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A STUDY OF FEEDING RESPONSES TO BACTERIAL PREY BY ESTUARINE CILIATES[1]

S. G. BERK, R. R. COLWELL, and E. B. SMALL
Department of Microbiology and Department of Zoology,
University of Maryland, College Park, Maryland 20742

BERK, S. G., COLWELL, R. R. & SMALL, E. B. 1976. A study of feeding responses to bacterial prey by estuarine ciliates. *Trans. Amer. Micros. Soc.*, 95: 514–520. Growth of two estuarine ciliates, *Uronema nigricans* and *Potomacus pottsi*, was inhibited when the bacterial food source was at a population level of $\leq 10^6$–10^7 cells/ml. Feeding rates of *Uronema* varied with the type and population density of bacteria. The feeding rate of *Uronema* was greater when a *Vibrio* sp. served as food source compared with a *Bacillus* sp. Significant differences noted in the ciliate population levels were related to the strains used as food source.

As predators of bacteria, ciliated protozoa can be significant in the ecology of aquatic environments. Marine protozoa grazing on bacteria are important agents in the regeneration of nutrients (Johannes, 1965). Protozoa, plentiful in activated sludge systems, are responsible for the quality of the sewage effluent since they graze on coliform bacteria associated with the effluent, thereby removing a high percentage of these bacteria (Curds & Cockburn, 1968). In turn, protozoa serve as prey for organisms of higher trophic levels, thus constituting a link in aquatic food chains.

It has been noted by Hamilton & Preslan (1969) that marine copepods feed on certain of the marine ciliates. In the present study, it has been observed that in estuarine water samples brought into the laboratory, copepod larvae attack and ingest *Pleuronema.* The feeding responses of ciliates to their estuarine bacterial prey, therefore, may have important consequences in the food web. Few investigations have been conducted on the feeding habits of estuarine bactivorous protozoa (Fenchel, 1968a,b; Hamilton & Preslan, 1969), although more observations have been made on fresh-water organisms (Barna & Weis, 1973; Curds & Cockburn, 1968; Hairston et al., 1968; Laybourn & Stewart, 1975; Proper & Garver, 1966; Seto & Tazaki, 1971). The present study examines some aspects of feeding responses of estuarine ciliates to their bacterial prey.

MATERIALS AND METHODS

The bacterial strains were isolated from the Rhode River, a sub-estuary of Chesapeake Bay. *Vibrio* sp. 10RR10 was isolated by Dr. T. Kaneko, and the

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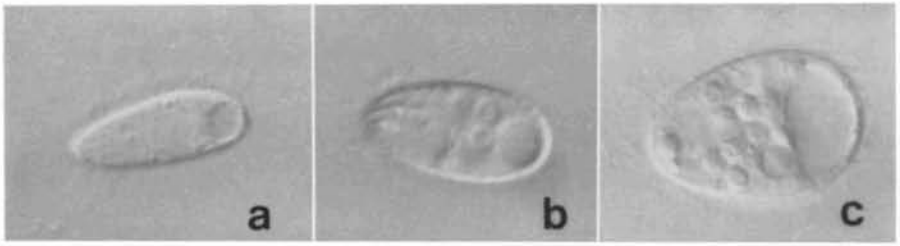

FIGS. 1a–c. Photographs of *Uronema* taken with a Nomarski contrast-interference microscope. Fig. 1a. Washed *Uronema*, starved for several hr, void of food vacuoles. Vacuole seen here is the contractile vacuole. Fig. 1b. After feeding on washed cells of *Vibrio* 10RR10 for 1 hr, *Uronema* half-filled with food vacuoles. Fig. 1c. After feeding for 2 hr on washed cells of *Vibrio* 10RR10, *Uronema* gorged with food vacuoles. × 400.

others, designated *Bacillus* sp. 8.20, *Vibrio* sp. 8.25, and unidentified sp. 36.17, were isolated by Dr. Jayne Carney. The bacteria were grown in ESWYE broth consisting, per liter, of: 0.18 g KCl; 1.75 g $MgSO_4 \cdot 7H_2O$; 5.8 g NaCl; 3 g yeast extract; 10 g proteose peptone; pH 7.2–7.4. Ciliated protozoa were isolated from a nearshore area of the Rhode River and were identified as *Uronema nigricans* and *Potomacus pottsi*. The protozoa were maintained on wheat germ or wheat grain infusion in .45 μm Millipore filtered Rhode River water (hereafter referred to as RRW). *Potomacus* was used only in population growth studies.

The ciliates were washed several hours with antibiotic solutions (50 μg/ml of chloramphenicol, polymyxin B, and tetracycline) to render them free of extraneous bacteria. Ca. 60 *Uronema* cells were placed in five replicate tubes containing 5 ml sterile RRW. To each tube was added a food source consisting of washed cells of the bacterial isolates, unidentified sp. 36.17, *Vibrio* sp. 8.25, *Vibrio* sp. 10RR10, and *Bacillus* sp. 8.20, in densities of 4×10^7, 1×10^7, 1.2×10^{10}, and 2.5×10^{10} cells/ml, respectively. Controls, consisting of replicate test tubes of ciliates without bacteria, were employed in all experiments. Test tubes were incubated at 25 C and counts of ciliates were made daily, until a decrease in number of ciliates was detected. A final bacterial count was made by plating on ESWYE agar at the end of the experiment in which *Uronema* was fed *Vibrio* 10RR10 at an initial density of 1×10^{10} bacteria/ml. The microstome forms of *P. pottsi* were washed, and 10 organisms were placed in duplicate test tubes containing 3 ml sterile RRW. Washed cells of *Bacillus* 8.20 were dispensed into test tubes, to final bacterial densities of 10^7, 10^5, and 10^3 bacteria/ml. The tubes were incubated at 15 C to prevent formation of macrostomes, a cannibalistic form of these ciliates. *Potomacus* was also fed washed *Vibrio* 10RR10, at densities of 10^7, 10^8, and 10^9 cells/ml.

Feeding rates were established from the total viable counts obtained by

TABLE I

Population growth responses of *Uronema* feeding on unidentified strain 36.17 and *Vibrio* strain 8.25 at densities of 10^7 cells/ml

Time (hr)	Average number of *Uronema*	
	Unidentified strain 36.17	*Vibrio* 8.25
0	6.0×10^{1}[1]	6.9×10^{1}[1]
67	2.5×10^{3}	—
80	5.6×10^{3}	5.0×10^{3}
104	1.9×10^{3}	3.3×10^{3}

[1] Numbers given are cells/ml.

TABLE II
Population growth responses of *Uronema* feeding on bacterial strains, *Vibrio* 10RR10 and *Bacillus* 8.20, at bacterial densities of 1.2×10^{10} bacteria/ml and 2.5×10^{10} bacteria/ml, respectively

Time (hr)	Average number of protozoa ($\times 10^4$/ml) feeding on		
	Bacillus	*Vibrio*	Control
68	0	5.75	0
75.5	.25	13.0	0
87.5	1.75	16.7	0
94.5	1.00	12.3	0

plating three dilutions, in triplicate, on ESWYE agar. Counts were made at the beginning and end of each experiment. A count of ciliates was also made for determination of feeding rate.

An experiment to detect presence of a lytic metabolite was also carried out, that is, ciliates were fed for 3 hr on washed bacteria (*Bacillus* 8.20 and *Vibrio* 10RR10) and the entire contents of each tube were subsequently filtered through a sterile 0.22 μm Millipore filter. The filtrate was added to a second test tube of washed bacteria. The amount of bacteria in the second tube approximated the amount in the tube from which the filtrate was made. For each experiment, plate counts were made at time "zero" and after 3 hr.

Ciliate concentrations were determined by diluting a 50 μl sample and examining a fraction of the dilution directly under a dissection microscope. Ciliates were fixed with a drop of Bouin's fixative for counting. Also, plate counts were made at intervals to determine whether the bacterial populations were altered in the sterile RRW.

Results

When *Uronema* was fed 10^7 cells/ml of *Vibrio* 8.25 and unidentified sp. 36.17, the number of protozoa never exceeded 5.6×10^3 cells/ml. The peak number of ciliates was 5.6×10^3/ml, for those ciliates fed strain 36.17, and 5.0×10^3/ml for those fed *Vibrio* 8.25 (Table I). In the control tubes, no ciliates were detected by direct count with a hemocytometer.

Uronema fed 1.2×10^{10} bacteria/ml of *Vibrio* 10RR10 and 2.5×10^{10} bacteria/ml of *Bacillus* 8.20, showed significant differences in total number of ciliates ($p \leq 0.05$). The *Vibrio* sp. supported higher ciliate population yields (Table II). At this bacterial density, the numbers of protozoa supported by *Vibrio* 10RR10 were large, 4.2×10^4 ciliates/ml. No ciliates were observed in samples taken from the control tubes.

The microstomes of *Potomacus* indicated very low levels of growth when fed *Bacillus* sp. 8.20 at densities of 10^7 bacteria/ml. The highest number of ciliates observed was ca. 1.4×10^3 cells/ml. No ciliate growth occurred in tubes containing lower concentrations of bacteria (i.e., 10^3 and 10^5 cells/ml). Higher

TABLE III
Feeding rates of *Uronema* given *Vibrio* and *Bacillus* strains

Bacterial strain	Bacterial density	Protozoan density	Ratio bact./prot.	Feeding rate (bact./hr-prot.)
Vibrio 10RR10	6.8×10^7/ml	5×10^5/ml	136	34
	5×10^8/ml	6.7×10^5/ml	746	244
Bacillus 8.20	5×10^8/ml	9.6×10^5/ml	530	73.3
	1.2×10^8/ml	1.6×10^6/ml	75	23

TABLE IV
Total viable counts of bacteria exposed to filtrate for 3 hr

Bacterial strain	TVC cells/ml t = 0	TVC cells/ml t = 3
Bacillus 8.20	1.75×10^8	2.0×10^8
	3.35×10^8	5.15×10^8
Vibrio 10RR10	6.3×10^7	9.85×10^7

levels of population growth occurred for microstomes feeding on *Vibrio* 10RR10 at a density of 10^8 bacteria/ml or greater. At 10^8 *Vibrio* cells/ml, the largest number of protozoa observed was 1.4×10^4 ciliates/ml.

When *Vibrio* sp. 10RR10 was provided as food for *Uronema*, at an initial bacterial density of 1×10^{10} cells/ml, the final bacterial density at the ciliate stationary phase was 4.0×10^7 cells/ml.

Feeding rates can be expressed as the number of bacteria consumed per ciliate per hour. Results of such calculations are given in Table III. At a bacterial density of 5×10^8 cells/ml, the feeding rate was 244 bacteria per hour per protozoon for *Vibrio* 10RR10. At 5×10^8 cells/ml of *Bacillus*, the rate was only 73.3 bacteria/protozoon per hour. At a density of 1.2×10^8 cells/ml, the rate was 23 bacteria/protozoon per hour. Bacteria-protozoon ratios for each experiment are given in Table III.

Results of experiments in which filtrates were added to the bacteria demonstrated that the decrease in viable bacteria was not due to an exogenous metabolite or to a lytic enzyme, since no decrease in bacterial numbers was noted after 3 hr (Table IV). Control experiments showed that *Vibrio* 10RR10 and *Bacillus* 8.20 did not decrease when held in RRW without protozoan predators (Table V).

Discussion

From the results of this study, it is concluded that there is a critical density of bacteria required for growth of ciliate populations. In the case of *Uronema* and *Potomacus*, the critical density was ca. 10^6–10^7 bacteria per ml. After the ciliate population that had been fed *Vibrio* 10RR10 reached stationary phase, ca. 10^7 bacteria/ml remained in the medium. Thus, at lower concentrations of bacteria, ciliates cannot obtain sufficient food to sustain population growth, although individual cells endure for long periods of time without additional food.

Seto & Tazaki (1971) have shown that lowest yield and slowest growth rate for *Tetrahymena* occurred at a bacterial concentration of 1.8×10^7 cells/ml, al-

TABLE V
Total viable counts of bacterial strains *Bacillus* 8.20 and *Vibrio* 10RR10 in Rhode River water

Bacterial strain	Time (hr)	Total viable counts
Bacillus 8.20	0	5.50×10^4 cells/ml
	4	5.90×10^4 cells/ml
	24	4.50×10^4 cells/ml
	0	4.50×10^8 cells/ml
	3	4.65×10^8 cells/ml
Vibrio 10RR10	0	1.70×10^4 cells/ml
	2	1.36×10^4 cells/ml
	24	3.46×10^4 cells/ml
	0	8.10×10^7 cells/ml
	4	8.80×10^7 cells/ml

though with concentrations to 2.3×10^9 bacteria/ml, growth rates of the protozoa were equal, but final yields differed. Approximately 10^7 bacteria/ml remained in flasks at stationary phase, regardless of the initial concentration of bacteria.

Recently, Danso & Alexander (1975) demonstrated that there is a critical bacterial density for amoebae populations. Interestingly, the bacterial density was also in the range of 10^6–10^7 bacteria/ml.

Bacterial numbers in the water column of the Rhode River estuary are normally in the order of 10^3–10^4 cells/ml (Kaneko & Colwell, 1973; Berk, unpublished data). In the sediment of the same estuary, the bacterial numbers range from 10^4–10^6 cells/g wet sediment (Kaneko & Colwell, 1973). *Uronema* in the natural environment, therefore, does not normally encounter such high densities of bacteria as those used in experiments described in this report. However, the laboratory conditions provide a useful model for determining the feeding potential of the ciliates. Since *Uronema* has been reported to be associated with bacteria on decaying *Ulva* (Hanna & Lilly, 1974), it may feed very efficiently in patches of decaying plants carrying very high, localized bacterial populations, thereby maintaining its own population density at a relatively high level.

Coleman & Laurie (1974) reported the existence of a lytic enzyme produced by a rumen ciliate that caused significant exogenous lysis of bacterial cells. Other ciliates tested, however, did not produce such a substance. To eliminate the possibility that the decrease in bacterial numbers noted in this study was due to factors other than engulfment by the ciliates, the filtrates were tested for lytic properties. Results showed that the filtrates had no effect on the bacterial populations (Table IV). Therefore, the rates of feeding are concluded to be valid measurements of rates of engulfment of the bacteria by the ciliates.

Uronema was found to have a higher feeding rate when fed *Vibrio* than when fed the *Bacillus* sp., viz., at densities of 5×10^8 cells/ml, the resulting rates of consumption were quite different, 244 *Vibrio* consumed/protozoon per hour versus 73.3 *Bacillus* consumed/protozoon per hour. The bacteria-protozoa ratios varied slightly, but, of the bacteria available to each ciliate, 32% of the *Vibrio* sp. was consumed per hour versus 13.8% of the *Bacillus* sp. The size or shape of bacteria may be important, since *Bacillus* spp. are larger and the Gram positive bacilli have a cell wall chemical structure that is different from that of the smaller, comma-shaped *Vibrio* cell.

Bacterial density had a marked effect on the feeding rate of the ciliate. For a 10-fold difference in numbers of *Vibrio*, a corresponding 7.2-fold difference in feeding rate was calculated. Low feeding rates were observed for densities of 6.8×10^7 bacteria/ml. Curds & Cockburn (1968) stressed the importance of the bacteria-protozoa ratio in determining feeding rates. From the data provided by Laybourn & Stewart (1975), *Colpidium* (a fresh-water ciliate much larger than *Uronema*) feeds at a rate of 5.2×10^4 bacteria/protozoon per hour. Bacterial densities employed in the study were much higher (1.25×10^9 cells/ml) and the ratio of bacteria to protozoa was significantly higher than in this study (2.5×10^6 bacteria/protozoon). However, *Colpidium* is larger with a more elaborate buccal ciliary apparatus than *Uronema* and, therefore, presumably can engulf more bacteria in a given time.

Feeding rates calculated from data obtained in the present study were lower than rates reported for other ciliates. Proper & Garver (1966) observed that *Colpoda* fed at a rate of 450 bacteria/protozoon per hour, when presented with an initial bacterial density of 1.0×10^7 cells/ml. From the data of Curds & Cockburn (1968), it is calculated that *Tetrahymena* can ingest 720 bacteria/protozoon per hour at an initial bacterial density of 3.7×10^7 cells/ml. Rates observed for *Uronema* fell within the range of those reported by Coleman & Laurie

(1974) for *Epidinium* spp. Given 10^9 bacteria/ml, *Epidinium* engulfed ca. 60–7,000 bacteria/protozoon per hour.

Uronema can undergo bursts of feeding after being starved for several hours and it will then feed almost continuously for at least 2 hr. Such a response by *Uronema* to high bacterial concentrations was also observed by Hamilton & Preslan (1969). Therefore, the calculated rates of engulfment, given as an average over 3 hr, is believed to be accurate. Figure 1a shows a ciliate devoid of food vacuoles after starvation for several hours. After feeding on washed cells of *Vibrio* 10RR10 for 1 hr, the ciliate appears to be about half full of food vacuoles (Fig. 1b), and after 2 hr of feeding, the ciliate is gorged with vacuoles (Fig. 1c).

These ciliates can exploit localized areas of high bacterial concentration. The morphological observations, taken in conjunction with data on feeding rates, demonstrate the potential of ciliates in a food web to serve as vehicles for transport of bacterial constituents, viz., environmental contaminants such as heavy metals, etc., concentrated by the bacteria, to higher trophic level organisms. A study is in progress which examines the role of ciliates in the transport of polychlorinated biphenyls from estuarine bacteria to higher forms.

In summary, *Uronema* and *Potomacus* were unable to maintain increases in population growth when bacterial populations were at or below 10^6–10^7 bacteria/ml. *Uronema* was found to yield significantly larger populations when fed *Vibrio* sp. 10RR10 compared with *Bacillus* sp. 8.20. A higher feeding rate was observed for the *Vibrio* as food source, compared with the *Bacillus* sp. Engulfment rates were found to be dependent on the density of the bacterial and protozoan populations, as well as the type of bacteria-protozoa in the system under study.

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USE OF THIN, FLEXIBLE PLASTIC COVERSLIPS FOR MICROSCOPY, MICROCOMPRESSION, AND COUNTING OF AEROBIC MICROORGANISMS[1]

DONALD M. SPOON

Department of Biology, Georgetown University, Washington, D. C. 20057

SPOON, D. M. 1976. Use of thin, flexible plastic coverslips for microscopy, microcompression, and counting of aerobic microorganisms. *Trans. Amer. Micros. Soc.*, 95: 520–523. A new method of microscopic slide preparation characterized by low toxicity and high gaseous exchange is described using nonwettable thin plastic film (Handiwrap©) as the coverslip and plastic slides. The preparation can be used with oil immersion (100 ×) lens with some modifications. Layers of high vacuum silicone grease are used to surround the film and reduce evaporation, creating a very long-lived, well-aerated preparation. Removing excess water pulls the plastic film down, creating a controllable microcompressor effect. Surprisingly, such thin preparations have excellent optical qualities. Various applications are discussed.

Slide preparations of samples from activated sludge have high oxygen demands (B.O.D.). In minutes after the coverslip is added, the enclosed organisms cease normal feeding activities. Their morphology and physiology change rapidly as anoxia brings on death. Even the thinnest commercially available glass and plastic coverslips allow little or no diffusion of gas through them. Thus, gaseous exchange is limited to the edges of the preparation. However, water evaporation occurs continually unless the coverslip is mounted with petroleum jelly, further limiting gaseous exchange. (The author has found that high vacuum silicone grease gives initially firmer coverslip support than petroleum jelly and allows superior prolonged preparations because silicone has better gaseous exchange.) Glass coverslips are charged surfaces which make them highly wettable as well as adherent to the polyelectrolytic molecules on the surfaces of microorganisms. Most commercially available plastic coverslips, though being nonwettable, usually exhibit a degree of toxicity.

The new technique described here was conceived to solve the special problems of studying the morphology, physiology, and ecology of activated sludge protozoa, especially peritrichs, and making rapid, repeatable counts of the numbers of each species. Its development was prompted by the problems with the standard Sedgwick-Rafter and Palmer counting cell glass preparations (Taras et al., 1971).

Activated sludge is a man-made continuous flow ecosystem, showing a worldwide uniformity in flora and fauna, dominated in biomass by the ciliates, in

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