Vol. 19: 237–246, 1984 | MARINE ECOLOGY – PROGRESS SERIES Mar. Ecol. Prog. Ser. | Published October 10

Estuarine and oceanic microflagellate predation of actively growing bacteria: estimation by frequency of dividing-divided bacteria

Paul G. Davis* and John McN. Sieburth**

Graduate School of Oceanography, University of Rhode Island, Kingston, Rhode Island 02881, USA

ABSTRACT: Rates of predation and clearance of heterotrophic microflagellates, isolated from estuarine and oceanic environments, were estimated using actively growing bacterial prey. In separate experiments, a significant relation (by linear regression) was found between bacterial growth rate and frequency of dividing-divided cells (FDDC) for a species of *Vibrio*. This relation allowed the estimation of flagellate predation rates on these bacteria by comparing net bacterial growth with expected growth, predicted by FDDC, relative to the enumerated flagellate populations. Cell measurements and counts also allowed the estimation of flagellate clearance rates. Results suggest that flagellates can effectively prey upon bacteria at environmental concentrations ranging from 10^5 to 10^7 bacteria ml^{-1} at average rates ranging from 30 to over 200 bacteria flagellate^{-1} h^{-1}. Environmental concentrations of flagellates further suggest that they play a dominant role as bacterial grazers in marine plankton.

INTRODUCTION

Over the last several decades advances in microbiological techniques including epifluorescence microscopy have elevated our estimates of bacterial numbers and biomass in the marine plankton by over 2 orders of magnitude (Sieburth, 1979). Similarly, evidence is mounting for the role of heterotrophic bacterioplankton as a dynamic population (Andrews and Williams, 1971; Azam and Hodson, 1977; Hobbie and Rublee, 1977; Fuhrman and Azam 1980, 1982; Burney et al., 1981, 1982) involved in the remineralization of 'dissolved' organic matter originating from primary producers. The transfer of this secondary production to the higher levels of the food web, however, is dependent upon predators capable of consuming the bacteria at *in situ* concentrations. Evidence for predation of bacteria by metazoans in marine planktonic systems has been minimal or negative, and although larvaceans may trap some bacteria on their fine filter nets (King et al., 1980), they do not appear to consume quantities which could affect bacterial population dynamics. Protozoans are well known bacterivores, but amoebae (and other sarcodines) and ciliates may have only minor roles as bacterial predators in the marine plankton due to relatively low abundance of amoebae (Davis et al., 1978) and ineffective ciliate predation at planktonic bacterial concentrations (Fenchel, 1980). Heterotrophic flagellates, however, are a major group of known bacterial phagotrophs (Haas and Webb, 1979; Kopylov et al., 1980; Fenchel, 1982a, b; Sherr et al., 1983) and have been observed in relatively high abundance in the marine plankton (Sorokin, 1979; Fenchel, 1982c; Davis and Sieburth, 1982, in prep.). Therefore, heterotrophic flagellates by implication appear to be the major predators of planktonic bacteria in aquatic ecosystems. In addition, considerable biomass of phototrophic cyanobacteria (Johnson and Sieburth, 1979) and chemotrophic bacteria such as the methane oxidizers (Sieburth et al., 1984) is available as bacterial prey for protozooplankton.

There exists some controversy over whether heterotrophic flagellates act as phagotrophs or osmotrophs, the latter having been suggested recently by Beers et al. (1975). Kopylov et al. (1980) have presented data suggesting significant osmotrophy as well as phagotrophy in the flagellate species, *Parabodo attenuatus*, in competition with bacteria, although methodological questions may limit data interpretation. However, Haas and Webb (1979) were unable to demonstrate any

* SEAMOcean, Inc. P.O. Box 1627, Wheaton, Maryland 20902, USA

** Addressee for reprint requests

0171-8630/84/0019/0237/$ 02.50

significant uptake of dissolved amino acids at low concentrations. Fenchel (1982a) has argued that at the low concentrations of organics found in seawater, uptake is diffusion limited and proportional to length of the cell, and therefore, flagellates should make poor competitors of the much smaller bacteria for organic uptake.

The purposes of this present study were twofold. The first was to develop a predictive relation between growth rate of a selected marine bacterial strain and frequency of its dividing cells; the second was to use this relation to estimate predation rates of heterotrophic marine microflagellates upon actively growing bacterial prey. Frequency of dividing cells was developed by Hagström et al. (1979) to estimate the *in situ* growth rates of mixed bacterial populations and has been further studied and utilized by Newell and Christian (1981), Christian et al. (1982), and Hanson et al. (1983).

Rates of flagellate predation for several species have been estimated previously by Daggett and Nerad (1982), Fenchel (1982b), and Sherr et al. (1983) using static bacterial populations. Kopylov et al. (1980) calculated similar predation rates for a single marine flagellate species, *Parabodo attenuatus*, using a growing bacterial population. In the present study, flagellate predation of an actively growing bacterial strain was estimated for a number of different flagellate species isolated from both oceanic and estuarine waters.

MATERIALS AND METHODS

Flagellate predators. Fourteen different isolates of bacterivorous microflagellates were used in predation experiments, half of which isolated from Narragansett Bay and half from the Sargasso Sea (Davis and Sieburth, in prep.). The species isolated from Narragansett Bay were *Actinomonas mirabilis*, *Paraphysomonas imperforata*, a species of *Monas* (2 isolates), *Acanthoeopsis unguiculata*, a species of *Bodo* and *Pseudobodo tremulans*. The species isolated from the Sargasso Sea were *Pseudobodo tremulans* (2 isolates), a species of *Monas*, a species of *Oikomonas*, *Bodo celer*, *B. designis*, and *Rhynchomonas nasuta*. The species of *Actinomonas*, *Paraphysomonas*, and *Monas* were all in the size range of 4 to 6 µm cell diameter. The species of *Acanthoeopsis* had a cell diameter of 4 to 5 µm within a 8 to 10 µm diameter silica lorica. The *Bodo* sp. from Narragansett Bay was approximately $5 \times 3 \times 2$ µm rectangular shaped, and *B. celer* and *B. designis* were both about 7×3 µm tubular shaped. The flagellate *Oikomonas* was of 2 to 3 µm diameter, and the flagellate *Rhynchomonas* was 5×3 µm tubular shaped.

Bacterial prey. Seven bacterial strains isolated from Narragansett Bay were initially tested for suitability for the predation experiments. Suitability required the determination of a significant linear relation between frequency of dividing-divided cells (FDDC) and growth rate as described below, and the ability to support actively growing bacterivorous flagellate populations. A species of *Vibrio* (Strain G1; Baxter and Sieburth, 1984) was used solely for the experiments as it met each of the criteria. The medium used for bacterial growth in all experiments was filtered natural seawater supplemented with 10 mg glucose l^{-1}, 10 mg NH_4Cl l^{-1}, and 1 mg $FePO_4$ l^{-1} and autoclaved prior to use. Narragansett Bay (NB) water was used for all experiments involving flagellates isolated from Narragansett Bay, and Sargasso Sea (SS) water was used with oceanic isolates. All experiments were conducted in 250 ml of autoclaved media. Experiments were conducted at 21 °C (± 1 C°) on an environmental shaker swirling the flasks at 100 rpm. Bacteria were preconditioned for all experiments by growing on fresh medium 24 to 48 h prior to inoculation.

Bacterial FDDC growth rate experiment. One ml of preconditioned bacterial culture (approximately 2×10^7 cells ml^{-1}) was added to 200 ml of sterile medium and sampled every 3 h for 24 h by withdrawing 10 ml of culture and preserving with 0.5 % (V/V) glutaraldehyde (final conc.). Three such successive experiments were performed with the bacterial culture subsequently used in the predation experiments: 2 with NB water, 1 with SS water as a medium base. Bacteria were enumerated by epifluorescence microscopy using the fluorochrome DAPI (Porter and Feig, 1980) and the frequency of dividing-divided cells (FDDC) was determined for all samples. The classification of a cell as a dividing-divided cell differed from the FDC (frequency of dividing cells) of Hagström et al. (1979) by including cells with complete separation, although such cells were included in the counts only if the separation was much smaller than the cell radius, and the cell orientation indicated recent division. This alteration of the procedure elevated the percentage of 'dividing' cells counted in a given sample (ranging from 13 to 38 % of the total number of cells present) over FDC counts and, therefore, reduced the total number of bacteria necessary to be counted.

Predation experiments. Predation experiments were begun by adding 10 ml of preconditioned bacterial culture (approximately 2×10^7 cells ml^{-1}) and 1 ml of a mature flagellate culture to 200 ml of media. Thus, bacteria in the 1 ml of flagellate culture (approximately 10^7 bacteria ml^{-1}) were considerably outnumbered by a bacterial strain preconditioned to a presumably lower nutrient medium (flagellate cultures were obtained and maintained on bacteria developing in a

rice grain enrichment). Cultures were allowed to grow approximately 12 h prior to the first sampling to allow for initial growth of the flagellate and bacterial populations. Sampling was conducted as in the FDDC-growth rate experiment but was performed at the beginning and end of a 3 h interval once per day for 4 to 5 d, during which time flagellate populations grew to maximum densities.

After the final sampling, a second phase of the experiments was begun in which the cultures were diluted with fresh medium to examine flagellate predation rates at lower bacterial densities. The grown cultures were diluted with fresh medium at a ratio of 1:5, 1:10, and 1:20 for NB flagellates, and at 1:4, 1:6, and 1:10 for SS flagellates, since the latter generally achieved lower final flagellate densities. Two ml of preconditioned bacteria culture were added to each flask to reduce further the chance that other bacteria present in the initial flagellate culture would interfere with the experiments. Cultures were given an adjustment period of 2 h prior to sampling and 3 samples were taken at 3 h intervals. All samples were counted by epifluorescence microscopy for Protozoa by staining with acridine orange (Davis and Sieburth, 1982) and for bacteria and FDDC as noted above. Four of the 14 cultures of flagellates did not reach sufficient final cell densities to allow accurate counting at the dilutions used in the second stage of the experiments, and these data are not graphed.

Bacterial counts of control flasks in which 1 ml of flagellate culture was inoculated without additional bacteria showed initial bacterial levels of less than 10^5 bacteria ml^{-1} which grew to levels of 10^6 to 5×10^6 bacteria ml^{-1} over a period of several days, after an initial lag period exceeding 6 h. It is believed that the higher initial inoculation of the G1 bacteria (approximately 2×10^6 ml^{-1}) in the experimental flasks, as well as their more rapid growth rates on the low substrate media, easily allowed the dominance of the G1 bacteria over bacteria introduced with the flagellate. Examination of bacteria from the experimental flasks during counting always showed an overwhelming predominance of morphologically uniform bacteria.

Experimental calculations. In FDDC/growth rate experiments for bacterial cultures, a linear relation was tested by linear regression analysis between estimated FDDC values and growth rate calculated between each 2 successive samples in the experiments. Growth rate $u = (\ln N_a/N_o)/t$, where N = number of bacteria ml^{-1} at times zero and a; 't' = time between samples (3 h). The FDDC value used for each paired observation was that value at time zero.

Using the linear regression developed between FDDC and 'u', the FDDC of the bacterial prey in the experimental flasks was used to estimate actual growth rates of the bacteria during predation by bacterivorous flagellates. This allows calculation of the bacteria consumed between successive sampling intervals by using formulas similar to those of King et al. (1980) and Kopylov et al. (1980). Since $N_t = N_o e^{(u-d)t}$ – where 'u' = bacterial growth rate (as estimated by FDDC), 'd' = grazing rate (both in the units h^{-1}) – $d = u - (\ln N_t/N_o)/t$. The number of bacteria grazed per hour is then equal to the grazing rate, times the average number of bacteria present during the sampling interval ($\overline{N}$), or $d\overline{N} = d(N_t - N_o)/(\ln N_t/N_o)$. Similarly, the average number of flagellates present during the sampling interval equals $\overline{X} = (X_t - X_o)/(\ln X_t/X_o)$, where 'X' = number of flagellates at times zero and 't'. Therefore, the average number of bacteria consumed per flagellate per hour is calculated as $(d\overline{N})/\overline{X}$. Clearance rate was estimated as the average number of bacteria grazed per flagellate per hour $(d\overline{N})/\overline{X}$ divided by the average number of bacteria present during that sampling interval ($\overline{N}$), or $d/\overline{X}$. These calculations assume that u(N) and $d(N_1X)$ are constant over a short time interval.

RESULTS AND DISCUSSION

FDDC-growth rate experiments

The results of experiments determining the relation between frequency of dividing-divided cells (FDDC) and bacterial growth rate for *Vibrio* strain G1 are shown in Fig. 1. Analysis by linear regression was highly significant ($\alpha < 0.0001$) and there was no significant difference between the use of Narragansett Bay or Sargasso Sea water as a medium base. The strength of this relation allows the prediction of growth

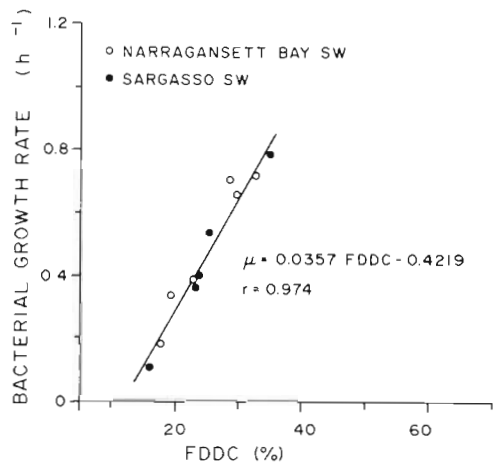

Fig. 1. *Vibrio* Strain G1. Relation between growth rate (u) and 'frequency of dividing-divided cells' (FDDC) in batch culture, using Narragansett Bay and Sargasso Sea waters as media bases. Linear regression equation given, with correlation coefficient (r). Significance of regression, $\alpha < 0.0005$

for this strain of *Vibrio* in the media over a 3 h sampling interval as conducted in these experiments. Prediction of growth rate does not require the knowledge of the time required for cell division as in the method used for phytoplankton (McDuff and Chisholm, 1982). The technique requires only that the population be continuously dividing at the specific growth rate over the sampling interval, and that the growth rate be linearly related to the FDDC of the initial sample.

Frequency of dividing cells as an index of growth has been used only recently for bacteria, primarily to estimate growth of environmental populations (Hagström et al., 1979; Christian et al., 1982; Larsson and Hagström, 1982; Hanson et al., 1983). FDDC as determined in the present study yielded significantly higher values (15 to 40 % FDDC) than those measured for FDC by other authors. This is a result of the intentional inclusion of cells as dividing which had complete separation between cells, though only when it was obvious that the cells had recently undergone division, as discussed in the 'Methods' section. FDC for several samples in the present study counted by the method of Hagström et al. (1979) yielded values averaging 26.1 % of the FDDC values obtained in the present study. It was felt that the increase in statistical accuracy from the larger number of dividing cells counted for a given number of bacteria was greater than the likelihood of mistakenly including cells which had not recently undergone division. This assumption is probably valid when working exclusively with a single bacterial strain rather than with mixed bacterial populations.

Another significant difference between the present use of FDDC and previous work on FDC is the estimation of FDDC and bacterial growth rates over several 3 h intervals within the growth curves. The growth rate of the G1 bacterial strain has been previously noted to change during its 'exponential' growth phase in batch culture (Baxter and Sieburth, 1984) and this observation was repeated several times during the present study. Exponential growth rates estimated between 3 h intervals, however, were found to be linearly related to FDDC and this fact allows prediction of bacterial growth in flagellate cultures over 3 h sampling intervals. The linear relation FDDC to growth rate broke down as the bacteria reached stationary phase in batch cultures, at a concentration of about 2×10^7 ml^{-1}; therefore, predation calculations were not made when bacterial concentrations were greater than 10^7 ml^{-1}. FDDC values during stationary phase tended to be high for low or negative growth rates. While the slope of the line in Fig. 1 would suggest a positive FDDC for some negative growth rates, where bacterial death would exceed growth, FDDC values lower than 12 % were not observed.

The strength of the FDDC to growth rate relation was further tested by examining the results of similar growth studies on the same bacterial strain using four different media: natural seawater alone or with glycerol, acetate, or casein added to concentrations of 10 mg l^{-1}. Bacterial growth rates as estimated by FDDC using the previously generated linear regression differed only slightly (average 3.5 %; standard deviation 2.9 %) from those calculated from the actual change in cell numbers over the 3 h intervals. Therefore, the relation of FDDC to growth rate may be expected to hold even as the concentration or type of available nutrients or substrate changes during the predation experiment, due to the influence of bacteria and flagellates.

Predation rates of flagellates on bacteria estimated by this method gave values ranging from 0 to 300 bacteria flagellate^{-1} h^{-1} over a range of bacterial concentrations of 10^5 to 10^7 ml^{-1}. These data are presented graphically in Fig. 2A–2E and 3A–3E for 10 of the flagellate cultures. Experiments for the 4 remaining flagellates tested (*Actinomonas mirabilis, Acanthoeopsis unguiculata* and the Sargasso Sea isolates of *Monas* sp. and *Bodo celer*) yielded four or fewer data points each (averaging 224, 136, 173, and 137 bacteria consumed h^{-1} flagellate^{-1}, respectively) and their values are not shown graphically.

In 9 of 121 predation rate estimates, the values exceeded 500 bacteria h^{-1} flagellate^{-1}; this greatly exceeds all other estimates and seems excessive from observations of flagellate feeding. In each case, these estimates were generated from samples taken early during the experiments when bacterial concentrations were close to 10^7 ml^{-1} and protozoan densities were low (about 10^3 ml^{-1} or less). The larger error in the protozoan counts due to fewer cells counted and the breakdown of the FDDC to growth rate relations at high bacterial densities probably contributed to the estimation of these high predation rates. They have not been included further in the data analysis since they are probably erroneous and would greatly skew the results.

Fenchel (1982b) estimated maximal predation rates of marine flagellates on non-growing bacteria for several of the same flagellate types and found values ranging from 27 to 254 bacteria h^{-1} flagellate^{-1}. Daggett and Nerad (1982) estimated slightly lower rates (25 bacteria flagellate^{-1} h^{-1}) for *Bodo edax* also using a static bacterial prey. In another study, predation rates of a *Monas* sp. on different bacterial species ranged from 10 to 75 bacteria consumed flagellate^{-1} h^{-1} (Sherr et al., 1983). These values fall within the range of predation rates estimated by the present study. Kopylov et al. (1980) calculated feeding rates for the flagellate species *Parabodo attenuatus* using formulas

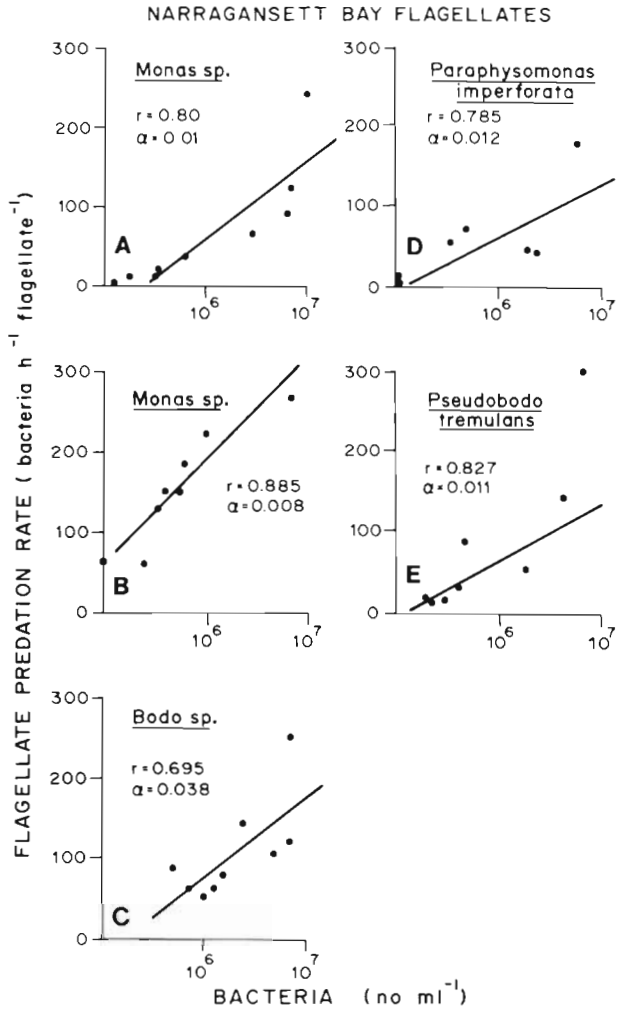

Fig. 2A–E. Plots of Narragansett Bay flagellate predation rates (bacteria consumed h^{-1} flagellate^{-1}) *vs.* bacterial concentration (log scale) for each species of flagellate. Correlation coefficient (r) and significance of regression (α) given for each plot

similar to those used in the present study. They found bacterial consumption ranging from 80 to 125 bacteria h^{-1} flagellate^{-1}. Their data also suggest that uptake of dissolved organic matter may account for 20 to 30 % of caloric intake. These data were generated by estimating bacteria growth rates and dissolved organic matter uptake in control flasks containing no flagellates, and, therefore, assumes that there is no effect of flagellate predation upon bacterial growth or activity, which seems unlikely. Such effects have been demonstrated by Kopylov and Moiseyev (1980), as well as by Sherr et al. (1982).

In general, the predation rates calculated in the present study show significant linear increases with log means of bacterial concentration (Fig. 2A–J; 3A–E). Fenchel (1980, 1982b) has argued that predation rates should increase as a hyperbolic with prey concentration and was able to fit his data to such curves to derive maximal rates of predation. The linear relation with log bacterial concentration in the present data suggests that the predation rate is approaching saturation. Sherr et al. (1983) reported a linear increase in predation rates of a *Monas* sp. with increasing bacterial density, and the effect of saturation is evidenced by their plot of clearance rate. The experiments of Sherr et al. (1983) were run at much higher bacterial concentrations, ranging from 2.5×10^7 to 8.5×10^8 cells ml^{-1}.

The recognition of trophic strategies among the different flagellate species is critical to comprehending their functional roles in the marine plankton. For example, 2 general trophic strategies appear to exist for the different flagellate species frequently observed in the plankton: (1) predation by 'filtration' of bacteria from the medium, (2) predation of bacteria by 'direct encounter' during flagellate movement. Flagellates in

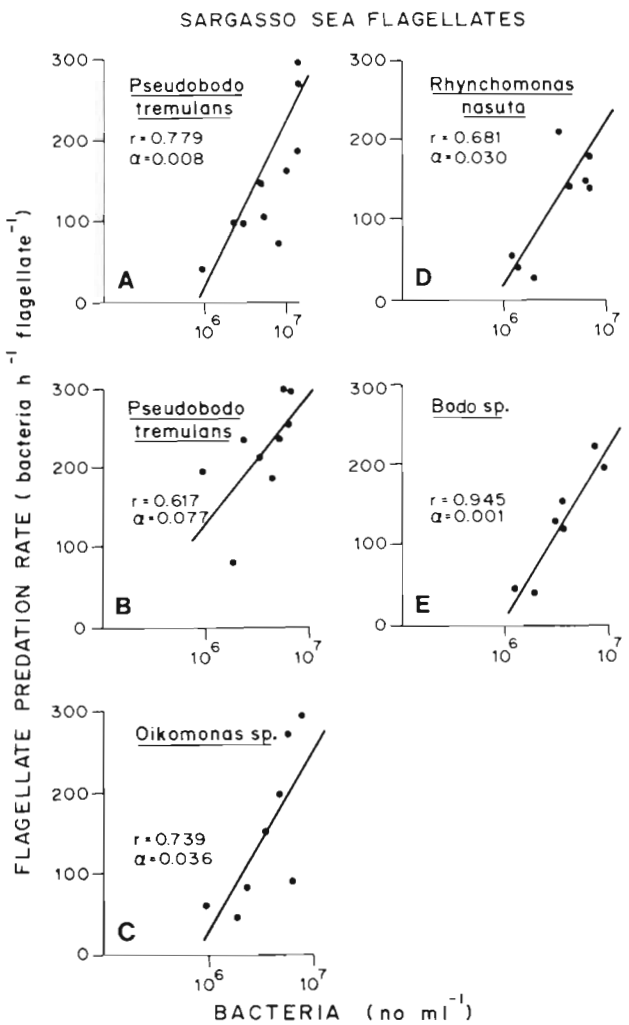

Fig. 3A–E. Plots of Sargasso Sea flagellate predation rates (bacteria consumed h^{-1} flagellate^{-1}) *vs.* bacterial concentration (log scale) for each flagellate species. Correlation coefficient (r) and significance of regression (α) given for each plot

the 'filtering' category would include the choanoflagellates and chrysomonad-like flagellates such as the bicoecids and the genera *Actinomonas, Paraphysomonas, Monas* and *Pseudobodo.* These flagellates establish currents by flagellar action which pass water and bacterial prey over the cell surface, and the bacteria are captured by various mechanisms.

Most of the remaining bacterivorous microflagellates, primarily bodonids, would fall into the 'encounter-feeder' group: those which rely upon movement of the flagellate towards the bacterial prey. Flagellar motion in this case plays a much smaller role in the movement of distant bacterial prey to the cell surface. For the higher bacterial levels in the present experiments (greater than 4.0×10^6 ml^{-1}), predation rates of the 'filterers' were significantly higher than those of the 'encounter feeders' ($\alpha = 0.005$ Wilcoxin Rank Sum Test) averaging 204 and 156 bacteria h^{-1} flagellate^{-1}, respectively. Such distinctions between flagellate types are of ecological importance since most microflagellates have a strong tendency to adhere to particles. 'Encounter-feeders' would more likely graze surface-adhering epibacteria whereas attached 'filterers' would continue to graze unattached planktonic bacteria. Flagellates which show no tendency toward attachment are rare, based on the strains cultured in concurrent studies (Davis, 1982). All flagellate isolates used in the present study, however, were chosen because the number of attaching cells in culture was low relative to non-attaching cells swimming free in the media. Caution should be used in selecting flagellate species for estimation of predation or clearance rates when only the free-swimming flagellates are measured. Attached flagellates may be dense relative to non-attached cells and possibly have a significant effect upon bacteria free in the medium. Predation rate estimates based on the disappearance of bacterial cells are over-estimated to the extent that flagellates attached to the experimental flask walls prey upon non-attached bacteria.

Fenchel (1982a) identifies 2 other trophic strategies which characterize the flagellates: flagellates may (1) sieve bacteria through projections of the cell membrane from the cell surface (e.g. species of *Actinomonas, Monosiga*), or (2) directly contact bacteria on their surface lacking such projections (e.g. bodonids, species of *Monas* and *Pseudobodo*). In the former category, the 'sieve' is essentially an adaptation which increases the cell surface area and thus increases the likelihood of cell contact and retention of bacterial prey. Such mechanisms may greatly increase the efficiency of predation on bacteria and play a role in defining the habitat limitations of the flagellates.

Differences among bacterial strains as food for bacterivorous flagellates may have effects on *in situ* populations of heterotrophic microflagellates. Sherr et al. (1983) demonstrated variation in growth rates, growth efficiencies and ammonium excretion for a species of *Monas* using 4 different bacterial prey. Studies which have estimated predation of growing or non-growing bacteria by flagellates (Kopylov et al., 1980; Fenchel, 1982b; Sherr et al., 1983; present study), even with different species of bacteria, have yielded generally similar ranges of predation rates, although the highest reported rates are from the present study. This suggests that predation rates may vary more due to variation of prey concentration and the species of flagellate being tested than due to the prey species.

Estimation of higher predation rates in the present study may in part be due to culture conditions and bacterial species used. Fenchel (1982a) has suggested that predation of bacteria by flagellates would increase with bacterial motility. The bacterium used in the present study was an actively growing and motile *Vibrio.* Fenchel (1982b) used a motile bacterial prey, but motility may have been affected since his experiments, as well as those of Sherr et al. (1983), were run under non-nutrient conditions. In addition, the predation rate estimates of these 2 studies both assume that the bacterial predation by flagellates and subsequent excretion do not result in undetected, 'cryptic', growth of the bacteria. Such 'cryptic' growth would result in an underestimation of predation rate. Alternatively, in the present study, if predation by flagellates affects the slope or relation of FDDC to growth rate, the estimated predation and clearance rates would be under- or over-estimated depending upon the direction of the effect.

Estimated flagellate clearance rates in this study ranged from 0.17 to 3.36×10^{-4} ml flagellate^{-1} h^{-1}. Since clearance rates are a function of predation rates, these values also tend to be somewhat higher than the maximal clearance rates estimated by Fenchel (1982b) (0.014 to 0.79×10^{-4} ml h^{-1} flagellate^{-1}) although the ranges overlap, and are higher than the clearance rates estimated by Sherr et al. (1983) (2 to 9.5×10^{-7} ml h^{-1}). Not unexpectedly, flagellate clearance rates tend to decrease with increasing prey density (Table 1; Fig. 3 and 4). Linear regression of this decrease with the log of bacterial concentration is highly significant ($\alpha = 0.00003$), although the variance remains large. Since predation rate is equal to clearance rate times bacterial concentration, clearance rate would be expected to decrease linearly with increasing log of bacterial concentration if predation rate increased linearly for the same values (Frost, 1972). Clearance rates for Narragansett Bay flagellates tend to be higher than those for Sargasso Sea flagellates at lower prey concentrations (less than 10^6 ml^{-1}). This may be due in part to the experiments for the Narragansett Bay flagellates being run at lower bacterial concentrations,

Table 1. Clearance rates (ml flagellate^{-1} h^{-1} × 10^{-4}) for Narragansett Bay and Sargasso Sea flagellates averaged for different ranges of bacterial concentrations

Flagellate species	Bacterial concentration (×10^6 m^{-1})				
	<0.5	0.5–1.0	1.0–4.0	4.0–7.5	>7.5
Narragansett Bay					
Actinomonas mirabilis	–	–	1.60	2.03	–
Monas sp. (1)	0.633	0.624	0.137	0.145	0.263
Monas sp. (2)	3.36	2.73	2.16	–	0.384
Paraphysomonas imperforata	1.11	1.45	0.18	0.28	–
Bodo sp.	–	1.20	0.50	0.19	0.32
Pseudobodo tremulans	0.84	1.65	0.25	0.39	–
Acanthoeopsis unguiculata	–	–	0.91	–	–
Weighted average	1.36	1.65	0.91	0.26	0.33
Sargasso Sea					
Pseudobodo tremulans (1)	–	0.40	0.40	0.20	0.30
Pseudobodo tremulans (2)	–	–	0.98	0.43	0.51
Monas sp. (3)	–	–	–	–	0.20
Oikomonas sp.	–	0.65	0.28	0.63	0.44
Bodo celer	–	–	–	0.39	–
Rhynchomonas nasuta	–	–	0.37	0.30	0.17
Bodo designis	–	–	0.32	0.57	0.41
Weighted average		0.52	0.48	0.43	0.32

rather than representing any real difference between oceanic and estuarine isolates. While clearance rates should theoretically decrease with the square of the cell diameter (Fenchel, 1982a), there was no such trend observed among the flagellates tested. The narrow range of flagellate sizes and variations in the predation mechanisms among the different strains and species of flagellates may account for this apparent lack of a relation.

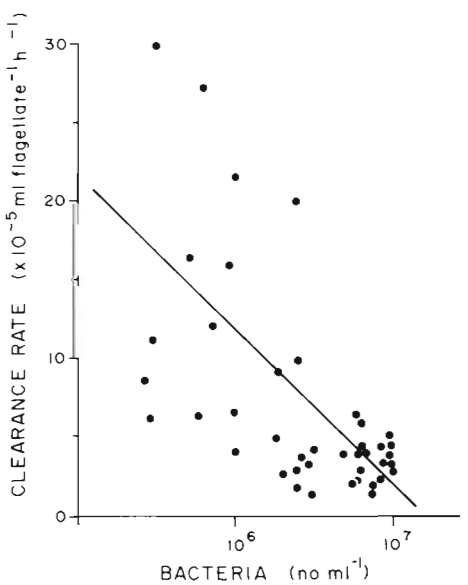

Fig. 4. Plot of clearance rates (ml flagellate^{-1} h^{-1}) for Narragansett Bay and Sargasso Sea flagellates *vs.* bacteria concentration (log scale). Correlation coefficient = −0.63; significance of regression = 0.00003

ENVIRONMENTAL CONCENTRATIONS OF HETEROTROPHIC MICROFLAGELLATES

Given these estimates of bacterial predation and clearance rates for heterotrophic microflagellates, it is possible to make some speculative estimates of the impact of these protozoans upon the bacterial populations in estuarine, shelf, and oligotrophic marine waters. As listed in Table 2, direct counts of heterotrophic nanoplankton (Davis and Sieburth, 1982) suggest flagellate populations of approximately 5×10^3, 3×10^3 and 0.5×10^3 organisms ml^{-1} for estuarine, shelf and Sargasso Sea, or Caribbean waters, respectively. Fenchel (1982c) reports similar flagellate populations for coastal waters. Bacterial populations are relatively constant for these same environments, being 2×10^6, 1×10^6, and 0.5×10^6 ml^{-1}, respectively. Average flagellate predation rates on bacteria presented for these environmental concentrations of flagellates and bacteria would yield, on a population basis, a consumption rate of bacteria of 0.6×10^6, 0.29×10^6, and 0.025×10^6 bacteria ml^{-1} h^{-1} for estuarine, shelf, and oceanic environments, respectively. Estimated bacterial volume per cell is approximately 60 % lower for oceanic planktonic bacteria than the bacteria used in the present experiments. The slight decrease in the linear dimensions of the oceanic bacteria would be expected to decrease the rate of predation somewhat (Fenchel, 1982a). Also, assuming an accompanying decrease in prey energy content with lessened size, flagellate growth rates would be expected to decrease.

For environmental concentrations of bacteria, and

Table 2. Average rates of flagellate predation and clearance for environmental concentrations of bacteria which are used to estimate bacterial growth rates for various marine environments

	Estuarine	Shelf	Oceanic	
			Water	Marine snow*
Bacteria populations ($\bar{x}$ ml^{-1})	2×10^6	1×10^6	0.5×10^6	1.0×10^7
Heterotrophic nanoplankton populations ($\bar{x}$ ml^{-1})	5×10^3	3×10^3	0.5×10^3	3.2×10^4
Predation rate ($\bar{x}$ bact flag^{-1} h^{-1})	121.0	96.2	50.0	210.1
Estimated bacteria consumed (ml^{-1} h^{-1})	0.605×10^6	0.289×10^6	0.025×10^6	6.72×10^6
Estimated bacterial growth rate (h^{-1})	0.30	0.29	0.05	0.51
Estimated bacterial doubling time (h)	2.3	2.4	13.9	1.3
Estimated clearance rate (ml h^{-1} flag^{-1})	6.05×10^{-5}	9.62×10^{-5}	1.0×10^{-4}	2.10×10^{-5}
Estimated No. ml cleared (h^{-1})	0.302	0.29	0.05	0.67

* Populations averaged from over 100 samples in Sargasso Sea and Gulf Stream (Caron et al., 1982, and Caron and Davis, unpubl.)

assuming a balanced system in which bacterial growth equals bacterial predation, and that all bacterial death is due to flagellate predation, then one can estimate the bacterial growth rates shown in Table 2 which would be equivalent to bacterial doubling times of 2.3, 2.4 and 13.9 h for estuarine, shelf, and oceanic environments, respectively. Clearance rates at this level of predation would suggest that 30 %, 29 % and 5 % of the water is cleared per hour for the same environments, respectively. Environmental concentrations of bacteria are assumed not to represent thresholds below which flagellates do not or cannot feed. While not detected in this study, such thresholds may exist. Planktonic bacterial populations are very stable over the long term, but may vary temporally on a diel basis (Meyr-Reil, 1979; Johnson et al., 1983). Flagellate predation could possibly be induced by such changes and, therefore, be discontinuous. The estimated bacterial doubling times would be overestimated to the degree that *in situ* predation was less than estimated by these experiments.

Marine snow is a specialized microenvironment present in marine waters and the site of enriched bacterial and heterotrophic flagellate populations (Caron et al., 1982). Since several of the flagellates used in this study were isolated from marine snow in the Sargasso Sea, estimates of predation impact, clearance, and bacterial growth were made for these microenvironments. Approximately 6.7×10^6 bacteria could be consumed ml^{-1} h^{-1} equaling a bacterial doubling time of 1.3 h assuming that the system is in balance. Almost 1 ml could be cleared by such flagellate populations per hour. However, this value is given with the caution that marine snow is in major part a surface environment and these estimates are made from rates determined for planktonic populations.

The preceding calculations all assume that the predation rate estimates for the cultured flagellates are representative of such rates for the populations of heterotrophic nanoplankton (Hnano) enumerated by epifluorescence microscopy, and that all of the Hnano are flagellates. Our data (Davis and Sieburth, 1983) suggest that 40 to 100 % of cells in the Hnano are flagellates, and that they are morphologically similar to cultured forms. The appearance of bacteria in the food vacuoles of flagellates as observed by transmission electron microscopy (Sieburth and Davis, 1982; Johnson and Sieburth, unpubl.) and epifluorescence microscopy (Sherr and Sherr, 1983) suggests that these flagellates are bacterivorous, although this is difficult to establish for all of the flagellates. Bacterial growth rates estimated by these considerations significantly exceed estimates by other authors. As summarized by Sorokin (1978), marine bacterial growth rates have been estimated to range between 0.3 to 1.0 d^{-1} (equivalent to a doubling time of 17 to 55 h). Using FDC-growth rate relation, Hagström et al. (1979), Newell and Christian (1981), and Hanson et al. (1983) have estimated bacterial growth rates for nearshore and oceanic waters in the same range, but occasionally with doubling times less than 10 h. Discrepancies between these bacterial growth rate estimates and those estimated in the present study may in part be reconciled if predation is not continuous throughout a diel cycle, but responsive to rapid increases in bacterial biomass resulting from pulsed phytoplankton release of zooplankton excretion of organic matter. While a cessation of feeding was not observed in the range of conditions of these experiments with the flagellate species used, it is possible that other uncultured species respond to threshold bacterial concentration in the marine plankton.

The results of this study indicate that apochlorotic microflagellates are capable of consuming actively growing bacteria at a range of environmental concentrations for both coastal and oceanic environments. Since populations of these micro-predators have been shown to be the most dominant protozoans in the

marine plankton (Davis 1982; Davis and Sieburth, in prep.), both numerically and in biomass, it seems evident that these protozoans represent the dominant bacterivores in the mixed layer of the sea. The consumption of these flagellates by larger planktivores (Kopylov et al., 1981; D. Caron, pers. comm.) including the tintinnids (Verity, 1984) undoubtedly represents the final link in the transfer of bacterial biomass to the higher trophic levels.

Acknowledgements. The authors are grateful to P. W. Johnson, J. Knapp, and J. Millar Davis for aid in manuscript preparation and to N. Hairston Jr. and D. A. Caron for critical review; A. Hagström provided helpful suggestions with the FDC procedure. This work was supported by the Biological Oceanography Program of the National Science Foundation (Grant OCE-8121881).

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This paper was submitted to the editor; it was accepted for printing on May 26, 1984