



Bacterivory by heterotrophic flagellates: community structure and feeding strategies

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Abstract

Heterotrophic flagellates (HF) are known as most important grazers of bacteria in many aquatic ecosystem. HF cannot be treated as a black box since HF generally contain a diverse community of species significantly differing in their feeding behaviour and other ecological properties. Today it seems that the dominant taxonomic groups among heterotrophic nano- and microflagellate communities within different marine, brackish and limnetic pelagic communities (heterokont taxa, dinoflagellates, choanoflagellates, kathablepharids) and benthic communities (euglenids, bodonids, thaumatomonads, apusomonads, cercomonads) are relatively similar. HF among protista *incertae sedis*, often neglected in ecological studies, are abundant bacterivores in all investigated habitats. Recent studies of flagellate feeding processes indicated that there are significant species-specific differences and individual variability regarding the food uptake and food selection of bacterivorous flagellates: Variability of bacterivory is discussed regarding the prevailing feeding modes, the energy budgets, the considerable importance of slight deviations in the time budgets of feeding phases, the ingestion rates and the feeding microhabitat, respectively. The significant flexibility of the grazing impact of bacterivorous flagellate communities creates a complex top-down pressure on bacteria which should have lead to the evolution of efficient predator avoidance mechanisms in bacteria and should be at least partly responsible for the diversity of present bacteria.

Introduction

The microbial components of aquatic food webs (bacteria, autotrophic picoplankton, heterotrophic and mixotrophic flagellates, and ciliates) can often be an important, and sometimes dominant, part of aquatic ecosystems. The significance of the 'microbial loop' for the carbon flow from primary producers to higher trophic levels (the classical food chain) has been hypothesised by Azam et al. (1983) and was investigated in detail by many successive studies. The 'microbial loop' concept was prepared by earlier conceptual works (e.g. Sorokin & Pavelyeva 1972; Pomeroy 1974; Williams 1981).

Heterotrophic flagellates (HF) have been recognised as consumers of suspended as well as of attached bacteria (for reviews see Fenchel 1986a; Sanders et

al. 1991; Laybourn-Parry & Parry 2000). In contrast to a variety of laboratory studies species-specific investigations of field communities are still rare (e.g. Sanders et al. 1989; Carrias et al. 1996; Cleven & Weisse 2001). Due to the paucity of characteristic HF features, identification based upon morphological features is difficult in fixed material using epifluorescence microscopy.

Bacteria are a relatively small prey for many bacterivores except the smallest ones – heterotrophic flagellates. HF may treat each prey item separately and therefore adaptations to a raptorial feeding or interception feeding mode could have been realised. Thus, specific morphological structures often support food gathering of bacterivorous flagellates. The aim of this mini review is to focus on the diversity of grazing interactions between flagellates and their bacterial prey

from the side of heterotrophic flagellates, while in another contribution to this volume Jürgens & Matz (2002) will illustrate these interactions from the side of bacteria.

Composition of flagellate communities

The evolutionary relationships between the various groups of flagellates are still under discussion (e.g. Patterson 1999; Cavalier-Smith 2000). Ecologists are faced with the problem that no comprehensive key for the determination of species exists. However, there are several memoir-like papers that can be of great help for identification (e.g. Patterson et al. 1989; Larsen & Patterson 1990; Patterson & Larsen 1991; Vørs 1992; Tong et al. 1998; Bernard et al. 2000). A rough idea of our present understanding of the relative importance of common taxa in pelagic and benthic habitats is summarized in Table 1. Autecological data are available only for a few species or strains.

There are some general methodological problems concerning the determination of abundance, biovolume and community structure of heterotrophic flagellates. The use of epifluorescence microscopy mostly applied in routine investigations does not allow in all cases a definite assignment of eukaryotic cells to 'HNF' since flagella are often invisible. In addition, many flagellates are disrupted during fixation and a significant non-uniform shrinkage of fixed flagellates makes estimates of biovolume difficult (e.g. Sonntag et al. 2000; Wirth & Arndt in prep.). Another important problem is that generally only about 50–70 flagellates are counted per filter. Thus forms that contribute only 1–2% to total abundance are easily overlooked. Though much less abundant than nanoflagellates, the contribution of large flagellates to total flagellate biomass in different ecosystems can be very significant (Sherr & Sherr 1994; Mathes & Arndt 1994). Heterotrophic microflagellates are often delicate forms (e.g. some chrysomonads and many representatives of Protista *incertae sedis*) difficult to quantify using routine fixatives. We strongly recommend to use the term microflagellates for flagellates equal to or larger than 15 μm , rather than to use 20 μm as the size separating nano- from microflagellates following the classification of plankton size fractions by Sieburth et al. (1978). HF larger than 15 μm are considerably less abundant than smaller forms and require a different method of quantification (cf. Arndt et al. 2000). Among the microflagellates

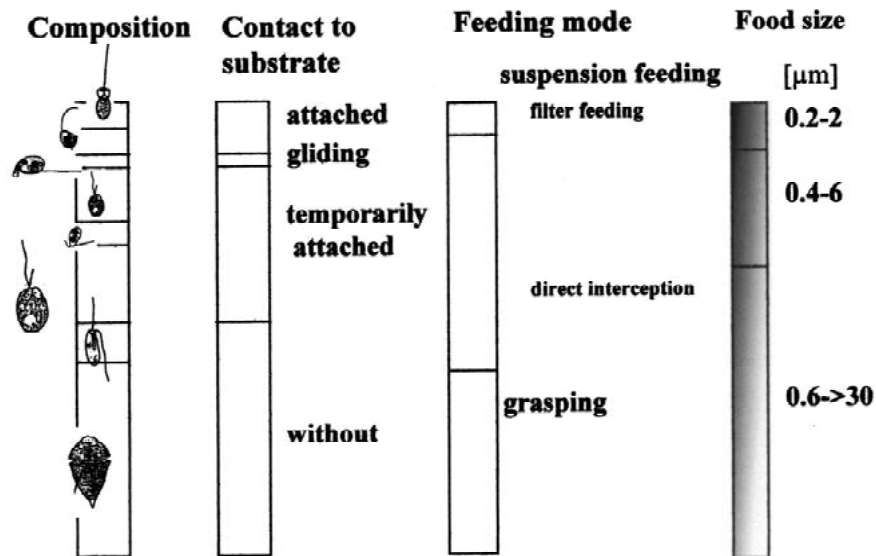
($\geq 15 \mu\text{m}$) the otherwise ignored flagellates in the size range between 15 and 20 μm form a considerable part. This change in size limits of nano- (>2–<15 μm)- and microflagellates ($\geq 15 \mu\text{m}$) will have no influence on the consideration of published 'HNF'-counts, since HF larger than 15 μm had generally not been considered. But this will have a very important influence on further studies of microflagellates which certainly deserve much more attention in routine investigations (for review see Arndt et al. 2000). Another size group, heterotrophic picoflagellates (<2 μm), will be a new research field since oceanic samples obviously contain a high number of very tiny flagellates (e.g. Moon-van der Stay et al. 2001).

Pelagic flagellate communities

The abundances of *heterotrophic nanoflagellates* (HNF) in different pelagic habitats can vary from about 20 to more than 20,000 HNF per ml but reach mostly 100 to 10,000 HNF/ml. There was observed a more or less close relationship of HNF abundances to the bacteria abundances (Sanders et al. 1992) depending on top-down and bottom-up effects (cf. Gasol 1994). The available information on the taxonomic composition of HNF from pelagic communities indicates relatively similar taxonomic groups forming the major part of the flagellate community in very different environments (Figure 1, left columns). Heterokont taxa as the dominant group contribute about 20–50% to average HNF biomass (mainly chrysomonads and bicosoecids), followed by choanoflagellates (5–40%) and kathablepharids (10–>25%). This seems to be similar for marine and freshwater pelagic communities (Vørs et al. 1995; Arndt et al. 2000). Though always present, kinetoplastids, small dinoflagellates, thaumatomonads, apusomonads, colourless cryptomonads and euglenids commonly form only minor parts of the HNF biomass. Recently, picoeukaryotes of different phylogenetic groups (heterokonts, alveolates, cercozoans) have been identified by 18S-rRNA gene cloning and sequencing (Diez et al. 2001; Lopez-Garcia et al. 2001; Moon-van der Stay et al. 2001). It seems that these heterotrophic picoeukaryotes are fundamental components at least of marine planktonic systems, however, their contribution to HF abundance and biomass and their possible effect on bacteria remain still unknown.

Regarding their biomass, *heterotrophic microflagellates* (≥ 15 –200 μm , HMF) seems to be as important as nanoflagellates (e.g. Sherr & Sherr 1989;

Pelagial



Benthic

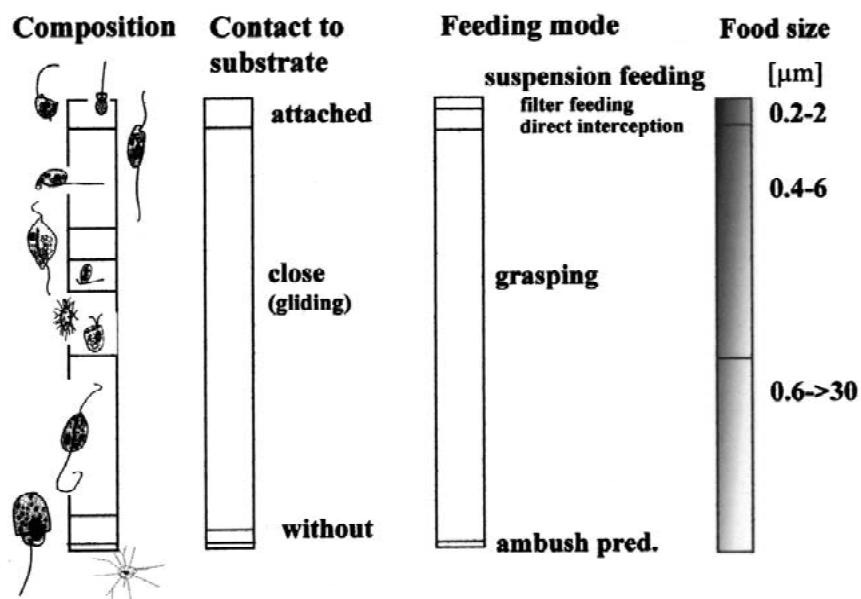


Figure 1. Comparison of the general taxonomic composition on the basis of mean biomass of pelagic and benthic flagellate communities (derived from Arndt et al. 2000). Typical taxa graphed on the left bars were for the pelagial (from top to bottom): choanoflagellates, bicosoecids, kinetoplastids, chrysomonads, thaumatomonads, large chrysomonads (and taxa of uncertain systematic position), kathablepharids, and dinoflagellates. For the benthic (from top to bottom): choanoflagellates and bicosoecids, kinetoplastids, apusomonads, thaumatomonads, cryptomonads and hemimastigids (as representatives of taxa of uncertain systematic position), euglenids, dinoflagellates, and ambush predators of different groups (e.g. *Massisteria* as a cercomonad). The right three bars give the percentage of total flagellate biomass that is formed by taxa that can be characterized either by their degree of contact to the substrate, by their feeding mode or by their preferred spectrum of food size, respectively. It means, for instance, that about two third of the flagellate biomass in the pelagial is formed by suspension feeding taxa.

Table 1. Major taxonomic groups of heterotrophic flagellates present in aquatic and benthic environments up to our present knowledge (compiled after Patterson & Lee 2000 and Arndt et al. 2000) □ and ■ indicate the relative importance regarding the abundance and biomass, respectively, in the different environments

Common taxonomic groups	Common taxa	Pelagic occurrence		Benthic occurrence	
		freshwater	marine	freshwater	marine
Euglenozoans					
Euglenids	<i>Petalomonas, Peranema</i>	□	□	■■■■■■■■□□	■■■■■■■■□□
Kinetoplastids	<i>Bodo, Caecitellus</i>	□	□	■■■■■■□□□□	■■■■□□□□
Cryptomonads	<i>Goniomonas</i>	□	□	■□	■□
Stramenopiles					
Chrysomonads	<i>Spumella, Paraphysomonas</i>	■■■■■■■■□□□□□□	■■■□□□? □	□	□
Bicosoecids	<i>Cafeteria, Bicosoeca</i>	■□□□	■□□□??	■□	■□
Alveolates					
Dinoflagellates	<i>Gymnodinium, Katodinium</i>	■■■□	■■■■■■■□ □		■■■□
Choanoflagellates	<i>Monosiga, Diaphanoeca</i>	■■■□□□	■■■□□□ □	□	□
Protista <i>incertae sedis</i>					
Cercomonads	<i>Cercomonas, Bodomorpha</i>	□	□	■□□□	■□□□
Kathablepharids	<i>Kathablepharis, Leucocryptos</i>	■□□□	■□□□	□	□
Thaumatomonads	<i>Protaspis, Thaumatomonas</i>	□	□	■□□□	■□□□
Apusomonads	<i>Amastigomonas, Apusomonas</i>	□	□	■□	■□
Ebriids	<i>Ebria</i>		■■■□		
Ancyromonads	<i>Ancyromonas</i>	□	□	■□	■□
diverse groups of uncertain systematic affiliation	<i>Metromonas, Diphyllia, Metopion, Telonema</i>	■■■□□	■□□□	■□□□	■□□□

Arndt & Mathes 1991). Most HMF are able to feed on bacteria, however, up to which extent is still largely unknown. Their abundances are about two to three orders of magnitude lower than that of HNF, however they may contribute significantly to annual mean HF biomass (Figure 1). The relative contribution of nano- and microflagellate biomass varies depending on the trophic status of lakes with a tendency for microflagellate biomass to prevail under hypertrophic conditions (Mathes & Arndt 1994). In temperate marine waters and in lakes HMF reach their largest percentage contribution to heterotrophic flagellate biomass during early spring (Smetaček 1981; Mathes & Arndt 1994). HMF communities are generally dominated by colourless dinoflagellates. In coastal marine waters ebriids and in freshwaters large heterokonts and Protista *incertae sedis* can occasionally form another substantial part of HMF biomass.

Biomasses of pelagic flagellates can change by a factor of 100 in the course of a year. Temperate regions are generally characterised by maxima in spring, due to an increased food supply, and minima in early summer due to intensive grazing pressure by metazoans (e.g. Weisse 1991). At times of high

metazoan grazing pressure HMF often disappear from pelagic communities (Arndt et al. 2000). Quantitative changes in the vertical distribution of HF are well documented for marine and freshwaters. Generally, maximum numbers occur at sites of highest food concentrations mostly at the surface (Fenchel 1986a).

Benthic flagellate communities

Abundances have been reported to be in the range of below 100 to several millions HF per ml sediment (Gasol 1993). While some authors found positive correlations between bacteria abundance, grain size and flagellate abundance (Hondeveld et al. 1994), other authors found inconsistent relationships (cf. Alongi 1991; Hamels et al. 2001). Quantitative data on the taxonomic composition are very sparse and often restricted to a few taxonomic groups. Both, HNF and HMF are considered to be important in benthic communities (for review see Arndt et al. 2000). Recent studies from the aerobic surface layer of marine, brackish and freshwater sediments (e.g. Dietrich & Arndt 2000; Lee 2001) indicate euglenids to be most important regarding their biomass contribution (20–

85%), followed by bodonids (5–20%), apusomonads and thaumatomonads (1–20%). Benthic dinoflagellates may contribute up to 20% of HF biomass. Occasionally of importance may be colourless cryptomonads, chrysomonads, choanoflagellates, cercomonads, bicosoecids, pedinellids and hemimastigids. Several genera of Protista *incertae sedis* may be temporarily abundant, but generally do not form a significant part of HF biomass (cf. Table 1). The knowledge regarding the specific requirements of the different taxa and the specific top-down and bottom-up effects, is still limited at present.

Except for areas where the sediment is covered with ice during longer periods, seasonal changes of benthic systems often seems to be reduced compared to the pelagial due to the supply of organic material in the sediment that allows for a relatively continuous bacterial production as a major food source. The vertical distribution of HF abundances is mainly related to the chemical properties of the different sediment layers and is not well understood. Highest numbers and biomasses are generally found in the upper layers of sediment (for review see Alongi 1991). At reductive conditions deep in the sediment the community structure changes significantly towards diplomonads, Protista *incertae sedis*, percolozoans, and several undefined forms. But also classically defined aerobes may occasionally occur (Fenchel & Finlay 1995).

Feeding ecology

Prevailing feeding modes

Feeding strategies of *pelagic* HF (Figure 1, upper panel) are clearly separated by the degree of contact to the substrate: (1) free-swimming forms (e.g. colourless katablepharids, dinoflagellates, large chrysomonads), (2) forms which are loosely and temporarily attached by protoplasmic threads (small chrysomonads, some choanoflagellates, pedinellids) or flagellum (e.g. bodonids, thaumatomonads, apusomonads, bicosoecids), and (3) attached forms (e.g. some loricate bicosoecids and choanoflagellates). At least half of the biomass of pelagic HF is composed of flagellates living in more or less close contact (group 2 and 3, see Figure 1, second bar from the left) to seston particles, e.g. algae or detritus ('snow'). Most pelagic flagellates feed on small food items (0.2–6 μm). Attachment of flagellates has been considered to be an important mechanism to increase the contact probability due to

hydrodynamic forces and consequently to increase ingestion rates (Fenchel 1986b). Only a few flagellates in the pelagial belong to raptorial feeders crawling over surfaces of detrital particles, where bacteria are significantly concentrated compared to the surrounding water. In contrast to these particle associated HF, free-swimming flagellates seem to feed preferably on relatively large food particles such as algae and other protists (Arndt et al. 2000).

In *benthic* (aerobic) communities bacterivores are to a minor extent suspension feeders (Figure 1, lower panel) grazing on bacteria of the pore water (bicosoecids, choanoflagellates, pedinellids) and forms that grasp more or less on attached bacteria (most bodonids, small euglenids, apusomonads, cercomonads, and several representatives of Protista *incertae sedis*). Bacteria concentrations in benthic systems are up to three orders of magnitude higher compared to the pelagial, thus grasping even on small bacteria is an efficient way of HF nutrition. Food consumption of benthic flagellates is often aided by specialised feeding organelles which is of importance especially for the detachment of closely attached bacteria. There are differences in the mobility of the HF also in the sediment which affects the feeding on bacteria. There are flagellates moving between the sand grains (e.g. bodonids and euglenids) and ambush predators (e.g. the cercomonad *Massisteria*, some thaumatomonads).

Combining food sources

Most HNF have been described as mainly or even exclusively bacterivores. However, the classification of flagellates into only one feeding type probably leads wrong. The feeding behaviour of HF can differ between the various groups of flagellates and the size spectrum of food particles is much larger than previously assumed (for reviews see Sanders 1991; Radek and Hausmann 1994; Sleigh 2000). HNF in general seem to be able to feed on all particle types they can get hold on if the particles can morphologically be ingested. The size of food particles can range from high molecular weight molecules (Sherr 1988; Christoffersen et al. 1996) and virus-sized particles (Gonzalez & Suttle 1993) up to >50 μm (in some dinoflagellates), thus deviating strongly from the original assumption that HF are exclusively bacterivorous. It is known that several nanoflagellates from marine and freshwaters also feed on nanophytoplankton (e.g. Sherr et al. 1991; Cleven 1995), and may also consume other flagellates, ciliates and even small

metazoans (cf. Sleight 2000). Besides bacterivory, HF can reach the same importance as predators of eukaryotic algae as ciliates in marine (Lessard and Swift 1985; Sherr and Sherr 1994) and in limnetic pelagic communities (Arndt et al. 1993). Figure 1 indicates the size spectrum of food particles for pelagic and benthic communities. It seems that the contribution of exclusively bacterivorous forms in pelagic habitats is only about 1/10 in limnetic sites (mainly choanoflagellates, bicosoecids, kinetoplastids), in marine and brackish sites the percentage may be significantly larger. In benthic habitats about one quarter of HF biomass consists of bacterivores (mainly kinetoplastids, bicosoecids, some Protista *incertae sedis*, cryptomonads, for more details see Fenchel 1986a and Arndt et al. 2000).

The majority of pelagic HF are omnivores feeding on different trophic levels. Cannibalism of some species (e.g. Goldman & Caron 1985; Jonsson 1986) has to be considered as an effective tool reducing the number of competing organisms as cells of the same species probably are the strongest competitors. In addition, bacterivory (feeding on *Aerobacter*) and carnivory (predation on a bacterivorous *Cercomonas*) was observed in the same strain of a small *Paraphysomonas* (diameter 3–4 μm) (Arndt et al. 2000). Algae are less abundant than bacteria and therefore the probability to capture algae is much lower compared to the probability to capture bacteria. However, due to the larger volume algae may contribute significantly to overall feeding (Cleven 1995). Most dinoflagellates, chrysomonads, some Protista *incertae sedis* and katablepharids are known to feed on bacteria, as well as on algae and most probably also on heterotrophic protists. Omnivory seems to be typical for all non-obligate bacterivores among HNF. Mixotrophy is known for many phagotrophic flagellates. Its relative importance can vary significantly within a genus and within a species depending on light and food conditions. This phenomenon will not be considered in detail here (for reviews see Sanders et al. 1990; Caron and Finlay 1994). High molecular weight polysaccharides can be ingested by flagellates (Sherr 1988). Its role in the nutrition of bacterivores probably is a minor one except for experimental conditions using high concentrations of organic nutrients which do normally not occur in nature (Fenchel 1987). However, in the pore water of benthic systems or in the anaerobic hypolimnion DOC concentrations can be several orders of magnitude higher compared to surface waters. Some species seem to be typical osmotrophs (Pringsheim 1963), how-

ever, the relative contribution of osmotrophy to the carbon budget of bacterivores is not well understood (for review see Sanders 1991).

Energetic considerations

To allow for a population growth the food concentration must exceed a critical minimum concentration which enables the flagellates to capture enough food items per time unit. This acquired food concentration depends on several factors such as efficiency of the capture mechanisms, species-specific conversion of prey carbon to predator carbon, basal metabolism, etc. Even though all these factors are surely species-specific some general statements seems to be possible.

The maximal water volume which can be processed by an individual (upper limit of the clearance rate) is in the range of about 10^5 units of predator cell volume per hour corresponding to 5–15 nl/h for a nanoflagellate (Fenchel 1986a, 1987; Boenigk & Arndt 2000a,b). This value is in accordance with specific clearance rates reported for HNF of different taxonomic groups and should be reliable even though, general specific clearance rates decreases with predator body size (Hansen et al. 1997). Using these values it can be easily calculated that HNF may capture at least about 5–10 bacteria per hour at a food concentration of about 10^6 bacteria per ml. At bacterial concentrations of less than $2\text{--}3 \cdot 10^5$ per ml, HNF can hardly capture one bacterium per hour. From these considerations it can be concluded that most HNF populations would need a food concentration of about 10^6 bacteria per ml to grow. This is in accordance with the general assumption that HNF are food limited at food concentrations below 10^6 bacteria per ml (Jürgens & DeMott 1995). However, HNF are able to adapt to very low food concentrations as realised for example in aquifers (e.g. Novarino et al. 1997; Süß et al. *subm.*). The mechanisms which allow HNF growth at such low food concentrations are not yet well understood. Eccleston-Parry & Leadbeater (1994) reviewed threshold bacteria concentrations for flagellates supporting HNF growth to be in the range of $10^5\text{--}10^6$ bacteria ml^{-1} . These threshold food concentrations represent the concentration at which energy uptake balances basal metabolic activity (cf. Lampert 1977).

Maximal ingestion rates reported for HNF may exceed 200 bacteria $\text{ind}^{-1} \text{h}^{-1}$ (e.g. Fenchel 1982b). For most HNF a maximal ingestion rate of about 30–80 bacteria per individual and hour seems reliable. It can

be calculated from the above considerations that a food concentration of at least about $3\text{--}5 \times 10^6$ bacteria per ml is required to show optimal ingestion rates and optimal growth. However, at these food concentrations the time budget of flagellates should be dominated by searching for prey particles rather than by handling and ingestion of the particles (Boenigk & Arndt 2000a). Therefore, highest ingestion rates and consequently highest growth rates are realised at food concentrations beyond $1\text{--}2 \times 10^7$ bacteria per ml.

Applying these considerations to the investigation of the flagellate feeding on other food particles (algae, protozoa) leads to the assumption that HF are relatively strong food-limited in most aquatic ecosystems. However, HF are found even in environments with very low food concentrations. Different strategies may have been adopted to survive periods of starvation. Such strategies comprise rapid encystment and excystment, changes in food sources used as well as dramatic changes in metabolic rates and other physiological and cytological changes (for review see Fenchel 1986a; Sleigh 2000).

Principal feeding types and phases of food uptake

The induction of phagocytosis was thought to be at least in part due to mechanical stimuli by the particles. This assumption was mainly based on the observation that food vacuole formation does not take place in particle-free water but inert particles were ingested (Fenchel 1987). Recent studies provide the assumption that the stimulus for ingestion can be influenced by the molecular surface characteristics of the food particles (Boenigk et al. 2002; Matz et al. 2002). Figure 2 gives an overview about the different mechanisms involved in the bacterivory of heterotrophic flagellates: Filter-feeding (choanoflagellates, pedinellids), sedimentation (choanoflagellate *Choanoeca*), interception feeding (chrysoomonads, bicosoecids, diplomonads, *Bodo saltans*), raptorial feeding supported by a pharynx (most bodonids and euglenids) or by pseudopods (apusomonads, cercomonads).

The ability of most flagellates to take up particles of different size aided by pseudopod-like structures (chrysoomonads, many dinoflagellates), a pharynx (e.g. euglenids, bodonids, kathablepharids), or by other mechanisms (e.g. pallium-feeding and myzocytosis outside the theca of dinoflagellates) seems to be an important feature to survive at low food concentrations in the pelagial. Most large flagellates seem to have two

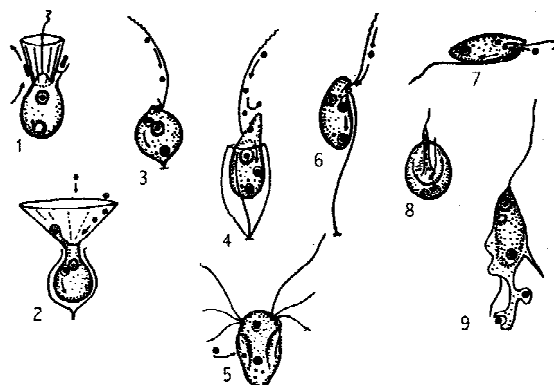


Figure 2. Feeding of different bacterivorous nanoflagellates (derived from Zhukov 1993) by filter-feeding (1, choanoflagellate *Monosiga*), sedimentation (2, choanoflagellate *Choanoeca perplexa*), interception feeding (3, chrysoomonad *Spumella*; 4, bicosoecid *Bicosoeca*; 5, diplomonad; 6 bodonid *Bodo saltans*), raptorial feeding by a pharynx (7, bodonid) or by pseudopod like structures (8, apusomonad *Apusomonas*; 9, cercomonad *Cercomonas*).

modes of feeding: grazing of large food particles upon occasional contacts and the uptake of small food items transported towards the cell by a filter current created by their flagella. This feeding behaviour seems to be a similar adaptation to the life in the diluted environment of the pelagial as the feeding behaviour of calanoid copepods which may switch between filter feeding of small and grasping of large food particles.

Classification of feeding strategies

While bacterivorous ciliates and metazoans principally capture their prey by a filtration structure, different predation mechanisms are realised in HNF. Fenchel (1986a, 1987) differentiated between 'filter feeding', 'direct interception', and 'diffusion feeding'. Filter feeding in the sense of Fenchel (1987) is characterised by the transport of water through a filter formed by cilia or pseudopodial tentacles and microvilli. Direct interception (which is used synonymously to 'raptorial feeder') is characterised by the capture of particles carried along the flow lines due to motility of the predator. Diffusion feeding is characterised by contacts of a mobile prey with a motionless consumer. Even though these definitions seem to be suitable for ciliates and large heterotrophic flagellates they seem somehow inconsistent for the description of the feeding behaviour of HNF. The morphological characteristics of a filter feeder may be coupled with capture mechanisms which are dominated by diffusional forces (Boenigk & Arndt 2000b). Brownian motion and diffusion (following Fenchel (1984) diffu-

Table 2. Comparison of parameters of the feeding process of six different bacterivorous nanoflagellates indicating the feeding mode, the ultrastructural properties aiding in the food uptake, the handling time comprising contact, processing, ingesting and refractory phase, and the speed of particles in the feeding current (summarised according to data from Boenigk & Arndt 2000a,b)

Flagellate species	Feeding mode	Food uptake	Handling time [s]	Particle speed [$\mu\text{m/s}$]
<i>Bodo saltans</i>	Interception feeding	Pharynx	33	91
<i>Rhynchomonas nasuta</i>	Raptorial feeding	Pharynx	3.7	–
<i>Cafeteria roenbergensis</i>	Interception feeding	Invagination of membrane	95	105
<i>Spumella spec.</i>	Interception feeding	Pseudopod formation	10	50
<i>Ochromonas spec.</i>	Interception feeding	Pseudopod formation	3.8	112
<i>Monosiga ovata</i>	Filter feeding	Pseudopod formation	~300	9

sion includes motility of the prey) becomes generally an important factor for such small organisms. Even so-called interception feeders may have some profit from diffusion. Motility of bacteria which may swim as fast as several hundred micrometers per second (Mitchell et al. 1995; Grossart et al. 2001) is a significant factor for interception feeding HNF which produce current velocities of up to only about $50\text{--}100 \mu\text{m s}^{-1}$ (Table 2). Thus the morphological characteristics defining a certain feeding type become inconsistent with the mechanisms involved. More appropriate seems the differentiation between morphological characteristics and behavioural/mechanistic features. Bacterivorous organisms may possess structures which enlarge the surface (which may be a 'filter'). Diffusional processes (including motility of the prey) on the one hand and the production of a flow field (regardless whether the water current flows towards a fixed predator or a mobile predator moving through more or less motionless water) are the principle mechanisms involved. It is important to note movement (swimming or creeping) and the production of a flow field may lead to a different potential prey spectrum. In conclusion, bacterivores may be categorised, e.g. due to their morphological features, but with regard to their feeding ecology these types contain each a variety of different adaptations. Rough generalisations seem not to match the variety of realised feeding mechanisms.

Sit-and-wait-foraging vs. mobile predation

Attachment and mobility are characterised by different advantages and disadvantages for the flagellate. Attachment should be advantageous for filter feeding organisms concerning the feeding efficiency (Fenchel 1986a). To a certain extent attachment seems also to be advantageous for interception feeders (in the narrow sense of Boenigk & Arndt 2000b) as these organisms tend to attach to surfaces in undisturbed media (see also Fenchel 1982a,b). Attachment allows the flagellates to stay in a certain micro-environment and to avoid drift once having found a suitable patch. Substrate-bound mobile predation is the only possibility to use attached bacteria as a food source. For flagellates feeding on suspended bacteria, however, mobility seems to be less important. But mobility allows searching for more suitable microenvironments. Attachment therefore should be expected under favourable food conditions whereas flagellates should tend to swim when food conditions become worse. We observed a detachment when starved *Cafeteria roenbergensis* cells were fed with 'unsuitable food' (latex particles, Boenigk et al. 2001). Detachment was not observed when *Cafeteria* was fed with 'suitable food' (bacteria). Satiated flagellates did also stay attached regardless of the food quality offered. Consistently, Fenchel (1982b) reported the occurrence of actively swimming cells ('swarmers') at the onset of starvation. Additional behavioural responses have been summarised by Sleigh (2000). The behavioural switch between attachment and swimming seems to be reg-

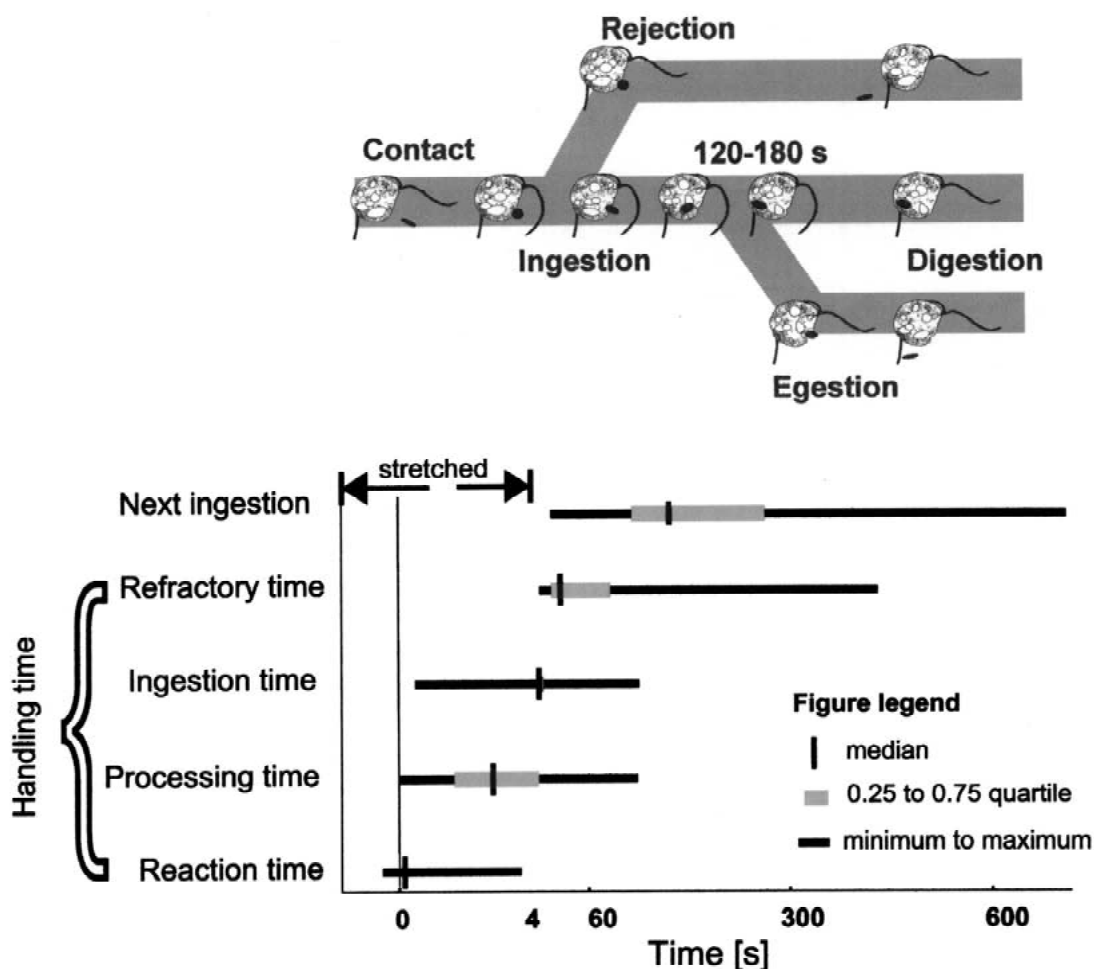


Figure 3. Time budget of the feeding phases for the bacterivorous bicoecid *Cafeteria roenbergensis* (lower panel) and illustration of the different fates of bacteria during the feeding process (upper panel) (according to data from Boenigk & Arndt 2000a; Boenigk et al. 2001).

ulated by the general physiological condition of the flagellate, i.e. starvation or satiation. Additionally, the particle quality seems to influence this behaviour, too. This complex behavioural response probably allows flagellates to maximise the time spent at patches of high food concentrations and to minimise the time spent at unsuitable sites (e.g. low food concentration, high suspended sediment concentrations).

Feeding phases

Video-microscopy allows the study of behavioural factors of the feeding process (Holen & Boraas 1991; Boraas et al. 1992; Ishigaki & Terazaki 1998). Following Boenigk & Arndt (2000a) the feeding process can be subdivided into the contact phase, processing phase, ingestion phase and refractory phase, which al-

together may be defined as the handling phase (Figures 3 and 4). The contact phase characterises the time-lag between the contact of the predator with the prey as well as the behavioural response of the predator. This phase should be short to allow efficient retention of particles. In fact, the contact phase may last several milliseconds only and may vary even among closely related HF species (see Figure 4). The processing phase is characterised by active particle handling by the predator prior to ingestion. During this phase the particle may be adjusted and food selection due to surface characteristics of the prey may take place (Figure 3, Matz & Jürgens 2001; Matz et al. 2002). The ingestion phase is characterised by food vacuole formation and the refractory time is a behavioural lag-phase after ingestion before the flagellate shows normal 'searching behaviour' again.

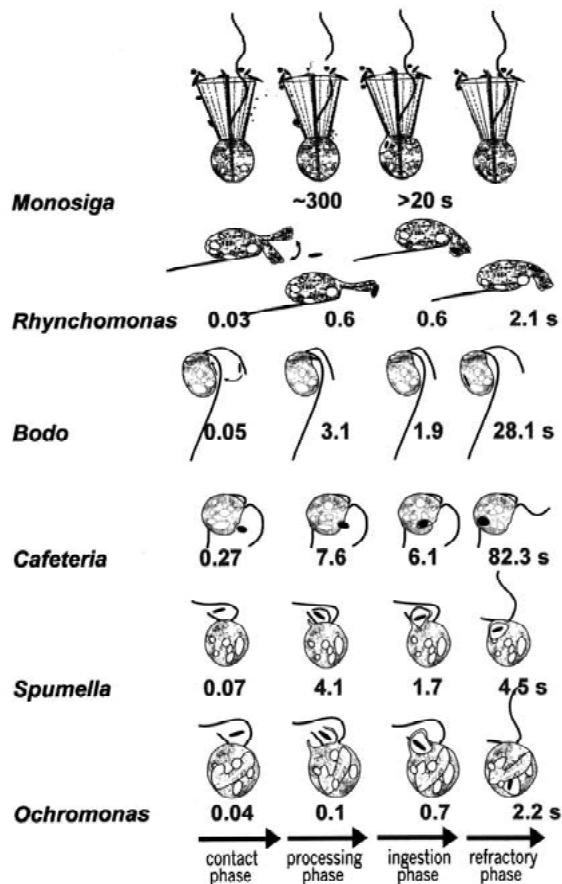


Figure 4. Comparison of average time budgets (in seconds) of feeding phases for six different bacterivorous flagellates (choanoflagellate *Monosiga ovata*, bodonids *Rhynchomonas nasuta* and *Bodo saltans*, bicosoecid *Cafeteria roenbergensis*, chrysomonads *Spumella* sp., *Ochromonas* sp.) (summary of data from Boenigk & Arndt 2000a,b).

It is important to note that the use of the term refractory phase (and with this the terms handling phase and handling time) may vary between different authors depending, e.g. on the methods and the behavioural or physiological characteristics. Fenchel (1986b) defined the time period to ingest a food particle (handling time) as the reciprocal of the maximal ingestion rate (I_{\max}). Behavioural studies showed that the mean time spent handling an individual particle (including a refractory phase after food ingestion) differs significantly from the time span as calculated from I_{\max} . This suggests that I_{\max} is not necessarily limited by the time spent handling particles but by general physiological processes. This problem counts especially for those HNF which possess filtration structures (e.g. choanoflagellates) as these organisms are able to handle

several food particles simultaneously. The handling of individual particles may last several minutes (Table 2) whereas the reciprocal I_{\max} would have been as short as several seconds.

Indigestible particles are removed from the cell by egestion generally at distinct places of the cell surface. This may happen within 2–3 min after ingestion (Boenigk et al. 2001). The average lifetime of a vacuole containing digestible bacteria seems to be about 30 minutes for flagellates (Zwart & Darbyshire 1992; own obs.).

Temporal aspects of feeding and selectivity

The time budget of a feeding flagellate can be subdivided into a time spent searching for a food particle and a time spent handling this particle. Obviously even at high particle concentrations the maximal ingestion rate is limited by the time used to handle a particle. At high particle concentrations handling time becomes dominant in the over-all time budget and the number of contacts with particles (and probably also of ingestions) is proportional to the reciprocal of the handling time (Figure 5, Boenigk & Arndt 2000a). The handling time becomes less important at low and moderate particle concentrations as the total time budget is dominated by the time spent handling. At low particle concentrations (and in flagellate species which are adapted to such conditions) one should generally expect a high efficiency in particle capture whereas the time spent handling a particle is unimportant. At high particle concentrations (and in flagellate species adapted to such conditions) capture efficiency might be lower as the capture rate can be maximised by decreasing the time spent handling individual particles (Boenigk & Arndt 2000a).

These general considerations can slightly be modified depending on whether or not active food selection is assumed. Handling a particle may include the 'decision' whether or not a particle is ingested. In this case short handling times should be even more advantageous as the flagellate is enabled to handle a high number of particles and reject the unsuitable ones (Figure 5, points A and B). Food selection in HNF has been explained mainly by passive forces such as contact probabilities or morphological limitations of the feeding apparatus. Recent studies gave evidence that also active food selection prior to food uptake can be of importance (Boenigk et al. 2001; Matz et al. 2002). Following optimal foraging the-

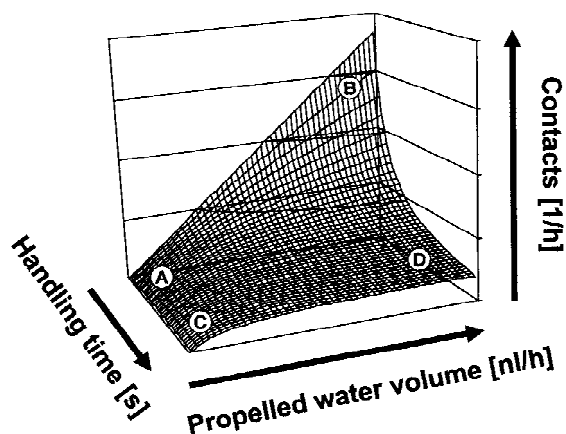


Figure 5. Model of the influence of handling time and propelled water volume on the number of contacts per hour during the feeding phases of heterotrophic flagellates. Letters point to extreme values of the time budgets (see text).

ory (for review see Stephens & Krebs 1986) short handling times and selective food uptake should be expected for high food concentrations whereas selectivity should decrease and short handling times seem not to be necessary at low food concentrations. Although a food concentration dependent selectivity has been described (Jürgens & DeMott 1995; Boenigk et al. 2002) the influence of particle concentration on handling time has not yet been investigated. However, comparative studies (see also Figure 4) hint to a rough correlation between handling time and preferred particle concentration: *Ochromonas* shows shorter handling times and a higher maximal clearance rate compared to *Spumella* and *Bodo*. Additionally, the freshwater species *Spumella* and *Bodo* show similar clearance rates as the marine species *Cafeteria*. The handling time of *Cafeteria* is significantly higher and this species seems to be less selective compared to the freshwater species. This might correlate with a more constant particle and food concentration in marine environments. The short handling times of the freshwater species should guarantee a fairly high ingestion rate on bacteria despite high concentrations of undigestible particles (e.g. clay). However, the overall significance of these considerations has not been proven. A comparison of the significant differences in the time budgets of the feeding phases even in closely related species with the effect of such variations on the feeding process (Figure 5) points to specific feeding niches of different heterotrophic flagellates.

Ingestion rates

Individual variability

Adaptations to prey abundance seem to last for several generations probably due to a time lag caused by adaptations of the cellular organelles to the new conditions: Eccleston-Parry & Leadbeater (1994) reported species-specific responses to long-term low prey densities. After starvation *Paraphysomonas imperforata* and *Bodo designis* showed lower maximum growth rates whereas *Stephanoeca diplocostata* showed a higher maximum growth rate.

Starvation and cell division have been discussed by several authors as a reason for the fact that parts of the investigated population show no food uptake (Hatzis et al. 1994; Bratvold et al. 2000; Cleven & Weisse 2001). Within one culture feeding rates can differ between individuals since freshly divided cells feed at lower rates compared to older cells (Boenigk & Arndt submitted). Bratvold et al. (2000) found subpopulations with different ingestion rates within one population of *Paraphysomonas* and *Rhynchomonas*. Flagellates should generally feed below their feeding capacity. Exposed to high food concentrations (e.g. micropatches) the flagellates can realise feeding rates which are much higher than the steady-state maximal ingestion rates. This is due to an initial phase during which food vacuole formation is not limited by digestive capabilities but only by the time used for the ingestion process itself (Boenigk et al. 2001, 2002).

Interspecific variability

Not only the size but also the concentration of food particles is known as an important factor influencing the co-occurrence of organisms. Data from literature about the incipient limiting concentration of food particles for HF are very variable for similar species and range between 10^4 and 10^7 bacteria per ml (cf. Eccleston-Parry & Leadbeater 1994). Large heterotrophic flagellates which depend on the frequency of contacts with large food items (e.g. algae) significantly increase in biomass and in their relative contribution to total HF biomass with increases in lake trophy (Mathes & Arndt 1994). Choanoflagellates which are known to be very effective filter feeders should have advantages in comparison to other flagellates when food concentrations are low. The relative contribution of choanoflagellates to HNF biomass increased from about 5% in hypertrophic lakes to about 11% in mesotrophic lakes (Auer & Arndt 2001). Bodonid

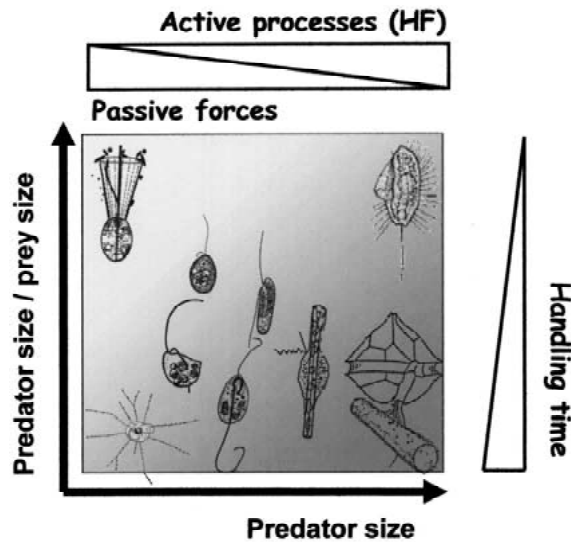


Figure 6. Hypothesis on preferable feeding mechanisms of heterotrophic flagellates in relation to predator size and the ratio of predator size to prey size (e.g. large flagellates without a filtration apparatus have to feed on large food items, flagellates have to be small when they feed on bacteria without active food concentration).

flagellates crawling on particles in the pelagial showed the opposite trend, 1% in mesotrophic and about 5% in hypertrophic lakes.

Small changes in the time budgets of feeding phases of even closely related species may lead to significant interspecific variability of feedings rates realised at certain food concentrations (cf. Fig. 5 and Table 2).

The importance of the feeding microenvironment

Even in the size range of several microns the environment consists of patches of different nutrient loadings created by substrate flocs, exsudation of organic compounds by autotrophic organisms, excretion of heterotrophic organisms, 'sloppy feeding' of metazoans and other factors. Consequently the distribution of bacteria is also patchy due to chemosensory behaviour (Blackburn et al. 1998) and their different growth rates. Bacterivorous organisms should be able to optimise their food uptake by searching for patches of high bacterial abundances. This may explain HNF survival in oligotrophic environments such as in aquifers (for review see Novarino et al. 1997) and in the deep sea (Arndt et al. *subm.*). Chemosensory behaviour of protozoa is known for a long time, but it has only recently been recognised to be important for the feed-

ing behaviour of bacterivores. Blackburn & Fenchel (1999) and Fenchel & Blackburn (1999) discussed the ability of protozoans to orient themselves in a gradient of an attractant by: (1) temporal gradient scaling, i.e. the ability to react on concentration differences before and after a certain time interval, and (2) by helical clinotaxis. Once found a patch a kinetic response of a flagellate, i.e. decreasing swimming speed, may be responsible for longer residence times in a patch. These mechanisms and combinations of them seem to allow an effective orientation over distances of a few centimetres (ciliates) or millimetres (flagellates) within a few minutes. This would allow the bacterivores to reach even short-term occurring bacterial patches. However, these mechanisms seem not to be 100% efficient and accumulation of bacterivorous protozoa may be best explained by satiation kinetics due to chemosensory attraction and 'diffusive' loss (Fenchel & Blackburn 1999). The significance of this 'searching behaviour' seems to increase when the cultures are starved (Bratvold et al. 2000; Boenigk et al. 2002). Furthermore, the food selectivity can change with the degree of starvation as could be shown by Jürgens & DeMott (1995).

Conclusions

Many earlier studies have treated bacterivorous protozoans as a 'black box'. Even more recent studies mostly report high taxonomic levels only, thus neglecting the fact that species of similar size possess species-specific ecological characteristics (Stensdotter-Blomberg 1998; Boenigk & Arndt 2000a; Cleven & Weisse 2001). The increasing knowledge on species-specific interactions between bacteria and protozoans indicates the necessity for species-specific investigations. The range of feeding responses is high. The complex micro-environments of HNF support the coexistence of many bacterivorous flagellate species (Figure 6). Handling time will be long when the size of the prey is large compared to the prey and specific filtration structures are necessary when handling time has to be reduced to obtain enough food. It is probably not possible to define a distinct food resource and a distinct 'feeding niche' for a flagellate species as feeding behaviour depends on biotic and abiotic environmental factors. Even the species-specific 'typical' microhabitat may be subject to active or passive (drift) changes. Figure 7 illustrates that the smallest heterotrophic eukaryotes possess a variety of not yet

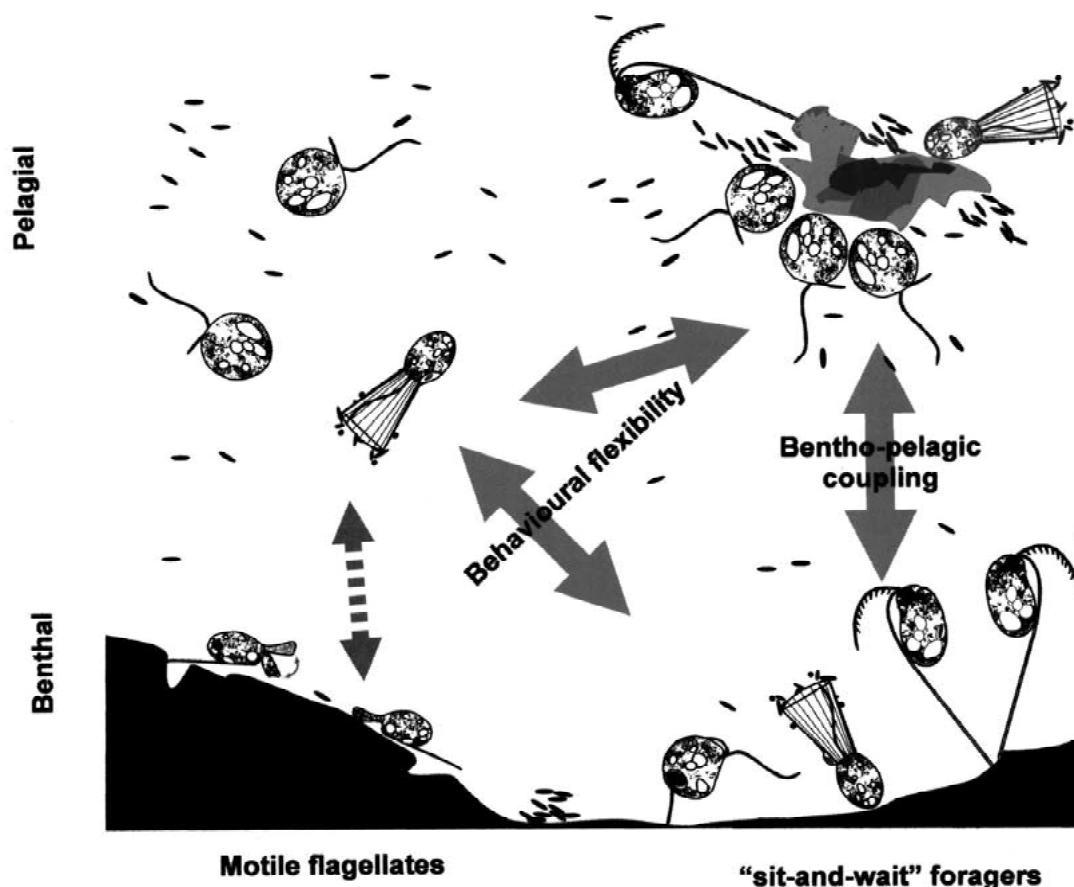


Figure 7. Feeding habits of bacterivorous flagellates (see text).

fully understood feeding mechanisms to select for specific bacteria, microhabitats can rapidly change from benthic to pelagic sites and vice versa (e.g. Garstecki et al. 2000). Behavioural flexibility may add to the diversity of bacterivory. The relatively low number of heterotrophic flagellate species known up to now (morphological approach) does not indicate a uniform grazing pressure on bacteria. The significant flexibility of the grazing impact of bacterivorous flagellate communities creates a complex top-down pressure on bacteria that should at least be partly responsible for the great diversity observed for bacteria. The great variety and flexibility of the effective flagellate bacterivory should be responsible for the evolution of effective predator avoidance mechanisms in bacteria (cf. Jürgens & Matz 2002). We are only at the beginning to get an inside into the 'arms races' on both sides.

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