BEYOND THE EDGE OF A NICHE: THE ROLE OF GRAZING ON *PRYMNESIUM PARVUM* BLOOM FORMATIONS IN TWO TEXAS LAKES

A Thesis

by

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ABSTRACT

Prymnesium parvum (golden algae) is a harmful algal bloom species that has caused tens of millions of dollars in natural resource damages in Texas as a result of massive fish kills. This species is present in many Texas lakes, but does not form blooms in all of them. Previous literature has suggested that predation, specifically by rotifers, may be an important loss factor to *P. parvum* populations. This research is focused on investigating whether grazing by rotifers is a significant factor in the prevention of blooms in some lakes. Three-day in-lake mesocosm experiments were conducted during times of bloom initiation and bloom development in two Texas lakes (Whitney and Somerville) where the former experiences *P. parvum* blooms and the latter does not. Controls and treatments consisted of *P. parvum* cultures in log- and stationary-growth phases, natural phytoplankton assemblages, and natural rotifer assemblages from each lake. Monitored parameters included *P. parvum* population density and population growth rate, toxicity, chlorophyll-*a*, and zooplankton biovolume.

Findings reveal that rotifers in Lake Somerville preferentially grazed *P. parvum* of all growth phases, while experiencing no negative effects from exposure. Some rotifers even exhibited trends of positive effects from exposure. The mesocosm experiments confirmed that rotifer assemblages in Lake Somerville effectively graze *P. parvum* with no observed negative effects. These findings support the theory that grazing of *P. parvum* by rotifers is an important contributing factor preventing blooms in Lake Somerville. On the other hand, rotifers from the winter Lake Whitney experiment

appeared to preferentially graze *P. parvum* populations inoculated from log-growth phase culture while significant reductions in rotifer biovolume were observed. *P. parvum* inoculated from stationary-growth phase culture were not significantly grazed, nor were other phytoplankton within the treatment suggesting that sub-lethal toxic effects from ingestion of stationary phase cells caused a decrease in zooplankton metabolic rates. These lake-specific relationships between *P. parvum* and rotifers may have been due to microevolutionary adaptation. For example, variation in biovolume responses of rotifers to *P. parvum* exposure between lakes is a result of rapid microevolutionary adaptation from constant low-level toxin exposure in Lake Somerville as a function of salinity.

DEDICATION

This thesis is dedicated to all struggling graduate students. There is an end to the madness… eventually.

A colleague and dear friend of mine once said in exasperation, “The things we do for science…” I have never heard a truer statement regarding research in my young and inexperienced life. Most people outside your field of study will never know, understand, or fully appreciate the enormous amount of time and effort you have devoted to your work. So refuge must be taken in the thought that you may discover something that no one else has ever known. Someday, someone or something might even benefit from your discovery.

I would also like to dedicate this thesis to my family and close friends. Without them, life would be meaningless and not nearly as fun.

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1. INTRODUCTION

In the field of aquatic ecosystem management, many countries have developed regulatory framework to help manage risk of chemical contaminants such as pesticides, fertilizers, and detergents. For example, phosphate concentrations have been reduced in many detergents to prevent eutrophication as a result of research by Schindler (1974). However, standard management approaches for toxins produced by harmful algal blooms (HABs) have been poorly developed to date (Brooks et al. 2011).

Algal blooms are a result of a period of rapid growth of algae in response to a variety of environmental factors that involve nutrient loads, salinity, temperature, pH, competition, etc. (Roelke and Buyukates 2001). Marine and freshwater HABs are increasing globally in frequency and magnitude, which has resulted in greater environmental and public health threats (Hallegraeff 1993, Fu et al. 2012). Thus, more preventative management approaches should be considered.

Many HABs produce toxins that negatively impact other organisms such as competing species of phytoplankton, predatory zooplankton, fish, shellfish, birds, small mammals, livestock, and even humans. Negative effects from HABs on aquatic organisms include reduced feeding, fecundity, growth, development, swimming ability, and increased mortality (Van Dolah et al. 2001, Landsberg 2002). Effects on mammals include decreased movement, paresis, cyanosis, tremors, and death when exposed to HAB toxins (Carmichael 2001). Poisoning of humans by HABs can occur from consumption of contaminated seafood, drinking water, or inhalation as some toxins from

species may be aerosolized or volatilized. Adverse effects of toxin ingestion include nausea, vomiting, diarrhea, sore throat, headache, seizures, par aesthesia, respiratory paralysis, tachycardia, permanent loss of short-term memory, and death (Carmichael 2001, Van Dolah et al. 2001, Landsberg 2002).

Unfortunately, the environmental factors causing HABs are widely variable and frequently species-specific (Roelke and Buyukates 2001, Brooks et al. 2011). Therefore, a universal approach to HAB management is not likely, and more focused management approaches that target specific organisms must be pursued. Some phytoplankton species bloom in particular bodies of water while not in others. The understanding of why blooms of certain species occur in specific lakes is a crucial piece of knowledge for stronger aquatic ecosystem management. A better comprehension of bloom dynamics for phytoplankton species may enable bloom management in impacted lakes, and prevent the spread of blooms into unimpacted lakes. One example of a problematic alga that requires more research for understanding bloom dynamics that could lead to effective management techniques is *Prymnesium parvum*.

Prymnesium parvum is a globally occurring mixotrophic haptophyte (Graneli et al. 2012), which was characterized as a harmful algal bloom (HAB) species by the Intergovernmental Oceanographic Commission of the United Nations Educational, Scientific, and Cultural Organization (Skovgaard and Hansen 2003). The alga is capable of forming large fish-killing blooms, and is tolerant of large variations of salinity, and temperature (Larsen and Bryant 1998, Baker et al. 2007, Baker et al. 2009). In the past 28 years this species of golden algae has invaded to southern regions of the USA with

the first documented bloom occurring in the Pecos River, TX, USA in 1985 (James and De La Cruz 1989). Currently, *P. parvum* is found in 18 U.S. states (i.e. AL, AR, AZ, CA, FL, HI, LA, ME, MS, NC, NM, OK, PA, SC, TX, WA, WV, WY) (Sager et al. 2008, Roelke et al. 2011).

Blooms of *P. parvum* typically occur in aquatic systems that are eutrophic and brackish (Kaartvedt et al. 1991, Guo et al. 1996, Roelke et al. 2007, Hambright et al. 2010). Late autumn-early winter is usually when blooms occur in North America, and they persist through the spring months (Roelke et al. 2007, Remmel et al. 2011). *P. parvum* is responsible for harmful blooms worldwide that result in large economic losses (Moestrup 1994). In the state of Texas alone, *P. parvum* blooms have caused tens of millions in U.S. dollars in natural resource damages, and is estimated to have killed 34 million fish (Southard et al. 2010, Brooks et al. 2011). Because of this, fisheries and recreation are threatened by seasonal *P. parvum* blooms in reservoirs (Brooks et al. 2011). Furthermore, Roelke et al. (2012) suggested that environmental conditions conducive to *P. parvum* blooms may become more common with climate change.

Toxicity is the main characteristic of *P. parvum* that causes fish kills and suppression of other aquatic organisms. Toxins produced by *P. parvum*, also called prymnesins, were shown to possess cytotoxic, hemolytic, neurotoxic, and ichthyotoxic properties (Yariv and Hestrin 1961, Parnas 1963, Paster 1973). Of the prymnesins studied, prymnesin-1 and prymnesin-2 were thought to be the main toxins contributing to toxicity of aquatic organisms (Igarashi et al. 1998, Igarashi et al. 1999). However, other research has pointed towards additional toxins that have not been characterized

(Schug et al. 2010) or other products of *P. parvum* cells such as fatty acids (Henrikson et al. 2010) and fatty acid amides (Bertin et al. 2012b, a).

The toxicity of *P. parvum* is harnessed through the phenomenon of allelopathy. Allelopathy is any process including secondary metabolites produced by algae, plants, fungi, and bacteria that impact the growth and development of biological systems (Graneli and Salomon 2010). *Prymnesium* spp. produce chemical substances that when released from the cell are hemolytic to other organisms, disrupting cellular membranes and causing lysis. Therefore these chemicals inhibit growth or kill bacteria and competing phytoplankton species, which then serve as prey to *P. parvum* (Graneli and Johansson 2003a, Skovgaard and Hansen 2003, Tillmann 2003). These toxins also cause a change in the selective permeability of gill epithelial cells, therefore inhibiting respiration of gill breathing organisms such as zooplankton and fish (Yariv and Hestrin 1961, Shilo 1967, Hallegraeff 1993). Currently, no cases of adverse effects to humans have been documented (Paster 1973, Lindholm et al. 1999). Production of allelopathic toxins is enhanced under nitrogen and phosphorus limited conditions (Graneli 2006, Roelke et al. 2007, Errera et al. 2008) as well as high turbulence or aeration (Igarashi et al. 1995).

There is no consensus on the effects of light on *P. parvum* toxin production due to diverse results from research studies. For example, Shilo (1967) concluded light was crucial to toxin production, while other studies determined that light intensity did not significantly influence *P. parvum* toxicity to fish or nauplii (Larsen and Bryant 1998, Baker et al. 2007). *P. parvum* toxicity was also found to be higher in low light conditions

and darkness (Hagstrom and Graneli 2005, Graneli and Salomon 2010). A more recent study found that photodegredation of *P. parvum* toxins occurred when cell free filtrates were exposed to full and partial sunlight for several hours, which resulted in decreased toxicity to fish (James et al. 2011a).

Research has shown inconsistent findings on the relationship between temperature and toxin production over the years as well. Results from Larsen and Bryant (1998) as well as Ulitzur and Shilo (1964) indicated that *P. parvum* toxin production was not dependent upon temperature. On the contrary, a general increase in *P. parvum* toxicity to fish was observed towards lower temperatures in the range of 5-35°C by Baker et al. (2007). Furthermore, Grover et al. (2007) observed a higher toxic effect on fish when *P. parvum* was grown at 20°C compared to 30°C and 10°C.

There is also no consensus on the relationship between toxin production and salinity due to dissimilar results from different studies. Shilo (1967) concluded that increasing salinity led to lower toxicity of *P. parvum* cells. Larsen and Bryant (1998) found salinity and toxicity to have no correlation. More recently, Baker et al. (2007) observed maximum toxicity of *P. parvum* at 7.5 and 35 psu with less toxic cells grown at intermediate salinities.

Early studies involving the influence of pH to *P. parvum* in marine/estuarine ecosystems noted that the algae was not ichthyotoxic at a pH of 7 or below, but became more toxic at higher pH levels (Shilo and Aschner 1953, Ulitzur and Shilo 1964). A recent study observed a similar relationship of pH and *P. parvum* toxicity in inland waters (Prosser et al. 2012). Overall, a review by Graneli and Salomon (2010) concluded

that *P. parvum* increases toxin production under conditions not optimal for growth (i.e. N or P deficiency, low light, or high turbulence).

Allelopathy of *P. parvum* is also influenced by growth phase. Observations from Shilo (1967) found that *P. parvum* hemolytic activity toward co-occurring algae was greatest in stationary phase growth and lowest in log or exponential growth. More recent studies have had similar findings (Johansson and Graneli 1999, Graneli and Johansson 2003b). While *P. parvum* itself is allelopathic, the harmful alga exhibited resistance to allelopathic effects of some cyanobacteria and dinoflagellates (Suikkanen et al. 2004, Tillmann et al. 2007, Neisch et al. 2012). The presence of *P. parvum* in many water bodies in Texas is known (Patiño et al. 2014). However, not all *P. parvum* inhabited lakes experience blooms. Speculation as to why blooms do not occur in certain lakes resulted in some studies, which suggested that algicidal bacteria and allelopathic phytoplankton might influence *P. parvum* bloom development (Grover et al. 2010, Roelke et al. 2010, James et al. 2011b). However, because there is no consensus as to the cause of this phenomenon, this topic merits further research.

Bloom dynamics may be influenced by top-down predation. But, the bulk of research on this topic has been conducted in marine environments. Research investigating this aspect of potential bloom control in brackish waters was conducted with zooplankton including cladocera, species of *Brachionus*, and other phytoplankton (Brooks et al. 2010, Remmel et al. 2011). Yet, observations from this prior research called for a closer look at the influence rotifers may have on *P. parvum* populations.

2. PURPOSE AND JUSTIFICATION

Some lakes are inhabited by *P. parvum*, but blooms do not form (Roelke et al. 2010). These lakes pose an interesting ecological scenario in which rotifer-*P. parvum* interactions might be a contributing factor preventing blooms. Therefore, in this research project I conducted experiments in a lake where *P. parvum* is present but does not bloom (i.e. Lake Somerville, TX, USA) as well as in a lake that experiences seasonal *P. parvum* blooms (i.e. Lake Whitney, TX, USA).

A study performed by (Schwierzke et al. 2010) showed rotifer-dominated zooplankton communities that prospered during toxic blooms of golden algae. These zooplankton communities were almost exclusively of the species *Notholca laurentiae*, suggesting that this species shows an attribute most zooplankton do not, i.e., resistance to toxins produced by golden algae. Therefore, it is reasonable to assume that communities with *N. laurentia* may have an influence on bloom initiation and development. Observations from Errera et al. (2008) suggested that grazing of *P. parvum* by rotifers might be an important loss factor for *P. parvum* populations during the fall under nutrient sufficient conditions. Thus, the objective of this research was to investigate the impact of rotifers on *P. parvum* bloom dynamics in lakes that experience and do not experience blooms.

3. RESEARCH OBJECTIVES

The overarching objective of this research was achieved by accomplishing the following specific objectives:

1. Collecting rotifer populations from a natural setting in Lakes Somerville and Whitney during the fall period of bloom initiation (Experiment 1) and the winter period of bloom development (Experiment 2).
2. For both periods, performing *in-situ* mesocosm-scale field experiments with naturally occurring phytoplankton and rotifer populations combined with lab grown *P. parvum* cultures to determine taxon-specific resistance to toxins produced by *P. parvum*, and ability to feed on toxic populations.

The methods, results and discussion of Experiment 1 from the fall are described in Appendix A. Due to inclement weather and equipment failures, findings from the fall experiment are limited. Consequently, the sections below focus on the winter experiment.

4. MATERIALS AND METHODS

4.1 Site Description

Lake Somerville is a United States Army Corps of Engineers (USACE) reservoir that was impounded in 1967. The lake is located on Yegua Creek in the Brazos River basin approximately 48 miles southwest of Bryan, TX, USA. Drainage area above the dam is approximately 1,619 km^2. Reservoir capacity at conservation storage elevation is approximately 1.97×10^8 m^3. Lake Somerville has an approximate surface area of 46 km^2, shoreline of 137 km, and maximum depth of 12 m (Bailes and Hudson 1982, TPWD 2013a, TSHA 2013). Lake Somerville is classified as a hypereutrophic lake (TCEQ 2011). Historical inorganic nutrient data from 1999 to 2002 indicates that soluble reactive phosphorus (SRP) ranged from around 0.05 to ~0.8 μmol L^{-1}, and dissolved inorganic nitrogen (DIN) ranged from 1 to ~25 μmol L^{-1} (Roelke et al. 2012).

Lake Whitney is also a USACE reservoir in the Brazos River basin. The lake was impounded in 1951 and is approximately 3 km west of Whitney, TX, USA. An area of approximately 42,107 km^2 drains into the lake. Reservoir capacity at conservation storage elevation is approximately 4.68×10^8 m^3. Lake Whitney has an approximate surface area of 95 km^2, shoreline of 362 km, and maximum depth of 33 m (Bailes and Hudson 1982, Breeding 2013, TPWD 2013b). Lake Whitney is classified as a hypereutrophic lake (TCEQ 2011). Data of inorganic nutrients sampled from 2008 to 2009 indicate that SRP ranged from around 0.2 to 1.6 μmol L^{-1}, and DIN ranged from 0.0 to 55 μmol L^{-1} (Roelke et al. 2004).

4.2 Methods

Objective 1 – Field collection of rotifers took place at Lake Whitney, where rotifer resistant taxa were previously observed, as well as Lake Somerville, where *P. parvum* is present but blooms do not occur. Collections took place during periods of bloom development (winter). Water samples at a depth of 0.5 m were collected from one location within each lake. These locations were in the open water regions of each lake near their respective dams.

To collect rotifer communities, a bucket volumetrically calibrated was used to collect 12 L (the volume of water collected in a standard Schindler trap, which was used in previous studies of these lakes). The water captured by this bucket was poured through a cod end equipped with 61 μm mesh. The retained contents of the cod end were then rinsed and passed through a 210 μm mesh net into another container. Thus, organisms within the 61 μm to 210 μm size range (primarily rotifers) were isolated. The container of the rotifer size fraction was brought to a larger volume and gently mixed. Aliquots from this well-mixed volume were used to initiate the appropriate treatments of the *in-situ* experiments. Aliquot volumes when added to the filtered water volumes in the experimental units resulted in restoration of the original rotifer population densities.

Objective 2 – Mesocosm experiments designed to focus on rotifer-*P. parvum* interactions consisted of 12 total treatments. Treatments 1 through 9 served as controls consisting of only phytoplankton contained in 2 L polycarbonate bottles. Treatments 10 through 12 consisted of rotifer and phytoplankton assemblages contained in 20 L polycarbonate carboys. Treatments in 2 L bottles were filled to 2 L and treatments in 20

L carboys were filled to 15 L during winter experiments with sufficient air left in the headspace of each experimental unit for neutral buoyancy. All treatments were performed in triplicate for a total of twenty-seven 2 L bottles and nine 20 L carboys. Details of the treatments are as follows:

Filtered lake water was used in all treatments except treatment 1 and 2. Lake water was passed through various filters of several sizes for different treatments. For example, rotifer-sized zooplankton were isolated from the natural assemblage by passing lake water through a 61 µm screen followed by a screening of the retained volume through a 210 µm mesh (as described previously). Nanoplankton were isolated by passing the 61 µm-filtered water through a 20 µm mesh net and keeping the retained volume. For the winter experiment, particle free lake water was achieved by using a 1.0 µm cartridge filter (GE Water and Process Technologies, Clearwater Drive, Minnetonka, MN, USA) in combination with a 0.2 µm capsule filter (Whatman GE Healthcare, Piscataway, NJ, USA).

Treatment 1 and 2: The first two treatments were designed to test if *P. parvum* cultures were able to grow under the light, turbulence, and temperature conditions present at each lake. These treatments consisted of reverse osmosis (RO) water that was brought to f/2 nutrient concentration (Guillard 1975). The salinity of the water was adjusted to be similar to each lake (i.e. 0.5 psu for Lake Somerville and 0.75 psu for Lake Whitney) with additions of artificial sea salt (Instant Ocean). *P. parvum* cultures in log (treatment 1) or stationary (treatment 2) growth phase were then added. *P. parvum* inoculation densities resulted in approximately 4,000 cells mL^{-1} for log-growth phase

cultures in the winter experiments. Inoculation of stationary-growth phase *P. parvum* cultures resulted in densities of approximately 200 cells mL^{-1} in Lake Whitney and 550 cells mL^{-1} in Lake Somerville. Log- growth phase *P. parvum* was inoculated into treatment 1 and stationary-growth phase *P. parvum* was used in treatment 2 (Figure 1).

Treatment 3 and 4: 0.2 µm filtered lake water was brought to f/2 nutrient concentration and inoculated with *P. parvum* cultures in log (treatment 3) or stationary (treatment 4) growth phase at densities described in treatment 1 and 2 (Figure 1). The filtered water in these treatments compared with treatments 1 and 2 allowed for the observation of the effect of unknown dissolved constituents (with any potential inorganic nutrient limitation effects removed) in the lake water, such as humic acids and pollutants, that might influence algal growth.

Treatment 5 and 6: 0.2 µm filtered lake water was inoculated with log (treatment 5) or stationary (treatment 6) growth phase *P. parvum* cultures at densities described in treatment 1 and 2 (Figure 1). These treatments combined with treatments 3 and 4 allowed the effects of nutrient additions on *P. parvum* cultures to be observed.

Treatment 7 and 8: 20 µm filtered lake water containing the natural phytoplankton assemblage was inoculated with log (treatment 7) or stationary (treatment 8) growth phase *P. parvum* cultures at densities described in treatment 1 and 2 (Figure 1). These treatments combined with treatments 5 and 6 allowed the effects of natural phytoplankton assemblages on *P. parvum* in both lakes to be observed.

Treatment 9: 20 µm filtered lake water containing only the natural phytoplankton assemblage of each lake (Figure 1). This treatment combined with treatments 7 and 8

allowed natural phytoplankton assemblage and *P. parvum* culture interactions to be observed.

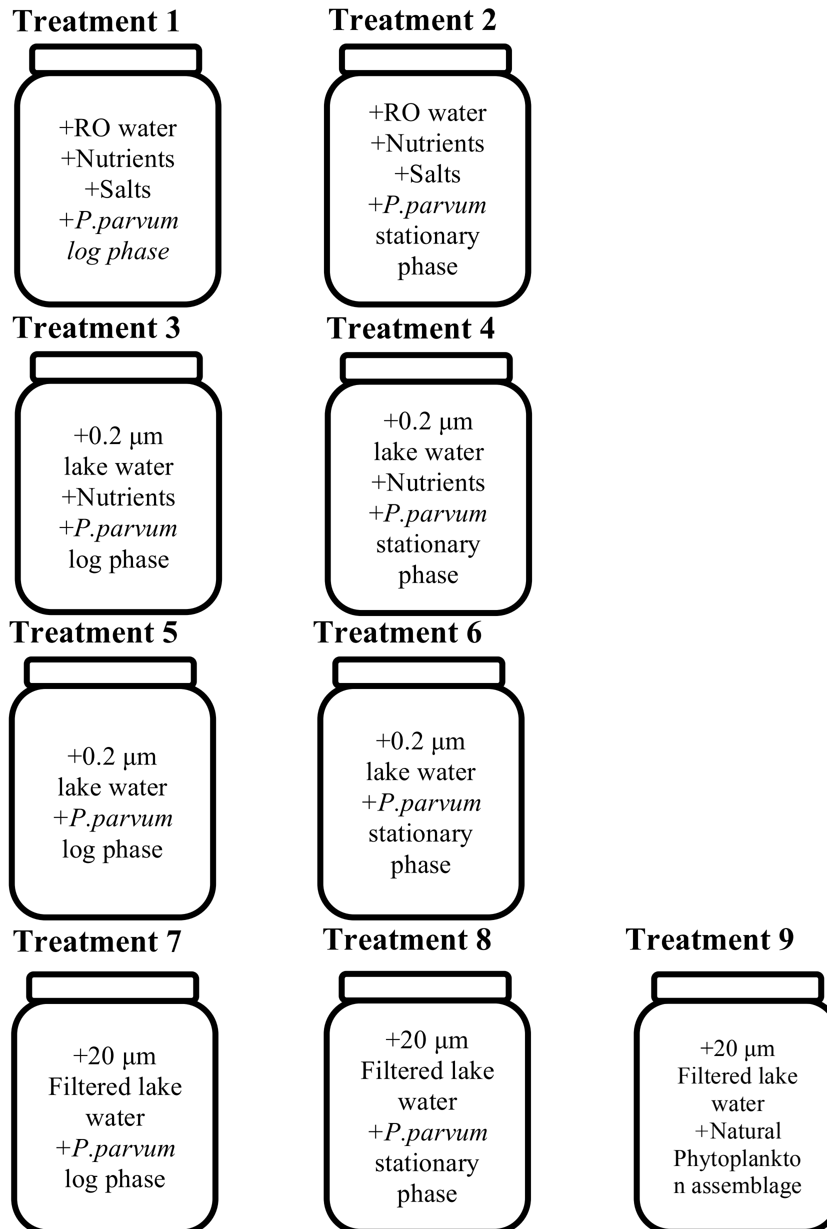

Figure 1: Treatments in triplicate consisting exclusively of phytoplankton in 2-liter bottles.

Treatment 10: 20 µm filtered lake water with added 61 – 210 µm rotifer size fraction obtained in objective 1 (Figure 2). This treatment combined with treatment 9 allowed for observation of how natural zooplankton assemblages in the 61 – 210 µm size range affect natural phytoplankton assemblages.

Treatment 11 and 12: 20 µm filtered lake water with added 61 – 210 µm rotifer size fraction and inoculated with log (treatment 11) or stationary (treatment 12) growth phase *P. parvum* at densities described in treatment 1 and 2 (Figure 2). These treatments combined with treatments 7, 8, and 10 allowed for observation of how log and stationary phase *P. parvum* cultures interact with natural zooplankton and phytoplankton assemblages.

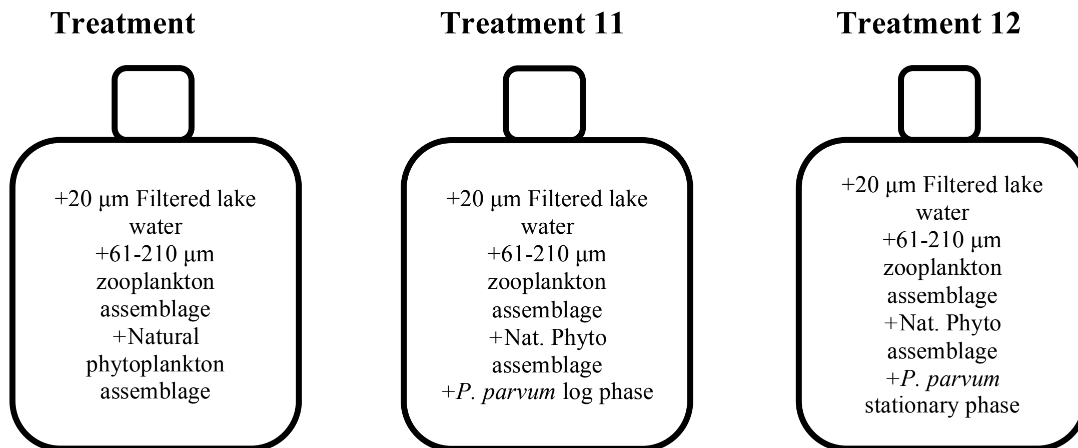

Figure 2: 20-liter carboy treatments in triplicate involving zooplankton in the 61 – 210 µm size range.

All 2 L bottles were floated in limno corrals on both lakes during *P. parvum* bloom development conditions (winter). Limno corrals were also used to house 20 L carboys. Limno corrals were covered with a neutral density screen in order to simulate the natural light environment of the euphotic zone at an approximate depth of 0.5 m. The limno corrals used in the Lake Somerville experiment were anchored in a cove directly west of Welch Park. During the Lake Whitney experiment, limno corrals were tethered to a boat dock belonging to the Little Rocky Lodge and Resort. Deploying the treatments in lake waters allowed each culture to experience ambient in lake environmental conditions (i.e. light, turbulence, and temperature). Each set of experiments floated in Lakes Somerville and Whitney for 3 days.

Treatments 1 through 8, 11, and 12 were each inoculated with a *P. parvum* culture in log- or stationary-growth phase. Odd number treatments contained log-growth phase *P. parvum* culture and even number treatments contained stationary-growth phase *P. parvum* culture. The culture of *P. parvum* was maintained in our laboratory at 1 psu salinity following methods reported by (Roelke et al. 2007). Treatments 9 and 10 only contained a natural phytoplankton assemblage. No additional *P. parvum* culture was inoculated into them.

4.3 Response Variables

Metrics and response variables that were monitored include zooplankton biovolume, chlorophyll-*a* concentrations, and *P. parvum* population density. Baseline water quality data (pH, salinity, and temperature) were taken at sampling locations of both lakes on the initial sampling day of the winter experiments using a Hydrolab

Quanta Multiparameter Sonde (Hach Company, Loveland, CO, USA). Sampling of phytoplankton and chlorophyll-*a* from each treatment occurred each day of all experiments starting at the time of deployment. Zooplankton were sampled once at deployment from the initial 61-210 μm isolated size fraction and on the third day from treatments 10, 11, and 12 when experiments were terminated. The initial zooplankton sample was equivalent to a 12 L sample concentrated to a volume between 35-45 mL depending on the total volume of the 61-210 μm size fraction isolated during each experiment. Upon experiment termination, a 12 L sample was taken from treatments 10, 11, and 12 and filtered through the cod end portion of a Schindler trap (61 μm), which was concentrated to 50 mL.

For chlorophyll-*a* analysis, a 50 mL water sample was vacuum pumped through GF/F 47 mm glass microfiber filters. Samples were then frozen until analysis (within 2 weeks of collection). Pigments were extracted using 90% acetone and centrifuging. A fluorometer was used to analyze the pigments following standard methods (APHA 2006).

A 100 mL phytoplankton sample was collected and preserved from each well-mixed bottle and carboy using glutaraldehyde (5% v/v). *P. parvum* population density was enumerated using a settling technique (Utermöhl 1958). Briefly, a subsample ranging from 1 to 5 mL, depending on density of materials in samples, was settled for a 24-h period, then counted using an inverted, phase-contrast light microscope (400x, Leica Microsystems, Bannochburn, Illinois). Around 20 randomly selected fields of view were counted depending on the density of the sample.

Zooplankton samples were preserved in 2% buffered formalin (10% v/v). Subsamples ranging from 5 to 13 mL, depending on material density, were settled for 24 hours, then counted using an inverted, phase-contrast light microscope (40x and 200x, Leica Microsystems). Dimensions for each individual counted were measured corresponding to best-fit geometric shapes in order to estimate biovolume (Wetzel and Likens 1991). Counted rotifers were grouped by genus. Copepods were grouped into copepod nauplii and total adult copepods. Zooplankton groups that made up greater than 5% of the average assemblage biovolume in any treatment, including the initial aliquot, were considered dominant, and all treatment biovolumes for that group were used for statistical analysis in the corresponding experiment.

P. parvum culture toxicity was determined via toxicity bioassays conducted at Baylor University, TX, USA. Juvenile *Pimephales promelas* was used as a model aquatic organism. Percent survival of organisms was calculated after 24 and 48 hours.

4.4 Data Analysis

A one-way ANOVA test was used for analyzing post experiment zooplankton biovolume, and *P. parvum* population density. *P. parvum* population growth rates (μ) were calculated using the following equation:

$$\mu = \left(LN \frac{\left(\frac{Y_f}{Y_{ia}}\right)}{T_d} \right)$$

where Y_f is the final population density, Y_{ia} is the initial average population density of like treatments in order to decrease error that may have been caused by variations in inoculation densities among treatments, and T_d is the time in days of the duration of the

experiment. *P. parvum* population growth rates were analyzed using a one-way ANOVA test as well. Population growth rates of zooplankton were not calculated since only like treatments with the same initial biovolumes were compared. The normality of data was not of concern because ANOVAS are known to be quite robust to non-normality (Underwood 1997).

A one-way repeated measures ANOVA test was used for analyzing differences in chlorophyll-*a* concentrations among all treatments. Only like treatments with the same initial chlorophyll-*a* concentrations were compared so no rates of change were used for statistical analysis. Percent change of chlorophyll-*a* concentrations was calculated using the initial average concentration of like treatments. If the result of any of the ANOVA tests were significant, then a Tukey's HSD post-hoc test was used to determine which treatments were significantly different ($\alpha < 0.05$). The statistics software package SPSS version 21 was used for all ANOVA tests.

5. RESULTS

5.1 Toxicity Bioassays

P. parvum cultures used in all experiments were virtually non-toxic to fish as percent survival of *Pimephales promelas* was 100% in almost all treatments after 48 hours. The exception was an 80% survival rating in one replicate from treatments 6, 9, and 12 of the winter Lake Somerville experiment. An 80% survival rating was also observed in one treatment 11 and 12 replicate from the winter Lake Whitey experiment.

5.2 Lake Somerville Winter Experiments

5.2.1 Initial lake conditions

During the winter experiment, water temperature in Lake Somerville was 14.75°C at a depth of 1 m. Salinity was 0.21 psu and pH was 7.21.

5.2.2 Population density and growth rates of **P. parvum** *and chlorophyll*-**a** *concentrations*

5.2.2.1 Effects of ambient lake conditions and water quality other than inorganic nutrients

Varied growth performance was observed between *P. parvum* populations inoculated from log- and stationary-growth phase cultures, with populations originating from log-growth phase culture accumulating densities under Lake Somerville conditions, when nutrients were not limiting due to f/2 additions, and populations originating from stationary-growth phase culture decreasing in density. *P. parvum* populations inoculated from log- and stationary-growth phase cultures decreased in density under Lake

Somerville conditions when nutrients were limiting. These observations are fully described in Appendix B.

5.2.2.2 Effects of (or influence of) natural phytoplankton assemblages and zooplankton grazers in the 61-210 µm size range

5.2.2.2.1 Treatments inoculated with log-growth phase **P. parvum**

Experimental results indicated that zooplankton assemblages from the winter season at Lake Somerville significantly grazed *P. parvum* inoculated from log-growth phase culture. The population growth rate of *P. parvum* inoculated from log-growth phase culture in filtered lake water containing organisms < 20 µm was 0.04 d^{-1} (treatment 7, Table 1, Figure 3A). On the other hand, *P. parvum* populations declined by -0.27 d^{-1} in the similar treatment that also contained zooplankton grazers in the 61-210 µm size range (treatment 11), and the population growth rates were statistically different between the two treatments (Tukey's HSD, $p < 0.05$; Table 1, Figure 3A). Chlorophyll-*a* concentrations increased by 16 ± 10% and 40 ± 13% in treatments 7 and 11 respectively, and concentrations were statistically different (Tukey's HSD, $p < 0.05$; Figure 3B). Prominent phytoplankton observed in the natural phytoplankton assemblages included cyanobacteria, chlorophytes, and chrysophytes.

Table 1: *Prymnesium parvum* population growth rates (day^{-1}) in winter experimental treatments. Odd numbered treatments were inoculated with log-growth phase *P. parvum* culture, and even numbered treatments were inoculated with stationary-growth phase *P. parvum* culture with the exception of treatments 9 and 10, which only contained assumed log phase *P. parvum* occurring in the natural phytoplankton assemblages. Experiments were conducted in early March 2013. Values shown are mean ± 1 SD, with n = 3.

	Lake Somerville		Lake Whitney	
Treatment	Log phase growth	Stationary phase growth	Log phase growth	Stationary phase growth
1 & 2	0.11 ± 0.08	-0.13 ± 0.06	0.05 ± 0.07	0.17 ± 0.11
3 & 4	0.01 ± 0.02	-0.35 ± 0.12	0.05 ± 0.08	0.09 ± 0.10
5 & 6	-0.10 ± 0.04	-0.71 ± 0.27	0.06 ± 0.03	-0.05 ± 0.05
7 & 8	0.04 ± 0.09	0.09 ± 0.05	0.07 ± 0.07	0.03 ± 0.07
9	0.07 ± 0.01	-	0.09 ±0.09	-
10	-0.21 ± 0.03	-	-0.12 ± 0.04	-
11 & 12	-0.27 ± 0.02	-0.25 ± 0.05	-0.13 ± 0.06	-0.01 ± 0.07

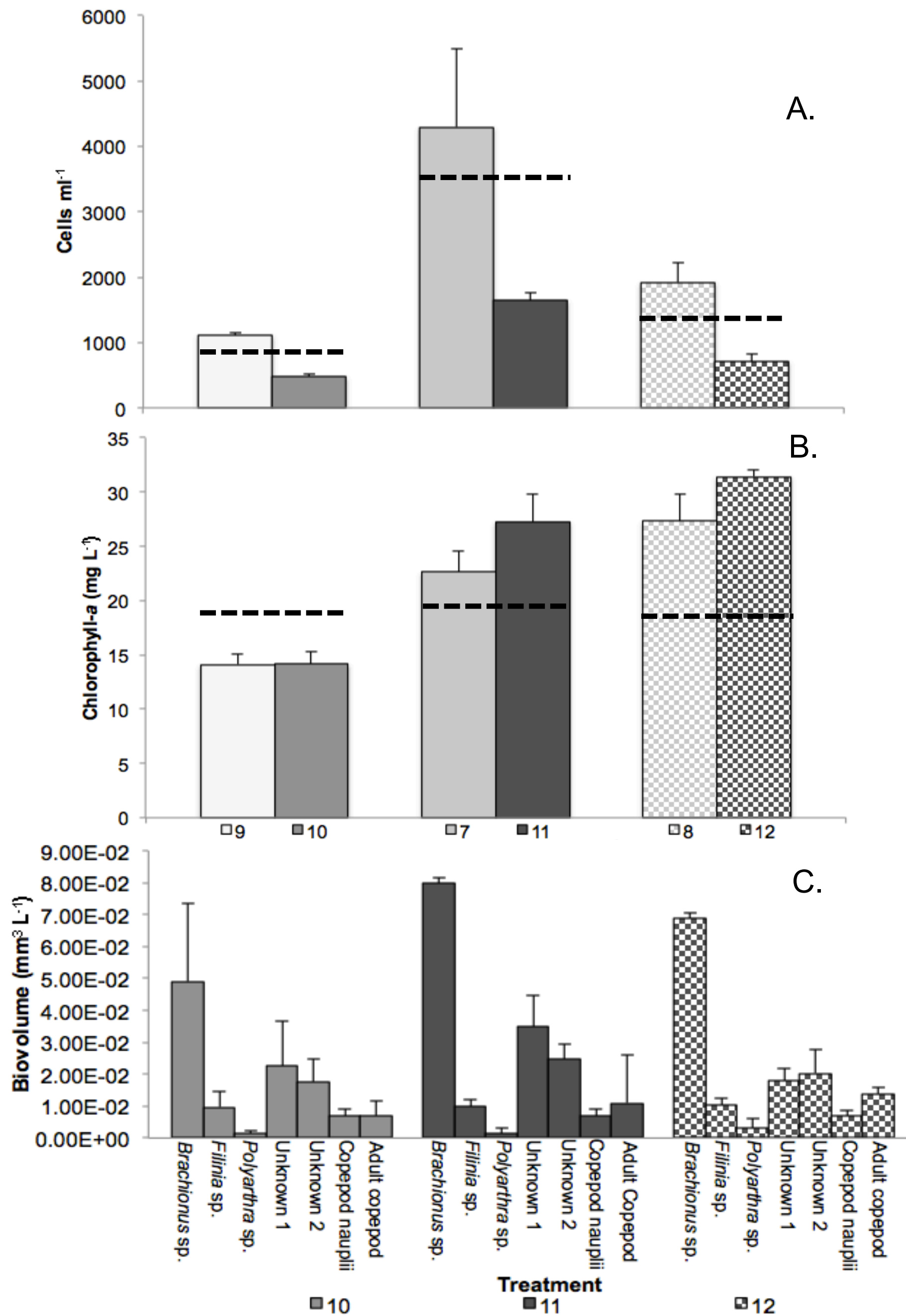

Figure 3: Treatment 7, 8, 9, 10, 11 and 12 final average ± 1 SD *P. parvum* population densities (A.), chlorophyll-*a* concentrations (B.), and biovolumes of dominant and sub-dominant zooplankton groups *Brachionus* sp., *Filinia* sp., *Polyarthra* sp., adult copepods, copepod nauplii, and the unidentified illoricate rotifers unknown 1 and unknown 2 (C.) for the Lake Somerville experiment conducted during winter 2013. The horizontal dashed lines represent the initial averages of like treatments. Treatments 9 and 10 only contained natural phytoplankton assemblages, while treatment 10 also contained zooplankton in the 61-210 µm size range. Treatments 7, 8, 11, and 12 contained natural phytoplankton assemblages while 7 and 11 were inoculated with *P. parvum* in log-growth phase, and 8 and 12 were inoculated with stationary-growth phase. Treatments 11 and 12 also contained zooplankton in the 61-210 µm size range.

5.2.2.2.2 Treatments inoculated with stationary-growth phase **P. parvum**

Results indicate that zooplankton assemblages from the winter season at Lake Somerville significantly grazed on *P. parvum* populations inoculated from stationary-growth phase culture. During the winter experiment, *P. parvum* population densities inoculated from stationary-growth phase culture in filtered lake water containing organisms < 20 µm (treatment 8) increased at a population growth rate of 0.09 d^{-1} (Table 1, Figure 3A). In contrast, *P. parvum* population densities in the treatment that contained zooplankton grazers in the 61-210 µm size range as well as organisms < 20 µm (treatment 12) declined by -0.25 d^{-1}, and the population growth rates were statistically different between treatments (Tukey's HSD, $p < 0.05$; Table 1, Figure 3A). Chlorophyll-*a* concentrations increased by 51 ± 13% and 74 ± 3% in treatments 8 and 12 respectively, and concentrations were statistically different between treatments (Tukey's HSD, $p < 0.05$; Figure 3B).

5.2.2.3 *P. parvum* cells occurring in the natural phytoplankton assemblages

Experimental results indicate that zooplankton assemblages from the winter season at Lake Somerville significantly grazed *P. parvum* occurring in the natural phytoplankton assemblages. The population growth rate of *P. parvum* densities present in the natural phytoplankton assemblage of Lake Somerville (treatment 9) was 0.07 d^{-1}, while population density decreased by -0.21 d^{-1} in the presence of zooplankton grazers in the 61-210 µm size range (treatment 10; Table 1, Figure 3A). Differences in *P. parvum* population growth rates were marginally insignificant between the two treatments (Tukey's HSD, $p = 0.056$). Unlike chlorophyll-*a* concentrations in treatments inoculated

with log or stationary phase growth *P. parvum*, chlorophyll-*a* concentrations declined in both treatments 9 and 10 by 25 ± 5% and 25 ± 6% respectively (Figure 3B). No significant difference in concentrations was observed between treatments (Tukey's HSD, $p > 0.05$).

5.2.4 Zooplankton biovolume and composition

All zooplankton treatments declined in final average total zooplankton biovolume below the initial total biovolume enumerated from the 61-210 µm zooplankton aliquot preserved during t_0 sampling, which was 2.41×10^{-1} mm^3 L^{-1}. Total zooplankton biovolume decreased in treatment 10, 11, and 12 to a mean of 1.25×10^{-1} mm^3 L^{-1}, 1.81×10^{-1} mm^3 L^{-1}, 1.49×10^{-1} mm^3 L^{-1} respectively. Total zooplankton biovolume did not change significantly when inoculated with log- or stationary-growth phase *P. parvum* as differences in final average total zooplankton biovolumes between treatments were not statistically significant (Tukey's HSD, $p > 0.05$). Yet an analysis of dominant and sub-dominant zooplankton biovolumes shows that some zooplankton groups increased in biovolume when treatments were inoculated with *P. parvum* culture.

Brachionus sp. was the dominant zooplankton group in the Lake Somerville winter experiment with an initial biovolume of 1.32×10^{-1} mm^3 L^{-1}. Sub-dominant zooplankton groups consisted of *Filinia* sp. (initial biovolume 1.69×10^{-2} mm^3 L^{-1}), *Polyarthra* sp. (initial biovolume 1.36×10^{-2} mm^3 L^{-1}), adult copepods (initial biovolume 5.48×10^{-3} mm^3 L^{-1}), copepod nauplii (initial biovolume 1.85×10^{-2} mm^3 L^{-1}), and two unidentified illoricate rotifers that will be referred to hereafter as unknown 1 (initial biovolume 5.50×10^{-3} mm^3 L^{-1}) and unknown 2 (initial biovolume 2.01×10^{-2} mm^3 L^{-1})

(Figure 3C). Zooplankton biovolumes were not significantly different when treatments were inoculated with moderate densities of log-growth phase *P. parvum* (treatment 11) or low densities of stationary-growth phase *P. parvum* culture (treatment 12) compared to when zooplankton were only exposed to the low *P. parvum* population densities occurring in the natural phytoplankton assemblage (treatment 10; Tukey's HSD, $p > 0.05$). Proportional differences in dominant zooplankton biovolumes between treatments were also not statistically significant (Tukey's HSD, $p > 0.05$).

Although not statistically significant at the 0.05 alpha level, the final biovolume of *Brachionus* sp. was greater in treatment 11 than in treatment 10, and the difference between treatments was statistically significant at the 0.20 alpha level (Tukey's HSD, $p = 0.17$; Figure 3C). The final average biovolume of *Brachionus* sp. was also greater in treatment 12 compared to treatment 10 (Figure 3C). Yet due to large within-group variations, differences were not statistically significant, even at the 0.20 alpha level (Tukey's HSD, $p = 0.42$).

5.3 Lake Whitney Winter Experiments

5.3.1 Initial lake conditions

Water temperature in Lake Whitney was 12.3 °C at a depth of 1 m. Salinity was 0.80 psu and pH was 7.65. No *P. parvum* blooms occurred in Lake Whitney during the experiment according to the Texas Parks and Wildlife Department.

5.3.2 Population density and growth rates of* P. parvum *and chlorophyll*-a *concentrations

5.3.2.1 Effects of ambient lake conditions and water quality other than inorganic nutrients

Similar growth performance was observed between *P. parvum* populations inoculated from log- and stationary-growth phase cultures, with populations accumulating densities under Lake Whitney conditions when nutrients were not limiting. *P. parvum* populations inoculated from log-growth phase culture also accumulated in density under Lake Whitney conditions when nutrients were limiting, while populations inoculated from stationary-growth phase culture did not. These observations are fully described in Appendix B.

5.3.2.2 Effects of (or influence of) natural phytoplankton assemblages and zooplankton grazers in the 61-210 µm size range

5.3.2.2.1 Treatments inoculated with log-growth phase **P. parvum**

Lake Whitney data indicated that zooplankton assemblages from the experiment significantly grazed *P. parvum* populations inoculated from log-growth phase culture. The population growth rate of *P. parvum* densities inoculated from log-growth phase culture in filtered lake water containing organisms < 20 µm was 0.07 d^{-1} (treatment 7, Table 1, Figure 4A). On the other hand, *P. parvum* populations declined by -0.13 d^{-1} in the similar treatment that also contained zooplankton grazers in the 61-210 µm size range (treatment 11, Table 1, Figure 4A). Differences in *P. parvum* population growth rates were marginally insignificant between the two treatments (Tukey's HSD, $p = 0.10$), but *P. parvum* population densities were statistically different (Tukey's HSD, $p < 0.05$). Chlorophyll-*a* concentrations increased by 47 ± 12% and 63 ± 14% in treatments 7 and 11 respectively, and concentrations were statistically different (Tukey's HSD, $p < 0.05$; Figure 4B). Prominent phytoplankton observed in the natural phytoplankton assemblages included cyanobacteria, chlorophytes, and chrysophytes.

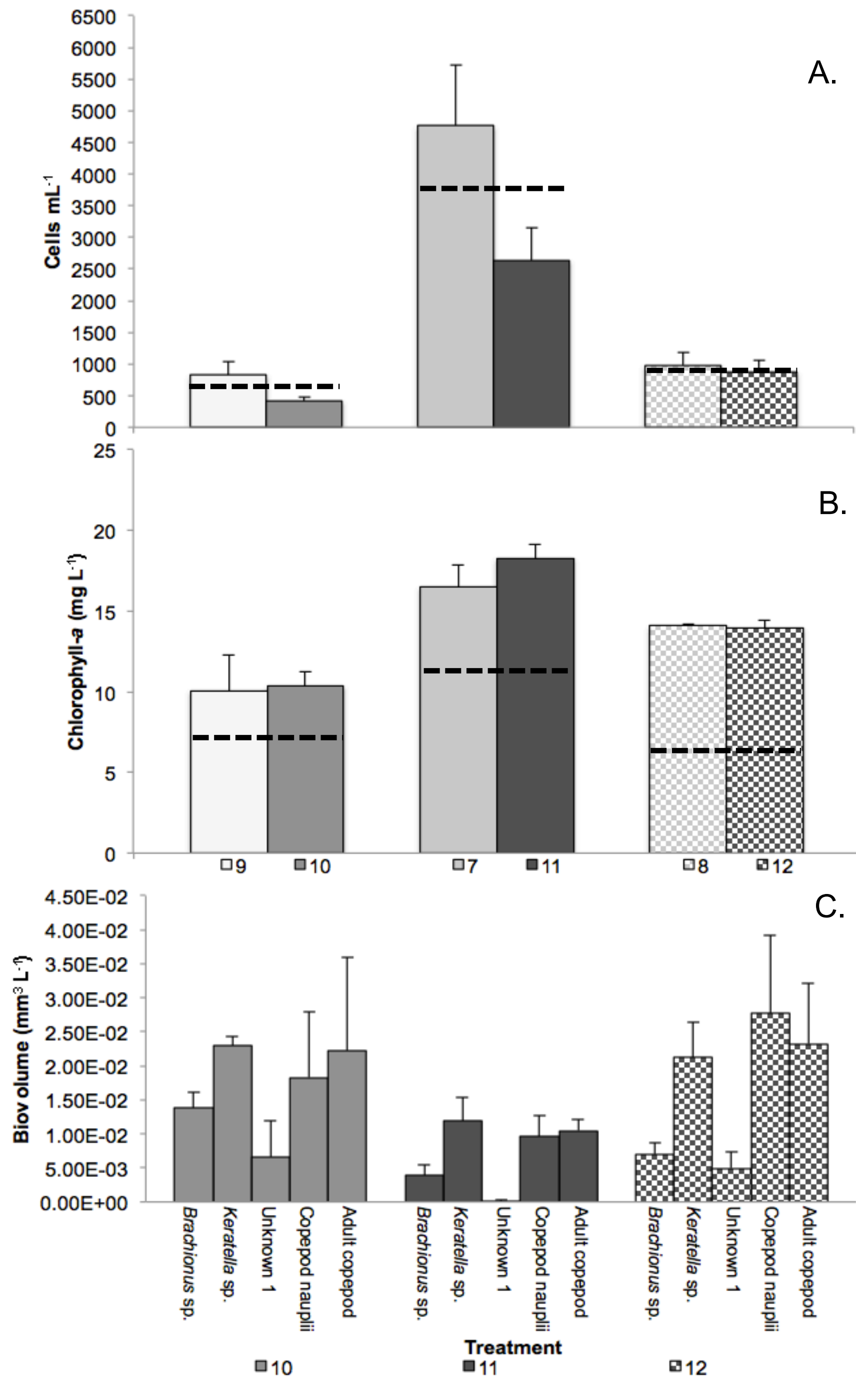

Figure 4: Treatment 7, 8, 9, 10, 11 and 12 final average ± 1 SD *P. parvum* population densities (A.), chlorophyll-*a* concentrations (B.), and biovolumes of dominant and sub-dominant zooplankton groups *Brachionus* sp., *Keratella* sp., copepod nauplii, adult copepods, and the unidentified illoricate rotifer unknown 1 (C.) for the Lake Whitney experiment conducted during winter 2013. The horizontal dashed lines represent the initial averages of like treatments. Treatments 9 and 10 only contained natural phytoplankton assemblages, while treatment 10 also contained zooplankton in the 61-210 µm size range. Treatments 7, 8, 11, and 12 contained natural phytoplankton assemblages while 7 and 11 were inoculated with *P. parvum* in log-growth phase, and 8 and 12 were inoculated with stationary-growth phase. Treatments 11 and 12 also contained zooplankton in the 61-210 µm size range.

5.3.2.2.2 Treatments inoculated with stationary-growth phase **P. parvum**

Experimental results indicate that zooplankton assemblages from the winter season at Lake Whitney did not significantly graze *P. parvum* populations inoculated from stationary-growth phase culture. During the winter experiment, *P. parvum* population densities inoculated from stationary-growth phase culture in filtered lake water containing organisms < 20 µm (treatment 8) increased at a population growth rate of 0.03 d^{-1} (Table 1, Figure 4A). In contrast, *P. parvum* population densities in the treatment that contained zooplankton grazers in the 61-210 µm size range as well as organisms < 20 µm (treatment 12) declined by -0.01 d^{-1}, and population growth rates were not statistically different between treatments (Tukey's HSD, $p > 0.05$; Table 1, Figure 4A). Chlorophyll-*a* concentrations increased by 128 ± 19% and 126 ± 20% in treatments 8 and 12 respectively, and concentrations were not statistically different between treatments (Tukey's HSD, $p > 0.05$; Figure 4B).

5.3.2.3 *P. parvum* cells occurring in the natural phytoplankton assemblages

Lake Whitney data indicated that zooplankton assemblages from the winter experiment significantly grazed *P. parvum* in the natural phytoplankton assemblage. In the winter experiment, the population growth rate of *P. parvum* densities present in the natural phytoplankton assemblage of Lake Whitney (treatment 9) was 0.09 d^{-1}, while populations decreased in density by -0.12 d^{-1} in the presence of zooplankton grazers in the 61-210 µm size range (treatment 10; Table 1, Figure 4A). Differences in *P. parvum* population growth rates were statistically significant between the two treatments (Tukey's HSD, $p < 0.05$). Chlorophyll-*a* concentrations increased in both treatments 9

and 10 by 41 ± 5% and 45 ± 21% respectively (Figure 4B). No significant difference in concentrations was observed between treatments (Tukey's HSD, $p > 0.05$).

5.3.3 Zooplankton biovolume and composition

All zooplankton treatments declined in final total zooplankton biovolume below the initial total biovolume enumerated from the 61-210 μm zooplankton aliquot preserved during t_0 sampling, which was 1.24 x 10^{-1} mm^3 L^{-1}. Total zooplankton biovolume decreased in treatment 10, 11, and 12 to a mean of 8.73 x 10^{-2} mm^3 L^{-1}, 3.68 x 10^{-2} mm^3 L^{-1}, and 8.46 x 10^{-2} mm^3 L^{-1} respectively. Differences in final total biovolumes between treatments only containing organisms < 20 μm (treatment 10) and were also inoculated with log-growth phase *P. parvum* culture (treatment 11) were marginally insignificant at the 0.05 alpha level (Tukey's HSD, $p = 0.054$). All other differences in total biovolumes among treatments were not statistically significant to a greater degree (Tukey's HSD, $p > 0.05$).

Keratella sp. was the dominant rotifer group in the winter Lake Whitney experiment with an initial biovolume of 3.04 x 10^{-2} mm^3 L^{-1}, while copepod nauplii was the overall initial dominant zooplankton group (initial biovolume 6.02 x 10^{-2} mm^3 L^{-1}). Sub-dominant zooplankton consisted of *Brachionus* sp. (initial biovolume 1.57 x 10^{-2} mm^3 L^{-1}), adult copepods (initial biovolume 1.58 x 10^{-2} mm^3 L^{-1}), and the unidentified illoricate rotifer unknown 1 (initial biovolume below detection level) (Figure 4C). The proportion of unknown 1 final biovolume in treatment 10 was 0.071 and 0.004 in treatment 11. These proportions were statistically different (Tukey's HSD, $p < 0.05$), while all other dominant zooplankton showed no significant proportional differences in

biovolume between treatments. Furthermore, the lower final unknown 1 biovolume in treatment 11 was statistically different compared to treatment 10 at the 0.20 alpha level (Tukey's HSD, $p = 0.12$; Figure 4C).

Significantly lower final biovolumes of *Brachionus* sp. and *Keratella* sp. were also observed in treatments inoculated with log-growth phase *P. parvum* culture (treatment 11) compared to the treatment that only contained the natural phytoplankton assemblage and other organisms < 20 μm (treatment 10; Tukey's HSD, $p < 0.05$, Figure 4C). *Brachionus* sp. final biovolume was also significantly lower in treatment 12, which was inoculated with stationary-growth phase *P. parvum* compared to treatment 10 (Tukey's HSD, $p < 0.05$; Figure 4C).

Final biovolumes of other zooplankton groups (i.e. adult copepods and copepod nauplii) were not statistically different between grazer treatments (Tukey's HSD, $p > 0.05$). *Keratella* sp. and unknown 1 also displayed no statistical difference in biovolume when exposed to low density *P. parvum* populations inoculated from stationary-growth phase culture combined with the natural phytoplankton assemblage (treatment 12) compared to when only exposed to the natural phytoplankton assemblage (treatment 10) (Tukey's HSD, $p > 0.05$).

5.4 *P. parvum* Growth Rate Comparisons Between Lakes

The following subsections compare *P. parvum* population growth rates from like treatments containing zooplankton grazers between Lake Somerville and Lake Whitney. Results from *P. parvum* monoculture treatments (treatments 1-6) are described in Appendix B. The results of treatments containing organisms < 20 μm and without the

addition of zooplankton grazers (treatments 7-9) are described and discussed in Appendix C.

5.4.1 **P. parvum** ***growth under exposure to natural phytoplankton assemblages and zooplankton grazers in the 61-210 µm size range***

5.4.1.1 Treatments inoculated with log-growth phase *P. parvum*

Growth rates of *P. parvum* populations inoculated from log-growth phase culture were not statistically different between winter lake treatments when exposed to natural phytoplankton assemblages and zooplankton grazers (treatment 11; Tukey's HSD, $p > 0.05$; Table 1).

5.4.1.2 Treatments inoculated with stationary-growth phase *P. parvum*

No statistical difference was observed in population growth rates of *P. parvum* inoculated from stationary-growth phase culture that were exposed to natural phytoplankton assemblages and zooplankton grazers (treatment 12; Tukey's HSD, $p > 0.05$; Table 1).

5.4.2 Growth of **P. parvum** ***cells occurring in the natural phytoplankton assemblages***

Population growth rates of *P. parvum* occurring in the natural phytoplankton assemblages were not significantly different between lakes in the winter when exposed to zooplankton grazers (treatment 10; Tukey's HSD, $p > 0.05$; Table 1).

6. DISCUSSION

6.1 *P. parvum* Effects on Other Phytoplankton

Similar to previous research, experimental results suggest that *P. parvum* affected other phytoplankton populations by means of phagotrophy, competition, and allelopathy in Lake Somerville and Lake Whitney treatments inoculated with log-growth phase culture (Graneli and Johansson 2003b, Roelke et al. 2007, Errera et al. 2008, Schwierzke et al. 2010). Details are described in Appendix D.

6.2 Decreases in Total Zooplankton Biovolumes

Many zooplankton have experienced negative impacts from exposure to *P. parvum* (Barreiro et al. 2005, Sopanen et al. 2006, Kozlowsky-Suzuki et al. 2009, Brooks et al. 2010). However, the decline in final mean total zooplankton biovolumes across all Lake Somerville and Lake Whitney treatments is considered to be a product of the experimental design. The exclusion of plankton in the 20-61 μm size range in the experimental design prevented organisms such as small ciliates and large dinoflagellates from inhabiting treatments. Therefore, raptorial feeding rotifers enumerated from samples (i.e. *Asplanchna* sp. and *Synchaeta* sp.) may have decreased in growth resulting from the absence of this plankton size fraction since their predator: prey size ratios can be as large as 1.5:1 to 5:1 (Hansen et al. 1994). In addition, predation by *Asplanchna* sp. and *Synchaeta* sp. on smaller rotifers may have also increased in some treatments due to the closed experimental design and exclusion of 20-61 μm organisms. The closed experimental design also prevented the replenishment of edible phytoplankton taxa in

the natural assemblages. Thus, potentially leaving unpalatable phytoplankton taxa for some zooplankton, which would also cause a decrease in zooplankton growth. Overestimation of initial zooplankton biovolumes is also a possibility since no replicate samples of t_0 aliquots were taken.

6.3 Zooplankton Community Grazing of, and Reactions to, *P. parvum*

6.3.1 Preferential grazing of **P. parvum** *and zooplankton response in Lake Somerville*

Some field experiments have suggested a possible grazer preference for *P. parvum* by rotifers, observations based on correlations (Errera et al. 2008, Schwierzke et al. 2010), here experimental results show this preference directly. *P. parvum* were preferentially grazed by zooplankton in Lake Somerville, as shown by decreases in *P. parvum* populations inoculated into natural assemblages without a corresponding decline in chlorophyll-*a*. The preference of *P. parvum* as a food source may be due to some nutritional benefit. Koski et al. (1999) suggested that copepod growth may benefit from the abundance of polyunsaturated acids in *Prymnesium patelliferum*, which is considered the same species as *P. parvum* (Larsen and Medlin 1997). This theory may also apply to rotifers as well. In addition, the size and shape of *P. parvum* may also play a role in selection by rotifers. Many filter feeding rotifers such as *Brachionus* sp. generally graze on micro-plankton in the 3 – 17 µm size range (Nogrady et al. 1993). The size of *P. parvum*, length 8-16 µm and width 4-10 µm, is right in the middle of this size range (Graneli et al. 2012). Also, the oval-like shape of a *P. parvum* cell makes it an easily ingested prey.

Zooplankton grazer treatments from Lake Somerville that only contained natural phytoplankton assemblages (treatment 10) most likely preferentially grazed *P. parvum* within those assemblages as well. Zooplankton preferential grazing of *P. parvum* was not indicated outright in treatment 10 as chlorophyll-*a* concentrations declined below initial averages. However, this is likely a result of the low initial average population density of *P. parvum* in these treatments and the large negative population growth rates seen in the grazer treatments. Meaning that grazers in Lake Somerville actively fed on *P. parvum* at a rate that caused *P. parvum* cells to become increasingly scarce to the point where zooplankton began to select other phytoplankton in order to supplement their preferred diet.

Zooplankton biovolumes and composition are variable in *P. parvum* inhabited waters (Errera et al. 2008, Michaloudi et al. 2009, Schwierzke et al. 2010). However, results from Lake Somerville agree with previous research that has suggested some rotifers may not be affected by exposure to *P. parvum* cells (Schwierzke et al. 2010). No negative effects to rotifer biovolume were observed. Furthermore, *Brachionus* sp. in Lake Somerville displayed positive effects from the presence of *P. parvum* populations inoculated from log-growth phase culture. This may indicate that *Brachionus* sp. benefits physiologically when moderate population densities of log-growth phase *P. parvum* are present. Additionally, the larger biovolumes suggest that *Brachionus* sp. is the main grazer that primarily selects *P. parvum* cells inoculated from log-growth phase culture as prey even when allelochemicals are present in the water, which could possibly prevent the harmful alga from reaching bloom densities. *Brachionus* sp. may also be the main

zooplankton that predominantly selects *P. parvum* cells inoculated from stationary-growth phase culture for food, but the low population densities used in the winter experiment provided inconclusive results. This may have been due to a lower initial population density of *P. parvum* cells in the grazer treatment inoculated with stationary phase *P. parvum*. Therefore, zooplankton food sources were not augmented to the same degree as they were in grazer treatments inoculated with log-growth phase *P. parvum*.

Some species of Brachionus have been shown to prey on small ciliates (Gilbert and Jack 1993). Thus, the absence of plankton in the 20-60 μm size range within treatments may have also impacted *Brachionus* sp. growth. Final average biovolumes of *Brachionus* sp. in each zooplankton treatment from the Lake Somerville experiment were lower than that of the initial aliquot taken during t_0 sampling. The decline in biovolumes could also be a result of increased competition for limited resources within the carboy in the absence of predation from organisms in higher trophic levels as final average biovolumes of other sub-dominant rotifers (i.e. unknown 3 during the fall and unknown 1 during the winter) increased beyond their initial levels in all grazer treatments. Unpalatable phytoplankton in the natural assemblages could have also caused a decrease in growth when *P. parvum* cells became scarce. Overestimation of initial zooplankton biovolumes may also be a contributing factor since no replicate samples were taken for enumeration.

6.3.2 Preferential grazing of **P. parvum** *and zooplankton response in Lake Whitney experiments*

Indications of zooplankton preferentially grazing on *P. parvum* in Lake Whitney experiments were varied. Zooplankton in winter treatments were found to preferentially graze on *P. parvum* populations inoculated from log-growth phase culture and *P. parvum* cells that inhabited the natural phytoplankton assemblage of Lake Whitney, as suggested by a decrease in *P. parvum* populations without a corresponding decline in chlorophyll-*a*.

A laboratory study performed by Barreiro et al. (2005) found the rotifer *Brachionus plicatilis* to experience a decreased growth rate when exposed to toxic *P. parvum* cell free filtrate. *Brachionus plicatilis* also did not select against toxic *P. parvum* when mixed with the non-toxic phytoplankton *Rhodomonas salina*, yet the growth rate of the rotifer declined (Barreiro et al. 2005). Similar rotifer behavior was observed during the winter Lake Whitney experiment. Effects of *P. parvum* populations inoculated from log-growth phase culture on *Brachionus* sp., *Keratella* sp., and the unknown 1 rotifer in Lake Whitney were deleterious, even though *P. parvum* was shown to be preferentially grazed by Lake Whitney zooplankton. This implies that toxic exudates produced by *P. parvum* did not act as feeding deterrents to the rotifers, but still caused biovolume to be affected. Furthermore, ingestion of *P. parvum* cells inoculated from log-growth phase culture did not have a significant impact on grazing like the *P. parvum* cells inoculated from stationary-growth phase culture did. Even though the toxicity bioassay indicated that the log-growth phase *P. parvum* culture was non-lethal to

fish, this negative impact on rotifer biovolume indicates that *P. parvum* was still toxic to zooplankton grazers.

Past research found some copepods to become inactive (Kozlowsky-Suzuki et al. 2009) when exposed to *P. parvum* and only ingest small amounts of the harmful alga even at concentrations of 2,000 cells mL^{-1} (Sopanen et al. 2006). So even though adult copepods and copepod nauplii showed no significant negative effect in biovolume from exposure to *P. parvum* inoculated from log- or stationary-growth phase culture, it is unlikely that they were the only zooplankton grazing on the harmful alga in the treatments inoculated with log-growth phase *P. parvum*. Furthermore, the negative *P. parvum* population growth rates were nearly identical between treatments inoculated with log-growth phase *P. parvum* and treatments only containing natural phytoplankton assemblages. This suggests that rotifers and copepods fed on *P. parvum* in both treatments in order for grazing rates to be so similar between treatments.

The phenomenon of slowed respiration and reduced fecundity has been observed in several rotifer species when food sources are stressed (Kirk 1997, Kirk et al. 1999, Ricci and Perletti 2006). Therefore, the ingestion of toxic *P. parvum* cells likely caused sub-lethal toxic effects resulting in decreased metabolic rates of zooplankton as well as reduced fecundity. Since *P. parvum* populations inoculated from stationary-growth phase culture were not significantly grazed by Lake Whitney zooplankton during the winter experiment, and little to no phytoplankton from the natural assemblage were grazed as indicated by the increase in chlorophyll-*a*. Thus, biovolumes of zooplankton exposed to *P. parvum* populations inoculated from stationary-growth phase (treatment

12) did not fluctuate significantly from those of zooplankton only exposed to the natural phytoplankton assemblage (treatment 10) within the three-day experiment duration even when grazing of all phytoplankton slowed. The decrease in *Brachionus* sp. biovolume indicates that survival of the rotifer was affected by ingestion of toxic cells inoculated from stationary-growth phase. Previous experiments have suggested that ingestion of toxic *P. parvum* cells may be how *Brachionus* sp. are primarily affected by the harmful alga (Barreiro et al. 2005). It is also possible that *Brachionus* sp. increased reproduction due to stressful conditions. The greater energy expenditure would increase mortality of the rotifer under the reduced grazing conditions. Although toxicity bioassay results indicated that the stationary-growth phase *P. parvum* culture was non-toxic to fish (see Section 5.1), the bioassay could not serve as an indicator of toxicity via ingestion. Consequently, the theory of toxic ingestion cannot be ruled out.

6.3.3 Salinity and microevolutionary adaptations of rotifers

Colin and Dam (2002) suggest that increasing grazer mortality due to frequent high toxicity algal blooms severely reduces the egg production of non-resistant individuals within the population, and therefore exerts selective pressure on grazers. This theory certainly applies to copepods, which exclusively reproduce sexually. However, I argue that more rapid adaptations may generally occur among cyclically parthenogenic rotifers under low-level toxin exposure as opposed to high-level toxicity. Dominant and sub-dominant rotifers from each lake experiment, including unknowns, were of the class Monogononta, which are cyclical parthenogens. Stress from habitat deterioration has been hypothesized to initiate sexual reproduction in cyclically parthenogenic rotifers

(Serra et al. 2008). Therefore, it is possible that the stress from sub-lethal toxin exposure may trigger more frequent sexual reproduction in cyclical parthenogens, and therefore rapid microevolutionary adaptations are able to develop via genetic variation. In contrast, rotifers present during periods of lethal toxicity levels (i.e. blooms) may experience much slower adaptations to *P. parvum* toxins as a result of mass mortality, since rotifers generally reproduce asexually at low population densities (Serra et al. 2008), which would limit the potential for genetic variation. Results from these experiments indicate that this process of rapid adaptation may have occurred in Lake Somerville.

While *P. parvum* population growth rates were not different in like grazer treatments between lakes, results from these experiments indicate that rotifers from Lake Somerville, where *P. parvum* is present but does not form toxic blooms, are superior to those of Lake Whitney, where toxic blooms occur, in regards to sustained grazing of, and interaction with, *P. parvum* cells. Lake Whitney rotifers were negatively affected by *P. parvum* populations inoculated into natural assemblages, and rotifers from Lake Somerville were not. Some rotifers, i.e. *Brachionus* sp. and unknown 1, were dominant in both lakes, but were affected differently. I surmise that the differences in *P. parvum* effects on rotifers between lakes is a result of microevolutionary adaptations *to P. parvum* toxins in Lake Somerville rotifers. Furthermore, I speculate that these adaptations occurred from continual exposure to sub-lethal toxin concentrations, and that low salinity is the cause for the perpetual low-level of *P. parvum* toxicity.

It is possible that *P. parvum* toxin production may be limited when salinity is below a certain threshold. Some studies have looked into the influence of salinity on toxicity (Shilo 1967, Larsen and Bryant 1998, Baker et al. 2007, Baker et al. 2009), but none have looked at salinity levels below ~0.5 psu. Preliminary results from a water quality meta-analysis by Patiño et al. (2014) indicated that salinity is the best predictor of *P. parvum* bloom occurrence among Texas reservoirs located in the Brazos River Basin. This basin is where Lake Somerville and Lake Whitney are located. Furthermore, the analysis indicated that toxic *P. parvum* bloom events in this basin are primarily associated with salinities greater than ~0.6 psu. This suggests that a salinity threshold for high toxin production may be around or slightly below 0.6 psu. Salinity levels of Lake Somerville during both seasonal experiments were below this threshold, while salinity levels from both seasonal Lake Whitney experiments were above it. Moreover, historical reservoir water quality data of fall and winter seasons (October to March) since the year 2000 indicates that the mean salinity of Lake Whitney is ~0.80 psu and the mean salinity of Lake Somerville is ~0.23 psu (TRWDP 2013). The historically lower salinity levels in Lake Somerville suggest that *P. parvum* cells inhabiting the lake may be living beyond the salinity niche for production of lethal concentrations of toxins. Thus, rotifers in Lake Somerville are exposed to sub-lethal concentrations of toxins, which would allow the rotifers that preferentially graze *P. parvum* to keep the alga at manageable population densities. The sub-lethal concentrations of toxins may also trigger frequent sexual reproduction and allow more rapid microevolutionary adaptations to develop in rotifers that succeed others during the winter. In contrast, the historically higher salinity level in

Lake Whitney may be above the threshold for *P. parvum* to produce lethal concentrations of toxins for blooms to occur. The toxic blooms are lethal to rotifers resulting in mass mortality and would prevent frequent sexual reproduction.

7. CONCLUSIONS

In summary, experimental results indicate that zooplankton preferentially grazed *P. parvum* in most treatments, regardless of effects from toxic exudates. Lake Somerville rotifers were unaffected by exposure to *P. parvum* populations inoculated into natural assemblages. In contrast, Lake Whitney rotifers were negatively affected by toxic exudates of *P. parvum* populations inoculated from log-growth phase culture even though preferential grazing of the harmful alga occurred. Lake Whitney zooplankton grazing was also affected by *P. parvum* populations inoculated from stationary-growth phase culture, likely from ingestion of the toxic cells.

I surmise that historically lower salinity levels in Lake Somerville prevented *P. parvum* from producing toxins at concentrations that would be lethal to grazers. Therefore, preferential grazing of *P. parvum* kept the alga at manageable population densities. The low level of toxicity during seasonal periods of bloom development and initiation allowed microevolutionary adaptation to toxins to develop more rapidly in rotifers via increased sexual reproduction. Whereas rotifers in Lake Whitney may undergo a much slower adaptation process due to reduced sexual reproduction caused by mass mortality from formation of highly toxic *P. parvum* blooms.

It is possible that this proposed phenomenon of rapid adaptation may also occur in other lakes of similar low level salinity that *P. parvum* currently inhabits. Of course, further research is needed to confirm such a hypothesis. Findings also imply that Lake Somerville rotifers adapted to *P. parvum* toxicity may have management implications in

other lakes. However, further research is needed to confirm this suggestion as well. Recommended future research includes similar experiments with log- and stationary-growth phase *P. parvum* at greater population densities to better determine toxin resistance of rotifers in Lake Somerville, lab experiments with log- and stationary-growth phase *P. parvum* and *Brachionus* rotifers isolated from each lake during fall and winter seasons in order to fine tune determination of toxin resistant species, and lab experiments investigating rotifer responses to ingestion of stationary-growth phase cells at various population densities.

Overall the mesocosm experiments confirm that rotifer assemblages in Lake Somerville effectively graze *P. parvum*. Significant declines of *P. parvum* populations inoculated from log- and stationary-growth phase cultures resulting from preferential grazing complimented by no observed negative effects to rotifer biovolume support the theory that grazing of *P. parvum* by rotifers is an important contributing factor preventing the harmful alga from reaching bloom densities in Lake Somerville. In contrast, observed negative effects on biovolume and grazing in Lake Whitney rotifers indicate that grazing of *P. parvum* would not prevent blooms from occurring.

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APPENDIX A

LAKE SOMERVILLE AND LAKE WHITNEY FALL MESOCOSM EXPERIMENTS

A-1. Methods

Lake Whitney and Somerville experiments conducted in the fall period of bloom initiation followed the same methodology of the winter experiments with differences described below.

Only odd numbered treatments inoculated with log-growth phase *P. parvum* culture (1, 3, 5, 7, 11) were deployed for fall experiments along with natural phytoplankton assemblage treatments (9 and 10). Inoculation densities of log-growth phase *P. parvum* culture consisted of approximately 3,000 cells mL^{-1}. Treatments in 20 L carboys were filled to 18 L instead of 15 L. Particle free 0.2 µm level filtration using cartridge filters (Cole-Parmer, Vernon Hills, IL, USA) was attempted with lake water for treatments 3 and 5, but not achieved due to equipment failure.

During fall experiments, all 20 L carboys were tethered to a square wooden frame with a perimeter of approximately 6 m, which floated in both lakes. Carboys were covered with a neutral density screen when tethered to the wooden frame. All 2 L bottles were floated in limno corrals on both lakes that were tethered to the wooden frame. Just like the winter experiment, the limno corral and wooden frame used in the fall Lake Somerville experiment were anchored in a cove directly west of Welch Park. The limno corral and wooden frame used in Lake Whitney for the fall experiments were initially

anchored in the southern part of the lake, just south of Walling Bend Island (where dense populations of *P. parvum* are known to occur). During the first night of the experiment, strong winds from a cold front unanchored the limno corrals and blew them against the southwest section of the dam. This incident resulted in damages to several treatments as well as prohibited the sampling of treatments during the first full day of the experiment (t_1). The mesocosm was recovered the next day (t_2) and transported to a cove in the southern region of the lake where it was tethered to a boat dock belonging to the Little Rocky Lodge and Resort.

A-1.1 Response variables

Metrics and response variables that were monitored in the winter experiments were also monitored during the fall experiments using the same methods. Salinity readings were also taken during the fall experiments.

A-1.2 Data analysis

Methods for fall experiment data analysis were identical to methods for the winter experiments.

A-2. Results

A-2.1 Toxicity bioassays

P. parvum cultures used in the fall Lake Whitney experiment were virtually non-toxic to fish as percent survival of *Pimephales promelas* was 100% in almost all treatments after 48 hours. The exceptions were 80% survival ratings in replicates from treatments 1, 3, 7, and 9. A bioassay was not conducted for the Lake Somerville experiment deployed in the fall due to insufficient preparation time.

A-2.2 Lake Somerville fall experiments

A-2.2.1 Initial lake conditions

During the fall experiment, salinity was 0.20 psu.

A-2.2.2 Population density and growth rates of *P. parvum* and chlorophyll-*a* concentrations

A-2.2.2.1 Effects of ambient lake conditions and water quality other than inorganic nutrients

See Appendix B.

A-2.2.2.2 Effects of (or influence of) natural phytoplankton assemblages and zooplankton grazers in the 61-210 µm size range

A-2.2.2.2.1 Treatments inoculated with log-growth phase *P. parvum*

Experimental results indicated that zooplankton assemblages from the fall season at Lake Somerville significantly grazed *P. parvum* inoculated from log-growth phase culture. During the fall experiment, treatment 7 and 11 displayed trends analogous to those of the winter experiment. Treatment 7 *P. parvum* population density increased at a population growth rate of 0.17 d^{-1}, while treatment 11 density decreased by -0.25 d^{-1} (Table 2, Figure 5A). *P. parvum* population growth rates were statistically different between treatments (Tukey's HSD, $p < 0.05$). Similar to the winter experiment, chlorophyll-*a* concentrations increased in both treatments by 8 ± 10% and 26 ± 10% respectively, and concentrations were statistically different (Tukey's HSD, $p < 0.05$; Figure 5B). Prominent phytoplankton observed in the natural phytoplankton assemblages included cyanobacteria, chlorophytes, and chrysophytes.

Table 2: *Prymnesium parvum* population growth rates (day^{-1}) in fall experimental treatments. Experiments only contained odd numbered treatments that were inoculated with log phase growth *P. parvum* culture with the exception of treatments 9 and 10, which only contained assumed log phase *P. parvum* occurring in the natural phytoplankton assemblages. Experiments were conducted in late November 2012. Values shown are mean ± 1 SD, with n = 3 unless otherwise stated.

	Lake Somerville	Lake Whitney
Treatment	Log phase growth	Log phase growth
1	0.06 ± 0.03	0.03 ± 0.07
7	0.17 ± 0.02	0.07 ± 0.03
9	0.06 ± 0.09	0.08 ± 0.02
10	-0.21 ± 0.08	-0.05[a]
11	-0.25 ± 0.05	-0.04[a]

[a] Treatments 10 and 11 only included one replicate.

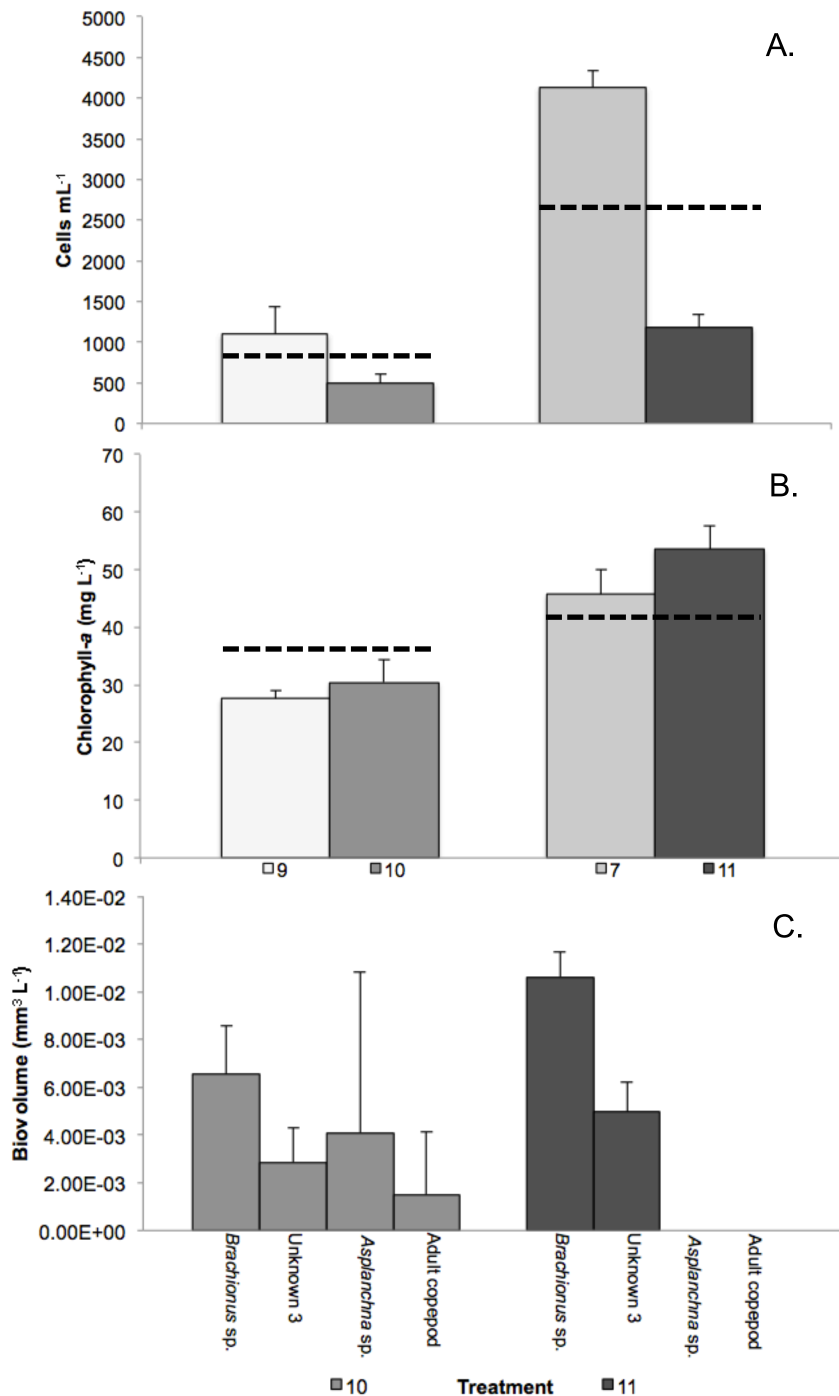

Figure 5: Treatment 7, 9, 10, and 11 final average ± 1 SD *P. parvum* population densities (A.), chlorophyll-*a* concentrations (B.), and biovolumes of dominant and sub-dominant zooplankton groups *Brachionus* sp., the unidentified illoricate rotifer unknown 3, *Asplanchna* sp., and adult copepods (C.) for the Lake Somerville experiment conducted during fall 2012. The horizontal dashed lines represent the initial averages of like treatments. Treatments 9 and 10 only contained natural phytoplankton assemblages, while treatment 10 also contained zooplankton in the 61-210 µm size range. Treatments 7 and 11 contained natural phytoplankton assemblages while 7 and 11 were inoculated with *P. parvum* in log-growth phase. Treatments 11 also contained zooplankton in the 61-210 µm size range.

A-2.2.2.2.2 *P. parvum* cells occurring in the natural phytoplankton assemblages

Results indicate that zooplankton assemblages from the fall season at Lake Somerville significantly grazed *P. parvum* occurring in the natural phytoplankton assemblages. During the fall experiment, treatment 9 and 10 showed trends parallel to those of the winter experiment with similar changes in *P. parvum* population densities and chlorophyll-*a* concentrations (Table 2, Figure 5A and 5B). Treatment 9 *P. parvum* population density increased at a population growth rate of 0.06 d^{-1}, while treatment 10 density decreased by -0.21 d^{-1}. *P. parvum* population growth rates were statistically different between these treatments (Tukey's HSD, $p < 0.05$). Chlorophyll-*a* concentrations decreased by 28 ± 4% and 21 ± 10% respectively, and were not statistically different between treatments (Tukey's HSD, $p > 0.05$).

A-2.2.2.2.3 Zooplankton biovolume and composition

All zooplankton treatments from the fall experiment declined in final average total zooplankton biovolume below the initial total biovolume enumerated from the 61-210 µm zooplankton aliquot preserved during t_0 sampling, which was 2.47×10^{-2} mm^3 L^{-1}. Total zooplankton biovolume in the fall experiment decreased in treatment 10 and 11 to a mean of 1.90×10^{-2} mm^3 L^{-1} and 1.82×10^{-2} mm^3 L^{-1}. Differences in final total biovolumes between treatments were not statistically significant (Tukey's HSD, $p > 0.05$).

Similar to the winter experiment, *Brachionus* sp. was a dominant zooplankton group during the fall experiment that had a greater final average biovolume when exposed to moderate population densities of *P. parvum* inoculated from log-growth phase culture along with the natural phytoplankton assemblage (treatment 11) compared to when the rotifer was only exposed to lower population densities of *P. parvum* present in the natural phytoplankton assemblage (initial biovolume 1.57×10^{-2} mm^3 L^{-1}) (treatment 10; Figure 5C). The difference in biovolumes between treatments was statistically significant (Tukey's HSD, $p < 0.05$). As mentioned previously, treatments inoculated with stationary phase *P. parvum* were not used in fall experiments.

An unidentified illoricate rotifer referred to hereafter as unknown 3 was a sub-dominant zooplankton in the fall experiment that also had a final average biovolume greater in treatment 11 than in treatment 10 (initial biovolume 2.41×10^{-3} mm^3 L^{-1}) (Figure 5C). Final biovolumes for unknown 3 were not statistically different between treatments at the 0.05 alpha level, but differences were significant at the 0.20 alpha level (Tukey's HSD, $p = 0.12$).

Other sub-dominant zooplankton from the fall experiment included *Asplanchna* sp. (initial biovolume 2.94×10^{-3} mm^3 L^{-1}) and adult copepods (initial biovolume below detection level) (Figure 5C). *Asplanchna* sp. and adult copepods fell below detection levels in treatment 11, yet due to large within-group variations, biovolumes were not statistically different compared to treatment 10 (Tukey's HSD, $p > 0.05$).

Additional aliquots of treatment 10 and 11 samples were settled and counted, but no *Asplanchna* sp. or adult copepods were observed. Parallel to the winter experiment, proportional differences in dominant zooplankton biovolumes were not statistically significant (Tukey's HSD, $p > 0.05$).

A-2.3 Lake Whitney fall experiments

A-2.3.1 Initial lake conditions

During the fall experiment, salinity was 0.76 psu. No *P. parvum* blooms occurred in Lake Whitney during the experiment according to the Texas Parks and Wildlife Department.

A-2.3.2 Population density and growth rates of *P. parvum* and chlorophyll-*a* concentrations

A-2.3.2.1 Effects of ambient lake conditions and water quality other than inorganic nutrients

See Appendix B for monoculture treatment (treatment 1) results.

A-2.3.2.2 Effects of (or influence of) natural phytoplankton assemblages and zooplankton grazers in the 61-210 µm size range

A-2.3.2.2.1 Treatments inoculated with log-growth phase *P. parvum*

During the fall experiment, treatment 7 and 11 displayed trends of zooplankton grazing on *P. parvum* populations inoculated from log-growth phase culture similar to the winter experiment. However, statistical differences in fall treatment data could not be calculated as only 1 replicate from treatment 11 was left uncompromised after weather damage (see Section 4.2). Treatment 7 *P. parvum* population density increased at a population growth rate of 0.07 d^{-1}, while treatment 11 density decreased by -0.04 d^{-1} (Table 2, Figure 6A). Unlike the winter experiment, treatment 7 reached a greater final chlorophyll-*a* concentration than treatment 11. Chlorophyll-*a* concentrations increased in both treatments by 110 ± 5% and 88% respectively (Figure 6B). Prominent phytoplankton observed in the natural phytoplankton assemblages included cyanobacteria, chlorophytes, and chrysophytes.

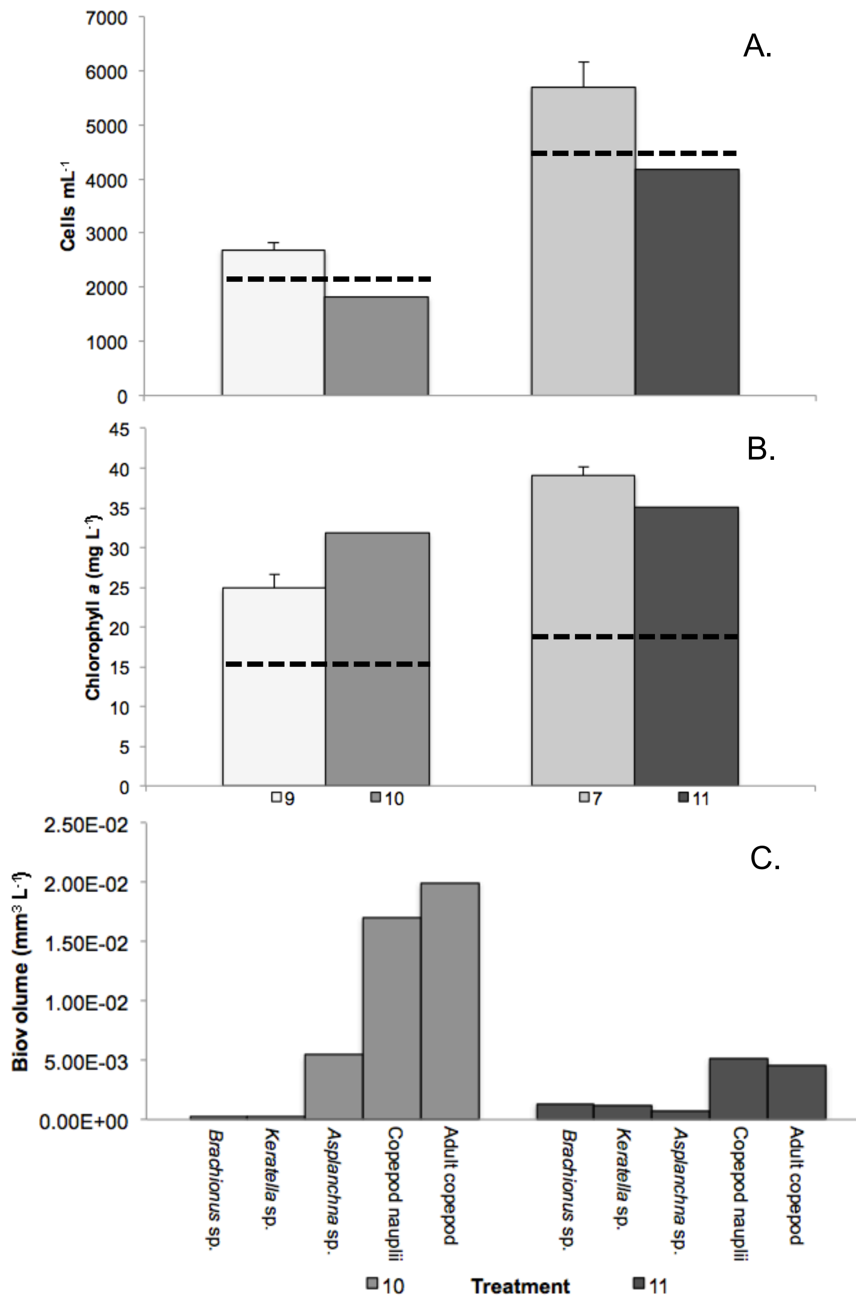

Figure 6: Treatment 7, 9, 10, and 11 final average ± 1 SD *P. parvum* population densities (A.), chlorophyll-*a* concentrations (B.), and biovolumes of dominant and sub-dominant zooplankton groups *Brachionus* sp., *Keratella* sp., *Asplanchna* sp., copepod nauplii, and adult copepods (C.) for the Lake Whitney experiment conducted during fall 2012. The horizontal dashed lines represent the initial averages of like treatments. Treatments 9 and 10 only contained natural phytoplankton assemblages, while treatment 10 also contained zooplankton in the 61-210 µm size range. Treatments 7 and 11 contained natural phytoplankton assemblages while 7 and 11 were inoculated with *P. parvum* in log-growth phase. Treatments 11 also contained zooplankton in the 61-210 µm size range.

A-2.3.2.2.2 *P. parvum* cells occurring in the natural phytoplankton assemblages

During the fall experiment, treatment 9 and 10 showed trends parallel to those of the winter experiment with similar changes in *P. parvum* population densities and chlorophyll-*a* concentrations, suggesting that zooplankton assemblages substantially grazed *P. parvum* in the natural phytoplankton assemblage (Table 2, Figure 6A and 6B). However, determination of statistical differences in fall treatment data was not possible as only 1 replicate from treatment 10 was left uncompromised after weather damage (see Section 4.2). Treatment 9 *P. parvum* population density increased at a population growth rate of 0.08 d^{-1}, while treatment 10 density decreased by -0.05 d^{-1}. Chlorophyll-*a* concentrations increased by 62 ± 11% and 107% in treatment 9 and 10 respectively.

A-2.3.2.2.3 Zooplankton biovolume and composition

All zooplankton treatments declined in final total zooplankton biovolume below the initial total biovolume enumerated from the 61-210 μm zooplankton aliquot preserved during t_0 sampling, which was 5.79×10^{-2} mm^3 L^{-1}. Just like the winter Lake Whitney experiment, total zooplankton biovolume decreased by a greater average percentage when exposed to moderate densities of *P. parvum* inoculated from log-growth phase culture (treatment 11) than when only exposed to the *P. parvum* population density present in the natural phytoplankton assemblage (treatment 10) in the fall, but statistical significance could not be determined. Total zooplankton biovolume in the fall experiment decreased in treatment 10 and 11 to a mean of 4.31×10^{-2} mm^3 L^{-1} and 1.39×10^{-2} mm^3 L^{-1}, compared to the initial 61-210 μm zooplankton aliquot preserved during t_0 sampling.

While *Brachionus* sp. and *Keratella* sp. biovolumes were significantly lower in treatments inoculated with log-growth phase *P. parvum* in the winter Lake Whitney experiment, trends from the Lake Whitney experiment conducted in the fall display a different scenario for these rotifers. Final biovolumes of *Brachionus* sp. (initial biovolume 7.69×10^{-4} mm^3 L^{-1}) and *Keratella* sp. (initial biovolume 2.57×10^{-3} mm^3 L^{-1}) were greater in the single treatment 11 replicate compared to the single treatment 10 replicate (Figure 6C). However due to weather damage, statistical differences in fall treatment data could not be calculated as only 1 replicate from treatments 10 and 11 were left uncompromised (see Section 4.2).

Copepod nauplii were the dominant zooplankton group from the fall experiment (initial biovolume 4.45×10^{-2} mm^3 L^{-1}), while sub-dominant zooplankton consisted of *Asplanchna* sp. (initial biovolume 5.64×10^{-3} mm^3 L^{-1}) and adult copepods (initial biovolume 3.47×10^{-3} mm^3 L^{-1}) (Figure 6C). Treatment 10 mean final biovolumes were greater than those in treatment 11 for all three of these zooplankton groups. Yet results are inconclusive since statistical analysis was not possible due to a lack of undamaged treatment replicates.

A-2.4* P. parvum *growth rate comparisons between lakes

P. parvum population growth rates of fall treatments from Lake Somerville and Whitney were not statistically compared as a result of weather damage to Lake Whitney treatment replicates during the. Results from *P. parvum* monoculture treatments (treatment 1) are described in Appendix B. The results of treatments containing

organisms < 20 μm and without the addition of zooplankton grazers (treatments 7 and 9) are described and discussed in Appendix C.

A-3. Discussion

A-3.1 **P. parvum** ***effects on other phytoplankton***

Similar to previous research, experimental results suggest that *P. parvum* affected other phytoplankton populations by means of phagotrophy, competition, and allelopathy in the Lake Somerville treatments inoculated with log-growth phase culture (Graneli and Johansson 2003b, Roelke et al. 2007, Errera et al. 2008, Schwierzke et al. 2010). Details are described in Appendix D.

A-3.2 Decreases in total zooplankton biovolume

Just like in the winter experiments, the decline in final mean total zooplankton biovolumes across all treatments is considered to be a product of the experimental design. As mentioned in Section 6.2, exclusion of plankton in the 20-61 μm size range, predation by *Asplanchna* sp. and *Synchaeta* sp. on smaller rotifers, the closed experimental design preventing the replenishment of edible phytoplankton taxa, and overestimation of initial zooplankton biovolumes may have all contributed to the decrease in total zooplankton biovolumes.

A-3.3 Zooplankton community grazing of, and reactions to, **P. parvum**

A-3.3.1 Preferential grazing of *P. parvum* and zooplankton response in Lake Somerville

Previous field experiments have suggested a possible grazer preference for *P. parvum* by rotifers, these observations have been based on correlations (Errera et al. 2008, Schwierzke et al. 2010), and here experimental results from the fall Lake Somerville experiment show this preference directly. Similar to the winter Lake Somerville data, *P. parvum* appeared to be preferentially grazed by zooplankton, as suggested by decreases in *P. parvum* populations inoculated into natural assemblages without a corresponding decline in chlorophyll-*a*. As discussed in Section 6.3.1, the preference of *P. parvum* as a food source may be due to some nutritional benefit as well as the phytoplankton's size and shape.

Zooplankton grazer treatments from the fall Lake Somerville experiment that contained only natural phytoplankton assemblages (treatment 10) most likely preferentially grazed *P. parvum* as well. Similar to the winter Lake Somerville experiment, zooplankton preferential grazing of *P. parvum* was not indicated outright in treatment 10 as chlorophyll-*a* concentrations declined below initial averages in both experiments. But again, this is likely a result of the large negative population growth rates seen in the grazer treatments and the low initial average population density of *P. parvum* in these treatments. Meaning that grazers in Lake Somerville actively fed on *P. parvum* at a rate that caused *P. parvum* cells to become increasingly scarce to the point

where zooplankton began to select other phytoplankton in order to supplement their preferred diet.

Similar to the winter Lake Somerville experiment, results from the fall Lake Somerville experiment agree with previous research that has suggested some rotifers may not be affected by exposure to *P. parvum* cells (Schwierzke et al. 2010). *Brachionus* sp. and the unidentified rotifer unknown 3 in Lake Somerville displayed positive effects from the presence of *P. parvum* populations inoculated from log-growth phase culture during the fall experiment. This suggests that these rotifers, particularly *Brachionus* sp., benefit physiologically when moderate population densities of log-growth phase *P. parvum* are present. This data is also supported by similar findings regarding *Brachionus* sp. from the winter experiment. Furthermore, the larger biovolumes suggest that these rotifers may be the main grazers that primarily select *P. parvum* cells inoculated from log-growth phase culture as prey, which could possibly prevent the harmful alga from reaching bloom densities even when allelochemicals are present in the water.

Similar to the winter experiment, final average biovolumes of *Brachionus* sp. in each zooplankton treatment from the Lake Somerville experiment were lower than that of the initial aliquot taken during t_0 sampling. As mentioned in Section 6.3.1, the decline in biovolumes is thought to be a product of the experimental design (i.e. increased competition for limited resources within the carboy, the absence of plankton in the 20-60 μm size range within treatments, unpalatable phytoplankton in the natural assemblages, and overestimation of initial zooplankton biovolumes).

A-3.3.2 Seasonal succession of rotifers and microevolutionary adaptations

Observations of rotifer biovolumes from fall and winter experiments conducted in Lake Whitney suggest that succession of less toxin resistant species or genotypes may have occurred in the wintertime. *Brachionus* sp. and *Keratella* sp. were sub-dominant during both fall and winter experiments. Yet *P. parvum* inoculated from log-growth phase culture had a significant negative impact on the rotifers during the winter experiment, while final biovolume of both rotifers in the fall was greater when exposed to moderate densities of *P. parvum* inoculated from log-growth phase combined with the natural phytoplankton assemblage (treatment 11) compared to only being exposed to the natural phytoplankton assemblage (treatment 10). Although the significance of the fall data could not be determined, the trend suggests that *Brachionus* sp. and *Keratella* sp. were not negatively affected by *P. parvum* inoculated from log-growth phase culture during the fall, even though *P. parvum* population densities were the largest of all experiments at this time. The potential difference in effects of *P. parvum* on both rotifers is possibly due to seasonal succession of different species and or genotypes within each genus. Papakostas et al. (2013) indicated that *Brachionus* species are able to adapt to different temperatures and therefore govern seasonal succession patterns between species and genotypes through this ecological specialization. This adaptation may also occur in other rotifers. Therefore, *Brachionus* sp. and *Keratella* sp. present during the fall may have been more adapted to *P. parvum* toxins than the species or genotypes that succeeded these rotifers during cooler winter temperatures. It is possible that *Brachionus* sp. and *Keratella* sp. from the fall experiment could have been more adapted to *P.*

parvum toxins because of their presence during periods of bloom development in Lake Whitney when toxicity is not at its peak.

Given the cyclical parthenogen microevolutionary adaptation speculation discussed in Section 6.3.3, it is possible that the stress from low-level toxin exposure during bloom development in the fall season may trigger more frequent sexual reproduction in cyclical parthenogens, and therefore rapid microevolutionary adaptations are able to develop via genetic variation. In contrast, *Brachionus* sp. and *Keratella* sp. that succeeded rotifers in the winter were present during the period of bloom initiation where maximum lethal toxicity levels are reached. Hence, much slower adaptations to *P. parvum* toxins may develop in winter rotifers as a result of mass mortality. Because low population densities generally cause rotifers to reproduce asexually (Serra et al. 2008), which would limit the potential for genetic variation. Similar to the winter Lake Somerville experiment (see Section 6.3.3), Results also indicate that this microevolutionary adaptation process may have occurred in Lake Somerville as a function of salinity during the fall as well.

A-4. Conclusions

Regardless of effects from toxic exudates, experimental results indicate that zooplankton preferentially grazed *P. parvum* in most treatments. Lake Somerville rotifers were unaffected by exposure to *P. parvum* populations inoculated into natural assemblages during the fall experiment. In contrast, one Lake Whitney rotifers appeared to be negatively affected by toxic exudates of *P. parvum* populations inoculated from log-growth phase culture even though preferential grazing of the harmful alga occurred.

However, results were inconclusive due to a lack of treatment replicates caused by weather damage. Different responses in biovolume from *Brachionus* and *Keratella* rotifers during fall and winter Lake Whitney experiments suggests that some species and/or genotypes of these rotifers may be better adapted to *P. parvum* toxins during the fall than their winter time counter-parts. This may be attributed to fall rotifer assemblages being exposed to lower toxin concentrations at the time of *P. parvum* bloom development compared to winter rotifer assemblages that are exposed to highly toxic fish killing blooms. The same concept may also be translated to rotifer assemblages in Lake Somerville as a function of salinity, which is summarized in Section 7.

APPENDIX B

RESULTS AND DISCUSSION FOR *P. parvum* MONOCULTURE TREATMENTS

B-1. Lake Somerville Winter and Fall Experiment Monoculture Treatments

B-1.2 Effects of ambient lake conditions and water quality other than inorganic nutrients

B-1.2.1 Treatments inoculated with log-growth phase *P. parvum*

During the winter, *P. parvum* growth rates of populations inoculated from log-growth phase culture in media made from RO water (treatment 1) and media made from filtered lake water (treatment 3) were 0.11 d^{-1} and 0.01 d^{-1} respectively (Table 1, Figure 7A), and the rate of increase was not statistically different between the treatments (Tukey's HSD, $p > 0.05$). Similarly, 11 ± 9% and 14 ± 20% increases in chlorophyll-*a* concentrations were not statistically different (Tukey's HSD, $p > 0.05$; Figure 7B). *P. parvum* density and chlorophyll-*a* concentration declined by -0.10 d^{-1} and 37 ± 4%, however, when inoculated into filtered lake water without nutrient additions (treatment 5; Table 1, Figure 7A-B).

During the fall experiment, *P. parvum* population density also increased by 0.06 d^{-1} in media made from RO water (treatment 1; Table 2, Figure 8A). However, a corresponding increase in chlorophyll-*a* concentration was not observed (Figure 8B). Due to failure of the 0.2 μm cartridge filter during the fall experiment, an evaluation of

P. parvum growth in waters with just dissolved constituents from Lake Somerville (comparison of treatments 1 and 3) was not possible.

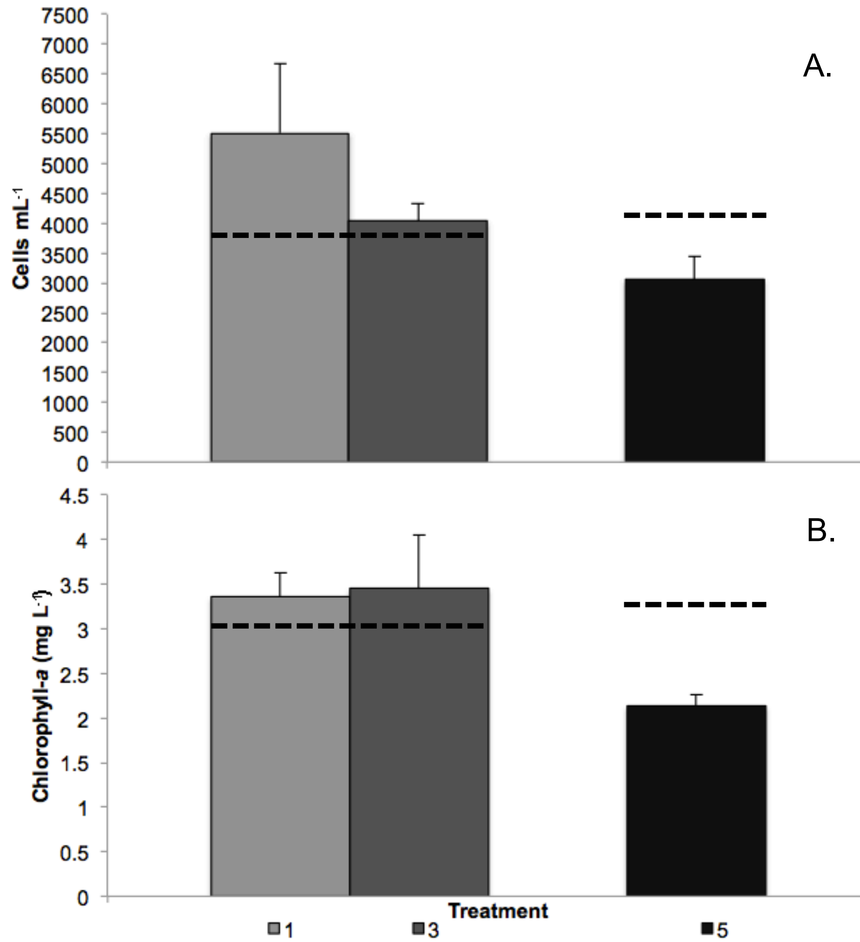

Figure 7: Treatment 1, 3, and 5 final average *P. parvum* population densities (A.) and chlorophyll-*a* concentrations (B.) ± 1 SD for the Lake Somerville experiment conducted during winter 2013. The horizontal dashed lines represent the initial averages of like treatments. Treatments 1 and 3 were inoculated with log-growth phase *P. parvum* and received f/2 nutrient additions. Treatment 1 contained RO water adjusted to 0.5 psu and treatment 3 contained 0.2 µm filtered lake water. Treatment 5 contained 0.2 µm filtered lake water and was also inoculated with log-growth phase *P. parvum*.

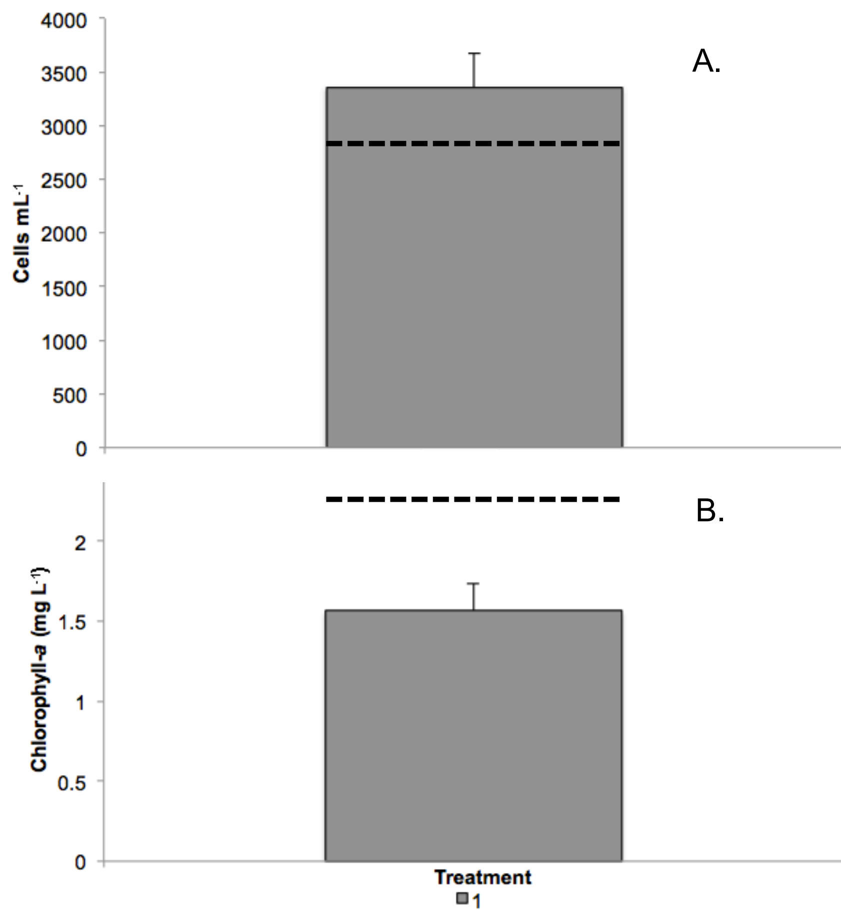

Figure 8: Treatment 1 final average *P. parvum* population density (A.) and chlorophyll-*a* concentrations (B.) ± 1 SD for the Lake Somerville experiment conducted during fall 2012. The horizontal dashed lines represent the initial averages. Treatments 3 and 5 were not included due to unsuccessful lake water filtration by the 0.2 µm cartridge filter. Treatment 1 was inoculated with log-growth phase *P. parvum* and contained RO water adjusted to 0.5 psu. The treatment was also brought to f/2 nutrient concentrations.

B-1.2.2 Treatments inoculated with stationary-growth phase *P. parvum*

Opposite to what was observed in treatments inoculated with log-growth phase culture, *P. parvum* growth rates of populations inoculated from stationary-growth phase culture were -0.13 d^{-1} in media made from RO water (treatment 2) and -0.35 d^{-1} in media made from filtered lake water (treatment 4), with the rate of decrease not statistically different between the two treatments (Tukey's HSD, $p > 0.05$; Table 1, Figure 9A). Chlorophyll-*a* concentrations also decreased (77 ± 6% and 76 ± 14%) in treatments 2 and 4 respectively, where again concentrations between the two treatments were not significantly different (Tukey's HSD, $p > 0.05$; Figure 9B). As with the no nutrient addition treatment inoculated with log-growth phase culture, *P. parvum* density and chlorophyll-*a* concentration declined by -0.71 d^{-1} and 73 ± 14% when inoculated into filtered lake water without nutrient additions (treatment 6; Table 1, Figure 9A-B).

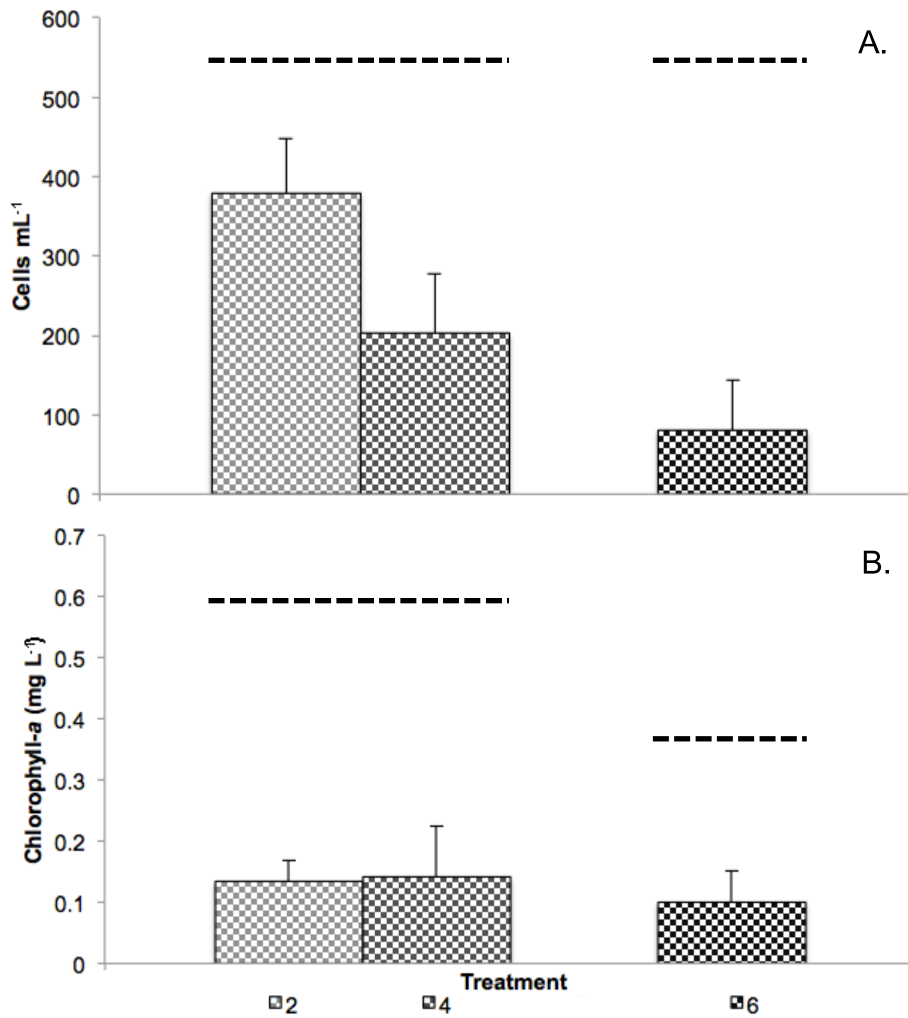

Figure 9: Treatment 2, 4, and 6 final average *P. parvum* population densities (A.) and chlorophyll-*a* concentrations (B.) ± 1 SD for the Lake Somerville experiment conducted during winter 2013. The horizontal dashed lines represent the initial averages of like treatments. Treatments 2 and 4 were inoculated with stationary-growth phase *P. parvum* and received f/2 nutrient additions. Treatment 2 contained RO water adjusted to 0.5 psu and treatment 4 contained 0.2 μm filtered lake water. Treatment 6 contained 0.2 μm filtered lake water and was inoculated with stationary-growth phase *P. parvum*.

B-2. Lake Whitney Winter and Fall Experiments

B-2.2 Effects of ambient lake conditions and water quality other than inorganic nutrients

B-2.2.1 Treatments inoculated with log-growth phase *P. parvum*

During the winter, the *P. parvum* growth rate of populations inoculated from log-growth phase culture in media made from RO water (treatment 1) and media made from filtered lake water (treatment 3) was 0.05 d^{-1} (Table 1, Figure 10A), and the rate of increase was not statistically different between the treatments (Tukey's HSD, $p > 0.05$). Similarly, 21 ± 7% and 7 ± 12% decreases in chlorophyll-*a* concentrations were not statistically different (Tukey's HSD, $p > 0.05$; Figure 10B). *P. parvum* density and chlorophyll-*a* concentration also increased by 0.06 d^{-1} and 0.60 ± 4% when inoculated into filtered lake water without nutrient additions (treatment 5; Table 1, Figure 10A-B).

During the fall experiment, *P. parvum* population density also increased by 0.03 d^{-1} in media made from RO water (treatment 1; Table 2, Figure 11A). A corresponding 35 ± 15% increase in chlorophyll-*a* concentration was observed as well (Figure 11B). Due to failure of the 0.2 µm cartridge filter during the fall experiment, an evaluation of *P. parvum* growth in waters with just dissolved constituents from Lake Whitney (comparison of treatments 1 and 3) was not possible.

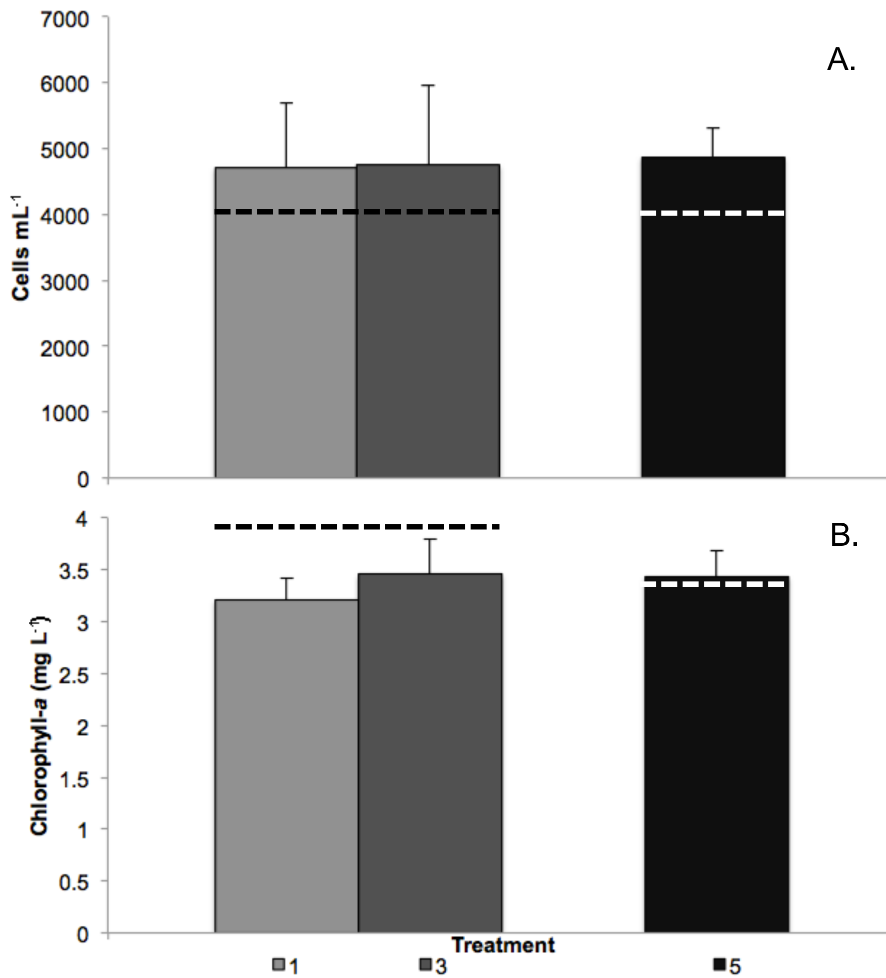

Figure 10: Treatments 1, 3, and 5 final average *P. parvum* population densities (A.) and chlorophyll-*a* concentrations (B.) ± 1 SD for the Lake Whitney experiment conducted during winter 2013. The horizontal dashed lines represent the initial averages of like treatments. Treatments 1 and 3 were inoculated with log-growth phase *P. parvum* and received f/2 nutrient additions. Treatment 1 contained RO water adjusted to 0.5 psu and treatment 3 contained 0.2 µm filtered lake water. Treatment 5 contained 0.2 µm filtered lake water and was inoculated with log-growth phase *P. parvum*.

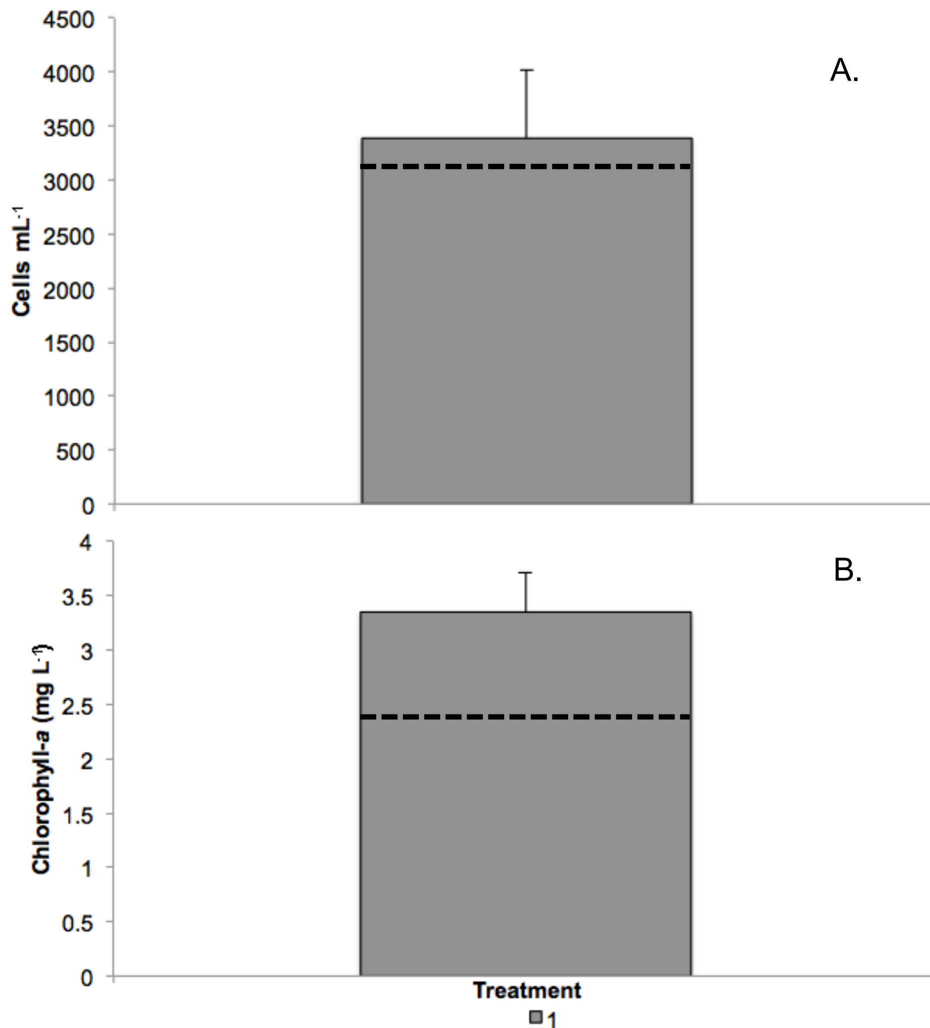

Figure 11: Treatment 1 final average *P. parvum* population density (A.) and chlorophyll-*a* concentrations (B.) ± 1 SD for the Lake Whitney experiment conducted during fall 2012. The horizontal dashed lines represent the initial averages of like treatments. Treatments 3 and 5 were not included due to unsuccessful lake water filtration by the 0.2 µm cartridge filter. Treatment 1 was inoculated with log phase growth *P. parvum* and contained RO water adjusted to 0.5 psu. The treatment was also brought to f/2 nutrient concentrations.

B-2.2.2 Treatments inoculated with stationary-growth phase *P. parvum*

P. parvum growth rates of populations inoculated from stationary-growth phase culture were 0.17 d^{-1} in media made from RO water (treatment 2) and 0.09 d^{-1} in media made from filtered lake water (treatment 4), with the rate of increase not statistically different between the two treatments (Tukey's HSD, $p > 0.05$; Table 1, Figure 12A). Chlorophyll-*a* concentrations decreased (47 ± 8% and 57 ± 2%) in treatments 2 and 4 respectively, where again concentrations between the two treatments were not significantly different (Tukey's HSD, $p > 0.05$; Figure 12B). As with the no nutrient addition treatment inoculated with log-growth phase culture, *P. parvum* density and chlorophyll-*a* concentration declined by -0.05 d^{-1} and 19 ± 37% when inoculated into filtered lake water without nutrient additions (treatment 6; Table 1, Figure 12A-B).

B-3. *P. parvum* Growth Rate Comparisons Between Lake Somerville and Lake Whitney

The following subsections compare *P. parvum* population growth rates from like monoculture treatments between lakes. All winter treatments were analyzed, but only treatment 1from experiments conducted in the fall were compared statistically as a result of filtration error to other monoculture treatments.

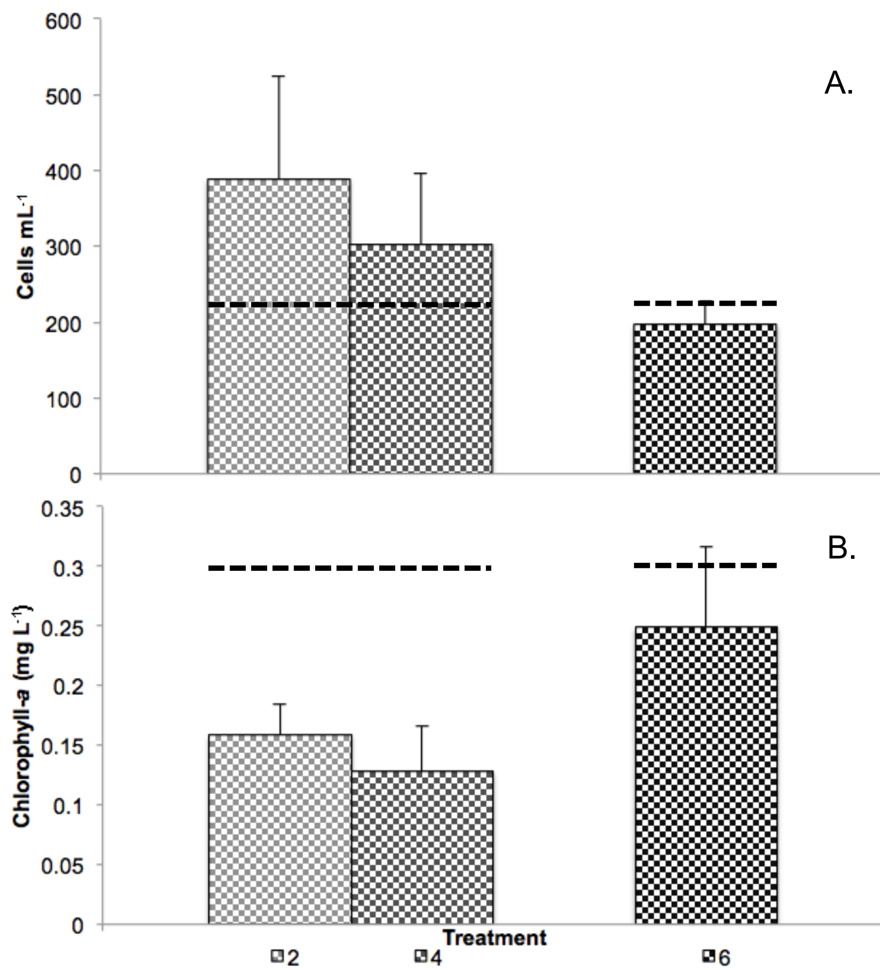

Figure 12: Treatments 2, 4, and 6 final average *P. parvum* population densities (A.) and chlorophyll-*a* concentrations (B.) ± 1 SD for the Lake Whitney experiment conducted during winter 2013. The horizontal dashed lines represent the initial averages of like treatments. Treatments 2 and 4 were inoculated with stationary-growth phase *P. parvum* and received f/2 nutrient additions. Treatment 2 contained RO water adjusted to 0.5 psu and treatment 4 contained 0.2 µm filtered lake water. Treatment 6 contained 0.2 µm filtered lake water and was inoculated with stationary-growth phase *P. parvum*.

B-3.1 **P. parvum** ***monoculture treatments***

B-3.1.1 Treatments inoculated with log-growth phase *P. parvum*

Population growth rates of *P. parvum* inoculated from log-growth phase culture from winter experiments were not statistically different between lakes in all monoculture treatments with f/2 nutrient additions (treatments 1 and 3) (Tukey's HSD, $p > 0.05$; Table 1). However, population growth rates were statistically different in filtered lake water treatments without nutrient additions (treatment 5) (Tukey's HSD, $p < 0.05$). Population growth rates from treatment 1 during the fall experiments did not differ significantly either (Tukey's HSD, $p > 0.05$; Table 2).

B-3.1.2 Treatments inoculated with stationary-growth phase *P. parvum*

Some Lake Whitney treatments inoculated with stationary-growth phase *P. parvum* displayed better growth compared to similar treatments from Lake Somerville. *P. parvum* cells grew at a significantly greater population growth rate when f/2 nutrients were added to RO water in Lake Whitney as well as 0.2 µm filtered Lake Whitney water compared to RO water in Lake Somerville and 0.2 µm filtered Lake Somerville water (treatments 2 and 4; Tukey's HSD, $p < 0.05$; Table 1). Treatment 6 average population growth rates of *P. parvum* were negative in both lakes, but to a significantly lesser degree in Lake Whitney (Tukey's HSD, $p < 0.05$; Table 1).

B-4. Discussion

B-4.1 Lake water quality and **P. parvum** ***growth***

Results reveal that population growth rates of *P. parvum* inoculated from log-growth phase culture from winter experiments were not statistically different between

lakes in all monoculture treatments with f/2 nutrient additions. This indicates that *P. parvum* inoculated from log-growth phase culture is able to grow in the ambient environmental conditions of both lakes when nutrients are not limiting. However, *P. parvum* inoculated from log-growth phase culture was shown to be unable to grow exclusively via photo-autotrophy in the ambient environmental conditions of Lake Somerville without the aid of nutrient additions (treatment 5), as population growth rates between lake monoculture treatments were significantly different. *P. parvum* growth declined in filtered Lake Somerville water, but increased in filtered Lake Whitney water. This indicates that the ambient water quality conditions of Lake Whitney are more conducive for *P. parvum* growth via photo-autotrophy.

P. parvum populations inoculated from stationary-growth phase culture also displayed better growth in some Lake Whitney treatments. Stationary phase *P. parvum* cells grew at a significantly greater population growth rate when f/2 nutrients were added to filtered Lake Whitney water compared to filtered Lake Somerville water (treatments 4) where population densities decreased. The decline in *P. parvum* population density as a monoculture in Lake Somerville is likely due to the low salinity of water in treatment 4, and the small number of stationary-growth phase *P. parvum* cells inoculated into the treatments. Therefore, the inoculation density of approximately 550 cells mL^{-1} might have been below the minimum viable population size for *P. parvum* to recover via photo-autotrophy in such a low salinity level even when nutrients were not limiting. This suggests that stationary phase *P. parvum* growth may be more affected by slight differences in salinity when nutrients are not limiting than growth of

log phase *P. parvum*. Data also indicates that low densities of stationary phase *P. parvum* are unable to grow exclusively via photo-autotrophy in either lake (treatment 6), but are less affected by ambient water quality conditions in Lake Whitney.

APPENDIX C

C-1. Results and Discussion of *P. parvum* Population Growth Rate Comparison Between Lake Somerville and Lake Whitney

The following subsections compare *P. parvum* population growth rates from like treatments containing organisms < 20 µm between lakes.

C-1.1 Treatments inoculated with log-growth phase **P. parvum**

Growth rates of *P. parvum* populations inoculated from log-growth phase culture were not statistically different between winter lake treatments when exposed to natural phytoplankton assemblages (treatments 7; Tukey's HSD, $p > 0.05$; Table 1). However, growth rates of *P. parvum* populations inoculated from log-growth phase culture from the Lake Somerville experiment conducted in the fall were significantly greater than those from Lake Whitney when exposed to natural phytoplankton assemblages (treatment 7; Tukey's HSD, $p < 0.05$; Table 2).

C-1.2 Treatments inoculated with stationary-growth phase **P. parvum**

No statistical difference was observed in population growth rates of *P. parvum* inoculated from stationary-growth phase culture that were exposed to natural phytoplankton assemblages (treatments 8; Tukey's HSD, $p > 0.05$; Table 1).

C-1.3 Growth of **P. parvum** *cells occurring in the natural phytoplankton assemblages*

Population growth rates of *P. parvum* occurring in the natural phytoplankton assemblages of both lakes during fall and winter experiments (treatments 9) were not statistically different (Tukey's HSD, $p > 0.05$; Tables 1 and 2).

C-2. Discussion of Lake Water Quality and *P. parvum* Growth in the Presence of Organisms Less Than 20 µm

P. parvum growth in winter experiments was not affected differently in either lake when populations had the opportunity to feed phagotrophically, despite evidence of inferior water quality conditions in Lake Somerville (see Section 5.2.1 and 5.3.1; Appendix B). *P. parvum* growth rates were not statistically different between winter lake experiments when exposed to natural phytoplankton assemblages and other organisms smaller than 20 µm (treatments 7 and 8). Furthermore, during the fall Lake Somerville experiment, growth rates of *P. parvum* populations inoculated from log-growth phase culture were significantly higher than those from Lake Whitney when exposed to natural phytoplankton assemblages and other organisms smaller than 20 µm. This could be a result of a large natural phytoplankton assemblage present in Lake Somerville, which was indicated by an average initial chlorophyll-*a* concentration that was more than double the initial averages from all other experiments at either lake. Previous experiments have shown that the phagotrophic feeding frequency of *P. parvum* increases when prey population densities increase (Skovgaard et al. 2003), and *P. parvum* growth is enhanced when cells feed phagotrophically (Martin-Cereceda et al. 2003). Therefore, the large natural phytoplankton assemblage would have increased *P. parvum* phagotrophy and consequently increased *P. parvum* growth.

APPENDIX D

D-1. Discussion of *P. parvum* Effects on Other Phytoplankton

Experimental results suggest that *P. parvum* affected other phytoplankton populations by means of phagotrophy, competition, and allelopathy (Graneli and Johansson 2003b, Roelke et al. 2007, Errera et al. 2008, Schwierzke et al. 2010). Chlorophyll-*a* concentrations were significantly greater in treatments with zooplankton grazers compared to treatments only containing phytoplankton in all Lake Somerville treatments inoculated with log- and stationary-growth phase *P. parvum*. The same occurrence was also observed in treatments inoculated with log-growth phase *P. parvum* from the winter Lake Whitney experiment. These greater increases in average chlorophyll-*a* concentrations of grazer treatments suggest that not only did the growth of other phytoplankton populations make up for the chlorophyll-*a* lost from the grazed *P. parvum*, but they also grew to population densities greater than those perceived in the no-grazer treatments likely because fewer *P. parvum* cells were present to prey on them and use allelopathy. Although no direct evidence of allelopathy on other phytoplankton was observed, it is still highly likely that allelopathic toxins were used by *P. parvum*. Previous studies have noted that *P. parvum* utilizes its allelochemicals to immobilize motile prey before phagotrophic ingestion can occur (Graneli and Johansson 2003b, Skovgaard and Hansen 2003, Tillmann 2003, Graneli et al. 2012). Since the significantly greater chlorophyll-*a* concentration of most grazer treatments compared to their similar no-grazer treatments containing natural phytoplankton assemblages suggests that *P.*

parvum cells preyed upon other phytoplankton, it is expected that allelopathic toxins were used by the harmful alga to some degree in all Lake Somerville treatments containing *P. parvum* and other microorganisms from fall and winter experiments. The same logic applies to treatments inoculated with log-growth phase *P. parvum* containing other microorganisms from the winter Lake Whitney experiment as well.

Unlike the winter Lake Whitney experiment, the final chlorophyll-*a* concentration of the grazer treatment inoculated with log-growth phase *P. parvum* (treatment 11) was not greater than the similar no-grazer treatment (treatment 7) in the fall Lake Whitney experiment. Therefore, no indication of zooplankton grazing contributing to a decrease in negative effects of competition, low-level allelopathy, and phagotrophy of *P. parvum* on other phytoplankton was observed. A greater initial *P. parvum* population density in the fall experiment may be a contributing factor to this. Meaning that *P. parvum* cells were abundant enough to still have an effect on other phytoplankton while being grazed by zooplankton.

P. parvum populations present in the natural phytoplankton assemblages of Lake Somerville during the fall and winter also displayed signs of negative effects on the natural phytoplankton assemblage. Average chlorophyll-*a* concentrations of treatments without grazers (treatment 9) declined below initial averages while *P. parvum* population densities increased. *P. parvum* is known to increase toxicity and phagotrophy under non-optimal growth conditions (Graneli and Salomon 2010). Hence, it is logical that phytoplankton in Lake Somerville would be negatively affect by *P. parvum* populations since the harmful alga was unable to grow as a monoculture in the filtered

lake water (Appendix B). Furthermore, no-grazer treatments inoculated with log- and stationary- growth phase *P. parvum* culture (treatments 7 and 8) were suggested to utilize phagotrophy. Thus, it is likely that *P. parvum* cells behaved similarly in treatment 9.

Indications of allelopathy and phagotrophy from the *P. parvum* population present in the natural phytoplankton assemblages of Lake Whitney were mixed. During the winter, chlorophyll-*a* concentrations were similar in grazer and no-grazer treatments suggesting that *P. parvum* populations did not considerably utilize allelopathy and phagotrophy. This was most likely a result of low competition for resources among phytoplankton. The natural phytoplankton assemblage in Lake Whitney during the winter appeared to be less dense than the assemblage in Lake Somerville as the initial average chlorophyll-*a* concentration for treatment 9 was less than half that of the winter Lake Somerville treatment 9. Also, The population density of *P. parvum* present in the natural phytoplankton assemblage was the lowest of all experiments during the winter experiment in Lake Whitney. Therefore, lower competition and improved water quality conditions (see Appendix B and C) likely prevented *P. parvum* cells from significantly exploiting their allelopathic and phagotrophic abilities.

On the other hand, a 45% greater average chlorophyll-*a* concentration in treatment 10 compared to treatment 9 in the fall Lake Whitney experiment suggests that *P. parvum* utilized phagotrophy and/or allelopathy to reduce competition of the natural phytoplankton assemblage in the no-grazer treatment. However, statistical significance could not be determined as only one replicate from the grazer treatment (treatment 10)

was left undamaged after severe weather conditions were encountered. This greater increase in treatment 10 chlorophyll-*a* is likely attributed to an initial average *P. parvum* population density that was more than double the winter average for these treatments. Findings from Legrand et al. (2003) showed that allelopathic effects of *P. parvum* are proportional to its population density. Hence, the larger *P. parvum* population in the fall experiment would have a greater negative impact on other phytoplankton populations in the absence of grazing pressure by zooplankton.

No negative impact of *P. parvum* populations inoculated from stationary-growth phase culture on other phytoplankton was suggested in treatment 8 and 12 data from Lake Whitney. However, this may be attributed to the low density of stationary-growth phase *P. parvum* cells used in the treatments. As mentioned in Section 4.2, the inoculation density of stationary-growth phase *P. parvum* was approximately 200 cells mL^{-1}. The inoculation density of stationary-growth phase cells in the Lake Somerville treatments was nearly 3 times greater than the Lake Whitney treatment. Therefore, competition, phagotrophy, and allelopathy of *P. parvum* would have had a greater influence on chlorophyll-*a* concentrations (i.e. other phytoplankton) in the Lake Somerville treatments, and results indicate that was so.