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Predation limitation in the pelagic microbial food web in an oligotrophic aquatic system

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ABSTRACT: The importance of predation as a limiting factor for net growth rates of organisms within the pelagic microbial food web was studied in a truncation experiment performed at a coastal station in the northern Baltic Sea. To remove potential predators, seawater was fractionated into 4 size groups: <0.8 µm (bacteria); <5 µm (bacteria + small flagellates + small phytoplankton); <10 µm (bacteria + flagellates + phytoplankton); and <90 µm (bacteria + flagellates + phytoplankton + ciliates). The samples were incubated *in situ* in dialysis bags with a cut-off of 12 to 14 kDa, allowing nutrients and macromolecules to pass in and out of the incubation bags. The development of the plankton community was followed over 8 d. Heterotrophic bacteria and flagellates were found to be predation-limited, as removal of grazing increased their initial net growth rates from 0 to 0.5 and 0.4 d^{-1}, respectively. Picoeukaryotic autotrophs increased their net growth rates from 0 to 0.6 d^{-1} when flagellates and ciliates were removed. Other phytoplankton and ciliates did not show any initial response to predator exclusion, indicating that they were not predation-limited. The main trophic links within the microbial food web seemed to be from heterotrophic bacteria to small heterotrophic flagellates, from small heterotrophic flagellates and autotrophic picoeukaryotes to intermediate protozoa (medium-sized flagellates and small ciliates) and from intermediate protozoa to large protozoa (large flagellates and large ciliates). Removal of predators caused no quick (<1 d) indirect response in the form of trophic cascades. The data indicate omnivory among flagellates and ciliates. A model of the microbial food web is presented, showing the main trophic links and interconnection between autotrophic and heterotrophic organisms.

KEY WORDS: Bacteria · Flagellates · Ciliates · Phytoplankton · Predation limitation · Growth rates · Direct and indirect effects · Trophic links

INTRODUCTION

Population dynamics in the microbial food web is influenced by resource availability and predator activity. Predation can influence population dynamics both directly by removing prey items and indirectly through complex trophic interactions. In the microbial food web, the main predators on heterotrophic bacteria and picoautotrophs are small phagotrophic nanoflagellates (Fenchel 1982, Sherr & Sherr 1994). The structure in the size range of 2 to 100 µm (e.g. flagellates, ciliates, phytoplankton) is less clear. Some studies indicate that there is a tightly size-structured predator-prey coupling within the nanoplanktonic food chain with at least 3 trophic levels (Rassoulzadegan & Sheldon 1986, Wikner & Hagström 1988, Sherr et al. 1991, Calbet et al. 2001). Other studies indicate that size cannot be used to divide organisms into different trophic levels, e.g. phagotrophic flagellates are capable of eating prey of their own size or even larger organisms (Tillmann 1998, Hansen & Calado 1999). Predation limitation has been defined as the degree to which the per capita growth rate is decreased by predation (Osenberg & Mittelbach 1995). Relatively few studies have been performed on predation limitation within the microbial food web and most of these often focus on predation limitation of single functional groups. Some theoretical theories hypothesize that predation and resource limitation

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alternate between adjacent trophic levels, while other theories assume simultaneous effects of resource and predation (Hairston et al 1960, Oksanen et al. 1981). A response to strong predation pressure may be the development of predator-resistant prey, which can result in resource-controlled population dynamics (Pace & Funke 1991, Balciunas & Lawler 1995, Morin & Lawler 1995).

An indirect effect of predation is trophic cascades (Paine 1980). This theory assumes predation limitation on all trophic levels (Polis & Strong 1996). In some studies, trophic cascades are indicated to be an important factor structuring the microbial food web, while in other studies, such interactions seem to be lacking (Güde 1988, Wikner & Hagström 1988, Pace & Funke 1991, Sanders et al. 1992, Reckermann & Veldhuis 1997, Calbet et al. 2001). There are several explanations for the lack of trophic cascades. For example, many prey are resistant to predators, and the predator is therefore not capable of reducing the prey enough for a cascade to occur. Another explanation for a lack of trophic cascades is omnivory, i.e. a high frequency of predation on more than 1 trophic level (Polis & Strong 1996). In earlier studies on the microbial food web showing the occurrence of trophic cascades down to the bacterial level, often only the response of the bacterial community has been studied. The results could therefore be explained by factors such as changes in resource level instead of changes in the trophic levels above. To demonstrate the occurrence of trophic cascades within the microbial food web, it may therefore be necessary to perform experiments in which all trophic levels are studied.

In this study, we wanted to identify the main predators on autotrophic and heterotrophic microorganisms of different trophic levels, and to test whether predation was an important factor limiting the net growth rates of these groups. In addition, we wanted to identify possible indirect effects of predation, such as trophic cascades. By doing so, we hoped to establish the main flows both within the heterotrophic food web and between the autotrophic and heterotrophic food web. A truncation experiment was performed with seawater from a coastal station in the northern Baltic Sea. The seawater was size-fractionated to obtain different functional groups of plankton: bacteria, flagellates and ciliates. The samples were incubated *in situ* and the responses of the different plankton organisms were studied over 8 d. To our knowledge, few contemporary studies on predation limitation at different trophic levels within the microbial food web have been performed. Since limiting factors to a high degree govern the structure and population dynamics of microbial communities, it is important to increase our knowledge on this subject.

MATERIALS AND METHODS

Experimental design. Seawater was sampled at a coastal station in the northern Baltic Sea, (63° 30.5′ N, 19° 48.0′ E) in late June 1998. At the location, the water depth is 25 m and salinity 2 to 5‰. Water was taken from 4 m depth (chlorophyll *a* [chl *a*] maximum) using a Ruttner water sampler. The water was immediately filtered through a 90 µm nylon net. At the laboratory, 3 additional fractionations were made, <0.8, <5 and <10 µm, by filtration through polycarbonate filters (Poretics). In addition, 0.8 µm filtered water was diluted in a 1/10 v/v relationship with 0.2 µm filtered water. A maximum filtration pressure of 100 mm Hg for the <0.2 and <0.8 µm filtration, and 30 mm Hg for the <5 and <10 µm filtrations was used. Water from each fraction was used to fill 400 ml dialysis bags (Spectra/Por 1, weight cut-off 12 to 14 kDa, 75 mm flat width). These dialysis bags allow nutrients and macromolecules to pass in and out, and where used to avoid differences in the chemical environment between treatments. Bags were closed by a knot, clamped to a steel frame and incubated *in situ* at 4 m depth. Daylight level varies from 20 to 130 µmol quanta m^{-2} s^{-1}at this depth. After 1, 2, 3, 4, 6 and 8 d, 3 replicate bags of each treatment were sampled. On each sampling occasion, triplicate samples were also taken from the free water mass at the station. The content of each bag was poured into acid-washed polycarbonate bottles and subsamples were taken for analysis of inorganic nutrients, chl *a* and biovolume concentration of pico-, nano- and micro-plankton (representing the size fractions 0.2 to 2, 2 to 10 and 10 to 100 µm, respectively). Samples from the free water mass were analyzed for the same parameters together with measurements of temperature and salinity.

Inorganic nutrients and chl *a*. Samples for determination of inorganic nutrients (PO_4^{-3}, NO_2^{-}, NO_3^{-2}, NH_4^{+}) were filtered through 0.2 µm Gelman Supor filters and stored at –20°C. After thawing, the samples were analyzed using an autoanalyzer (Technicon TRAACS 800) and standard analytic methods (Grasshoff et al. 1983). All filtration equipment and filters for measurement of inorganic nutrients were rinsed in 1.2 M HCl. For chl *a* measurements, 100 ml water were filtered onto 25 mm, 0.2 µm cellulose acetate filters (Sartorius) and chl *a* extracted in 5 ml of 95% EtOH for 24 h at room temperature in darkness. The concentration was measured fluorometrically (Perkin Elmer, fluorometer LS30) with an excitation wavelength of 433 nm and an emission wavelength of 674 nm, and chl *a* was calculated according to HELCOM (1998). The fluorometric readings were calibrated against a Shimadzu spectrophotometer (MPS-

2000). The chl *a* concentration was calculated using an adsorption coefficient of 83.4 l g^{-1} cm^{-1}.

Quantification of microorganisms. Water for enumeration of heterotrophic bacteria was fixed with 4% 0.2 µm filtered formaldehyde. After filtration onto 0.2 µm polycarbonate filters (Poretics) and staining with acridine orange (Hobbie 1977), the samples were examined by epifluorescence microscopy using blue excitation light (Zeiss Axiovert 100, 450 to 490 nm; Zeiss Filter Set No. 9, FT510 LP520). Estimates of cell numbers and bacterial cell volumes were acquired by image analysis (Blackburn et al. 1998). Cyanobacteria, pico- and nanoeukaryotes were enumerated from samples fixed with 2% glutaraldehyde (final conc.). A volume of 40 ml was filtered onto 0.6 µm polycarbonate filter (Poretics) and stained with proflavine (Sherr & Sherr 1993). Picocyanobacteria were counted with an epifluorescence microscope using green excitation light (510 to 560 nm; Zeiss Filter Set No. 15, BP546 FT580 LP 590) and pico- and nanoeukaryotes were counted using blue excitation light (450 to 490 nm; Zeiss Filter Set No. 9) at 1250× magnification. At least 300 cyanobacteria and 100 eukaryotes were counted for each filter. Eukaryotes <5 µm were counted in 100 fields and eukaryotes >5 µm in 2 transects scanned across the filter. Pigmented cells were distinguished from non-pigmented by their autofluorescence. After filtering and mounting on slides, all samples for epifluorescence analysis were stored at −20°C. Phagotrophic ciliates were counted from water samples fixed with alkaline Lugol's solution. A volume of 25 ml was settled in a sedimentation chamber for 20 h and counted on an inverted microscope using phase contrast and 400× magnification. For eukaryotes, 15 cells of each size group (1–5, 5–10, >10 µm) or taxonomic group were measured in each sample using an ocular grid and the volume was calculated using the geometries of the cells (Edler 1979). For unidentified autotrophic and heterotrophic flagellates, the cell geometry was assumed to be rotational ellipsoid. An average volume for each size class was calculated from all samples. For picocyanobacteria, a volume of 0.38 μm^3 was assumed (Kuosa 1988).

Growth and grazing rates. Exponential growth was assumed for the different groups of microorganisms and the net growth rates (µ) was calculated according to Eq. (1):

$$\mu = (\ln B_{t2} - \ln B_{t1})/(t_2 - t_1) \quad (1)$$

where B_{t1} and B_{t2} are the total biovolume concentrations of cells at t_1 and t_2, respectively. *In situ* grazing rates (d^{-1}) were calculated assuming that the initial increase in net growth rate, due to predator removal, was equal to the grazing rates in the free water mass. According to this, the grazing rates (G) on small (1 to 5 µm) flagellates by the 5–10, 10–90 and >90 µm size groups would be calculated according to Eqs. (2), (3) & (4), respectively:

$$G_{1-5,\,5-10\mu m} = \mu_{<5\mu m} - \mu_{<10\mu m} \quad (2)$$

$$G_{1-5,\,10-90\mu m} = \mu_{<10\mu m} - \mu_{<90\mu m} \quad (3)$$

$$G_{1-5,\,>90\mu m} = \mu_{<90\mu m} - \mu_{\text{free-water mass}} \quad (4)$$

where $\mu_{<5\ \mu m}$ is equal to the net growth rate of small flagellates in the <5 µm treatment and $\mu_{<10\mu m}$ is equal to net growth of small flagellates in the <10 µm treatment, and so on. Predation limitation was identified by using 2 criteria: (1) the initial growth rate of the prey organism should increase when predators were excluded; and (2) the biovolume concentration (yield) of the prey organism should be higher removing the predator. The initial response period was determined as the initial period with an exponential growth response. For heterotrophic bacteria, the initial response period was 48 h except for the <0.8 µm treatment were the growth decreased after 24 h. For picoautotrophs and nanoplankton, this period spanned over the first 72 h.

Statistical analyses. The initial growth rate was tested by linear regression (SYSTAT). If the slope was not significantly different from 0, the growth rate was set to 0. Differences between growth rates were tested with ANCOVA, using time as a covariate. The data used for analyzing differences in biovolume concentration did not fulfill the assumptions of a parametric test (homogeneity of variance and normal distribution). Therefore, the nonparametric Kruskal-Wallis test was used. The biovolume concentration was tested using data from the 2 or 3 days with the highest values. The net reproduction for heterotrophic microorganisms were calculated for each day and compared to physical-chemical parameters (temperature, salinity, inorganic nutrients) and biological parameters (concentrations of heterotrophic bacteria, heterotrophic flagellates and heterotrophic ciliates) by using multiple linear regressions. Ammonium, nitrate, temperature, picocyanobacteria and choanoflagellates were discarded from the analysis because they showed correlations to other independent parameters. Ammonium concentration showed a positive correlation to the biovolume concentration of small flagellates, and nitrate showed a negative correlation to temperature and positive correlation to salinity ($p \leq 0.05$). Temperature was also negatively correlated to salinity and positively correlated to the biovolume concentration of medium flagellates ($p \leq 0.05$). Picocyanobacteria showed a positive correlation to the biovolume concentration of medium flagellates, and choanoflagellates showed a positive correlation to large flagellates and ciliates ($p \leq 0.05$).

RESULTS

Variation of physical and chemical factors

Over the 8 d incubation, the seawater temperature increased from 11.6 to 14.7°C and the salinity varied from 2.5 to 2.8‰. There was a significant decrease in nitrate concentration during this period both in the free water mass and in the dialysis bags from 1.15 to 0.05 µM. No significant changes of ammonium (0.07 to 0.20 µM) or phosphate (0.014 to 0.025 µM) were measured over the period. In the dialysis bags, however, the ammonium concentration was significantly higher than in the surrounding water (0.16 to 0.66 µM), and this discrepancy increased with time (2-way ANOVA, $p \leq 0.05$). According to the Redfield-ratio, phosphorus limitation prevailed (DIN:DIP, molar ratio ~50) in our experimental system, indicating that the increased ammonium concentration in the incubation bags did not affect the growth of the microorganisms.

Initial distribution of protists

Seawater was filtered to obtain 4 size groups: (1) heterotrophic bacteria (<0.8 µm); (2) heterotrophic bacteria + unicellular cyanobacteria + picoeukaryotes + small flagellates + small phytoplankton (<5 µm); (3) bacteria + flagellates + phytoplankton (<10 µm); and (4) bacteria + flagellates + phytoplankton + ciliates (<90 µm). The filtrations worked well for heterotrophic bacteria and picoeukaryotes. Cyanobacteria was reduced in the <0.8 and <5 µm treatment to 3 and 53%, respectively. Small and medium flagellates partly slipped through the smallest filtration cut-offs, due to their amoebic or oval-shaped cell form. In the <0.8 µm treatment, they were reduced to 2 and 4%, respectively, and in the 5 µm filtration, medium flagellates were reduced to 40%. Ciliate biovolume concentration were reduced to <20% in the 10 µm filtration and to <0.5% in the 5 µm filtration. Ciliates were totally excluded in the 0.8 µm filtration. Even though some of the flagellates and ciliates slipped through, their initial concentrations were significantly reduced, allowing the effect of predation to be analyzed.

Heterotrophic flagellates mainly consisted of unidentified forms, choanoflagellates, cryptophytes (*Goniomonas* sp.), *Telonema* spp., *Leucocryptos* sp. and *Ebria tripartita* (Schumann) Lemmermann. All ciliates were observed to be >10 µm. Among the smaller forms (10 to 20 µm), *Lohmaniella* spp., *Balanion* sp. and unidentified forms were observed. Among larger ciliates (20 to 90 µm), *Vorticella* sp., *Strombidium* spp. and *Strobilidium* spp. were common. Autotrophic flagellates consisted of chrysophytes (*Chrysochromulina* spp., *Pseudopedinella* spp.), chryptophytes (*Plagioselmis prolonga* Butcher, *Teleaulax* spp.), chlorophytes (*Pyramimonas* spp.), unidentified dinoflagellates and unidentified forms.

Predation limitation

During the experiment, there were minor changes in the concentration of microorganisms in the surrounding free water mass (Table 1). The treatments,

Table 1. *In situ* concentrations, specific growth rates (mean ± SE) and grazing rates of heterotrophic (H) and autotrophic (A) microorganisms measured at the sampling station during the experiment

	Minimum to maximum concentration (10^3 cells l^{-1})	Specific growth rate (d^{-1})[a]	Grazing rate (d^{-1})	Predation (% of production d^{-1})	Predation (% of biovolume concentration d^{-1})
H bacteria	$1.9–2.7 \times 10^6$	0.49 ± 0.07	0.49	100	43
Small H flagellates	$2.2–4.8 \times 10^3$	0.37 ± 0.05	0.16	43	15
Medium H flagellates	$1.4–4.6 \times 10^2$	0.45 ± 0.08	0.24	53	22
Large H flagellates	$0.1–0.4 \times 10^2$	0.24 ± 0.08	0.24[b]	100	22
Choanoflagellates	$0.8–1.2 \times 10^2$	0.44 ± 0.10	0.44[b]	100	41
Ciliates	5.5–9.8	0	–	–	–
Picocyanobacteria	$1.0–1.8 \times 10^5$	0.30 ± 0.08	–/(0.30)[c]	–/(100)[c]	–/(27)[c]
Picoeukaryotes	$0.6–3.8 \times 10^3$	0.59 ± 0.19	0.26/(0.5)[c]	44/(100)[c]	23/(51)[c]
Cryptophyceans	$0.4–1.2 \times 10^3$	0	–	–	–
Small A flagellates	$0.2–2.0 \times 10^3$	0	–	–	–
Medium A flagellates	$0.4–4.2 \times 10^2$	0	–	–	–

[a]Calculated from treatments where predation was removed
[b]Calculated as the difference between <90 µm treatment and the free water mass
[c]Numbers in parentheses indicate grazing rates where the results from all included predators are not supported by changes in maximal biovolume concentration

however, resulted in changes in both net specific growth rates and total biovolume concentration. Removal of protozoan predators (0.8 µm filtration) increased the initial net growth rate of heterotrophic bacteria from 0 to 0.49 d^{-1} (ANCOVA, $p \leq 0.05$) and increased their total biovolume concentration by 42% compared to the <90 µm treatment (Kruskal-Wallis test, $p \leq 0.05$; Fig. 1A). In treatments where ciliates and large flagellates were removed (5 and 10 µm filtration), the initial net growth rate increased to 0.16 and 0.13 d^{-1}, respectively. The bacteria in the 90 µm treatment showed the same dynamic as in the free water mass. The largest increase in bacterial-specific growth rate was measured in the diluted <0.8 µm treatment, where the growth rate increased to 2.3 ± 0.15 d^{-1} (±1 SE, data not shown). Removal of protozoa larger than 5 µm caused an increased net growth rate of small flagellates from 0.22 to 0.37 d^{-1} and an increase of their total biovolume concentration by 69% relative to the <90 µm treatment (Kruskal-Wallis test, $p \leq 0.05$; Fig. 1B). Also, medium and large flagellates showed a clear initial response to removal of larger organisms, both in initial growth rates and total biovolume concentration (Fig. 1C,D). Initial growth rates increased from 0.24 to 0.45 d^{-1} and from 0 to 0.24 d^{-1} for medium and large flagellates, respectively, and biovolume concentration increased by 91 and 173%, respectively (Kruskal-Wallis test, $p \leq 0.05$). This indicates that all 3 size groups of flagellates were predation-limited, and possibly that there was a predator-prey chain within the heterotrophic flagellate community. The observed maximum biovolume concentrations further strengthen this interpretation (Fig. 1). Highest cell yield was assumed to occur when the main predator was excluded. For bacteria, the main predator was in the 1 to 5 µm size range (small flagellates);

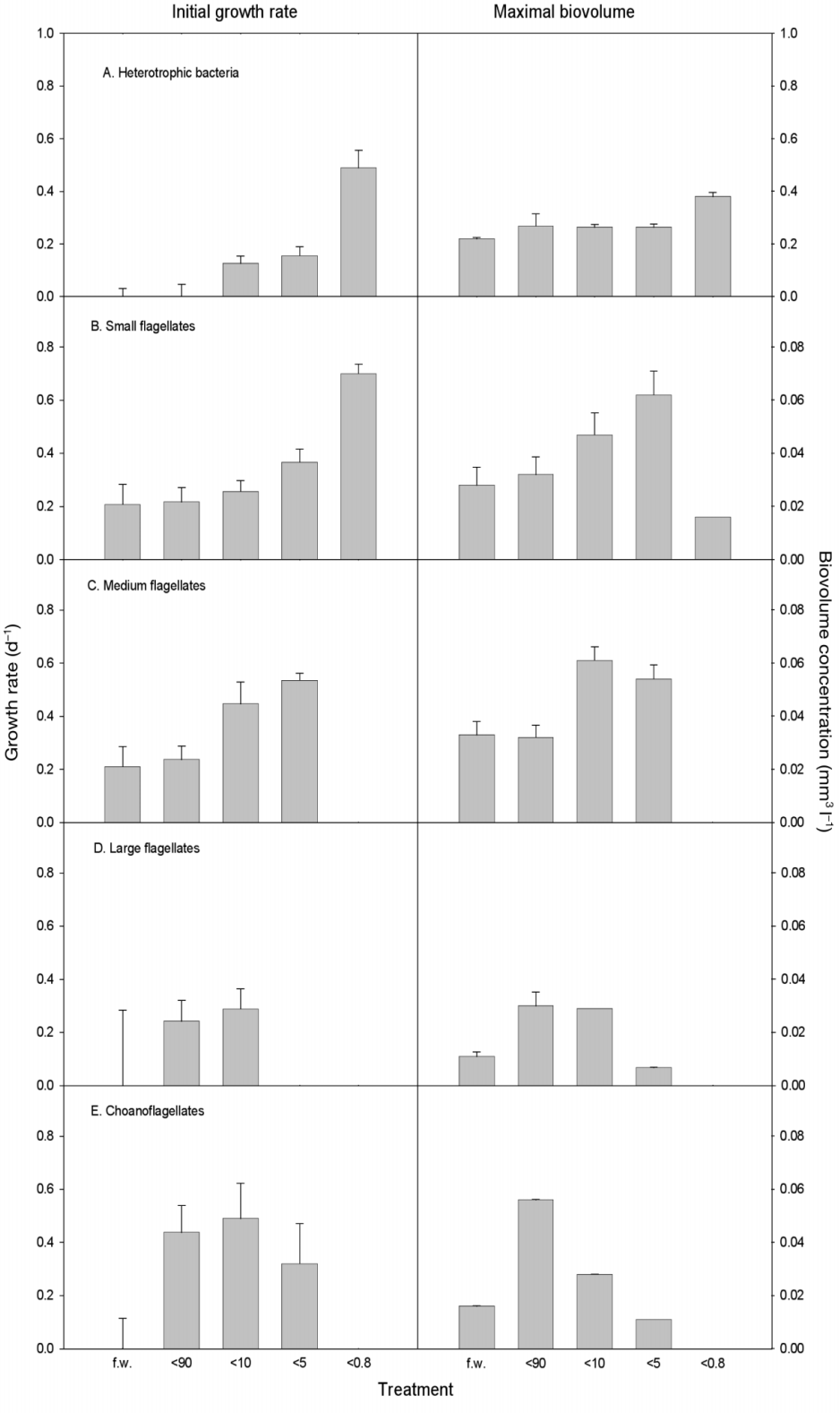

Fig. 1. Initial growth rates (d^{-1}) and maximal biovolume concentration ($mm^{-3}\ l^{-1}$) of heterotrophic organisms in the free water (f.w.) and in different treatments: filtration through 90 µm filter (<90), 10 µm filter (<10), 5 µm filter (<5) and 0.8 µm filter (<0.8). Growth rates are calculated over the first 24 to 48 h for heterotrophic bacteria and over the first 72 h for flagellates. Error bars show SE

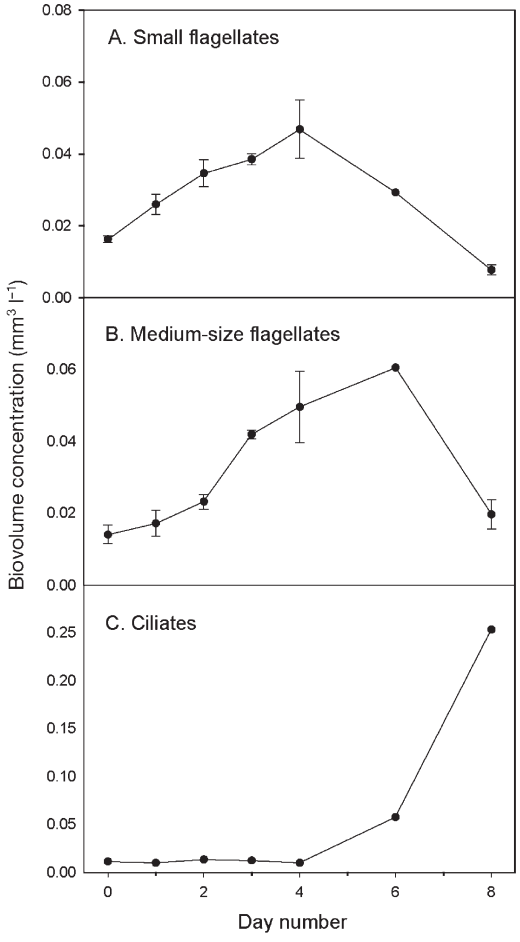

Fig. 2. Biovolume concentration (mm^{-3} l^{-1}). (A) Small heterotrophic flagellates (1 to 5 µm), (B) medium heterotrophic flagellates (5 to 10 µm) and (C) ciliates in the <10 µm treatment. Error bars show SE

for small flagellates, the main predator was found in the 5 to 10 µm size range (medium flagellates and small ciliates); and for medium flagellates, the main predator was observed in the 10 to 90 µm size range (large flagellates and ciliates). All these differences were significant (Kruskal-Wallis test, $p \leq 0.05$). Large flagellates increased their maximum cell concentration in the dialysis bags compared to the free water mass (Kruskal-Wallis test, $p \leq 0.05$). The same response was seen for choanoflagellates, increasing both initial net growth rates and biovolume concentrations (ANCOVA, $p \leq 0.05$; Kruskal-Wallis test, $p \leq 0.05$; Fig. 1E). This could be due to removal of a predator >90 µm or due to favorable effects of incubation in the dialysis bags.

The ciliates did not show any initial positive response to filtration, indicating that this organism group was not predation-limited (Fig. 2C). Instead, a marked positive growth response was observed when the flagellates, as a food resource, had increased in numbers (Fig. 2A,B). This occurred during the later part of the experiment between Days 4 and 8 (Fig. 2C). From the time course experiment, it seemed that the ciliates were feeding on all size groups of flagellates (Fig. 2). The food web was, thus, not completely linear.

The phytoplankton community, measured as chl *a*, showed no response to removal of potential predators (data not shown). In agreement with this result, there was no initial response of nano-sized autotrophic cells (cryptophyceans and unidentified chrysophyceans, 1 to 5 and 5 to 10 µm), indicating that these autotrophs did not constitute a major carbon flow into the heterotrophic microbial food web (Fig. 3C,D,E). On the contrary, picoeukaryotes increased their initial growth rate and biovolume concentration in the <5 µm treatment (ANCOVA, $p \leq 0.05$; Kruskal-Wallis test, $p \leq 0.05$; Fig. 3B). Picocyanobacteria also showed a positive response in initial net growth rates to exclusion of predators (5 and 10 µm filtration, Fig. 3A). The increase in net growth rate was, however, not followed by a significant increase in total biovolume concentration compared to the <90 µm treatment. The lack of increased total biovolume concentration may be explained by lower initial concentrations in the <5 µm treatment compared to the <90 µm treatment. Low grazing rates on picocyanobacteria was, however, also indicated by the lack of numerical response when potential predators (flagellates and ciliates) increased at the end of the experiment (data not shown). The results indicate that in the group of picoautotrophs, at least the eukaryotes entered directly into the heterotrophic microbial food web.

Trophic links

To determine which factor was most important for the net growth rates over the course of the experiment, statistical analysis of the data was performed (Table 2). The result is presented as partial *F*-values for the tested independent parameters, and a higher value indicates a relatively higher predictability of the dependent parameter. Small flagellates showed the best explanation for the variation of bacterial net growth rates, indicating that this group of flagellates was the primary predators on bacteria. The medium flagellates also showed a significant linear relationship to the bacterial net growth rates, but were of less importance. The growth rate of small flagellates showed a negative correlation to medium flagellates, indicating that there was a trophic link between these 2 organisms groups. Differences in growth rate of medium flagellates showed a low but significant negative relation to flagellates >10 µm. Large flagellates

and ciliates did not show any significant correlation to any biotic factor. Chemical and physical factors showed significant relationships to the net growth rates of small flagellates and ciliates.

Variations in initial growth rates in different filtration treatments were used to calculate grazing and to identify trophic links (Eqs. 3, 4 & 5). Calculations indicated that 68% of the bacterial loss was due to grazing by small flagellates, only 6% by medium flagellates and small ciliates, and 26% by large ciliates and large flagellates (Fig. 4). Small flagellates were predated by medium flagellates and small ciliates (69%), large protozoa (25%) and zooplankton >90 µm (6%). The main part of the production of medium flagellates (88%) was channeled to large flagellates and ciliates, while a smaller proportion (12%) was grazed by organisms >90 µm. Large flagellates were observed to be consumed by organisms >90 µm. Picoeukaryotes were grazed by medium flagellates and small ciliates. Data on initial growth rates also indicated that 10 to 90 µm protozoa were important predators on picoeukaryotes. This result was, however, not supported by data on total biovolume concentration (Fig. 3B). Similarly, there were possible links from cyanobacteria to small and medium protozoa.

An indirect response of predation removal was detected after 4 d in the form of decreased bacterial biovolume concentration when predators >5 µm was removed (Fig. 5). This was caused by a higher concentration of 1 to 5 µm flagellates. Also, a decrease of 1 to 5 µm flagellates were observed when predators >10 µm were removed (Fig. 2). This was a response of higher concentrations of 5 to 10 µm flagellates and ciliates in this treatment. No indirect effect on lower trophic levels were observed in the <90 µm treatment, where 10 to 20 µm flagellates increased (data not shown).

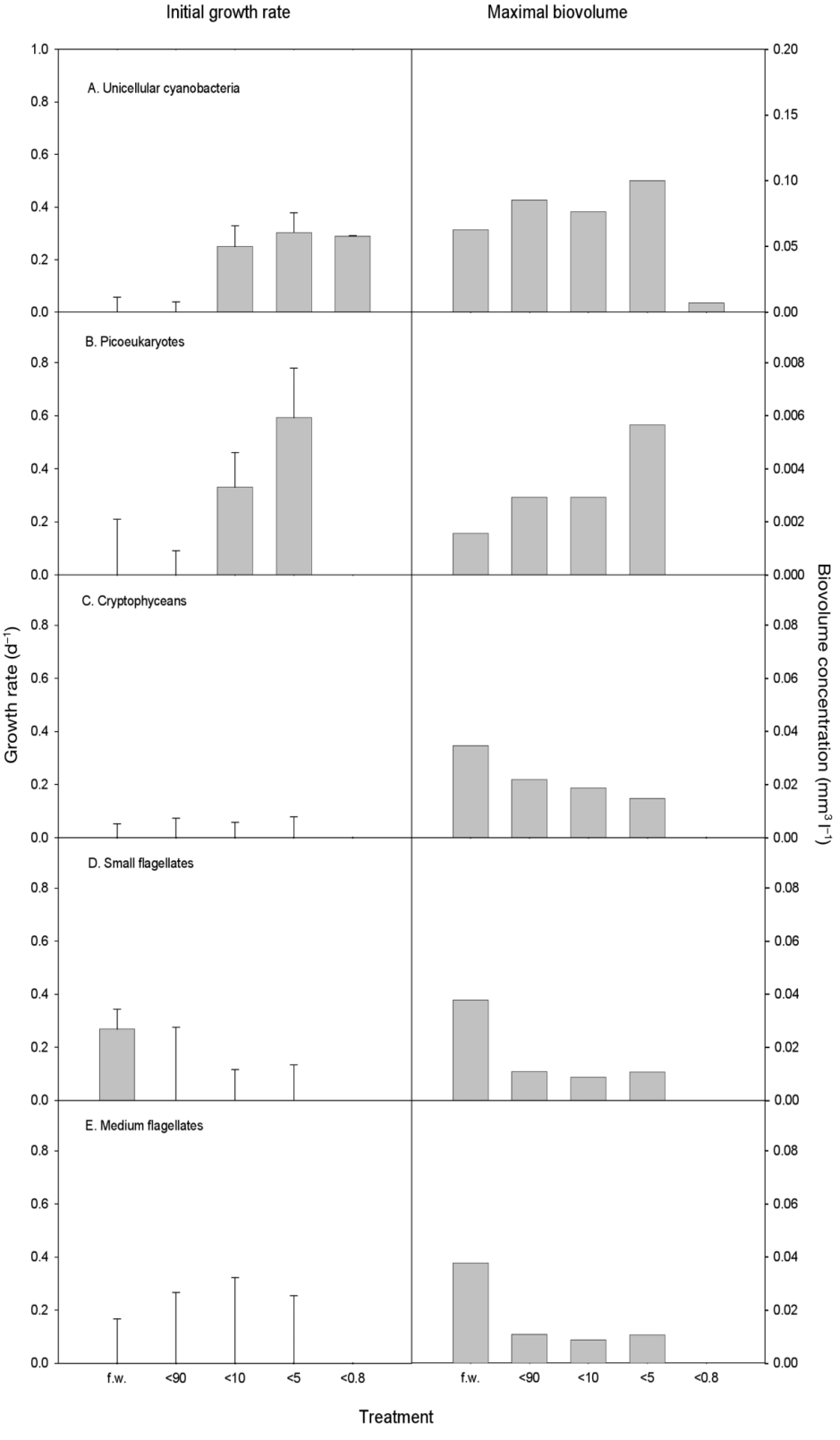

Fig. 3. Initial growth rates (d^{-1}) and maximal biovolume concentration ($mm^{-3}\ l^{-1}$) of autotrophic organisms in the free water and in the treatments (see Fig. 1). Growth rates are calculated over the first 72 h. Error bars show SE

Table 2. Results from multiple regressions presented as partial *F*-values. Linear relationship between growth rates of heterotrophic (H) bacteria, heterotrophic flagellates and phagotrophic ciliates (dependent parameters) were tested against salinity, phosphate, silicate, heterotrophic bacteria, heterotrophic flagellates and ciliates (independent parameters). Only statistically significant ($p \leq 0.05$) values are presented. A minus or a plus in parentheses indicates a negative or positive linear relationship

Dependent parameter (growth rate d^{-1})	Independent parameter	Partial *F*-values
Bacteria[a]	H flagellates 1–5 µm ($mm^3 l^{-1}$)	(–)33.8
	H flagellates 5–10 µm ($mm^3 l^{-1}$)	(–)10.7
H flagellates 1–5 µm[b]	H flagellates 5–10 µm ($mm^3 l^{-1}$)	(–)11.9
	Salinity (‰)	(+)6.9
	Phosphate (mg m^{-3})	(+)6.5
	H flagellates > 10 µm ($mm^3 l^{-1}$)	(–)6.4
H flagellates 5–10 µm[c]	H flagellates > 10 µm ($mm^3 l^{-1}$)	(–)4.4
Ciliates[d]	Phosphate (mg m^{-3})	(–)10.6
	Salinity (‰)	(–)6.3

[a]For the overall model adj $R^2 = 0.52$ and $p = 0.000$
[b]For the overall model adj $R^2 = 0.33$ and $p = 0.016$
[c]For the overall model adj $R^2 = 0.15$ and $p = 0.034$
[d]For the overall model adj $R^2 = 0.34$ and $p = 0.009$

DISCUSSION

Our results show that the direct effect of predation differed between size groups and between autotrophic and heterotrophic microorganisms. Autotrophs larger than 2 µm did not show any response to predator removal. This was seen both in cell count of nano-sized autotrophs and in total chl *a* concentrations. Similar results have been found in both limnic and marine systems (Pace & Funke 1991, Lignell et al. 1992, Kivi et al. 1993, 1996). In a study performed in the Gulf of Finland, only a small effect on the phytoplankton community was detected when predators were removed (Kivi et al. 1996). However, when nutrients were added to the experimental system, the importance of predation increased. This result suggests that the significance of predation as a structuring factor depends on the productivity of the system. Inorganic nutrient depletion, characterizing the summer period, could therefore be an explanation for the low response of the phytoplankton community observed in our experiment. Heterotrophs, however, could possibly maintain high growth rates during this period by retrieving nutrients via predation.

Many protozoan taxa, covering a wide size range, have the potential to graze on autotrophic picoplankton (Kuosa 1990). In this study, however, predation did not seem to be an important factor limiting picocyanobacteria. This was indicated by the lack of a significant increase in maximal biovolume concentration removing potential predators and the absence of response at the end of the experiment when potential predators, flagellates and ciliates, increased. Similar results are reported from the Mediterranean Sea, while strong predation limitation has been reported from other areas (Caron et al. 1991, Pernthaler et al. 1996, Dolan & Šimek 1999, Christaki et al. 2001). This distinction might be explained by differences in species composition or dissimilarity in system nutrient status. Experimental studies have shown that there are species-specific variations in the susceptibility of the picocyanobacteria *Synechococcus* to grazing and that all heterotrophic flagellates are not capable of digesting *Synechococcus* (Boenigk et al. 2001). In our study, picoeukaryotes seemed to be more sensitive to grazing than picocyanobacteria. We estimated a net grazing rate of 0.26 to 0.59 d^{-1} on picoeukaryotes, which is within the range of reported values (0.08 to 0.77 d^{-1}; Reckermann & Veldhuis 1997, Sanders et al. 2000). In the study by Reckerman & Veldhuis (1997), it was found that flagellates <10 µm were the main predators on small picoeukaryotes. Our results imply that medium protozoa (5 to 10 µm) are the dominating grazers of picoeukaryotes.

Estimates of grazing rates on bacteria indicate that grazing often is in the same magnitude as bacterial production (Kuuppo-Leinikki 1990, Wikner et al. 1990). Because no significant changes were measured

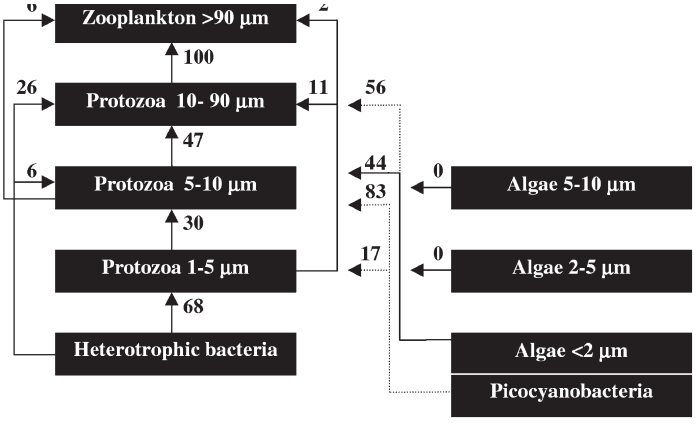

Fig. 4. Grazing rates (% of production grazed d^{-1}) for the different organism groups within the microbial food web. Dotted lines denote grazing rates where the result is not supported by proportional changes in maximal biovolume concentration

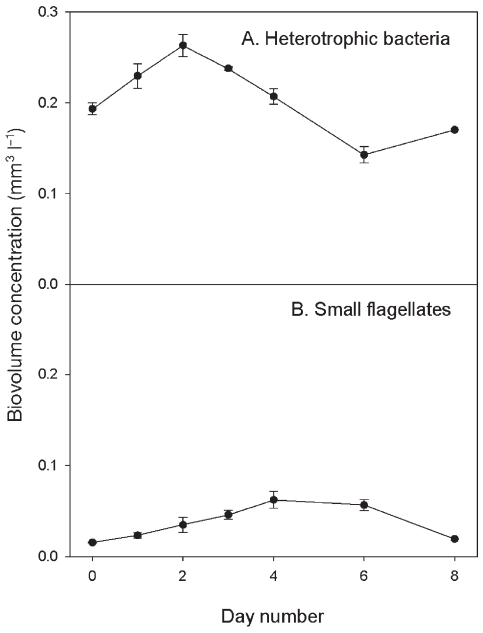

Fig. 5. Biovolume concentration (mm^{-3} l^{-1}). (A) Heterotrophic bacteria and (B) small heterotrophic flagellates (1 to 5 µm) in the <5 µm treatment. Error bars show SE

in the free water mass and in the 90 µm treatment, it seems that the growth rates and grazing rates were in balance also in our study. In accordance with other studies, the main response was seen when removing 1 to 5 µm predators. This was measured as increases in both initial growth rate and biovolume concentration. Our data on grazing rates equal a clearance rate for small flagellates on heterotrophic bacteria of 2 to 8.3 nl flagellate^{-1} h^{-1}, which is in agreement with earlier studies in the area reporting an average of 8.5 nl flagellate^{-1} h^{-1} (Wikner et al. 1990). Thus, heterotrophic flagellates might be an important factor controlling the bacterial number. An alternative explanation may be that the balance between growth and grazing is an effect of reduced resource competition. This hypothesis was tested by Gasol (1994), who showed that a high bacterial production coincided with periods of high concentrations of flagellates relative to bacteria. This suggest, when bacteria are consumed by flagellates they are able to increase their numbers up to the level where resource limitation takes place. This is in agreement with the observation that bacterial production in the northern Baltic Sea on an annual basis is mainly explained by variation in intrinsic growth rate limited by temperature, substrate and competition (Wikner & Hagström 1999). When the predator-free treatment in our study was diluted, the net bacterial growth rate increased from 0.49 to 2.29 d^{-1}, indicating higher importance of substrate competition than predation limitation.

Metazooplankton, rotifers, ciliates and flagellates appear to have the capacity to feed on and limit the number of heterotrophic flagellates (Weisse 1991, Pace et al. 1998). Data from the northern Baltic Sea of predation rates on heterotrophic nanoflagellates are few. In a study performed farther south in the Baltic Sea, the net growth rates showed positive response to predator exclusion (5 µm filtration) during different times of the year, indicating predation limitation of small heterotrophic flagellates (Kuosa 1991). Our results on grazing by predators >90 µm (metazooplankton), which removed 4% of the nanoflagellate community biovolume concentration daily, are in agreement with a study in the Gulf of Finland, where metazooplankton were estimated to remove on average 6% of the nanoprotozoan biomass per day (Uitto 1996). Available data including data from this study, thus indicate that heterotrophic flagellates are predation-limited in the studied system. Food limitation also seems to be an important factor regulating the number and growth rates of flagellates (Sanders et al. 1992, Weisse 1997, Caron et al. 1999). A simultaneous resource limitation would be possible in our experiment, but was not tested. According to a model based on the relative abundance of bacteria and heterotrophic nanoflagellates (Gasol 1994), the flagellates in this system should mainly be bottom-up regulated.

In this study, the ciliates did not respond to removal of predators and appeared to be limited by prey availability. Other studies in both limnic and marine environments have indicated that the ciliate communities were predation-limited (Pace & Funke 1991, Kivi et al. 1993, Nielsen & Kiorboe 1994, Kuuppo et al. 1998, Pace et al. 1998). Differences in resource level and species composition of the metazooplankton may explain differences in response. In the studied system, the potential ciliate predators are dominated by calanoid copepods (*Eurytemora affinis* and *Acartia bifilosa*), rotifers *Synchaeta* spp. and cladocerans *Bosmina longispina maritime* (Kuparinen et al. 1996). These zooplankton have shown the potential to influence the structure of the protozoan community. Kivi et al. (1996) found that addition of a 100-fold concentration of a natural metazooplankton community favored tintinnids and the dinoflagellate *Ebria tripartita*, while at low predation pressure (predators >100 µm were removed), aloricate oligotrich ciliates were favored. In our study, the ciliates community consisted of 70 to 80% aloricate oligotrich ciliates and only 4% tinntinids. This community composition further strengthens the conclusion that the grazing on ciliates was low and that ciliates were mainly resource-limited.

In all calculations of *in situ* grazing rates, we assumed that differences in growth rates after filtration were due to predator removal. There are however

possible experimental artifacts that could influence the result. Bacterial production could benefit from dissolved organic matter (DOM) released due to filtration of the water (Fuhrman & Bell 1985). Removal of large producers and grazers could result in decreased input rates of DOM. By using dialysis bags allowing transport of these small molecules, we hoped to minimize these effects (del Giorgio et al. 1996). Incubation in dialysis bags could also influence the growth rates of organisms by changing the light climate, the daily average temperature, turbulence or accumulation of nutrients. This did not seem to be the case for pico- and nanoplankton, of which most groups had similar initial growth rates in the free water mass and in the <90 µm treatment. The estimated grazing rates in this study in general are in the same range as results from other measurements (see above), which supports our assumption that changes in net growth rates was due to predation release. An additional factor is the effect of reduced competition within the flagellate community due to the filtrations. Several studies indicate that there are different trophic levels within the nanoflagellate community, and that medium flagellates and ciliates are predators primarily on smaller flagellates and thus, not competitors with bacterivorous flagellates (Wikner & Hagström 1988, Sherr et al. 1991). If the response in our experiment was due to decreased competition, another group of flagellates, the choanoflagellates, should react in the same way. This was not the case. The choanoflagellates, assumed to be resistant to flagellate and ciliate predation due to their lorica, showed the same growth rate in all treatments independent of filtration cut-off level. The results indicate that the response of aloricate flagellates was caused by differences in predation pressure and not competition.

The appearances of trophic cascades have previously been used to analyze the microbial food web structure (e.g. Wikner & Hagström 1988, Calbet & Landry 1999, Calbet et al. 2001). In these studies, a quick response of less than 1 d were measured. We did not find such a quick indirect response to removal of potential predators at the flagellate or bacterial levels. After 4 d, however, our results indicated a trophic cascade between ciliates, flagellates and heterotrophic bacteria. In a similar study performed in the Pacific Ocean, trophic cascades down to the bacterial level after 1 d were observed to depend on the initial level of bacteria and seemed to occur at high to intermediate biomass (Calbet et al. 2001). In our study, where no response was seen during the first 24 h, the bacterial biomass was as high or higher than values in the experiment in the Pacific Ocean. A large temperature difference (12°C) and different species composition may explain the discrepancy between the 2 studies. In contrast to our results, a previous study in the northern Baltic Sea indicated quick trophic cascades (<1 d) within the microbial food web (Wikner & Hagström 1988). In that study, changes in the grazing rate of bacteria due to different predator exclusions were analyzed. That method might be more sensitive for identifying trophic cascades than the method used in our study.

In conclusion, autotrophic picoeukaryotes were limited by predation from small and medium flagellates. Cyanobacteria and nano-autotrophs probably suffered from other factors, such as low nutrient levels. Top-down processes structured heterotrophic bacteria and flagellates, while ciliates seemed to be mainly resource-limited. The main flow within the heterotrophic food chain was between adjacent trophic levels. Omnivory was, however, indicated within the flagellate and ciliate groups. Removal of predators caused no rapid responses in non-adjacent trophic levels; however, a response after 4 d indicated indirect effects of predation in the form of trophic cascades. This study was performed in an oligotrophic aquatic system during summer. Since the same functional groups and similar concentrations of protists occur also in other temperate oligotrophic aquatic systems, we believe that the main results of this study are general and applicable to other nutrient-constrained environments.

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