Importance of pigmented and colourless nano-sized protists as grazers on nanoplankton in a phosphate-depleted Norwegian fjord and in enclosures

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ABSTRACT: Particulate food sizes of nano-sized colourless flagellates, ciliates, and mixotrophic dinoflagellates were investigated as well as the competition between mixotrophic and strictly heterotrophic protists. Samples were collected during 1 wk in July 1995 from the phosphate-depleted surface layer of the Hylsfjord, Norway, and from nutrient-manipulated enclosures. Grazing experiments conducted on the last day of the study period, using fluorescently labelled algae (FLA) and bacteria (FLB), suggested that phagotrophic protists, 10–20 μm in size, had a considerable grazing impact on nanoplankton, but not on picoplankton. In contrast, smaller colourless flagellates, 5–10 μm in size, ingested FLB significantly. In phosphate-enriched enclosures, the growth of strictly phototrophic protists and of heterotrophic bacteria was stimulated. At the end of the study period, the biomass of colourless flagellates and ciliates was also much higher in phosphate-enriched enclosures than in non-phosphate-enriched enclosures and the surface layer of the fjord. In contrast, mixotrophic dinoflagellates had a similar biomass regardless of phosphate enrichment. Mixotroph share of protist grazing on FLA-sized, 2–5 μm prey was on average 34% in non-phosphate-enriched environments and 12% in phosphate-enriched enclosures. The additional phototrophic mode of nutrition probably gave the mixotrophs better means to compete with strictly heterotrophic protists in environments where the prey density or production was low. The finding that both pigmented and colourless nanoprotoists had a considerable grazing impact on 2–5 μm protists (part of the nano size fraction) is important for the understanding of the relationships between organisms of the microbial food web.

KEY WORDS: Marine/brackish plankton · Bacteria · Pigmented organisms · Flagellates · Ciliates · Phagotrophic protists · Grazing · Mixotrophy · Microbial food web

INTRODUCTION

In recent years, it has become increasingly apparent that a large fraction of the bacterial and primary production in planktonic ecosystems is grazed by phagotrophic protists (for review see Capriulo 1990, Garrison 1991). Nano-sized (2–20 μm) phagotrophic protists have been reported as the major grazers of pico-sized (0.2–2 μm) bacteria (e.g. Rassoulzadegan & Sheldon 1986, Gonzalez et al. 1990), and micro-sized (20–200 μm) phagotrophic protists as important grazers of

nano-sized algae (e.g. Banse 1982, Rassoulzadegan et al. 1988, Burkill et al. 1993).

Recently, however, investigations have suggested that this compartmentalization is too broad: some micro-sized phagotrophs, e.g. dinoflagellates, have been reported to feed preferentially on other micro-sized organisms (e.g. Hansen 1992, Strom & Buskey 1993, Neuer & Cowles 1994, Nakamura et al. 1995). Similarly, for many larger nano-sized colourless flagellates, the optimum prey size seems to be in the nano size fraction (Sherr & Sherr 1991). Havskum & Riemann (1996) reported that the majority of the 10–20 μm colourless flagellates in the Bay of Aarhus (Denmark) did not ingest pico-sized heterotrophic bacteria.

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Instead, these nanoflagellates were frequently observed with nano-sized algae in their food vacuoles. Frequent observations of nano-sized prey in food vacuoles of larger nanoflagellates have also been made by Patterson et al. (1993) and Vørs et al. (1995). Uptake experiments using fluorescently labelled nano-sized prey (fluorescently labelled algae, FLA) also supported the idea that these were taken up not only by micro-sized, but also by nano-sized protists (Sherr et al. 1991). In addition, the division of protists as feeders on either heterotrophic or phototrophic food sources seems to be impossible: picoplankton has been reported to include not only pico-sized, heterotrophic bacteria but also, often to a considerable extent, <2 µm pigmented organisms, i.e. cyanobacteria, prochlorophytes, and small pigmented eukaryotes (e.g. Li et al. 1992, Sherr & Sherr 1994 and references therein). Hall et al. (1993) reported that most picoplankton grazers were feeding on both heterotrophic and phototrophic prey. Similarly, phagotrophic protists feeding on nano-sized pigmented protists have also been observed to ingest nano-sized colourless protists and detritus (e.g. Patterson et al. 1993). On the other hand, selectivity for prey quality by some phagotrophic protists has also been reported (e.g. Nygaard et al. 1988, Landry et al. 1991, Verity 1991, Neuer & Cowles 1995).

Another important finding that changed the current understanding of the role of specific protists in the microbial food web was the occurrence of mixotrophy among pelagic protists from various taxonomic groups (for review, see Riemann et al. 1995). Mixotrophic nanoflagellates, generally <10 µm in size, have been observed as major picoplankton grazers in several experiments conducted in both freshwater (e.g. Bird & Kalff 1986, Sanders et al. 1989) and marine environments (e.g. Nygaard & Tobiesen 1993, Havskum & Riemann 1996). The ecological importance of micro-sized, pigmented dinoflagellates as grazers of small ciliates (≤20 µm) has been reported by Bockstahler & Coats (1993a, b). While many investigations have focused on either <10 µm or >20 µm phagotrophic protists, still little is known about the prey size spectrum of the phagotrophic 10–20 µm protists.

The objective of this study was to examine the relationship between bacterioplankton, pigmented plankton, and various groups of nano-sized phagotrophic protists in a phosphate-depleted brackish environment and in phosphate-enriched enclosures. The study site, the Hylsfjord, is a part of the Sandsfjord system, Norway, where phosphorus in the surface layer is generally depleted in the summer (Aure & Rey 1992, Thingstad et al. 1993). Pronounced luxury consumption of added phosphate by the >1 µm and 0.2–1 µm size fractions in the brackish layer of the Sandsfjord led Thingstad et al. (1993) to suggest that the growth not

only of pigmented organisms but also of heterotrophic bacteria was phosphate-limited. In the present study, phosphate was added to stimulate the growth of primarily bacteria and pigmented organisms and secondarily of phagotrophic protists. Glycine was added to examine possible carbon limitation, and nitrate was added to all enclosures to prevent the complication of shifting the systems into nitrate limitation. We hypothesized that in this phosphate-depleted environment, mixotrophic protists could have a significant impact on the organic matter flow in the microbial food web, and that the success of the mixotrophs was controlled by a rapid succession of strict phototrophs and strict heterotrophs induced by phosphate enrichment. From short-term uptake experiments using fluorescently labelled bacteria (FLB) and algae (FLA), the significance of different groups of nano-sized colourless flagellates, mixotrophic flagellates, and ciliates as grazers on pico- and/or nano-sized organisms was determined, and the relative phagotrophic importance of mixotrophs and strict heterotrophs was evaluated.

MATERIAL AND METHODS

Study site. This study was carried out during the period 5 to 13 July 1995 from the Norwegian RV 'Håkon Mosby'. The study site was the Hylsfjord, Norway (59° 30' N, 6° 30' E). Water samples were collected from the brackish layer (7‰) of the fjord at 1 m depth and from enclosures, 1 m in diameter and 2 m deep, that were situated in the fjord and filled simultaneously with approximately 1600 l unfiltered surface water on 5 July between 07:00 and 08:00 h. For the reasons described above, 4 different nutrient manipulations were performed: [+N] addition of nitrate as NaNO₃, [+NG] addition of nitrate and glycine, [+NP] addition of nitrate and phosphate (phosphate as KH₂PO₄), and [+NPG] addition of nitrate, phosphate and glycine. The nutrients were added daily at 20:00 h, the first time on 5 July 1995. The following amounts were added: 157 µmol KH₂PO₄, 3140 µmol NaNO₃, and 6280 µmol glycine. The water temperature rose from 12.0°C at the beginning of the investigation period to 14.2°C at the end. Further details about sampling and chemical and physical analyses are presented by Thingstad et al. (unpubl.). Since the addition of glycine did not stimulate bacterial growth (T. F. Thingstad, B. Riemann, D. Lefèvre, H. Kaas, T. G. Nielsen unpubl.), the +N/+NG enclosures were considered as replicates in this study as were the +NP/+NPG enclosures. Water samples were collected without prefiltration on 6, 7, 9, 11, and 13 July at 08:00 h from 2 phosphate-enriched enclosures, from 2 non-phosphate-enriched enclosures and from the fjord at 1 m depth. On 5 July, water sam-

ples were only taken from the enclosures immediately after filling and before nutrient enrichment.

Determination of pico- and nanoplankton carbon biomass and of nanoprotist phagotrophy by microscopy. Heterotrophic bacteria and pigmented picoplankton organisms were grouped according to their size, enumerated, and measured using preparations made for epifluorescence microscopy only (see below). Pigmented organisms $>2\text{ }\mu\text{m}$ and colourless, nano-sized protists were identified using differently fixed and stained subsamples of the same sample prepared for ordinary light and for epifluorescence microscopy respectively. Samples fixed in Lugol's solution were viewed in an inverted microscope (Leica DM IRB) and in an ordinary light microscope (Leica DM RB) with 100 to 1000 $\times$ magnification (HMSO 1990). DAPI-stained and unstained preparations were made from glutaraldehyde-fixed subsamples (see below) and viewed in an Olympus BH2 epifluorescence microscope with 1250 $\times$ magnification. *Chaetoceros wighamii* cells were enumerated and measured in a 1 ml Sedgewick sedimentation chamber using a Leica DM RB microscope with 400 $\times$ magnification. Colourless nano-sized protists and pigmented organisms, except *C. wighamii*, were enumerated and measured on an Olympus BH2 epifluorescence microscope with 1250 $\times$ magnification. Water samples for epifluorescence microscopy were fixed with ice-cold 4% glutaraldehyde (quality as for electron microscopy) with the same salinity as the seawater in a 1:1 mixture with the seawater, resulting in a 2% final concentration. Unstained and DAPI-stained subsamples were filtered onto 0.2 μm and 0.8 μm black polycarbonate filters (diameter 25 mm) on the sampling day and stored at -30°C to preserve the chlorophyll autofluorescence (Porter & Feig 1980, Bloem et al. 1986, Sanders et al. 1989).

The nano-sized protists were assigned to 19 different groups according to their size, nutrition (see below), and taxonomic relationships. Pigmented picoplankton was always sparse ($<1\%$ of the picoplankton biomass) and not differentiated into prokaryotes and eukaryotes. The cell size of most organisms was expressed as equivalent spherical diameters, $\text{ESD} = (\text{cell volume}/0.524)^{1/3}$. Heterotrophic bacteria were differentiated into 3 groups: small picobacteria (length $<2\text{ }\mu\text{m}$, ESD $0.6\text{ }\mu\text{m}$), large picobacteria (length 2 to $8\text{ }\mu\text{m}$, average length $2.4\text{ }\mu\text{m}$, ESD $0.9\text{ }\mu\text{m}$), and filamentous bacteria (length $>8\text{ }\mu\text{m}$, average length 20 to $50\text{ }\mu\text{m}$).

At least 100 cells or colonies of each of these 23 microbial groups in each water sample were enumerated, and at least 20 of each group in each sample were measured. Cell volume (or colony volume) was calculated by assigning the organisms to simple geometric shapes and by measuring 1 or 2 dimensions. The dimensions of naked flagellates and of ciliates were

multiplied by a factor 1.1 to compensate for shrinkage due to fixation (Choi & Stoecker 1989). Heterotrophic bacteria were converted from cell volume to carbon biomass using a carbon content of $0.35\text{ pg C }\mu\text{m}^{-3}$ (Bjørnsen 1986); for pigmented picoplankton a conversion factor of $0.22\text{ pg C }\mu\text{m}^{-3}$ was used (Søndergaard et al. 1991). For all protists $>2\text{ }\mu\text{m}$, except diatoms, cell volume = plasma volume. For diatoms, plasma volume = cell volume - vacuole volume (Strathmann 1967). Protist plasma volume (μm^3) was converted to carbon biomass (pg) using a factor, $0.13\text{ pg }\mu\text{m}^{-3}$ for thecate dinoflagellates and $0.11\text{ pg }\mu\text{m}^{-3}$ for all other organisms (Mullin et al. 1966, BMEPC-HC 1988). Carbon biomass of the different groups was calculated from the cell density of each group and the carbon per cell content.

According to their mode of nutrition, the nanoprotists were classified as strictly heterotrophic protists (colourless flagellates, and colourless, non-plastid-retaining ciliates), mixotrophic protists (mixotrophy in the restricted sense, as a combination of phagotrophy and phototrophy), and other phototrophic protists. The mixotrophic protists were finally combined with other phototrophic nanoprotists and phototrophic picoplankton into the major group 'pigmented organisms'. The 2 other major groups were 'colourless nanoprotists' and 'heterotrophic bacteria'.

Phagotrophy (ingestion of pico- and/or nano-sized particles) was determined using food analogues according to the procedure described by Sherr et al. (1987) and Rublee & Gallegos (1989): fluorescently labelled, heat-killed pico-sized bacteria (FLB) and nano-sized algae (FLA) were prepared from cultures of *Serratia indica* and *Thalassiosira pseudonana* respectively. On average, the FLB cell volume was $0.44\text{ }\mu\text{m}^3$ (ESD $0.9\text{ }\mu\text{m}$, length $1.5\text{ }\mu\text{m}$), and the FLA cell volume was $21\text{ }\mu\text{m}^3$ (ESD $3.4\text{ }\mu\text{m}$). All food analogue uptake experiments were performed in duplicates. Subsamples of the raw water samples collected from the 4 enclosures and the fjord on 13 July 1995 were poured into clear, acid-washed 100 ml glass bottles, placed in containers on deck, covered by nets to mimic the approximate *in situ* light intensities, and incubated at *in situ* temperatures. The subsamples remained untreated for 1 h to let the microbial community recover from the handling shock. Subsequently FLB and FLA were added to different subsamples. FLB were added to samples from the enclosures at tracer densities (i.e. $\leq 1/3$ of similar-sized particles occurring in the samples), resulting in a final FLB biomass density of 5 to 11% of the large picobacterial (ESD $0.9\text{ }\mu\text{m}$) standing stock (in the samples from the fjord, the FLB accounted for 79% of the large picobacterial density). FLA were also added at tracer densities, resulting in final biomass densities of 11 to 34% of the standing

stock of protists with an ESD 2 to 5 μm . Incubations lasted 0 and 60 min for the FLB treatments, and 0 and 90 min for the FLA treatments. Particle digestion time by protists has previously been calculated to be between 55 and 95 min for FLB at 12 to 16°C (Sherr et al. 1988) and between 60 and 80 min for FLA at an unknown temperature (Ruble & Gallegos 1989). Preliminary uptake experiments with FLB and FLA lasting 20 and 30 min, and 30 and 60 min, respectively, showed insignificant or very low uptake of tracer particles by the phagotrophic protists present (data not shown). FLB and FLA samples were fixed and treated, as described above, with 4% ice-cold glutaraldehyde in a 1:1 mixture with the seawater sample, to avoid particle egestion due to fixation (Sanders et al. 1989). Uptake of FLB and FLA was enumerated using an Olympus BH2 epifluorescence microscope equipped with a blue-light filter set. Phagotrophic protist clearance rates were calculated from per cell FLB/FLA ingestion rates of phagotrophic groups and the FLB/FLA density in the sample. Net uptake of FLB/FLA was calculated by inspection of protists for association with FLB/FLA after 60/90 min of incubation and by subtracting time-zero inspections. Uptake was significant when the 95% confidence intervals of time zero and time t estimates did not overlap: $P_t - 1.96[P_t(1 - P_t)/n_t]^{1/2} > P_0 + 1.96[P_0(1 - P_0)/n_0]^{1/2}$, where P is the number of FLB/FLA associated with a cell, and n the number of cells inspected at respectively time t (60/90 min) and time zero (confidence interval of the mean; Rice 1988). At least 100 cells (maximum 800 cells) of each phagotrophic protist group were inspected in each sample (with at least 30 ingested FLB/FLA per group for the groups with significant uptake).

Uptake rates of tracer particles (FLB and/or FLA) were calculated on a group level, e.g. colourless flagellates 10–20 μm , since some individuals of potentially different species within a group could not always be separated with certainty from each other. The dominating 1 or 2 species accounted for >90% of the cells included in these groups. Cells of the dominating species displayed phagotrophy, but not necessarily all species included in these groups were feeding by phagotrophy *sensu stricto*: some pigmented dinoflagellate species with bright chloroplasts and some colourless flagellate and ciliate species did not seem to take up tracer particles, but it would have been either too time consuming or too difficult to split the groups into several subgroups. FLA ingestion rates of mixotrophic dinoflagellates in phosphate-enriched enclosures were compared with those in non-phosphate-enriched enclosures and in the brackish layer of the fjord using a Kolmogorov-Smirnov 2-sample test (Sokal & Rohlf 1995). Clearance rates were calculated from the per cell ingestion rates of

tracer particles within a phagotrophic group divided by the tracer densities. Volume-specific clearance rates were expressed in units grazer body volume (BV) cleared per grazer per unit time (Fenchel 1991). Volume-specific clearance rates of mixotrophic dinoflagellates were compared to those of similar-sized colourless flagellates and ciliates (taking all FLA uptake experiments into account) using a Bonferroni-adjusted Kolmogorov-Smirnov 2-sample test (Rice 1989, Sokal & Rohlf 1995). The grazing impact on prey organisms of similar size and shape as the tracers was calculated from the prey density, from the clearance rates of the predators and from the predator density (Sherr et al. 1991). The percentages of mixotroph grazing of the entire nanoprotozoan grazing impact on FLA-sized organisms in phosphate-enriched enclosures were compared with those in the non-phosphate-enriched enclosures and in the surface layer of the fjord using a Kolmogorov-Smirnov 2-sample test (Sokal & Rohlf 1995). The percentage of predator body carbon ingested per day was calculated from the grazing impact on prey organisms per predator per day and from the predator's body carbon content. Grazing impact on prey populations and prey carbon ingested per predator were expressed per day to facilitate comparisons with changes in microbial carbon biomass, which was calculated every 1 or 2 d.

RESULTS

Effects of nutrient addition on the microbial biomass

Effects on pigmented organisms. From the start to the end of the investigation period, nano-sized cells accounted for >99% of the pigmented biomass, pigmented picoplankton was always sparse (<0.4 $\mu\text{g C l}^{-1}$), and almost no micro-sized cells were observed (Fig. 1). Most organisms were single-celled, and only *Chaetoceros wighamii* formed chains (with long setae), most of which exceeded 20 μm . Throughout the study period, the pigmented organism composition did not change dramatically in any of the samples, i.e. the majority of all species were present throughout the period. Three species, *Katodinium rotundatum* (ESD 6.7 μm), *C. wighamii*, and the small centric diatoms (ESD 6.7 μm), accounted for the main part of the pigmented biomass in all samples (Fig. 1). The group of 10–20 μm pigmented dinoflagellates was dominated (>90%) by a *Gymnodinium/Gyrodinium* sp. that proved to be mixotrophic on 13 July in all samples (see below). Uptake of particulate food by these pigmented dinoflagellates was not investigated on other days, but judging from the species' size (ESD 15.8 μm), oval-

Fig. 1. Carbon biomass of pigmented organisms, heterotrophic bacteria, and colourless nanoprotists, for water samples taken on 5, 6, 7, 9, 11, and 13 July from the brackish layer of Hylsfjord, Norway, and from 4 nutrient-manipulated brackish water enclosures. N: nitrate; P: phosphate; G: glycine; ESD: equivalent spherical diameter. Notice different scale for the carbon biomass of colourless nanoprotists

round shape, and 8 to 12 pale plastids, it was the same species that dominated the group of 10–20 μm pigmented dinoflagellates in all samples throughout the study period.

A dramatic increase in the pigmented biomass, peaking on 9 July, was observed only in the phosphate-enriched enclosures (+NP) and (+NPG) (Fig. 1). The addition of nitrate alone or nitrate and glycine had no dramatic effect on the pigmented organism biomass, although a minor rise was observed in these enclosures on 9 July. The pigmented biomass peak was approximately $400 \mu\text{g C l}^{-1}$ in the phosphate-enriched enclosures, which was approximately 2 $\times$ as high as in the non-phosphate-enriched enclosures (Fig. 1). For the 3 species that accounted for the majority of the pigmented biomass, the following biomass increases were observed in the period 5 to 9 July in phosphate-enriched enclosures: *Katodinium rotundatum* 8 to 9 $\times$, *Chaetoceros wighamii* 5 to 7 $\times$, and the small centric diatoms (ESD 6.7 μm) 3 to 6 $\times$. In comparison, in the non-phosphate-enriched enclosures, *K. rotundatum* increased its biomass 3 to 5 $\times$, *C. wighamii* 2 to 3 $\times$, and the small centric diatoms 1 to 3 $\times$ in the same period (data partly shown in Fig. 1). Similarly, the Euglenophyceae (ESD 10.9 μm), the only single-celled strict phototrophs in the 10–20 μm fraction, increased their biomass on average 4 $\times$ in the phosphate-enriched enclosures and 1.7 $\times$ in the enclosures without phosphate enrichment (Fig. 1). In contrast, the biomass of the mixotrophic dinoflagellates (ESD 15.8 μm) declined in the phosphate-enriched enclosures in the period 5 to 9 July from on average 10.3 to $5.1 \mu\text{g C l}^{-1}$ but then rose to a level of on average $20.5 \mu\text{g C l}^{-1}$ on 13 July (Fig. 1). In the non-phosphate-enriched enclosures, the same pattern was observed for the mixotrophic dinoflagellates: in the period 5 to 9 July, a decline in the biomass from on average 8.6 to $6.3 \mu\text{g C l}^{-1}$, which was followed by a rise to on average $14.8 \mu\text{g C l}^{-1}$ on 13 July (Fig. 1). The maximum biomass increase of the mixotrophic dinoflagellates during the last part of the study period was 3 to 4 $\times$ within 2 d.

During the investigation period, diatoms, i.e. *Chaetoceros wighamii* and small centric diatoms, accounted for 1/3 to 3/4 of the pigmented biomass, with highest values in all samples on 9 July (Fig. 1, small centric diatoms included in the group pigmented organisms ESD 6.6–6.9 μm). Silicate was below the detection limit from 9 July to the end of the investigation period only in the phosphate-enriched enclosures (Thingstad et al. unpubl.), and a decline in pigmented organism biomass was observed in these enclosures during this period (Fig. 1). At the end of the experiment, on 13 July, the percentage of diatom biomass of the total pigmented organism biomass was on aver-

age 48% in the phosphate-enriched enclosures, where silicate was undetectable, and 58% in the non-phosphate-enriched enclosures (data not shown), where silicate was still found in measurable quantities (Thingstad et al. unpubl.). Non-motile pigmented protists other than diatoms (i.e. Chlorophyceae) accounted for <3% of the pigmented biomass in the samples from 13 July (data not shown). Pigmented, motile organisms (i.e. flagellates) accounted for on average 51% of the pigmented organism biomass in the phosphate-enriched enclosures and for on average 40% in the other enclosures (data not shown). The main part of pigmented flagellate biomass was mixotrophic Dinophyceae (ESD 15.8 μm), the dinoflagellate *Katodinium rotundatum* (ESD 6.7 μm), smaller Prymnesiophyceae (ESD 3.8 μm), and smaller Prasinophyceae (ESD 2.5 or 3.5 μm) (data not shown). A minor part was smaller (ESD 4.3 μm) and larger (ESD 6.9 μm) Cryptophyceae, larger Prasinophyceae (ESD 6.6 μm), and Pedinellophyceae (ESD 3.1 μm) (data not shown). Before 13 July, the biomass of Euglenophyceae (ESD 10.9 μm) and of larger Prymnesiophyceae (ESD 4.8 μm) was also determined, but also these accounted for a minor share of pigmented flagellate biomass (Fig. 1).

In the upper layer of the fjord, where samples were not taken from exactly the same water mass, no general trend in decline or rise in biomasses of pigmented organisms could be observed (Fig. 1). Although the biomass percentages of various pigmented groups were most variable in the fjord (Fig. 1), the same species and groups that accounted for the main part of the pigmented biomass in the enclosures also dominated the biomass of the pigmented organisms in the upper layer of the fjord, and groups with a minor share of the pigmented biomass in the enclosures also had a minor share in the fjord (Fig. 1).

Effects on heterotrophic bacteria. Three size classes of bacteria, i.e. small picobacteria (ESD 0.6 μm), large picobacteria (ESD 0.9 μm), and filamentous bacteria (average length 20 to 50 μm) were present before and after phosphate enrichment in all enclosures and in the surface layer of the fjord as well. From 5 to 13 July, the percentage of the small picobacteria of the total bacterial biomass declined in all enclosures from about 55% to between 17 and 30% (Fig. 1). Simultaneously in all enclosures, the biomass percentage of the large picobacteria increased from 5.2% to between 14 and 18%, and the biomass percentage of the filamentous bacteria increased from 40% to between 57 and 64% of the total bacterial biomass (Fig. 1). The highest bacterial biomasses were observed in the phosphate-enriched enclosures on 13 July after the decline of the pigmented organism bloom. The biomass accounted for between 410 and $490 \mu\text{g C l}^{-1}$, which was approxi-

mately 2× as high as in the non-phosphate-enriched enclosures (Fig. 1). In the brackish layer of the fjord, no general decline or rise in total bacterial biomass or in the percentages of small picobacteria, large picobacteria, or long filamentous bacteria of the total bacterial biomass was observed (Fig. 1).

Effects on colourless nanoprotists. A dramatic rise in colourless nanoprotist biomass from initially 15 and 21 $\mu\text{g C l}^{-1}$ to between 110 and 180 $\mu\text{g C l}^{-1}$ at the end of the study period was observed in the phosphate-enriched enclosures (+NPG and +NP), where dramatic increases in pigmented and bacterial biomasses were also observed (Fig. 1). In the other enclosures and in the brackish layer of the fjord, only a minor rise of colourless nanoprotist biomass was observed in the period 9 to 11 July, whereafter the biomass did not change or decreased and reached between 26 and 39 $\mu\text{g C l}^{-1}$ on 13 July (Fig. 1). Colourless flagellates 2–5 μm and 5–10 μm in size accounted for respectively between 1.5 and 18% and between 8.7 and 34% of the colourless nanoprotist biomass in all samples (Fig. 1). Colourless protists 10–20 μm in size accounted for between 54 and 90% of the colourless nanoprotist biomass in all samples (Fig. 1). The latter group included colourless flagellates and ciliates (mainly oligotrichs). All nano-sized ciliates seemed to be strictly heterotrophic. Strictly phototrophic or mixotrophic ciliates, i.e. ciliates with brightly fluorescent plastids (plastids without signs of degradation) were not observed in the size range 10–20 μm [among the ciliates >20 μm , however, species with brightly fluorescent plastids were observed; the biomass of ciliates >20 μm was not assessed in this investigation, but their density was low (<1% of that of ciliates 10–20 μm in size, data not shown)].

Towards the end of the period, the following maximum biomass increases over 2-day-periods were observed in the phosphate-enriched enclosures: 3× for 2–5 μm flagellates, 2.5× for 5–10 μm flagellates, 7× for 10–20 μm flagellates, and 3× for ciliates (Fig. 1). In the phosphate-enriched enclosures, the ciliate biomass was on average 67.5 $\mu\text{g C l}^{-1}$ on 13 July, which was on average 4× higher than in the enclosures without phosphate addition and 5× higher than in the fjord (Fig. 1). The 10–20 μm colourless flagellate biomass was on average 51.3 $\mu\text{g C l}^{-1}$ in the phosphate-enriched enclosures on 13 July, which was on average 12× higher than in the non-phosphate-enriched enclosures and 3× higher than in the fjord (Fig. 1). Similarly, on 13 July, the 5–10 μm and 2–5 μm colourless flagellates had respectively 2 to 5× and 2 to 4× higher biomasses in the phosphate-enriched enclosures than in the non-phosphate-enriched environments, where prey density or production was lower (Fig. 1).

Phagotrophic nanoprotist grazing on pico- and nanoplankton

Grazing by 10–20 μm phagotrophic protists. No significant uptake of FLB was observed for phagotrophic protists in the size range 10–20 μm on 13 July. Significant uptake of FLA was observed for the following protist groups in the size range 10–20 μm : colourless flagellates, pigmented dinoflagellates (mixotrophs), and ciliates (Table 1). Also organisms larger than FLA, i.e. *Katodinium rotundatum* (ESD 6.7 μm) or small centric diatoms (ESD 6.7 μm) were occasionally observed within the food vacuoles of both ciliates and colourless flagellates (data not shown). The FLA ingestion rates of

Table 1 FLA ingestion rates, volume-specific clearance rates, and FLA-sized prey carbon ingested per day (as a percentage of predator cell carbon) of phagotrophic protist groups in the size range 10–20 μm in different environments on 13 July. FLA: fluorescently labelled algae (ESD 3.4 μm); ESD: equivalent spherical diameter; BV: body volume. Standard deviation between replicates given in parentheses

Phagotrophic protist group, 10–20 μm	Fjord, 1 m depth	Non-phosphate-enriched enclosures	Phosphate-enriched enclosures
FLA ingestion rate (FLA protist ⁻¹ h ⁻¹)			
Ciliates	0.11 (± 0.03)	0.05 (± 0.03)	0.10 (± 0.01)
Colourless flagellates	0.12 (± 0.03)	0.17 (± 0.06)	0.12 (± 0.04)
Mixotrophic dinoflagellates	0.24 (± 0.07)	0.15 (± 0.08)	0.15 (± 0.08)
Volume-specific clearance rate (10 ⁴ BV protist ⁻¹ h ⁻¹)			
Ciliates	2.3 (± 0.7)	1.1 (± 0.6)	2.0 (± 0.3)
Colourless flagellates	3.8 (± 0.8)	5.5 (± 1.7)	3.9 (± 1.2)
Mixotrophic dinoflagellates	3.6 (± 1.0)	2.3 (± 1.1)	2.2 (± 1.3)
Percentage of own body carbon ingested per day through grazing on 2–5 μm protists			
Ciliates	12 (± 3.4)	8.3 (± 6.5)	21 (± 8.0)
Colourless flagellates	19 (± 4.0)	36 (± 5.5)	36 (± 3.7)
Mixotrophic dinoflagellates	18 (± 5.0)	15 (± 7.8)	19 (± 7.1)

Fig. 2. Colourless flagellate, colourless ciliate, and mixotrophic dinoflagellate share of the total grazing impact on FLA-sized, 2–5 µm prey by phagotrophic nanoplankton in different environments on 13 July (SD in parentheses). ESD: equivalent spherical diameter

mixotrophic dinoflagellates in the phosphate-enriched enclosures (0.03 to 0.23 FLA flagellate⁻¹ h⁻¹) were not significantly different from those measured in the non-phosphate-enriched enclosures and in the brackish layer of the fjord (0.04 to 0.30 FLA flagellate⁻¹ h⁻¹) (average ingestion rates shown in Table 1). The volume-specific FLA clearance rates (BV protist⁻¹ h⁻¹) of the phagotrophic protists 10–20 µm in size were in the following ranges: colourless flagellates 2.6×10^4 to 7.3×10^4 , mixotrophic dinoflagellates 1.1×10^4 to 3.6×10^4 , and colourless ciliates 0.8×10^4 to 2.3×10^4 (average rates shown in Table 1). The volume-specific clearance rates of the mixotrophic dinoflagellates (taking all FLA uptake experiments into account) were not significantly different from those of the colourless flagellates, or from those of the colourless ciliates in the size range 10–20 µm.

The daily grazing impact on the 2–5 µm protist standing stock by all phagotrophic protists in the size range 10–20 µm was on average 86% in the phosphate-enriched enclosures, 23% in the non-phosphate-enriched enclosures, and 28% in the brackish layer of the fjord (data not shown). The 10–20 µm mixotrophic dinoflagellates were responsible for 34% of the grazing impact on 2–5 µm prey in the non-phosphate-enriched environments (including the surface layer of the fjord), but their share was significantly lower, i.e. 12% ($p < 0.05$) in the phosphate-enriched enclosures (Fig. 2). Calculated from the grazing on 2–5 µm prey, the 10–20 µm phagotrophic protists ingested on average the following percentages of their own body carbon per day in the phosphate-enriched enclosures: ciliates 21%, colourless flagellates 36%, and mixotrophic dinoflagellates 19% (Table 1).

Grazing by 5–10 µm phagotrophic protists.

For protists in the size range 5–10 µm, no uptake of FLA was observed. Uptake of FLB was significant for colourless flagellates 5–10 µm in size (ESD 6.1 µm), but not for pigmented flagellates in this size-range. The FLB (ESD 0.9 µm) had approximately the same size as the large picobacteria (ESD 0.9 µm). On 13 July, in the phosphate-enriched enclosures, where the large picobacterial biomass was approximately 2× as high as in the non-phosphate-enriched enclosures (Fig. 1), the average FLB ingestion rate of the 5–10 µm colourless flagellates (0.16 FLB flagellate⁻¹ h⁻¹) was higher than the average FLB ingestion rate in the non-phosphate-enriched enclosures (0.05 FLB flagellate⁻¹ h⁻¹) (Table 2). In the water samples taken from the fjord, the final FLB density was 79% of the large picobacterial density, and thus higher than tracer densities in other samples (which were <1/3 of prey density). The average FLB ingestion rate of the 5–10 µm colourless flagellates was 0.29 FLB flagellate⁻¹ h⁻¹ (Table 2). In the enclosures with phosphate enrichment, where the highest increases of the 5–10 µm colourless flagellate

Table 2. Grazing on large picobacteria (ESD 0.9 µm) by colourless flagellates 5–10 µm (ESD 6.1 µm), as estimated using FLB (ESD 0.9 µm) uptake experiments, carried out on 13 July using water samples taken from the fjord at 1 m depth, from non-phosphate-enriched enclosures (–), and from phosphate-enriched enclosures (+). FLB: fluorescently labelled bacteria; ESD: equivalent spherical diameter; BV: body volume. Standard deviation between replicates given in parentheses

Sampling site (enclosure/fjord)	FLB ingestion rate (FLB flagellate ⁻¹ h ⁻¹)	Volume-specific clearance rate (10 ⁴ BV flagellate ⁻¹ h ⁻¹)	Percentage of flagellate body carbon ingested per day through grazing on large picobacteria
1 m depth	0.29 (±0.00)	10	11
– phosphate	0.05 (±0.02)	1.8	15
+ phosphate	0.16 (±0.07)	5.8	77

biomass were observed (Fig. 1), they ingested on average 77% of their own body carbon per day through feeding on the FLB-sized, large picobacteria (Table 2).

Grazing by 2–5 μm potentially phagotrophic protists. No uptake of FLB or FLA by potentially phagotrophic protists in the size range 2–5 μm , i.e. colourless flagellates (ESD 3 μm), and the potentially mixotrophic groups *Pedinellophyceae* (ESD 3.1 μm) and *Prymnesiophyceae* (ESD 3.8 μm) was observed on 13 July (data not shown).

DISCUSSION

Mixotrophic flagellates, colourless flagellates, and colourless ciliates in the size range 10–20 μm grazed on nano-sized prey, as demonstrated through the uptake of fluorescently labelled algae, FLA (Table 1). The strictly heterotrophic protists (colourless flagellates and ciliates) had the highest grazing impact on FLA-sized (2–5 μm) prey in all enclosures and in the fjord (Fig. 2). Mixotroph share of protist grazing on 2–5 μm prey was on average 34% in non-phosphate-enriched environments, but the share was significantly lower, i.e. 12% ($p < 0.05$), in the phosphate-enriched enclosures (Fig. 2), where both primary production and bacterial production were stimulated (Fig. 1).

Comparisons of growth rates between mixotrophs and strict phototrophs, and between mixotrophic and strictly heterotrophic protists were difficult to make, since the productivity of specific populations was not measured. Only changes in their biomasses could be considered.

While the pigmented biomass rose in the period 5 to 9 July in all enclosures, though most dramatically in those with phosphate enrichment, the percentage of the pigmented biomass comprised by the mixotrophic dinoflagellates was generally declining from between 9.9 and 13.4% to between 1.2 and 3.9% (Fig. 1). A faster growth of the strict phototrophs under improved growth conditions would be a possible explanation. A different grazing pressure on the strict phototrophs and the mixotrophs could be another explanation. In fact, most strict phototrophs were either considerably smaller than the mixotrophic dinoflagellates (ESD 15.8 μm) or considerably larger (Fig. 1). The 2–10 μm phototrophs were grazed by the mixotrophs themselves and by other phagotrophic nanoprotists (see above, Fig. 1) and may have been too small for potential grazers of the mixotrophic dinoflagellates. Similarly, *Chaetoceros wighamii*, a chain-forming diatom with long setae exceeding 20 μm , because of its size and shape, may have been bypassed by predators feeding on prey in the size range 10–20 μm . The only strict phototrophs similar in size to the mixotrophic

dinoflagellates were the *Euglenophyceae* (ESD 10.9 μm), and both were probably grazed by the same predators. While the *Euglenophyceae* only increased their biomass on average 1.7 $\times$ in the non-phosphate-enriched enclosures in the period 5 to 9 July, a 4 $\times$ biomass increase was observed in the phosphate-enriched enclosures (Fig. 1). In contrast, the growth of the similar-sized mixotrophic dinoflagellates did not seem to be stimulated by phosphate, since their biomass decreased to half of the initial amount in all enclosures during the first part of the study period (Fig. 1).

In environments where photosynthesis is not limited by mineral nutrients or light, one might expect a decreased need to ingest particles in mixotrophs that rely primarily on phototrophy. For instance, lower ingestion rates of particulate food were reported from cultures of the mixotrophic flagellate *Chrysochromulina brevifilum* and from natural communities of the mixotrophic flagellates *Chrysochromulina ericina* and *Prymnesium parvum* when phosphate was in excess compared to when phosphate was depleted (Jones et al. 1993, Nygaard & Tobiesen 1993). For the mixotrophic dinoflagellates in the present study, however, no decrease in FLA ingestion rate could be observed in phosphate-enriched enclosures (0.15 FLA flagellate⁻¹ h⁻¹) compared to non-phosphate-enriched enclosures and to the fjord (0.15 and 0.24 FLA flagellate⁻¹ h⁻¹) (Table 1).

For mixotrophs that rely primarily on heterotrophy, e.g. *Poterioochromonas malhamensis* and other ochromonads, considerably higher growth rates have been reported when prey availability was high than when it was low (Caron et al. 1990, Sanders et al. 1990, Rothhaupt 1996). In the present study, however, the mixotrophic dinoflagellates reached similar biomasses in environments with high and low prey productivity (Fig. 1). In the phosphate-enriched enclosures, where the prey availability increased towards the end the study period, the biomass of the 10–20 μm strictly heterotrophic protists, however, was considerably higher (on average 3 to 12 $\times$ for colourless flagellates and 4 to 5 $\times$ for ciliates) than in the non-phosphate-enriched enclosures and the surface layer of the fjord (Fig. 1).

The effects of nutrient and prey availability on the mixotrophic dinoflagellates in this study thus seemed to differ from those reported of the 2 different categories of mixotrophic flagellates represented by *Chrysochromulina brevifilum* and *Poterioochromonas malhamensis* respectively. The mixotrophic dinoflagellates in the present study may belong to a third category of mixotrophs, which rely primarily on phototrophy, but with a reduced capacity for uptake of dissolved inorganic nutrients (even in the presence of high concentrations of N and P). They may ingest par-

ticulate prey primarily for nutrients and for organic compounds that they are unable to synthesize, and their rate of phagotrophy may be proportional to their rate of photosynthesis as reported for mixotrophs belonging to this category (see Jones et al. 1995). The finding that FLA uptake rates were not different in the different environments was in accordance with the similar light conditions in all environments.

The volume-specific FLA clearance rates of the mixotrophic dinoflagellates did not deviate significantly from those of similar-sized colourless flagellates and ciliates (Table 1). Nevertheless, the mixotrophic dinoflagellates had a higher share of the protist grazing on 2–5 μm prey in the non-phosphate-enriched enclosures and in the fjord (average 34%) than in the phosphate-enriched enclosures (12%) (Fig. 2). The significant different shares in the phosphate-enriched and the non-phosphate-enriched environments can thus not be explained by differences in volume-specific clearance rates among the various protists in the size range 10–20 μm , but rather by differences in their assumed growth rates at high and low prey availability (Table 1, Fig. 1). The strictly heterotrophic protists reached considerably higher biomasses in those environments where the prey production was stimulated through phosphate enrichment (Fig. 1). In contrast, in the non-phosphate-enriched environments, where prey availability was low, the strictly heterotrophic protists 10–20 μm in size only sustained or decreased their biomass, whereas the mixotrophic dinoflagellates, apparently because of their dual mode of nutrition, increased their biomass 4 to 5 $\times$ during the last days of the study period (Fig. 1).

In the phosphate-enriched enclosures, where the most dramatic biomass increases of both phototrophic and heterotrophic microorganisms were observed (Fig. 1), the 10–20 μm phagotrophic protists grazed on average 86% of the 2–5 μm protist standing stock per day (data not shown). Nevertheless, on 13 July, ciliates ingested only 21% of their body carbon per day from this prey pool, colourless flagellates 36%, and mixotrophic dinoflagellates 19% (Table 1). From these amounts ingested per day, the 10–20 μm phagotrophic protists may only be able to sustain their biomass or increase it $1.1 \times \text{d}^{-1}$, assuming a 50% gross growth efficiency (Fenchel 1982, Jonsson 1986, Strom 1991), and assuming a low grazing pressure upon them. In contrast, a considerable biomass increase, similar to the 3 to 4 $\times$ biomass increase during the period 9 to 13 July, would not be possible for the 10–20 μm phagotrophic protists exclusively through feeding on FLA-sized, 2–5 μm prey.

Since the 10–20 μm protists also ingested larger pigmented prey (ESD 6.6 to 6.9 μm), their growth could be explained taking this food source into account: the bio-

mass of pigmented prey with ESD 6.6 to 6.9 μm was approximately 2 to 6 $\times$ higher than that of FLA-sized prey (ESD 2.5 to 4.8 μm) in the phosphate-enriched enclosures (Fig. 1) and could thus supply the need of the phagotrophic protists 10–20 μm in size. In addition to feeding on food sources larger than FLA, the mixotrophic dinoflagellates could supplement their needs through photosynthesis.

Potential prey for the 10–20 μm phagotrophic protists were also the long filamentous bacteria (length 8 to 200 μm). Carbon tracer experiments (D. Lefèvre pers. comm.) revealed that ^{13}C in the phosphate-enriched enclosures towards the end of the study period was mainly taken up by the 2–10 μm fraction (the filamentous bacteria), whereafter the tracer was transferred to the 10–53 μm fraction (including the phagotrophic protists 10–20 μm in size). In 1991, Inamori et al. reported that ciliates ingested filamentous bacteria at high rates. We do not know whether phagotrophic protists in the size range 10–20 μm ingested filamentous bacteria in the present study. Ciliates have been reported to feed preferentially on heterotrophic food sources, whereas gymnodinoid, colourless dinoflagellates were reported to select strongly for phototrophic food sources (Neuer & Cowles 1995).

It is also possible, however, that the FLA uptake technique underestimated the ingestion of the FLA-sized prey, and that enough 2–5 μm prey were ingested by the 10–20 μm phagotrophic protists to support the observed growth. Underestimation of grazing using heat-killed and stained food analogues compared to live prey have been reported by e.g. Landry et al. (1991).

Picobacteria, however, could probably be excluded as an important food source for 10–20 μm protists in the present study, since no significant uptake of FLB (ESD 0.9 μm) by mixotrophic dinoflagellates or by colourless flagellates and ciliates 10–20 μm in size was observed. For the 10–20 μm flagellates, this was in accordance with previous findings (Sherr & Sherr 1994, Havskum & Riemann 1996), whereas pelagic ciliates <20 μm have been reported as voracious consumers of picobacteria (Sherr & Sherr 1987). Although prey selection by phagotrophic protists on the basis of quality has been reported (Nygaard et al. 1988, Landry et al. 1991, Verity 1991), the fact that 5–10 μm flagellates took up FLB significantly (Table 2) showed that the FLB were edible, at least for other protists.

Hansen et al. (1994) proposed that the optimum predator:prey ESD ratio for (non-dino-)flagellates is 3:1, for oligotrichous ciliates 8:1, and for dinoflagellates 1:1. In the present study the 10–20 μm phagotrophic protist:FLB ratio (ESD:ESD) was 14:1 for colourless flagellates, 16:1 for ciliates, and 18:1 for mixotrophic

dinoflagellates, i.e. in all cases the ratio was considerably higher than the optimum predator:prey ESD ratio proposed by Hansen et al. (1994). The lack of FLB uptake by the 10–20 µm phagotrophic protists thus seems to be due to the relatively small ESD of the FLB rather than their quality. In comparison, the predator:prey ESD ratio between the 10–20 µm phagotrophic protists and the FLA, which were taken up significantly, was between 4:1 and 5:1. In addition, the prey size selectivity spectra have been proposed to be bell-shaped, with the top of the bell-shaped curve representing the optimum prey size (Hansen et al. 1994). Since both considerable uptake of FLA (ESD 3.4 µm) (Table 1) and occasional uptake of particles larger than FLA (e.g. pigmented organisms ESD 6.6 to 6.9 µm) have been observed (data not shown), it seems unlikely that the optimum prey size for the 10–20 µm protists in the present study should be in the pico size range.

Colourless flagellates in the size range 5–10 µm (ESD 6.1 µm) were the only nanoprotists in the samples that significantly ingested FLB (length 1.5 µm, ESD 0.9 µm). The biomass of the large picobacteria ingested per day corresponded on average to 77% of the 5–10 µm colourless flagellates' own body carbon on 13 July in the phosphate-enriched enclosures (Table 2). Assuming a net growth efficiency of 50% (Fenchel 1982, Goldman & Dennett 1992), the large picobacterial food source supplied them with approximately 40% of their body carbon per day. Since the 5–10 µm colourless flagellates increased their biomass on average 2× from 11 to 13 July (Fig. 1), they could similarly proceed to grow through ingestion of exclusively large picobacteria, assuming no strong grazing pressure upon the 5–10 µm colourless flagellates.

In the present study, the Pymnesiophyceae (ESD 3.8 µm) and the Pedinellophyceae (ESD 3.1 µm) did not take up FLB, which is in contrast to previous reports about mixotrophy in natural populations of Pymnesiophyceae and Pedinellophyceae (Nygaard & Tobiesen 1993, Thomsen et al. 1994, Havskum & Riemann 1996). The reason why uptake was not observed may be that not all species belonging to these 2 classes are mixotrophic. However, since colourless flagellates of similar size (ESD 3 µm) also did not take up FLB, the reason for the lack of FLB uptake may have been that the FLB size or quality was inappropriate for all 3 groups. Possible mixotrophy of the Pymnesiophyceae or the Pedinellophyceae could thus be neither ruled out nor proved by this study.

The size ratio between the small colourless flagellates and the FLB was 3:1, which was proposed to be the optimum predator:prey ESD ratio for flagellates other than dinoflagellates (Hansen et al. 1994), mainly on the basis of 1 investigation of optimum prey sizes of

bodonids by Chrzanowski & Šimek (1990). In view of the tremendous diversity of heterotrophic flagellates (Cavalier-Smith 1991), the proposed optimum predator:prey size ratio of 3:1 may not be universal. Sherr & Sherr (1991) reported that small (<5 µm) colourless flagellates were the most important grazers on picobacteria in several natural environments. These results were mainly based on experiments using smaller FLB (ESD 0.6 µm). The naturally occurring small picobacteria in the present study, which accounted for the main part of the picoplankton biomass (Fig. 1), also had an ESD of 0.6 µm. The smallest colourless flagellates (ESD 3 µm) in the present study may have been adapted to feed on the small picobacteria (predator:prey size ratio 5:1). Since the ESD of the smallest colourless flagellates in the present study was only 3× larger than that of the FLB, the relative FLB size may have been too large to ensure significant uptake. In contrast, colourless flagellates 5–10 µm in size (ESD 7× larger than that of the FLB) had significant uptake of FLB (Table 2) and could sustain their growth on FLB-sized particles (i.e. the large picobacteria in the phosphate-enriched enclosures; Fig. 1). The FLB uptake experiments seem to have reflected what was happening in nature: the 5–10 µm colourless flagellates were feeding on large picobacteria, but the flagellates <5 µm were not.

In conclusion, the results of the present investigation suggest that nano-sized phagotrophic protists prey on a variety of different food sources. FLB-sized (ESD 0.9 µm) prey seemed to be too small for 10–20 µm phagotrophic protists and too large for <5 µm potentially phagotrophic protists, but seemed to be an important food source for 5–10 µm phagotrophic protists. FLA-sized (ESD 3.4 µm) prey seemed to be an important food source only for phagotrophic protists 10–20 µm in size, i.e. mixotrophic dinoflagellates, colourless flagellates, and ciliates. In the phosphate-enriched enclosures, these 3 groups together grazed on average 86% of the 2–5 µm protist standing stock per day. Nevertheless, for none of the 3 groups did the carbon gained from FLA-sized organisms seem to be enough to ensure a growth similar to that observed in the phosphate-enriched enclosures in the period 9 to 13 July. The 10–20 µm phagotrophic protists could supplement their carbon requirement through feeding on other prey organisms, e.g. 5–10 µm protists and filamentous bacteria. Mixotrophic dinoflagellates could in addition supplement their carbon requirement through photosynthesis.

Since the mixotrophic dinoflagellates seemed to grow as well in phosphate-enriched as in phosphate-depleted environments, and their biomass was similar in environments with high and with low prey density or production, they seemed to differ from those categories of

mixotrophs to which *Poterioochromonas malhamensis* and *Chrysochromulina brevifilum* belong. Their apparent lack of response to factors such as prey availability or nutrient concentration rather placed the mixotrophic dinoflagellates within a category of mixotrophs that rely primarily on phototrophy, but take up particulate prey primarily for nutrients and organic compounds that they are unable to synthesize. Prey uptake rates in this category of mixotrophs are apparently proportional to the rate of photosynthesis (Jones et al. 1995).

In phosphate-enriched enclosures, growth of both bacteria and pigmented organisms was stimulated (Fig. 1). The increased prey density or production in these enclosures resulted in a considerable increase of the biomass of strictly heterotrophic nanoprotoists towards the end of the study period, whereas their biomass decreased or did not change in non-phosphate-enriched environments (Fig. 1). In contrast, the mixotrophic dinoflagellates increased their biomass similarly in all environments during the last days of the investigation period (Fig. 1). Consequently, the mixotrophic dinoflagellates accounted on 13 July for a higher share of the protist grazing on 2–5 µm prey in the non-phosphate-enriched environments including the fjord (34%) than in the phosphate-enriched environments (12%) (Fig. 2). These results suggest that the mixotrophs, because of their phototrophic nutrition, had a competitive advantage compared to the strictly heterotrophic protists in environments where prey availability was low.

The finding that organisms of the nano size fraction had a major grazing impact on other organisms of the same size fraction but not of the pico size fraction is an important observation in the present understanding of the nutrient dynamics within the microbial loop. Not only strictly heterotrophic but also mixotrophic nanoprotoists can be important nanoplankton grazers, especially when the density or production of suitable nano-sized food particles is low because of low inorganic nutrient concentration.

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