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The significance of inter- and intraspecific variation in bacterivorous and herbivorous protists

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Abstract

This paper reviews the emerging evidence on the significance of inter- and intraspecific variation in the feeding behaviour of aquatic protists. Small heterotrophic nanoflagellates (HNF) have been identified as the primary bacterial consumers in most aquatic environments. Recent research using novel techniques such as flow cytometry and high resolution video microscopy revealed that their feeding strategies and grazing rates are diverse. There is an important conceptual difference between uptake rates measured in short-term (min to h) experiments and grazing rates averaged over a longer-term (d). This is because the latter are strongly affected by digestion rates which are species-specific, i.e. the same bacterial prey may be digested differently by various grazers, and the same predator may selectively digest variable prey. Planktonic ciliates are the most important algal consumers in many lakes and marine systems. Large species-specific differences in their feeding behaviour and growth rates have been documented for closely related species. Intraspecific variation, which is, most likely, caused by varying clonal composition may be as important as interspecific variation. Finally, there is some evidence that the individual variability within a given population is generally large, both among bacterivorous HNF and among herbivorous ciliates. The consequences of this diversity becoming apparent at the levels of the species, population, clone and individual need to be considered by aquatic ecologists in their conceptual models.

Introduction

The significance of planktonic protists in pelagic food webs was recognized in the 1970s and the early 1980s (Pomeroy 1974; Williams 1981; Azam et al. 1983; Porter et al. 1985); it became apparent that bacterivorous and herbivorous protists were more numerous and thus played a large role for the cycling of matter. In the years following this conceptual change, investigations on heterotrophic protists focused on quantifying their significance for carbon flow and nutrient recycling (e.g. Sherr et al. 1983; Sherr & Sherr 1984; Caron et al. 1985; Güde 1986). Due primarily to insufficient techniques but also to arrive at general conclusions, many microorganisms that are, at best, remotely related were lumped together into presumed functional guilds such as ‘heterotrophic bacteria’, ‘heterotrophic nanoflagellates’ (HNF) or ‘microzooplankton’ in both field studies (Riemann et al. 1986; Sanders & Porter 1986; Nagata 1988; Dolan & Gallegos 1991) and ecosystem models (Ducklow 1991; Wylie & Currie 1991; Gaedke & Straile 1994). These field studies have been complemented by laboratory investigations on a few selected protozoan species (e.g. Fenchel 1982; Sherr et al. 1983; Caron & Dennett 1986).

With the increasing knowledge and the advent of novel techniques it became apparent that broad trophic categories such as HNF do not adequately describe the ecological complexity and the diversity of the ecological niches of aquatic protists (Arndt & Berninger 1995; Sherr & Sherr, this volume). It is now accepted that the nutritional ecology of HNF, dinoflagellates, and ciliates is diverse and in general poorly characterized (Arndt et al. 2000). The feeding strategies of these and other taxonomic groups have been reviewed extensively (Jacobson & Anderson 1986; Beaver & Crisman 1989; Lessard 1991; Sanders 1991; Pierce & Turner 1992; Schnepf & Elbrächter 1992; Laybourn-Parry 1992; Arndt 1993; Arndt et al. 2000) and shall not be repeated here. The primary goal of this paper is to illustrate the significance of species-specific differences in the feeding of bacterivorous and herbivorous protists using some typical case studies. The second aim is to draw attention to the potential significance of intraspecific ecophysiological variation in planktonic protists. The advent of sophisticated techniques, such as flow cytometry, not only permits the study of species with great precision in a relatively short time, but also allows for the assessment of the variation within a given population. Similar to the larger metazooplankton, it has become possible to measure properties of individual microorganisms, i.e. to study single cells and to assess the variation around the mean or median within a population. The potential and consequences of these novel possibilities have not yet been explored. I will, therefore, provide examples to elucidate that neglect of this type of natural variation will bias our understanding of microbial food web processes. For the sake of better understanding, and because different methods have been applied to study bacterivory and herbivory, I will discuss these aspects independently for bacterivorous and herbivorous protists.

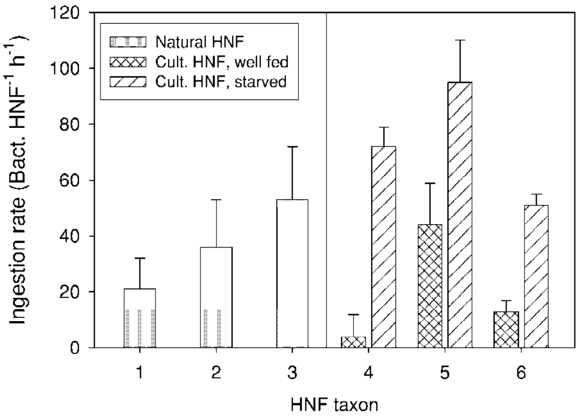

Figure 1. Cell-specific bacterial ingestion rates of heterotrophic nanoflagellates (HNF). The left panel (taxa 1 to 3) shows natural HNF taxa from a eutrophic reservoir; their bacterial grazing rates were measured by the uptake of fluorescently labeled bacteria (data from Šimek et al. 1997a). The right panel (taxa 4 to 6) denotes cultured species which were either starved or well fed with the bacterium *Pseudomonas putida*; bacterial ingestion rates were measured by video microscopy in this study (modified from Boenigk et al. 2001c). Error bars denote 1 standard deviation (SD). Numbers of taxa denote *Spumella*-like chrysomonads (1), Bodonids (2), choanoflagellates (3), *Cafeteria roenbergensis* (4), *Ochromonas* sp. (5) and *Spumella* sp. (6).

Bacterivorous protists

Intergeneric and interspecific differences among heterotrophic nanoflagellates

Various techniques have been used to study the uptake of planktonic bacteria by bacterivorous protists. A common method that detects species-specific differences uses fluorescently labeled bacteria (FLB) or cyanobacteria (Sherr et al. 1987; Sherr & Sherr 1993) in short term incubations: the number of FLB in the food vacuoles of a predator after a defined incubation period provides an uptake rate. This method is a refinement of an earlier approach which used inert fluorescent microspheres as an index of bacterial uptake rates by heterotrophic flagellates and ciliates (Børsheim 1984; McManus & Fuhrman 1986; Pace & Bailiff 1987). The major advantages of the FLB technique is that the labeled bacteria can be counted in the food vacuoles of the bacterial predators at the single cell level and that natural bacteria can be used. The pros and cons of this and other bacterial labeling techniques have been discussed elsewhere (McManus & Okubo 1991; Sherr & Sherr 1993; Landry 1994; Vaqué et al. 1994; Boenigk et al. 2001a) and shall not be repeated here.

Some differences in the bacterial uptake rates by various planktonic HNF taxa have been already noted by researchers using fluorescent latex beads and live observations during the 1980s (Table 1). Recent investigations conducted with the FLB technique further suggested the existence of taxon-specific differences among morphologically similar HNF (Hwang & Heath 1997; Šimek et al. 1997a; Cleven & Weisse 2001). Due to the large statistical error inherent in the FLB approach (Cleven & Weisse 2001; Cho et al. 2000, discussed below), caution is needed if studies from different environments, using different experimental protocols such as the ones listed in Table 1, are to be compared. Further, the taxonomic identification of HNF species is notoriously difficult (e.g. Arndt et al. 2000), and some taxa such as the genus *Spumella* are currently poorly described and seem to be underclassified (Preisig et al. 1991; Bruchmüller 1998). The various *Spumella* sp. listed in Table 1 are, therefore, not necessarily closely related to each other.

It appears, however, relatively safe to compare results obtained within a given study such as the one from eutrophic Římov reservoir, South Bohemia. Šimek & colleagues (Šimek et al. 1997a) found more than twofold differences in the average bacterial ingestion rate of choanoflagellates and small chrysomonads (Figure 1, left panel). Similar relative differences between

Table 1. Ingestion rates of heterotrophic nanoflagellate taxa obtained by various tracer methods and direct observations (FLB=fluorescently labeled bacteria, F=Freshwater, M=Marine). Ranking is based on taxonomy of the flagellates

Method	Taxon	Origin	Ingestion rate (bact. cell^{-1} h^{-1})	Reference
microspheres	*Spumella* sp.	F	3 – 23	Sanders et al. (1989)
live observations	*Spumella* sp.	F	14 – 38	Holen & Boraas (1991)
FLB	*Spumella* sp.	F	10 –15	Jürgens & Güde (1991)
FLB	*Spumella* sp.	F	10–14	Hwang & Heath (1997)
FLB	*Spumella*-like	F	21 ± 11$^{\#}$	Šimek et al (1997)
FLB	*Spumella* sp. (2–6 μm)	F	0 – 5	Cleven & Weisse (2001)
FLB	*Spumella* sp. >6 μm	F	6 – 31	Cleven & Weisse (2001)
microspheres	*Monas*-like$^{\$}$	F	1.6 – 27	Carrias et al. (1996)
microspheres	Chrysomonadida*	F	0.1 – 0.7	Pace & Bailiff (1987)
microspheres	*Ochromonas* sp.	F	2 – 53	Sanders et al. (1989)
cultured bact.	*Ochromonas* sp.	F	190§	Fenchel (1982)
cultured bact.	*Paraphysomonas vestita*	M	254§	Fenchel (1982)
cultured bact.	*Actinomonas mirabilis*	M	254§	Fenchel (1982)
cultured bact.	*Pseudobodo tremulans*	M	84§	Fenchel (1982)
microspheres	*Dinobryon bavaricum*	F	8 – 38	Sanders et al. (1989)
microspheres	*Dinobryon cylindricum*	F	6 – 12	Sanders et al. (1989)
FLB	*Katablepharis* sp.	F	0 – 5	Cleven & Weisse (2001)
FLB	Bodonids	F	36 ± 17$^{\#}$	Šimek et al (1997)
cultured bact.	*Pleuromonas jaculans*	F	54§	Fenchel (1982)
cultured bact.	*Monosiga* sp. (choanofl.)	M	107§	Fenchel (1982)
microspheres	Choanoflagellates	F	8 – 42	Sanders et al. (1989)
minicells	Choanoflagellates	F	13 – 73	Vaqué & Pace (1992)
microspheres	Choanoflagellates	F	1.7 – 33.6	Carrias et al. (1996)
FLB	Choanoflagellates	F	53 ± 19$^{\#}$	Šimek et al (1997)
FLB	Choanoflagellates	F	13 – 37	Hwang & Heath (1997)
FLB	Choanoflagellates	F	0 – 11	Cleven & Weisse (2001)

*Chrysomonadida = Heterokonta, e.g. *Spumella sp.*; $^{\$}$*Monas* syn. with *Spumella* (Preisig et al. 1991); §maximum value; $^{\#}$mean value ± 1 SD.

bacterial ingestion rates of major HNF taxa and some indication for seasonal shifts in bacterivory have been reported from mesoeutrophic Lake Constance, Germany (Cleven & Weisse 2001) and from eutrophic Lake Oglethorpe, Georgia (Sanders et al. 1989). Recent evidence using high resolution video microscopy also indicated species-specific differences in the average bacterial uptake rates among bacterivorous HNF (Boenigk & Arndt 2000a, b). The mean ingestion rate of several small HNF species was, however, highly variable and was dependent on both the nutritional status of the flagellates investigated and the species examined (Figure 1, right panel). Ingestion rates for the starved flagellates were calculated from the initial 5 min of the experiment. This study confirmed earlier findings obtained by the FLB technique (Jürgens & DeMott 1995). Small *Spumella*-like chrysomonads, although their mean per capita ingestion rates may be lower than those of choanoflagellates, seem to be the most important bacterial consumers in many lakes because they belong to the most abundant HNF taxa and feed, primarily or exclusively, on heterotrophic bacteria (Weisse 1997; Šimek et al. 1997a; Cleven & Weisse 2001).

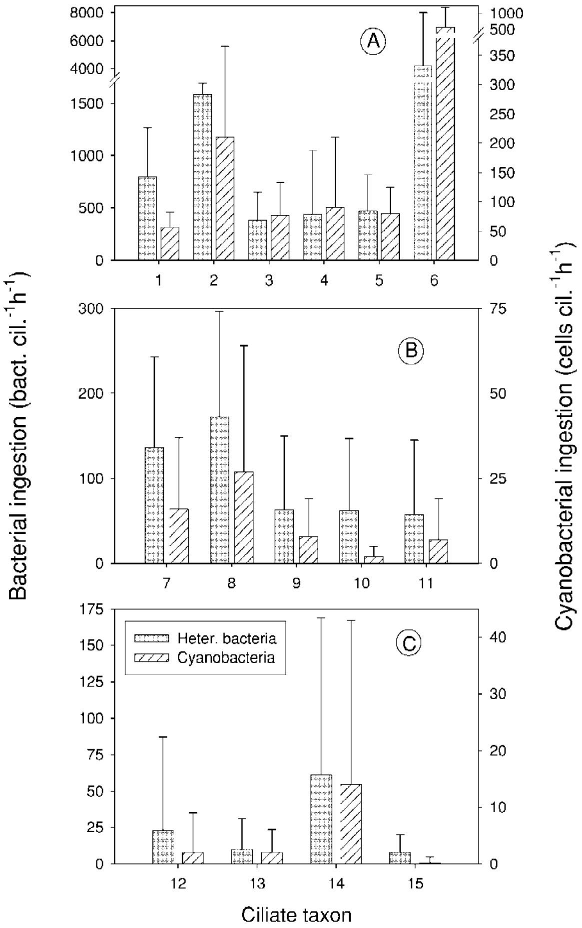

Figure 2. Cell-specific ingestion rates of bacteria (left *Y*-axis) and cyanobacteria (right *Y*-axis) of 15 natural ciliate taxa determined from the uptake of fluorescently labeled prey (data from Šimek et al. 1996). The ciliates were classified as highly efficient fine suspension feeders (A), less efficient fine suspension feeders (B), and raptorial feeders (C). Error bars denote 1 SD. Numbers of taxa denote *Pelagohalteria viridis* (1), *Halteria grandinella* (2), *Strobilidium hexakinetum* (3), an unidentified oligotrichous ciliate (4), *Cyclidium* sp. (5), *Vorticella aquadulcis* complex (6), *Pelagostrombidium fallax* (7), *Cyrtolophosis mucicola* (8), *Coleps* sp. (9), *Coleps spetai* (10), *Cinetochilum margaritaceum* (11), *Urotricha* spp. assemblage 1, composed to >90% of *U. furcata* (12), *Urotricha* spp. assemblage 2, composed to >60% of *U. furcata* (13), another small, unidentified *Urotricha* sp., (14), and *Balanion planctonicum* (15).

Other bacterivorous protists

Bacterivory by autotrophic flagellates in several algal classes is common in freshwater and marine systems (for review see Sander & Porter 1988; Caron 2000; Jones 2000). Wide ranges of bacterial uptake rates have also been reported for bacterivorous ciliates (Sherr & Sherr 1987; Sherr et al. 1989; Sanders et al. 1989; Šimek et al. 1995, 1996). The statistical reliability may be higher in FLB studies with ciliates because, in contrast to HNF, the frequency distribution of ingested FLB in ciliates does not seem to deviate significantly from a normal distribution, and the per capita ingestion of ciliates can be several orders of magnitude higher than that of HNF (Sanders et al. 1989; Šimek et al. 2000). Šimek & co-workers (1996) found large interspecific and intraspecific differences among picoplanktivorous freshwater ciliates (Figure 2). The ciliate species were grouped into three categories with decreasing ability to feed upon picoplankton-sized particles. Irrespective of the efficiency of the picoplankton ingestion, the standard deviation of the ingestion rates obtained for each taxon was large. In some cases, such as the small *Urotricha* spp. or *Halteria grandinella*, for which significantly different bacterial ingestion rates have been reported from various freshwater environments (Sanders et al. 1989; Šimek et al. 1995, 2000 and references therein), this may reflect species-specific differences; i.e., some similar species can not be identified unequivocally in fixed samples (Foissner et al. 1999; Šimek et al. 2000). However, the large scattering around the mean value remained even in cases where the species identity was unequivocal.

Bacterial feeding by other protists, such as the freshwater amoebae, has been studied only little (Weisse & Müller 1998), and, therefore, species-specific differences remain speculative. Selective use of certain bacterial strains has, however, been demonstrated for amoebae (Arndt 1993 and references therein). Most sarcodines such as the limnic heliozoans and the marine sarcodines, mainly radiolarians and foraminifera, seem to be omnivores and prefer larger food items than bacteria (reviewed by Swanberg 1983; Caron & Swanberg 1990; Arndt 1993).

Species-specific impact on the natural bacterial assemblage

Intense grazing by many flagellates and some ciliates may cause structural and genetic shifts in the bacterial assemblage (Turley et al. 1986; Šimek et al. 1997b; Pernthaler et al. 1997b; Šimek et al. 1999; Van Hannen et al. 1999). For instance, several bacterial strains respond to heavy grazing pressure by HNF by forming large filaments or colonies that cannot be ingested by HNF (Güde 1979; reviewed by Güde 1989; Jürgens et al. 1994; Hahn & Höfle 2001). Shifts in the phylogenetic bacterial composition may also occur when grazing pressure by bacterivores is relieved

(Suzuki 1999). Such bacterial responses to grazing pressure is apparently mutually taxon-specific; only some bacteria form grazing-resistant filaments and/or aggregates, and only certain protist species induce these changes. There is still little known about the specificity of the bacterial defense mechanisms. Evidence has only been obtained in laboratory cultures; no data on the influence of flagellate grazing on the morphology of single bacterial species are available from environmental studies (Hahn & Höfle 2001).

Taxonomic shifts in the natural bacterial assemblage have been documented using group-specific genetic markers, i.e. oligonucleotide probes (Pernthaler et al. 1997a; Šimek, et al. 1999; Jürgens et al. 1999), and other molecular techniques (see Hahn & Höfle 2001 for review). However, the resolution of these studies is currently hampered by the lack of specificity of the genetic bacterial markers. The development of strain-specific bacterial probes will undoubtedly enhance the understanding of the mutual effects between bacterivous protists and their bacterial prey in the near future.

Variability of bacterivores at the level of clones and individuals

The variability in the bacterial feeding of heterotrophic protists at the clonal and the cell level is virtually unknown. The large variations reported in almost every study that measured bacterial ingestion rates by flagellate and ciliate taxa, irrespective of the method used (Table 1), may indicate the significance of individual variability in the feeding process. Investigations using the FLB technique commonly find a large fraction of non-feeders among HNF species and a few cells which have ingested more than twice the average FLB number (Cleven & Weisse 2001; Bratvold et al. 2000 and references therein). These high values will have a significant effect on the calculated ingestion rate and their inclusion or rejection as outliers should be based on rigorous statistical methods (Cleven & Weisse 2001). The existence of those cells which deviate from the majority of the cells in the population is, however, an indication of individual variability. Most studies using bacterial prey surrogates report the calculated arithmetic mean values only. The mean value usually corresponds to the slope of the linear regression of ingested FLB per protist versus time. For theoretical reasons, in particular considering the non-normal distribution of the HNF with ingested FLB (McManus & Okubo 1991; Bratvold et al. 2000; Cleven & Weisse 2001), it is questionable if the mean bacterial uptake rate thus calculated is representative for the HNF population under study. The coefficient of variation of the slopes from duplicate experimental bottles ranged from 0.1 to 63% in an oligotrophic marine environment (Cho et al. 2000). Note that this variation denotes differences between replicate samples, it should not be confused with the individual variability within a given population (see below). Due to the large uncertainty involved in the calculation of mean ingestion rates (Cleven & Weisse 2001), it may be questioned if the reported large variation among populations of bacterivores (Figures 1 and 2) is real.

Microscopic observations from video microscopy support the large individual variability (Figure 1), which was apparent even within flagellate populations of comparable nutritional status and which could not be reduced by enlarging the sample size (Boenigk & Arndt 2000a, 2000b). Boenigk & Arndt (2000a) suggested that the high variability in size of prey and predator, ingestion rate and time budget of the feeding phases of HNF taxa may be typical of exponentially growing laboratory cultures which contain smaller, freshly divided cells and larger cells close to the next cell division. However, the variation in cell size is generally large among planktonic protists and is primarily dependent on their nutritional status and the ambient temperature (Jakobsen & Hansen 1997; Weisse & Kirchhoff 1997; Müller & Schlegel 1999; Weisse et al. 2001). We can, therefore, assume that the individual, intraspecific variability of protist feeding is even larger in the natural situation than under standardized laboratory conditions. It is, however, important to consider that most of the investigations that reported large individual variability used short-term experiments, ranging from minutes to hours. The individual variability may be considerably reduced if the grazing is averaged over a longer period.

Studies using flow cytometry further suggest that the scattering around the mean or median of the parameters such as forward scatter, side scatter and stain-induced fluorescence, commonly taken to characterize bacteria and bacterivores, is generally large (Vazquez-Dominguez et al. 1999; Bratvold et al. 2000; Lindström et al. 2002). The potential of flow cytometry to measure the variability of the uptake of bacteria by heterotrophic protists has not yet been explored. The few studies that have applied flow cytometry to measure bacterial grazing rates usually report mean uptake rates only (e.g. Lavin et al. 1990; Vazquez-Dominguez et al. 1999; Bratvold et al. 2000). This is surprising as

Gerritsen et al. (1987) over a decade ago used flow cytometry to analyze the individual feeding variability in the bacterivorous ciliate *Cyclidium* sp. A new protocol that has been published recently (Lindström et al. 2002) may facilitate the application of flow cytometry in future studies with cultured heterotrophic protists.

Are there principal differences in species-specific bacterivory between marine and freshwater ecosystems?

The existing evidence for species-specific differences of bacterial feeding reported above originated primarily from freshwater environments, although four out of the six species investigated by Fenchel (1982), in his pioneering work on HNF, were marine (Table 1). There is less information that compares marine flagellate or ciliate species in their ability to feed upon and digest bacteria and cyanobacteria. Using the FLB approach, Sherr et al. (1988) have reported differences in bacterial uptake and digestion rates between the scuticociliate *Uronema marina* and a small species of *Strombidium*. The two ciliate species had bacterial ingestion rates ranging from 380 to 1095 bact. cil^{-1} h^{-1}, depending on temperature. At the same experimental temperature (22 °C), the bacterial uptake of the strombidiid was 825 bact. cil^{-1} h^{-1}, compared to 710 bact. cil^{-1} h^{-1} of the scuticociliate. Bacterial ingestion rates of a mixed HNF assemblage ranged from 5.2 to 27.4 bact. HNF^{-1} h^{-1}, i.e. their per capita ingestion rates were several orders lower than those of the ciliates. Sherr et al. (1988) also found some differences in the digestion rate among the flagellates and the two ciliate species studied. Similarly, Nygaard et al. (1988) obtained a range of differences in the ability to discriminate between latex beads and bacteria (*Pseudomonas putida*) for eight species of marine HNF. It, therefore, appears that group- and species-specific differences occur to similar extents in marine and freshwater protists, although this has been better documented for freshwater taxa.

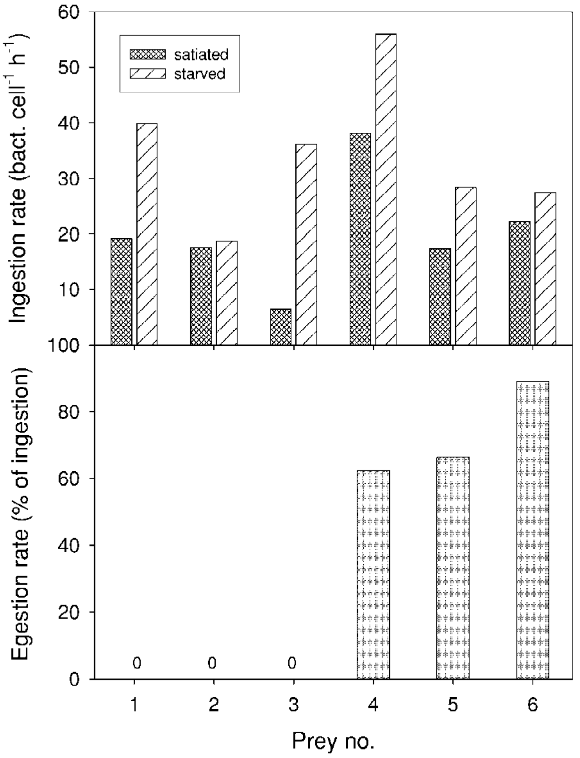

Figure 3. Cell-specific ingestion and egestion rates of the cultured heterotrophic nanoflagellate *Spumella* sp. (modified from Boenigk et al. 2001c). The upper panel shows ingestion rates for satiated (averaged over 0–15 min) and starved (0–5 min) flagellates, the lower panel the percentage of the ingested particles which were egested within the first 10 min of the experiments by the satiated flagellates. Prey numbers denote *Pseudomonas putida* (1), bacterial strain CM10 (2), *Bacillus subtilis* (3), *Synechococcus* sp. strain BO8809 (4), *Synechococcus elongatus* (5), and latex beads of 0.22 μm diameter (6).

What causes food selection by bacterivorous protists?

Although the mechanisms responsible for food selection are not yet well understood (Boenigk & Arndt 2000b), a suite of processes seems to be involved during the uptake and handling of prey particles. Heterotrophic nanoflagellates and ciliates may select their prey based upon size (Chrzanowski & Šimek 1990; Gonzales et al. 1990), motility (Monger & Landry 1992; Gonzalez et al. 1993), electrical charge (Hammer et al. 1999), surface hydrophobicity (Monger et al. 1999), and chemical cues. Many protists have chemosensory capabilities (e.g. Spero 1985; Sibbald et al. 1987; Bennett et al. 1988; Verity 1988) which they use to select different food items (Pace & Bailiff 1987; Nygaard et al. 1988; Landry et al. 1991). Application of video microscopy by Boenigk and co-workers showed that selection of bacterial prey by HNF species is based upon differences during the contact, processing, ingestion, and digestion phase. Digestibility seems to be a major criterion determining the fate of ingested particles (Gonzales, et al. 1990; Boenigk et al. 2001c). The example shown in Figure 3 illustrates that a freshwater *Spumella* sp. ingested three different bacterial strains, two cyanobacterial strains (*Synechococcus* spp.), and 0.22 μm^3-sized latex during the first 10 min of the experiment. The (cyano)bacterial strains ranged in size from 0.26 to 0.61 μm^3. Boenigk et al. (2001c) found some variation in the flagellate ingestion rates of the various food items offered, and initial uptake rates of starved *Spumella* were, in each case, higher than those of satiated specimens (Figure 3, upper panel). The ingestion rate of the starved cells decreased, however, with incubation time (not shown). While the bacterial strains were digested inside the food vacuoles, the majority of the cyanobacteria, and almost all of the beads were egested within the first 10 min of the experiment (Figure 3, lower panel). These findings challenge the accuracy of the FLB and other tracer techniques for measuring bacterial grazing rates; although prey surrogates such as FLB and latex particles may be ingested at rates comparable to those of natural bacteria, they were egested after a food vacuole passage time of only 2–3 min (Boenigk et al. 2001a–c).

The photoautotrophic chroococcoid cyanobacteria of the *Synechococcus* type are also ingested and digested by various protozoa (Bernard & Rassoulzadegan 1990, Šimek et al. 1995, 1996; Müller 1996; Dolan & Šimek 1997; Assmann 1998; Boenigk et al. 2001c). The mechanisms by which some protozoa are able to digest certain cyanobacterial strains which appear unpalatable or even harmful for other protozoa (Assmann 1998) are at present unknown. Similarly, it is an open question if the entire population of a given protist species is affected in a similar way by a harmful cyanobacterial strain (Assmann 1998).

Conclusions

It has become obvious that large, species-specific differences exist among the dominant bacterivorous protists in their ability to feed upon and digest heterotrophic bacteria and photoautotrophic cyanobacteria. Bacterial ingestion rates may vary by several orders of magnitude between protist species (Table 1, Figure 2). Heterotrophic nanoflagellates, in particular, are more selective than previously assumed. The same bacterial strain may be positively selected for by some protists and rejected by others. The assumption of a functional guild of bacterivorous flagellates that behave more or less identically with respect to their feeding impact on the planktonic bacteria is no longer valid. Among ciliates, there are some specialists such as scuticociliates and the oligotrich *Halteria* that can thrive on a picoplankton diet (Šimek et al. 1995, 1996, 2000) and may ingest bacteria and cyanobacteria at rates which are several orders of magnitude higher than those of the smaller HNF taxa. However, the majority of ciliate species prefers larger food items such as nanoplanktonic algae and HNF (see below). Large individual variability has become apparent using tracer techniques and direct observations for both HNF and ciliates. Intraspecific differences seem to be of similar magnitude or may be even larger than differences between similar bacterivorous species. The causes and consequences of this intraspecific variation are an area for future research.

Herbivorous protists

Numerous studies revealed the significance of planktonic protozoa as algal predators in lakes and in the ocean (summarized by Weisse & Müller 1998; Sherr & Sherr, this volume). Their quantitative importance can, permanently or seasonally, exceed that of the various metazooplankters (e.g. Verity 1986; Weisse et al. 1990; Edwards et al. 1999) with whom they compete for the same algal food. The feeding strategies are highly diverse among herbivorous protists (Jacobson & Anderson 1986; Fenchel 1987; Gaines & Elbrächter 1987; Lessard 1991; Sanders 1991; Arndt et al. 2000). In spite of the wealth of information documenting protozoan herbivory, however, there are relatively few studies that compared the feeding behaviour of several protozoan species in response to the same algal food.

Species-specific differences among ciliates and dinoflagellates

Species-specific differences of growth and grazing rates are convincing for ciliates and dinoflagellates. Among ciliates, naked oligotrichs, tintinnids, and prostome ciliates that dominate numerically in aquatic food webs (Laybourn-Parry 1992; Finlay & Fenchel 1996; Weisse & Müller 1998) have been studied in detail. It has become obvious that even a taxonomically well defined group such as the oligotrichs cannot be considered a single functional group in terms of growth and grazing rates (Montagnes 1996). Montagnes (1996) summarized results from 19 studies with 12 identified and several unidentified species in the genera *Strobilidium* and *Strombidium.* Growth parameters such as maximum growth rates and threshold food concentration, where population net growth is zero, differed by more than one order of magnitude among the species investigated. The

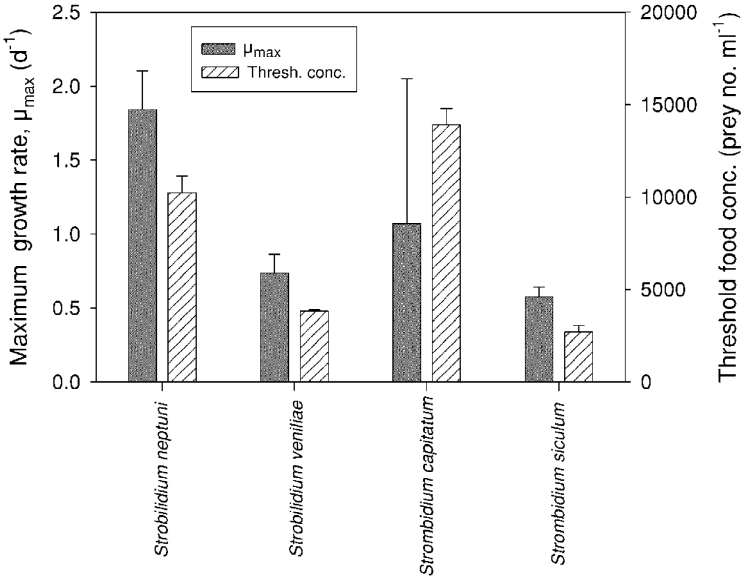

Figure 4. Maximum growth rates (left Y-axis) and threshold food concentrations (rigth Y-axis) of four oligotrichous marine ciliates maintained in laboratory cultures (data from Montagnes 1996). The ciliates were fed their preferred nanophytoflagellate prey. Error bars denote standard error (SE).

threshold concentrations reported for these oligotrichs covered the whole range of threshold concentrations known for heterotrophic dinoflagellates and planktonic ciliates (reviewed by Jakobsen & Hansen 1997). Montagnes (1996) also showed that the growth response of four species in the genera *Strobilidium* and *Strombidium*, fed three species of phytoflagellates, varied considerably under identical laboratory conditions. Even with the optimum food for each species, species-specific differences were significant (Figure 4).

Similar species-specific differences were found when the feeding behaviour of three common freshwater ciliates was investigated in response to cryptophyte and diatom prey (Müller & Schlegel 1999). The filter feeding oligotrichous ciliate *Strobilidium lacustris* (syn. *Rimostrombidium lacustris*, see Foissner et al. 1999), the prostome ciliate *Balanion planctonicum* and the diffusion feeding scuticociliate *Histiobalantium bodamicum*, which had been all isolated from the same mesoeutrophic lake (Lake Constance, Germany), could be cultivated on a small cryptophyte, *Cryptomonas* sp., whereas the similar sized centric diatom *Stephanodiscus hantzschii* did not support their growth. In spite of their similar feeding preference, ingestion rates, growth rates and threshold concentrations all varied species-specifically (Müller & Schlegel 1999). The obvious differences in major ecophysiological parameters of these three sympatric ciliate species had been interpreted earlier as examples of ‘r- and K-selection’ (Sommer 1981; DeMott 1989) among planktonic ciliates: along the r/K continuum, *B. planctonicum* and *S. lacustris* seem to be typical r-strategists with the ability to exploit short-lived algal blooms; the slower growing *H. bodamicum* is more of a K-strategist which may become a superior competitor during periods of low algal concentrations (Müller & Weisse 1994). The numerous studies on the ecology and ecophysiology of planktonic ciliates in Lake Constance have been summarized recently (Weisse & Müller 1998).

Planktonic heterotrophic dinoflagellates have adapted to various environmental conditions by developing sophisticated, and in some cases highly specialized, feeding strategies (Jacobson & Anderson 1986; Gaines & Elbrächter 1987; Lessard 1991; Sanders 1991; Schnepf & Elbrächter 1992). Species-specific differences in their nutritional ecology are, therefore, obvious. The small heterotrophic dinoflagellates (approximately 5 – 30 μm), in particular, may compete with ciliates for nanoflagellates (Strom 1991; Neuer & Cowles 1995; Jakobsen & Hansen 1997), but not much is known on their prey selectivity. Size-selectivity has been demonstrated both for marine (Strom 1991;

Hansen 1992; Hansen et al. 1996; Jakobsen & Hansen 1997) and for freshwater species (Weisse & Kirchhoff 1997).

Variability among populations and clones

There is increasing evidence that species-specific eco-physiological differences are pronounced even among closely related ciliate species (Weisse & Frahm 2001, 2002; Weisse et al. 2001). Recent research further suggests that intraspecific differences at the population or clone level are important among exclusively or primarily asexually reproducing ciliates. Differences in growth rates of geographically distant clones by a factor of two to three have been demonstrated both for planktonic marine (Perez-Uz 1995) and freshwater species (Weisse & Montagnes 1998; Montagnes & Weisse 2000, Weisse & Lettner 2002). The intraspecific differences seem to be of comparable magnitude as differences between closely related ciliate species of the same genus or even between similarly sized species belonging to different genera (Weisse & Montagnes 1998; Weisse et al. 2001; Weisse & Lettner 2002). Note that these intraspecific differences are real and do not reflect sub-clonal differences caused by clonal ageing in laboratory cultures (see Montagnes et al. 1996).

Evidence emerges that intraspecific variability is also common in grazing rates and feeding related parameters such as growth efficiency (Weisse et al. 2001). Feeding rates have been investigated recently for four small prostome ciliate species and four strains of the same species, *Balanion planctonicum* (Jakobsen & Hansen 1997; Müller & Schlegel 1999; Weisse et al. 2001). The maximum ingestion rates of three *B. planctonicum* strains isolated from the same lake (L. Constance, Germany) were highly variable when feeding on a small *Cryptomonas* species and comparable to feeding rates of another isolate of the same species from a different lake, and to grazing rates of the marine *Balanion comatum* and two other freshwater species of the genus *Urotricha* (Figure 5). This variation became apparent although all *B. planctonicum* strains had been investigated under virtually identical laboratory conditions, i.e. the food species, food level and experimental temperature used were the same or very similar (Weisse et al. 2001). This holds also true for two *Urotricha* species (Figure 5). The marine *B. comatum* was fed a marine cryptophyte, *Rhodomonas salina*, which is of comparable size to the freshwater *Cryptomonas* sp., and experiments were conducted at the same temperature (15 °C) used for the freshwater species. Note that estimates of the standard error of the maximum ingestion rates are unavailable for *Balanion* strains no. 1 and 5. In the first case, ingestion rates were recalculated from Müller's (1991) original data, without correcting for changes of prey concentration occurring in controls without ciliates during the experimental period. In all other experiments, maximum ingestion rates were derived from functional response curves reported in the respective studies; Jakobsen & Hansen (1997) did not report the standard error for the marine *B. comatum* in their study. The standard error of the maximum ingestion of *B. comatum* should, however, have been comparable to that of the other 5 prostome ciliate strains investigated, i.e. it probably was in the order of 10 – 25% of the estimate. The reason for the exceptionally high ingestion rate of the second *B. planctonicum* strain from Lake Constance is unknown. A possible explanation is that the *Balanion* investigated by Müller & Schlegel (1999) was larger than the original isolate from the same lake. The volume of *Balanion* changes with food supply and temperature (Müller 1991; Jakobsen & Hansen 1997) and may also change by a factor of two between clones (Weisse et al. 2001).

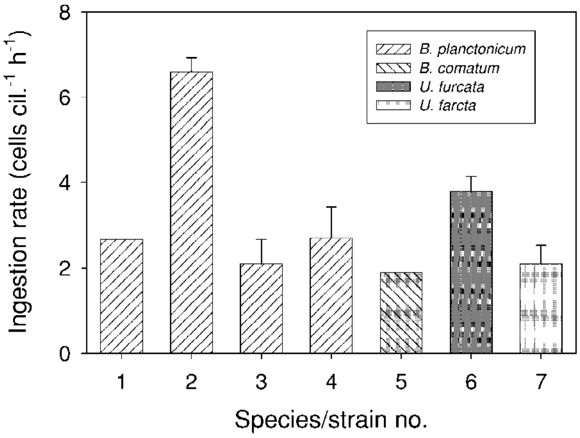

Figure 5. Maximum ingestion rates of four cultured herbivorous prostome species (data from Weisse et al. 2001). Error bars denote SE. For the freshwater species *Balanion planctonicum*, four different strains have been investigated (1 – 4) Strain numbers denote *B. planctonicum* isolated from Lake Constance (L. C.) in 1989 (Müller 1991; 1), *B. planctonicum* isolated from L. C. in 1993 (Müller & Schlegel 1999; 2); the same original isolate investigated by a different laboratory (Weisse et al. 2001; 3), *B. planctonicum* isolated from Lake Mondsee (Weisse et al. 2001; 4), the marine *B. comatum* isolated from Øresund (Jakobsen & Hansen 1997; 5), *Urotricha furcata* isolated from L. C. (Weisse et al. 2001; 6), *U. furcata* isolated from Lake Schöhsee (Weisse et al. 2001; 7).

The results shown in Figure 5 were obtained with cultures obtained from enrichment experiments. The original composition was, most likely, non-clonal, i.e. each isolate may have consisted of several to many clones. It appears, however, likely that individual, fast growing clones became dominant in the course of the culturing of each of the isolates and that the clonal composition of the *B. planctonicum* strains 1 – 4 was accordingly different during the experiments.

Although these results need to be supported by experiments with other taxa and by using alternative experimental techniques, the data suggest that intraspecific variation may be of similar magnitude to interspecific variation in herbivorous protists of similar cell size. The observed variation is, most likely, caused by morphological and physiological differences among clones.

The significance of individual variability

Ecophysiological variability will appear among (1) species, (2) populations, (3) clones and (4) individuals. The foregoing discussion considered variation occurring at the first three levels, and it should be noted that an unequivocal discrimination between the level of populations and clones is at present difficult for small protists. Genetically identical clones may behave ecologically differently, due to epigenetic effects. In the following I will consider individual variability of herbivorous protists. This is not just extending the issue of ecophysiological variability to the lowest level, but addresses a problem of fundamental biological significance. Variation among individuals is an important biological phenomenon, and reporting only population mean values lose potentially relevant information (Gerritsen et al. 1987), in particular with respect to the adaptive potential of a population or species to changing environmental conditions. Previous research was too much oriented towards mean values that were used to characterize populations, species, or even 'functional guilds'. In many grazing studies, for instance, the variation around the mean was neglected or completely unknown if the sample size was too small (see above). 'Standard' parameters such as threshold concentrations, half-saturation constants or growth efficiencies were used by modellers (e.g. Vézina & Platt 1988; Ducklow 1991; Moloney & Field 1991; Gaedke & Straile 1994). From a conceptual point of view, this approach is unsatisfactory because natural selection does not select for the average. The individuals that are best adapted to a given parameter will be favoured as long as the environmental conditions do not change; those individuals that are poorly adapted will first die out. A population which is composed of many clones with diverging reaction norms should be better adapted to fluctuating environmental conditions than a fairly uniform population. Disregard of this fundamental biological principle may in part explain why the predictive value of some ecological models is rather low.

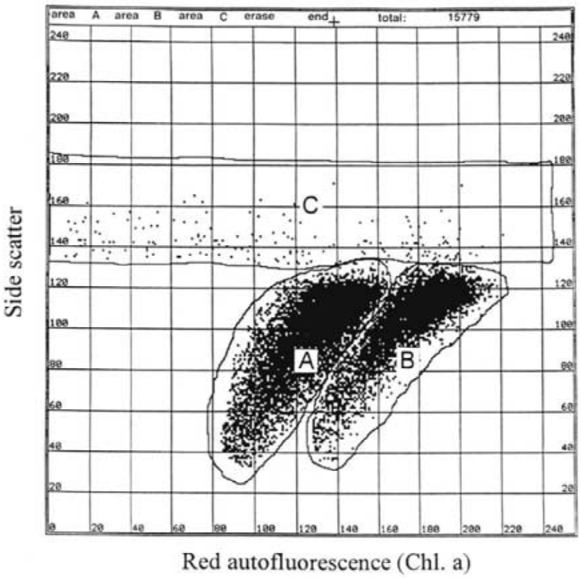

Figure 6. Flow cytometric analysis of the freshwater dinoflagellate *Peridiniopsis berolinense* (C) feeding on two cryptophyte species (A, B) . The plot shows the side scatter (SSC) vs. red autofluorescence histogram 21 min after the beginning of the experiment. The latter parameter is indicative of chlorophyll *a*, SSC corresponds to cell size. Each data point denotes one cell. Numbers along the axes denote relative units (channel numbers). Because red fluorescence of *P. berolinense* originates from algal pigments, the dinoflagellates are increasingly shifted along the *X*-axis to the right the more algae they have ingested (source: Weisse & Kirchhoff 1997).

As noted above, video microscopy and flow cytometry are promising tools to assess the individual variability within a given protist population. When flow cytometry was applied to measure the uptake of algae by herbivorous protist due to the increase of algal induced autofluorescence (Cucci et al. 1985, Gerritsen et al. 1987; Kenter et al. 1996) a high individual variability became apparent (Figure 6). At the beginning of the experiment shown in Figure 6, >90% of the dinoflagellates feeding on the cryptophyte algae were measured in channel numbers 0 to 20 of the *x*-axis, i. e. they were starved and had very low autofluorescence in their food vacuoles. After 21 min from the beginning of the experiment, the dinoflagellate autofluorescence covered almost the entire range from very low to very high autofluorescence (Figure 6). In this study, the increase of autofluorescence could be linked to the microscopically measured number of food vacuoles formed by the dinoflagellates (Weisse & Kirchhoff 1997). Mean ingestion rates derived from both techniques agreed well and suggested a fairly uniform behaviour of the dinoflagellate population. The flow cytometric analysis shown in Figure 6 demonstrated, however, that the population was, by no means, in a uniform nutritional state. In fact, the location of the individual cells in the SSC vs. red autofluorescence histogram and the range of each parameter can be used to estimate the nutritional status of the population which impacts measured uptake rates (Weisse & Kirchhoff 1997). The food vacuole autofluorescence is affected by ingestion as well as by beginning digestion. It is thus not surprising that the autofluorescence of predators shown in Figure 6 and measured in similar studies (Gerritsen et al. 1987, Kenter et al. 1996) represents a continuum, while uptake of particles is a discrete process. I infer from those results that, similar to the conclusion reached for bacterivorous flagellates (Gonzales et al. 1990; Boenigk et al. 2001c), digestability strongly affects uptake of algae by protozoa, and that variation in the individual ability to digest a given food item is highly variable.

General conclusions and recommendations for future research

There is a need in aquatic microbial ecology to study species-specific interactions that go beyond direct feeding relationships. Fuhrman's et al. (1994) conclusion that ignorance of bacterial species and their distributions is responsible for missing important ecological interactions such as competition holds true for many heterotrophic protists as well. Another important step is assessing the variation among a given population. The scattering around the mean, which seems to be an inherent property of many protist populations, is commonly interpreted as undesired 'noise' due to imperfect methods. From a conceptual point of view, this approach is unsatisfactory because natural selection and short-term environmental changes do not select for the average.

Tools for measuring the variation among bacterial and protist populations are already at hand. Image analysis in combination with epifluorescence microscopy (Sieracki & Webb 1991; Psenner 1993; Verity & Sieracki 1993), video microscopy (Boenigk & Arndt 200a, b) and flow cytometry (consult Davey & Kell 1996 and Reckermann & Colijn 2000 for review) are especially promising techniques for measuring various morphological, physiological, and biochemical properties of individual cells. The latter method has hitherto primarily been applied in marine phytoplankton ecology but is now increasingly being applied to feeding and growth studies on heterotrophic protists (Kenter et al. 1996; Hansen et al. 1996; Weisse & Kirchhoff 1997; Bratfold et al. 2000). Flow cytometry has been combined with immunofluorescence (reviewed by Ward 1990; Vrieling & Anderson 1996) to detect toxic marine dinoflagellates *in situ* (Vrieling et al. 1996 and references therein). Similarly, flow cytometry in combination with in situ hybridization has been used to identify marine flagellates and ciliates according to their fluorescence and size characteristics (Lim et al. 1993; Rice et al. 1997).

Although most if not all of the above mentioned techniques will need considerable refinement before they can be applied in routine investigations, they have collectively opened a new door to aquatic protist ecology. With the aid of species-specific immunofluorescent or oligonucleotide probes it should be possible to analyze population dynamics of the vast majority of unculturable microbes. For protists that can be identified with conventional and the novel techniques outlined above, the time has come to study population dynamics properly, i.e., to give up describing changes occurring within a population in inadequate terms (mean values only). Instead, the effect of selected environmental parameters should be analyzed on a cellular basis with reliable statistics. The diversity inherent at the levels of the species, population, clone and individual need to be considered in future conceptual and mathematical models.

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