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Do high concentrations of microcystin prevent *Daphnia* control of phytoplankton?

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ABSTRACT

Toxin-producing cyanobacteria have frequently been hypothesized to limit the ability of herbivorous zooplankton (such as *Daphnia*) to control phytoplankton biomass by inhibiting feeding, and in extreme cases, causing zooplankton mortality. Using limnocorral experiments in hyper-eutrophic ponds located in Alabama and Michigan (U.S.A.), we tested the hypothesis that high levels of cyanobacteria and microcystin, a class of hepatotoxins produced by several cyanobacterial genera, prevent *Daphnia* from strongly reducing phytoplankton abundance. At the start of the first experiment (Michigan), phytoplankton communities were dominated by toxic *Microcystis* and *Anabaena* (~96% of total phytoplankton biomass), and concentrations of microcystin were ~3 μg L^{-1}. Two weeks after adding *Daphnia pulicaria* from a nearby eutrophic lake, microcystin levels increased to ~6.5 μg L^{-1}, yet *Daphnia* populations increased exponentially ($r = 0.24$ day^{-1}). By the third week, *Daphnia* had suppressed phytoplankton biomass by ~74% relative to the no *Daphnia* controls and maintained reduced phytoplankton biomass until the conclusion of the five-week experiment. In the second experiment (Alabama), microcystin concentrations were greater than 100 μg L^{-1}, yet a mixture of three *D. pulicaria* clones from eutrophic lakes in southern MI increased and again reduced phytoplankton biomass, in this case by over 80%. The ability of *Daphnia* to increase in abundance and suppress phytoplankton biomass, despite high initial levels of cyanobacteria and microcystin, indicates that the latter does not prevent strong control of phytoplankton biomass by *Daphnia* genotypes that are adapted to environments with abundant cyanobacteria and associated cyanotoxins.

1. Introduction

Cyanobacteria are the most important taxa causing harmful algal blooms (HABs) in freshwater ecosystems, and cyanobacterial blooms are thought to be increasing in intensity and frequency as a consequence of the combined effects of eutrophication, global warming, and exotic species invasion (Smith and Schindler, 2009). Cyanobacterial blooms pose a serious threat to freshwater ecosystems by producing toxic secondary metabolites which have been shown to cause mortality in pets, livestock, and humans (Carmichael, 1992). Consequently, water resource managers have employed a variety of strategies aimed at controlling cyanobacterial blooms, including reducing nutrient inputs, using potent herbicides, disrupting stratification via mixing, and shading waterbodies with opaque liners or water-based stains (Schindler, 2006).

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Despite large-scale efforts to reduce nutrient enrichment, cultural eutrophication and concomitant HABs continue to be the leading cause of water pollution for many freshwater and coastal marine ecosystems and are a rapidly growing problem in the developing world (Smith and Schindler, 2009).

Biomanipulation – the alteration of a food-web to improve ecosystem health – has been studied in disparate communities since the concept was first introduced as an alternative approach to manage nutrient-rich freshwater lakes (Shapiro et al., 1975). In lakes, the basic premise is to manipulate higher trophic levels (adding piscivores or removing planktivores) to increase the size, abundance, and grazing pressure of herbivorous zooplankton and thus reduce algal abundance (Shapiro et al., 1975). The presence of the large generalist herbivore, *Daphnia*, seems to be critically important to the success of biomanipulation efforts (Leibold, 1989).

With respect to grazing, cyanobacteria have been shown to be poor quality food for most zooplankton, with most studies focusing on *Daphnia* (Wilson et al., 2006a, b; Wilson and Hay, 2007; Tillmanns et al., 2008). Numerous laboratory studies have also demonstrated that cyanobacteria can inhibit *Daphnia* feeding, growth, survival, and reproduction, and the production of toxic secondary metabolites has been hypothesized to mediate these negative effects (Gliwicz and Lampert, 1990; DeMott et al., 1991; Lürling and Van Der Grinten, 2003). Therefore, an important effect of cyanobacterial dominance with nutrient enrichment is that phytoplankton communities tend to be dominated by harmful species that are relatively resistant to zooplankton grazing (Sommer et al., 1986; Ghadouani et al., 2003). Extrapolating from past laboratory studies, it has been suggested that cyanobacteria are a major factor mediating declines in *Daphnia* with eutrophication, as a consequence of their negative effects on *Daphnia* fitness (Threlkeld, 1979; Sommer et al., 1986; Ghadouani et al., 2003). Therefore, the presence of toxic cyanobacteria may limit the success of biomanipulation efforts.

However, recent research has shown that local populations of *Daphnia* may evolve or otherwise adapt to tolerate toxin-producing cyanobacteria in the diet (Hairston et al., 1999; Gustafsson and Hansson, 2004; Sarnelle and Wilson, 2005). Genetically-based tolerance to toxic *Microcystis* has been observed within species of both North American and European *Daphnia* across habitats varying in nutrient enrichment (Sarnelle and Wilson, 2005) and over time in a eutrophied lake (Hairston et al., 1999, 2001). The evolution of tolerance to toxic cyanobacteria by *Daphnia* may have important consequences for the response of lakes to nutrient enrichment and for biomanipulation efforts to improve water quality in eutrophic lakes (Hairston et al., 2001; Sarnelle and Wilson, 2005).

To date, numerous studies demonstrate that *Daphnia* can greatly reduce the abundance of phytoplankton, including bloom-forming cyanobacteria, in eutrophic lakes, when freed from predation by planktivorous fish (Sarnelle, 1992, 2007; Mazumder, 1994). However, whether *Daphnia* can control toxic cyanobacteria continues to be a controversial question. Toxin-producing cyanobacteria have frequently been hypothesized to limit the ability of *Daphnia* to control phytoplankton (and cyanobacterial) biomass (Porter, 1977). However, recent meta-analyses of laboratory zooplankton-cyanobacteria experiments show that the role of cyanobacterial toxins as anti-herbivore chemical defenses is ambiguous, at best (Wilson et al., 2006a,b; Tillmanns et al., 2008). There is evidence that *Daphnia* may promote dominance by grazing-resistant cyanobacteria (Carpenter et al., 1995; Brett and Goldman, 1997; Ghadouani et al., 2003), but there are also several well-documented examples in which *Daphnia* have greatly reduced the abundance of cyanobacteria in the field (Lynch and Shapiro, 1981; Vanni et al., 1990; Sarnelle, 2007). The presence (or absence) of cyanobacterial toxins may help to explain the variable suppression of cyanobacterial abundance observed in previous studies. Here we describe two field experiments testing the hypothesis that high levels of toxic *Microcystis* (and microcystin) prevent a large species of *Daphnia* from suppressing cyanobacterial and total phytoplankton biomass. Most previous related field experiments were conducted prior to the advent of simple methods for measuring microcystin (but see Ghadouani et al., 2003).

2. Materials and methods

2.1. Study sites

The first experiment (Experiment 1) was conducted at Michigan State University, Michigan, in a small (~3 ha), man-made lake (MSU Lake 2). MSU Lake 2 is shallow (maximum depth = 2 m), polymictic, and highly eutrophic (total phosphorus concentrations average ~120 μg L^{-1} (O. Sarnelle unpubl.) by virtue of its initial use in a project to treat secondary sewage effluent (Spencer, 1981). Cyanobacteria begin to dominate the phytoplankton in late May, and *Microcystis* typically accounts for up to 90% of total phytoplankton biomass by August or September, although successional dynamics at the species level are variable from year-to-year (O. Sarnelle unpubl.).

The second experiment (Experiment 2) was conducted at the E.W. Shell Fisheries Research Station at Auburn University, Alabama, in two small, hypereutrophic ponds (F19 and F20). Both are highly productive and intensively managed for catfish aquaculture (e.g., mechanical aeration and daily feeding of fish). Furthermore, both ponds are shallow and polymictic with surface areas of approximately 0.1 ha, maximum depths of 2 m, and total phosphorus concentrations averaging about 650 and 1000 μg L^{-1}, respectively (Boyd and Shelton, 1984; M. F. Chislock and A. E. Wilson unpubl.). Cyanobacteria dominate phytoplankton communities throughout much of the year in both of these highly enriched systems, and *Microcystis* typically accounts for up to 100% of total phytoplankton biomass by August or September (M. F. Chislock and A. E. Wilson unpubl.).

2.2. Daphnia sources

Daphnia for both experiments originated from single adult females or hatched ephippial eggs that were collected from small (<0.3 km^2), eutrophic lakes in southern Michigan (Table 1). All clones were identified as *Daphnia pulicaria* on the basis of the postabdomen of the male (Hebert, 1995; Sarnelle and Wilson, 2005). However, this method is insufficient to distinguish between *D. pulicaria* and *D. pulicaria* × *pulex* hybrids, thus some of the clones could be hybrids. The *D. pulicaria* clone used in Experiment 1 originated from

Table 1 – Source lakes of the four *D. pulicaria* clones used in Experiments 1 and 2. All lakes are located in southern Michigan.

Experiment	Lake	Location (Lat. N, Long. W)	Total phosphorus ($\mu g\ L^{-1}$)
1	MSU Lake 1	42°40′53″, 84°28′57″	170–300
2	Baseline	42°25′24″, 85°51′8″	32
	Third Sister	42°16′34″, 83°48′22″	45
	Wintergreen	42°23′50″, 85°23′07″	50–70

a small, fishless eutrophic lake just upstream from MSU Lake 2 (MSU Lake 1) on 15 June 2007. In Experiment 2, we stocked equal densities of three *D. pulicaria* clones that were each descendants of one female isolated in 2010 from each of three larger eutrophic lakes in southern Michigan (Table 1). Prior to both field experiments, each *D. pulicaria* clone was mass cultured on a diet of the nutritious green alga, *Ankistrodesmus*, for several generations before being stocked into enclosures. Previous laboratory feeding studies have demonstrated that the *Daphnia* clones used in both experiments survive and grow on a diet containing growth-saturating concentrations (1 mg C L^{-1} – Lampert, 1977) of 100% toxic *Microcystis aeruginosa* (strain UTEX2667 – Sarnelle and Wilson, 2005; Wilson unpubl.).

2.3. Enclosure experiments

Experiment 1 was conducted in 4200-L, clear polyethylene enclosures that were sealed at the bottom, open to the atmosphere, and suspended from a floating platform (EZ-Dock) anchored in MSU Lake 2. Eight enclosures were filled on 2 July 2007 ('day 1') by pumping pond water through a 75-μm mesh net to initially exclude resident large zooplankton, including *Daphnia*. We then stocked half of the enclosures with *D. pulicaria* on 2 July 2007 to achieve initial densities of ~0.3 animals L^{-1}. Two *D. pulicaria* enclosures were damaged allowing planktivorous fish to invade. As a result, only two *D. pulicaria* enclosures were available for this experiment. We sampled the enclosures weekly from 2 July (prior to adding *Daphnia*) to 6 August 2007.

Experiment 2 was conducted in 160-L plastic enclosures that were secured to a floating PVC frame anchored in ponds F19 and F20. Four enclosures in each pond were filled on 22 September 2010 ('day 1') by pumping water through a 200-μm mesh net to initially exclude large resident zooplankton, including *Daphnia*, from treatments. We then stocked half of the enclosures with a mixture of equal densities of the three *D. pulicaria* clones (Table 1) on 1 October 2010 to achieve an initial total *Daphnia* density of ~0.15 animals L^{-1}. All enclosures in this experiment were fertilized biweekly with potassium nitrate and phosphate at a rate of 20% of the ambient total nitrogen and phosphorus concentrations to maintain high densities of toxic cyanobacteria. We sampled the enclosures on 29 September 2010 and then weekly from 10 November to 24 November 2010.

2.4. Sample collection and data analysis

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) for both experiments. Chlorophyll *a* concentrations were measured by extracting phytoplankton collected on Pall A/E filters in 90% ethanol for 24 h in the dark at 4 °C followed by measurement with a fluorometer (Sartory and Grobbelaar, 1984). Microcystin concentrations in particles were quantified using enzyme-linked immunosorbent assay (ELISA) (An and Carmichael, 1994) after extraction from filters with 75% aqueous methanol. Phytoplankton species abundance and composition were determined for selected dates via the inverted microscope technique (Utermöhl, 1958) using water samples preserved in 1% Lugol's solution. Biovolume for each species was calculated using cell counts and estimates of cell volume based on measurements of cell dimensions. We then converted biovolume ($mm^3\ L^{-1}$) to dry biomass ($\mu g\ L^{-1}$) 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). Using these data, we estimated microcystin content of the phytoplankton (i.e., dietary quota for *Daphnia*) or *Microcystis*, respectively, by dividing microcystin concentration by total phytoplankton dry biomass or *Microcystis* dry biomass and expressed both values as percentages. *Daphnia* were measured and counted at 40× 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).

For both experiments, the effects of *D. pulicaria* on chlorophyll *a* and microcystin concentrations were tested using repeated measures analysis of variance (ANOVA, sampling date = repeated measure) across all dates except the pretreatment sampling. ANOVA was used to analyze treatment effects on phytoplankton community composition for day 28 (30 July 2007) for Experiment 1 and day 50 (17 November 2010) for Experiment 2. We used a *t*-test to analyze treatment effects on mean microcystin content in the control and *Daphnia* enclosures for these same dates. Population growth rates for *D. pulicaria* in Experiment 1 were calculated as the slope of the linear regression of the natural logarithm of *D. pulicaria* density versus day of experiment during the period when populations were increasing (9 July to 23 July 2007). *Daphnia* population growth rates could not be reliably estimated in Experiment 2 because the early sampling intervals were too coarse. To compare *Daphnia* effects on total phytoplankton biomass for Experiments 1 and 2, we calculated effect sizes by dividing the mean chlorophyll *a* concentration (over the last three sampling dates) in control enclosures by the mean chlorophyll *a* concentration in enclosures with *D. pulicaria* (Algal Response Factor – ARF, Sarnelle, 1992). All data were checked for normality using quantile–quantile plots, homogeneity using residual plots, and log-transformed when necessary.

3. Results

3.1. Experiment 1

At the start of Experiment 1, the phytoplankton community in the enclosures was comprised almost entirely of cyanobacteria, and *Microcystis* and *Anabaena* accounted for >96% of

total phytoplankton biomass. Initial phytoplankton and cyanobacterial biomass (chlorophyll *a* = 130 μg L^{-1} and cyanobacterial biomass = 19,000 μg L^{-1}) were high relative to other eutrophic lakes in Michigan (Raikow et al., 2004), and microcystin concentration was above 3 μg L^{-1}. Initial microcystin content was 0.02% relative to total phytoplankton dry biomass, and 0.03% relative to *Microcystis* dry biomass. Initial phytoplankton in Experiment 1 were largely comprised of small colonies and filaments of edible size with respect to *Daphnia* (Burns, 1968; DeMott, 1995) with a mean (±standard deviation) equivalent spherical diameter (ESD) for *Microcystis* colonies of 28 (±5) μm.

Over the first two weeks of the experiment, microcystin levels increased to approximately 6 μg L^{-1} in enclosures with *D. pulicaria*, yet *D. pulicaria* populations increased exponentially ($r = 0.24$ day^{-1}, SE = 0.06 day^{-1}) (Fig. 1). By the third week, *D. pulicaria* had suppressed total phytoplankton biomass by 74% relative to no *D. pulicaria* controls (Fig. 1B), and this effect persisted until the conclusion of the experiment ($F_{1,4} = 20.634$, $P = 0.010$). The mean effect size of *Daphnia* on total phytoplankton biomass over the final three weeks was 3.7. In general, *D. pulicaria* strongly suppressed the biomass of all species of cyanobacteria on day 28, reducing *Microcystis*, *Oscillatoria*, and *Anabaena* species by factors of 4 ($F_{1,4} = 14.539$, $P = 0.019$), 56 ($F_{1,4} = 15.746$, $P = 0.017$), and 20 ($F_{1,4} = 5.857$, $P = 0.073$) (Fig. 2A), respectively, but the phytoplankton community was equally dominated by cyanobacteria in both treatments (Fig. 2B). *D. pulicaria* marginally reduced the concentration of microcystin, relative to no *D. pulicaria* controls over the course of the experiment ($F_{1,4} = 6.549$, $P = 0.063$, Fig. 1C). Mean microcystin content (relative to *Microcystis* dry biomass) for day 28 was similar (0.03%) for both control and *Daphnia* treatments ($t = 0.162$, $P = 0.879$, dF = 4).

3.2. *Experiment 2*

At the start of Experiment 2, enclosures in F19 and F20 had dense blooms of cyanobacteria, with *Microcystis* and *Oscillatoria* contributing >95% of total phytoplankton biomass. Initial phytoplankton biomass in F19 and F20 was very high (mean chlorophyll *a* = 710 and 1400 μg L^{-1}, respectively). Initial cyanobacterial biomass in F19 and F20 was 34,000 and 36,000 μg L^{-1}, and initial microcystin concentrations were 40 and 180 μg L^{-1}, respectively. Mean microcystin content, expressed as a percentage of total phytoplankton dry biomass, was 0.1% in F19 and 0.6% for F20. Toxic strains of *Microcystis* typically have microcystin content on the order of 0.1–0.3% dry mass (DeMott et al., 1991), and dietary quotas for both ponds were comparable or higher than these values. Further, initial phytoplankton were comprised of colonies and filaments of sizes that should be at least partly of edible size with respect to *D. pulicaria* (mean ± standard deviation ESD for *Microcystis* colonies = 70(±7) μm), as a closely related species *Daphnia pulex* with a mean carapace length of 1.25 mm effectively consumed *Microcystis* colonies with diameters between

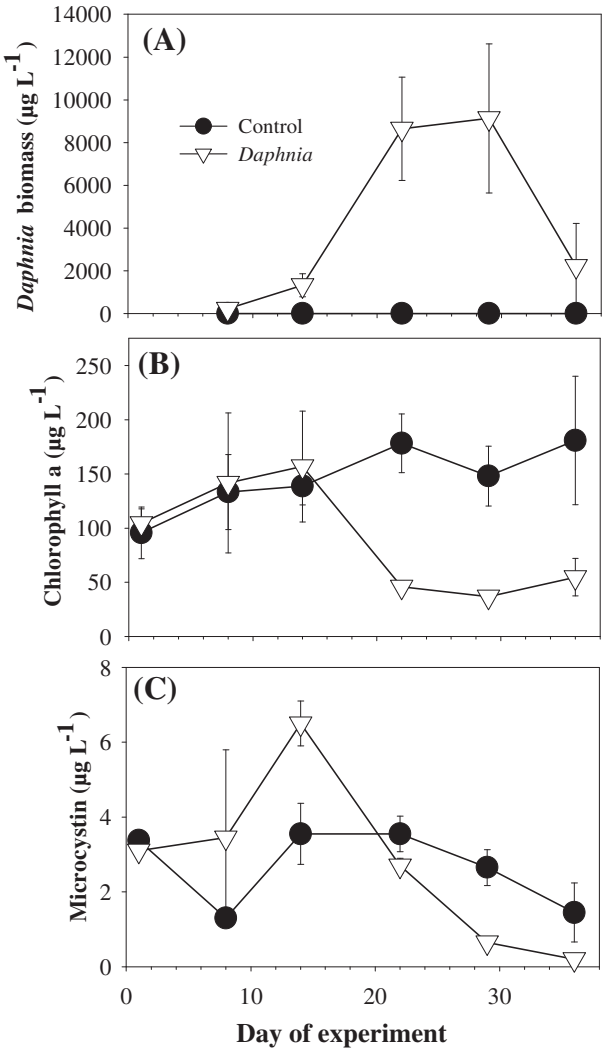

Fig. 1 – Dynamics of (A) *Daphnia pulicaria* biomass, (B) chlorophyll *a*, and (C) microcystin concentration for Experiment 1. Data represent means ± one standard error.

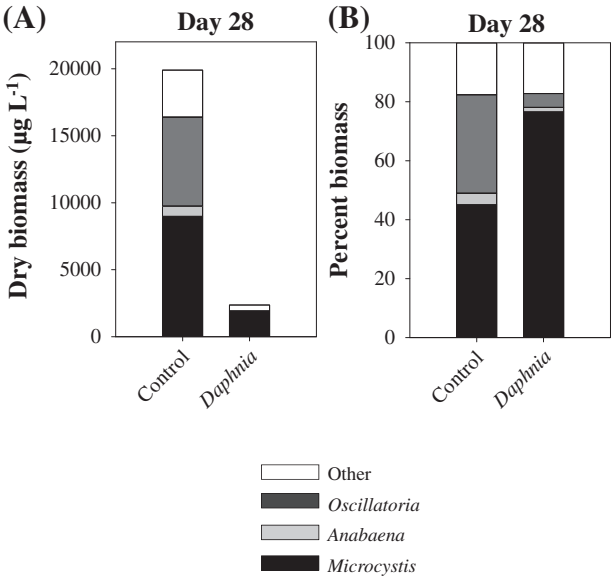

Fig. 2 – Mean absolute (A) and relative (B) biomass of dominant phytoplankton taxa on day 28 (30 July 2007) of Experiment 1. The "other" phytoplankton taxon included a mixture of flagellates and chlorophytes.

60 and 100 μm (Jarvis et al., 1987), and *D. pulicaria* can readily consume thin filaments of greater than 1 mm in length (DeMott, 1995).

D. pulicaria increased and reduced phytoplankton biomass in both ponds (Fig. 3A, Fig. 5A), with a mean effect size over the last three weeks of 4.5 in F19 ($F_{1,2} = 727.623$, $P = 0.001$) and 7.4 in F20 ($F_{1,2} = 34.244$, $P = 0.028$). By day 50, *D. pulicaria* had strongly reduced the biomass of cyanobacteria in both ponds (Fig. 4A, Fig. 6A), reducing *Microcystis* by a factor of 3.8 in F19 ($F_{1,2} = 266.799$, $P = 0.004$) and 19.6 in F20 ($F_{1,2} = 80.813$, $P = 0.012$). However, *D. pulicaria* had no effect on the relative biomass of *Microcystis* (F19 – $F_{1,2} = 6.974$, $P = 0.118$; F20 – $F_{1,2} = 0.444$, $P = 0.574$), and *Microcystis* spp. dominated the phytoplankton in all enclosures (Fig. 4B, Fig. 6B). Over the final three weeks, *D. pulicaria* significantly reduced microcystin concentration in both ponds (F19: $F_{1,2} = 37.074$, $P = 0.026$; F20: $F_{1,2} = 40.529$, $P = 0.024$; Fig. 3C, Fig. 5C). Mean microcystin content (relative to *Microcystis* dry biomass) for day 50 was marginally reduced for *Daphnia* treatments versus controls in F19 (0.02 vs 0.23%; $t = 3.529$; $P = 0.072$, dF = 2) but marginally elevated in *Daphnia* treatments versus controls in F20 (2.24 vs 0.32%; $t = 1.932$, $P = 0.193$, dF = 2).

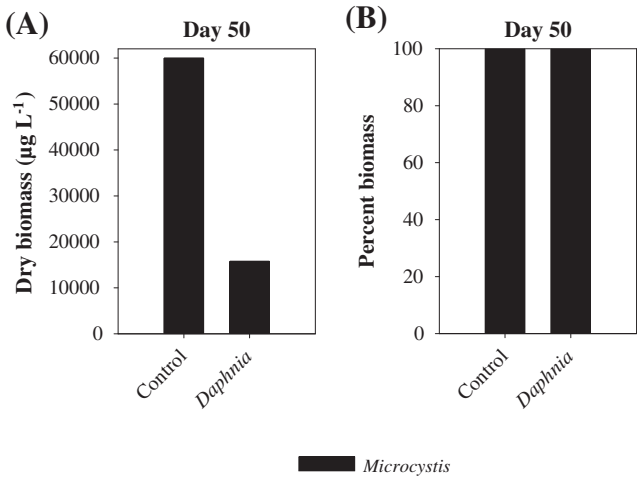

Fig. 4 – Mean absolute (A) and relative (B) biomass of dominant phytoplankton taxa on day 50 (17 November 2010) for enclosures in Pond F19.

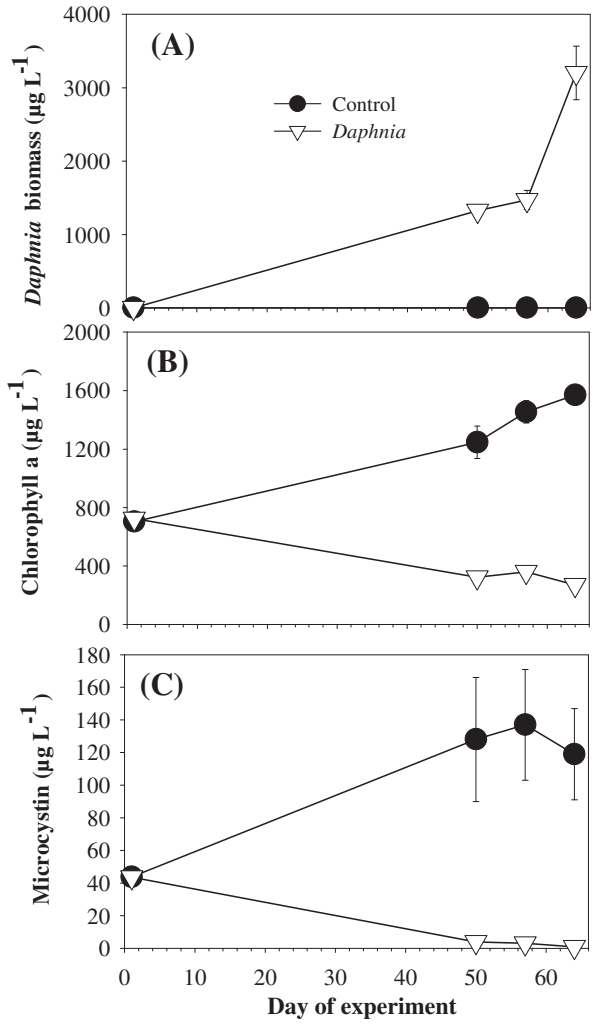

Fig. 3 – Dynamics of (A) *Daphnia pulicaria* biomass, (B) chlorophyll *a*, and (C) microcystin concentration for enclosures in Pond F19. Data represent means ± one standard error.

4. Discussion

High levels of cyanobacterial toxins are one explanation for the escape of phytoplankton from grazer control (Porter, 1977). Observational studies have documented negative correlations between cyanobacterial biomass and the abundance of large *Daphnia* both with eutrophication (Hansson et al., 2007) and seasonally within eutrophic lakes (Threlkeld, 1979). Furthermore, commonly-studied cyanobacterial toxins, including microcystin and anatoxin, have been shown to have negative effects on the fitness of zooplankton herbivores (including *Daphnia*) in laboratory studies where herbivores are maintained in lake water with dissolved toxins (DeMott et al., 1991; Reinikainen et al., 2001), fed cyanobacteria that produce microcystin or anatoxin (Gilbert, 1990; DeMott et al., 1991; Lürling and van der Grinten, 2003), or fed diets composed of dried green algae treated with dissolved microcystin-LR (Wilson and Hay, 2007). Consequently, it has been suggested that toxic cyanobacteria can drive declines of large daphnids with eutrophication and during seasonal succession and the concomitant escape of cyanobacteria from *Daphnia* control (Porter, 1977; Sommer et al., 1986). In direct contrast to these suggestions, our results show that *D. pulicaria* can increase from low density and strongly suppress both cyanobacterial and phytoplankton biomass despite high levels of one of the most commonly encountered and toxic of the cyanotoxins, microcystin.

Laboratory experiments have shown that microcystin concentrations of 1 μg L^{-1} significantly reduce *Daphnia* survival (Ferrão-Filho et al., 2000), and microcystin levels of 3.5 μg L^{-1} can result in 100% mortality of *Daphnia* (Lürling and van der Grinten, 2003). In our field experiments, initial microcystin levels were 3–6 μg L^{-1} (Experiment 1) and 40–180 μg L^{-1} (Experiment 2). Thus, microcystin

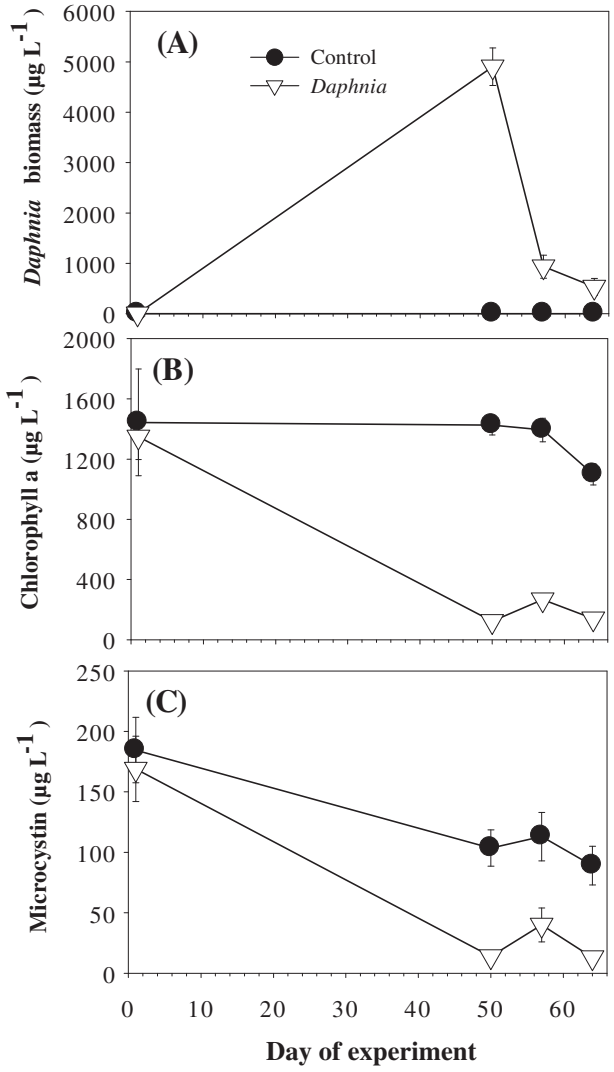

Fig. 5 – Dynamics of (A) *Daphnia pulicaria* biomass, (B) chlorophyll *a*, and (C) microcystin concentration for enclosures in Pond F20. Data represent means ± one standard error.

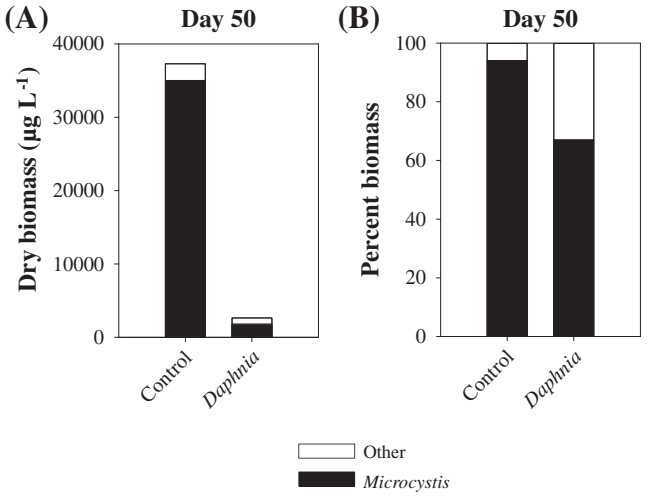

Fig. 6 – Mean absolute (A) and relative (B) biomass of dominant phytoplankton taxa on day 50 (17 November 2010) for enclosures in Pond F20. Data represent means for phytoplankton taxa for each treatment. The "other" phytoplankton taxon included a mixture of flagellates and chlorophytes.

concentrations in our field experiments were as high or much higher than in previous laboratory experiments reporting negative effects of diets containing microcystin-producing cyanobacteria on *Daphnia* fitness. Thus, we can conclude that the *D. pulicaria* clones that we chose increased and suppressed established blooms of toxic cyanobacteria despite microcystin levels that were at or far above thresholds that have caused 100% mortality for other *Daphnia* strains in previous laboratory assays (Lürling and van der Grinten, 2003).

One potential mechanism for *Daphnia* survival in environments with high microcystin levels is by avoiding ingestion of microcystin-producing cyanobacteria (Rohrlack et al., 2001). Previous laboratory experiments have demonstrated that variation in *Daphnia* survival when feeding on single-celled, toxic strains of *Microcystis* is correlated to microcystin ingestion rate, rather than to differences in susceptibility to microcystin (Rohrlack et al., 2001). However, more recent lab studies have demonstrated that populations of *Daphnia* may evolve to tolerate toxic cyanobacteria in the diet following prolonged exposure to cyanobacterial blooms (Hairston et al., 2001; Sarnelle and Wilson, 2005). Growth and survival rates for *Daphnia* clones from high-nutrient lakes are higher on a diet of 100% toxic *Microcystis* than for clones isolated from low nutrient lakes (Sarnelle and Wilson, 2005). In both of our field experiments, cyanobacterial species accounted for >95% of total phytoplankton biomass, and colonies and filaments were of edible sizes. Furthermore, microcystin content, relative to total phytoplankton biomass, in our study was within the range reported for toxic strains of single-celled *Microcystis* in lab studies (DeMott et al., 1991; 0.1–1%) in two of our three field experiments (F19 – 0.1%; F20 – 0.6%). It is well known that field populations of *Microcystis* are not genetically uniform and vary in terms of colony growth rates, morphology, and toxicity (Van Gremberghe et al., 2009; Wilson et al., 2005,2010). However, as *Daphnia* are relatively non-selective feeders other than with respect to particle size, it is unlikely that *Daphnia* were able to selectively feed on microcystin-lacking versus microcystin-producing colonies. Consequently, avoidance of toxin ingestion by *Daphnia* seems unlikely and it is difficult to explain the large suppression of cyanobacterial biomass and toxin concentrations by *Daphnia* without invoking consumptive effects.

There is an obvious incongruity between strong inhibition of *Daphnia* fitness by cyanobacteria in the laboratory (Wilson et al., 2006a,b) and repeated field observations of strong suppression of cyanobacteria by *Daphnia* grazing (Lynch and Shapiro, 1981; Vanni, 1984; Sarnelle, 1993, 2007; this study). One potential explanation is that laboratory experiments may use *Daphnia* clones that are evolutionarily naïve to toxic cyanobacteria. The source populations of *Daphnia* used in laboratory experiments are often not specified with respect to their evolutionary history. Recent laboratory experiments

support this explanation, as *Daphnia* from environments with frequent cyanobacterial blooms are less inhibited by toxic *Microcystis* than *Daphnia* from environments where cyanobacteria are rare (Hairston et al., 1999; Sarnelle and Wilson, 2005). Furthermore, phenotypic acclimation has been observed in *Daphnia* in response to exposure to toxic cyanobacteria in the diet (Gustafsson and Hansson, 2004). We do not claim that cyanobacteria are a high quality food for *Daphnia*, but rather that the documented negative effects of toxic cyanobacterial diets on *Daphnia* fitness in many laboratory experiments may reflect use of *Daphnia* strains that are not adapted to tolerate such a diet and cyanobacterial strains that are unusually toxic relative to genotypes that dominate in nature (Ferrão-Filho et al., 2000; Lürling and van der Grinten, 2003; Wilson et al., 2006a,b). Given the considerable variation in ecologically important traits observed for *Daphnia* (Tessier et al., 2000; Duffy, 2010) and cyanobacteria (Wilson et al., 2006a,b), an emphasis on intraspecific trait variation provides an interesting conceptual framework for the study of harmful cyanobacterial blooms, and several previous studies suggest that this approach may be profitable for plankton food webs (Hairston et al., 1999; Tessier et al., 2000; Sarnelle and Wilson, 2005; Post et al., 2008; Van Gremberghe et al., 2009; Duffy, 2010; Lemaire et al., 2011). In any case, the results of many laboratory experiments are not compatible with the outcome of the field experiments we conducted.

In addition to toxins, cyanobacteria have been shown to be poor food for zooplankton due to morphology and deficiencies in sterols and essential fatty acids (Wilson et al., 2006a,b). While cyanobacteria are of relatively poor quality food for planktonic herbivores in general, recent meta-analyses of laboratory studies have shown that cyanobacteria can support positive zooplankton population growth in some cases, and cyanobacteria that produce toxic secondary metabolites have variable effects on zooplankton population growth rates (Wilson et al., 2006a,b; Tillmanns et al., 2008).

Previous field experiments have shown that *Daphnia* grazing can prevent seasonal cyanobacterial succession (Sarnelle, 1993) and can greatly reduce phytoplankton and cyanobacterial biomass (Lynch and Shapiro, 1981; Vanni, 1984; Sarnelle, 2007). However, in the majority of cases, *Daphnia* attained high densities before cyanobacteria became dominant (but see Sarnelle, 2007) and toxin concentrations were not measured. In our study, microcystin levels were high, and cyanobacterial biomass was over two orders of magnitude higher than in previous experiments (e.g., Sarnelle, 2007), prior to *Daphnia* becoming abundant. Thus, our results extend previous findings about the ability of *D. pulicaria* to control total phytoplankton biomass to the case of extremely high initial concentrations of toxic cyanobacteria and microcystin. Microcystin concentrations observed at the start of both experiments (3–180 μg L^{-1}) were representative of the maximum microcystin concentrations reported in the recent National Lakes Assessment Report (225 μg L^{-1}; USEPA, 2009) and for midwestern lakes (4.5 μg L^{-1}; Graham et al., 2004), while also being significantly higher than levels suggested for safe drinking water (1 μg L^{-1} – WHO; Chorus and Bartram, 1999). Furthermore, chlorophyll *a* concentrations in both experiments (130–1400 μg L^{-1}) were comparable to the maximum levels observed for a wide range of temperate lakes in North America (596 μg L^{-1}; McCauley et al., 1989). Thus, our results are representative of the highest measured microcystin and chlorophyll *a* concentrations that occur in temperate lakes in North America.

A previous meta-analysis of field experiments showed that the magnitude of *Daphnia*'s effect on total phytoplankton biomass increases linearly with TP (Sarnelle, 1992), and provides a baseline of comparison for the effects we observed. Although effect sizes increased with increasing TP among our three experimental lakes, only the effect size from the MI site (Experiment 1) fell within the prediction limits of the linear model from that meta-analysis (Fig. 7). Effect sizes from the AL sites were clearly lower than predicted, suggesting a weakening of *Daphnia*'s effects at these very high nutrient levels. The AL sites represent an extrapolation beyond the range of the original meta-analysis, suggesting that given high enough nutrient levels (TP > 600 μg L^{-1}), *Daphnia*'s effects eventually start to depart from the linear expectations predicted by a two species Lotka–Volterra predator-prey model (Sarnelle, 1992). Specifically, the AL results provide some evidence for the effect of large (and consequently more resistant to grazing) algae in weakening *Daphnia*'s ability to suppress total phytoplankton biomass that was unavailable in Sarnelle (1992). Although the effect sizes from AL were smaller than would be predicted by the linear model, they still represented large suppressions (by factors of 4.5 and 7.4) of phytoplankton biomass by *Daphnia*, and so would result in major improvements in water quality relative to ungrazed conditions.

One potential explanation for effect sizes being lower than predicted is that some cyanobacterial colonies were too large to be consumed by *Daphnia* by the end of the experiment. In two of the three ponds, the average size of *Microcystis* colonies was significantly larger in the *D. pulicaria* treatment (Table 2). In the other pond (F19), colonies in both treatments were larger (90, 83 μm, Table 2) at the end than at the start (70 μm).

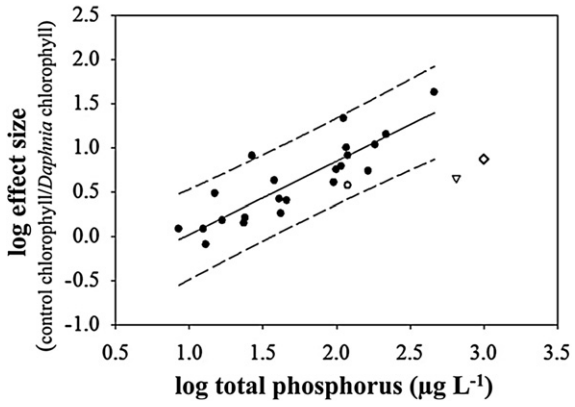

Fig. 7 – Comparison of effect sizes (log ratio of phytoplankton biomass in control versus *Daphnia* enclosures) from the three sites in this paper (open symbols) with a previous meta-analysis of field experiments (solid circles) of similar design (Sarnelle, 1992). Solid line is the regression between log-TP and log-effect size from the meta-analysis (log-effect size = 0.83 × log-TP – 0.81). Dotted lines are the 95% prediction bands for the meta-analysis regression.

Table 2 – Mean *Microcystis* colony size (mean equivalent spherical diameter – ESD ±1 standard error - SE) on day 28 for Experiment 1 and day 50 for Experiment 2.

Experiment	Pond	Control ESD (μm) mean ± 1 SE	*Daphnia* ESD (μm) mean ± 1 SE	P-value
1	MSU Lake 2	30 ± 1	39 ± 1	0.002
2	F19	90 ± 20	83 ± 9	0.762
	F20	66 ± 1	93 ± 4	0.017

This may reflect selective grazing by *Daphnia* on smaller colonies, and thus some escape from grazing by larger colonies. Although colony size may have contributed to grazing resistance of the phytoplankton in these experiments, it appears that microcystin did not.

Despite extremely high initial microcystin levels, we found that *D. pulicaria* populations increased dramatically in the absence of fish predation, to the point where they were able to strongly reduce phytoplankton biomass. Given the ability of these daphnid populations to increase under such conditions, our results suggest that negative relationships between lake productivity or cyanobacterial abundance and the abundance of large daphnids is probably less a function of phytoplankton composition and more likely driven by the increasing abundance of planktivorous fishes with nutrient enrichment (Sarnelle, 1992; Jeppesen et al., 1997).

5. Conclusions

Based on the results of our field experiments and synthesis of the available information, we conclude the following:

- High concentrations of microcystin do not prevent *Daphnia* from strongly suppressing phytoplankton biomass in nature.
- The current paradigm describing cyanobacteria as generally harmful to zooplankton needs to be reconsidered.
- As zooplankton can rapidly adapt to tolerate cyanobacteria and their associated toxins, studies of zooplankton-cyanobacteria interactions should consider grazer adaptations and the evolutionary history of populations.
- Given that most zooplankton-cyanobacteria studies have been conducted in the laboratory but the focus of these studies is on dynamics in nature, we encourage a greater emphasis on studies that determine if interactions in the laboratory can be extended to the field.

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