

Unexpected Effects of Prey Dimensions and Morphologies on the Size Selective Feeding by Two Bacterivorous Flagellates (*Ochromonas* sp. and *Spumella* sp.)

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ABSTRACT. Current models on protistan size-selective feeding assume that contact probability is the factor that largely explains observed food preferences. Contact probability is generally expected to be positively correlated with prey size and therefore to explain observed food selection for larger prey items. We critically tested these basic assumptions on size-selective feeding using the interception-feeding chryomonad nanoflagellates *Ochromonas* sp. and *Spumella* sp. Mechanisms of differential feeding were studied during distinct stages of the selection process (i.e. contact probability, capture efficiency, ingestion efficiency, and differential digestion) by means of high-resolution video microscopy. Food selection was investigated using a mixture of microspheres ranging from 0.3–2.2 μm in diam., as well as a mixed bacterial community. In contrast to current model assumptions, the contact probability was highest for microspheres of intermediate size (0.9–1.2 μm), but was not generally positively correlated with prey size over the whole prey size range. Capture and ingestion also proved to be involved in size selection: these patterns were also independent of the food concentration ($p = 0.968$ for *Ochromonas*, $p = 0.971$ for *Spumella*). Even though the capture rate was significantly higher for attached flagellates than for swimming flagellates ($p < 0.001$), size selectivity was not affected ($p > 0.05$). Our results indicate that: (i) size selection is not actively regulated by these flagellates, but is a passive process; (ii) contact probability is not generally positively correlated with prey size, but shows a maximum for intermediate-sized prey in the prey size spectrum of 0.3–2.2 μm ; and (iii) selection steps other than contact probability are crucial for size selection and should be integrated in models on size selection.

Key Words. Chryomonades, continuous cultivation system, feeding phases, Ochromonadaceae, optimal foraging.

SIZE selective grazing by protists contributes to the structure and dynamics of bacterioplankton. Most protists graze preferentially on medium-sized cells and can therefore induce a bi-directional shift of the formerly bell-shaped size distribution of a bacterial community towards larger and smaller cells. Small cocci and large bacteria with a complex morphology (e.g. filaments and microcolonies) are favored only when grazing pressure by protists is high (Hahn and Höfle 1999, 2000; Hahn, Moore, and Höfle 1999; Posch et al. 1999; Šimek and Chrzanowski 1992). The size-selective grazing by predators may also affect bacterial production and metabolic pathways (Posch et al. 1999).

Regarding the edible size classes, a positive correlation is generally assumed between prey size and the ingestion rate, up to an upper prey size where the morphology of the flagellate then limits ingestion (Fenchel 1984; González 1996; Shimeta and Jumars 1991; Šimek and Chrzanowski 1992). This basic positive correlation is thought to be due to an increasing contact probability of the flagellate cell in the case of larger particles. Several theoretical relationships between prey size and contact probabilities of interception-feeding flagellates have been described: The models of Fenchel (1984) and Shimeta and Jumars (1991) predicted that the clearance rate should be proportional to the square of the *prey radius* when the particles exactly followed the streamline of the flow produced by the flagella of the predator. González (1996) also predicted a direct proportionality between prey size and encounter rate. In contrast to the former models, González (1996) suggested a linear correlation to *prey volume* (i.e. the cube of prey radius). However, van der Waals forces and hydrodynamic boundary effects, neglected by the model, may alter this prediction. Monger and Landry (1991) proposed the ‘Force-Balance’ model in which clearance rates are proportional to the 0.7–1.0 power of *prey radius*. In summary, these models predict that contact probability is positively correlated with prey size over the whole prey-size range, and that phagotrophic nanoflagellates therefore should preferentially graze on large particles. The predicted strength of prey-size selection varies, however, among the analyzed models.

Investigations on the contact probabilities of rod-shaped or filamentous bacteria are rare. Contact probability has been seen as the most size-selective step in the feeding process, but recent investigations also showed that other feeding steps can be involved in the size selection of protistan feeding (Wu, Boenigk, and Hahn 2004). Even though size-selective feeding by heterotrophic nanoflagellates has already extensively been studied (e.g. Andersson, Larsson, and Hagstrom 1986; González 1996; Holen and Boraas 1991; Jürgens and DeMott, 1995; Kinner et al. 1998), the mechanisms responsible for food size selection are still debated. The diversity of theoretical models reflects this gap in our knowledge and emphasizes the need for specific investigations.

The feeding process of heterotrophic nanoflagellates can be subdivided into a sequence of feeding steps, each of which must be preceded by success of the previous one. These are as follows: 1) ‘contact’ of the prey (i.e. in the case of the investigated chryomonads contact with the sensitive region of the cell body) (Boenigk and Arndt 2000; Matz et al. 2002); 2) ‘capture’ (i.e. the long flagellum folded over the particle and pressed it against the short one) (Matz et al. 2002); 3) ‘ingestion’ (i.e. the formation of a food vacuole) (Boenigk and Arndt 2000; Matz et al. 2002); and 4) ‘digestion’. Selection may exist at any of the first three stages and digestion may be curtailed in many cases if the prey is unsuitable. The respective selection steps are ‘contact probability’ (i.e. the probability of certain particle classes contacting the flagellate in relation to their respective abundance in the environment), ‘capture efficiency’ (i.e. the proportion of particle-flagellate encounters that result in capture), and ‘ingestion efficiency’ (i.e. the fraction of captured particles that is subsequently ingested). The current study aimed to investigate the mechanisms of particle size selection during different steps of the feeding process (i.e. contact, capture, ingestion, and vacuole passage time) in two chryomonad nanoflagellate species (*Ochromonas* sp. and *Spumella* sp.). These flagellates are among the most widespread and abundant bacterivores in aquatic environments (Arndt et al. 2000), and therefore are suitable model organisms to investigate the mechanisms of size selection. We wanted to critically test the basic assumptions of the models 1) that contact probability is the selection step responsible for observed food selection and 2) that contact probability is generally positively correlated with

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prey size. Thus, differently-sized microspheres were offered as food particles, as well as a mixed bacterial community from a continuous cultivation system.

MATERIALS AND METHODS

Organisms and cultivation conditions. Two species of bacterivorous chrysomonad nanoflagellates, *Ochromonas* sp. DS (isolated from Lake Constance by D. Springmann) and *Spumella* sp. (isolated from Lake Schöhsee, Plön, by K. Jürgens), were cultured in 100-ml Erlenmeyer flasks containing WC-medium (Guillard and Lorenzen 1972) enriched with 15 mg yeast extract l⁻¹ under continuous shaking at room temperature.

Feeding and size selection of attached flagellates. Eight different sizes of fluorescent microspheres, ranging from 0.4–1.8 µm at 0.2 µm intervals, were purchased from Micromod (Rostock, Germany). Particle concentrations were determined using a flow cytometer (FacsCalibur, Beckton Dickinson). As each particle-size class consisted of particles of slightly different size (i.e. following a probability size distribution around the mean), these particles could be mixed yielding a bead suspension containing particles between roughly 0.3–2.2 µm, including the intermediate sizes at nearly equal densities. This particle mixture was offered as artificial food. The mixture of particles was added at a low (4.2×10^6 particles ml⁻¹) and a high concentration (4.2×10^7 particles ml⁻¹). As particle size distribution in the microenvironment of the flagellate (i.e. near the bottom of the cover slip) may differ from the mean particle size distribution in the medium, the prey size spectrum in the relevant water layer was analysed and used as reference for the analysis of size selection. Additionally, shifts in the size spectrum of the bead suspension over time due to sedimentation was tested. To determine the effect of sedimentation on the size distribution of microspheres, the sizes of 150 microspheres were measured near the bottom (i.e. the relevant water layer for feeding of the attached flagellates) after 0, 10, 20, and 30 min and their fractions calculated.

Observations were carried out according to Boenigk and Arndt (2000). A hole of 20-mm diam. was drilled in the bottom of a 55-mm diam. Petri dish and a cover slip was glued to the bottom. This observation chamber allowed us to use an inverted microscope (Zeiss Axiovert 200) equipped with oil immersion objectives with a high optical resolution in combination with a short distance condenser. Approximately 1 ml of flagellate culture was transferred to the center of the observation chamber and flagellates were allowed to rest for 30 min. During this time flagellates attached to the bottom cover slip and returned to normal feeding behavior. Immediately before the experiment was started, the suspension was pipetted off until a thin water film was left. Then, fresh WC-medium was added to wash off food bacteria. Depending on the bacterial concentration, the washing procedure was repeated two or three times. One attached flagellate at a time was chosen for detailed observation. About 6 ml of the microsphere suspension were added to the Petri dish. The large water volume allowed a long observation time without heating of the medium. Three individuals were observed for a defined time span: 30 min and 20 min for *Spumella* sp. at low and high food concentration, respectively; and 20 min and 10 min for *Ochromonas* sp. at low and high food concentration, respectively. Experiments were run in five replicates. All contacts, captures, and ingestions of beads were noted and feeding rates were back-calculated from the observed number of contacts, captures, and ingestions per time. Capture efficiency is defined as the fraction of particles contacted that were captured, and ingestion efficiency is defined as the fraction of captured particles that were ingested. In addition, vacuole passage times were determined for the ingested beads.

A Zeiss Axiovert 200 equipped with a 100× /1.3 oil objective was used for observation. The microscope was connected with a zoom adapter to a CCD camera (model XC-ST70CE, Hamamatsu). The output of the video camera was fed to a S-VHS recorder. The videotape analysis was carried out by using continuous and single-frame playback (Boenigk and Arndt 2000). For image analysis the particle size range was subdivided into 16 size categories, corresponding to a size difference of three pixels on the video monitor (0.11 µm).

Effect of flagellate swimming behavior and particle surface characteristics on feeding rates and size selection. Total feeding rates of swimming and attached flagellates were compared using 1-µm beads at a final concentration of 5×10^6 particles ml⁻¹. About 500 µl of flagellate culture already containing the bead suspension were transferred to the experimental chamber and covered with a cover slip resulting in a water column about 1 mm in height. This experimental setup left enough space for active movements of the flagellates, but the possibility for flagellates to swim out of the field of view was reduced. Alternately swimming and attached flagellates were recorded for 2 min and ingestions noted. Ten individuals were observed in total per replicate and the experiment was replicated 6 times. Attached flagellates were observed at a magnification of 1,000×, and swimming flagellates at 640× (*Ochromonas* sp.) and 1,000× (*Spumella* sp.). Differences in size selectivity between swimming and attached flagellates were investigated using the same general experimental system, but using a mixture of two microsphere sizes (i.e. 1 µm and 1.6 µm diam.).

In a set of additional experiments, the effect of surface fluorescent labeling of the beads on the flagellates' bacterivory was tested. Discrimination of fluorescently labeled vs. plain microspheres was determined for the ingestion rates and vacuole passage times of 1-µm beads. The setup of this experiment was the same as in the size selection experiment of attached flagellates. Experiments were initiated by adding either fluorescent or plain microspheres at a concentration of 5×10^6 particles ml⁻¹. Fifteen individual flagellates were observed for 10 min (*Ochromonas* sp.) and 20 min (*Spumella* sp.), respectively. Ingestion rates and vacuole passage times of prey particles were recorded.

Chemostat experiments. Flagellates and bacteria were cultured in a two-stage continuous cultivation system (Posch et al. 1999). The first stage (I-stage) was inoculated with a mixed bacterial community and the alga *Cryptomonas* sp. growing at a dilution rate of 0.35 d⁻¹. The alga *Cryptomonas* sp. was necessary to stimulate growth of the bacterial community, but did not affect grazing by *Ochromonas* sp. (Posch et al. 2001). A peristaltic pump (Ismatech, Switzerland) pumped bacteria and algae continuously from the first stage into the second stage vessels at a dilution rate of 0.31 d⁻¹. The second stage consisted of a control vessel with bacteria (II-stage control) and a vessel with bacteria and the flagellate *Ochromonas* sp. (II-stage flagellate). We used a WC medium with a reduced phosphorus content of 50 µg l⁻¹ for the system.

Chemostat samples were taken for six days after inoculation of the flagellates to determine the changes in bacterial cell dimensions (community level) due to grazing. Samples were fixed with Lugol's iodine (0.5% final concentration) and formaldehyde (2% f.c.), bleached by some drops of thiosulfate, stained with DAPI (2 µg ml⁻¹ f.c.), and filtered onto black polycarbonate filters (Osmonics) of 0.22-µm pore size. Images of cells were recorded using a highly sensitive CCD camera (Optronics ZVS-47EC) that was mounted on a Zeiss Axioplan microscope. Cell dimensions of approximately 400 bacteria and 100 flagellates were measured with an image analysis system (LUCIA

Table 1. Size selection coefficients (Chesson, 1983) for different particle sizes and concentrations (upper line = 4.2×10^6 particles ml^{-1} in total for all size classes; lower line = 4.2×10^7 particles ml^{-1} in total for all size classes). For each size class, three size categories as presented in Fig. 1 were pooled. The size selection coefficient indicates positive or negative selection of a particle size class in the respective step of feeding in comparison to the other size classes. $\alpha_1 = 0.16$ unselective feeding, $\alpha_1 > 0.16$ preference for this bead size (bold letters), $\alpha_1 < 0.16$ negative selection of a bead size. Values with * are significant different ($p < 0.05$) from unselective feeding (0.16).

	Feeding phase	<0.68 μm	0.68–1.01 μm	1.02–1.35 μm	1.36–1.69 μm	1.70–2.02 μm	>2.02 μm	n
<i>Spumella</i> sp.	Contact probability	0.01 \pm 0.02	0.21 \pm 0.04	0.45 \pm 0.05*	0.10 \pm 0.01*	0.04 \pm 0.03*	0.10 \pm 0.06	154
		0.13 \pm 0.02	0.19 \pm 0.04	0.35 \pm 0.09	0.13 \pm 0.04	0.07 \pm 0.02*	0.13 \pm 0.02	476
	Capture	0.06 \pm 0.06	0.20 \pm 0.05	0.20 \pm 0.07	0.24 \pm 0.03*	0.19 \pm 0.06	0.12 \pm 0.10	94
		0.06 \pm 0.03*	0.15 \pm 0.01	0.19 \pm 0.03	0.22 \pm 0.02	0.20 \pm 0.04	0.17 \pm 0.05	283
	Ingestion	0.21 \pm 0.05	0.27 \pm 0.07	0.24 \pm 0.00*	0.14 \pm 0.09	0.02 \pm 0.03	0.11 \pm 0.07	60
		0.23 \pm 0.09	0.19 \pm 0.01*	0.20 \pm 0.04	0.17 \pm 0.02	0.12 \pm 0.05	0.10 \pm 0.09	154
Clearance	0.04 \pm 0.04*	0.25 \pm 0.04	0.52 \pm 0.09*	0.12 \pm 0.05	0.01 \pm 0.02*	0.06 \pm 0.05	60	
	0.07 \pm 0.05	0.19 \pm 0.06	0.45 \pm 0.11*	0.15 \pm 0.01	0.05 \pm 0.03*	0.08 \pm 0.07	154	
<i>Ochromonas</i> sp.	Contact probability	0.14 \pm 0.03	0.19 \pm 0.04	0.36 \pm 0.07*	0.15 \pm 0.02	0.07 \pm 0.02*	0.09 \pm 0.03	250
		0.11 \pm 0.01*	0.22 \pm 0.02*	0.34 \pm 0.09	0.16 \pm 0.05	0.07 \pm 0.02*	0.10 \pm 0.05	422
	Capture	0.09 \pm 0.06	0.14 \pm 0.03	0.15 \pm 0.02	0.21 \pm 0.01*	0.22 \pm 0.03	0.19 \pm 0.01	149
		0.09 \pm 0.02*	0.11 \pm 0.05	0.14 \pm 0.06	0.23 \pm 0.05	0.19 \pm 0.02	0.23 \pm 0.04	247
	Ingestion	0.19 \pm 0.01	0.19 \pm 0.01	0.19 \pm 0.01	0.17 \pm 0.03	0.14 \pm 0.03	0.14 \pm 0.04	135
		0.21 \pm 0.01*	0.18 \pm 0.01*	0.10 \pm 0.02*	0.12 \pm 0.01*	0.23 \pm 0.03	0.16 \pm 0.02	197
Clearance	0.09 \pm 0.06	0.18 \pm 0.03	0.37 \pm 0.12	0.19 \pm 0.03	0.07 \pm 0.03*	0.09 \pm 0.03	135	
	0.08 \pm 0.05	0.18 \pm 0.10	0.32 \pm 0.09	0.20 \pm 0.08	0.08 \pm 0.01*	0.14 \pm 0.08	197	

G). Details of the image analysis are described in Posch et al. (1997).

Size selective feeding by individual flagellates on bacteria was investigated using video microscopy (as described above). As video microscopy is very time-consuming, experiments were run over a span of two days and the results were pooled. Chemostat subsamples were taken and experiments were conducted at day 5 and 6 of cultivation. For the experiment 0.5 ml of original sample with *Ochromonas* sp., taken from II-stage flagellate, was mixed with 1 ml of the II-stage control to yield a final bacterial abundance of 3×10^6 cells ml^{-1} . Samples were transferred to a Petri dish and six flagellates were observed for 5 min each. The 30-min of observation were defined as one replicate. Experiments were run in triplicate. Ingestions and cell dimensions of the ingested bacteria were recorded and analyzed.

Statistical analysis. For the statistical analysis of size selection, the 16 size categories obtained from the video analysis (one category corresponds to three pixels at the video monitor) were pooled into six size classes. As a measure of flagellate food size selectivity the Chesson index (Chesson 1983) was used. This index is a measure of food preference for a certain prey type during a feeding step and is independent of the relative abundance of the prey type:

$$\alpha_1 = \frac{I_a/C_a}{I_a/C_a + I_b/C_b + I_c/C_c + I_d/C_d + I_e/C_e + I_f/C_f}$$

where I_a is the number of handled microspheres of size class a, I_{b-f} the number of handled microspheres of the other size classes, and C_{a-f} the corresponding particle concentrations in the surrounding water. For the calculation of food selection during capture and ingestion, the Chesson coefficient was calculated from the actual number of particles handled in the previous feeding step and not from the particle concentration in the surrounding water. A value of $\alpha_1 = 0.16$ means non-selective feeding, $\alpha_1 > 0.16$ indicates preference for the bead size, and $\alpha_1 < 0.16$ means negative selection of the observed bead size. Further, a value of 0 indicates that the respective bead size has not been handled at all and a value of 1 indicates that only this

bead size class has been handled and that no particles of the other size classes were handled.

The overall effect of food particle size and food concentration on selectivity was tested using a two-way ANOVA. Selection within a distinct feeding step was tested using one-way ANOVA and subsequent Tukey test. In addition, the selectivity indices were tested against non-selectivity (0.16) using the *t*-test. Differences in ingestion rates of swimming and attached flagellates, as well as the effect of particle staining were statistically tested using the *t*-test. All tests were run using the software packages SigmaStat (version 2.0) and Statistica (version 6.1).

RESULTS

Selection of fluorescent and plain microspheres. The surface characteristics of artificial food particles are likely to play a major role in the selectivity of protists. In our study, the influence of fluorescently labeled and plain microspheres on the selective feeding by *Ochromonas* sp. and *Spumella* sp. was investigated to exclude artifacts due to particle surface characteristics. Contact probabilities, capture efficiency, ingestion efficiency, and vacuole processing times were not significantly different for fluorescently labeled and plain microspheres ($p > 0.05$ in all cases) (data not shown).

Size selection experiments. Over the whole prey size range, the flagellate *Ochromonas* sp. showed a total ingestion rate of 26.8 ± 2.6 particles h^{-1} cell $^{-1}$ (clearance rate of 6.4 ± 0.6 nl h^{-1}) and of 78.4 ± 7.7 particles h^{-1} cell $^{-1}$ (clearance rate of 1.9 ± 0.2 nl h^{-1}) at low and high food concentrations, respectively. For *Spumella* sp., ingestion rates were 7.4 ± 1.2 and 28.9 ± 2.7 particles h^{-1} cell $^{-1}$ and clearance rates were 1.8 ± 0.3 and 0.7 ± 0.06 nl h^{-1} for low and high food concentrations, respectively.

Both flagellates were generally selective for intermediate-sized particles during all investigated selection steps (i.e. contact probability, capture efficiency, and ingestion efficiency) (bold font Table 1, Fig. 1). Selectivity was generally stronger in *Spumella* sp. than in *Ochromonas* sp. Food size was a significant factor explaining food selection (two-way ANOVA, p

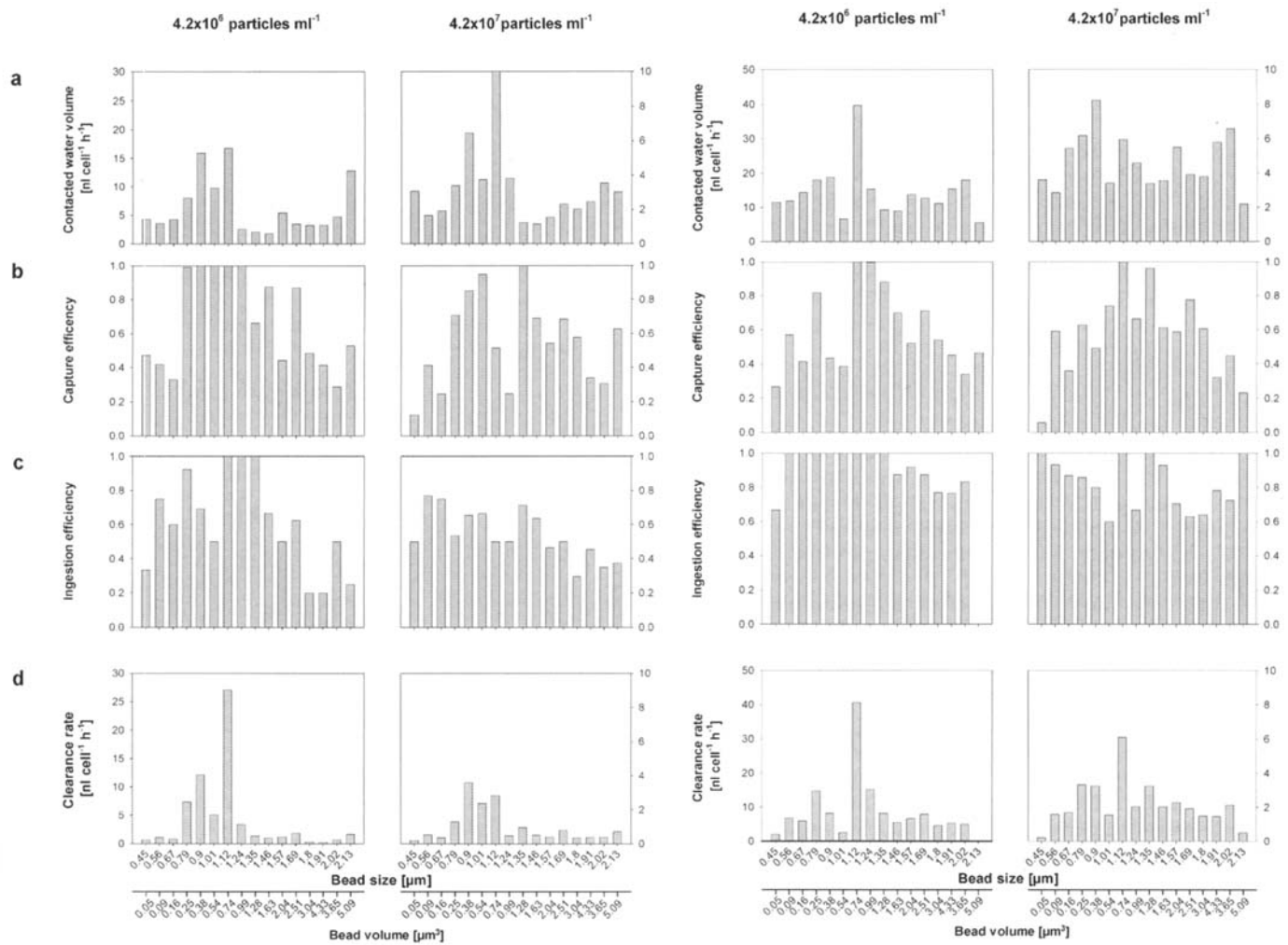


Fig. 1. Size selection of *Spumella* sp. and *Ochromonas* sp. during the different steps of feeding. **a)** Contacted water volume: Water volume in contact with the flagellate was back-calculated from observed contacts and particle concentration. The contacted water volume corresponds to the clearance rate, but refers to the contacted instead of ingested particles. **b)** Capture efficiency. **c)** Ingestion efficiency. **d)** Clearance rate (i.e. overall selection). Capture efficiency and ingestion efficiency are calculated from observed contacts, captures and ingestions. Note that capture efficiency and ingestion efficiency are probabilities and are therefore reported as values between 0 and 1. The volume contacted and the clearance rate refer to the processed water volume and are therefore given in nl. A corresponding probability cannot be calculated for these values, but the reported volumes permit comparison between different-sized particles as well.

< 0.001 in both species), whereas food concentration had no effect on food selection (two-way ANOVA, $p = 0.968$ for *Ochromonas* sp., $p = 0.971$ for *Spumella* sp.). However, there was a trend that the mean ingested particle size was positively correlated with the cell size of the individual flagellates for *Ochromonas* sp. (Spearman rank coefficient: 0.133, $p = 0.0126$, $n = 353$) whereas this was not significant for *Spumella* sp. (Spearman rank coefficient: 0.033, $p = 0.599$, $n = 250$).

In contrast to the assumptions of the current models, contact probability was not positively correlated with the prey size (Spearman rank correlation: $p > 0.05$ for both species at both food concentrations). Both species contacted preferentially intermediate beads of 0.68–1.35 μm diam. (Fig. 1). Accordingly, selection was positive even though only significant for *Ochromonas* and *Spumella* for 1.02–1.35 μm beads at the low prey concentration ($p = 0.039$ for *Ochromonas*, $p = 0.011$ for *Spumella*, Table 1) and for *Ochromonas* for 0.68–1.01 μm beads at the high prey concentration ($p = 0.035$, Table 1).

In the second phase of feeding (i.e. capturing of the beads of a particular size range that had been contacted), generally the intermediate sizes were captured more efficiently than the small and large beads (Fig. 1). Accordingly, selection of *Spumella* sp. was highest for particles of 1.36–1.69 μm for low and high particle concentrations ($p = 0.038$ for low particle concentration, $p = 0.052$ for high particle concentration), respectively. *Ochromonas* sp. showed a higher preference for larger beads of 1.36–> 2.00 μm , but also in this case selection was only significant for the capture of intermediate particles from 1.36–1.69 μm at low particle concentration ($p = 0.011$). Nearly all of the captured particles were ingested by *Ochromonas* sp. (Fig. 1). Only for *Spumella* sp. again intermediate beads were slightly positively selected (Table 1, Fig. 1).

Ingested latex beads were egested by *Spumella* sp. after 108 ± 45 and 113 ± 46 sec while *Ochromonas* sp. retained the beads for 592 ± 194 and 431 ± 108 sec in vacuoles at a low and high particle concentration, respectively. Differential di-

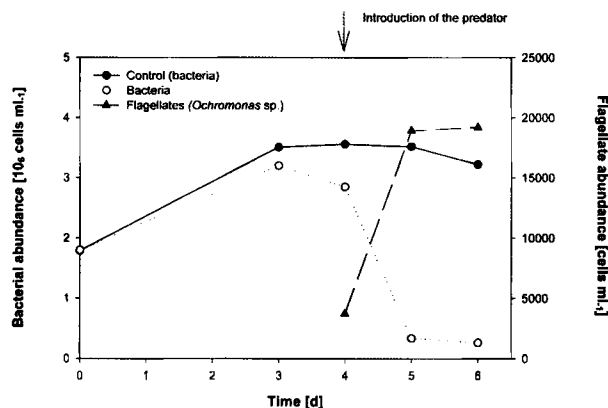


Fig. 2. Abundances of *Ochromonas* sp. and bacteria (control and grazed bacteria) in the chemostat vessels during 6 days of cultivation.

gestion was independent of particle size. For *Ochromonas* sp. the time budget of the feeding process was, however, affected by food concentration, as vacuole passage time was significantly shorter at a high particle concentration ($p = 0.01$). For *Spumella* sp. particle concentration had no effect on vacuole passage time ($p = 0.45$).

Feeding rates and size selection by swimming and attached flagellates. In both species, flagellates precultured at high food concentrations generally tended to attach within minutes, whereas flagellates precultured at low food concentrations hardly attached at all. The tendency to avoid attachment was extreme in starved *Ochromonas* sp. from the continuous culture system with a bacterial background concentration of around 5×10^5 bacteria ml^{-1} . Less than 5% of the cells attached, whereas nearly all of the cells attached during the first days of the experiment when bacterial food concentration was sufficiently high.

Spumella sp. tended to spin around in circles with a small diam. while *Ochromonas* sp. moved in large circles and straight ahead. The rate of successful captures was generally higher for the swimming *Ochromonas* sp. (54 ± 11.2 particles $\text{cell}^{-1} \text{h}^{-1}$) as compared to *Spumella* (22.8 ± 11.5 particles $\text{cell}^{-1} \text{h}^{-1}$). When swimming, ingestion rates were 25.2 ± 11.5 particles $\text{cell}^{-1} \text{h}^{-1}$ for *Ochromonas* sp. and 10.8 ± 6.6 particles $\text{cell}^{-1} \text{h}^{-1}$ for *Spumella* sp. Capture was significantly higher for attached as compared to swimming *Ochromonas* sp. and *Spumella* sp. ($p < 0.001$ for *Ochromonas*; $p < 0.001$ for *Spumella*), whereas ingestion rates of attached and swimming flagellates were similar for both, *Ochromonas* and *Spumella* ($p = 0.735$ for *Ochromonas*; $p = 0.115$ for *Spumella*). Attached *Ochromonas* sp. captured 129.6 ± 19.7 particles $\text{cell}^{-1} \text{h}^{-1}$ and ingested 27.6 ± 10.0 particles $\text{cell}^{-1} \text{h}^{-1}$ while attached *Spumella* sp. captured 92.4 ± 26.7 particles $\text{cell}^{-1} \text{h}^{-1}$ and ingested 22.8 ± 13.7 particles $\text{cell}^{-1} \text{h}^{-1}$. Size selection during capture, as well as during ingestion, was not significantly different between attached and swimming flagellates ($p = 0.943$ and $p = 0.819$ for *Ochromonas* sp.; $p = 1.000$ and $p = 0.249$ for *Spumella* sp.).

Size selective feeding on bacteria. Bacterial abundance in the continuous cultivation system increased slightly before the introduction of the predator and remained stable during the experiment in the II-stage control vessel without flagellates. Bacterial abundance was $3.3 \pm 0.7 \times 10^6$ cells ml^{-1} at a phosphorus content of $50 \mu\text{g l}^{-1}$. The inoculation of the predator *Ochromonas* sp. resulted in a decrease of bacterial abundance to a minimum abundance of 2.3×10^5 cells ml^{-1} (Fig. 2).

The initial mean cell volume of the flagellates was $124 \mu\text{m}^3$ and decreased within 1 day to $56 \mu\text{m}^3$ due to starvation. Bac-

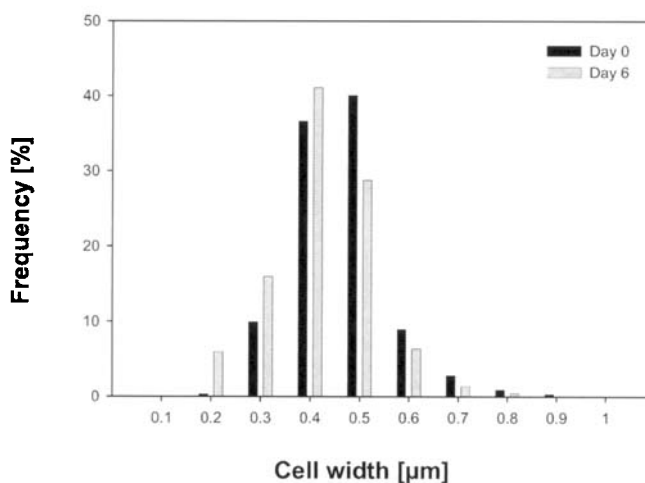
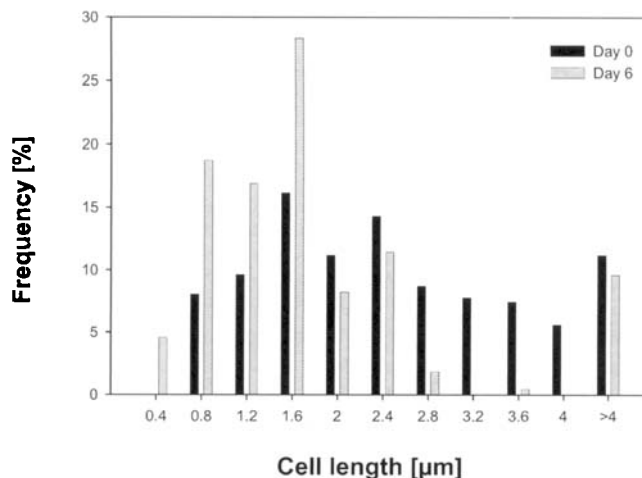


Fig. 3. Frequency distribution of bacterial cell dimensions in the continuous cultivation system at days 0 and 6 after inoculation of the predator *Ochromonas* sp. at a phosphorus concentration of $50 \mu\text{g P l}^{-1}$. Note that the bacterial size distribution shifts to smaller lengths and widths.

terial cell volumes of the II-stage control vessel were stable during the 6 days of cultivation. Mean bacterial cell volume (MCV_B) of the II-stage control was $0.3 \pm 0.013 \mu\text{m}^3$. In contrast, MCV_B in the II-stage flagellate vessel decreased from 0.31 to a minimum value of $0.16 \mu\text{m}^3$ after introduction of the flagellate. Mean bacterial cell widths decreased from 0.41 to $0.36 \mu\text{m}$ and mean bacterial cell lengths from 2.39 to $1.58 \mu\text{m}$ (Fig. 3).

For the investigation of size selection, flagellates were exposed to bacteria from the II-stage control treatment (final food concentration 3×10^6 bacteria ml^{-1}) and inspected video-microscopically. Ingestion rates of *Ochromonas* sp. were 31.7 ± 1.5 bacteria $\text{cell}^{-1} \text{h}^{-1}$. The size spectrum of ingested bacteria was similar to the size spectrum of the background bacteria (Fig. 4), and therefore no size-selective feeding by *Ochromonas* sp. could be detected as all size classes of bacteria were ingested in rates corresponding to the background bacteria ($p > 0.05$, Kolmogorov-Smirnov test).

DISCUSSION

Size selection is not actively regulated by the flagellate. The measured size-dependent differences in particle uptake in

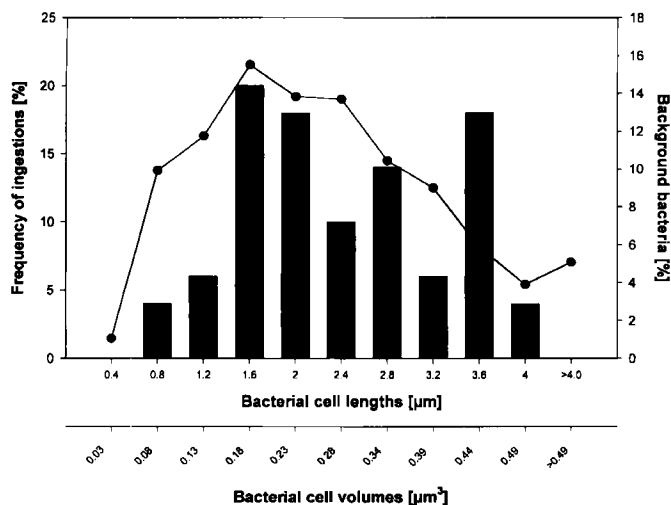


Fig. 4. Frequency distribution of size classes of bacteria in the size selection experiment (dots) and corresponding size-specific frequency of ingestions of *Ochromonas* sp. (bars). Corresponding bacterial cell volume was calculated from cell length assuming cylinders with half-spheres at both ends and a mean cell width of 0.4 µm. All size classes of bacteria were ingested in rates corresponding to the background bacteria (Kolmogorov-Smirnov test).

our experiments confirm that *Ochromonas* sp., as well as *Spumella* sp., have a food-size preference for particles between 0.9 µm and 1.2 µm (Andersson, Larsson, and Hagstrom 1986; Chrzanowski and Šimek 1990; Kinner et al 1998). According to optimal foraging theory, selection for food quality should be observed at high food concentrations, whereas selectivity should decrease at low food concentrations (e.g. Stephens and Krebs 1986). Accordingly, food concentration-dependent selectivity for *Ochromonas* sp. and *Spumella* sp. has been described in several studies (e.g. Boenigk et al. 2002; Holen and Boraas 1991; Jürgens and DeMott 1995). In contrast to selection for food quality, our data indicated that size-selective feeding was present at both low and high food concentrations and was mostly independent of particle concentration. Size selection of *Ochromonas* and *Spumella* seems to be mainly due to both, morphological limitations and threshold values and is therefore interpreted as passive selection (i.e. not actively regulated by the flagellate).

Ochromonas is generally a less efficient feeder of small bacteria as compared to larger bacteria (this study, Boenigk et al., in press; Posch et al. 1999). This is also supported by the initial decrease of bacterial size in our continuous cultivation system after inoculation of the flagellate. Regarding the realized bacterial size classes in the chemostat, however, clearance was similar and even the smallest bacteria (0.03 to 0.2 µm³) were ingested by *Ochromonas* sp. Accordingly, we found no strong size selection for beads. The low food concentrations in the chemostat probably led to starvation of the flagellates and consequently a decrease in flagellate cell vol. (from 124 to 56 µm³). When the size-selection experiment using organisms from the chemostat was started, flagellates were starved and able to feed at high rates even on the smallest bacteria. We conclude that starvation leads to a decrease in flagellate cell size, and as a result, small bacteria are now an edible size class for the predator. The food size optimum therefore shifts in relation to the flagellates' size, but is not actively regulated. This conclusion is also supported by a positive correlation between the flagellates size and the mean size of ingested particles. Ontogenetic

diet shifts as they have been reported for metazoans must therefore also be expected in flagellates.

Contact probability is not generally positively correlated with prey size. Mathematical models predict that contact probability increases with size and motility of the particles and subsequently grazing rates are expected to be positively correlated with prey size up to a certain size limit (Fenchel 1984; González 1996; Monger and Landry 1991; Shimeta and Jumars 1991). According to Fenchel (1984), a critical flow line exists that transports particles to the equator of the cell where they will be intercepted. These flow lines may be modified by viscous eddies as reported for choanoflagellates (Pettitt et al. 2002). We did, however, not observe deviance from smooth flow lines for the investigated chrysoomonads, but the current-field proceeds differently than assumed by Fenchel (1984) (see also Boenigk and Arndt 2000). Fenchel (1984) suggested a simple model for spherical grazers (radius R) feeding on particles (radius r) where the water flow delimited by the critical flow line corresponds to $2\pi Rr$, so that the specific clearance becomes $3/2R^{-2}r$. Van der Waals forces, particle rotation that may force the particles in a velocity gradient to migrate towards a surface, and to a lesser extent, Brownian movements may alter this model and lead to the interception of particles somewhat outside the critical flow lines. Our observations indicate that contrary to a basic assumption of this model, particles are captured in front of the cell as described by Sleigh (1964) and not at the equator as suggested by Fenchel (1984). Streamline compression immediately adjacent to the cell seems to be negligible because of gravitational and diffusional depositions (Shimeta and Jumars 1991). Shimeta and Jumars (1991) used the same equation as Fenchel (1984), but particles were assumed to fall directly on the flagellate. Therefore, the radius of the prey plays a minor role in their model.

Our data do not support, however, the generally positive correlation between prey size and contact probability as calculated by the models of Fenchel (1984) and Shimeta and Jumars (1991). A more likely model has been presented by González (1996) who predicted that the specific clearance rate should be proportional to prey volume. This model suggests a linear increase of the clearance rate with prey volume until a limit is reached at which clearance rates for large particles remain constant. In fact, our results clearly show that the contact probability is highest for intermediate-sized particles ranging from 0.9–1.2 µm. It seems that the contact probability increases up to a certain size limit, and then decreases again. Our results indicate that the feeding current does not catch large particles efficiently. Thus, they are transported to the flagellate cell at lower rates. However, none of the models can be rejected with absolute confidence, as they seem to fit the observed feeding behavior on small particles. As most models were developed using data on overall selection (i.e. food availability vs. ingested particles), it is not surprising that the models fit quite well to observed selection at least for small particles. The basic mechanistic assumptions must, however, be revised, and biological, as well as physical parameters, must be considered.

Selection steps other than contact probability play a crucial role in size selection. Contact probability has been regarded as the decisive feeding step for size selection (Fenchel 1984; González 1996; Monger and Landry 1991; Shimeta and Jumars 1991). Size selection also occurs during other steps of the feeding process. Our results indicate that size selection also occurs during capture and ingestion in the narrow sense. The preference of intermediate-sized particles during capture might be explained by the basic mechanical stimulus of the predator-prey contact subsequently inducing prey capture: Capture seems to be induced by mechanical stimuli: food particles are captured

after contacting the sensitive region of the cell, if a threshold value is reached. The threshold may be proportional to momentum and drag (i.e. mass and speed of the particle). Small particles do not or only rarely reach this threshold value, and are therefore captured at lower rates or not at all. Large microspheres reach this threshold, but were also not preferred (i.e. they frequently failed to induce a capture reaction by the flagellate). This indicates that there is also an upper threshold value above which the mechanical signal of the predator-prey contact is not interpreted as a contact with a prey organism but possibly as a contact with a non-food particle or even a predator, consequently a capture reaction is not triggered. In addition, it seems to be increasingly problematic for the flagellate to envelop large particles with the flagellum: capture and retention of large particles is probably more difficult than for small ones due to greater drag. Even though morphological limitations of the feeding process surely play a role in the negative selection of larger prey, we assume that negative selection is not solely due to morphological limitations but also due to the increasing contact strength, i.e. momentum, of the predator-prey contact.

The ingestion of captured particles seems to be less important with respect to food size selection for *Ochromonas* sp., which ingests nearly all of the captured particles. This may be an experimental artifact since only "optimal" food particles were offered. This was obviously not the case for *Spumella* sp. Small food particles may not cover the requirements of the flagellates and for this reason they may be ingested at lower rates, while large particles may be refused because of morphological limitations of food vacuole formation (Fenchel 1987).

Size selection depends on cell length, cell volume, and morphology. The appropriate size measure for size selection is currently debated (González 1996; Posch et al. 2001; Shimeta and Jumars 1991). Our data provide some new insights in the selection process and the relevant size measure.

Contact probabilities for spherical particles increased up to a certain size limit and then decreased again. For bacteria, we found no strong selection between the size classes present in the chemostat system. Regarding the prey volume, all bacteria in the chemostat system were at the lower end of the range of tested prey sizes, but with respect to cell length the bacteria covered the size range tested with beads. The comparison of size selection using beads and bacteria should therefore permit some conclusions on the relevant measure for size selection.

Wu, Boenigk, and Hahn (2004) reported no significant difference in **contact probability** between rod-shaped medium-sized bacteria and filamentous bacteria. In contrast to the spherical beads, the bacteria did not only differ in size, but also in morphology (i.e. the relative proportions of cell length to cell width). Neither length nor volume alone seem to fully explain observed contact probabilities, and we therefore suspect that, besides particle size in terms of length or volume, the morphology and shape played a crucial role in determining contact probability. The partitioning of the cell mass on the (filamentous) bacteria may significantly alter their susceptibility to the feeding current and consequently contact probability (Wu, Boenigk, and Hahn 2004).

Capture seemed to be triggered whenever a threshold contact momentum was reached. Small microspheres came in contact with the cell body, but did not initiate the capture reaction. Interestingly, similar observations were made by Wu, Boenigk, and Hahn (2004) for filamentous bacteria. Even though the mass of filamentous bacteria should have been high enough to initiate the capture reaction, it did not. This finding strengthens our hypothesis that momentum, or a combination of momentum and drag, not mass or length of the prey particle is responsible

for triggering capture. In filaments, the mass is distributed along the entire filament and the momentum may, therefore, not be accurately assessed by the flagellate so that the threshold value is not reached. For large beads, however, capture efficiency decreases again. This indicates that not only a lower limit, but also an upper limit exists. Particles inducing a stronger momentum may not be interpreted as prey, but as unusable or even as a predator and induce different reactions.

Within the investigated size range of microspheres and bacteria, size seemed to play a minor role for selection during ingestion in the narrow sense. For large and complex prey items, decreasing ingestion efficiency must, however, be assumed. It seems that during food vacuole formation (i.e. ingestion, the cell volume is limiting for spherical particles and the cell length for filamentous particles). The study of Wu, Boenigk, and Hahn (2004) indicated, however, that even larger bacteria can be successfully ingested.

Attachment increases capture rate, but does not change patterns of size selection. Fenchel (1986) suggested that attached flagellates create a large scale flow around themselves with their flagella, while during swimming the flagella only create small, local velocity fields. Subsequently, attachment should increase the contact rate of food particles. Recently, Christensen-Daalsgaard and Fenchel (2003) demonstrated that the fluid velocity in fact is higher for attached than for swimming flagellates. In agreement with these studies, contact rate was much higher for attached than for swimming flagellates in our experiments. In contrast, ingestion rates were not significantly different between swimming and attached flagellates. This might be due to the relatively high food concentration used in the experiments and reflect satiation. Irrespective of the similar ingestion rates, our results support the idea that attachment maximizes the contact rate.

Attachment might therefore be seen as an adaptation to low food concentrations. But in contrast to this assumption, mobility actually increased with decreasing food concentrations. Higher motility and a higher fraction of actively swimming cells have been reported at low prey levels (e.g. Boenigk and Arndt 2002; Christensen-Daalsgaard and Fenchel 2003; Fenchel 1982). These observations are consistent with our observations in the continuous cultivation system that *Ochromonas* tended to attach at high food concentration, but did not do so at a low food concentration. When food conditions become worse, mobility allows the flagellates to swim and search for more suitable microenvironments (Boenigk and Arndt 2002). Attachment must therefore be regarded as a mechanism to generally increase feeding rate, but cannot be regarded as an adaptation to low food conditions or starvation.

The optimal prey size may shift towards smaller prey when the flagellates' cell size decreases due to starvation. As starved flagellates tended to swim more actively than satiated cells this may have caused shifts in prey size selection between swimming and attached flagellates. We found, however, no principle difference in the patterns of size selection between swimming and attached flagellates. Even though optimal prey size depends on the flagellates' cell size and may systematically be linked to motility, we assume that the basic pattern of size selection holds true for both swimming and attached flagellates.

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LITERATURE CITED

- Andersson, A., Larsson, U. & Hagstrom, A. 1986. Size-selective grazing by a microflagellate on pelagic bacteria. *Mar. Ecol. Prog. Ser.*, **33**:51–57.
- Arndt, H., Dietrich, D., Auer, B., Cleven, E. J., Gräfenhan, T., Weitere, M. & Mylnikov, A. P. 2000. Functional diversity of heterotrophic flagellates in aquatic ecosystems. In: Leadbeater, B. S. C. & Green, J. C. (ed.), *The Flagellates*. Taylor & Francis, London. p. 240–268.
- Boenigk, J. & Arndt, H. 2000. Particle handling during interception feeding by four species of heterotrophic nanoflagellates. *J. Eukaryot. Microbiol.*, **47**:350–358.
- Boenigk, J. & Arndt, H. 2002. Bacterivory by heterotrophic flagellates: community structure and feeding strategies. *Anthony van Leeuwenhoek*, **81**:465–480.
- Boenigk, J., Matz, C., Jürgens, K. & Arndt, H. 2002. Food concentration-dependent regulation of food selectivity of interception-feeding bacterivorous nanoflagellates. *Aquat. Microb. Ecol.*, **27**:195–202.
- Boenigk, J., Stadler, P., Wiedroither, A. & Hahn, M. W. 2004. Microbial microdiversity: strain-specific differences in the grazing sensitivity of closely related ultramicrobacteria affiliated with the Polynucleobacter cluster. *Appl. Environ. Microbiol.*, **70**:5787–5793.
- Chesson, J. 1983. The estimation and analysis of preference and its relationship to foraging models. *Ecology*, **64**:1297–1304.
- Christensen-Dalsgaard, K. K. & Fenchel, T. 2003. Increased filtration efficiency of attached compared to free-swimming flagellates. *Aquat. Microb. Ecol.*, **33**:77–86.
- Chrzanowski, T. H. & Šimek, K. 1990. Prey-size selection by freshwater flagellated protozoa. *Limnol. Oceanogr.*, **35**:1429–1436.
- Fenchel, T. 1982. Ecology of heterotrophic microflagellates, III. Adaptations to heterogeneous environments. *Mar. Ecol. Prog. Ser.*, **9**:25–33.
- Fenchel, T. 1984. Suspended marine bacteria as a food source. In: Fasham, M. J. (ed.), *Flow of Material and Energy in Marine Ecosystems*. Plenum Press, New York. p. 301–315.
- Fenchel, T. 1986. The ecology of heterotrophic microflagellates. *Adv. Microb. Ecol.*, **9**:57–97.
- Fenchel, T. 1987. Ecology of Protozoa. *The Biology of Free-Living Phagotrophic Protists*. Springer Verlag, New York.
- González, J. M. 1996. Efficient size-selective bacterivory by phagotrophic nanoflagellates in aquatic ecosystems. *Mar. Biol.*, **126**:785–789.
- Guillard, R. R. L. & Lorenzen, C. J. 1972. Yellow-green algae with chlorophyllide C. *J. Phycol.*, **8**:10–14.
- Hahn, M. W. & Höfle, M. G. 1999. Flagellate predation on a bacterial model community: interplay of size-selective grazing, specific bacterial cell size, and bacterial community composition. *Appl. Environ. Microbiol.*, **65**:4863–4872.
- Hahn, M. W. & Höfle, M. G. 2000. Role of microcolony formation in the protistan grazing defense of the aquatic bacterium *Pseudomonas* sp. MWH1. *Microb. Ecol.*, **39**:175–185.
- Hahn, M. W., Moore, E. R. B. & Höfle, M. G. 1999. Bacterial filament formation, a defense mechanism against flagellate grazing, is growth rate controlled in bacteria of different phyla. *Appl. Environ. Microbiol.*, **65**:25–35.
- Holen, D. A. & Boraas, E. 1991. The feeding behavior of *Spumella* sp. as a function of particle size: implications for bacterial size in pelagic systems. *Hydrobiologia*, **220**:73–88.
- Jürgens, K. & DeMott, W. R. 1995. Behavioral flexibility in prey selection by bacterivorous nanoflagellates. *Limnol. Oceanogr.*, **40**:1503–1507.
- Kinner, N. E., Harvey, R. W., Blakeslee, K., Novarino, G. & Meeker, L. D. 1998. Size-selective predation on groundwater bacteria by nanoflagellates in an organic-contaminated aquifer. *Appl. Environ. Microbiol.*, **64**:618–625.
- Matz, C., Boenigk, J., Arndt, H. & Jürgens, K. 2002. Role of bacterial phenotypic traits in selective feeding of the heterotrophic nanoflagellate *Spumella* sp. *Aquat. Microb. Ecol.*, **27**:137–148.
- Monger, B. C. & Landry, M. R. 1991. Prey-size dependency of grazing by free-living marine flagellates. *Mar. Ecol. Prog. Ser.*, **74**:239–248.
- Pettitt, M. E., Orme, B. A. A., Blake, J. R. & Leadbeater, B. S. C. 2002. The hydrodynamics of filter feeding in choanoflagellates. *Europ. J. Protistol.*, **38**:313–332.
- Posch, T., Pernthaler, J., Alfreider, A. & Psenner, R. 1997. Cell-specific respiratory activity of aquatic bacteria studied with the tetrazolium reduction method, cyto-clear slides, and image analysis. *Appl. Environ. Microbiol.*, **63**:867–873.
- Posch, T., Jezbera, J., Vrba, J., Šimek, K., Pernthaler, J., Andreatta, S. & Sonntag, B. 2001. Size selective feeding in *Cyclidium glaucoma* (Ciliophora, Scuticociliatida) and its effects on bacterial community structure: a study from a continuous cultivation system. *Microb. Ecol.*, **42**:217–227.
- Posch, T., Šimek, K., Vrba, J., Pernthaler, J., Nedoma, J., Sattler, B., Sonntag, B. & Psenner, R. 1999. Predator-induced changes of bacterial size-structure and productivity studied on an experimental microbial community. *Aquat. Microb. Ecol.*, **18**:235–246.
- Shimeta, J. & Jumars, P. A. 1991. Physical mechanisms and rates of particle capture by suspension feeders. *Oceanogr. Mar. Biol. Annu. Rev.*, **29**:191–257.
- Šimek, K. & Chrzanowski, T. H. 1992. Direct and indirect evidence of size-selective grazing on pelagic bacteria by freshwater nanoflagellates. *Appl. Environ. Microbiol.*, **58**:3715–3720.
- Sleigh, M. A. 1964. Flagellar movement of the sessile flagellates *Actinomonas*, *Codonosiga*, *Monas* and *Poteriodendron*. *Q. J. Microsc. Sci.*, **105**:405–414.
- Stephens, D. W. & Krebs, J. R. 1986. *Foraging Theory*. Princeton University Press, New Jersey.
- Wu, Q. L., Boenigk, J. & Hahn, M. W. 2004. Successful predation by a nanoflagellate on filamentous bacteria challenges current models on flagellate bacterivory. *Appl. Environ. Microbiol.*, **70**:332–339.

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