

Evidence for a tightly coupled nanoplanktonic predator-prey link regulating the bacterivores in the marine environment

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ABSTRACT: A coupled predator-prey chain, starting with bacterivores, was investigated using the minicell recapture technique (MiniCap). Water samples were subjected to size fractionation with decreasing filter pore size, in order to obtain a successive truncation of the microbial food chain. Our results showed that the major bacterivores were flagellates in the size range of 1 to 3 μm . The truncation of the food chain caused increased or decreased predation on the bacteria, depending on whether the bacterivores were released from or subjected to increased predation pressure. We present a model describing trophic interactions between organisms less than 12 μm in size. This model suggests 4 trophic levels to form a regulatory chain, exerting a tight control on major bacterivores.

INTRODUCTION

Growth of heterotrophic bacteria in the pelagic ecosystem appears to be balanced mainly by mortality due to predation (Fuhrman & McManus 1984, Wright & Coffin 1984, Wikner et al. 1986). Nanoflagellates and small ciliates have been proposed to be the major grazers on aquatic bacteria (Haas & Webb 1979, Fenchel 1982a, b, Sherr et al. 1984, Andersen & Fenchel 1985, Davies et al. 1985, Gast 1985). However, the sizes of potential bacterivores range from microplankton (20 to 200 μm), such as dinoflagellates and nanociliates, to small organisms (picoflagellates) <0.6 μm (Fuhrman & McManus 1984, Cynar et al. 1985, Gast 1985, Lessard & Swift 1985).

Using prefiltration of natural samples and subsequent recording of changes in bacterial abundance, Wright & Coffin (1984) suggested 1 to 3 μm sized organisms to be the major grazers on bacteria. They assumed that a 1 μm filtrate was predator free and calculated the bacterial growth from these samples. Using prokaryotic and eucaryotic inhibitors, Fuhrman & McManus (1984) reported that 65 % of the predation on bacteria might be caused by organisms less than 0.6 μm in size. However, the use of 'predator free' controls and inhibitors when determining predation on bacteria have been questioned. Firstly, small bacterivores may pass a 1 μm filter (Cynar et al. 1985). Secondly, removal of nutrient excreting protozoans may affect bacterial growth

(Sherr et al. 1986). Hence, bacterial growth in filtered samples may not reflect the actual bacterial production. Thirdly, prokaryotic and eucaryotic inhibitors might give incomplete inhibition of the target organisms and unwanted effects on the non-target organisms, which make the interpretation of the results obtained with this technique difficult (Sherr et al. 1986, Taylor & Pace 1987). Thus, more direct measurements of the predation on pelagic bacteria are needed to determine predator-prey interactions in the microbial food web.

Investigations with this aim are scarce. However, one report by Rassoulzadegan & Sheldon (1986) suggests several trophic interactions, even within the nanoplanktonic size range. Based on the development of different populations following size fractionation of water samples, these authors concluded that at least 4 predator-prey interactions occurred among organisms less than 10 μm in size. Wright & Coffin (1984) also noticed changes in eucaryotic numbers depending on pore size used during the prescreening and suggested this to be due to rupture of the normal trophic interactions. Hence, removal of organisms in trophic levels higher than the bacterivores might also affect the rate of predation on bacteria.

The minicell recapture technique (MiniCap, Wikner et al. 1986) has made it possible to monitor predation on natural sized heterotrophic bacteria in short-term experiments, without the need to know which kind of predators are present or to use antibiotics. In this paper

we analyze the microbial community following size selective screening of water samples, in order to find the most important predators on pelagic bacteria and their regulation.

MATERIALS AND METHODS

Main sampling sites were the routine station Systarna in the Bothnian Sea (Hagström 1984) and Point B in the Bay of Villefranche-Sur-Mer in the Mediterranean Sea (Rassoulzadegan & Sheldon 1986). Water was collected in 10 l bottles from 4 m water depth; measurements were started within 2 h of sampling (about 10 am).

Size fractionations. Water samples of 1200 ml were filtered in each size fractionation (Nuclepore Corp. Pleasanton, Calif., 47 mm diam.). Filters were changed for every 200 ml of water. The 12 to 1 μm filters required only gravity force, while 50 and 100 mm Hg was applied during 0.6 and 0.2 μm filtrations respectively. Filtrations always began with the smallest pore size to avoid contaminations by larger organisms during handling.

Water samples (10 l) used for prolonged incubations were filtered as described above and incubated for 12 h (Mediterranean Sea) or 20 h (Bothnian Sea). As controls, the same volume of unfiltered water was incubated. Rate of predation on bacteria and bacterial production were measured simultaneously.

Rate of predation on bacteria. Filtrates (1200 ml) and 1 unfiltered sample of water were transferred to 2.5 l transparent polycarbonate bottles. Within 1 h of the start of the filtration procedure, genetically marked minicells, prepared from *Escherichia coli* M2141 containing the plasmid pACYC184, were added to water samples in concentrations of 0.9 to 3×10^5 cells ml^{-1} , depending on the specific activity of the minicells (Wikner et al. 1986). The minicells were carefully mixed by gently inverting the bottle 25 times. The bottles were then incubated at ambient temperature and light conditions. Samples of 200 ml were collected on 0.2 μm Nuclepore filters every hour during 4 h starting at time zero, and further treated as described by Wikner et al. (1986). Exceptions from this protocol were the use of Lumasolve (Lumac/3M, no. 1044) and Optiscint 'T' scintillation liquid (LKB, no. 1294) when extracting the marker proteins from the polyacrylamide gel, thus making the use of H_2O_2 and peroxidase unnecessary. Cpm-values were recalculated to give minicells ml^{-1} and the slope of the regression line provided the disappearance rate of minicells.

Bacterial production was measured in Bothnian Sea experiments 1986 and 1988, with the ^3H -thymidine method according to Fuhrman & Azam (1982). Incuba-

tions were started 1 h after filtration. Samples (triplicates) were incubated 1 h with ^3H -thymidine at a 10 nM final concentration.

Yield experiment. In the Bothnian Sea experiment 1986, water samples were filtered as described above. Triplicate samples of 10 ml from each filtrate were passed through a 0.2 μm (25 mm diam.) Nuclepore filter and transferred to 3 test tubes. One ml of brackish water, filtered first through a 1 μm and then through a 0.6 μm filter (in order to remove the major bacterivores) was added to each test tube (final conc. of natural bacteria 2×10^5 cells ml^{-1}). Tubes were incubated at room temperature for 48 h, and preserved using a formalin-ethidium bromide solution (Hagström 1984). The final concentration of heterotrophic bacteria was then determined from direct counts.

Cell counts. Heterotrophic bacteria and flagellates were stained using either DAPI (Porter & Feig 1980) or ethidium bromide (Hagström 1984) and counted in an epifluorescence microscope. One sample from each size fraction was filtered on a 0.2 μm and 0.6 μm Nuclepore filter, for the bacterial and flagellate counts respectively. Fifteen fields and 60 fields respectively, were counted to determine bacterial and flagellate abundance. Size of flagellates in the 1 and 3 μm size fractions was determined by comparison with an ocular grid (G1, Graticules LTD, Tonbridge, Kent, England) during microscopy.

RESULTS

Predation rates

Rates of predation on pelagic bacteria in size fractionated water samples with corresponding statistical data are tabulated in Table 1. Although the experiments cover different seasons (early summer to late autumn) and different locations (Bothnian Sea and Mediterranean Sea), a systematic effect of pore size used in the prescreening ($p < 0.001$, 2-way ANOVA on all fractions and experiments) emerged (Fig. 1). A major decrease in predation rates was observed when pore sizes of 1 μm and less were used in the prefiltration. This decline coincided with sharp decrease in flagellates (Table 3). The 5 and 3 μm size fractions, which preceded this decline, showed the highest predation rates observed during the experiments (up to 1.7 times the rate in unfiltered water). Rates of predation in the 0.2 μm fraction were below the detection limit of the method.

Predation rates in the 10 μm filtrates were also elevated compared to unfiltered samples, however less so than in the 5 to 3 μm filtrates. In the 8 μm filtrate the relative predation rate was dampened, as if the bac-

Table 1. Predation rates ($\times 10^4$ cells $\text{ml}^{-1} \text{h}^{-1}$) in size fractionated water samples from 4 experiments. Standard error (\pm SE), degrees of freedom (df), coefficient of determination (r^2) and relative predation rate (% of unfiltered sample) are shown for each estimate. Systematic variation in relative predation rate was statistically significant ($p < 0.02$) according to 2-way ANOVA (Statworks™)

	Size fraction (μm)								
	Unfilt.	< 12	< 10	< 8	< 5	< 3	< 1	< 0.6	< 0.2
Bothnian Sea 1985									
Predation rate	7.06	7.91	7.63	8.14	11.6	10.5	8.87	3.75	0.06
\pm SE	0.739	3.90	3.21	1.17	1.07	3.90	2.92	0.414	0.050
df	2	2	2	1	2	2	1	2	1
r^2	0.983	0.673	0.739	0.980	0.983	0.784	0.902	0.976	0.571
Rel. pred. (%)	100	112	108	115	165	149	126	53	9
Mediterranean 1986									
Predation rate	7.89	7.28	11.3	2.39	7.96	3.22	1.98	1.99	0
\pm SE	1.40	1.98	2.02	1.31	0.362	1.30	0.548	0.479	0.007
df	1	2	1	2	1	2	2	2	3
r^2	0.969	0.871	0.969	0.625	0.975	0.755	0.866	0.896	0.013
Rel. pred. (%)	100	92	143	30	101	41	25	25	0
Bothnian Sea 1986									
Predation rate	22.9	22.3	30.4	19.2	31.2	38.3	8.54	1.73	0.01
\pm SE	2.28	2.66	1.60	6.566	4.84	6.10	5.09	6.13	0.098
df	3	3	3	3	3	3	3	2	2
r^2	0.973	0.960	0.992	0.743	0.890	0.929	0.484	0.040	0.01
Rel. pred. (%)	100	97	133	84	136	167	37	7	0
Bothnian Sea 1988									
Predation rate	8.03	8.46	8.87	4.37	9.55	13.5	10.3	0	0
\pm SE	3.38	2.34	3.75	4.89	3.12	4.22	2.52	2.08	0.006
df	4	3	4	4	2	4	4	4	4
r^2	0.381	0.876	0.586	0.128	0.824	0.626	0.805	0.040	0.012
Rel. pred. (%)	100	105	111	54.4	119	168	128	0	0

terivores had been repressed. Hence, a bimodal response in rate of predation, was found in the $>3\mu\text{m}$ size fractions (Fig. 1). The systematic dependence of

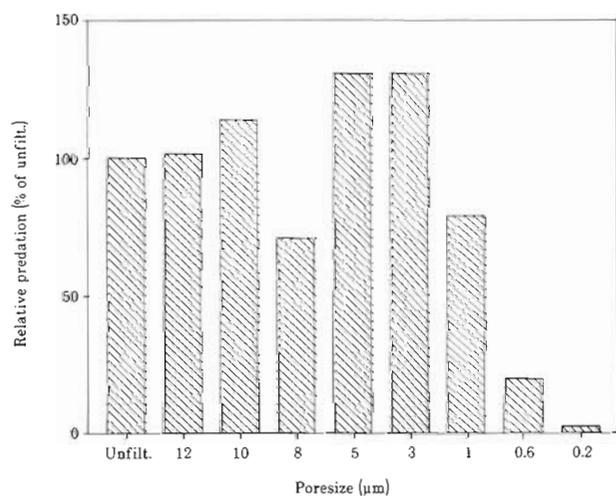


Fig. 1 Relative predation rates in size fractionated water samples compared to unfiltered water. Average rates in each of the size fractions from the 4 experiments (Table 1). Systematic dependence of pore size in the $>3\mu\text{m}$ size fractions was statistically significant ($p < 0.02$) according to 2-way analysis of variance (Table 2)

filter pore size found in the size fractions larger than $3\mu\text{m}$ was statistically significant ($p < 0.02$) according to a 2-way analysis of variance (Table 2).

Abundance of flagellates

Flagellate numbers were $6.7 \times 10^2 \text{ ml}^{-1}$ in unfiltered water in the Mediterranean Sea experiment (Table 3) and showed a significant decrease after a $1\mu\text{m}$ filtra-

Table 2. Analysis of variance obtained by 2-way ANOVA on all size fractions $>3\mu\text{m}$. Systematic dependence of the pore sizes used in prescreening (Fig. 1) was statistically significant ($p < 0.02$)

Source	Sum of squares	Degrees of freedom	Mean squares	F-ratio	α
Between experiments	2198	3	732	1.696	0.305
Between pore sizes	8768	4	2192	5.073	0.013
Error	5183	12	432		
Total	16 152	19			

Table 3. Initial concentration of nanoflagellates in water samples prescreened with filters of different pore sizes. Values for prefiltered water presented as percentage of the concentration in unfiltered water. Values in parentheses: 95% confidence interval for estimated mean value of unfiltered sample. NA: not available, ND: not determined

Location	Year	Unfiltered water (cells ml ⁻¹ × 10 ³)	Fraction (μm)				
			< 10	< 5	< 3	< 1	< 0.6
			(% of unfilt.)				
Mediterranean Sea	1986	0.68 (NA)	ND	100	60	16	ND
Bothnian Sea	1986	4.83 (± 0.63)	ND	80	48	10	< 0.7
Bothnian Sea	1988	10.1 (± 1.78)	51	19	18	8	< 0.7

tion, lowering the concentration to 24% of the unfiltered sample. In the 1986 Bothnian Sea experiment concentration of flagellates in unfiltered water was clearly higher, $4.8 \times 10^3 \text{ ml}^{-1}$. Filtration with successively smaller pore sizes resulted in a gradual decrease in flagellate numbers which was most marked in the 1 μm filtrate. In early summer 1988, the flagellate population was dense ($1.0 \times 10^4 \text{ ml}^{-1}$), with only 19% remaining in the <3 μm size fraction. The mean size of the organisms in the 1 and 3 μm size fractions was $1.55 \mu\text{m}$ (± SD 0.17, $n = 30$) and $2.28 \mu\text{m}$ (± SD 0.45, $n = 30$) respectively.

No organisms with flagellate characteristics were found in the 0.6 μm filtrate, although other organisms, larger than heterotrophic bacteria, were present. In the unfiltered samples ciliates and copepods were observed but not counted. Hence, several possible predators on pelagic bacteria were present during the experiments.

Bacterial production

Bacterial production was unaffected by removal of the >3 μm organisms in the early summer experiment 1988 (Bothnian Sea) (Fig. 2B). Using 3 to 0.6 μm filter pore sizes, the bacterial production was reduced to 75% of the rate in unfiltered water. Thus, the filter pore size used in the prescreening produced different effects on predation and growth of bacteria in this experiment.

In the autumn experiment 1986 (Bothnian Sea), rates of bacterial production showed a similar response to size fractionation as did the predation rate and was in absolute terms 2.5 times lower than the early summer experiment (Fig. 2A). However, in the 3 μm filtrate bacterial production decreased to a production rate corresponding to 60% of the activity in unfiltered water. No decrease in bacterial numbers or bacterivore activity was observed in this size fraction (Tables 1 and 4). Bacterial production decreased even further in the 1 and 0.6 μm filtrates, coinciding with the decline in bacterial numbers (Table 4). In accordance with the data on predation rates, we obtained increased bacterial

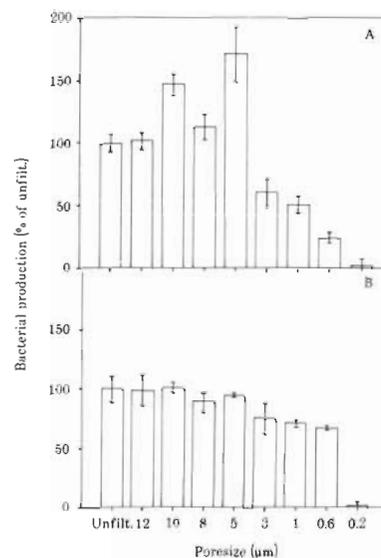


Fig. 2. Rate of bacterial production in water samples prescreened with different filter pore sizes. Bothnian Sea; (A) 1986, (B) 1988. Percentages of rates in unfiltered water (1.5×10^4 and $3.9 \times 10^4 \text{ cells ml}^{-1} \text{ h}^{-1}$, respectively). Differences found in the size fractions larger than 3 μm (1986) were significant on a 0.1% level, according to 1-way analysis of variance

production in the 10 and 5 μm filtrates, but not in the 8 μm filtrate. The variations in the >3 μm size fractions showed a statistical significance of $p < 0.1$ (1-way ANOVA). Thus, the effects of size fractionation on bacterial production and predation on bacteria with pore sizes larger than 3 μm appeared to be synchronized in this experiment.

Artificial release of utilizable DOM

The amount of utilizable dissolved organic matter (DOM) released during filtration for each filter pore size, was determined with bacterial growth as a bioassay (Fig. 3, Bothnian Sea 1986). The yield of bacterial biomass was higher in the size fractions between 10 and 1 μm than in the unfiltered water. Peak values were found in the 10 to 8 μm and the 3 μm

Table 4. Initial concentration of bacteria in water samples prescreened with filters of different pore sizes. Values for prefiltered water presented as percentages of the concentration in unfiltered water. Values in parentheses: 95% confidence interval for estimated mean value of unfiltered sample

Location	Year	Unfilt. water (cells ml ⁻¹ × 10 ⁶)	Fraction (µm) (% of unfilt.)								
			< 12	< 10	< 8	< 5	< 3	< 1	< 0.6	< 0.2	
Bothnian Sea	1985	1.27 (± 0.14)	112	106	105	108	116	70	64	0	
Mediterr. Sea	1986	0.93 (± 0.08)	99	97	99	75	67	49	49	0	
Bothnian Sea	1986	2.78 (± 0.31)	104	99	105	101	109	90	88	3	
Bothnian Sea	1988	1.56 (± 0.39)	97	93	88	79	73	65	65	1	

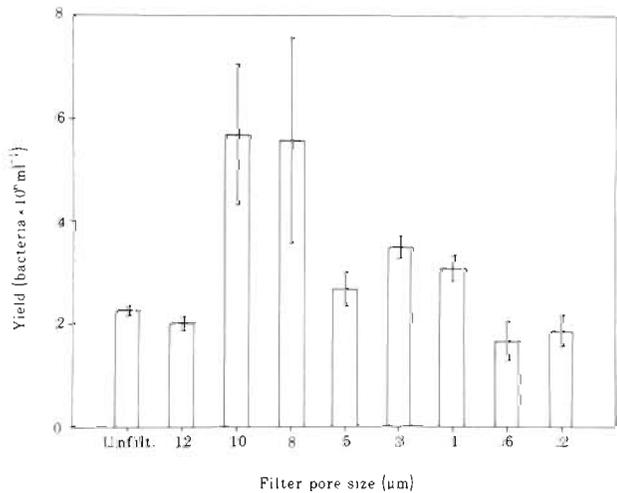


Fig. 3. Yield of bacterial biomass following a uniform inoculum of marine bacteria in water samples prescreened with different filter pore sizes. Bars: final concentration of bacteria; lines: standard error of estimated mean value

filtrates. In the 0.6 and 0.2 µm filtrates no increase in bacterial biomass were found, although a vacuum was applied during the filtrations (50 and 100 mm Hg respectively). The standard error for each triplet of samples was higher in the 10 and 8 µm filtrates indicating an uneven distribution of DOM sources in these filtrates. The size fractionation pattern obtained in the yield experiment showed a bimodal curve similar to that observed in the results of predation rate and bacterial production, however, peak heights and their location were shifted. Thus, release of DOM during the filtration procedure could not be clearly correlated with changes in bacterial production and predation rate in this experiment.

Effect of prolonged incubation of prescreened samples

In order to test if truncation of the microbial food chain could explain the effects observed on the bacterivore activity following prefiltration, the time between

Table 5. Rate of predation in water samples prescreened with different filter pore sizes 12 (Bothnian Sea) or 20 h (Mediterranean Sea) before measurement. Percentages of the rate in unfiltered water (5.6 × 10⁴ cells ml⁻¹ h⁻¹ and 3.1 × 10⁴ cells ml⁻¹ h⁻¹ respectively). Standard error (%) and degrees of freedom shown in parenthesis. Variations were different from zero (p < 0.01) according to 2-way analysis of variance

Location	Fraction (µm)		
	Unfilt.	< 10	< 3
Bothnian Sea	100 (43,3)	37 (54,3)	286 (40,3)
Mediterranean Sea	100 (25,1)	36 (3,3)	243 (58,2)

size fractionation and measurement was prolonged. Changes in predation rate on bacteria measured 20 h after size fractionation were in accordance with the results obtained 1 h after filtration (Table 5). That is, the bacterivore activity was repressed in the 10 µm filtrate while being clearly increased in the 3 µm filtrate. However, it was important to note that the observed responses were significantly greater after the prolonged incubation. This was probably caused by an increase in numbers of the top predator in the filtrate, as previously observed by Rassoulzadegan & Sheldon (1986).

Effects of size fractionation on bacterial production were also maintained for at least 20 h (Table 6). The remaining production in the 3 µm filtrate (60% of the

Table 6. Rate of bacterial production in water samples prescreened with different filter pore sizes 12 (Bothnian Sea) or 20 h (Mediterranean Sea) before measurement. Percentages of the rate in unfiltered water (13.0 × 10⁴ cells ml⁻¹ h⁻¹ and 4.3 × 10⁴ cells ml⁻¹ h⁻¹ respectively). Standard error (%) and number of replicates shown in parenthesis. Variations in each experiment were different from zero (p < 0.01) according to 1-way variance analysis

Location	Fraction (µm)		
	Unfilt.	< 10	< 3
Bothnian Sea	100 (5.7, 3)	81 (3.2, 3)	58 (4.7, 3)
Mediterranean Sea	100 (4.6, 3)	84 (2.9, 3)	172 (1.7, 3)

rate in unfiltered water) was of the same relative size as when measured directly after size fractionation (Fig. 2A). The 3µm filtrate of Mediterranean water showed a less pronounced uncoupling of bacterial production and predation.

Thus, prolonged incubations confirmed results obtained directly after size fractionation, by showing similar but pronounced responses to the successive truncation of the microbial food chain.

DISCUSSION

Food chain model

To formalize the results obtained in this study we outlined a model describing the trophic interactions between organisms less than 12µm in size (Fig. 4). Removal of each level of predators was thought to give a rapid response in the activity of its prey due to the release of predation pressure. This change was propagated through a predator-prey chain in less than 2 h after truncation. Our data agreed with the existence of 3 predator levels above the bacterivore, and that the bacterial biomass was transferred in a serial manner

through these trophic levels. Consumption of bacteria by the larger protozoa may have occurred, but seemed to be of minor importance.

Successive removal of organisms according to this model gave a response in the resulting bacterivore activity which was internally consistent with the observed results (Figs. 4 and 1). Thus, in our opinion a successive truncation of the microbial food chain was the most plausible explanation to the effects observed on the predation rate, as a result of the consecutive fractionation of water samples. Our experimental design did not allow monitoring of effects due to larger zooplankton or fish. However, the immediate short term control of the bacterivores did not seem to be influenced by other than nanoplankton. A successive transfer of the bacterial biomass through 4 trophic levels would mean that the contribution of bacterial carbon to the metazoan food chain was small. In one study supporting this scenario, Hagström et al. (1988) observed a daily transfer of 6% to higher trophic levels in an oligotrophic environment, where pelagic auto- and heterotrophic production was dominated by the picoplankton size fraction. Instead, the microbial community mainly influenced the rate of transformation of organic matter to CO₂ and inorganic nutrients.

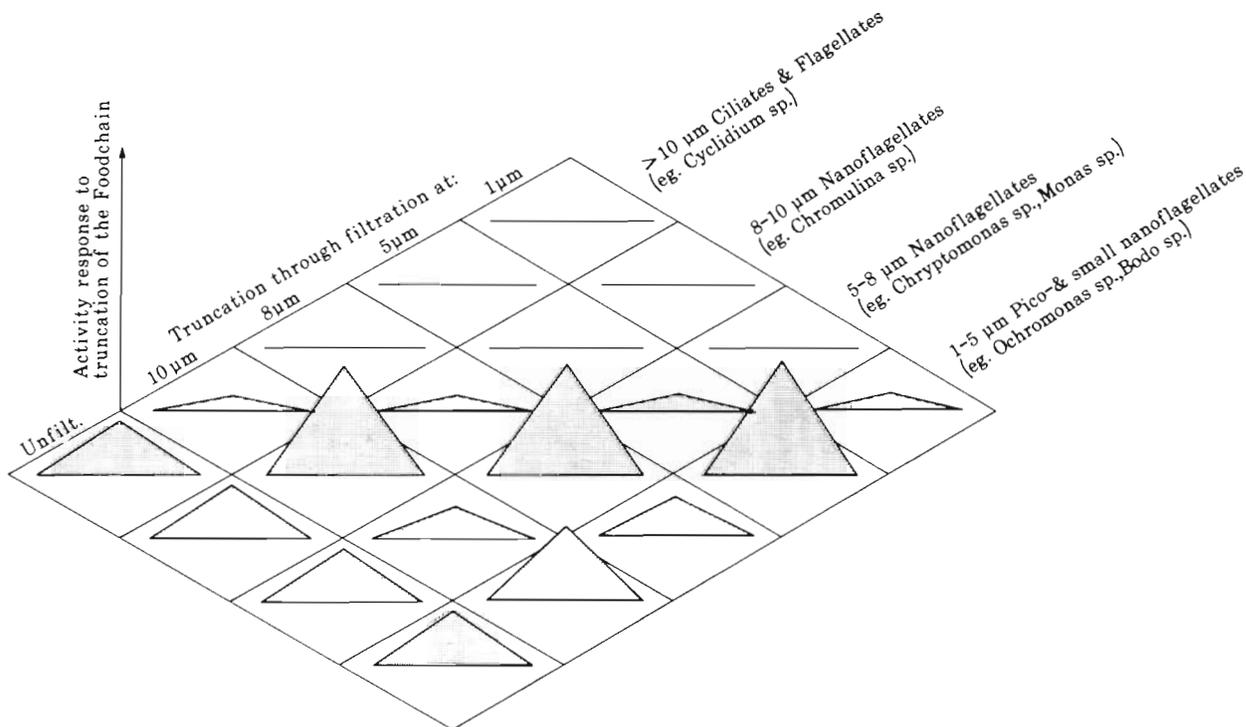


Fig. 4. Model of the microbial food chain. Height of symbols (z-axis): activity of organisms after truncation of food chain; triangles: protozoan predator-prey link; solid lines: organisms excluded by prefiltration. y-axis shows filter pore size used for truncation; maximum size range for each trophic level in these experiments is shown on x-axis. Species suggested for each trophic level have a suitable size and were known to be present in natural water samples during the time of experiments

Size of the bacterivore

Rate of predation on bacteria was not lowered compared to unfiltered water until pore sizes of 1 μm or less were used (Table 1, Fig. 1). Loss of predation could be expected if a bacterivore was totally retained by the filter or damaged during the passage. Since maximum predation rates observed were found in 3 μm screened water, the major bacterivore must have passed quite unaffected through these pores. Accordingly, mean size of flagellates in the 1 and 3 μm size fractions was 1.5 (\pm SD 0.17) and 2.3 (\pm SD 0.45) μm respectively. Thus, we conclude that the most important predators on the free living pelagic bacteria were in the size range of 1 to 3 μm . In agreement with this conclusion, most of the minicell ^{35}S -label have been observed to be incorporated into this size fraction (Wikner et al. unpubl.).

Rassoulzadegan & Sheldon (1986) argued that flagellates might pass pores half their cell width without damage. Therefore, the pore size used could slightly underestimate the size of the predator. However, even after 50 h of incubation of the filtered samples, populations developing in the filtrates had a size close to or smaller than the truncation point (Rassoulzadegan & Sheldon 1986). Also, since the only protozoans we observed in the <3 μm size fraction were 2.8 μm or smaller, we assume the larger organisms in this filtrate to be negligible in the time scale of our experiment.

The 1 to 3 μm size fraction coincides with Population II in the experiment of Rassoulzadegan & Sheldon (1986), suggested by the authors to be picoflagellates exerting a predatory control over the bacterial size fraction. Furthermore, Wright & Coffin (1984) reported that organisms 1 to 3 μm in size were responsible for most of the observed decrease in bacteria in their experiment. Taken together, these data support the findings presented here. Recent papers have emphasized the importance of ciliates as grazers on free living bacteria (Gast 1985, Sherr & Sherr 1987). However, our data unambiguously pointed towards nanoflagellates, in the size of 1 to 3 μm , as the most important predators on pelagic bacteria. Microprotozoa (20 to 200 μm) and larger nanoplankton are more likely to be important grazers on cyanobacteria and smaller nanoplankton as suggested by others (Iturriaga & Mitchell 1986, Sheldon et al. 1986).

Predatory activity in filtrates that have passed a 0.6 μm filter have been reported by Fuhrman & McManus (1984), who found more than 60 % of the grazing observed in unfiltered water in this size fraction. We also found bacterivore activity in this size fraction in 2 of our experiments (on average 16 % of the predation activity found in the 5 μm filtrate). Bacterivores of this size might be small flagellates that

have passed undamaged (Fuhrman & McManus 1984, Cynar et al. 1985, Rassoulzadegan & Sheldon 1986), predatory procaryotes (Guerrero et al. 1986) and/or bacteriophages. Although it was definitely important to acknowledge that such organisms may be present, we chose to regard this activity as an inevitable spill-over in the size fractionation process.

Predator-prey link

The unexpected increase in predation rate in the 10 and 5 to 3 μm filtrates indicated a derepression of predation on the bacterivores (Table 1, Fig. 1); that is, the bacterivores increased the grazing on bacteria due to a decrease in predation pressure on the bacterivores themselves. The increased grazing on bacteria could either be due to an increase in the per cell feeding rate of the bacterivores and/or number of bacterivores. In fact, assuming a generation time of 6 h for the bacterivores (Wikner et al. 1986), a 40 % increase in abundance could be achieved within 3 h if removing its predator. The bimodal dependence of the predation rate with decreasing filter pore size found in all 4 experiments, suggested that there were at least 3 higher trophic levels that influenced the activity of the bacterivores in a consecutive way. In other words, removal of organisms >10 μm seemed to affect the bacterivores through a predator-prey chain, consisting of 3 trophic links. Fewer trophic links between truncation site and bacterivore (smaller pore size in the pre-filtration) increased the strength of the measured effect on the bacterivore.

Effects on bacterial production

In the summer experiment (1988) a weak or no dependence between rate of predation on bacteria and amount of bacterial production in a given size fraction was observed (Fig. 2B). This showed that the pore size effect observed on the bacterivores was not caused by different rates of bacterial growth in the filtrates. However, the weak effects on bacterial growth in this experiment does not contradict the close dependence between predation and production found in the 1986 experiment (see below). In this experiment (autumn 1986, Bothnian Sea), bacterial production was clearly affected by the prefiltration, showing a similar response to size fractionation as did the predation rate (Fig. 2A). This result was also obtained in the prolonged experiments, where the effects were enhanced (Table 6).

Artificial increases in DOM, caused by the filtration, could theoretically have promoted bacterial growth

which in turn stimulated the bacterivores. However, the result from the yield experiment did not explain the increase in bacterial production since a poor correlation between the parameters was found (Figs. 2B and 3). Instead, it may be argued that an increase in bacterial growth yield reflected the presence of organisms susceptible to shear forces in filters with appropriate pore size.

Hence, the close coupling found between predation on bacteria and bacterial production ($r^2 = 0.94$, $n = 7$) in the autumn experiment 1986, was most likely due to organisms (3 to 5 μm) included in the predator-prey link, which excreted nutrients crucial for the bacterial growth. It has been shown in laboratory experiments that nanoflagellates release nutrients which promote bacterial growth (Johannes 1965, Andersson et al. 1985, Caron et al. 1985). This may be more important for the growth of heterotrophic bacteria at oligotrophic conditions than previously thought (Hagström et al. 1988) and might be the reason for the co-regulation found between the predation on bacteria and the bacterial production (Wikner et al. 1986, this paper).

Effects of prolonged incubation of prescreened samples

Following the predator-prey link hypothesis outlined above, we would expect the largest organisms remaining in the filtrates after the prescreening procedure to be released from predation pressure. In agreement with this, we observed magnified effects on growth of and predation on bacteria when extending the incubation time following size fractionation (Tables 5 and 6). The predation rate in the 3 μm filtrate was strongly elevated (2.5 times), compared to the unfiltered water, in both of the prolonged experiments, suggesting that a bacterivore close to, or smaller than, 3 μm had been released from predation pressure (Table 5). Predation rate in the 10 μm filtrate was lowered. The enhanced responses observed when measuring 12 h or more after size fractionation could be explained with greater changes in abundance of the remaining organisms in the filtrate (cf. Rassoulzadegan & Sheldon 1986). These results support the previous statements that the changes found in rate of predation and bacterial production rate, when size fractionating sea water, was due to rupture of the normal trophic interactions and that the bacterivores were exposed to strong predation by larger protozoans.

Hence, the results presented in this paper unambiguously show that the main bacterivores were 1 to 3 μm in size and controlled by predation rather than food supply. Furthermore, the scenario presented in our model advocates the bacterivore population to be maintained

in a balanced state in natural water, through close control between trophic levels. This tight regulation enables the organisms involved to adjust coordinately to changes in their environment. Finally, as a result of the existence of 4 trophic levels in the <12 μm size fraction, the bacterial biomass should be of minor importance as a food base for the metazoan food chain.

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LITERATURE CITED

- Andersen, P., Fenchel, T. (1985). Bacterivory by microheterotrophic flagellates in seawater samples. *Limnol. Oceanogr.* 30 (1): 198-202
- Andersson, A., Lee, C., Azam, F., Hagström, Å. (1985). Release of aminoacids and inorganic nutrients by heterotrophic marine microflagellates. *Mar. Ecol. Prog. Ser.* 23: 99-106
- Caron, D. A., Goldman, J. C., Andersen, O. K., Dennett, M. R. (1985). Nutrient cycling in a microflagellate food chain: II. Population dynamics and carbon cycling. *Mar. Ecol. Prog. Ser.* 24: 243-254
- Cynar, F. J., Estep, K. W., Sieburth, J. McN. (1985). The detection and characterization of bacteria-sized protists in 'protist-free' filtrates and their potential impact on experimental marine ecology. *Microb. Ecol.* 11: 281-288
- Davies, P. G., Caron, D. A., Johnson, P. W., Sieburth, J. McN. (1985). Phototrophic and apochlorotic components of picoplankton and nanoplankton in the North Atlantic: geographic, vertical, seasonal and diel distributions. *Mar. Ecol. Prog. Ser.* 21: 15-26
- Fenchel, T. (1982a). Ecology of Heterotrophic Microflagellates. II. Bioenergetics and growth. *Mar. Ecol. Prog. Ser.* 8: 225-231
- Fenchel, T. (1982b). Ecology of Heterotrophic Microflagellates. IV. Quantitative occurrence and importance as bacterial consumers. *Mar. Ecol. Prog. Ser.* 9: 35-42
- Fuhrman, J. A., Azam, F. (1982). Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine waters: Evaluation and field results. *Mar. Biol.* 66: 109-120
- Fuhrman, J. A., McManus, G. B. (1984). Do bacteria-sized marine eucaryotes consume significant bacterial production? *Science* 224: 1257-1260
- Gast, V. (1985). Bacteria as a food source for microzooplankton in the Schlei Fjord and Baltic Sea with special reference to ciliates. *Mar. Ecol. Prog. Ser.* 22: 107-120
- Guerrero, R., Pedrós-Alió, C., Esteve, I., Mas, J., Chase, D., Margulis, L. (1986). Predatory prokaryotes: Predation and primary consumption evolved in bacteria. *Proc. natl. Acad. Sci.* 83: 2138-2142
- Haas, L. W., Webb, K. L. (1979). Nutritional mode of several non pigmented microflagellates from York river estuary, Virginia. *J. exp. mar. Biol. Ecol.* 39: 125-134
- Hagström, Å. (1984). Aquatic bacteria: measurements and significance of growth. In: Klug, M. J., Reddy, C. A. (eds.) *Current perspectives in microbial ecology*. American Society for Microbiology, Washington, D.C., p. 495-501
- Hagström, Å., Azam, F., Andersson, A., Wikner, J., Rassoulzadegan, F. (1988). Microbial loop in an oligotrophic pelagic marine ecosystem: possible roles of cyanobacteria

- and microflagellates in the organic fluxes. *Mar. Ecol. Prog. Ser.* (in press)
- Iturriaga, R., Mitchell, B. G. (1986). Chroocoid cyanobacteria: a significant component in the food web dynamics of the open ocean. *Mar. Ecol. Prog. Ser.* 28: 291–297
- Johannes, R. E. (1965). Influence of marine protozoa on nutrient regeneration. *Limnol. Oceanogr.* 10: 434–442
- Lessard, E. J., Swift, E. (1985). Species-specific grazing rates of heterotrophic dinoflagellates in oceanic waters, measured with dual label technique. *Mar. Biol.* 87: 289–296
- Porter, K. G., Feig, Y. S. (1980). The use of DAPI for identifying and counting aquatic microflora. *Limnol. Oceanogr.* 25: 943–948
- Rassoulzadegan, F., Sheldon, R. W. (1986). Predator-prey interactions of nano-zooplankton and bacteria in an oligotrophic marine environment. *Limnol. Oceanogr.* 31 (5): 1010–1021
- Sheldon, R. W., Nival, P., Rassoulzadegan, F. (1986). An experimental investigation of a flagellate-ciliate-copepod food chain with some observations relevant to the linear biomass hypothesis. *Limnol. Oceanogr.* 31 (1): 184–188
- Sherr, B. F., Sherr, E. B., Newell, S. Y. (1984). Abundance and productivity of heterotrophic nanoplankton in Georgia coastal waters. *J. Plankton Res.* 6: 195–202
- Sherr, B. F., Sherr, E. B., Andrew, T. L., Fallon, R. D., Newell, S. Y. (1986). Trophic interactions between heterotrophic protozoa and bacterioplankton in estuarine water analyzed with selective metabolic inhibitors. *Mar. Ecol. Prog. Ser.* 32: 169–179
- Sherr, E. B., Sherr, B. F. (1987). High rates of consumption of bacteria by pelagic ciliates. *Nature, Lond.* 325: 710–711
- Taylor, G. T., Pace, M. L. (1987). Validity of eucaryotic inhibitors for assessing production and grazing mortality of marine bacterioplankton. *Appl. environ. Microbiol.* 53 (1): 119–128
- Wikner, J., Andersson, A., Normark, S., Hagström, Å. (1986). Use of genetically marked minicells as a probe in measurement of predation on bacteria in aquatic environments. *Appl. environ. Microbiol.* 52 (1): 4–8
- Wright, R. T., Coffin, R. B. (1984). Measuring microzooplankton grazing on planktonic marine bacteria by its impact on bacterial production. *Microb. Ecol.* 10: 137–149

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