ABSTRACT

Understanding Key Factors Influencing Habitat Quality for the Endangered Fountain Darter (*Etheostoma fonticola*) in the Comal River

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The research in this thesis was divided into 3 main studies that took place in Landa Lake: water column, sediment, and plant nutrient analyses, an herbivory and soil fertility experiment, and a spatial study of diel dissolved oxygen dynamics. Nutrient data were collected via water samples, nutrient diffusing substrata (NDS), sediment cores, and plant samples. The water samples and NDS results indicated a severe phosphorus limitation in the water column and some macrophyte species may be limited by phosphorus as well. Herbivory in Landa Lake, particularly by crayfish, appears to be much more severe than previously thought and increased soil fertility exacerbates the issue in *Ludwigia repens*. Diel dissolved oxygen varied across the lake, largely correlated with flow conditions. During drought, there may be several areas of the lake where DO is low enough to threaten fountain darter survival, but there is little cause for concern under current conditions.

Understanding Key Factors Influencing Habitat Quality for the Endangered Fountain Darter (*Etheostoma fonticola*) in the Comal River

by

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DEDICATION

To Katie- for the science that you never had the chance to do but would have loved.

CHAPTER ONE

Introduction

Comal River

The Comal River, located in New Braunfels, Texas, is fed by the Comal Springs. These springs are the largest naturally occurring freshwater springs in the Southwestern United States and result from water coming through the Edwards Aquifer (Lucas et al. 2016). Two dams impound the headsprings, creating Landa Lake. Downstream from the lake, the river splits into two channels - the historic "old channel" and the man-made "New Channel" - until it merges with the Guadalupe River. The Comal is the shortest navigable river in the state, running for only approximately 4 kilometers from the springs before it hits the Guadalupe (Johnson et al. 2012). The springs produce beautiful, clear water with a relatively stable temperature of around 23 degrees Celsius and a nearly neutral pH. The springs have a continuous flow with the notable exception of a 5-month period in 1956, in the height of a seven-year drought, in which the springs were completely dry. Although the Comal Springs have the greatest discharge of any springs in the Southwestern United States, these flows can diminish rapidly during drought conditions (Lucas et al. 2016).

The Edwards Aquifer, from which these waters flow, is a karst aquifer occupying almost 10 million hectares across the southern and the central portions of Texas. For centuries, this aquifer provided water to San Antonio and its surrounding cities, but the increasing human population in the area and subsequent growing water demands have

outstripped the aquifer's capacity to provide (Gibson et al. 2008). Because of this, many organisms, and the springs and rivers in which they live, have been severely altered or eliminated throughout the aquifers range across Texas. Along with this over-pumping of wells within the aquifer for personal use, this water is vital to Texas's agriculture and livestock industries, while also providing recreation and tourist attractions for millions of people annually. Additionally, exotic species have been introduced that have changed both the physical habitat of these waters as well as altered the prevailing food webs, posing significant threats to native and endemic species (Bowles and Arsuffi 1993).

Even with the ample withdrawals from the Edwards Aquifer and its extensive anthropogenic disturbance, this region is home to dozens of endemic and endangered species (Gibson et al. 2008). There are seven endangered species in the short Comal River alone, one of which is the fountain darter (*Etheostoma fonticola*). The Fountain Darter is a small, percid fish that only lives in the Comal River and the nearby spring-fed San Marcos River. A limited range, specific water quality needs, and increased sedimentation due to development along the rivers are the main factors that have led to the species becoming endangered (Alexander and Phillips 2012). These fish are also threatened by drought- both natural droughts due to a lack of rainfall and anthropogenic-induced droughts caused by over-pumping. The flow of the Comal and San Marcos rivers are critical to the water quality and darter habitat in the rivers, and it is significantly influenced by the amount of spring water that emerges from the Edwards Aquifer at the headwaters of each river (Olsen et al. 2016). When the springs completely dried up for 5 months in 1956, the fountain darters were very likely extirpated from the river entirely, and the physical separation between the Comal and the San Marcos darters prevented

natural recolonization. In 1975, ecologists captured 457 fountain darters from the San Marcos and transplanted them to the Comal, where they have since recolonized the river (Olsen et al. 2016).

Importance of Macrophytes to Fountain Darters

Fountain darters are rather sedentary creatures. Dammeyer et al. (2013) found that they only moved 10 meters (± 17) throughout the entire year. This limited ability to disperse has huge implications to the species, emphasizing the importance of habitat quality for the fish and even causing reproductively isolated populations to exist throughout the small Comal River (Olsen et al. 2016). Anthropogenic changes to the river - such as damming, sedimentation, fertilization, and urban development - have become a source of habitat fragmentation for the darters, in turn limiting movement and potential spawning habitat (Phillips et al. 2011; Olsen et al. 2016). Fountain darters inhabit vegetative areas that are characterized by algae, bryophytes, and macrophytes, preferring low, dense vegetation. The bryophytes provide a habitat for various macroinvertebrates and therefore constitute a reliable food source for the darters, while the macrophytes and algae provide refuge from predators and a possible substrate for egg deposition (Phillips et al. 2011). In order to sustain the endangered darters in the San Marcos and Comal rivers, the restoration and monitoring of habitat quality is vital (Alexander and Phillips 2012).

Since macrophytes are important to fountain darter survival, restoration ecologists have spent significant amounts of time removing invasive species from the Comal and replacing them with native species. *Hygrophila polysperma* is an invasive non-native macrophyte that has overtaken much of the Comal River, in part because it is

morphologically similar to a native plant, *Ludwigia repens*. *Ludwigia* and *Hygrophila* are both aquatic macrophytes commonly found in the Comal and San Marcos rivers, and they are frequently confused for one another based off of their similar branching structure, leaf morphology, and coloration. Both species can grow fully submersed, emergent, or even terrestrially and can produce adventitious roots from nodes along their stems. These adventitious roots allow the plants to reproduce successfully by fragmentation, making them fairly easy to transplant, both purposely and accidentally (Doyle et al. 2003). *Hygrophila polysperma* is native to South Asia and was introduced to North America via the aquarium trade in the late 1940s. *Hygrophila*, like *Ludwigia,* can reproduce both sexually and asexually, and has the ability to produce biomass at high rates. In the constant temperatures of the spring-fed Comal and San Marcos rivers, Hygrophila experiences very little seasonality and maintains its high growth rates year-round (Mukherjee et al. 2011; Doyle et al. 2003). By 1997, 20% of the Comal River was covered in dense Hygrophila, while the native Ludwigia covered less than 1% of the river's subsurface (Doyle et al. 2003).

Despite the close similarities between *Hygrophila polysperma* and *Ludwigia repens*, and the apparent competitive advantages *Hygrophila* exhibits, the endangered Fountain Darter (*Etheostoma fonticola*) significantly prefers to utilize *Ludwigia* for habitat. Figure 1 shows the results of twelve years of fountain darter habitat monitoring. The macrophyte that supports the greatest densities of darters is *Ludwigia repens.* Ludwigia supports almost twice the density of fountain darters that Hygrophila does (13.5/m^2 and 7.1/m^2, respectively) (BIO-West, Inc. 2013a). *Riccia fluitans*, a species of bryophyte commonly found in aquaria and naturally in the Comal River, supports the

highest densities of fountain darters of any vegetation type, but it is also the most susceptible to scouring events and fluctuated considerably over this 12-year study period. Both the bryophytes and the filamentous algae represented in Figure 1 are not rooted to the substrate and experience significant seasonality and year-to-year variations in density (BIO-West, Inc. 2013a). Additionally, the algae occasionally inhibits the growth of bryophytes when it clouds the water column and prevents sufficient light levels from reaching the bryophytes, so neither present a firm, consistent habitat for restoration ecologist to focus on. Instead, *Ludwigia* is good at recruiting bryophytes and their adventitious roots provide a relatively stable surface for the *Riccia* to attach to (BIO-West, Inc. 2013a). The combination of *Ludwigia repens,* the darters' preferred macrophyte, and *Riccia fluitans*, the darters' all-around preferred vegetation, creates exceptional fountain darter habitat. Phillips et al. (2011) found that fountain darters selectively deposit their eggs on the leaves of *Ludwigia* over every other macrophyte

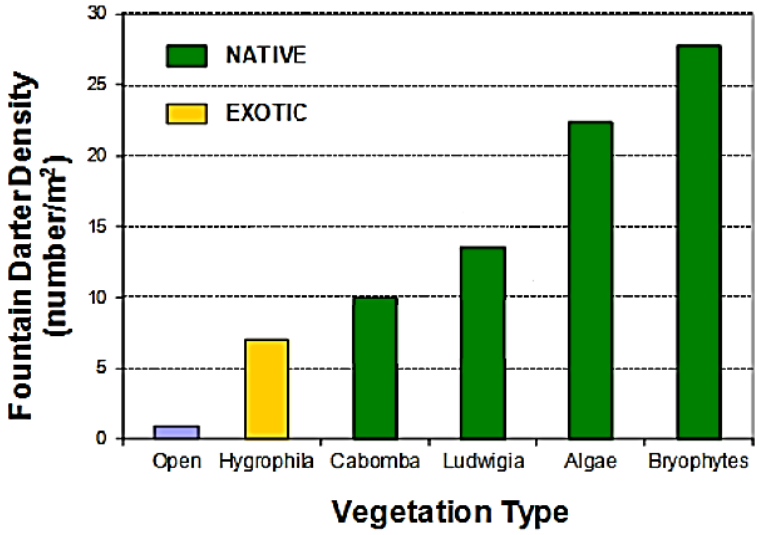

Figure 1. Density of Fountain Darters collected by vegetation type in the Comal River. Green indicates native vegetation types and yellow indicates non-native (modified from BIO-West, Inc. 2013a).

tested, and bryophytes are known to support high concentrations of aquatic microinvertebrates which the darters eat. This optimal habitat provides shelter, food, and spawning sites for both adult darters and their offspring.

In light of this, conservationists working in the Comal River have spent significant efforts removing the undesirable, exotic *Hygrophila* and planting *Ludwigia* in its place and along bare, scoured areas of the river. In order to grow the *Ludwigia* that will be planted in the river, BIO-West, an environmental consulting firm working with the city of New Braunfels and the Edwards Aquifer Authority, created an *in situ* propagation nursey using what they call a "MUPPT," or a mobile underwater plant propagation tray. The MUPPTs are constructed of steel covered in a rust-prevention coating and support four plastic nursery trays that can each hold 12 quart-sized pots (BIO-West 2013b). Each pot is loaded with silt/clay sediment thought to be nutrient rich from along a nearby island in the river before several small fragments of *Ludwigia* cut from populations in the Comal are added. The MUPPTs are then lowered into the water in a particularly shallow area in the Comal and left to grow for typically 3 to 4 weeks. After this growing period, the MUPPTs are lifted out of the water, the plants are removed from their pots, and the established *Ludwigia* is planted in areas bare of vegetation.

Ludwigia started in this fashion has generally grown fairly well, showing limited signs of herbivory, though snails, insect larvae, and crayfish have been observed living in and around the MUPPTs. However, these MUPPTs were also used in a plant competition study in 2015 that aimed to quantify the competition between Ludwigia and Hygrophila in the Comal and San Marcos rivers. After stocking the MUPPT with plants, it was left *in situ* for 8 weeks before being harvested. Unfortunately, whether due to the increased time

in the water or the decreased plant density in the MUPPT (only half of the pots in each MUPPT were utilized to minimize interactions between pots), many of the pots contained no plant, but only subsurface stems and roots left in the sediment. The main suspects for this destruction are crayfish, based off of personal observances and literature supporting this "clipping" behavior.

Crayfish and Fountain Darters

The ability of crayfish to greatly reduce the standing stock of macrophytes is well documented (Cronin et al. 2002). In some studies, such as Lodge and Lorman (1987), crayfish eliminated 100% of the macrophytes in their exclosure in only 12 weeks. Donana National Park in Spain saw an 80% reduction in macrophyte biomass after the introduction of *Procambarus clarkii* to the park. Though crayfish are generalist feeders and certainly eat macrophytes (which are the majority of an adult crayfish's diet), they have the tendency to shear plants off at the sediment surface or even uproot them for unknown reasons (Gherardi 2007). This non-consumptive plant clipping destroys more plant tissue than the crayfish can possibly eat and has been documented in many systems (Lodge 1991; Lodge et al. 1994; Nystrom and Strand 1996; Smart et al. 2002; Gherardi and Acquistapace 2007; Gherardi 2007).

There have been several theories proposed to explain this behavior. One theory suggests that crayfish may reduce macrophyte biomass to decrease habitat for their predators, causing them to find shelter away from the crayfish's burrow. This theory also predicts that destroying these macrophytes reduces the habitat for their prey, making prey such as small fish concentrate into smaller areas and easier to capture (Roth et al. 2007). Such behavior has been documented in rusty crayfish (*Orconectes rusticus*), which

actively evicted a close relative of the fountain darter, the johnny darter (Etheostoma nigrum), from its macrophyte shelter, increasing their activity levels and subjecting them to predation by small-mouth bass. The activity of the bass in the area then forced the darters back into the shelter now occupied by crayfish, subjecting them to potential predation by the crayfish (Jurcak et al. 2016; Rahel and Stein 1988).

Another theory for this behavior states that crayfish cut down the macrophytes so that they will sink to the sediment surface and begin decomposing, reducing their structural integrity and allowing the plant to be eaten more as detritus, which is more palatable, than living tissue. Crayfish base feeding decisions in part on the plant's morphology and structure, so this behavior may help explain why crayfish tend to destroy submersed plants more often than emergent ones - emergent plants have more structural material (Cronin et al. 2002). Based on this theory of crayfish behavior, *Ludwigia* may be particularly susceptible to clipping. In most submersed plants, structural materials are typically minimized, since the plant is supported by the buoyancy of water. The *Ludwigia* genus (and *Ludwigia repens* specifically), which contains plants that are primarily aquatic, actually has a cambium present in both emergent and submersed stems, making them unusually tough for submersed plants (Lytle 2003). Also, unlike most submersed species and the majority of its genus, *Ludwigia repens* has a thin layer of cuticle present on both sides of its leaves (Lytle 2003). Crayfish that want to eat *Ludwigia* may benefit from clipping leaves and stems off in hopes that they will decay and become easier to digest. Whatever the reason for it, this clipping behavior, along with intense grazing and crayfish preference for seedlings, causes significant declines in macrophyte biomass and can greatly reduce biodiversity in a system (Gherardi 2007).

Crayfish also cause macrophyte declines via bioturbation. Due to behaviors such as burrowing and uprooting plants, crayfish can cause a significant increase in the turbidity of water. This bioturbation reduces the water quality by increasing the suspended solids and decreases primary productivity by reducing light penetration (Gherardi 2007). Then, the reduction in macrophytes, which hold sediment in place and decrease erosion, leads to increased turbidity, amplifying the crayfish's effects on water turbidity. An increase in turbidity has recently been found to greatly affect fountain darters. In 2016, Swanbrow Becker et al. demonstrated that even a relatively minimal rise in turbidity can significantly decrease the amount of prey that fountain darters consume and increases the time it takes to initiate foraging. This is particularly important to the conservation of fountain darters because the Comal and San Marcos both experience high disturbance and turbidity levels during tourist season in the summer months due to high recreational use. Severe storm events, which are relatively frequent in Texas, can cause a significant increase in turbidity as well (Swanbrow Becker et al. 2016).

Dissolved Oxygen Dynamics

Dissolved Oxygen (DO) is the concentration of molecular oxygen dissolved in water and is essential for the survival of aquatic heterotrophs. Dissolved oxygen dynamics are influenced mainly by photosynthesis and respiration in the water, but can also be affected by water movements and reaeration from the atmosphere. Not only is DO necessary for many organisms' respiratory processes, the amount of oxygen in the water can have large effects on the solubility and availability of various nutrients, which consequently affects the productivity of the system (Lin 2010). Because DO concentrations are so sensitive, it is often used as an indicator of overall water quality in a

system. Reduced concentrations of DO can be fatal, and for most aquatic species this critical point is around 4 mg/L DO. Areas with DO concentrations under 2 mg/L are considered hypoxic, and anoxic conditions occur once concentrations reach 0 mg/L (Ganoe and De Young 2013).

Dissolved oxygen concentrations increase rapidly after sunrise due to organisms performing photosynthesis, but since photosynthesis is a light modulated process, concentrations decrease after nightfall, or on cloudy days when oxygen production ceases and oxygen consumption continues (Kaiser 2009). This process leads to large diel swings in dissolved oxygen, which can considerably stress fish and even cause behavioral abnormalities, body deformities, and larval death if the swings are drastic enough (Deromedi 1996). In Landa Lake, these diel swings are even more intense, since waters originating from an underground source, such as the Edwards Aquifer, are typically low in dissolved oxygen as a result of atmospheric isolation. Low dissolved oxygen can be the result of anthropogenic activities, such as agriculture and lawn fertilization, because the input of nitrogen to rivers and lakes leads to phytoplankton blooms and their subsequent decomposition that consumes oxygen (Patterson 2014). These conditions are also exacerbated by low flow or stagnant waters, which are commonly created by humans as well via the input of dams and structures to rivers.

The Comal River has been dammed in multiple locations and is surrounded by artificial lawns seeded with fertilizer from houses and businesses. There is even a large golf course bordering Landa Lake. As a result of these changes and natural occurrences, the Comal has notably variable flow conditions and depth along the river; stagnant conditions are prominent along channels near the golf course and lodges built upstream

of the lake in Upper Spring Run. Low DO or hypoxic conditions in only parts of a lake or river are not a challenge for most fish; they simply move to an area with sufficient oxygen. However, because fountain darters are such immobile creatures, they have the tendency to stay in an area with low DO and die, instead of moving. This behavior - or lack thereof - creates a need to establish where these conditions may occur in the Comal River and to monitor them for fountain darter survival.

Restoration of River Systems

Rivers are essential to our ecosystems in many different ways. For humans, rivers can provide food and clean water, process waste and runoff, offer recreational and aesthetical services, and be used for agriculture, navigational purposes, and power generation (Giller 2005; Malmqvist and Rundle 2002). However, rivers can only provide these services when they are operating appropriately - and degraded rivers are becoming the norm. In 2004, the U.S. EPA declared that almost half of all rivers in the US are considered so polluted or impaired that they are unsafe for human swimming or fishing (US EPA 2004). In terms of biological value, rivers contain diverse biota, including a high diversity of vertebrates, spanning many taxa, and an even greater diversity of invertebrates, plants, and algae (Malmqvist and Rundle 2002). Unfortunately, this diversity is expected to continually decrease; both current and predicted extinction rates are five times greater for freshwater biota than for terrestrial biota, which tend to receive the most attention (Ricciardi and Rassmussen 1999).

The scope and quantity of stressors to freshwater ecosystems are continually mounting (Giller 2005). These threats are largely anthropogenic and can include: habitat destruction and fragmentation, eutrophication and chemical pollution, invasive species,

overexploitation, and climate change (Yoshioka et al. 2013). Most rivers face one or more of these threats, which can cause them to function improperly and the ecosystem to degrade. A degraded river can mean pronounced loss of habitat, a decrease in species richness, declining water quality, and a loss of recreational space and food for humans (Kail et al. 2015; Chittoor Viswanathan et al. 2015; U.S. EPA 2004; Berndhart et al. 2005). These losses can have a profound effect on many organisms, ultimately leading to biodiversity loss (Yoshioka et al. 2013).

Ameliorating these threats, however, requires different approaches in every river, necessitating ecologists to adapt their strategies to each unique environment, often at great cost (Mehan et al. 2006). These knowledge gaps limit restoration efforts and drive fundamental river research, making river restoration one of the most prominent areas of applied water-resources science and supporting a multibillion dollar industry across the globe (Wohl et al. 2015). The success or failure of a river restoration project is subjective and highly debated, but a common "ideal" restoration creates geomorphical, hydrological, and ecological conditions that allow a river to be self-sustainable long term (Jahnig et al. 2011; Wohl et al. 2005).

One key way to help a degraded river become self-sustainable is to restore natural vegetation. Macrophytes, or vascular aquatic plants, occupy key positions in many biotic relationships within rivers and can significantly increase biodiversity in polluted rivers (Paice et al. 2016). Their foliage offers shelter and support for biota, and facilitates establishment by algae, mosses, and invertebrates (Sculthorpe 1985). Restoration of macrophytes has been associated with improved water quality, as their roots, stolons, and rhizomes bind to the soil to help reduce erosion and suspension of sediments within the

water (Larned et al. 2006; Davis et al. 2010; Sculthorpe 1985). They also generate a localized oxygen source, which in turn lures various microflora and fauna, which are a source of organic matter that improves soil fertility and overall abiotic sustainability (Sculthorpe 1985; Gómez-Aparicio 2009).

Macrophytes provide a food source, both directly and indirectly, for many organisms such as fish, invertebrates, mammals, and birds that frequent the habitat (Diehl and Kornijow 1998). The epiphytic algae that clings to the plant, along with the protozoans, bacteria, and rotifers they support, provide sustenance for the aquatic invertebrates upon which the vertebrates depend. Per unit of area, submerged macrophytes support 3 to 4 times more average biomass of animals than an equal area of silt and over 15 times greater than rock or gravel (Sculthorpe 1985).

The absence of these macrophytes in streams and rivers can become cyclic; once they are outed, it is more difficult for other macrophytes to establish. Low stream or river roughness results in low retention of plant shoots and the most important agent of plant retention is in-stream vegetation (Riis and Sand-Jensen 2006). Once there are no plants in the stream or riverbed, the overall river is degraded and it is more difficult for new plants to colonize (Riis et al. 2009).

The establishment of native macrophytes may also be impeded by non-native or invasive species. Invasive species are considered the second leading cause of global biodiversity loss, after habitat fragmentation, and pose a significant threat to the ecological integrity of native plants (Drake et al. 1989). Many non-native invasive plants out-compete native flora and change the species composition of a river, ultimately changing the structural and functional processes of an ecosystem (Vitousek et al. 1996).

They may alter soil chemistry, nutrient cycling, hydrology, and disturbance regimes of the infested ecosystem, as well as act as physical barriers to native seedling establishment (Jose et al. 2012). These changes to the ecosystem can have direct and indirect implications on resource availability, so that even if native seedlings are able to establish themselves in the invasive non-native plants, intense competition for resources can result in reduced robustness and biomass production (Jose et al. 2012). This makes the restoration of native macrophytes challenging in the presence of invaded rivers.

Invasive macrophytes have been introduced to aquatic ecosystems across the globe, both accidentally, like through release of contaminated ballast water, and intentionally, for ornamental or edible purposes (Larned et al. 2006). These non-native macrophytes are often early colonists and become invasive after physical disturbances in streams and river, where they rapidly attain high densities and become a physical barrier preventing native species from establishing (Barrat-Segretain 2001). After a river has been invaded by damaging non-native species and changes in the ecosystem properties and processes take place, active restoration has two basic directions. Top-down control of invasives involves the removal or elimination of the damaging macrophytes or reduction of their number and supply of propagules. Whereas bottom-up control highlights the restoration of underlying properties or process that contribute to the self-sustainability of the river (D'Antonio and Chambers 2006).

In practice, when restoring an invaded, damaged river, the top-down approach usually involves physical removal of non-natives, use of herbicides, or employing a biological control, but it can also include controlling the pathways by which the unwanted species are arriving to the restoration site (D'Antonio and Chambers 2006).

Bottom-up control ultimately involves different aspects of preventive management, and can include practices such as the amelioration of stressors to the rivers ecosystem that could affect the growth and establishment of the desired plant species and the management of disturbance regimes. It can also include altering soil conditions to reduce the growth of invasives or promote the growth of natives and directing seeding or planting to increase the likelihood of establishment and dominance of the desired species (D'Antonio and Chambers 2006). After reducing or eliminating the non-native plants, it is often necessary to plant desired natives, commonly at high densities, to ensure successful establishment (Larned et al. 2006).

Herbivory and Soil Fertility

A key component of any successful macrophyte restoration is the identification of factors that are contributing to the decline of the macrophytes or preventing them from recovering (Opperman and Merenlender 2000). The role of herbivory in regulating plant populations is being increasingly appreciated by plant ecologists (del-Val and Crawley 2005; Halpern and Underwood 2006; Maron and Crone 2006), as herbivores can decimate macrophyte populations even after they have been successfully planted, and on occasion, can render restoration efforts futile (Kauffman et al. 1997). Even in cases where herbivory does not lead to mortality in mature plants, herbivores can influence population trajectories through damage to reproductive tissues or by forcing plants to reallocate resources away from regrowth or defense (Strauss and Agrawal 1999). By selectively consuming certain palatable plants and avoiding others, herbivores commonly reduce plant biomass, both directly and indirectly alter community composition, ultimately influencing various ecosystem processes, such as detrital accumulation and geochemical

cycling (Lubchenco and Gaines 1981; Huntly 1991). Considering the importance of nutrient assimilation in macrophytes, it is probable that herbivory also influences changes in nutrient and energy dynamics in freshwater systems (Carlsson and Lacoursie`re 2005).

Herbivory can become more pronounced by the addition of non-native herbivores to the ecosystem (Parker et al. 2007). Generalist feeders often have a more profound effect on plant communities than specialists do, and non-native generalists such as crayfish substantially reduce macrophyte biomass and increase turbidity (Miller and Provenza 2007). These non-natives can even shift the plant community composition in favor of exotic or invasive plants and away from native plants, as exotic herbivores can present novel problems to native communities and selectively suppress native plants. This phenomenon is particularly common when herbivores differentially feed on grazing-intolerant native plants, or disperse or enhance germination of non-native species via gut passage (Parker et al. 2006; Yelenik and Levine 2010). In previous studies, exotic generalists have decimated native New World plants, allowing invasive "Old World" plants that were adapted to the herbivory to take over. This can have considerable implications for managers and conservationist, as it suggests that the eradication of exotic herbivores and the restoration of native generalists could aid in controlling non-native plant invasions (Parker et al. 2006).

The tolerance of plants to herbivory reflects the degree to which a plant can regrow and reproduce after damage from herbivores, and has been defined as the capacity of plants to reduce the negative effects of damage on fitness. This includes the fitness effects of traits expressed before, and the plastic phenotypic responses following, damage (Fornoni 2011; Strauss and Agrawal 1999). Compensatory ability is often associated with

tolerance and represents the most frequently studied plastic response to damage, where compensatory growth occurs when a plant attempts to replace damaged leaves, shoots, and roots (Fornoni 2011). Autoecological factors, as well as the presence of competitors and mutualists, can influence the degree of plant herbivory tolerance in individuals (Strauss and Agrawal 1999).

Traits that contribute to the herbivory tolerance of an organism can be divided into two main categories: traits that decrease the amount of damage to the plant (resistance), and traits that determine the amount of regrowth after damage occurs (compensation) (Marquis 1996). Within these traits, there are underlying mechanisms that can increase the tolerance of an organism. After damage has occurred, plants can increase their photosynthetic rate, increase branching or tillering (particularly after damage to the apical meristem), increase their growth rate, or shunt carbon store from their roots to their shoot. Before damage occurs, plants generally rely on morphology and chemical cues to prevent harm, but they may also have pre-existing high levels of carbon stored in their roots to allocate to above-ground reproduction in case herbivory transpires (Strauss and Agrawal 1999; Carlsson and Lacoursie`re 2005).

Plant architecture, and the related source-sink mechanism of resource movements, can have profound effects on an organism's ability to shuttle nutrients from one morphological unit of a plant to another. This aspect of plant morphology, commonly referred to as sectoriality, is not only key to carbon shunting from roots to shoots, but also to a plant's ability to increase growth rate and branching or tillering after damage has occur, which affects leaf surface area and the ability to increase photosynthetic rate (Marquis 1996). A plant's sectoriality can be viewed as both a contribution to and a

detriment to tolerance, with the distinction depending upon the plant tissue that is attacked. Because of this, it is important to identify the timing, location, and degree of plant tissue damage to herbivory in order to better understand the tolerance of a plant (Marquis 1996).

Considering the importance of nutrient shuttling abilities from roots to shoots in plants, soil fertility can play a particularly significant role in herbivory tolerance. Soil fertility alone can determine the success of some macrophyte restoration efforts and intentional fertilization of soils has long been a restoration practice because of its positive effects on plant growth and reproduction in many ecosystems (D'antonio and Chambers 2006). Adding fertilizer to the soil in order to help a plant establish and grow may seem like an ideal solution and, depending on the plant being restored and the level of herbivore activity in the system, it may be. The benefits of soil fertilization are well documented and studies have shown that it can increase plant biomass production, aid in root growth, and improve overall resilience in many plant species (Mizumachi et al. 2004; Peralta et al. 2003; Andersen et al. 2014). However, the addition of nutrients to the soil can have huge impacts on the intensity and type of herbivory a plant may experience.

For a long time, there was a general consensus among ecologists that plants growing in relatively resource-rich environments should be able to tolerate herbivory better than plants growing in more stressful environments, because greater nutrient availability should increase plant growth rate and allow plants to replace damaged tissues (Meyer 2000). This theory, first formalized by Maschinski and Whitman (1989), is now known as the Compensatory Continuum Hypothesis (Wise and Abrahamson 2005). While this prediction has strong intuitive appeal, and is an important assumption of a

handful of general theories of plant resistance (such as the resource availability hypothesis and the carbon-nutrient balance hypothesis), a range of scientific evidence is now available that actually shows the opposite may be true in some environments (Endara and Coley 2011; Hamilton et al. 2001).

Plants that are growing in soils with high nutrient availability typically have a greater concentration of nitrogen in their leaves, which can affect traits that are potential feeding cues (such as tissue nutrients and secondary chemicals) that attract herbivores (Lower et al.; Mizumachi et al. 2004). Plants growing under these more fertile conditions also tend to be able to support higher densities of herbivores than plant growing under less fertile conditions, due to their increased nutrient content (Meyer 2000). In some cases, the same degree of defoliation has had a greater impact on fertilized plants than on unfertilized plants, as high nutrient availability has been found to actually decrease plant tolerance to herbivory (Meyer and Root 1993). This could be due to the fact that higher nutrients tend to decrease the root-to-shoot ratio in plants, which is associated with reduced tolerance (Strauss and Agrawal 1999). However, an herbivore may increase its feeding rate if it is consuming a low-nitrogen diet, which could lead to greater impacts on plants growing in soils with lower nutrient availability (Mattson 1980; Meyer and Root 1993).

All of this contradicting evidence is presented to illustrate that plant-herbivore interactions are complex. The outcome of the interaction between soil fertility and herbivory changes with every species and ecosystem and can depend upon a number of factors, such as the feeding style of the herbivore, the duration of herbivory, the plant's morphology, and a plant's growth rate (Meyer 2000). Many ecologists have found that

the plants in their studied ecosystem tolerate herbivory better when they are fertilized (McNaughton et al. 1983; Verkaar et al. 1986; Maschinski and Whitman 1989), but many others have found that their plants overcome herbivory better when they are not fertilized (Meyer and Root 1993; Meyer 2000; Lower et al. 2003; Mizumachi at al. 2004). Every plant-herbivore-ecosystem combination may respond differently to various soil fertility levels, so ecologists and restoration mangers must determine what is best for the macrophyte they are trying to restore or promote growth in. Will increasing the soil fertility ameliorate the effects of herbivory by helping the plant compensate for the tissue damage it received, or will the higher nutrient content make it fed on too often for it to recover sufficiently?

Each of the factors discussed - crayfish herbivory, soil fertility, macrophyte restoration, removal of invasive species, dissolved oxygen dynamics, and anthropogenic effects on rivers - contribute not only to the survival of the fountain darters, but also to the overall health of the Comal River and the greater Edwards Aquifer ecosystem. Identifying and monitoring these issues is critical to the conservation and continual survival of fountain darters as well as the 6 other endangered species in the Comal.

Experiments

In order to further our understanding of several factors influencing the habitat quality of the endangered fountain darter (*Etheostoma fonticola*), we studied three separate but interrelated objectives. The first objective assessed the nutrients available in the sediment, water column, and in the macrophytes themselves in and around Landa Lake. This objective included three parts: water quality analyses, a nutrient diffusing substrata experiment, and a sediment and macrophyte tissue nutrient survey. The second

objective was to quantify the impacts of soil fertility and herbivory on *Ludwigia repens* via an *in situ* experiment involving crayfish exclosures in Landa Lake. The final objective was to characterize the daily variability in ambient dissolved oxygen (DO) throughout Upper Spring Run (USR) and Landa Lake and included two parts: a survey of spatial variability in DO, and a long-term study of vertical variation in DO.

CHAPTER TWO

Nutrient Assessment in Landa Lake

As discussed previously, nutrient availability within a river can have huge implications on the success of a macrophyte restoration effort. In a report to the Edwards Aquifer Authority, BIO-West, Inc. (2015a) highlighted the need to investigate the nutrient availability in the water column and sediment in the Comal and to learn how macrophytes are partitioning those nutrients in order to aid in habitat restoration efforts. Nutrient partitioning and shuttling are important processes in macrophytes that can drive biomass production and reproduction, but species respond to and use nutrients differently (Barko et al. 1991; BIO-West, Inc. 2015a).

Nutrients in the Water Column

Here we address the question of nutrient limitation of photosynthetic organisms that are dependent on the water column for nutrients. That is, unlike rooted aquatic macrophytes, these organisms do not have direct access to reservoirs of nutrients in the sediments. Organisms that are primarily dependent on water column nutrients include: algae in the water column, benthic mats of filamentous algae, epiphytic algae on rocks, bryophytes, and epiphytic algae growing on rooted macrophytes (although the algae attached to macrophytes may also benefit from nutrients potentially released by the macrophytes).Water quality is particularly important to fountain darters because of their sedentary nature and lack of ability to flee from situations detrimental to their health. The amount of algae in the system is also of great import to the fish. Too much algae can

cause a loss of sunlight, which means a loss of plants and an increase in decomposition, leading to a decrease in dissolved oxygen. Benthic algae mats, however, are the fountain darters second-most preferred habitat (Figure 1), and phytoplankton are the base of their food web, so it is imperative for fountain darter population management that we understand what factors could potentially affect algae mat growth.

The question of water column nutrient limitation was addressed in two ways. First, water samples were collected from the ecosystem during the summer of 2015 and the relative ratio of total and available (dissolved) nitrogen and phosphorus was analyzed. Second, we ran an experiment using nutrient diffusing substrata (NDS) placed across the lake and the upper spring run in the spring of 2016 and again in the winter of 2016 to directly evaluate nutrient limitation.

Methods

Total and dissolved forms of nitrogen and phosphorus in the water were monitored at four fixed points in Landa Lake and the upper spring run (USR) between March 28th and September 9th of 2015 (Heidelberg Lodge, Spring Island, Pecan Island and Fishing Pier, Figure 2). 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 (NO_3-N), and soluble reactive P (PO_4-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.

We measured nutrient limitation directly using nutrient diffusing substrata (NDS).

NDS are used for measuring whether the growth of biofilms is nutrient-limited in aquatic environments. NDS are generally constructed using small plastic tubes filled with nutrient-amended agar and topped with an inorganic surface (i.e., fritted glass disc) that allows for mainly algal colonization. Tubes are then incubated in the stream for 18–20 days. Preliminary studies have shown that the rate of nutrient diffusion from the plastic tubes, filled with agar and nutrient, is constant through 17 days and then declines only slightly to day 21 (Hauer and Lamberti 2007). Once inorganic surfaces have been colonized by algae, NDS are returned to the laboratory for analysis of algal colonization by measuring chlorophyll a standing stock. Variability between NDS within a treatment can sometimes be high and replicates allows for increased statistical power for determining treatment differences.

We followed the basic outline for constructing NDS as described in Methods in Stream Ecology (Hauer and Lamberti 2007). Our nutrient diffusing substrata were built first by boring a hole 2.23cm in diameter into the lid of 50 mL plastic centrifuge tube and placing a fritted glass disc (FGD) that was 2.5cm in diameter under the lid. The centrifuge tubes were filled with either plain (Control) or four combinations of nutrient-amended agarose gel. We amended agarose with Nitrogen (0.5 M NO3-N), Phosphorus (0.5 M PO4-P), Micronutrients (solution of Fe, Mg, and other micronutrients), or Phosphorus and Micronutrients (0.5 M PO4-P) and micronutrient broth) by dissolving salts or solution into the agar. A control treatment was constructed using plain agarose gel. Lids were secured so that there was no space between the lid and FGD. One of each of the 5 NDS treatments (Control, +N, +P, +Micro, +P&Micro) was attached, in a

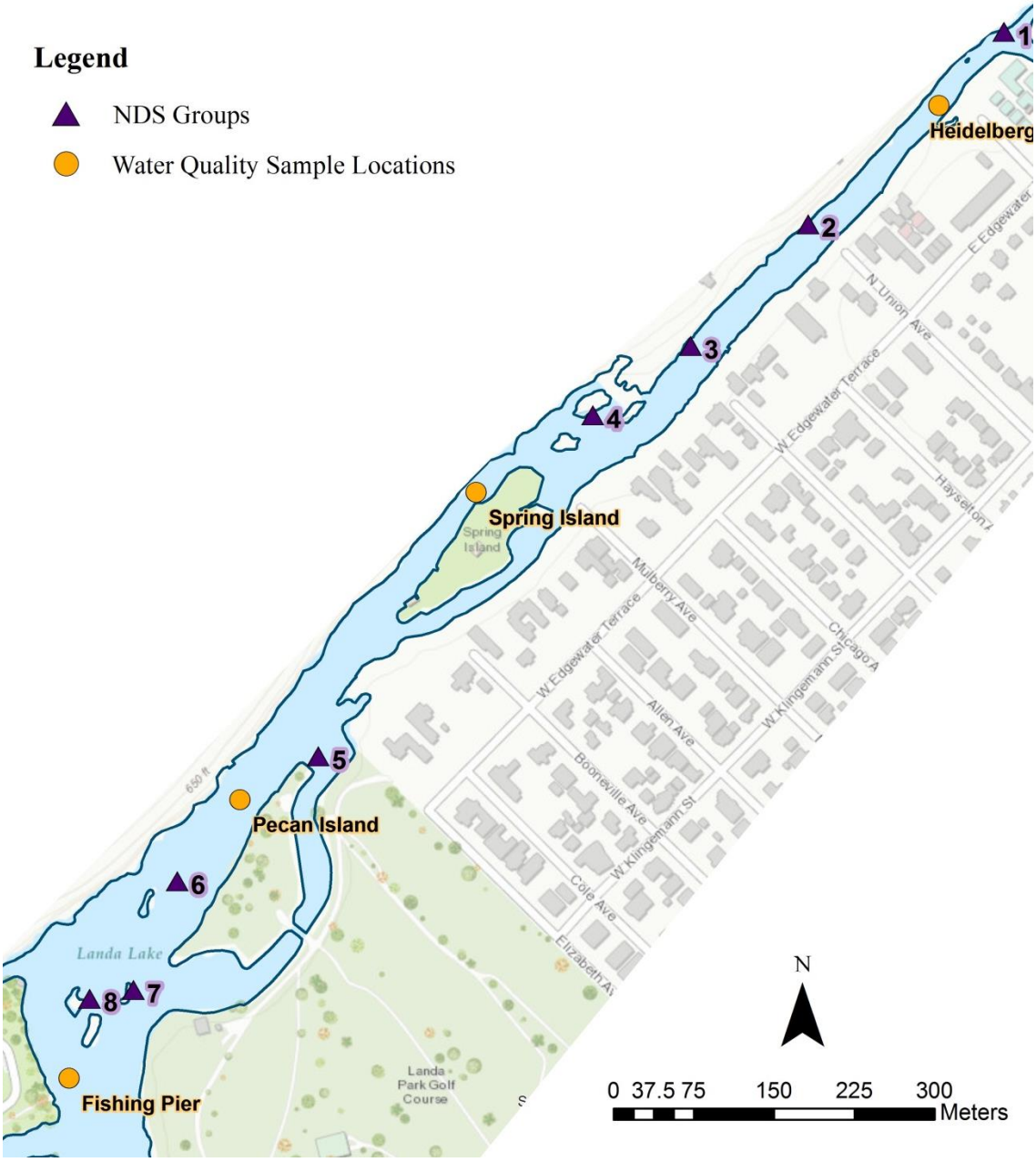

Figure 2. Map of NDS deployment locations in Landa Lake and the Upper Spring Run on the Comal River in New Braunfels, Texas. Each location and number corresponds to the group number used in further analyses of the NDS treatments. These locations were consistent across both NDS deployments. The orange circles correspond to the water quality sampling sites.

random order, to a metal bar that was anchored to the substrate in Landa Lake. Locations with good light availability were selected (that is, we avoided vegetation stands and riparian canopies). Eight of these groups of 5 were positioned in various locations along Landa Lake and the Upper Spring Run on February 18th, 2016 and collected on March 17th, 2016 (Figure 2).

After collection in the field, the NDS tubes were kept refrigerated until processed. Processing took place within four days of collection from the field. Chlorophyll-a extraction was made using the technique described in the New Zealand Stream Periphyton Monitoring Manual (Biggs and Kilroy 2000). The glass fiber fritted disks were carefully removed from the NDS tubes and placed in 10.0 ml of 90% ethanol in a polypropylene vial with tight-fitting top. Care was taken not to displace any periphyton layers accumulated on the fritted disks. Once all NDS tubes were processed, the vials were placed in a water bath at 78°C for 10 minutes. The racks were then allowed to cool in a dark location at laboratory temperature. After 2.5 hours, the vials were gently agitated to homogenize the extract, and the absorbance of the extracted solution was read on a Spectronic 20D+ at 665nm in 1.0 cm cylindrical glass tubes. The raw absorbance at 665 was then presented as a relative measure of Chlorophyll-a per disk.

This study was further expanded in December of 2016 to add 16 more groups of 5 tubes, in part to expand the sample size and partly because we observed snail herbivory on 2 of the 8 locations in the original run and wanted to attempt to ameliorate that effect. These tubes and treatment groups will be prepared in the same manner as the first set in this experiment. The tubes will be placed in the same locations as the first set in Landa Lake and the Upper Spring Run on December 17, 2016 and removed on January 14,

2017. Small rectangular cages were constructed of ¼" aluminum mesh that surrounded the tubes to protect them from most snail herbivory (see Figure A.1). Half of the tube groups were in a cage and half were just outside of one. To address issues with the cages shading the NDS, we took light measurements within and outside of the cages to compare.

These new tubes were analyzed differently than the first experimental set. Instead of a relative measure of Chlorophyll-a per disk, we measured the actual amount of Chl-a. Living algae contain mainly undegraded chlorophyll molecules (e.g., Chl-a alone or together with Chl-b or Chl-c), but because of cell death, samples also may include chlorophyll degradation products. The two most common degradation products of chlorophyll-a are phaeophytin-a and phaeophorbide-a. These can contribute up to 60% of the measured chlorophyll-a content and can absorb light in the same region of the spectrum as chlorophyll-a, so their presence can interfere with the spectrophotometric estimation of chlorophyll-a concentration. Thus, a measurement of actual chlorophyll-a requires absorbance to be measured both prior to, and following, acidification, in order to correct for the degradation products that may be present. The alternative spectrophotometric approach is simply to measure total chlorophyll pigments, estimating all chlorophyll pigments and degradation products that absorb at 665 nm, which is what we originally measured in the first round of NDS (Hauer and Lamberti 2007).

To measure the actual amount of Chl-a, the glass fiber fritted disks was first carefully removed from the NDS tubes and placed in 10.0 ml of 90% ethanol in a polypropylene vial with tight-fitting top. After inverting the vials a few times, they were placed in a water bath at 78°C for 10 minutes. The racks then cooled in a dark location at

laboratory temperature for 15 hours. After resting, the absorbance of 3.75mL of the extracted solution was read on a Perkin Elmer Lambda 35 UV/Vis Spectrophotometer at 630nm, 645nm, 665nm, and 750nm in cuvettes. The cuvette was removed and 200 µL of HCl was be added to acidify the sample and remove the phaeophytin pigments. Thirty seconds after acidification, the cuvettes were read again at the same wavelengths. This gives us data that portrays the actual Chlorophyll-a in the sample, reported below in micrograms Chl-a per cm^2.

Both sets of NDS data were analyzed using a two-way (nutrient treatment by location) analysis of variance (ANOVA) test to determine if there were any significant differences between the means of multiple treatment groups and locations. Since the ANOVA related a significant result, we then used a post- hoc test called Tukey's test, which is a multiple-comparisons procedure used to identify which sample means are significantly different from each other. Water quality data is reported as concentration (mg/L) with standard error values.

Results

The results from the water quality samples at the four fixed points across Landa Lake and the Upper Spring Run are presented in Table 1. This table includes seven measurements: mean total nitrogen ($\bar{X}$ TN), mean total phosphorus ($\bar{X}$ TP), mean nitrate ($\bar{X}$ NO_3), mean ammonium ($\bar{X}$ NH_4), mean phosphate ($\bar{X}$ PO_4), total nitrogen to total phosphorus ratio (TN:TP), and the dissolved nitrogen to dissolved phosphorus ratio (N:P). The dissolved nitrogen comprises of only the NO_3 and the NH_4 measurements, excluding any other forms of nitrogen that may have contributed to the total nitrogen

Table 1. Water quality data from 2015, showing mean (Standard Error) values for various forms of nitrogen and phosphorus from 13 samples collected at 4 fixed points in Landa Lake and Upper Spring Run. TN=Total Nitrogen, TP=Total Phosphorus, NO_3 = Nitrate, NH_4=Ammonium, PO_4=Phosphate. N:P represents the *available* N (NO_3 + NH_4) and the available P (PO_4). Concentration data shown in µg/L.

Location	$\bar{X}$ TN	$\bar{X}$ TP	$\bar{X}$ NO_3	$\bar{X}$ NH_4	$\bar{X}$ PO_4	TN:TP	N:P
Heidelberg	1794(33.7)	8.7(1.26)	1750(48.9)	8.0(1.15)	9.9(0.49)	205	178
Spring Island	1777(37.9)	8.7(1.04)	1717(49.4)	8.0(1.76)	9.3(0.33)	203	186
Pecan Island	1810(32.9)	9.5(1.62)	1735(41.8)	5.4(1.16)	10.0(0.35)	192	175
Fishing Pier	1789(37.7)	10.8(1.99)	1729(57.6)	7.0(1.38)	9.1(0.41)	165	190

measurement, and the dissolved phosphorus used in the ratio is only the PO_4 measured. The data are shown as micrograms (µg) per liter with standard error of the population mean in parentheses. Each datum presented is the mean of the 13 samples taken at that location. Note the high levels of dissolved nitrogen and low levels of dissolved phosphorus present in the water column at each of these locations.

Relative chlorophyll-a data for each tube in the first NDS deployment is shown in Figure 3. Locations 6 and 7 had atypical results compared to the other six locations, which we theorized could be due to the snail herbivory that was observed on the fritted disks at these two sites when the NDS tubes were collected. At these sites, there were no pronounced differences in Chl-a amounts among the treatments. At 4 of the 8 locations (2, 4, 5, 6), the +micronutrients treatment was considerably larger than the control and +nitrogen treatments. Each of the 8 locations were fairly variable in the amount of Chlorophyll-a found on the fritted disks, though one or both of the two treatments with added Phosphorus (+P and +P&Micro) generally produced the most, with the two exceptions mentioned (Figure 3).

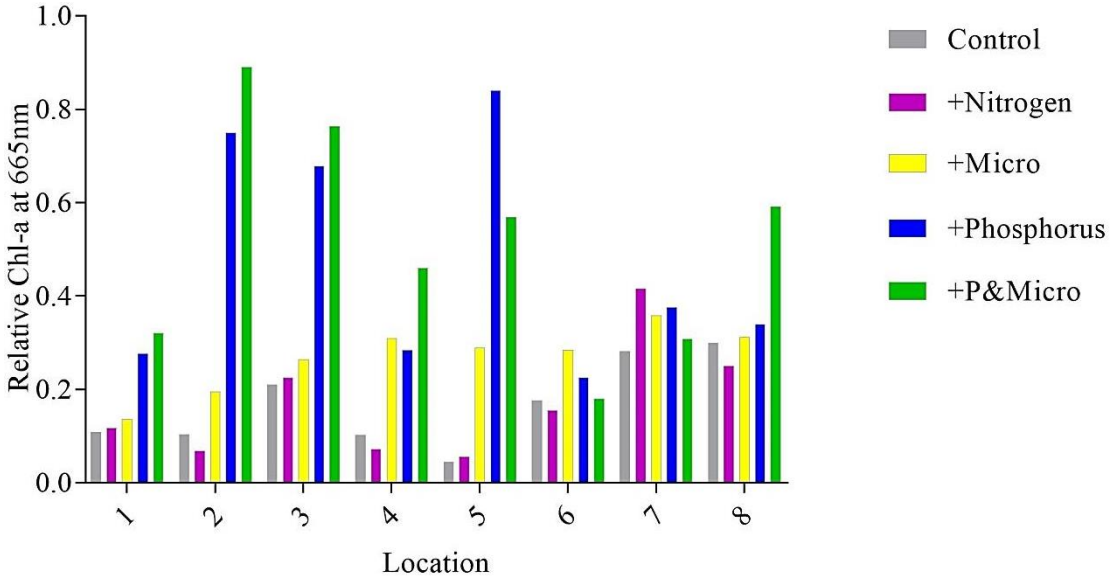

Figure 3. Relative Chl-a (absorbance at 665nm) at each location for the first NDS experiment, for all 5 treatments. Each bar represents a single NDS tube.

The ANOVA was significant (p=0.002) for treatment groups, showing significant differences among the 5 nutrient treatments, but not for location (p=0.19). The ANOVA assumed that the treatments had the same effect across all locations in all of the NDS tubes. The treatments accounted for 46% of the overall variation among the samples, and the p-value relates that there is only a 0.02% chance of randomly observing the magnitude of differences that we did among treatments in this data set, so the differences are considered highly significant. In this NDS deployment, location only accounted for 15% of the total variation and the p-value indicates that there is a 19% chance that this effect is random, so we do not consider it to be statistically significant (α=0.05).

The Tukey test showed that the +phosphorus and the +micronutrients and phosphorus treatments were statistically higher than the control, +nitrogen and +micronutrients treatments, which were not different among themselves (Figure 4). While the actual average of the +micronutrients treatment was about 30% higher than the

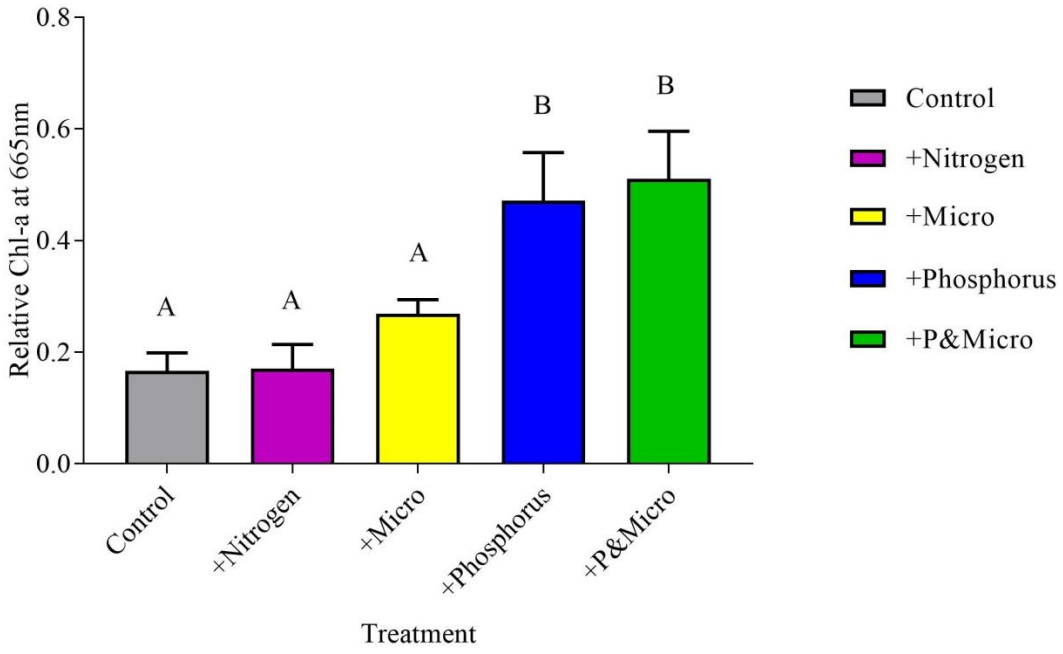

Figure 4. Mean relative Chlorophyll-a (n=8) from each NDS treatment in the first experiment with standard error bars. ANOVA was significant for differences in treatment ($p=0.002$), but not in location ($p=0.19$). Letters indicate significant differences among treatment means (Tukey Test, $p<0.05$).

controls and +nitrogen treatments, they were not significantly different. Figure 4 shows the differences among the means of each treatment group, with letters above the bars denoting the statistically different treatment means and standard error bars of each mean.

After analyzing the data for the second NDS experiment, patterns emerged that mimicked the first. Locations 6 and 7 still had atypical results- both the caged and the open- despite the presence of the cages that we had predicted would ameliorate the strange effect. We believed this anomaly in the first NDS deployment was due to snail herbivory, but this second set of data shows that was likely not the case. Figure 5 shows the actual Chl-a per cm^2 on each NDS tube across all 5 treatments, all 8 locations, and both types of deployment (caged and open). The added phosphorus and the added

phosphorus and micronutrient treatments still generally had more Chl-a than the other treatments.

Figure 6 depicts the mean *relative* Chl-a (the absorbance at 665nm) from the second NDS deployment- this data is directly comparable to the first NDS deployment information presented in Figure 4. Although we have data that is more realistic to the actual amount of live algae on the samples (presented in Figure 7), Figure 6 can be compared to the first deployment directly, because the same data (absorbance) is presented. Figure 7, then, shows the mean actual amount of Chl-a per cm^2 found on our fritted disks (Figure 8 uses this measurement as well). This measurement is obtain by taking the absorbance at 665nm before and after the sample is acidified, multiplying that difference by a constant (28.66), and then multiplying it again by the sample volume before being divided by the area of the fritted disk. The difference in the absorbance before and after the acidification represents the amount of currently living algae Chlorophyll-a pigments at the time of the reading.

Figure 7 shows the live Chl-a per cm^2 in the second NDS deployment, represented as the mean for each treatment along with standard error bars. The ANOVA was significant for both treatment ($p=0.0011$) and for location ($p<0.0001$), so Tukey tests followed to determine which means were significantly different. It is worth noting that this graph shows different levels of significance between treatments than the similar Figure 6. The letters above this figure relate that the +nitrogen and the +micronutrients treatments were not significantly different ($p>0.05$) from the control *or* from the +phosphorus treatment. However, the +phosphorus treatment was significantly higher than the control. The mean of the +phosphorus and micronutrients treatment was

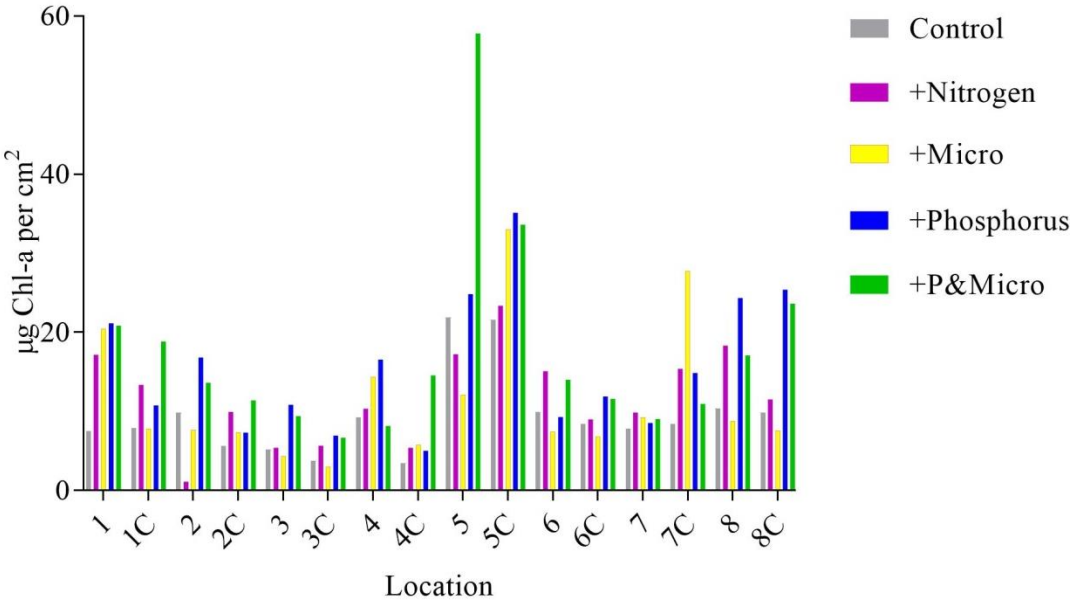

Figure 5. Micrograms of Chl-a (absorbance at 665nm) per centimeter squared on the fritted disks for the second NDS deployment at each location for all 5 treatments and both deployment types. The "C" denotes the NDS tubes enclosed within cages. Each bar represents a single NDS tube.

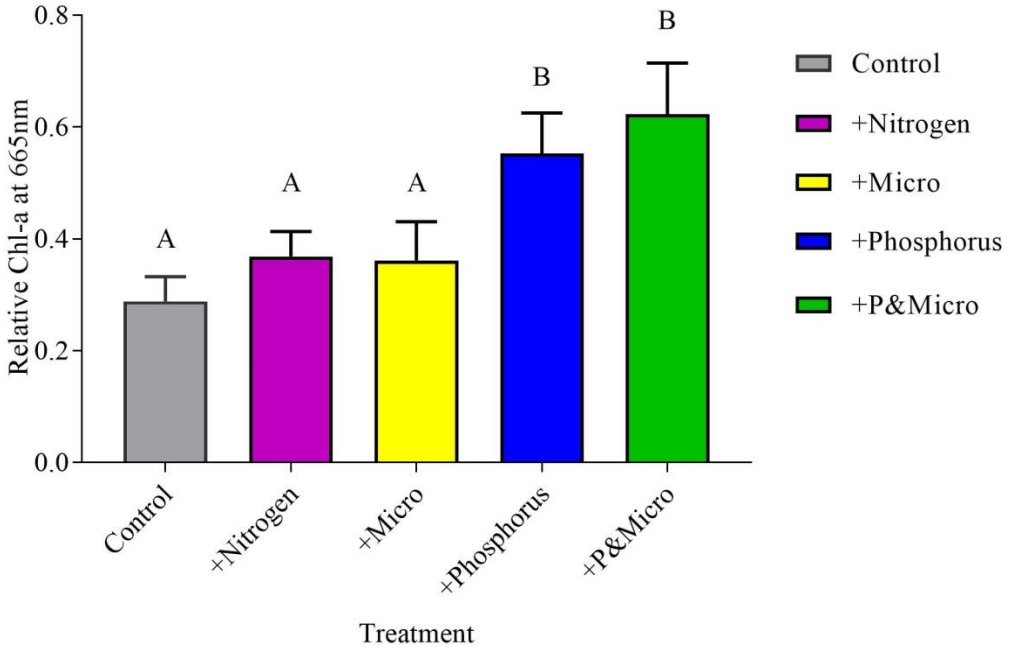

Figure 6. Mean relative Chlorophyll-a (n=16) from each NDS treatment in the second NDS experiment with standard error bars. ANOVA was significant for differences in treatment ($p=0.0011$), and in location ($p<0.0001$). Letters indicate significant differences among treatment means (Tukey Test, $p<0.05$). This graph presents the data from the second NDS experiment in the exact way we reported the first to allow for easier comparison between deployments.

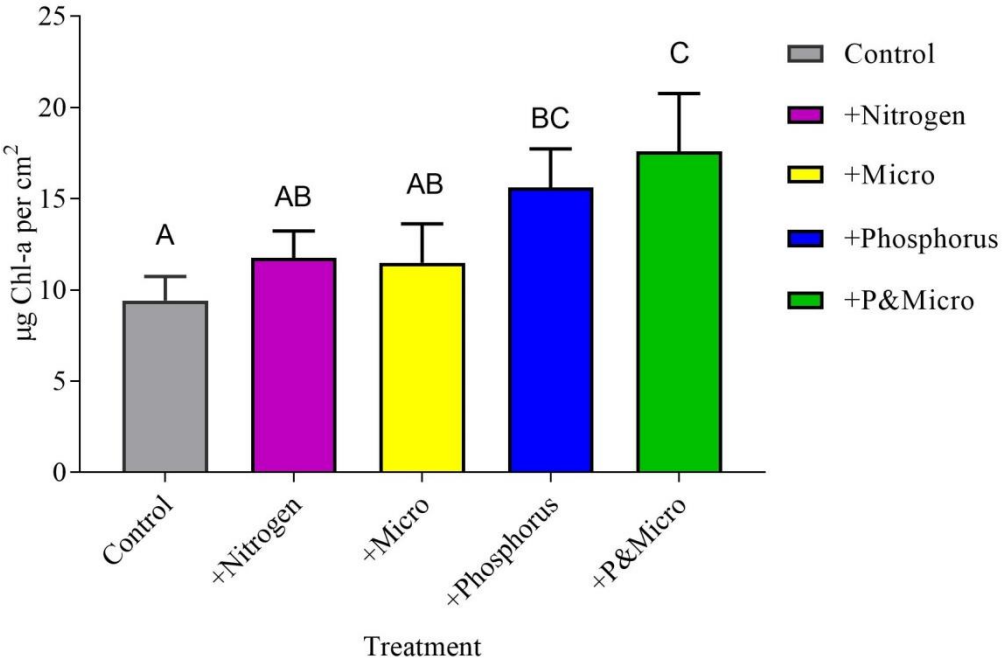

Figure 7. Mean micrograms Chlorophyll-a per centimeter squared (n=16) from each NDS treatment in the second experiment with standard error bars. ANOVA was significant for differences in treatment ($p=0.0011$) and in location ($p<0.0001$). Different letters indicate significant differences among treatment means (Tukey Test, $p<0.05$).

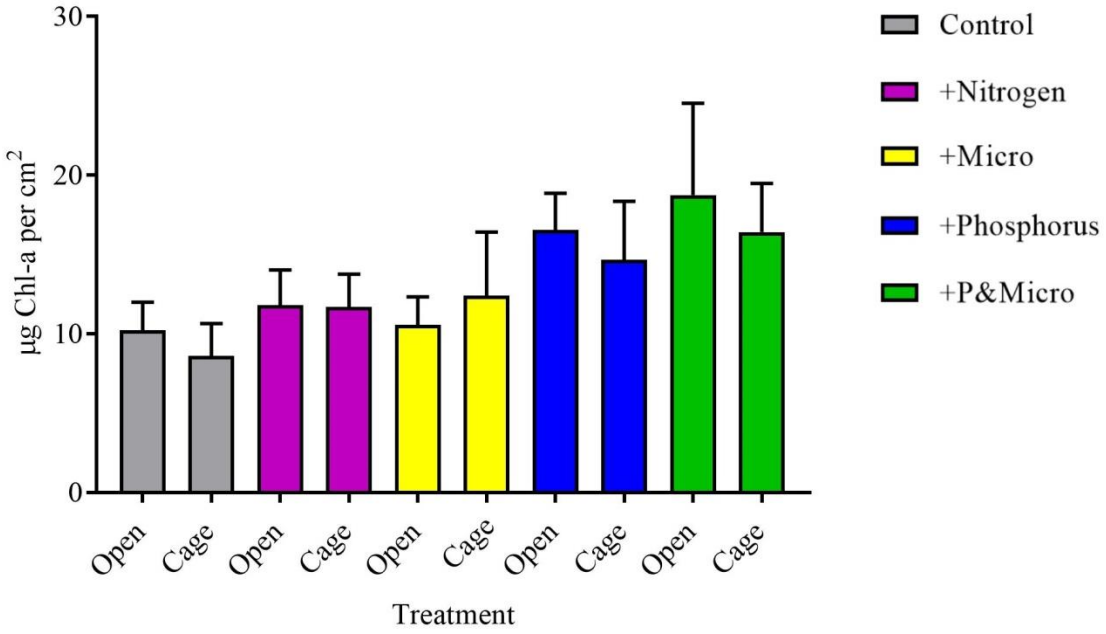

Figure 8. Mean micrograms Chlorophyll-a per centimeter squared (n=8) from each NDS location and deployment type in the second NDS experiment with standard error bars. This graph, and the ANOVA and Tukey Tests performed, confirm that there was no significant difference between the caged NDS tubes and the tubes not in cages (open).

significantly different than the control, nitrogen, and micronutrients treatments, but not different than the +phosphorus treatment.

In order to assess if herbivory or the presence of the cages around half of the NDS tubes were causing a significant difference in results, a two-way ANOVA was performed with subsequent Tukey's tests for multiple comparisons. Treatments were separated between "open" (NDS tubes not in cages) and "cage" (Figure 8). The Tukey test revealed that there were no significant differences between the caged and the open of each treatment (p-values were all greater than 0.98).

Discussion

One way to assess if nitrogen or phosphorus is potentially limiting algal growth in a body of water is to compare their relative abundance in the water column with the relative needs of the autotrophs growing in it. To gauge whether nutrient supplies are sufficient, biologist have long relied on the Redfield Ratio, which states that the typical alga requires 106 atoms of carbon for every 16 atoms of nitrogen for every 1 atom of phosphorus. This can translate to a mass ratio of 40:7:1 (C:N:P), and this ratio has subsequently been accepted as a general indicator for balanced growth with potential for near optimum growth rates. Therefore, an N:P ratio substantially above 7 is viewed as evidence that phosphorus is likely limiting algal growth while a ratio below 7 would indicate nitrogen limitation. The very high available N: available P ratios (average of 182:1) present in the water quality samples suggest there is a strong phosphorus limitation in the water column, based off of the accepted Redfield ratios (7:1).

If we had analyzed the first NDS deployment data for actual Chl-a per cm^2, we might expect different results than the simple absorbencies indicate, similar to the

differences between Figures 6 and 7. The fact that the raw absorbencies show that the added phosphorus treatment was significantly different than the +nitrogen and +micronutrients treatments, but the Chl-a per cm^2 data showed they were not is likely due to differing amounts of phaeophytin on the NDS tubes. If each treatment had accumulated the same proportion of phaeophytin to live algae, then the graph in Figure 7 would have looked the same as the graph in Figure 6, but with a larger scale on the y-axis. As it stands, it appears that the phosphorus treatment had a greater percentage of phaeophytin at the time of analysis than the nitrogen and micronutrients treatments. The +nitrogen and the +micronutrients treatments were not significantly different than the control, but the + phosphorus and +phosphorus and micronutrients treatments were different from the control. This makes some sense in that, since the phosphorus appeared to have the largest effect on algae growth, it was likely colonized faster than the control, nitrogen, and micronutrient treatments, and some of those algae would then be the first to die. The presence of this dead, decaying algae could then be a barrier and outcompete, so to say, the living algae for space on the fritted disk.

To ameliorate this effect, Methods in Stream Ecology (Hauer and Lamberti 2007) suggests that NDS are deployed for 17 to 21 days due to the rate of nutrient diffusion through the fritted disks slowing down and the phaeophytin having the opportunity to build up on the tubes, which is exasperated with time. Unfortunately, our first set of NDS tubes was left *in situ* for 28 days due to scheduling conflicts and, in an effort to keep the treatments as uniform as possible, the second set was deployed for 28 days as well. This delay in analysis of the NDS tubes and the possible accumulation of phaeophytin are the most likely causes of the variances in significant differences of treatment group means

found in Figures 6 and 7. Comparing the graphs leads us to suspect that there may have been a similar effect with the first NDS deployment, but we are not able to know for sure since we lack the actual Chl-a data for that set of tubes.

Regardless of the differences between the raw absorbencies and the actual Chl-a per cm^2, phosphorus appears to make the biggest impact on the algal growth, also suggesting (like the water quality data) that a phosphorus limitation exists. This result was not surprising for a few reasons. Soil, and subsequently water, tends to be phosphorus limited in temperate environments (as opposed to the tropics, where soil tends to be nitrogen limited). Additionally, the water that comes out of the springs is naturally high in nitrogen, which has recently been theorized to be due to nitrification of organic N within the aquifer, while the water is still underground (Musgrove et al. 2016). This is exasperated by the amount of nitrogen-rich fertilizer likely running off the artificial residential lawns and the large golf course that exist along the eastern side of the Upper Spring Run and Landa Lake.

However, the magnitude of the nitrogen-to-phosphorus ratio in the river was a bit surprising. As stated, an N:P ratio of 7:1 is ideal for phytoplankton, so this average of 182:1 (N:P) indicates that phosphorus could be strongly limiting the algal growth. This phosphorus limitation is important particularly in the face of human-induced eutrophication currently plaguing much of the industrialized world. The Comal Springs output clean, clear water into the Upper Spring Run and Landa Lake. This clear water at a constant temperature is the main reason there are so many rare and endemic species in the river, so any amount of eutrophication or clouding of the water column could severely mess with this ecosystem. Aquatic ecosystems are in danger of anthropogenic

eutrophication largely due to agricultural and urban runoff. Unfortunately, the Comal runs through an urban area with people living along its length, and land use within the Edwards Aquifer catchment is largely agricultural. Our water quality data combined with our NDS experiments show that phosphorus is limiting algal growth and that any increase in phosphorus availability could lead to a significant increase in algae. With light not being a limiting factor in this shallow, clear-water system, algal blooms can occur as a benthic mat, smothering any plants or animals unable to escape, or as surface algae blocking the light for the rest of the water column. This decrease in light would lead to less photosynthesis, which would mean lower dissolved oxygen in the water. The lack of light would also kill many plant species and their subsequent decay could lead to even lower dissolved oxygen, due to microbe respiration. Lower dissolved oxygen could then cause to an increase in fish mortality (Nylen 2015).

There is also some evidence of a minor micronutrient limitation. In the first NDS deployment, the average relative Chl-a of the +micronutrients treatment was over 30% higher than the control and +nitrogen treatments, though they were not significantly different. In both NDS deployments, the averages of the +phosphorus and micronutrient treatments were higher than the +phosphorus alone, though, again, this effect was not statistically significant. This may indicate that micronutrient additions are stimulating certain species of algae, but there is not a large enough community response to differentiate the mean from the other treatments. A future direction to expand our understanding of nutrient limitations in this system could include another set of NDS tubes with the micronutrients separated out, rather than having them all together in one, to figure which nutrient(s) could be stimulating this algal growth.

Methods

In order to better understand how nutrients are distributed across Upper Spring Run (USR) and Landa Lake, we collected 12 samples of both sediment and macrophytes across 10 locations. Macrophyte species sampled were: *Vallisneria americana* (Val), *Sagittaria platyphylla* (Sag), and *Ludwigia repens* (Lud). Sediment types included were characterized by silt, sand, and gravel. The location of each sample (Figure 9) was chosen to cover the most vegetation species – sediment type combinations. To obtain the sediment samples, the corer used was a Wildco® Hand Core Sediment Sampler. This device required driving into the soil by hand, draining the water from the corer, and then releasing the sediment core into a labeled container. Each core averaged approximately 8 inches deep (2 inches in diameter), to obtain the majority of the sediment used by macrophyte roots. Vegetation samples were harvested by hand as close to the sediment core as possible and, on occasion, within the core itself. In total, 10 sediment cores were obtained along with 3 Val samples, 4 Sag, and 3 Lud. Additionally we took 2 potted *Ludwigia* plants currently growing in a nursery MUPPT. The sediment that BIO-West ecologists use to plant the MUPPTs is a very soft, high-clay sediment located off of a small island and is thought to be high in nutrients. We wanted to include this sediment and the *Ludwigia* growing in it to determine if it was higher in essential nutrients than the other samples. All samples were analyzed at Baylor University.

Plants were thoroughly rinsed in DI water before being weighed for an initial fresh-weight of both the above and below ground materials. The plants were then dried in

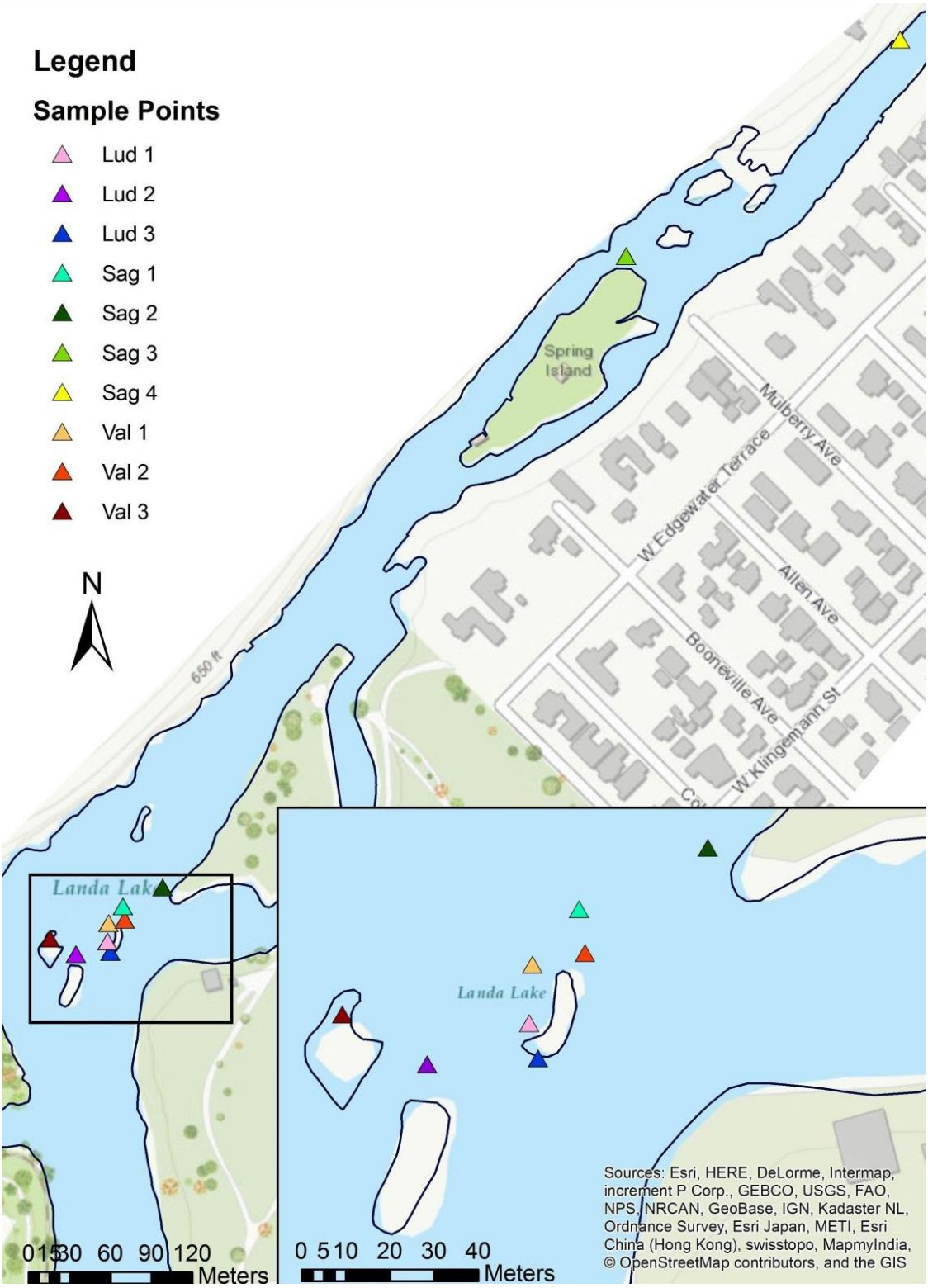

Figure 9. Location of the vegetation and sediment core samples from June 8, 2016. Each triangle represents one macrophyte and one core. Lud stands for *Ludwigia*, Sag for *Sagittaria*, and Val for *Vallisneria*.

a 50°C oven for one week before being weighed again for a dry-weight. They were then ground into a fine powder using a blender followed by one minute in a Mini-BeadBeater. To analyze carbon and nitrogen content in the samples, 1-3 mg of powder was placed in a small tin capsule. These capsules were then exposed to fuming HCl for 2 hours in a fumigation chamber in an air hood to destroy any inorganic carbon in the sample. After this, the tins were rolled, placed in a similarly sized silver tin, rolled again, and then analyzed in an elemental analyzer. C and N content were measured simultaneously with a Thermo-Finnegan Flash 1200 elemental analyzer (ThermoQuest, Milan, Italy). For phosphorus content, 2-20 mg were placed in a glass vial, capped with a lid containing a Teflon septum, and chemically digested in an autoclave for 1 h at 120°C by the method of Færøvig and Hessen (2003). For sample masses ≤2000 µg, 15 mL deionized (DI) water and 1.8 mL of digestion solution was used. For every 2000-µg increment, an additional 1.8 mL of digestion solution was used in place of 1.8 mL of DI water, up to a 22,000-mg maximum mass digested (19.8 mL digestion solution, no DI water). Phosphorus content was estimated via colorimetry by the ascorbic acid–molybdate method on a Lachat 8500 flow-injection autoanalyzer with an ASX-520 autosampler (Hach Co., Loveland, Colorado). Tissue standards of tomato leaf (SRM 1573a, 0.216% P) and dissolved inorganic standards were run for QA/QC (Back and King 2013).

The sediment in each core was homogenized and put into two small aluminum tins each before being dried for 1 week in a 50°C oven. One sediment sample tin from each pot was put into the furnace to burn the organic content from the samples, which was then weighed to obtain the ash-free dry weight. The other half of the samples was ground up in a blender before being strained through a 1mm sieve. The sediment that

could not pass through the sieve was labeled “course sediment” and set aside from the rest after being weighed. The finer sediment was then put into a Mini-BeadBeater before being weighed into tin capsules (8-10 mg) for carbon and nitrogen analyses and glass vials (2-20mg) for phosphorus analyses. The capsule and glass vials were then processed in the same way as the plant tissue nutrients were above. Nutrient concentrations are reported as a concentration (μg/mg), and nitrogen to phosphorus ratios separated into sediment, above-ground plant tissue, and below-ground plant tissue.

Results

The results of our brief nutrient survey conducted across Landa Lake are presented as nutrient concentrations in Table 2 and the above-ground nutrients are compared to literature in Figure 10. Overall, phosphorus was the nutrient that varied the most between plant species in both the above-ground (AG) and below-ground (BG) tissues, ranging from 1.22 μg/mg to 5.17 μg/mg, affecting the N:P ratios in more than nitrogen did. Carbon was rather constant (around 41%) across all the plant specimens, particularly in the AG tissues. Every nutrient measured- carbon, nitrogen, and phosphorus- was highly variable in the sediment collected. Phosphorus was always the lowest in quantity and occasionally there was too small of an amount to be measured, indicated by “BDL” (below detection limit) on Table 2. The sediment nutrients also did not necessarily translate into the plant tissues. For example, the sediment with the highest amount of phosphorus (Lud 1) actually had the *lowest* amount of phosphorus measured in the corresponding above-ground plant tissue. However, this plant did have the highest below-ground phosphorus concentration of all the *Ludwigia*- though that was still lower than all the other plant species BG phosphorus content. There is also a difference

Table 2. Nutrient concentration data collected from the 12 nutrient survey plants, divided into Lud (*Ludwigia repens*), PL (potted *Ludwigia* from the MUPPTs), Sag (*Sagittaria platyphylla*), and Val (*Vallisneria americana*). Phosphorus (P), nitrogen (N), carbon (C), and the nitrogen to phosphorus ratio (N:P) are reported. All concentrations are in µg/mg, and the N:P ratio is the ratio of this concentration (to obtain percent of each, divide by 10). "% Fine" under the sediment heading is the percent of sediment from the sample that, once dried, could fit through a 1mm sieve. Anything that could not fit- such as rocks and shells- were labeled as coarse sediment. BDL indicates that the sample phosphorus was below the method detection limit (MDL= 2.17 µg/L) of the instrument.

	Sediment					Plant Above Ground Tissue				Plant Below Ground Tissue			
Location	P	N	C	N:P	% Fine	P	N	C	N:P	P	N	C	N:P
Lud 1	0.591	1.48	56.51	2.5	81.3	1.22	23.28	404.41	19.1	1.46	20.40	422.15	14.0
Lud 2	0.081	---	---	---	44.5	1.37	24.54	445.05	18.0	0.92	17.89	303.78	19.5
Lud 3	BDL	1.93	98.28	---	47.1	1.36	24.20	406.20	17.8	0.53	15.39	297.58	29.1
PL 1	0.095	2.35	35.01	24.8	72.9	1.86	25.92	429.70	13.9	0.62	15.95	340.28	25.8
PL 2	0.448	3.06	52.08	6.8	54.1	2.73	30.03	452.17	11.0	0.59	14.18	379.34	24.0
Sag 1	BDL	1.00	75.29	---	46.3	4.18	33.82	425.39	8.1	2.19	28.88	377.19	13.2
Sag 2	0.100	2.96	86.89	29.7	84.9	4.37	30.68	363.31	7.0	4.38	39.01	379.53	8.9
Sag 3	0.051	3.60	74.37	70.8	88.2	2.35	27.45	425.06	11.7	1.84	27.96	374.30	15.2
Sag 4	0.295	10.99	171.29	37.3	36.2	5.17	35.12	429.99	6.8	3.27	35.29	417.91	10.8
Val 1	BDL	1.24	77.70	---	62.8	2.81	23.15	358.87	8.2	3.18	31.48	403.94	9.9
Val 2	BDL	1.10	62.89	---	77.7	1.70	27.69	413.68	16.3	2.99	25.95	428.93	8.7
Val 3	0.032	1.82	95.58	57.8	36.5	1.44	27.03	418.10	18.8	2.36	34.63	396.12	14.7

between the concentrations of nutrients in the sediment we measured and the sediment that the plants would realistically be using for nutrient uptake. The "coarse sediment" usually consisted of gravel or snail shells, which the plants are unlikely to use. Because of this, the percent fine sediment in each sample- what we obtained our nutrient concentrations from- is also reported in Table 2.

There was a fair amount of nutrient variability between the three species studied and a bit within each species as well. The naturally growing *Ludwigia* plants (not the Potted *Ludwigia*) averaged the highest N:P ratio in their above-ground tissues (18.3) and had the highest N:P ratio in the below-ground tissue of all samples (29.1, Lud 3). The first *Ludwigia* location (Lud 1) provides a bit of an anomaly. It had the highest N:P ratio in its AG tissues because it had the lowest amount of P, but the lowest *Ludwigia* N:P ratio in its BG tissue because it contained the *most* P. The plant may be storing phosphorus in its roots and not shuttling it to its AG tissues for some reason, even though, compared to the literature and the other Lud plants, its AG biomass appears to be deficient in P. *Ludwigia* at the third location did the opposite- the AG N:P ratio was the lowest of the species and the BG N:P was the highest. This pattern is commonly observed in plants experiencing herbivory; the plant is heavily investing its nutrients into above-ground growth and shuttling them away from the roots (Strauss and Agrawal 1999). The Potted *Ludwigia* (PL) plants showed similar patterns to Lud 3. Although the PL plants had lower above-ground ratios, they had below-ground N:P ratios much higher than the rest of the plants sampled, excluding the aforementioned Lud 3. Again suggesting that these plants are allocating their limited P resources away from their roots and to their above-ground

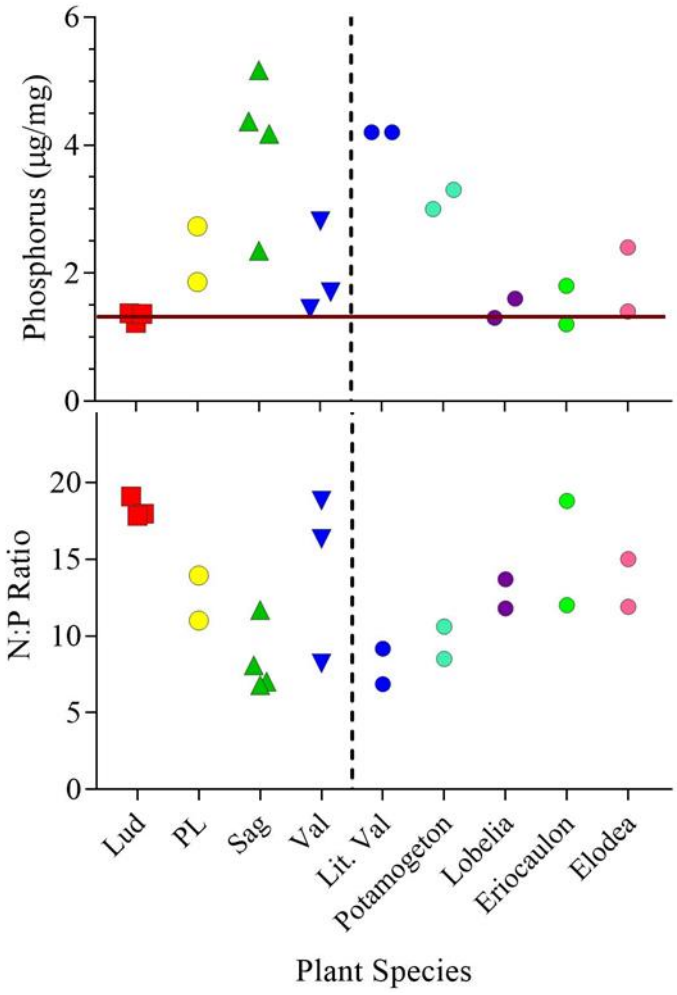

Figure 10. Visual representation of variation in above-ground tissue N:P ratios across and within species. The species and data points to the left of the dotted line were measured in our nutrient survey, while the points to the right of the line represent the AG N:P ratios and phosphorus in other freshwater macrophytes (Gerloff and Krombholz 1966). Lud (*Ludwigia*), PL (potted *Ludwigia*), Sag (*Sagittaria*), and Val (*Vallisneria*) were all taken from Landa Lake in New Braunfels, Texas. "Lit. Val" is *Vallisneria americana* collected from Lake Mendota in Madison, Wisconsin in June-July 1964. The others were collected in Lake Nebish in Wisconsin during the same time period (Gerloff and Krombholz 1966). The red line on the top graph represents the 1.3µg/mg critical concentration of phosphorus.

tissues, which is a sign of herbivory compensation; these plants were grown in the MUPPTs where there is frequently observed herbivory by crayfish.

The *Sagittaria* plant samples were surprising in that they had more nitrogen and much more phosphorus per mg than the other species and also the lowest N:P ratios, particularly in their above-ground tissues. The *Vallisneria* samples were polarized on N:P ratios- two samples (Val 2 and 3) had high N:P ratios in their AG tissues, while the third (Val 1) had one of the lowest ratios sampled (8.2). The low AG N:P ratio in Val 3 was largely due to it having more P than the others- twice the amount as Val 3- and slightly lower N. The amount of phosphorus in the *Vallisneria* BG tissue was also surprising in that it was above average for our data set and yet comes from some of the most P-deficient sediment (Val 1 and Val 2 sediment P was below the detection limit).

Figure 10 compares the above-ground nutrients collected in this experiment with previously published literature. The N:P ratios are plotted on the bottom of the graph, while the phosphorus concentration of each above-ground sample is plotted above it. The red line through the top graph represents the 1.3µg/mg critical concentration of phosphorus. The literature data presented in the graph all come from Gerloff and Krombholz (1966) and represent data taken from two lakes in the summer of 1965.

Discussion

The nitrogen to phosphorus ratio (N:P) is emphasized here and across much literature on plant nutrients because it can be used as a diagnostic tool to predict future community species compositions and physiological changes as well as total ecosystem function, which is particularly in the context of future climate changes and eutrophication (Tessier and Raynal 2003). Nitrogen and phosphorus concentrations are highly correlated

in plants because they are closely associated in plant biogeochemistry and protein synthesis in particular. Plants with skewed N:P ratios- much higher N than P, or vice versa- will be unable to produce the ATP, ADP, NADP, and amino acids in proper proportion required for rapid biomass growth (Duarte 1992).

The nutrient data collected in this survey can be better understood when it is compared to previously published literature. Critical nutrient concentrations are important to establish (in laboratory) to determine if growth in a plant species is nutrient-limited. Tissue nutrient contents below a critical concentration for a nutrient are considered deficient and result in less biomass production. Nutrient concentrations that are above their critical concentration in a species have no effect on biomass production and are referred to a "luxury uptake" (Gerloff and Krombholz 1966). For the most accurate determination of nutrient deficiencies, this value needs to be found for both N and P in each species studied. We did not determine critical nutrient concentrations ourselves, but we can directly compare them to others that have been published.

Most applicable to our study, Gerloff and Krombholz (1966) determined the critical nutrient concentrations for N and P in *Vallisneria Americana*- the same species we sampled. They determined that for maximal biomass production in Val, nitrogen needs to be approximately 1.3% (13µg/mg) and phosphorus around 0.13% (1.3µg/mg). Anything above these concentrations they considered to be luxury uptake and anything below was a deficiency. The critical concentrations for the other species they studied were approximately the same as the *Vallisneria* (Gerloff and Krombholz 1966). However, these concentrations and all of their nutrient data reported did not include below-ground tissues- only plant tissues that grew above the sediment surface. Our

measured AG N:P ratios are compared to some of the ratios reported in this study and visually represented in Figure 10.

Comparing our above-ground plant tissue data to this threshold reveals that none of our *Vallisneria* samples would be considered nutrient limited. However, the phosphorus content for Val 3 is fairly close to the critical concentration, and more samples taken from that area have the potential to be P-limited. The *Sagittaria* sampled was not even close- the average AG phosphorus was 3 times higher than this critical concentration. The magnitude of this luxury uptake, in both the AG and BG tissues, may mean that this species does not follow the critical concentrations Gerloff and Krombholz (1966) established for *Vallisneria* and a laboratory study of this species could be beneficial. Our naturally-growing *Ludwigia* (Lud 1, 2, and 3), however, may be phosphorus limited. Lud 1 was below the critical concentration (0.12%) and the other two samples, Lud 2 and Lud 3, were just above it (0.137% and 0.136%, respectively). The BG tissue phosphorus of these two samples was also under the concentration, though we do not have BG plant tissue data to compare it to.

The nutrient concentrations we report in Table 2 appear to be fairly common for freshwater angiosperms. In a review of nutrient concentrations published, Duarte (1992) reported the average C (n=104), N (n=104), and P (n=72) concentrations for various freshwater macrophytes. These averages (C: 38$\pm$7.4, N: 2.4$\pm$0.7, P: 0.29$\pm$0.23) are fairly close to our own (%C: 41$\pm$2.1, N: 2.8$\pm$0.4, P: 0.25$\pm$0.13), though our N is slightly more elevated and our P slightly less than the average Duarte published. This difference was mostly due to the lower % P we found in our *Ludwigia* and the larger amount of N in our *Sagittaria* samples. Both Duarte (1992) and Gerloff and Krombholz (1966) claim that

phosphorus is most often the limiting nutrient in freshwater macrophyte tissue, particularly in lakes that are not considered to be eutrophic.

Overall, there was the most evidence of a possible phosphorus limitation in naturally growing *Ludwigia* and two of the *Vallisneria* samples. The *Sagittaria* analyzed had surprisingly low and consistent N:P ratios compared to the others, but all the measurements were still within normal ranges published in comparable studies. Our knowledge of how the nutrients vary spatially across Landa Lake and the Upper Spring Run could benefit from an expansion of this study. The variability between species and within them- especially the *Vallisneria*- suggests that we need a larger sample size of organism and sediment to confidently identify trends in the lake. This expansion could provide us with a map of how nutrients are moving around Landa Lake and the USR in both the plants and the sediment and could be a useful tool for managers to base new plantings and restoration decisions on.

CHAPTER THREE

Herbivory by Fertility Interaction *in situ*

BIO-West ecologists have had issues establishing *Ludwigia* populations in bare, unvegetated areas, which typify much of the upper Comal River, known as Upper Spring Run, just upstream of Landa Lake. The sediment in this run is characterized by gravel and sand and has been planted with small patches of *Ludwigia* on multiple occasions, but these patches do not appear to last long. Fertilizing such sediment may aid in biomass production both above and below ground to allow the *Ludwigia* to establish, but it is unknown how this increased soil fertility will interact with the crayfish herbivory, as nutrient content appears to be a factor in their feeding decisions (Cronin et al. 2002; Gherardi 2007). In light of this, the overarching goal of this research is to quantify how herbivory and soil fertility interact to impact the ongoing restoration efforts of *Ludwigia repens* in the Comal River.

In this study, we explored the effects of crayfish herbivory on small *Ludwigia* plants and tested if fertilizing the soil would ameliorate some of those effects. There were, effectively, three questions that were answered:

1) Does herbivory impact *Ludwigia* biomass production?
2) Does enhanced soil fertilization impact *Ludwigia* biomass production?
3) Is there an interaction effect between herbivory and fertilization?

Methods

In order to study the interactive effects between herbivory and soil fertility, a two-by-two factorial design was used (Figure 11). This design results in 4 treatment groups, each of which was replicated 8 times (resulting in 32 individuals total): *Ludwigia* plants with no herbivory and no added nutrients (H-/F-), plants with herbivory and without fertilization (H+/F-), plants without herbivory and with fertilization (H-/F+), and plants with both herbivory and fertilization (H+/F+).

		Fertility	
		Ambient (F-)	Enriched (F+)
Herbivory	Open (H+)	8 Replicates	8 Replicates
	Closed (H-)	8 Replicates	8 Replicates

Figure 11. Two-by-two factorial design used in herbivory by fertility experiment.

This experiment was performed *in situ* in the Comal River in order to test herbivory-fertility interactions in the most natural setting possible. It is particularly important for herbivory experiments to use a plant's natural predator because simulated herbivory and natural herbivory evoke different responses in plants. The clear majority of studies on herbivory responses simulated herbivory damage. However, as Figure B.1 shows, simulated herbivory is usually a poor surrogate for real herbivory. Additionally, many studies of tolerance examine damage from grazing, which is likely to evoke different responses from herbivory by leaf- or root-feeding insects (Strauss and Agrawal

1999). Therefore, this experiment was expressly designed to produce data that will have the most meaning to restoration ecologists when they attempt to transplant *Ludwigia* into bare areas of the Comal. This data will accurately show the natural herbivory that occurs when you put a *Ludwigia* plant in an unvegetated area of the Comal with or without added soil fertilization.

The *Ludwigia* plants used in the experiment were pre-established in two MUPPTs from clippings of other *Ludwigia* plants in the Comal River. A standard 600 mL nursery pot was filled to approximately 500 mL with low-nutrient sediment from Landa Lake. This sediment was chosen from the sediment nutrient survey preformed prior and was used because it had low, but sufficient, nutrient for *Ludwigia* growth and was characterized by gravel and sand, like much of the river. Ten grams of a slow-release fertilizer- Scotts Natural Lawn Food (11:2:2 N:P:K)- was added to half of the MUPPT pots to create the "with fertilization" (F+) treatment of the experiment. This rate of fertilization (2.2 kg of Nitrogen per m^3 and 0.2 kg of Phosphorus per m^3) was decided upon based off comparable literature (Lower et al. 2003; Meyer and Root 1993; Peralta et al. 2003) and the past experiences of BIO-West ecologists. Five 20-centimeter-long segments of *Ludwigia repens* were then stuck into the sediment in each pot. In total, 64 pots were planted, with 32 pots having fertilizer added and the 32 left with the natural sediment nutrients. Sixteen of each treatment ultimately went into the experiment; planting double the number needed allowed us to remove 10 plants from each treatment that were either much bigger or much smaller than the others (or, in some cases, dead), which we refer to as "rejects," and then take out 6 plants to use for initial analyses. These

plants were grown in their respective soil fertility treatments in MUPPTs *in situ* for 30 days before beginning the experiment.

Since crayfish appear to be the most destructive herbivore feeding on *Ludwigia* in Landa Lake, they are the organism the experiment was designed to exclude in the "without herbivory" (H-) treatments. In order to keep half of the plants safe from crayfish herbivory, we designed individual exclosures made of ¼" aluminum screening and tomato cages. Both with and without herbivory treatments included screened cages

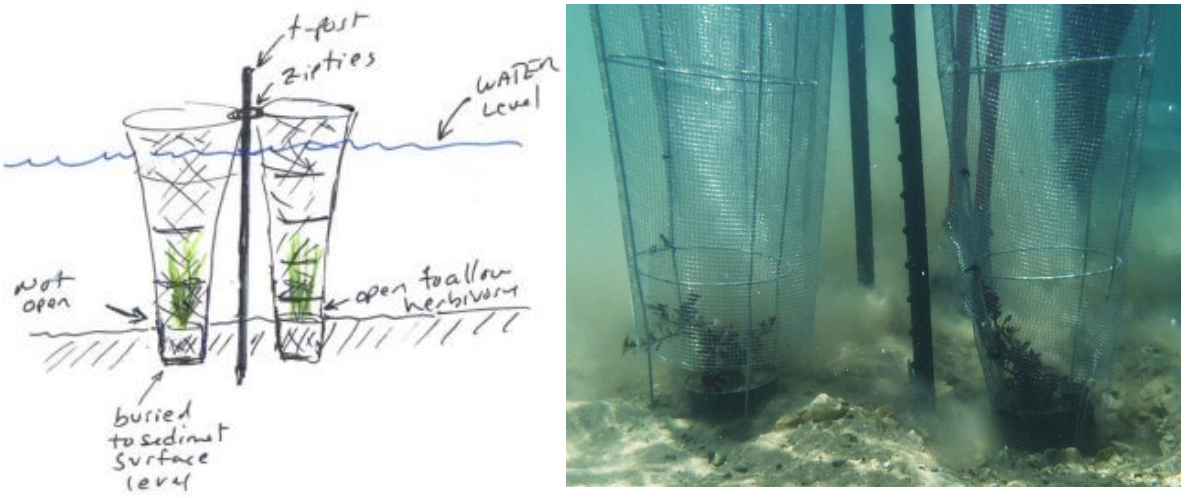

Figure 12. Depiction of exclosure cages used in the herbivory by fertility experiment. On the left is a conceptual drawing of what the cages looked like in the water, with one cage open to herbivory and the other closed. On the right is a picture of the cages after they were deployed in Landa Lake, with the cage open to crayfish on the left side and the closed cage on the right. After this picture was taken, sediment was built up along the sides of each pot to make it more level with the sediment surface.

around the plants, but "with herbivory" treatments had a 9-centimeter section near the sediment interface that was free of screening to allow for the movements of crayfish, but not the large tilapia and bass that frequent the areas nearby (Figure 12).

No top or "lid" was added to these exclosures because we were concerned that it would significantly limit the amount of light that reached the plants- the sides of the

exclosures already shaded the plants some. We considered adding a lid because of crayfish's ability to climb up the mesh sides, but ultimately decided against it. In order to monitor the light climate within the cages, two sensors measuring photosynthetically active radiation (PAR) sensors were added to cages 18 and 30 (Figure B.2). Temperature and dissolved oxygen in the water were monitored using 4 MiniDOTs (dissolved oxygen and temperature sondes) between the cages. The PAR and MiniDOT sensors were cleaned weekly over the course of the experiment to remove accumulated biofilm.

After their 30-day establishment period, the 32 *Ludwigia* plants selected for the experiment were transferred to new, hardier pots that fit better within the cage designs. These pots held about 1,100 mL, 500 mL more of sediment- from the same area the original sediment was harvested- was added to each pot. To keep the same fertilization rate in the F+ treatment plants, an additional 10 grams of fertilizer was added to the soil (bringing the total to 20 grams of fertilizer per pot). The 20 "rejects" were determined at this time, and the 12 "initials" were selected randomly from the remaining pool of plants and all 32 of these plants were brought back to Baylor to begin processing.

This experiment took place just above Pecan Island in Landa Lake and below Spring Island, near the golf course. This location was chosen for several reasons; first, it is out of the sight and reach of the public who frequent Landa Park. Second, it is easily accessible from the nearby golf course and shallow enough for the cages to emerge from the water (between 0.8 and 1 meter in depth). Third, the sediment in the area is relatively easy to dig the holes we needed for the cage pots and, most importantly, this area is bare of vegetation, and is similar to the places ecologists are trying to establish *Ludwigia*. Each experimental cage was attached to a T-post with one other cage, and two T-posts

and 4 cages made up a pod. Within every pod, there was one of each treatment (H-/F-, H+/F-, H-/F+, and H+/F+) randomly located within the group of 4, with there being 8 pods and 32 cages total (Figure B.2).

To place the cages, we first embedded a 6-foot T-post approximately two feet into the sediment. On either side of the post, we dug 6-inch deep hole and inserted the bottom of a cage. A *Ludwigia* plant in the 1,100-mL pot was then gently dropped down into the cage to settle along the bottom. If the pot was not level with the sediment surface, we built up sediment on either side of the pot to make it even. Each cage was then secured to its T-post with two zip-ties. The sediment surface of the plants fell between 80 cm and 100 cm in depth, fluctuating +/- 5cm throughout the experiment. A small label and a sign to discourage vandalism were added to each T-post in the experiment. The cages and plants were placed on August 24th, 2016 and removed on October 20th, 2016, 8 weeks later. During those 8 weeks, the experiment was monitored every 5-8 days.

After the experiment was pulled out of the river, the remaining plants from the cages were taken back to Baylor University for processing. These plants and the 12 plants used as "initials" be separated into above ground and below ground growth. Plants were thoroughly rinsed in DI water, before being weighed for an initial fresh-weight of both the above and below ground materials. The plants were then dried in a 50°C oven for one week before being weighed again for a dry-weight. Like the tissue and sediment nutrient analyses in Objective 1, the "initials" plants were then ground into a fine powder using a blender followed by one minute in a Mini-BeadBeater. To analyze carbon and nitrogen content in the samples, 1-3 mg of powder was placed in a small tin capsule. These capsules were then exposed to fuming HCl for 2 hours in a fumigation chamber in an air

hood to destroy any inorganic carbon in the sample. After this, the tins were rolled, placed in a similarly sized silver tin, rolled again, and then analyzed in an elemental analyzer. Carbon and Nitrogen content were measured simultaneously with a Thermo-Finnegan Flash 1200 elemental analyzer (ThermoQuest, Milan, Italy).

For phosphorus content, 1-2 mg were placed in a glass vial capped with a lid containing a Teflon septum, and chemically digested in an autoclave for 1 hour at 120°C by the method of Færøvig and Hessen (2003). For sample masses ≤2000 µg, 15 mL deionized (DI) water and 1.8 mL of digestion solution was used. For every 2000-µg increment, an additional 1.8 mL of digestion solution was used in place of 1.8 mL of DI water, up to a 22,000-mg maximum mass digested (19.8 mL digestion solution, no DI water). Phosphorus content was estimated via colorimetry by the ascorbic acid–molybdate method on a Lachat 8500 flow-injection autoanalyzer with an ASX-520 autosampler (Hach Co., Loveland, Colorado). Tissue standards of tomato leaf (SRM 1573a, 0.216% P), and bovine liver (SRM 1577c, 1.175% P) and dissolved inorganic standards were run for QA/QC (Back and King 2013).

The sediment in each pot was homogenized and put into two small aluminum tins each before being dried for 1 week in a 50°C oven. One sediment sample tin from each pot was put into the furnace to burn the organic content from the samples, which was then weighed to obtain the ash-free dry weight (AFDW). The other half of the "initials" sediment samples was ground up in a blender before being strained through a 1mm sieve. The sediment that could not pass through the sieve was labeled "course sediment" and set aside from the rest after being weighed. The finer sediment was then put into a Mini-BeadBeater before being weighed into tin capsules (8-10 mg) for carbon and nitrogen

analyses and glass vials (1-2 mg) for phosphorus analyses. These capsules were then exposed to fuming HCl for 2 hours in a fumigation chamber in an air hood to destroy any inorganic carbon in the sample. After this, the tins were rolled, placed in a similarly sized silver tin, rolled again, and then the carbon and nitrogen tins were analyzed in an elemental analyzer. For phosphorus content, 1-2 mg of powdered plant tissue was placed in a glass scintillation vial before being chemically digested and analyzed on a Lachat 8500 flow-injection autoanalyzer, as before.

The data from these analyses can be used in a variety of ways. First, we looked at the differences in the above-ground and below-ground biomass production by comparing the dry weights. For the "initials" plants, we also compared nutrient allocations between the fertilized and unfertilized treatments. Above-ground (AG) to below-ground (BG) ratios are commonly used to determine how an organism responded to herbivory – highly disturbed plants tend to shuttle nutrients from their roots to their stems and leaves in order to stimulate quick regrowth. The averages of these are easily tested for significant difference with a non-parametric t-test. We also compared the overall biomass of plants in each experimental treatment for both AG and BG tissues. To assess if there was a difference in survival between the H+/F+ treatments and the H+/F- treatments, we used a survivorship curve.

All data were first tested for a Gaussian distribution using the Shapiro-Wilk normality test. Each p-value presented for biomass and AG-to-BG ratios was calculated using unpaired t-tests (non-parametric and parametric as needed) and corrected for unequal variances with Welch's test when necessary. To evaluate the survivorship

curves, a log-rank (Mantel-Cox) test was used, as well as a Gehan-Breslow-Wilcoxon test that adds extra weight to hazards at early time-points.

Results

The biomass of the "reject" plants was the first data collected on this experiment. Presented in Figure 13, this data reflects the severe differences in plant growth observed in the MUPPTs. When we went to harvest the plants growing in the MUPPTs after 30-days, we were a bit surprised to observe an herbivory-by-fertility affect before the experiment really began. Many crayfish, like we usually observe, were living on our MUPPTs and consuming our plants- and consuming disproportionately. The fertilized plants were much more scrawny than the unfertilized and 4 of them were complete

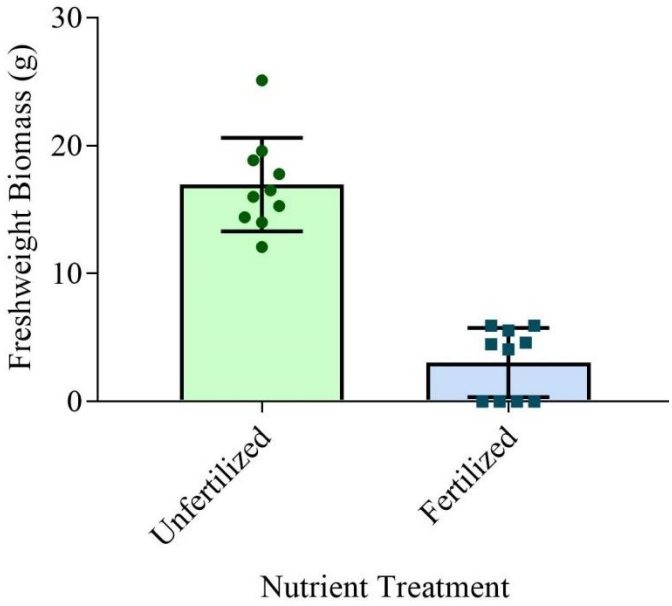

Figure 13. Mean biomass of the total plant tissue of the 20 "reject" plants with standard error bars, divided into the unfertilized (n=8) and fertilized (n=8) treatments. The mean biomass of plants in the unfertilized treatment was significantly higher ($p<0.0001$) than the biomass of the fertilized plants. The fertilized "reject" plants includes 4 pots completely devoid of plants.

gone (0 were missing from the unfertilized treatment). This effect is not likely to be due to location, as the fertilized and unfertilized plants were mixed within the MUPPTs and the dead plants were not located all in one area. Of the remaining 6 rejects, all of them were thrown out for being too small. The unfertilized plants fared better. None were dead and 4 out of the 10 were thrown out for being larger than the rest, while the remaining 6 were determined to be slightly smaller than the others. These observed differences translated to a statistically significant difference ($p<0.0001$) between the means of the fertilized and unfertilized plants (Figure 13). A Mann-Whitney test was used in place of a standard unpaired t-test, because the fertilized plant data was not normally distributed.

The biomass of the "initials" plants, which were separated into above-ground and below-ground tissues, is represented in Figure 14 and Figure 15. The means of the added above-ground and below-ground measurements are presented in Figure 14 as total biomass. The mean total biomass of the unfertilized plants was significantly higher than the mean total biomass of the fertilized plants ($p=0.0007$). The mean above-ground (AG) to below-ground (BG) ratio between the two treatments, however, was not significant (Figure 15). The graph still presents interesting data due to how the points are distributed within the overall mean; the unfertilized AG:BG were all similar to each other, while the fertilized AG:BG were scattered across a large range. We interpret these data for both the rejects and the initials as a measurement of possible herbivory during the 30-day plant establishment period.

The nutrient content of the "initials" sediment and plant tissues (separated between AG and BG) is presented in Table 3. In their soils, the two treatments had the same amount of phosphorus, but the fertilized soil had higher nitrogen content than the

unfertilized. While the fertilized plants were storing more phosphorus and much more nitrogen in their below-ground tissues than the unfertilized plants, they did not have more phosphorus in their above-ground tissues and only slightly less nitrogen. In the unfertilized treatment, the N:P ratios were pretty stable across both the above-ground and below-ground tissues, though the below-ground tissues have overall less phosphorus and much less nitrogen than the fertilized plants. The fertilized treatments also have much higher N:P ratios than the unfertilized, due to much more nitrogen in the below-ground tissues and slightly less phosphorus in the above-ground tissues.

Figure 16 shows the survivorship curves of the plants open to herbivory in the two treatments. When the experiment was checked each week, plant mortality was recorded for each cage. All of the plants in the H+ treatment were eaten or destroyed by crayfish within 26 days of the start of the experiment, with 87.5% of them disappearing within 14

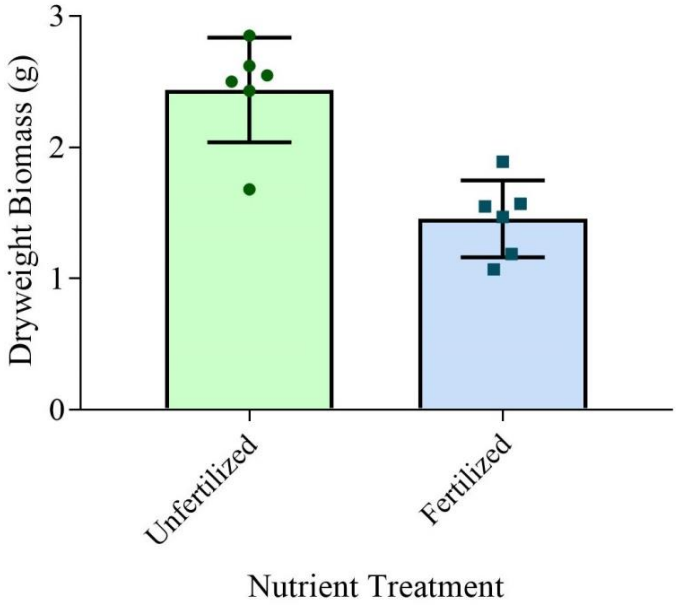

Figure 14. Mean biomass of the total plant tissue of the 12 “initials” plants with standard error bars, divided into the unfertilized (n=6) and fertilized (n=6) treatments. The mean of the unfertilized treatment was significantly higher than the fertilized treatment (p=0.0007).

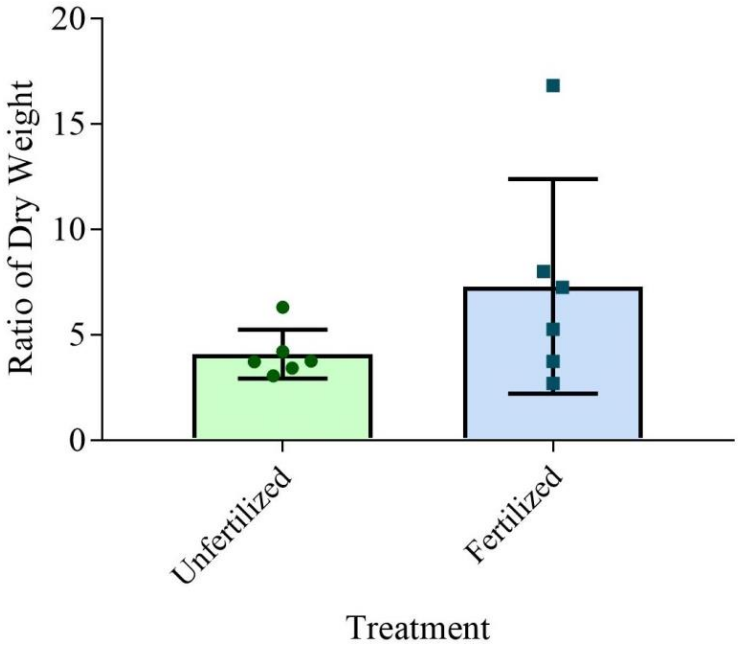

Figure 15. Mean ratio of above-ground plant tissue biomass to below-ground plant tissue biomass of the 12 "initials" plants with standard error bars, divided into the unfertilized (n=6) and fertilized treatments (n=6). The mean above-ground to below-ground ratio of the two treatments was not significantly different ($p>0.05$).

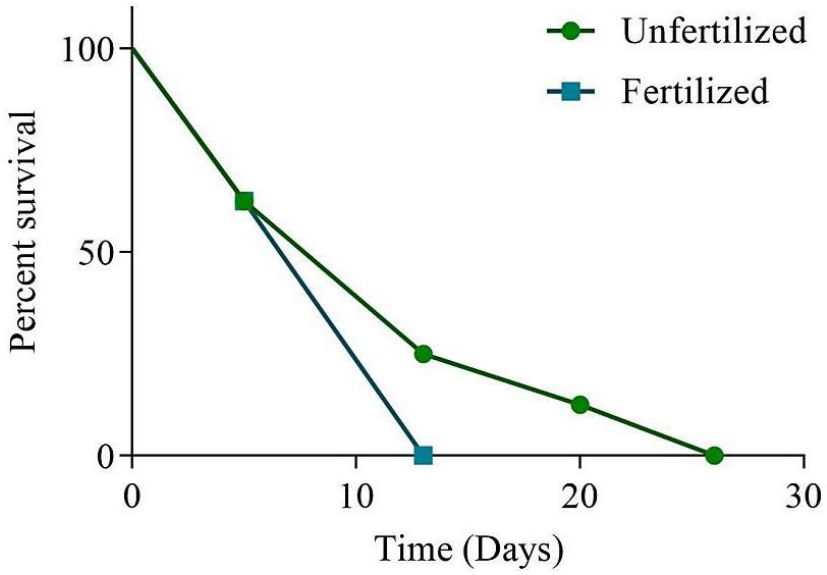

Figure 16. Percent survival by day of the plants in the H+ treatment, divided into the fertilized and unfertilized plants. The curves of each treatment were not significantly different ($p>0.05$).

Table 3. Nutrient concentration data collected from the 12 “initials” plants, divided into the fertilized and unfertilized treatments. Phosphorus (P), nitrogen (N), carbon (C), and the nitrogen to phosphorus ratio (N:P) are reported, as well as biomass in grams of the above-ground and below-ground plant tissues. All concentrations are in µg/mg, and the N:P ratios are the ratio of this concentration (to obtain percent of each, divide by 10).

		Sediment Nutrients			Plant Above Ground Tissue				Plant Below Ground Tissue			
	Plant	P	N	N:P	P	N	N:P	Biomass (g)	P	N	N:P	Biomass (g)
Fertilized	1	0.54	2.49	4.66	1.05	18.91	18.00	1.00	2.07	38.35	18.57	0.19
	2	0.58	2.51	4.34	1.07	19.29	18.07	1.16	1.55	28.49	18.32	0.31
	3	0.43	2.19	5.11	1.21	20.36	16.89	1.13	1.31	21.32	16.26	0.42
	4	0.62	3.22	5.20	1.09	20.06	18.33	1.01	1.16	27.82	23.97	0.06
	5	0.54	2.02	3.73	1.09	18.56	17.10	1.68	1.47	31.19	21.27	0.21
	6	0.47	2.23	4.76	1.07	17.84	16.73	1.38	1.32	26.89	20.40	0.19
Average		0.53	2.44	4.63	1.09	19.17	17.52	1.23	1.48	29.01	19.80	0.23
Unfertilized	1	0.46	1.78	3.86	1.20	15.74	13.07	2.02	1.06	12.97	12.24	0.48
	2	0.49	1.70	3.49	1.22	18.08	14.82	1.83	1.08	12.75	11.83	0.60
	3	0.69	1.74	2.51	1.34	17.66	13.21	2.01	0.81	11.81	14.51	0.54
	4	0.44	1.57	3.60	1.34	17.18	12.85	2.07	1.11	15.32	13.75	0.55
	5	0.61	1.63	2.68	1.66	20.00	12.02	2.46	1.15	12.26	10.65	0.39
	6	0.69	1.69	2.45	2.07	18.94	9.17	1.30	0.87	9.83	11.35	0.38
Average		0.56	1.68	3.10	1.47	17.93	12.52	1.95	1.01	12.49	12.39	0.49

days. The percent survival of each week is presented, separated into the unfertilized (H+/F-) and fertilized (H+/F+) plants that were in cages open to herbivory. Neither the log-rank (Mantel-Cox) test nor the Gehan-Breslow-Wilcoxon test used yielded a significant p-value. Even though the curves were not statistically significantly different from each other, we believe the graph provides valuable observations. The unfertilized plants lasted longer than the fertilized and overall looked healthier (less visibly eaten).

Additionally, the data points were only collected once a week, so there is a fair chance that this is not the most accurate representation of the order in which the plants disappeared. However, crayfish managed to climb up the sides of the cages to live in and eat the H- *Ludwigia* plants as well. Beginning in week 5 of the experiment, a few of the H- plants appeared to be losing small amounts of biomass. By week 8, when the experiment was collected, many of the plants were noticeably fed on. At the time of collection, at least 1 juvenile crayfish (*Procambarus clarkii*) was found in each cage (Figure B.3). Most cages held 1-2 very small, young crayfish living in the plants- no adult crayfish were observed, and no cage held more than 2 live and 1 dead crayfish. Because of this, we actually ended up with an herbivory-by-fertility experiment, despite the fact that all of our intentional H+ treatments were demolished early on.

Figures 17 and 18 show the biomass results of this sixteen plants that were originally H- (the only surviving at the end of the experimental period). The mean total biomass of each treatment is presented in Figure 17. The result of the Welch's t-test preformed was highly significant ($p=0.0082$), confirming our observations that the unfertilized plants were larger at the end of the experiment than the fertilized plants. The mean above-ground (AG) to below-ground (BG) ratio for each treatment is shown in

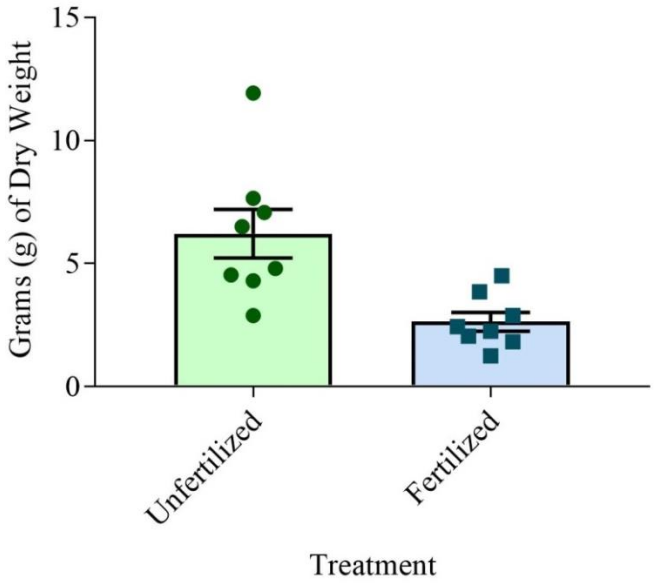

Figure 17. Mean biomass of the total plant tissue of the 16 experimental plants with standard error bars, divided into the unfertilized (n=8) and fertilized treatments (n=8). The mean total biomass of plants in the unfertilized treatment was significantly higher (p=0.0082) than the mean of the fertilized plants.

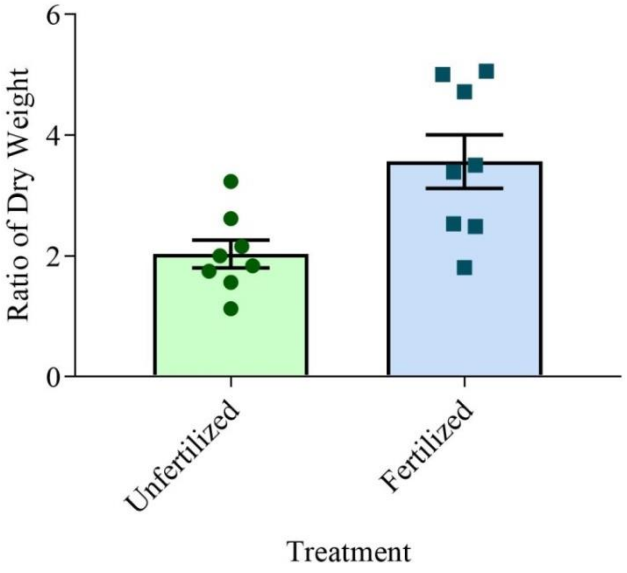

Figure 18. Mean ratio of above-ground plant tissue biomass to below-ground plant tissue biomass of the 16 experimental plants with standard error bars, divided into the unfertilized (n=8) and fertilized (n=8) treatments. The mean above-ground to below-ground ratio of the unfertilized plants is significantly lower (p=0.011) than that of the fertilized plants.

Figure 18. The unfertilized treatment had a lower overall AG:BG than the fertilized treatment ($p=0.011$). The differences in mean below-ground biomass were greater (Welch's t-test, $p=0.0003$) than the mean above-ground biomass (Welch's t-test, $p=0.032$), though both were statistically significant.

Discussion

This experiment presented many surprises throughout its duration. At its start, the blatant visual difference in the fertilized and unfertilized treatments of the plants grown in the MUPPTs was unexpected. Before we had completed setting up the cages in the lake, crayfish were observed exploring our plants. After the plants were in the cages for a week, we returned to check on them to discover that 6 of the 16 plants open to herbivory (H+) were completely gone and many of the remaining plants were visibly disturbed. When we returned on the second week, 87.5% were gone, and by day 26 every single H+ plant had disappeared. At week 4 of the experiment, we also observed an adult *Procambarus clarkii* scaling the side of one of the cages and, while we had our reasons for not adding a lid to the cages (pp. 53-54), it became apparent that not only could the crayfish climb up our cages, but that they would. By day 32 of the experiment, we began to see signs of herbivory on the H-, fully enclosed plants- including loss of leaves, bare stems, and floating plant fragments. The above-ground growth of the H- plants slowly began to visually decline from weeks 5-6 until the experiment was removed from the river at week 8. At the time of removal, all H- plants (8 H-/F- and 8 H-/F+) were still alive, but each plant had at least 1 small crayfish present in the cage with it. When the plants were brought back to the lab for processing, there was a noticeable difference in the sizes of the unfertilized and fertilized plants.

This sequence of events was not what we had expected. When we designed the experiment, we had no idea the herbivory would be as intense as it was. We expected the fertilized plants to be bigger going into the experiment than the unfertilized. Instead, they went through varying stages of biomass production throughout this process. They started in the cages smaller than the unfertilized plants, appeared to catch up to approximately the same above-ground biomass as the others around week 4, and then ended the experiment significantly smaller than their unfertilized counterparts. Additionally, if our H-cages had completely excluded crayfish as we had intended, we would not have observed an herbivory effect in our experiment, since the crayfish destroyed every H+ plant regardless of treatment. So although our cages failed their intended purpose, they failed in an informative manner.

We set out to answer the three questions outlined at the beginning of this chapter. The first question- whether or not herbivory impacts *Ludwigia* biomass- was effectively answered by our study. The *Ludwigia repens* plants in our study were certainly impacted by herbivory, which we suspect was primarily due to crayfish. Initially, there was concern that there would not be a large enough herbivory response to make a difference in our H+ and H- treatments, and we had considered creating enclosures to control the crayfish density, ensuring that crayfish would be present at our study area. However, as the point of this research was to inform restoration managers conserving the Comal ecosystem, we decided to keep as many factors as natural as possible. We realized our concerns were unfounded when we pulled our *Ludwigia* from the MUPPTs after are initial growing period. Figures 13 and 14 give a fairly good idea of what we saw when we did so; the unfertilized plants were bushy and flourishing, while the fertilized plants were

scrawny, chewed up, and in some cases completely dead. After beginning the experiment, we ended up with the opposite problem than we originally expected- we had *too large* of an herbivory response. Within two weeks of deployment, 14 out of the 16 H+ plants- 87.5%- were completely destroyed. The clipping behavior remarked upon in Chapter One was observed at every single plant in the experiment (Figure B.3). The differences in total biomass of the initials and the reject plants, as well as the total disappearance of every H+ plant, means that herbivory does significantly impact *Ludwigia* biomass.

Our second question- if soil fertility alone impacts *Ludwigia* biomass- was much less clear. Our experiment did not effectively answer this question because there was unplanned interference by herbivory, both when growing the initials and after the experimental cages were invaded by crayfish partway through. In order to address this question, we will need to run a separate experiment- likely in the laboratory- that will completely exclude intense herbivory. Our hypothesis would be that soil fertility would aid in *Ludwigia* biomass production, since the water column (Table 1) and likely the soil in the Comal (Table 2) is phosphorus limited, but we cannot tell from this experiment alone.

The last question- if there is an interaction effect between soil fertility and herbivory on *Ludwigia* in the Comal- was answered here as well. The initials and the reject plants displayed the interaction effect before the proper experiment even began (Figures 13 and 14), and Figures 17 and 18 show the same effect at the conclusion of the experiment. Soil fertility appears to have a negative effect on *Ludwigia* plants' susceptibility and tolerance to herbivory- at least when that herbivory is mainly crayfish-induced. In the "initials" we measured and the surviving experimental plants, the

unfertilized plants had significantly more biomass (Figures 14 and 17) and the fertilized experimental plants had significantly higher above-ground to below-ground biomass ratios- an indication of reduced tolerance. This result was not wholly unexpected, as crayfish have been documented to preferentially feed on more nutrient-rich plants (Cronin et al. 2002; Miller and Provenza 2006), however the intensity of herbivory was.

Based on field observations, ecologists in working in Landa Lake have suspected herbivory to be hindering some of the *Ludwigia* restoration efforts, but they were unsure if it really made a difference (BIO-West, Inc. 2015a). After herbivory destroyed an *in situ* experiment in 2015 (Chapter One, pp. 6-7), we decided it would be worth the effort to quantify how herbivory is affecting our planted *Ludwigia*. It appears that herbivory may be a bigger issue to restoring macrophytes in the Comal- specifically Landa Lake and the Upper Spring Run- than previously thought.

The behavior of crayfish to clip plants at the water-sediment interface is particularly destructive and can make whole *Ludwigia* plants disappear in a very short time period. Theories as to why crayfish may do this are presented in Chapter One (p. 7) and can include territorial behaviors, herbivory efforts, and even helpful to their carnivory. Whatever their reason, it is certainly detrimental to restoration efforts. To be certain crayfish are capable of destroying our plants, at the end of the experiment we brought two small, juvenile crayfish found in our cages back to the lab. These young *P. clarkii* were put into separate aquaria- one 20 gallon tall and one 10 gallon- filled with planted *Ludwigia repens* and water from Landa Lake and then observed for 6 weeks. After 3-4 weeks, the crayfish in the 10 gallon aquarium had clipped every one of the plants present (Figure B.4). The crayfish in the larger aquarium had clipped many of its

own plant stems, but appeared to focus on stripping whole leaves off of the stems- every plant in its aquarium was bare of leaves by week 4. This seems to confirm our suspicions that crayfish are capable of causing the damage observed in our experimental plants. These crayfish also molted twice each within the 6 week period we were observing them and grew rapidly, adding merit to our thoughts that they were very young individuals when we discovered them in our cages at the end of our experiment.

At week 5 of the experiment- after we had observed that every H+ plant had been destroyed- we decide to do a short, observational experiment to see if crayfish would destroy any plants we put out in that area. So we planted 16 plants that were approximately uniform in size into the soil near our ongoing cage experiment and added a roughly constructed cage of the same ¼" wire mesh around 8 of these. After 3 weeks we removed the cage and visually compared the plants' above-ground growth (a visual comparison is presented in Figure B.5). The uncaged plants were overall smaller than the caged and they had noticeably lower numbers of leaves on their stems. The caged plants fared better and appeared much more robust. (Note: after researchers returned two months later, all the planted *Ludwigia* from this short exercise was gone.) This suggests that it may benefit restoration managers to construct temporary cages around newly plant *Ludwigia* groups in Landa Lake to allow them time to establish before being exposed to herbivory.

One key aspect of herbivory tolerance that our experiment did not consider was genetic variability. Tolerance to herbivory has been shown by quantitative genetics to be a heritable trait (Fornoni 2010). Many of the *Ludwigia* fragments we used to plant the MUPPTs at the start of this experiment were taken from the same few established plants

nearby in the river, but the identity of the mother plants for each fragment was not recorded.

If this experiment was to be repeated, it would likely be of benefit to alter a few things. The density of crayfish would need to be controlled (and be lower than what we found to be natural) or lids should be added to the cages; additionally, this experiment could be run for a shorter amount of time. We would also need to remove all threats of herbivory in order to quantify how much soil fertility impacts *Ludwigia* biomass in the Comal- our second question. Future directions of this research could include an experiment determining how the size of the colony planted effects *Ludwigia* plants' ability to survive the intense herbivory. Moore et al. (2010) noted that large founder colonies may increase the plants' abilities to withstand herbivory, but that the potential effects of size and density of these colonies on reducing herbivory pressures is not well understood. It may also benefit restoration efforts to determine if some *Ludwigia repens* individuals in the Comal are better at withstanding herbivory than others, by virtue of their genetic adaptations.

In conclusion, it appears that herbivory may pose a much greater threat to *Ludwigia* restoration efforts in Landa Lake and the Upper Spring Run than previously assumed. When restoring plant communities in this system, not much thought has been given to how herbivores- and crayfish specifically- may be affecting the new plant communities. Temporarily caging these new colonies may help, but, according to this experiment, fertilizing the soil will be detrimental to the plants' herbivory tolerance.

CHAPTER FOUR

Dissolved Oxygen Dynamics of Landa Lake

In 2013, the Comal River had lower than average flow conditions, with the average monthly discharge ranging from 124 cubic feet per second (cfs) to 223 cfs. In 2014, the monthly mean discharge declined even further, to range from 80 cfs to 169 cfs, resulting in one of the lowest discharge conditions observed in the Comal in nearly 30 years (BIO-West, Inc. 2015b). During each of these summers, green filamentous algae increased in cover across the river and eventually became dominant as flows decreased. Possibly because of these algae coating the plant life along the river bottom, the upper spring run (USR) and Landa Lake saw significant decreases in bryophyte cover and some reduction macrophyte cover. As these filamentous algae died and floated to the water surface, floating vegetation mats increased in size and number. Other organic debris, both terrestrial and aquatic, began to accumulate in these mats and continued to do so as flows and water level decreased in Landa Lake over 2013 and 2014. The spring of 2015 brought with it substantial improvements in spring flow and discharge rates, with mean monthly discharge ranging from 154 to 386 from January through June (Figure C.1). Recent restoration of the Comal has played an important role in improving the overall robustness of the river in times of drought, however, the inevitable return of drought to South Texas and the resulting low-flow will no doubt cause the conditions witnessed from 2013 to 2014 to reoccur.

In light of these low flow conditions, and the fact that water in Landa Lake is likely to experience low dissolved oxygen due to the springs being isolated from atmospheric gas exchange, the experiment here was composed of two main parts: first, we characterized the spatial variability in dissolved oxygen (DO) across Landa Lake and the USR in a short study. In the second part, we studied how DO varies vertically in the water column. Our dissolved oxygen data presented here was collected in 2015 and will be able to represent DO under normal or good-flow conditions, so that when drought returns to the river, DO in the area can be reassessed and compared against this experiment.

Methods

Information on the spatial variation of dissolved oxygen (DO) in Landa Lake has rarely been collected. Currently, diel DO is only measured on a continuous basis at one location around mid-lake, and the data collected here have been used to make management decisions for the whole of Landa Lake (BIO-West, Inc. 2015b). To better understand the spatiotemporal distribution of DO, we measured DO and the corresponding water temperature at 14 locations in Landa Lake and Upper Spring Run (USR) from July 28, 2015 to August 5, 2015. DO measurements were collected with multiple MiniDOT DO sensors available from Precision Measurement Engineering (PME Inc. Vista, CA). These sensors utilize optical fluorescence technology and have been widely used by the United States Geological Survey across the United States. Before their deployment, the sensors were tested against the YSI brand data sonde (Xylem Inc.) by logging data of both instruments simultaneously through various stages of air-saturation and anoxia. The MiniDOT measurements were found to be equivalent to a YSI sonde

utilizing similar optical technology, as MiniDOT values were within a few percent of the YSI values at all measurement periods.

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

Measurement locations for the 8-day evaluation were selected during a reconnaissance investigation in late June. At this time, the Habitat Conservation Plan (HCP) aeration project water quality probe was in place and operating (a small part of the overall Edwards Aquifer Authority's restoration plan). We chose measurement sites in a variety of locations throughout Landa Lake to incorporate differing habitat types (deep, shallow, with current, stagnant, vegetated, non-vegetated, etc.) and to capture the wide range of conditions present throughout Landa Lake and the USR (Figure 19); Table 4 provides a description of the chosen sites. This study began on July 28, 2015 and

Figure 19. Locations of the 14 MiniDOT dissolved oxygen sensors during the initial, short-term spatial evaluation study.

continued for one week, after which the data from each sensor was downloaded. During this period, the total discharge in the Comal River ranged from approximately 300 to 340 cfs. From a historical perspective, this is slightly above the long-term historical average, which itself is considerably different from the very low-flow (65 cfs) experienced during the summer of 2014. The MiniDOTs were deployed at approximately 60% of water depth. At 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, and then tied to an anchor with a length of rope that allowed it to float at the appropriate depth. At each station, total water depth was measured along with water velocity at 20%, 60%, and 80% of the total depth. Vegetation type around the sensor and a GPS waypoint were also recorded for each MiniDOT.

The MiniDOTs were cleaned after 3 days of deployment in the lake, and at the end of the 8 days, data was downloaded from the sensor. To download the data, the sensors were first collected from the water, wiped dry, 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, and then wiped clean before being returned to the MiniDOT. The other 10 sensors, which were a year newer, were downloaded via a USB cable that was plugged directly into the sensor from the YUMA.

Building on the results of the 8-day spatial dissolved oxygen survey, we implemented a longer monitoring project (August 6, 2015 through October 6, 2015) at six locations in Landa Lake and the USR. We deployed the sensors so that, in addition to monitoring horizontal spatial variability like before, we could also compare variability at

Table 4. Descriptions of the 14 MiniDOT sensor locations in Landa Lake. Sensors were deployed at approximately the 0.6V depth.

Station #	Description	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

a vertical scale 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 are extremely important for the Comal River, since fountain darters almost exclusively live along the river-bottom, although DO is rarely measured there. A variety of vegetation types were included across the locations as well; the dominant macrophyte species in Landa Lake include *Vallisneria neotropicalis* (Vallisneria) and *Sagittaria platyphylla* (Sagittaria). Thick bryophytes- mostly composed of *Riccia fluitans*- were present along the sediment surface at some locations, aquatic macrophytes were present in others, and, in a few locations, vegetation was absent entirely (attributes of each location are provided in Table 5). Vegetation mats accumulated regularly on the surface of Landa Lake before and during this study.

Four MiniDOTs remained at previous sampling locations used in the initial one-week study in order to continue long-term data collection at these sites. Two large floating vegetation mats were identified and labeled as "Mat #1" and "Mat #2." Within each mat, two MiniDOTs were deployed, one on top of the other (totaling 4 sensors within vegetation mats), and another set of two sensors was deployed just outside of each mat (totaling four sensors considered to be outside a mat). Another pair of sensors was located at the HCP aerator deployed in the middle of Landa Lake (Figure 20). At these paired-sensor locations, a t-post was driven into the sediment and the sensors attached to the t-post; the top sensor was typically deployed 20-30 cm below the water surface, while the bottom sensor was deployed 2-10 cm from the sediment surface, depending on

Table 5. Attributes of MiniDOT locations selected for the prolonged DO 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, MiniDOT Depth	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 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. 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. Nuphar and Cabomba. Sensor at 20-30 cm below surface. Very rapid biofouling!	0.2V= 0.03	62	58	0.86

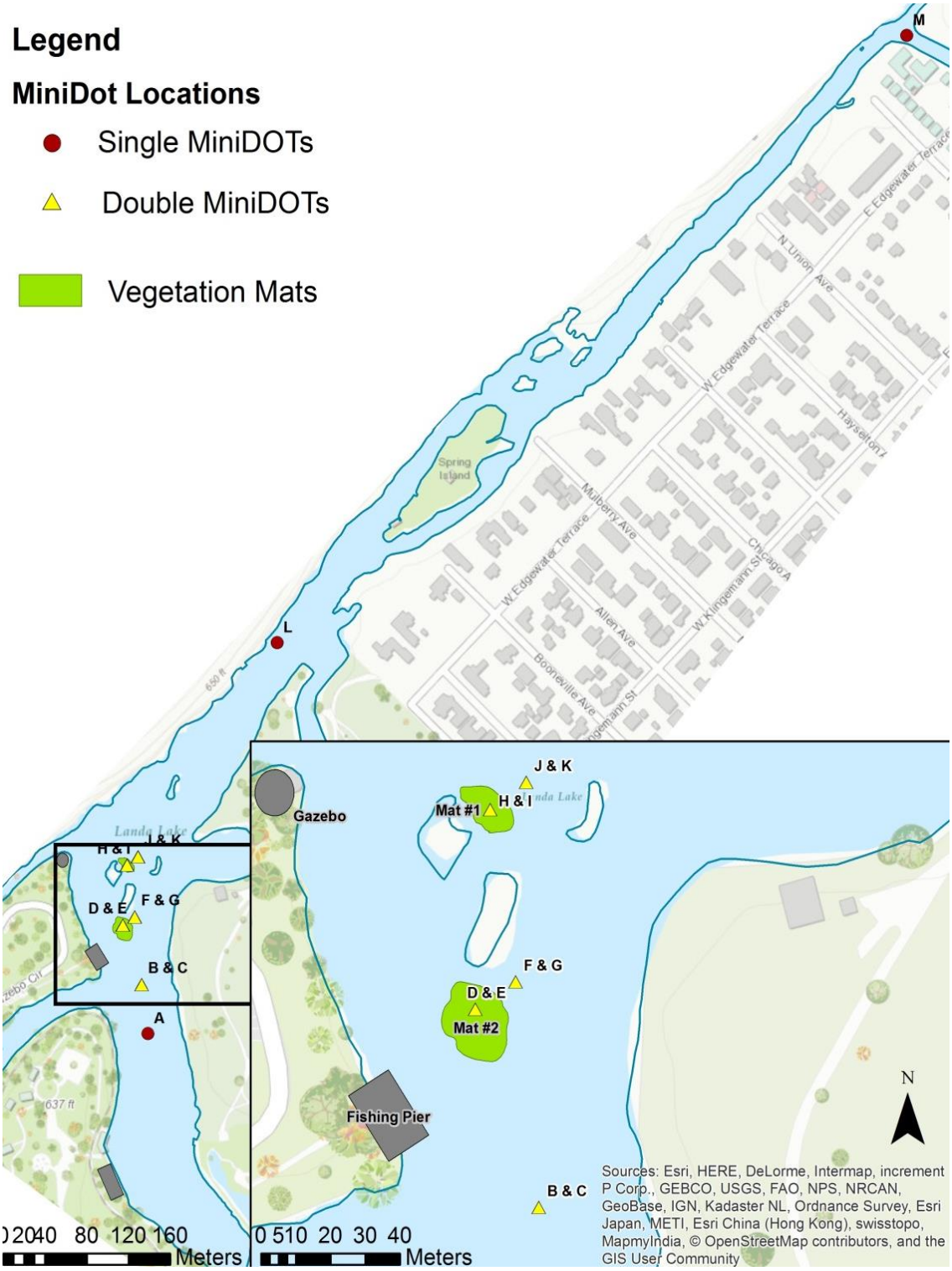

Figure 20. Long-term deployment locations of MiniDOT sensors. Two pairs were located inside floating vegetation mats (green polygons). The yellow triangles represent locations with two MiniDOTs, while the red circles represent locations with a single MiniDOT. presence or absence of photosynthetic benthic communities (most commonly, thick

bryophyte turf). Three stations received only one MiniDOT (Figure 20). The MiniDOTs were placed in a horizontal position, like before, to limit biofouling and were cleaned regularly after deployment (every 3 to 5 days); data was downloaded once per week. Over the course of this study, three of the MiniDOT sensors failed; human tampering flooded the internal components of one MiniDOT, while 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 and re-checked under air-saturated and anoxic conditions. All remaining sensors showed excellent performance.

Results

Short-term dissolved oxygen study. The results from the first DO study, the week-long spatial study, are presented in Figure 21. A solid red line was placed on each chart at 4.0 mg/L dissolved oxygen, representing the minimal DO goal set forth by the Edwards Aquifer Habitat Conservation Plan; survival of fish may be threatened at DO levels less than 4 mg/L (EARIP 2011). In general, diel DO ranged between approximately 4.0 and 9.0 mg/L, with the exception being backwater areas- such as stations 8, 12 and 14- where there is less flow. 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. This MiniDOT was located in a shallow, low-flow area surrounded by *Nuphar*, so low DO levels were not surprising. This area likely provides a glimpse of what might be expected when total system discharge conditions are considerably lower, like they are during drought, causing pockets of very low-flow.

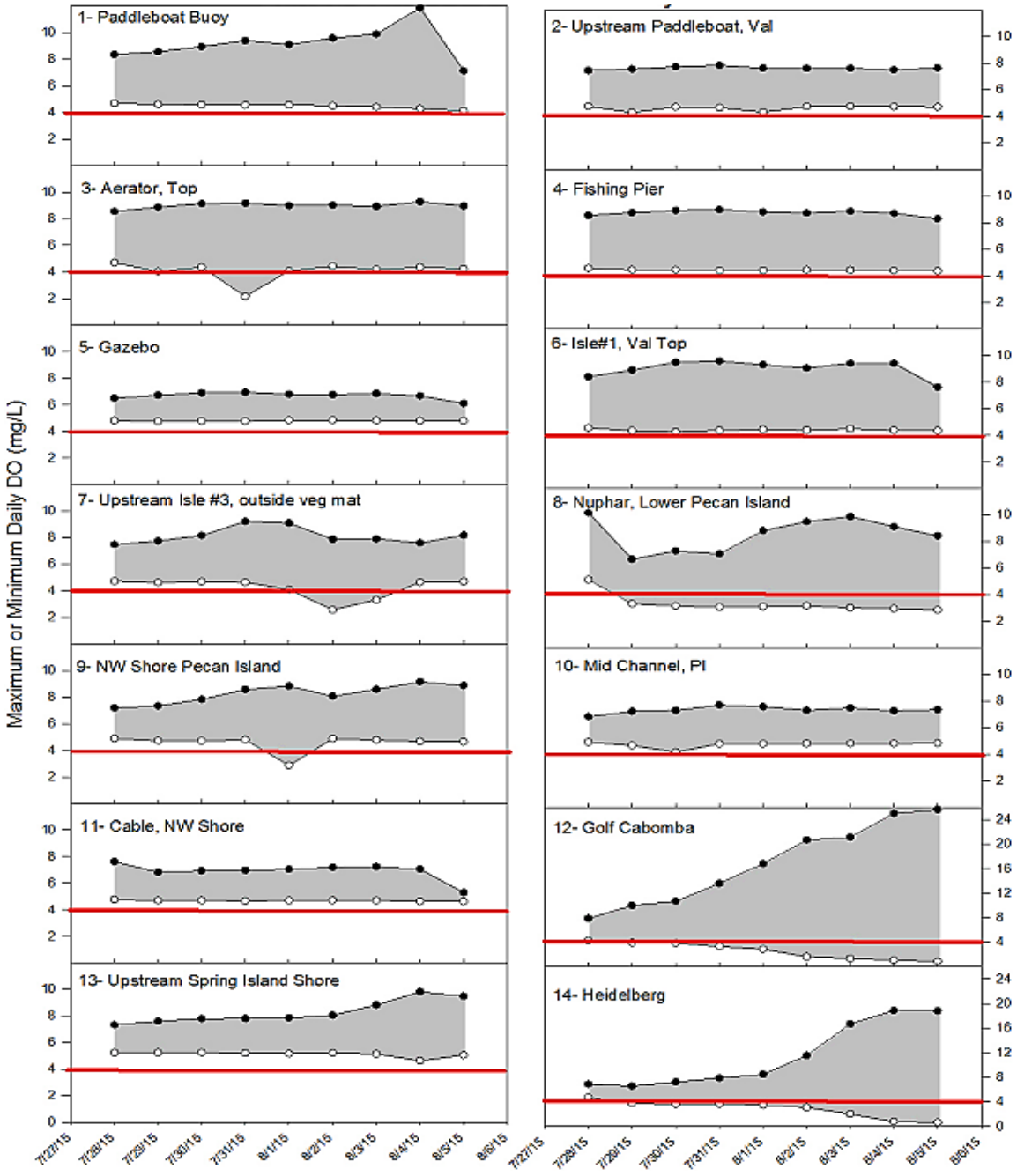

Figure 21. Maximum and minimum dissolved oxygen results (mg/L) from 14 MiniDOT sensors used during the spatial evaluation study (07/26/2015 to 08/05/2015). Data at stations 8, 12, and 14 were heavily affected by biofouling.

The MiniDOTs at sites 12 and 14 were also located in areas with low-flow would likely have experienced DO levels less than 4.0 mg/L similar to station 8 and on a daily basis. Though these conditions were experienced, biofouling of the MiniDOTs here occurred rapidly causing both extremely high and low measurements of DO, despite regular cleaning efforts (Figure 21). Further investigations later in the summer and fall improved our confidence that bio fouling was the culprit of the extreme DO levels at these locations. Because of this, we are not confident in the DO measurements reported in Figure 21 for these stagnant sites after more than two days of cleaning.

Although we had anticipated the 2014 drought conditions to continue into 2015, low-flow conditions did not continue into the summer of 2015. One large flooding event occurred in May of 2015 that increased the flow of the river significantly, so this spatial data represents what is to be expected during good and normal-flow 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.

Long-term dissolved oxygen study. The daily maximum and minimum DO recorded by each sensor at each of the locations is presented in Figure 22. The differences in diel DO between the top and bottom of the water column are notable, as DO measurements are often taken at the top of the water column, but fountain darters are much more impacted by dissolved oxygen conditions at the bottom of the water column. Five of our locations had MiniDOTs deployed at the top and at the bottom of the water column (Aerator station, inside mat 1, outside mat 1, inside mat 2, outside mat 2).

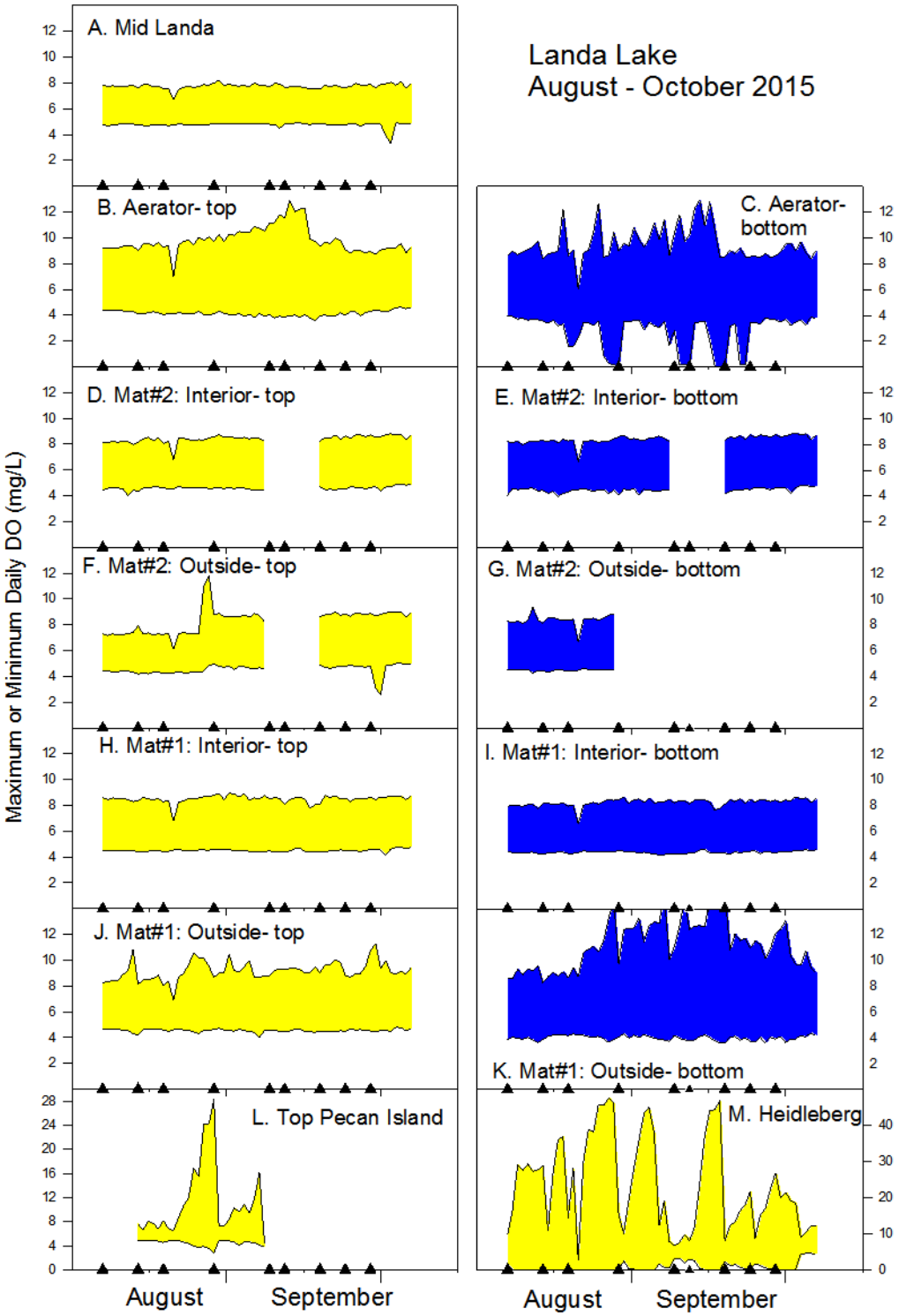

Figure 22. Maximum and minimum dissolved oxygen levels (mg/L) from MiniDOT sensors during long-term deployment at 13 locations across Landa Lake and the USR. Dates when sensors were serviced are shown as triangles along the date timeline. Yellow indicates sensors near the top or in the middle of the water column and blue indicates sensors located near the bottom.

The aerator 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 22, 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), though the magnitude of this difference was relatively modest (usually <1.0 mg/L higher). The overnight minima were lowest 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 5). 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 4.0 mg/L goal. During five separate periods the overnight lows were very low (< 1 mg/L, Figure 22, C), which are likely 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. The physical presence of the t-post and sensor may have facilitated this phenomenon.

The stations located within the floating vegetation mats were set at the approximate center of two selected mats, with MiniDOT sensors deployed at the top of the water column and the sediment-water interface. Two other locations were set outside and slightly upstream of these vegetation mats, again with two MiniDOT sensors deployed towards the top and bottom of the water column (Figure 20). These floating vegetation mats were primarily composed of bryophytes and algae, with some macrophyte pieces and terrestrial debris accumulation. The dominant vegetation beneath

both of the vegetation mats was *Vallisneria,* which filled the entire water column. The outside station was at the interface of the *Vallisneria* and an adjacent *Sagittaria* bed, where bryophytes heavily colonized between the plants and unvegetated areas.

At the station outside of Vegetation Mat #1 (Figure 22, J and K), the overnight minima at the top of the water column (Figure 22, J) averaged 4.55 mg/L over the 62 nights and never had an overnight minima below 4.0 mg/L DO (Table 5). The bottom sensor at this station recorded DO levels just under 4.0 mg/L on 29 of the 62 days of deployment, putting the average overnight DO at 3.99 mg/L. The absolute minimum DO recorded was 3.59 mg/L, and severe DO depletion (below 2 mg/L) was not observed at this location. At the top, daily maximum DO ranged from 7-10 mg/L (Figure 22, J), while at the bottom maximum values were often in the 10-13 mg/L range (Figure 22, K). Dissolved oxygen 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 fairly muted (Figure 22, 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, respectively.

The daily maxima and minima recorded at the top and bottom MiniDOTs within Vegetation Mat #1 are shown in Figure 23. These same data are shown in Figure 22 (H, I, J, K), but are plotted here to allow easier comparison of data between the inside the outside of the mats. Here, daily maxima were consistently higher outside the mat- at both the top and bottom of the water column (Figure 23). The difference in DO maxima were

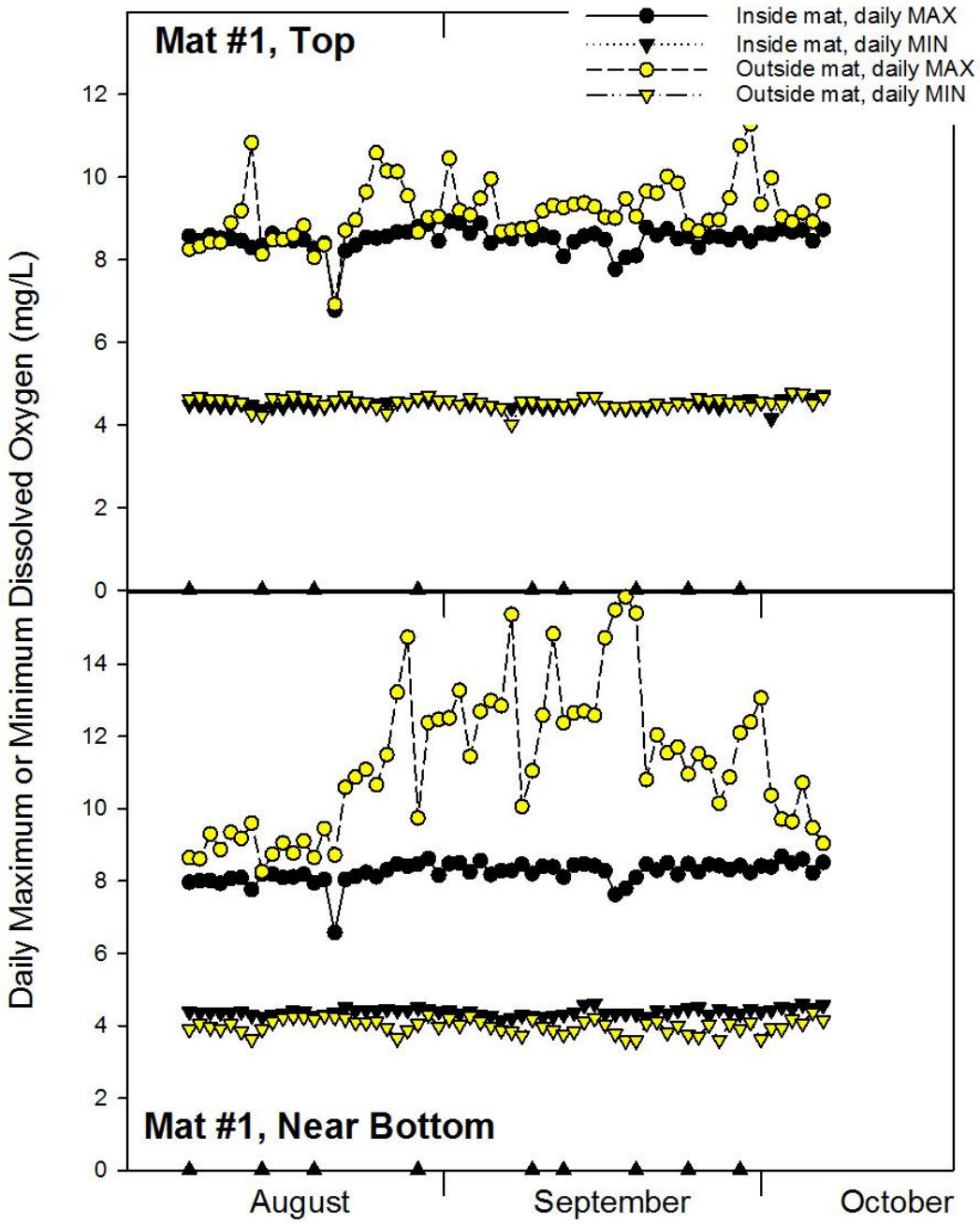

Figure 23. Daily maximum and minimum dissolved oxygen levels at Vegetation Mat #1. The top panel shows data from the top of the water column, while the bottom panel shows data for the sensor near the sediment surface. Yellow symbols are used for sensors outside the mat and black symbols for sensors inside the mat. Circle are used for sensors at the top of the water column while down triangles are used for the near-bottom sensors.

particularly pronounced near the bottom of the water column, which was likely due to the presence of an active bryophyte community outside the vegetation mat. However, daily minima at the top of the water column were virtually identical, and overnight minima at the bottom of the water column were actually slightly lower outside the mat than under the mat (likely due to the bryophyte community as well).

Vegetation Mat #2 was located just downstream of the three islands in Landa Lake and immediately in front of the fishing pier (Figure 20). This mat was composed of decomposing macrophytes, bryophytes, algae and terrestrial vegetation. The MiniDOTs were deployed in an identical manner to the first vegetation mat, and the dominant vegetation both inside and outside the mat was *Vallisneria*. At the location outside of Vegetation Mat #2, overnight minima at the top of the water column was usually well above 4.0 mg/L and averaged 4.57 mg/L over the 62 days (Figure 22, F). The two days with minimum DO levels 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, but after this the MiniDOT sensor was moved to allow continued monitoring at other locations after the sensor there was damaged (Figure 22, G). Minimum dissolved oxygen levels were never below 4 mg/L here at the bottom and averaged 4.51 mg/L.

The sensors deployed within Vegetation Mat #2 (Figure 22, D and E) showed a muted pattern similar to DO readings at the top and bottom of mat #1. Dissolved oxygen below 4.0 mg/L was recorded only once at each position, and both values were just barely below the level of concern (>3.9 mg/L). Daily maxima were typically just above

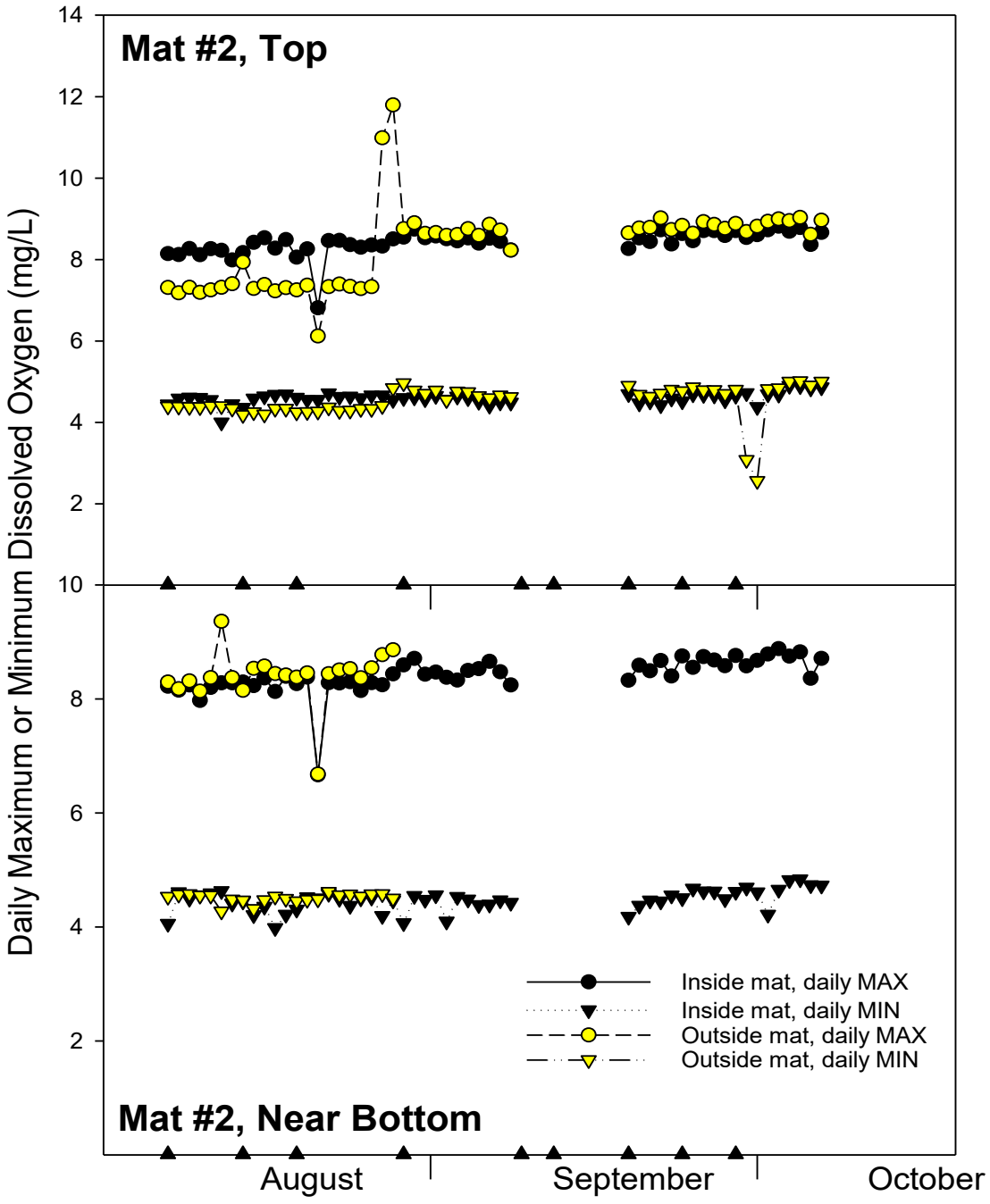

Figure 24. Daily maximum and minimum dissolved oxygen levels at Vegetation Mat #2. Top Panel shows data for the top of the water column, while the bottom panel shows data for the sensor near the sediment surface. Yellow symbols are used for sensors outside the mat and black symbols for sensors inside the mat. Circle are used for sensors at the top of the water column while down triangles are used for the near-bottom sensors.

8.0 mg/L; daily DO maxima and minima at the top and the near bottom at Vegetation Mat #2 are shown in Figure 24. These same data are shown in Figure 22 (D, E, F, G), but are re-plotted here to allow for easier comparison. 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 almost identical (Figure 24, top panel). Towards the end of August, the MiniDOT positioned at the bottom outside the mat was moved 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, making the maxima higher. Daily maxima and minima at the bottom of the water column (Figure 24, bottom panel) were virtually identical throughout the sampling period.

Water temperatures remained constrained between 23 and 25 °C at all stations except Heidelberg (location #14, MiniDOT M), which exceeded 25 °C initially in August (Figure C.3). Temperature data showed no significant differences between locations (top versus bottom; inside mat versus outside mat), indicating waters were well mixed throughout the water column as well underneath floating vegetation mats (Figure 25).

Discussion

The main areas of concern indicated by the prolonged dissolved oxygen monitoring in 2015 are low-flow locations with dense vegetation. These include the thick *Nuphar luteum* stand at the bottom of Pecan Island (Figure 21, Station #8) and the

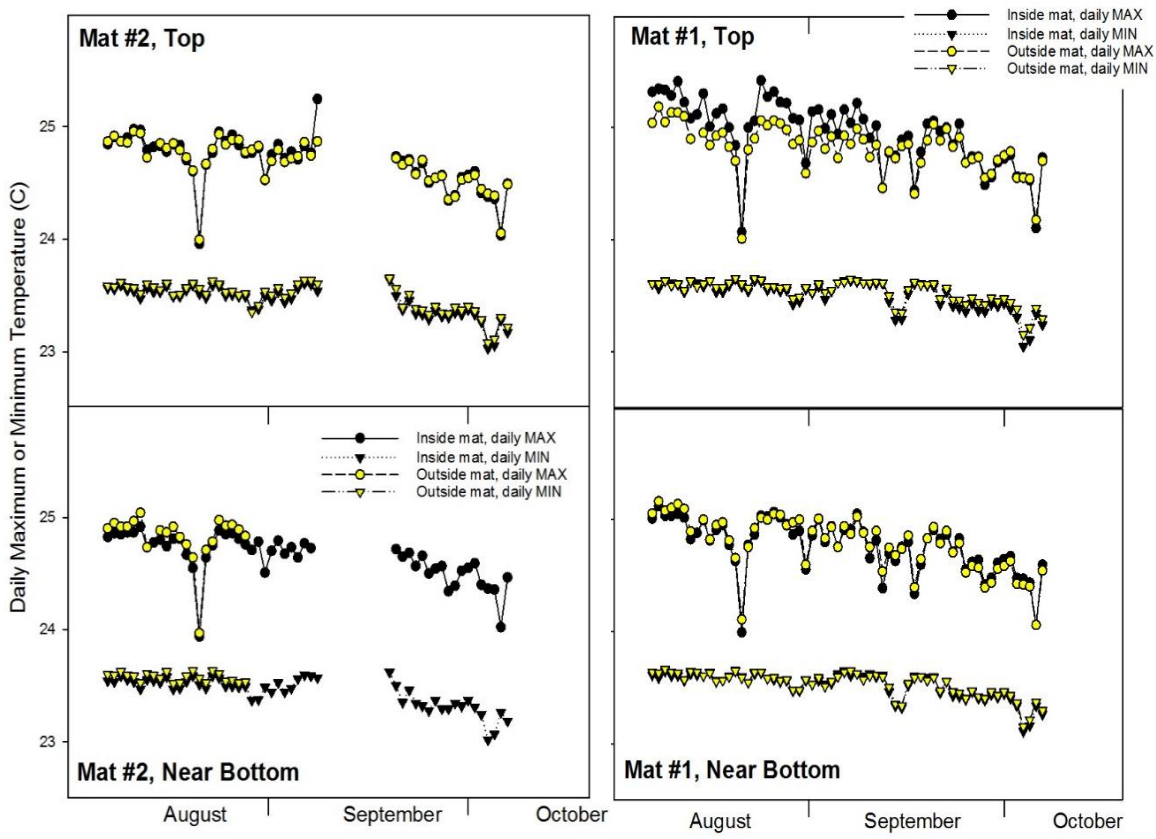

Figure 25. Maximum and minimum water temperatures (° C) from the MiniDOT sensors located within floating vegetation mats and outside the mats, at both the top and bottom of the water column.

Cabomba caroliniana in the channel behind Pecan Island, next to the golf course (Figure 21, Station #12). Though the MiniDOT sensors at these locations suffer from rapid biofouling, we believe the data support concern for low dissolved oxygen levels, as overnight DO fell well below 4 mg/L even immediately following sensor cleaning. These findings are cause for concern, because we expect to see an increase in these low-flow areas across Landa Lake and the USR during drought conditions. The location at Heidelberg Lodge (Figure 21, Station #14; Figure 22, M) experienced the highest degree of variability in dissolved oxygen levels, with hypoxic conditions routinely being measured. This site has been documented in the past to experienced severe hypoxic

conditions 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 the low DO continues regardless of flow conditions at this site. Additionally, fountain darters are routinely collected near this location, so if hypoxia occurs, the darters are apparently able to avoid it.

Comparing dissolved oxygen dynamics inside and outside of the vegetation mats showed few reasons for concern. Overall, these data indicate that the vegetation mats had unexpectedly little impact over diel DO patterns during 2015. In particular, there is no evidence that minimum DO levels under the mats fall to levels that provide a concern for fountain darters, as previously predicted; the comparably minimal vegetation mat formation during 2015 versus recent past year likely contributed to this finding (Figure C.4). We suspect that, as long as measurable flow continues beneath the mats, the DO minima are likely to present little problem to the fountain darters.

After evaluating these patterns, two general trends emerged. The first is that dissolved oxygen appears more variable outside the mats than underneath the mats. The diel variability in DO within the mats was remarkably constant at both the top and bottom of the water column (Figures 23 and 24). The more variable DO outside the mats is caused by two factors: apparent fluctuations caused by higher biofouling at the outside locations, and true DO fluctuations related to more metabolically active autotroph communities. The sensors outside the mats were much more prone to develop significant biofouling by periphyton, due to the higher light climate (the sensors not shaded by the mat). However, after this issue was identified we made frequent trips to service and clean the MiniDOT sensors, so the impact of biofouling is modest. The DO dynamics

underneath the mats are also generally free from localized swings caused by daytime photosynthesis (the mats shade the water column), so it is not too surprising that the DO maxima tend to be larger outside the floating vegetation mats. This trend is especially true at the bottom location outside of Vegetation Mat #1. This sensor was positioned just above a very active bryophyte community, which likely means that the large daily swings in DO measured reflect true variability at this site.

Although the low dissolved oxygen values occasionally recorded at the bottom sensors in this study are technically accurate, they likely do not reflect a concern for fountain darter habitat (see Figure 22, C). We believe these very low readings occurred when the benthic bryophyte layers expanded to engulf the sensor membranes on our MiniDOTs, so that the sensors are now within the mat matrix, instead of just above it as intended. Since the fountain darters should easily be able to avoid the low DO levels within the actual bryophyte layer, the low DO recorded is likely not a concern under current flow conditions. At the aerator site (Figure 22, C), the minimum DO measured, excluding five very low oxygen periods, was 3.34 mg/L, which likely reflects the true DO minima at the bottom of the water column above the benthic bryophyte community. While this indicates the area is routinely experiencing DO below the 4.0 mg/L goal, these values do not likely represent a serious threat to fountain darters survival.

The second general trend is that dissolved oxygen levels underneath the mats do not appear to be unfavorable for fountain darters. The DO minima at the locations beneath the mats were almost identical to those outside the mats, and in fact, the exception to that is the *lower* DO levels *outside* of Vegetation Mat #1, due to the high metabolic activity of the bryophytes, as explained above. In spite of this, fountain darters

are routinely observed and collected within this vegetation type. So if dissolved oxygen levels within bryophyte communities routinely drop below 4 mg/L, the question remains as to how fountain darters continually thrive in this habitat.

CHAPTER FIVE

Research Summary

Chapter Two contains the water quality data collected in 2015, two nutrient diffusing substrata (NDS) studies performed a year apart, and a brief sediment and plant nutrient survey across Landa Lake. Water quality data collected across Landa Lake and the Upper Spring Run indicated that there is a very strong phosphorus limitation in the water column. Nitrogen to phosphorus ratios averaged 182:1 across the 4 sampling sites- 26 times larger than the Redfield Ratio indicates that phytoplankton need for optimal growth rates.

Both NDS experiments confirm that attached algae growing in Landa Lake are phosphorus-limited. The attached algae grew significantly more on the treatments with added phosphorus (+phosphorus, +phosphorus and micronutrients) than the controls. The interpretation of the results from the second deployment was clouded a bit due to the unequal percentages of phaeophytin on the different treatment tubes, however the +nitrogen and +micronutrients were still not different from the control whereas the +phosphorus and +phosphorus and micronutrients were different. The NDS experiments also gave the suggestion of a minor micronutrient limitation (particularly in the first experiment). It may be beneficial to root out which micronutrient(s) is causing this effect and what algal species are responding to it.

The sheer magnitude of the phosphorus limitation in the water column indicates that there is strong potential for algal blooms when phosphorus gets added to the system.

This is especially problematic in the Comal River where the ecosystem depends on the cool, crystal clear water and high-light climate. Any significant shading of the river could spell disaster for many of the submersed plant and animal species, and particularly so for our slow-moving species of concern, the fountain darter.

The limited nutrient survey conducted across Landa Lake illustrates how bare of nutrients- especially phosphorus- much of the sediment is in the more gravel-dominated areas. Some of this translated into the plant tissue nutrients. There was a fair amount of variability between the three species studied and a bit within each species as well. The naturally growing *Ludwigia* plants (not the Potted *Ludwigia*) averaged the highest N:P ratio in their above-ground tissues (18.3) and the highest overall N:P ratio in their below-ground tissues (29.1, Lud 3).The Potted *Ludwigia* plants, although they had lower above-ground ratios, had below-ground N:P ratios much higher than the rest of the plants sampled, excluding the aforementioned Lud 3. This suggests that these plants are allocating their limited P resources away from their roots and to their above-ground tissues, which is a sign of herbivory compensation; these plants were grown in the MUPPTs where there is frequently observed herbivory by crayfish.

Overall, there was the most evidence of a phosphorus limitation in naturally growing *Ludwigia* and two of the *Vallisneria*. The *Sagittaria* analyzed had surprisingly low and consistent N:P ratios compared to the others, but all the measurements were still within normal ranges published in comparable studies. Our knowledge of how the nutrients vary spatially across Landa Lake and the Upper Spring Run could benefit from an expansion of this study. The variability between species and within them- especially the *Vallisneria*- suggest that we need a larger sample size of organism and sediment to

confidently identify trends in the lake. This expansion could provide us with a map of how nutrients are moving around Landa Lake and the USR in both the plants and the sediment and could be a useful tool for managers to base new plantings and restoration decisions on.

Chapter Three presented some interesting results of an herbivory-by-fertility experiment performed in Landa Lake. Herbivory was determined to be a much larger issue for our *Ludwigia* restoration efforts than we previously considered it to be. Every plant in the H+ treatment- the plants that were easily accessible to herbivores- were destroyed by crayfish within 26 days of the beginning of the experiment. The crayfish then climb up the cages and down through the top (which stuck up 5-12 cm above the water surface) to get to the caged, H- *Ludwigia* (supposedly inaccessible by herbivores) and began to feed on those. The experiment ended before these plants could be completely devastated as well. So are experiment that was initially intended to have 16 H+ plants, divided into 8 fertilized (F+) and 8 unfertilized (F-) and 16 H- plants, ended up having 8 F+ plants and 8 F-, along with 16 dead plants.

However, when the crayfish began destroying the caged (H-) plants, they did so discriminately. There were significant differences in mean total biomass and mean above-ground to below ground (AG:BG) ratios between the fertilized and unfertilized H- experimental treatment. The unfertilized plants had higher total biomass, indicating that the crayfish may be preferentially feeding on the fertilized plants, and lower overall AG:BG ratios- a sign of overall plant stability and reduced herbivory. A similar effect was also observed in the initials and reject plants before the experiment proper even began. Two short, unofficial experiments also confirmed that the crayfish were the

culprits of at least the majority of the damage done to our plants and that any *Ludwigia* planted in that area (just above Pecan Island) that was not protected would be destroyed (Figures B.5 and B.6).

Chapter Four reports two studies that sought to provide a better understanding of dissolved oxygen dynamics in the Landa Lake and Upper Spring Run region of the Comal River. Deployment of MiniDOT sensors throughout the Landa Lake and USR revealed little cause for concern related to low DO levels under the 2015 flow conditions, except for a three low-flow, stagnant portions of the system. While these low-flow locations covered relatively little of the system in 2015, they might be representative of a much larger portion of Landa Lake under low-flow or drought conditions.

Comparison of DO levels at the benthic surface versus the top of the water column showed that 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 levels that would threaten fountain darters. A few bottom sensors record very low DO measurements, but these ended up being *within* benthic bryophyte communities, which the fountain darters should be able to 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 underneath the mats. However, the lack of impact the vegetation mats made on dissolved oxygen levels may be directly related to the modest mat sizes formed in 2015. Additionally, good flow under the mats allowed the water column to mix thoroughly, but during a low-flow year with more extensive mats, the results are likely to 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 (Figure C.4).

APPENDICES

APPENDIX A

Additional Figures for Chapter Two Experiments

Figure A.1. The picture on the left is an NDS tube used in the experiments. The cap (blue) has a 2.23cm hole in the top to allow for algal growth on the fritted disk. The disk itself is placed under the cap and on top of the nutrient-amended agar. The picture on the right depicts tubes from the second NDS deployment after being removed from the river. Half of the tubes are inside the cage of ¼" wire mesh and half are attached outside of it. Note the snail hanging onto the cage and the snail feeding on an outside tube.

APPENDIX B

Additional Herbivory by Fertility Experiment Figures

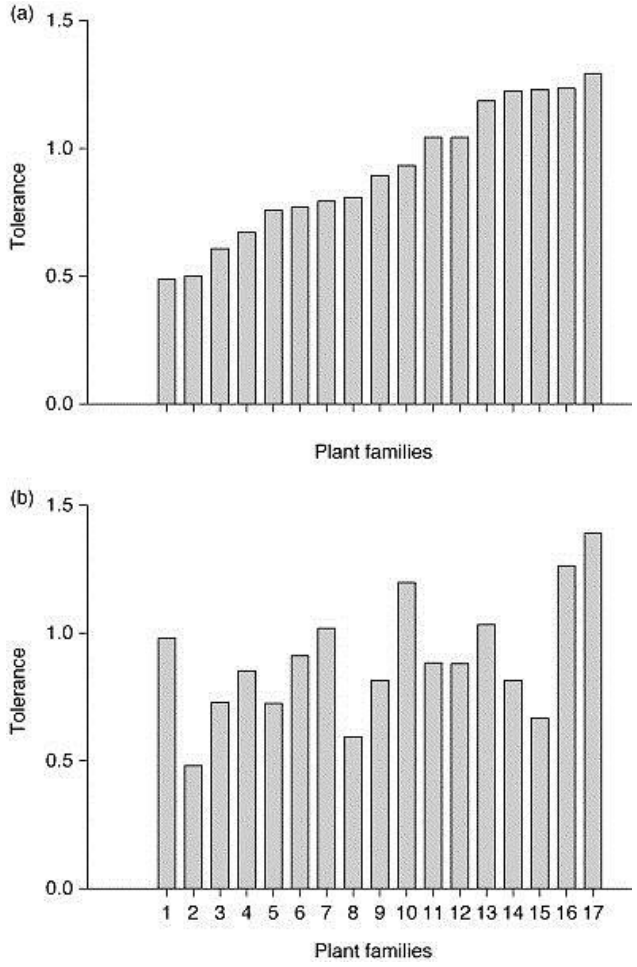

Figure B.1. Differences in intraspecific plant tolerance to herbivory in wild radish plants (*Raphanus raphanistrum*) using manual clipping and caterpillar damage. Tolerance is detected by a statistically significant interaction between herbivory treatment and plant family. Seventeen plant families are ranked based on their ability to tolerate 50% leaf–area removal. Tolerance is depicted as the ratio between mean fitness of damaged and undamaged siblings. (a) Herbivory by *Pieris rapae* butterfly larvae: there is a significant herbivory by family interaction indicating familial variation in tolerance to herbivory ($P = 0.046$). (b) Herbivory imposed by clipping with scissors: there is no herbivory by family interaction ($P = 0.225$). Several families have very different tolerance responses to the two types of damage (e.g. families 1 and 14). Had we used only simulated herbivory, we might have concluded that there was no evidence for a genetic basis to tolerance in this species (Strauss and Agrawal 1999).

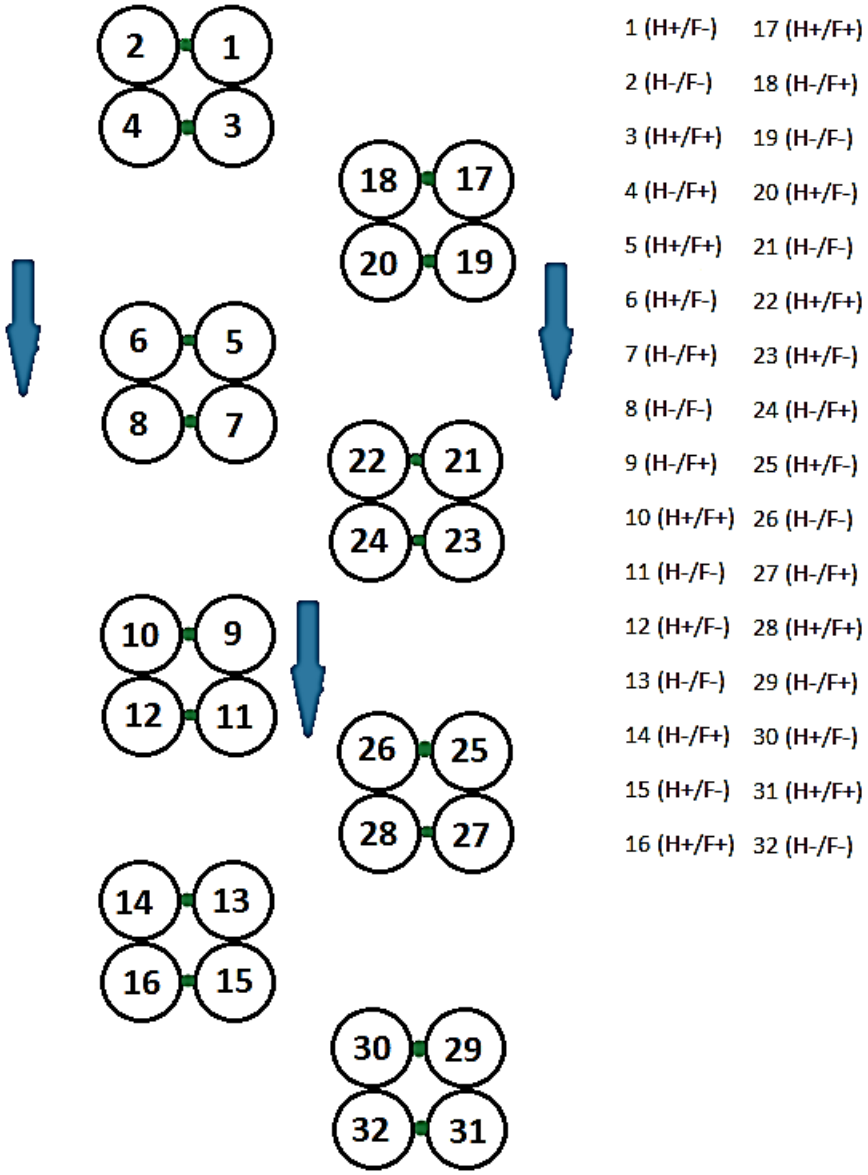

Figure B.2. Herbivory by fertility experimental cage configuration. Each treatment group was represented in each pod. The blue arrows show the direction of water flow. A fence was added just upstream of the cages to collect debris before it reached the experiment. PAR sensors were located within cages 18 and 30. MiniDOT sondes were located between cages 1 and 2, 13 and 14, 23 and 24, and 29 and 30.

Figure B.3. Fragments of *Ludwigia repens* clipped by crayfish. The first picture shows an experimental cage *in situ* with plant fragments floating at the top of the cage. The picture on the right is one taken in the lab just before the pieces were weighted. Note the chewed appearance of the leaves and the bottoms of the stems where they were raggedly cut.

Figure B.4. Young crayfish (*Procambarus clarkii*) in a 10-gallon aquarium. *Ludwigia repens* was planted across much of the aquarium before the crayfish was added. This picture was taken after 4 weeks- notice that the crayfish clipped each stem at the sediment surface and the fragments are floating at the top of the aquarium.

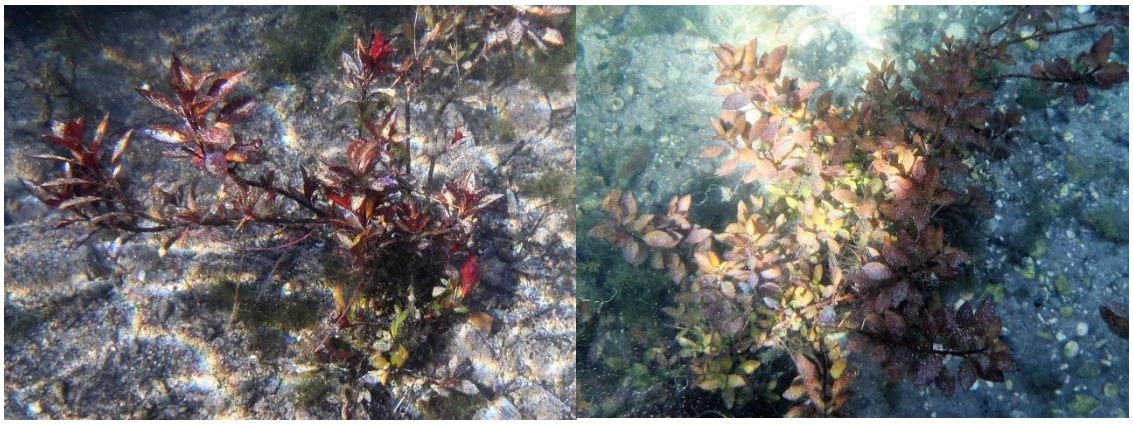

Figure B.5. Single plants from the short caged-vs-uncaged experiment. The plant on the left was not in a cage, while the plant on the right was caged. Not only were the caged plants bigger and had more leaves, they also appeared to recruit more bryophytes around their stems than the uncaged plants.

APPENDIX C

Additional Dissolved Oxygen Figures

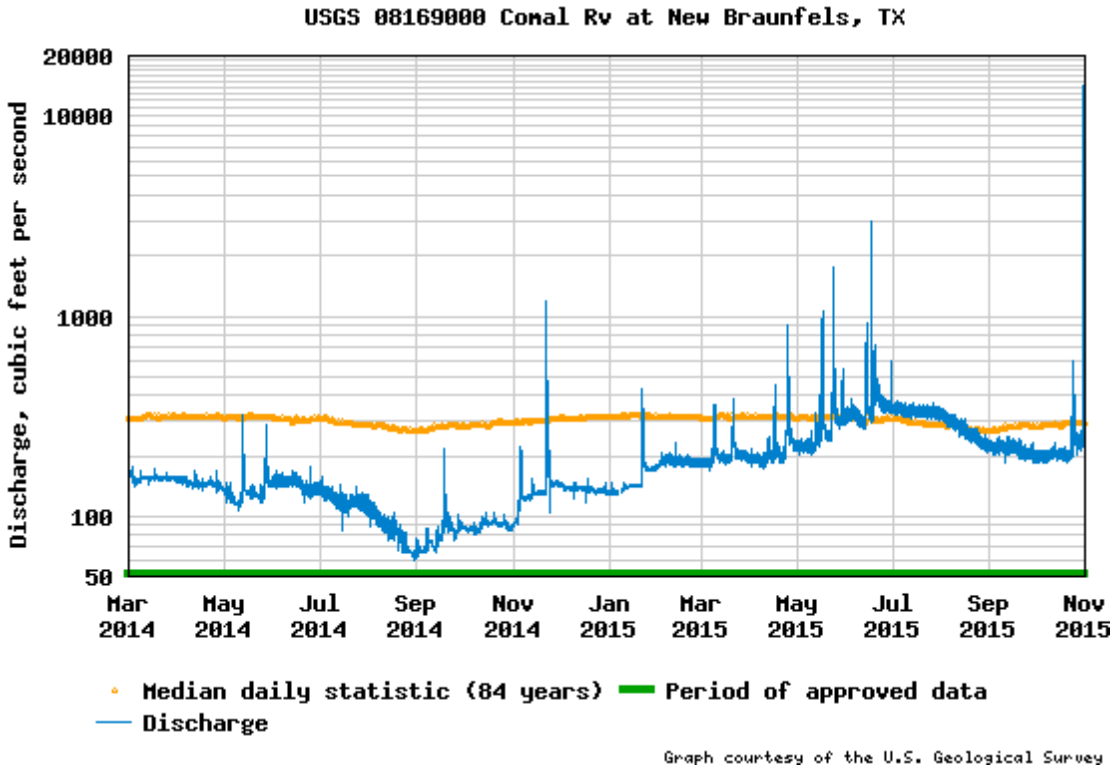

Figure C.1. Flow data on the Comal River from the spring of 2014 to the end of our study period in October 2015, represented in discharge ft^3/s. The orange line represents the 84-year average discharge for the system. Note the severe drought in 2014 and slightly above-average flow from May to August in 2015. Graph courtesy of the U.S. Geological Survey at https://waterdata.usgs.gov/nwis.

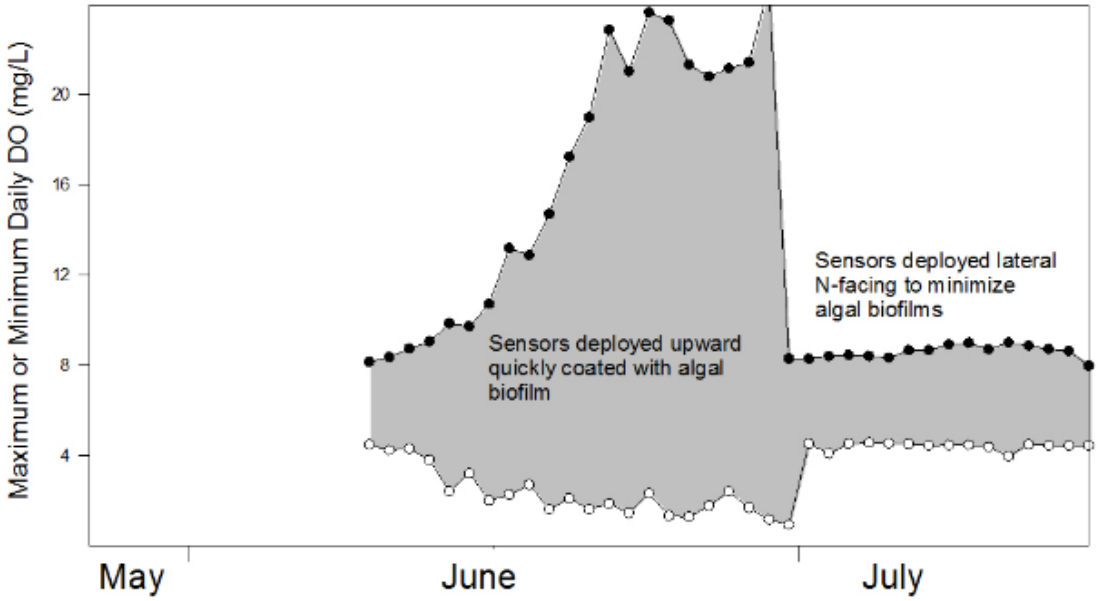

Figure C.2. MiniDOT sensor facing upward versus facing laterally. The upward deployment resulted in much quicker biofouling than lateral and north-facing deployment.

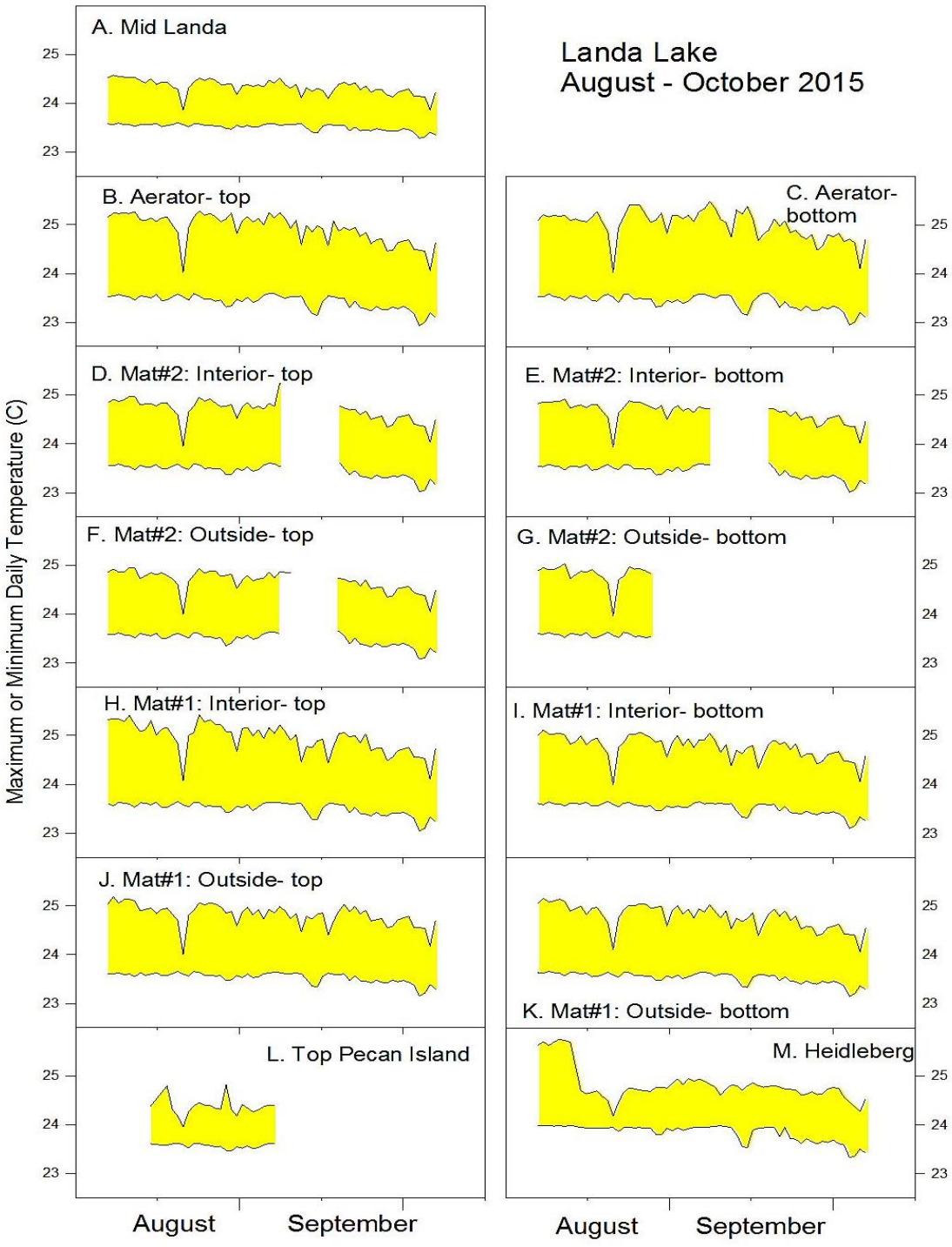

Figure C.3. Maximum and minimum water temperatures (° C) from the 13 MiniDOT sensors deployed long-term across Landa Lake and the Upper Spring Run.

Figure C.4. Map comparing cover of floating vegetation mats in September of 2014 (yellow) and September of 2015 (green) in Landa Lake.

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