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Effects of Temperature, Sulfide, and Food Abundance on Growth and Feeding of Anaerobic Ciliates

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The trophic role of ciliates in anaerobic food webs has not been assessed experimentally. In order to obtain basic information necessary to interpret field situations, we studied the effects of temperature, sulfide concentration, and food abundance on the growth and feeding activities of two anaerobic ciliates, *Plagiopyla nasuta* and *Metopus es*. The growth rate of *P. nasuta* increased with temperature from 8 to 18°C (Q_{10} = 2.0) and remained constant in the range between 18 and 24°C (0.22 day^{-1}). Sulfide concentrations of between 0 and 1 mM did not affect the feeding activities, but concentrations greater than 2 mM were inhibitory. The functional response of *P. nasuta* feeding on fluorescently labeled heterotrophic and phototrophic bacteria was investigated. In both cases, the parameters of the functional response were almost identical when expressed in terms of biovolume: the maximal uptake rate (U_m) was 1,800 μm^3 ciliate^{-1} h^{-1} and the half-saturation constant for ingestion (k) was 1.5 × 10^7 μm^3 ml^{-1}. The functional response of *M. es* feeding on heterotrophic bacteria was found to be similar to that of *P. nasuta*. These ciliates needed high bacterial abundances in order to maintain their growth (k of about 4 × 10^7 bacteria ml^{-1}), implying that they will frequently be food limited in planktonic environments. Both the maximal uptake rates and the maximal clearance rates were comparable to those of aerobic ciliates. By combining the growth and feeding data, we estimated gross growth efficiencies of 12 and 13% for *P. nasuta* and *M. es*, respectively. These results indicate that the feeding rates of anaerobic ciliates are similar to those of aerobic ciliates. Their slower growth must, therefore, be due to the lower gross growth efficiency (likely due to anaerobic metabolism).

Anoxic systems are colonized mostly by prokaryotic organisms displaying a considerable diversity of energy metabolisms (38). Among the eukaryotic organisms, only some protists can live their entire life cycle under such conditions. The presence of anaerobic ciliates in many anoxic aquatic systems, both in the plankton (13, 15) and in sediments (14, 36), has been described. We were interested in determining the trophic role of anaerobic ciliates in planktonic systems and assessing whether they were effective grazers on the bacterioplankton, comparable to aerobic ciliates (30).

The physiological aspects of the adaptation of these eukaryotic cells to anaerobiosis have been widely investigated. The principal catabolic process of anaerobic protozoa is fermentation, with CO_2, acetate, and other organic acids as end products (25). Some protozoa have special organelles, the hydrogenosomes, which produce hydrogen, and they often also have methanogenic bacteria, which produce methane, as endosymbionts (33). Nevertheless, very little has been done to elucidate the role of anaerobic ciliates in nature, and laboratory studies on their growth and feeding are a first step necessary to evaluate this role.

It is known that anaerobic ciliates grow more slowly than aerobic ciliates (11, 12). According to Fenchel and Finlay (11, 12), this is due to the lower growth efficiency of the anaerobic (fermentation) versus the aerobic (aerobic respiration) metabolic pathway. An alternative explanation, however, could be lower feeding rates among anaerobic than aerobic ciliates. The simultaneous determination of growth and feeding activities allowed us to answer this question and to estimate the gross growth efficiency of some anaerobic ciliates.

The relationship between uptake rate and prey abundance (functional response) has been widely studied. Holling (18) proposed three models of functional response: rectilinear (type I), hyperbolic (type II), and sigmoidal (type III). The same author (19) empirically deduced the disc equation to describe the type II hyperbolic model, and this equation turned out to be identical in form to the Michaelis-Menten model for enzyme kinetics and the Monod equation for bacterial growth. The disc equation is defined by two parameters: the maximal uptake rate, U_m, and the half-saturation constant, k. Both parameters have an ecological meaning: the maximal uptake rate relates to the maximal growth rate (through the gross growth efficiency [10]), and the half-saturation constant gives information on the prey abundance at which the predators are adapted to live (7). Once the functional response of a given predator is known, one can get a rough estimate of the uptake rate in any system if the food abundance is measured. The impact of predation on bacterial assemblages can then be estimated (23). The functional response has already been studied among aerobic protozoa, both ciliates (7, 21) and flagellates (9, 22), but it has not been studied among anaerobic ciliates.

In this article, we present data on the growth and feeding of two anaerobic ciliates, *Plagiopyla nasuta* and *Metopus es*, in laboratory cultures. First, the growth rate of *P. nasuta* was measured at different temperatures. Then, the functional response of *P. nasuta* feeding on fluorescently labeled heterotrophic (FLB) and phototrophic (FLC) bacteria was determined. The functional response of *M. es* feeding on FLB was also determined. Finally, the effect of sulfide concentration on

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the feeding rate of *P. nasuta* was studied. These three factors, food abundance, temperature, and sulfide concentration, are presumably the most important in determining the feeding rates in nature. In a separate article (23), we combine the results presented here with field experiments to assess the trophic role of a *Plagiopyla* population in the anaerobic meta- and hypolimnion of a small lake with large populations of heterotrophic and phototrophic bacteria.

MATERIALS AND METHODS

Isolation of ciliates. In the wastewater treatment facility of the Dekkerswald Hospital near Nijmegen, The Netherlands, we found an organic, anoxic sediment (sapropel) rich in organisms (between 10^2 and 10^3 ciliates cm^{-3}) and rich in diversity of anaerobic ciliates: *M. es*, *P. nasuta*, *Caenomorpha uniserialis*, *Caenomorpha medusula*, *Brachonella spiralis*, and *Brachonella darwinii*. This was the same system described previously by Wagener and coworkers (37). In the laboratory, 0.5 ml of sapropel was dispensed into a petri dish containing water from the same pond. The petri dish was screened under a dissecting microscope, and the ciliates swimming out of the sediment were picked up with a thin capillar pipette. About 20 ciliates were inoculated into 10 ml of culture medium (prepared as described below), and the bottles were incubated at 18°C in the dark.

Culture of anaerobic ciliates. The culture medium contained (in milligrams per liter): K_2HPO_4, 6; $(NH_4)_2SO_4$, 3; KCl, 8; $CaCl_2 \cdot 2H_2O$, 4; $MgSO_4 \cdot 7H_2O$, 5; $NaHCO_3$, 500; $Na_2S \cdot 9H_2O$, 36; and dried grass, 200; resazurine (0.1%, wt/vol) was also present (0.5 ml) (17). Before the addition of bicarbonate and sodium sulfide, between 10 and 20 ml of solution was dispensed into 60-ml serum bottles. The bottles were closed with rubber stoppers and aluminum seals, evacuated for 3 to 4 min, gassed with a mixture of 95% N_2–5% CO_2 (vol/vol) for 30 to 45 s at 40 kPa of pressure, and autoclaved. The bicarbonate and sodium sulfide solutions were prepared separately and added to each bottle through the stopper with a syringe. In order to maintain the desired pH in the medium (between 6.5 and 7.5), it was necessary to add some CO_2 gas to the headspace. Dried grass enhanced ciliate growth, especially of *Plagiopyla* species, both providing surfaces for grazing and allowing some bacterial growth (17). Resazurine served as a redox indicator. Complete anoxia was achieved only after the addition of sodium sulfide. Since part of the sulfide added was oxidized, the actual sulfide concentration was determined for some freshly prepared culture bottles and found to be 0.069 ± 0.009 mM (mean ± standard error [SE], $n = 9$). The sulfide concentration was measured by the methylene blue colorimetric method described by Golterman et al. (16).

The ciliates were fed a mixed bacterial enrichment made from the same sample from which the ciliates were isolated. These bacteria were grown in anaerobic nutrient broth at 37°C, concentrated by centrifugation for 12 min at 12,000 rpm in a GSA rotor (Sorvall Instruments), and stored in Eppendorf tubes at −30°C. The initial bacterial concentration added ranged between 5×10^7 and 1×10^8 bacteria ml^{-1}.

Ciliate growth was checked by observing the bottle directly with an inverted microscope. This gave an estimate of ciliate abundance without disturbing the culture. We succeeded in isolating and maintaining cultures of *M. es* and *P. nasuta*. Both ciliates had methanogenic bacterial endosymbionts which exhibited bluish fluorescence when excited with UV radiation (4, 33). The endosymbionts were not lost after more than 1 year in culture. Fixed specimens of the ciliates were measured in the microscope to obtain the linear dimensions: 96 by 55 μm for *P. nasuta* and 156 by 41 μm for *M. es*. It was difficult to determine their biovolume from these dimensions because of their irregular shape and their flattened bodies. Therefore, fixed ciliates were pressed between a slide and a coverslip until the sides were parallel. The length and the width were measured directly on the microscope, and the thickness was estimated by using the fine-focus knob of the microscope. The biovolume was 54,700 μm^3 for *P. nasuta* and 61,300 μm^3 for *M. es*. Biovolumes were converted to biomass by using conversion factors from the literature: 0.35 pg of C per μm^3 for bacteria (1) and 0.14 pg of C per μm^3 for ciliates (27).

Growth experiments. New cultures were started by inoculating 1 to 2 ml of a well-grown culture into 10 or 20 ml of fresh medium. Every 1 or 2 days, an aliquot (between 0.1 and 1 ml) was taken with a syringe and dispensed into a watch glass, and all the live ciliates were counted under a dissecting microscope. The natural logarithm of the ciliate concentration was plotted with respect to time. A linear regression was carried out with the values of the exponential phase, and the slope thus corresponded to the growth rate, μ (per day). Growth rates were determined at seven different temperatures: 8, 10, 13, 14, 18, 23, and 24°C. Normally, we had four or five cultures at each temperature except 18°C, for which we had 33 cultures. An exponential regression between growth rate (μ) and temperature (T) was performed, $\mu = A \times 10^{(B \times T)}$, where A and B are the coefficients of the regression. Subsequently, the Q_{10} was calculated by using the B coefficient: $Q_{10} = 10^{(10 \times B)}$ (3).

Determination of feeding rates. The feeding rates of ciliates were calculated by the incorporation of fluorescently labeled heterotrophic bacteria (FLB; 1.1 by 0.8 μm; biovolume, 0.42 μm^3), and fluorescently labeled *Chromatium* cells (FLC) prepared from a *Chromatium vinosum* culture (3.2 by 1.4 μm; biovolume, 4.2 μm^3).

For time course experiments, FLB or FLC were added directly to the ciliate culture, and aliquots were taken every 5 to 10 min. Under the dissecting microscope, a minimum of 20 ciliates were picked up from the aliquot. These ciliates were placed in a 200-μl drop of diluted Formalin (1.2% formaldehyde) and then transferred in a smaller volume (approximately 20 μl) to a slide. A coverslip (20 by 20 mm) was placed on the drop, and the whole area was examined by phase-contrast optics to detect the ciliates and by blue-light epifluorescence to count the ingested particles.

For the remaining experiments, 1 ml of ciliate culture was inoculated into a bottle containing 10 ml of fresh medium, and the appropriate concentration of fluorescent bacteria was added. After 5 min of incubation, the bottle was opened and the contents were fixed with 3 ml of formaldehyde solution (0.3% final concentration). This low final concentration of fixative facilitated the counting of ingested fluorescent particles, since the ciliates appeared relatively flat between the slide and the coverslip. The fixed sample was filtered through 37-μm nylon mesh in order to eliminate most of the fluorescent particles, while no ciliates were lost (23). The ciliates were then rinsed off the mesh with a small volume of particle-free water, stained with DAPI (4′,6-diamidino-2-phenylindole), and filtered through an 8-μm Nuclepore filter. The filter was mounted with immersion oil between a slide and a coverslip. The whole filter area was examined at low magnification (50×) with UV radiation in a Nikon epifluorescence microscope. When a ciliate was located, we changed to a higher magnification (500×) and to blue-light excitation in order to count the fluorescent particles ingested.

The mean number of particles ingested per ciliate (20 ciliates observed) represented the uptake within 5 min, and the

uptake rate, U (bacteria per ciliate per hour), was calculated by multiplying this value by 12 and by the ratio N/L, when applicable, where N was the total bacterial abundance and L was the tracer (FLB or FLC) concentration. The clearance rate, F (nanoliters per ciliate per hour), was calculated as U/N, where N was bacteria per nanoliter.

For the determination of sulfide effects on feeding activity, several bottles containing different sulfide concentrations were inoculated with 1 ml of culture. After 2 h of adaptation to the new medium, 5×10^6 FLB ml^{-1} were added to each bottle. After 5 min of incubation, 2 ml from each bottle was fixed for sulfide determination, and the remaining volume was fixed with formaldehyde. Clearance rates were determined as described above.

Methodological experiments. We did some methodological experiments to improve the determination of feeding rates for anaerobic ciliates. (i) We found the adaptation time needed by the ciliates to return to previous feeding rates after being transferred to a new medium (Fig. 1A). The clearance rate of *P. nasuta* determined immediately after transfer of the ciliates was half of the maximal rate. When the ciliates were left for 30 and 60 min in the new medium, the clearance rate dropped, and when they were left for 2 or 4 h, the clearance rate was maximal. From these results, we concluded that the ciliates needed at most 2 h to adapt to the new medium. (ii) We tested whether dried grass was necessary in the feeding experiments (Fig. 1B). This material was not lost in the filtration through 37-μm mesh and interfered with the the microscopic observation. However, it was necessary for ciliate growth (17; our results and data not shown). No significant differences were found by the t test between the clearance rates for *P. nasuta* obtained in medium with and without grass particles, either at the low (P = 0.540) or at the high (P = 0.308) FLB concentrations tested. (iii) We studied how the counting of ingested FLB could be improved in the experiments performed at high bacterial concentrations. In such cases, FLB appeared densely packed inside the food vacuoles, and the counting of individual particles was difficult. This problem would be solved if the stained particles (FLB) represented a minor percentage of the total particles added. Thus, we made an unstained particle suspension (NB, for nonstained bacteria) from the same bacterial culture from which FLB were prepared by the method of Sherr et al. (29) but without stain addition. To use a mixture of FLB and NB routinely in feeding experiments, we tested whether the ciliates demonstrated a preference between them. The clearance rate of *P. nasuta* was determined at different ratios of both particles giving total abundances of 8×10^6 bacteria ml^{-1}, and the clearance rate was similar at all the different ratios used (Fig. 1C). Since the slope of the regression line obtained in Fig. 1C was not significantly different from zero (P = 0.570), we concluded that there was no preference.

Determination of the functional response. In order to determine the functional response, we needed replicates of the same culture to which different amounts of bacteria were to be added. Also, the bacteria used as food had to represent a small fraction of the total bacteria (food bacteria plus fluorescent bacteria). Both problems were solved by inoculating 1 ml of culture into fresh medium without dried grass (Fig. 1B). We were able to obtain up to 10 replicates of the same culture, and the food bacteria were diluted 10-fold. The ciliates were allowed to adapt to the new medium for at least 2 h (Fig. 1A). Then, different amounts of bacteria were added to each bottle. In the case of heterotrophic bacteria, a ratio of 10 FLB to 90 NB was used (nonselectivity between FLB and NB, Fig. 1C), giving final concentrations of between 1×10^6 and 3×10^8 bacteria ml^{-1}. At this ratio, there were fewer ingested FLB to be counted and better dispersion within the food vacuoles. In the case of FLC, only the fluorescent particles were added, giving final concentrations of between 1×10^5 and 2×10^7 FLC ml^{-1}.

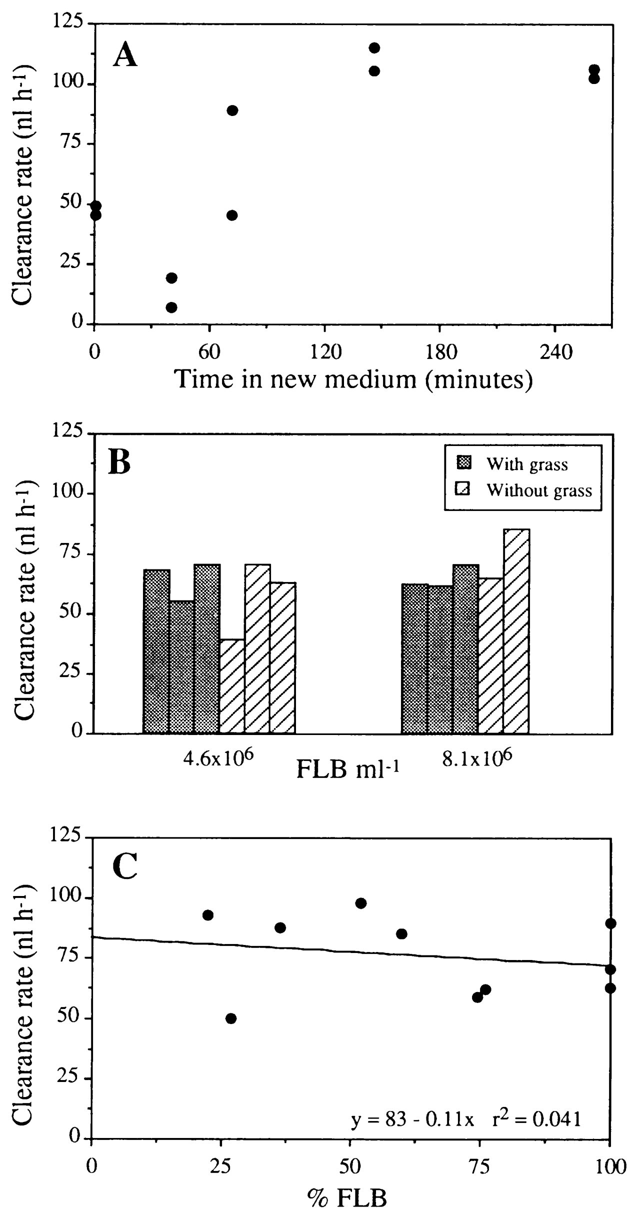

FIG. 1. (A) Clearance rates for *P. nasuta* determined when the ciliates were left for different times in fresh medium before starting the experiment. The FLB concentration was 4×10^6 ml^{-1}. (B) Clearance rates for *P. nasuta* determined in culture medium with and without dried grass. (C) Clearance rates for *P. nasuta* determined with different percentages of FLB and NB particles (FLB plus NB, 8×10^6 bacteria ml^{-1}).

The relationship between uptake rate and bacterial abundance was assessed, through nonlinear regression analysis, with the disc equation: $U = U_m \times [N/(N + k)]$, where U_m is the maximal uptake rate, and k is the half-saturation constant for ingestion (the bacterial abundance at which the uptake rate is half of the maximal rate). The maximal clearance rate (F_m) was

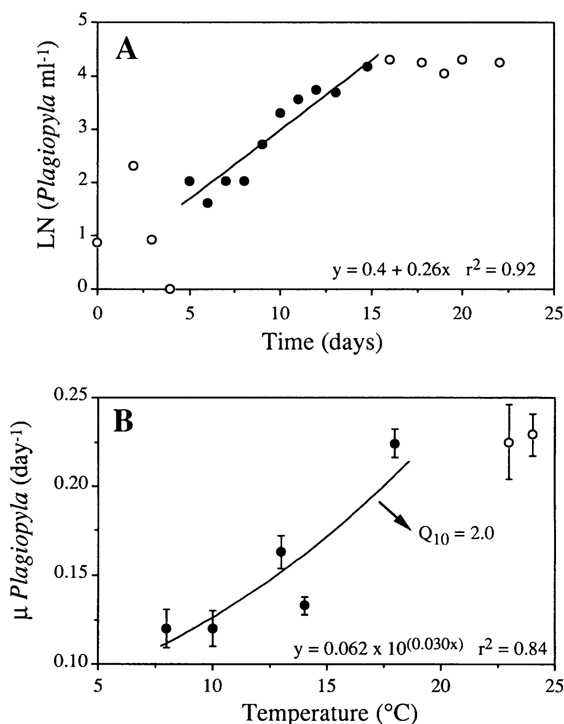

FIG. 2. (A) Growth curve for *P. nasuta* at 18°C. The slope of the linear regression between ciliate abundance (number of *Plagiopyla* ciliates ml^{-1} in natural logarithms [LN]) and time (only data represented by the solid circles were used) gives the growth rate directly: 0.26 day^{-1}. (B) Growth of *P. nasuta* versus temperature. The points represent the means for several cultures, and the vertical bars give the SE. The data represented by the solid circles were fitted to an exponential curve giving a Q_{10} of 2.0.

calculated as $F_m = U_m/k$. In the case of *P. nasuta* feeding on FLC, the regression made directly with the disc equation gave biased results, with an unrealistically high maximal clearance rate, because of the high dispersion of points. Therefore, the uptake rate values found at low prey abundance ($n = 13$) were used to calculate the maximal clearance rate (F_m) through linear regression. k was then calculated through nonlinear regression ($n = 31$) from a modification of the disc equation, in which U_m was substituted by the product of F_m and k.

RESULTS

Growth experiments. An example of a growth curve for *P. nasuta* shows the lag, exponential, and stationary phases (Fig. 2A). The linear regression was carried out with only the points corresponding to the exponential phase (solid circles), and the slope of this regression gave a growth rate of 0.26 day^{-1} (SE, 0.03). The determination of ciliate numbers at low abundance is rather imprecise, which causes a high dispersion of points in the lag phase. Nevertheless, the fit of the regression ($r^2 = 0.92$) was quite good.

The growth rate of *P. nasuta* increased with temperature: 0.12 day^{-1} at 8 and 10°C, 0.15 day^{-1} at 13 and 14°C, and 0.22 day^{-1} at 18, 23, and 24°C (Fig. 2B). The growth rate was highest at 18°C and did not seem to increase at higher temperatures. Figure 2B also shows the exponential regression made with the data for the range from 8 to 18°C (solid circles). The Q_{10} calculated for that interval was 2.0. Although the

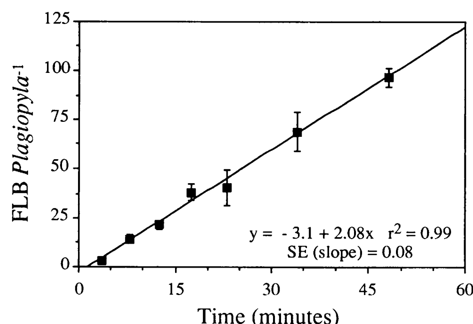

FIG. 3. Time course of *P. nasuta* feeding on FLB (10^6 FLB ml^{-1}). Incorporation was linear over the time interval observed (45 min). Vertical bars indicate SE.

growth rate for *M. es* was not estimated as carefully, it seemed to be in the same range as that of *P. nasuta* (data not shown).

Feeding experiments. (i) Time course. In a preliminary experiment, we monitored the incorporation of FLB by *P. nasuta* over time. We added 10^6 FLB ml^{-1} to a well-grown culture, and aliquots were taken at short time intervals. The mean number of ingested particles per ciliate was plotted versus time (Fig. 3). Uptake was linear throughout the time period tested (45 min). The clearance rate calculated from this experiment was 125 nl $ciliate^{-1}$ h^{-1} (SE, 5).

(ii) Functional response. The functional response of *P. nasuta* feeding on FLB (Fig. 4A) and on FLC (Fig. 4B) and the functional response of *M. es* feeding on FLB (Fig. 5) were

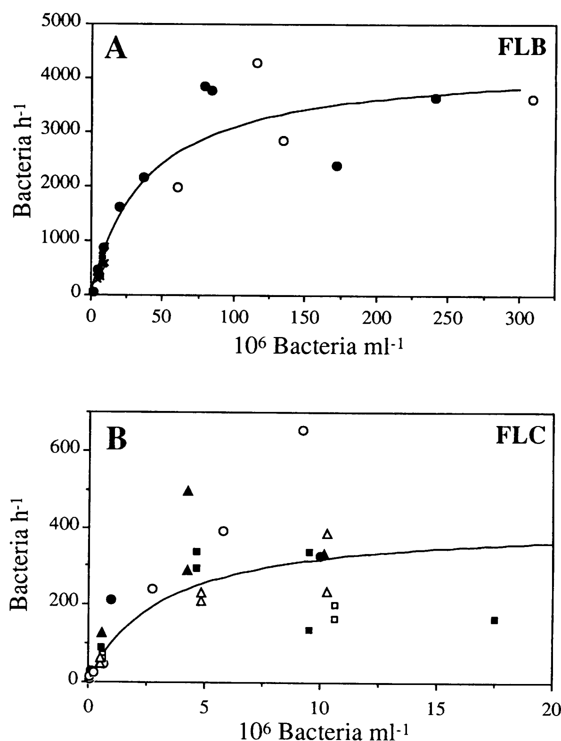

FIG. 4. Functional response of *P. nasuta* feeding on FLB (A) and on FLC (B). Different symbols represent different experimental sets. The curve plotted was obtained from the parameters U_m and k shown in Table 1.

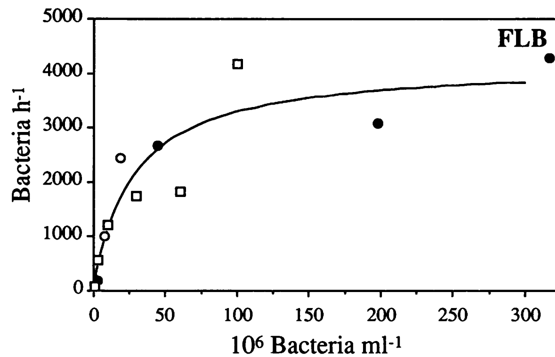

FIG. 5. Functional response of *M. es* feeding on FLB. Different symbols represent different experimental sets. The curve plotted was obtained from the parameters U_m and k shown in Table 1.

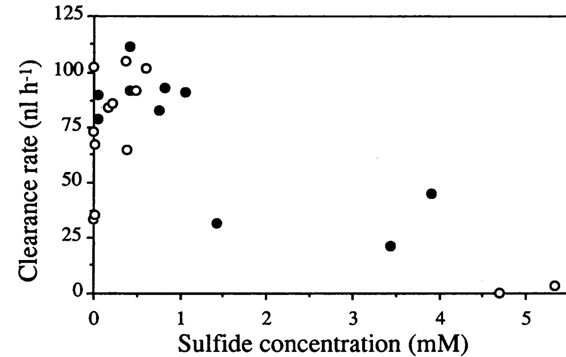

FIG. 6. Effect of sulfide concentration on clearance rate of *P. nasuta*. Open and solid circles represent two different experimental sets. Experiments were performed at 6×10^6 FLB ml^{-1}.

determined. These data were analyzed with the disc equation through a nonlinear regression to obtain the parameters U_m and k (Table 1). Figures 4 and 5 show the hyperbolic curves calculated from these parameters. The maximal uptake rate for *P. nasuta* was higher with FLB (4,295 FLB $ciliate^{-1}$ h^{-1}) than with FLC (418 FLC $ciliate^{-1}$ h^{-1}) (Fig. 4 and Table 1). When these data are converted to biovolumes, however, they are not significantly different (1,804 and 1,756 μm^3 $ciliate^{-1}$ h^{-1} for FLB and FLC, respectively). The same trend was observed in the half-saturation constant: higher values were found with FLB (3.95×10^7 FLB ml^{-1}) than with FLC (3.22×10^6 FLC ml^{-1}) but the values were similar when expressed in terms of biovolume (1.66×10^7 and 1.37×10^7 μm^3 ml^{-1} with FLB and FLC, respectively). Obviously, the maximal clearance rate was also similar in both cases: 109 and 130 nl $ciliate^{-1}$ h^{-1} for FLB and FLC, respectively. This means that *P. nasuta* exhibits the same functional response in terms of biovolume for particles as different in size as FLB and FLC. Both particles were ingested with the same efficiency, and both probably fell within the spectrum of optimal prey sizes.

The functional response of *M. es* feeding on FLB (Fig. 5 and Table 1) was similar to that of *P. nasuta*, with a maximal ingestion rate of 4,181 FLB $ciliate^{-1}$ h^{-1} and a slightly lower half-saturation constant, 2.74×10^7 FLB ml^{-1}. The maximal clearance rate was somewhat higher, 153 nl $ciliate^{-1}$ h^{-1}.

Effect of sulfide on feeding rates. The clearance rates for *P. nasuta* were determined in bottles with different sulfide concentrations (Fig. 6). No apparent differences in clearance rates were observed for a range of sulfide concentrations of between 0 and 1 mM, with values comparable to those found in previous experiments (mean, 83 nl $ciliate^{-1}$ h^{-1}; SE, 5). At sulfide concentrations above 1 mM, clearance rates decreased, and at sulfide concentrations above 4.5 mM, all feeding ceased.

DISCUSSION

Growth rate of anaerobic ciliates. Growth experiments were carried out at bacterial concentrations of 10^8 bacteria ml^{-1}. Since these concentrations are only 2.5 times the k value (4×10^7 bacteria ml^{-1}), the possibility of obtaining growth rates lower than the maximal possible rate existed. In order to evaluate this, we calculated the maximal growth rate, μ_m, from the equation $\mu_m = \mu \times [(N + k)/N]$, where μ was the experimentally found growth rate (0.22 day^{-1}), N was 10^8 bacteria ml^{-1} (assuming constant abundance through the experiment), and k was assumed to be the same half-saturation constant as for feeding, as suggested by Fenchel (10). The resultant μ_m was 0.30 day^{-1}, indicating that our experiments gave results very close to the maximal growth rate.

The highest growth rate obtained for *P. nasuta* was 0.22 day^{-1} at 18°C. Growth rates of between 0.20 and 0.47 day^{-1} have been reported for *Plagiopyla* and *Metopus* species (11, 12, 17, 32, 34). These values are clearly very low compared with the rates found for similarly sized aerobic ciliates. Fenchel and Finlay (11) postulated a gross growth efficiency for anaerobic ciliates of 10% (one-fourth of the value found for aerobic ciliates) because of the lower efficiency of anaerobic metabolism. Then, assuming that aerobic and anaerobic organisms have the same feeding rates, the growth rate of anaerobic ciliates should be 25% of that of aerobic ones. Recently, Müller and Geller (24) published an empirical multiple regression relating the growth rate of aerobic ciliates to ciliate biovolume and temperature. The growth rate of *P. nasuta* at different temperatures, multiplied by 4.0, fits well in that regression: at 13°C, the predicted and determined growth rates are almost identical. Below this temperature, the growth rates predicted are lower and above it they are higher than the experimentally determined growth rates. Thus, the growth rate

TABLE 1. Functional response of *P. nasuta* and *M. es* feeding on FLB and FLC, determined by nonlinear regression analysis[a]

Ciliate	Prey particles	U_m	k (10^6/ml)	n	r^2	F_m
Plagiopyla nasuta	FLB	4,295 (317)	39.5 (8.0)	29	0.897	109
Metopus es	FLB	4,181 (536)	27.4 (11.6)	12	0.855	153
Plagiopyla nasuta[b]	FLC	418	3.22 (0.39)	31		130 (34)

[a] U_m, maximal uptake rate (particles per ciliate per hour); k, half-saturation constant (particles per milliliter); n, number of cases; r^2, corrected r squared; F_m, maximal clearance rate (nanoliters per ciliate per hour). SEs, when available, are shown in parentheses.

[b] We first calculated the maximal clearance rate from the linear region of the curve (F_m = 130; SE = 34; n = 13; r^2 = 0.575). We then calculated k by fitting the whole data set (n = 31) with a modification of the disc equation, in which U_m was substituted by ($F_m \times k$) (r^2 = 0.515).

of *P. nasuta* is typically 25% of the rate obtained for a similarly sized aerobic ciliate.

As expected, the growth rate of *P. nasuta* increased with temperature. The Q_{10} calculated was 2.0, similar to values found for algae (Q_{10} = 1.9 [5]), microflagellates (Q_{10} = 2.5 [2] or 2.7 [3]), and aerobic ciliates (Q_{10} = 2.3 to 3.5 [6]). Most studies have reported an increase in protozoan growth rate up to at least 25°C, with the exception of some psychrophilic species (3). The growth rate of *P. nasuta*, however, did not increase between 18 and 24°C. In addition, we observed that the cell yield (final ciliate concentration) was generally lower at 23 or 24°C (47 ciliates ml^{-1}, n = 10) than at 14 or 18°C (86 ciliates ml^{-1}, n = 37). This agrees with the observations of a growth optimum for *P. nasuta* of between 15 and 20°C (17). Thus, it seems that these ciliates have a temperature optimum at about 18°C and are not well adapted to live at warmer temperatures. In the field, *Plagiopyla* species are normally restricted to the coldest part of the ecological system, bottom layers and sediments. In Lake Cisó, for example, the epilimnion can reach temperatures of 24°C during summer stratification. However, the anaerobic ciliates are restricted to the metalimnion and hypolimnion, with temperatures never exceeding 18°C (23).

Functional response. The functional response has attracted considerable attention from theoretical ecologists, since it has many applications in models of prey-predator dynamics (31). Earlier, some authors realized that the uptake rate could not increase indefinitely with increasing prey abundance, and Holling (18) proposed the rectilinear, hyperbolic, and sigmoidal models to describe such a saturating relationship. Most of the experimental data sets obtained for planktonic phagotrophs have been fitted to the rectilinear or the hyperbolic model, the latter described by the disc equation (19), or by the Ivlev equation (20). Since none of the three has been proven to be statistically better than the others (26), we decided to use the disc equation because its two parameters, U_m and k, could be interpreted easily. In addition, on theoretical grounds, Fenchel (7) deduced this equation from two assumptions: the existence of a maximal clearing rate and a finite time for making a food vacuole.

The functional responses of *P. nasuta* determined with FLB and FLC gave similar results in terms of biovolume. The data shown here suggest that *P. nasuta* does not select between heterotrophic and phototrophic bacteria, and thus the two prey are fed upon as a function of their abundance. This is important, since anaerobic ciliates normally encounter both bacterial types in the field (23).

The maximal uptake rates found were 1,804 and 1,756 μm^3 $ciliate^{-1}$ h^{-1} for *P. nasuta* and *M. es*, respectively. These values corresponded to specific uptake rates of 3.3 and 2.9% of body volume h^{-1}, respectively, which are at the lower end of the values reported for aerobic ciliates (8). Combining the growth and feeding data, we can estimate the gross growth efficiency of anaerobic ciliates. Given the mentioned maximal uptake rates, the maximal growth rate of 0.22 day^{-1} determined experimentally for *P. nasuta* (and assuming the same for *M. es*), and the biovolume and carbon content of the ciliates and bacteria (see Materials and Methods), the gross growth efficiency would be 12 and 13% for *P. nasuta* and *M. es*, respectively. Although several assumptions are involved in the calculation, it is clear that the gross growth efficiency is effectively lower in anaerobic ciliates than in aerobic ones. The values found here are very close to the theoretical value of 10% proposed by Fenchel and Finlay (11, 12).

The half-saturation constant (k) gives an estimate of the food concentration at which the organisms are adapted to live.

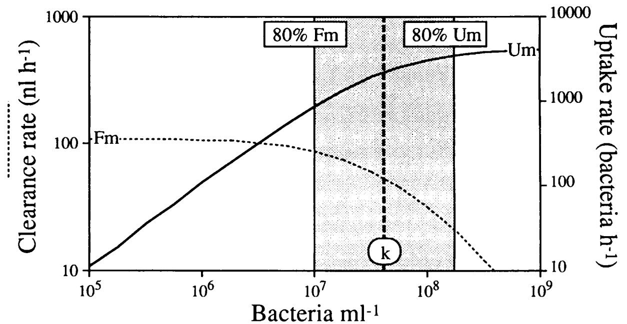

FIG. 7. Schematic representation of the functional response of *P. nasuta* feeding on FLB (U_m = 4,295 bacteria $ciliate^{-1}$ h^{-1}; $k = 4 \times 10^7$ bacteria ml^{-1}). The shaded region indicates bacterial abundances between the value corresponding to 80% of the maximal clearance rate and the value corresponding to 80% of the maximal uptake rate.

The values obtained in this study (ca. 2×10^7 μm^3 ml^{-1}) are similar to those found for other bacterivorous ciliates (8) and are seldom found in planktonic systems. The bacterial biomass of anoxic waters, however, is normally higher than that of oxic waters because of higher numbers and larger cells. For instance, in Lake Cisó, the peak of abundance of heterotrophic and phototrophic bacteria in anoxic layers reaches values close to k (23). Nevertheless, food limitation is likely to occur in planktonic anaerobic ciliates. On the other hand, the bacterial abundance in sediments is usually higher than in the plankton (28), with values well above k, and this suggests that anaerobic ciliates might be better adapted to the sediment. This is consistent with the fact that the highest abundances of anaerobic ciliates have been recorded to be in sediments (6, 14, 36, 37).

The maximal clearance rate gives an estimate of the efficiency with which organisms collect particles in a diluted environment. Keeping in mind the large error involved in the estimation of U_m and k (Table 1), the clearance rate for *M. es* (153 nl $ciliate^{-1}$ h^{-1}) is slightly higher than that of *P. nasuta* (109 nl $ciliate^{-1}$ h^{-1}), and this could indicate a greater adaptation of *M. es* to lower food levels. The specific clearance rates were 2.4×10^3 and 2.5×10^3 body volumes h^{-1} for *P. nasuta* and *M. es*, respectively, and both values fall within the range reported for aerobic ciliates (8).

The ecological usefulness of the functional response is in estimating the feeding rate of a predator in the field once the prey abundance is known. In such cases, we are assuming that prey abundance is the most important factor determining the uptake rate. We realize that other factors, such as temperature or oxygen concentration, can also affect feeding rates and that in situ determinations must be performed. The functional response, however, provides a good estimate of the values that can be expected in these experiments. In addition, predation impact can be assessed by combining the information on the functional response with in situ feeding rates (23).

The significance of the functional response for predator populations in the field is better understood from Fig. 7, which shows schematically the functional response curve for *P. nasuta* feeding on FLB (same as in Fig. 4A, but with the variables expressed logarithmically). A prey abundance range of between 10^5 and 10^9 bacteria ml^{-1} is shown, representing the range found in planktonic systems and bacterial cultures. The prey concentration at which the clearance rate is 80% of maximal (10^7 bacteria ml^{-1}) and at which the uptake rate is 80% of maximal (1.6×10^8 bacteria ml^{-1}) are also shown in

Fig. 7. The region between these two values (shaded region, with the center at $k = 4 \times 10^7$ bacteria ml^{-1}) delimits the range of prey abundance within which the determination of both the clearance and uptake rates requires use of the formula of the functional response. Below this region, the clearance rate is rather constant and maximal, and the uptake rate is given directly by the maximal clearance rate multiplied by the food concentration. Above this region, the clearance rate decreases proportionally to increases in prey abundance, and the uptake rate is rather constant and maximal. It is clear that we need the functional response in order to estimate the uptake rate for each field situation of prey abundance.

Effect of sulfide. Anaerobic ciliates are often found in environments with sulfide. This compound is extremely toxic to most eukaryotic organisms in nanomolar to micromolar concentrations (35), and we investigated whether it could also be toxic, above certain concentrations, to our cultured anaerobic ciliates. We found a decrease in feeding rates of *P. nasuta* at a concentration above 1 mM and total inhibition of feeding above 4.5 mM H_2S. *Metopus striatus* died at 2 mM H_2S (32), and *Metopus contortus* grew at 5 mM but not at 10 mM H_2S (34). Therefore, although anaerobic ciliates are mostly tolerant to sulfide, very high concentrations are toxic to them.

Conclusion. We studied the growth and feeding rates of anaerobic ciliates in laboratory cultures. Although their feeding rates were comparable to those determined for similarly sized aerobic ciliates, their growth rates were much lower, with a gross growth efficiency of around 10%. The ciliates need high bacterial abundances for growth, on the order of 5×10^7 bacteria ml^{-1}. Ciliates seem to be well adapted to live at temperatures of about 18°C and at sulfide concentrations of between 0 and 1 mM.

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