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Grazer control of nitrogen fixation: phytoplankton taxonomic composition and ecosystem functioning

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With 13 figures and 2 tables

Abstract: By differentially manipulating external nutrient inputs and grazer assemblages (through the presence or absence of fish that fed preferentially on daphniids) in replicate experimental ponds, we explored the potential for grazers to affect phytoplankton composition, particularly the abundance of N-fixing cyanobacteria, the concentration of fixed N, and the extent to which these effects were influenced by nutrient loading. Results indicate marked effects of both grazing and nutrient loading on phytoplankton abundance and composition, N-fixing cyanobacteria presence and N-fixing heterocyst density. When we manipulated N:P using two different N loading regimes at high P loading, grazer and N:P loading effects on cyanobacteria were similar in magnitude. In contrast when N:P loading was kept high while P loading was varied, grazer effects on cyanobacteria were small relative to nutrient loading effects. Aggregate grazer effects appeared to result exclusively from direct removal of cyanobacteria by grazing. Indirect alteration of available N:P by zooplankton was minor in magnitude compared with external and internal nutrient loading. Ecological interactions that structure zooplankton assemblages, such as fish predation, can have substantial cascading effects not only on primary producer biomass, but also on phytoplankton taxonomic composition and ecosystem functioning.

Key words: Cladocera, copepods, grazing, cyanobacteria, experimental ponds, nitrogen to phosphorus ratio.

Introduction

Nitrogen limitation of primary productivity is widespread in both terrestrial and aquatic ecosystems, even though N-fixing organisms are often abundant in both ecosystem types, and should be capable of ameliorating N-limiting conditions (Vitousek & Howarth 1991, Smith 1992). Indeed, this process is, at least in part, responsible for maintaining phosphorus limitation of algal growth in many lakes (Schindler 1977, Howarth et al. 1988b), as well as large expanses of the open ocean (Karl et al. 1997, Falkowski et al. 1998, Krom et al. 2003). Nitrogen fixation can contribute from 6 % to 82 % of all N inputs to eutrophic lakes (Howarth et al. 1988b). Nitrogen fixation by cyanobacteria is energetically expensive and is typically observed only when N is in relatively low supply compared with P (Howarth et al. 1988a, Vitousek et al. 2002). Other factors that may also contribute importantly to the control of N fixation in lakes have been debated, as have the reasons why planktonic N fixation is so much less important in coastal marine ecosystems than in lakes (reviewed by Howarth et al. 1988a, 1988b, Vitousek & Howarth 1991). Before the late 1990s, factors commonly hypothesized to limit N fixation in aquatic systems, like those invoked for terrestrial systems, were typically chemical, physical, or geographical in nature (Howarth et al. 1988b, Vitousek & Howarth 1991, Crews 1999). Over time, increasingly more attention has been paid to the potential role of trophic interactions in controlling N fixation (Sterner et al. 1992, Schaffner et al. 1994, Elser 1999, Fagan et al. 2002,

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Paterson et al. 2002) and to the interactions of biogeochemical and trophic factors (Howarth et al. 1999, Marino et al. 2002, Vitousek et al. 2002, Chan et al. 2004, Chan et al. 2006, Howarth & Marino 2006, Marino et al. 2006). In particular, effects of grazing zooplankton have been relatively little explored. Two processes are likely involved: direct consumption of N-fixing cyanobacteria, and alteration of the supply ratios of N and P through selective mineralization (Elser 1999, Howarth et al. 1999, Marino et al. 2006).

Any direct grazing effect is made more complex in natural systems by the fact that two of the major taxa of freshwater grazers – copepods and cladocerans – have distinct characteristics in how they capture and consume phytoplankton (Rothhaupt 1997, Sommer et al. 2001, Hambright et al. 2007). Copepods, especially calanoids in the Diaptomidae, are thought to be more selective grazers than cladocerans, and thus capable of avoiding consumption of cyanobacteria. By contrast, large cladocerans, such as *Daphnia* are considered non-selective "filter feeders" that accumulate all encountered particles in the water small enough to fit between the ventral carapace gap (e.g., Sarnelle 1992, 2003), including cyanobacteria, and can only non-selectively reject an entire bolus of food using its postabdominal claw (Lampert 1987, but see Bern 1990). In an accompanying paper, however, we discern differential feeding by *Daphnia* at the species level unrelated to size or major taxon (Hambright et al. 2007). Cladoceran zooplankton typically have comparatively higher *per capita* feeding rates than copepods (Lampert & Sommer 2007). Thus without considering the effects of poor food quality of many cyanobacteria, cladocerans may be expected to inflict larger *per capita* grazing mortalities on cyanobacteria compared with copepods.

In addition to direct impacts on primary producer mortality, grazers may indirectly affect the outcome of competition between primary producer taxa through differences in the rates and ratios at which they excrete and egest consumed nutrients back into the environment (Sterner & Elser 2002, Fagan & Denno 2004, Hambright et al. 2007). Freshwater zooplankton exhibit partial homeostatic control of tissue nutrient contents (Hessen & Lyche 1991, DeMott et al. 1998), their body tissue N:P should affect reciprocally the N:P returned to the water (reviewed by Sterner & Elser 2002). With a few exceptions, tied mostly to zooplankton ontogeny (e.g., Villar-Argaiz et al. 2002), the tissue content of cladocerans is relatively P-enriched compared with copepods, while copepods are relatively N-enriched. Thus, copepods by retaining a high N:P should release a low N:P to the water and favor N-fixation by cyanobacteria, whereas the opposite should be the case for cladocerans – inhibition of N-fixing cyanobacteria. Still, despite these observations, little evidence is available demonstrating that zooplankton effects on nutrient availability are of sufficient magnitude to affect phytoplankton species composition (Paterson et al. 2002, but see Sarnelle 1992).

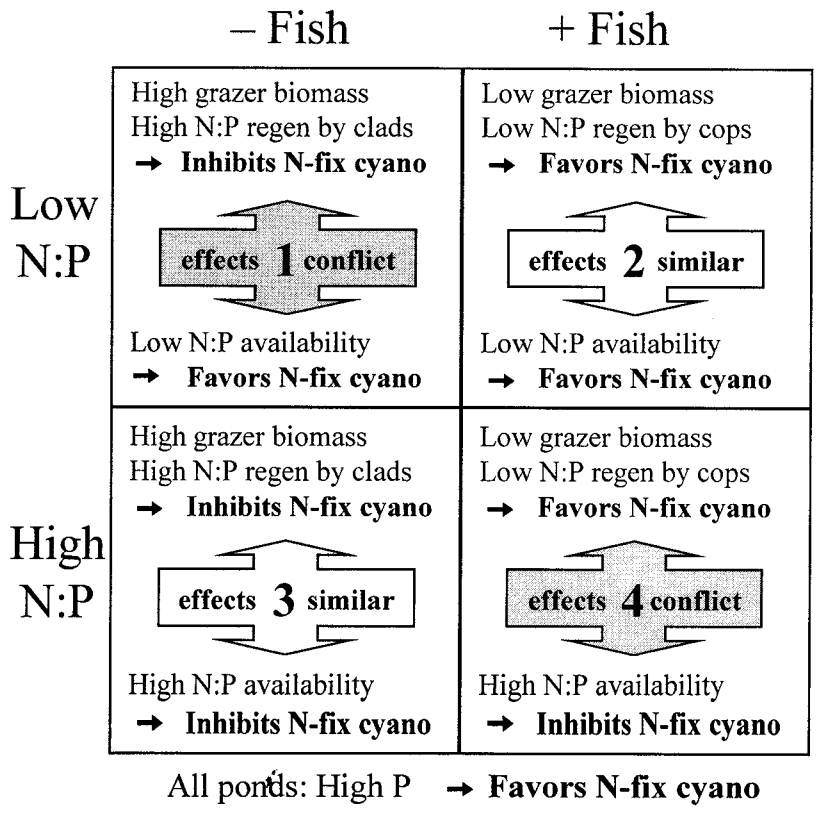
In the research reported here, we explored the potential role for grazers in controlling N-fixing cyanobacteria and the rates of planktonic N fixation in six-month manipulations of whole experimental ponds. The main thrust of our study was to discover if grazers play any role, whether through direct consumption or nutrient mineralization, in affecting the abundance of N-fixing cyanobacteria and the concentration of fixed N in a freshwater system and whether that role is influenced by nutrient loading.

Methods

Experimental design

We investigated the relative importances of different factors in controlling plankton community structure and ecosystem processes by manipulating critical regulation factors independently of each other in a series of replicated, cross-classified pond experiments. In the first experiment, we manipulated two factors, the N:P of supplied nutrients and the level of zooplanktivorous-fish predation (an indirect manipulation of the grazer assemblage), both at two treatments: high and low N:P, and presence (+) and absence (–) of fish (Fig. 1A). All treatment combinations received a high rate of P supply. For two of the treatment combinations (Low N:P +Fish; High N:P –Fish) the expected independent effects of nutrient addition and grazer manipulation are in the same direction (i.e., "compatible") and their relative impacts therefore indistinguishable. We hypothesized that the Low N:P +Fish ponds should contain the most N-fixing cyanobacteria; the High N:P –Fish ponds should contain the least. For the other two treatment combinations (Low N:P –Fish; High N:P +Fish), however, the nutrient loading and grazer biomass effects act in opposite directions allowing us to discern the relative strengths of the two factors (grazer mineralization stoichiometry versus direct grazer consumption) in controlling abundance and activity of N-fixing cyanobacteria. We hypothesized that if N:P mineralization effects dominate consumption effects, then Low N:P –Fish ponds should have greater abundances of N-fixing cyanobacteria than High N:P +Fish ponds, but if consumption dominates, the converse would be true.

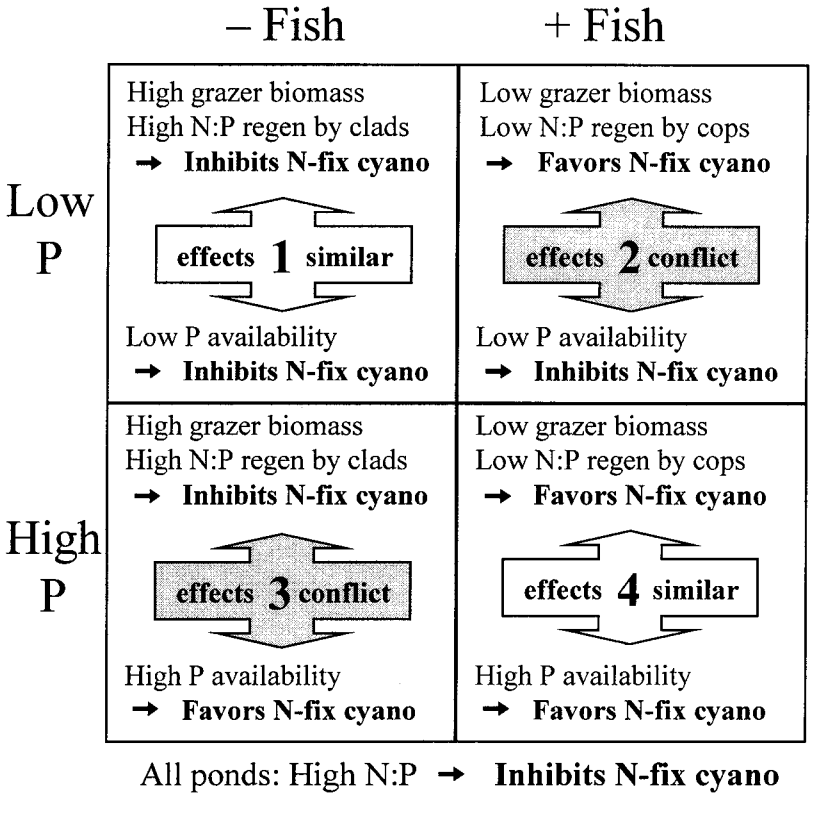
In the second experiment, we manipulated the supply rate of P (high versus low P loading) and the level of zooplanktivorous-fish predation (as in Experiment I), with all treatment combinations receiving high N:P nutrient supply (Fig. 1B). We chose this design for Experiment II in order to learn the extent of between-year effects in our system while simultaneously gaining knowledge about the importance of variable P loading. It would have been useful also to examine the relative roles of P loading and grazing under conditions of low N:P (thus applica-



A. Experiment I

Predicted N-fixing cyanobacteria and heterocyst abundances

a) N:P load effects dominate, 1, 2 > 3, 4
b) Grazer effects dominate, 2, 4 > 1, 3



B. Experiment II

Predicted N-fixing cyanobacteria and heterocyst abundances

a) P-load effects dominate, 3, 4 > 1, 2
b) Grazer effects dominate, 2, 4 > 1, 3

Fig. 1. Design of **(A)** whole-pond Experiment I and **(B)** whole-pond Experiment II, showing both the treatments and their anticipated effects on grazer biomass and regeneration and major predictions regarding cyanobacteria abundances.

ble to investigations relating to P-load reductions in eutrophic lakes), but this was not possible in our system because pond sediments were a major source of internal N loading (see below-Results: Experiment I). Thus, we chose to examine further the roles of P loading and grazing under conditions of high N:P. As in Experiment I, in this second experiment, the expected independent effects of fish predation level and nutrient addition in two of the treatment combinations were compatible and therefore indistinguishable. We hypothesized that High P +Fish ponds should contain the most N-fixing cyanobacteria and the Low P –Fish ponds should contain the least. For the other two treatment combinations (High P –Fish; Low P +Fish), however, nutrient loading and grazer biomass effects act in opposite directions allowing us to determine the relative strengths of these two factors in controlling abundance and activity of N-fixing cyanobacteria. We hypothesized that if P loading effects dominate grazer effects then High P –Fish ponds should have greater abundances of N-fixing cyanobacteria than Low P +Fish ponds, but if grazer effects dominate, the converse would be true.

Distinct plankton assemblages were created during two separate experiments over a three-year period each using two nutrient and two fish predation treatment levels (2×2 cross-classified design) in 16 ponds (4 ponds per treatment combination) at the Cornell University Experimental Ponds Facility. All ponds are 0.1 ha in surface area, 2.2 m deep, and contain 1,800 m^3 of water when full. In spring of the first year, all ponds were drained so that only about 10 cm of water remained. Rotenone was added to remove any fish present, and an herbicide (Diquat dibromide) was added to suppress macrophyte growth. After two weeks, the ponds were refilled with water pumped from an adjacent reservoir through a 500-µm mesh net to prevent introduction of fish. Treatment combinations for individual ponds in Experiment I were chosen using a block design based on sediment carbon, nitrogen and phosphorus data obtained from samples collected just prior to the addition of pesticides. In this way, the full range of sediment variation was present in each treatment combination at the start of the experiment.

Nutrients were added twice per week during spring and summer, and not at all during autumn and winter. Different fertilization regimes were applied in the two years of Experiment I. After information was accumulated in Year 1 on the effects of nutrient addition, treatments were altered in Year 2 to better achieve the desired effect of nitrogen-limitation in the low nitrogen addition ponds and to overcome substantial loss of P to the pond sediments. It was only in Year 2 that a complete data set relevant to our hypothesis was collected. In both years, all 16 ponds received the same phosphorus additions while eight ponds received substantial nitrogen additions and eight received little or no nitrogen. In Year 1 phosphorus was added weekly (June – October) as dilute H_3PO_4 at rate of 9.68 mmol P m^{-3} $year^{-1}$ (0.30 g P m^{-3} $year^{-1}$). Nitrogen was added to the ponds as NH_4NO_3, dissolved in the phosphoric acid, at a rate of 957 mmol N m^{-3} $year^{-1}$ (13.4 g N m^{-3} $year^{-1}$) in the "High N:P" treatments, and 95.7 mmol N m^{-3} $year^{-1}$ (1.3 g N m^{-3} $year^{-1}$) in the "Low N:P" treatments. In Year 2, P was added weekly (April – October) to all ponds at a rate of 18.1 mmol P m^{-3} $year^{-1}$ (0.56 g m^{-3} $year^{-1}$), N was added to the High N:P ponds at 886 mmol N m^{-3} $year^{-1}$ (12.4 g m^{-3} $year^{-1}$), and Low N:P ponds received no nitrogen addition.

Fathead minnows (*Pimephales promelas*), known to be effective zooplanktivores (Hambright & Hall 1992), were added in June of Year 1 to 8 ponds at 0.83 g fish m^{-3} and the remaining 8 ponds were maintained fishless. Fish density after the end of the experiment (all 16 ponds assayed by 3 whole-pond seine hauls each) averaged 9.44 g m^{-3} (± 3.78 sd) in the +Fish ponds and 0.0005 g m^{-3} (± 0.0013 sd) in the –Fish ponds. One of the –Fish ponds, fertilized with low N:P, was found partway through the study to contain fish. Because the date of fish introduction is unknown, this pond is dropped here from all analyses. There is some discussion in the literature that *P. promelas* also consumes phytoplankton, but intensive investigation of their feeding in our system indicated that algae were only consumed as a by-product of zooplankton consumption (Hambright & Hall 1992). In addition, we considered the potentially confounding possibility that excretion and defecation by *P. promelas* might influence nutrient availability for phytoplankton growth. Although studies have demonstrated the potential for planktivorous fish to affect phytoplankton indirectly via nutrient excretion (Schindler 1992, Vanni et al. 1997), this effect is unlikely to have been important in our system, given the high levels of external nutrient loading used. Moreover, allometric scaling of metabolic rates (e.g., Peters 1983, Brown et al. 2004) dictate that fish nutrient excretion rates would be substantially lower than those of zooplankton – as recently demonstrated in both oligotrophic and eutrophic systems (Attayde & Hansson 2001, Sarnelle & Knapp 2005, see also Hudson et al. 1999). Note also that fish added to mesocosm experiments such as ours can only be a net source of nutrients to the water column when they lose weight (Marino et al. 2006); in our experiments, fish population sizes and hence biomass increased over the period of study. Thus we are confident that the effects we attribute to zooplankton are not confounded by fish metabolism.

For Experiment II, the same 16 ponds were used. Eight ponds were randomly assigned to be stocked with fathead minnows, at the density present in the +Fish ponds at the end of Experiment I (9.44 g m^{-3} in each pond). The remaining eight ponds lacked fish. All ponds received nutrients at a high molar N:P of 50:1. Eight ponds were fertilized weekly at an annual rate of 13.9 mmol P m^{-3} $year^{-1}$ (0.43 g P m^{-3} $year^{-1}$) and 694 mmol N m^{-3} $year^{-1}$ (9.72 g N m^{-3} $year^{-1}$), and eight were fertilized at one tenth this rate: 1.39 mmol P m^{-3} $year^{-1}$ (0.043 g P m^{-3} $year^{-1}$) and 69.4 mmol N m^{-3} $year^{-1}$ (0.97 g N m^{-3} $year^{-1}$). The nutrient treatments were randomized with respect to the nutrient treatments in Experiment I.

Sampling and analyses

In Experiment I, zooplankton were sampled six times (May – September); phytoplankton were counted from collections made on seven dates (May – October). Nutrients and phytoplankton chlorophyll were sampled four times (April – September). In Experiment II, all ponds were sampled every three weeks (May – October).

Water for phytoplankton chlorophyll and cell counts and for nutrient analyses was collected with a 0.7-m long 3-L Scott bottle at two depths: 0 – 0.7 m and 0.8 – 1.5 m. Samples from the two depths were then combined in equal proportions for further analysis. Whole water was filtered through Whatman GF/C glass fiber filters. The filters were frozen and stored in the dark for subsequent (usually the next day) chlorophyll analysis. The material retained on the filter was extracted in 90 % acetone for 24 hr and analyzed spectrophotometrically according to the method of Lorenzen (1967). Phytoplankton samples (100 mL) were preserved with acid Lugol's solution. In the laboratory, phytoplankton samples were settled for at least 24 h and then counted at 400× under a Wild M40 inverted microscope (Lund

et al. 1958). Additional counts were made at 100× to estimate the abundances of larger, rarer forms. Typical cells of each taxon identified were measured using an eye-piece micrometer and phytoplankton cell biovolumes were calculated using standard volumetric formulae for the geometric shapes most closely matching those of the cells in question and then converted to biomass by assuming a specific gravity of 1 (e.g., Hillebrand et al. 1999). The filtrate was used for triplicate analyses for total soluble nitrogen (TSN), total soluble phosphorus (TSP), dissolved inorganic nitrogen (DIN), and soluble reactive phosphorus (SRP). Samples for TSN analysis were digested with alkaline potassium persulfate at 100 °C (D'Elia et al. 1977) and analyzed using the cadmium reduction method (American Public Health Association 1985). DIN was estimated as the sum of NH_4 (phenol method, American Public Health Association 1985) and NO_3-NO_2 (cadmium reduction). Samples for TSP (following digestion with potassium persulfate at 100 °C, Menzel & Corwin 1965) and SRP were analyzed using the ascorbic acid method (American Public Health Association 1985).

Zooplankton were collected later during the same week as the water chemistry and phytoplankton samples and from the same two depths using a 30-L Schindler-Patalas trap fitted with a 75 µm-mesh net. These samples were pooled and preserved with a 10 % sugar-formalin solution (Haney & Hall 1973). The process was repeated to provide duplicate zooplankton samples from each pond on each date. Zooplankton samples were subsampled (5 % to 50 % depending on density) and counted in a Bogorov-Litt tray at 25× under a Wild M8 stereomicroscope. Ten to 30 representatives of the major taxa were sorted onto nylon mesh, dried at 60 °C and weighed on a Sartorius ultramicrobalance in order to estimate biomass. Biomasses of less abundant species were estimated from measurements of species lengths and using standard length-weight regression equations (Culver et al. 1985).

Benthic flux-Sedimentation

Weekly sedimentation rates were measured using 4-L plastic jars anchored to the pond bottoms with cinder blocks. The jar openings were 9.1 cm diameter (area = 65.04 cm^2). There were two jars in each pond and three ponds per treatment combination. Each jar was covered with a mesh of hardware cloth (1/4 inch mesh) to keep fish out. Traps were deployed four times in ponds for one week each during late summer (July and August). At recovery, samples were placed in tared beakers and evaporated to dryness at ca. 60 °C. Cooled, dried samples were redried at ca. 60 °C for an additional hr, cooled to room temperature and weighed. Five mg from each dried sample were ashed at 550 °C, suspended in 20 ml deionized water and digested in HCl (4 mL 1 N HCl at 104 °C, for 2 hr). Samples were then filtered through Whatman 934-AH GF filter, diluted to 50 mL and analyzed for SRP (as detailed for pond samples above). Percent N and C were determined using a Carlo Erba model NA 1500 Carbon Nitrogen Analyzer at the Institute for Ecosystem Studies, Millbrook, NY.

Benthic flux-Sedimentary nutrient exchange

Sedimentary exchange of N and P were measured in the ponds using large benthic chambers (area = 0.12 m^2; volume = 22 L) constructed of linear polyethylene and deployed *in situ*. The chambers were dark so as to prevent photosynthesis. They were deployed by SCUBA or snorkeling. Small battery-operated pumps circulated water within the chambers and allowed sampling of the chambers over time from the surface. Fluxes were estimated from slopes of accumulation or loss of ammonium and TSP from the chambers during time-course measurements. Preliminary measurements demonstrated that 2–4 h incubations with sampling at 20–30 minute intervals yielded good results. An attached rubber bag compensated for water removed in sampling (Rutgers van der Loeff et al. 1984). Ammonium and TSP were analyzed as detailed for pond monitoring above. Flux measurements were made once during July and once during August in eight of the ponds (2 replicate ponds for each of the 4 treatment combinations). Two replicate chambers were used in each pond at the time of measurement.

Nitrogen fixation rates

In oxic water columns of lakes, nitrogen fixation (the biological reduction of molecular nitrogen to ammonia) is mediated almost exclusively by filamentous species of cyanobacteria, and rates tend to be well-correlated with abundances of heterocysts (Goldman & Horne 1983, Howarth et al. 1988b), the cyanobacterial cells specialized for nitrogen fixation. We obtained estimates of heterocyst densities in both experiments as part of our routine monitoring of phytoplankton densities in the ponds. To check for a relationship between heterocyst density and nitrogen fixation rate in our system, we measured rates of nitrogen fixation by the acetylene reduction method (Howarth & Cole 1985, Howarth et al. 1988b) and estimated heterocyst densities from phytoplankton samples in each of the 16 experimental ponds in August of Experiment II.

Statistical analyses

Effects of zooplankton type (as induced by fish predation) and N:P loading ratio in Experiment I, or P loading rate in Experiment II on phytoplankton and zooplankton assemblages were estimated using summer means (from 5–7 tri-weekly sampling dates) in each pond (Experiment I: n = 15; Experiment II: n = 16). These data were log-transformed, ln(x+1), and analyzed by ANOVA ($\alpha = 0.05$), except for sediment trap and sediment release data that were based on only 3 ponds per treatment combination for which α was set to 0.1 (Winer 1971). All results are presented as non log-transformed data.

Results

Experiment I

The biomass of grazing crustacean zooplankton present in the ponds was dominated (> 95 %) by calanoid copepods (mainly *Skistodiaptomus pallidus*) and cladocerans (mainly *Daphnia pulicaria*). Other smaller cladoceran and cyclopoid copepod species, present only in low biomass, made up the remainder of the grazing crustacean zooplankton community. We were successful in our use of fish density as a means to produce different zooplankton assemblages. Cladoceran biomass was strongly reduced in the presence of fish, due to a reduction in large species (*Daphnia*, *Di-*

Table 1. Results of pond Experiment I showing effect sizes of treatments (fish and N:P) for major zooplankton, nutrient and phytoplankton parameters. Bold indicates significant effects at $P \leq 0.05$ ([a]$P \leq 0.10$, see text). Edible and non-edible phytoplankton were defined based on results from grazing experiments (Hambright et al. 2007).

	Effect size			Effect size	
Parameter	Fish	N:P	Parameter	Fish	N:P
ZOOPLANKTON			TSP, μg L^{-1}	**–6.7**	1.8
Herb. crustaceans, μg L^{-1}	**–170.6**	**114.6**	TSN:TSP (molar ratio)	**19.8**	**76.1**
Lg. cladocerans, μg L^{-1}	**–125.6**	38.1	N Sed., mg N $m^{-2}d^{-1}$	**63.7**	**72.0**[a]
Sm. cladocerans, μg L^{-1}	0.0	0.7	P Sed., mg P $m^{-2}d^{-1}$	**4.6**	**10.3**
Herb. calanoids, μg L^{-1}	–38.6	**76.6**	N:P Sed. (molar ratio)	**2.9**[a]	0.1
Herb. cyclopoids, μg L^{-1}	**–9.3**	–2.0	N release, μg N $L^{-1}hr^{-1}$	**148.5**	**226.5**
Fraction lg. clad., %	**–37.4**	4.0	P release, μg P $L^{-1}hr^{-1}$	0.1	1.1
Pred. cyclopoids, μg L^{-1}	–25.6	**–31.2**	N:P release (molar ratio)	**96.8**	**265.4**
Rotifers, μg L^{-1}	**–49.9**	–27.0			
Chaoborus, No. m^3	**–203.9**	151.5	PHYTOPLANKTON		
			Total, μg mL^{-1}	**55.2**	30.0
NUTRIENTS			N-fixing cyano. , μg mL^{-1}	**13.5**	**14.3**
DIN, μg L^{-1}	–189.4	**697.4**	Heterocysts, No. L^{-1}	**52.5**	**–33.1**
SRP, μg L^{-1}	–1.6	–0.5	Other cyano., μg mL^{-1}	**9.5**	6.0
DIN:SRP (molar ratio)	**–68.1**	**856.1**	Edible, μg mL^{-1}	**28.8**	**21.4**
TSN, μg L^{-1}	–179.3	**807.0**	Non-edible, μg mL^{-1}	3.5	–0.2

aphanosoma, *Simocephalus*, *Ceriodaphnia*), whereas small cladoceran species (*Bosmina*, *Chydorus*) where unaffected by fish (Table 1, Fig. 2). The biomass of herbivorous calanoid copepods was not significantly affected by fish, but smaller herbivorous cyclopoids were reduced in the presence of fish. These combined effects resulted in a crustacean grazer assemblage composed of 40–50 % (by mass) of large cladocerans in the –Fish treatments, but less than 10 % in the +Fish treatments. Biomass of rotifers, the principal non-crustacean grazers, was also reduced in the presence of fish, as were densities of the large predatory invertebrate, *Chaoborus*. Manipulation of N:P by nitrogen addition produced few effects on the pond zooplankton assemblages. Herbivorous crustacean biomass was greater in the High N:P treatments, principally through increased calanoid copepod biomass, whereas biomass of predatory cyclopoid copepods was greatest in low N:P treatments. Only one statistically significant fish × N:P interaction was detected: predatory copepods were more abundant in low N:P treatments, but only in the absence of fish (Fig. 2).

As anticipated, the presence of fish had little or no direct positive statistical effect on nutrient concentrations in the ponds. There was no significant effect of fish on available nitrogen, either as DIN (sum of NH_4^+ and NO_3^-) or TSN, and concentrations of TSP were lower in +Fish treatments (Table 1, Fig. 3). The relative availability of N as DIN:SRP was lower in the presence of fish, but TSN:TSP was elevated. N:P loading did not affect P availability as SRP or TSP, but as expected, DIN, TSN, DIN:SRP and TSN:TSP were increased in High N:P treatments. Significant fish × N:P loading interaction effects on nutrients were common, with weaker impacts of nutrient manipulations when fish were present (i.e., domination by copepod grazers tended to reduce the N:P loading effects on DIN, TSN, TSP, and DIN:SRP) (Fig. 3).

Sedimentation of N and P was significantly higher in the presence of fish, as was the N:P of sedimenting material (Table 1, Fig. 4). Likewise, release of NH_4^+ from the sediments was elevated in the presence of fish. High N:P loading yielded enhanced sedimentation of both N and P, and sediment release of N. Overall sedimentation of organic mater (as C) was not affected by either fish or nutrient treatments (data not shown). As with many water column nutrients, the N:P loading impacts on N and P sedimentation were weaker in the presence of fish and there was a significant interaction (Fig. 4).

The presence of fish (and hence reduced crustacean biomass) produced significantly higher biomass of total phytoplankton, N-fixing and other cyanobacteria, and heterocyst densities, while High N:P loading yielded lower N-fixing cyanobacterial biomass and heterocyst densities (Table 1, Fig. 5). Results of mesocosm grazing experiments with *Skistodiaptomus* and *Daphnia* (Hambright et al. 2007) revealed no taxon-specific patterns in grazing across the major phytoplankton taxa present in ponds. Nevertheless, both grazers were

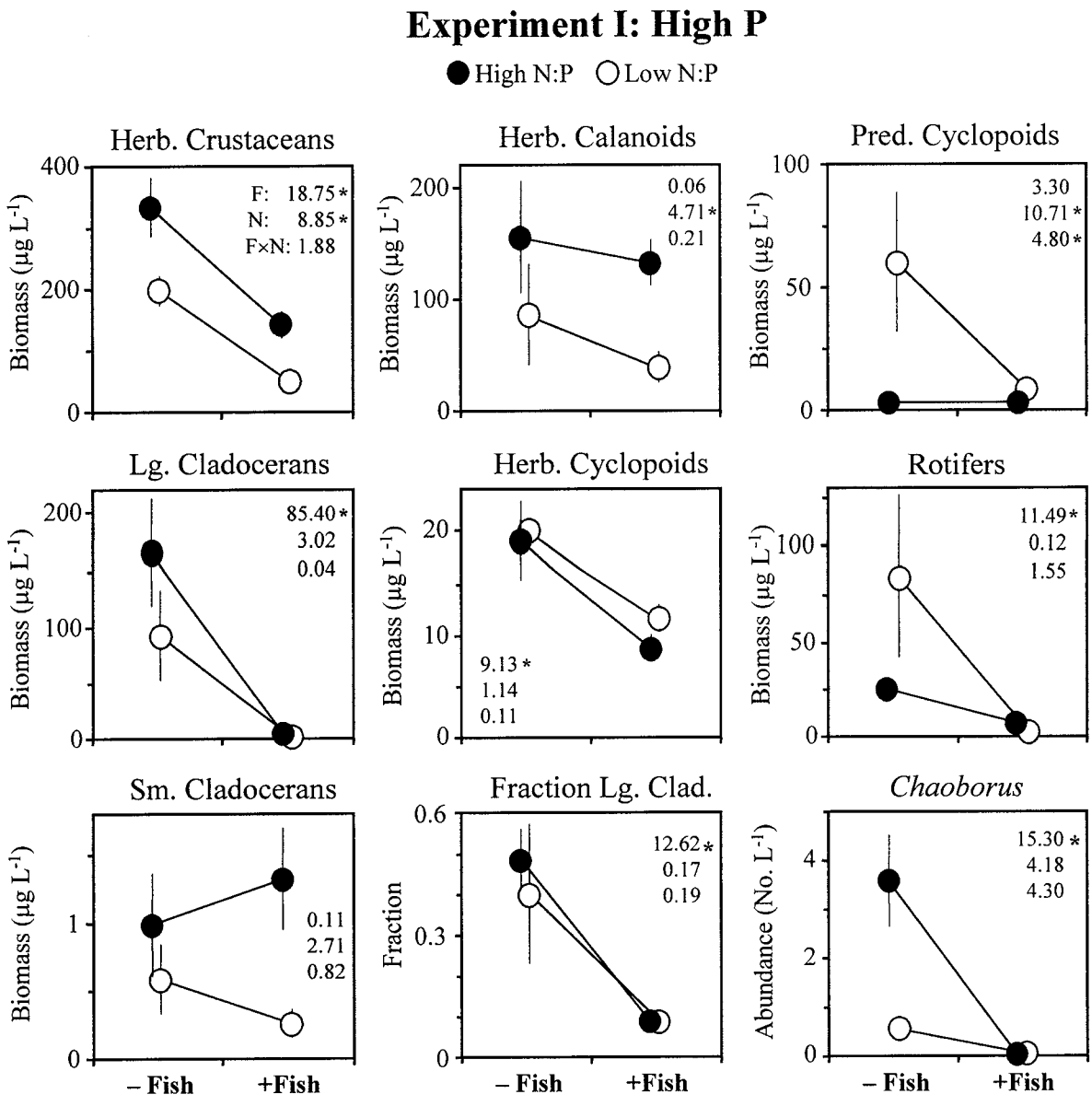


Fig. 2. Mean (± SE) biomass of major zooplankton groups in ponds during Experiment I showing main and interaction effects of fish and nutrient treatments. Numbers show results of 2-factor ANOVA as F-statistic values for fish (F), nutrient loading (N) and fish × nutrient loading interactions (F × N); asterisks indicate significant ($P \leq 0.05$) treatment and interaction effects.

selective for other characteristics allowing differentiation between "edible" and "non-edible" (i.e., grazed and non-grazed, see Hambright et al. 2007) algal taxa. Presence of fish (and hence copepod-dominated grazer assemblages) yielded significantly higher biomass of edible phytoplankton while these taxa were reduced under High N:P loading. In contrast to water column nutrient concentrations and nutrient sedimentation rates, significant fish × N:P interaction effects on phytoplankton indicate that the effects of nutrient loading were reduced in the absence of fish. This effect is primarily the result of severe reduction of total phytoplankton biomass and subsequent domination of the assemblages by both N-fixing (ca. 30 %) and non-N-fixing (ca. 23 %) cyanobacteria in *Daphnia*-dominated ponds (Fig. 5).

Rates of N fixation, measured by the acetylene reduction method (Howarth et al. 1993, Marino et al. 2006) on phytoplankton assemblages from 14 ponds in late summer during Experiment II, demonstrated a highly significant positive relationship between N fixation rate (as acetylene reduction, *AR*, 10^3 nmoles L^{-1} hr^{-1}) and heterocyst density, *HD*, 10^4 mL^{-1}, ($AR = 0.056\ HD - 28.48$, $r^2 = 0.98$, n = 14). Although the ef-

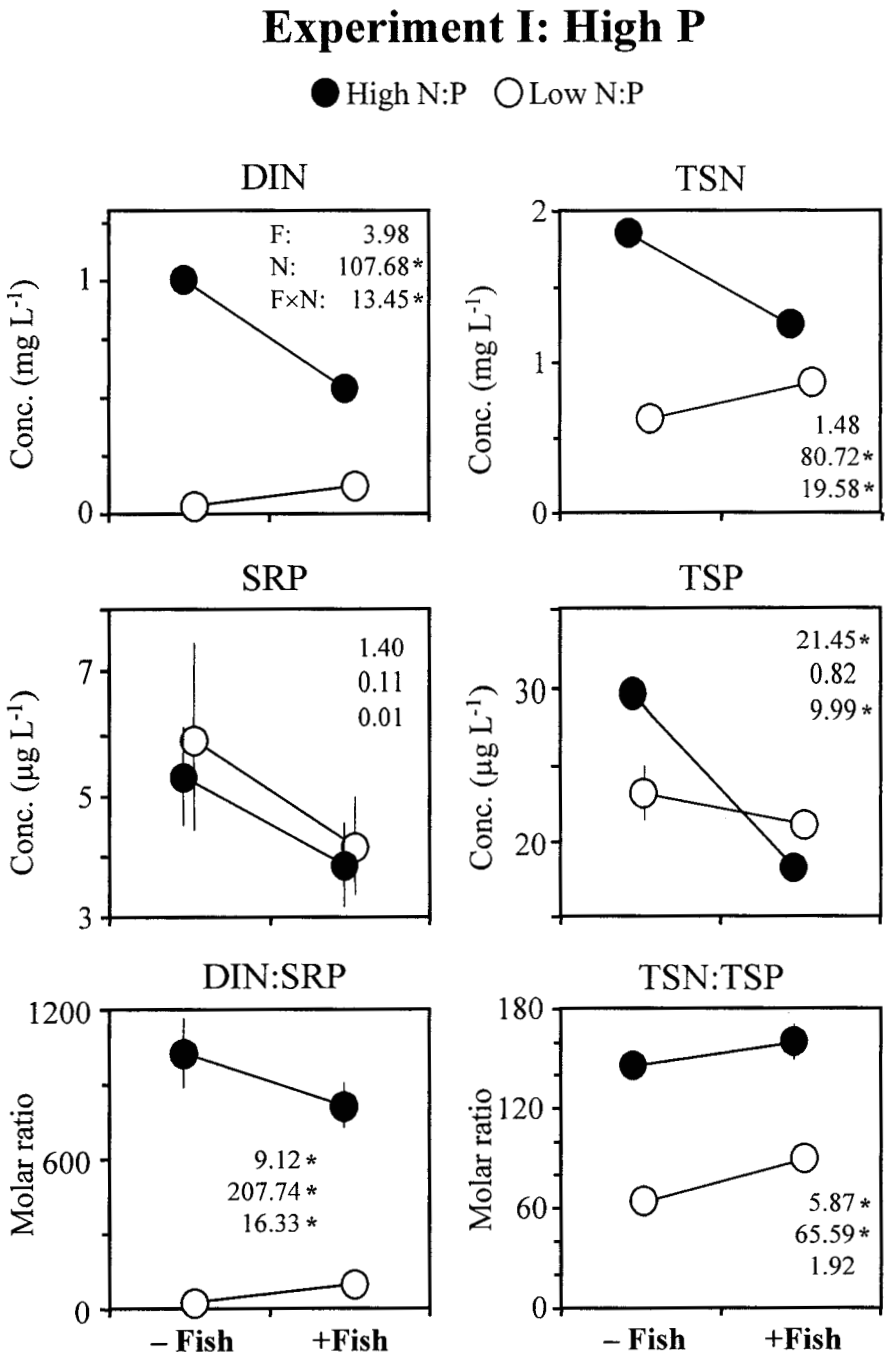


Fig. 3. Mean (± SE) concentrations of dissolved inorganic nitrogen (DIN = sum of ammonia + nitrate + nitrite), soluble reactive phosphorus (SRP), total soluble nitrogen (TSN) and total soluble phosphorus (TSP) and molar ratios of DIN:SRP and TSN:TSP in ponds during Experiment I showing main and interaction effects of fish and N:P loading. Labels as in Fig. 2.

fects of N fixation on water-column N levels can not be known precisely, a strong link is suggested by the relationships observed between cladoceran biomass, heterocyst density and N concentrations among treatments (Fig. 6). Under N limiting conditions (Low N:P) and in the absence of intense zooplankton grazing pressure (+Fish: few or no cladocerans), enhanced heterocyst formation led to a 10-fold increase in DIN and a 2-fold increase in TSN by September. Over the same period, experimental ponds exposed to the Low N:P treatment but having high cladoceran grazer biomass (–Fish) contained no or few heterocysts and showed no increase in water column N. In High N:P treatments with relatively high N availability due to the weekly additions of N, there was divergence in the N concentrations between ponds with and without fish. However, rather than inputs of N via N-fixation (which was effectively absent in these ponds), the el-

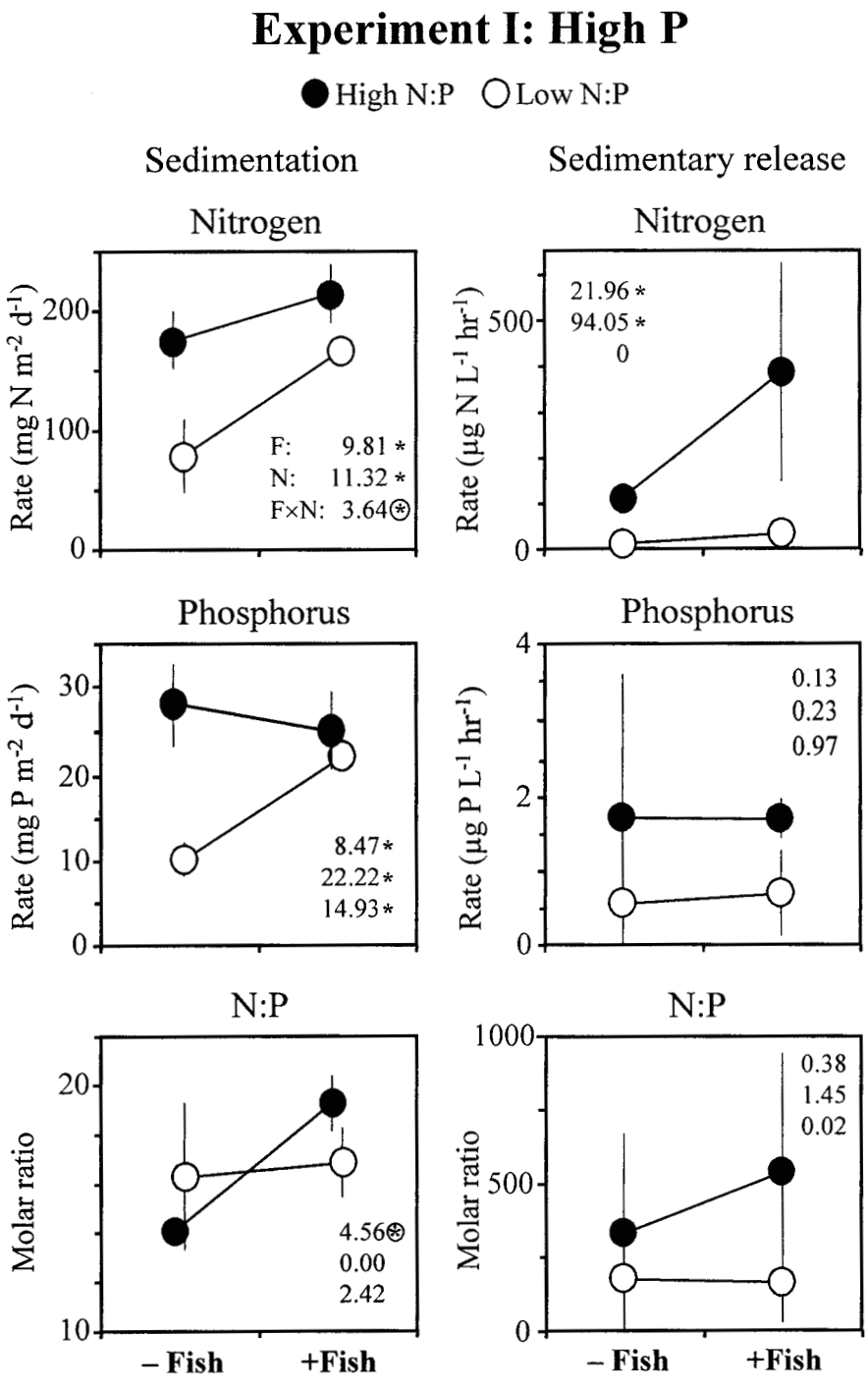


Fig. 4. Mean (± SE) sedimentation rates and sedimentary release rates of N and P and their ratios in ponds during Experiment I showing main and interaction effects of fish and N:P loading. Labels as in Fig. 2. Note: mean C sedimentation was 1.63 ± 0.18 g C m^{-2} d^{-1} with no significant differences among treatments (all $P > 0.15$). Circled asterisks indicate significant at $P \leq 0.10$, as sediment trap data are based on only four measurements from three ponds per treatment combination; sediment release data are based on two measurements from two ponds per treatment combination.

evated concentrations of N in High N:P –Fish ponds were related to lack of uptake by phytoplankton due to severe reductions in biomass, including cyanobacteria, as a result of elevated cladoceran grazing. Regardless of N:P loading, mean summer densities of N-fixing cyanobacteria and heterocysts in ponds (excluding two ponds in which 80 % or more of the cyanobacteria were non-heterocyst forming *Microcystis*) were significantly negatively associated with biomass of large cladocerans, regardless of N:P loading (Fig. 7).

Comparison of mean zooplankton nutrient excretion rates measured in mesocosm experiments (estimated to range from 0.2 to 1.5 g N m^{-2} d^{-1} and 0.2 to 2.0 mg P m^{-2} d^{-1} Hambright et al. 2007) with external loading rates (weekly nutrient additions), internal nutrient loading (estimated from benthic flux measure-

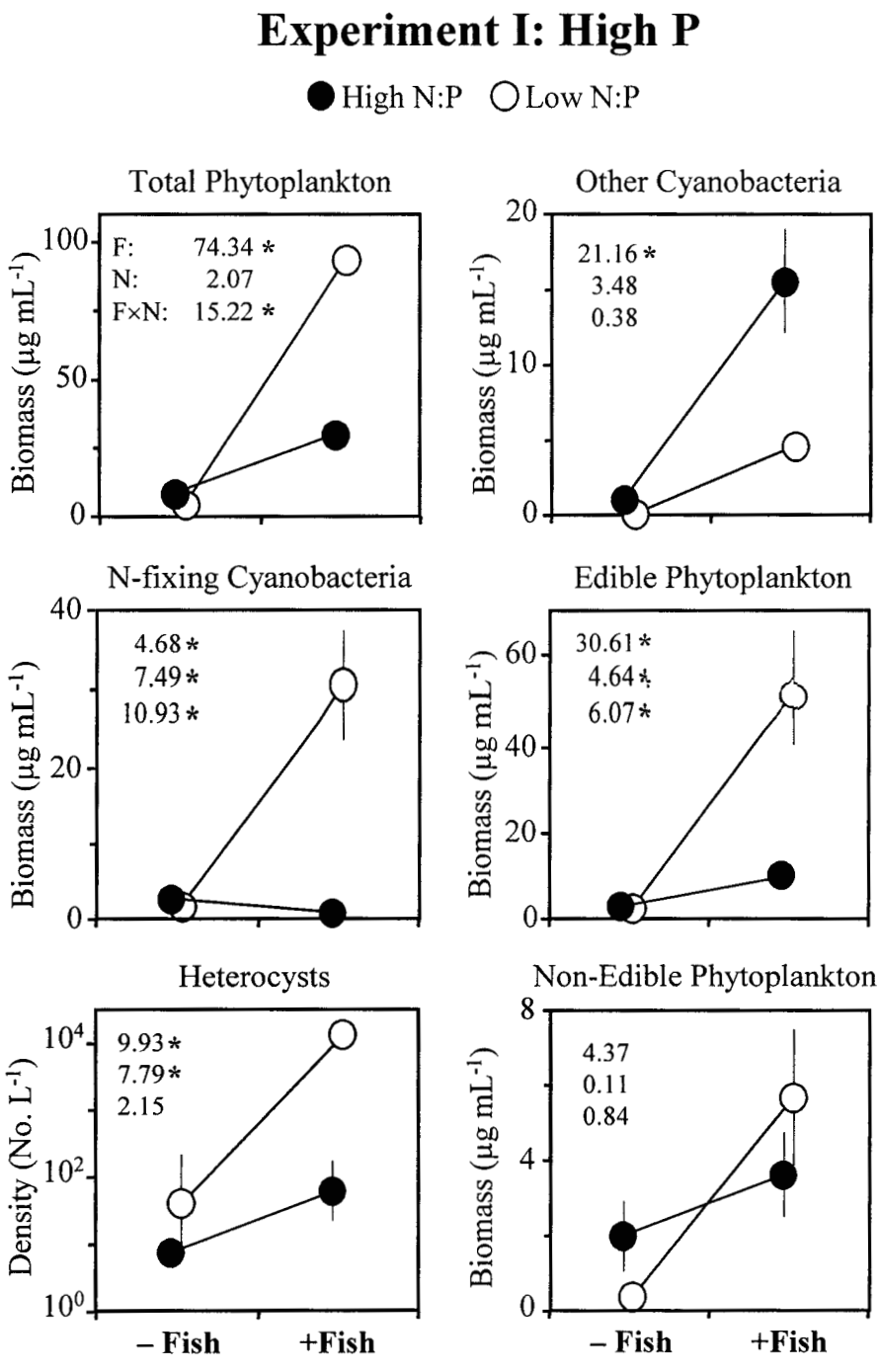


Fig. 5. Mean (± SE) biomass (based on cell counts and dimensions) of major phytoplankton groups in ponds during Experiment I showing main and interaction effects of fish and N:P loading. Labels as in Fig. 2. Edible and non-edible phytoplankton were defined based on results from grazing experiments (Hambright et al. 2007).

ments), and potential N-fixation rates (estimated from heterocyst densities and the acetylene reduction – heterocyst density relationship) revealed that zooplankton excretion accounted for an average of ca. 5 and 11 % of the available N and P in the ponds (Fig. 7).

Experiment II

In the second experiment, biomass of herbivorous crustaceans was slightly higher than in Experiment I, mostly due to a ca. 2-fold higher abundance of calanoid copepods, an effect similar to the High N:P effect seen in Experiment I. Effects of fish manipulations in the second experiment were similar to those of the first. Biomass of large cladocerans, carnivorous copepods, and rotifers and *Chaoborus* densities were reduced in the presence of fish (Table 2, Fig. 9). Unlike Experiment I, in which high N loading yielded only increased calanoid biomass, high P loading in Experi-

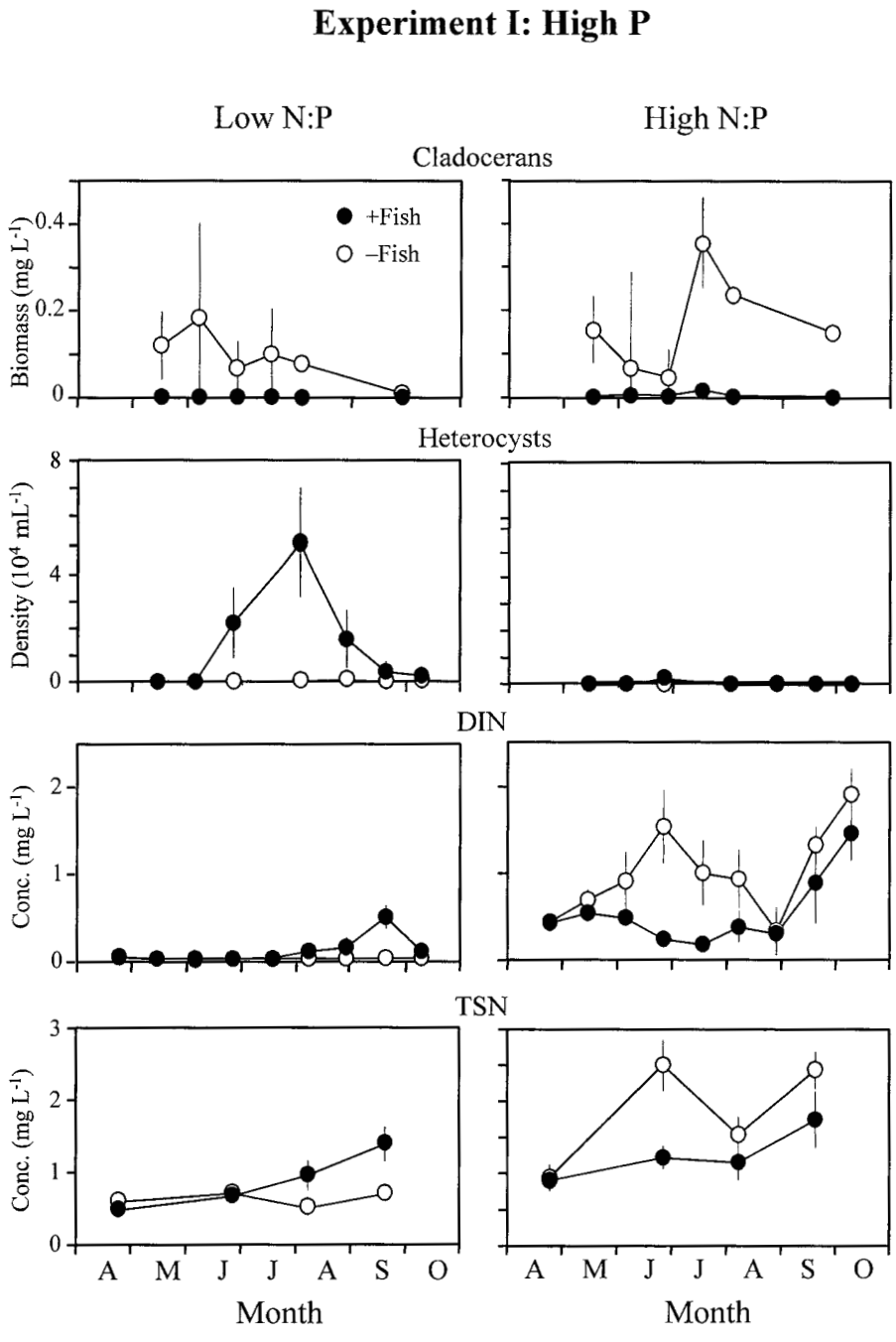


Fig. 6. Temporal trends in mean (± SE) cladoceran biomass, heterocyst density, DIN, and TSN during Experiment I in Low N:P (left panels) and High N:P (right panels) ponds.

ment II yielded increased biomass of large cladocerans and carnivorous cyclopoid copepods, but reduced biomass of small cladocerans. Overall, cladoceran biomass was similar across the two experiments, except that large cladocerans were slightly less abundant and dominated by *Ceriodaphnia, Diaphanosoma* and *Simocephalus* in the second experiment. In Experiment I, *Daphnia* constituted an average of 50 % of the large cladoceran biomass in the –Fish treatment, but only 26 % in Experiment II. In the case of large cladoceran biomass and the proportion of large cladocerans relative to total crustacean biomass, increased availability of P intensified the fish effect, as indicated by a significant Fish × P-load interaction due to enhancement of large cladocerans in –Fish, High P ponds compared with all others (Fig. 9).

Again as anticipated, the presence of fish did not affect available nitrogen (TSN) or phosphorus (TSP) but, as in Experiment I, did result in increased TSN:TSP (Table 2, Fig. 10). Manipulation of P at high

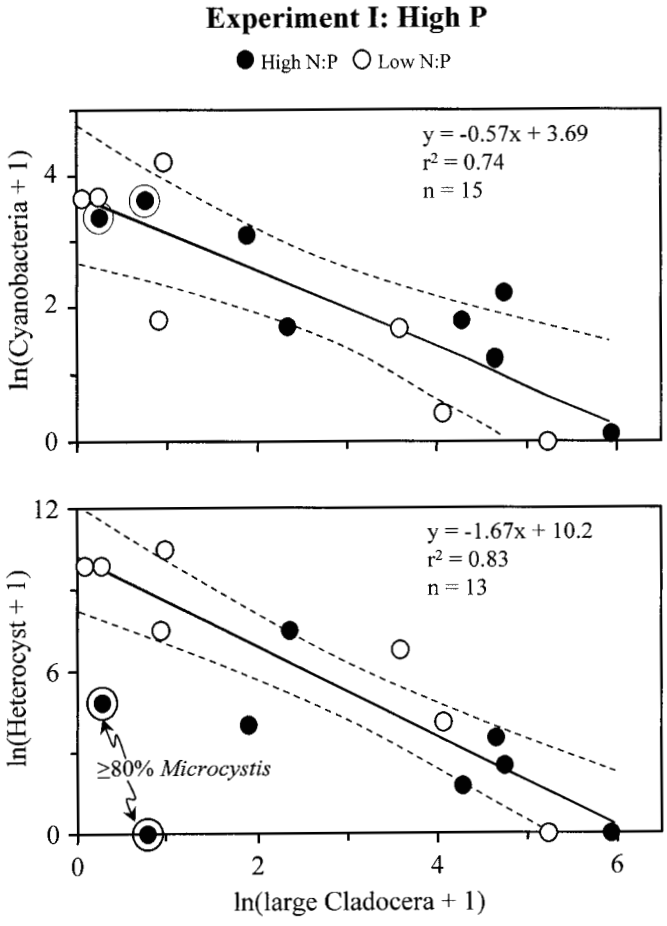


Fig. 7. Upper panel: relationship between mean summer N-fixing cyanobacteria biomass and mean summer biomass of large cladocerans in ponds with high (closed circle) and low (open circle) N:P loading during Experiment I. Lower panel: same as A but for mean summer heterocyst densities. Linear regression was fitted only to data from 13 of 15 total ponds used during the experiment, omitting two +Fish ponds in which the cyanobacteria assemblage was dominated (≥ 80 %) by *Microcystis* sp. which does not make heterocysts or fix N. *Microcystis* sp. averaged < 45 % of the cyanobacteria assemblages in all other ponds.

N:P strongly affected N and P availabilities. High P loading yielded increased availability of both TSN and TSP and lower TSN:TSP. The enhancement of TSP and reduction of TSN:TSP under High P loading were weakened in the presence of fish, as indicated by the significant Fish × P-load interaction (Fig. 10).

Sedimentation rate of N and the N:P of sedimenting material was significantly enhanced in +Fish treatments (Table 2, Fig. 11). High P loading yielded enhanced sedimentation of both N and P, and reduced N:P of sedimenting material. Unlike Experiment I, sedimentation of organic mater (as C) was strongly affected by both fish and nutrient treatments, with both the presence of fish and High P loading yielding 35 and 54 % increases in C sedimentation rate, respectively.

The effects of fish and P loading under conditions of high N:P produced some striking and even unexpected effects on the pond phytoplankton assemblages. As expected, both the presence of fish and high P loading resulted in increased phytoplankton biomass (Table 2, Fig. 12). As in Experiment I, the presence of fish produced increased biomass of non N-fixing cyanobacteria and edible algal taxa, but did not yield increased biomass of N-fixing cyanobacteria or heterocyst densities. However, even though all ponds were loaded weekly with nutrients at high N:P (50:1), N-fixing cyanobacteria and heterocysts occurred in all treatment combinations, especially those receiving high P loading, accounting for 64 and 57 % of the total phytoplankton biomass in High P ponds with and without fish, respectively. This effect contrasts with results of Experiment I in which the same levels of nutrient loading resulted in very low abundances of N-fixing cyanobacteria (3 and 12 %, respectively for High N:P ponds with and without fish) and heterocysts. One significant Fish × P-loading interaction was detected for phytoplankton in Experiment II – the enhancement of non N-fixing cyanobacteria at High P loading was intensified in the presence of fish (Fig. 12). Unlike Experiment I, we detected no significant negative relationship between cladoceran grazer biomass and either N-fixing cyanobacteria or heterocyst densities across P loading treatments (Fig. 13).

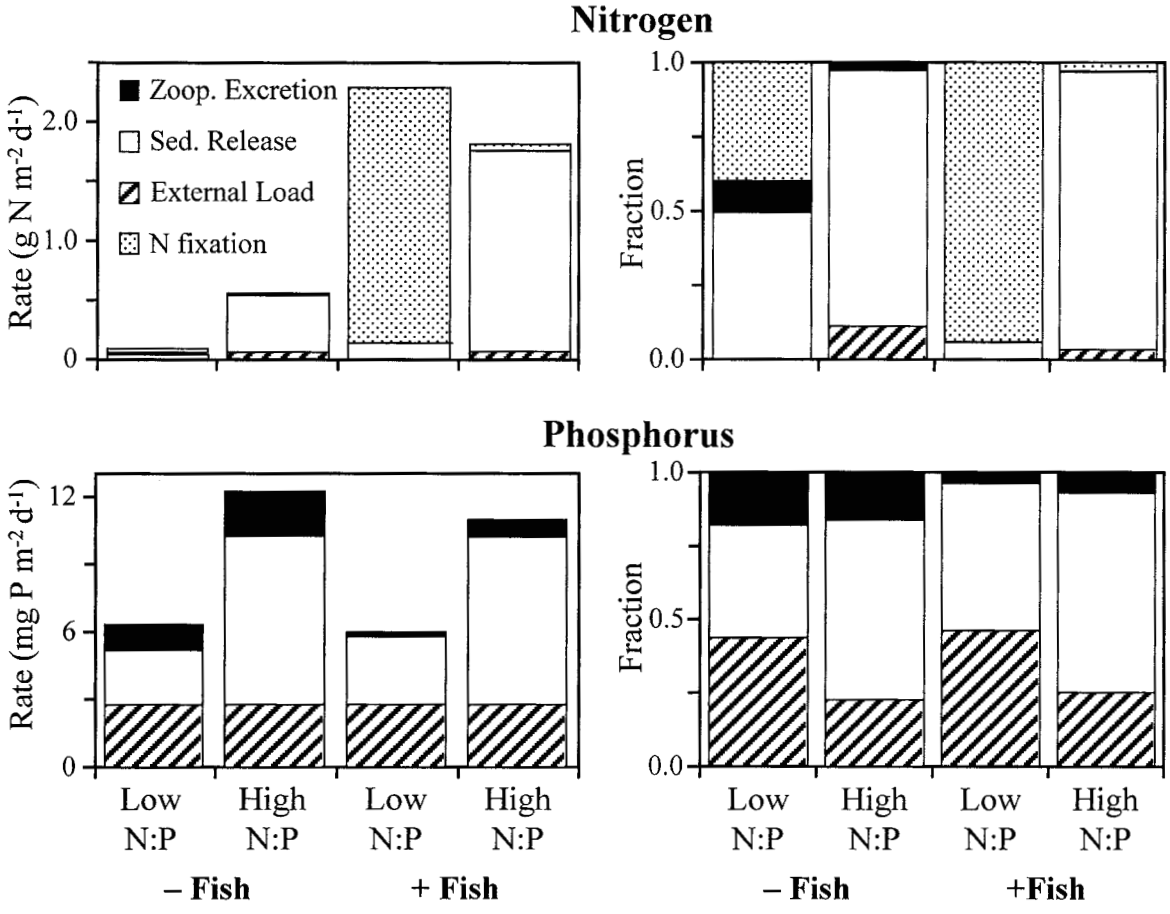


Fig. 8. Comparison of mean zooplankton mineralization of N and P (Hambright et al. 2007) with external and internal loading, and potential N fixation in Experiment I as absolute (g N and mg P $m^{-2}d^{-1}$; left-hand panels) and relative amounts (right-hand panels). External nutrient loading is the twice-weekly nutrient additions made as part of the experimental design; internal loading was estimated from July and August benthic nutrient flux measurements (see Fig. 4), and potential N fixation was estimated from heterocyst densities.

Table 2. Results of pond Experiment II showing effect sizes of treatments (fish and N:P) for major zooplankton, nutrient and phytoplankton parameters. Bold indicates significant effects at $P \leq 0.05$ ($^aP \leq 0.10$, see text). Edible and non-edible phytoplankton were defined based on results from grazing experiments (Hambright et al. 2007).

	Effect size	
Parameter	Fish	N:P
ZOOPLANKTON		
Herb. crustaceans, μg L^{-1}	–182.7	67.3
Lg. cladocerans, μg L^{-1}	**–93.4**	**58.1**
Sm. cladocerans, μg L^{-1}	3.5	**–3.6**
Herb. calanoids, μg L^{-1}	–93.0	13.2
Herb. cyclopoids, μg L^{-1}	0.1	–0.3
Fraction lg. clad., %	**–24.1**	9.8
Pred. cyclopoids, μg L^{-1}	**–13.4**	**8.5**
Rotifers, μg L^{-1}	**–79.1**	43.2
Chaoborus, No. m^3	**–110.4**	–40.0
NUTRIENTS		
TSN, μg L^{-1}	89.1	**484.1**
TSP, μg L^{-1}	–11.7	**32.2**

	Effect size	
Parameter	Fish	N:P
SN:TSP (molar ratio)	**17.6**	**–46.4**
N Sed., mg N $m^{-2}d^{-1}$	**102.7**[a]	**115.3**[a]
P Sed., mg P $m^{-2}d^{-1}$	6.4	**27.4**
C Sed., mg C $m^{-2}d^{-1}$	**0.9**[a]	**1.2**
N:P Sed. (molar ratio)	**2.5**	**–2.2**[a]
PHYTOPLANKTON		
Total, μg mL^{-1}	**29.4**	**41.5**
N-fixing cyano. , μg mL^{-1}	18.7	**28.1**
Heterocysts, No. L^{-1}	4.7	**335**
Other cyano., μg mL^{-1}	**7.4**	**8.2**
Edible, μg mL^{-1}	**6.2**	**7.7**
Non-edible, μg mL^{-1}	–0.7	1.8

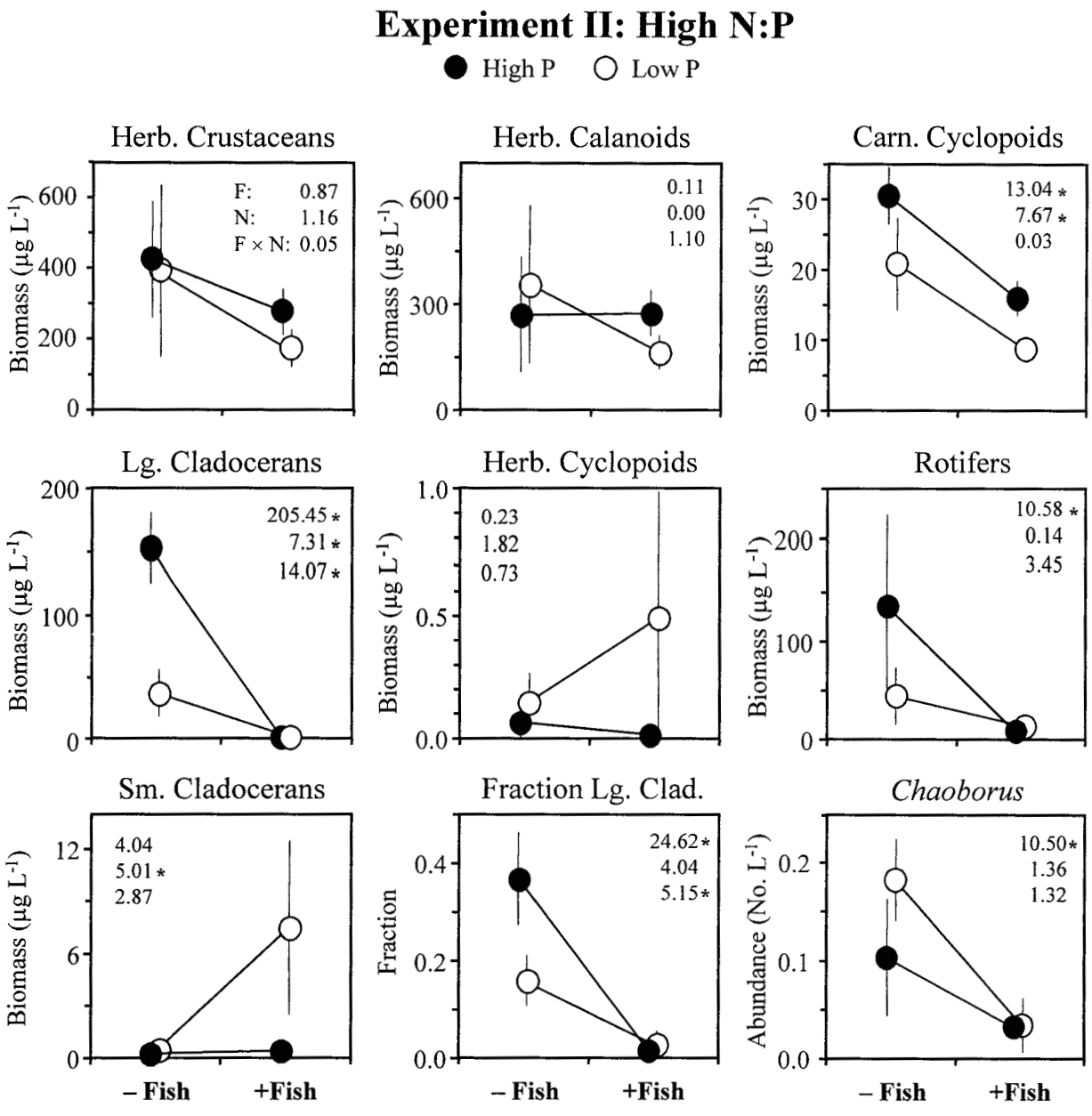


Fig. 9. Mean (± SE) biomass of major zooplankton groups in ponds during Experiment II. Numbers show results of 2-factor ANOVA as F-statistic values for fish (F), nutrient loading (N) and fish × nutrient loading interactions (F × N); asterisks indicate significant ($P \leq 0.05$) treatment and interaction effects.

Discussion

Overall results of our pond experiments indicated marked effects of both grazing and nutrient loading on the presence of N-fixing cyanobacteria and on the density of heterocysts in the experimental systems. In Experiment I, grazer and N:P loading effects were about equal in magnitude with respect to effects on cyanobacteria. There were more cyanobacteria and heterocysts in ponds dominated by copepods than in those dominated by cladocerans, and Low N:P ponds contained more cyanobacteria and heterocysts than High N:P ponds. The combined impacts of copepod dominance and low N:P loading (Effect 2 in Fig. 1A) yielded the highest densities of cyanobacteria and heterocysts (and hence likely N-fixation rates), while domination by large cladocerans and high N:P loading (Effect 3, Fig. 1A) both served to inhibit development by N-fixing cyanobacteria. There were few differences in cyanobacteria and heterocysts in ponds with con-

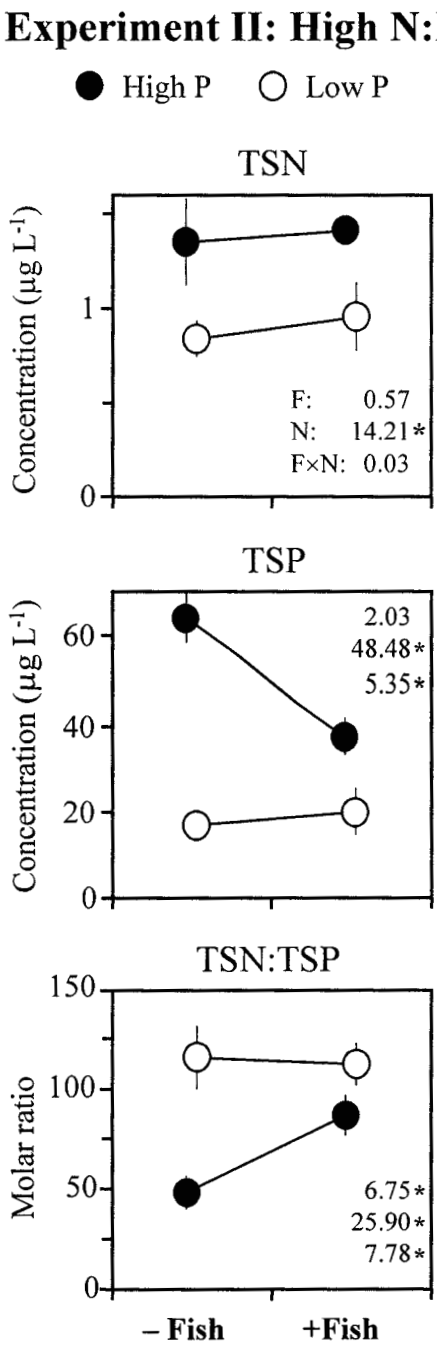


Fig. 10. Mean (± SE) concentrations of total soluble nitrogen (TSN) and total soluble phosphorus (TSP) and molar ratios of TSN:TSP in ponds during Experiment II showing main and interaction effects of fish and P loading. Labels as in Fig. 9.

flicting grazer and loading effects (i.e., Effects 1 and 4 were similar, Fig. 1A).

In Experiment II, although all ponds received high N:P loading, similar to the high N:P treatment in Experiment I, cyanobacteria and heterocysts occurred in all treatment combinations and grazer effects were less prominent. Nevertheless, the combined effects of high P loading and copepod domination (Effect 4 in Fig. 1B) contained the highest cyanobacteria and heterocyst abundances while low P loading and cladoceran domination (Effect 1, Fig. 1B) yielded the lowest cyanobacteria and heterocyst abundances. However, unlike Experiment I, grazer assemblages in Experiment II had little overall impact on cyanobacteria (i.e., Effects 2 and 4 were similar to Effects 1 and 3, Fig. 1B) and in ponds in which loading and grazer effects were conflicting, loading effects dominated (i.e., Effect 3 > Effect 2, Fig. 1B).

Direct control of a particular algal taxon by a herbivorous consumer is often considered of minor importance, and still relatively unpredictable in terms of species-species interactions in theoretical food web ecology and in application to fields like biomanipulation (Polis & Winemiller 1995, Drenner & Hambright 2002). However, Chan et al. (2004) demonstrated that N-fixing heterocystous cyanobacteria are far more vulnerable to the effects of grazing than are most other phytoplankton taxa; the ability to fix N is dependent upon having many photosynthetic cells to support the energy needs of the heterocyst; when grazing shortens filaments (i.e., filament clipping *sensu* Schaffner et al. 1994), rates of N fixation and growth of the cyanobacteria fall precipitously. On the other hand, many studies have considered filamentous N-fixing cyanobacteria to be generally resistant, even immune, to grazer control via a variety of mechanisms including, most notably toxicity. Thus, our findings and those of Chan et al. (2004) are striking. Moreover, the control of a major biogeochemical pathway, N-fixation, through the indirect effect of the presence or absence of planktivorous fish also is noteworthy. Consistent with the fact that N-fixation by cyanobacteria is energetically expensive and maintenance of non-photosynthetic cells that are not providing other valuable services (i.e., N supply) is also costly with respect to reproductive fitness (Howarth et al. 1988a, Vitousek et al. 2002, Berman-Frank et al. 2003), we found a direct relationship between heterocyst density and the potential for N-fixation. Thus N-fixation rates and subsequent N supply under N limiting conditions, was almost certainly reduced in the absence of fish and grazer dominance by large cladocerans. Our results therefore imply a strong cascading relationship between fish predation pressure and the prevalence of P limitation in freshwater systems.

The question then becomes: by what mechanism did large cladocerans suppress cyanobacteria abundances? We suspect that the primary mechanism was

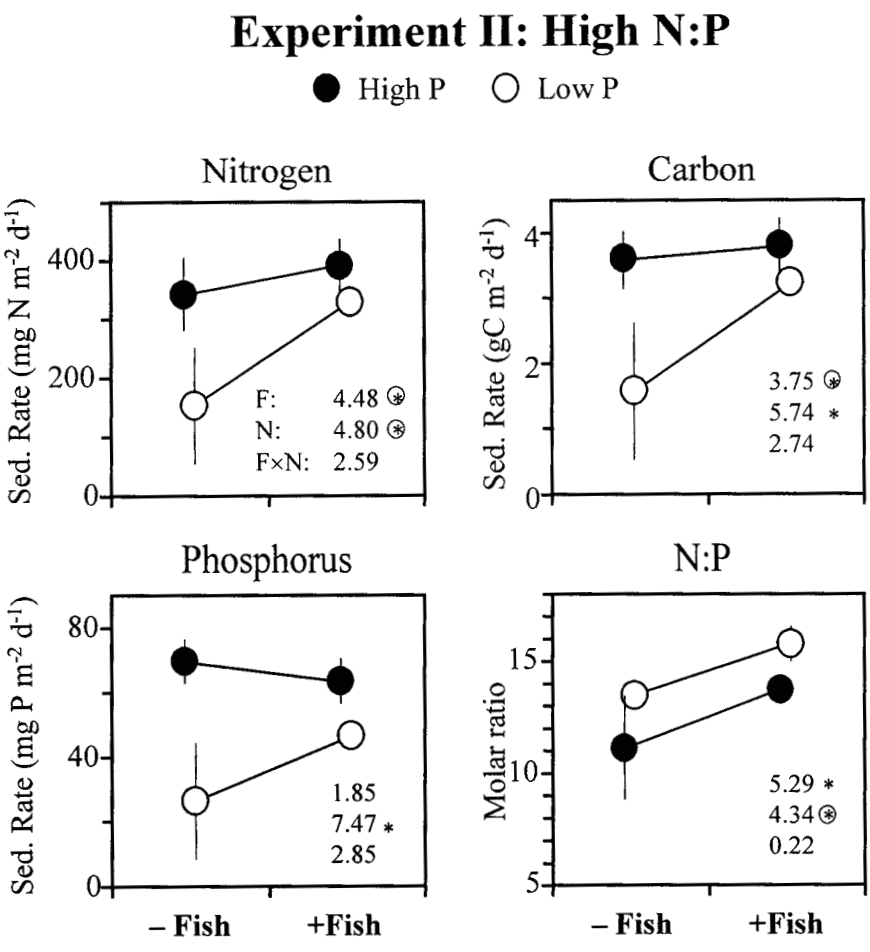


Fig. 11. Mean (± SE) sedimentation rates of nitrogen, phosphorus and carbon and the N:P ratio of sedimented material in ponds during Experiment II showing main and interaction effects of fish and P loading. Labels as in Fig. 9. Circled asterisks indicate significant main and interaction effects at $P \leq 0.10$, as data are based on only four measurements from three ponds per treatment combination.

that of elevated phytoplankton mortality, particularly during spring and early summer conditions prior to the initiation of cyanobacterial blooms. Results from our mesocosm grazing experiments (Hambright et al. 2007) showed that *Daphnia* taken from the experimental ponds readily consumed the filamentous cyanobacteria present in our study, imposing grazer-induced mortality rates equivalent in magnitude to those on other taxonomic groups more typically considered to be "good food" (e.g., chlorophytes and cryptophytes; Lampert 1987, see also Chan et al. 2004). Consistent with these observations, the pond densities of N-fixing cyanobacteria and heterocysts in Experiment I were significantly negatively associated with biomass of large cladocerans, regardless of N:P loading (Fig. 7). Thus in Experiment I, grazing zooplankton consumed N-fixing cyanobacteria, virtually eliminated N fixation, and as a consequence must have altered the water column concentration of an important nutrient for primary production. In Experiment II, cladoceran grazer biomass was similar to that in Experiment I, yet grazing was able only to reduce (and not eliminate) N-fixing cyanobacteria biomass. There was no statistical relationship between cladoceran biomass and either N-fixing cyanobacteria or heterocyst densities (Fig. 13). There were several key differences between the two experiments that likely contributed to the different grazer-cyanobacteria relationships. Presumably due to inter-annual differences in the onset of spring conditions, the cyanobacteria bloom started earlier (mid May) in Experiment II relative to the onset of the summer *Daphnia* population increase (early June) (data not shown) compared with Experiment I when the bloom began in June, and *Daphnia* were already abundant in May. Thus cyanobacteria were able to exceed a threshold beyond which population growth rate exceeded grazer-imposed mortalities (*sensu* Epp 1996, Howarth et al. 1999, Chan et al. 2004). This is a process we observed in mesocosm studies carried out concurrently with the pond experiments (Hambright et al. 2007). Alternatively, although cladoceran biomass was similar between the two experiments, its relative contribution to the grazer assemblage in the absence of fish was lower in Experiment II. Moreover, *Daphnia*

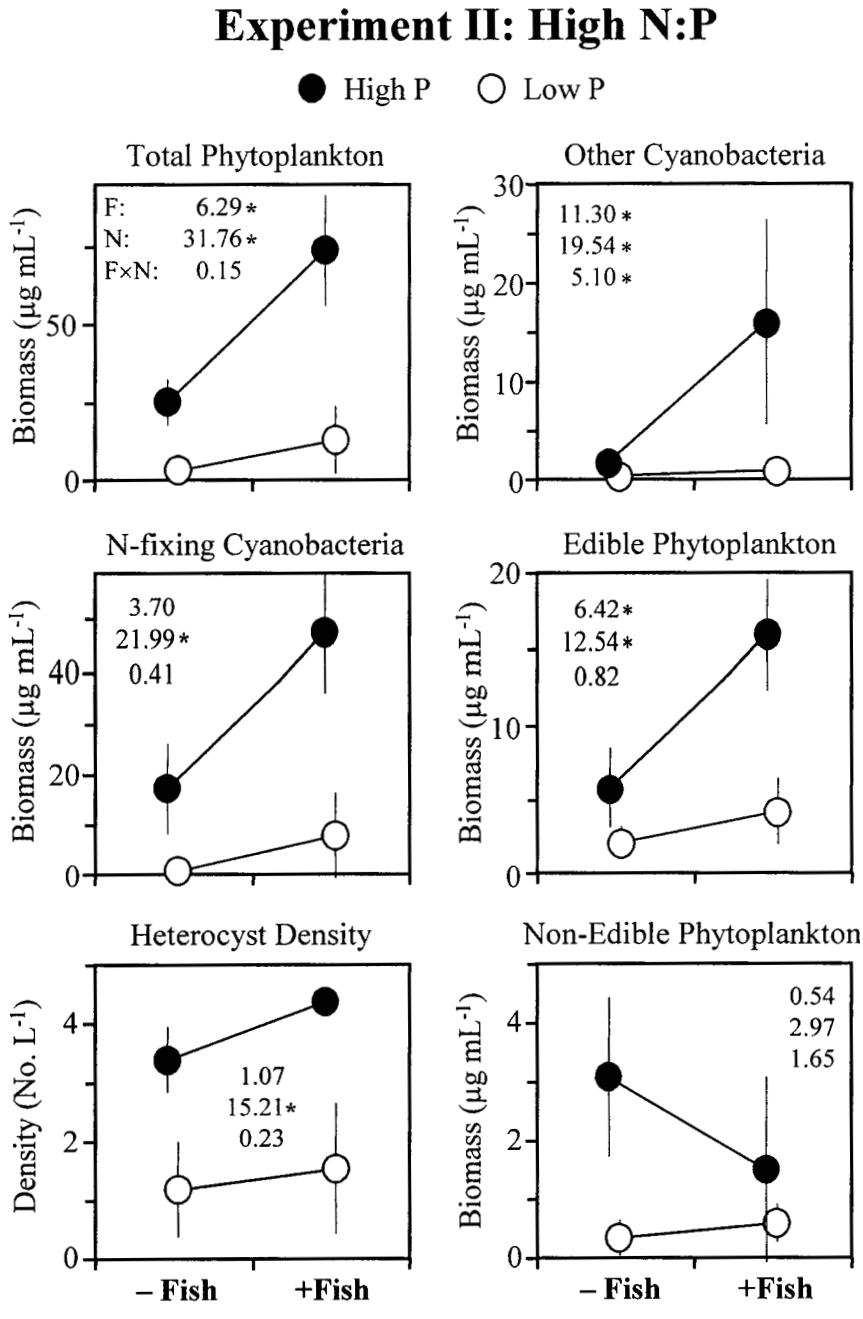


Fig. 12. Mean (± SE) biomass of major phytoplankton groups in ponds during Experiment II showing main and interaction effects of fish and P loading. Labels as in Fig. 9. Edible and non-edible phytoplankton were defined based on results from grazing experiments (Hambright et al. 2007).

were less abundant in Experiment II, accounting for only 26 % of the large cladoceran assemblage, compared with > 50 % in Experiment I. The remainder of the large cladoceran assemblages were comprised of *Ceriodaphnia*, *Diaphanosoma*, and *Simocephalus*, smaller species that were likely less able to consume filamentous cyanobacteria efficiently. Comparison of cyanobacterial abundances in Experiment II in High vs Low P loading ponds is consistent with this interpretation (Fig. 13). All ponds receiving High P loading (same treatment as High N:P loading in Experiment I) developed substantial N-fixing cyanobacteria abundances. In the presence of fish (i.e., at low cladoceran biomass) cyanobacteria and heterocyst densities were similar to those that developed in Experiment I. However, in the absence of fish (i.e., under high cladoceran biomass, but non-*Daphnia* species), cyanobacteria and heterocysts densities were reduced by only 37 and 23 %, respectively, falling above the Experiment I regression lines.

Direct consumption of cyanobacteria is not the only possible effect of grazers on N fixers. An alternative

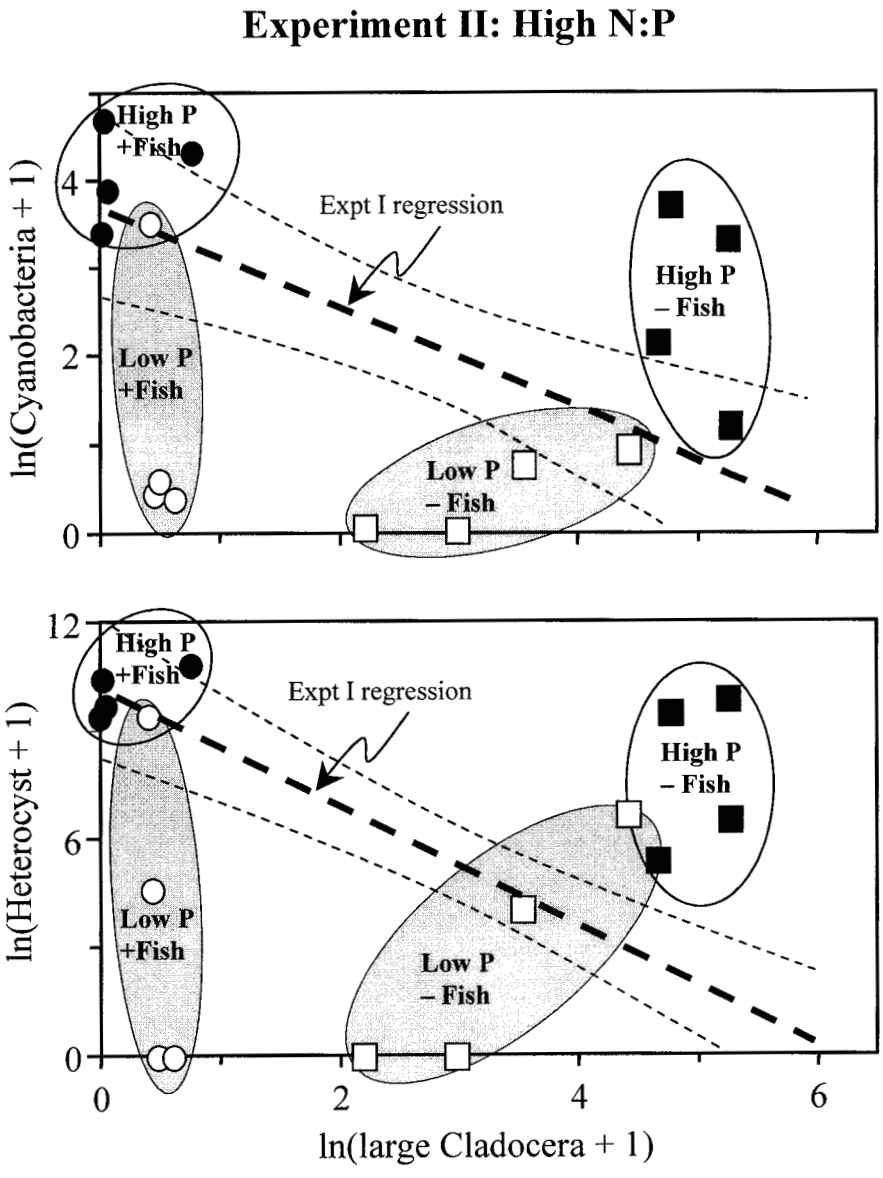


Fig. 13. Comparison of relationships between **(A)** summer mean N-fixing cyanobacteria and mean summer biomass of large cladocerans and **(B)** summer mean heterocyst densities and mean summer biomass of large cladocerans in ponds during Experiment II. Closed symbols are for ponds that received High P loading, with (circle) and without (square) fish and open symbols are for ponds that received Low P loading, with (circle) and without (square) fish. Dashed line indicates regression from Experiment I (Fig. 7).

is that cyanobacteria benefit indirectly from removal by grazing of competing algal taxa by the more selective copepods (Haney 1987, Shapiro 1990). In our system, however, the opposite appears to be the case. The general response by phytoplankton was virtually identical to the N-fixing cyanobacteria and heterocyst response. Total phytoplankton, whether edible or inedible (including non N-fixing cyanobacteria), were more abundant in Low N:P +Fish ponds compared with Low N:P –Fish ponds. Thus, if anything, competition from edible algae typically considered more edible would have been higher in the ponds containing high N-fixing cyanobacteria.

Elser (1999) presented a decisional flow chart illustrating that in cases of high nutrient, low N:P loading and appropriate hydrodynamic conditions (i.e., low turbulence, high light and temperature) the food web is the "last defense" against noxious cyanobacteria blooms. In other words, even if biogeochemical and physical conditions are favorable, the presence of *Daphnia* can prevent the formation of a noxious bloom of cyanobacteria, by creating high N:P availabilities through preferential recycling of N relative to P. Elser (1999) pointed out, however, that food-web interactions are highly complex and that underlying stoichiometric relations are likely non-linear and potentially chaotic (see Anderson 1997). Our results confirm that under appropriate biogeochemical and physical conditions, *Daphnia* can indeed prevent cyanobacterial blooms. Likewise, while the magnitude

of cyanobacteria development in our ponds was dependent on biomass of large cladoceran grazers in Experiment I, results from Experiment II clearly indicate that even when grazing zooplankton are present at high abundances, high P loading at High N:P can still favor the proliferation and community domination of N-fixing cyanobacteria, albeit at reduced densities. Interestingly, the presence of heterocysts in all ponds (though extremely scarce in some ponds) in both experiments suggests that N-fixation can occur irrespective of the N:P of external loading or of the taxonomic identity of the dominant grazer, as long as P availability is sufficiently high (Reynolds 1987, Howarth et al. 1988a, Reynolds 1997). Thus *Daphnia* can be the "last defense" hypothesized by Elser (1999), but its presence or absence is not a prerequisite for cyanobacterial bloom control or development, and its influence on cyanobacteria is more likely to be through direct grazing than indirect stoichiometric control (Chan et al. 2004, Howarth & Marino 2006, Marino et al. 2006).

Despite clear evidence in our experimental ponds that grazers can control cyanobacterial blooms under some conditions, evidence for or against such a process in natural lakes is difficult to find. One potential reason is simply that virtually all lakes likely to have cyanobacterial blooms also have large fish stocks and hence reduced grazer densities. One exception is lakes that occasionally have fish-kills (due to O_2 depletion either hypolimnetic in summer or under ice in winter). In at least two such instances, removal of zooplanktivorous fish from highly productive lakes were followed by significant increases in *Daphnia* biomass and significant reductions of cyanobacteria (Schindler & Comita 1972, Vanni et al. 1990). Smith (1983) cites a similar event in Lake Trummen, Sweden. It is still clear, however, from many studies that although cyanobacteria can be grazed, they are often not consumed by grazing zooplankton and stimulation of their populations in lakes via management efforts (e.g., biomanipulation) often fail to curtail cyanobacterial blooms (Drenner & Hambright 1999). One possible resolution is that blooms of cyanobacteria may be prevented when grazer density is already large early in the season before a cyanobacteria bloom starts (Dawidowicz et al. 1988, Gliwicz 1990, Howarth et al. 1999, Chan et al. 2004). Our mesocosm experiments (Hambright et al. 2007) showed that even potentially toxic, filamentous cyanobacteria can be consumed when they are a minor component of a largely edible algal assemblage and this is likely the mechanism ultimately behind our pond results. However, high nutrient availabilities can stimulate early cyanobacteria blooms thus reducing the likelihood of grazer control without simultaneous regulation of nutrient loading (Benndorf 1990, Drenner & Hambright 1999).

In nutrient-poor lakes, internal recycling can be a major, if not the dominant, source of nutrients supporting primary production (Caraco et al. 1992). However, it remains unclear if zooplankton nutrient mineralization can play an important role in the phytoplankton competitive arena in high nutrient loading systems as, for example, inferred in Elser's (1999) pathway to noxious cyanobacteria blooms. In debates on the importance of top-down predator control of food web dynamics, it has been argued that the strength of trophic cascades should depend on lake trophic status, the argument being that high nutrient availability can mask or overwhelm top down effects, particularly grazer control at the phytoplankton level (McQueen 1998). We suspect the same can be said of top-down effects of zooplankton nutrient mineralization, such that only if zooplankton are a major source of nutrients supporting production in an ecosystem will the rates and ratios of their excretion play a role in phytoplankton community dynamics (Elser et al. 2000). Under conditions of high nutrient loading (as in our ponds), we might not expect internal recycling to play a dominant role in nutrient supply (De Mazancourt et al. 1998, Daufresne & Loreau 2001). In our system, estimated total zooplankton nutrient release was a small fraction of the combined sources (external and internal loading plus excretion) of available N and P in the ponds (Fig. 8). Thus our results corroborate Paterson et al.'s (2002) conclusion, that the magnitude of any nutrient mineralization effect is likely small compared with other factors affecting nutrient availabilities (e.g., external and internal loading and N fixation). If low N:P mineralization by *Daphnia* was an important factor contributing to the reduction in cyanobacteria in our study, we would have expected that +Fish ponds that lacked *Daphnia* should have had higher phosphorus availabilities and lower N:P ratios than –Fish ponds where *Daphnia* were present. Although these ponds did have higher cyanobacteria abundances, we saw no such effect of grazer-induced changes in relative nutrient availabilities in either pond experiment. Indeed, the converse was found; the +Fish ponds had lower levels of TSP and higher TSN:TSP, even in the Low N:P treatments of Experiment I. This has also been observed in experimental studies of N-fixing cyanobacteria in estuarine mesocosms (Marino et al. 2006). Thus it seems that zooplankton-driven nutrient recycling, in shallow eutrophic systems may indeed be minor (Jeppesen et al. 1997, Moss et al. 1997). This conclu-

sion seems to contradict directly the findings of Hudson et al. (1999), who concluded that daily P recycling by complete planktonic assemblages in lakes ranging from oligotrophic and eutrophic (TP range: 4–80 µg P L^{-1}) increased logarithmically with total P concentration in the water column. However, these systems were chosen because they were relatively deep (maximum depth ranged 4.6–42 m) and stratified (mixing depth ranged 2–8.5 m) thereby minimizing benthic and littoral effects on pelagic nutrient dynamics (Hudson et al. 1999).

Conclusion

Our primary objective here has been to discover what role grazers play, whether directly through consumption (i.e., mortality) or indirectly through nutrient mineralization, in affecting the abundance of N-fixing cyanobacteria, the rate of N fixation, and the concentration of fixed N in a freshwater system, and further, the extent to which that role is influenced by nutrient loading. We found marked effects of both grazing and nutrient loading on the presence of N-fixing cyanobacteria and on the abundance of N-fixing heterocysts in our experimental system. In one experiment, using variable N additions to manipulate N:P at high P loading, grazer and N:P loading strongly affected cyanobacteria and were similar in the magnitude of these effects. However, when N:P loading was kept high while manipulating P loading, both grazing and P loading influenced cyanobacterial abundance, but overall grazer effects on cyanobacteria were small relative to effects of P loading. Although mesocosm experiments (Hambright et al. 2007) corroborated the known differences in N:P mineralization between copepods and cladocerans found by others (e.g., Rothhaupt 1997), the aggregate grazer effects in our experimental ponds appeared to result almost exclusively from the direct removal of cyanobacteria by grazing rather than by an alteration of the N:P of available nutrients by zooplankton mineralization. In *Daphnia*-dominated ponds, zooplankton P excretion amounted to about 23 % of total nutrient loading (sum of external and internal loading plus excretion), while in copepod-dominated ponds, zooplankton P excretion was only about 8 % of the total P supply; N excretion was even less important.

Zooplankton excretion can contribute to nutrient pools and availabilities, however, our results suggest that such effects will likely be small in systems where other nutrient sources such as external and internal loading are important. In our shallow systems in which benthic effects of nutrient availabilities were substantial, grazer effects on cyanobacteria were predominantly mortality-derived, rather from indirect effects of changing nutrient availabilities.

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