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The Role of Picophytoplankton in Lake Food Webs

BY

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Abstract

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Picophytoplankton (planktonic algae and cyanobacteria, $< 2 \mu\text{m}$) constitute an important component of pelagic food webs. They are linked to larger phytoplankton and heterotrophic bacteria through complex interactions including competition, commensalism and predation. In this thesis, field and laboratory studies on the competitive ability of picophytoplankton are reported.

Picophytoplankton were inferior competitors for inorganic phosphorus compared to heterotrophic bacteria. This may be due to the source of energy available for the heterotrophs, while cell-size was of minor importance. However, picophytoplankton were superior to large phytoplankton in the competition for nutrients at low concentrations.

Biomass of picophytoplankton was low in brownwater lakes and high in clearwater lakes, compared to the biomass of heterotrophic bacteria. The results suggest that picophytoplankton are inferior to heterotrophic bacteria in the competition for inorganic nutrients in brownwater lakes, where the production of heterotrophic bacteria is subsidized by humic dissolved organic carbon (DOC)

Relative to large phytoplankton, picophytoplankton were most important in lakes with intermediate water colour, despite the fact that the lowest nutrient concentrations were found in the clearwater lakes. Large phytoplankton in the clearwater lakes may be able to overcome nutrient competition with picophytoplankton by vertical migration.

In conclusion, changes in nutrient content, light availability and concentrations of DOC affect the interactions of heterotrophic bacteria, picophytoplankton and large phytoplankton and are therefore important factors for the structure of the food web in the pelagic zones of lakes.

Keywords: picophytoplankton, competition, heterotrophic bacteria, nutrients, humic DOC.

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This thesis is based on the following papers, which will be referred to in the text by their Roman numerals.

- I. Drakare S. Competition between picoplanktonic cyanobacteria and heterotrophic bacteria along crossed gradients of glucose and phosphate in a laboratory experiment. Accepted by Microbial Ecology.
- II. Blomqvist P., M. Jansson, S. Drakare, A.-K. Bergström & L. Brydsten. 2001. Effects of additions of DOC on pelagic biota in a clearwater system: Results from a whole lake experiment in northern Sweden. Microbial Ecology 42:383-394.
- III. Drakare S., P. Blomqvist, A.-K. Bergström & M. Jansson. 2002. Primary production and phytoplankton composition in relation to DOC input and bacterioplankton production in Lake Öträsket. Freshwater Biology 47(1): 41-52.
- IV. Drakare S., P. Blomqvist, A.-K. Bergström & M. Jansson. Relationships between picophytoplankton and their environment along a gradient of lakes of different water colour and nutrient content. Submitted to Freshwater Biology.

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Introduction

Picophytoplankton in the pelagic food web

Picoplankton, i.e. planktonic organisms 0.2 to 2 μm in diameter (Sieburth *et al.* 1978), are abundant in marine and fresh waters all over the world (Bird & Kalff 1984, Bell & Kalff 2001). This size class includes both prokaryotic and eukaryotic autotrophs as well as heterotrophs, the primary constituents being cyanobacteria and heterotrophic bacteria. All types of picoplankton are important components of the microbial food web, which also includes phagotrophic flagellates and ciliates (Stockner & Porter 1988). The microbial food web provides important pathways for carbon in aquatic systems (Azam *et al.* 1983). The interactions between organisms within the microbial food web (Figure 1) involve a wide range of interacting ecological relationships, including commensalism, competition and predation (Azam *et al.* 1983). Commensalism occurs in the production of dissolved organic carbon (DOC) by phytoplankton and its utilization by heterotrophic bacteria. The production of DOC is dependent on the availability of inorganic nutrients, for which phytoplankton and bacteria compete. Therefore, heterotrophic bacterial production is often related to primary production (Cole *et al.* 1988). Predation by phagotrophic flagellates and microzooplankton on bacteria influences the outcome of competition and provides a feedback route for some of the material flow within the food web. In marine systems, the production of DOC by phytoplankton is extremely important as a source of energy for bacterioplankton. Lakes may differ considerably in this respect since much of the DOC may be of terrestrial origin (Figure 1), imported from their respective catchment areas. In such systems, bacterial production can be more or less independent of phytoplankton production of DOC (Jansson *et al.* 1999), which reduces the importance of commensalism and makes the ability to compete for inorganic nutrients more important. Earlier studies of interactions between the organisms in the microbial food web often neglected picophytoplankton. However, these organisms can account for a substantial proportion of total primary production in lakes and oceans (see, e.g. Stockner 1988), and may therefore be important in competitive interactions for nutrients with both heterotrophic bacteria and

large autotrophic phytoplankton. They may also provide an important food resource for flagellates and larger grazers. In this thesis, the properties of the pelagic food web in lakes are discussed, focusing on the role of picophytoplankton.

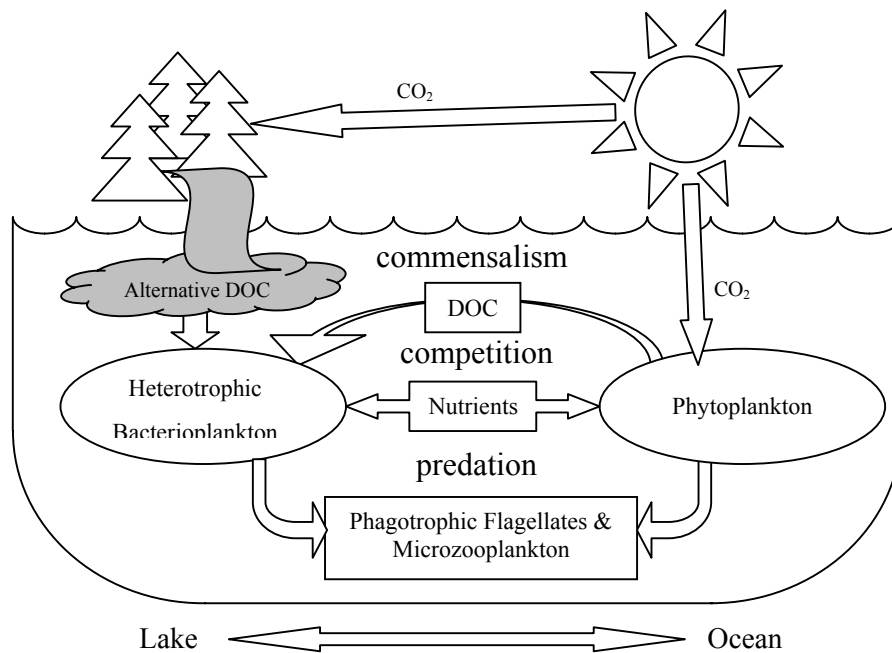


Figure 1. Simplified microbial food web showing the two main functional groups of base producers (heterotrophic bacterioplankton and autotrophic phytoplankton) and the proposed interactions between them in the open water of aquatic systems.

Interactions with other phytoplankton

Both the abundance and biomass of picophytoplankton increase with increases in lake trophic status, measured in terms of either total phytoplankton biomass or total phosphorus, whereas their relative contribution to total photosynthesis and phytoplankton biomass decreases (Søndergaard 1991, Burns & Stockner 1991, Takamura & Nojiri 1994). The latter correlation has been interpreted as a result of competition between phytoplankton of different sizes. A small size is advantageous if nutrients are present in low concentrations, because small cells have a large surface area to volume-ratio, which enhances metabolic rates relative to larger cells (Brock *et al.* 1994). Nutrient uptake is often diffusion-limited and small cells

have a competitive advantage when nutrient concentrations are low, since their small cell size also results in a thin boundary layer surrounding the cells (Fogg 1986, Raven 1986). Furthermore, growth rates of phytoplankton have been found to decrease with increasing cell size in a study by Smith & Kalff (1983), which seems highly relevant although picoplankton-sized cells were not included in their investigation. Uptake rates of phosphate are also size-dependent, with higher rates for smaller organisms (Currie & Kalff 1984). Thus, it seems reasonable to suggest that an important factor favouring picophytoplankton over larger phytoplankton at the oligotrophic end of the trophic gradient is that small phytoplankton have higher growth rates and uptake rates than large phytoplankton.

In relation to large phytoplankton, picophytoplankton are also believed to be favoured by low light conditions. From marine studies it has often been reported that picophytoplankton form deep abundance maxima, at a light level of ca. 1% of the surface light (Glover *et al.* 1986). Such deep maxima have also been reported from lakes at the metalimnion/hypolimnion boundary, again at depths corresponding to ca. 1% of surface light intensities (Fahnenstiel *et al.* 1991, Pick & Agbeti 1991, Stockner & Shortreed 1994, Padisák *et al.* 1997). It has been suggested that the deep oceanic abundance maxima are due to the picophytoplankton having greater photosynthetic and growth efficiency, in comparison to large phytoplankton, at low light levels and at the wavelengths (blue-violet and blue) prevailing at such depths (Glover *et al.* 1986, Raven 1998). A suggested advantage of inhabiting the dimly illuminated deeper water layers is that it allows access to the higher concentrations of nutrients found close to the thermocline (Glover *et al.* 1986). In contrast, when comparisons have been made between lakes, picophytoplankton seem to be favoured by good light conditions, i.e. the clearer the water the higher the abundance of picophytoplankton in the lake (Pick 1991). However, since Pick's evaluation did not include other phytoplankton, the relative importance of picophytoplankton in this relationship cannot be evaluated. Nevertheless, considering all of the above studies, it seems plausible to suggest that picophytoplankton are favoured by good light conditions, and their abundance is maximal in clearwater lakes and oceans. In such systems, they seem to be better than large phytoplankton at exploiting the high nutrient concentrations and low light prevailing in deeper strata.

At the time when the work towards this thesis was initiated, a conceptual understanding was emerging that the abundance of picophytoplankton increases with increases in light and/or nutrient concentrations, but their relative importance decreases.

Interactions with heterotrophic bacteria

The relationships between picophytoplankton and heterotrophic bacteria have not been intensively studied in the past, even though the nutrient and light conditions that seem to favour picophytoplankton have also been suggested to favour heterotrophic bacteria. One reason for this may be that in the stratified clearwater systems where picophytoplankton are important, bacteria are heavily dependent on DOC produced by phytoplankton. However, in many lakes the inflow of humic DOC from the drainage area can give heterotrophic bacterioplankton an alternative energy source. If the importance of commensalism is reduced by such inflows of humic DOC, the ability to compete for inorganic nutrients may become more important.

Competition experiments have shown that bacteria have similar phosphate uptake rates to phytoplankton, as long as they are dependent on organic carbon released by the phytoplankton (Jansson 1993). However, when the bacteria have access to a non-algal organic carbon source, they tend to have higher P-uptake rates than phytoplankton at both low and high phosphate concentrations (Jansson 1993). Other competition experiments have shown that phytoplankton growth is severely suppressed in the presence of bacteria supplied with glucose as an energy source, while the growth of bacteria is hardly affected by the phytoplankton (Rhee 1972). Thus, when the importance of commensalism is reduced by the availability of alternative energy sources for heterotrophic bacteria, phytoplankton appear to become inferior competitors for nutrients.

The competitive success of bacteria compared to phytoplankton has often been explained by their small size, which is said to enhance growth rates and nutrient uptake at low concentrations (Raven 1999, Suttle *et al.* 1990), i.e. the same reasons that have been proposed to favour picophytoplankton compared to large phytoplankton. It could be hypothesised that if cell size was the only factor of importance for the outcome of competition, phytoplankton and heterotrophic bacteria of the same size should be equally good competitors. However, heterotrophic bacterial uptake of nutrients requires access to an organic carbon source, while phytoplankton nutrient uptake depends on solar energy. Therefore, the presence of a suitable energy source could be more important for the outcome of competition than uptake ability. In principle, picophytoplankton and heterotrophic bacteria should compete on equal terms when both are limited more by nutrients than by the supply of energy. Bacteria could be expected to be favoured at high DOC supply rates and low light, whereas picophytoplankton could be expected to be favoured at high light and low DOC concentrations. In a lake situation this implies that picophytoplankton can be expected to have high importance relative to heterotrophic bacteria in clearwater systems, whereas the opposite should be true for brownwater systems.

Questions addressed in this thesis

Picophytoplankton are the main subject of this thesis, especially their relationship to other phytoplankton and heterotrophic bacteria. In this summary, the following questions are addressed:

- How competitive are picophytoplankton for nutrients compared to heterotrophic bacteria? (Paper I)
- How are picophytoplankton in a clearwater lake affected when heterotrophic bacteria are relieved of their dependence on phytoplankton-derived DOC? (Paper II)
- How do temporal changes in DOC loading influence picophytoplankton and other energy mobilizers in dystrophic lakes? (Paper III)
- How important are picophytoplankton along a gradient from clearwater to brownwater lakes? (Paper IV)

Methods

In all the studies included in this thesis, picophytoplankton biomass was determined by collecting picophytoplankton cells on filters, manually counting them and measuring their size using epifluorescence microscopy (MacIsaac & Stockner 1993). The primary autofluorescent pigments used for enumeration are chlorophylls (chlorophyll *a* and divinyl-chlorophyll *a*) and the phycobilin pigments (phycoerythrins and phycocyanins) (MacIsaac & Stockner 1993). Under blue excitation (450–490 nm), eukaryotic phytoplankton fluoresce deep red and cyanobacteria with type I phycoerythrin (PE) fluoresce yellow to orange (Pick 1991). Eukaryotic picophytoplankton were distinguished by localization of chlorophyll in the cell. Under blue excitation non-PE-containing cyanobacteria, containing phycoerythrin type II or phycocyanin, are either invisible or appear dull red (Pick 1991). To avoid this problem, counts were also made with yellow-green excitation (510–560 or 570 nm), which causes all cyanobacteria, including the non-PE-containing cells to fluoresce an intense red (Pick 1991). By measuring cell length and width the volume of each cell was calculated assuming appropriate geometric configurations. Wet weight was calculated assuming that the density of the organisms is the same as that of water (i.e. 1 mg ml⁻¹). The carbon content was assumed to be the same as for large phytoplankton, i.e. 22% and 16% of wet weight for picocyanobacteria and eukaryotic picophytoplankton, respectively (Olrik *et al.* 1998).

Primary production was measured *in situ* by H¹⁴CO₃-uptake (Wetzel & Likens 1991). Particles collected on 0.2 µm filters were assumed to account for total primary production, and the primary production attributable to

picophytoplankton was determined indirectly by subtracting ^{14}C collected on $2\mu\text{m}$ filters from the total primary production. The method has drawbacks since filters tend to clog during filtration, which reduces the effective pore size to below the nominal cut-off, causing the production of picophytoplankton to be underestimated. Their production can also be overestimated if large phytoplankton change in shape or get broken during filtration and thus pass through the filter. Thus, size-fractionated filtration is not a very precise method, but it is the only currently available way to estimate primary production (or chlorophyll biomass) of picophytoplankton.

Methods used for analyses of other plankton, chemical and physical parameters, as well as sampling details, are given in Paper I to IV.

Study sites

Five small and two larger lakes, situated within 20 km of each other, located approximately 100 km northwest of Umeå, in northern Sweden ($64^{\circ}10'\text{N}$, $18^{\circ}55'\text{E}$), were used for the field studies. The lakes represent a gradient from clearwater to brownwater systems (Table 1). This water colour gradient is caused by input of humic substances from the drainage areas which are dominated by mires and coniferous forests. Nutrient concentrations were low, and so were biomasses of phytoplankton.

Table 1. Lake characteristics and mean values of epilimnion chemistry and chlorophyll a of the seven lakes in the gradient. Data from summers (June – Sept) 1996 - 1998.

<i>Lake</i>	<i>Stor Sandsjön</i>	<i>Siholma-sjön</i>	<i>Örträsket</i>	<i>Lilla Björntjärn</i>	<i>Nedre Björntjärn</i>	<i>Byxriver-lidjärn</i>	<i>Övre Björntjärn</i>
Lake Area (km^2)	9.7	0.01	7.3	0.01	0.03	0.01	0.05
Epilimnion Depth (m)	6.6	4.3	12.8	2.6	2.8	1.8	2.4
DOC (mg l^{-1})	3	5	11	12	15	16	22
Absorbance (420 nm)	0.010	0.096	0.273	0.365	0.525	0.483	0.685
Secchi Depth (m)	7.8	4.3	2.4	1.6	1.4	1.8	0.9
Tot-N ($\mu\text{g l}^{-1}$)	208	269	393	419	475	441	558
Tot-P ($\mu\text{g l}^{-1}$)	5	14	20	22	25	21	27
pH	6.6	6.0	6.5	5.3	5.4	5.5	4.7
Chl. a (mg m^{-3})	1.4	2.3	1.6	4.3	0.8	1.0	0.8

Summary of the papers

How competitive are picophytoplankton for nutrients compared to heterotrophic bacteria? (Paper I)

In this study I assessed whether small organisms of the same size, but with different modes of energy uptake, were equally good competitors for phosphorus (P). Phytoplankton and heterotrophic bacterioplankton of similar size were tested because heterotrophic bacteria are suggested to be superior competitors for P, compared to phytoplankton, because of their small size. However, no competition experiments have been made between phytoplankton and heterotrophic bacterioplankton of equal size.

Large organisms were removed, by filtration through 2 μm pore-sized filters, from water collected in August 1996 from the uppermost 2 m of Lake Öträsket. The resulting picoplankton-sized community, dominated by *Cyanobium*-like cyanobacteria and rod-shaped heterotrophic bacteria, was grown in continuous light in the algal growth medium BG-11. Growth rates

Table 2. Maximum specific growth rates found for autotrophic and heterotrophic picoplankton in different treatments and between which days this occurred.

<i>Treatment</i>	<i>Start concentration of</i>		<i>Cyanobacteria</i>		<i>Bacteria</i>	
	<i>Phosphate</i> ($\mu\text{g P l}^{-1}$)	<i>Glucose</i> (mg C l^{-1})	μ (d^{-1})	<i>between</i> <i>days</i>	μ (d^{-1})	<i>between</i> <i>days</i>
P	10	0	0.87	2–4	1.67	1–2
PC	10	0.3	1.10	2–3	3.33	1–2
PCC	10	3	1.50	2–3	2.75	1–2
PP	25	0	0.79	2–4	2.28	1–2
PPC	25	0.3	1.10	2–3	3.60	1–2
PPCC	25	3	1.93	2–3	3.10	1–2
PPP	60	0	0.62	2–5	1.58	0–2
PPPC	60	0.3	1.14	2–3	3.02	1–2
PPPCC	60	3	0.76	3–5	2.65	1–3

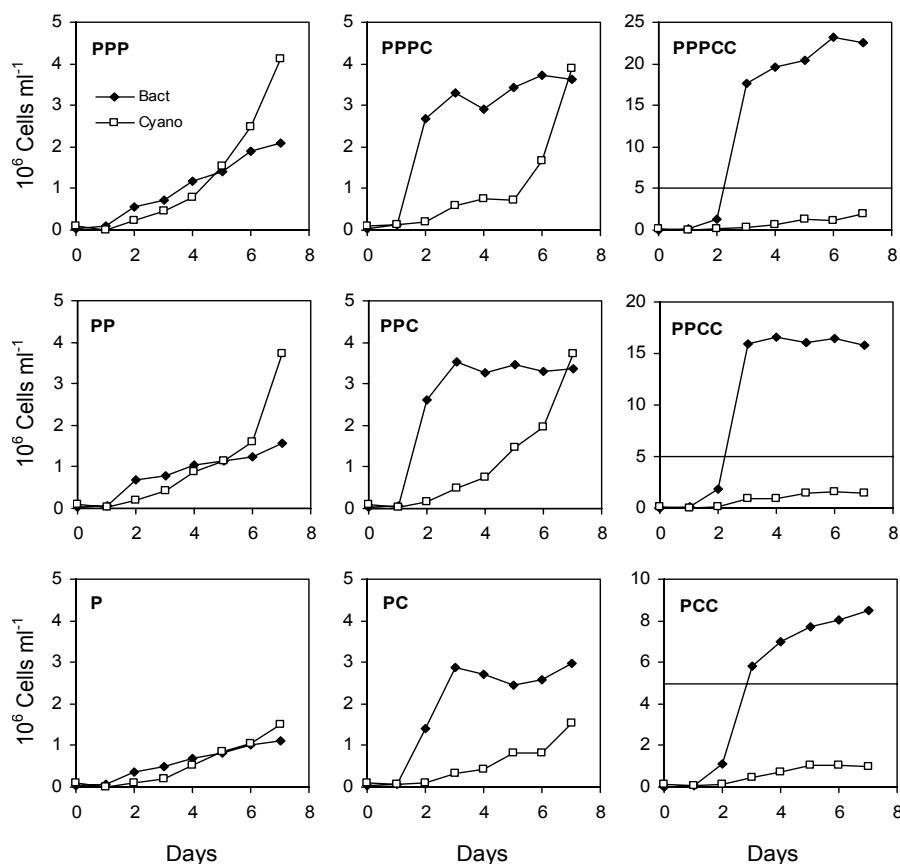


Figure 2. Development of picocyanobacteria and heterotrophic bacteria expressed as cell concentrations over time along crossed gradients of phosphorus (P) and glucose (C). Culture conditions are presented in table 2. Note the different scales of y-axes on the panels to the right. Help lines have been included to correspond with maximum values at the other panels.

and final yields of cells were studied in a crossed gradient of glucose and phosphate in a batch experiment with seven-day incubations. Phosphate-free BG-11 was used as the basal growth medium, to which a range of concentrations of glucose (0, 0.3, and 3 mg C l⁻¹) and phosphate (10, 25 and 60 µg P l⁻¹) were added in all possible combinations, giving nine different treatments. Samples were taken every day for analyses of cell concentration and to monitor soluble reactive phosphorus (SRP). When it was found that the size of the heterotrophic bacteria became considerably larger in some treatments, I decided to analyse the mean cell volume of the organisms on the last day of sampling.

The experiment showed that heterotrophic bacteria had higher maximum growth rates than the picocyanobacteria in all treatments (Table 2, Figure 2). There was also a difference in the timing of the maximum growth rates, bacteria having maximal rates between days one and two and picocyanobacteria after day two of the incubations. In addition, the concentrations of phosphate in the medium decreased below measurable values in all treatments on day two, coinciding with the first days of predominantly bacterial increase, suggesting that heterotrophic bacteria were responsible for most of the initial P-uptake.

Heterotrophic bacteria reached near final concentrations after only three days in all treatments with glucose. Furthermore, they reached approximately the same final concentration in all treatments with 0.3 mg C l^{-1} added as glucose (Figure 2). These results indicate that heterotrophic bacteria became energy-limited after three days. In treatments with no added glucose, growth of heterotrophic bacteria was slow and in these treatments cyanobacteria were able to reach and surpass the numbers of heterotrophic bacteria after five days. Also, in treatments with low additions of glucose, cyanobacteria were able to reach the numbers of heterotrophic bacteria by the end of the experiment, whereas this did not happen in treatments with high glucose additions. Thus, picophytoplankton were only able to compete for P with heterotrophic bacteria when the latter were dependent on phytoplankton-derived carbon as an energy source.

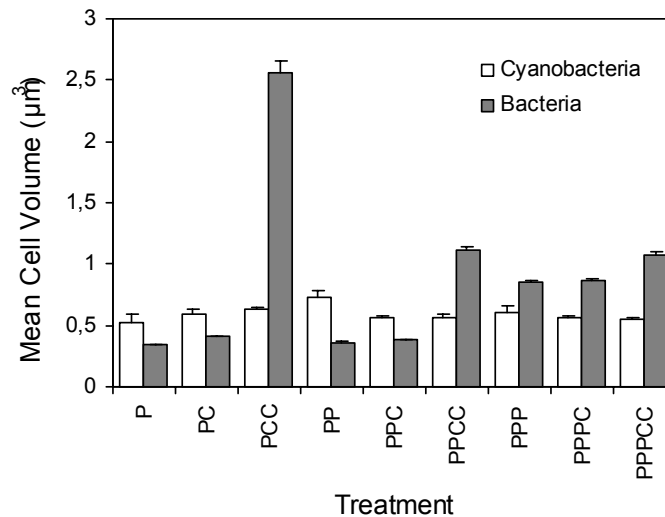


Figure 3. Final mean cell volume of picocyanobacteria and heterotrophic bacteria along crossed gradients of phosphorus (P) and glucose (C). Standard deviations are given as error bars. Culture conditions are presented in Table 2.

Picocyanobacteria did not vary in size between treatments, while heterotrophic bacteria became twice as large in treatments with the highest P additions and four times as large in treatments with the highest glucose and lowest phosphate additions (Figure 3). The increase in size did not seem to affect the competitive ability of the heterotrophic bacteria, suggesting that size is not important to be a successful competitor for P.

The variance in final yield of heterotrophic bacteria between treatments was best explained by the combined effect of glucose and phosphate ($R^2=0.93$). The final yield of picophytoplankton showed a positive response to P and a negative response to glucose ($R^2=0.72$). However, in the picophytoplankton model the effect of glucose was almost identical to the effect of bacteria ($R^2=0.70$). Hence, the negative response to glucose was most likely indirect and caused by the competition for P with heterotrophic bacteria.

Thus, it can be concluded that heterotrophic bacteria are superior competitors for P compared to picophytoplankton and that cell size is of minor importance for the outcome of this competition.

How are picophytoplankton in a clearwater lake affected when heterotrophic bacteria are relieved of their dependence on phytoplankton-derived DOC? (Paper II)

Humic substances alter the light regime for phytoplankton, while providing heterotrophic bacteria with an energy source that makes them independent of phytoplankton-derived carbon. The results in Paper I suggest that additions of an energy source for heterotrophic bacteria, without changes in the light regime, can be enough to change a picoplankton community from one dominated by picophytoplankton to one dominated by heterotrophic bacteria. To verify the results from Paper I, and to test whether the importance of humic substances in brownwater lakes is due to them providing an energy source, the plankton community of a clearwater lake was compared, before and after additions of uncoloured DOC, to that of a nearby brownwater lake.

The initial conditions of the lakes (Lakes Övre Björntjärn and Siholmasjön) were followed during the summer (June to September) of 1996, and additions of DOC in the form of white sugar (sucrose) were made regularly, to lake Siholmasjön, during the summers of 1997 and 1998 to increase the DOC concentration in the epilimnion by 0.5-0.6 mg C l⁻¹. This dose was added since it represents approximately 10-15% of the summer DOC loading of brownwater lakes in the area, which was assumed to be the proportion of the natural DOC input that can be exploited by bacteria (Tranvik 1988, 1998).

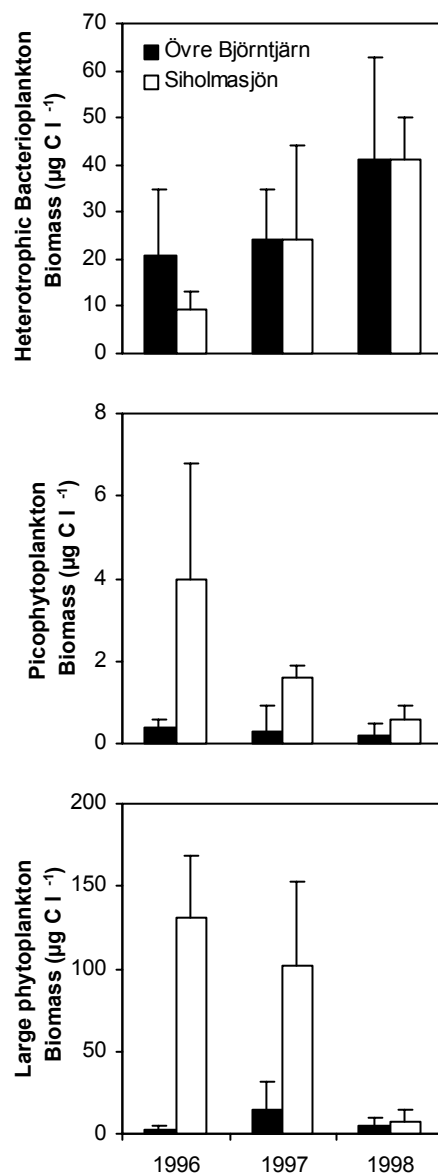


Figure 4. Biomass of heterotrophic bacterioplankton, picophytoplankton and large phytoplankton during summers of 1996–1998 in Lakes Övre Björntjärn (reference) and Siholmasjön (treated with sugar 1997 and 1998). Standard deviations are given as error bars.

The additions resulted in significant reductions in the picophytoplankton biomass, whereas the biomass of heterotrophic bacterioplankton increased (Figure 4). Also, the biomass of large autotrophic phytoplankton decreased, whereas the biomass of mixotrophic and heterotrophic flagellates increased. In terms of production (total primary production, picoplankton primary production and heterotrophic bacterioplankton production) the differences between years were insignificant.

The comparisons between the clearwater and humic lakes showed that the picophytoplankton biomass was higher in the clearwater lake, but they became quite similar in this respect after the additions of sugar to the clearwater lake (Figure 4). In terms of picoplankton primary production, the brownwater and clearwater lake differed markedly, both before and after the additions. Also, when comparing large phytoplankton and heterotrophic bacterioplankton biomass between the lakes, the lakes initially differed, but became quite similar after the sugar additions (Figure 4).

Bacteria utilized only 20% of the added DOC, indicating that the moderate DOC addition was sufficient to saturate the bacterial energy demands and that nutrient limitation prevented higher degrees of utilization. The additions of DOC caused a shift in nutrient allocation patterns in favour of heterotrophic bacteria and away from both pico-sized and large phytoplankton. The results also suggest that it is as an energy source that humic DOC influences the structure and function of the food web in brownwater lakes.

How do temporal changes in DOC loading influence picophytoplankton and other energy mobilizers in dystrophic lakes? (Paper III)

In the studies described in the third paper I tried to determine if, and under what circumstances, picophytoplankton could be important in a dystrophic lake with poor light climate and heterotrophic bacteria that are not dependent on phytoplankton for their energy supplies. The variations in primary production and heterotrophic bacterial production in relation to environmental factors in Lake Öträsket were followed during the summers (June-September) of 1994 to 1997 (picophytoplankton sampling started in 1996), to determine if seasonal and between-year variations in water flow and DOC input to the lake from its large drainage area could alter the relationships between picophytoplankton, heterotrophic bacterioplankton and large phytoplankton.

The total production (primary plus heterotrophic bacterial production) varied over the summer and was dominated by heterotrophic bacteria during the first half of the summer, coinciding with high DOC and high discharge values, while primary production dominated during the second half of the

summer, coinciding with high epilimnion temperature, shallow epilimnion depth and high pH values. Picophytoplankton developed in the second half of the summer, more or less simultaneously with large phytoplankton, and after the decline of the heterotrophic bacterioplankton. In 1996, picophytoplankton dominated primary production, accounting for 54 % of total primary production, whereas the corresponding proportion was only 16 % in 1997. In fact, picophytoplankton primary production was lower in 1997 than in 1996, while both large phytoplankton production and heterotrophic bacterial production were higher in 1997 than in 1996.

The major differences between the two years in which picophytoplankton were analysed were due to the low discharge and low input of humic material in 1996. These factors led to relatively low DOC concentrations and low water colour, which permitted higher light penetration and, thus, higher effective light climate. Another assumed effect of the low discharge is that the nutrient input was lower. In all years there were also differences between the early and late parts of the summer, i.e. the DOC concentrations and discharge were higher in early summer, and the light conditions were better in the first part of the summer.

In this dystrophic lake, it was the temporal differences in allochthonous DOC input that favoured heterotrophic bacteria and disfavoured phytoplankton. No evidence was found that small size was favourable to picophytoplankton compared to heterotrophic bacteria. Within the group of phytoplankton it could be favourable to be small, if the dominance of picophytoplankton among the primary producers in the summer of 1996 is interpreted as indicating that picophytoplankton are able to exploit low concentrations of nutrients better than large phytoplankton. The results were ambiguous regarding light, since picophytoplankton were favoured during the summer with relatively high light levels. On the other hand, both picophytoplankton and large phytoplankton had low biomass and the primary production was low during the time of the year, i.e. the first part of summer, with highest insolation and highest effective light climate.

How important are picophytoplankton along a gradient from clearwater to brownwater lakes? (Paper IV)

In this paper, seven lakes of varying water colour were chosen to test the importance of several environmental variables for explaining picophytoplankton biomass and production. Cross-system comparisons of picophytoplankton have shown that biomass and production increase with trophic status when measured in terms of either chlorophyll *a* or as total phosphorus concentrations (Stockner 1991, Bell & Kalff 2001). In brownwater lakes the relationship could be different as they could have low productivity despite

high concentrations of nutrients. The hypothesis tested was that picophytoplankton should have higher importance, both in absolute terms and relative to heterotrophic bacteria as well as large phytoplankton at the clearwater end of the gradient. In addition, two of the brownwater lakes were fertilized with inorganic phosphorus or nitrogen, and the data were used to test if inorganic nutrient competition was important for the success of picophytoplankton in brownwater lakes.

The lakes, five small (Siholmasjön, Lilla Björntjärn, Nedre Björntjärn, Övre Björntjärn and Byxriverlidjtjärn) and two large (Örträsket and Stor-Sandsjön), were sampled every second (1997, 1998) or every third (1996) week from June to September. Six lakes in 1996 (omitting Lake Stor-Sandsjön), all seven lakes in 1997, and five lakes in 1998 (omitting Lakes Stor-Sandsjön and Lilla Björntjärn) were sampled. Composite samples were taken representing the epilimnion or the whole lake when lakes were unstratified. Environmental variables analysed included nutrients (total P, total N, inorganic fractions of P and N), DOC, water colour, epilimnion depth, Secchi depth, chlorophyll *a*, plankton biomass (heterotrophic bacteria, picophytoplankton, phytoplankton, heterotrophic flagellates and zooplankton) and production (heterotrophic bacterial production and size fractionated primary production). Lake Nedre Björntjärn was fertilized with P (phosphoric acid) in 1997 and 1998, increasing the epilimnion orthophosphate concentrations by $10 \mu\text{g P l}^{-1}$ with each addition. Nitrogen in the form of dissolved ammonium nitrate was added to Lake Byxriverlidjtjärn in 1997 and 1998, each addition increasing the inorganic N by approximately $100 \mu\text{g N l}^{-1}$. The fertilization program is described in detail in Jansson *et al.* (2001).

Picophytoplankton biomass and production were highest in lakes with low DOC (Figure 5). Of all variables measured, DOC was also the most important for explaining variations in biomass ($R^2=0.42$, $p<0.0001$) and production ($R^2=0.38$, $p<0.0001$) amongst the picophytoplankton. Other important variables with negative influence on these parameters included light absorbance of the water and nutrient levels, while variables with an important positive effect included epilimnion depth, Secchi depth and pH. That picophytoplankton biomass and production decreased with increasing nutrient concentrations is contrary to general expectations (Stockner 1991). However, since N and P in all forms had a negative influence on the picophytoplankton it was not surprising that the additions of nutrients to the brownwater lakes induced a negative response in the picophytoplankton.

Picophytoplankton were more important than heterotrophic bacterioplankton in the clearwater lakes than in the brownwater lakes (Figure 6). The results were in agreement with the results in Papers I-III, showing that when heterotrophic bacteria rely mainly on non-algal carbon, as in brownwater lakes, they are superior competitors for nutrients compared

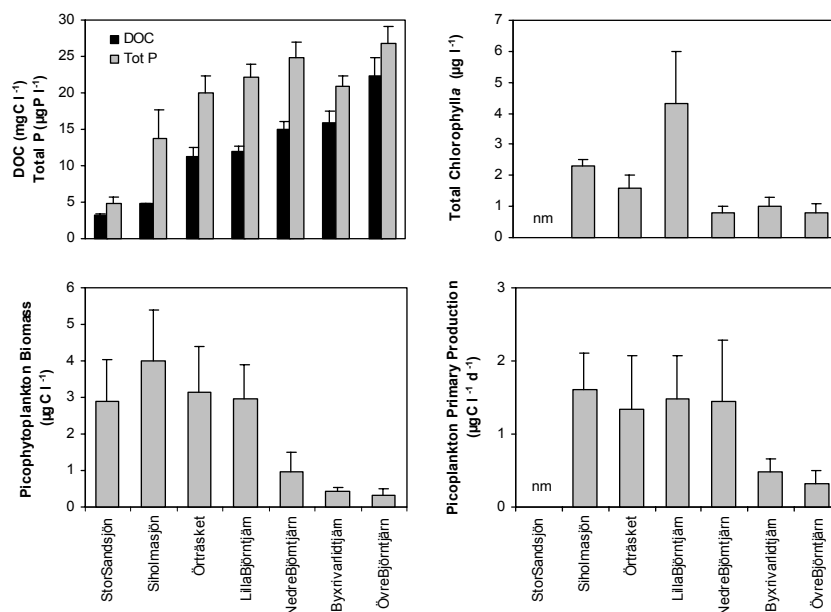


Figure 5. Concentration of DOC, total phosphorus, total chlorophyll a and picophytoplankton biomass and production in the lakes expressed as summer averages with standard deviations.

to picophytoplankton. However, the hypothesis that picophytoplankton should be of greater importance than larger phytoplankton in the clearwater lakes could not be verified. Picophytoplankton did best in lakes with intermediate DOC concentrations, while mixotrophs and other flagellated phytoplankton dominated in the brownwater lakes and large non-motile phytoplankton in the clearwater lakes (Figure 6). Compared to large phytoplankton, the advantages of being small were not sufficient to permit picophytoplankton to dominate over other phytoplankton in the clearwater lakes. In Lake Stor-Sandsjön a reason for the lack of picophytoplankton dominance was probably the fact that the lake was shallow enough to stratify only during calm weather in August. During the rest of the summer the phytoplankton community was dominated by diatoms, thus the low sedimentation rates of picophytoplankton and low tendency to settle out from the euphotic zone and epilimnion were important only during a short period in the year. In Lake Siholmasjön, the large, buoyant, and inedible *Botryococcus terribilis* dominated, further reducing the importance of losses due to sedimentation and grazing. In conclusion, this study showed that picophytoplankton were not better competitors for nutrients against heterotrophic bacteria than phytoplankton in general, and that small size was not an advantage for picophytoplankton in either brownwater or in clearwater lakes.

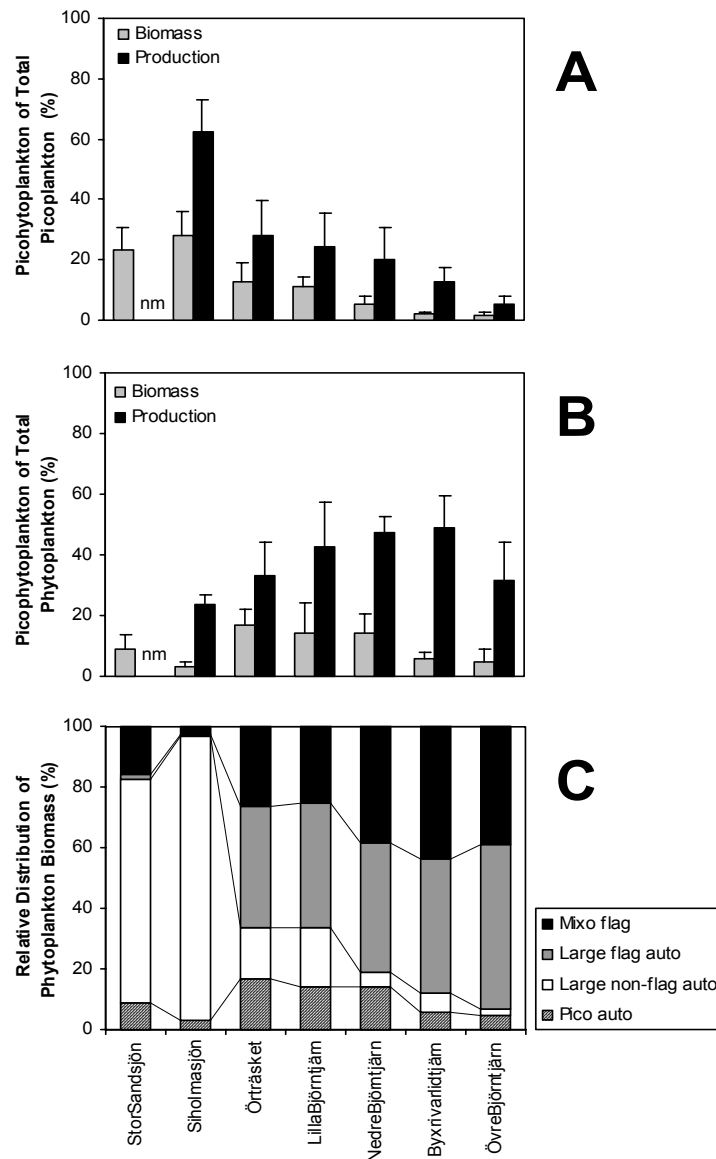


Figure 6. a) Relative distribution of picophytoplankton in relation to total picoplankton biomass and production (i.e. picophytoplankton + heterotrophic bacterioplankton); b) relative distribution of picophytoplankton in relation to total phytoplankton biomass and production; c) relative distribution of mixotrophic flagellates, large flagellated autotrophs, large non-flagellated autotrophs and pico-sized autotrophic phytoplankton in each lake. Error bars shows standard deviations.

General discussion

Overall, the results in this thesis indicate that in several respects picophytoplankton are not very different from other autotrophic phytoplankton. This means that they have a limited ability to compete with heterotrophic bacteria for nutrients when the latter are not relying on phytoplankton-derived energy. It also means that they lack the ability of mixotrophic phytoplankton to overcome competition with heterotrophic bacteria by preying upon them. As a result, picophytoplankton (and other non-motile autotrophs) are not major components of dystrophic lakes, especially relative to heterotrophic bacterioplankton and mixotrophs. In clearwater systems their importance is greater, especially relative to heterotrophic bacteria. The benefits of being small have been forwarded as an explanation for the success of picophytoplankton over larger phytoplankton in oligotrophic clearwater lakes and stratified parts of oceans. However, other groups of phytoplankton have also evolved strategies to help them stay buoyant and to overcome the scarcity of nutrients in the open water, e.g. perennation on the bottom and/or the ability to swim, that may be important in lakes. The success of such organisms may explain why the picophytoplankton in this study were most important in oligotrophic, slightly coloured lakes.

The findings that heterotrophic bacteria were superior competitors for nutrients compared to picophytoplankton, and that the benefits of being small were of minor importance for their success in different systems are central to this interpretation (Papers I, IV). Generally, small cells are suggested to have higher growth and metabolic rates than larger cells, but it has also been suggested that an autotroph should theoretically have a lower specific growth rate than a heterotroph of similar size, due to the large amounts of energy needed to build the photosynthetic apparatus (Raven 1999). It also appears, from data in Paper I, that heterotrophic bacteria in my studies that became much larger than the picophytoplankton were able to suppress the growth of the picophytoplankton. Thus, the competition for P between heterotrophic bacteria and phytoplankton are not size-dependent. Therefore, the suggestion that bacteria are superior competitors for P simply because they are small (Currie & Kalff 1984) was not verified. Instead, heterotrophic bacteria were superior competitors as long as they had access

to a non-algal carbon source: a conclusion supported by data from laboratory experiments on both pico-sized (Paper I) and large phytoplankton (Rhee 1972, Currie & Kalff 1984, Jansson 1993). Similar results could also be obtained for natural phytoplankton and bacterioplankton in our experiment with whole lake additions of DOC in the form of white sugar (Paper II). Furthermore, when heterotrophic bacteria were stimulated by natural additions of humic DOC they were able to suppress phytoplankton growth for several weeks (Paper III). The observed negative effects of DOC on picophytoplankton and other phytoplankton (Paper IV) may therefore mainly be indirect effects of competition with heterotrophic bacteria for nutrients.

Indirect effects of humic DOC on picophytoplankton may also be explained by its function as a light reducer. The presence of even uncoloured DOC can alter the relationship between picophytoplankton and heterotrophic bacteria, and make the food web of the clearwater lake very similar to that of a brownwater lake (Paper I and II). However, humic DOC can affect photosynthetic organisms since it reduces the light climate. Variables connected to good light conditions were important for explaining the increase of picophytoplankton in the comparison between lakes (Paper IV), as also was reported by Pick (1991). Furthermore, the growth of picophytoplankton declined, while that of both large phytoplankton and heterotrophic bacteria increased, during a summer with low effective light climate due to high inputs of humic DOC (Paper III). Thus, no evidence could be found to support the view that picophytoplankton can use low light levels more effectively than other phytoplankton, as suggested by Glover *et al.* (1986) and Raven (1998).

That cell size turned out to be of minor importance for the competition between picophytoplankton and heterotrophic bacteria does not necessarily mean that cell size is never important. The finding that picophytoplankton were less affected than large phytoplankton by whole lake additions of DOC (Paper II), together with similar results from mesocosm studies in lake Balaton (Shafik *et al.* 1998), indicate that small-celled species might have an advantage among phytoplankton. The reason for this may be that by inducing severe nutrient limitation (by additions of DOC) the nutrient competition also increases among phytoplankton, as does the importance of size (Fogg 1986, Raven 1986). Growth and uptake mechanisms related to cell size have also been suggested to explain why picophytoplankton are important relative to other phytoplankton in oligotrophic clearwater systems. However, in paper IV it was shown that, along the clearwater to brownwater gradient, picophytoplankton had their highest relative importance in lakes with intermediate nutrient and DOC concentrations, while mixotrophs and other flagellated phytoplankton dominated in the brownwater lakes and large non-motile phytoplankton in the clearwater lakes. The dominance of

flagellates in brownwater lakes could be a result of the poor ability of all autotrophs, including picophytoplankton, to compete with heterotrophic bacteria for inorganic nutrients (Rhee 1972, Currie & Kalff 1984, Jansson 1993, Paper I), while mixotrophic flagellates could be favoured by their ability to obtain nutrients by phagotrophy (Caron *et al.* 1990, Rothaupt 1996, Bergström *et al.* 2000). The finding that large autotrophs did better than picophytoplankton in the oligotrophic clearwater lakes was unexpected since picophytoplankton often dominate in such lakes (Stockner 1991, Weisse 1993). It also shows that some problems that can be solved by a small cell size, e.g. reduced sinking rate, can also be overcome by large phytoplankton (Paper IV). The lakes included in these studies are relatively small and shallow. It seems, therefore, that phytoplankton in such lakes can use other strategies to overcome nutrient limitation. For example, several phytoplankton species can actively move from nutrient-depleted epilimnia to nutrient-rich hypolimnion water or bottom sediments either by actively swimming (e.g. flagellates) or by the ability to regulate buoyancy with gas vesicles (e.g. many colonial cyanobacteria) and such depth movements can occur either daily or seasonally (Nauwerck 1963, Tilzer 1973, Jones 1991, Pettersson *et al.* 1993). In the oceans and large deep lakes, such adaptations may be less important since the distance to swim or sink is too large to make diurnal movements possible and seasonal movements may be less attractive if signals (e.g. temperature or light) regulating the re-colonization of the water column (Rengefors & Andersson 1998) do not reach the phytoplankton. In the oceans and large lakes the advantages of being small may be sufficient for picophytoplankton to dominate amongst phytoplankton. However, in the lakes evaluated in this thesis it was not an advantage to be small in either the clearwater or the brownwater lakes.

That picophytoplankton were inferior competitors for nutrients compared to heterotrophic bacteria, combined with the fact that most nutrients (e.g. P) were associated with humic DOC, may explain why picophytoplankton biomass and production in the lakes in these studies decreased with increasing nutrient concentrations – a finding that is opposite to the general view about picophytoplankton (Stockner 1991). For the above reasons it can also be suggested that the picophytoplankton fractions of total biomass and production, at the base of the food web, should be mainly a function of DOC. Including all energy mobilizers, i.e. both phytoplankton and heterotrophic bacteria, it may be suggested that if nutrient concentrations increase without increasing the input of humic DOC, picophytoplankton will decrease in proportion, while large phytoplankton increase, as suggested by Stockner (1991). Furthermore, heterotrophic bacterioplankton will also increase their biomass, as the concentrations of phytoplankton-derived DOC will increase. If nutrient concentrations increase because of high inputs of terrestrial DOC,

the findings in this thesis suggest that both picophytoplankton and large phytoplankton tend to decrease in proportion. This is because they are unable to compete with heterotrophic bacteria, with access to a combined carbon and nutrient source from the humic DOC. Therefore, the proportion of picophytoplankton is low at high nutrient concentrations at both high and low inputs of humic DOC. However, at low concentrations of nutrients and DOC, and especially in large stratified open waters with relatively little of littoral and benthic production, heterotrophic bacteria become dependent on phytoplankton-derived energy. This energy is mainly derived from picophytoplankton, which under such circumstances are the superior competitors for nutrients, compared to both heterotrophic bacteria and large phytoplankton. Thus, it can be concluded that picophytoplankton are the best-suited organisms to occupy open waters with low concentrations of nutrients and humic DOC.

The role of picophytoplankton in lake food webs is still far from being fully understood. The papers included in this thesis suggest that these organisms are affected by competition for nutrients with both heterotrophic bacteria and larger phytoplankton, whereas the importance of grazing as a regulatory mechanism on picophytoplankton in lake food webs remains unclear. It is also obvious that future picophytoplankton research must recognize the differences between picophytoplankton species in terms of their role in the pelagic food web. Tools for taxonomic identification of small organisms are rapidly developing. Therefore, studies of picophytoplankton and their role in aquatic food webs should continue to be a fascinating task in the future.

Svensk sammanfattning (Summary in Swedish)

I sjöars födovävar är det inte bara viktigt vem som äter vem. Det är minst lika viktigt att veta om det är konkurrens om näringsämnen eller tillgången på solljus, som begränsar tillväxten av organismer vid födovävens bas. Numera vet man att både växtplankton och bakterieplankton är viktiga organismer i denna bas. Mellan dessa organismtyper tror man att konkurrensen om näringsämnen kan vara hård, särskilt vad gäller oorganiskt fosfor och kväve. Man tror att växtplankton, för att de är relativt stora, är bra på att ta upp näringsämnen när dessa finns i höga koncentrationer och man vet att stora växtplankton därtill har möjlighet att lagra näring för senare bruk. Bakterieplankton, som är mycket små, är bättre på att ta upp näring i låga koncentrationer, men på grund av sin ringa storlek har de endast små möjligheter att lagra denna.

Växtplankton dominerar ofta basproduktionen i klarvattenssjöar, där de genom fotosyntes fixerar solenergin (koldioxid blir organiska energirika kolföreningar) och gör den tillgänglig för de andra organismerna i födoväven. De andra organismerna kan både vara plankton som äter växtplankton, t.ex. små kräftdjur, ciliater eller mixotrofa och heterotrofa flagellater, och bakterieplankton som tar upp kolföreningar från vattnet som läckt ut från växtplanktoncellerna. Även om klarvattenssjöar ofta är näringsfattiga och därför borde vara en gynnsam miljö för bakterieplankton tror man, på grund av att bakterierna är beroende av växtplankton för att få sitt energibehov tillgodosett, att de ändå inte kan dominera basproduktionen. Brunvattenssjöar är en mycket vanlig sjötyp i Sverige. Vattnet i dessa är färgat av humusämnen från skogs- och myrmarker i sjöarnas tillrinningsområden. Humusämnena bildas när organiskt material från träd, gräs och mossor bryts ner av marklevande organismer. I brunvattenssjöar dominerar ofta bakterieplankton basproduktionen på grund av sin förmåga att använda en del av energin från humusämnena. Detta gör att de kan vara oberoende av växtplanktonproduktionen av organiskt kol som i klarvattenssjöarna. Men man tror även att växtplankton i brunvatten sjöar missgynnas av att humusämnena minskar ljustillgången och därmed möjligheten till effektiv fotosyntes.

Relativt nyligen har man hittat växtplankton som är lika små som bakterieplankton och då har det med ens blivit oklart om de hittills giltiga förklarings sambanden mellan storlek och upptags- och tillväxthastigheter gäller. Växtplankton i bakteriestorlek borde ju också vara bra på att ta upp näring i låga koncentrationer. Jag har i mina studier av födoväven i klarvattens- och brunvattenssjöar koncentrerat mig på dessa små växtplankton som kallas pikoväxtplankton eller pikofytoplankton (fyto = växt). Ett pikoväxtplankton är ca 1 µm i diameter (0,001 mm) och namnet syftar på att vikten per cell är ca 1 pikogram (10^{-12} gram).

Laboratorieförsök (Artikel I) visade att konkurrensen mellan pikoväxtplankton och bakterieplankton var oberoende av deras cellstorlek. Bakterierna var de bättre konkurrenterna om näringen. Konkurrensen kontrollerades av om energikällan för bakterieplanktonen var växtplanktonproducerad eller oberoende av dessa genom tillsats av glukos som en alternativ energikälla. Resultaten kunde även verifieras till att gälla även då hela födoväven fanns representerad som i en sjö (Artikel II). Genom att tillsätta vanligt socker till en klarvattenssjö blev födoväven i denna mer lik födoväven i en brunvattenssjö trots att vattenfärgen inte ändrades, dvs. bakterieplankton ökade sin biomassa medan både små och stora växtplankton minskade sin. Även organismer som gärna äter bakterieplankton ökade i antal, t.ex. mixotrofa och heterotrofa flagellater. Pikoväxtplankton och stora växtplankton skiljer sig därför inte åt vad gäller förmågan att konkurrera med bakterieplankton.

Pikoväxtplankton kan, trots nackdelen med humusämnen i vattnet, ändå vara viktiga basproducenter i sjöar med måttligt brunt vatten (Artikel III). Detta till följd av att koncentrationerna av humusämnen i vattnet varierade kraftigt både säsongvis och mellan år. Under ett år med lågt tillflöde av humusämnen stod de tre basproducenterna bakterier, pikoväxtplankton och stora växtplankton för en ungefär lika stor andel var av produktionen. Under ett år med stort inflöde av humusämnen tredubblades produktionen av såväl bakterier som stora växtplankton medan produktionen av pikoväxtplankton istället halverades. Bakterierna gynnades av humusämnena och de stora växtplanktonen gynnades av att även inflödet av näringsämnen var stort. Pikoväxtplanktonen gynnades bara när inflödet var litet. Då blev bakterierna beroende av växtplankton för att få energi. Eftersom tillgången på näring var låg kunde pikoväxtplankton även konkurrera effektivt med stora växtplankton om denna. Den säsongsvisa variationen med stort inflöde av vatten och humusämnen under en mycket kort tid på året strax efter snösmältningen gynnar bakterierna och gör att de dominerar basproduktionen hela den första hälften av sommaren. Trots att ljusklimatet i vattnet är bäst på försommaren, då dagarna är längst runt midsommar, har inte några växtplankton möjlighet att dominera basproduktionen förrän

under andra hälften av sommaren. Ljuset verkar därför ha en liten betydelse för när på sommaren växtplankton, både små och stora, dominerar. Sammantaget tycks alltså den viktigaste effekten av humusämnen i brunvattenssjöar vara som energikälla för bakterieplankton, och att andra effekter är mindre viktiga som att reducera ljustillgången för växtplankton eller att minska tillgängligheten av näringsämnen.

Det visade sig också, när jämförelsen gjordes mellan sju sjöar med olika vattenfärg (Artikel IV), att pikoväxtplankton hade störst biomassa och produktion i sjöar med låg humushalt (DOC). Liksom i de tidigare studierna av enskilda sjöar (Artikel II, III) fanns det mycket pikoväxtplankton om humus- och näringshalten var låg och ljusklimatet var gott. Proportionen var också den förväntade mellan bakterieplankton och pikoväxtplankton, dvs. pikoväxtplankton dominerade i klarvattenssjöarna och bakterieplankton i brunvattenssjöarna. Även i jämfört med stora växtplankton borde pikoväxtplankton ha störst andel i klarvattenssjöarna som är näringsfattiga. Men det visade sig att de istället hade störst andel av växtplanktonbiomassan i de sjöar som var mitt i gradienten. Orsaken till att pikoväxtplankton inte dominerade i klarvattenssjöarna var att de andra växtplankton också hade anpassningar som minskade pikoväxtplanktonens konkurrensfördelar. Dessa anpassningar, t.ex. förmågan att röra sig i djupled för att hämta näring nära botten och ljus nära ytan, kan ha stor betydelse i sjöar som relativt sett är ganska grunda, som sjöarna i denna avhandling.

Denna avhandling visar att pikoväxtplankton påverkas av konkurrens om näring med både bakterier och andra växtplankton. I ett globalt perspektiv bör de vara mest gynnade relativt både bakterier och stora växtplankton i stora djupa sjöar och hav, med liten påverkan från land och botten. I dessa vatten är koncentrationerna av både humus och näringsämnen låga vilket i denna avhandling visat sig gynna pikoväxtplankton mest.

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