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

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Executive Summary

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

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

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

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

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

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

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

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

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

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

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

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

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

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

CHAPTER 1

ALGAE IDENTIFICATION

Material and Methods

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

Results

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

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

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

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

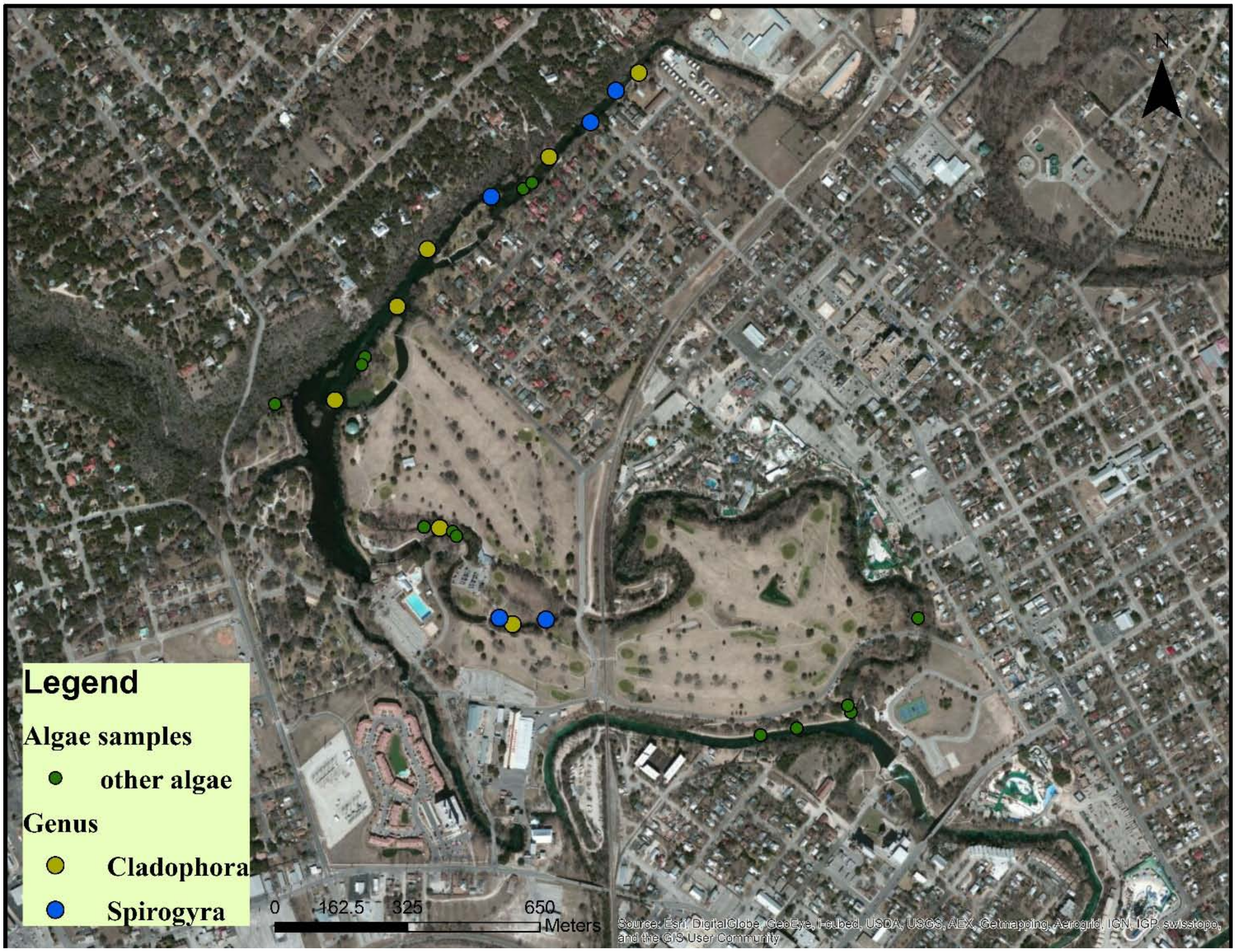

Figure 1.1 Map of algae grab samples collected from Upper Spring Run to the confluence of the Old and New Channels.

Table 1.1 Location attributes for each grab sample

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

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

Discussion and Conclusions

The most common algae found in grab samples were *Spirogyra*. Primarily a shallow water algae this genus is very much a generalist commonly found in ditches, slow moving streams, backwaters and littoral zones across all climatic gradients. It can grow loosely attached to substrate, macrophytes or other underwater structures where its filaments over winter and expand (Sheath and Cole, 1992). As *Spirogyra* filaments expand oxygen bubbles trapped within filaments can bring *Spirogyra* clumps to the surface where it is quite capable of existing as floating mats (Hillebrand, 1983). *Spirogyra* is not considered an indicator for water quality integrity due to its very general growth needs and wide habitat ranges. The other common genera of algae collected in grab samples were *Cladophora*. This algae is typically found in streams and rivers but is common in lakes as well. *Cladophora* filaments directly anchor to substrate such as rocks and gravel which allow it to prosper along shorelines of lakes and in faster flowing streams. However *Cladophora* filaments can detach from the anchoring holdfast during the growing season forming floating mats. Unlike *Spirogyra*, *Cladophora* tends to degrade once detached so that floating mats composed of *Cladophora* filaments can cause severe water quality issues such as elevated *E. coli* levels, odors and anoxic conditions (Vanden Heuvel et al., 2010). Excessive *Cladophora* growth can be indicative of degraded water quality and nutrient enrichment. Other filamentous algae genera were limited in distribution and warrant no further discussion in this report.

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

Works Cited and Recommended Readings

Bellinger, E. G. and D. C. Sigee. 2015. Freshwater algae: identification and use as bioindicators. John Wiley & Sons.

Dodds, W. K. 1991. Micro-environmental characteristics of filamentous algal communities in flowing freshwaters. Freshwater Biology, 25:199-209.

Hillebrand, H. 1983. Development and dynamics of floating clusters of filamentous algae. Periphyton of freshwater ecosystems. Springer Netherlands, p31-39.

Sheath, R. G. and K. M. Cole. 1992. Biogeography of stream macroalgae in North America. J. Phycol. 28:448-60.

Vanden Heuvel, A. C., C. R., McDermott, T. Pillsbury, J. Sandrin, J. Kinzelman, J. Ferguson, J., et al. 2010. The Green Alga, Promotes Growth and Contamination of Recreational Waters in Lake Michigan. Journal of environmental quality, 391:333-344.

CHAPTER 2

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

Materials and Methods

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

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

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

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

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

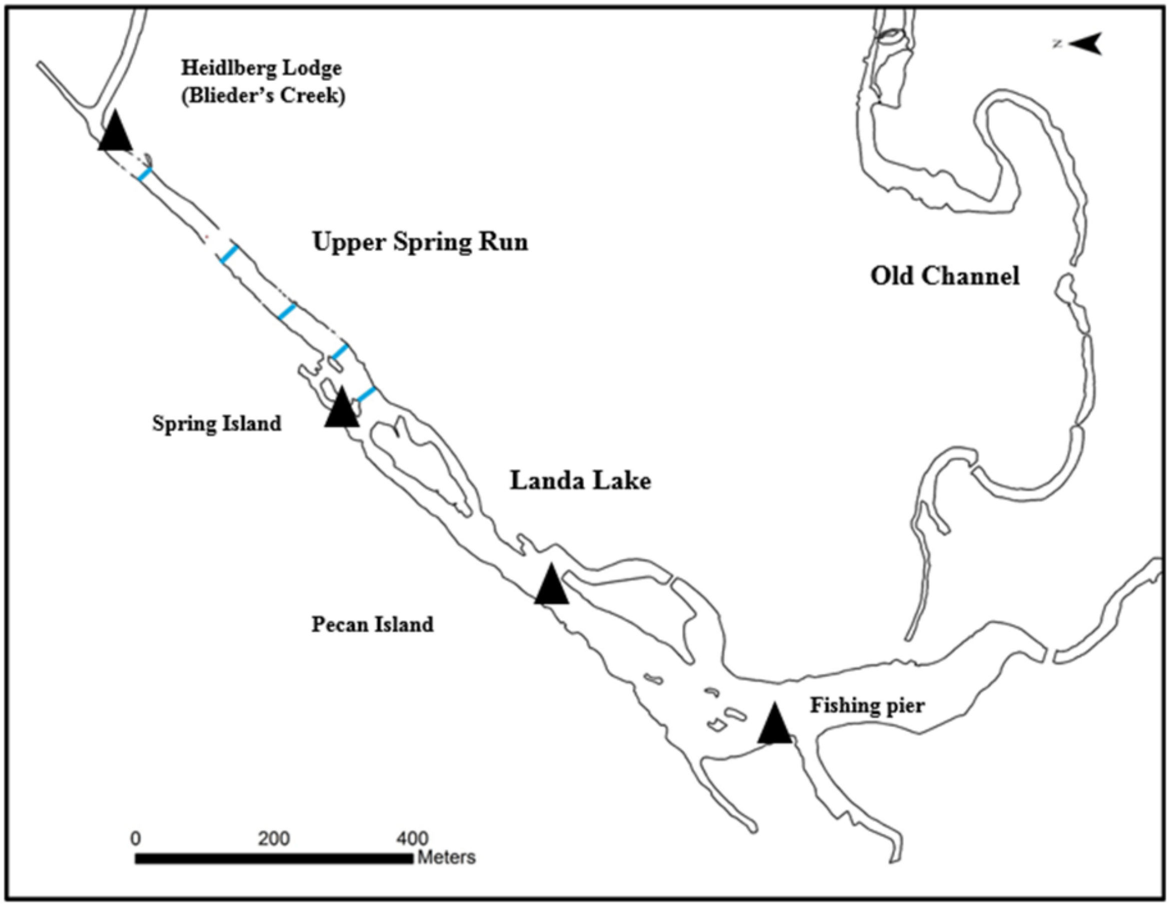

Figure 2.3 Map of Landa Lake and Upper Spring Run showing four routine sampling stations designated by triangles (Heidelberg, Spring Island, Upper Pecan Island, Landa Lake Fishing Pier). The location of the algae transects are indicated by blue lines.

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

Results

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

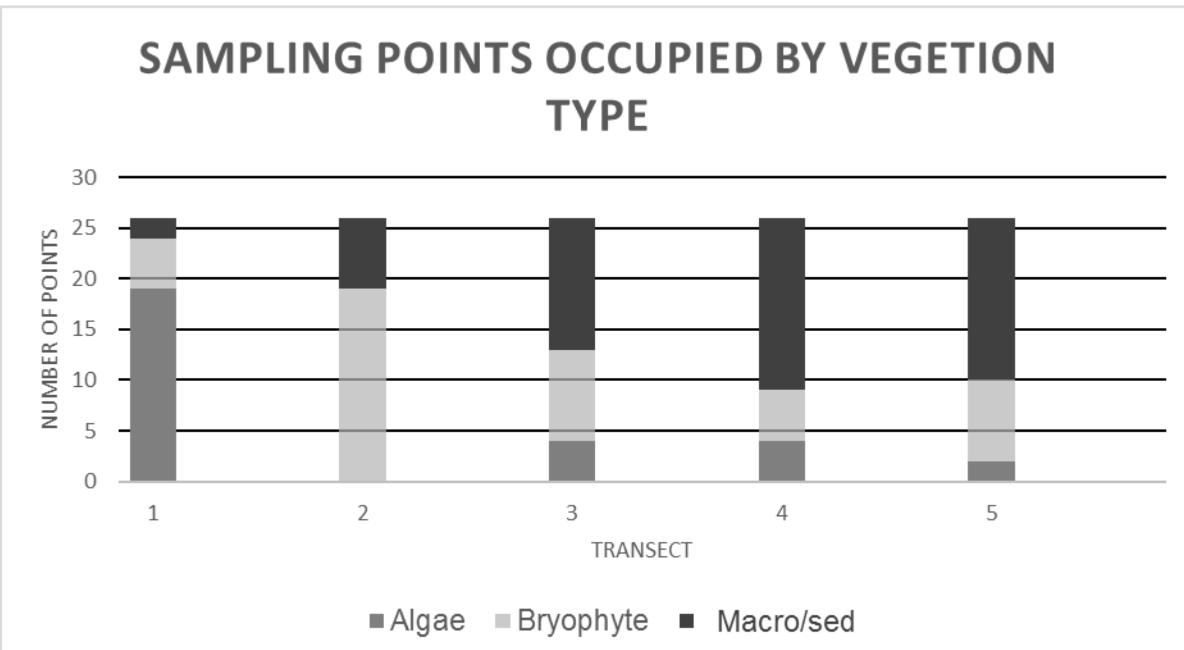

Figure 2.4 Total number of sampling points (n=26) occupied by vegetation type along each transect. Transect one had the highest number of points occupied by algae (grey) while all other transects were either dominated by bryophyte (light grey) or macrophyte and sediment (dark grey).

Mapping of plots showed relatively little coverage of *Spirogyra* turf in the USR compared to other vegetation types. Figure 2.5 shows the combined cover of each vegetation type per month. Though present in all plots at some point in time *Spirogyra* turf never dominated cover within the plots as a whole but instead expanded and retreated, by more than 50% over time. Bryophyte cover doubled from March to June while macrophyte expansion remained somewhat stable. Plots were covered with the most vegetation in July at which time algae expansion peaked, along with bryophyte and macrophyte cover, within the plots. When looking at vegetation cover within each individual plot only Plot 1 maintained cover of all vegetation types across all months while other plots were dominated by a single vegetation type, either bryophyte or macrophyte, but never algae (Figure 2.6). Plot 1 was the only plot in which turf algae persisted across all months while in other plots algae was only present occasionally. Again macrophytes were exclusively composed of *Sagittaria platyphylla* and *Chara* sp. A map set of plots is available in Appendix I.

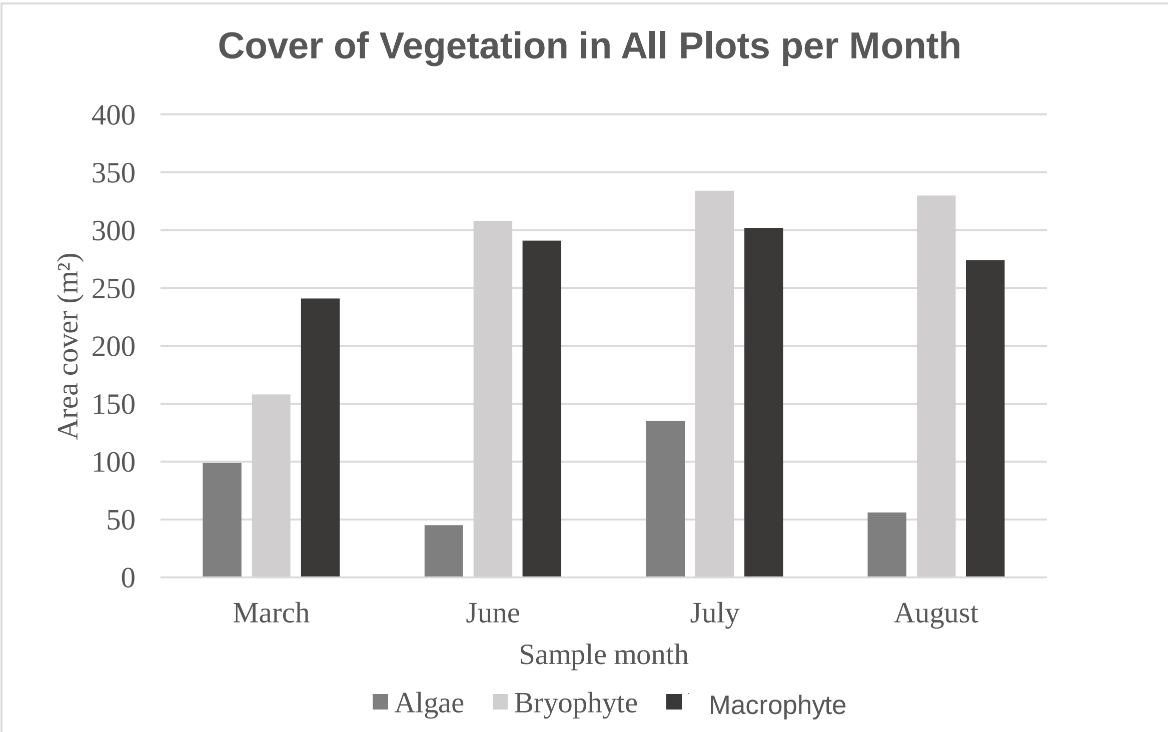

Figure 2.5 Cover of vegetation types by month when plots are combined.

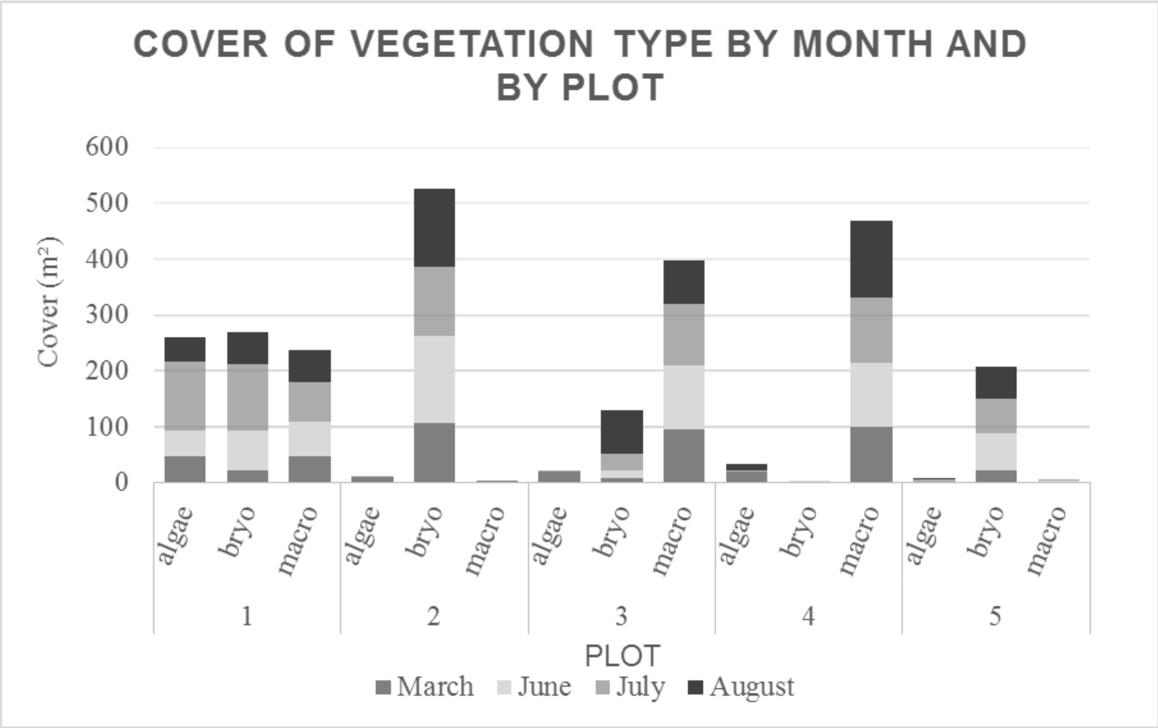

Figure 2.6 Cover of vegetation types by month and by plot. Total area of plots were plot 1: 304 m²; plot 2: 256m²; plot 3: 291m²; plot 4: 295m²; plot 5: 211m². Due to overlap of vegetation types total cover of vegetation can exceed the plot area.

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

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

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

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

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

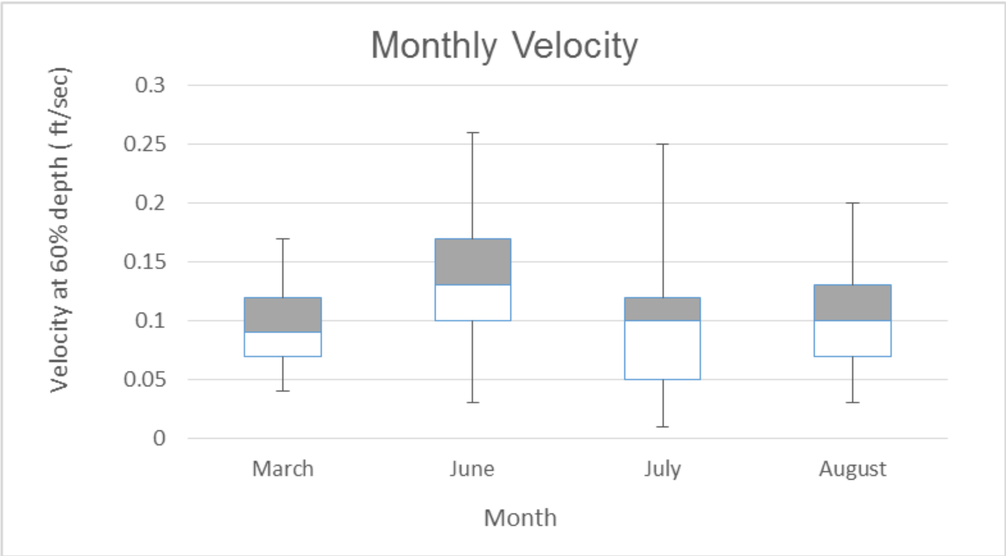

Figure 2.7 Average monthly water velocities measured at 60% of the total depth. Maximum velocity was highest in June and July.

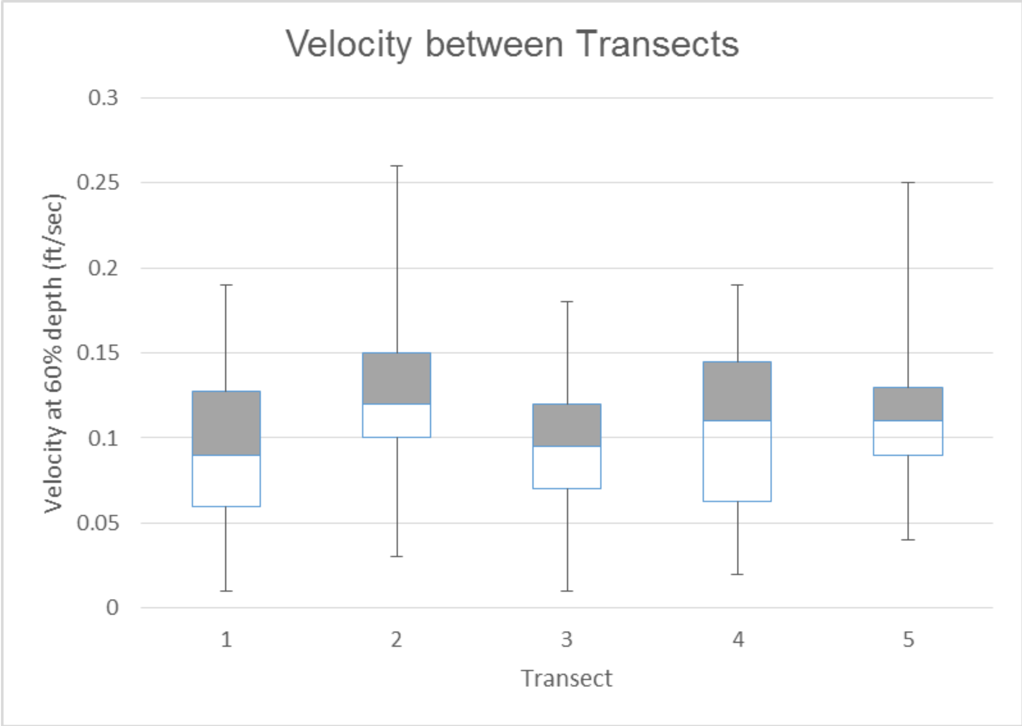

Figure 2.8 Average water velocities at each transect over the sampling period.

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

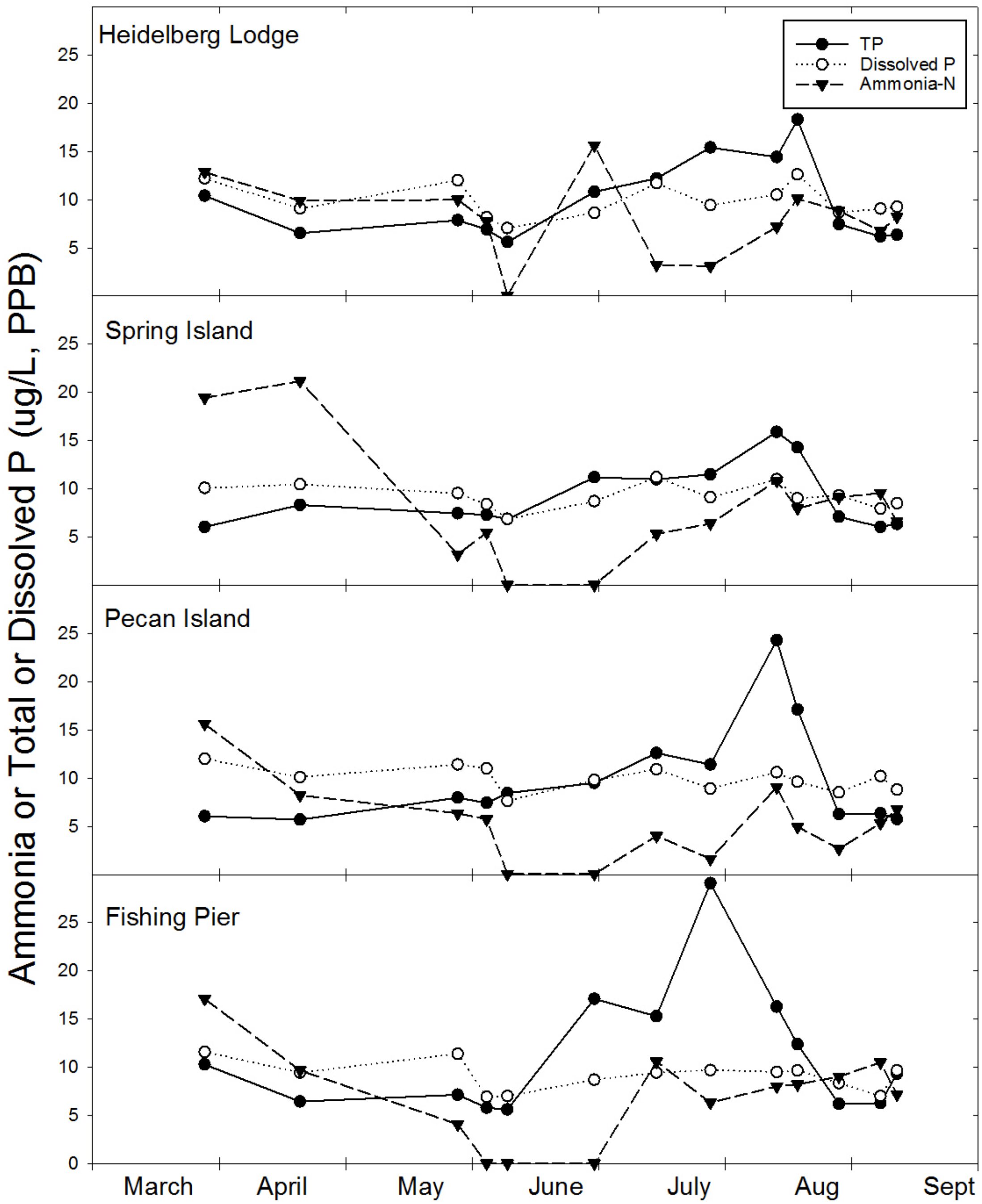

Figure 2.9 March-September 2015 NH3, Dissolved P and Total P at four locations on the Comal River, TX. The very low levels of TP and Dissolved P are notable, and are always very near the analytic detection limits. The occasional spikes in P observed are possibly related to recreation or large number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

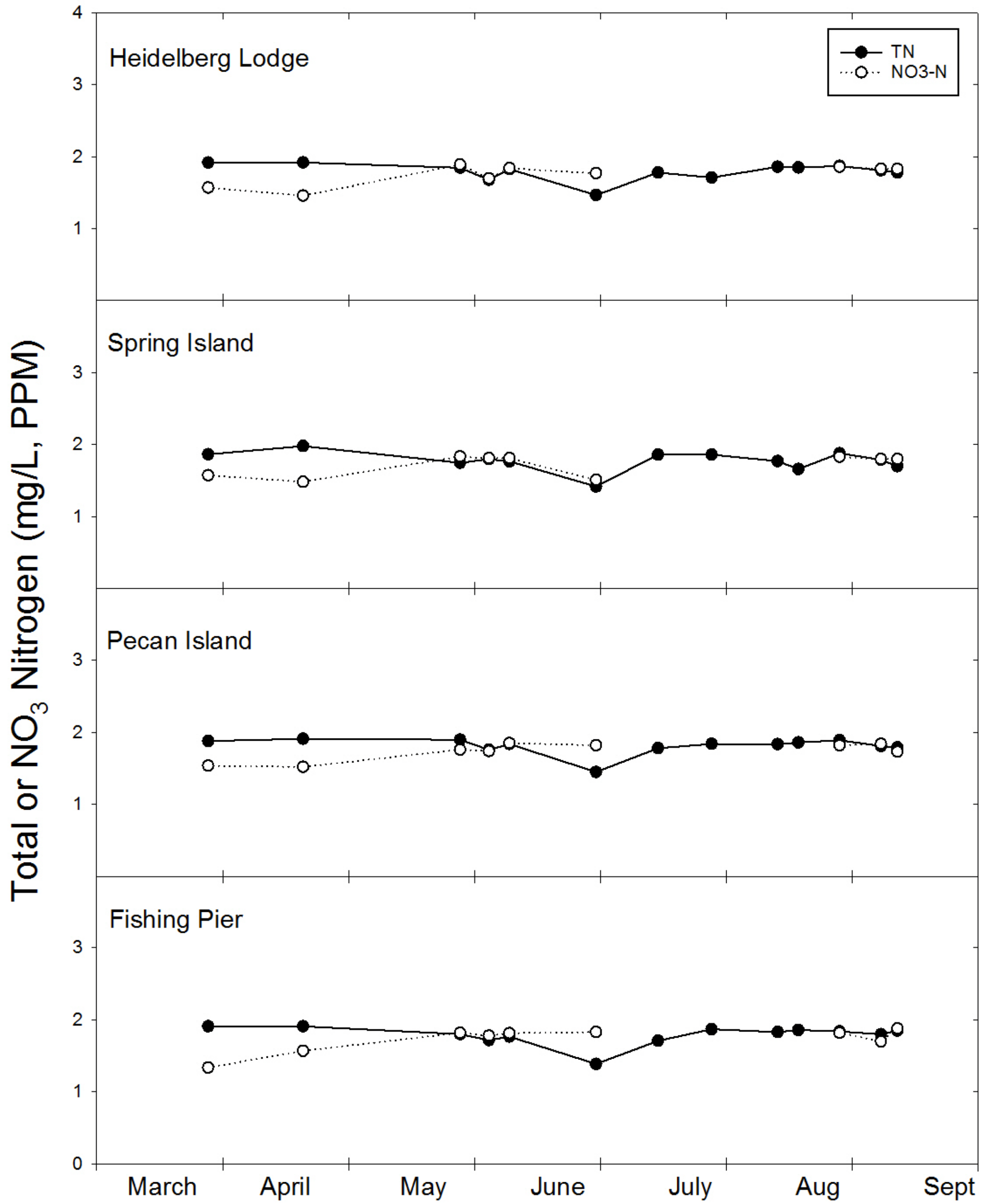

Figure 2.10 March-September 2015 NO_3 and Total N at four locations on the Comal River, TX. Total N is almost entirely composed on NO_3-N.

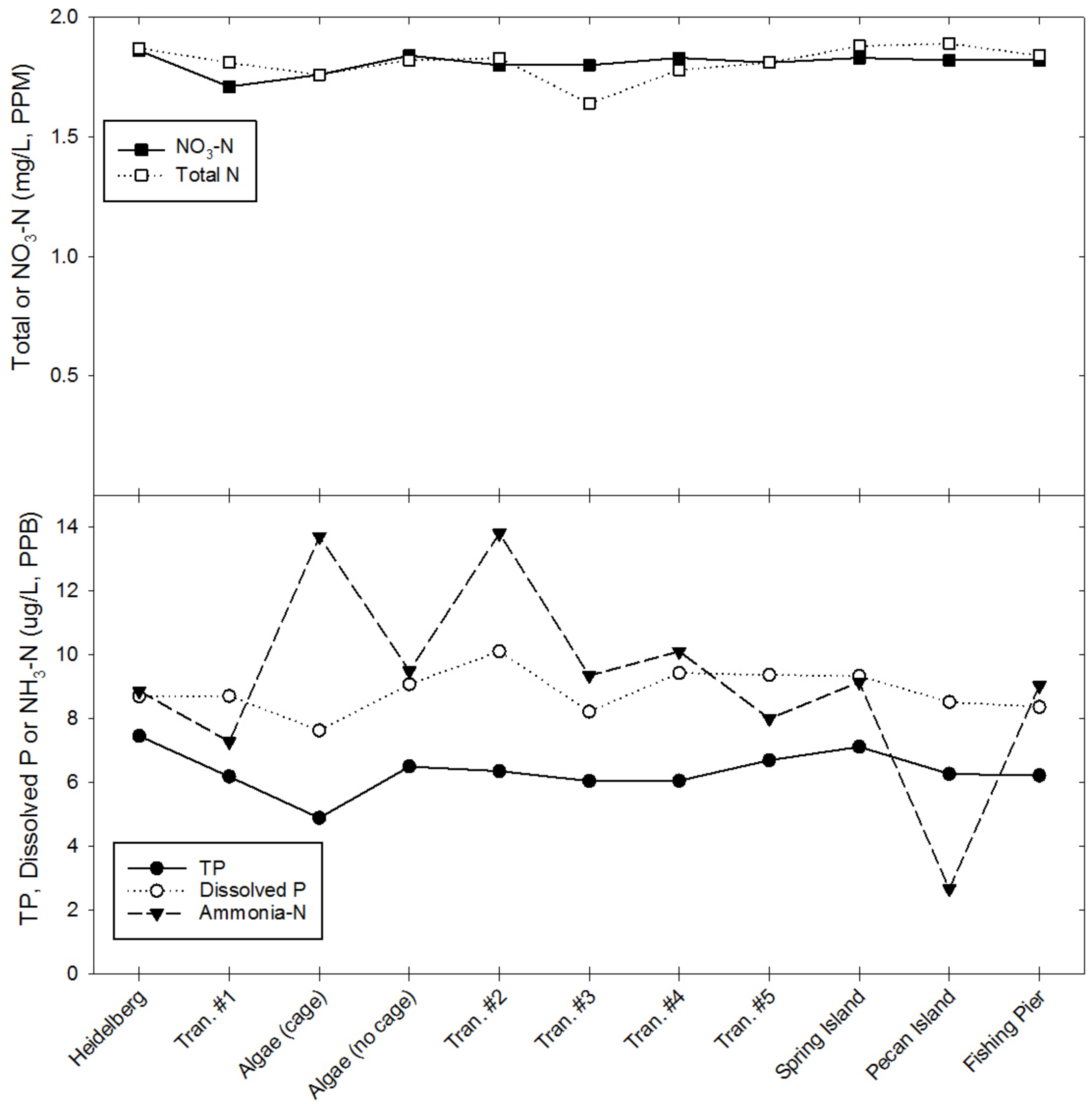

Figure 2.11 Spatial transect of water quality parameters from Heidelberg to the Landa Lake Fishing Pier made on August 28, 2015. The lack of variability among the locations is notable, indicating that in-lake processes are not happening quickly enough to impact the waters flowing through the system.

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

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

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

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

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

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

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

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

Discussion

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

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

Second, turf algae are present regardless of the season. In March multiple pockets of turf algae were mapped throughout the USR and pockets of algae have been observed to persist through the winter months as well and this persistence is probably due to *Spirogyra*'s ability to withstand cool water temperatures and lower light levels (Graham et al., 1995). As the growing season progresses these "mother colonies" eventually give rise to algae turf in multiple locations. For this study, one interesting note is how often algae turf translocated to different areas over the course of the mapping periods. In the USR and Landa Lake algae turf commonly disappeared only to colonize in another location. The Spring Island area of the USR was the only sampling location regularly occupied by turf algae and large scale mapping shows that algae in this location persisted, albeit fragmented, for the longest period of time.

Figure 2.16 Algae and bryophyte rarely intermixed.

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

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

Works Cited and Recommended Readings

Barbour, M.T., J. Gerritsen, B. D., Snyder and J. B. Striplings. 1999. Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates and fish, Second Edition. EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water; Washington, D.C.

Biggs, B. J. F., D. G. Goring, and V. I. Nikora. 1998. Subsidy and stress responses of stream periphyton to gradients in water velocity as a function of community growth form, Journal of Phycology, 34*:* 598–607

Collos, Y. 1986. Time-lag algae growth dynamics: biological constraints on primary production in aquatic environments. Mar. Ecol. Prog. Ser. 33:193-206.

Graham, J. M., C. A. Lembi, H. L. Adrian and D. F. Spencer. 1995. Physiological responses to temperature and irradiance in *Spirogyra* (ZYGNEMATALES, CHAROPHYCEAE), Journal of Phycology, 31:531-540.

CHAPTER 3

IMPACTS OF BENTHIC ALGAE MATS ON AQUATIC VEGETATION

Materials and Methods

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

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

Ludwigia repens, or Ludwigia, is a native aquatic plant that is easily propagated and used in ongoing aquatic habitat restoration in the Comal River. Due to these factors, Ludwigia was chosen to represent the vascular plant type for this study. Established Ludwigia was propagated by collecting 20cm long apical stem fragments from parent colonies located in Landa Lake. These stem fragments were planted into quart-sized nursery pots filled with native sediment also collected from Landa Lake. Ten stem fragments were planted per pot, and potted plants were allowed to establish in an in situ nursery in Landa Lake for three weeks. This was sufficient time for the plants to establish and root development to fill the nursery pots. Twelve established plants of equivalent size were selected for use in the study and divided between two plastic nursery trays which were assigned to the +Algae treatment or no-Algae site. Twelve new-growth Ludwigia plants were planted in the same manner as the established plants (ten 20cm apical fragments per pot) but given no pre-culture establishment period. Then, they were divided between two nursery trays and placed in either the treatment (+Algae) or reference (no-Algae) plot. Additional established Ludwigia plants and Ludwigia stem fragments were harvested to obtain initial biomass and morphometric parameters.

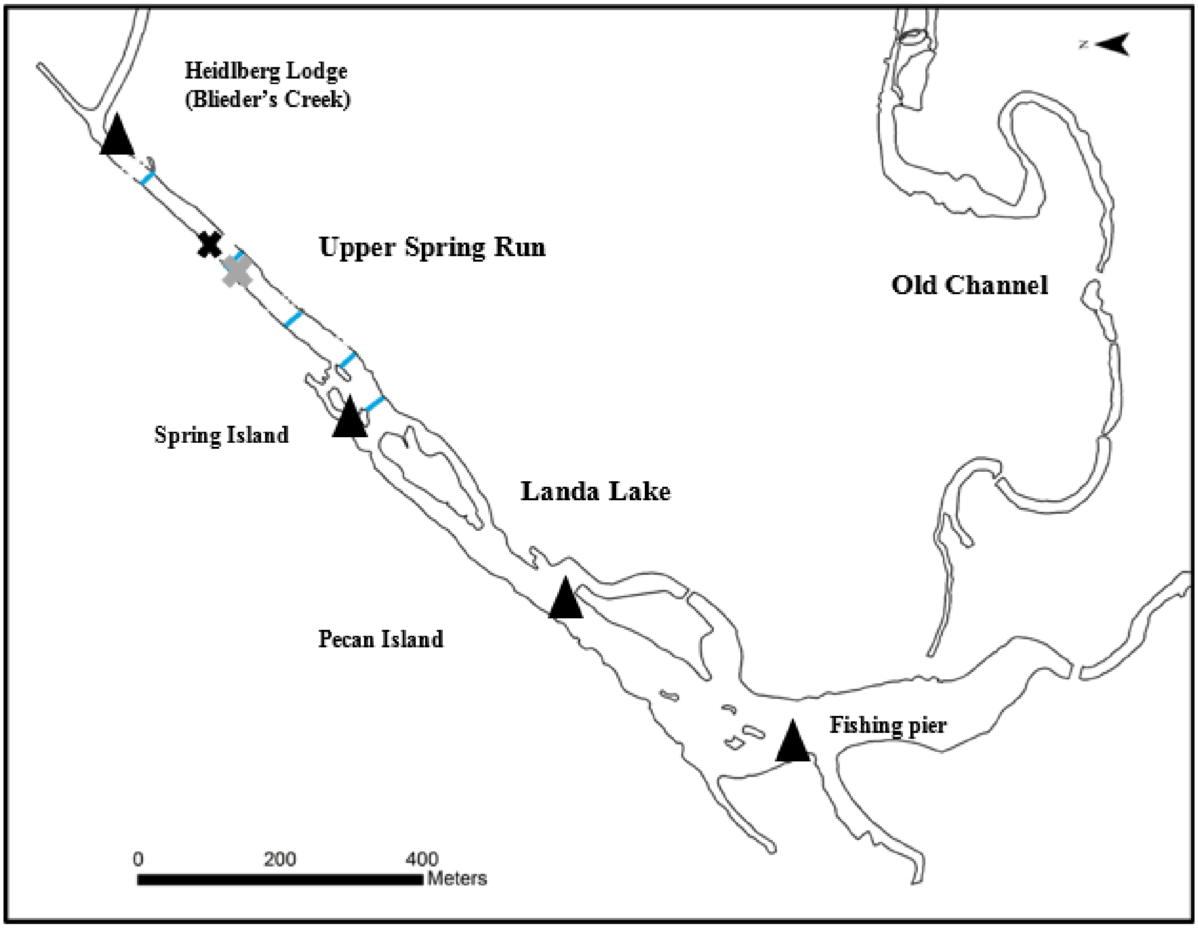

Figure 3.1 Location of the +Algae (black X) and no-Algae (grey X) plots in the Upper Spring Run in reference to algae transects (blue lines) and dissolved nutrients sampling stations (black triangles).

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

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

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

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

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

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

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

Results

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

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

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

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

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

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

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

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

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

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

Ludwigia stem lengths and the biomass of all vegetation classes are represented in Figures 3.5 and 3.6. The pattern of vegetation biomass over the experimental period shows that none of the vegetation types showed increases in biomass in either the treatment or the reference plot (Figure 3.6). Even so, differences existed between the +Algae and no-Algae plots by the end of the experiment for three of the four vegetation types. Established Ludwigia and established bryophytes maintained their initial biomass in the no-Algae plot, while their biomass in the +Algae treatment plot declined significantly by the end of the experiment. New-growth Ludwigia showed little variability through time as there was no significant difference in biomass between the +Algae and no-Algae plots. New-growth bryophytes declined in both plots, although they declined significantly more in the +Algae treatment plot than in the no-Algae plot. At the 32 day harvest established Ludwigia in the +Algae treatment exhibited more elongated stem growth then established Ludwigia in the no-Algae plot which were bushier with multiple secondary branching (Figure 3.5). At the conclusion of the study it appeared that herbivory or some other physical damage occurred to Ludwigia plants in the +Algae plot as stems of Ludwigia were found floating in the enclosure. These were gathered to include with biomass estimates but it is unknown how much biomass was lost or potentially consumed by herbivore activity. Furthermore, it is unclear if any biomass was also lost from the reference plot, since any broken stems would have floated away.

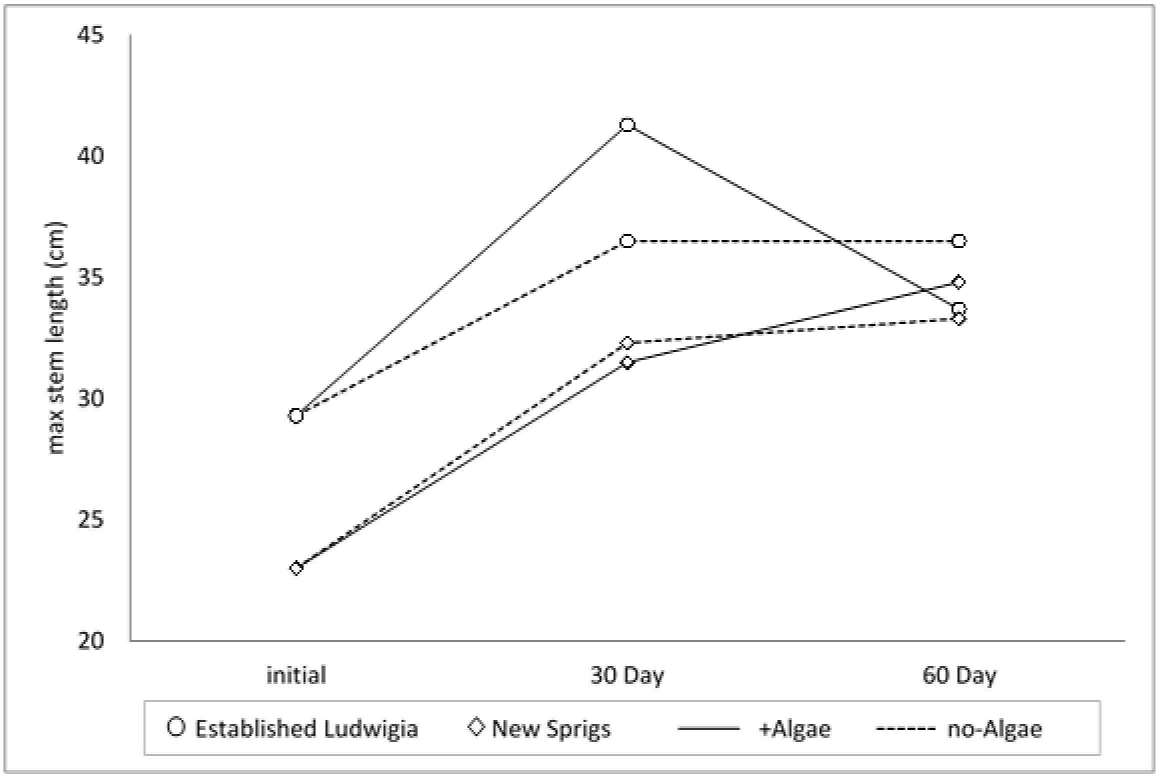

Figure 3.5 Stem lengths of established and new growth Ludwigia in the +Algae and reference plots. Established Ludwigia showed the greatest increase in stem length at the 32 day harvest but by the end harvest stem length was reduced by physical damage.

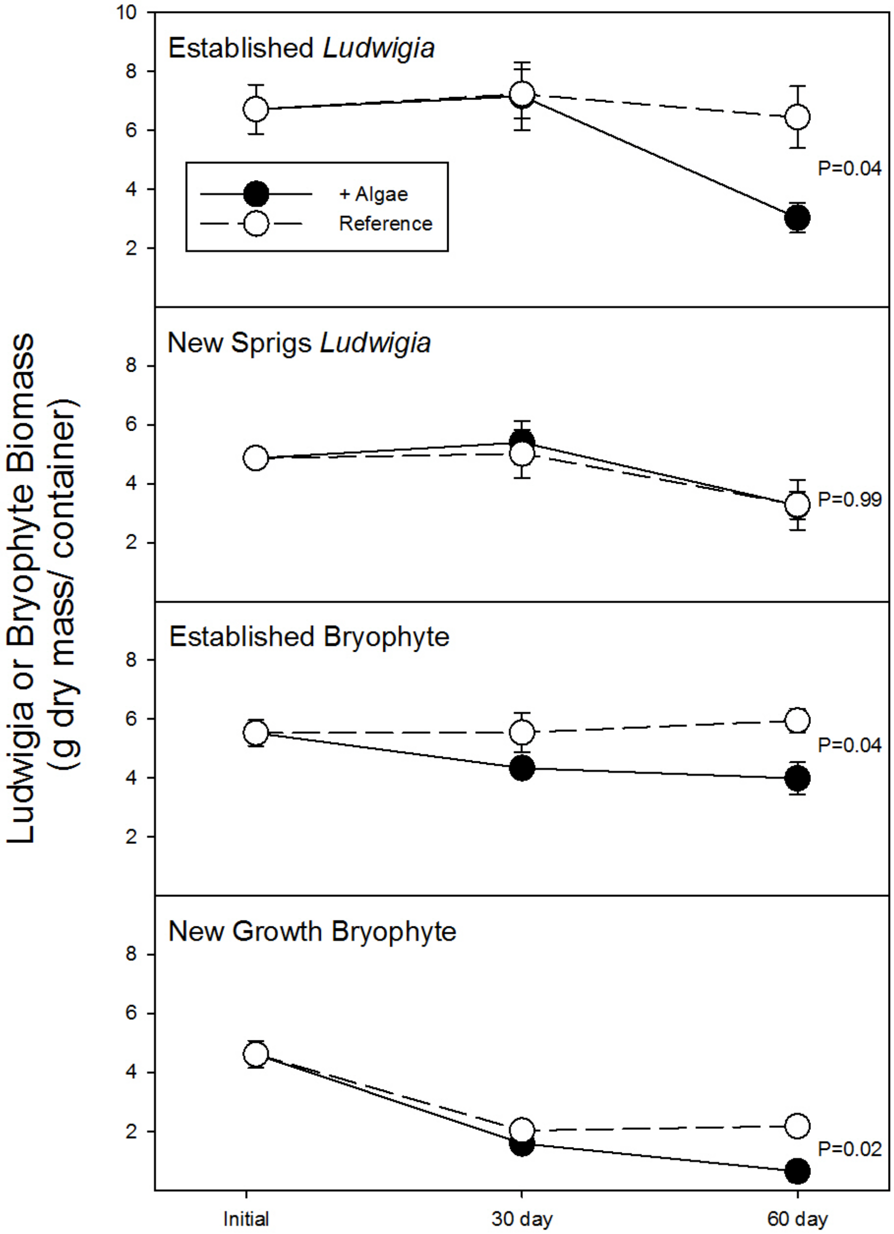

Figure 3.6 Vegetation biomass for each of the four vegetation types through the experimental growth period. The p-value of a Student *t-test* comparing the final biomass in the +Algae treatment and the Reference plot is shown.

Discussion

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

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

Works Cited and Recommended Readings

Gustafsson, C. and C. Bostrom. 2014. Algal mats reduce Eelgrass (*Zostera marina* L.) growth in mixed and monospecific meadows. J. Exp Mar Biol Ecol 461:85-92

Hauxwell, J., J. Cebrian, C. Furlong and I. Viliela. 2001. Macroalgal canopies contribute to Eelgrass (*Zostera marina*) decline in temperate estuarine ecosystems. Ecology 82:1007-1022

McGlathery, K. J. 2001. Macroalgal blooms contribute to the decline of Seagrass in nutrient-enriched coastal waters. J. Phycol. 37:453-456

Nakai S., M. Hosomi, M. Okada and A. Murakami. 1996. Control of algal growth by macrophytes and macrophyte-extrated bioactive compounds. Wat. Sci. Tech. 34:227-235

Parker, J. D., D. E. Burkepile, D. O. Collins, J. Kubanek and M. E. Hay. 2007. Stream mosses as chemically-defended refugia for freshwater macroinvertebrates. Oikos 116:302-312.

Pieczynska E. and A. Tarmanowska. 1996. Effect of decomposing filamentous algae on growth of *Elodea canadensis* Michx (a laboratory experiment). Aquatic Botany 54:313-319

Qiuying, H. and L. Dongyan. 2014. Macroalgae blooms and their effects on Seagrass ecosystems. J Ocean U China 13:791-798

Rasmussen J. R., B. Olesen and D. Krause-Jensen. 2012. Effects of filamentous macroalgae mats on growth and survival of Eelgrass, Zostera marina, seedlings. Aquatic Botany 99:41-48

Rasmussen J. R., M. F. Pedersen, Birgrit Olesen, S. L. Nielsen and T. M. Pedersen. 2013. Temporal and spatial dynamics of ephemeral drift-algae in Eelgrass, *Zostera marina*, beds. Estuarine, Coastal and Shelf Science. 119:167-175

Sand-Jensen, K. and M. Søndergaard. 1981. Phytoplankton and epiphyte development and their shading effect on submerged macrophytes in lakes of different nutrient status. Int. Revue Ges. Hydrobiol. 66:529–552.

Steinman, A. D. and H. L. Boston. 1993. The eco-logical role of aquatic bryophytes in a heterotro-phic woodland stream. Journal of the North American Benthological Society 12:17-26.

Stream Bryophyte Group. 1999. Roles of bryophytes in stream ecosystems. Journal of the North American Benthological Society p.151-184.

Tarmanowska A. 1995. Laboratory studies on the influence of living and decomposing filamentous algae on the growth of *Elodea canadensis* Michx. *Acta bot. Gallica* 142:685-692

CHAPTER 4

DISSOLVED OXYGEN DYNAMICS IN LANDA LAKE AND THE UPPER SPRING RUN

4.1 SPATIAL DISTRIBUTION OF DISSOLVED OXYGEN

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

Materials and Methods

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

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

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

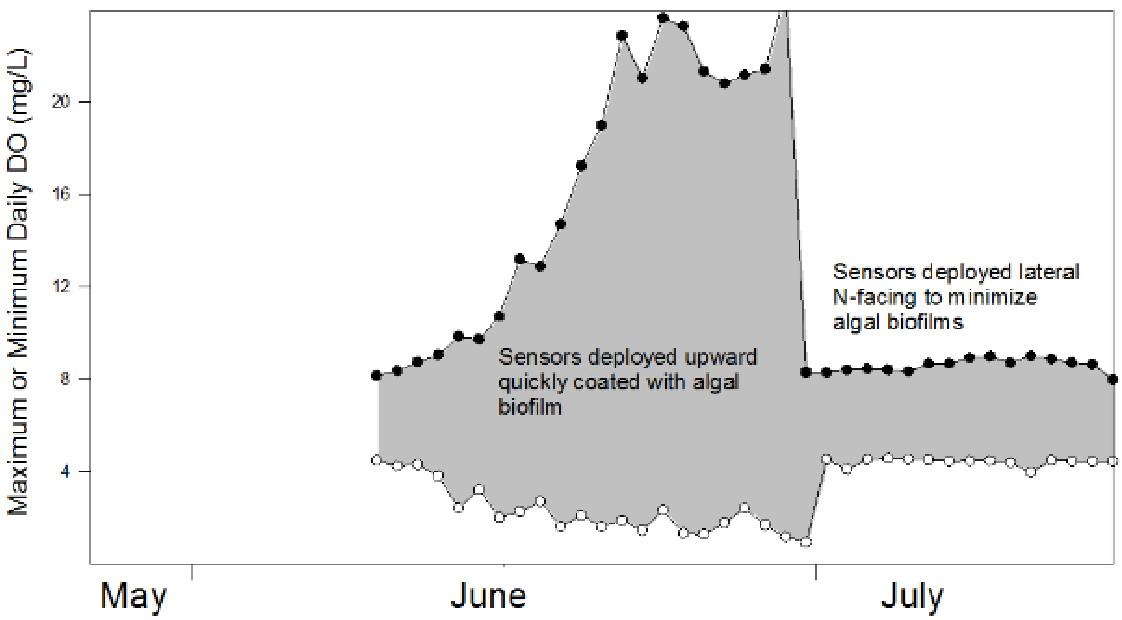

Figure 4.2 Upward versus horizontal deployment results of biofouling activity.

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

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

During deployment MiniDOTs were cleaned after 3 days. At the end of the 8 day deployment data was downloaded. To download the data, the sensors were collected, wiped down, opened, and turned off. Four of the MiniDOTs had SD cards that stored the data; these SD cards were removed, inserted into an SD card reader, downloaded onto a YUMA Tablet, then wiped clean and returned to the MiniDOT. The other 10 sensors were downloaded via usb cable that was plugged directly into the sensor from the YUMA.

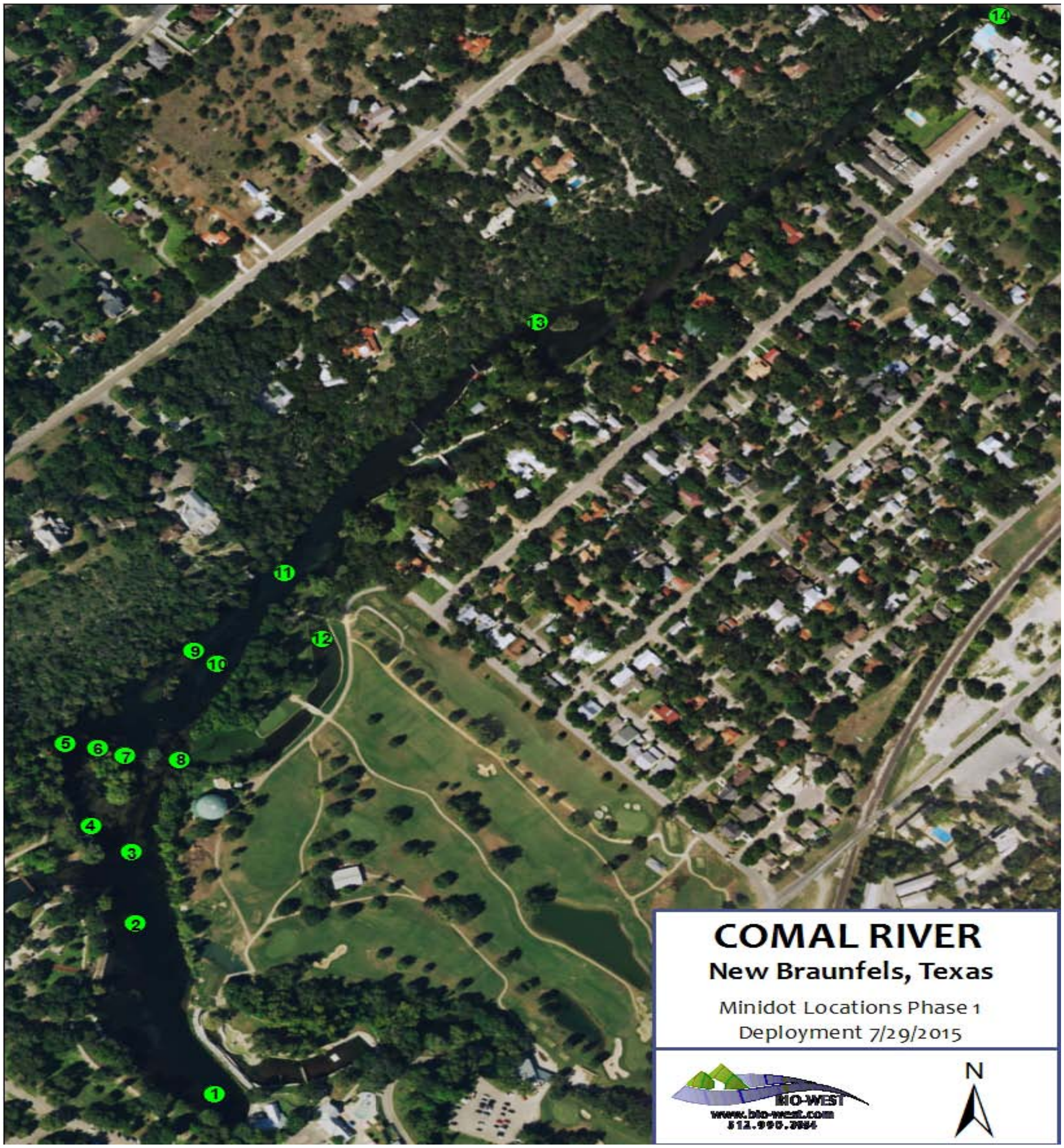

Figure 4.3 Location of 14 MiniDOT dissolved oxygen sensors during initial spatial evaluation study.

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

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

Results

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

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

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

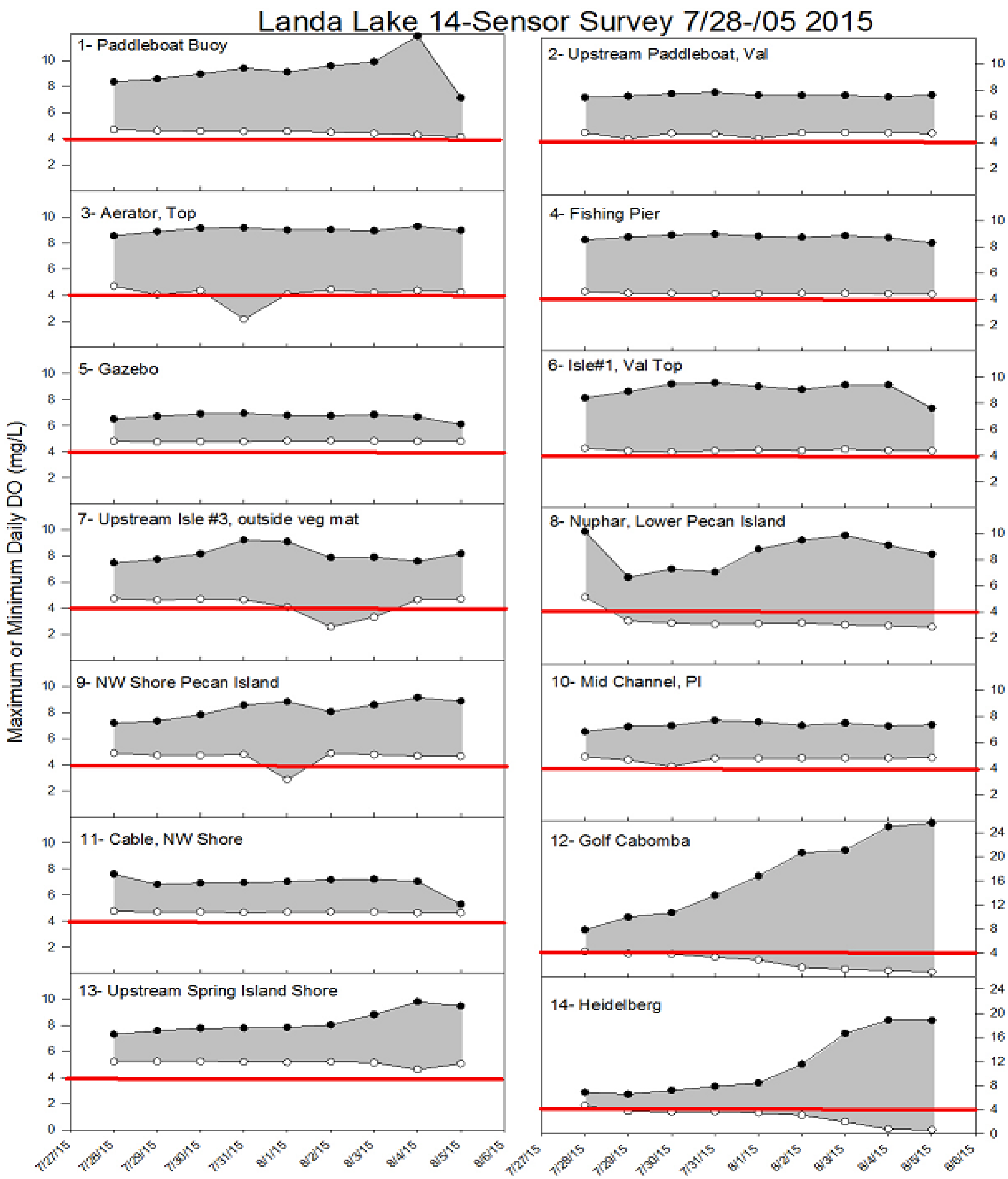

Figure 4.4 Maximum or Minimum dissolved oxygen results (mg/L) from 14 MiniDOT sensors during spatial evaluation study. Data at stations 8, 12 and 14 were heavily affected by biofouling.

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

Materials and Methods

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

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

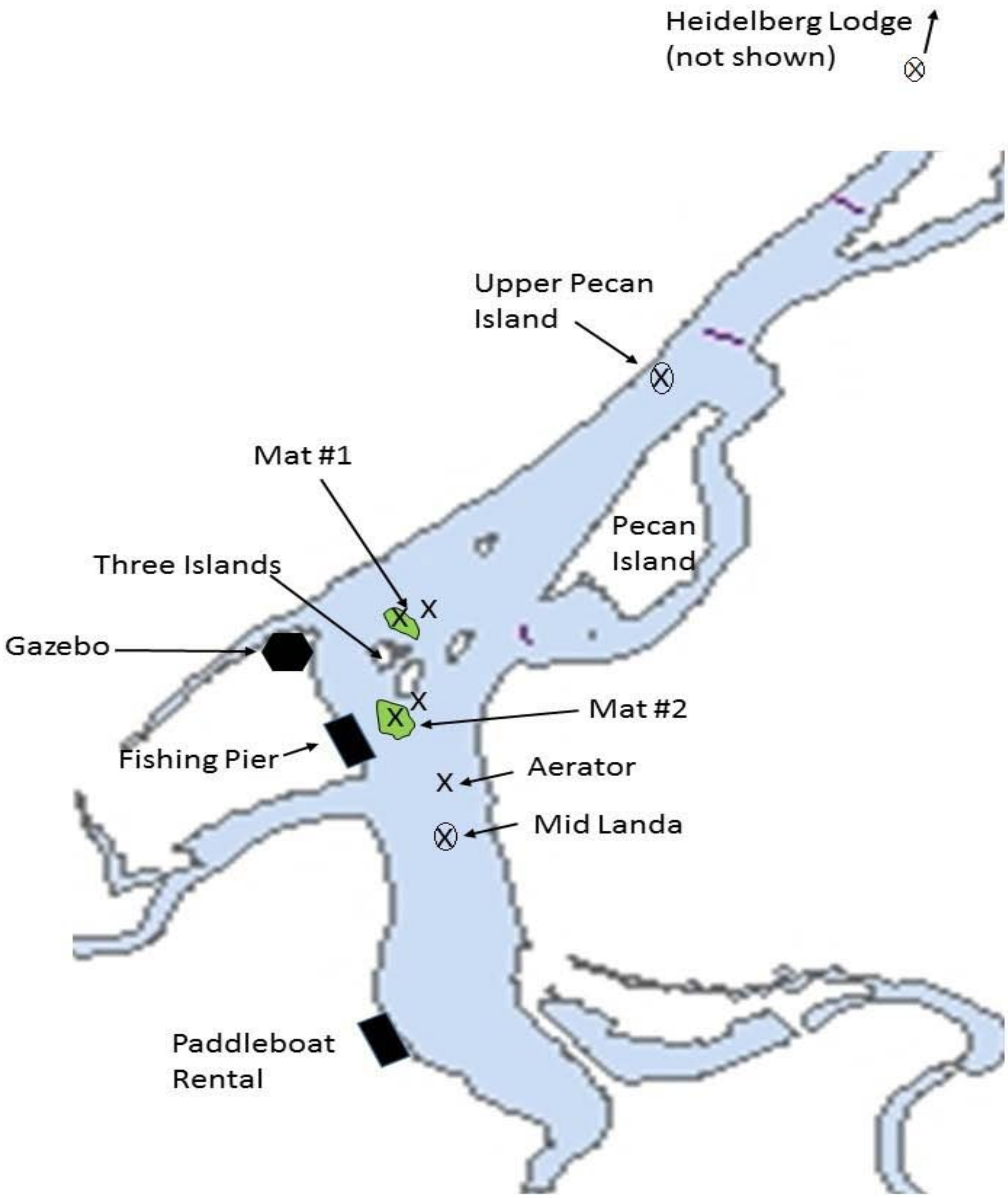

Figure 4.5 Location of eight MiniDOT dissolved oxygen stations selected for the August-October DO survey. Stations with X received one MINIDOT placed at mid-depth while locations with encircled X received two MINIDOTS placed at the sediment-water interface and just below the water surface. The Heidelberg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

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

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

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

Results

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

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

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

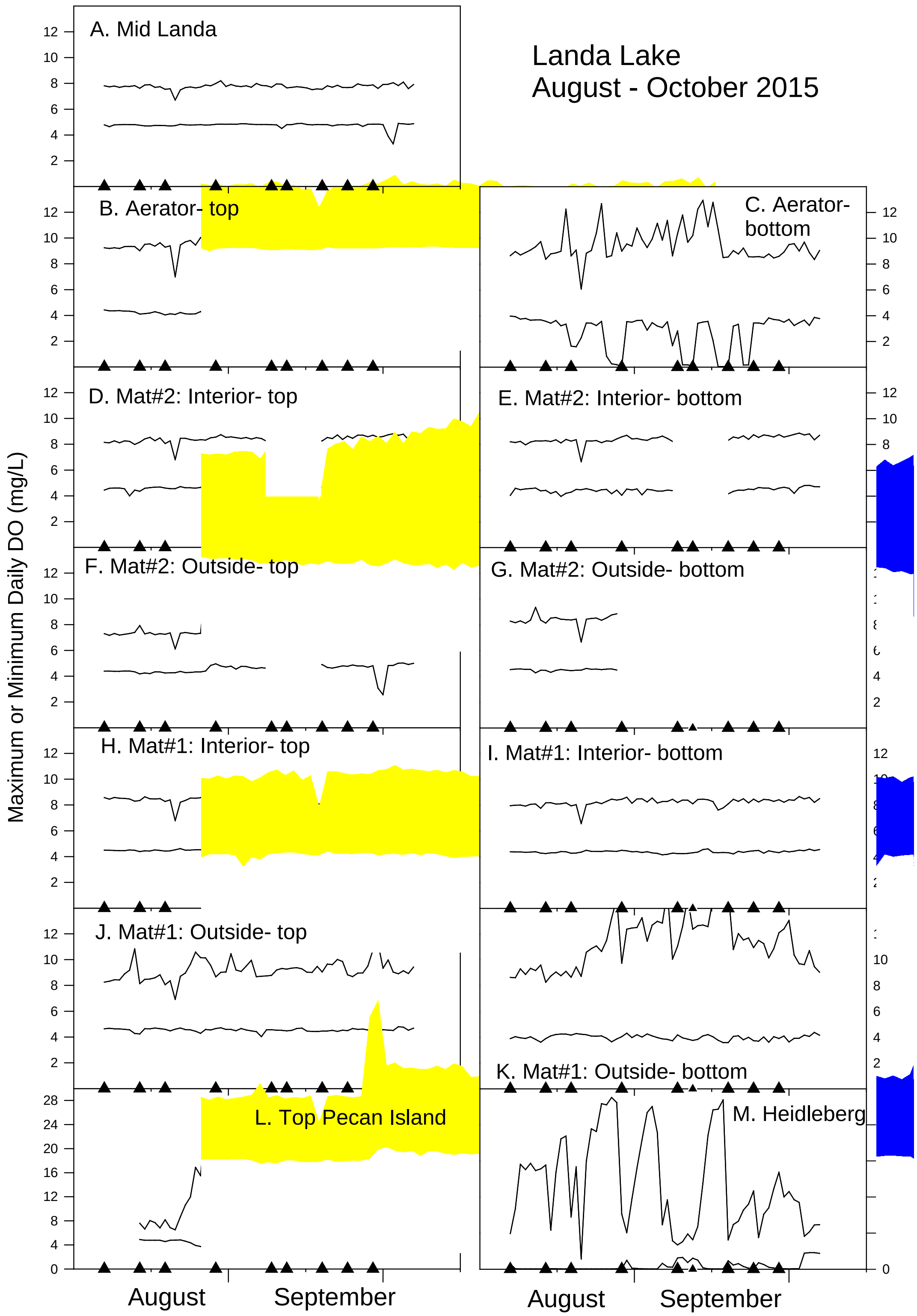

Figure 4.7 Maximum or Minimum dissolved oxygen results (mg/L) from MiniDOT sensors during deployment at the 13 locations. Dates when sensors were serviced are shown as triangles along the date timeline. Yellow= top sensors, Blue= near bottom.

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

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

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

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

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

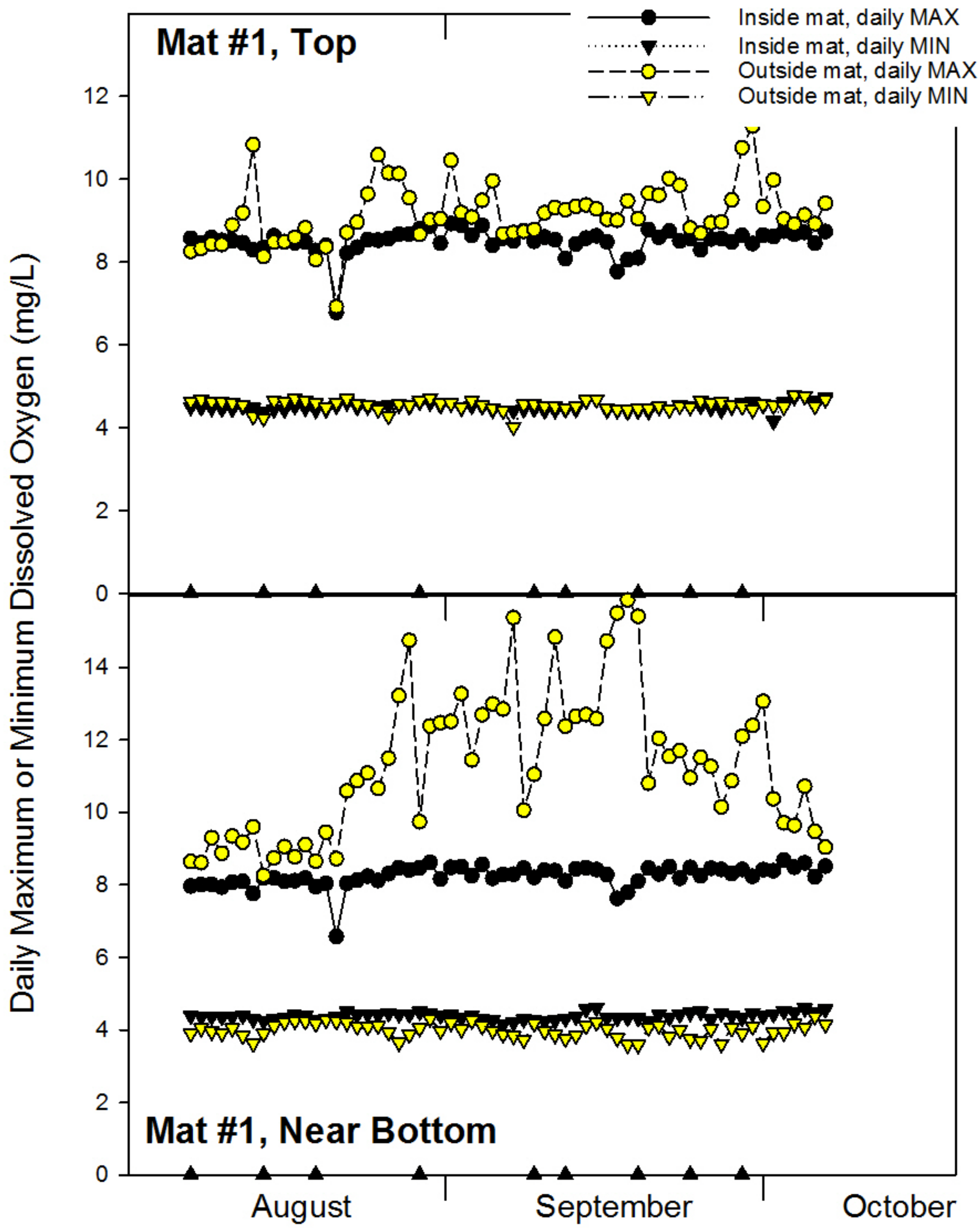

Figure 4.8 Daily maximum and minimum levels of dissolved oxygen at Mat #1. Top Panel shows data for the top of the water column, while the bottom panel shows data for the sensor near the sediment surface. Yellow symbols are used for sensors outside the mat and black symbols for sensors inside the mat.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

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

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

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

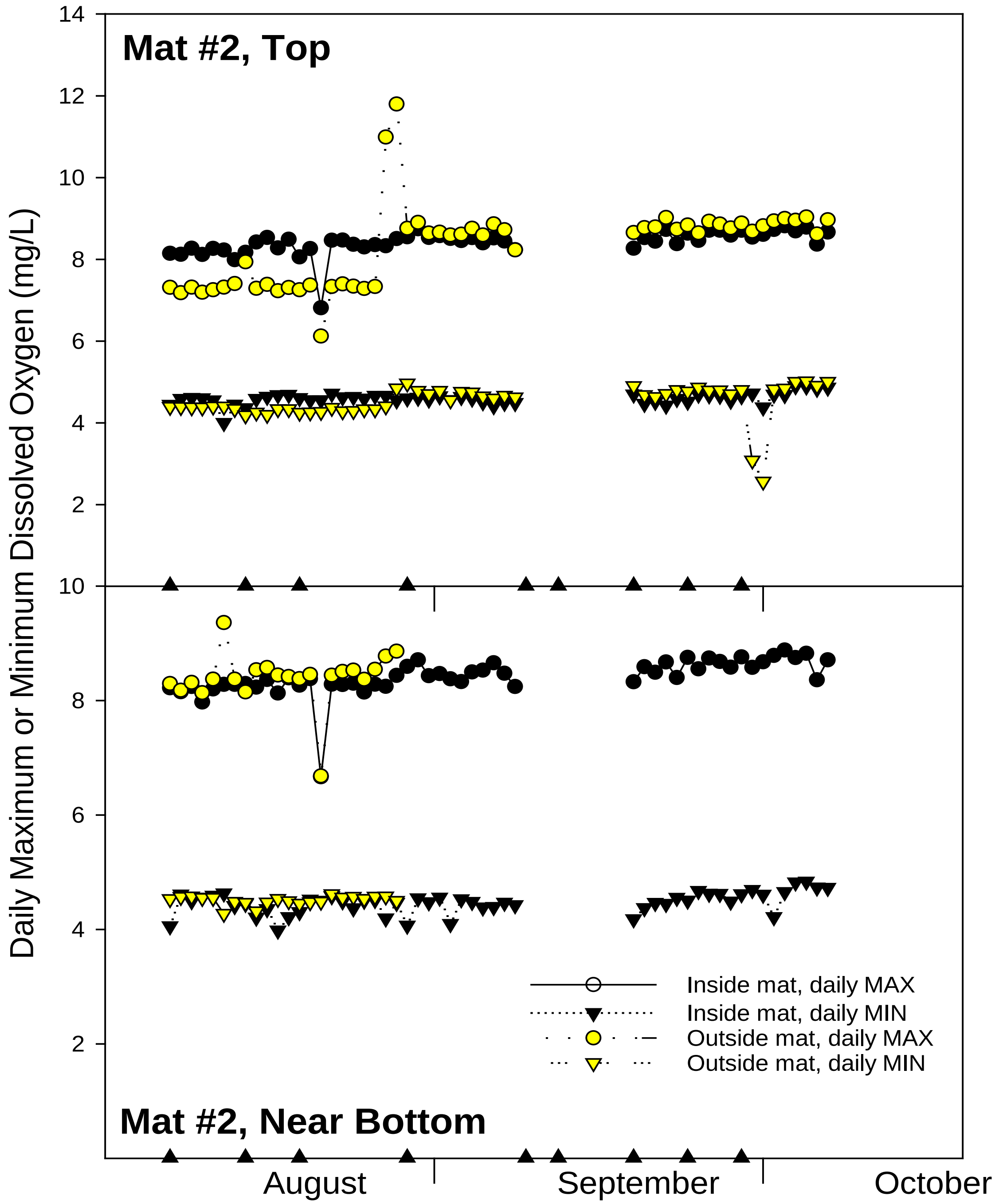

Figure 4.9 Daily maximum and minimum levels of dissolved oxygen at Mat #2. Top Panel shows data for the top of the water column, while the bottom panel shows data for the sensor near the sediment surface. Yellow symbols are used for sensors outside the mat and black symbols for sensors inside the mat.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

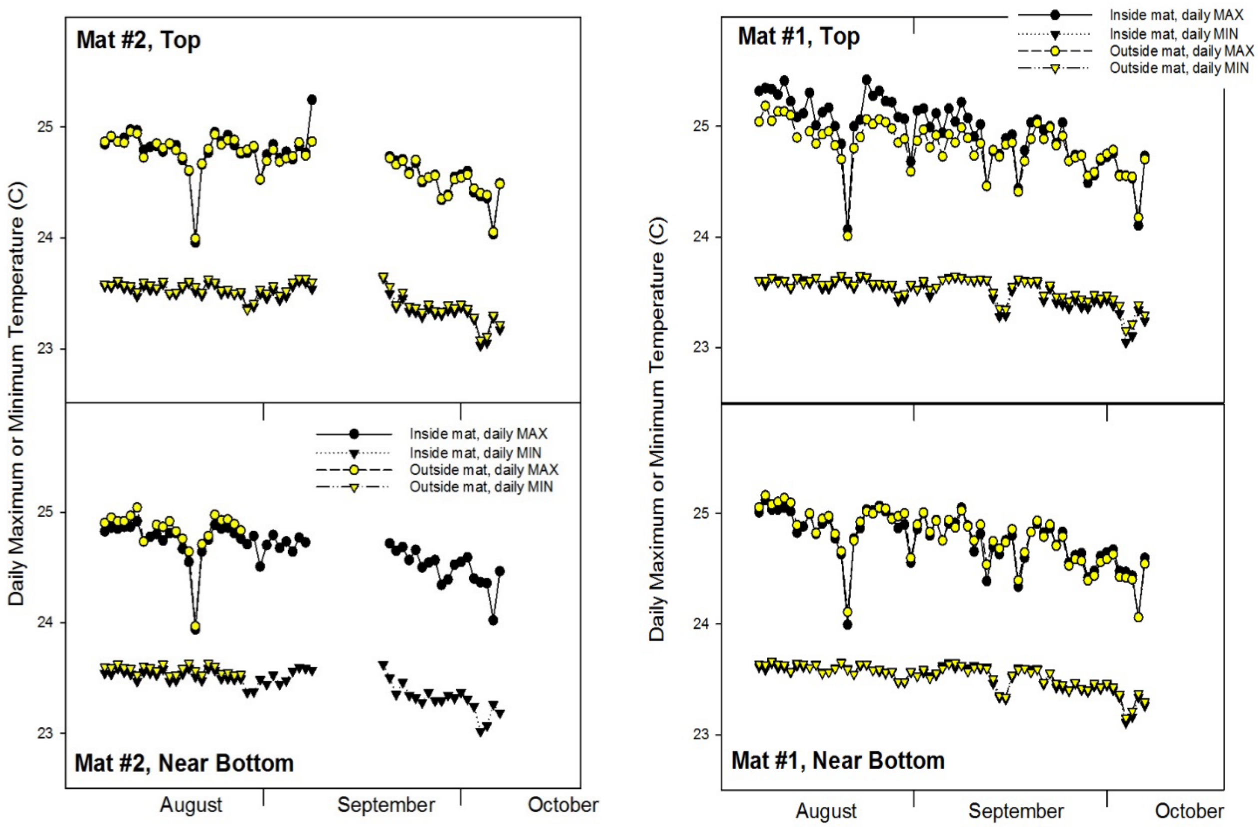

Figure 4.10 Maximum or Minimum water temperature results (° C) from MiniDOT sensors located within mats or outside of mats and at the top or bottom of the water column.

Discussion

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

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

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

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

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

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

4.2.1 COMPOSITION AND BIOMASS OF VEGETATION MATS

Materials and Methods

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

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

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

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

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

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

Results

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

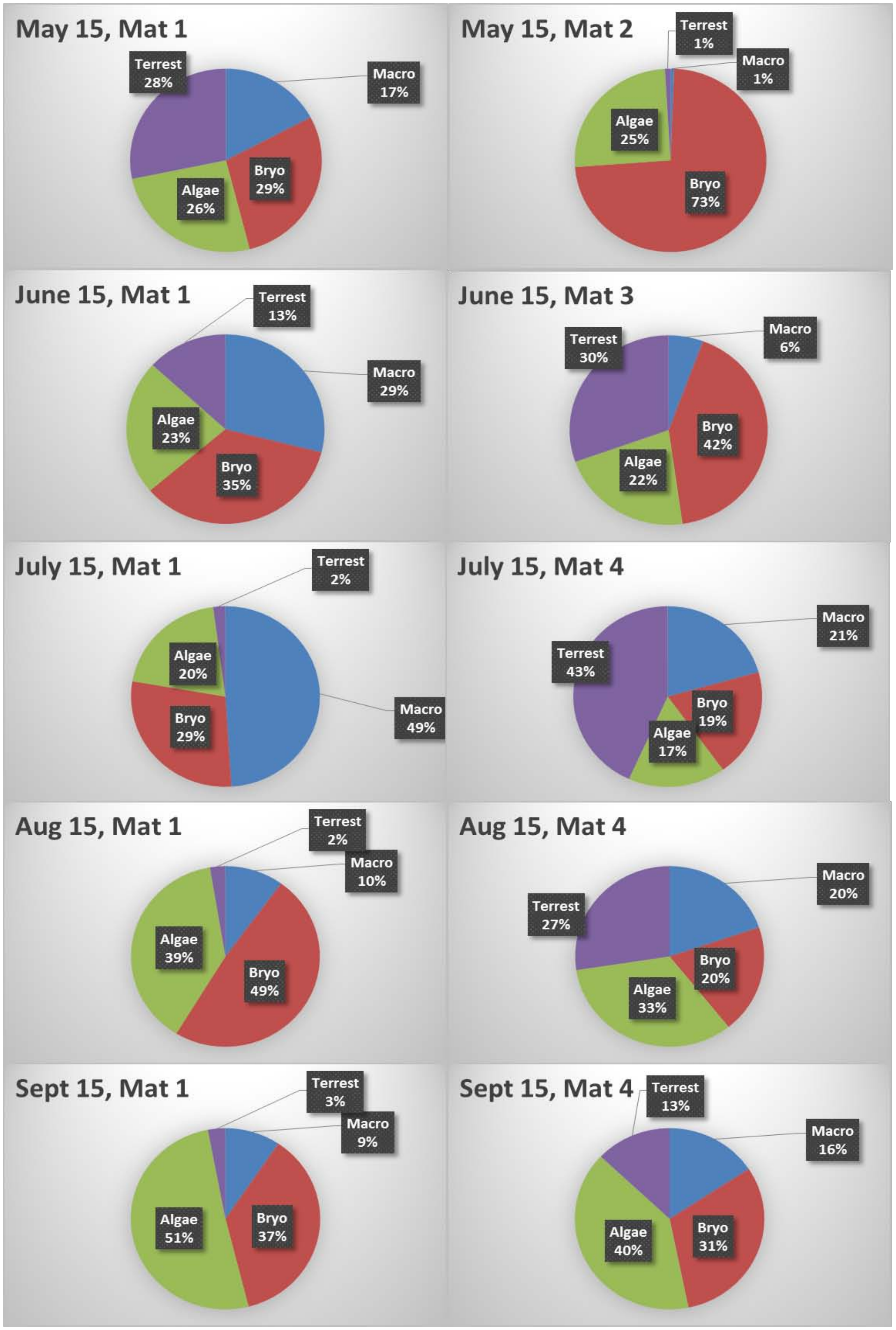

Figure 4.13 Composition of mats collected during each month. Shown are averages of dry mass samples for 3-4 quadrats collected at each mat site each month.

4.2.2 POTENTIAL BIOLOGICAL OXYGEN DEMAND OF VEGETATION MATS.

Materials and Methods.

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

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

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

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

Results

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

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

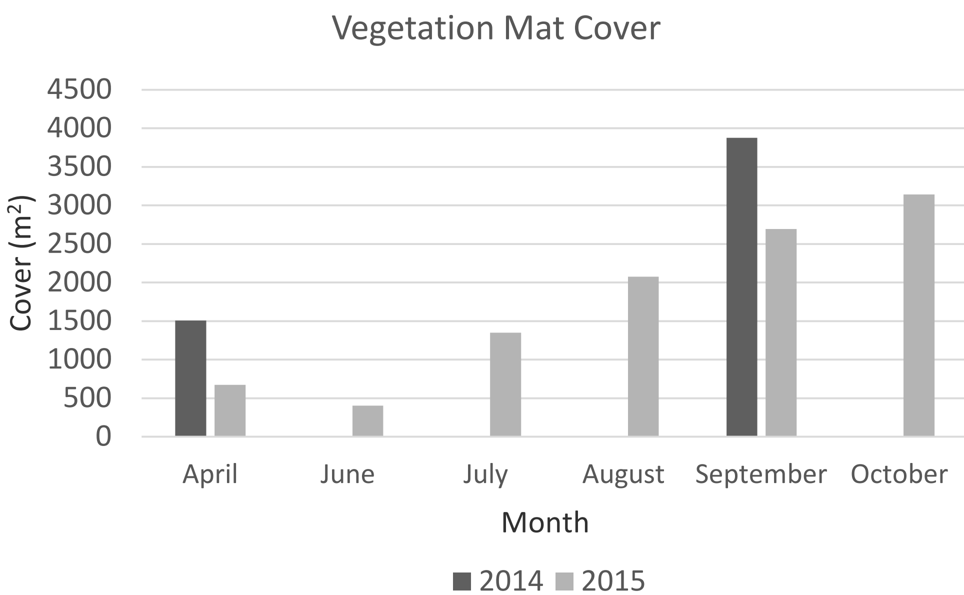

Figure 4.14 Total cover of vegetation mats during sampling months (2015) and compared to dates sampled in 2014.

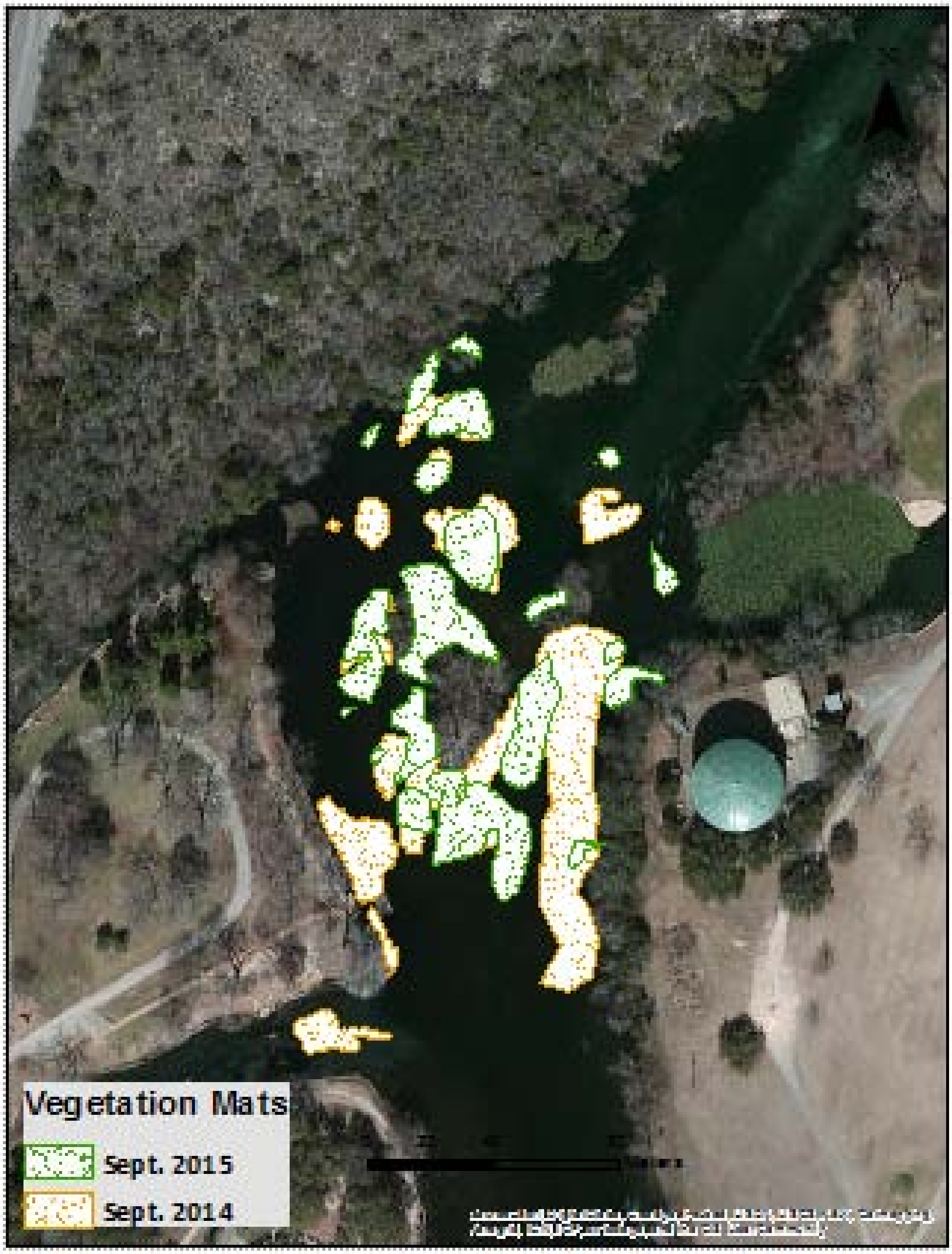

Figure 4.15 Map comparing cover of vegetation mat cover in September of 2014 and September of 2015.

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

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

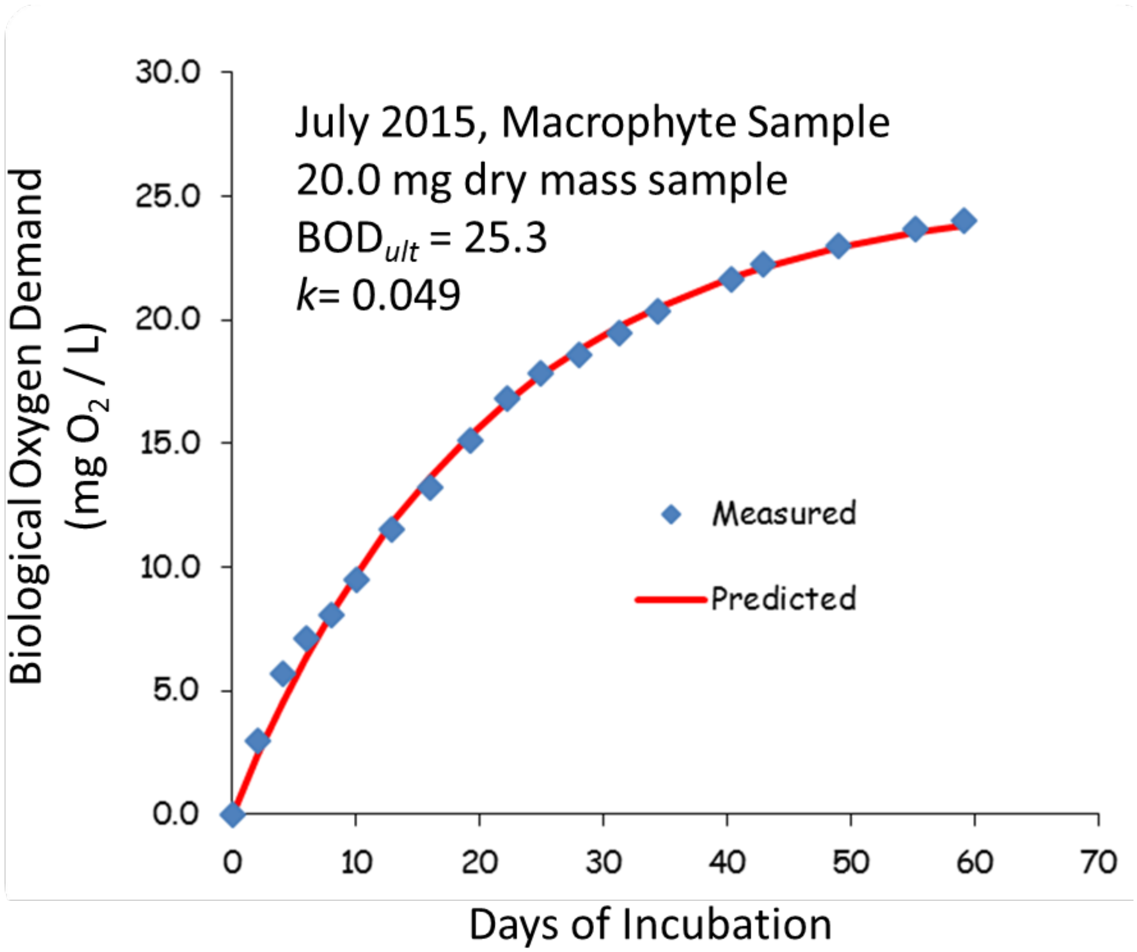

Figure 4.16 Measured Cumulative Biological Oxygen Demand through time (blue diamonds) and empirical curve fit to the data.

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

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

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

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

Discussion

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

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

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

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

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

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

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

It seems intuitive that the accumulation of floating vegetation constitutes a direct threat as they modify local conditions for the species around which they congregate and reduce light availability to submerged plants. One local experiment found that mats composed of drifting vegetation that became entangled and accumulated around artificial obstructions had negative effects on various growth parameters of endangered *Zizania texana*, including obvious mechanical damage to plant tissues (Power, 1996). The consequences of shading as well as diel DO and pH changes can be more pronounced where mat canopies are dense and broad (Frodge et al, 1990; Caraco et al, 2006). Mats composed of bryophyte and algal material might indirectly affect the food web as they attract invertebrates to the surface and away from benthic habitats (Power, 1990). Observational studies of floating islands of vegetation in Florida lakes found that mats collect sediment and develop more complex root systems over time, which could permanently alter the areas in which they form (Mallison et al, 2001).

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

Works Cited and Recommended Readings

Caraco, N., J. Cole, S. Findlay and C. Wigand.2006. Vascular plants as engineers of oxygen in aquatic systems. BioScience 56:219-225

Cherry, J. A. and L. Gough. 2005. Temporary floating island formation maintains wetland plant species richness: The role of the seed bank. Aquatic Botany 85:29-36

[EARIP] Edwards Aquifer Recovery Implementation Program. 2011. Habitat Conservation Plan. Prepared for the Edwards Aquifer Recovery Implementation Program.

Frodge, J. D., G. L. Thomas and G. B. Pauley. 1990. Effects of canopy formation by floating and submergent aquatic macrophytes on the water quality of two shallow Pacific Northwest lakes. Aquatic Botany 38:231-248

Mallison, C. T., R. K. Stocker and C. E. Cichra. 2001. Physical and vegetative characteristics of floating islands. J. Aquat. Plant Manage. 39:107-111

Power, M. 1990. Benthic turfs vs floating mats of algae in river food webs. Oikos 581: 67-79

Power, P. 1996. Direct and indirect effects of floating vegetation mats on Texas Wild Rice (*Zizania texana*). The Southwestern Naturalist 41:462-464

Somodi, I. and B. D. Zoltan. 2002. Determinant of floating island vegetation and succession in a recently flooded shallow lake, Kis-Balaton (Hungary). Aquatic Botany 79:357-366

Strosnider, W.H., and R. W. Nairn. 2010. Effects on the underlying water column by ecologically engineered floating vegetation mats. In Proceedings of the American Society of Mining and Reclamation National Conference 2010 pp. 1236-1257

Wang, S., X. Jin, Q. Bu, L.,Jiao, and F. Wu. 2008. Effects of dissolved oxygen supply level on phosphorous release from lake sediments. Colloids and Surfaces A: Physicochem Eng Aspects 316:245-252

CHAPTER 5

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

Materials and Methods

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

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

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

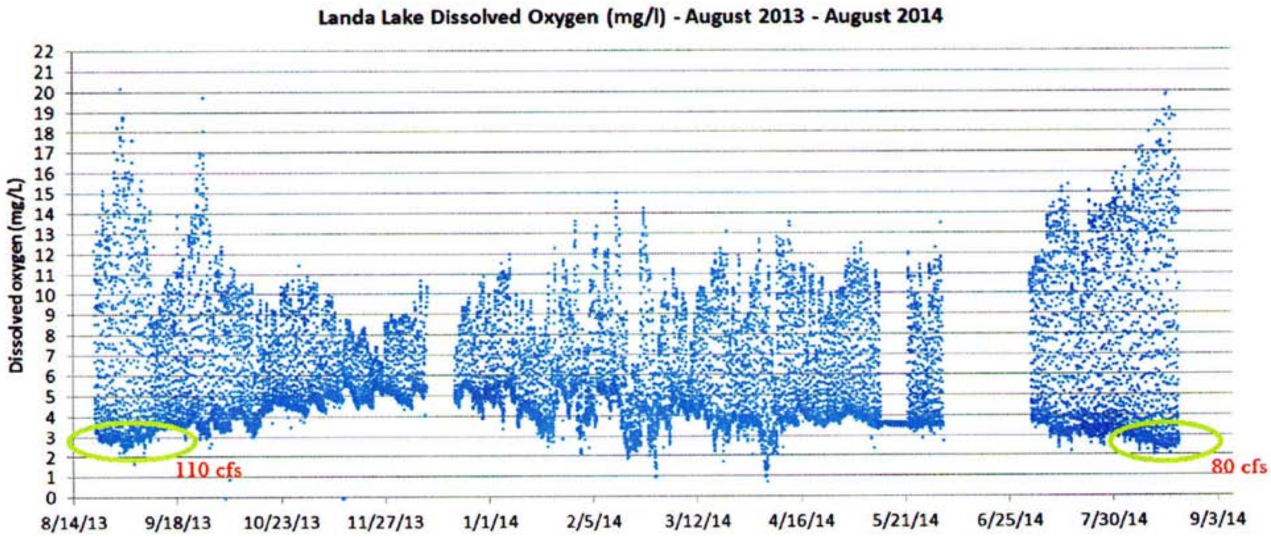

Figure 5.1 Dissolved oxygen time history in Landa Lake, 2013-2014 (SWCA 2013, 2014).

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

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

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

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

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

Table 5.1 Dissolved oxygen investigation scenarios.

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

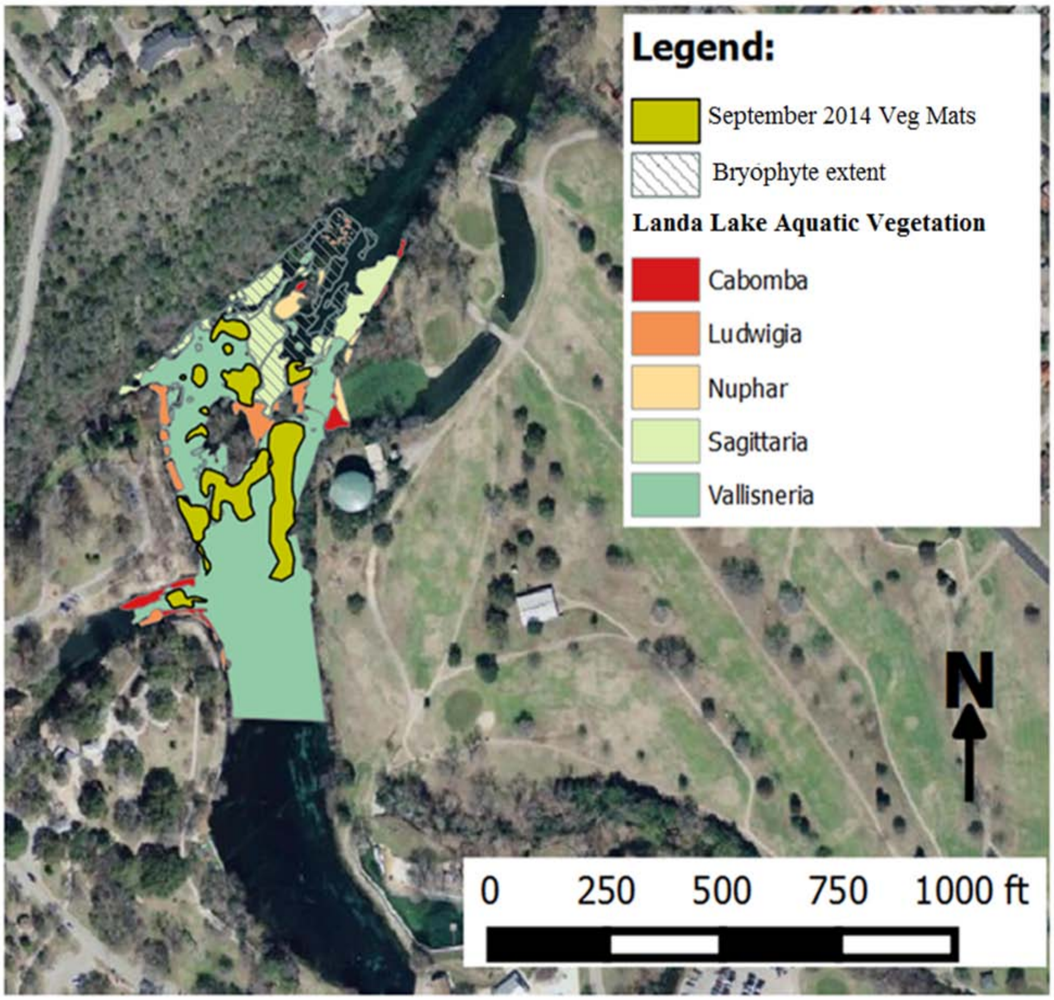

Figure 5.2 Landa Lake study area with vegetation mapping and maximum algal mat surface area in September 2014.

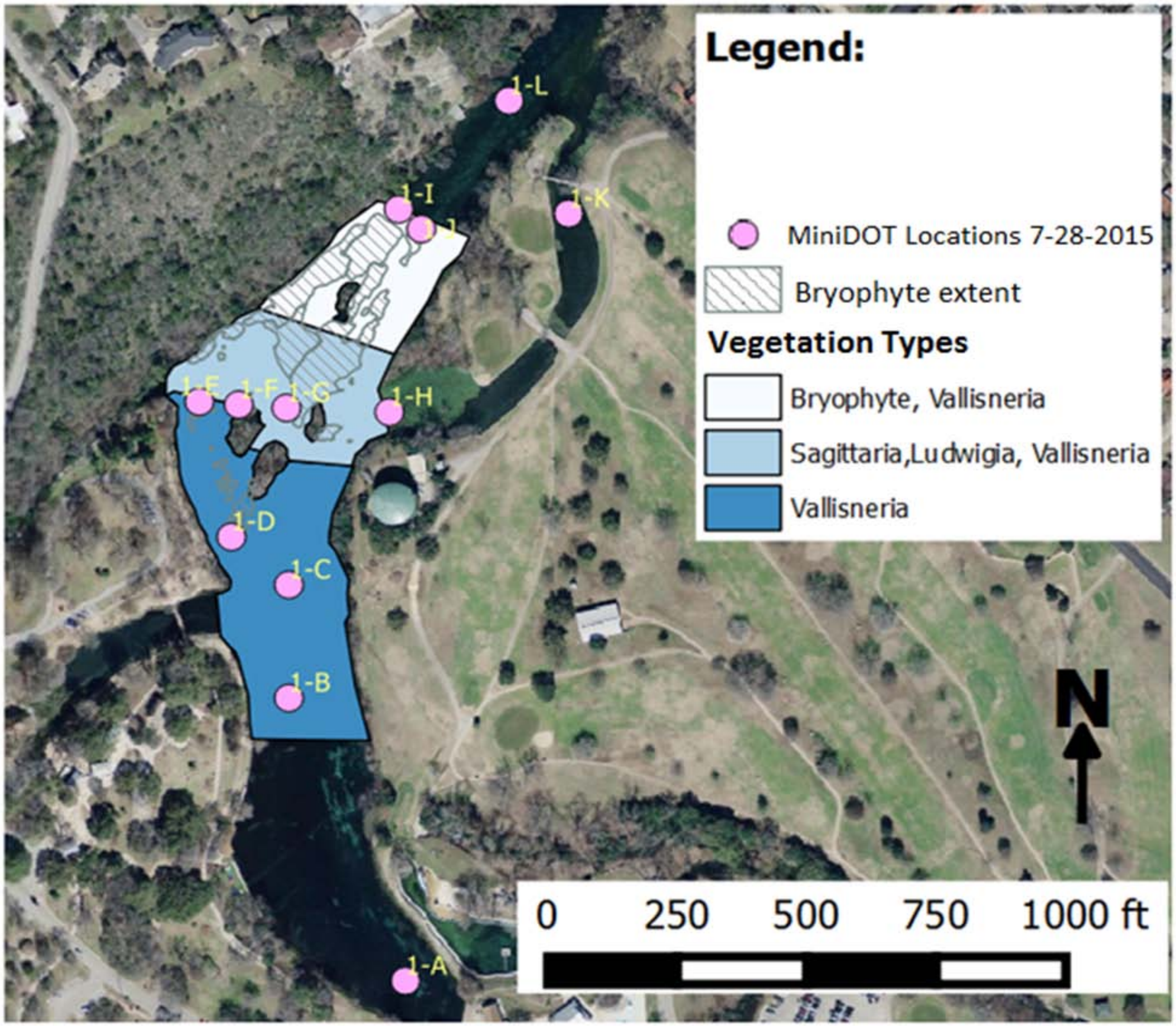

Figure 5.3 Landa Lake study area with generalized vegetation zones, initial MiniDOT locations and bryophyte areas.

Results

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

Scenario 1 represents a very simple condition where DO begins at 3 mg/L and is influenced only by atmospheric reaeration resulting from mixing caused by flow-induced water velocity. After 24 hours the DO concentration rises to 5.7 mg/L. After about 10 days the DO concentration approaches saturation concentration (Figure 5.4); the hydraulic reaeration exhibits the largest magnitude influence on DO until approximately day 10 when the increase resulting from reaeration is offset by the decrease from the SOD. Scenario 2 includes BOD from, for example, a storm runoff event; the DO concentration after 24 hours is lower (5.2 mg/L) indicating that the runoff BOD exhibits influence, but the reaeration remains a greater influence since DO rises from initial condition of 3 mg/L.

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

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

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

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

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

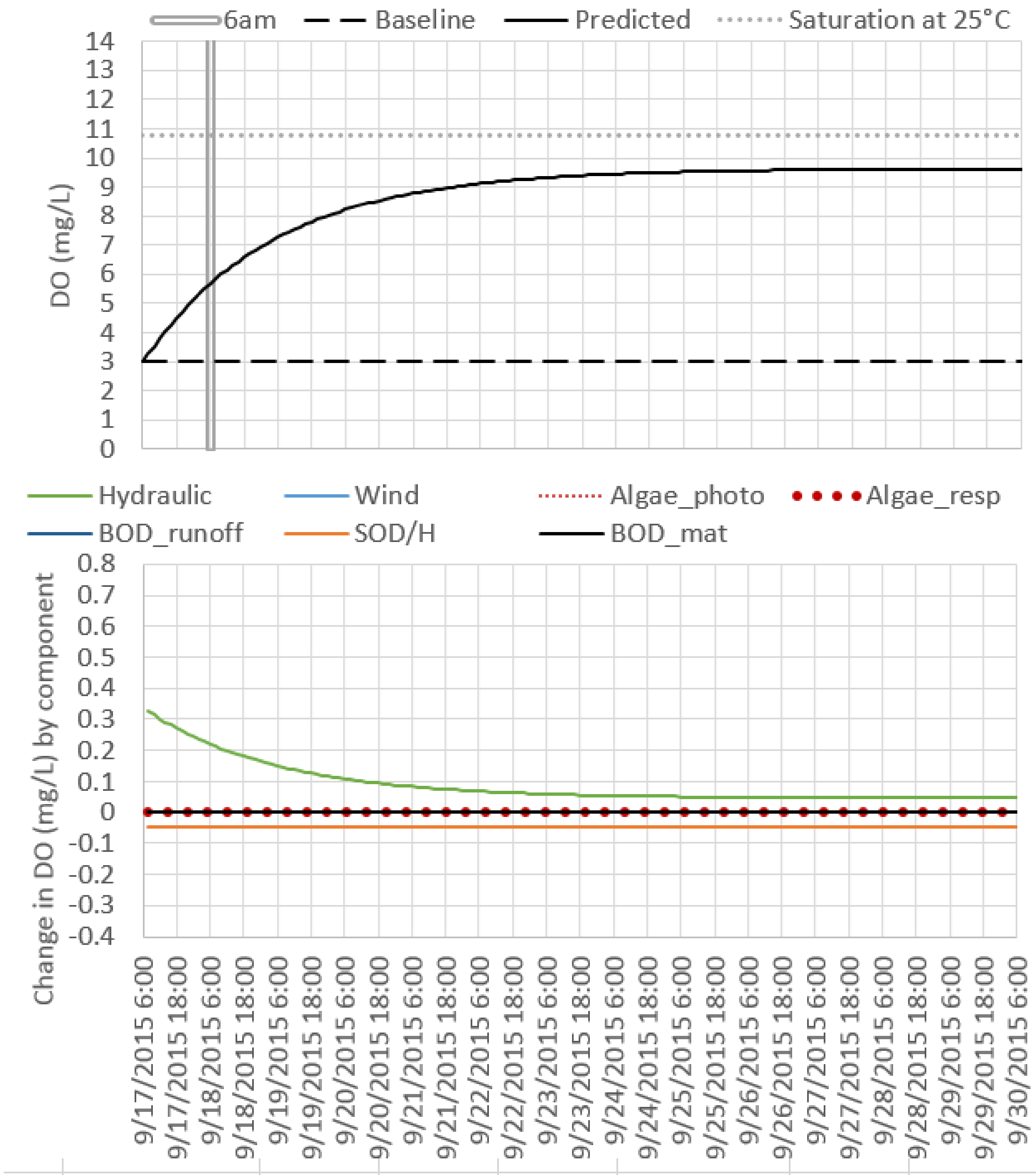

Figure 5.4 Scenario 1 – 212cfs with hydraulic reaeration and SOD only.

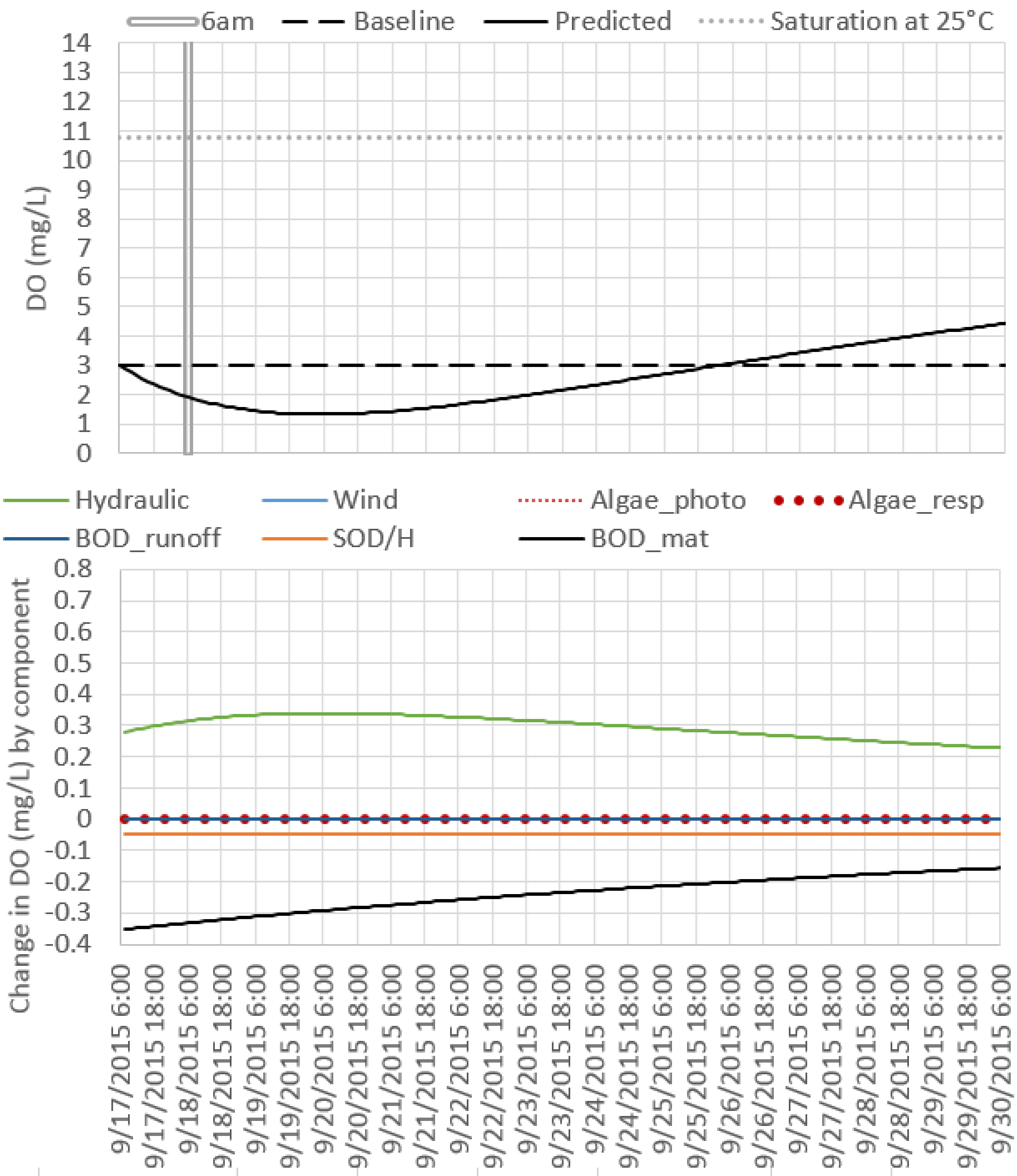

Figure 5.5 Scenario 3 - 212cfs with hydraulic reaeration, SOD and 100% of BOD_mat.

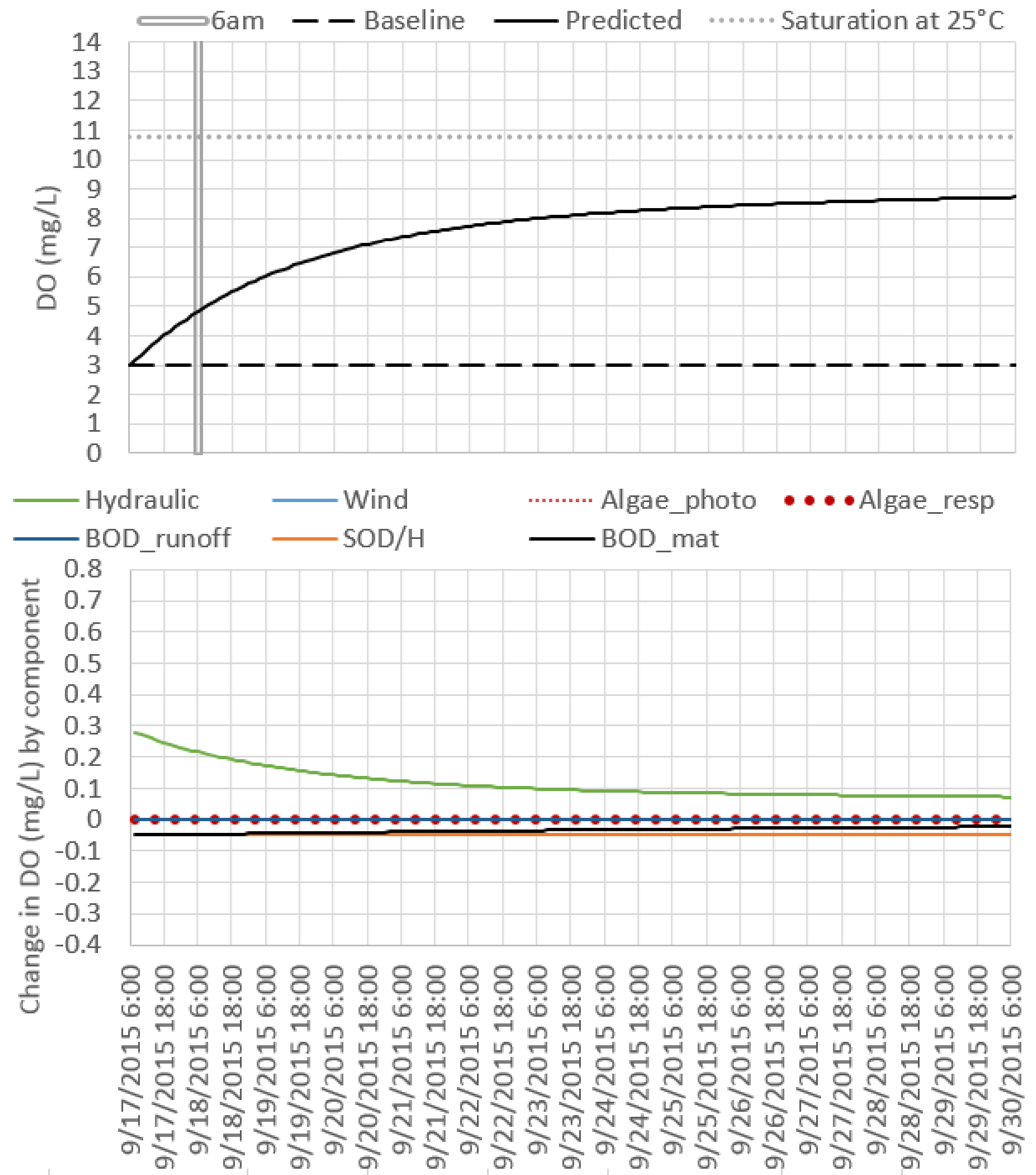

Figure 5.6 Scenario 4 – 212cfs with hydraulic reaeration, SOD and 14% of BOD_mat.

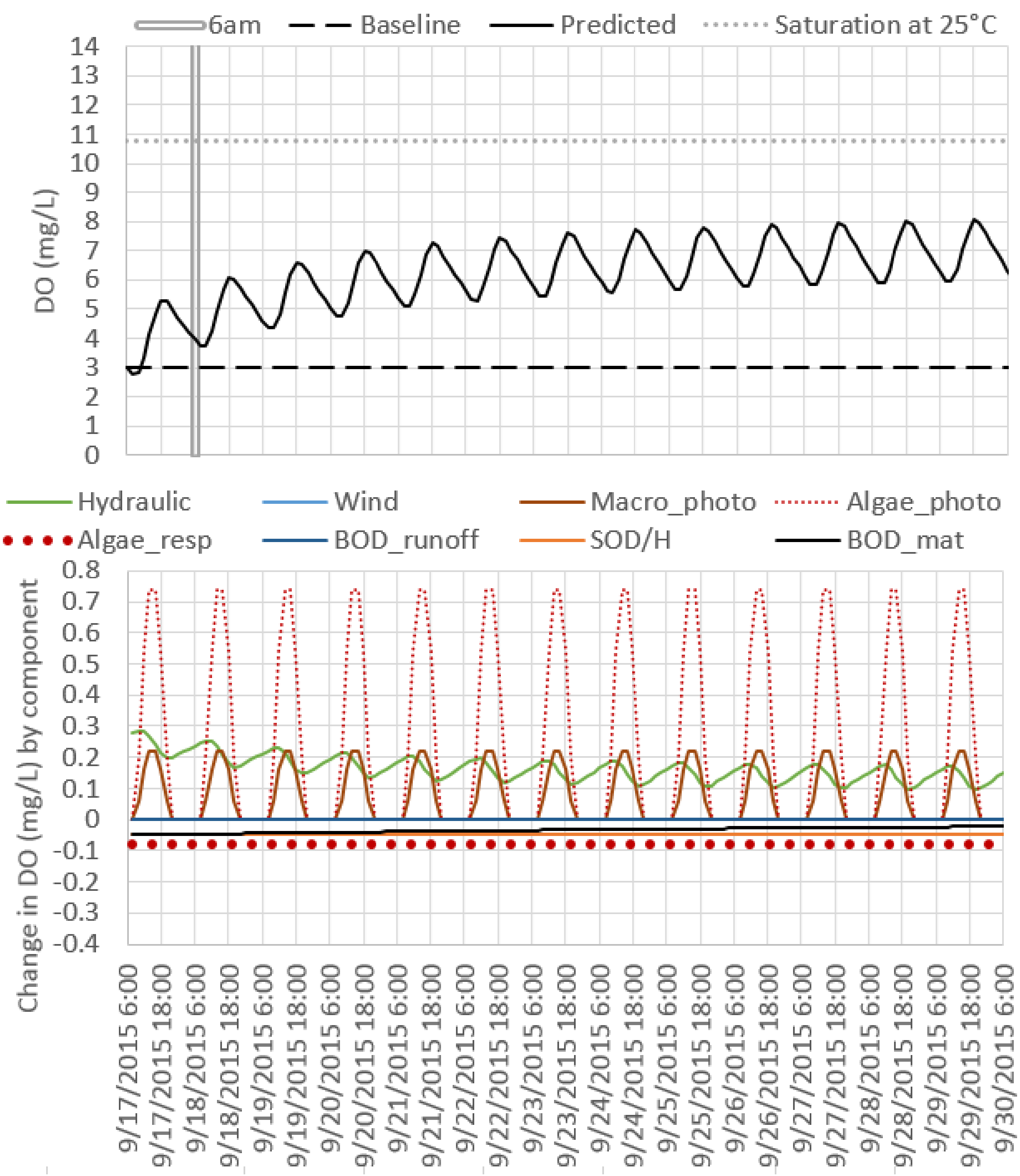

Figure 5.7 Scenario 6 - 212cfs with hydraulic reaeration, SOD, 14% of BOD_mat and assumed algae and macrophytes.

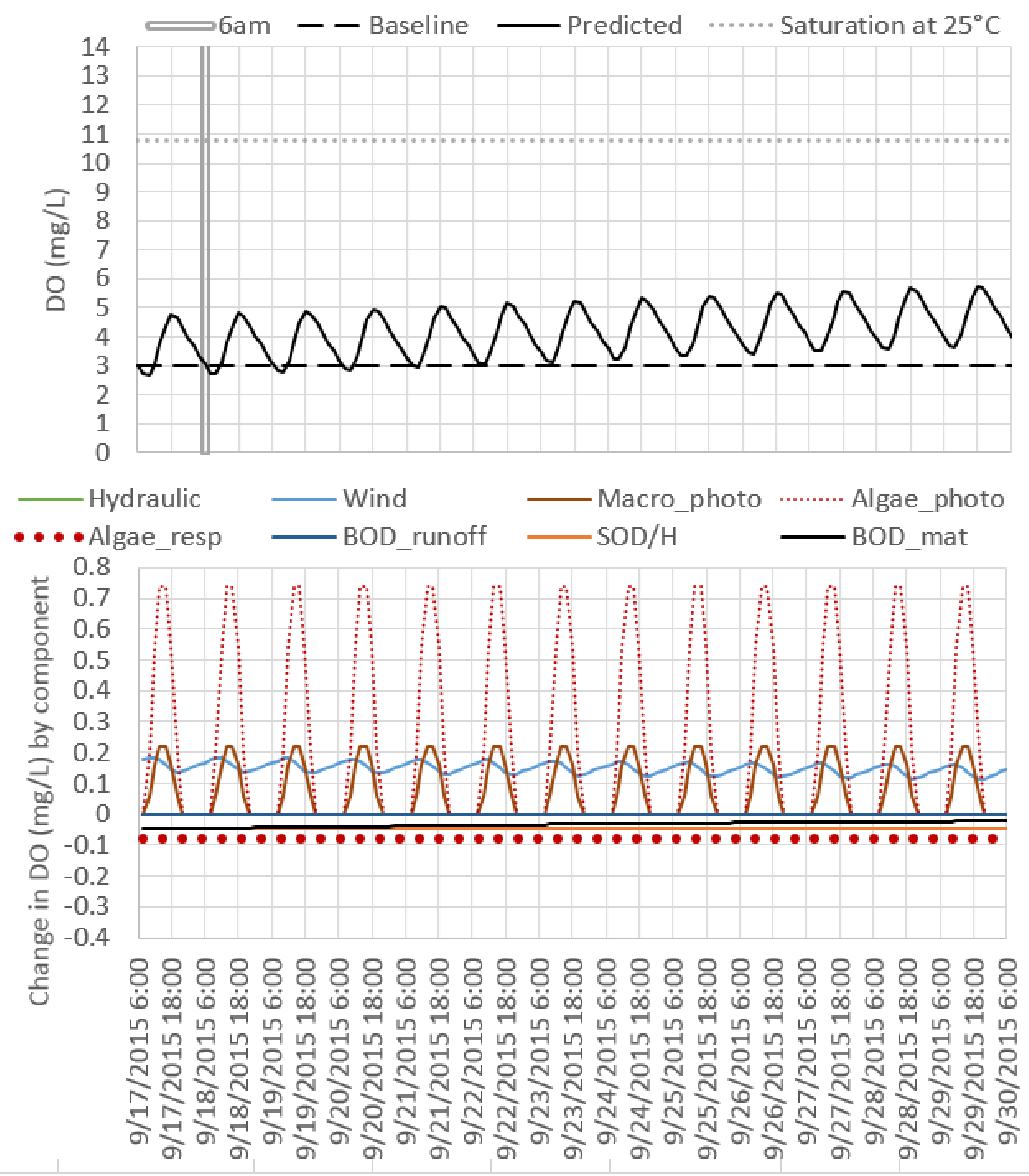

Figure 5.8 Scenario 11 - 30cfs with wind reaeration, SOD, 14% of BOD_mat and assumed algae and macrophytes.

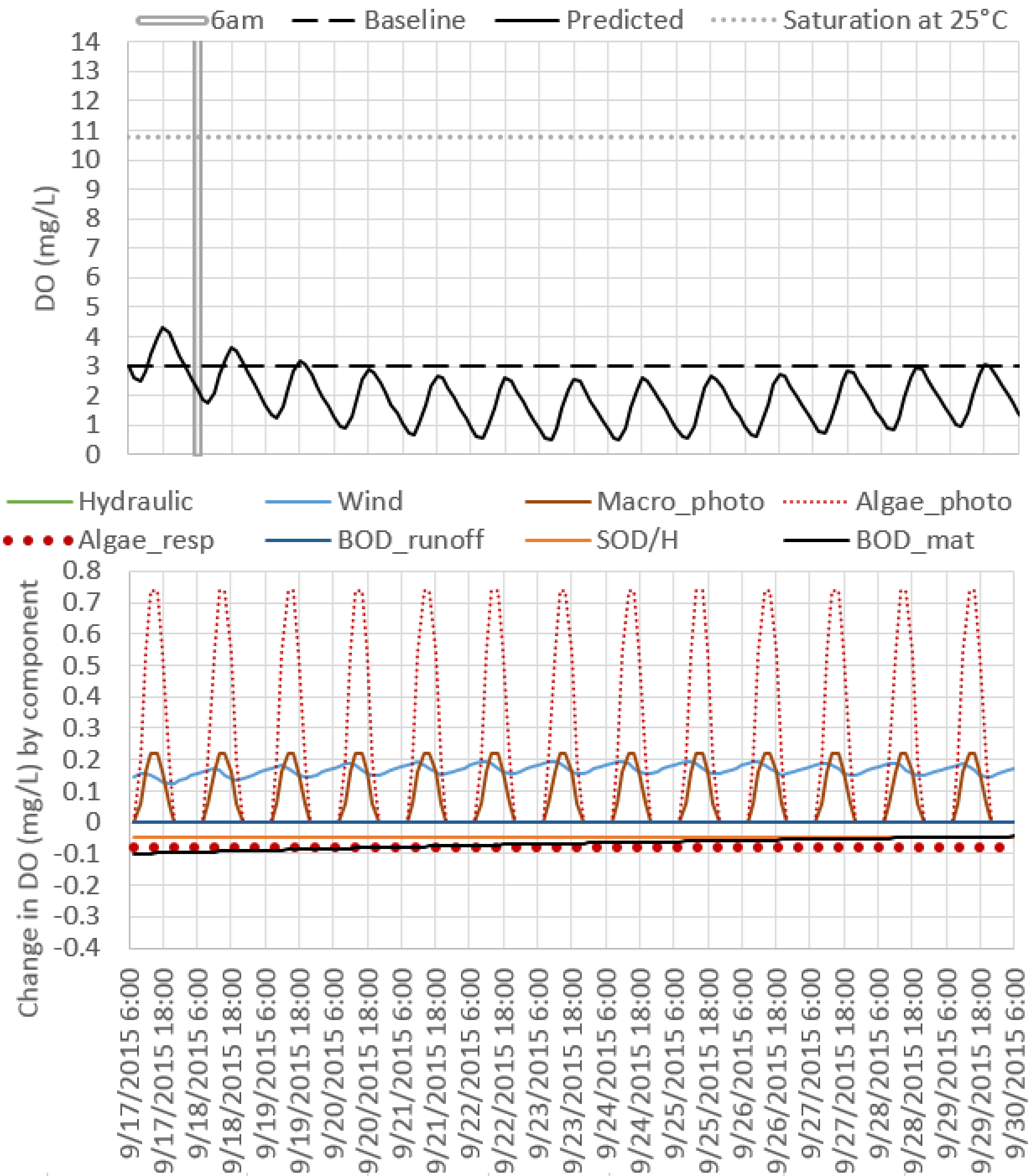

Figure 5.9 Scenario 13 - 30cfs with wind reaeration, SOD, 28% of BOD_mat (expanded area) and assumed algae and macrophytes.

Discussion

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

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

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

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

Works Cited and Recommended Readings

Caraco, N., J. Cole, S. Findlay and C. Wigand. 2006. Vascular Plants as Engineers of Oxygen in Aquatic Systems. BioSciennce 56:219-225

Cole, T. M. and S. A. Wells. 2011. CE-QUAL-W2: A Two-Dimensional, Laterally Averaged, Hydrodynamic and Water Quality Model, Version 3.71. User Manual. Instruction Report EL-11-1. Portland State University.

SWCA. 2013. Decaying Vegetation Removal and Dissolved Oxygen Mitigation 2013 Interim Report. Prepared for the City of New Braunfels. 9 p. plus Appendices.

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

Thomann, R. V. and J. A. Mueller. 1987. Principals of Surface Water Quality Modeling and Control. Harper Collins Publishers Inc. New York, NY.

CHAPTER 6

SUMMARY CONCLUSIONS AND FUTURE STUDY RECOMMENDATIONS

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

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

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

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

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

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

Chapter 3 reports efforts to conduct an experimental test of the impacts of benthic algae mats on Ludwigia and bryophytes. Originally we proposed to position actively growing pots of Ludwigia and racks of bryophytes in areas with and without benthic algal mats. However, due to the poor development of algae mats during 2015 this approach was not possible. Instead we attempted to create a single "dense algae" enclosure and compare growth of Ludwigia and bryophytes in that enclosure to that of replicates growing in an adjacent plot free of benthic algae mats. We used both established and new-growth (viable but not pre-established) cultures of both Ludwigia and mixed bryophytes.

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

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

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

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

Surprisingly, the DO dynamics do not appear to be strongly impacted by vegetation mats. Although DO is less variable under the mats than immediately outside the mats, there was no evidence of severe DO depletion beneath the mats. However, the lack of impact may be directly related to the modest mat formation in 2015. Additionally, good flow under the 2015 mats allowed the water column to mix thoroughly. During a low-flow year with more extensive mats, the results might be different. Maximum areal cover of floating vegetation mats was lower in 2015 than in 2014 and mats did not persist as long in 2015 as they did in 2014.

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

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

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

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

APPENDIX I

Figure I-1 March mapping of algae plots.

Figure I-2 June mapping of algae plots

Figure I-3 July mapping of algae plots

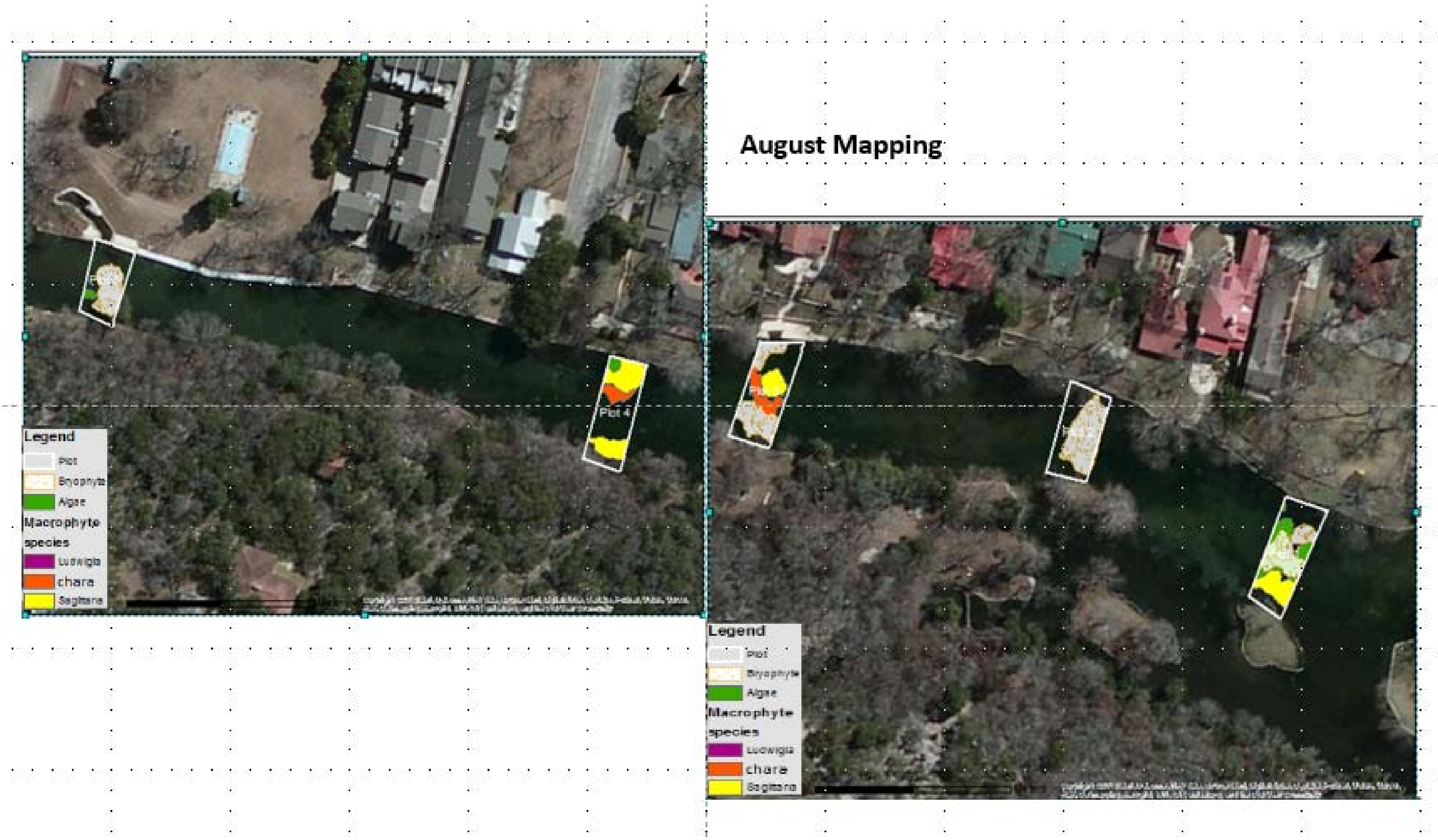

Figure I-4 August mapping of algae plots

APPENDIX II

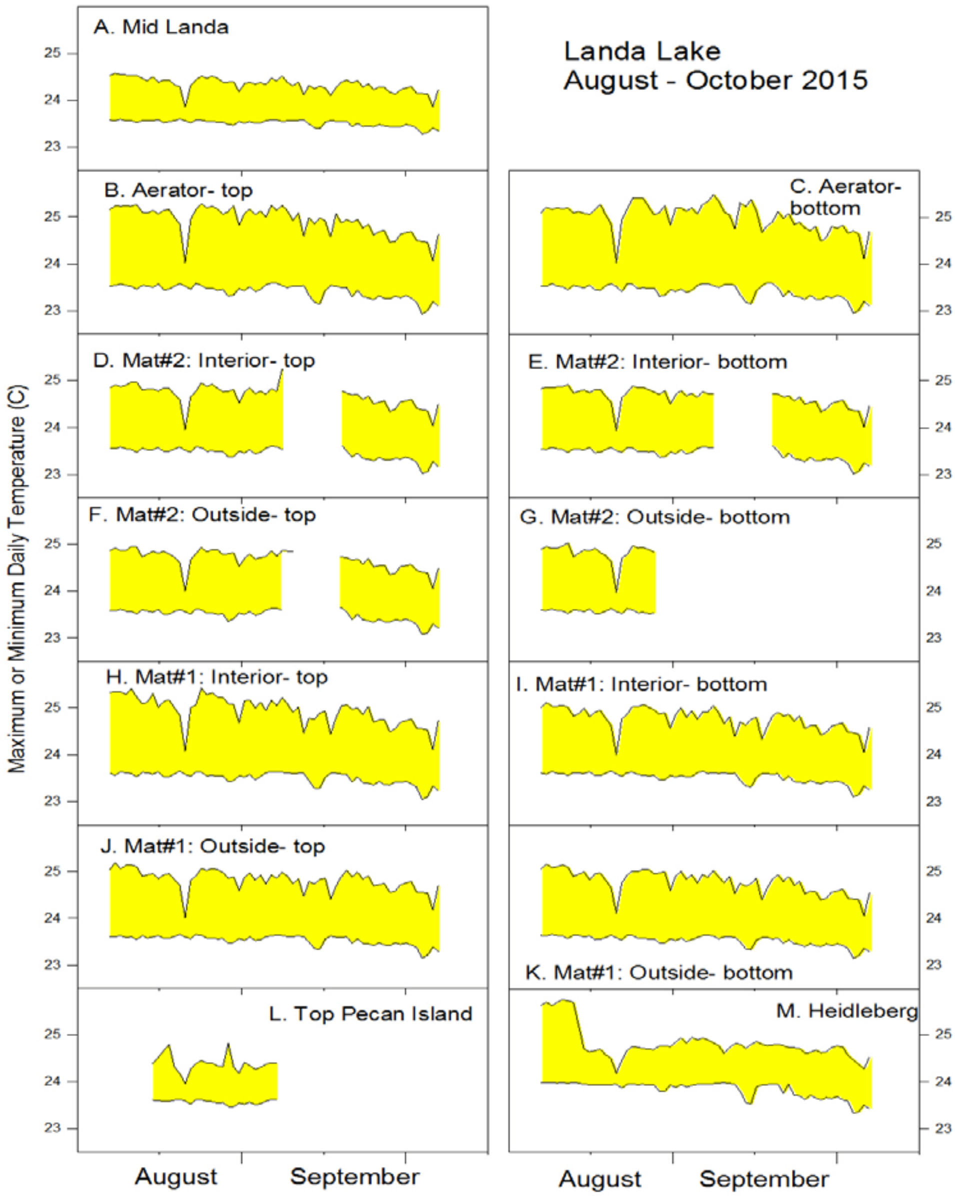

Figure II-1. Maximum or Minimum temperature results (°C) from MiniDOT sensors during deployment at the 13 stations.

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

Date	Sample Bottle ID	k (day^{-1})	BOD_{ult} ($g\ O_2\ g^{-1}$)
May-15	1A_Bryo	0.032	0.73
	1B_Bryo	0.030	0.77
	1C_Bryo	0.031	0.58
	2A_Bryo	0.026	0.53
	2B_Bryo	0.025	0.75
	2C_Bryo	0.025	0.76
	1A_Algae	0.036	0.65
	1B_Algae	0.041	0.38
	1C_Algae	0.038	0.50
	2A_Algae	0.025	0.41
	2B_Algae	0.011	0.46
	2C_Algae	0.020	0.37
June-15	54: 1A_Macro	0.050	0.59
	55: 1B_Macro	0.053	0.60
	56: 1C_Macro	0.046	0.63
	77: 2A_Macro	0.046	0.69
	78: 2B_Macro	0.050	0.56
	79: 2C_Macro	0.038	0.67
	90: 1A_Bryo	0.062	0.55
	91: 1B_Bryo	0.058	0.56
	92: 1C_Bryo	0.050	0.68
	98: 2B_Bryo	0.054	0.61
	99: 2C_Bryo	0.052	0.66
	101: 1A_Algae	0.059	0.55
	102: 1B_Algae	0.054	0.46
	113: 2A_Algae	0.052	0.64
	114: 2B_Algae	0.053	0.52
	115: 2C_Algae	0.060	0.57
July-15	1A_Macro	0.044	0.64
	1B_Macro	0.028	0.53
	1C_Macro	0.037	0.59
	1D_Macro	0.040	0.48
	2A_Macro	0.047	0.52
	2B_Macro	0.049	0.54
	2C_Macro	0.049	0.53
	1A_Bryo	0.043	0.50
	1B_Bryo	0.036	0.51
	1C_Bryo	0.038	0.51
	1D_Bryo	0.028	0.41
	2A_Bryo	0.050	0.59
	2B_Bryo	0.060	0.53
	2C_Bryo	0.043	0.43
	1A_Algae	0.052	0.43
	1B_Algae	0.029	0.38
	1C_Algae	0.026	0.40
	1D_Algae	0.028	0.40
	2A_Algae	0.052	0.50
	2B_Algae	0.047	0.38
	2C_Algae	0.048	0.45
	1A_Terr	0.021	0.28
	1B_Terr	0.026	0.28
	2A_Terr	0.020	0.41
	2B_Terr	0.019	0.59
	2C_Terr	0.022	0.40
August-15	1A_Macro	0.044	0.61
	1B_Macro	0.036	0.72
	1C_Macro	0.046	0.73
	1D_Macro	0.043	0.72
	2A_Macro	0.047	0.79
	2B_Macro	0.038	0.80
	2C_Macro	0.044	0.72
	2D_Macro	0.040	0.54
	1A_Bryo	0.050	0.59
	1B_Bryo	0.054	0.62
	1C_Bryo	0.046	0.50
	1D_Bryo	0.049	0.62
	2A_Bryo	0.046	0.65
	2B_Bryo	0.052	0.70
	2C_Bryo	0.051	0.51
	2D_Bryo	0.049	0.69
	1A_Algae	0.052	0.43
	1B_Algae	0.054	0.51
	1C_Algae	0.039	0.48
	1D_Algae	0.052	0.48
	2A_Algae	0.063	0.62
	2B_Algae	0.061	0.62
	2C_Algae	0.052	0.38
	2D_Algae	0.063	0.42
September-15	1A_Macro	0.019	0.83
	1B_Macro	0.021	0.70
	1C_Macro	0.023	0.69
	2A_Macro	0.048	0.54
	2B_Macro	0.038	0.56
	2C_Macro	0.046	0.52
	2D_Macro	0.050	0.58
	1A_Bryo	0.040	0.45
	1B_Bryo	0.035	0.47
	1C_Bryo	0.038	0.48
	2A_Bryo	0.048	0.66
	2B_Bryo	0.045	0.53
	2C_Bryo	0.050	0.60
	2D_Bryo	0.043	0.67
	1A_Algae	0.053	0.39
	1B_Algae	0.018	0.36
	1C_Algae	0.037	0.30
	2A_Algae	0.059	0.53
	2B_Algae	0.056	0.44
	2C_Algae	0.056	0.44
	2D_Algae	0.056	0.53

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

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

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

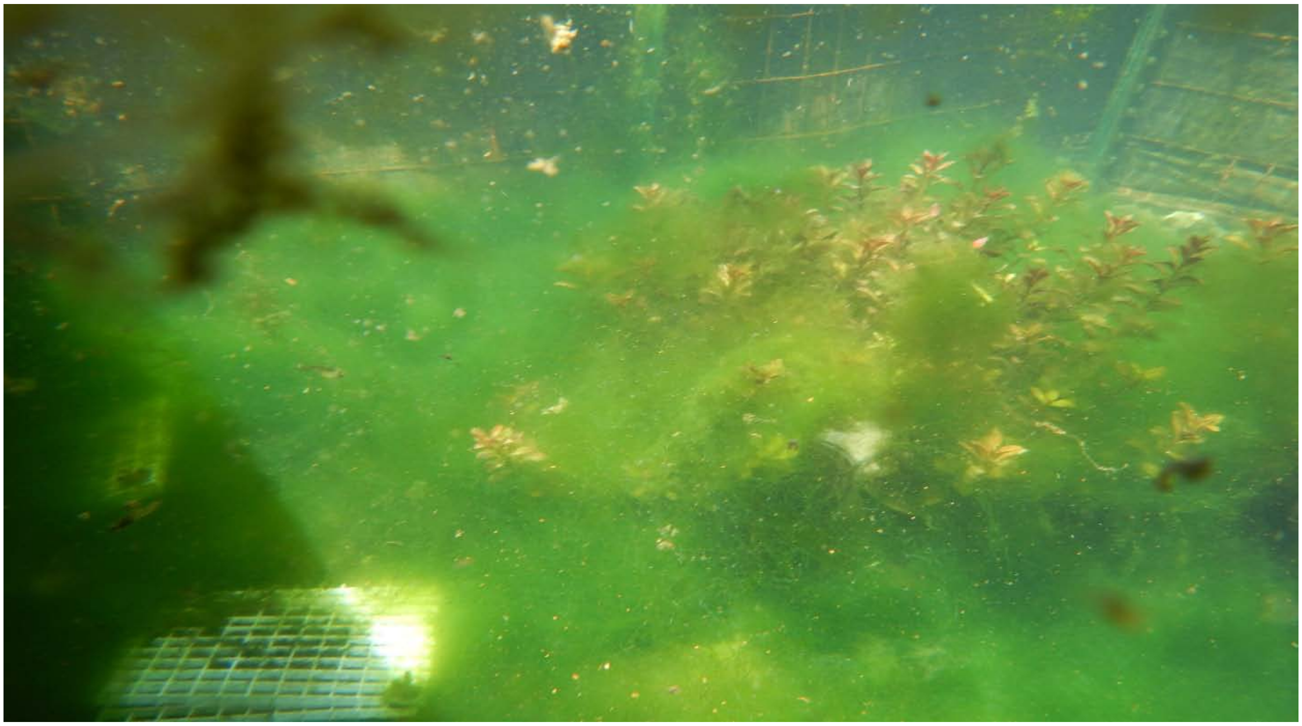

Figure II-5 Algae covering vegetation in the +Algae Plot.

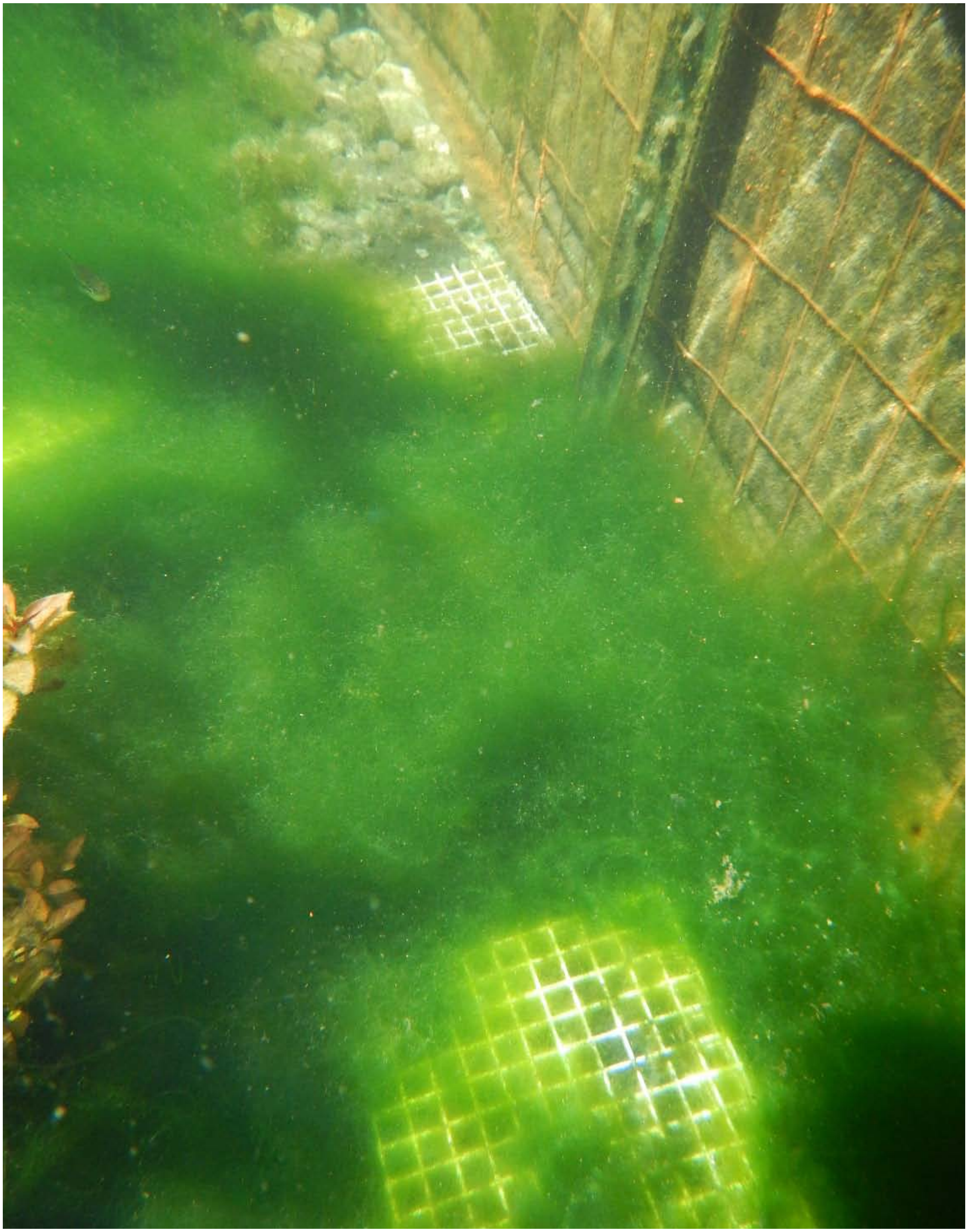

Figure II-6 Algae covering bryophyte cells in the +Algae Plot