A STUDY ON BIOLOGICAL THREATS TO TEXAS FRESHWATER RESOURCES

A Thesis

by

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ABSTRACT

This thesis consists of two separate experiments on unique biological threats to Texas freshwater ecosystems. The first experiment sought to understand the interaction between the harmful alga *Prymnesium parvum* and the cyanobacteria, *Anabaena sp*. The second experiment sought to determine the effectiveness of triploid grass carp, *Ctenopharyngodon idella*, as a biological control for two species of invasive macrophytes, which were giant salvinia, *Salvinia molesta*, and hygrophila, *Hygrophila polysperma.*

Prymnesium parvum blooms have become more frequent in the south-central United States, leading to significant ecological and economic impacts. Allelopathic effects from cyanobacteria were suggested as a mechanism that might limit the development of *P. parvum* blooms. This research focused on the effects of cultured cyanobacteria, *Anabaena* sp., on *P. parvum*. Over a 6-d period, daily additions of filtrate from the senescent *Anabaena* culture were made to *P. parvum* cultures growing in log phase. All treatments, including several types of controls, showed reductions in *P. parvum* biomass over the course of the experiment, but the treatments receiving *Anabaena* filtrate were reduced to a lesser degree, suggesting that filtrate from the senescent cyanobacteria culture was beneficial *to P. parvum* in some way.

Aquatic vegetation is an important component of most freshwater systems and provides numerous valuable ecosystem services, providing food, habitat and refuge for a variety of organisms. A significant threat to beneficial aquatic vegetation abundant in

many United States waterways is the introduction and spread of invasive macrophytes. Two of the newest invasive species, giant salvinia (*Salvinia molesta*) and hygrophila (*Hygrophila polysperma*), have quickly established in Texas waters. This research evaluated the potential use of triploid grass carp (*Ctenopharyngodon idella*) as a biological control agent for these two novel invasive species. Using a controlled mesocosm experiment, consumption rates and feeding preferences were measured. Giant salvinia and hygrophila were compared to six native and introduced species common in Texas and the Southern US. Grass carp were found to be potentially useful in controlling giant salvinia in the preliminary stages of an infestation but an overall poor control option for hygrophila.

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CHAPTER I

INTRODUCTION

Freshwater is understood as one of the most important resources on our planet. As freshwater environments have become increasingly important, so too is understanding the complex interactions that take place within these ecosystems. The research presented in this thesis is composed of two independent experiments focusing on biological threats to freshwater resources in Texas.

The first experiment, titled *Stimulating effect of Anabaena sp. exudate on Prymnesium parvum*, sought to understand the interaction between the harmful agla *Prymnesium parvum* and the cyanobacteria, *Anabaena*. *Prymnesium parvum* blooms have become more frequent in the south-central United States, leading to significant ecological and economic impacts. Allelopathic effects from cyanobacteria were suggested as a mechanism that might limit the development of *P. parvum* blooms. This research focused on the effects of cultured cyanobacteria, *Anabaena* sp., on *P. parvum*. Over a 6-d period, daily additions of filtrate from the senescent Anabaena culture were made to *P. parvum* cultures growing in log phase. All treatments, including several types of controls, showed reductions in *P. parvum* biomass over the course of the experiment, but the treatments receiving *Anabaena* filtrate were reduced to a lesser degree, suggesting that filtrate from the senescent cyanobacteria culture was beneficial *to P. parvum* in some way. This unexpected outcome may have resulted from stimulation of heterotrophic bacteria by the addition of *Anabaena* filtrate, which likely contained exudates rich in dissolved organic carbon compounds. *P. parvum* was then able to supplement its nutritional requirements

for growth by feeding on the elevated bacteria population. These findings coupled to previous observations suggest that interactions between cyanobacteria and *P. parvum* in natural environments are complex, where both allelopathic and growth-stimulating interactions are possible.

The second experiment, titled *Triploid grass carp feeding preferences for two novel invasive aquatic plants*, sought to determine the effectiveness of triploid grass carp as a biological control for giant salvinia and hygrophila. Aquatic vegetation is an important component of most freshwater systems and provides numerous valuable ecosystem services, providing food, habitat and refuge for a variety of organisms. A significant threat to beneficial aquatic vegetation abundant in many United States waterways is the introduction and spread of invasive macrophytes. Invasive aquatic plants degrade water quality, reduce species diversity, alter animal communities and suppress desirable native plants. Costs related to losses, damages and control of invasive macrophytes exceeds $110 million per year in the United States alone. Two of the newest invasive species, giant salvinia (*Salvinia molesta*) and hygrophila (*Hygrophila polysperma*), have quickly established in Texas waters. Current efforts to control giant salvinia and hygrophila are costly, time consuming and only moderately effective. This research evaluated the potential use of triploid grass carp (*Ctenopharyngodon idella*) as a biological control agent for these two novel invasive species. Using a controlled mesocosm experiment, consumption rates and feeding preferences were measured. Giant salvinia and hygrophila were compared to six native and introduced species common in Texas and the Southern US. Grass carp were found to be potentially useful in controlling giant salvinia in the preliminary stages of an infestation because of their propensity to

consume primary growth stage of the plant, but grass carp seemed an overall poor control

option for hygrophila as they scarcely consumed the plant.

CHAPTER II

STIMULATING EFFECT OF ANABAENA SP. EXUDATE ON PRYMNESIUM

PARVUM*

Introduction

Prymnesium parvum (N.Carter) is a haptophyte alga that occurs worldwide in a variety of environments. Under certain environmental conditions, such as nutrient scarcity (Uronen et al. 2005, Roelke et al. 2007, Errera et al. 2008), this alga produces toxins lethal to gill-breathing organisms. Since its first documentation in Texas in 1985, *P. parvum* blooms have increased in both scope and magnitude and are now observed in 33 reservoirs and 5 river basins in Texas, as well as 16 other states throughout the country (Southard et al., 2010, Roelke et al. 2011).

While there are many explanations for the increase in *P. parvum* fish kills, it is still unknown why particular lakes are plagued with frequent blooms and others are unaffected. Roelke et al. (2010) studied the effects of mixing water from Lake Waco, a reservoir in central Texas with no recorded *P. parvum* blooms, with water collected from Lake Whitney, which has a history of toxic blooms. Their research found that the introduction of Lake Waco water had a deleterious effect on *P. parvum*. Nuisance cyanobacteria, including *Anabaena* sp. and *Microcystis* sp., were abundant in water collected from Lake Waco at the initiation of the experiments. Both cyanobacteria are known to produce allelopathic chemicals capable of inhibiting *P. parvum* growth (Pflugmacher 2002, Legrand et al. 2003, James et al., 2011).

The findings from the studies mentioned above are limited in that linkages between cyanobacteria and *P. parvum* were based on either correlative association (i.e., Roelke et al. 2010) or exposure to a single chemical (microcystin-LR) produced by cyanobacteria (i.e., James et al. 2011). The purpose of this experiment was to further explore the interaction between cyanobacteria and *P. parvum*. In a controlled laboratory experiment, the Texas strain of *P. parvum* was exposed to cell free filtrates from a cultured strain of *Anabaena,* isolated from Lake Waco. Based on findings from Roelke et al. (2010) and James et al. (2011), we hypothesized that *P. parvum* growth would be suppressed by *Anabaena* filtrate.

Materials and Methods

This experiment was performed using a cultured strain of *P. parvum* from Texas (strain UTEX LL 2979, Culture Collection of Algae at the University of Texas at Austin), maintained in f/2 medium (Guillard, 1975) at 4 ppt salinity, 10°C and a 12:12 light:dark (L:D) cycle with irradiance of 200 μE m$^{-2} \cdot$ s^{-1} from a cool white fluorescent source. This *P. parvum* strain is maintained through a batch culturing method where the culture is inoculated monthly into fresh medium after stationary growth phase is reached at a 10:1 ratio (fresh media: stationary culture). The experiment was initiated using *P. parvum* culture 2-weeks after it was transferred to fresh media (i.e., in log growth phase based on our experience with maintaining this culture). Aseptic techniques were consistently utilized when handling the *P. parvum* culture.

This experiment also employed filtrate from a cyanobacteria culture. Anabaena was cultured from a sample collected from Lake Waco in January, 2009, maintained in f/2 medium at 4 ppt salinity, 24°C and a continuous light source. This culture was also maintained by monthly transferring a portion of the culture grown to stationary growth phase (determined by the saturating color intensity of the culture) into fresh media. The filtrates from the *Anabaena* culture, used for some of the treatments described below, were collected when the cyanobacteria culture was in stationary growth phase, ~30 days old.

Six treatments were tested in this experiment, two of which received *Anabaena* filtrate (treatments 5&6), while the others served as various controls (treatments 1-4). Treatment 1 received autoclaved reverse osmosis (RO) water brought to f/2 nutrient concentration and 4 ppt salinity. Treatment 2 received filtered Lake Waco water brought to f/2 nutrient concentration and 4 ppt salinity. Treatment 3 received stationary phase *P. parvum* filtrate (cell free) at 4 ppt salinity. Treatment 4 received stationary phase *P. parvum* filtrate (cell free) at 4 ppt salinity brought to f/2 nutrient concentration. Combined, these controls allowed for the assessment of experimental effects of differential nutrient additions (residual nutrients from cultures in stationary growth phase) and use of RO water. Treatment 5 received *Anabaena* filtrate (cell free) at 4 ppt salinity, and treatment 6 received *Anabaena* filtrate (cell free) at 4 ppt salinity brought to f/2 nutrient concentration. Cell free filtrates used in Treatments 3-6 were acquired by gently filtering the original culture (either *P. parvum* or *Anabaena* sp., respectively) through a GF/F filter (nominal pore size ~0.7 μm) at <100 mm Hg vacuum and were not autoclaved to prevent potential denaturing of organic compounds.

To each 125 mL flask, 50mL of *P. parvum* culture growing in log growth phase was added. Over a 6 day period, a 10% (5mL) solution exchange was made daily by withdrawing 5 mL of each flask's contents and adding 5 mL of treatment water. An experimental study duration of 6 days was selected because previous work over a 7 day period demonstrated *P. parvum* growth reduction by filtrates from cyanobacteria-rich Lake Waco water (Roelke et al., 2010). Each treatment was done in triplicate, for a total of 18 experimental units. The experiment was conducted at 10°C for a 12:12 light:dark (L:D) with irradiance of 200 μE m^{-2} s^{-1}, which is representative of winter conditions in which *P. parvum* blooms typically occur in Texas. Under these conditions, the expected maximum reproductive growth rate of *P. parvum* is about equal to the experimental dilution rate (~0.10 d^{-1}, Baker et al., 2009).

In-vivo fluorescence readings were taken daily using a Turner Designs 10-AU fluorometer at excitation-emission wavelengths optimized for chlorophyll *a* measurement (Sunnyvale, California, USA). Because we did not characterize cellular pigment packaging effects through an analysis of pigment content per cell, we normalized the fluorescence readings to relative units (i.e., the maximum observed fluorescence equaled 1 and all other readings became a proportion of this maximum reading).

In addition to the fluorescence readings, direct cell counts were performed. A 5mL sample was preserved in 5% gluteraldehyde at the conclusion of the experiment and enumerated microscopically. Cell densities were counted from a 1 mL subsample using a settling technique (Utermöhl, 1958) and a Leica DM IL (Wetzlar, Germany) inverted microscope at 400X, where fields of view were examined until ~200 cells were counted per sample.
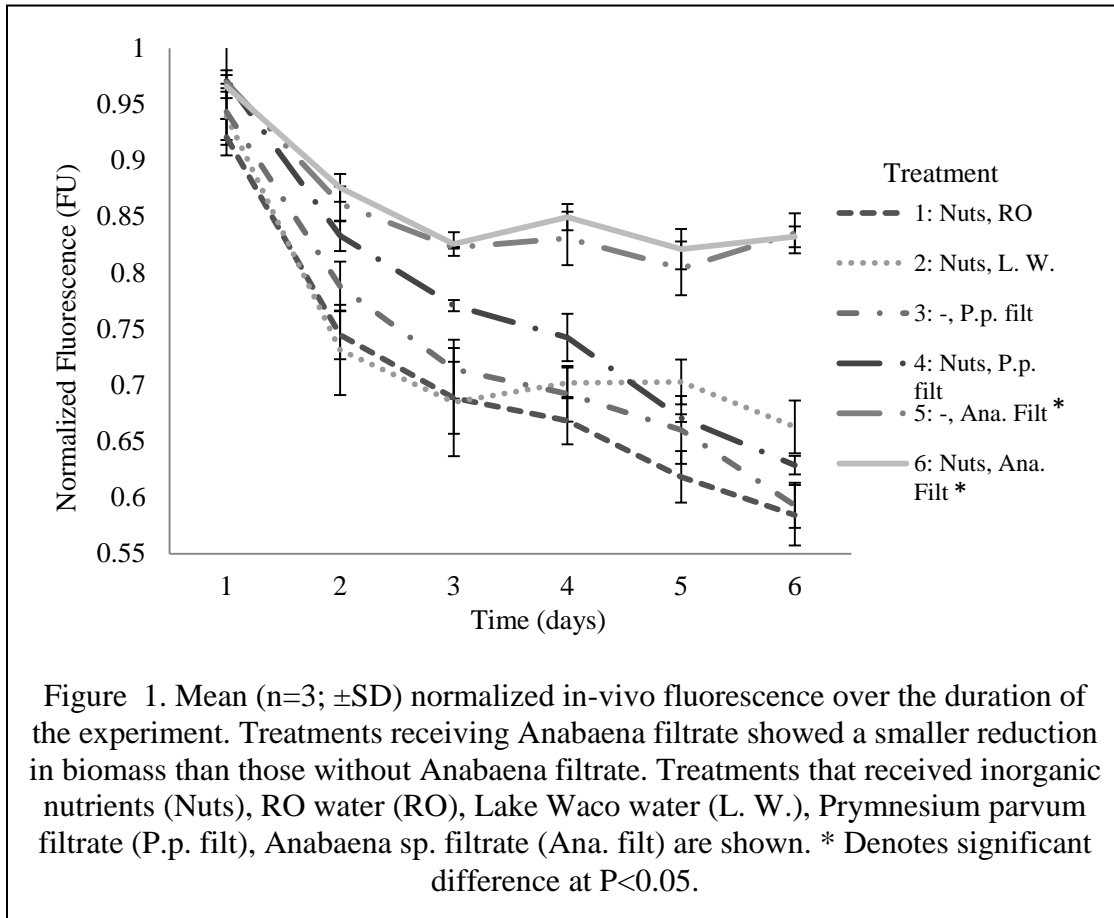
Fluorescence readings and cell count data were then used in combination. This was done by dividing the normalized fluorescence at the conclusion of the experiment by the final population densities, thereby giving us a first-order approximation of how the pigment content per cell changed in response to the experimental treatments. Live samples from day 6 were also observed under a Leica DM IL inverted microscope at 400X to observe *P. parvum* behavior.

Normalized fluorescence readings were analyzed by running one-way repeated-measures ANOVA in SPSS (Chicago, Illinois, USA) using Tukey's post-hoc test. In addition, the rate of change in the fluorescence readings (based on a negative exponential model, KaleidaGraph, Reading, Pennsylvania, USA), the final cell densities, and the relative fluorescence per cell were analyzed by running one-way ANOVAs in SPSS using Fisher's LSD post-hoc test.

Results

Assuming that changes in our measured *in-vivo* fluorescence were proportional to changes in biomass, then our observations suggest that all treatments experienced reduced biomass over the course of the experiment. In other words, the population growth rate of *P. parvum* was less than the dilution rate. Control treatments that did not receive *Anabaena* filtrate (treatments 1-4) suffered a greater reduction in biomass than did treatments that received *Anabaena* filtrate (treatments 5&6), with the difference between treatments significant at $p<0.05$ using both the repeated observations of *in-vivo* fluorescence and the calculated rate of change for *in-vivo* fluorescence (Figure 1).

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Figure 1. Mean (n=3; ±SD) normalized in-vivo fluorescence over the duration of the experiment. Treatments receiving Anabaena filtrate showed a smaller reduction in biomass than those without Anabaena filtrate. Treatments that received inorganic nutrients (Nuts), RO water (RO), Lake Waco water (L. W.), Prymnesium parvum filtrate (P.p. filt), Anabaena sp. filtrate (Ana. filt) are shown. * Denotes significant difference at $P < 0.05$.

Final cell densities were consistent with the normalized fluorescence readings, with the two cultures treated with *Anabaena* filtrate (treatments 5&6) having the highest densities, and again with the difference between treatments significant at $p < 0.05$ (Figure 2). As with the *in-vivo* fluorescence readings, there were no significant differences between treatments that did not receive *Anabaena* filtrate (treatments 1-4), nor were there significant differences between the treatments that received filtrate (treatments 5&6).

Figure 2 . Mean (n=3; ±SD) final cell density in cells liter-1 at day 6. Treatments that received Anabaena filtrate had a higher cell density than treatments that did not receive these additions. Treatments that received inorganic nutrients (Nuts), RO water (RO), Lake Waco water (L. W.), Prymnesium parvum filtrate (P.p. filt), Anabaena filtrate (Ana. filt) are shown. * Denotes significant difference at P<0.05.

The ratio of normalized *in-vivo* fluorescence per cell varied among the treatments, with *Anabaena* filtrate treatment (treatments 5&6) ratios lower than other treatments (Table 1). Despite large within treatment variation, treatments 5 and 6 were significantly different (p<0.05) from treatments 1 and 2, and different from treatments 3 and 4, but not significantly so (p>0.05). This suggests that the biomass estimates shown in Figure 1 for treatments 5&6 are underestimates of the actual population density, strengthening our observation that *P. parvum* performed better with the addition of *Anabaena* filtrate.

Treatment	Normalized ratio of *in-vivo* fluorescence per cell $(x10^{-8})$ $\pm$ 1 standard deviation $(x10^{-9})$
1	1.511 ± 4.908
2	1.048 ± 1.142
3	0.782 ± 1.066
4	0.941 ± 2.193
5*	0.758 ± 0.938
6*	0.683 ± 0.629

Table 1. Normalized in-vivo fluorescence per cell at day 6 of the experiment showing that cultures receiving Anabaena filtrate (treatments 5&6) had lower ratios of in-vivo fluorescence per cell than other treatments. * Denotes significant difference at $p<0.05$.

While we did not perform direct counts of bacteria, qualitative observations of bacteria clumps in the treatments receiving *Anabaena* filtrate indicated that bacteria densities were ~2 orders of magnitude greater compared to treatments not receiving *Anabaena* filtrate. In addition, *P. parvum* cells in the *Anabaena* filtrate treatments were observed to approach bacteria clumps and feed on them, with recently phagocytized bacteria cells observed inside *P. parvum* cells. We did not observe signs of phagotrophy in the other treatments. Instead, *P. parvum* cells from those treatments had well-developed chloroplasts (Figure 3). The photos of *P. parvum* cells shown here were representative of *P. parvum* cells from the respective treatments.
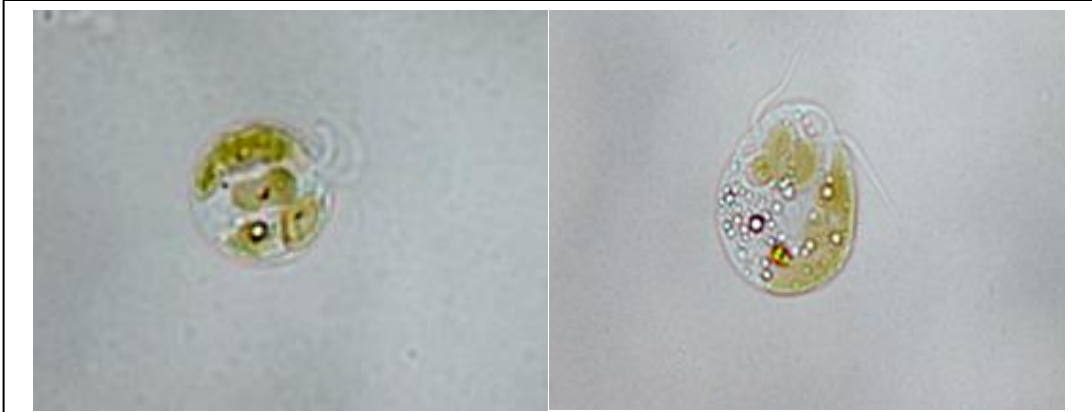
Figure 3. Representative views of P. parvum cells. Leftmost image displays a representative P. parvum cell from treatments without Anabaena filtrate addition (treatments 1-4), which was observed to have a well developed chloroplast while the image on the right displays a representative P. parvum cell from treatments that received Anabaena filtrate (treatments 5&6), which had a smaller chloroplast and noticeable ingested bacteria within.

Discussion

Our findings were not consistent with our hypothesis in that cultures treated with *Anabaena* filtrate performed better than our controls, rather than worse. A possible explanation for this is that *Anabaena* filtrate contained dissolved organic chemicals that promoted the growth of bacteria already present in the cultures and on which *P. parvum* cells could feed. It is also possible that a bacteria strain not normally present in the *P. parvum* cultures passed through the GF/F filter and was able to flourish in the presence of *P. parvum*, although to the best of our knowledge, this phenomenon has never been observed. Previous research showed that *P. parvum* phagocytizes bacteria as an alternative method of acquiring essential nutrients during periods of inorganic nutrient limitation (Nygaard and Tobiesen, 1993, Legrand et al. 2001). Under culture conditions creating intense competition with bacteria for inorganic phosphorus, Harman and Grover (2008) suggested that bactivory enabled *P. parvum* to persist, albeit at low population

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density. While we did not measure concentrations of inorganic nutrients, dissolved organic carbon or deduce the origin of the bacteria present in this experiment, we can surmise that bacterivory in treatments receiving *Anabaena* filtrate enhanced persistence of *P. parvum* at the low temperature employed in this experiment.

P. parvum fed on bacteria in an inorganic nutrient replete condition (treatment 6, which received supplemental inorganic nutrients and would not have become nutrient limited over a period of 6 days and declining algal biomass). Recent research showed that even under light and nutrient replete conditions, *P. parvum* can function heterotrophically, phagocytizing bacteria (Martin-Cereceda et al., 2003; Burkholder et al. 2008; Carvalho and Granéli 2010). In fact, *P. parvum* grew at significantly higher rates with the addition of bacteria to a nutrient rich culture (Martin-Cereceda et al., 2003). Our observations are consistent with these studies, in that treatments with high levels of observed bacteria (treatments 5&6) exhibited higher rates of growth than treatments without noticeable levels of bacteria (treatments 1-4). In addition, bacteria did not grow in the treatments receiving *P. parvum* extract, likely because the exudates from *P. parvum* growing in stationary phase also contained chemicals known to inhibit bacteria production (Fistarol et al., 2003).

Algae employing mixotrophic feeding strategies have variable chlorophyll *a* content per cell, with lower ratios occurring when cells are relying on phagotrophy and osmotrophy (Sanders et al., 1990). Our experimental findings are consistent with this observation in that the two treatments receiving *Anabaena* filtrate had the lowest ratio of *in-vivo* fluorescence per cell, suggesting that *P. parvum* cells adapted to increased bacterivory with less developed chloroplasts (Figure 3). Consequently, it is likely that the

actual difference in cell density was greater than suggested in Figure 1, as *in-vivo* fluorescence likely underestimated *P. parvum* biomass in the *Anabaena* treatments.

Conclusion

In summary, heterotrophic bacteria appeared to be stimulated by the addition of filtrate from the *Anabaena* culture, which likely contained exudates that increased concentrations of dissolved organic carbon compounds. In fact, cyanobacteria exude a complex mixture of chemicals, including volatile organic compounds and polyunsaturated fatty acids (Watson, 2003). In this study, it may have been that *P. parvum* supplemented its nutritional requirements for growth by feeding on elevated bacteria populations. Our findings coupled to previous observations suggest that interactions between cyanobacteria and *P. parvum* in natural environments are complex, where both allelopathic (Pflugmacher 2002, Legrand et al. 2003, Roelke et al. 2010, James et al., 2011) and growth-stimulating interactions are possible. To better understand these complex interactions, future research should focus on specific chemicals where the presence of allelopathic or growth-stimulating compounds are linked to the cell physiology of cyanobacteria, other phytoplankton, and ultimately *P. parvum* population dynamics. In addition, experiments are needed that employ multiple cyanobacteria cultures, as chemical production varies among taxa and strains.

CHAPTER III

TRIPLOID GRASS CARP FEEDING PREFERENCES FOR TWO NOVEL INVASIVE

AQUATIC PLANTS

Introduction

Aquatic vegetation is an important component of most freshwater systems and provides numerous valuable ecosystem services. Macrophytes provide, directly or indirectly, food, habitat and refuge for a variety of organisms, including waterfowl, invertebrates and fish. Macrophyte community structure is shown to have a direct impact on the associated freshwater assemblage, impacting periphyton distribution, macroinvertebrate abundance and overall food-web dynamics (Warfe and Barmuta 2006). Through decreased turbulence, aquatic vegetation also serves an important role in sediment stabilization, thereby improving water quality, and reducing turbidity, erosion and sediment resuspension (Barko et al. 1991, Van Nes 1999, Madsen et al. 2001).

The introduction and spread of invasive macrophytes is of significant concern in the United States. Invasive aquatic plants degrade water quality, reduce species diversity, alter animal communities and suppress desirable native plants (Dibble 2009). They also disrupt hydropower generation, impair commercial and recreational navigation, reduce property value and increase flood frequency, duration and intensity. Nationally, costs related to losses, damages and control of invasive macrophytes exceeds $110 million per year (Pimentel et al. 2000). As a result of human impact, primarily eutrophication, macrophyte invasions are expected to increase in both scope and magnitude (Verigin 1979).

One of the most recent and problematic invasive aquatic plants is giant salvinia (*Salvinia molesta* Mitchell), a floating fern of the Salviniaceae family native to South America. Giant salvinia reproduces exclusively by vegetative means, as stems fragment spontaneously as the plant matures. This reproductive strategy allows the plant to double in size weekly if environmental conditions are adequate (Oliver 1993). It grows to such high surface densities that it blocks sunlight and reduces dissolved oxygen levels to dangerously low levels (McFarland et al. 2004). The species was cultivated in the United States by the horticulture industry for several decades before its escape in South Carolina in 1995. Giant salvinia quickly spread, primarily by human movement, and is now considered naturalized in 10 states, with the most serious infestations affecting Texas and Louisiana (McFarland et al. 2004). The potential for this species to continue to spread poses the risk of increased economic and environmental damages.

Hygrophila (*Hygrophila polysperma* Anderson), commonly known as dwarf hygrophila, Miramar weed or Indian waterweed, is a fast growing member of Acanthaceace family native to India. Capable of growing both submerged and emergent, hygrophila was first introduced to the United States as an aquarium plant around 1945 as "oriental ludwigia" (Innes 1947). It was documented in the wild in 1965, and is now considered naturalized in many regions of Florida and south central Texas. Both giant salvinia and hygrophila are classified as a Federal Noxious Weed, making inter-state transport of the species illegal.

Current efforts to control giant salvinia and hygrophila include foliar application of EPA-approved aquatic herbicides and introduction of salvinia weevils (*Cyrtobagous salviniae* Calder & Sands), a USDA-approved biocontrol introduced from the plant's

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native range. While both methods have met with some success, they have their limits. Herbicide application is costly, time consuming and limited to the areas accessible by watercraft (McFarland et al. 2004). The salvinia weevil has thus far proven to be only moderately effective following field releases, in part due to questionable ability of the insect to overwinter in Texas in numbers sufficient to sustain giant salvinia control the following growing season (Tipping et al. 2008). Investigating additional control mechanisms for giant salvinia and hygrophila is therefore merited.

The grass carp (*Ctenopharyngodon idella* Valenciennes) is an herbivorous fish of the Cyprinidae family native to the large river systems of eastern Asia. The species was first introduced into the United States in 1963 as a method of controlling aquatic weeds and is now widely utilized as a biocontrol agent. Diploid grass carp carp have since escaped into the wild and established breeding populations in numerous river systems, including the Mississippi, Missouri and Trinity river drainages.

As a result of their potential invasive nature, only triploid grass carp are legal in most states for management use, as triploidy sterilizes the fish and prevents successful reproduction (Piferrer et al. 2009). The use of triploid grass carp to control macrophytes is an attractive choice in comparison to mechanical removal and chemical application as it is often less expensive and longer lasting (Pípalová 2006). Research has shown that triploid grass carp can be effective at controlling many types of submersed aquatic vegetation, including hydrilla (*Hydrilla verticillata*), chara (*Chara* spp.) and several species of pondweeds (*Potamogeton* spp.) (Pine & Anderson 1991, Bowers et al. 1987, Wiley et al. 1986). Though they have been reported to consume giant salvinia and hygrophila, no studies have focused on the preference for or consumption rate of the

plants by triploid grass carp. This research seeks to determine the consumption rate for giant salvinia and hygrophila, as well as the preference for the plants as compared to six native and introduced species common in Texas and the Southern US.

Materials and Methods

Research was conducted at the Texas A&M Aquaculture Research and Teaching facility near College Station, Texas. For this experiment, nine 4,000-L capacity concrete mesocosms measuring 2 meters wide and 1.4 meters deep covered in bird netting were utilized. Mesocosms were aerated using a centrifugal blower and supplied with water from a shallow water well at a rate allowing a complete 24 hour turnover. Input water supply temperature was recorded at a near constant 21°C and effectively buffered any rapid temperature changes from occurring within the mesocosms. Temperature and dissolved oxygen levels were measured weekly to ensure water quality within ranges which have been reported to not limit growth or feeding of grass carp (10-30°C, >4 mg/L, respectively).

Forty triploid grass carp, initial weight 277.8 g ± 58.3 g, were acquired October 2011 from a hatchery in Arkansas. Upon arrival, the fish were held in a 1/10th acre lined pond and fed a mixture of lettuce (*Lactuca sativa*) and a Rangen (Buhl, Idaho, USA) commercial fish feed *ad libitum*. Once appropriate water temperatures were reached in March 2012, three fish were randomly stocked per mesocosm.

Plants utilized for this experiment include six species common throughout the Southern United States; hydrilla (*Hydrilla verticillata*), Illinois pondweed *(Potamogeton illinoensis)*, coontail (*Ceratophyllum demersum)*, cabomba (*Cabomba caroliniana*), water

lettuce (Pistia stratiotes) and water hyacinth (*Eichhornia crassipes*); and two relatively recent invasive species, giant salvinia (*Salvinia molesta*) and hygrophila (*Hygrophila polysperma*). All plant species minus giant salvinia were collected from the San Marcos River, a spring-fed river located in Central Texas. Giant salvinia was collected at Caddo Lake, B.A. Steinhagen Lake and Lake Livingston as available. Plants were held in large mesocosoms and replaced weekly to ensure freshness.

This experiment was composed of three stages, single species consumption study; a paired comparison preference study; and finally a community selective consumption study. All trials were done in triplicate to account for natural feeding variation. Between each trial, fish were fed a mixture of lettuce (*Lactuca sativa*) and a Rangen commercial fish feed *ad libitum*, and then fasted for a 24 hour period to ensure complete gut evacuation. Plant species were presented in such a way to most closely imitate natural growth appearance, with benthic macrophytes weighted with a lead tie and floating macrophytes left unrestricted.

In the single species consumption trials, a non-limiting amount of a single plant species was presented to the grass carp for a 24 hour period. This study was conducted at low (18°C) and high temperature (25°C) to account for the effects of temperature on consumption. For the paired comparison preference study, a round-robin tournament method was utilized, where all possible combinations of the 8 plant species were presented to the grass carp (28 total trials), each for a 24 hour period. These trials were conducted in a random order and fell within the low and high temperatures (18°C and 25°C, respectively) from the single species consumption trials. In the community selective consumption trial, all eight plant species were presented to the grass carp

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simultaneously for a 24 hour period. An equal, non-limiting amount of plant matter (200 g ± 10 g) was introduced for each individual species in the paired comparison preference study and the community selective consumption study. This study was conducted exclusively at high temperature (25°C). Prior to introduction, plants were spin dried with a hand powered centrifuge to remove excess moisture and weight measured and recorded. At the conclusion of the feeding period, all unconsumed plant fragments greater than 1/8" were collected, separated by species, spin dried and weighed. For the purpose of this experiment, any change in plant biomass was attributed to fish consumption.

Due to various constraints, the same fish were utilized repeatedly for all experiments. It is possible that learned behavior during the trials might have biased observations, but steps were taken to minimize this effect, including the random assignment of trials to mesocosms and the use of a 24 hour trial period followed by a 24 hour fast.

The low temperature single species consumption data was analyzed in SPSS (Chicago, Illinois, USA) by running a one-way ANOVA using Tukey's post-hoc test. The high temperature single species consumption data was analyzed by running a one-way ANOVA in SPSS using Dunnet's T3 post-hoc test.

Ivlev's preference index (Ivlev 1961), which ranges from a preference index (PI) of -1 (plant not consumed at all) to +1 (only plant consumed) was utilized to determine the relationship between species in the paired comparison and community preference studies. A PI of 0 corresponds to a non-selected plant. Statistical significance of the differences was analyzed in SPSS using a one-way ANOVA with Tukey's post-hoc test.

Results

   While results varied somewhat between experiments, our observations indicate

that triploid grass carp exhibit a preferential feeding behavior. In the single species

consumption study, temperature and species had a significant effect ($p<0.05$) on total

consumption of plant matter per fish as well as a significant interaction effect. Overall

consumption at 25°C was greater than at 18°C for every species with the exception of

water lettuce. At the 18°C, Illinois pondweed was significantly different ($p<0.05$) than

hygrophila, giant salvinia, water hyacinth and cabomba. At 25°C, Illinois pondweed was

significantly different ($p<0.05$) than hygrophila, giant salvinia and water hyacinth. Other

observations were suggestive but not statistically significant (Figure 4).

Figure 4. Mean (n=3; ±SD) maximum single species consumption (g) per fish at 18°C and 25°C.
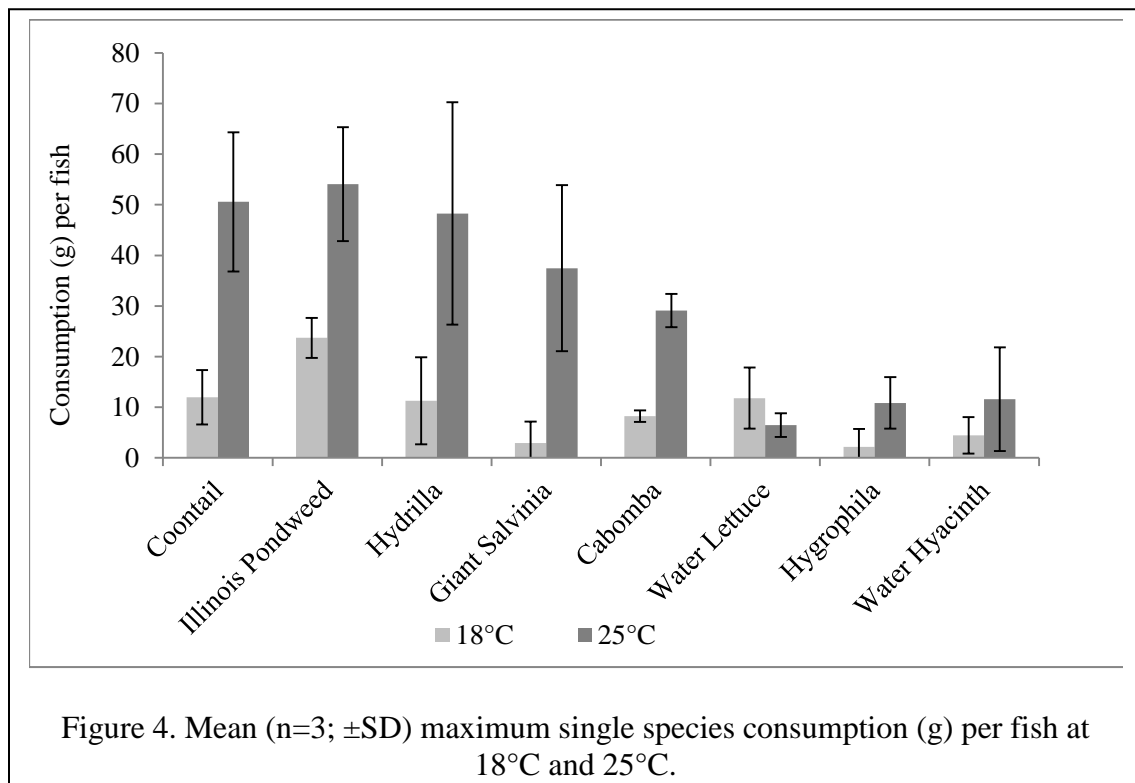
PI values for the paired comparison study differed significantly across the species and ranged from an average of -0.834 for hygrophila to 0.100 for Illinois pondweed (Figure 5). Tukey post-hoc comparisons of the eight species indicate three homogenous subsets with hygrophila (M=-0.834, 95% CI [-1.046, -0.623] and water lettuce (M=-0.5, 95% CI [-1.006,-0.72]) significantly different (p<0.05) than giant salvinia (M=-0.170, 95% CI [-0.530,-0.123]), hydrilla (M=0.000, 95% CI [-0.378,0.378]), cabomba (M=0.054, 95% CI [-0.262,0.371]), coontail (M=0.072, 95% CI [-0.101,0.246]), and Illinois pondweed (M=0.010, 95% CI [-0.090,0.289]).
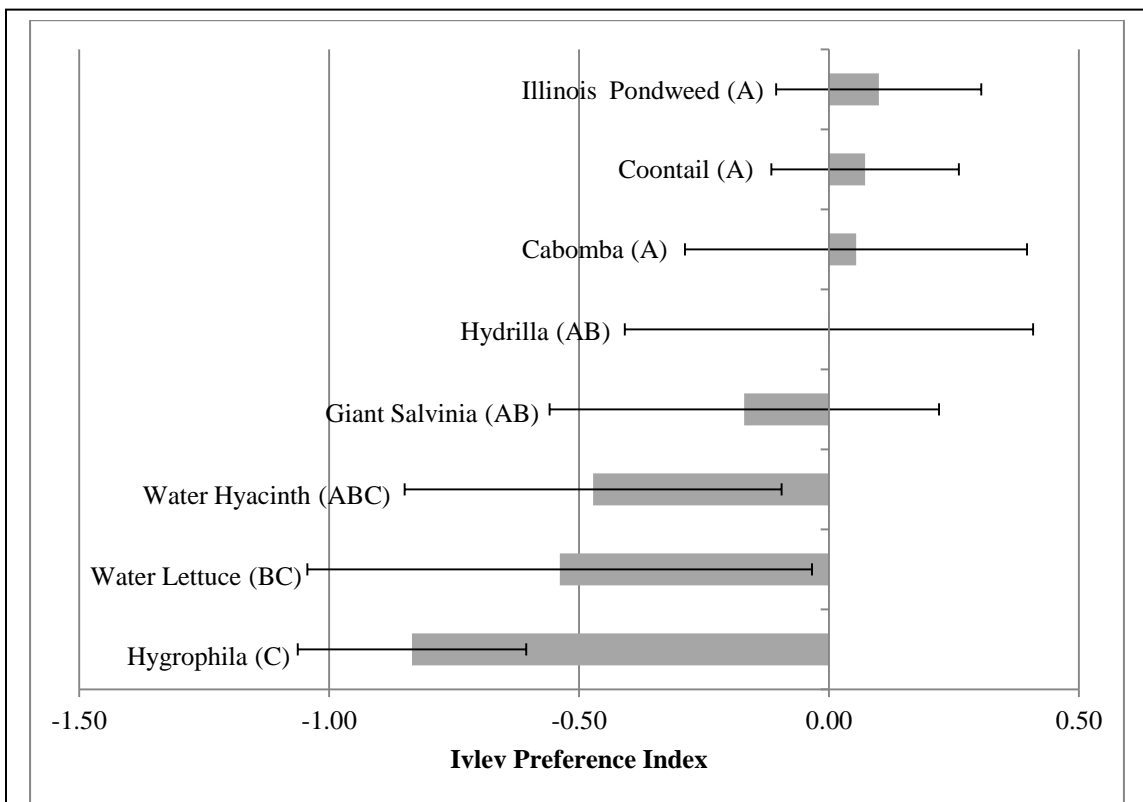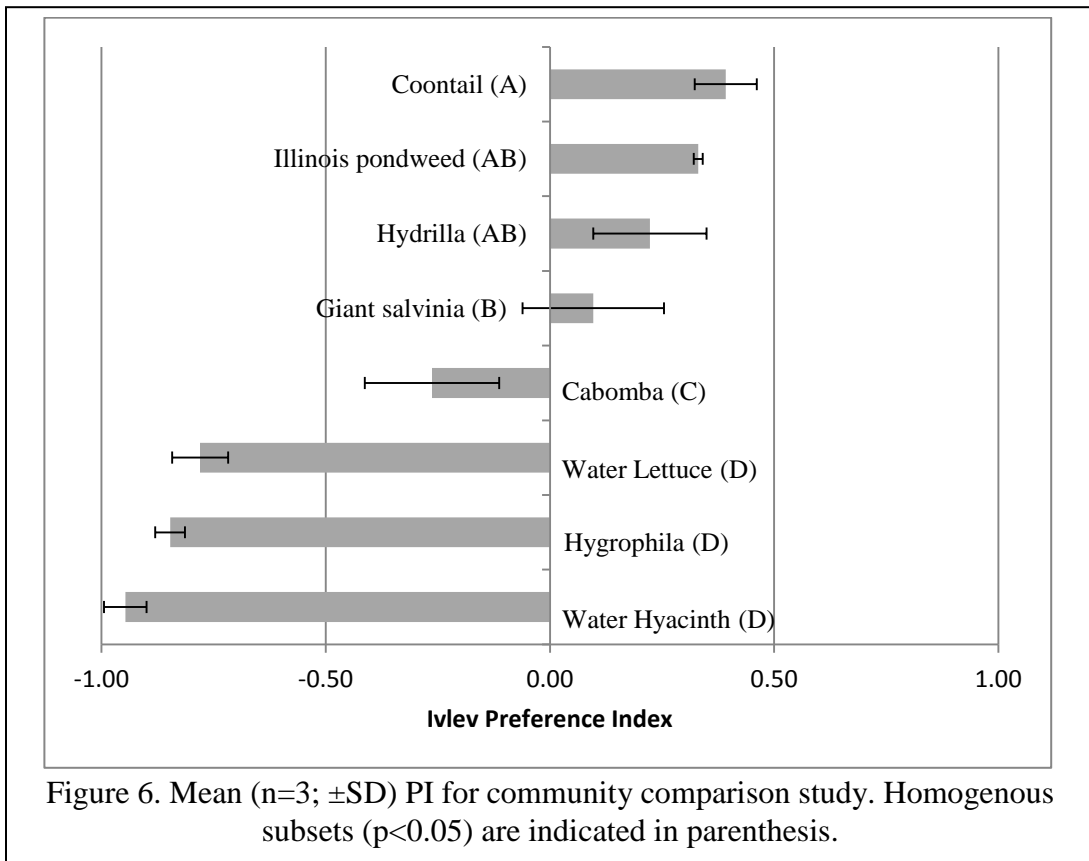

Figure 5. Mean (n=7; ±SD) PI for paired comparison study. Homogenous subsets (p<0.05) are indicated in parenthesis.

Community comparison results were similar to the paired comparison study, with PI values ranging from an average of -0.947 for water hyacinth to 0.392 for coontail (Figure 6). Tukey post-hoc comparisons of the eight species indicate four homogenous subsets, with water hyacinth (M=-0.947, 95% CI [-1.066,-0.829]), hygrophila (M=-0.847, 95% CI [-0.929,-0.765]), and water lettuce (M=-0.780, 95% CI [-0.936,-0.624]) significantly ($p<0.05$) different than cabomba (M=-0.263, 95% CI [-0.636,0.101]), giant salvinia (M=0.097, 95% CI [-0.294,0.488]), hydrilla (M=0.223, 95% CI [-0.091,0.537]), Illinois pondweed (M=0.331, 95% CI [0.306,0.355]), and coontail (M=0.392, 95% CI [0.221,0.564]); cabomba significantly ($p<0.05$) different than giant salvinia, hydrilla, Illinois pondweed and coontail; and giant salvinia significantly ($p<0.05$) different than coontail.

Figure 6. Mean (n=3; ±SD) PI for community comparison study. Homogenous subsets (p<0.05) are indicated in parenthesis.

Discussion

Our findings showed that triploid grass carp consumed giant salvinia, which is the first time this has been documented in a controlled setting. Previous research concluded that triploid grass carp would not consume giant salvinia even if it is the only plant available, most likely because the plant contains a metabolic inhibitor that is toxic to fish if consumed in quantity (Sanders, unpubl. data). Our research indicates that juvenile triploid grass carp will consume giant salvinia when presented as both a single food source as well as select for it in a community. It should be noted that giant salvinia utilized in our study was a mix of secondary and tertiary growth forms, and it is likely that consumption rate and overall preference varies between growth forms, with primary and secondary growth preferred over dense tertiary growth due to their relative ease of handling. Despite contradicting previous research, this conclusion is not entirely unexpected, as grass carp have been observed to feed on giant salvinia in a natural setting (Masser, personal communication), and during certain stages of growth is similar to other floating macrophyte species known to be consumed by grass carp.

Our experiments indicate that hygrophila is nearly unpalatable to triploid grass carp with the lowest and second to lowest PI in our experiments. This result is similar in conclusion to Cassani (1981) in which underyearling hybrids of grass carp and bighead carp (*Hypophthalmichthys nobilis*) fed very slightly on leaves of the plant. Observations by Cassani and Vandiver (1989) from grass carp stockings in two Florida urban canals had mixed results, with hygrophila heavily consumed in one canal section and unconsumed in another. Sutton (1995) found that grass carp successfully controlled hygrophila using fish larger than 0.45 kg at high stocking rates and only if more preferred

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plants are not present. The overall low preference and consumption of hygrophila observed in our experiments is likely due to the size of the fish utilized (less than 0.5 kg) as well as the presence of cystoliths, enlarged cells containing crystals of calcium carbonate, covering the stems and leaves of the plant (Cuda and Sutton 1999).

Our research also indicates that temperature has a significant effect on the consumption of plant material by grass carp. We observed not only a significant difference in consumption at low and high temperatures utilized in our experiments, but also an interaction effect between temperature and species. This finding is not entirely unexpected as numerous previous experiments have found that consumption rate and selectivity is moderately to highly dependent on temperature (Pine et al. 1990, Pipalova 2006)

Of much interest in this research was the order in which triploid grass carp would consume plants when multiplespecies were presented. While several previous experiments have studied the feeding preferences of diploid and triploid grass carp for various macrophytes (Cross 1969, Pine et al. 1990, Pine and Anderson 1991, Catarino et al. 1997), this is the first to include giant salvinia and hygrophila. We observed two distinct preference groups, with water lettuce, water hyacinth and hygrophila consistently different, and less preferred, than cabomba, giant salvinia, hydrilla, Illinois pondweed and coontail. Overall, the order of preference we perceived in our research was similar to other studies, with minor discrepancies likely due to age and size of the grass carp utilized as well as temperature.

From a management perspective, this research provides valuable insight regarding the potential impacts to plant communities from triploid grass carp. Although grass carp

are typically stocked to control nuisance hydrilla, out research found that their preference for the species is not significantly different than that of three other species, including two native and desirable species (Illinois pondweed and coontail). We also found that grass carp would moderately select for giant salvinia, even when given the option of a multitude of species. Not only that, but it was found that their preference for giant salvinia was significantly greater than four other species (water hyacinth, hygrophila, water lettuce and cabomba) and only significantly less than one (coontail). Based on grass carp consumption and preference for giant salvinia, it is probable that they could be used as a control mechanism, at least in a limited role.

On the other hand, our research indicates that grass carp are an extremely poor control option for hygrophila. Not only did the species have one of the consistently lowest preferences, grass carp would rarely consume the plant in any detectable fashion.

Conclusion

In summary, this research is the first to document the consumption rate of giant salvinia and hygrophila by triploid grass carp as well as its preference as compared to native and invasive macrophytes common in the Southeast United States. Hygrophila was scarcely consumed by grass carp and unpreferred to other species utilized in our experiment. Giant salvinia was consumed at a modest rate and was observed to be moderately preferred. Overall, triploid grass carp could be assumed to be a poor control option for hygrophila and could potentially control giant salvinia, keeping in mind their effects on the macrophyte community. Future research should more closely study the consumption rate and preference of the three distinct growth forms of giant salvinia. This

research indicates that it is possible grass carp could be effectively used in the early stages of a giant salvinia infestation, where primary growth is dominant, to prevent or slow the spread of the plant.

CHAPTER IV

CONCLUSIONS

Our findings in the *P. parvum* and *Anabaena* experiments indicate that interactions in nature are often more complex and intricate that we presume. Although *Anabaena* was predicted to have a negative impact on the growth of *P. parvum*, we found that it actually stimulated growth, likely due to an increase in heterotrophic bacteria that *P. parvum* could feed on. While this result was unanticipated, it reinforced the idea that *P. parvum* is an extremely adaptable species capable of modifying its feeding behavior in relatively short time.

Our findings in the grass carp feeding experiments were the first of their kind. Although previously observed in the wild, our research was the first controlled experimental confirmation of triploid grass carp consuming giant salvinia. This finding has the potential to impact how aquatic invasive plants are managed and provides an alternative to other control options for giant salvinia. By understanding the preference triploid grass carp have for both invasive and native species, we can better understand their overall effect on species diversity.

Overall, the research in this thesis has provided valuable insight into the functions and threats to freshwater resources, and it is our hope that it will be used in the protection and management of these important ecosystems.

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