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NOTE

Food concentration-dependent regulation of food selectivity of interception-feeding bacterivorous nanoflagellates

Jens Boenigk[1,*], Carsten Matz[2], Klaus Jürgens[2], Hartmut Arndt[3]

[1]Austrian Academy of Sciences, Institute for Limnology, Mondseestr. 9, 5310 Mondsee, Austria
[2]Max Planck Institute for Limnology, Department of Physiological Ecology, 24306 Plön, Germany
[3]Department of General Ecology and Limnology, Zoological Institute, University of Cologne, 50923 Cologne, Germany

ABSTRACT: The significance of food concentration for selectivity was analyzed by video microscopy for 3 species of interception-feeding bacterivorous nanoflagellates of the genera *Spumella*, *Ochromonas* and *Cafeteria*. Inert beads and live bacteria were offered simultaneously at 5 different concentrations. The fate of individual prey particles was recorded during the stages of the particle-flagellate interaction: capture, ingestion, digestion and egestion. The experiments revealed passive and active selection mechanisms that were regulated separately. Selective food uptake depended strongly on food concentration, whereas differential digestion was independent of the food concentration and independent of the number of previously ingested food particles. In addition to active selection of food items, passive selection occurred due to the different contact probabilities of prey. In contrast to the chrysomonads *Spumella* sp. and *Ochromonas* sp., the bicosoecid *Cafeteria* sp. showed no significant active selection, neither during food uptake nor during digestion. The results imply that it is more efficient for some interception-feeding flagellates to feed unselectively all particles that can be morphologically ingested and then to attempt to digest these particles. Active selection in advance may only be efficient when the particle concentration is sufficiently high such that vacuole formation becomes time limiting.

KEY WORDS: Ecology · Feeding process · Grazing · Microbial food web · Optimal foraging · Prey handling · Protists

In most aquatic ecosysytems, heterotrophic nanoflagellates (HNF) are among the most important bacterivorous organisms (Sanders et al. 1992, Arndt et al. 2000). Fluctuations in the bacterial community under grazing pressure including shifts in the species composition and morphological shifts within species have been demonstrated (Jürgens et al. 1999, Posch et al. 1999, Hahn & Höfle 2001). The responsible mechanisms behind these findings of selection became the focus of interest in recent years (Verity 1991, Dolan & Šimek 1998, Boenigk et al. 2001c). Besides HNF-mediated changes in the prey growth rate, viral lysis (Wommack & Colwell 2000) and feed back mechanisms on the bacterial level (e.g. Hahn et al. 1999, Hahn & Höfle 2001), selective feeding by bacterivorous and herbivorous protists is recognized as an important mechanism for the structuring of planktonic food webs (e.g. González et al. 1990b, Epstein & Shiaris 1992, Šimek et al. 1999). Size-selective feeding of HNF has been discussed as a major mechanism that shifts the size structure of bacterial communities (González et al. 1990b, Chrzanowski & Šimek 1993). Higher contact probability for larger particles has been assumed as a main reason for these findings (Fenchel 1987). Other properties of picoplankton besides size, such as motility (e.g. González et al. 1993) and hydrophobicity (Monger et al. 1999), can also mediate protist food selection. These factors probably alter contact probability between predator and prey. It is unclear to which extent these factors are, in addition, subject to active selection processes. However, chemical stimuli have been discussed to induce food uptake (Nisbet 1987) and may play a role in feeding and food selection (e.g. Hansen 1998, John & Davidson 2001).

Grazing experiments with HNF using bacteria and latex beads suggested that selectivity depended strongly on the food concentration and on the physiological state of the grazers (Jürgens & DeMott 1995): protists became more selective under satiating food conditions and then preferred bacteria over beads, whereas starved flagellates showed no selective feeding behavior. These findings were based on 'classical' grazing experiments, which quantify the number of tracer particles in the food vacuole of fixed flagellates after distinct time steps. However, such experiments do not give insight into the responsible selection mechanisms. As passive selection mechanisms (such as con-

*E-mail: jens.boenigk@oeaw.ac.at

tact probability and morphological limitations of the feeding structures) should be more or less independent of the food concentration, active selection mechanisms should be responsible for concentration-dependent shifts in selectivity.

Recent studies found that such active selection mechanisms play an important role in protozoan food selection: active selection has been reported during particle processing and by differential digestion (Nisbet 1987, Boenigk et al. 2001b). Chemotactic orientation (Fenchel & Blackburn 1999) may also alter the prey spectrum. As differential digestion seems to be relatively independent of the satiation of the flagellates (Boenigk et al. 2001c), selection during food processing (particle handling, ingestion, etc.) must be mainly responsible for the observed concentration-dependent shifts in food selection. This would suggest a receptor-mediated uptake mechanism that becomes active at higher particle concentrations. For our studies we used 3 interception-feeding species for which the principle mechanisms of feeding are relatively well investigated (*Spumella* sp.: e.g. Holen & Boraas 1991, Zwart & Darbyshire 1992; *Ochromonas* sp.: e.g. Aaronson 1974, Boraas et al. 1992; *Cafeteria* sp.: e.g. Ishigaki & Terazaki 1998). Interception feeding is a widespread and common feeding type in aquatic ecosystems (e.g. Fenchel 1987, Arndt et al. 2000). In continuation of former studies focussing on principle feeding mechanisms of these species and on the effect of physical and chemical prey characteristics (Boenigk & Arndt 2000b, Boenigk et al. 2001c), respectively, we now concentrated on the regulating effect of food concentration. The intention of this study was to investigate the principle ability of flagellates to adapt their selectivity to food concentration and to address the question of why such a phenomenon occurs. Feeding steps that may be actively regulated, i.e. the ingestion sensu stricto and prey processing in the food vacuole, were the focus of the investigations. Therefore, we studied the food capture and selectivity of bacterivorous nanoflagellates feeding on a mixture of 'high quality food' (*Pseudomonas putida* MM1) and inert latex beads. It has been suggested that selectivity should occur especially at high food concentrations (Jürgens & DeMott 1995). Thus, we tested different non-satiating and satiating food concentrations. Even though the obtained results cannot be directly applied to the interpretation of field experiments, we provide evidence for the principle mechanisms involved in food selection and their dependency on food concentration.

Materials and methods. *Ochromonas* sp. DS originated from Lake Constance, *Spumella* sp. from a mesotrophic lake in Plön and *Cafeteria roenbergensis* from the Baltic Sea at a salinity of 10‰ near Kloster (Hiddensee, Germany). As good quality food we chose the bacterium *Pseudomonas putida* MM1 (isolated by Christoffersen et al. [1997] from barley rhizosphere). *P. putida* is quite a large bacterium (0.61 ± 0.41 µm^3) compared to natural bacteria but within the size range of optimal prey size for the investigated flagellates (Matz & Jürgens 2001). It proved to be relatively good food, allowing for high ingestion and growth rates of the flagellates (Christoffersen et al. 1997, Boenigk & Arndt 2000b, M. W. Hahn pers. comm.). All 4 organisms have been used in previous studies and the general feeding behavior of the flagellates is known.

All organisms were cultivated in WC medium (Guillard & Lorenzen 1972) at 17°C and under constant light. *Cafeteria roenbergensis* was cultivated at a salinity of 3.5‰, which was obtained by adding NaCl to the basic medium. In order to reach high bacterial abundances, 100 mg glucose l^{-1} was added for cultivation of *Pseudomonas putida* MM1. For the experiments, bacteria in early stationary phase were used. Flagellates were pre-cultured at a food concentration of at least 5×10^6 bacteria ml^{-1}.

The experimental set-up followed generally the protocol by Boenigk & Arndt (2000a). Briefly, an aliquot of flagellate culture was transferred to a petri dish. Subsequently the food concentration was adjusted to 0.25×10^7 (0.75×10^7, 1.5×10^7, 2.5×10^7, 3.3×10^7) bacteria ml^{-1} by carefully adding the desired particle suspension. For the experiments with the lowest food concentration, the medium was carefully exchanged for fresh medium beforehand (see Boenigk & Arndt 2000b for details). Thirty minutes after the transfer, 1 attached flagellate was chosen for detailed observation. Finally, latex beads (Fluoresbrite Plain YG, 0.75 µm, Polysciences) were added. Preliminary experiments showed that the contact probability of the bacteria was higher than that of beads due to bacterial motility and size. Therefore, the concentration of beads (0.3×10^7, 0.9×10^7, 1.8×10^7, 3×10^7, 4×10^7 beads ml^{-1}) had to be about 20% higher than that of the bacteria to allow for similar capture probability of beads and bacteria by the flagellates. Two sets of control experiments were performed. In the first control experiment (Expt C1), the flagellates were treated as described above but no latex beads were added. In a second control experiment (Expt C2), the food concentration was adjusted to $<10^5$ bacteria ml^{-1} for 30 min (immediately after transfer to the petri dishes) before the experiment started. After this treatment nearly all ingested particles should be digested or egested by the flagellate and therefore food vacuoles are nearly empty or non-existent. At the start of this experiment, a mixture of bacteria and beads was added. Expt C2 was only done at the highest food concentration to obtain additional information on the influence of food vacuole content on food selectivity.

For video observations a Zeiss Axiovert S100 microscope equipped with a Plan Neofluar 100×/1.3 oil objective and connected to an MC-1009/S video camera (AVT Horn) was used. Bright field illumination at moderate intensity was used for observation. This moderate illumination avoids heating the medium and irritating the flagellates. Preliminary experiments gave evidence that the light level was far below a critical intensity that would cause irritation of the flagellates or strong cell damage (see also Boenigk et al. 2001a). The video signal was recorded by an S-VHS recorder (AG 7355, Panasonic). All contacts and ingestions were recorded during the first 5 min. After this observation time the flagellates were observed for another 10 min to study the fate of ingested latex beads. A contact between a bacterium and the sensitive region of the flagellate was defined as contact and the enclosure of the bacterium by the flagellate's flagellum was defined as capture. We observed 15 individual flagellate cells for each experiment. Beads and bacteria could easily be distinguished by using single-frame playback of the video sequences.

Food selectivity was calculated directly from the observed contacts (C) and ingestions (I), respectively, of bacteria (B) and beads (b) according to Chesson's α-index (Chesson 1983; $\alpha_I = 0.5$: unselective feeding, $\alpha_I > 0.5$: preference for beads, and $\alpha_I < 0.5$: preference for bacteria):

$$\alpha_I = \frac{I_b / C_b}{I_b / C_b + I_B / C_B} \quad (1)$$

Statistical analyses were carried out using the SPSS software (version 8.0.0). Food selectivity, α_I, was calculated using Eq. 1 and tested against non-selectivity ($\alpha_I = 0.5$) using the t-test. The influence of food concentration on the egestion of indigestible particles was tested by comparing the vacuole passage times of flagellates fed at different food concentrations for each flagellate species (ANOVA).

Results. All flagellate species generally stayed attached during the experiment. Contact probability for beads and bacteria was not significantly different (analysis of covariance [ANCOVA], $p > 0.05$ for all species) despite a significantly higher concentration of beads in the experimental vessels (see 'Materials and methods'). In addition we observed no significant difference in the capture efficiency of the flagellates for beads and bacteria prey (ANOVA, $p > 0.05$ for all species).

Ingestion rate and ingestion efficiency: Contact probability and the rate of successful captures were generally higher for bacteria than for beads but were not actively regulated dependent of the food concentration. In contrast, ingestion rates depended on the food concentration and showed a typical functional response. When a mixture of beads and bacteria was fed to the flagellates, ingestion rate (all particles) showed a similar functional response to that of flagellates feeding on bacteria only (Table 1, Fig. 1). The number of ingested bacteria per unit time (Table 1) did not differ significantly between flagellates feeding on bacteria and flagellates feeding on a mixture of bacteria and beads ($p > 0.05$ for *Spumella* sp. and *Ochromonas* sp.). The presence of indigestible particles (beads) had only a slight effect (not significant) on the ingestion of bacteria. Only in experiments involving *Cafeteria* sp. did we find significantly lower ingestion rates (bacteria) when beads were present ($p = 0.019$).

At low food concentrations, all the particles were ingested regardless of their nutritional suitability ($\alpha = 0.5$; Fig. 1). For medium food concentrations up to 1.5×10^7 particles ml^{-1}, Chesson's α-index did not differ significantly from 0.5 for all flagellate species, corresponding to non-selective food uptake (t-test, $p > 0.05$). When food concentration was increased, the flagellates started to reject captured food particles, and at increasing food concentrations, the flagellates started to select for bacteria ($\alpha < 0.5$). No beads were ingested by *Spumella* sp. and *Ochromonas* sp. ($\alpha = 0$) at the

Table 1. Ingestion rates (bacteria flagellate^{-1} h^{-1}) of *Spumella* sp., *Ochromonas* sp. and *Cafeteria* sp. feeding on bacteria and a mixture of bacteria and beads (mean and standard deviation [SD], n = 15 for each experiment). Total particle concentration in experiments with beads and bacteria was about twice as high as bacterial concentration. Ingestion rates are based on ingestions of bacteria only. IR = ingestion rate

Food conc. (bacteria) ml^{-1}	*Spumella* sp.			*Ochromonas* sp.			*Cafeteria* sp.		
	Bacteria	Bacteria + beads		Bacteria	Bacteria + beads		Bacteria	Bacteria + beads	
		IR (bacteria)	Total IR		IR (bacteria)	Total IR		IR (bacteria)	Total IR
0.25×10^7	7.2 ± 11.8	7.2 ± 8.8	12.8 ± 12.4	11.2 ± 9.6	12.8 ± 12.4	22.4 ± 11.9	4.0 ± 3.7	4.0 ± 4.8	6.4 ± 4.2
0.75×10^7	13.6 ± 10.0	12.0 ± 7.9	22.4 ± 11.9	22.4 ± 10.0	23.2 ± 14.7	48.0 ± 23.6	5.6 ± 7.7	4.8 ± 7.6	11.2 ± 5.0
1.5×10^7	22.4 ± 13.5	24.8 ± 12.4	32.8 ± 10.6	34.4 ± 11.0	42.4 ± 20.7	80.0 ± 23.4	12.8 ± 8.4	9.6 ± 8.1	19.2 ± 8.8
2.5×10^7	30.4 ± 18.6	29.6 ± 17.5	29.6 ± 17.5	72.0 ± 27.2	67.2 ± 26.4	88.0 ± 27.8	14.4 ± 8.1	12.0 ± 11.1	21.6 ± 9.3
3.3×10^7	32.8 ± 21.5	35.2 ± 15.4	35.2 ± 15.4	89.6 ± 30.7	92.0 ± 28.2	92.0 ± 28.2	18.4 ± 8.9	14.4 ± 12.2	24.0 ± 11.1

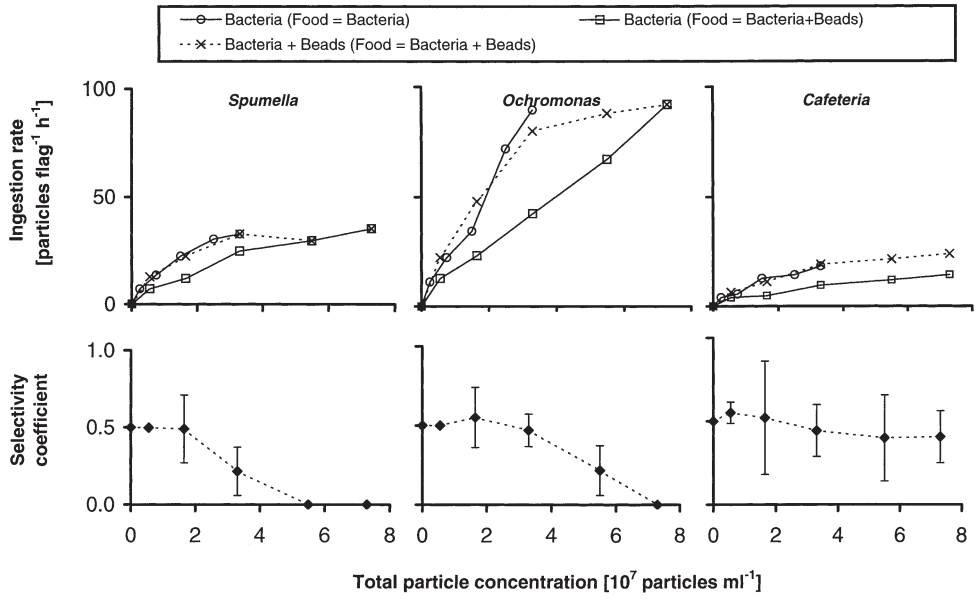

Fig. 1. Ingestion rates (particles flagellate^{-1} h^{-1}) and selectivity coefficient (Chesson's α-index) as functions of food concentration (bacteria) for 3 species of heterotrophic nanoflagellates. Ingestion rates for flagellates feeding on a mixture of beads and bacteria are given as total ingestion rates (including beads and bacteria) and ingestion rates of bacteria only. For comparison, ingestion rates are given for flagellates feeding on bacteria only. The concentration of beads was about 20% higher than bacterial concentration. Total particle concentration was therefore about twice as high as bacterial concentration. Ingestion rates (mean) and food selectivity (mean ± standard deviation [SD]) are calculated from 15 individual cells each. Note that at low food concentrations the flagellates ingested all captured particles and at high food concentrations *Spumella* sp. and *Ochromonas* sp. ingested no beads, both resulting in SD = 0

highest food concentration (3.3×10^7). This negative selection was due to rejection of captured beads by the flagellate at higher food concentrations. Rejection was also observed for *Cafeteria* sp., but selection was weak even at high food concentrations. *Cafeteria* sp. ingested latex beads (Fig. 1) even at the highest food concentrations.

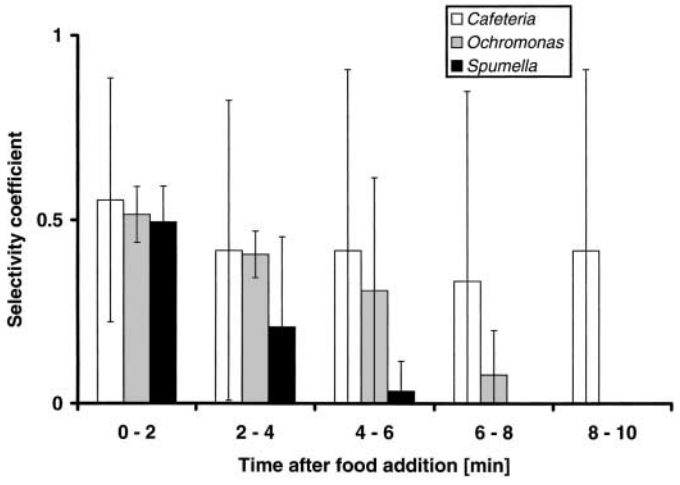

Fig. 2. Mean selectivity coefficient (Chesson's α-index) as calculated from 2 min intervals for starved flagellates (n = 15) exposed to high food concentrations (t = 0). Note the high standard deviation of α at intermediate time phases, which is due to differences between individuals

When starved flagellates were used (Expt C2), the ingestion rate was significantly higher during an initial feeding phase, as was expected ($p < 0.05$ for all flagellate species; see also Boenigk et al. 2001b). Nearly all captured particles were ingested by all species during the first minutes of an experiment and the flagellates showed no selectivity (α = 0.5; Fig. 2) despite high food concentrations. After this initial phase, *Spumella* sp. and *Ochromonas* sp. became more and more selective (Fig. 2) and finally showed strong food selection as observed for well-fed individuals.

Vacuole processing and differential digestion: Digestion of bacteria appeared to be complete as there was no indication of visible bacterial remains in food vacuoles. Egestion of intact bacterial cells was not observed. In contrast to bacteria, ingested latex beads were egested by *Spumella* sp. and *Ochromonas* sp. after a few minutes. *Cafeteria* sp. did not egest the beads after such short times but kept most of the beads for longer then 20 min in vacuoles. This species-specific vacuole processing was observed for all food concentrations and for starved as well as for satiated flagellates (see also Boenigk et al. 2001b). In contrast to

selection during food uptake, food concentration had no significant influence on vacuole passage time (ANOVA, $p > 0.05$ for all species). Active food selection in *Spumella* sp. and *Ochromonas* sp. is therefore composed of selective food uptake (only at high food concentration) and of differential digestion (independent of food concentration).

Discussion. We tested food selectivity of 3 bacterivorous nanoflagellates at different food concentrations. Recent studies on selection mechanisms showed that food selection in HNF is based on a combination of passive and active processes (Boenigk et al. 2001b), which seem to be regulated in different ways. Further, different HNF species show deviating selection behavior (e.g. Šimek & Chrzanowski 1992, Pernthaler et al. 2001) due to species-specific selection mechanisms (e.g. Hansen 1998, Boenigk et al. 2001b). In contrast to most previous studies, we tested selectivity by simultaneous application of the particles under view and subsequent live observation of the particle handling. Some general conclusions can be drawn from our experiments.

Overall food selection is composed of active (ingestion efficiency and differential digestion) and passive selection mechanisms (contact probability and capture efficiency): Selection in the feeding process of HNF is mainly due to contact probability, capture efficiency, ingestion efficiency and differential digestion. We observed a higher 'contact probability' for bacteria than for beads. Theoretical explanations suggest that particle size and physicochemical characteristics of the prey to increase contact probability and to be mainly responsible for prey selection (e.g. Fenchel 1982, Shimeta & Jumars 1991, Monger et al. 1999). In fact, the probability of a certain food particle being ingested by the flagellate has been shown to be correlated with prey size (Fenchel 1987, Kiørboe & Titelman 1998) and prey motility (Monger & Landry 1992, González et al. 1993). Larger particles and motile particles are therefore generally ingested at higher rates (e.g. González et al. 1990b, 1993, Chrzanowski & Šimek 1993). In contrast, 'capture efficiency' was shown to be lower for motile bacteria (Matz et al. 2002 this issue) as, with increasing motility of the prey, the escape probability may increase. However, when ingestion rates are corrected for contact probability, size and motility of the prey are not necessarily correlated with ingestion rate (Boenigk et al. 2001c) even though these parameters probably play a role during this feeding step. In this study, we observed no significant difference in capture efficiency between beads and bacteria. The swimming speed of *Pseudomonas putida* may be slow enough to allow for a capture efficiency as high as that for non-motile particles. The particle-specific 'efficiency of ingestion' of captured particles depends on food concentration. This corroborates the findings of Jürgens & DeMott (1995), who reported a dependency of food selection on prey concentration. That study as well as the present study suggest that this selection step is only significant at or near satiating food concentrations. Selection during this feeding step is likely to be active, may be receptor mediated and may therefore be due to 'signal substances' such as phospholipids on the surface of the prey (Nisbet 1987). Boenigk & Arndt (2000b) suggested an active food size selection during this step. However, other prey characteristics such as surface chemistry may mask size selection during this feeding step (Boenigk et al. 2001c). From our experiments, we cannot exclude prey size or prey shape as responsible selection factors. However, prey surface characteristics are likely to play a main role during this selection step. When a flagellate does not ingest a particle for ca. 10 min it starts to ingest beads (pers. obs.). Therefore, this selection step may be regulated by the amount of food vacuole content or on the ability to build new food vacuoles. In contrast to the ingestion process, selection during 'differential digestion' (González et al. 1990a, Boenigk et al. 2001b) was found to be relatively independent of the actual food concentration. It has been suggested that this selection mechanism depends on the digestibility of the particles and on the accessibility of digestive enzymes (Boenigk et al. 2001a). Studies on the effect of fluorochromes on the digestion process (Premke & Arndt 2000, Boenigk et al. 2001a) and on the processing of stained versus non-stained beads, and of clay particles by several flagellate species (Zwart & Darbyshire 1992, E. J. Cleven pers. comm., Boenigk unpubl.) provide evidence that the egestion of particles was due to the non-digestibility of the particles and not to surface characteristics (including fluorochromes). This may be different when high light intensities (especially UV or blue light) are used. Starvation of the flagellates may cause a slightly longer vacuole passage time possibly due to a generally slower metabolism (Boenigk et al. 2001b). Concentration-dependent variability in food selection seems therefore to be mainly based on prey handling prior to ingestion. Considering our earlier studies on food selection using the same organisms, we can show that the significance of different prey parameters for food selection depends not only on food concentration and satiation of the flagellates but also on the feeding step under view: focussing on contact probability prey size and motility seems to be of main importance (Boenigk & Arndt 2000b). During the processing phase, prey size may also play a role but motility and surface chemistry seem to become more important. This is, however, dependent on food concentration (this study) and may, at first sight, lead to contradicting results at one time, suggesting non-selectivity, while another time sug-

gesting selectivity during this feeding step (Boenigk et al. 2001b,c). The relative importance of different prey characteristics during this feeding step has to be subject to further investigations. Differential digestion could not be shown to depend significantly on the flagellates' satiation, even though shifts in selectivity during this selection step have been observed (C. Schygula pers. comm.). This also has to be studied in more detail.

Significance of food selection and the relative importance of distinct selection mechanisms are species specific: Generally, contact probability, capture efficiency and differential digestion seem to be relatively independent of the actual food concentration. In contrast, selection during particle handling prior to ingestion depends on the food concentration but seems to be realized only at very high food concentrations, which are usually not found in natural systems. All flagellate species have been cultured in the laboratory for years and may be adapted to higher food concentrations. Although we could show a general dependency of the ingestion efficiency on food concentration in HNF, its relative importance is questionable. Selection in advance, as is found in *Spumella* sp. and *Ochromonas* sp., may be an adaptation to environmental situations where 'non-suitable particles' become abundant. This may be important, for example, during flood events, when suspended sediment concentration increases. But it seems unlikely that this selection mechanism plays a major role in the selection between bacterial strains. However, for species that live in substrate flocs or in the benthos, chemoselection may also play an important role at low food concentrations. In contrast to *Spumella* sp. and *Ochromonas* sp., *Cafeteria* sp. only slightly selected (concerning selection in advance and differential digestion) even at high food concentrations. The strategy of *Cafeteria* sp. seems to be to minimize the efforts for searching and for selection. This behavior may be energetically favorable in environments where the concentration of unsuitable particles is low. The selection strategy of *Spumella* sp. and *Ochromonas* sp. may be to maximize the number of captures, and selection may be used to differentiate particle quality. Thus, this latter strategy may have higher costs but also greater benefits than the former strategy.

Significance of HNF food selection for field and laboratory studies: As mentioned above, the results of laboratory studies are not directly applicable to the interpretation of field data. However, they are a suitable tool for the investigation of principle mechanisms of predator-prey interactions. The rates of ingestion of bacteria by *Spumella* sp. and *Ochromonas* sp. were not significantly affected by the presence of beads. Therefore, the presence of naturally occurring particles in the size range of bacteria such as clay should also not affect the flagellates' feeding rates. These findings are in agreement with the study of Jack & Gilbert (1993), who found no negative effect of suspended clay on the growth rate of a bacterivorous ciliate. As illustrated above, food selection of HNF depends on morphological and physicochemical characteristics, motility, digestibility and molecular surface characteristics of the prey (e.g. González et al. 1990a, 1993, Monger et al. 1999, John & Davidson 2001, Matz et al. 2002). As selection is a combination of passive and active selection mechanisms, negative or positive selection can be measured relative to a reference particle but is neither static nor absolute. Furthermore, particle handling and selectivity are surely dependent on factors such as temperature, food concentration, and a variety of abiotic and biotic factors (e.g. Sherr et al. 1988, Tobiesen 1990). However, food concentration seems not to affect selectivity except very high particle concentrations (Jürgens & DeMott 1995, this study). Regarding the complexity of food selection, a high variability in ingestion rates and growth rates even within 1 species of bacterivores is not surprising (e.g. González et al. 1993, Christoffersen et al. 1997, Hansen 1998). In contrast, differences between species and between cultures of different physiological levels should be expected. Several factors responsible for food selection are linked to the physiological state of the prey (motility, surface characteristics, size; e.g. Hahn et al. 1999) and of the predator (chemical selectivity, differential digestion; e.g. Boenigk et al. 2001b, this study). Thus, food selection is the result of a multivariate predator-prey interaction, which is, in addition, regulated by feedback mechanisms. The treatment of HNF as a 'black box' may lead to misinterpretations, as the complexity of feeding interactions is not considered (e.g. Cleven & Weisse 2001).

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