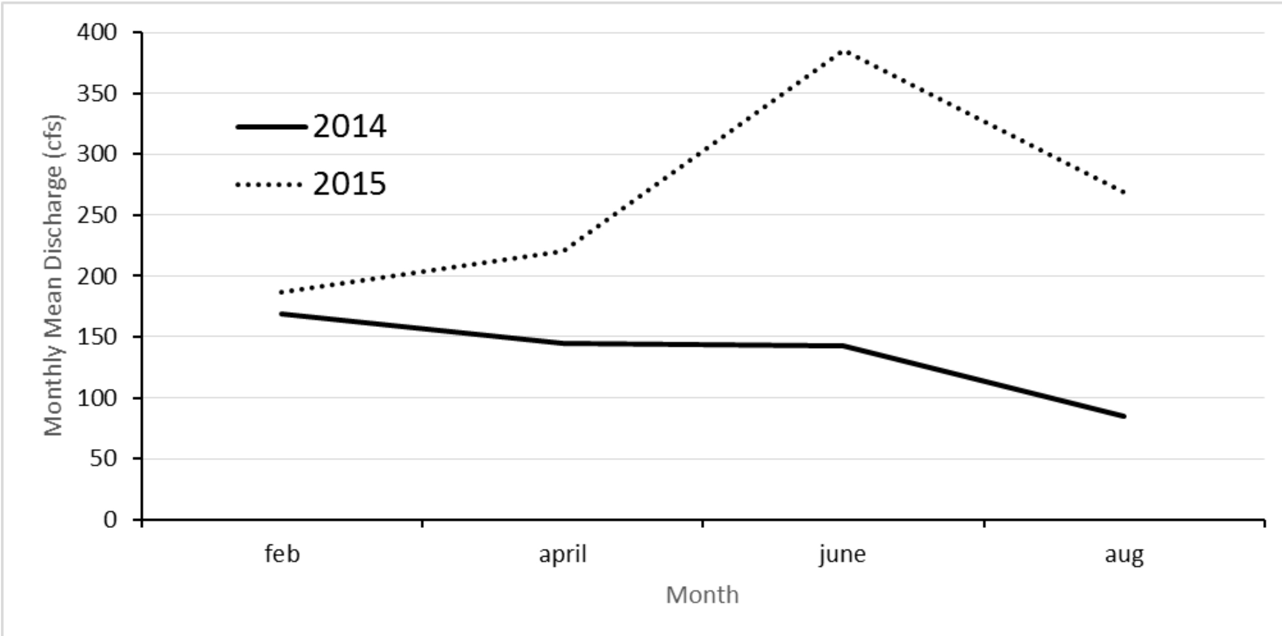


Figure 7. **Comparison of monthly mean discharge values recorded between February and August at the US Geological Survey gauge (# 08169000) on the Comal River illustrates differing flow regimes between 2014 and 2015 annual cercariae sampling periods.**

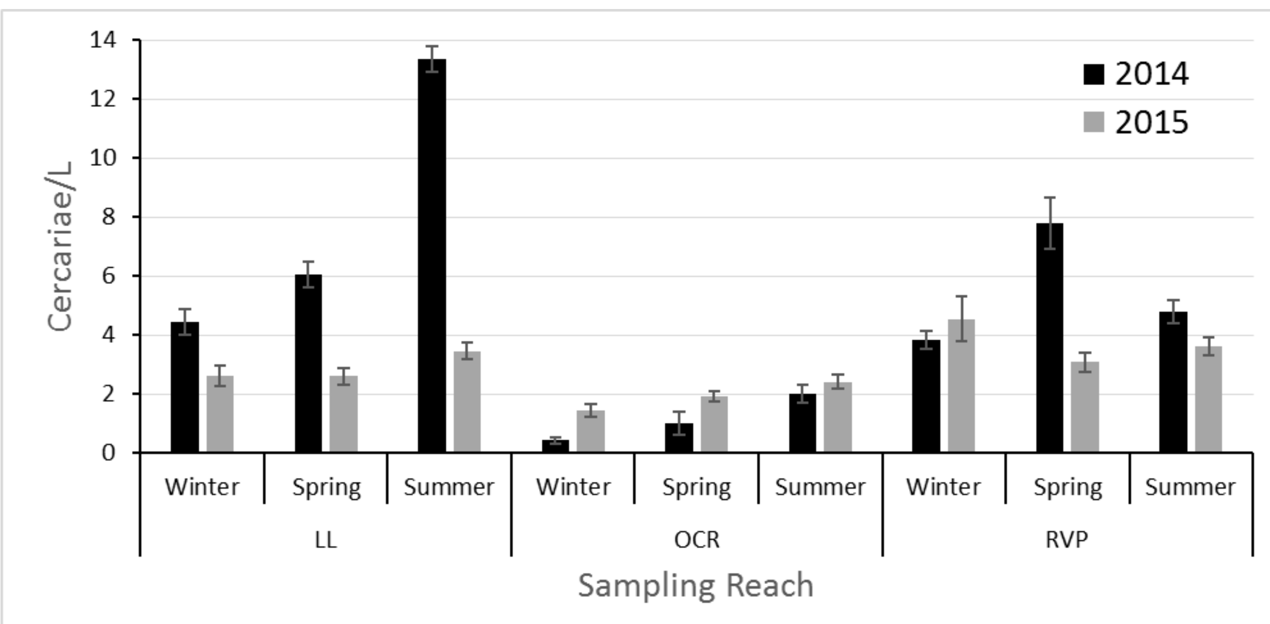


Figure 8. **Density (cercariae/liter) of gill parasite (*Centrocestus formosanus*) cercariae (error bars represent standard error of the mean) in samples taken from the water column at three sites during 2014 and 2015. The transects sampled included Landa Lake outflow (LL), Old Channel at Elizabeth Avenue (OCR) and New Channel at Landa RV Park (RVP).**

4.0 SNAIL INFECTION PREVALENCE

4.1 Methods

As part of this project in 2013, a snail-removal pilot study was conducted to assess the effectiveness of large-scale snail removal on reducing concentrations of *C. formosanus* cercariae in the water column. BIO-WEST removed an estimated 86,262 *M. tuberculatus* from a study reach located within the new channel. Based on pre- and post-drifting cercariae densities, it was determined that, in this case, the removal of snails did not have the desired effect of reducing parasite numbers. Site selection for this removal, however, was based on observed densities of host snails and drifting parasite cercariae as these were the only available data. Data on host snail infection prevalence were lacking. One likely explanation for the lack of success of that pilot removal study was that the snails removed were not those responsible for the high cercariae numbers seen; in other words, few or none of the snails may have been infected. Had the data been available such that a study site could have been selected based on the infection rate of the host snails, success would have been more probable. An attempt was made in 2013 to investigate snail infection prevalence through the commonly used method of visual observation of parasite shedding. This study suggested that infection rates of the samples were less than 2%. It was difficult to rectify the estimate with the abundance of cercariae observed in water column samples. This method was reliant on careful observation and may have been subjective in nature. It was also very slow, as it required many days of observation during which the snails remained segregated and occupied much lab space.

In 2014, based on the aforementioned management implications of infection prevalence data, additional effort was applied to systematically determining *C. formosanus* infection rates of host snail populations throughout the system. Rather than observing live snails for cercariae shedding, it was deemed more efficient to dissect snails and examine them for the parasite internally. Snails collected from each sample point were segregated in containers with sufficient system water and air to ensure survival during transport and labeled with collection date, time, and a unique site identifier. These samples were then transported in buckets to a Texas State University-San Marcos laboratory, where aerated, segregated areas were constructed to maintain optimal conditions for snail survival until testing while keeping individual samples separated (Figure 9).

To determine the infection status of host snails, cutters were used to remove the point of the shell, allowing removal of the digestive gland. The digestive gland was placed in a watch glass with a small amount of water and viewed under a lighted dissection microscope. As the parasites were still alive during this procedure, they were readily detected under the scope by movement, allowing the observer to zoom in for identification (Figure 10).

Figure 9. **Aerated containers used to maintain live *Melanoides tuberculatus* over the course of study and kept in water drawn from the Freeman Aquatic Building artesian well.**

Figure 10. **BIO-WEST biologist observing the digestive gland removed from a potential host snail to determine parasite infection (left). *Haplorchis pumilio* (upper right) and *Centrocestus formosanus* (lower right) cercariae found in the digestive glands of *Melanoides tuberculatus* individuals collected from the Comal system.**

4.2 Results and Discussion

A total of 274 snails were examined in 2015, much less than the 5,326 individuals examined in 2014. The more intensive initial effort in 2014 was necessary to generate an accurate baseline for snail infection in the system, while 2015 sampling was oriented toward monitoring and testing new hypotheses about the susceptibility of snail populations to infection. Location and number of samples collected in 2015 was influenced by results of 2014 studies, but was also determined by sampling needs for studies of population and life history as they relate to infection.

Two other species of parasites known from the system were also detected (*Haplorchis* sp. and *Philophthalmus* sp.), and two additional novel parasites previously unknown in the Comal system were discovered. One of these, presumed (confirmation is needed from genetic methods to confirm parasite ID at the species level) to be *Renicola sp.*, a kidney parasite of avian definitive hosts, was first observed in 2014 and additional specimens detected in 2015. The additional new species was recently detected in 2015, and is suspected to belong to the genus *Cloacitrema*, trematode bird eye flukes similar to *Philopthalmus*. *Centrocestus* mean infection rates for sample areas ranged from 0 to 42.9 % with an overall mean of all areas sampled of 19% (Table 4).

The maximum sample area mean observed in BIO-WEST 2014 infection data was 24.9 %, however, much higher rates were observed in some subsamples. These are illustrative of potential effects of sample size and sample error (chance) on variation between the two years data. Previous studies reported infection rates of 6.1–6.4% (Mitchell, et al., 2000; Tolley-Jordan & Owen, 2008), though these studies sampled varying areas and were conducted years earlier. There was an increase in infection rates observed in the upper portion of Landa Lake (SI) and USR in 2015. In 2014, under low flow conditions, conditions in these areas were drastically affected by for much of the year by drought. This may have increased the susceptibility of snail hosts to parasite infection. Density data also suggest that snail numbers may have been increasing in these areas since 2013. These data could make future snail removal efforts more successful in reducing parasite loading, as the areas of greatest infection prevalence could be located and targeted.

Table 4. Sample size (n) and infection rates (percent) of host snails (Melanoides tuberculatus) by Centrocestus formosanus and other detected parasites at high-host density areas in the Comal system. Data from the two study years is presented as: 2015 value (2014 value).

AREA[a]	*n*	PERCENT UNINFECTED	PERCENT *C. FORMOSANUS*	PERCENT OTHER PARASITES
LLA	21 (643)	42.9 (70.3)	42.9 (14.5)	14.3 (14.8)
LLB	50 (599)	26.0 (35.9)	14.0 (24.9)	60.0 (38.4)
NCA	40 (1,138)	37.5 (67.8)	12.5 (13.1)	50.0 (19.2)
OCR	22 (95)	100.0 (100.0)	0.0 (0.0)	0.0 (0.0)
SID	18 (968)	88.9 (89.8)	5.6 (6.5)	5.6 (3.5)
SIU	71 (771)	69.0 (88.7)	26.8 (6.7)	4.2 (4.5)
USR	52 (530)	69.2 (79.2)	21.1 (13.2)	9.6 (7.5)

[a] LLA, -B=Lake Landa sample areas A and B; NCA=New Channel Reach area A; OCR=Old Channel Reach; SID= Spring Island Downstream, SIU=Spring Island Upstream, USR=Upper Spring Run

5.0 FISH INFECTION PREVALENCE

5.1 Methods

Other pieces of the parasitic puzzle are the mechanisms and rate of transmittal of infection from *M. tuberculatus* to *E. fonticola*. While the levels of drifting cercariae in the water column can be quantified, little information is available on how these values relate to accumulation in wild *E. fonticola*. Previous researchers (Cantu, et al., 2013) have had difficulty in finding a relationship between these parameters. Consequently, in addition to performing studies on the host snails and drifting parasites, an effort was made in 2014 to quantify parasite concentrations in the gills of wild *E. fonticola*.

Previous data have shown infection rates in *E. fonticola* to be extremely variable and little data have been collected in recent years, despite the suggestion that drifting parasite concentrations are declining in some areas (Johnson, et al., 2012). To investigate this with minimal impact, gills of *E. fonticola* previously collected for HCP applied research fecundity studies were examined under microscopes and recently encysted *C. formosanus* metacercariae were counted. BIO- WEST biologists were trained by SMARC experts to identify encysted metacercariae of *C. formosanus* via microscopy of *E. fonticola* gill arches (Figure 11, Figure 12).

Recently encysted metacercariae (those with visible eye spots, *sensu* Cantu, et al. 2013) were the primary data desired, as these could be representative of water column cercariae and accumulation rates. Older encysted metacercariae were also counted, although as the age since encystment increases the ability to identify and classify cysts is reduced. Though researchers often extrapolate the number of cysts via various subsampling methodologies (Cantu, et al., 2013; Fleming, et al., 2011), complete enumeration (all arches observed on both sides of each fish) was used initially in 2014 in this study to reduce error. Examination of empirical data from 2014 supported that extrapolation using counts from one complete side was reasonably accurate (though further subsampling was shown to reduce accuracy), thus this procedure was adopted to increase efficiency in 2015. Only darters collected from the New Channel (RVP) and Old Channel (OCR) sites were counted in 2014, since these sites were also sampled for drifting cercariae as part of monitoring efforts. No correlation with drifting cercariae estimates was found. However, to provide a more complete picture of *E. fonticola* infection, fish sample from an additional site were counted in 2015.

Figure 11. BIO-WEST researchers receiving instruction from San Marcos Aquatic Resource Center (SMARC) staff on identification and classification of parasite metacercariae encysted in *Etheostoma fonticola* gills.

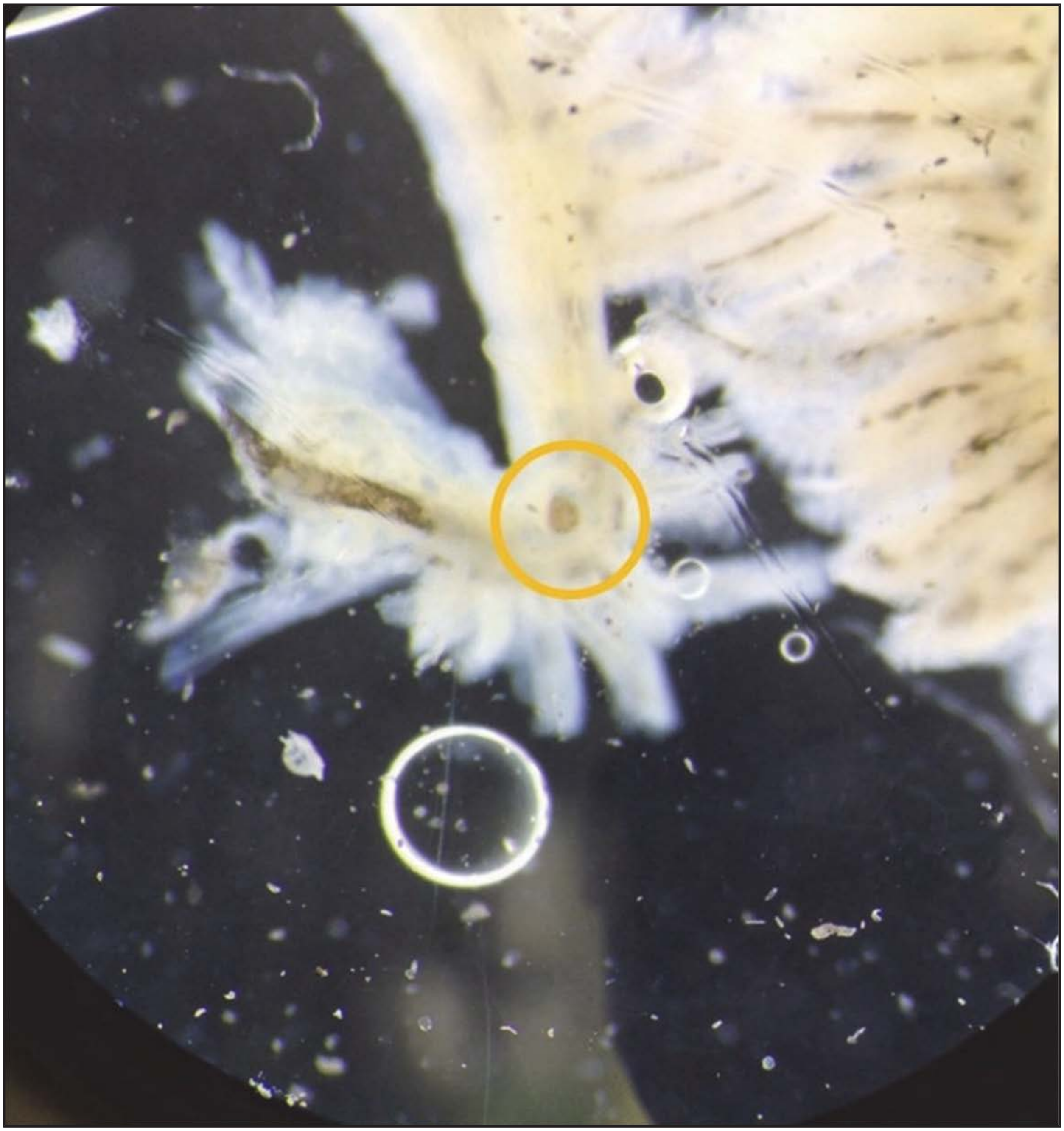


Figure 12. Dissected *Etheostoma fonticola* gill arch with encysted *Centrocestus formosanus* metacercariae (outlined by yellow circle).

5.2 Results and Discussion

Encysted parasite counts ranged from 2 to 52 per fish, with an overall mean of 17 parasites per infected fish in the New Channel (n = 25 infected). Encysted parasite counts in the Old Channel (OCR) ranged from 2 to 96 per fish, with an overall mean of 26 parasites per infected fish (n =23 infected). Encysted parasite counts in the Upper Spring Run (USR) ranged from 2 to 36 per fish, with a mean of 5 parasites per infected fish (n=20 infected). Mean values of both recently encysted and total encysted metacercariae in *E. fonticola* sampled were well under levels that are considered threatening to the species (Table 5).

Previous studies have concluded that approximately 800 or more encysted metacercariae are necessary to cause mortality in *E. fonticola* (Mitchell, et al., 2000), and laboratory experiments concluded that adult *E. fonticola* can survive accumulation of more than 600 parasites during an 8-h trial, while over 1,000 caused mortality in the same time period (McDonald, et al., 2006). It should be noted, however, that McDonald et al. (2006) also found that the lethal effects of metacercariae were correlated positively with fish length, and that an average of only 60.2 metacercariae caused mortality in larval *E. fonticola*.

It is apparent that there are mechanisms or processes that interfere with the transmittal of *C. formosanus* parasites to *E. fonticola*, such that the relationship of those parameters is less likely to be simply linear and easily defined. The benthic nature of *E. fonticola* and their tendency to use vegetative cover likely affect the ability of cercariae to encounter *E. fonticola* to varying degrees. It is also important to note that fish with infection levels high enough to significantly impact their behavior or survival may constitute an unobservable state. In other words, severely infected fish may have already been removed from the sample area through death or heightened susceptibility to predation, thus we cannot capture them and they are not included in infection estimates. We found that 96% of the fish from NCR and 100% from the OCR were infected, compared to 64% from USR. Relative infection rates of USR and NCR appear to reflect a similar pattern to snail infection, with higher rates in NCR and the lowest rates in USR. It is interesting to note, however, that OCR departs from this in that darter infection rates are high, while snail infection rates are low. This demonstrates that fish in OCR are being infected by parasites that are released in LL and drift down.

Table 5. **Summary of recently encysted *Centrocestus formosanus* metacercariae observed in *Etheostoma fonticola* gills at three locations on two sampling occasions in 2014, as well as mean (±SE) of the total number of encysted metacercariae. Location sample size are reported as (number of individuals recently infected, *number of individuals exhibiting old and recent infection*, total number of individuals sampled).**

RESULT	SAMPLING REACH[a]					
	NCR 21 FEB (10, *10*, **11**)	**NCR 23 MAY** (15, *16*, **16**)	**OCR 21 FEB** (11, *11*, **11**)	**OCR 23 MAY** (12, *14*, **14**)	**USR 21 FEB** (12, *13*, **18**)	**USR 23 MAY** (8, *12*, **21**)
MIN RECENT	2	2	6	2	2	2
MAX RECENT	50	52	96	64	36	4
MEAN RECENT (±SE)	16 (±4.2)	17 (±4.0)	33 (±7.0)	18 (±6.7)	6 (±2.7)	3 (±0.4)
MEAN TOTAL (±SE)	40 (±11.6)	47 (±13.6)	55 (±10.3)	30 (±7.7)	12 (±5.6)	4 (±0.5)

[a] NCR=New Channel Reach, OCR=Old Channel Reach, USR=Upper Spring Run Reach

6.0 SNAIL GENETICS AND MORPHOMETRICS

6.1 Introduction

Snail hosts such as *M. tuberculatus* are known to evolve and adapt rapidly, due in part to parthenogenetic reproduction, making them successful invaders in many environments. Because the species is parthenogenetic, the individuals in introduced populations do not differ genetically from every other individual, as with most species, but exist in the form of one or more genetic clones, each from a different geographic source. As infection data collected and analyzed by BIO-WEST has already shown, the distribution of highly infected snails occurs in patches, rather than being uniformly or randomly dispersed. One possible explanatory mechanism for this patchiness could be that the different clones prefer different microhabitats, and vary from other clones in their susceptibility to parasite infection.

In population and evolutionary biology, different lineages and subpopulations are identified by their genetics (DNA analysis) or by morphometrics analysis (the measurement of physical traits often augmented by the calculation of ratios and other metrics). These methods are used by taxonomists to define species, and can be scaled to delineate other hierarchical divisions. Identification of such traits would allow us to separate genotypically distinct clones of live snails by simple morphometric features or measurements. Live snails thus sorted into groups by morphometric criteria would allow us to then retrospectively determine patterns of micro-habitat associations for clones and also patterns of parasite susceptibility among the genetic clones, thus dramatically simplifying issues associated with mitigating the impact of the parasites brought in by the snails. Such simple means of assigning live snails to genetic clones would also allow us

to perform experiments on the clones in attempts to determine if there are differences between clones regarding low temperature tolerance, etc., which would simplify management issues associated with the recent spread of the snail into surface-fed waters.

After many hours of experimentation and study, about 40 anatomical measurements of the snail shell, and ratios based on these measurements, were selected as traits that potentially represent consistent patterns of low morphometric variation within a genetic clone, but potentially high morphometric variation between clones. To summarize, the use of genetic and morphological data along with parasite infection data *from the same individuals* will allow investigation into the underlying ecological processes producing the observed aggregations of highly infected snails. Discovery of these processes will potentially open the door to more efficient and targeted parasite-monitoring and management strategies.

6.2 Methods

6.2.1 *Morphometric analysis*

Snails are collected from various sites of interest and fixed in plastic containers of 95% EtOH marked with source locality codes. Prior to photographing, snails are individually scrubbed with brushes to remove silt, periphyton, and other accumulations. Each cleaned snail is then visually inspected and notes are recorded regarding color patterns and surface features such as grooves, ridges, and sculpturing, as well as subjective aspects of shape. Each snail is pressed into the surface of a block of modeling clay using the following orientation standards:

- The snail is positioned on a block of modeling clay close to a millimeter ruler also mounted on the block (Figure 13).
- The snail is rotated on its long axis (the columellar axis) until the lips of the apertural opening are parallel with the horizontal plane, as evidenced by the anal sulcus (Figure 13) pointing directly up.
- The tilt of the snail is then adjusted on the block such that the columellar axis (Figure 13) is horizontal, which forces it to be perpendicular to the optical axis of the camera lens.

Each snail is photographed with a Casio EX-FH25 digital camera mounted on a stable tripod with optical axis vertical and oriented directly over the middle of a snail. Lighting is provided by three LED bulbs having a “natural daylight” spectrum, a thermal color of 6500 °K, and arranged so as to minimize shadows. Unique serial numbers assigned by the camera to each digital photograph are then recorded and linked in a logbook with other data recorded for that snail, such as date of collection, site locality of collection, etc. After a snail has been described and photographed, it is set aside for DNA analysis, during which the shell is destroyed. Appendix 1 lists and explains the raw measurements collected on each snail and stored under the accession code for each snail. See Figure 14 for a visual key to the anatomical landmarks for the raw morphometric variables.

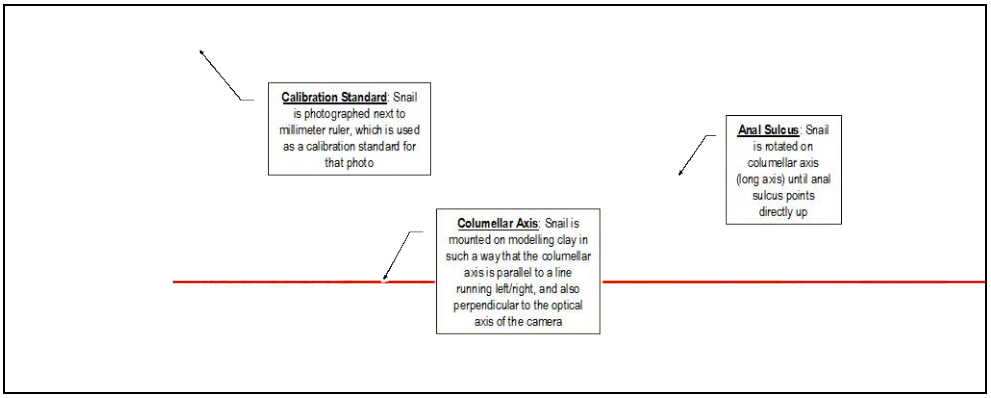


Figure 13. Orientation of snail on clay block prior to photography.

The cropped photographs of the snails were imported individually into a Windows-based program called Digimizer (v4.5.2; by MedCalc Software bvba, Acacialaan 22, 8400 Ostend, Belgium). Digimizer allows the user to import a photograph of a subject that includes a calibration reference that will be used to establish the units for its measuring tools. It provides several useful types of measurement tools that can be easily deployed once the calibration tool has been calibrated to the calibration reference in the photograph. The program also allows the user to define code names to each incidence of the tools and establish a data template that can then be applied to successive snail photographs. Figure 15 shows an example of a snail photograph and the Measurement List containing the variable codes associated with each tool deployed on the photograph.

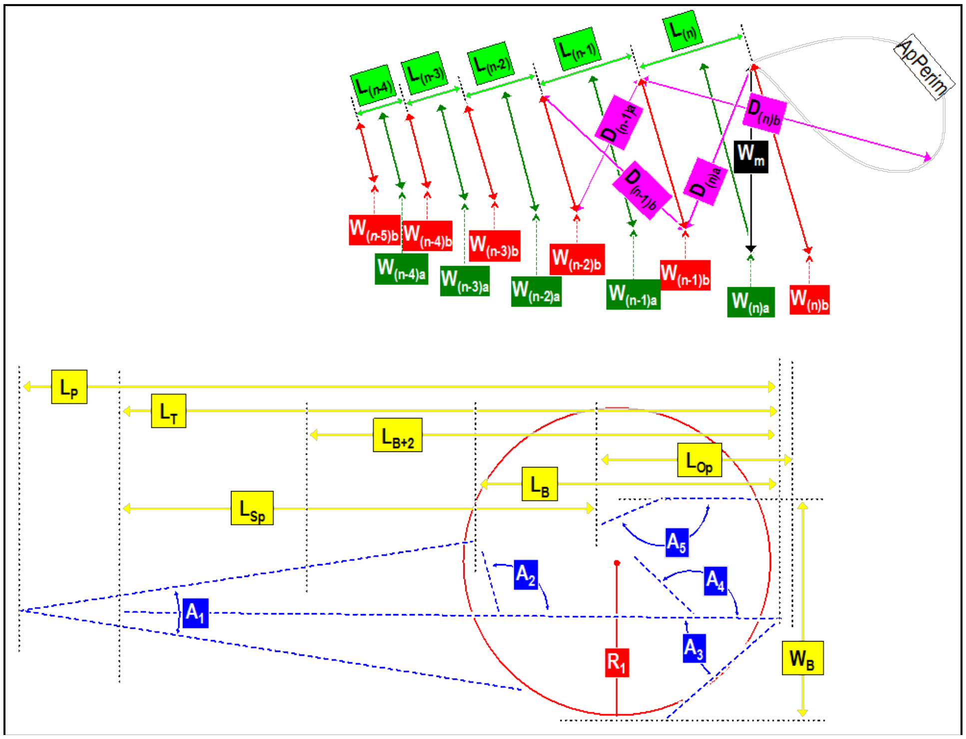


Figure 14. Anatomical key to morphometric variables.

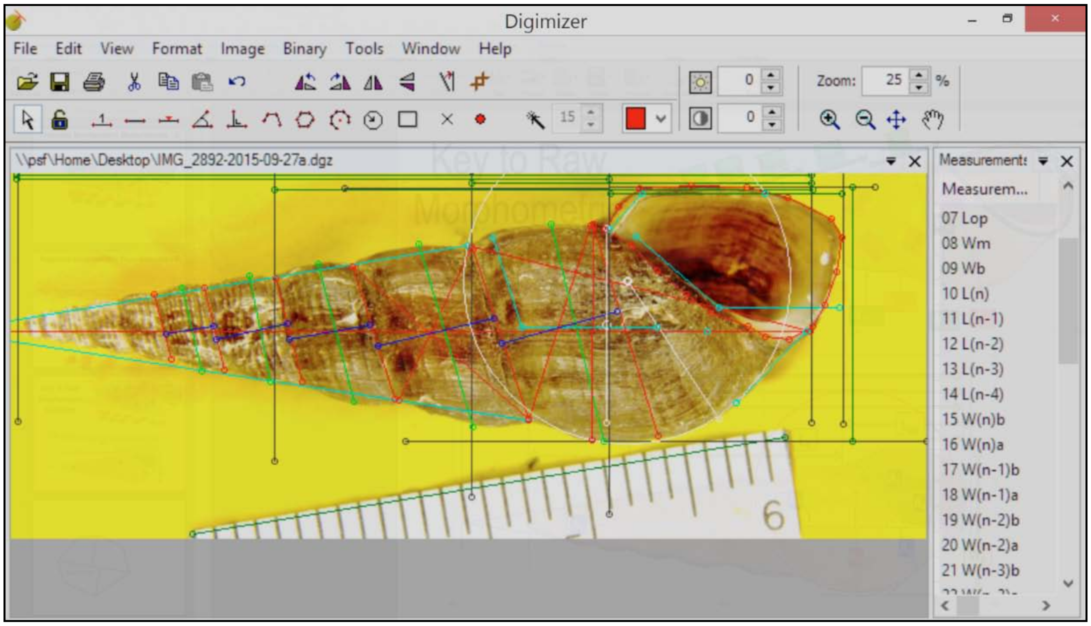


Figure 15. Screen shot from Digimizer showing photograph of a cropped snail and the scrollable Measurement List of variables associated with each tool.

As new data are entered into the master database, an array of synthetic variables is generated for each snail, with most of the synthetic variables based on ratios of two or more raw variables. These synthetic variables are designed to eliminate the dependence of the raw variables upon snail length, and are more likely to show variation related to genetic expression unrelated to growth. Formulae for these synthetic variables are described in Appendix 2. Additional synthetic variables may be added as intuition brings them to our attention as potentially useful.

6.2.2 Genetic Analysis

Snail DNA was isolated using a modified QIAGEN Gentra® Puregene® DNA purification protocol for Mouse Tail Tissue following manufacturers protocol. After DNA isolation and purification, all samples were diagnosed for successful isolation of high molecular weight DNA by agarose gel electrophoresis using a 1% Agarose gel and 0.5x TBE Buffer solution. A 5 µL aliquot of purified DNA from each snail sampled was mixed with 3 µL of loading dye and placed into a lane well in the agarose gel. The electrophoretic reaction was allowed to resolve at 91 volts for 35 minutes. After this time, the gel was viewed under high intensity UV light to determine if high molecular weight DNA was successfully extracted and purified.

Isolated DNA was subjected to amplification of desired markers using Polymerase Chain Reaction (PCR) procedures. All PCR amplifications followed standard protocols for a 25 µL reaction. Each reaction consisted of 21.125 µL of Nuclease Free H_2O, 0.25 µL of snail DNA, 2.5 µL GenScript 10x Taq Buffer, 0.25µL dntps (10mM each), 0.25 µL forward primer, 0.25 µL reverse primer, 0.25 µL BSA (400 ng/µL) and 0.125 µL GenScript Taq Polymerase (5u/µL). The cytochrome oxidase 1 mitochondrial gene (CO1) was amplified using HCO 2198 and LCO 1490 primers. The nuclear microsatellite loci were amplified using labelled Locus 1 F/R, Locus 9 F/R and Locus 10 F/R primers. The mtDNA CO1 locus was amplified using the following protocol: Initial denaturation: 95°C (60 sec), then 35 cycles of 95°C (30 sec), 40°C (60 sec), 72°C (60 sec) and a final elongation

at 72°C (5 mins). The nuclear microsatellite loci were amplified using the following protocol: Initial denaturation: 94°C (60 sec), then 35 cycles of 94°C (30 sec), 52°C (40 sec), 72°C (60 sec) and a final elongation at 72°C (10 mins). All amplicons were resolved on 2% agarose gels to determine if amplification was successful (Figure 17). After amplification, the mtDNA CO1 sequences were cleaned up following standard PCR product clean-up protocols and sequenced using an Applied Biosystems (ABI) 3500 Genetic Analyzer. All sequences were aligned and edited using Geneious© Bioinformatics Software. The CO1 reference sequence used during alignment and editing was a 658 bp fragment reported as Malaysian *Melanoides tuberculatus* Haplotype H7 (NCBI Accession: KP284136). After alignment and editing, the National Center for Biotechnology Information (NCBI) GenBank Basic Local Alignment Search Tool (BLAST) was used to compare the results to known *M. tuberculatus* CO1haplotypes (unique sequences) accessioned to NCBI databases by other researchers from around the world. The microsatellite loci were analyzed via a capillary electrophoresis.

6.3 Results

6.3.1 Morphometrics

To date, analysis of morphometric data is in the provisional stages and has only been attempted with limited data. Initial results are encouraging. The data were first standardized using a Wisconsin double standardization procedure as implemented in R v. 3.0.3 via the VEGAN R package. Subsequently, a partitioning around mediods (PAM) method was applied to estimate the number of clusters discernible in the data using the variables. Initial analysis on a limited set of data, and not yet including all variables eventually developed for use in this project, included *M. tuberculatus* from The Comal (n=9), San Marcos (n=2), and two outgroup populations: San Felipe Springs (Del Rio, TX, n=5) and Fort Clark Springs (Fort Clark, TX, n=3). These outgroup individuals are important in analysis of morphological data (as well as genetic data), their primary purpose being to properly scale inter- and intra-population variability for the taxa being studied.

The analysis effectively separated the Comal snails from all other snails, which is very encouraging given the small sample size in this analysis (Figure 16). Additional data is being compiled from samples taken using a design specifically for evaluating morphological and clonal variation in the Comal system and its relationship to parasite issues. Parasite infection and genetic data are being collected on the same individuals to allow analysis of the inter-relationships of these parameters.

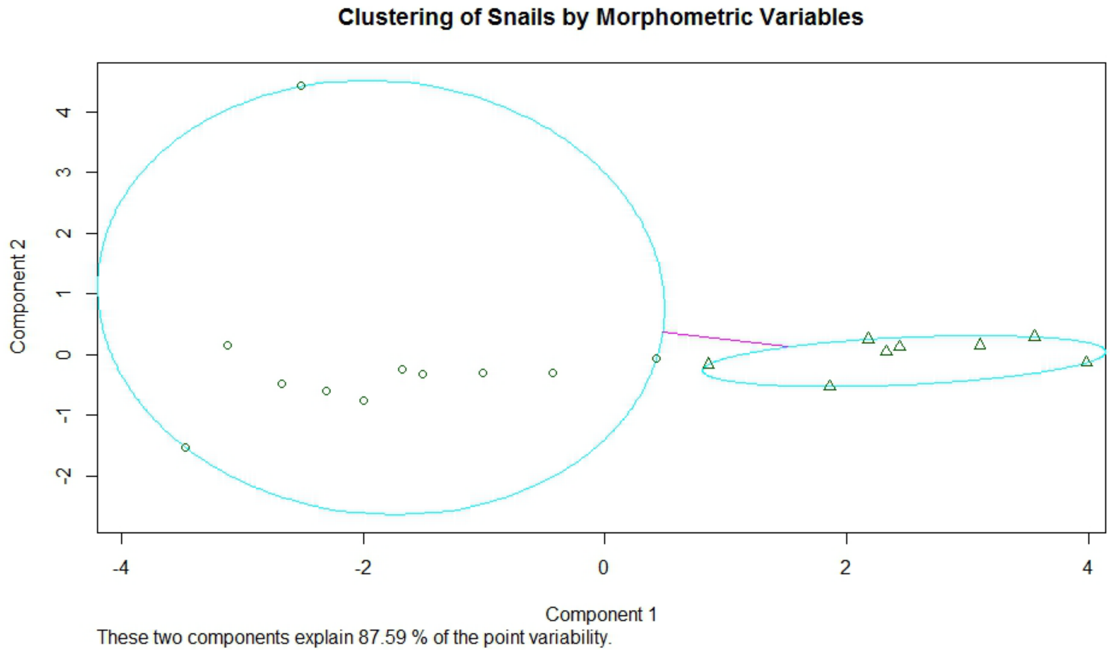


Figure 16. Results of initial trial analysis of multivariate morphological M. tuberculatus data. Even in this very limited data set, two clusters are clearly supported: snails from a sample in the Comal, and those from other outgroup drainages.

6.3.2 Genetics

Initial trials of mitochondrial DNA cytochrome oxidase subunit 1 gene (CO1) analysis consisted of 649 base pair sequences isolated from 12 individual snails (6 from the Comal River, 4 from San Felipe Springs, Del Rio, Texas, and 2 from Las Moras Springs, Fort Clark, Texas). The CO1 sequences were identical for all 12 individuals and were compared against known *M. tuberculatus* haplotypes available from the NCBI database. All sequences were found to be identical to a 658 base pair fragment isolated from Malaysian *Melanoides tuberculatus* Haplotype H9 (NCBI accession number KP284138.1) with a max score of 1153, 96% Query Coverage, 0.0 E-Value and 100% identity (Figure 18).

Secondary trials using 305 base pair sequences from a larger sample size of 90 snails yielded identical results. The shorter CO1 sequence length was used as a preliminary assessment of possible haplotype identity as the 90 snail sequences have not yet been fully edited. After the most recent sequencing attempt, 115 sequences that exceeded 600 base pairs have been isolated; however before any reasonable conclusions can be drawn, these sequences need to be edited to eliminate ambiguities in sequence data.

Initial trials of capillary electrophoretic analysis of variable nuclear DNA microsatellite genes consisted of locus 1, locus 9 and locus 10 isolates from 8 individual snails. Although most of the reactions did not succeed, there were 3 reactions that indicated two possible genotypes. There genotypic results among the Comal River snails consisted of one individual homozygous for the microsatellite fragment of 260 base pairs (includes labelled primer) in length and another that was heterozygous (Figure 19). The heterozygous individual had the 260 bp allele but also possessed a

280 bp allele. Complications in running nested PCR reactions to apply a label to microsatellite DNA primers were suspected to be the cause for inconsistent results and lack of success in yielding a more robust and reliable dataset. Additional trials are currently underway with factory labelled forward primers.

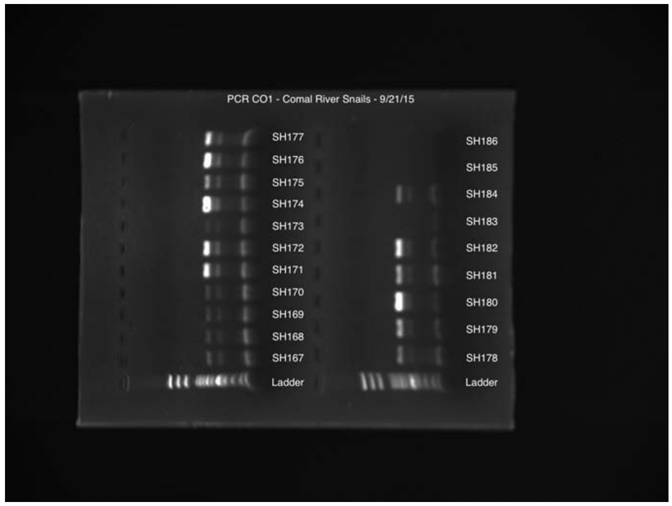


Figure 17. Visualization of polymerase chain reaction amplification of cytochrome oxidase 1 mitochondrial gene fragments via agarose gel electrophoresis.

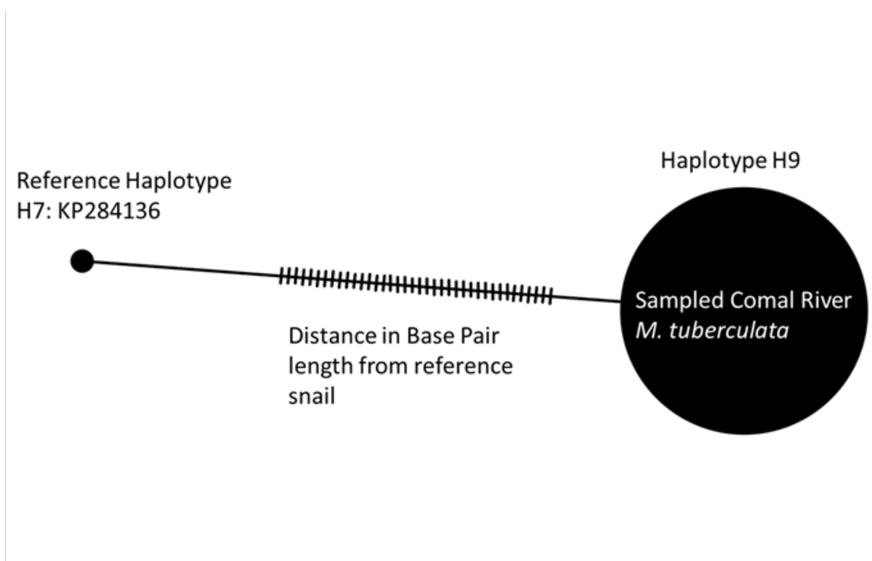


Figure 18. Haplotype network illustrating the number of nucleotide substitutions between the haplotype most similar to data from Comal M. tuberculatus and the next closest CO1 haplotype in NCBI GenBank. Reference haplotypes H7 and H9 differ by 38 substitutions.

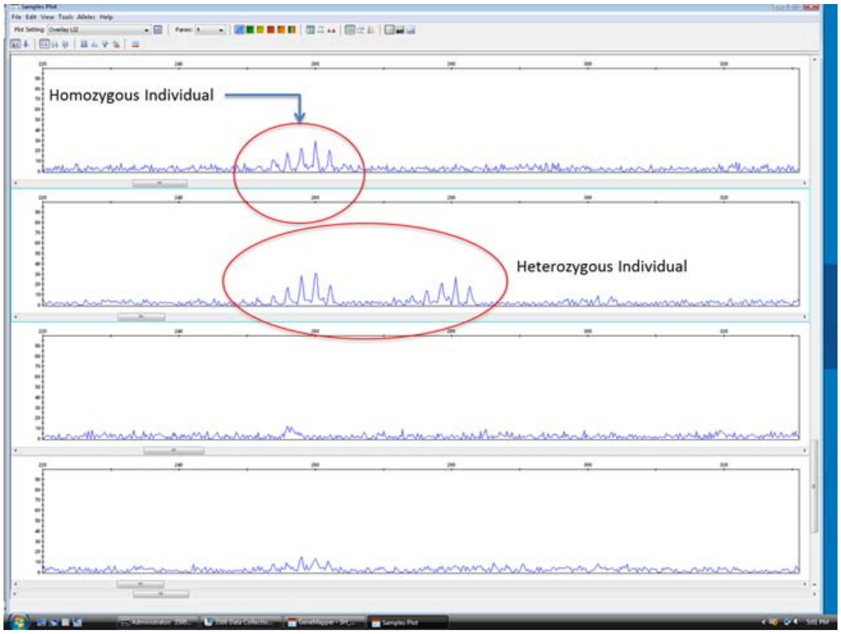


Figure 19. Example of preliminary microsatellite results. Presence of heterozygosity in the population suggests the presence of genetic variation that can be used to differentiate among groups of individuals.

6.4 DISCUSSION

While this line of investigation is not yet completed, great strides were taken in 2015 to conduct background research, methodological development and preliminary trials. This represents a monumental collaborative effort of BIO-WEST and Texas State University researchers, and will represent a great contribution to both academic understanding of these parasite – host relationships and information for the development of management techniques for this unique problem.

The methods developed in these 2015 preliminary trials will contribute greatly to our ability to investigate the management possibilities that may lie in other aspects of parasite/host biology, such as investigations into the avian hosts of the gill parasite. The final results will determine if there is sufficient variation in mitochondrial DNA sequences to be informative on our project scale. If not, this line of inquiry will be discontinued in 2016 in favor of increasing the number other genetic markers such as microsatellite loci.

7.0 SUMMARY AND CONCLUSIONS

Based on observations and previous studies in 2013 and 2014, various data were collected in 2015 to develop a more refined understanding the dynamics of *C. formosanus* and its impact on *E. fonticola* in the Comal system. Due to the complex life cycle of this particular parasite, which involves multiple hosts and life stages, data were collected on the first intermediate host (*M. tuberculatus*), the free-swimming cercariae, and the second intermediate host of concern (the *E. fonticola*). Collecting data on multiple life stages provides a more complete picture of parasite

dynamics within the system, and the data will be useful in assessing and mitigating effects of the parasite on *E. fonticola*, particularly during future low-flow scenarios.

Host snail (*M. tuberculatus*) distribution sampling results in 2015 were equivalent to previous years, with 41.6% of sites sampled being occupied by *M. tuberculatus*. *M. tuberculatus* remains abundant in Landa Lake and the New Channel above the old power plant, but is still relatively scarce in the OCR and lower portions of the NCR. Anecdotal observations made during dive and snorkel operations suggest *M. tuberculatus* to be abundant and ubiquitous in the majority of the Comal system, with the previously mentioned exceptions. Density estimates of snail hosts in 2015 appear to be lower at first glance, however this was undoubtedly affected by structural changes in some sample areas used in previous years resulting from construction projects in 2015. Due simply to chance, this happened to prevent sampling of some of the formerly most dense snail populations. The upper bound of density values observed is still similar in magnitude to previous years' samples. Changes in size-class structure within sampling reaches were observed in 2015 although mean snail length has remained similar across years. Increased frequency of larger snails (able to shed greater numbers of parasite cercariae) was apparent in some areas, and shifting of observed size class data in the NCR towards a more bimodal state. This form has been consistently observed in the USR since 2013, and may represent an equilibrium state consequent of biological processes. Continued observation of these populations will be important to relate these patterns to underlying causes and their effects on the host/parasite dynamic.

Estimates of density of drifting parasite cercariae in the water column at all three sampling sites were different in 2015 relative to comparable 2014 samples. Overall, observed 2015 densities were lower and more stable over time than in 2014, especially in the NCR and RVP, where large spikes in density observed in summer 2014 were lacking in 2015. Given that cercariae have been shown to survive up to 130 hours (>5 days) at 20°C (Lee & Lo, 1996) and are known to be present as far downstream as the confluence with the Guadalupe River under normal flow conditions (Fleming, et al., 2011), these data could support the supposition that higher flows in 2015 resulted in shorter residency time of cercariae (faster flushing of the system), preventing the cercariae from amassing in higher concentrations as observed in 2014. The alternative would be that in 2014, an underlying mechanism caused snail hosts to release more cercariae into the environment. Given the observations of snail abundance and infection rates in both years, this seems a less likely hypothesis. Additional year's data will provide enough at some point to test these hypotheses statistically, presuming observations can be made under sufficiently varying conditions.

Infection prevalence of *M. tuberculatus* with *C. formosanus* in 2015 remain higher than previously reported in the literature (Mitchell, et al., 2000; Tolley-Jordan & Owen, 2008).. Subsamples from within such areas were highly variable, suggesting that microhabitat scale factors may be important in determining snail infection. None of the *M. tuberculatus* collected from the OCR in 2014 or 2015 were infected with *C. formosanus*. Infection rates of darters in this reach, however, are among the highest observed. Though 2015 drifting cercariae densities in this reach seem low in relation to the other reaches, they are still non-trivial. Given a relatively constant base flow of 60 cfs, leading to a rough estimate through extrapolation of 3,228 cercariae in the volume of water that passes in one second through the channel (which is also substantially smaller than the other sampling sites). Our data suggest that most cercariae

production occurs upstream of the OCR in Landa Lake and few, if any, cercariae are likely produced from snails occupying the OCR. The USR, though it exhibited much higher snail host infection than the OCR, had much lower infection rates for fountain darters. These data therefore present that the OCR and the USR may represent areas of management opportunity for the gill parasite problem. Lowered darter infection rates in the USR are no doubt a result of the additive nature of the drifting cercariae as they drift downstream through the system, as long as flows is sufficient parasites should be quickly removed downstream from this area. If a barrier to cercariae transmission in the area of the OCR culverts could be designed with reasonable effectiveness, impact could be virtually eliminated in this reach.

8.0 MONITORING AND RESEARCH PROPOSED FOR 2016

Continuation of the distribution and density monitoring of both host snail and drifting cercariae is suggested for 2016, as in its present state it represents a cost effective means of monitoring temporal trends, such as those reported in 2015, of multiple species within the system and provide additional insight into the mechanisms behind such trends. As conditions in the system change over time, the utility of these data will only increase in its ability to inform management. Continued monitoring of parasite prevalence in the host snail should be included in 2016 activities as well, as it also represents a cost efficient monitoring method.

The foundation for incorporating advanced genetic and morphometric analysis methods was laid in 2015. The methods have been tested and refined, such that 2015 represents the bulk of the investment necessary to apply these tools. The presence of microsatellite marker variation and ability to separate host snail populations based on morphology are very promising results, especially when it is considered that these results are from limited preliminary data. The remaining samples from this 2015 study will be analyzed, allowing the results of this research to inform 2016 activities.

A recommended addition to 2016 activities is the reexamination of definitive hosts (birds) and host dynamics. Little information is available on avian host dynamics, and they may represent a unique management opportunity if one allows the perspective that they are the least numerous organism involved. Understanding more about the dynamics of the avian-to-snail infection pathway could certainly be beneficial for parasite management under all flow scenarios. Given the aggregated nature of highly infected populations, identification of definitive hosts would allow analysis of habitat conditions that promote transmittal of the parasite to snails in these areas. If such habitat features are determined, they could be viable opportunities for mitigation activities. Genetic markers developed and tested as part of 2015 research activities will allow determination of parasite infection of avian hosts without harming the birds, allowing for a larger sample size than would likely be permitted otherwise. This will also allow the tracking of individual infections over time. Preliminary plans for this research have been outlined by BIO-WEST in collaboration with leading parasitology and avian ecology researchers at Texas State University – San Marcos, with a focus on efficiency and using advanced methods in ecological study in the search for management opportunity.

Finally, the abundance of another exotic thiarid snail, *Tarebia granifera*, in the Comal system is surprising. Though this species has only rarely been reported to exhibit infection by *C. formosanus,* it has been known to occur in other countries. Currently, there is a paucity of data on trematode infection in this species in the study area. Likewise, there is little information on the many other species of parasites that have been found in the system as well as in fountain darters in the Comal system. It may be useful to consider gathering limited baseline data on these species proactively to help guide any future HCP concerns as we approach the next stages.

9.0 REFERENCES

Cantu, V., Brandt, T. M. & Arsuffi, T. L., 2013. An evaluation of three sampling methods to monitor a digenetic trematode *Centrocestus formosanus* in a spring-fed ecosystem. *Parasitology,* pp. 1–7.

EARIP, 2011. Edwards Aquifer Recovery Implementation Program Habitat Conservation Plan. *Prepared for the Edwards Aquifer Recovery Implementation Program.*

Fleming, P. F., Huffman, D. G., Bonner, T. H. & Brandt, T. M., 2011. Metacercarial distribution of *Centrocestus formosanus* among fish hosts in the Guadalupe River drainage of Texas. *Texas Journal of Aquatic Animal Health,* Volume 23, pp. 117–124.

Johnson, M. S. et al., 2012. Fluctuations in densities of the invasive gill parasite *Centrocestus formosanus* (Trematoda: Heterophyidae) in the Comal River, Comal County, Texas, U.S.A. *Journal of Parasitology,* 98(1), pp. 111–116.

Lee, C. T. & Lo, K. M., 1996. Pattern of emergence and the effects of temperature and light on the emergence and survival of Heterophyid cercariae (*Centrocestus formosanus* and *Haplorchis pumilio*). *The Journal of Parasitology,* 82(2), pp. 347–350.

McDonald, D. L., Brandt, T. M. & Trevino, G. H., 2006. Size susceptibility to treamtode-induced mortality in the endangered fountain darter (*Etheostoma fonticola*). *Journal of Freshwater Ecology,* 21(2), pp. 293–299.

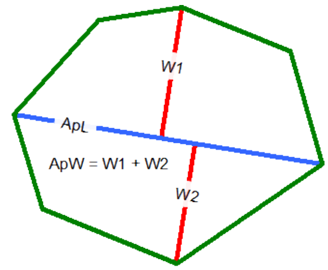
Mitchell, A. J. et al., 2000. Prevalence and pathogenicity of a heterophyid trematode infecting the gills of an endangered fish *Etheostoma fonticola* in two Texas spring fed rivers. *Journal of Aquatic Animal Health,* Volume 12, pp. 283–289.

Tolley-Jordan, L. R. & Owen, J. M., 2008. Habitat influences snail community structure and trematode infection levels in a spring-fed river, Texas, USA. *Hydrobiologia,* Volume 600, pp. 29–40.

APPENDIX A: RAW MORPHOMETRIC VARIABLES RECORDED FOR EACH SNAIL

Sequence	Code	Description
R01	Whorls	Number of Whorls; counted (or estimated) number of whorls (n).
R02	L_T	Total Length; length end-to-end along columellar axis for intact specimens (estimated for specimens with lost whorls).
R03	L_P	Projected Length; distance between point where lines forming angle A_1 intersect beyond the apex and a point representing the anterior end of the columella
R04	L_B	Body Length; distance parallel to columellar axis from dextral end of penultimate suture [$(n-1)^{th}$ suture] to a line perpendicular to the columellar axis and tangent to the extreme anterior end of aperture lip (estimated if chipped).
R05	L_{B+2}	Length of Body + 2 Whorls; same as BL, except extended two more whorls to dextral end of suture [$(n-3)^{th}$ suture].
R06	L_{Sp}	Length of Spire; distance between the extreme posterior end of first whorl (not counting protoconch; estimated if whorls are missing), and posterior margin of the anal sulcus (measured parallel to columellar axis).
R07	L_{Op}	Length of Opening; distance between posterior margin of anal sulcus to extreme anterior end of apertural lip, measured parallel to columellar axis. Compare with L_{Ap}.
R08	W_M	Maximum Width of Body Whorl; distance across body from terminus of last sutural groove near anal sulcus to opposite side of body whorl (ultimate, n^{th} whorl), measured perpendicular to columellar axis.
R09	W_B	Body Width; distance between two lines parallel to the columellar axis, one of which is tangent to the extreme dextral limit of shell, and the other of which is tangent to the extreme sinistral limit of shell.
R10	$L_{(n)}$	Length of Whorl n; (perpendicular to sutural grooves) of body whorl (ultimate, n^{th} whorl).
R11	$L_{(n-1)}$	Length of Whorl n-1; (perpendicular to sutural grooves) of penultimate whorl [$(n-1)^{th}$ whorl].
R12	$L_{(n-2)}$	Length of Whorl n-2; (perpendicular to sutural grooves) of antepenultimate whorl [$(n-2)^{th}$ whorl].
R13	$L_{(n-3)}$	Length of Whorl n-3; (perpendicular to sutural grooves) of whorl [$(n-3)^{th}$ whorl].
R14	$L_{(n-4)}$	Length of Whorl n-4; (perpendicular to sutural grooves) of whorl [$(n-4)^{th}$ whorl].
R15	$W_{(n)b}$	Sutural Width Anterior to Whorl n; distance from last sutural groove near anal sulcus to opposite side of body whorl (ultimate, n^{th} whorl), measured parallel to sutures.
R16	$W_{(n)a}$	Maximum Width of Whorl n; maximum distance across body whorl (ultimate, n^{th} whorl), measured parallel to sutures.
R17	$W_{(n-1)b}$	Sutural Width Anterior to Whorl n-1; minimum distance across penultimate suture, measured across posterior margin of body whorl (ultimate, n^{th} whorl).

R18	$W_{(n-1)a}$	Maximum Width of Whorl n-1; maximum distance across penultimate whorl [(n-1)th whorl], measured parallel to sutures.
R19	$W_{(n-2)b}$	Sutural Width Anterior to Whorl n-2; minimum distance across antepenultimate suture [(n-2)th suture].
R20	$W_{(n-2)a}$	Maximum Width of Whorl n-2; maximum distance across antepenultimate whorl [(n-2)th whorl], measured parallel to sutures.
R21	$W_{(n-3)b}$	Sutural Width Anterior to Whorl n-3; minimum distance across suture [(n-3)th suture].
R22	$W_{(n-3)a}$	Maximum Width of Whorl n-3; maximum distance across whorl [(n-3)th whorl], measured parallel to sutures.
R23	$W_{(n-4)b}$	Sutural Width Anterior to Whorl n-4; minimum distance across suture [(n-4)th suture].
R24	$W_{(n-4)a}$	Maximum Width of Whorl n-4; maximum distance across whorl [(n-4)th whorl], measured parallel to sutures.
R25	$W_{(n-5)b}$	Sutural Width Anterior to Whorl n-5; minimum distance across suture [(n-5)th suture].
R26	$D_{(n)a}$	Diagonal a of Body Whorl; diagonal distance across body whorl (ultimate, n^{th} whorl) from dextral end of last suture (ultimate, n^{th} suture) near anal sulcus to opposite (sinistral) end of penultimate suture [(n-1)th suture].
R27	$D_{(n)b}$	Diagonal b of Body Whorl; diagonal distance from dextral end of penultimate suture [(n-1)th suture] to anterior-most end of columella.
R28	$D_{(n-1)a}$	Diagonal a of Penultimate Whorl; diagonal distance from dextral end of penultimate suture [(n-1)th suture] to sinistral end of antepenultimate suture [(n-2)th suture].
R29	$D_{(n-1)b}$	Diagonal b of Penultimate Whorl; diagonal distance from dextral end of antepenultimate suture [(n-1)th suture] to sinistral end of penultimate suture [(n-1)th suture].
R30	A_1	Angle of Taper; acute angle between two lines, one of which intercepts the dextral ends of sutures $W_{(n-1)b}$ through $W_{(n-3)b}$ and the other intercepting the sinistral ends of these same sutures; the two lines converging posteriad.
R31	A_2	Angle 2; obtuse angle between the penultimate suture [$W_{(n-1)b}$] and the columellar axis.
R32	A_3	Angle 3; acute angle between a line along the columellar axis and a line along the sinistral edge of the shell between the extreme anterior end of the columella and the sinistral bulge of the body whorl.
R33	A_4	Angle 4; obtuse angle between a line along the columellar axis and a line along the parietal lip of the aperture.
R34	A_5	Angle 5; obtuse angle between a line parallel to the columellar axis and tangent to the dextral lip of the aperture, and a line following the apertural lip up from the anal sulcus.
R35	R_1	Radius of Body Whorl; radius of curvature of the sinistral margin of the body whorl (n^{th} whorl).
R36	ApArea	Aperture Area; area (mm^2) of the aperture outline (includes lips).
R37	ApPerim	Aperture Perimeter; perimeter of aperture outline (includes lips).
R38	L_{Ap}	Length of Aperture; maximum distance between any two points of the aperture outline (includes lips). Compare with L_{Op}.

R39	W_{Ap}	Width of Aperture; sum of longest perpendicular distance (W1) from long axis (ApL) of aperture outline to any anterior point on aperture outline, plus longest perpendicular distance (W2) between long axis (ApL) of aperture outline and any posterior point on aperture outline.	
R40	ApRnd	Roundness of Aperture; "index of roundness" of the apertural outline (including the lips), such that a perfectly round aperture would have an index value of 1.0, and very irregular or "unround" aperture margins would yield a value approaching zero. Calculated by $4\pi \times \text{Area}/\text{Perimeter}^2$.	

APPENDIX B: SYNTHETIC VARIABLE FORMULAE

S01, $\sqrt{L_T}$; S02, $\frac{W_{Ap}}{L_{Ap}}$, S03, $\frac{D_{(n)a}}{D_{(n)b}}$; S04, $\frac{D_{(n-1)a}}{D_{(n-1)b}}$; S05, $\frac{D_{(n)a}/D_{(n)b}}{D_{(n-1)a}/D_{(n-1)b}}$; S06, $\frac{L_{(n-1)}}{L_{(n)}}$; S07, $\frac{L_{(n-2)}}{L_{(n-1)}}$; S08, $\frac{L_{(n-3)}}{L_{(n-2)}}$; S09, $\frac{L_{(n-4)}}{L_{(n-3)}}$; S10, $\frac{W_{(n)b}}{W_{(n)a}}$; S11, $\frac{W_{(n-1)b}}{W_{(n-1)a}}$; S12, $\frac{W_{(n-2)b}}{W_{(n-2)a}}$; S13, $\frac{W_{(n-3)b}}{W_{(n-3)a}}$; S14, $\frac{W_{(n-1)b}}{W_{(n)b}}$; S15, $\frac{W_{(n-2)b}}{W_{(n-1)b}}$; S16, $\frac{W_{(n-3)b}}{W_{(n-2)b}}$; S17, $\frac{W_{(n-4)b}}{W_{(n-3)b}}$; S18, $\frac{L_{(n)}}{W_{(n)b}}$; S19, $\frac{L_{(n-1)}}{W_{(n-1)b}}$; S20, $\frac{L_{(n-2)}}{W_{(n-2)b}}$; S21, $\frac{L_{(n-3)}}{W_{(n-3)b}}$; S22, $\frac{L_{(n-4)}}{W_{(n-4)b}}$; S23, $\mathrm{Av}\left(\frac{L}{W_b}\right)$; S24, $\frac{W_{Ap}}{L_{(n)}}$; S25, $\frac{W_{Ap}}{D_{(n)a}}$; S26, $\frac{L_{(n-4)}}{L_{(n)}}$; S27, $\frac{W_{(n-5)b}}{W_{(n-1)b}}$;

S28, $\frac{\sum\left[L_{(n)}:L_{(n-4)}\right]}{\sum\left[W_{(n)a}:W_{(n-4)a}\right]}$; S29, $\frac{\left(W_{(n)b}-W_{(n-1)b}\right)}{W_{(n)b}}$; S30, $\frac{\left(W_{(n-1)b}-W_{(n-2)b}\right)}{W_{(n-1)b}}$;

S31, $\frac{\left(W_{(n-2)b}-W_{(n-3)b}\right)}{W_{(n-2)b}}$; S32, $\frac{\left(W_{(n-3)b}-W_{(n-4)b}\right)}{W_{(n-3)b}}$; S33, $\frac{\left(W_{(n-4)b}-W_{(n-5)b}\right)}{W_{(n-4)b}}$; S34, $\frac{\left(W_{(n)a}-W_{(n-1)a}\right)}{W_{(n)a}}$; S35, $\frac{\left(W_{(n-1)a}-W_{(n-2)a}\right)}{W_{(n-1)a}}$; S36, $\frac{\left(W_{(n-2)a}-W_{(n-3)a}\right)}{W_{(n-2)a}}$; S37, $\frac{\left(W_{(n-3)a}-W_{(n-4)a}\right)}{W_{(n-3)a}}$; S38, $\frac{\left(L_{(n)}-L_{(n-1)}\right)}{L_{(n)}}$; S39, $\frac{\left(L_{(n-1)}-L_{(n-2)}\right)}{L_{(n-1)}}$; S40, $\frac{\left(L_{(n-2)}-L_{(n-3)}\right)}{L_{(n-2)}}$; S41, $\frac{\left(L_{(n-3)}-L_{(n-4)}\right)}{L_{(n-3)}}$;

S42, Std Whl (#9) L; S43, Std Whl (#9) W; S44, $\frac{\text{Std Whl L*100}}{L_T}$; S45, $\frac{\text{Std Whl L}}{\text{Std Whl W}}$;

S46, $\frac{L_B}{L_{B+2}}$; and S47, $\frac{W_B}{L_B}$.

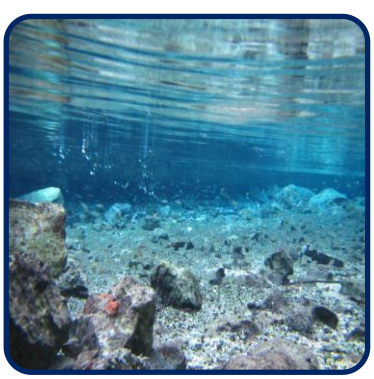
Appendix L7

2015 Final Report for Riffle Beetle Habitat Restoration Spring Run 3 and Landa Lake Shoreline

2015 Final Report for Riffle Beetle Habitat Restoration

Spring Run 3 and Landa Lake Shoreline

Date Submitted: November 13, 2015
Client: City of New Braunfels, Texas
Project Number: 13004.02



2015 Final Report for Riffle Beetle Habitat Restoration
Spring Run 3 and Landa Lake Shoreline

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November 6, 2015

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1 Executive Summary

This project supports the Edwards Aquifer Habitat Conservation Plan (HCP) which identified the need to maintain, improve and/or increase habitat of several endangered species, including the Comal Springs riffle beetle (*Heterelmis comalensis*), Peck's cave amphipod (*Stygobromus comalensis*), Comal Springs dryopid beetle (*Stygoparnus comalensis*), and fountain darter (*Etheostoma fonticola*). Comal Springs riffle beetles are found within 150 meters of spring outflows in gravel to cobble substrates with and without vegetation (Bowles et al. 2003).

Habitat restoration activities were conducted to benefit these species along Spring Run 3 and along the western shoreline of Landa Lake in New Braunfels, TX. Issues particular to the project area included encroachment by non-native vegetation, siltation around spring outflows, and erosion along the shoreline. Concepts to restore native shoreline habitat were identified by staff representatives from RPS Espey, BIO-WEST, Harkins Engineering Inc., United States Fish and Wildlife Service, Texas Parks and Wildlife Department and City of New Braunfels. A restoration work plan was developed (RPS Espey et. al 2013). Complementary work under a separate project suggested that reduction of fine sediment transport into the lake will benefit endangered beetles in their near-shore habitats.

The most effective restoration activity in 2015 was construction of additional shoreline erosion control measures. The erosion control sediment traps captured an estimated 4.2 cubic yards of sediment over the past calander year before sediment could enter Spring Run 3 or Landa Lake. Successful treatment the previous years resulted in a reduced stand of non-native elephant ear and allowed the use of limited mechanical removal of new plants in 2015. Shoreline areas were replanted with native grasses, sedges, as well as native trees, and all are having significant success. Non-native ligustrum trees were removed which allowed for more sunlight to penetrate some areas of the shoreline. The log and brush material from the ligustrum trees were utilized for on-site for construction of additional erosion control sediment traps. Planting and establishment of native ground cover and tree species were limited by the shallow rocky soils, wildlife and anthropogenic impacts, but was especially limited to the borders of the shoreline areas by the heavy canopy cover.

Future work should focus on: (1) maintaining existing sediment traps; (2) monitoring of riffle beetles in proximity to restoration areas with respect to silt; (3) consideration of planting a cool season cover; (4) reconnaissance survey to identify invasive exotics; and (4) reassess planting lists and site specific plantings to maximize beneficial shoreline restoration.

2 Introduction

The Edwards Aquifer Recovery Implementation Plan (EARIP) process led to development of the approved Habitat Conservation Plan (HCP) which identified the need to maintain, improve and/or increase habitat of several endangered species, including the Comal Springs riffle beetle (*Heterelmis comalensis*), that are found within 150 meters (492 feet) of spring outflows in gravel to cobble substrates with and without vegetation (Bowles et al. 2003). Concepts to restore native habitat were discussed and identified by RPS, BW, HEI, United States Fish and Wildlife Service (USFWS) staff, Texas Parks and Wildlife Department (TPWD) staff and City of New Braunfels (City) staff.

Additional monitoring studies are being completed to identify the range of riffle beetles, and results of those monitoring studies will be considered in future work at this site.

The project area encompassed Spring Run 3 and the adjacent 607 feet of western shoreline of Landa Lake (RPS 2013; **Error! Reference source not found.**). The project area did not change from 2014.

Figure 2.1 General location of project area (red box) and Comal River.

Issues particular to the project area included non-native vegetation, siltation around spring outflows, and erosion along the shoreline.

3 Project Area

3.1 Project Area Description

Landa Lake is located in New Braunfels, Texas, and is on the eastern edge of the Edwards Plateau. The shoreline in the project area is relatively steep, exhibiting grades between 20-40%, and the terrain is dominated by large limestone outcrops and steep slopes that create large hillside shelves (Figure 3.1).

Figure 3.1. General terrain of project area.

The soils of the project area are of the Eckrant-Rock outcrop complex (Batte et. al. 1984). They are a steep complex of shallow clayey soils and rock outcrops. Typically soils are very dark gray extremely stony clay limited to about 10 inches thick, thus the available rooting zone is shallow. The underlying layer is fractured limestone, which is also exposed as bare outcrops.

Riparian habitat along the shoreline is a heavily shaded woodland area with a combination of native and non-native flora. The native woodland vegetation includes anacua, live oak, black walnut, cedar elm, mexican buckeye, hackberry, and mountain laurel. The understory and ground cover of herbaceous species is sparse in the riparian zone and is mainly concentrated in

areas near the shoreline. Understory native vegetation species observed includes stinging nettle, false nettle, strangler daisy, Canada wildrye, streambed bristlegrass, switchgrass, bushy bluestem, iceplant, and wild onion.

Due to the steep terrain and of the shallow bedrock runoff is rapid and the available water capacity is very low. The soils themselves are moderatly alkaline and are calcareous throughout, with a high lime content in the soil profile which can cause nutrient unbalances that can limit the quality of forage (SCS Comal County, 1984). Deer grazing pressure, as well as disturbance from other wildlife, is high. Palatable species are browsed by deer. Other wildlife activities have also had an impact on establishing plugs survival in restoration areas (Figure 3.2). Due to the browsing, terrain, available water capacity, and shallow rooting zone, the potential for water erosion remains a severe on these soils.

Figure 3.2. Wildlife removal of a Cedar Sedge plug.

3.2 Restoration goals

Sedimentation along the shoreline was identified in the past as a risk for riffle beetle habitat reduction within the project area, and this is supported by no riffle beetles being found in silted areas (RPS Espey et al. 2014). Also, non-native plants, primarily elephant ear and japanese ligustrum, have replaced native vegetation in areas along the shoreline. Therefore, restoration

goals included: (1) removal and/or treatment of exotic vegetation; (2) construction and maintenance of erosion control structures; (3) revegetating the shoreline by planting native vegetation; and (4) sediment and vegetation monitoring.

The removal of ligustrum trees, chinese tallow, and chinaberry trees were retained in the restoration efforts. Ligustrums removed from the bank of Spring Run 3 were also used as raw materials to repair existing and make additional erosion control structures starting on February 18, 2015.

4 Restoration Site Work

The total length of the project area was approximately 1,105 feet, extending from the head of Spring Run 3 to a private property fence line on the western shoreline of Landa Lake. Restoration planting and erosion control activities continued to extend from the shoreline to approximately 45 feet up the hillside; planting lists from 2014 were refined and site specific planting plans were made in 2015 to attempt to increase overall plant survivability. Required maintenance and monitoring was conducted on all previous sites (Figure .1).

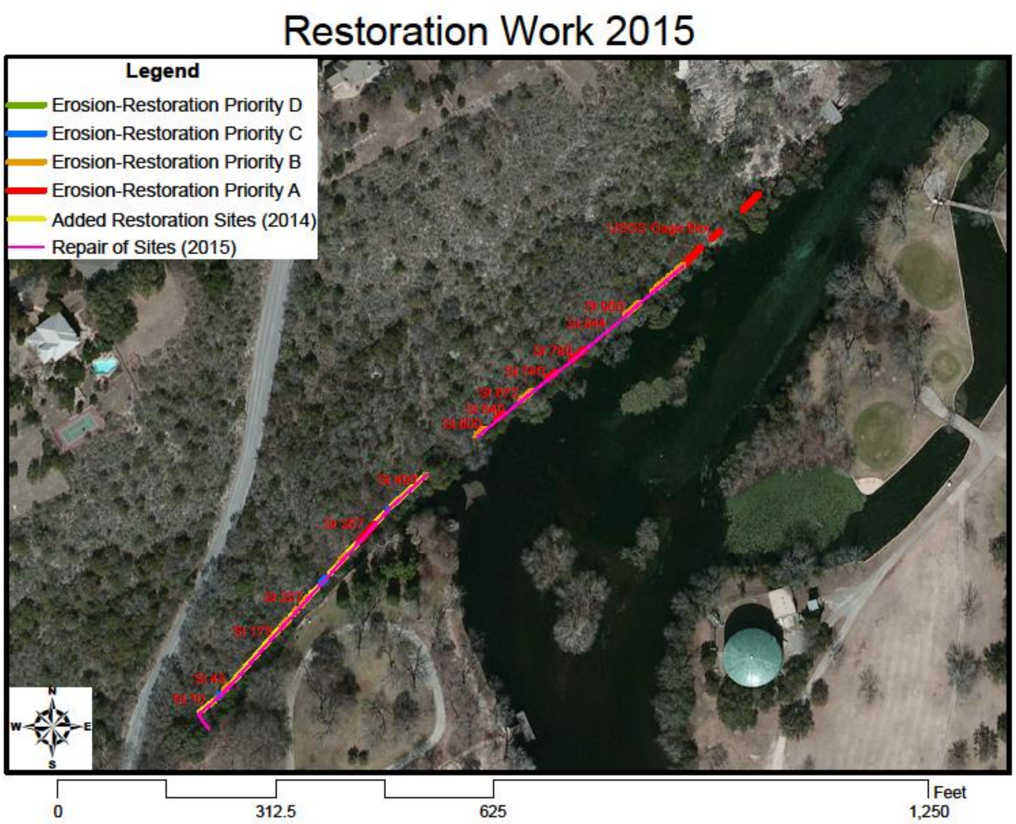


Figure 4.1. Map of locations of restoration activities in 2015

Signage that was originally erected in 2013 had deteriorated, and had to be replaced in 2015. Signage were placed at each pedestrian bridge, and a new sign was place at the springhead to further limit public access and reduce impact of foot traffic through the restoration areas (Figure 4.2). Despite the new signs, foot traffic continued. In April alone park visitors have torn out a new stacked rock wall at the springhead, torn down one sign, and disabled the irrigation system. These events have had impacts to both the restoration project area, as well as directly increasing sediment inputs to the springs themselves.

Figure 4.2. Signage to restrict public access to restoration area

Out of the 1,105 linear feet of shoreline within the project area, restoration efforts in 2015 were carried out in over 977 linear feet of shoreline. Erosion control structures made of natural materials sourced on-site were repaired or built in an orientatinon perpendicular to runoff pathways. Erosion control structures ranged from 3 feet long to 64 feet long and were placed along 740 feet of shoreline.

Riparian planting and monitoring were subsequently conducted in all restoration areas to determine the success and effectiveness of exotic species removal, riparian vegetation plantings, and erosion control measures.

4.1 Installation of Drip Irrigation System

Repairs the main supply line and to installation of a commercial grade drip irrigation system were started on March 30, 2015, and finished on April 2, 2015. One thousand feet of UV stabilized drip ½" drip line was installed along the erosion control structures. A series of quarter turn valves were added to the main line to allow park and restoration staff to isolate stubs or parts of the main in case of an irrigation system failure. Control and filtration equipment installed under the upstream bridge allowed the entire main to be shut down, as well as provided an inline pressure reducer and filter, to complete the drip irrigaton system. The system was monitored throughout the season to assure proper operation of the system, as well as the planted areas to verify the areas had soil moisture, but were not flooded.

4.2 Native Vegetation Planting Plans

On January 29, 2015 discussions were started with City of New Braunfels Watershed staff and Parks personnel, about identifying and proposing a plant species for 2015. An examination of existing native vegetation, native vegetation from nearby areas, and literature searches culminated in a list of species to be planted (Table 4.1). All vegetation plots installed in 2014 as well as the new sites for 2015 were planted.

Planting List 2015					
Common Name	**Scientific Name**	**Wetland Indicator Status**	**Sun**	**Life Cycle**	**Habitat**
Cedar Sedge	*Carex planostachys*	FACW	Shade	Perennial	Grows in shaded conditions in fast-draining soil, under deciduous or Juniper woodland.
Canada Wildrye	*Elymus canadensis*	FAC	Shade	Perennial	Exceptional seedling vigor. Is tolerant of drought and poor fertility. Grows along shaded stream banks.
Virignia wildrye	*Elymus virginicus*	FAC	Shade	Perennial	Prefers moist soils and heavier soil textures. Grows along shaded stream banks.
Frostweed	*Verbesina virginica*	FACU	Shade	Biennial	Open dryish woodlands and streambanks, usually is Perennial in Texas.
Indian Grass	*Sorghastrum nutans*	FACU	Shade/Partial	Perennial	Grows in floodplain soils, tolerant of alkaline conditions and clay. It can grow in the understory
Bristlegrass	*Setaria leucopila*	FACU	Partial	Perennial	Grows in bottomlands, alluvial flats, loamy and clay bottomlands.
Green Sprangletop	*Leptochloa dubia*	FACU	Partial	Perennial	Favors soils that are drained. It is adapted to rocky hills, canyons
Switchgrass	*Panicum virgatum*	FAC	Partial	Perennial	Moderately deep to deep soils that are somewhat dry to poorly drained. Can tolorate shade.
Inland Sea Oats	*Chasmanthium latifolium*	FAC	Partial	Perennial	Moderate to greater shade conditions in moist to well drained sites. Never found on droughty sites.
Meadow Sedge	*Carex granularis*	FACW	Partial/Sun	Perennial	Full to partial sun conditions. Wet to mesic soil conditions, of loam or clay-loam types.
Big Muhly	*Muhlenbergia lindheimeri*	FACW	Full	Perennial	Grows in full sun. Perfers well-drained sand, loam, clay, limestone.
Red Mulberry	*Morus rubra*	FACU	Shade/Partial	Perennial	Moist areas at edges of woods, very tolerant of shade. Usually an understory tree,

Table 4.1. Plant List 2015

Utilizing the species requirements and characteristics a site specific planting plan was developed to optimize placement of the plants in the landscape. Deer resistance was preferred in all species chosen due to the intense browsing pressure. Wetland indicator status (Reed, 1988) was utilized to locate plants vertically from near the waterline up the grade where soils become very dry during the summer. Plant indicator status is utilized by the Corps of Engineers to identify plant communities that prefer ecotypes such as wetland, or uplands. Thus plants that

were rated as being a part of the FACW (Facultative Wet) classification were planted near the water, and FACU (Facultative Upland) were planted further up the slope. Sun requirements were also utilized to refine the planting zones by inspection of each station. Finally, habitat notes were utilized to place plants in specific areas where they naturally would be found. Utilization of these characteristics defined the plant species, which allowed the formulation of a custom planting plan for each site based on the plant physiological requirements and specific site conditions. A summary of species planted by site is Table 4.2.

	Iceplant	Canada Wildrye	Virignia wildrye	Switchgrass	Bristlegrass	Green Sprangletop	Cedar Sedge	Indian Grass	Meadow Sedge	Big Muhly
	Seed	Seed	Seed	Seed	Seed	Seed	Plug	Plug	Plug	Plug
	FACU	FAC	FAC	FAC	FACU	FACU	FACW	FACU	FACW	FACW
Site Name	Shade	Shade	Shade	Partial	Partial	Partial	Shade	Shade/Part	Partial/Full	Full
10	x	x	x							
43	x	x	x				x			
49	x	x	x				x	x		
88	x	x	x				x	x		
135	x	x	x	x	x	x	x	x		
173	x	x	x	x	x	x	x	x	x	
208	x	x	x	x	x	x	x	x		
227	x	x	x	x	x	x	x	x	x	
253	x	x	x	x	x	x	x	x	x	
276	x	x	x	x	x	x	x	x		
335	x	x	x	x	x	x	x	x	x	
341	x	x	x	x	x	x	x	x	x	
341	x	x	x	x	x	x		x	x	
434	x	x	x	x	x	x				
490	x			x	x	x		x		x
600	x	x	x	x	x	x		x		
640	x	x	x	x	x	x	x	x	x	
672	x	x	x	x	x	x		x	x	x
740	x	x	x	x	x	x		x		
780	x	x	x	x	x	x		x	x	x
844	x	x	x	x	x	x		x	x	x
896	x	x	x	x	x	x	x			
903	x	x	x	x	x	x				
941	x	x	x	x	x	x	x			

Table 4.2. Planting by site 2015

Vegetative riparian restoration planting were done in 4 trips starting February 18, 2015 and was finished March 13, 2015. An additional reseeding was done on May 29, 2015. Trees were planted on April 20, 2015 and on July 16, 2015. All plantings were sprayed with an egg and cayenne solution (1 dozen eggs, 3 tsp cayenne to one gallon of water) to discourage deer, squirrels, and rodents, and the spraying was continued during visits for 3 months to allow establishment of plant roots. Protective fencing was installed around planted trees and consisted of 4-foot tall metal fencing surrounding each tree as is consistent with other deer fencing within Landa Park (Figure 4.3).

Figure 4.3. Protective fencing was installed around planted trees

Routine monitoring occurred on a biweekly to monthly schedule, and primarily focused on the integrity of the erosion control structures and restoration plantings. Additional riparian monitoring events were contractually agreed to be conducted in response to large rainfall events; specifically events with rainfall greater than 2 inches in the New Braunfels Landa Lake area. However, no rainfall events of this magnitude occurred in 2015 to 10/30/15.

4.3 Permits

All continued restoration work was completed in compliance with applicable permits. The following permits related to this project were acquired by the City and EAA:

1. Texas Parks and Wildlife Sand and Gravel Permit No. 2012-G042. Expires March 11, 2016.
2. United States Army Corp of Engineers, Nationwide Permit Condition 31, Project No. 2012-00240, Expires March 18, 2017.
3. United States Fish and Wildlife Service, Department of the Interior, Take Permit, Dated February 13, 2013.

The following permits, approvals, or notifications were completed by the project team before commencing on-site activities:

1. United States Fish and Wildlife Service, Department of the Interior, Federal Fish and Wildlife Permit, Native Endangered Species Recovery (BIO-WEST).
2. TPWD aquatic vegetation treatment authorization. Expires October 10, 2015.

4.4 Exotic Vegetation Removal

Along the shoreline (Figure 4.4), elephant ear (*Colocassia spp*.) concentrations were significantly reduced during 2014 with several herbicide treatments. Some re-growth of the elephant ear stands were noted during initial 2015 monitoring efforts; thus a follow up herbicide treatment was planned to occur during 2015. However, observation of the proposed treatment areas indicated that the areal extent of coverage was very small, and mechanical removal of isolated plants could be done without destabilizing the area, and without introduction of herbicide to the sensitive planting areas or nearby waters.

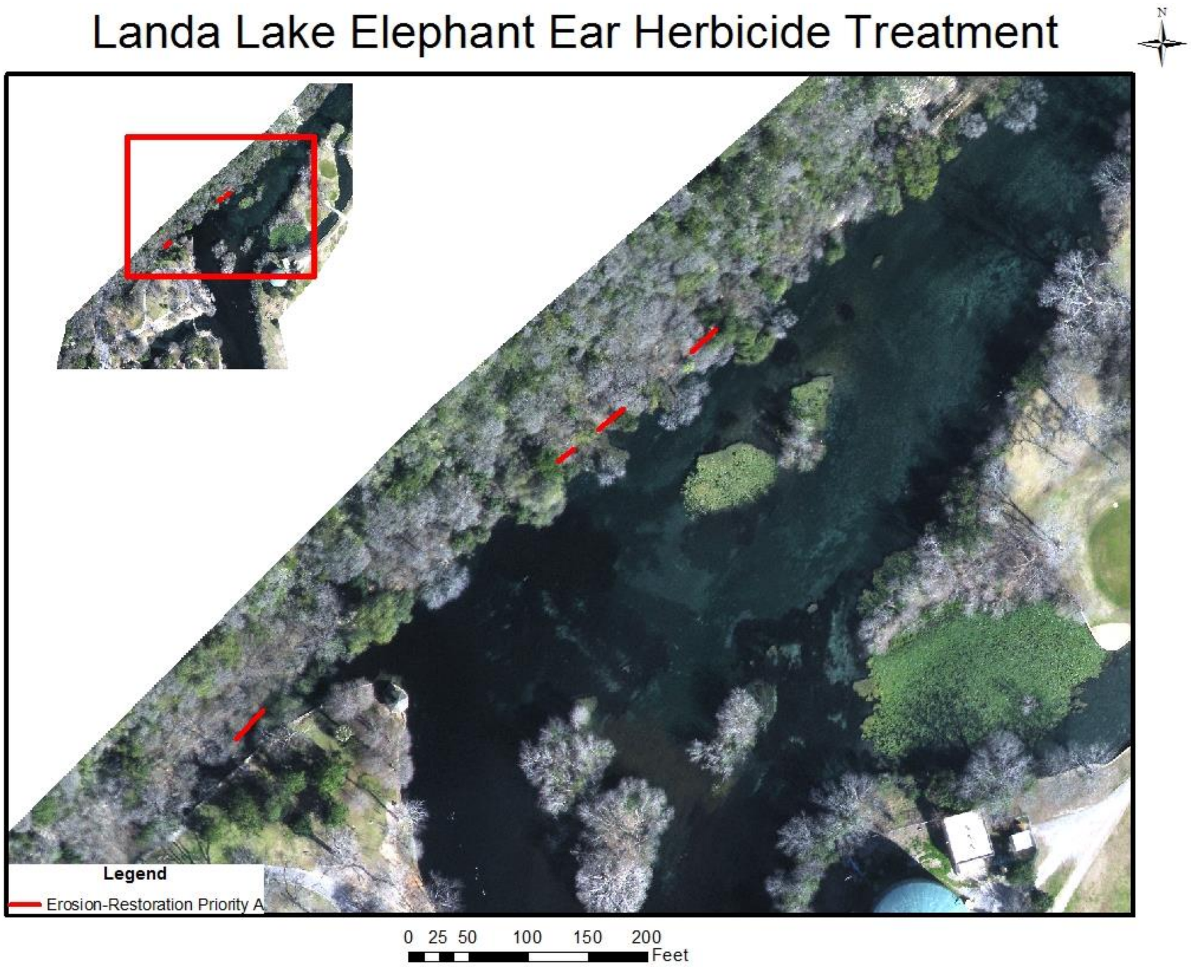


Figure 4.4. Landa Lake elephant ear (Colocasia spp.) 2014 herbicide treatment areas

In 2015, additional Japanese ligustrum (*Ligustrum japonicum*) were removed along the shoreline and used in the construction of erosion control structures (sediment traps). Ligustrum trees removed were between 2 and 6 inches (in) in diameter, and the trees were cut 6 to 12 inches from the ground to leave a stump to help anchor erosion control structures. The remaining stump was manually treated using a “brush on” technique to prevent re-growth. Limited re-growth was also observed from stumps left from the 2014 removals, which were also retreated. Larger diameter portions of each tree, the main trunk and large branches, were utilized to construct, and repair erosion control structures. The smaller ligustrum branches and twigs were used to form erosion prevention mats between the larger branches. The erosion control structures were also armored utilizing nearby rocks to prevent washout in high flows and to minimize browsing damage that was observed in 2014.

5 Vegetation Planting Monitoring Results

During the project term, broad soil moisture levels were hard to maintain even with the drip irrigation system. However, the irrigation system worked flawlessly from a system standpoint. The main line control valves were taped in the open position in June 2015 with duct tape after an incident where a park visitor shut off various stubs. The duct tape could be easily sliced by Park Staff if needed to stop a leak or a break, but proved to be sufficient to stop unauthorized use by park patrons. Although some significant rainfall events occurred during the project term (>0.75 inches), as is consistent with climatic norms the summer months were quite dry, and soil moistures became progressively lower. The temporary outage of the irrigation water due to the unauthorized shutdown of the system did precede a decline in quality of grass from the seeding, but it is unclear that this was actually the cause of the decline.

Success of vegetation plots was measured over the spring-summer-fall period (between late February first planting and the September monitoring). Success varied among project area locations as well as within each vegetation plot. Listing by plot location is in Appendix A. During monitoring, individual plug plant survival was recorded and survivability was calculated as the fraction of original plantings (**Error! Reference source not found.**).

	Percent Survivability				
Site Name	Cedar Sedge	Meadow Sedge	Indian Grass	Muhly Grass	Total
Station 43	33	NA	100	NA	71
Station 49	100	NA	100	NA	100
Station 88	100	NA	100	NA	100
Station 103	50	NA	100	NA	92
Station 135	100	NA	100	NA	100
Station 173	50	100	56	NA	58
Station 208	100	NA	100	NA	100
Station 227	100	50	62	NA	67
Station 253	100	100	100	NA	100
Station 276	100	NA	100	NA	100
Station 335	100	100	75	NA	86
Station 341	0	100	82	NA	77
Station 357	NA	100	67	NA	71
Station 383	NA	NA	NA	100	100
Station 386	NA	100	80	NA	83
Station 459	NA	100	100	NA	100
Station 490	NA	100	100	100	100
Station 600	NA	NA	56	NA	56
Station 640	67	100	40	NA	50
Station 672	NA	67	73	100	65
Station 740	100	NA	83	NA	71
Station 780	NA	50	75	100	63
Station 844	NA	100	67	100	81
Average	79	90	82	100	82

Table 5.1. Plant survival for project locations

Overall, survivability of plugs in 2015 was very high, and was much higher than in 2014 or 2013. Survivability for sedges in 2015 was 80 to 90 percent on average (depending on species), versus in 2014 when it was 36 percent and 60 percent in 2013. Grasses and sedges have also shown reproduction, and thick growth. (Figure 1), and have shown that they are retaining and stabilizing soils (Figure 5.2).

Figure 1. Thick coverage near spring run.

Figure 2. Grass retaining drift material.

While grass densities were not measured, the grass in plots which did poorly seemed to be due to the intensity of shading. Where there was intense shading, grass simply did not survive, and this was despite utilizing shade tolerant species. There were also some areas that appeared never to have recovered from the negatively impact of the lack of water due to issues with patrons cutting the irrigation system.

6 Erosion Controls

6.1 Sediment traps

Sediment traps were basically constructed according to methodologies set forth in the 2013 Final Report (RPS Espey et al. 2013). They also incorporated improvements of additional hardening and reinforcement using existing rocks and other on-site materials to avoid both structural failure in high flow areas and to reduce wildlife browsing impacts recognized in 2014 (RPS Espey et al. 2014). Sixteen erosion control structures totaling approximately 406 linear feet along the western shoreline were monitored for sediment capture volume over the sampling period from Febuary 18 to September 15, 2015. To monitor depth of captured sediment, a steel pin was driven just inside the erosion control structure approximately at the midway point along the structure length. Change in exposed height of the steel pin was used to calculate deposited material. Seven monitoring events were conducted to measure erosion pin height over the

sampling period. Over this period, several significant rainfall events occurred that filled the containment structures leaving wrack material behind (Figure 6.1). Station 335 had a steel pin that was removed presumbably by park patrons between 5/6/2015 and 5/29/2015.

Figure 6.1. Erosion control structure at Location 227 with rack line of leaf matter

6.2 Erosion Control Structures – results of post-rainfall monitoring

Daily rainfall amounts were obtained from a nearest weather station that had a complete record: Lake Dunlap KTXNEWBR14 (Weather Underground 2015). Table 6.1 shows daily rainfall events above three quarters of an inch and Figure 6.1 shows daily rainfall amounts.

Lake Dunlap Weather Station	
Date	Daily Precipation (in)
1/22/2015	0.86
3/9/2015	0.96
3/20/2015	0.76
3/21/2015	0.77
4/25/2015	1.24
5/15/2015	0.75
5/17/2015	1.39
5/23/2015	1.65
8/29/2015	0.94

Table 6.1. Daily rainfall amounts greater than 0.75" between January and September 2015

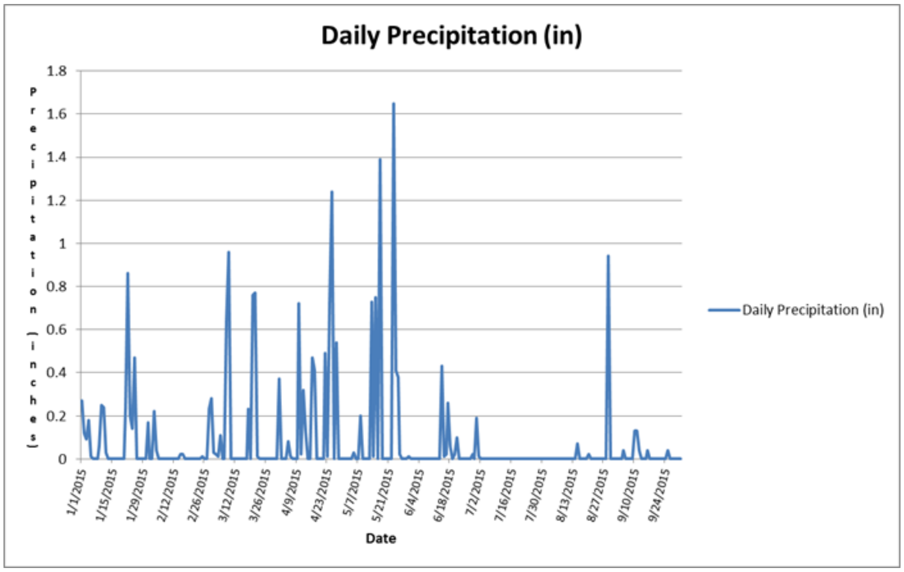


Figure 6.2 Graph of daily rainfall events at the nearby Lake Dunlap weather site

Pin height (**Error! Reference source not found.**). appeared to show general agreement between precipitation and the decline in pin height, where decline in pin height is equivalent to increase in accumulated sediment. It should be noted that all sediment control sites show sediment accumulation over the sampling period. Stronger declines in pin heights at most sites took place during the heaver late spring rain events.

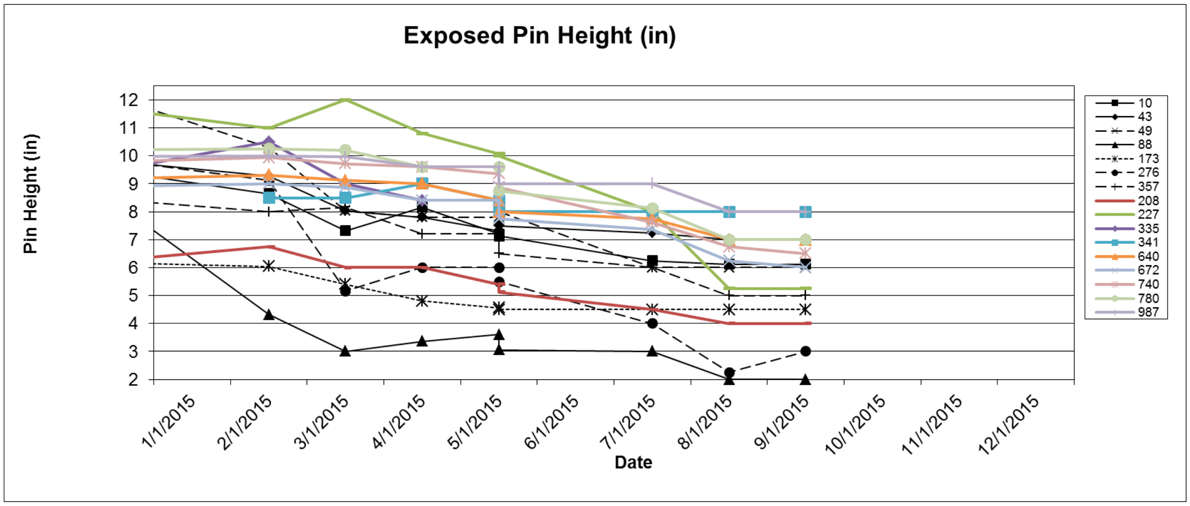


Figure 6.3 Decrease in pin height over time.

To quantify captured sediment runoff, a series of measurements were taken by dividing the selected control structures into equal segments (Figure). Cross-sectional area was calculated for each segment by assuming measured cross sections were parallel to each other, and the control structure was roughly triangular in shape (Figure ; 1/2 X base X height). This assumption is conservative and underestimated sediment accumulation behind the erosion control structures.

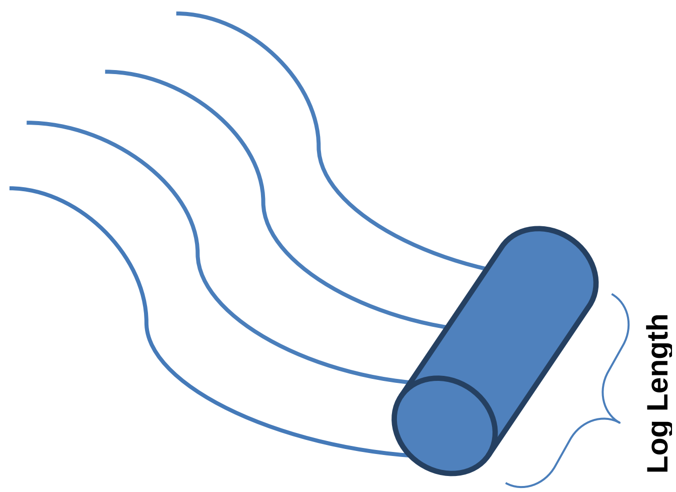


Figure 6.4. Illustrates equally divided cross sections monitored across the length of an erosion control structure

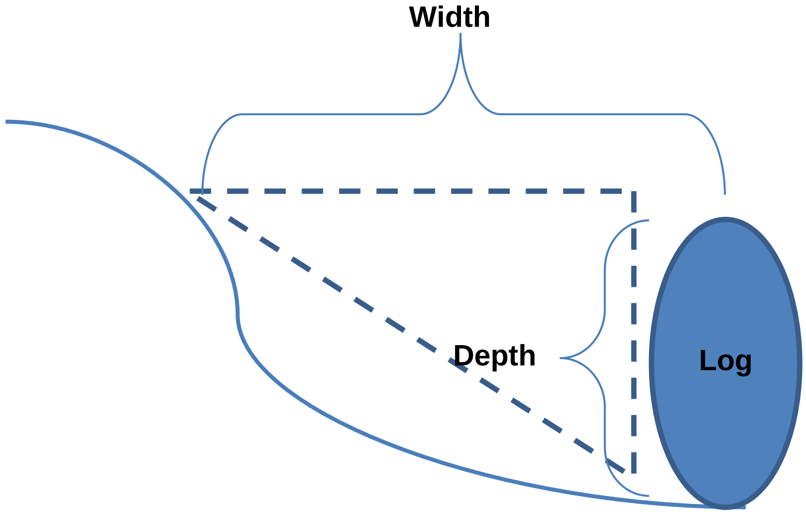


Figure 6.5. A typical erosion sediment trap, with cross section dimensions for monitoring

Captured sediment was estimated for the sampling period from February 18, 2015 to September 3, 2015 (Table). Total estimated sediment retained over this time period is estimated to be 4.2 cubic yards.

Soil Captured by Site	
Location	Total Soil Retained by Site (yrd^3)
10	0.2
43	0.1
49	0.7
88	0.2
173	0.1
208	0.3
227	0.5
276	0.8
335	0.6
341	0.0
357	0.8
640	0.2
672	0.5
740	0.2
780	0.2
987	0.1
Total Volume (yd^3)	4.2

Table 6.2. Total Sediment captured by site (cubic yards)

7 Conclusions

The efforts conducted under this project in 2013, 2014, and 2015 to reduce fine sediment accumulation in Landa Lake shoreline habitat areas have been successful, and continue to function, although stabilization of accumulated sediments with thick groundcover has not occurred in all sites. Additionally, exotic vegetation has been greatly reduced within the project area and native vegetation diversity and density have continued to increase.

7.1 Non-native vegetation removal

Reductions in exotic vegetation within the project area were observed throughout the restoration process and continued to progress in 2015. Elephant ear stands have nearly been eliminated. Ligustrum trees are greatly reduced within the project area. This reduction in tree canopy did

lead to somewhat greater infiltration of sunlight, but the area remains a closed canopy, which limits areas where native understory communities can be established.

7.2 Erosion control

The erosion control structures are effective in sediment retention and in the development of natural terraces. These natural terraces provide increses in soil depth, water retention, and a favorable habitat for permanent vegetation to re-establish along the shoreline (Figure).

Figure 7.1. Constructed erosion control structure with re-vegetation effort

Declines in pin height indicated that soil is being washed into the erosion control structures, and that the soil is retained. This retention of soil suggests that stabilized material has been prevented from deposition into Landa Lake. If vegetation can be established along the terraces created by these structures, it will help permanently stabilize the sediments in the basins and decrease the velocity of surface flows, which would result in restraining future soil movement. Activities during 2016 should assess and target areas most in need of increased sediment trap capacity, and attempt to stabilize these areas with vegetation.

7.3 Re-vegetation with native species

Several key obstacles impacted the overall survival of the vegetation planted during this year. The shallow soils do not retain much moisture. Irrigation was cut off in the spring due patrons of the park switching the shut off valves. The new grasses that were being established in many areas never really recovered, but it is unclear that soil moisture was the only causative factor. As with past years, planting efforts were hampered by wildlife grazing pressures on the herbaceous plantings (sedges, grasses, etc.). Although the repellent solution was not rigorously statistically tested on paired plots, grazing pressures did seem to be ameliorated to some extent by spraying of the egg mixture during planting and establishment. However, the overall main factor limiting the establishment of native understory communities continues to be the lack of sunlight. The area remains a closed canopy, and this severely limits areas where an understory can be established. In areas where partial shade can be achieved, the growth rate and survival of plants was much higher, such as areas in clearings, and along the open shores of Landa Lake. All of the listed factors impacted the survivability of vegetation planted in 2015, and reaffirms the selection of appropriate plants and trees for the environment seen along the spring run.

Year 3 plant selections were refined based on several onsite factors as identified above and the results from Years 1 and 2 restoration efforts. Perennial plant plugs and trees had a relatively high percent survivability. The establishment of grass was a high priority, and the grass did very well in the cool spring, but rapidly faded during the summer heat despite irrigation. Paradoxically this did not hold true in areas that had sun. The survivability documented should prompt efforts to continue and focus new plantings to species that can tolerate full shade and ones that cannot to be limited to areas adjacent to the shoreline that have partial sun to partial shade. Plantings should also include areas in front of the erosion control structures, but only to the edge of the high flow levels due to the suseptability of drowning and erosional loss.

Photographs taken during post-rainfall monitoring events provided documentation that the surviving vegetation were providing erosion protection. Although vegetation density is still

sparse in areas along many areas of the shoreline, reproduction of planted vegetation and growth of surviving vegetation was observed, and these communities have provided limited erosion prevention. **Error! Reference source not found.** shows leaf matter build-up behind grass and sedge plants. As established vegetation begins to reproduce and increase in density, the quantity of erosion prevention provided by the vegetation will also continue to increase.

Despite limited survivability exhibited by grasses in shaded areas, a fall planting of a cover crop such as native wildrye is still recommended. Wildryes have high shade tolerance, but typically burn out in the summer. However these stands can reproduce prolifically, and may help stabilize areas that have deep shade, and provide condusive conditions for the overseeding or natural extablishment of warm season covers. Other plantings should be focused on select areas in which there is sufficient sunlight for the species chosen. Due to the shallow soils, careful attention to season is also a critical factor for successful planting.

7.4 Summary and Recommendations

The most effective restoration activity in 2015 once again was the construction of shoreline erosion control measures; the sediment traps captured 4.2 cubic yards of sediment before it could enter Spring Run 3 or Landa Lake.

Exotic species removal continued to reduce onsite populations. Elephant ear was successfully reduced in the years 2013, and 2014. This year's elephant ear removal efforts have continued this trend along the shoreline. These shoreline areas were replanted with native species that are having success and have shown signs of reproduction (new sprouts and seed structures). The number of ligustrum trees have also been drastically reduced to the point that onsite availability of ligustrum logs and brush material, which was utilized on-site for construction of erosion control sediment traps, is becoming scarce and is becoming a limiting factor in building and reparing structures.

2015 shoreline plantings were quite successful, although many became hard to track when they became interspersed with taller false nettle and ice plant (which was also a planted species). Upon the upper benches behind the catchment structure the dominant covariant factors limiting the establishment of ground covers were intermittent water supply, wildlife grazing, and mainly the heavy shade from the closed canopy. These will likely continue to be limiting factors in the stabilization of the area.

Future work in 2016 should focus on maintenance of sediment traps and the associated restoration of native understory communities in these areas, and their effect on riffle beetle range. This work should include the following:

- Conduct monitoring to identify presence or absence of riffle beetles in different habitat areas in the resoration area;
  - Define sediment conditions where riffle beetles are found.
  - Incorporate and analyze long term riffle beetle dataset to determine if restoration activities are actually improving conditions for target species.
- Focus on shoreline areas;
  - Maintain erosion control structures
  - Assess and target areas in need of increased sediment trap capacity.
- Reassess exotic vegetation eradication and native vegetation establishment
  - Reassess this previous year as well as the past two years restoration work to promote success and increase diversity.
  - Treat regrowth of ligustrum and other non-native invasive species.
  - Replant areas as needed with native grasses, sedges, and native trees.
  - Consider planting native cool season cover.

8 References

Batte C.D. Trevino R., Divin J., and Bearden E., 1984. Soil Survey of Comal and Hays Counties, Texas . Guadalupe Soil and Water Conservation District.

Bowles DE, Barr CB, Stanford R. 2003. Habitat and phenology of the endangered riffle beetle Heterelmis comalensis and a coexisting species, Microcylloepus pusillus, (Coleoptera: Elmidae) at Comal Springs, Texas, USA. Archiv Fur Hydrobiologie 156: 361–383.

Gibson, J.R., S.J. Harden and J.N. Fries. 2008. Survey and distribution of invertebrates from selected Edwards Aquifer springs of Comal and Hays counties, Texas. Southwestern Naturalist 53: 74 – 84.

RPS Espey, HEI and BIO-WEST. 2013a. 2013 Work Plan for Riffle Beetle Habitat Restoration Spring Run 3 and Landa Lake Shoreline. Prepared for City of New Braunfels. April 1, 2013.

RPS Espey, HEI and BIO-WEST. 2014. 2014 Final Report for Riffle Beetle Habitat Restoration Spring Run 3 and Landa Lake Shoreline. Prepared for City of New Braunfels. December 22, 2014.

RPS Espey, HEI and BIO-WEST. 2013b. 2013 Final Report for Riffle Beetle Habitat Restoration: Spring Run 3 and Landa Lake Shoreline. Prepared for City of New Braunfels. December 17, 2013.

Weather Underground. 2015. PWS site Lake Dunlap Crossing KTXNEWBR14. Accessed 10/01/15.

Appendix A – Vegetation Plots monitoring

Planting areas were monitored for vegetation establishment in 2015. The primary monitoring activities occurred upon initial plantings February, with final monitoring in September.

The monitoring efforts summarized in these figures will be used to guide additional subsequent plantings in 2016.

Key for notations in figures of planting locations:

CS = Cedar Sedge
IG = Indian Grass
MS = Meadow Sedge
MG = Muhly Grass
Rye = Mix of Virginia and Canadian Wildrye
Iceplant = Verbesina virginica.
Light Green = Healthy
Dark Green = Distinguishes plants planted on that field visit (there were multiple plantings at some sites)
Yellow Background = Lost in vegetation, damaged, not healthy.
Red Background = Absent or dead
Grey background = Natural object such as rocks, bridges, or native large trees.

Site Stations

GPS_ID	Feature_Station	Lat	Long	Date/Time
3	10	29.71355572	-98.13700709	03-MAR-15 10:18:10AM
2	43	29.71366033	-98.13702662	03-MAR-15 10:16:21AM
4	49	29.71367441	-98.1369123	03-MAR-15 10:19:33AM
5	88	29.71374356	-98.13693903	03-MAR-15 10:23:13AM
6	103	29.71372018	-98.13687827	03-MAR-15 10:28:01AM
7	135	29.71382964	-98.13688849	03-MAR-15 10:34:02AM
8	173	29.71382688	-98.13672094	03-MAR-15 10:39:25AM
9	208	29.71391749	-98.13667031	03-MAR-15 10:51:30AM
13	227	29.71396443	-98.13663837	03-MAR-15 11:00:06AM
14	253	29.71405344	-98.13660217	03-MAR-15 11:10:42AM
15	276	29.71405956	-98.13657568	03-MAR-15 11:24:19AM
16	335	29.71423307	-98.13649588	03-MAR-15 11:37:44AM
17	341	29.71417607	-98.13644467	03-MAR-15 11:59:40AM
18	357	29.71429182	-98.13633503	03-MAR-15 12:00:38PM
19	383	29.71430331	-98.13630302	03-MAR-15 12:13:11PM
20	386	29.7143893	-98.13623688	03-MAR-15 12:15:07PM
21	443	29.71442283	-98.1361803	03-MAR-15 12:17:59PM
22	459	29.7143137	-98.13618357	03-MAR-15 12:21:14PM
23	490	29.71444722	-98.13614242	03-MAR-15 12:23:28PM
24	600	29.71467471	-98.13583966	03-MAR-15 12:36:34PM
25	640	29.71476347	-98.13583271	03-MAR-15 12:43:18PM
26	672	29.71478317	-98.13570874	03-MAR-15 1:58:56PM
27	740	29.71485215	-98.13551352	03-MAR-15 2:12:41PM
28	780	29.71493312	-98.13538956	03-MAR-15 2:26:38PM
29	900	29.71510495	-98.1351811	03-MAR-15 2:31:29PM
30	941	29.71521844	-98.13509334	03-MAR-15 2:37:04PM
31				End of seed

Planting_Keys

Planting Key

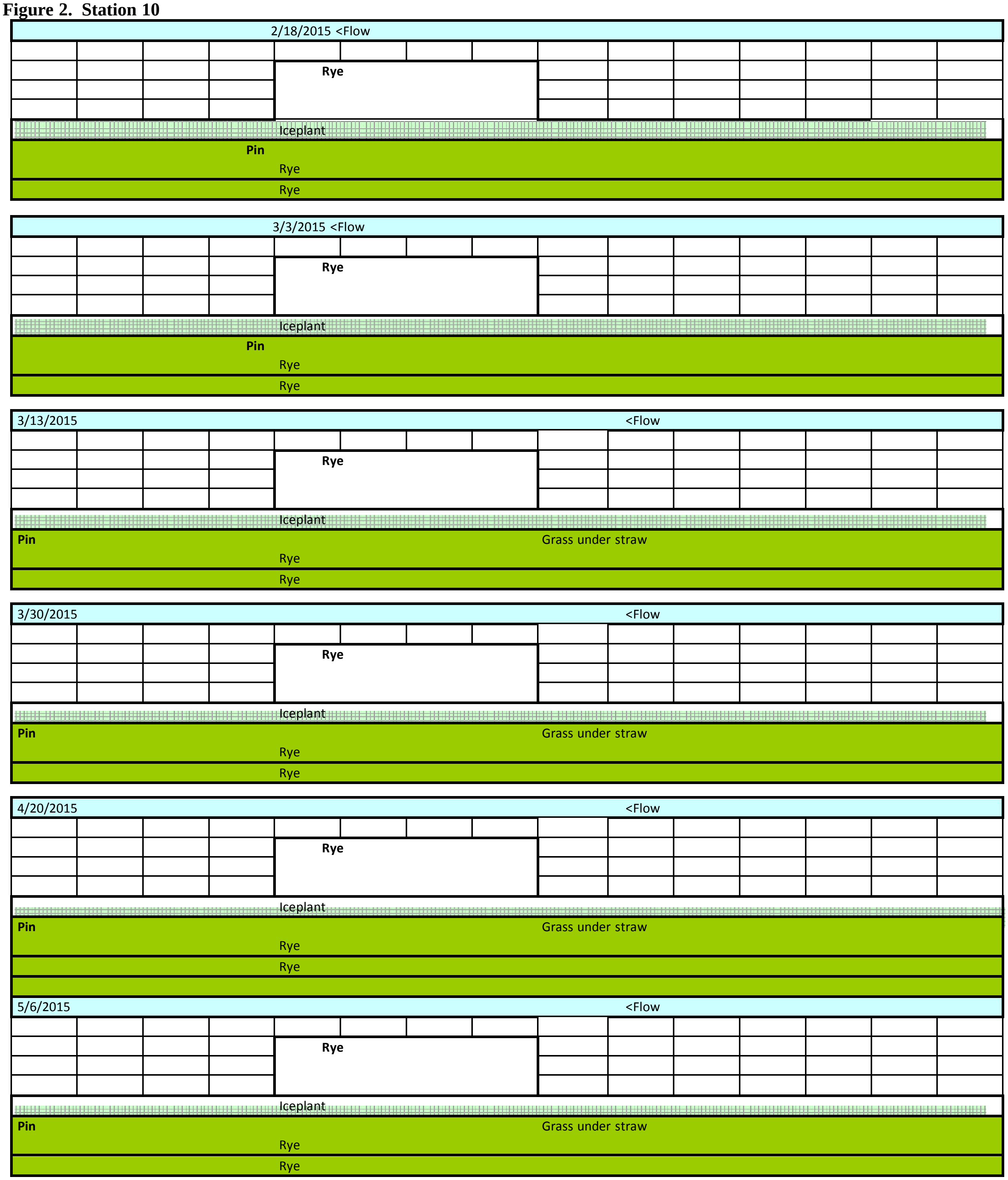
	Planted on 2/18/15
	Planted on 3/3/15
	Planted on 3/13/15
	Damaged but alive
	Mostlydead
	Dead or missing
	Natural Feature

St.0

Figure 1. Station 0 (Spring Head)

Date	Pics	Notes
3/13/2015	Pic 2389,2390	New rocks placed on spring head.
3/30/2015	Pic 2408	New wall stopping leaves and dirt.
4/20/2015	Pic 2342	Rocks thrown in springhead
5/6/2015	Pic 2343	Damaged wall
5/21/2015	Pic 2696	
5/29/2015	Pic 2747 and 2748	Rock in Springs
8/6/2015	Pic 2342	Pic of IG in Bloom
8/6/2015	Pic 2343	No wall left at spring head

Figure 2. Station 10



St.10

5/29/2015 <Flow

Rye

Iceplant

Pin Grass under straw

Rye

Rye

7/16/2015 <Flow

Rye

Iceplant

Pin Grass under straw

Rye

Rye

8/6/2015 <Flow

Rye

Iceplant

Pin Grass under straw

Rye

Rye

St.43

Figure 3. Station 43

St.43

5/21/2015										<Flow				
									IG		Rock		CS-DU	
				SeaOatSeeds				IG		IG		CS-DU		
									IG					
									Grass sprouting here			CS		
				Iceplant										
Pin								Grass growing up through straw						
				Rye										
				Rye										

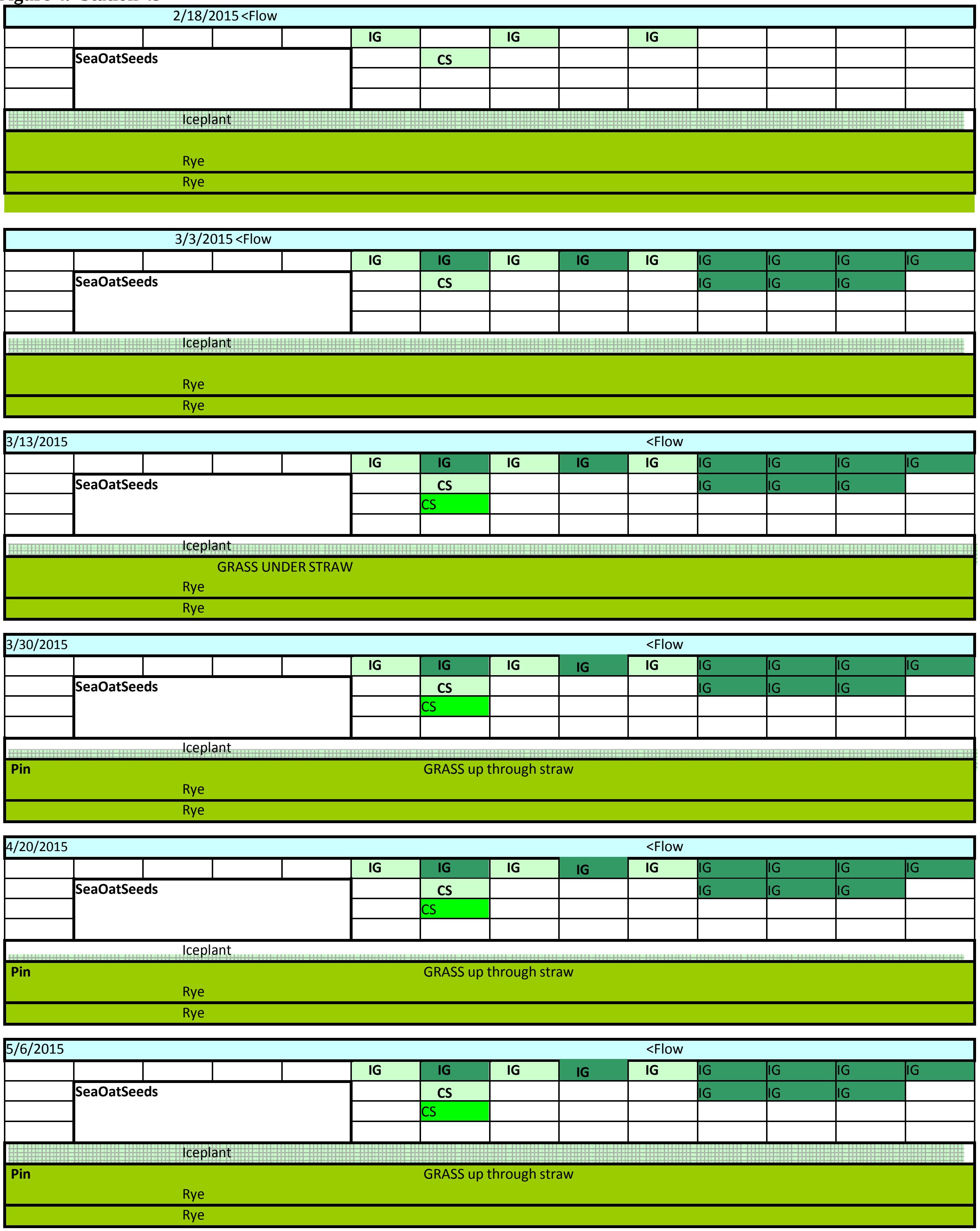
5/29/2015										<Flow				
									IG		Rock		CS-DU	
				SeaOatSeeds				IG		IG		CS-DU		
									IG					
									Grass good			CS		
				Iceplant										
Pin								Grass growing up through straw						
				Rye										
				Rye										

7/16/2015										<Flow				
									IG		Rock		CS-DU	
				SeaOatSeeds				IG		IG		CS-DU		
									IG					
									Grass good			CS		
				Iceplant										
Pin								Grass growing up through straw						
				Rye										

8/6/2015										<Flow				
									IG		Rock		CS-DU	
				SeaOatSeeds				IG		IG		CS-DU		
									IG					
									Grass good			CS		
				Iceplant										
Pin								Grass growing up through straw						
				Rye										
				Rye										

St.49

Figure 4. Station 49



St.49

5/21/2015									<Flow				
					IG	IG	IG	IG	IG	IG	IG	IG	IG
	SeaOatSeeds					CS				IG	IG	IG	
						CS							
		Iceplant											
Pin						GRASS up through straw							
		Rye											
		Rye											

5/29/2015									<Flow				
					IG	IG	IG	IG	IG	IG	IG	IG	IG
	SeaOatSeeds					CS				IG	IG	IG	
						CS							
		Iceplant											
Pin						GRASS up through straw							
		Rye											
		Rye											

7/16/2015									<Flow				
					IG	IG	IG	IG	IG	IG	IG	IG	IG
	SeaOatSeeds					CS				IG	IG	IG	
						CS							
		Iceplant											
Pin						GRASS up through straw							
		Rye											
		Rye											

8/6/2015									<Flow				
					IG	IG	IG	IG	IG	IG	IG	IG	IG
	SeaOatSeeds					CS				IG	IG	IG	
						CS							
		Iceplant											
Pin						GRASS up through straw							
		Rye											
		Rye											

St.88

Figure 5. Station 88

St.88

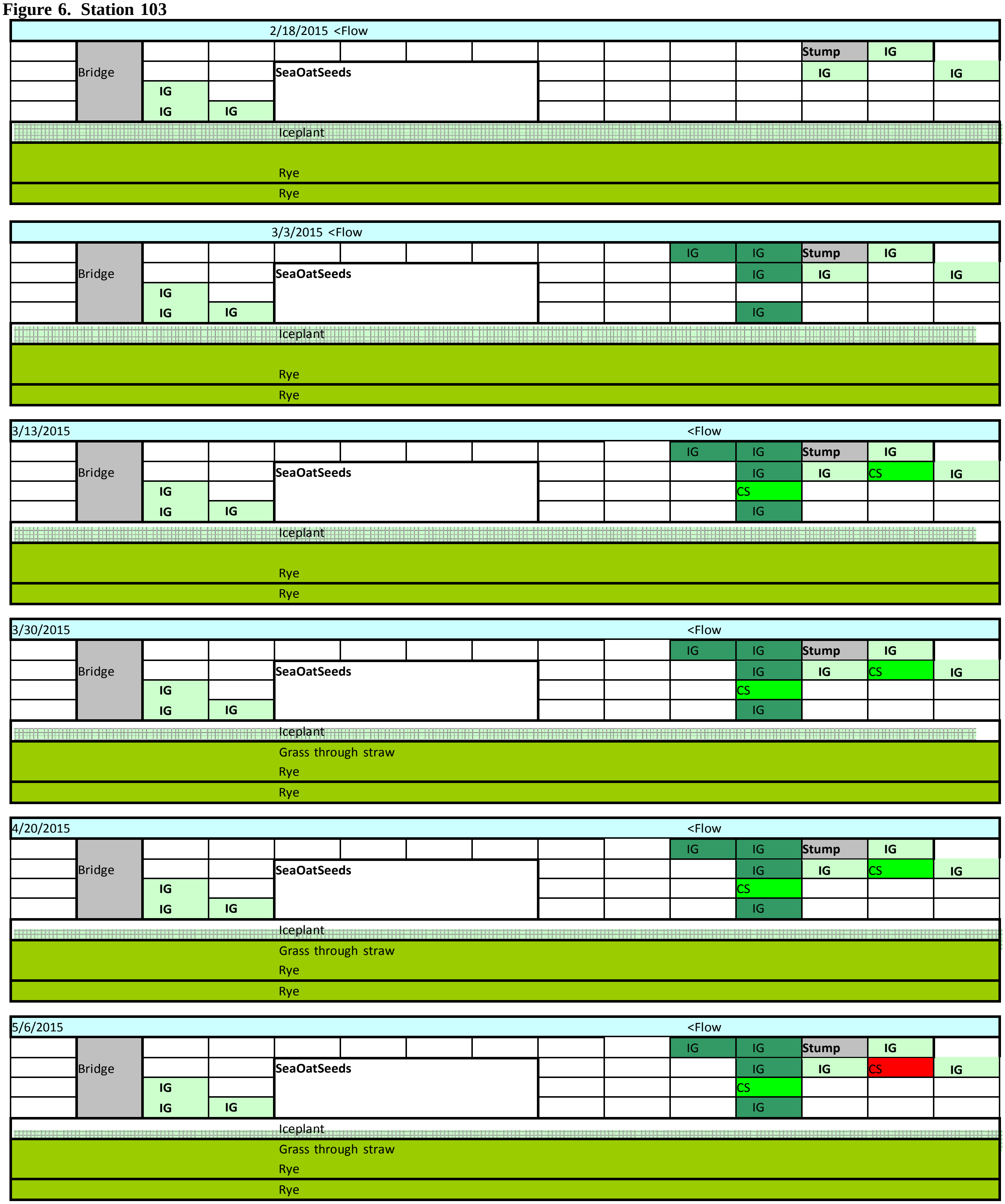
5/21/2015										<Flow				
	IG													
IG		IG		**SeaOatSeeds**					**CS**					
										IG	IG	**IG**	IG	IG
				Iceplant										
Pin								Grass Through Straw						
				Rye										
				Rye										

5/29/2015										<Flow				
	IG													
IG		IG		**SeaOatSeeds**					**CS**					
										IG	IG	**IG**	IG	IG
				Iceplant										
Pin								Grass Through Straw						
				Rye										
				Rye										

7/16/2015										<Flow				
	IG													
IG		IG		**SeaOatSeeds**					**CS**					
										IG	IG	**IG**	IG	IG
				Iceplant										
Pin								Grass Through Straw						
				Rye										
				Rye										

8/6/2015										<Flow				
	IG													
IG		IG		**SeaOatSeeds**					**CS**					
										IG	IG	**IG**	IG	IG
				Iceplant										
Pin								Grass Through Straw						
				Rye										
				Rye										

Figure 6. Station 103



St.103

5/21/2015										<Flow				
										IG	IG	Stump	IG	
	Bridge			SeaOatSeeds							IG	IG	CS	IG
		IG									CS			
		IG	IG								IG			
Iceplant														
Grass through straw														
Rye														
Rye														

5/29/2015										<Flow				
										IG	IG	Stump	IG	
	Bridge			SeaOatSeeds							IG	IG	CS	IG
		IG									CS			
		IG	IG								IG			
Iceplant														
Grass through straw														
Rye														
Rye														

7/16/2015										<Flow				
										IG	IG	Stump	IG	
	Bridge			SeaOatSeeds							IG	IG	CS	IG
		IG									CS			
		IG	IG								IG			
Iceplant														
Grass through straw														
Rye														
Rye														

8/6/2015										<Flow				
										IG	IG	Stump	IG	
	Bridge			SeaOatSeeds							IG	IG	CS	IG
		IG									CS			
		IG	IG								IG			
Iceplant														
Grass through straw														
Rye														
Rye														

St.135

Figure 7. Station 135

St.135

5/21/2015										<Flow				
							CS	IG		CS				Bridge
								IG	IG	IG				
				SeaOatSeeds						CS				
				Iceplant										
				sprangletop/switchgrass/bristle										
				Rye										

5/29/2015										<Flow				
							CS	IG		CS				Bridge
								IG	IG	IG				
				SeaOatSeeds						CS				
				Iceplant										
				sprangletop/switchgrass/bristle										
				Rye										

7/16/2015										<Flow				
							CS	IG		CS				Bridge
								IG	IG	IG				
				SeaOatSeeds						CS				
				Iceplant										
				sprangletop/switchgrass/bristle										
				Rye										

8/6/2015										<Flow				
							CS	IG		CS				Bridge
								IG	IG	IG				
				SeaOatSeeds						CS				
				Iceplant										
				sprangletop/switchgrass/bristle										
				Rye										

Figure 8. Station 173

St.173

				MS	5/21/2015			<Flow						
			IG	Rock	IG	Rock		Rock						
IG		IG					IG							
	IG		SeaOatSeeds					CS	IG	IG	IG			
		CS												
				Iceplant										
Sedges2014	Buckeye2014							Pin						
				sprangletop/switchgrass/bristle										
				Rye										

				MS	5/29/2015			<Flow						
			IG	Rock	IG	Rock		Rock						
IG		IG					IG							
	IG		SeaOatSeeds					CS	IG	IG	IG			
		CS												
				Iceplant										
Sedges201	Buckeye2014							Pin						
				sprangletop/switchgrass/bristle										
				Rye										

				MS	7/16/2015			<Flow						
			IG	Rock	IG	Rock		Rock						
IG		IG					IG							
	IG		SeaOatSeeds					CS	IG	IG	IG			
		CS												
				Iceplant										
Sedges2014	Buckeye2014							Pin						
				sprangletop/switchgrass/bristle										
				Rye										

				MS	8/6/2015			<Flow						
			IG	Rock	IG	Rock		Rock						
IG		IG					IG							
	IG		SeaOatSeeds					CS	IG	IG	IG			
		CS												
				Iceplant										
Sedges201	Buckeye2014							Pin						
				sprangletop/switchgrass/bristle										
				Rye										

St.208

Figure 9. Station 208

2/18/2015 <Flow

								IG	IG		IG	CS		
								CS		IG				

SeaOatSeeds

Iceplant

sprangletop/switchgrass/bristle

Rye

3/3/2015 <Flow

								IG	IG		IG	CS		
								CS		IG				

SeaOatSeeds

Iceplant

sprangletop/switchgrass/bristle

Rye

3/13/2015 <Flow

								IG	IG		IG	CS		
								CS		IG				

SeaOatSeeds

Iceplant

sprangletop/switchgrass/bristle

Rye

3/30/2015 <Flow

								IG	IG		IG	CS		
								CS		IG				

SeaOatSeeds

Iceplant

Pin

sprangletop/switchgrass/bristle

Rye

4/20/2015 <Flow

								IG	IG		IG	CS		
								CS		IG				

SeaOatSeeds

Iceplant

Pin

sprangletop/switchgrass/bristle

Rye

5/6/2015 <Flow

								IG	IG		IG	CS		
								CS		IG				

SeaOatSeeds

Iceplant

Pin

sprangletop/switchgrass/bristle

Rye

5/21/2015 <Flow

								IG	IG		IG	CS		
								CS		IG				

SeaOatSeeds

Iceplant

Pin

sprangletop/switchgrass/bristle

Rye

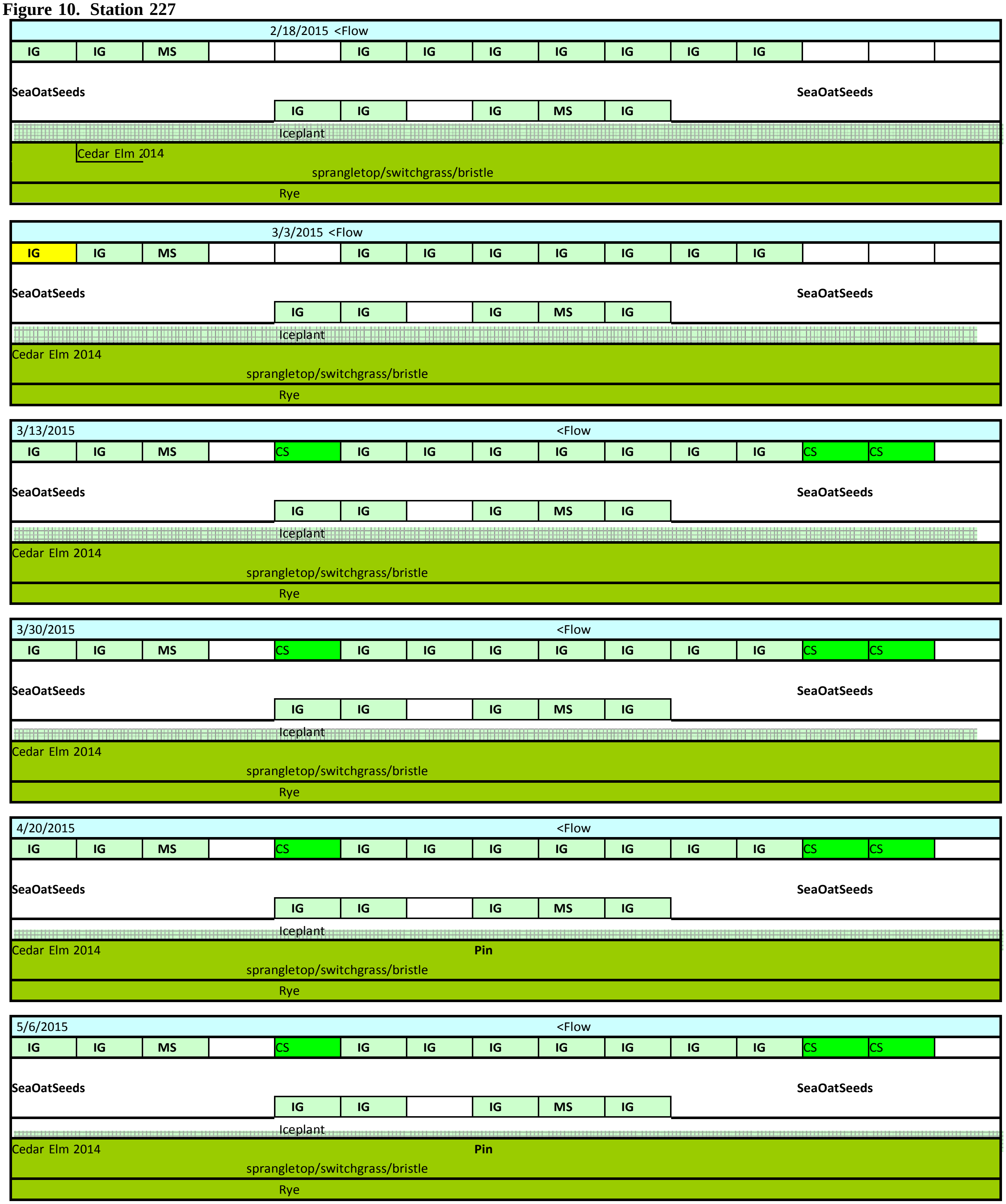
St.208

5/29/2015										<Flow				
								IG	**IG**		**IG**	CS		
								CS		**IG**				
SeaOatSeeds														
				Iceplant										
				Pin sprangletop/switchgrass/bristle										
				Rye										

7/16/2015										<Flow				
								IG	**IG**		**IG**	CS		
								CS		**IG**				
SeaOatSeeds														
				Iceplant										
				Pin sprangletop/switchgrass/bristle										
				Rye										

8/6/2015										<Flow				
								IG	**IG**		**IG**	CS		
								CS		**IG**				
SeaOatSeeds														
				Iceplant										
				Pin sprangletop/switchgrass/bristle										
				Rye										

Figure 10. Station 227



St.227

5/21/2015								<Flow						
IG	IG	MS		CS	IG	IG	IG	IG	IG	IG	IG	CS	CS	
SeaOatSeeds												SeaOatSeeds		
				IG	IG		IG	MS	IG					
				Iceplant										
Cedar Elm 2014							Pin							
			sprangletop/switchgrass/bristle											
				Rye										

5/29/2015								<Flow						
IG	IG	MS		CS	IG	IG	IG	IG	IG	IG	IG	CS	CS	
SeaOatSeeds												SeaOatSeeds		
				IG	IG		IG	MS	IG					
				Iceplant										
Cedar Elm 2014							Pin							
			sprangletop/switchgrass/bristle											
				Rye										

7/16/2015								<Flow						
IG	IG	MS		CS	IG	IG	IG	IG	IG	IG	IG	CS	CS	
SeaOatSeeds												SeaOatSeeds		
				IG	IG		IG	MS	IG					
				Iceplant										
Cedar Elm 2014							Pin							
			sprangletop/switchgrass/bristle											
				Rye										

8/6/2015								<Flow						
IG	IG	MS		CS	IG	IG	IG	IG	IG	IG	IG	CS	CS	
SeaOatSeeds												SeaOatSeeds		
				IG	IG		IG	MS	IG					
				Iceplant										
Cedar Elm 2014							Pin							
			sprangletop/switchgrass/bristle											
				Rye										

St.253

Figure 11. Station 253

2/18/2015 <Flow											
		IG	IG		IG			IG	IG		
			CS	SeaOatSeeds				IG			
					MS						
Iceplant											
				MS_2014							
sprangletop/switchgrass/bristle											
Rye											

3/3/2015 <Flow											
		IG	IG		IG			IG	IG		
		Rock	CS	SeaOatSeeds				IG			
					MS						
Iceplant											
				MG_2014							
sprangletop/switchgrass/bristle											
Rye											

3/13/2015								<Flow			
		IG	IG		IG			IG	IG	CS	
		Rock	CS	SeaOatSeeds				IG			
					MS						
Iceplant											
				MG_2014							
sprangletop/switchgrass/bristle											
Rye											

3/30/2015								<Flow			
		IG	IG		IG			IG	IG	CS	
		Rock	CS	SeaOatSeeds				IG			
					MS						
Iceplant											
				MG_2014							
sprangletop/switchgrass/bristle											
Rye											

4/20/2015								<Flow			
		IG	IG		IG			IG	IG	CS	
		Rock	CS	SeaOatSeeds				IG			
					MS						
Iceplant											
				MG_2014							
sprangletop/switchgrass/bristle											
Rye											

5/6/2015								<Flow			
		IG	IG		IG			IG	IG	CS	
		Rock	CS	SeaOatSeeds				IG			
					MS						
Iceplant											
				MG_2014							
sprangletop/switchgrass/bristle											
Rye											

St.253

5/21/2015							<Flow				
		IG	IG		IG			IG	IG	CS	
		Rock	CS	SeaOatSeeds				IG			
					MS						
		Iceplant									
				MG_2014							
		sprangletop/switchgrass/bristle									
		Rye									

5/29/2015							<Flow				
		IG	IG		IG			IG	IG	CS	
		Rock	CS	SeaOatSeeds				IG			
					MS						
		Iceplant									
				MG_2014							
		sprangletop/switchgrass/bristle									
		Rye									

7/16/2015							<Flow				
		IG	IG		IG			IG	IG	CS	
		Rock	CS	SeaOatSeeds				IG			
					MS						
		Iceplant									
				MG_2014							
		sprangletop/switchgrass/bristle									
		Rye									

8/6/2015							<Flow				
		IG	IG		IG			IG	IG	CS	
		Rock	CS	SeaOatSeeds				IG			
					MS						
		Iceplant									
				MG_2014							
		sprangletop/switchgrass/bristle									
		Rye									

St.276

Figure 12. Station 276

2/18/2015 <Flow											
									TREE		
		IG	IG	IG	IG	IG	IG	IG		IG	
		IG	CS	SeaOatSeeds							Rock
Iceplant											
sprangletop/switchgrass/bristle											
Rye											

3/3/2015 <Flow											
									TREE		
		IG	IG	IG	IG	IG	IG	IG		IG	
		IG	CS	SeaOatSeeds							Rock
Iceplant											
sprangletop/switchgrass/bristle											
Rye											

3/13/2015							<Flow				
									TREE		
		IG	IG	IG	IG	IG	IG	IG		IG	
		IG	CS	SeaOatSeeds							Rock
Iceplant											
sprangletop/switchgrass/bristle											
Rye											

3/30/2015							<Flow				
									TREE		
		IG	IG	IG	IG	IG	IG	IG		IG	
		IG	CS	SeaOatSeeds							Rock
Iceplant											
sprangletop/switchgrass/bristle											
Rye											

4/20/2015							<Flow				
									TREE		
		IG	IG	IG	IG	IG	IG	IG		IG	
		IG	CS	SeaOatSeeds							Rock
Iceplant											
Pin sprangletop/switchgrass/bristle											
Rye											

5/6/2015							<Flow				
									TREE		
		IG	IG	IG	IG	IG	IG	IG		IG	
		IG	CS	SeaOatSeeds							Rock
Iceplant											
Pin sprangletop/switchgrass/bristle											
Rye											

St.276

5/21/2015							<Flow				
									TREE		
		IG	IG	IG	IG	IG	IG	IG		IG	
		IG	CS	SeaOatSeeds							Rock
		Iceplant									
		Pin									
		sprangletop/switchgrass/bristle									
		Rye									

5/29/2015							<Flow				
									TREE		
		IG	IG	IG	IG	IG	IG	IG		IG	
		IG	CS	SeaOatSeeds							Rock
		Iceplant									
		Pin									
		sprangletop/switchgrass/bristle									
		Rye									

7/16/2015							<Flow				
									TREE		
		IG	IG	IG	IG	IG	IG	IG		IG	
		IG	CS	SeaOatSeeds							Rock
		Iceplant									
		Pin									
		sprangletop/switchgrass/bristle									
		Rye									

8/6/2015							<Flow				
									TREE		
		IG	IG	IG	IG	IG	IG	IG		IG	
		IG	CS	SeaOatSeeds							Rock
		Iceplant									
		Pin									
		sprangletop/switchgrass/bristle									
		Rye									

St.335

Figure 13. Station 335

2/18/2015 <Flow											
				IG		IG		IG		IG	
									MS		
Iceplant											
Pin				sprangletop/switchgrass/bristle							
Rye											

3/3/2015 <Flow											
				IG		IG		IG		IG	
									MS		
Iceplant											
Pin				sprangletop/switchgrass/bristle							
Rye											

3/13/2015						<Flow					
				IG		IG		IG	CS	IG	CS
									MS		
Iceplant											
Pin				sprangletop/switchgrass/bristle							
Rye											

3/30/2015						<Flow					
				IG		IG		IG	CS	IG	CS
									MS		
Iceplant											
Pin				sprangletop/switchgrass/bristle							
Rye											

4/20/2015						<Flow					
				IG		IG		IG	CS	IG	CS
									MS		
Iceplant											
Pin				sprangletop/switchgrass/bristle							
Rye											

5/6/2015						<Flow					
				IG		IG		IG	CS	IG	CS
									MS		
Iceplant											
Pin				sprangletop/switchgrass/bristle							
Rye											

St.335

5/21/2015						<Flow					
				IG		IG		IG	CS	IG	CS
									MS		
		Iceplant									
Pin				sprangletop/switchgrass/bristle							
		Rye									

5/29/2015						<Flow					
				IG		IG		IG	CS	IG	CS
									MS		
		Iceplant									
		Pin	sprangletop/switchgrass/bristle								
		Rye									

7/16/2015						<Flow					
				IG		IG		IG	CS	IG	CS
									MS		
		Iceplant									
		Pin	sprangletop/switchgrass/bristle								
		Rye									

8/6/2015						<Flow					
				IG		IG		IG	CS	IG	CS
									MS		
		Iceplant									
		Pin	sprangletop/switchgrass/bristle								
		Rye									

St.341

Figure 14. Station 341

2/18/2015 <Flow											
Bridge		IG		IG		IG		IG			
	CS		MS								
Iceplant											
sprangletop/switchgrass/bristle											
Rye											

3/3/2015 <Flow											
	IG	IG	IG	IG	IG	IG	IG				
Bridge		IG		IG		IG		IG			
	CS		MS								
Iceplant											
sprangletop/switchgrass/bristle											
Rye											

3/13/2015 <Flow											
	IG	IG	IG	IG	IG	IG	IG				
Bridge		IG		IG		IG		IG			
	CS		MS								
Iceplant											
sprangletop/switchgrass/bristle											
Rye											

3/30/2015 <Flow											
	IG	IG	IG	IG	IG	IG	IG				
Bridge		IG		IG		IG		IG			
	CS		MS								
Iceplant											
sprangletop/switchgrass/bristle											
Rye											

4/20/2015 <Flow											
	IG	IG	IG	IG	IG	IG	IG				
Bridge		IG		IG		IG		IG			
	CS		MS								
Iceplant											
Pin sprangletop/switchgrass/bristle											
Rye											

5/6/2015 <Flow											
	IG	IG	IG	IG	IG	IG	IG				
Bridge		IG		IG		IG		IG			
	CS		MS								
Iceplant											
Pin sprangletop/switchgrass/bristle											
Rye											

St.341

5/21/2015					<Flow						
	IG	IG	IG	IG	IG	IG	IG				
Bridge		IG		IG		IG		IG			
	CS		MS								
Iceplant											
Pin	sprangletop/switchgrass/bristle										
Rye											

5/29/2015					<Flow						
	IG	IG	IG	IG	IG	IG	IG				
Bridge		IG		IG		IG		IG			
	CS		MS								
Iceplant											
Pin	sprangletop/switchgrass/bristle										
Rye											

7/16/2015					<Flow						
	IG	IG	IG	IG	IG	IG	IG				
Bridge		IG		IG		IG		IG			
	CS		MS								
Iceplant											
Pin	sprangletop/switchgrass/bristle										
Rye											

8/6/2015					<Flow						
	IG	IG	IG	IG	IG	IG	IG				
Bridge		IG		IG		IG		IG			
	CS		MS								
Iceplant											
Pin	sprangletop/switchgrass/bristle										
Rye											

St.357

Figure 15. Station 357

2/19/2015 <Flow											
IG	IG	IG	IG	IG	IG	IG	IG	IG	IG	IG	
			IG		IG		IG		IG		
					MS						Bridge
				MS		MS					
Indian Grass Seed					MS	Indian Grass Seed					
Iceplant											
					Tree_2014						
sprangletop/switchgrass/bristle											
Rye											

3/3/2015 <Flow											
IG	IG-DU	IG	IG	IG	IG	IG	IG	IG	IG	G-Replace	d
		IG	IG-DU	IG	IG	IG	IG	IG	IG		
					MS						Bridge
			IG	MS		MS	IG				
Indian Grass Seed					MS	Indian Grass Seed					
				IG							
Iceplant											
					Tree_2014						
sprangletop/switchgrass/bristle											
Rye											

3/13/2015						<Flow					
IG	IG-DU	IG	IG	IG	IG	IG	IG	IG	IG	G-Replace	d
IG		IG	IG-DU	IG	IG	IG	IG	IG	IG		
IG					MS						Bridge
			IG	MS		MS	IG				
Indian Grass Seed					MS	Indian Grass Seed					
				IG							
Iceplant											
					Tree_2014						
sprangletop/switchgrass/bristle											
Rye											

3/30/2015						<Flow					
IG	IG	IG	IG	IG	IG	IG	IG	IG	IG	IG	
IG		IG	IG-DU	IG	IG	IG	IG	IG	IG		
IG					MS						Bridge
			IG	MS		MS	IG				
Indian Grass Seed					MS	Indian Grass Seed					
				IG							
Iceplant											
					Tree_2014						
sprangletop/switchgrass/bristle											
Rye											

St.357

4/20/2015						<Flow					
IG	IG	IG	IG	IG	IG	IG	IG	IG	IG	IG	
IG		IG	IG-DU	IG	IG	IG	IG	IG	IG		
IG					MS						Bridge
			IG	MS		MS	IG				
Indian Grass Seed					MS	Indian Grass Seed					
				IG							
Iceplant											
					Tree_2014	Pin					
sprangletop/switchgrass/bristle											
Rye											

5/6/2015						<Flow					
IG	IG	IG	IG	IG	IG	IG	IG	IG	IG	IG	
IG		IG	IG-DU	IG	IG	IG	IG	IG	IG		
IG					MS						Bridge
			IG	MS		MS	IG				
Indian Grass Seed					MS	Indian Grass Seed					
				IG							
Iceplant											
					Tree_2014	Pin					
sprangletop/switchgrass/bristle											
Rye											

5/21/2015						<Flow					
IG	IG	IG	IG	IG	IG	IG	IG	IG	IG	IG	
IG		IG	IG-DU	IG	IG	IG	IG	IG	IG		
IG					MS						Bridge
			IG	MS		MS	IG				
Indian Grass Seed					MS	Indian Grass Seed					
				IG							
Iceplant											
					Tree_2014	Pin					
sprangletop/switchgrass/bristle											
Rye											

5/29/2015						<Flow					
IG	IG	IG	IG	IG	IG	IG	IG	IG	IG	IG	
IG		IG	IG-DU	IG	IG	IG	IG	IG	IG		
IG					MS						Bridge
			IG	MS		MS	IG				
Indian Grass Seed					MS	Indian Grass Seed					
				IG							
Iceplant											
					Tree_2014	Pin					
sprangletop/switchgrass/bristle											
Rye											

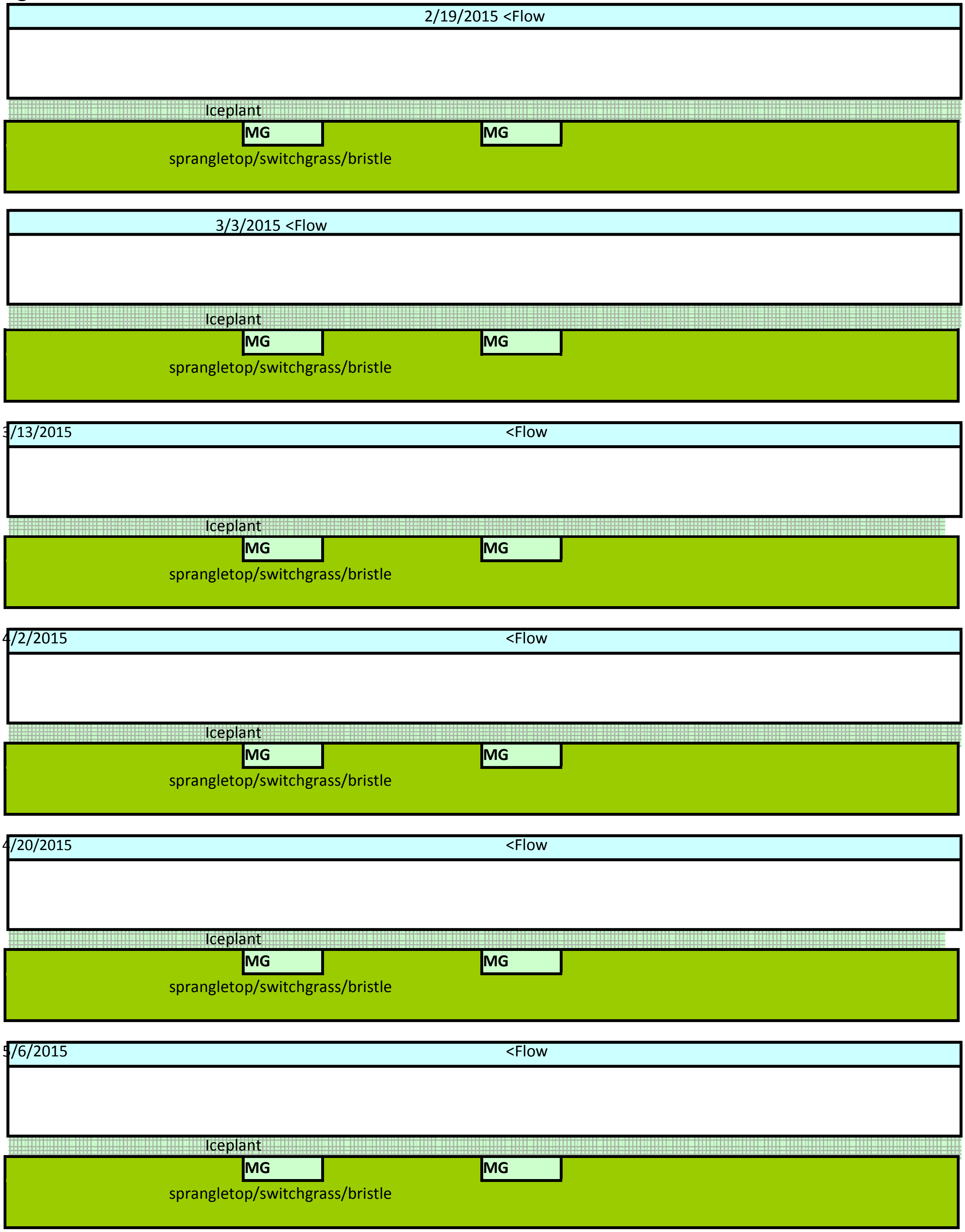
St.357

7/16/2015							<Flow				
IG	IG	IG	IG	IG	IG	IG	IG	IG	IG	IG	
IG		IG	IG-DU	IG	IG	IG	IG	IG	IG		
IG					MS						Bridge
			IG	MS		MS	IG				
Indian Grass Seed					MS	Indian Grass Seed					
				IG							
			Iceplant								
					Tree_2014	Pin					
		sprangletop/switchgrass/bristle									
			Rye								

8/6/2015							<Flow				
IG	IG	IG	IG	IG	IG	IG	IG	IG	IG	IG	
IG		IG	IG-DU	IG	IG	IG	IG	IG	IG		
IG					MS						Bridge
			IG	MS		MS	IG				
Indian Grass Seed					MS	Indian Grass Seed					
				IG							
			Iceplant								
					Tree_2014	Pin					
		sprangletop/switchgrass/bristle									
			Rye								

St.383

Figure 16. Station 383



St.383


5/21/2015
<Flow
Iceplant
MG
MG
sprangletop/switchgrass/bristle
5/29/2015
<Flow
Iceplant
MG
MG
sprangletop/switchgrass/bristle
7/16/2015
<Flow
Iceplant
MG
MG
sprangletop/switchgrass/bristle
8/6/2015
<Flow
Iceplant
MG
MG
sprangletop/switchgrass/bristle

St.386

Figure 17. Station 386

2/19/2015 <Flow											
				IG	IG	IG	IG	IG			
			MS								
Iceplant											
sprangletop/switchgrass/bristle											

3/3/2015 <Flow											
				IG	IG	IG	IG	IG			
			MS								
Iceplant											
sprangletop/switchgrass/bristle											

3/13/2015							<Flow				
				IG	IG	IG	IG	IG			
			MS								
Iceplant											
sprangletop/switchgrass/bristle											

4/2/2015							<Flow				
				IG	IG	IG	IG	IG			
			MS								
Iceplant											
sprangletop/switchgrass/bristle											

4/20/2015							<Flow				
				IG	IG	IG	IG	IG			
			MS								
Iceplant											
sprangletop/switchgrass/bristle											

5/6/2015							<Flow				
				IG	IG	IG	IG	IG			
			MS								
Iceplant											
sprangletop/switchgrass/bristle											

St.386

5/21/2015							<Flow				
				IG	IG	IG	IG	IG			
			MS								
			Iceplant								
			sprangletop/switchgrass/bristle								

5/29/2015							<Flow				
				IG	IG	IG	IG	IG			
			MS								
			Iceplant								
			sprangletop/switchgrass/bristle								

7/16/2015							<Flow				
				IG	IG	IG	IG	IG			
			MS								
			Iceplant								
			sprangletop/switchgrass/bristle								

8/6/2015							<Flow				
				IG	IG	IG	IG	IG			
			MS								
			Iceplant								
			sprangletop/switchgrass/bristle								

St.434

Figure 18. Station 434

St.434

5/21/2015 <Flow

Iceplant

sprangletop/switchgrass/bristle

5/29/2015 <Flow

Iceplant

sprangletop/switchgrass/bristle

7/16/2015 <Flow

Iceplant

sprangletop/switchgrass/bristle

8/6/2015 <Flow

Iceplant

sprangletop/switchgrass/bristle

Figure 19. Station 459

2/19/2015 <Flow											
	IG		IG	Stump	MS				MS		STUMP
		MS									
Iceplant											
sprangletop/switchgrass/bristle											
Rye											

3/3/2015 <Flow											
	IG		IG	Stump	MS				MS		STUMP
		MS									
Iceplant											
sprangletop/switchgrass/bristle											
Rye											

3/13/2015						<Flow					
	IG		IG	Stump	MS				MS		STUMP
		MS									
Iceplant											
sprangletop/switchgrass/bristle											
Rye											

4/2/2015						<Flow					
	IG		IG	Stump	MS				MS		STUMP
		MS									
Iceplant											
sprangletop/switchgrass/bristle											
Rye											

4/20/2015						<Flow					
	IG		IG	Stump	MS				MS		STUMP
		MS									
Iceplant											
sprangletop/switchgrass/bristle											
Rye											

5/6/2015						<Flow					
	IG		IG	Stump	MS				MS		STUMP
		MS									
Iceplant											
sprangletop/switchgrass/bristle											
Rye											

St.459

5/21/2015						<Flow					
	IG		IG	Stump	MS				MS		STUMP
		MS									
		Iceplant									
		sprangletop/switchgrass/bristle									
		Rye									

5/29/2015						<Flow					
	IG		IG	Stump	MS				MS		STUMP
		MS									
		Iceplant									
		sprangletop/switchgrass/bristle									
		Rye									

7/16/2015						<Flow					
	IG		IG	Stump	MS				MS		STUMP
		MS									
		Iceplant									
		sprangletop/switchgrass/bristle									
		Rye									

8/6/2015						<Flow					
	IG		IG	Stump	MS				MS		STUMP
		MS									
		Iceplant									
		sprangletop/switchgrass/bristle									
		Rye									

Figure 20. Station 490 (CLEAR PT)

2/19/2015 <Flow											
				TREE_2014	IG	IG	ROCK		MS		
		MG	STUMP				IG				
					NO BARRIER						
MG	MG	MG	MG	MG			MG	MG	MG	TREE	MS
							TREE	MG			
sprangletop/switchgrass/bristle											

3/3/2015 <Flow											
				TREE_2014	IG	IG	ROCK		MS		
		MG_DU	STUMP				IG				
					NO BARRIER						
MG	MG	MG	MG	MG			MG	MG	MG	TREE	MS
							TREE	MG			
sprangletop/switchgrass/bristle											

3/13/2015						<Flow					
MG	MG	MG	MG		MG	MG	MG				
				TREE_2014	IG	IG	ROCK		MS		
		MG_DU	STUMP				IG				
					NO BARRIER						
MG	MG	MG	MG	MG			MG	MG	MG	TREE	MS
							TREE	MG			
sprangletop/switchgrass/bristle											

4/2/2015						<Flow					
MG	MG	MG-DU	MG-DU		MG	MG	MG				
				TREE_2014	IG	IG	ROCK		MS		
		MG_DU	STUMP				IG				
					NO BARRIER						
MG	MG	MG	MG	MG			MG	MG	MG	TREE	MS
							TREE	MG			
sprangletop/switchgrass/bristle											

4/20/2015						<Flow					
MG	MG	MG	MG		MG	MG	MG				
				TREE_2014	IG	IG	ROCK		MS		
		MG_DU	STUMP				IG				
					NO BARRIER						
MG	MG	MG	MG	MG			MG	MG	MG	TREE	MS
							TREE	MG			
sprangletop/switchgrass/bristle											

5/6/2015						<Flow					
MG	MG	MG	MG		MG	MG	MG				
				TREE_2014	IG	IG	ROCK		MS		
		MG_DU	STUMP				IG				
					NO BARRIER						
MG	MG	MG	MG	MG			MG	MG	MG	TREE	MS
							TREE	MG			
sprangletop/switchgrass/bristle											

St.490

5/21/2015						<Flow					
MG	MG	MG	MG		MG	MG	MG				
				TREE_2014	IG	IG	ROCK		MS		
		MG_DU	STUMP				IG				
					NO BARRIER						
MG	MG	MG	MG	MG			MG	MG	MG	TREE	MS
							TREE	MG			
		sprangletop/switchgrass/bristle									

5/29/2015						<Flow					
MG	MG	MG	MG		MG	MG	MG				
				TREE_2014	IG	IG	ROCK		MS		
		MG_DU	STUMP				IG				
					NO BARRIER						
MG	MG	MG	MG	MG			MG	MG	MG	TREE	MS
							TREE	MG			
		sprangletop/switchgrass/bristle									

7/16/2015						<Flow					
MG	MG	MG	MG		MG	MG	MG				
				TREE_2014	IG	IG	ROCK		MS		
		MG_DU	STUMP				IG				
					NO BARRIER						
MG	MG	MG	MG	MG			MG	MG	MG	TREE	MS
							TREE	MG			
		sprangletop/switchgrass/bristle									

8/6/2015						<Flow					
MG	MG	MG	MG		MG	MG	MG				
				TREE_2014	IG	IG	ROCK		MS		
		MG_DU	STUMP				IG				
					NO BARRIER						
MG	MG	MG	MG	MG			MG	MG	MG	TREE	MS
							TREE	MG			
		sprangletop/switchgrass/bristle									

St.600

Figure 21. Station 600

2/19/2015 <Flow											
		IG		IG			IG		IG		
	Tree							IG		BLACK WALN UT	
IG		IG		IG			IG				
Iceplant											

3/3/2015 <Flow											
		IG		IG			IG		IG		
	Tree							IG		BLACK WALN UT	
IG		IG		IG			IG				
Iceplant											

3/13/2015						<Flow					
		IG		IG			IG		IG		
	Tree							IG		BLACK WALN UT	
IG		IG		IG			IG				
Iceplant											

4/2/2015						<Flow					
		IG		IG			IG		IG		
	Tree							IG		BLACK WALN UT	
IG		IG		IG			IG				
Iceplant											

4/20/2015						<Flow					
		IG		IG			IG		IG		
	Tree							IG		BLACK WALN UT	
IG		IG		IG			IG				
Iceplant											

5/6/2015						<Flow					
		IG		IG			IG		IG		
	Tree							IG		BLACK WALN UT	
IG		IG		IG			IG				
Iceplant											

St.600

5/21/2015						<Flow					
		IG		IG			IG		IG		
	Tree							IG		BLACK WALN UT	
IG		IG		IG			IG				
		Iceplant									

5/29/2015						<Flow					
		IG		IG			IG		IG		
	Tree							IG		BLACK WALN UT	
IG		IG		IG			IG				
		Iceplant									

7/16/2015						<Flow					
		IG		IG			IG		IG		
	Tree							IG		BLACK WALN UT	
IG		IG		IG			IG				
		Iceplant									

8/6/2015						<Flow					
		IG		IG			IG		IG		
	Tree							IG		BLACK WALN UT	
IG		IG		IG			IG				
		Iceplant									

St.640

Figure 22. Station 640

2/19/2015 <Flow

	IG		IG		IG		IG		IG	IG	IG	IG	IG	TREE
MS		CS			CS	IG	CS							

Iceplant

sprangletop/switchgrass/bristle

Rye

3/3/2015 <Flow

	IG		IG		IG		IG		IG	IG	IG	IG	IG	TREE
MS		CS			CS	IG	CS							

Iceplant

sprangletop/switchgrass/bristle

Rye

3/13/2015 <Flow

	IG		IG		IG		IG		IG	IG	IG	IG	IG	TREE
MS		CS			CS	IG	CS							

Iceplant

sprangletop/switchgrass/bristle

Rye

4/2/2015 <Flow

	IG		IG		IG		IG		IG	IG	IG	IG	IG	TREE
MS		CS			CS	IG	CS							

Iceplant

Pin

sprangletop/switchgrass/bristle

Rye

4/20/2015 <Flow

	IG		IG		IG		IG		IG	IG	IG	IG	IG	TREE
MS		CS			CS	IG	CS							

Iceplant

Pin

sprangletop/switchgrass/bristle

Rye

5/6/2015 <Flow

	IG		IG		IG		IG		IG	IG	IG	IG	IG	TREE
MS		CS			CS	IG	CS							

Iceplant

Pin

sprangletop/switchgrass/bristle

Rye

5/21/2015 <Flow

	IG		IG		IG		IG		IG	IG	IG	IG	IG	TREE
MS		CS			CS	IG	CS							

Iceplant

Pin

sprangletop/switchgrass/bristle

Rye

St.640

5/29/2015							<Flow							
	IG		**IG**		**IG**		**IG**		**IG**	**IG**	**IG**	**IG**	**IG**	TREE
MS		**CS**			**CS**	**IG**	**CS**							
		Iceplant												
			Pin											
sprangletop/switchgrass/bristle														
		Rye												

7/16/2015							<Flow							
	IG		**IG**		**IG**		**IG**		**IG**	**IG**	**IG**	**IG**	**IG**	TREE
MS		**CS**			**CS**	**IG**	**CS**							
		Iceplant												
			Pin											
sprangletop/switchgrass/bristle														
		Rye												

8/6/2015							<Flow							
	IG		**IG**		**IG**		**IG**		**IG**	**IG**	**IG**	**IG**	**IG**	TREE
MS		**CS**			**CS**	**IG**	**CS**							
		Iceplant												
			Pin											
sprangletop/switchgrass/bristle														
		Rye												

Figure 23. Station 672

2/19/2015 <Flow

	IG		IG	IG			IG		IG		IG		IG	IG	IG
Stump		IG					TREE								
	IG		MS	MS		MS		IG		IG		IG		IG	
Iceplant															
MG 14	Tree 14		MG 14	MG 14											
sprangletop/switchgrass/bristle															
Rye															

3/3/2015 <Flow

	IG		IG	IG			IG		IG		IG		IG	IG	IG
Stump		IG					TREE								
	IG		MS	MS		MS		IG		IG		IG		IG	
Iceplant															
MG 14	Tree 14		MG 14	MG 14											
sprangletop/switchgrass/bristle															
Rye															

3/13/2015 <Flow

	IG		IG	IG			IG		IG		IG		IG	IG	IG
Stump		IG			MG	MG	TREE								
	IG		MS	MS		MS		IG		IG		IG		IG	
Iceplant															
MG 14	**Tree 14**		MG 14	MG 14											
sprangletop/switchgrass/bristle															
Rye															

4/2/2015 <Flow

	IG		IG	IG			IG		IG		IG		IG	IG	IG
Stump		IG			MG	MG	TREE								
	IG		MS	MS		MS		IG		IG		IG		IG	
Iceplant															
MG 14	**Tree 14**		MG 14	MG 14	**Pin**										
sprangletop/switchgrass/bristle															
Rye															

4/20/2015 <Flow

	IG		IG	IG			IG		IG		IG		IG	IG	IG
Stump		IG			MG	MG	TREE								
	IG		MS	MS		MS		IG		IG		IG		IG	
Iceplant															
MG 14	Tree 15		MG 14	MG 14	**Pin**										
sprangletop/switchgrass/bristle															
Rye															

5/6/2015 <Flow

	IG		IG	IG			IG		IG		IG		IG	IG	IG
Stump		IG			MG	MG	TREE								
	IG		MS	MS		MS		IG		IG		IG		IG	
Iceplant															
MG 14	Tree 15		MG 14	MG 14	**Pin**										
sprangletop/switchgrass/bristle															
Rye															

5/21/2015 <Flow

	IG		IG	IG			IG		IG		IG		IG	IG	IG
Stump		IG			MG	MG	TREE								
	IG		MS	MS		MS		IG		IG		IG		IG	
Iceplant															
MG 14	Tree 15		MG 14	MG 14	**Pin**										
sprangletop/switchgrass/bristle															
Rye															

5/29/2015 <Flow

	IG		IG	IG			IG		IG		IG		IG	IG	IG
Stump		IG			MG	MG	TREE								
	IG		MS	MS		MS		IG		IG		IG		IG	
Iceplant															
MG 14	Tree 15		MG 14	MG 14	**Pin**										
sprangletop/switchgrass/bristle															

St.672

7/16/2015									<Flow						
	IG		**IG**	**IG**			**IG**		**IG**		**IG**		**IG**	**IG**	**IG**
Stump		**IG**			MG	MG	TREE								
	IG		**MS**	**MS**		**MS**		**IG**		**IG**		**IG**		**IG**	
Iceplant															
MG 14	Tree 15		MG 14	MG 14	**Pin**										
sprangletop/switchgrass/bristle															
Rye															

8/6/2015									<Flow						
	IG		**IG**	**IG**			**IG**		**IG**		**IG**		**IG**	**IG**	**IG**
Stump		**IG**			MG	MG	TREE								
	IG		**MS**	**MS**		**MS**		**IG**		**IG**		**IG**		**IG**	
Iceplant															
MG 14	Tree 15		MG 14	MG 14	**Pin**										
sprangletop/switchgrass/bristle															
Rye															

St.740

Figure 24. Station 740

2/19/2015 <Flow

				IG		IG		IG	IG	IG				
											IG			

Iceplant

sprangletop/switchgrass/bristle

Rye

3/3/2015 <Flow

				IG		IG		IG	IG	IG				
									CS		IG			

Iceplant

sprangletop/switchgrass/bristle

Rye

3/13/2015 <Flow

				IG		IG		IG	IG	IG				
									CS		IG			

Iceplant

sprangletop/switchgrass/bristle

Rye

4/2/2015 <Flow

				IG		IG		IG	IG	IG				
									CS		IG			

Iceplant

sprangletop/switchgrass/bristle

Rye

4/20/2015 <Flow

				IG		IG		IG	IG	IG				
									CS		IG			

Iceplant

Pin sprangletop/switchgrass/bristle

Rye

5/6/2015 <Flow

				IG		IG		IG	IG	IG				
									CS		IG			

Iceplant

Pin sprangletop/switchgrass/bristle

Rye

5/21/2015 <Flow

				IG		IG		IG	IG	IG				
									CS		IG			

Iceplant

Pin sprangletop/switchgrass/bristle

Rye

St.740

7/16/2015 <Flow

				IG		IG		IG	IG	IG				
									CS		IG			

Iceplant

Pin sprangletop/switchgrass/bristle

Rye

8/6/2015 <Flow

				IG		IG		IG	IG	IG				
									CS		IG			

Iceplant

Pin sprangletop/switchgrass/bristle

Rye

Figure 25. Station 780

2/19/2015 <Flow

IG	IG	IG	IG	MS		MS		MS		MS		MS			Tree
					MS		MS		MS		MS		MS		Tree
							SeaOats Seed								
Iceplant															
sprangletop/switchgrass/bristle															
Rye															

3/3/2015 <Flow

IG	IG	IG-DU	IG	MS		MS		MS		MS		MS			Tree
					MS		MS		MS		MS		MS		Tree
							SeaOats Seed								
Iceplant															
sprangletop/switchgrass/bristle															
Rye															

3/13/2015 <Flow

IG	IG	IG	IG	MS		MS		MS		MS		MS			Tree
					MS		MG	MG	MS		MS		MS		Tree
							SeaOats Seed								
Iceplant															
sprangletop/switchgrass/bristle															
Rye															

4/2/2015 <Flow

IG	IG	IG	IG	MS		MS		MS		MS		MS			Tree
					MS		MG	MG	MS		MS		MS		Tree
							SeaOats Seed								
Iceplant															
sprangletop/switchgrass/bristle															
Rye															

4/20/2015 <Flow

IG	IG	IG	IG	MS		MS		MS		MS		MS			Tree
					MS		MG	MG	MS		MS		MS		Tree
							SeaOats Seed								
Iceplant															
Pin sprangletop/switchgrass/bristle															
Rye															

5/6/2015 <Flow

IG	IG	IG	IG	MS		MS		MS		MS		MS			Tree
					MS		MG	MG	MS		MS		MS		Tree
							SeaOats Seed								
Iceplant															
Pin sprangletop/switchgrass/bristle															
Rye															

5/21/2015 <Flow

IG	IG	IG	IG	MS		MS		MS		MS		MS			Tree
					MS		MG	MG	MS		MS		MS		Tree
							SeaOats Seed								
Iceplant															
Pin sprangletop/switchgrass/bristle															
Rye															

5/29/2015 <Flow

IG	IG	IG	IG	MS		MS		MS		MS		MS			Tree
					MS		MG	MG	MS		MS		MS		Tree
							SeaOats Seed								
Iceplant															
Pin sprangletop/switchgrass/bristle															

St.780

7/16/2015	<Flow														
IG	IG	IG	IG	MS		MS		MS		MS		MS			Tree
					MS		MG	MG	MS		MS		MS		Tree
							SeaOats Seed								
Iceplant															
Pin sprangletop/switchgrass/bristle															
Rye															

8/6/2015	<Flow														
IG	IG	IG	IG	MS		MS		MS		MS		MS			Tree
					MS		MG	MG	MS		MS		MS		Tree
							SeaOats Seed								
Iceplant															
Pin sprangletop/switchgrass/bristle															
Rye															

St.844

Figure 26. Station 844

2/19/2015 <Flow															
		IG	IG						Big Tree	IG	IG	IG	IG		Rocks
					MS		MS				IG	IG	IG		Rocks
		SeaOats Seed				MS		Grapefruit		SeaOats Seed					
			Iceplant												
				Tree_2014		Tree_2014	sprangletop/switchgrass/bristle								
				Rye											

Hard to find IG next to cliff

3/3/2015 <Flow															
		IG	IG						Big Tree	IG	IG	IG	IG		Rocks
					MS		MS				IG	IG	IG		Rocks
		SeaOats Seed				MS		Grapefruit		SeaOats Seed					
			Iceplant												
				Tree_2014		Tree_2014	sprangletop/switchgrass/bristle								
				Rye											

3/13/2015 <Flow															
		IG	IG						Big Tree	IG	IG	IG	IG		Rocks
					MS		MS	MG			IG-DU	IG	IG		Rocks
		SeaOats Seed		MG	MG	MS-DU	MG	Grapefruit		SeaOats Seed					
			Iceplant												
				Tree_2014		Tree_2014	sprangletop/switchgrass/bristle								
				Rye											

4/2/2015								<Flow							
		IG	IG						Big Tree	IG	IG	IG	IG		Rocks
					MS		MS	MG			IG	IG	IG		Rocks
		SeaOats Seed		MG	MG	MS	MG	Grapefruit		SeaOats Seed					
			Iceplant												
				Tree_2014		Tree_2014	sprangletop/switchgrass/bristle								
				Rye											

4/20/2015 <Flow															
		IG	IG						Big Tree	IG	IG	IG	IG		Rocks
					MS		MS	MG			IG	IG	IG		Rocks
		SeaOats Seed		MG	MG	MS	MG	Grapefruit		SeaOats Seed					
			Iceplant												
				Tree_2014		Tree_2014	sprangletop/switchgrass/bristle								
				Rye											

5/6/2015								<Flow							
		IG	IG						Big Tree	IG	IG	IG	IG		Rocks
					MS		MS	MG			IG	IG	IG		Rocks
		SeaOats Seed		MG	MG	MS	MG	Grapefruit		SeaOats Seed					
			Iceplant												
				Tree_2014		Tree_2014	sprangletop/switchgrass/bristle								
				Rye											

5/21/2015 <Flow															
		IG	IG						Big Tree	IG	IG	IG	IG		Rocks
					MS		MS	MG			IG	IG	IG		Rocks
		SeaOats Seed		MG	MG	MS	MG	Grapefruit		SeaOats Seed					
			Iceplant												
				Tree_2014		Tree_2014	sprangletop/switchgrass/bristle								
				Rye											

5/29/2015 <Flow															
		IG	IG						Big Tree	IG	IG	IG	IG		Rocks
					MS		MS	MG			IG	IG	IG		Rocks
		SeaOats Seed		MG	MG	MS	MG	Grapefruit		SeaOats Seed					
			Iceplant												
				Tree_2014		Tree_2014	sprangletop/switchgrass/bristle								
				Rye											

St.844

			7/16/2015	<Flow											
		IG	**IG**						Big Tree	**IG**	**IG**	**IG**	**IG**		Rocks
					MS		**MS**	MG			**IG**	**IG**	**IG**		Rocks
		SeaOats Seed		MG	MG	**MS**	MG	Grapefruit		SeaOats Seed					
			Iceplant												
				Tree_2014		Tree_2014	sprangletop/switchgrass/bristle								
				Rye											

8/6/2015								<Flow							
		IG	**IG**						Big Tree	**IG**	**IG**	**IG**	**IG**		Rocks
					MS		**MS**	MG			**IG**	**IG**	**IG**		Rocks
		SeaOats Seed		MG	MG	**MS**	MG	Grapefruit		SeaOats Seed					
			Iceplant												
				Tree_2014		Tree_2014	sprangletop/switchgrass/bristle								
				Rye											

Figure 27. Station 956

4/20/2015 Planted Red Mulberry
5/6/2015 Pic 2366
5/21/2015 Pic 2732, 33
5/29/2015 Pic 2812

St.987

Figure 28. Station 987

St.987

8/6/2015 <Flow

Iceplant sprangletop/switchgrass/bristle

Appendix L8

2016 Landa Park Golf Course Integrated Pest Management Plan

2016

Landa Park Golf Course

Integrated Pest Management Plan

City of New Braunfels

1/1/2016

Table of Contents

Landa Park Golf Course
Integrated Pest Management Plan

Introduction

Landa Park Golf Course recognizes the importance of sound environmental stewardship and the sensitivity to surrounding areas. That is why we are committed to optimizing the golf course management practices to protect the environment within, and surrounding the golf course. The epicenter of environmental stewardship at Landa Park Golf Course is the philosophy of Integrated Pest Management (IPM).

Several examples of cultural methods to control pests include optimizing turf health through best management practices to enhance natural plant resistance to pest infestations, optimizing plant health and vigor, and minimizing turf damage resulting from routine golf course operations. When cultural practices are not fully effective at controlling pests that exceeds established pest threshold levels, the use of pesticides to minimize and manage pest damage may be required. An essential part of an Integrated Pest Management plan is to coordinate the use of cultural practices and pest management to minimize the amounts of pesticide applications.

Included in this plan, you will find defined managements areas, cultural practices, anticipated pests and their timing, monitoring practices, pest damage threshold levels, anticipated actions and control measures, and an explanation of control measures with their benefits. The plan also serves as an anticipated operational management plan that contains details regarding management practices on and around the course. As a result, this document is to be viewed as a functional document that will evolve over time and be updated on an annual basis. This will ensure updated environmental concerns and industry standards continue to be exceeded as we provide the highest quality product possible to our golfing clientele and the City of New Braunfels.

Integrated Pest Management Definition

There are numerous definitions of Integrated Pest Management. In detail, we believe the best summarized definition is provided by the Golf Course Superintendents Association of America.

"Integrated pest management is a continuous system by which pests (weeds, diseases, insects or others) are identified, action thresholds are considered, all possible control options are evaluated and control(s) are implemented. Control options – which include biological, chemical, cultural, manual and mechanical methods – are used to prevent or remedy unacceptable pest activity or damage. Choice of control option(s) is based on effectiveness, environmental impact, site characteristics, worker/public health and safety, and economics."

"The goal of an IPM system is to manage pests and the environment to balance benefits of control, environmental quality, costs, public health and site specific requirements. IPM takes advantage of all appropriate pest management options."

IPM Objectives

- Minimize potential hazards to human and environmental components
- Providing optimal course playing conditions
- Minimize the amount of chemical applications and optimize application efficacy
- Enhance communication with co-workers, golfers, golf course management, and outside agencies regarding agronomic and pest management practices
- Control operating costs and maximize budget restrictions with proper planning and execution
- Provide site specific planning to develop a detailed plan on specific management sites
- Easy access to essential information on pest biology, control, agronomic guidelines, and monitoring tools and references

IPM Structure

The outline of the Integrated Pest Management plan is structured such that it targets specific management zones and the anticipated pests in those zones. It targets those pests that pose the largest threat to the overall agronomic health of the golf course and its environment. The structure of the IPM plan is outlined below:

- Define the management zones that require specific maintenance and monitoring intensity in each specific area
- Identify likely pests that will be encountered and anticipated timeline those pests are expected
- Identify and implement cultural practices that increase turf health and vigor, increasing pest tolerance and resistance
- Establish threshold levels for each pest while outlining corrective action if thresholds are exceeded
- Outline all possible preventative and corrective actions that address maintenance practices, chemical application options, and explanation of chemical selection based on efficacy and environmental impact
- Document monitoring, treatments, and treatment results

Area Definition

The Landa Park Golf Course is a municipal golf course originally opened in 1938 and consisting of a nine hole layout confined to the west side of the railroad tracks that currently splits the golf course. The second nine holes was added and opened in 1971 and expanded the course by creating another nine holes to the east of the railroad track division. The golf course was constructed using native soil for fairway, rough, and tee construction which consists of a silty clay (heavier soil). Based on cup cutting operations as well as aerification outcomes, it is expected that a sand material was used or supplemented in the use of the push up greens when they were renovated and/or constructed in the 1970's. The golf course went through a full reconstruction starting in the fall of 2013 and reopened in September of 2014. The construction of the course is still primarily the same as far as native soils. However, the tees now have sand found in the top four inches and the greens were built to USGA spec.

The course is located the town of New Braunfels, Texas and sits between the 8th and 9th plant hardiness zones as outlined by the USDA. By most estimates, this locates the course south of what is perceived, as the turfgrass transition zone.   The management zones of this course consist of turfgrass areas, non-turfgrass areas, aquatic areas, and native areas.

A. Turfgrass Areas

The turfgrass areas are broken into three to four different management sub-zones due to the diversity of features and management requirements. These turfgrass area sub-zones include greens, tees/fairways, rough, and native areas. The greens consist of an ultra dwarf bermudagrass called Miniverde. The turfgrass found on the tees, fairways, and rough consist of a hybrid bermudagrass called Tifway 419, except for three tee boxes on the course that have a type of Zoysia grass on them called Cavalier. The turfgrass in the native areas include wildflowers, shrubs, trees, and a variety of other mixes depending on location. Many of these areas are in native surrounds of the course and provide a buffer between the course and its bordering features.

TABLE 1> Landa Park Municipal Golf Course Turfgrass Area Maintenance Requirements

Area	Total Acreage	Fertilizer Requirement	Irrigation Requirement	Mowing Frequency	Cultural Frequency
Greens	3	High	High	High	High
Tee Surface	2	Medium	Medium	Medium	Low
Fairway	21	Medium	Medium	Medium	Low
Rough	60	Low	Low - N/A	Low	Low
Native	18	N/A	N/A	N/A	N/A

B. Non-Turfgrass Areas

These areas consist of bunkers, flower beds, aquatic areas, and natural areas. These will play a less significant role in the overall environmental health of the property. However, they play a significant role in the overall aesthetic and operational aspects of the course.

Bunkers

There are thirty-five bunkers that are strategically placed throughout the entire golf course. Bunker maintenance will involve raking bottoms with sand pro, hand raking edges, edging bunker outlines, and removal of debris.

Flower Beds

A number of flower beds exist around the clubhouse grounds and hitting cages. These areas are aesthetically significant and grab the eye of patrons upon approach to the property. High standards of maintenance will be expected and performed in these areas.

Aquatic areas

Four irrigation ponds and one aesthetic pond exist on the course. All five play an important role in the playability and design of the current course layout.

Buffer Zones

Buffer zones currently exist adjacent to the waterways and watershed locations that receive no fertilizer or pesticide applications. Currently we strive to maintain a minimum of 25 foot buffer zone at all points that surround the course with the exception of some greens and tee boxes that lie within the 25 foot boundary. In these areas, special care and consideration are given when making fertilizer and pesticide applications.

Cart Paths and Service Roads

The entire course has an infrastructure of cart path that includes a continuous concrete path that extends from tee to green and back to tee of the next hole.

Turfgrass Management Practices

Turfgrass area management involves the largest demand on the labor force. The primary objective of the IPMp is to optimize turfgrass quality and health utilizing best management practices to reduce pest infestation and turfgrass resistance to stress. The primary cultural practices include mowing, fertilization, and irrigation. Secondary cultural practices include practices such as aeration, topdressing, verticutting, and overseeding.

A. Primary Cultural Practice

1. Mowing

   Mowing is performed as needed based on growth conditions and playability needs. During the peak growing season, mowing will occur as defined below:

Area	Frequency of Mowing	Mowing Height (inches)
Greens	Daily	.110 - .175
Tees, Collars & Fairways	2-3 Times a Week	.400 - .600
Rough Areas	Once Weekly	1 - 3

2. Fertilization

Management of nutrient availability is crucial in maintaining turfgrass health and vigor. Management of turf fertility involves the understanding of soil composition, plant nutrient requirements, fertility management history, use of soil test information, and applications of the appropriate fertilizer with the proper application timing. The objective of the fertilizer program is to provide optimal nutrient availability to the turf while simultaneously avoiding the application of excess nutrients to avoid nutrient runoff/leaching, disease development and weed infestation.

a. Soil Testing
Testing for proper soil nutrient levels will be conducted on a minimum of a bi-annually basis to insure that current practices are providing adequate soil nutrient levels key for optimized turf quality. Testing also gives insight to specialized nutrient applications that can minimize blanket applications and provide a focused approach to any deficiencies that may occur. The use of tissue testing may also be involved to access the accuracy of available nutrients in the soil for plant utilization.

b. Turfgrass Nutrient Requirements
The major nutrients for turfgrass health are nitrogen, phosphorus, potassium (NPK) along with calcium, magnesium, and sulfur. Essential minor nutrients include iron, boron, copper, manganese, molybdenum, zinc, chlorine, and nickel. The availability of nutrients to turfgrass is influenced markedly by the pH of the soil. Consequently, maintenance of the appropriate pH is an important component of the fertilization program. Whenever possible, slow release fertilizers will be used as the primary source of nutrients, with adjustments being made for special needs and conditions. Greens fertilization programs may also include light applications of soluble foliar-adsorbed applied on a frequent basis.

Major Nutrients

1) Nitrogen
The management of nitrogen levels is critical owing to the high turf demand for this nutrient and the potential for excess nitrogen to enter into surface water and/or groundwater. As a result, the

amount of nitrogen delivered to turfgrass will be the minimum amount necessary to promote turf vigor. In general, nitrogen rates and formulations will be determined based on turf condition, soil test results, season, weather, and other information. In certain instances when turf and/or climate conditions dictate, rates of application will be adjusted (either higher or lower) at the discretion of the Superintendent.

Nitrogen formulation consists of water insoluble (slow release) and water soluble (quick release) types. Slow release nitrogen sources include methylene urea, sulfur-coated urea, IBDU, polymer coated fertilizers, and organic fertilizers processed and formulated as slow release products. Examples of quick release nitrogen sources include ammonium sulfate, ammonium nitrate, potassium nitrate, and urea. "Bridge" fertilizers combine the best qualities of synthetic and organic fertilizers providing both quick and slow release of nutrients. Where appropriate, organic formulations will be considered for providing sustainable slow release nutrients, soil organic matter, and potentially higher soil biological activity. To maximize plant uptake and minimize nitrogen leaching or storm water runoff (e.g., nitrate), slow release nitrogen sources and/or light applications of soluble nitrogen ("spoonfeeding") will be used whenever possible.

2) Phosphorus
Turf requirements for phosphorus are relatively low and phosphorus is relatively immobile in soil. As a result, application rates tend to be correspondingly low, which minimizes the possibility of leaching or storm water runoff carrying residual phosphorus off-site.

3) Potassium
Turf requirements for potassium are intermediate to high in relation to nitrogen and phosphorus levels. Although applied to maximize efficiency of uptake, potassium does not pose the extent of environmental risk that excess nitrogen and phosphorus levels represent. Proper levels of potassium are an important component of plant disease resistance and contribute to the ability of turf to withstand wear and traffic.

Minor Nutrients
In general, turfgrass requirements for the minor nutrients iron, boron, copper, manganese, molybdenum, and zinc are substantially lower than those for nitrogen, phosphorus, and potassium. Minor nutrients are essential for optimal turf performance and are typically available in soils

in sufficient quantities to support healthy turf. However, when turf conditions or soil testing results indicate deficiencies, these nutrients will be applied at the discretion of the Superintendent.

pH
Maintenance of the proper soil pH is essential in optimizing the availability of nutrients, and is important in minimizing overall turfgrass stress. When the soil pH requires adjustment to the more alkaline pH, lime will be added until the targeted pH is obtained. When soil requires adjustments to a more acidic pH, ammonium sulfate or other acidifying product will be added until the targeted pH is obtained.

c. Fertilizer Treatment Areas
The rate and frequency of fertilizer application is area and situation dependent. A typical area-specific fertilizer application frequency, and corresponding total yearly nitrogen applied (lbs/1000 ft^2) is shown in Table 2. Fertilizer application is most frequent on greens with less frequent applications being made to tees and fairways, and the least frequent application being made to the rough.

TABLE 2> Landa Park Municipal Golf Course:
Fertilizer Application Areas and Typical Yearly Applications

Area	Total Acreage	Applications per Year	Total Nitrogen per Year
Greens	**3**	**10-14**	**4 - 6 lbs**
Tee Surface	**2**	**3-4**	**2 - 4 lbs**
Fairway	**21**	**3-4**	**2 - 4 lbs**
Immediate Rough	**25**	**2-3**	**2 - 3 lbs**
Secondary Rough	**35**	**1-2**	**1 - 2 lbs**

d. Fertilizer Application
Fertilizer application equipment will be calibrated prior to use to ensure proper rate of application. Fertilizer will not be applied if heavy rain is forecasted following the potential application event.

e. Fertilizer Storage
All fertilizer will be maintained in a dedicated moisture free, well ventilated, approved storage area.

f. Fertilizer Documentation
   All fertilizer applications will be documented on a fertilizer application form. Information recorded will include date of application, location of application, total area treated, formulation of fertilizer, rate of application expressed as lbs. of N/1000 ft2, total quantity of product applied, and the applicator name.

3. Irrigation

   The distribution of adequate water onto turf via irrigation, without over-watering, is essential to turf health. In addition to providing optimal moisture levels for turf, irrigation practices are designed to conserve water whenever possible. Wetting agents will be used when necessary to improve water infiltration for localized dry spots and other hydrophobic areas of turf. Wetting agents will be applied in accordance with label rates and recommendations.

   a. Water Source
      Landa Park Municipal Golf Course currently has 300 acre feet of water diversion rights from the old channel of the Comal river. Irrigation water is currently diverted from a point adjacent to number sixteen fairway and behind number six green. From there the water is pumped to the holding ponds located adjacent number eleven fairway and number three fairway.

   b. Irrigation System
      The irrigation system is a computer integrated automated system that operates twenty irrigation satellite boxes in the field. The system was installed during the 2014 reconstruction period. The new system is put together with HDPE pipe and therefore, can withstand greater pressure and sustain and longer life period. The new irrigation system also stretches to all boundaries of the golf course, therefore providing healthier turf wall to wall. Daily water use is still determined using resources such as local weather stations that determine sports turf ET rates, as well as, on site scouting for any additional needs.

   c. Irrigation Water Quality
      No irrigation quality problems are anticipated with the use of the water from current irrigation sources. However, continued testing to evaluate any changes in water quality will be performed. It is recommended to take water samples from the sprinkler head to get an accurate reading of water quality landing on the turf and not taking from source water in the pond.

   d. Water Conservation
      The irrigation system and program is designed to prevent over-application of water as a means of optimizing turf vigor and conserving water. The

area needing the most frequent irrigation is the greens. Because it does represent a larger area of coverage, fairways, roughs and native areas are irrigated using a deeper, less frequent method to help increase water efficiency.

The primary means of determining turfgrass irrigation requirements will be the evapotranspirational losses determined by computer supplemented data along with daily observations and monitoring by the Superintendent and staff. Data obtained from a local weather station is entered into the computer controlled irrigation system to establish site-specific irrigation duration and frequencies.

B. Secondary Cultural Practice

1. Aerification

Aerification is the practice of removing soil cores from turf and is performed to reduce turf compaction. This practice enhances the movement of air, water and nutrients in the soil. Aerification will occur primarily on greens and tee surfaces on a regular basis, at least twice a year. Aerification is typically performed during periods of active turf growth in the mid-spring thru early fall. Additional aeration may occur at the discretion of the Superintendent. Aerified greens will be topdressed with sand to fill aerification holes and improve water and air infiltration.

2. Thatch Management

Thatch is a layer of organic debris and the roots, crowns, and stems of grass that exist between the soil and the turf canopy. In the absence of cultural management, this layer becomes thicker over time, resulting in sub-optimal turf growth. This also has a negative effect on ball roll and greens conditions which make playability suffer and condition to deteriorate. It can also cause the turfgrass to be more susceptible to disease. Thatch management will include hollow core aerification, topdressing, and vertical mowing.

3. Topdressing

The practice of topdressing consists of the application of sand to the greens complex and is used to assist in the thatch layer management. It also assists in maintaining a smooth and fast putting surface. Topdressing applications typically follow the aerification or verticutting of greens, and will also be made in the absence of aerification ("light" topdressing). Light brushing may be used after topdressing to work sand into the turf surface.

4. Overseeding

Overseeding in late fall provides a course that is playable and green year-round. Fall overseeding consists of perennial ryegrass on tees and possibly fairways. Rates may vary depending on playing surface desirability and quality necessary. With the greens being renovated to Miniverde there is no longer and need to overseed them. This is because of the much greater turf density the Miniverde consist of compared to the older mutated greens that were believed to originate from either Tifgreen 328 or Tifdwarf.

Tree Management

A variety of trees are located at Landa Park Golf Course and require routine maintenance. Best management practices will be performed and are listed below. Many of these assessments will be performed with the assistance of the Urban Forester, a certified arborist, to ensure proper technique and evaluation.

A. Tree Maintenance

Routine evaluation and monitoring for influence on playing characteristics, overall health, influence on turfgrass and surrounding environment, and any safety concerns. Current tree populations that are established on the golf course do not require supplemental irrigation except for special considerations during periods of extreme drought. Trees will be pruned during the late fall and winter months to avoid potential disease and pest encroachment on open wounds. These will be pruned to optimize health, allow passage of light, minimize hazard, and manage pests. The use of pruning paint and wound treatment will be adhered to on sensitive trees to promote health while performing pruning practices.

B. Tree Removal

With the consultation of the Urban Forester and the Golf Course Superintendent, the need for tree removal may be necessary based on disease, age, and potential safety issues.

C. Tree Planting

During the 2013- 2014 renovation an estimated number of 200 trees were planted. These trees were planted in planting holes appropriate for the root ball/root mass and planting holes were backfilled with native material. The planted areas will receive irrigation as required through the first two to three growing seasons. Anymore possible planting will occur during the fall.

Composting and Organic Materials Management

A. Grass Clippings and Aerification Cores

Grass clippings and/or aerification cores will be spread on site as a mulch type product.

Materials will be spread thin as to not damage any underlying plants and will be kept clear of any buffer zones to ensure runoff is not a threat.

B. Woody Brush

Tree limbs and other woody material will be processed through a chipper and mulch produced will be stockpiled for use in flower beds, tree wells, steep slopes, and natural areas as desired.

C. Logs, Stumps, and Large Woody Debris

These items will be stockpiled in a suitable location and periodically processed with a wood grinder to generate wood fiber landscape mulch. The goal is to mulch this stockpile on an annual basis.

Pest Population Definition

A summary of the potential pests at Landa Park Golf Course is shown in Table 3.

Table 3> Expected Pest Outline at Landa Park Golf Course

Category	Pests
Fungal Disease	*Cyanobacteria* (Algae)
	Sclerontinia homoeocarpa (Dollar Spot)
	Rhizoctonia solani (Large Patch)
	Ophiosphaerella korrae/herpotricha (Spring Dead Spot)
	Gaeumannomyces graminis var. graminis (Bermuda Decline and/or Take-all patch)
Insects	Fire Ants
	Nuisance Ants
	Cutworms
	Fall Armyworms
Weeds	Poa Annua
	Goosegrass
	Nutsedge/Killinga
	Dallisgrass/Crabgrass
	Broadleaves
Other	Localized Dry Spots

Pest Threshold Levels

The damage threshold levels for specific pest types are shown in Table 4. Damage threshold level is defined as the number of pests detected within a specified area that may lead to corrective action to reduce the density of the specific pest below the damage threshold level.

Table 4> Damage Threshold Limits for Specific Pest Categories

Pest	Greens	Tees	Fairways	Rough
Fungal Disease	0.1%[a,b]	15%[a,b]	25%[a]	N/A
Weeds	1/1000ft^2	3-5/1000ft^2	10-20/1000ft^2	20/1000ft^2
Insects				
Fire Ants	Any	1/1000ft^2	1/1000ft^2	3-5/1000ft^2
Worms[c]	5-10/1000ft^2	10-30/1000ft^2	N/A	N/A
Other				
LDS	5-10%[a,b]	25%[a]	N/A	N/A

a % of area affected

b when condition dictate, preventative measures will be considered

c Includes Armyworms and Cutworms

Pest Monitoring and Pest Control

All golf course staff will be trained in golf course IPM to monitor for evidence of pest infestation. The intensity and frequency of monitoring will be adjusted based on the likelihood or presence of pest infestation (i.e. seasonal) or in situational/site specific instances. All observation of potential pest infestation will be reported directly to the Superintendent on the same day of the observation. Appropriate corrective action will be implemented as necessary.

The pest control strategy is sequential and consists of using cultural practices as the first line of defense. Pest control strategy will be developed on a case by case basis with all potential control options given consideration. The decision to implement chemical pest control measures beyond cultural, biological, or mechanical practices will be based on the review of relevant safety, scientific, economic, and environmental information. All products used for pest control will be those approved for use by the Environmental Production Agency for the specific indication.

A. Fungal Disease

Within the overall spectrum of pest management, fungal disease represents the most serious and consistent threat to turfgrass health at Landa Park Golf Course, and is of concern primarily on greens and tees. Greens and tees will be inspected regularly for symptoms of fungal disease. The primary means of identifying fungal disease will be diagnosis by the Superintendent. However, in some instances symptoms consistent with fungal disease may have alternative causes (nutrient deficiency, insects, etc.) When uncertainty regarding potential fungal disease is encountered, samples will be sent to a plant pathology laboratory for confirmation of the presence of fungal pathogens. More frequent monitoring of greens and tees will occur when conditions know to favor the development of these pathogens occur.

An essential aspect of preventing the development of fungal disease is the optimization of turf vigor through routine cultural practice. In addition, fungal disease control is dependent on the understanding of disease cycle and conditions that promote disease development, the correct recognition of disease symptoms, and the selective use of the appropriate fungicide agents when necessary. Specific cultural practices will be employed to minimize the potential for fungal disease, which are described below. In general, if these measures fail and symptoms of fungal infestation exceeds defined damage thresholds, fungicide applications may be necessary to control disease. Numerous factors including season, weather, and turf health/vigor contribute to the determination whether fungicide treatment may or may not be implemented.

A description of conditions favoring disease development, symptoms of disease, and specific control measures for each type of fungal disease that requires pest management follows:

1. Algae[1]

[Cyanobacteria]

SYMPTOMS

Although they do not infect grasses, blue-green algae are a significant pest problem in the turfgrass industry. These organisms contain chlorophyll just like plants, but they grow by producing chains of thread-like cells similar to fungi. Symptoms of algae appear in areas where the turf canopy has been thinned by poor growing conditions or other pest activity. In these areas, a green or black mat of fuzzy growth is evident in the turf canopy or on the surface of the thatch. During periods of dry weather, this algal growth forms a dry, cracking crust on the thatch surface that repels water and impedes turf recovery.

Host grass species: all turfgrasses; most problematic on putting green turf

FACTORS AFFECTING DISEASE DEVELOPMENT

Algae may develop whenever thinning of the turf canopy permits sufficient air, light, and water to reach the thatch surface. Algal growth is most aggressive during the late spring, summer, and early fall when warm,

[1] Disease profile information provided by NC State University's Turf files at **www.truffiles.ncsu.edu**

humid conditions are conducive to algae growth and turf thinning. Low mowing heights, shady conditions, poor soil drainage, and frequent irrigation also encourage algal growth in the turf canopy.

Algae have historically been thought of as secondary colonizers, meaning that they only fill-in areas where turf density has been reduced by some other problem. However, mounting evidence indicates that high levels of algae activity can directly cause thinning of putting green turf, possibly by production of toxins or competition for air, water, and nutrients. An aggressive algae management program can greatly increase the density and overall quality of putting greens during periods of warm and humid weather.

CULTURAL CONTROL

Maintenance of dense, healthy turf is the most effective way to prevent algae invasions. Avoid establishing turf in areas that are heavily shaded or poorly drained, or take steps to correct these problems in established turf. Mow at the recommended height for each turfgrass species, and increase mowing heights in shady areas to compensate for the reduced light levels. Irrigation should be applied deeply and infrequently; apply sufficient water to wet the entire root zone, and then reapply as needed when the turf shows signs of wilt. Putting greens and other heavily trafficked areas must be cultivated regularly to maintain soil drainage and aeration.

CHEMICAL CONTROL

Ammonium sulfate, hydrated lime, or other materials can be applied to “burn” the algae in infested areas. Extreme caution is needed when doing this, especially on golf course putting greens, as these materials can also burn the turf or cause nutritional imbalances in the soil.

The fungicides chlorothalonil and mancozeb are also effective algaecides. These products will control algae on a preventative or curative basis, but preventative applications are much more effective. Repeat applications on a 10 to 14 day interval during warm, humid weather, provides excellent algae control and significantly increases the density of putting green turfgrasses. Note that chlorothalonil and mancozeb are not approved for application to residential lawns. Fludioxonil (Medallion) provides moderate algae suppression and may be useful in areas where chlorothalonil and mancozeb cannot be applied.

Fungicides containing copper hydroxide should be used with caution, as copper can accumulate in the soil to toxic levels after repeated applications. For this reason, copper hydroxide should only be used under extreme circumstances to bring severe algae infestations under control.

Once a severe algae infestation has occurred, fungicide applications alone will not provide acceptable control. Additional steps must be taken to physically break-up the mat of algal growth so that the turf can recover. Spiking, aerification, verticutting, topdressing, or combinations thereof are effective ways to accomplish this.

2. **Dollar Spot**[2]

[Sclerotinia homoeocarpa]

SYMPTOMS

On putting green turf, dollar spot appears as small spots, approximately the size of a dollar coin, that are bleached-white or light tan in color. On turf mowed at heights greater than 0.5”, the spots may expand in size up to 6” or more in diameter. The affected leaves typically remain upright and are characterized by having white or light-tan lesions with light reddish-brown margins. As the lesions expand, the leaves are girdled and the upper part of the leaves dies slowly. Distinct lesions are sometimes not evident on close-cut turfgrasses;

[2] Disease profile information provided by NC State University’s Turf files at **www.truffiles.ncsu.edu**

instead, the leaves die back from the tip and turn white or light tan in color. The grass in the spots may be killed to the soil surface if the disease continues to develop, and many spots may merge to produce large blighted areas. Short, fuzzy white mycelium is often observed on affected turf in the morning when dew is present.

Characteristic	Description
Host Grass Species	all
Month(s) with symptoms	February to November
Stand Symptoms	spots, patches (4 to 12 inches)
Foliar Symptoms - Location/Shape	round leaf spots, leaf lesions
Foliar Symptoms - Color	tan or white
Root/Crown Symptoms	none
Fungal Signs	mycelium or none

<-dollar spot leaf lesions on Kentucky bluegrass

FACTORS AFFECTING DISEASE DEVELOPMENT

The dollar spot fungus begins to grow and infect susceptible grasses in the spring when night temperatures exceed 50°F, even though symptoms of the disease may not appear until later in the spring or early summer. In addition, the pathogen requires extended periods of leaf wetness, 10 to 12 continuous hours. Heavy dews that often form during cool nights in the late spring or early summer are most conducive to the disease. Extended periods of wet, overcast weather can also lead to severe dollar spot epidemics on susceptible grasses. Dollar spot remains active throughout the summer in many areas, but disease activity typically slows when high temperatures consistently exceed 90°F.

Turfgrasses that are deficient in nutrients, especially nitrogen, are more prone to dollar spot and also recover from the damage more slowly than well-fertilized turf. The disease is also encouraged by drought stress, low mowing, excessive thatch accumulation, frequent irrigation, and low air movement. Certain cultivars of creeping bentgrass, perennial ryegrass, and Kentucky bluegrass are very susceptible to dollar spot, while others are fairly tolerant.

CULTURAL CONTROL

Use of resistant cultivars is one of the most effective means of dollar spot management. This is particularly important for creeping bentgrass, perennial ryegrass, and Kentucky bluegrass, as cultivars vary widely in their susceptibility to the disease. Base turfgrass selection on University recommendations or regional cultivar trials operated by the National Turfgrass Evaluation Program or local universities. When planting cool-season grasses, use blends and mixtures of multiple species and varieties whenever possible..

Adequate nitrogen fertilization will help to prevent dollar spot, and will also encourage plants to recover quickly from the disease if it occurs. Select nitrogen sources, rates, and timings based on local University recommendations for your turfgrass species and climate. In general, golf course putting greens established with creeping bentgrass or annual bluegrass should be fertilized with 0.5 lb N/1000 ft^2 per growing month. More or less nitrogen may be required for your location depending on soil type, rainfall amounts, traffic intensity, and other management practices. Deficiencies in other nutrients that limit foliar growth may also

exacerbate dollar spot problems. Use soil test results to apply the recommended amounts of phosphorus, potassium, lime, and micronutrients.

Dollar spot is encouraged by drought stress and leaf wetness. Proper irrigation timing is needed to balance these factors. Irrigate based on the moisture status of the soil, not on a calendar schedule. When irrigation is necessary, it should be applied early in the morning, between midnight and 6 AM, to keep leaf wetness periods as short as possible. Mowing, dragging, or whipping the turf in the morning to remove dew can help to prevent dollar spot, but these practices can spread the disease if it is actively developing. Improve air movement and reduce humidity by pruning trees, clearing unwanted vegetation, or relocating desirable plants.

Excessive thatch accumulations greatly encourage dollar spot activity. Remove excess thatch by vertical mowing or power raking. Golf course putting greens should be aerified regularly and topdressed with sand to reduce thatch buildup.

Dollar spot is readily spread in leaf tissue or clippings from infected areas. Avoid spreading the disease by washing equipment before entering an uninfected area, by encouraging golfers to clean their shoes between rounds, and by removing and disposing of clippings taken from infected areas.

CHEMICAL CONTROL

Many fungicides control dollar spot, but preventative applications are most effective. A preventative program should be implemented in the early spring when night temperatures consistently exceed 50°F. When applied on a curative basis, fungicides must be applied at high rates and short application intervals.

Uniform spray coverage is important for maximizing fungicide performance; even small gaps in coverage may allow dollar spot to develop. Nozzle type, nozzle pressure, and dilution rate have the greatest impact on the uniformity of fungicide applications. Nozzles that produce coarse to extremely coarse droplets, such as TurfJet or Raindrop nozzles, dramatically reduce the performance of fungicides for dollar spot control. Air-induction or flat fan nozzles that produce fine to medium droplets are recommended. In order to provide thorough coverage of the turfgrass foliage, fungicides should be applied in 2 gallons of water per 1000 ft^2; lower carrier volumes reduce the performance of fungicides for foliar disease control.

The fungus that causes dollar spot develops resistance to fungicides very quickly. To prevent or delay the onset of fungicide resistance, use integrated management to minimize fungicide use, rotate among fungicide classes after each application, and tank-mix systemic fungicides with a contact fungicide.

3. **Large Patch**[3]

[Rhizoctonia solani]

SYMPTOMS

Large patch is a new name for an old disease of warm-season turfgrasses. This disease was formerly called brown patch, the same disease that affects cool-season grasses during hot weather. Other than the fact that they affect different grasses, there are several important differences between brown patch and large patch that necessitated a name change: they occur at different times of the year, produce distinct symptoms, are caused by different strains of the fungus *Rhizoctonia solani*, and require very different control strategies.

Large patch appears in roughly circular patches that are yellow, tan, or straw-brown. The patches are initially 2 to 3 feet in diameter, but can expand in size rapidly up to 10 feet or more in diameter, hence the name “large patch”. Multiple patches may coalesce to encompass even larger areas of turf. When the disease is actively developing, the outer edge of the patches are often red, orange, or bronze in color. Close examination of individual plants reveals the presence of reddish-brown or gray lesions on the leaf sheaths.

[3] Disease profile information provided by NC State University’s Turf files at **www.truffiles.ncsu.edu**

It may be necessary to peel away the older, dead leaves in order to reveal the lesions on the younger leaf sheaths below.

Characteristic	Description
Host Grass Species	bermudagrass, centipedegrass, seashore paspalum, St. Augustinegrass,zoysiagrass
Month(s) with symptoms	August to May
Stand Symptoms	patches (1 foot to greater than 3 feet)
Foliar Symptoms - Location/Shape	lesions on leaf sheaths
Foliar Symptoms - Color	tan, yellow, orange, red
Root/Crown Symptoms	None
Fungal Signs	None

large patch sheath lesion in St. Augustinegrass

FACTORS AFFECTING DISEASE DEVELOPMENT

Large patch begins to develop when soil temperatures decline to 70°F in the fall, but the symptoms do not necessarily appear at this time. The symptoms of large patch are most evident during periods of cool, wet weather in the fall and spring. In many cases, symptoms may not become evident until early spring when the warm season grasses are greening up.

Large patch is favored by excessive nitrogen in the fall and spring, poor soil drainage, over-irrigation, excessive thatch accumulations, and low mowing heights. Centipedegrass and seashore paspalum are most susceptible to large patch, followed by zoysiagrass, and then St. Augustinegrass. Bermudagrass, rarely affected by large patch, recovers very quickly when the disease does occur.

CULTURAL CONTROL

Establishment of a disease-resistant turfgrass species is the most effective means for management of large patch. Bermudagrass rarely sustains significant damage from large patch, and grows of out the symptoms quickly when the disease does occur. In contrast, centipedegrass, seashore paspalum, St. Augustinegrass, and zoysiagrass often sustain serious damage and recovery can take several weeks or months. Fescues and bluegrasses are immune to large patch and are also an option in areas where cool-season turfgrasses can be maintained.

Do not apply nitrogen to warm-season grasses in the fall and spring. These grasses are growing slowly during this time and do not require a significant amount of this nutrient. In general, nitrogen should not be applied to the warm-season grasses within 6 weeks before dormancy in the fall or within 3 weeks after green-up begins in the spring. Warm-season grasses vary in their fertility requirements, so refer to local University recommendations for more specific recommendations for timing and rates.

Avoid establishing warm-season grasses in low lying areas that remain saturated for extended periods of time from surface runoff. If this is unavoidable, install subsurface drainage to remove excess water from the soil. Irrigate only as needed to prevent severe drought stress in the fall and spring. Control traffic patterns to prevent severe compaction, and aerify as needed to maintain soil drainage and aeration. Mow at recommended heights, and power rake or vertical mow as needed to control thatch accumulations.

CHEMICAL CONTROL

Fungicides are available for large patch control, but must be applied on a preventative basis. Applications should be initiated in the fall when soil temperatures decline to 70°F, regardless of when symptoms have appeared in the past. One or two well-timed applications provide season-long control of large patch in many situations. In severely affected sites, repeat applications should be made on 4 to 6 week intervals as long as soil temperatures are between 40°F and 70°F. Mapping of affected areas in the spring for spot-treatment in the fall can substantially reduce fungicide expenditures.

4. Spring Dead Spot[4]

[Ophiosphaerella korrae & Ophiosphaerella herpotricha]

SYMPTOMS

Spring dead spot symptoms appear in circular patches from 6 inches to several feet in diameter that remain dormant as the turf greens up in the spring. These patches eventually die and collapse to the soil surface. The roots, stolons, and rhizomes are dark and rotten in affected areas. Spring dead spot patches recur in the same spot each year and increase in size by up to several inches each season. As the patches expand, the centers are sometimes re-established with bermudagrass or weedy species, resulting in a ring-like appearance. Recovery of the patches occurs by spread of the bermudagrass from the outside. This process is very slow, taking the entire growing season in severe situations. The spring dead spot patches greatly detract from the uniformity of the playing surface and are frequently invaded by weeds. Spring dead spot may also occur in certain varieties of zoysiagrass, such as ‘Meyer’ and ‘El Toro’.

FACTORS AFFECTING DISEASE DEVELOPMENT

Spring dead spot is most evident on intensely managed bermudagrass, such as athletic fields and golf courses. The disease typically takes 3 to 5 years to become established in a new bermudagrass stand. Unlike take-all patch, spring dead spot does not decline in severity as the turf matures. It becomes more severe if left unmanaged.

The spring dead spot fungus attacks the roots, rhizomes, and stolons of bermudagrass during the fall and winter. This activity does not directly kill the plant, but instead makes the bermudagrass more susceptible to freezing injury. As a result, spring dead spot is most severe in the northern range of bermudagrass adaptation and is usually more severe after extremely cold winters.

Any factor that restricts bermudagrass root growth or increases its susceptibility to winter injury will also enhance the disease. Excessive nitrogen, potassium deficiencies, poor soil drainage, over-irrigation, excessive thatch accumulation, and soil compaction have been shown to encourage disease development. The impact of soil pH on spring dead spot development in bermudagrass is not well understood.

Characteristic	Description
Host Grass Species	bermudagrass, zoysiagrass
Month(s) with symptoms	April to September
Stand Symptoms	spots, circles, patches (6 inches to greater than 3 feet), rings
Foliar Symptoms - Location/Shape	blighting of entire leaves
Foliar Symptoms - Color	tan, yellow, orange
Root/Crown Symptoms	roots, stolons, rhizomes, and/or crowns dark brown or black
Fungal Signs	none

[4] Disease profile information provided by NC State University’s Turf files at **www.truffiles.ncsu.edu**

spring dead spot root and stolon rot

CULTURAL CONTROL

Fertilize to meet the nutritional needs of the turf, but do not apply excessive rates of nitrogen. Do not apply nitrogen within 6 weeks of winter dormancy, and do not exceed more than 1 pound of nitrogen per 1,000 square feet per application at any time during the growing season. Reduce thatch buildup and relieve soil compaction through aggressive aerification and vertical mowing. Areas that are severely affected by spring dead spot should be hollow-tine aerified at least three times per year, during the summer when bermudagrass is most actively growing. Golf greens should also be topdressed along with aerification to control thatch accumulation.

The impact of soil pH on spring dead spot development is unclear at this time. Past recommendations focused on the use of acidifying nitrogen sources like ammonium sulfate to manage this disease. However, recent research at NC State University has shown that different spring dead spot pathogens respond differently to nitrogen sources. *Ophiosphaerella korrae*, the most common pathogen in the eastern US was controlled effectively by application of calcium nitrate as the sole nitrogen source. On the other hand, *O. herpotricha*, the most common pathogen in midwestern states, was suppressed by ammonium sulfate. Fall applications of potassium, which have been frequently recommended for spring dead spot management, had no effect on the disease in our research.

Once the symptoms of spring dead spot appear, the only means of control is to encourage the spread of bermudagrass into the affected patches. Frequent spiking or aerification is recommended to break up the mat of dead turf in affected patches. Applying extra nitrogen to encourage recovery is not recommended, as this can enhance the disease in the following year. Dinitroaniline (DNA) herbicides, which are commonly used for preemergent control of annual grasses, can slow the recovery of bermudagrass from spring dead spot injury and should not be used in sites with a history of the disease.

CHEMICAL CONTROL

Fungicides are available for spring dead spot control, but they must be applied preventatively in the fall. Fenarimol and tebuconazole have been the most effective and consistent fungicides for spring dead spot control. Applications are most effective when soil temperatures are between 60 and 80°F. To move the fungicide into the root zone, apply in a high volume of water (5 gallons per 1,000 square feet) or water in with ¼” of irrigation immediately after application. Repeat applications at high label rates may be necessary in severely affected areas. Affected areas should be mapped in the spring for treatment in the fall to reduce fungicide expenditures.

5. Bermuda Grass Decline[5]

[Gaeumannomyces graminis var. graminis]

SYMPTOMS
Circular patches .5 to 3 ft. diameter; initially yellowish; gradually turning brown and thinning; roots darkened; chlorotic leaf blades may develop next to green shoots at margins of diseased area; roots brown and without feeder roots and root hairs; root surface appears as dark brown hypal runners; runner hyphae may be visible during microscopic examination.

HOST GRASSES: Major hosts: Bermudagrass, St. Augustinegrass Other hosts: Zoysiagrass, Centipedegrass

DISEASE CYCLE:
Symptoms most evident in late summer (hot, wet periods) and early fall-fungus organism most active during fall, winter and spring in moderate temperatures and abundant moisture; root attacking fungus; spread by mechanical means and plant-to-plant contact; fungus grows on the surface of roots, stolons, rhizomes, crown and leaf sheaths-and then penetrates and infects the tissues; pathogen survives on infected debris and infected perennial parts of living grass plants

FACTORS THAT MAY PROMOTE DISEASE DEVELOPMENT:
Cool, moist weather are conditions which favor growth of the fungus; symptoms increase in summer months; stressed turfgrasses-low mowing heights, water stress, excessive thatch, excessive fertilization, compaction, alkaline soils, etc… promote the disease.

CULTURAL CONTROL:
Raise cutting height; maintain moderate nitrogen; control excessive thatch; positive plant water balance, soil acidifiers in alkaline soils; use of ammonium sulfate to decrease pH; aerification program; some research has been done using higher rates of ammonium sulfate and moderate aerification on bermudagrass greens-Dr. Richard White.

CHEMICAL CONTROL:
Fungicide applications are not very effective; proper cultural practices, especially in the infected areas, are a must.

B. Insects

Central Texas offers a variety of pests and potential pest infestation problems that vary from golfer safety to turfgrass damage. The highest pressure for potential pest problems comes from cutworm and fall armyworm damage where little damage can be tolerated on greens surfaces. Fire ants can pose a potential safety issue to patrons and guests at the facility and also have the potential of negatively influencing customer satisfaction in multiple aspects. Monitoring for insects will consist of routine visual inspection of susceptible areas on a weekly basis. General turfgrass cultural practices leading to optimal turf vigor are the primary means of minimizing the potential for insect infestation. If cultural practices are ineffective at preventing damage thresholds for a specific pest form being exceeded, the selective use of insecticides will be employed. Rotational strategies will be employed as necessary to reduce insect resistance to specific products.

[5] Disease profile provided by http://aggieturf.tamu.edu/answers4you/disease/bermdecline.html

A description of specific insect pests, symptoms of infestation, and corresponding control measures follows:

1. Fire Ants[6]

***[Solenopsis invicta* Buren]**

Red imported fire ants infest approximately 300 million acres in the United States. Every year these ants cause hundreds of millions of dollars in damage. These costs affect everyone from small property homeowners to large landowners and ranchers to managers of golf courses and commercial properties. Fire ants are the single most prolific pest in turfgrass. Although they do not damage the turf directly, the mounds are unsightly and can cause damage to mowing equipment. Fire ants hinder outdoor recreation, which thus affects tourism. Although less than one percent of the human population has extreme reactions to fire ants stings, they can pose a serious medical threat to visitors of public lands. Serious incidents can be costly to managers of these areas, which can be held liable for such an event. Golf courses are especially attractive to fire ants due to the ideal conditions that exist there. Their mounds can ruin a good shot. In the south, the 'unofficial ruling' is that you may remove your ball from the mound the distance of one club length. Golf courses are man-made ecosystems that include natural habitats; wildlife population's (permanent and migratory) ground and surface water, managed turf, and provide recreation for millions of people each year. Additionally, grasses release oxygen and reduce glare and noise. Irrigation water applied to golf courses provides an ideal habitat for fire ants. Fire ants are attracted to areas of high moisture content, fertile soil, and open sunny areas.

Chemical Control

Many golf course superintendents indicate their budget does not allow for fire ant treatments exclusively. However, for the ideal situation, a combination of two approaches should be able to provide acceptable levels of fire ant control at a reasonable cost.

The Two-Step Method: This method is suitable for larger turfgrass areas and provides relatively long-term control, but rarely provides 100 percent control:

Step 1) Make an annual or semi-annual (once or twice per year) broadcast application* of a baitformulated insecticide. Conventional baits (i.e., Amdro® or Seige®, Award®, Ascend® or Varsity®, or Extinguish™) are applied at 1 to ½ pounds of product per acre. Other products (i.e., FireStar®) require different rates. Periodic broadcast applications of fire ant baits provide roughly 90% suppression of ants when properly applied (using fresh bait, applied in late evening/mid morning). The speed and duration of ant suppression differ with the product used. A late summer or early fall application can produce fewer ants by the following spring.

Step 2) Wait several days after the bait is applied, and then treat nuisance ant colonies (in high traffic areas) using an individual mound treatment such as products formulated as dusts, granules, granules drenched with water after application, liquid drenches, baits, or aerosol injections.

The Ant Elimination Method: This program controls nearly all ants in treated areas. Its effects are more rapid than those of other programs, and re-invasion of treated areas by migrating colonies and mated queen ants is minimized as long as the contact insecticide remains active on the treated surface. However, it is relatively more expensive and uses more insecticide, requiring more frequent treatments. For high use areas in golf courses such as putting greens and tee boxes where maximum fire ant control is required, this program would be preferable.

*Step 1***)** (Optional) Broadcast a bait-formulated insecticide in areas where there are more than 20 per acre. Wait at least 2 to 3 days before conducting the next step.

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[6] Disease profile information provided by Texas A&M University at **http://fireant.tamu.edu/materials/fact-sheets/**

Step 2) Apply a contact insecticide (i.e., pyrethroid products like those containing bifenthrin, permethrin, lambda-cyhalothrin or others, or products containing fipronil granules like Chipco® Choice™ or Chipco® TopChoice™) to turf periodically as directed (i.e., generally every 4 to 8 weeks, or when ant activity is detected). Liquid or granular products that can be evenly applied to an area are appropriate for this treatment. Areas treated must be watered soon after application to wash the insecticide below the surface. Although surface treatment may not initially kill ants located deep in mounds, routine re-application will eventually eliminate colonies.

Individual Mound Treatments: In areas with just a few fire ant mounds, use of an individual mound treatment product may be all that is needed. This approach may help preserve native ant colonies that are left untreated.

Program combinations: The three programs described above can be used on specific sites within a managed area where different levels of fire ant control are desired. On golf courses, for instance, The Ant Elimination Method may be suitable for high use areas such as putting greens and tee boxes. In fairways and rough areas, The Two-Step Method may be sufficient. Careful monitoring may document the absence of imported fire ants in some areas, or the presence of competitor ants that are not pestiferous. These areas can remain untreated. Furthermore, imported fire ants migrating from nearby untreated areas can be detected and treatments applied only as barrier zones to prevent movement of colonies into the managed areas from these reservoirs.

Cultural Control

Practices used by golf course managers for managing the imported fire ant management involves mowing frequency and height. The more times areas are mowed, the less time fire ants can establish colonies. Constant disturbance of ant colonies nesting in frequently mowed areas usually causes them to move to less disturbed areas. As an example of a management program, greens can be mowed seven times a week, tee boxes and fairways moved three times a week, and roughs can be mowed once each week. Spot treatments for fire ants may only be needed on roughs. Watering turfgrass and bodies of water will attract ant colonies. Conversely, minimizing watering may result in reduced ant nesting activity.

2. Cutworms[7]

General Information

Cutworms are caterpillars that feed on the stems and leaves of young plants and often cut them off near the soil line, hence their common name. Although there are many important species of cutworms, the black, granulate, and variegated cutworms are the ones most commonly encountered on turfgrass.

Distribution -- Cutworms are found throughout the United States.

Host Plants -- Besides turf, cutworms attack many field and vegetable crops.

Damage -- Many cutworms prefer wilted plant material and sever the plants sometime prior to feeding. Cutworms in turf often burrow in the thatch or ground. At night they emerge and chew stems and blades near the soil. The damage may appear as circular spots of dead grass, finger-sized brown crescents or ball

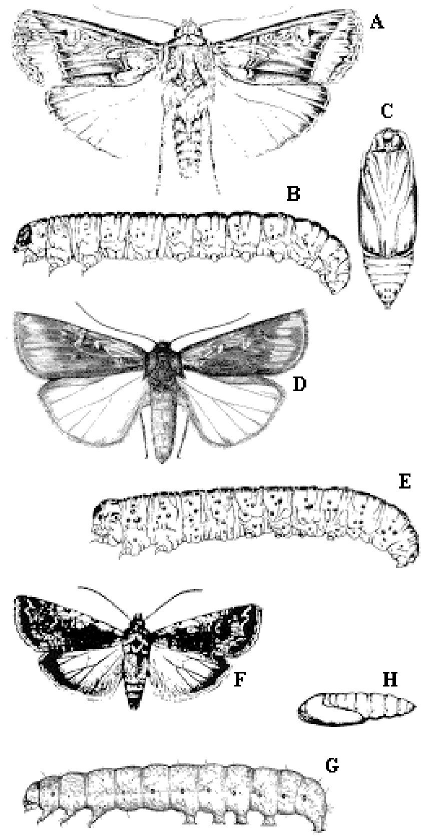
[7] Insect Profile provided by http://www.turffiles.ncsu.edu/insects/Cutworms.aspx

marks on a golf green. Bronzed cutworms are active in spring and fall and may strip large areas of turf at ground level.

Life Cycle

Adult -- When resting, cutworm moths hold their wings back in a triangular position. The moths are generally stocky and have a wingspan of about 1.5 inches (40 mm). The forewings are dark brown and mottled or streaked; the hindwings are lightly colored and unmarked.
Egg -- The eggs are usually white (becoming darker prior to hatching), round, and smaller than a pin head (0.5 to 0.75 mm) in diameter.
Larva -- If disturbed, the larvae usually curl into a C-shaped ball. Cutworms are plump, smooth, dull-colored caterpillars that measure about 1.75 inches (45 mm) when fully grown.
Pupa -- Pupae are brown and 15 to 22 mm long.

Each cutworm species differs slightly from the others in details of habits and appearance, but their life histories are generally similar. Adults and larvae are nocturnal and hide during the day but may also become active on cloudy days. Larvae are very active and may crawl 60 feet or more during the night. Cutworms overwinter in the soil either as pupae or mature larvae. In the spring, the hibernating larvae pupate. Adults begin to appear in the middle of March. Female moths deposit eggs singly or in clusters, and each female can lay as many as 500 eggs. Under optimum conditions, the eggs hatch in 3 to 5 days, and larvae develop in 3 to 4 weeks passing through 6 instars. Pupae mature in 2 weeks during the summer and as many as 9 weeks in the fall. Some of the cutworms can produce as many as four generations each year.



Black cutworm. A, Adult. B, Larva. C, Pupa. Granulate cutworm. D, Adult. E, Larva. Variegated cutworm. F, Adult. L, Larva. H, Pupa.

Scouting
Since cutworms may be difficult to detect, soap flushing solutions can be helpful for detection. Use 1 oz of lemon-scented liquid dishwashing detergent in 2 gal of water. Dispense with a watering can within a 3ft x 3ft grid. Larvae should appear in five to ten minutes. Treatment threshold is five to ten larvae per square yard. Threshold may be lower on finer turf such as golf greens. Irrigate the grid with plain water afterwards. Commercial pheromone traps that attract male moths can be used to determine treatment timing but are not indicators of larval densities in the turf. Damage can be expected 2 weeks after peak flight so time pesticide applications accordingly.

Nonchemical Control Strategies
Kentucky bluegrass is a non-preferred host, for black cutworm, compared to creeping bentgrass and perennial rye and may reduce infestation. This may be used in mixed turf or in border areas. The nematode, *Steinernema carpocapsae* is moderately effective against black cutworm. Follow label instructions exactly. *Bacillus thuringiensis* products work best while larvae are young. Collection and removal of clippings from the area may be helpful.

Chemical Control- For chemical control, liquid formulations should be used, applied as late in the day as possible, and not watered in for at least 24 h. Treating a 20-30 ft buffer zone around greens and tees will reduce migration from outlying areas. Precede chemical treatments with mowing 24 hours prior to treatment. Apply chemicals in the evening for optimal night time contact.

C. Weeds

Several weeds stand out as hard to control in bermudagrass stands depending on season. During the cool season, poa annua continues to be a nuisance in bermuda grass stands and is easily controlled in non-overseeded environments, however, when other desirable cool season grass are incorporated, its management can be a challenge. During the growing season, dallisgrass as well as goosegrass, can provide a challenge in control and overall quality of bermudagrass stands. Many of these pests can be controlled with proper preventative action, leaving only an isolated need for post emergent control.

1. Annual Bluegrass

[Poa Annua]

Poa annua, also known as annual bluegrass, is a cosmopolitan weed in all turfgrass situations. The vast majority of the annual bluegrass is the true winter annual (*Poa annua* var.*annua*) that germinates in the fall, grows throughout the winter season, flowers profusely in the spring during March and April, and then dies as the summer temperatures rise. The seed will remain in the soil all summer long and will germinate again early during the next fall. The annual form of *Poa annua* produces stemmy seedheads that often grow in a circular pattern around the leaves, especially when the turf is mowed regularly. In the summer, when bermudagrass grows well and is manicured regularly, the bermudagrass greens will squeeze out most of the *Poa,* both annual and perennial forms. The hot weather stress is not conducive for *Poa* growth.
The perennial form of "annual" bluegrass, *Poa annua* var. *reptans,* occurs much less frequently. The perennial types of *Poa annua* essentially survive only in very close-cut turfs like golf course greens. In higher cut turfs on tees, fairways, and roughs, the annual type will be the more common weed. Weed control programs in turfgrasses generally are targeted against the annual form of *Poa* and consideration should be given to strategies based on the type of herbicides to apply at different growth stages of *Poa* in different turfgrasses.

Preemergence Control of *Poa annua*

There are many options available if a winter turfgrass is not overseeded into the bermudagrass. Several preemergence grass herbicides will easily control *Poa* by preventing seedling emergence. Treat bermudagrass turf before late September. If these herbicides were applied in the previous spring for summer annual grass weed control, these chemicals will not last long enough in the soil to be effective against *Poa* in the fall season.

When bermudagrass **will** be overseeded with a winter turf (ryegrass), the selection of an herbicide is limited and timing of application of a preemergence herbicide is very critical. In this case, ryegrass must be able to emerge safely after overseeding and at the same time try to prevent *Poa* establishment. Most preemergence herbicides will also prevent the ryegrass from emerging. However, a properly timed and a very early application of a preemergence herbicide is one option. Another option is to use selective chemicals that control the *Poa* while being safe on the emerging winter turf.

Postemergence Control of *Poa annua*

The *Poa* is emerged and exists as a seedling or established plant. The size and age of the weed and the "background" turfgrass are important considerations when applying postemergence herbicides for a *Poa* control program. When ryegrass is overseeded and established as a winter turfgrass, the safety to ryegrass and the underlying dormant bermudagrass is critical.

In situations when the bermudagrass is not overseeded with a winter turf, there are many options available. Many of the herbicides are non-selective and so it is critical to be sure that the underlying bermudagrass is dormant before making applications. The *Poa* will begin to flower from late January to mid-April with profuse flowering in March. It is better to eliminate the *Poa* before flowering.

2. Grassy Weeds-(Dallisgrass/Crabgrass/Goosegrass)

Cultural Control

The presence of these weeds can be eased by the optimization of turf health through standard cultural practices. Proper fertilization, irrigation, and insect/disease control produces a dense vigorous turf that optimizes resistance to many weeds. Normally the weed pressure from common grassy weeds is so great, that a preventative herbicide application is necessary to reduce and/or eliminate the need for multiple post-emergent applications.

Chemical Control

Pre-emergent herbicides will be applied in early spring, prior to germination period for Crabgrass/Dallisgrass. It is recommended that the application rate of this application be split into two separate applications, February and May to ensure prolong control of both early germination varieties that tend to become active in early spring, as well as, boost control for those weeds that prolong germination into early summer. This “booster” application should help provide quality goosegrass control.

Post-emergent control will be done based on weekly scouting performed during weed pressure timeframes and decisions made based off of threshold requirements. If proper pre-emergent applications are made, only small broadcast, and spot applications will be necessary to keep turf relatively weed free throughout the growing season.

3. Nutsedge

The nutsedges are grass-like, colony-forming, perennial weeds that grow actively during the frost-free season, spread by rhizomes, and propagate from year to year by small, starchy tubers (sometimes called “nutlets," which give the weeds their common name). Nutsedges, also called nutgrasses, are difficult to manage because of their tolerance to heat, drought, and flooding; their prolific underground vegetative reproduction; and their ability to regrow after cultivation.

Control
An effective pre-emergent control has not been found for nutsedge in a bermudagrass turf environment. It is recommended that a post-emergent application be made that focuses on sites that are medium to heavily infested with nutsedge to keep turfgrass quality at a reasonable level.

Pesticides

1. Pesticide Definition

A pesticide is any substance that is used to control pests including insects (insecticide), weeds (herbicide), fungi (fungicide), nematodes (nematicide), and algae (algaecide). The mechanism of action of most pesticides is to eliminate the pest by suppressing, weakening, or eradicating the target pest.

2. Pesticide Use Determination

The ideal pesticide is highly potent (requires minimal application), is target-specific (is safe for non-target species), and is compatible with the environment. While pesticide manufacturers pursue these properties, the degree of cross-toxicity and environmental compatibility in pesticides approved for use by the Environmental Protection Agency can vary considerably. As a result, if avoidable, pesticides will not be used. In the event that pesticide application is necessary, pesticides will be applied according to label.

The primary strategy for pest management as defined in this Integrated Pest Management plan is to optimize turf vigor through cultural practices to optimize turf resistance to, or tolerance of pests. In the event that cultural practices do not contain pest populations below damage thresholds, chemical control measures will be employed as necessary. Pesticides applied to control pests will be selected by the Superintendent based on their safety, efficacy, economic impact, toxicology, and environmental compatibility. In addition, the Superintendent will monitor developments in pesticide research and development; and he/she will incorporate the use of newly developed, tested, and improved pesticides approved by the EPA where appropriate.

3. Projected Pesticide Use

The location of pesticide use and the projected frequencies are shown in Table 5.

Table 5> Landa Park Golf Course:
Pesticide Application Areas and Typical Application Frequencies

Area	% Total Area	Pesticide Applications per Year	Pesticide Category
Greens	2.5%	20-30	Fungicide, Herbicide, Insecticide
Tee Surface	3%	10-15	Herbicide, Insecticide
Fairway	21%	10-15	Herbicide, Insecticide
Immediate Rough	25%	4-8	Herbicide, Insecticide
Secondary Rough	35%	3-6	Herbicide, Insecticide
Native	13.5%	0-2	Insecticide

The pesticides that have potential for use at Landa Park Golf Course include five fungicides, five herbicides, three insecticides, and one wetting agent (Table 6). To minimize the development of resistance, pesticides in different families with different mechanisms of action will be rotate as frequently as practical and necessary. In addition, if pest resistance to one or more of these pesticides does develop, or if unanticipated circumstances arise, the Superintendent may use alternative pesticides that are EPA approved for treatment of the specific indication.

Table 6> Pesticide Selection for Potential Application at Landa Park GC

Pesticide Trade Name	Pesticide Chemical Name	Pesticide Category
Fore	Mancozeb	Fungicide
Daconil Action	Chlorothanlonil	Fungicide
Heritage	Azoxystrobin	Fungicide
Cleary 3336	Thiophanate-methyl	Fungicide
Cleary 26/36	Iprodione/Thiophananate-M	Fungicide
Triple Crown	Bifenthrin	Insecticide
Award	Fenoxycarb	Insecticide
Advion	Indoxacarb	Insecticide
Monument	Trifloxysulfuron	Herbicide
Revolver	Foramsulfuron	Herbicide
Illoxan	diclofop-methyl	Herbicide
Barricade	Prodiamine	Herbicide
Ronstar	Oxadiazon	Herbicide
Soaker Plus	Modified Alkylated Polyol	Wetting Agent

4. Pesticide Storage

All pesticides will be maintained in a dedicated, dry, well-ventilated, approved storage area that has restricted access and meets the requirements of the Texas Department of Agriculture.

5. Pesticide Mixing

The entire pesticide product label will be read and understood prior to the use of any pesticide prior to pesticide mixing; the Superintendent will determine that local weather conditions are suitable for pesticide application. All pesticides will be mixed according to manufacturer's labeling instructions by a licensed pesticide applicator. Personnel will wear proper personal protective equipment during the entire mixing process, as instructed on the label of the pesticide being mixed.

6. Application

All pesticides will be applied by a licensed pesticide applicator or personnel properly trained in the safe application of these agents by a licensed pesticide applicator. Applicators will wear appropriate personal protective equipment appropriate for the pesticide being applied. All pesticide application equipment will be properly calibrated prior to the addition of the pesticide formulation to the equipment and application to the golf course.

The areas of the golf course requiring pesticide application will be specifically

defined by the superintendent. Whenever possible, applications will be selective and limited to localized, targeted areas to minimize the amount of pesticide being applied. No pesticide spray application will occur if wind speeds exceed 10 mph or if wind direction or activity will carry pesticides toward, or deposit them upon open water. Pesticides will not be applied if heavy rain is forecast following the potential application event. No pesticide applications will be made in defined buffer zones.

7. Clean Up and Disposal

Pesticide containers, mixing tanks, and equipment will be rinsed n accordance with recommended procedures and rinse water will be disposed of in accordance with state and local ordinances.

8. Pesticide Documentation

All pesticide applications and usage will be documented to the specification required by the Texas Department of Agriculture. Information recorded will include date of application, location of application, the type of pesticide applied, rate of application, weather conditions including wind and temperature, target pest, the equipment used for application, and indentifying the person making the application. In addition, current pesticide labels and MSDS sheets will be compiled and maintained in a location accessible to all employees. All pesticide documentation will be in accordance with state and federal regulations.

Facilities Description

1. Maintenance Building

Current maintenance functions are performed in a modified stone building adjacent to the golf course property. The building is segregated into three main areas. The first area is an open space for crew lockers, postings, and picnic style seating. The second area consists of a semi-enclosed storage area for small tool and janitorial storage as well as a small climate controlled office space that has been converted to staff quarters and irrigation control office. The third area consists of mower and fertilizer storage and occupies the back half of the golf section of the building. This building is also shared with park operations as a joint use facility to accommodate centralized park operations.

2. Petroleum and Fuel Storage and Disposal

Hydraulic and engine oil will be stored in a dedicated area to keep a level of confinement around stored petroleum. Used fluids will be stored in separate dedicated containers labeled with the type of fluid and in appropriate containers for that type of fluid. Fluids will be disposed of according to state and local regulations.

3. Fuel Storage

With no current on site fueling depot, fuel is maintained on site in two to five gallon cans for both diesel and unleaded fuel. These containers are to be kept in a flammable safety cabinet and secured when not in use. Fueling and transport is minimized by using the closest fueling station to the maintenance shop facility.

4. Equipment Washing

All equipment is to be washed on two centralized wash stations centrally located within the two nine hole sections of the course. This eliminates any contamination to adjacent waterways from grass clippings and petroleum rinsate. Equipment will be washed with water only, in the exception of the occasional pressure wash that may include the use of a mild degreaser.

5. Pesticide Storage

Current pesticide storage is located behind the maintenance building in a dedicated, locked chemical storage shed. This building has limited access to ensure adequate safety and security.