Consumer adaptation mediates top-down regulation of ecosystems across a nutrient gradient

by

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Abstract

Individuals within a species frequently differ in important traits, and these differences are known functions of genetic, epigenetic, and environmental influences. While evolutionary biologists have been studying genetically-based trait variation within species since Darwin, the ecological consequences of this variation in nature have remained largely unexplored. For my dissertation research, I have investigated the ecosystem-level consequences of local adaptation using a model system consisting of toxic algae (i.e., cyanobacteria) and a keystone herbivore (i.e., the microcrustacean *Daphnia pulicaria*) that adapts to the presence of toxic cyanobacteria in its environment. I use a combination of large-scale field experiments and mechanistic smaller-scale laboratory experiments to address my questions. While the presence of *Daphnia* is essential for a strong trophic cascade in lakes, my research shows for the first time that: 1) the effect of different *D. pulicaria* genotypes on ecosystem function can be as large as the effect of presence/absence of this species; 2) the mechanism is keyed to a superior numerical response of adapted *D. pulicaria* genotypes not a difference in functional response; 3) the effect of *D. pulicara* on primary producer biomass increases with productivity, even in the presence of toxic and resistant prey; 4) *D. pulicaria* and its adaptation to toxic prey can mediate effects on primary producer biomass across a productivity gradient; 5) that tolerance to toxic cyanobacteria by *D. pulicaria* may be costly in terms of performance in low-nutrient environments; and 6) that *D. pulicaria* adaptations to toxic cyanobacteria may mediate the response of a toxic and invasive cyanobacterium (*Cylindrospermopsis raciborskii*) to nutrient enrichment and climate change. In

general, human activities have had far-reaching effects on most ecosystems, and these effects are predicted to escalate. The results presented here suggest that the long-term responses of ecosystems to eutrophication (a major agent of global change) may depend not only on the presence of particular consumer species but also on local adaptations by consumers in response to these environmental changes. Given the threat the eutrophication poses to water resources around the world, our findings have important implications for natural resource managers.

Acknowledgments

I dedicate this dissertation in memory of my mom and to my family who have always given me their love, support, faith, and encouragement. I love you more than I can possibly explain here. –Michael

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Chapter 1

Large effects of consumer offense on ecosystem structure and function

Abstract

Study of the role of within-species adaptation in ecological dynamics has focused largely on prey adaptations that reduce consumption risk (prey defense). Few, if any, studies have examined how consumer adaptations to overcome prey defenses (consumer offense) affect ecosystem structure and function. We manipulated two sets of genotypes of a planktonic herbivore (*Daphnia pulicaria*) in a highly productive ecosystem with abundant toxic prey (cyanobacteria). The two sets of consumer genotypes varied widely in their tolerance of toxic cyanobacteria in the diet (i.e., sensitive vs. tolerant). We found a large effect of tolerant *D. pulicaria* on phytoplankton biomass and gross primary productivity but no effect of sensitive genotypes, this result stemming from genotype-specific differences in population growth in the presence of toxic prey. The former effect was as large as effects seen in previous *Daphnia* manipulations at similar productivity levels. Thus, we demonstrated that the effect of consumer genotypes with contrasting offensive adaptations was as large as the effect of consumer presence/absence.

Introduction

Traditionally, ecologists have assumed that adaptive evolutionary change is much slower than ecological dynamics (Thompson 1998). This assumption implies that species traits can be treated as fixed with respect to understanding how species affect community and ecosystem dynamics (Chase and Leibold 2003). The concept of species as packages of more or less fixed traits has been fundamental to ecological research on trophic cascades (Leibold 1989; Brett 1994; Cottingham et al. 2004), biodiversity-ecosystem function (Hooper et al. 2005), and food-web analysis (Tilman 1982).

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In contrast, accumulating evidence shows considerable malleability in the ecological traits of many species over short time scales (Grant and Grant 2002; Hairston et al. 1999). Much of this evidence comes from the study of phenotypic plasticity (Tollrian and Harvell 1999), but more recently ecologists have begun to appreciate how heritable adaptive traits within a species can influence community and ecosystem dynamics (Pennisi 2012; Weis and Post 2013). To date, the consequences of intraspecific adaptive variation for trophic interactions have been examined largely from the perspective of prey (both plants and animals) responding to changes in consumption or consumption risk (i.e., prey defense) (Rhoades 1985; Karban and Agrawal 2002). In contrast, adaptations by consumers to overcome prey defenses (i.e., consumer offense) are less well-studied (Sotka and Whalen 2008) and the consequences of consumer adaptations for ecosystem dynamics are relatively unknown. This bias, in part, follows from the "life-dinner" argument: asymmetrical fitness outcomes of consumer-prey encounters should promote stronger selection on prey than on consumers (Dawkins and Krebs 1979). One important exception to this argument, however, is "dangerous" prey that can produce toxins that can harm or even kill consumers (Brodie and Brodie 1999; DeMott et al. 1991; Hansson et al. 2007). The most profitable systems in which to pursue the consequences of consumer offense should be those in which prey can have negative effects on consumer fitness. Thus, we have chosen to investigate the ecosystem-level consequences of adaptation by *Daphnia pulicaria*, a consumer that adapts to prey that are dangerous (toxic cyanobacteria) (Hairston et al. 1999; Sarnelle and Wilson 2005). *Daphnia* is well-known to have dramatic effects on the total biomass and productivity of primary producers (phytoplankton), with effects being larger in more productive systems (Sarnelle 1992; Mazumder 1994). Indeed, the presence of *Daphnia* is essential for a strong trophic cascade in lakes (Leibold 1989; Carpenter et al. 1985).

Currently, the few experiments that have measured the effects of intraspecific variation in consumer traits on ecosystem structure or function have reported effects that are statistically significant but smaller than the effects of species presence/absence (Post et al. 2008; Bassar et al. 2010; Walsh et al. 2012; Pennisi 2012; Weis and Post 2013). In none of these studies, however, was the consumer adaptation offensive in nature. We asked whether the effect of different *D. pulicaria* genotypes having contrasting offensive ability (i.e., tolerant or sensitive to toxic prey; Appendices A, B; Table B1) was as large as the effect of *D. pulicaria* presence/absence, with a field experiment conducted in an environment with high concentrations of toxic cyanobacteria.

Methods

We assessed the effects of sensitive versus tolerant *D. pulicaria* genotypes on ecosystem structure and function (phytoplankton biomass and productivity) with a limnocorral experiment. We then conducted smaller-scale experiments to determine whether 1) toxic cyanobacteria were driving the poor performance of sensitive genotypes (cubitainer experiment) and 2) to compare the relative abilities of sensitive and tolerant genotypes to graze on phytoplankton (phytoplankton mortality experiment).

Limnocorral experiment

The experiment was conducted in limnocorrals (3,100 L) suspended from a floating PVC frame anchored in an aquaculture pond at Auburn University (Auburn, AL). Limnocorrals were filled on 28 August 2012 ('day 1') by pumping water from a nearby highly enriched pond through a 75-µm mesh net to exclude any resident large zooplankton, including native *Daphnia*. Three treatments were established in the limnocorrals with four replicates of each: no-*Daphnia* control, sensitive *D. pulicaria*, and tolerant *D. pulicaria*. Two enclosures were damaged at the

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beginning of the experiment (1 control, 1 tolerant). As a result, there were only three replicates for the no-*Daphnia* control and tolerant *D. pulicaria* treatments.

To start the experiment, we stocked three sensitive or three tolerant *D. pulicaria* genotypes (in approximately equal proportions) into each corresponding limnocorral at very low densities (~0.05 animals L^{-1}). A low initial density insured that *Daphnia* populations would have to increase greatly to suppress phytoplankton biomass, as would be the case in any natural system. All enclosures were fertilized approximately biweekly with sodium nitrate ($NaNO_3$) and sodium phosphate (Na_2HPO_4) at a rate of 10% of the ambient total nitrogen (TN) and phosphorus (TP) concentrations (TN ~3500 µg L^{-1}, TP ~ 500 µg L^{-1}) to maintain high densities of toxic cyanobacteria. The experiment was terminated after *Daphnia* populations had stabilized (Fig. 1.1; 24 October 2012).

Depth-integrated water samples for total phytoplankton biomass (as chlorophyll *a*), phytoplankton species composition, microcystin, and macrozooplankton biomass and species composition were collected with a tube sampler (inside diameter = 51 mm, 0.5 m deep) weekly from 30 August 2012 to 24 October 2012 and analyzed using standard limnological protocols (Chislock et al. 2013; Appendix A). We measured diel oxygen flux (measurements made at dawn, dusk, and the following dawn) at the beginning (there were no pre-treatment differences; ANOVA: $F_{2,7} = 0.192$, $P = 0.829$) and end of the experiment to estimate gross primary productivity (GPP; Wojdak 2005). We used repeated measures analysis of variance (ANOVA, sampling date = repeated measure) across all dates except the pre-treatment sampling to compare *D. pulicaria* biomass, chlorophyll *a*, and microcystin concentrations for all treatments. We also used ANOVA to compare treatment effects on GPP at the conclusion of the experiment.

Cubitainer experiment

To verify that the relatively poor performance of sensitive *D. pulicaria* genotypes (Fig. 1.1) was driven by the phytoplankton rather than some other environmental factor, we conducted a small-scale field experiment toward the end of the limnocorral experiment. Juveniles of the same sensitive *D. pulicaria* genotypes were stocked into replicate 5-L cubitainers filled with either unaltered limnocorral water ('pond water') or limnocorral water from which all phytoplankton were removed and replaced with a comparable biomass of the highly nutritious phytoplankter, *Ankistrodesmus falcatus* ('good food', Appendix A). Microcystin concentrations (< 35 µm) for the 'good food' and 'pond water' treatments were ~0.01 and 3.0 µg L^{-1}, respectively, at the start of the experiment. Cubitainers were incubated in the pond for 8 days at which point all *D. pulicaria* were counted, measured, and scored for fecundity (Appendix A).

Phytoplankton mortality experiment

We conducted a 24-hour grazing experiment at the end of the limnocorral experiment to compare the abilities of adult sensitive and tolerant *D. pulicaria* genotypes to inflict mortality on the phytoplankton present in the limnocorrals (Appendix A). Three treatments were established in replicate 100-mL glass bottles: no-*Daphnia* control, sensitive *D. pulicaria*, and tolerant *D. pulicaria*. Bottles were incubated for 24 hours in the dark on a plankton wheel in a climate controlled room (25ºC), and we used ANOVA to compare treatment effects on chlorophyll *a* at the conclusion of the experiment.

Results

Limnocorral experiment

At the start of the experiment, enclosures contained high levels of toxic cyanobacteria and the cyanobacterial toxin microcystin (Fig. 1.1C), with the genera *Oscillatoria*, *Microcystis*, and

Cylindrospermopsis accounting for >98% of total phytoplankton biomass. All three of these genera are known producers of microcystin (Carmichael 1992). Tolerant *D. pulicaria* increased more rapidly than sensitive genotypes and equilibrated at about 10-fold higher biomass (Tukey's test: $P < 0.0001$; Fig. 1.1). As a consequence of higher *D. pulicaria* biomass, total phytoplankton biomass (as chlorophyll *a*) during the last three weeks of the experiment was ~95% lower in the tolerant-*Daphnia* treatment than in either the no-*Daphnia* control (Tukey's test: $P < 0.002$) or the sensitive-*Daphnia* treatments ($P < 0.0001$). Phytoplankton biomass in the sensitive-*Daphnia* treatment was not significantly different from controls ($P > 0.500$). By the conclusion of the experiment, tolerant *Daphnia* had suppressed the biomass of all the phytoplankton (Appendix C - Fig. C1) relative to the other two treatments and resulted in a shift in phytoplankton species composition towards cryptophytes and cyanobacteria (Appendix C - Fig. C1). The effect of tolerant *Daphnia* on phytoplankton biomass was as large as effects seen in previous *Daphnia* manipulations at comparable productivity levels (Sarnelle 1992). Thus, the magnitude of the *Daphnia*-genotype effect on primary producer biomass in our experiment was essentially the same as the effect of *Daphnia* presence/absence. The response of gross primary production was analogous to that of primary producer biomass (Fig. 1.2), although smaller in overall magnitude likely due to higher periphyton growth on limnocorral walls in the tolerant-*Daphnia* treatment (since light penetration was higher).

Cubitainer experiment

Survival and fecundity of sensitive *D. pulicaria* were both much lower in cubitainers containing unaltered limnocorral water relative to the treatment with *Ankistrodesmus* (Fig. 1.3). Thus, the phytoplankton in the limnocorrals was highly inhibitory to neonates of the sensitive genotypes, which can explain the relatively poor performance of sensitive *D. pulicaria* in the

limnocorrals. Sensitive neonates showed extremely poor survival in the small-scale experiment (Fig. 1.3), yet in the limnocorrals there was some population growth by sensitive genotypes (although much lower than by tolerant genotypes) despite high concentrations of toxic cyanobacteria. This contrast probably stemmed from the fact that the initial inocula in the limnocorrals contained adults, combined with the demonstrated ability of adult *Daphnia* to phenotypically acclimate to toxic cyanobacteria and transfer some degree of tolerance to their offspring (Gustafsson et al. 2005).

Phytoplankton mortality experiment

Interestingly, the inability of sensitive genotypes to suppress phytoplankton biomass in the presence of toxic cyanobacteria (Fig. 1.1) did not seem to be driven by an inability of adults to graze on the phytoplankton in the limnocorrals. Sensitive and tolerant *D. pulicaria* genotypes inflicted statistically indistinguishable mortality on the phytoplankton as a whole, reducing chlorophyll *a* by >75% (Fig. 1.4). Thus, the inability of sensitive *Daphnia* to control phytoplankton biomass was probably more the result of poor neonate survival (and perhaps reduced fecundity) leading to reduced population growth, not an inability of individuals to kill toxic phytoplankton. In support of this conclusion, another limnocorral experiment conducted in a productive pond with low levels of toxic cyanobacteria showed no differences in the ability of these same *D. pulicaria* genotypes to increase and greatly suppress phytoplankton biomass (Appendix A; Appendix D - Fig. D1).

Discussion

Our results may help to explain an apparent paradox with respect to phytoplankton responses to fertilization in low-nutrient systems versus responses to top-down manipulation in high nutrient systems. When released from the predation pressure of zooplanktivores, *Daphnia*

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typically strongly suppress cyanobacteria-dominated phytoplankton in high nutrient lakes (Lynch and Shapiro 1981; Shapiro and Wright 1984; Vanni 1984; Reinertsen et al. 1990; Sanni and Waervagen 1990; Vanni et al. 1990; Sarnelle 1993). In contrast, *Daphnia* often fail to prevent phytoplankton biomass from increasing when low-nutrient systems are experimentally enriched, with failure attributed to escape from *Daphnia* control by dangerous cyanobacteria (Carpenter et al. 1995; Brett and Goldman 1997; Carpenter et al. 2001; Ghadouani et al. 2003). This contrast might be explained by postulating the presence (or absence) of sensitive versus tolerant *Daphnia* genotypes in low- versus high- nutrient systems. Enrichment studies (and global change studies, in general) tend to measure short-term responses (Schindler et al. 2008; Carlsson et al. 2009) relative to the time scales of heritable adaptive change (Weider et al. 1997). The long-term responses of ecosystems to eutrophication (a major agent of global change) may depend not only on the presence of particular consumer species (such as *Daphnia*) but also on the evolution of consumer adaptations to overcome prey defenses. The response of primary producers to enrichment has been a major focus in food-web research (Leibold 1989; Sarnelle 1992; Mazumder 1994; Rosenzweig 1971; Oksanen et al. 1981; McCauley et al. 1989; Persson et al. 1988) but the role of consumer adaptation in regulating that response remains to be understood.

It has been suggested that intraspecific trait variation be incorporated into studies of species interactions only when that variation has large consequences for communities/ecosystems (Agrawal 2003). In our experiment, the effect of contrasting genotypes of a single species on ecosystem structure and function was of the same magnitude as the effect of presence/absence of that species. Thus, in this system, adaptation can be as important a driver of ecosystem dynamics as species identity. This result has major implications for community and ecosystem ecology since adaptation (particularly by consumers) is usually ignored in studies of food-web

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interactions. Our results add to accumulating evidence about the importance of adaptation in ecological dynamics and argue for more attention to adaptation in studies of species responses to global change (Hairston et al. 1999; Sarnelle and Wilson 2005; Lohbeck et al. 2012; Parmesan 2006).

Acknowledgments

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Figure Legends

Figure 1.1. Dynamics of (A) *Daphnia pulicaria* biomass, (B) chlorophyll *a*, and (C) <35 µm microcystin concentration. Data represent means ± one standard error. Open symbols denote controls (no *Daphnia*), gray symbols indicate *D. pulicaria* genotypes that are sensitive to toxic cyanobacteria, and black symbols indicate *D. pulicaria* genotypes that are tolerant to toxic cyanobacteria. ANOVA: (*Daphnia* biomass: $F_{2,7}$ = 313.927, $P < 0.0001$; chlorophyll *a*: $F_{2,7}$ = 32.378, $P < 0.0001$; <35 µm microcystin: $F_{2,7}$ = 1.640, $P > 0.250$).

Figure 1.2. Mean gross primary productivity (GPP) at the conclusion of the experiment (day 57), ±1 standard error. Lowercase letters indicate significant treatment differences using Tukey's multiple comparisons ($P < 0.05$). ANOVA: $F_{2,7}$ = 7.407, $P < 0.02$.

Figure 1.3. (A) Survival and (B) fecundity of *Daphnia pulicaria* genotypes sensitive to toxic cyanobacteria in mesocosms containing either pond water or pond water filtered to remove toxic cyanobacteria and replaced with a similar quantity of the nutritious green alga *Ankistrodesmus*. t-test: (survival: T_{14} = 8.203, $P < 0.0001$; fecundity: T_{14} = 10.820, $P < 0.0001$). Zero reproduction in the pond-water treatment was a consequence of zero neonates reaching adult size.

Figure 1.4. Chlorophyll *a* concentrations at the conclusion of the lab experiment comparing the ability of high densities (>300 L^{-1}) of cyanobacterial-sensitive versus cyanobacterial -tolerant *Daphnia pulicaria* to kill phytoplankton from the pond. Lowercase letters indicate significant treatment differences using Tukey's multiple comparisons ($P < 0.05$). ANOVA: $F_{2,18}$ = 111.580, $P < 0.0001$.

Figure 1.1
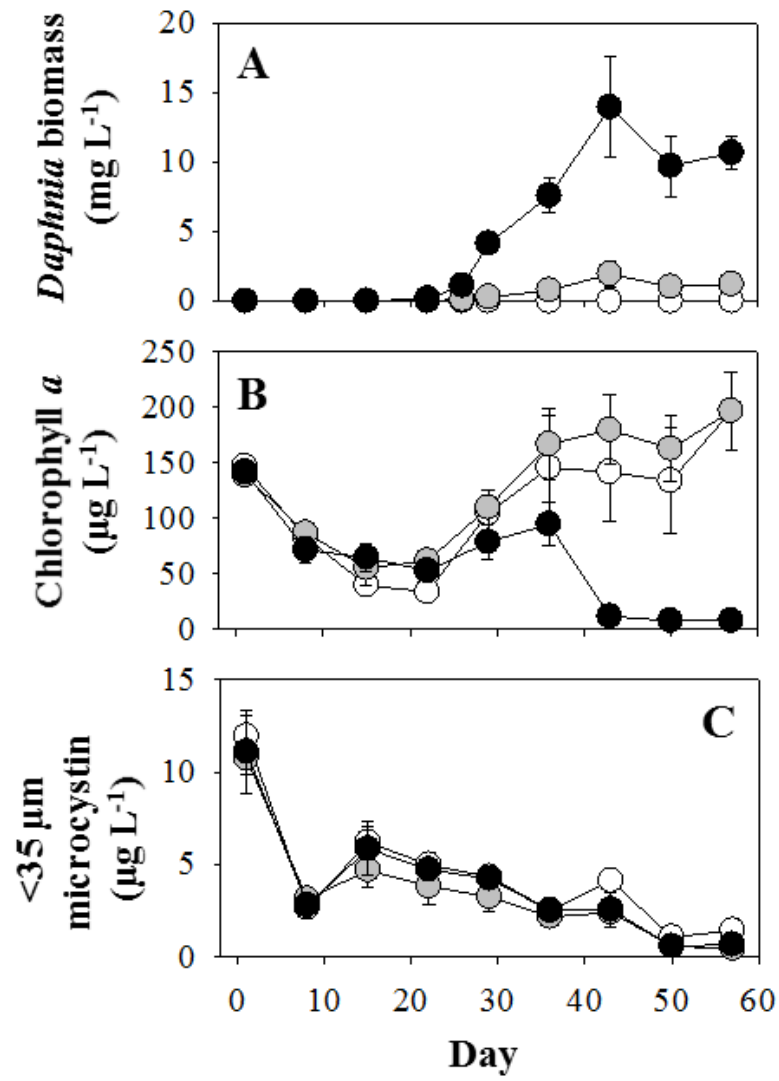
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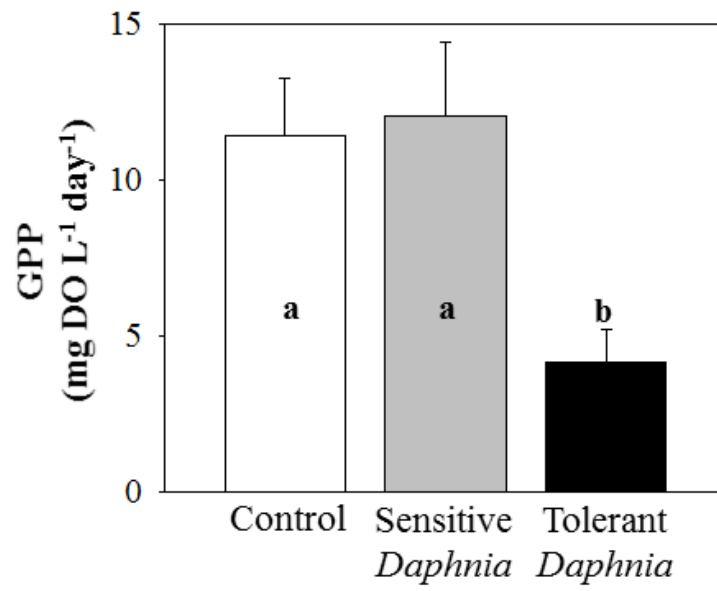
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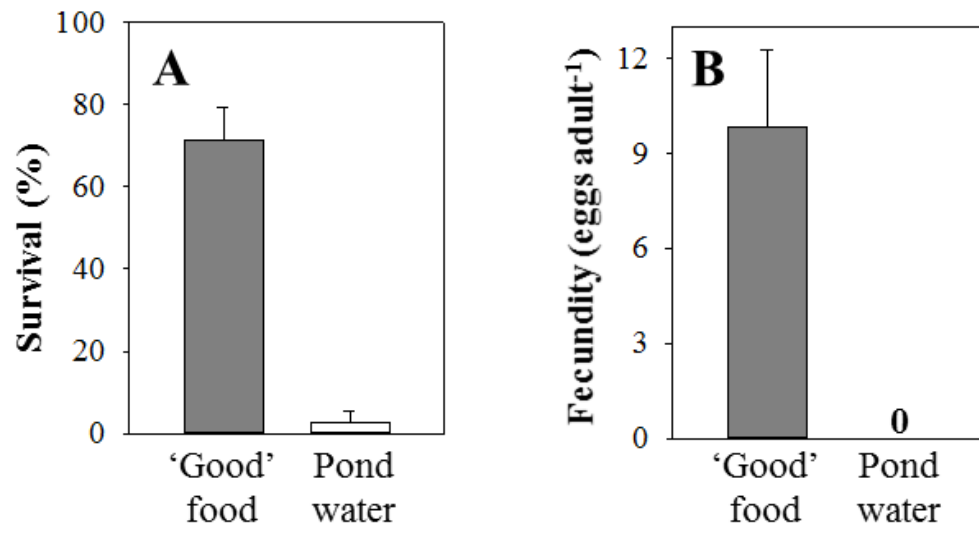
Figure 1.4
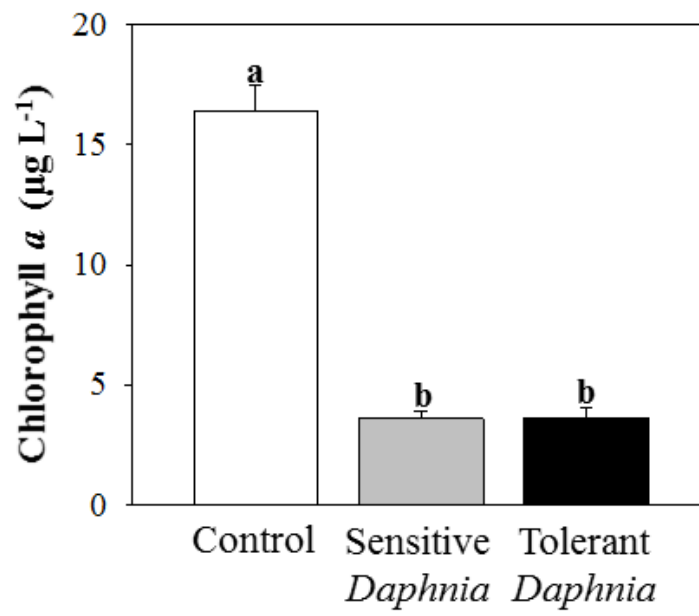
Chapter 2

Consumer adaptation mediates top-down regulation of ecosystems across a productivity gradient

Abstract

Humans have artificially enhanced the productivity of terrestrial and aquatic ecosystems on a global scale by increasing nutrient loading. While the consequences of eutrophication are well-known, the potential role of adaptation by organisms in stabilizing the response of ecological systems to such perturbations is unknown, since most studies tend to examine short-term responses relative to the time scales of heritable adaptive change. Here we test the hypothesis that adaptation by a generalist consumer (*Daphnia pulicaria*) to toxic prey (cyanobacteria) can mediate the response of lake ecosystems to nutrient enrichment. Overall, the effect of *Daphnia* on primary producer biomass increased with productivity. However, these effects were contingent on consumer genotype (i.e., tolerant vs sensitive to toxic cyanobacteria). Tolerant *Daphnia* strongly suppressed toxic cyanobacteria in nutrient-rich ponds, but sensitive *Daphnia* did not. Elucidating the role of organismal adaptation is critical for understanding and predicting ecosystem-level consequences of anthropogenic environmental perturbations.

Introduction

Human activities have had far-reaching effects on most ecosystems, and these effects are escalating. In particular, eutrophication is perhaps the most widespread of all human perturbations, and humans have artificially enhanced the productivity of ecosystems spanning the globe (i.e., cultural eutrophication) (Vitousek et al. 1997; Pimentel and Edwards 1982). Furthermore, cultural eutrophication has resulted in large-scale effects on biodiversity, species composition, and ecosystem function (Carpenter et al. 1998; Hautier et al. 2015; Isbell et al. 2013; Vitousek et al. 1997b). Although the long-term response of ecosystems to perturbations is

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likely to depend on the evolutionary adaptations of resident organisms, most studies of these effects tend to measure short-term responses relative to the time scales of heritable adaptive change. Consequently, we know little about how the response of communities and ecosystems to environmental perturbations is mediated by adaptation. Here we test the hypothesis that the long-term responses of ecosystems to eutrophication (a major agent of global change) may depend not only on the presence of particular consumer species but also on their adaptations that have evolved in response to past environmental changes.

The response of ecosystems to nutrient enrichment is affected by the interaction of bottom-up (resource) and top-down (consumer) control of primary productivity. Strong control of primary production and community structure by resources (e.g., nutrients) is well-documented (Schindler 1974). The importance of top-down control of ecosystems (Hairston et al. 1960) is illustrated by the dramatic cascading effects of top consumers across a wide variety of environments (Estes et al. 2011; Paine 1966), in addition to ecosystem regime shifts as a result of global declines in top consumers (Daskalov et al. 2007; Young et al. 2015). Consumers can sometimes dampen the positive response of primary producers to nutrient inputs (Hairston et al. 1960); however, complex interactions among resource availability, factors that deter herbivores (e.g., plant defenses), and herbivory are all known to regulate primary production and the distribution of plant biomass (Leibold et al. 1997; Oksanen et al. 1981; Polis 1999).

Recent research has highlighted a wide array of adaptations by species in response to global changes (Carlsson et al. 2009; Lohbeck et al. 2012; Parmesan 2006; Sarnelle and Wilson 2005; Urban et al. 2014), and shown that the ecological traits of many species can be quite malleable over short time scales (Grant and Grant 2002; Hairston et al. 1999). Contemporary evolution can also have important effects on species interactions, community and ecosystem dynamics, and the

feedback between ecology and evolution (Bassar et al. 2010; Pennisi 2012; Post et al. 2008; Walsh et al. 2012). Therefore, a fundamental question is whether intraspecific adaptive trait variation can in turn modulate the response of ecosystems to global change. Several studies have provided evidence that consumer evolution can have ecosystem-level consequences (Chislock et al. 2013; Urban 2013), even over short time scales (Harmon et al. 2009).

Globally, eutrophication is the leading cause of impairment of freshwater and coastal marine ecosystems (Smith and Schindler 2009). In freshwaters, nutrient-rich ecosystems are frequently dominated by bloom-forming cyanobacteria (i.e., blue-green algae), with many of these taxa producing toxic secondary metabolites that can poison fishes, pets, and humans (Carmichael 1992). The general paradigm is that these species are resistant to grazing by herbivorous zooplankton, thus preventing top-down control (Sommer et al. 1986). However, recent research has revealed that populations of the microcrustacean herbivore *Daphnia* can adapt in response to toxic cyanobacteria in their environment (Hairston et al. 1999; Hairston et al. 2001; Pennisi 2012; Sarnelle and Wilson 2005). *Daphnia* can have dramatic effects on algal biomass and ecosystem productivity and function in lentic environments (Carpenter et al. 1985; Chislock et al. 2013; Leibold 1989). In this paper, we focus on the effects of different *Daphnia* genotypes (i.e., tolerant or sensitive to toxic prey) (Appendix E) on algal biomass across a nutrient enrichment gradient (Mazumder 1994; Sarnelle 1992). *Daphnia pulicaria* genotypes sensitive and tolerant to toxic cyanobacteria were isolated from lakes with low and high levels of bloom-forming cyanobacteria during the summer, respectively (i.e., oligotrophic versus eutrophic lakes) (Appendix F - Table F1).

Methods

Here, we present the results from two sets of randomized field experiments conducted in the spring and fall in ten freshwater ponds that span a large productivity gradient as a result of cultural eutrophication. Across the experimental ponds, the prevalence of cyanobacteria varied both as a function of nutrient concentration and season. During the spring experiment, grazing-resistant cyanobacteria were rare across all ponds, while cyanobacteria were common in the fall experiment, particularly in the most nutrient-rich ponds (Appendix G – Table G1, Table G2). The experiments were designed to explicitly address (i) the effect of *D. pulicaria* on algal biomass across a productivity gradient; (ii) if effect size depended on *D. pulicaria* adaptation to toxic cyanobacteria; and (iii) whether the magnitude of these effects varied seasonally as a function of cyanobacterial presence. Three treatments were established in the enclosures (150 L) within each pond: no-*Daphnia* control (2 replicates), sensitive *D. pulicaria* (3 replicates), and tolerant *D. pulicaria* (3 replicates). We were interested in the effect of *Daphnia* on algal biomass (measured as chlorophyll *a*) at equilibrium (i.e., when *Daphnia* populations had stabilized), which can be defined as algal biomass in the absence of *Daphnia* divided by algal biomass with *Daphnia* (i.e., algal response factor; ARF) (Leibold et al. 1997; Sarnelle 1992) (Appendix E). Specifically, we hypothesized that consumer adaptation within *D. pulicaria* is a critical driver of total phytoplankton biomass in the presence of cyanobacteria, the dominant taxa causing harmful algal blooms in freshwaters impaired by eutrophication. Thus, we predict that ARF will be greater with tolerant *Daphnia* than with sensitive *Daphnia*

Results

Total phosphorus (TP) concentration across ponds ranged from 20 to 334 μg L^{-1}, with cyanobacteria and associated cyanotoxins being rare in the spring (Appendix G - Table G1) and

prevalent only in the fall (Appendix G - Table G2). In the spring experiments, the effect of *Daphnia* on algal biomass (ARF) ranged from ~2.2 to 161, was a positive function of TP, and was independent of *Daphnia* genotype (Figure 1A; Appendix H - Table H1). Furthermore, both tolerant and sensitive *D. pulicaria* increased rapidly in the spring experiments, with equilibrium biomasses proportional to TP (Figure 1B; Appendix H - Table H2).

Similar to the spring experiments, *Daphnia* effects on algal biomass also increased with TP in the fall (Figure 1C), but effects across the TP gradient were highly contingent on *D. pulicaria* genotype. Effects of the two *Daphnia* types were similar in magnitude at low TP (TP < 22 µg L^{-1}) but tolerant *D. pulicaria* genotypes had up to a 10-fold larger effect than sensitive genotypes at high TP (Appendix H - Table H3). The increasingly larger effects of tolerant *D. pulicaria* with increasing TP were the direct result of higher biomass of tolerant *D. pulicaria* (Figure 1D; Appendix H - Table H4). The results of these experiments clearly indicate that *Daphnia* adaptation, resulting from prior natural selection for tolerance to cyanobacteria, can have a large effect on the abundance of primary producers across a productivity gradient, and that the genotype effect is a function of phytoplankton composition.

Discussion

Lower biomass of sensitive *D. pulicaria*, and hence their inability to control phytoplankton biomass, in the fall was likely a direct consequence of reduced fecundity and neonate survival in the presence of grazing-resistant, toxic prey (Chislock et al. 2013). In ponds with extreme levels of toxic cyanobacteria and their cyanobacterial toxin, microcystin, the effect of tolerant versus sensitive *D. pulicaria* genotypes becomes as large as the effect of *D. pulicaria* presence/absence (Harmon et al. 2009). In general, overall *Daphnia* effects in the fall (ARF: 0.5 – 22) were nearly an order of magnitude lower than in the spring (2 – 166). There are at least two potential non-

mutually exclusive explanations for lower *Daphnia* effect sizes in the fall. First, if at a given TP concentration, total phytoplankton biomass in the absence of *Daphnia* is lower in the fall, this could account for smaller effects. However, phytoplankton biomass at a given TP concentration was comparable for the spring and fall, thus we found no support for this mechanism. Secondly, grazing-resistant taxa were more abundant in the fall, and some cyanobacterial filaments and colonies are too large to be effectively consumed by *Daphnia*. While we found no effects of *D. pulicaria* genotype on the relative abundance of phytoplankton taxa, mean *Microcystis* colony size was significantly larger in the presence of tolerant *D. pulicaria* than in either of the other two treatments in four of the five ponds (Appendix I - Table I1, Table I2). A similar pattern was also observed for mean *Cylindrospermopsis* filament lengths in the two ponds where this taxon was present. These data suggest that tolerant *D. pulicaria* can promote larger colony size by grazing down smaller colonies.

Previous studies have reached contrasting conclusions regarding the magnitude of consumer effects as productivity increases. Simple predator-prey models predict that the magnitude of consumer effects should increase with productivity, and these predictions have been supported by manipulative experiments in lentic environments (Sarnelle 1992). However, several other studies have suggested that consumer effects should be largest at low productivity, with weaker effects at higher productivities due to species turnover and changes in prey composition favoring resistant taxa (Chase et al. 2000; Hatton et al. 2015; Leibold et al. 1997). We found that the magnitude of top-down control is a function of prey species composition, with weakened effects when grazing-resistant taxa were abundant. However, we also found that consumer effects tended to increase with productivity, with the magnitude of consumer effects being mediated by consumer adaptation to resistant prey. While the general importance of prey compositional shifts

versus consumer adaptation in regulating top-down effects is subject to debate, our data suggest that both can have effects similar in magnitude.

The study of prey resistance to consumers has a rich history, and traditionally, much emphasis has been placed on understanding the role of prey adaptations that reduce consumption risk (i.e., prey defense) (Karban and Agrawal 2002; Rhoades 1985). Here, we demonstrate that the local adaptations by consumers to overcome these defenses (i.e., consumer offense) (Chislock et al. 2013; Hairston et al. 1999; Sarnelle and Wilson 2005) can mediate the magnitude of top-down effects by that consumer across a nutrient gradient. Past studies of consumer offenses have emphasized the importance of specialist herbivores (Karban and Agrawal 2002; Rhoades 1985). Our work contrasts with this previous research as we studied a generalist consumer and its offensive adaptation, which can lead to community- and ecosystem-level effects. Thus, adaptations by consumers cannot be ignored in studies of food web interactions and may play an increasingly important role, particularly in disturbed ecosystems. Rapid adaptive evolution by consumers may provide an important feedback mechanism mediating the net effects of large-scale environmental perturbations on community- and ecosystem-level processes (Hairston et al. 1999).

Acknowledgments

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Figure Legends

Figure 2.1. Relationship between total phosphorus (μg L^{-1}) and standardized *Daphnia pulicaria* effects on phytoplankton biomass as chlorophyll *a* (i.e., algal response factor) and *Daphnia* biomass (μg L^{-1}) for the spring experiment when cyanobacteria were rare (A, B) and fall experiment when cyanobacteria were common (C, D). Gray symbols indicate *D. pulicaria* genotypes that are sensitive to toxic cyanobacteria, and black symbols indicate *D. pulicaria* genotypes that are tolerant to toxic cyanobacteria. Effects are presented for the final two weeks of the experiment when *Daphnia* populations had stabilized. Separate regression lines are shown for sensitive and tolerant *D. pulicaria* in the spring (A, B) to facilitate comparison. However, there was no statistically significant effect of *Daphnia* genotype or the interaction between total phosphorus and genotype on the observed relationships in the spring experiment. Regression lines for sensitive *D. pulicaria* are included on both panels (C, D) for the fall experiment to facilitate comparison but were not statistically significant.

Figure 2.1
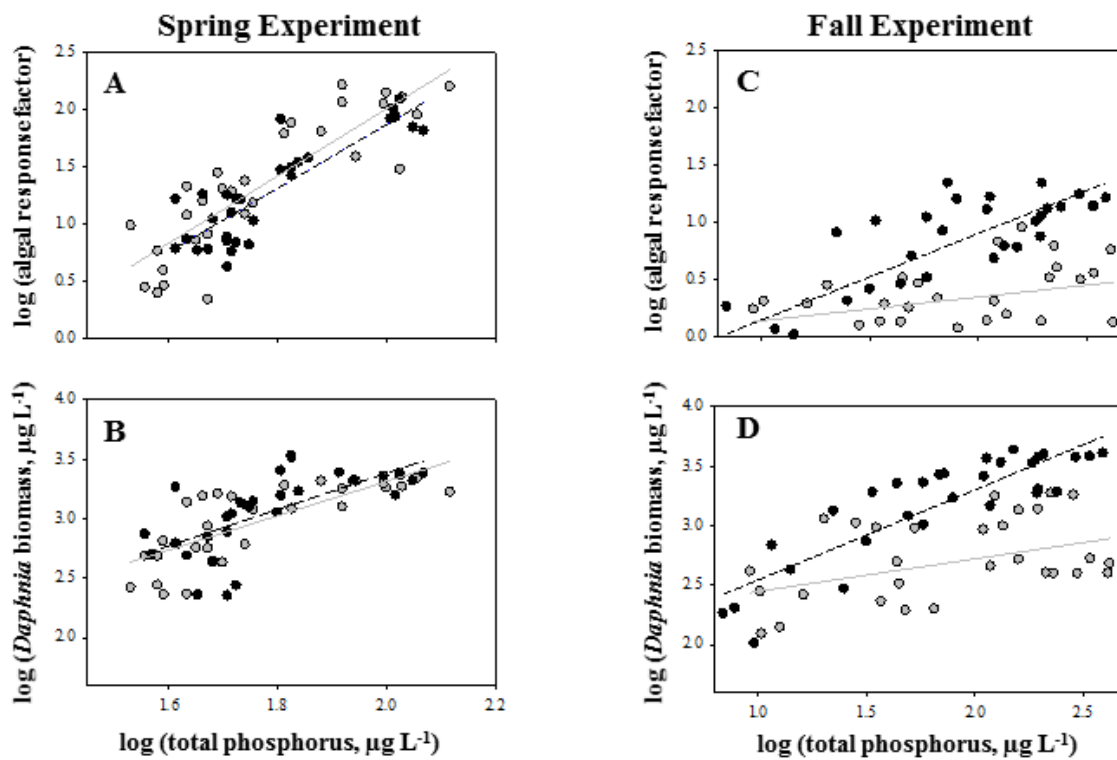
Chapter 3

Cylindrospermopsis raciborskii dominates under very low and high nitrogen-to-phosphorus

ratio: implications for its control

Abstract

In freshwater ecosystems, a variety of factors mediate phytoplankton community structure,

including herbivore community structure, light availability, temperature, mixing, and absolute

and relative nutrient concentrations (total nitrogen (TN), total phosphorus (TP)). Ecological

stoichiometry examines how the nutrient content of organisms and their environment may

mediate population-, community-, and ecosystem-level processes (Elser et al 2007). The

manipulation of N:P ratios is a widely regarded tool for managing phytoplankton species

composition given that nitrogen-fixing cyanobacteria should dominate algal communities under

low N:P ($< 64:1$, by atoms) given their ability to convert dissolved dinitrogen gas into organic

nitrogen. However, due to the physiological expense of nitrogen fixation, diazotrophs should be

outcompeted by non-nitrogen fixing phytoplankton under higher N:P when other environmental

factors are similar. We tested this hypothesis in a field experiment using 2500-L limnocorrals

installed in a eutrophic lake (ambient N:P ~40:1 (by atoms); TN ~1360 μg L^{-1}; TP ~75 μg L^{-1}).

At the start of the experiment, we randomly assigned limnocorrals among the ambient (40:1) and

low (7:1) or high (122:1) N:P treatments (n = 4 replicates/treatment), which were established by

adding P or N at the start of the experiment, respectively. The phytoplankton community in the

enclosures at the start of the experiment was diverse (i.e., 18 phytoplankton genera) and

dominated by chlorophytes (including *Coelastrum* and *Scenedesmus* (30% and 13% of total

biomass, respectively)) and cyanobacteria (including *Anabaena* and *Cylindrospermopsis* (23%

and 17% of total biomass, respectively)). In contrast to predictions based on ecological

stoichiometry, the phytoplankton community in all N:P treatments increased in abundance and was almost entirely comprised by the nitrogen-fixing cyanobacterium, *Cylindrospermopsis raciborskii*, by the conclusion of the study. Moreover, concentrations of the cyanobacterial neurotoxin, saxitoxin, were enhanced under the two highest N:P conditions. The ability of *C. raciborskii* to dominate phytoplankton communities under such extreme N:P shows that short-term management of nutrient stoichiometry is not likely to be effective for controlling blooms of this noxious cyanobacterium and may help to explain the rapid expansion of this invasive species to temperate latitudes.

Introduction

   Ecological stoichiometry, the study of the effects of the elemental composition of organisms and their environment on food web interactions and ecosystem function, has played an important role in basic and applied research by relating the physiology of organisms to ecosystem-level processes, such as biogeochemical cycles, nutrient recycling, and limiting nutrients (Sterner et al. 1992; Sterner and Elser 2002). In particular, the study of freshwater and marine phytoplankton has played a key role in the development of the field of ecological stoichiometry (Pearsall 1932; Tilman 1977; Klausmeier et al. 2008). One classical assumption in ecological stoichiometry studies of marine phytoplankton is the relative constancy of the carbon: nitrogen: phosphorus ratio (i.e., the Redfield ratio; carbon-to-nitrogen-to-phosphorus (C:N:P) = 106:16:1, by atoms), and the Redfield ratio is frequently used in understanding biogeochemical cycles of these elements and nutrient limitation in aquatic systems, in general (Redfield 1958; Healey and Hendzel 1980; Howarth 1988). Observational studies of freshwater lakes have shown that low nitrogen-to-phosphorus ratios (N:P < 64:1, by atoms) tend to favor dominance by nitrogen-fixing cyanobacteria (Schindler 1977; Smith 1983; Sterner et al. 1992; Downing et al. 2001; Ferber et

al. 2004; but see Nõges et al. 2008, Kosten et al. 2009) through their ability to convert dissolved dinitrogen gas into organic nitrogen. These findings are further supported by field N:P manipulations, which tended to study the effects of reduced N:P (Schindler 1974; Barica et al. 1980; Barica 1994; Paterson et al. 2002, Vrede et al. 2009; Finlay et al. 2010; Donald et al. 2011). Although other environmental factors are known to promote cyanobacterial dominance, such as reduced light penetration and turbulence, elevated temperature or pH, or small-bodied zooplankton (Ferber et al. 2004; Paerl and Paul 2012; Chislock et al. 2013), controlling nutrient supply and stoichiometry have been central foci of lake management and restoration efforts (Smith and Schindler 2009) given that eutrophication and concomitant cyanobacterial blooms are the leading cause of water quality impairment of many freshwater and coastal marine ecosystems around the world.

Human activities have accelerated the rate and extent of eutrophication through both point and non-point discharges of limiting nutrients, such as nitrogen and phosphorus (Carpenter 1998; Paerl et al. 2011; Paerl and Paul 2012), and numerous studies in the ecological literature demonstrate that eutrophication can be controlled by decreasing nutrient inputs (Edmondson 1970; Schindler 1974; Jeppesen et al. 2005). However, determining which nutrient is ultimately limiting algal biomass and primary productivity is often a point of contention for basic research and applied management decisions (Schindler et al. 2008; Conley et al. 2009; Paerl 2009; Scott and McCarthy 2010), especially considering that some systems are co-limited by both N and P (Elser et al. 2007). Numerous studies have documented the successful management and restoration of freshwater lakes affected by eutrophication by controlling phosphorus inputs, since the classic study by Schindler (1974). The emphasis on controlling eutrophication has been strongly influenced by the phosphorus (P)-limitation paradigm, as phosphorus can be readily

removed from pollution sources and possibly because this nutrient was first identified as the mechanism driving eutrophication (Paerl and Scott 2010). However, there has also been a recent (renewed) surge of interest and advocacy for nitrogen or dual nitrogen and phosphorus control in abatement efforts (Paerl and Scott 2010; Scott and McCarthy 2010).

Advocates of phosphorus control of eutrophication argue that phosphorus reductions also reduce harmful cyanobacterial blooms caused by nitrogen-fixing species by altering the N:P ratio in favor of other non-nitrogen fixing species (Barica et al. 1980; Tilman et al. 1982; McQueen and Lean 1987; Stockner and Shortread 1988; Jeppesen et al. 2005; Schindler et al. 2008). Alternatively, the P-limitation paradigm also predicts that blooms of N-fixing cyanobacteria are made worse if nitrogen levels are reduced without also decreasing phosphorus concentrations (Schindler 1977; Smith 1983). These predictions have received some support from lake survey data and whole-lake experiments (Barica et al. 1980; Findley and Kasian 1987; Schindler 1977; Schindler et al. 2008). Several studies have even advocated the addition of nitrogen fertilizers at the whole-lake scale to shift the N:P ratio in favor of more innocuous, non-nitrogen fixing species (Smith 1983). However, these predictions have rarely been tested using replicated, manipulative field experiments (but see Barica et al. 1980; Vrede et al. 2009; Finlay et al. 2010; Donald et al. 2011). Here we explicitly test the hypothesis that nitrogen-fixing cyanobacteria should dominate under low N:P (< 64:1, by atoms) but are outcompeted by non-nitrogen fixing species under high N:P using a limnocorral experiment in a eutrophic pond.

Methods

During a 49-day field experiment, we manipulated N:P ratio in 2,500-L, clear polyethylene enclosures that were sealed at the bottom, open to the atmosphere, and suspended from a floating platform (EZ-Dock) anchored in Pond S1 at the Auburn University E.W. Shell Fisheries

Research Station in Auburn, Alabama. S1 is shallow (maximum depth = 3 m), polymictic, and eutrophic (TN ~1360 µg L^{-1}; TP ~75 µg L^{-1}, SRP ~9 µg L^{-1}; N:P ~40:1, by atoms, Secchi depth ~0.5 m). Twelve enclosures were filled on 11 June 2010 ('day 1') by pumping water through a 60-µm mesh net to exclude fish and large zooplankton. We then randomly assigned limnocorrals to each of the three N:P treatments: (1) ambient (40:1, by atoms), (2) low (7:1), and (3) high (122:1) with four replicates per treatment. The high N:P treatment was almost double the N:P threshold observed in Smith (1983). Ambient enclosures received no initial nutrient additions, low N:P enclosures were fertilized with sodium phosphate to establish an N:P ratio of 7:1, and sodium nitrate was added to high N:P enclosures to create an N:P ratio of 122:1. Given that this field experiment aimed to test the importance of three N:P ratios on phytoplankton community structure, we attempted to maintain treatment N:P using weekly fertilizations at a rate of 10% of the initial mean TN and TP concentration for their corresponding N:P treatment (Fig. 3.1). Thus, the low and ambient N:P treatments received the same weekly N additions, and the ambient and high N:P treatments received the same weekly P additions. Soluble reactive phosphorus (SRP) was readily available in the low N:P treatment over the course of the experiment but <5 µg L^{-1} in the ambient and high N:P treatments (Fig. 3.1D). In contrast, nitrogen fixation occurred in the ambient and low N:P treatments (Fig. 3.1B; 3.4B). Thus, despite the high TN and TP in this eutrophic pond, contrasting nutrients were likely limiting in the three N:P ratio treatments over the course of the experiment.

We sampled the pond and enclosures approximately biweekly from 11 June to 29 July 2010. To avoid contaminating enclosures, Secchi depth was recorded and temperature was measured across depth (0.2 to 2.0 m) in the pond next to the enclosures during each sampling event. Secchi depth in the pond varied from 0.4 to 0.6 m during the experiment. Although transparency was

not directly measured in the enclosures, chlorophyll *a* concentrations in the pond declined from 55 to 44 µg L^{-1} during the experiment while chlorophyll increased 4 to 6 fold in the enclosures by the conclusion of the experiment (data not shown). At such dense chlorophyll *a* concentrations, estimated Secchi depths for the enclosures at the conclusion of the experiment ranged from 0.24 to 0.30 m in the N:P treatments (Appendix K - Fig. K1; A.E. Wilson unpublished data). Light is important for phytoplankton stoichiometry (Sterner et al. 1997; Hessen 2006), and phytoplankton were clearly light limited in the enclosures during the experiment (Appendix K - Fig. K2; Appendix L) with the lowest mean transparency being estimated for the high N:P treatment (Appendix K - Fig. K2).

Depth-integrated water samples for nutrients (TN, TP, and SRP), total phytoplankton biomass and species composition, microcystin, saxitoxin, cylindrospermopsin, and zooplankton biomass and species composition were collected with a tube sampler (inside diameter = 51 mm). TN, TP, and SRP were analyzed using standard methods (colorimetric assays for TP/SRP; ultraviolet spectrophotometry for TN; Gross and Boyd 1998). Microcystin, saxitoxin, and cylindrospermopsin 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 via the inverted microscope technique (Utermöhl 1958) using water samples preserved in 1% Lugol's solution. Biovolume for each species was calculated using cell counts (25-50 fields per magnification from 100x-1000x) and estimates of cell volume based on measurements of cell dimensions (400x-1000x). We then converted biovolume (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 0.4 (Riemann et al. 1989; Sarnelle et al. 2005; Knoll et al. 2008). *Cylindrospermopsis raciborskii* was primarily

observed as straight, heterocystic filaments and was enumerated as individual filaments given faint to nonexistent cell divisions in each filament. Zooplankton 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 dominant taxa and species (Culver et al. 1985). Given that the pond was not fertilized weekly like the enclosures, pond water quality dynamics and community structure varied significantly from all N:P treatments and are not further discussed relative to dynamics observed in the enclosures.

The effects of N:P treatment on nutrient concentrations, phytoplankton and *C. raciborskii* dry biomass, microcystin, saxitoxin, and cylindrospermopsin concentrations were tested using repeated measures analysis of variance (ANOVA, factors = N:P treatment, sampling date = repeated measure) across all dates except the pre-treatment sampling. Mean phytoplankton biomass measured over time was compared across treatments using ANOVA and Tukey's multiple comparison test. Using arcsine-transformed data, we also used repeated measures ANOVA to compare the effects of N:P treatment on the relative abundance of *C. raciborskii* over time. We used a mass balance approach to compare the relative change in TN and TP across treatments from the beginning to the end of the experiment. We calculated the predicted TN and TP concentration for each treatment at the conclusion of the study based on the total amount of fertilizer added and initial nutrient concentrations. Relative change (%) in TN or TP was then calculated by subtracting the predicted TN or TP concentration at the conclusion of the experiment (day 49) from the actual TN or TP concentration on day 49, dividing by the predicted concentration, and multiplying by 100. We then calculated a 95% confidence interval for each treatment mean to compare the relative change to 0.

Results

   At the start of the experiment, the phytoplankton community was diverse (i.e., 18

phytoplankton genera) and comprised primarily of chlorophytes (~50%) and cyanobacteria

(~40%), with the N-fixing cyanobacterium, *Cylindrospermopsis raciborskii,* accounting for

~17% of total phytoplankton biomass (Appendix M - Fig. M1). Average initial phytoplankton

biomass was 3 mg L^{-1} and significantly increased in all three N:P treatments over time (6-16×

increase; Fig. 3.2A). Phytoplankton biomass was significantly higher in the high N:P treatment

(122:1) than for the other two treatments over the course of the experiment (Tukey's test: $P \leq$

0.008), with phytoplankton biomass being ~60% and 70% higher than in the 40:1 and 7:1

treatments, respectively, by the conclusion of the experiment (Fig. 3.2A). In contrast,

phytoplankton biomass was not significantly different between the 40:1 and 7:1 treatments

(Tukey's test: $P = 0.540$). By day 15, all N:P treatments were becoming dominated by *C.*

raciborskii (~40-75%), and, as expected for an N-fixing species, the low N:P treatment (7:1)

contained a higher absolute biomass of *C. raciborskii* than the other two N:P treatments (Fig.

3.2B). Similar patterns were obtained for *C. raciborskii* relative biomass (i.e., *C. raciborskii*

relative biomass was negatively related to N:P). However, regardless of N:P treatment, regular

nutrient additions resulted in a rapid loss of phytoplankton species diversity and complete

dominance by *C. raciborskii* (~99-100%) by the conclusion of the experiment (day 49, Fig.

3.2C).

   Interestingly, saxitoxin concentrations were elevated in the two highest N:P treatments ($F_{2,7} =$

50.83, $P = 0.0001$; Fig. 3.3A) and were significantly highest in the high N:P treatment (Tukey's

test: $P \leq 0.002$). Furthermore, mean saxitoxin content at the conclusion of the experiment,

expressed as a percentage of total phytoplankton dry biomass, was nearly an order of magnitude

higher at high N:P than for the other two N:P treatments. In contrast, N:P treatment had no effect

on microcystin concentration ($F_{2,8} = 0.90$, $P = 0.44$) (Fig. 3.3B). *Anabaena* was the only other

abundant, toxigenic cyanobacterium present during the experiment, and *Anabaena* abundance

followed declining microcystin production over time. Cylindrospermopsin concentrations were

below detection limit over the course of the experiment, regardless of treatment.

Although N:P ratio was maintained with weekly nutrient additions in the high (122:1) N:P

treatment (+1% change from start to end), large increases in N:P were observed in the two lower

N:P treatments (7 N:P = +69% change; 40 N:P = +97% change) during the 49-day study (Fig.

3.1A). Mass balance calculations showed large P losses in all three treatments (range = -19 – -

34%) (Fig. 3.4A) and a loss of N in the high N:P treatment (-19%) (Fig. 3.4B). Interestingly, the

two treatments with limiting N showed large N gains (+92-114%), presumably through nitrogen

fixation (Fig. 3.4B).

Discussion

While controlling phosphorus has played a central role in the management of eutrophication

in lakes, limnologists continue to argue whether nitrogen inputs should be controlled as well as

(or even instead) of phosphorus (Schindler et al. 2008). Smith (1982, 1983) used resource-based

competition theory to support the hypothesis that alterations of N:P ratio in lakes can have strong

effects on phytoplankton biomass and species composition. In particular, Smith (1983)

hypothesized that nitrogen-fixing cyanobacteria dominate systems with low N:P (< 64:1, by

atoms), but lose their competitive advantage at higher N:P when nitrogen is readily available.

While controlling nutrient supply and stoichiometry have been central to decades of lake

management and restoration efforts, the importance of stoichiometry for controlling

cyanobacteria has typically only been evaluated using short-term, laboratory bioassays (reviewed

by Lewis and Wurtsbaugh 2008) or large-scale lake surveys (Downing et al. 2001; Smith 1983) and has been less tested via manipulative field experiments (but see Barica et al. 1980; Stockner and Shortreed 1988; Paterson et al. 2002; Vrede et al. 2009; Finlay et al. 2010; Donald et al. 2011). In our experiment, the phytoplankton community at the conclusion of the experiment was almost entirely comprised by the nitrogen-fixing cyanobacterium, *C. raciborskii*, regardless of N:P treatment. Thus, we suggest that short-term management of nutrient stoichiometry is not likely to be effective for improving water quality and controlling blooms of this noxious cyanobacterium.

A large literature shows that phytoplankton abundance increases with concentrations of total phosphorus (Jones et al. 1998; Brown et al. 2000; Schindler et al. 2008), total nitrogen (Paerl 1988, 2009; Finlay et al. 2010), or both total nitrogen and total phosphorus (Downing et al. 2001; Dolman et al. 2012). Similarly, absolute and/or relative abundance of cyanobacteria have also been shown to increase with nutrients (Watson et al. 1997; Downing et al. 2001; Elser et al. 2007; Vrede et al. 2009; Finlay et al. 2010; Millie et al. 2011; Donald et al. 2011). While N:P stoichiometry has played a central role in lake management, whether cyanobacterial abundance is more strongly affected by TP, TN, or N:P is poorly understood (Smith 1983; Downing et al. 2001; Catherine et al. 2008). In particular, stoichiometric effects on phytoplankton composition are expected under nutrient-stressed conditions (i.e., when nutrient concentrations decrease below phytoplankton cell quotas), thus affecting the growth and response of organisms but are likely less important if the total amount of nutrients is high. In our experiment, weekly nutrient fertilizations promoted algal growth in all N:P treatments, with the highest phytoplankton biomass in the high N:P treatment at the conclusion of the experiment. SRP was low in two of the treatments (ambient and high). Furthermore, we found large increases of N in the ambient

and low N:P treatments, presumably through nitrogen fixation. Thus, we observed evidence of phosphorus versus nitrogen limitation in our contrasting N:P treatments despite the elevated total nutrient concentrations in our study system. Our results, combined with the documented ability of *C. raciborskii* to tolerate wide variations in nutrient availability (Isvanovics et al. 2000; Moisander et al. 2012), suggest that controlling nitrogen and phosphorus (i.e., dual control) may be necessary to reduce phytoplankton biomass, when this species is prevalent (Isvanovics et al. 2000). Future experiments simultaneously manipulating N:P ratio and nutrient concentrations are necessary to determine the potential interaction between these factors for phytoplankton community structure.

Given the ability of many cyanobacterial species to fix atmospheric nitrogen and that many species are thought to be poor competitors for inorganic phosphorus (Tilman et al. 1982; Stockner and Shortreed 1988), the results of our experiment did not agree with theoretical predictions as N:P had no effect on phytoplankton composition. Previous manipulative experiments have found that increased N:P ratio may actually favor cyanobacterial species lacking the ability to fix nitrogen (Barica et al. 1980; Finlay et al. 2010) (e.g., *Merismopedia*, *Phormidium*, *Microcystis*) but capable of producing toxins (Finlay et al. 2010; Donald et al. 2011). However, in our experiment, *C. raciborskii* accounted for ~100% of total phytoplankton biomass under very low (7:1) and very high (122:1) N:P ratios, and microcystin concentrations decreased and saxitoxin concentrations increased over time. While prior studies suggest that N:P can be important for structuring phytoplankton communities, our results indicate that *C. raciborskii* presence may supersede N:P influence.

Indeed, blooms of *C. raciborskii* are becoming increasingly prevalent in tropical, subtropical, and temperate systems around the world (Padisák 1997). The rapidly expanding geographical

range of this invasive phytoplankton species, potential ecosystem-level effects, and human health concerns (Chorus and Bartram 1999) have stimulated much recent ecological interest in *C. raciborskii* (Fig. 3.5). In general, ecologists have attributed the success of *C. raciborskii* to resistance to grazing by zooplankton (Panosso et al. 2003), high P uptake and storage capacity (Isvanovics et al. 2000), high ammonium (and nitrate) uptake affinity (Presing et al. 1996), tolerance of low light levels (Briand et al. 2004; de Tezanos Pinto and Litchman 2010), ability to fix nitrogen (Moisander et al. 2012), and tolerance of a wide range of temperatures (including temperatures in excess of 30ºC, Butterwick et al. 2005; Bonilla et al. 2011). We did not measure light attenuation in the enclosures to avoid contamination. However, light availability was minimal given the dense algal biomass in the enclosures (Figs. 3.2A,B; Appendix K - Fig. K1,2). Moreover, calanoid copepods dominated the zooplankton community in our experiment, regardless of N:P treatment (data not shown). Given the inability of calanoid copepods to reduce the biomass of several species of cyanobacteria, including *C. raciborskii*, in laboratory feeding assays (Panosso et al. 2003), it is likely that the grazing resistance and tolerance of high water temperature (Fig. 3.6) or low light by *C. raciborskii* mediated the effects of N:P in our study. Future experiments are encouraged to test these hypotheses.

Acknowledgments

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Figure Legends

Figure 3.1. Dynamics of (A) N:P ratio (by atoms), (B) total nitrogen (TN), (C) total phosphorus (TP), and (D) soluble reactive phosphorus (SRP) over the 49-day experiment. Data represent means ± one standard error. Open symbols denote the ambient (40:1, by atoms) N:P treatment, gray symbols indicate the low (7:1) N:P treatment, and closed symbols indicate the high (122:1) N:P treatment.

Figure 3.2. Dynamics of (A) phytoplankton dry biomass, (B) *Cylindrospermopsis raciborskii* dry biomass, and (C) relative biomass (%) of *C. raciborskii* over the 49-day experiment. Data represent means ± one standard error. Open symbols denote the ambient (40:1, by atoms) N:P treatment, gray symbols indicate the low (7:1) N:P treatment, and closed symbols indicate the high (122:1) N:P treatment.

Figure 3.3. Dynamics of the cyanotoxins, microcystin (A) and saxitoxin (B), during the 49-day experiment. Data represent means ± one standard error.

Figure 3.4. Relative change in (A) total phosphorus (TP) and (B) total nitrogen (TN) from the beginning to the end of the experiment. Using a mass balance approach, we calculated the predicted TN and TP concentration for each treatment at the conclusion of the study based on the total amount of fertilizer added and initial nutrient concentrations. Relative change in TN or TP was calculated by subtracting the predicted TN or TP concentration at the conclusion of the experiment (day 49) from the actual TN or TP concentration on day 49 and then dividing by the predicted concentration. Data represent means ± 95% confidence interval (CI). If the 95% CI does not include 0, there was a significant change in nutrient concentration.

Figure 3.5. Number of "*Cylindrospermopsis*" publications listed in ISI Web of Science from 1980 to 2011.

Figure 3.6. Water temperature dynamics in the pond over the 49-day experiment. Closed circles denote water temperature at 0.2 m, gray circles indicate water temperature at 0.5 m, open circles indicate water temperature at 1 m, gray triangles indicate water temperature at 1.5 m, and closed triangles indicate water temperature at 2.0 m.

Figure 3.1
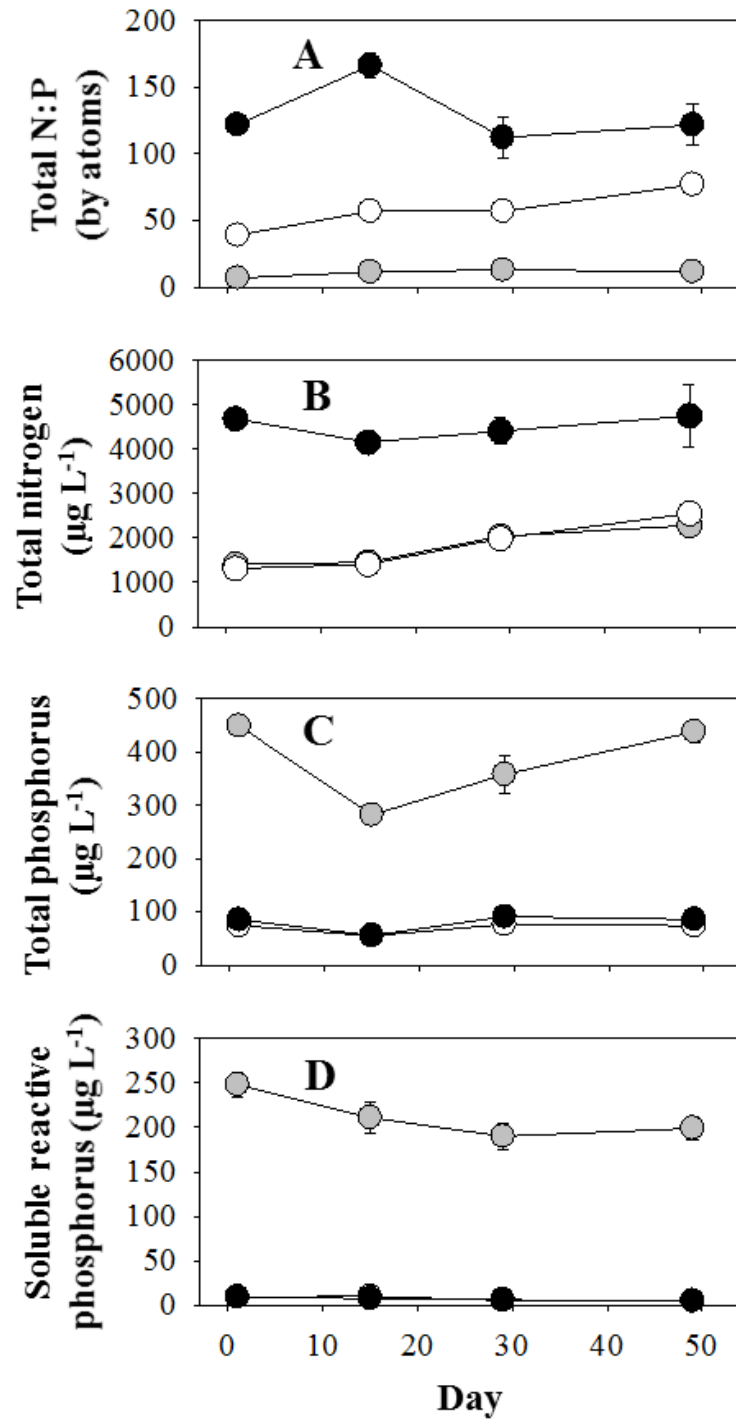
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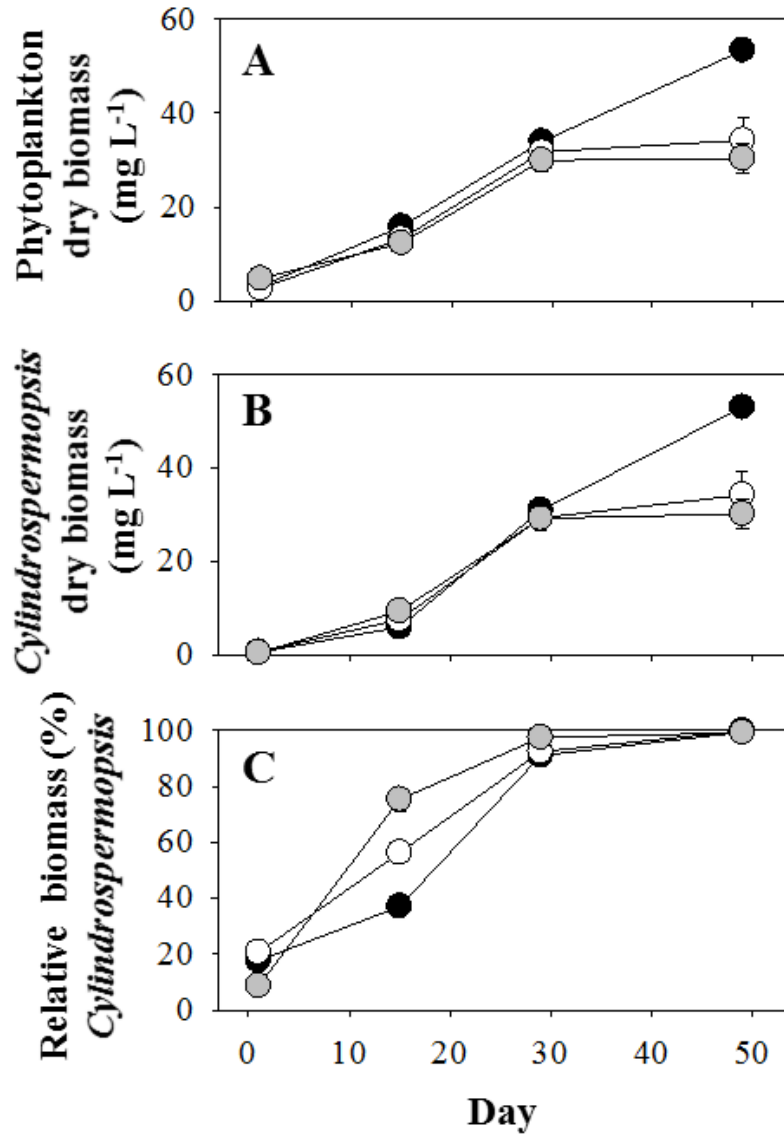
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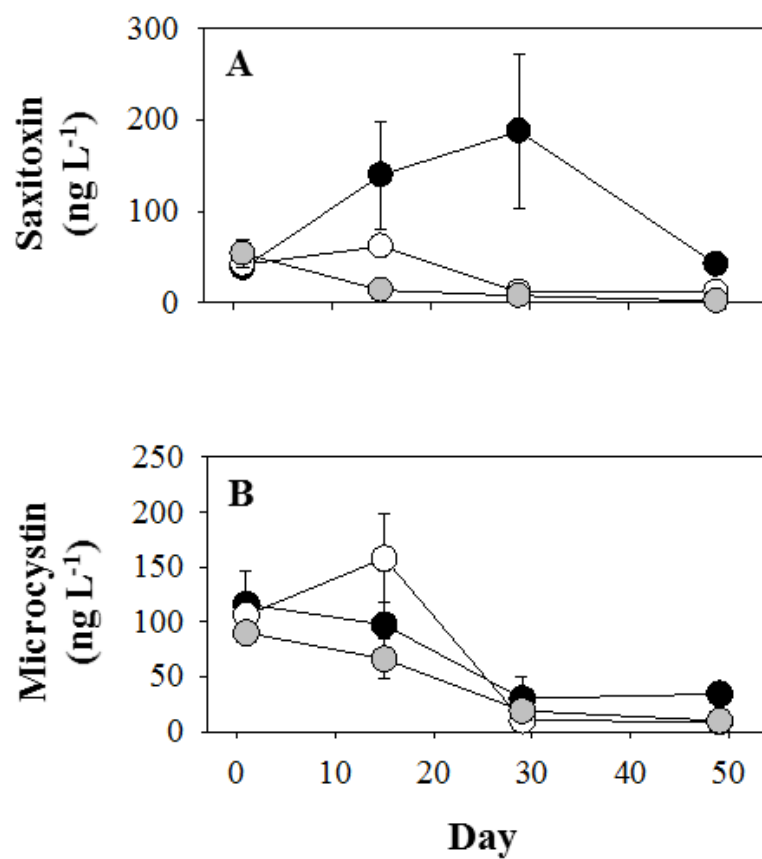
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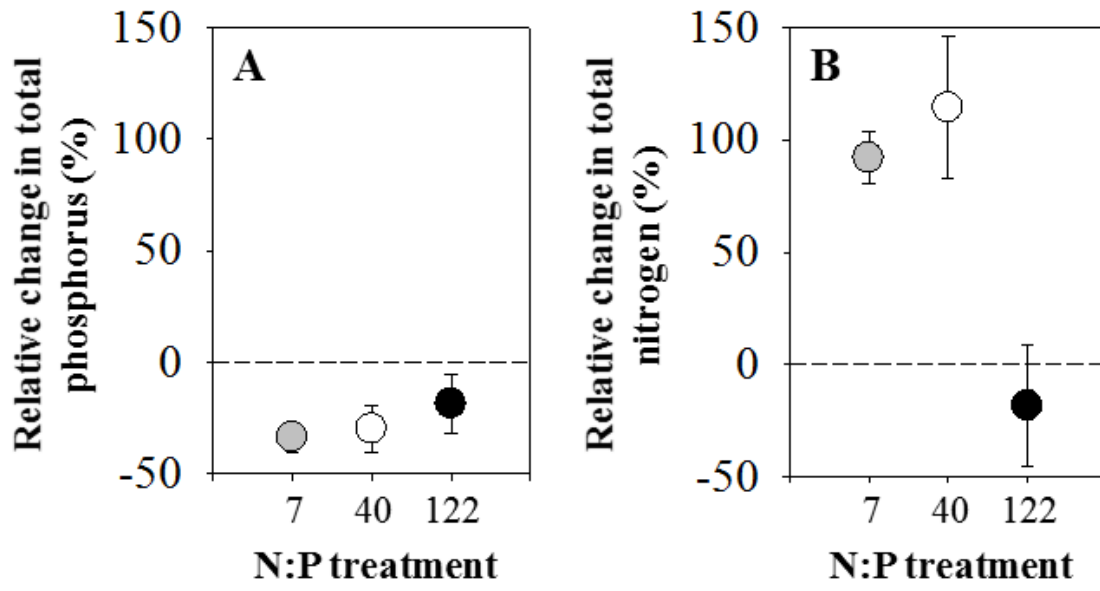
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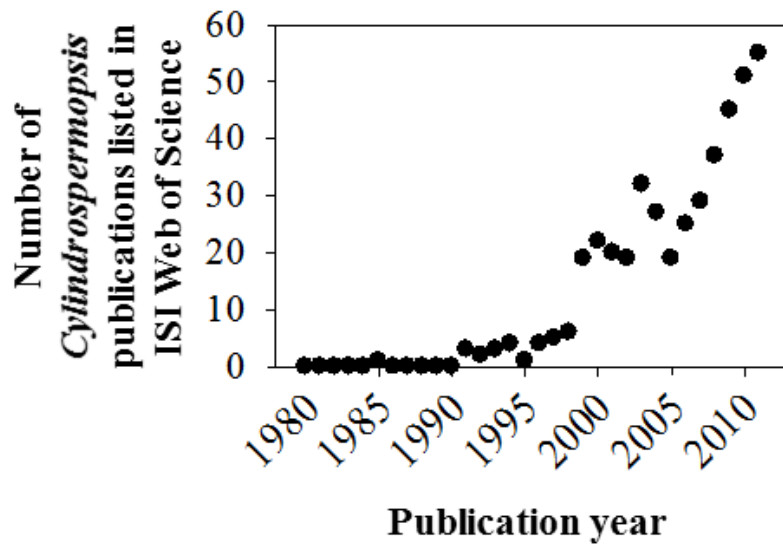
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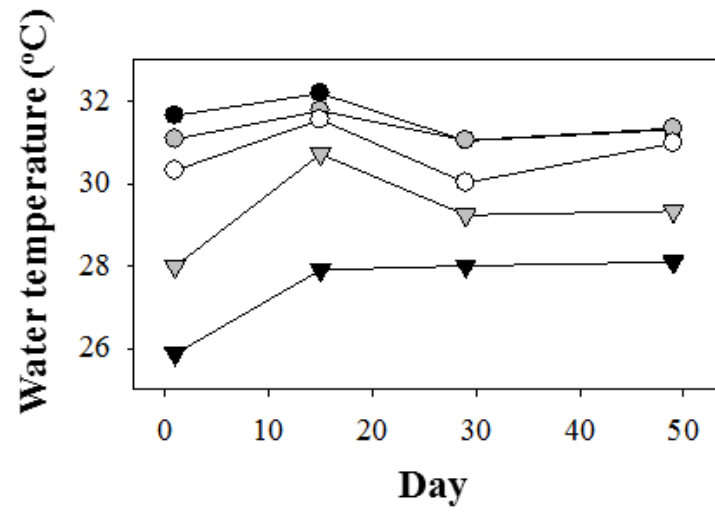
Chapter 4

Intraspecific variation in lake *Daphnia* affects the response of phytoplankton biomass and the

neurotoxin, saxitoxin, to fertilization

Abstract

Primary producer biomass can be affected by bottom-up (e.g., nutrient) and top-down (e.g.,

herbivore) effects. Reducing inputs of nutrients has been widely successful in controlling

eutrophication in disparate ecological systems. However, biomanipulation efforts to enhance

herbivory have had mixed effects, and ecologists have suggested numerous potential

mechanisms to explain these results, including the presence of well-defended, resistant, and/or

toxic prey species. In particular, food web manipulations in lentic freshwater environments to

enhance grazing by large-bodied zooplankton (i.e., *Daphnia*) are not always successful at

reducing phytoplankton biomass, and blooms of grazing-resistant and toxic species of

cyanobacteria (i.e., blue-green algae) are thought to be a deterrent to the ultimate success of top-

down control in eutrophic systems.

Here, we test the hypothesis that the effect of nutrient enrichment and herbivory depends on

the presence of *Daphnia* genotypes that have adapted to tolerate toxic cyanobacteria in their

environment. We conducted a large-scale field experiment in limnocorrals where we

manipulated nutrients (treatments: ambient, fertilized) and *Daphnia* (treatments: no *Daphnia*

control, cyanobacterial-sensitive *Daphnia*, cyanobacterial-tolerant *Daphnia*) using a two-way,

fully factorial design. Cyanobacterial-tolerant *Daphnia* genotypes were collected from eutrophic

lakes and have adapted to become more tolerant of toxic cyanobacteria in their diet. In contrast,

cyanobacterial-sensitive *Daphnia* genotypes were collected from oligotrophic lakes, where

cyanobacteria and their associated toxins are rare. Both *Daphnia* types had comparable effects on

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phytoplankton biomass in unfertilized enclosures, with grazing by cyanobacterial-sensitive *Daphnia* resulting in marginally-lower phytoplankton biomass. However, in the fertilized treatment, cyanobacterial-sensitive *Daphnia* did not significantly reduce phytoplankton, while grazing by cyanobacterial-tolerant *Daphnia* resulted in a greater than 80% reduction in phytoplankton biomass, relative to the no *Daphnia* control. The interactive effects of fertilization and local adaptation by *Daphnia* on phytoplankton biomass were mediated by the positive response of the toxic cyanobacterium *Cylindrospermopsis raciborskii* and an associated cyanotoxin (saxitoxin) to nutrient enrichment. As a direct consequence, cyanobacterial-sensitive *Daphnia* had a lower population growth rate (~70% lower) and attained a lower biomass at equilibrium (~80% lower), relative to cyanobacterial-tolerant *Daphnia*, in the fertilized treatment.

Our data show that the response of cyanobacteria and phytoplankton biomass in general to nutrient enrichment depends on which *Daphnia* genotypes are present. *Daphnia* that are adapted to tolerate cyanobacteria can control phytoplankton biomass despite the presence of the toxic and grazing-resistant cyanobacterium *C. raciborskii*. Thus, the success of biomanipulation efforts in lakes may depend not only on zooplankton community composition but also on grazer adaptation to cyanobacteria and genotypic composition.

Introduction

Human activities have accelerated nutrient loading into terrestrial, freshwater, and marine ecosystems (Vitousek et al. 1997), resulting in large-scale effects on biodiversity, community structure, and ecosystem function (Carpenter et al. 1998; Hautier et al. 2015). Management of cultural eutrophication has focused on both bottom-up (i.e., resource-based) (Schindler 1974) and top-down (i.e., consumer-based) mechanisms (Carpenter et al. 1987). Remediation efforts

that focus on reducing external and internal supplies of nutrients have been successful at controlling eutrophication (Schindler et al. 2008). In contrast, food-web manipulations that focus on enhancing herbivory are not always successful (Benndorf 1987; Hovick and Carson 2015), and ecologists have been studying mechanisms thought to attenuate top-down effects as productivity increases for decades (Duffy et al. 2015; Leibold et al. 1997; Polis 1999; Stein et al. 1995).

In particular, nutrient enrichment is a leading cause of declines in global water quality, and harmful algal blooms (HABs) are a common associated risk (Smith and Schindler 2009). Nutrient-rich lentic freshwater environments are characterized by noxious scums of colonial and filamentous cyanobacteria (Downing et al. 2001), with several of these genera (e.g., *Microcystis*, *Oscillatoria*, *Anabaena*, and *Cylindrospermopsis*) being known producers of cyanotoxins (e.g., microcystin, anatoxin, cylindrospermopsin, and saxitoxin) (Carmichael 1992). In particular, the hepatotoxin microcystin is particularly well-studied and can be prevalent in waterbodies across the United States and Canada (Graham et al. 2010; Orihel et al. 2012). A large body of literature has demonstrated that cyanobacteria and associated cyanotoxins can have strong negative effects on zooplankton survival and reproduction (DeMott et al. 1991) but that large variation exists for sensitivity to toxic cyanobacteria within and across zooplankton species (Wilson et al. 2006; Wilson and Hay 2007). While cyanobacterial toxins are frequently cited as a reason for cyanobacteria escaping herbivore control in nature (Ghadouani et al. 2003), this paradigm is being overturned (Hairston et al. 1999; Sarnelle and Wilson 2005; Chislock et al. 2013; Ekvall et al. 2014; Urrutia-Cordero et al. 2015).

Studies of zooplankton-cyanobacterial interactions and the responses of ecosystems to nutrient enrichment, in general, tend to focus on short-term effects relative to the time scale of

local adaptations by organisms (Hairston et al. 1999). However, the last decade has seen a rapid rise in the number of studies aimed at understanding the community- and ecosystem-level consequences of heritable adaptations (Harmon et al. 2009; Post et al. 2008; Urban et al. 2014). These studies have demonstrated that evolution can occur on ecological time scales (Grant et al. 2002; Hairston et al 1999), and that this contemporary evolution can have large effects on species interactions and ecosystem processes (Driscoll, Hackett, and Ferriere 2016; Bassar et al. 2010). Recent research has shown that populations of the generalist herbivore, *Daphnia*, can evolve to tolerate toxic cyanobacteria in the diet following prolonged exposure to cyanobacterial blooms (Hairston et al. 1999; 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) and across populations of North American *D. pulicaria* across lakes of varying nutrient enrichment (Sarnelle and Wilson 2005). Furthermore, local adaptation by *Daphnia* in response to toxic prey can have large effects on phytoplankton biomass and ecosystem functioning in hypereutrophic freshwater systems (Chislock et al. 2013). The observation that *Daphnia* can adapt to toxic cyanobacteria likely has important consequences for food web interactions. However, the effects of these adaptations for understanding the response of aquatic ecosystems to nutrient enrichment are completely unexplored.

A key factor for understanding the role of these consumer adaptations in modulating ecosystem-level responses to nutrient enrichment is whether cyanobacterial-tolerant genotypes exist at high enough densities in oligotrophic lakes to enable control of phytoplankton biomass within a single growth season. Past studies (e.g., Hairston et al. 2001; Sarnelle and Wilson 2005)

have suggested that tolerant genotypes may be rare in oligotrophic environments. This suggests that the cost of tolerance to toxic cyanobacteria is high, and that there is a tradeoff that accompanies being tolerant. One potential tradeoff is the ability to exploit high food conditions vs. the ability to depress resources to low levels (R^*) (Tessier et al. 2000; Tessier and Woodruff 2002). As tolerant genotypes are more prevalent in eutrophic environments, such a tradeoff seems possible. However, no studies have examined tradeoffs with respect to consumer tolerance of dangerous (i.e., toxic) prey. Tolerance to microcystin by *Daphnia* may be affected by the expression of transporter genes that prevent uptake of the toxin (Schwarzenberger et al. 2014). If cellular mechanisms that reduce assimilation of toxins also reduce assimilation of key nutrients, a tradeoff associated with tolerance may be pronounced in oligotrophic environments with low quantities of digestion-resistant algae.

   Strong trophic cascades in lakes are well-documented in lakes with *Daphnia* (Leibold 1989). Furthermore, the magnitude of top-down control is thought to be affected by ecosystem fertility (Sarnelle 1992; Carpenter et al. 1995). We conducted a field enclosure experiment in which three *D. pulicaria* genotype treatments (no *Daphnia* control, cyanobacteria-tolerant *D. pulicaria*, sensitive *D. pulicaria*) were crossed with two fertilization treatments (ambient nutrients vs. fertilized) in an oligotrophic pond. In the fertilized treatment, we predicted that both *D. pulicaria* genotype treatments would reduce phytoplankton biomass, relative to the control. However, we expected grazing by *D. pulicaria* genotypes adapted to toxic cyanobacteria (and eutrophic conditions, in general) to result in larger reductions in phytoplankton biomass. In contrast, we hypothesized that the sensitive *D. pulicaria* genotype treatment would result in a larger reduction of phytoplankton biomass in the unfertilized, ambient enclosures, possibly due to a tradeoff between tolerance to toxic cyanobacteria and grazing ability in oligotrophic conditions.

Methods

Limnocorral experiment

During a 52-day field experiment, we manipulated nutrients (ambient vs fertilized) and

Daphnia genotype (no *Daphnia*, cyanobacteria-sensitive *D. pulicaria*, and cyanobacteria-tolerant

D. pulicaria) using a fully factorial design. We conducted the experiment in 2,500-L, clear

polyethylene enclosures that were sealed at the bottom, open to the atmosphere, and suspended

from a floating PVC frame anchored in the center of Pond 202 at the Auburn University South

Research Station in Auburn, Alabama. 202 is shallow (maximum depth = 2.5 m), polymictic, and

oligotrophic (total nitrogen ~150 µg L^{-1}, total phosphorus ~6-10 µg L^{-1}). Twenty-four enclosures

were filled on 5 April 2013 ('day 0') by pumping water through a 60-µm mesh net to exclude

fish and large zooplankton. We then randomly assigned limnocorrals to each of the six treatment

combinations: (1) ambient nutrients/no *Daphnia*; (2) ambient nutrients/sensitive *D. pulicaria*; (3)

ambient nutrients/tolerant *D. pulicaria*; (4) fertilized/no *Daphnia*; (5) fertilized/sensitive *D.*

pulicaria; (6) fertilized/tolerant *D. pulicaria*, with four replicates per treatment. 'Ambient'

enclosures received no nutrient additions, while we added sodium nitrate and sodium phosphorus

(NaH_2PO_4) to 'fertilized' enclosures to increase the total nitrogen concentration to ~4000 µg L^{-1}

and the total phosphorus concentration to ~200 µg L^{-1} on 6 April 2013 ('day 1'). *Daphnia*

treatments were established on 17 April 2013 ('day 12') by stocking either three sensitive or

three tolerant *D. pulicaria* genotypes (in approximately equal proportions) into each

corresponding limnocorral at very low densities (~0.015 animals L^{-1}). All enclosures for the

'fertilized' treatment received approximately biweekly additions of sodium nitrate and phosphate

at a rate of 400 µg L^{-1} total nitrogen and 20 µg L^{-1} total phosphorus (~10% post-treatment

nutrient concentrations) to maintain elevated phytoplankton biomass.

We sampled the enclosures approximately weekly-to-biweekly from 8 April to 20 May 2013. Depth-integrated (1.5 m depth) water samples for nutrients (TN, TP, and SRP), phytoplankton biomass as chlorophyll *a*, microcystin, saxitoxin, phytoplankton species composition and *Daphnia* biomass were collected with a tube sampler (inside diameter = 51 mm). TN, TP, and SRP were analyzed using standard methods (colorimetric assays for TP/SRP; ultraviolet spectrophotometry for TN; Gross and Boyd 1998). Chlorophyll *a* concentrations were measured by extracting phytoplankton collected on Pall A/E filters in 90% ethanol for 24 h in the dark at 4ºC followed by measurement with a fluorometer (Sartory and Grobbelaar 1984). Microcystin and saxitoxin concentrations in cells were quantified for selected dates using enzyme-linked immunosorbent assay (ELISA) following methanol extraction from algal cells collected on Pall A/E filters (An and Carmichael 1994). Phytoplankton species composition was also determined for selected dates via the inverted microscope technique (Utermöhl 1958) using water samples preserved in 1% Lugol's solution. Biovolume for each phytoplankton species was calculated using cell counts (25-50 fields per magnification from 100x-1000x) and estimates of cell volume based on measurements of cell dimensions (400x-1000x). We then converted biovolume (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 0.4 (Sarnelle et al. 2005). *Cylindrospermopsis raciborskii* was primarily observed as straight, heterocystic filaments and was enumerated as individual filaments given faint to nonexistent cell divisions in each filament. *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* (Culver et al. 1985).

The effects of fertilization, *Daphnia* treatment, and their interaction on chlorophyll *a*, *Daphnia* biomass, and saxitoxin concentration were tested using repeated measures analysis of

variance (RM-ANOVA, sampling date = repeated measure) across all dates post-*Daphnia* treatment (i.e., day 12). We used Tukey's post hoc test to compare treatment means across each of the six treatment combinations for chlorophyll *a*, *Daphnia* biomass, and saxitoxin concentration. We also estimated *Daphnia* per capita population growth rates (*r*) for '*Daphnia*' enclosures using animal densities during the period of time when *Daphnia* populations were increasing approximately exponentially (29 April to 9 May – days 24 to 31). We then used factorial ANOVA and Tukey's post hoc tests to compare *D. pulicaria* per capita population growth rates across treatments.

Daphnia pulicaria *genotypes used in the experiments*

The six *D. pulicaria* genotypes used in experiments were descendants of single females isolated from three oligotrophic (based on total phosphorus concentration, Appendix O - Table O1) lakes with few toxic cyanobacteria and three eutrophic lakes with more prevalent cyanobacteria. All genotypes were grown in the laboratory for several generations under common garden conditions to homogenize maternal effects (Sarnelle and Wilson 2005) before use in feeding assays and the field experiment. Each of the six genotypes has been genetically discriminated using microsatellites, and their survivorship and performance on a diet of 100% toxic *Microcystis* diet has been characterized in past studies (Chislock et al. 2013).

Cylindrospermopsis *feeding assays*

Juvenile survivorship assays were conducted in the laboratory to estimate the sensitivity of each *D. pulicaria* genotype to the toxic cyanobacterium *Cylindrospermopsis*. Neonates (<24 hours old) were exposed to two dietary treatments: growth-saturating (>1 mg C/L, (Lampert 1977)) quantities of 1) a strain of *Cylindrospermopsis raciborskii* (collected from a hypereutrophic pond at Auburn University) that produces the toxic secondary metabolite

saxitoxin and 2) the nutritious chlorophyte *Ankistrodesmus falcatus* as a control (Sarnelle and Wilson 2005). Cultured *Cylindrospermopsis* was sieved (30-μm) to remove large colonies to ensure that it could be readily consumed by all size classes of *Daphnia*, concentrated, and re-suspended in A/E filtered and autoclaved pond water. A control food treatment of the nutritious green alga *Ankistridesmus falcatus* was also prepared. Experiments were conducted at 20°C in sealed 20-mL bottles that were attached to a slowly rotating plankton wheel to keep food in suspension. Three replicate bottles were employed per diet × genotype combination. Food and medium were replaced daily to prevent depletion. Each replicate bottle was stocked with five neonates to initiate the experiment. *Daphnia* survival was assessed after two days. While *Microcystis* and microcystin have been shown to inhibit the survival and fecundity of *Daphnia* (Lürling and Van Der Grinten 2003), the effects of the invasive cyanobacterium *Cylindrospermopsis* and its associated cyanotoxins (e.g., saxitoxin) on zooplankton are less well-studied (but see Costa et al. 2013; Ferrao Filho et al. 2008; Soares et al. 2009). Furthermore, it is unknown whether tolerance to toxic *Microcystis* (and microcystin) is correlated with tolerance to other toxic cyanobacterial genera (and associated cyanotoxins).

Results

At the start of the experiment, all enclosures contained a mixture of edible green algae and diatoms, with phytoplankton composition remaining relatively unchanged in ambient enclosures. However, fertilization resulted in a rapid increase in phytoplankton, in general, including the filamentous cyanobacterium *Cylindrospermopsis raciborskii* and the associated toxin, saxitoxin, (Fig. 1) with the cyanobacterial genera *Cylindrospermopsis* and *Anabaena* accounting for ~30% of total phytoplankton biomass. In the ambient treatment, both sensitive and tolerant *D. pulicaria* increased (Fig. 1). Sensitive *D. pulicaria* increased marginally more rapidly than tolerant

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genotypes ($r = 0.092$ vs. 0.069) and attained a higher peak biomass (Fig. 1A) ($P = 0.027$).

However, the magnitude of this difference decreased over the final two weeks of the experiment.

Both sensitive and tolerant genotypes suppressed total phytoplankton biomass (as chlorophyll *a*)

over the final three weeks of the experiment (P < 0.0001), with ~60% lower chlorophyll *a* in

Daphnia treatments than the no-*Daphnia* control (Fig. 1B).

In the fertilized treatment, tolerant *D. pulicaria* increased more rapidly than sensitive

genotypes and attained a ~4-fold higher peak biomass (Tukey's test, $P < 0.0001$; Fig. 1D). As a

direct consequence of higher *D. pulicaria* biomass, chlorophyll *a* during the final three weeks of

the experiment was ~83% lower in the tolerant-*Daphnia* treatment than in either the no-*Daphnia*

control (Tukey's test, $P < 0.0001$) or the sensitive-*Daphnia* treatments ($P = 0.003$). Chlorophyll

a in the sensitive-*Daphnia* treatment was marginally lower, but not statistically different from the

fertilized no-*Daphnia* controls ($P = 0.321$). Tolerant *D. pulicaria* also reduced levels of the

neurotoxin saxitoxin in fertilized enclosures by greater than 60%, relative to the control ($P =$

0.034) and sensitive *D. pulicaria* treatments (0.074), over the course of the experiment. Survival

of tolerant *D. pulicaria* clones on a laboratory diet of toxic *Cylindrospermopsis* was over 300%

higher than sensitive *D. pulicaria* clones.

Discussion

Our study represents a first step in assessing the role of adaptive evolution and consumer

offense by a generalist herbivore in the responses of lake ecosystems to fertilization and food-

web perturbations. Previous research has focused on effects of environmental changes on trait

variation within species. A growing number of studies have demonstrated that these trait shifts

can profoundly affect ecosystem functioning (Chislock et al. 2013). Consumer adaptation to

toxic cyanobacteria has primarily been shown for toxic *Microcystis* (and other microcystin

producers). However, we know considerably less about the potential implications of these adaptations for other genera of cyanobacteria that produce a different suite of toxins. Here we show that the presence of adapted consumers can modulate the positive response of algal biomass (i.e., *Cylindrospermopsis raciborskii*) and the cyanobacterial toxin saxitoxin to nutrient enrichment. As the prevalence *C. raciborskii* has increased and its range is predicted to increase dramatically as a result of climate change (Sinha et al. 2012), understanding ecological interactions between native zooplankton and this species is particularly important.

Invasive primary producers (i.e., algae and true plants) are frequently hypothesized to have an advantage over native species with nutrient enrichment (Chislock et al. 2014; Seabloom et al. 2015). However, the role of consumers in mediating these responses is poorly understood. In freshwater lakes, blooms of the toxic cyanobacterium, *Cylindrospermopsis raciborskii*, are becoming increasing prevalent in temperate and subtropical systems. Its prevalence is attributed to grazing-resistance (Panosso et al. 2003) and toxicity (Ferrao Filho et al. 2008; Soares et al. 2009) to zooplankton and competitive ability under a wide range of nutrient and temperature conditions (Isvanovics et al. 2000; Presing et al. 1996; Moisander et al. 2012; Bonilla et al. 2012). It is well-established that the generalist herbivore *Daphnia* can adapt to tolerate toxic cyanobacteria. Previous research has shown that *Daphnia* collected from eutrophic lakes are more tolerant of the toxic cyanobacterium *Microcystis aeruginosa* than *Daphnia* collected from oligotrophic lakes (Sarnelle and Wilson 2005; Chislock et al. 2013). However, whether *Daphnia* identified to be tolerant to toxic *M. aeruginosa* are more tolerant of other toxic cyanobacteria has not previously been explored. Many strains of *M. aeruginosa* are well known for their production of the cyclic peptide microcystin (a hepatotoxin). Contrastingly, other strains of different cyanobacterial genera are known producers of other toxins, including alkaloid neurotoxins (e.g.,

Anabaena and anatoxin; *Cylindrospermopsis* and saxitoxin). Our results indicate that *Daphnia* genotypes from eutrophic lakes previously identified to be tolerant to toxic *Microcystis* also have increased tolerance to toxic *Cylindrospermopsis*, relative to *Daphnia* genotypes from oligotrophic lakes (Appendix O - Table O1). Consequently, *Daphnia* suppression of phytoplankton biomass and levels of saxitoxin under high nutrient conditions was strongly dependent on the identity of *Daphnia* genotypes and their functional traits. While the physiological mechanisms responsible for zooplankton tolerance to different cyanobacterial toxins are unknown, one hypothesis is that tolerance by *Daphnia* may be affected by the expression of transporter genes that prevent uptake of cyanotoxins (Schwarzenberger et al. 2014) Thus, variation in assimilation and feeding efficiency associated with tolerance to toxins is one possible explanation.

Protecting diminishing water quality is a challenging environmental issue due to the interactive effects of climate change, species invasions, and cultural eutrophication. The potential for consumer adaptations to mediate the long-term response of ecosystems to eutrophication likely depends on several factors including 1) the presence of particular consumer species (such as *Daphnia*), 2) trade-offs associated with consumer adaptations, 3) the prevalence of adaptations across different consumer species, and 4) the ability to reduce planktivory/enhance zooplankton grazing. The presence of *Daphnia* is thought to be essential for strong suppression of phytoplankton following biomanipulation. The results of our research additionally demonstrate that the effect of different *Daphnia* genotypes on ecosystem function can be as large as the presence/absence of this species (Chislock et al. 2013). A comparison of tolerant versus sensitive *D. pulicaria* performance in our unfertilized enclosures is suggestive of a potential tradeoff exhibited under low food conditions. However, additional research is needed to further support

this hypothesis. While the traditional paradigm in limnology is that grazing-resistant and toxic cyanobacteria cannot be strongly suppressed by zooplankton herbivores, there has been a recent surge of interest in understanding variation in zooplankton tolerance among and within species (Chen et al. 2015; Ferrao Filho et al. 2008; Ger et al. 2014; Hairston et al. 1999; Kuster and Von Elert 2013; Pham et al. 2015; Soares et al. 2009). Indeed, adaptations by zooplankton consumers in response to toxic prey appear to be more common than previously hypothesized (Karban 2002). Future research is needed to understand existing genetic variation within *Daphnia* populations in oligotrophic systems as well as other potential costs of adaptations to tolerate toxic cyanobacteria. Such research will aid in determining the potential for rapid evolution in response to global changes such as eutrophication to help stabilize ecosystems and affect the response of systems to future perturbations.

Acknowledgments

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Figure Legends

Figure 4.1. Dynamics of *Daphnia pulicaria* biomass, chlorophyll *a*, and <35 µm saxitoxin concentration for the ambient (unfertilized) (A, B, C) and fertilized (D, E, F) treatments, respectively. Open symbols denote controls (no *Daphnia*) gray symbols indicate *D. pulicaria* genotypes that are sensitive to toxic cyanobacteria, and black symbols indicate *D. pulicaria* genotypes that are tolerant of toxic cyanobacteria.

Figure 4.1
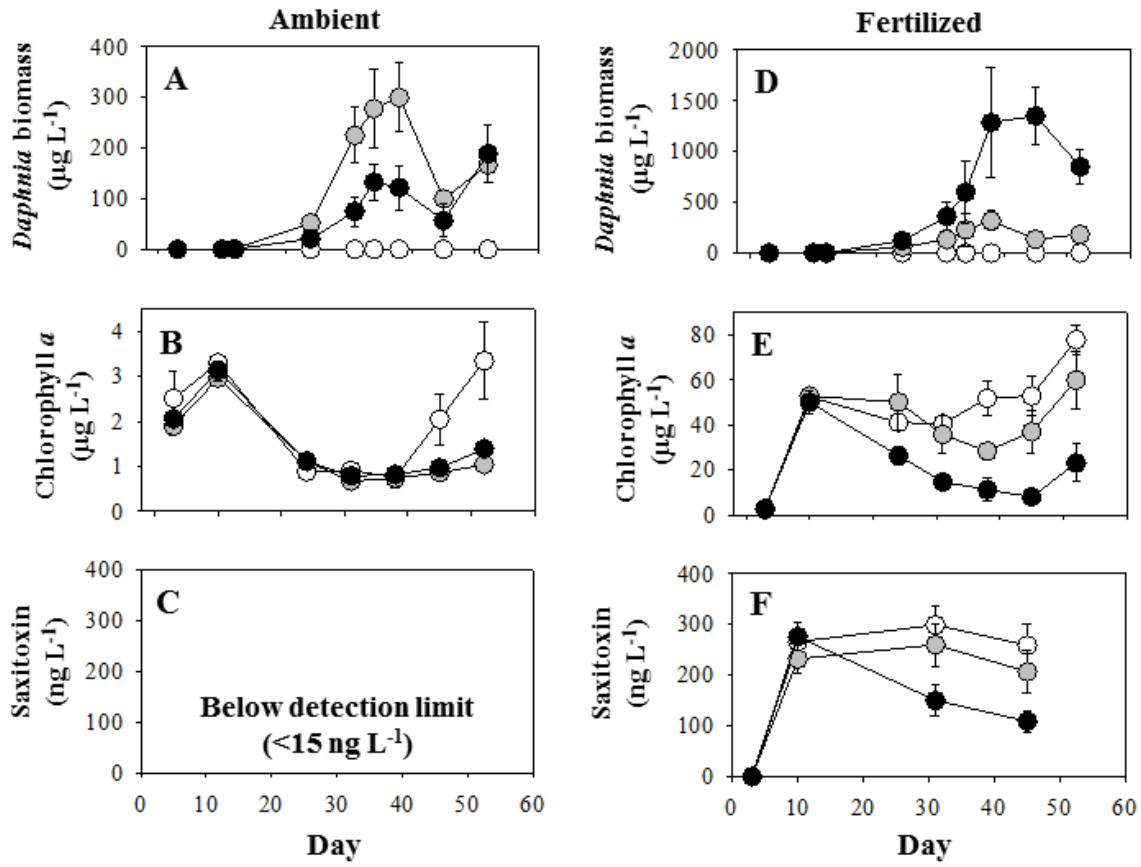
Appendix A. Detailed methods (chapter 1)

Daphnia pulicaria *genotypes used in the experiments*

The six *Daphnia pulicaria* genotypes used in experiments were descendants of single females isolated in 2009 from each of six small glacial lakes (<0.3 km^2) in southern Michigan. Three of the lakes are oligotrophic (based on total phosphorus concentration, Table B1) with few toxic cyanobacteria, while three lakes are moderately to highly eutrophic with variable cyanobacterial abundance during the summer. Each of the six genotypes used in the experiment were genetically discriminated using variation in 7 microsatellite loci (Figure B1) (Colbourne et al. 2004; Wilson and Hay 2007). All genotypes were maintained for several generations in the laboratory under common garden conditions (Sarnelle and Wilson 2005) before use in survivorship assays and field experiments. Consequently, we conclude that the trait differences shown in Table B1 are genetically based.

Juvenile survivorship assays were conducted to estimate the sensitivity of each *D. pulicaria* genotype to toxic cyanobacteria (Sarnelle and Wilson 2005). Neonates (<24 hours old) were fed a diet consisting solely of a strain of *Microcystis aeruginosa* (UTEX 2667) that produces the toxic secondary metabolite, microcystin (Carmichael 1992). *Microcystis* and microcystin have both been shown to inhibit the survival and fecundity of *Daphnia* (Lürling and Van Der Grinten 2003). A matched set of neonates were fed the highly nutritious chlorophyte *Ankistrodesmus falcatus* as a control (Sarnelle and Wilson 2005). Three genotypes with high survivorship on the *Microcystis* diet ("tolerant") and three with low survivorship ("sensitive") were used in the experiments (Table B1). Prior to the limnocorral experiment, each *D. pulicaria* genotype was inoculated into a 20-L tank filled with water from a low-nutrient lake that was filtered through a 35-µm sieve. We added high densities of *Ankistrodesmus* to the tanks as a food source. All *D. pulicaria* genotypes grew rapidly on this high-quality food source. These tank cultures were allowed to grow for several weeks to provide animals to stock into the limnocorrals.

Limnocorral experiment

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 and cylindrospermopsin concentrations in the size fraction edible to *Daphnia* (i.e., <35 µm) (Burns 1968; DeMott 1995) was quantified using enzyme-linked immunosorbent assay (ELISA) (An and Carmichael 1994). Cylindrospermopsin concentrations were below the detection limit (< 0.1 µg L^{-1}) at the beginning of the experiment, and *Cylindrospermopsis* was not present at later sampling dates. Phytoplankton species abundance and

composition were determined at the start and end of the experiment via the inverted microscope technique (Utermöhl 1958) using water samples preserved in 1% Lugol's solution. Biovolume for each species was calculated using cell counts and estimates of cell volume based on measurements of cell dimensions. We then converted biovolume (mm 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* (Culver et al. 1985).

Cubitainer experiment

We stocked 6 juveniles of the sensitive *D. pulicaria* genotypes (equal proportions) into replicate 5-L cubitainers filled with enclosure water either containing or lacking cyanobacteria (n=8 replicates per treatment). The cyanobacteria-containing cubitainers ('pond water' treatment) were filled with water taken from the sensitive-*Daphnia* enclosures that was poured through a 75-µm sieve to exclude *Daphnia*. To establish the cyanobacteria-lacking treatment ('good food'), we filtered enclosure water to remove all phytoplankton (flow-through 1 µm glass-fiber filter followed by vacuum filtration using Pall A/E filters) and added back a similar biomass of cultured *Ankistrodesmus falcatus*. The total concentration of phytoplanktonic food was far above that needed to saturate *Daphnia* feeding rate in both treatments (chlorophyll *a* > 200 µg L^{-1}) (Sarnelle and Wilson 2005). Cubitainers were incubated in the pond for 8 days at which point *D. pulicaria* survivorship and fecundity were assessed. There were some "new" neonates in the 'good food' treatment after 8 days as a result of high reproductive rates. These were ignored in assessing treatment effects. We used a t-test to compare mean *Daphnia* survivorship and fecundity for the two treatments.

Phytoplankton mortality experiment

We conducted a 24-hour experiment at the end of the limnocorral experiment (day 57) to compare the abilities of sensitive and tolerant *D. pulicaria* genotypes to inflict mortality on the phytoplankton present in the limnocorrals. Three treatments were established in 100-mL glass bottles with seven replicates of each: no-*Daphnia* control, sensitive *D. pulicaria*, and tolerant *D. pulicaria*. We screened water from the control limnocorrals through a 75-µm sieve (to remove any large zooplankton) to create the phytoplankton suspension. To obtain readily-detectable phytoplankton responses, we employed high *Daphnia* densities and diluted the phytoplankton suspension with A/E filtered water to reduce chlorophyll *a* concentrations to less than 25 µg L^{-1} (Sarnelle 1993). We stocked ~30 adult

Daphnia (>1 mm, approximately 10 of each genotype) from laboratory cultures into each bottle according to treatments. Bottles were incubated in the dark on a plankton wheel in a climate controlled room (25°C). This procedure prevented phytoplankton from settling out or dividing at different rates across the treatments. After 24 hours, we measured phytoplankton biomass as chlorophyll *a* extracted from filters, counted and measured all *D. pulicaria* in each bottle, and calculated *Daphnia* biomass and filtration rate using standard regression equations (Culver et al. 1985). Mean *Daphnia* biomass and population filtration rates for the sensitive treatment (2797 µg L^{-1}, 2217 mL day^{-1}) were not significantly different than for the tolerant treatment (2175 µg L^{-1}, 1751 mL day^{-1}, biomass: $T_{12} = 1.281$, $P > 0.22$; filtration: $T_{12} = 1.247$, $P > 0.23$).

Limnocorral experiment – low levels of cyanobacterial toxins

Laboratory experiments have shown that microcystin concentrations near 3 µg L^{-1} tend to reduce survival of *Daphnia* (Lürling and Van Der Grinten 2003). Thus, we compared the limnocorral results obtained at Auburn (microcystin 5-10 µg L^{-1}) (Fig. 1.1) with a limnocorral experiment of the same treatment design conducted in a productive ecosystem in Michigan in which microcystin concentrations were substantially lower (<1 µg L^{-1}). Sample collection protocols were identical to the Auburn experiment. In this experiment, we observed a large effect of presence/absence of *D. pulicaria* on phytoplankton biomass, irrespective of *Daphnia* genotype, with both sensitive and tolerant *Daphnia* reducing chlorophyll *a* concentrations by 90% (relative to the no *D. pulicaria* control) (Appendix C - Fig. C1).

Appendix B. Juvenile survivorship and genetic discrimination using microsatellites for the six *Daphnia pulicaria* genotypes used in the experiments.

Table B1. Juvenile survivorship (after 5 days) assays for the six genotypes of *Daphnia pulicaria* fed a diet of 100% microcystin-producing *Microcystis aeruginosa*. Survivorship in controls (*Ankistrodesmus* as food) was near 100% for all genotypes. All six genotypes were isolated in 2009 from each of six small glacial lakes in southern Michigan. Three of the lakes are oligotrophic (based on total phosphorus (TP) concentration), while three lakes are moderately to highly eutrophic. All lakes were surveyed in 2009 and 2011, and TP represents the mean total phosphorus concentration ($\mu g \ L^{-1}$) measured in the spring of 2009 and 2011.

Source Lake (TP_{spring})	Isolate designation	Survival on *Microcystis* diet (SE)
Sensitive genotypes		
Sherman (11)	Dp-Sh-7	0.04 (0.04)
Bassett (10)	Dp-Ba-6	0.05 (0.05)
Lawrence (9)	Dp-La-6	0.11 (0.06)
Tolerant genotypes		
3rd Sister (29)	Dp-3S-2	0.93 (0.07)
Baseline (36)	Dp-Bs-13	0.73 (0.18)
Wintergreen (33)	Dp-Wi-6	0.93 (0.07)

Genetic Distance

0.600   0.450   0.300   0.150   0.000

Dp-BA-6 (sensitive)
Dp-SH-7 (sensitive)
Dp-3S-2 (tolerant)
Dp-WI-6 (tolerant)
Dp-BS-13 (tolerant)
Dp-LA-6 (sensitive)
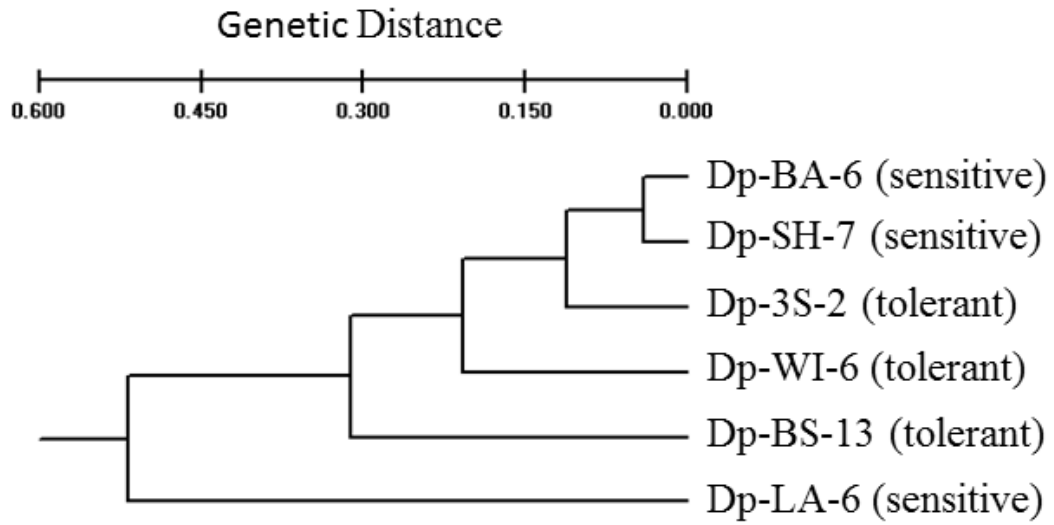
Figure B1. Dendrogram showing relationships among the six *Daphnia pulicaria* clones that are sensitive (n=3) or tolerant (n=3) to cyanobacteria in the diet (Table B1) using arithmetic averages (UPGMA) and Nei's genetic distance of microsatellite data (Dp3, Dp337, Dp339, Dp464, Dp496, Dp502, Dp1486; Colbourne et al. 2004).

Appendix C. Phytoplankton biomass and species composition for major taxa at the conclusion of the experiment.

Figure C1. Mean absolute and relative biomass of dominant phytoplankton taxa at the conclusion of the experiment. ANOVA: (Cyanophyta dry biomass: $F_{2,7} = 15.301$, $P = 0.003$; Cyanophyta percent biomass: $F_{2,7} = 7.710$, $P = 0.017$). Mean equivalent spherical diameter for *Microcystis* colonies: (Control: 89 µm; Sensitive: 53 µm; Tolerant: 103 µm)

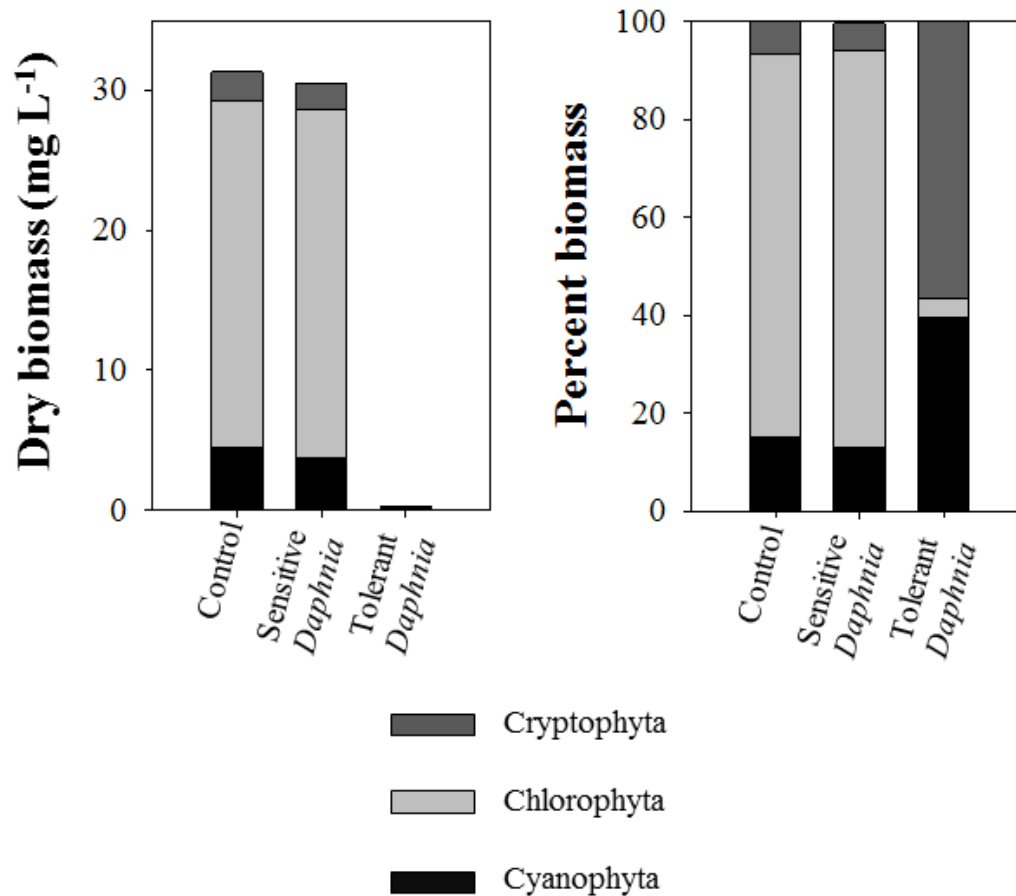**Appendix D.** *Daphnia* and chlorophyll data from a related experiment where cyanobacteria and microcystin were lower in abundance.
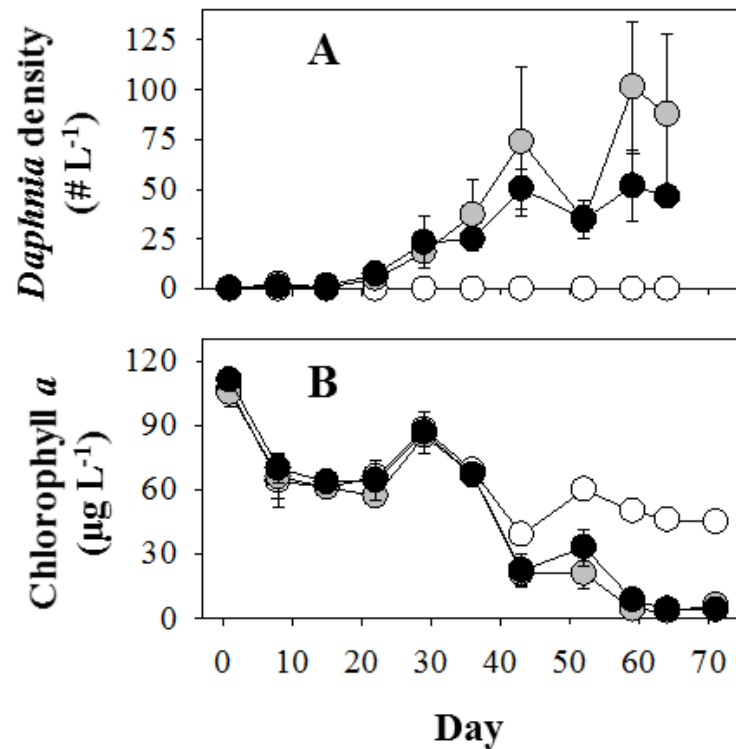
Figure D1. Dynamics of **(A)** *Daphnia pulicaria* density and **(B)** chlorophyll *a* in an experiment conducted in Michigan where cyanobacteria and microcystin were significantly lower. Data represent means ± one standard error. Open symbols denote controls (no *Daphnia*), gray symbols indicate *D. pulicaria* genotypes that are sensitive to toxic cyanobacteria, and black symbols indicate *D. pulicaria* genotypes that are tolerant to toxic cyanobacteria. ANOVA: (*Daphnia* biomass: $F_{2,8} = 9.642$, $P = 0.010$; chlorophyll *a*: $F_{2,8} = 10.026$, $P = 0.007$).

Appendix E. Detailed methods (chapter 2)

Recent research has shown that *Daphnia* populations exposed to high concentrations of cyanobacteria and associated toxins can adapt to become more tolerant of toxic cyanobacteria in the diet. This observation is supported by temporal trends between the eutrophication status of lakes and shifts in *Daphnia* cyanobacterial tolerance (Hairston et al. 1999; Hairston et al. 2001) and across lakes with widely varying levels of nutrient enrichment (Sarnelle and Wilson 2005). We were interested in the community- and ecosystem-level consequences of these adaptations by *Daphnia*. We assessed the effects of cyanobacterial-sensitive vs. tolerant *D. pulicaria* genotypes on phytoplankton biomass across a productivity gradient using 2 sets of mesocosm experiments spanning contrasting seasons with different prey diversity.

Mesocosm experiments

We conducted simultaneous six-week mesocosm experiments in five ponds that spanned a large total phosphorus gradient (49-167 µg L^{-1}) at the E.W. Shell Fisheries Research Station at Auburn University, Alabama, during the spring (March-April). We then conducted a similar set of simultaneous mesocosm experiments in a similar set of five ponds (TP = 20-334 µg L^{-1}) during the fall (October-November). All ponds are shallow and polymictic with surface areas ranging from 0.1 to 1 ha and maximum depths of 3 m (Boyd and Shelton 1984). Cyanobacteria are common in most ponds in late May and can account for up to 90% of total phytoplankton biomass by September in the most productive ponds (Chislock et al. 2013). Thus, grazing-resistant phytoplankton should be more common in the fall vs. spring.

We stocked a mixture of either three sensitive or three tolerant *D. pulicaria* clones (in approximately equal proportions) into each corresponding enclosure at very low densities (0.1 animals L^{-1}) in both experiments, and each clone originated from single adult females or hatched ephippial eggs that were collected from six small (<0.3 km^2) lakes of varying productivity. Three of the lakes are oligotrophic (based on TP) with few toxic cyanobacteria, while three lakes are moderately to highly eutrophic with variable cyanobacterial abundance during the summer (Appendix F - Table F1). Juvenile growth rates of each genotype on diets consisting of 100% *Ankistrodesmus falcatus* (a nutritious green alga) or 100% *Microcystis aeruginosa* (UTEX 2667; toxic) were compared for each of the genotypes in a laboratory experiment (Sarnelle and Wilson 2005). Somatic growth rates for neonates (<24 hours old) was assessed for each diet over three days. Instantaneous somatic growth was calculated as (ln W$_f$ − ln W$_i$)/3, where W$_i$ and W$_f$ are initial and final (day 3) masses, respectively. We then calculated a relative index of growth

inhibition by *Microcystis* for each clone as $(g_a - g_m)/g_a$, where g_a and g_m are the instantaneous somatic growth rates on *Ankistrodemus* and *Microcystis*, respectively. Larger values of this index indicate greater inhibition by *Microcystis* relative to *Ankistrodesmus* (values greater than 1 occur if animals gain weight when fed *Ankistrodesmus* but lose weight when fed *Microcystis*). All genotypes were maintained for several generations in the laboratory before use in experiments. Prior to the mesocosm experiment, each *D. pulicaria* genotype was inoculated into a 20-L tank filled with water from a low-nutrient lake that was filtered through a 35-µm sieve. We added high densities of a nutritious green alga (*Ankistrodesmus*) to the tanks as a food source. These tank cultures were allowed to grow for 1 month to provide animals to stock into the mesocosms.

Experiments were conducted in 160-L plastic enclosures that were secured to a floating PVC frame anchored in each pond. Eight enclosures in each pond were filled by pumping water through a 100-µm mesh net to initially exclude resident *Daphnia* and planktivorous fish from treatments. We then stocked an equal mixture of either three sensitive or three tolerant genotypes into each of three replicate mesocosms for treatments containing *Daphnia* within each pond. The remaining two enclosures within each pond served as 'no *Daphnia*' controls. We sampled all enclosures immediately prior to adding *Daphnia* and then weekly until the end of the experiments. Depth-integrated water samples for total phytoplankton biomass (as chlorophyll *a*), phytoplankton species composition, and *Daphnia* biomass were collected 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). Phytoplankton species composition was determined at the beginning and end of both experiments via the inverted microscope technique (Utermohl 1958) using water samples preserved in 1% Lugol's solution (Chislock et al. 2013; Chislock et al. 2014). Microcystin concentrations in whole-water samples were quantified using enzyme-linked immunosorbent assay (ELISA) (An and Carmichael 1994).

Data analysis

To standardize Daphnia effects on total phytoplankton biomass across all ponds, we calculated effect sizes for both experiments (algal response factor – ARF) (Sarnelle 1992). ARF was calculated by dividing the mean chlorophyll a concentration in the two controls by the chlorophyll a concentration for each of the six Daphnia enclosures, within each pond, for each of the final two weeks of the experiment when Daphnia populations had stabilized (Appendix J). We were interested in the magnitude of Daphnia effects on phytoplankton biomass across a

nutrient (TP) gradient. Therefore, we used a mixed model to determine the effects of TP, Daphnia genotype, and their interaction on ARF. We log-transformed TP and ARF to meet the assumptions of the model (normality and homogeneity of variance). For our model, we had clustered-longitudinal data with three-level nesting (week of the experiment = level 1; Daphnia genotype = level 2; pond = level 3). We also included a quadratic term for TP to test for evidence of an asymptote in the TP-ARF relationship as evidence of increased grazing resistance of phytoplankton at higher TP (Sarnelle 1992). We analyzed the spring and fall experiments separately versus including another categorical predictor (i.e., season) to simplify the analysis. We used the Johnson-Neyman approach to determine the TP concentration where ARF for the two Daphnia types was significantly different.

Appendix F. Source lakes for the eight genotypes of *Daphnia pulicaria* used in the experiments.

Table F1. Information for source lakes for the eight genotypes of *Daphnia pulicaria* used in the experiments. All eight genotypes were isolated in 2009 from each of six small glacial lakes in southern Michigan. Three of the lakes are oligotrophic (based on total phosphorus (TP) concentration), while three lakes are moderately to highly eutrophic. All lakes were surveyed in 2009 and 2011, and TP represents the mean total phosphorus concentration measured in the spring. Chlorophyll *a* and microcystin are mean values for the summer. *D. pulicaria* genotypes were confirmed to be sensitive or tolerant to toxic cyanobacteria by comparing juvenile growth when fed diets consisting of the nutritious green alga (i.e., *Ankistrodesmus falcatus*) vs. toxic cyanobacteria (*Microcystis aeruginosa*; UTEX 2667). Relative growth inhibition was calculated for each genotype following the methods of Sarnelle and Wilson (2005). Larger values of this index indicate greater inhibition by *Microcystis* relative to *Ankistrodesmus* (values greater than 1 occur if animals gain weight when fed *Ankistrodesmus* but lose weight when fed *Microcystis*).

Source Lake	TP (μg L^{-1})	Chl *a* (μg L^{-1})	Microcystin (μg L^{-1})	Relative growth inhibition
Sensitive genotypes:				
Bassett Lake	10	8	0.005	1.51
Eagle	10	5	0.006	1.40
Lawrence Lake	13	4	0.004	1.30
Sherman Lake	8	5	0.010	*N/A
Tolerant genotypes:				
MSU Lake 1	71	60	0.500	0.92
Kent Lake	25	10	0.024	1.20
Duncan Lake (clone 1)	62	37	0.016	1.12
Duncan Lake (clone 2)				0.75

*Relative growth inhibition could not be calculated for the Sherman Lake genotype as all animals died when fed *Microcystis*

Appendix G. Description of ponds used in the spring and fall experiments.

Table G1. Nutrient concentrations (total phosphorus, TP), chlorophyll a, relative and absolute cyanobacterial abundance, and levels of the cyanotoxin, microcystin, for each of the five ponds at the beginning of the spring experiment. Ponds are listed in rank order of increasing productivity.

Pond	TP ($\mu g\ L^{-1}$)	Chl a ($\mu g\ L^{-1}$)	Percent cyanobacteria	Cyanobacterial biomass ($\mu g\ L^{-1}$)	Microcystin ($\mu g\ L^{-1}$)
S4	49	19	0	0	< 0.1
S29	50	16	0	0	< 0.1
Asheton	87	26	0	0	< 0.1
S12	114	47	0.2	22	< 0.1
S11	167	53	0	0	< 0.1

Table G2. Nutrient concentrations (total phosphorus, TP), chlorophyll a, relative cyanobacterial abundance, and levels of the cyanotoxin, microcystin, for each of the five ponds at the beginning of the fall experiment. Ponds are listed in rank order of increasing productivity.

Pond	TP ($\mu g\ L^{-1}$)	Chl a ($\mu g\ L^{-1}$)	Percent cyanobacteria	Cyanobacterial biomass ($\mu g\ L^{-1}$)	Microcystin ($\mu g\ L^{-1}$)
FP14	10	9	0	0	< 0.1
S22	59	33	65	1,187	0.6
F9	129	51	20	406	0.36
F20	169	78	11	127	0.38
S9	334	53	98	5480	1.43

Appendix H. Statistical results for the spring and fall experiments.

Table H1. Statistical results for the spring experiment for the mixed model examining the effects of *Daphnia pulicaria* genotype, total phosphorus, and their interaction on the algal response factor (ARF), which compares the magnitude of *Daphnia* effects on phytoplankton biomass as chlorophyll *a*. Data were analyzed over the final two weeks of the experiment when *D. pulicaria* populations had stabilized.

Fixed effects	Estimate	SE	df	T	p-value
Daphnia treatment	0.10	0.10	4	0.98	0.38
log (total phosphorus)	1.27	0.48	39	2.67	0.01
Interaction	0.16	0.73	38	0.22	0.82

Random effects	σ	Percent of total variance
Pond	0.29	56
Daphnia treatment in pond	0.12	10
Day in treatment in pond	0.11	8
Residual	0.20	26

Table H2. Statistical results for the spring experiment for the mixed model examining the effects of *Daphnia pulicaria* genotype, total phosphorus, and their interaction on *Daphnia* biomass. Data were analyzed over the final two weeks of the experiment when *D. pulicaria* populations had stabilized.

Fixed effects	Estimate	SE	df	T	p-value
Daphnia treatment	0.001	0.053	4	0.02	0.98
log (total phosphorus)	0.75	0.38	39	1.99	0.05
Interaction	0.06	0.37	38	0.17	0.87

Random effects	σ	Percent of total variance
Pond	0.21	52
Daphnia treatment in pond	2.64×10^{-6}	0
Day in treatment in pond	2.90×10^{-6}	0
Residual	0.20	48

Table H3. Statistical results for the fall experiment for the mixed model examining the effects of *Daphnia pulicaria* genotype, total phosphorus, and their interaction on the algal response factor (ARF), which compares the magnitude of *Daphnia* effects on phytoplankton biomass as chlorophyll *a*. Data were analyzed over the final two weeks of the experiment when *D. pulicaria* populations had stabilized.

Fixed effects	Estimate	SE	df	T	p-value
Daphnia treatment	0.48	0.37	4	1.32	0.26
log (total phosphorus)	0.66	0.14	38	4.78	< 0.0001
Interaction	-0.52	0.19	38	-2.75	0.01

Random effects	σ	Percent of total variance
Pond	1.99×10^{-5}	0
Daphnia treatment in pond	2.05×10^{-7}	0
Day in treatment in pond	0.23	65
Residual	0.17	35

Table H4. Statistical results for the fall experiment for the mixed model examining the effects of *Daphnia pulicaria* genotype, total phosphorus, and their interaction on *Daphnia* biomass. Data were analyzed over the final two weeks of the experiment when *D. pulicaria* populations had stabilized.

Fixed effects	Estimate	SE	df	T	p-value
Daphnia treatment	0.39	0.29	4	1.35	0.25
log (total phosphorus)	0.62	0.16	38	3.78	0.0005
Interaction	-0.48	0.15	38	-3.20	0.0028

Random effects	σ	Percent of total variance
Pond	0.19	32
Daphnia treatment in pond	0.04	1
Day in treatment in pond	0.05	2
Residual	0.27	65

Appendix I. Cyanobacterial colony/filament sizes at the conclusion of the fall experiment.

Table I1. Mean *Microcystis* colony size (mean equivalent spherical diameter − ESD ± 1 standard error − SE) at the end of the fall experiment. Ponds are listed in rank order of increasing productivity.

Pond	Control ESD (μm) mean ± 1 SE	Sensitive ESD (μm) mean ± 1 SE	Tolerant ESD (μm) mean ± 1 SE
FP14	109 ± 4	101 ± 6	109 ± 12
S22	66 ± 9	95 ± 11	119 ± 6
F9	76 ± 11	72 ± 3	114 ± 9
F20	64 ± 1	105 ± 7	118 ± 5
S11	36 ± 6	66 ± 3	82 ± 3

Table I2. Mean *Cylindrospermopsis* filament length (length ± 1 standard error − SE) at the end of the fall experiment. Ponds are listed in rank order of increasing productivity. N/A indicates ponds where *Cylindrospermopsis* was not found.

Pond	Control ESD (μm) mean ± 1 SE	Sensitive ESD (μm) mean ± 1 SE	Tolerant ESD (μm) mean ± 1 SE
FP14	NA	NA	NA
S22	72 ± 4	82 ± 8	106 ± 4
F9	NA	NA	NA
F20	NA	NA	NA
S9	85 ± 9	159 ± 13	170 ± 10

Appendix J. Dynamics of *Daphnia pulicaria* biomass and chlorophyll *a* for each pond in the spring and fall experiments.
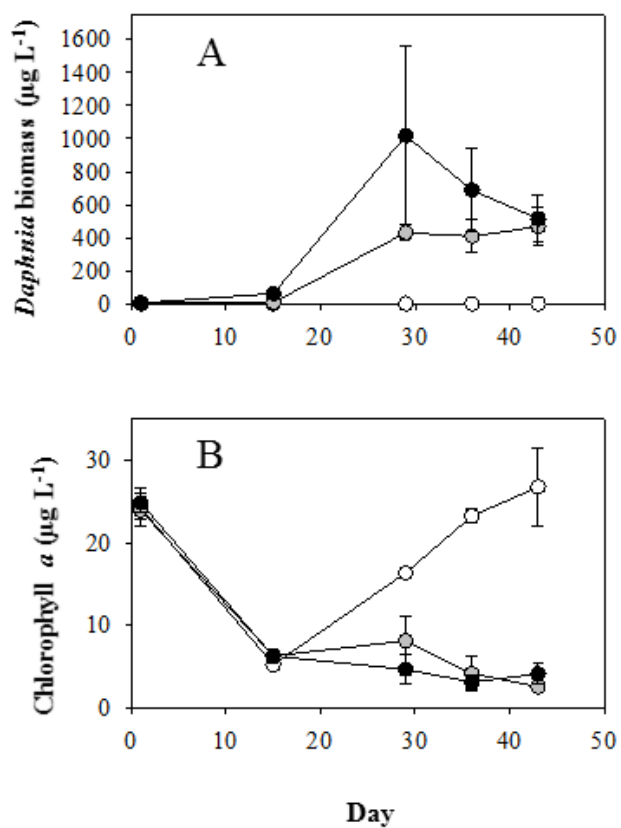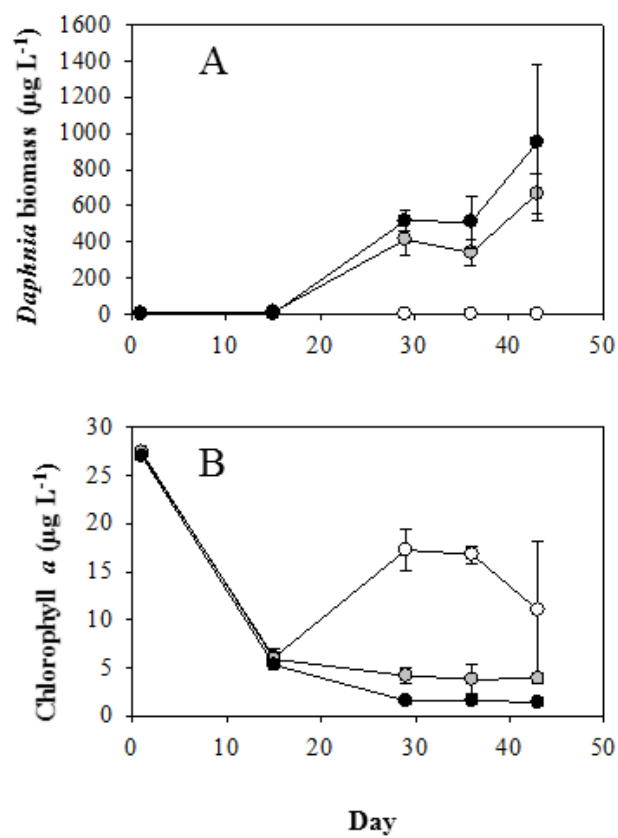

Figure J1. Dynamics of (A) *Daphnia pulicaria* biomass and (B) algal biomass as chlorophyll *a* in pond S4 (spring experiment). Data represent means ± SE. Open symbols denote controls (no *Daphnia*), gray symbols indicate *D. pulicaria* genotypes that are sensitive to toxic cyanobacteria, and black symbols indicate *D. pulicaria* genotypes that are tolerant of toxic cyanobacteria.
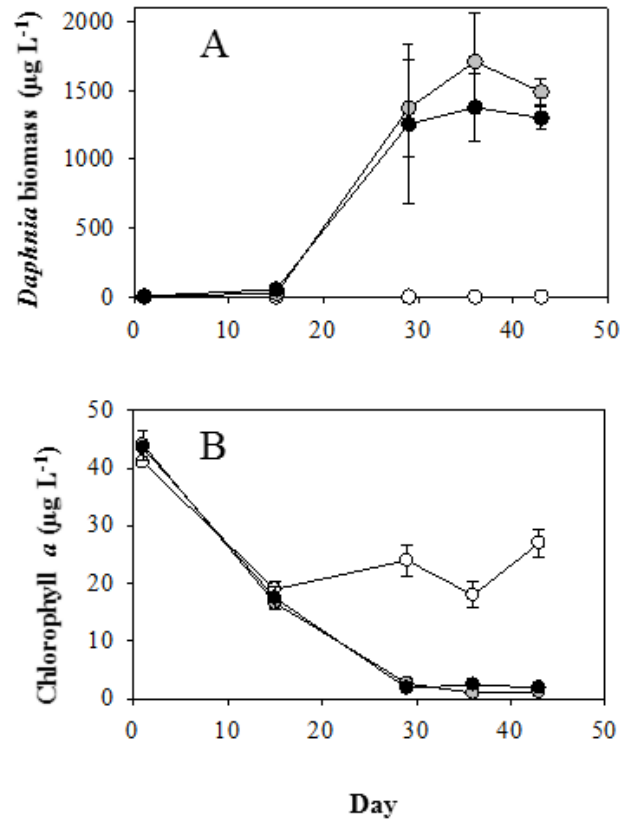
Figure J2. Dynamics of (A) *Daphnia pulicaria* biomass and (B) algal biomass as chlorophyll *a* in pond S29 (spring experiment). Data represent means ± SE. Open symbols denote controls (no *Daphnia*), gray symbols indicate *D. pulicaria* genotypes that are sensitive to toxic cyanobacteria, and black symbols indicate *D. pulicaria* genotypes that are tolerant of toxic cyanobacteria.
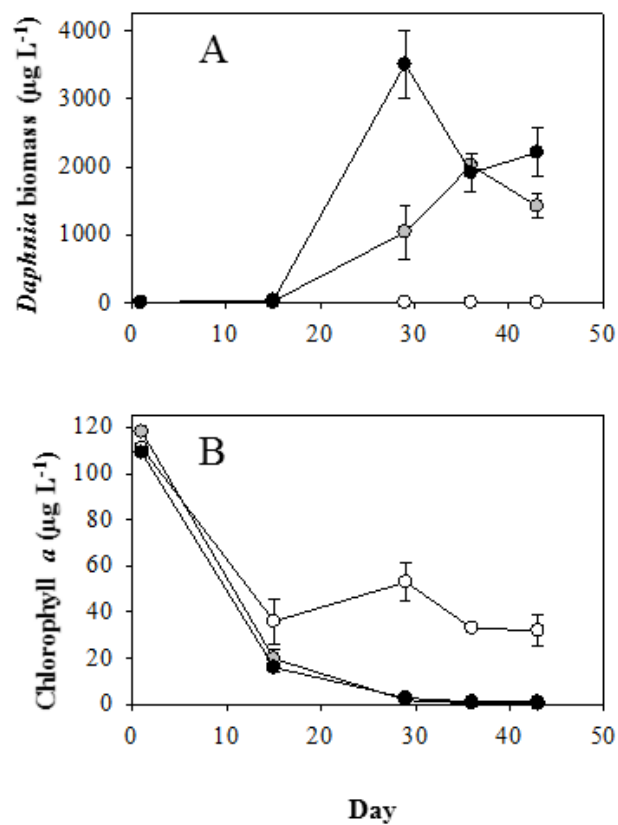
Figure J3. Dynamics of (A) *Daphnia pulicaria* biomass and (B) algal biomass as chlorophyll *a* in Asheton Lake (spring experiment). Data represent means ± SE. Open symbols denote controls (no *Daphnia*), gray symbols indicate *D. pulicaria* genotypes that are sensitive to toxic cyanobacteria, and black symbols indicate *D. pulicaria* genotypes that are tolerant of toxic cyanobacteria.
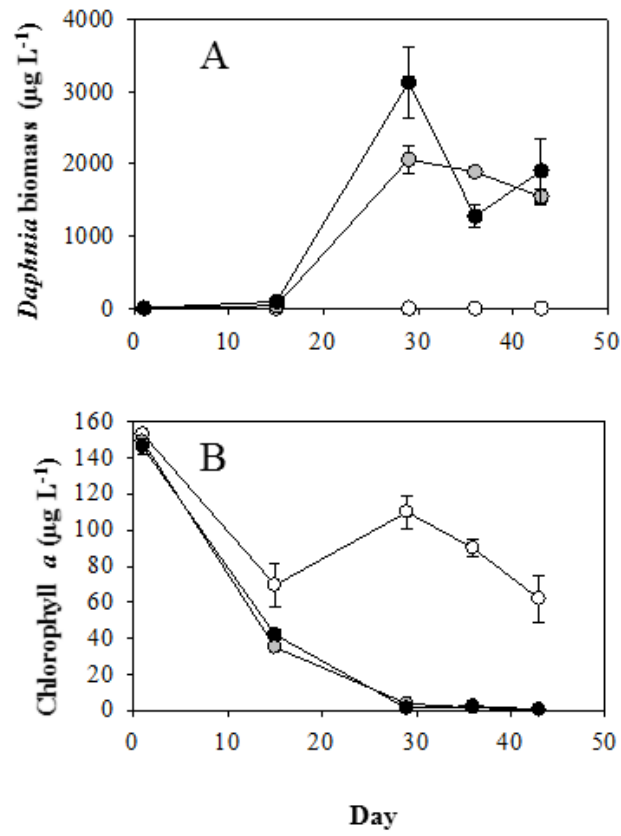
Figure J4. Dynamics of (A) *Daphnia pulicaria* biomass and (B) algal biomass as chlorophyll *a* in pond S12 (spring experiment). Data represent means ± SE. Open symbols denote controls (no *Daphnia*), gray symbols indicate *D. pulicaria* genotypes that are sensitive to toxic cyanobacteria, and black symbols indicate *D. pulicaria* genotypes that are tolerant of toxic cyanobacteria.

Figure J5. Dynamics of (A) *Daphnia pulicaria* biomass and (B) algal biomass as chlorophyll *a* in pond S11 (spring experiment). Data represent means ± SE. Open symbols denote controls (no *Daphnia*), gray symbols indicate *D. pulicaria* genotypes that are sensitive to toxic cyanobacteria, and black symbols indicate *D. pulicaria* genotypes that are tolerant of toxic cyanobacteria.
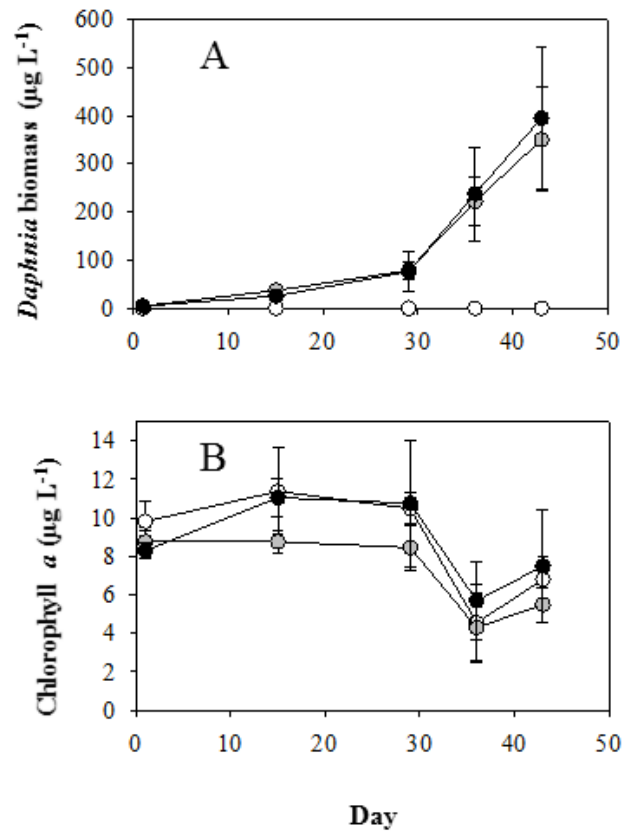
Figure J6. Dynamics of (A) *Daphnia pulicaria* biomass and (B) algal biomass as chlorophyll *a* in FP14 (fall experiment). Data represent means ± SE. Open symbols denote controls (no *Daphnia*), gray symbols indicate *D. pulicaria* genotypes that are sensitive to toxic cyanobacteria, and black symbols indicate *D. pulicaria* genotypes that are tolerant of toxic cyanobacteria.
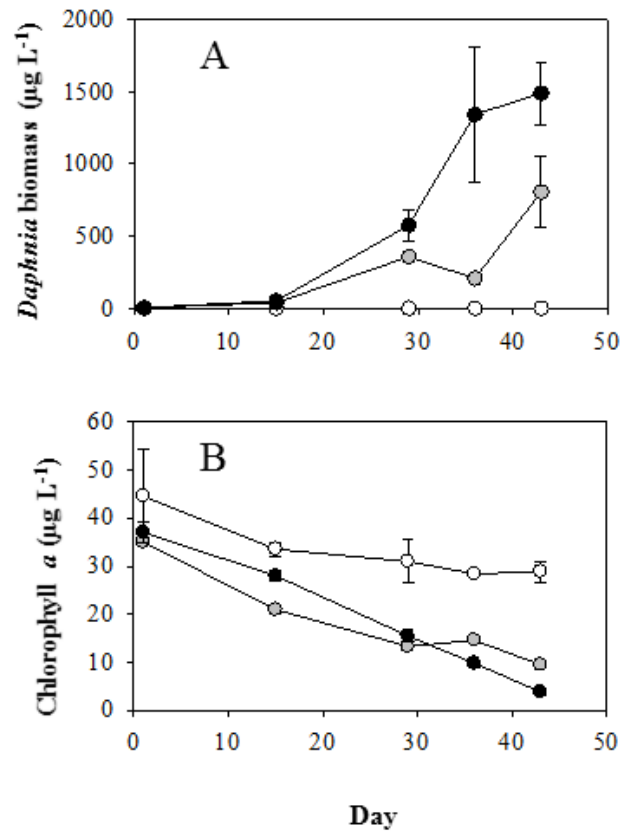
Figure J7. Dynamics of (A) *Daphnia pulicaria* biomass and (B) algal biomass as chlorophyll *a* in pond S22 (fall experiment). Data represent means ± SE. Open symbols denote controls (no *Daphnia*), gray symbols indicate *D. pulicaria* genotypes that are sensitive to toxic cyanobacteria, and black symbols indicate *D. pulicaria* genotypes that are tolerant of toxic cyanobacteria.
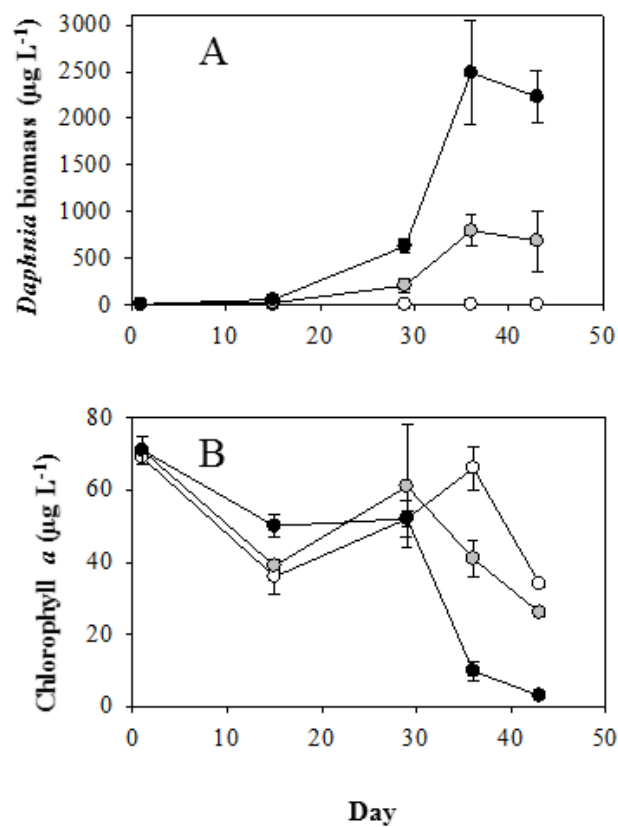
Figure J8. Dynamics of (A) *Daphnia pulicaria* biomass and (B) algal biomass as chlorophyll *a* in pond F9 (fall experiment). Data represent means ± SE. Open symbols denote controls (no *Daphnia*), gray symbols indicate *D. pulicaria* genotypes that are sensitive to toxic cyanobacteria, and black symbols indicate *D. pulicaria* genotypes that are tolerant of toxic cyanobacteria.
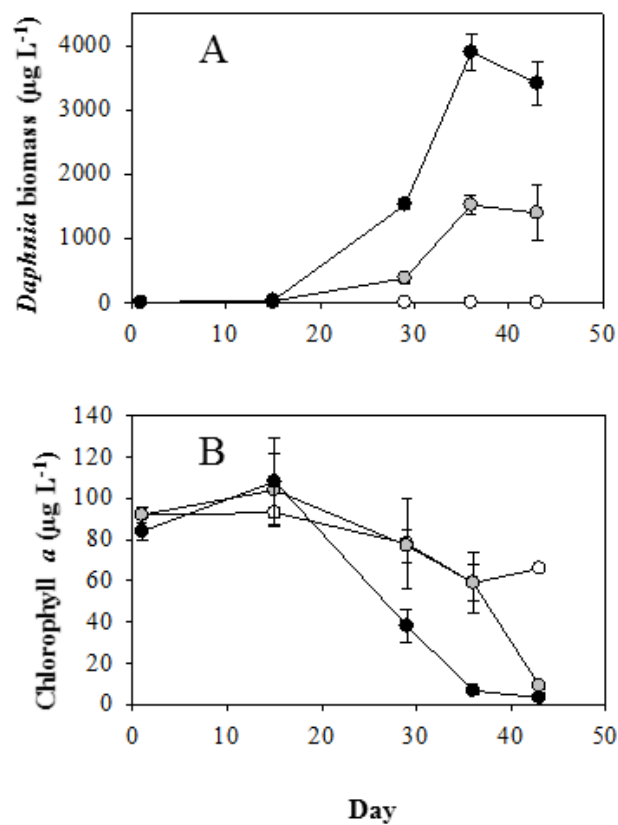
Figure J9. Dynamics of (A) *Daphnia pulicaria* biomass and (B) algal biomass as chlorophyll *a* in pond F20 (fall experiment). Data represent means ± SE. Open symbols denote controls (no *Daphnia*), gray symbols indicate *D. pulicaria* genotypes that are sensitive to toxic cyanobacteria, and black symbols indicate *D. pulicaria* genotypes that are tolerant of toxic cyanobacteria.

Figure J10. Dynamics of (A) *Daphnia pulicaria* biomass and (B) algal biomass as chlorophyll *a* in pond S9 (fall experiment). Data represent means ± SE. Open symbols denote controls (no *Daphnia*), gray symbols indicate *D. pulicaria* genotypes that are sensitive to toxic cyanobacteria, and black symbols indicate *D. pulicaria* genotypes that are tolerant of toxic cyanobacteria.
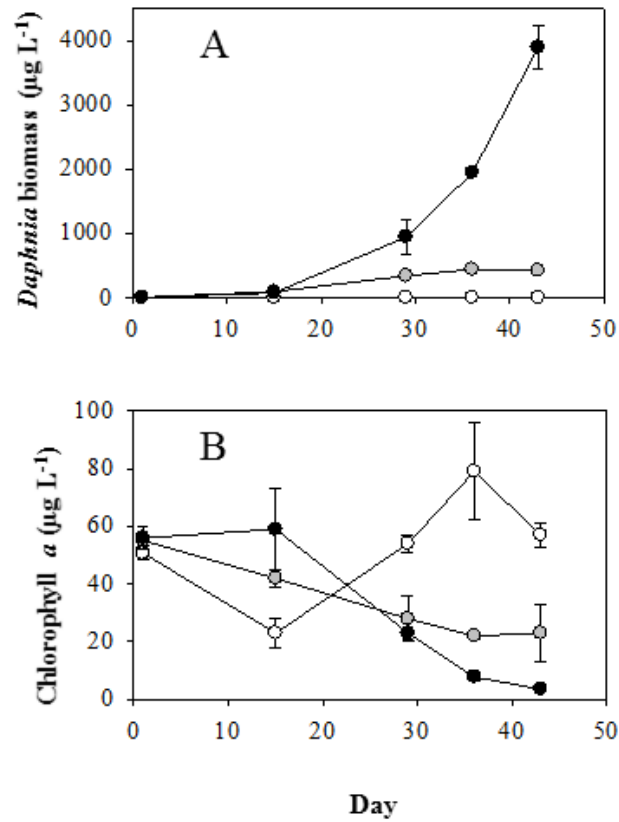
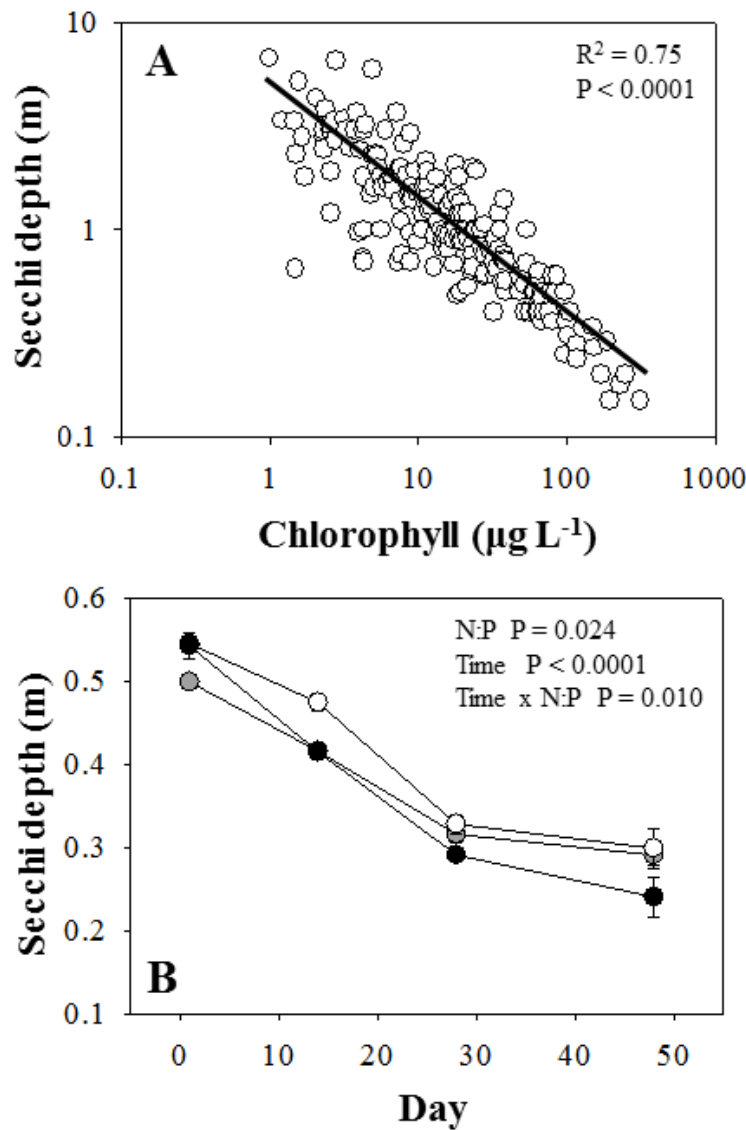**Appendix K. Secchi depth patterns in southeastern reservoirs and the enclosures during the N:P experiment.**

Figure K1. (A) Relationship between Secchi depth (m) and chlorophyll concentration ($\mu g\ L^{-1}$) in southeastern ponds and reservoirs. Log Secchi = (-0.5319 x log chlorophyll) + 0.6561 (unpublished data, A. E. Wilson). (B) Estimated Secchi depth (m) in the three N:P treatments during the experiment. Open symbols denote the ambient (40:1, by atoms) N:P treatment, gray symbols indicate the low (7:1) N:P treatment, and closed symbols indicate the high (122:1) N:P treatment. Repeated measures ANOVA results presented in panel.

Appendix L. Photos of enclosures during the experiment.

A **Start of experiment 10 June 2010**

B **End of experiment 29 July 2010**

40 N:P   100 N:P   7 N:P

Figure L1. Photos of the enclosures at the beginning (A) and end (B) of the experiment showing dense *Cylindrospermopsis* blooms in the higher N:P treatments.

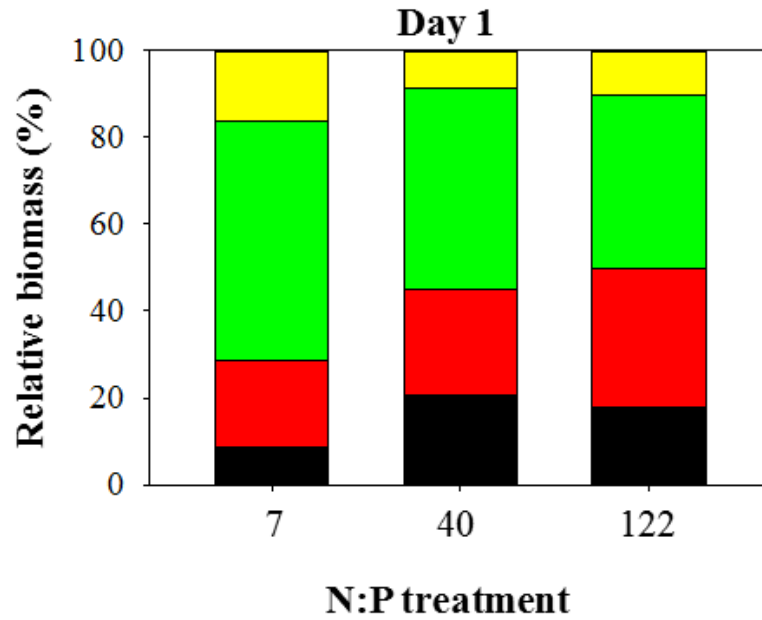**Appendix M. Initial phytoplankton composition in the enclosures**

Figure M1. Relative biomass of dominant phytoplankton taxa at the beginning of the experiment (day 1). Bar heights denote means for phytoplankton taxa. Black bars denote *Cylindrospermopsis*, red bars denote *Anabaena*, green bars denote chlorophytes, and yellow bars denote miscellaneous flagellates.

Appendix N. Statistical results (chapter 4).

Table N1. Repeated measures ANOVA tables for statistical results comparing the effects of *Daphnia pulicaria* genotype treatment, fertilization treatment, and their interaction across all sampling post-*Daphnia* treatment for (A) *Daphnia* biomass, (B) chlorophyll *a*, and (C) saxitoxin.

(A) *Daphnia* biomass data

Source of variation	F	P
Daphnia treatment	60.36	<0.0001
Fertilization treatment	23.81	0.001
Interaction	17.10	<0.0001

(B) Chlorophyll *a* data

Source of variation	F	P
Daphnia treatment	15.93	<0.0001
Fertilization treatment	172.74	<0.0001
Interaction	15.07	<0.0001

(C) Saxitoxin data

Source of variation	F	P
Daphnia treatment	8.50	0.018
Fertilization treatment	146.72	<0.0001
Interaction	17.10	0.018

Appendix O. Juvenile survivorship on *Cylindrospermopsis raciborskii* for the six genotypes of *Daphnia pulicaria* used in the field experiment.

Table O1. Juvenile survivorship (after 2 days) assays for the six genotypes of *Daphnia pulicaria* fed a diet of 100% saxitoxin-producing *Cylindrospermopsis raciborskii*. Survivorship in controls (*Ankistrodesmus* as food) was 100% for all genotypes. All six genotypes were isolated in 2009 from each of six small glacial lakes in southern Michigan. Three of the lakes are oligotrophic (based on total phosphorus (TP) concentration), while three lakes are moderately to highly eutrophic. All lakes were surveyed in 2009 and 2011, and TP represents the mean total phosphorus concentration (μg L^{-1}) measured in the spring of 2009 and 2011.

Source Lake (TP$_{spring}$)	Isolate designation	Survival on *Cylindrospermopsis* (SE)
Oligotrophic lake genotypes		
Bassett (10)	Dp-Ba-6	0.07 (0.07)
Lawrence (9)	Dp-La-6	0.13 (0.07)
Sherman (11)	Dp-Sh-7	0.47 (0.07)
Eutrophic lake genotypes		
Baseline (36)	Dp-Bs-13	0.93 (0.07)
3rd Sister (29)	Dp-3S-2	0.93 (0.07)
Wintergreen (33)	Dp-Wi-6	1.00 (0.00)