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High Motility Reduces Grazing Mortality of Planktonic Bacteria

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We tested the impact of bacterial swimming speed on the survival of planktonic bacteria in the presence of protozoan grazers. Grazing experiments with three common bacterivorous nanoflagellates revealed low clearance rates for highly motile bacteria. High-resolution video microscopy demonstrated that the number of predator-prey contacts increased with bacterial swimming speed, but ingestion rates dropped at speeds of >25 μm s^{-1} as a result of handling problems with highly motile cells. Comparative studies of a moderately motile strain (<25 μm s^{-1}) and a highly motile strain (>45 μm s^{-1}) further revealed changes in the bacterial swimming speed distribution due to speed-selective flagellate grazing. Better long-term survival of the highly motile strain was indicated by fourfold-higher bacterial numbers in the presence of grazing compared to the moderately motile strain. Putative constraints of maintaining high swimming speeds were tested at high growth rates and under starvation with the following results: (i) for two out of three strains increased growth rate resulted in larger and slower bacterial cells, and (ii) starved cells became smaller but maintained their swimming speeds. Combined data sets for bacterial swimming speed and cell size revealed highest grazing losses for moderately motile bacteria with a cell size between 0.2 and 0.4 μm^3. Grazing mortality was lowest for cells of >0.5 μm^3 and small, highly motile bacteria. Survival efficiencies of >95% for the ultramicrobacterial isolate CP-1 (≤0.1 μm^3, >50 μm s^{-1}) illustrated the combined protective action of small cell size and high motility. Our findings suggest that motility has an important adaptive function in the survival of planktonic bacteria during protozoan grazing.

Bacteria in most aquatic and terrestrial microbial communities are exposed to grazing by bacterivorous protozoans. Predation by protozoans is a significant control factor of bacterial biomass and production and an important selective force for bacterial community structure (for a review see reference 17). Whereas bacterial grazing losses have been extensively quantified in laboratory and field studies for more than two decades, the actual mechanisms of interaction between bacteria and protozoa and potential protective adaptations have received increasing attention only in recent years.

One important feature of protozoan grazing is its size selectivity, which results in the reduction of the average cell size in grazed bacterial assemblages (10, 36). As a consequence size-selective protozoan grazing is widely believed to contribute to the small average cell size of bacterioplankton communities. Moreover, bacterial assemblages can develop complex inedible morphologies—such as cell filaments and microcolonies—in the presence of heterotrophic nanoflagellates which may ultimately lead to changes in the structural and taxonomic community composition (14, 18, 37). Large bacterial morphotypes are an effective defense mechanism against nanoplankton protozoans but imply the tradeoff of being part of the prey size spectrum of larger predators (e.g., micro- and mesozooplankton).

For that reason size-unrelated protective mechanisms, which include biochemical cell composition, cell surface parameters, and motility characteristics, may be of particular importance for the survival of planktonic bacteria. In a recent study, for instance, we demonstrated the lethal impact of bacteria containing the secondary metabolite violacein on bacterivorous flagellates (21). Furthermore, extremely negative surface charges and the biochemical surface composition of bacterial cells were shown to reduce flagellate feeding rates (20). Indigestible prey was found to be prematurely egested by nanoflagellates (3), and it is well known that some bacterial pathogens survive in protozoan food vacuoles (19). Initial evidence that bacterial motility may also influence interactions between bacteria and protozoa came from chemostat experiments in which highly motile bacteria accumulated during increased grazing pressure by heterotrophic nanoflagellates (24).

To date, bacterial motility has been interpreted mainly as an adaptive trait that allows bacteria to reach nutrient patches and optimal growth conditions in a heterogeneous and otherwise nutrient-poor environment. To achieve this, many planktonic bacteria are able to respond to chemical signals by chemotaxis (2). Although motile bacteria can be isolated from diverse aquatic and terrestrial habitats, the quantification of motile bacteria in natural environments is still in its infancy. For marine bacterioplankton, for instance, it has only recently been estimated that 20 to 80% of bacteria are motile (6, 13). Additionally swimming speeds cover a wide range among motile marine bacteria: in a phylogenetically diverse collection of 84 marine bacterial isolates (γ-proteobacteria, α-proteobacteria, cytophaga, and gram positives) mean swimming speeds ranged from 11.3 to 38.5 μm s^{-1} (16). Mitchell and coworkers reported mean community swimming speeds of around 45 μm s^{-1} for marine bacterioplankton and maximum swimming speeds exceeding 200 μm s^{-1} for some marine isolates (27, 28).

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Such a pronounced inter- and intraspecific variability among planktonic bacteria—from immobility to extremely high swimming speeds—calls for a thorough examination of the impact of bacterial motility and swimming speed on interactions between bacteria and protozoa. Based on the indications from a recent chemostat study (24), we hypothesized that heterotrophic nanoflagellates feed not only size selectively but also speed selectively. As a consequence we expected that highly motile bacteria would exhibit a better survival due to lower flagellate ingestion rates. Moreover, we focused special attention on the interdependence of bacterial cell size and speed and the combined effect of small cell size and high swimming speed on survival rates of planktonic bacteria.

MATERIALS AND METHODS

Organisms and growth conditions. The bacterial strains used in this study were isolated from a mesotrophic lake in northern Germany (Schöhsee). While five strains (KB6, KB9, KB23, MM1, and SG81R1) were isolated directly from field samples, four strains (CM10, CP-1, CP-2, and CP-17) were obtained from field samples that had been cultivated under severe grazing pressure. Strains CP-1, CP-2, and CP-17 were isolated from a carbon-limited chemostat where highly motile bacteria accumulated during increased flagellate grazing (24). Similarities of 16S rRNA gene sequences to the closest related strain are given in Table 1. Bacterial strains were routinely maintained in continuous culture under carbon-limited growth conditions: The growth medium contained inorganic WC medium with a phosphorus concentration of 300 μg of $P\text{-}PO_4$ liter^{-1} and was supplemented with 7.4 mg of C liter^{-1} (glucose, serine, glycine, threonine, alanine, and aspartate) (24). The growth medium was pumped into the culture vessels at a dilution rate of $D = 0.02$ h^{-1}. Strain CP-1 was not successfully cultivated under these conditions and was therefore grown in batch cultures on nutrient broth (4 g liter^{-1}).

The predators used in this study were three interception-feeding nanoflagellates that are commonly found in lake plankton: *Spumella* sp., *Ochromonas* sp., and *Bodo saltans* were isolated from the same lake as the bacteria (Schöhsee) and have been examined in a number of previous studies (e.g., references 20 and 23). Axenic cultures of *Ochromonas* sp. were established by G. Corno (Plön) and were maintained on suspensions of heat-killed *Pseudomonas putida* MM1. Stock cultures of the flagellates *Spumella* sp. and *B. saltans* were kept on live *P. putida* MM1 suspended in WC medium with a glucose concentration of 100 mg liter^{-1}. For all experiments, flagellates were taken from 5-day-old stock cultures when bacteria were reduced below 10^4 cells ml^{-1} and flagellate abundances reached approximately 10^5 cells ml^{-1}.

Experimental design. (i) The first experiment compared the clearance rates of three nanoflagellates (*Spumella* sp., *Ochromonas* sp., and *B. saltans*) on three bacterial strains (strains KB6, KB23, and SG81R1) with different motilities but similar cell sizes. Bacterial cell sizes and swimming speeds were measured before the bacteria were added to flagellate cultures at a final concentration of 10^7 cells ml^{-1}. After an incubation for 15 min at room temperature flagellate cells were fixed with ice-cold glutaraldehyde (2% final concentration). In each of the three replicates 100 flagellate cells were inspected for ingested bacteria by means of immunofluorescence microscopy as detailed below.

(ii) The direct impact of bacterial swimming speed on flagellate feeding behavior was studied by means of high-resolution video microscopy. Six bacterial strains (strains KB9, MM1, KB6, KB23, SG81R1, and CP-2) with similar cell sizes but covering an average swimming speed range from <1 to 60 μm s^{-1} were offered separately to the flagellate *Spumella* sp. Heat-immobilized cells (60°C for 1 h) were used as control treatment for each strain. The experimental setup followed the one previously described (20). Briefly, bacteria were added at a concentration of 2×10^7 ml^{-1} to *Spumella* sp. cultures in an observational chamber, which was constructed out of a 55-mm-diameter petri dish fitted with a hole and a coverslip at the bottom (3). For each bacterial strain, the feeding behavior of 12 individual flagellate cells was video recorded for 15 min and later analyzed.

(iii) We used the moderately motile strain CP-17 and the highly motile strain CP-2 to evaluate bacterial speed distributions in response to flagellate grazing. Samples from bacterial chemostat cultures were divided into triplicates before the flagellate *Ochromonas* sp. was added. Bacterial swimming speeds were measured after 0, 5, and 10 h of grazing.

(iv) Subsequently the chemostats were used to compare the survival of the moderately motile strain CP-17 and the highly motile strain CP-2 during grazing under continuous growth conditions. Bacteria were grown for 10 days at $D = 0.02$ h^{-1} to reach steady state before the flagellate *Ochromonas* sp. was added. Bacterial numbers, cell size, and motility parameters and flagellate numbers were analyzed before and after the introduction of the grazer.

(v) Chemostat cultures were used to test the influence of a 10-fold increase in bacterial growth rate (from $D = 0.02$ h^{-1} to $D = 2.0$ h^{-1}) and starvation conditions on swimming speed and cell size. Three strains (CP-2, CP-17, and CM10) were compared at steady state for each growth rate. Starvation conditions were simulated by incubating 200-ml chemostat samples for 20 days after centrifugation and washing of bacteria with WC medium.

(vi) Survival of the ultramicrobacterium CP-1 in the presence of the flagellate *Spumella* sp. was studied in three 200-ml batch cultures without the addition of an organic carbon source. Precultures of strain CP-1 were grown overnight on nutrient broth and diluted in WC medium to a concentration of 2×10^7 ml^{-1}. Instantly after the addition of the flagellate on day 1, culture subsamples were evaluated by high-resolution video microscopy as described below. Throughout the experiment bacterial and flagellate numbers were determined on a daily basis. In both experiments we used heat-immobilized CP-1 cells as control treatment.

Video microscopy. For high-resolution video microscopy we employed an inverted microscope, a standard video camera, and a videocassette recorder. We used the same definitions for the main steps in the flagellate feeding process as mentioned in the work of Matz et al. (20). Events were defined as "contact" when bacteria encountered the flagellate's sensitive "oral" region near the base of the long flagellum. Once the flagellate folded the long flagellum over the bacterium, feeding events were scored as "capture." The subsequent inclusion in a food vacuole was scored as "ingestion."

Cell size and number. Samples for the enumeration of bacteria and flagellates were preserved with formaldehyde (final concentration, 2%). Cell numbers were counted microscopically after staining with 4′,6′-diamidino-2-phenylindole (DAPI; Sigma). These preparations were also used to determine bacterial cell size with an automated image analysis system (SIS GmbH, Münster, Germany).

Bacterial swimming speed. Bacterial swimming behavior was documented microscopically by means of a standard video camera and videocassette recorder (20, 24). Chemostat samples were instantly transferred to the observational chamber, which was constructed out of a glass slide and a coverslip separated by adhesive tape. Three to five subsamples of each sample were examined. Recordings were performed at dark-field illumination with 200× magnification (20× objective and 10× magnification ring). Observations were made midchamber for 1 min at maximum light intensity and contrast. Videotapes were analyzed automatically using MedeaLab 3.1 Tracking System (1994 to 1997; Medea AV GmbH, Erlangen, Germany).

Immunofluorescence microscopy. Ingested bacteria were detected in flagellate food vacuoles by rabbit polyclonal antibodies that were raised against six strains (Eurogentec, Seraing, Belgium). Ingested cells were visualized by the binding of Cy3-conjugated goat anti-rabbit immunoglobulin G (Jackson ImmunoResearch Laboratories) and by being stained nonspecifically with DAPI (see references 21 and 23).

Data analysis. Flagellate clearance rates (F) were determined from the ingestion rate (I) (bacterial flagellate^{-1} h^{-1}) and the initial bacterial concentration (B) (bacteria ml^{-1}) by using the formula $F = I/B$ = (nanoliters flagellate^{-1} h^{-1}). Flagellate feeding efficiencies were calculated as defined in the work of Matz et al. (20): "capture efficiency" describes the proportion of bacterium-flagellate encounters that were captured, whereas "ingestion efficiency" describes how many

TABLE 1. Bacterial strains used in this study and similarities of 16S rRNA gene sequences with the nearest relatives from GenBank

Strain	Closest relative	Similarity (%)	Reference; GenBank accession no.
KB6	*Pseudomonas pavonaceae*	99.5	1
KB9	*Flavobacterium columnare*	96.7	1
KB23	*Pseudomonas rhodesiae*	99.0	1
SG81R1	*Pseudomonas aeruginosa*	NA[a]	12
CM10	*Pseudomonas fluorescens*	99.9	22
MM1	*Pseudomonas putida*	99.6	This study; AY623928
CP-2	*Acidovorax* sp.	99.1	This study; AY623930
CP-17	*Pseudomonas rhodesiae*	99.7	This study; AY623931
CP-1	Uncultured ultramicrobacterium Um1	97.7	This study; AY623929

[a] NA, not available.

TABLE 2. Size and motility characteristics of the bacterial strains used in video microscopy experiments[a]

Bacterial strain	Cell length (μm)	Cell vol (μm³)	Mean speed (μm s⁻¹)	Max speed (μm s⁻¹)
KB9	1.24 ± 0.29	0.26 ± 0.03	0.6 ± 0.1	0.8 ± 0.2
MM1	1.23 ± 0.20	0.24 ± 0.03	6.7 ± 1.3	40.5 ± 8.6
KB6	1.27 ± 0.25	0.25 ± 0.05	17.1 ± 4.2	72.2 ± 24.2
KB23	1.28 ± 0.27	0.26 ± 0.04	28.7 ± 5.4	82.7 ± 35.9
SG81R1	1.23 ± 0.27	0.24 ± 0.05	43.9 ± 3.4	144.9 ± 44.4
CP-2	1.25 ± 0.24	0.24 ± 0.01	59.3 ± 4.6	160.9 ± 36.0

[a] Bacteria were taken from chemostat cultures grown to steady state at a dilution rate of $D = 0.02\ h^{-1}$. The data (presented as means ± standard deviations) are based on 12 subsamples.

of the captured bacteria were subsequently ingested. Bacterial survival efficiency describes the proportion of bacteria that were contacted by the flagellate but did not end up in the flagellate food vacuole.

One-way analyses of variance were used to test for significant differences of bacterial swimming speeds and cell sizes and flagellate feeding rates against bacterial strains. Absolute values were ln transformed, and percentages were arcsine-square root transformed. Post hoc comparisons of means were provided by Tukey tests. Pearson product-moment correlations were used to test for significant relationships between bacterial swimming speed and flagellate-bacterium contact rates, flagellate capture failure, and bacterial survival efficiency.

Nucleotide sequence accession numbers. The 16S rRNA gene sequences have been deposited in the GenBank database under the accession numbers given in Table 1.

RESULTS

High bacterial motility affects clearance rates of three nanoflagellates. The impact of bacterial motility on feeding rates of *Spumella* sp., *Ochromonas* sp., and *B. saltans* was studied in grazing experiments with three morphologically comparable bacterial isolates: *Pseudomonas pavonaceae* KB6 had mean swimming speeds of 17 μm s^{-1}, *Pseudomonas rhodesiae* KB23 had speeds of 29 μm s^{-1}, and *Pseudomonas aeruginosa* SG81R1 had speeds of 44 μm s^{-1} (Table 2). Ingestion measurements performed by immunofluorescence microscopy revealed that clearance rates of all three flagellates consistently decreased from moderate to high bacterial motility (Fig. 1). Differences in clearance rates between the highly motile isolate *P. aeruginosa* SG81R1 and the moderately motile strain *P. pavonaceae* KB6 were significant ($P \leq 0.005$ for *Ochromonas* sp., $P \leq 0.001$ for *Spumella* sp., and $P \leq 0.002$ for *B. saltans*). *B. saltans* had the lowest clearance rates (0.5 to 0.9 nl flagellate^{-1} h^{-1}), and *Ochromonas* sp. had the highest (1.1 to 1.9 nl flagellate^{-1} h^{-1}).

High bacterial swimming speed increases predator-prey contacts but facilitates bacterial survival. High-resolution video microscopy was used to allow a detailed analysis of the influence of bacterial swimming speed on flagellate feeding success and bacterial survival efficiencies. Six bacterial strains with comparable cell sizes but mean swimming speeds ranging from <1 to 60 μm s^{-1} (Table 2) were tested in grazing experiments with *Spumella* sp. We consistently observed that flagellates failed to capture highly motile bacteria subsequent to cell contact, which is illustrated in Fig. 2.

Figure 3A shows that contact rates between flagellates and bacteria significantly increased with mean bacterial swimming speed ($P < 0.001$, $r = 0.97$). The mean contact rate for bacteria with an average swimming speed of 60 μm s^{-1} (38.5 ± 8.4 bacteria flagellate^{-1} 15 min^{-1}) was approximately threefold higher than for immotile bacteria (12.6 ± 2.3 bacteria flagellate^{-1} 15 min^{-1}). In contrast, flagellate ingestion rates increased only until a mean bacterial swimming speed of about 20 μm s^{-1} was reached. Ingestion rates for bacteria swimming at this speed were significantly higher than for bacteria with lower or higher speeds ($P \leq 0.05$). At bacterial swimming speeds exceeding 25 μm s^{-1}, the number of ingested bacteria dropped significantly despite steadily increasing rates of contact with the flagellate ($P < 0.01$, $r = -0.98$). Ingestion rates for bacteria swimming at 60 μm s^{-1} were significantly lower (4.4 ± 1.6 bacteria flagellate^{-1} 15 min^{-1}) than those recorded for immotile bacteria (8.2 ± 1.9 bacteria flagellate^{-1} 15 min^{-1}, $P < 0.04$).

Figure 3B demonstrates that low ingestion rates at high bacterial swimming speeds resulted from increasing flagellate capture failure and bacterial survival success. Flagellate capture failure, which describes how many of the contacted bacteria could not be captured, showed a significant correlation with bacterial swimming speed ($P < 0.001$, $r = 0.98$). Capture failures of heat-immobilized bacteria remained in the range from 11 to 18% and were always significantly lower than those for the corresponding motile bacteria ($P < 0.001$, data not shown). Total bacterial survival success (number of bacteria that survived contact, capture, and handling) increased with increasing bacterial swimming speed from about 35 to about 90% ($P = 0.01$, $r = 0.92$). Total survival success of bacteria faster than 25 μm s^{-1} was significantly higher than flagellate capture failure on the same strain ($P < 0.001$), which indicates additional escape of highly motile bacteria during prey handling.

Changes in bacterial swimming speed distributions due to speed-selective grazing. We compared the effect of flagellate grazing on bacterial speed distribution in monocultures of the moderately motile isolate *P. rhodesiae* CP-17 and the highly

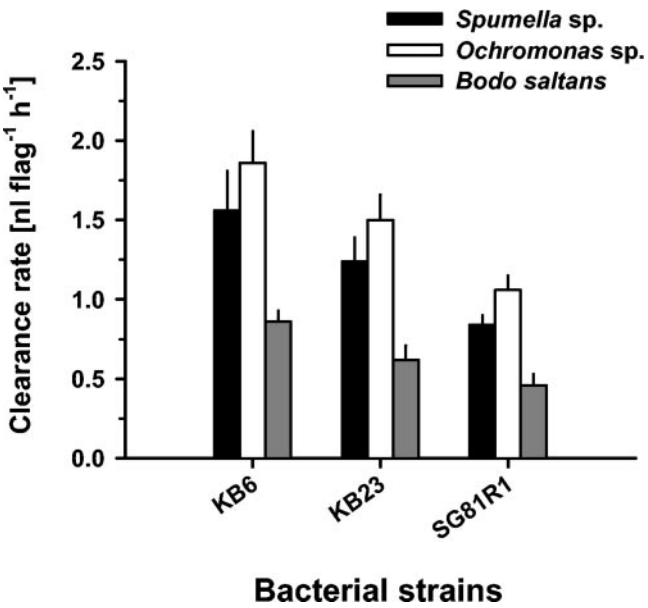

FIG. 1. Clearance rates of three flagellates on bacterial strains with different motility. *P. pavonaceae* KB6 showed mean swimming speeds of 17 μm s^{-1}, *P. rhodesiae* KB23 showed speeds of 29 μm s^{-1}, and *P. aeruginosa* SG81R1 showed speeds of 44 μm s^{-1}. Bacteria were sorted in the order of increasing swimming speed. Error bars indicate standard deviations of three replicates.

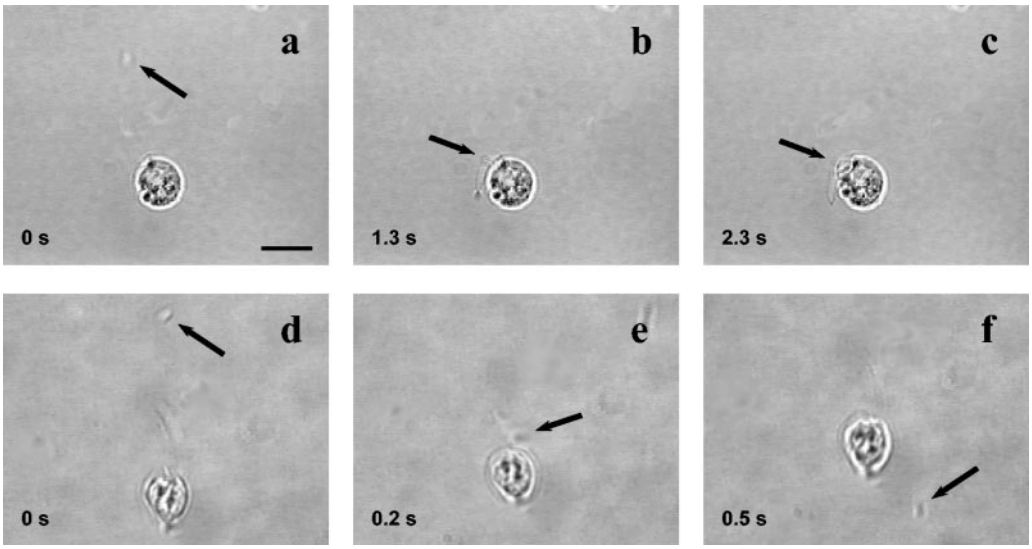

FIG. 2. Interaction between *Spumella* sp. and moderately motile *P. pavonaceae* KB6 (a to c) and highly motile *Acidovorax* sp. strain CP-2 (d to f). After the prey bacterium is caught in the feeding current of the flagellate (a and d), the moderately motile KB6 is captured by the long flagellum of the flagellate (b) and forced into a food vacuole after 2.3 s (c). Note that the highly motile CP-2 escapes the capture reaction of the flagellate after cell contact (e) and moves out of reach within less than 0.5 s (f). The arrow indicates the location of the prey bacterium. Light video microscopy was used (1,200×, oil immersion). Bar = 5 μm.

motile isolate *Acidovorax* sp. strain CP-2 (Fig. 4). Before the flagellate *Ochromonas* sp. was added, the unimodal speed distribution of *P. rhodesiae* CP-17 was characterized by a frequency peak at 5 to 10 μm s^{-1}, a median of 15.3 ± 2.1 μm s^{-1}, and a mean of 22.0 ± 3.7 μm s^{-1}. In contrast, *Acidovorax* sp. strain CP-2 showed a bimodal speed distribution peaking at 5 to 10 μm s^{-1} and at 60 to 65 μm s^{-1}, which resulted in a significantly higher median (41.4 ± 6.2 μm s^{-1}, $P < 0.001$) and mean (40.5 ± 4.2 μm s^{-1}, $P < 0.002$) swimming speed compared to *P. rhodesiae* CP-17.

Five hours after the flagellate had been added, the initial total cell number of moderately motile *P. rhodesiae* CP-17 bacteria was reduced by about 66%. Under grazing, the median swimming speed was slightly lower (10.3 ± 2.7 μm s^{-1}), indicating a preferred reduction of moderately motile bacteria. Bacteria swimming between 15 and 50 μm s^{-1} were reduced by about 75%. After 10 h of grazing total bacterial counts were reduced below 4% of the initial number and the median swimming speed was at 9.8 ± 3.2 μm s^{-1}. Feeding on highly motile *Acidovorax* sp. strain CP-2, *Ochromonas* sp. reduced bacterial numbers by only 43% in the first 5 h. Speed-selective flagellate feeding was indicated by a pronounced reduction of the lower-speed classes and the significant increase of the median swimming speed (61.0 ± 2.0 μm s^{-1}, $P < 0.002$). While bacteria swimming at speeds below 50 μm s^{-1} were reduced by more than 60%, bacteria exceeding 50 μm s^{-1} lost less than 30%. After 10 h of flagellate grazing, 22% of the total number of bacteria was still left and the median swimming speed was significantly higher (57.5 ± 2.4 μm s^{-1}, $P < 0.003$) than before the introduction of the grazers.

High cell numbers of highly motile bacteria in the presence of flagellate grazing. Chemostats were used to examine the role of high bacterial motility in the survival of grazed bacterial populations at continuous growth conditions. Continuous cultures of moderately motile *P. rhodesiae* CP-17 and highly motile *Acidovorax* sp. strain CP-2 revealed comparable steady-state bacterial numbers and cell sizes before the flagellate *Ochromonas* sp. was added (Table 3). The mean swimming speed of *Acidovorax* sp. strain CP-2 was considerably higher than that of *P. rhodesiae* CP-17 (47.0 ± 4.5 versus 21.6 ± 3.7 μm s^{-1}, respectively).

After the introduction of the flagellates, *P. rhodesiae* CP-17 was reduced from 13.4 × 10^6 ± 1.14 × 10^6 cells ml^{-1} to a minimum threshold concentration of 0.32 × 10^6 ± 0.09 × 10^6 cells ml^{-1}. In contrast, highly motile *Acidovorax* sp. strain CP-2 showed a fourfold higher minimum concentration (1.2 × 10^6 ± 0.14 × 10^6 cells ml^{-1}) in the presence of grazers. In comparison to the pregrazing condition, mean swimming speeds under the impact of grazing decreased for *P. rhodesiae* CP-17 (15.3 ± 4.6 μm s^{-1}) and increased for *Acidovorax* sp. strain CP-2 (54.2 ± 5.1 μm s^{-1}). Maximum flagellate numbers were about 50% higher on moderately motile *P. rhodesiae* CP-17 (39.1 × 10^3 ± 3.00 × 10^3 cells ml^{-1}) than on highly motile *Acidovorax* sp. strain CP-2 (25.6 × 10^3 ± 0.55 × 10^3 cells ml^{-1}). Furthermore flagellate growth rates during the initial exponential growth phase were about 30% higher on moderately motile *P. rhodesiae* CP-17 (1.4 ± 0.09 day^{-1}) than on highly motile *Acidovorax* sp. strain CP-2 (1.1 ± 0.06 day^{-1}).

Impact of growth and starvation on bacterial swimming speed and cell size. Continuous bacterial cultures were used to evaluate the influence of a 10-fold increase in bacterial growth rate on the swimming speeds and cell size of two moderately motile isolates, *Pseudomonas fluorescens* CM10 and *P. rhodesiae* CP-17, and the highly motile isolate *Acidovorax* sp. strain CP-2 (Fig. 5A). At a growth rate of μ = 0.02 h^{-1}, the three strains showed comparable cell sizes (0.24 ± 0.01 $μm^3$ for strain CP-2, 0.25 ± 0.03 $μm^3$ for *P. fluorescens* CM10, and 0.21 ± 0.04 $μm^3$ for *P. rhodesiae* CP-17). The mean swimming speed of *Acidovorax* sp. strain CP-2 (51.2 ± 5.9 μm s^{-1}) was higher than the speed of *P. fluorescens* CM10 (20.0 ± 5.2 μm

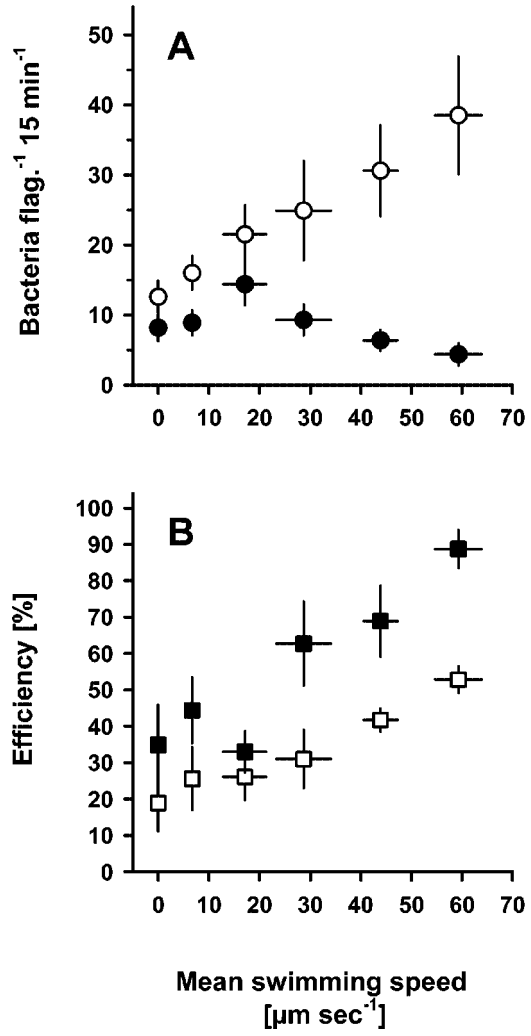

FIG. 3. Flagellate feeding and bacterial survival parameters at different bacterial swimming speeds. The upper graph (A) shows contact rates between bacteria and flagellates (○) and ingestion rates (●). The lower graph (B) illustrates bacterial escape efficiencies after contact with the flagellate (□) and total survival efficiencies (■). Values are given as means ± standard deviations based on the evaluation of 12 replicates.

s^{-1}) and *P. rhodesiae* CP-17 (23.4 ± 2.5 µm s^{-1}). By increasing the growth rate in the chemostats to $\mu = 0.2\ h^{-1}$, an increase in cell size by about 50% was observed for *Acidovorax* sp. strain CP-2 (0.38 ± 0.05 μm^3) and *P. fluorescens* CM10 (0.35 ± 0.04 μm^3). At the same time mean swimming speeds dropped for *Acidovorax* sp. strain CP-2 (40.3 ± 1.3 µm s^{-1}) and *P. rhodesiae* CP-17 (12.1 ± 2.3 µm s^{-1}). *P. rhodesiae* CP-17 responded to higher growth rates with a higher swimming speed (39.9 ± 3.1 µm s^{-1}), while its cell size remained fairly constant. Likewise, maximum swimming speeds of *P. rhodesiae* CP-17—which may serve as a measure for acceleration capability—were also considerably increased by higher growth rates (from 78.7 ± 3.6 to 129.1 ± 0.7 µm s^{-1}).

Starvation conditions were simulated by incubating the three bacterial strains without external nutrient supply for 20 days (Fig. 5B). All three strains responded to starvation by a decrease in cell size (0.18 ± 0.01 μm^3 for *Acidovorax* sp. strain CP-2, 0.21 ± 0.04 μm^3 for *P. fluorescens* CM10, and 0.19 ± 0.01 μm^3 for *P. rhodesiae* CP-17). Mean swimming speeds of the three strains, however, were not affected by starvation conditions. Maximum swimming speeds of *Acidovorax* sp. strain CP-2, however, were reduced by about 30% after 20 days of starvation (from 145.4 ± 21.9 to 98.0 ± 8.4 µm s^{-1}).

Combined effect of swimming speed and cell size on bacterial survival efficiency. Pooled data sets from the experiments presented above and a previous study which followed the same experimental design (20) were used to examine the combined effect of bacterial cell size and swimming speed on the probability of surviving encounters with the bacterivorous nanoflagellate *Spumella* sp. (Fig. 6). Video microscopy analysis of a total of 23 bacterial size-speed categories provided the following trends: (i) the lowest survival efficiencies were observed for moderately motile bacteria swimming at 10 to 20 µm s^{-1} with a cell volume between 0.2 and 0.4 μm^3, (ii) within the same size range (0.2 to 0.4 μm^3) a steady increase of bacterial survival efficiencies up to 80% was observed with increasing swimming speed, (iii) survival efficiencies of the size class from 0.1 to 0.2 μm^3 were consistently higher than for the size class from 0.2 to 0.3 μm^3, (iv) bacteria swimming at 0 to 10 µm s^{-1} generally exhibited higher survival efficiencies than cells swimming at 10 to 20 µm s^{-1}, (v) swimming speeds of bacteria larger than 0.4 μm^3 did not exceed 20 µm s^{-1}, and (vi) the highest survival efficiencies (>90%) were found for either bacteria larger than 0.5 μm^3 or the smallest and fastest bacteria.

Grazing protection of the ultramicrobacterial strain CP-1 due to high motility and small cell size. Based on our findings of high survival rates for small, highly motile bacteria, we tested the grazing mortality of the ultramicrobacterial strain CP-1. This bacterium was characterized by a cell volume of 0.1 ± 0.01 μm^3 and an average swimming speed of 58.8 ± 7.2 µm s^{-1} before the nanoflagellate *Spumella* sp. was added (Table 4). Video microscopy analysis in the first hours after the addition of the flagellate revealed a bacterial survival efficiency of 96.7% ± 2.9% while heat-immobilized CP-1 cells had a significantly lower survival efficiency (57.1% ± 4.8%, $P < 0.001$). After 6 days cell numbers of CP-1 had decreased only slightly from $19.4 \times 10^6 \pm 2.7 \times 10^6$ cells ml^{-1} to $16.3 \times 10^6 \pm 1.4 \times 10^6$ cells ml^{-1}. In contrast, heat-immobilized CP-1 cells dropped by more than 90% and provided fourfold-higher flagellate growth rates.

DISCUSSION

Bacteria in natural planktonic communities have been reported to swim at speeds around 45 µm s^{-1} and with individual bursts of more than 200 µm s^{-1} (27, 28). A recent large-scale screening of 84 marine isolates from different phylogenetic lineages found mean bacterial swimming speeds of up to 40 µm s^{-1} and maximum speeds of up to 75 µm s^{-1} (16). In accordance with these findings the bacterial strains used in our study covered a wide spectrum of bacterioplankton swimming speeds from mean speeds of <1 to 60 µm s^{-1} and maximum speeds of up to 160 µm s^{-1}. Our selection of bacterial strains was based on the assessment of a total of 45 strains isolated from freshwater sources under nongrazing and enhanced grazing conditions. In this selection process high phylogenetic diversity was less important than comparable cell morphologies

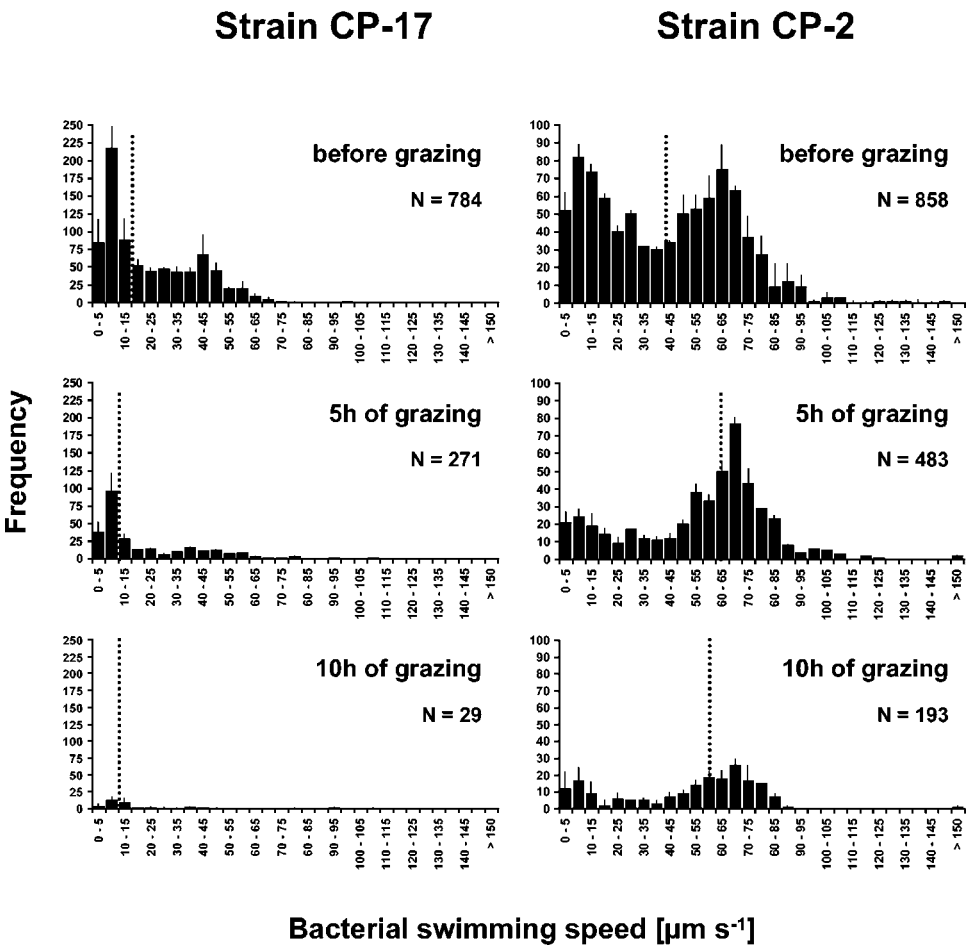

FIG. 4. Grazing-mediated changes of the swimming speed distribution of moderately motile *P. rhodesiae* CP-17 and highly motile *Acidovorax* sp. strain CP-2. The top panels show speed distributions before the addition of the flagellate *Ochromonas* sp. The lower graphs show swimming speed distributions after 5 and 10 h of grazing. The dotted line indicates the median swimming speed. Values of each speed class are given as means ± standard deviations of three replicates. N gives the average number of bacterial cells counted by the motion analysis software.

and sizes and a wide range of swimming speeds among the bacterial strains (Table 2).

Effect of bacterial motility on flagellate feeding. Within this speed range we obtained a clear positive correlation for predator-prey contact rates and bacterial swimming speed as a result of the higher encounter probability of faster-moving prey (Fig. 3A). This finding verifies the correlation observed in a multivariate data set on the role of various bacterial properties on nanoflagellate feeding efficiencies (20). The present model of protozoan bacterivory suggests that higher contact rates would consequently result in higher ingestion rates. This was concluded from two grazing studies where motile bacterial cells were compared with heat-killed and fluorescently labeled cells (9, 30). Our data confirm such a relationship for bacterial swimming speeds up to 20 µm s^{-1} but clearly demonstrate that ingestion rates decrease for highly motile bacteria (>25 µm

TABLE 3. Bacterial characteristics before (PRE-GRAZ) and after (GRAZ) the addition of *Ochromonas* sp. to chemostat cultures of moderately motile *P. rhodesiae* CP-17 and highly motile *Acidovorax* sp. strain CP-2[a]

Bacterial strain and sampling time	Bacteria			*Ochromonas* sp.	
	Mean speed (µm s^{-1})	Cell vol (µm^3)	Cell no. (10^6 ml^{-1})	Cell no. (10^3 ml^{-1})	Max growth rate (day^{-1})
CP-17					
PRE-GRAZ	21.6 ± 3.7	0.23 ± 0.05	13.4 ± 1.14		
GRAZ	15.3 ± 4.6	0.21 ± 0.04	0.32 ± 0.09	39.1 ± 3.00	1.40 ± 0.09
CP-2					
PRE-GRAZ	47.0 ± 4.5	0.24 ± 0.01	11.5 ± 0.85		
GRAZ	54.2 ± 5.1	0.21 ± 0.03	1.20 ± 0.14	25.6 ± 0.55	1.08 ± 0.06

[a] Values were averaged from 5 days of steady state and are given as mean ± range from two replicate reactors.

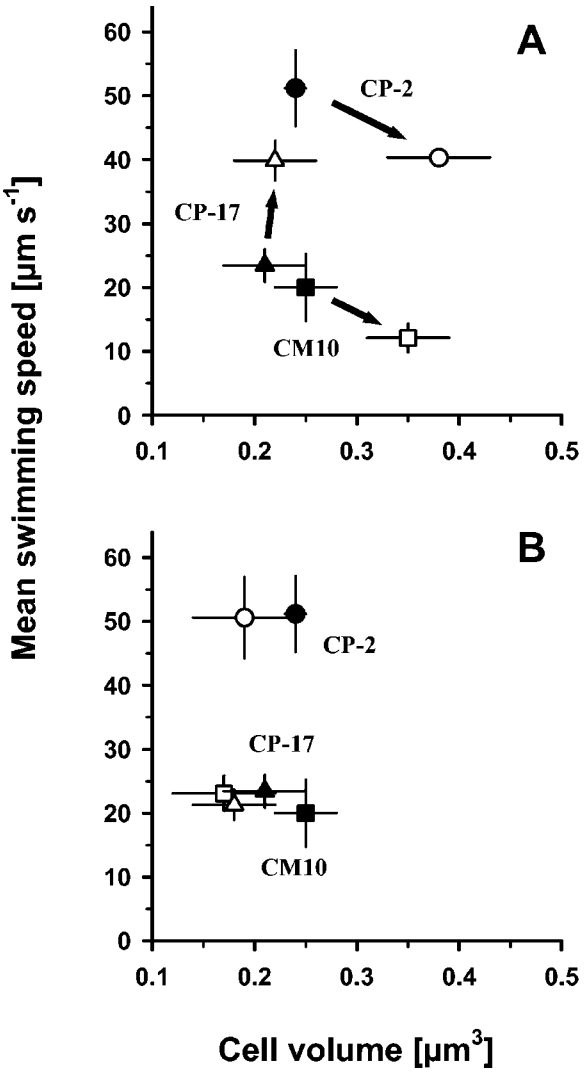

FIG. 5. Impact of growth rate and starvation on bacterial swimming speed and cell size. Three bacterial isolates (*Acidovorax* sp. strain CP-2, *P. rhodesiae* CP-17, and *P. fluorescens* CM10) were grown in continuous culture at two different growth rates (A), and culture subsamples were kept without nutrient supply for 20 days (B). The upper graph (A) shows the effect of a 10-fold increase in bacterial growth rate μ from 0.02 h^{-1} (filled symbols) to 0.2 h^{-1} (open symbols). The lower graph (B) illustrates changes of cultures which were grown at $\mu = 0.02$ h^{-1} (filled symbols) and starved for 20 days (open symbols). Values are given as means ± standard deviations on three consecutive days.

s^{-1}) despite rising contact rates. In contrast to the two previous studies our experiments were based on the examination of a wide range of bacterial swimming speeds and the application of noninvasive methods such as postingestional staining by fluorescent antibodies, direct live observation by high-resolution video microscopy, and moderate heat treatment for cell immobilization.

Our live observations revealed further that low ingestion rates for highly motile bacteria resulted from relatively long reaction times of the flagellates subsequent to contact with highly motile prey and concomitant frequent capture failure. In addition, captured bacteria with high motility caused handling problems and higher loss rates. As a consequence of low capture and handling success, survival efficiencies were highest for the fastest bacteria (Fig. 3B). Accordingly, we found low clearance rates on highly motile bacteria for three common freshwater nanoflagellates (*Spumella* sp., *Ochromonas* sp., and *B. saltans*) as well as lower growth rates of *Ochromonas* sp. on highly motile *Acidovorax* sp. strain CP-2 compared to moderately motile *P. rhodesiae* CP-17. This finding is in agreement with lower growth yields reported for *B. saltans* when feeding on a motile bacterium compared to yields on nonmotile bacteria (25).

Effect of speed-selective feeding on bacterial speed distribution. Currently, protozoan bacterivory is viewed as a primarily size-selective process. Small bacterial cells are subjected to a lower grazing mortality (4, 10, 29), so that their relative proportion in a bacterioplankton assemblage increases during enhanced protozoan grazing (36). Reduced ingestion efficiencies for large bacteria (20) may also result in an accumulation of inedible morphologies such as filaments or aggregates (14, 17, 24, 31, 38). Similar to the grazing-mediated shifts in bacterioplankton size distributions, we observed bidirectional changes in the swimming speed distribution of bacterial populations in the presence of grazing (Fig. 4): (i) the proportion of highly motile cells increased during flagellate grazing (*Acidovorax* sp. strain CP-2), and (ii) immotile cells were reduced at lower rates than moderately motile cells (*P. rhodesiae* CP-17). The preferred elimination of moderately motile bacteria resulted from increased encounter rates compared to immotile cells and lower escape probabilities compared to highly motile bacteria. The gradual shift of an *Acidovorax* sp. strain CP-2 population to a higher mean swimming speed under grazing pressure also occurred in the chemostat experiments (Table 3). Similarly, in a previous chemostat study on a mixed bacterial community we observed the enrichment of highly motile bacteria in the presence of grazers leading to an increase in the mean community speed from 23 to 87 $\mu m\ s^{-1}$ within 41 days (24). Both the community study and the present study of two bacterial isolates indicate a similar impact of bacterial motility on bacterial community structure under grazing as it is known for bacterial cell size.

Linking the effect of bacterial cell size and swimming speed. Within the scope of the 23 bacterial strains tested in the present and a previous study (20), we observed the lowest survival efficiencies for moderately motile cells with a size between 0.2 and 0.4 μm^3 (Fig. 6). Chemostat studies of *Acidovorax* sp. strain CP-2, *P. rhodesiae* CP-17, and *P. fluorescens* CM10 illustrated that this is the size range of growing and dividing bacteria (Fig. 5). In fact, field studies have shown that the actively growing portion of the bacterial community is highly susceptible to grazing and is preferentially cropped by protozoan grazers (5, 7, 34).

It is generally known that growth rate is a major determinant of bacterial cell size (39). The increase of growth rates for *Acidovorax* sp. strain CP-2 and *P. fluorescens* CM10, however, resulted not only in larger cell sizes ($>0.35\ \mu m^3$) but also in lower swimming speeds. Interestingly, among the 23 bacterial isolates tested, bacteria of $>0.4\ \mu m^3$ never had swimming speeds of $>20\ \mu m\ s^{-1}$ (Fig. 6). Although some studies could not confirm a correlation of bacterial cell size and swimming speed (16), others have repeatedly suggested such a relationship. Mitchell (26), for instance, discussed the necessity for

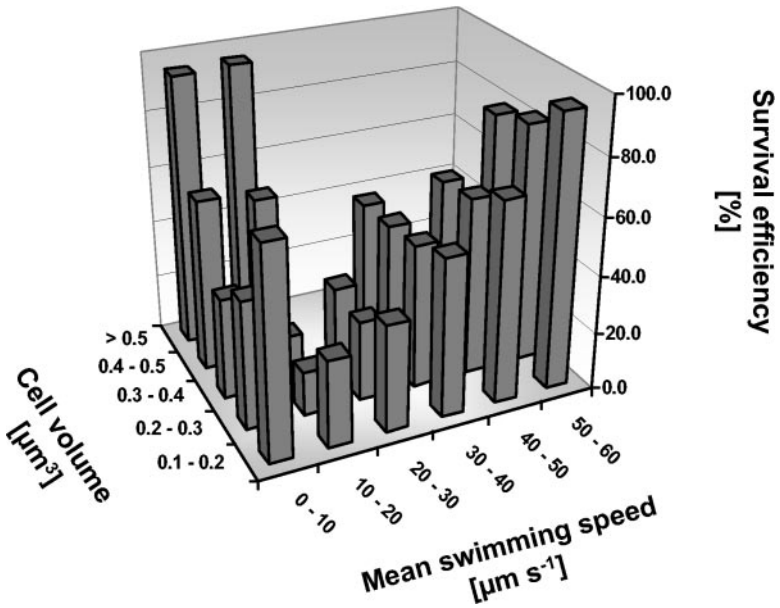

FIG. 6. Bacterial survival efficiencies in the presence of grazing in relation to bacterial cell size and swimming speed. The data presented were pooled from this study and an earlier study which followed the same experimental design (20) to give a total of 23 data points. Each value is the mean of 12 individual observations of flagellate feeding behavior evaluated by high-resolution video microscopy.

small marine bacteria to swim at high speeds in order to maintain chemotaxis. In addition, our data support the notion of a limited susceptibility of bacterial motility to low-nutrient or starvation conditions (Fig. 5). Therefore, the evaluation of bacterial motility as a function of cell morphology, growth conditions, and chemical gradients remains an important challenge for future studies.

In our study we found the highest survival efficiencies for oversized bacteria (>0.5 μm^3) and for small, highly motile bacteria (<0.2 μm^3, >50 $\mu m\ s^{-1}$; Fig. 6). While the handling problems of bacterivorous flagellates with oversized prey have been well characterized (e.g., references 20 and 24), the combined action of small cell size and high motility adds an important new dimension to our understanding of protozoan size selectivity and bacterial grazing protection.

Grazing protection of the ultramicrobacterium CP-1. In freshwater and marine bacterioplankton many cells are <0.1 μm^3 in size, so-called ultramicrobacteria. Their identification and isolation have become a central area of interest in aquatic microbial ecology (e.g., reference 33). In freshwater ecosystems, for instance, recent studies have demonstrated the widespread occurrence of small-sized actinobacteria (8, 15, 40, 41). Interestingly, one study reported the enrichment of actinobacteria during grazing of the flagellate *Ochromonas* sp (32). Likewise, Hahn and coworkers found that cell numbers of an ultramicro-sized actinobacterial isolate remained stable in the presence of the same flagellate and speculated that small cell size alone could not account for the findings (15).

Our data clearly demonstrate the important role of high swimming speed in the survival of the ultramicrobacterial isolate CP-1 during flagellate grazing. Mean swimming speeds of more than 55 $\mu m\ s^{-1}$ allowed CP-1 cells to reach survival efficiencies of >95% while only 57% of the heat-immobilized cells survived the contact with the flagellate *Spumella* sp. Cocultivation of live and heat-immobilized CP-1 cells with *Spumella* sp. over 7 days showed that small cell size alone was not efficient grazing protection for CP-1. Rather the combination of small cell size and high swimming speed resulted in stable bacterial numbers. The successful persistence of ultramicro-sized, highly motile bacteria is further underlined by the fact that CP-1 was isolated from a bacterioplankton community where highly motile bacteria accumulated during increased flagellate grazing pressure (24).

TABLE 4. Survival of the ultramicrobacterial strain CP-1 in the presence of the bacterivorous flagellate *Spumella* sp.[a]

Day	Strain CP-1					*Spumella* sp., max growth rate (day^{-1})
	Heat immobilization	Cell vol (μm^3)	Mean speed ($\mu m\ s^{-1}$)	Survival efficiency (%)	Cell no. ($10^6\ ml^{-1}$)	
1	−	0.10 ± 0.01	58.8 ± 7.2	96.7 ± 2.9	19.4 ± 2.7	
	+	0.09 ± 0.01	5.0 ± 4.3	57.1 ± 4.8	17.0 ± 0.2	
7	−	0.08 ± 0.02	60.1 ± 5.3	ND[b]	16.3 ± 1.4	0.05 ± 0.01
	+	ND	ND	ND	1.5 ± 0.9	0.22 ± 0.03

[a] Bacterial survival efficiencies were evaluated by video microscopy instantly after the addition of the flagellate on day 1. All values are given as means ± standard deviations.
[b] ND, not determined.

Implications for bacterioplankton communities. Apparently, ecological benefits of bacterial motility are not confined to the chemotactic localization of nutrient micropatches. Our finding of speed-selective grazing and the reduced grazing mortality of highly motile bacteria may have substantial implications for predator-prey interactions in microbial food webs. The small average cell size of bacterioplankton has been suggested to be at least partly a result of selective feeding of bacterivorous protozoa on larger cells, although heterotrophic nanoflagellates were shown to ingest even virus-like particles (11) and colloids (35). Our data may shed light on this paradox by suggesting the involvement of bacterial motility and swimming speed in the grazing-mediated changes in bacterioplankton size structure. Specifically, the survival of ultramicrobacterioplankton ($<0.1\ \mu m^3$) might be assigned to the combined action of small cell size and high motility. Therefore, the role of bacterial motility in natural communities, its relation to bacterial cell size, and the interactions with the prevalent selective forces need to be considered in future studies. Such studies are anticipated to improve our understanding of the functioning of microbial food webs, bacterioplankton community structure, and inherent biogeochemical processes.

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