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Initial conditions mediate the interaction between *Daphnia* and bloom-forming cyanobacteria

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Abstract

To assess whether *Daphnia* populations in eutrophic lakes can increase when bloom-forming cyanobacteria dominate the phytoplankton assemblage and whether such an increase can result in strong suppression of phytoplankton biomass, I created contrasting initial conditions (high *Daphnia pulicaria*, low cyanobacteria vs. low *D. pulicaria*, high cyanobacteria) via fish manipulation in large enclosures, then removed fish from some enclosures and subsequently monitored zooplankton and phytoplankton abundance for 48 days. After being released from fish predation, *D. pulicaria* was apparently able to reduce cyanobacteria and total phytoplankton biomass to very low levels despite the fact that the phytoplankton assemblage was initially composed of about 90% *Microcystis aeruginosa*, a species that has inhibited *Daphnia* growth and reproduction in many laboratory studies. Thus, it appears possible for *Daphnia* to graze down an established bloom of cyanobacteria. In contrast, in enclosures where fish were never present, *M. aeruginosa* was eventually able to increase from low levels despite initially high *D. pulicaria* biomass. As a result, the apparent effect of *D. pulicaria* on *M. aeruginosa* at the end of the experiment was very different across enclosures with different initial conditions.

Bloom-forming cyanobacteria (species in the genera *Anabaena*, *Aphanizomenon*, *Microcystis*, and *Oscillatoria*, hereafter referred to as cyanobacteria) increasingly dominate phytoplankton assemblages as lakes are enriched (Trimbee and Prepas 1987; Downing et al. 2001), at times comprising close to 100% of phytoplankton biomass during the summer (Sarnelle 1993). This is an important trend in the context of freshwater trophic interactions because cyanobacteria as a group tend to be less vulnerable to grazing (Sterner 1989; Sarnelle 1993; Sarnelle 2003) and of low food quality for herbivorous zooplankton (Lampert 1987; DeMott 1989). In addition, cyanobacteria often produce toxins that can endanger public health (Chorus and Bartram 1999).

Increasing dominance by grazing-resistant cyanobacteria with eutrophication, and the succession toward cyanobacteria that occurs seasonally within eutrophic lakes, have been postulated as major factors leading to declines in *Daphnia* across and within lakes (Threlkeld 1979; Sommer et al. 1986; Ghadouani et al. 2003). Mechanistic studies in the laboratory have shown that *Daphnia*, by virtue of its relatively non-selective feeding behavior, is more negatively affected by the presence of cyanobacteria than other more-selective herbivore species (Richman and Dodson 1983; Lampert 1987; Hawkins and Lampert 1989). Most notably, the addition of cyanobacteria to laboratory cultures reverses the outcome of competition between *Daphnia* and smaller herbivores and sometimes lead to *Daphnia* extinction (Gilbert 1990). Thus, there is considerable evidence that cyanobacteria negatively effect *Daphnia*, at least under laboratory conditions. Negative correlations between cyanobacterial biomass and *Daphnia* biomass across lakes with varying nutrient inputs may be a reflection of these effects (Ghadouani et al. 2006). All of the above suggest that dominance of a phytoplankton assemblage by cyanobacteria may interfere with the ability of *Daphnia* to suppress total phytoplankton biomass, as has been suggested many times (Sommer et al. 1986; Benndorf and Henning 1989; Ghadouani et al. 2003). Recent studies indicate that *Daphnia* may adapt to tolerate cyanobacteria in the diet (Hairston et al. 2001; Gustafsson and Hansson 2004; Sarnelle and Wilson 2005), but the significance of these findings with respect to *Daphnia* control of cyanobacteria has yet to be determined.

At the same time, there is considerable evidence that *Daphnia* can sometimes have large negative effects on cyanobacterial abundance in eutrophic lakes, despite the relative grazing-resistance of these phytoplankton. Observations of the effects of both natural and experimental perturbations of zooplanktivorous fish density have revealed that in the absence of fish predation, the biomass of large species of *Daphnia* increases and the biomass of cyanobacteria often decreases (Vanni et al. 1990; Sarnelle 1993; Paterson et al. 2002). Direct manipulations of *Daphnia* have confirmed that strong decreases in cyanobacteria biomass can be driven primarily by *Daphnia* (Sarnelle 1993; Paterson et al. 2002; Sarnelle 2003), rather than by other indirect effects of fish (Vanni 2002). Suppression of grazing-resistant cyanobacteria by *Daphnia* is counterintuitive and not fully understood, but it has been suggested that changes in light attenuation and nutrient ratios driven by high *Daphnia* grazing may reduce the

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competitive advantage of cyanobacteria and make it more difficult for cyanobacteria to invade phytoplankton assemblages (Spencer and King 1987; MacKay and Elser 1998; Paterson et al. 2002).

To date, experiments showing that *Daphnia* can suppress cyanobacteria in eutrophic lakes have been limited to cases were *Daphnia* were able to achieve high densities before cyanobacteria became dominant (Christoffersen et al. 1993; Sarnelle 1993; Paterson et al. 2002), which is typical of the seasonal sequence in temperate lakes (Sommer et al. 1986). Thus, it is not clear whether *Daphnia* can overcome the inhibitory effects of high cyanobacterial abundance on its competitive ability (Gilbert 1990; Ghadouani et al. 2003) and invade a cyanobacteria-dominated assemblage. The experiment described in this article was aimed at determining if *Daphnia* could invade when the phytoplankton assemblage was initially dominated by cyanobacteria and subsequently drive phytoplankton biomass down to clear-water conditions. To my knowledge, no previous replicated field experiments aimed at assessing the effects of *Daphnia* on phytoplankton have directly manipulated initial conditions.

Methods

Study site—The experiment was conducted in Zaca Lake, a small, eutrophic lake located at an elevation of 730 m in the San Rafael mountains of southern California (Sarnelle 1993). The lake resides in a steep-sided basin and consequently has a small littoral zone (75% of the lake bottom is >5 m deep, maximum depth = 13 m). Total phosphorus (TP) concentrations in the mixed layer average about 300 μg P L^{-1} during winter mixing periods and range from 50 μg P L^{-1} to 250 μg P L^{-1} during thermally stratified periods (Sarnelle 1992). Previous enclosure experiments in Zaca Lake revealed large negative effects of *Daphnia* on total phytoplankton biomass and cyanobacterial dominance (Sarnelle 1993; Sarnelle 2003), but these experiments were initiated before cyanobacteria were a significant fraction of total phytoplankton biomass. A previous study of seasonal succession in the lake also showed that when zooplanktivorous fish abundance is high (and *Daphnia* grazing pressure low), cyanobacteria begin to dominate the phytoplankton in late April to early May (Sarnelle 1993).

Enclosure experiment—The experiment was conducted in large enclosures (diameter, 2.3 m; depth, 8 m) constructed of clear polyethylene sheeting that was heat-sealed into tubes. Each enclosure was fitted with coarse netting on the bottom to exclude fish as the enclosure bottom was lowered. Otherwise, enclosure bottoms were open to the anoxic hypolimnion and the sediment, although they did not extend to the lake bottom. Enclosures were open to the atmosphere at the top, suspended from rafts, and held open by attaching a weight to a plastic hoop attached to the bottom edge of the polyethylene tube. Nine enclosures were deployed in the middle of Zaca Lake on 11 April 1995.

The experimental design comprised two phases. The purpose of Phase 1 was to create a set of replicate cyanobacteria-dominated systems with low *Daphnia* abundance, alongside a set of enclosures with high *Daphnia* abundance and low cyanobacteria. To initiate Phase 1, 6–10 (depending on size) zooplanktivorous bluegill (*Lepomis macrochirus*) were added to each of six enclosures on 24 April, leaving three enclosures as fishless controls. Fish were stocked at a rate of 100 g wet mass per enclosure, which translates to a fish biomass of 24,000 kg km^{-2}. This stocking density was chosen to approximate the prediction of an empirical model relating fish biomass to TP across temperate lakes (Hanson and Leggett 1981) for a TP of 200 μg P L^{-1}. Fish were initially stocked into cages hung within each enclosure (with identical empty cages in fishless enclosures) to facilitate fish removal at the end of Phase 1. Cages were 60 cm × 60 cm × 180 cm deep and made of 6-mm mesh nylon seine netting stretched over frames of 1.25-cm plastic pipe. Despite low mortality (<5%) and rapid growth (>1 g d^{-1} $fish^{-1}$), fish in cages were ineffective at reducing *Daphnia* biomass during the first 40 days of Phase 1, perhaps because cages created a refuge for *Daphnia*. Consequently, fish were released from cages, and cages were removed from all enclosures on 06 June. One of the fish enclosures was damaged during Phase 1 and was dropped from the experiment.

Phase 2 of the experiment was initiated when *Daphnia* had declined sufficiently in some of the fish enclosures to result in dominance by cyanobacteria. To enable the strongest test of whether *Daphnia* could invade (i.e., increase from low density) a cyanobacteria-dominated plankton community and reduce phytoplankton biomass to low levels, the three fish enclosures with the lowest *Daphnia* and the highest phytoplankton biomass were assigned to the invasion treatment (fish → no fish) of Phase 2. All fish were removed from these three enclosures on 29 June (day 0) using a large fish net pulled vertically through each enclosure. The two remaining fish enclosures served as controls for the fish removal treatment. Thus, Phase 2 consisted of three treatments: never-fish (n = 3), always-fish (n = 2), and fish → no-fish (n = 3). The first and last treatments both lacked fish and so enabled a comparison of the effects of *Daphnia* on phytoplankton (relative to the always-fish treatment) across differing initial conditions (low *Daphnia*, high cyanobacteria vs. high *Daphnia*, low cyanobacteria).

Enclosures were sampled weekly during Phase 2 for dissolved nutrients, phytoplankton, and zooplankton. Depth-integrated water samples were taken from the euphotic zone of each enclosure with a tube sampler (51-mm inside diameter). The contents of multiple casts of the tube sampler were pooled in a plastic barrel and subsampled for chlorophyll *a* (Chl *a*), phytoplankton enumeration, and nutrients. Samples for Chl *a* were filtered on to Gelman A/E filters in the field. The filtrate was collected for analysis of soluble reactive phosphorous (SRP) and NH_4^+. Chl *a* and nutrient samples were held on ice in the field and frozen upon return to the laboratory. Water samples for phytoplankton enumeration were preserved in Lugol's solution. Macrozooplankton (crustaceans) were sampled with vertical hauls of a 13-cm-diameter, 102-μm-mesh net, which was assumed to have

an efficiency of 60% based on previous empirical experience (Sarnelle 1992). Four net hauls from each enclosure were pooled into a single sample, and samples were preserved in sucrose-formalin (Haney and Hall 1973).

Chl *a* concentrations were measured by extracting phytoplankton collected on A/E filters in 90% ethanol overnight at 4°C, followed by spectrophotometric analysis with acid correction (Sartory and Grobbelaar 1984). Phytoplankton species abundances were determined via the inverted microscope technique (Utermöhl 1958). Cell counts were combined with estimates of cell volume (based on measurements of cell dimensions) to estimate the biovolume of each species ($mm^3\ L^{-1}$), which was converted to dry biomass ($\mu g\ L^{-1}$) assuming that phytoplankton have a specific gravity of 1 and a dry mass : wet mass ratio of 0.10 (Riemann et al. 1989). Zooplankton were counted and measured at 40× in a Sedgwick-Rafter cell. Measurements of body length were made with a digitizer and drawing tube and used to calculate dry biomass using equations developed from dried Zaca Lake specimens (O. Sarnelle, unpubl. data).

Results from Phase 2 of the experiment were statistically analyzed with repeated-measures multivariate analysis of variance (MANOVA) using data from all dates except day 0. The conservative Greenhouse–Geisser probability criterion was applied in all cases (Von Ende 2001). Repeated-measures analysis provides tests of overall treatment, time, or treatment × time effects, but cannot distinguish between effects occurring early or late in the experiment. The objective was to determine cyanobacterial responses after *Daphnia* had time to increase from low density in the fish → no fish treatment (i.e., toward the end of Phase 2), so response variables averaged over the last three sampling dates were also analyzed using one-way ANOVA. The Tukey–Kramer honestly significant difference (HSD) was used to test for differences among the three treatments. Relationships between *Daphnia* and phytoplankton biomass were analyzed with least-squares linear correlation. Response variables were log transformed to homogenize treatment variances and linearize relationships.

Results

Suppression of *Daphnia* biomass by fish was variable during Phase 1 of the experiment, as only three of the five fish enclosures had low *Daphnia* (<300 $\mu g\ L^{-1}$) and high phytoplankton biomass (>300 $\mu g\ L^{-1}$) by the end of Phase 1. However, there were strong negative relationships between *Daphnia* biomass and total phytoplankton and cyanobacterial biomass across the enclosures on the last date, indicating that where *Daphnia* was suppressed by fish, phytoplankton, including cyanobacteria, responded positively (Fig. 1). In contrast, the biomass of *Ceriodaphnia affinis* and *Diaptomus siciloides*, the two other common herbivorous species in the enclosures, were either positively or not related to phytoplankton and cyanobacteria biomass at the end of Phase 1 (data not shown, $r = 0.85$ and 0.85, both significant at $p < 0.01$ for *C. affinis*; $r = 0.44$ and 0.38, both nonsignificant at $p > 0.05$ for *D. siciloides*).

The three enclosures with the lowest *Daphnia* and highest phytoplankton biomass were selected to be fish → no-fish treatments for Phase 2 of the experiment to create the strongest challenge to the ability of *Daphnia* to invade. As a result of this selection, there was significantly lower *Daphnia* biomass and significantly higher Chl *a*, phytoplankton biomass, and cyanobacterial biomass in the fish → no-fish than in the never-fish enclosures at the start of Phase 2 (Figs. 2 and 3; one-way ANOVA *F*-tests, $p < 0.03$). Thus, the intended treatments were successfully established, in a statistical sense, at the start of Phase 2. In contrast, *Ceriodaphnia* biomass was highest in the fish → no-fish enclosures at the start of Phase 2 (Fig. 2). However, there were no statistical differences among the treatments in either *Ceriodaphnia* or *Diaptomus* biomass at the start of Phase 2 (one-way ANOVA *F*-tests, $p > 0.10$). Individual *Daphnia* were also significantly smaller in the fish → no-fish enclosures at the start of Phase 2 (Fig. 2; one-way ANOVA *F*-tests, $p < 0.001$). Mean cyanobacterial biomass (a mixture of *Anabaena flos-aquae* and *Microcystis aeruginosa*) was 300 $\mu g\ L^{-1}$ and comprised 89% of total phytoplankton biomass in the fish → no-fish enclosures on day 0 (Fig. 3). By day 7, mean cyanobacterial biomass and percent dominance had increased slightly to 333 $\mu g\ L^{-1}$ and 92%, respectively, and was entirely composed of *M. aeruginosa*.

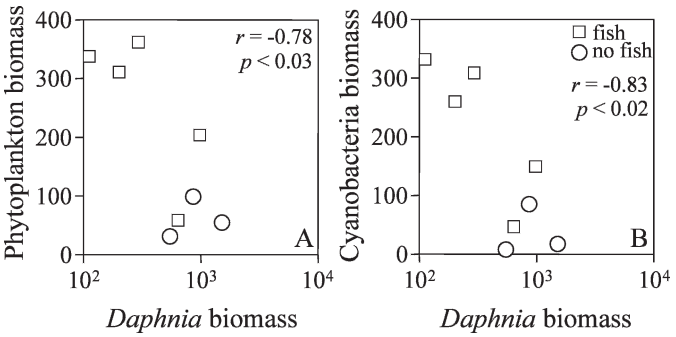

Fig. 1. Relationships between *Daphnia* biomass (μg dry mass L^{-1}) and the biomass (μg dry mass L^{-1}) of (A) phytoplankton and (B) cyanobacteria (mixture of *Anabaena flos-aquae* and *Microcystis aeruginosa*) at the end of Phase 1 of the experiment (day 0 of Phase 2). Squares, enclosures with fish; circles, enclosures lacking fish. Statistics refer to linear correlations of y versus $\log_{10} x$.

Repeated-measures MANOVA of data from days 8–48 revealed significant treatment × time interactions for *Daphnia* biomass ($p < 0.005$), Chl *a* ($p < 0.01$), phytoplankton biomass ($p < 0.001$), cyanobacteria biomass ($p < 0.001$), and *Microcystis* biomass ($p < 0.001$) and a significant overall effect of treatment on *Ceriodaphnia* biomass ($p < 0.05$). There were no treatment or treatment × time effects on *Diaptomus* biomass ($p > 0.05$). Repeated-measures analysis also revealed a significant influence of time on all the above response variables ($p < 0.01$).

Daphnia was eventually driven to near extinction, and both phytoplankton and cyanobacteria biomass increased in the always-fish treatment (Figs. 2 and 3). A somewhat similar sequence of events occurred in the never-fish enclosures, although these had higher *Daphnia* and lower phytoplankton than the always-fish enclosures toward the end of the experiment (Figs. 2 and 3). In contrast, there was

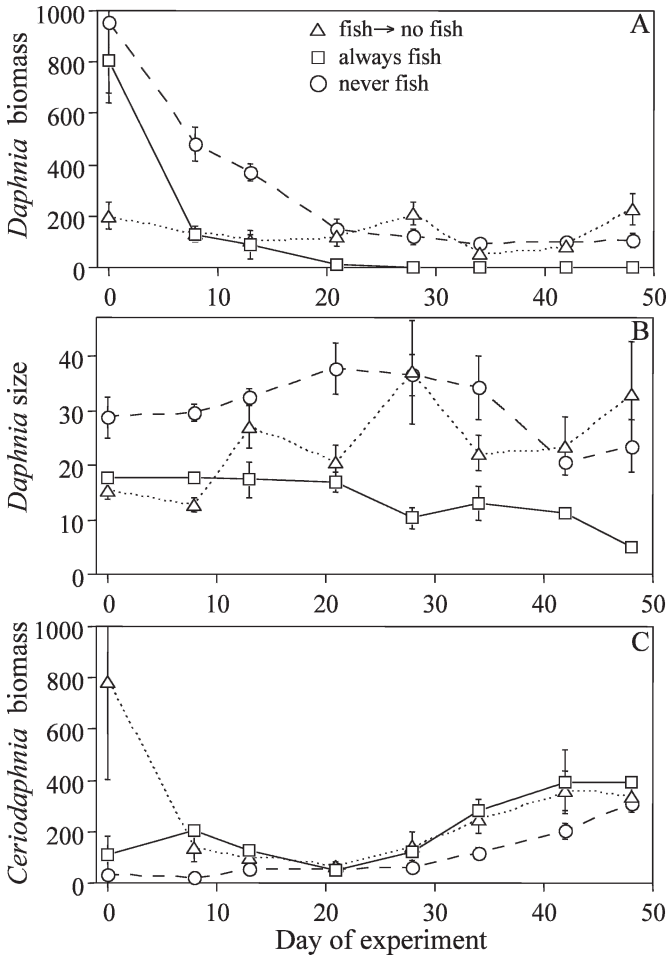

Fig. 2. Dynamics of (A) *Daphnia* biomass (μg dry mass L^{-1}), (B) *Daphnia* individual size (μg), and (C) *Ceriodaphnia* biomass (μg dry mass L^{-1}) during Phase 2 of the experiment. Error bars represent 1 SE.

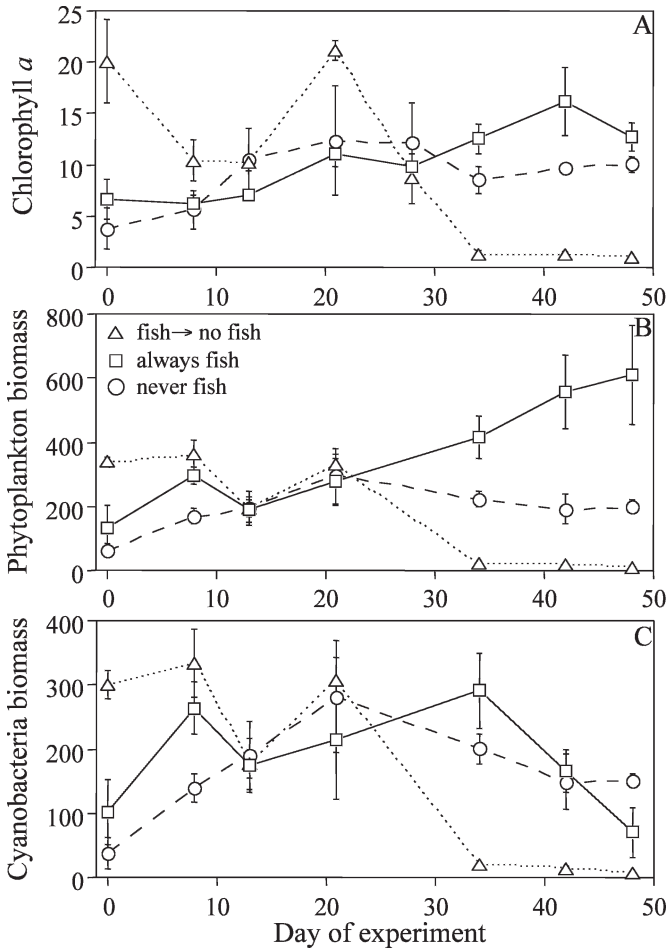

Fig. 3. Dynamics of (A) Chl *a* (μg L^{-1}), (B) total phytoplankton biomass (μg dry mass L^{-1}), and (C) total cyanobacteria biomass (μg dry mass L^{-1}) during Phase 2 of the experiment. Error bars represent 1 SE.

a small increase in *Daphnia* biomass and a large increase in *Daphnia* size (mean individual mass) in the fish → no-fish enclosures (Fig. 2), and by day 34, phytoplankton and cyanobacteria biomass had decreased to extremely low levels (Fig. 3). In contrast to *Daphnia*, *Ceriodaphnia* biomass was highest in the always-fish treatment and lowest in the never-fish treatment (Fig. 2) toward the end of the experiment.

During the last three sampling days of Phase 2, Chl *a* averaged 1.2 μg L^{-1} in the fish → no-fish enclosures, about an order of magnitude less than in enclosures with fish (Fig. 3). The strong decline in phytoplankton and cyanobacteria biomass in the fish → no-fish enclosures during the last half of the experiment (Fig. 3) occurred despite higher levels of dissolved nutrients in this treatment (Fig. 4). Mean *Daphnia* biomass was similar in the two fishless treatments, (Fig. 5; fish → no-fish and never-fish means not significantly different, Tukey-Kramer HSD, $p > 0.05$), whereas *Daphnia* biomass was much lower in the always-fish treatment (Tukey-Kramer HSD, $p < 0.05$). In contrast, phytoplankton biomass was significantly different among all three treatments at this time (Fig. 5; Tukey-Kramer HSD, $p < 0.05$). The biomass of *M. aeruginosa* was significantly lower in the fish → no-fish treatment (Fig. 5; Tukey-Kramer HSD, $p < 0.05$), but nearly identical between the always-fish and never-fish treatments (Tukey-Kramer HSD, $p > 0.05$). There were no significant treatment effects on *Ceriodaphnia* or *Diaptomus* biomass during the last three sampling dates (one-way ANOVA *F*-tests, $p > 0.20$).

As seen as the start of Phase 2, there was a strong negative relationship between *Daphnia* biomass and total phytoplankton biomass across the enclosures at the end of Phase 2, but no significant relationships between phytoplankton biomass and the biomass of either *Ceriodaphnia* or *Diaptomus* (Fig. 6; $p > 0.20$, *Diaptomus* data not shown). As a consequence of the contrasting effects of the Phase 2 treatments on *Daphnia* and *M. aeruginosa* biomass (Fig. 5), there were very different relationships between *Daphnia* and *M. aeruginosa* biomass at the end of the experiment when comparing the low-*Daphnia*, always-fish enclosures to either the high-*Daphnia* fish → no-fish enclosures or the high-*Daphnia* never-fish enclosures (Fig. 6). This difference was not related to differences in

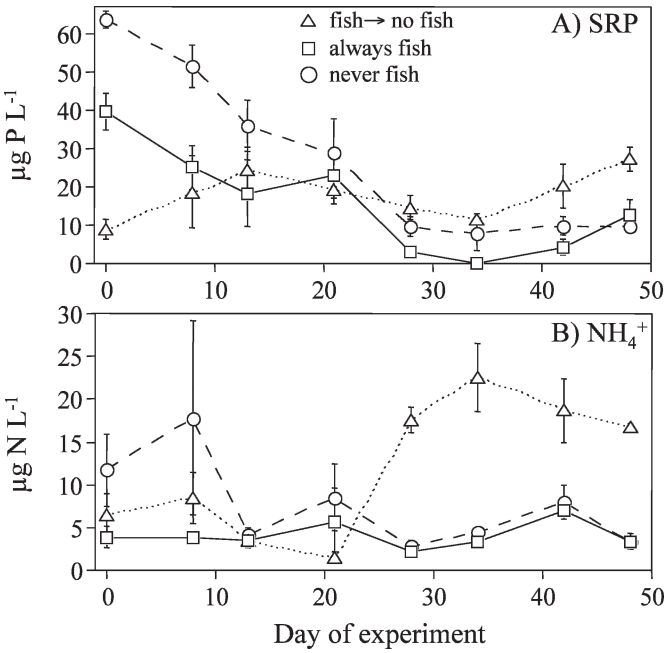

Fig. 4. Dynamics of (A) SRP and (B) NH_4^+ during Phase 2 of the experiment. Error bars represent 1 SE.

Daphnia size between the two treatments that lacked fish (Fig. 2; ANOVA *F*-test, $p > 0.05$).

Discussion

The results of Phase 2 showed that *D. pulicaria* can invade a cyanobacteria-dominated community with a concomitant large decrease in phytoplankton and cyanobacterial biomass (an order of magnitude lower than in the enclosures with fish), with the transition being completed in 34 days (Figs. 2 and 3). The transition to low phytoplankton biomass in the fish → no-fish enclosures occurred despite higher levels of phytoplankton resources (Fig. 4), indicating that the sharp decline in phytoplankton biomass was more likely a consequence of consumptive (top-down) effects of grazers. *D. pulicaria* was the only common crustacean that was affected by the treatments over the last three sampling dates when phytoplankton and cyanobacteria biomass were greatly reduced (Figs. 3 and 4). Although *Ceriodaphnia* biomass was greater than *Daphnia* biomass in all of the enclosures at this time, *Ceriodaphnia* biomass was not negatively correlated with phytoplankton biomass (Fig. 6), as would be expected if *Ceriodaphnia* grazing was driving the striking differences in phytoplankton and cyanobacteria biomass. In contrast, phytoplankton biomass was negatively correlated with *Daphnia* biomass at the end of the experiment (Fig. 6), just as at the start (Fig. 1). It is reasonable to conclude that the strong reduction in phytoplankton and cyanobacteria after 30 days in the fish → no-fish treatment was primarily driven by *Daphnia* grazing given that: phytoplankton resources were higher in fish → no-fish enclosures (Fig. 4); the treatments affected the abundance of *Daphnia* but not that of other common grazers; phytoplankton

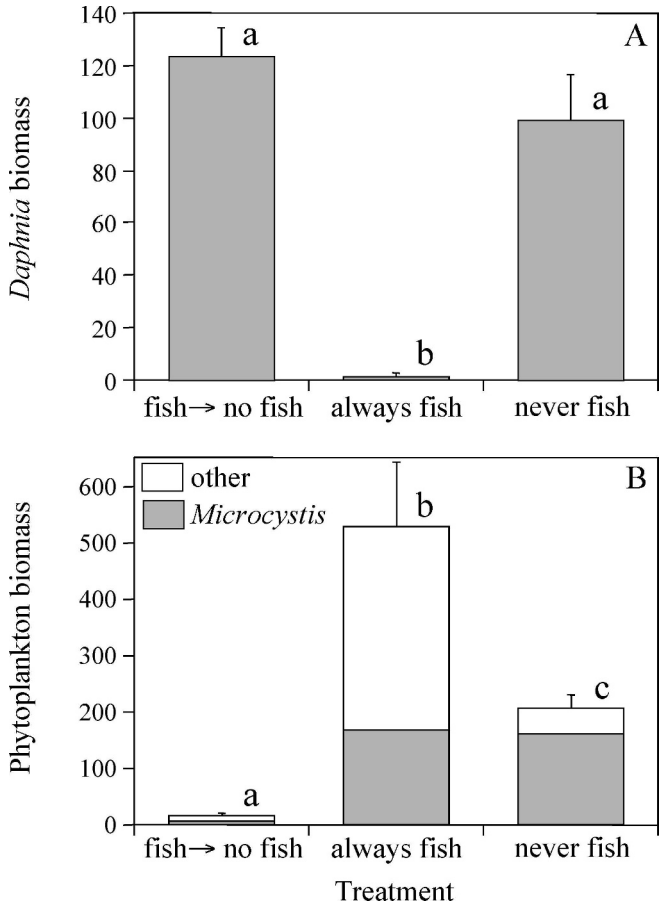

Fig. 5. Mean biomass (μg dry mass L^{-1}) of (A) *Daphnia* and (B) phytoplankton over the last three sampling dates of the experiment. *Ceratium hirundinella* dominated the "other" category in the always-fish treatment. Error bars represent 1 SE. Significantly different means (Tukey-Kramer HSD, $p < 0.05$) are indicated with different lowercase letters.

biomass was negatively correlated with *Daphnia* biomass but not with the biomass of other common grazers (Fig. 6); and *Daphnia* has been shown, via direct manipulation in enclosures, to strongly suppress cyanobacteria in previous experiments in this lake (Sarnelle 1993; Sarnelle 2003). However, it cannot be completely ruled out that the order of magnitude difference in phytoplankton biomass across the enclosures in this experiment was the result of other unmeasured factors that were correlated with *Daphnia* biomass.

The apparent *Daphnia* grazing effect was associated primarily with an increase in *D. pulicaria* size (mean individual mass tripled during the experiment), as there was only a small increase in *Daphnia* biomass (Fig. 2). Larger *Daphnia* have a broader diet (DeMott 1989) and so may have been better able to consume colony-forming cyanobacteria like *M. aeruginosa*, the biomass dominant. Individual *D. pulicaria* were able to grow, and the population was able to have a seemingly large grazing effect on total phytoplankton biomass, even though early in the experiment (day 7), *M. aeruginosa*, a species of very low food quality that is at least potentially toxic to *Daphnia*

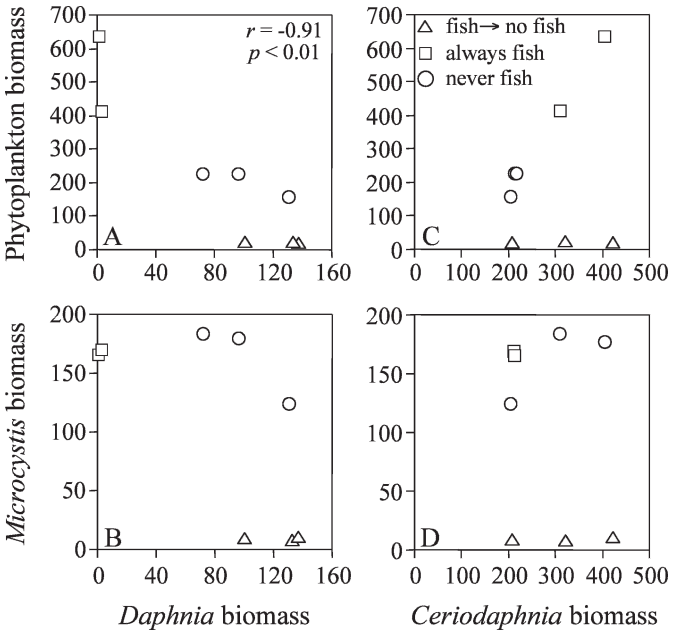

Fig. 6. Relationships between *Daphnia* biomass (μg dry mass L^{-1}) and the biomass (μg dry mass L^{-1}) of (A) phytoplankton and (B) *Microcystis aeruginosa*, and between *Ceriodaphnia* biomass and the biomass of (C) phytoplankton and (D) *M. aeruginosa* at the end of Phase 2 of the experiment. Each data point represents biomass averaged over the last three sampling dates (days 34–48) for each of eight enclosures (two with fish, six without fish). Statistics refer to linear correlations.

(Lampert 1987; Lurling 2003; Wilson et al. 2006) comprised 92% of phytoplankton biomass.

The conclusion that *Daphnia* can reduce the biomass of a cyanobacteria-dominated phytoplankton assemblage must be qualified by the fact that initial cyanobacteria biomass in the fish → no-fish enclosures (Fig. 3) was well below maximum levels observed in the lake (1,260–1,450 μg L^{-1}) during years with high fish biomass (Sarnelle 1993). Thus, it can only be said that *Daphnia* was able to invade and reduce cyanobacteria when *M. aeruginosa* initially comprised ~90% of a total phytoplankton biomass of ~300 μg L^{-1}. This may help to explain why these results contrast somewhat with those of Ghadouani et al. (2003), who reported that *D. pulicaria* declined in shallow enclosures to which very high amounts of phosphorus were added to stimulate a large bloom of cyanobacteria (TP > 600 μg L^{-1}). However, it should be noted that the experiments of Ghadouani et al. (2003) only allowed 29 days for *D. pulicaria* to respond to an order of magnitude increase in nutrients. In this experiment, strong suppression of phytoplankton biomass by *D. pulicaria* was not apparent until 34 days after fish were removed (Fig. 3). It is also interesting to note that Ghadouani et al. (2003) found little effect of massive nutrient addition on total phytoplankton biomass in deep enclosures in the same experiment despite an increase in *M. aeruginosa*, an observation that is more consistent with the results reported here. It is certainly reasonable to postulate that the ability of *Daphnia* to suppress an existing bloom may be a function of initial cyanobacterial biomass (among other factors), and future experiments might profitably examine how a gradient of initial cyanobacterial conditions affects the ability of *Daphnia* to suppress blooms.

An apparent negative effect of *Daphnia* on an established population of *M. aeruginosa* is somewhat surprising given the strong negative effects of cyanobacteria in general (Lampert 1987), and of *M. aeruginosa* specifically (Lampert 1987; Lurling 2003), on *Daphnia* survival, growth, and reproduction in laboratory experiments (Wilson et al. 2006). There are several possible explanations for this seeming lack of congruence. The simplest perhaps is to postulate that *M. aeruginosa* in Zaca Lake is non-toxic and so less inhibitory to *Daphnia* than strains used in laboratory experiments. This explanation seems unlikely as most *M. aeruginosa* genotypes in nature appear to be capable of producing microcystin, the best known of the cyanobacterial toxins (Chorus and Bartram 1999). For example, Wilson et al. (2005) found microcystin-capable genotypes of *M. aeruginosa* in every lake they sampled, and Knoll et al. (in press) report a strong positive correlation between particulate microcystin concentrations and *M. aeruginosa* biomass across lakes. However, no measurements of toxin levels in Zaca Lake have been made, so the hypothesis that the cyanobacteria were not toxic cannot be excluded.

Another possible explanation for the lack of congruence between field and laboratory results may involve a difference in the fundamental nature of the cyanobacteria-grazer interaction in these two experimental settings. In order to maintain the effectiveness of a cyanobacteria-containing treatment in the laboratory, cyanobacteria are commonly added at frequent intervals (every 2 days in the case of Gilbert 1990). This introduces donor control (DeAngelis 1992) into the experimental system in that the population dynamics of the cyanobacteria in the experiment are strongly driven by external input (i.e., immigration). As a consequence, grazers in laboratory containers cannot influence the primary source of cyanobacterial production (immigration rate), which should minimize an important aspect of the phytoplankton-grazer interaction as it occurs in nature, namely grazer control over phytoplankton biomass and species composition (Sarnelle 1999). Large-scale field experiments permit both the effects of cyanobacteria on *Daphnia* and the effects of *Daphnia* on cyanobacteria to be more fully expressed.

Lastly, the lack of congruence between field and laboratory results could be related to the recently documented ability of *Daphnia* to adapt to the presence of toxic cyanobacteria in their diet (Hairston et al. 2001; Gustafsson and Hansson 2004; Sarnelle and Wilson 2005). Specifically, *D. pulicaria* genotypes isolated from lakes with abundant cyanobacteria are better able to grow on a diet of 100% toxic *M. aeruginosa* than genotypes from lakes with few cyanobacteria (Sarnelle and Wilson 2005). In addition, exposing *Daphnia* to toxic cyanobacteria can lead to increased tolerance by individuals and their offspring (Gustafsson and Hansson 2004; Gustafsson et al. 2005).

In contrast to the apparent negative effect of *D. pulicaria* on *M. aeruginosa* in the fish → no-fish enclosures, *Daphnia* biomass declined and phytoplankton

biomass approximately doubled in the never-fish enclosures durings the same period (Figs. 2 and 3). Thus, although *Daphnia* became abundant in the never-fish treatment well before cyanobacteria appeared (O. Sarnelle, unpubl. data), the suppression of cyanobacteria by *Daphnia* seemed to weaken during the experiment (Fig. 5). As a result of these contrasting dynamics, relationships between *Daphnia* and *M. aeruginosa* biomass appeared very different by the end of the experiment across treatments with different histories (Fig. 6). *D. pulicaria* was seemingly both able and not able to suppress *M. aeruginosa* (Fig. 6), an unexpected result. Individual grazer size is an important determinant of the ability of *Daphnia* populations to suppress phytoplankton biomass (Pace 1984; Carpenter et al. 2001), but variable suppression of *M. aeruginosa* by the end of the experiment was not related to differences in *D. pulicaria* size across the two fishless treatments with different histories (Fig. 2).

One general explanation that might account for the seemingly variable ability of *D. pulicaria* to control *M. aeruginosa* is to postulate that the ability of *M. aeruginosa* to resist *D. pulicaria* grazing, the ability of *D. pulicaria* to utilize *M. aeruginosa*, or both, changed with time. Such changes could have been adaptive responses to high levels of *D. pulicaria* grazing or *M. aeruginosa* biomass. Laboratory studies have reported evidence of phenotypic and/or evolutionary adaptations of phytoplankton and *Daphnia* that can make the phytoplankton more resistant to grazing or enhance the ability of *Daphnia* to feed on grazing-resistant cyanobacteria (Van Donk et al. 1999; Gustafsson and Hansson 2004; Sarnelle and Wilson 2005). To date however, no studies have specifically demonstrated that *M. aeruginosa* can rapidly adapt to grazing pressure by becoming more resistant to consumption by zooplankton (Van Donk et al. 1999). Future experiments would be profitably directed at comparing the grazing resistance of cyanobacteria exposed to varying levels of prolonged *Daphnia* grazing. The prospect of variable traits within species greatly complicates efforts to understand trophic interactions.

The primary objective of this study was to determine if initial conditions matter, specifically whether initial dominance by cyanobacteria would affect the ability of *Daphnia* to suppress phytoplankton under eutrophic conditions. Although phytoplankton suppression was slowed compared to previous experiments in Zaca Lake that were initiated before the appearance of cyanobacteria (Sarnelle 1993), the end result was the same in that *Daphnia* eventually seemed able to reduced phytoplankton biomass to levels typical of oligo-mesotrophic lakes (Fig. 3). Thus, initial cyanobacterial dominance did not appear to prevent strong grazer control (subject to the qualifications noted above). Unexpectedly, evidence was found suggesting effects of initial conditions in a different context—the seemingly variable ability of *D. pulicaria* to control *M. aeruginosa* across treatments with different histories (Fig. 6). This result might be a signal that rapid adaptation (i.e., fast enough to occur within the time frame of a field experiment) may lead to complex trophic dynamics in lakes.

This study contributes to a better understanding of biomanipulation, the applied strategy of controlling phytoplankton biomass and increasing water clarity in eutrophic lakes via top-down (i.e., fish) manipulation (Hansson et al. 1998). Bloom-forming cyanobacteria have often been postulated as major obstacles to successful biomanipulation by virtue of their resistance to zooplankton grazing and their negative effects on *Daphnia* in particular (Sommer et al. 1986; Benndorf and Henning 1989; Ghadouani et al. 2003). Experimental studies have shown that phytoplankton and cyanobacterial biomass can be greatly reduced by increasing *Daphnia* grazing, but these have been limited to cases where *Daphnia* attained high densities before cyanobacteria (Christoffersen et al. 1993; Sarnelle 1993; Paterson et al. 2002). This study suggests that *D. pulicaria* can also suppress an established cyanobacteria population, although this result was not apparent until 30+ days after fish removal and may be limited by the magnitude of the established bloom. In addition, however, this study suggests that *Daphnia* control may eventually weaken after 2–3 months, perhaps as a result of increased resistance to grazing by cyanobacteria exposed to high grazing for extended periods. Thus, even where high *Daphnia* grazing can be maintained throughout the growth season by the elimination of zooplanktivorous fish, blooms of cyanobacteria, although reduced, may not be completely eliminated.

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