LIMNOLOGY
AND
OCEANOGRAPHY

July 2001
Volume 46
Number 5

Limnol. Oceanogr., 46(5), 2001, 1001–1012

The relative importance of fish predation and excretion effects on planktonic communities

José Luiz Attayde and Lars-Anders Hansson[1]
Department of Limnology, University of Lund, Ecology Building, S-223 62, Lund, Sweden

Abstract

The effects of planktivorous fish on lower trophic levels through predation on zooplankton and nutrient excretion were experimentally separated and their relative importance quantified in a eutrophic humic lake. The experiment was performed in 12 enclosures (3 m^3), which initially were identical with respect to all components except fish. At the start of the experiment, caged fish not able to feed on zooplankton were added to four of the enclosures (excretion treatment), and free swimming fish to four enclosures (excretion plus predation treatment). Four enclosures were left as controls. Samples for nutrients and all major groups of organisms from bacteria to zooplankton were taken after 14 and 28 d. The effect sizes of fish excretion and predation were calculated for each variable. Our results suggest that in eutrophic lakes fish predation on zooplankton may be more important than nutrient excretion by fish for the structure and dynamics of planktonic communities. Fish predation on zooplankton was the most important mechanism accounting for fish effects on nutrient concentrations in the water, on phytoplankton biovolume, on rotifers and total zooplankton biomass, as well as on protozoan densities. However, our results suggest that nutrient excretion by fish may have important indirect effects on zooplankton. Hence, the effects of planktivorous fish through both predation on zooplankton and nutrient excretion act in concert and may be quantitatively important to shape the structure and dynamics of planktonic communities.

Although the effects of planktivorous fish on planktonic communities have been well demonstrated in many aquatic systems, the mechanisms accounting for these effects are still not clear. The classical interpretation is that these effects result from size-selective predation processes throughout the food chain. Planktivorous fish selectively consume large herbivores and often shift zooplankton structure toward dominance by rotifers, small cladocerans, and copepods (reviewed by Gliwicz and Pijanowska 1989), which have a lower grazing impact on phytoplankton and higher mass-specific rates of nutrient recycling (reviewed by Sterner 1989). The increase in phytoplankton biomass arising from fish predation on large zooplankton results in increased densities of smaller zooplankton species that, owing to their small size, are less vulnerable to predation by planktivorous fish (Vanni 1987). Similarly, size-selective predation by fish may also have indirect effects on microbial communities, since bacteria and protozoa interact with both phytoplankton and zooplankton through many direct and indirect pathways (Porter 1996).

Recently, it has been recognized that the trophic cascade effect of planktivorous fish on phytoplankton can also arise through mechanisms other than size-selective predation on zooplankton, such as through excretion of limiting nutrients (Vanni and Findlay 1990; Carpenter et al. 1992; Schindler 1992; Persson 1997*a;* Vanni and Layne 1997). Many studies have considered nutrient release by fish to be an important source of nutrients to phytoplankton (e.g., Brabrand et al. 1990; Carpenter et al. 1992; Schindler et al. 1993), and a few studies have provided experimental evidence that direct nutrient recycling by fish affects phytoplankton community structure (Reinertsen et al. 1986; Vanni and Findlay 1990; Schindler 1992; Vanni and Layne 1997; Attayde and Hansson 1999). Hence, it may be hypothesized that nutrient excretion by fish affects zooplankton community composition indirectly by changing the quantity and quality of their major food resource—phytoplankton. Moreover, since bacteria are often limited by phosphorus (Morris and Lewis 1992), which

[1] Corresponding author.

Acknowledgments

The Brazilian Government, through CAPES (Post-Graduate Federal Agency), has awarded J.L.A. with a Ph.D. grant and has financed this research. Funding was also received from the Swedish Natural Science Research Council (NFR) to L.A.H. and from the Swedish Foundation for International Cooperation in Research and Higher Education (STINT) to J.L.A. We thank Marie Svensson, Ola Svensson, Renata Panosso, Alexandre Anesio, Maurício Petrúcio, and Ramunas Stepanauskas for their assistance in the field or lab. We also thank Renata Panosso, Christer Brönmark, Wilhelm Granéli, Gary Mittelbach, and two anonymous reviewers for valuable comments and suggestions to improve the paper.

at times is supplied mainly by fish excretion (Brabrand et al. 1990; Persson 1997*b*), it may be hypothesized that nutrient recycling by fish increases bacterial growth and indirectly increases the growth of their protozoan consumers.

Here we present results of a press perturbation experiment in a Swedish temperate lake designed to examine the relative importance of the predation and excretion effects of planktivorous fish on natural planktonic communities. Our goal was to compare the relative magnitude of these effects on phytoplankton, zooplankton, and microbial communities as well as on nutrient concentrations. We have done this by assessing the effect size of fish predation and excretion on the per unit net change of each response variable. With this approach, the relative importance of predation and excretion could be quantified by comparing the size of one effect relative to that of the other.

Material and methods

Study site—Dagstorpssjön is a eutrophic and humic lake situated in the middle of Scania, southern Sweden. The lake area is 0.48 km^2, maximum depth is 5 m, and mean depth is 2.8 m (Romare et al. 1999). Sparse rooted vegetation grows in the shallow parts of small bays, but most of the shorelines are stony and steep. Perch (*Perca fluviatilis*) is one of the most important species of fish in the lake and can have strong effects on zooplankton and phytoplankton dynamics (Romare et al. 1999). During our experiment, the chlorophyll *a* concentration in the lake ranged from 29 to 77 $\mu g\ L^{-1}$ (mean = 50; SD = 20), the total phosphorus (TP) concentration from 39 to 47 $\mu g\ L^{-1}$ (mean = 43; SD = 4), the total nitrogen (TN) concentration from 850 to 1,320 $\mu g\ L^{-1}$ (mean = 1,117; SD = 241), and the dissolved organic carbon (DOC) concentration ranged from 8.78 to 9.54 mg L^{-1} (mean = 9.02; SD = 0.31).

Experimental design—We conducted an enclosure experiment from 12 August to 10 September 1997 to determine the relative importance of predation and excretion by planktivorous fish on planktonic communities. Enclosures were made of thin, clear polyethylene formed into a cylindrical tube and suspended from a wooden frame buoyed by styrofoam floats. Enclosures were sealed at the bottom and open to the atmosphere at the top. The diameter of the enclosures was 1.6 m and the depth 1.5 m, yielding a volume of approximately 3 m^3. We employed three treatments in this experiment: (1) enclosures containing natural plankton densities without addition of fish (control treatment, C), (2) enclosures containing natural plankton densities plus three perch (*Perca fluviatilis*), 7–9 cm long, placed in 1.57 m^3 cages with 500-μm net to prevent fish predation on large zooplankton but permit fish excretion to reach the whole enclosure (excretion treatment, E), and (3) enclosures containing natural plankton densities plus three perch of the same size free to feed on zooplankton (excretion plus predation treatment, EP). Each treatment was replicated four times.

Enclosures were placed in Dagstorpssjön on 9 August 1997 and fish were added to the fish enclosures on 11 August 1997. Fish were obtained from Dagstorpssjön by electrofishing. Although perch density in Dagstorpssjön has not been directly estimated, the stocking rate used here (1 fish m^{-3}) is within the range of planktivorous fish density in Swedish lakes (Persson et al. 1993). Three 7–9-cm perch constitute a stocking biomass of approximately 70 kg ha^{-1}, a reasonable amount of fish for a lake with TP $\approx$ 40–50 $\mu g\ L^{-1}$ according to the regression equation of Hanson and Leggett (1982, fig. 4).

Twice a week, enclosures and cages were brushed to reduce colonization by periphyton and carefully checked for dead fish, since decaying fish could affect the outcome of the experiment (Threlkeld 1987). The potential artifact resulting from the lack of cages in the control and the PE treatment is that cages collect periphyton and periphyton can be a substantial sink for nutrients. However, there was no significant difference in the biovolume of periphytic filamentous algae between the treatments (Kruskal-Wallis, $p > 0.10$), which indicates that brushing the cages twice a week was effective to smooth out any differences in periphyton biomass among treatments.

A 500-μm mesh was used to separate fish from zooplankton in the excretion treatment because this has been reported to be the minimum size of prey for 7–8-cm perch consumption (Lessmark 1983, p. 133). This mesh was impermeable to all zooplankton prey species except *Bosmina* and *Ceriodaphnia*, which had a mean body (carapace) length in the excretion treatment of about 400 μm. However, the mean length of these cladoceran prey in the excretion (E) treatment did not differ from their mean length in the control during the experiment ($p > 0.10$), which suggests there was little fish predation on zooplankton in the excretion treatment. On the other hand, the mean lengths of *Diaphanosoma*, *Ceriodaphnia*, and *Bosmina* in the predation plus excretion (PE) treatment were significantly lower than their mean lengths in the control ($p < 0.05$), which suggests that fish were feeding on these cladocerans in the PE treatment. However, the mean length of *Eudiaptomus* and *Cyclops* did not differ among the treatments ($p > 0.10$). Since copepods are better than cladocerans at avoiding predators, small perch (one year old, 1+) have higher capture rate and lower handling time when fed cladocerans than when fed copepods of similar size (Persson 1987). Therefore, copepods and cladocerans smaller than 500 μm are more likely to respond positively to fish predation due to their low electivities by perch.

Fish in the excretion enclosures were fed with 3 g (wet weight) of chironomid larvae every 3 d to avoid starvation. Similar amounts of chironomids were added to all other enclosures to avoid biased enrichment of the excretion treatment. We added chironomids instead of zooplankton prey because many fish under natural conditions (including *Perca fluviatilis*) eat benthic prey and excrete nutrients from benthic habitats into pelagic habitats, which may have important consequences for planktonic communities (Schindler et al. 1996; Vanni 1996). Chironomids were the only source of food in the E treatment, whereas in the PE treatment fish fed both zooplankton and chironomids. We estimated from length–weight regressions that three 7–9-cm perch have about 6 g of dry-weight (unpubl. data). The maintenance ratio size, i.e., the ratio that covers metabolic demand, for *Perca fluviatilis* at 20°C (i.e., the temperature in the enclo-

sures) is about 2% of fish weight per day respectively (Lessmark 1983, p. 38). Therefore, the maintenance ratio for 6 g of perch during a period of 3 d at 20°C would be about 0.36 g (= 0.12 × 3). Assuming a dry to wet weight percentage of 15% for chironomids (Frank 1982), we estimate that the amount of chironomids given to the fish every 3 d was about 0.45 g. Therefore, we assume that fish growth rate in the E treatment was close to zero, whereas in the PE treatment it was positive.

Sampling and analytical methods—Samples were collected on day 1 (12 August), day 14, and day 28 for total nitrogen (TN), total phosphorus (TP), dissolved organic carbon (DOC), phytoplankton, zooplankton, protozoa, and bacteria analysis. Water samples were taken from surface to bottom of each enclosure with a Plexiglas tube (diameter 70 mm) at five different positions within the enclosure and pooled. From each pooled sample, 20-ml subsamples were taken for bacteria, 20 ml for flagellates, 100 ml for nutrients, and 100 ml for phytoplankton analysis. For zooplankton analysis, 1-liter subsamples were taken and filtered through a 10-μm mesh to concentrate the organisms. Phytoplankton and zooplankton samples were preserved with acid Lugol's solution. Bacteria samples were preserved with 0.2-μm filtered formaldehyde at a final concentration of 2%, and flagellates samples were preserved with a mixed solution of formalin, lugol, and thiosulfate (Sherr and Sherr 1993).

Total phosphorus (TP) was analyzed as soluble reactive phosphorus after digestion with potassium persulfate. Total nitrogen (TN) was analyzed as nitrite after digestion with potassium persulfate and sodium hydroxid and after nitrate reduction by a copper-cadmium reductor column. These analyses were done with a Technicon autoanalyzer II according to Technicon protocols. Dissolved organic carbon (DOC) concentration was determined with a Shimadzu TOC-5000 analyzer.

Bacterial abundance was determined with a Nikon Labophot-2 epifluorescence microscope after staining with acridine orange and filtration onto 0.2-μm filters with gentle vacuum. At least 300 cells and 10 fields were counted at 1,000 × magnification for each sample. Direct image analysis was used to estimate the biovolume of bacterial cells. Cell edge detection was performed using a Marr-Hildreth operator and thresholding the image to zero according to Ramsing et al. (1996). Cell volume was calculated as a cylinder with hemispherical ends according to Blackburn et al. (1998). Total bacterial biovolume in a given sample was estimated as the product of bacterial abundance and mean biovolume in that sample.

Flagellate protists were counted by epifluorescence microscopy after staining with DAPI and filtration onto 0.8-μm filters with gentle vacuum. Counts were made at 1,000 × magnification by scanning strips until at least 200 cells were counted for each filter. We did not discriminate heterotrophic from mixotrophic and autotrophic cells, but small heterotrophic flagellates were far more abundant. Ciliates were counted at 200 × magnification, and rotifers, cladocerans, and copepods were counted at 100 × magnification with an inverted microscope, after sedimentation in 10-ml chambers for at least 2 h. At least 200 organisms of each group were counted per zooplankton sample. For each zooplankton sample, the length of 20 individuals of each rotifer, cladoceran, and copepod taxa was measured and the respective biomass calculated using specific length–weight regression equations reported in Dumont et al. (1975) and Bottrell et al. (1976). Bias due to logarithmic transformation of some length–weight regression models was corrected according to Bird and Prairie (1985). The biomass of each taxa in a given sample was estimated as the product of its mean biomass and density in that sample. Total zooplankton biomass was estimated as the sum of the total biomass of rotifers, cladocerans, and copepods.

Phytoplankton were counted at 250 × magnification on permanent slides made using HPMA (Crumpton 1987). In each sample a minimum of 10 fields or 200 cells were counted. The length and width of 20 individuals of each algal species were measured, and the biovolumes were calculated using different formulae according to their geometric shape. Algal species with greatest axial linear dimension (GALD) smaller than 30 μm were categorized as edible algae, whereas larger species were categorized as inedible algae.

Data analysis—The total effect of fish on a given variable was assessed as the difference between the per unit net changes of that variable in the PE and control treatments [$\ln(N_{t,\mathrm{PE}}/N_{0,\mathrm{PE}}) - \ln(N_{t,\mathrm{C}}/N_{0,\mathrm{C}})$; Fig. 1]. The effect of fish excretion was assessed as the difference between the per unit net changes of the variable in the excretion (E) and control treatments [$\ln(N_{t,\mathrm{E}}/N_{0,\mathrm{E}}) - \ln(N_{t,\mathrm{C}}/N_{0,\mathrm{C}})$; Fig. 1], and the effect of fish predation was assessed as the difference between the per unit net changes of the variable in the PE and E treatments [$\ln(N_{t,\mathrm{PE}}/N_{0,\mathrm{PE}}) - \ln(N_{t,\mathrm{E}}/N_{0,\mathrm{E}})$; Fig. 1]. $N_{t,\mathrm{PE}}$, $N_{t,\mathrm{E}}$, and $N_{t,\mathrm{C}}$ (or $N_{0,\mathrm{PE}}$, $N_{0,\mathrm{E}}$, and $N_{0,\mathrm{C}}$) are the concentrations, densities, or biomasses of the target variable in the PE, E, and control treatments respectively after t d (or at the start; $t = 0$) of the experiment (modified from Osenberg et al. 1997). The ratios of abundances (N_t/N_0) were log transformed before the effect size metric was calculated in order to stabilize variances in the effect size data as well as provide a symmetrical scale. In order to express the full range of variation in our experiment, we calculated effect sizes based on all possible combinations of replicates for each treatment comparison (e.g., E1-C1, E2-C1, E3-C1, E4-C1, . . . , E1-C4, E2-C4, E3-C4, E4-C4). From these 16 effect size estimates (4 × 4 replicates) we calculated the mean and standard deviation of the effect.

We used ANOVA to test for differences between treatment effects and, when significant differences were found, a Tukey test was used for multiple comparison of means. The nonparametric Kruskal-Wallis test was used when the ANOVA assumptions were violated. A significant difference ($p < 0.10$) between the per unit net changes of a given variable in the PE and control treatments [$\ln(N_{t,\mathrm{PE}}/N_{0,\mathrm{PE}}) \neq \ln(N_{t,\mathrm{C}}/N_{0,\mathrm{C}})$] indicates that the total effect of fish on that variable was significantly different from zero. Likewise, a significant difference between the per unit net changes of the variable in the E and control treatments [$\ln(N_{t,\mathrm{E}}/N_{0,\mathrm{E}}) \neq \ln(N_{t,\mathrm{C}}/N_{0,\mathrm{C}})$] indicates that the fish excretion effect on that variable was significantly different from zero. On the other hand, the fish predation effect was considered significantly different from

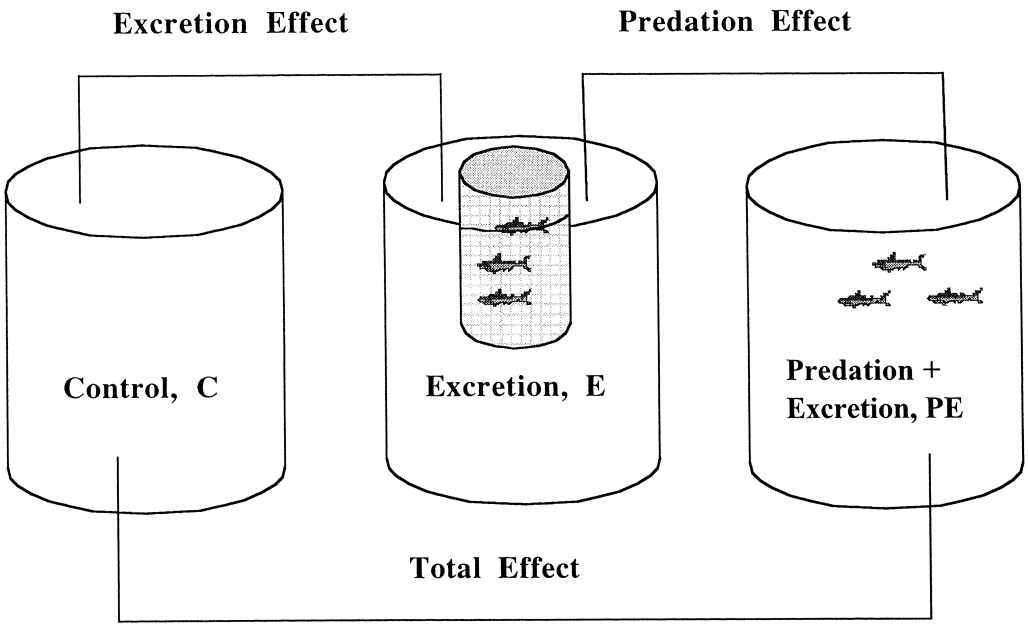

Fig. 1. Schematic representation of the experimental design. The total effect of fish on a given variable was calculated as the difference between the per unit net change of the variable in the PE and C treatments. The fish excretion effect was calculated as the difference between the per unit net change of the variable in the E and C treatments, whereas the fish predation effect was calculated as the difference between the per unit net change in the PE and E treatments (*see* material and methods section for more details).

zero when there was a significant difference between the per unit net changes of the target variable in the PE and E treatments [$\ln(N_{t,\mathrm{PE}}/N_{0,\mathrm{PE}}) \neq \ln(N_{t,\mathrm{E}}/N_{0,\mathrm{E}})$].

Note that statistical significance does not measure the biological importance of a factor or mechanism. The relative importance of a factor can only be ascertained by comparing the effect size of this factor (which is a quantitative measure of magnitude, not statistical significance) relative to the effect size of other factors. Recently, many ecologists have advocated the use of effect size estimates to quantify treatment effect and compare the relative importance of different ecological processes (e.g., Osenberg and Mittelbach 1996; Osenberg et al. 1997). The aim of our study was to assess the relative importance of fish predation and excretion effects. Therefore, we provide measures of effect sizes (and their standard deviations) for these factors.

Results

Effects on nutrients—The average concentration of TP, TN, and DOC increased in all treatments during the experiment (Fig. 2). However, the increases in TN and DOC concentrations were significantly higher in the PE treatment than in the control (Table 1), which indicates that fish had a significant overall effect on TN and DOC concentrations. Results also show that the increases in TP and TN concentrations were significantly higher in the PE treatment than in the E treatment (Table 1), which indicates that fish predation on zooplankton had significant effects on TP and TN concentrations. The sizes of the total effect of fish on TP, TN, and DOC concentrations were all positive during the experiment, and fish predation on zooplankton accounted for most of these effects (Table 2).

Effects on phytoplankton—The average biovolume of total phytoplankton and inedible algae (GALD $>$ 30 μm) decreased in the control and excretion treatments but increased in the PE treatment during the experiment (Fig. 3). On the other hand, the average biovolume of edible algae (GALD $<$ 30 μm) increased in all treatments during the experiment (Fig. 3). The changes in phytoplankton biovolume were significantly higher in the PE treatment than in the control or excretion treatments, indicating that fish, particularly fish predation on zooplankton, had significant effects on phytoplankton (Table 1). The total effects of fish on the biovolume of total phytoplankton and both edible and inedible algae were positive during the experiment, and fish predation on zooplankton accounted for most of these effects (Table 2).

Effects on bacteria and protozoa—During the experiment, the average biovolume of bacteria decreased in all treatments, whereas the average densities of flagellates increased in the PE treatment (Fig. 4). In the control and excretion treatments, the average densities of ciliates and flagellates showed no clear trends (Fig. 4). The changes in densities of ciliates and flagellates were significantly higher in the PE treatment than in the excretion treatment after 14 d, which indicates that fish predation on zooplankton had significant effects on protozoa densities (Table 1). The size of the total effect of fish on densities of flagellates was positive during the experiment, and fish predation on zooplankton accounted for most of these effects (Table 3). Fish predation also ac-

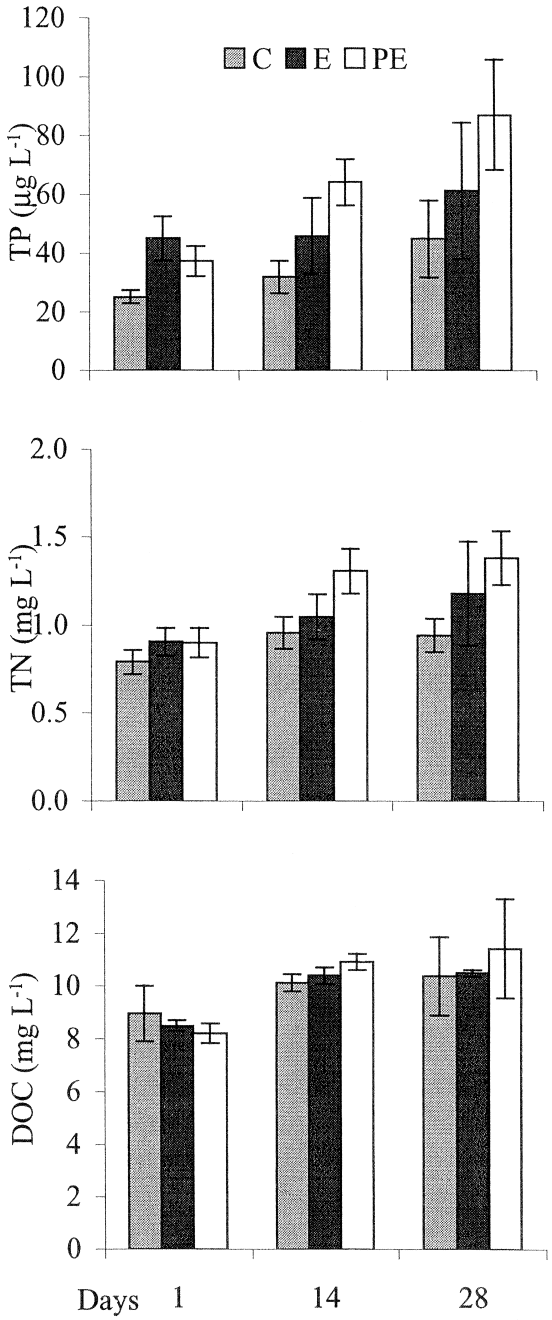

Fig. 2. Mean (±1 SD) concentration of total phosphorus (TP), total nitrogen (TN), and dissolved organic carbon (DOC) in the control (C), excretion (E), and predation plus excretion (PE) treatments after 1, 14, and 28 d of experiment.

counted for most of the fish effects on ciliate densities after 14 d (Table 3). However, after 28 d, the effects of fish excretion on ciliate densities and bacteria biomass were stronger than the effects of fish predation (Table 3).

Effects on zooplankton—The average biomass of copepods and cladocerans increased in all treatments during the experiment (Fig. 5). The average biomass of total zooplankton increased in the PE treatment but showed no clear trend in the other treatments, whereas the mean biomass of rotifers showed decreasing trends in the control and excretion treatment but no clear trend in the PE treatment during the experiment (Fig. 5). The changes in rotifer biomass were significantly higher in the PE treatment than in the excretion or control treatments, which indicates that fish, particularly fish predation on zooplankton, had significant effects on rotifers (Table 1). Fish also had significant effects on total zooplankton, cladocerans, *Bosmina*, and *Cyclops* after 28 d (Table 1). Except for copepods after 14 d, all major zooplankton groups and total zooplankton biomass responded positively to fish (Table 3). A comparison of the average magnitude of the fish predation and excretion effects indicates that fish predation had stronger effects on rotifers and total zooplankton biomass, whereas fish excretion had stronger effects on the total biomass of cladocerans during the experiment (Table 3). Fish excretion also had stronger effects on the total biomass of copepods after 14 d of experiment, whereas fish predation accounted for most of the fish effects on copepods after 28 d (Table 3).

Examining the response of the zooplankton community to fish predation and excretion in more detail reveals a positive trend in biomass development for *Ceriodaphnia* and *Cyclops* in all treatments and for *Bosmina* in the E and PE treatments (Fig. 6). *Diaphanosoma* showed an increase followed by a decrease in mean biomass in all treatments during the experiment, whereas *Eudiaptomus* showed the opposite pattern (Fig. 6). Overall, fish had positive effects on *Bosmina* and *Cyclops* and negative effects on *Diaphanosoma* and *Eudiaptomus* during the experiment (Table 4). Fish predation had positive effects on *Cyclops* and negative effects on biomass of *Ceriodaphnia* and *Diaphanosoma* but mixed effects on *Bosmina* and *Eudiaptomus* during the experiment (Table 4). Except for *Eudiaptomus*, all major zooplankton genera included in this study responded positively to fish excretion (Table 4). A comparison of the average magnitude of these two effects indicates that fish predation had stronger effects on the biomass of *Diaphanosoma*, whereas fish excretion had stronger effects on the biomass of *Eudiaptomus* during the experiment (Table 4). In addition, fish excretion had stronger effects on *Ceriodaphnia* and *Cyclops* after 28 d and on *Bosmina* after 14 d of experiment (Table 4).

Discussion

It has been repeatedly demonstrated in lakes that planktivorous fish increase phytoplankton biomass, change phytoplankton community structure (*see* Carpenter and Kitchell 1993), and increase the concentration of total phosphorus (TP) and total nitrogen (TN) in the water column (e.g., Schindler and Eby 1997; Vanni et al. 1997). However, the relative importance of the mechanisms by which fish can affect phytoplankton communities and influence the dynamics of limiting nutrients is still not clear. Our results suggest that fish predation on zooplankton can be the most important mechanism accounting for the higher phytoplankton biomass and higher TP and TN concentrations in the presence of fish (Table 2).

Most studies addressing the impact of planktivorous fish

Table 1. Mean and standard deviation of the net changes in the abundance of the response variables in the control (C), excretion (E), and predation plus excretion (PE) treatments after 14 and 28 d of experiment. The net change was estimated for each replicate as the log of the ratio between the abundance of the variable after *t* days and at the start of the experiment [i.e., ln (N_t/N_0)]. Results of ANOVA and Tukey test for differences between treatment effects on the net changes of the variables. Note that only variables showing significant differences (at the 10% level) are shown.

	C		E		PE		ANOVA	
	Mean	SD	Mean	SD	Mean	SD	*p* value	Tukey
14 days								
TP	0.23	0.18	0.00	0.34	0.54	0.07	0.025	PE > E
TN	0.19	0.03	0.14	0.07	0.37	0.10	0.003	PE > E = C
DOC	0.13	0.11	0.20	0.03	0.29	0.07	0.060	PE > C
Edible algae	1.12	0.60	1.71	0.55	2.43	0.43	0.022	PE > C
Flagellates	−0.44	0.52	−0.80	0.52	0.23	0.39	0.038	PE > E
Ciliates	−1.83	0.96	−1.59	1.05	1.20	1.41	0.009	PE > E = C
Rotifers	−1.98	1.46	−2.06	1.23	0.39	0.82	0.028	PE > E = C
28 days								
TP	0.55	0.28	0.26	0.34	0.84	0.26	0.065	PE > E
Total algae	−0.99	0.61	−1.10	1.21	0.66	0.28	0.021	PE > E = C
Inedible algae	−1.81	0.74	−1.42	1.22	0.33	0.31	0.013	PE > E = C
Edible algae	2.12	1.12	1.86	1.09	3.66	0.29	0.044	PE > E
Total zooplankton	−0.33	0.75	0.04	0.65	0.73	0.19	0.083	PE > C
Cladocerans	0.87	0.95	2.17	0.98	3.03	1.47	0.074	PE > C
Rotifers	−3.25	0.24	−3.11	1.23	−1.06	0.83	0.010	PE > E = C
Bosmina	−0.86	0.88	0.10	2.13	3.02	1.22	0.029	PE > C
Cyclops	0.19	2.01	2.31	1.82	3.13	0.70	0.079	PE > C

on planktonic communities use an absence of fish as the experimental control for fish and, therefore, do not distinguish the effects of fish predation from those of fish excretion. Hansson and Carpenter (1993) used fish placed inside cages as a control treatment for fish, and with this design they were able to address the effects of fish predation on phytoplankton without the confounding effects of fish excretion. They found that fish predation on zooplankton alone had significant effects on phytoplankton, which is also confirmed by our study (Table 1). However, as they did not have a treatment without fish, the effects of fish excretion on phytoplankton could not be assessed.

Table 2. Mean effect size and standard deviation of excretion, predation, and total effects of fish on nutrient concentrations and algal biovolume after 14 and 28 d of experiment.

	Total effect		Excretion effect		Predation effect	
	Mean	SD	Mean	SD	Mean	SD
14 days						
TP	0.31	0.17	−0.23	0.35	0.55	0.31
TN	0.18	0.09	−0.05	0.07	0.23	0.11
DOC	0.16	0.12	0.07	0.11	0.08	0.07
Total algae	0.86	0.64	0.28	0.80	0.58	0.90
Inedible algae	0.82	0.74	0.15	1.01	0.66	1.09
Edible algae	1.31	0.66	0.59	0.73	0.72	0.63
28 days						
TP	0.29	0.34	−0.29	0.40	0.58	0.39
TN	0.25	0.16	0.07	0.20	0.18	0.25
DOC	0.18	0.22	0.07	0.17	0.11	0.14
Total algae	1.66	0.60	−0.10	1.22	1.76	1.11
Inedible algae	2.15	0.72	0.39	1.27	1.76	1.12
Edible algae	1.54	1.04	−0.27	1.40	1.80	1.01

Persson (1997*a*) found that phytoplankton biomass was significantly enhanced when fish predation and excretion acted together but not when they acted alone and suggested that both are important mechanisms by which fish affect phytoplankton. However, the effect of fish predation in his study was quantified by artificially removing zooplankton with a net, which resulted in a significant decrease in total phosphorus concentration. Thus, it is not clear from his results if algal biomass failed to increase with the artificial removal of zooplankton because nutrients were unavailable or because algae were unaffected by zooplankton. To circumvent this problem, we estimated the effect of fish predation in Persson's experiment by subtracting the excretion effect from the total effect of fish (the E bar from the PE bar in his fig. 5). Quantifying the fish predation effects in his study in this way suggests that fish predation on zooplankton had much stronger effects on total phytoplankton biomass than did nutrient excretion by fish. This is in accordance with the results from our study, which suggests that the indirect effect of fish predation on zooplankton can account for most of the fish effects on phytoplankton biomass (Table 2).

On the other hand, Vanni and Layne (1997) provided experimental evidence suggesting that nutrient excretion by fish is an important mechanism controlling phytoplankton communities, but it is not clear from their results whether the effects of fish excretion were more important than the

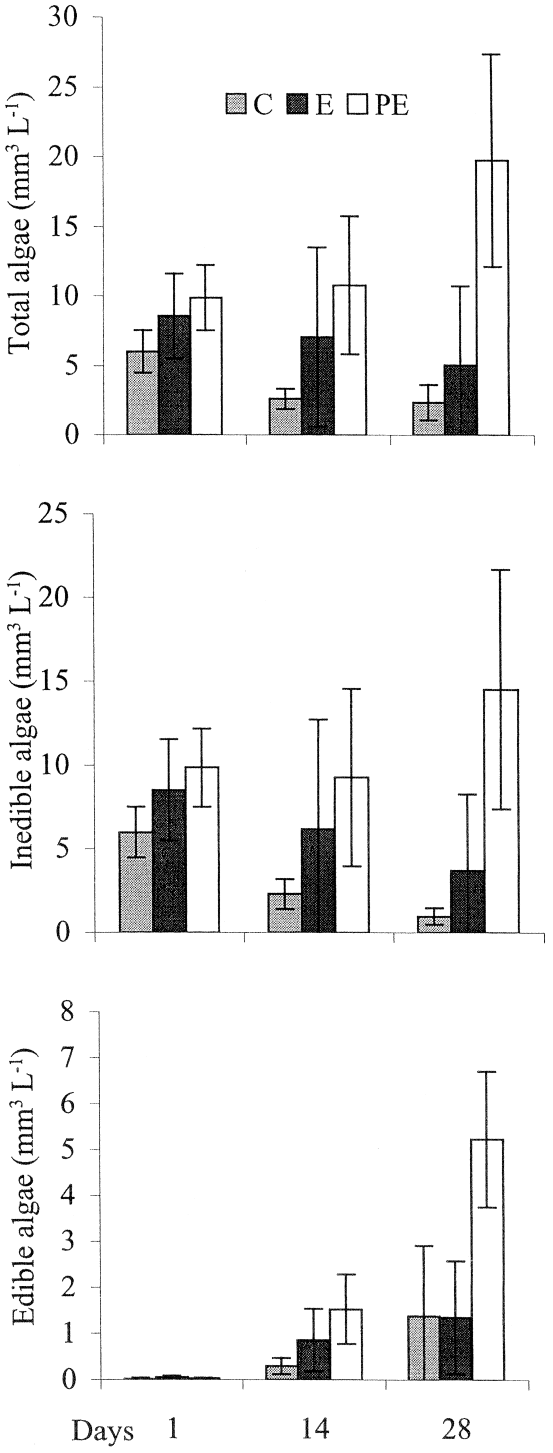

Fig. 3. Mean (±1 SD) biovolume of total phytoplankton, edible (GALD < 30 μm) algae, and inedible (GALD > 30 μm) algae in the control (C), excretion (E), and predation plus excretion (PE) treatments after 1, 14, and 28 d of experiment.

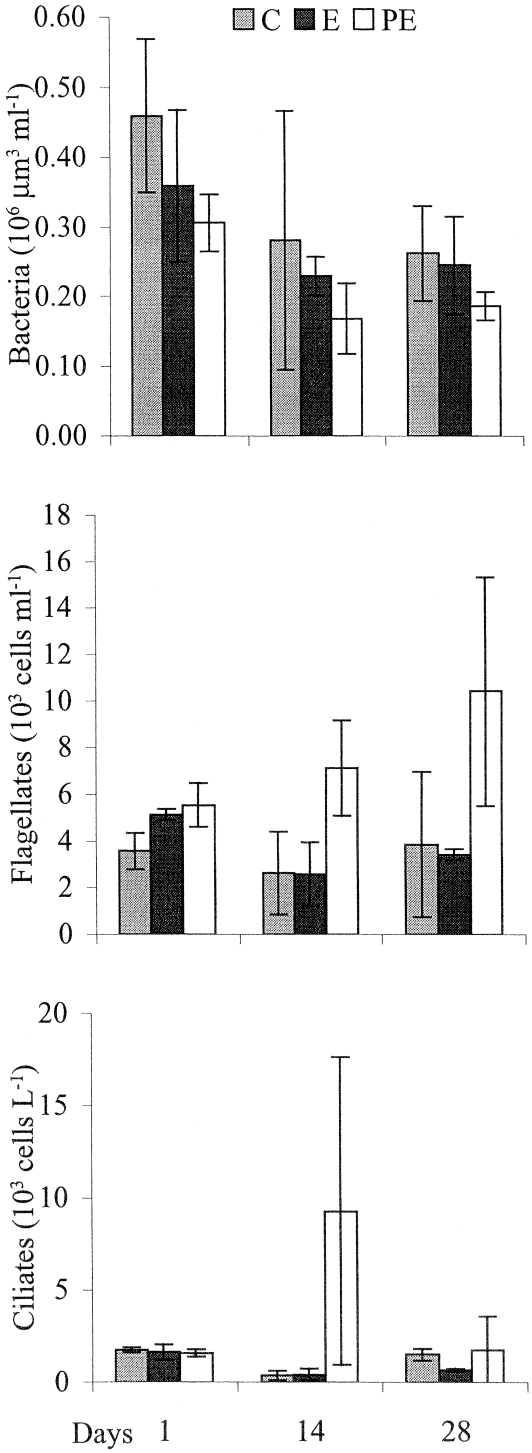

Fig. 4. Mean (±1 SD) biovolume of bacteria and density of flagellates and ciliates in the control (C), excretion (E), and predation plus excretion (PE) treatments after 1, 14, and 28 d of experiment.

effects of fish predation on zooplankton. In their experiment, several phytoplankton taxa showed increased abundance in nutrient permeable chambers (excluding herbivores) incubated in enclosures with fish compared to chambers incubated in enclosures without fish, which suggests that these taxa responded to fish even when separated from direct herbivory but exposed to nutrients recycled by fish and zooplankton. In separate enclosures without fish, phytoplankton were exposed to contrasting zooplankton assemblages shaped by fish predation. Since the phytoplankton response

Table 3. Mean effect size and standard deviation of excretion, predation, and total effects of fish on biovolume of bacteria, densities of flagellates and ciliates, and biomass of rotifers, cladocerans, copepods, and total zooplankton after 14 and 28 d of experiment.

	Total effect		Excretion effect		Predation effect	
	Mean	SD	Mean	SD	Mean	SD
14 days						
Bacteria	−0.01	0.62	0.20	0.56	−0.20	0.39
Flagellates	0.68	0.58	−0.36	0.66	1.03	0.58
Ciliates	3.03	1.53	0.24	1.27	2.79	1.58
Rotifers	2.36	1.50	−0.08	1.70	2.44	1.32
Cladocerans	0.33	1.01	0.67	0.89	−0.35	1.22
Copepods	−0.26	1.94	−0.56	1.70	0.30	2.51
Total zooplankton	0.60	0.79	−0.28	0.67	0.88	0.94
28 days						
Bacteria	−0.22	0.27	−0.18	0.31	−0.04	0.25
Flagellates	0.76	0.93	−0.20	0.79	0.96	0.51
Ciliates	−0.11	0.98	−0.70	0.39	0.60	1.00
Rotifers	2.19	0.77	0.14	1.12	2.05	1.33
Cladocerans	2.16	1.57	1.30	1.22	0.86	1.58
Copepods	1.37	1.16	0.34	1.42	1.03	0.98
Total zooplankton	1.05	0.70	0.36	0.89	0.69	0.61

Table 4. Mean effect size and standard deviation of excretion, predation, and total effects of fish on biomass of the major zooplankton taxa after 14 and 28 d of experiment.

	Total effect		Excretion effect		Predation effect	
	Mean	SD	Mean	SD	Mean	SD
14 days						
Bosmina	0.78	1.70	1.15	1.19	−0.37	1.64
Ceriodaphnia	−0.66	1.58	1.06	1.00	−1.72	1.32
Diaphanosoma	−1.16	1.68	0.07	2.25	−1.23	2.53
Cyclops	0.87	2.26	0.23	2.04	0.64	2.93
Eudiaptomus	−0.48	0.94	−0.43	1.05	−0.05	1.39
28 days						
Bosmina	3.12	1.80	0.95	2.06	2.17	2.50
Ceriodaphnia	0.26	1.96	1.09	0.95	−0.83	2.00
Diaphanosoma	−0.44	1.82	0.19	2.66	−0.63	2.29
Cyclops	2.94	1.90	2.12	2.42	0.82	1.74
Eudiaptomus	−0.21	1.43	−0.51	0.59	0.30	1.39

was weaker in chambers (excluding herbivores) incubated in these enclosures than in chambers incubated in the enclosures with fish, Vanni and Layne (1997) concluded that direct nutrient recycling by fish was an important mechanism by which fish affected phytoplankton.

Several factors might explain why the effects of fish excretion on phytoplankton were more important in the study of Vanni and Layne (1997) than in our study. First, our experiment was performed in a eutrophic lake, whereas the experiment in Vanni and Layne (1997) was conducted in a low-nutrient system. The relative importance of fish predation and excretion should change along the trophic gradient. Nutrient excretion by fish may be more important in lakes with low P inputs (and high seston C : P) and a relatively high biomass of planktivorous fish, which may arise when piscivore fish are absent or rare and when planktivorous fish consume considerable amounts of littoral/benthic resources (Vanni and Layne 1997). Therefore, it is possible that nutrient excretion by fish was not so important in our study because phytoplankton may have been less limited by nutrients in the eutrophic lake where we conducted our experiment.

Second, we might not have observed a stronger response of phytoplankton to fish excretion because any increase in phytoplankton biomass resulting from nutrient excretion by fish in the E treatment is likely to have been obscured by zooplankton grazing on phytoplankton. In the study of Vanni and Layne (1997), the effect of fish excretion on phytoplankton was estimated inside chambers that excluded zooplankton. We have also shown in a previous study that nutrient excretion by fish has substantial effects on phytoplankton community structure in the absence of herbivores (Attayde and Hansson 1999). However, when zooplankton are abundant, any increase in productivity and/or phytoplankton biomass resulting from nutrient enrichment (i.e., fish excretion) is likely to be consumed by zooplankton. In fact, Schaus and Vanni (2000) demonstrated that the effects of fish excretion on phytoplankton biomass were only observed when zooplankton were suppressed by fish. Indeed, food chain theory predicts that when herbivores are not controlled by carnivores, nutrient enrichment should increase the equilibrium biomass of herbivores, leaving the equilibrium biomass of primary producers unchanged (Oksanen et al. 1981). The distinction between fish-mediated nutrient effects on algal growth versus on ''equilibrium'' algal biomass highlights the limitation of sampling phytoplankton at 2-week intervals in this type of study.

Finally, as pointed out by Vanni and Layne (1997), it is likely that their excretion effects were somewhat overestimated by their experimental design. On the other hand, our experimental design could possibly result in underestimation of excretion effects, and this potential bias needs to be addressed. Fish excretion is strongly dependent on fish feeding and growth pattern (Threlkeld 1987; Mather et al. 1995). Because fish were also fed zooplankton in the PE treatment, they might have excreted more nutrients in the PE than in the E treatment, where they were fed only chironomids. This was unavoidable given the necessity of preventing the fish from feeding on enclosure zooplankton in the E treatment. Unfortunately, we do not know how conservative our excretion estimates are, since fish growth was not estimated in our study. Therefore, our predation effect estimates also include an excretion component from the zooplankton diet in the PE treatment, and this may have caused a bias toward a greater predation effect of fish compared to the excretion effect. Thus, we must exercise restraint when concluding that predation effects were stronger than excretion effects.

Numerous studies have demonstrated that when planktivorous fish are abundant, zooplankton communities are dominated by ciliates, rotifers, small cladocerans, and copepods, whereas in the absence of fish, zooplankton is dominated by large taxa (reviewed by Gliwicz and Pijanowska 1989). This effect of planktivorous fish on zooplankton communities is often attributed to size-selective predation on larger zoo-

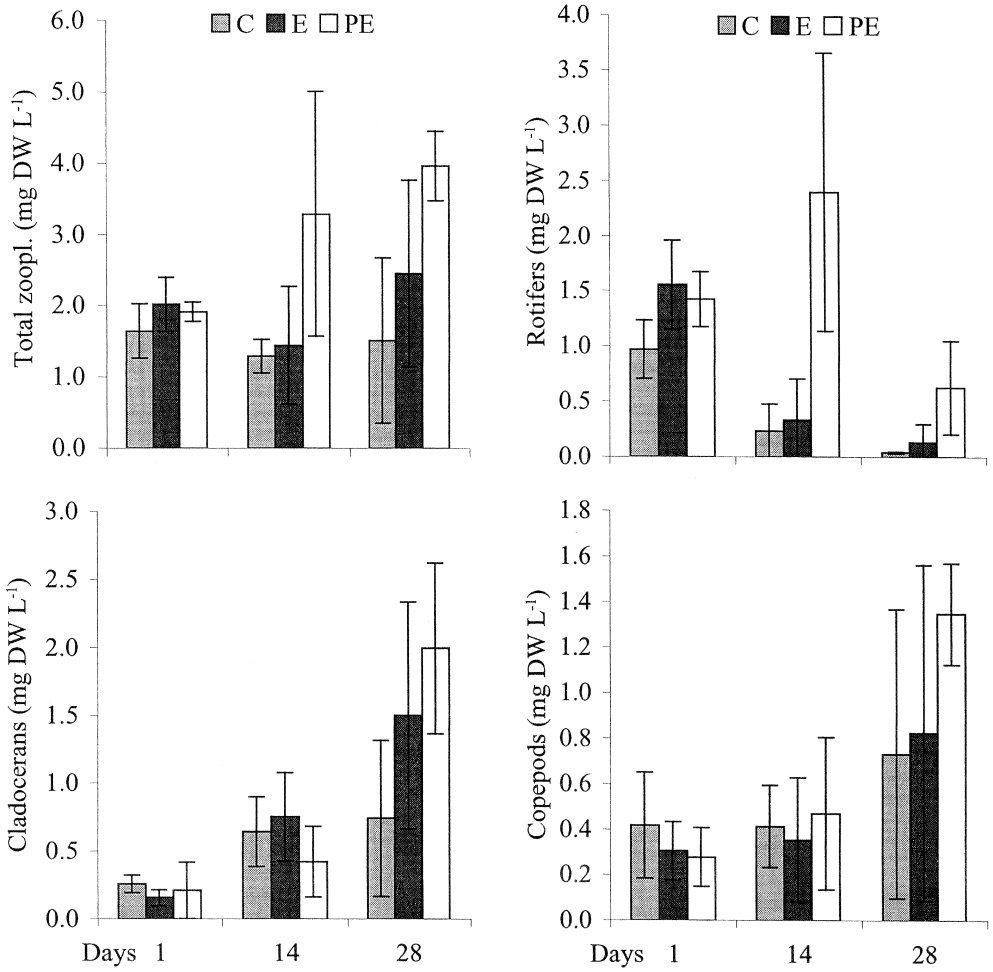

Fig. 5. Mean (±1 SD) biomass of rotifers, cladocerans, copepods, and total zooplankton in the control (C), excretion (E), and predation plus excretion (PE) treatments after 1, 14, and 28 d of experiment.

plankton. Indeed, our study suggests that fish predation on large cladocerans (>500 μm) was the most important mechanism accounting for the observed increase in protozoans and rotifers in the presence of fish (Table 3). A plausible explanation is that the selective predation of perch on large cladocerans may have released the microzooplankton from the negative effects of these large herbivores, such as mechanical interference and exploitative competition for food (Sarnelle 1997; Pace et al. 1998). In spite of their strong positive effect on protozoans and rotifers after 14 d, planktivorous fish had no effect on bacteria biomass (Table 3). In this case, the increase in microzooplankton may have suppressed bacterial biomass by the time our first samples were taken.

It has been shown that the increase in phytoplankton availability arising from fish predation on large zooplankton results in increased survival and/or reproduction of the smaller zooplankton individuals that manage to avoid predation (Vanni 1987). Likewise, any increase in food (phytoplankton) availability arising from nutrient excretion by fish may also have indirect effects on zooplankton communities. The only study we know of that has investigated the effects of fish excretion on zooplankton suggests that fish excretion can have substantial effects on zooplankton communities (Schindler 1992), but it is not clear whether such excretion effects can be stronger than the effects of fish predation. Obviously, the extent to which fish excretion influences the zooplankton community will depend on how phytoplankton respond to fish excretion and how the coexisting zooplankton populations respond to these changes in food availability and quality. In our study, all major zooplankton taxa, except *Eudiaptomus*, responded positively to fish excretion (Table 4). Even though we have not found any statistical difference in zooplankton biomass between the control and excretion treatment, our effect size estimates suggest that nutrient excretion by fish may have important indirect effects on zooplankton that could be of equal or greater magnitude than the effects of fish predation (Table 4).

This reinforces our suggestion that zooplankton may have prevented their resources (phytoplankton and bacteria) from accumulating in response to a fish-induced stimulation of growth rates. Fish excretion may have strongly influenced algal growth, but this response may have been masked by day 14 due to the stimulation of grazers. The long time scale

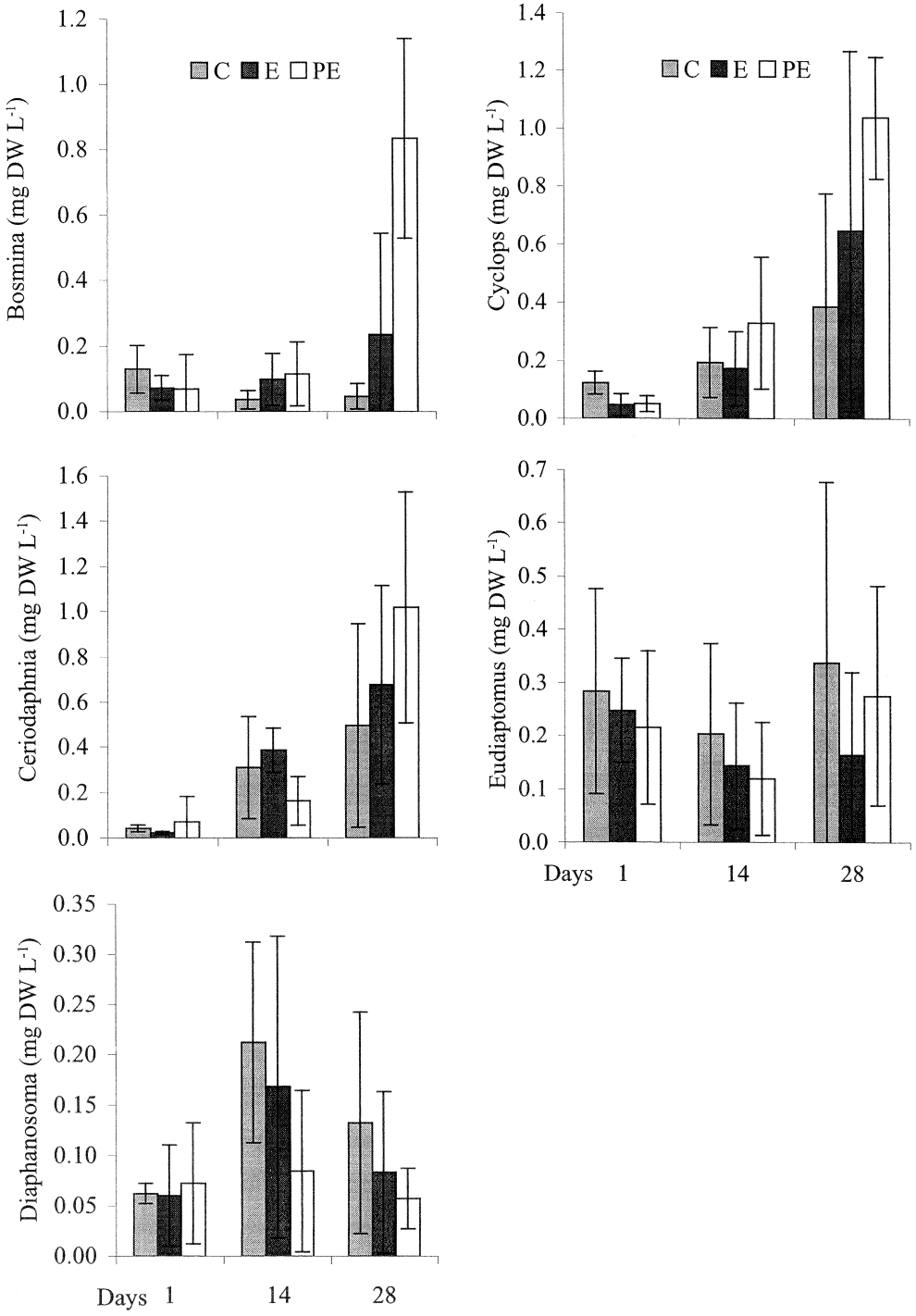

Fig. 6. Mean (±1 SD) biomass of the most important crustacean zooplankton in the control (C), excretion (E), and predation plus excretion (PE) treatments after 1, 14, and 28 d of experiment.

of the experiment (relative to the dynamics of bacteria and phytoplankton) may explain why fish excretion may have had a stronger influence on certain zooplankton than on phytoplankton or bacteria.

Obviously, no single study can claim to yield general results that hold for the full diversity of species, communities, and ecosystems or can tell us whether a process operating in a single location and season occurs at different sites and seasons. Hence, generality can only be inferred by comparing the results of many experiments conducted in a variety of systems (Morin 1998). Owing to their small size, mesocosms necessarily exclude some processes that may be important at larger scales. Under natural conditions, many fish feed in the littoral zones of lakes, migrate, and excrete nutrients into the pelagic habitats (Schindler et al. 1996; Vanni 1996). Although we have tried to simulate this process of

nutrient transport by fish by adding chironomids to the enclosures, we might expect that under natural conditions the effects of fish excretion on planktonic communities may be more important than in our mesocosm study. Even though great care should be taken in generalizing or extrapolating results of small-scale experiments to larger scales, this is an important step for understanding mechanisms acting in natural systems. Thus, the results of our mesocosm study should help us understanding the mechanisms by which planktivorous fish may affect planktonic communities in lakes.

In summary, our results suggest that in eutrophic lakes fish predation on zooplankton may be more important than nutrient excretion by fish for the structure and dynamics of planktonic communities. These results, when contrasted with the results of Vanni and Layne (1997), suggest that fish excretion may be less important in eutrophic than in oligotrophic lakes. However, even with conservative estimates of excretion effects, we show here that nutrient excretion by fish can have important indirect effects on zooplankton. The time scale of the experiment may explain why fish excretion had stronger effects on zooplankton than on phytoplankton or bacteria. Hence, planktivorous fish affect planktonic communities directly and indirectly through both predation on zooplankton and nutrient excretion. Therefore, the relative importance of direct versus indirect effects in pelagic food webs and the relative importance of top-down versus bottom-up control in planktonic communities can only be acknowledged if we understand how consumer-mediated nutrient recycling affects the structure and dynamics of planktonic communities.

References

ATTAYDE, J. L., AND L. A. HANSSON. 1999. Effects of nutrient recycling by zooplankton and fish on phytoplankton communities. Oecologia **121:** 47–54.

BIRD, D. F., AND Y. T. PRAIRE. 1985. Practical guidelines for the use of zooplankton length-weight regression equations. J. Plankton Res. **7:** 955–960.

BLACKBURN, N., Å. HAGSTRÖM, L. WIKNER, R. C. HANSSON, AND P. K. BJORNSEN. 1998. Rapid determination of bacterial abundance, biovolume, morphology, and growth by neural-network based image analysis. Appl. Environ. Microbiol. **64:** 3246–3255.

BOTTRELL, H. H., AND OTHERS. 1976. A review of some problems in zooplankton production studies. Nor. J. Zool. **24:** 419–456.

BRABRAND, A., B. A. FAAFENG, AND J. P. M. NILSEN. 1990. Relative importance of phosphorus supply to phytoplankton production: Fish excretion versus external loading. Can. J. Fish. Aquat. Sci. **47:** 364–372.

CARPENTER, S. R., AND J. F. KITCHELL [EDS.] 1993. The trophic cascade in lakes. Cambridge Univ. Press.

———, C. E. KRAFT, R. WRIGHT, X. HE, P. A. SORANNO, AND J. R. HODGSON. 1992. Resilience and resistance of a lake phosphorus cycle before and after food web manipulation. Am. Nat. **140:** 781–798.

CRUMPTON, W. 1987. A simple and reliable method for making permanent mounts of phytoplankton for light and fluorescence microscopy. Limnol. Oceanogr. **32:** 1154–1159.

DUMONT, H. J., I. VAN DE VELDE, AND S. DUMONT. 1975. The dry weight estimate of biomass in a selection of cladocera, copepoda and rotifera from the plankton, periphyton and benthos of continental waters. Oecologia **19:** 75–97.

FRANK, C. 1982. Ecology, production and anaerobic metabolism of *Chironomus plumosus* L. larvae in a shallow lake I. Ecology and production. Arch. Hydrobiol. **94:** 460–491.

GLIWICZ, Z. M., AND J. PIJANOWSKA. 1989. The role of predation in zooplankton succession, p. 253–296. *In* U. Sommer [ed.], Plankton ecology: Succession in plankton communities. Springer.

HANSON, J. M., AND W. C. LEGGETT. 1982. Empirical prediction of fish biomass and yield. Can. J. Fish. Aquat. Sci. **39:** 257–263.

HANSSON, L. A., AND S. R. CARPENTER. 1993. Relative importance of nutrient availability and food chain for size and community composition in phytoplankton. Oikos **67:** 257–263.

LESSMARK, O. 1983. Competition between perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*) in south Swedish lakes. Ph.D. thesis, University of Lund, Sweden.

MATHER, M. E., M. J. VANNI, T. E. WISSING, S. A. DAVIS, AND M. H. SCHAUS. 1995. Regeneration of nitrogen and phosphorus by bluegill and gizzard shad: Effect of feeding history. Can. J. Fish. Aquat. Sci. **52:** 2327–2338.

MORIN, P. J. 1998. Realism, precision, and generality in experimental ecology, p. 50–70. *In* W. J. Resetarits Jr. and J. Bernardo [eds.], Experimental ecology: Issues and perspectives. Oxford Univ. Press.

MORIS, D. P., AND W. M. LEWIS. 1992. Nutrient limitation of bacterioplankton growth in Lake Dillon, Colorado. Limnol. Oceanogr. **37:** 1179–1192.

OKSANEN L., S. D. FRETWELL, J. ARRUDA, AND P. NIEMELA. 1981. Exploitation ecosystems in gradients of primary productivity. Am. Nat. **118:** 240–261.

OSENBERG, C. W., AND G. G. MITTELBACH. 1996. The relative importance of resource limitation and predation limitation in food chains, p. 134–148. *In* G. A. Polis and K. O. Winemiller [eds.], Food webs: Integration of patterns and dynamics. Chapman and Hall.

———, O. SARNELLE, AND S. D. COOPER. 1997. Effect size in ecological experiments: The application of biological models in meta-analysis. Am. Nat. **150:** 798–812.

PACE, M. L., J. J. COLE, AND S. R. CARPENTER. 1998. Trophic cascades and compensation: Differential responses of microzooplankton in whole-lake experiments. Ecology **79:** 138–152.

PERSSON, A. 1997*a*. Effects of fish predation and excretion on the configuration of aquatic food webs. Oikos **79:** 137–146.

———. 1997*b*. Phosphorus release by fish in relation to external and internal load in a eutrophic lake. Limnol. Oceanogr. **42:** 577–583.

PERSSON, L. 1987. The effects of resource availability and distribution on size class interactions in perch, *Perca fluviatilis.* Oikos **48:** 148–160.

———, L. JOHANSSON, G. ANDERSSON, S. DIEHL, AND S. F. HAMRIN. 1993. Density dependent interactions in lake ecosystems: Whole lake perturbation experiments. Oikos **66:** 193–208.

PORTER, K. G. 1996. Integrating the microbial loop and the classic food chain into a realistic planktonic food web, p. 51–59. *In* G. A. Polis and K. O. Winemiller [eds.], Food webs: Integration of patterns and dynamics. Chapman and Hall.

RAMSING, N. B., H. FISSING, T. G. FERDELMAN, F. ANDERSEN, AND B. THAMDRUP. 1996. Distribution of bacterial populations in a stratified fjord (Mariager Fjord, Denmark) quantified by in situ hybridization and related to chemical gradients in the water column. Appl. Environ. Microbiol. **62:** 1391–1404.

REINERTSEN, H., A. JENSEN, A. LANGELAND, AND Y. OLSON. 1986. Algal competition for phosphorus: The influence of zooplankton and fish. Can. J. Fish. Aquat. Sci. **43:** 1135–1141.

ROMARE, P., E. BERGMAN, AND L. A. HANSSON. 1999. The impact of larval and juvenile fish on zooplankton and algal dynamics. Limnol. Oceanogr. **44:** 1655–1666.

SARNELLE, O. 1997. Daphnia effects on microzooplankton: Comparisons of enclosure and whole-lake responses. Ecology **78:** 913–928.

SCHAUS, M. H., AND M. J. VANNI. 2000. Effects of gizzard shad on phytoplankton and nutrient dynamics: Role of sediment feeding and fish size. Ecology **81:** 1701–1719.

SCHINDLER, D. E. 1992. Nutrient regeneration by sockeye salmon (*Oncorhynchus nerka*) fry and subsequent effects on zooplankton and phytoplankton. Can. J. Fish. Aquat. Sci. **49:** 2498–2506.

———, S. R. CARPENTER, K. L. COTTINGHAM, X. HE, J. R. HODGSON, J. F. KITCHELL, AND P. A. SORANNO. 1996. Food web structure and littoral zone coupling to pelagic trophic cascades, p. 96–105. *In* G. A. Polis and K. O. Winemiller [eds.], Food webs: Integration of patterns and dynamics. Chapman and Hall.

———, AND L. A. EBY. 1997. Stoichiometry of fishes and their prey: Implications for nutrient recycling. Ecology **78:** 1816–1831.

———, J. F. KITCHELL, X. HE, S. R. CARPENTER, J. R. HODGSON, AND K. L. COTTINGHAM. 1993. Food web structure and phosphorus cycling in lakes. Trans. Am. Fish. Soc. **122:** 756–772.

SHERR, E. B., AND B. F. SHERR. 1993. Preservation and storage of samples for enumeration of heterotrophic protists, p. 207–212. *In* P. F. Kemp, B. F. Sherr, E. B. Sherr, and J. J. Cole [eds.], Handbook for methods in aquatic microbial ecology. Lewis.

STERNER, R. W. 1989. The role of grazers in phytoplankton succession, p. 107–170. *In* U. Sommer [ed.], Plankton ecology: Succession in plankton communities. Springer.

THRELKELD, S. T. 1987. Experimental evaluation of trophic-cascade and nutrient-mediated effects of planktivorous fish on plankton community structure, p. 161–173. *In* W. C. Kerfoot and A. Sih [eds.], Predation: Direct and indirect impacts on aquatic communities. University Press of New England.

VANNI, M. J. 1987. Effects of food availability and fish predation on a zooplankton community. Ecol. Monogr. **57:** 61–88.

———. 1996. Nutrient transport and recycling by consumers in lake food webs: Implications for algal communities, p. 81–95. *In* G. A. Polis and K. O. Winemiller [eds.], Food webs: Integration of patterns and dynamics. Chapman and Hall.

———, AND D. L. FINDLAY. 1990. Trophic cascade and phytoplankton community structure. Ecology **71:** 921–937.

———, AND C. D. LAYNE. 1997. Nutrient recycling and herbivory as mechanisms in the ''top-down'' effect of fish on algae in lakes. Ecology **78:** 21–40.

———, ———, AND S. E. ARNOTT. 1997. ''Top-down'' trophic interactions in lakes: Effects of fish on nutrient dynamics. Ecology **78:** 1–20.

Received: 21 September 2000
Amended: 22 January 2001
Accepted: 14 February 2001