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Rotifer–*Prymnesium parvum* interactions: role of lake bloom history on rotifer adaptation to toxins produced by *P. parvum*

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ABSTRACT: *Prymnesium parvum* is a harmful algal bloom species present in many inland water bodies of the southcentral USA, but does not form fish-killing blooms in all of them. The present study tested the hypothesis that rotifer grazing of *P. parvum* might influence the incidence of blooms. Three-day in-lake experiments, which focused on the size fraction of zooplankton dominated by rotifers and natural phytoplankton assemblages inoculated with *P. parvum*, were conducted during the time of bloom development in 2 reservoirs of the southcentral USA: Lakes Somerville and Whitney, where the latter experiences *P. parvum* blooms and the former does not. Toxicity at a level lethal to fish was only occasionally observed during these experiments, so our experimental treatments are considered to be at a low-toxicity level. As a whole, rotifers in Lakes Somerville and Whitney selectively grazed *P. parvum.* Rotifers in Lake Somerville appeared to benefit from this selective grazing, while rotifers in Lake Whitney did not. The differences between rotifer communities from these lakes might be because rotifers from Lake Somerville historically have only been exposed to low levels of toxins produced by *P. parvum* and were able to develop resistance to these toxins, thus enabling them to persist and perhaps contribute to the suppression of blooms there. The opportunity for this type of microevolutionary adaptation may not occur in lakes where *P. parvum* blooms and waters reach high toxicity levels, such as those which have occurred historically in Lake Whitney.

KEY WORDS: *Prymnesium parvum* · Rotifer · Selective grazing · Microevolutionary adaptation

INTRODUCTION

Harmful algal blooms (HABs) are increasing globally in frequency and magnitude, which has resulted in greater environmental and public health threats (Smayda 1990, Hallegraeff 1993, Fu et al. 2012). Thus, proactive management approaches to reducing HABs are appealing. Unfortunately, the environmental factors enabling HABs are widely variable and frequently species-specific (Roelke & 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 will need to be pursued. For example, some phytoplankton species bloom in particular water bodies while not in others. Understanding why this happens might provide insights into potential proactive management options. One exam-

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ple of a HAB species that is problematic in some water bodies, but not in others that are nearby, is *Prymnesium parvum*.

P. parvum, commonly known as golden algae, is a globally occurring mixotrophic haptophyte that is capable of forming large fish-killing blooms, and is tolerant of large variations of salinity and temperature (Larsen & Bryant 1998, Baker et al. 2009, Granéli et al. 2012). In the past 29 years *P. parvum* blooms have spread throughout southern regions of the USA, and more recently into northern areas, with the first documented bloom occurring in the southcentral USA (Pecos River, Texas, in 1985) (James & De La Cruz 1989). Currently, *P. parvum* is found in 23 US states (Sager et al. 2008, Roelke et al. 2011, Hambright 2012, Patiño et al. 2014).

Blooms of *P. parvum* typically occur in aquatic systems that are eutrophic and brackish (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, and for the period of 2001 to 2008, *P. parvum* blooms caused losses conservatively estimated at tens of millions in US dollars in natural resource damages, and were estimated to have killed at least 34 million fish (Southard et al. 2010, Brooks et al. 2011). Fisheries activities and recreation were impaired by these seasonal *P. parvum* blooms (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 in this region.

Identification of the chemicals responsible for toxic effects from *P. parvum* is still an area of emerging science (Henrikson et al. 2010, Bertin 2012a,b, 2014, Blossom et al. 2014a,b). One pathway of toxicity is associated with allelopathy. *Prymnesium* spp. can produce chemicals that are believed to be released from the cell, producing deleterious effects to other phytoplankton, suppressing growth or causing lysis (Granéli & Johansson 2003a, Granéli et al. 2012). Immobilization of bacteria and suppression of zooplankton feeding and reproduction, even zooplankton death, have also been observed as a result of chemicals produced by *P. parvum* (Granéli & Johansson 2003a, Skovgaard & Hansen 2003, Tillmann 2003). Some chemicals (possibly the same chemicals responsible for deleterious effects to plankton) disrupt cellular membranes of fish and shellfish, leading to mortality (Yariv & Hestrin 1961, Shilo 1967, Hallegraeff 1993). Production of allelopathic and toxic chemicals is enhanced under conditions of nutrient limitation (Granéli 2006, Roelke et al. 2007, Errera et al. 2008), low salinity and temperature (Baker et al. 2007, 2009), and high turbulence or aeration (Igarashi et al. 1995, Vidyarathna et al. 2014). Toxicity by *P. parvum* also varies depending on growth phase, with stationary-growth phase cells being more toxic than log-growth phase cells (Shilo 1967, Johansson & Granéli 1999, Granéli & Johansson 2003b).

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 (Roelke et al. 2010). Several studies suggested that algicidal bacteria and allelopathic phytoplankton might prevent *P. parvum* blooms in some lakes (Grover et al. 2010, Roelke et al. 2010, James et al. 2011). Another study showed that rotifer-dominated zooplankton communities prospered during toxic blooms of *P. parvum*, and suggested that top-down control by rotifers might influence *P. parvum* bloom dynamics (Schwierzke et al. 2010). 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 *P. parvum*. Therefore, it is reasonable to assume that communities with *N. laurentiae* may have an influence on bloom initiation and development. Observations from Errera et al. (2008) also suggested that grazing of *P. parvum* by rotifers might be an important loss factor for *P. parvum* populations under nutrient sufficient conditions during the autumn.

Lakes inhabited by, but not experiencing blooms of, *P. parvum* pose an interesting ecological scenario in which rotifer–*P. parvum* interactions might be a contributing factor preventing blooms. Therefore we tested the hypothesis that grazing of *P. parvum* by rotifers might be a significant factor in the prevention of blooms in some lakes, and that some rotifer taxa may be more resistant to *P. parvum* toxicity and more efficient at grazing *P. parvum* compared to others. In the present study we report findings from field experiments conducted during the winter of 2013 in a lake where *P. parvum* is present but does not form blooms (i.e. Lake Somerville, Texas, USA) and in a lake that experiences seasonal *P. parvum* blooms (i.e. Lake Whitney, Texas, USA). The focus of the experiments was on the influence rotifers have on *P. parvum* bloom development.

MATERIALS AND METHODS

Site description

Lake Somerville (30° 19′ 21.2″ N, 96° 32′ 09.9″ W) 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 approx. 77 km southwest of Bryan, Texas, USA. Drainage area above the dam is approx. 1619 km^2. Reservoir capacity at conservation storage elevation is approx. 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 & Hudson 1982, TPWD 2013a, TSHA 2013). Lake Somerville is classified as a hypereutrophic lake (TCEQ 2011). Historical inorganic nutrient data from 1999 to 2002 indicate that soluble reactive phosphorus (SRP) ranged from ~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). Historical reservoir water quality data for the months of December to March since the year 2000 indicate that the minimum and maximum salinity values for Lake Somerville are 0.14 and 0.55 psu, respectively (raw data obtained from Burley et al. 2011, http://pubs.usgs.gov/ds/594/). *Prymnesium parvum* inhabits Lake Somerville, but toxic blooms have not been documented.

Lake Whitney (31° 52′ 10.2″ N, 97° 22′ 28.1″ W) is also a USACE reservoir in the Brazos River basin. The lake was impounded in 1951 and is approx. 3 km west of Whitney, Texas, USA. An area of approx. 42 107 km^2 drains into the lake. Reservoir capacity at conservation storage elevation is approx. 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 & Hudson 1982, Breeding 2013, TPWD 2013b). Lake Whitney is also classified as a hypereutrophic lake (TCEQ 2011). Data of inorganic nutrients sampled from 2008 to 2009 indicate that SRP ranged from ~0.2 to 1.6 µmol l^{-1}, and DIN ranged from 0.0 to 55 µmol l^{-1} (Roelke et al. 2004). Minimum and maximum salinity values of winter seasons since the year 2000 are 0.46 and 0.90 psu, respectively (raw data obtained from Burley et al. 2011, http://pubs.usgs.gov/ds/594/). Lake Whitney has consistently experienced *P. parvum* HABs over the past decade.

Field methods and experimental treatments

We conducted experiments in Lake Somerville and Lake Whitney during 2013 (initiated March 6th and March 11th, respectively); each experiment lasted 3 d, which is an adequate experiment duration given known rotifer grazing rates (Hansen et al. 1997, Barreiro et al. 2005). Our methods involved collecting rotifer populations from a natural setting in Lakes Somerville and Whitney during the winter, a period of *P. parvum* bloom development in many lakes of the region, and performing in-field experiments with naturally occurring phytoplankton and rotifer populations combined with lab grown *P. parvum* cultures. Both log- and stationary-growth phase *P. parvum* cultures were used because of their differences in toxicity (Shilo 1967, Johansson & Granéli 1999, Granéli & Johansson 2003b).

Field collection of rotifers took place at a depth of 0.5 m in the open water regions of each lake near their respective dams. A bucket volumetrically calibrated was used to collect 12 l of water filtered successively through a cod end equipped with 61 µm mesh and a 210 µm mesh net. The retained contents were thus organisms within the 61 to 210 µm size range (primarily rotifers). The container of the rotifer size fraction was brought to a larger volume that could be gently mixed and easily divided into equal parts. Aliquots from this well-mixed volume were used to initiate the appropriate treatments of the *in situ* experiments (discussed below). Aliquot volumes were added to the experimental units so that the original rotifer population densities that occurred in each lake were restored. The zooplankton isolated in the 61 to 210 µm size range are hereafter referred to as 'zooplankton'.

In situ experiments designed to focus on rotifer–*P. parvum* interactions consisted of 12 total treatments deployed at each lake. Treatments 1 through 9 served as controls and consisted only of phytoplankton. Consequently, these treatments were conducted in 2 l polycarbonate bottles (Table 1). Treatments 10 through 12 consisted of combined rotifer and phytoplankton assemblages. Consequently, these treatments were conducted in larger-volume experimental units, i.e. 20 l polycarbonate carboys (Table 1). The large number of control treatments was necessary to interpret the observations within the treatments containing rotifers. Treatments in 2 l bottles were filled to 2 l and treatments in 20 l carboys were filled to 15 l during experiments with sufficient air left in the headspace of each experimental unit for buoyancy. Bottles (2 l) were used for logistical purposes, and have been used previously in similar experiments (Errera et al. 2008). All treatments were performed in triplicate for a total sample size of 72 experimental units (fifty-four 2 l bottles and eighteen 20 l carboys).

Table 1. Description of experimental treatments of *Prymnesium parvum* conducted in triplicate. Treatments 1–9 consisted exclusively of phytoplankton in 2 l polycarbonate bottles. Treatments 10–12 involved zooplankton in the 61–210 µm size range in 20 l polycarbonate carboys. Twenty µm filtered lake water contained naturally occurring nanoplankton. Addition or no addition of *P. parvum* culture into treatments is indicated by – (no culture added), + (log-growth phase culture added), and ++ (stationary-growth phase culture added). Water filtered by reverse osmosis is referred to as RO water. Treatments were deployed in limnocorrals on Lake Somerville and Lake Whitney, Texas, USA, during early March 2013

Treatment	RO water + f/2	0.2 µm filtered lake water + f/2 nutrients	0.2 µm filtered lake water	20 µm filtered lake water	20 µm filtered lake water + zooplankton
1	+				
2	++				
3		+			
4		++			
5			+		
6			++		
7				+	
8				++	
9				–	
10					–
11					+
12					++

Filtered lake water was used in all treatments except Treatments 1 and 2. Lake water was passed through various filters of several sizes for different treatments. 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 <20 µm were isolated by passing the 61 µm-filtered water through a 20 µm mesh net and keeping the retained volume. Particle-free lake water was achieved by using a 1.0 µm cartridge filter (GE Water and Process Technologies) in combination with a 0.2 µm capsule filter (Whatman GE).

Treatments 1 and 2. The first 2 treatments were designed to test if *P. parvum* was able to grow under the light, turbulence, and temperature conditions present at each lake. Artificial sea salt (Instant Ocean) was added to the reverse osmosis filtered (RO) tap water in both treatments to match the salinity of each lake (i.e. 0.5 psu for Lake Somerville and 0.75 psu for Lake Whitney). Inorganic nutrients were also added at growthsaturating levels (f/2 concentration; Guillard 1975). *P. parvum* laboratory cultures in log- (Treatment 1) or stationary- (Treatment 2) growth phase were then added to observe differences in cell toxicity due to growth phase (Table 1). *P. parvum* inoculation densities resulted in approx. 4000 cells ml^{-1} for log-growth phase cultures in the *in situ* experiments. Inoculation of stationary-growth phase *P. parvum* cultures resulted in densities of approx. 200 cells ml^{-1} in Lake Whitney and 550 cells ml^{-1} in Lake Somerville in experimental treatments. The difference in stationary-growth phase culture inoculation densities between lake experiments was a result of lack of available *P. parvum* culture at the time of Lake Whitney experiment deployment. All laboratory cultures of *P. parvum* used in the experiments were maintained at 1 psu salinity and 13 ± 1°C prior to inoculation into treatments (Roelke et al. 2007).

Treatments 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 for Treatments 1 and 2 (Table 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 environmental contaminants, that might influence algal growth.

Treatments 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 for Treatments 1 and 2 (Table 1). These treatments combined with Treatments 3 and 4 allowed the effects of nutrient additions on *P. parvum* cultures to be observed.

Treatments 7 and 8. 20 µm filtered lake water containing the natural phytoplankton assemblage in the nanoplankton size range was inoculated with log- (Treatment 7) or stationary- (Treatment 8) growth phase *P. parvum* cultures at densities described in Treatments 1 and 2 (Table 1). These treatments combined with Treatments 5 and 6 allowed the effects of natural phytoplankton assemblages in the nano-

plankton size range on *P. parvum* in both lakes to be observed.

Treatment 9. 20 µm filtered lake water containing only the natural phytoplankton assemblage in the nanoplankton size range of each lake was used for this treatment (Table 1). *P. parvum* culture was not inoculated into Treatment 9, but the nanoplankton contained *P. parvum* at a low concentration as a part of the naturally occurring assemblage. This treatment combined with Treatments 7 and 8 allowed natural phytoplankton assemblages in the nanoplankton size range and *P. parvum* culture interactions to be observed.

Treatment 10. 20 µm filtered lake water containing the natural phytoplankton assemblage in the nanoplankton size range with added zooplankton was used for this treatment (Table 1). This treatment combined with Treatment 9 allowed for observation of how natural zooplankton assemblages in the 61–210 µm size range affected natural phytoplankton assemblages in the nanoplankton size range.

Treatments 11 and 12. 20 µm filtered lake water containing the natural phytoplankton assemblage in the nanoplankton size range with added zooplankton, and also inoculated with log- (Treatment 11) or stationary- (Treatment 12) growth phase *P. parvum* at densities described for Treatments 1 and 2 was used for this treatment (Table 1). These treatments combined with Treatments 7, 8, and 10 allowed for observation of how log- and stationary-growth phase *P. parvum* cultures interacted with natural zooplankton and phytoplankton assemblages.

All 2 l bottles and all 20 l carboys were deployed within enclosures in surface waters on both lakes during *P. parvum* bloom development conditions. Enclosures 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 enclosures used in the Lake Somerville experiment were anchored in a cove directly west of Welch Park. During the Lake Whitney experiment, enclosures were tethered to a boat dock belonging to the Little Rocky Lodge and Resort (Clifton, TX, USA). Deploying the experiment in lake waters allowed each treatment to experience environmental conditions in each lake (i.e. light fluctuation, wave action, and temperature).

Response variables

Response variables included *P. parvum* population density from which population growth rate was estimated, ambient water toxicity, chl *a*, and biovolumes of zooplankton taxonomic groups (mostly to the level of genera). Baseline water quality data (pH, salinity, and temperature) were taken at sampling locations of both lakes on the initial sampling day of the experiments using a Hydrolab Quanta Multiparameter Sonde (Hach Company). Samples of phytoplankton and chl *a* were taken every day of the experiments from each treatment. Zooplankton were sampled once at deployment from the initial 61 to 210 µm isolated size fraction and on the third day from Treatments 10, 11, and 12 when experiments were terminated. Upon experiment terminations, 12 l samples were 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 *P. parvum* population densities, 100 ml phytoplankton samples were collected and preserved from each well-mixed bottle and carboy. For preservation, a 25% glutaraldehyde solution (5% v/v) was used. *P. parvum* population densities were 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 (400×, Leica Microsystems). Around 20 randomly selected fields of view were counted depending on the density of the sample.

P. parvum population growth rates (µ) were calculated from the population density data using:

$$\mu = \frac{LN\left(\frac{Y_f}{Y_{ia}}\right)}{T_d} \quad (1)$$

where Y_f is the final population density within each experimental unit, Y_{ia} is the initial average population density of like treatments, and T_d is the experimental duration.

Prior to the initiation of *in situ* experiments, acute aquatic toxicity of log and stationary phase *P. parvum* lab cultures was determined using juvenile fathead minnow *Pimephales promelas* following standard methods (US EPA 2002). Organisms were fed *Artemia salina* nauplii (brine shrimp) to satiation 2 h prior to initiation of bioassays, which were performed in climate-controlled chambers at 25°C on a 16:8 h light dark cycle, and mortality was recorded after 24 and 48 h (US EPA 2002). At the conclusion of the 3 d *in situ* experiments, samples were collected and stored on ice in the dark during transport to the laboratory, where additional fish toxicity assays were initiated for each experimental unit within 24 h (US EPA 2002).

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

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 h, then counted using an inverted, phase-contrast light microscope (40× and 200×, Leica Microsystems). Dimensions for each individual counted were measured corresponding to best-fit geometric shapes in order to estimate biovolume (Wetzel & Likens 1991). Counted rotifers were grouped by genus. Copepods were grouped into copepod nauplii and total adult copepods. Zooplankton groups that made up >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 statistics in the corresponding analyses.

Data analysis

One-way ANOVA tests were used for analyzing *P. parvum* population density and growth rates. Final zooplankton biovolumes (absolute and relative) were also analyzed using 1-way ANOVA tests. Population growth rates of zooplankton were not calculated since only like treatments with the same initial biovolumes were compared. For chl *a* concentrations (absolute and relative), 1-way repeated measures ANOVA tests were used. If results of any of the ANOVA tests were significant, then Tukey's HSD post-hoc tests were used to determine which treatments were significantly different ($\alpha < 0.05$). The statistics software package SPSS v. 21 was used for all ANOVA tests.

When interpreting our experimental observations, we partially relied on taxonomic-specific size relationships between predator and prey. For this, we calculated the equivalent spherical diameters (ESDs) for the most common biovolume of each dominant and sub-dominant zooplankton group in both Lake Somerville and Lake Whitney experiments. Predator:prey ratios were calculated based on the ESDs of the zooplankton groups and the calculated ESD of a moderately sized *P. parvum* cell using cell measurements described in Granéli et al. (2012). *P. parvum* biovolume was calculated using an ellipsoid equation (Wetzel & Likens 1991). Zooplankton selection probability of *P. parvum* cells was then estimated by applying the calculated predator:prey ratios to the size selectivity spectra from Hansen et al. (1994).

RESULTS

Toxicity bioassays

Prymnesium parvum cultures used in all experiments were mostly non-toxic to fish, as survival of *Pimephales promelas* was 100% in almost all treatments, including lab controls, after 48 h. The exception was 80% survival in 5 experimental units: one from each of Treatments 6, 9, and 12 in the Lake Somerville experiment, and one from each of Treatments 11 and 12 in the Lake Whitey experiment. This suggests modest production of toxins from this nutrient-sufficient culture when the *in situ* experiments were initiated.

Abiotic conditions in lakes

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. In Lake Whitney, water temperature was 12.3°C at a depth of 1 m, while the salinity was 0.80 psu and pH measured 7.65.

Lake Somerville experiment

P. parvum population growth in filtered lake waters from Lake Somerville varied depending on whether they were inoculated from cultures in log- or stationary-growth phase, and whether or not inorganic nutrients were added. These data are not shown here, but are reported in Davis (2014). Briefly, under nutrient-replete conditions, *P. parvum* populations originating from log-growth phase culture grew at rates of 0.11 d^{-1} (with salinity adjusted, Treatment 1) and 0.01 d^{-1} (without salinity adjusted, Treatment 3). *P. parvum* populations originating from stationary-growth phase culture decreased at rates of –0.13 d^{-1} (with salinity adjusted, Treatment 2) and –0.35 d^{-1} (without salinity adjusted, Treatment 4). With no nutrient additions, *P. parvum* populations inoculated from log- and stationary-growth phase cultures decreased at rates of –0.10 and –0.71 d^{-1} (Treatments 5 and 6, respectively).

Zooplankton assemblages at Lake Somerville grazed the added *P. parvum*. This was apparent from

Table 2. *Prymnesium parvum* population growth rates (d^{-1}) in winter experimental treatments at Lake Somerville and Lake Whitney, Texas, USA. Treatment descriptions are provided in Table 1. Experiments were conducted in early March 2013. Values shown are mean ± 1 SD, with n = 3

Treatment	Size-fraction of lake water included	*P. parvum* growth phase	Lake Somerville	Lake Whitney
7	<20 µm	Log	0.04 ± 0.09	0.07 ± 0.07
8	<20 µm	Stationary	0.09 ± 0.05	0.03 ± 0.07
9	<20 µm	Naturally occurring	0.07 ± 0.01	0.09 ± 0.09
10	<20 µm + 61–210 µm	Naturally occurring	−0.21 ± 0.03	−0.12 ± 0.04
11	<20 µm + 61–210 µm	Log	−0.27 ± 0.02	−0.13 ± 0.06
12	<20 µm + 61–210 µm	Stationary	−0.25 ± 0.05	−0.01 ± 0.07

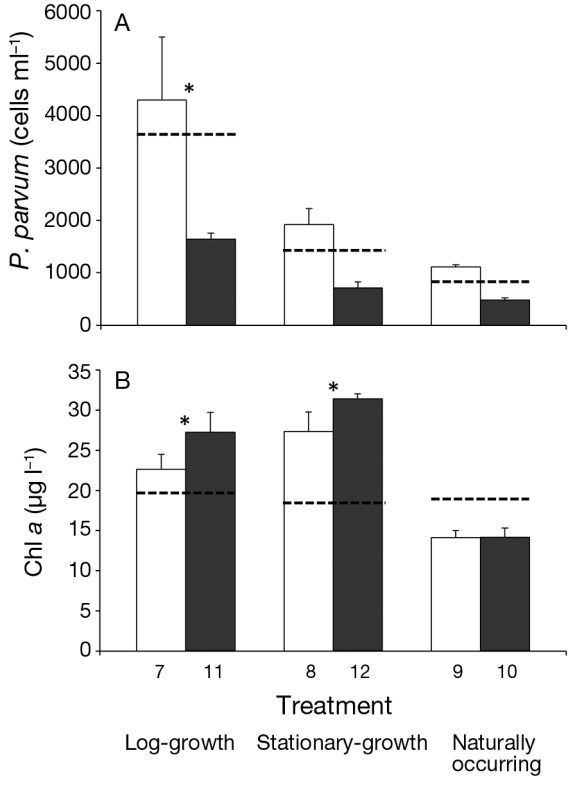

Fig. 1. Treatments 7, 8, 9, 10, 11 and 12 final average ±1 SD *Prymnesium parvum* (A) population densities and (B) total chlorophyll *a* concentrations for the Lake Somerville, Texas, USA, experiment conducted during winter 2013. Horizontal dashed lines represent the initial averages of like treatments. Black bars contained zooplankton in the 61–210 µm size range while white bars did not. Treatments 9 and 10 contained natural phytoplankton assemblages. 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 *P. parvum*. *Significant difference at $p < 0.05$

comparisons between Treatments 7 and 11, both receiving *P. parvum* additions from log-growth phase culture. In these treatments, *P. parvum* population growth rate was 0.04 d^{-1} in lake water containing nanoplankton and decreased −0.27 d^{-1} in lake water containing nanoplankton and zooplankton grazers (Table 2, Fig. 1A). These population growth rate differences were statistically significant (Tukey's HSD, $p < 0.05$; Tables 2 & 3, Fig. 1A). Comparisons between Treatments 8 and 12, both receiving *P. parvum* additions from stationary-growth phase culture, showed the same trend. In these treatments, *P. parvum* population growth rate was 0.09 d^{-1} in lake water containing nanoplankton and decreased −0.25 d^{-1} in lake water containing nanoplankton and zooplankton grazers (Table 2, Fig. 1A). These population growth rate differences were statistically significant (Tukey's HSD, $p < 0.05$; Tables 2 & 3, Fig. 1A).

Zooplankton assemblages at Lake Somerville also grazed the naturally occurring *P. parvum*. This was apparent through comparisons of Treatments 9 and 10. Here, *P. parvum* population growth rate was 0.07 d^{-1} in lake water containing nanoplankton and decreased −0.21 d^{-1} in lake water containing nanoplankton and zooplankton grazers (Table 2, Fig. 1A). Differences in *P. parvum* population growth rates between these treatments were marginally insignificant between the 2 treatments (Tukey's HSD, $p = 0.056$).

In treatments where *P. parvum* was added, chl *a* concentrations increased during the experiment; even more so when nanoplankton and zooplankton were present, a difference that was statistically significant (Tukey's HSD, $p < 0.05$; Fig. 1B). In treatments where no *P. parvum* culture was added, chl *a*

Table 3. Significance results from Tukeys post-hoc tests (1-way ANOVA) comparing *Prymnesium parvum* population growth rates (d^{-1}) between treatments, which examined the effect of zooplankton additions in the 61–210 µm size-range on growth of log- and stationary-growth phase *P. parvum*, as well as naturally occurring *P. parvum,* during experiments at Lake Somerville and Lake Whitney, Texas, USA

Treatments compared	*P. parvum* growth phase	Lake Somerville	Lake Whitney
7 & 11	Log	$p = 0.023$	$p = 0.102$
8 & 12	Stationary	$p = 0.013$	$p = 1.000$
9 & 10	Naturally occurring	$p = 0.056$	$p = 0.046$

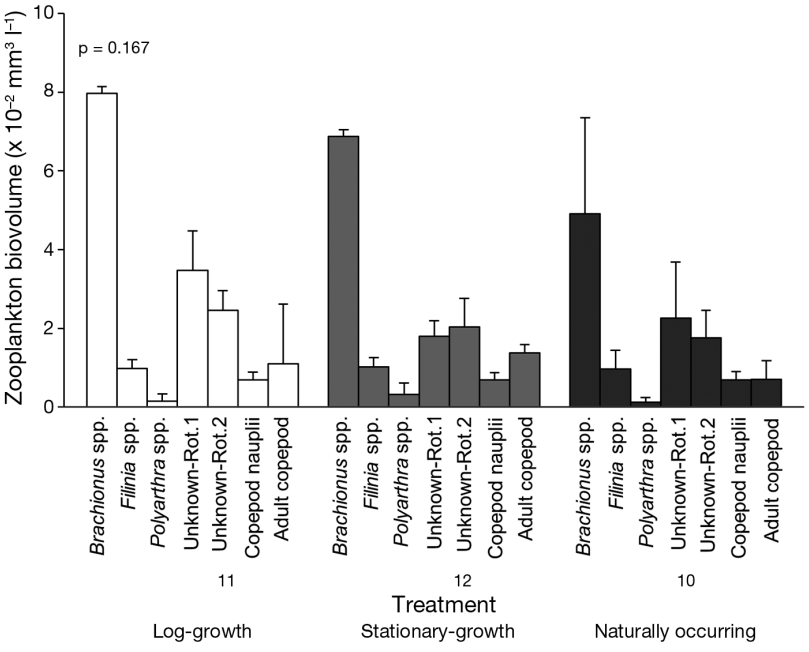

Fig. 2. Treatments 10, 11, and 12 final average ±1 SD biovolumes of dominant and sub-dominant zooplankton groups for the Lake Somerville, Texas, USA, experiment conducted during winter 2013. Treatments 10, 11, and 12 contained natural phytoplankton assemblages and zooplankton in the 61–210 µm size range. Treatment 11 was also inoculated with *Prymnesium parvum* in log-growth phase, while Treatment 12 was inoculated with stationary-growth phase *P. parvum*. Final zooplankton biovolumes in Treatments 11 and 12 were compared to final zooplankton biovolumes in Treatment 10. Unknown-Rot.1 and -Rot.2: unidentified illoricate rotifers

decreased. Prominent nanophytoplankton observed in these treatments included cyanobacteria, chlorophytes, and chrysophytes.

In all treatments with zooplankton, total zooplankton biovolume declined. The initial total biovolume was 2.41×10^{-1} mm^3 l^{-1}. By the termination of the experiments, total zooplankton biovolume was 1.81×10^{-1}, 1.49×10^{-1} and 1.25×10^{-1} mm^3 l^{-1} in Treatments 11 (log-growth phase culture added), 12 (stationary-growth phase culture added) and 10 (no culture added), respectively. The differences between Treatments 11 and 12 were not statistically significant (Tukey's HSD, $p > 0.05$). A taxonomic analysis showed that *Brachionus* spp. (initial biovolume of 13.2×10^{-2} mm^3 l^{-1}), *Filinia* spp. (1.69×10^{-2} mm^3 l^{-1}), *Polyarthra* spp. (1.36×10^{-2} mm^3 l^{-1}) and copepod nauplii (1.85×10^{-2} mm^3 l^{-1}) contributed to this overall decline (Fig. 2). A comparison between these treatments showed that these taxa declined less when *P. parvum* culture was added, with the exception of copepod nauplii.

The taxonomic analysis of zooplankton also showed that some groups increased in numbers. For example, adult copepods of orders Cyclopoida and Calanoida (initial biovolume of 5.48×10^{-3} mm^3 l^{-1}) and 2 unidentified illoricate rotifers, referred to hereafter as unknown-Rot.1 (5.50×10^{-3} mm^3 l^{-1}) and unknown-Rot.2 (2.01×10^{-2} mm^3 l^{-1}), increased in numbers. For adult copepods and unknown-Rot.1, this increase occurred in all 3 treatments, but was greater in treatments receiving addition of *P. parvum* culture (Fig. 2). Unknown-Rot.2 biovolume increased in treatments with added *P. parvum*, but was greater when added *P. parvum* culture was in log-growth phase (Fig. 2).

Predator:prey calculations of dominant and sub-dominant zooplankton in Lake Somerville revealed that *Brachionus* spp. and the unknown-Rot.1 had the highest estimated selection probability on *P. parvum* at ~96% (Table 4). *Polyarthra* spp. had the lowest selection probability at ~60% (Table 4).

Lake Whitney experiments

P. parvum population growth in filtered lake waters from Lake Whitney also varied depending on whether they were inoculated from cultures in log- or stationary-growth phase, and depending whether or not inorganic nutrients were added. But they varied in ways different from what was observed in Lake Somerville. Under nutrient-replete conditions, *P. parvum* populations originating from log-growth phase culture grew at rates of 0.05 d^{-1} in treatments with and without salinity adjustments (Treatments 1 and 3). *P. parvum* populations originating from stationary-growth phase culture grew at rates of 0.17 d^{-1} (with salinity adjusted, Treatment 2) and 0.09 d^{-1} (without salinity adjusted, Treatment 4). With no nutrient additions, *P. parvum* populations inoculated from log-growth phase cultures grew at a rate of 0.06 d^{-1} (Treatment 5), while stationary-growth phase cultures decreased at a rate of –0.05 d^{-1} (Treatment 6).

Zooplankton assemblages at Lake Whitney grazed the added *P. parvum*, but only when inoculated from log-growth phase cultures. This was apparent from comparisons between Treatments 7 and 11, both receiving *P. parvum* additions from log-growth phase culture. *P. parvum* population growth rate in these treatments was 0.07 d^{-1} in lake water contain-

Table 4. Predator:prey ratios, based on volume, and estimated selection probability of the most abundant dominant and sub-dominant zooplankton sizes in Lake Somerville and Lake Whitney, Texas, USA, for a moderately sized *Prymnesium parvum* cell (equivalent spherical diameter ≈ 8 µm). Double entries of some zooplankton indicate 2 different size groups that were highly abundant. *P. parvum* cell measurements were taken from Granéli et al. (2012). Selection probability was estimated based on calculated predator:prey ratios and size selectivity curves from Hansen et al. (1994). Unknown-Rot.1 and -Rot.2: unidentified illoricate rotifers

Zooplankton	Predator:prey ratio	Selection probability (%)
Lake Somerville		
Brachionus spp.	13	70
Brachionus spp.	20	96
Filinia spp.	11	66
Polyarthra spp.	10	60
Unknown-Rot.1	16	88
Unknown-Rot.1	20	96
Unknown-Rot.2	27	88
Adult copepod	27	88
Copepod nauplii	16	87
Copepod nauplii	15	85
Lake Whitney		
Brachionus spp.	18	96
Keratella spp.	11	64
Keratella spp.	12	68
Unknown-Rot.1	23	97
Adult copepod	22	97
Copepod nauplii	20	96

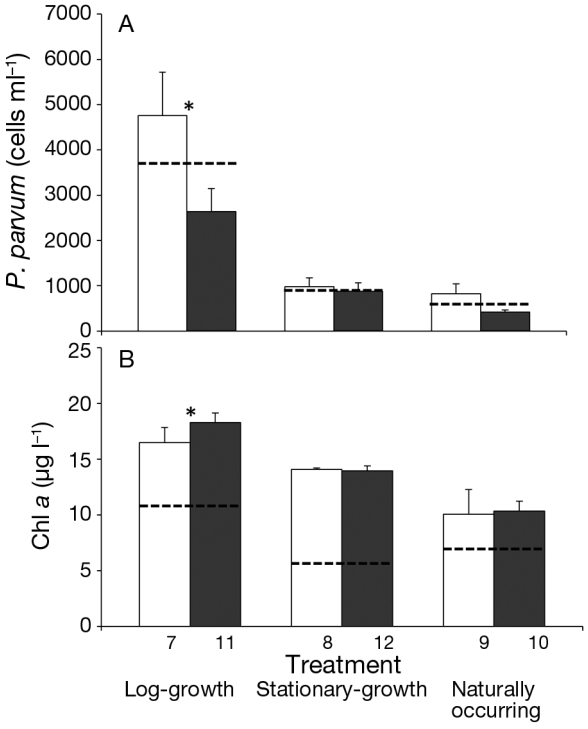

Fig. 3. Treatments 7, 8, 9, 10, 11 and 12 final average ±1 SD *Prymnesium parvum* (A) population densities and (B) total chlorophyll *a* concentrations for the Lake Whitney, Texas, USA, experiment conducted during winter 2013. Horizontal dashed lines represent the initial averages of like treatments. Black bars contained zooplankton in the 61–210 µm size range while white bars did not. Treatments 9 and 10 contained natural phytoplankton assemblages. Treatments 7, 8, 11, and 12 contained natural phytoplankton assemblages while Treatments 7 and 11 were inoculated with *P. parvum* in log-growth phase, and Treatments 8 and 12 were inoculated with stationary-growth phase *P. parvum*. *Significant difference at $p < 0.05$

ing nanoplankton and decreased –0.13 d^{-1} in lake water containing nanoplankton and zooplankton grazers (Table 2, Fig. 3A). Comparisons between Treatments 8 and 12, both receiving *P. parvum* additions from stationary-growth phase culture, showed little difference.

Zooplankton assemblages at Lake Whitney grazed the naturally occurring *P. parvum*. This was apparent through comparisons of Treatments 9 and 10. In these treatments, *P. parvum* population growth rate was 0.09 d^{-1} in lake water containing nanoplankton and decreased –0.12 d^{-1} in lake water containing nanoplankton and zooplankton grazers (Table 2, Fig. 3A). Differences in *P. parvum* population growth rates between these treatments were statistically significant (Tukey's HSD, $p < 0.05$).

Chl *a* concentrations increased during the Lake Whitney experiment in treatments where *P. parvum* was added, even more so when nanoplankton and zooplankton were present with log-growth phase *P. parvum* culture, a difference that was statistically significant (Tukey's HSD, $p < 0.05$; Fig. 3B). In treatments where no *P. parvum* culture was added, chl *a* still increased, but to a lesser degree. Prominent nanophytoplankton observed in these treatments included cyanobacteria, chlorophytes, and chrysophytes.

In all treatments with zooplankton, total zooplankton biovolume declined in the Lake Whitney experiment. The initial total biovolume was 12.4×10^{-2} mm^3 l^{-1}. By the termination of the experiment, total zooplankton biovolume was 8.73×10^{-2}, 3.68×10^{-2} and 8.46×10^{-2} mm^3 l^{-1} in Treatments 11 (log-growth phase culture added), 12 (stationary-growth phase culture added) and 10 (no culture added), respectively. The differences between these treatments were not statistically significant (Tukey's HSD, $p > 0.05$). A taxonomic analysis showed that copepod nauplii (initial biovolume of 6.02×10^{-2} mm^3 l^{-1}), *Keratella* spp. (3.04×10^{-2} mm^3 l^{-1}) and *Brachionus* spp. (1.57×10^{-2} mm^3 l^{-1}) contributed to this overall

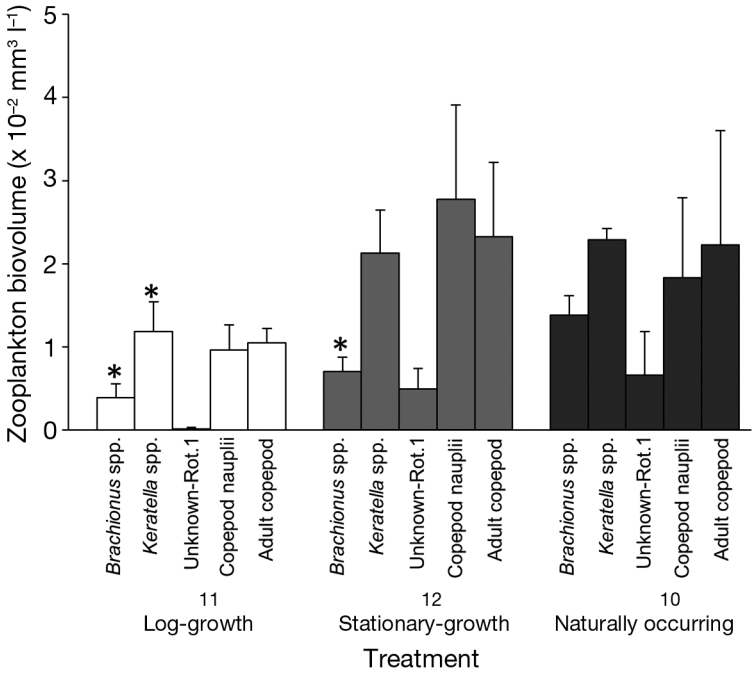

Fig. 4. Treatments 10, 11, and 12 final average ±1 SD biovolumes of dominant and sub-dominant zooplankton groups for the Lake Whitney, Texas, USA, experiment conducted during winter 2013. Treatments 10, 11, and 12 contained natural phytoplankton assemblages and zooplankton in the 61–210 µm size range. Treatment 11 was also inoculated with *Prymnesium parvum* in log-growth phase, while Treatment 12 was inoculated with stationary-growth phase *P. parvum*. Final zooplankton biovolumes in Treatments 11 and 12 were compared to final zooplankton biovolumes in Treatment 10. *Significant difference at $p < 0.05$

decline, with adult copepods of orders Cyclopoida and Calanoida (initial biovolume of 1.58×10^{-2} mm^3 l^{-1}) sometimes contributing (Fig. 4). A comparison between these treatments showed that *Keratella* spp. declined more when log-growth phase *P. parvum* culture was added compared to the treatment receiving no *P. parvum* inoculation. *Brachionus* spp. declined both in the treatment receiving log-growth phase *P. parvum* and in the treatment receiving stationary-growth phase *P. parvum*, compared to the treatment receiving no *P. parvum* inoculation (Tukey's HSD, $p < 0.05$; Fig. 4).

The taxonomic analysis of zooplankton also showed that one group increased in numbers. The biovolume of unknown-Rot.1 was initially below detection levels but the rotifer became sub-dominant as its biovolume increased in all treatments. The final biovolume of unknown-Rot.1 was significantly lower when log-growth phase *P. parvum* culture was added (Treatment 11) compared to biovolumes observed when stationary-growth phase *P. parvum* culture was added (Treatment 12) and when there was no addition of *P. parvum* culture (Treatment 10). This difference was observed at the 0.20 alpha level (Tukey's HSD, $p = 0.118$; Fig. 4), whereas no statistical difference in final biovolume was observed between Treatments 12 and 10 (Tukey's HSD, $p > 0.05$).

An analysis of predator:prey calculations for dominant and sub-dominant zooplankton in Lake Whitney revealed that unknown-Rot.1 and adult copepods had the highest estimated selection probability at ~97 % for a moderately sized *P. parvum* cell (Table 4). *Brachionus* spp. and copepod nauplii had the second highest selection probability at ~96 %. *Keratella* spp. had the lowest selection probability at ~64 %.

DISCUSSION

Our tests revealed only occasional toxicity in our experimental units. It is likely, however, that toxins were produced by *Prymnesium parvum* throughout our experiments, just at a level lower than mortality thresholds of a juvenile fish model. Low-level toxicity is known to occur for *Prymnesium* spp. even while growing in log-phase (Granéli et al. 2012). In addition, our observations of deleterious effects to other phytoplankton in treatments where the same cultures of *P. parvum* were added supports the notion of low-level toxicity (Davis 2014). Also, in many other studies the *P. parvum* culture used in this research negatively influenced other plankton by means of allelopathy and toxicity (Roelke et al. 2007, Errera et al. 2008, Schwierzke et al. 2010).

Declines of zooplankton populations in our experiments occurred across all treatments and in both Lake Somerville and Lake Whitney experiments. Therefore, these declines were likely a product of our experimental design. For example, the experimental units were closed to the ambient lake waters, which prevented the migration of additional phytoplankton. Over the course of the experiment, this would have limited the availability of more edible phytoplankton taxa, possibly negatively impacting zooplankton growth. In addition, the exclusion of plankton in the 20 to 61 µm size fraction might have excluded important prey organisms for some zooplankton, again possibly negatively influencing growth.

Previously, it was suggested that some rotifers might have a grazing preference for *P. parvum* (Errera et al. 2008, Schwierzke et al. 2010). Those

suggestions were based on observations from field experiments not designed to test that notion directly. Our experimental design enabled closer observation of that hypothesis. We conclude that the zooplankton communities dominated by rotifers (Lake Somerville) and co-dominated by copepods (Lake Whitney) impact *P. parvum* populations more than the total phytoplankton community. During the Lake Somerville and Lake Whitney experiments, selective grazing was observed by decreases in *P. parvum* populations without corresponding declines in chl *a*. The preference of *P. parvum* as a food source may be due to some nutritional benefit. For example, the abundance of polyunsaturated acids in *Prymnesium patelliferum*, which is considered the same species as *P. parvum* (Larsen & Medlin 1997), appeared to promote growth of copepods (Koski et al. 1999). This may be true for rotifers as well. In addition, the size and shape of *P. parvum* may also play a role in selection by rotifers, where for some of the rotifer taxa, i.e. unknown-Rot.1, estimated selection probability based on sizes was nearly 100%.

The consequences of exposure to and grazing on *P. parvum* varied between lakes. For example, in Lake Somerville, selective grazing of added *P. parvum* culture in both log- and stationary-growth phases led to positive effects on zooplankton populations. In this case, those populations increased (unknown-Rot.1 and -Rot.2, and adult copepods) or declined to a lesser degree (*Brachionus* spp., *Filinia* spp., *Polyarthra* spp., and copepod nauplii) with the addition of *P. parvum* culture. But these apparent benefits of selective grazing on *P. parvum* were not observed in Lake Whitney zooplankton. For example, some zooplankton populations showed lesser increases (unknown-Rot.1) or greater population declines (*Keratella* spp. and *Brachionus* spp.) with the addition of *P. parvum* culture in log-growth phase. This suggests that in the Lake Whitney experiment toxins produced by *P. parvum* were not at high enough levels to deter grazing, but were still high enough to cause other negative effects, a phenomenon observed previously with other zooplankton feeding on *P. parvum* of low toxicity (Sopanen et al. 2006, Kozlowsky-Suzuki et al. 2009).

The differences in rotifer responses to added *P. parvum* culture between the 2 lakes might be linked to microevolutionary adaptations of rotifers. For example, Colin & Dam (2002) suggested that increasing grazer mortality due to frequent high-toxicity algal blooms severely reduces the egg production of non-resistant individuals within a population, and therefore exerts selective pressure on grazers. This idea certainly applies to copepods, which exclusively reproduce sexually. However, we 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 the unknown-Rot.1 and -Rot.2, were of the class Monogononta, which are cyclical parthenogens. It was postulated that stress from habitat deterioration initiated 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. Clearly, adaptive mechanisms and responses to *P. parvum* and other HABs deserve future study.

Our experimental results indicate that rotifers from Lake Somerville, where *P. parvum* is present but does not form toxic blooms, are more fit when exposed to *P. parvum* populations of low toxicity compared to rotifers of Lake Whitney, where toxic blooms occur. Of particular note is that *Brachionus* spp. and unknown-Rot.1 were dominant in both lake experiments, but were affected by addition of *P. parvum* culture differently, depending on which lake these species resided. We suggest that the differences in *P. parvum* effects on rotifers between lakes resulted from microevolutionary adaptations to *P. parvum* toxins in Lake Somerville rotifers. Furthermore, we speculate that these adaptations occurred from continual exposure to sub-lethal toxin concentrations. But why Lake Somerville and not Lake Whitney? *P. parvum* toxin production appears limited when salinity is below a certain threshold (Larsen & Bryant 1998, Baker et al. 2007, 2009), and salinity represents a basic requirement for *P. parvum* growth and toxicity (Brooks et al. 2011). In addition, results from a recent meta-analysis by Patiño et al. (2014) indicated that salinity is the best predictor of *P. parvum* bloom occurrence in the southcentral USA. Although salinity was similar between the 2 lakes at the time of these experiments, salinity in Lake Somerville has been lower than in Lake Whitney historically (raw data obtained from Burley et al. 2011,

http://pubs.usgs.gov/ds/594/). This lower salinity is what ultimately might have allowed for rotifer populations in Lake Somerville to become better adapted for grazing on *P. parvum*. In turn, these *P. parvum*-adapted rotifer populations likely contribute to the suppression of blooms in Lake Somerville.

In summary, results from this study support the notion that grazing by rotifers may be a contributing factor in the prevention of blooms in some lakes inhabited by *P. parvum*. Our experimental results showed that zooplankton selectively grazed *P. parvum* in most treatments, with Lake Somerville rotifers benefiting from this selective grazing and Lake Whitney rotifers experiencing negative effects. We speculate that historically lower salinity levels in Lake Somerville prevented *P. parvum* from producing toxins at concentrations that would be lethal to grazers, thus enabling microevolutionary adaptation to toxins. In contrast, rotifers in Lake Whitney may undergo a slower adaptation process due to reduced sexual reproduction caused by mass mortality from formation of highly toxic *P. parvum* blooms. Grazing of *P. parvum* by rotifers may be 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* may be suppressed at that particular lake.

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