Protozoan grazing on planktonic bacteria and its impact on bacterial population

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ABSTRACT: Grazing by heterotrophic nanoflagellates and < 100 μm microzooplankton on planktonic bacteria was followed during a mesocosm experiment in the Baltic Sea between 23 July and 12 August 1988 on the SW coast of Finland. During the succession of the planktonic community in one mesocosm, 4 grazing experiments were run with a size-fractionation technique. The size fractions used were: < 1 μm (bacterioplankton), < 5 μm (bacteria and heterotrophic nanoflagellates), and < 100 μm (bacteria, heterotrophic nanoflagellates and ciliates). Clearance rate of < 5 μm flagellates was 0.6 to 5.3 nl flag.$^{-1}$ h^{-1} Grazing in the < 5 μm size fraction was 34 to 134 % of grazing in the < 100 μm fraction. The < 5 μm and < 100 μm protozoa harvested hourly on average 75 and 90 % of bacterial production, respectively. Nonetheless, heterotrophic nanoflagellates could not satisfy their carbon demand from bacteria. Grazing by protozoa altered bacterial size distribution, and reduced bacterial cell number and production. When the water temperature was ca 20 °C, < 100 μm microzooplankton could consume about 50 to 100 % of flagellate standing stock daily. During the 3 wk mesocosm experiment, protozoan clearance rate fluctuated in the < 5 and < 100 μm filtrates by a factor of 9 and 3, respectively. A fall of water temperature from 18 to 14 °C was the main factor affecting the activity of the microbial community in the mesocosm.

INTRODUCTION

In the microbial loop (Azam et al. 1983) planktonic bacteria convert dissolved organic substrates to particulate form. Whether the organic carbon is transferred effectively from bacterioplankton to other trophic levels depends on the subsequent steps in the microbial loop.

Heterotrophic nanoflagellates are claimed to be the most important grazers of bacteria and controllers of bacterial populations in both marine (e.g. Fenchel 1982b, Andersen & Fenchel 1985, McManus & Fuhrman 1988) and freshwater (Bloem et al. 1989, Sanders et al. 1989) habitats, together with ciliated protozoa (e.g. Fenchel 1980, Gast 1985, Turley et al. 1986, Sherr & Sherr 1987). Fluctuations of bacterial and flagellate populations show coupled oscillations (Andersen & Fenchel 1985, Bjørnsen et al. 1988, Wikner & Hagström 1988), implying close interaction between the 2 groups. The carbon from heterotrophic nanoflagellates is subsequently transported further in the pelagial food web by ciliates and mesozooplankton (Porter et al. 1979, Azam et al. 1983, Rassoulzadegan & Sheldon 1986). The complexity and effectivity of these processes are

still obscure, as are the diverse factors influencing the dynamics of the microbial loop.

In the Baltic Sea heterotrophic flagellates are grazers of autotrophic pico- and nanoflagellates, picoplanktonic cyanobacteria and planktonic bacteria (Kuosa & Marcussen 1988, Wikner 1989, Kuosa 1990). Kuosa & Kivi (1989) have emphasized the link between bacteria and heterotrophic nanoflagellates in the carbon flow in the northern Baltic. Little is known however, about the variability of that link, for instance its importance in different nutritional conditions or temperatures. Moreover, the direct influence of flagellate grazing on bacterial populations in the Baltic Sea is largely unknown.

In this study, protozoan grazing was studied during a succession of the planktonic community in an enclosure which was manipulated with nutrient and top predator additions. The following questions were asked: What are the apparent grazing rates of heterotrophic nanoflagellates and ciliates, and how large are their short-term (< 1 wk) fluctuations? What part of bacterial grazing is due to small heterotrophic nanoflagellates compared to the rest of the protozoan community? What is the grazing pressure by < 100 μm

protozoa (mainly ciliates) on heterotrophic nanoflagellates? Do heterotrophic nanoflagellates regulate bacterial population by grazing and does flagellate grazing alter the structure of bacterial population?

MATERIAL AND METHODS

Protozoan grazing experiments were carried out as a joint experiment on plankton dynamics between 23 July and 12 August 1988, in the Tvärminne sea area on the SW coast of Finland, northern Baltic Sea. Pelagial water was enclosed in plastic mesocosms (volume ca 30 m^3, diameter 2.15 m, depth 8 m). The enclosures were manipulated by sequential additions of inorganic nutrients (PO_4-P: 4 mg m^{-3} d^{-1} and NH_4-N: 16 mg m^{-3} d^{-1}) and young three-spined sticklebacks *Gasterosteus aculeatus* as predators (90 ind. enclosure^{-1}). Each manipulation period lasted for 5 d, and the whole experiment lasted for 21 d. The sequence of manipulations in the enclosure used for protozoan grazing experiments is presented in Fig. 1.

Water temperature was about 20 °C in the beginning of the enclosure experiment. It dropped drastically several degrees on the 8th experimental day, shortly before grazing Expt III, and remained between 12.5 and 14.5 °C during the rest of the experiment (Fig. 1). At the beginning of the grazing Expts I, II, III and IV, water temperatures were 21.3, 18.0, 13.1 and 14.5 °C, respectively.

The salinity of the water in the enclosure was ca 5‰.

Experiments. The protozoan grazing experiments were run using the size-fractionation technique (Wright & Coffin 1984, Kuuppo-Leinikki & Kuosa 1990) with 5 d intervals (Fig. 1). Water samples were taken with a Ruttner-type sampler from 0, 1, 2, 3, 4, and 5 m. An integrated water sample was collected in an autoclaved polycarbonate flask (8 l) and transported to the
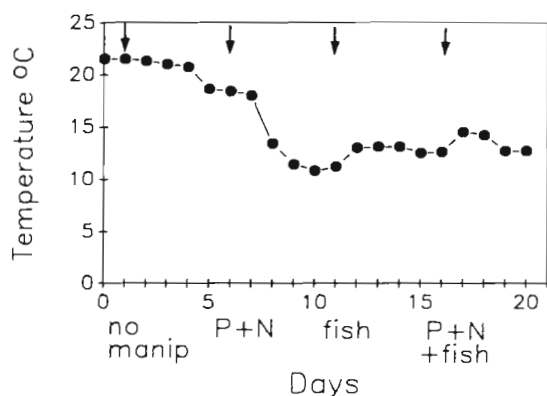

Fig. 1. Water temperature and manipulation periods in the mesocosm experiment, 23 July to 12 August 1988. Arrows: start of grazing experiments

laboratory where the water was filtered through a 100 μm plankton net and 5 μm and 1 μm Nuclepore polycarbonate filters (diam. 50 mm) with a sterilized Nalgene filtering device. The < 100 and < 5 μm subsamples were filtered by gravity and < 1 μm samples with a vacuum < 2 kPa. Expts I and IV were started in the evening and Expts II and III in the morning.

Duplicate < 100, < 5 and < 1 μm batch cultures were incubated in a shaker in acid-washed sterile and darkened glass flasks (1 l) in the laboratory for 48 h and sampled every 12 h. The temperature was adjusted according to the in situ temperature: 18 °C in Expts I and II, and 10 °C in III and IV.

Bacterial cell production was measured with the ^{3}H-thymidine incorporation technique (Fuhrman & Azam 1980, 1982). Duplicate samples and an absorption control killed with formalin (final conc. 0.5 %) were incubated for 30 min in identical conditions with fractionated batches using 10 nM methyl-^{3}H-thymidine (40 to 42 Ci mmol^{-1}; Radiochemical Centre, Amersham, UK). Incubations were terminated by adding formalin (final conc. 0.5 %). The samples were extracted in ice-cold TCA, and the particulate material was collected by filtering the sample through a 25 mm diameter 0.2 μm pore size Sartorius membrane filter. The samples were stored at 6 °C for 3 to 4 d before the filtration procedure.

The effect of preservation (Bell et al. 1983, Riemann 1985) on ^{3}H-thymidine samples was checked. The radioactivity of the samples decreased 7 to 14 % from the original in 4 d. A correction factor to compensate for the decrease was applied in calculations of bacterial cell production.

Conversion factors to estimate bacterial cell production from ^{3}H-thymidine incorporation were calculated from the < 1 μm size fraction.

Subsamples for cell counts were fixed immediately after sampling with unbuffered glutaraldehyde (final conc. 1 %). Heterotrophic nanoflagellates and bacteria were stained with proflavine (Haas 1982) and examined with a Leitz Dialux microscope fitted with epifluorescence light with a 100 × oil immersion objective under blue excitation light.

The cell counts were done on the same or the next day after sampling. Flagellates were enumerated from 100 fields, the number of cells counted varying between 30 to 320. The number of counted bacterial cells was 150 to 450. Flagellates were counted from duplicate filters. Average difference in flagellate counts between the duplicates was 18, 19 and 38 % in the < 100, < 5 and < 1 μm size fractions, respectively.

The maximum dimension of 100 flagellates from each sample was measured using the New Porton ocular graticule (May 1965). One hundred bacteria were measured with the New Porton graticule. Rod-shaped and coccoid forms were differentiated.

Total number of microzooplankton was counted from integrated 0 to 5 m samples taken a day before each grazing experiment. These samples were fixed with Lugol's iodine solution and counted with the Utermöhl technique.

Calculations. Flagellate cell volumes (V) were estimated by the equation of an ellipsoid:

$$V = (\pi\, l\, d^2)/6 \qquad (1)$$

where l = cell length (diameter of the circle in the grid); d = cell width (microscopically estimated, on average $2/3 \times$ length).

Bacterial cell volume was estimated by:

$$V = (\pi/4)\, d^2\, (l-d/3) \qquad (2)$$

where l = cell length; d = cell width.

The volumes were converted to carbon units by using the factors 0.22 pgC μm^{-3} for heterotrophic nanoflagellates (Børsheim & Bratbak 1987) and 0.35 pgC μm-3 for bacteria (Bjørnsen 1986).

The generation times (G) of bacteria in the < 1 μm size fraction and heterotrophic nanoflagellates in the < 5 μm size fraction were calculated by:

$$G = (\ln 2\, /\, (\ln n_2 - \ln n_1))\, t \qquad (3)$$

where n_1 = cell number at the beginning of the incubation; n_2 = cell number at the end of the incubation; t = duration of the incubation (h).

The conversion factors to estimate bacterial cell production from ^{3}H-thymidine incorporation in the < 1 μm fraction were calculated according to Riemann et al. (1987):

$$CF = (n_2 - n_1)\, /\, {}^3H\ inc. \qquad (4)$$

where n_1 = cell number at the beginning of the incubation; n_2 = cell number at the end of the incubation; ^{3}H inc.= integrated thymidine incorporation rate during the period.

Grazing rates on bacteria in the < 5 and < 100 μm fractions were calculated from the difference between bacterial cell production measured with the ^{3}H-thymidine technique, and the observed number of bacteria (Kuuppo-Leinikki & Kuosa 1990)

$$Grazing = \Sigma\ (n_{2est} - n_{2obs.})\, /\, s \qquad (5)$$

$$n_{2est.} = n_{1obs.} + P_1 \qquad (6)$$

where n_1 = bacterial number at time t_1; n_2 = bacterial number in time t_2; P_1 = bacterial cell production at time t_1, using the conversion factor determined in each experiment; s = number of samplings − 1 (applicable only if sampling periods are of the same length).

In both the < 5 and < 100 μm fractions, grazing and clearance rates were expressed as bacteria eaten per flagellate per unit time, because the number of other protozoa in the < 100 μm fraction was not known.

Grazing rate by < 100 μm protozoa on heterotrophic nanoflagellates was estimated from the difference in flagellate growth between ungrazed (< 5 μm) and grazed (< 100 μm) samples:

$$Grazing = BB\ (5\mu m) - BB\ (100\ \mu m) \qquad (7)$$

where BB = $N_o\, e^{kt}$; k = flagellate growth constant; t = time (h).

Statistical analysis. In order to study the impact of grazing on bacterial number and production, linear regression was fitted into the values from the < 1 and < 5 μm size fractions. The regression coefficients between different size fractions were compared statistically. Regressions with an r^2 value of > 50 % were analysed further.

RESULTS

Bacterial number

Virtually all the planktonic bacteria were unattached in the experiments, and the effect of filtration on bacterial number was negligible. The differences in bacterial densities between < 100, < 5, < 1 μm and filtrates were always within the error of microscopical counts.

Generation times of the bacterial population in the < 1 μm fraction were 3 to 5 d (Table 1).

The number of bacteria after filtration was 3.5 to 7.5 $\times\ 10^6$ cells ml^{-1}, and it varied during the 48 h incubation from 2.8 to 10.9 $\times\ 10^6$ cells ml^{-1} (Fig. 2). Bacterial numbers increased in the < 1 μm size fraction, but in the presence of protozoan grazers they remained more or less constant (Expts III and IV) or decreased (I and II). The control of bacterial abundance by heterotrophic nanoflagellates in the < 5 μm size fraction in Expt II was statistically significant (Table 2).

Bacterial production

The average conversion factor for bacterial cell production, calculated from the < 1 μm size fraction, was 0.1 $\times\ 10^9$ cells $nmol^{-1}$ (range 0.7 to 1.4 $\times\ 10^9$).

At the beginning of each experiment, bacterial cell production was comparable in all size fractions (Fig. 3). In the < 1 μm size fractions bacterial production increased, reaching values of 4.3 to 7.9 $\times\ 10^4$ cells ml^{-1} h^{-1} (Fig. 3C). In the < 5 and < 100 μm size fractions there was a decline in cell production in Expts II and IV but an increase in Expts I and III, which in Expt I exceeded the < 1 μm size fraction (Fig. 3).

Protozoan grazing in the < 5 μm size fraction significantly reduced bacterial production in Expts II and III (Table 2).

Table 1. Bacterial and protozoan parameters at the beginning of grazing incubations during the mesocosm experiment between 23 July and 12 August 1988. All values are from the < 100 μm size fraction unless otherwise mentioned

Parameter	Expt I	Expt II	Expt III	Expt IV
Bacterial density (cells ml^{-1})	6.5×10^6	6.8×10^6	3.9×10^6	6.4×10^6
Average volume of bacteria (μm^3)[a]	0.049	0.043	0.032	0.038
Bacterial production (cells ml^{-1} h^{-1})	3.7×10^4	6.6×10^4	1.3×10^4	8.0×10^4
HNAN density (cells ml^{-1})	2.4×10^3	2.1×10^3	4.8×10^3	1.8×10^3
Average volume of HNAN (μm^3)	25.9	17.0	23.6	39.2
Microzooplankton density, total (cells l^{-1})[b]	1.9×10^4	2.5×10^4	8.1×10^3	2.0×10^4
Generation time of bacteria (d)[c]	5	3	5	3
Generation time of HNAN (d)[a,c]	1	0.7	8	21

[a] Value from the < 5 μm size fraction
[b] Value from the enclosure
[c] Value from 48 h incubation

Table 2. Effect of flagellate grazing on bacterial density and production: regression coefficients of bacterial cell number and production against time in < 1 and < 5 μm filtrates were compared statistically. · Regressions with r^2 < 50 %; were not analyzed

	Regression coefficient (r)	
	of bacterial cell number	of bacterial production
Expt I		
< 1 μm	4.0×10^4	2.0×10^2·
< 5 μm	-3.9×10^3·	3.4×10^2
Significance	not tested	not tested
Expt II		
< 1 μm	8.0×10^4	3.0×10^2
< 5 μm	-5.3×10^4	-5.0×10^2
Significance	$p < 0.001$	$p < 0.001$
Expt III		
< 1 μm	3.6×10^4	1.0×10^3
< 5 μm	1.3×10^4·	4.9×10^2
Significance	not tested	$p < 0.001$
Expt IV		
< 1 μm	8.3×10^4	2.6×10^2·
< 5 μm	7.4×10^3·	-6.8×10^2
Significance	not tested	not tested

Bacterial size distribution

At the beginning of the incubations, average volume of bacteria was 0.041 μm^3 (Table 1). The bacteria were largest in the first 2 experiments. Bacterial size difference was statistically insignificant between the experiments, as well as before and immediately after the filtration through the 1 μm filter.

The percentage of cells < 0.05 μm^3 was 80 to 91 % in the beginning of the experiments, and it decreased to 65 to 76 % during the incubation in the < 1 μm fraction. In the grazed samples (< 5 μm) the proportion of the smallest cells remained 83 to 86 %, but decreased in Expt III to 62 %.

Average bacterial volume in the < 1 μm size fraction increased during the 48 h incubation, but significantly only in Expts II and III (Table 3). In the presence of heterotrophic nanoflagellates (< 5 μm) the mean cell size decreased in the first 2 experiments, but increased in Expt III (Table 3).

The proportion of rods increased during incubations in the < 1 μm size fraction, but decreased in the < 5 μm fraction (Fig. 4). The elongation of the cells was most pronounced in Expt III, coinciding with low bacterial number and intensive cell production, probably caused by weak grazing pressure. In contrast, the proportion of rods in the < 1 μm fraction decreased in Expt II, when the grazing rate of protozoa was highest.

Number of heterotrophic nanoflagellates

The taxonomical composition of the community of heterotrophic flagellates in the enclosure was studied (Vørs unpubl.). About 40 taxa of heterotrophic nanoflagellates were detected in the enclosure.

Immediately after the filtration, the number of heterotrophic nanoflagellates varied between 0.9×10^2 and 4.8×10^3 cells ml^{-1}. In the < 5 μm fraction the flagellate number was 55 to 100 %, and in the < 1 μm fraction 9 to 28 % of that in the < 100 μm fraction (Fig. 5).

During the incubation, the flagellate number remained constant in the < 1 μm size fraction (Fig. 5C). It was 2.4 to 11.0 % of the number in the < 100 μm size fraction. Without grazers (< 5 μm size fraction) flagellate density increased 5- and 10-fold in the first 2 experiments, but remained more or less constant in Expts III and IV (Fig. 5B). In the < 100 μm filtrate, flagellate growth was slower than in the < 5 μm filtrate in Expts I and II, which was attributed to microzooplankton grazing. In the last 2 experiments the growth was equal in the < 5 μm and < 100 μm fractions (Fig. 5A).
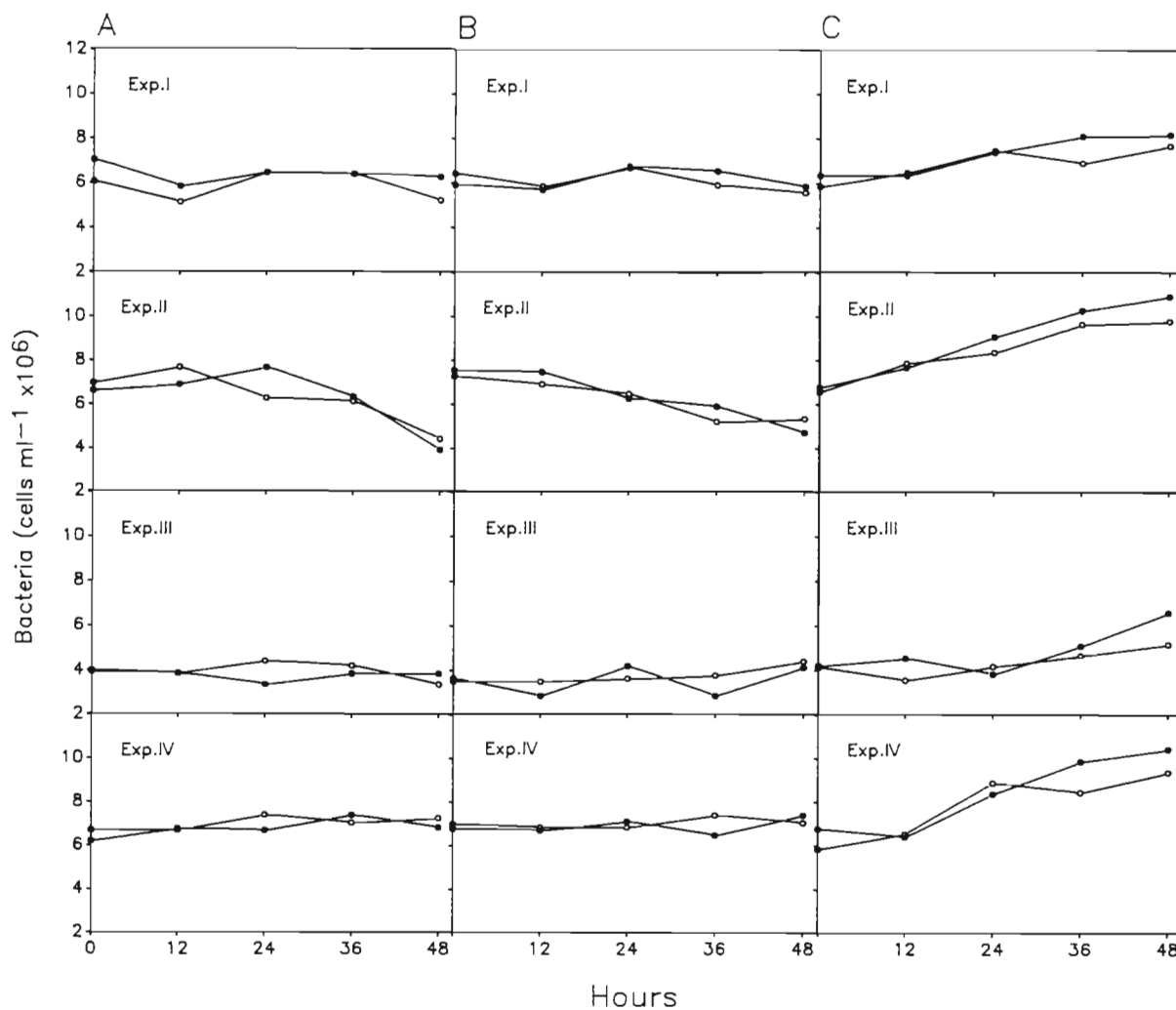
Fig. 2. Development of bacterial number in grazing Expts I, II, III and IV. (●, ○) Duplicates. Size fractions: (A) < 100 µm, (B) < 5 µm and (C) < 1 µm

Generation time of heterotrophic nanoflagellates varied between 0.7 and 21 d (Table 1). It seemed to be affected by the incubation temperature.

Size distribution of heterotrophic nanoflagellates

The mean volume of heterotrophic nanoflagellates at the beginning of the grazing experiments varied between 17.0 and 39.2 µm^3 (Table 1). The average flagellate volume decreased during the incubations, except in the < 100 µm filtrate in the first experiment.

In the < 100 µm size fraction, flagellate size distribution varied significantly between the experiments (1-way ANOVA: $p < 0.001$, Tukey's a posteriori test: all experiments different), but filtration through 5 µm equalized the cell size distribution between the experiments (1-way ANOVA: $p < 0.001$, Tukey's a posteriori test: only Expt I different), as the largest flagellates

were retained on the < 5 µm filter. Taking the whole set of flagellate cell sizes during the incubation, the difference between the < 100 and < 5 µm size fractions remained statistically significant in all experiments except Expt I (Table 4).

Microprotozoa

The total cell numbers of < 100 µm microzooplankton (ciliates) in the enclosure, on the day before grazing experiments, was 7.3×10^3 to 2.3×10^4 cells l^{-1}, which is fairly high compared to the results of Kivi (1986).

The microzooplankton that passes the < 100 µm net consists mainly of ciliates (K. Kivi unpubl.), of which *Lohmanniella oviformis*, *Strombidium* sp. and *Urotrichia* sp. were dominant. Nano-sized (< 20 µm) unidentified ciliates were found before Expts I, II and IV in densities between 3.0 and 1.9×10^2 cells l^{-1}.
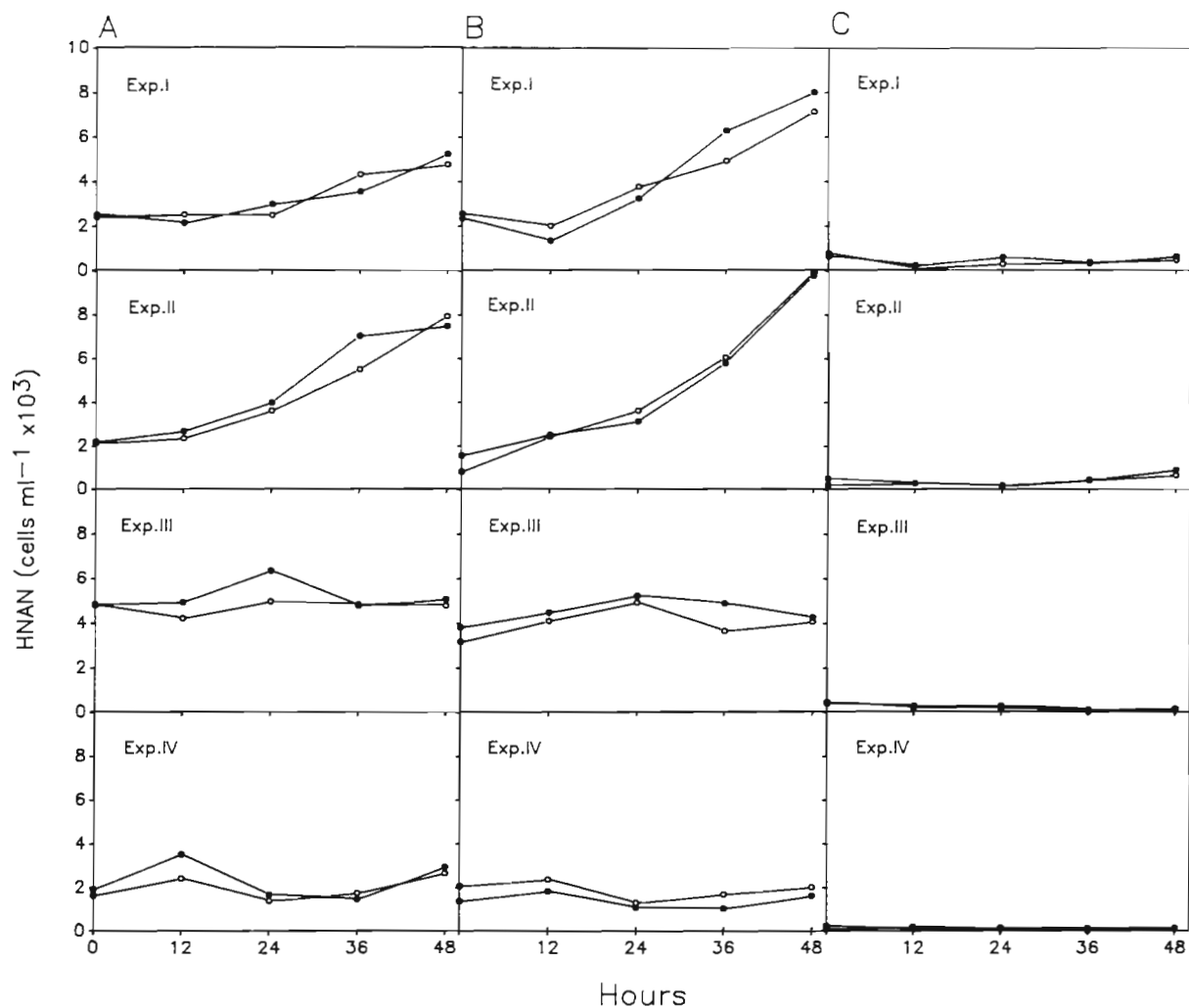
Fig. 3. Bacterial cell production in grazing Expts I to IV (●, ○) Duplicates. Size fractions: (A) < 100 μm, (B) < 5 μm and (C) < 1 μm

Table 3. Effect of flagellate grazing on bacterial cell volume, tested with Mann-Whitney's U-test

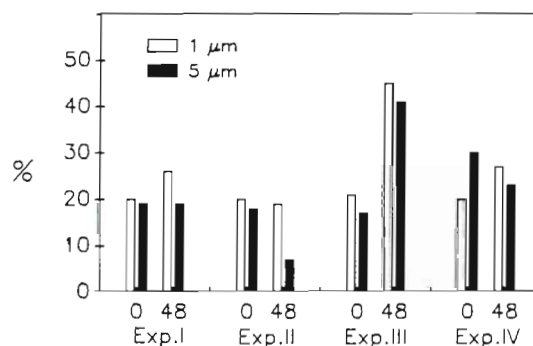|	Mean cell volume (SD) (μm^3)		Significance
	0 h	48 h	
Expt I			
< 1 μm	0.024 (0.027)	0.086 (0.418)	p = 0.0811
< 5 μm	0.049 (0.124)	0.042 (0.091)	p = 0.9047
Expt II			
< 1 μm	0.028 (0.050)	0.057 (0.086)	p = 0.0045
< 5 μm	0.043 (0.089)	0.030 (0.057)	p = 0.1498
Expt III			
< 1 μm	0.024 (0.052)	0.084 (0.139)	p = 0.0000
< 5 μm	0.032 (0.080)	0.095 (0.174)	p = 0.0151
Expt IV			
< 1 μm	0.031 (0.048)	0.049 (0.081)	p = 0.0693
< 5 μm	0.038 (0.061)	0.034 (0.065)	p = 0.0661

Fig. 4. Proportion of rod-shaped bacteria at the beginning (0) and at the end (48) of the grazing experiments

Grazing by protozoa

The ingestion rate varied from 2.0 to 36.8 bacteria flagellate^{-1} h^{-1} and clearance rate from 0.6 to 5.3 nl flagellate^{-1} h^{-1} (Table 5). The ingestion and clearance values in the < 5 μm filtrate were from 34 to 134 % of that in the < 100 μm filtrate. In Expt IV, grazing was more intensive in the fraction with only small flagellates than in the fraction with large flagellates and other protozoa. After the temperature drop (Expt III), the contribution of < 5 μm flagellates to grazing was
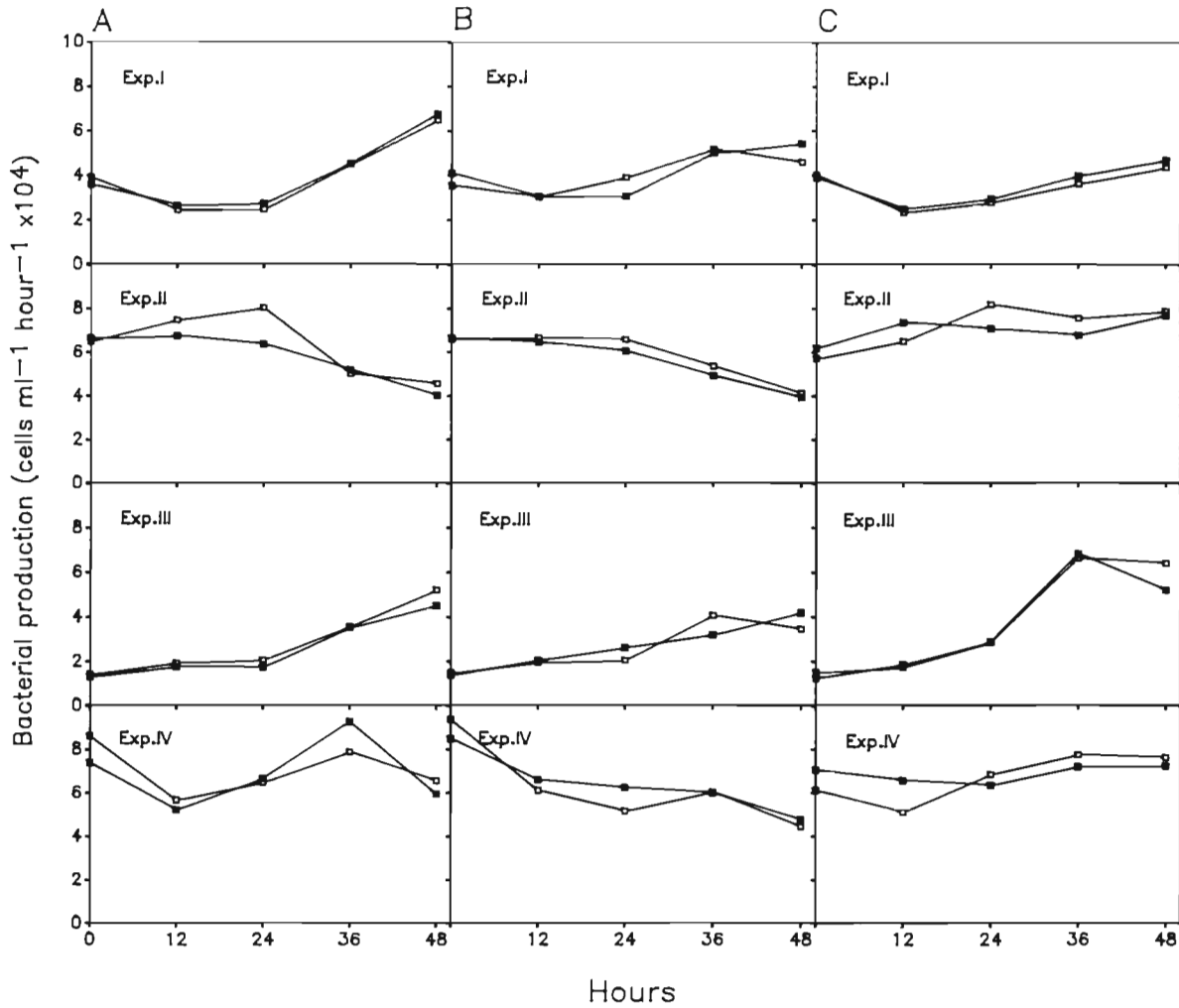
Fig. 5. Number of heterotrophic nanoflagellates in the grazing experiments. (●, ○) Duplicates. Size fractions: (A) < 100 μm, (B) < 5 μm and (C) < 1 μm

smallest. Volume-specific clearance in the < 5 μm size fraction was 0.4 to 2.9 × 10^5 h^{-1} (Table 5). Clearance rate of bacteria by the protozoan community varied from 1.0 to 6.6 × 10^4 bacteria ml^{-1} h^{-1}, which corresponded to between 5 and 23 % of the water column per day. The protozoa cleared on average 83 % of bacterial cell production (Table 5).

Bacterial carbon consumption per flagellate biomass varied from 1 to 12 % h^{-1} (Table 5). The value was largest when grazing rates were high.

Apparent gross growth efficiency of heterotrophic nanoflagellates was from 0.6 to 105 % (Table 5). Lowest values were obtained when grazing rate, bacterial number and cell production were low and bacteria were small (Table 5).

The drop in water temperature from 18 to 13 °C between Expts II and III was accompanied by decreased bacterial density, biomass and production (Table 1), increased protozoan density, biomass and flagellate generation time and decreased protozoan grazing efficiency (Tables 1 and 5). In Expt IV the

microbial community probably had changed, and consisted of species that were adapted to lower temperature.

DISCUSSION

Bacterial numbers and production

The number and biovolume of bacteria were in accordance with previous studies from the Tvärminne area (e.g. Väätänen 1981, Virtanen 1985, Kuparinen 1987). The high proportion of small cocci in the Baltic Sea especially in summer has also been reported by Virtanen (1985) and Andersson et al. (1986). It has been suggested as a consequence of size selective grazing by nanoflagellates (Andersson et al. 1986).

The small cell size may also refer to the dormant state of the bacteria (e.g. Bell et al. 1983), which can also cause the long doubling time of the population (Moriarty & Pollard 1982, Riemann et al. 1984). As the

enclosure experiment started during a period of out-flow of less saline water from an inner archipelago, a high proportion of dormant bacteria is possible. Yet, the generation times of the bacterial population in this study (3 to 5 d) were not exceptionally long (Fuhrman & Azam 1982, Riemann et al. 1984, Moriarty et al. 1985).

In the grazing experiments practically all the bacteria were free-living. Moreover, Väätänen (1981) observed that a maximum of 4 to 6 % of bacteria were attached to planktonic algae and detritus particles in the Tvärminne area during summer. This fact decreases the risk of obtaining underestimated grazing values with the fractionation method, caused by retention of particles on the 5 μm filter, and so excluding any particle-associated flagellates (Fenchel 1982b, Caron 1987, Sibbald & Albright 1988).

Table 4. Difference in size distribution of heterotrophic nano-flagellates between <5 and <100 μm filtrates, tested with Student's t-test

	Mean diameter of HNAN (SD) (μm)		Significance
	<100 μm	<5 μm	
Expt I	4.219 (1.483)	4.144 (1.449)	p = 0.3018
Expt II	3.966 (1.223)	3.870 (1.130)	p = 0.0396
Expt III	4.513 (1.400)	4.228 (1.208)	p = 0.0005
Expt IV	4.897 (1.673)	4.346 (1.305)	p = 0.0000

The conversion factors for bacterial cell production obtained in this study (0.7 to 1.4 × 10^9 cells nmol^{-1}) were in the lower end of the spectrum reported in the literature (Kirchman et al. 1982, Riemann et al. 1987, Coveney & Wetzel 1988, Smits & Riemann 1988). However, they were in accordance with the theoretical value (Fuhrman & Azam 1980) and values previously obtained in the Tvärminne area (Kuparinen 1987). Therefore, the factors of this study were used instead of literature values for calculations of bacterial production.

Heterotrophic nanoflagellates in the filtrates

The number and size of heterotrophic nanoflagellates were in the same range as values from the study of Kuosa & Marcussen (1988) and Kuuppo-Leinikki & Kuosa (1990) from the Tvärminne area. The proportion of heterotrophic nanoflagellates that passed the 5 and 1 μm filters is in agreement with the results of Wikner & Hagström (1988). In their study, however, filtration reduced bacterial counts, which did not occur in these experiments.

The number of heterotrophic nanoflagellates in the <1 μm filtrate did not increase during the incubation, which implies that the flagellates either were damaged after being squeezed through 1 μm pores, or were

Table 5. Results from the protozoan grazing incubations during mesocosm experiment between 23 July and 12 August 1988. All values are from 48 h incubations

	Expt I	Expt II	Expt III	Expt IV
Ingestion rate (bact. flag.$^{-1}$ h^{-1})				
<5 μm	11.6	24.4	2.0	36.8
<100 μm	15.3	26.7	5.8	27.6
% (<5 μm/<100 μm)	75	91	34	134
Clearance rate (nl flag.$^{-1}$ h^{-1})				
<5 μm	1.9	3.9	0.6	5.3
<100 μm	2.5	4.2	1.5	4.0
Volume-specific clearance (flag. h^{-1})				
<5 μm	1.1×10^5	2.8×10^5	0.4×10^5	2.8×10^5
<100 μm	1.3×10^5	2.9×10^5	0.7×10^5	1.4×10^5
Predation rate (bact. ml^{-1} h^{-1})				
<5 μm	2.8×10^4	5.1×10^4	1.0×10^4	6.6×10^4
<100 μm	3.7×10^4	5.6×10^4	2.8×10^4	5.0×10^4
Predation/production				
<5 μm	68 %	89 %	38 %	105 %
<100 μm	93 %	93 %	104 %	71 %
Carbon consumption per flagellate biomass (in 1 h)	5 %	11 %	1 %	12 %
Gross growth efficiency of HNAN[a]	105 %	39 %	0.6 %	3 %
Microzooplankton ingestion rate of HNAN (flag. protoz.$^{-1}$ h^{-1})	2.5	4.7	0	0

[a] Value calculated only from <5 μm size fraction

dormant stages that might start dividing after 48 h (Kuuppo-Leinikki & Kuosa 1990). Multiplying the ingestion rate in the < 5 µm fraction by the flagellate number in the < 1 µm fraction, the estimated loss of bacteria via flagellate grazing throughout the experiments was maximally between 1 and 15 % of the production of new bacterial cells. Therefore, the effect of heterotrophic nanoflagellates on bacteria, e.g. the conversion factor for bacterial production in the < 1 µm filtrate (Fuhrman & McManus 1984, Cynar et al. 1985), was minor. The study of Wikner & Hagström (1988) from the Baltic Sea also showed that the flagellate predation rate decreases sharply in < 1 µm water sample. Lysis of bacterial cells by viruses (Bergh et al. 1989) could have caused loss of bacteria in the < 1 µm filtrate, which probably lowered the conversion factor. However, it is reasonable to assume that the rate of viral infection was equal in < 1, < 5 and < 100 µm filtrates, and it had no effect on the apparent grazing rates.

Grazing on bacteria by heterotrophic flagellates

Ingestion and clearance rates of heterotrophic nanoflagellates were low compared to values obtained with labeled bacteria as food tracers for natural assemblages (e.g. Sherr et al. 1986, Wikner et al. 1986, Wikner & Hagström 1988, Wikner et al. unpubl.). They were, however, in accordance with several other studies done with natural water samples (Wright & Coffin 1984, Coffin & Sharp 1987, Bjørnsen et al. 1988, Kuosa & Marcussen 1988, Kuuppo-Leinikki & Kuosa 1990). The fractionation method tends to give somewhat lower grazing values than the use of labeled bacteria.

The volume-specific clearances (average 1.8×10^5 h^{-1}) were in accordance with the values of e.g. Fenchel (1982a), McManus & Fuhrman (1988) and Nygaard et al. (1988), which implies that the low grazing values of these experiments could be due to the small size of flagellates.

In the experiments the < 5 µm flagellates were able to graze, on average, 75 %, and the < 100 µm protozoa 90 % of bacterial production, which agrees with the level reported in the literature (Wikner et al. 1986, McManus & Fuhrman 1988, Roman et al. 1988, Wikner et al. unpubl.). It seems that also in the Baltic Sea protozooplankton, especially heterotrophic nanoflagellates < 5 µm (Wright & Coffin 1984, Wikner & Hagström 1988, Wikner et al. unpubl.), are mainly responsible for bacterial grazing.

In the northern Baltic Sea the effective harvesting of the bacterial population cannot satisfy the carbon demand of heterotrophic nanoflagellates (Kuosa & Kivi 1989). In this study, the flagellates < 5 µm derived only 1 to 12 % hourly of their carbon biomass from bacteria.

Most probably the heterotrophic flagellates used carbon sources other than bacteria: pico- and nanoplanktonic eucaryotes (Parslow et al. 1986, Kuosa & Marcussen 1988) and picoplanktonic cyanobacteria (Campbell & Carpenter 1986, Kuosa 1990) or possibly high molecular weight DOC (Sherr 1988).

Apparent flagellate growth yield (gross growth efficiency 0.6 to 105 %) varied widely in these experiments. The values were calculated on the basis of bacterial carbon only. The value exceeding 100 % also implied that flagellates used other carbon sources in addition to bacterioplankton. When water temperature was low, growth efficiency was low and heterotrophic flagellates likely used the carbon only for maintenance. According to Caron et al. (1986), low water temperature does not affect flagellate gross growth efficiency. The result of this study, however, was a property of the whole flagellate community, in which the growth efficiency of a single species was not necessarily changed. On the other hand, volume estimates and carbon conversion factors used for bacteria and their grazers may severely influence the growth yield estimates (Bratbak 1987).

Effect of grazing on bacterial population

Heterotrophic nanoflagellates not only grazed most of the bacterial production, but the predators also reduced bacterial cell production. However, the increased concentration of substrates and nutrients released by filtration (Fuhrman & Bell 1985) in the < 1 µm filtrate probably masked the effect of reduced nutrient regeneration (Sherr et al. 1983, Goldman et al. 1985, Andersen et al. 1986) and substrate release (Andersson et al. 1985, Turley et al. 1986) by protozoa, which normally enhances bacterial production.

Heterotrophic nanoflagellates showed apparent size selectivity towards larger rod-shaped bacteria, a feature that has been observed among protozoa (Andersson et al. 1986, Turley et al. 1986, Krambeck 1988). The cell volume of bacteria in the ungrazed fraction increased significantly, whereas flagellate grazing kept the cell volume small. Also the change in the proportion of rods showed the effect of predation. In all experiments, the percentage of rods in the fraction with predators remained constant as opposed to the fraction without predators. When predation pressure was weak due to low temperature, the number of rods, as well as large cells in general, increased considerably. The increase was not as pronounced in grazed as in ungrazed fractions. It is possible that small cells, which dominated the bacterial community, were below the threshold size for the flagellates (Andersson et al. 1986).

Effects of temperature

Water temperature had a major effect on the activity of the protozoan community, e.g. on grazing rates and the generation time of heterotrophic nanoflagellates (Caron et al. 1986, Sherr et al. 1988), but also on the gross growth efficiency. Despite the low temperature, the ingestion and clearance rates of < 5 μm heterotrophic nanoflagellates in Expt IV were high. The flagellate community probably consisted of species that were adapted to low temperature and fed effectively on bacteria, as also the volume-specific clearance was high.

Bacterial cell number and production declined with decreasing temperature (Expt III), but were recovered in Expt IV. However, the generation time of bacteria was not affected by changes in temperature. According to Scavia & Laird (1987) bacterial growth rate is controlled by temperature only below +10°C, which agrees with this study.

Grazing by microzooplankton

Dominant ciliates in the enclosure had a minor role in the grazing of bacteria, except in Expt III. It is possible that ciliates could withstand fall in water temperature better than heterotrophic nanoflagellates, and still consume the whole bacterial production. The bacterial density in the experiments was high enough to sustain effective grazing by ciliates (Gast 1985, Albright et al. 1987, Sherr & Sherr 1987), but the average size of bacteria was probably below threshold for ciliates (Fenchel 1980).

The proportion of microzooplankton that passed through 100 μm sieve in this study was a rough estimate based on the previous studies from this area (Kivi unpubl.). Therefore the results give only an idea of the level of grazing on heterotrophic nanoflagellates. With the ingestion values obtained in Expts I and II, the < 100 μm protozoa used 48 and 108 % of the flagellate standing stock in a day, respectively. The doubling times of flagellate population (1 and 0.7 d) were short enough to keep the population growing. After the temperature decrease ciliate grazing on heterotrophic nanoflagellates was extremely weak.

CONCLUSIONS

In this study small heterotrophic nanoflagellates were the most important bacterial grazers, controlling bacterial numbers, production and population structure.

Temperature changes affected the activity of heterotrophic nanoflagellates and microprotozoa either directly or through changes in the bacterial population. Adaptation of the microbial community to low temperature took place in a few days. Also the inorganic nutrients added to the enclosure before the last grazing experiment could have enhanced the bacterial growth and production, and thereby the grazing intensity of protozoa.

The importance of heterotrophic nanoflagellates as the pathway of carbon to the next trophic level could only be suggested in the light of this study. It seemed, however, that a major part of the flagellate carbon was channelled to the grazing food web via ciliates, when favorable conditions for effective predation prevailed.

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