A Comparison of the Growth Characteristics and Spatial Distribution of Hypolimnetic Ciliates in a Small Lake and an Artificial Lake Ecosystem

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Within the anaerobic hypolimnion of a small eutrophic lake, a well defined and stable community of large ciliates was present throughout the period of summer stratification. Peak ciliate densities occurred at the oxic/anoxic interface; ciliate numbers then declined in an approximately exponential manner with increasing depth. Two thermally stratified 2 m high artificial lake ecosystems were constructed in which an anaerobic hypolimnion developed. Within these model lakes, populations of hypolimnetic ciliates were maintained for many months. Community structure, growth characteristics and spatial distribution of the ciliates were similar to those in the natural environment.

INTRODUCTION

The work described here forms part of a larger study on the ciliated protozoa present in the anaerobic hypolimnia and sediments of the Shropshire/Cheshire Meres. The Meres comprise about 60 small fertile eutrophic lakes occupying hollows in the glacial drift which overlies much of the Shropshire/Cheshire Plain in the North West Midlands of England. The lakes range from 1–70 ha in area and from 1–27.5 m in depth. In general, those meres which exceed about 5 m in depth thermally stratify during the summer and develop an anaerobic hypolimnion.

In the 17 meres we have examined, a community of large ciliates ($> 150 \mu\text{m}$), dominated by *Loxodes magnus*, *Loxodes striatus*, *Frontonia leucas*, *Spirostomum teres* and *Prorodon* sp., occurs in the deep water sediments during the period of winter mixing. Once thermal stratification occurs and the bottom water becomes anaerobic, these ciliates leave the sediments and move up into the water column. They then progressively colonize the hypolimnion in phase with the upward spread of anoxic conditions, returning to the sediment following autumnal destratification. A similar hypolimnetic ciliate community has been reported from other eutrophic lakes by Goulder (1972), Bark (1981) and Finlay (1981).

With the primary aim of helping to understand the factors affecting ciliate distribution within the water column and to test putative correlations derived from field studies, two artificial lake systems, or microcosms, were constructed. These microcosms were designed to mimic a thermally stratified eutrophic lake as closely as possible. This paper describes the construction and operation of the microcosms. Ciliate distribution and growth rates in the artificial lakes are compared with data on population growth and distribution in Kettle Mere.

METHODS

Field study site. All field data presented here were obtained from Kettle Mere (location UK Grid Reference SJ 418341). This is one of the smaller meres with an area of only 1.7 ha. It is, however, relatively deep with a maximum depth of 7.3 m. It is one of the more productive sites and was probably formed as a kettle hole during the retreat of the late Devensian ice fronts. The mere is surrounded by trees on some 75% of its perimeter and the remaining edge is flanked by a steep slope. As a result of its extremely sheltered location Kettle Mere exhibits very stable thermal stratification. The lake normally stratifies thermally in April and does not become fully mixed again

until late November. For 3–4 months each summer, during June, July, August and September, some 75% of the total lake volume is anoxic, the oxygenated epilimnion being little more than 1 m in depth.

Field methods. Sampling of Kettle Mere was carried out throughout the spring, summer and autumn of 1983 at the deepest point in the mere, which was marked by a buoy. Sampling was undertaken at 7–10 d intervals, although for short periods more frequent samples were obtained. The normal procedure was to use a pHOX 63TE oxygen meter equipped with a Mackereth probe to determine the oxygen and temperature profile. Water samples were collected using a pump-operated tube sampler with a flat disc aperture at its lower end, at either 10, 20 or 25 cm intervals in the region of the oxycline and normally at 50 cm intervals throughout the rest of the hypolimnion. In the epilimnion, samples were taken at either 50 cm or 1 m intervals. Samples collected in glass bottles were fixed in the field for subsequent Winkler dissolved oxygen titration (Mackereth *et al.*, 1978) so that oxygen profiles precisely related to ciliate distribution could be plotted. Samples in plastic bottles collected for counting were immediately placed in insulated boxes in order to minimize temperature changes and exposure to light. Ciliate counts were completed within 6 h of sample collection. Tests showed that population levels changed little in this time if care was taken to prevent temperature shock and an air space was left at the top of the bottle.

After preliminary examination of the samples to identify the species present, subsamples were fixed and ciliate numbers determined by direct counting using a binocular dissecting microscope at $\times 16$ magnification. After

Fig. 1. Diagram of the artificial lake ecosystem or microcosm designed to simulate a stratified eutrophic lake.

shaking the sample, 2 or 5 ml subsamples were pipetted into 5 cm grid marked Petri dishes and fixed with Lugol's iodine. Usually, five replicate subsamples were counted and cell density l^{-1} was calculated.

A detailed bathymetric survey was also undertaken and areas and volumes contained by each 0.5 m contour were determined. Whole lake populations of the various ciliate species could then be calculated.

Design and management of the artificial lake. The basic features of the laboratory microcosm are illustrated in Fig. 1. The lake system consisted of a 2 m high, 20 cm diameter perspex tube (total capacity approximately 60 l) with 36 sampling ports, set at 5 cm intervals, down one side of the column. The columns were filled with Kettle Mere water overlying approximately 20 cm of deep water sediment and were maintained in a cold room with an air temperature of 12 °C. Thermal stratification of the columns was achieved by circulating warmer water through water jackets surrounding the lower, middle and upper regions of the column, establishing a temperature gradient from 22 °C at the top to 12 °C at the bottom. Heat loss and consequent mixing was avoided by insulating the water column, in between the water jackets, with 'Bubble Glaze' (Transatlantic Plastics, Garden Estate, Ventnor, Isle of Wight, UK). The upper region of the column was illuminated for 16 h per day to create a photic zone and maintain aerobic conditions. The rest of the column was kept dark with black polythene sheeting.

Within the natural habitat, the two species of *Loxodes* and *Prorodon* sp. feed principally on algae which sediment out, or actively move down, from the upper oxygenated water. *Frontonia leucas* and *Spirostomum teres* filter feed on smaller particles, principally bacteria. In order to provide a comparable food source within the microcosms, *Euglena gracilis* was added to the top of the columns. Axenically cultivated *Euglena* were centrifuged at 2000 r.p.m. for 2 min and resuspended in Kettle Mere water to give a final concentration of approximately 1×10^8 cells l^{-1} , and then fed into the columns by means of a peristaltic pump at a rate of approximately 0.5 l d^{-1} . Addition of *Euglena* was discontinuous and was controlled by a time clock giving four 45 min 'on' periods. This intermittent, relatively high speed dosing was used to prevent clogging of the feed lines. No other nutrients were added to the microcosms. Either the top sampling port, or one near the bottom, was used as an outflow.

Oxygen and temperature profiles within the microcosms were normally determined by progressively lowering a pHOX 63TE combined oxygen and temperature probe down through the column. However, agitation of the probe, necessary for its correct functioning, clearly caused a small degree of mixing within the column. When more precise oxygen determinations were required, these were obtained by Winkler titration of samples drawn from the sampling ports. Water drawn from the ports was also used to determine redox potential with a bright platinum electrode and to determine sulphide colorimetrically with a Hach Hydrogen Sulphide Test Kit (Cam Labs, Cambridge, UK).

Two methods were used to measure ciliate populations within the columns. Integrated samples were taken using a tube sampler consisting of a 1 cm diameter rubber tube with a weight and a length of string attached to its lower end. This was lowered into the column until its end was just above the mud surface. A bung was then inserted in the top end of the tube and the tube was withdrawn from the column by means of the string attached to its lower end. The water sample could then be run from the tube into a bottle. This method of sampling was rapid and simple, and could be used to determine mean cell density or total column population but gave no information on ciliate distribution. If distribution profiles were required, samples could be drawn from individual sampling ports. Ciliate numbers were determined in the same manner as those obtained from the lakes.

RESULTS

Field data

Fig. 2 illustrates the vertical distribution of the hypolimnetic ciliates in relation to oxygen and temperature in Kettle Mere, one of the richer meres, on August 16, 1983. Peak population densities occurred at the oxic/anoxic boundary, above which there was a sharp cut off. Ciliate numbers then declined more or less exponentially with increasing depth, the ciliates penetrating 2.5 m into the anaerobic water. These results are typical of any of the meres at any time during the period of summer stratification, although peak ciliate densities may be as low as 10^3 – 10^4 cells l^{-1} in some sites and exceed 10^6 cells l^{-1} in others. Up to 5 m of anoxic water may be colonized.

Fig. 3 is a depth–time chart for oxygen in Kettle Mere from April to November 1983. It shows that anoxic water was first present at the bottom of the mere in mid-April and that the volume of anoxic water increased rapidly until June 5. The anoxic zone then expanded less rapidly until it reached a peak on July 17. A period of dull warm calm weather between August 16 and 21 caused another increase in the volume of anoxic water. Cold windy weather in late August/early September caused considerable mixing and a sharp reduction in the volume of anoxic water, which was followed by another rapid increase between September 5 and 9 when more settled

Fig. 2. Oxygen, temperature and ciliate distribution profiles for Kettle Mere on August 16, 1983. Peak cell densities are given as no. organisms l⁻¹ ± the range.

weather returned. Further unsettled weather in September pushed the oxic/anoxic boundary to below 2.5 m but the lake was again anoxic from 1.5 m in late October and early November, the end of the sampling period.

Throughout the sampling period, the spatial distribution of the hypolimnetic ciliates in relation to the oxygen concentration always followed the pattern shown in Fig. 2. Upward and downward movements of the oxic/anoxic boundary were mirrored by corresponding vertical movements of the ciliate community.

When total lake populations for the various ciliate species were computed for each sampling date a striking correlation with the oxygen chart in Fig. 3 became apparent. Fig. 4 shows changes in the total lake population of *Loxodes magnus*, *L. striatus* and *Spirostomum teres*.

During the expansion of the anoxic zone between April 21 and July 17, all three species steadily increased in numbers. Mean population doubling times during this period, calculated from the regression line, were 7.7 d for *L. magnus*, 10.7 d for *L. striatus* and 10.3 d for *S. teres*. Numbers of all three species then declined slightly but increased rapidly between August 16 and 21 when the oxycline moved upwards. Population doubling times during this period were 3.24 d for *L. magnus*, 1.98 d for *L. striatus* and 3.03 d for *S. teres*. Rapid depression of the thermocline in early September, when ciliates were presumably carried up into the epilimnion by water currents, caused a marked decline in population numbers. A further period of rapid population expansion occurred between September 7 and 9. On this occasion, the population doubling time for *L. magnus* was 2.29 d, for *L. striatus* 2.87 d and for *S. teres* 3.27 d.

Fig. 5 shows the ciliate population distribution in Kettle Mere on September 2 during one of the periods of unsettled weather. On this occasion, the ciliate community did not show its usual sharp cut off at the oxic/anoxic boundary as seen in Fig. 3. Rather, the organisms spread some distance into the aerobic zone presumably as the result of water mixing. This figure does, however, show the characteristic overlap of the ciliate community with the distribution pattern of the main algal species present. *Cryptomonas* appeared to form the main food of *L. magnus*, *L. striatus* and *Prorodon* sp. but the larger *L. magnus* was also seen to consume *Coelosphaerium* and filamentous algae. Fig. 5 also shows sulphide and redox profiles. Sulphide was first detected in the bottom water in May, gradually spreading upwards so that by August sulphide was present almost to the top of the anoxic zone. Sulphide, produced by sulphate reducing bacteria, never exceeded 5 mg l⁻¹ at any point in the water column. Small populations of the photosynthetic

Fig. 3. Oxygen depth-time chart for Kettle Mere during 1983. Oxygen isopleths are given in mg l^{-1} .

Fig. 4. Total lake populations of *Spirostomum teres*, *Loxodes magnus* and *Loxodes striatus* in Kettle Mere during 1983. Population doubling times for the periods April 21–July 17, August 16–21 and September 7–9 are also given.

Fig. 5. Vertical distribution and species composition of the hypolimnetic ciliates and main algal species in Kettle Mere on September 2, 1983 in relation to profiles of oxygen, temperature, redox potential, sulphide and ammonia. Peak cell densities are given as no. organisms l⁻¹ ± the range.

bacteria *Thiopodia rosea* and *Pelodictyon clathratiforme* were present just below the oxic/anoxic interface and these also formed a component of the ciliate diet. Smaller suspended particles such as bacteria formed the main component of the diet of *F. leucas* and *S. teres* although *F. leucas* was occasionally seen to contain larger objects in food vacuoles.

Artificial lake

Fig. 6 shows the typical pattern of colonization of one of the microcosms, column 2, during the 42 d period from November 23, 1983 until January 4, 1984. The column was filled on November 22. During the first 4 d no ciliates were present in the water column but the hypolimnetic oxygen concentration dropped steadily. On day 5, November 26, the oxygen concentration at the bottom of the microcosm had dropped to below 5% and the ciliates had left the sediment and colonized the bottom 30 cm of the water column. The ciliates then progressively colonized the water column in phase with the upward spread of anoxic conditions, increasing steadily in numbers. After 42 d the peak population density at the level of port 10 was 554 000 cells l⁻¹. Oxygen profiles were determined using the oxygen electrode (after removing samples for ciliate counts) so as to avoid removing excessive quantities of water from the column. The true oxygen profile therefore probably displayed a much sharper oxic/anoxic interface than is indicated in Fig. 6. On filling a column, this same pattern of progressive colonization in line with the prevailing oxygen regime was always observed, providing at least a few ciliates were present in either the mud or water.

Fig. 7 shows typical distribution patterns of the major ciliate species in relation to oxygen, temperature and sulphide profiles, for column 1 on March 7, 1984. Oxygen was determined by Winkler titration of samples withdrawn from the ports and there was a very sharp oxycline, similar to that seen in Kettle Mere. Ciliate distribution also showed a markedly similar pattern to that shown for Kettle Mere in Fig. 2, with peak ciliate densities just below the oxic/anoxic interface, cell numbers then declining with increasing depth. Very low numbers of ciliates were, however, present in the oxygenated water. This was probably caused by slight mixing taking place in the epilimnion of the column. Surface temperatures were in fact slightly lower than

Fig. 6. Development and migration of the hypolimnetic ciliate community in relation to the changing oxygen concentration profile in column 2 from November 23, 1983–January 4, 1984 after filling on November 22, 1983. Days 2–7 show a progressive drop in oxygen concentration in the hypolimnion. On day 5 oxygen had dropped to $< 5\%$ at port 33 and ciliates first appeared in the water column having previously been confined to the sediment layer. ■, Oxygen concentration; ●, ciliate population density.

those at the levels of ports 4–6, possibly enough to give rise to slight convection currents (see Fig. 7). The sulphide plot in Fig. 7, again markedly similar to that seen in Kettle Mere itself, shows that sulphate reducing bacteria were also active in the microcosms. Furthermore, a stable community of copepods and cladocerans was present in the aerobic zone (not shown).

Once established, peak ciliate population densities within a column were normally in the range of 5×10^5 – 10^6 cells l^{-1} , similar to peak cell densities observed in Kettle Mere. Populations remained stable within a column for between 4 and 6 weeks, whereupon numbers slowly declined. The maximum population density achieved in either of the columns was in column 1 on October 12, 1983 when 1.53×10^6 ciliates l^{-1} were present at the level of port 9.

Hypolimnetic ciliates can be maintained within these microcosms for many months. Column 1 was originally filled on August 23, 1983. In early May 1984, all of the original ciliate species making up the community were still present, in proportions similar to those found within Kettle Mere. In mid-December 1983, circulation in the water jackets in this column was stopped, allowing the whole water mass to cool to $12^\circ C$ and mix. The upper part of the column was also aerated for a few days, simulating winter mixing, and as a result the ciliate community left the water column and recolonized the sediment. On January 20, 1984, the column was restratified and the normal sequence of colonization of the hypolimnion and population increase once more

Fig. 7. Ciliate vertical distribution and population density in column 1 on March 7, 1984 in relation to oxygen, temperature and sulphide profiles. Peak cell densities are given as no. organisms $l^{-1} \pm$ the range.

occurred. The column was put through another cycle of mixing in late March followed by restratification in late April 1984, the ciliates again showing an identical response. The addition of *Euglena* was discontinued during the two periods of simulated winter mixing.

Ciliate growth rates within the microcosms were also determined on a number of occasions. Total microcosm populations were determined on eight occasions between January 25 and February 13, 1984 in column 1 upon restratification after the first period of simulated winter mixing. Growth rates were obtained from the regression of population against time. The mean population doubling time during this period for *L. magnus* was 8.36 d, for *L. striatus* 4.45 d and for *F. leucas* 4.64 d. Similar sustained population doubling times were recorded on other occasions in the two columns. Typical population doubling times for *Prorodon* sp. were 3–4 d and for *S. teres* 4–5 d.

As was observed in the field, short periods of more rapid growth occurred within the columns. For instance, the minimum population doubling time observed for *L. magnus* was 2.1 d, for *L. striatus* 2.5 d, for *Prorodon* sp. 0.9 d and for *F. leucas* 1.5 d.

DISCUSSION

In all of the meres examined, a stable community of hypolimnetic ciliates dominated by *Loxodes magnus*, *L. striatus*, *Spirostomum teres*, *Frontonia leucas* and *Prorodon* sp. occurred in the anaerobic hypolimnion and the extreme lower limits of the aerobic zone during summer stratification. Ciliate densities were greatest at the oxic/anoxic interface, but declined in an approximately exponential manner with depth. Using the laboratory model lake ecosystems, this community of ciliates could be easily grown and maintained. Within the microcosms, ciliate community structure and spatial distribution was identical to that in the natural environment. The five ciliate species clearly formed a stable, balanced and robust community. The microcosms were open to the atmosphere, yet no loss or gain of species occurred over many months. After initial filling, other ciliate species occasionally developed. They were, however, always replaced by the typical hypolimnetic community within a few days.

It would appear that the five species occupy complementary environmental niches and are not

in direct competition within either the natural or the laboratory systems. Within the natural lake, a variety of particulate food is available and coexistence might be explained by different feeding preferences. Niche separation, however, cannot be simply a function of available food within the artificial environment. In the microcosms, *L. magnus*, *L. striatus* and *Prorodon* sp. all appeared to feed exclusively on the added *Euglena*; *F. leucas* did so in part, although both *Frontonia* and *Spirostomum* have an alternative food source as they are predominantly bacterial filter feeders. The continued input of *Euglena* cells would, either directly or following their consumption, provide nutrients for a decomposer community, this community in turn sustaining *Frontonia* and *Spirostomum*.

We have shown that population growth characteristics in both Kettle Mere and the microcosms are comparable. Within both there was a period of sustained population increase which eventually reached a relatively stable and similar plateau. The longer period of population increase within Kettle Mere was undoubtedly associated with low initial populations and a continual increase in the physical dimensions of the habitat of the ciliates as the oxycline moved upwards between May and July. Within the microcosms too, the period of population increase was considerably extended if the initial population was very small (<100 cells l^{-1}).

During the period of population increase, population doubling times within the microcosms were two to three times faster than those observed in Kettle Mere. This could be associated with either greater or more constant supplies of food being available within the artificial habitat, or with unaccounted population losses, possibly predation, within the Mere. Short periods of more rapid growth, with population doubling times of 1–2 d depending on species occurred in both the natural and artificial lakes.

Growth rates obtained within the columns may superficially appear to be low. Reported minimum generation times for many ciliates are in the range of 2–3 h for small species and 10–30 h for large ones (Fenchel, 1968; Finlay, 1977; Taylor, 1978). However, these rapid growth rates are invariably achieved in highly artificial situations with high nutrient levels. It is recognized (Finlay, 1982) that such laboratory based estimates of generation times probably overestimate *in situ* growth rates. Despite this, most protozoan production studies have been based on laboratory derived growth rates (Finlay, 1978), and this probably results in overestimates of productivity. A few workers (Heller, 1977 and Weiler & Chisolm, 1976 for dinoflagellates; Hagstrom *et al.*, 1979 for bacteria) have used the frequency of dividing cells to estimate *in situ* growth rates. Schonborn (1977) used changes in the numbers of empty and occupied tests to estimate production rates of testate amoebae. Such studies suggest that mean generation time of a few days may be expected for protozoa in nature. However, ciliates are highly opportunistic and are capable of very high feeding rates (Fenchel, 1980*a, b*) when sufficient concentrations of food particles are available, so that short and intermittent periods of rapid growth can be expected. Consequently, the rates of population increase obtained in the microcosms are probably typical of the natural habitat.

In the last 15 years, there has been an increasing interest in the use of microcosms in both aquatic and terrestrial ecology and whole volumes have been produced on their use, design and application (Geisy, 1978). In the great majority of recent studies, microcosms have been designed and used to study the environmental fate of hazardous materials such as pesticides (Giddings *et al.*, 1979; Pritchard *et al.*, 1979; Cole *et al.*, 1976). Older studies, however, were primarily concerned with fundamental ecology (McConnell, 1962; Beyers, 1963). Some authors (Harte *et al.*, 1978) claim close resemblance to the natural system and the achievement of good replicability. Others (Slater & Hardman, 1982) have expressed grave doubts concerning the claims of reproducibility and correspondence to the natural environment.

Lake microcosms have been designed for many different purposes and in many shapes and sizes ranging from simple sediment cores with overlying water (Pritchard *et al.*, 1979) to large deep tanks (Strickland *et al.*, 1969) up to *in situ* cylindrical lake enclosures of 45.5 m diameter, which create a complete thermally stratified lake within a lake (Lund, 1978). We have not, however, found reports of a simple laboratory model lake system which incorporates thermal stratification and an anoxic hypolimnion such as the one described here. The system was cheap to construct and is easy to maintain. Furthermore, at least as far as the hypolimnetic planktonic

ciliates are concerned, it appears in many ways to mimic the natural environment. The ultimate aim in the construction of the model lake systems was to study ciliate community behaviour and species interactions and to test putative correlations derived from field data.

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