Vol. 27: 137–148, 2002 | AQUATIC MICROBIAL ECOLOGY
Aquat Microb Ecol | Published March 15

Role of bacterial phenotypic traits in selective feeding of the heterotrophic nanoflagellate *Spumella* sp.

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

[1]Department of Physiological Ecology, Max Planck Institute for Limnology, PO Box 165, 24302 Plön, Germany
[2]Department of General Ecology and Limnology, Zoological Institute, University of Cologne, 50923 Cologne, Germany

ABSTRACT: The influence of different bacterial phenotypic traits on the feeding selectivity of a bacterivorous nanoflagellate was investigated in laboratory experiments. Twelve bacterial isolates from freshwater habitats were characterized in terms of cell size, morphology, capsule formation, surface hydrophobicity and charge, and swimming behavior. Mechanisms of differential flagellate feeding on these isolates were studied in short-term grazing experiments by high-resolution video microscopy using the nanoflagellate *Spumella* sp. as a model interception-feeding predator. The differentiation into distinct stages of the feeding process (contact, capture, ingestion) revealed a complex selection behavior illustrated by the relatively small proportion of ingested bacteria over cell contacts. We found that bacterial swimming speed increased contact rates but also decreased flagellate capture efficiency, which had a compensating effect on overall ingestion rates. Bacterial cell size revealed no correlation with flagellate contact rates but an effective inhibition of ingestion rates when exceeding a critical size limit. Correlation analysis presented no bacterial property to account for the residual variability of flagellate ingestion efficiencies. Experiments with specifically coated artificial particles provided evidence for the relative importance of the biochemical surface composition in flagellate food selection compared to physicochemical interaction forces. Only extreme charges beyond −45 mV reduced flagellate ingestion rates. Our results reinforce the idea that bacterial cell size strongly affects the feeding success of bacterivorous flagellates, and further implicates size-independent bacterial traits such as swimming speed and biochemical surface composition in this feeding success.

KEY WORDS: Selective flagellate feeding · Bacterial properties · Size · Swimming speed · Cell surface · Video microscopy

INTRODUCTION

In marine and freshwater systems, bacterial biomass and production are transferred to higher trophic levels via bacterivorous protists, mainly by heterotrophic nanoflagellates (HNF) (Fenchel 1982b, Azam et al. 1983, Sanders et al. 1992). Moreover, protozoan grazing can be viewed as an important structuring factor giving rise to situational changes in the taxonomical and morphological composition of bacterial communities (Šimek et al. 1997, Jürgens et al. 1999, Hahn & Höfle 2001). Generally, survival of bacteria under changing grazing conditions necessitates phenotypic and genotypic flexibility. Given the high species diversity (e.g. Giovannoni et al. 1990, DeLong et al. 1993) and substantial phenotypic variation found in bacteria (Roszak & Colwell 1987, Rainey et al. 1993), natural bacterial populations are assumed to harbor a huge potential for an effective response to protozoan grazing.

*E-mail: matz@mpil-ploen.mpg.de
**Present address: Austrian Academy of Sciences, Institute of Limnology, Gaisberg 116, 5310 Mondsee, Austria

Most studies on the predator-prey relationship between bacteria and HNF have focused on the dominant role of bacterial cell size (Chrzanowski & Šimek 1990, González et al. 1990b, Hahn et al. 1999, Posch et al. 1999), while other bacterial traits remain barely investigated. Jürgens & Güde (1994) reviewed the possible mechanisms that bacterial adaptation may have acquired to avoid protozoan grazing, including motility, exopolymer formation and chemical surface properties. In view of the increasing interest in qualitative facets of bacterivory dynamics, the elucidation of the functional role of phenotypic traits other than size is overdue.

Motility is a common characteristic of bacteria in natural environments. A large fraction of the bacteria in aquatic habitats has been reported to be motile (Grossart & Azam 1998, Fenchel 2001). Swimming of the bacterial cell is considered to be a response to the environment. For instance, motility is beneficial in finding substrate-rich micropatches (Azam & Ammerman 1984, Blackburn et al. 1998), thus resulting in higher growth rates of motile bacteria in oligotrophic systems (Lauffenburger 1991). Unlike its importance in reaching nutrient microzones, motility in bacteria has rarely been discussed as a behavioral component in the vulnerability to grazers (Monger & Landry 1992, González et al. 1993).

Exopolymeric capsules can be exhibited by about 65% of the intact suspended bacteria in marine bacterioplankton (Heissenberger et al. 1996). Whether bacterial encapsulation affects selective protistan grazing is still an open question. Some indication, however, may be gathered from analogous studies on pathogenic bacteria in mammalian immune systems: there, for instance, capsule-deficient *Escherichia coli* were efficiently ingested by phagocytes whereas capsulated *E. coli* were similarly attached but not ingested (Horwitz & Silverstein 1980).

Immunological studies have described several cellular recognition mechanisms in non-opsonic phagocytosis mediated by lectin-carbohydrate, protein-protein or hydrophobic interactions (see Ofek et al. 1995). They raised the attractive issue that chemical surface properties of bacteria might matter in natural microbial predator-prey interactions in an analogous fashion (Jürgens & Güde 1994). However, recent experiments focusing on the role of a physicochemical mechanism (hydrophobicity) in flagellate feeding have not brought forth consistent results (Monger et al. 1999, Matz & Jürgens 2001). This suggests either a non-uniform importance among protozoan species or some complex interference of other, more specific bacterial surface variables.

Subtle mechanisms of bacterial grazing resistance on the one side may implicate pronounced selective feeding of the flagellate predator on the other. It is known that HNF and other bacterivorous protozoans do not consume bacteria of different species with equal efficiency (Taylor & Berger 1976, Sherr et al. 1983, Mitchell et al. 1988). Many of these studies, however, could not yield direct conclusions, as some prey variables had not been measured or completely controlled. Most planktonic flagellates belong to the feeding type 'interception feeder' (Fenchel 1986), which capture and process single prey particles at a sensitive region near the flagellar base (Boenigk & Arndt 2000b), seemingly without elaborate feeding structures. Since some evidence for a complex selection behavior in interception-feeding flagellates has been provided (e.g. Landry et al. 1991, Jürgens & De Mott 1995, Boenigk et al. 2001b, Matz & Jürgens 2001), it is assumed that bacterial properties may influence different steps in the process of food acquisition.

We addressed the question of the mechanistic impact of different phenotypic traits on bacterial grazing mortality and on flagellate feeding behavior by live observations of a common interception-feeding flagellate. This technique provides detailed observational data of every step (encounter, capture, ingestion) in the process of food uptake (Boenigk & Arndt 2000a, Boenigk et al. 2001a) and circumvents a manipulation of bacterial cells prior to the experiment. Previous experiments have rendered the colorless chrysomonad *Spumella* sp. an ideal model organism (Boenigk & Arndt 2000b, Boenigk et al. 2001a, b).

The purpose of this study was to determine the influence of common phenotypic traits in bacteria (cell motility, size, surface properties) on the susceptibility to ingestion by an interception-feeding flagellate in a comprehensive overview. In particular, we tested non-morphological properties and hypothesized (1) that bacterial motility influences encounter and capture probabilities; and (2) that flagellate ingestion efficiencies are affected by bacterial cell surface properties (capsule, hydrophobicity, charge, biochemical composition). In this context, we tried to elucidate with specific artificial microspheres whether selective flagellate feeding is based on mere physicochemical interaction forces (non-specific mechanism) or on a more specific discrimination between biochemical constituents of the prey surface.

MATERIALS AND METHODS

Organisms. Twelve strains of bacteria exhibiting comparable cell sizes within the flagellate prey spectrum as well as a wide range of surface characteristics and swimming speeds were selected from a set of 41 isolates. They had been obtained from freshwater con-

Table 1. Phenotypic characteristics of the 12 bacterial isolates used. Cell dimensions (excluding and including capsule), hydrophobicity (as measured by the bacterial adhesion to hydrocarbon [BATH] assay and the hydrophobic interaction chromatography [HIC] assay) and surface charge (measured as zeta potential) are given as mean ± standard deviation. ND: not determined

Strain	Cell length (µm)	Capsule	Cell length incl. capsule (µm)	Cell width incl. capsule (µm)	Motility	BATH (%)	HIC index	Zeta potential (mV)
KB9	1.30 ± 0.33	–	1.32 ± 0.42	0.54 ± 0.07	–	95.3 ± 0.1	0.23 ± 0.15	3.2 ± 0.7
CM10	1.28 ± 0.23	–	1.33 ± 0.28	0.60 ± 0.06	+	51.2 ± 10.1	0.19 ± 0.08	–20.9 ± 0.0
CM28	1.26 ± 0.27	+	1.49 ± 0.42	0.68 ± 0.05	–	95.5 ± 2.3	0.11 ± 0.00	–24.9 ± 0.5
SG81R1	1.32 ± 0.28	+	1.53 ± 0.39	0.71 ± 0.04	+	67.2 ± 6.8	0.62 ± 0.01	–24.7 ± 1.1
KB6	1.47 ± 0.31	+	1.61 ± 0.35	0.73 ± 0.11	–	86.2 ± 0.1	0.04 ± 0.04	–23.4 ± 1.6
KB23	1.29 ± 0.28	+	1.71 ± 0.42	0.90 ± 0.05	+	93.1 ± 1.0	0.66 ± 0.02	–29.3 ± 1.8
CM20	1.30 ± 0.32	+	1.79 ± 0.37	0.93 ± 0.06	–	1.6 ± 2.7	0.45 ± 0.08	–16.6 ± 3.0
KB10	1.89 ± 0.46	+	2.11 ± 0.46	0.79 ± 0.10	–	97.0 ± 0.2	0.24 ± 0.12	–25.7 ± 1.3
KB12	1.53 ± 0.31	+	2.54 ± 0.68	1.35 ± 0.06	–	6.0 ± 2.5	0.09 ± 0.02	–36.1 ± 2.8
KB16	1.02 ± 0.29	+	2.54 ± 0.53	1.74 ± 0.08	+	43.8 ± 13.8	0.24 ± 0.03	3.1 ± 0.2
KB27	2.72 ± 1.05	+	3.30 ± 1.14	1.08 ± 0.06	+	37.3 ± 4.9	0.63 ± 0.06	–6.8 ± 2.9
CM32	2.88 ± 1.23	+	3.85 ± 1.35	1.31 ± 0.17	–	73.0 ± 14.5	ND	ND

tinuous cultures featuring grazing and non-grazing conditions (Matz & Jürgens 2001). Stock preparations were stored at –70°C until further cultivation. For phenotypic characterization and feeding experiments, the bacteria were first grown on nutrient broth medium. Then they were transferred to WC medium (Guillard & Lorenzen 1972) supplemented with 100 mg glucose l^{-1} and grown to stationary growth phase.

The strain of the common bacterivorous flagellate *Spumella* sp. had been isolated from Lake Schöhsee (Plön, Germany). It was cultivated at 17°C on the bacterium *Pseudomonas putida* MM1 (Christoffersen et al. 1997) in WC medium supplemented with 100 mg glucose l^{-1}. For ingestion experiments, flagellates were grown in triplicate batch cultures and continuously transferred to fresh bacterial suspensions. Culture subsamples were taken from flagellate exponential growth phase; additional food supply provided satiated test organisms throughout the feeding experiments.

Bacterial characteristics. Each bacterial isolate was analyzed for the phenotypic features under examination (Table 1) covering cell size, morphology (including capsule formation), motility and physicochemical cell surface parameters (hydrophobicity and charge). Physicochemical surface properties had been previously assessed for these strains under identical culture conditions (Matz & Jürgens 2001). These measurements were included in this study, as repeated experiments revealed stable surface characteristics during stationary growth phase (coefficients of variation [CV] ≤5.7%). The methods that were used in the cell surface analysis were the bacterial adhesion to hydrocarbon (BATH) assay and hydrophobic interaction chromatography (HIC), both determining surface hydrophobicity, and zeta potentials were measured with a Zetasizer 3000 (Malvern Instruments). Bacterial cell size was determined from 4′,6-diamidino-2-phenylindole (DAPI) preparations (Porter & Feig 1980) of formalin (2%) fixed samples with an automated image analysis system (SIS).

The presence of capsules was detected by the use of a modified negative staining technique after Plante & Shriver (1998). Ten microliters of a formalin-fixed bacterial suspension was mixed with 10 µl of 0.2 µm filtered Congo Red (1% aqueous) on a clean glass slide, covered with a coverslip and allowed to dry at room temperature. Maneval's stain (Carolina Biological Supply) was added laterally to flood the preparation for approximately 1 min. Preparations were gently blotted dry and were examined under phase-contrast at 1000× magnification. *Azotobacter vinelandii* DSM85 served as positive and *A. vinelandii* DSM86 as negative control for capsule formation. Preparations were also used to measure directly length and width of capsulated cells with an image analysis system.

Bacterial swimming behavior was documented in dark field situations by means of a standard video camera and videocassette recorder (VCR). Recordings were performed at 2000× magnification in an observational chamber, which was constructed of a glass slide and a coverslip separated by adhesive tape. Observations were made midchamber at maximum light intensity and contrast. Swimming behavior of motile strains was quantified from videotapes using MedeaLab 3.1 Tracking System (1994–97, Medea AV). Cells moving out of the plane of focus were sorted out of the series of digitized tracks.

Table 2. Polystyrene particles (non-fluorescent, 0.75 µm in diameter) with specific coatings and surface charge (measured as zeta potentials). Different densities of carboxyl groups are termed Plain, COOH low, COOH medium and COOH high. Zeta potentials are given as mean ± standard deviation

Particle loading	Zeta potential (mV)
Plain	−4.7 ± 0.7
COOH low	−46.2 ± 1.1
COOH medium	−49.4 ± 0.6
COOH high	−53.1 ± 0.3
Protein A	−37.9 ± 0.5
Bovine serum albumin	−39.7 ± 0.4
Polyethylenglycol-300	−37.3 ± 0.9
Polygalacturonic acid	−39.8 ± 0.4
Starch	−39.5 ± 2.0

Artificial particles. In our uptake experiments, we also used monosized polystyrene beads (non-fluorescent, 0.75 µm in diameter; micromod Partikeltechnologie) as a device for testing the relative importance of physicochemical interaction forces (by means of surface charge determination) and the biochemical surface composition in flagellate-prey interactions. Nine different particles were provided, which were covalently coated with specific surface layers (Table 2). Four particles exhibited different densities of carboxyl groups (plain, COOH low, COOH medium, COOH high) and were used to mimic a surface charge gradient (zeta potentials from −4.7 to −53.1 mV) without concomitant change in surface composition. The other 5 particles (protein A, bovine serum albumin [BSA], polyethylenglycol-300, polygalacturonic acid, starch) differed in their surface composition but exhibited similar zeta potentials (from −37.3 to −39.8 mV).

Feeding experiments. The experiments were performed by means of high-resolution video microscopy employing an inverted microscope, a standard video camera and a VCR. The experimental design largely follows the one previously described (Boenigk & Arndt 2000a, Boenigk et al. 2001a). Prior to the experiments, flagellates from triplicate cultures were transferred to the observational chamber and were allowed to attach to the bottom for 30 min. After washing away the indigenous bacteria (*Pseudomonas putida* MM1), the test particle was added to a concentration of 2×10^7 ml^{-1} and the flagellates were allowed to adapt to the new food situations for 2 min. One attached flagellate per chamber was selected for video observations, which did not exceed 15 min per individual flagellate cell. In total, 12 individuals from different chambers were observed for each food particle. Randomly selected individuals were checked for abnormalities in particle digestion during another 15 min period.

Table 3 summarizes the definition of crucial steps observed in flagellate feeding behavior. Events were defined as 'contact' when particles directly encountered the sensitive region near the base of the long flagellum. If the long flagellum folded over the particle and pressed it against the short one, feeding events were scored as 'capture'. The subsequent formation of a food vacuole was scored as 'ingestion'. The difference between particle capture and particle ingestion was scored as 'rejection' and was based on the behavior of unfolding the long flagellum in order to release the prey. Feeding efficiencies were defined as the following: 'capture efficiency' describes the proportion of particle-flagellate encounters that was captured and 'ingestion efficiencies' describes how many of the captured particles were subsequently ingested.

Data analysis. Pearson product-moment correlations were used to test for significant relationships between single bacterial phenotypic properties (hydrophobicity, surface charge, cell size, swimming speed) and

Table 3. Definition of the major feeding interactions observed between the flagellate predator *Spumella* sp. and prey particles

Events in flagellate feeding behavior	Microscopic observation	Abbreviation
Contact	Direct encounter of prey particle with sensitive region near the flagellar base	*Con* = *Esc* + *Cap* (*Rej* + *Ing*)
Escape	Contacted prey particle that is not captured	*Esc* = *Con* – *Cap*
Capture	Folding of long flagellum over the prey particle	*Cap*
Rejection	Captured prey particle that is not ingested but released by unfolding of the flagellum	*Rej* = *Cap* – *Ing*
Ingestion	Captured prey particle that is enclosed by pseudopodia (food vacuole)	*Ing*
Capture efficiency	Proportion of captured over encountered prey particles	*Capeff* = *Cap*/*Con* × 100 [%]
Ingestion efficiency	Proportion of ingested over captured prey particles	*Ingeff* = *Ing*/*Cap* × 100 [%]

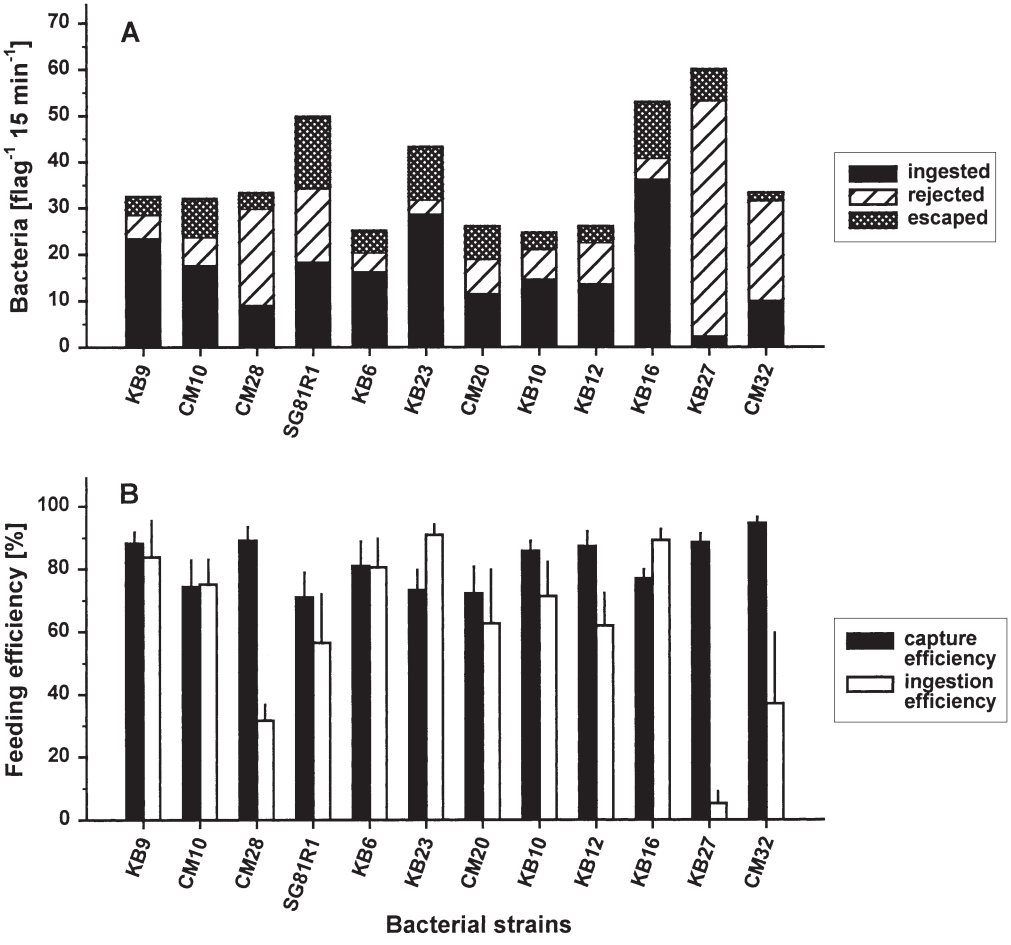

Fig. 1. Flagellate (flag) feeding on bacterial isolates exhibiting different phenotypic properties. (A) Average numbers of escaped, rejected and ingested bacteria of the total number of flagellate-bacterium contacts. (B) Flagellate feeding efficiencies (capture and ingestion efficiency) are given as mean ± standard deviation. The data are based on 12 individual flagellate cells for each bacterial strain within an observation time of 15 min

flagellate feeding parameters. We employed multiple regression analysis to evaluate the relative importance of bacterial phenotypic properties in explaining variation in flagellate feeding rates. As the sample size was limited, we used only the bacterial variables that alone yielded a significant correlation. One-way ANOVAs were used to test for significant differences between swimming speeds of the 5 motile strains and between flagellate feeding rates on all 12 strains. Likewise, significant effects of capsule formation, different coatings of artificial particles and heat-killed bacteria, respectively, on flagellate feeding efficiencies were tested by 1-way ANOVAs. Absolute values were ln transformed and percentages arcsine-square root transformed. Post hoc comparisons of means were provided by Tukey tests. All statistical tests were performed using STATISTICA, Version 5.0 (StatSoft).

RESULTS

The process of food uptake in interception-feeding flagellates can be subdivided into several steps, starting with the encounter or contact of flagellate and bacterium, followed by capture and particle handling, and ending up in either ingestion or rejection of the particle (cf. Boenigk & Arndt 2000b). All steps in the process of phagocytosis were found to be affected by at least one of the bacterial properties under examination, which consequently determined strain-specific bacterial mortality.

Flagellate feeding rates varied significantly between the 12 bacterial strains tested in our grazing experiments ($F \geq 18.67$, $p < 0.001$). Fig. 1A shows strain specifically the fractions of escaped, rejected and ingested cells in the total number of contacted bacteria within an observation time of 15 min. Contact

Table 4. Pearson product-moment correlation matrix between the phenotypic characteristics of the bacterial isolates and feeding parameters of *Spumella* sp. *p-values of significant relationships. Correlation coefficient r is given in parentheses

	Hydrophobicity BATH	Hydrophobicity HIC	Surface charge zeta potential	Speed	Cell volume	Cell length	Cell length incl. capsule
Contact rate	0.836 (–0.067)	0.028* (0.658)	0.171 (0.445)	0.010* (0.706)	0.837 (–0.067)	0.631 (0.155)	0.440 (0.247)
Capture rate	0.916 (–0.034)	0.084 (0.543)	0.119 (0.499)	0.132 (0.461)	0.690 (0.129)	0.221 (0.382)	0.164 (0.429)
Ingestion rate	0.551 (0.192)	0.957 (–0.019)	0.362 (0.305)	0.157 (0.436)	0.035* (–0.610)	0.023* (–0.648)	0.116 (–0.478)
Capture efficiency	0.486 (0.223)	0.182 (–0.434)	0.745 (0.111)	0.016* (–0.673)	0.077 (0.530)	0.025* (0.640)	0.047* (0.583)
Ingestion efficiency	0.568 (0.184)	0.457 (–0.251)	0.918 (0.035)	0.635 (0.153)	0.104 (–0.492)	0.011* (–0.700)	0.081 (–0.523)

rates varied from 25 to about 60 bacteria flagellate^{-1} 15 min^{-1} while ingestion rates ranged from 2.5 bacteria flagellate^{-1} 15 min^{-1} for strain KB27 to 36.4 bacteria flagellate^{-1} 15 min^{-1} for strain KB16. A proportional relationship between contact rates and ingestion rates was not evident. Rather, the differences between contacted and eventually ingested prey particles indicated a selection process prior to phagocytosis. The portions of escaped bacterial cells and rejected cells varied significantly between the strains. This is illustrated by the calculated feeding efficiencies shown in Fig. 1B. Both capture and ingestion efficiency exhibited significant strain-specific differences (F = 22.72, p < 0.001 and F = 58.96, p < 0.001, respectively). However, capture efficiencies were relatively constant (all values ≥ 70%) compared to the significant variability of ingestion efficiencies (from <10 to 90%) during particle handling (F = 53.56, p < 0.001). Correlation analyses were used to determine significant relationships between bacterial phenotypic properties and flagellate feeding rates (Table 4). We found no significant correlation between bacterial concentration and the contact rates between flagellates and bacteria in these experiments (p = 0.837). This was expected because there was little variability in the concentrations of bacteria in the observational chamber (1.9 ± 0.20 × 10^7 cells ml^{-1}).

Bacterial cell size

The cells of all bacterial strains were rod shaped and differed significantly in size, spanning a range from 1.0 to 2.9 µm for the average cell length. Strains KB27 and CM32 exhibited significantly larger cells (Tukey test, p < 0.001) and strain KB16 significantly smaller cells (p < 0.001) than the rest of the strains. The highest contact rate was reported for strain KB27, which was one of the largest bacteria used and also motile. However, contact rates did not correlate with bacterial cell size even among the non-motile strains (p = 0.646). Instead, there was a significant negative correlation between cell size and flagellate ingestion rate, which is illustrated by the highest ingestion rate found for the smallest bacterium (strain KB16) and the lowest for the second largest (strain KB27). Additional F-tests revealed that although the largest bacteria (strains CM32 and KB27) were captured with significantly higher efficiencies (F = 34.93, p < 0.001), their ingestion efficiencies were some of the lowest recorded (F = 107.87, p < 0.001). Generally, the impact of bacterial cell volume was less pronounced than cell length.

Capsule formation

The majority of the bacterial strains (10 of 12) showed capsule formation under the experimental culture conditions (Table 1). However, the presence or absence of a capsule did not affect flagellate feeding rates (F ≤ 1.06, p ≥ 0.327). Capsule thickness varied between the strains, so that cell sizes as measured by DAPI staining were corrected by capsule dimensions. Correlation analysis of these values confirmed the positive relationship of cell size with flagellate capture efficiency and the negative relationship with ingestion efficiency but at a lower level of significance (p = 0.047 and p = 0.081, respectively).

Bacterial motility

Five of 12 strains were motile and differed significantly in their swimming speed (Table 5; F = 48.52, p < 0.001) with an average speed from 14.6 to 35.1 µm s^{-1}. All motile bacteria were found to exhibit movement

patterns previously described as 'run and reversals' by Mitchell et al. (1995a). Motile cells in all treatments made up more than 70% of the total number of bacteria. The swimming speed of strain SG81R1 was the highest (Tukey test, $p < 0.001$) with an average speed of 35.1 µm s^{-1} and a maximum speed up to 140 µm s^{-1}. While bacterial cell size did not correlate with the number of cell contacts, the average swimming speed of the bacterial strains showed a significant positive relationship with contact rates ($r^2 = 0.502$; Table 4). Multiple regression analysis with motility and bacterial hydrophobicity (as measured by HIC) as independent variables yielded a slightly better correlation with flagellate contact rates ($r^2 = 0.571$). While contact rates of motile bacteria were significantly higher than for non-motile strains (Fig. 1A; $F = 63.44$, $p < 0.001$), the proportion of escapes in the total number of cell contacts increased for the motile strains. Consequently, capture efficiencies of the flagellate were significantly lower for motile strains ($F = 41.01$, $p < 0.001$) but still exceeded 70% (Fig. 1B). Correlation analysis revealed that increasing bacterial swimming speed reduced flagellate capture efficiencies significantly ($r^2 = 0.453$). Taking into account the influence of bacterial cell length, multiple regression analysis revealed an even better correlation ($r^2 = 0.689$). For ingestion efficiencies, no significant influence of bacterial motility was observable ($p = 0.747$).

The influence of bacterial motility was specified by the immobilization of the highly motile strain SG81R1 by heat incubation (60°C for 1 and 2 h, respectively; Fig. 2) prior to the feeding experiment. The experiment revealed a significant increase of capture efficiencies for non-motile, heat-killed bacteria ($F = 60.0$, $p < 0.001$). In contrast, ingestion efficiencies were not affected by the immobilization procedure and remained constant for 0 and 1 h of heat incubation and denaturation. After 2 h of heat incubation, however, the ingestion efficiency decreased significantly ($F = 11.64$, $p < 0.001$).

Table 5. Swimming speed of the 5 motile bacterial strains as measured by an automated tracking system. Mean swimming speed is given as mean ± standard deviation. N: number of observations

Strain	Mean speed (µm s^{-1})	Maximum speed (µm s^{-1})	N
CM10	21.9 ± 11.2	57.0	102
KB16	20.5 ± 11.9	79.8	210
KB23	26.6 ± 13.8	113.6	310
KB27	14.6 ± 5.6	45.9	42
SG81R1	35.1 ± 15.7	140.3	262

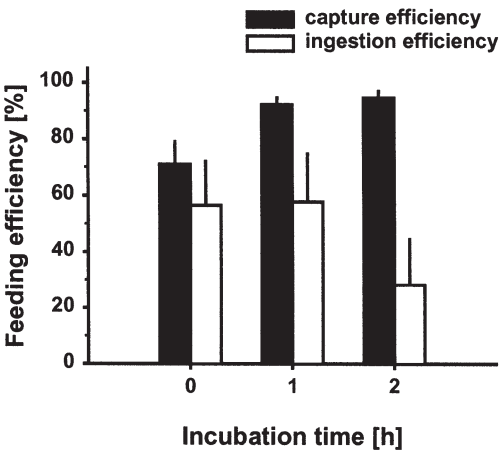

Fig. 2. Influence of immobilization of the highly motile bacterium SG81R1 on flagellate feeding rates. Bacteria were incubated for 1 and 2 h at 60°C prior to the feeding experiment. Flagellate feeding efficiencies (capture and ingestion efficiency) are given as mean ± standard deviation. The data are based on 12 individual flagellate cells for each bacterial strain within an observation time of 15 min

Bacterial surface properties

The considerable strain-specific variability of capture, rejection and ingestion rates did not correlate significantly with any of the measured physicochemical surface parameters assessed for the 12 bacterial strains (hydrophobicity, charge; Table 4). The feeding experiments revealed only a slight positive correlation between the number of cell contacts and bacterial hydrophobicity as measured by the HIC assay and a marginal correlation with flagellate capture rates. At the step of processing captured bacteria, neither bacterial hydrophobicity nor surface charge could account for the differences in flagellate ingestion efficiencies between the 12 strains. This was not even the case between strains of comparable cell size, which differed about 3-fold (from 30 to 90% for strains CM28 and KB23) in their ingestion efficiencies.

In order to uncouple the influence of physicochemical surface parameters from the influence of the chemical surface composition, artificial particles with specific coatings were fed to the flagellates. Particles that had been loaded by an increasing density of carboxyl groups resulted in an increase of surface charge measured as zeta potential and comparable chemical composition at the same time. Flagellate feeding rates were significantly related to this surface charge gradient (Fig. 3A): contact rates decreased significantly (data not shown, $F = 8.22$, $p < 0.001$) for the most negatively charged particles (COOH medium, COOH high). A significant decrease in capture efficiencies ($F = 10.15$, $p < 0.001$) and ingestion efficiencies ($F =$

9.43, $p < 0.001$) was also evident, which resulted in reduced uptake rates ($F = 12.00$, $p < 0.001$).

In contrast, particles with comparable surface charges (mean zeta potential of –38.8 ± 1.16 mV, CV = 0.03) but distinct differences in chemical surface composition (protein A, BSA, polyethylenglycol-300, polygalacturonic acid, starch) were ingested with different efficiencies ($F = 10.96$, $p < 0.001$) (Fig. 3B). Particle capture efficiencies, however, did not differ significantly ($F = 1.43$, $p < 0.2377$). Ingestion rates were lowest on starch surfaces where 90% of the captured particles were rejected, whereas the highest efficiencies were observed on BSA loaded particles (>50%). Thus, there was an electrostatic effect of prey surfaces observable, leading to reduced contact, capture and ingestion rates, on the one hand, and a compositional effect resulting in unaltered capture efficiencies but a significant variation in particle handling on the other.

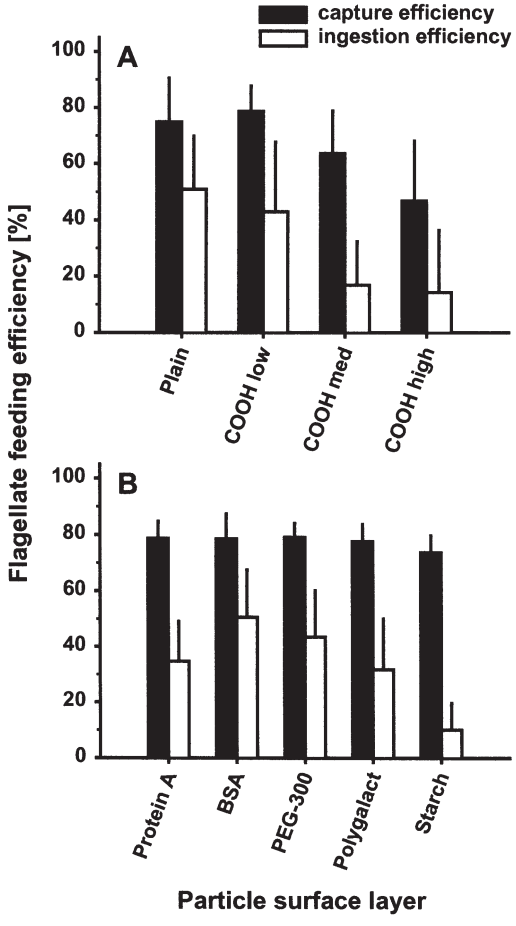

Fig. 3. Differential flagellate feeding on artificial particles (0.75 µm in diameter) exhibiting a physicochemical force gradient versus a chemical composition spectrum. (A) Four particles with different densities of carboxyl groups (plain, COOH low, COOH medium, COOH high) simulated a surface charge gradient (zeta potentials from –4.7 to –53.1 mV) without concomitant change in surface composition. (B) Five particles (protein A, bovine serum albumin, polyethylenglycol-300, polygalacturonic acid, starch) differing in their surface composition but with similar zeta potentials (from –37.3 to –39.8 mV). Flagellate feeding efficiencies (capture and ingestion efficiency) are given as mean ± standard deviation. The data are based on 12 individual flagellate cells for each bacterial strain within an observation time of 15 min

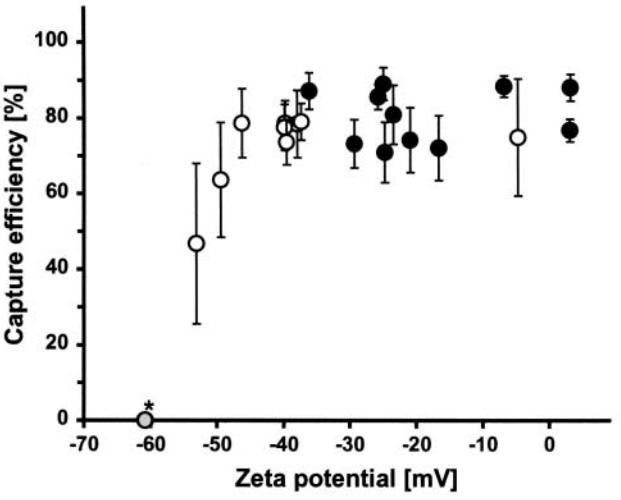

Fig. 4. Relationship between surface charge (measured as zeta potential) of bacteria (closed symbols) and coated microspheres (open symbols) and flagellate capture efficiency. Values are given as mean ± standard deviation. The data are based on 12 individual flagellate cells for each bacterial strain within an observation time of 15 min. Marked data point (*) refers to data of the dinoflagellate *Oxyrrhis marina* feeding on carboxylated 1 µm microspheres (Hammer et al. 1999)

Plotting the relationship between flagellate capture efficiencies and bacterial and particle surface charge (Fig. 4), we achieved the complete range of zeta potentials from 0 to –55 mV. Prey surface charges between 0 and –45 mV, including bacteria as well as artificial particles, showed no effect on capture efficiencies. Only extreme particle charges beyond –45 mV severely reduced flagellate feeding efficiency.

DISCUSSION

The experimental results presented in this paper provide the first comprehensive comparison of the influence of different bacterial properties (covering morphological, behavioral and chemical aspects) in the food selection of an interception-feeding nanoflagellate. Our data demonstrate the importance of evaluating different stages of the feeding process in order to understand the mechanisms of food selectivity and grazing protection in microbial predator-prey interactions. Selective flagellate feeding on several bacterial strains was illustrated by substantial differences be-

tween contact and ingestion rates. In contrast to largely comparable capture efficiencies, ingestion efficiencies were variable and strain specific, which suggests that handling of captured particles is a most decisive step in the process of flagellate food selection. The feeding experiments provided some insight into the bacterial properties affecting prey uptake of the flagellate.

Bacterial cell size

Size-selective feeding of bacterivorous flagellates has been viewed as an interaction between encounter probabilities and morphological limitations of the predator. Mathematical models predict that encounter rates generally increase with prey size (Fenchel 1982a, Monger & Landry 1990), so that grazing rates increase (González et al. 1990b, Šimek & Chrzanowski 1992) until a certain size limit. Our study found no significant overall relationship between bacterial cell size (volume and length) and contact rate. This might be due to the fact that prey sizes in our experiments spanned only a comparably narrow size range, as the main goal of our study was the examination of non-morphological traits. However, the efficiency of capturing a contacted prey particle was higher for the longest bacteria (≥90%). We also provided data for the effective mechanism of bacterial cell length to reduce the ingestion probability of captured cells. Ingestion efficiencies and rates were lowest for the longest bacteria. The repeated observation of initialized pseudopodia formation even for oversized bacteria ending in a subsequent stop of food vacuole formation (cf. Boenigk & Arndt 2000b) and the fact that at the same time capture efficiencies were highest for the largest bacteria imply morphological limitations of the predator at the handling process. Obviously, a critical prey size sets the morphological constraints for internalization, whereas prey size below this limit does not affect flagellate food selection. This is supported by the non-correlation of cell length and ingestion efficiency when the 2 largest bacteria (KB27 and CM32) were excluded from the analysis ($p = 0.668$).

Bacterial capsules

Many studies have suggested a protective function of exopolymeric capsules against digestion by protozoans (Decho 1990, González et al. 1990a, Jürgens & Güde 1994, Plante 2000). Their role in the uptake process, however, has not been investigated so far. In this study, we found that bacterial strains were ingested regardless of capsule formation. Likewise, capsulate bacterial strains exhibited no reduced susceptibility to flagellate digestion (data not shown). Hence, the mere presence of a capsule does not seem to imply a general protective function in bacteria-flagellate interactions (cf. Plante & Shriver 1998). Instead, future studies should focus on capsule quality and quantity, which are known to vary interspecifically as well as within strains according to activity level or physiological state (Sutherland 1977). In this context, we should remark that bacterial cell size measurements based on intracellular stains (e.g. DAPI) generally might underestimate the effective prey size, as the dimensions of bacterial capsules differed strain specifically. On the other hand, we need more information on the reliability of capsule dimensions when using Maneval's stain.

Bacterial motility

Based on the findings of Monger & Landry (1992) and González et al. (1993) it has been proposed that higher encounter rates would result in higher feeding rates on motile cells. In our experiments, this was indeed observed when the swimming speed of bacterial strains increased the encounter (contact) rate with the interception-feeding flagellate. However, we also observed the compensation of higher contact rates by reduced capture efficiencies, which may result in comparable ingestion rates for motile and non-motile bacterial strains. Escape of bacterial cells was recognized to be not only a result of high swimming speed but also of the 'run and reversal' swimming pattern described for marine bacteria (Mitchell et al. 1995a,b). Compared to the 'run and tumble' model known for *Escherichia coli* and other enteric bacteria (Berg & Brown 1972), this mechanism allows a rapid retreat after encountering a predator, whose reaction time was found to be comparably long (data not shown). Since average swimming speeds recorded in this study did not exceed 40 µm s^{-1}, we propose that bacteria with an even higher swimming speed would further decrease flagellate capture efficiencies, resulting in lower ingestion rates and thus in a higher survival rate of the bacterium. Our assumption is supported by the findings of Mitchell et al. (1995a), who reported maximum community speeds of up to 144 µm s^{-1} and a maximum individual burst velocity of 407 µm s^{-1}. Motility might provide an active mechanism of 'encounter avoidance' such as responsive acceleration to chemical gradients or physical obstacles and an independent movement relative to the feeding current of the predator. However, as bacterial swimming is assumed to be nutrient controlled (Mitchell 1991), 'escape mechanisms' based on swimming speed might be restricted to nutrient-rich situations.

Bacterial surface properties

One major hypothesis in this study was that bacterial surface properties influence the ingestion probability. Bacterial cell surfaces as a site of diverse constituents (Hammond et al. 1984) give rise to specific biochemical structures and physicochemical interaction forces. Some immunological studies (Van Oss 1978, Absolom 1988, Ofek et al. 1995) along with one ecological study (Monger et al. 1999) have proposed the importance of hydrophobic interaction forces in the process of phagocytosis, so that hydrophilic bacteria would exhibit lower uptake rates. Here, we found no significant effect of bacterial hydrophobicity and charge to account for the low ingestion rates. This is in accordance with earlier results on the influence of extreme bacterial hydrophobicity on 3 interception-feeding nanoflagellates (Matz & Jürgens 2001). Moreover, we found that only artificial particles with highly negatively charged (hydrophilic) surfaces could significantly reduce flagellate capture and ingestion efficiencies and even contact probabilities. This is supported by the predictions of the colloid theory (Monger & Landry 1990) and findings for the heterotrophic dinoflagellate *Oxyrrhis marina* (Hammer et al. 1999). However, zeta potentials of these artificial particles were outside of the range commonly found in natural bacterial isolates (Van der Mei et al. 1987, Gannon et al. 1991, Matz & Jürgens 2001). Within the natural range (0 to –40 mV) we observed no impact of bacterial and artificial surface charge, so that we can infer a minor importance of hydrophobicity and charge in the selection and feeding process of interception-feeding flagellates. The linear relationship between particle charge and flagellate feeding rate proposed by Hammer et al. (1999) may be supplemented by our data, which suggest a rather rectangular model with a critical value for flagellate feeding (Fig. 4). A small variation in the plateau area might be due to other surface factors.

In contrast, feeding on polystyrene beads coated with specific biochemical components resulted in significantly different ingestion efficiencies, although other factors such as charge, size and motility were kept constant. Our results indicate that differential feeding on similar-sized food items was not based on a physicochemical interaction mechanism but on the actual biochemical surface composition. Similarly, latex particles with distinct monosaccharide coatings but comparable zeta potentials determined variability in phagocytosis rates of polymorphonuclear leucocytes (Yamada et al. 1993). Although many protozoans apparently lack elaborate feeding structures, convincing evidence has already been presented for the complexity of protozoan feeding behavior and the significance of chemical cues. Ciliates and flagellates possess chemosensory abilities (Sibbald et al. 1987, Bennett et al. 1988, Snyder 1991) and have been reported to discriminate between similar-sized prey for some chemical reason (Stoecker et al. 1986, Sanders 1988, Landry et al. 1991, Verity 1991, Jürgens & De Mott 1995, Christaki et al. 1998, Wolfe 2000). This has been confirmed by live video observations, which revealed the assessment and active rejection of captured prey particles (Taniguchi & Takeda 1988, Boenigk & Arndt 2000b). Accordingly, our compilation of bacteria showed significant differences in ingestion efficiencies that could not be attributed to bacterial cell size or any other factor directly assessed in our experiments. Moreover, reduced ingestion efficiencies of heat-killed strain SG81R1 may imply surface discrimination to some degree (see also Landry et al. 1991). We suggest that, along with prey size, specific surface components might be relevant to account for the active rejection of already captured bacterial cells by bacterivorous protozoans. However, since selective feeding in heterotrophic flagellates was found to be prey concentration-dependent according to optimal foraging models (Jürgens & De Mott 1995), the importance of the 'taste factor' might be restricted to feeding of well-fed flagellates and high bacterial abundances, respectively. Planktonic microorganisms live in a spatially and temporarily heterogeneous microenvironment. As a consequence, it will be important to examine the regulation and significance of chemically mediated prey selection in field studies and direct selection experiments.

General considerations

Our results give new insights into the selective feeding behavior of a bacterivorous nanoflagellate and the bacterial phenotypic properties involved. Given the need for abstraction and simplification, experimental studies like this one provide conclusions that might be restricted to the flagellate species and the experimental conditions used. For practical reasons, this study could not focus on low bacterial concentrations nor on free-swimming individuals, and thus all conclusions are based only on behavior of the attached part of the flagellate population. Also, more information is needed on the selection behavior of other feeding types to understand the impact of bacterial traits on bacterial grazing mortality in natural communities. Nevertheless, our finding that bacterial cell size effectively determines the handling success of the predator is in accordance with studies on the importance of size-selective bacterivory in natural bacterial populations (for a review see Hahn & Höfle 2001). Moreover, our data revealed the potential of size-independent traits in bacteria-flagellate interactions, such as bacterial

motility and surface composition. Non-morphological traits might account for the situational occurrence of high proportions of freely suspended cells observed in bacterioplankton despite severe protozoan grazing pressure (Matz & Jürgens unpubl.). However, the identification of the specific mechanisms governing chemically mediated food selection remains a technical challenge. Future work should specify the effectiveness of such size-independent mechanisms and their contribution to the divergent ways of bacterial grazing resistance.

Acknowledgements. We are grateful to Sandra Diederichs and Marlene Willkomm for technical assistance during this study, and to Jens-Ulrich Siebmann and Bryan F. J. Manly for comments on the statistics. Financial support for this research was provided by grant Ju 367/2-1 and Ar 288/3-1 from the Deutsche Forschungsgemeinschaft.

LITERATURE CITED

Absolom DR (1988) The role of bacterial hydrophobicity in infection: bacterial adhesion and phygocytic ingestion. Can J Microbiol 34:287–298

Azam F, Ammerman JW (1984) Cycling of organic matter by bacterioplankton in pelagic marine ecosystems: microenvironmental considerations. In: Fasham MJR (ed) Flows of energy and materials in marine ecosystems. Plenum Press, New York, p 345–360

Azam F, Fenchel T, Field JG, Gray JS, Meyer-Reil LA, Thingstad F (1983) The ecological role of water column microbes in the sea. Mar Ecol Prog Ser 10:257–263

Bennett SJ, Sanders RW, Porter KG (1988) Chemosensory responses of heterotrophic and mixotrophic flagellates to potential food sources. Bull Mar Sci 43:764–771

Berg HC, Brown DA (1972) Chemotaxis in *Escherichia coli* analysed by three-dimensional tracking. Nature 239: 500–504

Blackburn N, Fenchel T, Mitchell JG (1998) Microscale nutrient patches in planktonic habitats shown by chemotactic bacteria. Science 282:2254–2256

Boenigk J, Arndt H (2000a) Comparative studies on the feeding behavior of two heterotrophic nanoflagellates: the filter-feeding choanoflagellate *Monosiga ovata* and the raptorial-feeding kinetoplastid *Rhynchomonas nasuta*. Aquat Microb Ecol 22:243–249

Boenigk J, Arndt H (2000b) Particle handling during interception feeding by four species of heterotrophic nanoflagellates. J Eukaryot Microbiol 47:350–358

Boenigk J, Matz C, Jürgens K, Arndt H (2001a) Confusing selective feeding with differential digestion in bacterivorous nanoflagellates. J Eukaryot Microbiol 48:425–432

Boenigk J, Matz C, Jürgens K, Arndt H (2001b) The influence of preculture and food quality on the ingestion and digestion process of three species of heterotrophic nanoflagellates. Microb Ecol 42:168–176

Christaki U, Dolan JR, Pelegri S, Rassoulzadegan F (1998) Consumption of picoplankton-size particles by marine ciliates: effects of physiological state of the ciliate and particle quality. Limnol Oceanogr 43:458–464

Christoffersen K, Nybroe O, Jürgens K, Hansen M (1997) Measurement of bacterivory by heterotrophic nanoflagellates using immunofluorescence labelling of ingested cells. Aquat Microb Ecol 13:127–134

Chrzanowski TH, Šimek K (1990) Prey-size selection by freshwater flagellated protozoa. Limnol Oceanogr 35: 1429–1436

Decho AW (1990) Microbial exopolymer secretions in ocean environments: their role(s) in food webs and marine processes. Oceanogr Mar Biol Annu Rev 28:73–153

DeLong EF, Franks DG, Allredge AL (1993) Phylogenetic diversity of aggregate-attached vs. free-living marine bacterial assemblages. Limnol Oceanogr 38:924–934

Fenchel T (1982a) Ecology of heterotrophic microflagellates. I. Some important forms and their functional morphology. Mar Ecol Prog Ser 8:211–223

Fenchel T (1982b) Ecology of heterotrophic microflagellates. IV. Quantitative occurrence and importance as bacterial consumers. Mar Ecol Prog Ser 9:35–42

Fenchel T (1986) The ecology of heterotrophic microflagellates. Adv Microb Ecol 9:57–97

Fenchel T (2001) Eppur si muove: many water column bacteria are motile. Aquat Microb Ecol 24:197–201

Gannon JT, Manilal VB, Alexander M (1991) Relationship between cell surface properties and transport of bacteria through soil. Appl Environ Microbiol 57:190–193

Giovannoni SJ, Britschgi TB, Moyer CL, Field KG (1990) Genetic diversity in Sargasso Sea bacterioplankton. Nature 35:60–63

González JM, Iriberri J, Egea L, Barcina I (1990a) Differential rates of digestion of bacteria by freshwater and marine phagotrophic protozoa. Appl Environ Microbiol 56: 1851–1857

González JM, Sherr EB, Sherr BF (1990b) Size-selective grazing on bacteria by natural assemblages of estuarine flagellates and ciliates. Appl Environ Microbiol 56:583–589

González JM, Sherr EB, Sherr BF (1993) Differential feeding by marine flagellates on growing versus starving, and on motile versus nonmotile, bacterial prey. Mar Ecol Prog Ser 102:257–267

Grossart HP, Azam F (1998) Motility of natural bacterial assemblages in coastal waters. EOS Trans (Suppl) 79: 807–809

Guillard RRL, Lorenzen CJ (1972) Yellow-green algae with chlorophyllide c. J Phycol 8:10–14

Hahn MW, Höfle MG (2001) Grazing of protozoa and its effects on populations of aquatic bacteria. FEMS Microbiol Ecol 35:113–121

Hahn MW, Moore ERB, Höfle MG (1999) Bacterial filament formation, a defense mechanism against flagellate grazing, is growth rate controlled in bacteria of different phyla. Appl Environ Microbiol 65:25–35

Hammer A, Grüttner C, Schumann R (1999) The effect of electrostatic charge of food particles on capture efficiency by *Oxyrrhis marina* Dujardin (dinoflagellate). Protist 150: 375–382

Hammond SM, Lambert PA, Rycoft AN (1984) The bacterial cell surface. Croom Helm, London

Heissenberger A, Leppard GG, Herndl GJ (1996) Relationship between the intracellular integrity and the morphology of the capsular envelope in attached and free-living bacteria. Appl Environ Microbiol 62:4521–4528

Horwitz MA, Silverstein SC (1980) Influence of the *Escherichia coli* capsule on complement fixation and on phagocytosis and killing by human phagocytes. J Clin Invest 65:82–94

Jürgens K, De Mott WR (1995) Behavioral flexibility in prey selection by bacterivorous nanoflagellates. Limnol Oceanogr 40:1503–1507

Jürgens K, Güde H (1994) The potential importance of graz-

ing-resistant bacteria in planktonic systems. Mar Ecol Prog Ser 112:169–188
Jürgens K, Pernthaler J, Schalla S, Amann R (1999) Morphological and compositional changes in a planktonic bacterial community in response to enhanced protozoan grazing. Appl Environ Microbiol 65:1241–1250
Landry MR, Lehner-Fournier JM, Sundstrom JA, Fagerness L, Selph KE (1991) Discrimination between living and heat-killed prey by a marine zooflagellate, *Paraphysomonas vestita* (Stokes). J Exp Mar Biol Ecol 146:139–151
Lauffenburger DA (1991) Quantitative studies of bacterial chemotaxis and microbial population dynamics. Microb Ecol 22:175–185
Matz C, Jürgens K (2001) Effects of hydrophobic and electrostatic cell surface properties of bacteria on feeding rates of heterotrophic nanoflagellates. Appl Environ Microbiol 67: 814–820
Mitchell GC, Baker JH, Sleigh MA (1988) Feeding of a freshwater flagellate, *Bodo saltans*, on diverse bacteria. J Protozool 35:219–222
Mitchell JG (1991) The influence of cell size on marine bacterial motility and energetics. Microb Ecol 22:227–238
Mitchell JG, Pearson L, Bonazinga A, Dillon S, Khouri H, Paxinos R (1995a) Long lag times and high velocities in the motility of natural assemblages of marine bacteria. Appl Environ Microbiol 61:877–882
Mitchell JG, Pearson L, Dillon S, Kantalis K (1995b) Natural assemblages of marine bacteria exhibiting high-speed motility and large accelerations. Appl Environ Microbiol 61:4436–4440
Monger BC, Landry MR (1990) Direct-interception feeding by marine zooflagellates: the importance of surface and hydrodynamic forces. Mar Ecol Prog Ser 65:123–140
Monger BC, Landry MR (1992) Size-selective grazing by heterotrophic nanoflagellates: an analysis using live-stained bacteria and dual-beam flow cytometry. Arch Hydrobiol Beih 37:173–185
Monger BC, Landry MR, Brown SL (1999) Feeding selection of heterotrophic marine nanoflagellates based on the surface hydrophobicity of their picoplankton prey. Limnol Ocenogr 44:1917–1927
Ofek I, Goldhar J, Keisari Y, Sharon N (1995) Nonopsonic phagocytosis of microorganisms. Annu Rev Microbiol 49: 239–276
Plante CJ (2000) Role of bacterial exopolymeric capsules in protection from deposit-feeder digestion. Aquat Microb Ecol 21:211–219
Plante CJ, Shriver A (1998) Differential lysis of sedimentary bacteria by *Arenicola marina*: examination of cell wall structure and exopolymeric capsules as correlates. J Exp Mar Biol Ecol 229:35–52
Porter KG, Feig YS (1980) The use of DAPI for identifying and counting aquatic microflora. Limnol Oceanogr 25:943–948
Posch T, Šimek K, Vrba J, Pernthaler J, Nedoma J, Sattler B, Sonntag B, Psenner R (1999) Predator-induced changes of bacterial size-structure and productivity studied on an experimental microbial community. Aquat Microb Ecol 18: 235–246
Rainey PB, Moxon ER, Thompson IP (1993) Intraclonal polymorphism in bacteria. Adv Microb Ecol 13:263–300
Roszak DB, Colwell RR (1987) Survival strategies of bacteria in the natural environment. Microb Rev 51:365–379
Sanders RW (1988) Feeding by *Cyclidium* sp. (Ciliophora, Scuticociliatida) on particles of different sizes and surface properties. Bull Mar Sci 43:446–457
Sanders RW, Caron DA, Berninger UG (1992) Relationships between bacteria and heterotrophic nanoplankton in marine and fresh waters: an inter-system comparison. Mar Ecol Prog Ser 86:1–14
Sherr BF, Sherr EB, Berman T (1983) Grazing, growth, and ammonium excretion rates of a heterotrophic microflagellate fed with four species of bacteria. Appl Environ Microbiol 45:1196–1201
Sibbald MJ, Albright LJ, Sibbald PR (1987) Chemosensory response of a heterotrophic microflagellate to bacteria and several nitrogen compounds. Mar Ecol Prog Ser 36: 201–201
Šimek K, Chrzanowski TH (1992) Direct and indirect evidence of size-selective grazing on pelagic bacteria by freshwater nanoflagellates. Appl Environ Microbiol 58: 3715–3720
Šimek K, Vrba J, Pernthaler J, Posch T, Hartman P, Nedoma J, Psenner R (1997) Morphological and compositional shifts in an experimental bacterial community influenced by protists with contrasting feeding modes. Appl Environ Microbiol 63:587–595
Snyder RA (1991) Chemoattraction of a bacterivorous ciliate to bacterial surface compounds. Hydrobiologia 215: 205–213
Stoecker DK, Cucci TL, Hurlbert EM, Yentsch CM (1986) Selective feeding by *Balanion* sp. (Ciliata: Balanionidae) on phytoplankton that best support its growth. J Exp Mar Biol Ecol 95:113–130
Sutherland IW (1977) Bacterial exopolysaccharides: their nature and production. In: Sutherland IW (ed) Surface carbohydrates of the procaryotic cell. Academic Press, New York
Taniguchi A, Takeda Y (1988) Feeding rate and behavior of the titinnid ciliate *Favella taraikaensis* observed with a high speed VTR system. Mar Microb Food Webs 3:21–34
Taylor WD, Berger J (1976) Growth responses of cohabiting ciliate protozoa to various prey bacteria. Can J Zool 54: 1111–1114
Van der Mei HC, Weerkamp AH, Busscher HJ (1987) A comparison of various methods to determine hydrophobic properties of streptococcal cell surfaces. J Microbiol Methods 6:277–287
Van Oss CJ (1978) Phagocytosis as a surface phenomenon. Annu Rev Microbiol 32:19–39
Verity PG (1991) Feeding in planktonic protozoans: evidence for non-random acquisition of prey. J Protozool 38:69–76
Wolfe GV (2000) The chemical defense ecology of marine unicellular plankton: constraints, mechanisms, and impacts. Biol Bull 198:225–244
Yamada T, Muramatsu N, Kondo T (1993) Phagocytosis of monosaccharide-binding latex particles by guinea-pig polymorphonuclear leucocytes. J Biomat Sci Polymer Ed 4:347–355

Editorial responsibility: Karel Šimek, České Budějovice, Czech Republic

Submitted: September 25, 2001; Accepted: December 18, 2001
Proofs received from author(s): February 22, 2002