APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 1994, p. 1325–1334
0099-2240/94/$04.00+0

Vol. 60, No. 4

Role of Anaerobic Ciliates in Planktonic Food Webs: Abundance, Feeding, and Impact on Bacteria in the Field

RAMON MASSANA* AND CARLOS PEDRÓS-ALIÓ

Institut de Ciències del Mar, Consejo Superior de Investigaciones Científicas, Passeig Joan de Borbó s/n, E-08039 Barcelona, Spain

Received 4 August 1993/Accepted 19 January 1994

We studied the dynamics of two populations of anaerobic ciliates, *Plagiopyla* sp. and *Metopus* sp., and of their potential prey, heterotrophic and phototrophic purple bacteria, in Lake Cisó throughout a 1-year cycle. The abundance of both ciliates was very low (less than 2 individuals per ml). During mixing, *Plagiopyla* ciliates exhibited high clearance rates (about 100 nl ciliate^{-1} h^{-1}), its integrated abundance increased with a net doubling time of 47 days, and its potential doubling times, as calculated from the number of bacteria consumed, ranged between 5 and 8 days. During stratification, the activity of *Plagiopyla* ciliates was reduced and the population decreased; this was related to the higher amounts of sulfide present. The impact of predation by the *Plagiopyla* population on bacterioplankton was found to be insignificant, less than 0.1% of bacterial biomass consumed per day. Thus, anaerobic ciliates cannot control the bacterioplankton in Lake Cisó because of both the low abundance over the period studied and the low feeding rates during certain periods. A review of available field studies suggests that this conclusion can be extrapolated to most other anoxic systems.

Anaerobic ciliates are widely distributed in anoxic environments (9), but very little is known about their trophic role in anaerobic food webs. They are mainly bacterivorous, feeding on both heterotrophic and phototrophic bacteria (6), and often represent the main bacterial consumers in anoxic environments. Fenchel and Finlay (8) suggested that predation by anaerobic protozoa could control the bacterial abundance in some systems, as is frequently the case in aerobic communities (1). They also argued for short food chains in anoxic systems because of the low growth efficiency of anaerobic metabolism, involving essentially grazing of bacteria by protozoans. Studies on the trophic role of anaerobic ciliate populations in the plankton have seldom been carried out (12, 18). A complete and quantitative assessment of this trophic role requires the analysis of four parameters: abundance, in situ feeding activities, impact on natural bacterial assemblages, and potential growth rate derived from this trophic relationship.

Ciliates have been found to be present at moderate to low abundance in anoxic planktonic systems compared with the numbers in oxic systems with similar food levels (10, 12, 24). The abundance can be 10 times higher in sediments and sapropel than it is in the plankton (11, 41). The feeding rates of anaerobic ciliates are comparable to those for similarly sized aerobic ciliates. We demonstrated this point in a separate study (28), in which we described the functional response of *Plagiopyla nasuta* and *Metopus es* cultures to feeding on heterotrophic and phototrophic purple bacteria. A similarity in feeding activities between aerobic and anaerobic protozoa has already been suggested (8, 9). The feeding impact of ciliates on bacterioplankton, expressed as the percentage of the bacterial assemblage consumed per day, depends on the abundance of ciliates and their feeding activities and indicates whether the bacterioplankton is significantly grazed by ciliates. The importance of predation for the predator population, expressed as its potential growth rate calculated from the amount of food taken by predation, depends on prey abundance, predator feeding activities, and predator growth efficiency. The calculated potential growth rate indicates whether the predator can grow and persist by depending only on the studied trophic relationship.

We analyzed these four questions in the anaerobic community of Lake Cisó (31), a sulfurous lake which harbors anaerobic ciliates, mostly *Plagiopyla* sp. and *M. es*. First, we determined the vertical and temporal distribution of both ciliates and of their potential prey, heterotrophic and phototrophic purple bacteria. On several occasions, the clearance rate of *Plagiopyla* ciliates was determined in situ by using fluorescently labeled phototrophic bacteria. Total predation by the *Plagiopyla* population on bacterioplankton was calculated by applying an algorithm (27) which considers the vertical distributions of prey and predator and the functional response of the predator. The functional response of *P. nasuta* had been determined previously in the laboratory (28). Finally, the impact of this trophic relationship was estimated for both prey and predator. In addition, we analyze here some other systems under the same perspective and discuss the role of ciliates in anaerobic planktonic food webs.

MATERIALS AND METHODS

Lake Cisó. Lake Cisó is a small holomictic lake (maximal depth, about 7 m) located in the karstic region of Banyoles, Girona, Spain. The biology of the lake is largely influenced by the high amounts of sulfide present in the water (15, 17, 31). The water has a high sulfate content because water percolates through a gypsum-rich layer before entering the lake by seepage. Sulfate is reduced to sulfide by bacterial activities, and because of the bathimetry of the lake (high depth-surface ratio) and the protection from wind provided by the surrounding trees, sulfide diffuses throughout the water column during winter holomixis (October to March), and the whole lake becomes anoxic. Prokaryotes dominate (15), but some anaerobic ciliates are present (5, 14). During stratification (April to September), the epilimnion becomes oxic, and there is a small region in the metalimnion where oxygen and sulfide coexist.

* Corresponding author. Phone: 34-3-2216450. Fax: 34-3-2217340.

During this period, three different ciliate communities can be recognized: aerobic in the epilimnion, microaerophilic in the upper metalimnion (26), and anaerobic (similar to that found during holomixis) in the lower metalimnion and the hypolimnion (5, 15). The last community is the one analyzed in the present study.

Sampling and physicochemical parameters. Temperature was measured with a YSI-33 temperature conductivity probe (Yellow Springs Instruments). Water samples were taken in the region of maximal depth with a polyvinyl chloride cone connected through tubing to a pump (for a better description of the sampling device, see reference 29). The cone was lowered to the desired depth, and samples were pumped out from different depths. A 250-ml biological oxygen demand bottle was filled for oxygen determination with the Winkler titration as modified by Ingvorsen and Jørgensen (20). Screw-capped tubes (13-ml capacity) containing 1 ml of zinc acetate (37%, wt/vol) were filled for sulfide determination by the methylene blue colorimetric method (16). For counts of organisms, 125-ml samples were fixed with formaldehyde (2% final concentration) and stored at 4°C in the dark until processed 2 or 3 days later.

Counts of organisms. To count accurately all the relevant organisms for this study, three different preparations were made from each sample. Subsamples of 1 ml were stained with DAPI (4′,6-diamidino-2-phenylindole; 0.1 μg/ml final concentration) and filtered through a 0.2-μm black-stained Nuclepore filter (33). The filter was examined at 1,250× magnification with a Nikon epifluorescence microscope under UV radiation. The bacterioplankton was divided into three categories: two morphologically distinguishable phototrophic purple bacteria (*Chromatium* sp., probably *Chromatium minus*, and *Amoebobacter* sp., probably *Amoebobacter purpureus*) and the remaining bacteria, which included heterotrophic, chemolithoautotrophic, and phototrophic bacteria with typical bacterial morphologies (rods and cocci). These are referred to as "other bacteria" for convenience in this article. Heterotrophic flagellates were counted on the same filters as the bacterioplankton.

For small ciliates and algae, 15-ml subsamples were allowed to settle for 2 h in a chamber (25 mm in diameter and 20 mm high), and the organisms were counted at 100× or 400× magnification by phase contrast under a Nikon inverted microscope. Finally, 100-ml subsamples were filtered through a 37-μm nylon net to remove the bacteria and algae from the sample. The organisms retained were resuspended in 15 ml of filtered water, allowed to settle, and observed as above. Large ciliates and copepods were counted in these subsamples by examining the entire preparation at 40× or 100× magnification. We demonstrated that the 37-μm mesh net was the largest which would retain all *Metopus* and *Plagiopyla* individuals (Fig. 1A). The classification of anaerobic ciliates follows the descriptions of Kahl (22), Jankowski (21), and Bick (3).

Integrated abundance. On every sampling date, samples were taken from n depths ($z_1, \ldots, z_i, \ldots, z_n$; centimeters) and the abundance of population N ($N_1, \ldots, N_i, \ldots, N_n$; cells per milliliter) was determined at each depth. We chose the sampling depths that best described the vertical heterogeneity of the system. We always considered a water column between 0 and 500 cm in depth. When the limits of this interval (0 or 500 cm) were not sampled, they were assumed to have the same concentration as the closest depth. The integrated abundance, N_I (cells per square centimeter), was determined by using the following equation:

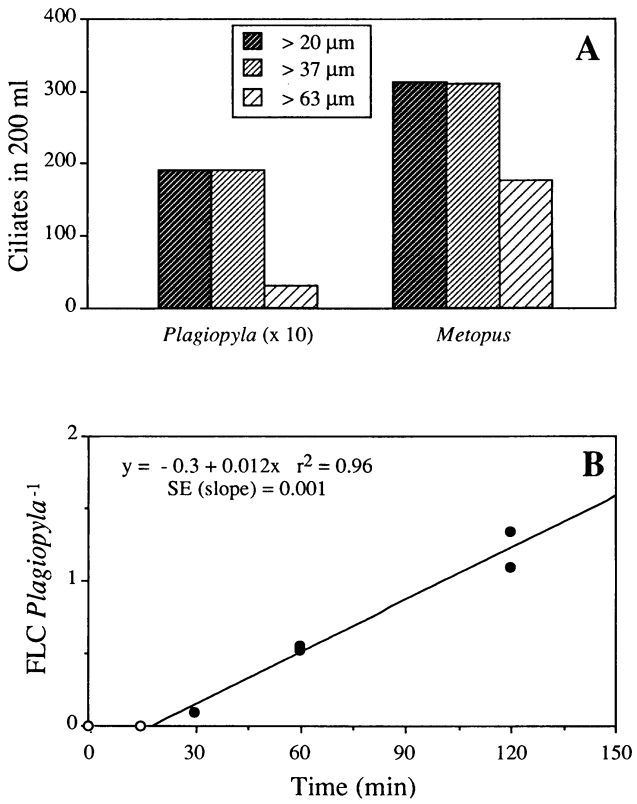

FIG. 1. (A) Number of *Plagiopyla* and *Metopus* ciliates retained by nylon mesh with pore sizes of 20, 37, and 63 μm. Samples were obtained on 14 March 1991 at a 2.0-m depth (formaldehyde-fixed sample). (B) Time course of *Plagiopyla* ciliates feeding on FLC in situ. The experiment was done on 19 February 1991 at a 2.0-m depth. Ciliates started to feed after 30 min of incubation. Only the data represented by the solid circles were used in the regression analysis.

$$N_I = \sum_{i=1}^{i=n-1} \left(\frac{N_i + N_{i+1}}{2} (z_{i+1} - z_i) \right) \quad (1)$$

Biovolume and biomass estimations. Phototrophic purple bacteria were sized directly in DAPI-stained preparations by epifluorescence microscopy, and other bacteria were measured by examining scanning electron microscopy (SEM) pictures. Sizes were converted to biovolumes by assuming that the bacteria were cylinders with hemispherical caps. Because of the shrinkage of bacteria in SEM preparations, a correction factor of 2.3 (30) was applied to correct the biovolume values for other bacteria. Ciliates were sized under an inverted microscope, but a large error might be introduced by assuming a certain geometrical shape for these flattened cells. Their biovolume was deduced from the biovolume of similar ciliates in cultures, carefully estimated by measuring the length and the width independently of the thickness (28). For *Plagiopyla* sp. in Lake Cisó, the biovolume of *P. nasuta* in culture was assumed. For *Metopus* sp. in Lake Cisó, the biovolume of *M. es* in culture was modified accordingly because the cultured cells were slightly smaller. Biovolumes were converted to biomass by using conversion factors from the literature: 0.35 pg of C per μm^3 for bacteria (4) and 0.14 pg of C per μm^3 for ciliates (35). Table 1 summarizes the size, biovolume, and biomass findings for the organisms included in this study.

TABLE 1. Mean size, biovolume, and biomass of predator and prey microorganisms

Organism	Size (μm)	Biovolume (μm³)	Biomass (pg of C)	k^a (cells/ml)
Predators				
Plagiopyla sp.	99 × 45	54,700	7,660	NA
Metopus es	155 × 56	127,000	17,780	NA
Prey				
Chromatium sp.	5.3 × 3.3	38.5	13.5	3.6×10^5
Amoebobacter sp.	2.0 × 2.0[b]	4.2	1.47	3.3×10^6
Other bacteria	1.8 × 0.7	0.58[c]	0.20	2.4×10^7

[a] Half-saturation constants for ingestion (k) of *Plagiopyla* sp. feeding on these prey cells, taken from reference 28. NA, not applicable.
[b] Sizes shown correspond to individual cells. These bacteria, however, form aggregates of 11.6 cells on average (32).
[c] Mean bacterial biovolume of several anoxic samples.

TABLE 2. Functional response of *Plagiopyla* sp. for several sampling dates in 1991[a]

Date	F (nl h⁻¹)	V (μm³ ml⁻¹)	F_m (nl h⁻¹)	Maximal uptake (cells h⁻¹)		
				U_m-Chr	U_m-Amo	U_m-Bac
19 February	75	9.6	127	45.2	417	3,007
14 March	83	8.8	136	47.2	435	3,136
4 April	107	10.7	191	67.7	624	4,503
11 June	13	11.7	25	8.7	80	581
20 September	10	16.1	22	7.7	71	512

[a] F, in situ-determined clearance rate; V, total food abundance in the experimental bottle (the three bacterial groups plus FLC); F_m, maximal clearance rate, calculated from equation 4; U_m-Chr, U_m-Amo, and U_m-Bac, maximal uptake rates for *Chromatium* sp., *Amoebobacter* sp., and other bacteria, respectively. The values of k appear in Table 1 and are assumed to be the same for all dates.

Ingestion experiments. In situ clearance rates of anaerobic ciliates were determined in experiments in which the ingestion of fluorescently labeled *Chromatium* cells (FLC) was monitored over time. The FLC suspension was obtained by heat-killing and staining a culture of *Chromatium vinosum* (3.2 by 1.4 μm; biovolume, 4.2 μm³), as described by Sherr et al. (37). In each experiment, seven 250-ml screw-capped bottles were gassed with nitrogen and filled completely (to preserve anoxic conditions during incubations) with water pumped from the experimental depth. A small amount of tracer was added to obtain concentrations of 1×10^4 to 6×10^4 FLC ml⁻¹. One bottle (time zero) was immediately fixed with formaldehyde (2% final concentration). The other six bottles were incubated in the dark in an ice box with water from the same depth as the samples to preserve the in situ temperature. At different times (typically 15, 30, and 60 min), the contents of two bottles (replicates) were fixed with formaldehyde.

In the laboratory, 100-ml subsamples were allowed to settle as described above, and the ciliates were observed with a Nikon inverted epifluorescence microscope. Predators were located by phase contrast at low magnification (40× or 100×), and the number of particles inside the predator was determined with blue-light epifluorescence at higher magnification (400×). Between 15 and 20 cells were examined for each time point, and the average number of particles ingested was plotted versus time (Fig. 1B). The slope of the straight line obtained was the ingestion rate, I (FLC ingested per ciliate per minute). A lag was often observed during which the ciliates did not feed, probably because of the stress of sampling and manipulation. In these cases, only the points in which the incorporation was linear were used (solid circles in Fig. 1B). The clearance rate, F (nanoliters per ciliate per hour), was obtained according to the formula $F = (I/L) \times 6 \times 10^7$, where L was the tracer concentration (FLC per milliliter) in the experimental bottle at time zero and 6×10^7 was the unit conversion factor.

Integrated predation. Populations are sharply stratified in Lake Cisó, and predator and prey are usually slightly segregated with respect to depth. Predation impact should thus be determined for the whole population, considering areal integrated numbers. We have proposed an algorithm to calculate integrated predation, G_I (prey eaten per square centimeter per hour), from the distribution of prey and predator populations and the functional response of the predator (27). The functional response was described by the disc equation (19), analogous to the Michaelis-Menten model for enzyme kinetics, and defined by two parameters: the maximal uptake rate, U_m (prey eaten per ciliate per hour), and the half-saturation constant for ingestion, k (prey per milliliter). We developed an integral equation which incorporated U_m, k, and the changes in prey and predator abundance with depth. With the algorithm FS-B (27), this integral was solved for intervals limited by sampling depths. Integrated predation in the first interval [$G_{I(1,2)}$], between depths z_1 and z_2, is calculated as:

$$G_{I(1,2)} = U_m\left(\frac{N_1 + N_2}{N_1 + N_2 + 2k}\right)\left(\frac{P_1 + P_2}{2}\right)(z_2 - z_1) \quad (2)$$

where N_1 and P_1 are the abundances of prey and predator, respectively, at z_1 (cells per milliliter) and N_2 and P_2 are the abundances of prey and predator, respectively, at z_2. Integrated predation in the whole water column is calculated by adding up the results in each interval:

$$G_I = \sum_{i=1}^{i=n-1} [G_{I(i,i+1)}] \quad (3)$$

To use this algorithm with our field data, we first needed to determine U_m and k for each sampling date from the in situ-determined clearance rates. For dates when in situ experiments were not conducted, we used the average clearance rate between the preceding and following dates. In laboratory experiments, we determined the functional response of *P. nasuta* ciliates feeding on FLC (28). The half-saturation constant found in the laboratory (1.37×10^7 μm³ ml⁻¹) is considered to be the same for all sampling dates and is equivalent to 3.6×10^5 *Chromatium* cells ml⁻¹, 3.3×10^6 *Amoebobacter* cells ml⁻¹, and 2.4×10^7 other bacteria ml⁻¹ (Table 1). From the in situ-determined clearance rates (F), this constant value of k, and the total food present in the experimental bottle, V (cubic micrometers per milliliter), we can calculate the maximal clearance rate (F_m) according to a modification of the disc equation:

$$F_m = F\frac{V + k}{k} \quad (4)$$

Finally, U_m is calculated for each date by multiplying k times this maximal clearance rate: $U_m = k \times F_m$. The results of these calculations for the five dates when the clearance rates were determined are shown in Table 2.

Impact studies. By using the calculated value for integrated predation, the impact of predation for both prey and predator

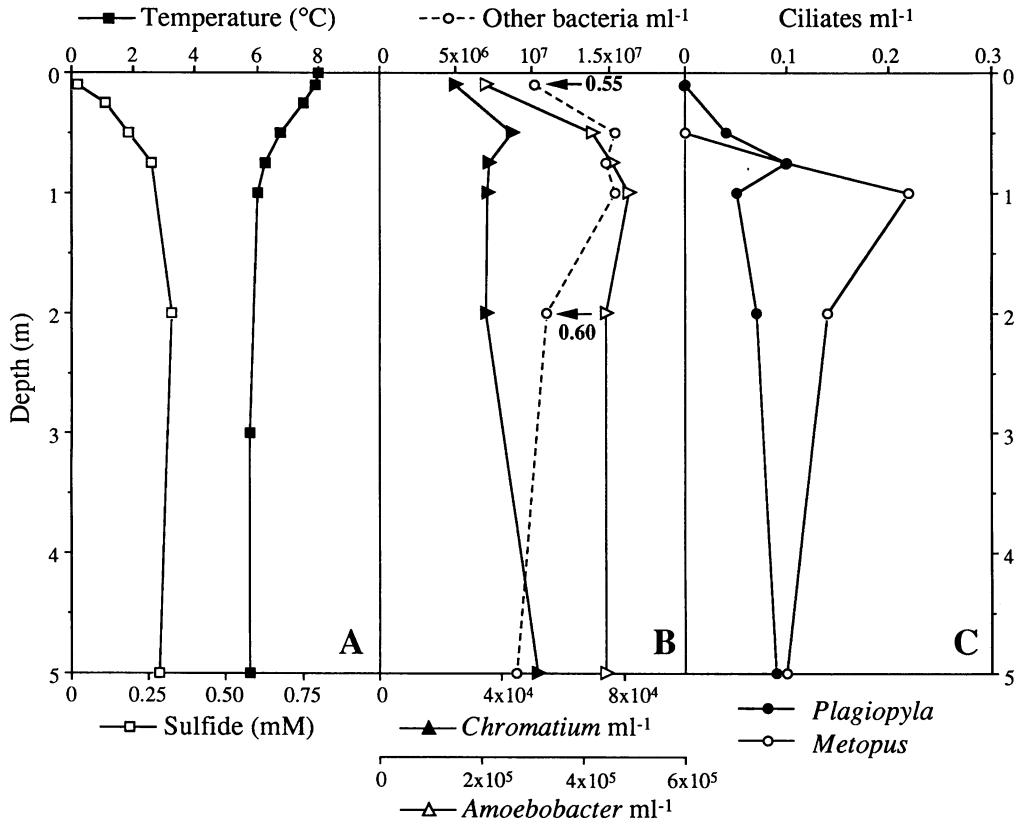

FIG. 2. Vertical distribution of several elements in Lake Cisó during holomixis (19 February 1991). (A) Temperature and sulfide concentration; (B) prey species concentrations. The mean biovolume of other bacteria (at the depths indicated by arrows) is also shown (in cubic micrometers). (C) *Plagiopyla* and *Metopus* concentrations.

populations was estimated for each date. The impact on the bacterial assemblages could be expressed as the percentage of the bacterial biomass eaten per day: $[G_I \times 24)/N_I] \times 100$, where G_I (prey eaten per square centimeter per hour) is the integrated predation and N_I (prey per square centimeter) is the integrated abundance of bacteria.

The impact of predation for the ciliate population could be estimated as its potential growth rate, μ (per hour), considering this trophic step as the only one providing food to the predator:

$$\mu = \ln\left(1 + \frac{G_I}{P_I}\frac{B_N}{B_P}\frac{C_N}{C_P}0.1\right) \quad (5)$$

where G_I is the integrated predation, P_I is the integrated abundance of the ciliate (cells per square centimeter), B_N and B_P are the bacterial and ciliate individual biovolumes, respectively (cubic micrometers), C_N and C_P are the carbon content of the bacterial and ciliate biovolume, respectively (0.35 and 0.14 pg of C per μm^3, respectively), and 0.1 is the gross growth efficiency for anaerobic ciliates (28). Potential doubling times of the ciliate populations, D_t (days), were calculated as follows: $D_t = [\ln 2/(\mu \times 24)]$.

RESULTS

Vertical distribution of organisms. The vertical distribution of several parameters for a typical holomixis date (February 19, 1991) presented a slightly superficial stratification from 0 to 0.75 m (Fig. 2A), with a daily shallow thermocline which disappeared during the night (13). All the elements studied were distributed homogeneously below this thermocline, with sulfide concentrations of about 0.3 mM (Fig. 2A). The lake did not have aerobic organisms and flagellates were below detectable levels (<200 cells ml^{-1}), but it had abundant populations of the purple phototrophic bacteria *Chromatium* and *Amoebobacter* sp. as well as other bacteria (Fig. 2B). The mean biovolume of other bacteria, determined at two depths, seemed to be relatively constant throughout the water column, and it was larger than the biovolume of typical aerobic bacteria, as has been described before for anaerobic bacterioplankton (4a, 34). *Plagiopyla* and *Metopus* ciliates were present in low numbers (between 0.1 and 0.2 ml^{-1}) throughout the water column except for the superficial region (Fig. 2C). A slight maximum could be seen just below the thermocline. Other anaerobic ciliates smaller than 37 μm (and thus lost during filtration) were not detected in the unfiltered 15-ml sample (detection level of 0.2 to 2 cells ml^{-1}). This potentially low abundance and their lower biovolume than that of *Plagiopyla* sp. indicated that, if they had been present, they would not have played any role in the anaerobic food web.

On a typical stratification sampling date (June 11, 1991), a sharp thermocline was established from 18°C at 0.75 m to 12°C at 1.75 m (Fig. 3A). In this region, gradients of oxygen and sulfide formed, and different populations stratified. In the upper metalimnion, there was a microaerophilic community (Fig. 3D). The main populations on this date were the alga *Cryptomonas phaseolus* (peak of abundance, 5×10^4 cells

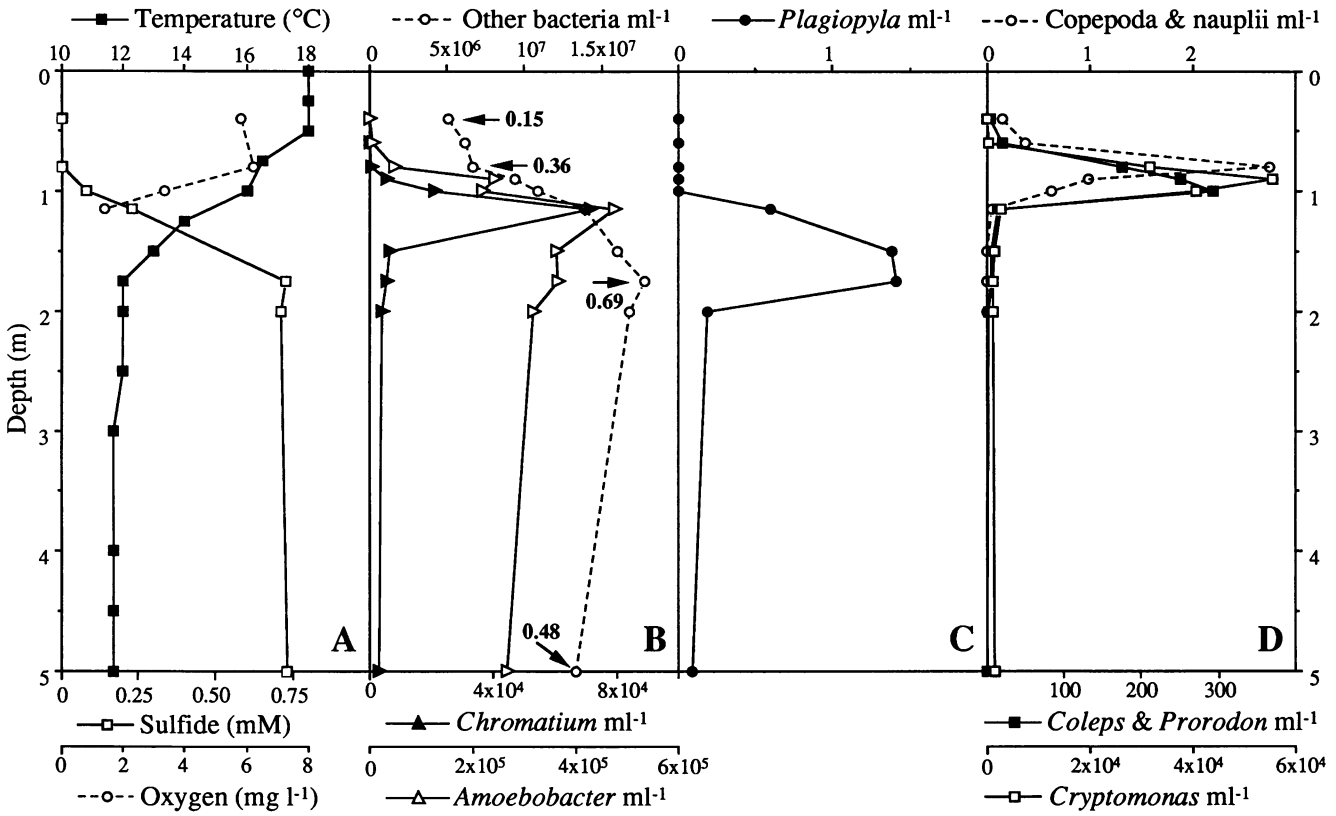

FIG. 3. Vertical distribution of several elements in Lake Cisó during stratification (11 June 1991). See the legend to Fig. 2 for details.

ml^{-1}) and the ciliates *Coleps* sp. and *Prorodon* sp. (up to 300 cells ml^{-1}). The only copepod (*Thermocyclops dybowskii*) and its nauplii also accumulated in the upper metalimnion (Fig. 3D). In the lower metalimnion, with sulfide present, purple phototrophic bacteria appeared (Fig. 3B): *Chromatium* cells formed a sharp peak of abundance in that region, whereas *Amoebobacter* cells were distributed quite homogeneously throughout the hypolimnion. Other bacteria were present throughout the water column, with higher numbers in the meta- and hypolimnion than in the epilimnion. The accumulation of biomass at the meta- and hypolimnion was even larger because the anaerobic bacteria were bigger than the aerobic ones (Fig. 3B). Finally, *Metopus* ciliates were absent from and *Plagiopyla* ciliates accumulated in the lower metalimnion, under the *Chromatium* peak, with maximal abundances 10 times higher than those found on winter sampling dates (Fig. 3C).

Population changes with time. Figure 4 shows the vertical distribution for populations (lower panels) and their integrated abundance (upper panels) over the year. *Plagiopyla* ciliates were homogeneously distributed in low numbers during February and March (Fig. 4A). During the establishment of stratification, the population grew and accumulated in the lower metalimnion, below the *Chromatium* peak. After July, the population decreased and the peak disappeared. *Metopus* ciliates had a distribution similar to that of *Plagiopyla* ciliates at the beginning of the year, but it disappeared at the beginning of stratification (Fig. 4B).

The *Chromatium* population was distributed homogeneously throughout the water column at the beginning of the year (Fig. 4C). The numbers of this bacterium clearly decreased from February to March. At the beginning of stratification (May to July), the population grew and accumulated in the metalimnion, forming a sharp peak (about 10^6 cells ml^{-1}), which remained until the stratification disappeared in October. The integrated abundance reached in July remained constant for the rest of the year. The *Amoebobacter* population was distributed homogeneously throughout the water column (about 10^5 cells ml^{-1}) all year except in the epilimnion during stratification (Fig. 4D). Its integrated abundance remained essentially constant over the year. Finally, other bacteria were homogeneously distributed during holomixis and tended to accumulate in the hypolimnion during stratification (Fig. 4E). Their integrated abundance remained relatively constant throughout the year.

Particle ingestion experiments. The clearance rate of *Metopus* ciliates was determined on only one date (32 nl ciliate^{-1} h^{-1} on February 19) because the low abundances found on the remaining dates made further measurements impossible. Clearance rates for *Plagiopyla* ciliates were determined on five dates and showed two different periods (Fig. 5). During the mixed period (February to April), the values were moderately high (about 100 nl ciliate^{-1} h^{-1}) and coincided with the net growth of the population (Fig. 4A). During the second period (summer stratification, June to September), the clearance rates were low (10 to 20 nl ciliate^{-1} h^{-1}) and coincided with the decrease in the population (Fig. 4A). On one date, we studied the possible effects of day-night cycles on the feeding activities of *Plagiopyla* ciliates. The clearance rates determined for day and for night were found not to be significantly different (Fig. 5).

Impact of predation. We determined the impact of preda-

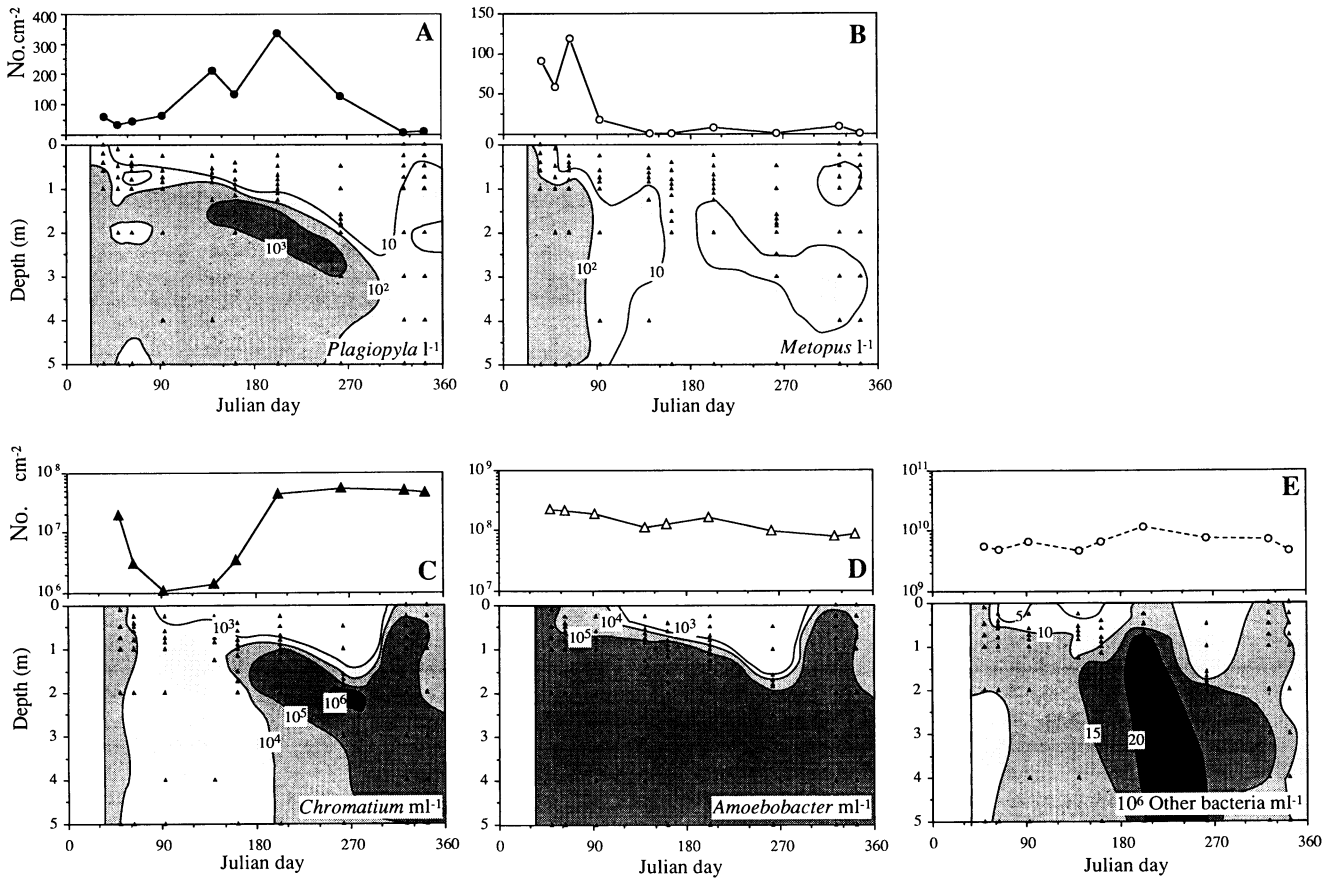

FIG. 4. Changes with depth and time of several populations (bottom panels) and evolution with time of their integrated abundance (upper panels) in Lake Cisó. The horizontal scale is the Julian day, beginning 1 January 1991. Different scales (either linear or logarithmic) were chosen for convenience. Solid triangles in the lower panels indicate sampling depths. The upper part of each panel shows the number of the indicated population per square centimeter; values shown inside differently shaded areas in the lower part of each panel show the concentrations of the indicated population per liter (predators) or per milliliter (prey).

tion by the *Plagiopyla* population (*Metopus* ciliates were almost absent during most of the period studied) on the bacterioplankton. From the clearance rates determined and the functional response found in the laboratory (28), we found the U_m and k for each date (Table 2). These values were then used, together with the vertical profiles of prey and predator abundance, to calculate integrated predation from equations 2 and 3. The impact of *Plagiopyla* ciliates on bacterial assemblages, as the percentage of bacterial standing stock consumed per day, was always extremely small: the highest value never exceeded 0.1% day^{-1} for any of the three bacterial groups considered (Fig. 6A). The same calculations can be done using values only for the upper layer of phototrophic bacteria, which are presumably actively growing (17), during the stratification period. Even in this case, less than 0.1% of bacterial standing stock was consumed per day. This indicates that the bacterioplankton of Lake Cisó was not significantly grazed upon by anaerobic ciliates. In addition, other bacterial grazers, such as heterotrophic nanoflagellates and rotifers, must play no role in the anaerobic food web of Lake Cisó because of their very low abundances.

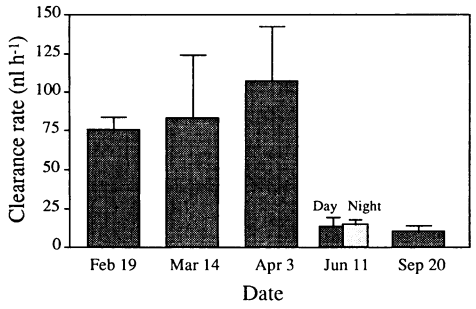

FIG. 5. Clearance rates determined in situ for the *Plagiopyla* population on different sampling dates with FLC as the tracer. Vertical bars indicate the standard errors.

The potential growth rates (from equation 5) and potential doubling times of the *Plagiopyla* population at each sampling date were also calculated (Fig. 6B). During holomixis, potential doubling times were shorter (range, 5 to 8 days) than they were later in the year (range, 25 to 38 days). This indicates a potential growth of the *Plagiopyla* population which is not reflected in the observed dynamics of the population in the field (Fig. 4A): the net doubling time derived directly from changes in the integrated abundance is 47 days at the beginning of the year and negative during stratification.

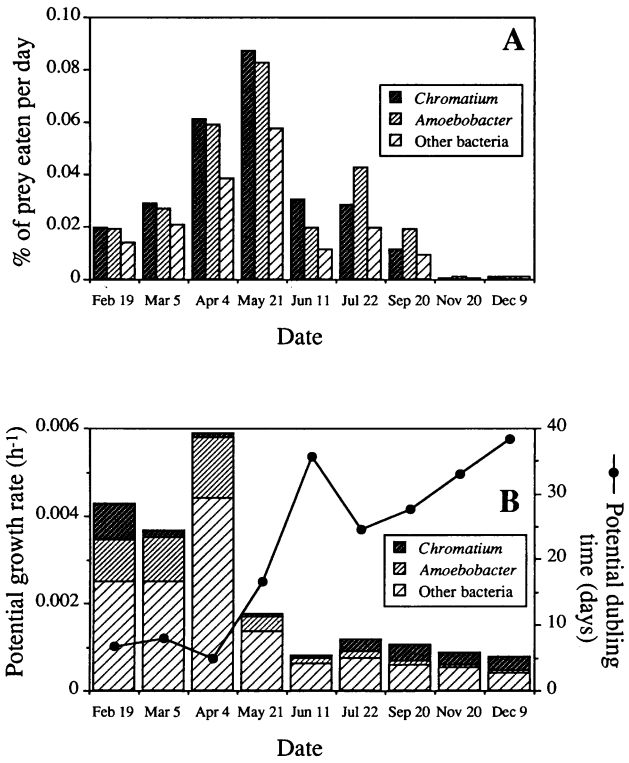

FIG. 6. (A) Predation impact of the *Plagiopyla* population feeding on bacterioplankton, expressed as the percentage of bacterial standing stock consumed per day, and considering each prey group separately. (B) Potential growth rates (histograms) and potential doubling times (●) determined for the *Plagiopyla* population feeding on natural bacterioplankton for each sampling date. The participation of each bacterial prey group to the growth rate is also shown by different shadings of the histograms.

We also calculated the contribution of each bacterial prey group to the growth rate of the *Plagiopyla* population (Fig. 6B). On all dates tested, other bacteria accounted for more than half of the diet (mean, 65%; maximum, 78% on May 21), whereas *Chromatium* sp. (mean, 18%; maximum, 38% on December 9) and *Amoebobacter* sp. (mean, 17%; maximum, 28% on March 5) represented a smaller portion.

DISCUSSION

Abundance of anaerobic ciliates. The presence of anaerobic ciliates in Lake Cisó has already been reported. Dyer et al. (5) detected "low abundances" of *Plagiopyla* and *Metopus* spp. in the sulfide-rich hypolimnion and grew both ciliates in enrichment cultures. Gasol et al. (14) found a group of anaerobic ciliates accumulating in the lower metalimnion during a summer day, with maximal abundances of between 1 and 3 ciliates per ml for *Plagiopyla* and *Metopus* spp. and an unidentified odontostomatid. Our work, however, is the first quantitative study of their vertical distribution and evolution during an annual cycle.

A few genera of anaerobic ciliates were identified in Lake Cisó during the studied period: *Plagiopyla* and *Metopus* were the most abundant, and individuals of the genera *Brachonella*, *Caenomorpha*, and *Lacrymaria* and an unidentified odontostomatid were found sporadically. *Plagiopyla* sp. was the most abundant ciliate throughout the year, and it was the only one which could potentially have a significant trophic role in the anaerobic food web. The *Plagiopyla* population presented two clearly different periods. During holomixis and the onset of stratification (from February to May), the population grew and accumulated gradually in the lower metalimnion (Fig. 4A). During this period, the integrated abundance increased (Fig. 4A), the clearance rates were highest (Fig. 5), and the potential doubling times were shortest (Fig. 6B). During the second period (from June to December), *Plagiopyla* abundance decreased, as did the clearance rates, while the doubling times were longer.

The maximal abundance recorded (1.5 *Plagiopyla* ciliates per ml) and the maximal integrated abundance (335 *Plagiopyla* ciliates per cm^2) seem to be low. This is surprising in such a eutrophic lake, which presents some of the highest abundances of other organisms recorded in natural, undisturbed water bodies (15). During the period studied, for example, we recorded up to 10^5 *C. phaseolus* cells, 10^3 *Coleps* sp. cells, and 10^3 *Anuraeopsis jissa* per ml. We may have missed an important part of the anaerobic ciliate community by sampling, fixing, or preserving ciliates in a nonoptimal way. Only ciliates smaller than 37 μm and less abundant than 0.2 to 2 cells per ml could have been missed in the present study. At such a cell size and abundance, their contribution to total ciliate biomass would have been minor. The fixative used here, formaldehyde, seemed to be adequate for Lake Cisó samples: the ciliates had the correct shape and their food vacuoles were intact. When live counts were compared with counts of formaldehyde-fixed samples (data not shown), we never detected significant differences. The filtration of fixed ciliates through a nylon net did not result in ciliate loss, as similar counts were obtained in filtered and unfiltered samples (data not shown). Finally, different members of our group have studied the biomass of all the organisms present in Lake Cisó (5, 14, 15). In all these cases, the concentrations of anaerobic ciliates have been similar to those reported here, and in no case have other small ciliates been found in significant numbers. Taking all this together, we are quite confident that our estimates of ciliate abundance are satisfactory.

We collected data from other karstic lakes in northeastern Spain during summer stratification and found that the abundance of anaerobic ciliates was very low in all of them (Table 3). The scarce data for other systems show abundances higher than (Arcas-2 and Priest Pot) or similar to (Esthwaite, Lake Rudushku, and Lake Okaro) those for Lake Cisó (Table 3). The highest abundances of anaerobic ciliates have been found in sediments: 50 ciliates per cm^3 in Priest Pot (11); 300 ciliates per cm^3 in Lake Cadagno (40); 900 ciliates per cm^3 in Esthwaite (24); and 650 ciliates per ml in the sapropel of a sludge backing pond (41). In some lakes, the populations colonize the sediment in winter, and when anoxia appears in the water during summer stratification, the whole population (18) or a part of it (24) migrates to the water column. In other lakes (Arcas-2 [12]), the clay sediments do not allow colonization by ciliates, and this is probably the case in Lake Cisó. Despite the similarity between Lake Cisó and Arcas-2 (both are thermally stratified karstic lakes with metalimnetic populations of *Cryptomonas* spp. and purple bacteria), both the species composition and abundance of ciliates were very different. The most abundant genera of ciliates in Arcas-2 (*Lagynus*, *Caenomorpha*, and *Lacrymaria*) were never important in Lake Cisó, whereas the genera found in Lake Cisó (*Plagiopyla* and *Metopus*) were found in low numbers in Arcas-2 (12). With respect to the vertical distribution, the ciliates accumulated near the sediment in Arcas-2, whereas in

TABLE 3. Abundance of planktonic anaerobic ciliates in different freshwater systems

System[a]	Abundance[b] (ciliates/ml)						Reference
	Plagiopyla	*Metopus*	*Caenomorpha*	*Lacrymaria*	Scuticociliates	Odontostomatids	
Cisó	1.6	1.5	++	+		+	This study
C-III		+	+++				This study
C-IV			++				This study
C-VI			+	+			This study
Vilar			++				This study
Estanya	++	+	++	+			This study
Arcas-2	*	*	19.2	8.3	*	3.6	12
Priest Pot			12.4		11.5		17a, 18
Esthwaite	6.6	1.1	0.4			*	17a, 24
Rudushku	0.3	1.4	0.5		2.0		23
Okaro			*	*	1.5		20a
Kulatá	*	*	*				39
Velká Karasí	11.0	*	*				39

[a] C-III, C-IV, and C-VI are three different basins of Lake Banyoles.
[b] Symbols: *, present but abundance not reported; +, 0.01 to 0.1; ++, 0.1 to 1; +++, 1 to 3.

Lake Cisó they accumulated in the lower metalimnion (14; this study).

In conclusion, despite the small number of planktonic systems for which the anaerobic ciliate community is known, the low abundance in the plankton of Lake Cisó is not very different from that in other systems and cannot be considered an exception.

Feeding rates. Clearance rates determined in situ in Lake Cisó for *Plagiopyla* ciliates ranged between 10 and 107 (mean, 51) nl ciliate^{-1} h^{-1} (Fig. 5). These values corresponded to maximal clearance rates (F_m), when correcting for in situ food abundance, of between 22 and 191 nl ciliate^{-1} h^{-1} (Table 2). Clearly, there were two periods in the feeding activities. In the first, corresponding to winter mixing, the maximal clearance rates were high, near the value found for *Plagiopyla* cultures (130 nl ciliate^{-1} h^{-1} [28]), and comparable to values for similarly sized bacterivorous aerobic ciliates (2, 7, 38). During the second period, corresponding to summer stratification, the maximal clearance rates dropped to only 18% (mean of the values of the two last dates) of the laboratory value. Since we are comparing maximal clearance rates, differences cannot be due to different food abundance. The growth rate of *Plagiopyla* ciliates in culture, and presumably also the feeding rate, increases with temperature (28). In our case, however, clearance rates determined in winter, at low temperatures (6 to 10°C), were higher than rates determined in summer, at warmer temperatures (13 to 18°C). Thus, temperature could not explain the lower activity during the second period. On the other hand, sulfide concentrations above 1 mM inhibit feeding rates (28). Clearance rates were determined at 0.3 to 0.5 mM H_2S in the first period and at 0.6 mM H_2S (in the metalimnion) during the second period. During the stratification period, however, the hypolimnetic sulfide concentration increased up to 1.5 mM H_2S, and the flux of sulfide to the metalimnion was probably significant. The higher concentration of sulfide in the lake could thus explain the lower feeding rates in the second period as well as the metalimnetic distribution of *Plagiopyla* sp., which were avoiding the sulfide-rich hypolimnion. In any case, our results show that feeding rates determined in the laboratory cannot be extrapolated to field situations in order to carry out calculations of feeding impact. Calculations that assume laboratory values for the in situ feeding will most likely overestimate the predatory impact.

Impact on bacteria. In the impact studies (Fig. 6), we were assuming that the sizes of the three bacterial groups studied fit into the range of optimal prey size for *Plagiopyla* sp. and that FLC was a good tracer for them. This assumption probably holds for other bacteria (0.58 μm^3), since laboratory experiments showed that both FLC (4.2 μm^3) and fluorescently labeled heterotrophic bacteria (0.42 μm^3) are optimal food particles (28). Although *Chromatium* sp. (38 μm^3) are much bigger than FLC and *Amoebobacter* sp. form aggregates (mean size, 48 μm^3), we also considered these two as prey of optimal size. In fact, both heterotrophic and phototrophic bacteria have been observed in the food vacuoles of *Plagiopyla* ciliates (6), and we also observed large amounts of *Amoebobacter* aggregates inside the vacuoles of *Plagiopyla* ciliates.

The predation impact of the *Plagiopyla* population on bacterioplankton of Lake Cisó was very low, never exceeding 0.1% of biomass consumed per day (Fig. 6A). Considering this maximal value (0.1% day^{-1}), a specific loss rate of 0.001 day^{-1} can be calculated, which is much smaller than the specific loss rates calculated for Lake Cisó for *Chromatium* sp. (0 to 0.025 day^{-1}) and *Amoebobacter* sp. (0 to 0.015 day^{-1}) (25). It seems, thus, that predation is an insignificant loss factor for phototrophic bacteria compared with other loss factors (sedimentation, decomposition, and washout). In principle, two reasons could explain the low predation impact on bacterioplankton: low feeding rates and low predator abundance. The in situ clearance rates were lower than the laboratory rates only during summer stratification. If we did the same calculations for this period with the maximal laboratory rates, the impact would be only slightly larger: 0.16, 0.24, and 0.11% day^{-1} for *Chromatium* sp. (June 6), *Amoebobacter* sp. (July 22), and other bacteria (July 22), respectively.

The other reason, low predator abundance, has two components: the real abundances of the predator and their overlap with the distribution of prey population. In our case, the overlap was always large and thus did not play a role. It could have some influence during stratification, when the *Plagiopyla* population accumulated just below the *Chromatium* peak (Fig. 3). Even in this case, however, if we made the calculations considering artificially complete overlapping of both populations, the impact on the *Chromatium* population would be only slightly higher, increasing from 0.030 to 0.035% day^{-1}. The other component is the low abundance of anaerobic ciliates in Lake Cisó. Fenchel and Finlay (8) found that the biomass ratio between predator and their prey in anoxic systems was 0.25 (0.15 in two Spanish karstic lakes, Arcas-2 and Laguna de la

Cruz), as opposed to a ratio of close to 1 for aerobic communities. In Lake Cisó, this ratio was 100 times lower (average, 0.0012; range, 0.0001 to 0.0039). Using a predator-to-prey ratio of 0.15 for Lake Cisó would represent an integrated *Plagiopyla* abundance of 12,000 ciliates per cm^2 on June 11 instead of the 136 ciliates per cm^2 found. Applying this maximal abundance value to the distribution shown in Fig. 3, we could obtain a new profile, with a metalimnetic peak of 125 ciliates per ml and hypolimnetic abundances of between 8 and 16 ciliates per ml. The impact on the bacterioplankton with such a potential profile would be 2.7, 1.7, and 1.0% day^{-1} for *Chromatium* sp., *Amoebobacter* sp., and other bacteria, respectively. In addition, if we use the feeding rates from the laboratory, these values would become 14.3, 9.2, and 5.3% day^{-1}, respectively, which are significant for the community food web. It is clear, therefore, that both factors, low feeding rates and low abundance, are responsible for the low predation impact on bacterioplankton.

We did the same impact calculations with the vertical profiles of purple bacteria (mainly *Chromatium* spp.) and anaerobic ciliates for Arcas-2 (12). We assumed that the bacterivorous ciliates (mainly *Caenomorpha* spp. and small odontostomatids) had the same functional response as *P. nasuta* in the laboratory (28). The *Chromatium* stock consumed in a day was 4.3%. Finlay et al. (12) calculated roughly the integrated predation on the *Chromatium* population to be 0.9×10^7 cells cm^{-2} h^{-1}, corresponding to 6% of the bacterial stock consumed per day. These values are similar to the maximal consumption rate hypothetically calculated for Lake Cisó but are still far from the 100% day^{-1} postulated for oxic systems (1). This indicates that the abundance of anaerobic bacterial assemblages, unlike that of aerobic ones, is generally not controlled by predation. This is consistent with the larger sizes and higher numbers of bacteria in anaerobic than in aerobic communities.

On the other hand, bacterial production could be controlled by grazing if the growth rate were also very low, comparable to the low grazing measured. The maximal doubling times of 11 days for *Chromatium* sp. and 19 days for *Amoebobacter* sp. calculated in reference 25 suggest that the consumption of 0.1% of biomass per day is insignificant. Thus, the anaerobic ciliates in Lake Cisó do not control bacterial production either. Therefore, such bacterial assemblages seem to be controlled by bottom-up mechanisms: they can apparently grow to their carrying capacity, and the large biomass attained is maintained by low growth rates and low predation losses.

Potential growth of the *Plagiopyla* population. The potential doubling times of *Plagiopyla* sp. in Lake Cisó, calculated from the feeding data, showed two periods (Fig. 6B). During mixing, the calculated doubling times (range, 5 to 8 days; mean, 6.5 days) were close to the shortest value found in the laboratory (3 days [28]). During stratification, the doubling times were much longer. The factors responsible for this could be the low feeding rates and the low prey abundance. The doubling times of the *Plagiopyla* population calculated by considering the feeding rates from the laboratory ranged from 4 to 10 days (mean, 6.2 days). Thus, it seems clear that the low doubling times during stratification were due to the low feeding rates, while there was enough food all year to maintain reasonable growth of the *Plagiopyla* population. This is confirmed by the high abundance in the field of each bacterial group, which can be similar to (or even higher than) the half-saturation constant.

During the mixing period, there was a clear discrepancy between the net doubling time calculated from changes in integrated abundance (47 days) and the potential doubling time calculated from feeding experiments (5 days). This discrepancy could be explained by an unknown but important loss factor for the *Plagiopyla* population or by the possibility that some of the considered food was in fact not ingested or not assimilated (36).

Conclusion. In Lake Cisó, neither the abundance nor the production of anaerobic bacterioplankton is controlled by ciliate grazing. This conclusion seems to be applicable to other systems as well. The reasons for this are the low feeding activities during certain periods and, mostly, the low ciliate abundance. Whereas the lower feeding rates in the field than in the laboratory can be explained by the increased concentration of sulfide as stratification develops, the low abundance of anaerobic ciliates, despite the sufficient food supply available, remains to be explained. The carbon flux between bacteria and protozoans is very low in the anaerobic food web of Lake Cisó.

ACKNOWLEDGMENTS

We thank Juan I. Calderón-Paz for help with sampling, Josep M. Gasol for suggestions and comments on the manuscript, and Maira Martínez and Isabel Esteve for kindly providing a *Chromatium vinosum* culture.

This work was supported by DGICYT grants PB87-0183 and PB91-075. R.M. held a fellowship from the Spanish Ministry of Education and Science.

REFERENCES

1. **Azam, F., T. Fenchel, J. G. Field, J. S. Gray, L. A. Meyer-Reil, and F. Thingstad.** 1983. The ecological role of water-column microbes in the sea. Mar. Ecol. Prog. Ser. **10:**257–263.
2. **Barcina, I., B. Ayo, A. Muela, L. Egea, and J. Iriberri.** 1991. Predation rates of flagellate and ciliate protozoa on bacterioplankton in a river. FEMS Microbiol. Ecol. **85:**141–150.
3. **Bick, H.** 1972. Ciliated protozoa. World Health Organization, Geneva.
4. **Bjørnsen, P. K.** 1986. Automatic determination of bacterioplankton biomass by image analysis. Appl. Environ. Microbiol. **51:**1199–1204.

4a. **Cole, J. J., M. L. Pace, N. F. Caraco, and G. Steinhart.** 1992. Bacterial biomass and cell size are greatest in anoxic hypolimnia. Abstr. Am. Soc. Limnol. Oceanogr. 1992. American Society of Limnology and Oceanography, Santa Fe, N.Mex.

5. **Dyer, B. D., N. Gaju, C. Pedrós-Alió, I. Esteve, and R. Guerrero.** 1986. Ciliates from a fresh water sulfuretum. BioSystems **19:**127–135.
6. **Fenchel, T.** 1968. The ecology of marine microbenthos. II. The food of marine benthic ciliates. Ophelia **5:**73–121.
7. **Fenchel, T.** 1980. Suspension feeding in ciliated protozoa: feeding rates and their ecological significance. Microb. Ecol. **6:**13–25.
8. **Fenchel, T., and B. J. Finlay.** 1990. Anaerobic free-living protozoa: growth efficiencies and the structure of anaerobic communities. FEMS Microbiol. Ecol. **74:**269–276.
9. **Fenchel, T., and B. J. Finlay.** 1991. The biology of free-living anaerobic ciliates. Eur. J. Protistol. **26:**201–215.
10. **Fenchel, T., L. D. Kristensen, and L. Rasmussen.** 1990. Water column anoxia: vertical zonation of planktonic protozoa. Mar. Ecol. Prog. Ser. **62:**1–10.
11. **Finlay, B. J., K. J. Clarke, A. J. Cowling, R. M. Hindle, A. Rogerson, and U.-G. Berninger.** 1988. On the abundance and distribution of protozoa and their food in a productive freshwater pond. Eur. J. Protistol. **23:**205–217.
12. **Finlay, B. J., K. J. Clarke, E. Vicente, and M. R. Miracle.** 1991. Anaerobic ciliates from a sulphide-rich solution lake in Spain. Eur. J. Protistol. **27:**148–159.
13. **García-Cantizano, J.** 1992. Análisis funcional de la comunidad microbiana en ecosistemas planctónicos. Ph.D. thesis. Universitat Autònoma de Barcelona, Bellaterra, Spain.
14. **Gasol, J. M., J. García-Cantizano, R. Massana, F. Peters, R. Guerrero, and C. Pedrós-Alió.** 1991. Diel changes in the microstratification of the metalimnetic community in Lake Cisó. Hydrobiologia **211:**227–240.

15. **Gasol, J. M., F. Peters, R. Guerrero, and C. Pedrós-Alió.** 1992. Community structure in Lake Cisó: biomass allocation to trophic groups and differing patterns of seasonal succession in the meta- and epilimnion. Arch. Hydrobiol. **123:**275–303.
16. **Golterman, H. L., R. S. Clymo, and M. A. M. Ohnstad.** 1978. Methods for physical and chemical analysis of fresh waters. IBP Handbook No. 8. Blackwell Scientific Publications, Oxford.
17. **Guerrero, R., E. Montesinos, C. Pedrós-Alió, I. Esteve, J. Mas, H. Van Gemerden, P. A. G. Hofman, and J. F. Bakker.** 1985. Phototrophic sulfur bacteria in two Spanish lakes: vertical distribution and limiting factors. Limnol. Oceanogr. **30:**919–931.
17a. **Guhl, B. E.** Unpublished data.
18. **Guhl, B. E., and B. J. Finlay.** 1993. Anaerobic predatory ciliates track seasonal migrations of planktonic photosynthetic bacteria. FEMS Microbiol. Lett. **107:**313–316.
19. **Holling, C. S.** 1959. Some characteristics of simple types of predation and parasitism. Can. Entomol. **91:**385–398.
20. **Ingvorsen, K., and B. B. Jørgensen.** 1979. Combined measurement of oxygen and sulfide in water samples. Limnol. Oceanogr. **24:**390–393.
20a. **James, M. R.** Unpublished data.
21. **Jankowski, A. W.** 1964. Morphology and evolution of ciliophora. III. Diagnoses and phylogenesis of 53 sapropelebionts, mainly of the order Heterotrichida. Arch. Protistenkd. **107:**185–294.
22. **Kahl, A.** 1930. Urtiere oder Protozoa. I. Wimpertiere oder Ciliata (Infusoria), eine Bearbeitung der freilebenden und ectocommensalen Infusorien der Erde, unter Ausschluss der marinen Tintinnidae, p. 1–886. *In* F. Dahl (ed.), Die Tierwelt Deutschlands. G. Fischer, Jena, Germany.
23. **Lavrentyev, P. J., and V. V. Maslevtsov.** 1988. Protozoa in lakes of various types, p. 207–221. *In* V. G. Drabkova (ed.), The changes of the lake ecosystem structure under increasing nutrient load. Science, Leningrad. (In Russian.)
24. **Laybourn-Parry, J., J. Olver, and S. Rees.** 1990. The hypolimnetic protozoan plankton of a eutrophic lake. Hydrobiologia **203:**111–119.
25. **Mas, J., C. Pedrós-Alió, and R. Guerrero.** 1990. In situ specific loss and growth rates of purple sulfur bacteria in Lake Cisó. FEMS Microbiol. Ecol. **73:**271–281.
26. **Massana, R., J. M. Gasol, and C. Pedrós-Alió.** Interrupted succession of ciliate communities in sharply stratified metalimnia. Mar. Microb. Food Webs, in press.
27. **Massana, R., and C. Pedrós-Alió.** A method to determine integrated predation in stratified waters. Limnol. Oceanogr., in press.
28. **Massana, R., C. K. Stumm, and C. Pedrós-Alió.** 1994. Effects of temperature, sulfide, and food abundance on growth and feeding of anaerobic ciliates. Appl. Environ. Microbiol. **60:**1317–1324.
29. **Miracle, M. R., E. Vicente, and C. Pedrós-Alió.** 1992. Biological studies in Spanish meromictic and stratified lakes. Limnetica **8:**59–77.
30. **Montesinos, E., I. Esteve, and R. Guerrero.** 1983. Comparison between direct methods for determination of microbial cell volume: electron microscopy and electronic particle sizing. Appl. Environ. Microbiol. **45:**1651–1658.
31. **Pedrós-Alió, C., and R. Guerrero.** 1993. Microbial ecology in Lake Cisó. Adv. Microb. Ecol. **13:**155–209.
32. **Pedrós-Alió, C., J. Mas, J. M. Gasol, and R. Guerrero.** 1989. Sinking speeds of free-living phototrophic bacteria determined with covered and uncovered traps. J. Plankton Res. **11:**887–905.
33. **Porter, K. G., and Y. S. Feig.** 1980. The use of DAPI for identifying and counting aquatic microflora. Limnol. Oceanogr. **25:**943–948.
34. **Psenner, R., and K. Schlott-Idl.** 1985. Trophic relationships between bacteria and protozoa in the hypolimnion of a meromictic mesotrophic lake. Hydrobiologia **121:**111–120.
35. **Putt, M., and D. K. Stoecker.** 1989. An experimentally determined carbon:volume ratio for marine "oligotrichous" ciliates from estuarine and coastal waters. Limnol. Oceanogr. **34:**1097–1103.
36. **Schulz, S., S. Wagener, and N. Pfennig.** 1990. Utilization of various chemotrophic and phototrophic bacteria as food by the anaerobic ciliate *Trimyema compressum*. Eur. J. Protistol. **26:**122–131.
37. **Sherr, B. F., E. B. Sherr, and R. D. Fallon.** 1987. Use of monodispersed, fluorescently labeled bacteria to estimate in situ protozoan bacterivory. Appl. Environ. Microbiol. **53:**958–965.
38. **Sherr, E. B., F. Rassoulzadegan, and B. F. Sherr.** 1989. Bacterivory by pelagic choreotrichous ciliates in coastal waters of the NW Mediterranean Sea. Mar. Ecol. Prog. Ser. **55:**235–240.
39. **Straskrabová, V.** 1959. Hydrobiological research in two naturally polluted pools in the woody inundation area of the Elbe. Acta Soc. Zool. Bohemoslovenicae **23(1):**34–69.
40. **Wagener, S., S. Schulz, and K. Hanselmann.** 1990. Abundance and distribution of anaerobic protozoa and their contribution to methane production in Lake Cadagno (Switzerland). FEMS Microbiol. Ecol. **74:**39–48.
41. **Wagener, S., C. K. Stumm, and G. D. Vogels.** 1986. Electromigration, a tool for studies of anaerobic ciliates. FEMS Microbiol. Ecol. **38:**197–203.