Journal of Plankton Research Vol.18 no.3, pp.335–353, 1996

Comparison of ciliate communities in the anoxic hypolimnia of three lakes: general features and the influence of lake characteristics

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Abstract. The ciliate communities and their food sources were investigated in the anoxic hypolimnia of three lakes with differing water chemistry. Bacterial biomass and, as a result, the biomass of bacterivorous ciliates were correlated with lake trophy. Additionally, high sulfate and sulfide concentrations led to high bacterial biomass of sulfate reducers and anaerobic phototrophic and heterotrophic bacteria, which in turn sustained large ciliate populations. The anaerobic ciliate communities of the lakes shared many characteristics. They were comprised of the same or closely related species; this was attributed to a low diversity of food sources. Ciliate to prey biomass ratios were 1.2–3.8% which is consistent with a low theoretical growth efficiency of anaerobic metabolism. Grazing pressure on anaerobic ciliates by metazoa was insignificant. In all three lakes, ciliate populations showed distinct vertical non-random distribution patterns which were often correlated with the distribution of the corresponding food sources. It is suggested that the microbial communities in anoxic water bodies are largely influenced by few common environmental conditions and are therefore often inhabited by similarly structured ciliate communities.

Introduction

Anoxic water bodies harbor characteristic microbial communities. If, for example, light reaches sulfide-containing water, dense, often closely stratified populations of anaerobic phototrophic bacteria develop (Montesinos *et al.*, 1983; Miracle *et al.*, 1992). Anaerobic microbial communities also consist of specialized heterotrophic bacteria (Caldwell and Tiedje, 1975a; Gast and Gocke, 1988) and a variety of protozoa which, unique amongst phagotrophic organisms, are capable of completing their life cycle under anoxic conditions (Fenchel and Finlay, 1995). Anaerobic ciliates are frequently described as the main bacterial consumers in anoxic freshwater and marine environments (Fenchel *et al.*, 1990; Madoni, 1990; Massana and Pedrós-Alío, 1994), but the functioning of the anaerobic microbial food web is still largely unknown. Studies on hypolimnetic ciliate communities have concentrated on the investigation of species composition (e.g. Webb, 1961; Dyer *et al.*, 1986; Madoni, 1990) and, more rarely, on the seasonal development (Fenchel *et al.*, 1990; Laybourn-Parry *et al.*, 1990). Some studies focusing on the vertical distribution of hypolimnetic ciliates noticed distinct non-random distribution patterns (Fenchel *et al.*, 1990; Laybourn-Parry *et al.*, 1990; Zubkov *et al.*, 1992; Guhl *et al.*, 1994) for which no generally accepted explanation has been offered. While Guhl *et al.* (1994) found a correlation between the distribution of ciliates and their food sources, Fenchel *et al.* (1990) suggested that bacterial growth would determine ciliate distri-

bution. Very little is known about the relationship between anaerobic ciliates and their food under *in situ* conditions. Laboratory cultures of anaerobic ciliates have low gross growth efficiencies (Fenchel and Finlay, 1990; Schulz *et al.*, 1990). The low yields in the cultures are attributed to the strictly fermentative metabolism of anaerobic ciliates. Under natural conditions, the abundance of anaerobic ciliates is always relatively low and they seem to have long *in situ* doubling times (Guhl *et al.*, 1994; Massana and Pedrós-Alío, 1994). This may be related to the anaerobic metabolism (Fenchel and Finlay, 1990).

In our comparison of ciliate communities in the anoxic hypolimnia of three lakes, we were interested in the functional relationship between anaerobic ciliates and their food. We hypothesized that this relationship would be shaped by the anoxic environmental conditions and the anaerobic metabolism of predators and prey. At the same time, we wanted to examine the influence of lake characteristics, such as morphology and trophy, on ciliate community structure and biomass. We therefore chose two adjacent lakes of different morphometry and trophic status, and one small solution lake for which the water chemistry is determined by the composition of the surrounding rock. The seasonal and spatial patterns of the ciliate community in Priest Pot, one of the lakes in this study, have been described elsewhere (Guhl *et al.*, 1994).

Method

Study sites

Two of the lakes investigated, Priest Pot and Esthwaite Water, are situated adjacent to each other in the southeastern part of the English Lake District (Cumbria). Arcas-2 is one of many small solution lakes near Cuenca, ~150 km east of Madrid in central Spain.

Priest Pot has an area of 1 ha and a maximum depth of 3.9 m. The lake has very high nutrient levels, with for example, total phosphorus concentrations of up to 3 μM. Priest Pot stratifies thermally from April to October. The hypolimnion is anoxic from May until the autumn overturn. At the time of its maximum development, the anoxic hypolimnion extends up to 2 m above the sediment and comprises ~30% of the lake volume. During the stratification period, dense populations of anaerobic phototrophic bacteria, mainly the purple sulfur bacterium *Thiopedia* sp. and the green sulfur bacterium *Clathrochloris hypolimnica*, develop (Davison and Finlay, 1986).

Esthwaite Water has an area of 1000 ha, a maximum depth of 14.5 m and lower nutrient levels than Priest Pot (e.g. total phosphorus up to 1.0 μM), but is still eutrophic. Esthwaite Water begins to stratify in May. An anoxic hypolimnion usually develops between June and October. It extends up to 5.5 m above the sediment and fills ~10% of the lake volume. Anaerobic photosynthetic bacteria never reach significant abundances.

Arcas consists of two round basins, formed from the dissolution of gypsum-rich marls from the Tertiary Paleocene. Each of the basins has an area of ~0.2 ha and a maximum depth of 14 m. Continuous contact between the lake and the water table provides the lake with a nearly constant water level and the lake water with a high

sulfate concentration of ~16 mM (Miracle *et al.*, 1992). Total phosphorus concentrations reach values around 0.5 μM. Arcas stratifies each year from March until the end of October. During this period, an anoxic hypolimnion extends to fill the bottom 6 m (~40% of the lake volume), with sulfide concentrations of up to 8 mM near the bottom. Arcas supports dense microstratified plates of *Oscillatoria* sp., *Cryptomonas erosa* and *Chromatium weissei* (Finlay *et al.*, 1991). Further information on Priest Pot is given in Finlay *et al.* (1988) and Guhl *et al.* (1994), on Esthwaite Water in Jones (1978), Finlay (1981) and Heaney *et al.* (1986), and on Arcas in Vicente *et al.* (1991), Finlay *et al.* (1991) and Esteban *et al.* (1993).

Sampling procedure and quantification of organisms

The water column of the two English lakes was investigated during the stratification period of 1991 at weekly (Priest Pot) or monthly (Esthwaite Water) sampling intervals. In 1992, sampling was carried out every 3 weeks on Priest Pot and on one occasion (17 August 1992) on Esthwaite Water. Water samples from Arcas were collected between 14 September and 16 September 1992.

All water samples were taken at the deepest point of each lake. The anoxic hypolimnion was defined as the part of the water column where dissolved oxygen concentrations (as measured with an oxygen electrode) were ≤ 0.5 mg O_2 l^{-1}. On Priest Pot and Esthwaite Water the hypolimnion was sampled at 20–25 cm intervals using a 1 l Friedinger bottle. On Arcas water samples were taken from a boat fixed to cross wires, using an electrically powered peristaltic pump connected to a special inlet device for sampling narrow water strata (Jørgensen *et al.*, 1979). The steady position of the boat allowed vertical sampling at intervals of <10 cm.

Prior to sampling, an oxygen and temperature profile was recorded using a Yellow Springs Instrument (Priest Pot, Esthwaite Water) or a WTW instrument (Arcas). For the English lakes, pH was measured immediately after the return to the laboratory using a Radiometer PM1 meter. On Arcas, the pH was measured during the sampling process in a flow-through cell, using Ross (Orion 80-05) reference electrodes (Finlay *et al.*, 1991). For each sampled depth the following subsamples were collected: (i) 100 ml (Arcas: 10 ml) were fixed immediately with formaldehyde (final concentration 5%) for quantification of microzooplankton; (ii) 10 ml were fixed with glutaraldehyde in 0.1 M cacodylate buffer pH 7.0 (final concentration 4% v/v) for enumeration of bacteria and phytoplankton; 100 ml were prepared for sulfide analysis by precipitating the sulfide in the field with 0.2 ml 1 M zinc acetate; additionally, untreated lake water was sampled and transported in an insulated box to the laboratory for observation of living organisms and further chemical analyses.

Ciliates and bacteria were quantified as described in Guhl *et al.* (1994). Ciliates were enumerated from 10–40 ml subsamples, gently centrifuged and reduced to a volume of <1 ml. The cells were counted in a counting chamber at ×40 magnification. Potential ciliate food was quantified for Priest Pot on four occasions in 1991 and on seven occasions in 1992, for Esthwaite Water on 19 September 1991 and on 17 August 1992, and for Arcas on 15 September 1992. Bacteria were stained with DAPI and enumerated by epifluorescence microscopy. Bacteria were sized using an ocular micrometer. Three size categories were distinguished: >1 μm maximum

dimension, ~1 μm and <0.5 μm. Large prominent bacteria, e.g. phototrophic bacteria, were counted separately. At Priest Pot on three occasions additionally heterotrophic nanoflagellates (i.e. flagellates of 2–20 μm length) were quantified from filter preparations. Ciliate biovolume was calculated assuming geometric shapes and measuring the appropriate cell dimensions under the microscope. For conversion of ciliate biovolume into biomass a wet weight of 1 g cm^{-3} was assumed and a carbon conversion factor of 140 fg μm^{-3} recommended for formalin-fixed samples (Putt and Stoecker, 1989) was used. The biovolume of flagellates, phytoplankton, and large abundant bacteria of distinct shape, e.g. phototrophic bacteria, was determined similarly to that of ciliates. The carbon conversion factors for flagellates and phytoplankton were 220 and 160 fg C μm^{-3}, respectively (Morgan and Kalff, 1979; Børsheim and Bratbak, 1987). Based on the measurements of 100 bacterial cells for each size class from Priest Pot and Esthwaite Water, the carbon content of the different bacteria size categories was calculated assuming a decreasing dry weight with increasing bacterial size (Lee and Fuhrman, 1987; Simon and Azam, 1989): <0.5 μm = 10 fg C $cell^{-1}$ (1 μm^3 = 550 fg C; mean cell volume = 0.018 ± 0.007 μm^3); 1 μm = 30 fg C $cell^{-1}$ (1 μm^3 = 250 fg C; mean cell volume = 0.12 ± 0.09 μm^3); >1 μm = 80 fg C $cell^{-1}$ (1 μm^3 = 220 fg C; mean cell volume = 0.36 ± 0.19 μm^3). The carbon content of large bacteria was assumed to be 220 fg C μm^{-3} (Bratbak and Dundas, 1984). While 1 μm bacteria varied in their cell shapes, small and large bacteria displayed a relatively uniform cell morphology. Small bacteria were almost always spherical. Bacteria >1 μm predominantly had a cylindrical shape with a diameter of 0.25–0.4 μm and a length of 2–4 μm. The mean cell carbon content for the bacterial size classes derived from Priest Pot and Esthwaite Water bacterioplankton was also used to calculate bacterial biomass for Arcas, but as the bacterioplankton of Arcas was clearly dominated by populations of distinct large heterotrophic and phototrophic bacteria which were measured separately, only a minor error was introduced by this generalization.

Chemical analyses

Sulfide and ammonium were measured according to Davison and Lishman (1983) and Weatherburn (1967), respectively. Total phosphorus was determined for Priest Pot on 11 August and 24 November 1991, and for Esthwaite Water on 28 August and 17 November 1991 following the method of Mackereth *et al.* (1978).

Results

Species composition

Table I lists the common species in the hypolimnia of the three lakes investigated. Species were regarded as common when they were present on >30% of the sampling occasions or on one occasion with >5 cells ml^{-1} at any depth. Ciliate community composition was remarkably similar in the three lakes. The anaerobic ciliate communities were dominated by the same taxonomic groups, often down to genus level. For example, members of the genus *Caenomorpha* were abundant in all three lakes. Also, members of the genus *Cyclidium* were common. Within the Prostomatida, one species always dominated. This species belonged to a different

Table I. Species list of abundant anaerobic ciliates in Priest Pot, Esthwaite Water and Arcas

Priest Pot	Esthwaite Water	Arcas
Armophorida		
Caenomorpha medusula	*Caenomorpha medusula* *Caenomorpha uniserialis*	*Caenomorpha lata* *Caenomorpha corlissi*
Spirotrichea		
Tropidoatractus acuminatus *Bothrostoma undulans*	*Metopus striatus* *Metopus daphnides* *Metopus laminarius*	
Prostomatida		
Prorodon sp.	*Metacystis* sp.	*Holophrya bicoronata* *Prorodon corpulentissimum*
Haptorida		
Lacrymaria sapropelica		*Lacrymaria sapropelica* *Lacrymaria elegans*
Scuticociliatida		
Cyclidium porcatum *Dexiotricha plagia* *Cristigera* sp.	*Cyclidium* sp. *Dexiotricha* sp.	*Cyclidium dilectissimum* *Cyclidium* sp. *Cristigera* sp.
Odontostomatida		
Saprodinium mimeticum *Epalxella* sp.		*Saprodinium difficile* *Epalxella spinosa* *Epalxella oligotricha*
Trichostomatida		
Plagiopyla nasuta	*Plagiopyla nasuta*	*Plagiopyla nasuta*

genus in each lake. There were, however, some exceptions to this similarity of community structure. Representatives of the Odontostomatida, which formed relatively large populations in Priest Pot and especially Arcas, occurred only sporadically in Esthwaite Water. The latter, in turn, had high abundances of *Metopus* species, which were rare in the other two lakes. During the investigation, altogether 25 anaerobic ciliate species were found in Priest Pot and 21 in Esthwaite Water. On the single sampling occasion at Arcas, 15 anaerobic ciliate species were registered. Most of the recorded species are considered to be bacterivorous (Foissner *et al.*, 1992). Prostomatida are adapted to feed on larger particles (Fenchel, 1987). The haptorid *Lacrymaria* is capable of feeding on other protozoa as well as on phytoplankton (Fenchel, 1968).

Community abundance and biovolume

In Table II average community abundances and biomass of ciliates in the anoxic part of the water column are compiled. For Priest Pot and Esthwaite Water data are presented for when ciliate communities were maximally developed. Additionally, mean values for the stratification period were calculated where possible. In Arcas sampling was limited to one occasion. Ciliate abundances were ~8–10 times higher in Priest Pot than in Esthwaite Water. In Esthwaite Water anaerobic ciliates

Table II. Mean abundance and biomass of planktonic ciliates in the anoxic hypolimnia at the time of maximum community development, and mean values for the stratification period

Anaerobic ciliates	Priest Pot				Esthwaite Water			Arcas
	29.8.91	Mean for 1991	3.9.92	Mean for 1992	19.8.91	Mean for 1991	17.8.92	15.9.92
abundance (ml^{-1})	44.4	16.3	42.8	32.0	4.7	2.3	5.9	20.8
biomass (ng wet wt ml^{-1})	360	143	423	240	22	8	20	173

were on average smaller than in Priest Pot. The biomass of the two communities differed by a factor of 15–20. In Arcas mean ciliate concentrations and biomass were about half of the values from Priest Pot. Maximum cell concentrations of anaerobic ciliates were ~5 cells ml^{-1} in Esthwaite Water, 30 cells ml^{-1} in Priest Pot, and, due to a very dense stratification, >100 cells ml^{-1} in Arcas.

In both summers investigated the anaerobic ciliate communities developed in a similar way. Maximum abundances as well as maximum biomass varied only slightly between the years. In Priest Pot differences between mean values for the two stratification periods were more pronounced than for the maxima. The anaerobic ciliate community in 1992 had on average about twice the cell number and slightly less than twice the biomass of the ciliate community in 1991.

Food composition and biomass ratios

The compositions of potential food sources at the time of maximum community development are presented in Figure 1. As mentioned above most anaerobic ciliates are specialized on bacterial food. Prostomatida and Haptorida are raptorial feeders and can engulf particles up to a half of their own body length. In the lakes investigated the major food source for these ciliates were *Cryptomonas* populations which undertook migrations into the hypolimnion and, to a lesser extent, sedimenting phytoplankton. Therefore, during the investigation, food sources were differentiated into phototrophic bacteria, heterotrophic bacteria subdivided into size categories, and phytoplankton which includes *Cryptomonas* sp. The very abundant green sulfur bacterium *Clathrochloris hypolimnetica* in Priest Pot was disregarded. The small cells (~0.2 μm) are contained in large mucous aggregates (Davison and Finlay, 1986) which can probably not be ingested by ciliates.

While in Priest Pot the ciliate food consisted of several food sources with varying fractions, in Esthwaite Water bacteria of ~1 μm length comprised the highest biomass. In Esthwaite Water in both years most large bacteria could be assigned to five distinct populations. Small bacteria were nearly absent from this lake. Additionally, *Cryptomonas* and anaerobic phototrophic bacteria never developed significant numbers and the sedimenting phytoplankton consisted mainly of *Asterionella* and *Ceratium*, which could not be ingested by the hypolimnetic ciliates present. In Priest Pot *Cryptomonas* and anaerobic phototrophic bacteria, mainly the

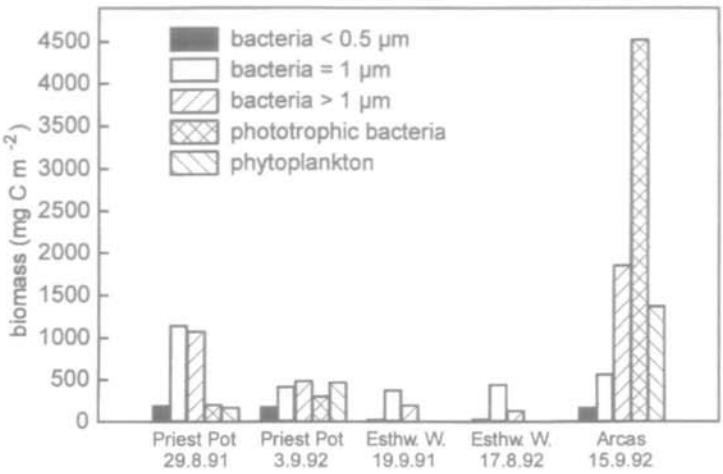

Fig. 1. Biomass of potential food sources in Priest Pot, Esthwaite Water and Arcas for selected sampling occasions

purple sulfur bacterium *Thiopedia* sp., contributed to the ciliate diet, particularly in 1992.

In Arcas, ciliate food composition differed markedly from the other lakes. The most important food source by far was phototrophic bacteria. These consisted chiefly of the purple sulfur bacterium *Chromatium weissei.* Arcas also supported large populations of *Cryptomonas erosa.* As opposed to the other lakes, the biomass of the heterotrophic bacterial community in Arcas was dominated by large bacteria. The population biomass of small bacteria was comparable to that in Priest Pot and the biomass of 1 µm bacteria was similar in all three lakes. Total food biomass concentrations were ~1.0–1.5 µg ml^{-1} in Priest Pot, 1.4 µg ml^{-1} in Arcas and 0.1 µg ml^{-1} in Esthwaite Water.

In Esthwaite Water the food composition varied little between years. In Priest Pot in September 1992, due to a cold autumn, heterotrophic bacteria were already declining. For the same reason phytoplankton had started to sediment into the hypolimnion which led to a higher availability of this food source to anaerobic ciliates. Food populations displayed only slow changes over the stratification period (unpublished observations; compare Figure 3). In Priest Pot the variation of the food composition may have led to a higher ciliate biomass in 1992 (Table III). At the time of maximum ciliate community development, total food biomass was markedly higher in 1991 than in 1992 but ciliate biomass was higher in 1992. This resulted in a ciliate to food biomass ratio of 5.6% in 1992 compared to 3.1% in 1991. Ciliates which select for large particles, such as phototrophic bacteria and phytoplankton, were more abundant in Priest Pot 1992 than in 1991 (Guhl *et al.*, 1994).

Generally, ciliate to food biomass ratios ranged between 1.0 and 5.6% (Table III). Highest values were calculated for Priest Pot. This study did not address the investigation of hypolimnetic flagellates, but in Priest Pot the flagellate community biomass was determined three times, to get an impression of the approximate size of this community. On 3 September 1992 flagellate biomass was about four times

Table III. Biomass ratio between anaerobic ciliates and their potential prey. For Priest Pot and Esthwaite Water times of maximum ciliate community development are presented

	Priest Pot		Esthwaite Water		Arcas
	29.8.91	3.9.92	19.9.91	17.8.92	15.9.92
Food biomass (mg C m^{-2})	2794	1851	612	619	8493
Ciliate biomass (mg C m^{-2})	88	104	6	13	133
Flagellate biomass (mg C m^{-2})	nd	400	nd	nd	nd
Ratio ciliate: food biomass (%)	3.1	5.6 27.2*	1.0	2.1	1.6

nd, not determined.
* Ratio between protozoan (i.e. ciliate and flagellate) biomass and food biomass.

higher than that of ciliates. Total protozoan biomass, i.e. ciliate and flagellate biomass together, comprised 27.2% of food biomass. Flagellates were rare in Arcas [~20 mg C m^{-2}; recalculated from Fenchel and Finlay (1990)]. Most flagellates in Priest Pot and Esthwaite Water are <5 μm and therefore feed on small bacteria (Berninger, 1990). It is likely that in these two lakes hypolimnetic ciliates had to compete with flagellates, at least for smaller bacteria. We therefore compared predator and prey biomass for individual ciliate populations which were the sole predators of distinct food sources. The major food sources of the prostomatids *Prorodon* sp. and *Holophrya bicoronata* were *Cryptomonas* populations and sedimenting phytoplankton. This was confirmed by epifluorescence microscopy which revealed intact and partly digested phytoplankton inside the food vacuoles of ciliates (Figure 2). In Priest Pot *Caenomorpha medusula* fed mainly on *Thiopedia* sp., as was demonstrated by Guhl and Finlay (1993). In Arcas, *Caenomorpha lata* did not feed on phototrophic bacteria but on large heterotrophic bacteria. In Esthwaite Water this food source was exploited by two *Metopus* species. *Caenomorpha* and *Metopus* are both filter feeders, filtering the water with an adoral zone of membranelles (AZM). The distance between the membranelles within the AZM determines the size of particles which are retained. The distance can be measured after silver staining and is around 1 μm for both ciliates (Figure 2). As most bacteria in the food vacuoles of *Caenomorpha* and *Metopus* were larger (4 μm; Figure 2) the ciliate food source was defined by the length of bacteria rather than the bacterial diameter which is difficult to measure accurately.

Mean biomass ratios over the season and for the water column ranged from 1.2 to 3.8% (Table IV). Biomass ratios increased over the summer and on particular sampling occasions they could rise to >8% (Figure 3). In Priest Pot the population of *Caenomorpha medusula* developed parallel to that of its food source with no indication of a 'top–down control' of the bacteria (Figure 3). The development of the *Prorodon* population followed that of its food, with a time lag of 2–3 weeks

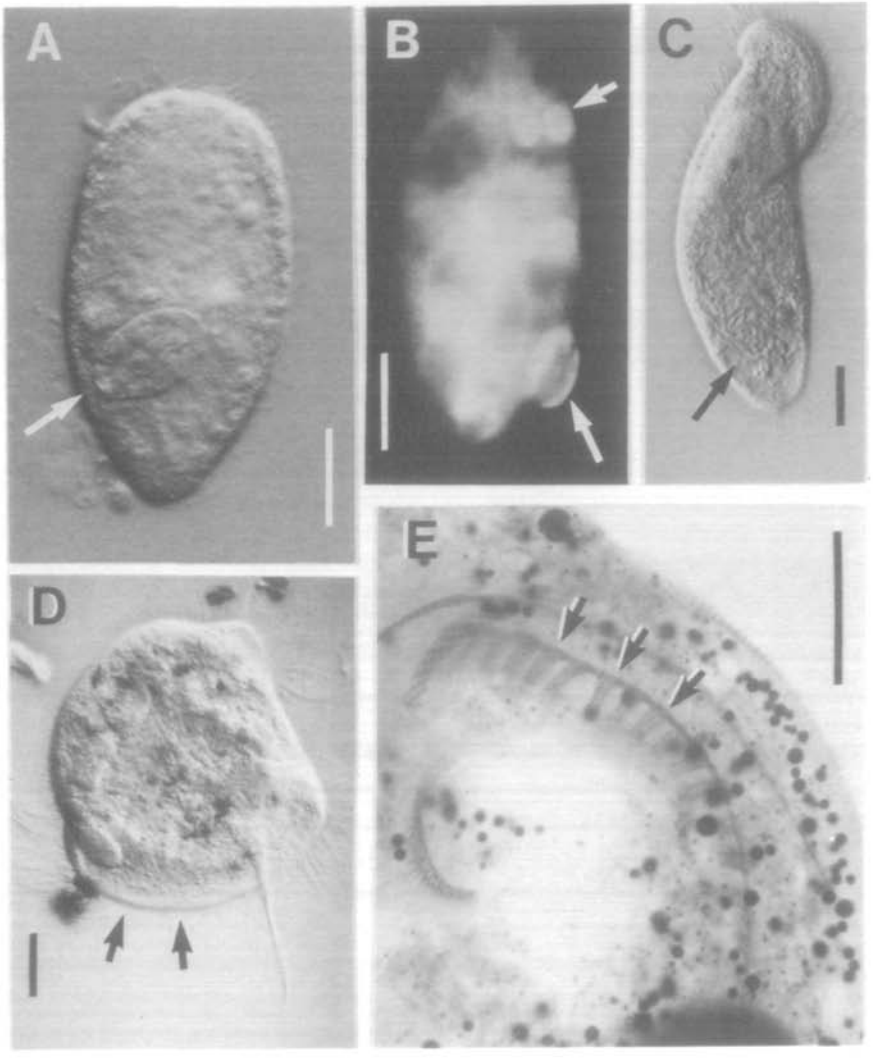

Fig. 2. Anaerobic ciliates from the lakes investigated. Material fixed with formaldehyde. (**A**) *Holophrya bicoronata* from Arcas with ingested *Cryptomonas* (arrow). (**B**) Autofluorescence of partly digested *Cryptomonas* inside the food vacuoles of *Holophrya bicoronata* (small arrow) after excitation with violet light (peak 420 nm). The large arrow points towards a *Cryptomonas* cell outside the ciliate. (**C**) *Metopus daphnides* from Esthwaite Water with ingested bacteria inside the food vacuole (arrow). (**D**) *Caenomorpha medusula* from Priest Pot. Arrow indicates the location of the adoral zone of membranelles (AZM). (**E**) Silver preparation of a *Caenomorpha* cell showing individual membranelles of the AZM as short parallel rows. All scale bars represent 10 μm.

between ciliates and their prey in spring. Highest ciliate biomass occurred shortly after the peak of prey biomass which apparently implies a strong grazing impact on the food populations. However, the sudden decrease of phytoplankton, mainly *Cryptomonas*, in August was probably due to changing weather conditions rather

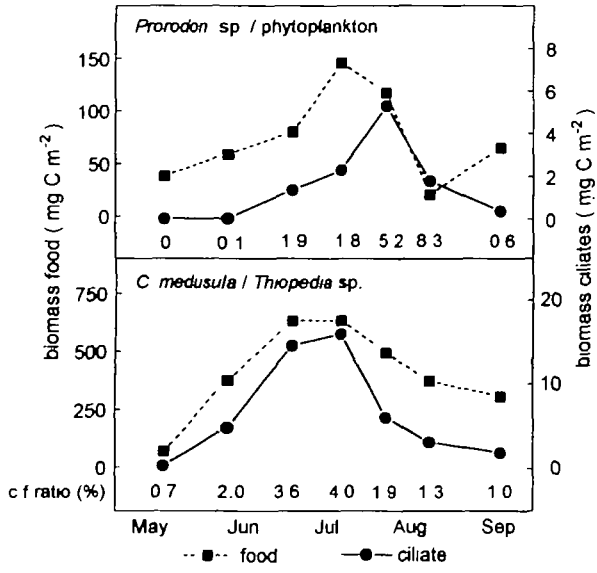

Fig. 3. Seasonal development of ciliate biomass and biomass development of the corresponding food source in Priest Pot in 1992. **Upper panel:** comparison of biomass development for *Prorodon* sp. and phytoplankton. **Lower panel:** comparison for *Caenomorpha medusula* and *Thiopedia* sp. Numbers below the graphs are biomass ratios between ciliate and food populations.

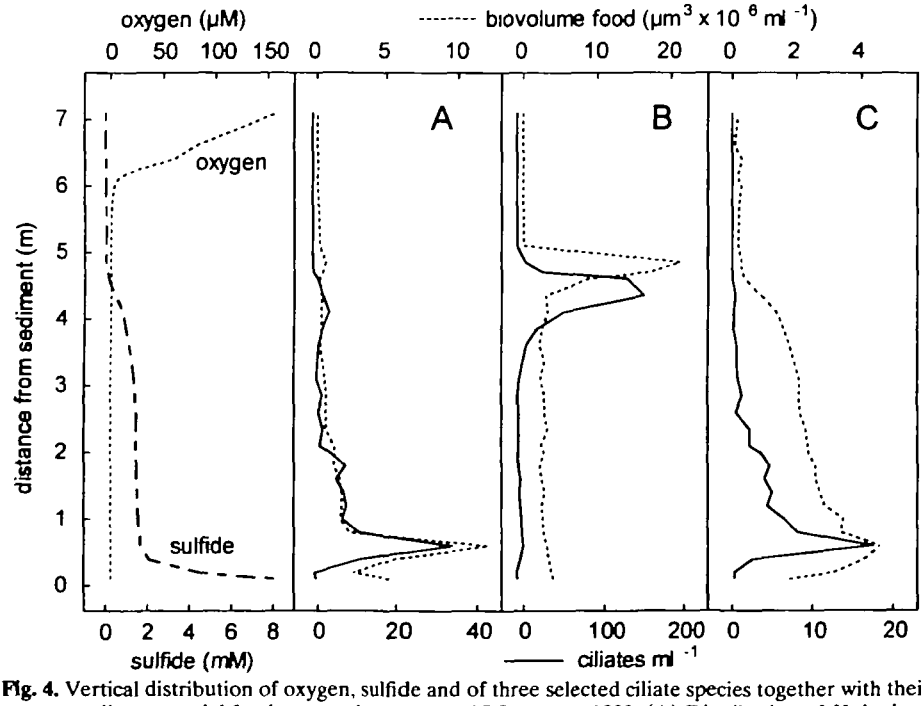

Fig. 4. Vertical distribution of oxygen, sulfide and of three selected ciliate species together with their corresponding potential food sources in Arcas on 15 Septemer 1992. (**A**) Distribution of *Holophrya bicoronata* and *Cryptomonas erosa*. (**B**) Distribution of scuticociliates and *Chromatium weissei*. (**C**) Distribution of *Caenomorpha lata* and large bacteria.

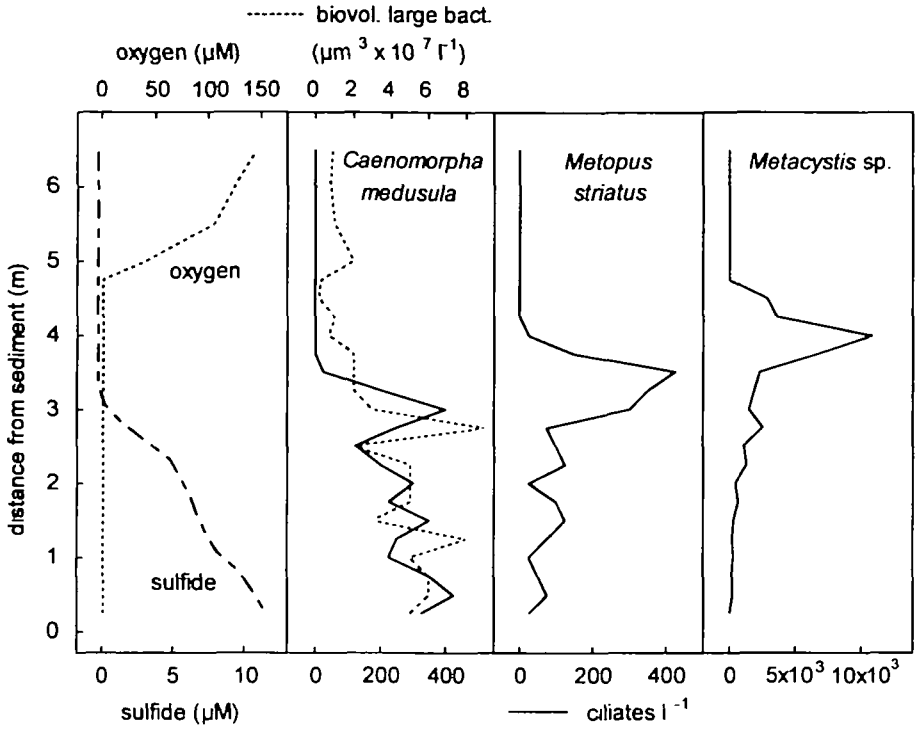

Fig. 5. Vertical distribution of oxygen, sulfide and of three selected ciliate species in Esthwaite Water on 17 August 1992. Together with the profile of *Caenomorpha medusula* the distribution of large bacteria, i.e. the corresponding potential food source, is shown.

than overgrazing by *Prorodon*. The control of *Cryptomonas* populations by anaerobic ciliates is unlikely because part of the population is always in the oxic part of the water column and therefore not accessible to the ciliates.

Vertical distribution patterns

The ciliate communities of Arcas and Esthwaite Water displayed distinct vertical non-random distributions (Figures 4 and 5). The same phenomenon has already been described for Priest Pot (Guhl *et al.*, 1994). In most cases the distribution patterns did not correlate with any known chemical gradients. Instead, the vertical distribution of certain ciliates could be clearly related to the distribution of major food sources. In Arcas, for example, the prostomatid *Holophrya bicoronata* fed on *Cryptomonas erosa* (Figure 4). Similarly the distribution of *Caenomorpha lata* followed that of bacteria >4 µm. In Esthwaite Water the distribution of *Caenomorpha medusula* correlated with that of large bacteria (Figure 5). The pronounced distribution patterns of other ciliates, e.g. the two abundant species *Metopus striatus* and *Metacystis* sp. in Esthwaite Water, did not match the distribution of distinct food sources although bacterial food was discernible in the food vacuoles of *Metopus*. Owing to the rigid pellicle of *Metacystis* sp. no food sources could be identified from food vacuole contents. Figure 5 might imply an impact by the sulfide gradient

Table IV. Biomass ratios between ciliate populations and their major food source, calculated per m^2 lake surface

Lake	Ciliate	Ciliate biomass (mg C m^{-2})	Food organism	Food biomass (mg C m^{-2})	Biomass ratio (%)
Arcas (15.9.92)	*Caenomorpha lata*	39	Bacteria >4 μm	1306	3.0
Arcas (15.9.92)	*Holophrya bicoronata*	48	*Cryptomonas erosa*	1320	3.6
Priest Pot (mean for 1992)	*Caenomorpha medusula*	6.7	*Thiopedia* sp.	411	1.6
Priest Pot (mean for 1992)	*Prorodon* sp.	2.1	Phytoplankton*	81	2.6
Esthwaite Water (19.9.91)	*Metopus striatus*	1.7	Bacteria >4 μm	139	1.2
Esthwaite Water (17.8.92)	*Metopus striatus* + *Metopus daphnides*	4.6	Bacteria >4 μm	120	3.8

* Mainly *Cryptomonas* spp.

on the vertical distribution of *Metopus striatus* and *Metacystis* sp. However, on other sampling occasions both species had distinct population maxima in water containing high sulfide concentrations.

In Arcas, small scuticociliates (i.e. <30 μm length) accumulated immediately below the dense plate of *Chromatium weissei* (Figure 4). These ciliates did not feed on the purple sulfur bacteria because the *Chromatium* cells were too large for their filter apparatus. Neither did the distribution of scuticociliates correspond to that of 1 μm bacteria nor of small bacteria. In this case alone it is possible that the ciliate distribution was influenced by the sulfide gradient as the scuticociliates accumulated where sulfide concentrations decreased sharply.

Discussion

Influence of physical and chemical lake characteristics on the hypolimnetic microbial community

The lakes investigated differ in many ways, e.g. nutrient levels and sulfide concentrations. One of the aims of our study was the investigation of the influence of these factors on the hypolimnetic ciliate communities. In Table V, data for ammonium, sulfide, total phosphorus and epilimnetic chlorophyll *a* concentrations are compiled from our own measurements and from the literature. For Priest Pot and Esthwaite Water the range of values during the stratification period 1992 is given. The chemistry of these lakes has been extensively investigated by Berninger (1990).

Table V. Concentrations of chlorophyll *a* in the epilimnion, and of ammonium, sulfide, and total phosphorus in the hypolimnion of Priest Pot, Esthwaite Water and Arcas

	Priest Pot	Esthwaite Water	Arcas
Chlorophyll (μg l^{-1})	90.8[a]	25.4[a]	5.2[b]
Ammonium (μM)	1–210	12–53	15–350[c]
Sulfide (μM)	2–23	2–12	10–8000
Total P (μM)	0.8–3.2	0.4–1.0	0.05–0.5[c]

[a] Berninger (1990), [b] Vicente *et al.* (1991), [c] Miracle *et al.* (1992).

Priest Pot had the highest nutrient levels, as expressed for example by total phosphorus concentrations. Higher nutrient levels correlated with higher bacterial numbers and biomass. This correlation has been found in many fresh and marine waters (Bird and Kalff, 1984; Cole *et al.*, 1988). Additionally, owing to the shallow basin of Priest Pot, light penetrates into the hypolimnion thus allowing the development of anaerobic phototrophic bacteria.

For the anaerobic microbial food web of Arcas, lake trophy was of secondary importance. The water chemistry of this lake is determined mainly by the geology of the surrounding area. Dissolution of calcium sulfates, together with subterranean water flow, provides a sulfate concentration of ~16 mM (Finlay *et al.*, 1991) and therefore an abundant supply of terminal electron acceptor for sulfate reducers in the anoxic hypolimnion. The sulfide produced serves as an electron donor for anaerobic phototrophic bacteria. These processes led to the high bacterial biomass recorded in Arcas.

The estimation of bacterial biomass based partly on size categories yields results of limited accuracy. Also, the conversion factors for calculating the biomass of bacteria are still controversial (e.g. Simon and Azam, 1989). However, the bacterial communities investigated differed considerably with regards to biomass and composition. The composition of the bacterial communities in Priest Pot and Esthwaite Water varied only slightly between the years. This may have been fortuitous, especially since sampling of the bacterial community in Esthwaite Water was limited to two occasions; but a comparison with earlier investigations of the two lakes (Jones, 1978; Davison and Finlay, 1986; Finlay *et al.*, 1988) suggests that the hypolimnetic bacterial community does not display rapid changes. In Esthwaite Water Jones (1978) identified (as demonstrated by photographs) the same distinct morphotypes of large bacteria that we found more than a decade later. Also for Arcas a similarly structured bacterial community dominated by the same two phototrophic bacteria has been found in previous years (Finlay *et al.*, 1991; Vicente *et al.*, 1991).

Higher bacterial biomass supported a larger ciliate community. Ciliate abundances and biomass were lowest in Esthwaite Water and highest in Priest Pot. The densely stratified ciliate community of Arcas reached maximum cell concentrations which are comparable to values from the epilimnion of meso- or eutrophic

Table VI. Abundant ciliate taxa reported from anoxic freshwater and marine water bodies

Locality	Ciliate taxon						Reference
	Caenomorpha	*Metopus*	*Plagiopyla*	*Lacrymaria*	Scuticociliates	Odontostomes	
Velká Karasí (FW)	+	+	+				Straškrabová (1959)
Cadagno (FW)	+	+	+	+			Wagener *et al.* (1990)
Laguna de la Cruz (FW)	+						Fenchel and Finlay (1990)
Cisó (FW)	+	+	+			+	Massana and Pedrós-Alió (1994)
Estanya (FW)	+	+	+	+			Massana and Pedrós-Alió (1994)
Kinneret (FW)	+	+	+		+	+	Madoni (1990)
Black Sea (M)					+		Zubkov *et al.* (1992)
Mariager Fjord (M)	+	+	+	+		+	Fenchel *et al.* (1990)
Limfjord (M)		+	+		+	+	Fenchel *et al* (1990)

FW, freshwater habitat; M, marine habitat.

lakes (e.g. Šimek and Straškrabová, 1992; Müller and Weisse, 1994). It is worth mentioning that the differences between the ciliate biomass in the sediment of Priest Pot and Esthwaite Water are similar to those between the planktonic populations (Finlay, 1982; Guhl *et al.*, 1994).

There are indications of an influence of the composition of food sources on ciliate species composition. As an example, *Lacrymaria* species predominantly feed on large particles. During this study large food particles were scarce in Esthwaite Water and no anaerobic *Lacrymaria* were found in the water column. We also suppose that the absence of odontostomatids from the hypolimnion of Esthwaite Water was related to the lack of suitable food. Odontostomatids are bacterivores of ~20–40 μm length (Foissner *et al.*, 1992) and presumably specialize on small particles. As they are difficult to cultivate, their food particle range has yet to be tested in feeding experiments. During our investigations, bacteria <0.5 μm were rare in Esthwaite Water but abundant in Priest Pot and Arcas where odontostomatids were present in considerable numbers.

General features of the anaerobic ciliate communities

The anaerobic ciliate communities of the water bodies investigated had many similarities. The differences between the species composition in the three lakes should not hide the fact that the ciliate communities were basically similarly structured. All three lakes supported populations of *Caenomorpha* species, *Plagiopyla nasuta* and scuticociliates. *Metopus* species were also present in each lake, but occurred in Arcas and Priest Pot only in small numbers. Odontostomatida have been reported from the sediment of Esthwaite Water (Finlay, 1982). In contrast to the findings for the hypolimnetic ciliates, the species composition of the epilimnetic ciliate communities in the lakes investigated are reported to differ considerably. In Esthwaite Water *Strombidium*, vorticellids and tintinnids dominate (Laybourn-Parry *et al.*, 1990), while in Priest Pot *Hypotrichidium*, *Halteria grandinella* and zoochlorellae-bearing ciliates, such as *Coleps hirtus*, *Prorodon* spp. and *Disematostoma bütschlii*, are abundant (Finlay *et al.*, 1988). The epilimnetic ciliate community of Arcas is comprised mainly of *Coleps hirtus* and scuticociliates and seems to harbour only few species (Finlay *et al.*, 1991).

The similarity of the structures of the anaerobic ciliate communities may reflect similar available food sources. The same or closely related ciliate species have been reported from a variety of anoxic freshwater environments and even marine habitats, and they seem to have a wide geographical distribution (Table VI). It is possible that anoxic environments offer only a limited number of habitat niches and a lower diversity of food sources than oxic habitats.

It is curious that heterotrophic nanoflagellates are apparently not a major food source for anaerobic ciliates. In oxic habitats heterotrophic nanoflagellates are the major bacterivores and ciliates are regarded as consumers of phototrophic and heterotrophic nanoplankton (Fenchel, 1988; Sanders *et al.*, 1992). Bacterivorous ciliates often require bacterial concentrations of $>5 \times 10^6$ cells ml^{-1} to sustain growth (Fenchel, 1980). The anoxic hypolimnia investigated with up to 2.5×10^7 bacteria ml^{-1} certainly provided suitable food concentrations for many ciliates. Additionally, bacterivorous ciliates are known to select for larger particles (Šimek

et al., 1994) and in anoxic environments may gain a competitive advantage over flagellates due to the often larger cell size of anaerobic bacteria compared to aerobic bacteria (Gast and Gocke, 1988; Cole *et al.*, 1993). Anaerobic prostomatids of course feed to some extent on heterotrophic flagellates as they tend to engulf most of the particles in a suitable size range. However, judged from the distribution patterns (see below), we did not find prostomatids which were specialized on flagellates other than *Cryptomonas*. These findings are supported by theoretical considerations on the structure of anaerobic food webs. In anaerobic food chains there is a low energy transfer from one trophic level to the next. Ciliates predominantly feeding on heterotrophic nanoflagellates would have to establish themselves as an additional trophic level. Assuming a gross growth efficiency of 10% (Fenchel and Finlay, 1990), the population biomass of these ciliates would be ~1% of the bacterial biomass present which may not be sufficient to support a population.

The predator to prey biomass ratios recorded during our investigation were between ~1 and 4%, regardless of whether food sources were identified down to species level or roughly categorized as bacteria of a certain size group. Calculations based on other published carbon conversion factors (e.g. Massana and Pedrós-Alío, 1994) lead to similar results. It is likely that biomass ratios were influenced by the different nutritional values of different bacteria. This effect has been documented for laboratory cultures of anaerobic ciliates (Schulz *et al.*, 1990; Yamada *et al.*, 1994) which yielded biomass ratios between 4 and 6%. The biomass ratios for individual ciliate populations and their food sources tended to increase over the stratification period until the autumn mixing of the lake forced the anaerobic microbial community to move back into the sediment. However, the results from laboratory experiments suggest that even under constant environmental conditions biomass ratios do not rise much above 10% (Fenchel and Finlay, 1990).

The low *in situ* biomass ratios were not caused by metazoan predation on hypolimnetic ciliates. Massana *et al.* (1994) reported a case where the invasion of a *Daphnia* population efficiently decimated a metalimnetic microbial community, but due to the reducing conditions and the presence of sulfide, metazoa are normally excluded from exploiting food sources of anoxic water bodies. We have demonstrated that in Priest Pot the metazoan grazing impact on anaerobic ciliates is insignificant (Guhl *et al.*, 1994). The same holds true for the other two lakes investigated. Metazoa were not encountered in any of the samples from the anoxic hypolimnion in Esthwaite Water. Sampling in Arcas was limited to one occasion, but the high sulfide concentrations in the anoxic water column of Arcas render the presence of metazooplankton very unlikely.

The most conspicuous feature of the hypolimnetic ciliate communities was the presence of distinct non-random distribution patterns in the water column. These patterns have been described in many studies about hypolimnetic ciliates (Fenchel *et al.*, 1990; Laybourn-Parry *et al.*, 1990; Zubkov *et al.*, 1992). Guhl *et al.* (1994) have shown that in Priest Pot these vertical patterns are far more pronounced than horizontal patchiness and that the distribution patterns of many ciliates can be directly related to that of the corresponding food source. Also, in Arcas and Esthwaite Water, certain ciliates followed the distribution of their food. We could not dem-

onstrate similar correlations for all abundant ciliates although many of them had distinct distribution maxima within the water column. This may be due to our classification of heterotrophic bacteria based merely on morphological features which certainly underestimated the diversity of bacterial taxa present at any sampling depth. We are unable to decide whether in Arcas the distribution of scuticociliates followed bacterial populations associated with the *Chromatium* plate or whether it was determined by a chemical gradient, such as sulfide concentration. However, it should be mentioned that none of the six species of anaerobic ciliates we managed to cultivate was growth inhibited by sulfide concentrations of 0.5–1 mM (unpublished observation). In Arcas sulfide concentrations were 1–1.5 mM for the largest part of the hypolimnetic water column.

The vertical distribution patterns are evidence of the near to steady-state conditions in the anoxic hypolimnia. In Priest Pot these patterns changed only slowly over weeks (Guhl *et al.*, 1994). There seems to be no 'top–down control' of food sources. Massana and Pedrós-Alió (1994) investigated the feeding impact of *Plagiopyla* sp. on anaerobic bacteria in Lake Cisó and calculated that during the investigation period the ciliate population never removed >0.1% of the standing stock of bacteria present in the water column. They found, as we did, long doubling times in the range of weeks.

We conclude that despite the limited number of sampling occasions on two lakes and despite the few investigated habitats, we have described typical anoxic water bodies. The ciliate communities of these lakes share many characteristics with each other and with other freshwater habitats. Anoxic hypolimnia seem to be governed mainly by a few factors, such as low metazoan grazing pressure, relatively stable physicochemical environments, low growth efficiency of heterotrophic bacteria as well as anaerobic protozoa, and low food diversity. These will often lead to the establishment of similarly structured ciliate communities.

Acknowledgements

This work received financial support from Studienstiftung des deutschen Volkes and the Natural Environmental Research Council (UK). We would like to thank Ruth Hindle and Sabine Stelter for technical help and Eduardo Vicente and Maria R.Miracle for their co-operation during our sampling programme at Arcas. Genoveva Esteban kindly provided the silver preparations of ciliates.

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Received on June 28, 1995; accepted on October 18, 1995