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Top-down effects on the size-biomass distribution of a freshwater bacterioplankton community

Jakob Pernthaler[1,*], Birgit Sattler[1], Karel Šimek[2], Angela Schwarzenbacher[1], Roland Psenner[1]

[1]Institute of Zoology and Limnology, University of Innsbruck, Technikerstraße 25, A-6020 Innsbruck, Austria
[2] Hydrobiological Institute of the Czech Academy of Sciences, Na sádkách 7, Ceské Budejovice 37005, Czech Republic

ABSTRACT: Protistan size-selective grazing on aquatic bacteria has frequently been demonstrated in the laboratory, but its postulated effects on the size distribution of natural bacterioplankton communities remain uninvestigated. For a period of 5 wk we followed the spring development of the epilimnetic microbial community in an oligo-mesotrophic lake. Changes in bacterial size distributions and growth parameters were quantified and compared with the abundance, biomass and bacterivory of the heterotrophic nanoflagellate (HNF) community. We observed pronounced shifts within 2 distinct size ranges of the bacterioplankton. Short, rod-shaped cells between 0.4 and 1.2 µm cell length formed between 30 to 70% of total bacterial biomass; cells >2.4 µm were responsible for another 20 to 50%. High HNF community grazing rates (>100% of newly produced cells) were reflected in a biomass drop of bacteria between 0.4 and 1.2 µm cell length, whereas large cells (>2.4 µm) were almost half of total bacterial biomass during this period. When HNF abundance and bacterivory decreased, the opposite pattern was observed. [^{3}H]Thymidine and [^{14}C]leucine uptake per respiring bacterial cell were maximal during the period of rapid increase of bacterial cells between 0.4 and 1.2 µm. Both flagellate total grazing and individual clearance rates were negatively correlated with growth per active bacterial cell. The biomass of 'grazing-resistant' bacteria was correlated with flagellate bacterivory and biomass, and size-selective protistan grazing influenced the size structure of bacterioplankton as well as the mean per-cell growth of the active bacterial fraction. We suggest a model that divides freshwater bacterioplankton into 4 size ranges of different vulnerability to size-selective protistan grazing.

KEY WORDS: Bacterioplankton size structure · Bacterial biomass · Image analysis · Grazing-resistant bacteria · Protistan size selectivity · Cell-specific activity

INTRODUCTION

Aquatic bacterial assemblages exhibit morphological properties that differ from those of bacterial communities in continuous cultures. The numerical dominance of very small cells (0.01 to 0.1 µm^3) seems to be a general feature in marine and freshwater pelagic systems, but bacterial cell sizes often cannot be predicted from abiotic factors alone. For example, decreases in mean bacterioplankton cell size have been associated with the warm (Chrzanowski et al. 1988, Bjørnsen et al. 1989) and cold seasons (Albright & McCrae 1987).

Alternative hypotheses have considered the feeding ecology of the most important bacterial predators. Studies of heterotrophic nanoflagellates (HNF) (Chrzanowski & Šimek 1990, Gonzáles et al. 1990, Monger & Landry 1992) and small ciliates (Šimek et al. 1994, 1995) indicate that protists can influence the size structure of laboratory bacterial communities by selectively eliminating larger cells. Such foraging should leave its mark on natural bacterioplankton size distributions. In particular, the hypothesis predicts that the biomass of the smallest bacterial cells should be only weakly related to changes in protist predation, whereas slightly larger bacteria should reflect protist bacterivory much more clearly. Cells above a certain size range are expected to be so subjected to size-

*E-mail: jakob.pernthaler@uibk.ac.at

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selective feeding that they are grazed to very low densities even if grazing is weak.

As bacterial cell size increases during the cell cycle, size-selective predators are expected to graze more intensively on bacteria shortly before or during cell division (Sherr et al. 1992), i.e. on the fastest-growing fraction within the bacterioplankton. If grazing pressure on the bacterioplankton increases, the hypothesis predicts that the mean growth activity per bacterial cell should decline, as the most actively growing cells suffer highest mortality rates.

In contrast to marine systems, there is increasing evidence that large thread-like bacteria may play an important role in the bacterioplankton of lakes as well. They show a high seasonal variability in cell size (Jürgens & Stolpe 1995) and mean biovolume (Sime-Ngando et al. 1991), and their abundance is commonly 1 to 2 orders of magnitude below the number of small cells. Such large cells, however, contain 50 to 100 times more organic carbon than the 'average' bacterium (Sommaruga & Psenner 1995). A doubling in the abundance of large bacteria is likely to be overlooked in routine counting, but might completely alter the actual size distribution of bacterial biomass, thus favouring predator species with different foraging behaviour. Large bacteria may be resistant to protist grazing (Jürgens & Güde 1994), but are vulnerable to a different type of predation, e.g. by *Daphnia* spp. (Güde 1988, Jürgens et al. 1994). Laboratory experiments (Shikano et al. 1990) and enclosure studies (Jürgens et al. 1994) suggest that strong protistan grazing pressure can cause the appearance of thread-like bacteria in freshwater communities, and Sommaruga & Psenner (1995) confirmed the permanent presence of such bacterial morphotypes in a hypertrophic lake that was lacking cladoceran grazers. Up to now, however, most data on 'grazing-resistant' bacteria originate from short-term manipulative studies or correlations with HNF abundance on a seasonal level. The actual formation or disappearance of 'protist-inedible' bacterial cells in the context of changing protistan bacterivory has not yet been described in an unmanipulated system.

We followed the spring development of the microbial community in an oligo-mesotrophic lake during a 5 wk period. In particular, we tried to replace the black box concept of 'bacterial biomass' with a detailed analysis of biomass shifts within the bacterioplankton, and thus evaluate the 2 current hypotheses that predict biomass shifts as a consequence of protistan grazing (Gonzáles et al. 1990, Jürgens & Güde 1994). In order to test another prediction of the size-selective grazing hypothesis (Sherr et al. 1992), we combined 2 well-established methods that measure bacterial secondary production with one that defines the size of the active fraction of bacterioplankton and analyzed the correlation between bacterial mean per-cell growth and bacterivory.

MATERIAL AND METHODS

Between 4 May and 1 June 1994 we collected samples 3 times a week from the epi- and metalimnion of the oligo-mesotrophic Piburger See (Tyrol, Austria, 915 m above sea level) with a 5 l Schindler-Patalas sampler. For details of the study site and sampling procedures see Pechlaner (1979) and Pernthaler et al. (1996). The lake was thermally stratified, with a phytoplankton maximum below the thermocline, and temperatures ranged from 13.5 to 16.5°C. As this study focusses on very specific aspects of a larger investigation (Macek et al. 1996, Pernthaler et al. 1996, Šimek et al. 1996, Vrba et al. in press), only data from epilimnion samples are considered.

Bacteria were enumerated by epifluorescence microscopy (Porter & Feig 1980). Formalin-fixed, 50 µm prescreened samples were stained with 2,4-diamidinophenyl-indol (DAPI), filtered on black 0.2 µm Poretics filters and counted at 1600× magnification under UV excitation (365/395/397 filter set). Size measurement of 400 to 800 stained bacterial cells was performed with a semi-automated image analysis system. It consisted of a microscope (Zeiss Axioplan) equipped with a highly sensitive camera (Hamamatsu Photonics, Japan) which was linked to a personal computer (software: LUCIA, Laboratory Imaging, Prague, Czech Republic). Three size classes of fluorescent latex beads (Polyscience Inc., USA) were used to calibrate the system (M. Krößbacher, J. Mikes & R. Psenner unpubl.), and individual cell volumes were calculated from cell length and width according to Psenner (1993). The allometric relationship between cell volume and carbon content taken from Simon & Azam (1989) in its recalculated version by Norland (1993) was used for biomass estimation.

As the variation of bacterial width was often below the minimal size difference that can be detected reliably by image analysis and showed little variability beyond a certain size range (Fig. 1), we chose the variation of cell length to subdivide bacterioplankton into size classes.

For detailed analysis, data from image analysis was split into 7 classes of cell length at 0.4 µm intervals, although the above described system was able to detect much smaller size differences (0.1 to 0.2 µm).

For each size class we calculated a mean cell carbon content (MCC) from the pooled data of all 13 sampling dates (between 230 and 2600 cells measured per size class). The biomass (BM) fraction in each size

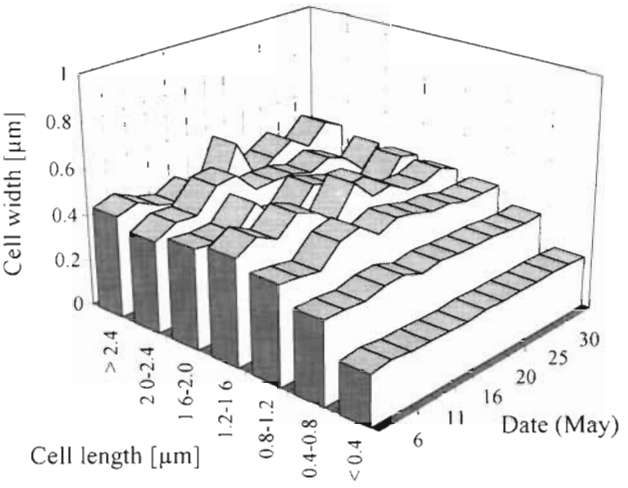

Fig. 1. Distribution of bacterial cell width in size classes of cell length between 4 May and 1 June 1994 in the epilimnion of Piburger See

class (i) was calculated separately for each sampling date as

$$BM_i = MCC_i \times F_i \times N \quad (1)$$

where F_i is the fraction of cells in the size class i and N is the total bacterial abundance at the respective sampling date.

The fraction of bacterioplankton with detectable respiration was estimated by incubation of 10 ml subsamples with 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride (INT, Sigma Chemicals) for 1 h at *in situ* temperature (Zimmermann et al. 1978). Incubation was stopped by the addition of buffered formalin (final conc.: 2% w/v), and bacterial cells containing red formazan crystals (400 to 600 cells) were quantified on white cellulose nitrate filters at 1600× magnification. Bacterial secondary production was estimated in parallel via incorporation of radioactively labeled [^{3}H]thymidine (5 nmol) and [^{14}C]leucine (20 nmol, Amersham) (modified from Simon & Azam 1989). We did not convert incorporation rates into production rates of new bacterial cells (Bell 1990) or biomass (Simon & Azam 1989), but interpreted tracer uptake as a measure of bacterial growth and division activity. In order to estimate the productivity of the 'average active cell', we calculated the ratio of the respective tracer incorporation rate and the abundance of INT-reducing bacteria for each sampling date (Dufour et al. 1990).

Colorless HNF were stained with DAPI and quantified on black membrane filters (Poretics, 1 µm pore size) by epifluorescence microscopy (Sherr & Sherr 1993). A total of 40 to 90 individuals per sampling date were measured with an eyepiece micrometer. HNF grazing was estimated in short-term direct uptake experiments with fluorescently labeled bacteria (FLB). Bacteria were concentrated from 20 l of untreated lake water with an AMICON hollow fiber filtration apparatus and stained with 5-([4,6,-dichlorotriazin-yl]amino) fluorescein (DTAF) (Sherr & Sherr 1993). Uptake experiments (20 to 30 min) were performed in 500 ml of 50 µm prescreened lake water. At least 50 individuals were evaluated, and hourly uptake rates (UR) of lake bacteria per HNF were estimated assuming linear ingestion rates (Pernthaler 1996). HNF bacterivory was calculated as total HNF grazing (TGR) and clearance rates (CR).

$$TGR = n_{HNF} \times UR \ (\text{bacteria l}^{-1}\ \text{h}^{-1}) \quad (2)$$

$$CR = \frac{UR}{n_{bact}} \ (\text{nl HNF}^{-1}\ \text{h}^{-1}) \quad (3)$$

where n_{HNF}: HNF abundance; n_{bact}: bacterial abundance.

Statistical evaluation was carried out on a personal computer with the STATISTICA™ software package. We calculated non-parametric Spearman rank correlation coefficients (r_S) between the measured parameters of the bacterial and protist communities. However, instantaneous bacterial per-cell growth rate is not necessarily correlated with bacterial standing stock, but rather with changes in bacterial standing stock. Therefore we additionally calculated the changes of bacterial biomass in each size class between 2 consecutive sampling dates and compared these data with mean per-cell growth of active bacteria.

RESULTS

Bacterial abundance and biomass were highest at the beginning and towards the end of the investigation, and the mean cell volume showed maximal values during the first half of May (Table 1). Mean bacterial widths ranged from 0.2 to 0.5 µm (Fig. 1), and no significant variations in width were detectable for bacteria >0.8 µm.

Bacteria between 0.4 and 1.2 µm cell length and cells >2.4 µm together constituted about 80% of total bacterial biomass throughout the study period (Figs. 2 & 3B). The 2 size classes showed a pronounced inverse trend of increase and decrease. Large cells showed a distinct biomass maximum during the first half of the investigation, when the biomass fraction of cells between 0.4 and 1.2 µm was minimal. The opposite pattern was found during the second half of May (Fig. 2). There was a significant negative correlation between bacterial biomass in the cell ranges 0.4 to 1.6 with the biomass of bacteria >2.4 µm (Table 2). The abundance of cells <0.4 µm and between 1.2 and 2.4 µm cell length remained fairly constant during the whole investiga-

Table 1 Total bacterial abundance (N), mean cell volume (MCV), biovolume (BV), carbon per cell (CPC), biomass (BM), and % INT-active cells (INT-active)

Date	N ($\times 10^6$ ml^{-1})	MCV (μm^3)	BV (mm^3 l^{-1})	CPC (fg)	BM (μg C l^{-1})	INT-active (%)
4 May	2.35	0.099	0.233	19.0	44.6	2.8
6 May	1.87	0.127	0.237	21.8	40.7	10.3
9 May	2.14	0.111	0.237	19.9	42.6	8.8
11 May	1.96	0.142	0.279	24.0	47.1	3.7
13 May	1.43	0.194	0.277	29.1	41.5	5.7
16 May	1.37	0.117	0.161	21.7	29.7	9.5
18 May	1.73	0.123	0.213	22.1	38.3	4.7
20 May	1.95	0.084	0.163	17.5	34.1	3.8
23 May	2.27	0.099	0.224	19.9	45.1	3.2
25 May	3.54	0.078	0.276	17.4	61.7	1.8
27 May	2.75	0.074	0.204	16.4	45.0	2.5
30 May	3.10	0.075	0.232	16.7	51.6	6.0
1 Jun	2.85	0.074	0.210	16.7	47.6	3.2

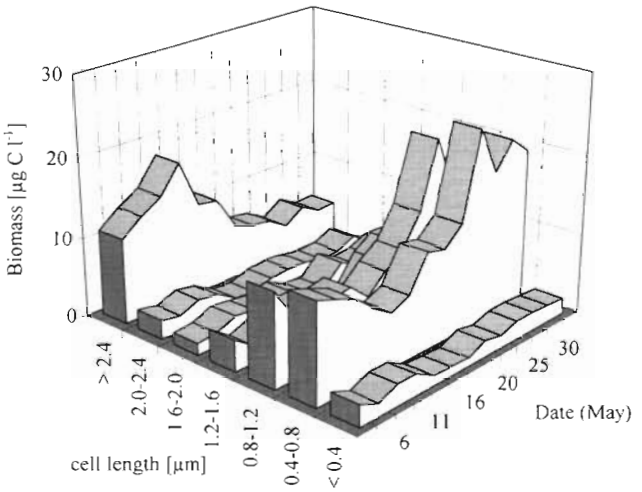

Fig. 2. Distribution of bacterial biomass (organic carbon) in size classes of cell length

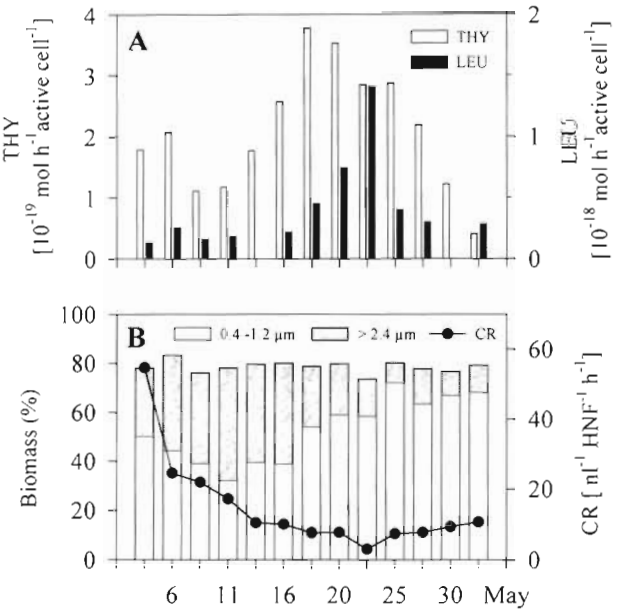

Fig. 3. (A) Incorporation of [^{3}H]thymidine (THY) and [^{14}C]leucine (LEU) divided by the abundance of INT-reducing bacteria (see 'Results'). (B) Bars: fractions of total bacterial biomass that are formed by small (0.4 to 1.2 µm) and large (>2.4 µm) bacteria. Line: heterotrophic nanoflagellate clearance rate (CR) on bacteria

tion period, and the contribution of these size fractions to total biomass was small (Fig. 2).

The size of the active bacterial fraction and secondary production parameters showed pronounced fluctuations (Tables 1 & 3, Sattler unpubl.). A very similar pattern of tracer uptake per active cell was found for both tracers, with maximal incorporation rates during the second half of the investigation (Fig. 3A). Rates of per-cell [^{14}C]leucine incorporation lagged behind those of [^{3}H]thymidine incorporation. The only statistically significant relationship between per-cell growth and changes of bacterial biomass was found between [^{3}H]thymidine incorporation per active cell and biomass changes of the size fraction 0.4 to 0.8 µm (r_S = 0.622, p = 0.03, n = 12). Per-cell [^{14}C]leucine incorporation was negatively correlated with the biomass of cells >2.4 µm (Table 2), but not with biomass changes of any size fraction.

The abundance and biomass of HNF decreased continuously during the study period (Table 3, Pernthaler et al. 1996). Both parameters were negatively correlated with bacterial biomass in the size range 0.4 to 1.6 µm and positively with the biomass of cells >2.4 µm (Table 2). HNF clearance rate decreased sharply after an initial maximum, and increased slightly towards the end of the study (Fig. 3B). CR and TGR showed significant correlations with the development of bacterial biomass in the size range >2.4 µm, and with both

Table 2. Spearman rank correlations for heterotrophic nanoflagellates (HNF), total grazing rate (TGR), clearance rate (CR), abundance (ABU) and biomass (BM), [^{3}H]thymidine (THY pc) and [^{14}C]leucine (LEU pc) incorporation per active bacterial cell, and bacterial biomass in different size classes (<0.4 to >2.4 µm). Numbers of observations: n = 13 (n = 11 for LEU pc). $^{\bullet}p < 0.05$, $^{\bullet\bullet}p < 0.01$, $^{\bullet\bullet\bullet}p < 0.001$, –: not significant

	THY pc	LEU pc	<0.4	0.4–0.8	0.8–1.2	1.2–1.6	1.6–2.0	2.0–2.4	>2.4
TGR	–0.637$^{\bullet}$	–0.855$^{\bullet\bullet}$	–	–	–	–	–	–	0.643$^{\bullet}$
CR	–0.654$^{\bullet}$	–0.945$^{\bullet\bullet\bullet}$	–	–	–	–	–	–	0.714$^{\bullet\bullet}$
HNF ABU	–	–	–	–0.720$^{\bullet\bullet}$	–0.720$^{\bullet\bullet}$	–0.885$^{\bullet\bullet\bullet}$	–	–	0.659$^{\bullet}$
HNF BM	–	–0.709$^{\bullet}$	–	–0.615$^{\bullet}$	–0.637$^{\bullet}$	–0.758$^{\bullet\bullet}$	–	–	0.758$^{\bullet\bullet}$
THY pc		0.736$^{\bullet\bullet}$	–	–	–	–	–	–	–
LEU pc			–	–	–	–	–	–	–0.673$^{\bullet}$
<0.4				–	–	–	–	0.643$^{\bullet}$	–
0.4–0.8					0.907$^{\bullet\bullet\bullet}$	–	–	–	–0.863$^{\bullet\bullet\bullet}$
0.8–1.2						0.824$^{\bullet\bullet}$	–	–	–0.885$^{\bullet\bullet\bullet}$
1.2–1.6							–	–	–0.775$^{\bullet\bullet}$
1.6–2.0								–	–
2.0–2.4									–

Table 3. Total bacterial [^{3}H]thymidine incorporation (THY), heterotrophic nanoflagellate abundance (N), biomass (BM) and total grazing rate (TGR). Data from Pernthaler et al. (1996)

Date	THY (pmol l^{-1} h^{-1})	N (× 10^3 ml^{-1})	BM (µg C l^{-1})	TGR (× 10^6 l^{-1} h^{-1})
4 May	11.6	1.66	47.57	30.8
6 May	40.2	2.80	80.35	35.5
9 May	20.8	2.45	34.68	58.7
11 May	8.5	2.25	31.86	31.4
13 May	14.4	2.06	29.22	23.9
16 May	33.6	2.96	34.05	25.8
18 May	30.6	1.69	19.43	11.5
20 May	26.4	2.29	26.32	8.0
23 May	20.9	1.58	15.42	5.0
25 May	18.3	1.22	11.93	15.8
27 May	15.0	1.06	10.33	7.3
30 May	22.9	1.73	24.60	26.6
1 Jun	3.7	1.77	25.16	22.3

radiotracer uptake rates per INT-reducing cell (Table 2).

DISCUSSION

Changes of bacterial morphology

As cell widths were surprisingly similar, about 0.4 µm, in all length classes >0.8 µm length, we morphologically classify bacteria ≤0.4 µm length as predominantly coccoid, and longer cells as rod-shaped (Fig. 1). Presently, however, we can only speculate on the underlying ecological constraints, which might include selective foraging as well as bottom-up effects (Krambeck 1988).

Our observation that some size fractions of bacterial biomass available for protists can vary considerably (Fig. 2) indicates that the mean cell size of a bacterial assemblage may be a rather misleading parameter and does not correspond to the size classes of bacterioplankton which dominate biomass. Approximately one quarter (15 to 33%) of all bacteria, but only 3 to 6% of total biomass, was found in the fraction of small coccoids (≤0.4 µm) (Fig. 2). This fraction plays a prominent role in any evaluation of microbial food web relationships based on numbers (e.g. Berninger et al. 1991), which is not justified in terms of biomass. It has even been proposed that small DAPI-stainable particles are not even living bacterial cells (Zweifel & Hagström 1995), and there is strong evidence for the presence of similar-sized virus-like particles (Sommaruga et al. 1995). The rather constant amount of biomass of cocci was statistically unrelated to the pronounced changes in per-cell activity and bacterivory (Table 2). This supports the conclusion that this fraction of the bacterioplankton was only marginally involved in growth and predation processes. Bird & Kalff (1993) reached a similar conclusion from observed [^{3}H]thymidine uptake patterns in different bacterial size classes. They found a 2- to 10-fold range of growth rates depending on cell size, and the smallest bacterial cells (<0.4 µm) were growing most slowly. On the other hand, we know from direct uptake studies that small, starving bacteria are grazed upon at significantly lower rates than larger, growing cells (González et al. 1993). The pool of very small cells may thus constitute a refuge, where bacteria are able to escape predation, but at the same time may represent a state of low physiological turnover (Kjelleberg et al. 1987).

Both HNF community grazing and CR indicated intensive predation on bacteria between 0.4 and

1.2 µm length during the first half of the study (Fig. 3B, Table 3). This was reflected clearly in the biomass development of this size fraction (Fig. 2), and we found significant negative correlations of HNF abundance, biomass and CR with bacterial biomass between 0.4 and 1.2 µm cell length (Table 2). In this context, we have to consider the results presented by Sanders et al. (1992), who were first to show a relationship between planktonic bacteria and their predators across numerous systems. The relatively large scatter in the data they present has attracted criticism (Gasol & Vaqué 1993). However, our refined analysis of predator-prey relations from a single system basically supports the general conclusion of Sanders et al. (1992), a tight link between bacterioplankton and their predators (Fig. 2). Bacterial and HNF abundances might not have been the most sensitive parameter for such a correlation, because different fractions of the bacterioplankton appear to be affected differently by predation (Fig. 2, Table 2). Changes of total bacterial abundance and biomass (Table 1) were much less pronounced and did not correlate significantly with parameters of HNF bacterivory (data not shown).

An almost equal amount of bacterial biomass was found in cells from 0.4 to 0.8 µm and cells between 0.8 and 1.2 µm (Fig. 2). Why did bacteria with an average cell volume of 0.123 μm^3 (0.8 to 1.2 µm) develop as much biomass and undergo almost identical fluctuations as cells with an average volume of only 0.045 μm^3 (0.4 to 0.8 µm) (Fig. 2)? According to the principles of size-selective grazing, cells of ~1 µm length should suffer considerably higher mortality rates than cells of half this size (González et al. 1990). Yet those 15 to 20% of pelagic bacteria in the size range between 0.8 and 1.2 µm accounted for approximately one third of total bacterial biomass (Fig. 2). This indicates that the relationship between bacterial cell sizes and elimination rates is not linear. Protist size-selective grazing probably defines a threshold size for bacterial cells, above which loss rates increase substantially. The 'trough' of bacterial biomass in the size classes between 1.2 and 2.4 µm (Fig. 2) even during periods of intensive growth and reduced grazing suggests a permanently strong grazing pressure on this fraction of bacterioplankton. This suggestion is supported by the results of Pernthaler et al. (1996), who observed highly selective HNF predation on fluorescently labeled picocyanobacteria (size 1 to 2 µm) in the same system. Nutrient limitation as an alternative explanation for the lack of biomass in this size range cannot be totally ruled out, as we do not know if all size classes of the bacterioplankton are limited by the same nutrients. However, both smaller (<1.2 µm) and larger (>2.4 µm) cells were found to increase to high biomass levels (Fig. 2) during the same period. Surprisingly, any single size fraction of cells >1.2 µm contributed as much or more to total bacterial biomass than small coccoid cells did. On average, between 14 and 24% of total bacterial carbon was found in the small number of cells between 1.2 and 2.4 µm (Fig. 2). So when discussing the question why the bacterioplankton is dominated by small cells, we must also consider that these far less abundant larger bacteria, regarded as the 'losers' of size-selective grazing, play a much more important role in carbon transfer than it is commonly assumed. The observed low abundances in these size classes ($1–2.5 \times 10^5$ cells ml^{-1}) are probably a consequence of protist foraging, but such cells, on the other hand, contain considerable biomass and are perhaps able to grow into size ranges beyond the particle uptake capacity of their predators (Jürgens & Güde 1994).

We observed pronounced biomass changes in the fraction of large bacteria (>2.4 µm, average cell size: 5.0 to 8.4 µm). Most of the biomass in this size class is probably not available to bacterivorous HNF (Jürgens & Güde 1994). The development of 'edible' and 'non-edible' bacterial biomass in the context of changing HNF bacterivory (Fig. 3B) and grazing pressure (Table 2) is strong support for the hypothesis of Jürgens & Güde (1994), which predicts the formation of grazing-resistant bacterial morphotypes during intense protist predation. We showed that the biomass of (presumably) protist-inedible bacteria is positively correlated with HNF abundance, biomass and bacterivory during the spring development of a natural planktonic community. Until now the appearance of large bacterial cells was either induced experimentally (e.g. Shikano et al. 1990, Jürgens et al. 1994) or deduced from observed standing stock changes of protists and *Daphnia* spp. (Güde 1988, Jürgens & Stolpe 1995).

Bacterial cell specific activity

For our analysis of grazing effects on growing bacterial cells, we have introduced a new parameter of 'weighted' mean per-cell growth, which requires a short theoretical justification: Bacterioplankton secondary production measured via the incorporation of radioactively labeled tracer substances (Fuhrman & Azam 1980, Simon & Azam 1989) is the consequence of the changes in individual cell growth and division activity and the size of the active fraction of bacteria. Currently there are several well-established methods to determine the size of this active fraction (e.g. Zimmermann et al. 1978, Rodriguez et al. 1992). Estimates range from 1 to 100% for different water bodies and seasons (Tabor & Neihof 1982, Šimek 1986, Dufour et

al. 1990, Šimek et al. 1990, Sommaruga 1995), and there is evidence that the active fraction of the bacterioplankton increases with the trophic state of the system (del Giorgio & Scarborough 1995). Since the majority of bacteria in numerous aquatic systems cannot be shown to be actively respiring (del Giorgio & Scarborough 1995), it is misleading to calculate the mean per-cell uptake of radiolabeled tracer substances by dividing total tracer uptake by total bacterial abundances. Why should we not assume, however, that predominantly active, i.e. respiring, cells are responsible for the incorporation of labeled tracer substances, and thus combine methods that measure complementary parameters of bacterial physiology? INT-uptake is regarded as such a measure of the active fraction of bacterioplankton (Dufour et al. 1990). Therefore, we have related the changes of total [^{3}H]thymidine and [^{14}C]leucine incorporation specifically to the fraction of INT-reducing bacteria in order to follow the specific growth of the 'mean active bacterial cell' (Fig. 3A). To assign radiotracer uptake exclusively to the INT-reducing fraction of bacterioplankton is probably not totally correct, as other cells might be viable as well, but below the detection limit of the INT method (Šimek 1986). Bacteria with a very low respiration rate, such as dormant cells, might not produce visible formazane crystals during the incubation period. It is safe to assume, however, that most physiological turnover will take place in the most intensively respiring fraction of an aerobic bacterial community (Dufour et al. 1990). Moreover, we know from autoradiography that only a small fraction of pelagic bacteria show high uptake rates of organic macromolecules, whereas the majority incorporate labeled tracer substances at a rate close to zero (Šimek 1989). So even if the absolute values in Fig. 3A may well be an overestimation of radiotracer uptake per individual cell (as a few percent might be incorporated into non-INT active cells), the general pattern of the curves remains unchanged.

We observed a consistent, positively correlated uptake pattern of thymidine and leucine per respiring bacterial cell (indicating DNA and protein synthesis rates, Table 2). The cell specific incorporation maxima during the second half of the investigation preceded the biomass maxima of bacteria between 0.4 and 1.2 µm cell length (Figs. 2 & 3B) by 1 to 3 d. The observed negative correlation between mean per-cell tracer uptake and HNF bacterivory (Table 2) indicates that the active fraction of bacterioplankton was negatively influenced by top-down effects, e.g. through the selective suppression of the fastest-growing or dividing cells during periods of high grazing (Šimek et al. 1990, Sherr et al. 1992, González et al. 1993).

There was a positive correlation between changes of bacterial biomass in the size class 0.4 to 0.8 µm and mean per-cell thymidine uptake. It would be too speculative, however, to assign bacterial activity to 1 size fraction of the bacterioplankton from this result, as there was no correlation of the second radiotracer and the biomass change of any size class.

CONCLUSION

In this study we compared processes (bacterial per-cell growth and bacterivory) and standing stocks (biomasses per size class and protistan abundance/biomass) of a natural predator-prey system. The results from both these analyses are consistent (Fig. 3, Table 2) with the predictions of 2 suggested regulation mechanisms of bacterial morphology: size-selective grazing (González et al. 1990, Šimek & Chrzanowski 1992) and the appearance of grazing-resistant bacteria (Güde 1988, Jürgens & Güde 1994). We suggest that radiotracer estimates can be used to estimate mean bacterial per-cell growth and division rates only if they are assigned to the active, e.g. INT-reducing, fraction of the bacterioplankton. Using this parameter, we showed that an increase in protistan grazing negatively correlates with the mean growth per active bacterial cell, which confirms another prediction of the size-selective grazing hypothesis (Sherr et al. 1992) and agrees with laboratory studies on protistan foraging (González et al. 1993).

Size-selective grazing on bacteria has been studied in order to explain the 'small average cell size of *in situ* bacterioplankton' (González et al. 1990). We want to extend this view by considering that different size classes of the bacterioplankton suffer different mortalities. The studied bacterial community was found to respond quickly to changes in predation intensity, but mainly through reallocation of biomass into different size classes. This process buffered the changes of total bacterial abundance and biomass, which therefore appeared to be only weakly related to HNF abundance or grazing. Fluctuations of bacterial standing stock were limited to cell sizes at the upper and lower end of the size range most vulnerable to protist predation (0.4 to 2.4 µm, Fig 2).

These observations encourage us to operationally define at least 4 functional size fractions within bacterial communities: small cells, weakly affected by protist grazing, 'grazing-vulnerable' bacteria, 'grazing-suppressed', and 'grazing-resistant' fractions of the bacterioplankton. The biomass dynamics of particular bacterioplankton size classes reflect changes in community structure, cell specific activity and protist bacterivory better than the development of total bacterial abundance or biomass.

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