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Response of phytoplankton and bacteria to nutrients and zooplankton: a mesocosm experiment

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Abstract. Although both nutrient inputs and zooplankton grazing are important to phytoplankton and bacteria in lakes, controversy surrounds the relative importance of grazing pressure for these two groups of organisms. For phytoplankton, the controversy revolves around whether zooplankton grazers, especially large cladocerans like *Daphnia*, can effectively reduce phytoplankton populations regardless of nutrient conditions. For bacteria, little is known about the balance between possible direct and indirect effects of both nutrients and zooplankton grazing. However, there is evidence that bacteria may affect phytoplankton responses to nutrients or zooplankton grazing through direct or apparent competition. We performed a mesocosm experiment to evaluate the relative importance of the effects of nutrients and zooplankton grazing for phytoplankton and bacteria, and to determine whether bacteria mediate phytoplankton responses to these factors. The factorial design crossed two zooplankton treatments (unsieved and sieved) with four nutrient treatments (0, 0.5, 1.0 and 2.0 μg phosphorus (P) l^{-1} day^{-1}, together with nitrogen (N) at a N:P ratio of 20:1 by weight). Weekly sieving with 300 μm mesh reduced the average size of crustacean zooplankton in the mesocosms, decreased the numbers and biomass of *Daphnia*, and increased the biomass of adult copepods. Nutrient enrichment caused significant increases in phytoplankton chlorophyll *a* (4–5×), bacterial abundance and production (1.3× and 1.6×, respectively), *Daphnia* (3×) and total zooplankton biomass (2×). Although both total phytoplankton chlorophyll *a* and chlorophyll *a* in the <35 μm size fraction were significantly lower in unsieved mesocosms than in sieved mesocosms, sieving had no significant effect on bacterial abundance or production. There was no statistical interaction between nutrient and zooplankton treatments for total phytoplankton biomass or bacterial abundance, although there were marginally significant interactions for phytoplankton biomass <35 μm and bacterial production. Our results do not support the hypothesis that large cladocerans become less effective grazers with enrichment; rather, the difference between phytoplankton biomass in sieved versus unsieved zooplankton treatments increased across the gradient of nutrient additions. Furthermore, there was no evidence that bacteria buffered phytoplankton responses to enrichment by either sequestering P or affecting the growth of zooplankton.

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Introduction

Both nutrients and zooplankton are important to phytoplankton in lakes. For example, total phytoplankton biomass tends to increase with increased nutrients (Schindler, 1977), but decrease with increased zooplankton grazing, especially when the grazers are predominantly large cladocerans as compared to rotifers, copepods or small cladocerans (Pace, 1984; Carpenter *et al.*, 1991). However, we do not yet understand the extent to which these two opposing factors—nutrients and zooplankton—interact to influence phytoplankton.

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Carney and Elser (1990), McQueen (1990) and Elser and Goldman (1991) have hypothesized that the coupling between zooplankton and phytoplankton weakens as nutrient availability increases. This weakening is attributed to several factors, including the tendency for eutrophic systems to develop blooms of large, grazing-resistant phytoplankton (Lynch and Shapiro, 1981; Elser and Goldman, 1991; Reynolds, 1994; Benndorf, 1995). Large cladocerans such as *Daphnia* are particularly vulnerable to these blooms (Webster and Peters, 1978), and may be unable to graze effectively on them (Gliwicz, 1990). If large cladocerans become less effective grazers under conditions of high nutrients, then we would expect to see increased similarity between systems dominated by large cladocerans and those dominated by other zooplankters with enrichment. Evidence to support this expectation comes from a variety of sources, including short-term enclosure experiments (<100 l; e.g. Elser and Goldman, 1991), simulation models (Scheffer, 1991; Carpenter, 1992), comparative studies (Hansson, 1992) and whole-lake experiments (e.g. Benndorf, 1987, 1990, 1995; Jeppesen *et al.*, 1990).

However, there is also evidence that large cladocerans, especially *Daphnia*, are more effective than smaller zooplankton at reducing algal biomass at a wide range of nutrient conditions (e.g. Leibold, 1989; Sarnelle, 1992; Mazumder, 1994). Can large zooplankton control phytoplankton more effectively than small zooplankton regardless of nutrient conditions? Fundamental concepts about the functioning of lake ecosystems and the applicability of food web manipulation for water quality management depend on the answer to this question (Gulati *et al.*, 1990; Carpenter and Kitchell, 1993).

Bacteria represent an added complexity to understanding nutrient–phytoplankton–zooplankton relationships. Few studies have evaluated bacterial responses to experimental manipulations of both grazers and resources [but see Riemann and Sondergaard (1986), Pace and Funke (1991) and Christoffersen *et al.* (1993)]. In comparative studies, bacterial production and abundance tend to track phytoplankton production and abundance, presumably because phytoplankton supply organic substrates for bacteria (e.g. Cole *et al.*, 1988), especially phosphorus (P) (Pace and Funke, 1991; Pace and Cole, 1996). Increasing nutrients should, therefore, increase resources for bacteria both directly through nutrient supply (Pace and Funke, 1991) and indirectly through increased phytoplankton.

Bacterivorous organisms (such as flagellates and *Daphnia*, which can consume a large fraction of bacterial production; Riemann and Christoffersen, 1993) may also have both direct and indirect effects on bacteria. For example, *Daphnia* may decrease bacteria by direct grazing (Jurgens, 1994). Alternatively, large zooplankton such as *Daphnia* may graze flagellates, releasing bacteria from flagellate grazing. Although increased nutrients should lead to increased bacteria, the effects of increased zooplankton grazing are more difficult to predict.

Phytoplankton responses to nutrients and zooplankton grazing may depend in part on bacteria. Bacteria are effective competitors for P (Currie and Kalff, 1984), and may sequester P or delay its availability to phytoplankton. Because bacteria are grazed by zooplankton (Riemann and Christoffersen, 1993; Jurgens, 1994), phytoplankton may suffer increased grazing losses through apparent competition

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(Holt, 1977) if microbial activity stimulates growth of zooplankton. Therefore, bacteria dampen phytoplankton responses to increased nutrients through both direct and indirect effects.

We performed a mesocosm experiment to evaluate the net effects of nutrients and zooplankton grazing, as well as their interaction, on phytoplankton and bacteria in lake ecosystems, and to test whether bacteria might dampen phytoplankton responses to nutrients or grazers. The factorial experiment crossed two zooplankton treatments (unsieved and sieved) with four levels of nutrient enrichment (0, 0.5, 1.0 and 2.0 μg P l^{-1} day^{-1}; or 0, 1, 2 and 4 mg m^{-2} day^{-1} on an areal basis). The sieved versus unsieved zooplankton treatments represent communities dominated by cyclopoid copepods versus *Daphnia*, respectively, while the nutrient loading rates span a range of daily nutrient loading rates typical of mesotrophic to eutrophic lakes. We therefore focus on the relative ability of these contrasting zooplankton communities to control the responses of phytoplankton biomass and bacterial abundance and production to daily nutrient additions at rates 5–20 times higher than background.

Method

Site description

Our experiment was conducted in the relatively shallow (mean depth 2.3 m) central basin of oligo-mesotrophic Long Lake (University of Notre Dame Environmental Research Center, Gogebic County, Michigan; 46°14′N, 89°30′W). Fish were rare and *Daphnia* very abundant in Central Long Lake during our experiment. Summer average conditions in 1992 were thermocline depth of 3.1 m and Secchi depth of 2.6 m. Epilimnetic temperatures ranged from 15 to 22°C, with average epilimnetic chlorophyll *a* of 6 μg l^{-1} and total phosphorus (TP) of 11 μg P l^{-1}. Background P loading into Long Lake was ~0.1–0.2 μg l^{-1} day^{-1} (Carpenter *et al.*, 1996).

Experimental design

The mesocosm experiment was a factorial design that crossed four nutrient loading rates with two zooplankton treatments. Each combination of nutrient loading and zooplankton treatment was conducted in triplicate. The 24 mesocosms were suspended from six rafts floating in 2 m of water in Central Long Lake. Treatments were assigned randomly to locations on the rafts. Mesocosms were constructed of translucent plastic formed into open-ended cylinders with a 1 m^2 cross-sectional area and a volume of 2000 l. Each mesocosm had an iron hoop at the bottom which was embedded 0.3 m into the sediments, and plastic hoops at the middle and top of the cylinder to maintain mesocosm shape. Mesocosms were filled with lake water filtered through 100 μm mesh to remove adult crustacean zooplankton. The experiment ran for 9 weeks from 12 June to 7 August 1992.

Each day, we added a concentrated solution of Na_2HPO_4 and NH_4NO_3 to obtain daily loading rates of 0.5 μg P l^{-1} with 10 μg nitrogen (N) l^{-1}, 1 μg P l^{-1} with

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20 μg N l^{-1}, or 2 μg P l^{-1} with 40 μg N l^{-1}. This N:P ratio approximated the ratio in the lake. A fourth set of mesocosms with no added nutrients served as a control. After addition of the nutrient concentrate, we gently mixed the water column in all mesocosms (including controls) by lowering a Secchi disk to 1.25 m and slowly pulling it back towards the surface. Visual inspection indicated that this method did not disturb the sediments. Hereafter, we identify these treatments by their addition rate of P (0, 0.5, 1.0 and 2.0 μg P l^{-1} day^{-1}), since P is the limiting nutrient in this and nearby lakes (Carpenter and Kitchell, 1993; S.R.Carpenter *et al.*, unpublished data).

Our two zooplankton treatments were characteristic of north temperate lakes with moderate versus low levels of size-selective zooplanktivory by fish. Lakes with moderate zooplanktivory tend to be dominated by smaller-bodied species, such as rotifers and copepods, while lakes with little to no zooplanktivory tend to be dominated by larger-bodied cladocerans such as *Daphnia*. In this experiment, treatments were initiated with equal biomasses of zooplankton taken from either the high-planktivory east or low-planktivory west basin of Long Lake (Christensen *et al.*, 1996). At the time of zooplankton collection, East Long Lake was dominated by rotifers (especially *Asplanchna* spp.), *Bosmina longirostris* and cyclopoid copepods (*Mesocyclops edax, Orthocyclops modestus* and nauplii). In contrast, West Long Lake was dominated by large cladocerans (*Daphnia rosea, Daphnia pulex* and *Holopedium gibberum*) with some cyclopoid copepods (*Orthocyclops modestus* and *Cyclops varicans rubellus*). Zooplankton from each basin (collected with vertical tows of an 80 μm conical net at the deepest part of the basin) were pooled into a single container, *Chaoborus* larvae were removed with a pipette, and subsamples of the homogenate were distributed to the 12 mesocosms receiving that zooplankton treatment. The biomass of added zooplankton was standardized to 44 μg dry weight l^{-1}, 50% of the mean 1991 midsummer zooplankton biomass in the east and west basins of Long Lake.

These zooplankton communities were quickly supplemented by additional organisms (especially *Daphnia* spp.) recruiting into the enclosures from the sediments. However, we successfully maintained differences in size structure and community composition between zooplankton treatments by mimicking size-selective zooplanktivory with a mesh sieve. Once each week (4 days before sampling of zooplankton), zooplankton >300 μm were removed from the mesocosms which had begun with zooplankton from East Long Lake; sieved zooplankton were returned to Long Lake and were not included in any of our analyses. Because all mesocosms were mixed daily as part of the nutrient treatment, sieving had little effect on mesocosms aside from zooplankton removal. For simplicity, we refer to the zooplankton treatments as sieved and unsieved throughout the rest of this paper. Overall, sieved mesocosms contained smaller zooplankton, more copepods, fewer *Daphnia*, and somewhat lower total zooplankton biomass than unsieved mesocosms (see Results).

Because the morphometry of our mesocosms over-represented surfaces for attached algae relative to natural lakes, we scrubbed the inner sides of the mesocosms on 6 July to reduce build-up of periphyton. This resulted in a short-term pulse of total nutrients, but no increase in water column chlorophyll.

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In sampling the mesocosms, we focused on the responses of phytoplankton and bacteria to our experimental treatments on a weekly time scale. This provided us with sufficient information to meet our goal of understanding the net effects of nutrient addition and sieving on phytoplankton and bacterial growth, death, sinking and grazing processes.

Manipulated variables—nutrients and zooplankton

Mesocosms were sampled weekly using a 1.5 m long PVC pipe (5 cm diameter) with a rubber stopper. We used this depth-integrating sampler to collect water for chemical, chlorophyll *a* and zooplankton analyses.

Samples for dissolved nutrient analyses [soluble reactive phosphorus (SRP), NH_4 and NO_3 (DIN)] were filtered through Whatman GF/F filters into a flask and frozen for 3 days before analysis. TP samples were frozen and later digested with ammonium persulfate prior to analysis. Total nitrogen (TN) samples were preserved with 300 µl 18 N H_2SO_4 and refrigerated prior to Kjeldahl digestion with a block heater. All concentrations (SRP, DIN, TP, TN) were then determined with an autoanalyzer. TP and SRP were measured every week, and TN every other week during the experiment. We measured DIN weekly for the last 4 weeks of the experiment.

Zooplankton samples were obtained by filtering 9–15 l of water through an 80 µm mesh in the field. Each week, samples for total zooplankton biomass were concentrated onto a dried and tared Whatman GF/F filter, dried and reweighed. On 12 June, 19 June, 10 July and 31 July, additional samples for zooplankton identification and counts were preserved with 8% buffered formalin. Adult crustaceans were identified and enumerated to genera (some to species), while copepodites, nauplii and rotifers were enumerated as aggregate categories. The biomass of each identified zooplankton taxon was calculated using the mean length of that taxon in East or West Long Lake in 1992 (East Long for sieved mesocosms, West Long for unsieved mesocosms; S.R.Carpenter *et al.*, unpublished data) and the length–dry mass regressions of Downing and Rigler (1984). Because rotifers contributed <5% of the biomass in both treatments throughout the experiment, we focus on crustacean responses in the results. Crustacean mean length was calculated in each mesocosm on each of the four sampling dates as described by Elser *et al.* (1987).

Response variables—phytoplankton and bacteria

Two samples for phytoplankton biomass (as chlorophyll *a*) were taken from each mesocosm. One sample was filtered directly onto a Whatman GF/F filter (for total chlorophyll *a*), while the second was pre-filtered through a 35 µm screen before being collected on a GF/F filter. This smaller fraction (<35 µm) of phytoplankton is referred to below as edible chlorophyll *a*. All chlorophyll *a* samples were frozen for at least 24 h, then extracted with methanol and analyzed with a fluorometer (Marker *et al.*, 1980).

Weekly samples were taken to estimate bacterial abundance and production in

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each mesocosm. A single sample was taken for abundance, and four replicate samples were taken for production by gently mixing the water column and removing 10 ml samples at the surface. Bacterial abundance was determined using the acridine orange direct count method of Hobbie *et al.* (1977). At least 400 cells were enumerated from every sample.

Bacterial production was measured using the [^{3}H]leucine incorporation method of Kirchman *et al.* (1985). [^{3}H]Leucine was added to achieve a final, saturating concentration of 17 nM (specific activity 2220 GBq mmol^{-1}; Pace and Cole, 1994). Bacterial productivity was calculated by converting leucine incorporated into units of μg carbon (C) l^{-1} day^{-1} following Simon and Azam (1989). We assumed that the added radioactive leucine was 'diluted' by an equal concentration of unlabeled leucine based on our prior measurements (i.e. an isotope dilution factor of 2.0; Pace and Cole, 1994).

Statistics

Analyses of variance were performed using Systat (Wilkinson, 1989). Nutrient concentrations, phytoplankton total and edible chlorophyll *a*, total zooplankton biomass, and bacterial abundance and production were analyzed by two-way (nutrient loading rate × zooplankton treatment) repeated measures analysis of variance (Winer, 1971; Gurevitch and Chester, 1986) for all nine sampling dates. Crustacean mean length, *Daphnia* density, and biomasses of adult copepods, copepodites, nauplii, *Daphnia* and other cladocerans were analyzed by two-way repeated measures ANOVA for the sampling dates on which they were measured. The significance level for effects was set at $P \leq 0.05$, with $0.05 < P \leq 0.10$ as the level for marginal significance.

Residuals of all ANOVAs were checked for normality using normal probability plots. All variates except for crustacean mean length were log transformed to normalize residuals and equalize variance among treatments.

Results

Zooplankton

Weekly sieving maintained substantial differences in zooplankton size structure and community composition throughout the experiment (Figure 1, Table I). Sieved treatments were dominated by cyclopoid copepods, while unsieved treatments were dominated by *Daphnia*. There were twice as many *Daphnia* ($P = 0.041$) and more than three times as much *Daphnia* biomass ($P = 0.002$) in the unsieved treatment than in the sieved treatment (Figure 1A, Table I). In contrast, sieved treatments had significantly higher biomass of adult copepods ($P = 0.001$) and marginally higher biomasses of both copepodites and nauplii ($P = 0.067$ and $P = 0.057$, respectively). The biomass of non-daphnid cladocerans (Figure 1) and rotifers (not shown) was not significantly different between treatments ($P > 0.5$). Mean crustacean length was significantly ($P = 0.045$) smaller in sieved mesocosms than in unsieved mesocosms (at the end of the experiment, mean ± 1 SD was 0.66 ± 0.04 versus 0.84 ± 0.05 mm).

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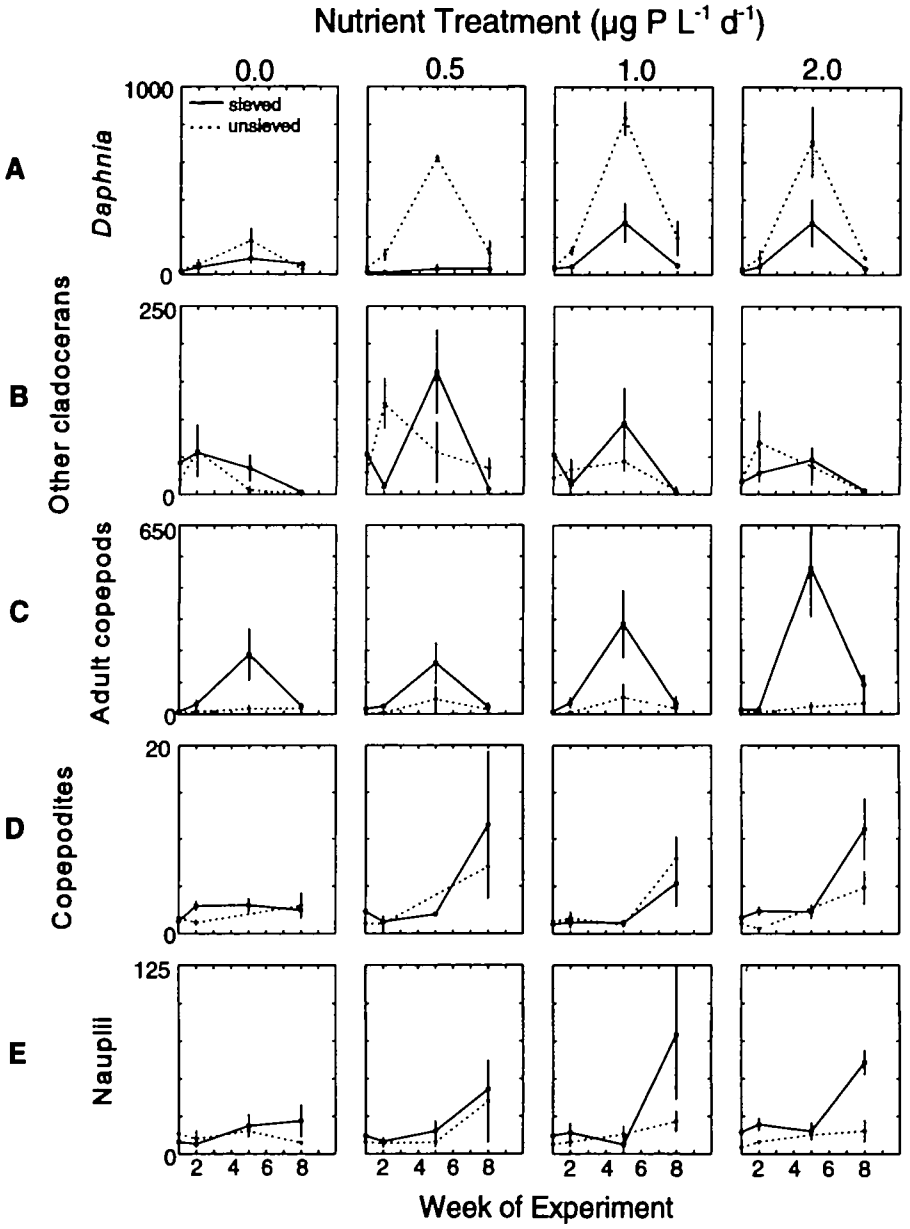

Fig. 1. Biomass (µg l^{-1}) of different crustacean zooplankton during the experiment. Nutrient loading treatments are represented by columns; dotted lines indicate unsieved treatments and solid lines represent sieved treatments. Error bars are ± 1 SD; note the different *y*-axis scales for the different groups. (**A**) *Daphnia*, including *D.pulex* and *D.rosea*. (**B**) Other cladocerans, including *Alona* spp., *Bosmina longirostris*, *Ceriodaphnia* spp., *Chydorus* spp. and *Diaphanosoma birgei*, *Holopedium gibberum* and *Polyphemus* spp. (**C**) Adult cyclopoid copepods, mostly *Mesocyclops edax*, *Orthocyclops modestus*, *Tropocyclops prasinus mexicanus*, *Diacyclops thomasi* and *Cyclops varicans rubellus*. (**D**) Copepodites. (**E**) Nauplii.

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Table I. Treatment effects on zooplankton, phytoplankton, and bacteria (mean from weeks 2–9, ± 1 SD)

(A) Effect of sieving

Variate		Sieved	Unsieved
Total zooplankton[a]		421.0 ± 144.0	509.6 ± 256.7
Daphnia	Biomass[b]	81.0 ± 93.7	261.5 ± 284.8
	Density[c]	14.6 ± 16.3	29.9 ± 31.7
Other cladocerans	Biomass[b]	38.6 ± 48.0	38.9 ± 35.0
	Density[c]	11.6 ± 10.9	12.2 ± 14.2
Adult copepods	Biomass[b]	114.8 ± 139.0	20.8 ± 16.0
	Density[c]	14.7 ± 15.6	8.1 ± 6.7
Copepodites	Biomass[b]	3.9 ± 3.6	3.1 ± 2.8
	Density[c]	8.9 ± 8.2	8.8 ± 7.9
Nauplii	Biomass[b]	25.6 ± 23.2	13.3 ± 8.1
	Density[c]	28.5 ± 25.8	14.0 ± 8.6
Total chlorophyll[d]		14.5 ± 14.8	7.9 ± 4.8
Edible (<35 μm) chlorophyll[d]		12.0 ± 13.4	6.1 ± 3.8
Bacterial abundance[e]		7.7 ± 2.0	7.5 ± 1.8
Bacterial production[f]		5.0 ± 2.4	4.5 ± 1.6

(B) Effect of nutrient addition

Variate		None	0.5 μg l^{-1} day^{-1}	1 μg l^{-1} day^{-1}	2 μg l^{-1} day^{-1}
Total zooplankton[a]		281.8 ± 56.5	467.2 ± 144.6	572.4 ± 216.7	539.9 ± 249.6
Daphnia	Biomass[b]	72.8 ± 56.1	151.5 ± 234.1	254.2 ± 297.6	206.7 ± 261.7
	Density[c]	10.0 ± 6.2	18.3 ± 24.9	33.4 ± 33.8	27.6 ± 29.9
Other cladocerans	Biomass[b]	26.2 ± 26.8	65.3 ± 63.4	31.9 ± 34.9	31.5 ± 25.4
	Density[c]	5.6 ± 5.1	17.8 ± 18.3	11.4 ± 10.9	12.7 ± 11.6
Adult copepods	Biomass[b]	48.3 ± 68.8	45.5 ± 57.9	71.7 ± 105.6	105.8 ± 177.9
	Density[c]	8.0 ± 6.7	7.6 ± 7.2	13.5 ± 12.1	16.4 ± 19.4
Copepodites	Biomass[b]	2.5 ± 0.8	4.6 ± 4.6	3.0 ± 2.9	4.2 ± 4.1
	Density[c]	6.2 ± 1.8	11.3 ± 10.9	7.8 ± 7.9	10.2 ± 9.4
Nauplii	Biomass[b]	13.2 ± 6.3	19.3 ± 15.7	23.5 ± 27.8	21.8 ± 19.5
	Density[c]	14.4 ± 7.1	21.0 ± 17.2	25.8 ± 31.1	23.9 ± 21.9
Total chlorophyll[d]		4.4 ± 1.5	8.1 ± 4.3	13.6 ± 7.9	18.7 ± 18.4
Edible (<35 μm) chlorophyll[d]		3.6 ± 1.2	6.4 ± 3.7	10.6 ± 7.2	15.5 ± 16.9
Bacterial abundance[e]		6.7 ± 1.4	7.7 ± 1.7	7.7 ± 2.0	8.5 ± 2.1
Bacterial production[f]		3.6 ± 1.5	4.9 ± 2.3	5.1 ± 2.1	5.9 ± 2.1

[a]Biomass on filters; μg dry mass l^{-1}.
[b]Biomass in counts; μg dry mass l^{-1}.
[c]μg l^{-1}.
[d]Density; number of animals l^{-1}.
[e]10^9 cells l^{-1}.
[f]μg C l^{-1} day^{-1}.

In addition to these differences in size structure and species composition, total zooplankton biomass (as determined from the net increase in filter weight) was slightly higher in unsieved mesocosms than in sieved mesocosms (P = 0.096; Figure 2A, Table IA). In all mesocosms, zooplankton biomass was relatively high, with maxima typical of eutrophic lakes.

In both sieved and unsieved zooplankton treatments, total zooplankton biomass increased significantly with nutrient loading rate (P = 0.002; Figure 2A), and there was no significant interaction between nutrients and sieving. Of the

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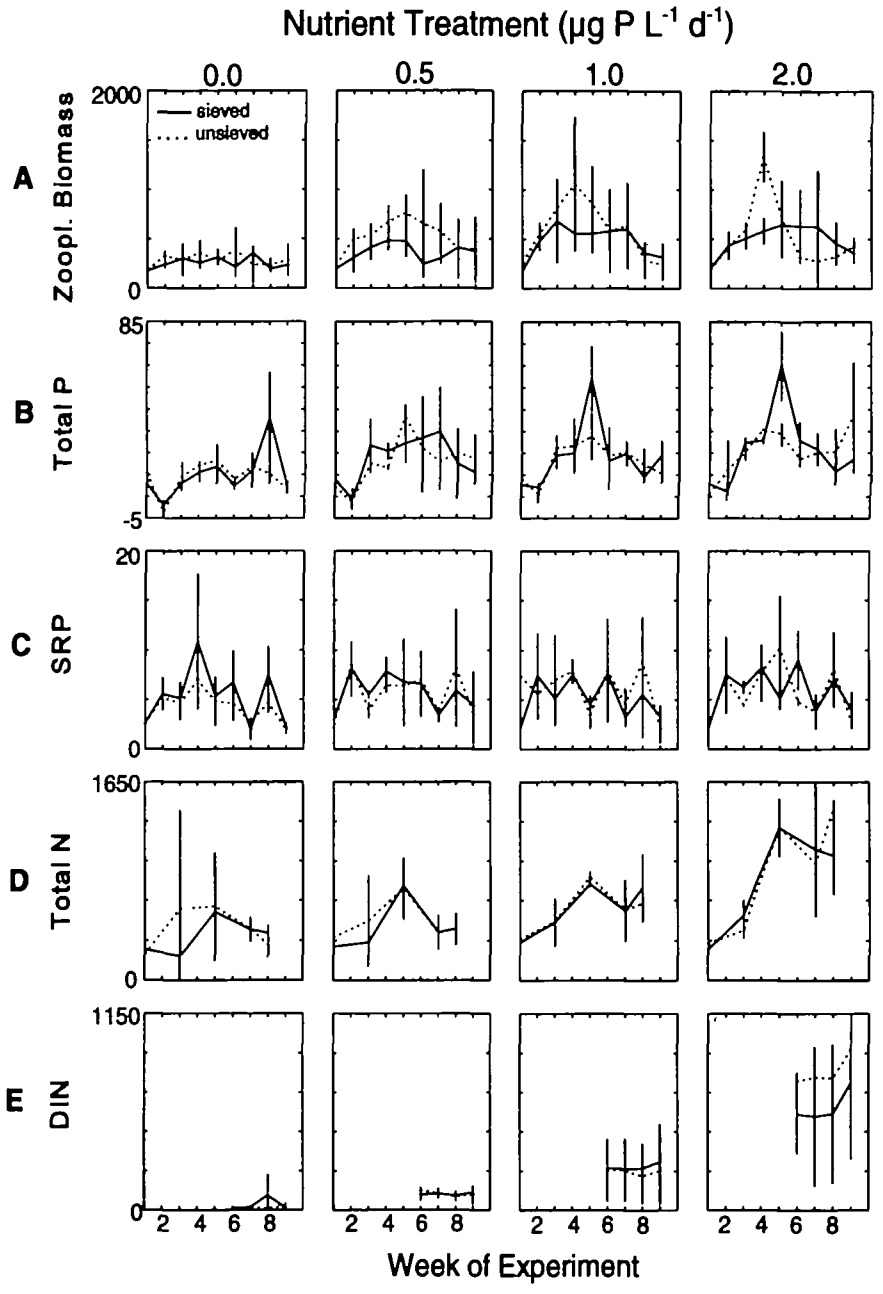

Fig. 2. Changes in total zooplankton biomass and nutrients over the course of the experiment. Nutrient loading treatments are represented by columns; dotted lines indicate unsieved treatments and solid lines represent sieved treatments. Error bars are ± 1 SD. All units are µg l^{-1}. (**A**) Zooplankton biomass. (**B**) Total phosphorus. (**C**) Soluble reactive phosphorus (SRP). (**D**) Total nitrogen. (**E**) Dissolved inorganic nitrogen (DIN).

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zooplankton taxa enumerated, only *Daphnia* increased significantly with nutrient additions over the whole experiment (Figure 1A): *Daphnia* density increased 4-fold over the nutrient addition range ($P = 0.013$), while biomass increased 3-fold ($P = 0.099$). Ephemeral increases in other zooplankton taxa, especially adult copepods (Figure 1C), were noted during individual sampling events, but did not persist long enough for significance over the whole experiment (all $P > 0.2$). Marginally significant nutrient × sieving interactions were detected for *Daphnia* density ($P = 0.093$), but not for other zooplankton variates.

Nutrients

Nutrient addition significantly increased water column TP ($P = 0.002$; Figure 2B) and TN ($P = 0.001$; Figure 2D) over the course of the experiment. However, nutrient loading did not increase SRP ($P = 0.454$; Figure 2C). Zooplankton treatments had no effect on TP ($P = 0.343$), TN ($P = 0.8$) or SRP ($P = 0.362$), and there was no significant nutrient × zooplankton interaction for TP ($P = 0.997$), TN ($P = 0.9$) or SRP ($P = 0.454$).

DIN was measured for only a few weeks and was not analyzed statistically, but appeared to accumulate in treatments with high nutrient loading (Figure 2E). This accumulation of DIN (but not SRP) indicates that the mesocosms were primarily P limited at all loading rates. This is consistent with conditions in Long Lake (S.R.Carpenter *et al.*, unpublished data) and many other lakes (Schindler, 1977). It is unlikely, then, that there were any significant shifts in N:P ratios as a consequence of changes in nutrient supply ratios or in zooplankton community composition (Sterner *et al.*, 1992).

Chlorophyll

Phytoplankton chlorophyll *a* responded significantly to both nutrients ($P = 0.001$; Figure 3A) and sieving ($P = 0.001$; Figure 3A). There was a larger increase in water column chlorophyll *a* with nutrient enrichment in sieved mesocosms as compared to unsieved mesocosms, especially during weeks 3–6 (Figure 3A). However, over the entire period of the experiment, the interaction between nutrient addition rate and sieving was not statistically significant ($P = 0.222$). Zooplankton in the unsieved mesocosms controlled phytoplankton throughout the experiment, while zooplankton in the sieved mesocosms lost control of the phytoplankton during the early part of the experiment. Interestingly, phytoplankton abundance during the latter part of the experiment was quite similar between sieved and unsieved treatments (Figure 2A), suggesting that after week 5 there was little difference between zooplankton treatments. These short-term nutrient × zooplankton interactions contributed to a statistically significant time × nutrient × zooplankton interaction ($P = 0.015$).

Chlorophyll *a* in the <35 μm size fraction ('edible' phytoplankton) closely tracked total water column chlorophyll *a* throughout the experiment (Figure 3B). Although the mean percent chlorophyll <35 μm was significantly lower in unsieved mesocosms than in sieved mesocosms (*t*-test $P = 0.003$, 75.5% versus

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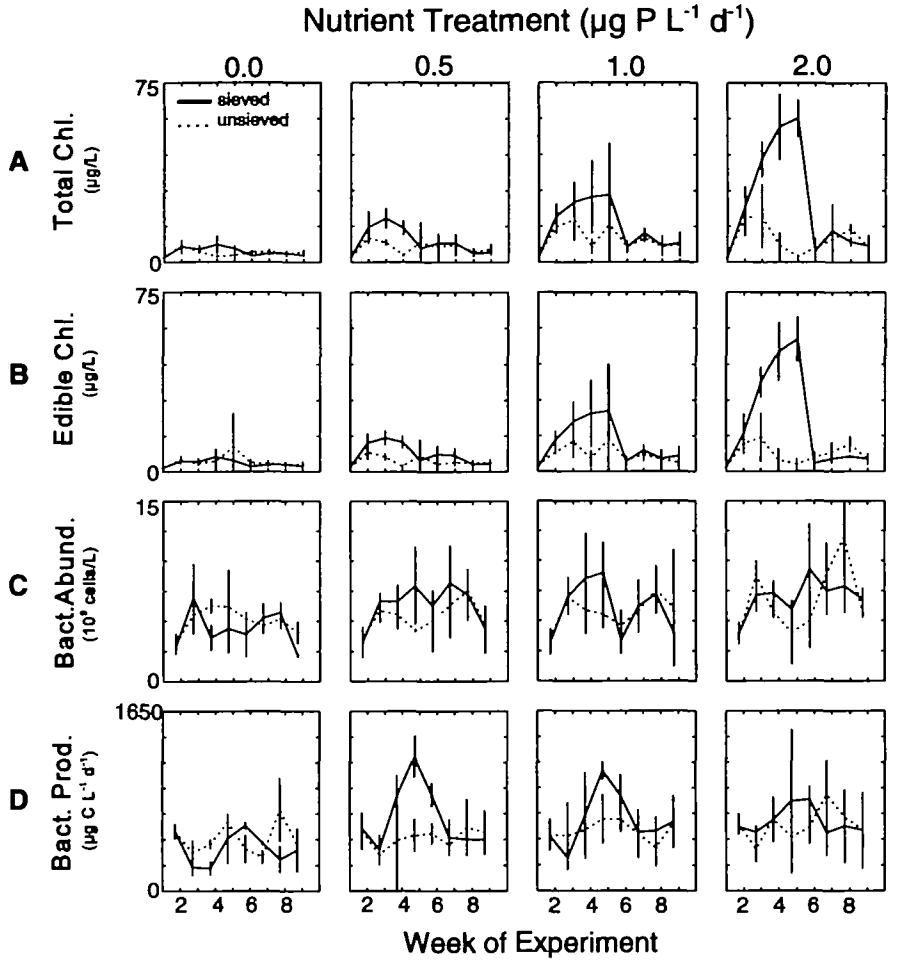

Fig. 3. Responses of phytoplankton and bacteria to experimental treatments. Nutrient treatments are represented by columns; dotted lines indicate unsieved treatments and solid lines represent sieved treatments. Error bars are ± 1 SD. (**A**) Total phytoplankton chlorophyll *a*. (**B**) Phytoplankton chlorophyll *a* in the <35 µm size fraction. (**C**) Bacterial abundance. (**D**) Bacterial production. The midsummer declines in chlorophyll *a* also occurred in Central Long Lake (S.R.Carpenter *et al.*, unpublished data), and are not thought to be treatment effects.

81.4%), there was no shift to large, bloom-forming taxa in response to either enrichment or sieving. The main effects of nutrients ($P = 0.001$) and sieving ($P = 0.001$) on phytoplankton chlorophyll *a* <35 µm were highly significant, and the interaction between effects was marginally significant ($P = 0.063$).

Bacteria

Bacterial abundance was marginally higher in enriched mesocosms ($P = 0.058$), but differences in abundance among treatments were not large (Figure 3C). There was no significant difference in bacterial abundance between sieved and

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unsieved zooplankton treatments (P = 0.433), and no significant interaction between nutrient and zooplankton treatments (P = 0.354), although there was a tendency for abundances to be higher in the sieved mesocosms with nutrients, especially in weeks 3–6 (Figure 3C).

Bacterial production, as measured by leucine incorporation into protein, was significantly higher in nutrient-enriched mesocosms (P = 0.023; Figure 3D), but there was no significant effect of zooplankton treatment (P = 0.416) and a marginally significant interaction between nutrient and zooplankton treatments (P = 0.100). The maximum mean difference among treatments was between sieved treatments at control (no) and low (0.5 μg P l^{-1} day^{-1}) nutrient loading, where average bacterial production was 3.4 and 5.7 μg C l^{-1} day^{-1}, respectively. This represented a 70% increase in mean production.

Discussion

Phytoplankton responses

As expected, phytoplankton biomass increased with increased nutrients in all enriched mesocosms. However, increases were much smaller in mesocosms with unsieved zooplankton populations, especially during the first half of the experiment (Table I). On average, there was no evidence that unsieved mesocosms became more like sieved mesocosms as nutrient loading rates increased. In fact, the difference in chlorophyll between the sieved and unsieved zooplankton treatments increased, not decreased, with increased nutrient loading: sieved mesocosms had 43.6% more chlorophyll than unsieved mesocosms at 0.5 μg P l^{-1} day^{-1}, 54.5% more chlorophyll at 1.0 μg P l^{-1} day^{-1}, and 155.3% more chlorophyll at 2.0 μg P l^{-1} day^{-1}. Thus, on the time scale of the entire experiment, the *Daphnia* in the unsieved mesocosms clearly reduced net phytoplankton responses to enrichment much more effectively than the copepods in the sieved mesocosms at all three nutrient addition rates tested.

In contrast to the large differences among enriched mesocosms caused by sieving of large zooplankton, there was little difference in chlorophyll *a* between sieved and unsieved control mesocosms. Consistent with this result, enrichment commonly amplifies grazer effects in mesocosm experiments in these lakes (Elser *et al.*, 1987; Pace and Funke, 1991). On the other hand, whole-lake food web manipulations without nutrient amendments have affected phytoplankton chlorophyll *a* in lakes (e.g. Gulati *et al.*, 1990; Carpenter and Kitchell, 1993), perhaps because manipulations of predation on zooplankton by fish cause even more extreme differences in zooplankton than the contrast employed in our experiment. Fish manipulations also change both the allocation of P among trophic levels and P recycling rates (Carpenter *et al.*, 1992). Our experiment was not intended to represent the changes in nutrient cycling that can occur when fish are manipulated.

From a budgetary perspective, added nutrients accumulated at different trophic levels in the different zooplankton treatments (Figures 1 and 3). Nutrients accumulated as algal and, to some extent, copepod biomass in the sieved mesocosms, but as *Daphnia* biomass in unsieved mesocosms. Since TN and TP

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concentrations were not affected by sieving, we suggest that the efficiency with which added nutrients were transferred through the food web was greater in the unsieved, high-*Daphnia* treatment. This result is consistent with other mesocosm experiments (Hansson and Carpenter, 1993), comparative studies (Carpenter *et al.*, 1991; Persson *et al.*, 1992) and whole-lake experiments (Reinertsen and Langeland, 1982; Carpenter *et al.*, 1996). The pattern also corroborates the conclusion of Carpenter *et al.* (1992) that *Daphnia* can limit algal increases following increased nutrient availability.

When we consider the average responses of phytoplankton to our experimental treatments, the results of this experiment do not support the hypothesis that the ability of large cladocerans to control phytoplankton biomass declines at high nutrient loading rates (Benndorf, 1987; Carney and Elser, 1990; McQueen, 1990; Elser and Goldman, 1991). We saw no evidence of a decrease in zooplankton control of phytoplankton in unsieved, enriched mesocosms, particularly during the first part of the experiment. This result is consistent with other mesocosm studies (Vanni, 1987; Hansson and Carpenter, 1993) and multi-lake comparisons (Pace, 1984; Carpenter *et al.*, 1991; Sarnelle, 1992; Mazumder, 1994) which indicate that large cladocerans are effective grazers under a variety of nutrient conditions. Taken together, these studies indicate that biomanipulation, a lake management strategy that seeks to increase large cladocerans, can be an effective approach for reducing total phytoplankton biomass at nutrient loading rates <2 μg P l^{-1} day^{-1}.

However, the observation that sieved and unsieved mesocosms became quite similar at all nutrient loading rates during the second half of our experiment suggests that differences due to sieving could be transient, non-equilibrium dynamics. After 6 weeks, the copepod-dominated zooplankton communities in the sieved mesocosms were as effective at controlling phytoplankton biomass as the *Daphnia*-dominated communities in the unsieved mesocosms. The long-term effectiveness of biomanipulation may therefore depend on maintaining non-equilibrium dynamics (Shapiro, 1990; Carpenter and Kitchell, 1993).

It is also important to recognize that small-scale experiments like this one employ simple model systems (Scheffer and Beets, 1994). Mesocosms are useful for testing hypotheses under controlled, replicated conditions, but may not accurately reflect whole-ecosystem responses.

Bacterial responses

On a weekly time scale, bacterial abundance and production tracked nutrient loading and increases in phytoplankton (Figure 3). Neither bacterial abundance nor bacterial production responded significantly to sieving, although it is quite likely that bacterial mortality was strongly affected by *Daphnia* in all mesocosms. Log–log regressions between mean chlorophyll and bacterial abundance (R^2 = 0.42, P = 0.001) and mean chlorophyll and bacterial production in each mesocosm (R^2 = 0.36, P = 0.005) were highly significant, supporting the hypothesis of Pace (1993) that zooplankton effects on bacteria are mediated by phytoplankton.

However, changes in bacteria were quite modest compared to changes in

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phytoplankton biomass. Based on models from comparative studies (Bird and Kalff, 1984; Cole *et al.*, 1988), we would have predicted much larger increases in bacterial abundance and productivity (4× and 7×, respectively) than those we observed. Grazing by *Daphnia* may explain the modest response of bacteria. We can compare estimated specific rates of grazing by *Daphnia* to estimated specific rates of bacterial growth for all dates and treatments where we measured the abundance of *Daphnia* ($n = 32$). Consumption of bacteria can be estimated from the equation of Porter *et al.* (1983) that relates clearance rates to *Daphnia* body size. These estimates of *Daphnia* clearance rates average 0.54 ml animal^{-1} h^{-1} (range 0.38–0.60). Specific clearance rates based on these estimates and the abundance of *Daphnia* average 0.23 l l^{-1} day^{-1} (range 0.027–0.90). These estimates of consumption greatly exceed our estimates of specific bacterial growth (average 0.085 day^{-1}, range 0.038–0.16). Clearly, the estimation of both consumption and growth rates involves considerable uncertainty. Nevertheless, this comparison indicates that grazing by *Daphnia* was potentially very important in limiting the biomass of bacteria in all of the mesocosms. Thus, our results may be most representative of lakes during seasonal peaks of *Daphnia*, in systems that sustain high *Daphnia* abundances throughout the year, and in enclosure experiments such as ours where high biomasses of *Daphnia* develop (e.g. Christoffersen *et al.*, 1993).

Could bacteria have affected phytoplankton responses to enrichment? Bacteria are extremely effective competitors for P (Currie and Kalff, 1984) and are grazed by many zooplankters (Jurgens, 1994). We had expected that bacteria might dampen phytoplankton responses to enrichment by sequestering P and/or supporting increased grazer biomass. However, there was no compelling evidence for either mechanism. The modest response of microbial biomass to enrichment indicates that bacteria were not a major sink for nutrients added to the mesocosms. Although turnover of bacterial biomass may involve a significant flow of nutrients to zooplankton and flagellate grazers (Riemann and Christoffersen, 1993), changes in microbial production were relatively small and do not suggest that bacterivory was important in augmenting grazer biomass in enriched mesocosms. We conclude that bacteria are unlikely to have affected the response of phytoplankton to enrichment or sieving.

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