Consumer adaptation mediates top-down regulation across a productivity gradient

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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 (e.g., harmful algal blooms, toxic cyanobacteria), most studies tend to examine short-term responses relative to the time scales of heritable adaptive change. Thus, the potential role of adaptation by organisms in stabilizing the response of ecological systems to such perturbations is largely unknown. We tested the hypothesis that adaptation by a generalist consumer (*Daphnia pulicaria*) to toxic prey (cyanobacteria) mediates the response of plankton communities to nutrient enrichment. Overall, the strength of *Daphnia*'s top-down effect on primary producer biomass increased with productivity. However, these effects were contingent on prey traits (e.g., rare vs. common toxic cyanobacteria) and 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. In contrast, both tolerant and sensitive *Daphnia* genotypes had comparable effects on producer biomass when toxic cyanobacteria were absent. Our results demonstrate that organismal adaptation is critical for understanding and predicting ecosystem-level consequences of anthropogenic environmental perturbations.

Key Words: Eutrophication, nutrient enrichment, toxic cyanobacteria, microcystin, management, harmful algal blooms (HABs), nitrogen, phosphorus, top-down, bottom-up, consumer offense, evolution

Introduction

Humans have artificially enhanced the productivity of ecosystems (cultural eutrophication) across the globe (Vitousek et al. 1997; Pimentel and Edwards 1982). Indeed, cultural eutrophication is perhaps the most widespread of all human perturbations and 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 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 adaptations in response to past environmental changes (for example, in response to lake acidification (Gray and Arnott 2009)).

The response of primary productivity 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). In contrast, the importance of top-down control of natural 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), and the ecosystem regime shifts resulting from global declines in top consumers (Daskalov et al. 2007; Young et al. 2015). Moreover, consumers can sometimes dampen the positive response of primary producers to nutrient inputs (Carpenter et al. 1985; 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). Furthermore, the potential role of organismal adaptations in response to past environmental changes in mediating consumer versus resource control is largely unknown (but see Frisch et al. 2017, Weider et al. 2018).

Recent research has highlighted a wide array of adaptations by species in response to global changes (Carlsson et al. 2009; Lohbeck et al. 2012; Monchamp et al. 2017; 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 (Des Roches et al. 2017; Grant and Grant 2002; Hairston et al. 1999; Yousey al. 2018). 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). 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). Therefore, a fundamental question is whether intraspecific adaptive trait variation can in turn modulate the response of ecosystems to global change.

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 colonial and filamentous cyanobacteria (i.e., blue-green algae), the dominant taxa causing harmful algal blooms, with many of these taxa producing toxic secondary metabolites (Carmichael 1992). The general paradigm has been that cyanobacteria are largely resistant to grazing by herbivorous zooplankton, thus preventing effective 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). Most importantly, *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 extend our earlier findings documenting strong trait-based effects of *Daphnia* on toxic cyanobacteria (Chislock et al. 2013) to focus on the relative effects of different *Daphnia* genotypes (i.e., tolerant or sensitive to toxic prey) on algal biomass across a nutrient enrichment gradient, mimicking earlier meta-analytic studies of the effects of food-web structure (Mazumder 1994; Sarnelle 1992).

Our experiments were designed as a specific test of predator-prey theory as described in Sarnelle (1992). The difference in algal biomass in the presence versus absence of grazers such as *Daphnia* is expected to increase with system productivity when algae are edible, as *Daphnia* should reduce edible algae to similarly low levels regardless of nutrient enrichment. In contrast, the effect of *Daphnia* should be weaker when grazing-resistant algae are included in the model. In the previous meta-analysis (Sarnelle 1992), most *Daphnia* treatments were established indirectly via planktivorous fish manipulation.

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 induced by eutrophication (Table 1). Cyanobacteria tend to increase in dominance with nutrient enrichment (Downing et al. 2001) and during seasonal succession in eutrophic waterbodies (Sarnelle 2007). As expected, the prevalence of cyanobacteria varied both as a function of nutrient concentration and season across the ponds. During the spring experiment, grazing-resistant cyanobacteria were generally rare across all ponds, while cyanobacteria were common in the fall experiment, particularly in the most nutrient-rich ponds (Table 1). The experiments were designed to explicitly address (i) the magnitude of *D. pulicaria*'s effect on algal biomass across a productivity (i.e., TP) gradient; (ii) if this effect depended on *D. pulicaria* adaptation to toxic cyanobacteria; and (iii) whether the magnitude of these effects varied seasonally as a function of cyanobacterial presence. We were also interested in comparing the relationship between *D. pulicaria* biomass and productivity for the contrasting *D. pulicaria* treatments, as a means of assessing food-web structure.

Methods

Daphnia pulicaria *genotypes used in the experiments*

Based on past work by our research group and others (e.g., Chislock et al. 2013; Hairston et al. 2001; Sarnelle and Wilson 2005), we expected that *Daphnia* genotypes isolated from nutrient-poor lakes with low chlorophyll *a* (and few cyanobacteria) would be inherently more sensitive to toxic cyanobacteria than genotypes from nutrient-rich lakes with high chlorophyll *a* (and moderate to high levels of cyanobacteria). We validated this assumption with laboratory experiments measuring the sensitivity of *Daphnia* neonates to a diet of toxic cyanobacteria (Hairston et al. 2001; Sarnelle and Wilson 2005).

Each of the eight *D. pulicaria* genotypes used in these experiments originated from a single adult female or hatched ephippial egg collected from seven small (<0.3 km^2) lakes of varying productivity (Table 2). Two of the tolerant *D. pulicaria* clones used in experiments were from the same lake (Duncan: clones 1 and 2). Each of the seven lakes was surveyed during the spring (May-June) of 2009 and 2011 to measure total phosphorus (TP) in the mixed layer to estimate potential productivity and again during the summer (August) of 2009 and 2011 to quantify algal biomass (as chlorophyll *a*) and microcystin levels. Four of the lakes are oligotrophic (based on TP) with few cyanobacteria, while three lakes are moderately to highly eutrophic with variable cyanobacterial abundance during the summer (Table 2).

We subsequently assessed the sensitivity of these clones by comparing their somatic growth rates when fed edible green algae (*Ankistrodesmus*) vs. potentially toxic cyanobacteria (*Microcystis*). Juvenile growth rates of each genotype on diets consisting of 100% *Ankistrodesmus falcatus* (a nutritious green algal) or 100% *Microcystis aeruginosa* (UTEX 2667; toxic) were compared for each of the genotypes in a laboratory experiment using previously published methods (Sarnelle and Wilson 2005). Briefly, somatic growth rates for neonates (<24 hours old) were 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*). T-tests were used to compare growth rates on both diets and relative growth inhibition of *D. pulicaria* genotypes collected from the two lake types.

All *Daphnia* 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 to remove competing zooplankton and large phytoplankton. 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.

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 = 10-334 µg L^{-1}) during the fall (October-November). For logistical reasons, we were unable to use the same ponds in the fall experiment as in the spring experiment. 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 by 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, and were, more common in the fall vs. spring.

Experiments were conducted in mesocosms (160-L) which consisted of polyethylene barrels (0.5 m diameter × 0.8 m deep) that were open to the atmosphere, closed at the bottom, and 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 four sensitive or four tolerant *D. pulicaria* clones (Table 2) at very low densities (total density per enclosure: 0.1 animals L^{-1}) into each of three replicate mesocosms for treatments containing *Daphnia*. 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*), total phosphorus, 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 4ºC 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). Biovolume for dominant taxa was calculated using cell counts and estimates of cell volume based on measurements of cell dimensions. 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 0.40 (Chislock et al. 2014). Microcystin concentrations in whole-water samples at the start of the experiment 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 A). We then calculated mean ARF for each enclosure over the final two weeks. Larger ARF values represent greater reductions in algae compared to the control treatments without grazers.

We were interested in the magnitude of *Daphnia* effects on phytoplankton biomass across a nutrient (TP) gradient. For our model, we had clustered data with two-level nesting (*Daphnia* genotype = level 1; pond = level 2). Therefore, we used a mixed modeling approach, which accounted for this nesting, to determine the effects of mean TP (average TP in each mesocosm over the final two weeks), *Daphnia* type (sensitive or tolerant), and their interaction on ARF, absolute chlorophyll *a*, and *Daphnia* biomass. We log-transformed TP and each of the three response variables to meet the assumptions of the model (normality and homogeneity of variance). We also initially 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), but this factor was not significant. We initially fit a full model with season of experiment as a factor and found that the effect of season of experiment was statistically significant ($P = 0.0042$). For ease of interpretation and to simplify the analysis, we thus present separate models for the spring and fall experiments.

To simplify our data for visualization (i.e., Figure 1), we averaged values across replicates over the final two weeks of the experiment when *Daphnia* populations had stabilized and plotted these means ±1 standard error. Power functions ($y = ax^b$) were then fit for each treatment, with x representing TP and y corresponding responses. Regression lines are shown when statistically significant ($P < 0.05$).

Results

Relative growth inhibition of Daphnia pulicaria *genotypes used in the experiments*

Somatic growth rates of *D. pulicaria* neonates fed a diet of the nutritious green alga *A. falcatus* were similar for genotypes collected from high-nutrient lakes versus low-nutrient lakes ($T_6 = -0.717$, $P = 0.500$). In contrast, *D. pulicaria* genotypes from high-nutrient lakes had higher juvenile somatic growth rates ($T_5 = 3.106$, $P = 0.027$) and showed less relative growth inhibition ($T_5 = 3.117$, $P = 0.026$) on the potentially toxic diet of *M. aeruginosa*, when compared to *D. pulicaria* genotypes from low-nutrient lakes. Therefore, *D. pulicaria* genotypes from high-nutrient lakes were more tolerant of toxic *Microcystis* in the diet than genotypes from low-nutrient lakes. Two of the “tolerant” genotypes did have negative somatic growth rates on a 100% *Microcystis* diet. However, growth rates of these two genotypes on *Microcystis* were still higher and relative growth inhibition lower than for all of the four “sensitive” genotypes. In nature, phytopankton communities at high TP are generally *dominated* by toxic cyanobacteria rather than being 100% toxic *Microcystis*, so our laboratory assessments are an extreme test of tolerance.

Mesocosm experiments.

Total phosphorus (TP) concentration across ponds ranged from 10 to 334 μg L^{-1}, with cyanobacteria and associated cyanotoxins being rare in the spring (Table 2A) and prevalent in the fall (Table 2B). In the spring experiments, the effect of *Daphnia* on algal biomass (ARF) ranged from ~2.2 to 161, was a positive function of TP (Table 3A, $P < 0.0001$), and was independent of *Daphnia* genotype (Figure 1A; Table 3A; $P = 0.16$). In the absence of *Daphnia,* algal biomass measured as chlorophyll *a* increased linearly with TP (Table 3B; Figure 1B; $P = 0.0065$). In contrast, chlorophyll *a* was depressed to a flat line of similarly low concentrations across the TP gradient in the presence of both sensitive and tolerant *D. pulicaria* (Table 3B; Figure 1B). Both sensitive and tolerant *D. pulicaria* biomass similarly increased with TP until reaching an asymptote at the highest TP concentrations (~100 ug/L) in the spring (Figure 1B; Table 3B; Genotype P = 0.61; TP P = 0.05).

Daphnia effects on algal biomass also increased with TP in the fall (Figure 1A; Figure 1B; Table 4A; $P < 0.0001$), but effects across the TP gradient were highly contingent on consumer genotypes. Effects of the two *Daphnia* types were similar in magnitude at low TP (TP < 22 $\mu g\ L^{-1}$) but tolerant *D. pulicaria* genotypes had up to a 10-fold larger effect than sensitive genotypes at high TP (Table 4A; Genotype x TP interaction P = 0.0081). In the fall experiment, chlorophyll *a* increased with TP in both the control and sensitive *D. pulicaria* treatments (Fig. 1E). In contrast, tolerant *D. pulicaria* depressed chlorophyll *a* to similarly low levels across the TP gradient (Fig. 1E; Table 4B). Increasingly larger effects of tolerant *D. pulicaria* with increasing TP were associated with higher biomass of tolerant *D. pulicaria* at high TP. In contrast, biomass of sensitive *D. pulicaria* was similarly low across the TP gradient (Figure 1F; Table 4; Genotype x TP interaction P = 0.0048).

At the conclusion of the experiment, *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 (Table 5A). A similar pattern was also observed for mean *Cylindrospermopsis* filament length in the two ponds where this taxon was present (Table 5B).

Discussion

The results of these two 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 interacts with phytoplankton composition (comparing spring and fall results). For example, lower biomass of sensitive *D. pulicaria*, and hence their inability to control phytoplankton biomass, in the fall was likely a direct consequence of reduced neonate survival (and perhaps adult fecundity) in the presence of grazing-resistant, toxic prey (Chislock et al. 2013). In ponds with extreme levels of toxic cyanobacteria and the cyanobacterial toxin, microcystin, the effect of tolerant versus sensitive *D. pulicaria* genotypes becomes as large as the effect of *D. pulicaria* presence/absence (Chislock et al. 2013).

Daphnia effects on algal biomass in spring and fall were standardized, relative to no *Daphnia* controls, by calculating algal response factors (ARF), with larger ARF values indicating greater reductions in algae compared to no *Daphnia* controls. 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 as *Daphnia* should reduce phytoplankton biomass to similar levels (i.e., *Daphnia's* R*, Tilman 1982; Gliwicz 1990), assuming that all phytoplankton are edible (Sarnelle 1992). However, phytoplankton biomass at a given TP concentration in controls was comparable for the spring and fall (Figure 1B, E), thus we found no support for this mechanism. Secondly, grazing-resistant taxa were more abundant in the fall, and some cyanobacterial filaments and colonies were 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. A similar pattern was also observed for mean *Cylindrospermopsis* filament lengths. These data suggest that tolerant *D. pulicaria* can shift the colony size spectrum upward through selective grazing. Larger overall colony size as a result of grazing by tolerant *D. pulicaria* may have important long-term implications for trophic structure and ecosystem function in lakes as these size-resistant phytoplankton could lead to decreased zooplankton:phytoplankton biomass (Higgins et al. 2014; Birtel and Matthews 2016; Heathcote et al. 2016). Future studies examining subsequent cyanobacterial responses (e.g., increased size and growth rates) to increased grazing pressure by adapted zooplankton will likely be informative in exploring the longer term consequences of this potential arms race.

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 lakes (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). In both fall and spring experiments, the negative effects of *Daphnia* on algal prey increased across the productivity gradient (i.e., larger ARF at higher TP; Figure 1). However, *Daphnia* adaptation as well as algal prey composition influenced the magnitude of these effects. In the spring, when there were few cyanobacteria (Table 1), overall *Daphnia* effects (i.e., ARF at a given TP) were larger than in the fall, when cyanobacteria were abundant. Therefore, our results also support the prediction that 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 within each season (i.e., spring and fall) increased with productivity even in the presence of grazing-resistant prey (i. e., colonial and filamentous cyanobacteria), with the magnitude of consumer effects during the fall 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 of similar 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) 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, which, in contrast to specialist consumers, can have ecosystem-level effects. Thus, adaptations by generalist consumers cannot be ignored in studies of food web interactions and may play an increasingly important role, particularly in disturbed ecosystems. 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).

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Tables

Table 1. Enclosure 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 (A) spring and (B) fall experiments. Ponds are listed in rank order of increasing productivity

(A)

Pond	TP ($\mu g\ L^{-1}$)	Chl *a* ($\mu g\ L^{-1}$)	Percent cyanobacteria	Cyanobacterial dry 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

(B)

Pond	TP ($\mu g\ L^{-1}$)	Chl *a* ($\mu g\ L^{-1}$)	Percent cyanobacteria	Cyanobacterial dry 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

Table 2. 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 seven small glacial lakes (Bassett, Eagle, Lawrence, Sherman, MSU, Kent, and Duncan) in southern Michigan. Four 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 ($\mu g\ L^{-1}$)	Chl *a* ($\mu g\ L^{-1}$)	Microcystin ($\mu g\ L^{-1}$)	Growth Rate Ank, Micro (d^{-1})	Relative growth inhibition
Sensitive genotypes:					
Bassett Lake	10	8	0.005	0.47, -0.24	1.51
Eagle	10	5	0.006	0.34, -0.14	1.40
Lawrence Lake	13	4	0.004	0.36, -0.11	1.30
Sherman Lake	8	5	0.010	0.41, all died	*N/A
Tolerant genotypes:					
MSU Lake 1	71	60	0.500	0.81, 0.07	0.92
Kent Lake	25	10	0.024	0.30, -0.06	1.20
Duncan Lake (clone 1)	62	37	0.016	0.35, -0.04	1.12
Duncan Lake (clone 2)				0.46, 0.11	0.75

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

Table 3. Statistical results for the spring experiment for the mixed model examining the effects of *Daphnia pulicaria* genotype, total phosphorus, and their interaction on (A) the algal response factor (ARF), which compares the magnitude of *Daphnia* effects on phytoplankton biomass as chlorophyll *a*, (B) absolute chlorophyll *a* concentrations, and (C) *Daphnia* biomass. Data were averaged over the final two weeks of the experiment when *D. pulicaria* populations had stabilized. Models for (A) and (C) compared sensitive and tolerant *Daphnia* treatments. The mixed model for (B) compared control, sensitive, and tolerant *Daphnia* treatments

(A) ARF

Fixed effects	Estimate	SE	df	T	p-value
Genotype treatment	0.15	0.09	4	1.72	0.16
log (total phosphorus)	2.48	0.33	19	2.67	<0.0001
Interaction	1.17	0.73	18	1.60	0.13

Random effects	σ	Percent of total variance
Pond	0.08	25
Genotype treatment in pond	1.06×10^{-5}	0
Residual	0.24	75

(B) Chlorophyll *a*

Fixed effects	Estimate	SE	df	T	p-value
Tolerant *Daphnia* treatment	1.15	1.00	8	1.15	0.28
Sensitive *Daphnia* treatment	3.10	0.85	8	3.63	0.0067
log (total phosphorus)	1.25	0.42	22	3.01	0.0065
Interaction (Tolerant)	-1.22	0.55	22	-2.22	0.037
Interaction (Sensitive)	-2.39	0.46	22	-5.17	<0.0001

Random effects	σ	Percent of total variance
Pond	0.16	37
Genotype treatment in pond	0.098	22
Residual	0.18	41

(C) *Daphnia* biomass

Fixed effects	Estimate	SE	df	T	p-value
Genotype treatment	-0.03	0.05	4	-0.56	0.61
log (total phosphorus)	0.76	0.37	19	2.06	0.05
Interaction	0.20	0.33	18	0.59	0.56

Random effects	σ	Percent of total variance
Pond	0.19	58
Genotype treatment in pond	4.81×10^{-7}	0
Residual	0.14	42

Table 4. Statistical results for the fall experiment for the mixed model examining the effects of *Daphnia pulicaria* genotype, total phosphorus, and their interaction on (A) the algal response factor (ARF), which compares the magnitude of *Daphnia* effects on phytoplankton biomass as chlorophyll *a*, (B) absolute chlorophyll *a* concentrations, and (C) *Daphnia* biomass. Data were averaged over the final two weeks of the experiment when *D. pulicaria* populations had stabilized. Models for (A) and (C) compared sensitive and tolerant *Daphnia* treatments. The mixed model for (B) compared control, sensitive, and tolerant *Daphnia* treatments

(A) ARF

Fixed effects	Estimate	SE	df	T	p-value
Genotype treatment	0.51	0.34	4	1.49	0.21
log (total phosphorus)	0.81	0.14	18	5.95	<0.0001
Interaction	-0.52	0.17	18	-2.97	0.0081

Random effects	σ	Percent of total variance
Pond	0.07	22
Genotype treatment in pond	0.12	38
Residual	0.13	40

(B) Chlorophyll *a*

Fixed effects	Estimate	SE	df	T	p-value
Tolerant *Daphnia* treatment	0.62	0.27	8	2.28	0.05
Sensitive *Daphnia* treatment	-0.05	0.28	8	-0.18	0.86
log (total phosphorus)	0.40	0.12	22	3.32	0.0031
Interaction (Tolerant)	-0.74	0.14	22	-5.22	<0.0001
Interaction (Sensitive)	-0.13	0.14	22	-0.94	0.36

Random effects	σ	Percent of total variance
Pond	0.20	48
Genotype treatment in pond	0.091	21
Residual	0.13	31

(C) *Daphnia* biomass

Fixed effects	Estimate	SE	df	T	p-value
Genotype treatment	0.32	0.26	4	1.23	0.29
log (total phosphorus)	0.74	0.17	18	4.41	0.0003
Interaction	-0.43	0.13	18	-3.22	0.0048

Random effects	σ	Percent of total variance
Pond	0.18	47
Genotype treatment in pond	0.04	11
Residual	0.16	42

Table 5. (A) Mean *Microcystis* colony size (mean equivalent spherical diameter – ESD ± 1 standard error – SE) and (B) mean *Cylindrospermopsis* filament length (length ± 1 standard error – SE) at the end of the fall experiment. *Cylindrospermopsis* was absent in ponds FP14, F9, and F20. Ponds are listed in rank order of increasing total phosphorus

(A)

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
S9	36 ± 6	66 ± 3	82 ± 3

(B)

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

Figure Legends

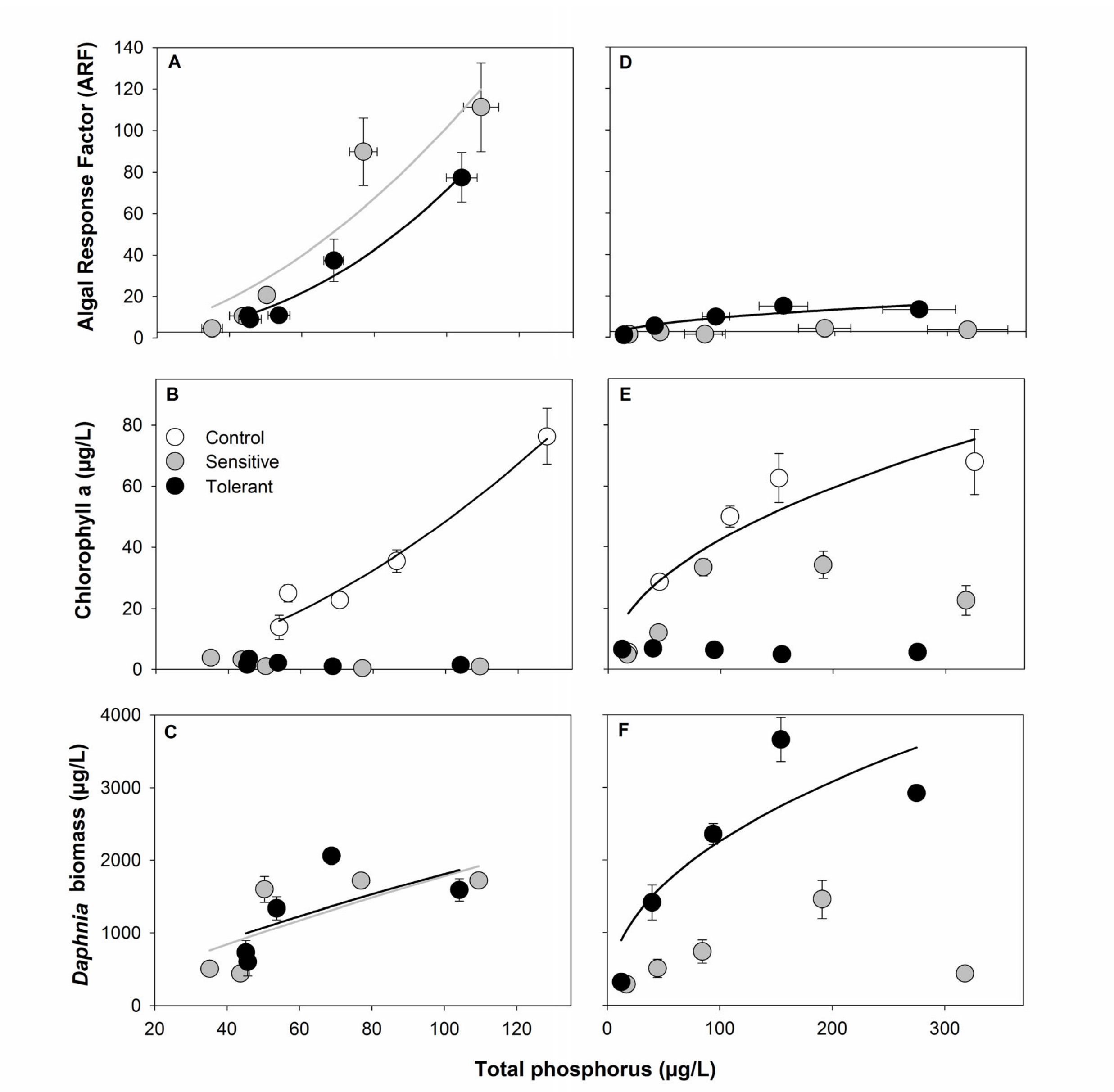
Fig. 1 Relationship between total phosphorus (μg L^{-1}) and (A) the magnitude of *Daphnia pulicaria* effects on phytoplankton biomass as chlorophyll *a* (i.e., algal response factor), (B) absolute chlorophyll *a* concentrations (μg L^{-1}), and (C) *Daphnia* biomass (μg L^{-1}) for the spring experiment when cyanobacteria were rare, and the fall experiment when cyanobacteria were common (D, E, F). White symbols (B and D panels only) indicate no *Daphnia* controls, 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. Data were averaged across replicates over the final two weeks of the experiment when *Daphnia* populations had stabilized, with points representing pond means ±1 standard error for each treatment. Standard errors for TP of treatments are indicated in the horizontal direction for panels (A) and (D) for simplicity. Power functions ($y = ax^b$) were fit for each treatment, and regression lines are shown when statistically significant ($P < 0.05$). Separate regression lines are shown for sensitive and tolerant *D. pulicaria* in the spring (A, C) 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.

<u>Regression equations for spring experiment:</u>

$ARF_{sensitive} = 0.022 \times TP^{1.84}$, $R^2 = 0.89$; $ARF_{tolerant} = 0.0016 \times TP^{2.33}$, $R^2 = 0.97$
$Chlorophyll_{control} = 0.013 \times TP^{1.79}$, $R^2 = 0.97$
$Daphnia_{sensitive} = 40.83 \times TP^{0.82}$, $R^2 = 0.58$; $Daphnia_{tolerant} = 55.63 \times TP^{0.76}$, $R^2 = 0.43$

<u>Regression equations for fall experiment:</u>
$ARF_{tolerant} = 0.97 \times TP^{0.50}$, $R^2 = 0.83$
$Chlorophyll_{control} = 4.55 \times TP^{0.49}$, $R^2 = 0.86$
$Daphnia_{tolerant} = 289.1 \times TP^{0.44}$, $R^2 = 0.77$

Figure 1


A
B
C
D
E
F
Algal Response Factor (ARF)
Chlorophyll a (µg/L)
Daphnia biomass (µg/L)
Control
Sensitive
Tolerant
Total phosphorus (µg/L)