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Size-Selective Grazing on Bacteria by Natural Assemblages of Estuarine Flagellates and Ciliates†

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The small average cell size of in situ bacterioplankton, relative to cultured cells, has been suggested to be at least partly a result of selection of larger-sized cells by bacterivorous protozoa. In this study, we determined the relative rates of uptake of fluorescence-labeled bacteria (FLB), of various cell sizes and cell types, by natural assemblages of flagellates and ciliates in estuarine water. Calculated clearance rates of bacterivorous flagellates had a highly significant, positive relationship with size of FLB, over a range of average biovolume of FLB of 0.03 to 0.08 μm³. Bacterial cell type or cell shape per se did not appear to affect flagellate clearance rates. The dominant size classes of flagellates which ingested all types of FLB were 3- to 4-μm cells. Ciliates also showed a general preference for larger-sized bacteria. However, ciliates ingested a gram-positive enteric bacterium and a marine bacterial isolate at higher rates than they did a similarly sized, gram-negative enteric bacterium or natural bacterioplankton, respectively. From the results of an experiment designed to test whether the addition of a preferentially grazed bacterial strain stimulated clearance rates of natural bacterioplankton FLB by the ciliates, we hypothesized that measured differences in rates of FLB uptake were due instead to differences in effective retention of bacteria by the ciliates. In general, clearance rates for different FLB varied by a factor of 2 to 4. Selective grazing by protozoa of larger bacterioplankton cells, which are generally the cells actively growing or dividing, may in part explain the small average cell size, low frequency of dividing cells, and low growth rates generally observed for assemblages of suspended bacteria.

Marine pelagic bacteria are characterized by relatively low and uniform standing stock abundances, on the order of 10^5 to 10^6 ml^{-1} (7, 34), and by small mean cell size, on the order of 0.02 to 0.12 μm^3, equivalent to an average effective cell diameter of 0.3 to 0.6 μm (1, 11, 17, 22). Since grazing by phagotrophic protozoa is the main source of bacterial mortality in the sea (3, 9, 30), predation by protozoa may not only control cell abundance, but, via selective grazing of larger-sized bacteria, also affect the size distribution of bacterioplankton (1).

Results of previous studies suggest that bacterivorous protozoa preferentially consume larger bacterial cells within a size spectrum of available cell sizes. Andersson et al. (2) reported that a heterotrophic microflagellate, an *Ochromonas* sp., selectively grazed bacterial cells larger than 0.2 μm^3 (0.7-μm effective spherical diameter). Turley et al. (33) showed that, during feeding by two species of bacterivorous ciliates, larger rod-shaped bacteria were removed before smaller coccoid bacteria. Krambeck (14) reported similar decreases in average cell size of grazed bacterioplankton in lakewater. In these studies, effects of grazing by protozoa on bacterial cell size were monitored by examining changes in bacterial biovolumes or cell shape over periods of days to weeks.

Here we report evidence for size-selective grazing on bacteria by natural assemblages of bacterivorous flagellates and ciliates present in waters of a salt marsh estuary. Preferential grazing was determined by comparing short-term (10 to 20 min) rates of clearance of tracer amounts of fluorescence-labeled bacteria (FLB) prepared from natural bacterioplankton and from isolated strains of bacteria of various biovolumes and cell shape. Our results confirm those of the long-term studies discussed above by showing that size-selective grazing of bacteria by natural assemblages of flagellates and ciliates occurs on time scales relevant to individual unicellular organisms.

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MATERIALS AND METHODS

Preparation of FLB. FLB were made via simultaneous heat killing and staining with 5-([4,6-dichloro-triazin-2-yl] amino) fluorescein as described in Sherr et al. (25). Various FLB preparations were made, either using intact estuarine bacterioplankton assemblages or from monospecific strains of bacteria. Bacterioplankton were obtained by concentrating 20 liters of 0.8-μm prescreened tidal creek water to 200 ml in an Amicon hollow-fiber filter apparatus, using filters of 0.1-μm pore size. Aliquots of the mixed-species assemblage of bacterioplankton-FLB were suspended in 0.2-μm filtered estuarine water and then screened through 0.6-μm-pore-size Nuclepore filters to obtain bacterioplankton-FLB of smaller average cell size. In the later experiments, we found that repeated sonication also resulted in smaller cell size in working stocks of the bacterioplankton-FLB due to fragmentation, and we caution others who use FLB to be aware of this problem.

Two strains of marine bacteria were isolated from cultures of a mixotrophic *Ochromonas* sp., clone IC1 of the Provasoli-Guillard Culture Center. One strain, long-rod isolate, was characterized by cells 0.3 ± 0.08 μm wide by 0.9 ± 0.6 μm long in suspension and produced white colonies on nutrient agar. The other marine strain, short-rod isolate, was characterized by cells 0.4 ± 0.08 μm wide by 0.6 ± 0.16 μm long in suspension and grew as yellow colonies on agar. Two strains of enteric bacteria were obtained from the American Type Culture Collection: *Enterococcus faecalis* ATCC 19433, and *Escherichia coli* ATCC 11775. Monospecific

bacterial strains were grown up overnight in nutrient broth and harvested by centrifugation prior to FLB preparation (25).

Bacterial cell size was determined by measuring the length and width of 50 randomly selected bacteria in each FLB preparation at a magnification of ×2,000, using a Zeiss Universal epifluorescence microscope. Cell biovolumes were calculated by the equation $V = (4/3)[\pi(W/2)^3] + [\pi(W/2)^2 \times (L - W)]$, where W is cell width and L is cell length.

FLB selectivity experiments. Rates of clearance of the various types of FLB by in situ assemblages of estuarine flagellates and ciliates were assayed in a series of short-term FLB uptake experiments in which protozoan uptake rates of two or three types of FLB were compared. For each experiment, water was collected from a salt marsh tidal creek and returned to the laboratory. Water temperature and salinity (measured with a refractometer) were determined for each sample. Fifty- to 100-ml aliquots of the water were poured into replicate 400-ml Whirl-pak bags which had been soaked in 10% HCl and copiously rinsed with deionized water. The bags were placed in several hundred milliliters of tidal creek water in 1-liter plastic beakers to maintain uniform temperature during the experiments. The samples were allowed to sit for 30 min to allow the protozoan assemblage to stabilize after handling. Because of an order of magnitude difference in filtration capability of bacterivorous flagellates and ciliates (8, 9, 25), flagellate and ciliate FLB uptake rates were determined in separate treatments in which FLB were added to yield a final concentration of 0.5×10^6 to 1.0×10^6 ml^{-1} for flagellates or 1×10^5 to 3×10^5 ml^{-1} for ciliates. For each series of experiments, equal concentrations of different types of FLB were added to individual Whirl-pak bags. Experiments were run in the laboratory at a temperature of 27 to 28°C.

After addition of FLB, 5-ml subsamples were taken from each experimental bag at 2-min intervals for 12 min and immediately fixed by adding 0.5% (final concentration) alkaline Lugol solution followed by 3% (final concentration) borate-buffered Formalin to preserve the cells and decolorize the Lugol solution. This method of fixation reduces dissolution of naked ciliates and prevents significant flagellate egestion of food vacuole contents (26). The preserved subsamples were stained with 4',6'-diamidino-2-phenylindole, filtered onto 0.8-μm-pore-size Nuclepore filters, and inspected via epifluorescence microscopy (25) to determine the average number of FLB per cell for each time sampled. A total of 20 to 150 ciliates or nonpigmented flagellates were inspected for FLB ingestion in each subsample. Each flagellate was also scored for cell size during the counts.

Aliquots, 0.2 to 0.5 ml, of water from each experiment were filtered onto 0.2-μm-pore-size Nuclepore membrane filters and inspected at ×1,250, using blue light fluorescence to determine the average number of FLB per ml. Separate 100-μl aliquots were taken to enumerate total bacterial numbers via acridine orange direct counts (13).

To compare regression coefficients for the two or three uptake rates determined in each individual experiment, FLB per cell values at each time point were divided by the FLB concentration (FLB per nanoliter) determined for each bag, to yield cumulative effective per-cell clearance (nanoliters per cell). Variations in FLB concentration between bags in any one experiment ranged from 10% up to a twofold difference. Protozoan clearance rates were calculated from the change in cumulative clearance rate (nanoliters per cell) with time over the linear portion of the uptake curve (27), using linear regression analysis (31). The slopes of the regression lines (nanoliter per cell per minute) were multiplied by 60 to yield hourly clearance rates for comparison with other literature values.

The regression lines obtained in individual experiments were tested for significant differences in slope at the $P = 0.05$ level, using statistical methods presented in Sokal and Rohlf (31). For experiments comparing uptake of two types of FLB, the F-test for difference between two regression coefficients was made (reference 31 [p. 505]). For cases in which three uptake slopes were compared, the regression coefficients were tested by the GT2 analysis for unplanned comparisons of multiple slopes, using Gabriel's approximate method (31 [p. 507–508]).

To test whether variations in ciliate clearance rate were a result of differences of retention efficiency of various types of FLB or were due to increased rate of filtration stimulated by the presence of a "preferred" bacterial prey, we compared clearance of bacterioplankton-FLB by ciliates in treatments with or without the addition of 2×10^5 unstained, heat-killed *E. faecalis* ml^{-1}.

Several samples of tidal creek water used in the experiments were preserved with Bouin fixative and subsequently silver stained via the quantitative protargol staining method of Montagnes and Lynn (21) to facilitate taxonomic identification of ciliates.

RESULTS

During the late summer period in which the experiments were carried out, the average temperature of the tidal creek water was 28 ± 1.6°C and the salinity ranged from 21 to 28‰. The average standing stock of bacterioplankton in creek water was $(1.6 \pm 0.7) \times 10^7$ cells ml^{-1} ($n = 15$). The flagellate population was composed largely of 2- to 10-μm-diameter monads, bodonids, and choanoflagellates. The ciliate population was dominated (50 to 75% of cells) by a single species of choreotrich, a *Strobilidium* sp. 10 to 12 μm wide by 15 to 20 μm long. A second *Strobilidium* sp., 15 to 20 by 35 to 50 μm, composed 15 to 40%, and a scuticociliate, 15 to 20 by 35 to 40 μm, composed 10 to 20% of the ciliate assemblage in creek water. Two *Strombidium* spp., one 25 μm and the other 35 to 40 μm long, occurred rarely in the population. Since these sizes are based on inspection of fixed cells, it is likely that the dimensions of the living cells were somewhat greater, as recent studies indicate that preservation decreases the average cell biovolume of flagellates and ciliates by about twofold (4–6).

Comparison of protozoan clearance of bacterioplankton-FLB and of FLB made from the two strains of enteric bacteria. In the first series of experiments, we determined ciliate and flagellate grazing rates on their natural bacterioplankton prey and on two exotic species of bacteria with an average cell size 1 order of magnitude larger than that of the bacterioplankton assemblage (Table 1). Plots of FLB per cell over time for the experiment of 9/16/87, in which both ciliate and flagellate grazing rates were assessed for both types of enteric bacteria, are shown in Fig. 1. Nonpigmented flagellates ingested both types of enteric bacteria-FLB with clearance rates two to four times greater than for the smaller-sized bacterioplankton-FLB (Table 1). Ciliates, however, grazed *E. faecalis*-FLB at a higher rate than they did either *Escherichia coli*- or bacterioplankton-FLB (Table 1).

Comparison of protozoan grazing rates on bacterioplankton-FLB of various average cell sizes and on two marine bacterial isolates with differing average cell lengths. The

TABLE 1. Comparisons of protozoan clearance rates based on uptake of FLB made from estuarine bacterioplankton (0.08 ± 0.11 μm^3) or from two strains of enteric bacteria, *E. faecalis* (0.66 ± 0.45 μm^3) and *Escherichia coli* (0.68 ± 0.49 μm^3)[a]

Date (1987)	FLB	Clearance rate[b]			
		Flagellates		Ciliates	
		nl $cell^{-1}$ min^{-1}	nl $cell$ h^{-1}	nl $cell^{-1}$ min^{-1}	nl $cell^{-1}$ h^{-1}
8/29	Bacterioplankton	0.043 (0.005)	2.58	1.93 (1.79)	116
	E. faecalis	0.084 (0.005)*	5.01	6.18 (1.79)*	371
9/7	Bacterioplankton	0.014 (0.002)	0.86	3.29 (0.36)	197
	E. faecalis	0.036 (0.002)*	2.13	6.76 (0.36)	406
9/13	Bacterioplankton	0.007 (0.001)	0.43	2.30 (0.11)	128
	Escherichia coli	0.017 (0.007)*	1.04	2.13 (0.11) NS	138
9/16	Bacterioplankton	0.005 (0.005)*	0.32	0.90 (0.16)*	54
	Escherichia coli	0.020 (0.005) NS	1.23	1.93 (0.22)*	116
	E. faecalis	0.022 (0.005) NS	1.30	3.43 (0.16)*	206

[a] Standard errors of the regression coefficients (nanoliters per cell per minute) are presented in parentheses (see text for details on statistical methods).
[b] Asterisks indicate a significant difference between slopes at the $\alpha = 0.05$ level. NS, Not significantly different from other slope(s).

second block of experiments was designed to assay variations in protozoan clearance rates of FLB prepared from marine bacteria, including natural assemblages of estuarine bacterioplankton and two marine bacterial isolates which differed morphologically. Plots of FLB per cell versus time for a flagellate and a ciliate experiment comparing uptake of unscreened bacterioplankton-FLB and 0.6-μm prescreened bacterioplankton-FLB are shown in Fig. 2. Flagellates had significantly higher clearance rates on the larger-sized FLB in each experiment (Table 2).

Although there were significant differences between uptake of unscreened bacterioplankton-FLB and 0.6-μm prescreened bacterioplankton-FLB by ciliates (Table 3), the difference in ciliate clearance rate was only about twofold for bacterioplankton-FLB of differing size. However, ciliates did show a markedly higher grazing rate on the short-rod marine isolate, even though the average cell size of this strain was about the same as that of the long-rod isolate and of bacterioplankton-FLB (Table 3).

Plots of clearance rate as a function of the average cell volume of FLB for flagellates and ciliates in these experiments (Fig. 3) demonstrate that flagellate grazing rate was apparently influenced only by the average bacterial cell size, while ciliate clearance rates were affected by both average size and type of bacteria. Regression analysis of the two plots (31) indicated that >99% of the variation in clearance rates for flagellates could be explained by variation in FLB cell volume, while for ciliates, >80% of the variation in clearance rates of bacterioplankton-FLB was due to FLB cell volume variations.

In the ciliate experiment (9/20/87) in which we compared uptake rates of bacterioplankton-FLB by estuarine ciliates with or without the addition of unstained *E. faecalis*, there was no difference in ciliate clearance rates of bacterioplankton-FLB in the two treatments (Table 3).

The hourly clearance rates, which ranged from 0.19 to 5.01 nl $cell^{-1}$ h^{-1} for flagellates and from 49 to 406 nl $cell^{-1}$ h^{-1} for ciliates (Tables 1 to 3), were comparable to other values reported in the literature for rates of clearance of bacterium-sized particles by natural assemblages of flagellates and ciliates (4, 18, 19, 24, 26, 29).

Size spectrum analysis of flagellate bacterivores. To determine whether the assemblage of nonpigmented flagellates in the estuary showed resource partitioning by size class for differently sized bacterial prey, we plotted the percentage of flagellates with ingested FLB in 1-μm size classes from 2 to

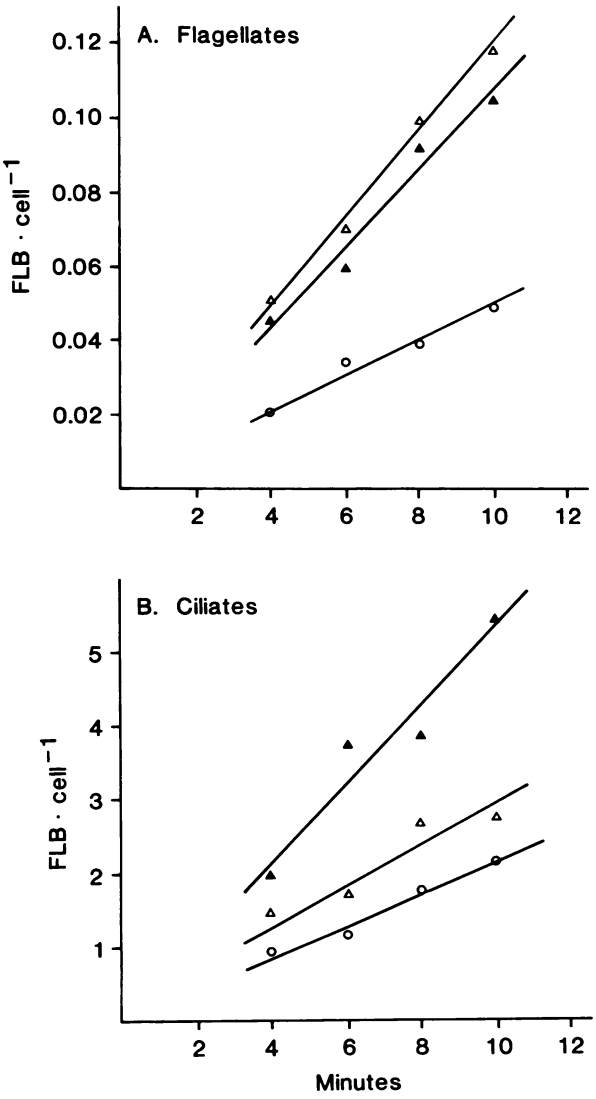

FIG. 1. Comparison of the increase in enteric bacteria-FLB per cell and in bacterioplankton-FLB per protozoan cell as a function of time for (A) flagellates and (B) ciliates. Symbols: ○, bacterioplankton-FLB; △, *Escherichia coli*-FLB; ▲, *E. faecalis*-FLB.

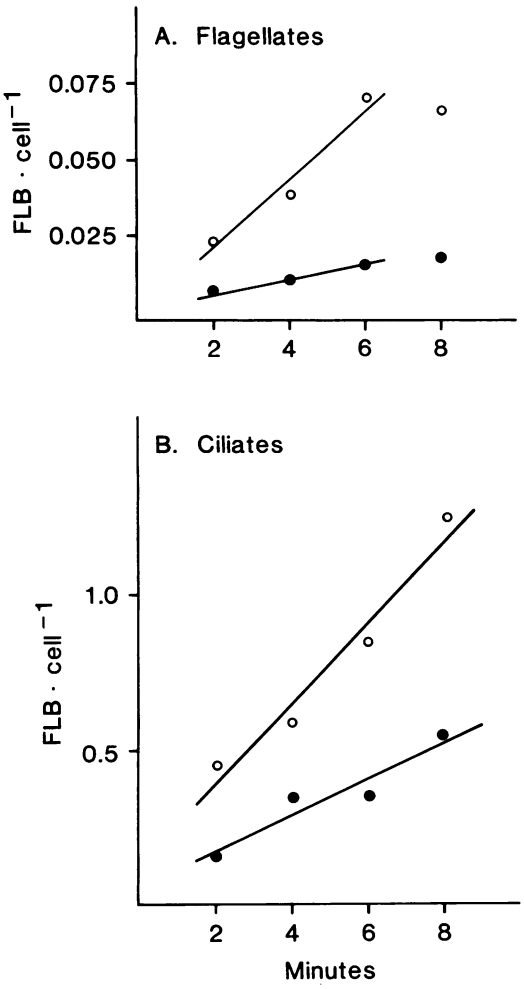

FIG. 2. Comparison of the increase in bacterioplankton-FLB per protozoan cell as a function of time for (A) flagellates and (B) ciliates. Symbols: ○, 0.08- to 0.10-μm^3 average biovolume bacterioplankton-FLB; ●, 0.03-μm^3 average biovolume bacterioplankton-FLB.

10 μm for the various experiments (Fig. 4). For each of the three size classes of bacterial prey, >70% of flagellates with ingested FLB were in the 3- to 4-μm size classes. However, for the 0.08- and 0.7-μm^3 size classes of prey, 5 to 7% of FLB-ingesting flagellates were in the >6-μm size class, while only 1% of >6-μm flagellates ingested FLB with an average size of 0.03 to 0.06 μm.

DISCUSSION

The results of our short-term FLB uptake experiments confirm the idea, based on previous speculation (1) and long-term incubation studies (2, 32), that bacterivorous protozoa, both flagellates and ciliates, preferentially ingest the larger bacterial cells in a mixed population.

The flagellates had the most pronounced response to bacterial cell size, with a threefold-higher grazing rate on larger versus smaller cells (Tables 1 and 2; Fig. 3). Fenchel (9) has shown that, for direct interception or contact feeding, which is common in flagellates, such as relationship can be explained in part by the probability that larger prey cells will be intercepted more frequently than smaller prey cells. The

TABLE 2. Comparison of flagellate clearance rates based on uptake of FLB made from bacterioplankton of various average cell size and from two marine bacterial isolates[a]

Date (1987)	FLB		Clearance rate	
	Type	Vol (μm^3)	nl $cell^{-1}$ min^{-1}	nl $cell^{-1}$ h^{-1}
9/22	Bacterioplankton	0.08 ± 0.11	0.019 (0.001)	1.13
	<6-μm bacterioplankton	0.04 ± 0.03	0.008 (0.001)*	0.46
9/25	Bacterioplankton	0.06 ± 0.09	0.015 (0.003)	0.91
	<6-μm bacterioplankton	0.03 ± 0.04	0.004 (0.003)	0.23
9/28	Bacterioplankton	0.04 ± 0.04	0.007 (0.002) NS	0.44
	Long-rod isolate	0.056 ± 0.04	0.010 (0.002) NS	0.61
	Short-rod isolate	0.065 ± 0.04	0.014 (0.002)*	0.83
10/5	Bacterioplankton	0.03 ± 0.03	0.003 (0.001)*	0.19
	Long-rod isolate	0.055 ± 0.02	0.012 (0.001)*	0.56
	Short-rod isolate	0.063 ± 0.03	0.009 (0.001)*	0.74

[a] Mean biovolume ± 1 standard deviation. Standard errors of the regression coefficients (nanoliters per cell per minute) are given in parentheses. *Slope(s) different at the α = 0.05 level. NS, Not significantly different from other slope(s).

regression equation of FLB biovolume versus flagellate clearance rates (Fig. 3) gave an x-intercept of 0.016 μm^3, equivalent to an average effective spherical cell diameter of 0.3 μm. This could indicate that bacteria with <0.3-μm effective spherical diameter might be protected from flagellate grazing due to their small size. However, recent information that some flagellates can ingest high-molecular-weight organic molecules which are 10^7 times smaller in mass than the average suspended bacterial cell (28) calls into question the idea that bacteria could have a refuge from predation based on size.

Flagellates did not show any preferential grazing based on cell type or cell shape, either between the enteric bacterial strains (Table 1; Fig. 1) or between the marine isolates and similarly sized bacterioplankton (Table 2; Fig. 3). However, in two previous studies with freshwater flagellates, a *Monas* sp. (24) and *Bodo saltans* (20), differential growth of flagel-

TABLE 3. Comparisons of ciliate clearance rates based on uptake of FLB made from bacterioplankton of various average cell size and from two marine bacterial isolates[a]

Date (1987)	FLB		Clearance rate[b]	
	Type	Vol (μm^3)	nl $cell^{-1}$ min^{-1}	nl $cell^{-1}$ h^{-1}
9/20	Bacterioplankton	0.08 ± 0.11	1.28 (0.07)	77
	Bacterioplankton + unstained *E. faecalis*	0.08 ± 0.11	1.01 (0.07)*	61
10/5	Bacterioplankton	0.03 ± 0.03	0.82 (0.11)*	49
	Long-rod isolate	0.055 ± 0.02	1.26 (0.16)*	76
	Short-rod isolate	0.063 ± 0.04	3.15 (0.16)*	189
10/7	Bacterioplankton	0.10 ± 0.12	1.16 (0.09)	70
	<6-μm bacterioplankton	0.03 ± 0.04	0.82 (0.09)*	49

[a] Mean biovolume ± 1 standard deviation. Standard errors of the regression coefficients (nanoliters per cell per minute) are given in parentheses.
[b] Asterisks indicate slope(s) different at the α = 0.05 level.

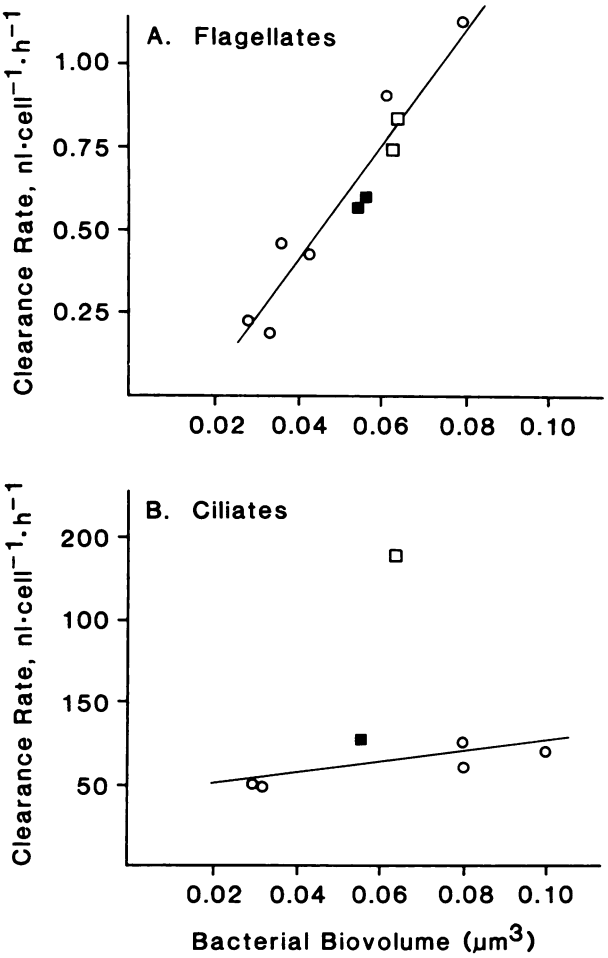

FIG. 3. Relationship of protozoan clearance rate to average cell biovolume of bacterial prey for bacterioplankton-FLB and for FLB made from two marine isolates for (A) flagellates and (B) ciliates. Symbols: ○, bacterioplankton-FLB; □, short-rod isolate; ■, long-rod isolate. Regression equations are (A) $y = -0.27 + 17.0x$, $r = 0.96$; (B) (bacterioplankton-FLB only) $y = 44.5 + 307x$, $r = 0.70$.

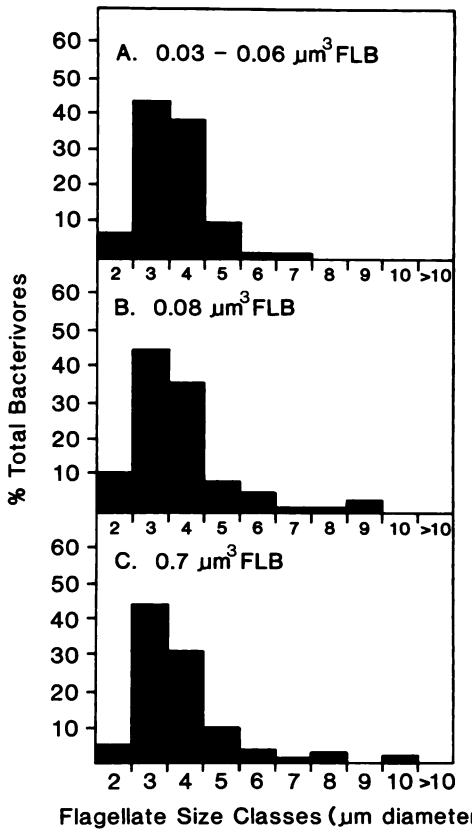

FIG. 4. Size frequency distribution of flagellates observed with ingested FLB for three different average bacterial cell sizes: (A) 0.03- to 0.06-μm^3 FLB (bacterioplankton and marine isolates); (B) 0.08-μm^3 bacterioplankton-FLB; (C) 0.7-μm^3 enteric bacteria-FLB.

lates fed various monoxenic bacterial strains was reported. For various strains of bacteria, flagellates might display similar grazing rates but different growth efficiencies (24). It is also possible that the heat-killing and staining procedure used to prepare FLB altered some of the characteristics of the individual bacterial strains (such as motility) which might affect flagellate grazing rate. For example, Mitchell et al. (20) found that *B. saltans* displayed a lower growth rate on flagellated compared with nonflagellated bacteria. Preparation of FLB might also affect the cell surface chemistry of the bacteria to render the cells more or less susceptible to binding on contact with a flagellate cell membrane. The present results, based on FLB uptake by mixed assemblages of flagellates, indicated that grazing rates varied only with respect to average bacterial cell size. This result may suggest that preparation of FLB minimizes other characteristics of individual bacterial strains which could affect flagellate grazing rate.

It is possible that size selection of bacterial prey is a function of flagellate cell size, i.e., that the smallest phagotrophic flagellates tend to graze the smaller bacterial cells, while the largest bacterial cells are consumed primarily by larger-sized flagellates. To test this idea, we examined the size frequency distribution of flagellates observed ingesting various size classes of FLB. The results (Fig. 4) showed virtually no shift in the size frequency distribution of bacterivorous flagellates over an order of magnitude difference in prey biovolume. Thus, it appears that there is little partitioning of bacterial prey with respect to size among the different size classes of phagotrophic flagellates.

The filter-feeding ciliates showed a different pattern of selective grazing compared with that of the flagellates. The ciliates had only slightly higher grazing rates on larger bacterioplankton cells (Table 3; Fig. 3). Ciliate grazing rates were much greater on FLB prepared from cultured bacteria, and ciliates showed differential grazing between individual strains of cultured bacteria of similar biovolume (Tables 1 and 3). Ciliates grazed the gram-positive *E. faecalis* at a higher rate in comparison to the gram-negative *Escherichia coli* and the short-rod marine isolate at a higher rate than the long-rod isolate. For ciliate grazing, then, preparation of FLB did not minimize all of the characteristics, other than cell size, of individual bacterial strains (e.g., type of cell wall and cell shape) which could affect protozoan clearance rate.

Under the uniform conditions of food concentration and temperature which prevailed in our experiments, an increase in protozoan clearance rate can be explained by either an increased rate of processing of water by the protozoan in response to the presence of preferred food (35) or more effective retention or capture of prey (8, 9, 20, 29). To test which mechanism might have resulted in the observed

variations in ciliate grazing rate, we carried out an experiment in which we compared ciliate uptake of bacterioplankton-FLB with or without addition of unstained preferred bacterial prey. If the ciliates responded to the presence of preferred prey by increasing their absolute clearance rate, then, assuming no between-treatment difference in ciliate retention of bacterioplankton-FLB, we would expect a higher rate of ingestion of FLB. However, we found no difference in clearance rate of the bacterioplankton-FLB between the two treatments (Table 3). Thus, we concluded that the variations in clearance rate observed for ciliates ingesting FLB of various average cell size appeared to be a result of differences in effective retention of the bacterial cells rather than of differences in rates of processing of water. Clearance rates based on FLB uptake should probably be interpreted for both flagellates and ciliates as an effective grazing rate on a particular prey rather than as an absolute rate of clearance of water.

The combined results of this study and of the reports of Andersson et al. (2), Turley et al. (33), and Krambeck (14) make a strong case for the selective grazing by bacterivorous protozoa on the larger-sized cells within a given bacterioplankton assemblage. Flagellates and ciliates both appear to have proportionally higher clearance rates for larger-sized bacterial prey. For contact-feeding flagellates, such selectivity is due in part to size-dependent prey contact rates, as noted above; for filter-feeding ciliates, it could be based on a more effective retention of larger-sized cells. Flagellates and ciliates may also select for live bacteria based on other factors, e.g., motility and chemosensory responses (20, 35).

It has been repeatedly observed that, when actively growing bacterioplankton populations are removed from predation, the average bacterial cell size increases, usually by a factor of 2 or more (e.g., see references 1, 16, and 32). Turley and Lochte (32) reported that increases in bacterial cell size preceded increase in bacterial numbers in the surface waters of the Irish Sea. Larsson and Hagstrom (16) found a strong positive correlation between the frequency of dividing cells, an indicator of population growth rate, and average bacterial cell size for bacterioplankton cultures taken from the Baltic Sea.

If the larger-sized bacteria in a bacterioplankton population are mainly cells which are actively growing and dividing (15), preferential grazing on these cells suggests that protozoa are specifically cropping bacterial production rather than simply grazing some randomly selected fraction of the bacterioplankton standing stock. The notion that grazers are consuming the production rather than the standing stock was proposed to us (B. and E. Sherr) by F. Rassoulzadegan several years ago, but we did not then recognize that a potential mechanism for selective cropping of bacterial production might exist. The basic idea has already been proposed by C. Krambeck (14) for the protozoan-bacterial trophic link in a German lake: ". . . grazing obviously imposes an upper limit to increases in cell size and thereby specific growth rate and keeps specific bacterial properties constant from a certain productivity level on."

This concept of protozoan bacterivory implies that protozoa would not have to graze an equivalent amount of bacteria corresponding to the cell number produced in the absence of grazing to balance bacterioplankton production. A lower rate of bacterial mortality due to protozoan grazing, which featured intense and selective grazing pressure on those bacterial cells which are near to, or in, division, could also effectively control bacterial abundance. If this is true, then apparent discrepancies between bacterial production and lower bacterial mortality due to grazing reported by various investigators (18, 19, 23, 26) might be resolved. Such selective grazing might also at least partially explain the small average cell size, low growth rates, and low frequency of dividing cells of most bacterioplankton assemblages in natural waters (3, 7, 12, 34).

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