Toxic Cyanobacteria and Plant Defenses Against a Generalist Herbivore

by

Michael F. Chislock

A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
August 6, 2011

Keywords: Eutrophication, harmful algal blooms, toxic cyanobacteria,
chemical defenses, arms race, *Daphnia*

Copyright 2011 by Michael F. Chislock

Approved by

Alan Wilson, Chair, Assistant Professor of Fisheries and Allied Aquacultures
Dennis DeVries, Professor of Fisheries and Allied Aquacultures
James Stoeckel, Assistant Professor of Fisheries and Allied Aquacultures

Acknowledgments

First and foremost, I would like to thank my family for their love, support, and encouragement. I don't get to see you as often as I'd like, but I'd do anything in the world for y'all. I'd also like to thank all of my friends for keeping me sane – life as a graduate student certainly wasn't easy, and I owe y'all more than I'll ever be able to give back.

Thank you to Susan Smith, Karen Booker, Jeanie Harry, Carolyn Jones ("C.J."), T. Speir, and the rest of the folks working in the Fisheries main office – y'all are awesome!! Thank you so much for fixing all of my mistakes – I appreciate your time and efforts, and I'll try not to cause too many headaches as a doctoral student!

I would also like to thank my research mentors and friends, Andy Turner and Alan Wilson. As a "budding field ecologist," I'm sure I helped contribute to all of Andy's gray hair! When I interviewed to be in Alan's lab at Auburn, I'm sure I was probably the shyest and quietest student that Alan had met. I'm still definitely not an extrovert but am working on it! I appreciate the opportunity to continue working in Alan's lab as a doctoral student. I would also like to thank my graduate committee, Jim Stoeckel and Dennis DeVries, for their advice and guidance. Finally, I would like to thank Rusty Wright for insight on teaching, experiments, and pond management (I truly am sorry about the fish kill on S1 during my first year at Auburn…).

I thank Gary Mittelbach for loaning his pump to fill enclosures, Eric Park, Kristin Adamson, Jonathan Buck, and Jessica Davenport for assistance with experimental set-up, sampling, and

laboratory analyses, Cova Arias, Oscar Olivares-Fuster, RajReni Kaul, and Lauren Jernigan for help and support with genetic analyses, and the staff of the Auburn University E. W. Shell. Fisheries Research Center for logistical support. My thesis was supported by an Auburn University Graduate School Thesis and Dissertation Research Award, a Sigma Xi Grant-in-Aid of Research, and by NSF grants DEB-0841864 and DEB-0841944.

Lastly, I would like to thank all of my lab mates for hanging out, going fishing, and for subway/pizza/ice cream runs. I would especially like to thank Steve Cason, Matt Ferrell, and Lauren Jernigan for their help and support. I am sure that I could never have gotten a fellowship allowing me to stay on as a doctoral student at Auburn without all of your help.

Table of Contents

Acknowledgments……………………………………………………………………………......ii

List of Tables……………………………………………………………………………...v

List of Figures………………………………………………………………………………vi

Chapters

1 Do High Levels of Cyanobacterial Toxins Prevent *Daphnia* Control of Phytoplankton Biomass?...1

Abstract…………………………………………………………………………...1

Introduction…………………………………………………………………………2

Methods……………………………………………………………………………...4

Results………………………………………………………………………….....9

Discussion……………………………………………………………………………11

References……………………………………………………………………………15

2 Nitrogen-to-Phosphorus Ratio Mediates the Outcome of Intraspecific Competition in *Daphnia*……………………………………………………………………………..27

Abstract……………………………………………………………………………27

Introduction………………………………………………………………………...28

Methods……………………………………………………………………………30

Results……………………………………………………………………………...35

Discussion……..37

References……………………………………………………………………………42

List of Tables

Table 1.1. Source lakes and genetic characterizations of the three *Daphnia pulicaria* clones. 21

Table 2.1. Source lakes and genetic characterizations of the six *Daphnia pulicaria* clones. …...47

Table 2.2. Effect of N:P ratio on the relative abundance of each of the six *Daphnia pulicaria* genotypes at the conclusion of the experiment (10 December 2009). ……………….47

List of Figures

Figure 1.1. Dynamics of (A) *Daphnia pulicaria* biomass, (B) chlorophyll a, and (C) microcystin concentration for Experiment 1. ……………………………………23

Figure 1.2. Relative biomass of dominant phytoplankton taxa on day 28 (30 July 2007) of Experiment 1. ………………………………………………………………24

Figure 1.3. Dynamics of (A) *Daphnia pulicaria* biomass and (B) chlorophyll a for Experiment 2. ……………………………………………………………...25

Figure 1.4. Relative biomass of dominant phytoplankton taxa and microcystin concentration on day 28 (5 May 2008) of Experiment 2. …………………………..26

Figure 2.1. Mean juvenile somatic growth rates for the six *Daphnia pulicaria* genotypes on two diets: a nutritious green alga (100% *Ankistrodesmus*) and toxic cyanobacteria (100 % *Microcystis aeruginosa*). …………………………50

Figure 2.2. Dynamics of (A) chlorophyll a and (B) cyanobacterial dry biomass over the ten-week enclosure experiment. ……………………………………...51

Figure 2.3. Concentration of the toxin microcystin for each nitrogen-to-phosphorus (N:P) treatment immediately prior to stocking of *Daphnia pulicaria* genotypes (16 October 2009). ……………………………………………………52

Figure 2.4. Relative biomass of phytoplankton taxa at the beginning (16 October 2009), middle (3 November 2009), and end (10 December 2009) of the ten-week enclosure experiment. ………………………………………………...53

Figure 2.5. *Daphnia pulicaria* (A) density and (B) biomass for each nitrogen-to-phosphorus (N:P) treatment at the conclusion of the experiment (10 December 2009). ………………………………………………………………54

Figure 2.6. Relative abundance of (A) each *Daphnia pulicaria* genotype and (B) *D. pulicaria* genotypes from oligotrophic and eutrophic lakes at the conclusion of the experiment (10 December 2009). ……………………………55

Chapter 1

Do High Levels of Cyanobacterial Toxins Prevent *Daphnia* Control of Phytoplankton biomass?

ABSTRACT

Toxin-producing cyanobacteria have frequently been hypothesized to limit the ability of herbivorous zooplankton (such as *Daphnia*) to control phytoplankton biomass by inhibiting *Daphnia* feeding, population growth, and in extreme cases, causing *Daphnia* mortality. Using two limnocorral experiments in eutrophic ponds, we tested the hypothesis that high levels of cyanobacterial toxins prevent *Daphnia* from strongly reducing phytoplankton abundance. At the start of the first experiment, phytoplankton communities were dominated by toxic *Microcystis* and *Anabaena* (~96% of total phytoplankton biomass), and concentrations of the toxin microcystin were ~3 μg L^{-1}. *Daphnia pulicaria* were collected from a eutrophic, fishless lake, cultured in the laboratory, and stocked at a low density (~0.3 L^{-1}) into half (n = 4) of the enclosures. Over the first two weeks after adding *Daphnia*, toxin levels increased to ~6.5 μg L^{-1}, yet *Daphnia* populations increased. By the third week, *Daphnia* had suppressed phytoplankton biomass by ~74% relative to "no *Daphnia*" controls, and reduced phytoplankton biomass persisted until the conclusion of the experiment (6 August 2007). In the second experiment, phytoplankton communities were dominated by *Anabaena* and *Microcystis*, and our four treatments included a no *Daphnia* control and each of three genetically-distinct *D. pulicaria* clones that varied in tolerance to toxic *Microcystis* in a previously published laboratory experiment. We found no differences among the three *D. pulicaria* genotypes in their population growth rates and carrying capacities, and all three *D. pulicaria* genotypes reduced phytoplankton biomass similarly relative to controls. By the conclusion of the experiment (19 May 2008), *D. pulicaria* had completely eliminated cyanobacteria, regardless of their genotype. The ability of

Daphnia to increase and suppress phytoplankton biomass, despite high levels of cyanobacteria and associated toxins, suggests that toxin-producing cyanobacteria do not prevent strong *Daphnia* control of phytoplankton biomass.

INTRODUCTION

Within freshwater ecosystems, cyanobacteria are the most important phytoplankton taxa associated with harmful algal blooms (HABs) (Paerl 1988). Toxigenic cyanobacteria, including *Anabaena*, *Cylindrospermopsis*, *Microcystis*, and *Oscillatoria* (*Planktothrix*), tend to dominate nutrient-rich freshwater systems due to their superior competitive abilities under high nutrients, low nitrogen-to-phosphorus ratios, low light levels, reduced mixing, and high temperatures (Reynolds et al. 1987; Downing et al. 2001; Paerl and Huisman 2009). Despite large-scale efforts to limit nutrient enrichment, such as the Clean Water Act (Carpenter et al. 1998), cultural eutrophication (and concomitant HABs) continues to be the leading cause of water pollution for most freshwater and coastal marine ecosystems in the world and is a rapidly growing problem in the developing world (Smith and Schindler 2009).

Cyanobacteria pose one of the most serious threats to nutrient-rich freshwater ecosystems by producing toxic secondary metabolites that can poison fishes, pets, livestock, and humans (Paerl 1988; Carmichael 1992). In addition to posing significant public health risks, cyanobacteria have been shown to be poor quality food for most zooplankton grazers in laboratory studies (Wilson et al. 2006; Wilson and Hay 2007; Tillmanns et al. 2008). Numerous studies have demonstrated that cyanobacteria can inhibit zooplankton feeding, growth, and reproduction through several mechanisms including (1) colonial or filamentous morphologies, (2) nutritional deficiencies, and (3) production of toxins (Porter 1977; Lampert 1987; Wilson et al. 2006). Therefore, an important consequence of cyanobacterial dominance with nutrient enrichment is that

phytoplankton communities tend to be dominated by harmful species that are relatively resistant to zooplankton grazing (Sommer et al. 1986; Ghadouani et al. 2003).

However, recent research has shown that local populations of *Daphnia* may evolve tolerance to toxin-producing cyanobacteria in the diet under eutrophic conditions (Hairston et al. 1999; Gustafsson and Hansson 2004; Sarnelle and Wilson 2005). Genetically-based tolerance to toxic *Microcystis* has been observed within species of both European and North American *Daphnia* across habitats varying in nutrient enrichment (Sarnelle and Wilson 2005) and over time in a eutrophied lake (Hairston et al. 1999; Hairston et al. 2001). It is well known that the presence of the generalist herbivore *Daphnia* is critically important to the success of biomanipulation efforts to improve water quality in eutrophic lakes (Leibold 1989; Schindler 2006), and the evolution of tolerance to toxic cyanobacteria may have important consequences for the response of lakes to nutrient enrichment (Hairston et al. 2001; Sarnelle and Wilson 2005).

To date, numerous studies demonstrate that *Daphnia* can greatly reduce the abundance of non-resistant phytoplankton in eutrophic lakes when freed from predation by planktivorous fish (Sarnelle 1992; Mazumder 1994). However, whether *Daphnia* can control grazing-resistant cyanobacteria continues to be a controversial question. There is considerable evidence that *Daphnia* may promote the dominance of grazing-resistant cyanobacteria (Carpenter et al. 1995; Brett and Goldman 1997; Ghadouani et al. 2003), but there are also several well-documented examples in which *Daphnia* have greatly reduced the abundance of cyanobacteria (Lynch and Shapiro 1981; Vanni et al. 1990; Sarnelle 2007). The presence (or absence) of cyanobacterial toxins may help explain the variable suppression of cyanobacterial abundance observed in previous studies. Toxin-producing cyanobacteria have frequently been hypothesized to limit the ability of *Daphnia* to control phytoplankton (and cyanobacterial) biomass (Porter 1977).

Furthermore, laboratory experiments have demonstrated that toxic cyanobacteria may inhibit *Daphnia* feeding, population growth, and in extreme cases, cause *Daphnia* mortality (Gliwicz and Lampert 1990; Lürling and van der Grinten 2003; reviewed by Wilson et al. 2006). Here we conduct two field experiments to test the hypothesis that high levels of toxic *Microcystis* (and the toxin microcystin) prevent *Daphnia* from suppressing cyanobacterial abundance. To our knowledge, no field experiments have examined the detrimental effects of cyanobacterial toxins on *Daphnia*, and previous field experiments were conducted prior to the advent of simple methods for measuring cyanobacterial toxins.

METHODS

Study sites

The first experiment (Experiment 1) was conducted at Michigan State University, Michigan, in a small, eutrophic pond (MSU Lake 2). MSU Lake 2 was created as part of a project to treat secondary sewage effluent in 1973 (Spencer 1981) and is a shallow, polymictic pond with a surface area of approximately 3 ha, maximum depth of 2 m, and total phosphorus concentrations in the mixed layer averaging about 200 μg L^{-1} (O. Sarnelle unpubl.). Cyanobacteria begin to dominate phytoplankton communities in MSU Pond 2 in late May, and *Microcystis* typically comprises greater than 90% of total phytoplankton biomass by August each year (O. Sarnelle unpubl.).

The second experiment (Experiment 2) was conducted at the E.W. Shell Fisheries Research Station at Auburn University, Alabama, in a small, eutrophic reservoir pond (Pond S1). This pond is shallow and polymictic with a surface area of approximately 8 ha, maximum depth of 3.5 m, and total nitrogen (TN) and total phosphorus (TP) concentrations in the mixed layer averaging about 1900 μg L^{-1} and 100 μg L^{-1}, respectively (Boyd and Shelton 1984; A. E. Wilson

and M. F. Chislock unpubl.). Cyanobacteria begin to dominate phytoplankton communities in the pond during late spring (April), and cyanobacterial blooms typically persist into early fall (September) (A. E. Wilson and M. F. Chislock unpubl.).

Daphnia *sources*

D. pulicaria for both experiments were collected from three small (<0.3 km^2), eutrophic lakes in southern Michigan that have high cyanobacterial abundance during the summer months (Sarnelle and Wilson 2005; Table 1). *D. pulicaria* for Experiment 1 were collected from a nearby eutrophic pond lacking fish (MSU Lake 1) on 15 June 2007 and mass cultured in the laboratory in a 20-L glass carboy filled with autoclaved lake water, bubbled with an airstone, and fed a nutritious green algae (*Ankistrodesmus*) prior to the start of the experiment. The three *D. pulicaria* clones used in Experiment 2 were descendants of one female isolated in 2004 from each of three eutrophic lakes in southern Michigan and were shown to be tolerant to a diet of toxic *Microcystis aeruginosa* in a previous growth experiment (Sarnelle and Wilson 2005; Table 1). Prior to the field experiment, each *D. pulicaria* clone was first maintained in the laboratory (20-25°C, 12h light: 12h dark) in 20-L glass carboys filled with autoclaved lake water, bubbled with an airstone, and fed a nutritious green algae (*Chlorella*) grown in a nutrient-rich medium (modified BG-11 medium – Vanderploeg et al. 2001). Each *D. pulicaria* clone was then transferred to separate outdoor 160-L tanks filled with lake water filtered through a 35-µm sieve and supplemented with *Chlorella* as a food source several weeks before the start of the field experiment. Therefore, all three *D. pulicaria* clones were grown under common-garden conditions to provide animals for stocking the limnocorrals.

Daphnia *genetic analyses*

D. pulicaria genotypes for each of the three clones used in the second experiment were characterized using variation in two microsatellite loci (Dp464, Dp496 – Colbourne et al. 2004) that have proven to be highly polymorphic for *D. pulicaria* collected from several of our study lakes in Michigan (Wilson and Hay 2007, A. E. Wilson unpubl.) (Table 1). We subsequently characterized *D. pulicaria* genotypes from each enclosure two weeks after initial stocking and at the conclusion of the experiment to confirm establishment of each *D. pulicaria* genotype treatment and that we had maintained single-genotype treatments of *D. pulicaria* in our enclosures. We genetically characterized 20-25 randomly selected *D. pulicaria* individuals from 95% ethanol-preserved macrozooplankton samples for all *Daphnia* enclosures for both dates (detailed methods in Wilson and Hay 2007).

Enclosure experiments

Experiment 1 was conducted in 4200-L, clear polyethylene enclosures that were sealed at the bottom, open to the atmosphere at the top, and suspended from a floating platform (EZ-Dock) anchored in MSU Pond 2. Eight enclosures were filled on 2 July 2007 by pumping pond water through a 75-µm mesh net to initially exclude resident *Daphnia*. We then randomly assigned half of the enclosures as no *D. pulicaria* controls, and half of the enclosures were stocked with *D. pulicaria* on 2 July 2007 to achieve initial densities of ~0.3 animals L^{-1}. Two enclosures *D. pulicaria* enclosures were damaged allowing planktivorous fish (bluegill) to invade. We therefore only included the remaining 2 *D. pulicaria* enclosures in all statistical analyses. We sampled all enclosures weekly beginning on 2 July 2007 (immediately prior to *Daphnia* addition), and the experiment was concluded on 6 August 2007.

Experiment 2 was conducted in 2500-L, clear polyethylene enclosures that were sealed at the bottom, open to the atmosphere at the top, and suspended from a floating platform (EZ-Dock) anchored in the middle of Pond S1. Twelve enclosures were filled on 30 March 2008 by pumping pond water through a 75-μm mesh net to initially exclude resident *Daphnia* from treatments. Our four treatments included a no *Daphnia* control and each of three genetically-distinct *D. pulicaria* clones (Table 1), and we randomly assigned each enclosure to one of the four treatments. Enclosures for each *D. pulicaria* genotype treatment were stocked with *Daphnia* on 6 April 2008 to achieve initial densities of ~0.3 animals L^{-1}. We sampled all enclosures weekly beginning on 5 April 2008, and the experiment was concluded on 19 May 2008.

Sample collection and data analysis

Depth-integrated water samples for chlorophyll a, phytoplankton biomass and species composition, microcystin, and macrozooplankton biomass and species composition were collected from the mixed layer of each enclosure with a tube sampler (inside diameter = 51 mm) for both experiments. Chlorophyll a concentrations were measured by extracting phytoplankton collected on Pall A/E filters in 90% ethanol for 24 h in the dark at 4°C followed by measurement with a fluorometer (Sartory and Grobbelaar 1984). Microcystin concentrations in particles were quantified using enzyme-linked immunosorbent assay (ELISA) (An and Carmichael 1994) after extraction from filters with 75% aqueous methanol. Phytoplankton species abundance and composition were determined for selected dates via the inverted microscope technique (Utermöhl 1958) using water samples preserved in 1% Lugol's solution. Biovolumes for each species were calculated using cell counts and estimates of cell volume based on measurements of cell dimensions. We then converted biovolumes (mm^3 L^{-1}) to dry biomass (μg L^{-1}) assuming a specific gravity of 1 g cm^{-3} and a dry biomass: wet biomass ratio of 0.40 (Riemann et al. 1989;

Sarnelle et al. 2005; Knoll et al. 2008). *Daphnia* were measured and counted at 40X in a Sedgwick-Rafter cell, and total body lengths were converted to biomass using a length-weight regression for *D. pulicaria* (O. Sarnelle unpubl.).

For Experiment 1, the effect of *D. pulicaria* on chlorophyll a and microcystin concentrations was tested using repeated measures analysis of variance (ANOVA, sampling date = repeated measure) across all sampling dates for the five-week experiment. *Daphnia* effects on phytoplankton community composition were examined for samples collected on day 28 (30 July 2007) of the experiment. Population growth rates for *D. pulicaria* were calculated as the slope of the linear regression of the natural logarithm of *D. pulicaria* density versus day of experiment during the period when populations were increasing (9 July to 23 July 2007). For Experiment 2, we used repeated measures ANOVA to analyze treatment effects on chlorophyll a across all sampling dates for the six-week experiment. ANOVA was used to analyze treatment effects on phytoplankton community composition and microcystin concentrations on day 28 (5 May 2008). Tukey's post hoc test was then used to assess pairwise differences across the three *D. pulicaria* genotypes treatments. Population growth rates for each *D. pulicaria* genotype in each replicate enclosure were calculated as in Experiment 1, using *D. pulicaria* densities from 14 April to 28 April 2008. We then used ANOVA to compare mean population growth rates across the three *D. pulicaria* genotype treatments. To compare the *Daphnia* effects on phytoplankton abundance for Experiments 1 and 2, we calculated effect sizes by dividing the mean chlorophyll concentration in control enclosures by the mean chlorophyll concentrations in enclosures with *D. pulicaria* (Sarnelle 1992). All data were checked for normality and homogeneity and log-transformed when necessary.

RESULTS

Experiment 1

At the start of Experiment 1, phytoplankton communities in the enclosures were dominated almost entirely by toxic cyanobacteria, and *Microcystis* and *Anabaena* contributed over 96% of total phytoplankton biomass. Initial phytoplankton and cyanobacterial biomass were high across all enclosures (mean chlorophyll a = ~130 μg L^{-1} and mean cyanobacterial biomass = ~19,000 μg L^{-1}), and concentrations of the toxin, microcystin, were above 3 μg L^{-1} (Figure 1). Over the first two weeks of the experiment, toxin levels increased to approximately 6 μg L^{-1} in enclosures with *D. pulicaria* (Figure 1C), yet *D. pulicaria* populations were increasing (r = 0.24 day^{-1}, SE = 0.06 day^{-1}) (Figure 1A). By the third week, *D. pulicaria* had suppressed phytoplankton biomass by ~74% relative to no *D. pulicaria* controls (Figure 1B), and this reduced phytoplankton biomass persisted until the conclusion of the experiment (6 August 2007) ($F_{1,4} = 20.634$, $P = 0.010$). *D. pulicaria* reduced chlorophyll a concentrations by a factor of 3.3, relative to no *D. pulicaria* controls, at the conclusion of the experiment. In general, *D. pulicaria* strongly suppressed the biomass of all species of cyanobacteria (Figure 2) relative to no *D. pulicaria* controls by day 28 (30 July 2007), reducing *Microcystis*, *Oscillatoria*, and *Anabaena* species by factors of ~4 ($F_{1,4} = 14.539$, $P = 0.019$), 56 ($F_{1,4} = 15.746$, $P = 0.017$), and 20 ($F_{1,4} = 5.857$, $P = 0.073$), respectively.

Experiment 2

Initial phytoplankton biomass was high across all treatments for Experiment 2 (mean chlorophyll a = 75 μg L^{-1}) (Figure 3B), and phytoplankton communities were dominated by a mixture of the cyanobacteria *Anabaena circinalis*, *Microcystis aeruginosa*, and *Chroococcus* sp., which comprised approximately 85% of total phytoplankton biomass on 5 April 2008.

Characterization of *D. pulicaria* genotypes from each enclosure two weeks after initial stocking and at the conclusion of the experiment confirmed that the appropriate genotypes became established in each enclosure and that we were able to maintain single-genotype treatments of *D. pulicaria* throughout the experiment. 100% of the 20-25 randomly selected *D. pulicaria* individuals from each enclosure were the correct genotype two weeks after stocking and at the conclusion of the experiment.

All three *D. pulicaria* genotypes rapidly increased in abundance and biomass during the first three weeks of the experiment (Figure 3A), and population growth rates did not differ across the three *D. pulicaria* genotypes ($F_{2,6} = 0.235$, $P = 0.798$). Initial microcystin concentrations were ~0.04 μg L^{-1} for controls and enclosures for the three *D. pulicaria* genotypes. By day 28 (5 May 2008), microcystin concentrations had increased to greater than 1 μg L^{-1} in control enclosures (Figure 4B), while concentrations of the toxin were less than 1 ng L^{-1} in enclosures for all *D. pulicaria* genotype treatments ($F_{3,8} = 333.398$, $P < 0.0001$). Over the course of the experiment, *D. pulicaria* abundance and biomass were similar for the three *D. pulicaria* genotype treatments (Abundance: $F_{2,6} = 0.513$, $P = 0.623$; Biomass: $F_{2,6} = 1.434$, $P = 0.310$), and by the final two weeks of the experiment, *D. pulicaria* populations had declined from their peak and leveled off (Figure 3A).

In general, all three *D. pulicaria* genotypes significantly reduced chlorophyll a concentration over time (Figure 3B; $F_{3,8} = 94.677$, $P < 0.0001$). *D. pulicaria* from Baker Lake had the largest effect on chlorophyll a over time (Figure 3B; Tukey's test: Baker vs. Wintergreen, $P = 0.007$; Baker vs. MSU Pond 1, $P = 0.079$). However, by the conclusion of the experiment (19 May 2008), all three *D. pulicaria* genotypes reduced chlorophyll a concentrations similarly relative to the control treatment (Tukey's test for final date: $P > 0.90$), and *D. pulicaria* reduced chlorophyll

a concentrations by a factor of 26.1, relative to no *D. pulicaria* controls. Furthermore, all three *D. pulicaria* genotypes strongly suppressed the biomass of cyanobacteria ($F_{3,8} = 82.669$, $P < 0.0001$) and flagellates ($F_{3,8} = 80.801$, $P < 0.0001$) but did not affect the biomass of chlorophytes ($F_{3,8} = 2.623$, $P = 0.122$) and diatoms ($F_{3,8} = 0.743$, $P = 0.556$) (Figure 4A).

DISCUSSION

High levels of phytoplankton toxins are one explanation for the escape of phytoplankton from grazer control (Porter 1977). Within freshwater ecosystems, bloom-forming cyanobacteria (including *Anabaena*, *Microcystis*, and *Oscillatoria*) are the most important toxin-producing phytoplankton taxa (Carmichael 1992). Cyanobacterial toxins have frequently been shown to have negative effects on the fitness of zooplankton herbivores (including *Daphnia*) in laboratory studies (Gilbert 1990; DeMott et al. 1991, Lurling and van der Grinten 2003). Therefore, toxin production by cyanobacteria is hypothesized to drive declines of large daphnids with eutrophication and the concomitant escape of cyanobacteria from *Daphnia* control (Jeppesen et al. 2000; Porter 1977; Sommer et al. 1986). However, our results show that *D. pulicaria* can strongly suppress both cyanobacterial and phytoplankton biomass despite high levels of one of the most commonly measured and toxic of the cyanotoxins, microcystin.

Recent laboratory experiments have revealed intraspecific variation within *Daphnia* species for traits conferring tolerance to toxic *Microcystis* (Hairston et al. 1999; Hairston et al. 2001; Sarnelle and Wilson 2005). *Daphnia* from environments with infrequent cyanobacterial blooms are more inhibited by *Microcystis* than *Daphnia* from environments with recurring cyanobacterial blooms (Sarnelle and Wilson 2005). Furthermore, phenotypic plasticity has been observed in *Daphnia* in response to exposure to toxic cyanobacteria in the diet (Gustafsson and Hansson 2004). Therefore, cyanotoxins are likely strong selective forces that potentially shape

Daphnia population genetics seasonally within lakes and across lakes of varying trophic status. For Experiments 1 and 2, *D. pulicaria* were collected from environments that have high cyanobacterial abundance during the summer months. Therefore, the ability of *D. pulicaria* to increase in abundance and suppress toxic cyanobacteria may have been driven by local adaptation to recurring cyanobacterial blooms.

In Experiment 2, we compared the ability of three *D. pulicaria* clones, identified to be tolerant to a laboratory diet of toxic *Microcystis* in a previously published laboratory experiment (Sarnelle and Wilson 2005), to reproduce and control toxic cyanobacteria in the field. Numerous studies have demonstrated that the results of laboratory microcosm experiments are not always consistent with the results of manipulative field experiments and whole-lake studies (Carpenter 1996). Consequently, several recent studies have examined the relevance of generalizations based on microcosms to communities and ecosystems (Levin 1992; Sarnelle 1997; Spivak et al. 2011). Our results show that *D. pulicaria* genotypes identified to be tolerant to *Microcystis* in laboratory experiments can increase dramatically and eliminate cyanobacteria in the field. Therefore, the presumed role of cyanobacteria in driving seasonal declines of large daphnids may need to be re-evaluated in light of these results. The negative correlation between lake productivity and the abundance of large daphnids is more likely driven by the increasing abundance of planktivorous fish with nutrient enrichment (Jeppesen et al. 1997). As the *Microcystis*-tolerant *D. pulicaria* genotypes in our experiment were collected from three lakes spanning a large total phosphorus gradient (21 to 300 µg L^{-1}), local adaptation in response to increased cyanobacterial dominance may be a common occurrence in nature, particularly in lakes with a long history of eutrophication (Hairston et al. 1999).

D. pulicaria strongly suppressed cyanobacterial and phytoplankton abundance in both Experiments 1 and 2. However, *D. pulicaria* had a much larger effect on phytoplankton abundance in Experiment 2, reducing chlorophyll a concentrations by a factor of 26.1 (compared to 3.3 in Experiment 1). Furthermore, *D. pulicaria* completely eliminated all cyanobacterial species from enclosures by the conclusion of Experiment 2, while cyanobacteria comprised approximately 75% of the phytoplankton biomass on day 28 of Experiment 1 (data not shown). One potential explanation for *D. pulicaria* failing to eliminate all cyanobacteria in Experiment 1 is that the remaining cyanobacterial colonies were too large to be consumed by *Daphnia*. In Experiment 2, the majority of *Microcystis* colonies were edible (<10 cells per colony). Contrastingly, colonies with greater than ten cells (> 40μm) contributed over 80% of *Microcystis* biomass in Experiment 1. Therefore, larger colony size likely contributed to the greater overall grazing-resistance in the phytoplankton in Experiment 1. However, the equivalent diameter of *Microcystis* colonies in control and *D. pulicaria* enclosures did not differ on day 28 ($F_{1,4} = 0.969$, $P = 0.381$) for Experiment 1. In addition, the proportion of edible *Microcystis* (i.e., single cells and colonies of less than 10 cells) did not differ between control and *D. pulicaria* enclosures ($F_{1,4} = 0.784$, $P = 0.426$). Therefore, *D. pulicaria* presence did not favor larger *Microcystis* colonies.

Concentrations and dynamics of the cyanotoxin microcystin also differed between Experiments 1 and 2. In Experiment 1, microcystin concentrations were greater than 3 μg L^{-1} immediately prior to the addition of *D. pulicaria*. In contrast, initial microcystin concentrations for Experiment 2 were nearly two orders of magnitude lower (~0.04 μg L^{-1}) than in Experiment 1. Although microcystin concentrations increased to greater than 1 μg L^{-1} in enclosures lacking *D. pulicaria* in Experiment 2, microcystin concentrations decreased to less than 1 ng L^{-1} in enclosures with *D. pulicaria* as *Daphnia* were able to suppress cyanobacterial biomass before

toxin levels could increase. High levels of cyanobacterial toxins (microcystin > 3 μg L^{-1}) certainly do not prevent *D. pulicaria* from suppressing phytoplankton and cyanobacterial abundance. However, higher initial concentrations of the toxin microcystin may have contributed to the greater overall grazing-resistance in the phytoplankton in Experiment 1. It is also possible that nutritional deficiencies and the toxicity of undescribed (and unmeasured) cyanobacterial compounds mediated differences in the grazing-resistance of phytoplankton in Experiments 1 and 2.

One reason why the world is green (Hairston et al. 1960) may be that many plants are chemically defended against herbivores (Hay and Fenical 1988). In green lakes, toxin-producing cyanobacteria have frequently been hypothesized to limit the ability of herbivorous zooplankton (such as *Daphnia*) to suppress phytoplankton biomass (Porter 1977). However, our study demonstrates that high levels of the cyanotoxin microcystin do not prevent strong *Daphnia* control of phytoplankton biomass and adds to a growing body of literature demonstrating that *Daphnia* can have large negative effects on the abundance of cyanobacteria in eutrophic lakes, when freed from predation by planktivorous fish (Vanni et al. 1990; Sarnelle 1993; Sarnelle 2007). In both experiments, we used *Daphnia* collected from lakes with recurring cyanobacterial blooms, and Experiment 2 demonstrated that adaptations by *D. pulicaria* to increased prevalence of cyanobacteria in three different eutrophic lakes seem to have similar community- and ecosystem-level effects. As *Daphnia* from eutrophic lakes tend to be more tolerant to toxic cyanobacteria than *Daphnia* from oligotrophic lakes, local adaptation by *Daphnia* in response to toxic cyanobacteria may play an important role in the response of lake ecosystems to cultural eutrophication and food-web manipulations (Hairston et al. 1999).

REFERENCES

An, J. S., and W. W. Carmichael. 1994. Use of a colorimetric protein phosphatase inhibition assay and enzyme-linked-immunosorbent-assay for the study of microcystins and nodularins. Toxicon **32:** 1495-1507.

Boyd, C. E., and J. L. Shelton. 1984. Observations on the hydrology and morphometry of ponds on the Auburn University Fisheries Research Unit. Alabama Agricultural Experiment Station Bulletin**:** 1-64.

Brett, M. T., and C. R. Goldman. 1997. Consumer versus resource control in freshwater pelagic food webs. Science **275:** 384-386.

Carmichael, W. W. 1992. Cyanobacteria secondary metabolites - the cyanotoxins. Journal of Applied Bacteriology **72:** 445-459.

Carpenter, S. R. 1996. Microcosm experiments have limited relevance for community and ecosystem ecology. Ecology **77:** 677-680.

Carpenter, S. R., D. L. Christensen, J. J. Cole, K. L. Cottingham, X. He, J. R. Hodgson, J. F. Kitchell, S. E. Knight, and M. L. Pace. 1995. Biological control of eutrophication in lakes. Environmental Science & Technology **29:** 784-786.

Carpenter, S. R., N. F. Caraco, D. L. Correll, R. W. Howarth, A. N. Sharpley, and V. H. Smith. 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. Ecological Applications **8:** 559-568.

Colbourne, J. K., B. Robison, K. Bogart, and M. Lynch. 2004. Five hundred and twenty-eight microsatellite markers for ecological genomic investigations using *Daphnia*. Molecular Ecology Notes **4:** 485-490.

DeMott, W. R., Q. X. Zhang, and W. W. Carmichael. 1991. Effects of toxic cyanobacteria and

purified toxins on the survival and feeding of a copepod and 3 species of *Daphnia*. Limnol. Oceanogr. **36:** 1346-1357.

Downing, J. A., S. B. Watson, and E. McCauley. 2001. Predicting cyanobacteria dominance in lakes. Canadian Journal of Fisheries and Aquatic Sciences **58:** 1905-1908.

Ghadouani, A., B. Pinel-Alloul, and E. E. Prepas. 2003. Effects of experimentally induced cyanobacterial blooms on crustacean zooplankton communities. Freshwater Biology **48:** 363-381.

Gilbert, J. J. 1990. Differential effects of *Anabaena affinis* on cladocerans and rotifers: mechanisms and implications. Ecology **71:** 1727-1740.

Gliwicz, Z. M., and W. Lampert. 1990. Food thresholds in *Daphnia* species in the absence and presence of blue-green filaments. Ecology **71:** 691-702.

Gustafsson, S., and L.-A. Hansson. 2004. Development of tolerance against toxic cyanobacteria in *Daphnia*. Aquatic Ecology **38:** 37-44.

Hairston, G. G., F. E. Smith, and L. B. Slobodkin. 1960. Community structure, population control, and competition. The American Naturalist **94:** 421-425.

Hairston, N. G., W. Lampert, C. E. Caceres, C. L. Holtmeier, L. J. Weider, U. Gaedke, J. M. Fischer, J. A. Fox, and D. M. Post. 1999. Lake ecosystems - Rapid evolution revealed by dormant eggs. Nature **401:** 446-446.

Hairston, N. G., C. L. Holtmeier, W. Lampert, L. J. Weider, D. M. Post, J. M. Fischer, C. E. Caceres, J. A. Fox, and U. Gaedke. 2001. Natural selection for grazer resistance to toxic cyanobacteria: Evolution of phenotypic plasticity? Evolution **55:** 2203-2214.

Hay, M. E. and W. Fenical. 1988. Marine plant-herbivore interactions: The ecology of chemical defense. Annual Review of Ecology and Systematics **19:** 111-145.

Knoll, L. B. S., O.; Hamilton, S. K.; Kissman, C. E. H., Wilson, A. E., Rose, J. B., and Morgan, M. R. 2008. Corrigendum: Invasive zebra mussels (*Dreissena polymorpha*) increase cyanobacterial toxin concentrations in low-nutrient lakes. Canadian Journal of Fisheries and Aquatic Sciences **65:** 2552-2552.

Lampert, W. 1987. Laboratory studies on zooplankton-cyanobacteria interactions. New Zealand Journal of Marine and Freshwater Research **21:** 483-490.

Leibold, M. A. 1989. Resource edibility and the effects of predators and productivity on the outcome of trophic interactions. American Naturalist **134:** 922-949.

Levin, S. A. 1992. The problem of pattern and scale in ecology. Ecology **73:** 1943-1967.

Lürling, M., and E. Van Der Grinten. 2003. Life-history characteristics of *Daphnia* exposed to dissolved microcystin-LR and to the cyanobacterium *Microcystis aeruginosa* with and without microcystins. Environmental Toxicology and Chemistry **22:** 1281-1287.

Lynch, M., and J. Shapiro. 1981. Predation, enrichment, and phytoplankton community structure. Limnol. Oceanogr. **26:** 86-102.

Mazumder, A. 1994. Patterns of algal biomass in dominant odd- vs. even-link lake ecosystems. Ecology **75:** 1141-1149.

Paerl, H. W. 1988. Nuisance phytoplankton blooms in coastal, estuarine, and inland waters. Limnol. Oceanogr. **33:** 823-847.

Paerl, H. W., and J. Huisman. 2009. Climate change: a catalyst for global expansion of harmful cyanobacterial blooms. Environmental Microbiology Reports **1:** 27-37.

Porter, K. G. 1977. The plant-animal interface in freshwater ecosystems. American Scientist **65**: 159-170.

Reynolds, C. S., R. L. Oliver, and A. E. Walsby. 1987. Cyanobacterial dominance - the role of

buoyancy regulation in dynamic lake environments. New Zealand Journal of Marine and Freshwater Research **21:** 379-390.

Riemann, B., P. Simonsen, and L. Stensgaard. 1989. The carbon and chlorophyll content of phytoplankton from various nutrient regimes. Journal of Plankton Research **11:** 1037-1045.

Sarnelle, O. 1992. Nutrient enrichment and grazer effects on phytoplankton in lakes. Ecology **73:** 551-560.

Sarnelle, O. 1993. Herbivore effects on phytoplankton succession in a eutrophic lake. Ecological Monographs **63:** 129-149.

Sarnelle, O. 1997. *Daphnia* effects on microzooplankton: comparisons of enclosure and whole-lake responses. Ecology **78:** 913-928.

Sarnelle, O. 2007. Initial conditions mediate the interaction between *Daphnia* and bloom-forming cyanobacteria. Limnol. Oceanogr. **52:** 2120-2127.

Sarnelle, O., and A. E. Wilson. 2005. Local adaptation of *Daphnia pulicaria* to toxic cyanobacteria. Limnol. Oceanogr. **50:** 1565-1570.

Sarnelle, O., A. E. Wilson, S. K. Hamilton, L. B. Knoll, and D. F. Raikow. 2005. Complex interactions between the zebra mussel, *Dreissena polymorpha*, and the harmful phytoplankton, *Microcystis aeruginosa*. Limnol. Oceanogr. **50:** 896-904.

Sartory, D. P., and J. U. Grobbelaar. 1984. Extraction of chlorophyll-a from fresh-water phytoplankton for spectrophotometric analysis. Hydrobiologia **114:** 177-187.

Schindler, D. W. 2006. Recent advances in the understanding and management of eutrophication. Limnol. Oceanogr. **51:** 356-363.

Smith, V. H., and D. W. Schindler. 2009. Eutrophication science: where do we go from here? Trends Ecol Evol **24:** 201-207.

Sommer, U., Z. M. Gliwicz, W. Lampert, and A. Duncan. 1986. The PEG-model of seasonal succession of planktonic event in fresh waters. Arch. Hydrobiol. **106:** 433-471.

Spencer, C. N. 1981. Regulation of algae and macrophytes in eutrophic ponds by interacting biological, chemical, and physical factors. M.S. thesis. Michigan State University.

Spivak, A. C., M. J. Vanni, and E. M. Mette. 2011. Moving on up: can results from simple aquatic mesocosm experiments be applied across broad spatial scales? Freshwater Biology **56:** 279-291.

Tillmanns, A. R., A. E. Wilson, F. R. Pick, and O. Sarnelle. 2008. Meta-analysis of cyanobacterial effects on zooplankton population growth rate: species-specific responses. Fundamental and Applied Limnology **171:** 285-295.

Utermöhl, H. 1958. Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. Mitt. int. Ver. theor. angew. Limnol. **9:** 1-38.

Vanderploeg, H. A., J. R. Liebig, W. W. Carmichael, M. A. Agy, T. H. Johengen, G. L. Fahnenstiel, and T. F. Nalepa. 2001. Zebra mussel (*Dreissena polymorpha*) selective filtration promoted toxic *Microcystis* blooms in Saginaw Bay (Lake Huron) and Lake Erie. Canadian Journal of Fisheries and Aquatic Sciences **58:** 1208-1221.

Vanni, M. J., C. Luecke, J. F. Kitchell, Y. Allen, J. Temte, and J. J. Magnuson. 1990. Effects on lower trophic levels of massive fish mortality. Nature **344:** 333-335.

Wilson, A. E., and M. E. Hay. 2007. A direct test of cyanobacterial chemical defense: Variable effects of microcystin-treated food on two *Daphnia pulicaria* clones. Limnol. Oceanogr. **52:** 1467-1479.

Wilson, A. E., O. Sarnelle, and A. R. Tillmanns. 2006. Effects of cyanobacterial toxicity and morphology on the population growth of freshwater zooplankton: Meta-analyses of laboratory

experiments. Limnol. Oceanogr. **51:** 1915-1924.

TABLES

Table 1.1. Source lakes and genetic characterizations of the three *D. pulicaria* clones. Each *D. pulicaria* clone was characterized using two microsatellite markers (Dp464 and Dp496), and microsatellite nucleotide lengths were determined for both loci.

Lake	Location Lat. N, Long. W)	Total Phosphorus (μg l^{-1})	Dp464 length (bp)	Dp496 length (bp)
Baker	42°26'27", 85°21'03"	21-40	147, 147	201, 216
MSU Pond 1	42°40'53", 84°28'57"	170-300	148, 148	198, 202
Wintergreen	42°23'50", 85°23'07"	50-70	149, 149	196, 202

FIGURE LEGENDS

Figure 1. Dynamics of (A) *Daphnia pulicaria* biomass, (B) chlorophyll a, and (C) microcystin concentration for Experiment 1. Data represent means ± one standard error for each treatment over the five-week enclosure experiment.

Figure 2. Relative biomass of dominant phytoplankton taxa on day 28 (30 July 2007) of Experiment 1. Data represent means for phytoplankton taxa for each treatment. The “other” phytoplankton taxon included a mixture of flagellates and chlorophytes.

Figure 3. Dynamics of (A) *Daphnia pulicaria* biomass and (B) chlorophyll a for Experiment 2. Data represent means ± one standard error for each treatment over the six-week experiment.

Figure 4. Relative biomass of dominant phytoplankton taxa and microcystin concentration on day 28 (5 May 2008) of Experiment 2. Data represent means for phytoplankton taxa and means ± one standard error for microcystin concentration for each treatment.

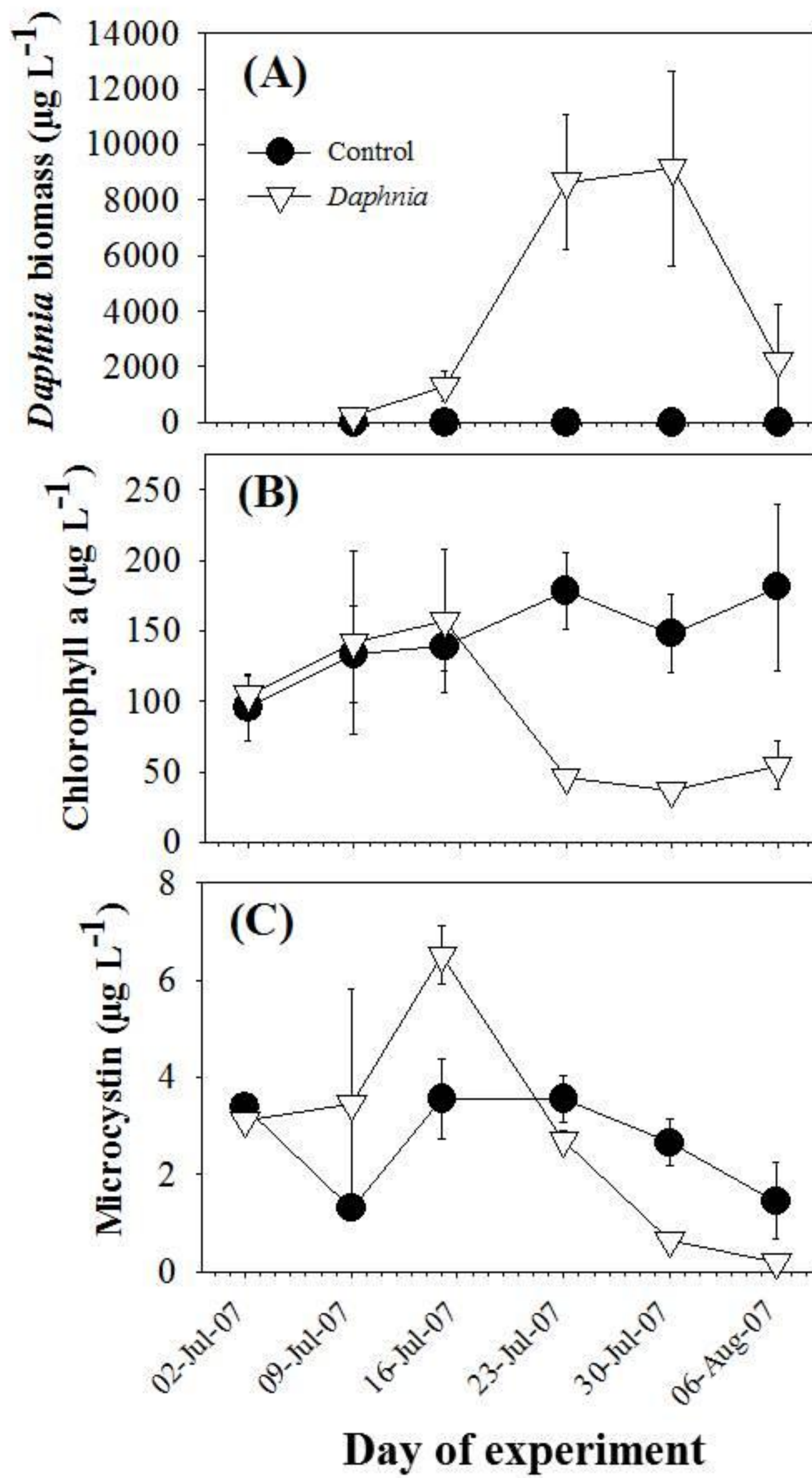


Figure 1.1

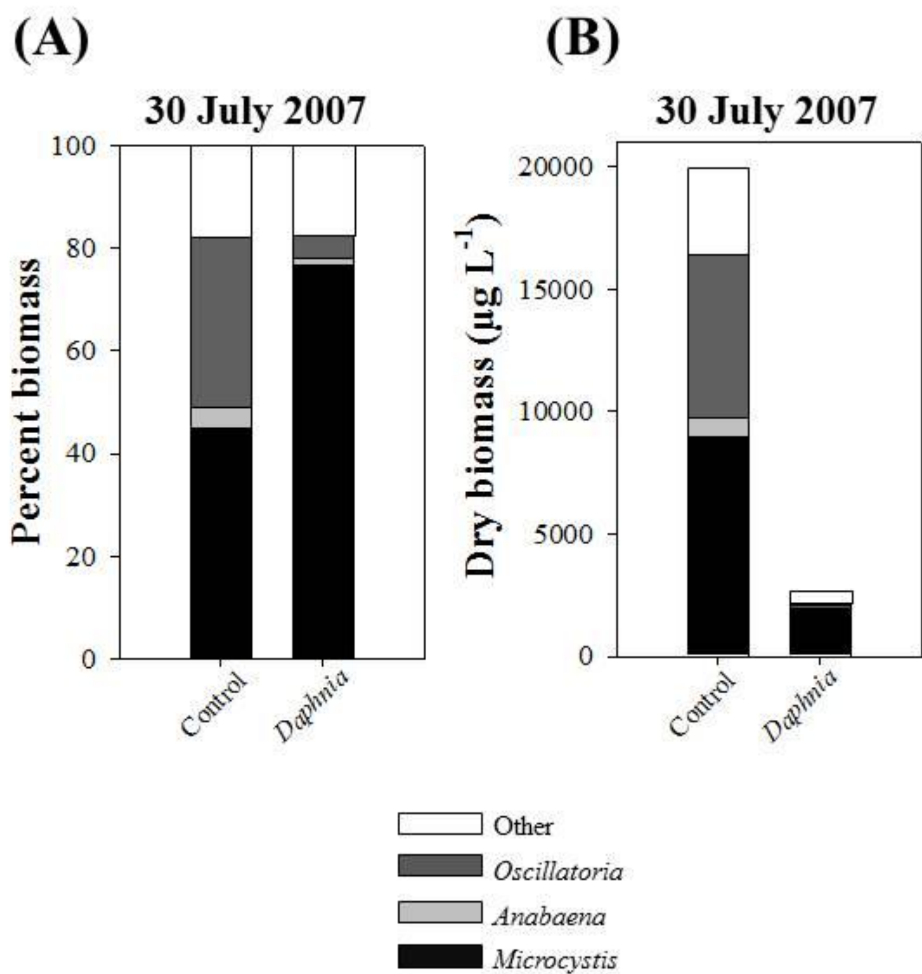


Figure 1.2

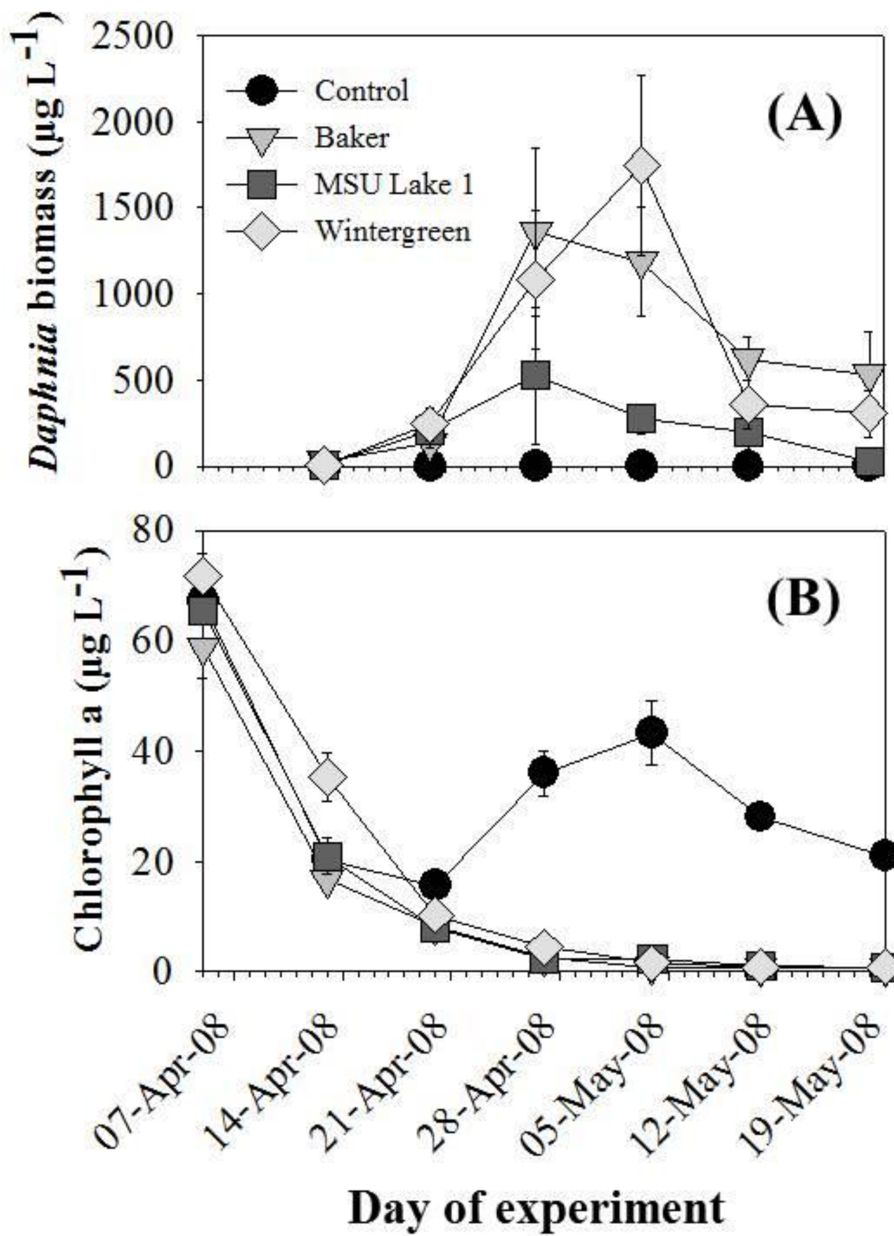


Figure 1.3

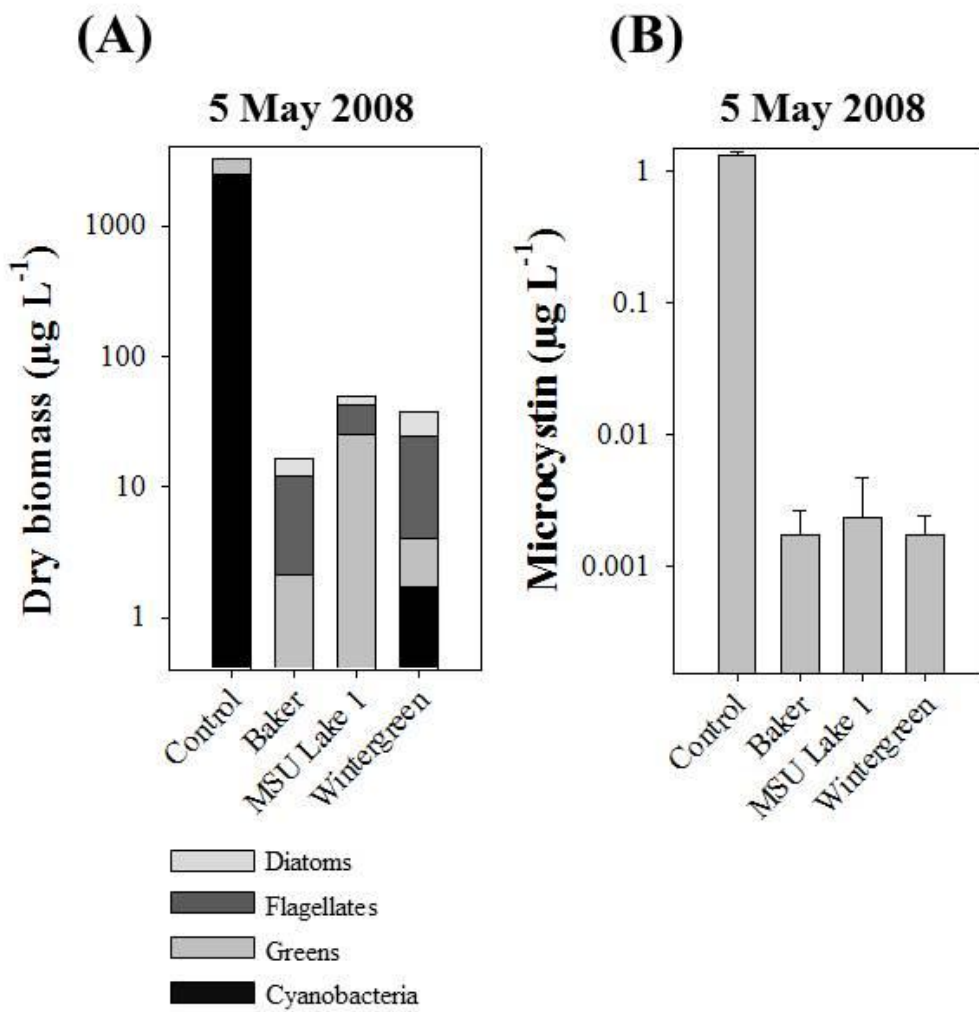


Figure 1.4

Chapter 2

Nitrogen-to-Phosphorus Ratio Mediates the Outcome of Intraspecific Competition in *Daphnia*

ABSTRACT

Despite the traditional emphasis on plant defenses in plant-animal arms races, herbivore offenses may have important consequences for community structure and ecosystem function. Cyanobacteria produce toxins that may reduce the fitness of herbivorous zooplankton (such as *Daphnia*). However, it has recently been discovered that *Daphnia* populations in eutrophic lakes can adapt to tolerate toxic cyanobacteria. We use lab-measured variation in tolerance to toxic cyanobacteria and juvenile growth rates on high quality food for *D. pulicaria* populations from eutrophic versus oligotrophic lakes to examine intraspecific competition among *Daphnia* and potential trade-offs associated with these adaptations in a ten-week limnocorral experiment. Enclosures were fertilized at high or low nitrogen-to-phosphorus (N:P) ratios to manipulate phytoplankton and cyanobacterial abundance (and concentrations of the toxin microcystin). We then stocked a mixture of six genetically-distinct *D. pulicaria* clones from oligotrophic and eutrophic lakes into half of the enclosures. High N:P resulted in over four-fold higher chlorophyll a concentrations and eight-fold higher cyanobacterial biomass relative to low N:P enclosures at the start of the experiment. By the conclusion of the experiment, relative abundance of *Daphnia* genotypes from eutrophic lakes was nearly 100% for high N:P enclosures, while all six *Daphnia* genotypes were equally represented at low N:P. However, these results were driven by the dominance of a single *Daphnia* genotype at high N:P. Thus, our results indicate that N:P ratio can mediate the outcome of intraspecific competition in *Daphnia* by affecting phytoplankton abundance and species composition.

INTRODUCTION

A central goal in community ecology is to understand how consumer-resource interactions influence the dynamics of populations, communities, and ecosystems (Tilman 1982). Despite the traditional assumption of phenotypic homogeneity within a species (Tilman 1982; Leibold et al. 1997), a growing body of studies suggests that intraspecific trait variation may have important ecological consequences (Thompson et al. 2001; Whitham et al. 2006; Post et al. 2008). We now know that genetically-based phenotypic variation, phenotypic plasticity, and inducible defenses can all be important in mediating species interactions (Turner and Mittelbach 1990; Tollrian and Harvell 1999; Werner and Peacor 2003). While numerous studies have documented trade-offs in the competitive abilities of different consumer species for resources (Desmarais and Tessier 1999; Tessier et al. 2000; Tessier and Woodruff 2002), the consequences of similar trade-offs within species are less studied.

Recent research has revealed that there is a large amount of inter- and intraspecific variation in the ability of herbivores to tolerate plant defenses (Karban and Agrawal 2002). Theory and evidence in support of plant defenses have accumulated over the past several decades, and plant chemical defenses against herbivory have been documented for terrestrial and freshwater vascular plants (Howe and Westley 1988), freshwater and marine macroalgae (Hay and Fenical 1988), and some phytoplankton taxa (Carmichael 1992; Kirk and Gilbert 1992). In contrast, the study of herbivore offenses, the adaptations that increase herbivore fitness by countering plant defenses has received less emphasis (Karban and Agrawal 2002). Here we use intraspecific variation within a generalist herbivore in freshwater lakes (i.e., *Daphnia*) to examine the relative competitive ability of *Daphnia* that vary in tolerance to cyanobacteria and their associated toxins (Sarnelle and Wilson 2005).

Cyanobacteria pose one of the most serious threats to nutrient-rich freshwater ecosystems by producing toxic secondary metabolites that can poison fishes, pets, livestock, and humans (Paerl 1988; Carmichael 1992). In addition to posing significant public health risks, cyanobacteria have been shown to be poor quality food for herbivorous zooplankton (such as *Daphnia*) in laboratory studies (Wilson et al. 2006; Wilson and Hay 2007; Tillmanns et al. 2008). However, recent research has shown that local populations of *Daphnia* may evolve to tolerate toxic cyanobacteria following prolonged exposure to cyanobacterial blooms (Hairston et al. 1999; Hairston et al. 2001; Sarnelle and Wilson 2005). Genetically-based tolerance to toxic cyanobacteria has now been documented within *Daphnia* species using common-garden laboratory experiments comparing populations of European *D. galeata* before and after a major eutrophication event (Hairston et al. 1999; Hairston et al. 2001) and populations of North American *D. pulicaria* across lakes of varying nutrient enrichment (Sarnelle and Wilson 2005). It is well known that the presence of the generalist herbivore *Daphnia* is critically important to the success of biomanipulation efforts to improve water quality in eutrophic lakes (Leibold 1989; Schindler 2006), and the evolution of tolerance to toxic cyanobacteria may have important consequences for the response of lakes to nutrient enrichment (Hairston et al. 2001; Sarnelle and Wilson 2005).

Nutrient enrichment of freshwater ecosystems leads to a simultaneous increase in total phytoplankton biomass and the relative abundance of cyanobacteria (Smith 1983; Watson et al. 1997; Downing et al. 2001). It is well known that many herbivores (including *Daphnia*) often trade-off the ability to exploit high resource levels (i.e., high food quality and quantity) with the ability to depress resources to low levels (Tessier et al. 2000; Tessier and Woodruff 2002). However, the costs and competitive consequences of tolerance to toxic cyanobacteria for *Daphnia* (and for herbivore adaptations to cope with plant chemical defenses, in general) are

unexplored (Karban and Agrawal 2002). *Daphnia* from eutrophic lakes are more tolerant of toxic cyanobacteria than *Daphnia* from oligotrophic lakes (Sarnelle and Wilson 2005). Adaptation to toxic cyanobacteria is likely beneficial when cyanobacterial biomass is high. However, tolerance to toxic cyanobacteria may be costly in environments where cyanobacteria are rare. Here we use variation in tolerance to toxic cyanobacteria for *D. pulicaria* populations from eutrophic versus oligotrophic lakes to examine intraspecific competition among *Daphnia* and potential trade-offs associated with these adaptations. We manipulated nitrogen-to-phosphorus (N:P) ratios in a limnocorral experiment to favor dominance by toxic cyanobacteria in half of the enclosures. We then evaluated the effect of N:P ratio on intraspecific competition among six genetically-distinct *D. pulicaria* clones that were collected from oligotrophic and eutrophic lakes and varied in tolerance to toxic cyanobacteria in a previously published laboratory experiment (Sarnelle and Wilson 2005).

METHODS

Daphnia *clones*

The six *D. pulicaria* clones used in this experiment were descendants of one female isolated in 2004 from each of six small glacial lakes (<0.3 km^2) in southern Michigan and were shown to vary in tolerance to a laboratory diet of toxic *Microcystis aeruginosa* and in juvenile growth rates on high quality food (the green alga *Ankistrodesmus*) in a previous growth experiment (Figure 1; Sarnelle and Wilson 2005). Three of the *D. pulicaria* source lakes (Lawrence, Sixteen, and Warner) are oligotrophic and cyanobacteria are rare, while the other three lakes are eutrophic and have high cyanobacterial abundance during the summer months (Table 1; Sarnelle and Wilson 2005). Prior to the field experiment, each *D. pulicaria* clone was first maintained in the laboratory (25°C, 12h light: 12h dark) in 1-L glass beakers filled with autoclaved lake water and

fed a nutritious green algae (*Chlorella*) grown in a nutrient-rich medium (modified BG-11 medium – Vanderploeg et al. 2001). Each *D. pulicaria* clone was then transferred to separate outdoor 160-L tanks filled with lake water filtered through a 35-μm sieve and supplemented with *Chlorella* as a food source several weeks before the start of the experiment. Therefore, all six *D. pulicaria* clones were grown under common-garden conditions to provide animals for stocking the enclosures.

Daphnia *genetic analyses*

D. pulicaria genotypes for each of the six clones used in the experiment were genetically discriminated using variation in two microsatellite loci (Dp3, Dp339 – Colbourne et al. 2004) that have proven to be highly polymorphic for *D. pulicaria* collected from several of our study lakes in Michigan (Table 1; A. E. Wilson unpubl.). *D. pulicaria* from ethanol-preserved samples (see "Sample collection and data analysis" section) were rinsed thoroughly with distilled water to remove attached bacteria and phytoplankton. Genomic deoxyribonucleic acid (DNA) was extracted by heating individual *D. pulicaria* to 95°C in 10 μL Lyse-N-Go PCR reagent (Pierce Chemical Co., Rockford, IL). Forward primers were modified with (-29)/IRDye labeled 19-mer M13 primer sequence in order to visualize polymerase chain reaction (PCR) products on a Li-Cor 4300 DNA Analyzer (Li-Cor Biosciences, Lincoln, NE). Amplification of microsatellite alleles was performed using polymerase chain reaction (PCR) in 12.5 μL volumes (~40 ng of DNA, 1X buffer [Promega Go Green Colorless Buffer], 1.5 mM $MgCl_2$, 0.2 mM dNTP, 1 pmol M13 labeled forward microsatellite primer, 2 pmol reverse microsatellite primer, 0.5 pmol IRDye labeled M13 primer, and 0.5 units of *Taq* DNA polymerase). Each locus was separately analyzed for each *D. pulicaria* individual. PCR used a touchdown protocol under the following conditions: 95°C for 3 min followed by 10 cycles of 94°C for 35 s, 65°C (-1°C/cycle) for 35 s,

72°C for 45 s, followed by an additional 30 cycles with a constant annealing temperature of 55°C with a final extension at 72°C for 10 min. Reactions were stopped with 6 μL stop buffer, diluted as necessary with deionized water, denatured for 3 minutes at 90°C, and snapped cold before loading on a 6.5% polyacrylamide gel.

Study site

The experiment was conducted at the E.W. Shell Fisheries Research Station at Auburn University, Alabama, in a small, eutrophic reservoir pond (Pond S1). Pond S1 is a shallow, polymictic pond with a surface area of approximately 8 ha, maximum depth of 3.5 m, and total nitrogen (TN) and total phosphorus (TP) concentrations in the mixed layer averaging about 1000 μg L^{-1} and 150 μg L^{-1}, respectively in the fall (Boyd and Shelton 1984; A. E. Wilson and M. F. Chislock unpubl.). Cyanobacteria begin to dominate phytoplankton communities in Pond S1 during late spring (April), and cyanobacterial blooms typically persist into early fall (September (A. E. Wilson and M. F. Chislock unpubl.). Immediately prior to the initiation of the experiment (5 October 2009), chlorophyll a in Pond S1 was approximately 30 μg L^{-1}, and cyanobacteria comprised less than 10% of total phytoplankton biomass.

Enclosure experiment

We manipulated nitrogen-to-phosphorus (N:P) ratio in 2500-L, clear polyethylene enclosures that were sealed at the bottom, open to the atmosphere at the top, and suspended from a floating platform (EZ-Dock) anchored in the middle of the pond. Twenty-four enclosures were filled on 5 October 2009 by pumping pond water through a 75-μm mesh net to initially exclude resident *Daphnia*. All enclosures were enriched at a rate of 400 μg L^{-1} phosphorus added as K_2HPO_4. Low N:P enclosures received no addition of nitrogen, while high N:P enclosures were also enriched with 7000 μg L^{-1} nitrogen added as NH_4Cl (n=12 enclosures per N:P ratio). Therefore,

the ratio of total nitrogen to total phosphorus (TN:TP) in low N:P enclosures was ~2:1, by mass, and high N:P enclosures had a TN:TP ratio of ~14:1, by mass. We subsequently stocked approximately equal densities of six genetically-distinct *D. pulicaria* clones from oligotrophic and eutrophic lakes (n=3 clones per lake type) into half (n=12) of the enclosures at a total density of 0.1 L^{-1} on 17 October 2009, and the remaining twelve enclosures served as no *D. pulicaria* controls. Thus, there were six replicate enclosures for each N:P ratio/*D. pulicaria* treatment. During *D. pulicaria* addition, we preserved two subsamples of the *Daphnia* inoculum in 95% ethanol for genetic analysis to confirm that we stocked approximately equal densities of each *D. pulicaria* genotype. We sampled all enclosures biweekly-to-monthly beginning on 5 October 2009, and the experiment was concluded on 10 December 2009.

Sample collection and data analysis

Depth-integrated water samples for chlorophyll a, phytoplankton biomass and species composition, and microcystin were collected from each enclosure with a tube sampler (inside diameter = 51 mm) on each sampling date. On the final sampling date (10 December 2009), we also collected depth-integrated samples for *D. pulicaria* density, biomass, and genotypic composition. Chlorophyll a concentrations were measured by extracting phytoplankton collected on Pall A/E filters in 90% ethanol for 24 h in the dark at 4°C followed by measurement with a fluorometer (Sartory and Grobbelaar 1984). Microcystin concentrations in particles were quantified using enzyme-linked immunosorbent assay (ELISA) (An and Carmichael 1994) after extraction from 75% aqueous methanol. Phytoplankton species abundance and composition were determined via the inverted microscope technique (Utermöhl 1958) using water samples preserved in 1% Lugol's solution. Biovolumes for each species were calculated using cell counts and estimates of cell volume based on measurements of cell dimensions. We then converted

biovolumes (mm^3 L^{-1}) to dry biomass (μg L^{-1}) assuming a specific gravity of 1 g cm^{-3} and a dry biomass: wet biomass ratio of 0.40 (Riemann et al. 1989; Sarnelle and Wilson 2005; Knoll et al. 2008). We characterized *D. pulicaria* genotypes for the inoculum used to stock the enclosures to confirm that we added approximately equal densities of each clone and for all *D. pulicaria* enclosures at the conclusion of the experiment to determine the outcome of intraspecific competition among the clones. We genetically characterized 20-25 randomly selected *D. pulicaria* individuals from ethanol-preserved macrozooplankton samples for each inoculum subsample and for all *Daphnia* enclosures at the conclusion of the experiment. *D. pulicaria* were measured and counted at 40X in a Sedgwick-Rafter cell, and *D. pulicaria* lengths were converted to biomass using a length-weight regression (O. Sarnelle unpubl.).

We used analysis of variance (ANOVA) to compare chlorophyll a, cyanobacterial dry biomass, relative abundance of dominant phytoplankton taxa, and microcystin concentrations for low and high N:P ratios immediately prior to the addition of *D. pulicaria* (16 October 2009). The effects of N:P ratio and *D. pulicaria* presence on chlorophyll a and cyanobacterial dry biomass over time were tested using repeated measures ANOVA (sampling date = repeated measures), and pairwise differences among treatments were assessed with Tukey's test. We used ANOVA to analyze the effects of N:P ratio and *D. pulicaria* presence on the relative abundance of dominant phytoplankton on 3 November and 10 December 2009. The effect of N:P ratio on *D. pulicaria* density and biomass at the conclusion of the experiment was tested using ANOVA. We then compared the relative abundance of each *D. pulicaria* clone initially stocked into the enclosures to its relative abundance at the conclusion of the experiment for low and high N:P ratios using ANOVA and Tukey's test for pairwise comparisons. ANOVA and Tukey's test were also used to compare the relative abundance of *D. pulicaria* clones from oligotrophic and

eutrophic lakes stocked into the enclosures versus their relative abundance at the conclusion of the experiment for low and high N:P ratios. Relative abundance data for dominant phytoplankton taxa and *D. pulicaria* genotypes were arcsine-square-root-transformed prior to all statistical analyses. All other data were checked for normality and homogeneity and log-transformed when necessary.

RESULTS

N:P ratio had a large and significant positive effect on chlorophyll a, cyanobacterial biomass, and concentrations of the toxin microcystin over the first eleven days of the experiment (Figures 2 and 3). Mean chlorophyll a and cyanobacterial biomass for high N:P enclosures were approximately four- and eight-times higher, respectively, than for low N:P enclosures (chlorophyll a: $F_{1,22} = 19201.816$, $P < 0.0001$; cyanobacterial biomass: $F_{1,22} = 62.285$, $P < 0.0001$), immediately prior to stocking of *D. pulicaria* genotypes (16 October 2009). Furthermore, the toxic cyanobacterium *Microcystis aeruginosa* contributed over 20% of total phytoplankton biomass (Figure 4) and mean concentrations of microcystin were over three-times higher at high N:P than low N:P on 16 October 2009 ($F_{1,22} = 8.002$, $P = 0.010$) (Figure 3). In contrast, *M. aeruginosa* comprised approximately 2% of total phytoplankton biomass and the filamentous cyanobacterium *Anabaena circinalis* contributed less than 10% of total phytoplankton biomass at low N:P on 16 October 2009 (Figure 4). In addition, the relative abundance of dinoflagellates was significantly higher at low N:P on 16 October 2009 ($F_{1,22} = 22.769$, $P < 0.0001$). By the fourth week of the experiment (~two weeks after *D. pulicaria* addition - 3 November 2009), *D. pulicaria* had suppressed cyanobacterial biomass by ~96% relative to no *D. pulicaria* controls at high N:P (Tukey's test: $P = 0.009$). The relative abundance of *M. aeruginosa* increased to approximately 36% of total phytoplankton biomass in the no *D.*

pulicaria control while *D. pulicaria* reduced the relative abundance of *M. aeruginosa* to 2% of total phytoplankton biomass at high N:P (Tukey's test: $P < 0.0001$). In contrast, the relative abundance of chlorophytes was increased in *D. pulicaria* enclosures at high N:P, relative to no *D. pulicaria* controls at high N:P (Tukey's test $P = 0.010$). *D. pulicaria* had no effect on cyanobacterial biomass at low N:P by 3 November 2009 (Figure 4 - Tukey's test: $P = 1.000$). Furthermore, the relative abundance of *A. circinalis* and other cyanobacteria (*Cylindrospermopsis* and *Chroococcus* species) did not differ with and without *D. pulicaria* at low N:P on 3 November 2009 (Tukey's test: *Anabaena* – $P = 0.997$; other cyanobacteria – $P = 0.866$). On 10 December 2009, the relative abundance of *Anabaena* and *Microcystis* was significantly higher for the *D. pulicaria*/low N:P treatment than for each of the other three treatments (Tukey's test: $P < 0.05$ for each pairwise comparison). In general, high N:P enclosures were largely dominated by chlorophytes at the conclusion of the experiment (~88% of total phytoplankton biomass) and *M. aeruginosa* contributed less than 5% of total phytoplankton biomass regardless of *D. pulicaria* presence. Repeated-measures ANOVA over the 10-week experiment revealed significant effects of N:P ratio ($F_{1,20} = 31.837$, $P < 0.0001$), *Daphnia* presence ($F_{1,20} = 8.635$, $P = 0.008$), and the interaction of N:P ratio and *Daphnia* presence ($F_{1,20} = 8.241$, $P = 0.0009$) on cyanobacterial biomass. Furthermore, N:P ratio ($F_{1,20} = 519.876$, $P < 0.0001$), *D. pulicaria* presence ($F_{1,20} = 9.931$, $P = 0.005$), and the interaction of N:P ratio and *D. pulicaria* presence ($F_{1,20} = 6.221$, $P = 0.022$) all had significant effects on chlorophyll a over time. At the conclusion of the experiment, *D. pulicaria* had reduced chlorophyll a by approximately 46%, relative to the no *D. pulicaria* control at high N:P (Tukey's test: $P = 0.005$). In contrast, *D. pulicaria* had no effect on chlorophyll a at low N:P (Tukey's test: $P = 0.963$).

By the conclusion of the experiment, N:P ratio had large effects on the relative abundance of all six *D. pulicaria* genotypes (Figure 6A; Table 2). Tukey's test revealed that the relative abundance of Lawrence Lake ($P = 0.188$), Lake Sixteen ($P = 0.832$), Warner Lake ($P = 0.990$), Baker Lake ($P = 0.517$), MSU Pond 1 ($P = 0.166$), and Wintergreen Lake ($P = 0.723$) *D. pulicaria* genotypes at low N:P did not differ from their relative abundance during stocking. In contrast, the relative abundance of Lawrence Lake, Lake Sixteen, Warner Lake, and Wintergreen Lake *D. pulicaria* genotypes was significantly lower at high N:P when compared to their relative abundance during stocking (Tukey's test - Lawrence: $P = 0.0187$; Sixteen: $P = 0.0250$; Warner: $P < 0.0001$). The relative abundance of the Baker Lake *D. pulicaria* genotype was significantly higher at low N:P than at high N:P (Tukey's test: $P = 0.024$); however, the relative abundance of the Baker Lake genotype at high N:P did not differ from its relative abundance during stocking (Tukey's test: $P = 0.541$). In contrast, the relative abundance of the MSU Pond 1 *D. pulicaria* genotype was significantly higher at high N:P than during stocking, and the MSU Pond 1 genotype contributed over 95% of *D. pulicaria* in high N:P enclosures at the conclusion of the experiment. In general, the relative abundance of *Daphnia* genotypes from eutrophic lakes (Baker, MSU Pond 1, and Wintergreen) was nearly 100% for high N:P enclosures, while all six *D. pulicaria* genotypes were equally represented at low N:P (Figure 6B).

DISCUSSION

It is well known that species frequently trade-off high maximum growth rates for low minimum resource requirements (Tessier et al. 2000). *Daphnia* species from environments with low mortality risk and low food conditions (e.g., deep lakes) tend to have low minimum resource requirements (i.e., R^*) but do poorly in resource-rich environments. In contrast, *Daphnia* species from environments with high mortality risk and abundant food (e.g., shallow lakes) typically

have high per capita reproductive rates but also high minimum resource requirements (Tessier and Woodruff 2002). In addition to variation in food quantity observed across the pond-size and permanence gradient, food quantity and quality also differ across lakes of contrasting trophic status. It is well known that nutrient enrichment of freshwater ecosystems leads to an increase in total phytoplankton biomass (i.e., food quantity) (Dillon and Rigler 1975). Furthermore, food quality may also decline with nutrient enrichment, particularly during the summer months, as phytoplankton communities are increasingly dominated by toxic cyanobacteria with eutrophication (Trimbee and Prepas 1987; Watson et al. 1997; Downing et al. 2001). The interplay between increasing phytoplankton biomass and cyanobacterial dominance with nutrient enrichment creates an interesting framework to study the consequences of the trade-off between ability to exploit resource-rich (with potentially toxic food) versus resource-poor environments. By manipulating N:P ratio, we created two contrasting environments – one characterized by increased food quantity but also higher levels of cyanobacteria and associated toxins at high N:P (i.e., lower food quality). In contrast, food quantity, cyanobacterial biomass, and toxins were significantly lower at low N:P.

The trade-off in resource exploitation across different species of daphnids has been shown to have important consequences for phytoplankton communities in previous studies (Tessier et al. 2000). Our results build on these previous studies by examining a similar trade-off within a single species of *Daphnia*. We demonstrate that *Daphnia* may trade-off ability to exploit high resource environments and tolerate toxic cyanobacteria for low minimum resource requirements. Furthermore, the outcome of intraspecific competition among *Daphnia* genotypes also had important consequences for phytoplankton communities. *Daphnia* genotypes from eutrophic lakes became dominant at high N:P and had large effects on chlorophyll a and cyanobacterial

biomass. In contrast, *Daphnia* genotypes from oligotrophic and eutrophic lakes were equally represented at low N:P and had no effect on chlorophyll a and cyanobacterial biomass.

While *Daphnia* genotypes from eutrophic lakes became dominant at high N:P, this was driven by the relative abundance of a single genotype (MSU Pond 1) that comprised greater than 90% of all *Daphnia* genotypes at the conclusion of the experiment. Immediately prior to stocking *D. pulicaria* genotypes, the toxic cyanobacterium *Microcystis aeruginosa* contributed over 25% of total phytoplankton biomass. However, the phytoplankton community at high N:P was dominated by relatively nutritious phytoplankton (i.e., small, edible chlorophytes). Therefore, selection for *Daphnia* genotypes may have been driven by high maximum growth rates on high quantities of good quality food versus tolerance to toxic cyanobacteria. The Baker Lake genotype was most tolerant of toxic cyanobacteria in a previously published laboratory experiment. However, the relative abundance of the Baker Lake genotype was less than 1% of all *Daphnia* genotypes at the conclusion of the experiment. As we only extensively sampled *Daphnia* populations at the conclusion of the experiment to minimize contamination of *Daphnia* genotypes, the dynamics of relative abundances for *Daphnia* genotypes over time are unknown.

Experiments examining how population growth rates and minimum resource requirements of *Daphnia* are affected by food quantity and quality (or toxicity) would be interesting and could help to identify potential mechanisms driving the results observed here. While *Daphnia* from environments with low food quantity (e.g., oligotrophic lakes) tend to have low minimum resource requirements, it would be interesting to see if cyanobacteria in the diet reverse the outcome of resource competition in *Daphnia*. Cyanobacteria have repeatedly been shown to be poor quality food for most zooplankton grazers (including *Daphnia*) in laboratory studies (Wilson et al. 2006; Wilson and Hay 2007; Tillmanns et al. 2008). Numerous studies have

demonstrated that cyanobacteria can inhibit zooplankton feeding, growth, and reproduction through several mechanisms including (1) colonial or filamentous morphologies, (2) nutritional deficiencies, and (3) production of toxins (Porter 1977; Lampert 1987; Wilson et al. 2006). While experimental examinations of resource competition typically manipulate food quantity (Tilman 1982), it is possible that the presence of poor quality food (e.g., cyanobacteria) may also affect the outcome of resource competition. Toxin-producing cyanobacteria have frequently been hypothesized to limit the ability of herbivorous zooplankton (such as *Daphnia*) to control phytoplankton biomass by inhibiting *Daphnia* feeding, population growth, and in extreme cases, causing *Daphnia* mortality (Gilbert 1990; DeMott et al. 1991, Lurling and van der Grinten 2003). Understanding the effects of cyanobacteria that are poor quality and potentially dangerous food for *Daphnia* consumers on minimum resource requirements may help explain the evolution of *Daphnia* tolerance to toxic cyanobacteria.

While evolutionary biologists have been studying intraspecific trait variation since Darwin, we still have a poor understanding of what maintains this variability within a population. In addition to the intraspecific variation in tolerance to cyanobacteria between *Daphnia* populations in eutrophic versus oligotrophic environments, there is also considerable variation for traits conferring tolerance to cyanobacteria within eutrophic lakes (Sarnelle and Wilson 2005). Trade-offs associated with the presence (or absence) of cyanobacteria in the environment may help to explain the maintenance variability within *Daphnia* populations. Assuming tolerance to cyanobacteria is costly in the absence of cyanobacteria, it would be interesting to determine whether there are seasonal changes in *Daphnia* tolerance to cyanobacteria as a result of phytoplankton succession.

Ecological genetics is a rapidly growing field which merges aspects of population genetics and community ecology to understand the consequences of intraspecific variation for communities and ecosystems (Whitham et al. 2006; Wade 2007). In particular, intraspecific variation within keystone and foundation species is expected to have large effects on ecological interactions (Whitham et al. 2006; Post et al. 2008). In freshwater ecosystems, rapid adaptive evolution by major consumers may play an important role in the response of lake ecosystems to cultural eutrophication and food web manipulations (Hairston et al. 2001; Sarnelle and Wilson 2005). Hairston et al. (1999) made the seminal observation that a generalist herbivore in freshwater lakes (*Daphnia*) can evolve to tolerate toxic cyanobacteria in the diet in response to nutrient enrichment. *Daphnia* tolerance to toxic cyanobacteria has now been observed for a *D. galeata* population following eutrophication of Lake Constance in Europe (Hairston et al. 2001) and for *D. pulicaria* populations in North America (Sarnelle and Wilson 2005). Understanding the causes and consequences of intraspecific variation within *Daphnia* may aid in the future management of eutrophic lakes, and we can now accurately characterize intraspecific genetic variation within and across populations of *Daphnia* (Colbourne et al. 2004). An emphasis on intraspecific trait variation provides an interesting conceptual framework for linking diversity to ecosystem function, and previous studies suggest that this approach may be profitable, particularly for plankton food webs (Tessier et al. 2000; Post et al. 2008; Duffy et al. 2010).

REFERENCES

An, J. S., and W. W. Carmichael. 1994. Use of a colorimetric protein phosphatase inhibition assay and enzyme-linked-immunosorbent-assay for the study of microcystins and nodularins. Toxicon **32:** 1495-1507.

Boyd, C. E., and J. L. Shelton. 1984. Observations on the hydrology and morphometry of ponds on the Auburn University Fisheries Research Unit. Alabama Agricultural Experiment Station Bulletin**:** 1-64.

Carmichael, W. W. 1992. Cyanobacteria secondary metabolites - the cyanotoxins. Journal of Applied Bacteriology **72:** 445-459.

Colbourne, J. K., B. Robison, K. Bogart, and M. Lynch. 2004. Five hundred and twenty-eight microsatellite markers for ecological genomic investigations using *Daphnia*. Molecular Ecology Notes **4:** 485-490.

DeMott, W. R., Q. X. Zhang, and W. W. Carmichael. 1991. Effects of toxic cyanobacteria and purified toxins on the survival and feeding of a copepod and 3 species of *Daphnia*. Limnol. Oceanogr. **36:** 1346-1357.

Desmarais, K. H., and A. J. Tessier. 1999. Performance trade-off across a natural resource gradient. Oecologia **120:** 137-146.

Dillon, P. J. and F. H. Rigler. 1974. The phosphorus-chlorophyll relationship in lakes. Limnol. Oceanogr. **19**: 767-773.

Downing, J. A., S. B. Watson, and E. McCauley. 2001. Predicting cyanobacteria dominance in lakes. Canadian Journal of Fisheries and Aquatic Sciences **58:** 1905-1908.

Duffy, M. A. 2010. Ecological consequences of intraspecific variation in lake Daphnia. Freshwater Biology **55:** 995-1004.

Gilbert, J. J. 1990. Differential effects of *Anabaena affinis* on cladocerans and rotifers: mechanisms and implications. Ecology **71:** 1727-1740.

Hairston, N. G., W. Lampert, C. E. Caceres, C. L. Holtmeier, L. J. Weider, U. Gaedke, J. M. Fischer, J. A. Fox, and D. M. Post. 1999. Lake ecosystems - Rapid evolution revealed by dormant eggs. Nature **401:** 446-446.

Hairston, N. G., C. L. Holtmeier, W. Lampert, L. J. Weider, D. M. Post, J. M. Fischer, C. E. Caceres, J. A. Fox, and U. Gaedke. 2001. Natural selection for grazer resistance to toxic cyanobacteria: Evolution of phenotypic plasticity? Evolution **55:** 2203-2214.

Hay, M. E., and W. Fenical. 1988. Marine plant-herbivore interactions: the ecology of chemical defense. Annual Review of Ecology and Systematics **19:** 111-145.

Howe, H. F. and L. C. Westley. 1988. Ecological relationships of plants and animals. Oxford Univ. Press.

Karban, R., and A. A. Agrawal. 2002. Herbivore offense. Annual Review of Ecology and Systematics **33:** 641-664.

Kirk, K. L., and J. J. Gilbert. 1992. Variation in herbivore response to chemical defenses: zooplankton foraging on toxic cyanobacteria. Ecology **73:** 2208-2217.

Knoll, L. B. S., O.; Hamilton, S. K.; Kissman, C. E. H., Wilson, A. E., Rose, J. B., and Morgan, M. R. 2008. Corrigendum: Invasive zebra mussels (*Dreissena polymorpha*) increase cyanobacterial toxin concentrations in low-nutrient lakes. Canadian Journal of Fisheries and Aquatic Sciences **65:** 2552-2552.

Lampert, W. 1987. Laboratory studies on zooplankton-cyanobacteria interactions. New Zealand Journal of Marine and Freshwater Research **21:** 483-490.

Leibold, M. A. 1989. Resource edibility and the effects of predators and productivity on the

outcome of trophic interactions. American Naturalist **134:** 922-949.

Leibold, M. A., J. M. Chase, J. B. Shurin, and A. L. Downing. 1997. Species turnover and the regulation of trophic structure. Annual Review of Ecology and Systematics **28:** 467-494.

Lürling, M., and E. Van Der Grinten. 2003. Life-history characteristics of *Daphnia* exposed to dissolved microcystin-LR and to the cyanobacterium *Microcystis aeruginosa* with and without microcystins. Environmental Toxicology and Chemistry **22:** 1281-1287.

Paerl, H. W. 1988. Nuisance phytoplankton blooms in coastal, estuarine, and inland waters. Limnol. Oceanogr. **33:** 823-847.

Porter, K. G. 1977. The plant-animal interface in freshwater ecosystems. American Scientist **65**: 159-170.

Post, D. M., E. P. Palkovacs, E. G. Schielke, and S. I. Dodson. 2008. Intraspecific variation in a predator affects community structure and cascading trophic interactions. Ecology **89:** 2019-2032.

Riemann, B., P. Simonsen, and L. Stensgaard. 1989. The carbon and chlorophyll content of phytoplankton from various nutrient regimes. Journal of Plankton Research **11:** 1037-1045.

Sarnelle, O., and A. E. Wilson. 2005. Local adaptation of *Daphnia pulicaria* to toxic cyanobacteria. Limnol. Oceanogr. **50:** 1565-1570.

Sartory, D. P., and J. U. Grobbelaar. 1984. Extraction of chlorophyll-a from fresh-water phytoplankton for spectrophotometric analysis. Hydrobiologia **114:** 177-187.

Schindler, D. W. 2006. Recent advances in the understanding and management of eutrophication. Limnol. Oceanogr. **51:** 356-363.

Smith, V. H. 1983. Low nitrogen to phosphorus ratios favor dominance by blue-green-algae in lake phytoplankton. Science **221:** 669-671.

Tessier, A. J., and P. Woodruff. 2002. Trading off the ability to exploit rich versus poor food quality. Ecology Letters **5:** 685-692.

Tessier, A. J., M. A. Leibold, and J. Tsao. 2000. A fundamental trade-off in resource exploitation by *Daphnia* and consequences to plankton communities Ecology **81:** 826-841.

Thompson, J. N., O. J. Reichman, P. J. Morin, G. A. Polis, M. E. Power, R. W. Sterner, C. A. Couch, L. Gough, R. Holt, D. U. Hooper, F. Keesing, C. R. Lovell, B. T. Milne, M. C. Molles, D. W. Roberts, and S. Y. Strauss. 2001. Frontiers of ecology. BioScience **51:** 15-24.

Tilman, D. 1982. Resource competition and community structure. Princeton Univ. Press.

Tillmanns, A. R., A. E. Wilson, F. R. Pick, and O. Sarnelle. 2008. Meta-analysis of cyanobacterial effects on zooplankton population growth rate: species-specific responses. Fundamental and Applied Limnology **171:** 285-295.

Tollrian, R., and C. D. Harvell. 1999. The ecology and evolution of inducible defenses. Princeton Univ. Press.

Trimbee, A. M., and E. E. Prepas. 1987. Evaluation of total phosphorus as a predictor of the relative biomass of blue-green algae with emphasis on Alberta lakes. Canadian Journal of Fisheries and Aquatic Sciences **44:** 1337-1342.

Turner, A. M., and G. G. Mittelbach. 1990. Predator avoidance and community structure: interactions among piscivores, planktivores, and plankton. Ecology **71:** 2241-2254.

Utermöhl, H. 1958. Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. Mitt. int. Ver. theor. angew. Limnol. **9:** 1-38.

Vanderploeg, H. A., J. R. Liebig, W. W. Carmichael, M. A. Agy, T. H. Johengen, G. L. Fahnenstiel, and T. F. Nalepa. 2001. Zebra mussel (*Dreissena polymorpha*) selective filtration promoted toxic *Microcystis* blooms in Saginaw Bay (Lake Huron) and Lake Erie. Canadian

Journal of Fisheries and Aquatic Sciences **58:** 1208-1221.

Wade, M. J. 2007. The co-evolutionary genetics of ecological communities. Nat. Rev. Genet. **8:** 185-195.

Watson, S. B., E. Mccauley, and J. A. Downing. 1997. Patterns in phytoplankton taxonomic composition across temperate lakes of differing nutrient status. Limnol. Oceanogr. **42:** 487-495.

Werner, E. E., and S. D. Peacor. 2003. A review of trait-mediated indirect interactions in ecological communities. Ecology **84:** 1083-1100.

Whitham, T. G., J. K. Bailey, J. A. Schweitzer, S. M. Shuster, R. K. Bangert, C. J. Leroy, E. V. Lonsdorf, G. J. Allan, S. P. DiFazio, B. M. Potts, D. G. Fischer, C. A. Gehring, R. L. Lindroth, J. C. Marks, S. C. Hart, G. M. Wimp, and S. C. Wooley. 2006. A framework for community and ecosystem genetics: from genes to ecosystems. Nat. Rev. Genet. **7:** 510-523.

Wilson, A. E., and M. E. Hay. 2007. A direct test of cyanobacterial chemical defense: Variable effects of microcystin-treated food on two *Daphnia pulicaria* clones. Limnol. Oceanogr. **52:** 1467-1479.

Wilson, A. E., O. Sarnelle, and A. R. Tillmanns. 2006. Effects of cyanobacterial toxicity and morphology on the population growth of freshwater zooplankton: Meta-analyses of laboratory experiments. Limnol. Oceanogr. **51:** 1915-1924.

TABLES

Table 2.1. Source lakes and genetic characterizations of the six *D. pulicaria* clones. Three *D. pulicaria* clones were collected from oligotrophic lakes where cyanobacteria were absent (Lawrence, Sixteen, and Warner), and three clones were collected from eutrophic lakes with abundant cyanobacteria (Baker, MSU Pond 1, and Wintergreen). Each *D. pulicaria* clone was characterized using two microsatellite markers (Dp3 and Dp339), and microsatellite nucleotide lengths were determined for both loci.

Lake	Location Lat. N, Long. W	Total Phosphorus (μg l^{-1})	Dp3 length (bp)	Dp339 length (bp)
Lawrence	42°26’27”, 85°21’03”	8-10	282, 291	175, 180
Sixteen	42°33’90”, 85°36’80”	9-12	282, 286	175, 180
Warner	42°28’16”, 85°31’30”	12-14	282, 282	175, 180
Baker	42°26’27”, 85°21’03”	21-40	291, 291	175, 180
Wintergreen	42°23’50”, 85°23’07”	50-70	282, 282	175, 175
MSU Pond 1	42°40’53”, 84°28’57”	170-300	282, 286	175, 190

Table 2.2. Effect of N:P ratio on the relative abundance of each of the six *Daphnia pulicaria* genotypes at the conclusion of the experiment (10 December 2009).

D. pulicaria genotype	Lake type	DF	F	*P*
Lawrence Lake	Oligotrophic	2	23.757	<0.0001
Lake Sixteen	Oligotrophic	2	28.210	<0.0001
Warner Lake	Oligotrophic	2	13.807	0.001
Baker Lake	Eutrophic	2	4.912	0.030
MSU Pond 1	Eutrophic	2	330.668	<0.0001
Wintergreen Lake	Eutrophic	2	20.553	<0.0001
Error		11		

FIGURE LEGENDS

Figure 1. Mean juvenile somatic growth rates for the six *Daphnia pulicaria* genotypes on two diets: a nutritious green alga (100 % *Ankistrodesmus*) and toxic cyanobacteria (100% *Microcystis aeruginosa*). Error bars represent one standard error. Data are from Sarnelle and Wilson 2005.

Figure 2. Dynamics of (A) chlorophyll a and (B) cyanobacterial dry biomass over the ten-week enclosure experiment. Data represent means ± one standard error for each treatment.

Figure 3. Concentration of the toxin microcystin for each nitrogen-to-phosphorus (N:P) treatment immediately prior to stocking of *Daphnia pulicaria* genotypes (16 October 2009). Data represent means ± one standard error.

Figure 4. Relative biomass of phytoplankton taxa at the beginning (16 October 2009), middle (3 November 2009), and end (10 December 2009) of the ten-week enclosure experiment. Data represent means for phytoplankton taxa. Since *Daphnia pulicaria* were added on 17 October 2009, relative biomass of phytoplankton taxa for "No *Daphnia*" and "*Daphnia*" enclosures were pooled for each N:P ratio on 16 October 2009.

Figure 5. *Daphnia pulicaria* (A) density and (B) biomass for each nitrogen-to-phosphorus (N:P) treatment at the conclusion of the experiment (10 December 2009). Data represent means ± one standard error for each N:P treatment.

Figure 6. Relative abundance of (A) each *Daphnia pulicaria* genotype and (B) *D. pulicaria* genotypes from oligotrophic and eutrophic lakes at the conclusion of the experiment (10 December 2009). Data represent means ± one standard error for each N:P treatment.

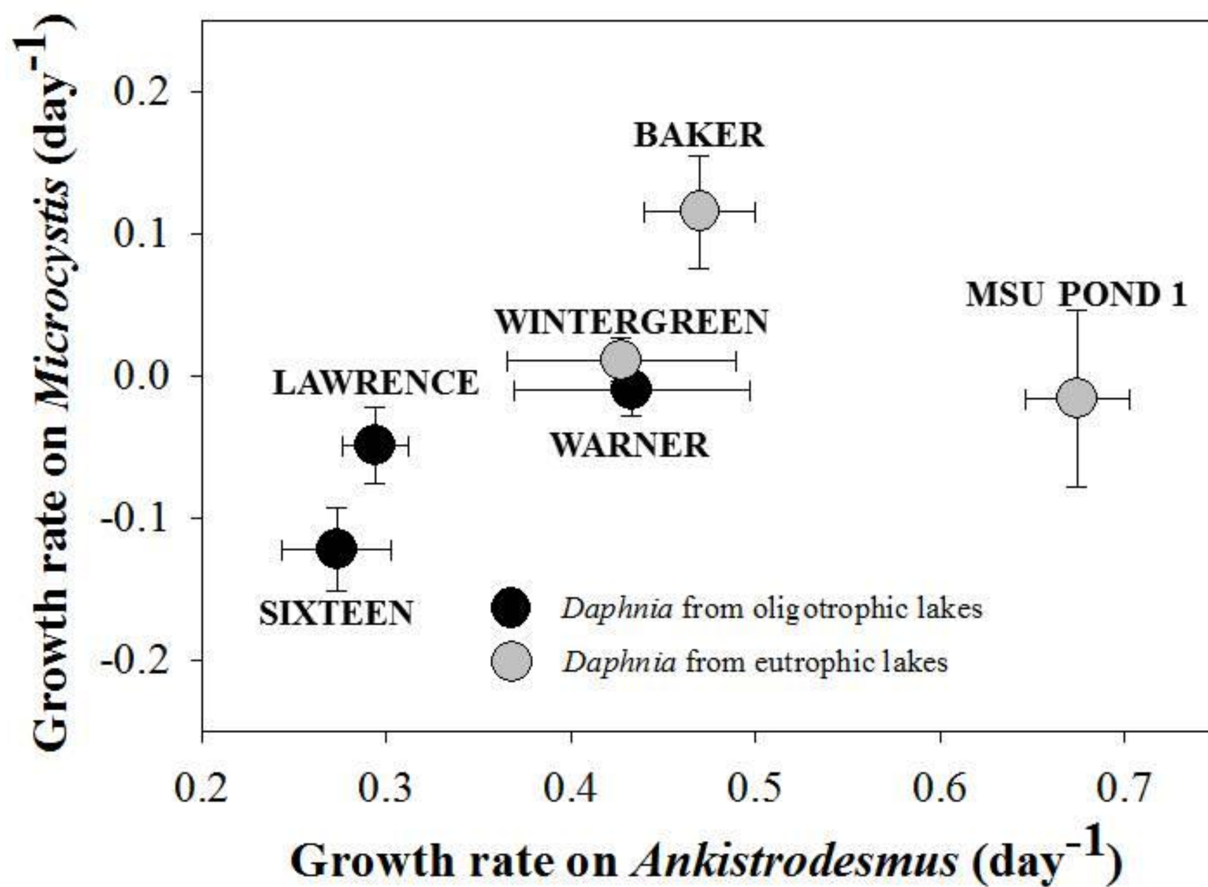


Figure 2.1

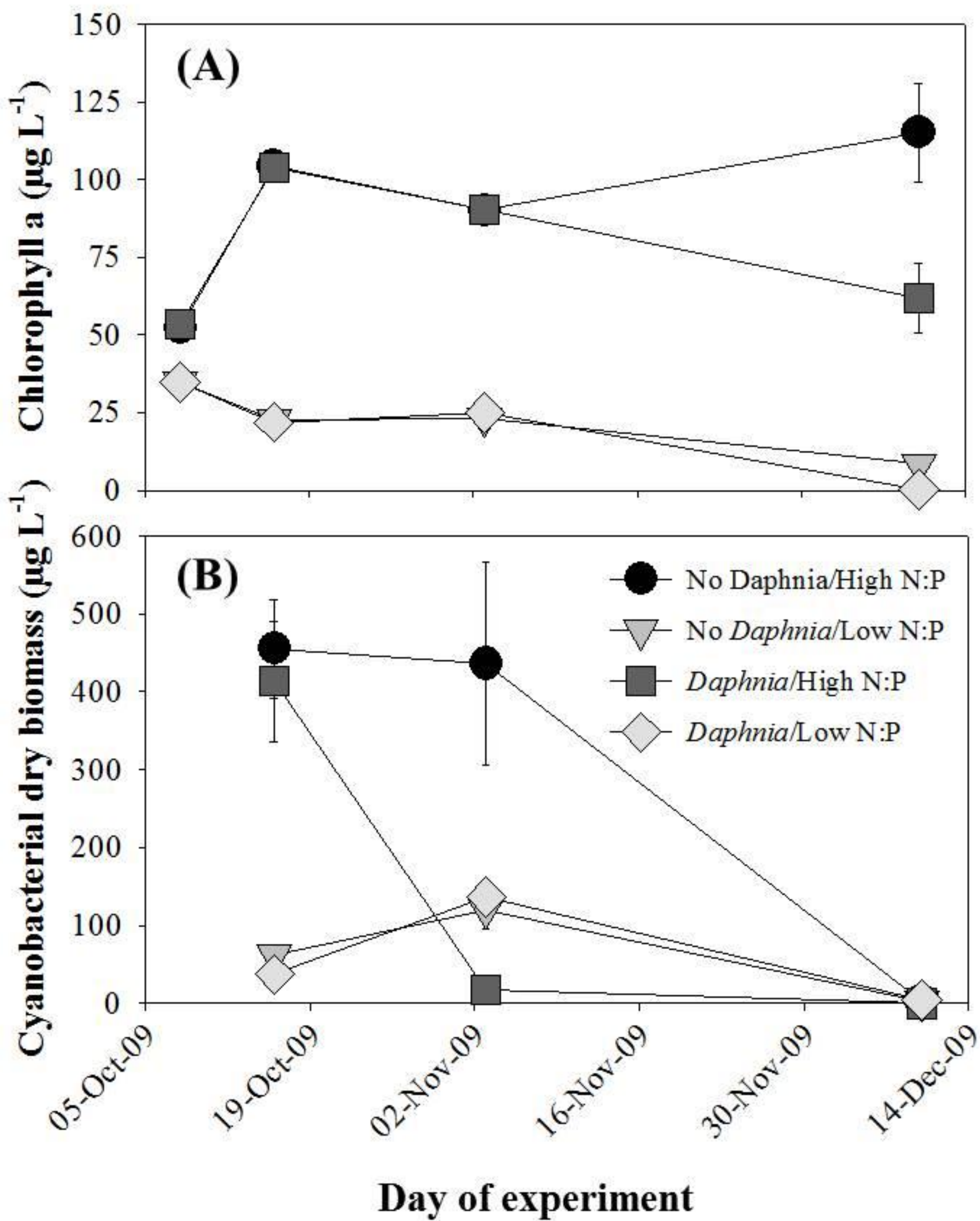


Figure 2.2

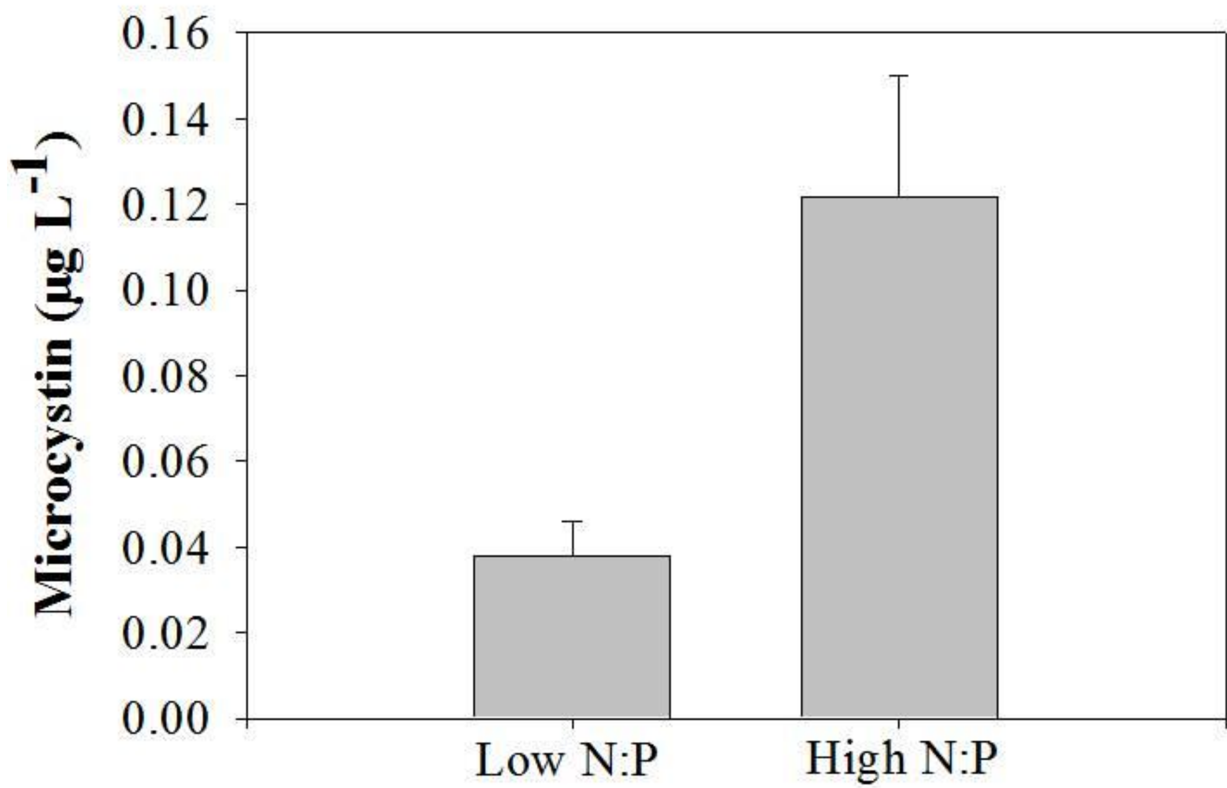


Figure 2.3

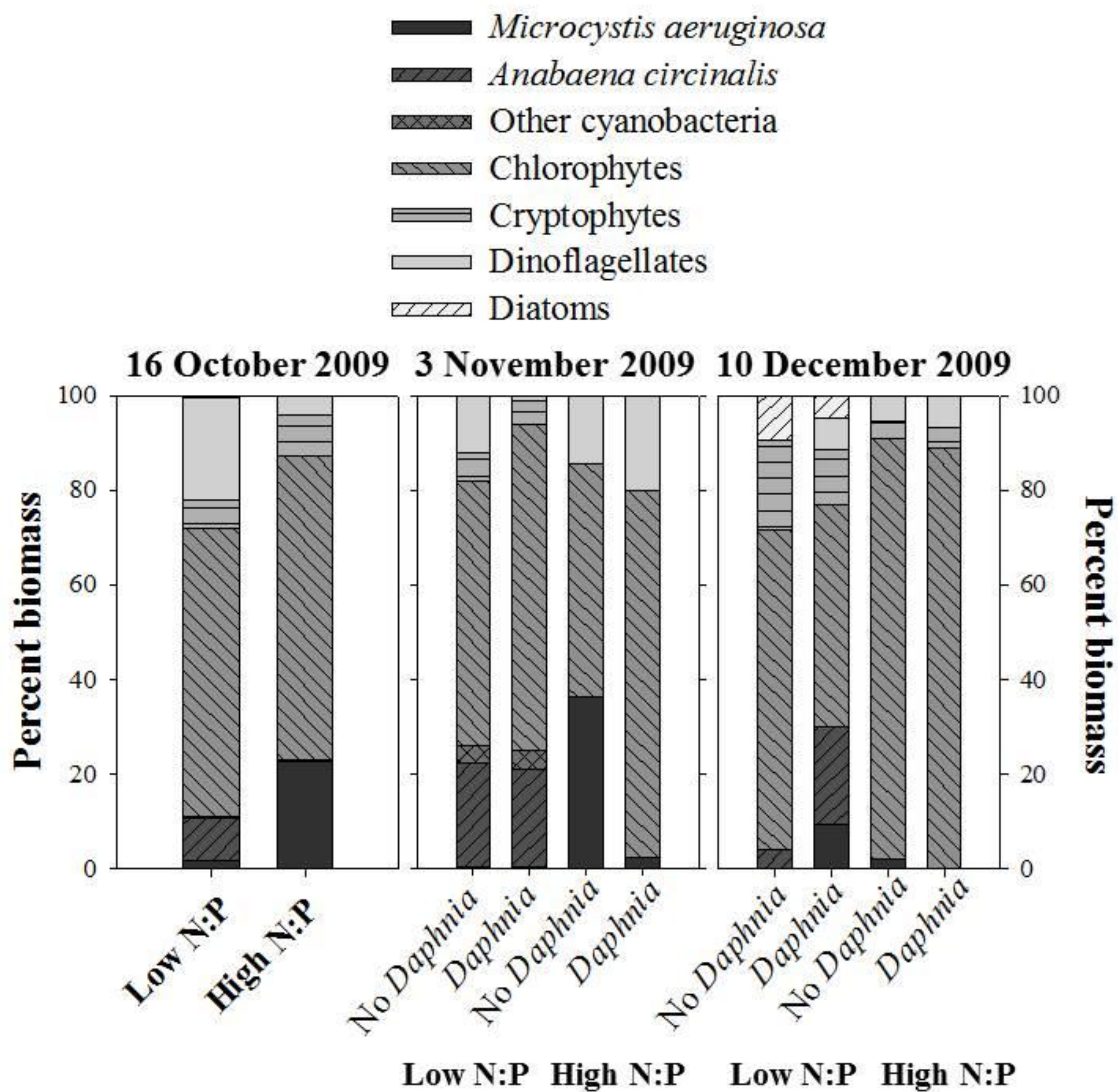


Figure 2.4

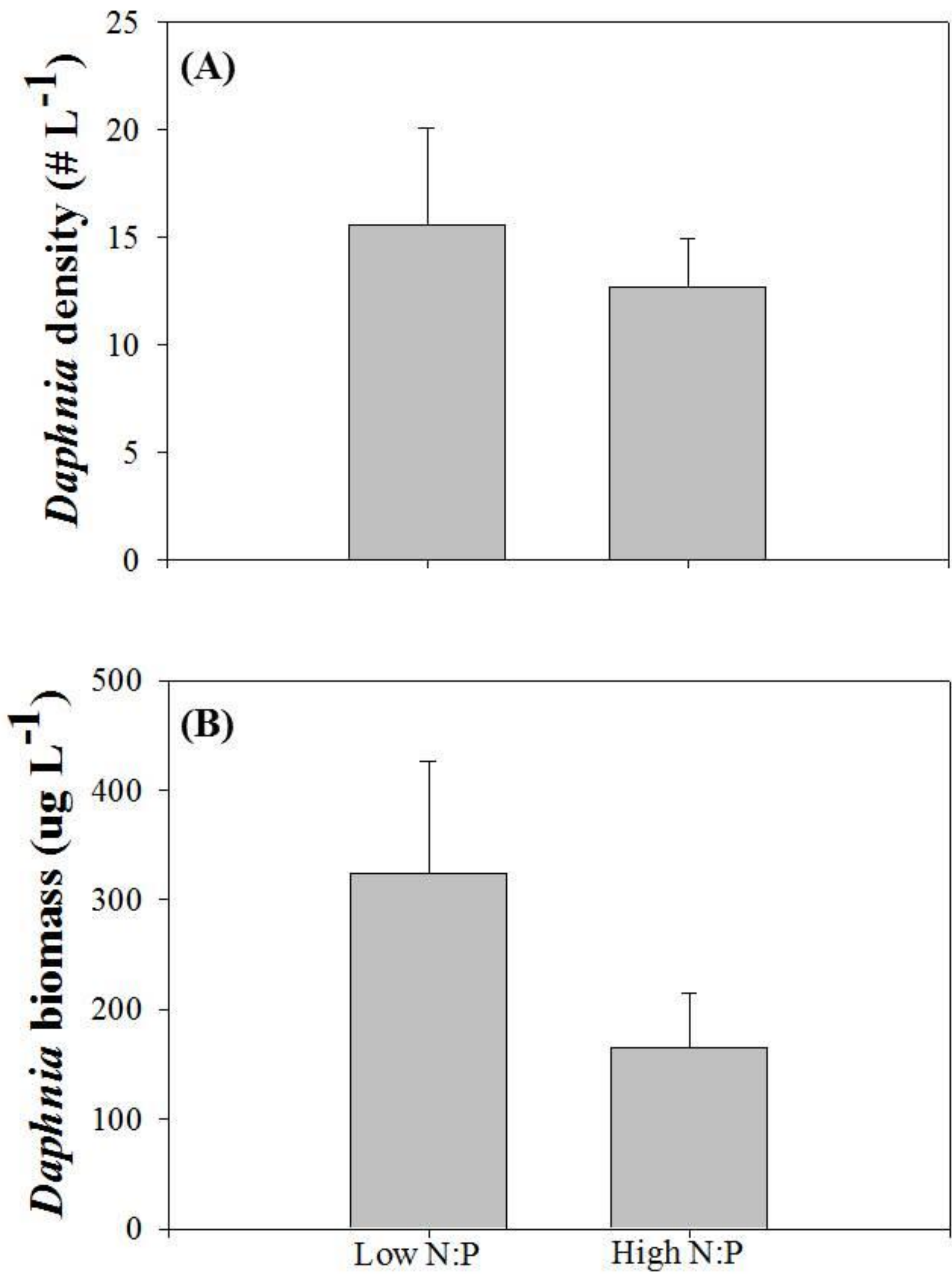
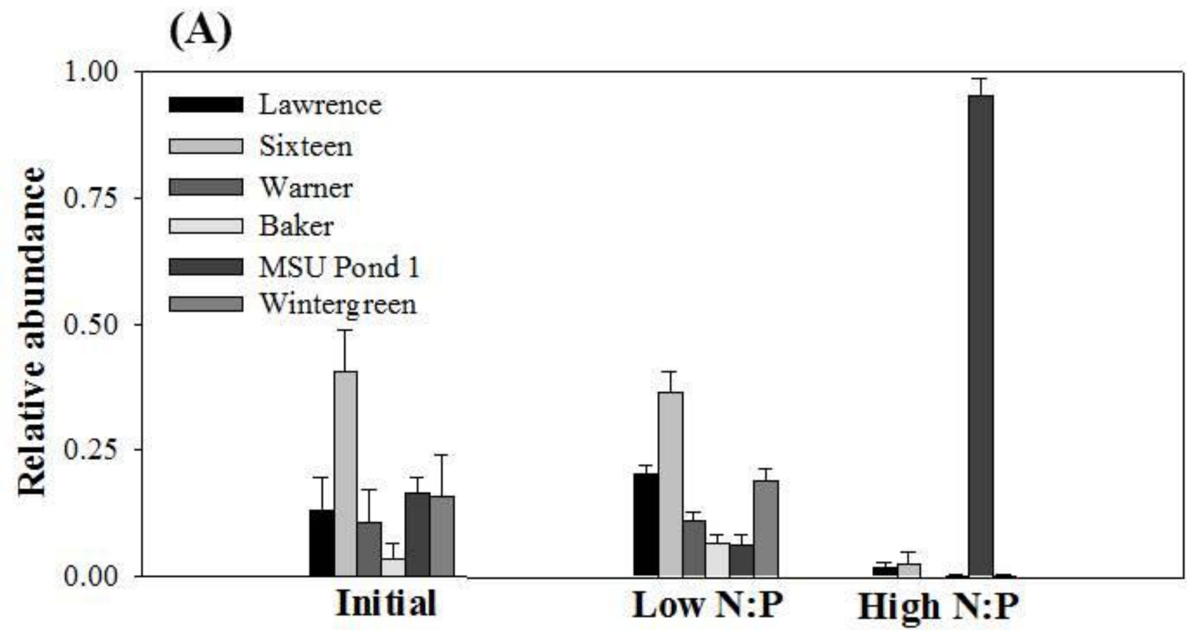


Figure 2.5



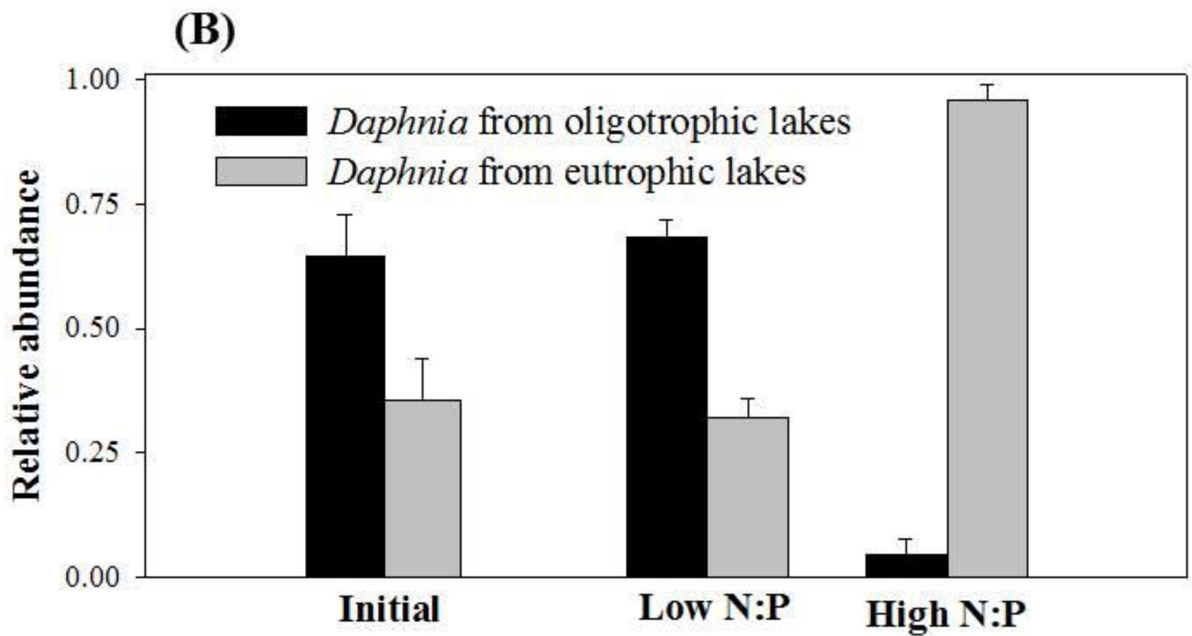


Figure 2.6