

# Predator-induced changes of bacterial size-structure and productivity studied on an experimental microbial community

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**ABSTRACT:** The grazing impact of 3 different protozoan species on a mixed bacterial community was studied by means of a simplified and functionally reproducible experimental microbial food web in a 2-stage flow-through system. In the first stage the algae *Rhodomonas* sp. was grown on an inorganic medium with its accompanying bacterial community (BC) growing on algal exudates. This mixture of algae and bacteria was transferred into 4 second stage vessels: (1) a control, and 3 vessels inoculated with (2) a heterotrophic nanoflagellate, *Bodo saltans*, (3) a scuticociliate, *Cyclidium glaucoma*, and (4) a mixotrophic flagellate, *Ochromonas* sp. Using image analysis techniques we followed the changes in bacterial size distributions and bacterial to protozoan total biovolume ratios over an experimental period of 15 d. In addition, productivity of the grazed and ungrazed BC was measured using [<sup>3</sup>H]thymidine and [<sup>14</sup>C]leucine. As a consequence of total grazing rates and size-selective feeding we observed 3 different responses of the initially identical BC to grazing of the 3 protists. (1) Low grazing by *B. saltans* caused a slow decrease of bacterial cell numbers from 14 to  $5.9 \times 10^6$  cells ml<sup>-1</sup>, but no significant shift of the mean cell volume (MCV, average 0.107 μm<sup>3</sup>) and bacterial production. (2) Higher grazing rates of *C. glaucoma* resulted in the decline of bacterial abundance to  $3.3 \times 10^6$  cells ml<sup>-1</sup> in parallel with a doubling of the MCV to 0.207 μm<sup>3</sup> and high DNA and protein synthesis rates. Due to the ciliate's ability to graze also on small prey (<1.5 μm) an increase in MCV seemed to provide higher grazing resistance or at least decreased vulnerability. (3) *Ochromonas* sp. showed the highest grazing rates and reduced bacterial numbers by 20 times within 2 d. Only the smallest — obviously more grazing-protected — bacteria (<0.9 μm) survived, which increased thereafter to almost initial bacterial densities. Although the bacterial MCV dropped to 0.038 μm<sup>3</sup>, [<sup>3</sup>H]thymidine uptake rates per cell were greatly enhanced and highly variable. Our results reflect the potential of BC responses to different predation regimes and the advantages of phenotypic traits in order to coexist with various grazers. This should be seen in the context of influencing bottom-up effects and the varying potential of individual bacterial species to change morphology, growth strategies, and activity patterns.

**KEY WORDS:** Continuous flow systems · Grazing resistance · Bacterial growth and size-structure · Image analysis · *Bodo saltans* · *Cyclidium glaucoma* · *Ochromonas* sp.

## INTRODUCTION

Heterotrophic nanoflagellates, mixotrophic flagellates, and small ciliates are known as the major con-

sumers of heterotrophic bacterioplankton in freshwaters (Porter 1988, Berninger et al. 1991, Riemann et al. 1995, Šimek et al. 1995). Consequently, protozoan feeding is thought to have a strong impact (Güde 1989) on the taxonomic diversity, apparent morphotypes and secondary production patterns of bacterial

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communities (BC). The interactions of protozoa and bacteria perhaps represent the oldest predator-prey relationships we may study in nature (Jürgens & Güde 1994), representing thus highly complex and multiple strategies of bacteria as a reaction to grazing and of protists to overcome such strategies (coevolution).

The introduction of more sophisticated methods to analyze parameters at the single bacterial cell level (Zimmermann et al. 1978, Sieracki et al. 1985, Šimek & Fuksa 1989, Hicks et al. 1992) allowed more precise descriptions of the bacterial strategies to reduce grazing mortality or escape into resistance (Jürgens & Güde 1994, and references therein). The most prominent and obvious effects are changes in the bacterial size distribution to inedible or less vulnerable morphologies (e.g. small cocci, filaments, prosthecate cells). These morphological shifts have been described in both field and laboratory experiments. They can be related to changes in taxonomic composition (Güde 1979, Pernthaler et al. 1997a, Šimek et al. 1997) as well as to phenotypic plasticity of individual species (Shikano et al. 1990, Hahn & Höfle 1998), and to the activity patterns of the whole BC (Bird & Kalff 1993, Gasol et al. 1995).

Although laboratory experiments modeling these interactions always represent a simplification of the more complex relationships occurring in a natural system, they allow a detailed analysis of individual mechanisms that might otherwise be obscured by the simultaneous acting of various top-down and bottom-up effects (Psenner & Sommaruga 1992). However, a comprehensive comparison of published data on phenotypic responses of bacterial cells to grazing is strongly limited by the wide range of experimental conditions applied: different protozoan predators have been fed, e.g. with single bacterial species, very large bacteria or mixed BC of unknown taxonomy and/or activity status.

Therefore we set up a continuous flow system to establish a simplified, but functionally reproducible microbial food web based on a single algal species, its accompanying BC which mainly depended on exudates of the growing algae (Šimek et al. 1997, Vrba et al. unpubl.) and 3 different protozoan predators (*Bodo saltans*, *Cyclidium glaucoma* and *Ochromonas* sp.). We investigated the potential shifts in the BC considering the bacterial size distribution, activity and taxonomic composition (Pernthaler et al. unpubl.) as a consequence of grazing pressure, size-selective feeding, and growth strategies of protozoa. Image analysis was used for a detailed investigation of cell morphologies and biomasses of both predators and prey. Moreover bacterial activity as well as mortality rates of the microbial assemblages were determined.

## MATERIALS AND METHODS

**Design of the continuous flow system.** Two large glass bottles (5 l and 10 l) were connected to store a sufficient volume of the inorganic WC (MBL) medium (Guillard & Lorenzen 1972). The phosphorus content of the medium was reduced to  $200 \mu\text{g P l}^{-1}$ . For the first stage of the system a vessel with a filling volume of 2.3 l was used and aerated from the bottom (for details see Fig. 1). On top of the glass vessel were 5 openings—for de-aeration, for the medium inflow, for inoculation and sampling, and 2 for the connecting lines to the second stages. Special pear-shaped bacterial traps were used to disrupt the flows and to avoid a possible upstream migration of organisms. The second stage vessels with a filling volume of 750 ml were also aerated from the bottom and had a lateral outflow (Fig. 1). We used 3 l Erlenmeyer bottles sealed with rubber stoppers and sterile de-aeration for the outflow of medium. Silicon tubes with an inner diameter of 2 to 5 mm were utilized for the connections. Longer connections were divided into several smaller lines allowing a fast change of possibly damaged parts without changing the whole line. Two peristaltic pumps (Digital Drive MCP with cartridge pump heads, Ismatec, Switzerland) were applied for the transportation of the medium. We used pumping tubes with inner diameters of 1.02 mm and 2.06 mm (Ismatec, Switzerland) which have a long lifetime even in 24 h use and allowed an exact calibration of the peristaltic pumps (down to low flow-rates of  $270 \mu\text{l min}^{-1}$ ).

**Set-up of the system.** All parts were cleaned with distilled water and sterilized before the system was assembled in a walk-in chamber at  $18 \pm 1^\circ\text{C}$ . The WC medium was pumped into the first and the second stages until all vessels were half-filled. Additionally, the aeration of all vessels was started. The first stage was inoculated with 50 ml of a pure *Rhodomonas* sp. stock culture (strain 26.28 from the algal culture collection in Goettingen, Germany) with its accompanying bacteria. Pumping was stopped for 4 d until algae reached numbers of  $10^3 \text{ ml}^{-1}$  in the first stage. Thereafter, pumps were restarted again and algae were pumped into the second stage vessels. The dilution rate was set at  $0.38 \text{ d}^{-1}$  for the first stage and  $0.25 \text{ d}^{-1}$  for the second stages. When the abundance of *Rhodomonas* sp. was nearly equal in all stages we started sampling and considered this day as Day 0, still without addition of protozoa.

Three different protozoan species were inoculated into the second stages: a heterotrophic nanoflagellate (*Bodo saltans*), a mixotrophic flagellate (*Ochromonas* sp.) and the scuticiliate *Cyclidium glaucoma*. All species were originally cultured in tap water on autoclaved wheat grains with the accompanying bacteria.

Before the experiment, the protozoa were pre-cultivated in batch cultures of *Rhodomonas* sp. inoculated with protozoa that were washed free of bacteria by rinsing over 1  $\mu\text{m}$  filters (Šimek et al. 1997). These protistan-*Rhodomonas* sp. stock cultures were held under continuous light at 18°C. Twenty to 50 ml of the cultures were used to inoculate the second stages at the end of Day 0.

**Sampling and sample preparation.** One hundred ml subsamples were collected from each vessel into pre-cleaned sterile 250 ml Schott bottles every morning for a period of 2 wk. All subsamples were prefixed with Lugol's solution (0.5% final concentration = f.c.) immediately followed by formaline (3% f.c.) and decolorized by several drops of sodium thiosulfate (3%). Live subsamples were used for the determination of grazing via uptake rates of fluorescently labeled bacteria (FLB) (Sherr et al. 1987, Šimek et al. 1994). The surrogates amounted to <10% of the actual bacterial abundance and protists showed linear uptake rates within the first 20 min (data not shown). With regard to the optimal number of tracer particles per protozoan cell, hourly uptake rates (total grazing rates) were calculated from 5 min and 10 min feeding periods for ciliates and flagellates, respectively. Bacteria for the preparation of FLB were taken from the first stage of a previous experiment.

Bacterial numbers and cell dimensions were determined from 4',6-diamidino-2-phenylindole (DAPI, Sigma) stained cells (2  $\mu\text{g ml}^{-1}$  f.c.) on black 0.2  $\mu\text{m}$  pore sized polycarbonate filters (Poretics, USA). Staining conditions and time (7 min) were kept constant during the experiment to obtain comparable signal intensity for counting and image processing. We used Zeiss Axioplan, Axiovert, and Axiophot 2 microscopes with optovars (up to 2.5 $\times$ ) and 40 $\times$  and 100 $\times$  oil objectives. Bacterial abundances were always determined by the same person during the whole experiment to reduce counting error (~5%, data not shown, cf. Kirchman et al. 1982). Between 500 and 700 cells

were counted per sample at a magnification of 1250 $\times$ . Images (524  $\times$  752 pixels, 8-bit, pixel size: 0.065  $\mu\text{m}$ ) of stained cells were recorded at a magnification of 125 $\times$  with a highly sensitive CCD camera (Optronics ZVS-47EC) and processed with the image analysis software LUCIA D (Version 3.52ab, Laboratory Imaging Prague, Czech Republic, <http://www.lim.cz>). At least 500 bac-

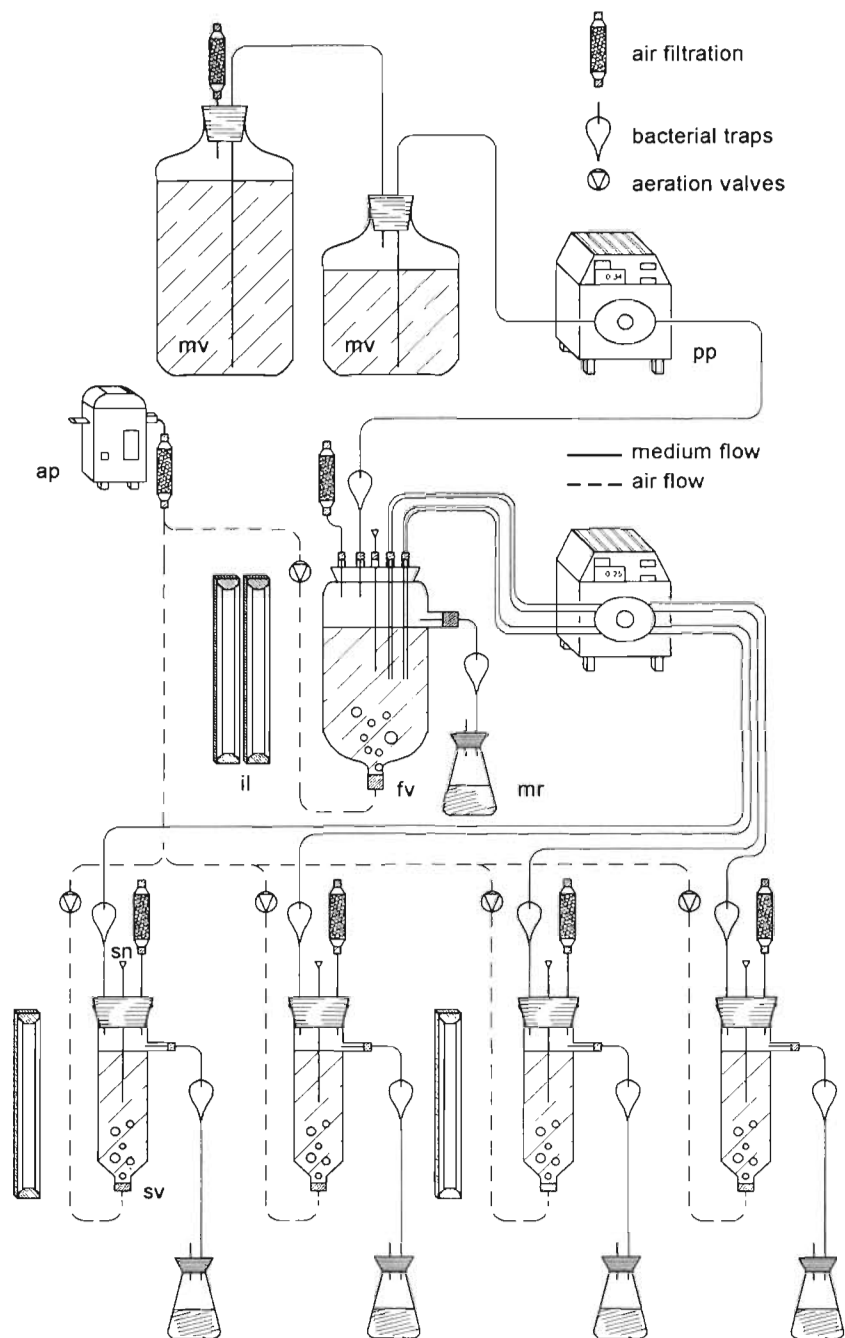


Fig. 1. Scheme of the 2-stage continuous flow system. (ap: aeration pump; fv: first stage vessel; il: illumination; mv: medium vessel; mr: medium fluid recipient; pp: peristaltic pump; sn: steel needle for sampling; sv: second stage vessel)

teria were analyzed per sample and the most reliable parameters of pixel measurement were chosen: cell area and cell perimeter. We calculated cell dimensions (length, width, volume) according to algorithm 3 published by Massana et al. (1997). Details of the image processing (gray transformation, edge finding) are described in Pernthaler et al. (1997b) and Posch et al. (1997).

Algae and protozoa were filtered onto 1.0  $\mu\text{m}$  pore sized black polycarbonate filters (Poretics, USA), stained with DAPI (0.5 to 1  $\mu\text{g ml}^{-1}$  f.c.) and counted at a magnification of 400 $\times$ . Cell dimensions were measured interactively with a computer mouse on the screen using the same image analysis software as described above. We measured lengths and widths of at least 100 protozoan cells per sample and calculated the cell volume using simple geometrical formulas like those for half ellipsoids or spheres. Bacterial and protozoan total biovolumes ( $\text{mm}^3 \text{l}^{-1}$ ) were calculated from the observed abundances ( $\text{cells l}^{-1}$ ) times the mean cell volume (MCV) ( $\mu\text{m}^3 \text{cell}^{-1}$ ). To follow the development of all organisms in the experimental set-up all samples were analyzed on the same day as sampling.

**Determination of bacterial production.** The modified method of Simon (1990) was applied for a combined measurement of cell multiplication rates via [ $^3\text{H}$ ]thymidine incorporation (specific activity 70 to 90 Ci  $\text{mmol}^{-1}$ , Amersham) and protein synthesis via [ $^{14}\text{C}$ ]leucine incorporation (specific activity 310 mCi  $\text{mmol}^{-1}$ ). Five ml of triplicate samples were incubated with [ $^3\text{H}$ ]thymidine (5 nM f.c.) and [ $^{14}\text{C}$ ]leucine (20 nM f.c.) for 60 min at 18°C. After Day 2 the incubation time was reduced to 30 min because of increasing bacterial growth. Uptake of radiochemicals was stopped by the addition of 0.5 ml formaline (4 % f.c.). Blanks were pro-

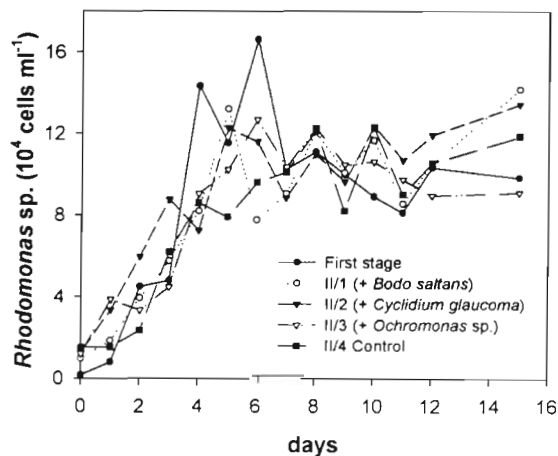


Fig. 2. Abundances of *Rhodomonas* sp. in the first stage, control and all other vessels of the second stage during the experimental period of 15 d

duced by fixing the sample with formaline and adding the radiotracers after 15 min to correct for abiotic absorption of [ $^3\text{H}$ ]thymidine and [ $^{14}\text{C}$ ]leucine. Filters were rinsed twice with ice-cold trichloric acetic acid (TCA) and then with particle-free distilled water before samples were filtered onto white polycarbonate filters (0.2  $\mu\text{m}$  pore size, Poretics). Five ml of TCA were used for disruption of cells and the extraction of macromolecules (DNA and proteins). After filtration the filters were washed again 3 times with TCA to remove all remaining cell fragments. Afterwards, filters were dried and 10 ml of a scintillation cocktail (Beckman Ready Safe) were added. After 5 to 6 h, i.e. after complete disintegration of the filters, samples were counted twice for 10 min, with internal quench correction (Beckman Liquid Scintillation Counter LS 6000 IC).

## RESULTS

### Development of bacterial abundances and MCV

During the first 5 d of the experiment *Rhodomonas* sp. increased from 2 to  $8 \times 10^4$  cells  $\text{ml}^{-1}$ , then stabilized at 8 to  $12 \times 10^4$  cells  $\text{ml}^{-1}$ , with slight fluctuations over the investigation period in all experimental vessels (Fig. 2). MCV of algae in all second stages except with *Ochromonas* sp. lay in the range of 273 to 288  $\mu\text{m}^3$  during the 15 d. In that vessel *Rhodomonas* sp. cells showed a higher mean volume of 338  $\mu\text{m}^3$ .

(1) Bacterial abundance in the first stage increased slowly during the 15 d, likely associated with rising algal numbers reaching mean values of  $8.5 \times 10^6$  cells  $\text{ml}^{-1}$  (Fig. 3). These bacterial cells had a very constant MCV of  $0.110 \pm 0.015 \mu\text{m}^3$  (mean  $\pm$  SD), mostly rod-shaped with mean cell dimensions of  $1.25 \times 0.36 \mu\text{m}$ .

(2) The addition of *Bodo saltans* in one of the second stages resulted in an unexpected slow development of protozoan numbers instead of logarithmic growth, especially during the first days of the experiment. A maximum number of  $1.8 \times 10^4$  cells  $\text{ml}^{-1}$  and a total grazing rate of  $2.2 \times 10^5$  cells  $\text{ml}^{-1} \text{h}^{-1}$  was reached at the last day (Fig. 3). During the experiment a mean individual grazing rate of  $13.8 \pm 3.6$  bacteria  $\text{h}^{-1}$  flagellate $^{-1}$  was observed. Although we still do not know the reasons for the slow protozoan growth, we decided to include this data set as an example of lower grazing in comparison to the 2 other protistan-influenced variants. Bacterial abundance in this vessel decreased from 14.0 to  $5.9 \times 10^6$  cells  $\text{ml}^{-1}$  whereas the MCV fluctuated around  $0.107 \pm 0.016 \mu\text{m}^3$  during the test period. At the end of the experiment, bacteria had increased in numbers with a slight trend towards smaller cells (MCV at Day 15:  $0.091 \mu\text{m}^3$ ).

(3) The inoculation of *Cyclidium glaucoma* caused a bacterial decrease to  $3.3 \times 10^6$  cells  $\text{ml}^{-1}$  (Fig. 3). Ciliates showed fast exponential growth and reached up to 2000 cells  $\text{ml}^{-1}$ . A mean individual grazing rate of  $548 \pm 172$  bacteria  $\text{h}^{-1}$  ciliate $^{-1}$  was measured. The total grazing rate increased until Day 6 to a maximum of  $5.8 \times 10^5$  cells  $\text{ml}^{-1} \text{h}^{-1}$  and the grazing pressure caused an obvious shift in bacterial cell dimensions. The MCV nearly doubled to  $0.207 \mu\text{m}^3$  (mean volume during the experiment:  $0.160 \pm 0.032 \mu\text{m}^3$ ).

(4) The addition of *Ochromonas* sp. resulted in a 20-fold decrease of bacterial numbers within 2 d (Fig. 3). Bacterial abundance dropped from  $12.0$  to  $0.7 \times 10^6$  cells  $\text{ml}^{-1}$  with a simultaneous strong decrease in MCV to  $0.038 \mu\text{m}^3$ . During the whole experiment a bacterial MCV of  $0.059 \pm 0.023 \mu\text{m}^3$  was calculated. *Ochromonas* sp. grew constantly until Day 15 and reached cell numbers of  $8.2 \times 10^4$  cells  $\text{ml}^{-1}$ . This protist showed the highest total grazing rates resulting in a maximal value of  $10.9 \times 10^5$  cells  $\text{ml}^{-1} \text{h}^{-1}$ . We observed a mean individual grazing rate of  $14.5 \pm 4.8$  bacteria  $\text{h}^{-1}$  flagellate $^{-1}$  for the whole experiment.

(5) Bacterial abundances in the control, predator-free vessel of the second stage remained quite stable during the experiment, with slightly higher numbers (on average  $1.2 \times 10^7$  cells  $\text{ml}^{-1}$ ) and MCV ( $0.130 \pm 0.015 \mu\text{m}^3$ ) than in the first stage (Fig. 3). This phenomenon was often observed during earlier experiments (Šimek et al. 1997), presumably caused by a slower dilution rate of  $0.25 \text{ d}^{-1}$  in comparison to  $0.38 \text{ d}^{-1}$  of the first stage which allowed bacteria to grow up and accumulate.

The bacterial MCV in the vessels with *Ochromonas* sp. and *Cyclidium glaucoma* were significantly different from each other and all other stages (ANOVA, Scheffé post hoc comparison,  $p < 0.05$ ). However we found no significant differences between the bacterial MCV of the first stage, the vessel inoculated with *Bodo saltans* and the control (ANOVA, Scheffé post hoc comparison,  $p < 0.05$ ).

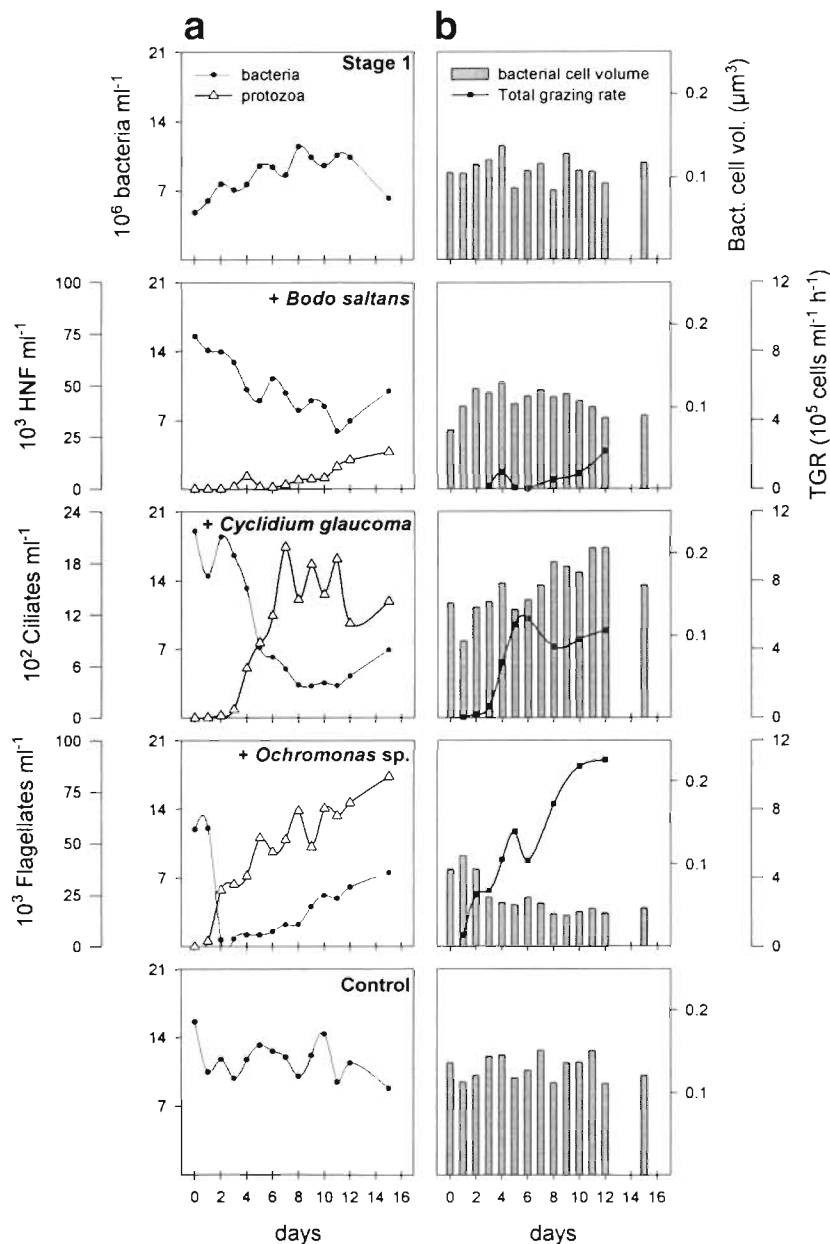


Fig. 3. (a) Changes in bacterial and protozoan cell numbers in the first stage and the 4 second stage vessels. (b) Development of bacterial cell volumes and total grazing rates after inoculation of *Bodo saltans*, *Cyclidium glaucoma* and *Ochromonas* sp.

#### Development of bacterial size classes associated with grazing

We split the image analysis data into 5 distinct size classes to differentiate selective feeding by the 3 protozoan species (Fig. 4). Cell lengths were chosen as decisive factors because cell widths remained quite stable during the experiment at all stages ( $0.36 \pm 0.02 \mu\text{m}$ ) except after addition of *Ochromonas* sp. ( $0.28 \pm$

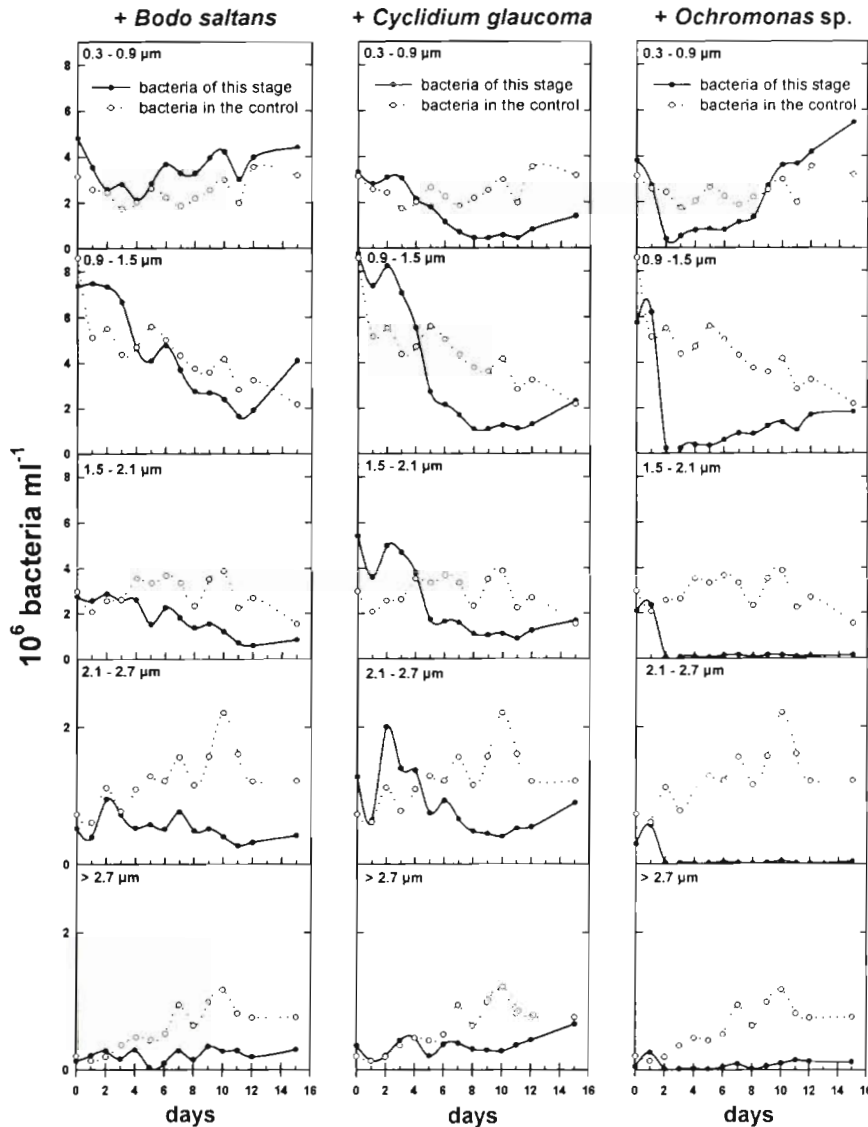


Fig. 4. Changes of the bacterial size class distributions after inoculation with 3 different protistan predators. The cell-size data were split into 5 size classes and the development of each class in the predator stages is compared with the control stage (predator-free)

0.04 μm). This data treatment allowed a detailed description of bacterial size shifts, which are not obvious when only the MCV is considered (compare for instance Fig. 4 with Fig. 3). Abundances of smallest (0.3 to 0.9 μm) and medium-sized cells (1.5 to 2.1 μm) remained stable in the control stage over the observed period whereas the abundance of larger cells increased slightly with time. We do not know which smaller cells were growing up during the experiment and therefore contributed to larger size classes. The addition of *Bodo saltans* caused an increase of smaller bacteria and a moderate decrease of larger cells. The scuticociliate *Cyclidium glaucoma* reduced the abundance of all size classes within 0.3 to 2.7 μm cell length but caused a slight increase of the larger cells (>1.5 μm) in contrast with the other 2 predator systems. Only *C. glaucoma* had a permanent negative effect on

the abundance of the smallest bacteria. *Ochromonas sp.* eliminated nearly all cells larger than 1.5 μm, reducing numbers down to 10<sup>4</sup> cells ml<sup>-1</sup>, but the smallest forms survived this strong grazing pressure and became dominant at the end of the experiment.

#### Cell-specific uptake rates of labeled thymidine and leucine

Bacteria in the first stage showed constant uptake rates after 5 d. At that time numbers and biovolumes of algae, the only substrate suppliers for bacteria, reached their maxima, then kept similar values throughout the experiment (Fig. 2). We observed only slight fluctuations of [<sup>14</sup>C]leucine uptake in the control vessel whereas the uptake of [<sup>3</sup>H]thymidine nearly

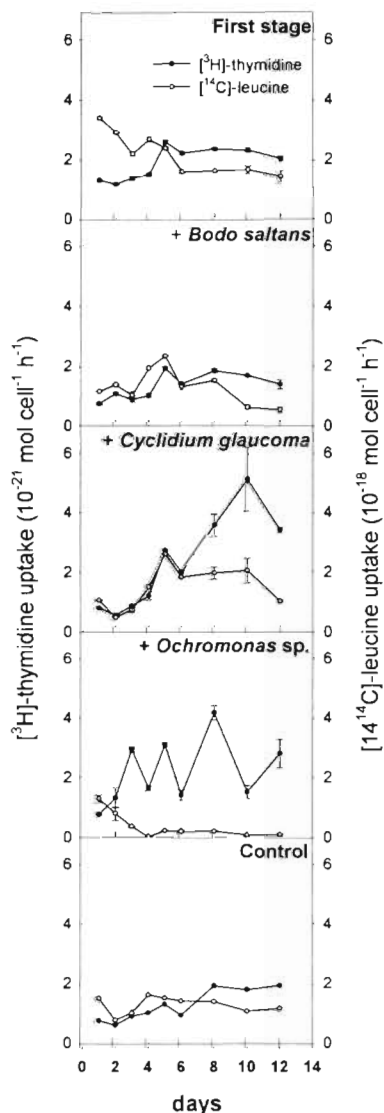


Fig. 5. Cell-specific uptake rates of [<sup>3</sup>H]thymidine and [<sup>14</sup>C]leucine in the first stage, after addition of protozoan predators, and in the control stage

doubled with time (Fig. 5). Bacterial production in the first stage and the control showed nearly equal patterns after Day 8.

Protozoan grazing led to tremendous changes of secondary production, resulting in 2 contrasting bacterial responses. After addition of *Cyclidium glaucoma*, uptake rates of [<sup>3</sup>H]thymidine and [<sup>14</sup>C]leucine increased in parallel until Day 5. Specific uptake of [<sup>3</sup>H]thymidine increased furthermore up to  $5.1 \times 10^{-21}$  mol cell<sup>-1</sup> h<sup>-1</sup>. Bacteria showed the highest leucine uptake rates of all second stage vessels (up to  $2.6 \times 10^{-18}$  mol cell<sup>-1</sup> h<sup>-1</sup>), which suggests that bacteria reacted on grazing pressure by replicating faster and growing larger (Figs. 3 & 4).

On the other hand, immediately after inoculation of *Ochromonas* sp. bacterial thymidine uptake rates increased extremely fast whereas the uptake of leucine dropped to very low values (Fig. 5). While DNA synthesis rates showed strong and regular fluctuations after Day 3, leucine uptake rates remained extremely low until the end of the experiment. The abundance of cells <math>1.5 \mu\text{m}</math> increased constantly, parallel with rising uptake rates of [<sup>3</sup>H]thymidine (compare Figs. 4 & 5). The low feeding activities of *Bodo saltans* caused no distinct production pattern in comparison to the control stage.

### Development of bacterial and protozoan total biovolumes

The MCV of protists over the experiment were as follows: *Bodo saltans* ( $43 \pm 12 \mu\text{m}^3$ ), *Cyclidium glaucoma* ( $1264 \pm 324 \mu\text{m}^3$ ) and *Ochromonas* sp. ( $120 \pm 51 \mu\text{m}^3$ ). Especially in the phase of intensive protistan growth we observed higher variations of the MCV mainly caused by high percentages of dividing cells. Although *B. saltans* developed very slowly during the experiment, it caused a slight decrease of total bacterial biovolume. Due to high total grazing rates of *C. glaucoma*, bacterial biovolume dropped to  $0.60 \text{ mm}^3 \text{ l}^{-1}$ . An increase in the MCV (see Fig. 3) compensated this effect and the proportion of bacterial to protozoan total biovolume moved to a 1:1 ratio (Fig. 6). The addition of *Ochromonas* sp., which developed the highest biovolumes of the 3 tested organisms (up to  $16 \text{ mm}^3 \text{ l}^{-1}$ ), caused a reduction of bacterial biovolume down to  $0.05 \text{ mm}^3 \text{ l}^{-1}$ . The ratio of protozoan versus bacterial biovolume reached on some days up to 7000% (Fig. 6). As a consequence of the steep increase in numbers of the smallest size class (see Fig. 4) bacterial biovolumes increased at the end of the experiment.

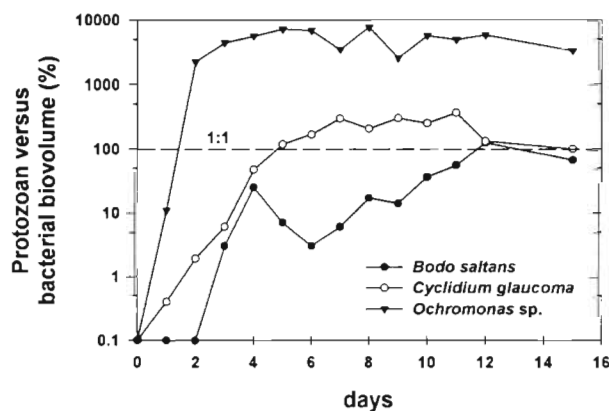


Fig. 6. Time course of protozoan versus bacterial total biovolume (%) during the experiment. Note the logarithmic scale to present the high biovolume ratios of *Ochromonas* sp.

## DISCUSSION

Although we worked with a simplified experimental microbial food web, we could observe relatively high phenotypic and genotypic plasticity of the microbial community reflected in bacterial size distributions (Fig. 4) and the contrasting patterns of production (Fig. 5).

The initially identical bacterial composition in the second stage vessels, as revealed by oligonucleotide probing (Pernthaler et al. unpubl.), developed 3 distinct 'responses' to grazing pressure. Low grazing by *Bodo saltans* only caused a decline of bacterial numbers and the total grazing rate per hour (TGR) never exceeded 3% of the actual bacterial standing stock (average: 0.9%). However, bacterial numbers decreased while the mean cell size did not, and DNA and protein synthesis rates did not show patterns different from those observed in the control vessel. All presented total grazing rates in this study were determined by uptake rates of FLB. We know that this method has some real drawbacks as we had to use the same surrogates during the whole experiment independent of the observed size shifts in the BC.

Grazing by *Cyclidium glaucoma* increased until Day 6 and the TGR reached 12% of bacterial standing stock hourly removed (average: 6.3%). The BC reacted by doubling of the mean cell size as reflected by higher abundances of cells >1.5  $\mu\text{m}$ , which did not happen in

the other 2 predator-exposed variants. Due to increasing bacterial cell sizes during the 14 d, the initial decrease of total bacterial biovolume was compensated over time and nearly reached the predator's biovolume. According to the literature, this scuticiliate can also ingest smaller prey, even particles down to a diameter of 0.2  $\mu\text{m}$  (Fenchel 1980a). In our study *C. glaucoma* was the only predator able to reduce the smallest size class of 0.3 to 0.9  $\mu\text{m}$  throughout the experiment. However, it is difficult to define the optimal prey size range for this scuticiliate (Table 1). Šimek et al. (1994) reported highest ingestion rates (up to 24 bacteria ciliate<sup>-1</sup> min<sup>-1</sup>) for a single bacterial species with a volume of 0.377  $\mu\text{m}^3$  whereas maximal clearance rates for latex beads of 0.36  $\mu\text{m}$  diameter (~0.024  $\mu\text{m}^3$ ) were described by Fenchel (1980a). Grazing activities of *C. glaucoma* had a strong impact on the cell-specific DNA and protein synthesis rates, which hints at how close top-down and bottom-up effects may be interrelated. Size-selective feeding by this ciliate caused a shift to larger, less vulnerable morphotypes with higher division rates (top-down effect), and since this BC was not apparently limited by substrates, it could react on grazing pressure by increasing the cell-specific activity (bottom-up effect).

The mixotrophic flagellate *Ochromonas* sp. grew to a total biovolume 20-fold larger than the bacterial standing stock, obviously profiting from 24 h illumination (and potentially from residual nutrients of the inor-

Table 1. Ingestion rates of *Cyclidium* sp. for different-sized prey particles. FLB: fluorescently labeled bacteria

Particle volume ( $\mu\text{m}^3$ )	Particle type	Particle morphology ( $\mu\text{m}$ )	Particle concentration (cells ml <sup>-1</sup> )	Ingestion (particles ciliate <sup>-1</sup> min <sup>-1</sup> )	Preferred size ( $\mu\text{m}^3$ )	Source
0.007	Latex beads	0.23 spherical	0.2 – 58 × 10 <sup>4</sup>	2.6 – 7.8		Fenchel (1980a)
0.024	Latex beads	0.36 spherical	4.0 – 96 × 10 <sup>4</sup>	7.0 – 216.2		Fenchel (1980a)
0.065	Bacterioplankton + FLB	Cocci + rods	0.3 – 11 × 10 <sup>6</sup>	0.6 – 10.6	0.082 – 0.096	Šimek et al. (1994)
0.106	Bacterioplankton + FLB	Cocci + rods	0.5 – 1.4 × 10 <sup>6</sup>	2.3 – 8.8	0.105 – 0.111	Šimek et al. (1994)
0.113	Carboxylated fluorescent microspheres	0.60 spherical	0.5 – 4.8 × 10 <sup>6</sup> 7.0 – 13.0 × 10 <sup>7</sup>	0.4 – 2.9 40.7 – 93.9		Sanders (1988)
0.113	Protein treated fluorescent microspheres	0.60 spherical	0.6 – 59 × 10 <sup>6</sup> 23 – 49 × 10 <sup>7</sup>	0.4 – 36.1 139.3 – 134.5		Sanders (1988)
0.202	<i>Alcaligenes xylosoxidans</i>	0.97 × 0.57 rods		17.0	0.275	Šimek et al. (1994)
0.377	<i>Alcaligenes xylosoxidans</i>	1.43 × 0.62 rods		24.4	0.352	Šimek et al. (1994)
0.421	Carboxylated fluorescent microspheres	0.93 spherical	0.3 – 10 × 10 <sup>6</sup> 10 × 10 <sup>7</sup>	0.03 – 2.6 9.0		Sanders (1988)
0.421	Protein treated fluorescent microspheres	0.93 spherical	0.3 – 18 × 10 <sup>6</sup> 13 × 10 <sup>7</sup>	0.07 – 6.2 9.5		Sanders (1988)
0.678	Latex beads	1.09 spherical	3 – 40 × 10 <sup>7</sup>	0.4 – 1		Fenchel (1980a)



ganic WC medium). Although the numbers of the *Rhodomonas* sp. population in the second stages were continuously stabilized by influx from the first stage (Fig. 2), *Ochromonas* sp. could successfully establish a dense population in association with the autotroph. This can only be explained by the ability of the chryso-phyte to acquire limiting nutrients by phagotrophy (Rothhaupt 1996). The total hourly grazing rates of *Ochromonas* sp. reached up to 50% of the bacterial standing stock (average 32%). We could observe a prominent impact of total grazing on bacterial cell-specific activity (Fig. 5) and on the size distribution of the total BC. All bacterial size classes were reduced by nearly 2 orders of magnitude within 2 d after inoculation with *Ochromonas* sp, and bacteria  $>0.9 \mu\text{m}$  never recovered from grazing pressure. The smallest size class ( $0.3$  to  $0.9 \mu\text{m}$ ) seemed to be less grazing-vulnerable and these bacteria, very uniform in shape, started to increase in number already after the second day as indicated by image analysis data and high uptake rates of [ $^3\text{H}$ ]thymidine. At the end of the experiment bacterial abundance (but not biomass) was similar to the original density on Day 0. Bacteria thus reacted to grazing by high division rates compensating for high mortality, but not by an enlargement of their mean cell size, as indicated by low per cell protein synthesis rates. When we compare activity patterns of the protistan-influenced BC with the control stage we should consider feedback mechanisms of intensive grazing, e.g. remineralization of phosphorus or carbon. In our former experiments we could always observe an increase in *Rhodomonas* sp. biomasses as a consequence of intensive protistan grazing on bacteria (Vrba et al. unpubl.) and this phenomenon is also described by several authors (Caron et al. 1988, Rothhaupt 1992). On the other hand feeding on bacteria and simultaneous remineralization of nutrients could have a direct positive feedback on the bacterial prey itself. This effect would consequently not appear in our control vessel.

When comparing the effects of the 3 tested protists on the BC, several important aspects of grazing must be considered, such as the total grazing rate, prey-size selection and how rapidly these parameters change over time (Fig. 7). The magnitude of total grazing and its increase over time is probably a decisive factor for how rapidly bacteria have to react to grazing induced mortality, and consequently which fraction of the bacterial standing stock with respect to species composition, numbers and cell sizes will be reduced.

Each protozoan species shows highest clearance rates for a distinct prey-size class (Fenchel 1980b), and for larger ciliates this range may even change with the size of the predator during its life cycle (Fenchel 1980a). However, we may assume a rather constant

pattern of size selectivity of our small tested organisms during their cell cycles. There is increasing evidence that natural protozoan communities selectively feed on the larger-sized fraction of bacterioplankton (Andersson et al. 1986, Chrzanowski & Šimek 1990, Gonzalez et al. 1990, Sherr et al. 1992, Bird & Kalff 1993, Jürgens & Güde 1994). Such a preference for distinct size classes would not only cause a general reduction of bacterial biomass but also an above-average loss of the active and dividing cells if they fall into the vulnerable size range. Protozoan grazing will therefore sometimes have a stronger impact on bacterial production (Gonzalez et al. 1990, Sherr et al. 1992) or the most active bacterial fraction (Gasol et al. 1995) than on total standing stock. However, bacteria within the edible size range may compensate loss rates by an increased cell division without conspicuous morphological changes (Fig. 7). This has been suggested for members of the alpha-Proteobacteria in a comparable experimental community (Pernthaler et al. 1997a). Alternatively, cells could also keep their original size if they are indigestible or even toxic for their predators.

Another possibility to escape grazing is a shift to inedible or less vulnerable size classes (Fig. 7). Some bacteria may be able to decrease in size and, consequently, reduce ingestion rates (Andersson et al. 1986). As the removal of large edible bacteria probably caused a shift in competition within the BC in the presence of *Ochromonas* sp., the initially minor fraction of small bacteria were therefore released from limitation by substrate or nutrients. They could thus enhance division rates (Fig. 5) and compensate for high mortalities. This can also be deduced from the initial reduction of abundances in this size class after the addition of *Ochromonas* sp. and the subsequently observed regrowth (Fig. 4). Bacteria coexisting with the mixotroph stabilized at an average cell size of  $0.04 \mu\text{m}^3$ , which falls into the size range of the majority of bacteria in freshwater or marine planktonic systems (Billen et al. 1990). This observation raises the question of whether larger bacteria are indeed the only or predominant fraction of a planktonic community that may show high growth and division rates. Andersson et al. (1986) observed very similar effects of grazing activities by a marine *Ochromonas* sp. The offered BC decreased its MCV by 47% and the abundance of smaller cells ( $<0.05$  to  $0.1 \mu\text{m}^3$ ) increased during the experimental period of 14 d.

A frequently discussed strategy of acquiring grazing resistance is the formation of filaments (Güde 1979, Shikano et al. 1990, Sommaruga & Psenner 1995, Šimek et al. 1997), cells with a complex morphology (e.g. prosthecae cells, Bianchi 1989) or aggregates (Jürgens & Güde 1994). Threadlike bacteria are usually not important numerically yet in terms of biomass

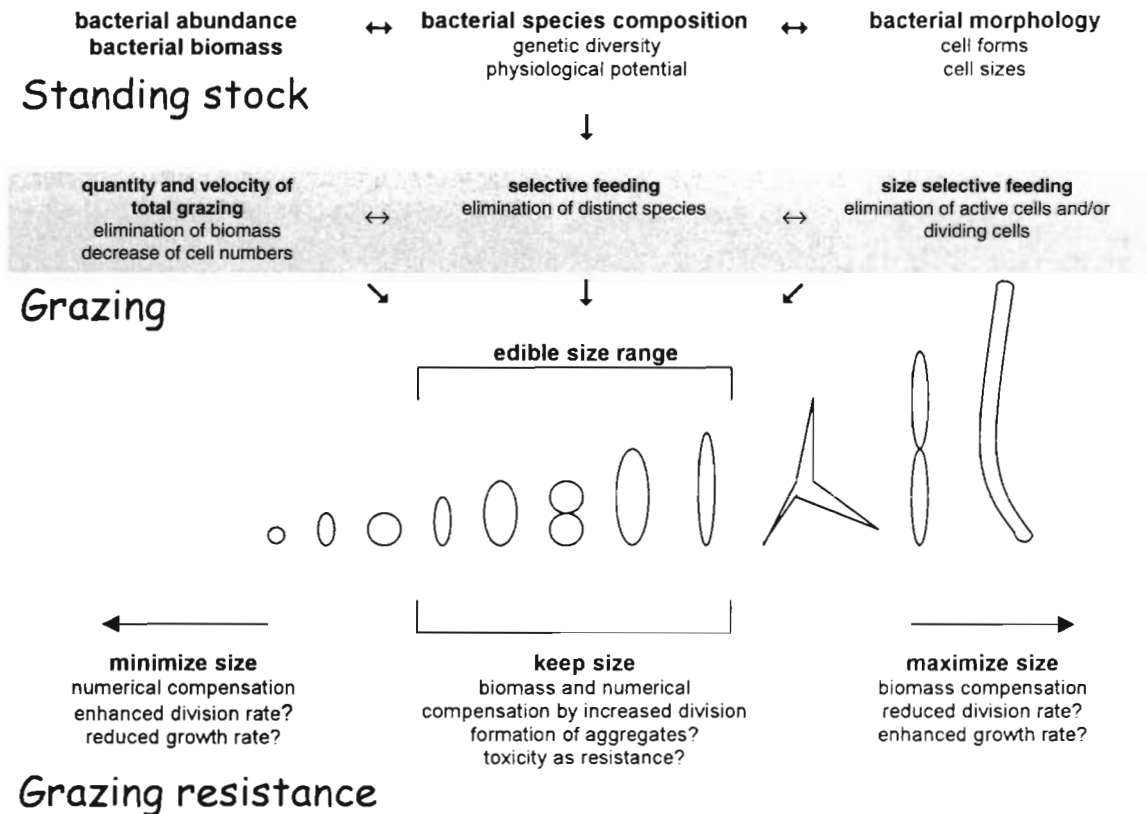


Fig. 7. Possible responses of bacterial communities to protozoan grazing. The impact of grazing pressure can be seen as a combination of several factors (e.g. selective feeding, amount and velocity of total grazing). The reactions on protozoan grazing should be linked with the species composition and/or bottom-up effects

they may fully compensate for grazing losses (Šimek et al. 1997). Filamentation of *Comamonas acidovorans* and other bacteria has been observed as a consequence of increased growth rate related to grazing pressure (Hahn & Höfle 1998, M. Hahn pers. comm.). In contrast to an earlier experiment in a comparable set-up (Šimek et al. 1997), the formation of filamentous morphotypes as a consequence of predation was not found during the present study (Fig. 4). This is a clear indication that this particular response to grazing depends on the presence or absence of species which have the genetic potential to change their morphology accordingly (Hahn & Höfle 1998).

However, it is still open to discussion if observed changes in the BC are an 'active' response to the presence of a predator. Chemical stimuli released by protozoan predators might induce shifts in the bacterial growth patterns and consequently in their size distribution or morphology. Such phenomena have been described for ciliate interspecific relationships (Kuhlmann & Heckmann 1985, Kusch & Kuhlmann 1994), and chemical cross talk between bacteria and eukaryotes has been observed before. Alternatively we may only observe 'passive' responses as a reaction on shifts

in the competition within the BC (Hahn & Höfle 1998).

In summary we should consider planktonic protozoa not only as the eliminators of the 'active' fraction of bacterial communities, but also as the driving force of changes in bacterial growth and division patterns, and as agents influencing interspecific competition. These shifts caused by top-down effects may occur at short time scales and can be closely related to limiting bottom-up effects (Psenner & Sommaruga 1992). Last but not least, the individual changes in bacterial cell size, species composition and production will give feedback on the presence and the wellbeing of their protistan predators. This aspect should be considered in a series of experiments which allow the study of feedback effects of prey consumption on predators. We hope, thus, that our work will draw more attention to the characteristic growth and feeding patterns of individual protistan predators and their conspicuous implications for bacterial communities.

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