

Beyond bulk properties: Responses of coastal summer plankton communities to nutrient enrichment in the northern Baltic Sea

Risto Lignell¹

University of Helsinki, Department of Limnology, P.O. Box 27, FIN-00014 Helsinki University, Finland

Jukka Seppälä, Pirjo Kuuppo, and Timo Tamminen

Finnish Environment Institute, Impacts Research Division, P.O. Box 140, FIN-00241 Helsinki, Finland

Tom Andersen

Norwegian Institute for Water Research, P.O. Box 173, N-0411 Oslo, Norway

Ingrid Gismervik

University of Oslo, Department of Biology, P.O. Box 1064, Blindern, N-0316 Oslo, Norway

Abstract

Enclosure experiments with all combinations of NH_4 and PO_4 treatments were carried out in the northern Baltic coastal zone during the post-spring bloom growth season. Empirical models were used to identify the important 3-d treatment effects on the state and process variables governing the main plankton compartments. Moreover, dilution experiments revealed the main nutrient sources for algal growth (external pools, intracellular stores, remineralization) and estimated phytoplankton growth and feeding loss rates. Maximal (nutrient-replete) algal community growth rates were $0.1\text{--}0.7\text{ d}^{-1}$. Small ($2\text{--}10\ \mu\text{m}$) algae grew fast ($0.5\text{--}1.5\text{ d}^{-1}$), whereas the abundant trichal blue-green algae grew very slowly. Ciliates showed a clear increase in units with positive algal response at minimum grazing estimates of $0.05\text{--}0.3\text{ d}^{-1}$. Initial inorganic N:P ratios were consistently low (<4 , wt/wt), and internal stores and remineralization were the most important nutrient sources for algae. During P-replete early summer, phytoplankton showed N limitation of biomass, rather than growth rate, in all (<2 , $2\text{--}10$, $>10\ \mu\text{m}$) size fractions. During the mineral N- and P-deplete late summer bloom of trichal blue-green algae, the positive P responses of chlorophyll *a*-normalized $^{14}\text{CO}_2$ fixation and growth rates indicated physiological P deficiency of the whole algal assemblage, but only a combined N and P addition evoked an increase in algal biomass. In early summer, low inorganic N:P ratios reflected N limitation, as commonly found in estuaries, but later, the indicative value of this ratio evaporated, evidently because of gaseous N_2 fixation by blue-green algae.

Because of the dynamic nature of coastal systems, eutrophication effects of nutrient discharges are immediately perceived in the plankton realm. A typical eutrophication monitoring program is focused on concentrations of dissolved and, more infrequently, particulate nutrients (C, N, P, and Si). These extensive programs often provide statistically relevant information for management of water resources, and the ratio of inorganic N to P in estuaries turns out to be a good predictor of limitation of both productivity and growth (e.g., see a recent broad overview in Anonymous 2000).

However, the problem with this approach is that planktonic algae are known to show appreciable growth in nutrient concentrations well below normal detection limits (Goldman et al. 1979), and the measured mineral nutrient pools can grossly overestimate those truly available for phytoplankton and bacterioplankton (Thingstad et al. 1993). Thus, concentrations and ratios of essential elements neither permit precise identification of the limiting nutrient nor allow quantification of the flow of nutrients through the plankton food web. Moreover, differentiation between physiological nutrient limitation of phytoplankton growth rate (Blackman 1905) and systemic (Liebig-type) biomass limitation is usually not conceivable because algal intracellular C:N:P:Si ratios are masked by those of detritus and herbivore pools (cf. Goldman 1980; Thingstad and Sakshaug 1990; Cullen et al. 1992).

Nutrient enrichment experiments with confined natural plankton communities meet these problems by measuring the net result of the network of anabolic and catabolic processes on individual, population, or community levels. Thus, factors controlling plankton systems are frequently studied with active experiments, including appropriate treatment of enclosed samples (e.g., nutrient additions and removal of large zooplankton by prescreening, see Hecky and Kilham 1988;

¹ Present address: Finnish Institute of Marine Research, P.O. Box 33, FIN-00931 Helsinki, Finland.

Acknowledgments

Sincere and honest thanks are due to Harri Kuosa for counting the phytoplankton samples and for his valuable comments on phytoplankton autecology. Veli-Matti Ollilainen and Andrei Tauber cooperated in pigment labeling analysis. The positive comments of Frede Thingstad and Seppo Niemelä are also acknowledged.

This study was conducted at the Tvärminne Zoological Station (University of Helsinki); it was included in the EU project BASYS (MAS3-CT96-0058) and was funded in part by EU projects COMWEB (MAS3-CT96-0052), and DANLIM (EVK3-CT2001-00049).

This study is a contribution of project PELAG.

Elser et al. 1990). With the proper factorial experimental design (with all combinations of treatments), empirical analysis of these experiments reveals the important factors that regulate the structure and function of the system, including the biologically often most relevant combined treatment effects (e.g., Lignell et al. 1992; Breitburg et al. 1999; Seppälä et al. 1999). What is lost in naturalism in the enclosure experiments (including nutrient flows mediated by eddy diffusion, advection, macrozooplankton, and higher trophic levels) is in turn gained by control of the external factors (most important, the introduction of clear physical boundaries, allowing mass balance calculations, comparison of methods, etc.).

Extrapolation of the treatment responses in enclosures to natural conditions presumes caution to a variable degree (e.g., Schindler 1988; Breitburg et al. 1999), but evidently unicellular plankton organisms with their short generation times are among the most meaningful objects in these short-term studies (Schindler 1988). Moreover, one can claim that understanding what is going on in enclosures is a prerequisite to understanding the functioning of complex natural plankton ecosystems. On the one hand, active enclosure experiments can be a fruitful testing ground for plankton ecological theories and, on the other hand, might provide response patterns, including strange or unexpected ones, to be met by the theory.

There is a concern that confinement of phytoplankton at ambient densities alters the nutrient supply regime naturally experienced by phytoplankton (inputs by water transport and remineralization) and, hence, that conventional enclosure experiments with undiluted sample water could overestimate the occurrence and severity of nutrient limitation *in situ* (Elser and Kimmel 1986). To meet the problems associated with sample confinement, Sommer (1988) and Sterner (1994) suggested that nutrient treatments should be applied to diluted (with 0.1–0.4 fraction of undiluted) sample water. They argued that this approach would prevent enclosed phytoplankton from depleting ambient nutrients and yield a more realistic estimate of the growth status of prevailing algal assemblage. However, Elser and Frees (1995) showed that the interpretation of these “single point dilution experiments” is not straightforward. They found changes in the primary limiting element, depending on the dilution, and they argued that these shifts occurred because algal growth rates responded differently to single N and P additions, depending on the relative importance of internal stores, remineralization, and the external pool for the two nutrients.

To overcome the confusion caused by the undefined role of different phytoplankton nutrient sources in enrichment bioassays, Andersen et al. (1991) developed a modification of a dilution experiment used by Landry and Hassett (1982), which was then used as a basis for the above argument of Elser and Frees (1995). The theoretical framework of the Andersen modification allows identification of three main sources of mineral nutrients to support algal growth during incubation: (1) an external free-mineral nutrient pool, (2) intracellular stores of algae, and (3) mineral nutrients recycled via mineralization by zooplankton.

Schindler (1988) carried out extensive investigations covering temporal and spatial scales, from short-term bottle in-

cubations to multiannual experiments that included whole lakes and their drainage areas, showing that ecosystem- and community-level variables (e.g., total standing stocks, production, and respiration) are much more robust in plankton ecosystems at meeting external stress than the biotic structure and functioning of the food webs themselves. To understand in more detail the structural and functional responses of coastal Baltic plankton communities to nutrient load, we combined enclosure experiments (nutrient enrichment of <100- μm prescreened samples) with dilution experiments (Landry and Hassett 1982), which again followed the modification of Andersen et al. (1991, *see above*). Moreover, the time courses of inorganic and organic nutrients, size-fractionated chlorophyll *a* (Chl *a*), and biomasses of main autotrophic and heterotrophic compartments, as well as the processes governing them, were followed for 3 d. In particular, dilution experiments allowed estimation of feeding pressure on phytoplankton and maximum (N- and P-replete) algal growth rates (Landry and Hassett 1982). These directly measured growth rate values (based on net Chl *a* change over the dilution gradient) were compared with those recorded by the pigment labeling method (Redalje and Laws 1981). Finally, a sharper picture was provided by rates of change of particle size groups in dilution bottles.

Materials and methods

The study area was located in the outer coastal zone off the southwest coast of Finland in the northern Baltic Sea. Local physical and chemical characteristics and the succession of autotrophic and heterotrophic plankton, as well as the main processes governing plankton, are well documented (e.g., Niemi 1975; Kivi 1986; Lignell 1990; Kivi et al. 1993; Heiskanen et al. 1998 and references therein). Upwellings of nutrient-rich deep water (generated by moderate northwest winds, persisting for 2–3 d) typically occur in the Tvärminne area in summer (Niemi 1975). The area is not directly affected by large sewage outlets.

In 1997, water was sampled from the surface in a free-floating boat in the open sea 2 km south of the southernmost island, Långskär, in the Tvärminne archipelago (59°47'N, 23°19'E). Prescreened (100- μm plankton net) samples were placed in 200-liter transparent plastic bags, which were mounted inline on a floating pontoon in a bay at nearby Tvärminne Zoological Station (University of Helsinki). The enclosures were covered with plastic, and extra units at both pontoon ends ensured that all units received similar solar irradiation. Nutrient enrichment experiments (NE), lasting for 3 d, were conducted four times: twice during the summer minimum period (2–5 and 16–19 June), once during the blue-green algal bloom (14–17 July), and once during the early autumn upwelling (15–18 September). Each NE experiment was supplemented with six 24-h dilution (DIL) experiments.

The nutrient limiting phytoplankton growth was studied in NE experiments using all combinations of mineral N and P treatments (pulsed additions at the start or on Day 0: C unit, control [no additions]; N unit, N addition; P unit, P addition; NP unit, N and P additions) or a 2² factorial design

Table 1. Design of the 3-d nutrient enrichment experiments. Nitrogen ($80 \mu\text{g NH}_4\text{-N L}^{-1}$; N_{ad}) and phosphorus ($20 \mu\text{g PO}_4\text{-P L}^{-1}$; P_{ad}) were added in all combinations to enclosures ($<100\text{-}\mu\text{m}$ prescreened surface samples) at the start of the experiment (Day 0). C, control unit (no additions); NP/2, center point replication (50% of N_{ad} and P_{ad}).

Enclosure	N addition	P addition
C	0	0
N	N_{ad}	0
P	0	P_{ad}
NP	N_{ad}	P_{ad}
NP/2a	$N_{\text{ad}}/2$	$P_{\text{ad}}/2$
NP/2b	$N_{\text{ad}}/2$	$P_{\text{ad}}/2$

with center point replication (NP/2; Table 1). Silicon was not included in these investigations because Si concentrations remain consistently high ($\sim 100 \mu\text{g SiO}_2 \text{L}^{-1}$) after vernal blooms of diatoms and dinoflagellates in our study area. Accordingly, we did not find any appreciable Si effect in a 3-week nutrient enrichment study carried out in mesocosms in summer (unpubl. data).

The enclosures were sampled at 0700–0800 h by taking surface samples after careful mixing of the upper 1 m of the water column (total enclosure depth ≈ 1.5 m). The temporal development of nutrients (inorganic and organic), size-fractionated Chl *a* and $^{14}\text{CO}_2$ fixation, bacterial productivity and biomass, picoalgae (cyanobacteria and picoeucaryotes), large ($2\text{--}100 \mu\text{m}$) algae, heterotrophic nanoflagellates, and ciliates were followed daily (from Day 0 to Day 3, four samplings altogether).

Algal growth rates and feeding pressure, as well as algal nutrient sources, were estimated with the dilution method. On Day 0, prescreened $<100\text{-}\mu\text{m}$ and $<20\text{-}\mu\text{m}$ (minimum period) or $<40\text{-}\mu\text{m}$ (bloom and autumn) DIL samples were taken from the control unit. After that, DIL samples were taken from N and P units on Day 2 and from C and NP units on Day 3 (cf. Table 1). On Days 2 and 3, the same water samples were used for NE and DIL measurements and incubations, except for the bloom experiment, where extra $<40\text{-}\mu\text{m}$ prescreening was applied to DIL samples in an attempt to get rid of clumps of trichal blue-green algae (*but see Results*). DIL samples were also used to estimate algal growth rates from time courses of particle size groups (over the entire dilution gradient) and by the pigment-labeling method (undiluted samples).

Primary productivity—Algal $^{14}\text{CO}_2$ fixation was measured by the ^{14}C method (Steemann Nielsen 1952). $\text{NaH}^{14}\text{CO}_3$ ($1 \mu\text{Ci}$; specific activity of the undiluted stock = $57.8 \text{mCi mmol}^{-1}$, C14 Centralen, VKI, Hørsholm, DK) was added to 20-ml glass scintillation vials. Duplicate light bottles were taken from each enclosure, and duplicate dark bottles were taken from C and NP units. The in vitro incubations (in situ temperature, irradiance $\approx 80 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) were started at 1000–1100 h and lasted ~ 6 h.

Total $^{14}\text{CO}_2$ fixation (particulate plus dissolved organic ^{14}C) was measured by allowing an acidified 4-ml subsample ($\text{pH} < 2$) to stand open for 24 h (no bubbling) before taking radioactivity measurements (Niemi et al. 1983). Algal $^{14}\text{CO}_2$

fixation was measured in whole and in $<10\text{-}$ and $<2\text{-}\mu\text{m}$ prescreened samples (by gravity, acid-washed polycarbonate filters, Poretics).

The radioactivity measurements were performed using HiSafe III scintillation cocktail (Wallac) and an LKB Wallac Rackbeta 1215 liquid scintillation counter with the external standard channel ratio method. In all ^{14}C primary productivity calculations, the dark $^{14}\text{CO}_2$ fixation values were subtracted from the corresponding light values. Dissolved inorganic carbon was measured with an infrared carbon detector (Elektro-Dynamo carbon analyzer).

Bacterial productivity—Net bacterial productivity was measured from tritiated thymidine incorporation (TTI) into material precipitable with cold trichloroacetic acid (TCA) (Fuhrman and Azam 1982). A saturating (10nmol) concentration of ^3H -thymidine (specific activity $38\text{--}44 \text{Ci mmol}^{-1}$, Amersham) was added to 10-ml scintillation vials (duplicate living samples and one formalin-killed blank from each enclosure). The TTI samples were incubated for about 1 h at in situ temperature; they were started simultaneously with the ^{14}C incubations and terminated with formalin ($200 \mu\text{l}$ 38% formalin to a 10-ml sample). Subsamples (5 ml) were extracted for 0.5–1 h with 5 ml 10% TCA in ice-cold conditions and then filtered on $0.2\text{-}\mu\text{m}$ cellulose acetate filters (Sartorius). The filters were rinsed with 5% ice-cold TCA before radioactivity measurements with Insta Gel scintillation cocktail.

Net bacterial productivity was calculated using mean bacterial cell volumes of $0.063 \mu\text{m}^3$ (R. Autio pers. comm.), a ^3H -thymidine conversion factor of $1.1 \times 10^{18} \text{cells mol}^{-1}$ (Riemann et al. 1987), and a carbon content of $0.35 \text{pg C } \mu\text{m}^{-3}$ (Bjørnsen 1986).

Biomass determinations—Large ($>2 \mu\text{m}$) algae and ciliates were preserved with acid Lugol's solution (final concentrations ~ 0.3 and 2%, respectively). The samples were counted according to the method of Utermöhl (1958) using phase contrast microscopy. Nanoflagellates and picosized ($<2 \mu\text{m}$) cyanobacteria and eukaryotes were preserved with unbuffered glutaraldehyde (final conc. 1%) and enumerated with an epifluorescence microscope. Nanoflagellates were stained with proflavine (Haas 1982), and they were counted with an epifluorescence microscope; heterotrophic and autotrophic organisms were distinguished on the basis of the red autofluorescence of Chl *a* (Davis and Sieburth 1982). Bacteria were preserved with formaldehyde (final conc. 1%) and stained with acridine orange (Hobbie et al. 1977).

Wet weight values were transformed to carbon values using a coefficient of 0.35 for bacteria (Bjørnsen 1986), 0.22 for flagellates (Børsheim and Bratbak 1987), 0.19 for ciliates (Putt and Stoecker 1989), 0.2 for $<2\text{-}\mu\text{m}$ picoalgae, and 0.1 for large algae. The nomenclature of phytoplankton follows Edler et al. (1984).

Nutrients and Chl a—Dissolved ammonium-N, nitrate-N, total N, and phosphate-P were determined according to Grashoff et al. (1983). In particulate organic carbon (POC), phosphorus (POP), and nitrogen (PON) measurements, all filtration glassware and Whatman GF/F glass fiber filters

were acid-washed and precombusted (4 h at 500°C). The POC and PON filters were dried and stored at room temperature until analysis with a Heraeus CHN-O-RAPID analyzer. Total, particulate, and dissolved (less than GF/F, <GF/F) organic phosphorus fractions were measured spectrophotometrically after wet oxidation according to Solorzano and Sharp (1980). Dissolved (<GF/F) organic N was measured according to Grasshoff et al. (1983).

Chl *a* was filtered on Whatman GF/F glass fiber filters (100-ml duplicate samples), extracted in 96% ethanol for 24 h in darkness at room temperature (Jespersen and Christoffersen 1987), and measured fluorometrically (Shimadzu RFPC 5001; calibrated with pure Chl *a*, Sigma).

Dilution experiments—In DIL experiments, the initial samples were first prescreened (<100 μm, but <40 μm in the bloom experiment) to remove large zooplankters and clumps of trichal blue-green algae. These samples were then diluted with particle-free (<0.2-μm tangential flow filtration of <20-μm prescreened samples) sample water to form a linear gradient (nine equal 10% dilution steps ranging from the undiluted sample to a 90% dilution sample; altogether 10, 1-liter acid-washed polycarbonate bottles). Three dilution bottle sets (10 bottles in each) with different nutrient treatments were used (single N, single P, and combined N and P additions; the same additions as used in enclosures; Table 1). The bottles were incubated for ~24 h in a rotating wheel (1 rpm, in situ temperature, 18:6 light:dark [LD] period, irradiance ~100 μmol photons m⁻² s⁻¹).

The Chl *a* response patterns (algal net growth) over the dilution gradients were used for estimation of maximal (N- and P-replete) algal growth rates, the feeding pressure on algae (Landry and Hassett 1982), and the nutrient sources for algal growth (Andersen et al. 1991).

Apparent (net) algal growth rate (m_x) in each dilution bottle was calculated from the starting and endpoint Chl *a* concentrations (Chl *a*(x , 0) and Chl *a*(x , t), respectively) as

$$m_x = \frac{1}{t} (\ln[\text{Chl } a\{x, t\}] - \ln[\text{Chl } a\{x, 0\}]) \quad (1)$$

where t is the incubation time and x is the fraction of undiluted sample.

Algal net growth rate (m_x) in dilution bottles was determined by the balance between average algal growth rate over the incubation period ($\bar{\mu}(x, t)$) and grazing losses (g). Grazing rate (product of grazer abundance and clearance rate) is again assumed to be constant; hence, it is only dependent on the dilution factor (x). Thus, we get

$$m_x = \bar{\mu}(x, t) - x \cdot g \quad (2)$$

When the m_x values over the dilution gradient ($x = 10$ –100%) are plotted against x , using the bottle set with the combined N and P addition, the slope of the regression line gives $-g$, whereas its constant term gives a maximal algal growth rate under prevailing conditions (“infinite” dilution, or no grazing pressure).

Sources for algal nutrients (remineralization, internal stores, external free pool) were again identified from the two dilution bottle sets with single N or P addition. Patterns in the m_x response over the dilution gradient were used to

agnose the sources of the nutrient not added to the bottle set through the following principles. Remineralization depends on the encounter frequency of protozoans and their algal prey; hence, it increases in a quadratic fashion with decreasing dilution (i.e., the numbers of both predators and prey increase along with increasing x). The effect of intracellular stores on m_x increases again linearly with decreasing dilution, whereas the effect of the ambient free-mineral nutrient pool is independent of dilution. After further formal arrangements, the importance of the three different nutrient sources in evoking the realized m_x patterns was extracted by multiple linear regression analysis, as outlined by Andersen et al. (1991).

Particle counts—The number of particles and their size distribution were measured with an Elzone particle counter (Particle Data Europe) from the DIL bottles, including the initial undiluted samples and, after 24 h of incubation, the DIL bottle set (dilution gradient) with combined N and P addition (NP series). A 38-μm-diameter orifice was used to enumerate smaller particles from 1.9 to 28.2 μm. The measurements in the upper range were excluded, however, because these samples were prescreened through a 20-μm net to prevent blockage of the orifice. A 190-μm-diameter orifice was used for larger particles from 14.2 to 67.4 μm (no extra prescreening). Both orifices gathered data with 128 channels, following a logarithmically evenly spaced diameter distribution. In both cases, the 19 smallest channels contained mostly noise and were excluded. To smooth the response pattern and improve its precision (increase the number of counts), particle counts in eight adjoining channels were always summed together to give a response of size groups over the channels.

The maximum growth rate for each size group was calculated by fitting a linear regression to the relationship between apparent growth rates and NP dilution series according to Landry and Hassett (1982). Those size groups with zero counts in one or more dilution levels were excluded from the analysis.

Phytoplankton pigment labeling—Phytoplankton growth rates also were estimated in DIL experiments with the pigment labeling method introduced by Redalje and Laws (1981). Undiluted replicate samples with combined N and P treatments (as in the DIL experiments; *see above*) were spiked with NaH¹⁴CO₃ (50 μCi in 1-liter samples) and incubated in a rotating wheel simultaneously with the DIL samples. After that, the samples were filtered onto Whatman GF/C filters and extracted in ethanol for 2–3 d at –20°C. Then the pigment extract was stored (without filter) under N₂ at –80°C.

The separation/purification protocol of the ¹⁴C-labeled Chl *a* (¹⁴C-Chl *a*) pigment followed the procedure outlined by Goericke and Welschmeyer (1993). First, the ¹⁴C-Chl *a* was separated from the pigment matrix with high-performance liquid chromatography (HPLC, Hewlett Packard 1090), then the ¹⁴C-Chl *a* was converted to pheophytin *a* and subsequently rechromatographed on the HPLC system (to get rid of possible colorless ¹⁴C lipid contaminants). The pigment concentration in the samples was measured spectropho-

metrically by the diode array detector (DAD) of the HPLC against pure standards. The pigment samples were collected from the outlet of the HPLC, and their radioactivity was measured with a Wallac Winspectral 1410 liquid scintillation counter. Phytoplankton growth rates were calculated from the C-specific radioactivity of ^{14}C -pheophytin *a* according to Goericke and Welschmeyer (1993).

Statistical analysis of treatment responses—The nutrient (N and P) treatments followed a 2^2 factorial design with center point replication (altogether six experimental units; Table 1). The statistical analysis of important treatment effects was done by fitting polynomial curves to the temporal responses with orthogonal regression analysis (see Lignell et al. 1992; Seppälä et al. 1999 and references therein) on normalized data ($\log[x + 1]$ transformation, except for $\arcsin\sqrt{p}$ transformations with percent values and square root transformations with bacterial growth rates; $p > 0.9$, Wilk-Shapiro normality test).

The experimental design allowed us to construct a regression model for each variable in which all single and combined effects of the N and P treatments were included (altogether three regression coefficients plus the mean of the responses; Box and Draper 1987). In turn, the center point replication allowed calculation of an independent estimate of the experimental error variance (F -criteria; Khuri and Cornell 1987).

The enclosures were sampled four times at uniform (1-d) intervals, which resulted in a 4×2^2 experimental design with center point (NP/2) replication. This allowed the effect of each treatment (C, N, P, NP, and NP/2; Table 1) to be represented by its mean and three orthogonal polynomials of degree 1–3, describing the response over time (Draper and Smith 1981). That of degree 1 is the best fitting linear regression for the temporal effect of the treatment, whereas those of degree 2 and 3 represent the improvement in fit achieved by including the quadratic and third-degree (oscillating) temporal terms. This is demonstrated in Fig. 1B, where the main treatment effect (here, N addition on Chl *a*) was extracted as an important regression coefficient representing the combination of N treatment and the linear time term (Nt). Thus altogether, 19 orthogonal regression coefficients (plus the mean of the responses) were obtained for the model. The significance of the variance-normalized regression coefficients was tested using F -statistics ($F_{1,1}$, $p < 0.10$).

The limited degrees of freedom included in our experimental design ($F_{1,1}$) resulted in a conservative test for the significant effects (yet, assuming normal distribution, the experimental error might be large at random). Thus, the important coefficients extracted with F -criteria were compared with those determined with normal probability plots (Box and Draper 1987). The latter method is often a more robust approach because it deals with the main treatments only (center point replicates are excluded), giving simpler models for identification of the currency running the system (i.e., limiting nutrient).

Because of the orthogonality of the experimental matrix (Table 1), the regression coefficients, representing main treatments, are independent. Those corrected contrasts (var-

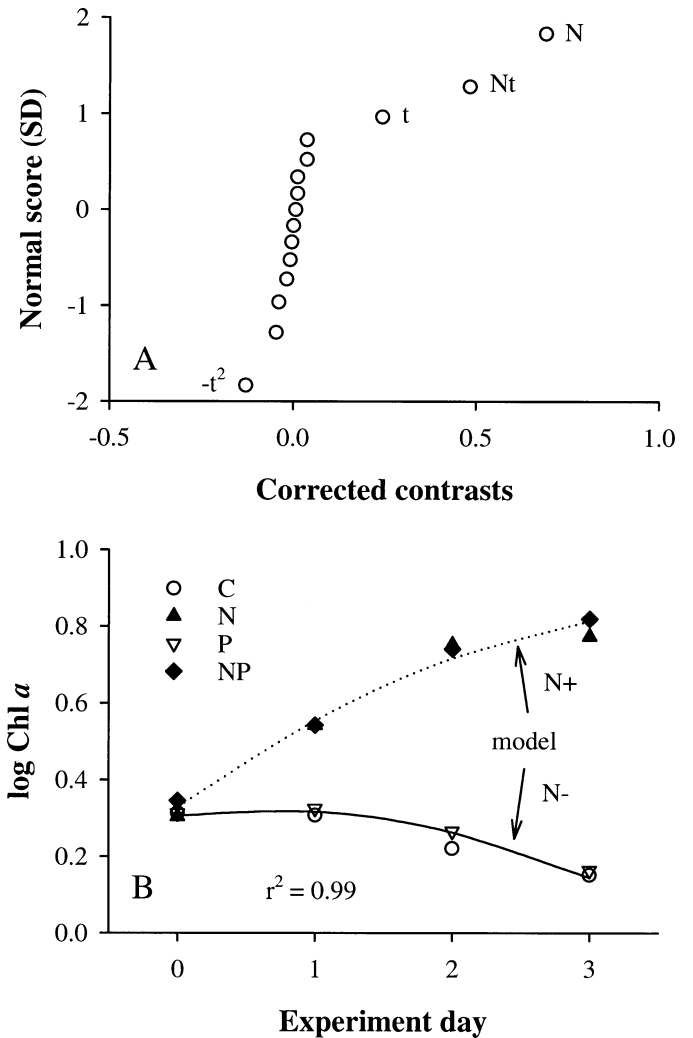


Fig. 1. (A) Estimation of important treatment effects by normal probability plots and (B) the corresponding regression model fit to observations (Chl *a* response with an important temporal N effect in mid-June 1997). Those variance-normalized regression coefficients (corrected contrasts), included in experimental noise, form a straight line, whereas important treatment effects deviate from this line. The clear N effect (N and NP units) is extracted here as an important coefficient for the combination of the linear time term and N treatment (Nt). Treatment design is in Table 1.

iance-normalized regression coefficients) that follow normal distribution (i.e., the corresponding effects are small and included in experimental noise) form a straight line on the normal probability plot, whereas those coefficients that deviate from this line represent important treatment effects (Fig. 1A). In borderline cases, those coefficients whose contribution to the information and adequacy of the regression model was small were disregarded (also, a diagnostic check was applied to the residuals). The resulting empirical models (polynomial response curves) were then examined to identify the important nutrient treatment effects on the state and process variables governing the plankton communities (Box and Draper 1987).

Table 2. Starting dates and initial values of temperature (°C), salinity (‰) and nutrient ($\mu\text{g N}$ or P L^{-1}) and Chl *a* ($\mu\text{g L}^{-1}$) concentrations in 3-d enclosure experiments with pulsed nutrient enrichment in Tvärminne, off the southwest coast of Finland. The experiments were carried out during periods of summer minimum, blue-green algal bloom, and autumn upwelling, POC, PON, POP = particulate organic C, N, and P, respectively.

Date	Period	Temperature	Salinity	Nutrient ($\mu\text{g L}^{-1}$)						
				NH ₄	NO ₃	PO ₄	POC	PON	POP	Chl <i>a</i>
2 Jun 97	Minimum	12.0	5.8	2.4	1.6	6.3	701	114	22	4.3
16 Jun 97	Minimum	16.6	5.4	1.4	1.0	6.7	417	75	10	2.0
14 Jul 97	Bloom	18.4	5.1	1.5	1.7	1.0	882	160	16	7.7
15 Sep 97	Autumn	14.0	6.2	44.5	30.2	20.5	426	76	11	4.2

Results

Initial conditions—On 2 and 16 June, the experiments took place during the early summer minimum period following the decline of the spring bloom. The surface concentrations of mineral N and Chl *a* were low, whereas free mineral P was still relatively abundant (Table 2). Picosized ($<2 \mu\text{m}$) cyanobacteria and the trichal blue-green alga *Aphanizomenon flos-aquae* (L.) Ralfs ex Borneo et Flahault clearly predominated in the early summer phytoplankton communities (Table 3).

During the bloom of trichal blue-green algae (*Anabaena lemmermannii* P. Richter and *A. flos-aquae*), elevated Chl *a* and particulate organic nutrient concentrations were found

(Tables 2, 3). By then, the ambient PO₄ pool had also declined close to the detection level. In turn, the autumn experiment took place during an upwelling, when strong north-west winds had removed the surface water layer, giving way to nutrient-rich and more saline deep water with a low mineral N:P ratio. This event was associated with a diverse phytoplankton community, where picocyanobacteria, cryptophytes, and dinoflagellates predominated. Unfortunately, two enclosures (NP and NP/2a) started to leak after Day 0, and they were not sampled further.

Fate of nutrient treatments—During the summer minimum period, N additions were exhausted from the enclosures within 3 d, whereas appreciable mineral P depletion

Table 3. Predominant algal species and total algal biomass by treatment and day of sampling in Tvärminne nutrient enrichment experiments. Samples were prescreened to $<100 \mu\text{m}$, except on Days 2 and 3 in the bloom experiment, where $<40\text{-}\mu\text{m}$ samples were extrapolated to $<100 \mu\text{m}$ by corresponding Chl *a* fractions. In autumn, the NP/2 treatment enclosure instead of the NP enclosure was sampled on Day 3. ND, not determined. Other explanations in Tables 1 and 2.

Period (date)	Predominant species	Total biomass (%)				
		C Day 0	N Day 2	P Day 2	C Day 3	NP Day 3
Minimum (2 Jun 97)	<i>Aphanizomenon flos-aquae</i>	64	32	42	43	33
	<i>Dinophysis acuminata</i>	9	24	17	14	16
	<i>Mesodinium rubrum</i>	2	3	5	3	10
	<i>Synechococcus</i> spp. ($<2 \mu\text{m}$)	20	35	34	36	35
	Total biomass ($\mu\text{g C L}^{-1}$)	170.6	174.4	84.2	80.1	176.7
Minimum (16 Jun 97)	<i>A. flos-aquae</i>	19	14	21	29	16
	Chrysophytes and prymnesiophytes	11	11	16	19	9
	<i>Eutreptiella gymnastica</i>	0	7	0	0	13
	Small (2–10 μm) cells	6	16	11	5	6
	<i>Synechococcus</i> spp. ($<2 \mu\text{m}$)	46	35	28	33	27
Total biomass ($\mu\text{g C L}^{-1}$)	70.3	145.8	59.5	46.9	171.6	
Bloom (14 Jul 97)	<i>Anabaena lemmermannii</i>	33	ND	37	18	26
	<i>A. flos-aquae</i>	23	ND	30	26	13
	Chrysophytes and prymnesiophytes	4	ND	2	13	3
	<i>Monoraphidium contortum</i>	1	ND	5	7	14
	<i>Synechococcus</i> spp. ($<2 \mu\text{m}$)	19	ND	18	12	9
Total biomass ($\mu\text{g C L}^{-1}$)	188.8	ND	202.1	63.5	93.3	
Autumn (15 Sep 97)	<i>A. flos-aquae</i>	12	6	12	23	15
	<i>Heterocapsa rotundata</i>	5	15	12	13	8
	<i>Heterocapsa triquetra</i>	8	7	6	6	0
	Cryptophytes	17	19	20	5	20
	Chrysophytes and prymnesiophytes	4	4	7	8	6
	<i>Pyramimonas</i> spp.	4	9	3	2	7
	<i>Synechococcus</i> sp. ($<2 \mu\text{m}$)	22	21	24	21	20
Total biomass ($\mu\text{g C L}^{-1}$)	39.5	98.5	54.2	23.2	64.1	

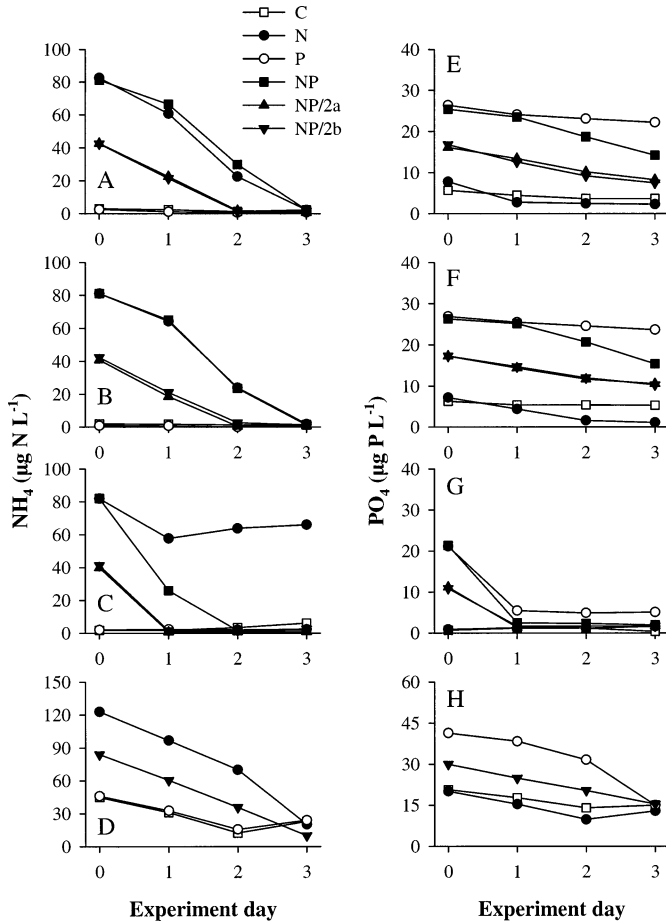


Fig. 2. Time courses of mineral nutrients (NH_4 and PO_4) in 3-d enclosure experiments with nutrient enrichment off the southwest coast of Finland in 1997. The experiments were carried out during the periods of summer minimum (A, E: 2 June; B, F: 16 June), blue-green algal bloom (C, G), and autumn upwelling (D, H). Other explanations are in Tables 1 and 2.

took place only in units with N addition (Fig. 2). In contrast, during the blue-green algal bloom, the single P addition was virtually exhausted within 1 d; in the NP unit, the added P was depleted faster than N as well. At the same time, only 20% of the single N addition was used within 3 d.

In autumn, the upwelling of deep water led to high initial

mineral N and P concentrations in the surface layer (Table 2). These large free-mineral nutrient pools evoked efficient depletion of both single N and P additions in the enclosures. It is noteworthy, however, that by Day 2, the NH_4 -N and PO_4 -P concentrations in the control unit and in respective P and N units seemed to have declined to their minimum or “background” levels ($10\text{--}15 \mu\text{g L}^{-1}$), since no further decline took place by Day 3 in these enclosures (Fig. 2). Moreover, on Day 3, similar background NH_4 -N and PO_4 -P concentrations were recorded in all enclosures. At the same, the NO_3 concentrations remained high in all enclosures (initially $30 \mu\text{g N L}^{-1}$; Table 2), showing an equal decrease of $\sim 10\%$ within 3 d.

Chl *a*-normalized ammonium depletion rates over Days 0–1 (N debt) ranged from 3.8 to $8.5 \mu\text{g N } (\mu\text{g Chl } a)^{-1} \text{ d}^{-1}$ in N units (Table 4). During the minimum and autumn periods, the corresponding phosphate depletion rates (P debt) were about $1 \mu\text{g P } (\mu\text{g Chl } a)^{-1} \text{ d}^{-1}$ in P units. During the blue-green algal bloom, P debt was clearly higher ($2.6 \mu\text{g P } [\mu\text{g Chl } a]^{-1} \text{ d}^{-1}$), and even this value probably underestimated the P depletion potential because phosphate was exhausted within the first 24 h in P units (Fig. 2).

Sedimentation losses of N were estimated as the rate of decrease in total N concentrations over the 3-d experiments in enclosures. They were consistently low (a few percentage per day) irrespective of treatment or study period (Table 4). P sedimentation losses were correspondingly estimated from total P depletion, except in the bloom experiment, when more accurate (but more scarce) estimates based on depletion of summed total dissolved P and POP were used because of the poor quality of total P data. In general, P sedimentation losses were also small, but they tended to be higher than those of N.

The initial dissolved organic nitrogen (DON) pools were $260\text{--}280 \mu\text{g N L}^{-1}$, or 2.5–3.5 times as high as the corresponding particulate (PON) pools (cf. Table 2). In summer and autumn, the dissolved organic phosphorus (DOP) pools were $18\text{--}24$ and $7 \mu\text{g P L}^{-1}$, respectively, being 0.7–1.8 times as high as the corresponding particulate (POP) pools. Net changes in DON and DOP pools were usually relatively small and inconsistent, but in the bloom experiment, a consistent DON increase of $20\text{--}35 \mu\text{g N L}^{-1}$ took place in all units after Day 0. Nutrient treatment effects were manifested in corresponding PON and POP responses analogously with those of Chl *a* (data not shown, cf. Fig. 4).

Table 4. Chl *a*-specific depletion rates of ammonium (N debt, $\mu\text{g N } [\mu\text{g Chl } a]^{-1} \text{ d}^{-1}$) and phosphate (P debt, $\mu\text{g P } [\mu\text{g Chl } a]^{-1} \text{ d}^{-1}$) over Days 0–1 in respective N and P units; average (\pm SD) mineral nutrient depletion ratios (N:P depl., by weight) in N- and P-replete samples over Days 0–1; and sedimentation losses over Days 0–3 in all units. In the bloom experiment, mineral P was exhausted from both P and NP units within 1 d (corresponding P debt and N:P depletion values are probably underestimates), and P sedimentation was estimated from the sum of total dissolved P and particulate organic P. Other explanations in Tables 1 and 2.

Date	Period	N debt ($\mu\text{g N } [\mu\text{g Chl } a]^{-1} \text{ d}^{-1}$)	P debt ($\mu\text{g P } [\mu\text{g Chl } a]^{-1} \text{ d}^{-1}$)	N:P depl. (wt/wt)	Sedimentation losses	
					N (% total d^{-1})	P (% total d^{-1})
2 Jun 97	Minimum	4.5	0.6	6.2(1.7)	2(1)	7(2)
16 Jun 97	Minimum	6.1	0.7	9.1(3.5)	2(1)	2(1)
14 Jul 97	Bloom	3.8	2.6	2.9(—)	2(1)	6(1)
15 Sep 97	Autumn	8.5	1.1	5.5(0.4)	5(3)	11(6)

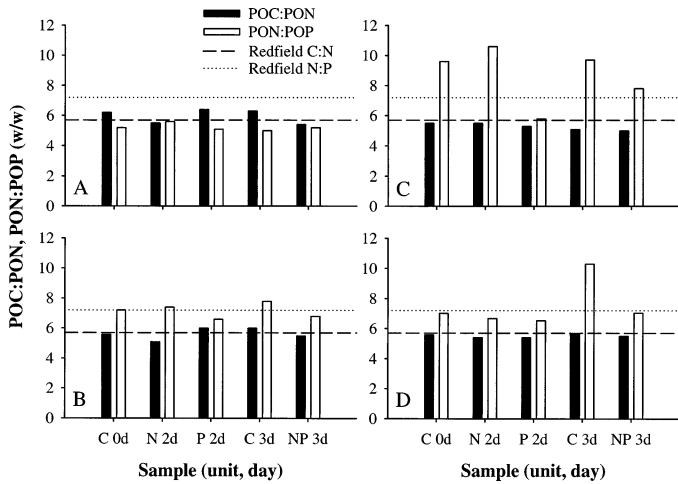


Fig. 3. Particulate organic nutrient (C, N, and P) ratios (by weight) in 3-d enclosure experiments with nutrient enrichment off the southwest coast of Finland. Dashed and dotted lines are the respective Redfield C:N and N:P ratios. The experiments were carried out during the periods of summer minimum (A: 2 June; B: 16 June), blue-green algal bloom (C), and autumn upwelling (D). Samples are given by experimental unit treatment and day. Other explanations are in Tables 1 and 2.

The initial particulate organic C:N ratios (POC:POP, wt/wt) were close to the Redfield ratio in all experiments (5.7; Redfield et al. 1963; Fig. 3). This seemed to be a robust feature because, irrespective of the treatment, POC:POP ratios also remained close to the Redfield ratio in enclosures over all four 3-d experiments (Fig. 3).

During the summer minimum period, the PON:POP ratios varied. In early June, they were consistently relatively low in all enclosures (5.0–5.6), whereas in mid-June, they were reasonably close to that of Redfield (7.2; Fig. 3). During the blue-green algal bloom, the initial PON:POP ratio was relatively high (10.3 and 9.6 in total and <40- μ m samples, respectively). The PON:POP ratios also remained at a high level in units without P addition, whereas in P and NP units, this ratio dropped to 5.8 and 7.8, respectively (Fig. 3). During the autumn upwelling, the PON:POP ratios were again close to that of Redfield, except for the high value (10.3) in C unit, on Day 3.

In both minimum period experiments, the initial mineral N:P depletion ratios (from Days 0–1) were, in general, reasonably close to that of Redfield in N- and P-replete units (Table 4). As an exception, in mid-June, this depletion ratio was 14 in the NP unit. During the bloom, when the initial PON:POP ratios were relatively high, the initial mineral N:P depletion ratio (2.9) was clearly below the Redfield ratio in the NP enclosure. At the same time, both mineral N and P concentrations were exhausted from the replicate NP/2 enclosures within 1 d; thus, these units were not included in the calculation of the N:P depletion ratio.

Biomass responses to nutrient enrichment—During the summer minimum period, the phytoplankton communities were clearly N-limited in both experiments (Table 3; Fig. 4), as was confirmed by fitting polynomial curves to the nutrient

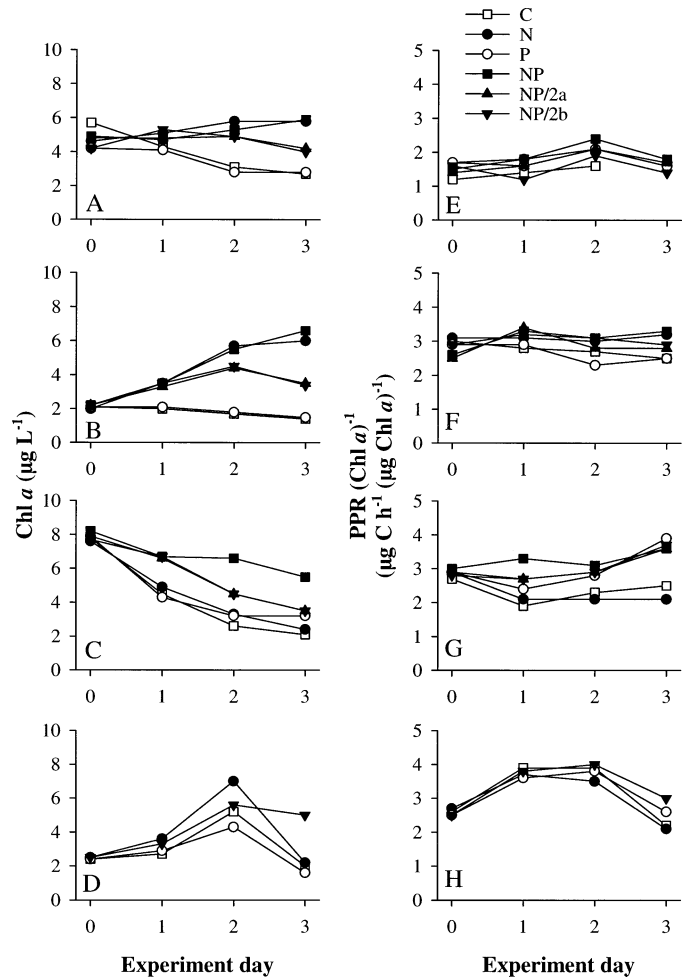


Fig. 4. Time courses of total Chl *a* and Chl *a*-normalized $^{14}\text{CO}_2$ fixation rate ($\mu\text{g C L}^{-1} \text{h}^{-1} [\mu\text{g Chl } a \text{ L}^{-1}]^{-1}$) in 3-d enclosure experiments with nutrient enrichment off the southwest coast of Finland. Other explanations are in Tables 1 and 2 and Fig. 2.

treatment responses of Chl *a* by orthogonal regression analysis (Table 5). This result is in accordance with preferential mineral N depletion compared to that of P in the enclosures (Fig. 2).

On 2 June, *A. flos-aquae* predominated (64% of total algal biomass); subsequently, its biomass declined during the 3-d study in all enclosures (Table 3). In contrast, 2 weeks later, *A. flos-aquae*, accounting for 19% of the initial total algal biomass, showed consistent biomass over 3 d in all enclosures (Table 3). This evidently explains the difference in the overall temporal Chl *a* development patterns in these experiments. In early June, a sharper Chl *a* decline took place in the C unit and, accordingly, N treatment evoked a smaller net increase in Chl *a*. Thus, the important treatment effects were diagnosed as the Chl *a* difference between enclosures with and without N addition (Fig. 4; cf. Fig. 1B).

In early June, the initial proportion of picoalgal (<2 μm) biomass was 20% of the total, and the poor overall success of *A. flos-aquae* was compensated for by almost a doubling of the share of picoalgae in the enclosures (Fig. 5; Table 3). At the same time, small (2–10 μm) nanoalgae benefited from

Table 5. Important responses of plankton communities to nutrient treatments during different periods of phytoplankton growth in 1997. The effects of nutrient additions (all combinations of mineral N and P additions) were followed for 3 d in prescreened ($<100 \mu\text{m}$) enclosed surface samples. The effects are given in the order of their quantitative importance, and they were extracted using orthogonal regression analysis of the 4×2^2 experimental design. Single time terms (t) represent “bag effects” common to all enclosures, whereas the combination of the nutrient (N, P or both) and first degree time effect (e.g., Nt) is interpreted as an important nutrient treatment effect (trend). Second and third degree temporal effects (t^2 and t^3) represent improvements of the polynomial fit by mimicking quadratic (parabolic) and oscillating patterns, respectively. The main effects were identified with normal probability plots (Fig. 1), and the r^2 value below is the coefficient of determination for the corresponding polynomial regression model. Effects in *italics* are missing data from Day 3 (3×2^2 design). PPR, $^{14}\text{CO}_2$ fixation; HNF, heterotrophic nanoflagellates.

Variable	Main effects (r^2)			
	Minimum 2 Jun 97	Minimum 16 Jun 97	Bloom 14 Jul 97	Autumn 15 Sep 97
Total Chl <i>a</i> ($\mu\text{g L}^{-1}$)	Nt, -t (0.92)	Nt, t, - t^2 (0.99)	-t, NPt, t^2 (0.98)	- t^2 , - t^3 (0.76)
Total PPR ($\mu\text{g C L}^{-1} \text{ h}^{-1}$)	Nt (0.91)	Nt, t (0.97)	t^2 , -t, Pt (0.96)	- t^2 , - t^3 (0.83)
$<2\text{-}\mu\text{m}$ PPR ($\mu\text{g C L}^{-1} \text{ h}^{-1}$)	t, Nt, NPt (0.84)	Nt, -N t^2 , -t (0.97)	t^2 , -t (0.91)	- t^2 , - t^3 (0.59)
$<2\text{-}\mu\text{m}$ PPR (% total PPR)	t (0.77)	-t, t^2 , -Nt (0.97)	t^2 , -t, -Nt (0.90)	t^2 (0.59)
2–10- μm PPR ($\mu\text{g C L}^{-1} \text{ h}^{-1}$)	- t^2 , -N t^2 , Nt (0.98)	Nt, -N t^2 , - t^2 , t (0.99)	-t, t^2 , Pt (0.91)	- t^2 , - t^3 (0.82)
2–10- μm PPR (% total PPR)	None (0.00)	Nt, t^3 (0.81)	t, NPt (0.42)	- t^2 (0.73)
$>10\text{-}\mu\text{m}$ PPR ($\mu\text{g C L}^{-1} \text{ h}^{-1}$)	Nt, -t, - t^2 , -N t^2 (0.95)	t, Nt, N t^2 (0.91)	t^2 , -t, Pt (0.98)	-t, - t^2 , NPt (0.82)
$>10\text{-}\mu\text{m}$ PPR (% total PPR)	t^2 , -t (0.86)	t, N t^2 , NPt 3 (0.72)	N t^2 (0.38)	t^2 (0.50)
Total PPR (Chl <i>a</i>) $^{-1}$	t, NPt (0.53)	Nt (0.71)	t^2 , Pt (0.82)	- t^2 (0.89)
Bacterial biomass ($\mu\text{g C L}^{-1}$)	- t^2 (0.74)	t^2 , -t, -NPt 3 (0.86)	<i>None</i> (0.00)	<i>None</i> (0.00)
Bacterial productivity ($\mu\text{g C L}^{-1} \text{ h}^{-1}$)	- t^2 , t (0.76)	Nt (0.76)	t^3 , - t^2 , -t (0.94)	- t^2 , t (0.62)
Bacterial growth rate (h^{-1})	N t^2 , t, Pt (0.66)	<i>None</i> (0.00)	- t^2 (0.92)	<i>None</i> (0.00)
HNF biomass ($\mu\text{g C L}^{-1}$)	Data missing	<i>None</i> (0.00)	-t (0.75)	<i>None</i> (0.00)
Ciliate biomass ($\mu\text{g C L}^{-1}$)	t, Nt (0.78)	t, Nt (0.91)	t, - t^2 (0.92)	- t^3 (0.43)

N treatment at the expense of larger ($>10 \mu\text{m}$) algae. In contrast, in mid-June, the high initial shares (46%) of picoalgae declined in all enclosures, whereas $>10\text{-}\mu\text{m}$ algae (including *Eutreptiella gymnastica* Thronsen and chrysophytes) increased their shares. Again, N treatment seemed to favor small nanoalgae, judging from their smaller proportions in contemporary units without N additions (Fig. 5).

During the bloom, phytoplankton biomass exhibited a decreasing trend in all units (Fig. 4). As in early June, the biomass of trichal blue-green algae approximately halved by Day 3 (Table 3). Only the combined addition of N and P evoked a clear Chl *a* response (Chl *a* difference between NP and C units, Table 5), evidently reflecting the low ambient concentrations of both mineral N and P (Table 2). Nevertheless, the single P addition was virtually exhausted within 1 d (Fig. 2), with no concomitant positive response in biomasses of autotrophs (Fig. 4) or heterotrophs (Figs. 6, 7). This indicated a luxury P uptake in P units, which was also reflected in a marked decrease of 3.8 (wt/wt) units in the PON:POP ratio (Fig. 3).

In autumn, both single N (Day 2) and combined N and P additions (Day 3) seemed to evoke a positive Chl *a* response (Fig. 4) despite high ambient mineral N and P concentrations. However, the adequate ($r^2 \approx 0.8$) polynomial regression model that mimicked the Chl *a* responses did not extract any important treatment effects (Table 5).

No clear biomass responses to nutrient treatments were recorded with bacteria or heterotrophic nanoflagellates (Figs. 6, 7; Table 5). In contrast, ciliates showed similar, though damped and lagged, responses with phytoplankton to the nutrient treatments (Fig. 7). Moreover, during the bloom, when the initial ciliate biomass was exceptionally high, picoalgae and nanosized autotrophs and heterotrophs declined sharply in all units during the 3-d study, whereas this was not the case with bacteria (cf. Figs. 4–7; Table 5).

Process responses to nutrient enrichment—In general, total $^{14}\text{CO}_2$ fixation and Chl *a* showed similar responses (Table 5). The only exception to this was the bloom experiment, where the single P treatment was the only one to evoke an

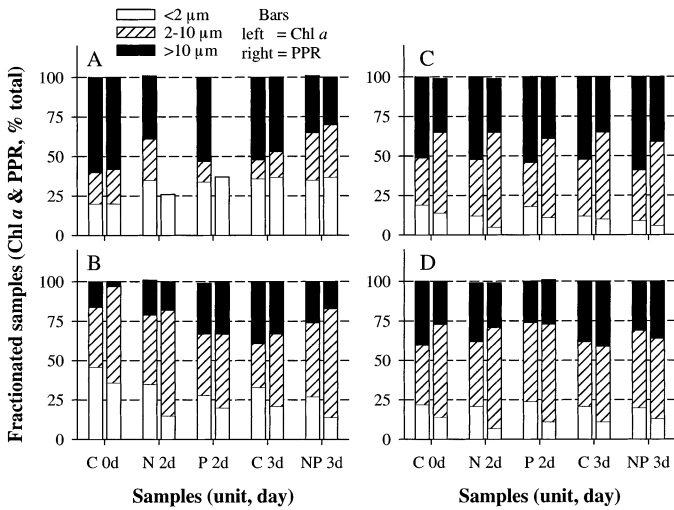


Fig. 5. Size distribution of Chl *a* and phytoplankton ¹⁴CO₂ fixation (PPR; from whole sample and the <10- and <2-μm pre-screened samples) in 3-d enclosure experiments with nutrient enrichment off the southwest coast of Finland. Other explanations are in Tables 1 and 2 and Fig. 3.

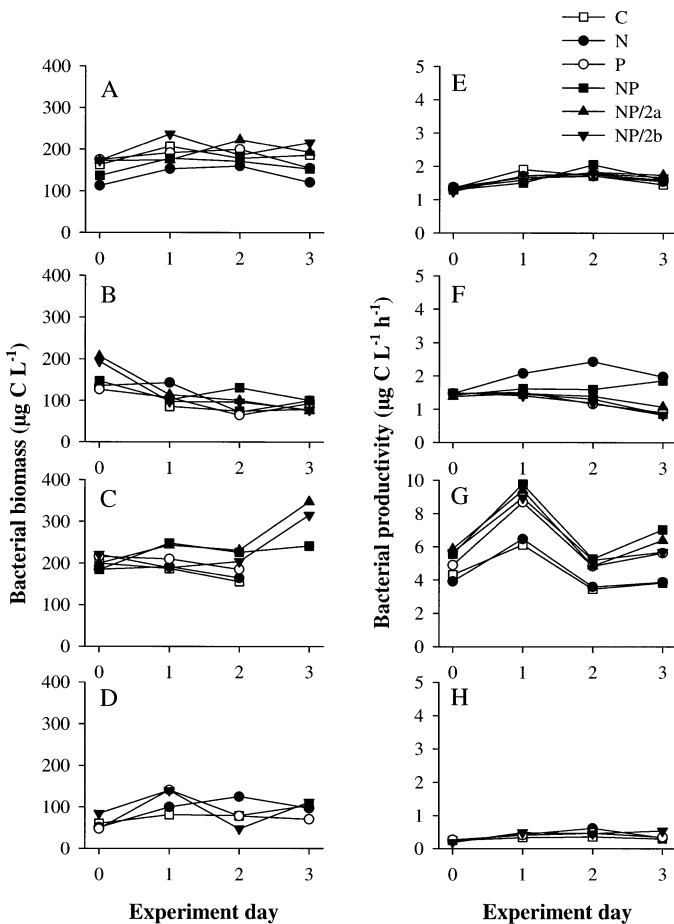


Fig. 6. Time course of bacterioplankton biomass and net productivity (³H-thymidine incorporation) in 3-d enclosure experiments with nutrient enrichment off the southwest coast of Finland. Other explanations are in Tables 1 and 2 and Fig. 2.

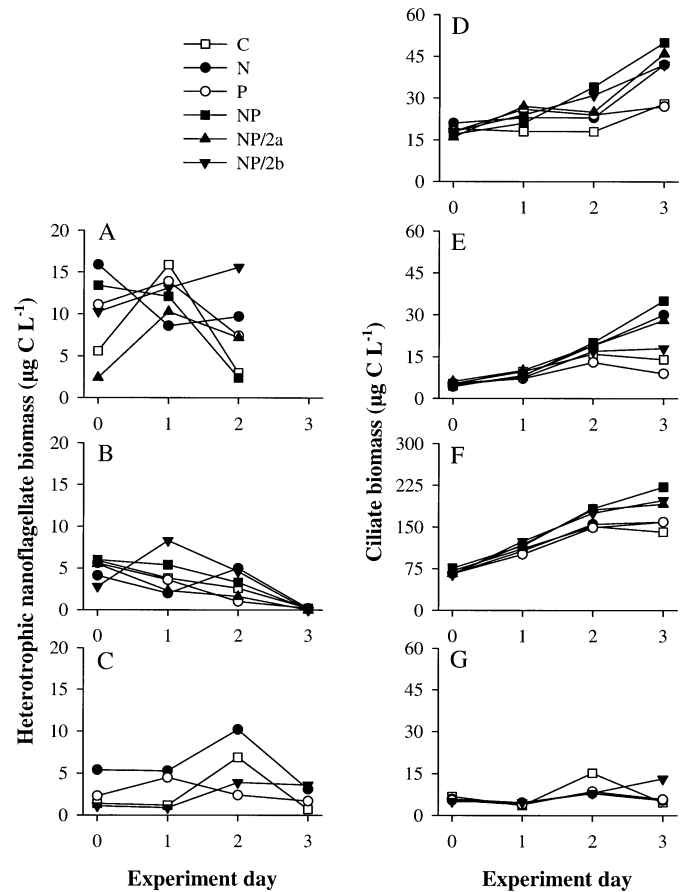


Fig. 7. Time courses of the biomass of nanosized (2–20 μm) heterotrophic flagellates and ciliates in 3-d enclosure experiments with nutrient enrichment off the southwest coast of Finland. Data originates from the periods of summer minimum (D: 2 June; A, E: 16 June), blue green algal bloom (B, F), and autumn upwelling (C, G). Other explanations are in Tables 1 and 2.

important effect on ¹⁴CO₂ fixation, whereas a combined N and P addition was needed for a significant Chl *a* response (Table 5). Accordingly, during the blue-green algal bloom, the Chl *a*-normalized ¹⁴CO₂ fixation rate (ratio of ¹⁴CO₂ fixation to Chl *a*) increased significantly by Day 3 in units with P treatment (Fig. 4; Table 5). Similar significant responses of 30–50% increases in Chl *a*-normalized ¹⁴CO₂ fixation evoked by P treatment (vs. the contemporary unit without P addition) were found in >10- and <10-μm size fractions (all standard deviation-normalized differences or responses >2), suggesting that the whole algal assemblage was P-limited (data not shown). In the minimum period and autumn experiments, these values were not affected by the nutrient treatments.

The most active phytoplankton compartment in all experiments was the small (2–10 μm) nanoalgae, showing in all enclosures consistently higher percent ¹⁴CO₂ fixation values (of total) than their corresponding percent Chl *a* shares (experimentwise, 1.2–1.7-fold higher on average; cf. Fig. 5; *p* < 0.001, paired *t*-test, *n* = 18). In general, ¹⁴CO₂ fixation in <2-, 2–10-, and >10-μm size fractions showed similar responses as corresponding total values (Table 5). Accord-

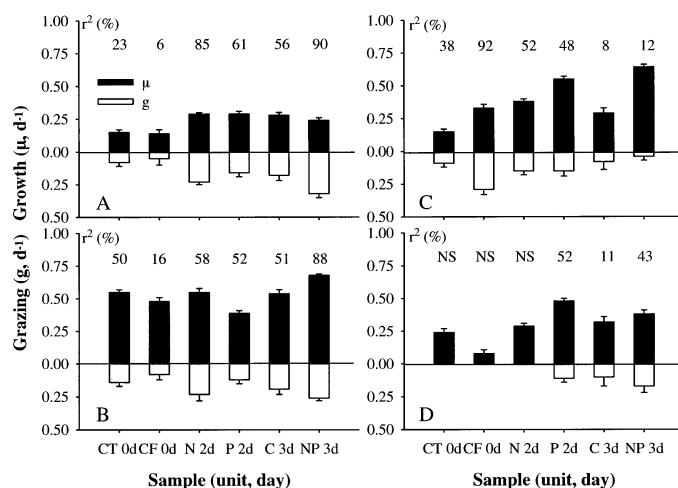


Fig. 8. Maximal (N- and P-replete) phytoplankton growth rates and protozooplankton feeding rates on algae in 3-d enclosure experiments with nutrient enrichment off the southwest coast of Finland. Both processes were estimated by linear regressions fitted to the net algal biomass (Chl *a*) response over a dilution gradient (Landry and Hassett 1982). Error bars + SE; on Day 0, both the <100- μ m (T) and <20- μ m (minimum period) or <40- μ m (bloom, autumn) prescreened (F) samples were studied. Other explanations are in Tables 1 and 2 and Fig. 3.

ly, except for the apparent positive N effect on the share of $^{14}\text{CO}_2$ fixation in the 2–10- μ m size fraction in mid-June, no quantitatively important nutrient treatment effects on the percent $^{14}\text{CO}_2$ fixation in different size fractions were evident. However at times, there were clear temporal trends in the proportions of size-fractionated $^{14}\text{CO}_2$ fixation concerning all enclosures (i.e., bag effects; Table 5).

During the minimum period, bacterial productivity was 1–2 $\mu\text{g C L}^{-1} \text{h}^{-1}$, whereas during the bloom and autumn upwelling, these values were ~ 5 and 0.3 $\mu\text{g C L}^{-1} \text{h}^{-1}$, respectively (Fig. 6). Only in mid-June did bacterial productivity appear to be affected by the nutrient (N) treatments, but even then, no important biomass or growth rate (productivity per biomass) responses to treatments were recorded (Table 5; cf. Fig. 5).

Phytoplankton growth rates—Maximal (N- and P-replete) phytoplankton growth rates obtained with the dilution method (μ_{DIL} ; Landry and Hassett 1982) varied between 0.1 and 0.7 d^{-1} during the study, with highest rates during the summer minimum period (mid-June; Fig. 8). In general, μ_{DIL} seemed to be higher on Days 2 and 3 than on Day 0, possibly reflecting selection of the species thriving in the enclosed communities, as exemplified by the decline of trichal blue-green algae in the early June and bloom experiments (Table 3; see below).

The microprotozoan grazing rate (g) varied between 0.05 and 0.3 d^{-1} when estimated from the slope of realized net Chl *a* change over dilution gradients (Fig. 8). These grazing rates were highest in the summer minimum experiments, and N treatments (with their positive phytoplankton response) seemed to evoke elevated g values.

The algal N- and P-replete growth rate estimates obtained

by the dilution (Landry and Hassett 1982) and pigment labeling methods (the latter one in undiluted samples, Redalje and Laws 1981; Goericke and Welschmeyer 1993) fit together reasonably well. The latter method gave, on average, $\sim 20\%$ smaller values (respective means \pm SD in all dilution experiments $0.41 \pm 0.16 \text{ d}^{-1}$ and $0.34 \pm 0.15 \text{ d}^{-1}$; $p < 0.08$, $n = 11$, paired *t*-test). In most cases, the two methods gave similar results, two presently unexplainable outliers accounting for most of the difference in the mean values.

For comparison, algal net growth rate values were calculated for N- and P-replete enclosures, in which nutrient treatments evoked a clear positive Chl *a* response within 3 d (Fig. 4; Eq. 1, contemporary control enclosures were used as a reference). In general, these values, ranging from 0.3 to 0.6 d^{-1} , were similar to those found with the other two methods. The NP unit in the bloom experiment seemed to be the only exception, with a rate of Chl *a* net change of $\sim 50\%$ of that recorded with the dilution method. However, the pre-screening treatments were exceptionally different between enclosures and DIL samples (<100 μm and <40 μm , respectively). About half of the trichal blue-green biomass was retained on 40- μm filters. Because the predominant trichal blue-green algae did not show appreciable growth in enclosures or DIL bottles (Table 3; Fig. 9C), their lower share in DIL samples evidently resulted in higher biomass-specific algal growth rates than those found in enclosures.

The initial maximal growth rates of different size groups were recorded with the particle counter from their time courses in nutrient (N and P)-replete DIL samples. In summer, nanosize groups (studied with the small orifice, covering a range of 2–14 μm) in initial samples showed similar maximal growth rates with a range from 0.5 to 1.5 d^{-1} (Fig. 9). In autumn, the nanoalgal growth rates were lower (around 0.5 d^{-1}). In the experiments with trichal blue-green algae as the predominant species (early June and bloom), microsize groups (14–60 μm) showed clearly lower maximal growth rates than nanosize groups (0–0.5 d^{-1}), whereas in the other two experiments, nano- and microsize growth rates were more similar (Fig. 9).

Nutrient sources for algal growth—The dilution experiments showed that internal stores were frequently the most important P source for phytoplankton growth under mineral P-replete conditions, including the summer minimum experiments and all units with single P treatment (Fig. 10). In mid-June, the samples from P units on Day 2 seemed to be an exception to this. However, actual analysis by the multiple linear regression on net growth (m_x) response over the N-replete dilution gradient gave almost as good a fit for internal stores as for recycling for the dominant algal P source (cf. Andersen et al. 1991). It is hence possible that both sources were important, especially since remineralization also was a significant P supply in early June.

Internal stores and recycling via remineralization were the most important N sources for algae. Interestingly, in the N-deplete conditions of the summer minimum period, N stores also were important in units without N addition. Unfortunately, in several cases, our data were too noisy for identification of algal nutrient sources in the autumn experiment.

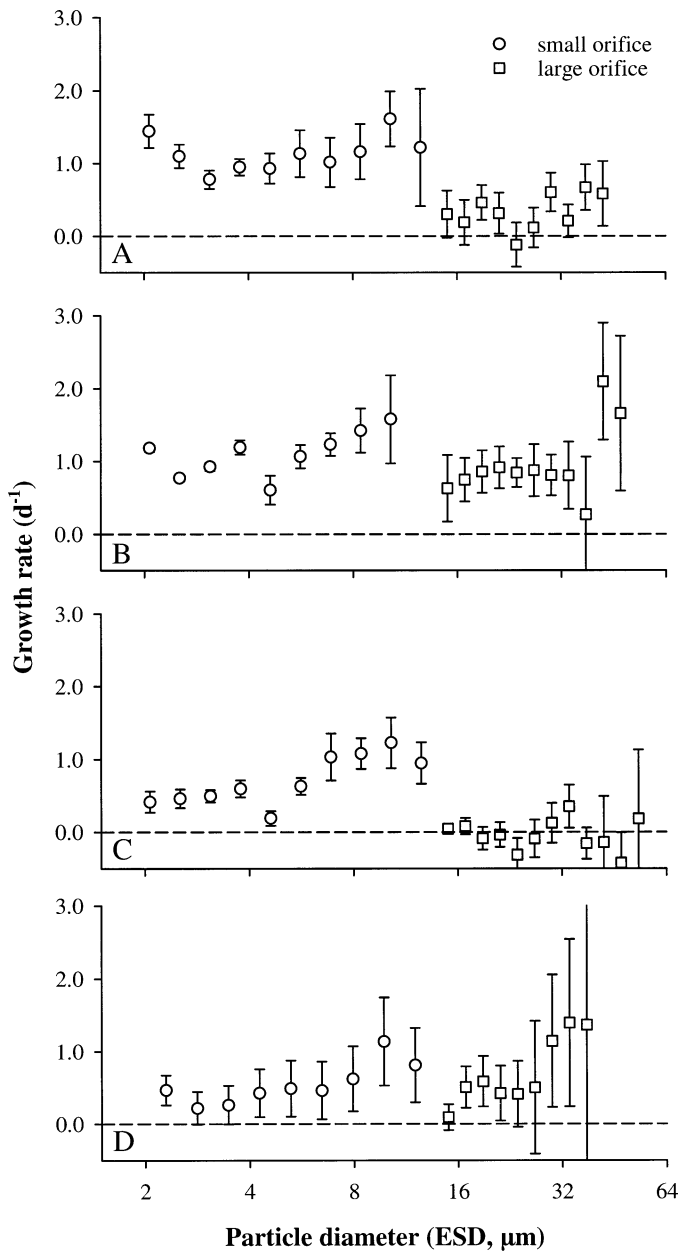


Fig. 9. Maximal (N- and P-replete) rates of change of particle size groups in initial plankton communities off the southwest coast of Finland. Nanosized (2–14 μm) particles were counted with the small orifice, and microsized (14–70 μm) particles were counted with the large orifice. The rates of change over a dilution gradient were estimated by a linear regression (Landry and Hassett 1982). Note the logarithmic scale of the x-axis; error bars, 95% C.L. Other explanations are in Tables 1 and 2 and Figs. 3 and 8.

Statistical considerations—Normal probability plots (NPP) extracted the same main nutrient treatment effects (Table 5) as those identified with F -criteria in orthogonal regression analysis. The only exception to this was the positive ciliate biomass response to combined N and P treatment in the bloom experiment (Fig. 7). NPP gave an excellent polynomial fit ($r^2 = 0.92$) with single time terms only (t and $-t^2$), mimicking the overall bag effect or ciliate biomass

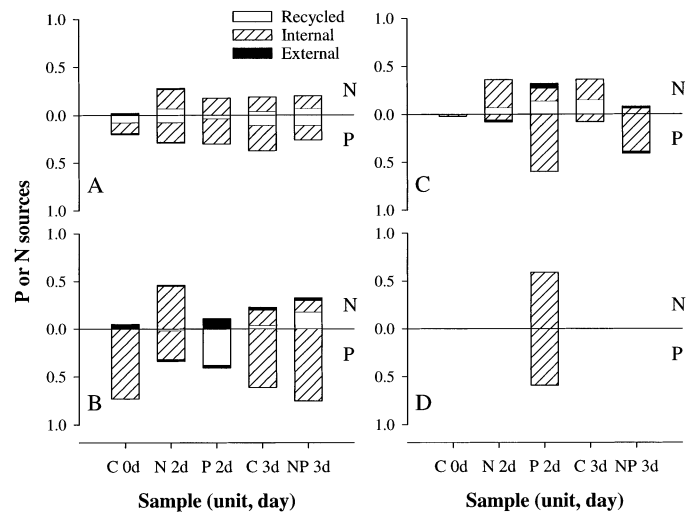


Fig. 10. Nutrient sources for phytoplankton growth (external, internal, and recycled supplies) in 3-d enclosure experiments with nutrient enrichment off the southwest coast of Finland. The relative contributions of these sources (scaled to initial Chl a) to potential production (grazing-corrected net growth in undiluted samples) were estimated by a stepwise multiple linear regression on N- or P-replete dilution gradients (revealing P or N sources, respectively; Andersen et al. 1991). Samples were prescreened ($<100 \mu\text{m}$), except for the $<40\text{-}\mu\text{m}$ prescreening on Days 2 and 3, during the bloom (C). Upward and downward bars denote N and P sources, respectively. Other explanations are in Tables 1 and 2 and Fig. 3.

increase in all units. However, the F -criterion suggested that the $\sim 50\%$ increase in ciliate biomass by Day 3 in NP units (vs. units without combined N and P addition) was significant as well. We also have found earlier that the polynomial curves fitted by the NPP method to the 3-d experiments are not always able to mimic clear responses that first emerge at the end (on Day 3; Lignell et al. 1992).

In some cases, treatment effects found important with NPP were not confirmed with F -criteria (Table 5). In some cases, this indeed might have reflected excessive noise in the data (e.g., possibly caused by prefractionation of $^{14}\text{CO}_2$ fixation samples). However, often the most likely explanation for this discrepancy was the conservative $F_{1,1}$ -criterion, in turn reflecting the limited degrees of freedom in our experimental design. On the other hand, in several cases, the F -criterion included so many terms in the regression model that the model evidently started to explain the noise in the data along with the treatment effects. We decided to use NPP as a more robust approach to identify the main effects, which were then confirmed by the F -criteria.

Discussion

Our study covered the three major scenes (early summer minimum period, late summer blue-green algal bloom, autumn upwelling) characterizing the post-spring bloom season of phytoplankton growth in the northern Baltic coastal zone. The summer minimum experiments took place during the period of low mineral N and Chl a concentrations (Table 2). Picosized ($<2 \mu\text{m}$) cyanobacteria and the trichal blue-

green alga *A. flos-aquae* clearly predominated the phytoplankton communities (Table 3). In early summer, *A. flos-aquae* is often abundant in our study area. This time, contrary to their late-summer blooms, they do not possess heterocysts (sites for anoxic N₂ fixation), evidently reflecting the good availability of mineral N in winter and early spring (H. Kuosa pers. comm.).

Contrary to the mineral N pool, the PO₄ pool was not exhausted from the mixed layer by the end of the 1997 spring bloom. This availability of excess PO₄ is a new phenomenon, starting in the 1990s, in our study area (cf. Niemi 1975; Lignell 1990). Possibly, it reflects the recently recorded increase of anoxic bottom areas in the Gulf of Finland with subsequent release of the enormous PO₄ reserves adsorbed in oxic sediments.

During the bloom of trichal blue-green algae (*A. lemmermannii* and *A. flos-aquae*), elevated Chl *a* and particulate organic nutrient concentrations were found (Table 2). By then, the ambient PO₄ pool had declined close to the detection level. This decline might have been due in part to the N₂-fixing capability of the predominant diazotrophic blue-green algae, which relaxes N limitation constraints in their P uptake.

The autumn experiment took place during an upwelling, being associated with a diverse phytoplankton community (Table 3). Upwellings frequently occur in our study area (and the rest of the Baltic Sea as well) when northwest winds remove the relatively shallow (10–15 m) surface mixed layer, giving way to colder, nutrient-rich, and more saline deep water with a low mineral N:P ratio (Table 2; Niemi 1975).

The initial biomasses of the main heterotrophic groups (bacteria, nanoflagellates, and ciliates), as well as bacterial productivity, were generally within the range commonly found during summer–autumn off the southwest coast of Finland (Figs. 6, 7; Kivi 1986; Lignell 1990; Kivi et al. 1993 and references therein). The only exception seemed to be the high ciliate abundance and bacterial productivity during the bloom, probably reflecting, at least in part, good food availability among blue-green clumps (cf. Hoppe 1981). On the other hand, field observations indicate that natural ciliate communities often grow close to maximal rates (Nielsen and Kjørboe 1994). Thus, the abundant blue-green clumps probably also promoted high ciliate numbers by providing refuges for ciliates against mesozooan grazing.

Nutrient dynamics—Sedimentation losses of total N over the 3-d experiments were consistently low (a few percentage per day) irrespective of treatment or study period (Table 4). With the exception of the autumn upwelling event, sedimentation losses of total P were ~20% or less over the 3-d studies. These results suggest that the added nutrients were efficiently retained in the water column, hence being available for the enclosed plankton food web. Moreover, the control units with no extra treatments seemed to serve as true controls for treatment responses because the sedimentation losses in them were low as well and showed similar proportions as in other units (data not shown).

During the summer minimum period, the low initial mineral N:P ratios of <1 (wt/wt), the abundant ambient PO₄ pool, and the mineral N concentrations close to detection

level (Table 2) indicated N-limited growth of phytoplankton. Nevertheless, the N:P ratios of mineral nutrients depleted over the first day of the experiment were reasonably close to that of Redfield (7.2; Redfield et al. 1963) in N- and P-replete enclosures (Table 4). The NP unit in mid-June was the only clear exception, with an N:P depletion ratio of 14, but in contemporary NP/2 units, this ratio was ~8 (cf. Fig. 2). Thus, the N:P ratios in nutrient uptake generally indicated balanced growth by algae (cf. Goldman et al. 1979; Sakshaug and Olsen 1986).

The initial particulate organic C:N ratios (POC:PON) were close to that of Redfield (5.7, Table 2; Fig. 3) in all experiments, indicating again that phytoplankton growth was reasonably balanced with respect to N (Fig. 4; cf. Goldman et al. 1979; Guildford and Hecky 2000). These results are in line with our earlier studies, which showed that the major part (~50%) of fixed ¹⁴CO₂ is channeled into proteins over the succession of summer phytoplankton in our study area (Lindqvist and Lignell 1997).

Irrespective of the treatment, POC:PON ratios also remained close to that of Redfield over all 3-d experiments, conforming to the recorded robustness of this ratio in natural plankton communities. It should be noted, however, that although algae are able to adjust their stoichiometric C:N ratios according to the relative availability of C and N (e.g., Goldman 1980), this ratio is much more stable in heterotrophs (see Gismervik et al. 1996) and detritus (cf. Heiskanen et al. 1998). Phytoplankton accounted only for 10–25% of total POC in the enclosures; hence, the possible adjustments in algal C:N ratios were masked when judged from bulk POC:PON ratios.

In P-replete early June, when *A. flos-aquae* accounted for 85 and 56% of phytoplankton biomass and total POC, respectively, in the >10- μ m size fraction, the initial >10- μ m POC:POP ratio was 27 (data not shown, cf. Fig. 3). Two weeks later, when free mineral P was still abundant (Table 2), the biomass of *A. flos-aquae* was clearly lower (15% of >10- μ m POC), and the initial >10- μ m POC:POP ratio was in turn higher (36). In both June experiments, the initial POC:POP ratios in the <10- μ m size fraction were close to that of Redfield (41). Thus, our results seem to support the findings of Larsson et al. (2001), who found trichal blue-green algae to show low C:P ratios of ~20 in early summer P-replete conditions in the northern Baltic Sea. These results most likely reflected the high capacity for luxury P storage by *A. flos-aquae* (Turpin 1988; Larsson et al. 2001).

Larsson et al. (2001) found trichal blue-green algae to show extremely high (~150) C:P ratios during P-deplete, late summer blue-green algal blooms in the northern Baltic. Our results from the bloom seem to support this finding, as the POC:POP ratio in the >10- μ m size fraction (the predominant trichal blue-green algae accounted for 25% of the >10- μ m POC) was 72 (data not shown), or almost three times as high as the corresponding value in early June.

Conforming to the above findings, there was a striking difference between the effects of P additions (P and NP enclosures) on PON:POP ratios in early June and bloom experiments (the contemporary POC:PON ratios remained consistent; Fig. 3). In both cases, trichal blue-green algae were predominant in the initial phytoplankton assemblages,

with an ~60% share of the total algal biomass (Table 3). During the minimum period, nutrient treatments, including single P addition, evoked only relatively small changes in PON:POP ratios, which remained reasonably close to that of Redfield. In contrast, during the bloom, the initial total PON:POP ratio of 10.3 exceeded the criterion of 9.9 given to indicate physiological P deficiency of phytoplankton (Guildford and Hecky 2000), and the single P treatment resulted in a marked decrease of 3.8 (wt/wt) units in this ratio in the <40- μm samples. This decline in PON:POP ratios evidently reflected refilling of algal P stores because no concomitant biomass response took place (Fig. 4). The single P addition was channeled into the >2- μm POP fraction (data not shown), excluding the possibility of concomitant bacterial luxury P uptake (cf. Gismervik et al. 1996).

During the bloom, the low mineral N:P ratio indicated relatively low availability of N, but the concentrations of both mineral N and P were close to detection level (Table 2). The mineral N:P depletion ratio in NP units was <3, supporting the above results of P-deficient phytoplankton growth (Table 4; cf. Sakshaug and Olsen 1986). A similar picture was found in nutrient enrichment experiments during late summer blue-green algal blooms in the southern Gulf of Riga (*A. flos-aquae* predominant, Seppälä et al. 1999) and at the Gotland Deep, the central Baltic (*A. flos-aquae* and *Nodularia spumigena* Mertens ex Bornet et Flahault predominant; unpubl. data). These results suggest that the initially low mineral N:P uptake ratios in combined N and P pulse exploitation, POC:PON ratios close to that of Redfield, and high initial PON:POP ratios (~10) with a sharp decline in units with P addition are characteristic for late summer blue-green algal blooms with low ambient mineral N and P concentrations in the Baltic Sea.

Responses of biological compartments to nutrient enrichment—Phytoplankton: During the summer minimum, the ambient mineral N concentrations were close to detection level while mineral P was abundant (Table 2). Judging from the time courses of mineral N and P depletion (Fig. 2) and Chl *a* (Fig. 4), algal biomass (Table 3), and $^{14}\text{CO}_2$ fixation (Table 5), the phytoplankton communities were clearly N-limited.

Guildford and Hecky (2000) suggested that N and P debt values (defined as Chl *a*-normalized ammonium and phosphate depletion rates over 24 h in respective samples with single additions of 5 $\mu\text{mol N or P L}^{-1}$) of >2.3 $\mu\text{g N or P} (\mu\text{g Chl } a)^{-1} \text{ d}^{-1}$ indicate nutrient-limited algal growth rates. Thus, the N debt values of 4.5 and 6.1 $\mu\text{g N} (\mu\text{g Chl } a)^{-1} \text{ d}^{-1}$ indicated algal N deficiency during the minimum period (Table 4). However, no other quantitatively important signs of physiological (Blackman-type) N limitation of growth rate were found, including the N effect on mineral N:P depletion ratios, Chl *a*-normalized $^{14}\text{CO}_2$ fixation, or algal growth rates (Fig. 4; Tables 4–7; cf. Goldman 1980; Sakshaug and Olsen 1986; Cullen et al. 1992; Graziano et al. 1996; Guildford and Hecky 2000).

Algal biomass has frequently been found to be N-limited in other nutrient enrichment studies off the southwest coast of Finland (Kivi et al. 1993), in other parts of the Baltic Sea (the low-salinity Bothnian Bay excluded, e.g., Granéli et al.

Table 6. Corrected maximal nutrient (N+P)-replete algal growth rates (μ_{DIL} , d^{-1}) in nutrient enrichment experiments. Growth rates were estimated by the dilution method on <100- μm prescreened samples (<40- μm samples in bloom experiment). Trichal blue-green algae are omitted, assuming zero growth for them. Only during the bloom was a consistently significant treatment (P) effect found in contemporary samples (*t*-test, $p < 0.01$ and 0.02 on Days 2 and 3, respectively, $\text{df} = 16$). Other explanations in Tables 1 and 2.

Treatment, sample	Algal growth rate (SE, d^{-1})			
	Minimum 2 Jun 97	Minimum 16 Jun 97	Bloom 14 Jul 97	Autumn 15 Sep 97
C, Day 0	0.40(0.06)	0.70(0.02)	0.57(0.03)	0.29(0.03)
N, Day 2	0.45(0.03)	0.64(0.03)	0.69(0.05)	0.39(0.02)
P, Day 2	0.50(0.03)	0.51(0.02)	1.03(0.05)	0.55(0.03)
C, Day 3	0.49(0.04)	0.78(0.02)	0.50(0.08)	0.55(0.05)
NP, Day 3	0.36(0.03)	0.79(0.02)	0.91(0.02)	0.48(0.03)

1990; Pitkänen and Tamminin 1995; Seppälä et al. 1999), and in most other estuarine and coastal areas (see Anonymous 2000). The issue of nutrient limitation of marine phytoplankton is not settled, however, in part because of the different approaches used. For example, Guildford and Hecky (2000) seldom found algal growth rates to be nutrient limited in a study covering a broad range of ocean sites when examining particulate organic nutrient ratios, alkaline phosphatase activity, and N and P debt values as indicators. In contrast, Graziano et al. (1996) found both systemic and physiological nitrogen limitation of the predominant picoplankton across much of the North Atlantic Ocean when using variable fluorescence methods supplemented with nutrient bioassays.

All algal size groups responded similarly to the nutrient treatments, and accordingly, no quantitatively important treatment effects were generally found on the percent distribution of Chl *a* and $^{14}\text{CO}_2$ fixation in different size fractions (<2, 2–10, and >10 μm , Fig. 5; Table 5). This seemed to contradict the theory and empirical findings, which suggest that picoalgae show consistent biomass over coastal zones with a varying load of new nutrients (Thingstad and Sakshaug 1990; Søndergaard et al. 1991). The mechanism behind this consistency would be that picoalgae grow at close to maximal rates in natural coastal (sufficiently eutrophic) systems, their biomass being again controlled by flagellate grazing. However, channeling of large nutrient pulses through the food web affects the complex interaction network of the whole plankton community, which could lead to transient relaxation of picoalgal grazing as well. We have found, in longer lasting mesocosm experiments with smaller daily nutrient (N and P) additions used up overnight, that although picoalgal biomass and productivity might show an initial increase, these variables tend to return to their initial states within a couple of days (Kuuppo-Leinikki et al. 1994; unpubl. data).

During the blue-green algal bloom, the combined addition of N and P was needed to evoke a clear Chl *a* response (Fig. 4; Table 5). This evidently reflected the low ambient concentrations of both mineral N and P (Table 2), preventing a

Table 7. Indications of nutrient (N or P) limitation during different phytoplankton growth periods and the magnitude of important biological nutrient treatment responses. Traditional nutrient limitation indicators based on initial inorganic (DIN:DIP) and particulate organic (PON:POP) ratios (by weight) are compared to those based on Chl *a*-normalized inorganic nutrient depletion rates (N debt and P debt) and ratios and on other variables reflecting important responses of autotrophic and heterotrophic compartments to N and P treatments are given experiment days (compiled from Tables 2 and 4–6). N or P in front of initial DIN:DIP and PON:POP ratios (in brackets) denotes N or P limitation (respective ratio <4.5 or >9.9). N debt and P debt values >2.3 $\mu\text{g element } (\mu\text{g Chl } a)^{-1} \text{ d}^{-1}$ indicated physiological N or P limitation, respectively (denoted by N or P; Table 4). Statistically important N or P effects on the other biological variables are also denoted by N or P, respectively (Tables 5, 6); subsequent values in brackets represent the corresponding quantitative effect (as percent increase compared to the value in the contemporary control unit or a unit without this treatment). Ranges given for the summer minimum period represent the values recorded in the two June experiments. Indicator criteria are based on Goldman et al. (1979) and Guildford and Hecky (2000); for more details, see text.

Indicator variable	Response interval (d)	Summer minimum	Bloom of blue-green algae	Autumn upwelling
DIN:DIP*	Initial value	N (0.4–0.6)	NP(3.4)	None(3.6)
PON:POP	Initial value	None (5.2–7.2)	P(10.3)	None(6.9)
N debt	0–1	N	N	N
P debt	0–1	None	P	None
DIN:DIP depletion ratio	0–1	None	P	None
Chl <i>a</i>	0–3	N (120–320%)	NP(170%)	None
Algal $^{14}\text{CO}_2$ fixation	0–3	N (140–430%)	P(140%)	None
$^{14}\text{CO}_2$ fixation (Chl <i>a</i>) ⁻¹	0–3	None	P(60%)	None
Corrected μ_{DIL} **	0–2 or 0–3	None	P(50%)	None
Bacterial biomass	0–3	None	None	None
Bacterial productivity	0–3	None–N(130%)	None	None
HNF biomass	0–3	None	None	None
Ciliate biomass	0–3	N(50–110%)	NP (60%)	None

* During the bloom, the concentrations of both DIN and DIP were low (close to detection level), but during the autumn upwelling these concentrations were high (Table 2).

** Corrected μ_{DIL} = maximal phytoplankton growth rates, excluding slowly growing trichal blue-green algae (Table 6). Other explanations in Tables 2 and 4–6.

build-up of new biomass with single N or P additions. Accordingly, the high N and P debt values (3.8 $\mu\text{g N}$ and 2.6 $\mu\text{g P } (\mu\text{g Chl } a)^{-1} \text{ d}^{-1}$) suggested poor availability or physiological deficiency (Guildford and Hecky 2000) of both these nutrients (Table 4). Still, P debt was most likely underestimated because mineral P was virtually exhausted in P units within Days 0–1 (Fig. 2).

The added P was most likely channeled to P stores by algae in P units because no corresponding biomass responses of autotrophs or heterotrophs were observed (Figs. 4, 6, 7; cf. Turpin 1988; Gismervik et al. 1996). However, P treatment evoked a significant increase in Chl *a*-normalized $^{14}\text{CO}_2$ fixation rates by Day 3 (Fig. 4; Table 5). Both the >10- and <10- μm size fractions responded similarly, the P treatment evoking a significant 30–50% increase in these values. This indicated that, contrary to the situation in early summer, the major part of the phytoplankton community was physiologically P-limited during the bloom (cf. Cullen et al. 1992; see also below).

The P-limited bloom situation did not fit the generally found correlation between low inorganic N:P ratios (<4) and N limitation of marine plankton communities (Goldman et al. 1979; Anonymous 2000). In late summer, the predominant trichal blue-green algae are able to fix gaseous N_2 ; at the same, they show extremely low P quotas (Larsson et al. 2001). Thus, the blue-green algae supplied organic matter with a high N:P ratio to the plankton food web (see Anonymous 2000; Larsson et al. 2001), evidently explaining the

loss as an indicator value of the low inorganic N:P ratio in the productive surface layer.

During autumn upwelling, both single N (on Day 2) and combined N and P additions (on Day 3) seemed to evoke a positive Chl *a* response (Fig. 4; $^{14}\text{CO}_2$ fixation responded similarly, data not shown). If this effect was real (*but see Table 5*), it was surprising as well because the ambient mineral N and P concentrations were high (Fig. 2). By Day 2, the $\text{NH}_4\text{-N}$ and $\text{PO}_4\text{-P}$ concentrations had declined to 10–15 $\mu\text{g L}^{-1}$ in P and N units, respectively, and in the control unit; by Day 3, similar “background” concentrations were recorded in all units. When comparing the time courses of nutrients to those of Chl *a* and $^{14}\text{CO}_2$ fixation, a possible explanation would seem to be that these high-background NH_4 and PO_4 pools were not readily available for algae. The upwelling probably brought deep-water detritus and resuspended matter from nearby bottom deposits to surface layer (detritus accounted for about two thirds of total POC; Tables 2, 3; Figs. 6, 7; cf. Heiskanen et al. 1998). If so, then the apparently low availability of the recorded NH_4 and PO_4 pools might again have reflected their adsorption to dissolved or particulate organic matter (cf. Mackin and Aller 1984; Thurman 1985).

In general, the quantitatively important phytoplankton biomass responses conformed to those reported by Breitburg et al. (1999), who found that species-level nutrient effects vary in the magnitude, not the direction, of the response (Table 3). However, trichal blue-green algae did not generally seem

to respond to the nutrient treatments. When they were initially predominant (early June and bloom), their biomass showed an overall declining trend in all enclosures over the 3-d experiments, whereas in other cases, their biomass was consistent. Also Lehman and Sandgren (1985) found in nutrient bioassays carried out in two lakes that a large proportion of the phytoplankton species did not respond to nutrient additions, and 20% of the species showed negative net growth rates not accounted for by grazing losses.

In part, our results might reflect unfavorable growth conditions for trichal blue-green algae in the enclosures (altered irradiance, turbulence, etc.) or poor physiological condition (especially during the bloom). Trichal blue-green algae are difficult to count quantitatively because of uneven trichome lengths, incomplete sedimentation in settling chambers, and nonhomogeneous distribution of clumps of various sizes. Thus, these algae might have been growing, but too slowly for any clear nutrient treatment responses to show within the 3-d experimental period.

Finally, some opportunistic responses were also evident at the species level in our experiments. In mid-June, *Eutrepitella gymnastica* Thronsen benefited from N treatment, possibly reflecting initial physiological N deficiency (and low relative growth rate) of this particular species (cf. Cullen et al. 1992). Moreover, during the bloom, *Monoraphidium contortum* (Thuret) Komárkova-Legenerová exhibited an overall biomass increase, suggesting the benefit of enclosure conditions (bag effect).

Heterotrophs—In general, no clear biomass responses to nutrient treatments were recorded with bacteria or heterotrophic nanoflagellates (Figs. 6, 7; Table 5). In contrast, ciliates, being the top predators in our <100- μm prescreened samples, showed similar responses with their phytoplankton prey to the nutrient treatments (Fig. 7; Table 5), which followed the algal response with about a 1-d lag (cf. Fig. 4). The fast numerical response of ciliates is in line with the growth rates reported for ciliates ($\geq 1 \text{ d}^{-1}$, Heinbokel 1978, 1988).

In the bloom experiment, picoalgae and nanosized autotrophs and heterotrophs declined dramatically in all units, whereas this was not the case with bacteria (cf. Figs. 4–7; Tables 3, 5). Prescreening (<100 μm) of the enclosed plankton community removed most of the blue-green algal clumps, which are inhabited by dense and dynamic microbial food webs (Hoppe 1981). The initial biomass and the temporal response of the exceptionally abundant ciliate community remaining free after prescreening was predominated by a strobilid (45 μm) and a hypotrich (benthic surface feeder, 37 μm), which evidently accounted for the decline of their major prey, nanoplankton (see Burkill et al. 1987). The ciliate community was subdominated by a small strombidid (16 μm) and a small strobilid (9–15 μm), which also might have been able to prey on picoalgae. Moreover, even if ciliates were not effective grazers of picosized prey (see Burkill et al. 1987), they could have affected picoalgae indirectly by removing heterotrophic nanoflagellates—hence releasing grazing pressure on bacteria. This might have favored bacteria in competition for nutrients with picoalgae (cf. Pengerud et al. 1987) and resulted in decreased growth rates of picoalgae and hence their biomass decline under the pre-

vailing feeding pressure. This hypothesis seems to be supported by the high contemporary bacterial productivity (Fig. 6) and the clear signs of physiological P limitation of small (<10 μm) algae (see also Fig. 9).

Bacterial growth rates were usually not affected by the nutrient treatments, but in mid-June, bacterial productivity appeared to be N-limited (Fig. 6; Table 5). This was in line with earlier observations of occasional mineral nutrient limitation of bacteria off the southwest coast of Finland (Kivi et al. 1993) and with consistent findings in several marine systems (e.g., Thingstad et al. 1998; Caron et al. 2000). However, addition of both mineral N and P and a labile carbon source has generally been needed to evoke a significant bacterial productivity response in our study area (Lignell et al. 1992; Kivi et al. 1993). At least in part, this probably reflects the relatively strict stoichiometric nutrient demands of bacteria (see Gismervik et al. 1996) on the one hand and a reasonably balanced supply of bacterial C, N, and P substrates within the plankton food web on the other.

Phytoplankton growth rates—During the summer minimum period, no clear signs of Blackman-type physiological growth rate limitation were found (consistently high N debt values excluded; Tables 2, 4–7). This suggested that the major part of active algal cells were growing close to their maximal rates, determined mainly by the prevailing temperature and irradiance (cf. Goldman 1980; Thingstad and Sakshaug 1990; Cullen et al. 1992).

Measurement of algal growth rates in natural communities is not a trivial task because of the overlapping size spectra of autotrophs and heterotrophs and their complex interactions (including grazing and viral lysis-related remineralization pathways of the nutrient supply). In our opinion, the dilution and pigment labeling methods used here are among the most promising because the entire original plankton community is included in incubations, and minimal extra manipulations are carried out in addition to sample confinement. In dilution experiments, growth was measured directly from the temporal net change of a robust and general algal biomass indicator (Chl *a*); at the same time, the potentially harmful and only partially effective prescreening step to remove the masking effect of top predators was avoided. In the pigment labeling method the phytoplankton growth rate could again be measured directly from the carbon-specific ^{14}C radioactivity of Chl *a*.

The maximal (N- and P-replete) community growth rate values of algal assemblages were similar (ranging from 0.1 to 0.7 d^{-1}) when measured with dilution (μ_{DIL}) and pigment labeling methods. For comparison, net Chl *a* change rates over 3 d in N- and P-replete enclosures were generally similar as well, showing that extra grazing pressure from enhanced food availability (i.e., a combined N and P effect on grazing) was relatively small in these short-term experiments.

Our maximal growth rate values were at the lower end of those reported for natural phytoplankton communities (~ 1 – 2 d^{-1} , see Furnas 1990) and for those found by the dilution method within a temperature and irradiance range similar to our study in Oslo fjord (Andersen et al. 1991). On the other hand, our μ_{DIL} values were similar to those found with the

dilution method in the open sea and a tidally mixed bay (Burkill et al. 1987) and in a mesoeutrophic lake (Elser and Frees 1995). Moreover, our results were in line with the species-specific growth rates found in enclosed phytoplankton communities in two lakes (Lehman and Sandgren 1985).

Natural phytoplankton communities are diverse, and during annual succession, their components can show different physiological states and respective high or low relative growth rates (cf. Goldman 1980; Lehman and Sandgren 1985; Burkill et al. 1987). The fast-growing algal assemblages in the study of Andersen et al. (1991) were mostly virtually unialgal populations (and in one case a mixed flagellate community), which evidently were in a physiologically healthy condition.

In contrast, in our early June and bloom experiments, the phytoplankton communities were predominated by trichal blue-green algae (*A. flos-aquae* and *A. lemmermannii*), which did not show appreciable net growth in any enclosure, irrespective of treatment (Table 3). The presence of a large, passive Chl *a* pool showing very slow turnover rate could partly explain our low maximal growth rate values based on bulk Chl *a* measurements (dilution and pigment labeling methods, net growth). When the >10- μm Chl *a* accounted for by trichal blue-green algae (estimated from the >10- μm algal biomass distribution; cf. Table 3) was omitted, assuming zero growth of blue-green algae in dilution incubations, the corrected algal μ_{DIL} values for the remaining algal assemblage varied between 0.3 and 1.0 d^{-1} (Table 6).

Contrary to other experiments with no clear nutrient effects, during the bloom, P addition evoked a significant increase in the corrected μ_{DIL} value, representing the piconanoalgal community (*t*-test, $p < 0.01$, $df = 16$; Table 6; cf. also Fig. 8). In support of this conclusion, the time courses of large (>14 μm) particle size groups indeed showed close to zero growth in DIL bottles from P units on Day 2 (data not shown). Thus, these results conform to the view of physiological (Blackman-type) P limitation of phytoplankton growth rate, as judged from the increase in Chl *a*-normalized $^{14}\text{CO}_2$ fixation rates recorded in units with P treatment (Fig. 4; Table 5).

Although the corrected algal μ_{DIL} values (Table 6) were clearly higher than the original ones found by the dilution method, they still are on the lower end of those reported by Andersen et al. (1991; see also Furnas 1990). In several dilution experiments (those in mid-June excluded), the algal net growth rate (m_x) was not linearly related to dilution, which would be the ideal case, if grazing is unsaturated at all dilution levels (Landry and Hassett 1982). Instead, m_x was at times much higher in the largest dilutions. Possibly, the microprotozoan feeding rates were saturated within the dilution Chl *a* gradient, in which case, the algal μ_{DIL} values would be underestimates (Gallegos 1989; Elser and Frees 1995). Moreover, in the autumn experiment, the apparent growth rate showed a maximum of $\sim 0.5 \text{ d}^{-1}$ in samples with small dilution and decreased with increasing dilution. Thus, linear regression gave a μ_{DIL} estimate of 0.29 d^{-1} and a negative grazing coefficient (impossible). Gallegos (1989) suggested that this type of violation of the assumptions behind the dilution technique might emerge, if within the prevailing microprotozoan community some groups prey on those that

feed on algae. Then, algal feeders would be released from grazing pressure with increasing dilution and hence allowed to feed on algae more effectively.

The maximal growth rates of different size groups measured with the particle counter over the dilution gradient (samples with combined N and P addition) support the above considerations. In all summer experiments, nanoalgal size groups (spanning 2–14 μm) showed similar maximal growth rates with a range from 0.5 to 1.5 d^{-1} (Fig. 9). In autumn, the nanoalgal growth rates were lower ($\sim 0.5 \text{ d}^{-1}$). In the experiments with trichal blue-green algae as the predominant species (early June and bloom), microalgal size groups (14–60 μm) showed clearly lower maximal growth rates than nanoalgae (0–0.5 d^{-1}), whereas in the other two experiments, nano- and microalgal growth rates were more similar (Fig. 9).

Our results on Chl *a*-normalized $^{14}\text{CO}_2$ fixation rates seem to fit this picture, as small (2–10 μm) algae exhibited consistently significantly higher rates (on average, 1.2–1.7-fold higher) than larger algae. However, judging from lack of corresponding temporal changes in their Chl *a* proportions (Fig. 5), the higher photosynthetic activity of small nanoalgae seems to have been compensated for by more intensive grazing. Analogously, Burkill et al. (1987) found those algal taxa preferentially grazed to show the highest specific growth rates.

The particle counts from the large orifice (14–60 μm diameter), covering the microalgae, are more difficult to interpret than those concerning nanoalgae. Trichal blue-green algae, especially *A. flos-aquae* could have formed trichome clumps of various sizes, which makes it impossible to differentiate these algae from other microalgae by size distribution of particle counts (where the particle sizes are given as equivalent spherical diameter, their shapes being overlooked). Nevertheless, the clearly lower growth rates of microalgal size groups compared to those of nanoalgae in the initial samples predominated by trichal blue-green algae seem to support the view of very slow growth of trichal blue-green algae, as was judged from the lack of their appreciable biomass response in all experimental units (Table 3). The assumption that *Dinophysis acuminata* Clapèrede et Lachman and the autotrophic ciliate *Mesodinium rubrum* Lohman (which accounted for $\sim 15\%$ of algal biomass in the >20- μm fraction; Table 3) showed maximal growth rates of $\sim 1 \text{ d}^{-1}$, as is generally found in the nanoalgal size fraction in early June, would suggest that trichal blue-green algae indeed grew very slowly in these samples.

A sharper picture emerges from the bloom experiment, where the microsized compartment of the initial phytoplankton community consisted virtually completely of the predominant trichal blue-green algae *A. lemmermannii* and *A. flos-aquae* (Table 3). During this period, the time courses of microsize particle counts in nutrient (N and P)-replete DIL samples revealed that the predominant trichal blue-green algae did not show appreciable growth (Fig. 9).

Nutrient sources for algal growth—The dilution experiments showed that internal stores were frequently the most important P source for phytoplankton growth under the mineral P-replete natural conditions during the summer mini-

mum and in units with P treatment (Fig. 10; note that in mid-June, a multiple linear regression gave statistically almost as good a fit for internal stores as for recycling for the P source in P unit on Day 2). In addition, recycling of P was important in all units in early June. Overall, these results support the above considerations based on changes in bulk particulate organic nutrient ratios, where phytoplankton appeared to store abundantly available P as polyphosphates, allowing luxury P consumption in excess of immediate demands of cell growth (cf. Turpin 1988).

Internal stores and recycling of N via remineralization were the most important N sources for algae. Interestingly, under the N-deplete conditions during the summer minimum, N stores also were important in units without N addition. Phytoplankton is able store N as well, mainly as amino acids, but the capacity for luxury N storage in algal cells is small (Dortch et al. 1984), as evidenced by the consistency of POC: PON ratios over the experiments (Fig. 3). Evidently these results reflected intracellular restructuring of all large intracellular N pools, including macromolecules (proteins), rather than the mere use of extra storage products (cf. Dortch et al. 1984).

It might seem surprising that algae resorted relatively strongly to their internal N stores under N-deficient conditions. In the longer run, N-limited algae evidently need external N sources to support their growth and to compensate for nutrient losses. It is generally believed that remineralization by protozoans is the key process in supplying nutrients for the turnover of pelagic phytoplankton standing stocks in summer (e.g., Glibert 1982; Nielsen and Kiørboe 1994). Bacteria might act as remineralizers as well, depending on their gross growth efficiency and the C:N ratio of their substrate (e.g., Goldman et al. 1987). However, this option is overlooked in the theoretical frame used for interpretation of the dilution method (Andersen et al. 1991), potentially biasing our results. Like the effect of intracellular stores, the number of bacteria (and therefore their remineralization effect) increases linearly with decreasing dilution; hence, the possible contribution of bacteria to nutrient supply would be allocated to that of intracellular stores in our multiple regression examinations (see above).

Thus, our results, presenting internal stores as a major N source for algae (Fig. 10), might in part have reflected the importance of bacterial remineralization. On the other hand, the C:N ratio of labile dissolved organic matter, depleted in 2 d in particle-free ($<0.2 \mu\text{m}$) samples inoculated with $<0.8\text{-}\mu\text{m}$ natural bacterial assemblages, ranges from 12 to 15 in summer in our study area (unpubl. data). Goldman et al. (1987) cultured natural marine bacterial communities on labile C and N sources, and their results showed no net release of ammonium with a substrate C:N > 10 . These results again suggest that bacteria were not important remineralizers in our samples.

Assuming that our results on algal nutrient sources were not seriously biased by experimental shortcomings (confinement, altered turbulence, etc.), the major role of internal N stores under N limitation might in part have reflected the importance of external N inputs, which were excluded from our samples. These inputs include "new" nutrients from the frequently occurring upwellings in our study area (as in Sep-

tember before the experiment, Table 2; see Niemi 1975) and from riverine, atmospheric, and groundwater sources. Finally, remineralization by mesozooplankton (removed in the present study by the $<100\text{-}\mu\text{m}$ prescreening) could be an important N source. An inverse solution of unknown flows in two comprehensive data sets suggested that mesozooplankton, rather than microzooplankton, accounted for the major part of N regeneration in summer plankton food webs near the English coast (Vézina and Platt 1988).

Microprotozoan grazing—The microprotozoan grazing rate (g) estimates from dilution experiments varied between 0.05 and 0.3 d^{-1} (Fig. 8), showing that in summer, these predators exert a substantial feeding pressure on phytoplankton. The grazing rates were highest in the summer minimum experiments, and N treatment with its positive algal and subsequent ciliate biomass responses seemed to evoke an increase in g values as well. Altogether, our results showed that during this period, the microbial food web is very active, with its small biotic nutrient compartment turning over quickly.

Our grazing values are at the lower end of those of $0.4\text{--}1.0 \text{ d}^{-1}$ found with the dilution technique in the Celtic Sea by Burkill et al. (1987), but they are similar to corresponding values reported by Andersen et al. (1991) and Elser and Frees (1995). It is worth noting, however, that our g values should be considered minimum estimates. First, in several cases when the net algal growth rate (m_x) was plotted against dilution (x) (Eq. 2), deviation from a linear relationship was suggested with clearly higher m_x values in the most diluted samples. If this pattern reflected saturated feeding response within the dilution gradient, then our g values would be underestimates (cf. Gallegos 1989; Elser and Frees 1995; see above). Second, the implicit assumption in the interpretation of dilution experiment responses (Landry and Hassett 1982) is that the grazed Chl *a* is disintegrated immediately. This is not necessarily the case, as some of the ingested Chl *a* could be relatively long-lived within the food vacuoles of the protozoans (e.g., Dolan and Simek 1997). Finally, the trichomes of the abundant blue-green algae were most likely not markedly consumed by the ciliate community (Hoppe 1981; Kivi and Setälä 1995), representing top predators in our $<100\text{-}\mu\text{m}$ prescreened dilution samples. Correcting our grazing values by omitting the trichal blue-green algae in these experiments (analogously with estimation of corrected μ_{DIL} values, Table 6) suggested an average increase of 50% (10% C.V.) in grazing pressure on the rest of the algal community.

Conclusions—Traditional nutrient limitation indicators (initial inorganic and particulate organic N:P ratios) along with supplementary information provided by statistically and biologically (quantitatively) important physiological and biomass nutrient responses are compiled in Table 7. During the early summer minimum period, the inorganic N:P ratio was low, and Chl *a* and $^{14}\text{CO}_2$ fixation were clearly limited by the availability of N. Thus, these results agreed with the general view of N limitation of coastal and estuarine systems and supported the conclusions of a recent extensive overview, where the inorganic N:P ratio turned out to be a good

predictor of the limitation of phytoplankton growth (Anonymous 2000).

Later in summer, a conspicuous bloom of trichal blue-green algae emerged, which is a typical phenomenon for the brackish Baltic Sea but is found only in a few other major estuarine or coastal areas (e.g., Anonymous 2000; Larsson et al. 2001). The whole phytoplankton community showed clear signs of physiological P limitation despite a low inorganic N:P ratio (Table 7). In part, this apparent discrepancy evidently reflected the ability of trichal blue-green algae to fix gaseous N₂, providing the plankton system extra organic N input via the production of blue-green algal biomass with a low P quota (cf. Anonymous 2000; Larsson et al. 2001). Moreover, in late summer, the concentrations of both inorganic N and P were close to the detection level; hence, their ratio was very sensitive to methodological uncertainties (especially those of the denominator).

Interpretation of the traditional nutrient limitation indicators based on state variables is further hampered by methodological problems, such as the lack of measurement accuracy and a knowledge of the share of measured mineral nutrient pools truly available for phytoplankton growth (Goldman et al. 1979; Thingstad et al. 1993). Moreover, the diagnostic criteria for nutrient limitation might need to be re-established. Usually these criteria are based on deviations from the Redfield N:P ratio or the N:P range shown by phytoplankton cells under optimal nutrient-replete conditions (~4–10; Goldman et al. 1979; Guildford and Hecky 2000). However, based on a review on marine microalgal biochemistry, it has recently been argued that this approach is not valid (Geider and La Roche 2002). The critical N:P ratio, marking the transition between N and P limitation of phytoplankton growth (based on respective cell quotas sensu Droop), seems to lie in the range of ~7–14.

Identification of the limiting nutrient is important for cost-efficient management and evaluation of the effects of an anthropogenic nutrient load. To overcome the potential sources of confusion discussed above, information on the nutritional status of the prevailing phytoplankton community is needed to supplement routine monitoring programs. This information can be extracted from measurements on physiological nutrient limitation (e.g., Graziano et al. 1996) or phytoplankton responses in nutrient bioassays, as demonstrated in the present study.

References

- ANDERSEN, T., A. K. L. SCHARTAU, AND E. PAASCHE. 1991. Quantifying external and internal nitrogen and phosphorus pools, as well as nitrogen and phosphorus supplied through remineralization, in coastal marine plankton by means of a dilution technique. *Mar. Ecol. Prog. Ser.* **69**: 67–80.
- ANONYMOUS. 2000. Clean coastal waters: Understanding and reducing the effects of nutrient pollution. U.S. National Academy of Sciences: National Research Council. National Academy Press.
- BJØRNSSEN, P. K. 1986. Automatic determination of bacterioplankton biomass by image analysis. *Appl. Environ. Microbiol.* **51**: 1199–1204.
- BLACKMAN, F. F. 1905. Optima and limiting factors. *Ann. Bot.* **19**: 281.
- BØRSHEIM, K. Y., AND G. BRATBAK. 1987. Cell volume to cell carbon conversion factors for a bacterivorous *Monas* sp. enriched from seawater. *Mar. Ecol. Prog. Ser.* **36**: 171–175.
- BOX, G. E. P., AND N. R. DRAPER. 1987. Empirical model—building and response surfaces. Wiley.
- BREITBURG, D. L. AND OTHERS. 1999. Variability in responses to nutrients and trace elements, and transmission of stressor effects through an estuarine food web. *Limnol. Oceanogr.* **44**: 837–863.
- BURKILL, P. H., R. C. F. MANTOURA, C. A. LLEWELLYN, AND N. J. P. OWENS. 1987. Microzooplankton grazing and selectivity of phytoplankton in coastal waters. *Mar. Biol.* **93**: 581–590.
- CARON, D. A., E. L. LIM, R. W. SANDERS, M. R. DENNETT, AND U.-G. BERINGER. 2000. Responses of bacterioplankton to organic carbon and inorganic nutrient additions in contrasting oceanic ecosystems. *Aquat. Microb. Ecol.* **22**: 175–184.
- CULLEN, J. J., X. YANG, AND H. L. MACINTYRE. 1992. Nutrient limitation of marine photosynthesis, p. 69–88. In P. G. Falkowski and A. Woodhead [eds.], Primary productivity and biogeochemical cycles in the sea. Plenum.
- DAVIS, P. G., AND J. MCN. SIEBURTH. 1982. Differentiation of phototrophic and heterotrophic nanoplankton populations in marine waters by epifluorescence microscopy. *Ann. Inst. Oceanogr. (suppl.)* **58**: 249–260.
- DOLAN, J. R., AND K. SIMEK. 1997. Processing of ingested matter in *Strombidium sulcatum*, a marine ciliate (Oligotrichida). *Limnol. Oceanogr.* **42**: 393–397.
- DORTCH, Q., J. R. CLAYTON, S. S. THORESEN, AND S. I. AHMED. 1984. Species differences in accumulation of nitrogen pools in phytoplankton. *Mar. Biol.* **81**: 237–250.
- DRAPER, N. R., AND H. SMITH. 1981. Applied regression analysis, 2nd ed. Wiley.
- EDLER, L., G. HÄLLFORS, AND Å. NIEMI. 1984. A preliminary check-list of the phytoplankton of the Baltic Sea. *Acta Bot. Fenn.* **128**: 1–26.
- ELSER, J. J., AND D. L. FREES. 1995. Microconsumer grazing and sources of limiting nutrients for phytoplankton growth: Application and complications of a nutrient-deletion/dilution-gradient technique. *Limnol. Oceanogr.* **40**: 1–16.
- , AND B. L. KIMMEL. 1986. Alteration of phytoplankton phosphorus status during enrichment experiments: Implications for interpreting nutrient enrichment bioassays. *Hydrobiologia* **133**: 217–222.
- ELSER, J. J., E. R. MARZOLF, AND C. R. GOLDMAN. 1990. Phosphorus and nitrogen limitation of phytoplankton growth in the freshwaters of North America: A review and critique of experimental enrichments. *Can. J. Fish. Aquat. Sci.* **47**: 1468–1477.
- FUHRMAN, J., AND F. AZAM. 1982. Thymidine incorporation as a measure of bacterioplankton production in marine surface waters: Evaluation and field results. *Mar. Biol.* **66**: 109–120.
- FURNAS, M. J. 1990. In situ growth rates of marine phytoplankton: Approaches to measurement, community and species growth rates. *J. Plankton Res.* **12**: 1117–1151.
- GALLEGOS, C. L. 1989. Microzooplankton grazing on phytoplankton in the Rhode River, Maryland: Non-linear feeding kinetics. *Mar. Ecol. Prog. Ser.* **57**: 23–33.
- GEIDER, R. J., AND J. LA ROCHE. 2002. Redfield revisited: Variability of C:N:P in marine microalgae and its biochemical basis. *Eur. J. Phycol.* **37**: 1–17.
- GISMERVIK, I., T. ANDERSEN, AND O. VADSTEIN. 1996. Pelagic food webs and eutrophication of coastal waters: Impact of grazers on algal communities. *Mar. Poll. Bull.* **33**: 22–35.
- GLIBERT, P. M. 1982. Regional studies of daily, seasonal and size fraction variability in ammonium remineralization. *Mar. Biol.* **70**: 209–222.

- GOERICKE, R., AND N. A. WELSCHMEYER. 1993. The chlorophyll-labeling method: Measuring specific rates of chlorophyll *a* synthesis in cultures and in the open ocean. *Limnol. Oceanogr.* **38**: 80–95.
- GOLDMAN, J. C. 1980. Physiological processes, nutrient availability, and the concept of relative growth rate in marine plankton ecology, p. 179–194. *In* P. G. Falkowski [ed.], *Primary productivity in the sea*. Plenum Press.
- , J. J. MCCARTHY, AND D. G. PEAVEY. 1979. Growth rate influence on the chemical composition of phytoplankton in oceanic waters. *Nature* **279**: 210–215.
- , D. A. CARON, AND M. R. DENNETT. 1987. Regulation of gross growth efficiency and ammonium regeneration in bacteria by substrate C:N ratio. *Limnol. Oceanogr.* **32**: 1239–1252.
- GRANÉLI, E., K. WALLSTRÖM, U. LARSSON, W. GRANÉLI, AND R. ELMGREN. 1990. Nutrient limitation of primary production in the Baltic Sea area. *Ambio* **19**: 142–151.
- GRASSHOFF, K., M. ERHARDT, AND K. KREMLING [EDS.]. 1983. *Methods of seawater analysis*. Verlag Chemie.
- GRAZIANO, L. M., R. J. GEIDER, W. K. W. LI, AND M. OLAIZOLA. 1996. Nitrogen limitation of North Atlantic phytoplankton: Analysis of physiological condition in nutrient enrichment experiments. *Aquat. Microb. Ecol.* **11**: 53–64.
- GUILDFORD, S. J., AND R. E. HECKY. 2000. Total nitrogen, total phosphorus, and nutrient limitation in lakes and oceans: Is there a common relationship? *Limnol. Oceanogr.* **45**: 1213–1223.
- HAAS, L. W. 1982. Improved epifluorescence microscopy for observing planktonic micro-organisms. *Ann. Inst. Oceanogr.* **58**: 261–266.
- HECKY, R. E., AND P. KILHAM. 1988. Nutrient limitation of phytoplankton in freshwater and marine environments: A review of recent evidence on the effects of enrichment. *Limnol. Oceanogr.* **33**: 796–822.
- HEINBOKEL, J. F. 1978. Studies on the functional role of tintinnids in the Southern California Bight. I. Grazing and growth rates in laboratory cultures. *Mar. Biol.* **47**: 177–189.
- . 1988. Reproductive rates and periodicities of oceanic tintinnid ciliates. *Mar. Ecol. Prog. Ser.* **47**: 239–248.
- HEISKANEN, A.-S., J. HAAPALA, AND K. GUNDERSEN. 1998. Sedimentation and pelagic retention of particulate C, N and P in the coastal northern Baltic Sea. *Estuar. Coast. Shelf Sci.* **46**: 703–712.
- HOBBIE, J. E., R. J. DALEY, AND S. JASPER. 1977. Use of Nuclepore filters for counting bacteria by epifluorescence microscopy. *Appl. Environ. Microbiol.* **33**: 1225–1228.
- HOPPE, H.-G. 1981. Blue-green algae agglomeration in surface water: A microhabitat of high bacterial activity. *Kieler Meeresforsch. Sonderh.* **5**: 291–303.
- JESPERSEN, A.-M., AND K. CHRISTOFFERSEN. 1987. Measurements of chlorophyll-*a* from phytoplankton using ethanol as extraction solvent. *Arch. Hydrobiol.* **109**: 445–454.
- KHURI, A. I., AND J. A. CORNELL. 1987. *Response surfaces*. ASQC Quality Press.
- KIVI, K. 1986. Annual succession of pelagic protozoans and rotifers in the Tvärminne Storfjärden, SW coast of Finland. *Ophelia Suppl.* **4**: 101–110.
- , AND O. SETÄLÄ. 1995. Simultaneous measurement of food particle selection and clearance rates of planktonic oligotrich ciliates (Ciliophora: Oligotrichina). *Mar. Ecol. Prog. Ser.* **119**: 125–137.
- , S. KAITALA, H. KUOSA, J. KUPARINEN, E. LESKINEN, R. LIGNELL, B. MARCUSSEN, AND T. TAMMINEN. 1993. Nutrient limitation and grazing control of Baltic plankton community during annual succession. *Limnol. Oceanogr.* **38**: 893–905.
- KUUPPO-LEINIKKI, P., R. AUTIO, S. HÄLLFORS, H. KUOSA, J. KUPARINEN, AND R. PAJUNIEMI. 1994. Trophic interactions and carbon flow between picoplankton and protozoa in pelagic enclosures manipulated with nutrients and a top predator. *Mar. Ecol. Prog. Ser.* **107**: 89–102.
- LANDRY, M. R., AND R. P. HASSETT. 1982. Estimating the grazing impact of marine microzooplankton. *Mar. Biol.* **67**: 283–288.
- LARSSON, U., S. HAJDU, J. WALVE, AND R. ELMGREN. 2001. Baltic Sea nitrogen fixation estimated from the summer increase in upper mixed layer total nitrogen. *Limnol. Oceanogr.* **46**: 811–820.
- LEHMAN, J. T., AND C. D. SANDGREN. 1985. Species-specific rates of growth and grazing loss among freshwater algae. *Limnol. Oceanogr.* **30**: 34–46.
- LIGNELL, R. 1990. Excretion of organic carbon by phytoplankton: Its relation to algal biomass, primary productivity and bacterial secondary productivity in the Baltic Sea. *Mar. Ecol. Prog. Ser.* **68**: 85–99.
- , S. KAITALA, AND H. KUOSA. 1992. Factors controlling phyto- and bacterioplankton on a late spring salinity gradient in the northern Baltic. *Mar. Ecol. Prog. Ser.* **84**: 121–131.
- LINDQVIST, K., AND R. LIGNELL. 1997. Intracellular partitioning of ¹⁴CO₂ in phytoplankton during a growth season in the northern Baltic. *Mar. Ecol. Prog. Ser.* **152**: 41–50.
- MACKIN, J. E., AND R. C. ALLER. 1984. Ammonium adsorption in marine sediments. *Limnol. Oceanogr.* **29**: 250–257.
- NIELSEN, T. G., AND T. KJØRBOE. 1994. Regulation of zooplankton biomass and production in a temperate, coastal ecosystem. *Limnol. Oceanogr.* **39**: 508–519.
- NIEMI, Å. 1975. Ecology of phytoplankton in the Tvärminne area, SW coast of Finland. II. Primary production and environmental conditions in the archipelago and the sea zone. *Acta Bot. Fenn.* **105**: 1–73.
- NIEMI, M., J. KUPARINEN, A. UUSI-RAUVA, AND K. KORHONEN. 1983. Preparation of algal samples for liquid scintillation counting. *Hydrobiologia* **106**: 149–156.
- PENGERUD, B., E. F. SKJOLDAL, AND T. F. THINGSTAD. 1987. The reciprocal interaction between degradation of glucose and ecosystem structure. Studies in mixed chemostat cultures of marine bacteria, algae, and bacterivorous nanoflagellates. *Mar. Ecol. Prog. Ser.* **35**: 111–117.
- PITKÄNEN, H., AND T. TAMMINEN. 1995. Nitrogen and phosphorus as production limiting factors in the estuarine waters of the eastern Gulf of Finland. *Mar. Ecol. Prog. Ser.* **129**: 283–294.
- PUTT, M., AND D. K. STOECKER. 1989. An experimentally determined carbon: volume ratio for marine “oligotrichous” ciliates from estuarine and coastal waters. *Limnol. Oceanogr.* **34**: 1097–1103.
- REDALJE, D. G., AND E. A. LAWS. 1981. A new method for estimating phytoplankton growth rates and carbon biomass. *Mar. Biol.* **62**: 73–79.
- REDFIELD, A. C., B. H. KETCHUM, AND F. A. RICHARDS. 1963. The influence of organisms on the composition of sea-water, p. 26–77. *In* M. N. Hill [ed.], *The sea*, v. 2. Interscience.
- RIEMANN, B., P. K. BJØRNSSEN, S. NEWELL, AND R. FALLON. 1987. Calculation of cell production of coastal bacteria based on measured incorporation of 3H-thymidine. *Limnol. Oceanogr.* **32**: 471–476.
- SAKSHAUG, E., AND Y. OLSEN. 1986. Nutrient status of phytoplankton blooms in Norwegian waters and algal strategies for nutrient competition. *Can. J. Fish. Aquat. Sci.* **43**: 389–396.
- SCHINDLER, D. W. 1988. Experimental studies of chemical stressors on whole lake ecosystems. *Verh. Internat. Verein. Limnol.* **23**: 11–41.
- SEPPÄLÄ, J., T. TAMMINEN, AND S. KAITALA. 1999. Experimental

- evaluation of nutrient limitation of phytoplankton communities in the Gulf of Riga. *J. Mar. Syst.* **23**: 107–126.
- SOLORZANO, L., AND J. H. SHARP. 1980. Determination of total dissolved phosphorus and particulate phosphorus in natural waters. *Limnol. Oceanogr.* **25**: 754–758.
- SOMMER, U. 1988. Does nutrient competition among phytoplankton occur in situ? *Int. Ver. Theor. Angew. Limnol. Verh.* **23**: 707–712.
- SØNDERGAARD, M., L. M. JENSEN, AND G. ÆRTEBJERG. 1991. Picoalgae in Danish coastal waters during summer stratification. *Mar. Ecol. Prog. Ser.* **79**: 139–149.
- STEMMANN NIELSEN, E. 1952. The use of radioactive carbon (^{14}C) for measuring organic production in the sea. *J. Cons. Perm. Int. Explor. Mer* **18**: 117–140.
- STERNER, R. W. 1994. Seasonal and spatial patterns in macro- and micronutrient limitation in Joe Pool Lake, Texas. *Limnol. Oceanogr.* **39**: 535–550.
- THINGSTAD, T. F., AND E. SAKSHAUG. 1990. Control of phytoplankton growth in nutrient recycling ecosystems. Theory and terminology. *Mar. Ecol. Prog. Ser.* **63**: 261–272.
- , E. F. SKJOLDAL, AND R. A. BOHNE. 1993. Phosphorus cycling and algal–bacterial competition in Sandsfjord, western Norway. *Mar. Ecol. Prog. Ser.* **99**: 239–259.
- , U. L. ZWEIFEL, AND F. RASSOULZADEGAN. 1998. P limitation of heterotrophic bacteria and phytoplankton in the north-west Mediterranean. *Limnol. Oceanogr.* **43**: 88–94.
- THURMAN, E. M. 1985. Organic geochemistry of natural waters. Dr W Junk Publ.
- TURPIN, D. H. 1988. Physiological mechanisms in phytoplankton resource competition, p. 316–368. *In* C. D. Sandgren [ed.], Growth and reproductive strategies of freshwater phytoplankton. Cambridge Univ. Press.
- UTERMÖHL, H. 1958. Zur Vervollkommung der quantitativen Phytoplanktonmethodik. *Mitt. Int. Ver. Theor. Angew. Limnol.* **9**: 1–38.
- VÉZINA, A. F., AND T. PLATT. 1988. Food web dynamics in the ocean. I. Best-estimates of flow networks using inverse methods. *Mar. Ecol. Prog. Ser.* **42**: 269–287.

Received: 3 December 2001

Accepted: 3 September 2002

Amended: 17 September 2002