

## Effects of nutrients (phosphorous, nitrogen, and carbon) and zooplankton on bacterioplankton and phytoplankton—a seasonal study

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### Abstract

The effects of inorganic nutrients (P and N), organic C, and metazoan zooplankton on bacterioplankton production and abundance and on phytoplankton biomass were studied in five experiments (from May to September) in Lake Erken. In addition, the seasonal dynamics of bacterioplankton and phytoplankton were followed in the lake from April to November.

Bacterioplankton production was P limited from May to August. N alone never stimulated bacterioplankton production, but bacterioplankton growth was close to colimited by P and N in July and August. Organic C stimulated bacterial production in June and September. Zooplankton enhanced bacterioplankton production in June, when bacterioplankton production was limited by P and C and the phytoplankton biomass in the lake was low. N alone stimulated phytoplankton growth in all experiments. In addition, P alone stimulated phytoplankton growth in May and July, and the combination of P and N stimulated phytoplankton growth in July and August. Zooplankton additions resulted in a decrease in phytoplankton biomass in May and September, mainly owing to grazing on Cryptophyceae.

The experimental results indicate that bacterioplankton and phytoplankton growth were uncoupled during most of the open-water period because P primarily limited bacterioplankton growth and N limited phytoplankton growth. The response of the bacterioplankton community was most likely a direct effect of nutrient additions. Primary production and bacterioplankton production were correlated during the season, but partial correlations analysis indicates that this relationship can be attributed to the fact that both primary production and bacterioplankton production showed strong positive correlations with temperature. We suggest that uncoupling of bacterioplankton production and phytoplankton production may be a common phenomenon in lakes.

Microbial food webs and the interactions between bacterioplankton and phytoplankton have been intensely studied during the past few decades. However, few attempts have been made to simultaneously determine how the nutrient limitation of bacterioplankton and phytoplankton production varies seasonally, and whether phytoplankton and bacterioplankton are limited by the same nutrient.

Bacterioplankton biomass and production are positively correlated with the biomass and production of phytoplankton both between and within lakes (e.g., Bird and Kalff 1984; Cole et al. 1988; Schweitzer and Simon 1995; Jeppesen et al. 1997). These correlations can be ascribed to the dependence of bacteria on organic carbon (C) derived from phytoplankton. However, the correlations may also reflect an indirect relationship mediated via dependence on common regulating factors such as temperature and nutrients (Currie 1990; Coveney and Wetzel 1995).

Phosphorus (P) has been suggested to be the primary nutrient limiting phytoplankton production in lakes (Schindler

1977), but colimitation by P and nitrogen (N) is not uncommon (Elser et al. 1990; Jansson et al. 1996). In general, bacterial growth has been considered to be primarily limited by organic C (Rheinheimer 1992). However, several lacustrine studies have shown that bacterial growth can be stimulated by additions of inorganic P alone (e.g., Toolan et al. 1991; Elser et al. 1995) or inorganic N, either alone or in combination with organic C or inorganic P (e.g., Morris and Lewis 1992; Elser et al. 1995).

Additions of inorganic nutrients have been reported to stimulate bacterioplankton growth directly in several studies (Toolan et al. 1991; Coveney and Wetzel 1992; Le et al. 1994), but indirect stimulation via increased phytoplankton growth and increased release rates of organic C from phytoplankton has also been found (Riemann and Søndergaard 1986; Wang et al. 1992). Taken together, these findings indicate that the correlation between bacterioplankton and phytoplankton growth can be attributed either to bacterial dependence on organic C released from phytoplankton or to the regulation of both bacterioplankton and phytoplankton growth by the same factor.

Metazoan zooplankton affect phytoplankton both directly, by grazing, and indirectly, by nutrient regeneration (Sterner 1986). Inorganic nutrients released by zooplankton can constitute a considerable fraction of the total nutrient supply supporting phytoplankton growth (Lehman 1980). The impact of zooplankton on the biomass and production of bacterioplankton is more multifaceted because zooplankton feed not only on bacteria, but on bacterivorous protozoans and phytoplankton as well, and release both organic and inorganic nutrients. Considering the complex nature of the direct

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Table 1. Design of the 2<sup>4</sup> factorial experiments. Each treatment was performed in duplicate.

Treatment	P	N	C	Z
l	-	-	-	-
p	+	-	-	-
n	-	+	-	-
np	+	+	-	-
c	-	-	+	-
pc	+	-	+	-
nc	-	+	+	-
pnc	+	+	+	-
z	-	-	-	+
pz	+	-	-	+
nz	-	+	-	+
pnz	+	+	-	+
cz	-	-	+	+
pcz	+	-	+	+
ncz	-	+	+	+
pncz	+	+	+	+

Manipulations: p, 30  $\mu\text{g P L}^{-1}$  added as  $\text{KH}_2\text{PO}_4$ ; n, 220  $\mu\text{g N}$  added as  $\text{NH}_4\text{Cl}$ ; c, 1,230  $\mu\text{g C L}^{-1}$  added as glucose; and z, zooplankton added at approximately ambient concentration.

and indirect interactions between zooplankton and bacterioplankton, it is not surprising that the net effect of zooplankton on bacterial biomass or production has been reported to be negative, none, or positive (reviewed by Vrede 1998).

The objective of this study was to assess the effects of inorganic nutrients (P and N), organic C, and zooplankton on bacterioplankton production and biomass as well as on phytoplankton biomass. Five mesocosm experiments were performed from May to September in Lake Erken. In addition, the seasonal dynamics of bacterioplankton and phytoplankton in the lake were followed from April to November and related to both the experimental results and to environmental variables.

## Methods

**Study site**—Lake Erken (59°51'N, 18°35'E) is a naturally eutrophic, temperate, dimictic lake with a total phosphorus concentration of 25  $\mu\text{g P L}^{-1}$ , a total nitrogen concentration of 700  $\mu\text{g N L}^{-1}$ , and a water color of 15 mg Pt  $\text{L}^{-1}$  (Pettersson 1985; Pettersson unpubl. data). The lake has a surface area of 24 km<sup>2</sup>, a maximum depth of 21 m, and an average depth of 9 m (Håkanson 1978).

**Experiments**—Five 2<sup>4</sup> factorial experiments were performed in which inorganic phosphorus (P), inorganic nitrogen (N), organic carbon (C), and metazoan zooplankton (Z) were manipulated (i.e., added or not added) in all possible combinations (Table 1). Each treatment was duplicated. Natural planktonic communities, from which large zooplankton had been removed by sieving with a 120- $\mu\text{m}$  net, were incubated in 20-liter transparent polyethylene containers. Water was pumped from approximately 2 m depth (upper circulating layer). Treatments were assigned in random order to the containers, and P (30  $\mu\text{g P L}^{-1}$  as  $\text{KH}_2\text{PO}_4$ ), N (220

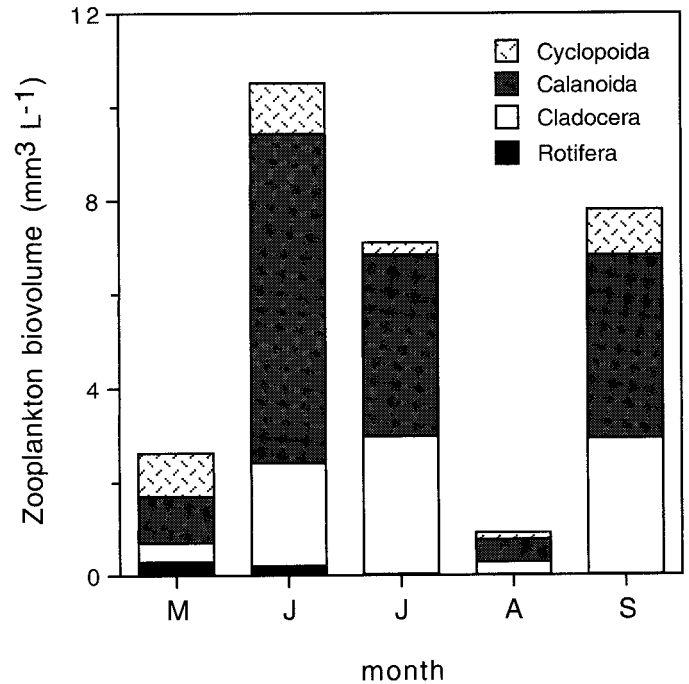


Fig. 1. Zooplankton biovolume at the start of the experiments in treatments with zooplankton addition ( $n = 4$ ).

$\mu\text{g N L}^{-1}$  as  $\text{NH}_4\text{Cl}$ ), C (1,230  $\mu\text{g C L}^{-1}$  as glucose), and Z (approximately ambient concentration) were added. Eight additional containers were used in each experiment and were manipulated as follows: no addition, Z alone, P alone, N alone, C alone, P + Z, N + Z, and C + Z. Samples from these eight containers were analyzed immediately to obtain initial values. The other 32 containers were incubated hanging from two floating racks (one full set of 16 treatments on each rack) 1.5 m deep. Water for the five experiments was collected on 27 May, 8 June, 7 July, 2 August, and 8 September. In May, August, and September, the experiments were started on the same day as the water was collected. In June and July, the manipulations had to be carried out in the morning of the day after the water had been sampled, owing to strong winds. On these occasions, the containers were stored in near-shore water at night. All experiments were terminated 4 d after the water had been collected. Samples for analyses of bacterioplankton abundance and production, phytoplankton species composition and biomass, zooplankton species composition and biovolume, and water chemistry were taken from all containers.

We chose not to include silica (Si) in our experimental design. Although it is a potentially important nutrient for the limitation of diatom growth, both Pechlaner (1970) and Eriksson and Pettersson (1984) have shown that Si concentrations are high enough throughout the spring (minimum 0.49 and 1.7 mg Si  $\text{L}^{-1}$ , respectively) not to limit diatom growth. The lack of Si limitation is further evidenced by the fact that diatoms occur frequently throughout the summer (Fig. 2; Vrede et al. unpubl. data).

**Lake study**—Samples for analyses of bacterioplankton abundance and production, autotrophic picoplankton abun-

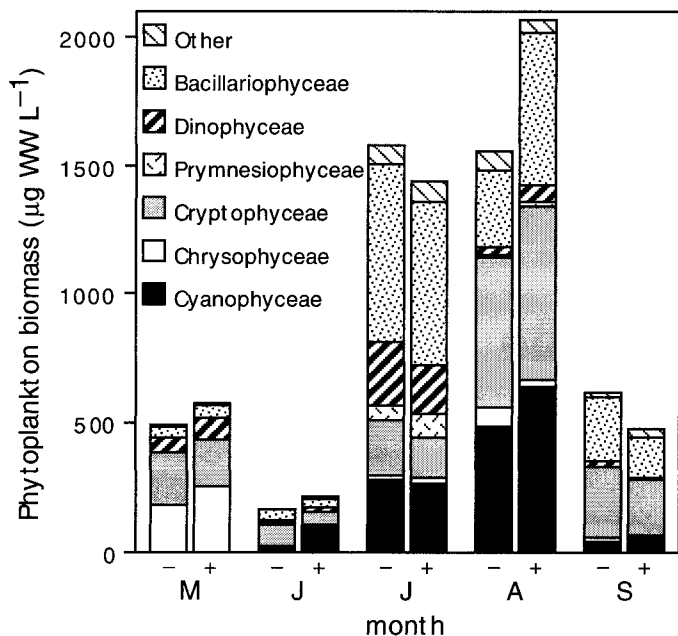


Fig. 2. Phytoplankton biomass (wet weight) at the start of the experiments ( $n = 4$  for each bar). -, without zooplankton; +, with zooplankton.

dance, chlorophyll *a* (Chl *a*), primary production, phosphate, ammonium, and nitrate were taken weekly in the lake from April (shortly after ice break) to September in 1992. In addition, we collected two samples in October and one in November. Samplings were carried out between 0800 and 1000 h. Before any water samples were taken, the temperature was measured in 0.5–2-m intervals to determine whether the lake was stratified. If it was, then the depth of the epilimnion was determined. A vertically integrated composite sample from the upper circulating layer was taken with a 2-m tube sampler in different (2-m) layers in proportion to the layers' proportional contribution to the total lake volume. During circulation, a composite sample from the upper 10 m was taken. Samples for determination of Chl *a* concentration, zooplankton biovolume, and water chemistry were taken at five stations in the main basin of the lake and pooled. All other analyses were made on water taken above the deepest point in the lake.

**Analyses**—Bacterioplankton production was determined by measuring  $^3\text{H}$ -thymidine incorporation into macromolecules (Bell 1993). Triplicate 5-ml samples and one formaldehyde-killed blank were incubated with 20 nM (final concentration)  $^3\text{H}$ -methylthymidine (Amersham 40–60 Ci mmole $^{-1}$ ) for 30–60 min at in situ temperature. The dependence of the bacterial thymidine uptake rate on thymidine concentration was tested in June, and it was found that the uptake rate did not increase further at concentrations above 20 nM. The incubations were stopped by adding formaldehyde (2% final concentration). Macromolecules were precipitated with trichloroacetic acid and filtered onto cellulose acetate filters. The radioactivity of the filters was determined by liquid scintillation counting. Samples for determination

of bacterial abundance and autotrophic picoplankton abundance were preserved with formaldehyde (4% final concentration). Bacteria were stained with acridine orange, filtered onto 0.2- $\mu\text{m}$  black polycarbonate filters, and counted in an epifluorescence microscope (Hobbie et al. 1977). At least 400 cells were counted in at least 20 fields. Autotrophic picoplankton were filtered onto 0.2- $\mu\text{m}$  black polycarbonate filters and counted with an epifluorescence microscope using blue and green excitation filters (Waterbury et al. 1979). At least 400 cells or 40 fields were counted.

Chl *a* concentrations were measured according to Strickland and Parsons (1968). Primary production was measured according to Schindler et al. (1972) with modifications described below. Stopped glass flasks (120 ml) were filled with lake water, and 12  $\mu\text{Ci}$  of  $\text{NaH}^{14}\text{CO}_3$  (Amersham) was added to each flask. Duplicate samples were incubated for 4–7 h at 0, 0.2, 0.5, 1, 1.5, 2, 3, and 4 m depth at the deepest point in the lake. Dark bottles were incubated at 4 m depth. The incubations were started between 1000 and 1100 h and were stopped by putting the flasks in darkness. From each flask, a 1.5-ml subsample was acidified with 150  $\mu\text{l}$  of 0.5 M HCl and put on a shaking table for 1 h. Scintillation cocktail was added and the  $^{14}\text{C}$  activity was measured in a liquid scintillation counter.

Zooplankton was concentrated by filtering 10–18 liters of water through a 120- $\mu\text{m}$  net. Phytoplankton and zooplankton were preserved with acid Lugol's solution and counted in an inverted microscope. Phytoplankton biovolume was estimated using geometrical formulae and converted to biomass assuming a density of 1 mg wet weight  $\text{mm}^{-3}$ . Zooplankton biovolume (excluding large, carnivorous Cladocera) was calculated by using average biovolumes for each species, previously determined by Nauwerck (1963). Standard methods were used for determining concentrations of soluble reactive phosphorus (SRP) (Murphy and Riley 1962), ammonium-N (Chaney and Marbach 1962), and nitrate-N (Wood et al. 1967). In each experiment, nutrient additions (phosphate and ammonium) were estimated as the differences between the average concentrations of SRP or ammonium in containers to which the nutrient had been added and the average concentrations in containers to which the nutrient had not been added. The analyses were performed immediately after nutrient addition.

**Statistical analyses**—For the eight start-day containers, differences in bacterial, phytoplankton, and nutrient variables between those that had received zooplankton and those that had not were analyzed with *t*-tests.

The results from the experiments were analyzed with factorial analysis of variance (ANOVA) after  $\log_{10}$ -transformation in order to stabilize the variance. Effect sizes were calculated with Yate's algorithm (Box et al. 1978). Response parameters were bacterial production, bacterial abundance, and total phytoplankton biomass. Responses of phytoplankton were also analyzed for groups constituting  $>5\%$  of the total biomass at the start of the experiment. In May and June, Dinophyceae were excluded from analysis owing to very low counts per sample, even though they constituted 13 and 6% of total phytoplankton biomass, respectively. We chose to regard effects with  $P < 0.02$  as significant. This level of

probability is justified because it keeps large and biologically important effects, but excludes some higher-order interactions, which may be related to differences between incubation racks or the variation introduced in connection with adding zooplankton. Comparison with analysis of normal probability plots of effect size (25 factorial design, incubation rack as the fifth factor) (Daniel 1976; Box et al. 1978) showed that the effects judged significant at  $P < 0.02$  by ANOVA were also the effects that were greater than any effect including the incubation rack factor.

Both single factor effects and interaction effects were analyzed. In some experiments, there was a significant interaction effect of two nutrients, but only one of the nutrients had an effect on its own. In such cases, only the nutrient that had an effect on its own was regarded as primarily limiting. The combined effect of the two nutrients was interpreted as though the nutrients were close to colimiting, even though only one of them was primarily limiting. The combined effect of two nutrients was regarded as a "true colimitation" when there only was a combined effect of two nutrients or when each element in the combined effect also had a significant effect of its own.

Relationships between bacterial production, bacterial abundance, primary production, Chl *a* concentration, autotrophic picoplankton abundance, and water temperature in the lake were examined by calculating Spearman rank correlation coefficients and rank partial correlation coefficients (Conover 1980).

## Experimental results

*Manipulations and initial conditions*—The average ( $\pm$  standard deviation) addition of phosphate and ammonium in all experiments was  $25 \pm 2 \mu\text{g P L}^{-1}$  and  $217 \pm 29 \mu\text{g N L}^{-1}$ , respectively. The zooplankton additions ranged between  $0.92 \pm 0.04 \text{ mm}^3 \text{ L}^{-1}$  (August) and  $10.5 \pm 0.01 \text{ mm}^3 \text{ L}^{-1}$  (June) (Fig. 1), and the zooplankton additions corresponded to 0.3, 1.8, 0.8, 0.2, and 2.2 times the ambient zooplankton biovolume in the lake from May to September. In containers that did not receive zooplankton, the total zooplankton biovolume was  $<0.06 \pm 0.03 \text{ mm}^3 \text{ L}^{-1}$  in all experiments. Calanoid copepods (mainly *Eudiaptomus graciloides*) were always the largest group in containers that had received zooplankton. Cyclopoid copepods were dominated by *Thermocyclops oithonoides* and *Mesocyclops leuckartii*. Cladocerans were dominated by *Bosmina coregoni* sensu lato (May and June), *Diaphanosoma brachyurum* (July and August), and *Daphnia* spp. (September). Zooplankton additions did not result in significant changes in initial bacterial production, total phytoplankton biomass,  $\text{PO}_4$  concentration, or  $\text{NH}_4$  concentration (*t*-tests,  $P > 0.05$ ). Although large phytoplankters (Cyanophyceae and Bacillariophyceae) appeared to be overrepresented in containers receiving zooplankton in the August experiment, the variation was high between replicates, and as a result the overall difference was not statistically significant (Fig. 2). In September, initial bacterial abundance was 24% higher in treatments with zooplankton added than in those without zooplankton (*t*-test,  $P = 0.04$ ), but this type of difference was not found in the other experiments.

Dominating phytoplankton classes (together constituting  $>70\%$  of the total biomass) at the start of the experiments were Chrysophyceae and Cryptophyceae (May); Cryptophyceae, Cyanophyceae, and Bacillariophyceae (June); Bacillariophyceae, Cyanophyceae, Dinophyceae, and Cryptophyceae (July); Cryptophyceae, Cyanophyceae, and Bacillariophyceae (August); and Cryptophyceae and Bacillariophyceae (September) (Fig. 2).

*Bacterioplankton*—P alone stimulated bacterioplankton production in all experiments except in September, whereas N alone never had any significant effect (Fig. 3A). In June, C, P, and Z, as well as PC and PN, had positive effects on bacterial production. In July and August, P and PN stimulated bacterioplankton production. In September, C alone and CZ had positive effects on bacterial production. In contrast to production, bacterial abundance only responded in a few cases (Fig. 3B). In June and August, P alone had a positive effect on bacterial abundance. In September, PZ had a negative effect on bacterial abundance, and PCZ had a positive effect.

*Phytoplankton*—Phytoplankton differed from bacteria in their response to the nutrient additions. N alone increased total phytoplankton biomass significantly in all five experiments, and the addition of N always resulted in the strongest effect (Fig. 3C). In May, N alone and P alone enhanced phytoplankton biomass. In July, N alone, P alone, and PN had positive effects. In August, additions of N alone and PN resulted in increased phytoplankton biomass. In addition, there were three significant higher-order interactions, which were small compared with the effects of N and PN. Zooplankton had significant (negative) effects only in May and September.

Chrysophyceae, Cryptophyceae, Prymnesiophyceae, and Bacillariophyceae had the strongest responses to the additions of N among the phytoplankton (Table 2). Cyanophyceae, which was dominated by the nitrogen-fixing genera *Gloeostrictia* and *Anabaena*, never responded to N alone but was stimulated by P or PN in July and August. Bacillariophyceae responded positively to P alone, N alone, and PN in May. From June to September, Bacillariophyceae responded in a way similar to that of Cyanophyceae, the main difference being that the former was also positively affected by N alone in July and August. Neither Cyanophyceae nor Bacillariophyceae responded to any of the treatments in June and September. Cryptophyceae, which was dominated by *Cryptomonas* spp. in May and by *Cryptomonas* spp. and *Rhodomonas lacustris* in the other experiments, always responded positively to N. Cryptophyceae was also the only class affected by Z alone, which reduced populations in May and September. Chrysophyceae, dominated by the mixotrophic genus *Dinobryon*, were only abundant in May and June. In May, both N alone and P alone had a positive effect, whereas Chrysophyceae did not respond to nutrients in June. Prymnesiophyceae (dominated by the potentially mixotrophic *Chrysochromulina parva*) responded only to N additions, whereas Dinophyceae (mainly *Ceratium hirundinella*) did not respond to any of the treatments.

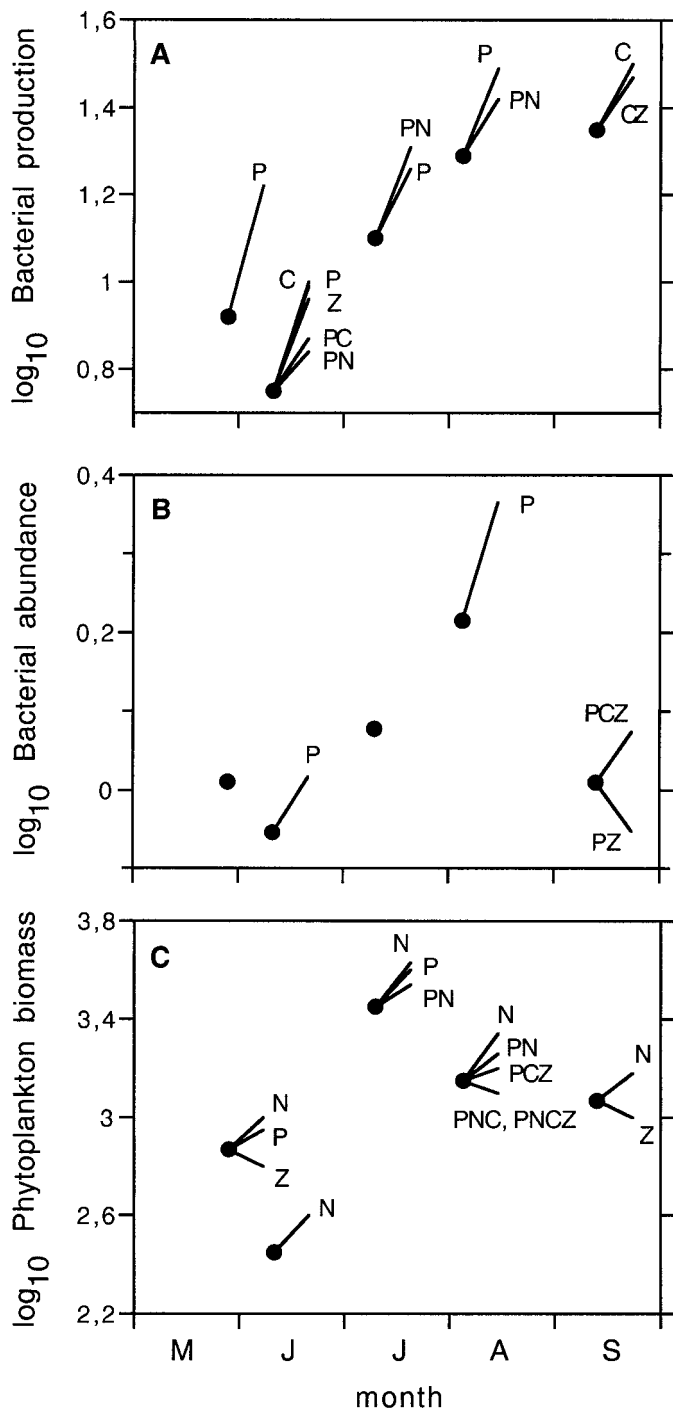


Fig. 3. Significant effects in the factorial experiments. Filled circle, average of all treatments on the stop day; (A)  $\log_{10}$  bacterial production (pmole  $L^{-1} h^{-1}$ ); (B)  $\log_{10}$  bacterial abundance ( $\times 10^9$  cells  $L^{-1}$ ); (C)  $\log_{10}$  phytoplankton biomass ( $\mu g$  WW  $L^{-1}$ ). Factors: phosphorus (P), nitrogen (N), organic carbon (C), and zooplankton (Z).

#### Lake study

Bacterioplankton abundance averaged  $1.2 \times 10^9$  cells  $L^{-1}$  and ranged from  $0.7$  to  $2.1 \times 10^9$  cells  $L^{-1}$  (Fig. 4A). Bac-

terioplankton production increased during spring and reached a maximum in late May (Fig. 4A). Thereafter it declined before reaching a second maximum in late June. From July to September bacterial production fluctuated, and in late September it declined. The Chl *a* concentration decreased during April and May and reached its minimum,  $1.6 \mu g L^{-1}$ , in mid-June (Fig. 4B). Thereafter, the Chl *a* concentration increased to a maximum of  $11.8 \mu g L^{-1}$  in early August. The average abundance of autotrophic picoplankton was  $3.9 \times 10^7$  cells  $L^{-1}$ , and its maximum,  $1.8 \times 10^8$  cells  $L^{-1}$ , occurred in late June (Fig. 4B). Primary production was low in April and May, increased in June, and reached its maximum in July (Fig. 4C).

The temperature of the upper circulating layer was low ( $<2^\circ C$ ) in April (Fig. 5A). In May it increased, and from late May to mid-September (the period when the experiments were carried out), the temperature varied between 15 and  $20^\circ C$ . The SRP concentration was below  $6 \mu g P L^{-1}$  from May to August (Fig. 5A). The  $NO_3^-$  concentration was approximately  $50 \mu g N L^{-1}$  in early April and decreased to below  $20 \mu g N L^{-1}$  in June and July, with a minimum of  $3 \mu g N L^{-1}$  in late July and early August (Fig. 5B). The  $NH_4^+$  concentration only occasionally reached levels higher than  $10 \mu g N L^{-1}$  during the period from April to mid-August (Fig. 5B). In late August and in September, concomitant with decreasing temperature and mixing of the lake, concentrations of SRP,  $NO_3^-$ , and  $NH_4^+$  increased to levels higher than those measured earlier in the summer.

Bacterial production, primary production, autotrophic picoplankton abundance, and water temperature were positively correlated with each other (Table 3). Neither bacterial abundance nor Chl *a* concentration was correlated with any of the other parameters. Partial rank correlation coefficients between bacterial production or bacterial abundance and primary production, Chl *a*, or autotrophic picoplankton abundance were all below 0.2 (Table 3). The highest partial correlations were those between primary production and temperature and between bacterial production and temperature. The partial correlation between Chl *a* and autotrophic picoplankton abundance was negative.

#### Discussion

*Effects of nutrients*—Bacterioplankton production was primarily limited by P availability in four of the five experiments (Fig. 3A). This shows that the P limitation of bacterioplankton in Lake Erken prevails during most of the growing season rather than being restricted to the spring bloom, as observed by Eriksson and Pedrós-Alió (1990). It was only in September, when ambient concentrations of inorganic P were high owing to mixing (Fig. 5A), that P did not limit bacterioplankton production. In July and August, P was primarily limiting, but P and N were close to colimiting bacterioplankton production. Organic C limited bacterioplankton production in June and September. The experiment in June was carried out during the early summer clearwater period (Fig. 4B). The C limitation could thus have been due to low release rates of organic compounds from the phytoplankton community (even though primary production was

Table 2. Significant ( $P < 0.02$ ) positive (+) and negative (-) effects on phytoplankton biomass ( $\log_{10}$ -transformed wet weight). Effects are sorted within cells in descending order with regard to absolute effect size. Effects: phosphorus addition (P), nitrogen addition (N), carbon addition (C), and zooplankton addition (Z).\*

Experiment	Cyanophyceae		Chrysophyceae		Cryptophyceae		Prymnesio- phyceae		Dinophyceae		Bacillariophyceae	
	+	-	+	-	+	-	+	-	+	-	+	-
May	ND	ND	N, P	NS	N	Z	ND	ND	ND	ND	P, N, PN	NS
Jun	NS	NS	NS	PNCZ	N	NS	ND	ND	ND	ND	NS	NS
Jul	P, PN	NS	ND	ND	N	NS	N	NS	NS	NS	N, P, PN	PC
Aug	PN	PNCZ	ND	ND	N	P, PC	ND	ND	ND	ND	N, PN	PNCZ
Sep	NS	NS	ND	ND	N, PNC, PNZ	Z, PCZ	ND	ND	ND	ND	NS	NS

\* ND, no statistical analysis performed (biomass <5% of total biomass); NS, no significant effect.

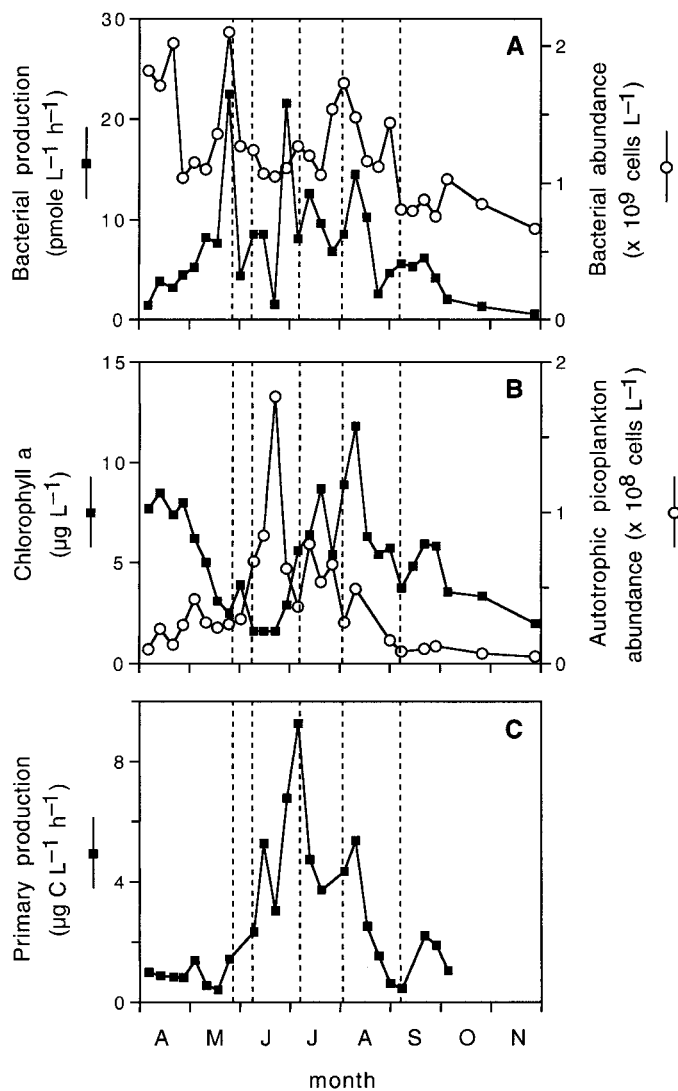


Fig. 4. Bacterial and phytoplankton parameters in the upper circulating layer of Lake Erken 1992. (A) Bacterial production and abundance; (B) Chl *a* concentration and abundance of autotrophic picoplankton; (C) primary production. Broken vertical lines indicate start dates of the experiments.

increasing). In addition, low phytoplankton biomass may have resulted in a relatively low release of organic compounds from metazoan zooplankton grazing. The other occasion on which C availability was found to limit bacterioplankton production was in September, after the onset of the autumn turnover, i.e., when the concentrations of both inorganic P and N were high (Fig. 5).

In contrast to bacterioplankton growth, phytoplankton growth was consistently limited by N (Fig. 3C). In May and July, P alone was also limiting for phytoplankton growth. Other studies in Lake Erken have shown that P availability

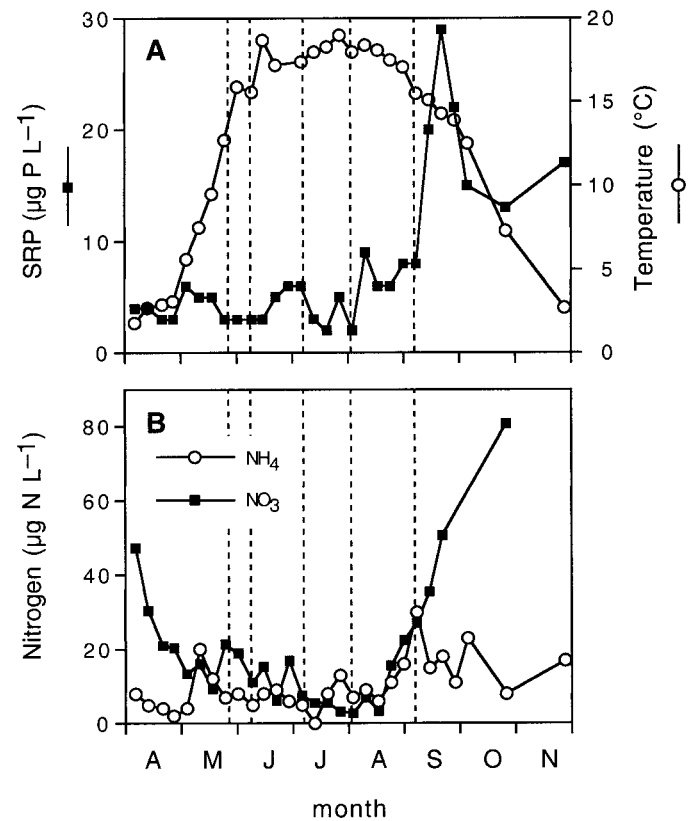


Fig. 5. Abiotic parameters in the upper circulating layer of Lake Erken in 1992. (A) Temperature and soluble reactive phosphorus (SRP) concentration; (B)  $\text{NH}_4$  and  $\text{NO}_3$  concentrations. Broken vertical lines indicate start dates of the experiments.

Table 3. Spearman rank correlation coefficients and rank partial correlation coefficients for bacterioplankton production (BP), bacterioplankton abundance (BA), primary production (PP), chlorophyll *a* concentration (Chl *a*), autotrophic picoplankton abundance (PICO), and temperature (TEMP) in the upper circulating layer in Lake Erken ( $n = 20$ ). Spearman rank correlation coefficients significantly different from 0 are indicated with \* ( $P < 0.05$ ), \*\* ( $P < 0.01$ ), or \*\*\* ( $P < 0.001$ ).

	Spearman rank correlation coefficients					Rank partial correlation coefficients				
	BP	BA	PP	Chl <i>a</i>	PICO	BP	BA	PP	Chl <i>a</i>	PICO
BA	0.12					0.31				
PP	0.47*	-0.01				-0.13	0.11			
Chl <i>a</i>	-0.03	0.22	0.12			-0.01	0.20	0.35		
PICO	0.45*	-0.03	0.67**	-0.23		0.10	0.06	0.48	-0.40	
TEMP	0.64**	-0.17	0.76***	-0.04	0.62**	0.53	-0.35	0.57	-0.06	0.08

can strongly limit phytoplankton production during the early stratification period, but, in general, the level of P limitation is only moderate to low in July and August (Istvánovics et al. 1992, 1994). In addition, the dominance of N<sub>2</sub>-fixing cyanobacteria in July and August indicates that P is not the main factor limiting primary production in the lake at the end of the summer. Among the phytoplankton groups, Cryptophyceae was always N limited. It has been suggested that cryptophytes are poor competitors for P and therefore can only dominate when the P supply is relatively good (Sommer 1983). This seems to contradict the observed N limitation, but the apparent contradiction could be explained if the N requirements of Cryptophyceae exceed their P demand.

In the present study, bacterioplankton production, which is a more sensitive measure of the bacterial community response compared with bacterial abundance (Pace 1993), responded much more strongly to the manipulations than bacterial abundance did. The generation time of bacterioplankton ( $g = \ln 2/r$ , where  $r$  is the specific growth rate, calculated as production divided by abundance using a conversion factor of  $2 \times 10^{18}$  cells produced per mole thymidine incorporated; Bell 1990) varied between 1.3 and 2.9 d in the lake at the start of the experiments. Thus, 3–4 d should have been long enough for bacterial abundance to respond to the nutrient manipulations. Furthermore, similar experiments have shown that both bacterioplankton and phytoplankton biomass and production are consistently able to respond to nutrient enrichment in 4-d experiments (Jansson et al. 1996). The weak response of bacterial abundance in the present study may be explained by grazing, because bacteria are effectively grazed by a wide range of organisms (heterotrophic and mixotrophic flagellates, ciliates, and some metazoan zooplankton, e.g., Sanders et al. 1989). Primary production (which was not measured in the experiments) is a more sensitive measure of phytoplankton community response compared with phytoplankton biomass. However, by removing the metazoan zooplankton in one parallel set of containers, the potential phytoplankton grazers were substantially reduced. Therefore, it can be concluded that the response of the phytoplankton community in these containers was mainly due to added nutrients.

Strong nutrient limitation of one or several species may not be detected in a community average, if most or all species are not limited by the same element (Sommer 1988). The degree of nutrient limitation may be exaggerated in enclosure experiments because nutrient entrainments from the

hypolimnion are cut off. Furthermore, if P, N, and organic C are supplied at different rates in the mixing processes, the system may be forced toward limitation by one or another nutrient. On the other hand, this effect is not likely to be important if more than one nutrient is limiting in the same experiment, as frequently was the case in the present study.

*Effects of zooplankton*—Zooplankton alone stimulated bacterioplankton production only in June (during the clear-water period), and the presence of zooplankton never resulted in increased bacterial abundance. Markager et al. (1994) showed that during the clearwater period, organic compounds released by zooplankton were an important C source for bacterioplankton. A positive effect of metazoan zooplankton could also be attributed to top-down control of planktonic bacterivorous protists (Brett et al. 1994; Stensdotter-Blomberg 1998), which may result in lower grazing pressure on bacterioplankton. This makes it difficult to identify the mechanism by which zooplankton enhanced bacterioplankton growth.

The presence of zooplankton resulted in reduction in total phytoplankton biomass in May and September. In both these cases, it was Cryptophyceae that responded negatively. Larger and less edible phytoplankton were neither grazed nor stimulated in the presence of grazers. The fact that neither bacterioplankton nor phytoplankton responded to zooplankton in August may be explained by the very low zooplankton biomass added in that experiment (Fig. 1). However, zooplankton had a negative effect on total phytoplankton biomass and the biomass of Cryptophyceae in May, when the zooplankton addition also was low. The situation in August was rather similar to that in July, and the lack of zooplankton effect may reflect the high level of adaptation of microbial communities to metazoan grazing during this period.

*Uncoupling between bacterioplankton and phytoplankton*—It can be concluded that bacterioplankton growth and phytoplankton growth were uncoupled, since the bacterioplankton community responded directly to nutrient additions and bacterioplankton growth was primarily limited by P on most occasions, while N always had the largest effect on phytoplankton. In laboratory experiments, Wang and Priscu (1994) concluded that bacterioplankton was indirectly stimulated by inorganic nutrients via phytoplankton when organic C was in short supply. The results from Lake Erken do not confirm this, because when bacterioplankton production

was primarily limited by organic C (June and September), the treatment in which phytoplankton responded differed from that in which bacterioplankton responded. If bacterioplankton were dependent on organic C from phytoplankton, one would have expected both bacteria and phytoplankton to respond in the same treatment. Furthermore, our results are in agreement with studies in which algae have been excluded or reduced in numbers through filtration (Toolan et al. 1991; Coveney and Wetzel 1992; Morris and Lewis 1992). In experiments with only bacteria present, Schweitzer and Simon (1995) found that the combination of P and organic C increased bacterial growth during the end of the phytoplankton spring bloom and during the following clear-water period, which is also consistent with our results. Pace (1993) and Le et al. (1994) reported that bacteria and phytoplankton differed in their growth responses to P and N additions. However, those studies were carried out on single occasions rather than during the entire growing season as in the present study.

Bacterioplankton could have been stimulated indirectly by autotrophic picoplankton, which were not included in the experimental analyses. However, the abundance of picoplankton was approximately one order of magnitude lower than in other lakes with similar total phosphorus concentrations (Stockner 1991), which indicates that they may not be important in Lake Erken. The highest abundance of autotrophic picoplankton occurred on a date with comparatively low primary production (Fig. 4B,C). Based on the observation that the abundances of autotrophic picoplankton were considerably lower on all other sampling dates in June, July, and August, coupled with the fact that primary production was higher on most of these occasions, we conclude that autotrophic picoplankton production did not account for a major part of the total primary production. It should also be noted that primary production was measured as total incorporation of  $^{14}\text{C}$ , thus including organic C produced by autotrophic picoplankton.

The experimental results indicating that bacterial and phytoplankton growth were uncoupled are strengthened by the correlation analysis in which bacterial and phytoplankton parameters were related to water temperature in the lake. Even though positive significant correlations were found between primary production, autotrophic picoplankton abundance, and bacterioplankton production in the Spearman rank correlation analysis, the partial correlations indicate that these relationships originate from correlations of these parameters with temperature. Coveney and Wetzel (1995) found a similar relationship between bacterial and phytoplankton production and temperature in oligotrophic Lawrence Lake. They concluded that the apparent coupling between phytoplankton and bacterioplankton was a result of similar responses by each component to external common regulating factors (primarily temperature and phosphorus), rather than being a consequence of a direct coupling. Furthermore, in a study of 36 North American lakes, Currie (1990) showed that P, as well as another factor (probably temperature), directly influenced both phytoplankton and bacterioplankton abundance. Jeppesen et al. (1997) found that bacterial parameters were coupled less strongly to Chl *a* and primary production in hypertrophic Lake Søbygård than in oligotro-

phic lakes. They argued that this reflected the fact that only a small part of the autochthonous primary production was channelized via bacteria in the hypertrophic system. We suggest that this lack of coupling between phytoplankton and bacterioplankton may also be common in less eutrophic systems.

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