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# Species-specific differences in phytoplankton responses to N and P enrichments and the N:P ratio in the Archipelago Sea, northern Baltic Sea

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*A nutrient enrichment experiment was conducted in order to study the role of nitrogen (N), phosphorus (P) and the N:P ratio on the early summer phytoplankton community in the Archipelago Sea, northern Baltic Sea. The phytoplankton community was, in terms of chlorophyll a and total biomass, primarily N-limited, but the individual species varied in their responses to the nutrient supply. The recorded overall N limitation was due to fast growth responses of a few N-limited species such as the diatom Chaetoceros wighamii (Brightwell) and the mixotrophic chrysophyte Uroglena sp. Another dominating diatom, Skeletonema costatum (Greville) Cleve was most clearly P-limited. The N:P ratio had the strongest effect on Uroglena sp., which grew exponentially in the enrichments with a high N:P ratio. This can be explained by the ability of the species to feed on P-rich bacteria, which gives it a competitive advantage in P-limited conditions. The species-specific differences in the responses to the nutrient enrichments can generally be explained by differences in the species physiology and they were consistent with the theory of resource competition.*

## INTRODUCTION

According to Liebig's law of the minimum, the yield of any organism is limited by the factor present in the lowest amount in relation to its requirements (de Baar, 1994). Of the nutrients, nitrogen (N) or phosphorus (P) most commonly limits the primary production in freshwater, estuarine and coastal ecosystems [e.g. (Hecky and Kilham, 1988; Downing, 1997)]. Since phytoplankton cells on average have a ratio of C, N and P of approximately 106:16:1 (by atoms), the Redfield ratio (Redfield, 1958), it has been suggested that the nutrient that potentially limits primary production can be predicted from total or dissolved nutrient concentrations of water (Forsberg *et al.*, 1978; Smith, 1984; Kirkkala *et al.*, 1998). A deviation from the Redfield ratio is then used as an indication of which nutrient is limiting. However, the Redfield ratio is only an average optimal ratio for the whole phytoplankton community. Species differ in their kinetics of nutrient uptake, assimilation and storage capacities and may, therefore, have different nutrient requirements as well as different cellular composition of N and P (Rhee and Gotham, 1980; Hecky and Kilham, 1988; Quigg *et al.*, 2003). Moreover, the species-specific optimum nutrient ratios may vary depending on different factors, e.g. growth rate (Terry *et al.*, 1985; Elrifi and Turpin, 1985; Turpin, 1986), temperature (Tilman *et al.*, 1986) light conditions (Healey, 1985), $CO_2$ availability (Burkhardt and Riebesell, 1997) or nutrient concentrations (Flynn, 2002).

Competition for limiting nutrients is seen as an important factor in the determination of phytoplankton community composition (Tilman *et al.*, 1982; Sommer, 1989a; Grover, 1997). Tilman's resource competition theory states that under nutrient limitation in equilibrium conditions, those species which have either the lowest requirement for the limited resource or the highest ability to utilize it, will succeed in competition (Tilman, 1977, 1982; Tilman *et al.*, 1982). The ability of the algae to compete for nutrients is determined by its physiological properties, e.g. half saturation constants,

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growth rate, transport rates and storage capacities (Flynn, 2002). In nature the outcome of nutrient competition is affected by several other factors, like mortality rates, and by the continuously changing environmental conditions like temperature (Tilman *et al.*, 1986) or light conditions (Sommer, 1994). Zooplankton may affect the outcome of phytoplankton resource competition either by selective grazing or by differential regeneration of nutrients (Sterner, 1986, 1990; Elser and Urabe, 1999).

Additional nutritional strategies by phytoplankton, like nitrogen fixation and mixotrophy, seem to be especially advantageous in nutrient-limited conditions. It has been hypothesized that diazotrophic cyanobacteria, which are capable of fixing molecular nitrogen, will have a competitive advantage during N limitation (Smith, 1983, 1986; Levine and Schindler, 1999). Mixotrophic phytoplankton have on the other hand been thought to be favoured in nutrient-limited conditions, because of their ability to utilize nutrients from particulate food (Sanders, 1991; Nygaard and Tobiesen, 1993; Isaksson, 1998; Stibor and Sommer, 2003).

An extension of Tilman's resource competition theory is the resource ratio hypothesis, which predicts that the relative abundances of coexisting species depend on the ratio of the limiting resources, not on their absolute concentrations (Tilman, 1982; Tilman *et al.*, 1982). Superior competitors are expected to be dominant at their optimal resource ratios and to be succeeded by others with different optimal resource ratios, if the resource ratios in the environment change (Tilman, 1982). Evidence supporting the importance of resource ratios is restricted to nitrogen-fixing cyanobacteria, which have been shown to be enhanced by a low N:P ratio [e.g. (Smith, 1983; Levine and Schindler, 1999; Smith and Bennett, 1999)] and to diatoms, which tend to outcompete non-siliceous algae at high Si:P and Si:N ratios [e.g. (Sommer, 1983, 1994; Tilman *et al.*, 1986)]. Moreover, results from culture experiments with freshwater algae showed that diatoms dominated at high N:P ratios while green algae were favoured by intermediate N:P ratios (Tilman *et al.*, 1986).

Resource competition has been verified in laboratory chemostat experiments both with species from clonal cultures [e.g. (Tilman, 1977, 1981; Fujimoto *et al.*, 1997) and with natural phytoplankton assemblages [e.g. (Sommer, 1983; Suttle and Harrison, 1988; Grover, 1989a)]. Rothhaupt (Rothhaupt, 1996) recently applied the resource competition theory to explain the outcome of competition experiments between heterotrophic, mixotrophic and autotrophic flagellates. In some instances the results of laboratory experiments have been confirmed by correlative field studies [e.g. (Carney *et al.*, 1988; Sommer, 1993; Interlandi and Kilham, 2001)]. However, only a few experimental studies with plankton communities in more natural conditions have been focusing on resource competition [e.g. (Schindler, 1977; Carney *et al.*, 1988; Findlay *et al.*, 1999; Levine and Schindler, 1999)]. They are important, however, to be able to estimate the role of resource competition in natural phytoplankton communities.

We conducted an enclosure experiment in the brackish Archipelago Sea, northern Baltic Sea, in order to study the roles of N, P, the N:P ratio and zooplankton in regulating the early summer phytoplankton community. We asked the following questions: (i) which one of the nutrients limits phytoplankton yield, (ii) are there species-specific differences in phytoplankton responses to nutrient limitation and the N:P ratio, (iii) if so, could the differences be explained according to the species' physiological properties or according to our knowledge from laboratory experiments, (iv) are mixotrophic species favoured by nutrient limitation, (v) is the zooplankton community affected by the nutrient enrichments, and (vi) does the zooplankton affect the outcome of nutrient competition among the phytoplankton?

The experiment was designed so that the phytoplankton community in the different treatments would be either potentially N-limited, P-limited or supplied with N and P in an optimal ratio, according to the Redfield ratio. To be able to separate the N:P ratio effect from the direct resource effect, three N:P ratios were supplied in two different concentrations. The natural zooplankton community was included in all but one treatment, which was used to estimate the effect of zooplankton grazing on the phytoplankton community.

## METHOD

### Study area

The experiment was carried out in a small, sheltered bay close to the island of Seili (60°13′N and 21°58′E, Figure 1) in the mesotrophic middle part of the Archipelago Sea, northern Baltic Sea. The bay is about 3 m deep and representative of other shallow water areas close to the shorelines of the about 25 000 islands in the Archipelago Sea. There are no tides in the Archipelago Sea, the mean water depth is only 23 m and the salinity is about 6‰. Summer temperature of the seawater reaches +20°C, and there is an ice cover during winter. During the last decades the Archipelago Sea has been severely eutrophicated by nutrients from diffuse loading, fish farming and municipal waste waters (Helminen *et al.*, 1998). At the same time as the concentrations of nitrogen and phosphorus have increased, the N:P ratio has decreased, indicating that the primary production has shifted from a co-limitation of N and P towards a clearer N-limitation during the last decades (Tamminen, 1990; Kirkkala *et al.*, 1998).

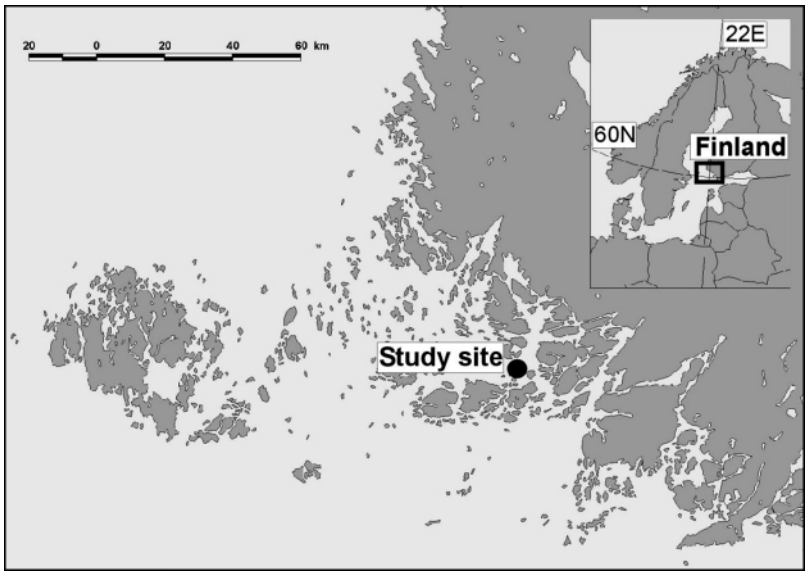


**Fig.1.** Map of the study area.

The phytoplankton succession in the Archipelago Sea follows the general pattern for the Baltic Sea. After ice-melting in April–May there is a spring bloom dominated by diatoms and dinoflagellates. After the spring bloom the inorganic nutrients are depleted, which leads to a summer phytoplankton minimum. A second bloom dominated by nitrogen-fixing cyanobacteria usually occurs in late summer (Kauppila and Lepistö, 2001).

## Experimental design

The mesocosm experiment was conducted on 7–18 June 1999, after the collapse of the spring bloom. The dissolved nutrients were almost completely depleted from the surface water, and the phytoplankton biomass was low. The 300 L transparent enclosures were made of 0.15 mm thick polyethylene and were 1.5 m deep. The enclosures were mounted on three floating wooden racks, which were anchored in the sea in a row in E–W direction. Ten enclosures were placed in each rack, but the enclosures at each end of the rack were not used in the experiment, because of different exposure to radiation. The enclosures were protected from bird faeces with a plastic roof, but the exchange of gases between air and the sea was not prevented.

The experimental design consisted of daily additions of N (as $NH_4Cl$) and P (as $KH_2PO_4$) in three different molar N:P ratios; N:P = 6, N:P = 40, and N:P = 15 (close to Redfield ratio) crossed with two nutrient levels (low and high) (Table I, Figure 2). This resulted in six different nutrient enrichments which included totally three different N and P doses. The doses were for nitrogen: 0.214 μM (low), 0.542 μM (medium) and 1.428 μM (high), and for phosphorus: 0.014 μM (low), 0.036 μM (medium) and 0.094 μM (high). In addition, there was a control treatment with no nutrient addition and a filtering treatment with removal of mesozooplankton (Table I). The controls received the same amount of distilled water as a substitute for the nutrient treatments. The zooplankton removal treatment was supplied with the same amounts of nutrients as the highest nutrient enrichment (Table I, Figure 2). The treatments were performed in triplicate and arranged in the three racks according to the randomized complete blocks design (Sokal and Rohlf, 1995).

*Table I: Mesocosm treatments and the daily additions of N and P*

| Treatment code | N dose μM | P dose μM | N:P ratio | Zooplankton removal |
|---|---|---|---|---|
| Control | 0 | 0 | – | No |
| 6L; $N_{low}P_{med}$ | 0.214 | 0.036 | 5.94 | No |
| 6H; $N_{med}P_{high}$ | 0.542 | 0.094 | 5.77 | No |
| 15L; $N_{med}P_{med}$ | 0.542 | 0.036 | 15.06 | No |
| 15H; $N_{high}P_{high}$ | 1.428 | 0.094 | 15.19 | No |
| 40L; $N_{med}P_{low}$ | 0.542 | 0.014 | 38.71 | No |
| 40H; $N_{high}P_{med}$ | 1.428 | 0.036 | 39.67 | No |
| 15H-Z; $N_{high}P_{high}$ | 1.428 | 0.094 | 15.19 | Yes |

The enclosures were filled with surface water from the study site in the evening prior to the start of the experiment. In the zooplankton removal treatment, the water was filtered through a 100 μm net during filling.

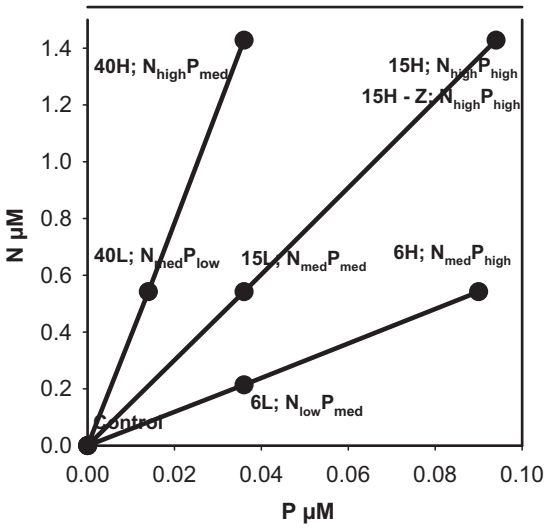


**Fig. 2.** The experimental design. The different treatments according to their doses of N and P. The treatments were control and three different N:P ratios at two levels (H = high, L = low). In addition, zooplankton removal was performed in one treatment receiving the highest doses of both N and P (15H; $N_{high}P_{high}$).

## Sampling and sample analysis

Sampling took place between 6 and 7 a.m., before the daily nutrient additions. The enclosures were manually mixed both before sampling and after nutrient additions. Chlorophyll *a* (Chl *a*) samples were taken daily from all enclosures, except for days 2, 4 and 10 when only one replicate was sampled. Samples for nutrient analyses [total N, total P, ($NO_2$ + $NO_3$)-N, $NH_4$-N and $PO_4$-P] were taken on days 0, 1, 3, 6, 8 and 10. Phytoplankton and autotrophic picoplankton (APP) were sampled on days 0, 6 and 10. Zooplankton samples were taken at the start and at the end of the experiment.

The concentrations of total nitrogen (TN), ammonium-nitrogen ($NH_4$-N), nitrite- and nitrate-nitrogen ($NO_2$-N + $NO_3$-N), total phosphorus (TP) and phosphate-phosphorus ($PO_4$-P) were determined within 8 h from sampling, according to standard methods (Koroleff, 1976, 1979; National Board of Waters, 1981). For Chl *a* analyses, water samples were filtered on glass-fibre filters (Whatman GF/C, 47 mm). The filters were air-dried, stored frozen, and extracted in ethanol and measured spectrophotometrically. Phytoplankton samples were preserved in acid Lugol's solution and analysed with an inverted microscope (Nikon Eclipse) using the Utermöhl technique (Utermöhl, 1958). However, at day 0, samples from only the three zooplankton removal enclosures and from six others, randomly chosen enclosures, were analysed, since there were no differences among the enclosures in other measured variables.

At least 100 units of each of the dominant species were counted, which yields a precision of ±20% within 95% confidence limits if the algae were randomly distributed (Lund *et al.*, 1958). Cell volumes were calculated from cell geometry (Edler, 1979). The relative importance of a species was expressed by its contribution to the total biovolume of each sample. Autotrophic picoplankton samples were preserved with ice-cold 2% glutaraldehyde. Subsamples were filtered on black-stained Nuclepore filters (pore size 0.2 µm). The filters were stored in −24°C and counted later with a Leica Dialux epifluorescence microscope using Leica M2 filter set with green excitation rate (BP 546/14). Mesozooplankton samples (30 L) were concentrated on a 25 µm mesh net and fixed in 70% ethanol. Zooplankton were identified and numerated with an inverted microscope and the biomasses were calculated according to average species-specific biomass values (Hernroth, 1985).

## Statistical tests

To reveal the relative importance of N, P and the N:P ratio, forward stepwise multiple regression analysis was performed (the significance level to enter in the model was 0.15) [Proc REG (SAS Institute Inc., 1996)]. The predicting variables in the model were N, P, $N^2$, $P^2$, N × P and N $P^{-1}$. Quadratic terms of the variables were entered in order to account for nonlinearities. The effect of zooplankton removal on the different phytoplankton groups was analysed separately by comparing the zooplankton removal treatment with the corresponding nutrient treatment with repeated measures ANOVA [Proc GLM (SAS Institute Inc., 1996)]. In the analyses, zooplankton removal and block were treated as between-subject factors and time (day 0, 6 and 10) was treated as a within-subject factor.

The normal distribution of each variable was verified with the normal probability plot of residuals and Kolmogorov–Smirnov's test (Sokal and Rohlf, 1995). When required, the data were log (x + 1) or square root transformed. Algal relative biomasses were arcsin square-root transformed. However, untransformed data are presented in all figures.

# RESULTS

## Temperature and nutrients

At the start of the experiment the water temperature was 14°C. Due to sunny and warm weather the temperature increased steadily up to 19°C at the end of the experiment. There were no differences in water temperature among the enclosures.

The initial concentrations of inorganic N and P were low. The concentrations of $NH_4$-N and ($NO_2$ + $NO_3$)-N

were about 0.1 μM and that of $PO_4$-P about 0.07 μM (Figure 3). The ratio of inorganic N to inorganic P (DIN:DIP) was 3.5 (by atoms), indicating the potential for N limitation of the phytoplankton community. The added nutrients were depleted rapidly and the concentrations of inorganic N and P were close to the detection level most of the time in almost all treatments (Figure 3). However, ammonium accumulated exponentially in the treatment with a high N:P ratio and a high N dose (40H; $N_{high}P_{med}$) until day 8. During the last two days the concentration decreased. Also in the other treatment with a high N:P ratio, but a medium N dose (40L; $N_{med}P_{low}$), the ammonium concentration increased in the middle of the experiment, but decreased after that (Figure 3). Phosphate, on the other hand, accumulated only in the treatment with a low N:P ratio and a high P dose (6H; $N_{med}P_{high}$). The concentration of ($NO_2$ + $NO_3$)-N was below 0.2 μM in all treatments. The DIN:DIP ratio remained below 9 in all other treatments, except in the treatment with a high N:P ratio and a high N dose (40H; $N_{high}P_{med}$), where the ratio increased to a maximum of 43 on day 8. The concentrations of total N and P changed in the different treatments corresponding to the daily additions of N and P (Figure 3).

## Chlorophyll *a*

The Chl *a* concentration increased during the experiment in all other treatments except in the control (Figure 3). During the first part of the experiment, the increase in the different treatments was best related to the amounts of N added. According to the stepwise regression analysis, the N dose alone explained 72% ($r^2$) of the variance in Chl *a* concentration on day 6 (Table II). In the treatments with the highest N dose, the Chl *a* concentration increased additionally when also P was added in a high dose (15H; $N_{high}P_{high}$), seen as a significant effect of the crossproduct of N and P on day 6 (Figure 3, Table II). After day 6, however, the Chl *a* concentration increased most in the treatment with a high dose of N but with a medium dose of P (40H; $N_{high}P_{med}$). In the three treatments with a medium dose of N but with a low, medium or high dose of P, the increase in the Chl *a* concentration was almost identical until day 6. After that the concentration increased most when the P dose was lowest (Figure 3). At the end of the experiment the N:P ratio explained most of the variance ($r^2$ = 68 %) in Chl *a* concentration (Table II). This was due to an exponential increase in the concentration of Chl *a* in the two treatments with a high N:P ratio during the last four days.

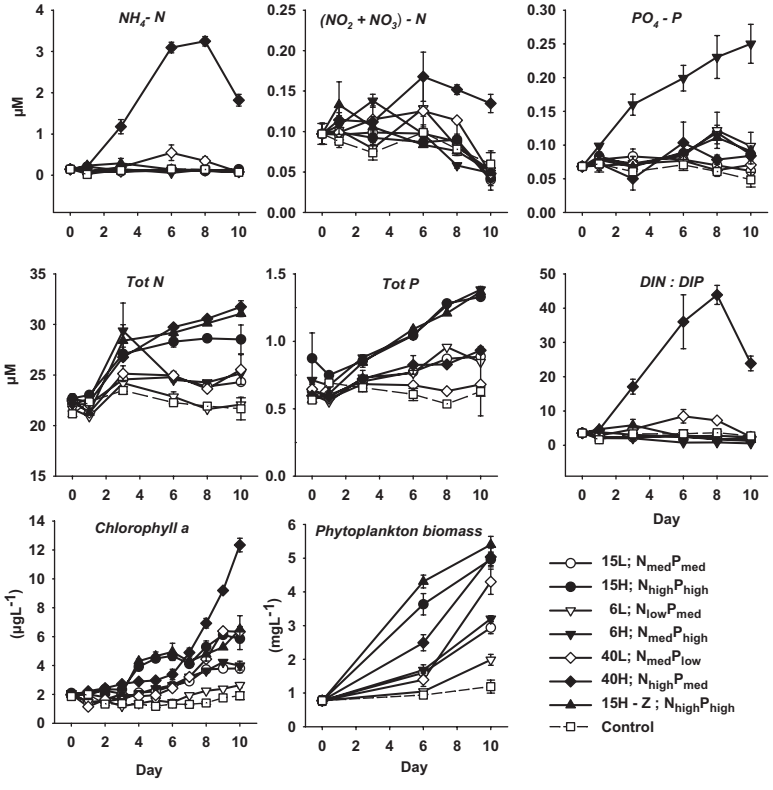


**Fig. 3.** Concentrations (treatment mean ±1 SE) of $NH_4$, $NO_2$ + $NO_3$, $PO_4$, total N, total P, DIN:DIP ratio, Chl *a* concentration and total phytoplankton biomass in the different treatments during the experiment.

*Table II: Results of stepwise regression analysis for the absolute biomasses of the main phytoplankton and zooplankton groups and species and Chl a*

| | Day 6 | | | | | Day 12 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Step variable entered | Parameter estimate $\times 10^{-2}$ | $r^2$ | $F$ | $P$ | Step variable entered | Parameter estimate $\times 10^{-2}$ | $r^2$ | $F$ | $P$ |
| *Dinobryon faculiferum* | no variable met the 0.15 significance level | | | | | 1. N × P | 42.09 | 0.40 | 10.46 | 0.0052 |
| *Pseudopedinella* spp.[b] | 1. N × P | 167.48 | 0.73 | 42.28 | <0.0001 | 1. N × P | 332.25 | 0.83 | 76.14 | <0.0001 |
| | 2. N:P | −0.22 | 0.77 | 3.17 | 0.0951 | | | | | |
| | 3. P | −130.69 | 0.85 | 6.96 | 0.0194 | | | | | |
| *Uroglena* sp.[a] | 1. N:P | 0.10 | 0.64 | 28.14 | <0.0001 | 1. N:P | 0.89 | 0.91 | 158.50 | <0.0001 |
| | 2. N × P | 9.83 | 0.70 | 3.04 | 0.1019 | 2. $N^2$ | −3.11 | 0.94 | 6.65 | 0.0209 |
| *Chrysochromulina* spp. | 1. N × P | 1.71 | 0.16 | 3.11 | 0.0967 | 1. N:P | 0.07 | 0.14 | 2.55 | 0.1296 |
| *Chaetoceros* spp.[a] | 1. $N^2$ | 13.41 | 0.89 | 127.77 | <0.0001 | 1. N:P | 0.45 | 0.76 | 49.57 | <0.0001 |
| | 2. $P^2$ | −79.12 | 0.94 | 13.80 | 0.0021 | | | | | |
| *Skeletonema costatum*[a] | 1. N × P | 427.38 | 0.88 | 120.70 | <0.0001 | 1. P | 390.33 | 0.83 | 77.72 | <0.0001 |
| | 2. $N^2$ | −8.89 | 0.95 | 19.56 | 0.0005 | | | | | |
| | 3. $P^2$ | −13.57 | 0.98 | 28.13 | 0.0001 | | | | | |
| *Nitzschia* spp.[a] | 1. P | 9.49 | 0.44 | 12.50 | 0.0027 | 1. $P^2$ | 45.23 | 0.60 | 24.31 | 0.0002 |
| | | | | | | 2. $N^2$ | −0.91 | 0.68 | 3.48 | 0.0819 |
| Pennate diatoms (others) | 1. N × P | 3.23 | 0.16 | 2.94 | 0.1060 | 1. N:P | 0.32 | 0.36 | 8.91 | 0.0087 |
| | | | | | | 2. $N^2$ | −2.69 | 0.47 | 3.18 | 0.0949 |
| Cryptophyta | 1. N × P | 3.52 | 0.15 | 2.89 | 0.1086 | 1. $N^2$ | 1.55 | 0.29 | 6.70 | 0.0198 |
| | | | | | | 2. $P^2$ | −17.87 | 0.43 | 3.56 | 0.0789 |
| Dinophyta | 1. N | 18.06 | 0.65 | 29.30 | <0.0001 | 1. N × P | 267.65 | 0.37 | 9.48 | 0.0072 |
| | 2. P | 130.23 | 0.74 | 5.12 | 0.0390 | | | | | |
| *M. rubra* (big)[a] | 1. N | 14.86 | 0.93 | 56.02 | 0.0017 | 1. P | 83.40 | 0.43 | 12.08 | 0.0030 |
| | 2. P | 57.87 | 0.98 | 5.38 | 0.1031 | | | | | |
| *M. rubra* (small) | 1. N × P | 128.43 | 0.56 | 20.03 | 0.0004 | 1. P | 35.11 | 0.60 | 23.78 | 0.0002 |
| | | | | | | 2. $P^2$ | −11.09 | 0.71 | 6.00 | 0.0271 |
| APP | 1. N | −3.16 | 0.90 | 37.88 | 0.0035 | 1. N | −6.39 | 0.57 | 21.15 | 0.0003 |
| | | | | | | 2. $N^2$ | 2.85 | 0.77 | 13.17 | 0.0025 |
| Total phytoplankton | 1. N | 17.23 | 0.90 | 148.04 | <0.0001 | 1. N | 34.66 | 0.70 | 36.92 | <0.0001 |
| biomass[a] | 2. N × P | 95.46 | 0.95 | 13.64 | 0.0022 | 2. $N^2$ | 0.54 | 0.82 | 9.70 | 0.0071 |
| | | | | | | 3. N:P | −18.84 | 0.88 | 7.60 | 0.0154 |
| | | | | | | 4. N × P | 167.77 | 0.93 | 8.17 | 0.0134 |
| Chl *a*[a] | 1. N | 66.11 | 0.72 | 41.11 | <0.0001 | 1. N:P | 0.77 | 0.68 | 33.93 | <0.0001 |
| | 2. N × P | 119.75 | 0.81 | 6.57 | 0.0216 | 2. N | 37.13 | 0.90 | 31.28 | <0.0001 |
| | 3. $N^2$ | –29.83 | 0.88 | 8.75 | 0.0104 | 3. N × P | −327.54 | 0.91 | 2.63 | 0.1273 |
| | | | | | | 4. P | 304.55 | 0.95 | 7.91 | 0.0147 |
| Total zooplankton | | | | | | 1. N | 53.22 | 0.79 | 15.20 | 0.0176 |
| biomass[a] | | | | | | 2. P | 141.53 | 0.93 | 6.20 | 0.0885 |
| | | | | | | 3. $N^2$ | −19.88 | 0.98 | 6.29 | 0.1290 |
| Copepoda, adults and copepodites[a] | | | | | | no variable met the 0.15 significance level | | | | |
| Copepoda naupiles[a] | | | | | | no variable met the 0.15 significance level | | | | |
| Cladocera[a] | | | | | | 1. P | 856.65 | 0.45 | 3.25 | 0.1459 |
| | | | | | | 2.$N^2$ | −23.71 | 0.88 | 10.86 | 0.0459 |
| | | | | | | 3. N:P | 0.75 | 0.97 | 5.95 | 0.1350 |
| *Synchaeta baltica*[a] | | | | | | 1. N | 120.79 | 0.88 | 29.93 | 0.0054 |
| | | | | | | 2. $N^2$ | −47.66 | 0.99 | 94.36 | 0.0023 |
| *Syncaheta* spp. (others)[a] | | | | | | 1. N | 74.61 | 0.88 | 29.18 | 0.0057 |

The significance level to enter in the model was 0.15. [a]$\log_{10}(x+1)$ transformed; [b] transformed.

## Phytoplankton biomass

The initial phytoplankton biomass was low, only 0.7 mg $L^{-1}$ (Figures 3 and 4). The phototrophic ciliate *Myrionecta rubra* (Jankowski, 1976) [= *Mesodinium rubrum* (Lohmann, 1908)] seemed to be the main primary producer. With a mean density of $1.2 \times 10^5$ cells $L^{-1}$ and a biomass of 0.46 mg $L^{-1}$, *M. rubra* corresponded to 62% of the total autotrophic biomass (Figure 4). Autotrophic picoplankton (APP) was the second largest group, making up about 20% of the total autotrophic biomass. The rest of the autotrophic biomass consisted mainly of dinoflagellates (dominated by *Dinophysis acuminata*), diatoms, chrysophytes and cryptophytes. Of cyanobacteria a few chroococcal species and *Aphanizomenon* sp. occurred only randomly in the samples, and because of their low abundances they are included in the group 'others' (Figure 4). Until the middle of the experiment, the total autotrophic biomass increased in the different treatments almost in the same way as the Chl *a* concentration (Figure 3). During the last four days the biomass increased most in the two treatments with a high N:P ratio (40H and 40L) due to a high growth of the mixotrophic chrysophyte *Uroglena* sp. However, different from the Chl *a* concentration, the biomass in the treatment with a high N:P ratio and a high dose of N (40H; $N_{high}P_{med}$) only reached the same level as in the highest nutrient enrichment with a Redfield N:P ratio (15H; $N_{high}P_{high}$). Thus, the N dose explained most of the biomass variation even though the P dose also contributed to the biomass increase (Table II). At the end of the experiment the total phytoplankton biomass was at the same level as that during the vernal bloom in the study area [(Kauppila and Lepistö, 2001) J. Suomela, unpublished data]

## Chrysophytes

Of chrysophytes, *Dinobryon faculiferum* (Willén), *Pseudopedinella* spp. and *Uroglena* sp. were dominating. *Dinobryon faculiferum* grew equally well in all nutrient enrichments during the first half of the experiment (Figure 5). This resulted in a negative relationship between the relative biomass of *D. faculiferum* and the N dose, since the total phytoplankton biomass at this time was N-limited (Figure 6, Table III). In the second half of the experiment the biomass of *D. faculiferum* was highest in the treatments with the highest doses of both N and P (significant N × P–effect, Figure 5, Table II). The relative biomass was then poorly ($r^2 = 21\%$) positively related to the P dose (Table III).

Also *Pseudopedinella* spp. grew best in the treatment with the highest doses of both N and P in a Redfield ratio (significant N × P–effect, Figure 5, Table II). The relative biomass of *Pseudopedinella* followed the same pattern as the absolute biomass, but was more clearly also negatively related to the N:P ratio and also positively related to the N dose at the end of the experiment (Figures 5 and 6, Table III).

*Uroglena* sp. differed from the other species in being most clearly affected by the N:P ratio of the nutrient enrichments (Tables II and III). During the last four days *Uroglena* sp. grew exponentially in the two treatments with the highest N:P ratio (40L and 40H) (Figure 5). In these treatments it made up 35–40% of the total phytoplankton biomass at the end of the experiment (Figures 4 and 6). Then the N:P ratio alone explained 91% of the variance in absolute biomass and 88% of the relative biomass of *Uroglena* (Tables II and III).

## Prymnesiophytes

Of prymnesiophytes, *Chrysochromulina* spp. was present in low numbers during the experiment. During the last days the abundances increased especially in the treatments with a high or medium N dose (Figure 5) and was slightly positively ($r^2 = 14\%$) related to the N:P ratio. The relative abundance of *Chrysochromulina* was not

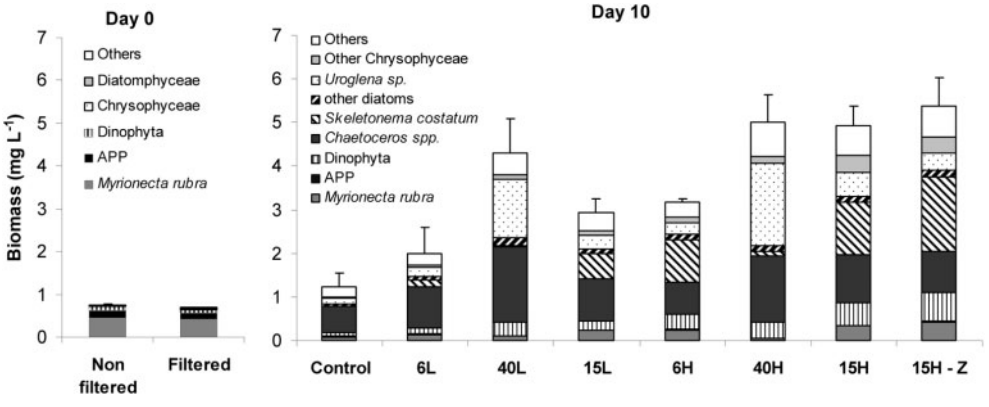


**Fig. 4.** Phytoplankton community composition at the start (Day 0) and at the end (Day 10) of the experiment. At day 0, only the means of the zooplankton removal treatment (filtered) and the non removal (non filtered) treatments are shown, since there were no differences among the other treatments. For treatment codes, see Table I, Figure 2.

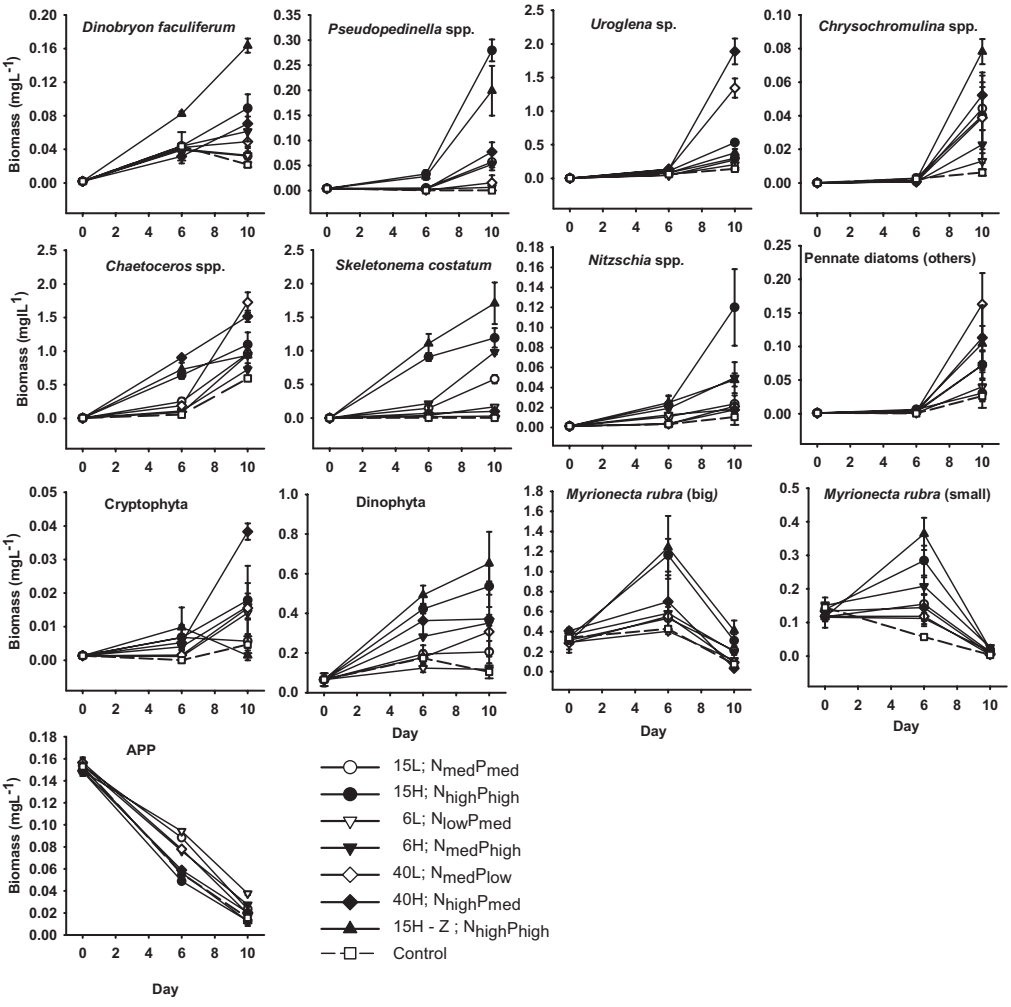


**Fig. 5.** Absolute biomasses of dominating phytoplankton species/groups (Treatment mean ±1 SE) in the different treatments during the experiment.

significantly explained by any factor (Table III), probably due to the low overall abundance of the species.

## Diatoms

Although the biomass of diatoms was low at the start of the experiment, they responded quickly to the nutrient addition and became the dominant algal group. However, the different species responded differently to the nutrient enrichments. Two fastgrowing centric species, *Chaetoceros wighamii* (Brightwell) and *Skeletonema costatum* (Greville) Cleve made up 32–57% of total phytoplankton biomass at the end of the experiment in all treatments (Figures 4 and 6). During the first part of the experiment the biomass of *Chaetoceros* spp. (dominated by *C. wighamii*) was highest in the treatments with the highest N dose (Figure 5). During the later part of the experiment the biomass increased rapidly also in the other treatments, especially in the treatment with a high N:P ratio (40L; $N_{med}P_{low}$) in which ammonium started to accumulate (Figure 5). In the middle of the experiment both the absolute and the relative biomass of *Chaetoceros* spp. was best related to the N dose (Tables II and III). The effect of P was negative (Table II), since in the treatments with a high N dose, *Chaetoceros* was more abundant when the P dose was moderate (40H; $N_{high}P_{med}$) as compared with when it was high (15H; $N_{high}P_{high}$). At the end of the experiment the N:P ratio explained most of the variance in the biomass, since the biomass was highest in both the treatments with high N:P ratios (40L and 40H) (Figure 5, Table II). The relative biomass of *Chaetoceros* was on the other hand highest in the control and in the treatments with the lowest nutrient enrichments, resulting in a negative relation to both the P dose and the product of N and P (Figure 6, Table III).

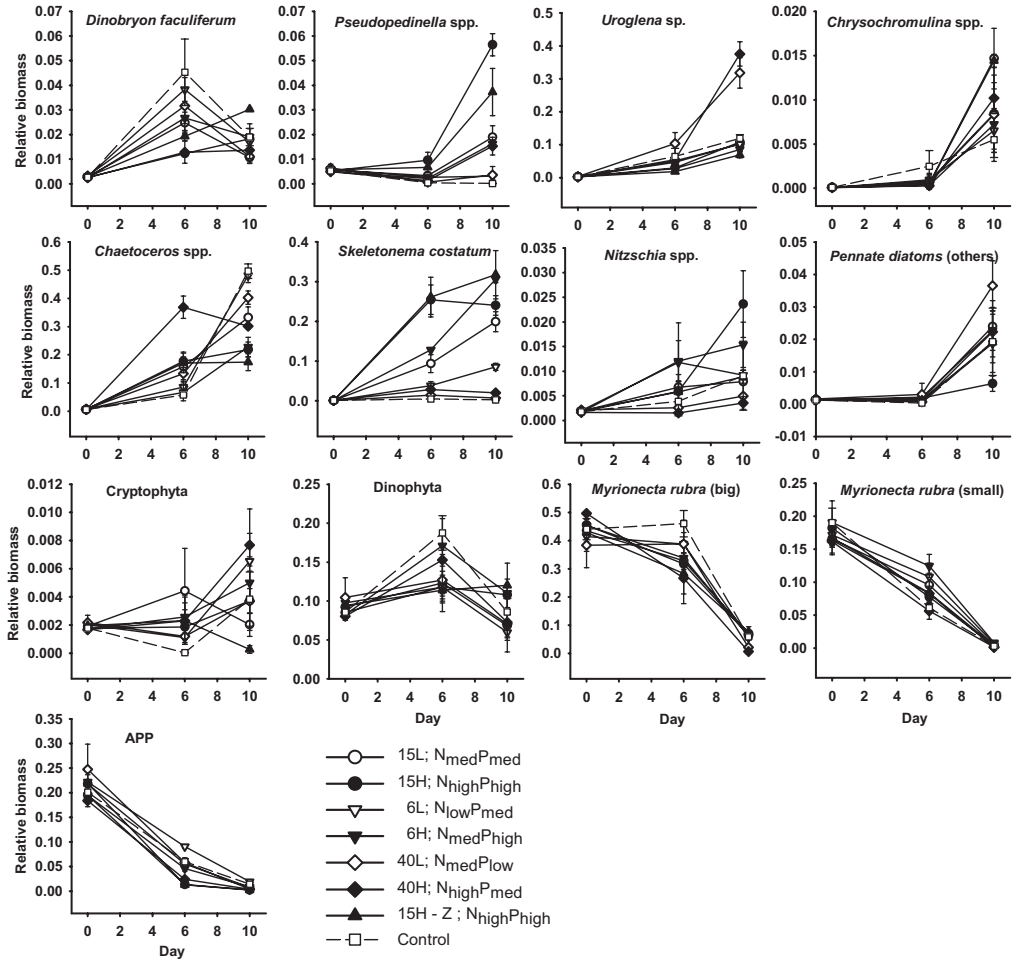


**Fig. 6.** Relative biomasses of dominating phytoplankton species/groups (Treatment mean ±1 SE) in the different treatments during the experiment.

The biomass of the other dominant diatom, *Skeletonema costatum*, increased rapidly in the treatment with the highest doses of both N and P (Figure 5). Consequently, the crossproduct of N and P explained most of the variation in biomass at day 6 (Table II). During the later part of the experiment, the biomass of *S. costatum* increased also in the treatments with a medium N dose in combination with a high or medium P dose and the P dose explained most of the biomass variance (Figure 5, Table II). The relative abundance of *S. costatum* responded almost in the same way as the absolute biomass, but was also negatively correlated to the N:P ratio, due to the relatively high growth in both treatments with low N:P ratios (6L and 6H) and the lowest growth in the treatments with high N:P ratios (40L and 40H) (Figure 6, Table III). The relative biomasses of *Chaetoceros* spp. and *S. costatum* were negatively correlated with each other at the end of the experiment (Spearman's rank correlation, $r^2 = -0.73$, $P < 0.001$).

The biomass of diatoms other than *Chaetoceros* and *S. costatum* was low (Figures 4 and 5).The results of these other diatoms are based on a lower number of counted cells than in the other species, which probably also resulted in the lower coefficient of determination (= the proportion of the total variation in the dependent variable that is explained by the independent variables in the model) (Tables II and III). *Nitzschia* spp. responded on day 6, in terms of absolute biomass, primarily to the P dose, whereas the relative abundance was negatively related to the N:P ratio (Figures 5 and 6, Tables II and III). During the last part of the experiment the biomass increased, especially in the treatment with the highest doses of both N and P, but also in the treatment with a high P dose and a medium N dose. This resulted in a positive relation to the P dose of both the absolute and relative biomass of *Nitzschia*.

The other pennate diatoms were combined in one group and consisted probably also of periphyton that had

*Table III: Results of stepwise regression analysis for the relative biomasses of the main phytoplankton groups and species*

| | Day 6 | | | | | Day 12 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Step variable entered | Parameter estimate | $r^2$ | $F$ | $P$ | Step variable entered | Parameter estimate $\times 10^2$ | $r^2$ | $F$ | $P$ |
| *Dinobryon faculiferum* | 1. 1. N | −3.78 | 0.73 | 43.69 | <0.0001 | 1. P | 0.21 | 0.21 | 4.21 | 0.0570 |
| *Pseudopedinella* spp. | 1. N × P | 42.95 | 0.45 | 13.26 | 0.0022 | 1. N | 0.08 | 0.44 | 12.37 | 0.0029 |
| | 2. N:P | −0.09 | 0.62 | 6.57 | 0.0216 | 2. N:P | −0.16 | 0.76 | 20.04 | 0.0004 |
| | 3. P | −40.23 | 0.72 | 4.95 | 0.0431 | 3. N × P | 150.64 | 0.84 | 7.77 | 0.0145 |
| *Uroglena* sp. | 1. P | −361.73 | 0.52 | 17.49 | 0.0007 | 1. N:P | 0.26 | 0.88 | 116.31 | <0.0001 |
| | 2. $P^2$ | 2445.80 | 0.65 | 5.74 | 0.0301 | 2. N × P | −246.19 | 0.93 | 10.35 | 0.0058 |
| *Chrysochromulina* spp. | no variable met the 0.15 significance level | | | | | no variable met the 0.15 significance level | | | | |
| *Chaetoceros* spp. | 1. $N^2$ | 12.95 | 0.53 | 18.39 | 0.0006 | 1. P | −2.15 | 0.56 | 20.01 | 0.0004 |
| | 2. N × P | −155.77 | 0.82 | 22.86 | 0.0002 | 2. N × P | −2.13 | 0.72 | 9.01 | 0.0089 |
| *Skeletonema costatum* | 1. N × P | 143.18 | 0.66 | 30.66 | <0.0001 | 1. P | 26.46 | 0.68 | 34.69 | <0.0001 |
| | 2. N:P | −0.30 | 0.87 | 23.60 | 0.0002 | 2. N:P | −0.88 | 0.85 | 17.40 | 0.0008 |
| | 4. $N^2$ | −16.88 | 0.89 | 3.11 | 0.0997 | 3. N × P | 858.58 | 0.88 | 2.77 | 0.1182 |
| | 5. N | 30.26 | 0.93 | 6.82 | 0.0215 | 4. $P^2$ | −179.93 | 0.92 | 6.59 | 0.0235 |
| | | | | | | 5. N | −0.21 | 0.98 | 32.79 | <0.0001 |
| *Nitzschia* spp. | 1. N:P | −0.10 | 0.52 | 17.14 | 0.0008 | 1. $P^2$ | 4.51 | 0.56 | 20.22 | 0.0004 |
| Pennate diatoms (others) | 1. N × P | 42.96 | 0.45 | 13.26 | 0.0022 | 1. P | −0.45 | 0.24 | 5.04 | 0.0390 |
| | 2. N:P | −0.09 | 0.62 | 6.57 | 0.0216 | | | | | |
| | 3. P | −40.26 | 0.72 | 4.95 | 0.0431 | | | | | |
| Cryptophyta | no variable met the 0.15 significance level | | | | | no variable met the 0.15 significance level | | | | |
| Dinophyta | no variable met the 0.15 significance level | | | | | 1. $P^2$ | 5.85 | 0.28 | 6.28 | 0.0234 |
| *M. rubra* (big) | 1. N | −4.62 | 0.16 | 3.10 | 0.0976 | 1. N:P | −0.21 | 0.71 | 39.40 | <0.0001 |
| | | | | | | 2. N × P | 203.88 | 0.85 | 13.70 | 0.0021 |
| *M. rubra* (small) | 1. N:P | −0.10 | 0.40 | 10.66 | 0.0049 | 1. N × P | −0.78 | 0.74 | 45.97 | <0.0001 |
| | 2. $N^2$ | −1.37 | 0.56 | 5.35 | 0.0353 | | | | | |
| APP | 1. N | −14.99 | 0.87 | 108.90 | <0.0001 | 1. N | −0.07 | 0.64 | 27.88 | <0.0001 |
| | 2. $P^2$ | −230.19 | 0.93 | 11.23 | 0.0044 | 2. $N^2$ | 0.03 | 0.88 | 31.91 | <0.0001 |
| | 3. $N^2$ | 4.71 | 0.95 | 7.98 | 0.0135 | 3. N × P | −61.74 | 0.91 | 3.38 | 0.0875 |
| | | | | | | 4. N:P | 0.06 | 0.96 | 19.88 | 0.0006 |
| | | | | | | 5. P | −0.18 | 0.98 | 7.55 | 0.0177 |

The significance level to enter in the model was 0.15.

detached from the enclosure walls. At the first half part of the experiment the biomass of pennate diatoms was low and was best related to the crossproduct of N and P (Figures 5 and 6, Tables II and III). The biomass increased mostly during the last four days. The increase was highest in the treatment with a high N:P ratio and with the lower nutrient level (40L; $N_{med}P_{low}$) (Figure 5). At the end of the experiment the absolute biomass of pennate diatoms was positively related to the N:P ratio and negatively related to the N dose, whereas the relative biomass was negatively related to the P dose (Tables II and III). However, the coefficients of determination were low.

## Cryptophytes

Cryptophytes were not abundant during the experiment, but increased during the last few days, especially in the treatment with a high N:P ratio and with the highest addition of ammonium (40H; $N_{high}P_{med}$) (Figure 5). Thus, the biomass of cryptophytes was best related to the N dose at the end of the experiment (Table II).

## Dinoflagellates

Because of the low overall number of dinoflagellates and since many of them were unidentified, they were

combined in one group. Unfortunately, phagotrophic species could not be distinguished from autotrophic or mixotrophic ones. The biomass of dinoflagellates increased in all treatments (Figure 5). The increase was highest in the highest nutrient enrichments, resulting in a positive relation to both the N and P dose (Figure 5, Table II). The relative abundance of dinoflagellates increased until the middle of the experiment, but decreased after that in all treatments (Figure 6). At day 6, the relative biomass was not significantly affected by the nutrient enrichments, but at the end of the experiment it was slightly ($r^2$ = 28%) positively related to the P dose (Figure 6, Table III).

## Myrionecta rubra

Because of the large size differences in *Myrionecta rubra*, the species was divided into two size classes; small (average length 17 μm, range 13–23 μm) and large (average length 27 μm, range 23–33 μm) cells. In the first half of the experiment, both size classes responded positively to the doses of both N and P, which was seen as a high growth in the highest nutrient enrichment (15H; $N_{high}P_{high}$) (Figure 5, Table II). During the second half of the experiment the abundance of *M. rubra* declined abruptly in all enclosures. At the end both size classes were primarily positively related to the P dose (Table II). At day 6, the relative biomass of the large *M. rubra* was highest in the treatments with the lowest nutrient enrichments, resulting in a negative relation to the N dose. The relative biomass of the small individuals was highest in both the treatments with low N:P ratios (6L and 6H) and thus negatively related to the N:P ratio (Figure 6, Table III). Although the relative biomasses were low at the end of the experiment, the large individuals were then negatively related to the N:P ratio and positively related to the product of N and P, whereas the small individuals were negatively related to the product of N and P (Figure 6, Table III).

## APP

Autotrophic picoplankton (APP) were abundant at the start of the experiment, but declined drastically during the experiment in all treatments (Figure 5). Both the absolute and relative abundances of APP were all the time best negatively related to the N dose, since the biomass decreased least in the treatment with the lowest N dose (6L; $N_{low}P_{med}$) (Figures 5 and 6, Tables II and III).

## Zooplankton and effects of zooplankton exclusion

The initial zooplankton community was dominated by the rotifers *Synchaeta baltica* and *Synchaeta* spp. (mainly *S. litoralis* and *S. monopus*), which made up 70% of the total zooplankton biomass (Figure 7). Cladocerans (dominated by *Podon polyphemoides* and *Bosmina coregoni maritima*) and calanoids (*Eurytemora affinis* and *Acartia bifilosa*) were present in low numbers (Figure 7). In the zooplankton removal treatment, the crustacean zooplankton and the rotifer *Synchaeta baltica*, were successfully removed (Figure 7). Smaller *Synchaeta* species and calanoid nauplii were not affected by the filtering. However, because of the low overall number of crustaceans, statistical differences between the zooplankton removal and corresponding non-removal treatments were recorded only for *S. baltica* at the beginning of the experiment (ANOVA, $P < 0.05$).

The zooplankton biomass increased in all treatments during the experiment (Figure 7). At the end of the experiment the zooplankton biomass was highest in the treatments with the highest phytoplankton biomass, resulting in a primary positive relation to the N dose (Figure 7, Table II). Of the zooplankton groups, copepods increased in all treatments but there were no statistically significant effects due to the enrichment of N or P. The cladocerans were on the other hand positively related to the P dose and negatively to

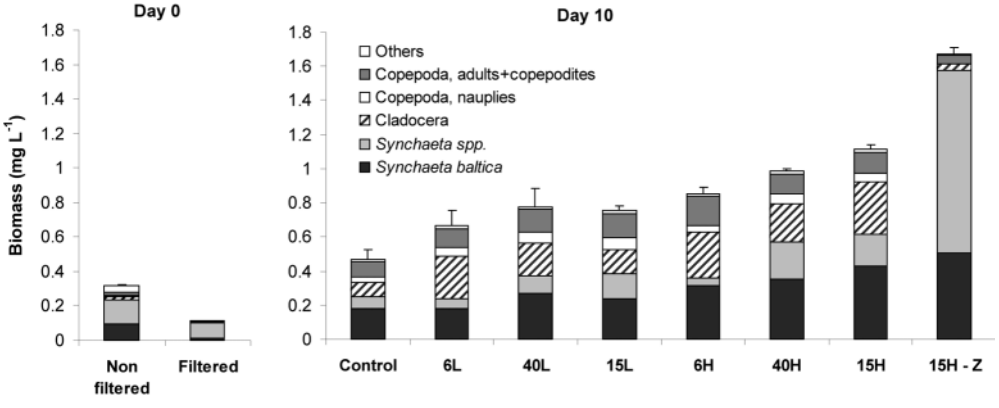


**Fig. 7.** Biomasses of main zooplankton groups at the start (Day 0) and at the end (Day10) of the experiment. At day 0, only the means of the zooplankton removal treatment (filtered) and the non removal (non filtered) treatments are shown, since there were no differences among the other treatments. For treatment codes, see Table I, Figure 2.

the N dose, whereas *Synchaeta baltica* and *Synchaeta* spp. were positively related to the N dose at the end of the experiment.

In the mesozooplankton removal treatment the abundances of copepods and cladocerans increased slightly, but were still low at the end of the experiment. The biomass of *S. baltica* increased to the same level as in the corresponding non-removal treatment, but did not statistically differ from this. The biomass of *Synchaeta* spp. (*S. baltica* not included) increased on the other hand five-fold more in the removal treatment than in the corresponding nutrient enrichment treatment (repeated measures ANOVA, $P < 0.05$) (Figure 7). This resulted in a higher total zooplankton biomass in the mesozooplankton removal than in the non-removal treatment at the end of the experiment (Figure 7).

Exclusion of large mesozooplankton did not affect the Chl *a* concentration (Figure 3). The total phytoplankton biomass was somewhat higher in the zooplankton removal treatment than in the corresponding non removal treatment, even though the difference was not statistically significant (Figure 3). Zooplankton removal affected, however, the composition of the phytoplankton community. The biomasses of *Dinobryon faculiferum* and small *Myrionecta rubra* were significantly higher in the treatment with removal of mesozooplankton (repeated measures ANOVA, $P < 0.01$, Figure 5). The biomasses of *S. costatum*, pennate diatoms and *Chrysochromulina* spp. were also higher in the zooplankton removal treatment than in the corresponding non-removal treatment, but the differences were not statistically quite significant (Figure 5, repeated measures ANOVA, $0.05 < P < 0.2$). Mesozooplankton removal affected negatively the biomasses of *Nitzschia* spp. and *Pseudopedinella* spp. (Figure 5, repeated measures ANOVA, $P = 0.01$).

## DISCUSSION

### Phytoplankton nutrient limitation

The phytoplankton community was N-limited in terms of Chl *a* and total biomass. This is in accordance with predictions based on the relatively low N:P ratio of the water in the area (Kirkkala *et al.*, 1998) and with the generally observed N-limitation of the northern Baltic Sea (Granéli *et al.*, 1990; Kivi *et al.*, 1993). The N-deficiency was clearly observable in the treatments with a medium N dose in which the dose of P (low, medium or high) had no additive effect on the autotrophic growth. When the N dose was high, a high P dose increased the phytoplankton biomass additionally compared with the medium P dose, indicating that also the availability of P started to limit phytoplankton growth.

Although the total phytoplankton biomass and Chl *a* concentration indicated N-limitation, the individual species varied in their responses to the nutrient supply. Even though most species at the first part of the experiment responded both to the N and P supply, there were species that were clearly N-limited and others that were more P-limited. The N-limitation of the total phytoplankton biomass was due to fast growth responses of a few N-limited species. Only the diatom *Chaetoceros* spp. responded clearly primarily to the N dose, whereas the mixotrophic chrysophyte *Uroglena* sp. was positively related to the N:P ratio, also indicating N-limitation. Later in the experiment the differences among the groups became stronger. At the end of the experiment *Uroglena* and *Chaetoceros* spp., were still N-limited, whereas the other chrysophytes and the dinoflagellates were best related to the addition of both N and P. *Skeletonema costatum*, *Nitzschia* spp. and *Myrionecta rubra* responded primarily to the addition of P and seemed thus to be P-limited.

Phytoplankton biomass and Chl *a* concentration responded somewhat differently to the nutrient enrichments. During the later part of the experiment the Chl *a* concentration increased exponentially in the treatment with a high N:P ratio and a high N dose (40H; $N_{high}P_{med}$) and were, from day 7 onwards, higher in this treatment than in the one with the same amount of N but with a higher P dose (15H; $N_{high}P_{high}$). The total algal biomass did not, however, differ between these two treatments. The increase in Chl *a* in the treatment with a high N:P ratio must thus be due either to species-specific differences in cellular Chl *a* content or to an increase in the cellular quota of Chl *a*. The treatments with high N:P ratios became dominated by the mixotrophic chrysophyte *Uroglena* sp., but it is not in our knowledge whether this species can have higher contents of Chl *a* than the other species. Previous studies have shown that if nitrogen is added to N-limited phytoplankton cells, the Chl *a* content of the cells will increase almost immediately, even if there may not be a corresponding response in cell growth or division (Rhee, 1978) [(Geider and Osborne, 1992) p. 176]. This may be an explanation for the high Chl *a* concentrations in the treatments with high N:P ratios, in which ammonium accumulated in the middle of the experiment. The result suggests that Chl *a* concentration is not always a reliable measure of either nutrient limitation or phytoplankton biomass.

### Species-specific responses and resource competition

The experiment started at the first week of June when the vernal bloom was over and the total biomass of phytoplankton was low. The diatoms responded fast to the added nutrients and became the dominant group. Of the diatoms, two species, *Chaetoceros wighamii* and *Skeletonema costatum* became dominating and made up 32–57% of the

total biomass in all treatments at the end of the experiment. Centric diatoms are known for their fast growth responses to nutrient enrichments also from other enclosure experiments (Harrison and Davis, 1979; Sanders *et al.*, 1987; Kuosa *et al.*, 1997) and dominate often in natural eutrophic waters. The two dominating species differed, however, in their responses to the nutrient enrichments. *Chaetoceros* spp. (mainly *C. wighamii*) was positively related to high amounts of ammonium. During the first half of the experiment the growth was highest in the treatments with the highest N dose, but when the ammonium concentrations increased in the treatment with a high N:P ratio and with a medium N dose (40L; $N_{med}P_{low}$), the biomass of the species increased rapidly also in this treatment. *Skeletonema costatum* was, on the other hand, P-limited, even though the growth was most stimulated by high additions of both N and P.

The relative biomasses of *Chaetoceros* spp. and *S. costatum*, which reflect their competitive success, were negatively correlated with each other at the end of the experiment. *Chaetoceros* spp. was in terms of relative biomass highest in the control and in the treatments with the lowest doses of N and P, and negatively related to the P dose. This can be explained by the species' high affinity to low nutrient concentrations (Turpin and Harrison, 1979). Accordingly, both a high growth rate (Sommer, 1989b) and very low half saturation constants for both ammonium and phosphate have been measured for *Chaetoceros* species (Eppley *et al.*, 1969; Finenko and Krupatkina, 1974). The fact that the biomass started a fast increase in the treatment with a high N:P ratio and with a moderate N dose only after the ammonium concentration started to increase, indicates, however, that the species was not the best competitor for N. This may be due to differences in the uptake rates of ammonium of the dominant species (Flynn, 1998). Consequently, in continuous culture experiments *S. costatum* has been shown to have a higher uptake rate (Conway and Harrison, 1977; Turpin and Harrison, 1979; Quarmby *et al.*, 1982; Stolte *et al.*, 1994) but also a higher requirement for ammonium than *Chaetoceros* species (Mickelson *et al.*, 1979). In addition, *S. costatum* is able to modify its uptake rates to changing nutrient regimes (Harrison *et al.*, 1976; Conway *et al.*, 1976). The uptake rate is high especially at high nutrient concentrations and the species has consistently been shown to be favoured by a pulsed nutrient supply (Conway and Harrison, 1977; Quarmby *et al.*, 1982; Turpin and Harrison, 1979). Turpin and Harrison (Turpin and Harrison, 1979) found that ammonium-limited continuous cultures were dominated at equilibrium by *Chaetoceros* species, whereas cultures with one pulse per day were dominated by *S. costatum*, and cultures with eight pulses per day contained both taxa. *Skeletonema costatum* was probably also in our experiment a better competitor for ammonium than *Chaetoceros* at high concentrations, but had also a higher requirement for the nutrient which gave it a competitive disadvantage at low nutrient concentrations. Moreover, *S. costatum* seemed to have a higher requirement for phosphorus than *Chaetoceros*, which is also in agreement with earlier studies (Sakshaug and Andersen, 1986). *S. costatum* has been thought to require more phosphate because it has a high content of ATP associated with a rapid growth rate (Sakshaug and Andersen, 1986). Consistently, Sakshaug and Olsen (Sakshaug and Olsen, 1986) reported an optimum N:P ratio of 9:1 (molar) for *S. costatum* during nutrient saturation. The results suggest that of the two species *Chaetoceros*, during nutrient competition, should be dominant at low nutrient concentrations and at high N:P ratios, whereas *S. costatum* is favoured by high nutrient concentrations and lower N:P ratios.

The low biomass of pennate diatoms in our experiment may be due to the fact that they generally have lower growth rates than the centric species (Grover, 1989b; Sommer, 1989b). This probably explains the biomass increase during the few last days only. Many species of the genus *Nitzschia*, and also other pennate diatoms, have a very low half saturation constant for P and tend to dominate in P-limited habitats (Sommer, 1985; Grover 1989a, 1989b). Grover (Grover, 1989c) demonstrated that an elongate shape with a high surface:volume ratio may be advantageous in the uptake of nutrients over smaller spherical cells. Also in our experiment, the pennate diatoms seemed to be good competitors for P since the relative biomass was negatively related to the P dose at the end of the experiment. Contrary to the other pennate diatoms, *Nitzschia* spp. was positively related to the P dose. This is in agreement with the study of Suttle and Harrison (Suttle and Harrison, 1988), in which *Nitzschia* became dominant in N-limited cultures. Tilman *et al.* (Tilman *et al.*, 1986) found that the competitive ability of diatoms for P decreases at temperatures >17°C, whereas the temperature in our study increased to 19°C in the end of the experiment. However, the genus *Nitzschia* is large, and it is known that there exist species-specific differences in their ability to compete for P (Grover, 1989a).

Although we only studied the effects of N and P, some other nutrients or trace elements may have became limiting during the experiment and affected the competition among the species. Growth of diatoms can be limited by the availability of silica (Conley *et al.*, 1993), but silica is normally not considered as a potentially limiting nutrient in the Baltic Sea. Due to the high growth of diatoms we do not suggest that silica limited phytoplankton growth in our experiment. However, we

cannot rule out the possibility that the availability of Si became limited and affected the competition among the diatom species (Kuosa *et al.*, 1997). Of the two dominating diatom species, data from the literature suggests that Si-limitation would have favoured *Chaetoceros*, since it is more efficient at taking up silicate than *Skeletonema* (Conway and Harrison, 1977; Harrison and Davis, 1979).

According to resource competition theories, picoplankton should be favoured over larger phytoplankton in nutrient limited conditions because of their higher nutrient affinity associated to their small size and higher ratios of surface area to volume (Smith and Kalff, 1982; Fogg, 1986; Raven, 1998). This appears to be so during the nutrient-limited situation at the start of the experiment, when the APP made up 20% of the total autotrophic biomass. This is also in the common range of abundances of APP in the northern Baltic during the season of regenerated production in summer (Kuosa, 1988, 1991). We suggest that the overall decline of both the absolute and even more the relative biomass of APP during the experiment was due to increased predation by both heterotrophic and mixotrophic flagellates and the rotifer *Synchaeta* spp. A decline of the relative contribution of picoplankton to the total autotrophic biomass as the total production increases has also been observed in other studies, both experimentally after nutrient additions, and in field conditions (Agawin *et al.*, 2000). APP decreased least, however, in the N-depleted nutrient enrichments with the lowest N dose (6L; $N_{low}P_{med}$), which could be due to their competitive advantage in taking up N at low concentrations.

The dominance of the autotrophic ciliate *Myrionecta rubra* under the nutrient-limited condition at the start of the experiment indicates that the species is an especially good competitor for nutrients. The species has developed such a strong dependence on its cryptophyte endosymbiont that it has generally been considered as an obligate autotroph (Lindholm, 1985, 1992). However, Gustafson *et al.* (Gustafson *et al.*, 2000) found recently that *M. rubra* is capable of ingesting cryptophyte prey and steal their organelles. In our study area the species usually has a biomass peak during the phytoplankton minimum in the beginning of June. The species has been shown to have a high demand for inorganic N, but also one to four times higher uptake rate for nitrate than diatoms and dinoflagellates (Wilkerson and Grunseich, 1990). The competitive advantage during nutrient limitation is probably increased by the species' very high swimming speed, which allows it to exploit resources from deeper water strata as well as from nutrient patches. *Myrionecta rubra* responded in the first half of the experiment to the nutrient enrichments as a true autotroph, with a high biomass increase in the treatment with the highest doses of both N and P. The relative biomass on the other hand was highest in the treatment with the lowest nutrient additions (significant negative relation to N and the N:P ratio), which confirm the assumption of the good ability of the species to compete for nutrients. The abundance of *M. rubra* decreased drastically in all enclosures during the last four days of the experiment. The smaller *M. rubra* cells were affected by predation of copepods, seen as a significant positive response to the zooplankton removal. However, the larger individuals were not affected by the zooplankton removal. Thus the overall decrease of *M. rubra* was probably due to other things than an increased predation pressure. The species is very fragile and known to be difficult to maintain in cultures (Stoecker *et al.*, 1991).

## Growth of mixotrophic phytoplankton

One mixotrophic species, the chrysophyte *Uroglena* sp., became dominant during the experiment. *Uroglena* sp. responded strongest to the N:P ratio of the treatments, due to its exponential growth in both the enrichments with high N:P ratios whereas the growth was lowest in the treatments with the lowest N:P ratios. In the two treatments with high N:P ratios *Uroglena* made up 30–40% of the total phytoplankton biomass at the end of the experiment. The result is consistent with other experimental studies in which enrichment of N alone has stimulated growth of other mixotrophic flagellates (Jansson *et al.*, 1996). *Uroglena americana* is also known to form dense blooms in Lake Biwa, Japan, under P-limited conditions (Urabe *et al.*, 1999) and is dominant in Japanese lakes with low DIP concentrations and high DIN:DIP ratios (Yoshida *et al.*, 1995). The species is an obligate bacterivore, which by ingesting bacteria receives phospholipids that are essential for its growth (Kimura and Ishida, 1985; Kimura *et al.*, 1986).

Jansson *et al.* (Jansson *et al.*, 1996) suggested that N-limitation in mixotrophic species may be induced by grazing on P-rich bacteria, since both the C:P and N:P ratios are generally lower in bacteria than in phytoplankton (Fagerbakke *et al.*, 1996). Compared with flagellates, bacteria have a higher affinity for phosphate at low concentrations (Currie and Kalff, 1984; Bratbak and Thingstad, 1985; Güde, 1985) and they are also better competitors for dissolved organics, due to their higher surface-to-volume ratio (Sieburth and Davis, 1982; Fenchel, 1986). Thus, under P-limitation, algae that are able to feed upon P-rich bacteria would have a competitive advantage. Consistently, Urabe *et al.* (Urabe *et al.*, 1999) found in a feeding experiment, that the bacterivory of *U. americana* increased when the P concentrations in the lake water decreased.

The abundances of *Dinobryon faculiferum* and *Pseudopedinella* spp., the other potentially mixotrophic chrysophytes present in our experiment, were not positively related to either the N dose nor the N:P ratio of the

nutrient additions. The high growth of these species in the highest nutrient enrichment (15H; $N_{high}P_{high}$) indicates that the species had a high requirement of nutrients. Of prymnesiophytes the potential mixotrophic *Chrysochromulina* spp. was present during the experiment but did not reach high numbers in any treatment. The cell numbers increased during the last four days, especially in the enrichments with a medium or high N dose, resulting in a weak ($r^2 = 0.14$) positive relation to the N:P ratio. In another experiment in the same area in late summer, *Chrysochromulina* spp. bloomed in a treatment with a high ammonium addition in a high N:P ratio (Lagus *et al.*, 2003). *Chrysochromulina* species have also in other studies been found to be favoured by high nitrogen concentrations (Hajdu *et al.*, 1996), and have recently been proved to be able to gain P through bacterivory at low dissolved P concentrations (Stibor and Sommer, 2003).

Dinoflagellates are normally included in the group of potentially mixotrophic phytoplankton, even though the group is poorly known and includes both autotrophic, heterotrophic and mixotrophic species. Generally about half of the species are estimated to be unpigmented heterotrophs (Gaines and Elbrächter, 1987). Most mixo- and phagotrophic species rather prey on other phytoplankton and even on ciliates than on bacteria (Jacobson and Anderson, 1986; Li *et al.*, 1996). In our experiment the growth of dinoflagellates was favoured by the highest nutrient enrichments and seemed to follow the same pattern as the total phytoplankton biomass. The positive growth response to the highest N and P doses could thus be due both to direct autotrophic growth and to predation on autotrophs, which increased in the same treatments.

## Zooplankton

The zooplankton removal treatment decreased the amount of large zooplankton. This in turn resulted in a decreased predation pressure on the smaller zooplankton, seen as a manifold increase in the rotifer *Synchaeta* spp. in the removal treatment. At the end of the experiment the total zooplankton biomass was actually higher in the zooplankton removal treatment than in the corresponding non removal treatment.

The zooplankton removal did not affect the Chl *a* concentrations, but the total phytoplankton biomass was slightly higher in the removal treatment even though the difference was not statistically significant. This is consistent with other studies from the Baltic Sea, where the total phytoplankton biomass has been shown to be primarily nutrient-limited during the summer minimum (Kivi *et al.*, 1993). However, although mesozooplankton did not affect the total phytoplankton biomass, they did have an effect on species composition. This agrees with results from other mesocosm experiments and can be explained by the fact that the suppression of species preferred by the large zooplankton is compensated by growth of other species that are released from grazing by smaller zooplankton and protozoa (Sommer *et al.*, 2003). In the present experiment, zooplankton removal favoured growth of *Dinobryon faculiferum*, small *Myrionecta rubra, Skeletonema costatum*, pennate diatoms and *Chrysochromulina* spp., but depressed *Nitzschia* spp. and *Pseudopedinella* spp. We suggest decreased grazing by copepods and cladocerans as a reason for the increases in the zooplankton removal treatment. Increased grazing by *Synchaeta* spp. or increased competition among the phytoplankton species were on the other hand the probable causes for the decrease of *Nitzschia* and *Pseudopedinella* in the zooplankton removal treatment. Since *Synchaeta* spp. was the dominating zooplankter overall it is probable that it also depressed the biomass of those species in the other treatments and thus affected the results on their responses to the nutrient enrichments. For example, since *Synchaeta* spp. responded primarily to the N dose, the positive response of *Nitzschia* spp. to the P dose could be due to intense predation by *Synchaeta* in the treatments with a high N dose.

We do not think that grazing by copepods was responsible for the differences in phytoplankton growth in the different nutrient enrichment treatments, since the overall abundance of copepods was low and no significant difference in copepod biomass due to the nutrient enrichments was recorded. Overall we do not suggest that the large zooplankton significantly altered the nutrient availability for the algae. The species that were most favoured by the zooplankton removal treatment, were those that grew best in the corresponding nutrient enrichment without removal.

Of the zooplankton, the cladocerans responded positively to the P addition and the rotifers *Synchaeta baltica* and *Synchaeta* spp. to the N-enrichment. This may result from differences in the species nutrient demand and elemental composition of C, N and P (Andersen and Hessen, 1991; Elser and Urabe, 1999; Elser *et al.*, 2003). In freshwater systems, the cladoceran *Daphnia* is known to have a high P content and low body N:P ratio and thus is usually P-limited (Urabe *et al.*, 1997). Copepods generally have a lower P content and higher N:P ratios and are expected more often to be N-limited (Andersen and Hessen, 1991; Sterner and Hessen, 1994). In the Baltic Sea, clear differences in the C:N:P stoichiometry between the zooplankton species have not been recorded (Walve and Larsson, 1999; Pertola *et al.*, 2002), but Walve and Larsson (Walve and Larsson, 1999) reported a lower content of N in the cladocerans *Bosmina longispina maritima* and *Evadne nordmanni* than in the copepod *Acartia* sp. On the other hand, Pertola *et al.* (Pertola *et al.*, 2002) suggested that most of the mesozooplankton in the Baltic Sea are P-limited based

on comparisons between seston and zooplankton N:P ratios. It is thus possible that the cladocerans in our experiment (dominated by *Podon intermedius* and *Bosmina longispina maritima*) responded directly to the enhanced P-availability in accordance with the stoichiometric theory. Alternatively, the positive effect of the P dose can be explained by the increase of preferred food species. This is indicated by the fact that of the phytoplankton species that seemed to be the preferred food for the larger zooplankton (since they were favoured by the zooplankton removal) *D. faculiferum*, *M. rubra* and *S. costatum* were at the end of the experiment primarily related to the P dose as were also the cladoceran zooplankton. On the other hand, N-addition led to an increase in mainly inedible algae, such as *Chaetoceros*.

The elemental composition of rotifers is poorly known, but a high N content has been recorded in the freshwater species *Brachionus rubens* (Rothhaupt, 1995). The positive response to the N dose of the rotifers in this study could thus be due to their higher demand of N compared with other zooplankton. However high abundances of rotifers have also been found to correlate with increased eutrophication (Johansson, 1983) which suggest that the genera were favoured by the overall increased production in the treatments with the highest N doses.

### Role of nutrient competition in determining phytoplankton species composition

In spite of the fact that factors regulating phytoplankton communities have been studied intensively for decades, the role of nutrient limitation, resource competition and the resource-ratio hypothesis in natural phytoplankton communities is still a subject of debate [e.g. (Bothwell, 1985; Harris, 1986; Bulgakov and Levich, 1999; Reynolds, 1999; Sommer, 1999)]. The widespread occurrence of a sestonic stoichiometry near C:N:P = 106:16:1 (Redfield ratio) has sometimes been considered as evidence that phytoplankton growth is nutrient-saturated (Goldman *et al.*, 1979; Harris, 1986). In our study, the fast growth responses due to the nutrient enrichments and the fast depletion of the added nutrients indicate that the phytoplankton was nutrient-limited.

It has been pointed out that competitive exclusion proceeds slowly, especially when the resource supply is variable, and therefore experiments must be run for several weeks to allow competitive dynamics to be discerned (Sommer, 1989a, 1999; Grover, 1990). Such long term experiments can be conducted in controlled flow-through systems, but in mesocosms such as ours, enclosure effects will become prevailing during long incubation times. Since such equilibrium conditions will not occur in natural aquatic ecosystems, it has been suggested that any pattern in phytoplankton community structure that might result from resource competition is destroyed by the high variability in nature (Harris, 1986). However, despite the short duration (10 days) of our experiment, and the dominance of fast-growing opportunistic species, we were able to explain the species-specific responses to the nutrient enrichments by resource competition theories. This indicates that resource competition may be an important factor in determining the phytoplankton community structure also in natural aquatic ecosystems.

Moreover, our results confirm the hypothesis that mixotrophy is a competitively advantageous strategy under certain poor nutrient conditions. High abundances of mixotrophs are usually recorded in humic lakes with low nutrient concentrations and low light conditions (Jones, 2000), but the role of mixotrophs in brackish and marine environments is poorly known. Mixotrophic phytoplankton may have an especially important role in the regeneration of phosphorus in the food web during phosphorus limitation.

Our results suggest that the N:P supply ratio in addition to the absolute nutrient concentration is an important factor structuring the phytoplankton community in the Archipelago Sea. Therefore the phytoplankton community in this system may be sensitive to changes in the N:P supply ratios. A decrease in the water N:P ratio has been assumed to cause an increase of toxic cyanobacterial blooms in the Baltic Sea (Niemi, 1979; Granéli *et al.*, 1990). Cyanobacteria are common only during late summer in the area, and were present in very low numbers during our early summer experiment. On the other hand, we suggest that an increase in the water N:P ratio may increase the abundances of mixotrophic species such as *Uroglena*, but also other species of which some might be harmful. An indication of this occurred in 1988, when a bloom of the toxic *Chrysochromulina polylepis* occurred along the Scandinavian coast during a time when the N:P ratio of the water was high (Dahl *et al.*, 1989). The role of nutrient limitation, resource competition and the N:P ratio in structuring the phytoplankton communities probably varies considerably both spatially and temporally among different systems.

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## REFERENCES


Agawin, N. S. R., Duarte, C. M. and Agusti, S. (2000) Nutrient and temperature control of the contribution of picoplankton to phytoplankton biomass and production. *Limnol. Oceanogr.*, **45**, 591–600.

Andersen, T. and Hessen, D. O. (1991) Carbon, nitrogen and phosphorus content of freshwater zooplankton. *Limnol. Oceanogr.*, **36**, 807–814.

Bothwell, M. L. (1985) Phosphorus limitation of lotic periphyton growth rates: an intersite comparison using continuous-flow troughs (Thompson River system, British Columbia). *Limnol. Oceanogr.*, **30**, 527–542.

Bratbak, G. and Thingstad, T. F. (1985) Phytoplankton-bacteria interactions: Analysis of a model system with both competition and commensalisms. *Mar. Ecol. Progr. Ser.*, **25**, 23–30.

Bulgakov, N. G. and Levich, A. P. (1999) The nitrogen: phosphorus ratio as a factor regulating phytoplankton community structure. *Arch. Hydrobiol.*, **146**, 3–22.

Burkhardt, S. and Riebesell, U. (1997) $CO_2$ availability affects elemental composition (C:N:P) of the marine diatom *Skeletonema costatum*. *Mar. Ecol. Progr. Ser.*, **155**, 67–76.

Carney, H. J., Richerson, P. J., Goldman, C. R. and Richards, R. C. (1988) Seasonal phytoplankton demographic processes and experiments on interspecific competition. *Ecology*, **69**, 664–678.

Conley, D. J., Schelske, C. L. and Stoermer, E. F. (1993) Modification of the biogeochemical cycle of silica with eutrophication. *Mar. Ecol. Progr. Ser.*, **101**, 179–192.

Conway, H. L., Harrison, P. J. and Davis, C. O. (1976) Marine diatoms grown in chemostats under silicate or ammonium limitation. II. Transient response of *Skeletonema costatum* to a single addition of the limiting nutrient. *Mar. Biol.*, **35**, 187–199.

Conway, H. L. and Harrison, P. J. (1977) Marine diatoms growth in chemostats under silicate or ammonium limitation. IV. Transient response of *Chaetoceros debilis*, *Skeletonema costatum* and *Thalassiosira gravida* to a single addition of the limiting nutrient. *Mar. Biol.*, **43**, 33–43.

Currie, D. J. and Kalff, J. (1984) A comparison of the abilities of freshwater algae and bacteria to acquire and retain phosphorus. *Limnol. Oceanogr.*, **29**, 298–310.

Dahl, E., Lindahl, O., Paasche, E. and Throndsen, J. (1989) The *Chrysochromulina polylepis* bloom in Scandinavian waters during spring 1988. In Cosper, E. M., Bricelj, V. M. and Carpenter, E. J. (eds), *Novel Phytoplankton Blooms*, Springer Verlag. pp. 383–405.

de Baar, H. J. W. (1994) von Liebig's law of the minimum and plankton ecology (1899–1991). *Prog. Oceanogr.*, **33**, 347–386.

Downing, J. A. (1997) Marine nitrogen:phosphorus stoichiometry and the global N:P cycle. *Biogeochemistry*, **37**, 237–252.

Edler, L. (ed.) (1979) Recommendations for marine biological studies in the Baltic Sea. Phytoplankton and chlorophyll. *Baltic Mar. Biol. Publ.*, **5**, 1–35.

Elrifi, I. R. and Turpin, D. H. (1985) Steady-state luxury consumption and the concept of optimum nutrient ratios:a study with phosphate and nitrate limited *Selenastrum minutum* (Chlorophyta). *J. Phycol.*, **21**, 592–602.

Elser, J. J. and Urabe, J. (1999) The stoichiometry of consumer-driven nutrient recycling: theory, observations, and consequences. *Ecology*, **80**, 745–751.

Elser, J. J., Acharya, K., Kyle, M., Cotner, J., Makino, W., Markow, T., Watts, T., Hobbie, S., Fagan, W., Schade, J., Hood, J. and Sterner, R. W. (2003) Growth rate-stoichiometry couplings in diverse biota. *Ecol. Lett.*, **6**, 936–943.

Eppley, R. W., Rogers, J. N. and McCarthy, J. J. (1969) Half-saturation constants for uptake of nitrate and ammonium by marine phytoplankton. *Limnol. Oceanogr.*, **14**, 912–920.

Fagerbakke, K. M., Heldal, M. and Norland, S. (1996) Content of carbon, nitrogen, oxygen, sulfur and phosphorus in native aquatic and cultured bacteria. *Aquat. Microb. Ecol.*, **10**, 15–27.

Fenchel, T. (1986) The ecology of heterotrophic microflagellates. In Marshall, K. C. (ed.) *Adv. Microb. Ecol.* Plenum, New York, pp 57–98.

Findlay, D. L., Hecky, R. E., Kasian, S. E. M., Stainton, M. P., Hendzel, L. L., Schindler, E. U. (1999) Effects on phytoplankton of nutrients added in conjunction with acidification. *Freshwater Biol.*, **41**, 131–145.

Finenko, Z. Z. and Krupatkina, D. K. (1974) Effect of inorganic phosphorus on the growth rate of diatoms. *Mar. Biol.*, **26**, 193–201.

Flynn, K. J. (1998) Estimation of kinetic parameters for the transport of nitrate and ammonium into marine phytoplankton. *Mar. Ecol. Progr. Ser.*, **169**, 13–28.

Flynn, K. J. (2002) How critical is the critical N:P ratio? *J. Phycol.*, **38**, 961–970.

Fogg, G. E. (1986) *Picoplankton*. Proceedings of the Royal Society (London), **228**, 1–30.

Forsberg, C., Ryding, S.-O., Claesson, A. and Forsberg, Å. (1978) Water chemical analyses and/or algal assay? Sewage effluent and polluted lake water studies. *Mitt. In. Verein. Limnol.*, **21**, 352–363.

Fujimoto, N., Sudo, R., Sugiura, N. and Inamori, Y. (1997) Nutrient-limited growth of *Microcystis aeruginosa* and *Phormidium tenue* and competition under various N:P supply ratios and temperatures. *Limnol. Oceanogr.*, **42**, 250–256.

Gaines, G. and Elbrächter, M. (1987) Heterotrophic nutrition. In Taylor, F. J. R. (ed.), *The Biology of Dinoflagellates*. Blackwell, Boston, pp. 224–268.

Geider, R. J. and Osborne, B. A. (1992) *Algal Photosynthesis: the Measurement of Algal Gas Exchange*. Chapman and Hall, New York.

Goldman, J. C., McCarthy, J. J. and Peavey, D. G. (1979) Growth rate influence on the chemical composition of phytoplankton in oceanic waters. *Nature*, **279**, 210–215.

Granéli, E., Wallström, K., Larsson, U., Granéli, W. and Elmgren, R. (1990) Nutrient limitation of primary production in the Baltic Sea area. *Ambio*, **19**, 142–151.

Grover, J. P. (1989a). Effects of Si:P supply ratio, supply variability, and selective grazing in the plankton: an experiment with a natural algal and protistan assemblage. *Limnol. Oceanogr.*, **34**, 349–367.

Grover, J. P. (1989b) Phosphorus-dependent growth kinetics of 11 species of freshwater algae. *Limnol. Oceanogr.*, **34**, 341–348.

Grover, J. P. (1989c) Influence of cell shape and size on algal competitive ability. *J. Phycol.*, **24**, 402–405.

Grover, J. P. (1990) Resource competition in a variable environment: phytoplankton growing according to Monod's model. *Am. Nat.*, **136**, 771–789.

Grover, J. P. (1997) *Resource Competition*. Chapman and Hall, London, UK.

Güde, H. (1985) Influence of phagotrophic processes on the regeneration of nutrients in two-stage continuous culture systems. *Microb. Ecol.*, **11**, 193–204.

Gustafson, D. E. Jr., Stoecker, D. K., Johnson, M. D., Van Heukelem, W. F. and Sneider, K. (2000) Cryptophyte algae are robbed of their organelles by the marine ciliate *Mesodinium rubrum*. *Nature*, **405**, 1049–1052.

Hajdu, S., Larsson U. and Moestrup, Ø. (1996) Seasonal dynamics of *Chrysochromulina* species (Prymnesiophyceae) in a coastal area and a nutrient-enriched inlet of the northern Baltic proper. *Botanica Marina*, **39**, 281–295.

Harris, G. P. (1986) *Phytoplankton Ecology: Structure, Function, and Fluctuation*. Chapman and Hall, London.

Harrison, P. J. and Davis, C. O. (1979) The use of outdoor phytoplankton continuous cultures to analyze factors influencing species selection. *J. Exp. Mar. Biol. Ecol.*, **14**, 19–23.

Harrison, P. J., Conway, H. L. and Dugdale, R. C. (1976) Marine diatoms grown in chemostats under silicate or ammonium limitation. 1. Cellular chemical composition and steady-state growth of *Skeletonema costatum*. *Mar. Biol.*, **35**, 177–186.

Healey, F. P. (1985) Interacting effects of light and nutrient limitation on the growth rate of *Synechococcus linearis* (Cyanophyceae). *J. Phycol.*, **21**, 134–146.

Hecky, R. E. and Kilham, P. (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.

Helminen, H., Juntura, E., Koponen, J., Laihonen, P. and Ylinen, H. (1998) Assessing of long-distance background nutrient loading to the Archipelago Sea, northern Baltic, with a hydrodynamic model. *Environ. Model. Softw.*, **13**, 511–518.

Hernroth, L. (1985) *Recommendations on Methods for Marine Biological Studies in the Baltic Sea. Mesozooplankton Biomass Assessment.* Baltic Mar. Biol. Publ. **10**, 1–32.

Interlandi, S. J. and Kilham, S. (2001) Limiting resources and the regulation of diversity in phytoplankton communities. *Ecology*, **82**, 1270–1282.

Isaksson, A. (1998) Phagotrophic phytoflagellates in lakes – a literature review. *Arch. Hydrobiol. Spec. Issues. Advanc. Limnol.*, **51**, 63–90.

Jacobson, D. M. and Anderson, D. M. (1986) Thecate heterotrophic dinoflagellates: feeding behavior and mechanisms. *J. Phycol.*, **22**, 249–258.

Jansson, M., Blomqvist, P., Jonsson, A. and Bergström, A.-K. (1996) Nutrient limitation of bacterioplankton, autotrophic and mixotrophic phytoplankton, and heterotrophic nanoflagellates in Lake örträsket. *Limnol. Oceanogr.*, **41**, 1552–1559.

Johansson, S. (1983) Annual dynamics and production of rotifers in an eutrophication gradient in the Baltic Sea. *Hydrobiologia*, **104**, 335–340.

Jones, R. I. (2000) Mixotrophy in planktonic protists: an overview. *Freshwater Biol.*, **45**, 219–226.

Kauppila, P. and Lepistö, L. (2001) Changes in phytoplankton. In Kauppila, P. and Bäck, S. (eds), *The State of Finnish Coastal Waters in the 1990s. The Finnish Environment*, **472**, pp. 61–70.

Kimura, B. and Ishida, Y. (1985) Photophagotrophy in *Uroglena americana*, Chrysophyceae. *Jpn. J. Limnol.*, **46**, 315–318.

Kimura, B., Ishida, Y. and Kadota, H. (1986) Effect of naturally collected bacteria on growth of *Uroglena americana*, a freshwater red tide Chrysophyceae. *Bull. Jap. Soc. Sci. Fish.* /NISSUISHI., **52**, 691–696.

Kirkkala, T., Helminen, H. and Erkkilä, A. (1998) Variability of nutrient limitation in the Archipelago Sea, SW Finland. *Hydrobiologia*, **363**, 117–126.

Kivi, K., Kaitala, S., Kuosa, H., Kuparinen, J., Leskinen, E., Lignell, R., Marcussen, B. and Tamminen, T. (1993). Nutrient limitation and grazing control of the Baltic plankton community during annual succession. *Limnol. Oceanogr.*, **38**, 893–905.

Koroleff, F. (1976) Determination of nutrients. In Grasshoff, K. (ed.), *Methods of Seawater Analysis*. Verlag Chemie. Weinheim, New York, pp. 117–133.

Koroleff, F. (1979) *The General Chemical Analysis Methods of Sea Water*. Institute of Marine Research, Finland. Meri, no., **7**, 60 pp. (in Finnish).

Kuosa, H. (1988). Occurrence of autotrophic picoplankton along an open sea-inner archipelago gradient in the Gulf of Finland, Baltic Sea. *Ophelia*, **28**, 85–93.

Kuosa, H. (1991) Picoplanktonic algae in the northern Baltic Sea: seasonal dynamics and flagellate grazing. *Mar. Ecol. Progr. Ser.*, **73**, 269–276.

Kuosa, H., Autio, R., Kuuppo, P., Setälä, O. and Tanskanen, S. (1997) Nitrogen, silicon and zooplankton controlling the Baltic Spring bloom: an experimental study. *Estuar. Coast. Shelf Sci.*, **45**, 813–821.

Lagus, A., Silander, M. and Suomela, J. (2003) Influence of nutrient enrichments on cyanobacteria in the Archipelago Sea, Northern Baltic. *Verh. Internat. Verein. Limnol.*, **28**, 607–612.

Levine, S. N. and Schindler, D. W. (1999) Influence of nitrogen to phosphorus supply ratios and physiochemical conditions on cyanobacteria and phytoplankton species composition in the Experimental Lakes Area, Canada. *Can. J. Fish. Aquat. Sci.*, **56**, 451–466.

Li, A., Stoecker, D. K., Coats, D. W. and Adam, E. J. (1996) Ingestion of fluorescently labeled and phycoerythrin-containing prey by mixotrophic dinoflagellates. *Aquat. Microb. Ecol.*, **10**, 139–147.

Lindholm, T. (1985) *Mesodinium rubrum*- a unique photosynthetic ciliate. *Adv. Aquat. Microbiol.*, **3**, 1–48.

Lindholm, T. (1992) *Mesodinium rubrum*- a photosynthetic ciliate. In Reisser, W. (ed.), *Algae and Symbioses*. Biopress Limited, Bristol, pp. 501–514.

Lund, J. W., Kipling, C. and Le Cren, E. D. (1958) The inverted microscope method for estimating algal numbers and statistical basis of estimations by counting. *Hydrobiologia*, **11**, 143–170.

Mickelson, M. J., Maske, H. and Dugdale, R. C. (1979) Nutrient determined dominance in multispecies chemostat cultures of diatoms. *Limnol. Oceanogr.*, **24**, 298–315.

National Board of Waters (1981) *The Analytical Methods used by National Board of Waters and the Environment*. National Board of Waters, Finland, Report, **213**, 136 pp. Helsinki (in Finnish).

Niemi, Å. (1979) Blue-green algal blooms and N:P ratios in the Baltic Sea. *Acta Bot. Fenn.*, **110**, 57–61.

Nygaard, K. and Tobiesen, A. (1993) Bacterivory in algae: a survival strategy during nutrient limitation. *Limnol. Oceanogr.*, **38**, 273–279.

Pertola, S., Koski, M. and Viitasalo, M. (2002) Stoichiometry of mesozooplankton in N- and P-limited areas of the Baltic Sea. *Mar. Biol.*, **140**, 425–434.

Quarmby, L. M., Turpin, D. H. and Harrison, P. J. (1982) Physiological responses of two marine diatoms to pulsed additions of ammonium. *J. Exp. Mar. Biol. Ecol.*, **63**, 173–181.

Quigg, A., Finkel, Z. V., Irwin, A. J., Rosenthal, Y., Ho T-Y., Reinfelder, J. R., Schofield, O., Morel, F. M. M. and Falkowski, P. G. (2003) The evolutionary inheritance of elemental stoichiometry in marine phytoplankton. *Nature*, **425**, 291–294.

Raven, J. A. (1998) The twelfth Tansley Lecture. Small is beautiful: the picophytoplankton. *Funct. Ecol.*, **12**, 503–513.

Redfield, A. C. (1958) The biological control of chemical factors in the environment. *Am. Sci.*, **46**, 205–221.

Reynolds, C. S. (1999) Non-determinism to probability, or N:P in the community ecology of phytoplankton. *Arch. Hydrobiol.*, **146**, 23–35.

Rhee, G-Y. (1978) Effects of N:P atomic ratios and nitrate limitation on algal growth, cell composition, and nitrate uptake. *Limnol. Oceanogr.*, **23**, 10–25.

Rhee, G-Y. and Gotham, I. J. (1980) Optimum N:P ratios and coexistence of planktonic algae. *J. Phycol.*, **16**, 486–489.

Rothhaupt, K. O. (1995) Algal nutrient limitation affects rotifer growth rate but not ingestion rate. *Limnol. Oceanogr.*, **40**, 1201–1208.

Rothhaupt, K. O. (1996) Laboratory experiments with a mixotrophic chrysophyte and obligately phagotrophic and phototrophic competitors. *Ecology*, **77**, 716–724.

Sakshaug, E. and Andersen, K. (1986) Effect of light regime upon growth rate and chemical composition of a clone of *Skeletonema costatum* from the Trondheimfjord, Norway. *J. Plankton Res.*, **8**, 619–637.

Sakshaug, E. and Olsen, Y. (1986) Nutrient status of phytoplankton blooms in Norwegian waters and algal strategies for nutrient competition. *Can. J. Fish. Aquat. Sci.*, **43**, 389–396.

Sanders, R. W. (1991) Mixotrophic protists in marine and freshwater ecosystems. *J. Protozool.*, **38**, 76–81.

Sanders, J. G., Cibik, S. J., D'Elia, C.- F. and Boynton, W. R. (1987) Nutrient enrichment studies in a coastal plain estuary: changes in phytoplankton species composition. *Can J. Fish. Aquat. Sci.*, **44**, 83–90.

SAS Institute Inc. (1996) SAS/STAT Users guide, version 6.12. SAS Institute Inc., Cary, NC, USA.

Schindler, D. W. (1977) Evolution of phosphorus limitation in lakes. *Science*, **195**, 260–262.

Sieburth, J. Mc. N. and Davis, P. G. (1982) The role of heterotrophic nanoplankton in the grazing and nurturing of planktonic bacteria in the Sargasso and Caribbean seas. Ann. *Inst. Oceanogr.*, **58**, 285–296.

Smith, R. E. H. and Kalff, J. (1982) Size-dependent phosphorous uptake kinetics and cell quota in phytoplankton. *J. Phycol.*, **18**, 275–284.

Smith, S. V. (1984) Phosphorus versus nitrogen limitation in the marine environment. *Limnol. Oceanogr.*, **29**, 1149–1160.

Smith, V. H. (1983) Low nitrogen to phosphorus ratios favor dominance by blue-green algae in lake phytoplankton. *Science*, **221**, 669–671.

Smith, V. H. (1986) Light and nutrient effects on the relative biomass of blue-green algae in lake phytoplankton. *Can. J. Fish. Aquat. Sci.*, **43**, 148–153.

Smith, V. H. and Bennett, S. J. (1999) Nitrogen:phosphorus supply ratios and phytoplankton community structure in lakes. *Arch. Hydrobiol.*, **146**, 37–53.

Sokal, R. R. and Rohlf, F. J. (1995) *Biometry*. Freeman, New York

Sommer, U. (1983) Nutrient competition between phytoplankton species in multispecies chemostat experiments. *Arch. Hydrobiol.*, **96**, 399–416.

Sommer, U. (1985) Comparison between steady state and nonsteady state competition: experiments with natural phytoplankton. *Limnol. Oceanogr.*, **30**, 335–346.

Sommer, U. (1989a) The role of competition for resources in phytoplankton succession. In Sommer, U. (ed), *Plankton Ecology: Succession in Plankton Communities*. Springer, Berlin, pp. 57–107.

Sommer, U. (1989b) Nutrient status and nutrient competition of phytoplankton in a shallow, hypertrophic lake. *Limnol. Oceanogr.*, **34**, 1162–1173.

Sommer, U. (1993) Phytoplankton competition in Plussee: a field test of the resource-ratio hypothesis. *Limnol. Oceanogr.*, **38**, 838–845.

Sommer, U. (1994) The impact of light intensity and daylength on silicate and nitrate competition among marine phytoplankton. *Limnol. Oceanogr.*, **39**, 1680–1688.

Sommer, U. (1999) A comment on the proper use of nutrient ratios in microalgal ecology. *Arch. Hydrobiol.*, **146**, 55–64.

Sommer, U., Sommer, F., Santer, B., Zöllner, E., Jürgens, K., Jamieson, C., Boersma, M. and Gocke, K. (2003) *Daphnia* versus copepod impact on summer phytoplankton: functional compensation at both trophic levels. Oecologia, **135**, 639–647.

Sterner, R. W. (1986) Herbivores' direct and indirect effects on algal populations. *Science*, **231**, 605–607.

Sterner, R. W. (1990) The ratio of nitrogen to phosphorus resupplied by herbivores: zooplankton and the algal competitive arena. *Am. Nat.*, **136**, 209–229.

Sterner, R. W. and Hessen, D. O. (1994) Algal nutrient limitation and the nutrition of aquatic herbivores. *Annu. Rev. Ecol. Syst.*, **25**, 1–29.

Stibor, H. and Sommer, U. (2003) Mixotrophy of a photosynthetic flagellate viewed from an optimal foraging perspective. *Protist*, **154**, 91–98.

Stoecker, D. K., Putt, M., Davis, L. H. and Michaels, A. E. (1991) Photosynthesis in *Mesodinium rubrum*: species-specific measurements and comparison to community rates. *Mar. Ecol. Progr. Ser.*, **73**, 245–252.

Stolte, W., McCollin, T., Noordeloos, A. A. M. and Riegman, R. (1994) Effect of nitrogen on the size distribution within marine phytoplankton populations. *J. Exp. Mar. Biol. Ecol.*, **184**, 83–97.

Suttle, C. A. and Harrison, P. J. (1988) Ammonium and phosphate uptake rates, N:P supply ratios, and evidence for N and P limitation in some oligotrophic freshwater lakes. *Limnol. Oceanogr.*, **33**, 186–202.

Tamminen, T. (1990) *Eutrophication and the Baltic Sea: Studies on Phytoplankton, Bacterioplankton and Pelagic Nutrient Cycles*. University of Helsinki, 21 pp.

Terry, K. L., Laws, E. A. and Burns, D. J. (1985) The critical ratio concept: Growth rate variation in the N:P requirement ratio of phytoplankton. *J. Phycol.*, **21**, 323–329.

Tilman, D. (1977) Resource competition between planktonic algae: an experimental and theoretical approach. *Ecology*, **58**, 338–348.

Tilman, D. (1981) Tests of resource competition theory using four species of Lake Michigan algae. *Ecology*, **62**, 802–815.

Tilman, D. (1982) *Resource Competition and Community Structure*. University Press, Princeton.

Tilman, D., Kiesling, R. L., Sterner, R., Kilham, S. S., and Johnson, F. A. (1986) Green, bluegreen and diatom algae: taxonomic differences in competitive ability for phosphorus, silicon, and nitrogen. *Arch. Hydrobiol.*, **106**, 473–485.

Tilman, D., Kilham, S. S. and Kilham, P. (1982) Phytoplankton community ecology: the role of limiting nutrients. *Annu. Rev. Ecol. Syst.*, **13**, 349–372.

Turpin, D. H. (1986) Growth rate dependent optimum ratios in *Selenastrum minutum* (Chlorophyta): implications for competition, coexistence and stability in phytoplankton communities. *J. Phycol.*, **22**, 94–102.

Turpin, D. H. and Harrison, P. J. (1979) Limiting nutrient patchiness and its role in phytoplankton ecology. *J. Exp. Mar. Biol. Ecol.*, **39**, 151–166.

Urabe, J., Clasen, J. and Sterner, R. W. (1997) Phosphorus limitation of *Daphnia* growth: is it real? *Limnol. Oceanogr.*, **42**, 1436–1443.

Urabe, J., Gurung, T. B. and Yoshida, T. (1999) Effects of phosphorus supply on phagotrophy by the mixotrophic alga *Uroglena americana* (Chrysophycea). *Aquat. Microb. Ecol.*, **18**, 77–83.

Utermöhl, H. (1958) Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. *Mitt. Int. Verh. Limnol.*, **9**, 1–38.

Walve, J. and Larsson, U. (1999). Carbon, nitrogen and phosphorus stoichiometry of crustacean zooplankton in the Baltic Sea: implications for nutrient recycling. *J. Plankton Res.*, **21**, 2309–2321.

Wilkerson, F. P. and Grunseich, G. (1990) Formation of blooms by symbiotic ciliate *Mesodinium rubrum*: the significance of nitrogen uptake. *J. Plankton Res.*, **12**, 973–989.

Yoshida, Y., Miyahara, K. and Nakahara, H. (1995) Relationships between the dominant phytoplankton and DIN:DIP ratios in inland waters. *Fish. Sci.*, **61**, 396–400.