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Grazing, Growth, and Ammonium Excretion Rates of a Heterotrophic Microflagellate Fed with Four Species of Bacteria†

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We studied aspects of the population growth of a microflagellate, *Monas* sp., isolated from Lake Kinneret, Israel. The protozoan growth rates, rates of ingestion of bacteria, and final population yields generally increased with increasing bacterial concentrations, although the exact relationship varied depending on the species of bacteria used as food. Grazing rates decreased hyperbolically with increasing food density. Gross growth efficiencies and ammonia excretion rates were similar over a range of food densities among the four species of bacteria. Population doubling times and ammonia excretion rates were lowest, and growth efficiencies were highest, at temperatures between 18 and 24°C. Under optimum conditions, the microflagellates had average population doubling times of 5.0 to 7.8 h, average growth efficiencies of 23.7 to 48.7%, and average ammonia excretion rates of 0.76 to 1.23 μmol of NH_4^+ per mg (dry wt) per h.

Bacteriovorous microprotozoa (2 to 20 μm in diameter) appear to be a ubiquitous and important part of the carbon cycle of aquatic ecosystems (9, 10, 27, 33, 34). These organisms, most of which are microflagellates in the subphylum Mastigophora, have a short intrinsic generation time and can respond rapidly to blooms of bacteria (15, 16). Microflagellates are often associated with decomposing organic matter in eutrophic systems; as microbial grazers, they are thought to stimulate bacterial growth and thus the rate of decomposition (1, 6, 10, 18). Since the efficiency of conversion of microbial carbon into protozoan biomass is high (e.g., 8, 24, 27), microflagellates may also be an important link in the aquatic food web between bacteria and larger zooplankton.

We have begun investigations of the ecological roles of microflagellates in the carbon cycle of Lake Kinneret, Israel. In Lake Kinneret, only a small portion (10 to 20%) of the intense spring bloom of the dinoflagellate *Peridinium cinctum* subsp. *westii* is directly consumed by herbivores; most of the algal biomass, in the form of detritus, is degraded within the water column (31). During *Peridinium* blooms, colorless microflagellates have been observed in the lake. We found experimentally that the presence of microflagellates native to the lake specifically enhanced the process of decomposition of the carbohydrate thecae (cell walls) of dead *Peridinium* cells (32). This effect appeared to result from a stimulation of thecal hydrolyzing bacteria by microflagellate grazing.

In this report, we present results of a study designed to analyze the growth potential of a Lake Kinneret microflagellate isolated from degrading *P. cinctum* when fed with various concentrations of four species of bacteria. Two of the bacterial species were native to the lake, and two were not. Our main objective was to determine to what extent bacterial grazing (clearance and ingestion rates) and growth (growth rate and gross growth efficiency) of the microflagellate were affected by food concentration and food source. We also analyzed microflagellate growth with respect to temperature and measured the ammonia excretion rates of the microflagellates under various experimental conditions. It should be noted that although there is abundant literature on the ecological energetics, grazing rates, and population growth potentials of ciliates and amoebae (e.g., 3, 5, 17, 19, 28, 35), the number of such studies on heterotrophic microflagellates is much smaller (8, 16, 24, 34).

MATERIALS AND METHODS

A microflagellate culture was obtained by a series of enrichment and dilution steps beginning with a suspension of dead freeze-dried *Peridinium* cells added to 10 μm of screened Lake Kinneret water. The microflagellate we isolated (diameter, 3 to 5 μm) was identified as

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Monas sp. by B. S. C. Leadbeater, University of Birmingham, England. The microflagellates were maintained in an encysted state at 5°C; the addition of bacteria resulted in prompt excystment of the cells.

Experiments were designed to analyze growth responses of the microflagellates when fed with four species of bacteria. Two of the bacterial species were native to Lake Kinneret: *Chlorobium phaeobacteroides*, a photosynthetic sulfur bacterium which blooms in the metalimnic zone from July to September (2), and isolate 1, a bacterium which we obtained from degrading *P. cinctum*. The other species were the common potential pathogens *Escherichia coli* and *Salmonella typhimurium* 635B+. *C. phaeobacteroides*, *E. coli*, and *S. typhimurium* are large bacteria with cell volumes of about 1.0 μm^3; by microscopic inspection, we judged isolate 1 to be about one-third that size, or 0.3 μm^3. The bacteria were maintained on nutrient agar slants at 19 to 20°C. Before an experiment, concentrated suspensions of the bacteria were obtained either by growing an inoculum overnight in 50 ml of initially sterile nutrient broth or (for *C. phaeobacteroides*) by incubating a culture in liquid Pfennig medium for 7 days at medium light intensity (2). The bacteria were centrifuged out of the growth medium, washed twice with artificial Kinneret water (AKW; Table 1), and suspended in a few milliliters of AKW. Known amounts of the washed bacterial concentrate were then added to the experimental flasks. At the beginning of each experiment, three 0.2-ml samples of the bacterial concentrate were filtered onto preweighed Millipore filters (pore size, 0.45 μm; Millipore Corp., Bedford, Mass.) which were dried in a desiccator and reweighed to obtain the milligrams (dry wt) of the concentrate per milliliter.

For the growth experiments, we used replicate sterile 250-ml Erlenmeyer flasks containing 100-ml samples of filter-sterilized AKW. To each flask we added a known number of microflagellates from the stock culture (10^5 to 10^6 cells per flask) and various amounts of bacterial concentrate. In each experiment, one flask with the maximum added bacteria but no microflagellates was used as a control. The flasks were stoppered with sterile cotton and gauze plugs and were incubated with slow shaking at 19 to 20°C. At various time intervals up to 45 to 60 h, subsamples were taken with sterile pipettes for analysis of microflagellate abundance, bacterial biomass, and ammonia concentration. Flagellate numbers were assayed by preserving 1 ml of culture with acid Lugols solution, making appropriate dilutions with AKW, and counting eight replicate 0.1-mm^3 blocks on a hemocytometer at ×200. The minimum number of cells which could be accurately counted was 10^4 cells per ml. The change in bacterial biomass was determined spectrophotometrically at 420 nm. The bacterial biomass as dry weight per milliliter was calculated from regression equations of optical density versus dry weight per milliliter of culture, empirically determined at the start of each experiment. Because the microflagellates were essentially transparent, they did not contribute to the readings of optical density. Ammonia concentrations were determined by the indophenol method of Koroleff (25) on 1-ml subsamples of the cultures diluted 1:20 with NH_4^+-free deionized water.

A separate experimental series was run to determine the growth response of the microflagellates over a range of temperatures. Eight flasks containing 100 ml of AKW with 0.055 mg (dry wt) of isolate 1 per ml were inoculated with the microflagellate culture (8×10^5 cells per flask). Duplicate flasks were incubated at 3, 18, 23.5, and 30°C; all except the flasks incubated at 3°C were slowly shaken. Samples were withdrawn from the flasks over a period of 160 h (only the microflagellates at 3 and 30°C continued to grow after 50 h). The change in numbers of flagellates, bacterial biomass, and ammonia concentration were analyzed as described above.

TABLE 1. Composition of AKW used in the growth experiment

Salt	Amt (mg l^{-1} of distilled water)[a]
$NaHCO_3$	197
NaCl	155
$CaCl_2$	130
$MgCl_2$	124
$MgSO_4$	65
KCl	13
$CaSO_4$	9.5

[a] Total salts, 693.5 mg l^{-1}. Total solids in Lake Kinneret water range from 640 to 714 mg l^{-1} (30).

We monitored microflagellate grazing and growth by following the disappearance of bacterial biomass and the appearance of microflagellates in the cultures. The microflagellates were assumed to be growing exponentially at a constant rate throughout a given sampling interval, so that the specific growth rate, r, could be calculated for each experiment as $r = (\ln N_t - \ln N_o)/t$ where N_o and N_t are the observed concentrations of flagellates at the beginning and end of a time interval of t h. Because r was found to vary with food concentration, we calculated r for time intervals when > 50% of the initial bacterial biomass remained in the flasks. The specific growth rate r was used to calculate the population doubling time, T (in hours), as $T = 0.693/r$. We also determined the gross growth efficiency, E, by calculating the amount of protozoan biomass produced from the added microbial biomass: $E = [P_f/(B_i - B_f)] \times 100$ where P_f is the maximum microflagellate biomass in milligrams (dry wt) per milliliter (we empirically determined that the average microflagellate biomass was 7×10^{-12} g [dry wt] per cell), B_i is the biomass of added bacteria expressed as milligrams (dry wt) per milliliter, and B_f is the final biomass of bacteria per milliliter in the flask.

In addition, we calculated the grazing rate, F (volume swept clear, 10^{-7} ml per flagellate per h), and ingestion rate, I (picograms [dry wt] per flagellate per hour), by the equations of Frost (12) as modified by Heinbokel (19) to account for the growth of the grazer during the sampling intervals. Time intervals were chosen during the logarithmic part of the growth curve so that the number of flagellates increased by 50% between t_o and t_f, and an average number of flagellates, $\bar{N}$, was calculated by the equation $\bar{N} = (N_t - N_o)/(\ln N_t - \ln N_o)$. The biomass of bacteria, B, was determined by interpolation at the midpoint of the chosen time interval, and these values of N and B were used in the equations of Frost (12) to determine F and I.

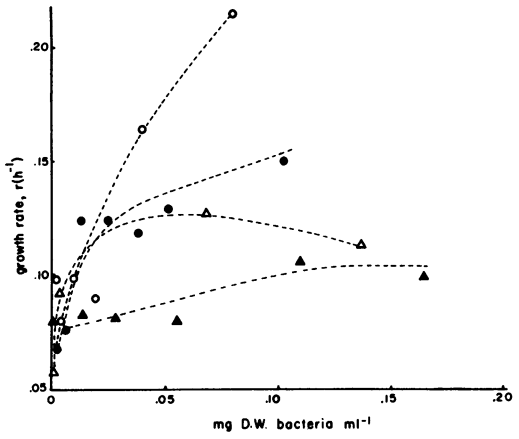

FIG. 1. Specific growth rates of a microflagellate (*Monas* sp.) fed with four bacterial species as a function of initial food density. Symbols: ○, *C. phaeobacteroides*; ●, isolate 1; △, *E. coli*; ▲, *S. typhimurium* 635B+; D.W., dry wt.

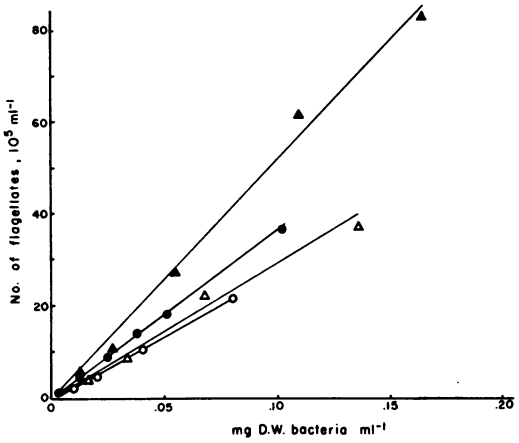

FIG. 2. Microflagellate yields (final density) as a function of initial bacterial density. Symbols as in Fig. 1.

The rates of ammonium excretion were calculated during the log phase of growth over short time intervals during which flagellate numbers increased by 50% or less. The total amount of ammonium accumulated in the cultures during this time period was divided by the calculated biomass (dry wt) of the protozoa to yield an ammonia excretion rate as micromoles of NH_4^+ per milligram (dry wt) per hour.

RESULTS AND DISCUSSION

The amount of initial bacterial biomass added to the flasks at the beginning of the growth experiments ranged from 0.005 to 0.17 mg (dry wt) per ml. We calculated that the average biomass of *C. phaeobacteroides*, *E. coli*, and *S. typhimurium* was 2×10^{-13} g (dry wt) per cell and that of isolate 1 was 6×10^{-14} g (dry wt) per cell. Thus, the amount of added bacteria ranged from 2.5×10^7 to 8.5×10^8 cells per ml for the larger bacteria, and from 0.8×10^8 to 3×10^9 cells per ml for isolate 1. These concentrations of bacteria are one to two orders of magnitude greater than the average abundance of microbial populations in most natural waters, including Lake Kinneret (2, 11, 20, 34). However, bacterial populations in sediment, in highly eutrophic waters, and in oxidation ponds are in the range of 10^7 to 10^8 cells per ml (7, 27, 34). Also, bacterial concentrations on detritus particles are frequently high, about 10^9 to 10^{10} cells per g (dry wt) (10). Heterotrophic microflagellates are typically abundant in eutrophic waters and around detrital particles (10, 32, 34). Thus, our experiments may be relevant to microflagellate growth under such naturally eutrophic conditions, both in Lake Kinneret and elsewhere.

The population growth responses of the *Monas* sp. varied, depending on both food concentration and the bacterial species used as the food source. For each strain of bacteria, the specific growth rate, r, of the microflagellate increased from low to high food density (Fig. 1). Flagellate growth rates in cultures with isolate 1 and *E. coli* reached a maximum at about 0.01 mg (dry wt) of bacteria per ml. Growth rates of microflagellates fed the *Chlorobium* species increased rapidly up to 0.22/h with increasing food concentration up to 0.07 mg (dry wt) of bacteria per ml; on the *Salmonella* species, the flagellate had population growth rates below 0.10/h even at a bacterial concentration of 0.17 mg (dry wt) per ml (Fig. 1). These growth rates correspond to population doubling times of 3.2 to 8.7 h (Table 1).

The maximum population abundances, or yields, of the *Monas* sp. were linearly related to the initial biomass of each species of bacteria (Fig. 2). In terms of the final number of microflagellates, *S. typhimurium* and isolate 1 produced

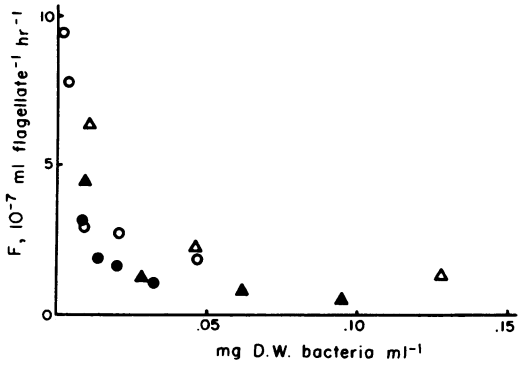

FIG. 3. Microflagellate clearance rates as a function of bacterial density. Symbols as in Fig. 1.

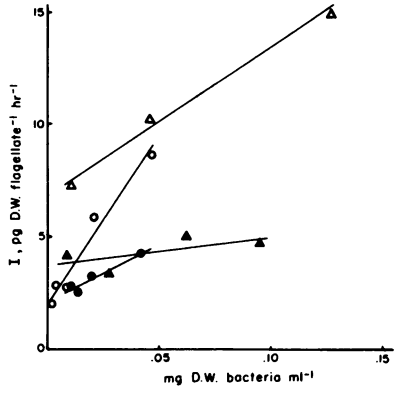

FIG. 4. Rates of ingestion of bacteria by the microflagellates for various densities of the four species of bacteria. Symbols as in Fig. 1.

the highest yields, and *C. phaeobacteroides* and *E. coli* produced the lowest yields, for a given initial amount of bacteria. Another way of expressing this relationship is the gross growth efficiency, *E* (Table 1); the mean *E* was highest for isolate 1 (44.9%) and lowest for *E. coli* (23.3%). Because *E* varied so much within each range of bacterial concentrations, however, no significant difference was found by analysis of variance among the mean *E* values for the various bacterial species.

Except with *S. typhimurium* as a food source, the microflagellate cell yield was essentially constant within the range of measured growth rates. This suggests that the energy required for growth dominated the energy budget of *Monas* sp. and that the maintenance energy (basal metabolism) was a small fraction of the total budget. Similar results have been obtained for several species of marine and freshwater microflagellates grown on *Pseudomonas* sp. (8). The phenomenon is also well known in bacteria and in yeasts (26) and appears to be characteristic of small organisms whose populations exist in only one of three states: actively growing, declining (starving), or encysting (in response to low food levels). Below a critical food concentration, the *Monas* sp. used in this study changed into an encysted form which remained dormant until food was available, at which time excystment occurred and population growth commenced.

Among the four bacterial species used as food sources, there was generally an inverse relationship between microflagellate growth rates and cell yields, i.e., the highest growth rates and lowest cell yields occurred with *C. phaeobacteroides*, and the lowest growth rates and highest cell yields occurred with *S. typhimurium* (Fig. 1 and 2). The reasons for this relationship are unclear, but it may be related to a difference in the nutritional quality of the bacterial species. Gorjacheva et al. (14) also found that the generation time of laboratory-cultured microflagellates varied, depending upon food quality.

The grazing rate *F* and ingestion rate *I* of the microflagellates were also functions of food concentration. The grazing rates had an inverse hyperbolic relation to bacterial density (Fig. 3): above 0.1 mg (dry wt) per ml, the flagellates cleared less than 2×10^{-7} ml per cell per h, whereas below 0.1 mg (dry wt) per ml, a larger volume was cleared, up to 9.5×10^{-7} ml per cell per h. The ingestion rate varied according to the bacterial species and also had a positive linear relation to food concentration (Fig. 4). The greatest amount of bacteria ingested per unit of time occurred with *E. coli*; for the other bacteria, ingestion rates were similar below 0.02 mg (dry wt) of bacteria per ml but diverged beyond that amount. Based on the estimated dry weight per cell for the four bacterial species, we calculated that the microflagellates ingested from 10 to 75 bacteria per flagellate per h, which corresponded to between 30 and 200% of the average microflagellate body weight per hour. For six species of microflagellates grown on *Pseudomonas* sp., Fenchel (8) determined maximum grazing (clearance rates) of 1.4×10^{-6} to 10^{-5} ml per flagellate per h and maximum ingestion rates of 27 to 254 bacteria per flagellate per h. The higher grazing and ingestion rates reported by Fenchel were ascribed to microflagellates with cell volumes three- to sixfold greater than the average cell volume of our *Monas* sp. (30 μm^3); the grazing and ingestion rates which he found for microflagellate species with average cell vol-

TABLE 2. Growth data for a microflagellate (*Monas* sp.) grown on various concentrations of four species of bacteria

Food bacterium	Population doubling time (h)		Gross growth efficiency (%)		Ammonia excretion rate[a]	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
C. phaeobacteroides	6.3 ± 2.1	3.2–8.6	27.6 ± 3.2	24–31	0.83 ± 0.19	0.71–1.10
Isolate 1	5.4 ± 0.5	4.6–5.9	45.0 ± 13.3	30–63	0.89 ± 0.30	0.63–1.50
E. coli	5.8 ± 0.9	5.0–7.4	23.7 ± 2.6	20–28	1.23 ± 0.51	0.60–2.13
S. typhimurium	7.8 ± 0.9	6.5–8.7	36.4 ± 9.5	26–49	0.76 ± 0.19	0.59–1.03

[a] Micromoles of NH_4^+ per milligram (dry wt) per hour.

TABLE 3. Growth data for a microflagellate (*Monas* sp.) grown at four different temperatures[a]

Temp (°C)	Maximum growth (10^5 cells per ml)	Population doubling time (h)	Gross growth efficiency (%)	Ammonia excretion rate[b]
3	5.3 ± 0.0	21.3 ± 0.2	12.0 ± 2.0	0.84 ± 0.16
18	25.7 ± 0.3	5.4 ± 0.05	47.1 ± 0.6	0.46 ± 0.01
23.5	29.1 ± 1.5	5.0 ± 0.1	48.7 ± 2.9	0.51 ± 0.01
30	7.2 ± 0.7	13.1 ± 2.3	11.7 ± 0.9	3.63 ± 0.20

[a] Microflagellate was grown on 0.055 mg (dry wt) of isolate 1 per ml at four temperatures. Mean values ± 1 standard deviation from duplicate flasks are shown.
[b] Micromoles of NH_4^+ per milligram (dry wt) per hour.

umes of 20 and 50 μm^3 were similar to the rates reported here.

There were no differences in mean rates of ammonia excretion among microflagellates grown on the different species of bacteria (Table 2). The excretion rates ranged from 0.76 to 1.23 μmol of NH_4^+ per mg (dry wt) of microflagellate per h (Table 2). These values are similar to rates of ammonia excretion previously reported for marine ciliates (P. L. Johansen, Abstr. 5th Int. Meet. Protozool. 1977, 363) and are one to two orders of magnitude higher than those measured for net zooplankton in Lake Kinneret and other systems (4, 13, 22). Protozoa have already been cited as having an extremely important role in phosphorus regeneration in aquatic ecosystems (1, 23). There has been recent speculation that microzooplankton, specifically heterotrophic protozoa, are similarly important for in situ nitrogen remineralization (21). The high specific rates of ammonia excretion measured in the present study suggest that this could indeed be the case.

Incubation temperature also affected microflagellate growth. The optimum temperatures for the microflagellate were 18 and 23.5°C, which yielded the greatest population densities, the shortest doubling times, and the highest growth efficiencies (Table 3). The lowest population yield and growth rate occurred at 3°C. At 30°C, microflagellate growth appeared to be stressed; this was particularly apparent in ammonia excretion rates fivefold greater than at the lower temperatures. The average annual water temperature of Lake Kinneret (30) falls within the optimum temperature range we found for growth of the *Monas* sp.

Although growth responses of the *Monas* sp. varied depending on the bacterial food source, the overall growth rates (0.05 to 0.20 h^{-1}), population doubling times (3.2 to 8.7 h), and gross growth efficiencies (20 to 63%) found here were within the ranges of values previously reported for other bacteriovorous microflagellates (8, 16) as well as for ciliates and heterotrophic bacteria (17, 19, 26). However, from the results of our experiments, it appears that future laboratory studies of the ecophysiology of bacteriovorous microflagellates will need to consider the relationship of food quality to the population growth response.

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LITERATURE CITED

1. **Barsdate, R. J., T. Fenchel, and R. T. Prentki.** 1974. Phosphorus cycle of model ecosystems: significance for decomposer food chains and effect of bacterial grazers. Oikos **25**:239–251.
2. **Bergstein, T., Y. Henis, and B. Z. Cavari.** 1979. Investigations on the photosynthetic sulfur bacterium *Chlorobium phaeobacteroides* causing seasonal blooms in Lake Kinneret. Can. J. Microbiol. **25**:999–1007.
3. **Berk, S. G., R. R. Colwell, and E. B. Small.** 1976. A study of feeding responses to bacterial prey by estuarine ciliates. Trans. Am. Microsc. Soc. **95**:514–520.
4. **Conover, R. J., and E. D. S. Corner.** 1968. Respiration and excretion in zooplankton. J. Mar. Biol. Assoc. U.K. **48**:49–57.
5. **Curds, C. R.** 1977. Microbial interactions involving protozoa, p. 69–105. *In* F. A. Skinner and J. N. Shewan (ed.), Aquatic microbiology. Academic Press, Inc., New York.
6. **Fenchel, T.** 1970. Studies on the decomposition of organic detritus derived from the turtlegrass *T. testudinum*. Limnol. Oceanogr. **15**:14–20.
7. **Fenchel, T.** 1975. The quantitative importance of the benthic microflora of an Arctic tundra pond. Hydrobiologia **46**:445–464.
8. **Fenchel, T.** 1982. Ecology of heterotrophic microflagellates. II. Bioenergetics and growth. Mar. Ecol. Prog. Ser. **8**:225–231.
9. **Fenchel, T.** 1982. Ecology of heterotrophic microflagellates. IV. Quantitative occurrence and importance as consumers of bacteria. Mar. Ecol. Prog. Ser. **9**:35–42.
10. **Fenchel, T., and P. Harrison.** 1976. The significance of bacterial grazing and mineral cycling for the decomposition of particulate detritus, p. 285–299. *In* J. M. Anderson and A. Macfadyen (ed.), The role of terrestrial and aquatic organisms in decomposition processes. Blackwell Scientific Publications, Ltd., Oxford.
11. **Ferguson, R. L., and P. Rublee.** 1976. Contribution of bacteria to standing crop of coastal plankton. Limnol. Oceanogr. **21**:141–145.
12. **Frost, B. W.** 1972. Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod *Calanus pacificus*. Limnol. Oceanogr. **17**:805–815.
13. **Gophen, M.** 1976. Temperature dependence of food intake, ammonia excretion, and respiration in *Ceriodaphnia reticulata* (Jurine) (Lake Kinneret, Israel). Freshwater Biol. **6**:451–455.
14. **Gorjacheva, N. V., B. F. Zukov, and A. P. Mylnikov.** 1978. Biology of free living Bodonides, p. 29–50. *In* Biology and systematics of lower organisms. Transactions

of the Institute of Biology of Inland Waters, vol. 35 (In Russian).
15. **Güde, H.** 1979. Grazing by protozoa as selection factor for activated sludge bacteria. Microb. Ecol. **5**:225–237.
16. **Haas, L. W., and K. L. Webb.** 1979. Nutritional mode of several non-pigmented micro-flagellates from the York River estuary, Virginia. J. Exp. Mar. Biol. Ecol. **39**:125–134.
17. **Hamilton, R. D., and J. E. Preslan.** 1970. Observations on the continuous culture of a planktonic protozoan. J. Exp. Mar. Biol. Ecol. **5**:94–104.
18. **Harrison, P. G., and K. H. Mann.** 1975. Detritus formation from eelgrass (*Zostera marina* L.): the relative effects of fragmentation, leaching, and decay. Limnol. Oceanogr. **20**:924–934.
19. **Heinbokel, J. F.** 1978. Studies on the functional role of tintinnids in the Southern California Bight. I. Grazing and growth rates in laboratory cultures. Mar. Biol. **47**:177–189.
20. **Hobbie, J. E.** 1979. Activity and bacterial biomass. Arch. Hydrobiol. Beih. Ergebn. Limnol. **12**:59–63.
21. **Jackson, G. A.** 1980. Phytoplankton growth and zooplankton grazing in oligotrophic oceans. Nature (London) **284**:439–441.
22. **Jawed, M.** 1973. Ammonia excretion by zooplankton and its significance to primary productivity during summer. Mar. Biol. **23**:115–120.
23. **Johannes, R. E.** 1965. Influence of marine protozoa on nutrient regeneration. Limnol. Oceanogr. **10**:434–442.
24. **Kopylov, A. I., T. I. Mamayeva, and S. F. Batsanin.** 1980. Energy balance of the colorless flagellate *Parabodo attenuatus*. Oceanology **20**:705–708.
25. **Koroleff, F.** 1976. Determination of ammonia, p. 126–133. *In* K. Grasshoff (ed.), Methods of seawater analysis. Verlag Chemie GmbH, New York.
26. **Payne, W. J.** 1970. Energy yields and growth of heterotrophs. Ann. Rev. Microbiol. **24**:17–52.
27. **Pomeroy, L. R.** 1980. Microbial roles in aquatic food webs, p. 85–109. *In* R. R. Colwell (ed.), Proceedings of the American Society for Microbiology Conference on Aquatic Microbial Ecology. National Oceanographic and Atmospheric Administration, Washington, D.C.
28. **Rogerson, A.** 1981. The ecological energetics of *Amoeba proteus* (protozoa). Hydrobiologia **85**:117–128.
29. **Rublee, P., and B. E. Dornseif.** 1978. Direct counts of bacteria in the sediments of a North Carolina salt marsh. Estuaries **1**:188–191.
30. **Serruya, C.** 1978. Water chemistry, p. 185–204. *In* C. Serruya (ed.), Lake Kinneret. Dr. W. Junk, B.V., The Hague.
31. **Serruya, C., M. Gophen, and U. Pollingher.** 1980. Lake Kinneret: carbon flow patterns and ecosystem management. Arch. Hydrobiol. **88**:265–302.
32. **Sherr, B. F., E. B. Sherr, and T. Berman.** 1982. Decomposition of organic detritus: a selective role for microflagellate protozoa. Limnol. Oceanogr. **27**:765–769.
33. **Sieburth, J. M.** 1979. Sea microbes. Oxford University Press, Oxford.
34. **Sorokin, Y. I.** 1981. Microheterotrophic organisms in marine ecosystems, p. 293–342. *In* A. R. Longhurst (ed.), Analysis of marine ecosystems. Academic Press, Inc., New York.
35. **Taylor, W. D.** 1978. Growth responses of ciliate protozoa to the abundance of their bacterial prey. Microb. Ecol. **4**:207–214.