A STUDY OF THE FRESHWATER DINOFLAGELLATES CERATIUM HIRUNDINELLA AND CERATIUM FURCOIDES WITH SPECIAL REFERENCE TO THEIR TAXONOMY AND RECENT HISTORY IN THE ~~ENGLISH~~ LAKE DISTRICT

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by

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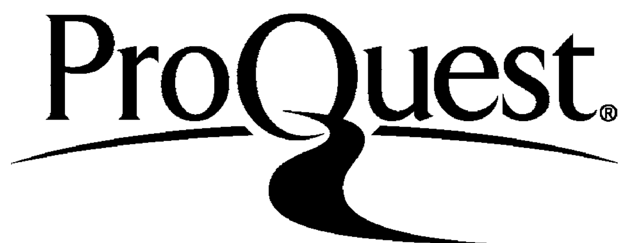

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To my parents

ERRATA

Page	Line	Corrected Text
32	23	... = $\Sigma \frac{(x-\bar{x})^2}{\bar{x}}$
35	6	as above
103	21	... of equal length for C. hirundinella ...
108	8	... is calculated ...
110	23	$U = n_1 . n_2 + \frac{n_1(n_1+1)}{2} - R_1$
110	24	$U = n_1 . n_2 + \frac{n_2(n_2+1)}{2} - R_2$
140	15	... may thus illustrate ...
145	15	... not between ...
151	24	... low cyst counts ...
164	18	... observed in May.

ABSTRACT

Motile cells and cysts of *Ceratium* were studied using light and scanning electron microscopy. Two species, *C. hirundinella* and *C. furcoides*, were distinguished on the basis of the cell length to breadth ratio, the shape of the epitheca and the arrangement of apical plates. Cysts differed in shape and in the length of the horns. Analytical scanning electron microscopy demonstrated the presence of silicon in the multi-layered granular wall of the cysts of both species.

The vertical distribution of viable cysts of *C. furcoides* and *C. hirundinella* was studied in 8 cm cores taken over three seasons from Esthwaite Water, Cumbria. The diatom *Stephanodiscus parvus* was used as a marker species, to attribute a time scale to the cores. Some agreement was demonstrated between cyst numbers and past populations of *Ceratium* spp., although the majority of cysts occurred in the upper 4 cm of the cores. A study of the proportion of *C. hirundinella* to *C. furcoides* cells from 1946-1986 showed that the ratio of each species changed markedly over this period. It was concluded that the relative numbers of each species were determined by the proportion of cysts germinating in the spring, with parasitism an important factor in controlling cyst viability.

Germination of the cysts of both species was induced in the laboratory. The excystment of *C. furcoides* was achieved down to a depth of 5½ cm, from cysts with an equivalent age of approximately 7 years. Cysts from sediment which had been left

to dry out failed to germinate.

The *Ceratium* populations of several southern sites were also studied. The number of *Ceratium* cells was shown to decline when the water column was disturbed, either by the input of water through high velocity jets, or the failure to form a stable thermocline. *C. hirundinella* was observed to be the more numerous of the two species in the reservoirs studied, but in Virginia Water Lake *C. furcoides* occurred in greater numbers.

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List of Symbols and Abbreviations

Symbol	Meaning
d	Difference between the ranks of two variables
F.B.A.	Freshwater Biological Association
g	Acceleration due to gravity
H_o	Null hypothesis
I	Index of dispersion
N	Total number of samples
N^2	Brunt-Väisälä frequency
n	Number of individuals
n	Taxa found only in net hauls
p	Samples taken from pontoon
r_s	Spearman's Rank Correlation Coefficient
s	Samples taken from shore
s^2	Variance
S.E.M.	Scanning Electron Microscope
T	Term used to adjust for tied ranks
U	Mann-Whitney U Test statistic
v	Degrees of freedom
$\bar{x}$	Mean value of variable x
z	Depth
α	Unknown probability of occurrence of calculated data
$\delta\rho$	Change in water density over the relevant depth interval
δz	Change in depth over the relevant depth interval
ρ	Density of water
$\bar{\rho}$	Average density of water over the relevant depth interval
Σ	Sum of terms
σ	Standard deviation
χ^2	Chi squared value
$\chi^2 r$	Approximation to the χ^2 value using the Friedman Two-Way Analysis of Variance by Ranks
+	Taxa in which less than 20 cells/colonies were counted

CHAPTER 1

INTRODUCTION

The large motile cells of the dinoflagellate genus Ceratium are a conspicuous and often major component of the summer phytoplankton of many temperate lakes and reservoirs. For more than half a century a large number of studies have centred on the taxonomy of the genus and on various aspects of the ecology and physiology of its constituent freshwater species. In recent years it has become apparent that, despite this extensive background of work, there are difficulties concerning the taxonomic separation of certain species. The freshwater species generally recognised are Ceratium hirundinella (O.F.Müller) Bergh., C. furcoides (Levander) Langhans, C. brachyceros Daday, C. cornutum (Ehrenberg) Claparède and Lachmann and C. carolinianum (Bailey) Jorgensen, of which the first two and their form types have given rise to the greatest confusion. An additional species, C. rhomvoides Hickel, has recently been proposed by Hickel (1988b).

The present work concerns only C. hirundinella and C. furcoides, which often occur in the same body of water. The classical work of Langhans (1925) and Entz (1927) clearly describe the features of the motile and cyst stages of both species. However, there has been a tendency amongst ecologists and some taxonomists to combine the two species as C. hirundinella. A possible cause of this may be the great variation of cell size and shape (e.g. Huber-Pestalozzi, 1950;

Hauge, 1958; Dottne-Lindgren and Ekbohm, 1975; Krupa, 1981b). The phenomenon of cyclomorphosis, differences associated with seasonal changes of one morphological form to another, shown by this genus (e.g. Pearsall, 1929; Hutchinson, 1967) has also tended to cause confusion between the species. For the purpose of this study it was essential to establish the number of Ceratium species present and to be able to distinguish between them. A detailed comparison of the taxonomic features involved are considered in a later chapter.

The life cycles of C. hirundinella and C. furcoides in temperate lakes and reservoirs have been well documented (e.g. Hauge, 1958; Chapman, 1981; Heaney, Chapman and Morison, 1983; Hickel, 1985). The motile cells of both species appear in the water column in late spring, increasing in numbers by binary fission until late summer when extensive "blooms" may be formed. To date, sexual reproduction has been recorded in only three of the freshwater species, C. cornutum (Stosch, 1965), C. furcoides (Hickel, 1988a) and C. rhomvoides (Hickel, 1988b). During September and October the Ceratium phytoplankton population declines, usually accompanied by the formation of cysts which sink to the bottom of the water basin. The cysts overwinter in the surface sediment and provide an inoculum for growth the following year. Excystment occurs in spring, generally when water temperatures have exceeded 4°C (Huber and Nipkow, 1923; Heaney, Chapman and Morison, 1983). However, in some temperate lakes the water temperature never falls below 4°C, suggesting that other factors must be responsible for the timing of

excystment (Heaney, Lund, Canter and Gray, 1988). In tropical and sub-tropical lakes C. hirundinella, with its narrow tolerance range for water temperature, may form oversummering cysts in order to survive when the temperature exceeds 25°C, as observed by Pollingher (1986a) in Lake Kinneret, Israel.

The ecology of Ceratium has been the subject of extensive study over the last 20 years. The majority of workers have compared seasonal abundance of Ceratium for one or two years with physical and chemical environmental factors (e.g. Hauge, 1958; Pfiester, 1971; Dottne-Lindgren and Ekbohm, 1975; Harris, Heaney and Talling, 1979; Heaney and Talling, 1980a, b; Hickel, 1985). A few studies have recorded changes over more extended periods of time (e.g. Heaney and Butterwick, 1985; Heaney, Lund, Canter and Gray, 1988).

The distribution of Ceratium within the water column is influenced by the possession of flagella, which enable the motile cells to undertake depth regulation through vertical migration in response to internal physiological changes and environmental factors (Talling, 1976). One such variable is the diel light regime (Talling, 1971). Motile cells avoid the water surface layers in the morning (Heaney and Talling, 1980a, b) and sub-surface maxima are produced (Harris, Heaney and Talling, 1979). Marked horizontal patchiness and local discolouration of the surface layers may be created within lakes as a consequence of such vertical migrations coupled with horizontal water movements (Heaney and Talling, 1980a, b).

Observations on the rate of population increase (e.g.

Heaney and Talling, 1980a, b) and the determination of growth rates by the use of phased cell division (e.g. Heller, 1977; Frempong, 1982; Sommer, Wedemeyer and Lowsky, 1984) have shown that populations of Ceratium are relatively slow growing with a minimum population doubling time of about 5 days (Heaney, 1976). Culture work (Heaney and Butterwick, 1985) and fieldwork (Heaney, Chapman and Morison, 1983) have shown that the rate of growth slowed at temperatures below 10°C. The slow growth rate prevents the rapid replacement of cells lost through predation, disease, parasitism or via the lake outflow. Predation on Ceratium is generally regarded as low (Reynolds, 1986), due to the large size of the motile cell, but fungal parasites have recently been recognised as exerting a major influence on Ceratium population dynamics (Heaney, Lund, Canter and Gray, 1988). Aphanomycopsis cryptica Canter and Lagenidium species parasitise the motile cells of C. furcoides and C. hirundinella, and Rhizophydium nobile Canter the cysts of C. furcoides (Canter and Heaney, 1984). Inspite of these constraints very large populations of Ceratium can develop. For example, in Esthwaite Water during the 1970s the Ceratium population exceeded 600 cells ml^{-1} (F.B.A. data). It was suggested by George and Heaney (1978) that in populations of this density self-shading may be the limiting factor.

Although a great deal of ecological and physiological work had been undertaken on the motile cells of Ceratium, until recently the cyst stage had been largely overlooked by biologists with the exception of Entz (1925) and Huber and

Nipkow (1923) who recognised the importance of overwintering cysts in providing an inoculum of motile cells to the phytoplankton. Since then observations have been rare, possibly because of low numbers of cysts in the sediment and the difficulties involved in extracting them. Fossil marine dinoflagellate cysts have long been studied by palynologists. Biological interest was renewed when a link between excystment and the timing and extent of red-tides was established (Steidinger, 1975; Anderson and Morel, 1979).

The vertical (Livingstone, 1979) and horizontal (Chapman, 1981; Heaney, unpublished data) distribution of Ceratium cysts within lake sediments had been studied previously, but in the past no distinction was made between C. furcoides and C. hirundinella. The present work sought to compare the vertical distribution of Ceratium cysts within the sediment and past populations of motile cells, making use of long term phytoplankton records and historic samples collected from Esthwaite Water in the Lake District. Diatom assemblages were studied in an attempt to assess the rate of sedimentation and ascribe a date to sections of the core. In order to establish the viability of cysts below the surface sediment a series of excystment experiments were conducted in the laboratory.

The original aim of this project had been to study the population dynamics of C. hirundinella and C. furcoides within a Thames Valley reservoir and determine the importance of recruitment of motile cells from the overwintering cyst population. However, during 1984/5 a series of sites, many the

subject of extensive work, were lost due to permanent or temporary drainage of water, as a consequence of changes in management policy by Thames Water. Phytoplankton counts were continued on one reservoir in order to monitor the effects of disturbance of the ecosystem due to water drainage. In addition a qualitative survey of the proportion of *C. hirundinella* to *C. furcoides* in several reservoirs and a lake in the Thames Valley was conducted.

CHAPTER 2

MATERIALS AND METHODS

2.1 Routine Sampling of Island Barn Reservoir

General Sampling

Weekly water samples and ecological measurements were taken over a two year period from a boat moored at Buoy F, towards the centre of the reservoir (maximum depth at Buoy F = 8.5 metres). Temperature and oxygen profiles were measured with a combined thermistor and oxygen electrode (Yellow Springs Instruments). Water samples were taken at regular intervals (1 m, 3 m, 5 m, 7 m) through the water column and transferred to the Thames Water Biological Laboratories at Wraysbury where chlorophyll, carbon and nutrient levels were assessed using standard techniques. Zooplankton samples were collected by taking two vertical net hauls, the contents of which, together with two "washings", were retained. A finer phytoplankton net was trawled beside the boat, just below the surface of the water, for between 30 seconds and 3 minutes depending on the abundance of the phytoplankton. The contents of the net and two "washings" were retained.

When conditions prevented a boat being launched, samples were taken from the end of a wooden pontoon extending out into deep water (maximum depth at pontoon = 5 metres). Low water levels during the period of drainage (October 1985 to April 1986) made even this restricted form of sampling impossible (see Chapter 7, page 258). Between these dates dip samples and a

single temperature and oxygen reading were taken from the shore.

Collection of Samples for Phytoplankton Counts

Weekly water samples were collected from the upper 5 metres of the reservoir using either a Patalas Sampler (Patalas, 1954), or a weighted five metre long tube for the collection of integrated samples (Lund, 1949). The former is a rectangular, perspex box, with an upper and lower lid which close when the apparatus is pulled back up to the surface of the water. It has the advantage of collecting large volumes of water at a time (7 litres). The samples, of between 3 litres and 15 litres depending on the time of year, were fixed immediately with Lugol's Iodine before returning to the laboratory.

Lugol's Iodine, with added acetic acid, was chosen as a fixative. The cells stained brown/yellow to black, which enabled them to be easily distinguished for counting purposes. However, overstaining did on occasions obscure surface structures and made identification more difficult. The addition of iodine increased the weight of the cells, thereby decreasing the settling time necessary to concentrate samples by sedimentation. Samples preserved with iodine needed to be monitored, as in time the iodine became colourless, a process slowed by storage in the dark.

Preparation of Samples for Phytoplankton Counts

For most of the year the number of dinoflagellates was

less than 1 cell ml^{-1}. Therefore it was necessary to concentrate the phytoplankton samples to enable a statistically significant number ($\geqslant$ 20) to be counted in a reasonable period of time. Several methods for concentrating phytoplankton were investigated and for various reasons rejected. Filtration of several litres of water through a Millipore filter successfully concentrated the phytoplankton. The filter was inverted into a petri dish of water and any remaining material was removed with a paint brush in a jet of distilled water. The "washings" of the paint brush were added to the petri dish. Careful rinsing ensured that the amount of material lost on the filter and the paint brush was low (< 5%). However, to obtain statistically significant numbers of Ceratium, particularly during the spring and early summer when numbers of cells were low, it was necessary to filter large volumes of water, which resulted in rapid blocking of the filter when the number of other phytoplankton species was high. The use of two or more filters increased the loss of material to an unacceptable level so other techniques were investigated. The Inverted Microscope technique of concentrating samples in sedimentation chambers (Lund, Kipling and LeCren, 1958), was considered as an alternative, but since it prevented the re-orientation of cells to assist identification and did not allow individual cells to be removed for other purposes, (e.g. scanning electron microscopy), it was also rejected.

A settling method (Sukhanova, 1978) was selected to concentrate the phytoplankton samples. On returning to the

laboratory, the water sample, preserved with Lugol's Iodine, was vigourously shaken to reincorporate any organisms that had already sunk to the bottom of the container. Sub-samples were immediately transferred to 1 litre stoppered measuring cylinders, 40 cm in height. The sample was allowed to settle for one week. A rubber tube of appropriate length was used to siphon water off to within 4 cm of the base of the cylinder. A clamp restricted the flow of water to a few drops a second. The remaining sample was poured into a 100 ml cylinder, 20 cm in height. The larger cylinder was rinsed twice with distilled water and the "washings" added to the smaller vessel. The sample was allowed to settle for 2 days when the water was again siphoned off to within 2 cm of the bottom of the cylinder. The remaining sample was transferred into a calibrated glass sample tube. The cylinder was rinsed twice with distilled water and these "washings" were added to the sample together with a few drops of Lugol's Iodine where necessary. The sample was made up to 20 ml with distilled water, stoppered and stored in the dark.

The main advantage of this technique was that large samples could be concentrated using simple apparatus and allowing other work to be undertaken whilst samples were settling or being siphoned. The prime disadvantage was loss of material through either failure of cells to sink, where the specific weight of the cells was less than unity, or cells sticking to the walls of the settling cylinder. The rinsing of the cylinders with distilled water reduced the possibility of the latter, but the former may have resulted in the

underestimation of some algal groups, in particular buoyant cyanophytes.

For this reason net haul samples were also examined. On returning to the laboratory (approximately 1 hour after collection), the live phytoplankton net haul samples were examined in a petri dish using an Olympus SZH binocular microscope. The genera present were recorded and their relative abundance noted (i.e. abundant, common, frequent, occasional or rare). This provided a useful guideline when the preserved, quantitative samples were counted. It also gave an indication of those genera present in numbers too low to register in the less concentrated samples. A 20 ml sub-sample of the net haul was preserved with Lugol's Iodine and retained for further reference.

<u>Counting the Phytoplankton Samples</u>

Although a range of alternative techniques are now available for estimating phytoplankton numbers, visual counting remains the most efficient and the most informative (Butterwick, Heaney and Talling, 1982). Although more time consuming, the accuracy of the counts, particularly at lower densities, and the knowledge of the species composition of the sample gained, cannot be equalled by electronic or chemical estimations. In this study the numbers of each species, particularly of the two dinoflagellate genera, was of prime importance and so a visual counting technique was adopted.

The concentrated phytoplankton sample was resuspended by

inverting the sample tube 20 times. A 1 ml aliquot was immediately withdrawn using a new, disposable calibrated pipette and introduced into a Sedgewick-Rafter Chamber (McAlice, 1971). The rectangular shaped chamber (50 mm x 20 mm) has a depth of 1 mm and a volume of 1 ml. The base is sub-divided into 1000 squares each with an area of 1 mm^2. The depth of the chamber enabled the largest components of the phytoplankton to be studied, which was an important consideration in the case of Ceratium cells. However, the increased depth also prevented the chamber being viewed at magnifications greater than x20, since stronger objectives were too great in length, making it impossible to identify smaller components of the phytoplankton. When a large percentage of the phytoplankton population was composed of small organisms a few drops of the sample were examined on a slide under a coverslip for identification purposes.

It was essential that the contents of the aliquot were distributed randomly throughout the chamber, to enable only a fraction of the slide to be counted when dense samples were encountered. Filling the chamber from one corner with the remainder of the coverslip in place, the APHA method (McAlice, 1971), failed to give the required random distribution of cells and often resulted in the production of a large air bubble in the corner of the chamber. The use of an uncovered chamber (Ricker, 1937) was considered impractical, since a count could take up to 2 hours and evaporation would have distorted the results. A random distribution was produced by introducing the

sample gradually along the length of the chamber, simultaneously sliding the coverslip along to cover the sample already deposited (for statistical analysis, see page 36).

Identification of Phytoplankton

Although this study was primarily concerned with variation in Ceratium numbers, the importance of other components of the phytoplankton was recognised. Dinoflagellates were identified, if possible to species level, using Huber-Pestalozzi (1950). Ceratium species were determined using variations in morphology discussed in Chapter 4 (including the shape of the epitheca, the cell length to breadth ratio and, where visible, the arrangement of the apical plates), and by reference to Entz (1927) and the Fritsch collection (held at the F.B.A.). The remaining phytoplankton groups were identified to generic level, with species defined in some cases. Belcher and Swale (1976, 1979) and Bellinger (1980) were used as general guides. Some groups proved difficult to identify without more detailed observations, for example small flagellates. For the purpose of this study it was not regarded as necessary to separate these genera. Therefore, in such cases a single term includes many taxa, for example small pennate diatoms.

Recording Phytoplankton Counts

A large proportion of the taxa observed take the form of individual cells, for example C. hirundinella, Staurastrum sp. and Stephanodiscus astraea. Counting was therefore

straightforward with the number of individuals present in a given volume recorded.

Colonial taxa require more data to be noted, i.e. the number of colonies and the number of cells in a colony. In the case of genera like *Asterionella* and *Tabellaria* with only a few cells (< 20) it is not too difficult to count the cells individually. However, when the number of cells in a colony rises above 30 compromises have to be made to shorten the counting process. Numbers of some genera, for example *Fragillaria*, *Melosira*, *Anabaena* and *Tribonema*, were assessed by counting 10 cells and using their combined length to estimate the total number of cells. Ideally the length of the chain (or ribbon) of cells would be measured with an eyepiece graticule, however, the convoluted position in which many colonies were arranged, particularly *Anabaena*, made this very difficult. Those colonies formed of very large numbers of cells and not arranged in a chain, for example *Microcystis* and *Aphanizomenon* were recorded as number of colonies and the percentage of the slide (or section of the slide) that they occupied.

The Number of Cells Counted

The distribution of organisms within a sample, or sub-sample, is often contagious, making estimates of population size based on only low cell counts unreliable. In order to ensure an accurate approximation of the total population large samples would appear necessary. However, a slight increase in statistical accuracy will not always justify the extra time

taken. A compromise has to be reached between statistical accuracy and the time available (Elliott, 1983).

The problem of how many cells to count has been considered by a number of authors and a range of criteria proposed (e.g. Venrick, 1978). Table 2.1 shows the number of cells from a random distribution that need to be counted in order to achieve the required level of accuracy (Lund, Kipling and Le Cren, 1958).

Table 2.1 Size of counts with the corresponding accuracy obtained

Number of Cells	Approximate 0.95 Confidence Limits (% of Count)	(Range)
4	100	0 - 8
16	50	8 - 24
100	20	80 - 120
400	10	360 - 440
1,600	5	1,520 - 1,680

For the purposes of this study it was decided that variation of ± 50% was acceptable (i.e. the doubling in numbers of a species could still be recognised), but where possible variation would be reduced to ± 20%. Therefore, the aim was to count 100 cells of the most numerous species with at least 20 cells of the remaining species. In some samples this was not possible without a substantial increase in labour and in such cases only the presence or absence of a species was noted.

The Variation between Samples from Different Locations within the same Reservoir

It was considered necessary to assess the extent to which a sample from a single location is representative of the whole reservoir. Water samples of 1 litre were taken in the usual way from 8 sites selected at random on Island Barn Reservoir. On returning to the laboratory, samples were concentrated in the usual way (page 25). The total number of colonies for the three most numerous genera (Anabaena, Eudorina and Oscillatoria), were assessed in each of three Sedgewick-Rafter Chambers for each location. The mean values for each site are shown in Table 2.2.

The variance to mean ratio, also known as the index of dispersion (I), gives an indication of the distribution pattern where:-

$$I = \frac{s^2}{\bar{x}}$$

If colonies are distributed randomly between the sites, i.e. a Poisson distribution, the sample variance (s^2) would be expected to be equal to the sample mean ($\bar{x}$). The expression I(n-1) has been shown to give a good approximation to χ^2 (Elliott, 1983) and was used to calculate the χ^2 values in this study.

$$\therefore \chi^2 = I(n-1) = \frac{s^2(n-1)}{\bar{x}} = \frac{(x-\bar{x})^2}{\bar{x}}$$

Table 2.2 The mean number of colonies for three phytoplankton species taken from random sites on the same reservoir

Site	Anabaena	Eudorina	Oscillatoria
	Genus		
1.	40.3	3.0	4.7
2.	29.7	8.0	2.0
3.	22.7	7.3	2.0
4.	63.7	3.7	9.3
5.	99.0	4.7	26.7
6.	116.0	5.7	30.0
7.	117.7	4.7	35.3
8.	79.3	7.3	16.0
$\bar{x}$	71.0	5.5	15.7
s^2	1442.1	3.4	178.2

The null hypothesis (H_o) is that $s^2 = \bar{x}$, i.e. a random distribution prevails. The degrees of freedom, in all three calculations, is represented by (n-1), which equals 7. The χ^2 values for Anabaena and Oscillatoria ($\chi^2_{\alpha,7}$ = 142.09 and $\chi^2_{\alpha,7}$ = 79.2, respectively) both exceed the value of $\chi^2_{0.01,7}$ = 18.4753 obtained from tables (White, Yeats and Skipworth, 1985). Thus the null hypothesis is rejected at the 99% probability level, and the distribution must be regarded as non-random. In the case of Eudorina the value of $\chi^2_{\alpha,7}$ = 4.3 is less than the

tabulated value of $\chi^2_{0.05,7}$ ($\chi^2_{0.05,7}$ = 14.0671). The null hypothesis cannot therefore be rejected at the 95% probability level and the presence of a random distribution cannot be disproved.

All three genera, the only ones numerous at the time of sampling, were of a colonial habit. However, it has been shown by some authors (e.g. George and Heaney, 1978) that Ceratium is non-randomly distributed within a lake, although this is disputed by Dottne-Lindgren and Ekbohm (1975). The time available allowed for the collection and counting of only one set of samples each week. Therefore the non-random distribution of some taxa suggests that the data collected, although appropriate for seasonal comparisons, cannot be regarded as representative of the whole reservoir, and therefore should not be used as a basis for estimates of total populations.

The Variation between Sub-Samples from the same Initial Sample

In some instances only a single sub-sample was counted for a given sample. It was therefore important to determine that the sub-sample was representative, i.e. that cells were distributed randomly in the concentrated sample. Ten sub-samples from the same concentrated sample, taken at the time of the summer maxima from Queen Mother reservoir, were counted in the usual way. The distribution pattern of the counts obtained was assessed as previously, using the index of dispersion.

$H_0 : s^2 = \bar{x}$

$H_1 : s^2 \neq \bar{x}$

$\Sigma x = 501$

$n = 10$

$\bar{x} = 50.1$

$$s^2 = \frac{(x-\bar{x})^2}{(n-1)} = 58.77$$

$$\chi^2 = \frac{s^2(n-1)}{\bar{x}} = \frac{58.77(10-1)}{50.1} = 10.56$$

Degrees of freedom = (n-1) = (10-1) = 9

$\chi^2_{\alpha,9} = 10.56$

from tables $\chi^2_{0.05,9} = 16.9190$

$\therefore \chi^2_{\alpha,9} < \chi^2_{0.05,9}$

$\therefore$ H_0 cannot be rejected at the 95% level of probability.

Therefore the existence of a random distribution cannot be disproved. A single sub-sample can thus be regarded as being representative of the whole concentrated sample. To ensure that the sample mean ($\bar{x}$) was representative of the population mean (μ), the 95% confidence limits of the mean were calculated. The following calculation establishes the upper and lower values of a range within which the true population mean would be expected to be on 95 out of 100 occasions (Elliott, 1983).

$$\bar{x} \pm t\sqrt{\frac{\bar{x}}{n}}$$

where t is obtained from tables (when 2 Q = 0.05 and v = n-1).

Therefore, for this data:-

$$t\sqrt{\frac{\bar{x}}{n}} = 2.262\sqrt{\frac{50.1}{10}} = 5.063$$

Confidence limits of the mean = 50.1 ± 5.1

The population mean would be expected to lie between 45.0 and 55.2 in 95 out of 100 occasions. Therefore, the 95% confidence limits indicate a possible deviation of $\pm$ 10% from the sample mean. This is statistically acceptable and indicates low variation in the number of cells between sub-samples

The Variation Within a Sub-Sample

In those samples in which the entire Sedgewick-Rafter Chamber was counted a non-random distribution was unimportant, provided it did not result in cells becoming obscured. However, some species were frequently too numerous to count throughout the whole chamber. The concentration of a sample was determined by the number of Ceratium cells. For example, during the late summer when Ceratium numbers would be expected to be at their maximum only low volumes of water (e.g. 1 litre) would be collected, whilst during the spring and early summer when Ceratium numbers would be expected to be low, large volumes of water would be collected (e.g. > 5 litres). Large numbers of diatoms would often be present in spring samples.

Before counting fractions of a Sedgewick-Rafter Chamber it was necessary to ensure that cells/colonies were distributed randomly. Four genera were selected, that represented both colonial (Eudorina and Anabaena) and single cell habits

(Ceratium and Stephanodiscus) and were present in sufficient numbers. The number of each genera was recorded in 10 random squares, from up to 10 samples taken from the same sub-sample. Table 2.3 shows the χ^2 values, derived from the index of dispersal, obtained for each chamber.

Table 2.3 The χ^2 values for four phytoplankton species counted in ten random squares of a Sedgewick-Rafter Chamber

Genera	χ^2 Value for each Chamber									
Ceratium	4.9	10.0	15.1	9.9	7.0	9.0	12.6	5.1	24.1*	15.7
Stephano.	10.2	11.2	2.2	9.0	9.8	9.3	18.4*	14.1	16.3	-
Eudorina	35.1*	17.7*	14.6	10.6	9.4	7.7	16.3	7.4	-	-
Anabaena	4.2	7.7	14.6	10.6	9.4	7.7	17.8*	23.8*	-	-

The index of dispersion shows that cells, or colonies, were distributed randomly in the majority of the slides ($\chi^2_{0.05,9}$ = 16.92), i.e. H_o cannot be rejected at the 95% level of probability. Each genus was distributed non-randomly on at least one occasion (indicated by *), but these deviations usually occurred in different chambers. The presence of a random distribution cannot therefore be disproved at the 95% level of probability in most of the counts. Therefore, the phytoplankton cells/colonies within 10 random squares of a Sedgewick-Rafter Chamber can be regarded as being representative of the whole chamber.

2.2 Sediment Sampling from Esthwaite Water and Thames Valley Reservoirs

Introduction

Diatoms are the most widely studied group of algae within the sediment. The high silica content of their frustules enables them to remain intact for centuries. As such they are often used as an indication of previous phytoplankton populations. Diatom stratigraphy used in the present study is detailed later (page 55).

Less attention has been paid to other taxa. The biggest difficulty to overcome when counting algae or algal remains within the sediment is how to prevent other core material obscuring the individuals to be viewed. In order to avoid identification problems some authors have used chlorophyll derivatives in surface sediment as an indication of lake productivity (e.g. Gorham, 1960; Gorham, Lund, Sanger and Dean, 1974). Livingstone (1979) diluted sediment with water prior to examining sub-samples under a light microscope. Anderson, Aubrey, Tyler and Coats (1982) sonicated sediment samples, in order to break up particles, and then passed material through two sieves. This effectively removed larger and finer material from the sample. Heaney (1978) utilised the auto-fluorescence property of chlorophyll-a to count algal cultures. Chapman (1981) used the same technique to enumerate _Ceratium_ cysts.

Collection of Sediment Cores

In order to study the vertical distribution of

dinoflagellate cysts within the sediment, it was essential to obtain sediment cores with minimum disturbance. The Jenkin Surface-Mud Sampler (Ohnstad and Jones, 1982) illustrated in Figure 2.1, was used to obtain undisturbed sediment cores from the mud-water interface. Care was taken to retain only undisturbed cores. Any cores in which the interstitial water was cloudy were discarded. The cores were transferred to the laboratory intact, retained in the collecting tubes, and stored at 4°C prior to extrusion to prevent the cysts becoming active and excysting.

Hydraulic Extrusion of Sediment Cores

Although the use of the Jenkin Surface-Mud Sampler is well documented (e.g. Mortimer, 1971) the technique for hydraulic extrusion of the cores (Ohnstad and Jones, 1982) is less well known and is therefore described in detail here. The extrusion process can be divided into three stages:-

(i) Loading of the driving piston into the sample tube

(ii) Loading the sample tube into the extruder block

(iii) Extrusion of the core by introducing water under pressure into the extruder block

The upper lid (d in Figure 2.2) was removed and the interstitial water siphoned off, taking care not to remove any of the sediment. The depression in the base of the driving piston (i) was aligned with the dimple on the dummy piston of the loading unit. Similarly the screw protruding from the lower lid (h) of the sample tube was aligned with the depression at

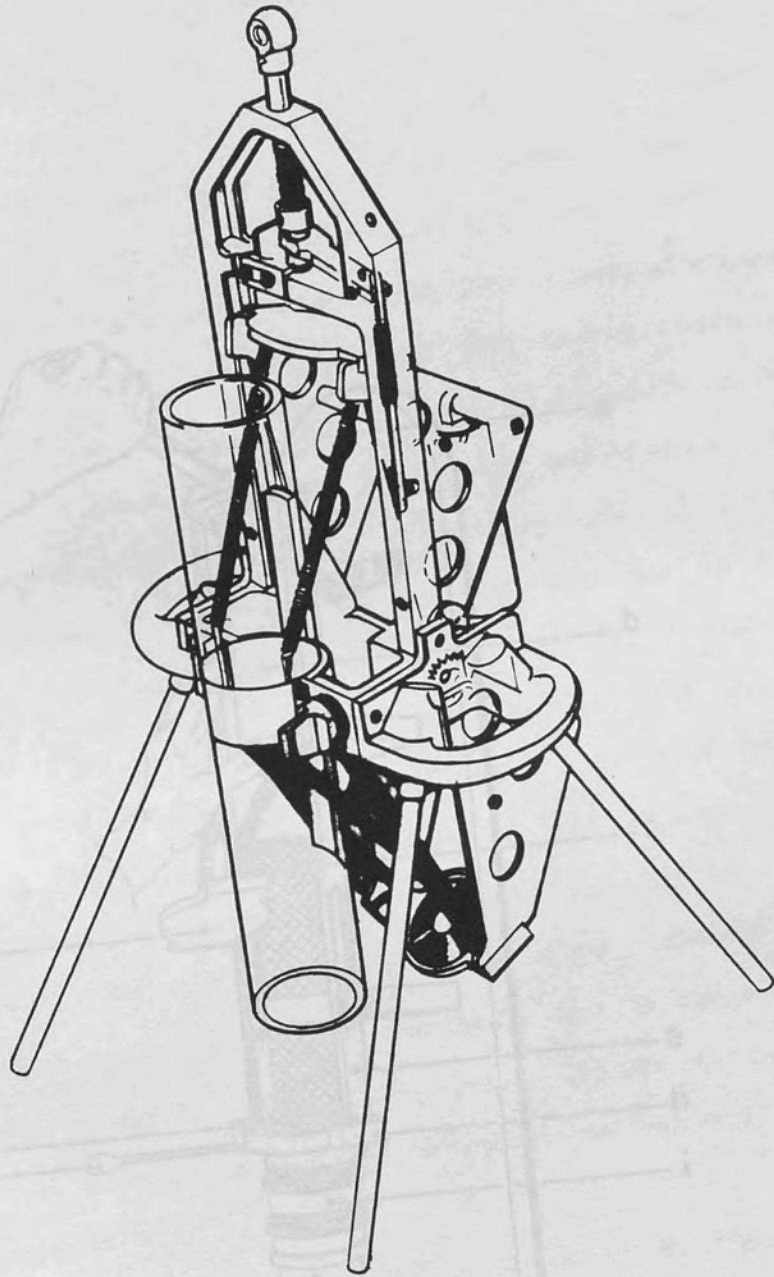


Figure 2.1 The Jenkin Surface-Mud Sampler
(after Ohnstad and Jones,1982)

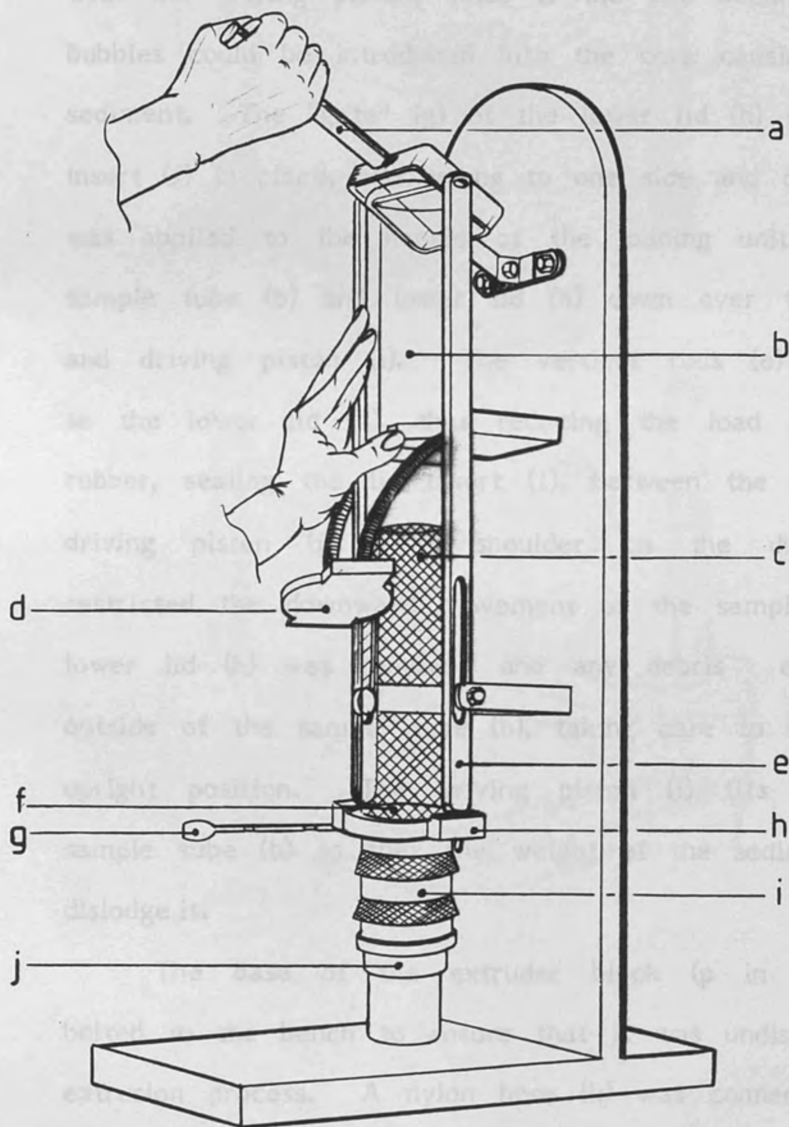
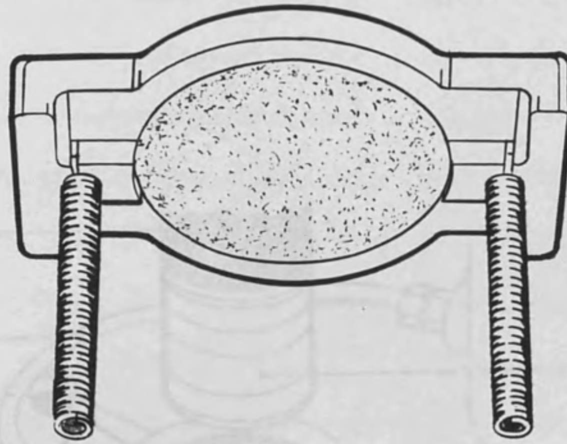


Figure 2.2 Loading the Driving Piston into the Sample Tube (after Ohnstad and Jones, 1982)

the top of the driving piston (i). The lower lid (h) differs from the upper lid (d) in the possession of a removable insert (f), illustrated in Figure 2.3. This passes up the sample tube (b) with the driving piston (i) and prevents any loss of sediment (c). It was important to align the insert correctly with the driving piston, since if the two became disengaged air bubbles could be introduced into the core causing mixing of the sediment. The "gate" (g) of the lower lid (h) which secured the insert (f) in place, was swung to one side and downward pressure was applied to the handle of the loading unit (a), forcing the sample tube (b) and lower lid (h) down over the lid insert (f) and driving piston (i). The vertical rods (e) applied pressure to the lower lid (h), thus reducing the load and trapping the rubber, sealing the lid insert (f), between the tube (b) and the driving piston (i). A shoulder on the dummy piston (j) restricted the downward movement of the sample tube (b). The lower lid (h) was removed and any debris cleaned from the outside of the sample tube (b), taking care to maintain it in an upright position. The driving piston (i) fits tightly into the sample tube (b) so that the weight of the sediment (c) will not dislodge it.

The base of the extruder block (p in Figure 2.4) was bolted to the bench to ensure that it was undisturbed during the extrusion process. A nylon hose (k) was connected between the extruder block (m) and a water tap. The handle on the lid of the extruder block (l) was opened by approximately a half turn anti-clockwise. The sample tube (b) was lowered into the



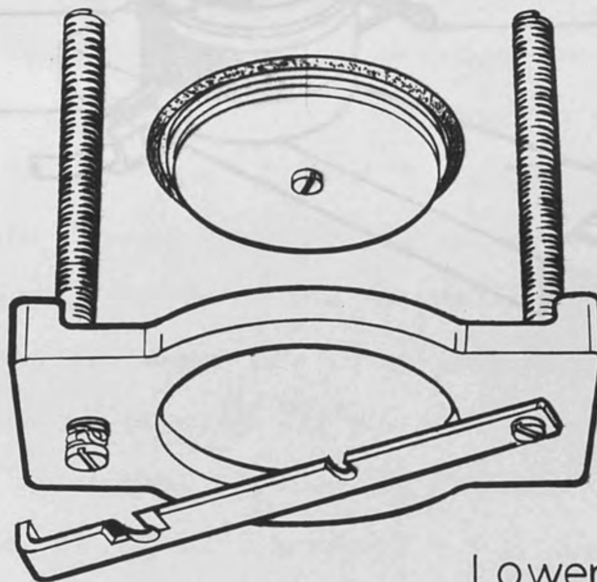


Figure 2.3 Upper and Lower Core Tube Lids Detailing Lower Lid Insert (after Ohnstad and Jones,1982)

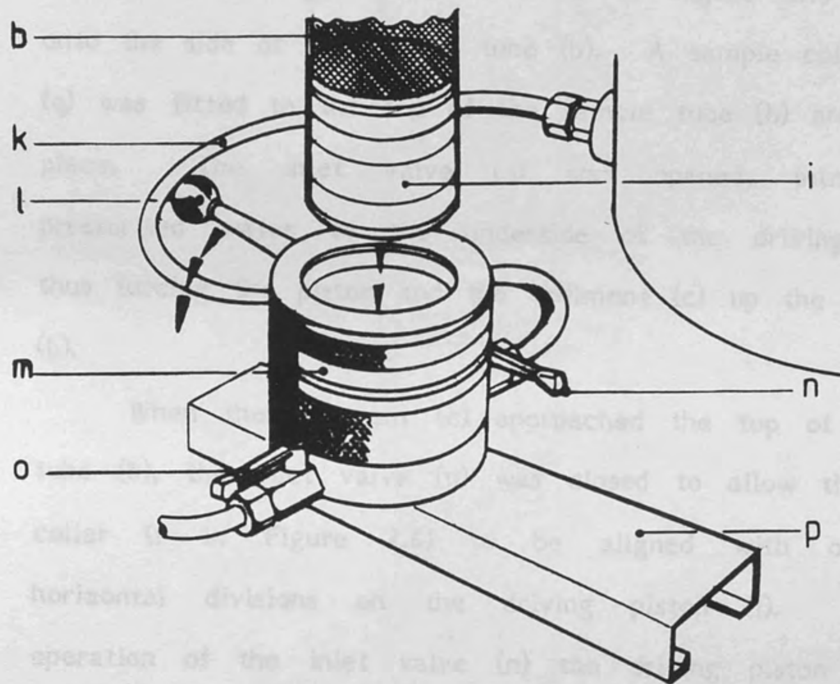


Figure 2.4 Loading the Sample Tube into the Extruder Block (after Ohnstad and Jones, 1982)

extruder block (m) and locked into place by turning the handle (l) in a clockwise direction. The water tap was turned on and both the inlet valve (n) and the outlet value (o) were opened to release any air trapped in the base of the extruder block (m). Both valves were closed when air-free water flowed out.

A plastic graduated collar (r in Figure 2.5) was clipped onto the side of the sample tube (b). A sample collection spout (q) was fitted to the top of the sample tube (b) and secured in place. The inlet valve (n) was opened, introducing the pressurised water to the underside of the driving piston (i), thus forcing the piston and the sediment (c) up the sample tube (b).

When the sediment (c) approached the top of the sample tube (b), the inlet valve (n) was closed to allow the graduated collar (r in Figure 2.6) to be aligned with one of the horizontal divisions on the driving piston (i). By careful operation of the inlet valve (n) the driving piston (i) can be moved up the sample tube (b) by fixed amounts, using the scale shown on the collar (r). In this way the sediment was removed in 0.5 cm sections. The driving piston (i) was advanced by 0.5 cm, the sediment (c) accumulated in the collection spout (q) and was removed into a screwtopped jar (t) with a scraper (s). Care was taken to remove all sediment from the collection spout before advancing the piston to extrude the next section. This procedure was repeated until the top 8 cm of the core had been removed. The upper 8 cm of sediment was judged to represent the deposition of the previous 9-10 years (Pennington, Cambray and

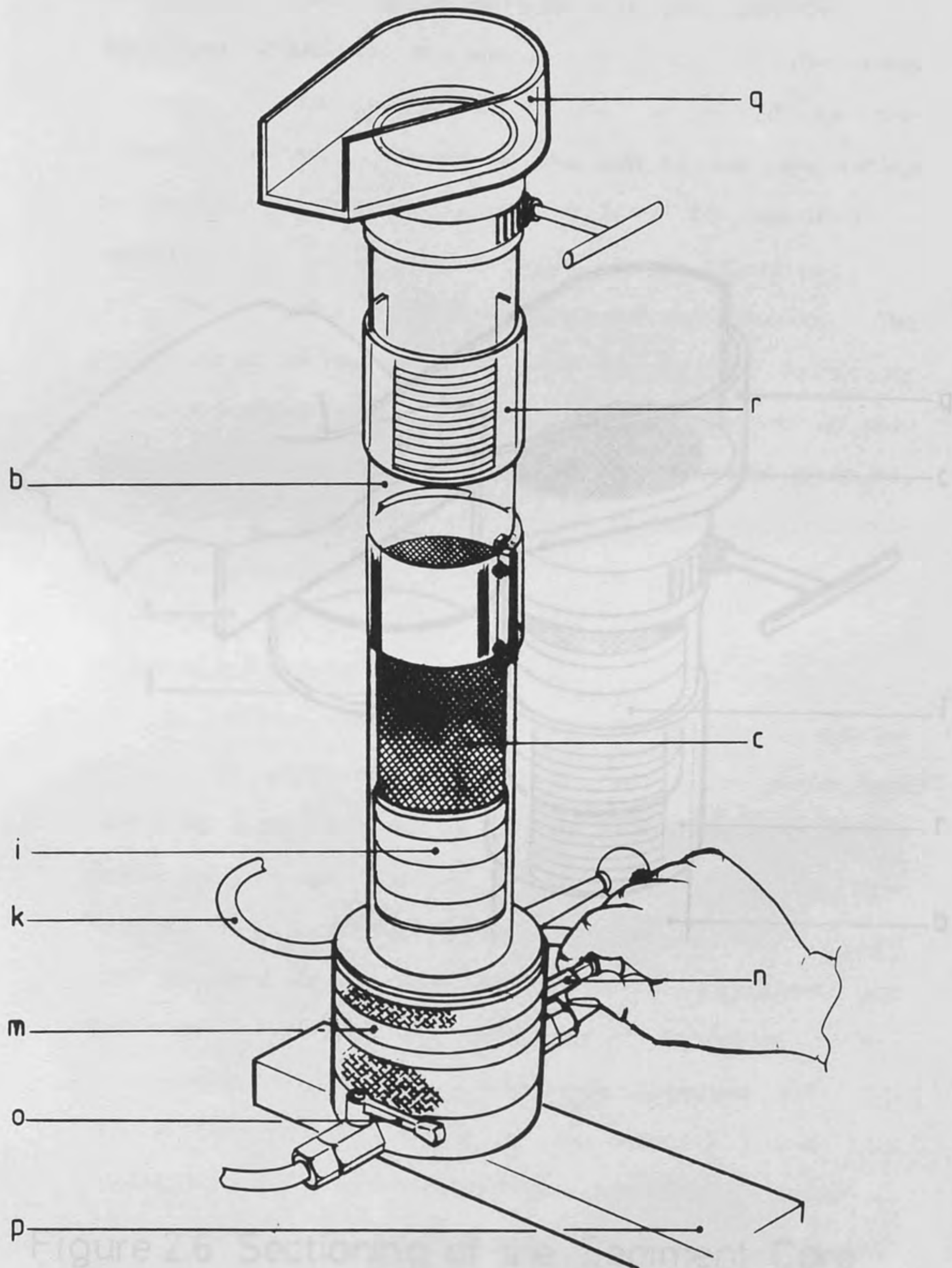


Figure 2.5 Extrusion of the Core by the Introduction of Water under Pressure
(after Ohnstad and Jones, 1982)

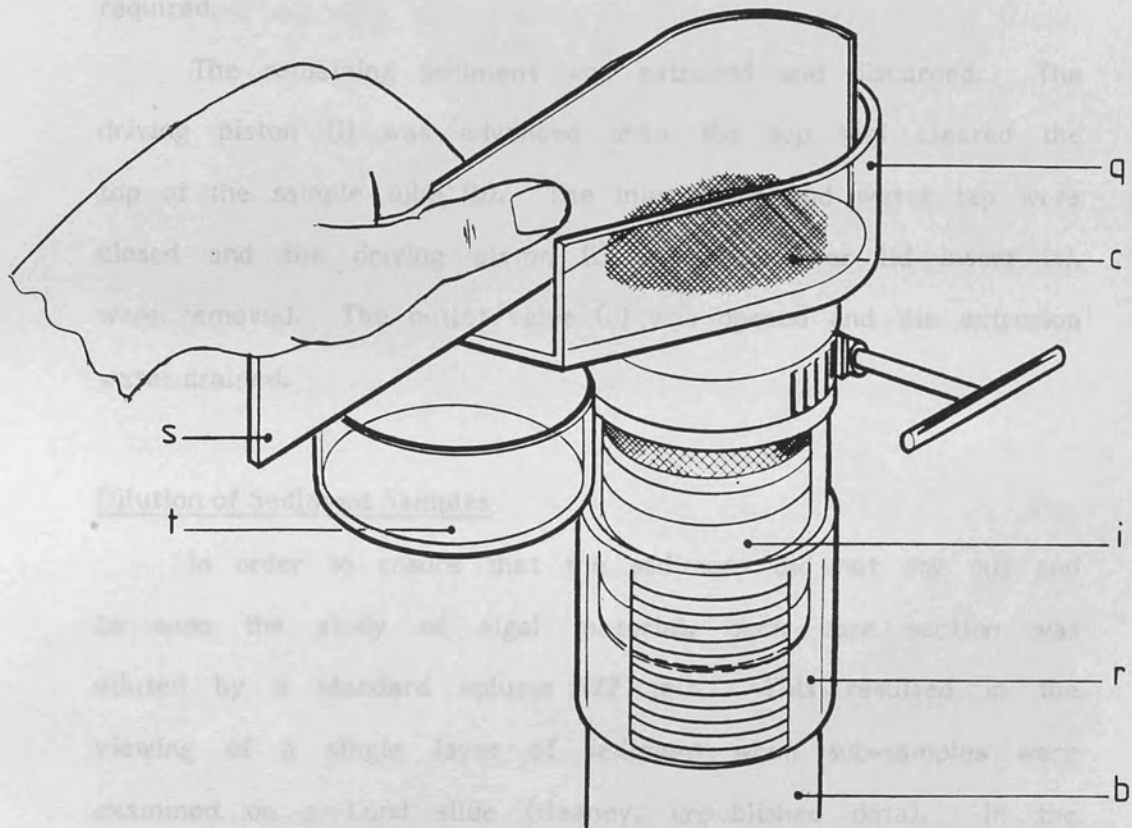


Figure 2.6 Sectioning of the Sediment Core (after Ohnstad and Jones, 1982)

Fisher, 1973), considered to be a suitable time span for study. This depth of sediment was also easy to obtain from the chosen locations and did not necessitate the use of all the core material collected. Sediment at the base of the core, subject to disruption by the closure of the lower lid, was thus not required.

The remaining sediment was extruded and discarded. The driving piston (i) was advanced until the top seal cleared the top of the sample tube (b). The inlet valve and water tap were closed and the driving piston (i) and the lower lid insert (f), were removed. The outlet valve (o) was opened and the extrusion water drained.

Dilution of Sediment Samples

In order to ensure that the sediment did not dry out and to ease the study of algal material, each core section was diluted by a standard volume (77 ml). This resulted in the viewing of a single layer of sediment when sub-samples were examined on a Lund slide (Heaney, unpublished data). In the case of cores to be used for excystment experiments this dilution was achieved by the addition of distilled water. Core sections intended for cyst counts were preserved with 40% formalin (14 ml) and diluted to the standard volume with distilled water (53 ml) and glycerol (10 ml), which helped to prevent slides drying out during counting. Unpreserved core sections were then stored at a temperature of 4°C, to prevent premature germination of cysts. Preserved cores were stored in

the dark, in order to delay loss of autofluoresence.

Enumeration of Ceratium Cysts

In order to obtain an accurate assessment of cyst numbers, cysts had to be viewed unobscured. Initial experiments to reduce the quantity of unwanted sediment by wet sieving (Dale, 1979) had proved unsuccessful. The upper 4 cm of an undiluted sediment core from Kempton Park West Reservoir was well mixed before withdrawing 3-5 g of sediment. The sample was made up to 20 ml with distilled water. The suspension was stirred thoroughly then sonicated gently for 1 minute to break up sediment aggregates. Two sieves were used, an upper sieve with a large mesh (90 μm) which removed large particles, and a lower small mesh (45 μm) through which the fine silts passed. The material collected on the lower sieve was rinsed into a collecting vessel, made up to 20 ml, and examined on a gridded petri dish under an Olympus SZH binocular microscope. A comparison of sieved and unsieved samples revealed the presence of a significantly lower number of cysts in sieved samples (77% of the number present in unsieved samples). The diameter of the cysts was less than 90 μm, but the horns of the cysts, particularly in the case of C. furcoides, increased the length and presumably prevented the passage of cysts through the upper sieve, unless the horns were oriented at 90° to the mesh. Samples were examined from which the smallest particles had been removed, with the 45 μm sieve, but visibility was impaired by the presence of larger particles.

The Density Gradient Centrifugation technique (Bowen, St Onge, Colton and Price, 1972; Price and Guillard, 1978) separates the various components of a suspension according to density. Initial experiments were made using a series of glucose solutions of increasing concentration, to ensure that the technique was successful before investing in the more costly recommended solutions. The technique was a partial success in that particles were separated into distinct bands. However, retrieval of the appropriate band of sediment for examination under the microscope was awkward and messy and it was felt that there would be a loss of material. It would appear to be a suitable technique for concentrating cells but not for quantitative counts.

The counting of cysts from diluted, unsieved sediment with a light microscope (Livingstone, 1979) would have been tedious and time consuming, and inaccuracies could have arisen through some cysts being obscured by other particles. However, this technique was used to assess numbers of unviable cysts, which could not be counted by the same method used for viable cysts (see page 54).

Viable Ceratium cysts contain chloroplasts which exhibit red auto-fluoresence when excited with blue light (Heaney, 1978; Chapman, 1981). This facility enabled a combination of fluorescence and light microscopy to be used in the counting process. Sub-samples of 0.25-0.50 cm^3 from each section of the core, thoroughly mixed using a magnetic flea, were introduced onto a Lund slide (Lund, 1959, 1962). A wide bored pipette was

used to ensure sediment particles did not cause blockages. The previous dilution of the sediment ensured that only one layer of sediment was viewed on the slide. The slide was examined with a Leitz Diaplan Fluorescence Microscope using the x25 objective. Two filter blocks were required; a blue narrow peak excitation filter with maximum transmission at 425 nm and a red, sharp cut-off emission filter with maximum transmission at 687 nm (Heaney, 1978). Cysts were searched for using fluorescent light, and, once located, were identified to species level by switching to transmitted light. In this way even cysts bound in faecal pellets or obscured by other particles could be observed. A comparison was made of the two techniques, counting the number of cysts present on a slide by light and then fluorescence microscopy. The result showed an average of 31% more cysts using the fluorescence technique.

Identification of Ceratium Cysts

Ceratium cysts were identified using Entz (1927) and by reference to the Fritsch collection. Species were distinguished on the basis of morphological features, for example, the length and shape of the cyst horns, and the ratio of cyst width to length (see Chapter 4, page 88). No other species were determined.

The Number of Cysts Counted

The same criteria used to determine the number of cells to be counted were applied to determine the number of cysts (see

page 30). In order to achieve 95% confidence limits of ± 20% 100 cysts had to be counted for each sample. In samples containing a very low number of cysts, the time taken to achieve a count of this size did not justify the increased statistical accuracy. In such cases the initial aim was to count at least 20 cysts. However, in a few core sections, difficulty was experienced in attaining this number.

<u>Variation between Samples from Different Locations within the same Lake</u>

A study of the total cyst population of the lake was beyond the scope of the present study, since too few cores were taken at any given time to establish statistically whether cysts were randomly distributed.

<u>Variation between Sub-Samples from the same Initial Sample</u>

If a single sub-sample contained at least 100 cysts it would not theoretically be necessary to count any more. It was therefore essential to establish whether a single sub-sample was representative of the whole core section, i.e. were the cysts randomly distributed within the container? Thirty sub-samples each of 0.25 cm^3, from a single core section, were counted in the usual way (see page 50). The total number of Ceratium cysts for each were compared using the index of dispersion.

$H_0 : s^2 = \bar{x}$
$H_1 : s^2 \neq \bar{x}$

$\Sigma x = 4663$

$n = 30$

$\bar{x} = 155.4$

$s^2 = 155.0$

$\therefore \chi^2 = \frac{s^2(n-1)}{\bar{x}} = \frac{155.0(30-1)}{155.4} = 28.92$

Degrees of freedom = (n-1) = (30-1) = 29

from tables $\chi^2_{0.05,29} = 42.5570$

$\therefore \chi^2_{\alpha,29} < \chi^2_{0.05,29}$

$\therefore H_o$ cannot be rejected at the 95% level of probability

Therefore the existence of a random distribution cannot be disproved. Thus a single sample can be considered representative of the entire core section.

In order to establish the variation between sub-samples the 95% confidence limits were calculated. Since agreement with the Poisson series could not be disproved the following calculation was used:-

$$\bar{x} \pm t\sqrt{\frac{\bar{x}}{n}}$$

when 2 Q = 0.05, v = n-1, t = 2.045

$$\bar{x} \pm 2.045\sqrt{\frac{155.4}{30}}$$

$$\bar{x} \pm 4.654$$

$\therefore$ The population mean lies between 150.7 and 160.0 on 95% of

occasions. This indicates very low variation between counts of sub-samples (< 3%).

No analysis was required of the variation within a sub-sample, since entire slides were always counted.

<u>Counts of Non-Viable Cysts</u>

The fluorescence technique, although efficient, allows only viable cysts to be enumerated. In order to establish the numbers of non-viable and empty cysts an alternative technique was required. A sub-sample of 0.25 cm^3 of diluted sediment was made up to 20 ml with distilled water. The resulting suspension was thoroughly mixed and 1 ml withdrawn immediately and introduced into a Sedgewick-Rafter Chamber. Counts of <u>Ceratium</u> cysts were made of the whole slide, recording viable, non-viable and empty cells separately.

<u>Determination of the Dry Weight of Sediment Samples</u>

Since this study was primarily concerned with the "in situ" distribution of <u>Ceratium</u> cysts, it was not considered necessary to express these figures as numbers of cysts per unit dry weight of sediment. However, in order to gauge the relative change in water content with depth (Chapter 5, page 130) measurements were made on a single core. As such they are not intended to be representative of the lake as a whole.

An empty porcelain crucible (with lid) was weighed on a balance accurate to 4 decimal places. Diluted sediment from one core section was added until this weight was increased by

5 grammes. This procedure was repeated for the remaining core sections. The crucibles were then transferred to a fume cupboard. Reweighing occurred at intervals until a constant weight was attained. A comparison of the two sets of figures enabled the water content to be calculated.

Preparation of Slides for Diatom Counts

In some lakes sediment is deposited in easily discernable layers (varved sediment). These correspond to variation in the material deposited or to changing conditions at the sediment surface at different times of the year (e.g. Lake Zurich, Huber and Nipkow, 1922). In other lakes further investigation reveals annual or seasonal banding. Bodbacka (1986) used X-ray radiography to reveal the laminated sediment of Lakes Lilla Ullfjärden and Stora Ullfjärden in Sweden.

However, many lakes, including Esthwaite Water, do not produce sediment in distinct layers. Therefore, in order to assess the sedimentation rate in these lakes, other parameters need to be measured. Sediment trapping assesses the rate of input of material to the sediment. Although widely used (e.g. Pennington, 1974), the technique can take no account of compression of the sediment once deposited, and thus the annual increment could be over estimated. Re-suspended material may also be collected at times of lake mixing increasing the sediment collected. ^{137}Caesium has successfully been used as a tracer by several authors (e.g. Pennington, Cambray and Fisher, 1973; Ritchie, McHenry and Gill, 1973; Pennington, Cambray,

Eakins and Harkness, 1976). However, cores covering the past 25 to 35 years are required in order to include the two peaks of ^{137}Caesium production in 1954 and 1963. Since this study was concerned with only the upper 8 cm of a core, a technique concentrating on this part of the core was considered more appropriate.

Diatom frustules remain well preserved in the sediment due to their high silica content. This facility has made them useful to authors studying long term changes in the phytoplankton of a lake (e.g. Pennington, 1943), in much the same way as pollen analysis indicates past populations of terrestrial plants (e.g. Franks and Pennington, 1961). Diatom stratigraphy in recent sediment has also been used in conjunction with phytoplankton records (e.g. Haworth, 1980).

In a similar way diatoms can be used as "marker species" in the sediment of lakes whose phytoplankton is regularly monitored. If a new species arises in the phytoplankton in a particular year, or appears in unusually large numbers, the appearance of the same species in the sediment will be indicative of that year (e.g. Haworth, 1979). Although a regular sedimentation rate can only be surmised, the presence of a band of cells of known age does give an indication of the accumulation of sediment between given years. In this study use was made of the diatom **Stephanodiscus parvus** (Grunow) Stoermer and Håkansson (Stoermer and Håkansson, 1984) to determine the date of strata within the sediment core (see Chapter 5, page 158).

Cleaned coverslips were placed on a labelled sheet of paper. A new, disposable wide-ended pipette was used to place 0.003 cm^3 of sediment from each well mixed, diluted section of the core onto a coverslip. Three or four drops of distilled water were added to disperse the sample. Samples were covered with petri dishes to protect from dust and allowed to dry out overnight. Storax was used as a mountant, to enhance contrast of the silica in the frustules. Two or three drops of storax were added to a labelled slide which was inverted over the coverslip to avoid loss of sediment. The righted slide was examined under the oil immersion lens of an Olympus BH2 microscope using Nomarski interference phase contrast. Numbers of **Stephanodiscus parvus** and **Cyclotella** spp. were noted. Initially numbers were counted for the whole slide but this was found to be too time consuming, and so only 3 random transects were counted from each slide. The number of frustules was recorded for each taxa. Fragments greater than half a frustule were included.

Determination of Cyst Viability

Two techniques were employed in order to establish the ability of cysts throughout the sediment core to germinate. The first used slurries of sediment and culture medium in order to determine whether excystment occurred at a particular depth, whilst the second enabled observations to be made on specific isolated cysts.

(i) Slurry Technique

Modified Chu 10 culture medium (Appendix 2.1) was introduced into a new petri dish. A 1 cm^3 aliquot of diluted fresh sediment was withdrawn from the upper section of the core with a sterile, disposable pipette, and transferred to the petri dish. The covered dish was agitated gently in order to distribute the sediment evenly, and was then left to settle out under controlled light and temperature conditions (24 hours light at 20°C). The procedure was repeated for all sections of the core. Dishes were examined twice a day, using an Olympus SZH binocular microscope, to monitor the appearance of motile Ceratium cells. Observations were continued for 2 weeks, or until fungal growth infected the dish.

(ii) Watchglass Technique

A diluted sub-sample of sediment from each core section was examined in a petri dish using an Olympus SZH binocular microscope. A maximum of 10 viable Ceratium cysts of a single species (i.e. those in which the cytoplasm filled the cyst and displayed Brownian motion) were removed with a micropipette and transferred to a watchglass containing culture medium (modified Chu 10). Each watchglass was enclosed in a sterile petri dish in order to reduce infection and limit evaporation. Chambers were examined twice daily for emergent motile cells. The number of empty cysts and the number of cells were recorded in order to assess the rate of cell division. Examination was continued for 2 weeks, or until fungal growth prevented further observations.

Additional culture medium was added where necessary to prevent dessication.

<u>Viability of Cysts following Dessication</u>

An additional experiment was conducted to assess the ability of cysts to withstand a period of drying. Diluted sediment (1 cm^3) was introduced into a petri dish and left, exposed to the air, until the sediment appeared dry. Culture medium was introduced, the sediment resuspended and then left to settle. The sediment was examined daily in the usual way (see page 58) for the presence of any motile cells.

2.3 Prepartion of Samples for the Scanning Electron Microscope

A 1 cm^3 sub-sample of sediment was added to a petri dish of distilled water, allowed to settle and examined under an Olympus SZH binocular microscope. Individual <u>Ceratium</u> cysts were removed using a micropipette and transferred to a Nuclepore filter supported in a suitable holder. Motile cells were collected in a similar way, with individuals removed from net samples. The isolated samples were taken through an acetone series, to dehydrate them, prior to drying to the critical point for liquid carbon dioxide. The filters were then transferred from the holder, fixed on to stubs and coated with gold palladium. Stubs were examined using a Jeol 25S or a Cambridge S100 scanning electron microscope (S.E.M.). In the case of material to be used for analysis, cysts (or cells) were first transferred through a series of watchglasses containing

distilled water. This sought to clean them of any attached material. The above procedure was then followed, with the exception of the gold palladium used to coat the stubs, which was replaced by carbon.

2.4 Separation of the Thecal Plates of Ceratium Cells

The arrangement of the thecal plates of *Ceratium* motile cells were examined in order to assist in the identification of the two species. Individual *Ceratium* cells were removed from preserved net samples from Esthwaite Water with a micropipette, placed onto separate slides in a drop of distilled water, and covered with a coverslip. The slide was transferred to an Olympus BH2 microscope and the cell photographed. Blotting paper was used to draw first Lugol's Iodine across the slide, to stain the cells, and then sodium hypochlorite (50%) to break down the plate sutures (Taylor, 1978). Pressure was then applied to the slide and the cell, with its thecal plates now disassociated, was photographed again.

APPENDIX 2.1 Modified Chu 10 Culture Medium

Stock Solutions

1.	$Ca(NO_3)_2 4H_2O$	20.0 gl^{-1}
2.	$MgSO_4 7H_2O$	25.0 gl^{-1}
3.	Na_2SO_3	25.0 gl^{-1}
4.	KH_2PO_4	6.2 gl^{-1}
5.	Na_2CO_3	20.0 gl^{-1}
6.	N.HCl	
7.	EDTA Fe Complex -	
	EDTA Na_2 2g + $FeCl_3$ 1g l^{-1}*	
8.	Trace Mix	
	H_3BO_2	2.48 gl^{-1}
	$MnCl_2 4H_2O$	1.39 gl^{-1}
	$(NH_4)_6Mn_4O_{24}4H_2O$	1.0 gl^{-1}
9.	Vitamin B_{12}	1.0 mgl^{-1}
10.	Thiamin B_1	1.0 mgl^{-1}
11.	Biotin	1.0 mgl^{-1}

* if using $FeCl_3 6H_2O$ then require 1.66 gl

Method

1. Add to 1 litre of distilled water:-
   (a) 2 ml each stock solution 1 - 5.
   (b) 2 ml each stock solution 7 - 11.
   (c) 0.5 ml stock solution 6.
   (d) 50 ml soil extract.
2. Make up to 2 litres with distilled water.
3. Put into the oven at 100°C to achieve a pale brown colour

(approximately 1 hour $litre^{-1}$).

4. Filter through a GFC filter with the aid of a vacuum pump.

5. Filter through a 0.22 μm membrane filter in the same way.

6. Transfer into small flasks (approx. 250 ml), plug with cotton wool and cover with aluminium foil secured with autoclave tape.

7. Autoclave at 15 lb square inch for approximately 15 minutes.

8. Cool before use. Store, sealed as above, in a cool place.

CHAPTER 3

GENERAL FEATURES OF SITES SAMPLED

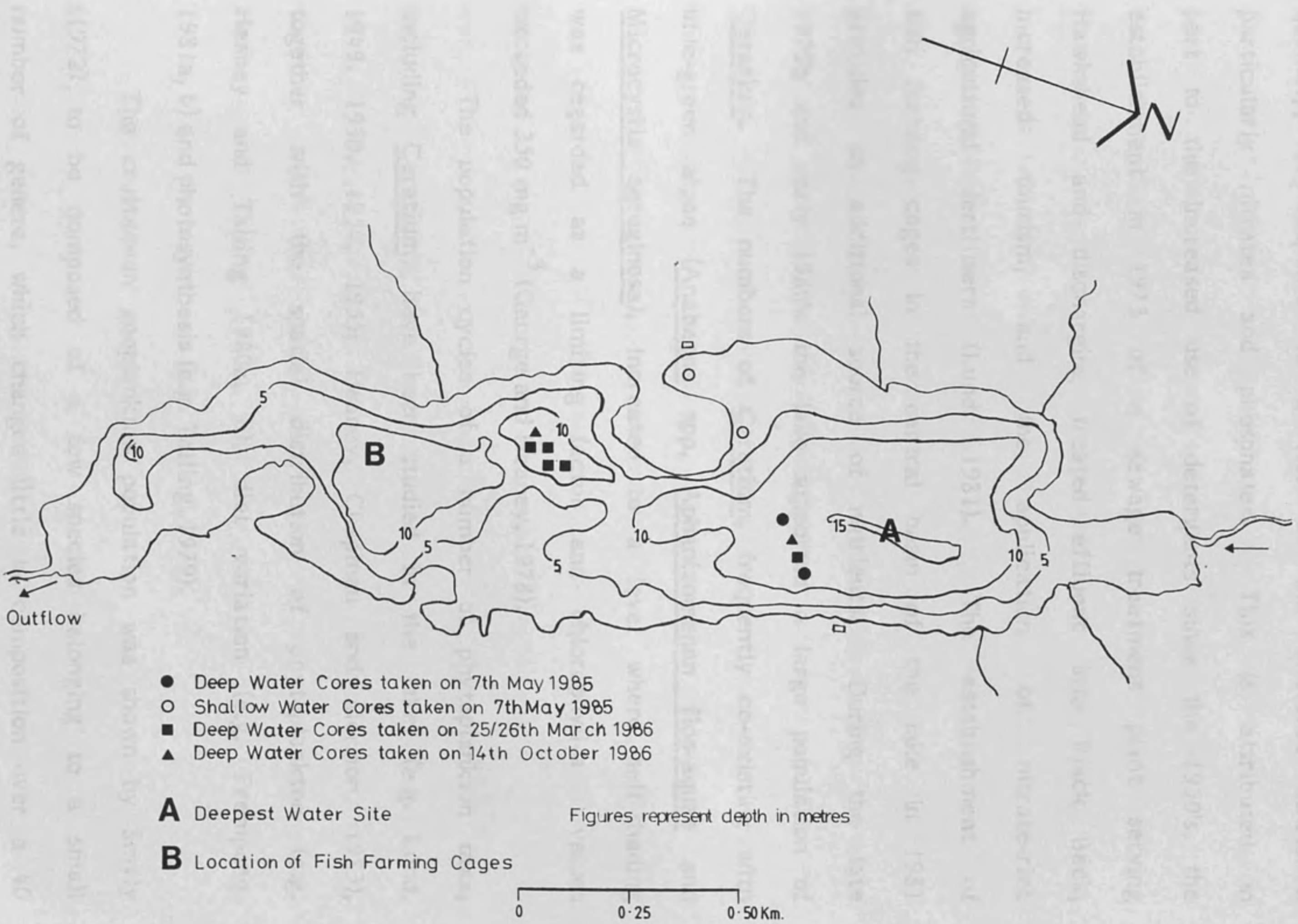
3.1 Esthwaite Water

Esthwaite Water (54°21'N, 3°O'W) is a small glacial lake situated at an altitude of 65 metres in the English Lake District, an area with a cool oceanic climate and high annual rainfall (185 cm). The lake (area 1.0 km^2, maximum depth 15.5 m, mean depth 6.4 m) is composed of three basins of unequal size (see Figure 3.1), with a mean retention time of 13 weeks (Heaney and Butterwick, 1985). It is served by six inflow streams, of which Black Beck, entering at the head of the lake, supplies half of the total input. The drainage basin of 17.1 km^2, composed of gently sloping pastureland and grassland based on rich alluvial soil, contributes to its position as one of the most productive lakes in the area (Macan, 1970). The underlying slates and grits of the hills are younger than the silurian rocks below the lake (Pearsall and Pennington, 1973). Further bathymetric details are provided in Ramsbottom (1976).

In general the lake is thermally stratified from April until October with a temperature difference of up to 7-9°C between the surface and the bottom waters. Anoxic conditions can prevail in the bottom waters during the summer (Mortimer, 1941, 1942). In winter the lake may be partially or totally frozen for several weeks.

The phytoplankton population has been regularly sampled since 1945 (e.g. Lund, 1979). The data collected has shown that

Figure 3.1 Esthwaite Water, Cumbria



since 1963 the summer phytoplankton population has increased in quantity, in response to a rise in the dissolved nutrients, particularly nitrates and phosphates. This is attributed in part to the increased use of detergents since the 1950's, the establishment in 1973 of a sewage treatment plant serving Hawkshead and discharging treated effluent into Black Beck, increased tourism, and the application of nitrate-rich agricultural fertilisers (Lund, 1981). The establishment of fish farming cages in the central basin of the lake in 1981 provided an additional source of nutrients. During the late 1970's and early 1980's the lake supported a large population of Ceratium. The numbers of Ceratium, frequently co-existing with blue-green algae (Anabaena spp, Aphanizomenon flos-aquae and Microcystis aeruginosa), increased to a level where self-shading was regarded as a limiting factor and chlorophyll-a values exceeded 350 mg m^{-3} (George and Heaney, 1978).

The population cycles of a number of phytoplankton taxa, including Ceratium, have been studied in the lake, (e.g. Lund, 1949, 1950, 1954, 1955; Heaney, Chapman and Morison, 1983), together with the spatial distribution of phytoplankton (e.g. Heaney and Talling 1980a, b), diel variation (e.g. Frempong, 1981a, b) and photosynthesis (e.g. Talling, 1979).

The crustacean zooplankton population was shown by Smyly (1972) to be composed of a few species belonging to a small number of genera, which changed little in composition over a 40 year period. Studies on benthic fauna have included the ecology of chironomids (Mundie, 1965) and tubificids (Reynoldson, 1983).

The rate of sedimentation has been assessed using diatom stratigraphy (e.g. Haworth, 1985) and by ^{137}Caesium (Pennington, Cambray and Fisher, 1973), whilst studies of diatom assemblages within the sediment have also been conducted (e.g. Round, 1961).

The chemical processes in the lake and its sediment include the classical studies by Mortimer (1941, 1942) and Mackereth (1965, 1966). Recent work includes an analysis of the release of phosphorus from littoral sediments (Drake and Heaney, 1987) and the determination of iron and manganese cycles (Davison, Woof and Rigg, 1982). The interaction between biological, chemical and physical factors operating within the lake are extensively discussed in Heaney, Smyly and Talling (1986), which also includes a comprehensive review of the biological research undertaken on Esthwaite Water.

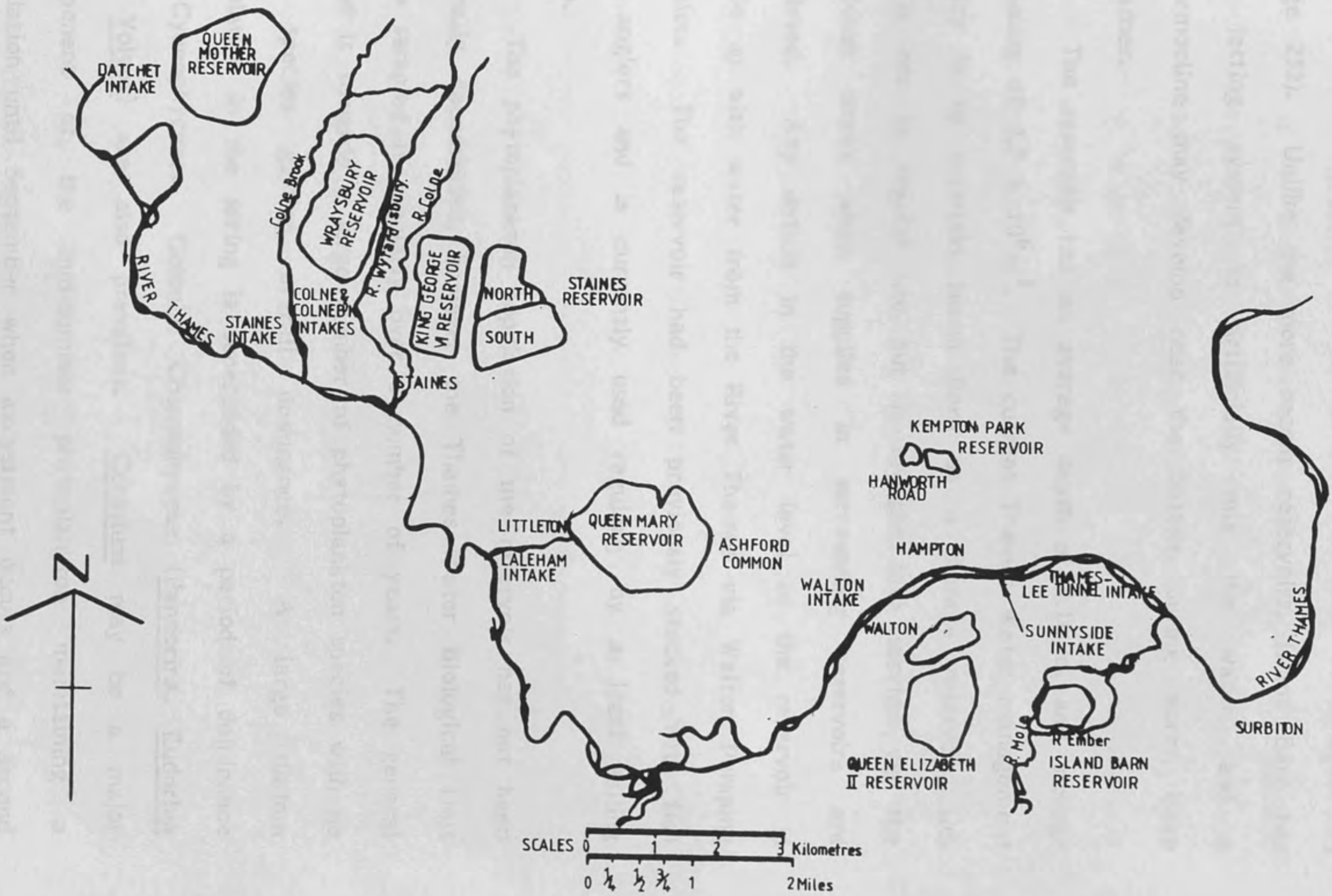
The wealth of information associated with this lake, particularly the existence of long term phytoplankton records, was a key factor in selecting it as a sampling site. The ecology of Ceratium within the lake had been well documented (e.g. Heaney, 1976; Heaney and Talling, 1980a, b; Heaney, Chapman and Morison, 1983) and provides a sound background to further work.

3.2 Thames Valley Reservoir Sites

Island Barn Reservoir

The Island Barn Reservoir (51°24'N, O°24'W) is a comparatively small reservoir of 48.97 hectares situated in the Thames Valley to the east of Walton (see Figure 3.2). Completed

Figure 3.2 Location of Reservoirs in the Thames Valley



in 1911, the reservoir is constructed in a similar way to other purpose built reservoirs in the area (detailed in Chapter 7, page 252). Unlike the more recent reservoirs, Island Barn has no jetting system to artificially mix the water, and a thermocline may develop near the bottom during warm, calm weather.

The reservoir has an average depth of 9.14 m and storage capacity of $3.7 \times 10^6 m^3$. The current Thames Water management policy is to maintain Island Barn as a storage reservoir, i.e. it is not in regular use but is brought into service via the Surbiton works when supplies in surrounding reservoirs are depleted. Any deficit in the water level of the reservoir is made up with water from the River Thames via Walton Pumping Station. The reservoir had been previously stocked with fish for anglers and is currently used regularly by a local sailing club.

The phytoplankton population of the reservoir has not been formally documented, however the Thames Water Biological Unit have sampled it regularly over a number of years. The general trend is towards a large number of phytoplankton species with no one species gaining overall dominance. A large diatom population in the spring is superceded by a period of dominance by Cyanophyceae. Colonial Chlorophyceae (*Pandorina*, *Eudorina* and *Volvox*) are also prevalent. *Ceratium* may be a major component of the mid-summer phytoplankton maintaining a population until September when excystment occurs and a second diatom population dominates.

The site was selected with the intention of undertaking a study of the Ceratium life cycle (discussed fully in Chapter 7, page 257). It had supported an extensive population of Ceratium in the previous year (1984) and unlike many more recent reservoirs had a continuous layer of sediment, enabling the cyst population to be sampled. The reservoir was drained by several metres during the summer of 1984. Levels were restored by early November and sampling was commenced the following February. Water levels were lowered again in October 1985, with full capacity restored by April 1986 (detailed in Chapter 7, page 258).

Kempton Park West Reservoir

This eutrophic reservoir (51°26'N, 0°24'W) is smaller (combined area of west and east reservoirs is 25.09 hectares) and shallower (maximum depth = 6.1 m) than the other reservoirs in this study. Water levels are maintained from the Queen Mary Reservoir. This was the site originally chosen for a quantitative study, since previous work (Chapman, 1981) had shown the presence of a large Ceratium population. Permanent drainage of the reservoir in April 1984 prevented further sampling.

Queen Mary Reservoir

This reservoir (51°25'N, 0°28'W), completed in 1925, has the largest area (286 hectares) of the reservoirs in this study, but a lower maximum depth of 11.6 metres means that its total

capacity is less than either Queen Mother or Wraysbury Reservoirs. Water levels are maintained from the River Thames at Laleham. In common with Island Barn and Queen Mother Reservoirs, Queen Mary Reservoir is used by a local sailing club.

Queen Mother (Datchet) Reservoir

The Queen Mother Reservoir (51°29'N, 0°31'W), completed in 1974, is the most recently constructed of the reservoirs studied, and has the greatest water capacity ($38.0 \times 10^6 m^3$). With a maximum depth of 22.9 metres the reservoir had a tendency to become thermally stratified during the summer. To overcome this, air diffusion pumps were installed to ensure that the water column remained mixed (detailed in Chapter 7, page 254). The reservoir has been stocked with trout for local anglers and is used for sailing. Ceratium generally forms an important component of the summer phytoplankton, often reaching large populations. Other important algal groups include colonial Volvocales (e.g. Eudorina and Pandorina) and Cyanophyceae (e.g. Aphanizomenon and Anabaena).

In this recent reservoir, insufficient time had elapsed for a substantial layer of sediment to accumulate. Thus this site was unsuitable for sampling cyst populations.

Staines North and South Reservoirs

These two reservoirs (51°27'N, 0°29'W) are generally operated together. Staines South reservoir is the larger (99.6

hectares) and shallower (9 metres). Water levels are maintained from the Rivers Thames and Wyrardisbury (Wraysbury) and Colne Brook. Ceratium has been observed to reach large populations in both reservoirs. However, during the period of this study both reservoirs were periodically drained (almost totally), and refilled, making it impossible to sustain a regular sampling programme.

3.3 Virginia Water Lake

Virginia Water Lake, located at the southern edge of Windsor Great Park (51°25'N, 0°37'W), was created by the raising of the water level in a stream connecting a series of small ponds, and the construction of the outlet falls. The resulting lake (see Figure 3.3) is less than 2 km in length and shallow, never exceeding 3 m in depth. The east shore and most of the north shore is formed of Bagshot sand, whilst the remainder of the north and northwest is of London clay. The main drainage into the lake is from the north and west, whilst the waterfall affords the only outlet. Retention time has varied from 10 days to several months (J.H. Evans, personal communication). The lake was drained between 1939-45, and was considerably lower during the drought of 1976. Ceratium has been observed regularly in the lake, but has not produced large populations in recent years (J.H. Evans, personal communication).

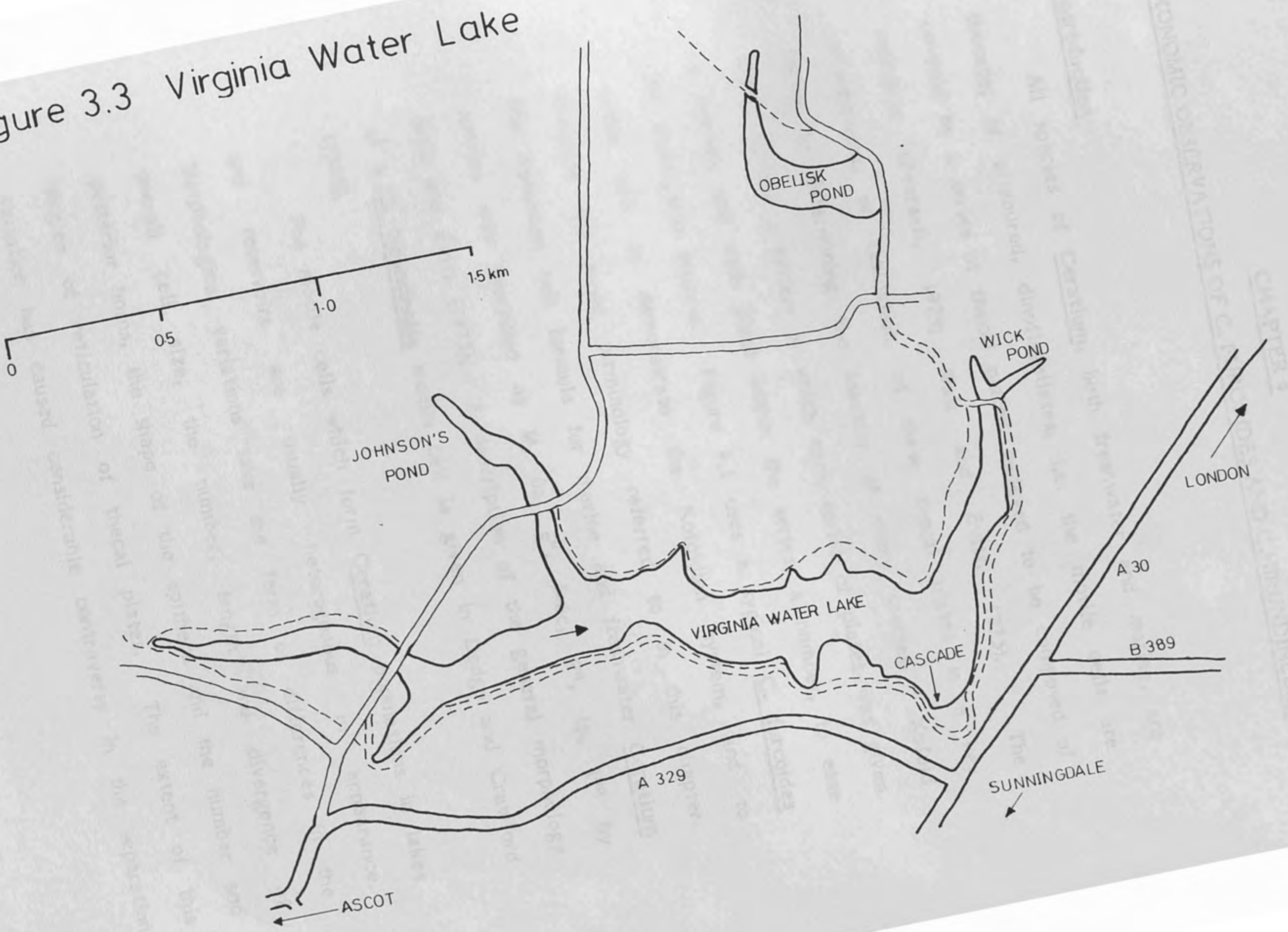


Figure 3.3 Virginia Water Lake

CHAPTER 4

TAXONOMIC OBSERVATIONS OF C. FURCOIDES AND C.HIRUNDINELLA

Introduction

All species of Ceratium, both freshwater and marine, are thecate, or armoured, dinoflagellates, i.e. the motile cells are covered by a series of thecal plates, believed to be composed of cellulose (Pearsall, 1929; Wall and Evitt, 1975). The arrangement, or tabulation, of these thecal plates is a key factor in determining the identity of many species. Kofoid (1907) devised a system in which each series of plates was given a symbol, and each plate within the series, a number, to ease the description process. Figure 4.1 uses a typical C. furcoides motile cell to demonstrate the Kofoidian system, and to illustrate the basic terminology referred to in this chapter. The Kofoidian cell formula for marine and freshwater Ceratium species was described as 4', 0a, 6'', 5-6c, 6''', 1p, 1'''' by Wall and Evitt (1975). A description of the general morphology of a C. hirundinella motile cell is given in Dodge and Crawford (1970).

The motile cells which form Ceratium populations in lakes and reservoirs are usually heterogenous in appearance. Morphological variations take the form of differences in the overall cell size, the number, length and divergence of posterior horns, the shape of the epitheca and the number and degree of reticulation of thecal plates. The extent of this variation has caused considerable controversy in the separation

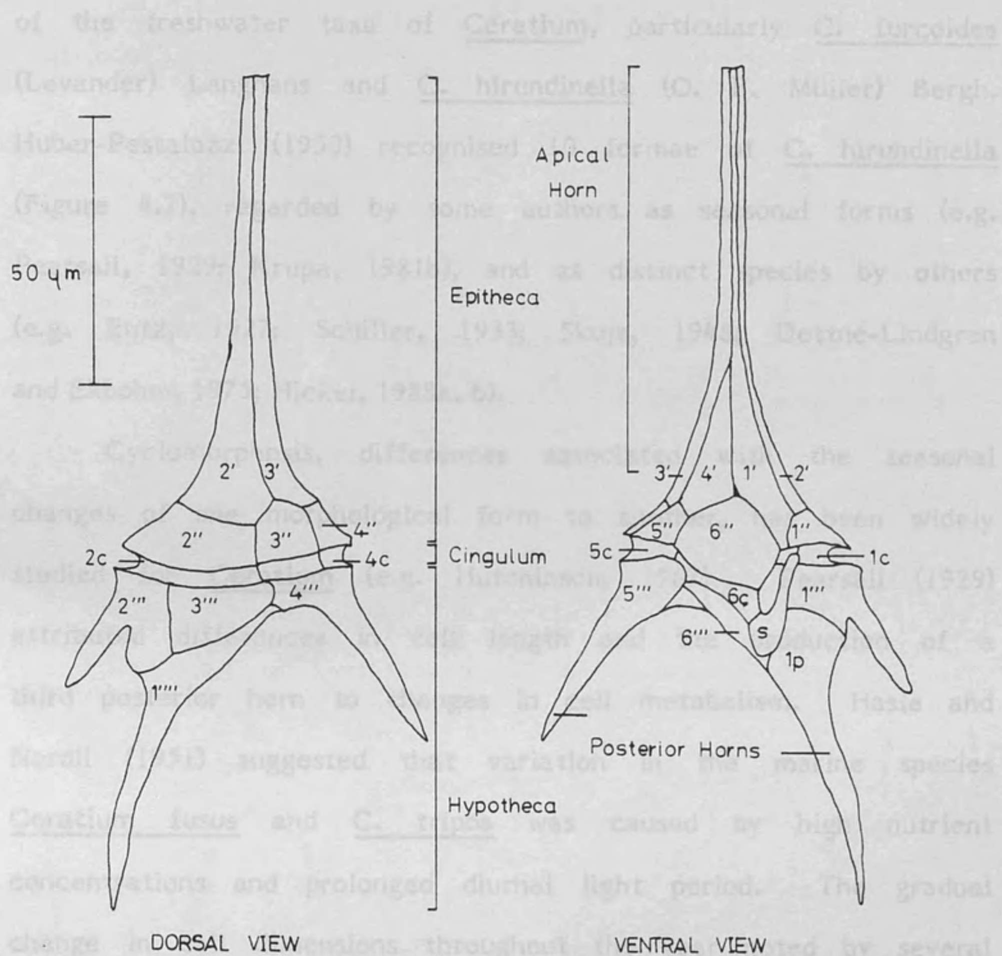


Figure 4.1 Tabulation and Basic Terminology of C.furcoides

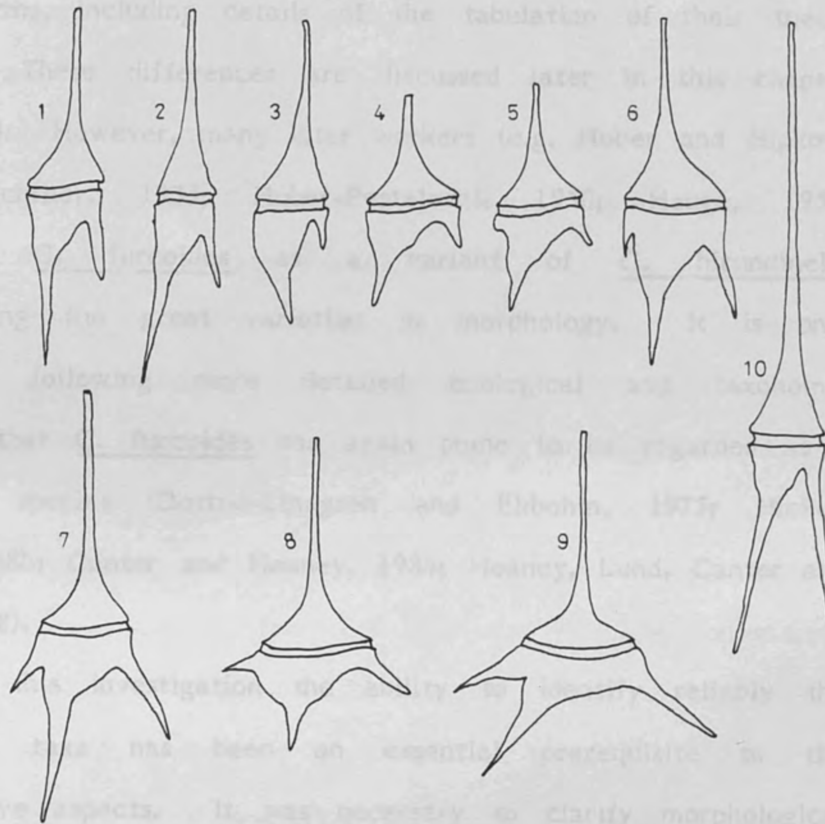
(after Wall and Evitt, 1975)

of the freshwater taxa of Ceratium, particularly C. furcoides (Levander) Langhans and C. hirundinella (O. F. Müller) Bergh. Huber-Pestalozzi (1950) recognised 10 formae of C. hirundinella (Figure 4.2), regarded by some authors as seasonal forms (e.g. Pearsall, 1929; Krupa, 1981b), and as distinct species by others (e.g. Entz, 1927; Schiller, 1933; Skuja, 1948; Dottne-Lindgren and Ekbohm, 1975; Hickel, 1988a, b).

Cyclomorphosis, differences associated with the seasonal changes of one morphological form to another, has been widely studied for Ceratium (e.g. Hutchinson, 1967). Pearsall (1929) attributed differences in cell length and the production of a third posterior horn to changes in cell metabolism. Hasle and Nordli (1951) suggested that variation in the marine species Ceratium fusus and C. tripos was caused by high nutrient concentrations and prolonged diurnal light period. The gradual change in cell dimensions throughout the year noted by several authors (Pearsall, 1929; Dottne-Lindgren and Ekbohm, 1975; Krupa, 1981b) has been interpreted as being caused by seasonal rather than genetic factors. Dottne-Lindgren and Ekbohm (1975) recognised three types of C. hirundinella with corresponding cysts. These forms occurred together at the same time, discounting the possibility of cyclomorphosis as the cause of variation.

The differences between C. furcoides and C. hirundinella were emphasised by Langhans (1925). He presented outline drawings of the two taxa and proposed that C. hirundinella var. furcoides Levander, be regarded as a separate species. The

Figure 4.2 Form Variation in C.hirundinella (from Huber-Pestalozzi,1950)



1 C.hirundinella furcoides-typus
2 C.hirundinella brachyceroides-typus
3 C.hirundinella silesiacum-typus
4 C.hirundinella carinthiacum-typus
5 C.hirundinella austriacum-typus
6 C.hirundinella gracile-typus
7 C.hirundinella scotticum-typus
8 C.hirundinella robustum-typus
9 C.hirundinella piburgense-typus
10 C.hirundinella yünnanense-typus

comprehensive account of Entz (1927) clearly illustrated the characteristics of both C. furcoides and C. hirundinella, and their forms, including details of the tabulation of their thecal plates. These differences are discussed later in this chapter (page 78). However, many later workers (e.g. Huber and Nipkow, 1922; Schiller, 1933; Huber-Pestalozzi, 1950; Hauge, 1958) regarded C. furcoides as a variant of C. hirundinella emphasising the great variation in morphology. It is only recently, following more detailed ecological and taxonomic studies, that C. furcoides has again come to be regarded as a separate species (Dottne-Lindgren and Ekbohm, 1975; Hickel, 1985, 1988b; Canter and Heaney, 1984; Heaney, Lund, Canter and Gray, 1988).

In this investigation the ability to identify reliably the Ceratium taxa has been an essential prerequisite to the quantitative aspects. It was necessary to clarify morphological differences and to establish whether these could be interpreted as cyclomorphosis or whether they were indicative of different species or sub-species. For this purpose a detailed study using light and scanning electron microscopy was made of both the motile cells and the cysts of Ceratium from Esthwaite Water.

Methods

Motile cells of Ceratium collected in net samples from Esthwaite Water, throughout the growing season, were examined with an Olympus BH2 light microscope. Observations were made of cell morphology, ensuring that differences were real and not a

consequence of cell orientation. Linear measurements were made of cells collected at the time of the summer maxima, using an eyepiece graticule. The thecae of cells preserved in Lugol's Iodine were difficult to observe as the individual plates tended to be obscured by the heavily stained cell contents. Therefore, the thecal plates of individual cells were separated using the technique described in Chapter 2 (page 60).

Ceratium cysts were isolated from diluted sediment samples from Esthwaite Water, as outlined in Chapter 2 (page 54). Their morphology was also studied and length and breadth measurements noted.

Both motile cells and cysts were also studied with a Cambridge S100 and a Jeol 25S Scanning Electron Microscope (S.E.M.). A description of the method used to isolate and prepare material is included in Chapter 2 (page 59). Particular attention was paid to the fine detail of the cyst wall. An analysis probe was used in conjunction with the Cambridge S100 S.E.M. in order to establish the composition of both cell and cyst walls.

Results

(i) Observations of Ceratium Motile Cells

Light Microscopy Observations

Two distinct forms of motile cells could be distinguished. Cells resembling C. hirundinella (Entz 1927) were characterized by a marked increase in diameter around the cingulum, and a strongly tapered epitheca which formed a convex "shoulder" above

the cingulum (Plate 4.1, 1-3). Cells corresponding to C. furcoides (Entz 1927) were also observed. These cells were characterised by a more slender appearance, and an epitheca which tapered gradually, and lacked a "shoulder" (Plate 4.1, 4-6).

Length and breadth of individual cells varied considerably throughout the year and between years but were generally consistent within a sample. Figure 4.3 shows the variation in the dimensions of cells of both species measured at the time of the summer maxima. The cells can be seen to be separated into two distinct groups corresponding to the two species. This apparently negative correlation between cell length and cell breadth was shown to be statistically significant using Spearman's Rank Correlation Coefficient (Appendix 4.1). Length, breadth and the length to breadth ratio of both taxa were compared using the Mann-Whitney U test (Appendix 4.2). The difference between all three variables was also shown to be statistically significant. Cells of C. furcoides are longer and narrower (mean length = 248 μm, mean breadth = 46.5 μm) than C. hirundinella cells (mean length = 222.7 μm, mean breadth = 63.7 μm).

Although both taxa appeared in the same sample, to ensure that they did not represent seasonal forms, net samples for an entire season were studied (referred to in detail in Chapter 5, page 165). It was shown that generally, in samples taken during three separate years from Esthwaite Water (1972, 1977 and 1982) the proportion of the two taxa following excystment was

Plate 4.1 Form Variation in Cells of C. hirundinella and C. furcoides

All photographs are light micrographs of preserved material.

1-3 C. hirundinella

Cells broader and shorter, with epitheca forming a convex "shoulder" above the cingulum.

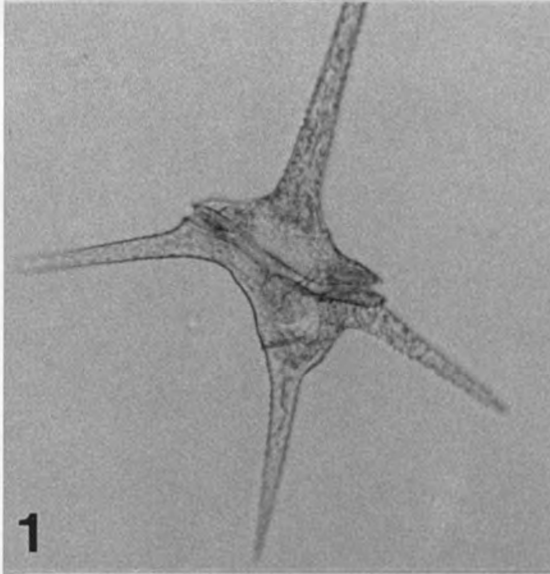
1. Dorsal view of cell with three posterior horns (total cell length = 136 μm; width = 40 μm). Collected from Island Barn Reservoir, 31.7.1985.
2. Dorsal view of cell with shortened third posterior horn (total cell length = 132 μm; width = 44 μm). Collected from Esthwaite Water, 18.8.1954.
3. Dorsal view of cell with greatly reduced third posterior horn (total cell length = 140 μm; width = 36 μm). Collected from Esthwaite Water, 26.8.1957.

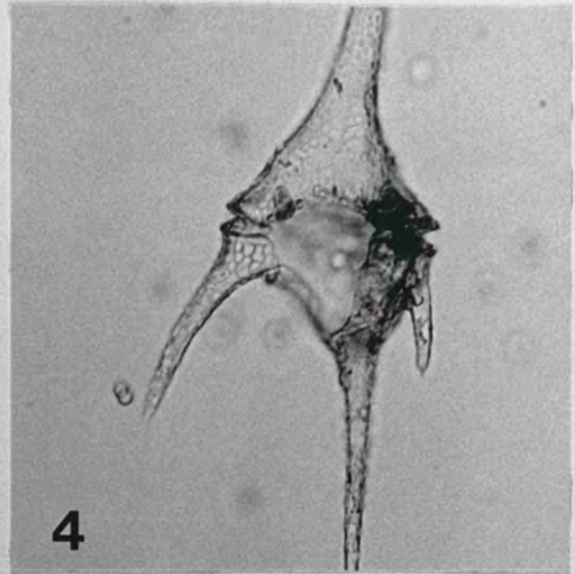
4-6 C. furcoides

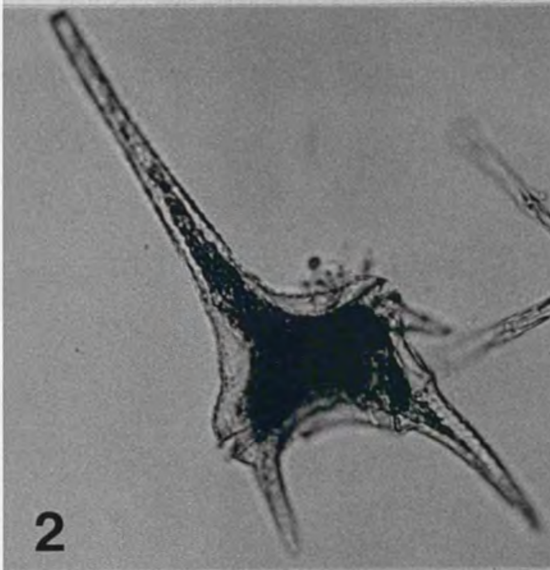
Cells more slender, with epitheca tapering gradually to form a concave angle above the cingulum. Collected from Esthwaite Water during August and September.

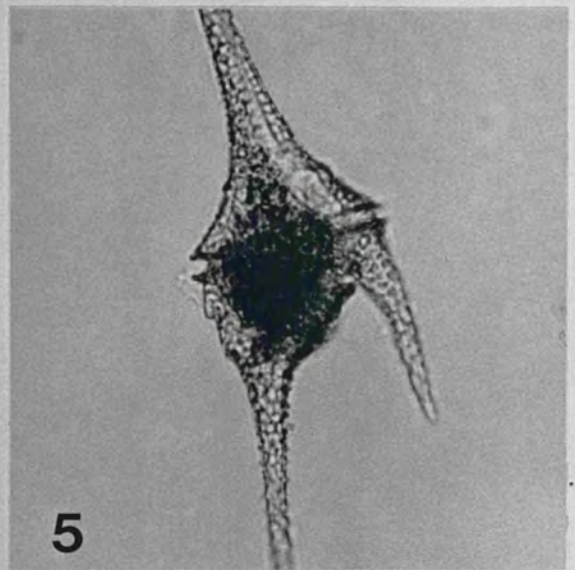
4. Ventral view of cell with three posterior horns (total cell length = 168 μm; width = 40 μm).
5. Ventral view of cell lacking third posterior horn (total cell length = 152 μm; width = 36 μm).
6. Ventral view of cell lacking third posterior horn (total cell length = 234 μm; width = 60 μm).

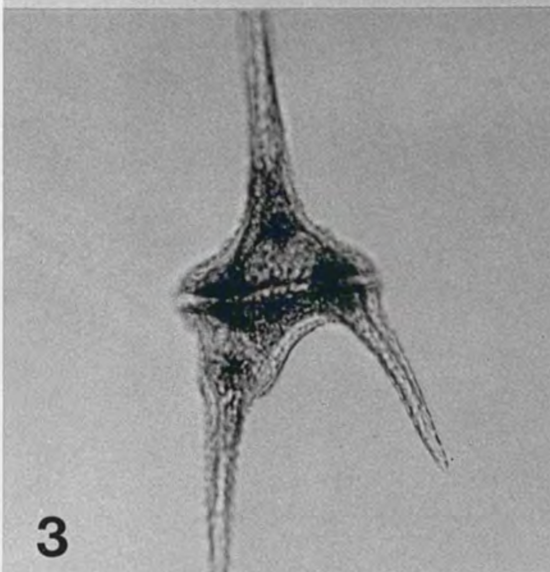
Plate 4.1











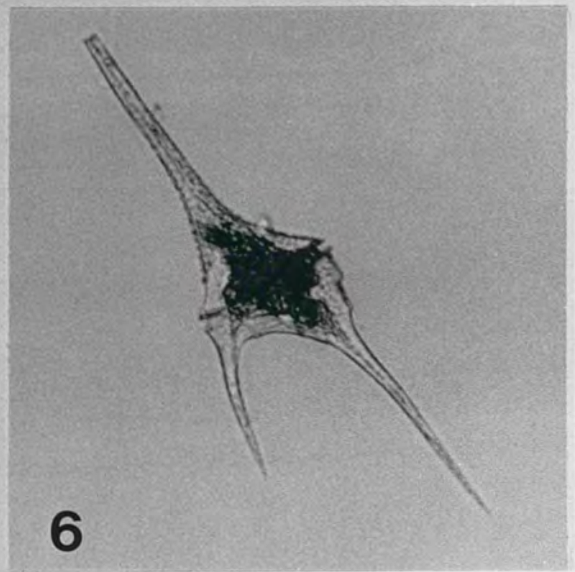
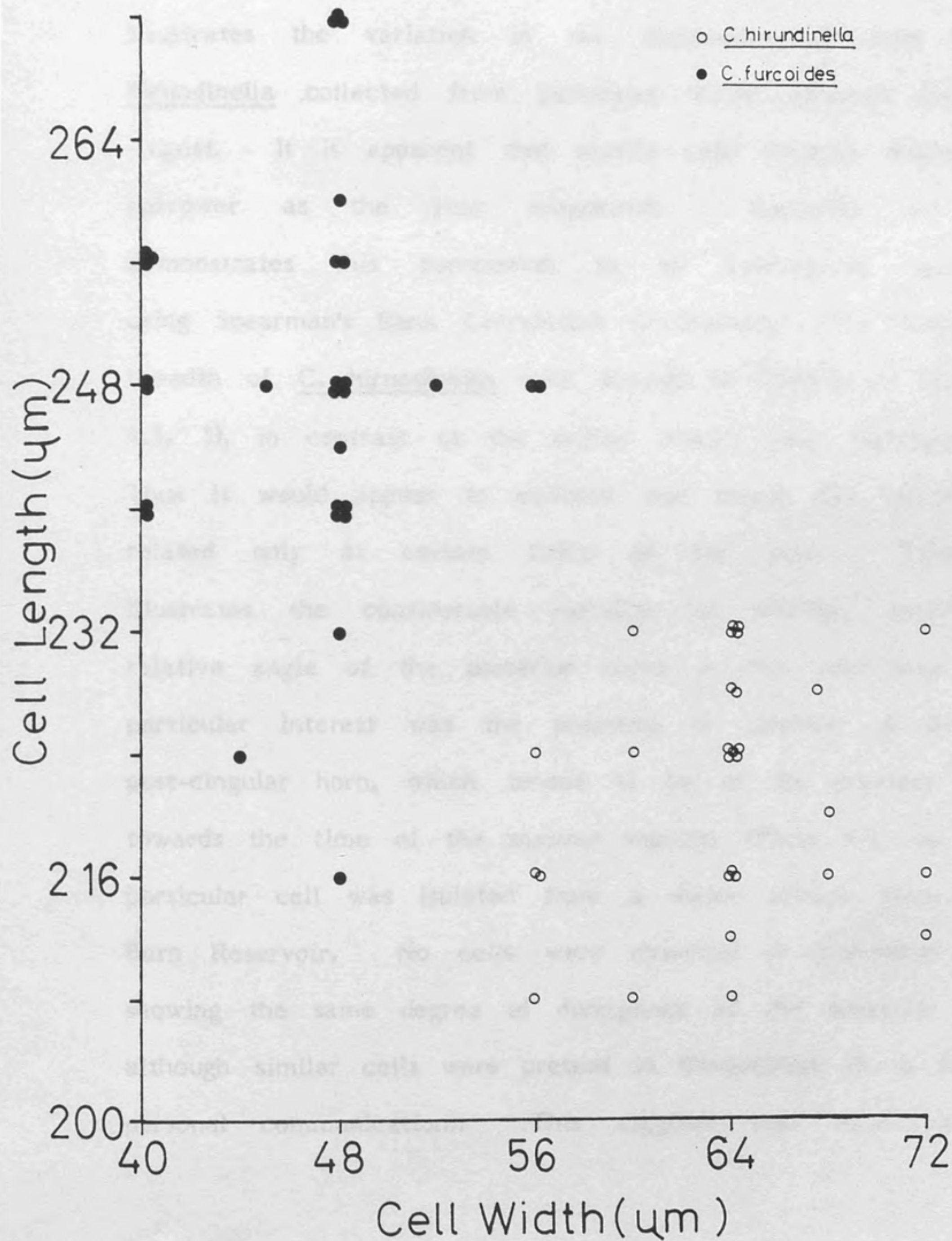


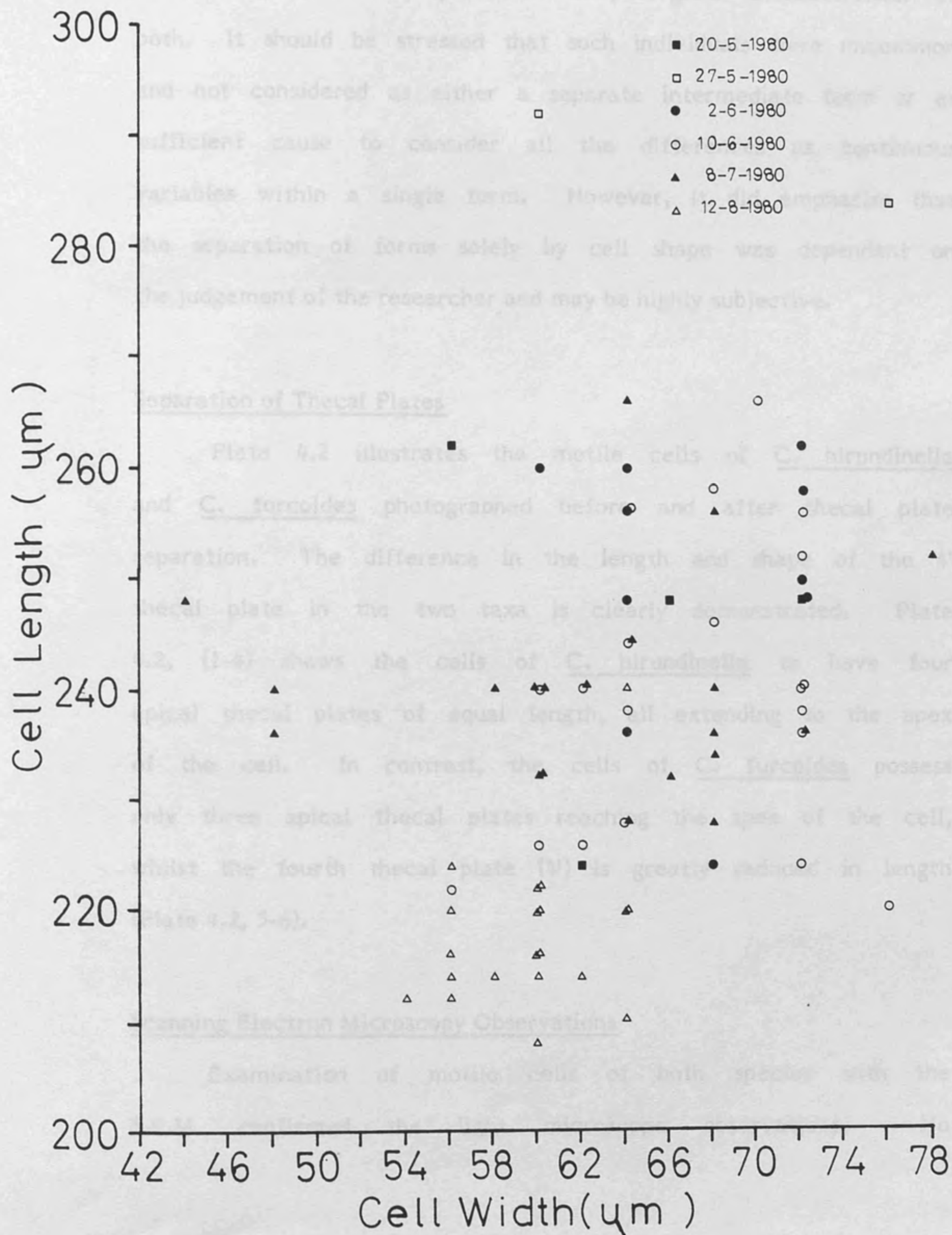
Figure 4.3 Variation in the Size of Ceratium Cells at the Time of the Maxima



maintained throughout the year (illustrated in Figure 5.10, page 166).

In previous studies concerning seasonal form variation in C. hirundinella most attention was focused on overall length and breadth of the cell and the number of posterior horns. Although this was not the main aim of this investigation a brief quantitative study was made over one season. Figure 4.4 illustrates the variation in the dimensions of cells of C. hirundinella collected from Esthwaite Water between May and August. It is apparent that motile cells became shorter and narrower as the year progressed. Appendix 4.3 (1-2) demonstrates this correlation to be statistically significant using Spearman's Rank Correlation Coefficient. The length and breadth of C. hirundinella cells showed no correlation (Appendix 4.3, 3), in contrast to the earlier results (see Appendix 4.1). Thus it would appear to indicate that length and breadth are related only at certain times of the year. Plate 4.1 illustrates the considerable variation in number, length and relative angle of the posterior horns in the two taxa. Of particular interest was the presence or absence of the left post-cingular horn, which tended to be at its greatest length towards the time of the summer maxima (Plate 4.1, 1). This particular cell was isolated from a water sample from Island Barn Reservoir. No cells were observed in Esthwaite Water showing the same degree of divergence of the posterior horns, although similar cells were present in Windermere (S. I. Heaney, personal communication). This suggests that local variation

Figure 4.4 Variation in the Size of <u>C.hirundinella</u> Cells from May to August



exists in cell morphology.

Although the variation in the characteristics used to separate the taxa was discrete and not continuous, it should be noted that a few cells could not be confidently assigned to either taxon, as they possessed morphological characteristics of both. It should be stressed that such individuals were uncommon and not considered as either a separate intermediate form or as sufficient cause to consider all the differences as continuous variables within a single form. However, it did emphasise that the separation of forms solely by cell shape was dependent on the judgement of the researcher and may be highly subjective.

Separation of Thecal Plates

Plate 4.2 illustrates the motile cells of C. hirundinella and C. furcoides photographed before and after thecal plate separation. The difference in the length and shape of the 4' thecal plate in the two taxa is clearly demonstrated. Plate 4.2, (1-4) shows the cells of C. hirundinella to have four apical thecal plates of equal length, all extending to the apex of the cell. In contrast, the cells of C. furcoides possess only three apical thecal plates reaching the apex of the cell, whilst the fourth thecal plate (4') is greatly reduced in length (Plate 4.2, 5-6).

Scanning Electron Microscopy Observations

Examination of motile cells of both species with the S.E.M. confirmed the light microscope observations. No

Plate 4.2 Separation of the Apical Thecal Plates of the Cells of C. hirundinella and C. furcoides

All photographs are light micrographs of preserved material.

1-4 C. hirundinella

Separation of the thecal plates illustrates the presence of four apical plates reaching the top of the epitheca.

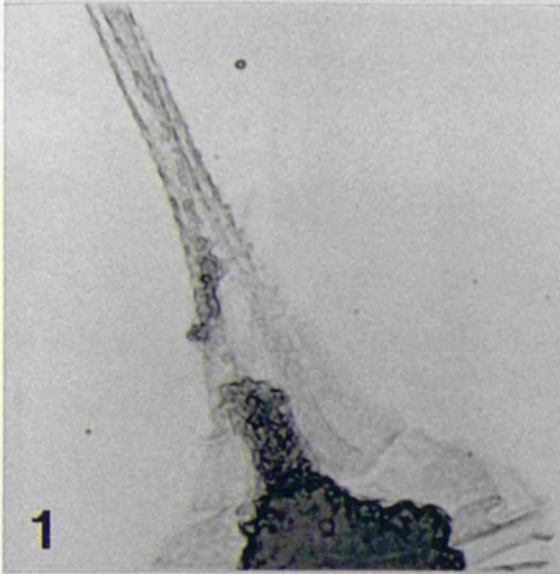
1. Epitheca of cell prior to thecal plate separation (total cell length = 228 μm; width = 78 μm).
2. Epitheca of cell following thecal plate separation, showing the meeting of the four apical plates at the cell apex.
3. Cell prior to thecal plate separation, shown under phase contrast (total cell length = 242 μm; width = 72 μm)
4. Epitheca of cell after thecal plate separation, illustrating the presence of four apical plates.

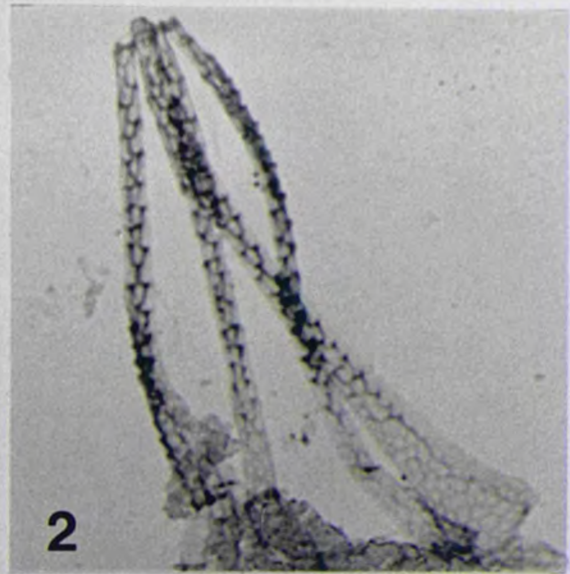
5-6 C. furcoides

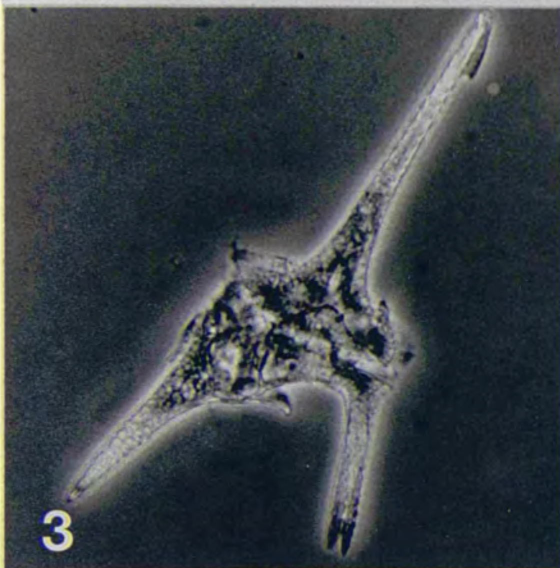
Separation of the thecal plates illustrates the presence of three apical plates reaching the top of the epitheca and a fourth plate much reduced in length.

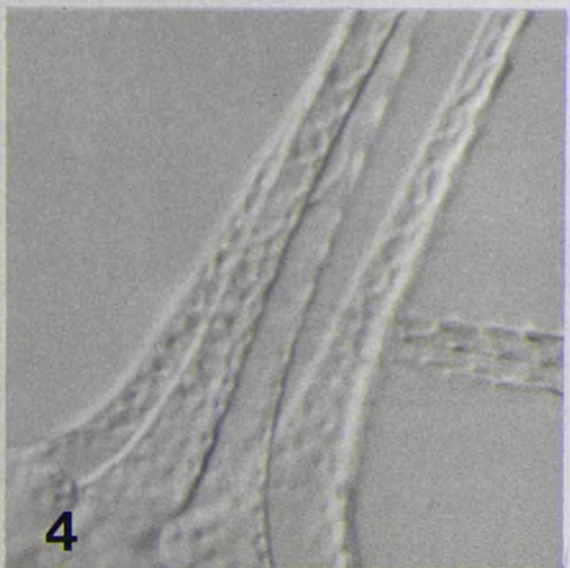
5. Cell prior to thecal plate separation (total cell length = 168 μm; width = 44 μm).
6. Epitheca of cell showing the reduction in the length of the fourth apical thecal plate (4'), shown under phase contrast.

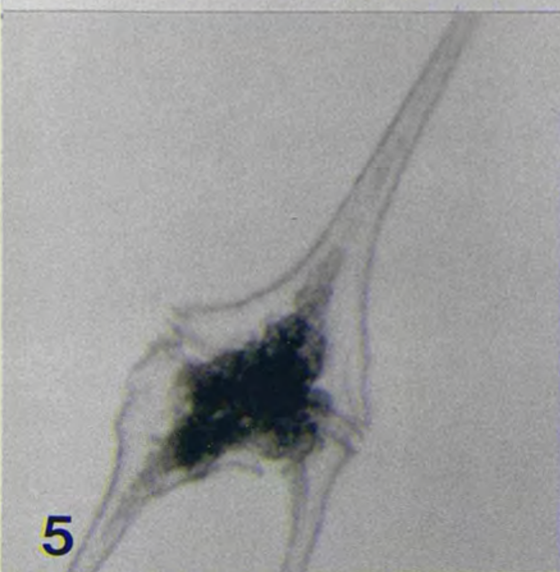
Plate 4.2

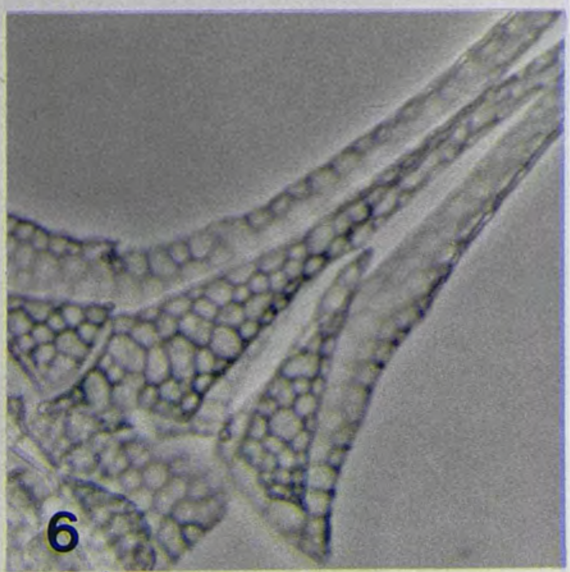












additional differences were observed.

In addition, motile cells of both taxa were examined with an X-ray micro-analysis probe linked to the S.E.M, in order to establish any differences in chemical composition of the cell wall. Figure 4.5 shows examples of typical spectra produced by cells of the two taxa. Considerable variation was observed between different locations on the same cell, and between cells of the same species. Differences between the two taxa are thus difficult to isolate. Calcium registered in both types of cell (Figure 4.5, 1 and 4) and sulphur (Figure 4.5, 1, 3 and 4). Silicon, present in all four traces, was more plentiful in C. hirundinella (see page 94).

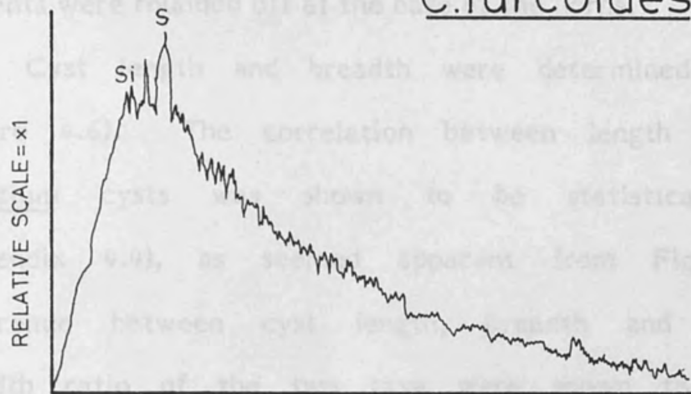
(ii) Observations of Ceratium Cysts

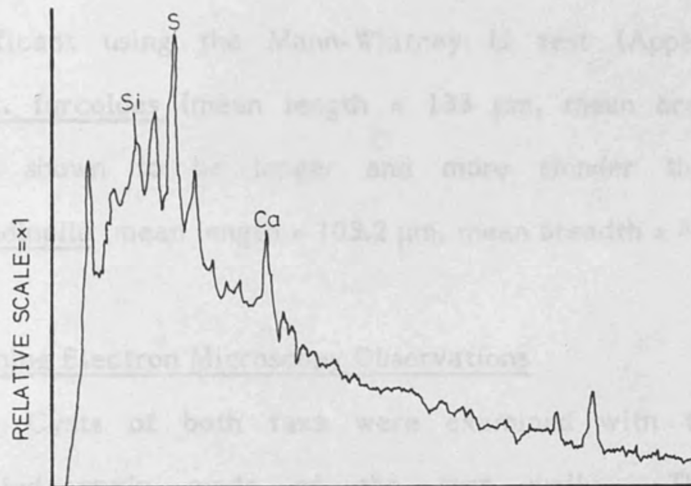
Light Microscopy Observations

Cysts of freshwater Ceratium reflect the shape of the motile cells. Two distinct forms of cyst could be clearly identified. Excystment experiments, discussed in detail in Chapter 6, revealed that each cyst type produced motile cells with the characteristics described previously. Cysts of C. hirundinella were nearly spherical in shape with shorter horns (Plate 4.3, 1). C. furcoides cysts were more tetrahedral in appearance with extended horns (Plate 4.4, 1). The number of posterior horns varied, as in the motile cells. The most frequent arrangement was a single apical horn and two posterior horns, although three posterior horns was not uncommon. In the cysts of C. hirundinella the cyst contents were observed to

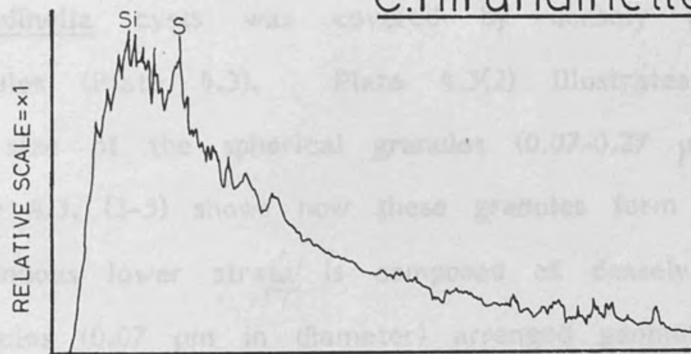
Figure 4.5 Analysis Spectra for <u>Ceratium</u> Cells

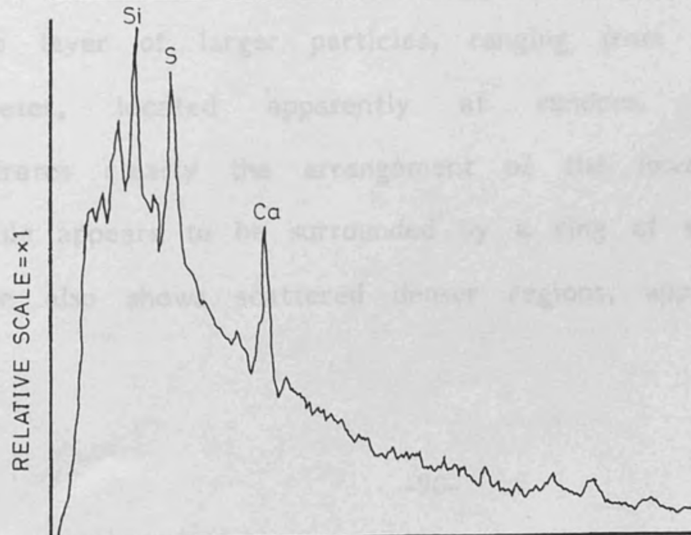
<u>C.furcoides</u>





<u>C.hirundinella</u>





extend into the horns, whilst in cysts of C. furcoides the cyst contents were rounded off at the base of the horns.

Cyst length and breadth were determined for both taxa (Figure 4.6). The correlation between length and breadth of Ceratium cysts was shown to be statistically insignificant (Appendix 4.4), as seemed apparent from Figure 4.6. The difference between cyst length, breadth and the length to breadth ratio of the two taxa were shown to be statistically significant using the Mann-Whitney U test (Appendix 4.5). Cysts of C. furcoides (mean length = 133 µm, mean breadth = 40.3 µm) were shown to be longer and more slender than those of C. hirundinella (mean length = 103.2 µm, mean breadth = 46.1 µm).

Scanning Electron Microscopy Observations

Cysts of both taxa were examined with the S.E.M and a detailed study made of the cyst wall. The wall of C. hirundinella cysts was covered by densely packed spherical granules (Plate 4.3). Plate 4.3(2) illustrates differences in the size of the spherical granules (0.07-0.27 µm in diameter). Plate 4.3, (3-5) shows how these granules form two layers. A continuous lower strata is composed of densely packed smaller particles (0.07 µm in diameter) arranged geometrically, with an upper layer of larger particles, ranging from 0.11-0.22 µm in diameter, located apparently at random. Plate 4.3(3) illustrates clearly the arrangement of the lower layer. Each granule appears to be surrounded by a ring of six others. This figure also shows scattered denser regions, apparently replacing

Figure 4.6 Variation in the Size of <u>Ceratium</u> Cysts from Esthwaite Water

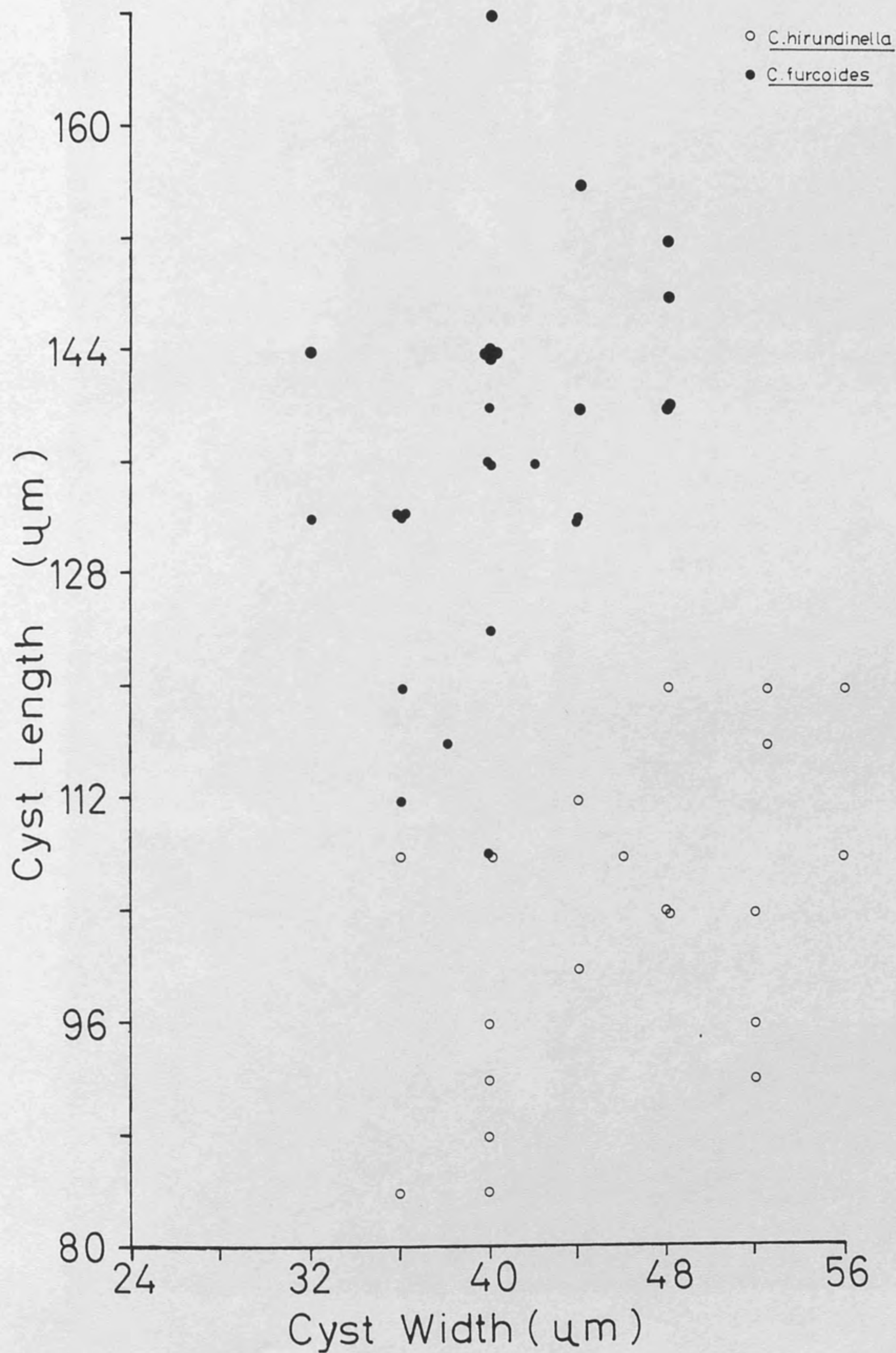
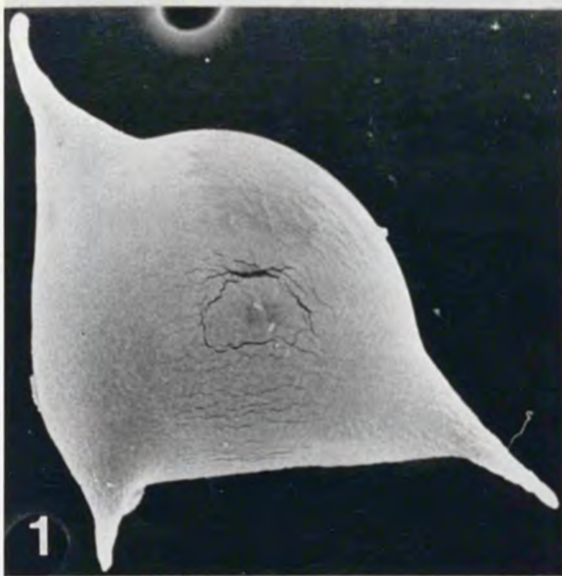


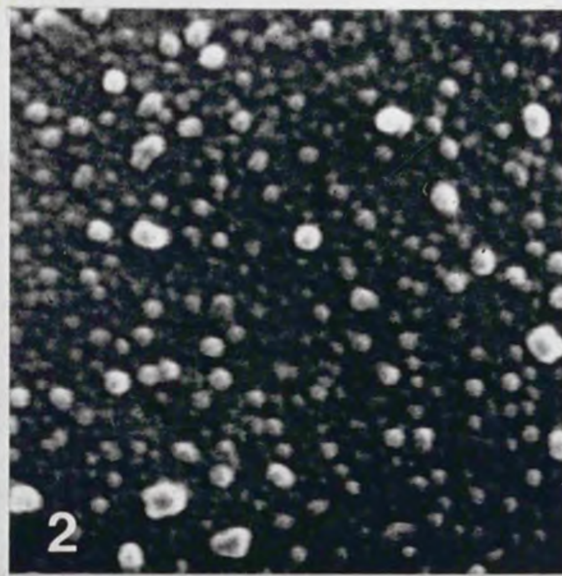
Plate 4.3 Morphological Details of the Cyst Wall of C. hirundinella

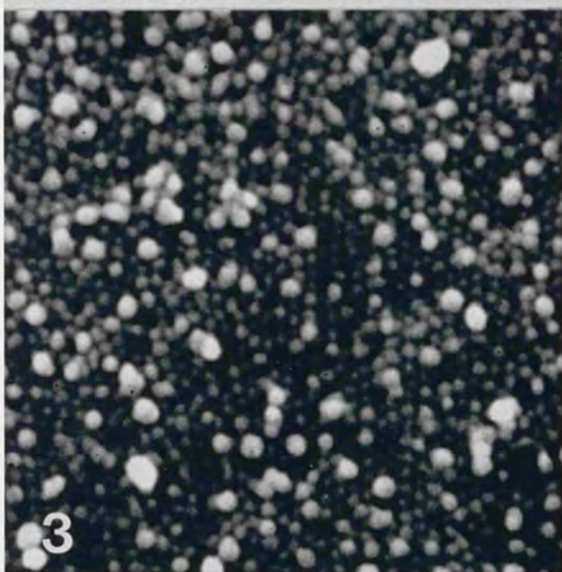
All photographs are scanning electron micrographs of preserved material.

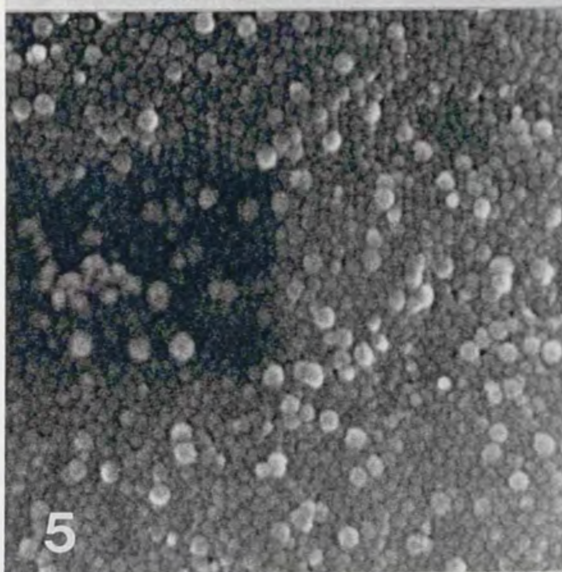
1. C. hirundinella cyst (maximum cyst length = 94.55 μm).
2. Details of the cyst wall showing the differences in size of granules (diameter = 0.077-0.27 μm). Magnification = x 26,000.
3. Further details of cyst wall showing the geometric arrangement of granules. Magnification = x 27,000.
4. Further details of cyst wall showing the arrangement of granules into a lower layer of densely packed smaller granules (diameter = 0.07 μm) and a discontinuous upper layer of larger granules (diameter = 0.11-0.22 μm). Magnification = x 27,500.
5. Additional view of both layers. Magnification = x 20,000.
6. Details of the lower granular layer. The broad, dark irregular line corresponds to cracks in the cyst shown in (1). Magnification = x 31500.

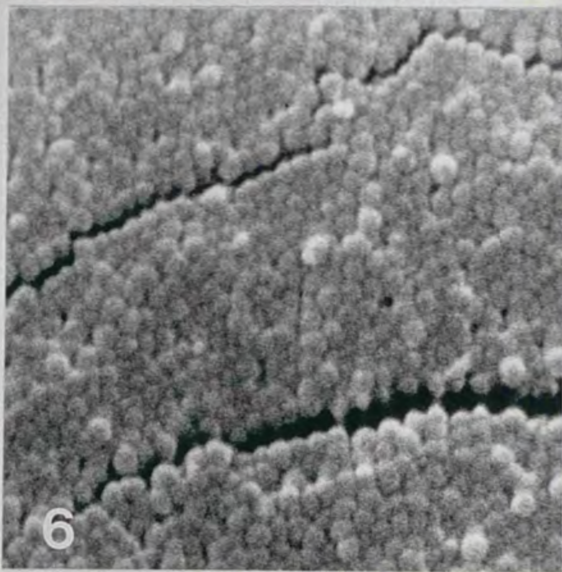
Plate 4.3











granules on the lower levels. These may represent granules which have been lost. Plate 4.3(6) shows still greater detail of the cyst wall of C. hirundinella. The broad dark irregular bands correspond to the cracks in the cyst wall, visible in Plate 4.3(1). These are caused by prolonged exposure to the electron beam. It is interesting to note that the cracks form between granules. Particles on either side of the divide match like jigsaw pieces.

Plate 4.4 illustrates a similar study of C. furcoides cysts. Although a double granular layer was also present (Plate 4.4, 3-4), the granules in the lower layer appeared less densely packed and lacked the geometric arrangement shown by C. hirundinella cysts. Granules were of comparable size to those observed in C. hirundinella (0.07-0.22 μm in diameter).

An X-ray micro-analysis probe linked to the S.E.M. was used to determine differences in the composition of the cyst wall. "Spot analysis" enabled specific areas of the cyst to be selected. Figures 4.7-4.8 illustrate the range of analysis spectra obtained for three sites on a cyst of C. hirundinella. All traces show that the most significant element present is silicon. Values varied considerably over the surface of a cyst and between cysts, indicating that silicon was not necessarily present in a uniform layer.

Figure 4.9 shows the equivalent spectra for C. furcoides cysts. Although still present, silicon is observed in substantially reduced amounts. Variation was less marked than in C. hirundinella.

Plate 4.4 Morphological Details of the Cyst Wall of *C. furcoides*

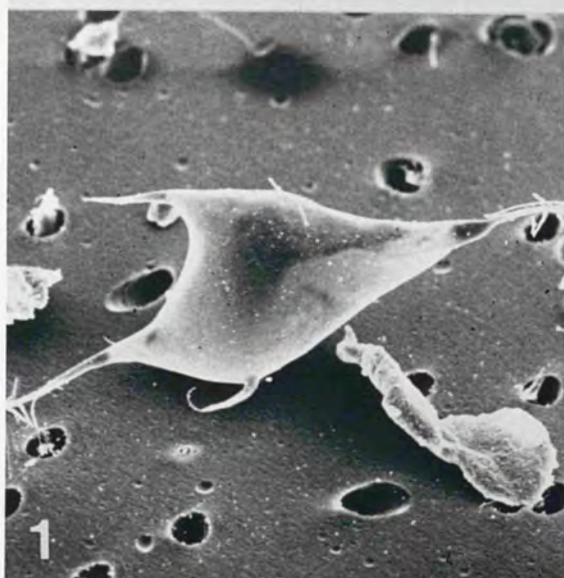
All photographs are scanning electron micrographs of preserved material.

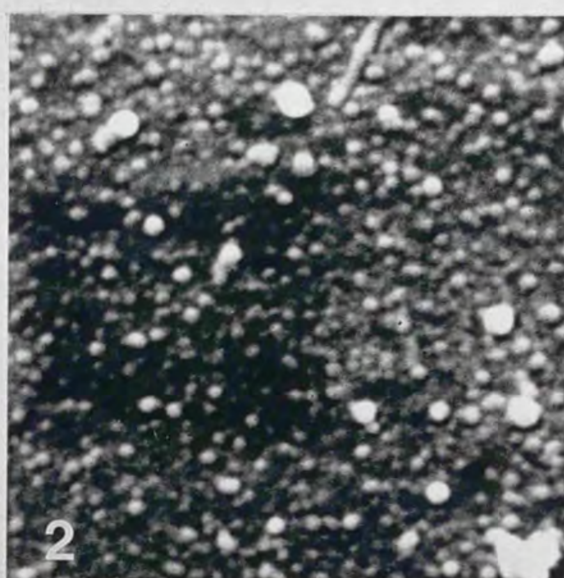
1. *C. furcoides* cyst (maximum cyst length = 132 µm).

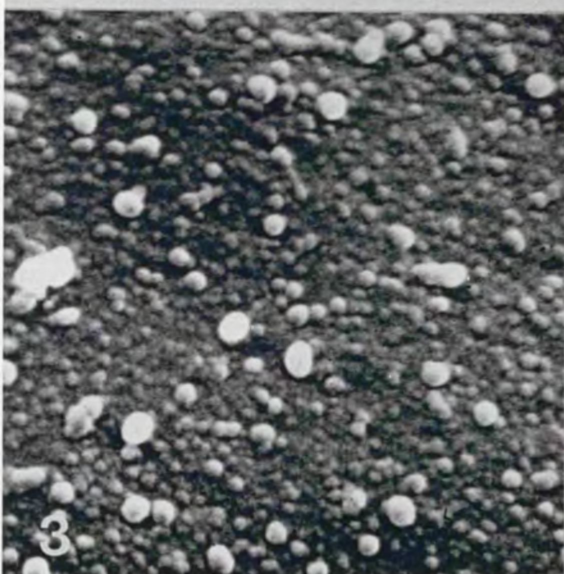
2-6 Details of cyst wall showing the presence of a granular layer, but lacking the geometric structure of the cyst wall of *C. hirundinella*.

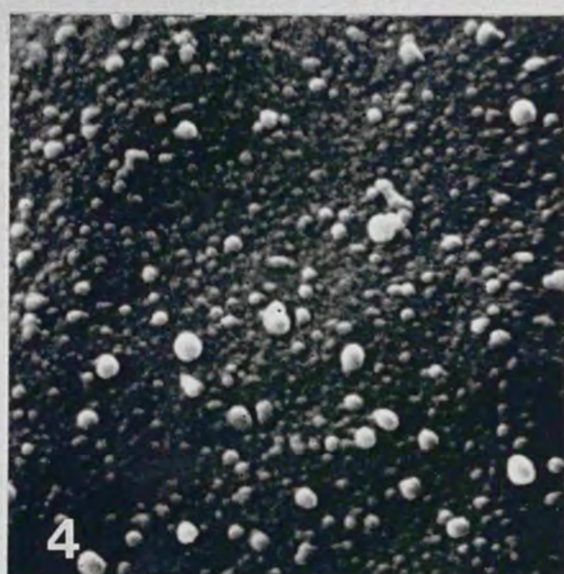
2. Magnification = x 26,500
3. Magnification = x 27,000
4. Magnification = x 26,750
5. Magnification = x 27,250
6. Magnification = x 29,500

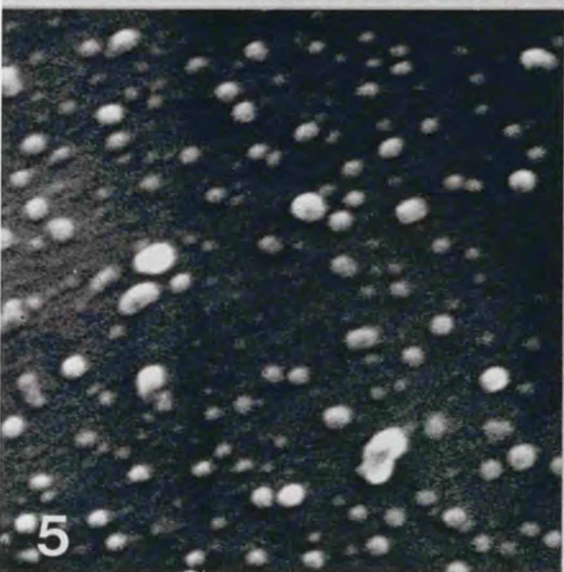
Plate 4.4











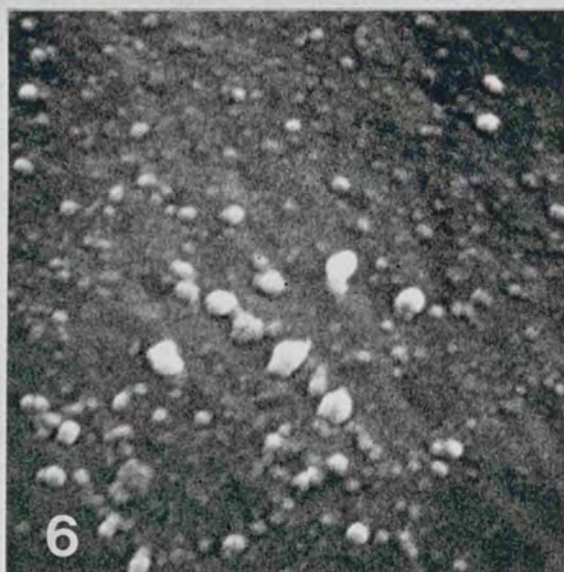
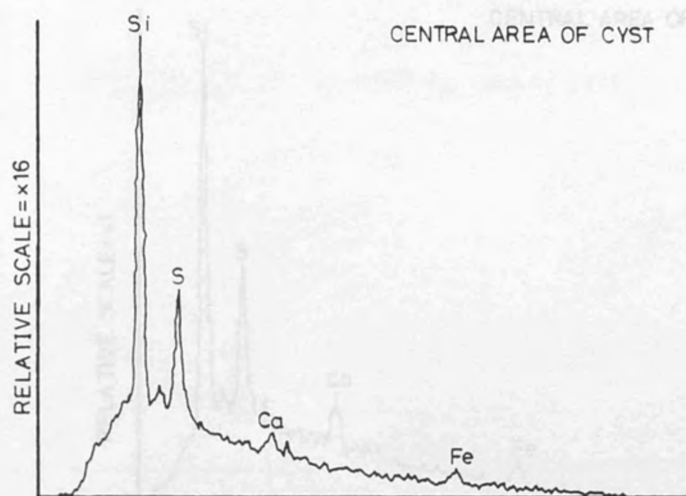
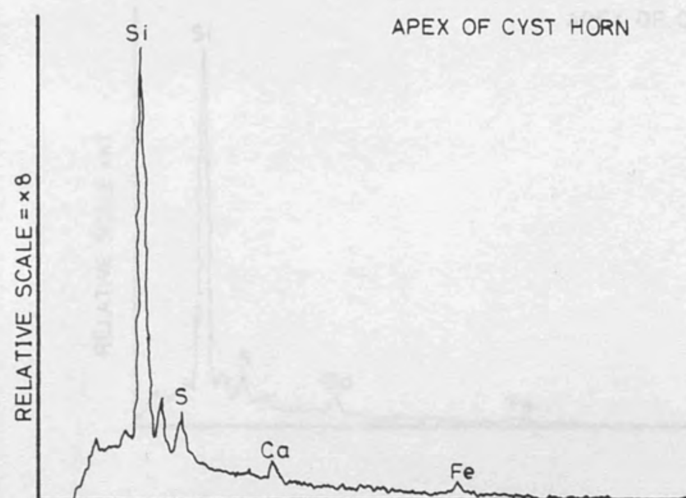


Figure 4.7 Analysis Spectra for C.hirundinella Cysts (1)





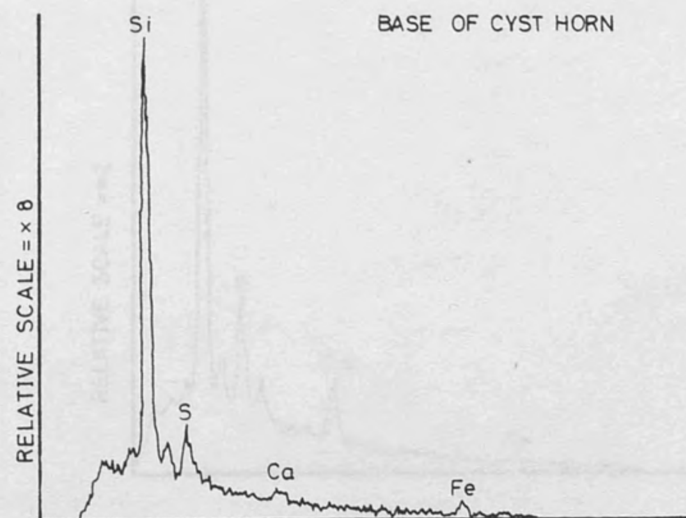
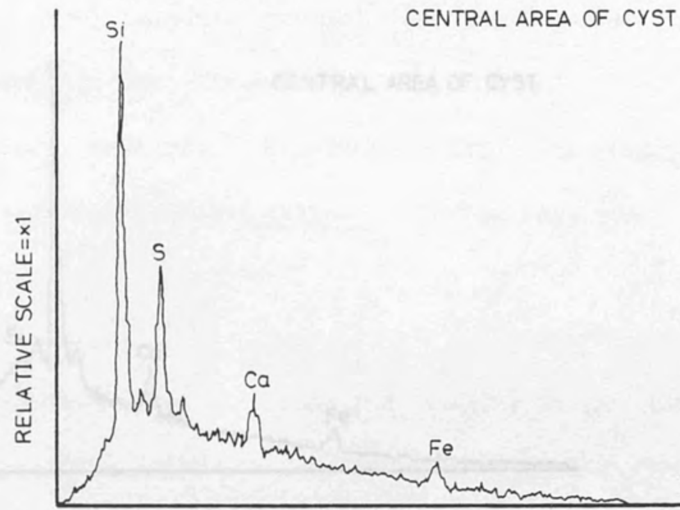
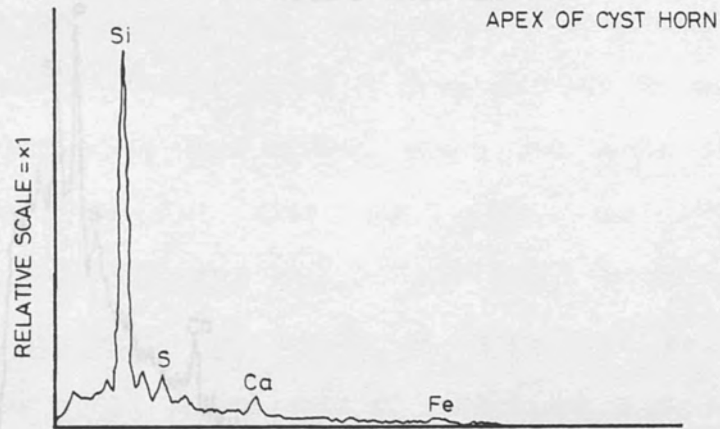


Figure 4.8 Analysis Spectra for *C.hirundinella* Cysts (2)





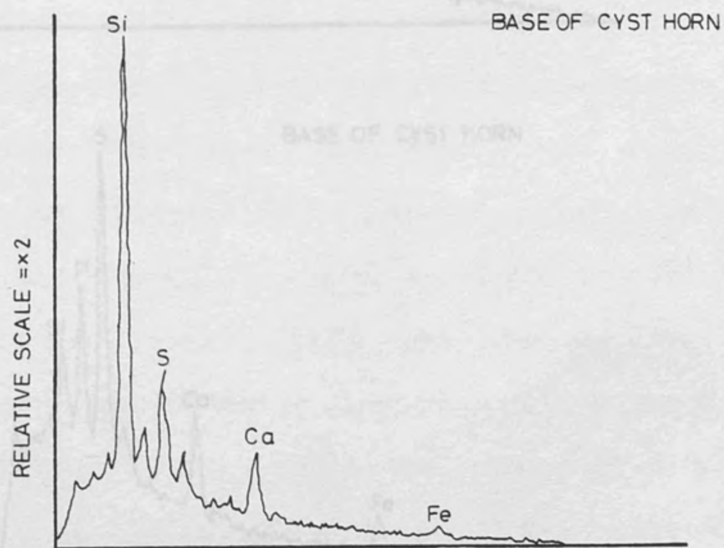
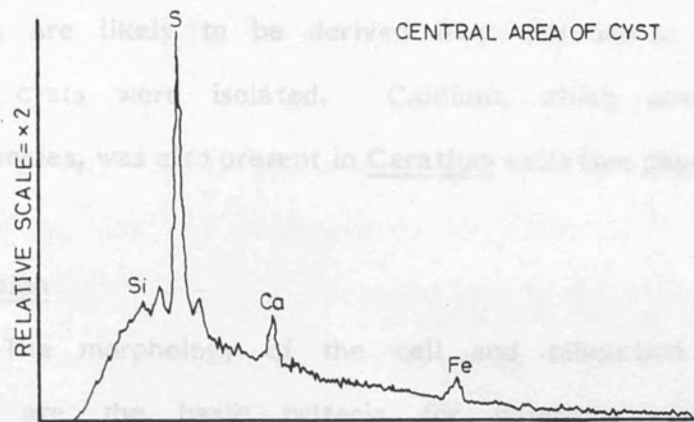
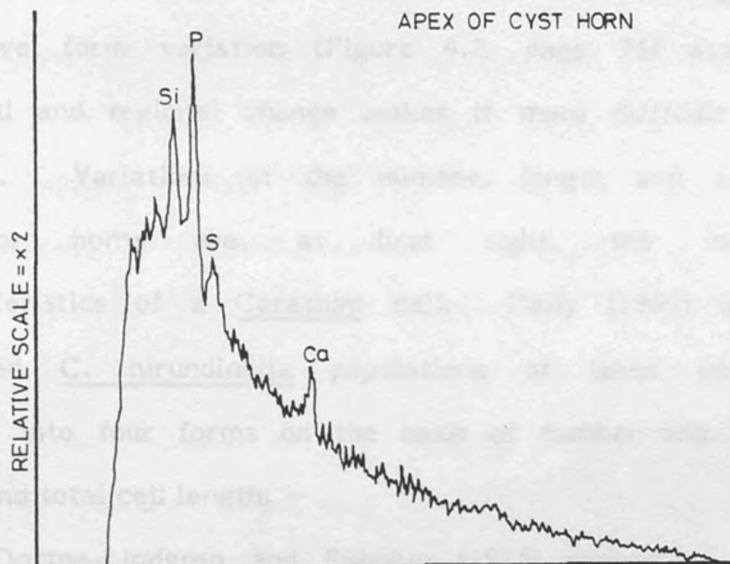
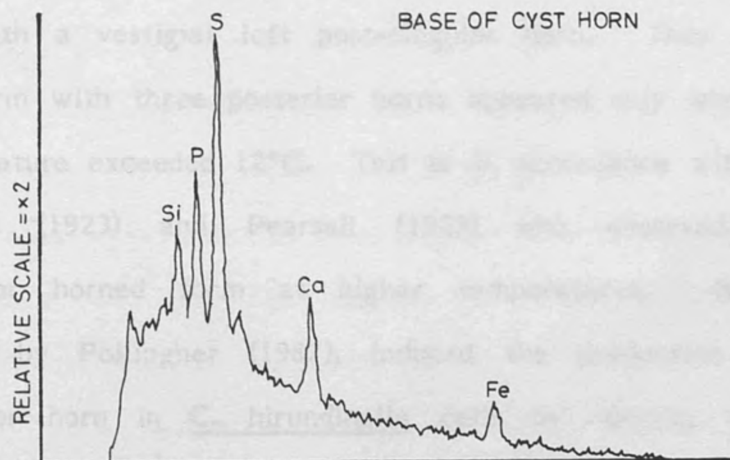


Figure 4.9 Analysis Spectra for C.furcoides Cysts







The iron and sulphur present in the spectra of both species are likely to be derived from the anoxic sediments from which cysts were isolated. Calcium, which also registered in both species, was also present in Ceratium cells (see page 88).

Discussion

The morphology of the cell and tabulation of the thecal plates are the basic criteria for determining the species of dinoflagellate (Dodge, 1984). In the case of Ceratium the extensive form variation (Figure 4.2, page 76) associated with seasonal and regional change makes it more difficult to separate species. Variations in the number, length and angle of the posterior horns are, at first sight, the most obvious characteristics of a Ceratium cell. Daily (1960) separated the so-called C. hirundinella populations of lakes and ponds of Indiana into four forms on the basis of number and shape of the horns and total cell length.

Dottne-Lindgren and Ekbohm (1975) recognised three forms of C. hirundinella, with and without a left post-cingular horn and with a vestigial left post-cingular horn. They showed that the form with three posterior horns appeared only when the water temperature exceeded 12°C. This is in accordance with Huber and Nipkow (1923) and Pearsall (1929) who observed the three posterior horned form at higher temperatures. Bruno (1975), quoted by Pollingher (1987), induced the production of a third posterior horn in C. hirundinella cells by varying temperatures. At 15°C a large percentage of cells had three posterior horns,

corresponding with the results of the previous workers. However, at 21-25°C all cells in a culture had two posterior horns. The superficial study undertaken here showed that the form with three posterior horns appeared in the phytoplankton towards the summer maxima, at temperatures of 15-20°C. Temperatures in excess of 21°C were not recorded and it was thus impossible to observe whether the form with 2 posterior horns was restored at higher temperatures.

Dottne-Lindgren and Ekbohm (1975) maintained that the three forms in their study were independent of each other and hatched from specific cysts. All three were present at the same time, implying that this was not a consequence of cyclomorphosis. However, Happach Kasan (in a poster presented at the Hexrose Conference on modern and fossil dinoflagellates, Tubingen, West Germany, 1981) isolated four types of cell from a single clonal culture of C. furcoides, distinguished by the number of posterior horns and the angles between them. This suggests that one form evolved from another as outlined in the earlier work (e.g. Pearsall, 1929; Hutchinson, 1967). Heaney, Jaworski and Cranwell (paper presented at the "Dino III" Conference on modern and fossil dinoflagellates, Egham, Surrey, 1985) showed that a similar sequence of events occurs in cysts. An isolated cyst with 3 posterior horns excysted to produce a cell which was in turn induced to encyst. The cyst thus produced had only two posterior horns. The differences in these features would appear to be seasonal or environmental in nature and therefore not a suitable basis for identification.

Seasonal changes in the linear dimensions of cells are also striking. Krupa (1981b) showed that the length of the left post-cingular horn was the most variable dimension in cells measured throughout the year. Dottne-Lindgren and Ekbohm (1975) and Krupa (1981b) both observed that cell length of C. hirundinella decreased from a spring maximum to produce short individuals during the summer, which corresponds with the limited data presented in the present study. In contrast Morling (1979) noted that in two other species of Ceratium, C. carolinianum and C. cornutum, there was an increase in length during the summer.

Dottne-Lindgren and Ekbohm (1975), like Pearsall (1929) before, suggested that the longer winter form of C. hirundinella was a consequence of metabolic changes in the cell. Chapman, Dodge and Heaney (1985) in a study of ultrastructural changes within C. hirundinella noted a decrease in starch, lipid, pyrenoids and accumulation bodies in cells collected between April and September, after which abundance once again increased. Pollingher (1987) suggested that decreasing cell length is a consequence of the increased division rate brought about by an increase in temperature. Earlier observations by Serruya and Pollingher (1977) had noted a reduction in the body size of Peridinium cinctum in response to an increased division rate in cells from Lake Kinneret, Israel.

Pearsall (1929) compared the length to breadth ratios of motile cells of C. hirundinella through the year. During the spring the average ratio was 3.77, falling sharply to 3.4 or

less, in the summer. In the present study values reached a maximum of 4.3 in spring, falling to 3.6 in early summer, but increasing again in subsequent months. This rise indicates that the late summer cells measured in this study were either longer or more slender than those observed by Pearsall. With such diverse seasonal changes it is clear that only when morphological characteristics remain constant through the year can they be used as a basis for identification.

The morphological features used in the present study to distinguish between the two taxa have been employed for the same purpose by several previous authors (Entz, 1927; Skuja, 1948; Canter and Heaney, 1984; Hickel, 1985), all of whom identified *C. furcoides* as a separate species.

The separation of the thecal plates revealed a major difference in the tabulation of the two taxa. The illustrations of *C. furcoides* in Entz (1927) correspond to the observations of the present study, but those of *C. hirundinella* show the presence of only three apical plates. However, other authors (Hurst and Strong, 1931; Huber-Pestalozzi, 1950; Bourrelly, 1968) all clearly demonstrate the presence of four apical plates of equal length *C. hirundinella*, as shown here. Wall and Evitt (1975) illustrated the disassociated thecal plates of a freshwater *Ceratium* cell described as *C. hirundinella*. However, the presence of a shortened 4' plate and the overall shape of the cell are indicative of *C. furcoides*. A relabelled version of the same cell appeared in a later work (Evitt, 1985).

Excystment experiments in the present study (Chapter 6)

and by Heaney and Jaworski (unpublished data) demonstrate that cysts of characteristic form excyst to produce motile cells of the respective taxa. Entz (1925) established that C. hirundinella and C. furcoides produced specific cysts which resembled the shape of the cell. He also reported that the range in cyst size is less than that of the motile stage. This is in agreement with the data presented here (compare Figure 4.3 and 4.6). Huber and Nipkow (1922) also distinguished three morphologically different cysts, but interpreted these as representing forms of the same species.

Chapman, Dodge and Heaney (1982) reported the walls of C. hirundinella cysts to be covered with a uniform layer of closely packed silicon-containing granules. The present investigation, has confirmed the presence of a granular wall. Examination at a higher magnification enabled further details to be elucidated. X-ray micro-analysis indicated the occurrence of high silicon values in C. hirundinella cysts. A second cyst type, believed by Chapman, Dodge and Heaney (1982) to be a "temporary" cyst, was smooth walled, and apparently lacking in silicon and such granules. Canter and Heaney (1984) interpreted such cysts as the overwintering cysts of C. furcoides. In this study C. furcoides was also shown to have a granular wall, but not as complex as that of C. hirundinella.

X-ray micro-analysis showed the presence of silicon, in cysts of C. furcoides, but in relatively lower quantities to C. hirundinella. This suggests the presence of some silicon-containing granules within the cyst wall of both

species. The closely packed lower granular layer of C. hirundinella may contribute to this higher silicon value. However, it is unclear whether the silicon containing granules correspond to the lower, or the upper layers described. Chapman, Dodge and Heaney (1982) showed that silicon is present in specialised vesicles in the motile cells of C. hirundinella prior to encystment. Following cyst formation the silicon granules are mobilised and become deposited as a single outer layer of the cyst wall. Silicon has also been identified in the cyst wall of Peridinium species and the fossil cyst Peridinites (Evitt, 1985). The purpose of the incorporation of silicon into the cyst wall is unknown.

Chapman, Dodge and Heaney (1982) suggested that possession of a silicon layer enabled the cysts of C. hirundinella to survive in the sediment for prolonged periods. However, in Chapter 6 of the present work it will be shown that cysts of both C. hirundinella and C. furcoides remain viable in the sediment for several years, even though the latter species appear to have a reduced silicon layer. An alternative explanation is that the silicon granules protect C. hirundinella cysts from parasitic attack (Chapman, Dodge and Heaney, 1982). The chytrid Rhizophydium nobile Canter is believed to be specific to C. furcoides cysts (Canter and Heaney, 1984; Heaney, Lund, Canter and Gray, 1988), but there are other fungal parasites which attack the cysts of C. hirundinella (Heaney, Lund, Canter and Gray, 1988).

The differences in the morphology of motile cells and

cysts, the arrangement of thecal plates, the composition of cyst walls and the specificity of some fungal parasites all indicate the existence of two genetically distinct taxa. It is more difficult to establish whether two species, or two forms of the same species are involved. The existence of morphologically different cysts, which excyst to produce motile cells that are morphologically true to form, and the fact that, of the two taxa, sexual reproduction has only been observed in C. furcoides (Hickel, 1988a), suggests that the two taxa have independent life cycles. Entz (1924) observed chain formation in C. hirundinella and suggested that this could be evidence of sexual reproduction, however, no similar observations have been made in recent studies. The difficulty of maintaining a laboratory culture of C. hirundinella compared with the relative ease of growing C. furcoides (G. Jaworski, personal communication) suggests a difference in nutrient requirements or sensitivity to handling.

In all, this information would suggest that the two taxa are sufficiently distinct to warrant the growing acceptance that they represent different species. In some previous studies such differences have been overlooked and both taxa collectively referred to as C. hirundinella. For example, Dodge and Crawford (1970) used collections of wild material, which were probably C. hirundinella, in addition to cultured material from the Freshwater Biological Association, which has since been identified as C. furcoides. In addition the diagrams of cysts found in Felt Lake, California, by Wall and Evitt (1975) are

representative of C. furcoides and not C. hirundinella as labelled.

Recently, Hickel (1988b) has proposed that a taxon of Ceratium, similar in appearance to C. furcoides and C. furcoides f. gracile (Bachmann) Entz, should be recognised as a separate species, C. rhomvoides Hickel. Cells of this species differ from those of C. furcoides in size, shape and reticulation of the theca, and in the size and shape of the apical thecal plates. A corresponding cyst was also identified which was of "rounded-triangular" appearance with very short horns. However, no evidence was given that the motile cells shown excysted from these cysts. Sexual reproduction was also observed to occur. It is considered that the present study may have included cells of C. rhomvoides. However, although cells of this species bear a close resemblance to C. furcoides, they are markedly shorter, the length of C. rhomvoides is 123-203 µm, whilst the length of C. furcoides is 162-322 µm. (Hickel, 1985), and it is felt that such a striking characteristic could not have been overlooked.

Clearly, in future studies of freshwater Ceratium care should be taken to identify species correctly before proceeding with experimental work.

APPENDIX 4.1 Calculation of the Spearman's Rank Correlation Coefficient to compare cell dimensions

Use of Spearman's Rank Correlation Coefficient

Spearman's Rank Correlation Coefficient (r_s) is a non-parametric test, used to compare paired observations. Each set of data is ranked, from the lowest to the highest value. The difference between the ranks of each pair of observations (d) is calculation and squared (d^2). The following formula is generally used:-

$$r_s = 1 - \frac{6 \Sigma d^2}{n(n^2-1)}$$

However, since the data in this study included many tied observations the alternative formula was often used, which made an adjustment for ties:-

$$r_s = \frac{\Sigma x^2 + \Sigma y^2 - \Sigma d^2}{2\sqrt{\Sigma x^2 . \Sigma y^2}}$$

where $\Sigma x^2 = \frac{N^3 - N}{12} - \Sigma Tx$

$\Sigma y^2 = \frac{N^3 - N}{12} - \Sigma Ty$

N = no. of observations

$\Sigma Tx = \frac{(\text{no. of ties at given rank})^3 - \text{no. of ties}}{12}$

$\Sigma Ty = \frac{(\text{no. of ties at given rank})^3 - \text{no of ties}}{12}$

To test the significance of r_s the following formula was used:-

$$t = r_s \sqrt{\frac{N-2}{1-r_s^2}}$$

The figure obtained was compared with tables (Siegel, 1956).

<u>Is there a Correlation between the Length and Breadth of Ceratium Cells?</u>

$$\Sigma x^2 = \frac{N^3-N}{12} - \Sigma Tx$$

$$\Sigma Tx = \frac{(2^3-2)+3(3^3-3)+(5^3-5)+(7^3-7)+(17^3-17)+(18^3-18)}{12}$$

$$= 937$$

$$\Sigma x^2 = \frac{60^3-60}{12} - 937 = 17058$$

$$\Sigma y^2 = \frac{N^3-N}{12} - \Sigma Ty$$

$$\Sigma Ty = \frac{(2^3-2)+3(3^3-3)+(5^3-5)+(6^3-6)+2(7^3-7)+(9^3-9)+(11^3-11)}{12}$$

$$= 260$$

$$\Sigma y^2 = \frac{60^3-60}{12} - 260 = 17735$$

$$r_s = \frac{\Sigma x^2+\Sigma y^2-\Sigma d^2}{2\sqrt{\Sigma x^2 . \Sigma y^2}} = \frac{17058+17735-56873}{2\sqrt{17058 \times 17735}} = -0.63$$

$$t = r_s \sqrt{\frac{N-2}{1-r_s^2}} = 0.63 \sqrt{\frac{60-2}{1-(0.63)^2}} = 6.178$$

Degrees of freedom = (n-2) = 60-2 = 58

from tables $t_{0.01,60} = 2.39$

$\therefore t_{\alpha,58} > t_{0.01,60}$

The correlation between the two variables is statistically significant at the 99% level of probability. Therefore a negative correlation exists between length and breadth of cells of C. hirundinella.

APPENDIX 4.2 **Calculation of the Mann-Whitney U Test to compare cell dimensions**

Use of the Mann-Whitney U Test

The Mann-Whitney U Test is used to assess whether two independent samples come from the same population (Siegel, 1956). This non-parametric test is equivalent to the parametric Student's t-test. The null hypothesis (H_0) states that variable A and variable B have the same distribution. Data from the two samples are ranked as one sample. The standard deviation (σ_u) is calculated where:-

$$\sigma_u = \sqrt{\left(\frac{n_1 . n_2}{N(N-1)}\right)\left(\frac{N^3-N}{12} - \Sigma T\right)}$$

The inclusion in the calculation of the term ΣT provides an adjustment for tied ranks. Two possible values of U exist where:-

$$U = n_1.n_2 + n_1(n_1+1) - R_1$$

$$\text{or } U = n_1.n_2 + n_2(n_2+1) - R_2$$

The lower of the two values of U is then applied to the following formula:-

$$z = \frac{1}{\sigma_u}\left(U - \frac{n_1 . n_2}{2}\right)$$

Tables are used to find the probability of the occurrence of H_0 for the value of z obtained. If this tabulated probability value is less than $\alpha = 0.01$, H_0 can be rejected.

__Is there a Statistically Significant Difference between the Length of Cells of the two *Ceratium* taxa?__

H_0 : The length of cells of the two __*Ceratium*__ taxa are not significantly different.

H_1 : The length of cells of the two __*Ceratium*__ taxa differ significantly.

$$\Sigma T = 274$$

$$\sigma_u = \sqrt{\left(\frac{30x30}{60(60-1)}\right)\left(\frac{60^3-60}{12} -274\right)} = 67.1$$

$$U = 30 \times 30 + \frac{30(30+1)}{2} - 1327.5 = 37.5$$

$$z = \frac{1}{67.1}\left(37.5 - \frac{30x30}{2}\right) = 6.15$$

From tables $z \geqslant 6.15$ has a 1-tailed probability under H_0 of $p<0.00003$. Therefore, since this value is less than $\alpha = 0.01$, H_0 can be rejected at the 99% level of significance. Therefore, the difference between the length of __*Ceratium*__ cells of the two taxa is statistically significant.

Is there a Statistically Significant Difference between the Breadth of Cells of the two *Ceratium* taxa?

H_0 : The breadth of cells of the two *Ceratium* taxa are not significantly different.

H_1 : The breadth of cells of the two *Ceratium* taxa differ significantly.

$$\Sigma T = \frac{8(2^3-2)+2(3^3-3)+3(4^3-4)+(5^3-5)+(6^3-6)+(7^3-7)}{12} = 78.5$$

$$\sigma_u = \sqrt{\left(\frac{30x30}{60(60-1)}\right)\left(\frac{(60^3-60)}{12} - 78.5\right)} = 67.49$$

$$U = 30x30 + \frac{30(30+1)}{2} - 1361 = 4.0$$

$$z = \frac{1}{67.49}\left(4.0-\frac{30 \times 30}{2}\right) = -6.61$$

From tables $z \geqslant 6.61$ has a 1-tailed probability under H_0 of $p<0.00003$. Therefore, since this value is less than $\alpha = 0.01$, H_0 can be rejected at the 99% level of significance. Therefore, the difference between the breadth of *Ceratium* cells of the two taxa is statistically significant.

Is there a Statistically Significant Difference between the Length to Breadth Ratio of Cells of the two Ceratium taxa?

H_0 : The length to breadth ratio of cells of the two *Ceratium* taxa are not significantly different.

H_1 : The length to breadth ratio of cells of the two *Ceratium* taxa differ significantly.

$$\Sigma T = \frac{8(2^3-2)+3(3^3-3)+2(4^3-4)+(5^3-5)+(6^3-6)+(7^3-7)}{12} = 75.5$$

$$\sigma_u = \sqrt{\left(\frac{30 \times 30}{60(60-1)}\right)\left(\frac{60^3-60}{12} - 75.5\right)} = 67.5$$

$$U = 30 \times 30 + \frac{30(30+1)}{2} - 1365 = 0$$

$$z = \frac{1}{67.5}\left(0 - \frac{30 \times 30}{2}\right) = -6.67$$

From tables $z \geqslant 6.67$ has a 1-tailed probability under H_0 of $p<0.00003$. Therefore, since this value is less than $\alpha = 0.01$, H_0 can be rejected at the 99% level of significance. Therefore the difference between the length to breadth ratio of the *Ceratium* cells of the two taxa is statistically significant.

APPENDIX 4.3 Statistical analysis of C. hirundinella cells sampled throughout the year using Spearman's Rank Correlation Coefficient

Does the Length of C. hirundinella Cells Change Significantly through the Year?

$$\Sigma Tx = \frac{(2^3-2)+(4^3-4)+(10^3-10)+3(20^3-20)}{12} = 2083$$

$$\Sigma x^2 = \frac{76^3-76}{12} - 2083 = 34492$$

$$\Sigma Ty = \frac{7(2^3-2)+5(3^3-3)+3(4^3-4)+(5^3-5)+2(6^3-6)+(11^3-11)}{12}$$

$$= 183.5$$

$$\Sigma y^2 = \frac{76^3-76}{12} - 183.5 = 36391.5$$

$$r_s = \frac{34492 + 36391.5 - 118831.5}{2\sqrt{34492 \times 36391.5}} = 0.68$$

$$t = 0.68\sqrt{\frac{76-2}{1-0.68^2}} = 7.98$$

Degress of freedom = (n-2) = 76-2 = 74

from tables $t_{0.01,60} = 2.39$

$\therefore t_{\alpha,74} > t_{0.01,60}$

The correlation between the two variables is statistically significant at the 99% level of probability.

Therefore the difference in the length of C. hirundinella cells through the year is statistically significant.

Does the Breadth of C. hirundinella Cells Change Significantly through the Year?

$\Sigma Tx = 2083$

$$\Sigma x^2 = \frac{76^3-76}{12} - 2083 = 34492$$

$\Sigma Ty = 860.5$

$$\Sigma y^2 = \frac{76^2-76}{12} - 860.5 = 35714.5$$

$$r_s = \frac{34492 + 35714.5 - 106395.5}{2\sqrt{34492 \times 35714.5}} = -0.516$$

$$t = 0.516 \sqrt{\frac{76-2}{1-0.516^2}} = 5.18$$

Degress of freedom = (n-2) = 76-2 = 74

from tables $t_{0.01,60} = 2.39$

$\therefore t_{\alpha,74} > t_{0.01,60}$

The correlation between the two variables is statistically significant at the 99% level of probability.

Therefore the difference in the breadth of C. hirundinella cells through the year is statistically significant.

Is Cell Length Correlated with Cell Breadth in Cells of C. hirundinella throughout the Year?

$$\Sigma x^2 = \frac{76^3-76}{12} - 860.5 = 35714.5$$

$$\Sigma y^2 = \frac{76^3-76}{12} - 183.5 = 36391.5$$

$$r_s = \frac{35714.5 + 36391.5 - 84591}{2\sqrt{35714.5 \times 36391.5}} = -0.173$$

$$t = 0.173\sqrt{\frac{76-2}{1-0.173^2}} = 1.46$$

Degress of freedom = (n-2) = 76-2 = 74

from tables $t_{0.01,60} = 2.39$

$\therefore t_{\propto,74} < t_{0.01,60}$

The correlation between the two variables is not statistically significant at the 99% level of probability.

Therefore, no correlation exists between length and breadth in cells of C. hirundinella.

APPENDIX 4.4 Statistical analysis of Ceratium cysts using Spearman's Rank Correlation Coefficient

Is there a Correlation between Length and Breadth of Ceratium Cysts?

$$\Sigma\, Tx = \frac{(2^3-2)+(6^3-6)+2(7^3-7)+(16^3-16)}{12} = 414$$

$$\Sigma\, x^2 = \frac{48^3-48}{12} - 414 = 8798$$

$$\Sigma\, Ty = \frac{5(2^3-2)+2(3^3-3)+2(4^3-4)+(5^3-5)+2(6^3-6)}{12} = 61.5$$

$$\Sigma\, y^2 = \frac{48^3-48}{12} - 61.5 = 9150.5$$

$$r_s = \frac{8798 + 9150.5 - 19440}{2\sqrt{8798 \times 9150.5}} = -0.08$$

$$t = 0.08\sqrt{\frac{48-2}{1-0.08^2}} = 0.544$$

Degrees of freedom = (n-2) = 48-2 = 46

from tables $t_{0.01,40} = 2.42$

$\therefore t_{\alpha,46} < t_{0.01,40}$

The correlation between the two variables is not statistically significant at the 99% level of probability.

Therefore, no correlation exists between cyst length and breadth.

APPENDIX 4.5 Comparison of cyst dimensions using the Mann-Whitney U Test

Is there a Difference in the Length of Cysts of the two *Ceratium* taxa?

H_0 : Length of cysts of the two *Ceratium* taxa are the same.

H_1 : Length of cysts of the two *Ceratium* taxa differ significantly.

$\Sigma T = 61.5$

$$\sigma_u = \sqrt{\left(\frac{28\text{x}20}{48(48-1)}\right)\left(\frac{48^3-48}{12} - 61.5\right)} = 47.67$$

$$U = 28 \times 20 + \frac{28(28+1)}{2} - 949.5 = 16.5$$

$$z = \frac{1}{47.67}\left(16.5 - \frac{28\text{x}20}{2}\right) = -5.53$$

From tables $z \geqslant 5.53$ has a 1-tailed probability under H_0 of $p < 0.00003$. Therefore since this value is less than $\alpha = 0.01$, H_0 can be rejected.

Therefore, there is a statistically significant difference between the length of Ceratium cysts of the two taxa.

Is there a Difference in the Breadth of Cysts of the two Ceratium taxa?

H_0 : Breadth of cysts of the two Ceratium taxa are the same.

H_1 : Breadth of cysts of the two Ceratium taxa differ significantly.

$$\Sigma T = \frac{2(2^3-2)+(5^3-5)+(6^3-6)+2(7^3-7)+(16^3-16)}{12} = 424$$

$$\sigma_u = \sqrt{\left(\frac{28 \times 20}{48(48-1)}\right)\left(\frac{48^3-48}{12} - 424\right)} = 46.7$$

$$U = 28 \times 20 + \frac{20(20+1)}{2} - 628.5 = 141.5$$

$$z = \frac{1}{46.7}\left(141.5 - \frac{28 \times 20}{2}\right) = -2.96$$

From tables $z \geqslant 2.96$ has a 1-tailed probability under H_0 of $p < 0.0015$. Therefore, since the value is less than $\alpha = 0.01$, H_0 can be rejected.

Therefore, there is a statistically significant difference between the breadth of Ceratium cysts of the two taxa.

Is there a Difference in the Length to Breadth Ratio of Cysts of the two *Ceratium* taxa?

H_0 : The length to breadth ratio of cysts of the two *Ceratium* taxa are the same.

H_1 : The length to breadth ratio of cysts of the two *Ceratium* taxa differ significantly.

$$\Sigma T = \frac{7(2^3-2)+3(3^3-3)+2(4^3-4)+2(5^3-5)}{12} = 39.5$$

$$\sigma_U = \sqrt{\left(\frac{28 \times 20}{48(48-1)}\right)\left(\frac{48^3-48}{12} - 39.5\right)} = 47.72$$

$$U = 28 \times 20 + \frac{28(28+1)}{2} - 961 = 5.0$$

$$z = \frac{1}{47.72}\left(5 - \frac{28 \times 20}{2}\right) = -5.76$$

From tables $z \geqslant 5.76$ has a 1-tailed probability under H_0 of $p < 0.00003$. Therefore, since the value is less than $\alpha = 0.01$, H_0 can be rejected.

Therefore there is a statistically significant difference between the length to breadth ratio of *Ceratium* cysts of the two taxa.

CHAPTER 5

THE STRATIGRAPHY AND POPULATION DYNAMICS OF CERATIUM IN ESTHWAITE WATER

Introduction

Of the 2,000 species of living dinoflagellates only 10% are known to have a dormant cyst stage in their life cycle (Dale, 1983). In the genus Ceratium, no cysts have been identified for any of the large number of marine species, although they have been recorded for several freshwater species - C. hirundinella, C. furcoides (Entz, 1925, 1927), C. rhomvoides (Hickel, 1988b), C. cornutum and C. carolinianum (Entz, 1925).

Observations of encystment in Ceratium have been made by several authors (e.g. Entz 1925, 1927; Chapman, Dodge and Heaney, 1982). Cysts form within the motile cell. During the stationary phase of growth, motile cells of Ceratium show a marked increase of starch and lipids, the former causing cells to become strongly stained by Lugol's Iodine. At the same time chloroplasts become reduced and condensed. Chapman, Dodge and Heaney (1982) observed many vesicles containing electron-dense granules in cells with increased storage products, which may be indicative of cells about to encyst. The flagella are shed prior to encystment and the cytoplasm withdrawn from the cell horns. The theca then separates to release the cyst.

The newly formed cyst is easily identified by a depression around the centre, corresponding to the position of the

cingulum, and by the rounded ends to its horns, which take time to condense to the characteristic points. Cysts are generally brown in colour, with orange pigmented bodies throughout the cytoplasm, which are thought to correspond to the accumulation bodies in motile cells (Chapman, Dodge and Heaney, 1982). At this stage the ultrastructure of the cyst resembles that of the motile cell at the stationary phase, but without a theca. Bounded by two membranes, the cyst contains an abundance of starch and lipids. Chapman, Dodge and Heaney (1982) distinguished granular and smooth walled cysts. In the former granule-containing vesicles were present in cells prior to encystment. Small spherical granules (60 nm in diameter) were deposited in a uniform layer on the outer membrane of such cysts, beneath which a thick wall layer developed. The cyst contents undergo some reorganisation, until, when fully dormant, the brown pigmentation is condensed at the centre of the cyst, with lipid globules towards the edge.

Anderson, Lively, Reardon and Price (1985) observed that encystment of the marine dinoflagellates Gyrodinium uncatenum, Gonyaulax tamarensis and Scrippsiella trochoidea produced cysts with higher densities than the motile cells. This increase was attributed to the thick multi-layered cellulose cell walls and a dense accumulation of starch. The increased density enabled cysts to sink rapidly to the sediment. The same authors calculated that in 31% sea water at 22°C the sinking rate of the cysts listed previously, based on Stokes Law, was 0.008-0.013 cm s^{-1} (equivalent to 6.91-11.23 m day^{-1}). In addition, cysts

covered with many short spines (e.g. Scrippsiella trochoidea) were observed to descend more rapidly than cysts with no spines. However, the adaptive significance of surface ornamentation is, as yet, unknown.

The conditions triggering the onset of encystment in C. hirundinella have yet to be determined. Chapman, Dodge and Heaney (1982) reported the "spontaneous production" of smooth-walled cysts (i.e. C. furcoides) in laboratory culture. Some authors have successfully induced the sexual formation of cysts in other dinoflagellates by transferring motile cells to a nitrogen-free culture medium, e.g. Peridinium cinctum (Pfiester, 1975, 1976, 1977) and Gonyaulax tamarensis (Turpin, Dobell and Taylor, 1978); whilst others have achieved the same result using a nitrogen and phosphorus-free culture medium, e.g. Peridinium cunningtonii (Sako, Ishida, Kadota and Hata, 1984), Gyrodinium uncatenum (Anderson, Coats and Tyler, 1985) and Gonyaulax tamarensis (Anderson, Kulis and Binder, 1984). Details of the inclusion of a sexual stage in the dinoflagellate life cycle are given in Chapter 6 (page 240).

Anderson and Wall (1978) observed that pellicle (temporary) cysts of Gonyaulax tamarensis and G. excavata, could be produced by exposing motile cells to a temperature reduction (from 16°C to 0°C), "nutrient starvation" (transference to culture media lacking nitrogen, phosphorus, silicon and vitamins), or toxic copper concentrations ($\geqslant 10^{-9.7}$M copper). Schmitter (1979) observed that cells of G. excavata in culture remained motile under a wide range of temperature and light

conditions, including darkness at 3-4°C for 20 hours. However, when exposed to temperatures of 1-2°C in darkness for 20 hours 87-100% of cells became non-motile, with 70-90% forming temporary cysts. A maximum of 26% of non-motile cells regained their motility within 70 hours of normal conditions being restored. Anderson, Kulis and Binder (1984) induced encystment in G.tamarensis by limiting phosphorus and nitrogen. They observed optimum cyst production over a narrow temperature range, and deduced that the onset of encystment is more sensitive to temperature change than is the division of motile cells. No cysts were formed at those temperatures at which growth of motile cells were observed.

The formation of dormant cysts enables some dinoflagellate species to survive periods unfavourable for growth, thus providing an inoculum for growth in subsequent years. In temperate lakes and reservoirs Ceratium cells overwinter as cysts when temperatures are too low ($< 10°C$) for significant growth (e.g. Huber and Nipkow, 1923; Heaney, Chapman and Morison, 1983). However, in sub-tropical lakes, e.g. Lake Kinneret in Israel, C. hirundinella oversummers in cyst form (Pollingher, 1986a), avoiding the very high summer temperatures ($> 26°C$), which are also unfavourable for growth.

Not all the viable cysts produced in a given season will germinate the following year once the threshold temperature has been reached (Heaney, Chapman and Morison, 1983). It is still uncertain whether excystment occurs within the sediment or following the resuspension of cysts into the water column

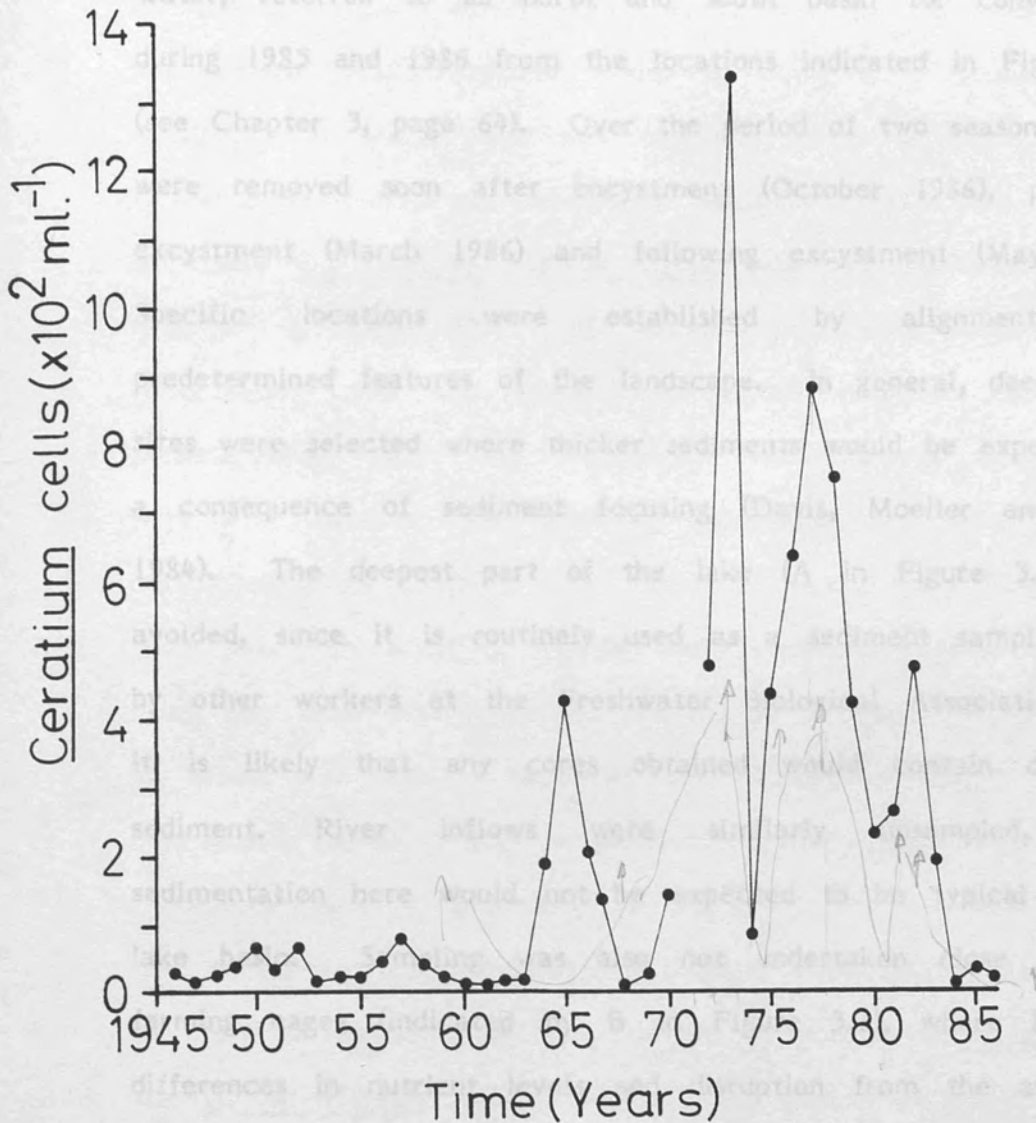
(e.g. by wave action). The cysts which fail to excyst may correspond to those which are not resuspended. They may remain within the sediment and excyst at a later date, compensating for a poor production of cysts in a particular year. Viable cysts, buried several centimetres below the sediment surface, may be reintroduced to the sediment water interface as a consequence of water turbulence, or the activity of benthic animals. Huber and Nipkow (1922) sucessfully germinated C. hirundinella cysts up to 6½ years old from the varved sediments of Lake Zurich, Switzerland. Chapter 6 of the present study demonstrates the successful germination of cysts from 5.5 cm, interpreted as 7 years old. Huber and Nipkow only assessed the viability of cysts and did not attempt an estimate of cyst numbers. Livingstone (1979) studied the vertical distribution of Ceratium cysts in several water bodies. In Esthwaite Water he observed that less than 7% of the cysts occurred below the upper 3 cm of sediment. The loss of some cysts would be expected from the sediment as a result of degeneration, loss through lake outflows and predation. The importance of parasitism in the determination of the Ceratium population has been recently established (Canter and Heaney, 1984; Heaney, Lund, Canter and Gray, 1988) and is discussed in detail later. In neither of these previous studies was any distinction made between C. hirundinella and C. furcoides.

A major part of this study was involved in establishing the vertical distribution of C. hirundinella and C. furcoides cysts within the upper 8 cm sediment of cores taken from

Esthwaite Water. The sediment of Esthwaite Water is not laminated and therefore the age of a core section cannot be directly established. The determination of core section age based on the average rate of sedimentation can be highly speculative and should be treated with caution (see page 180). Therefore, in this study changes in diatom stratigraphy were used to attribute a date to the sediment core. Cyst viability throughout the sediment core was assessed by inducing excystment in the laboratory, and this is discussed in Chapter 6.

Comparatively few research establishments have monitored phytoplankton changes over a long period of time (i.e. decades). In 1945 Lund instigated a sampling programme of several lakes in the Lake District, including Esthwaite Water. Regular monitoring of the phytoplankton and water chemistry has been carried out since then. Figure 5.1 uses this data to show the Ceratium population in Esthwaite Water from 1946-1986. The large increase in cell numbers in the late 1970's is attributed to increasing nutrient enrichment and the subsequent decline in the 1980's to parasitism (see Chapter 3.1, page 65). Initially all motile cells of Ceratium were recorded as C. hirundinella, but after 1983 C. furcoides and C. hirundinella were recorded separately. The present study sought to establish retrospectively the relative proportions of C. furcoides and C. hirundinella for the period prior to 1983 using preserved phytoplankton net samples. In addition to the discussion of long term changes in the proportion of the two species, the results obtained were used to compare the number of Ceratium

Figure 5.1 Maximum Population of Ceratium Cells in Esthwaite Water



cells in the phytoplankton with the cyst population over the period of time covered by the sediment cores.

Methods

Cores were taken from the two main basins of Esthwaite Water, referred to as north and south basin for convenience, during 1985 and 1986 from the locations indicated in Figure 3.1 (see Chapter 3, page 64). Over the period of two seasons, cores were removed soon after encystment (October 1986), prior to excystment (March 1986) and following excystment (May 1985). Specific locations were established by alignment with predetermined features of the landscape. In general, deep water sites were selected where thicker sediments would be expected as a consequence of sediment focusing (Davis, Moeller and Ford, 1984). The deepest part of the lake (A in Figure 3.1), was avoided, since it is routinely used as a sediment sampling site by other workers at the Freshwater Biological Association, and it is likely that any cores obtained would contain disturbed sediment. River inflows were similarly unsampled, since sedimentation here would not be expected to be typical of the lake basin. Sampling was also not undertaken close to fish farming cages (indicated by B in Figure 3.1), where localised differences in nutrient levels and disruption from the anchoring of the cages may have given atypical results.

Cores were withdrawn from the sediment using a Jenkin Surface-Mud Sampler (Ohnstad and Jones, 1982) and transferred to the laboratory, where they were stored at 4°C prior to extrusion

and sectioning at 0.5 cm intervals (detailed in Chapter 2, page 39). In general, half of the cores were preserved with 40% formaldehyde and the remainder, to be used for excystment experiments, were stored at 4°C.

Estimates of viable cyst numbers were made on sub-samples of 0.25-0.50 cm^3 which were withdrawn with a wide bored micropipette and introduced onto a Lund slide (Lund, 1959, 1962). The whole slide was examined under a Leitz Diaplan fluorescence microscope using blue excitation light, and numbers of viable C. furcoides and C. hirundinella cysts determined (see Chapter 2, page 50). If necessary, further counts were undertaken until the number of cysts counted reached a predetermined value of 100 cysts (detailed in Chapter 2, page 51). Counts were made of non-viable cysts from selected cores, using an Olympus BH2 light microscope (see Chapter 2, page 54). In addition, counts were made of diatom numbers, using the technique described in Chapter 2 (page 55), in order to assess the rate of sedimentation. The dry weight of each core section was determined for one core (Chapter 2, page 54).

Net samples, preserved in Lugol's Iodine or formaldehyde and stored at the Freshwater Biological Association, were used to make a retrospective study of the proportion of C. furcoides and C. hirundinella motile cells in the phytoplankton. Samples taken at the time of the summer maximum were examined for each of the years between 1946 and 1986, excepting 1971 for which no sample could be traced. A few drops of the sample were examined on a slide and the number of each species in random fields of

view was recorded, until at least 100 cells had been counted. The two species were distinguished on the basis of the morphological features discussed in Chapter 4 (page 88). It was necessary to ensure that the proportion of species during the summer maximum was representative of the year as a whole. Therefore three years were selected to be studied in detail. In one (1972) C. furcoides occurred in relatively greater numbers, in another (1977) C. hirundinella was proportionally more numerous and in the third (1982) the two species were found in approximately the same numbers. For each year one sample a month, from May to September, was studied as outlined above.

Results

(i) The Vertical Distribution of Ceratium Cysts within Sediment Cores

General Variation between the Cores

If the sediment had remained undisturbed from the time the cysts had been deposited, the base of the core would be expected to contain the oldest cysts and sediment nearest to the water column, the most recent cysts. Numbers of cysts would be expected to be greatest in the upper sediment, particularly in those cores collected in October soon after encystment. Conversely, numbers of viable cysts should decline towards the base of the core since losses (e.g. ingestion, disease, parasitism, loss of viability) would have occurred during the intervening years. Those years in which the production of cysts was high would be most likely to register in the sediment.

However, disruption of the sediment does occur particularly in the upper 2 cm of the core. Here the sediment is highly flocculent and cysts are liable to resuspension as a consequence of wave action. Subsequent redeposition of the cysts may be at a different level. In addition, the action of burrowing invertebrates may be responsible for the redistributiton of cysts to higher or lower horizons within the sediment.

Figure 5.2 illustrates the only assessment of water content within the cores that was undertaken. The proportion of water throughout the core (Core 2.4) is shown to be high (> 97%). This would be expected as the determination of dry weight was made on samples following dilution (see Chapter 2, page 54). However, this figure does demonstrate that water content decreases with depth. This suggests that a sub-sample of given volume from a section of the core near the surface, for example 0-0.5 cm, will contain less sediment (0.46%) than a sub-sample of the same volume from a deeper core section, for example 7.5-8.0 cm (2.57%). This indicates that the number of cysts near the surface of the core may be underestimated and those towards the base of the core overestimated. The use of this data in conjunction with observed numbers of cysts is discussed later (see page 178).

The vertical distribution of cysts within the upper 8 cm of the sediment cores from Esthwaite Water are illustrated in Figures 5.3-5.5. The considerable variation shown is discussed with respect to certain variables. The Friedman Two-Way Analysis of Variance by Ranks (Siegel, 1956) was used to

Figure 5.2 Proportion of Sediment in each Section of an 8cm Core

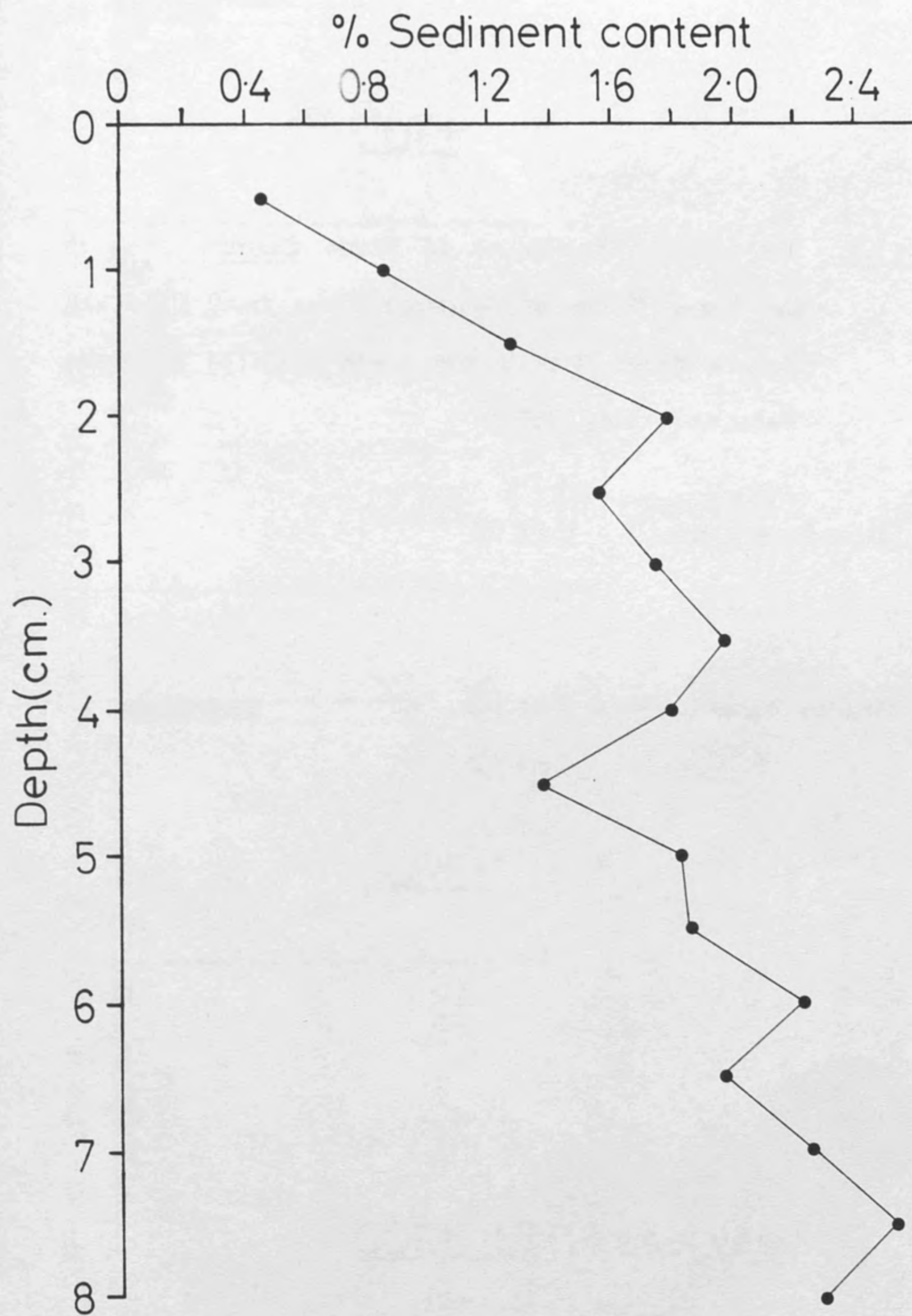


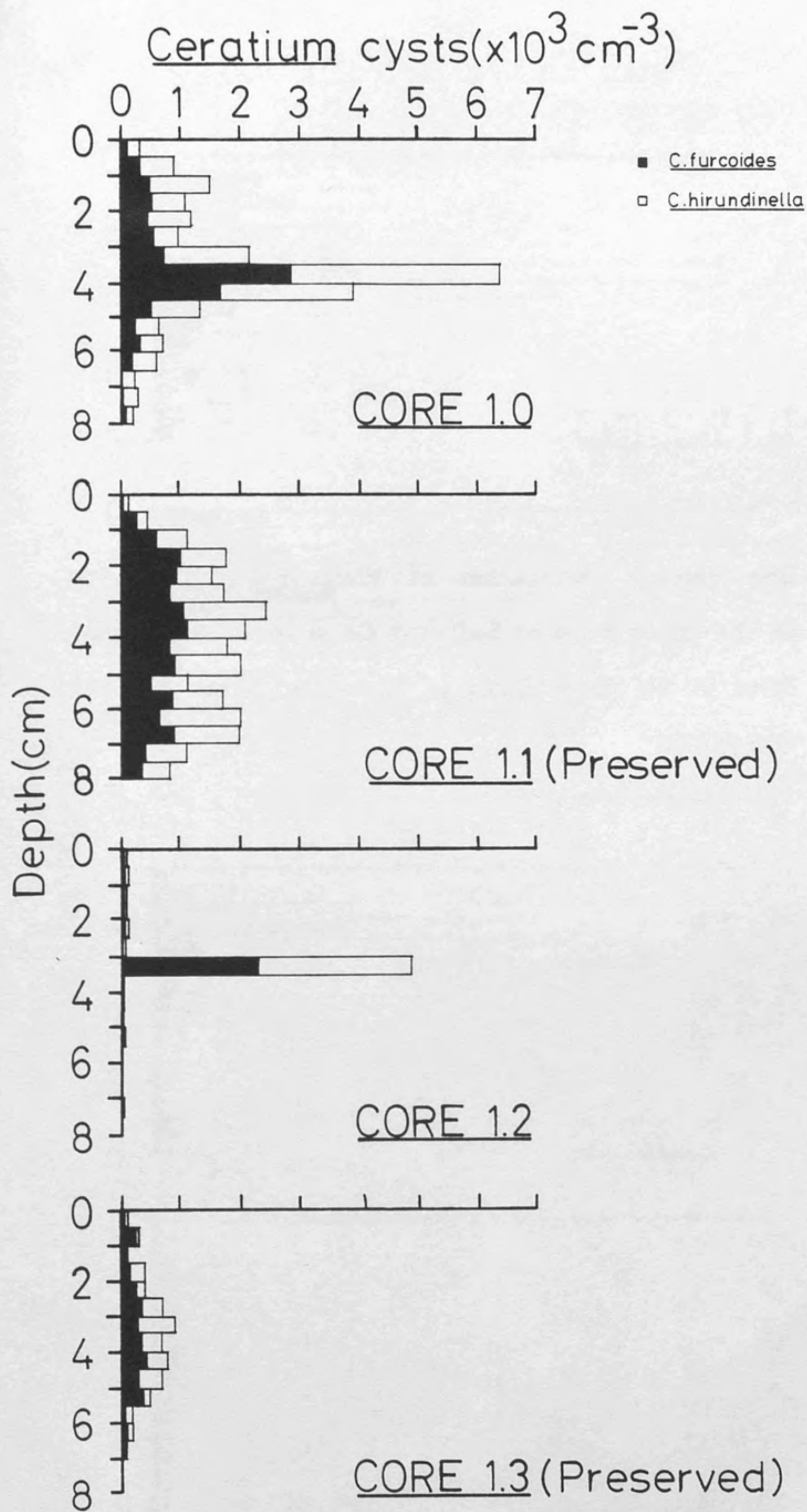
Figure 5.3 The Vertical Distribution of Viable *Certium* Cysts in the Upper 8 cm of Sediment Cores from Deep and Shallow Water Sites in the North Basin of Esthwaite Water on 7th May 1985

Deep Water Cores - Core 1.0

Core 1.1

Shallow Water Cores - Core 1.2

Core 1.3



Ceratium cysts(x10^3 cm^-3)
0 1 2 3 4 5 6 7
C.furcoides
C.hirundinella
Depth(cm)
0 2 4 6 8
CORE 1.0
CORE 1.1 (Preserved)
CORE 1.2
CORE 1.3 (Preserved)

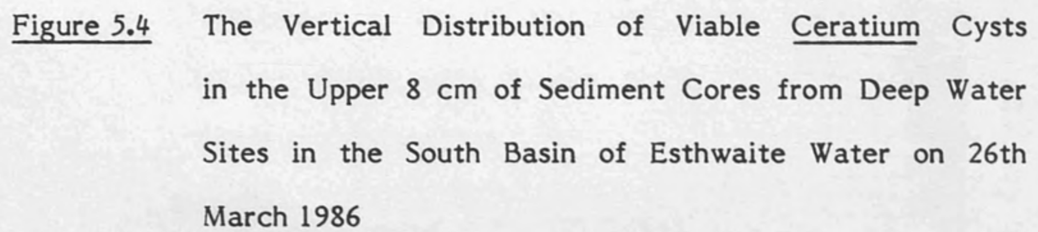
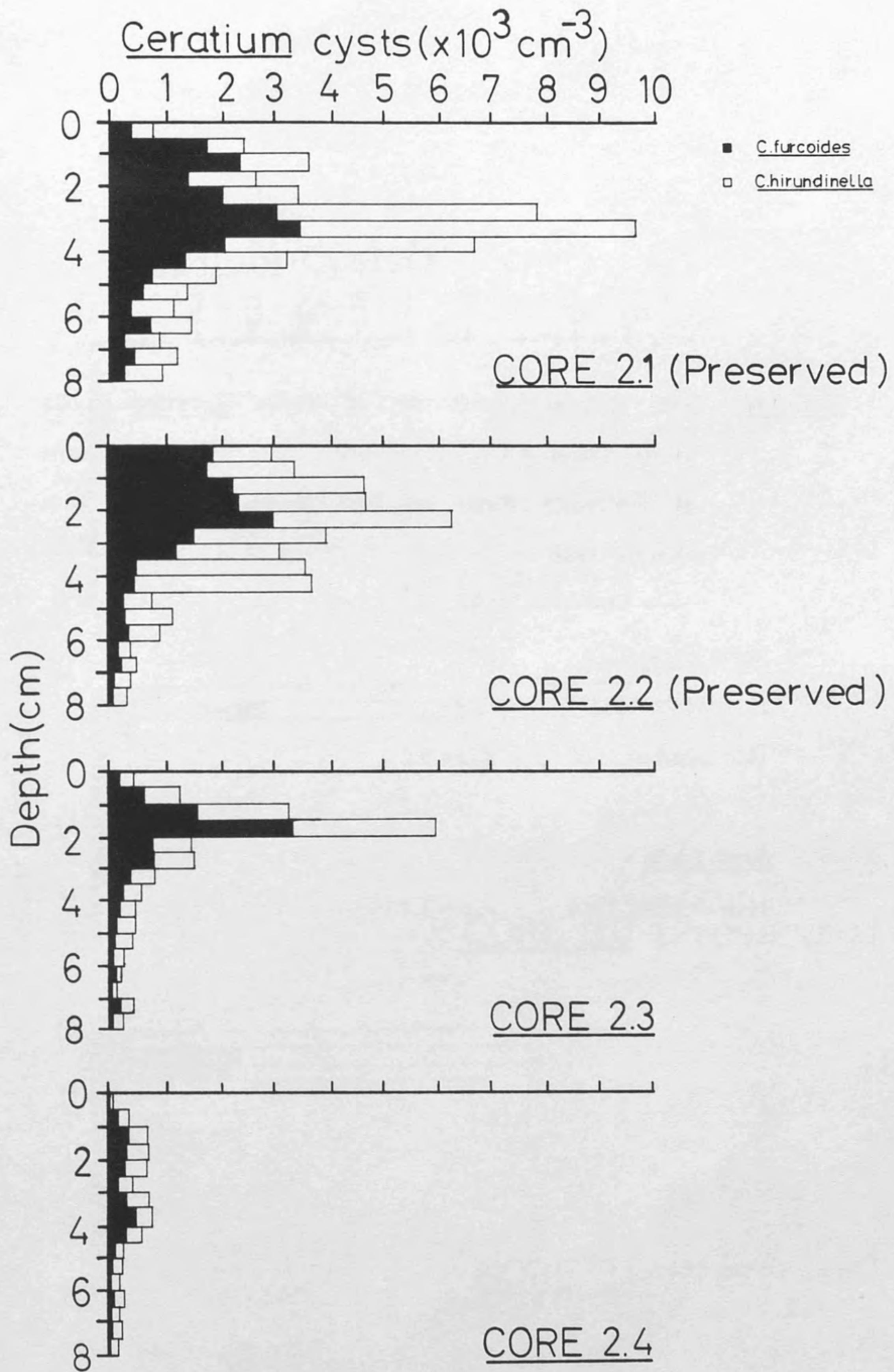


Figure 5.4 The Vertical Distribution of Viable *Ceratium* Cysts in the Upper 8 cm of Sediment Cores from Deep Water Sites in the South Basin of Esthwaite Water on 26th March 1986



Ceratium cysts(x10^{3}cm^{-3})
0 1 2 3 4 5 6 7 8 9 10
C.furcoides
C.hirundinella
CORE 2.1 (Preserved)
CORE 2.2 (Preserved)
CORE 2.3
CORE 2.4
Depth(cm)
0 2 4 6 8

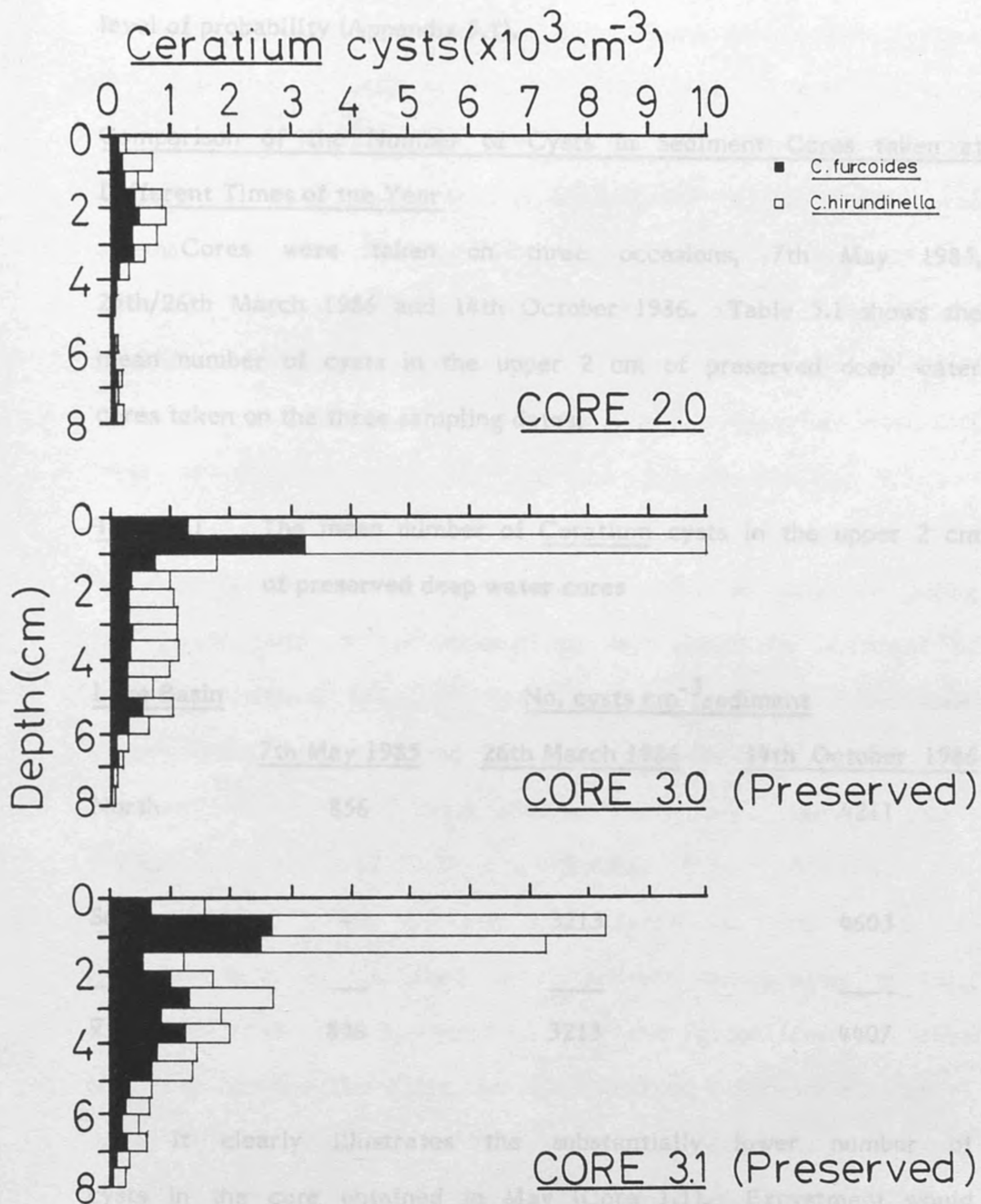
Figure 5.5 The Vertical Distribution of Viable *Ceratium* Cysts in the Upper 8 cm of Sediment Cores from both Basins of Esthwaite Water on 25th March 1986 and 14th October 1986

North Basin

25th March 1986	Core 2.0
14th October 1986	Core 3.0

South Basin

14th October 1986	Core 3.1



Ceratium cysts(x10^3 cm^-3)
0 1 2 3 4 5 6 7 8 9 10
C. furcoides
C.hirundinella
CORE 2.0
CORE 3.0 (Preserved)
CORE 3.1 (Preserved)
Depth(cm)
0 2 4 6 8

determine whether variation was statistically significant. Cores collected from similar locations on the same occasion (Cores 2.1 and 2.2; Cores 2.3 and 2.4) were compared and any differences between them shown to be insignificant at the 99% level of probability (Appendix 5.1).

Comparison of the Number of Cysts in Sediment Cores taken at Different Times of the Year

Cores were taken on three occasions, 7th May 1985, 25th/26th March 1986 and 14th October 1986. Table 5.1 shows the mean number of cysts in the upper 2 cm of preserved deep water cores taken on the three sampling dates.

Table 5.1 The mean number of *Ceratium* cysts in the upper 2 cm of preserved deep water cores

Lake Basin	No. cysts cm^{-3} sediment		
	7th May 1985	26th March 1986	14th October 1986
North	856	-	4211
South	-	3213	4603
$\bar{x}$	856	3213	4407

It clearly illustrates the substantially lower number of cysts in the core obtained in May (Core 1.1). Excystment would be expected to have occurred in this core since the temperature

at the sediment surface was already in excess of 4°C (8.4°C, F.B.A. data). Therefore a lower number of cysts would be anticipated in the upper few centimetres of the core. In comparison, cores taken in March (prior to excystment) and October (following encystment) should contain more cysts. Since the proportion of cells encysting in each year is unknown, it is not possible to say to what extent this shortfall in cyst numbers in samples taken in May 1985 is due to excystment. The calculation of the Friedman Two-Way Analysis of Variance (see Appendix 5.2) showed the existence of a statistically significant difference between samples from the same lake basin collected at different times of the year at least at the 95% level of significance (Cores 1.1 and 3.0; Cores 2.1, 2.2 and 3.1; Cores 1.0 and 2.0).

Some reduction in cyst numbers would be expected during the period between encystment and excystment as a result of death, parasitism, predation and redeposition in lower horizons. These losses are reflected to some extent in the difference between the number of cysts observed in samples taken in March (Cores 2.1, 2.2 and 2.3) and October (Cores 3.0 and 3.1), illustrated in Figures 5.4 and 5.5 respectively. The number of Ceratium cells at the time of encystment was greater in 1985 than in 1986 (F.B.A. data). If the proportion of cells encysting remains the same, the formation of a greater number of cysts could be expected in 1985 than in 1986. Thus if no cyst losses were experienced the cores removed in March 1986 would be expected to contain more cysts than those taken in October 1986.

The chytrid <u>Rhizophydium nobile</u> was prevalent in the autumn of 1985 and may have been responsible for a reduction in the <u>C. furcoides</u> cyst population (Heaney, Lund, Canter and Gray, 1988).

The extent of mixing within the sediment is evident by a comparison of the cores. None of those cores taken in May 1985 (Cores 1.0, 1.1, 1.2 and 1.3) show a peak in cyst numbers within the upper 3 cm, which characterises the remainder of the cores (Figures 5.3-5.5). The cores removed in October 1986 (Cores 3.0 and 3.1) show the most marked increase in numbers close to the surface as would be expected. The upper 1-2 cm of the sediment in Esthwaite Water is flocculent and liable to resuspension, and is generally regarded as representing the previous years sedimentation (Gorham, 1958). The vertical distribution of the cysts may thus illustrates the extent of this mixing. The cysts within the cores taken in October, soon after encystment, had little time to be reworked in the sediment. The distinct peak in cyst numbers close to the sediment surface reflects this. Cores obtained in March, just prior to excystment had been exposed to winter mixing. Distinct peaks in cyst numbers can still be observed in three of the cores (Cores 2.1, 2.2 and 2.3). However, these modal depths are several centimetres below the sediment water interface, for example, 3.0-3.5 cm in Core 2.1. Those sediment cores taken in May 1985 (Cores 1.0, 1.1, 1.2 and 1.3) show no distinct peak in cyst numbers in the upper 3 cm of the core. This may reflect mixing which has occurred since the previous autumn. However, since sampling

followed excystment, a maxima in cyst numbers close to the surface would be expected to have been reduced.

Comparison of the Number of Cysts in Sediment Cores taken from Different Basins of the same Lake

The total number of cysts found within each 8 cm core is detailed in Table 5.2. A comparison was made between the unpreserved cores from each basin taken in March 1986 (Cores 2.0, 2.3 and 2.4) and preserved cores taken from each basin in October 1986 (Cores 3.0 and 3.1). The average value for each basin indicated that only 38% of all cysts observed in these cores were found in the north basin. However, if the less reliable figures for unpreserved cores (where some cysts are liable to have excysted) are discounted, the proportion of cysts in the north basin is raised to 44%. This represents only a ± 6% deviation from parity, a difference within the range of acceptable experimental error. The relative sedimentation rates for the two basins are undocumented, and it is thus not possible to deduce whether the difference between them is representative of the relative size of the phytoplankton population prior to encystment, the rate of sediment accumulation, or to topographical features. The spatial distribution of encysting cells will also affect the location of the cysts. Although Dottne-Lindgren and Ekbohm (1975) observed that the horizontal distribution of Ceratium cells in Lake Erken, Sweden was random, other authors (Heaney, 1976; George and Heaney, 1978; Heaney and Talling, 1980a, b) have demonstrated a non-uniform distribution

Table 5.2 The total number of cysts in cores from the north and south basins of the lake

		No. of Cysts cm^{-3} sediment ($x10^4$)	
		North Basin	South Basin
May 1985			
Deep Water Sites			
Unpreserved	- Core 1.0	2.2	
Preserved	- Core 1.1	2.3	
Shallow Water Sites			
Unpreserved	- Core 1.2	0.5	
Preserved	- Core 1.3	0.6	
March 1986			
Deep Water Sites			
Unpreserved	- Core 2.0	0.6	
	- Core 2.3		1.8
	- Core 2.4		0.7
Preserved	- Core 2.1		5.0
	- Core 2.2		3.7
October 1986			
Deep Water Sites			
Preserved	- Core 3.0	2.6	
	- Core 3.1		3.3

in Esthwaite Water. Thus the deposition of cysts would also be expected to be non-random. In order to establish any significant differences between the two basins it would be necessary to undertake a more extensive survey than was possible within the current study. With the limited data available, the calculation of the Friedman Two-Way Analysis of Variance (Appendix 5.3) showed the existence of a statistically significant difference in the vertical distribution of cysts between otherwise similar cores from different basins of the lake (Cores 3.0 and 3.1; Cores 2.0, 2.3 and 2.4).

Comparison of the Number of Cysts in Sediment Cores taken from Deep and Shallow Sites

Only two cores were taken from shallow water sites (Cores 1.2 and 1.3), both from the north basin of the lake. Figure 5.3 illustrates the number of cysts found through these cores (one of which, Core 1.3, was only 7 cm in length) and allows comparison with cores taken at the same time from deep water sites in the north basin (Cores 1.0 and 1.1). In general fewer viable cysts were observed through the cores from shallow water sites. The mean total for the two shallow cores was 5,797 cysts cm^{-3} sediment, compared with 22,636 cysts cm^{-3} sediment for the two deeper cores.

In Core 1.2 there was a substantial increase in cyst numbers at a depth of 3.0-3.5 cm (4,851 cysts cm^{-3} sediment), which contrasted markedly with the remainder of the core, containing very few cysts (mean number of cysts in a core

section = 41 cysts cm^{-3} sediment). It is possible that this peak may represent a sudden increase in cyst production, or a failure to excyst. However, such a sharp rise in numbers, coupled with very low numbers in the remainder of the core, suggests an accumulation of cysts at this depth due to some factor, possibly topographical. This is discussed in detail in a later section (page 172).

The application of the Friedman Two-Way Analysis of Variance (Appendix 5.4) showed that a statistically significant difference existed between the vertical distribution of cysts of Cores 1.0 and 1.2 (unpreserved cores), but not between Cores 1.1 and 1.3 (preserved cores). This variation in numbers between shallow and deep cores could be due to the differences in the volume of water above the sediment, in the rate of sedimentation, or to variation in the degree of sediment mixing. The larger column of water above the sediment in deep water sites will evidently have a greater capacity to provide material to the sediment than the shorter water column in shallow sites. In addition, sediment accumulation in shallow waters is less than for deep water sites, due to sediment resuspension focusing to deeper regions (see page 173). Therefore, in each year less sediment will be deposited below shallow water. Thus an 8 cm sediment core taken from such a shallow site will represent a greater time span than a core of similar length taken from deeper water. It follows that cysts at the base of an 8 cm core from a shallow water site will be older, and thus more likely to be non-viable, than cysts from a corresponding depth in a deep

water core.

Comparison of the Number of Cysts in Preserved and Unpreserved Sediment Cores

Figures 5.3-5.5 clearly show the reduction in the number of viable cysts in the majority of unpreserved cores. The difference in cyst numbers between preserved and unpreserved cores taken at the same location on the same day showed great variation. The number of cysts in Core 1.0 represented 94% of the total for Core 1.1 (May 1985), whilst Core 2.4 contained only 15% of the number of cysts in Core 2.1 representing the biggest difference between the cores collected in March 1986. The calculation of the Friedman Two-Way Analysis of Variance showed a statistically significant difference in cyst distribution between Cores 2.1-2.4 and Cores 1.2 and 1.3, but lnot between Cores 1.0 and 1.1 (see Appendix 5.5).

Unpreserved samples, packed in ice, were transferred from the Lake District to Royal Holloway and Bedford New College, where all fluorescence counts were undertaken. It is possible that during this journey exposure to an increase in temperature (in excess of 4°C) may have initiated excystment. In order to investigate the extent of this possible excystment a comparison was made of the number of viable, and non-viable and empty cysts from both preserved and unpreserved cores. Empty Ceratium cysts are not always easy to observe since they have a tendency to collapse and lose their characteristic shape. In addition the frequent loss of horns makes it difficult to separate the two

species with any reliability.

Table 5.3 shows that a higher proportion of non-viable and empty cysts was noted in the upper 4 cm of the two unpreserved cores studied (mean proportion of non-viable and empty cysts in a 2 cm core section = 93%) than for the cores preserved with formaldehyde (mean proportion of non-viable and empty cysts in a 2 cm core section = 60%). Below 4 cm the proportion of

Table 5.3 The number and proportion of viable, and non-viable and empty cysts in cores from the south basin of the lake

	No. Cysts cm^{-3} sediment ($x10^3$)								
Depth (cm)	Preserved Cores			Unpreserved Cores					
	Core 2.1			Core 2.0			Core 2.4		
	a	b	%	a	b	%	a	b	%
0-2	5.2	3.7	70	1.0	11.3	95	0.8	5.2	93
2-4	8.4	27.5		0.7	19.8		1.0	18.7	
4-6	2.6	200.0	99	0.1	24.8	99	0.4	27.9	98
6-8	1.8	13.4		0.3	9.9		0.4	18.3	

where a = the number of viable cysts

b = the number of non-viable and empty cysts

% = the proportion of non-viable and empty cysts to the total number of cysts in a 4 cm section

non-viable and empty cysts was similar for preserved and unpreserved cores (99%). This depth corresponds to the level to which cysts are able to excyst (see Chapter 6, page 245). This suggests that in unpreserved cores the greater number of non-viable and empty cysts may correspond to those cysts which have excysted in transit.

Comparison of the Vertical Distribution of Ceratium Cysts through the Sediment Core

There is considerable variation between the cores in the number of cysts observed at each depth (Figures 5.3-5.5). However, in all cores there is a marked decline in cyst numbers at the sediment surface. In the case of cores taken in May 1985, this reduction may represent those cysts which have already excysted (page 138). This explanation cannot, however, be applied to cores taken in March 1986, prior to excystment, or October 1986, following encystment. The occurrence of resuspension and deposition of cysts in the flocculent upper 2 cm of the sediment will cause mixing of the horizons (page 174), and is a possible cause for the reduction of numbers at the surface. This may be the major factor responsible for the distribution of cysts in the upper few centimetres of the core taken in March 1986 (Cores 2.1, 2.2, 2.3 and 2.4). The depth of sediment above the maxima value is too great to have been deposited within six months. It therefore seems more likely that cysts may have been resuspended and deposited at a lower level. However, the cysts within the cores collected in

October 1986 (Cores 3.0 and 3.1) are unlikely to have been disturbed by mixing, as they have only recently been deposited.

It is also possible that the fall in the number of cysts at the sediment surface may represent only an apparent change. The higher water content of the sediment at the mud-water interface will result in the upper 0.5 cm section of the core being more dilute than those below. The sediment content of the 1.5-2.0 cm core section was shown to be four times greater, and the 7.0-7.5 cm core section five to six times greater, than the 0-0.5 cm core section of Core 2.4 (see Figure 5.2, page 131). Thus a corresponding reduction in the number of cysts within the upper section of the core would be expected, if the proportion of sediment was the only factor responsible (i.e. the 0-0.5 cm core section would be expected to contain 25% of the cysts present in the 1.5-2.0 cm core section). In Cores 3.0 and 3.1 the observed number of cysts in the upper core section exceeded that in the 1.5-2.0 cm core section. This indicated that if sediment content was also taken into consideration, the difference in the number of cysts between the two core sections would be even greater (difference between observed values = 61% of total; difference between values corrected for sediment content = 88% of total). The observed number of cysts in the 0-0.5 cm section of Cores 2.1 and 2.2 was less than in the 1.5-2.0 cm section. However, when values for the upper section of both cores were corrected for sediment content, the number of cysts present was shown to be greater than for the 1.5-2.0 cm core section. This indicates that the lower sediment content in

the 0-0.5 cm core section was responsible for an apparent decrease in cyst numbers. In Core 1.1 the number of cysts in the upper 0.5 cm remained lower than the remaining core sections even when figures were adjusted to allow for changes in sediment content. This implies that an additional factor(s) was responsible for the decrease in cyst numbers at the sediment surface. The number of cysts present in these cores, adjusted to incorporate the increased sediment content with depth, are illustrated in Figures 5.13 and 5.14 and the use of this data discussed further in the accompanying text (see page 183). Thus it would seem that the position of the maxima just below the surface sediment and the sharp reduction in cyst numbers at the surface is, in some instances, due to an apparent change to the high water content of the uppermost section of the core.

All cores also show a decrease in the numbers of viable cysts in the lower portion of the core, particularly below 5 cm. Below the upper 2-3 cm of the core it would generally be expected that the cyst population would experience losses rather than gains, although some migration would be probable through the activities of burrowing invertebrates. Parasitism, ingestion, degeneration of cysts contents and redeposition to lower horizons due to burrowing animals would all reduce the population. With little new input, the number of viable cysts would be expected to decline with increasing depth as the proportion of non-viable cysts increased. Large residual populations of cysts would be expected to take a longer period of time to be reduced to low numbers and would therefore remain

prominent for some time.

In Chapter 6 (page 244) there is an assessment of the ability of cysts from sub-surface sediment to excyst. Viable cysts isolated from sediment below 5.5 cm rarely germinated in the laboratory. Thus, although apparently viable cysts were observed below this depth (i.e. fluoresced red under blue light), their ability to germinate is questionable. Excystment from such depths under natural conditions is in doubt, due to the increased distance from the water column. It has not been determined whether cysts are capable of germination "in situ" at depths in excess of 2 cm below the sediment surface. If this was possible newly excysted cells would have to rise through the sediment to the base of the water column. Alternatively germination of cysts from such depths would necessitate the resuspension of cysts in to the water column.

In addition to a large peak in cyst numbers near to the surface, representing cysts produced the previous autumn, it was hoped that a second, reduced peak would be found corresponding to those cysts, deposited the year before, which had failed to excyst in the following spring. There was no clear evidence of such a distribution pattern. In Core 1.1 (Figure 5.3) there appears to be two peaks in cyst numbers. However, with just one lower value separating the two groups it may only represent local variation. Cores 1.0 and 1.2 (Figure 5.3) have large peaks of cyst numbers below 3 cm in the sediment column. It is possible that these peaks may also represent cysts deposited in previous years. However, the virtual absence of cysts in the

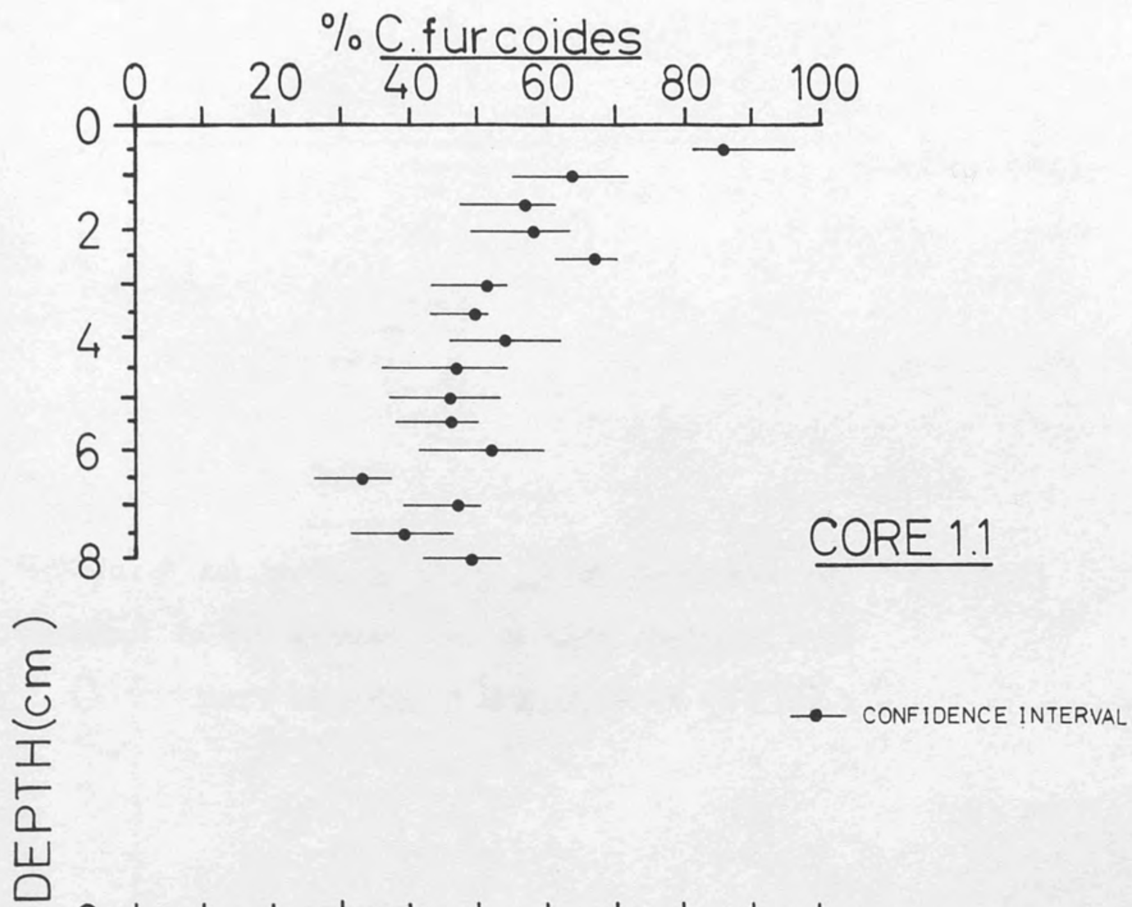
remainder of Core 1.2 (unpreserved) suggests that either cysts in the remaining core sections have excysted, or that they have accumulated at between 3.0 and 3.5 cm.

The cores collected in October 1986 (Cores 3.0 and 3.1), illustrated in Figure 5.5, would be expected to show the most distinct bimodal distribution of cyst numbers, if such a phenomenon exists, since the time between deposition of cysts and observation is at its shortest. However, no second peak is evident, since as in the remaining cores, cyst numbers reach a peak and then steadily decline. Therefore the validity of those cores displaying two peaks is questionable. It would appear that mixing activity within the sediment coupled with cyst losses distorts the vertical distribution of cysts, making it difficult to distinguish maximum values.

Comparison of the Proportion and Numbers of C. furcoides and C. hirundinella Cysts within the Sediment Cores

The proportion of C. furcoides cysts for five of the cores sampled is shown in Figures 5.6 and 5.7 as a percentage of the total number of identified cysts. The 95% confidence limits are also shown, and give an indication of the accuracy of the count. These values were calculated using the formula proposed by Moore and Webb (1978), detailed in Appendix 5.6. Those cores not illustrated (Cores 1.1, 1.2, 1.3, 2.0, 2.3, 2.4), many based on low cell counts were shown to be too variable. The relative proportion of the cysts of each species was not constant through the length of the core. Peaks in the actual numbers of

Figure 5.6 The Proportion of C. furcoides Cysts (as % of the total Ceratium spp.) in the Upper 8 cm of Sediment Cores from the North Basin of Esthwaite Water


% C.furcoides
0
20
40
60
80
100
0
2
4
6
8
DEPTH(cm)
CORE 1.1
CONFIDENCE INTERVAL

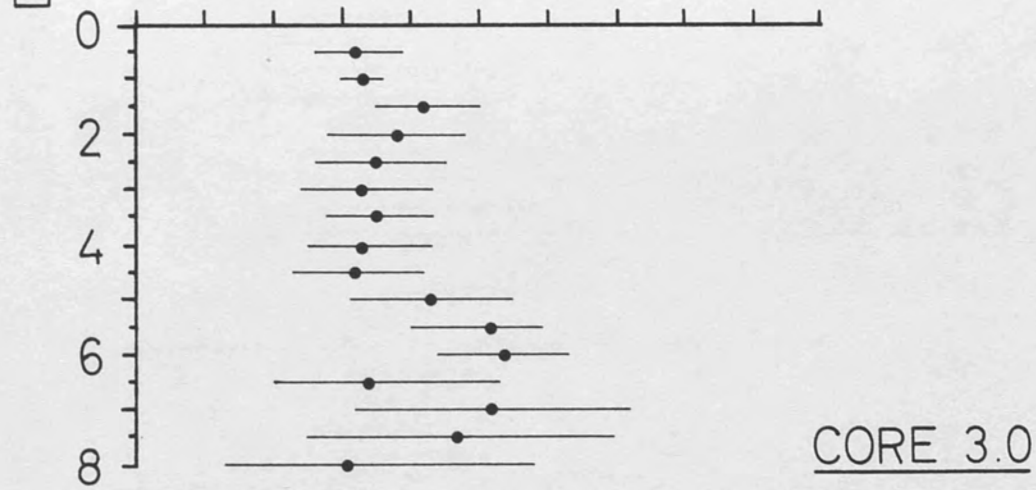
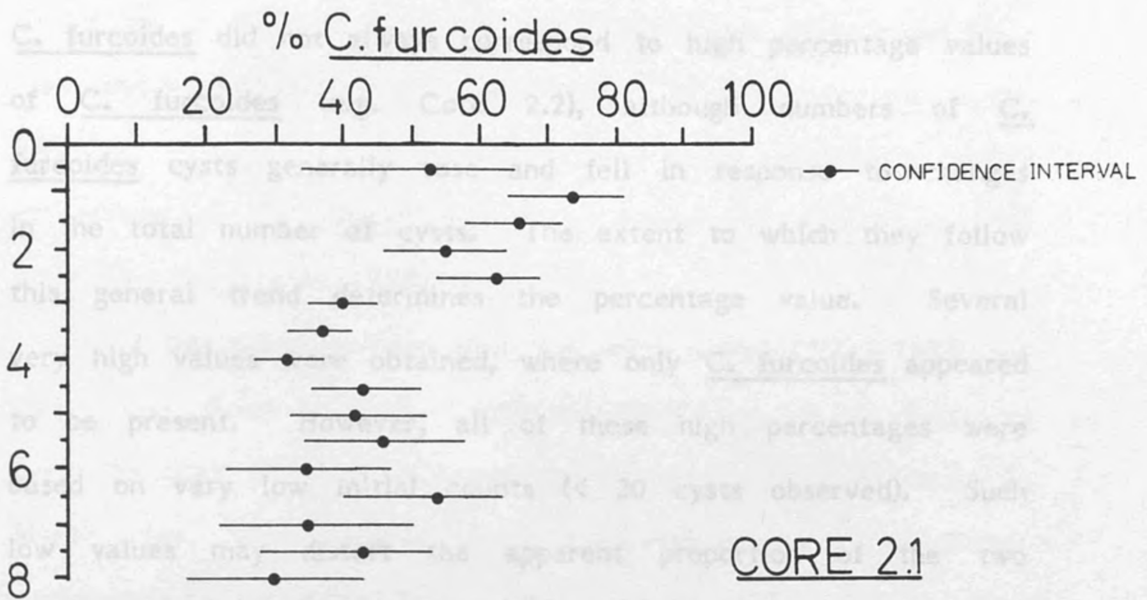
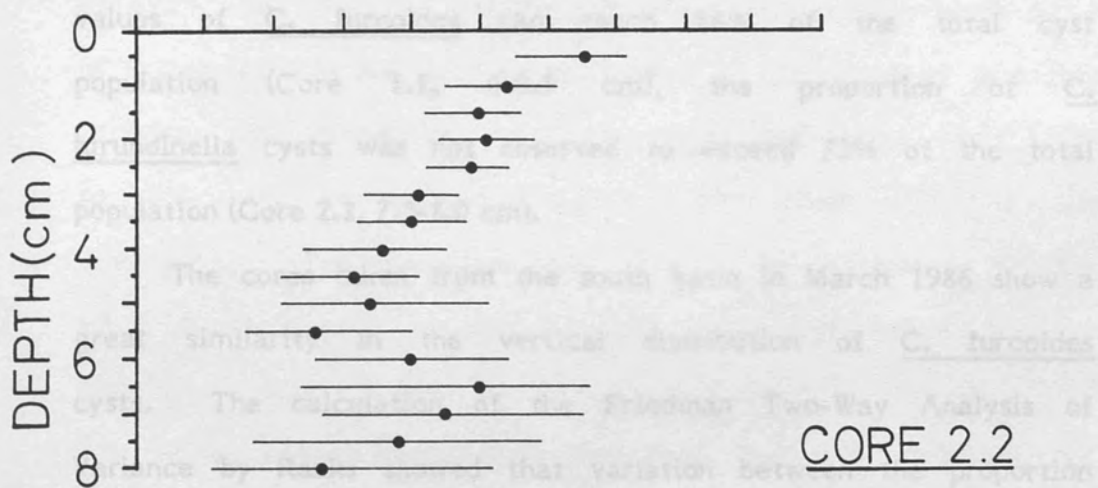

0
2
4
6
8
CORE 3.0

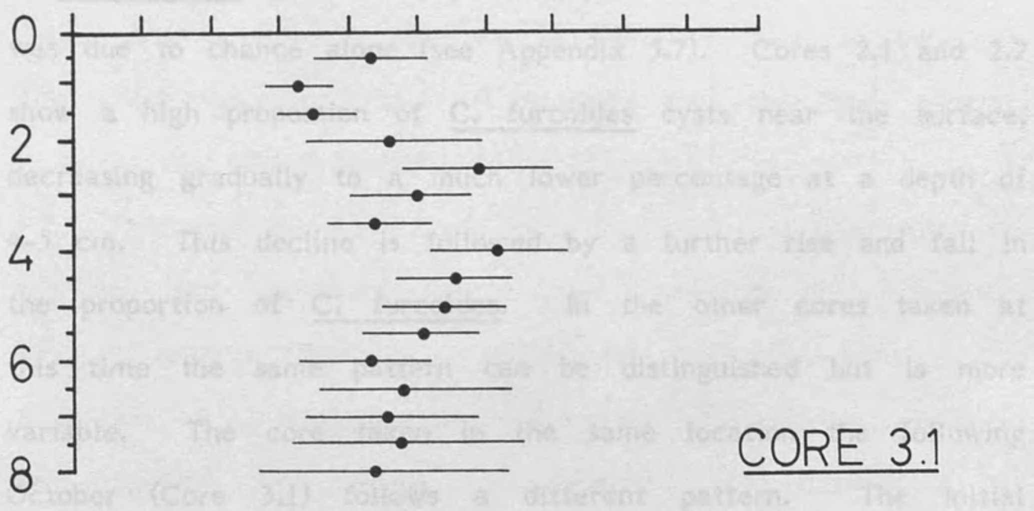
Figure 5.7 The Proportion of *C. furcoides* Cysts (as % of the total *Ceratium* spp.) in the Upper 8 cm of Sediment Cores from the South Basin of Esthwaite Water



% C.furcoides
0 20 40 60 80 100
0 2 4 6 8
CONFIDENCE INTERVAL
CORE 2.1



DEPTH(cm)
0 2 4 6 8
CORE 2.2



0 2 4 6 8
CORE 3.1

C. furcoides did not always correspond to high percentage values of C. furcoides (e.g. Core 2.2), although numbers of C. furcoides cysts generally rose and fell in response to changes in the total number of cysts. The extent to which they follow this general trend determines the percentage value. Several very high values were obtained, where only C. furcoides appeared to be present. However, all of these high percentages were based on very low initial counts (< 20 cysts observed). Such low values may distort the apparent proportion of the two species, placing undue importance on a few cysts. Although values of C. furcoides can reach 86% of the total cyst population (Core 1.1, 0-0.5 cm), the proportion of C. hirundinella cysts was not observed to exceed 73% of the total population (Core 2.2, 7.5-8.0 cm).

The cores taken from the south basin in March 1986 show a great similarity in the vertical distribution of C. furcoides cysts. The calculation of the Friedman Two-Way Analysis of Variance by Ranks showed that variation between the proportion of C. furcoides cysts in Cores 2.1 and 2.2 and Cores 2.3 and 2.4 was due to chance alone (see Appendix 5.7). Cores 2.1 and 2.2 show a high proportion of C. furcoides cysts near the surface, decreasing gradually to a much lower percentage at a depth of 4-5 cm. This decline is followed by a further rise and fall in the proportion of C. furcoides. In the other cores taken at this time the same pattern can be distinguished but is more variable. The core taken in the same location the following October (Core 3.1) follows a different pattern. The initial

proportion of C. furcoides is lower (< 45%) and is followed by a rise and then fall in the proportion of C. furcoides cysts. This suggests that the newly deposited cysts contain a higher proportion of C. hirundinella than those remaining from the previous spring (see page 169).

Of the cores removed from the north basin in May 1985, one (Core 1.1) shows a similar vertical distribution of C. furcoides cysts to that observed in cores taken from the south basin in March 1986. The remainder show a greater degree of variability, particularly those cores from shallow water sites where numbers of cysts were low. The single core removed from the north basin in March 1986 (Core 2.0) to be studied in this context is highly variable, with proportions of C. furcoides fluctuating from 100% to 0% and, as confidence limits were high, it was not illustrated. The variation can largely be explained by the small numbers used to calculate the percentage values, and as such is not totally reliable.

The core removed from the north basin in October 1986 (Core 3.0) shows a dominance of C. furcoides towards the base of the core (e.g. 5.5-6.0 cm). As in the core taken at the same time from the south basin it suggests that the cysts recently deposited have a lower proportion of C. furcoides.

The similarity of Cores 2.1, 2.2, 2.3 and 2.4 (the last two were not illustrated due to high confidence limits) suggests that in general there is no significant difference between the proportion of C. hirundinella in preserved and unpreserved cores (see Appendix 5.7). Thus, if excystment had occurred in the

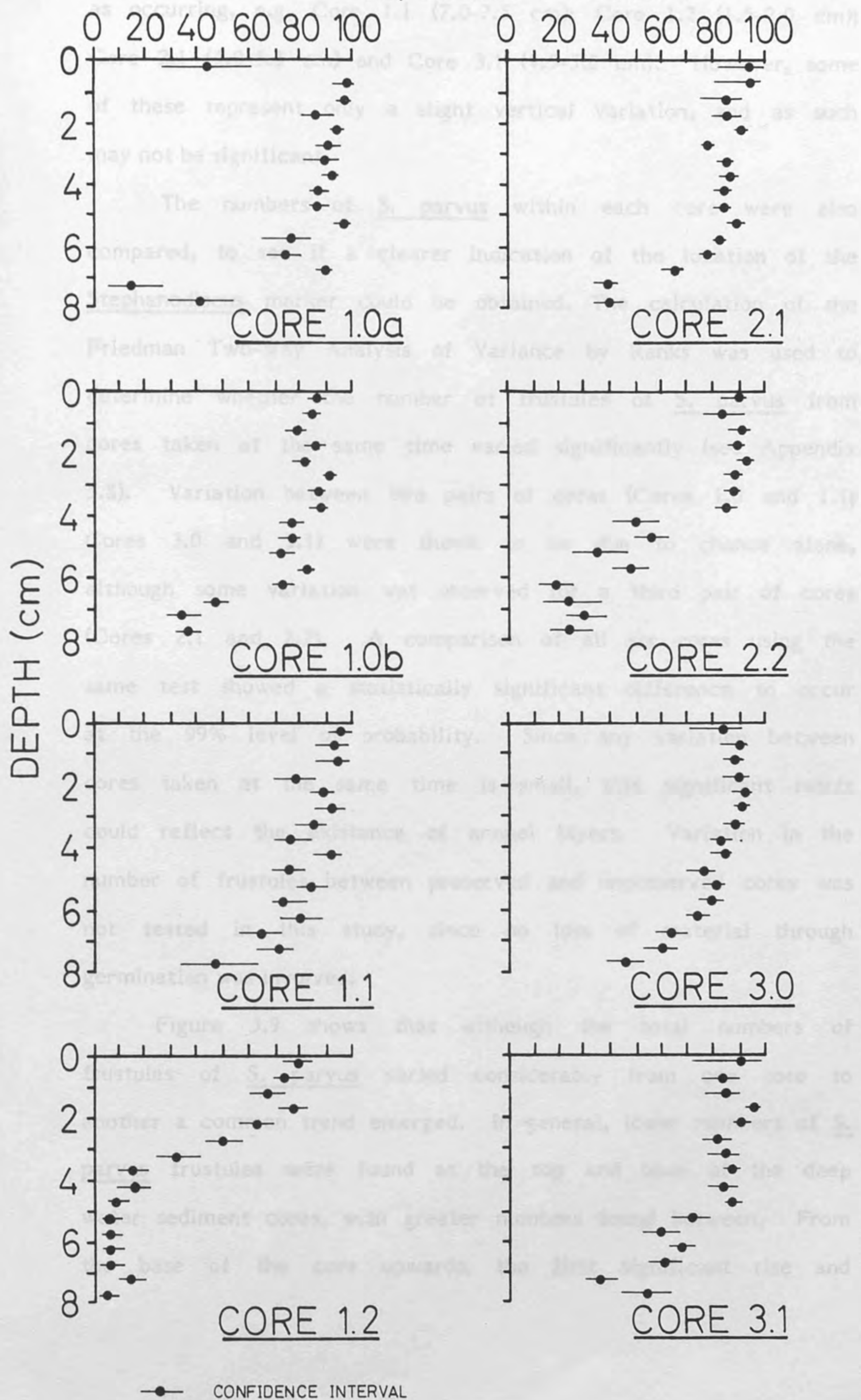
unpreserved cores it can be assumed that it took place at a comparable rate in both species. This is in agreement with laboratory experiments discussed in Chapter 6 (page 245) of the present study, in which the germination rate of the two species was compared and found to be the same.

A comparison of Cores 3.0 and 3.1 suggested that cores taken at the same time from different basins of the lake are dissimilar (see page 141). Core 3.0 has a peak of C. furcoides cysts at a depth of about 6 cm, whilst Core 3.1 peaks between 2-4 cm. This suggests some local variation in the distribution of cysts of different species, as is the case for overall cyst numbers.

(ii) An Analysis of Diatom Stratigraphy within the Sediment Cores

Figure 5.8 illustrates the observed proportion of Stephanodiscus parvus frustules, as a percentage of the total number of S. parvus and Cyclotella spp. The accuracy of the counts was tested by the calculation of the 95% confidence limits, which are also illustrated in Figure 5.8. Variation was shown to be small, generally less than ± 10% and the counts can thus be regarded as statistically reliable. The general trend was for a decrease in the proportion of S. parvus towards the base of the sediment core. It was difficult to identify a prominent maxima in the proportion of S. parvus, which would correspond with the large population known to have occurred in 1978 (see Chapter 2, page 56). Such peaks could be interpreted

Figure 5.8 The Proportion of Stephanodiscus parvus Frustules in the Upper 8 cm of Sediment Cores from Esthwaite Water



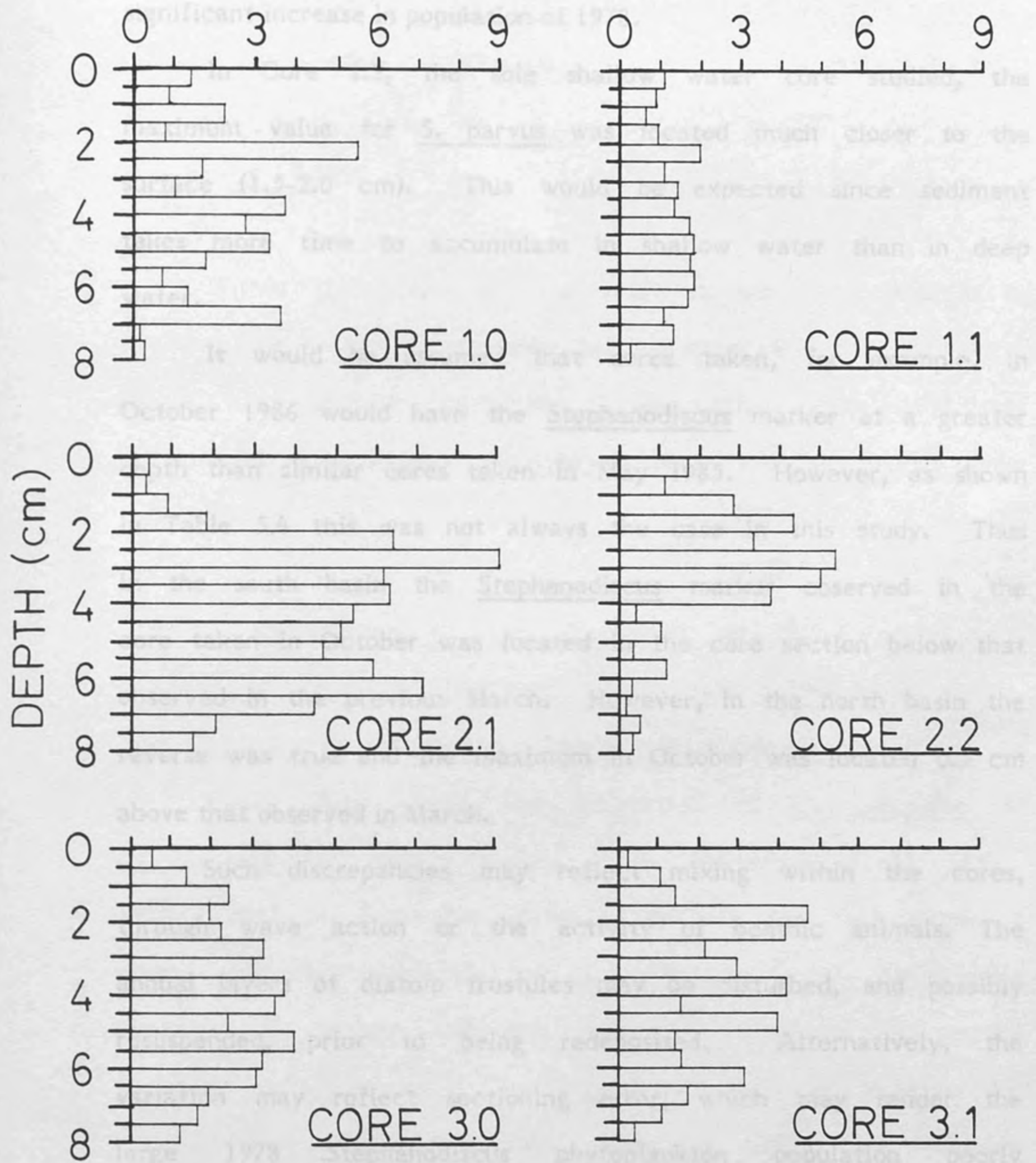
% S.parvus frustules
0 20 40 60 80 100
DEPTH (cm)
0
2
4
6
8
CORE 1.0a
CORE 2.1
CORE 1.0b
CORE 2.2
CORE 1.1
CORE 3.0
CORE 1.2
CORE 3.1
CONFIDENCE INTERVAL

as occurring, e.g. Core 1.1 (7.0-7.5 cm); Core 1.2 (1.5-2.0 cm); Core 2.1 (5.0-5.5 cm) and Core 3.1 (4.5-5.0 cm). However, some of these represent only a slight vertical variation, and as such may not be significant.

The numbers of S. parvus within each core were also compared, to see if a clearer indication of the location of the Stephanodiscus marker could be obtained. The calculation of the Friedman Two-Way Analysis of Variance by Ranks was used to determine whether the number of frustules of S. parvus from cores taken at the same time varied significantly (see Appendix 5.8). Variation between two pairs of cores (Cores 1.0 and 1.1; Cores 3.0 and 3.1) were shown to be due to chance alone, although some variation was observed for a third pair of cores (Cores 2.1 and 2.2). A comparison of all six cores using the same test showed a statistically significant difference to occur at the 99% level of probability. Since any variation between cores taken at the same time is small, this significant result could reflect the existence of annual layers. Variation in the number of frustules between preserved and unpreserved cores was not tested in this study, since no loss of material through germination was involved.

Figure 5.9 shows that although the total numbers of frustules of S. parvus varied considerably from one core to another a common trend emerged. In general, lower numbers of S. parvus frustules were found at the top and base of the deep water sediment cores, with greater numbers found between. From the base of the core upwards, the first significant rise and

Figure 5.9 The Number of Stephanodiscus parvus Frustules in the Upper 8 cm of Sediment Cores from Esthwaite Water


S. parvus frustules (x10^{7}cm^{-3} sed.)
0 3 6 9
0 3 6 9
DEPTH (cm)
0
2
4
6
8
CORE 1.0
CORE 1.1
CORE 2.1
CORE 2.2
CORE 3.0
CORE 3.1

fall in S. parvus was located within 1 cm of the 6.0-6.5 cm section. This does not necessarily represent the greatest value of S. parvus but is most likely to correspond to the first significant increase in population of 1978.

In Core 1.2, the sole shallow water core studied, the maximum value for S. parvus was located much closer to the surface (1.5-2.0 cm). This would be expected since sediment takes more time to accumulate in shallow water than in deep water.

It would be assumed that cores taken, for example, in October 1986 would have the Stephanodiscus marker at a greater depth than similar cores taken in May 1985. However, as shown in Table 5.4 this was not always the case in this study. Thus in the south basin the Stephanodiscus marker observed in the core taken in October was located in the core section below that observed in the previous March. However, in the north basin the reverse was true and the maximum in October was located 0.5 cm above that observed in March.

Such discrepancies may reflect mixing within the cores, through wave action or the activity of benthic animals. The annual layers of diatom frustules may be disturbed, and possibly resuspended, prior to being redeposited. Alternatively, the variation may reflect sectioning error, which may render the large 1978 Stephanodiscus phytoplankton population poorly resolved in the sediment cores. Differences between sites would also be expected to account for some variation.

Table 5.4 The section of the core representing the *Stephanodiscus parvus* population of 1978

Basin	May 1985	Date March 1986	October 1986
North	5.5 - 6.0	-	5.0 - 5.5
South	-	5.5 - 6.0	6.0 - 6.5

Within the scope of this study it was only possible to examine the diatom stratigraphy of the upper 8 cm of sediment. It is also possible that the sedimentation since 1978 has exceeded 8 cm and that the *Stephanodiscus* line may occur below this level.

(iii) A Comparison of the Proportion of C. furcoides and C. hirundinella in Preserved Net Samples from 1946-1986

Variation in the Proportion of the Two Species within a Year

Figure 5.10 shows the proportion of *C. furcoides* to *C. hirundinella* cells through three individual years (1972, 1977, 1982) in Esthwaite Water. It indicates that within a given year the proportion of *C. furcoides* to *C. hirundinella* appeared to remain constant, despite fluctuations in overall *Ceratium* cell numbers. The largest variation was observed for 1982, when both species were found in similar but variable numbers. However, the calculation of the Spearman's Rank Correlation Coefficient showed that the variation was statistically insignificant at the 5% level in 1977 and 1982. A

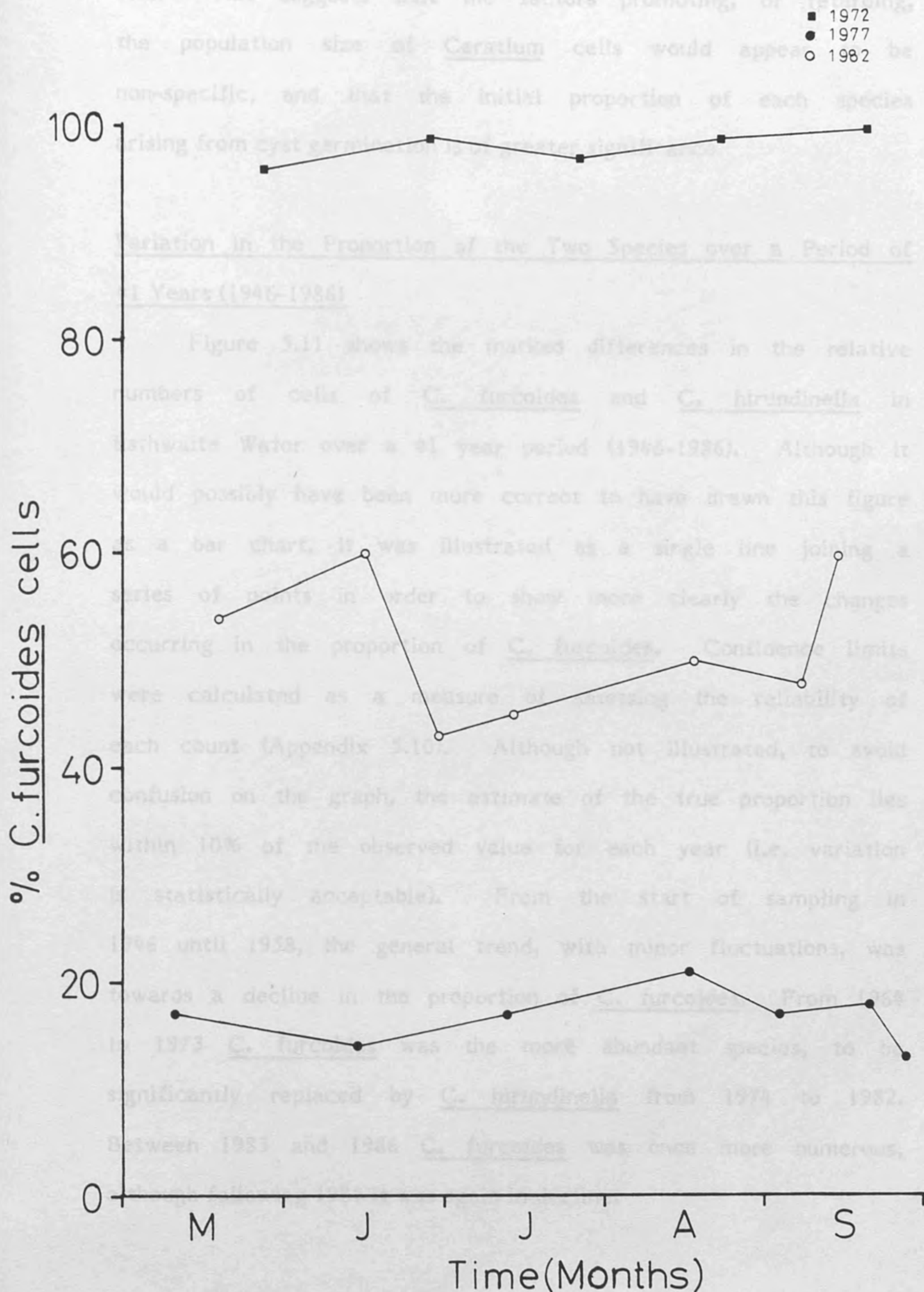


Figure 5.10 Proportion of *C.furcoides* cells (as % of total *Ceratium* spp.) Through 3 Years in Esthwaite Water

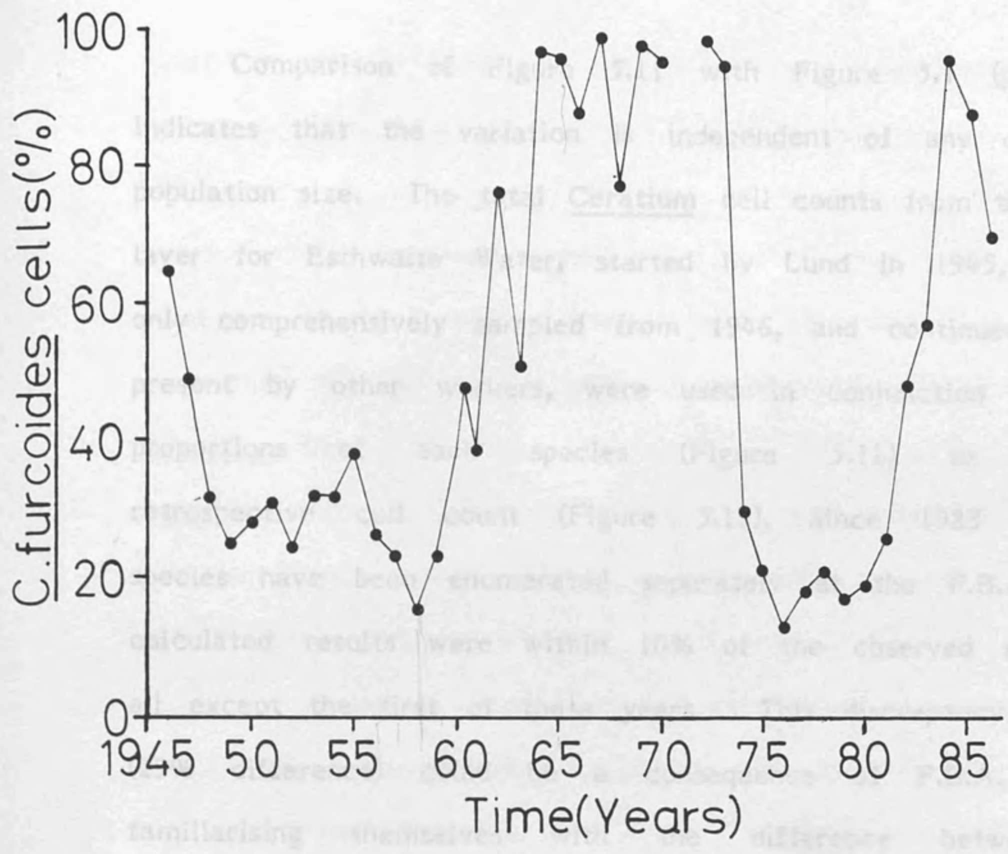
statistically significant result was observed for 1972, but all the figures were still within 2% of the mean value (Appendix 5.9). This suggests that the factors promoting, or retarding, the population size of Ceratium cells would appear to be non-specific, and that the initial proportion of each species arising from cyst germination is of greater significance.

Variation in the Proportion of the Two Species over a Period of 41 Years (1946-1986)

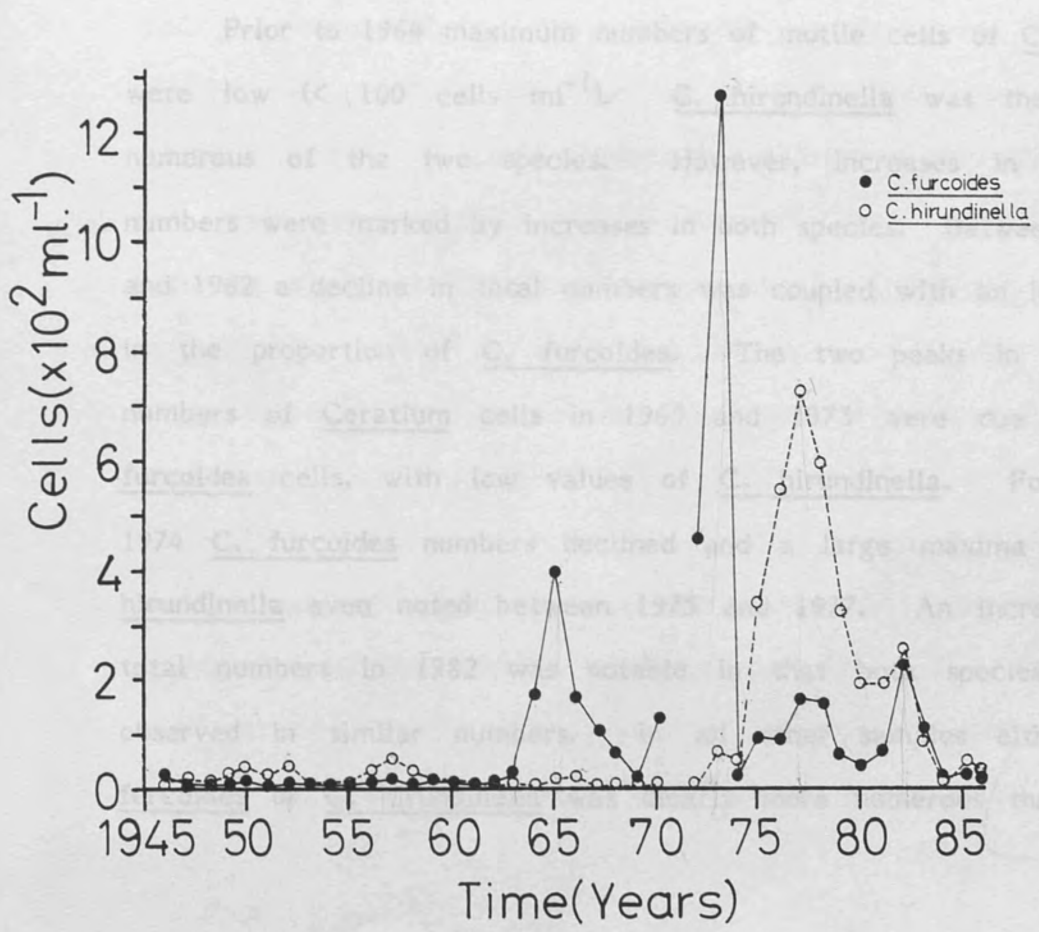
Figure 5.11 shows the marked differences in the relative numbers of cells of C. furcoides and C. hirundinella in Esthwaite Water over a 41 year period (1946-1986). Although it would possibly have been more correct to have drawn this figure as a bar chart, it was illustrated as a single line joining a series of points in order to show more clearly the changes occurring in the proportion of C. furcoides. Confidence limits were calculated as a measure of assessing the reliability of each count (Appendix 5.10). Although not illustrated, to avoid confusion on the graph, the estimate of the true proportion lies within 10% of the observed value for each year (i.e. variation is statistically acceptable). From the start of sampling in 1946 until 1958, the general trend, with minor fluctuations, was towards a decline in the proportion of C. furcoides. From 1964 to 1973 C. furcoides was the more abundant species, to be significantly replaced by C. hirundinella from 1974 to 1982. Between 1983 and 1986 C. furcoides was once more numerous, although following 1984 it was again in decline.

Figure 5.11 The Proportion of *C. furcoides* Cells (as % of total *Ceratium* spp.) in Esthwaite Water (1946-1986)

Figure 5.12 The Number of *C. furcoides* Cells in Esthwaite Water (1946-1986)



C.furcoides cells(%)
100
80
60
40
20
0
1945
50
55
60
65
70
75
80
85
Time(Years)



Cells(x10² ml⁻¹)
12
10
8
6
4
2
0
C.furcoides
C.hirundinella
1945
50
55
60
65
70
75
80
85
Time(Years)

Comparison of Figure 5.11 with Figure 5.1 (page 126) indicates that the variation is independent of any change in population size. The total Ceratium cell counts from the 0-5 m layer for Esthwaite Water, started by Lund in 1945, although only comprehensively sampled from 1946, and continued to the present by other workers, were used in conjunction with the proportions of each species (Figure 5.11) to give a retrospective cell count (Figure 5.12). Since 1983 the two species have been enumerated separately at the F.B.A. The calculated results were within 10% of the observed results in all except the first of these years. This discrepancy in 1983 (29% difference) could be a consequence of F.B.A. workers familiarising themselves with the difference between the species.

Prior to 1964 maximum numbers of motile cells of Ceratium were low (< 100 cells ml^{-1}). C. hirundinella was the more numerous of the two species. However, increases in overall numbers were marked by increases in both species. Between 1958 and 1962 a decline in total numbers was coupled with an increase in the proportion of C. furcoides. The two peaks in overall numbers of Ceratium cells in 1965 and 1973 were due to C. furcoides cells, with low values of C. hirundinella. Following 1974 C. furcoides numbers declined and a large maxima of C. hirundinella even noted between 1975 and 1977. An increase in total numbers in 1982 was notable in that both species were observed in similar numbers. In all other samples either C. furcoides or C. hirundinella was clearly more numerous than the

other, indicating that changes in relative abundance occurred rapidly. The total number of Ceratium fell sharply following 1982, with C. hirundinella declining more than C. furcoides, as shown by the increase in the proportion of the latter. In 1984 total cell numbers reached their lowest value since 1968 and the proportion of C. hirundinella, although still lower than C. furcoides was increasing. In addition, whilst on occasions C. furcoides cells represented 99% of the total identified Ceratium cells (1967 and 1972), C. hirundinella only reached a maximum of 87% (1976).

The timing of encystment and excystment in both species is similar. It is therefore difficult to identify the factor(s) responsible for the observed, almost cyclical, rise and fall in the proportion, and numbers, of C. furcoides if it is presumed that the proportion of cells encysting is the same for both species. It has already been established that, in general, relative numbers of each species are maintained throughout the year (see page 165). This suggests that those factors initiating a change in the proportion of C. hirundinella and C. furcoides are likely to be acting on cyst production, survival or germination, and not on the phytoplankton production. The relative number of cysts produced, their capacity to survive in the sediment and subsequently excyst, will govern the proportion of each species in the phytoplankton. Both cyst production and germination can be affected by fungal parasites. Some parasites may attack motile cells prior to encystment, whilst other species parasitise the cysts. The proportion of each species

will only be affected if one species is more susceptible than the other. For example, the chytrid Rhizophydium nobile is believed to be specific to the cysts of C. furcoides (Canter and Heaney, 1984). The effects of parasitism are outlined more fully in the discussion (page 195).

Discussion

The sediment deposited within a given lake basin originates from two sources, autochthonous material, produced within the lake, and allochthonous material, derived from sediment transported into the lake by streams and tributaries. The loading capacity of these streams, and the location of the lake (either close to the river source or near to the estuary) will govern the extent of the contribution of all allochthonous material (Håkanson and Jansson, 1983).

Deposition of sediment does not occur evenly and thus the depth of sediment will vary throughout the lake basin. Bloesch and Uehlinger (1986) noted large differences between sites, in sediment trapping investigations conducted in Lake Hallwil, Switzerland, whilst Davis (1973) measured variation of up to a factor of 10 in Lake Frains, a stratified lake in Michigan. Pennington, Cambray, Eakins and Harkness (1976) observed changes in depth of sediment between sites by a factor of two within Blelham Tarn, in the English Lake District.

Sedimentation is related to the topography of the lake basin and to hydrodynamic flow patterns (Davis, 1973). Promontories, embayments and the gradient of shorelines will

influence the accumulation of sediment. Water depth and the effects of the prevailing wind on water current are also of major importance. A tendency has been recognised for sediment to accumulate below deeper water sites in contrast to shallow water sites within the same lake basin. Likens and Davis (1975) introduced the term "sediment focusing" to describe this phenomenon. Davis, Moeller and Ford (1984) identified two mechanisms responsible for this process. Continuous sediment focusing involves the resuspension of the flocculent superficial sediment in the shallows and subsequent deposition in deeper water. Episodic sediment focusing is associated with particularly strong winds which cause the formation of strong currents. Erosion is concentrated in the shallows and the sediment redistributed in the deeper parts of the lake. Davis (1973) compared the extent of resuspension in a stratified (Frains Lake, Michigan) and non-stratified lake (Sayles Lake, Michigan). In the former resuspension occurred during the spring and autumn mixing, with the sediment well incorporated into the water column. In the latter irregular resuspension occurred through the year, although mixing with the water column was poor.

Hilton, Lishmann and Allen (1986) recognised a minimum of ten processes responsible for sediment distribution in small lakes. In a study of Esthwaite Water these authors compared the thickness of sediment from a common zinc horizon with water depth, in order to identify the major mechanisms controlling the pattern of sedimentation. They observed a major trend towards

increased thickness of sediment with an increased depth of water, i.e. sediment focusing.

Intermittent complete mixing was determined to be the dominant process in sediment distribution. This mechanism involves the resuspension of material from throughout the lake bed, followed by total mixing within the water column. The subsequent resedimentation will result in increased sediment accumulation at deep water sites, due to the increased number of particles held in the water column. In addition, peripheral wave action was also considered to be an important determining factor, in which the action of waves created turbulence, which in turn resulted in the resuspension, mixing and transference of material to deep water sites. Riverine plume sedimentation was observed to be responsible for localised increases in sediment accumulation. An unaccountable proportion of the total variance was believed to correspond to random resuspension processes.

The position of the cores in the present study correspond closely to four of those used by Hilton, Lishmann and Allan - 8D, 12B, 13G and 14H. They showed that in all four sites there was a close linear relationship between sediment and water depth, indicating that sediment focusing type activities are the major influences on sediment distribution. These particular sites appeared to be uninfluenced by the localised differential sedimentation created by inlet streams and slopes with steep gradients.

The extent of resuspension of material from shallow to deeper water sites is likely to involve the flocculent upper

1-2 cm of sediment (Gorham, 1958). Thus it is possible that resuspended material can be redeposited down to a depth of 2 cm. Resuspension and redeposition will mix sediments of different ages and from different parts of the lake, with no sorting of redeposited material (Davis, 1973). The action of resuspension may be an essential prerequisite to the process of excystment. It has yet to be determined whether germination of Ceratium cysts occurs in the surface sediment or following resuspension into the water column.

Resuspension is only one of the forces acting upon the sediment. Davis (1974) calculated that 36% of surface pollen in a study core was greater than 30 years old, and that 5% was more than 90 years old. Stockner and Lund (1970) observed viable cells to a depth of 35 cm in Esthwaite Water, which they interpreted as a consequence of disruption of the sediment core. This suggests that additional forces are responsible for the migration of particles up, or down, through the core.

Bioturbation would be expected to be one of the factors creating disturbance within the sediment column. This involves the mechanical mixing of the sediment by foraging fishes and benthic fauna under aerobic conditions. Different benthic organisms, including polychaetes, oligochaetes, bivalves, crustaceans, insect larvae and micro-organisms may be involved, all unevenly distributed in space and time throughout the lake basin and with different behavoural activities (Håkanson and Jansson, 1983). In addition, the production of gas bubbles under anaerobic conditions may cause further disturbances. This

system is thus highly complex and as such difficult to quantify. Håkanson and Jansson (1983) refer to the work of Fisher and others who used caesium-137 to study the activity of tubificids in the laboratory. They observed that tubificids alone were responsible for significant mixing of the sediment. The extent of mixing decreased with depth, down to the bioturbation limit. However, Gorham (1958) noted that tubificids and the production of gas bubbles did not have a significant influence on the stratigraphy of Esthwaite Water. Pennington, Cambray and Fisher (1973) deduced that disturbance of the sediment column in Esthwaite Water was little influenced by bioturbation, or other factors, based on the well defined peaks of caesium-137.

Other factors will specifically affect the number of Ceratium cysts in the sediment. The action of both resuspension and bioturbation may result in the redeposition of cysts below the surface sediment, thus reducing the possibility of germination. A reduction in numbers could be expected through the failure of some cysts to reach the sediment surface. A number of recently formed cysts may be transported from the lake basin via the outflow, although this figure is judged to be low due to the rapid passage of cysts to the sediment (Heaney, Smyly and Talling, 1986). The process of excystment inevitably results in the loss of cysts from the sediment. If encystment fails to occur in any given year, excystment may still occur the following spring, through the resuspension of viable cysts into the water column, further depleting cyst numbers from previous years. Ingestion by zooplankton and benthic organisms may also

occur (Krupa, 1981b). In some sediment samples, Ceratium cysts were observed closely bound together in what appeared to be faecal pellets (see Chapter 2, page 51).

Parasitism is now known to have the potential to be a major factor controlling Ceratium populations. Sommer, Wedemeyer and Lowsky (1984) observed a reduction in the population density of C. hirundinella in Lake Constance, Federal Republic of Germany, induced by a fungal parasite, whilst Boltovskoy (1984) detailed the parasitism of Peridinium willei by another fungal parasite, Aphanomycopsis peridiniella. Canter and Heaney (1984) described a new species of chytrid, Aphanomycopsis cryptica, which parasitised Ceratium cells, and was responsible for a marked reduction in their numbers. The chytrid Rhizophydium nobile has been identified as a specific parasite of C. furcoides, while other, as yet undescribed parasites, have been observed for Ceratium cysts within the sediment (Canter, 1968; Canter and Heaney, 1984; Heaney, Lund, Canter and Gray, 1988). Unlike A. cryptica, R. nobile appears to have little effect on reducing the size of subsequent phytoplankton populations (see page 195) but its influence on cyst numbers is largely unknown. Whilst incidents of parasitism and grazing would be expected to be greatest in the sub-surface sediment, loss of cysts through degeneration would be expected to increase with depth. A reduction in numbers of Ceratium cysts at the base of the majority of the cores would appear to reflect this.

Loss of material through procedural errors could

reasonably be expected to be more or less constant through the length of the core. The act of sectioning a core introduces artificial boundaries which may distort the results. For example, a peak in numbers occurring at the edge of two core sections will be divided between them and the discrete maximum lost. The cutting of thinner sections would alleviate this, but would create additional sources of error, since it is more difficult to cut sections of less than 0.5 cm accurately.

A major difficulty in interpreting core data involves assigning a time scale to the vertical profile. In this project the use of diatom stratigraphy assessed the average deposition rate for the two basins over a two year period as 0.8 cm year^{-1}. It should be stressed that this figure represents the average through the core. Compaction of the sediment with depth, and a higher water content towards the surface will result in 1 cm of sediment at the base of the core representing a greater period of time than 1 cm of surface sediment (Hilton and Gibbs, 1984).

The extent of compression is best assessed by the comparison of wet and dry weight measurements. This investigation was primarily concerned with the "in situ" vertical distribution of cysts and so no comprehensive survey of dry weights was undertaken. The single core that was analysed showed a marked increase in the proportion of sediment with depth, from 0.46% at the surface to 2.57% at the base of the core (Figure 5.2, page 131). Gorham (1958) observed that the upper 1.5 cm of sediment taken from Esthwaite Water was less

than half as dense as the following 2.0 cm sections down to a depth of 9.5 cm (0.06 g cm^3 at the surface compared to a mean value of 0.15 g cm^3 for the lower core sections). A similar decrease was observed in the present study, with the upper 1.5 cm representing an average of 46% of the dry weight mean value for each remaining 2.0 cm section.

A decrease in the number of individuals per unit volume may not represent a real decline in numbers. Either an increase in the deposition of the mineral faction, or, an increase in water content could be responsible. In order to quantify the extent of the former it is necessary to express the diatom content over an interval of time, for example, the number of cells per 10 year period (Round, 1964). The latter can be assessed by the calculation of dry weight values for each section of the core.

The use of diatom stratigraphy to assess the sedimentation rates relies upon the deposits for a given year forming a discrete layer. As such it not only enables a speculative date to be attributed to a point in the core, but also provides an indication of the degree of disturbance within the sediment column. It is possible that the diatoms themselves may be susceptible to relocation further down the core. Haworth (1976) studied the vertical distribution of a species of Stephanodiscus new to Blelham Tarn, Cumbria. Frustules of S. astraea var. minutula were found down to a depth of 5 cm, having only appeared in the plankton the previous February. It was suggested that the spherical shape of the diatoms enabled them

to pass easily through the sediment, thus rendering them unsuitable as a marker species. However, a further study (Haworth, 1979) was undertaken when S. astraea var. minutula had been present in the spring blooms for three seasons. The results indicated that the maximum concentrations of S. astraea var. minutula were maintained within the sediment profile. Comparison with phytoplankton populations indicated that the annual increments had not remained entirely discrete, and that some material from the initial population was present in more recent sediments, suggesting that resuspension had occurred. This may account for the lack of a prominent Stephanodiscus maxima in some of the cores in the present study e.g. Core 3.0. Round (1961) studied the diatom stratigraphy of a 5.15 metre core from Esthwaite Water. He observed the mixing of surface deposits with the water column and suggested that cells may be lost from the sediment or redeposited at a lower level.

Estimates of annual sedimentation rate in Esthwaite Water by previous authors have differed by tenfold. Tutin (1955) used sediment traps to determine the annual increment in Windermere. The mean value of 0.26 cm $year^{-1}$ was also believed to be representative of Esthwaite Water. Stockner and Lund (1970) studied the diatom stratigraphy of 50 cm cores taken from Esthwaite Water. They estimated a deposition rate of 0.19 cm $year^{-1}$ in the upper 5 cm and 0.12-0.15 cm $year^{-1}$ through the whole core. Pennington, Cambray and Fisher (1973) monitored the vertical and horizontal distribution of caesium-137 in cores from the same lake. They obtained an average sedimentation rate

of 0.9 cm year^{-1} calculated from the caesium peak associated with 1963. A later study by Pennington (1974) again utilized sediment trapping, this time obtaining an average value of 2.16 cm year^{-1}. This substantially higher figure was attributed to the collection of resuspended material, thus effectively doubling the amount of sediment actually deposited.

Some of the variation between the counts can be accounted for by local differences in sediment accumulation (Gorham, Lund, Sanger and Dean, 1974). Meriläinen (1971) observed an uneven distribution of frustules in the recent sediment of four lake basins. In addition, later estimates of sediment accumulation in Esthwaite Water, based on the upper horizons of the sediment column, would be expected to be greater than earlier ones due to the increased productivity in the lake associated with a rise in nutrient levels in the mid to late 1970's (see Chapter 3.1, page 65). The resuspension of the flocculent upper 2 cm of the sediment (Gorham, 1958), apparently greater than a single year's deposition, suggests that an overlap is likely to occur between the sediment of successive years (Haworth, 1979), thus effectively "averaging out" the material deposited over a couple of years. Nevertheless, the average estimate of the sedimentation rate obtained in this study, 0.8 cm year^{-1}, is comparable with the figure of 0.9 cm year^{-1} obtained by Pennington, Cambray and Fisher (1973), and generally regarded as the most reliable of the values obtained for Esthwaite Water (e.g. Livingstone, 1979; Pennington, 1978).

The figure of 0.8 cm year^{-1} can be used in conjunction

with the vertical distribution of cysts in order to establish the length of time cysts remain viable within the sediment. Observation of the cores in this study (Figures 5.3-5.5) suggested that the number of viable Ceratium cysts declined at a depth of about 6 cm. This indicates either a decrease in the number of cysts originally deposited, or, an increase in the mortality of cysts from this depth downwards. The former explanation is explored shortly when phytoplankton populations are compared with cyst numbers. In this case, however, it would seem likely that loss of viability is responsible for the decline in the cyst population, which occurs at around the same point in the core regardless of when the core was extracted.

If it is assumed that sediment accumulation occurs at an average rate of 0.8 cm $year^{-1}$, sediment at a depth of 6 cm can thus be tentatively dated at about 7½ years. If the estimate of 0.9 cm $year^{-1}$ for sediment deposition in Esthwaite Water is used (Pennington, Cambray and Fisher, 1973), sediment at 6 cm would be expected to have been laid down approximately 6½ years previously. Both these figures correspond with the observations of Huber and Nipkow (1922), who noted that cysts removed from varved sediments of up to 6½ years were still capable of germination. (The ability of cysts from the present study to excyst is investigated further in Chapter 6). In contrast with these results, Livingstone (1979) observed viable Ceratium cysts in only the upper 3 cm of cores from Esthwaite Water, Cumbria and Rostherne Mere, Cheshire although non-viable and empty cysts were found below this depth, down to a maximum of 70 cm in the

latter. Stockner and Lund (1970) cultured algal species (Ceratium was not mentioned) from Esthwaite Water sediment down to a maximum depth of 35 cm. The relative viability of C. furcoides and C. hirundinella are discussed in detail in a later section (see page 189).

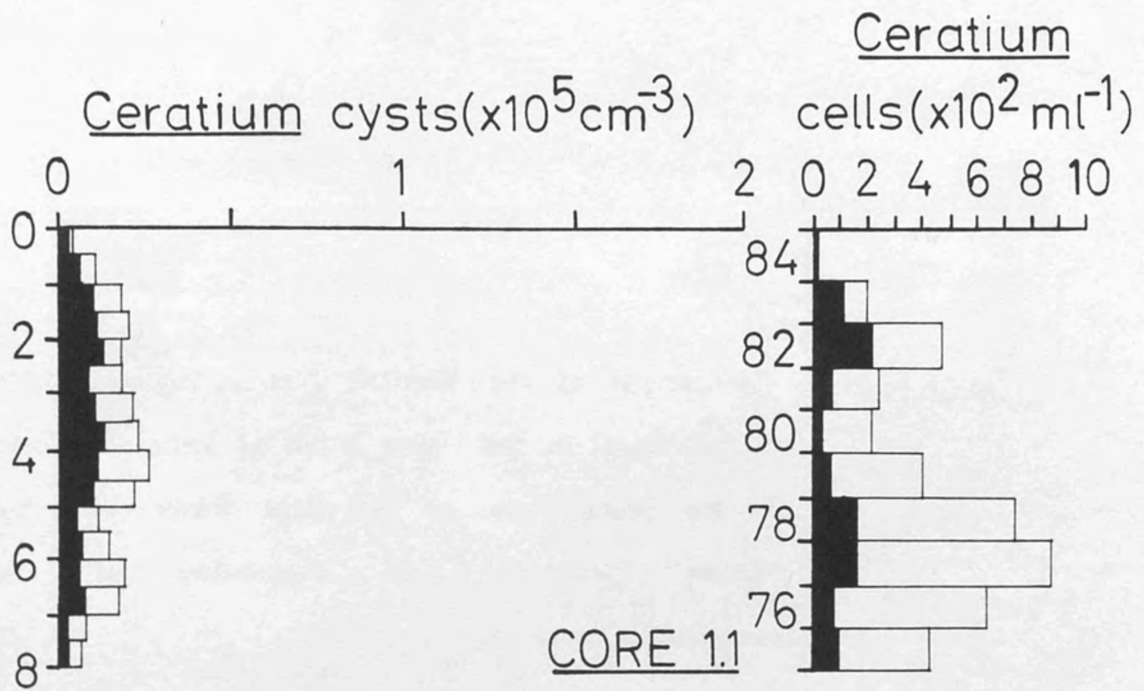
Figures 5.13-5.14 illustrate the vertical distribution of viable Ceratium cysts from preserved, deepwater cores, from the two basins of Esthwaite Water, with the corresponding maximum cell counts for that year. In contrast to Figures 5.3-5.5, cyst numbers were adjusted, using the values obtained in Figure 5.2 (page 131), to allow for differences in the proportion of sediment in each core section. Although based on only one analysis of sediment content it was deemed worthwhile to compare the adjusted data with the straightforward plots. The distribution pattern remains largely unchanged although the low number of cysts at the base of the cores are emphasised. The annual sedimentation rate of 0.8 cm $year^{-1}$, estimated from the diatom stratigraphy, was used to place the phytoplankton values in relation to the number of cysts. Diatom numbers were also adjusted in line with sediment content. However, since no significant change was observed (adjusted figure = 0.87 cm $year^{-1}$), the original figure of 0.8 cm $year^{-1}$ was retained. The difficulty in comparing data of this kind is that each uses a different time interval as a basis for a single count. Phytoplankton cells represent the maximum single count for an individual year, whilst cyst counts are undertaken throughout a 0.5 cm core section representing less than an annual increment

Figure 5.13 A Comparison of the Vertical Distribution of Viable *Ceratium* Cysts in the Upper 8 cm of Sediment Cores from the North Basin of Esthwaite Water with the Maximum *Ceratium* Cell Population of the Corresponding Year

Key to vertical axes

Cysts - depth in cm

Cells - time in years



■ C.furcoides cysts
□ C.hirundinella cysts

■ C.furcoides cells
□ C.hirundinella cells

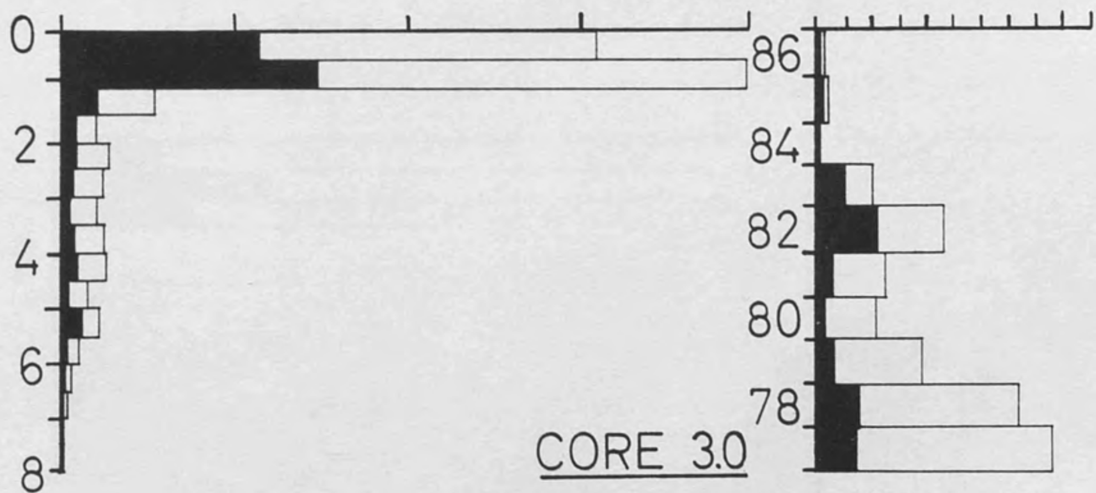
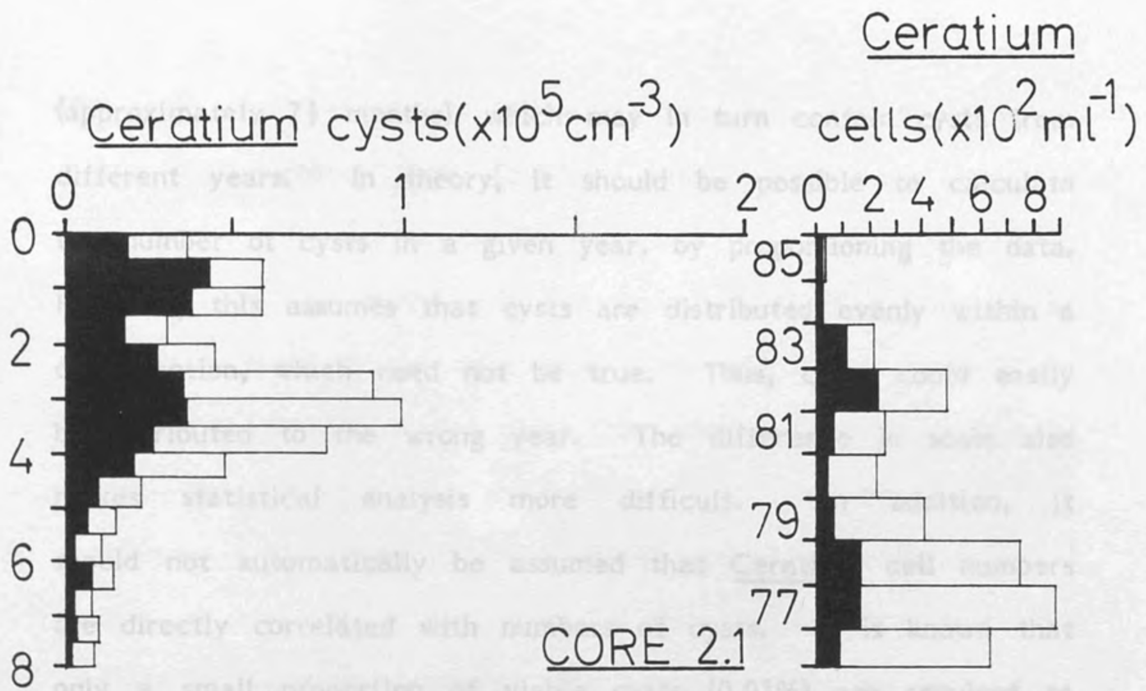


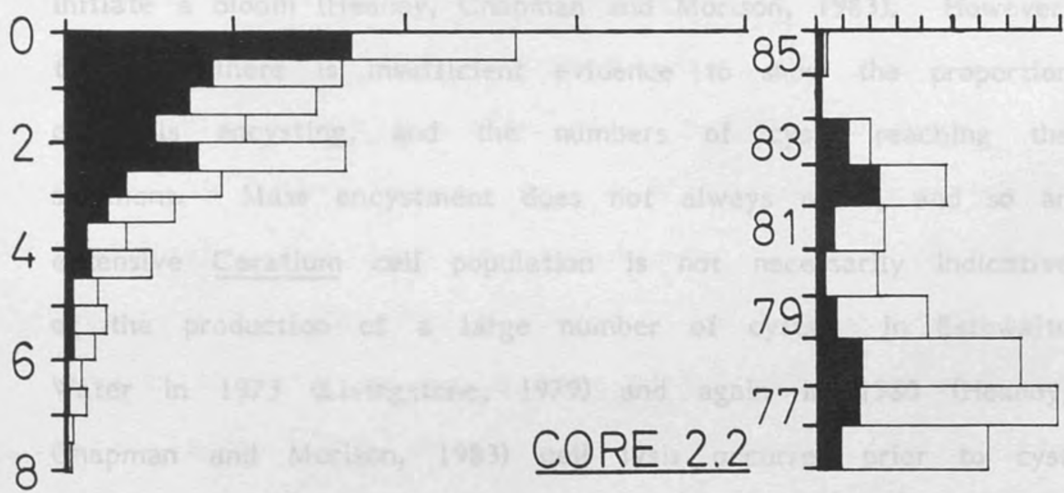
Figure 5.14 A Comparison of the Vertical Distribution of Viable *Ceratium* Cysts in the Upper 8 cm of Sediment Cores from the South Basin of Esthwaite Water with the Maximum *Ceratium* Cell Population of the Corresponding Year

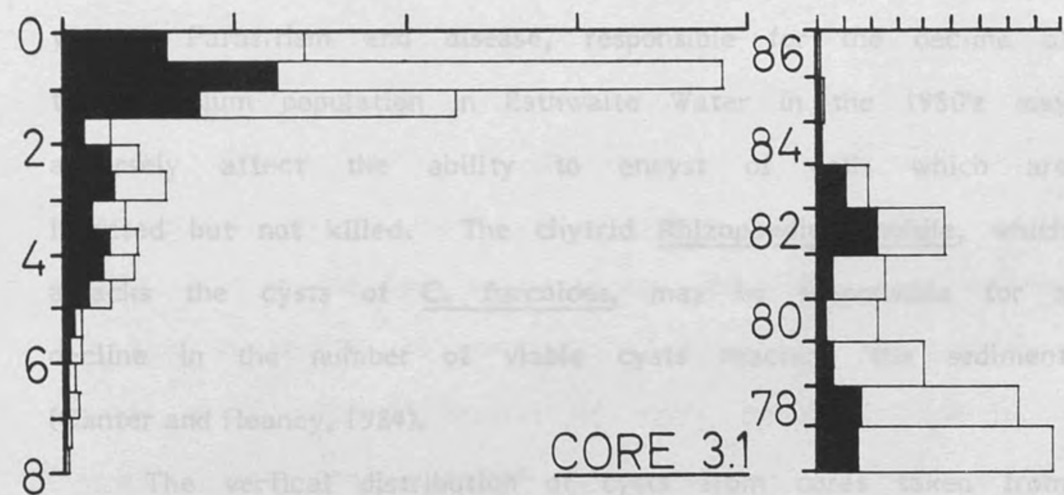
Key to vertical axes

Cysts - depth in cm

Cells - time in years







■ C. furcoides cysts

□ C. hirundinella cysts

■ C. furcoides cells

□ C. hirundinella cells

(approximately 7½ months), which may in turn contain cysts from different years. In theory, it should be possible to calculate the number of cysts in a given year, by proportioning the data. However, this assumes that cysts are distributed evenly within a core section, which need not be true. Thus, cysts could easily be attributed to the wrong year. The difference in scale also makes statistical analysis more difficult. In addition, it should not automatically be assumed that Ceratium cell numbers are directly correlated with numbers of cysts. It is known that only a small proportion of viable cysts (0.03%) are required to initiate a bloom (Heaney, Chapman and Morison, 1983). However, to date, there is insufficient evidence to show the proportion of cells encysting, and the numbers of cysts reaching the sediment. Mass encystment does not always occur, and so an extensive Ceratium cell population is not necessarily indicative of the production of a large number of cysts. In Esthwaite Water in 1973 (Livingstone, 1979) and again in 1980 (Heaney, Chapman and Morison, 1983) cell lysis occurred prior to cyst formation, thus presumably reducing cyst production in that year. Parasitism and disease, responsible for the decline of the Ceratium population in Esthwaite Water in the 1980's may adversely affect the ability to encyst of cells which are infected but not killed. The chytrid Rhizophydium nobile, which attacks the cysts of C. furcoides, may be responsible for a decline in the number of viable cysts reaching the sediment (Canter and Heaney, 1984).

The vertical distribution of cysts from cores taken from

the south basin appear to follow the increases and decreases in motile cell numbers more faithfully than cores taken from the north basin. In the latter little variation in cyst numbers was noted through the core, with the exception of high numbers of sub-surface cysts in the core taken soon after encystment (Core 3.0). The upper half of the cores from the south basin appear to reflect the changes in the motile cells of the Ceratium population. All three of these deep water cores (Figure 5.14) showed an increase in cyst numbers corresponding with the 1982 increase in Ceratium numbers. The subsequent decline in numbers of motile cells was also well represented in the cyst profile. However, the second cell maximum associated with 1977/8 was not evident in the cyst population, which continued to decline. It is possible that mixing, by water currents and benthic invertebrates, may have transported cysts to lower or higher horizons within the sediment column. However, it would seem more likely that cysts corresponding to the cell maximum are no longer viable. This would appear to corroborate the previous suggestion that cyst mortality increases below 6 cm.

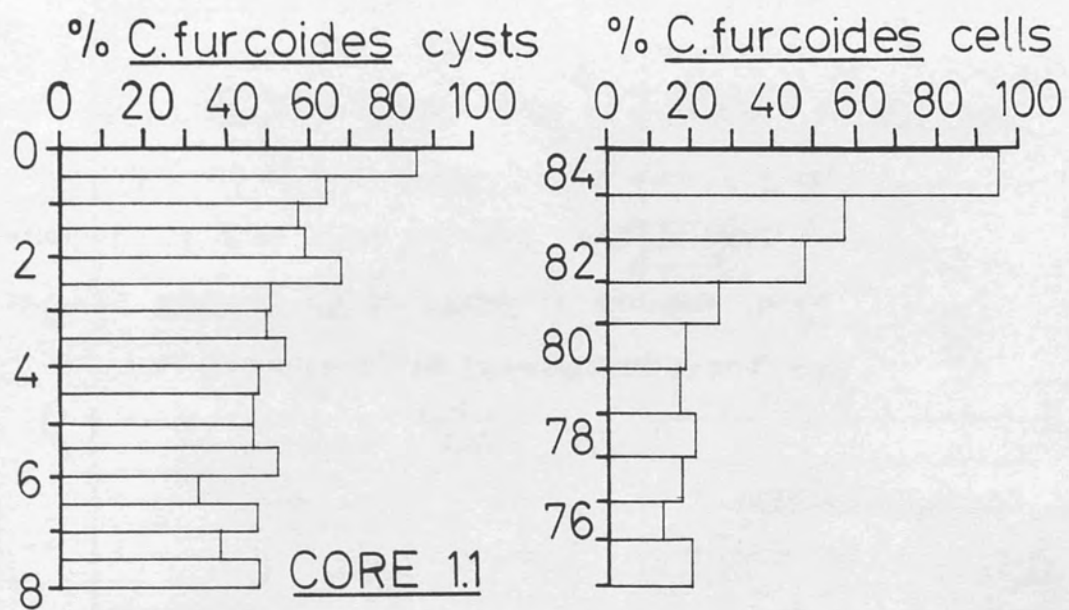
Figures 5.15-5.16 show the proportion of C. furcoides cysts through the sediment column, similarly adjusted for sediment content, with the proportion of C. furcoides as a percentage of the maximum total Ceratium cell population in the phytoplankton of the corresponding year. When comparing data of this kind it has to be assumed, in addition to the conditions already noted, that production of cysts occurs in the same proportion, with regard to the number of cells in both species,

Figure 5.15 A Comparison of the Proportion of *C. furcoides* Cysts (as % of the total *Ceratium* spp.) in the Upper 8 cm of Sediment Cores from the North Basin of Esthwaite Water with the Proportion of *C. furcoides* Cells at the Time of the Maxima of the Corresponding Year

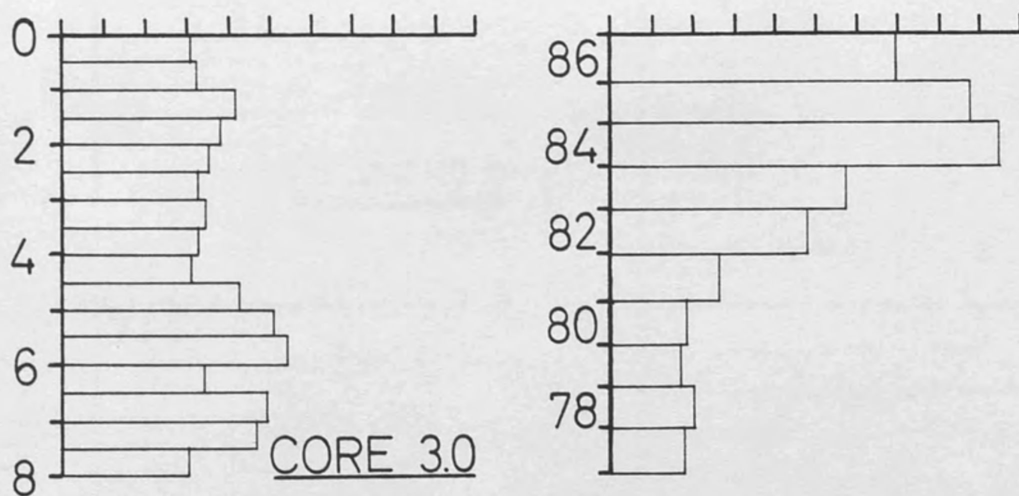
Key to vertical axes

Cysts - depth in cm

Cells - time in years



% C.furcoides cysts
0 20 40 60 80 100
0
2
4
6
8
CORE 1.1
% C.furcoides cells
0 20 40 60 80 100
84
82
80
78
76



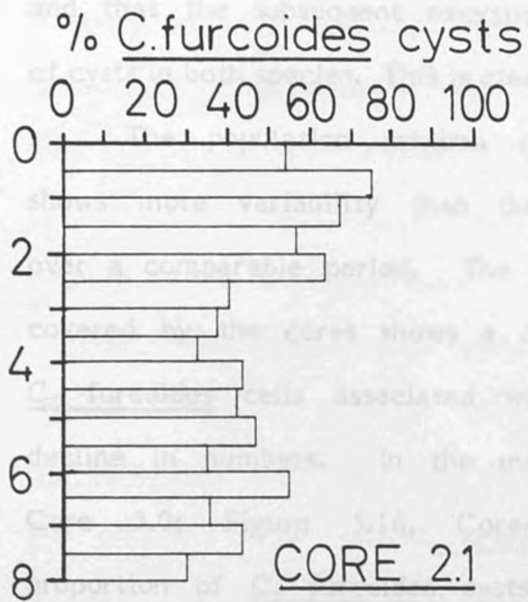
0
2
4
6
8
CORE 3.0
86
84
82
80
78

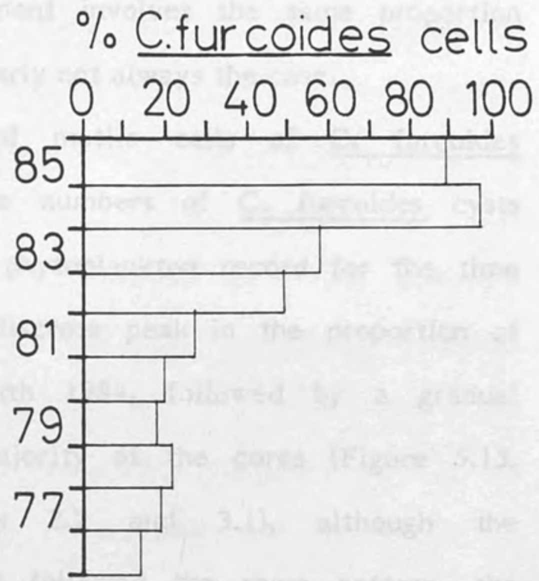
Figure 5.16 A Comparison of the Proportion of *C. furcoides* Cysts (as % of the total *Ceratium* spp.) in the Upper 8 cm of Sediment Cores from the South Basin of Esthwaite Water with the Proportion of *C. furcoides* Cells at the Time of the Maxima of the Corresponding Year

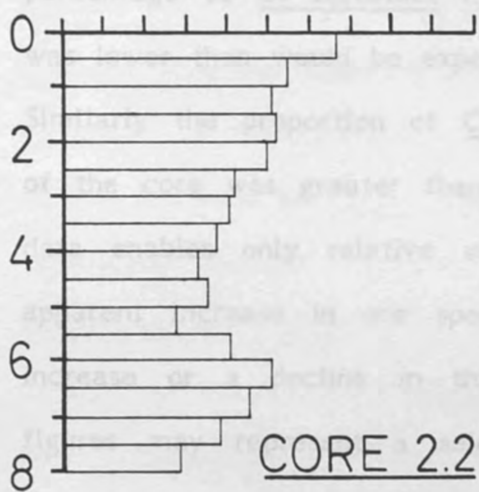
Key to vertical axes

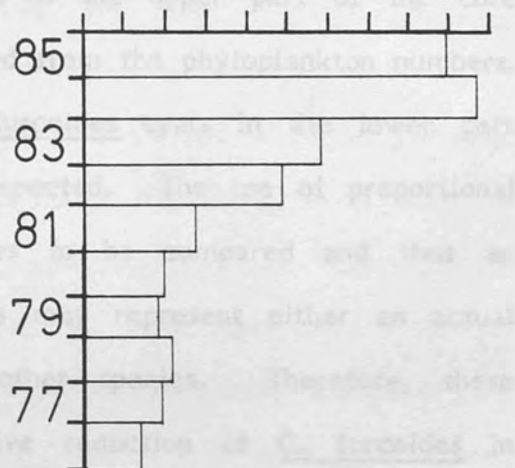
Cysts - depth in cm

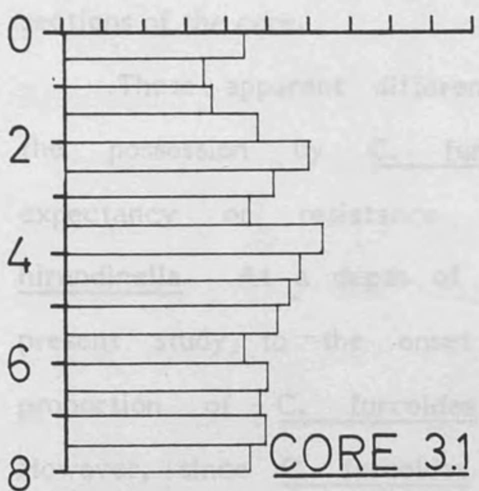
Cells - time in years

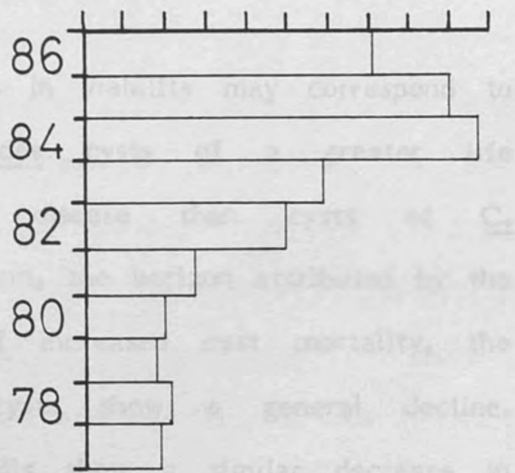

% C.furcoides cysts
0 20 40 60 80 100
0
2
4
6
8
CORE 2.1


% C.furcoides cells
0 20 40 60 80 100
85
83
81
79
77


0
2
4
6
8
CORE 2.2


85
83
81
79
77


0
2
4
6
8
CORE 3.1


86
84
82
80
78

and that the subsequent excystment involves the same proportion of cysts in both species. This is clearly not always the case.

The population maxima of motile cells of C. furcoides shows more variability than the numbers of C. furcoides cysts over a comparable period. The phytoplankton record for the time covered by the cores shows a discrete peak in the proportion of C. furcoides cells associated with 1984, followed by a gradual decline in numbers. In the majority of the cores (Figure 5.15, Core 3.0; Figure 5.16, Cores 2.2 and 3.1), although the proportion of C. furcoides cysts followed the same pattern, the percentage of C. furcoides cysts in the upper part of the core was lower than would be expected from the phytoplankton numbers. Similarly the proportion of C. furcoides cysts in the lower part of the core was greater than expected. The use of proportional data enables only relative values to be compared and thus an apparent increase in one species may represent either an actual increase or a decline in the other species. Therefore, these figures may represent a selective reduction of C. furcoides in the upper part of the core, and of C. hirundinella in the lower sections of the core.

These apparent differences in viability may correspond to the possession by C. furcoides cysts of a greater life expectancy or resistance to disease than cysts of C. hirundinella. At a depth of 6 cm, the horizon attributed by the present study to the onset of increased cyst mortality, the proportion of C. furcoides cysts show a general decline. However, since C. furcoides cells show a similar decrease in

numbers in the corresponding year, this is more likely to reflect the phytoplankton than a tendency to be less resistant than *C. hirundinella* cysts. The chytrid *Rhizophydium nobile* is known to be specific to cysts of *C. furcoides* (Canter and Heaney, 1984; Heaney, Lund, Canter and Gray, 1988), although originally described as a parasite of *C. hirundinella* (Canter, 1968). The long term changes in the proportion and numbers of *C. furcoides* cells in the present study, discussed earlier in this chapter, show that *R. nobile* occurs when the number of *C. furcoides* is high (Heaney, Lund, Canter and Gray, 1988), and appears to have little effect on the size of the *Ceratium* population of the following year. The most recent occurrence of *R. nobile* in 1983 does not appear to have had a major influence on the numbers of *C. furcoides* cysts in the core, which show no significant changes at the corresponding depth. The chytrid *Aphanomycopsis cryptica* is known to have contributed to the decline in the numbers of *Ceratium* in Esthwaite Water in the early 1980's (Heaney, Lund, Canter and Gray, 1988), but is not believed to act selectively on either species. Although several so far undescribed parasites have been recognised, to date none have been observed to be specific to *C. hirundinella* (Canter and Heaney, 1984). Thus it is not possible to attribute the low proportion of *C. hirundinella* in the lower part of the cores to parasitism. It is also possible that the numbers represent a greater tendency for cysts of *C. hirundinella* to excyst, although this is contrary to laboratory experiments (see Chapter 6, page 245).

Alternatively the relative distribution of C. furcoides and C. hirundinella may be accounted for by the occurrence of mixing within the sediment. Stockner and Lund (1970) also observed less distinct peaks in the numbers of Tabellaria in sediment cores from Esthwaite Water, than from phytoplankton samples from a corresponding period, and attributed this to mixing. However, Pennington, Cambray and Fisher (1973) deduced that disturbance of the sediment of Esthwaite Water was limited (see page 176). Disturbance of the sediment created by water currents and the activity of benthic invertebrates may be responsible for the downward movement of cysts to lower sediment horizons, thus reducing the proportion of C. furcoides cysts in the surface sediment and increasing the percentage further down the core.

The majority of previous studies involving algal remains within the sediment have concentrated on diatoms, the siliceous frustules of which usually remain well preserved for long periods of time. Several authors have shown the existence of a positive correlation between the vertical distribution of diatoms and the plankton record. Stockner and Benson (1967) established a relationship between the distribution and species composition of diatoms within the sediment of Lake Washington, Seattle, U.S.A., with the pattern of sewage enrichment over 80 years. Stockner and Lund (1970) showed that a close agreement existed between the proportion of four diatom species within the sediment and the corresponding plankton record in Esthwaite Water. Haworth (1980) correlated diatom stratigraphy with

phytoplankton over a 35 year period in Blelham Tarn. In contrast Stoermer, Wolin, Schelske and Cowley (1985) observed no relationship between the vertical distribution of diatoms in a 40 cm core from Lake Ontario, Canada/U.S.A. border and the corresponding phytoplankton population.

Few equivalent studies have compared the vertical distribution of dinoflagellate cysts with past plankton populations. Most studies, both freshwater and marine, have investigated the capacity for the cyst population to "seed" the phytoplankton population of subsequent years (e.g. Anderson and Morel, 1979; Heaney, Chapman and Morison, 1983; Lewis, Tett and Dodge, 1985). Dale (1976) recognised that a connection existed between the number of cysts in the sediment and cells in the plankton, recommending that cores representing at least five years were necessary for any meaningful comparison. Anderson, Aubrey, Tyler and Coates (1982) observed a wide range of vertical distributions in the cysts of marine dinoflagellates <u>Gonyaulax tamarensis</u>, <u>G. scrippsae</u>, <u>Gyrodinium uncatenum</u>, <u>Gyrodinium</u> sp. and <u>Heterocapsa triquetra</u>, in sediment cores of up to 11 cm from Perch Pond (Cape Cod), Massachusetts; Buzzard Bay, Massachusetts and the estuary of the Potomac River, Maryland. Pollingher (1986b) observed no dinoflagellate cysts in sediment samples from Lake Kinneret, Israel, which supports large populations of <u>Peridinium cinctum</u> fa. <u>westii</u> and <u>C. hirundinella</u>. However, preparation of sediment for examination involved treatment with hydrochloric acid, hydrofluoric acid and acetolysis, which would be expected to destroy the largely

cellulose cyst wall of these species.

Livingstone (1979) compared the sedimentary remains of several algal species, including Ceratium, with past phytoplankton records. The stratigraphy of algae in sediment cores from Rostherne Mere, Cheshire, was shown to reflect accurately the past fluctuations in phytoplankton. However, the sedimentary record from Esthwaite Water was interpreted as showing little resemblance to the known phytoplankton history. Despite the presence of large numbers of Ceratium cells in previous years, viable Ceratium cysts declined rapidly from the surface sediment, with no viable cysts observed below 3 cm.

Table 5.5 shows the mean results of five of Livingstone's cores, with the figures extrapolated to number of cysts cm^{-3}, in order to aid comparison with results from cores in the present study taken at a similar time of year (October).

It can be seen that despite an overall greater total, Livingstone's cores contained far fewer cysts at lower depths. An average of 84% of cysts were contained in the upper 2 cm, dropping sharply to 0.1% in the lowest core section, compared to 60% in the upper 2 cm and a steady fall to nearly 2%, in the current study. This rapid reduction of cysts with depth, with no viable cysts below the surface sediments, was attributed by Livingstone to the decomposition of cysts in the oxidised microzone. However, the presence of viable cysts in reduced, but still sizeable numbers in the present study, through the entire 8 cm of the cores suggests an alternative explanation. Livingstone enumerated cysts from small sub-samples (0.05 cm^3)

Table 5.5 Comparison of the number of Ceratium cysts in the upper 8 cm of cores taken in 1976/77 and 1986 from Esthwaite Water

Depth (cm)	Mean No. of Cysts cm^{-3}			
	1976/77	% of total	1986	% of total
0-1	6856	40.3	5987	41.0
1-2	7464	43.9	2828	19.4
2-3	1540	9.1	1655	11.3
3-4	736	4.3	1536	10.5
4-5	84	0.5	1141	7.8
5-6	120	0.7	780	5.3
6-7	172	1.0	420	2.9
7-8	24	0.1	247	1.7

where 1976/77 values are from Livingstone's cores taken between October and January

1986 values are from cores taken in October during the course of the present study

by light microscopy. The larger sample size (0.25-0.50 cm^3) and use of fluorescence microscopy in the current study may have made it easier to observe cysts present in low numbers.

A high number of Ceratium cells in the phytoplankton was not always indicative of the production of a large number of cysts (see page 183). However, a comparison of the Ceratium phytoplankton population 10 years prior to the present study and that of Livingstone may help to explain the differences between

the cores, assuming sedimentation rates are comparable in the two cores (Figure 5.1, page 126). In the present study, the <u>Ceratium</u> phytoplankton population at the time of the summer maxima was very low (1986: 15 cells ml^{-1}), but had been preceded in previous years by a large population (e.g. 1982: 470 cells ml^{-1}). Of greatest importance, the years corresponding to the base of the cores had supported very large phytoplankton populations of <u>Ceratium</u> (1977: 880 cells ml^{-1}). In total contrast, the earlier study by Livingstone had been conducted at a time when the summer maximum of <u>Ceratium</u> was high (1976: 632 cells ml^{-1}), following a year of low <u>Ceratium</u> (e.g. 1974: 79 cells ml^{-1}), whilst those years corresponding to the base of the core had very low <u>Ceratium</u> phytoplankton populations (e.g. 1968: 3 cells ml^{-1}). Thus the number of cysts in the earlier study would be expected to decline from the surface, corresponding to the fall in <u>Ceratium</u> cells in the plankton, whilst in the present study numbers would be expected to rise with depth, if no other factors were involved. The number of cysts in the upper 2 cm of the sediment cores described in Livingstone's study would be expected to be greater than in this one since cell numbers were considerably greater. However, the difference between the two sets of data is less than would be expected if the ratio of cysts produced to the number of cells remained the same, i.e. the <u>Ceratium</u> cell population of 1986 represents only 2.4% of that of 1976, whilst the cyst population of the 0-2cm section of the 1986 core corresponds to 61.6% of the same stratum of the earlier core. It may be that the fluorescence

microscope technique used here made it easier to observe viable cysts than the previous method.

The absence of viable cysts near the base of the earlier core could correspond with the very low Ceratium cell numbers associated with 1967-1970. However, this does not account for the very sharp decline in numbers and lack of viable cysts below 3 cm. High numbers of Ceratium were recorded in the phytoplankton in 1972 and 1973 and these do not appear to be reflected in the cyst population. In 1973 a mass cell lysis was observed in the Ceratium population just prior to encystment (Heaney, Chapman and Morison, 1983) and consequently few cysts would be expected to be produced. This would necessitate the recruitment of cysts from previous years to provide the inoculum for the following years phytoplankton. Thus a further reduction of the 1971 and 1972 cyst population may occur as viable cysts, which had not excysted the previous year, seed the 1974 Ceratium population, possibly due to resuspension. In addition, 1972 and 1973, both years with a high proportion of C. furcoides, were associated with occurrences of Rhizophydium nobile, which attacks and causes the death of C. furcoides cysts (Canter and Heaney, 1984). Thus although a large Ceratium cell population was produced in 1973 (maximum population = 1332 cells ml^{-1}) and a reduced one in 1974 (maximum population = 79 cells ml^{-1}) the cyst population would be expected to be severely depleted. The lack of viable cysts between 3-6 cm in Livingstone's cores may correspond to this period of time. Below this depth the present study has shown a reduction in the number of viable cysts.

The alternative explanation for the presence of viable cysts throughout the cores in the current work is that a greater degree of mixing has occurred, resulting in the transference of recent cysts to greater depths. However, if extensive disturbance had taken place the vertical distribution of cysts would be expected to be more homogenous in contrast to the data presented here, which shows a distinct reduction in cyst numbers towards the base of the core.

Some correlation was shown to exist between the distribution pattern of Ceratium cysts within a sediment core and the Ceratium cell population at the time of the summer maxima. Variation in the number of cysts was less marked, with the exception of the upper 1-2 cm of the sediment, than differences between the number of cells. This may be a consequence of bioturbation and resuspension, causing the overlap of cysts deposited in different years and making the distinction of annual layers more difficult. Nevertheless, the identification of a peak in cyst numbers corresponding to the 1982 cell maximum in sediment cores from the south basin of the lake, indicated that despite such disturbances within the sediment the cyst population still gave some indication of past phytoplankton populations.

To gain a fuller understanding of the way in which cyst numbers reflect past Ceratium cell populations a more comprehensive study would be necessary with more cores taken on a given occasion. The amount of work entailed was beyond the scope of the present project but is currently being undertaken

at the F.B.A. Further investigation is also required to establish any variation in the life cycles of C.hirundinella and C. furcoides. The presence of a sexual stage in the life cycle has been established in C. furcoides (Hickel, 1988a), but it is unknown whether the same proportions of the two species encyst/excyst, or whether one has a greater life expectancy.

APPENDIX 5.1 Comparison of the number of viable cysts in sediment cores taken on the same occasion using the Friedman Two-Way Analysis of Variance by Ranks

Use of the Friedman Two-Way Analysis of Variance by Ranks

This non-parametric test determines whether samples come from the same population. The test compares paired (or multiple) data and so was a suitable choice for the current work, where it was necessary to study core sections from corresponding depths. The main disadvantage of this test is that the use of ranks obscures the extent of variation between two samples, i.e. it may show that a is greater than b, but does not show by how much.

The data was arranged in a contingency table with N rows (corresponding to depth of core section) and k columns (corresponding to the number of sediment cores). The test required the data in each row to be ranked separately. The squared sums of the ranks for each column were then used in the following formula:-

$$\chi^2_r = \frac{12}{Nk(k+1)} \cdot \Sigma (Rj)^2 - 3N(k+1)$$

where N = no. of rows

k = no. of columns

Rj = sum of ranks in jth column

The figure obtained was then compared with tabulated values to determine the probability of occurrence.

Comparison of Preserved Sediment Cores

H_0 : All samples originated from the same population.

H_1 : The samples originated from different populations.

CORE 2.1		CORE 2.2	
No. of Cysts (cm^{-3})	Rank 1	No. of Cysts (cm^{-3})	Rank 2
828	1	3035	2
2483	1	3448	2
3701	1	4713	2
2759	1	4736	2
3471	1	6368	2
7885	2	3977	1
9770	2	3149	1
6782	2	1632	1
3287	2	1747	1
2000	2	805	1
1448	2	1149	1
1218	2	942	1
1517	2	414	1
920	2	506	1
1264	2	391	1
988	2	345	1
	27		21

$$\chi^2 r_{\alpha,1} = \frac{12}{Nk(k+1)} . \Sigma(Rj)^2 - 3N(k+1)$$

$$\chi^2 r_{\alpha,1} = \frac{12}{16x2(2+1)} \cdot 1170 - 3x16(2+1) = 2.25$$

from tables $\chi^2_{0.05,1} = 3.84$

$\therefore \chi^2 r_{\alpha,1} < \chi^2_{0.05,1}$

$\therefore H_0$ cannot be rejected at the 95% level of probability.

Therefore, all samples originated from the same population.

Comparison of Unpreserved Sediment Cores

H_0 : All samples originated from the same population.

H_1 : The samples originated from different populations.

CORE 2.3		CORE 2.4	
No. of Cysts (cm^{-3})	Rank 1	No. of Cysts (cm^{-3})	Rank 2
230	2	115	1
1287	2	414	1
3287	2	782	1
6046	2	759	1
1540	2	736	1
1586	2	483	1
851	2	759	1
598	1	805	1
506	1	644	2
506	2	322	2
437	2	276	1
299	2	230	1
253	1	322	2

138	1	230	2
437	2	253	1
253	2	207	1
	28		20

$$\chi^2 r_{\alpha,1} = \frac{12}{Nk(k+1)} . \Sigma(Rj)^2 - 3N(k+1)$$

$$= \frac{12}{16x2(2+1)} .1184 - 3x16(2+1) = 4.0$$

from tables $\chi^2_{0.05,1} = 3.84$, $\chi^2_{0.01,1} = 6.64$

$\therefore \chi^2_{0.05,1} < \chi^2 r_{\alpha,1} < \chi^2_{0.01,1}$

$\therefore H_0$ can be rejected at the 95%, but not at the 99%, level of probability.

Therefore, samples cannot conclusively be shown to have originated from different populations.

APPENDIX 5.2 **Comparison of the number of viable Ceratium cysts in sediment cores taken at different times of the year, using the Friedman Two-Way Analysis of Variance by Ranks**

Comparison of Preserved Cores from the North Basin of Esthwaite Water

H_0 : All samples originated from the same population.

H_1 : The samples originated from different populations.

CORE 1.1		CORE 3.0	
No. of Cysts (cm^{-3})	Rank 1	No. of Cysts (cm^{-3})	Rank 2
98	1	4133	2
433	1	9931	2
1138	1	1770	2
1757	2	1012	1
1489	2	1046	1
1685	2	1115	1
2204	2	1111	1
2092	2	1138	1
1771	2	977	1
1980	2	724	1
1076	2	1069	1
1729	2	621	1
1994	2	333	1
2008	2	241	1
1067	2	172	1
798	2	149	1
	29		19

$$\chi^2 r_{\alpha,1} = \frac{12}{Nk(k+1)} . \Sigma(Rj)^2 - 3N(k+1)$$

$$= \frac{12}{16x2(2+1)} .1202 - 3x16(2+1) = 6.25$$

from tables $\chi^2_{0.05,1} = 3.84$, $\chi^2_{0.01,1} = 6.64$

$\therefore \chi^2_{0.05,1} < \chi^2 r_{\alpha,1} < \chi^2_{0.01,1}$

$\therefore H_0$ can be rejected at the 95%, but not at the 99%, level

of probability.

Therefore, samples cannot conclusively be shown to have originated from different populations.

Comparison of Unpreserved Cores from the North Basin of Esthwaite Water

H_0 : All samples originated from the same population.

H_1 : The samples originated from different populations.

CORE 1.0		CORE 2.0	
No. of Cysts (cm^{-3})	Rank 1	No. of Cysts (cm^{-3})	Rank 2
322	2	230	1
874	2	667	1
1448	2	460	1
1057	2	851	1
1172	2	920	1
966	2	759	1
2092	2	575	1
6414	2	322	1
3908	2	69	1
1310	2	69	1
598	2	46	1
621	2	115	1
575	2	92	1
207	2	184	1
230	2	138	1
161	1	207	2
	31		17

$$\chi^2 r_{\alpha,1} = \frac{12}{Nk(k+1)} . \Sigma(Rj)^2 - 3N(k+1)$$

$$= \frac{12}{16x2(2+1)} .1250 - 3x16(2+1) = 12.25$$

from tables $\chi^2_{0.05,1} = 3.84$, $\chi^2_{0.01,1} = 6.64$

$\therefore \chi^2 r_{\alpha,1} > \chi^2_{0.01,1}$

$\therefore H_0$ can be rejected at the 99% level of probability.

Therefore, all samples originated from the different populations.

Comparison of Preserved Cores from the South Basin of Esthwaite Water

H_0 : All samples originated from the same population.

H_1 : The samples originated from different populations.

CORE 2.1		CORE 2.2		CORE 3.1	
No. Cysts	Rank 1	No. Cysts	Rank 2	No. Cysts	Rank 3
828	1	3035	3	1609	2
2483	1	3448	2	8276	3
3701	1	4713	2	7287	3
2759	2	4736	3	1241	1
3471	2	6368	3	1701	1
7885	3	3977	2	2758	1
9770	3	3149	2	1874	1
6782	3	1632	1	2023	2
3287	3	1747	2	1459	1
2000	3	804	1	1402	2

1448	3	1149	2	724	1
1218	3	942	2	707	1
1517	3	414	1	783	2
919	3	506	1	621	2
1264	3	391	2	379	1
988	3	345	2	287	1
	40		31		25

$$\chi^2 r_{\alpha,1} = \frac{12}{Nk(k+1)} . \Sigma(Rj)^2 - 3N(k+1)$$

$$= \frac{12}{16x3(3+1)} .3186 - 3x16(3+1) = 7.125$$

from tables $\chi^2_{0.05,2} = 5.99$, $\chi^2_{0.01,2} = 9.21$

$\therefore \chi^2_{0.05,2} < \chi^2 r_{\alpha,2} < \chi^2_{0.01,1}$

$\therefore H_0$ can be rejected at the 95%, but not at the 99%, level of probability.

Therefore, the samples cannot conclusively be shown to have originated from different populations.

APPENDIX 5.3 **Comparison of the number of viable cysts in sediment cores taken from different basins of the lake, using the Friedman Two-Way Analysis of Variance by Ranks**

Comparison of Cores taken in March 1986

H_0 : All samples originated from the same population.

H_1 : The samples originated from different populations.

CORE 2.0		CORE 2.3		CORE 2.4	
No. Cysts	Rank 1	No. Cysts	Rank 2	No. Cysts	Rank 3
230	2.5	230	2.5	115	1
667	2	1287	3	414	1
460	1	3287	3	782	2
851	2	6046	3	759	1
920	2	1540	3	736	1
759	2	1586	3	483	1
575	1	851	3	759	2
322	1	598	2	805	3
69	1	506	2	644	3
69	1	506	3	322	2
46	1	437	3	276	2
115	1	299	3	230	2
92	1	253	2	322	3
184	2	138	1	230	3
138	1	437	3	253	2
207	1.5	253	3	207	1.5
	23.0		30.5		42.5

$$\chi^2 r_{\alpha,1} = \frac{12}{Nk(k+1)} . \Sigma(Rj)^2 - 3N(k+1)$$

$$= \frac{12}{16x3(3+1)} .3265.5 - 3x16(3+1) = 12.09$$

from tables $\chi^2_{0.05,2} = 5.99$, $\chi^2_{0.01,2} = 9.21$

$\therefore \chi^2 r_{\alpha,2} > \chi^2_{0.01,1}$

∴ H_0 can be rejected at the 99% level of probability.

Therefore, the samples originated from different populations.

Comparison of Cores taken in October 1986

H_0 : All samples originated from the same population.

H_1 : The samples originated from different populations.

CORE 3.0		CORE 3.1	
No. of Cysts (cm^{-3})	Rank 1	No. of Cysts (cm^{-3})	Rank 2
4133	2	1609	1
9931	2	8276	1
1770	1	7287	2
1012	1	1241	2
1046	1	1701	2
1115	1	2759	2
1111	1	1874	2
1138	1	2023	2
977	1	1460	2
724	1	1402	2
1069	2	724	1
621	1	707	2
333	1	483	2
241	1	621	2
172	1	379	2
149	1	287	2
	19		29

$$\chi^2 r_{\alpha,1} = \frac{12}{Nk(k+1)} . \Sigma(Rj)^2 - 3N(k+1)$$

$$= \frac{12}{16x2(2+1)} .1202 - 3x16(2+1) = 6.25$$

from tables $\chi^2_{0.05,1} = 3.84$, $\chi^2_{0.01,1} = 6.64$

$\therefore \chi^2_{0.05,1} < \chi^2 r_{\alpha,1} < \chi^2_{0.01,1}$

$\therefore H_0$ can be rejected at the 95%, but not the 99%, level of probability.

Therefore, the samples cannot conclusively be shown to have originated from different populations.

APPENDIX 5.4 Comparison of the number of viable cysts in sediment cores from deep and shallow sites, using the Friedman Two-Way Analysis of Variance by Ranks

Comparison of Preserved Cores from the North Basin of Esthwaite Water

H_0 : All samples originated from the same population.

H_1 : The samples originated from different populations.

CORE 1.1		CORE 1.3	
No. of Cysts (cm^{-3})	Rank 1	No. of Cysts (cm^{-3})	Rank 2
98	1	112	2
432	2	335	1
1138	2	140	1
1757	2	391	1

1489	2	391	1
1685	2	752	1
2204	2	893	1
2092	2	725	1
1771	2	781	1
1980	2	697	1
1076	2	488	1
1729	2	195	1
1994	2	195	1
2008	2	56	1
	27		15

$$\chi^2 r_{\alpha,1} = \frac{12}{Nk(k+1)} . \Sigma(Rj)^2 - 3N(k+1)$$

$$= \frac{12}{14x2(2+1)} .954 - 3x14(2+1) = 10.3$$

from tables $\chi^2_{0.05,1} = 3.84$, $\chi^2_{0.01,1} = 6.64$

$\therefore \chi^2 r_{\alpha,1} > \chi^2_{0.01,1}$

$\therefore H_0$ can be rejected at the 99% level of probability.

Therefore, the samples originated from different populations.

Comparison of Unpreserved Cores from the North Basin of Esthwaite Water

H_0 : All samples originated from the same population.

H_1 : The samples originated from different populations.

CORE 1.0		CORE 1.2	
No. of Cysts (cm^{-3})	Rank 1	No. of Cysts (cm^{-3})	Rank 2
322	2	46	1
874	2	138	1
1448	2	69	1
1058	2	69	1
1172	2	92	1
966	2	69	1
2092	1	4851	2
6414	2	23	1
3908	2	23	1
1310	2	23	1
598	2	46	1
621	2	0	1
575	2	0	1
207	2	0	1
230	2	23	1
161	2	0	1
	31		17

$$\chi^2 r_{\alpha,1} = \frac{12}{Nk(k+1)} . \Sigma(Rj)^2 - 3N(k+1)$$

$$= \frac{12}{16x2(2+1)} . 1250 - 3x16(2+1) = 12.25$$

from tables $\chi^2_{0.05,1} = 3.84$, $\chi^2_{0.01,1} = 6.64$

$\therefore \chi^2 r_{\alpha,1} > \chi^2_{0.01,1}$

$\therefore H_0$ can be rejected at the 99% level of probability.

Therefore, the samples originated from different populations.

APPENDIX 5.5 Comparison of the number of viable cysts in preserved and unpreserved sediment cores, using the Friedman Two-Way Analysis of Variance of Ranks

Comparison of Deep Cores from the North Basin of Esthwaite Water

H_0 : All samples originated from the same population.

H_1 : The samples originated from different populations.

CORE 1.0		CORE 1.1	
No. of Cysts (cm^{-3})	Rank 1	No. of Cysts (cm^{-3})	Rank 2
328	2	98	1
874	2	432	1
1448	2	1138	1
1058	1	1757	2
1172	1	1489	2
966	1	1685	2
2092	1	2204	2
6414	2	2092	1
3908	2	1771	1
1310	1	1980	2
598	1	1076	2
621	1	1729	2
575	1	1994	2

207	1	2008	2
230	1	1067	2
161	1	798	2
	21		27

$$\chi^2 r_{\alpha,1} = \frac{12}{Nk(k+1)} . \Sigma(Rj)^2 - 3N(k+1)$$

$$= \frac{12}{16x2(2+1)} .1170 - 3x16(2+1) = 2.25$$

from tables $\chi^2_{0.05,1} = 3.84$, $\chi^2_{0.01,1} = 6.64$

$\therefore \chi^2 r_{\alpha,1} < \chi^2_{0.05,1}$

$\therefore H_0$ cannot be rejected at the 95% level of probability.

Therefore, the samples originated from the same population.

<u>Comparison of Shallow Cores from the North Basin of Esthwaite Water</u>

H_0 : All samples originated from the same population.

H_1 : The samples originated from different populations.

CORE 1.2		CORE 1.3	
No. of Cysts (cm^{-3})	Rank 1	No. of Cysts (cm^{-3})	Rank 2
46	1	112	2
138	1	335	2
69	1	140	2
69	1	390	2
92	1	390	2

69	1	725	2
4851	2	893	1
23	1	725	2
23	1	781	2
23	1	697	2
46	1	488	2
0	1	195	2
0	1	195	2
0	1	56	2
	15		27

$$\chi^2 r_{\alpha,1} = \frac{12}{Nk(k+1)} . \Sigma(Rj)^2 - 3N(k+1)$$

$$= \frac{12}{14x2(2+1)} .954 - 3x14(2+1) = 10.3$$

from tables $\chi^2_{0.05,1} = 3.84$, $\chi^2_{0.01,1} = 6.64$

$\therefore \chi^2 r_{\alpha,1} > \chi^2_{0.01,1}$

$\therefore H_0$ can be rejected at the 99% level of probability.

Therefore, the samples originated from different populations.

Comparison of Deep Cores from the South Basin of Esthwaite Water

H_0 : All samples originated from the same population.

H_1 : The samples originated from different populations.

CORE 2.1 No. Cysts	Rank 1	CORE 2.2 No. Cysts	Rank 2	CORE 2.3 No. Cysts	Rank 3	CORE 2.4 No. Cysts	Rank 4
828	3	3034	4	230	2	115	1
2483	3	3448	4	1287	2	414	1
3701	3	4713	4	3287	2	782	1
2759	2	4736	3	6046	4	759	1
3471	3	6368	4	1540	2	736	1
7885	4	3977	3	1586	2	483	1
9770	4	3149	3	851	2	759	1
6782	4	1632	3	598	1	805	2
3287	4	1747	3	506	1	644	2
2000	4	805	3	506	2	322	1
1448	4	1149	3	437	2	276	1
1218	4	942	3	299	2	230	1
1517	4	414	3	253	1	322	2
920	4	506	3	138	1	230	2
1264	4	391	2	437	3	253	1
988	4	345	3	253	2	207	1
	58		51		31		20

$$\chi^2 r_{\alpha,1} = \frac{12}{Nk(k+1)} . \Sigma(Rj)^2 - 3N(k+1)$$

$$= \frac{12}{16x4(4+1)} .7326 - 3x16(4+1) = 34.7$$

from tables $\chi^2_{0.01,3} = 11.34$

$\therefore \chi^2 r_{\alpha,3} > \chi^2_{0.01,3}$

$\therefore H_0$ can be rejected at the 99% level of probability.

Therefore, the samples originated from different populations.

APPENDIX 5.6 **Estimation of the 95% Confidence Limits for *C.furcoides* cysts**

The confidence interval provides a measure of sampling error and as such the reliability of a count. Variation is assessed by the variance of the data, and in order to estimate this, replicate counts are generally required. However, in this example, the probability distribution of the counts can be regarded as binomial (Moore and Webb, 1978). Therefore, the variance can be estimated for the cyst counts for each core section. Limits give an indication of the true percentage on 95 out of 100 occasions.

$$95\% \text{ Con. Limits} = \frac{\hat{p} + \left[\frac{(1.96)^2}{2n}\right] \pm 1.96\sqrt{\left[\frac{\hat{p}(1-\hat{p})}{n}\right] + \left[\frac{(1.96)^2}{4n}\right]}}{1 + \left[\frac{(1.96)^2}{n}\right]}$$

where $\hat{p} = \frac{x}{n}$

x = no. of *C. furcoides* cysts

n = total no. of cysts

APPENDIX 5.7 Comparison of the proportion of C. furcoides cysts from similar cores using the Friedman Two-Way Analysis of Variance by Ranks

Comparison of Preserved Cores from the South Basin of Esthwaite Water

H_0 : All samples originated from the same population.

H_1 : The samples originated from different populations.

CORE 2.1		CORE 2.2	
% C. furcoides	Rank 1	% C. furcoides	Rank 2
53	1	65	2
74	2	54	1
66	2	50	1
55	2	51	1
63	2	49	1
40	1	41	2
37	1	40	2
32	1	36	2
43	2	32	1
42	2	34	1
46	2	26	1
35	1	40	2
54	2	50	1
35	1	45	2
43	2	38	1
30	2	27	1
	26		22

$$\chi^2 r_{\alpha,1} = \frac{12}{Nk(k+1)} . \sum(Rj)^2 - 3N(k+1)$$

$$= \frac{12}{16x2(2+1)} . 1160 - 3x16(2+1) = 1$$

from tables $\chi^2_{0.05,1} = 3.84$

$\therefore \chi^2 r_{\alpha,1} < \chi^2_{0.05,1}$

$\therefore H_0$ cannot be rejected at the 95% level of probability.

Therefore, the samples originated from the same population and any variation in the proportion of C. furcoides between the two cores is statistically insignificant.

Comparison of Unpreserved Cores from the South Basin of Esthwaite Water

H_0 : All samples originated from the same population.

H_1 : The samples originated from different populations.

CORE 2.3		CORE 2.4	
% C. furcoides	Rank 1	% C. furcoides	Rank 2
100	2	40	1
51	1	56	2
52	1.5	52	1.5
57	2	52	1
52	1.5	52	1.5
56	2	43	1
56	2	53	1
50	1	68	2

48	1	58	2
36	1	43	2
37	1	38	2
38	1	43	2
54	2	43	1
40	2	33	1
47	1	62	2
36	1	57	2
	23.0		25.0

$$\chi^2 r_{\alpha,1} = \frac{12}{Nk(k+1)} . \Sigma (Rj)^2 - 3N(k+1)$$

$$= \frac{12}{16x2(2+1)} .1154 - 3x16(2+1) = 0.25$$

from tables $\chi^2_{0.05,1} = 3.84$

$\therefore \chi^2 r_{\alpha,1} < \chi^2_{0.05,1}$

$\therefore H_0$ cannot be rejected at the 95% level of probability.

Therefore, the samples originated from the same population and any variation in the proportion of C. furcoides between the two cores is statistically insignificant.

Comparison of Preserved and Unpreserved Cores from the South Basin of Esthwaite Water

H_0 : All samples originated from the same population.

H_1 : The samples originated from different populations.

CORE 2.1		CORE 2.2		CORE 2.3		CORE 2.4	
% C.f.	Rank 1	% C.f.	Rank 2	% C.f.	Rank 3	% C.f.	Rank 3
53	2	65	3	100	4	40	1
74	4	54	2	51	1	56	3
66	4	50	1	52	2.5	52	2.5
55	3	51	1	57	4	52	2
63	4	49	1	52	2.5	52	2.5
40	1	41	2	56	4	43	3
37	1	40	2	56	4	53	3
32	1	36	2	50	3	68	4
43	2	32	1	48	3	58	4
42	3	34	1	36	2	43	4
46	4	26	1	37	2	38	3
35	1	40	3	38	2	43	4
54	3.5	50	2	54	3.5	43	1
35	2	45	4	40	3	33	1
43	2	38	1	47	3	62	4
30	2	27	1	36	3	57	4
	39.5		28.0		46.5		46.0

$$\chi^2 r_{\alpha,1} = \frac{12}{Nk(k+1)} . \Sigma(Rj)^2 - 3N(k+1)$$

$$= \frac{12}{16x4(4+1)} .6622.5 - 3x16(4+1) = 8.34$$

from tables $\chi^2_{0.05,3} = 7.82$, $\chi^2_{0.01,3} = 11.34$

$\therefore \chi^2_{0.05,3} < \chi^2 r_{\alpha,3} < \chi^2_{0.01,3}$

$\therefore H_0$ can be rejected at the 95%, but not at the 99%, level of probability.

Therefore, the samples cannot conclusively be shown to have originated from different populations.

APPENDIX 5.8 Comparison of the number of *Stephanodiscus parvus* frustules from cores taken on the same occasion, using the Friedman Two-Way Analysis of Variance by Ranks

Comparison of Cores taken from Esthwaite Water in May 1985

H_0 : All samples originated from the same population.

H_1 : The samples originated from different populations.

CORE 1.0		CORE 1.1	
No. frustules	Rank 1	No. frustules	Rank 2
12	1	81	2
69	2	63	1
189	2	45	1
60	1	72	2
479	2	150	1
145	2	84	1
87	1	108	2
319	2	102	1
234	2	129	1
290	2	138	1

158	2	132	1
59	1	138	2
129	1	135	2
312	2	84	1
6	1	99	2
23	2	21	1
	26		22

$$\chi^2 r_{\alpha,1} = \frac{12}{Nk(k+1)} . \Sigma(Rj)^2 - 3N(k+1)$$

$$= \frac{12}{16x2(2+1)} .1160 - 3x16(2+1) = 1$$

from tables $\chi^2_{0.05,1} = 3.84$

$\therefore \chi^2 r_{\alpha,1} < \chi^2_{0.05,1}$

$\therefore H_0$ cannot be rejected at the 95% level of probability.

Therefore, the samples originated from the same population.

Comparison of Cores taken from Esthwaite Water in March 1986

H_0 : All samples originated from the same population.

H_1 : The samples originated from different populations.

CORE 2.1		CORE 2.2	
No. frustules	Rank 1	No. frustules	Rank 2
33	1	132	2
30	1	90	2
66	1	222	2

138	1	330	2
513	2	258	1
990	2	414	1
477	2	387	1
483	2	288	1
420	2	54	1
402	2	102	1
384	2	27	1
456	2	90	1
558	2	30	1
348	2	12	1
159	2	36	1
117	2	24	1
	28		20

$$\chi^2 r_{\alpha,1} = \frac{12}{Nk(k+1)} . \Sigma(Rj)^2 - 3N(k+1)$$

$$= \frac{12}{16x2(2+1)} .1184 - 3x16(2+1) = 4$$

from tables $\chi^2_{0.05,1} = 3.84$, $\chi^2_{0.01,1} = 6.64$

$\therefore \chi^2_{0.05,1} < \chi^2 r_{\alpha,1} < \chi^2_{0.01,1}$

$\therefore H_0$ can be rejected at the 95%, but not the 99%, level of probability.

Therefore, the samples cannot conclusively be shown to have originated from different populations.

Comparison of Cores taken from Esthwaite Water in October 1986

H_0 : All samples originated from the same population.

H_1 : The samples originated from different populations.

CORE 3.0		CORE 3.1	
No. frustules	Rank 1	No. frustules	Rank 2
42	2	15	1
132	2	84	1
186	2	108	1
147	1	363	2
174	1	324	2
249	2	162	1
234	2	228	1
288	1	324	2
273	2	117	1
186	1	312	2
315	2	111	1
246	2	114	1
243	1.5	243	1.5
147	2	135	1
126	2	84	1
93	2	57	1
	27.5		20.5

$$\chi^2 r_{\alpha,1} = \frac{12}{Nk(k+1)} . \Sigma(Rj)^2 - 3N(k+1)$$

$$= \frac{12}{16x2(2+1)} .1176.5 - 3x16(2+1) = 3.1$$

from tables $\chi^2_{0.05,1} = 3.84$

$\therefore \chi^2 r_{\alpha,1} < \chi^2_{0.05,1}$

$\therefore H_0$ cannot be rejected at the 95% level of probability.

Therefore, the samples originated from the same population.

Comparison of All Cores from Esthwaite Water

H_0 : All samples originated from the same population.

H_1 : The samples originated from different populations.

CORE 1.0	CORE 1.1	CORE 2.1	CORE 2.2	CORE 3.0	CORE 3.1
1	5	3	6	4	2
3	2	1	5	6	4
5	1	2	6	4	3
1	2	3	5	4	6
5	1	6	3	2	4
2	1	6	5	4	3
1	2	6	5	4	3
4	1	6	2.5	2.5	5
4	3	6	1	5	2
4	2	6	1	3	5
4	3	6	1	5	2
1	4	6	2	5	3
2	3	6	1	4.5	4.5
5	2	6	1	4	3
1	4	6	2	5	3
2	1	6	3	5	4
45.0	37.0	81.0	49.5	67.0	56.5

In order to accommodate the table on one page only the ranked data is shown.

$$\chi^2 r_{\alpha,1} = \frac{12}{Nk(k+1)} . \Sigma(Rj)^2 - 3N(k+1)$$

$$= \frac{12}{16x6(6+1)} . 20086.5 - 3x16(6+1) = 22.69$$

from tables $\chi^2_{0.05,5} = 11.07$, $\chi^2_{0.01,5} = 15.09$

$\therefore \chi^2 r_{\alpha,1} > \chi^2_{0.01,5}$

$\therefore H_0$ can be rejected at the 99% level of probability.

Therefore, the samples originated from different populations.

APPENDIX 5.9 Comparison of the proportion of C. furcoides cells from net samples taken in three separate years from Esthwaite Water using Spearman's Rank Correlation Coefficient

1. 1972

Date (1)	% C. furcoides (2)	Rank 1	Rank 2	d	d^2
25-4	96	1	1.5	0.5	0.25
23-5	96	2	1.5	0.5	0.25
27-6	99	3	4.5	1.5	2.25
25-7	97	4	3.0	1.0	1.00
22-8	99	5	4.5	0.5	0.25
19-9	100	6	6.5	0.5	0.25
3-10	100	7	6.5	0.5	0.25
					4.50

$$\Sigma x^2 = \frac{N^3-N}{12} - \Sigma Tx = \frac{7^3-7}{12} - 0 = 28$$

$$\Sigma y^2 = \frac{N^3-N}{12} - \Sigma Ty = \frac{7^3-7}{12} - \frac{3(2^3-2)}{12} = 26.5$$

$$r_s = \frac{\Sigma x^2+\Sigma y^2-\Sigma d^2}{2\sqrt{\Sigma x^2 . \Sigma y^2}} = \frac{28+26.5-4.5}{2\sqrt{28x26.5}} = 0.92$$

Degrees of freedom = N-2 = 5

from tables $r_{s0.05,5} = 0.9$

since $r_{s\alpha,5} > r_{s0.05,5}$, the calculated value of r_s can be regarded as statistically significant at the 95% level of probability.

2. 1977

Date (1)	% C. furcoides (2)	Rank 1	Rank 2	d	d^2
23-3	33	1	9	8	64
12-4	58	2	10	8	64
10-5	17	3	4	1	1
14-6	14	4	2	2	4
12-7	17	5	4	1	1
16-8	21	6	7	1	1
3-9	17	7	4	3	9
20-9	18	8	6	2	4
27-9	13	9	1	8	64
18-10	32	10	8	2	4
					216

Since no tied ranks were involved the following formula was used:-

$$r_s = 1 - \frac{6\Sigma d^2}{n(n^2-1)} = 1 - \frac{6x216}{10(100-1)} = -0.31$$

Degrees of freedom = n-2 = 8

from tables $r_{s0.05,8} = 0.643$

$r_{s\alpha,8} < r_{s0.05,8}$

The difference between the proportion of C. furcoides cells through the year is not statistically significant at the 95% level of probability.

3. 1982

Date (1)	% C. furcoides (2)	Rank 1	Rank 2	d	d^2
20-4	50	1	5.5	4.5	20.25
18-5	54	2	7.0	5.0	2.50
15-6	60	3	8.5	5.5	30.25
29-6	43	4	1.0	3.0	9.00
13-7	45	5	2.5	2.5	6.25
17-8	50	6	5.5	0.5	0.25
7-9	48	7	4.0	3.0	9.00
14-9	60	8	8.5	0.5	0.25
5-10	45	9	2.5	6.5	42.50
					142.50

$$\Sigma x^2 = \frac{N^3-N}{12} - \Sigma Tx = \frac{9^3-9}{12} - 0 = 60$$

$$\Sigma y^2 = \frac{N^3-N}{12} - \Sigma T y = \frac{9^3-9}{12} - 3\frac{(2^3-2)}{12} = 58.5$$

$$r_s = \frac{\Sigma x^2 + \Sigma y^2 - \Sigma d^2}{2\sqrt{\Sigma x^2 . \Sigma y^2}} = \frac{60+58.5-142.5}{2\sqrt{60 \times 58.5}} = -0.20$$

Degrees of freedom = N-2 = 7

from tables $r_{s0.05,7} = 0.714$

since $r_{s\kappa,7} < r_{s0.05,7}$, the calculated value of r_s cannot be regarded as statistically significant at the 95% level of probability.

APPENDIX 5.10 The 95% Confidence Interval of *C. furcoides* cells in net samples from Esthwaite Water

Year	% *C. furcoides*	95% Confidence Limits	
		Upper Limit	Lower Limit
1946	65	72	57
1947	49	56	41
1948	32	51	42
1949	25	28	22
1950	28	33	23
1951	31	36	25
1952	24	28	20
1953	32	36	27
1954	32	37	27
1955	38	41	33
1956	26	32	22
1957	23	29	19

1958	17	19	11
1959	23	28	18
1960	39	45	32
1961	39	46	33
1962	77	82	71
1963	51	55	46
1964	97	98	95
1965	96	98	91
1966	88	91	84
1967	99	100	97
1968	77	81	73
1969	98	99	95
1970	96	98	93
1971	-	-	-
1972	99	100	97
1973	95	97	90
1974	30	32	25
1975	21	27	17
1976	13	17	10
1977	18	21	15
1978	21	23	19
1979	17	20	14
1980	19	22	16
1981	26	29	23
1982	48	52	44
1983	57	60	54
1984	95	97	92

1985	88	91	85
1986	70	75	64

CHAPTER 6

THE EXCYSTMENT OF C. FURCOIDES and C. HIRUNDINELLA IN THE LABORATORY

Introduction

In temperate lakes and reservoirs the overwintering cysts of Ceratium excyst during the spring to produce motile cells. It has yet to be determined whether excystment occurs in the surface sediment or following the resuspension of cysts into the water column (see Chapter 5, page 150). Early workers (Huber and Nipkow, 1922; Entz, 1931) collected cysts and induced them to germinate in the laboratory by raising the ambient temperature. Their light microscopy observations were later confirmed and expanded by an electron microscopy study (Chapman, Livingstone and Dodge, 1981).

Cysts preparing to excyst can be identified by the presence of brown colouration throughout and Brownian movement at the base of the horns. The first stage of cell development (gymnoceratium) forms within the cyst before emerging through a slit-like aperture. At this stage the cingulum is shallow, diagonally encircling the cell. During the following two hours the cell assumes a more "triangular" shape, the cingulum becomes more distinct and the horns start to develop (preceratium). In the late preceratium stage thecal plates can be distinguished.

Newly formed Ceratium cysts require a period of dormancy before germination can occur (Huber and Nipkow, 1923; Livingstone, 1979). The main factor initiating excystment in

dinoflagellates appears to be an increase in temperature. The threshold temperature, responsible for triggering excystment, is species specific (Anderson, in a paper presented at the "Dino III" Conference on modern and fossil dinoflagellates, Egham, Surrey, 1985). Huber and Nipkow (1923) and Heaney, Chapman and Morison (1983) showed that Ceratium cysts from temperate lakes germinated in the laboratory once temperatures had exceeded 4°C. Higher temperatures enabled excystment to occur more rapidly. At 7-9°C germination took 7 days, whilst at 23-26°C, the optimum temperature, only 36 hours was required. At higher and lower temperatures abnormalities in cell morphology, including split horns, have been shown to occur in Ceratium (e.g. Huber and Nipkow, 1923). Similar divided posterior horns were noted by Okada (1933) in natural populations of C. hirundinella cells found in Hokkaido, Japan.

Sako, Ishida, Kadota and Hata (1985) observed a much higher temperature threshold value, 15°C, for excystment in Peridinium cunningtonii. The optimum temperature for germination was shown to be 22°C, initiating excystment in 90% of cysts. At 18°C and 26°C excystment success was lower, 30% and 44% respectively. Eren (1969) showed that excystment in Peridinium cinctum occurred at temperatures of 15°C or greater (no upper limit was given). Anderson (1980) stored Gonyaulax tamarensis cysts at 5°C and 22°C prior to incubation at 15°C. Cysts kept at a constant temperature remained dormant, indicating that a temperature change is more important than a temperature increase. However, it is possible that anaerobic

conditions in the sediment inhibited the earlier growth of cysts stored at 22°C.

In tropical lakes, and in temperate lakes where the temperature never falls below 4°C, the situation is different. Temperature may determine the time of excystment relative to the period between cyst formation and maturation (Heaney, Lund, Canter and Gray, 1988). In Lake Kinneret, Israel, where summer temperatures exceed 26°C, C. hirundinella forms oversummering cysts (Pollingher, 1986a).

Unlike some dinoflagellates, Ceratium cysts do not require light to germinate (Huber and Nipkow, 1923; Krupa, 1981a). In Scrippsiella trochoidea this requirement can be fulfilled by low photon fluences (0.2 μm lm^{-2}) with an exposure time as short as one second, with green light producing the best response (Binder and Anderson, 1986). Anderson, Taylor and Armbrust (1987) demonstrated that the cysts of Gonyaulax polyedra also require light before excystment will occur. Three other species G. tamarensis, G. verior and Scrippsiella sp. (undetermined), germinated more rapidly in the light, but were still able to excyst in darkness. One species, G. rugosum, was unaffected by light conditions. Sako, Ishida, Kadota and Hata (1985) reported that germination in Peridinium cunningtonii was similarly independent of the light regime.

Anaerobic conditions have also been cited as adversely affecting excystment. Huber and Nipkow (1923) successfully germinated C. hirundinella from culture medium stripped of oxygen and carbon dioxide. However, excystment took twice as

long, four days instead of the two days in aerobic conditions. Krupa (1981a) observed that the addition of "hydrogen sulphide water" to sediment did not impair the ability of cysts to germinate. However, Anderson, Taylor and Armbrust (1987) demonstrated that germination in all five species studied (G. polyedra, G. tamarensis, G. rugosum, G. verior and Scrippsiella sp.) was inhibited by anaerobiosis. The authors suggested that atmospheric oxygen may not have been totally excluded in the previous studies.

In recent years the existence of a sexual life cycle has been confirmed in 10 of the approximately 230 species of freshwater dinoflagellates (Hickel, 1988a). In most studies sexual stages were induced using nitrogen - deficient culture medium, or short light period (e.g. Pfiester, 1975, 1976), as detailed in Chapter 5 (page 122). The topic was comprehensively reviewed by Beam and Himes (1980).

Figure 6.1 (from Dale, 1983) shows a schematic diagram of the basic sexual cycle of dinoflagellates producing cysts as hypnozygotes. The motile vegetative cell stage (1) produces gametes (2), which fuse to form a planozygote (3). In time, the planozygote loses its motility and a cyst is formed within the cell (4). The cyst (hypnozygote) expands causing the disintegration of the cell (5), and sinks to the sediment surface (6). Excystment (7) may produce a planozygote (8), comparable with (3), which by a reduction division re-establishes the vegetative cell stage (1). In other species the reduction division is completed during encystment, and so

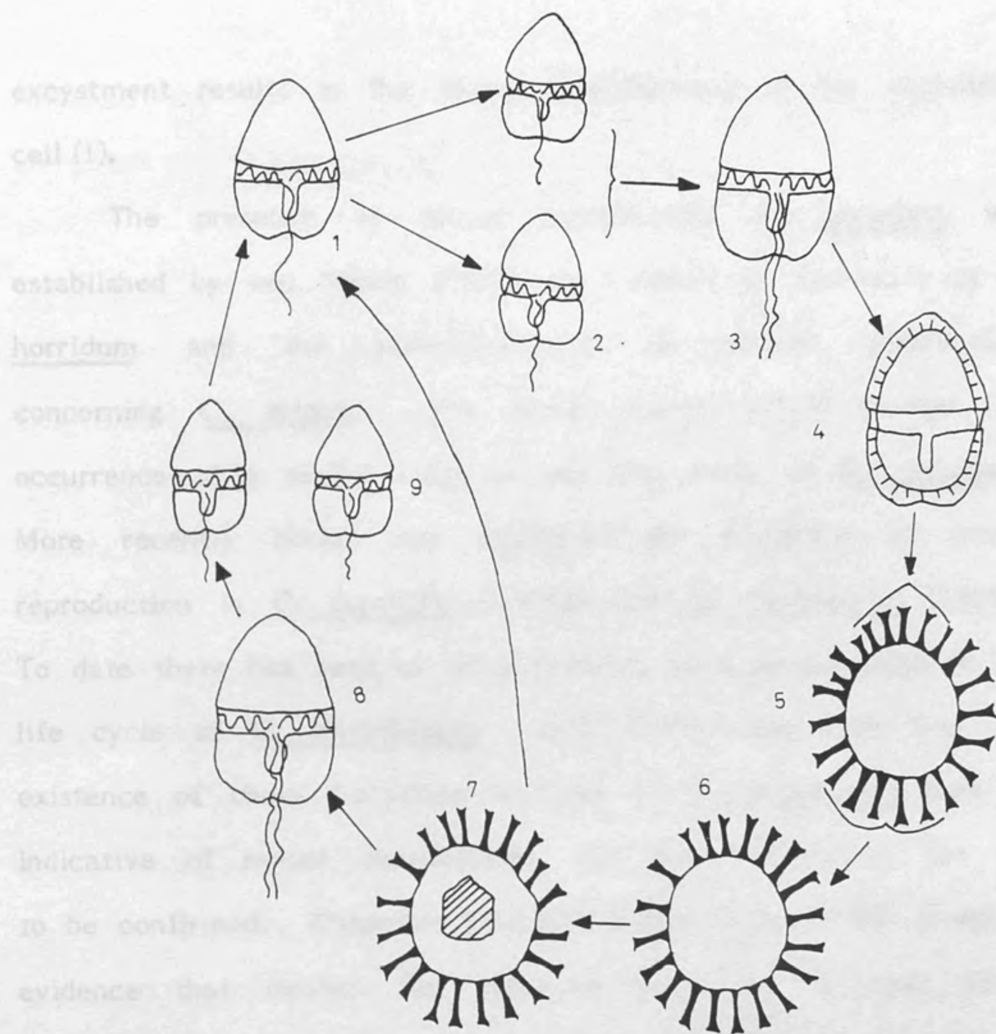


Figure 6.1 The Basic Dinoflagellate Sexual Cycle (from Dale,1983)

excystment results in the direct establishment of the vegetative cell (1).

The presence of sexual reproduction in Ceratium was established by von Stosch (1964), as a result of the study of C. horridum and the reinterpretation of earlier observations concerning C. tripos. The same author (1965) showed the occurrence of a sexual stage in the life cycle of C. cornutum. More recently Hickel has described the existence of sexual reproduction in C. furcoides (1988a) and C. rhomvoides (1988b). To date there has been no firm evidence of a sexual stage in the life cycle of C. hirundinella. Entz (1924) suggested that the existence of chain formation in cells of C. hirundinella may be indicative of sexual reproduction, but this observation has yet to be confirmed. Chapman, Livingstone and Dodge (1981) found no evidence that meiosis had occurred prior to, or soon after, excystment in Ceratium. Only single cells were observed to emerge from cysts. These authors studied only "granular walled cysts" (see Chapter 4, page 104).

Excystment has been used by some authors to establish the relationship between living and fossil dinoflagellates (e.g. Wall and Dale, 1968) and could be used for taxonomic purposes to distinguish between species with similar cysts and different motile stages.

One of the objectives of this study was to establish how long the cysts remained viable in the sediment, using excystment experiments in conjunction with the estimates of the sedimentation rate obtained in Chapter 5 (page 158). In

addition, the relative germination success of C. furcoides was compared with C. hirundinella.

Methods

Initial experiments were undertaken to compare the excystment of C. furcoides and C. hirundinella. Individual cysts were isolated from sectioned sediment cores from Esthwaite Water, which had been stored at 4°C to prevent premature excystment. Cysts were placed in microslides or petri dishes, to which culture medium had been added and transferred to the culture room, where temperature (20°C) and light regime (24 hour light) could be maintained. Observations were made daily for up to two weeks, or until fungal growth had engulfed the cyst.

In order to establish the viability of cysts with depth, sediment/culture medium slurries were set up. Sub-samples of 1 cm^3 of diluted sediment were withdrawn from alternate 0.5 cm depth intervals of the core from Esthwaite Water, and transferred to the culture room. Daily observations were made and the number of motile cells were noted. This technique, although quick to set up, had the disadvantage of not indicating the initial number of cysts, or the species composition. Therefore, cysts of each species were isolated from the sediment and transferred into small petri dishes containing culture medium. Cysts of different species and from different depths of sediment were kept separate (details in Chapter 2, page 58).

An additional experiment assessed the ability of cysts to remain viable following desiccation. Sub-samples of 1 cm^3 of

diluted sediment were introduced into petri dishes and exposed to the air for several days until the sample appeared dry. Culture medium was added and observations made over the course of the subsequent fortnight.

Results

Germination of the cysts of both C. furcoides and C. hirundinella was achieved. In both species an average of 3 days elapsed before mature motile cells were observed. Greater difficulty was experienced in obtaining motile cells of C.hirundinella. Excystment occurred, as in C. furcoides, but development frequently failed to progress beyond the gymnoceratium stage, with cells dying before reaching maturity. This phenomenon was observed only once in C. furcoides.

The germination of cysts of both C. hirundinella and C. furcoides was achieved from sub-surface sediment. Table 6.1 shows that in slurry experiments the motile cells of C. furcoides were observed to a depth of 5.0-5.5 cm. It is possible that below 5.5 cm cysts remained viable but required a longer period of time to excyst. The germination time was approximately the same at all depths. No C. hirundinella motile cells were observed. It is possible that C. hirundinella cysts may have germinated, but failed to mature. The large amount of sediment in the samples precluded the observation of the gymnoceratium stage.

Several other organisms successfully grew from slurries including diatoms, colonial Chlorophyceae and Pediastrum. Cysts

Table 6.1 Germination of dinoflagellate cysts in sediment culture slurries

Species	Core Section (0.5 cm Intervals)															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1.	+	+	+	+	+	+	+	+	-	+	+	-	-	-	-	-
2.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3.	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-

where species 1. = C. furcoides
2. = C. hirundinella
3. = Peridinium cinctum

Table 6.2 % Germination of isolated Ceratium cysts from the upper 5.0 cm of sediment cores

Species	Maximum Depth of Core Section (cm)									
	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0
1.	100	50	50	-	100	33	100	50	-	50
2.	-	-	-	-	-	-	-	-	-	-

where species 1. = C. furcoides
2. = C. hirundinella

of Peridinium cinctum excysted to a depth of 5.0-5.5 cm. Chlamydomonas was observed to grow in sediment from a depth of 7.0-7.5 cm, often in very large numbers.

In the experiments involving the isolated cysts, C. hirundinella failed to germinate (see Table 6.2). Cysts in core sections below 5.0 cm failed to form motile cells, and are therefore not included in the table. One C. furcoides cyst germinated from the 7.0-7.5 cm section of the core, but did not develop beyond the gymnoceratium stage. Motile cells were produced as a result of excystment, to a depth of 4.0-4.5 cm.

In general, it was not possible to continue the germination experiments for longer than a week, when fungal growth set in. However, on one occasion a culture was maintained for 18 days. The cyst germinated within 5 days and the motile cell thus produced divided within a further 4 days. Ten days after the excystment of the initial cell, 7 motile cells had been formed, an average doubling time of 3 days. After another 4 days, during which no further division occurred, the culture medium was renewed, however, cells still failed to divide.

All cysts failed to germinate following air drying of the sediment. The experiment was repeated with the same result. It is possible that the comparatively thin, mainly cellulose, cyst walls (Entz, 1925; Pearsall, 1929; Wall and Evitt, 1975; Evitt, 1985) were unable to withstand the pressure imposed by dehydration. Alternatively the cyst protoplast may have been rendered unviable during the period of air drying. It may be

that the cysts remained viable but required longer periods in favourable conditions before germination could occur. It was not possible to make observations after 14 days due to extensive fungal growth.

Germination did not always occur when isolated cysts were introduced into a culture medium and provided with constant light and temperature conditions. In some cases the cysts concerned may not have been viable. When selecting cysts those in which Brownian motion occurred in the cytoplasm at the base of the horns were selected preferentially, as an indication of viability (Chapman, Livingstone and Dodge, 1981). However, in some samples particularly in the lower sections of the core, the number of cysts were so low that all observed "full" cysts were used.

Discussion

Germination success is difficult to assess for these experiments. In the upper core sections, where cysts were numerous, those most likely to excyst were preferentially selected. Lower down the core, where few cysts were present, all full cysts were included, whether or not they displayed Brownian motion. Under natural conditions not all the viable cysts from the previous year would be expected to germinate. Heaney, Chapman and Morison (1983) showed that some viable cysts remain in the sediment for longer than one year.

The difficulty in obtaining mature motile cells of C. hirundinella from cysts compared to C. furcoides suggests some

difference in requirements, either physical or chemical. An increase in temperature triggers excystment in both species. However, in C. hirundinella maturation of motile cells does not always continue beyond the gymnoceratium stage. This suggests either additional nutrient requirements or a greater intolerance of "in vitro" conditions. Heaney and Jaworski (personal communication) have been unable to maintain C. hirundinella in culture, despite growing C. furcoides in culture for a number of years (Chapter 4, page 106).

The failure of cysts to germinate from air-dried sediment confirms the observation of Huber and Nipkow (1922 or possibly 1923) as reported by Livingstone (1979). They observed that cysts were unable to withstand drought or long periods of frost. Evans (1958, 1959) conducted drying experiments and made observations on a large number of algae. Motile cells of Glenodinium pulvisculus, which had survived in an encysted form, were noted 8 weeks after the rewetting of an air-dried sample. It was deduced that longer periods spent as a cyst required an extended amount of time for germination once conditions were favourable. Motile cells of Peridinium were also observed, but there was no evidence that their cysts were drought resistant.

The long-term viability of Ceratium cysts within the sediment has been open to question (see also Chapter 5, page 146). Huber and Nipkow (1922) germinated cysts from the varved sediments of Lake Zurich, Switzerland. They observed excystment to occur in C. hirundinella cysts of up to 6½ years old, whilst cysts of Peridinium cinctum remained viable for 16½

years. Wall and Evitt (1975) sought to confirm the potential life expectancy of Ceratium cysts by determining the depth at which cysts were no longer found in the varved sediment of the same lake. Livingstone (1979) noted that only 7% of cysts (full and empty), from various lake basins, including Esthwaite Water, occurred below the upper 3 cm of the sediment. In Esthwaite Water he observed no viable cysts below 5 cm. Only cysts from the upper few centimetres of the core were germinated.

In this study apparently viable Ceratium cysts (i.e. those which fluoresced when excited with blue light) were present throughout the entire 8 cm of the sediment cores, although less numerous below 4 cm (see Chapter 5, pages 132-137). However, the successful germination of Ceratium and Peridinium cysts was almost completely confined to the upper 5.5 cm of the core. Pennington (1978) assessed that the rate of sedimentation in Esthwaite Water was equivalent to 10 cm in 11 years, a mean accumulation of 0.9 cm each year (see Chapter 5). Thus the upper 5.5 cm of the core would represent 6 years deposition. An investigation of diatom stratigraphy, included in the present work, indicated an average sedimentation rate in Esthwaite Water of 8.8 cm in 11 years, representing an annual increment of 0.8 cm (see Chapter 5, page 165). Therefore, cysts would appear to be able to germinate between 6 years (based on Pennington's observations) and 7 years (based on diatom stratigraphy in this study). Both these figures are subjective and liable to some errors. However, they correspond well with the figures of 6½ years obtained by Huber and Nipkow (1922) from varved

sediments, which can be reliably dated.

Full and apparently still viable cysts were present below the 5.5 cm level, although fewer in number (Chapter 5, pages 132-137), suggesting that many had decomposed. The failure of these cysts to germinate may be due to a requirement for a longer period prior to excystment. However, this appears unlikely since all cysts above 5.5 cm germinated within the same period of time (3-4 days). Cysts at this depth may require a stronger "trigger" to initiate germination. It is more probable that cysts below this depth had lost the ability to germinate, whilst retaining viable chloroplasts. Other apparently full cysts may have become unviable as a consequence of parasitism (see Chapter 5, page 195).

CHAPTER 7

THE POPULATIONS OF C. FURCOIDES AND C. HIRUNDINELLA IN THAMES VALLEY SITES

7.1 Periodicity of Phytoplankton in a Thames Valley Reservoir Preceding and Following Water Drainage

Introduction

Reservoirs serve two main functions, that of direct supply and storage prior to water purification (Ridley, 1970; Steel, 1975), and that of river regulation, where flow is seasonal. In addition reservoirs can provide a valuable source of hydro-electric power (e.g. Llyn Celyn, Gwynedd), can be used for fishing and other recreational pursuits (e.g. Queen Mother Reservoir), and provide valuable roosting and breeding sites for water-fowl (e.g. Staines South Reservoir).

Reservoirs can be separated into three groups on the basis of their construction. Impounding reservoirs (e.g. Thirlmere), are formed by constructing a dam across an existing watercourse, retaining and controlling the flow. Direct supply reservoirs of this type are generally situated in upland areas, from which the water is transferred to the demand area by aqueduct. Impoundment reservoirs used for river regulation and compensation are much scarcer. All their water is released into the river in a controlled manner in order to overcome low summer flows. During the winter, in their partly drained state, these reservoirs are able to accommodate flood water.

Pumped storage reservoirs (e.g. Queen Mary Reservoir), are

used almost exclusively for direct supply. They are purpose built to accommodate water from a nearby river, or other source, and store it prior to pumping to a water treatment plant.

A third category of reservoir are those formed by an estuarine barrage (e.g. once proposed for Morecambe Bay). These reservoirs are still rare in this country, partly because of the miles of aqueducts or underground mains required to distribute impounded water to areas of shortage and partly due to their high initial cost.

All Thames Valley sites sampled in the present study are pumped storage reservoirs located in the lower Thames Valley (Figure 3.2, page 67). The design of the reservoirs utilises the water holding capacity of London clay (Ridley, 1964; Steel, 1975). Ballast removed from the site is formed into a continuous embankment enclosing a central region of clay from the same site. The clay extends from below ground level to near to the top of the embankment, thus uniting it with the underlying clay. These inner slopes are protected from erosion by concrete slabs in the recent reservoirs (e.g. Queen Mother Reservoir) and by small stone blocks in the older reservoirs (e.g. Island Barn Reservoir). The outer slopes of the embankment are generally covered with top soil and turfed. Reservoirs are filled from the River Thames, the water being retained for between 10 days to many months before being pumped to the treatment works. The water is then subjected to rapid sand filtration or microstraining followed by slow sand filtration and chlorination.

The ever increasing demand for water by industrial and domestic consumers in the London area has led to the construction of three new reservoirs (Queen Elizabeth II, Queen Mother and Wraysbury Reservoirs) within the last 26 years. The high cost and limited availability of land in the region, coupled with the need for increased capacity has resulted in these recent reservoirs being deeper than those built previously (Table 7.1).

Table 7.1 Details of the key reservoirs in the Thames Valley (from Steel, 1975)

Reservoir	Completed	Area	Mean Depth	Maximum Depth	Vol.
		ha	m	m	$10^6 m^3$
Queen Mother	1974	192	19.3	22.9	38.0
Wraysbury	1971	202	16.8	21.6	33.9
Queen Elizabeth II	1962	128	15.3	17.8	19.6
King George VI	1947	142	14.2	16.0	20.2
Queen Mary	1925	286	10.6	11.6	30.4
Island Barn	1911	49	7.6	9.2	3.7
Bessborough	1907	30	10.3	12.8	3.1
Knight	1906	21	10.0	12.8	2.1
Staines South	1902	100	7.9	9.0	7.9
Staines North	1902	72	10.0	11.9	7.2

In these three reservoirs submerged jetting systems were installed during construction in order to impose a circulatory system of turbulence. Under certain circumstances growth of phytoplankton, particularly Cyanophyceae, can become very dense. Algal blooms may develop as a consequence of either the onset or the destruction of the thermocline. When algal growth becomes excessive, sand filters in the water treatment process may become blocked, toxic substances may be produced and an increased deoxygenation of the lower water layers may be induced by dead and decaying algal cells. In addition, an unpleasant odour may be imparted to the water, and discolouration of water and the production of an unsightly scum may result. A system of artificial water turbulence was introduced in an attempt to maintain a mixed water column throughout the year and to reduce blooms of certain phytoplankton, particularly Cyanophyceae and Dinophyceae (Ridley, 1964, 1970; Ridley, Cooley and Steel, 1966; Ridley and Symons, 1972; Steel, 1975). In some cases turbulence may reduce growth and prevent the formation of algal blooms (Ridley, Cooley and Steel, 1966; Lorenzen and Mitchell, 1975). In other situations it encourages growth, possibly by introducing substances to the photic zone that were previously absent or by the resuspension of cells into the photic zone. Toetz (1981) and Steinberg (1983) showed that algal biomass remained the same, or increased, after artificial destratification as a result of diatoms and cryptophytes replacing taxa that had been reduced in number. Reynolds, Wiseman, Godfrey and Butterwick (1983) observed that

Sphaerocystis and Anabaena in Blelham Tarn, Cumbria grew relatively faster when the water column was unmixed. Artificial mixing promoted the growth of diatoms (e.g. Fragilaria). Further work at the same site (Reynolds, Wiseman and Clarke, 1984) showed that Eudorina and Sphaerocystis declined in number during periods of artificial mixing, whilst although growth rates of Anabaena, Ceratium, Volvox and Microcystis were reduced, biomass was generally maintained. McGill (1969) studied the initial effects of the jetting systems installed in Queen Elizabeth II Reservoir, whilst Haffner (1974) and Hardy (1977) studied the phytoplankton populations in Wraysbury Reservoir, which was also subjected to the high or low velocity input of water from the River Thames. It was observed that mixing produced a vertically homogenous temperature and dissolved oxygen concentration profile and that on most occasions the distribution of phytoplankton was also homogenous. Resuspension of the non-productive components of the seston from the River Thames was shown to be an indirect result of mixing, increasing turbidity of the water column and thus limiting production.

In reservoirs with no artificial system for circulating water (e.g. Island Barn Reservoir) there is usually a natural development of a temperature gradient (thermocline) during the summer months and the supression of vertical water movements, resulting in the deoxygenation of the lower water layers. The surface of water bodies heats up more rapidly than the underlying water, especially during the summer months. Fast

flowing streams remain isothermal throughout the year as the water movement breaks down any temperature gradient. However in sluggish streams, and in lakes and reservoirs, wind and water movements are often insufficient during the warmer months of the year to completely break down this temperature gradient. Wind mixes the heated surface water layers downwards, but may not be strong enough to extend them to the bottom of the basin. This results in an upper warmer isothermal layer (epilimnion) of a few metres, or tens of metres, in depth, which floats on the cooler, denser, lower layer (hypolimnion).

A thermocline is detectable when differences between the two layers equal or exceed 1°C. Direct thermal stratification ceases when the water column is thoroughly mixed. In some shallow lakes, where a slight increase in wind action will remix the water layers, it may only be short lived (1-2 days). In other temperate lakes the condition persists for the warmer months of the year, whilst in tropical areas it may continue throughout the year. In temperate lakes the cooling of the water surface and increased wind activity associated with autumn combine to destroy any temperature gradients. The existence of a thermocline isolates water in the hypolimnion from the atmosphere. Photosynthetic oxygen production below the thermocline may become very low when the thermocline is located below the photic zone and numbers of healthy algae are reduced. However respiration of bacteria, associated with detritus or sediment, and benthic animals continues. Therefore the concentration of carbon dioxide is increased and that of oxygen

reduced, or completely depleted in some circumstances. In a reservoir, deoxygenation of the hypolimnion renders it unusable for supply.

The abundance and diversity of the phytoplankton population within the Thames Valley reservoirs contrasts markedly with that of the River Thames, from which their water levels are maintained. The phytoplankton of the river is dominated by diatoms in the spring and autumn and Chlorophyceae in the summer, usually in association with a low zooplankton population. In contrast, the storage reservoirs suppport the abundant and varied flora and fauna associated with an enriched lake (Ridley, 1970). Thames Valley reservoirs as bodies of water fed by a eutrophic river, provide the potential for high phytoplankton productivity.

The original aim of this investigation was to study the life cycle of the Ceratium population in a Thames Valley reservoir over a two year period, paying particular attention to the extent that excystment influences the abundance of the phytoplankton population. However, a succession of sites, some of which had been the subject of extensive study, were lost through temporary or permanent drainage, as a consequence of changes in Thames Water management policy and a particularly dry summer.

Island Barn Reservoir was selected as a suitable site (see Chapter 3, page 66), since it had supported a very large Ceratium population in the summer of 1984, prior to the lowering of the water level by several metres (see Chapter 3, page 69).

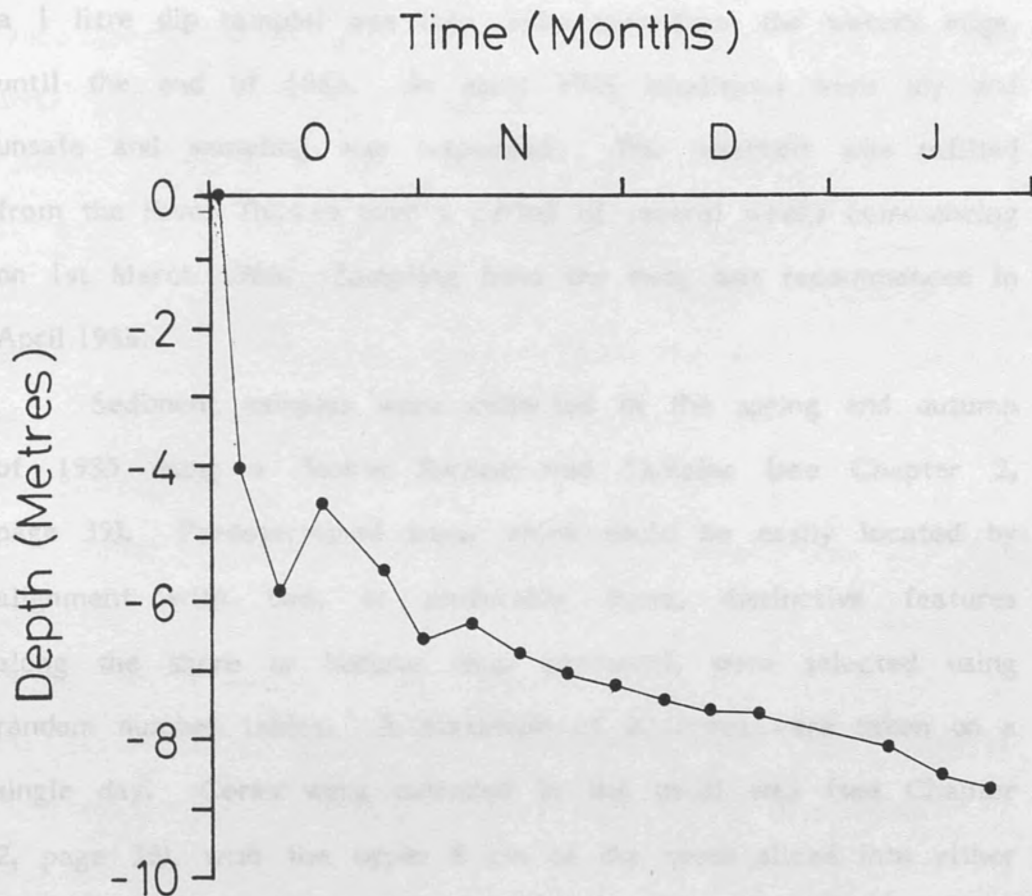
A sampling programme was initiated early in 1985. On 1st October 1985 Thames Water started draining this reservoir in order to undertake repairs. Initially the water level was lowered rapidly (Figure 7.1) from a valve located in the middle of the water column (4.5 metres below the surface). Subsequent drainage was from a valve close to the bottom of the water column. The centre of the reservoir remained covered by some water throughout. Refilling commenced on 1st March 1986 and by 1st April 1986 the reservoir was restored to full capacity. At this stage it was too late to change site again and it was decided to continue monitoring the reservoir and to examine the effect of drainage and subsequent refilling on the phytoplankton in general and on the Ceratium population in particular.

Methods

(i) Collection of Samples

The Island Barn Reservoir was sampled routinely over a two year period. Sampling was conducted at approximately the same time each week, in order to eliminate any variation due to diurnal changes. Weekly water samples, for nutrient analysis and determination of algal numbers, and vertical profiles of temperature and oxygen were taken from a marker buoy located towards the centre of the reservoir (sampling details in Chapter 2, page 23). Phytoplankton net tows were taken from the side of a slowly moving boat. When inclement weather made it unsafe to launch a boat, samples were taken from the end of a pontoon which enabled only half the water column to be examined. These

Figure 7.1 Decrease in the Water Level of Island Barn Reservoir (October 1985 - January 1986)



until a final volume of 20 ml was produced (detailed in Chapter 2, page 25). Sub-samples were removed and viewed in a Sedgewick-Rafter Chamber, using an Olympus BH2 microscope. Counts of Ceratium were made over the whole chamber, whilst those of other species were made from squares selected at random. Where feasible at least 100 Ceratium cells, and 100 units (cells or filaments) of the most numerous taxa were counted. Details of the technique involved and guidelines used in determining the number of cells to count are given in Chapter 2 (page 30). The number of individuals, expressed as units ml^{-1}, are given in Appendix 7.1. The '+' symbol denotes species for which fewer than 20 organisms were present in a particular sample.

Live net samples were examined in conjunction with the preserved quantitative samples, as a useful method of detecting species which either do not preserve well, or fail to sink in the sedimentation process (see Chapter 2, page 26). Species observed in these samples, but not subsequently are denoted by 'n' in Appendix 7.1.

The duration and extent of the thermal statification of the water column are important factors affecting the size of the Ceratium population in a given lake (Heaney and Butterwick, 1985; Heaney, Lund, Canter and Gray, 1988). Mortimer (1974) showed how the stability of the epilimnion, or upper mixed layer, can be made using the Brunt-Väisälä frequency (N).

Weekly temperature figures for the upper 6 metres of the water column were obtained for the period from mid-June to late

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Weekly temperature figures for the upper 6 metres of the water column were obtained for the period from mid-June to late

August from vertical profile data. These were converted into water density values using the table in Kell (1967). Weekly values of N^2 were calculated using the following equation:-

$$N^2 = \frac{g}{\bar{\rho}} \times \frac{\delta\rho}{\delta z}$$

where N = Brunt-Väisälä frequency

g = acceleration due to gravity

ρ = density of water

$\bar{\rho}$ = average density of water over the relevant depth interval

z = depth

Mean monthly and seasonal values were subsequently calculated (see Appendix 7.2). Analysis of nutrients and the determination of carbon and chlorophyll concentrations were carried out by Thames Water using standard methods. Sediment samples were analysed using the technique of sonication and sieving detailed in Chapter 2 (page 49).

Results

(i) 1985

The reservoir supported a very large diatom population until mid-April, comprised mainly of *Stephanodiscus* spp. and *Asterionella formosa*, (see Appendix 7.1, 1). A maxima in mid-March was followed by a steady decline as silica became depleted. *Fragilaria* spp. and *Stephanodiscus* cf. *hantzschii* maintained high numbers until the end of May, in association with *Tribonema*, *Anabaena* and several Chlorophyceae (*Staurastrum*,

Ankistrodesmus, Pandorina and Eudorina). Thermal stratification of the water column developed in early June and oxygen concentrations towards the base of the water column became low (2.2 mg l^{-1}). Anabaena, Aphanizomenon, Eudorina and Tribonema were all numerous. Volvox dominated the phytoplankton from late June and through July, with Eudorina and Tribonema as the only other algae observed in significant numbers. The water column became remixed in early August as the weather became cool and unsettled. Possibly as a consequence of this Melosira and Microcystis increased in number from mid-August until early October. Numbers of Stephanodiscus cf. hantzschii increased in late September, followed by a decline in early October coinciding with the commencement of water drainage. By late November, when the water level had stabilised at 7.5 metres below normal, high numbers (4×10^5 cells ml^{-1}) of S. cf. hantzschii were recorded

C. furcoides and Peridinium cinctum were observed in the water column in low numbers towards the end of February. By mid-April C. hirundinella and Glenodinium sp. had also appeared in the phytoplankton. Numbers of Ceratium remained low until late June when a maximum of 13 cells ml^{-1} was followed by a sharp fall in numbers. There was no evidence to suggest that this decline corresponded to cyst formation. C. hirundinella and C. furcoides were observed throughout the summer, although numbers remained low. Cysts of C. hirundinella were observed in the phytoplankton in late August, but their appearance was followed by a rise in numbers of C. hirundinella motile cells.

A second lower maximum of 1.6 cells ml^{-1} was achieved in late September. A sharp decline in numbers of C. hirundinella corresponded with the commencement of water drainage. No cysts were observed in the phytoplankton and it is possible that none were formed prior to the onset of the lowering of the water level. A certain amount of the variability in cell numbers could be attributed to spatial heterogeneity (e.g. Heaney, 1976; George and Heaney, 1978). Thus if the weekly figures obtained from one sampling station were used to estimate total reservoir production, large errors would be expected.

The number of Ceratium cysts found in the sediment was very low (e.g. 50 cells in 1 g sediment). Only one or two cysts were present in any sub-sample counted. In order to assess accurately the size and seasonality of the cyst population, to the same extent as in Chapter 5, counts of 20-100 cysts would have been required (Lund, Kipling and Le Cren, 1958) from several sampling sites both after excystment and following encystment. With such low numbers involved it would have been necessary to count a considerable number of sub-samples. Some of the data acquired is used in the second part of this chapter in discussing the occurrence of the two Ceratium species in reservoirs in the Thames Valley.

(ii) 1986

The water column was dominated by diatoms (Stephanodiscus cf. hantzschii, S. astraea and Asterionella formosa) for most of the year, (see Appendix 7.1, 2). Thermal stratification of the

water column occurred in mid-June, accompanied by a large growth of Anabaena, Tribonema and Eudorina. The refilling of the reservoir was resumed briefly in early July, and, coupled with a cool and unsettled summer, contributed to the premature remixing of the water column in late July. The phytoplankton continued to be dominated by Anabaena, together with Melosira, until late August. Various Chlorophyceae were present in low numbers (e.g. Eudorina and Pandorina). Stephanodiscus cf. hantzschii increased once more in late August.

Numbers of C. hirundinella and C. furcoides cells remained low through the entire year. Peridinium cinctum and Glenodinium sp. were not observed in the water column. Ceratium was present in net samples from early April, but did not appear in quantitative samples until early July. No dinoflagellate cells were observed after early October. Cysts were not observed in the water column.

(iii) Comparison of the Two Years

The changes in abundance and composition of phytoplankton species, within a given body of water, over a 12 month period are often repeated in subsequent years, although this is not always the case (Bailey-Watts, 1978). Although the timing may vary from one year to another and an individual species may show considerable variation, the assemblage of species at a given time of the year tend to remain the same.

In this study water drainage and subsequent refilling will inevitably have disrupted this annual cycle. Figure 7.2

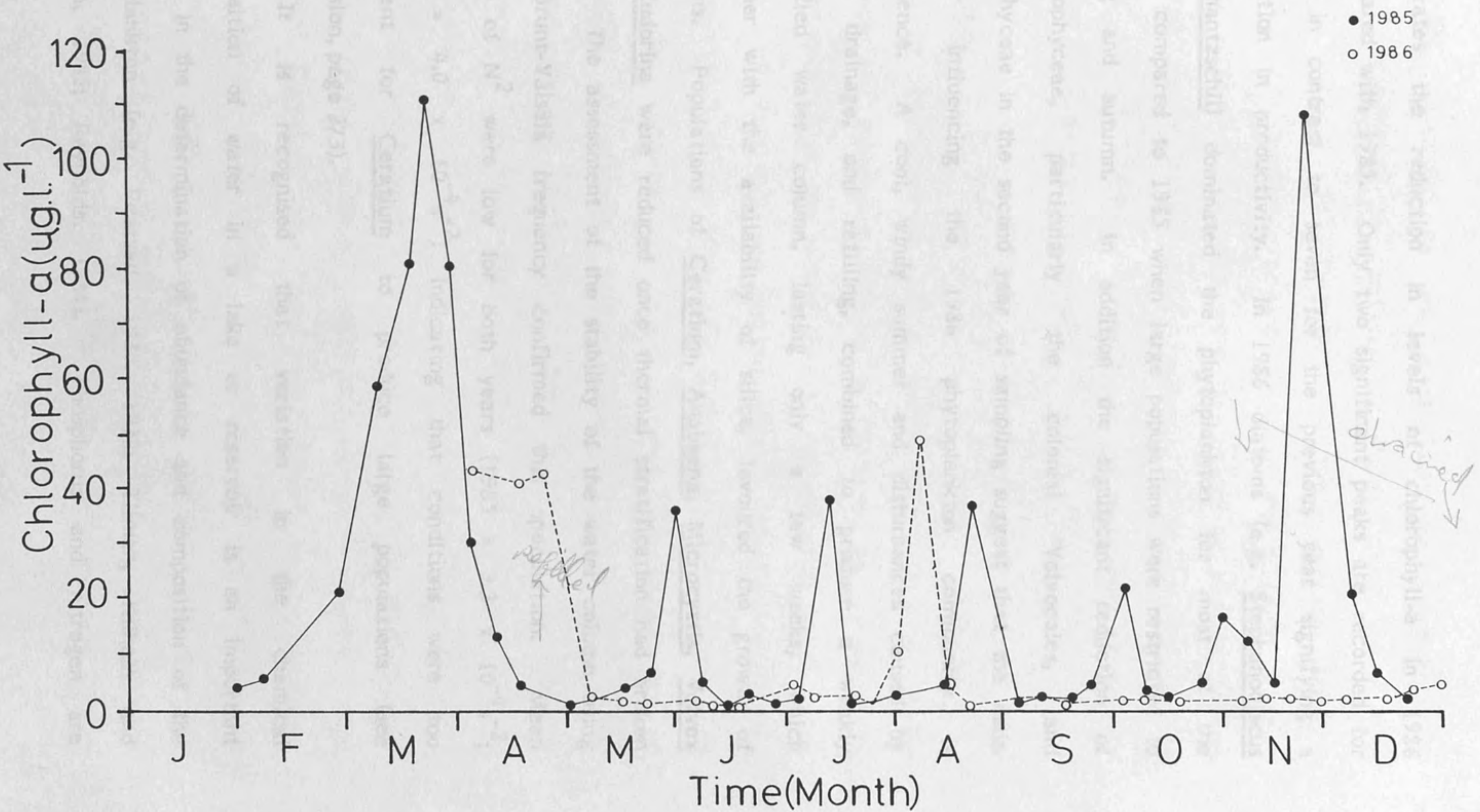


Figure 7.2 Mean Chlorophyll-a Values for Island Barn Reservoir (1985 - 1986)

illustrates the reduction in levels of chlorophyll-a in 1986 compared with 1985. Only two significant peaks are recorded for 1986 in contrast to seven for the previous year signifying a reduction in productivity. In 1986 diatoms (e.g. Stephanodiscus cf. hantzschii) dominated the phytoplankton for most of the year, compared to 1985 when large populations were restricted to spring and autumn. In addition the significant reduction of Chlorophyceae, particularly the colonial Volvocales, and Dinophyceae in the second year of sampling suggest that the main factor influencing the 1986 phytoplankton community is turbulence. A cool, windy summer and disturbances caused by water drainage, and refilling, combined to produce a weakly stratified water column, lasting only a few weeks, which together with the availability of silica, favoured the growth of diatoms. Populations of Ceratium, Anabaena, Microcystis, Volvox and Eudorina were reduced once thermal stratification had broken down. The assessment of the stability of the water column using the Brunt-Väisälä frequency confirmed this speculation. Mean values of N^2 were low for both years (1985 = $3.3 \times 10^{-4}s^{-2}$; 1986 = $4.0 \times 10^{-4}s^{-2}$) indicating that conditions were too turbulent for Ceratium to produce large populations (see discussion, page 273).

It is recognised that variation in the chemical composition of water in a lake or reservoir is an important factor in the determination of abundance and composition of the phytoplankton (e.g. Pearsall, 1930, 1932; Tilman, Kilham and Kilham, 1982; Reynolds, 1984). Phosphorus and nitrogen are

generally recognised as the most important nutrients, with silicon important in diatoms. Figure 7.3 shows that concentrations of orthophosphate and total available nitrogen, although not constant, remained high in the reservoir in both years, suggesting that they were not acting as limiting factors. However, silica levels, greatly depleted in March 1985 by the spring diatom bloom, are likely to have brought about the collapse of the diatom population.

Discussion

The phytoplankton of a water body following partial drainage and subsequent refilling is likely to be dominated by species common in the source used to restore water levels. The Island Barn Reservoir was refilled from a eutrophic river, the Thames, which at the time, near Walton, supported species which would be expected in the reservoir (*Stephanodiscus* cf. *hantzschii*, *Asterionella formosa* and *Aphanizomenon*). In addition, species which are able to survive unfavourable environmental conditions as a resting phase in the sediment would also be expected to be prevalent, provided that sufficient cysts/spores had been produced before the onset of drainage, and that comparable conditions were restored after refilling. The cysts of *Ceratium* have been well studied (e.g. Livingstone, 1979; Chapman, 1981; Heaney, Chapman and Morison, 1983) and are discussed in detail in Chapter 5. In addition, *Anabaena* and *Aphanizomenon* produce akinetes (asexual spores), *Eudorina* and *Volvox* sexual zygotes and Chrysophytes silicious cysts, which

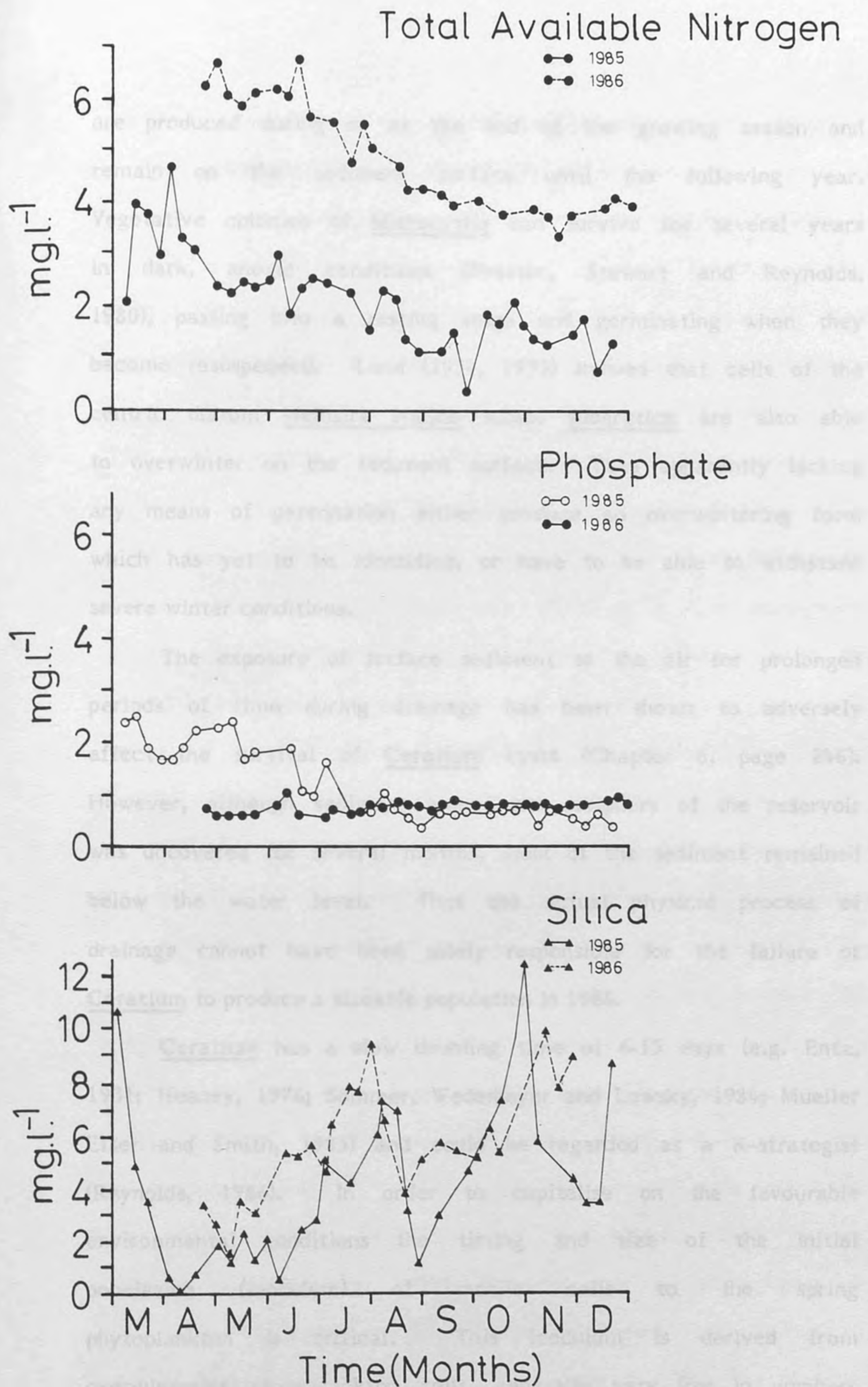


Figure 7.3 Mean Values of Nutrients for Island Barn Reservoir (1985-1986)

are produced during or at the end of the growing season and remain on the sediment surface until the following year. Vegetative colonies of Microcystis can survive for several years in dark, anoxic conditions (Preston, Stewart and Reynolds, 1980), passing into a resting stage and germinating when they become resuspended. Lund (1954, 1955) showed that cells of the centric diatom Melosira italica subsp. subarctica are also able to overwinter on the sediment surface. Taxa apparently lacking any means of perennation either produce an overwintering form which has yet to be identified, or have to be able to withstand severe winter conditions.

The exposure of surface sediment to the air for prolonged periods of time during drainage has been shown to adversely affect the survival of Ceratium cysts (Chapter 6, page 246). However, although sediment around the periphery of the reservoir was uncovered for several months, most of the sediment remained below the water level. Thus the actual physical process of drainage cannot have been solely responsible for the failure of Ceratium to produce a sizeable population in 1986.

Ceratium has a slow doubling time of 6-15 days (e.g. Entz, 1931; Heaney, 1976; Sommer, Wedemeyer and Lowsky, 1984; Mueller Elser and Smith, 1985) and could be regarded as a K-strategist (Reynolds, 1986). In order to capitalise on the favourable environmental conditions the timing and size of the initial population (inoculum) of motile cells to the spring phytoplankton is critical. This inoculum is derived from overwintering phytoplankton cells, generally very low in numbers

and of limited importance, and/or overwintering cysts. Ceratium is found only rarely in the River Thames and thus the number of cells or cysts introduced into the reservoir during refilling is likely to have been negligible. The introduction of phytoplankton cells from other sources by birds has been suggested by Atkinson (1980). However, although viable cells of Asterionella formosa have been recovered from faeces, it has still to be proved that this is a mechanism for dispersal. In spite of being sensitive to desiccation (Chapter 6, page 246), it is possible that Ceratium cysts could be thus transported, although it seems likely that this would be a rare occurrence. In this study it is considered that the contribution from the overwintering cyst population would outweigh any cysts/cells introduced in this way. Therefore, it is reasonable to assume that the main contribution to the inoculum came from excystment.

The timing of excystment, dependent on water temperature, is important, since a delay in the production of Ceratium cells will enable other summer species to become established. The earlier the cells of Ceratium are established the greater the time available for nutrient uptake and growth once sufficiently high temperatures have been reached (Heaney, Lund, Canter and Gray, 1988). In general, excystment does not occur until temperatures exceed 4°C and vegetative growth does not occur until temperatures reach 10°C (Huber and Nipkow, 1923; Heaney, Chapman and Morison, 1983), although this situation does not apply in some temperate lakes with an oceanic climate and in tropical lakes (Chapter 6, page 239). Even at 20°C, the optimum

temperature, the growth in culture of C. furcoides, based on cell counts, was shown to be slower than Oscillatoria bourrellyi or Asterionella formosa (Heaney and Butterwick, 1985). Therefore, prolonged winters will delay excystment until late spring and would be expected to reduce the chance of a large Ceratium population being produced. In this study temperatures exceeded 4°C in early March in 1985, and had already reached 7°C at the commencement of sampling in 1986. In neither year did numbers increase markedly once temperatures had reached 10°C, which in 1986 was not until early May, following a particularly cold winter. Heaney, Lund, Canter and Gray (1988) suggested that to achieve a mean weekly cell concentration in excess of 50 cells ml^{-1} in the upper 5 metres of the water column, a cell density of 0.1 cells ml^{-1} must be achieved before June, although an early population does not guarantee a large number of cells in the summer phytoplankton. In Island Barn Reservoir in 1985 the number of cells exceeded 0.1 cells ml^{-1} before the end of May, although a large population maxima was not attained. In 1986 the inoculum was very low. Cells did not appear in net samples until early July, compared to late February the previous year.

Heaney, Chapman and Morison (1983) calculated that only 0.03% of the total cyst population was required to excyst to produce an inoculum of approximately 0.1 cells ml^{-1}, the number considered as a basis for producing a viable population. Even taking consideration of the losses through grazing, parasitism (Canter and Heaney, 1984) and washout during drainage, only a

small proportion of the total cyst population would be required to excyst to produce viable phytoplankton numbers. In Island Barn Reservoir the 0.1 cells ml^{-1} value was not reached in 1986. The low 1985 Ceratium population, coupled with the possibility that drainage had commenced before mass encystment had occurred, may have resulted in few cysts reaching the sediment. Cysts produced in previous years remain viable in the sediment and should be able to compensate for low cyst production in a given year (Chapter 6, page 245). Examination of surface sediment samples (upper 1 cm) revealed low numbers of cysts despite the high phytoplankton population in 1984. This suggests that the lowering of the water level in 1984 may have commenced before encystment had occurred, or that cysts were lost through parasitism or had become buried at greater depths as a result of disturbance during drainage. A study of the upper 4 cm of sediment did not reveal an increase in the number of cysts and it is unlikely that redistribution would have located them below this level. Low cyst numbers would reduce recruitment to the phytoplankton and the inoculum would take longer to form, thus delaying growth (Heaney, Lund, Canter and Gray, 1988).

Local climatic conditions can profoundly affect the extent of a Ceratium maxima by disrupting, or strengthening, the thermal stratification of the water column. Reynolds, Wiseman and Clarke (1984) observed a reduction in the growth rate of Anabaena, Ceratium, Volvox and Microcystis during intermittent periods of artificial mixing of Blelham Tarn. Heaney and Butterwick (1985) used the Brunt-Väisälä frequency (Mortimer,

1974) in order to determine the stability of the epilimnion during thermal stratification in Esthwaite Water. During cool, windy summers, with only weak thermal stratification (with mean N^2 values $< 5 \times 10^{-4} s^{-2}$), Ceratium failed to produce large populations and the phytoplankton was dominated by summer diatoms. When summers were warm and a well developed thermal stratification occurred (with mean N^2 values $> 5 \times 10^{-4} s^{-2}$), Ceratium was abundant and diatoms virtually absent. In this study mean N^2 values for both years were less than $5 \times 10^{-4} s^{-2}$ ($N^2 = 3.3 \times 10^{-4} s^{-2}$ for 1985; $N^2 = 4.0 \times 10^{-4} s^{-2}$ for 1986), indicating that conditions were too turbulent for Ceratium to produce large numbers. The possession of flagella enables Ceratium cells to regulate their depth within the water column to favourable light and nutrient conditions (Heaney and Talling, 1980a, b). Any increase in water turbulence may prevent cells from maintaining their preferred position (Heaney and Butterwick, 1985). Cells may be drawn into deeper water due to the downward movement of cooler, denser water, where light levels are reduced and temperatures are lower. Ceratium cells respond less favourably to a reduction in light levels than either Oscillatoria or Asterionella (Heaney and Butterwick, 1985). Taxa able to function successfully in low light conditions would thus be expected to increase in numbers, whilst those unable to do so would be reduced. Turbulence may also act directly on the cell by disrupting cell division, demonstrated in Peridinium cinctum by Pollingher and Zemel (1981). The dominance of the phytoplankton of 1986 by diatoms confirms the

observations of Toetz (1981) and Steinberg (1983). These authors noted that following the artificial destratification of the water column the phytoplankton was dominated by diatoms and cryptophytes.

Nevertheless, other factors must also contribute to the production of high cell numbers. Heaney, Lund, Canter and Gray (1988) showed that low numbers of Ceratium in Esthwaite Water could be correlated with a weak thermal stratification. However, in another more sheltered lake (Blelham Tarn) in the same drainage basin, warm calm summers, with thermally stratified water columns, occurred in all but one of the 25 years studied, when artificial mixing was induced, but large Ceratium populations were less frequent than in Esthwaite Water.

Parasitism of Ceratium cells and cysts (Canter, 1968; Canter and Heaney, 1984; Heaney, Lund, Canter and Gray, 1988) is now recognised as exerting a major influence on populations of Ceratium. However, none of the documented parasites were observed on either cells or cysts of Ceratium from Island Barn Reservoir over the two year period.

Shortage of the two major nutrients (phosphorus and nitrogen) would not appear to be a factor limiting growth of Ceratium as both remained high through the two years of the study. Ceratium, like most algae takes up nitrogen in the form of NH_4-N in preference to NO_3-N (Heaney, Smyly and Talling 1986). Pfiester (1971) found that nitrate and phosphate had no significant effect on the population of Ceratium between October and April, whilst Reynolds and Reynolds (1985) noted that

dissolved phosphorus and nitrate were at their lowest annual levels (1 μg $SRPl^{-1}$, 2 μg Nl^{-1}) when Ceratium dominated the water column (400 cells ml^{-1}). Moore (1981) observed that the nutrient availability controlled the intensity but not the timing of the Ceratium populations of six sub-arctic Canadian lakes. However, Bruno and McLaughlin (1977) have shown that at high levels (> 7 mg N l^{-1}) NH_4 compounds become toxic to cultures of the so-called C. hirundinella (the photograph suggests that this species is C. furcoides). In the present study concentrations of NH_4 in the reservoir never exceeded 0.8 mg N l^{-1} and would not therefore be expected to be toxic. Bruno and McLaughlin also showed that Ceratium growth was limited by levels of phosphorus less than 0.01 mg l^{-1}. They observed that inorganic and organic sources of phosphorus promoted growth, the optimum being achieved with the addition of ATP at 3-5 mg P l^{-1}. In this study recorded levels of orthophosphate occurred between these two values, suggesting that this was not a limiting factor. Harris, Heaney and Talling (1979) showed that a fall in nutrient availability during thermal stratification was reflected in a decrease in cellular chlorophyll contents in Ceratium. Serruya and Pollingher (1977) observed a reduction in numbers of Peridinium cinctum following the lowering of the water level in Lake Kinneret, Israel from 26 metres to 3 metres. They suggested that nutrients were thus distributed in a smaller volume of water, resulting in a net increase in their concentration. Therefore, the slow growing P. cinctum, which can survive at lower nutrient concentrations, was unable to

compete with faster growing taxa which were able to increase due to the rise in nutrients. In this study, drainage occurred after the decline of the dinoflagellate population (October 1985). Increases in values of total available nitrogen and silica were observed following the onset of drainage (see Figure 7.3). A subsequent decline in silica resulted from a substantial increase in numbers of centric diatoms.

It is interesting to note that in 1987, after a late start (motile cells were first noted in the phytoplankton in July), numbers of Ceratium in Island Barn Reservoir reached comparable populations to 1985 (Thames Water, personal communication). Since the Ceratium population of 1986 was very small any contribution of cysts to the sediment would have been negligible. Thus it seems likely that the 1987 phytoplankton population, which reached a population maximum in August (no quantitative data available), was derived from cysts from previous years. Water drainage and subsequent refilling at the end of 1986 may have disrupted the sediment and brought buried cysts to the surface, enabling them to germinate the following year. The extent of cyst viability is discussed in Chapter 6 (page 247).

The production of a large population of the slow growing Ceratium would seem to be dependent on the existence of certain factors to enable it to compete successfully with phytoplankton species with a faster growth rate. In addition to the usual light, temperature and nutrient requirements, the availability of sufficient viable cysts is necessary, in order to produce a

sizeable inoculum of motile cells to the phytoplankton, coupled with a mild spring allowing early excystment and subsequent growth. Finally, a well stratified water column persisting through the summer enables the motile cells to regulate their position and undertake cell divisions without disruption. Mass excystment at the end of the growing season ensures the availability of viable cysts the following spring.

7.2 The Proportion of C. furcoides to C. hirundinella in Thames Valley Sites

Introduction

Ceratium is commonly represented in the freshwater phytoplankton and has been recorded in many studies (e.g. Sebestyén, 1959; Pfiester, 1971; Heaney and Talling, 1980b; Chapman, 1981; Krupa, 1981a, b). Comparatively few authors, however, have attempted to distinguish between C. furcoides and C. hirundinella (e.g. Dottne-Lindgren and Ekbohm, 1975; Canter and Heaney, 1984; Hickel, 1985; Heaney, Lund, Canter and Gray, 1988).

As part of this investigation the ratio of C. furcoides to C. hirundinella was determined for several lakes and reservoirs in the Lake District and the Thames Valley. Results from the former location (Esthwaite Water) are discussed in Chapter 5 and only the southern sites, descriptions of which are included in Chapter 3 (page 66), are detailed here.

Methods

Net haul samples were examined in a Sedgewick-Rafter Chamber under an Olympus BH2 microscope, and the number of Ceratium cells of each species recorded. The total number of cells observed was often low (< 20 cells in a sub-sample) as species of Ceratium were rarely dominant in the phytoplankton of some sites. Sediment samples from various sites were also examined. Samples were sonicated and sieved (Chapter 2, page 49) and the number of cysts of each species noted. Numbers of cysts were often low (< 10 cysts in a sub-sample), as in the phytoplankton samples.

Results

In four of the reservoirs, Island Barn, Queen Mary, Queen Mother and Staines South, cells of C. hirundinella were consistently more numerous than C. furcoides (Table 7.2). In one observation from the Queen Mother reservoir (4.9.1986) this situation was reversed, possibly due to the earlier encystment of C. hirundinella. It is possible that the ratio between the two species may vary through the year, although the results obtained from Esthwaite Water (Chapter 5, page 166) would suggest that relative frequencies remain the same. However, the same data shows how markedly proportions can change from year to year indicating that comparisons cannot be drawn between sites sampled in different years (Chapter 5, page 166).

In Virginia Water Lake, even making allowances for seasonal differences, it was evident that the Ceratium

Table 7.2 The proportion of C. furcoides and C. hirundinella cells from Thames Valley Sites

Site	Date	No. of Cells C.h.	C.f.	Proportion C.f. (%)
Reservoirs				
Island Barn	30-5-1985	195	3	2
	14-6-1985	19	1	5
	20-6-1985	15	3	2
	27-6-1985	1948	1	0.05
	12-7-1985	191	1	0.5
	14-6-1986	19	1	5
	15-8-1986	121	-	-
Queen Mary	4-10-1984	88	14	14
	8-10-1984	390	28	7
Queen Mother	25-9-1984	81	8	9
	7-8-1986	238	146	38
	14-8-1986	365	277	43
	28-8-1986	84	37	30
	4-9-1986	38	68	64
Staines South	3-7-1984	118	72	38
	10-7-1984	1220	175	12
Lake				
Virginia Water	4-10-1984	5	137	96

population differed markedly from any of the four reservoirs sampled. Three of the four reservoirs (Queen Mary, Queen Mother and Staines South) were sampled in the autumn of 1984 and all showed a low percentage of C. furcoides (7-38%). In Virginia Water Lake the situation was reversed with C. hirundinella almost absent (Table 7.2).

Table 7.3 demonstrates that a similar situation exists for the cyst population, with low proportions of C. furcoides in the reservoirs sampled (Island Barn, Kempton Park West, Queen Mother and Staines South), and a high ratio of C. furcoides in Virginia Water Lake. The percentage of C. furcoides cysts in the lake corresponds to the proportion of C. furcoides cells in the previous year, although this conclusion is based on only a low cyst count (23 cysts).

Discussion

The most obvious feature of the data collected is the very great difference in the proportion of species between the reservoirs and the lake. Without a long term study of a given water body it is impossible to establish whether the observed proportions are merely transitory. A study of the changes of the Ceratium population in Esthwaite Water over a 41 year period, detailed in Chapter 5 (page 169) of the present study and Heaney, Lund, Canter and Gray (1988), suggests that the relative numbers of the two species are unlikely to remain constant. A study by Hickel (1985) of the Ceratium population in a small eutrophic lake (Plusssee, West Germany) showed that

Table 7.3 The proportion of C. furcoides and C. hirundinella cysts from Thames Valley Sites

Site	Date	No. of Cysts C.h.	C.f.	Proportion C.f. (%)
Reservoirs				
Island Barn	8-3-1985	8	1	11
	Spring 1985	144	29	17
Kempton Park West	7-12-1983	11	3	21
Queen Mother	17-10-1983	3	1	25
	21-11-1983	7	5	42
	5-12-1983	26	12	32
Staines South	3-7-1984	5	1	17
Lake				
Virginia Water	Autumn 1984	1	22	96

the relative numbers of three species (*C. hirundinella*, *C. furcoides* and *C. furcoides* f. *gracile*, now *C. rhomvoides*, Hickel 1988b), varied during the year and between years. Observations were made during the summers of two successive years (June-August 1981 and July-October 1982), throughout which *C. furcoides* remained the most numerous species. A decline in total numbers in 1982 was accompanied by a relative increase in *C. furcoides* f. *gracile*.

All the reservoirs sampled in the present study were originally supplied from a common source, the River Thames, whilst Virginia Water is fed by a series of small streams. *Ceratium* is uncommon in the river, which would suggest that the initial inoculum of cells, or cysts, to each water body would be very low. The *Ceratium* populations of the streams is unknown. Thus the proportion of each species in the small initial population would be important, assuming requirements for growth are the same for each species. Atkinson (1980) proposed that birds could act as agents of dispersal for phytoplankton (see Chapter 7.1, page 271). However, transference of cysts or cells between sites in this way is likely to be uncommon.

It is possible that numbers of *C. furcoides* in the reservoirs were originally higher and have declined as a result of disease or parasitism. The fungal parasite *Rhizophydium nobile* is known to be specific to *C. furcoides* (Canter and Heaney, 1984) and to have an influence on population numbers (Heaney, Lund, Canter and Gray, 1988). However, in the reservoir populations no parasitised cysts were observed. It is

possible that in the reservoirs the C. furcoides populations are recovering following a fungal attack, although this is unlikely to have occurred simultaneously in all reservoirs.

The stability of the water column may have some influence on the ratio of the two species. During the early summer of 1986 the Queen Mother Reservoir was strongly stratified, compared to the weaker stratification in Island Barn Reservoir (detailed in Chapter 7.3, page 288). This would seem to account for the greater proportion of C. furcoides cells (mean number of cells = 44%) in the Queen Mother Reservoir, in comparison to only 2.5% in Island Barn Reservoir. However, comparison of water stability in Esthwaite Water (Heaney and Butterwick, 1985) with the proportion of C. furcoides cells in the phytoplankton (Chapter 5, page 169), showed little correlation between the ratio of C. furcoides and a strongly stratified water column. Moreover, it would not account for the high proportion of C. furcoides cells in Virginia Water Lake, which is shallow and thus unlikely to be strongly thermally stratified during the summer.

7.3 Observations on a Ceratium Population in a Thames Valley Reservoir

Introduction

Although algal "blooms" are generally associated with the Cyanophyceae, dinoflagellates can, under favourable conditions, attain very large populations. The annual occurrence of a sizeable dinoflagellate population at a particular location has

enabled detailed ecological studies to be undertaken on Peridinium cinctum in Lake Kinneret, Israel (e.g. Serruya and Pollingher, 1971; Pollingher and Serruya, 1976) and on Ceratium in Plusssee, West Germany (Hickel, 1985, 1988a, b) and Esthwaite Water, Cumbria (e.g. Talling, 1971; Heaney, 1976; Heller, 1977; George and Heaney, 1978; Harris, Heaney and Talling, 1979; Chapman, 1981; Frempong, 1981a, 1982, 1984; Heaney, Chapman and Morison, 1983, Canter and Heaney, 1984).

The size of such "blooms" can be extensive, discolouring the water and imparting an unpleasant odour. The Ceratium population in Esthwaite Water during the 1970's exceeded 600 cells ml^{-1} during the late summer maxima. Nicholls, Kennedy and Hammett (1980) observed a dense population of cells they referred to as C. hirundinella (1,300 cells ml^{-1}) in Heart Lake, Ontario, which reduced the oxygen concentration, instigating a fish kill. Reservoirs frequently support still larger populations. Herrgesell, Sibley and Knight (1976) recorded a maximum of 2,880 cells ml^{-1} of Peridinium penardii f. californicum in a Californian reservoir (Lake Berryessa) during the spring "bloom". Pollingher (1987), in a review of freshwater dinoflagellates, cites Nakomoto (1975) who described densities of dinoflagellates, mainly Peridinium cinctum, reaching 93,000 cells ml^{-1} in a Japanese reservoir.

During the course of the present investigation one reservoir, Queen Mother (Datchet) Reservoir, supported a large Ceratium population (during the summer of 1986). The late summer population decline was noted and a comparison made with

physical and chemical variables operating in the reservoir. Partial drainage and refilling of the reservoir was undertaken by Thames Water during this period. The water level was lowered by 3-4 metres commencing on 4.8.1986. River water was pumped back in via the jetting system between 13.8.1986 and 21.8.1986.

Methods

Sampling commenced when it became evident that a large Ceratium population was going to develop in the Queen Mother Reservoir (Thames Water - personal communication). Water samples of 1 litre were collected at intervals from July to October from the end of a pontoon, using a 5 metre long weighted tube. Samples were immediately preserved with Lugol's Iodine and, on returning to the laboratory, concentrated to 20 ml using the settling technique described in Chapter 2 (page 25). Counts of cell numbers were made in the usual way (detailed in Chapter 2, page 27). Nutrient analysis data and chlorophyll values were obtained from Thames Water. Weekly temperature data (collected by Thames Water) from mid-June to late August was used to calculate the mean Brunt-Väisälä frequency (see Appendix 7.3), in order to establish the stability of the epilimnion (detailed in Chapter 7.1, page 261).

Results

Figure 7.4 shows that during the period of sampling the Ceratium population reached a maxima of 257 cells ml^{-1} in mid-August, followed by a marked decline in numbers. Cells

Figure 7.4 Ceratium Population of Queen Mother Reservoir (1986)

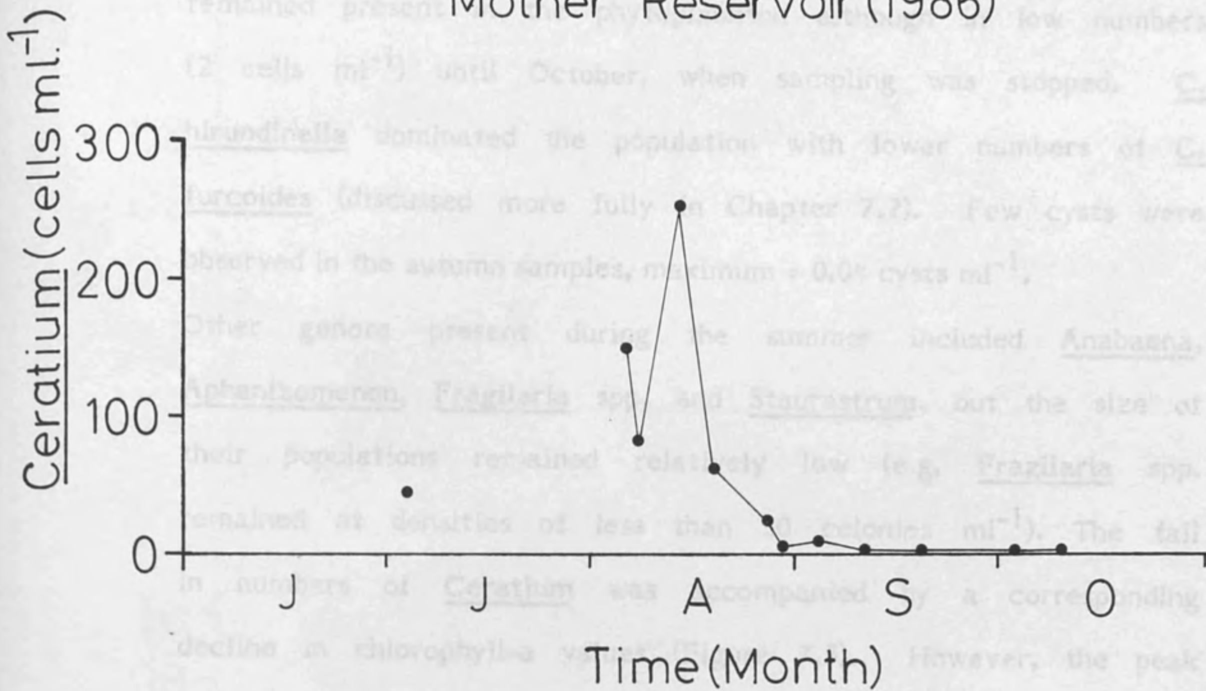
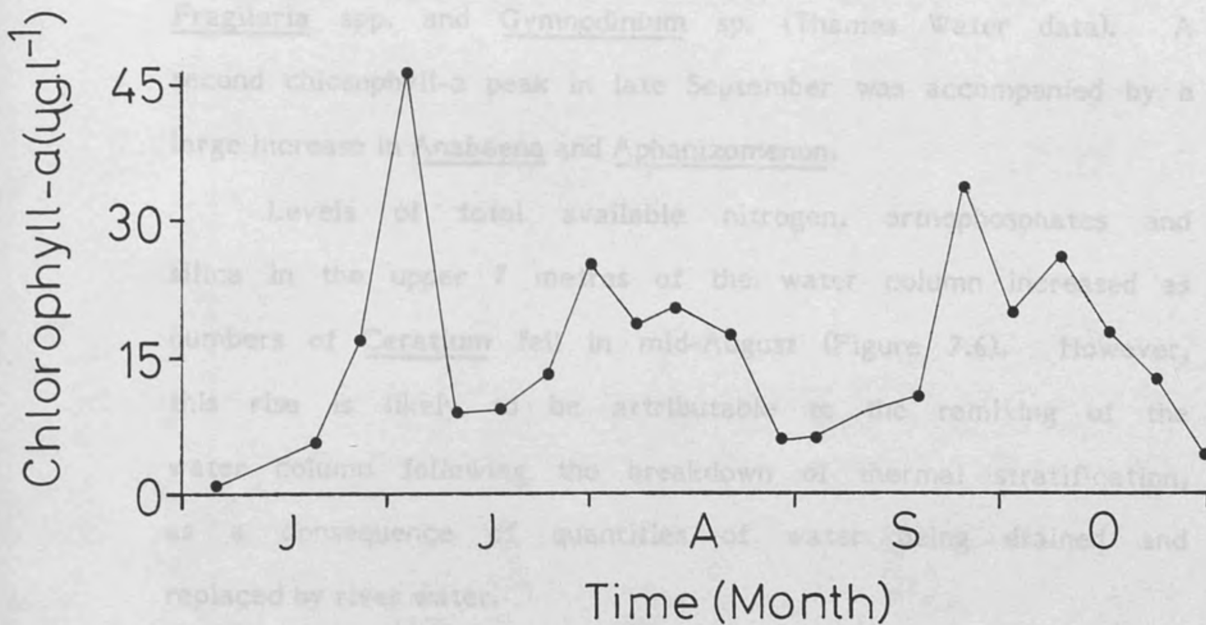


Figure 7.5 Mean Chlorophyll-a Values for Queen Mother Reservoir (1986)



remained present in the phytoplankton although in low numbers (2 cells ml^{-1}) until October, when sampling was stopped. C. hirundinella dominated the population with lower numbers of C. furcoides (discussed more fully in Chapter 7.2). Few cysts were observed in the autumn samples, maximum = 0.04 cysts ml^{-1}.

Other genera present during the summer included Anabaena, Aphanizomenon, Fragilaria spp. and Staurastrum, but the size of their populations remained relatively low (e.g. Fragilaria spp. remained at densities of less than 20 colonies ml^{-1}). The fall in numbers of Ceratium was accompanied by a corresponding decline in chlorophyll-a values (Figure 7.5). However, the peak in numbers of Ceratium was not coupled with a marked increase in chlorophyll-a. The highest chlorophyll-a value (48.0 $\mu g\ l^{-1}$) corresponded to high numbers of Eudorina and Aphanizomenon, both of which declined in the subsequent week to be replaced by Fragilaria spp. and Gymnodinium sp. (Thames Water data). A second chlorophyll-a peak in late September was accompanied by a large increase in Anabaena and Aphanizomenon.

Levels of total available nitrogen, orthophosphates and silica in the upper 7 metres of the water column increased as numbers of Ceratium fell in mid-August (Figure 7.6). However, this rise is likely to be attributable to the remixing of the water column following the breakdown of thermal stratification, as a consequence of quantities of water being drained and replaced by river water.

A mean N^2 value of $7.4 \times 10^{-4} s^{-2}$ was obtained as a measure of stability (see Appendix 7.3), indicating that the upper 6

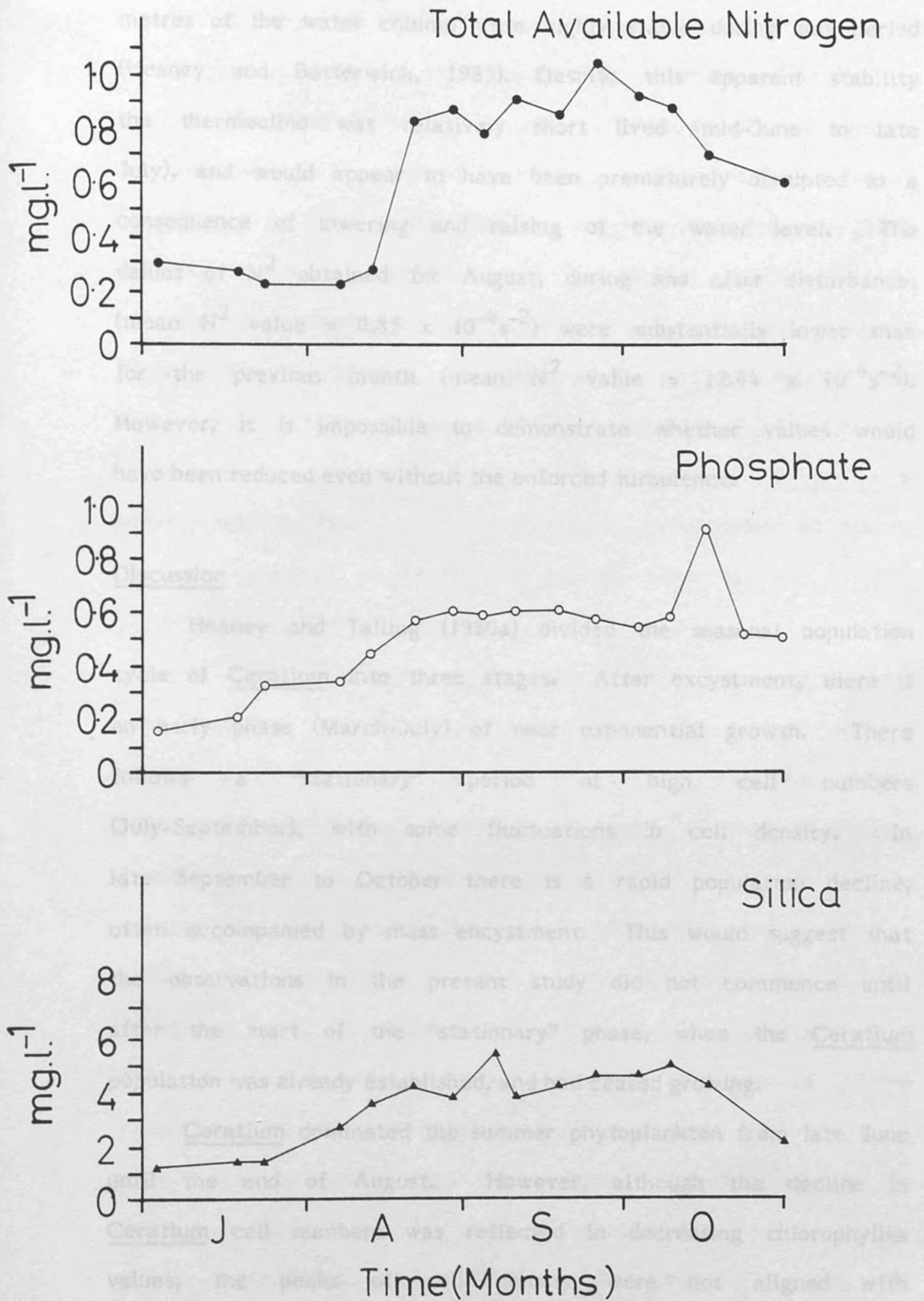


Figure 7.6 Mean Values of Nutrients for Queen Mother Reservoir (1986)

metres of the water column were highly stable during this period (Heaney and Butterwick, 1985). Despite this apparent stability the thermocline was relatively short lived (mid-June to late July), and would appear to have been prematurely disrupted as a consequence of lowering and raising of the water level. The values of N^2 obtained for August, during and after disturbance, (mean N^2 value = $0.85 \times 10^{-4}s^{-2}$) were substantially lower than for the previous month (mean N^2 value = $12.44 \times 10^{-4}s^{-2}$). However, it is impossible to demonstrate whether values would have been reduced even without the enforced turbulence.

Discussion

Heaney and Talling (1980a) divided the seasonal population cycle of Ceratium into three stages. After excystment, there is an early phase (March-July) of near exponential growth. There follows a "stationary" period of high cell numbers (July-September), with some fluctuations in cell density. In late September to October there is a rapid population decline, often accompanied by mass encystment. This would suggest that the observations in the present study did not commence until after the start of the "stationary" phase, when the Ceratium population was already established, and had ceased growing.

Ceratium dominated the summer phytoplankton from late June until the end of August. However, although the decline in Ceratium cell numbers was reflected in decreasing chlorophyll-a values, the peaks of cell density were not aligned with corresponding increases in chlorophyll-a (Figure 7.5). It

should be stressed that Thames Water obtained their water samples at a different location on the reservoir (close to the inlet tower), and from deeper water. Although Dottne-Lindgren and Ekbohm (1975) observed a random spatial distribution of *C. hirundinella* in Lake Erken, Sweden, other authors (Heaney, 1976; George and Heaney, 1978; Heaney and Talling, 1980a, b), have recorded a non-uniform distribution for *Ceratium* in Esthwaite Water. Spatial clumping was governed by actively moving cells and passive movement created by wind currents. Pollingher and Berman (1975) also noted a patchy distribution of *Peridinium* in Lake Kinneret, Israel. It is therefore unreasonable to assume that the *Ceratium* population will be the same at two sampling points on Queen Mother Reservoir.

In addition, samples obtained by Thames Water for analysis were collected at discrete intervals (alternate metres) with a patalas sampler, whilst the samples for phytoplankton counts were continuous, collected with a 5 metre weighted tube (see Chapter 2, page 24). These differences would be expected to be reflected in the data. The extent of such variation can be pronounced. Heaney (1976) noted a 100% difference between the number of cells at different sampling sites in the same lake (Esthwaite Water). No comparisons can be made here as Thames Water do not undertake quantitative phytoplankton counts. It is evident that the data should be treated with consideration for such variations. Clearly emphasis should not be placed on weekly comparisons, but attention paid instead to seasonal changes.

The rapid decline in cell numbers occurred soon after sampling had started. Numbers of Ceratium usually remain high until late August. Therefore the reduction in numbers was considerably earlier than would be expected. The stability of the epilimnion from mid-June to the end of August, as assessed using the Brunt-Väisälä frequency (mean N^2 value = $7.4 \times 10^{-4}s^{-2}$), indicated a strongly stratified water column which might be expected to promote the growth of Ceratium (Heaney and Butterwick, 1985). The existence of a stable epilimnion appears to be inconsistent with the expected disturbance created by the refilling of the reservoir following partial drainage. However, a comparison of N^2 values before (mean N^2 value for July = $12.44 \times 10^{-4}s^{-2}$) and during drainage (mean N^2 value for August = $0.85 \times 10^{-4}s^{-2}$) indicates that the stability of the water column was greatly reduced in the late summer, earlier than would normally be expected. The mean N^2 value, for the same period for Island Barn Reservoir, where growth of Ceratium was limited, (see Chapter 7.1, page 267) was lower ($4.0 \times 10^{-4}s^{-2}$). However, it was still substantially higher than the August value for the Queen Mother Reservoir.

The lowering of the water level occurred before the commencement of regular sampling and so the impact cannot be judged. Refilling of the reservoir, via high velocity input jetting system (13.8.1986 to 21.8.1986), appears to have prematurely mixed the water column, breaking down the thermal stratification. This resulted in the destabalisation of the water column and may have caused a decline in cell numbers of

Ceratium. This reduction in algal numbers following a period of mixing corresponds with the observations of Ridley, Cooley and Steel (1966) and Lorenzen and Mitchell (1975), who determined that algal biomass declined following the onset of artificial destratification (see page 254).

In addition to its influence on turbulence, water temperature is generally regarded as a major factor associated with Ceratium numbers. A threshold value of 10°C needs to be reached before rapid population increases can occur. Heaney, Chapman and Morison (1983) showed that the optimal temperature for growth of C. hirundinella (reidentified as C. furcoides) in culture is 20°C. In this investigation high Ceratium cell numbers were not associated with the highest temperatures. The one Ceratium count taken in July occurred when the temperature at 1 metre was high (20.6°C) but cell numbers were relatively low, 45 cells ml^{-1} compared to a later peak of 257 cells ml^{-1} when temperatures were lower (16.3°C). Both Pfiester (1971) and Moore (1977) positively correlated Ceratium numbers with temperature. Pfiester compared the onset of excystment in Ceratium with a fall in temperature. In Queen Mother Reservoir cell numbers dropped more steeply than the decline in temperature.

Encystment would be expected to cause a reduction in the number of cells. The first cysts appeared in August, during the period of refilling of the reservoir. However, cyst production appeared to be low, a maximum of 0.04 cysts ml^{-1} was recorded in early October. Heaney (1976) observed that numbers of cysts

increased in water samples collected after the autumnal mixing until November when they equalled the number of cells. However, in the Queen Mother Reservoir both cells and cysts were low in numbers following the decline of cell production. Mass encystment does not always occur (Heaney, Chapman and Morison, 1983), which may be the case here, either as a result of a decline in the number of cells prior to the onset of encystment or due to another undetermined factor. Alternatively, the rapid fall in cell numbers may have been accompanied by mass production of cysts which sank out of the water column before being detected.

Levels of nitrates and orthophosphates remained high throughout the year and it is unlikely that these nutrients were ever growth limiting. The increase in availability of nutrients, as a result of the mixing of the water column in August, although not high enough to be toxic to Ceratium (Bruno and McLaughlin, 1977), may have enabled the growth of other species, with which Ceratium, with its slower doubling rate (7 days, Heaney, 1976) was unable to compete.

Underwater light levels were not determined. However, the density of Ceratium cells, the most numerous genera, was well below those recorded in the late 1970's in Esthwaite Water (e.g. Heaney and Talling, 1980b) and would thus not apparently be limited by self-shading.

It has been shown that large populations of Cyanophytes may secrete dialysable metabolites, which can inhibit or kill algal species in culture (Reynolds, 1984). However, Krupa

(1981a) observed that although C. hirundinella cells did not occur in large numbers when the phytoplankton was dominated by Cyanophyceae, the sub-dominance of C. hirundinella and Cyanophyceae in Bialskie Lake was followed by the dominance of the phytoplankton by C. hirundinella. In the present study there was no corresponding increase in either Anabaena or Aphanizomenon, the two most numerous Cyanophyceae genera, at the time of the Ceratium population decline.

Predation by zooplankton on Ceratium is generally regarded as being low, due to the large size and awkward shape of Ceratium cells (Reynolds, 1986), although Sebestyén (1959) recorded the cells referred to as C. hirundinella in the bodies of the rotifer Asplanchna brightwellii. At the time of the Ceratium decline in Queen Mother Reservoir, numbers of zooplankton showed no significant change, 235.6 mg $haul^{-1}$ in comparison to 295.1 ml $haul^{-1}$ the previous week (Thames Water data).

It would thus appear that the refilling of the reservoir in mid-August (13.8.1986 to 21.8.1986) was the chief factor responsible for the premature decline of the Ceratium population. The mean N^2 value ($7.4 \times 10^{-4} s^{-2}$) indicated a strongly stratified water column with the potential to produce a large Ceratium population (Heaney and Butterwick, 1985). However, this brief period of mixing, caused by the introduction of water via high velocity jets, destabalised the epilimnion. The changes in the physical and chemical conditions created by mixing could thus be assumed to have either an adverse effect on

Ceratium cells (e.g. prevents them from maintaining their position in the water column, see Chapter 7.1, page 274), or a favourable influence on other species usually unable to develop under the prevailing conditions (e.g. redistribution of nutrients or the resuspension of cells). The reduction in chlorophyll-a values at the time of the *Ceratium* decline suggests that the former explanation applies in this example.

Key to Appendix 7.1

Number	Taxa
1.	C. furcoides
2.	C. hirundinella
3.	Glenodinium sp.
4.	Peridinium cinctum
5.	Actinastrum
6.	Ankistrodesmus
7.	Botryococcus
8.	Chlorella
9.	Closterium
10.	Coelastrum
11.	Cosmarium
12.	Eudorina
13.	Microspora
14.	Pandorina
15.	Pediastrum
16.	Scenedesmus
17.	Staurastrum
18.	Volvox
19.	Tribonema
20.	Asterionella formosa
21.	Cyclotella
22.	Diatoma
23.	Fragilaria capucina
24.	Fragilaria crotonensis
25.	Melosira spp.
26.	Stephanodiscus astraea
27.	Stephanodiscus cf. hantzschii
28.	Tabellaria
29.	Small penate diatoms
30.	Cryptomonas
31.	Anabaena
32.	Aphanocapsa
33.	Aphanizomenon
34.	Chroococcus
35.	Merismopedia
36.	Microcystis spp.
37.	Oscillatoria
38.	Small flagellates

Figures represent the number of cells in 1 ml^{-1}. In colonial forms the additional figures in brackets represent the mean number of cells in a colony.

+	denotes counts of less than 20 organisms
n	denotes taxa found only in net samples
p	denotes those samples taken from the pontoon
s	denotes those samples taken from the shore

APPENDIX 7.1 Phytoplankton counts from Island Barn Reservoir

1. February to December 1985

Taxa	Date					
	7-2	28-2	8-3	14-3	28-3	4-4	11-4
						P	P
1.	-	+(0.4)	-	-	-	-	-
2.	-	-	-	-	-	-	+
3.	-	-	+(1.5)	-	-	-	-
4.	-	+(0.4)	-	-	-	-	+
6.	-	-	-	-	-	-	+(0.1)
7.	-	-	-	-	-	-	-
8.	n	n	n	-	+	n	+(0.4)
9.	-	-	n	+(2.5)	+	+(5)	+(0.6)
10.	-	-	-	-	-	-	-
11.	-	-	-	-	-	-	-
12.	-	-	-	-	-	-	-
13.	-	-	-	-	+	+	-
14.	-	-	-	-	n	-	-
15.	+	+(1.2)	+(1)	n	-	-	-
16.	-	-	-	-	-	-	-
17.	+	n	+(0.5)	n	-	-	-
18.	-	-	-	-	-	-	-
19.	0.8	n	+(1)	n	+	+	-
20.	0.4	300	387(7)	388(5)	+(12)	+(2.5)	3
21.	-	-	n	-	-	-	-
23.	-	-	-	-	-	-	-
24.	+	+(2.4)	+(3)	+(28)	+	+(22)	17
25.	n	n	+(7.5)	n	n	n	+(0.1)
26.	0.3	132	376	1965	252	225	75
27.	-	-	764	1808	312	1035	-
28.	-	-	-	-	-	n	-
29.	-	-	n	-	n	-	-
30.	-	125	82	+(2.5)	-	-	-
31.	-	n	+(1)	-	n	+(28)	+(0.8)
32.	-	-	-	-	-	-	-
33.	+	n	20	n	n	-	n
34.	-	-	-	-	-	-	-
35.	-	-	-	-	-	-	-
36.	-	-	-	-	-	-	-
37.	-	-	-	-	-	-	-
38.	-	-	-	-	-	-	-

APPENDIX 7.1(1) continued

Taxa	Date						
	18-4	25-4	2-5	9-5	16-5	25-5 p	30-5
1.	-	-	-	-	-	n	-
2.	-	-	-	-	-	+	0.2
3.	-	-	-	-	-	-	-
4.	-	-	-	-	+(2)	-	-
6.	-	-	3	-	262	+	+(14)
7.	-	-	-	-	-	-	-
8.	8	+	+	n	-	n	-
9.	+	+(1.4)	+	+(0.1)	+(2)	-	-
10.	-	-	-	-	-	-	-
11.	-	-	-	-	-	-	+
12.	-	5	+	-	130	+(60)	+(4)
13.	-	-	-	-	-	-	-
14.	-	+(1)	+	29	72	+(5)	n
15.	+	+(0.2)	+	-	+(2)	n	+
16.	-	-	-	-	134	-	-
17.	+	+	-	-	+(8)	+	+
18.	-	-	-	-	-	-	-
19.	+(5)	+	+(11)	n	n	-	-
20.	+(2.2)	+(0.4)	+	-	-	+(15)	+
21.	-	-	-	-	-	-	-
23.	+(0.3)	-	+	-	+(6)	-	+
24.	100(13)	+(4.3)	+	+(0.7)	210(1)	+(35)	+(14)
25.	+(0.6)	+(4)	+(25)	n	-	-	-
26.	+(2.5)	+(16)	+	1	+(18)	+(10)	-
27.	+(5.9)	15	4	-	+(2)	1562	+(0.9)
28.	-	-	-	-	-	-	-
29.	-	-	-	-	-	-	-
30.	-	-	-	-	-	-	-
31.	+	+(0.2)	+(11)	+(1)	16(18)	+(90)	+(9)
32.	-	-	-	-	-	-	-
33.	-	+	-	-	n	n	n
34.	-	-	-	-	-	-	-
35.	-	-	-	-	-	-	-
36.	-	-	-	-	-	-	-
37.	-	+(0.2)	+(2)	-	16(18)	+(45)	+(4)
38.	+(0.9)	-	1	-	-	+(15)	-

APPENDIX 7.1(1) continued

Taxa	Date						
	7-6	14-6	20-6 p	27-6	4-7	12-7	18-7
1.	-	-	-	+	-	+	+
2.	0.02	+	+(0.1)	13	0.9	1	0.1
3.	-	+	+	-	-	-	-
4.	-	-	-	-	-	-	-
6.	-	+	-	-	-	+	-
7.	-	-	-	-	-	-	-
8.	-	-	n	-	-	-	-
9.	+	-	-	-	-	-	-
10.	+	+(1.4)	+(4)	-	-	-	-
11.	-	-	-	-	-	-	-
12.	23	2	435	+(13)	2	-	+(0.6)
13.	-	-	+	-	-	-	-
14.	+	+(0.1)	+	+	+(4)	+(4)	n
15.	+	+(1.4)	n	+	+	+(2)	n
16.	-	-	-	-	-	-	-
17.	+	+(0.1)	+(9)	n	n	n	n
18.	-	-	n	+(36)	1	++++	n
19.	+	-	-	84(7)	80(11)	12	+(1)
20.	+	+(1.4)	+(9)	+(4.4)	n	+	-
21.	-	-	-	-	-	-	-
23.	+	+(0.7)	+(13)	-	+(33)	-	-
24.	37(17)	-	+(4)	+(40)	n	+	-
25.	+	+	-	+(122)	-	-	+(1)
26.	+	+(1.3)	+(80)	-	+(3)	+(2)	+(0.6)
27.	+	1	+(22)	+(9)	+(15)	-	+(0.6)
28.	n	-	-	-	-	-	-
29.	+	-	-	-	-	-	-
30.	-	-	-	-	-	-	-
31.	27(14)	+(0.2)	218	+(13)	1(16)	-	-
32.	-	-	-	-	-	-	-
33.	8	n	-	+	-	-	n
34.	-	-	-	-	-	-	-
35.	-	-	-	-	-	-	-
36.	+	-	-	-	-	-	+
37.	+	+(0.1)	+(27)	151(11)	-	-	-
38.	-	-	-	-	-	-	-

APPENDIX 7.1(1) continued

Taxa	Date						
	25-7	31-7	8-8	15-8	22-8	29-8	4-9
	p				p		
1.	-	-	n	-	-	+	+
2.	0.05	0.4	n	0.4	0.4	1.0	0.2
3.	-	-	-	-	-	-	-
4.	-	-	-	-	-	-	-
6.	-	-	-	-	+	-	-
7.	-	n	n	-	-	-	-
8.	-	-	-	-	-	-	-
9.	-	-	-	-	-	-	-
10.	n	-	-	-	n	-	-
11.	-	-	-	-	-	-	-
12.	8	+(8)	-	+	+(7)	-	-
13.	-	-	-	-	-	-	-
14.	+(0.6)	+	n	-	+	+(0.1)	+
15.	-	n	n	+	n	+(0.1)	+
16.	-	-	-	-	-	-	-
17.	n	n	-	+	+(33)	-	+
18.	n	n	-	n	+	n	+
19.	31(16)	n	n	-	-	-	n
20.	-	n	-	-	-	-	-
21.	-	-	-	-	-	-	-
23.	-	-	-	n	-	-	-
24.	-	-	-	n	-	-	-
25.	+(0.6)	+(5)	n	+	183(8)	117	1.4
26.	+(0.3)	+(0.6)	-	n	+(7)	n	n
27.	+(0.3)	-	-	-	-	-	-
28.	-	-	-	-	-	-	-
29.	-	-	-	-	-	-	+
30.	-	-	-	-	-	-	-
31.	+(1)	+(5)	n	+	+(60)	+	-
32.	-	-	-	-	-	-	n
33.	+(2)	n	n	-	-	n	+
34.	-	-	-	-	-	-	-
35.	-	-	-	-	-	n	-
36.	n	+	n	-	n	+	+
37.	-	+(1)	n	-	-	-	-
38.	-	-	-	-	-	-	-

APPENDIX 7.1(1) continued

Taxa	10-9	19-9	26-9	3-10 P	9-10 P	15-10	24-10
1.	-	+	n	+	-	-	-
2.	0.4	1.0	1.6	0.2	+	-	-
3.	-	-	-	-	-	-	-
4.	0.5	-	-	-	-	-	-
6.	-	-	-	-	-	-	-
7.	n	-	-	-	-	-	-
8.	-	-	-	+	-	-	-
9.	-	-	+	+	-	-	-
10.	-	-	-	-	-	-	-
11.	+	+	+(1)	+	+	+(2)	n
12.	+(0.8)	+(0.2)	+(2)	-	-	+	-
13.	-	-	-	-	-	-	-
14.	-	+	-	+	-	-	-
15.	n	+(0.2)	n	+	+(2)	-	+
16.	-	-	+(1)	-	-	-	-
17.	+	n	+	+	+(3)	-	-
18.	n	+(0.2)	n	-	-	-	-
19.	-	+	n	+(22)	+(9)	-	n
20.	-	-	-	-	-	-	-
21.	-	-	-	-	-	-	-
23.	-	-	-	-	-	-	-
24.	-	+(0.2)	-	-	+(1)	+(3)	-
25.	+(3.6)	+(3)	20(7)	48(1)	32(4)	+	+
26.	+(1)	+(0.7)	+(10)	+	+(2.5)	+(7)	-
27.	+(3)	+(0.5)	550	145	+(1.2)	-	-
28.	-	-	-	-	-	-	-
29.	-	-	-	-	-	+(2)	-
30.	-	-	-	-	-	-	-
31.	+(0.4)	+	+(1)	+	+(4)	+	+
32.	-	-	-	-	-	-	-
33.	n	n	n	-	-	-	-
34.	-	-	-	-	-	-	-
35.	n	-	n	-	-	-	-
36.	n	+	n	-	-	-	-
37.	-	+(2)	-	-	-	+(2)	-
38.	-	-	-	-	-	+(8)	-

APPENDIX 7.1(1) continued

Taxa	Date		
	28-11 s	5-12 s	19-12 s
1.	-	-	-
2.	-	-	-
3.	-	-	-
4.	-	-	-
6.	-	-	-
7.	-	-	-
8.	-	-	-
9.	-	-	-
10.	-	-	-
11.	+	+	-
12.	-	-	-
13.	-	-	-
14.	-	-	-
15.	+	+	-
16.	-	-	-
17.	-	-	-
18.	-	-	-
19.	+(255)	+	-
20.	-	-	-
21.	-	-	-
23.	-	-	+
24.	-	-	-
25.	+(10)	+(20)	-
26.	+(5)	+(60)	+
27.	41400	18300	+
28.	-	-	+
29.	+	-	-
30.	-	-	-
31.	+	-	-
32.	-	-	-
33.	-	-	-
34.	-	-	-
35.	-	-	-
36.	-	-	-
37.	-	-	-
38.	-	-	-

APPENDIX 7.1

2. April to October 1986

Taxa	Date						
	3-4	17-4	26-4	1-5	7-5	15-5	22-5
1.	-	-	-	-	-	-	-
2.	n	-	n	n	-	-	-
5.	-	-	-	-	-	-	-
6.	-	+	-	-	-	-	-
7.	-	-	-	-	-	-	-
8.	-	-	-	-	-	-	-
9.	n	-	+	-	-	-	-
10.	-	-	-	-	-	-	-
11.	-	-	-	-	-	-	+
12.	-	-	-	-	-	-	+
14.	-	-	-	-	-	-	+
15.	-	-	+	-	-	-	+
16.	n	-	n	-	-	n	n
17.	-	-	n	-	-	-	-
18.	-	-	-	-	-	-	-
19.	-	-	n	-	-	-	-
20.	n	+(4)	115(2)	n	-	n	-
22.	n	-	-	-	-	-	-
23.	n	n	-	n	-	-	-
24.	-	+(2)	n	n	-	-	-
25.	-	n	-	-	-	-	-
26.	n	+	435	-	n	-	-
27.	n	75	6025	n	-	+	85
28.	n	-	n	-	-	-	-
29.	-	-	-	-	-	+	-
31.	-	-	-	-	-	-	-
32.	-	-	-	-	-	-	-
33.	-	-	-	-	-	-	-
34.	n	-	-	-	-	-	-
35.	-	-	-	-	-	-	-
36.	-	-	-	n	-	-	-
37.	-	-	-	-	-	-	-
38.	-	-	-	-	-	+	-

APPENDIX 7.1(2) continued

Taxa	Date						
	29-5	5-6	10-6	17-6	26-6	3-7	8-7
1.	-	-	-	-	-	-	+
2.	n	n	n	n	-	-	-
5.	+	-	-	+	+	+	+
6.	-	-	n	n	-	-	-
7.	-	-	-	-	-	n	n
8.	-	-	+	-	-	-	n
9.	-	+	-	-	+	-	-
10.	-	-	-	-	-	-	n
11.	+	+	-	-	-	-	n
12.	+	-	-	+	+	+	13
14.	-	+	-	+	-	+	+
15.	+	-	n	+	-	+	-
16.	-	-	-	-	+	+	+
17.	+	-	-	-	-	-	-
18.	-	-	-	-	-	-	n
19.	-	-	-	43(2)	+(12)	+(8)	-
20.	+	-	-	-	-	-	-
22.	-	-	-	-	-	-	-
23.	-	-	-	-	-	-	-
24.	-	-	-	-	-	-	-
25.	-	-	n	-	-	-	+(10)
26.	-	-	-	+	-	-	-
27.	173	128	164	158	-	93	129
28.	-	-	-	-	-	-	-
29.	-	-	-	-	-	-	-
31.	-	-	+(10)	11(22)	262(22)	285(12)	+(13)
32.	-	+	-	-	-	-	n
33.	-	+	-	-	-	n	-
34.	+	-	-	+	-	-	-
35.	-	-	-	-	-	-	-
36.	-	-	+	+	-	-	-
37.	-	-	-	-	-	-	-
38.	-	18	+		-	-	-

APPENDIX 7.1(2) continued

Taxa	17-7	24-7	31-7	7-8	14-8	28-8	4-9
1.	-	-	-	+	+	-	-
2.	-	-	-	-	+	-	n
5.	-	-	-	-	-	-	-
6.	-	-	+	-	-	-	-
7.	n	n	-	n	-	-	-
8.	-	n	+	-	-	-	-
9.	-	-	-	-	-	-	-
10.	+	-	-	-	-	-	-
11.	-	-	n	+	-	+	-
12.	-	-	n	-	+	-	n
14.	n	n	n	+	-	-	n
15.	+	-	+	-	+	+	n
16.	+	-	-	-	-	-	-
17.	-	-	-	-	+	-	+
18.	n	n	n	n	n	n	n
19.	-	-	-	+	-	-	-
20.	-	-	-	-	-	-	-
22.	-	-	-	-	-	-	-
23.	-	-	-	-	-	-	-
24.	-	-	-	-	-	-	-
25.	+(21)	-	15(14)	364	106	76	+(6)
26.	142	-	-	-	-	-	+
27.	+	-	+	+	-	590	67
28.	-	-	-	-	+	-	-
29.	+	-	-	-	-	-	-
31.	+(35)	n	87(29)	40(16)	+(8)	+(18)	+
32.	n	n	-	n	-	-	n
33.	-	-	-	-	-	-	-
34.	-	-	-	-	-	-	-
35.	-	-	-	-	-	-	-
36.	-	-	-	-	+	-	n
37.	-	-	-	n	-	-	-
38.	-	-	+	-	-	-	+

APPENDIX 7.1(2) continued

Taxa	Date			
	11-9	18-9	2-10	9-10
1.	-	-	-	-
2.	-	n	n	-
5.	-	-	-	-
6.	-	+	+	+(11)
7.	-	-	n	-
8.	+	n	-	-
9.	-	-	-	-
10.	-	-	-	-
11.	+	+	+	+
12.	+	+	-	n
14.	n	+	+	n
15.	+	+	+	+
16.	-	-	-	-
17.	-	-	-	-
18.	+	n	-	-
19.	+(38)	-	12(21)	-
20.	-	-	-	-
22.	-	-	-	-
23.	-	-	-	-
24.	-	-	-	-
25.	+	4	+(18)	n
26.	+	+	-	-
27.	21	47	16	+
28.	-	-	-	-
29.	-	-	+	-
31.	21(21)	6(11)	33(19)	+(9)
32.	+	-	n	-
33.	-	n	-	-
34.	-	-	-	-
35.	-	-	-	-
36.	-	n	+	-
37.	-	-	-	-
38.	+	-	-	-

APPENDIX 7.2 Assessment of water turbulence in Island Barn Reservoir using the Brunt-Väisälä Frequency

1. 1985

Date	Temperature (°C)			Water density ($x10^{-5}$)		N^2($x10^{-4}s^{-2}$)
	0m	6m	Mean	$\delta\rho$	$\bar{\rho}$	
14-6	14.8	14.4	14.6	5.7	99918.7	0.93
27-6	15.7	15.5	15.6	11.0	99903.5	1.79
4-7	19.4	16.1	17.9	60.0	99864.3	9.79
12-7	19.7	16.9	19.2	52.7	99839.5	8.60
18-7	19.2	16.7	18.9	45.9	99845.4	7.49
31-7	18.5	18.2	18.3	5.7	99856.8	0.93
8-8	17.2	17.2	17.2	0.0	99876.8	0.00
15-8	17.2	17.1	17.1	1.8	99878.6	0.29
28-8	17.5	17.1	17.2	7.2	99876.8	1.18

Σ = 30.00

$\bar{x}$ = 3.30

2. 1986

Date	Temperature (°C)			Water density ($x10^{-5}$)		N^2($x10^{-4}s^{-2}$)
	0m	6m	Mean	$\delta\rho$	$\bar{\rho}$	
17-6	19.6	14.3	16.8	91.6	99883.8	14.9
26-6	19.1	16.4	18.3	49.0	99856.8	8.0
3-7	20.5	18.9	19.9	32.6	99805.3	5.3
8-7	19.3	18.9	19.1	7.9	99841.5	1.3
17-7	20.7	19.3	20.1	28.9	99821.3	4.7
24-7	18.7	18.5	18.6	3.8	99851.1	0.6
31-7	17.6	18.1	18.1	9.1	99860.6	1.5
14-8	18.5	18.0	18.4	9.5	99854.9	1.6
21-8	17.5	17.5	17.5	0.0	99871.4	0.0
28-8	15.5	15.7	15.7	11.0	99901.9	1.8

Σ = 39.70

$\bar{x}$ = 3.97

APPENDIX 7.3 Assessment of water turbulence in Queen Mother Reservoir using the Brunt-Väisälä Frequency

Date	Temperature (°C)			Water density (x10^{-5})		N^2(x10^{-4}s^{-2})
	0m	6m	Mean	$\delta\rho$	$\bar{\rho}$	
19-6	17.0	13.4	15.2	5.5	99909.8	9.0
26-6	16.4	13.8	15.6	3.9	99903.5	6.4
3-7	20.6	13.8	17.3	11.9	99875.0	19.5
10-7	18.1	15.4	17.0	4.6	99880.4	7.5
17-7	21.0	14.3	17.8	12.1	99866.1	19.7
23-7	18.8	14.6	16.5	7.1	99888.8	11.6
31-7	16.6	15.1	16.2	2.4	99893.8	3.9
7-8	15.3	15.4	15.4	0.2	99906.6	0.3
13-8	16.3	15.4	15.8	1.4	99900.3	2.3
21-8	16.2	16.0	16.1	0.3	99895.5	0.5
28-8	15.7	15.6	15.6	0.2	99903.5	0.3
					Σ =	81.1
					$\bar{x}$ =	7.4

CHAPTER 8

SUMMARY

It was established that two species of Ceratium, C. furcoides and C. hirundinella, were present in the phytoplankton of Esthwaite Water, Cumbria, and in various sites in the Thames Valley. Identification of cells was based on the shape of the epitheca, the overall linear dimensions and the number of thecal plates reaching the apex of the cell. The number and size of the posterior horns were deemed unreliable taxonomic characteristics, as they displayed seasonal variation. Cell length and breadth also varied seasonally, but C. furcoides cells were shown to always remain longer and more slender.
Cysts were similarly distinguished on the basis of morphological characteristics. In addition, scanning electron microscopy indicated that, in both species, the cyst wall was formed of a continuous, and a discontinuous, granular layer, found to contain silicon. In C. hirundinella this structure appeared more highly organised, with granules tightly packed in a geometric arrangement, and registering high silicon values. The cyst walls of C. furcoides included granules which were less densely spaced, and silicon values which were considerably lower. The possible advantages of the incorporation of silicon were discussed.

A study was made of the Ceratium phytoplankton population of samples covering a period of 41 years (1946-1986) taken from the same lake. It was established that the relative proportion

of C. hirundinella and C. furcoides, although remaining constant through a single season, varied considerably over a longer period of time. The resulting change from dominance of one species to the other was shown to occur rapidly. This situation is believed to result not from direct competition, but from the relative ability of cysts of the two species to survive. Parasitism of the cyst (for example, by Rhizophydium nobile) or of the cells prior to encystment (for example, by Aphanomycopsis cryptica), will result in a decline in the cyst population and may affect one species more than the other.

A study of the Ceratium species present in the phytoplankton of various Thames Valley sites was conducted over only one or two years and in view of the previous observations cannot be regarded as a permanent situation. However, it is interesting to note that the greater proportion of C. hirundinella in the reservoir sites was replaced by a large C. furcoides population in Virginia Water Lake. No parasites were observed in any of the southern sites. The size of the Ceratium cell population in the Thames Valley sites, (no equivalent study was made of the phytoplankton in Esthwaite Water), was shown to be adversely affected by instability within the water column. The Ceratium populations from two sites were demonstrated to decline in numbers when turbulence caused the premature breakdown of the thermocline, as a result of cool unsettled weather or the high velocity input of water. It is uncertain whether the decrease in the number of Ceratium cells was due directly to the physical effects of mixing, or indirectly to the

redistribution of nutrients, which may favour the growth of other genera.

A study of viable Ceratium cysts concentrated on their vertical distribution in 8 cm cores from Esthwaite Water. Preserved cores were shown to contain more cysts than fresh cores, stored at below 4°C. The shortfall in numbers in the latter was taken as representing cysts which had excysted whilst cores were transferred from the Lake District to the south of England. Variation in cyst numbers was also noted between cores taken at different times of the year. Clearly cores taken soon after encystment would be expected to contain more cysts than cores taken the following spring, where cysts might have been lost through disease, predation or redistribution to a lower level. Further differences were shown between cores taken from different basins of the lake, and from shallow and deep water sites. As would be expected, deeper water locations yielded cores with more cysts, possibly as a consequence of sediment focusing.

An approximation to the rate of sedimentation was determined using the diatom Stephanodiscus parvus as a "marker" species. The average annual accumulation of sediment was estimated to be 0.8 cm $year^{-1}$. Viable cysts were induced to germinate in the laboratory when introduced into a culture medium and exposed to an increase in temperature. Although fluorescing cysts were present throughout the 8 cm cores germination only occurred to a depth of 5.5 cm. These cysts would thus be expected to be up to 7 years old.

The vertical distribution of cysts of both species was compared with the numbers of cells in the phytoplankton of what was judged to be the corresponding period. Some agreement was shown between the total number of cysts and the number of cells. However, a correlation between the proportion of C. furcoides cysts and cells was less evident.

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LITERATURE CITED

Anderson, D.M. (1980). Effects of temperature conditioning on development and germination of Gonyaulax tamarensis (Dinophyceae) hypnozygotes. Journal of Phycology, 16, 166-172.

Anderson, D.M., Aubrey, D.G., Tyler, M.A. and Coats, D.W. (1982). Vertical and horizontal distribution of dinoflagellate cysts in sediments. Limnology and Oceanography, 27(4), 757-765.

Anderson, D.M., Coats, D.W. and Tyler, M.A. (1985). Encystment of the dinoflagellate Gyrodinium uncatenum: Temperature and nutrient effects. Journal of Phycology, 21, 200-206.

Anderson, D.M., Kulis, D.M. and Binder, B.J. (1984). Sexuality and cyst formation in the dinoflagellate Gonyaulax tamarensis: cyst yield in batch cultures. Journal of Phycology, 20, 418-425.

Anderson, D.M., Lively, J.J., Reardon, E.M. and Price, C.A. (1985). Sinking characteristics of dinoflagellate cysts. Limnology and Oceanography, 30(5), 1000-1009.

Anderson, D.M. and Morel, F.M.M. (1979). The seeding of two red tide blooms by the germination of benthic Gonyaulax tamarensis hypnocysts. Estuarine and Coastal Marine Science, 8, 279-293.

Anderson, D.M., Taylor, C.D. and Armbrust, E.V. (1987). The effects of darkness and anaerobiosis on dinoflagellate cyst germination. Limnology and Oceanography, 32(2), 340-351.

Anderson, D.M. and Wall, D. (1978). Potential importance of benthic cysts of Gonyaulax tamarensis and G. excavata in initiating toxic dinoflagellate blooms. Journal of Phycology, 14(2), 224-234.

Atkinson, K.M. (1980). Experiments in dispersal of phytoplankton by ducks. British Phycological Journal, 15, 49-58.

Bailey-Watts, A.E. (1978). A nine year study of the phytoplankton of the eutrophic and non-stratifying Loch Leven (Kinross, Scotland). Journal of Ecology, 66, 741-771.

Beam, C.A. and Himes, M. (1980). Sexuality and meiosis in dinoflagellates. pp. 171-206. In: Biochemistry and Physiology of Protoza (Second Edition), 3, (eds.) M. Levandowsky and S.H. Hutner, Academic Press, London.

Belcher, J.H. and Swale, E.M.F. (1976). A Beginners Guide to Freshwater Algae. H.M.S.O., London. 48 pages.

Belcher, J.H. and Swale, E.M.F. (1979). An Illustrated Guide to River Phytoplankton. H.M.S.O., London. 64 pages.

Bellinger, E.G. (1980). A Key to Common British Algae. Institute of Water Engineers and Scientists. 94 pages.

Binder, B.J. and Anderson, D.M. (1986). Green light-mediated photomorphogenesis in a dinoflagellate resting cyst. Nature, 322, 659-661.

Bloesch, J. and Uehlinger, U. (1986) Horizontal sedimentation differences in a eutrophic Swiss lake. Limnology and Oceanography, 31(5), 1094-1109.

Bodbacka, L. (1986). Sediment accumulation in Lakes Lilla Ullfjärden and Stora Ullfjärden, Sweden. Hydrobiologia, 143, 337-342.

Boltovskoy, A. (1984). Relacion huesped-parasito entre el quiste de Peridinium willei y el oomicete Aphanomycopsis peridinella. Limnobios, 2(8), 635-645.

Bourrelly, P. (1968). Notes sur les Péridiniens d'eau Douce. Protistologica, 4(1), 5-16.

Bowen, R.A., St Onge, J.M., Colton, J.B. and Price, C.A. (1972). Density-gradient centrifugation as an aid to sorting phytoplanktonic organisms. Marine Biology, 14, 242-247.

Bruno, S.F. (1975). Cultural Studies on the Ecology of Ceratium hirundinella (O.F.M.) Bergh, with Notes on Cyclomorphism. Ph.D. Thesis, Fordham University.

Bruno, S.F. and McLaughlin, J.J.A. (1977). The nutrition of the freshwater dinoflagellate Ceratium hirundinella. Journal of Protozoology, 24(4), 548-553.

Butterwick, C., Heaney, S.I. and Talling, J.F. (1982). A comparison of eight methods for estimating the biomass and growth of planktonic algae. British Phycological Journal, 17, 69-79.

Canter, H.M. (1968). Studies on British chytrids. XXVIII. Rhizophydium nobile sp. nov., parasitic on the resting spore of Ceratium hirundinella O.F. Müller from the plankton. Proceedings of the Linnean Society London, 179(2), 197-201.

Canter, H.M. and Heaney, S.I. (1984). Observations on zoosporic fungi of Ceratium spp. in lakes of the English Lake District; Importance for phytoplankton population dynamics. New Phytologist, 97, 601-612.

Chapman, D.V. (1981). An Ultrastructural and Ecological Study of the Dinoflagellate Ceratium hirundinella. Ph.D. Thesis, University of London. 226 pages.

Chapman, D.V., Dodge, J.D. and Heaney, S.I. (1982). Cyst formation in the freshwater dinoflagellate Ceratium hirundinella (Dinophyceae). Journal of Phycology, 18, 121-129.

Chapman, D.V., Dodge, J.D. and Heaney, S.I. (1985). Seasonal and diel changes in ultrastructure in the dinoflagellate Ceratium hirundinella. Journal of Plankton Research, 7(2), 263-278.

Chapman, D.V., Livingstone, D. and Dodge, J.D. (1981). An electron microscope study of the excystment and early development of the dinoflagellate Ceratium hirundinella. British Phycological Journal, 16, 183-194.

Daily, W.A. (1960). Forms of Ceratium hirundinella (O.F. Müller) Schrank in lakes and ponds of Indiana. Proceedings of Indiana Academy of Science, 70, 213-215.

Dale, B. (1976). Cyst formation, sedimentation and preservation: factors affecting dinoflagellate assemblages in recent sediments from Trondheimsfjord, Norway. Review of Palaeobotany and Palynology, 22, 39-60.

Dale, B. (1979). Collection, preparation and identification of dinoflagellate resting cysts. pp. 443-452. In: Toxic Dinoflagellate Blooms. (eds.) D.L. Taylor and H.H. Seliger, Developments in Marine Biology, 1, Elsevier, Amsterdam. 505 pages.

Dale, B. (1983). Dinoflagellate resting cysts: Benthic plankton. pp. 69-136. In: Survival Strategies of the Algae. (ed.) G.A. Fryxell, Cambridge University Press, Cambridge. 144 pages.

Davis, M.B. (1973). Redeposition of pollen grains in lake sediment. Limnology and Oceanography, 18, 45-52.

Davis, M.B., Moeller, R.E. and Ford, J. (1984). Sediment focusing and pollen influx. In: Lake Sediments and Environmental History. (eds.) E.Y. Haworth and J.W.G. Lund, Leceister University Press, Leicester. 411 pages.

Davis, R.B. (1974). The stratigraphic effects of tubificids in profundal lake sediments. Limnology and Oceanography, 19, 466-488.

Davison, W., Woof, C. and Rigg, E. (1982). The dynamics of iron and manganese in a seasonally anoxic lake; direct measurements of fluxes using sedimentation traps. Limnology and Oceanography, 27, 987-1003.

Dodge, J.D. (1984). Dinoflagellate taxonomy. pp. 17-42. In: Dinoflagellates. (ed.) D.L.Spector, Academic Press, London. 545 pages.

Dodge, J.D. and Crawford, R.M. (1970). The morphology and fine structure of Ceratium hirundinella (Dinophyceae). Journal of Phycology, 6, 137-149.

Dottne-Lindgren, A. and Ekbohm, G. (1975). Ceratium hirundinella in Lake Erken: Horizontal distribution and form variation. Internat. Revue ges. Hydrobiol., 60(1), 115-144.

Drake, J.C. and Heaney, S.I. (1987). Occurrence of phosphorus and its potential remobilization in the littoral sediments of a productive English lake. Freshwater Biology, 17, 513-523.

Elliott, J.M. (1983). Some Methods for the Statistical Analysis of Samples of Benthic Invertebrates. Second Edition (Revised). Freshwater Biological Association Scientific Publication, 25. 160 pages.

Entz, G. (1924). On chain formation in Ceratium hirundinella. Biologica Hung., 1(3), 1-5.

Entz, G. (1925). Über cysten und encystierung der süsswasser Ceratien. Archiv für Protistenkunde, 51, 131-183.

Entz, G. (1927). Beiträge zur kenntnis der Peridineen. II. resp. VII. Studien an süsswasser-Ceratien. (Morphologie, Variation, Biologie). Archiv für Protistenkunde, 58, 344-440.

Entz, G. (1931). Cytologische beobachtungen an zwei auchim balaton vorkommenden Dinoflagellaten. Arb. der I. Abtl. des Ungarischen Biol. Forschungsinstitutes, 4, 1-9.

Eren, J. (1969). Studies on the development cycle of Peridinium cinctum f. westii. Verh. Internat. Verein. Limnol., 17, 1013-1016.

Evans, J.H. (1958). The survival of freshwater algae during dry periods. Part I. An investigation of the algae of five small ponds. Journal of Ecology, 46, 149-167.

Evans, J.H. (1959). The survival of freshwater algae during dry periods. Part II. Drying experiments. Part III. Stratification of algae in pond margin litter and mud. Journal of Ecology, 47, 55-81.

Evitt, W.R. (1985). Sporopollenin Dinoflagellate Cysts: Their Morphology and Interpretation. American Association of Stratigraphic Palynologists Foundation. 333 pages.

Franks, J.W. and Pennington, W. (1961). The late glacial and post glacial deposits of the Esthwaite Basin, North Lancashire. The New Phytologist, 60, 27-42.

Frempong, E. (1981a). Diel periodicity in the chemical composition of lake phytoplankton. Archiv für Hydrobiologie, 92, 457-495.

Frempong, E. (1981b). Diel variation in the abundance, vertical distribution and species composition of phytoplankton in a eutrophic English lake. Journal of Ecology, 69, 919-939.

Frempong, E. (1982). The space-time resolution of phased cell division in natural populations of the freshwater dinoflagellate Ceratium hirundinella. Internat. Revue ges. Hydrobiol., 67(3), 323-339.

Frempong, E. (1984). A seasonal sequence of diel distribution patterns for the planktonic dinoflagellate Ceratium hirundinella in a eutrophic lake. Freshwater Biology, 14, 401-421.

George, D.G. and Heaney, S.I. (1978). Factors influencing the spatial distribution of phytoplankton in a small productive lake. Journal of Ecology, 66, 133-155.

Gorham, E. (1958). Observations on the formation and breakdown of the oxidised microzone at the mud surface in lakes. Limnology and Oceanography, 3, 291-298.

Gorham, E. (1960). Chlorophyll derivatives in surface muds from the English Lakes. Limnology and Oceanography, 5, 29-33.

Gorham, E., Lund, J.W.G., Sanger, J.E. and Dean, W.E.D., Jr. (1974). Some relationships between algal standing crop, water chemistry and sediment chemistry in the English Lakes. Limnology and Oceanography, 19, 601-617.

Haffner, G.D. (1974). Seston Distribution and Phytoplankton Production in a New, Eutrophic Reservoir Subject to Artificial Mixing. Ph.D. Thesis, University of London.

Håkanson, L. and Jansson, M. (1983). Principles of Lake Sedimentology. Springer-Verlag, Berlin. 316 pages.

Hardy, D.M. (1977). An Investigation of the Phytoplankton and Environmental Conditions in a New Thames Valley Reservoir Subject to Artificial Turbulence. Ph.D. Thesis, University of London.

Harris, G.P., Heaney, S.I. and Talling, J.F. (1979). Physiological and environmental constraints in the ecology of the planktonic dinoflagellate Ceratium hirundinella. Freshwater Biology, 9, 413-428.

Hasle, G.R. and Nordli, E. (1951). Form variation in Ceratium fusus and C. tripos populations in cultures and from the sea. Avhandlinger Utgitt av det Norske Videnskaps. 1. Mat. Naturv. Klasse, 4, Akademi I Oslo.

Hauge, H.V. (1958). On the freshwater species of Ceratium. Nytt. Magasin for Botikk., 6, 97-119.

Haworth, E.Y. (1976). The changes in the composition of the diatom assemblages found in the surface sediments of Blelham Tarn in the English Lake District during 1973. Annals of Botany, 40, 1195-1205.

Haworth, E.Y. (1979). The distribution of a species of Stephanodiscus in the recent sediments of Blelham Tarn, English Lake District. Nova Hedwigia, 64, 395-410.

Haworth, E.Y. (1980). Comparison of continuous phytoplankton records with the diatom stratigraphy in the recent sediments of Blelham Tarn. Limnology and Oceanography, 25(6), 1093-1103.

Haworth, E.Y. (1985). The highly nervous system of the English Lakes: Aquatic ecosystem sensitivity to external changes, as demonstrated by diatoms. Freshwater Biological Association Annual Report, 53, 60-79.

Heaney, S.I. (1976). Temporal and spatial distribution of the dinoflagellate Ceratium hirundinella O.F. Müller within a small productive lake. Freshwater Biology, 6, 531-542.

Heaney, S.I. (1978). Some observations on the use of the in vivo fluorescence technique to determine chlorophyll-a in natural populations and cultures of freshwater phytoplankton. Freshwater Biology, 8, 115-126.

Heaney, S.I. and Butterwick, C. (1985). Comparative mechanisms of algal movement in relation to phytoplankton production. pp. 114-134. In: Migration: Mechanisms and Adaptive Significance. (ed.) M.A. Rankin, Contributions in Marine Science Supplement, 27.

Heaney, S.I., Chapman, D.V. and Morison, H.R. (1983). The role of the cyst stage in the seasonal growth of the dinoflagellate Ceratium hirundinella within a small productive lake. British Phycological Journal, 18, 47-59.

Heaney, S.I., Lund, J.W.G., Canter, H.M. and Gray, K. (1988). Population dynamics of Ceratium spp. in three English lakes, 1945-1985. Hydrobiologia, 161, 133-148.

Heaney, S.I., Smyly, W.J.P. and Talling, J.F. (1986). Interactions of physical, chemical and biological processes in depth and time within a productive English lake during summer stratification. Internat. Revue ges. Hydrobiol., 71(4), 441-494.

Heaney, S.I. and Talling, J.F. (1980a). Dynamic aspects of dinoflagellate distribution patterns in a small productive lake. Journal of Ecology, 68, 75-94.

Heaney, S.I. and Talling, J.F. (1980b). Ceratium hirundinella - Ecology of a complex, mobile and successful plant. Freshwater Biological Association Annual Report, 48, 26-40.

Heller, M.D. (1977). The phased division of the freshwater dinoflagellate Ceratium hirundinella and its use as a method of assessing growth in natural populations. Freshwater Biology, 7, 527-533.

Herrgesell, P.L., Sibley, T.H. and Knight, A.W. (1976). Some observations on dinoflagellate population density during a bloom in a California reservoir. Limnology and Oceanography, 21(4), 619-624.

Hickel, B. (1985). The population structure of Ceratium in a small eutrophic lake. Verh. Internat. Verein. Limnol., 22, 2845-2849.

Hickel, B. (1988a). Sexual reproduction and life cycle of Ceratium furcoides (Dinophyceae) in situ in the Lake Plusssee (F.R.). Hydrobiologia, 161, 41-48.

Hickel, B. (1988b). Morphology and life cycle of Ceratium rhomvoides nov. sp. (Dinophyceae) from the Lake Plusssee (Federal Republic). Hydrobiologia, 161, 49-54.

Hilton, J. and Gibbs, M.M. (1984). The horizontal distribution of major elements and organic matter in the sediment of Esthwaite Water, England. Chemical Geology, 47, 57-83.

Hilton, J., Lishman, J.P. and Allen, P.V. (1986). The dominant processes of sediment distribution and focusing in a small, eutrophic, monomictic lake. Limnology and Oceanography, 31(1), 125-133.

Huber, G. and Nipkow, F. (1922). Experimentelle Untersuchungen über die Entwicklung von Ceratium hirundinella O.F.M. Zeitschrift für Botanik, 14, 337-371.

Huber, G. and Nipkow, F. (1923). Experimentelle untersuchungen über entwicklung und formbildung von Ceratium hirundinella O.F.M. Flora, 116, 114-215.

Huber-Pestalozzi, G. (1950). Das Phytoplankton des Süsswassers. Systematik und Biologie, 3 Tiel, Cryptophyceen, Chloromonadinen, Peridineen. pp. 252-284. E. Schweizerbart'sche Verlagsbuchlandlung, Stuttgart. 310 pages.

Hurst, C.T. and Strong, D.R. (1931). Studies on the plates of the freshwater Ceratium, the so-called C. hirundinella. Archiv für Protistenkunde, 73, 104-110.

Hutchinson, G.E. (1967). A Treatise on Limnology. Volume II. Introduction to Lake Biology and the Limnoplankton. pp. 910-921. Wiley, New York. 1115 pages.

Kell, G.S. (1967). Precise representation of volume properties of water at one atmosphere. Journal of Chemical Engineering Data, 12, 66-69.

Kofoid, C.A. (1907). The plates of Ceratium with a note on the unity of the genus. Zoologischen Anzeiger, 32(7), 177-183.

Krupa, D. (1981a). Ceratium hirundinella (O.F. Müller) Bergh in two trophically different lakes. I. Population dynamics (with the cysts taken into account). Ekologia Polska, 29(4), 545-570.

Krupa, D. (1981b). Ceratium hirundinella (O.F. Müller) Bergh in two trophically different lakes. II. Development and morphological variation of active forms and cysts. Ekologia Polska, 29(4), 571-583.

Langhans, V.H. (1925). Gemischte populationen von Ceratium hirundinella (O.F.M.) Schrank und ihre deutung. Archiv für Protistenkunde, 52, 585-602.

Lewis, J.M., Tett, P. and Dodge, J.D. (1985). The cyst-theca cycle of Gonyaulax polyedra (Lingulodinium machaerophorum) in Creran, a Scottish west coast sea-loch. pp. 85-90. In: Toxic Dinoflagellates. Proceedings of the Third International Conference on Toxic Dinoflagellates. (eds.) D.M. Anderson, A.W. White and D.G. Baden, Elsevier, Amsterdam. 561 pages.

Likens, G.E. and Davis, M.B. (1975). Post glacial history of Mirror Lake and its watershed in New Hampshire, U.S.A.: An initial report. Verh. Internat. Verein. Theor. Angew. Limnol., 19(2), 982-993.

Livingstone, D. (1979). Algal Remains in Recent Lake Sediments. Ph.D. Thesis, University of Leicester. 247 pages.

Lorenzen, M.W. and Mitchell, R. (1975). An evaluation of artificial destratification for control of algal blooms. Journal of American Waterworkers Association, 67, 373-376.

Lund, J.W.G. (1949). Studies on Asterionella. I. The origin and nature of the cells producing seasonal maxima. Journal of Ecology, 37, 389-419.

Lund, J.W.G. (1950). Studies on Asterionella formosa Hass. II. Nutrient depletion and the spring maximum. Part I. Observations on Windermere, Esthwaite Water and Blelham Tarn. Journal of Ecology, 38, 1-14.

Lund, J.W.G. (1954). The seasonal cycle of the plankton diatom Melosira italica (Ehr.) Kütz. subsp. subarctica O.F. Müll. Journal of Ecology, 42, 151-179.

Lund, J.W.G. (1955). Further observations on the seasonal cycle of Melosira italica (Ehr.) Kütz. subsp. subarctica O.F. Müll. Journal of Ecology, 43(1), 90-102.

Lund, J.W.G. (1959). A simple counting chamber for nannoplankton. Limnology and Oceanography, 4(1), 57-65.

Lund, J.W.G. (1962). Concerning a counting chamber for nannoplankton described previously. Limnology and Oceanography, 7(2), 261-262.

Lund, J.W.G. (1978). Changes in the phytoplankton of an English lake 1945-1977. Hydrobiological Journal, 14(1), 6-21.

Lund, J.W.G. (1981). Investigations on Phytoplankton with Special Reference to Water Usage. Freshwater Biological Association Occasional Publication, 13. 64 pages.

Lund, J.W.G., Kipling, C. and Le Cren, E.D. (1958). The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting. Hydrobiologia, 11(2), 143-170.

McAlice, B.J. (1971). Phytoplankton sampling with the Sedgewick Rafter Cell. Limnology and Oceanography, 16, 19-28.

McGill, S.M. (1969). The Ecology of the Phytoplankton of a New Reservoir in the Thames Valley 1965-1969. Ph.D. Thesis, University of London. 215 pages.

Macan, T.T. (1970). Biological Studies of the English Lakes. Longman, London. 260 pages.

Mackereth, F.J.H. (1965). Chemical investigation of lake sediments and their interpretation. Proceedings of the Royal Society (B), 161, 295-309.

Mackereth, F.J.H. (1966). Some chemical observations on post-glacial lake sediments. Phil. Trans. R. Soc. (B), 250, 165-213.

Mërilainen, J. (1971). The recent sedimentation of diatom frustules in four meromictic lakes. Ann. Bot. Fennici., 8, 160-176.

Moore, J.W. (1977). Some factors influencing the density of sub-arctic populations of Bosmina longirostris, Holopedium gibberum, Codonella cratera and Ceratium hirundinella. Hydrobiologia, 56, 199-207.

Moore, J.W. (1981). Seasonal abundance of Ceratium hirundinella (O.F. Müller) Schrank in lakes of different trophy. Archiv für Hydrobiologie, 92(4), 535-548.

Moore, P.D. and Webb, J.A. (1978). An Illustrated Guide to Pollen Analysis. pp. 83-89, Hodder and Stoughton, London.

Morling, G. (1979). Ceratium carolinianum and related C. carolinianum var. elongatum and C. cornutum. A study of the distribution and morphology. Nova Hedwigia, 31(4), 937-968.

Mortimer, C.H. (1941). The exchange of dissolved substances between mud and water in lakes. I and II. Journal of Ecology, 29, 280-329.

Mortimer, C.H. (1942). The exchange of dissolved substances between mud and water in lakes. III and IV. Journal of Ecology, 30, 147-201.

Mortimer, C.H. (1971). Chemical exchanges between sediments and water in the Great Lakes - Speculations on probable regulatory mechanisms. Limnology and Oceanography, 16, 387-404.

Mortimer, C.H. (1974). Lake hydrodynamics. Mitt. Internat. Verein. Limnol., 20, 124-197.

Mueller Elser, M. and Smith, W.O. (1985). Phased cell division and growth rate of a planktonic dinoflagellate, **Ceratium hirundinella**, in relation to environmental variables. Archiv für Hydrobiologie, 104(4), 477-491.

Mundie, J.H. (1965). Data in Director's Report. Freshwater Biological Association Annual Report, 33, 39.

Nakomoto, N. (1975). A freshwater red tide on a water reservoir. Japanese Journal of Limnology, 36, 55-64.

Nicholls, K.H., Kennedy, W. and Hammett (1980). A fish-kill in Heart Lake, Ontario, associated with the collapse of a massive population of **Ceratium hirundinella** (Dinophyceae). Freshwater Biology, 10, 553-561.

Ohnstad, F.R. and Jones, J.G. (1982). The Jenkin Surface-Mud Sampler User Manual. Freshwater Biological Association Occasional Publication, 15. 45 pages.

Okada, Y. (1933). Abnormalities in **Ceratium hirundinella** found in Hokkaido, Japan. Annotationes Zoologicae Japanenses, 14(2), 191-192.

Patalas, K. (1954). Povownawcze badania nad nowym tynem samoczynnego czerpacza planktonowego i hydrochemiczneg. Ekologia Polska (A), 2, 231-242.

Pearsall, W.H. (1929). Form variation in **Ceratium hirundinella** (O.F.M.) Proceedings of the Leeds Philosophical Society, 1(9), 432-439.

Pearsall, W.H. (1930). Phytoplankton in the English Lakes. I. The proportions in the waters of some dissolved substances of biological importance. Journal of Ecology, 18(2), 306-320.

Pearsall, W.H. (1932). Phytoplankton in the English Lakes. II. The composition of the phytoplankton in relation to dissolved substances. Journal of Ecology, 20, 241-261.

Pearsall, W.H. and Pennington, W. (1973). The Lake District: A Landscape History. The New Naturalist, Collins, London.

Pennington, W. (1943). Lake sediments: The bottom deposits of the North Basin of Windermere, with special reference to the diatom succession. The New Phytologist, 42(1), 1-27.

Pennington, W. (1974). Seston and sediment formation in five Lake District lakes. Journal of Ecology, 62, 215-251.

Pennington, W. (1978). Responses of some British lakes to past changes in land use on their catchments. Verh. Internat. Verein. Limnol., 20(1), 636-641.

Pennington, W., Cambray, R.S., Eakins, J.D. and Harkness, D.D. (1976). Radionuclide dating of the recent sediments of Blelham Tarn. Freshwater Biology, 6, 317-331.

Pennington, W., Cambray, R.S. and Fisher, E.M. (1973). Observations on lake sediment using fallout ^{137}Cs as a tracer. Nature, 242, 324-326.

Pfiester, L.A. (1971). Periodicity of **Ceratium hirundinella** (O.F.M.) Dujardin and **Peridinium cinctum** (O.F.M.) Ehrenberg in relation to certain ecological factors. Castanea, 36(4), 246-257.

Pfiester, L.A. (1975). Sexual reproduction of **Peridinium cinctum** f. **ovoplanum** (Dinophyceae). Journal of Phycology, 11, 259-265.

Pfiester, L.A. (1976). Sexual reproduction of Peridinium willei (Dinophyceae). Journal of Phycology, 12(2), 234-238.

Pfiester, L.A. (1977). Sexual reproduction of Peridinium gatunense (Dinophyceae). Journal of Phycology, 13, 92-95.

Pollingher, U. (1986a). Phytoplankton periodicity in a subtropical lake (Lake Kinneret, Israel). Hydrobiologia, 138, 127-138.

Pollingher, U. (1986b). Non-siliceous algae in a five metre core from Lake Kinneret. Hydrobiologia, 143, 213-216.

Pollingher, U. (1987). Ecology of dinoflagellates. B. Freshwater ecosystems. pp. 502-529. In: The Biology of Dinoflagellates. (ed.) F.J.R. Taylor, Blackwell Scientific Publications, Oxford. 785 pages.

Pollingher, U. and Berman, T. (1975). Temporal and spatial patterns of dinoflagellate blooms in Lake Kinneret, Israel (1969-1974). Verh. Internat. Verein. Limnol., 19, 1370-1382.

Pollingher, U. and Serruya, C. (1976). Phased division of Peridinium cinctum f. westii (Dinophyceae) and development of the Lake Kinneret (Israel) bloom. Journal of Phycology, 12, 162-170.

Pollingher, U. and Zemel, E. (1981). In situ and experimental evidence of the influence of turbulence on cell division processes of Peridinium cinctum forma. westii (Lemm.) Lefevre. British Phycological Journal, 16, 281-287.

Preston, T., Stewart, W.D.P. and Reynolds, C.S. (1980). Bloom-forming cyanobacterium Microcystis aeruginosa overwinters on the sediment surface. Nature, 288, 365-367.

Price, C.A. and Guillard, R.R.L. (1978). Collection of dinoflagellates and other marine microalgae by centrifugation in density gradients of a modified silica solution. Limnology and Oceanography, 23(3), 548-553.

Ramsbottom, A.E. (1976). Depth Charts of the Cumbrian Lakes. Freshwater Biological Association Scientific Publication, 33. 39 pages.

Reynolds, C.S. (1984). The Ecology of Freshwater Phytoplankton. Cambridge University Press, Cambridge. 384 pages.

Reynolds, C.S. (1986). Experimental manipulation of the phytoplankton periodicity in large limnetic enclosures in Blelham Tarn, English Lake District. Hydrobiologia, 138, 43-64.

Reynolds, C.S. and Reynolds, J.B. (1985). The atypical seasonality of phytoplankton in Cross Mere, 1972: an independent test of the hypothesis that variability in the physical environment regulates community dynamics and structure. British Phycological Journal, 20, 227-242.

Reynolds, C.S., Wiseman, S.W. and Clarke, M.J.O. (1984). Growth and loss-rate responses of phytoplankton to intermittent artifical mixing and their potential application to the control of planktonic algal blooms. Journal of Applied Ecology, 21, 11-39.

Reynolds, C.S., Wiseman, S.W., Godfrey, B.M. and Butterwick, C. (1983). Some effects of artificial mixing on the dynamics of phytoplankton populations in large limnetic enclosures. Journal of Plankton Research, 5, 203-234.

Reynoldson, T.B. (1983). The Population Dynamics of the Aquatic Oligochaeta in the English Lake District. Ph.D. Thesis, University of Lancaster. 245 pages.

Ricker, W.E. (1937). Statistical treatment of sampling processes useful in the enumeration of plankton organisms. Archiv für Hydrobiologie, 31(1), 68-84.

Ridley, J.E. (1964). Thermal stratification and thermocline control in storage reservoirs. Proceedings of the Society for Water Treatment, 13, 275-297.

Ridley, J.E. (1970). The biology and management of eutrophic reservoirs. Water Treatment and Examination, 19, 374-399.

Ridley, J.E., Cooley, P. and Steel, J.A.P. (1966). Control of thermal stratification in Thames Valley reservoirs. Proceedings of the Society for Water Treatment and Examination, 15, 225-244.

Ridley, J.E. and Symons, J.M. (1972). New approaches to water quality control in impoundments. pp. 389-412. In: Water Pollution Microbiology. (ed.) R. Mitchell, John Wiley & Son, Chichester.

Ritchie, J.C., McHenry, J.R. and Gill, A.C. (1973). Dating recent reservoir sediments. Limnology and Oceanography, 18, 254-263.

Round, F.E. (1961). The diatoms of a core from Esthwaite Water. The New Phytologist, 60, 43-59.

Round, F.E. (1964). The diatom sequence in lake deposits: Some problems of interpretation. Verh. Internat. Verein. Limnol., 15, 1012-1020.

Sako, Y., Ishida, Y., Kadota, H. and Hata, Y. (1984). Sexual reproduction and cyst formation in the freshwater dinoflagellate Peridinium cunningtonii. Bulletin of the Japanese Society of Scientific Fisheries, 50(5), 743-750.

Sako, Y., Ishida, Y., Kadota, H. and Hata, Y, (1985). Excystment in the freshwater dinoflagellate Peridinium cunningtonii. Bulletin of the Japanese Society of Scientific Fisheries, 51(2), 267-272.

Schiller, J. (1933). Dinoflagellatae (Peridineae). Monographischer Behandlung, 10(2), Akademische Verlagsgesell schaft. M.B.H., Leipzig.

Schmitter, R.E. (1979). Temporary cysts of Gonyaulax excavata: Effects of temperature and light. pp. 123-126. In: Toxic Dinoflagellate Blooms. (eds.) D.L. Taylor and H.H. Seliger, Developments in Marine Biology, 1, Elsevier, Amsterdam. 505 pages.

Sebestyén, O. (1959). The ecological niche of Ceratium hirundinella (O.F. Müller) Schrank in the plankton community and in lacustrine life in general. Acta Biologica-Academiae Scientarum Hungaricae, 10, 235-244.

Serruya, C. and Pollingher, U. (1971). An attempt at forecasting the Peridinium bloom in Lake Kinneret (Lake Tiberias). Mitt. Internat. Verein. Limnol, 19, 277-291.

Serruya, C. and Pollingher, U. (1977). Lowering of water level and algal biomass in Lake Kinneret. Hydrobiologia, 54, 73-80.

Siegel, S. (1956). Non-parametric Statistics for the Behavioural Sciences. International Student Edition. McGraw-Hill Book Company Incorporated, New York. 312 pages.

Skuja, H. (1948). Taxonomie des Phytoplanktons einiger Seen in Uppland, Schweden. pp. 373-375. Symbolae Botanicae Upsalienses, 9(3), 1-399.

Smyly, W.J.P (1972). The crustacean zooplankton, past and present, in Esthwaite Water. Verh. Internat. Verein. Limnol., 18, 320-326.

Sommer, U., Wedemeyer, C. and Lowsky, B. (1984). Comparison of potential growth rates of **Ceratium hirundinella** with observed population density changes. Hydrobiologia, 109, 159-164.

Steel, J.A. (1975). The management of Thames Valley reservoirs. pp. 371-407. In: The Effects of Storage on Water Quality. Papers and Proceedings of the Water Research Centre Symposium, Reading. 588 pages.

Steidinger, K. (1975). Basic factors influencing red tides. pp. 153-162. In: Toxic Dinoflagellate Blooms. Proceedings of the First International Conference of Toxic Dinoflagellate Blooms. (ed.) V.R. LoCicero, Massachusetts Science and Technology Foundation, Wakefield, Massachusetts. 541 pages.

Steinberg, C. (1983). Effects of artificial destratification on the phytoplankton populations in a small lake. Journal of Plankton Research, 5(6), 855-864.

Stockner, J.G. and Benson, W.W. (1967). The succession of diatom assemblages in the recent sediments of Lake Washington. Limnology and Oceanography, 12, 513-532.

Stockner, J.G. and Lund, J.W.G. (1970). Live algae in post glacial lake deposits. Limnology and Oceanography, 15, 41-58.

Stoermer, E.F. and Håkansson, H. (1984). Stephanodiscus parvus: validation of an enigmatic and widely misconstrued taxon. Nova Hedwigia, 39, 497-511.

Stoermer, E.F., Wolin, J.A., Schelske, C.L. and Cowley, D.J. (1985). An assessment of ecological changes during the recent history of Lake Ontario based on siliceous algal microfossils preserved in the sediments. Journal of Phycology, 21, 257-276.

Stosch, H.A., von (1964). Zum problem der sexuellen Fortpflanzung in der Perideengattung Ceratium. Helgol. Wiss. Meeresunters, 10, 140-152.

Stosch, H.A., von (1965). Sexualitat bei Ceratium cornutum (Dinophyta). Naturwissenschaften, 52, 112-113.

Sukhanova, I.N. (1978). Settling without the inverted microscope. pp. 97. In: Phytoplankton Manual. (ed.) A Sournia, U.N.E.S.C.O., Paris. 337 pages.

Talling, J.F. (1971). The underwater light climate as a controlling factor in the production ecology of freshwater phytoplankton. Mitt. Internat. Verein. Theor. Angew. Limnol., 19, 214-243.

Talling, J.F. (1976). The depletion of CO_2 from lake water by phytoplankton. Journal of Ecology, 64, 79-121.

Talling, J.F. (1979). Factor interactions and implications for the prediction of lake metabolism. Archiv für Hydrobiologie, 13, 96-109.

Taylor, F.J.R. (1978). Dinoflagellates. pp. 143-147. In: Phytoplankton Manual. (ed.) A Sournia, U.N.E.S.C.O., Paris. 337 pages.

Tilman, D., Kilham, S.S. and Kilham, P. (1982). Phytoplankton community ecology: The role of limiting nutrients. Ann. Revue Ecol. Syst., 13, 349-372.

Toetz, D.W. (1981). Effects of whole lake mixing on water quality and phytoplankton. Water Research, 15, 1205-1210.

Turpin, D.H., Dobell, P.E.R. and Taylor, F.J.R. (1978). Sexuality and cyst formation in Pacific strains of the toxic dinoflagellate **Gonyaulax tamarensis.** Journal of Phycology, 14(2), 235-238.

Tutin, W. (1955). Preliminary observations on a year's cycle of sedimentation in Windermere, England. Mem. Ist. Ital. Idrobiol., 8, 467-484.

Venrick, E.L. (1978). How many cells to count? pp. 167-180. In: Phytoplankton Manual. (ed.) A Sournia, U.N.E.S.C.O., Paris. 337 pages.

Wall, D. and Dale, B. (1968). Modern dinoflagellate cysts and evolution of the Peridiniales. Micropaleontology, 14, 265-304.

Wall, D. and Evitt, W.R. (1975). A comparison of the modern genus **Ceratium** Schrank, 1793, with certain Cretaceous marine dinoflagellates. Micropaleontology, 21(1), 14-44.

White, J., Yeats, A. and Skipworth, G. (1985). Tables for Statisticians. Third Edition. Stanley Thorne (Publishers) Limited, Cheltenham. 70 pages.