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"The Survival of Fresh Water Algae During
Dry Periods"

By
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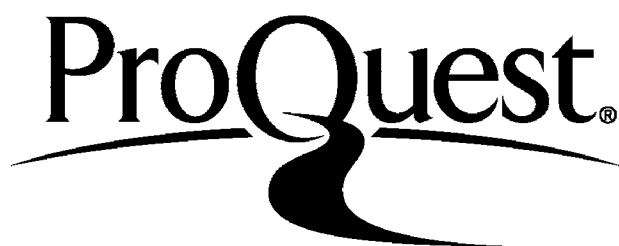
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The survival of fresh water algae during dry periods.

Abstract.

An investigation which has tended to be extensive rather than intensive, of the survival of fresh water algae during dry periods has been carried out.

From April 1953 to June 1955, five small ponds, two in Hertfordshire, two in Middlesex and one in Surrey, have been visited regularly. At these ponds, water levels, water temperatures and the pH of the water and marginal litter or mud were recorded. Litter and mud samples were taken for the estimation of moisture content. Water samples and samples of litter and mud exposed above the water level were collected and examined for algae. In addition, throughout the period of the whole investigation, samples of water, litter and mud have been taken from various other habitats at irregular intervals.

A series of large- and small-scale drying experiments have been carried out to investigate the reactions of various algae to drought and the survival of species through drought periods.

Some investigation was made of the stratification of algae in pond margin litter and mud. Two methods were employed, a buried-slide technique and a micro-sampling technique.

The observations made suggest that certain algae are capable of surviving dry periods by methods other than by the existence of resistant spores. It has been found that more or less obviously modified vegetative cells of a number of algae will survive drought. Further, certain algae, both motile and non-motile, have been found to survive in the deeper layers of litter and mud at pond margins during exposure and drying.

While an attempt has been made to cover an extensive field, more detailed observations have been made on a number of individual species of algae.

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The Survival of Fresh Water Algae During Dry Periods.

B. Methods and Results Contents.

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It is incorrect to generalise about algae, as species of a genus may differ widely in their reactions to any given conditions.

An initial difficulty is to define a resting stage, satisfactorily, for the purpose of discussion in the present account. Physiologically, a resting stage may be defined as a cell in which growth, temporarily at least, has ceased and in which the metabolic rate has slowed down. In addition one might define a resting stage as any cell, however formed (i.e. sexually, asexually or by modification of a vegetative cell),

I. Introduction.

The information available regarding the survival of fresh water algae during dry periods is somewhat meagre. Text books (Fritsch, 1935; Smith, 1938 & 1951; West and Fritsch, 1927) refer to "resting cells", "cysts", "akinetes", and "resistant stages" but, with a few exceptions, little appears to be known, in detail, about the drought survival value of these stages. Strom (1924), referring to the overwintering of algae, said that the importance of zygospores and resting spores has been greatly exaggerated. Strom (1924) has also pointed out that it is incorrect to generalise about algae, as species of a genus may differ widely in their reactions to any given conditions.

An initial difficulty is to define a resting stage, satisfactorily, for the purpose of discussion in the present account. Physiologically, a resting stage may be defined as a cell in which growth, temporarily at least, has ceased and in which the metabolic rate has slowed down. In addition one might define a resting stage as any cell, however formed (i.e. sexually, asexually or by modification of a vegetative cell), describe as resistant. Petersen (1935) stated that

which will assist the survival of a species during adverse conditions. In the present investigation, the adverse condition particularly considered has been that of drought.

Bristol (1920) found that algae surviving in old stored soils (26 - 73 years old) included members of the Cyanophyceae, and the Chlorophyceae and one diatom, Nitzschia palea. Some terrestrial algae have been investigated with regard to the survival of vegetative stages during drought by Fritsch (1922) and Fritsch and Haines (1923). Various references have been made to the accumulation of granular bodies or oil globules in algal cells exposed to periods of desiccation (see Introduction to Appendix 1, p.179). Little work has been attempted, however, on the survival of pond algae during exposure to drought.

Rao (1953) carried out some work on the algae of a pond which eventually dried up, but found no resting stages. Lund (1942) observed an immediate decrease in the algal flora of exposed pond-bottom deposits when the surface of the mud areas began to dry but found no stages of the normally aquatic algae which he could describe as resistant. Petersen (1935) stated that

hydrophytic algae, unlike eu-aerial and soil algae, will never, in the vegetative condition, be able to survive a desiccation. He considered that only resting spores, especially adapted for the purpose (often zygospores and oospores), could withstand desiccation. One of the aims of the present investigation has been to test this view. In this matter it seems likely that too little attention has been paid to the nature of the cell wall and to the formation of protective mucilage sheaths.

Fritsch (1944) observed what appeared to be a true cuticle in a terrestrial species of the Cladophorales, and the existence of a fatty pellicle bounding filaments of Ulothrix and Hormidium has been demonstrated by Jane and Woodhead (1941). In an investigation of the chemical composition of algal cell walls, Wurdach (1923) reported a mucilaginous sheath of pectic acid in Zygnema cruciatum, and an outer wall layer of chitin in Cladophora glomerata and Oedogonium irregulare. Strom (1924) has stated that even algae which often produce zygospores, will survive the winter in a vegetative condition "surrounded by thick, mucous sheaths or with cellular contents strongly condensed and often filled

with starch or oil". By similar stages algae might also survive drought periods, and it is possible that structures of this nature may afford a greater degree of protection against desiccation than has so far been realized.

There is no sharp dividing line between aquatic and terrestrial algae and some species will live and even thrive in either habitat, and it is perhaps the ecological elasticity, so to speak, of some species, which allows them to survive during drought periods. Attention has been paid to this in the present investigation, as well as to other possible ways in which fresh water algae may survive dry periods.

Partly because the literature relating to the survival of aquatic algae upon exposure and drought is meagre, and partly due to the great complexity of the subject, the present investigation has tended to be extensive rather than intensive. In view of this, each of the following Sections has been treated more or less as a separate entity (though numerous cross-references have been made in the course of discussions) and each, for the sake of clarity, has been prefaced with its own introduction, avoiding what would have

been a long, general introduction to the whole investigation.

While investigating the survival of fresh water
This investigation was carried out during the tenure of a post-graduate studentship at Royal Holloway College. Thanks are due to the College authorities for awarding the studentship and to the Ministry of Education for supplementing the award.

I should like to express my profound gratitude to Professor F.W. Jane for suggesting the problem and for his interest, encouragement and constructive criticism throughout this investigation. I should also like to thank Dr. M.A.P. Madge for much advice and many helpful suggestions.

In addition, thanks are due to the following:-

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Miss T. Ergis, B.A., for reading and criticising the text of this account.

II. An Investigation of Five Small Ponds.

A. Introduction.

While investigating the survival of fresh water algae during dry periods, five ponds, differing from one another in various ways to be described below, were studied for a period of rather more than two years. The first collection of samples was made in March, 1953, and the last in July, 1955. Altogether a total of 73 collections were made from each of the five ponds, each being visited usually every fortnight and, during very dry periods, every few days.

1) A description of the ponds.

Pond I, Englefield Green pond. (Figures 1,A; 2,A i and ii).

This pond is oval in shape, approximately 40 metres long and 34 metres wide, and is several metres deep in the centre. There is no tree cover but the pond is surrounded by grasses, sedges and rushes. Lemna minor is present, at times extensively. During wetter periods water runs into the pond from the roadside ditch but there is apparently no outlet for the water, which escapes only slowly by soaking into the soil and by evaporation. The slope of the pond margin tends to be concave so that when the water level is high (figure 1,B c),

considerable drop in this level exposes only small areas of litter and mud, whereas when the water level is low (as was the case in the course of the present investigation only between June and November 1953) a slight drop exposes relatively large areas of litter and mud.

At about 5 metres from the high water level margin the bottom of the pond drops steeply down into the deep centre, which has not been exposed in the course of this investigation.

Pond II, Stanmore pond. (Figures 1,B and 2,B).

This pond is considerably larger than the other four, being approximately 80 metres long, 50 metres wide, and several metres deep even near the margin. There is a small copse to the north-east consisting of Betula pubescens, Acer pseudoplatanus, Crataegus monogyna, Salix caprea and Sambucus nigra. In the water are Nymphaea alba, Lemna minor and a population, dense at times, of Stratiotes aloides. At times, water runs into this pond via a small stream from a neighbouring pond situated a metre or so higher and about 30 metres to the north. There is no apparent outlet for the water. Except for a swampy area to the east the edges of the pond slope very steeply and at the position, (Figure 1,B c),

where most samples were collected the edge is almost vertical. However, during sufficiently prolonged dry periods (this occurred in the course of the present investigation only between August and October 1953) a horizontal shelf less than 1 metre wide was exposed and, from its surface, samples were collected.

Pond III, Stanmore Common pond. (Figures 1,C; 2,Di and ii).

This "pond" is part of a series of ponds and streams making up an extensive swampy area in Stanmore Common. The part considered as "the pond" was, in May 1953, about 1 metre deep at the deepest point. There is a dense tree canopy provided mainly by Betula pendula but there are other trees present including Quercus robur, Acer pseudo-platanus, Fagus sylvatica and Salix caprea. Glyceria fluitans and Juncus effusus are present and reach their maxima during the summer. Water drains into this pond from the nearby road which is about 1 metre above the highest water level of the pond. Water escapes via small streams and by seepage. At the position where samples were taken (Figure 1,C c) the margin slopes fairly steeply, dropping about 60 centimetres over a horizontal distance of about 2 metres. This pond almost dried up even during the comparatively wet summer of 1954, due,

Pond V, Rowley Green "acid" pond. (Figures 1,B; 2,E i and ii)
probably, to the large surface area of water relative
to the depth.

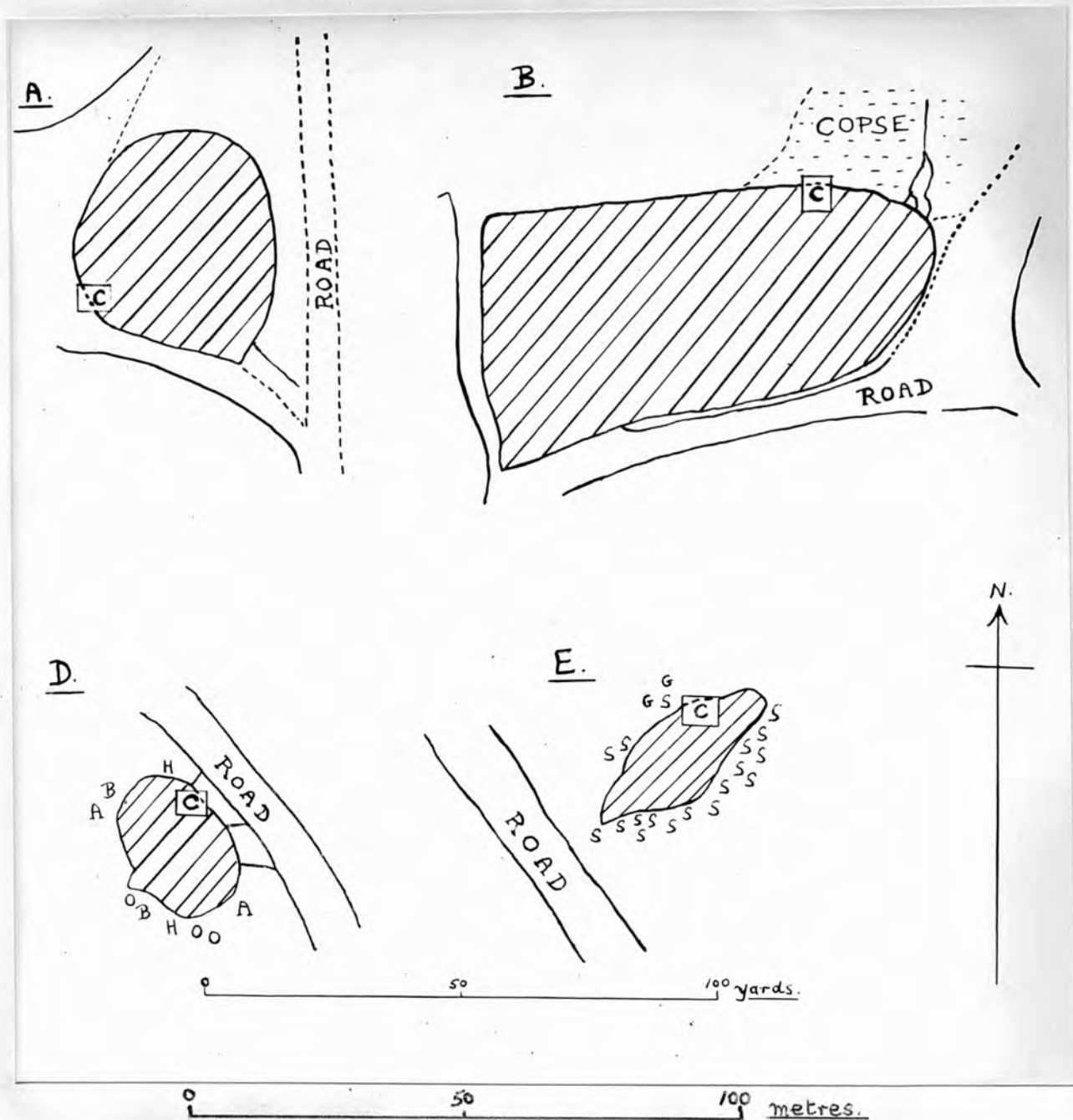
Pond IV, Rowley Green "normal" pond. (Figures 1,D; 2,C).

Approximately oval in shape, this pond is about 25 metres long, 15 metres wide, and is several metres deep in the centre. On all except the north-east side some tree cover is provided by Quercus robur, Fagus sylvatica, Crataegus monogyna and Fraxinus excelsior. Grasses and rushes grow luxuriantly at the margin. Also present are Rumex acetosella, Epilobium Angustifolium, E.hirsuta, Iris pseudacorus and Lemna minor. Water drains into the pond from the adjacent road. The water level fluctuated irregularly, but the maximum total drop was only about 8 centimetres. This suggests the existence of an underground spring which, however, local council surveyors and engineers have been unable to confirm or refute. This pond is limited at the north-east side by an almost vertical bank, but throughout the course of the investigation the edge of the water has remained 60 - 180 centimetres from the base of this bank. Litter and mud samples have been taken from the almost flat shelf sloping gently from the base of the bank into the water.

Pond V, Rowley Green "acid" pond. (Figures 1,E; 2,E i and ii).

This pond is irregular in outline, about 36 metres long, 15 metres wide and nowhere much more than 1 metre deep. There is some shrub cover, consisting mainly of Ulex europeaus, to the north-west, and a fairly dense stand of Salix caprea to the south and south-east. Grasses grow to the edge of the pond and Juncus effusus, J.articulatus, Rumex acetosella and Epilobium angustifolium are also present. Sphagnum occurs abundantly in places. There is no obvious source of, nor outlet for, water, but the pond is shallow for its size so that rainfall is sufficient to keep the water level up in the wetter seasons, while the water tends to drain and evaporate away in the summer. The margin slopes gently and evenly at the point from which most samples were taken, so that large areas of litter and mud were exposed with only small drops in the water level.

Figure 1. Plans of the Five Ponds.



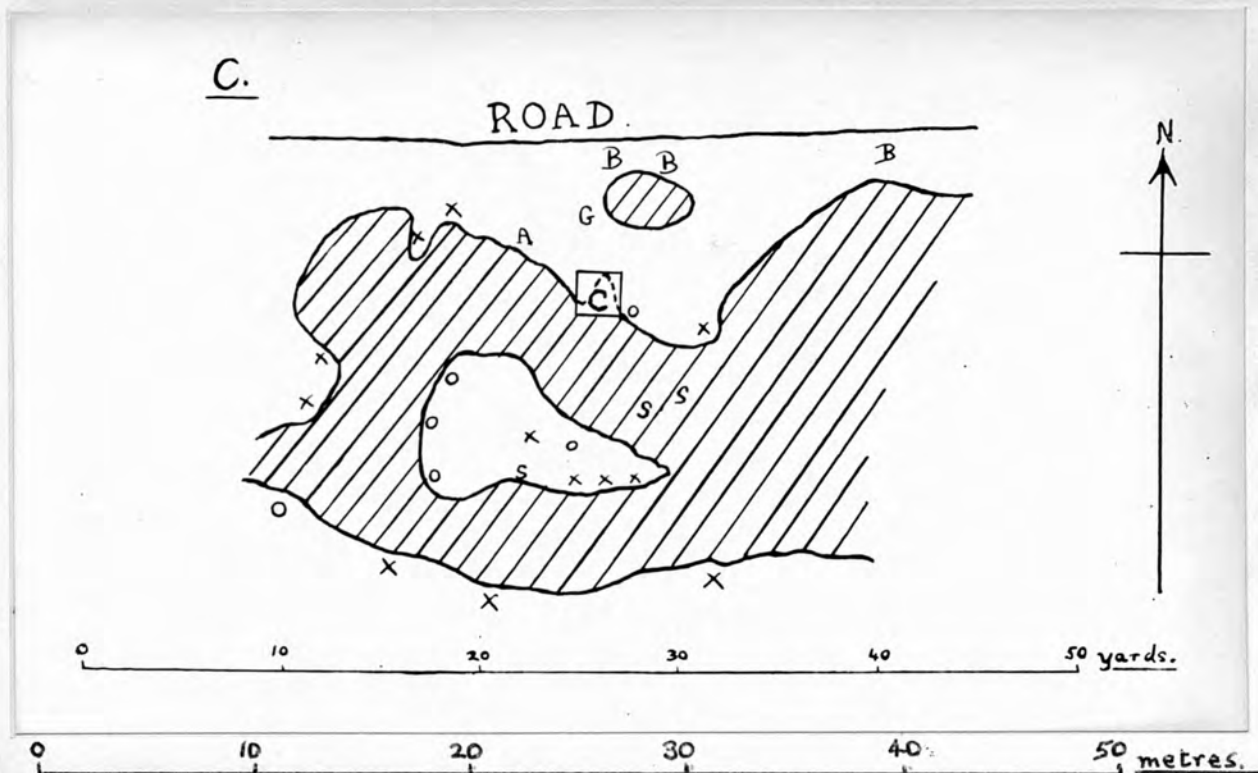
A. I, Englefield Green pond.

B. II, Stanmore pond.

D. IV, Rowley Green "normal" pond.

E. V, Rowley Green "acid" pond.

Figure 1. (Continued).



C. III, Stanmore Common pond.

- c = position where most samples taken.
- X = *Betula pendula*.
- B = *Fagus sylvatica*.
- G = *Ulex europaeus*.
- O = *Quercus robur*.
- S = *Salix caprea*.
- H = *Crataegus monogyna*.
- A = *Fraxinus excelsior*.

Note. Plans of A, B, D and E are based upon those obtained from the Surveyors' and Engineers' departments of local councils, A from Egham, B from Harrow and D and E from Barnet. Plan C is based upon a prismatic compass survey.

Figure 2. The Five Ponds.

A. i.

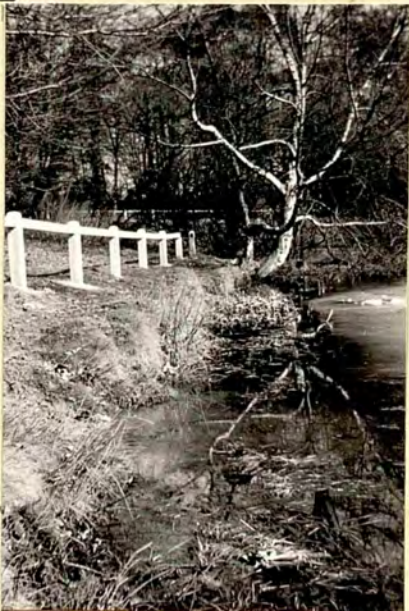


ii.



A. Englefield Green pond; i. high-water level, 17.3.55.
ii. after a fall of 8cm. in the water level, 14.7.55.

B.



B. Stanmore pond.

C.



C. Rowley Green "normal" pond.

Figure 2. The Five Ponds. (continued).

D. i.



ii.



D. Stanmore Common pond; i. high-water level, 17.3.55.
ii. after a fall of 30cm. in the water level, most of
the pond bottom exposed, 2.8.55.

E. i



ii.



E. Rowley Green "acid" pond; i. high-water level, 17.3.55.
ii. after a fall of 15cm. in the water level, marginal
area of the pond exposed, 2.8.55.

2) Methods of Investigation.

Water samples were taken, in one-litre, wide-mouthed, screw-capped bottles, from the surface, about 1 metre from the margin of each pond, and included epiphytes scraped from any macro-vegetation present. From time to time additional water samples were collected by squeezing the water from marginal litter and/or mud into bottles, in which case both water samples from each pond were examined for algae and estimated for total dissolved solids. Litter and/or mud samples were taken by scraping the surface with a sterile scalpel and placing portions so obtained in 2" x $\frac{1}{2}$ " glass tubes. The samples were taken from various zones at the margins of each pond:-

- Zone 1) below the water, 2.5cm. from edge of water.
- Zone 2) above the water, 2.5cm. from edge of water.
- Zone 3) above the water, 5.0cm. from edge of water.
- Zone 4) above the water, 15.0cm. from edge of water.
- Zones 5) to 7) above the water, 30cm., or more, from edge of the water.

Zones 2), 3) and 4) were regarded, for the purposes of this investigation, as the "transition zones" and zones 5) to 7) as the "drying zones". The lighter algal cells (e.g. small diatoms), if present, were sampled

From the samples, frequency estimates of algae were made as follows: drops of the water samples were mounted and examined, counting the number of cells of each species present in a total of 50 high-power microscope fields (using a x15 ocular and a 1/6th" objective). These estimates were made in triplicate, the means calculated and the results so obtained entered in tables. A complete survey of the mount was also made listing other species found, as present. From each of the litter and/or mud samples a small portion was removed and mixed with sterile, distilled water on a slide. This mount was examined, the number of cells of each species present in a total of 25 high-power fields being noted. To the remainder of each of the litter and/or mud samples was added sterile, distilled water, the tubes being almost topped-up, then shaken vigorously. After allowing sedimentation to occur for a few minutes, drops were taken from each tube from the water surface and from the surface of the settled solid matter and again an estimate was made of the algae in 25 high-power fields. This latter method was employed in order to take into account algal cells which might have been missed by the coarser sampling method. The lighter algal cells (e.g. small diatoms), if present, were sampled

from the water surface, and algal cells adhering to the lighter particles of litter and/or mud were sampled from the surface of the sediment. The sum of the algae being estimated in a total of 50 high-power fields of each litter and/or mud sample for each species was calculated and recorded.

3) Meteorological and other factors.

Various meteorological and other factors, listed below, have been recorded and have been referred to or discussed in this or succeeding sections.

1. Rainfall and humidity. Records were obtained from the Meteorological Office at Harrow, Middlesex.

2. Water temperatures.

3. Total dissolved solids. Estimates were made by evaporation after the method described by Thresh, Beale and Suckling in an attempt to determine whether correlation exists between this factor and the water level. As found by Hodgetts (1921), this relationship is not necessarily an inverse one, other factors, such as the decay of vegetation, having some effect. Too few estimates were made for accurate curves to be constructed of the results (Table 1) but the initial object in mind was achieved and, in addition, it was found that the concentration of the

water at the extreme edge of the pond is usually, but not always, higher than the concentration of the free water. This may be due to a number of factors. During rain, salts may be leached from the marginal litter and/or mud and be carried into the water at the pond edge. Subsequent diffusion of those salts into the free water may be slow. Furthermore, evaporation, relative to the volume of water involved, would occur more rapidly from the thin films of water on the marginal litter than from the surface of the free water away from the pond margin. On two occasions the concentration of dissolved solids in the water at the extreme edge of the smallest of the ponds investigated (III, the Stanmore Common pond) was considerably higher than the concentration in the free water (Table 1). This may be correlated with the negligible wave action occurring in such a small and well protected pond. In each of the other four ponds, which are larger and/or more exposed than the Stanmore Common pond, the differences in concentration between the edge and the free water are less marked. The results obtained are too few to allow definite conclusions to be reached but they do suggest that the

ratio between the concentration of dissolved solids in the water at the extreme edge of the ponds and that of the free water is associated with pond size (Table 1). There is a tendency for this ratio to increase with decreasing water volume. This ratio may, however, be associated with other factors. In the Rowley Green "normal" pond (IV), for instance, an underground spring, the existence of which is suspected (see above), might lower this ratio by leaching from the soil salts which are carried into the free water of the pond.

All the five ponds investigated are to be regarded as of the eutrophic type. The largest of these ponds (II, the Stanmore pond), however, would appear to have undergone less eutrophication than the other ponds. This pond is larger in surface area (and probably deeper, though as no measurements of depth were made this cannot be stated with certainty) and the water is relatively more transparent. The margin slopes steeply, in places vertically, into the water. The Englefield Green pond (I) shows similar, though less marked, tendencies away from the extreme eutrophic type. The ratio between concentration of dissolved solids at the edge of the ponds and in the free water may, then, be associated

Table 1.

with the degree of eutrophication. The greater this is, the higher the ratio tends to be. If the Stanmore pond (and to a lesser extent the Englefield Green pond) are to be considered less eutrophic in type than the two acid ponds (Stanmore Common and Rowley Green "acid" ponds) then it should be noted that the former ponds approach to the oligotrophic type is morphometric in nature rather than edaphic.

4. pH Values. have been recorded regularly since March 1954, that of the water being estimated with a Lovibond comparator and checked occasionally with a Cambridge pH meter. The pH of litter and/or mud samples was estimated, from time to time, by the latter method only. The pH ranges in various samples, obtained in the course of the present investigation, are recorded in Table 4.

5. Moisture contents of mud and litter were estimated by oven-drying (at 105°C.) samples of known weight (each, usually, of about 5gm., dry-weight) for 24 hours and re-weighing. The results of moisture estimations are expressed throughout as the percentage of the wet weight. This unusual method of recording moisture content may be justified as follows: if the moisture content is estimated

Table 1. Total dissolved solids in water samples, expressed as degrees (grams per 100,000 ml.).

Pond No.	Area of pond in sq. ft.		1953			1954		1955		Mean	
			Aug. 30	April 6	May 4	August 3	12	Nov. 17	Jan. 25		May 9
I	12,800	W.L.	-10.0	0	0	-1.0	-2.5	0	0	0	
		F.W.	19.5	23.3	21.8	23.0	14.6	18.1	10.2	24.0	19.1
		E.W.	20.8	28.8	26.0	21.2	20.3	19.6	17.4	28.0	23.1
		R.	1.05	1.23	1.19	0.92	1.39	1.21	1.70	1.16	1.28
II	32,900	W.L.			-2.5	0	0	0	0	0	
		F.W.			18.1	16.4	16.6	14.3	14.0	18.4	16.3
		E.W.			19.8	16.8	16.3	16.1	18.8	27.5	19.2
		R.			1.09	1.02	0.98	1.12	1.34	1.49	1.17
III	2,400	W.L.			-5.0	-23.0	-18.0	-25.0	0	0	
		F.W.			19.4	22.3	11.2	16.6	11.1	22.8	17.2
		E.W.			51.7	14.6	13.2	13.1	15.3	68.9	29.9
		R.			2.66	0.65	1.18	0.91	1.42	3.00	1.63
IV	4,200	W.L.				-5.0	0	0	-2.5	-2.5	
		F.W.				39.5	26.4	27.2	17.2	29.6	26.0
		E.W.					34.3	28.8	24.3	34.0	30.3
		R.					1.29	1.05	1.41	1.15	1.25
V	3,900	W.L.			-2.5	-18.0	-2.5	-5.0	-2.5	-10.0	
		F.W.			37.2	25.6	21.0	21.2	20.8	20.4	24.4
		E.W.			37.5	43.1	28.4	28.6	25.4	24.0	31.1
		R.			1.01	1.68	1.35	1.35	1.24	1.17	1.30

N.B. The areas of the ponds are approximate estimates obtained by the squared paper method.

W.L. = water level in centimetres.

F.W. = concentration of dissolved solids in the free water away from the pond margin.

E.W. = concentration of dissolved solids in the water at the extreme edge of the pond.

R. = ratio between E.W. and F.W.

and recorded as the percentage of the dry weight, as is the usual practice, then the figures recorded will, theoretically, range from 0 (zero) to a very high figure (i.e. approaching $\frac{100}{0}$). In the present investigation this range, in practice, would be 2% to nearly 2000%. By estimating and recording moisture content as the percentage of the wet weight, the theoretical range is 0 to a figure approaching 100% and the range in practice about 2 - 95%. Results which are recorded in this way are easily compared with one another and it is mainly for comparative purposes that such estimates have been made.

6. Moisture equivalent estimations (Table 2) were made after the method of Boyoucos (1929) to determine the resistance of marginal mud and litter to loss of water by suction.

7. Loss-on-ignition estimations (Table 3) were made from time to time, but not regularly, by placing air-dry samples of marginal litter and mud in a muffle furnace at 650°C. for 4½ hours and re-weighing.

For these last three factors, since March 1954, estimates were made, in each case, from the upper layer (0 - 0.5cm.) and the lower layer (0.5 - 3 or 4cm.) of

litter and/or mud in order to gain some insight of the vertical movements of water during wet and dry conditions and the reasons for these water movements.

Regarding moisture equivalents (Table 2), no more than approximate estimates, for comparative purposes, were obtained and the results are highly variable. The results of the loss-on-ignition estimates also vary somewhat but the following general tendencies are observable almost throughout:-

i. Moisture equivalent and loss-on-ignition is higher in the surface layer than in the lower layers of litter and/or mud which indicates that the upper layer, while losing moisture to the atmosphere by evaporation, is able to regain moisture from below.

ii. The higher the loss-on-ignition the higher the moisture equivalent and, usually, the higher the moisture content at any given time.

iii. At the Stanmore pond, loss-on-ignition, moisture equivalent and moisture contents of the mud at any given time were lower generally than at the other ponds. There is also a lower ratio between the upper and lower layers for all three factors at this pond.

Table 2. Moisture equivalents of marginal litter and mud expressed as percentages of air-dry weights.

Pond No.	Litter or mud Layer.	1954				1955	
		May 4	June 15	July 29	Oct. 12	Jan. 19	April 25
I	Upper		59	41	55	102	81
	Lower		34	20	34	74	18
II	Upper	8		38		27	29
	Lower			37		31	22
III	Upper	208	135	152	143	101	166
	Lower	152	129	88	129	64	75
IV	Upper	92		139	328	59	120
	Lower	58		90	193	78	91
V	Upper	460		156	184	222	133
	Lower	294		198	340	121	47

Table 3. Loss on ignition of marginal litter and mud expressed as percentages of air-dry weights.

Pond No.	Litter or mud Layer.	1954				1955	
		March 24	April 6	June 29	Nov. 30	March 30	June 27
I	Upper		32	17	18	40	38
	Lower		9	13	7	14	43
II	Upper	24	16	13	11	19	4
	Lower	24	16	11	11	15	9
III	Upper	78	64	76	66	42	63
	Lower	81	54	75	58	14	68
IV	Upper		87	55	40	30	28
	Lower		38	49	22	38	31
V	Upper		47	50	79	76	69
	Lower		70	71	32	60	69

B. The Species List.

A complete species list has been prepared, summarising the results obtained for the five ponds investigated during the two years April 1953 - July 1955. In table 4, the figures for percentage loss on ignition are approximate means of all the estimates made for the upper surface layers of litter and/or mud samples from each pond. The maximum frequency for each species is represented by a symbol:- a, abundant and r, rare. Where the maximum frequency of the species was ^{somewhat} ~~somewhat~~ between the two extremes the symbol + is used. The results obtained justified such broad estimates of maximum frequency but probably did not justify the use of such categories as "frequent" or "common". As the primary purpose of this investigation was not, however, the determination of algal periodicity, the broad categories of frequency listed are felt to be sufficient. For certain of the species investigated (e.g. Microspora floccosa) periodicity is considered in more detail, and for most of these species, not symbols but actual counts are quoted.

The pH ranges recorded and the algal species observed are listed for three regions of each pond. These regions are represented in table 4 by the symbols W, T and M.

W = water samples; T = transition zone samples, 2.5 - 15.0 cm. away from the water edge; M = samples from the region above the transition zones.

Where the species name is in brackets in the list (Table 4), it indicates an element of doubt regarding the identification.

Sp. No.	"acid" ponds				
	I field	II	IV Green	III Common	V Rowley Green acid
	W T M	W T M	W T M	W T M	W T M
pH range	7.2-8.8	7.5-8.3	7.4-7.8	6.0-7.0	6.5-7.2
	7.6-7.7	7.6-7.7	7.7-7.8	6.6-7.0	6.6-7.2
	0.3-0.6	0.0-0.2	0.5-0.9	0.0-0.0	0.4-0.4
% loss on ignition of exposed litter	30	14	48	65	64
<i>Pyramimonas deliculata</i>	r				
<i>Chlamydomonas</i> spp.	a + r	a r r	a r r	r + +	a a
<i>Gonium pectorale</i>	r	r		r	
<i>G. sociale</i>		r			
<i>Eudorina morum</i>	a r	+ r	r	r	+ r
<i>Eudorina elegans</i>	a r r			r	r r
<i>Carteria (globosa)</i>	r				
<i>Chlorogonium suchlorum</i>					a a
<i>Volvox aureus</i>	+				
<i>Chlorococcum infusionum</i>	r r r	r	r r r	r r r	r r r
<i>Characium Pringsheimii</i>	r		a	+ + r	r
<i>Dactylococcus bicaudatus</i>	r				+
<i>Ankistrodesmus falcatus</i>	r				+
<i>Scenedesmus quadricauda</i>	a + a	r a r			
<i>S. obliquus</i>	r r				
<i>Ulothrix subtilis</i>	a a	+ + +	+ + +	a a a	a a a
<i>U. zonata</i>		+		+ +	
<i>Microspora floccosa</i>	r r r	a	a + +	a a a	a a a
<i>M. stegnum</i>	+ + +	+ + +	+ +	a + +	a + +
<i>M. williana</i>				r	
<i>Cladophora fracta</i>		a r			

Table 4. The Species List (continued).

Table 4. The Species List.		"acid" ponds			
Pond.	I Engle- field Green	II Stanmore	IV Rowley Green "normal"	III Stanmore Common	V Rowley Green "acid"
Zone.	W T M	W T M	W T M	W T M	W T M
<i>Polonium tenue</i>					
<i>Chaetophora incrustata</i>					
<i>Microthamnion Kutzringianum</i>					
<i>M. stricklandianum</i>					
<i>Chaetete repens</i>					
<i>Chaetete viridis</i>					
<i>Chaetete scutata</i>					
<i>Sedogonium</i> sp. A.	7.2 6.6 7.6	7.6 6.6 7.2	7.4 7.1 7.6	6.2 6.0 7.0	6.6 6.4 7.2
pH range.	- - -	- - -	- - -	- - -	- - -
<i>Nectria oblongum</i> var. <i>cylindricum</i>	6.0 6.0 4.6	6.0 6.0 3.2	6.5 6.4 6.2	4.0 4.8 3.0	4.4 5.4 2.4
% loss on ignition of exposed litter.	30	14	48	65	64
<i>Pyramimonas deliculata</i>	r				
<i>Chlamydomonas</i> spp.	a + r	a r r	a r r	+ + +	a a
<i>Gonium pectorale</i>	r	r		r	
<i>G. sociale</i> sp. A.		r			
<i>Pandorina morum</i>	a r	+ r	r	r	+ r
<i>Eudorina elegans</i>	a r r			r	r r
<i>Carteria (globosa)</i>	r				
<i>Chlorogonium euchlorum</i>					a a
<i>Volvox aureus</i>	+				
<i>Chlorococcum infusionum</i>	r r r	r r r	r r r	r r r	r r r
<i>Characium Pringsheimii</i>	r		a	+ + r	r
<i>Dactylococcus bicaudatus</i>	r				+
<i>Ankistrodesmus falcatus</i>	r				+
<i>Scenedesmus quadricauda</i>	a + a	r a r			
<i>S. obliquus</i>	r r				
<i>Ulothrix subtilis</i>	a a	+ + +	+ + +	a a a	a a a
<i>U. zonata</i>		+		+ +	
<i>Microspora floccosa</i>	r r r	a	a + +	a a a	a a a
<i>M. stagnorum</i> var. <i>elegans</i>	+ + +	+ + +	+ +	a + +	a + +
<i>M. Willeana</i>				r	
<i>Cladophora fracta</i>		a r			

Table 4. The Species List (continued).

Pond.	I			II			IV			III			V		
	W	T	M	W	T	M	W	T	M	W	T	M	W	T	M
Stigeoclonium tenue		+	+				+			+	+				r
Chaetophora incrassata				r	r		r								
Microthamnion Kutzingianum	r			r	r		r	r		+	+	r		a	a
M.strictissimum										+					+
Aphanochaete repens				r			r								
Protoderma viride				+											
Coleochaete scutata				a											
Oedogonium sp.A.	+	r	r												
Oedogonium sp.B.					+	r	r								
Netrium oblongum var. cylindricum											+	+			
Zygnema sp.A.							a								
Zygnema sp.B.										+	+	a			
Spirogyra cylindrica										a	+				
S.nitida	a	+													
Spirogyra spp.	+			r			+			r				r	
Mougeotia sp.A.	a	+	a	a	+		a	+						+	a
M.parvula	+	+		+	r					a	a	a		a	a
Closterium archerianum	r						r								
C.Braunii	r														
C.cornutum															+
C.costatum	r	r					r							r	r
C.Cynthia	r	r			r									r	a
C.dianae					r	r	r								
C.eboracense	r	r			+	+	r								
C.Ehrenbergii	r	r					+	r							
C.intermedium	r	r													
C.Kutzingii	r	r					r							r	r
C.lanceolatum	r	r													
C.lineatum	r														
C.macilentum														r	
C.malinvernianiforme	+	r						r							
C.striolatum	+	r					r	r							
C.strigosum var.elegans							r	r							

Table 4. The Species List (continued).

Pond.	I			II			IV			III			V		
	W	T	M	W	T	M	W	T	M	W	T	M	W	T	M
Pleurotaenium trabecula	+	+					r								
Tetmemorus laevis	r			r	r					a	+		a	+	
Cosmarium cucurbitinum		r	r	r	r		r			r			r		
C. impressulum				r	r										
C. praemorsum	r	r		r	r		+	r							
C. botrytisum	r	r													
Ophiocytium arbuscula	+	+	+	+	a	+	r	r	r	+	+	+	a	+	+
Tribonema viride	a	+	+	r	+	+	a	+	r	+	a	a	a	+	a
T. vulgare	r	+		+	+	+				+	+	+	+	a	+
T. subtilissimum	a	+		r	r		+			+					
Chromulina ovalis	r	r	r	r	+	r	r								
Chrysococcus rufescens										r					
Mallomonas longiseta				r											
Synura agg.	+	r		+	r		r			a	r		a	+	
Dinobryon sp.	+			r	r					r				+	
Melosira varians															r
Naviculoid diatoms	+	a	a	+	a		+	a	a	+	a	a	+	a	a
Pinnularia viridis	+	+		+	r		a	a	+	a	a	+	a	a	+
P. major										r	r				
Stauroneis phoenicenteron	r			r	r		r	r							
Gyrosigma acuminatum				r	r		r								
Gomphonema acuminatum				r			+			+					
Cymbella lanceolatum							r	+							
Epithemia sp.							a	a	a						
Nitzschia palea	+	+	+	+	+	+	a	a	a						r
N. acicularis				r											
N. linearis							a	+	+	a	a	a	a	a	r
Hantzschia amphioxys		r						r			+	a			r
Cryptomonas curvata	+			a			r			a	r		a		
C. ovata	+	r		r			r			+	r		+	r	

Table 4. The Species List (continued).

Pond.	I			IV			IV			III			V		
	W	T	M	W	T	M	W	T	M	W	T	M	W	T	M
Glenodinium pulvisculus	r			r						a	+		a		
G.(uliginosum).							r						r		
Peredinium cinctum										a					
P.Willei	a	r					+	r					a		
P.(palatinum).	r														
oscillatoria tenuis	a	a		a	a		a	a							
Euglena viridis	+	+	+	+	a	+	r	r	r	+	+	+	a	+	+
E.mutabilis	+	+	+	+	+	+	r	r	r	+	a	a	+	a	a
E.deses	+	+		+	+	+				+	+	+	+	a	+
E.spirogyra	r			r	r					+					
E.acus	r	r								a			+	r	
E.minima	r	r	r	+	+	r								r	
E.Ehrenbergii	r			r											
E.proxima		r												r	
E.tripteris	r	r		r			r								
E.agilis	r			r											
E.variabilis				r											
E.oxyuris	r														
E.sanguinea				+											
E.inflata										+	+	r			
E.fusca							r	r		+	r				
E.velata	r														
Phacus triqueter	r	r		r	r		+								
P.pyrum	r	r								+			r		
P.longicauda	r			r	r					r					
P.parvula										r					
P.pleuronectes										r			r		
P.oscillans							r								
Lepocinclis texta													a		
L.teres										a	a		a	a	r
L.reeuwikiana	r									r					
L.Butschlii						r									
L.hetero chila										r	r				
L.ovum var.globula										a	r		r	r	

C. Discussion of some of the results obtained for
certain algal groups and species.

1) Pandorina morum and Eudorina elegans.

These species were observed only in the Englefield Green pond and the Rowley Green "acid" pond with any marked frequency. Pandorina morum was found from April to November in 1953 and 1954, reaching a maximum in the August of each year, but failed to appear during 1955. In the Rowley Green "acid" pond Eudorina elegans occurred in August 1953, August to November 1954 and, like Pandorina morum, failed to appear in 1955. In the Englefield Green pond Eudorina elegans occurred from April to October 1953 and from March 1954 to June 1955, reaching a high maximum (an average of 17 coenobia being estimated in 50 high-power microscope fields) in February 1955. Coenobia were occasionally found in the "transition zones" (5.0 - 15cm. away from the free water) which were usually more or less normal in appearance but sometimes lacked flagella and were, as a consequence, non-motile. Coenobia were rarely found above the "transition zones" but one such coenobium, found 60 cm. away from the waters edge of the Englefield Green pond in May 1953, had retained its flagella which began to beat sluggishly when the coenobium was mounted

in water. This suggests that Eudorina elegans is not killed off immediately upon exposure, but may survive for sufficient time, provided that drying does not occur too rapidly, for more permanent resistant stages to be formed. In the Rowley Green "acid" pond a considerable fall in the water level occurred in the late summer and autumn leaving little free water. That Eudorina elegans is rarely found above the transition zones in this pond suggests that prolonged exposure and drought may be survived by single cells (possibly zygotes). This has been supported, for Eudorina elegans, to some extent, by experimental work (Sections III and V). As far as Pandorina morum is concerned, it may be that this species will not survive from one season to the next where complete drying up of a body of water occurs. This might help to explain the non-appearance of this species in the Stanmore Common pond.

2) Chlorogonium euchlorum.

This species was found only in the Rowley Green "acid" pond, occurring from November 1953 to May 1954 and from December 1954 to June 1955, with maxima in December 1953, April 1954 and February 1955.

Vegetative cells were found occasionally in the

"transition zones" but never above. It was shown, however, by long-term drying experiments (Section III), that this species is capable of surviving prolonged exposure and severe drought. It may have survived by means of zygotes, as was suggested by Droop (1953) for Chlorogonium elongatum.

3) Microspora floccosa.

Figures 4A and 4B show the occurrence of this species at the Stanmore Common and Rowley Green "acid" ponds. The monthly mean cell frequency was calculated from the results obtained by the method described above (p.16). The periodicity shown for this species in the water compares closely with the results obtained by earlier work (Fritsch and Rich, 1913; Hodgetts, 1921) but the prolonged presence of vegetative stages on exposed litter deposits ("transition zones" and/or the litter above the "transition zones") during the summer and autumn is a remarkable feature, when one considers the generally accepted view that this species is at its maximum during the colder part of the year.

In the two ponds investigated, vegetative stages may survive for several months on exposed marginal deposits, but it should be noted that akinetes were found to occur

more frequently in the dry, warm summer of 1953 than during the relatively wet, cool summer of 1954.

An indication of the way in which the vegetative stages may survive has been obtained by drying litter samples in the laboratory. After 18 days drying from 91% to 13% moisture (expressed as % of the wet weight) the filaments had thickened cell walls, a mucilage layer, and cell contents including dark globules which stained with the Sudan stains (Figure 3).

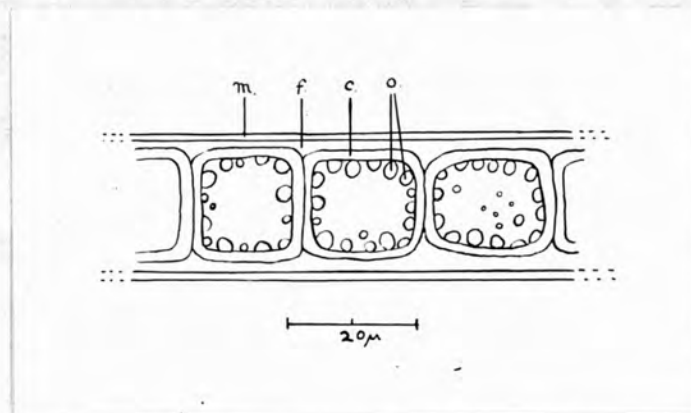
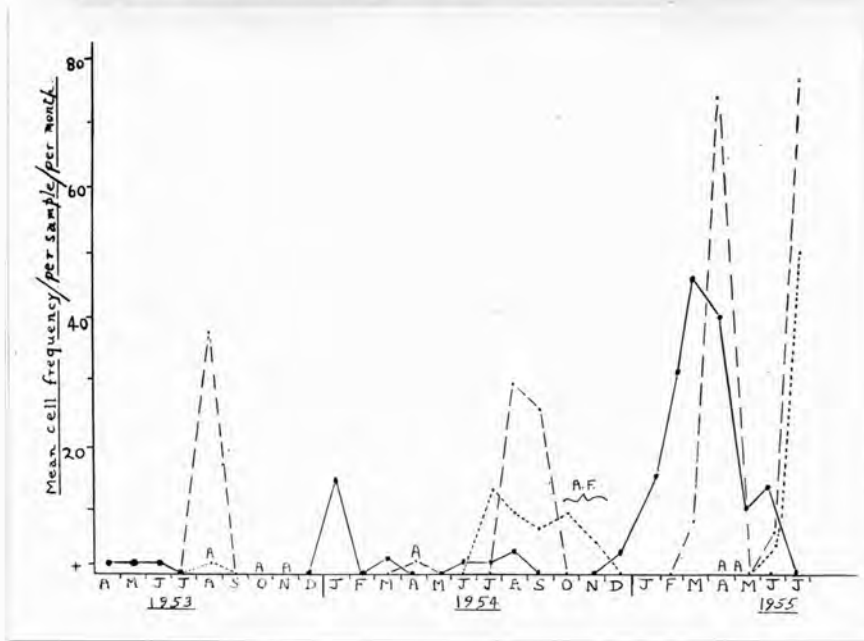


Figure 3. Microspora floccosa. Part of a filament dried for 18 days in the laboratory. m, mucilage layer; f, mother cell wall; c, cell wall; o, fat globules.

Upon culturing such filaments in soil solution normal vegetative growth re-commenced within 14 days. Similar drought resistant filaments were found in the large-scale experiments (Section III).

Figure 4. Microspora floccosa.

A. Stanmore Common pond.



B. Rowley Green "acid" pond.

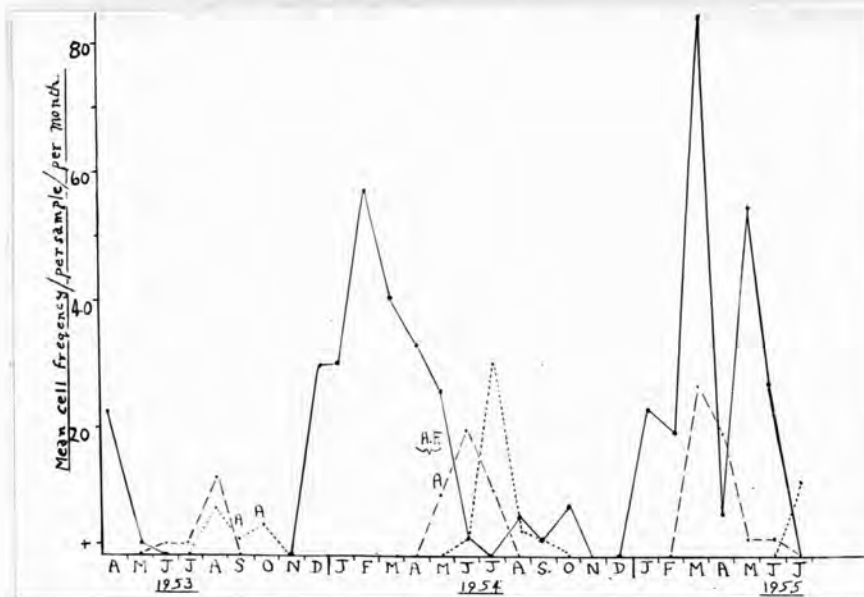


Figure 4. The periodicity of Microspora floccosa at

the Stanmore Common pond (A) and at the Rowley Green "acid" pond (B).

Unbroken line = cell frequency in water samples.

Broken line = cell frequency in the "transition zone" samples.

Dotted line = cell frequency in samples from above the "transition zones".

A = the occurrence of akinetes.

A.F. = akinete formation, i.e. the occurrence of filaments which, upon direct observation in the laboratory, were found to break up to form akinetes.

The cells of the filaments which survived artificial drought might, perhaps, be regarded as akinetes. It is, however, remarkable that although breaking-up of filaments to form separate akinetes was observed several times in nature, such observations were never made of filaments subjected to artificial drought.

Stages in the formation of drought resistant filaments have been observed after drying normal filaments on agar in the laboratory, and have also been found on exposed litter deposits in nature. In both cases the first observed change was usually the thickening of the cell wall, but the accumulation of globules in the cells may occur at the same time or very soon afterwards. Mucilage formation, where observed, seems to be the final development.

Similar changes to those above described may occur in response to conditions other than drought. In water samples from the Stanmore Common pond collected in early May 1955, many cells showed an accumulation of large globules. This may possibly have been related to a marked and fairly rapid increase in temperature through the preceding month.

4) *Microspora stagnorum*.

This species was found in all five ponds investigated,

but occurred with the greatest frequency in the two acid ponds (Stanmore Common and Rowley Green "acid" pond), as did M.floccosa. The periodicity of M.stagnorum was found to be somewhat similar to that of M.floccosa, though the former was rather less frequent. The filaments of M.stagnorum encountered in the course of the present investigation broke up and formed akinetes more readily than the filaments of M.floccosa, and the species was found to survive very prolonged and severe drying (Section III). In drought-resistant filaments which remained intact during drying, cells were found to accumulate considerable quantities of oily matter as globules (see Appendix 1, p.196 and Table 60).

5) Zygnema spp.

The filaments found in the course of the present investigation were of two distinct types, neither of which could be identified as reproductive stages were never observed. One type, to be referred to as Zygnema sp.A, occurred only in the water of the Rowley Green "normal" pond and was at times, particularly in the spring and early summer, abundant. Filaments exposed on litter deposits by a drop in the water level did not survive for long, and this type would probably not

survive from one season to the next in a body of water liable to complete drying up. The other type, Zygnema sp.B, was abundant at the Stanmore Common pond, at, or about, the high water mark of the region where most samples were taken. These filaments, unlike those of Zygnema sp.A, spend the major part of each year (late March to mid December in 1954) above the water level and exposed to desiccation. The filaments, during this time possess thick walls and, towards the end of the period of exposure (October and November of 1954), the cells accumulated globules of oily matter. Initially, upon exposure, some of the filaments break up to form thick-walled, separate cells or akinetes. When the thick-walled, drought-resistant filaments are placed in liquid culture (soil solution or Beijerinck 0.05% solution) the thin-walled aquatic form develops. In nature, similarly, when the water level rises and swamps over the thick-walled, drought-resistant filaments (as occurred between mid-January and mid-April 1955) the thin-walled, aquatic form was found to develop. Thus, Zygnema sp.A and Zygnema sp.B are two contrasting forms of the same genus, the former entirely aquatic and unable to survive exposure and drying in the vegetative state

while the latter is well adapted to prolonged exposure and spends more time as a terrestrial than as an aquatic alga. This conclusion has been confirmed by drying and rewetting experiments described in Section III (see Table 37). The way in which dispersal of Zygnema sp.A occurs is unknown. Zygosporos were not found, but as this may have been due to rarity rather than absence, dispersal might occur by means of such zygosporos.

6) Spirogyra cylindrica.

This species was abundant in the summer of 1953 at the Stanmore Common pond (Table 5). It is, like Zygnema sp.A, an aquatic species, filaments exposed on the litter surface above the water level soon dying.

Table 5. The occurrence of Spirogyra cylindrica at the Stanmore Common pond in 1953.

Month.		May	June	July	Aug.	Sep.
No. of Samples		3	4	3	6	2
No. of cells in 50 high power fields of:-	Water	-	4	70	43	
	Transition Zones			150	93	
	Drying litter				17	3

By early October 1953 the Stanmore Common pond had dried up almost completely, but Spirogyra cylindrica had produced zygosporos in July and in this form the species survived the drought to re-appear the following year,

germinating zygospores being found in mid-May 1954.

Vegetative filaments were present in the water from May to August 1954, but the cells were never abundant and no reproductive stages were found. In 1955 filaments were found in the water from May to July (after which time no further collections were made), though, again, the species was not frequent, but zygospores were produced in mid-June.

Vegetative cells were also found not to survive when dried experimentally, while earlier-formed zygospores were capable of surviving prolonged and severe drought (Section III). This species, then, apparently relies entirely upon the zygospores for survival through dry periods from one season to the next.

7) Mougeotia parvula.

The results obtained in a study of the periodicity of this species in the Stanmore Common and Rowley Green "acid" ponds (Figures 5A and B) agrees with those of Fritsch and Rich (1913), the cells being most frequent in the summer and tending to persist into the winter. Of the three points to be considered, only the last is directly related to the problem of survival during dry periods, though the first two are of interest in relation to rare in the Rowley Green "acid" pond in 1953 appeared, or increased in frequency, in 1954.

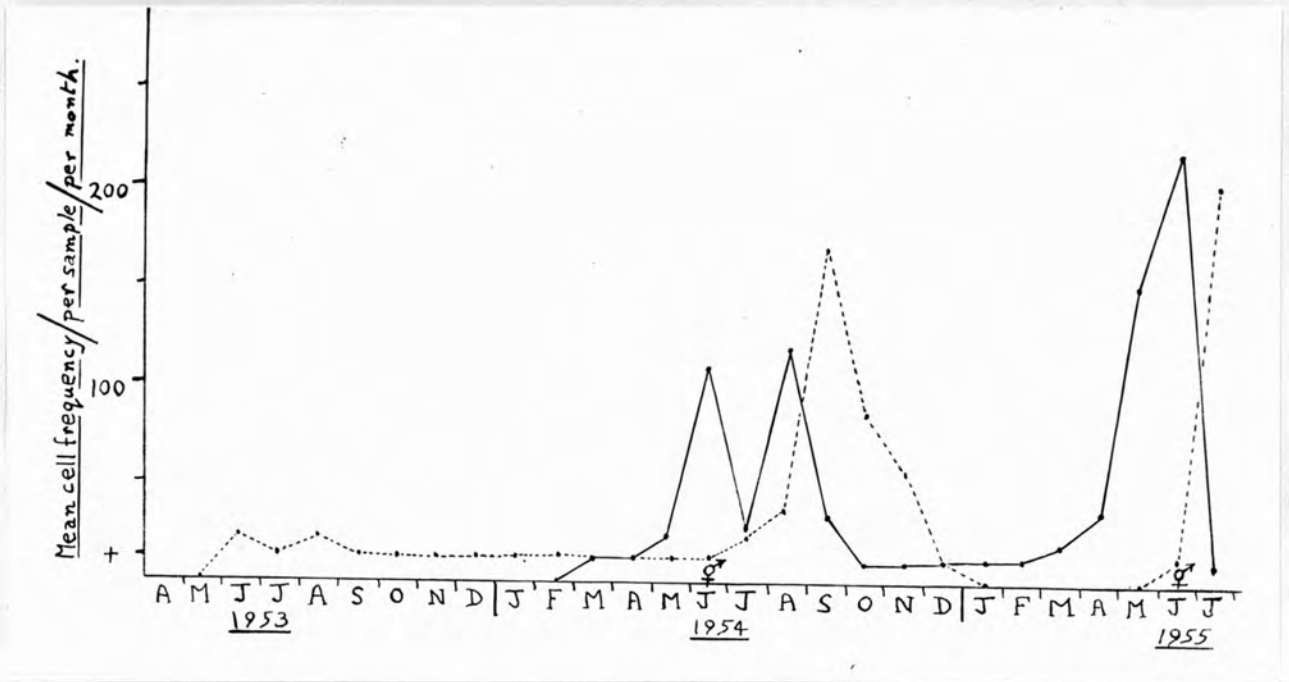
to the problem of survival in general.

Firstly, the species persisted throughout the period of the investigation at the Stanmore Common pond, but it was not found at the other pond during each of the two winters included in this period. This may have been due to the greater exposure of the north side of the Rowley Green "acid" pond.

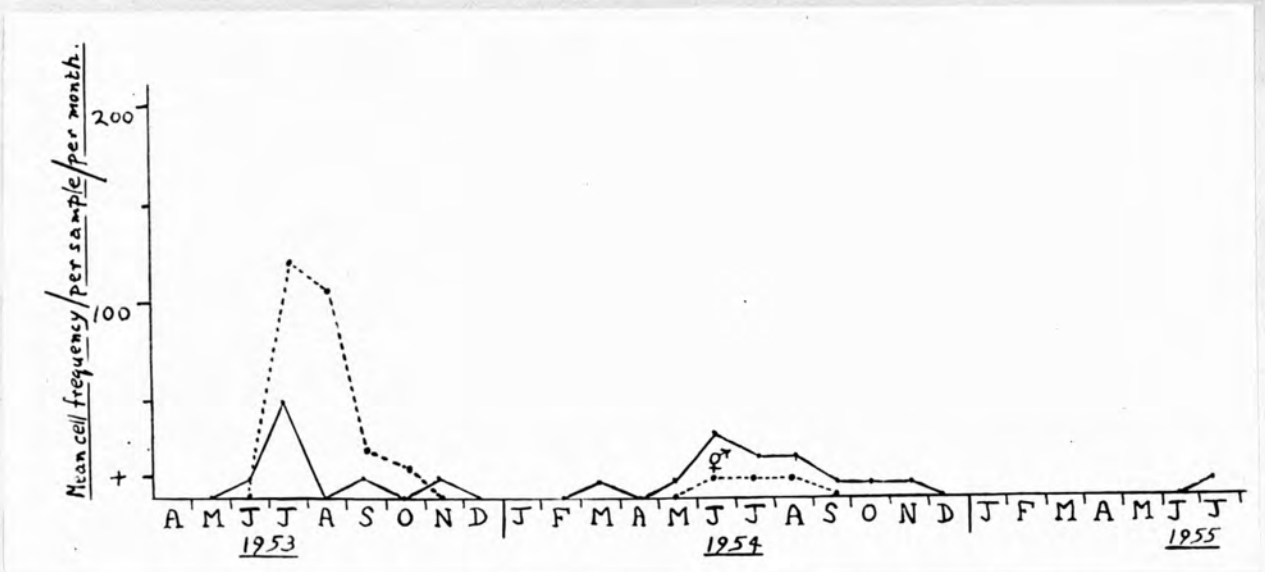
Secondly, there seemed to be an increase in this species at the Stanmore Common pond, and a decrease at the other pond during the period of the investigation. These apparent changes were possibly minor reflections of local and/or seasonal fluctuations in the weather. A contributory factor may have been increasing competition from other species of algae in the Rowley Green "acid" pond, and decreasing competition at the Stanmore Common pond. In 1953 Spirogyra cylindrica was abundant at the Stanmore Common pond, while it had decreased considerably in the following two years. Microspora floccosa was more frequent in the Rowley Green "acid" pond than in the Stanmore Common pond during the two winters 1953-54 and 1954-55 (Figures 4A & B). In addition, certain species of algae, including Ulothrix spp., Microthamnion Kutzingianum and Tribonema viride, which had been absent or rare in the Rowley Green "acid" pond in 1953 appeared, or increased in frequency, in 1954.

Figure 5. Mougeotia parvula.

A. Stanmore Common pond.



B. Rowley Green "acid" pond.



Unbroken line = water samples; Dotted line = samples from above the transition zone; ♂ = conjugation.

Thirdly, and most important in this investigation, filaments of Mougeotia parvula appeared on the litter nearly a metre above and several metres away from the water's edge during late summer and autumn after exposure of a number of months with little, if any, change in the appearance of the cells from those found in the water. That this species is able to survive severe and prolonged drought has been shown by experiment (Section III). Conjugation occurred in 1954 at both ponds and in 1955 in the Stanmore Common pond only. This species, however, seems able to survive dry periods without the production of zygotes, no conjugation having been observed during the relatively dry, warm summer of 1953 in either pond. For the Stanmore Common pond evidence has been obtained that cells descend into the deeper litter as the water level falls (Section IV) and results of culturing experimentally-dried litter tends to confirm this (Section III) and suggests that the same thing occurs at the Rowley Green "acid" pond in the exposed litter. This descent of cells into deeper layers of litter would increase their chance of survival in the event of a prolonged and severe drought occurring in nature. It might also be the means by which the species survives other

extreme conditions associated with exposure on the litter surface, and may explain the apparent absence of cells from the Rowley Green "acid" pond in winter.

8) Mougeotia Sp.A.

This species, unidentified, as conjugation was never observed nor zygotes found, is mentioned only as a contrast to the other, drought-resistant species, M. parvula.

Mougeotia sp.A occurred in the three less-acid ponds (Englefield Green, Stanmore and Rowley Green "normal" ponds) usually between March and September. Cells stranded above the water level and exposed to drying showed little resistance to desiccation and rapidly died. The species showed some survival of experimental drying (Section III), but this may have been due to the existence of previously formed zygosporangia in the samples.

9) The Desmidiaceae.

²³
22 Species of Desmids (see species list p.25), have been identified but no single species has occurred with sufficient frequency for any conclusions regarding its periodicity to be reached. Desmids have been found in each of the five ponds investigated, though more species were found in the Englefield Green and Rowley Green "normal" ponds than in the others. They occurred with

the greatest frequency in the Englefield Green pond. In this pond cells of one species or another were found throughout the period of the investigation and there was a tendency for a maximum to be reached in the early autumn (September 1953 and October 1954).

The vegetative cells of most species were confined entirely, or almost so, to the water, though cells were occasionally found on the litter or mud surface of the "transition zones" (2.5 - 15cm. away from the edge of the water) but with a few exceptions the species of desmids encountered seemed unable to survive exposure and drying. One distinct exception is the Saccoderm desmid, Netrium oblongum var. cylindricum, which occurred at the Stanmore Common pond, cells of which were found most frequently in August and September 1954, on the litter surface about 2 metres away from and 30cm. above the edge of the water. Drying was occurring during this time, the estimates of moisture contents in early August and late September being, respectively, 84% and 55% (of the wet weights), but, although the water did not rise to this region of the litter until late December, vegetative cells were found throughout the period of exposure. Cells were not found again until the water

level began to fall in June 1955 and this species might be said, as it multiplied upon exposure, not only to survive, but to thrive upon, exposure and drying.

In addition to the above, a number of species of Cosmarium and Closterium have been found to possess, in varying degrees, the ability of surviving drought (Section III) either by resistant vegetative cells or by previously formed zygospores. of the Chrysophyceae were found (see 10) Synura agg. 25), but were never as frequent as Synura.

For the purpose of this investigation the species of Synura encountered have been grouped together as Synura agg. With the exception of the Rowley Green "normal" pond, in which it was found only very infrequently, Synura was present in all of the ponds investigated. In the years 1953 to 1955 colonies were most frequent during the spring, attaining a maximum between early March and late April. Some colonies, it was observed, would remain motile in very little free water but upon exposure above the water level, and subsequent drying, the colonies rapidly broke up, the cells dying or, provided drying occurred slowly, encysting (see Section V, p.158 -159).

That cysts have been found to survive from one season to the next in the deeper layers of pond margin litter (see

Section IV) and that Synura disappears after prolonged and severe experimental drying (Section III), suggests that cysts below the surface may withstand drought more successfully than those at the surface. It is possible to conclude from this that it is only by passive carriage of cysts into the deeper litter that Synura may survive a serious drought in nature.

Other species of the Chrysophyceae were found (see species list (p.25), but were never as frequent as Synura. Dinobryon sp. also forms resistant cysts which descend into the deeper layers of litter (Section IV) and might, hence, be assisted in its survival of an extreme drought in nature.

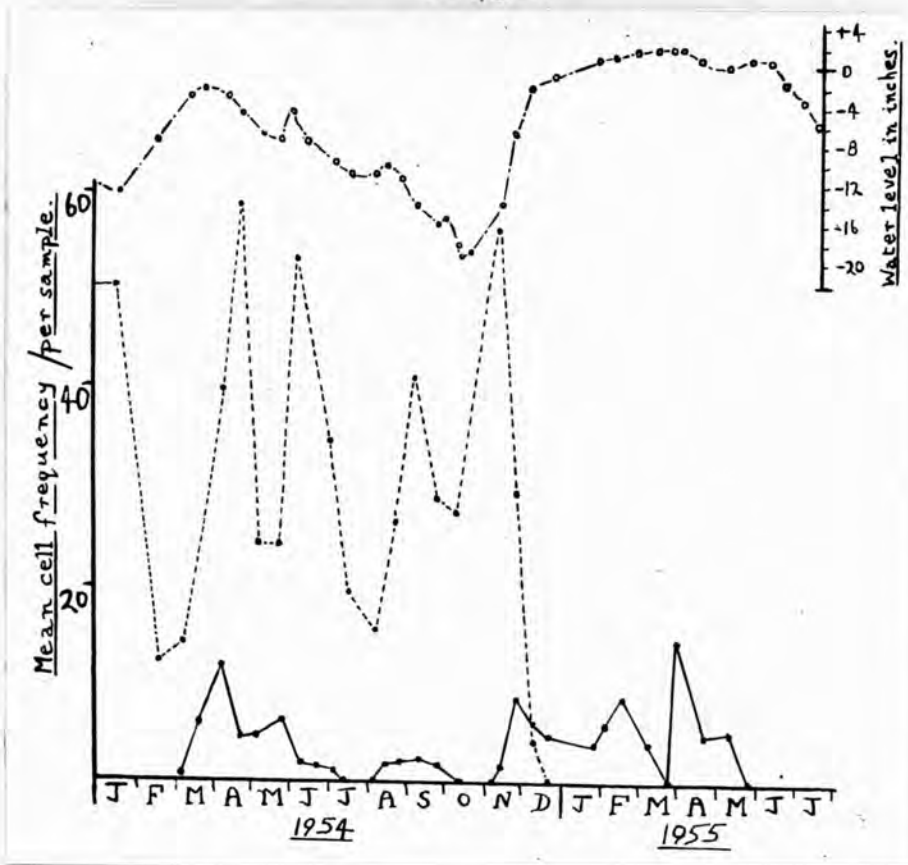
11) the Diatoms.

Twelve species of diatoms have been identified (see species list (p.25), but what is probably a large number of species has been grouped, and will be discussed, as naviculoid diatoms. In this investigation a wide survey has been attempted which has involved counting large numbers of individual cells from each sample taken. To have identified every cell observed of such a group as the naviculoid diatoms would have necessitated a drastic reduction in the number of samples dealt with. Consequently, it was decided, for this group, to sacrifice

taxonomic detail in order to observe the reactions of the group, as a whole, to exposure and drought.

The diatoms observed tended to reach a maximum in the spring or early summer in the water of the five ponds investigated, and to some extent this periodicity was reflected on the "transition zones".

The naviculoid diatoms showed preference for litter surfaces well above the water levels, subject to long exposure, and only appeared in water samples with any frequency when the water rose and flooded these litter surfaces. At the Stanmore Common pond, for instance, naviculoid diatoms were frequent on the litter surface during the long period of exposure in 1954 (April to late November) (Figure 6). After the rise in the water level, fewer cells were found in the water samples than had previously been found on the exposed litter (Figure 6). Although this may have been due to the different types of samples, two observations should be considered:- In the water samples, more cells were found after the rise in the water level than before; secondly, no cells were found in samples of submerged litter. The cells survive exposure for the greater part of the year and, during this time, may resist considerable drying.



Broken line =
samples from
the exposed litter
above the "transi-
tion zones".

Unbroken line =
water samples.

Figure 6. Naviculoid diatoms at the Stanmore Common pond.

That some at least of the cells of naviculoid diatoms from the Rowley Green "normal" pond are able to withstand prolonged and severe drought was observed in the course of the large-scale drying experiments (Section III). Cells may also descend into the deeper layers of litter and/or mud and remain viable (Section IV), and this might assist survival during a drought.

The identified species of diatoms, of which the most frequent were Pinnularia viridis and Nitzschia palea, were also found on exposed mud and litter surfaces but

were, in nature, usually more frequent in the water and on the exposed, but still very moist, surfaces of litter or mud close to the edge of the water (i.e. the "transition zones", 2.5cm. to 15.0cm. from the water margin). By experimental drying (Section III), Pinnularia viridis has been found capable of surviving exposure and severe, though not prolonged, drought. The descent of cells of this species into the deeper litter layers (Section IV) may help the species to survive a prolonged drought in nature, while the active downward movements of cells through the uppermost millimetres of litter (Section IV) might also assist the species in avoiding the consequences of unfavourable conditions in general, and drought in particular. This species, then, though from my observations seemingly more aquatic than those grouped as the naviculoid diatoms, is none-the-less capable of surviving during dry conditions.

It has been found by observation and experiment that the only visible difference between the usual form of the cells in the water and those cells that withstand drying is the accumulation, by the latter, of oily matter in the cells (See Appendix 1, p.186). Nitzschia palea was found in all the ponds investigated

except the Stanmore Common pond. It occurred with varying frequency, being rare in the Rowley Green "acid" pond, more frequent in the Englefield Green and Stanmore ponds, and, at times, very abundant in the Rowley Green "normal" pond. In one water sample, of early February 1955, from this last pond a mean of 70 cells was calculated for each of the three series of 50 high-power microscope fields surveyed. This species was found to be considerably more resistant to exposure and drying than Pinnularia viridis and usually showed an increase, at least initially, under these conditions. At the Rowley Green "normal" pond, for example, the species showed a marked increase during a three months exposure of the litter surface above the "transition zones" (Table 6). This was also found to occur in drying experiments (Section III).

Table 6. Nitzschia palea on the exposed litter of that species, of the Rowley Green "normal" pond.

Date (1953)	May 19	June 16	August 7	August 11	August 15
<u>Time exposed in days</u>	6	34	86	90	94
<u>Number of cells in 50 high-power fields</u>	1	32	37	38	218

Observations made in the course of this investigation, and experiments carried out, suggest, as with Pinnularia viridis, that drying may be accompanied by an

accumulation of oil in the cells (see Appendix 1, P.194)

That Nitzschia palea is able to survive severe and prolonged drought was established by large-scale drying experiments (Section III) and an investigation of pond-margin microstratification by a slide technique (Section IV) suggests that descent into the deeper layers of litter and/or mud may assist in this survival.

An interesting feature regarding Nitzschia palea is that during 1953 and 1954 cells appeared, and were abundant upon the exposed marginal areas of the Stanmore pond between June and August. These results coincide closely with those of Lund (1942) for similar areas of the Clay Pit, Richmond Park.

Nitzschia palea, then, thrives upon exposure and this conclusion, also, agrees with Lund's observations (1942) of that species, Pinnularia viridis and certain other diatoms which, he found, became more abundant on exposed mud than they were as a bottom-living flora. In addition, Nitzschia palea, at least, is capable of surviving prolonged and severe drought.

Hantzschia amphioxys was never found at the Stanmore pond, was rare at three of the other ponds investigated, and appeared in abundance only during December 1953 -

January 1954 on the exposed litter of the Stanmore Common pond. However, the species appeared upon re-wetting of litter and mud samples, from the Englefield Green and Rowley Green "normal" ponds, which had undergone prolonged and severe drying (Section III).

Bearing in mind that relatively very few diatoms have been specifically identified and investigated regarding their survival during dry periods, a tentative sub-division of diatoms into five "survival types" is suggested, as follows:-

- a) Species found in the water only and apparently unable to survive exposure and drying.
E.g. Gomphonema acuminatum, Gyrosigma acuminatum, Frustulia rhomboides, Nitzschia acicularis, Epithemia sp. and Melosira varians.
- b) Species found mainly in the water but able to survive some exposure and drying.
E.g. Pinnularia viridis, P. major, Cymbella lanceolata, Nitzschia linearis.
- c) Species found in the water and on the litter surface with approximately equal frequency and able to survive prolonged and severe drought.
E.g. Nitzschia palæa and Stauroneis phoenicenteron
(see Section III)

d) Species showing preference for exposed litter surfaces and able to survive prolonged and severe drought. In this group might be included some of the naviculoid diatoms, unidentified in the present investigation.

(see e) Species usually only becoming frequent upon re-wetting after drought and able to survive prolonged and severe drought.

E.g. Hantzschia amphioxys.

12) The Dinophyceae.

Cells of one or another species of the Dinophyceae were found in each of the five ponds investigated, but for the Stanmore and Rowley Green "normal" ponds were usually rather rare. Five species have been identified, two of Glenodinium and three of Peridinium.

Motile vegetative cells of Peridinium lead a planktonic existence, but were also found, though rarely, in water films on the surface of exposed litter immediately after a fall in the water level. Oil-containing cysts of Peridinium have also been found, both in the water and, occasionally, on the litter surface of the "transition zones", but no evidence has been obtained that these cysts are drought-resistant.

Altogether, 11 species of the Euglenineae have been identified in the course of the present investigation (see species list p. 25), and a number of these species are discussed, taxonomically, in Section VI. Ten of these

Cells of Glenodinium pulvisculus were, usually, also planktonic, and occurred most commonly in the Stanmore Common pond in winter. Motile cells were also found amongst dead leaves on the exposed pond bottom after the onset of relatively damp, cool weather in November 1954 (see Section IV). Rounded-off cysts of this species, previously found on the pond bottom, gave rise to motile cells when placed in soil solution culture, suggesting that it is by means of the cysts that this species of the Dinophyceae survives exposure and drying. This species was also found, by experiment (Section III), to survive prolonged and severe drought and to re-appear in large-scale, outdoor, drying experiments at times which closely paralleled the times of appearance of the species in nature. A most interesting feature regarding this species is that the cysts, although formed while the cells are still in the water, and, therefore, in response to conditions other than drought, are, none the less, highly drought-resistant.

13) The Euglenineae.

Altogether, 31 species of the Euglenineae have been identified in the course of the present investigation (see species list p. 25), and a number of these species are discussed, taxonomically, in Section VI. Ten of these to be equally sensitive to drying, and, again, the search

species occurred in one or another of the ponds only, while each of the other species was common (to two or more of the ponds). Only ⁴5 species were common to all five ponds investigated which is possibly a reflection of the varying nature of the ponds. As was found by Lund (1942), there seemed to be a tendency towards an autumn maximum, though cells were also often frequent in the spring and/or early summer. Only 8 of the 31 species identified were ever abundant (90 or more cells counted in 50 high-power microscope fields) while 14 species were referred to as rare (4 or fewer cells in 50 fields) and the remaining 9 species fall between these two extremes.

None of the 6 species of Phacus identified was ever found on the exposed mud or litter surface above the moist "transition zones", and 3 of the species:- P. parvula, P. pleuronectes and P. oscillans, were found only in water samples. This suggests that these species are, in the normal vegetative state, particularly sensitive to desiccation and will not long survive exposure. All attempts at finding truly drought-resistant stages in nature, or producing them experimentally, failed. The 3 species of Trachelomonas identified were found to be equally sensitive to drying, and, again, the search

for truly drought-resistant stages met with failure.

One species of Lepocinclis, L.teres (see Section VI.) occurred, at times abundantly, on the litter surface of the "transition zones" (2.5 - 15cm. away from the edge of the water) of the Stanmore Common and Rowley Green "acid" ponds, and cells were also found, though rarely, above the "transition zones". These cells differed from those in the water only by the lack of flagella and their loss of motility, and may have been resting cells though they could not be induced to revive in liquid culture in the laboratory. The other species of Lepocinclis, like those of Phacus and Trachelomonas, were very sensitive to drying.

Amongst the species of Euglena, 5 were found to be capable of withstanding some exposure in nature:- E.deses, E.viridis, E.mutabilis, E.minima and E.inflata. Of these species, one, E.mutabilis, appeared to thrive upon exposure occurring abundantly at times on the litter surface above the "transition zones" of the Stanmore Common and Rowley Green "acid" ponds. E.viridis and E.deses were found to survive mild experimental drought (Section III) but no species survived prolonged and severe experimental drying. E.deses and E.mutabilis were found in the deeper layers of

The vegetative cells of the above named blue-green algae can exist in the absence of free water, and survive here we have some indication of the way in which these species, at least, of the Euglenineae, survive drought in nature. The cells of Nostoc commune are, probably, saved from

14) The Cyanophyceae

The Cyanophyceae were rare in the two acid ponds but in the other three ponds, particularly the Stanmore pond, were, at times, abundant. Maximum frequency occurred in the summer months, May to September, which agrees with the observations of Fritsch and Rich (1913), Hodgetts (1921) and, to a large extent, with the results of Rao (1949).

For the same reason as that put forward regarding the grouping of "naviculoid diatoms" (p.46), all specimens of Anabaena encountered have been considered generically as Anabaena spp.

Anabaena spp. and Oscillatoria tenuis were the most frequent of the blue-green algae found in the course of the present investigation. They were often found on the exposed mud or litter surfaces of, and above, the "transition zones" of the Englefield Green, Stanmore and Rowley Green "normal" ponds.

Nostoc commune was also found at times on the exposed margins of the Englefield Green and Stanmore ponds.

The vegetative cells of the above named blue-green algae can exist in the absence of free water, and survive exposure and drying with little or no apparent change.

The cells of Nostoc commune are, probably, saved from desiccation, to a large extent, by the gelatinous envelope which has been observed, during severe drying, to shrink round the cells.

Filaments of Anabaena spp. and Oscillatoria tenuis have been found by experiment to survive prolonged and severe drought (Section III). They have also been found in the deeper layers of the litter and mud of pond margins (Section IV). This may help to account for their survival of very severe drought.

state in the "wet" than in the "dry" soil. This would appear to be a reasonable conclusion, and the observations and experiments carried out in the course of the present investigation agree with Rao's observations and tend to support his conclusion.

Rao (1953) found no resting stages in his cultures, either because they were not present or because they were so rare as to be overlooked.

In the present investigation it was realized that liquid cultures of dried litter and/or mud, although

Section III. Drying Experiments.

A. Introduction.

During an investigation by Rao (1953) of the distribution of algae in small ponds, one of the ponds dried up. Rao took two sets of samples from the margin of the pond, one set when the soil was still sufficiently moist to be described as "wet", and a second set, five days later, of samples which were dried for a further two days in the laboratory and were described as "dry" samples. These samples were cultured in various media and it was found that the "wet" soil gave rise to an initial sharp rise in the "total" algae. The explanation put forward for this occurrence was that there were more algae in the vegetative state in the "wet" than in the "dry" soil. This would appear to be a reasonable conclusion, and the observations and experiments carried out in the course of the present investigation agree with Rao's observations and tend to support his conclusion.

Rao (1953) found no resting stages in his cultures, either because they were not present or because they were so rare as to be overlooked.

In the present investigation it was realized that liquid cultures of dried litter and/or mud, although

revealing what species are present in any sample, may give little, or in most cases no, indication of the mode of existence of the algae in that sample. With this in mind a good deal of attention has been paid in the present investigation to the direct, microscopical observation of samples taken from pond margin litter and/or mud.

Prolonged periods of warm, dry weather are relatively rare in Britain, and although the water level in small ponds may drop considerably during the spring and summer, the more or less frequent occurrence of rain and formation of dew tends to keep exposed marginal deposits of most ponds rather damp and, particularly during wet, cool years, little or no drying of the marginal litter and mud may occur.

To ensure eventual drying up of some part of the marginal zones of the ponds investigated, it was realised that it would be necessary to bring about artificial drought by protecting samples of marginal litter from rainfall. This was brought about by allowing small samples of litter to dry in the laboratory at room temperature. As the conditions under which these earlier drying experiments took place were so artificial, they must be regarded merely as preliminary experiments.

In order that a closer approximation to conditions in the nature might be realised, a series of "large-scale" drying experiments have also been carried out, drying several cubic feet of litter and/or mud from the margins of each of four ponds. Additional experimental work has included further drying experiments, and the culturing of dried samples.

B. Methods.

1) Preliminary experiments.

In all but one of the preliminary experiments the pond margin litter and/or mud samples consisted of portions approximately 2cm. deep with a surface of 8 x 8 cm. These portions were placed in shallow, square, white dishes and allowed to dry slowly in the air. The samples were taken from the margins of four of the ponds described in Section II, i.e. ponds I (Englefield Green), III (Stanmore Common), IV (Rowley Green "normal") and V (Rowley Green "acid" pond).

There were three main series of preliminary experiments, commenced respectively in late May, late June and mid-July 1953, and these small samples of litter and/or mud, dried in the laboratory, were all treated in more or less the same way, as follows: an initial investigation was made

of the wet samples as soon as they were brought into the laboratory, a surface scrape being placed on a slide, thoroughly mixed with sterile, distilled water and surveyed, listing the species of algae present. The samples were then allowed to dry slowly at room temperature, and from time to time, usually every two or three days, scrapes were again treated as described above and the algae present listed.

A few approximate estimations of moisture content were made during the preliminary experiments by weighing a portion of each litter sample, drying in an oven at 105°C. for 24 hours, and re-weighing. The results of moisture estimations are throughout expressed as the percentage of the wet weight (for an explanation of this unusual method of recording moisture content see Section II, page 20).

Although, as has been pointed out earlier, the shortcomings of liquid culture in an investigation of this nature have been considered, this method has still been used to test the viability of algae in dried litter and/or mud. Towards the end of each preliminary experiment, when the samples were becoming very dry, litter surface scrapes, which had been observed microscopically

on a slide, were placed in Beijerinck (1895) 0.05% culture solution and the liquid culture itself examined for algae every week, for up to four weeks.

In a further preliminary drying experiment, the samples, which consisted of a green scum scraped from the mud surface at the margin of pond I (Englefield Green) in mid-September 1953, were dried in watch glasses. Two separate samples had been collected, one which was originally very wet and the other which had already begun to dry before the collection was made. These samples were treated in much the same way as in the other preliminary experiments, but when the observations for algae were made the number of cells of each species present was counted in a total of 50 high-power (x 875) fields. Again, small portions were placed in liquid culture when the green scum became dry.

2) Large-scale drying experiments.

In early August 1953, very large samples, each several square feet in area, of litter and/or mud, were collected in shallow sinks from the margins of the four ponds mentioned above. The sample from pond I (Englefield Green) was about 7.5 cm. deep, the samples from the other ponds about 15 cm. deep. The four sinks containing

the initially wet litter and/or mud were placed in a cold frame, each sink being tilted slightly to allow excess free water to run out of the drain hole. These sinks were kept under glass, and hence protected from rain, from August 1953 until October 1954, for a total of 434 days.

From each of the sinks, surface samples of the litter and/or mud were taken to a depth of about 0.5cm. at intervals and the moisture content estimated. Examinations for the presence and frequency of living algae were also made, as follows: small, glass tubes (2" x 1") were approximately half-filled with litter and/or mud scraped from the surface; the tubes were then almost topped-up with sterile, distilled water and shaken vigorously. After allowing these to settle, 2 drops of liquid were withdrawn from each tube, one from the liquid surface and one from immediately above the sediment. The 2 drops were placed on a slide and examined, the number of cells of each algal species being counted in a total of 50 fields at x ⁸⁷⁵975.

The sequence of events and the sampling methods employed in the large-scale drying experiments are to be considered in three main phases as described below:-

Phase A: For the first 7 sets of samples (i.e. until the 19th day of drying) litter and/or mud was taken from 4

(surface) and lower layers of litter and/or mud with regard to their moisture contents and the living algae (if any) different regions in each sink:

- 1) the area originally nearest to the free-water.
- 4) " " " " farthest from " "

2) and 3) intermediate positions.

After the samples had been taken on the 14th day (mid-October 1954), the glass plates were removed from the cold frame exposing the slabs of litter and/or mud to the weather, and especially to the sun. The surface litter and/or mud retained considerable moisture throughout this initial period (see Tables 24 to 27, p.109).

Phase B: From mid-September 1953 until mid-March 1954, during which time minimum moisture contents were reached, only two samples were taken from each sink at each of nine collections. One sample was taken from region 1) and one from region 4). At the close of this period (mid-February to mid-March 1954) it was found that moisture contents had increased, even though rain was still being excluded, due to re-absorption of moisture from the atmosphere. As in the preliminary experiments, liquid cultures were started from time to time to find out what species of algae, if any were still viable. The first liquid cultures were set up in triplicate, using three different media: sterile distilled water, Knop's 0.7% solution and soil solution. As it was found that more species appeared in the soil solution cultures, this medium only

Phase C: Subsequent to the "atmospheric re-wetting" (from mid-April 1954) samples were taken from each sink as follows:

- i) to a depth of about 0.5cm.
- ii) from about 0.5cm. to 3.0cm. in depth.

These cultures were then examined microscopically for algae at the end of a fortnight, a month,

(surface) and lower layers of litter and/or mud with regard to their moisture contents and the living algae (if any) or, where applicable, as abundant. The samples of litter contained therein.

After the samples had been taken on the 434th day (mid-October 1954), the glass lights were removed from each sink: i) from the surface layer to a depth of about 0.5cm. and ii) from the lower layer (about 0.5 - 3.0cm.) to the weather, and especially to precipitation. The samples taken subsequently were taken in order to investigate re-wetting of the litter and/or mud, and liquid culture of the lower layer samples only. In the tables of results, the symbol of presence in brackets, thus: (+), indicates the appearance of what species in the re-appearance of algae.

In November 1954, after the sinks had been exposed to the weather, free water began to accumulate in two of them, that one containing mainly litter from pond III (Stagnant Common) and that one containing litter and mud from pond V (Rowley Green "acid" pond). Since then, the water also has been examined for algae. As it was found that more species appeared in the soil solution cultures, this medium only has since been used. Liquid cultures were started after

3) Additional experiments.
a) Slow and rapid drying.
Samples of litter and mud, collected in April 1955 from the margins of the five ponds described in Section II, solution culture, approximately 1.0gm. of litter and/or mud from the surface of each of the areas 1) and 4) of dry relatively slowly at room temperature to air dryness. These cultures were then examined microscopically for algae at the end of a fortnight, a month, The samples of the second series were drained artificially

and two months, the species found being recorded as present or, where applicable, as abundant. The samples of litter and/or mud for starting liquid cultures were taken on, and have largely been taken since, the 244th day of drying at each sink; i) from the surface layer to a depth of about 0.5cm. and ii) from the lower layer (about 0.5 - 3.0cm.) In the tables of results, the symbol of presence in brackets, thus: (+), indicates the appearance of that species in liquid culture of the lower layer samples only.

In November 1954, after the sinks had been exposed to the weather, free water began to accumulate in two of them, that one containing mainly litter from pond III (Stanmore Common) and that one containing litter and mud from pond V (Rowley Green "acid" pond). Since then, the water also has been examined for algae.

3) Additional experiments.

a) Slow and rapid drying.

Samples of litter and mud, collected in April 1955 from the margins of the five ponds described in Section II, were divided into two series. One series was allowed to dry relatively slowly at room temperature to air dryness, the time to reach this condition being about 110 hours. The samples of the second series were drained artificially

of their free water by placing each on a filter paper disc, and subjecting each to 10 minutes suction in a Büchner funnel. After this treatment these latter samples became air dry at room temperature within 20 hours. Portions of the dried samples of both series were placed in soil solution culture and these cultures were examined for algae after one week, a fortnight and one month. Control cultures, of soil solution inoculated with moist samples as collected from the pond margins, were also set up.

b) Re-wetting experiment.

Samples of litter and mud, collected in June 1955, from the margins of the five ponds described in Section II, were dried at room temperature to air dryness, and then portions of each dried sample were re-wetted in one of two ways. In one series of cultures (A) the portions were completely immersed in soil solution by placing them under inverted watch glasses in crystallizing dishes half-full of soil solution. In another series of cultures (B) the portions of dried litter or mud were placed, initially, in shallow soil solution in dishes and not totally immersed. After 7 days, during which time slow re-wetting of the dried litter or mud occurred, more soil solution was added, half-filling each crystallizing dish of this second series of

cultures (B). The cultures were examined for algae after 1 week, 1 month, and 6 weeks.

C. Results and discussion.

In this Section, as each experiment is not discussed individually, and as the results are presented mainly in the form of tables which are referred to frequently by number in the following account, these tables have been placed together at the end of the Section (pp. 103 - 119).

In almost all the preliminary drying experiments and liquid cultures, species of algae have been recorded merely as present or absent. Where, in the preliminary experiments (Tables 7 - 19), the symbol for presence is enclosed in brackets, thus: (+), this indicates some obvious difference, in the cells observed, from the normal condition. In the case of the usually motile algae, the flagellates and biraphid diatoms, it indicates the loss of motility in the individuals observed; in other species it indicates some other change (E.g. slight contraction of the protoplast in Cosmarium praemorsum).

In the tables of results 32 - 35, showing the presence of algal species in the liquid cultures derived from the large-scale drying experiments, symbols of presence are placed in brackets, thus: (+), where the species was found

only in the culture derived from the lower layer (0.5 - 3.0cm.) of litter and/or mud. It should be noted that samples derived from the lower layer were placed in liquid culture only from the 4th series of cultures (sample No.17) onwards.

For the last of the preliminary experiments (table 22), and the large-scale drying experiments, cell counts were made of the algal species found at each collection. This method, despite its faults, which have been pointed out by Lund (1942), is at least objective.

The large-scale drying experiments were treated in three main phases, and the following points should be noted regarding the results as presented in the tables 24 - 31.

Moisture estimations: in phase A (samples 2 - 7) the figures are averages of 4 separate estimations; in phase B (samples 8 - 16) they are averages of 2 estimations; in phase C, during the investigation of the upper (0 - 0.5cm.) and lower(0.5 - 3.0cm.) layers of litter and/or mud, the results of the moisture estimations of both layers are presented when there is a difference between them.

Frequency counts of algae: in phase A, the figures for each species are the total numbers of cells counted in a

total of 200 high-power fields of the microscope (i.e. 50 fields surveyed from each of the 4 samples taken); in phase B the figures are the total numbers of cells counted in 100 high-power fields; in phase C they are the total numbers of cells counted in only 50 fields. In table 25 the count of Ulothrix subtilis cells for sample 21 is placed in brackets, indicating that the cells were found in the sample from the lower layer (0.5 - 3.0cm.) of litter. In the tables 28 - 31, showing the results of exposing the sinks of litter and/or mud to the weather, the frequency counts are mainly of cells found in the upper layers of litter and/or mud, but where algae were also found in the lower layer the frequency count for the sample from this layer is expressed as the denominator of a fraction. In the tables 30 - 31, showing the results of exposure to the weather of the sinks containing the litter and/or mud from, respectively, ponds III (Stanmore Common) and V (Rowley Green "acid" pond), are included counts of algae found in the free water which accumulated in these two sinks. The frequency of the species in the free water, samples of which were taken in 2" x 1" glass tubes, was estimated as described in Section II for field collections of pond water, three drops being mounted on a slide and

examined with the high-power of the microscope recording the total numbers of cells of each species present in 50 fields.

A general feature of the drying experiments is that there is no initial decrease in algal frequency or number of species. In fact, some species, E.g. Nitzschia palea and Oscillatoria tenuis, show an apparent increase during the early stages of drying (Tables 24 and 26). Later, there is a marked decrease in the number of species and in algal frequency, and this seems to occur below a moisture content of about 50%. Lund (1942) observed an immediate and marked decrease in the algal flora of exposed marginal mud which had begun to dry, and although he gave no moisture estimations, my observations suggest that it is not unreasonable to assume that this decrease also occurred when the moisture content was at about 50%.

Initially, as was also found by Lund (1942), species of Euglena were found creeping over the mud surface and some Chlamydomonas species were actively motile in the thin films of water surrounding particles of litter and/or mud. Trachelomonas was uncommon in all the drying samples, but T.hispida appeared in a liquid culture of litter from pond I (Table 11), which had been dried for 12 days,

indicating some degree of resistance to desiccation by this species. But neither this species, nor any other species of Trachelomonas, appeared in liquid cultures of the large-scale drying experiment samples, which indicates non-survival of the prolonged and severe artificial drought. In agreement with Lund, again, no species of Cryptomonas were found on the drying litter or mud. Cryptomonas ovata, however, appeared in liquid culture of dried litter and/or mud nine times during the course of the experiments (Tables 11, 20, 23, 32, 33 and 37), indicating a high degree of survival, at least of this species of Cryptomonas, during exposure and drying. In one instance (Table 32) C.ovata had survived 244 days in one of the large-scale drying experiments, and although the mud had initially been wet (i.e. during phase A) and although there was the "atmospheric" wetting in February and March 1954, this period included a relatively prolonged and very dry period extending over at least 92 days during phase B. This is a longer period than any likely to be encountered in nature in this country. Despite many observations, however, no resting stages have ever been found of this species. If a resting stage does exist it may be formed, like that of Glenodinium pulvisculus, well as Pinnularia viridis, were found to survive drying.

in the water of the pond itself (C.f. Section II, p.54 and Section IV, p.135), or at least, very soon after exposure of marginal litter and/or mud, before any appreciable drying has occurred. In the last of the preliminary experiments (Tables 22 and 23) it was found that upon drying rapidly in the laboratory there is a tendency for algae which had already undergone some drying in nature to survive, while those algae collected from very wet areas are killed off. This suggests that drought-resistant cells might be more likely to be formed in nature during the relatively slow drying upon exposure, than during the relatively rapid drying induced artificially in the laboratory. That, in the laboratory, rapid drying kills more species of algae than slow drying to the same degree of dryness (i.e. air dry) has been shown by experiment (see pp. 98 - 100 and Table 37).

In the large-scale drying experiments (Tables 24 - 27) some species, including all the members of the Euglenineae originally found, apparently disappeared completely, never being found after a time by direct observations and never appearing in liquid cultures of samples. But some of these species, including Euglena viridis and E. deses as well as Pinnularia viridis, were found to survive drying,

for short periods at least, in the preliminary experiments (Table 11). Some species, although disappearing from one or more of the large-scale drying experiments, appeared in liquid culture of samples taken from one or more of the other large-scale drying experiments. Nitzschia palea, for example, which was originally found in all four of the large-scale drying experiments, though more frequently in the non-acid ponds, I (Englefield Green) and IV (Rowley Green "normal" pond), than in the acid ponds, III (Stanmore Common) and V (Rowley Green "acid" pond), apparently disappeared from all four experiments during the drying. This species, however, eventually re-appeared in the sample from pond I after 123 days of exposure to the weather (Table 28) and also has appeared twice in liquid cultures of the pond I samples (Table 32) and four times in liquid cultures of the pond IV samples (Table 35).

The non-appearance of Nitzschia palea in the liquid cultures of the "acid" pond samples may be related to the pH in these cultures. The pH of the soil solution used as a culture medium is 6.6 approximately, and it has been found that within one month of commencing culture of the litter and/or mud from the large-scale drying experiments, the pH in the cultures of samples from ponds I

and IV goes up to 8.2 and 7.1 respectively, while the pH in the cultures of samples from ponds III and V, the "acid" ponds, goes down to 6.0 approximately.

Some of the species which disappeared during drying and were never found in liquid cultures of dried litter and/or mud have been found never to thrive in laboratory culture, even when the original inoculum consisted of wet material containing living algae. In all cases, growth was arrested and, usually within a very short time, a week at most, the algae died. In this investigation the following algae have been found to fall into this category: Volvox aureus, Closterium eboracense, Pleurotaenium trabecula, Phacus triqueter, P. caudata and Trachelomonas volvocina; and for these species it cannot be said definitely that excessive drought killed them off. Only those species that do appear in laboratory cultures at times, but which disappeared completely during drying and never appeared in liquid cultures of dried litter and/or mud, can be said, and then only tentatively, to have been killed off by the artificial drought. Of the algae investigated, only two species fall into this group, Pandorina morum and Euglena mutabilis. But both these species have been found to withstand some exposure in the course of the present investigation is apparently

in nature. Non-motile coenobia of Pandorina morum, with fairly extensive layers of surrounding mucilage, were found 7.5cm. away from the water edge on the litter surface at the margin of the Rowley Green "acid" pond in August 1953, and a single such coenobium, placed in Beijerinck (1898) 0.05% culture solution, revived and had multiplied within 2 days. This species will, then, survive brief periods of exposure at least, and it may be that it would survive more prolonged exposure and drying, provided the initial drying occurred more slowly than that induced artificially. Regarding Euglena mutabilis, it has been found that in nature this species rarely occurs in the free water of the ponds investigated, but is often found in some abundance on exposed marginal litter, particularly where the pH is low. It has been observed at the Stanmore Common pond on exposed litter several metres away from, and more than 30cm. above, the free water, during the period of low water level in autumn. In liquid cultures in the laboratory, this species, although found initially, disappears after a week or ten days. Euglena mutabilis has also been found living on very sandy soil (John, 1942) which would, at times, suffer considerable drying; but the form of E. mutabilis found in the course of the present investigation is apparently

a hydro-terrestrial form which will not survive either prolonged drying or, conversely, immersion in liquid. It will, however, thrive upon damp, pond margin litter provided the pH is sufficiently low (the pH range of the litter of the Stanmore Common pond is 3.00 - 7.00 with most readings within the range 4.01 - 5.00).

In the large-scale drying experiments there was an increase in the moisture content of the litter and/or mud during February - March 1954 (Tables 24 - 27). No algae re-appeared in the litter from pond V, where the moisture increase was small, but algae did re-appear in the other experiments. In the mud and litter of pond IV (Rowley Green "normal" pond), in which there was only a slightly greater increase of moisture, three species appeared: Chlamydomonas sp., Ulothrix subtilis and a naviculoid diatom. In the litter of pond III (Stanmore Common pond), the moisture content of which increased considerably, only two species appeared: Ulothrix subtilis and Glenodinium pulvisculus; while in the mud and litter of pond I (Englefield Green pond), although the increase of moisture content was not as great as for pond III, six species appeared: Chlamydomonas sp., Ulothrix subtilis, Microthamnion Kutzingianum, Anabaena sp. and Oscillatoria

tenuis. The appearance of relatively so many species in the mud and litter of pond I may be associated, at least in part, with the presence of more algae in the original sample.

It is not at all surprising that Ulothrix subtilis should have re-appeared in three of these large-scale experiments upon the increase in moisture content, as this species has been found living in nature in relatively exposed habitats such as pond margins and stream banks well above the general water level, and has also been found to survive prolonged periods of severe drought (see Section V). The re-appearance of this species at a moisture content of 28% (Table 25), and its non-appearance at a moisture content of 29% (Table 27), suggests that the critical level of moisture content for U. subtilis is in the region of 30%.

Regarding the flagellates that appeared during the moisture increase, it has been found that these compare with the flagellates which occurred in nature in the water of the corresponding ponds. Chlamydomonas appeared in ponds I (Englefield Green) and IV (Rowley Green "normal" pond) at this time, and in the former reached a definite maximum in March. Chromulina ovalis was only found once in pond I, and that was on 9th March

1954, which was in the same week as it was found in the corresponding large-scale drying experiment. Glenodinium pulvisculus has often been found in pond III (Stanmore Common) water samples and was abundant in March 1954. The occurrence of these flagellates in the large-scale drying experiments would appear to be due, then, to meteorological conditions and the "atmospheric re-wetting" of the litter and/or mud. These species, in the form of resting stages, had passed through a prolonged and severe drought. Although actual resting stages were not found at the time, it is likely that Chlamydomonas sp. survived as zygospores, while Chromulina ovalis and Glenodinium pulvisculus may have survived as resistant cysts similar to those found elsewhere (C.f. Section IV, p. 135 and Section V, p. 157). These species required only a very small amount of free water for the resting stages to become the motile, flagellate stages once again. With the exception of Chlamydomonas sp. from pond I (Englefield Green) these flagellates were not found in the original samples collected for the large scale drying experiments in early August 1953; thus they were either so rare as not to be observed in the original samples, or they were already in the form of resting stages at this time.

On October 22nd 1954, the glass lights on the cold frames in which the experimental sinks had been kept were removed to expose the litter and mud to the weather after a total of 434 days of protection. The subsequent increase in moisture content and the appearance of algae are recorded in the tables 28 - 31. The moisture increase was no greater initially in the mud and litter of ponds I (Englefield Green) and IV (Rowley Green "normal" pond) than it was for the other two ponds. More algae, however, appeared during the first six weeks of exposure to the weather on the mud and litter of ponds I and IV. This was probably due to the initial resistance to re-wetting of the litter of ponds III (Stanmore Common) and V (Rowley Green "acid" pond), the earlier estimations of moisture content being, in the case of these two ponds merely an indication of the free water on the litter surface and occupying the larger crevices therein. In January 1955, freezing occurred, the resistance to re-wetting was broken down, and there was a subsequent increase in the algae on the litter of these two ponds.

At the end of December 1954, relatively large volumes of free water began to collect in the sinks containing the litter and mud from ponds III and V, and the algae which

are merely lists of species found in the soil

appeared in the water are listed under "W" in the tables 30 and 31. The occurrence of Glenodinium pulvisculus again coincides with its occurrence in nature, a maximum being reached by this species in pond III at the end of December 1954. Chlorogonium euchlorum, which was very abundant in the free water of the sink containing the litter and mud from pond V (Rowley Green "acid") in early February, reached a maximum in nature at this pond at about the same time. The appearance of the flagellates in the large-scale experiments in early 1955 would, then, seem to be due to a similar combination of optimum conditions as that which brought about the appearance of flagellates in early 1954, except that the more recent moisture increase was due to exposure of the litter and/or mud to rain rather than to absorption of atmospheric moisture. There was a considerable delay in the second appearance of the flagellates, indicating that the longer the species remain in the resting stage the longer is the interval between availability of water and the production of the motile form from the resistant stage. This conclusion receives some support from the observations made on soil solution cultures of the drying litter and/or mud. Although, for the sake of brevity, the tables 32 - 35 are merely lists of species found in the soil solution

cultures, it was found that in the earlier cultures most, or in some cases all, of the species eventually present appear within the first 14 days, while in subsequent cultures species take progressively longer periods of time to appear. In liquid culture of sample No.26 taken from the pond I (Englefield Green) large-scale experiment, for instance, Ulothrix subtilis, Stigeoclonium tenue and Microthamnion Kutzingianum, did not appear until one month had elapsed, while Anabaena sp. and Scenedesmus quadricauda did not appear until after two months.

The two species of Microspora that appear in the large-scale experiments after exposure to the weather also show parallels with their occurrence in nature, these cold-water forms not appearing until after the cold spell in January 1955 (Tables 30 and 31).

Progressively fewer species of algae appeared in the liquid cultures of litter and/or mud during the course of the first part of the large-scale drying experiments (Tables 32 - 35), while there was protection from rainfall (August 1953 - October 1954). Liquid cultures of ponds I (Englefield Green) and IV (Rowley Green "normal") mud and litter set up since the protective glass lights of the cold frames were removed show an

that there was no increase in the number of species

increase in the number of species. One possible conclusion to be made from these observations is that a sudden immersion in a relatively large volume of liquid may inhibit the germination of the resting stages of certain species as effectively as continued drought. To confirm this, re-wetting experiments were performed (see p. 68.) and the results obtained are summarised in table 36. With the exception of the Rowley Green "normal" pond samples there was a definite tendency for more species to appear upon slow, than upon rapid, re-wetting of the dried litter or mud. It is worthy of note that species of naviculoid diatoms were completely inhibited by total immersion and that three of the four species of Closterium appearing upon slow re-wetting of the Englefield Green pond sample failed to appear upon total immersion. The zygospore of Spirogyra nitida, although surviving the drying, apparently failed to germinate when immersed in soil solution. This may help to explain the non-appearance of certain other species in liquid culture, and the results suggest that a more accurate idea of the species present in dried mud or litter may be obtained if the sample is allowed to re-wet slowly, and not suddenly immersed in liquid. That there was no increase in the number of species

appearing in the first liquid cultures of the other two ponds (III and V) after exposure to the weather may be associated with the initial resistance to, and delay in, the re-wetting of the litter of these ponds.

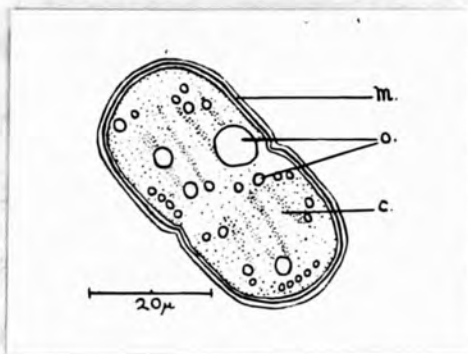
The appearance of a high proportion of Chlorophyceae in the liquid cultures is probably associated with the high proportion of this group occurring in the algal flora of the ponds in nature. Similarly a high proportion of Chlorophyceae are included in those algae re-appearing in the large-scale experiments after exposure to the weather, and eventual re-wetting of the dried litter and/or mud (Tables 28 - 31).

The algae which appeared in liquid culture of the lower layer (0.5 - 3.0cm.) only of litter or mud, indicated in the tables 32 - 35 by (+), show some increase for the litter and mud samples of ponds I (Englefield Green) and IV (Rowley Green "normal") from the start of this part of the investigation (sample No.17) onwards, particularly after exposure of the sinks to rainfall in October 1954. This agrees with Petersen's account (1935) of the passive carriage of algae into deeper layers of soil by water seepage. Too few species appeared in cultures of the ponds III (Stanmore Common) and V (Rowley Green "acid")

pond) litter and/or mud samples, for any such conclusion to be reached.

As far as individual species are concerned, it is not possible to discuss in detail each and every one encountered, but certain species, of which relevant and important observations have been made, and which have not been discussed earlier, will be considered below.

A number of species have been found, after prolonged drying in the large-scale experiments, which have survived the drying with apparently little or no modification of the vegetative stages, the usual modification being that of an accumulation of oil in the cells. Cosmarium cucurbitinum was found on the pond I (Englefield Green) drying experiment after 54 days drying, when the moisture content was estimated as 4.5%, the single cell found (Figure 7) possessing a somewhat discoloured chromatophore, prominent oil globules and a mucilage sheath.



m = mucilage sheath.

o = oil globules.

c = chromatophore.

Figure 7. Cosmarium cucurbitinum, vegetative cell after 54 days drying.

This cell was found to be alive by placing it in soil solution culture, when divisions occurred resulting in a number of cells which contained distinct chromatophores, and in which the oil had been replaced by starch. This drought-resistant cell is, then, in some respects, functionally comparable with the zygospore of the Conjugatae, i.e. it stores oil which, at germination, is replaced by, or converted into, starch, and it has a protective sheath. This species appeared in the first three liquid cultures of dried litter and mud from the pond I large-scale experiment, i.e. up to 54 days of drying, but not since then, indicating that there is a limit to the time which such a drought-resistant stage will survive severe drying. But another species of Cosmarium, C.praemorsum, cells of which were found in some of the preliminary experiments (Tables 12 and 16) with slightly contracted protoplasts containing oil globules, has appeared in all the liquid cultures of the pond I (Englefield Green) large-scale experiments (Table 32), and has also re-appeared on the mud itself since exposure to rainfall (Table 28). This species may have survived by means of zygospores, but as zygospores were never found, it is possible that C.praemorsum did, in fact, survive very prolonged drought, by a modified vegetative cell even more drought-resistant

than that of C.cucurbitinum. In the most recent liquid culture (Table 32) from the pond I large-scale experiment, Cosmarium botrytis appeared, a species which had not been found previously. In this case it is not possible to say whether survival was by means of zygospores, or highly drought-resistant vegetative cells.

Filaments of Ulothrix were also found after prolonged drying in the large-scale experiments, U.subtilis (Figure 8) from pond III (Stanmore Common) and other filaments, which may have been of U.subtilis, from pond V (Rowley Green "acid" pond) (Figure 9). They both showed differential survival of cells which is in accord with the experiments and observations of Fritsch and Haines (1923). In the former there was no apparent modification within the living cells and only slight thickening of the cell walls, while in the latter the living cells contained oil globules and the cell walls were considerably thickened. These cells had survived 54 days of drying and recommenced active growth in liquid culture. Ulothrix subtilis appeared in almost all the liquid cultures of dried litter and/or mud and re-appeared in the large-scale experiments in early 1954 during the moisture increase. It also appeared after exposure of the experiments to rain in October, 1955, and, obviously, is able to resist

Figure 8

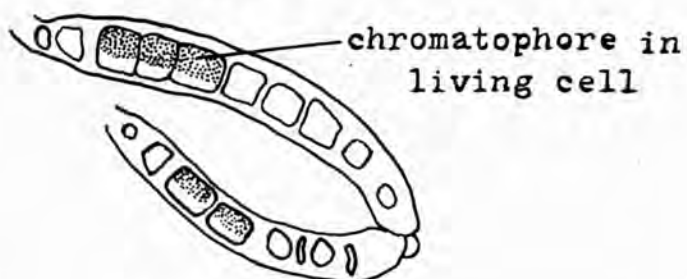


Figure 9

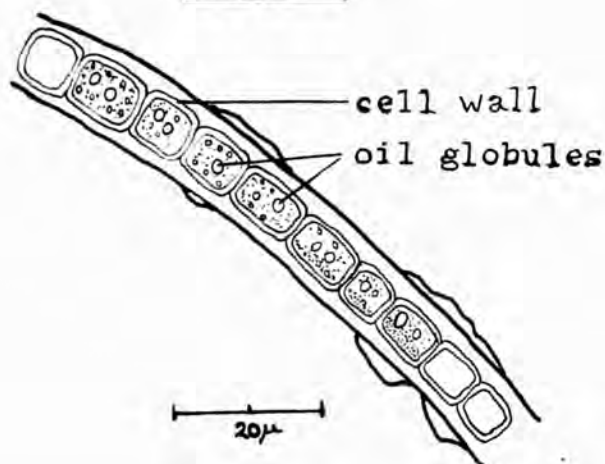
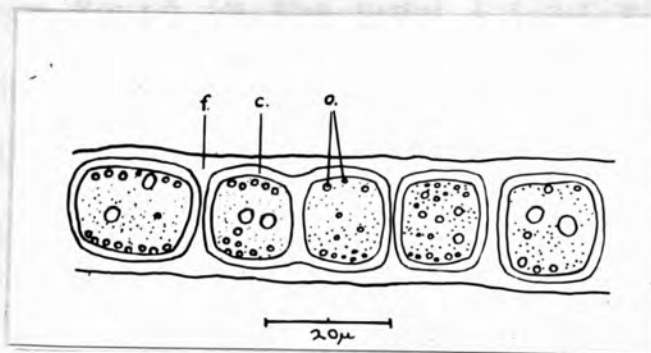


Figure 8. Ulothrix subtilis, part of a filament found after prolonged experimental drying. Originally collected from the Stanmore Common pond.

Figure 9. Ulothrix subtilis, part of a filament found after prolonged experimental drying. Originally collected from the Rowley Green "acid" pond.

severe desiccation merely by modification of the vegetative form.

After 69 days of drying, a short filament of Microspora floccosa was found in the large-scale experiment of the pond V (Rowley Green "acid" pond) litter and mud (Figure 10) which was very similar in appearance, with thick cell walls and with cells containing oil globules, to drought-resistant stages induced in a much shorter time (18 days) in the laboratory (C.f. Section II).



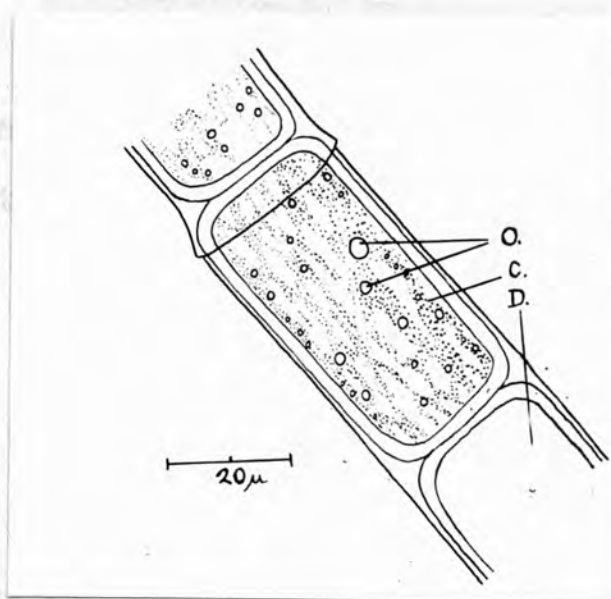
f = old cell wall, thickened.
c = young " " "
o = oil globules.

Figure 10. Microspora floccosa, part of a filament after prolonged experimental drying.

This filament resumed active growth within 14 days in soil solution culture. This alga, then, may be regarded as another species which is able to survive drought by a modification of the vegetative stage. The cells might be regarded as akinetes which are retained within the filament sheath. This species and M. stagnorum reappeared in the large-scale experiments during the cold spell early

in 1955 (Tables 30 and 31) and, therefore, it may be concluded, survived a prolonged drought. No stage of M.stagnorum has been found in the large-scale experiments comparable with the drought-resistant stages of M.floccosa, but comparable stages have been found in nature during dry periods (C.f. Section II) when akinetes of these species have also been found, and it seems that filaments of M.stagnorum break up more readily into separate akinetes.

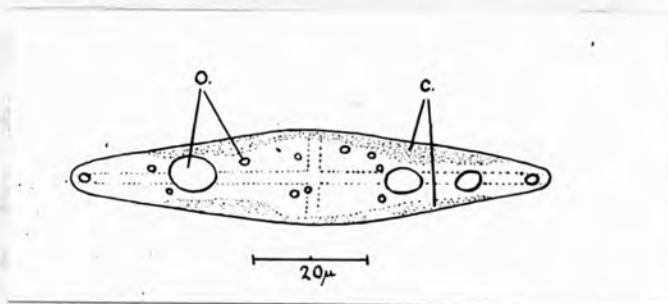
A few cells of an unidentified species of Oedogonium were found in the pond I (Englefield Green) large-scale experiments after 69 days of drying (Figure 11). The cell walls were somewhat thickened and the cells contained oil.



- O = oil globules.
- C = chromatophore.
- D = dead cell.

Figure 11. Oedogonium sp., part of a filament found after prolonged experimental drying.

In soil solution culture normal vegetative growth occurred within 14 days, so it may be concluded that this species had survived a severe and prolonged drought in the vegetative stage. Oedogonium sp. also appeared, within 14 days, in soil solution culture of a sample taken from the pond I large-scale experiment after 244 days of drying; but this time the species appeared only in the liquid culture of the lower layer (0.5 - 3.0cm.) of mud. This suggests, if survival was by means of a vegetative stage, that survival may be prolonged if the cells are below the surface. It may be, however, that this species had only survived for such a long time by means of an oospore. Oedogonium sp. also survived at least the first 15 days of the rather more rapid laboratory drying of the preliminary experiments (Table 11). A diatom, Stauroneis phoenicenteron (Figure 12), that found in the pond I (Englefield Green) large-scale experiment after 54 days of drying contained large oil globules



It was mentioned earlier that Pandorina species apparently
drought. Large other
candace (Pandorina elegans,
o = oil globules.
appeared, however, from
c = chromatophores.
dried litter and mud

Figure 12. Stauroneis phoenicenteron, cell found after prolonged experimental drying.

comparable with those found in Pinnularia viridis and other diatoms (see Appendix), but an attempt to induce normal growth, by placing the cell in soil solution, failed. Growth of this species was twice observed, however, in soil solution cultures of dried mud and litter from the Englefield Green pond (Table 32, page 115). One culture was of mud and litter which had been dried for 37 days to an approximate moisture content of 10%. The other culture was of mud and litter which had been dried for 69 days to a moisture content of about 7% (Table 24, page ¹⁰⁹93). Stauroneis phoenicenteron is then, capable of surviving fairly prolonged artificial drought which indicates that it could survive a natural drought. That the single cell found by direct observation of dried mud and litter could not be induced to grow, may have been due to laboratory conditions at the time. It may be that such a cell, apparently modified only by an accumulation of oil, is the means by which drought is survived.

It was mentioned earlier that Pandorina morum apparently does not survive severe artificial drought. Three other species of the coenobial Chlamydomonadeae (Eudorina elegans, Gonium pectorale and G. sociale) appeared, however, from time to time, in liquid cultures of dried litter and mud from the Englefield Green and Rowley Green "normal" ponds (Tables 11, 32 and 35). Eudorina elegans, has appeared

twice in liquid cultures of dried litter and mud from pond I (Englefield Green). In one of the preliminary experiments non-motile coenobia of this species were transferred from a drying experiment (Table 9), after 12 days of drying, to liquid culture (Table 11) and regained their motility and resumed growth, but coenobia did not survive 15 or more days of laboratory drying. Eudorina elegans survived 54 days of drying in the large-scale experiments (Table 32), possibly in the form of zygospores, but did not appear again after this time. Consequently, this species, although showing a higher degree of survival during drought than Pandorina morum, will not, apparently, survive such a severe drought as that induced artificially, for much longer than 54 days, even as zygospores. The two species of Gonium encountered survived a more prolonged artificial drought than did Eudorina elegans. G. pectorale survived a year of drying in the mud and litter of the Englefield Green and Rowley Green "normal" ponds (Tables 32 and 35). G. sociale appeared in cultures from the Englefield Green pond mud and litter after 54 days of drying and in February 1955, after 94 days of re-exposure of the large-scale samples to re-wetting (Table 32). Survival of these species of Gonium is possibly by means of zygospores.

Scenedesmus quadricauda has been found to survive drying in the laboratory (Tables 11, 16, 20, 22, 23, 36 and 37). It has also survived prolonged drought in the large-scale experiments (Table 32), appearing initially in cultures of the upper layer (0 - 0.5cm.) of litter and mud and appearing later in the cultures of the lower layer (0.5 - 3.0cm.) only. This suggests that a prolonged drought, while killing those cells at the surface, does not kill the cells which were carried passively into the lower layer, probably in the early stages of the experiments while considerable moisture was still present. Survival during dry periods in nature could occur in this way, the re-appearance of the species being dependent, to some extent, upon a chance disturbance of the litter surface.

Filaments of Spirogyra have often been found in liquid cultures of the drying experiments, and it is possible that the means of survival during drought is by zygospores. Spirogyra cylindrica, for example, from the Stanmore Common pond, conjugates, and forms zygospores readily in nature and in laboratory culture. It has also been found to survive for 367 days of drying in the large-scale experiments (Table 33) and has appeared in the large-scale

experiments after exposure to the weather and eventual re-wetting by rain (Table 30). Unidentified filaments (designated as species A, B and C in the tables) from the other ponds, may also have survived prolonged drying by earlier formed zygospores. Direct evidence is lacking, however, as zygospores were not found. It is not suggested that conjugation and zygospore formation occurs in response to the onset of dry conditions in nature or experimentally, but there is no doubt that zygospores, possibly formed while conditions were still very wet, may survive prolonged droughts, only very short periods of which are sufficient to kill the vegetative cells.

Two forms of Mougeotia have been found, one identified as M. parvula, and the other, of which no reproductive stages have been found, and which is, hence, unidentified, is designated as Mougeotia sp.A. in the tables. This latter has been found four times in the drying experiments of pond I (Englefield Green) mud and litter (Tables 9, 12, 16 and 24), but has never appeared in liquid culture of the mud, and it may be that the vegetative cells have been killed by artificial drying, as filaments placed in culture from a wet medium do survive. The same, or a similar, species appeared in soil solution culture of pond IV

(Rowley Green "normal" pond) mud and litter which had been dried for 69 days (Table 35) in the course of the large-scale experiments. This species was not found originally in the pond IV sample, but does occur at times in the water of the pond, and it seems likely that prolonged survival in the drying experiment was by means of a zygospore. The vegetative cells of Mougeotia parvula survive longer periods of drying than those of the other species (Tables 19, 25 and 27) though their reappearance in soil solution culture after prolonged drying (Tables 33 and 35), and in the pond V large-scale experiment (Table 31), may have been due to zygospores. The filaments of Mougeotia parvula have been found to withstand considerable periods of exposure in nature (Section II) and this species is not apparently so completely hydrophytic as Mougeotia sp.A. Pinnularia viridis occurs frequently at the margins of the ponds investigated, and was found often in the drying experiments. This species survived the relatively short periods of drying in some of the preliminary experiments (Tables 7 - 11) but, apparently, did not survive the prolonged drought of the large-scale experiments. Some idea of the effect of drought upon a

population of this species may be obtained from the Table 21. A large proportion of the cells die, leaving a few which usually, it has been found, contain large quantities of oil (C.f. Appendix 1, p.178).

Hantzschia amphioxys appears to be of a rather different type from Pinnularia viridis, never, or very rarely, having been found in the very wet samples collected from the pond margins, but appearing in liquid cultures after drying for short (Table 11) or long (Tables 32 and 35) periods of time, and appearing also in the large-scale drying experiments after exposure to rain (Tables 28 and 29). The form of Hantzschia amphioxys observed, shows a high degree of survival of drought conditions, but it differs markedly from Nitzschia palea, mentioned earlier, in that its maximum growth occurs after the drought, upon re-availability of water, rather than in the initial stages of the drought itself.

To investigate the reaction of similar populations of algae to more or less rapid laboratory drying, a series of drying, and soil solution culture, experiments were carried out as described above (p.67). The results, shown in table 37, suggest clearly that more species will survive a relatively slow drying than will survive a

rapid one in the laboratory, and this is probably true also of drying in nature. It should be noted that the samples from which free water was not suctioned off (i.e. "slow drying" samples), although not becoming air dry for about 110 hours, lost most of the free water fairly rapidly by evaporation, each of the samples estimated as containing less than 50% moisture (of the wet weight) within 40 hours. This may help to explain the disappearance of some of the species from the samples dried relatively slowly. Oedogonium sp., for example, disappeared from the pond I (Englefield Green) samples after "slow" as well as after "rapid" drying in the laboratory, but this species was found to survive severe, and rather prolonged, drought in the large-scale experiments (Tables 24 and 32) where the initial drying occurred more slowly than in the laboratory and, probably, more closely imitated conditions in nature.

It is interesting to see how naviculoid diatoms, from three of the pond margins at least, survived "slow" drying (i.e. within 110 hours) but not rapid drying (within 20 hours), suggesting that during a drought in nature, in which the initial drying would occur relatively slowly, some cells of certain species of diatoms may of the conclusions arrived at are necessarily tentative and perhaps over-generalized.

become modified to resist further, and possibly more severe, drying. It may be that one of the more important of these modifications is the formation of oil within the cells (C.f. Appendix 1, p. 178). Also, a relatively slow initial drying may allow cells the time to descend below the surface of the exposed litter or mud before desiccation occurs (C.f. Section IV).

The appearance of Cryptomonas ovata in the culture of the pond V (Rowley Green "acid" pond) slowly-dried sample is difficult to explain, as the species did not appear in the control culture. It may be, however, that the slow-drying, followed by the re-immersion, created optimum conditions for initiating the growth of a resistant stage of this species.

D. Some general conclusions.

The foregoing observations and experiments were carried out in an attempt to determine the way in which, and for how long, certain species of algae might survive artificial drought. From the results obtained, it was hoped to reach more or less definite conclusions regarding the ways in which survival may occur during dry periods in nature. The whole of the investigation has tended to be extensive rather than intensive and for this reason most of the conclusions arrived at are necessarily tentative and perhaps over-generalized.

- 1) For most algae the number of species found and the frequency of individuals decrease during drought, particularly below a moisture content of about 50% (of the wet weight).
- 2) Two species, Nitzschia palea and Oscillatoria tenuis, show an increase during the initial stages of drought.
- 3) Of all the species investigated only two, Pandorina morum and Euglena mutabilis, seem definitely not to have survived the severe artificial droughts, but even for these species there is evidence that they will survive some exposure in nature.
- 4) Although it may be concluded that some species rely upon earlier formed zygospores for survival during severe drought (e.g. Spirogyra cylindrica) it has been established that some filamentous algae and possibly two species of Cosmarium, which are normally hydrophytic in the ponds concerned, may survive considerable periods of drought by some modification of the vegetative stage, usually including one or more of the following: accumulation of oil in the protoplast; thickening of the cell wall; the protective action of a surrounding layer of mucilage.
- 5) For some species at least, it may be said that the longer the resting period, the longer it will take for the resting stage to re-adjust its metabolism and commence normal growth when water is again available.

- 6) The resistance of pond margin litter to re-wetting after a severe and prolonged drought, will be a factor itself causing the delayed, or perhaps, non-appearance of algal species. This is certainly the case for pond margins where considerable litter is present on the surface (e.g. ponds III, the Stanmore Common pond (see Tables 30 and 31 and compare with Tables 28 and 29). Conversely, however, sudden complete immersion may delay, or inhibit, the appearance of certain species of algae (Table 36).
- 7) During severe drought species are more likely to survive if they are below the surface of the litter or mud.
- 8) That most of the algae investigated survive severe droughts induced experimentally suggests that many will survive in nature.

Table 10.

Algae observed in a litter sample, from the Rowley Green "acid" pond, during drying in the laboratory. Date of collection of sample- 31.5.53.

Table 10.

Date and days of drying	June					
	4	5	8	10	12	15 18
<i>Chlamydomonas</i> sp.	+	+	+	+	+	
<i>Chlorococcum infusionum</i>	+	+				
<i>Ulothrix subtilis</i>				+	+	+
<i>Microspora floccosa</i>	+					
<i>Microthamnion Kützingerianum</i>	+	+				
<i>Pinnularia viridis</i>	+	+	+	+	+	(+)
<i>Euglena viridis</i>	+	+	+			

Table 11.

Algae appearing in liquid cultures (Beijerinck (1895) 0.05% solution) of dried litter and/or mud.

Table 11.

Pond No. Number of days drying before liquid culture	I			III	IV				V
	12	15	18	14	12	19	22	26	10
<i>Chlamydomonas</i> sp.			+						
<i>Gonium pectorale</i>				+					
<i>Eudorina elegans</i>	+								
<i>Scenedesmus quadricauda</i>	+								
<i>Ankistrodesmus falcatus</i>	+								
<i>Ulothrix subtilis</i>									+
<i>Microspora stagnorum</i>	+	+	+	+	+				
<i>M.floccosa</i>	+								
<i>Microthamnion Kützingerianum</i>			+						+
<i>Closterium Ehrenbergii</i>	+								
<i>Cosmarium praemorsum</i>	+	+							
<i>Oedogonium</i> sp.	+	+							
<i>Pinnularia viridis</i>	+	+	(+)	+	+	+	+	+	
<i>Nitzschia pelea</i>	+	+			+				
<i>Hantzschia amphioxys</i>							+	+	
<i>Cryptomonas ovata</i>			+						
<i>Euglena viridis</i>	+								
<i>E.deses</i>	+								
<i>Trachelomonas hispida</i>	+								
<i>Anabaena</i> spp.	+						+		
<i>Oscillatoria tenuis</i>	+	+	+						

Table 12.

Percentage moisture estimates of, and algae observed in, mud and litter from the Englefield Green pond during drying in the laboratory. The samples were collected in June 1953.

Table 13.

Ditto, Stanmore common pond sample.

Table 14.

Ditto, Rowley Green "normal" pond sample.

Table 15.

Ditto, Rowley Green "acid" pond sample.

Table 12.

Date	June		July
	23	26	13
<u>Days of drying</u>	0	3	20
<u>% moisture</u>	83	85	24
<i>Eudorina elegans</i>	+	+	
<i>Scenedesmus quadricauda</i>	+	+	+
<i>Microspora stagnorum</i>	+	+	+
<i>M.floccosa</i>	+	+	
<i>Mougeotia sp.A.</i>	+	+	
<i>Closterium acerosum</i>	+	+	+
<i>Cosmarium praemorsum</i>	+	+	+
<i>Oedogonium sp.</i>	+	+	(+)
<i>Pinnularia viridis</i>	+	+	(+)
<i>Nitzschia palea</i>		+	+
<i>Euglena viridis</i>	+	+	
<i>Oscillatoria tenuis</i>	+	+	+

Table 13.

	91	86	14
<u>% moisture</u>			
<i>Chlamydomonas sp.</i>	+	+	
<i>Ulothrix subtilis</i>	+	+	+
<i>Microspora floccosa</i>	+	+	
<i>Mougeotia parvula</i>	+	+	
<i>Pinnularia viridis</i>	+	+	(+)
<i>Euglena deses</i>	+	+	

Table 14.

	90	91	14
<u>% moisture</u>			
<i>Microspora stagnorum</i>	+	+	
<i>Pinnularia viridis</i>	+	+	(+)
<i>Nitzschia palea</i>	+	+	
<i>Anabaena sp.</i>	+	+	

Table 15.

	91	91	13
<u>% moisture</u>			
<i>Chlamydomonas sp.</i>	+	+	
<i>Ulothrix subtilis</i>	+	+	+
<i>Microspora floccosa</i>		+	+
<i>Pinnularia viridis</i>	+	+	(+)
<i>Euglena viridis</i>	+	+	
<i>E.mutabilis</i>	+	+	

Table 16.

Percentage moisture estimates of, and algae observed in, mud and litter from the Englefield Green pond during drying in the laboratory. The sample was collected in July 1953.

Table 16.

Date	July				August		
	14	20	23	28	1	5	10
Days of drying	0	6	9	14	18	22	27
% moisture	78	70		51		9	5
<i>Chlamydomonas</i> sp.	+	+	+	(+)	(+)		
<i>Scenedesmus quadricauda</i>	+				(+)		
<i>Microspora floccosa</i>	+	+					(+)
<i>M. stagnorum</i>	+					+	
<i>Wougeotia</i> sp.A.	+	+	+	(+)			
<i>Cosmarium praemorsum</i>	+	+	+	+	+	(+)	(+)
<i>Pleurotaenium trabecula</i>	+	+				(+)	
<i>Stauroneis phoenicenteron</i>		+	+	+	+		
<i>Pinnularia viridis</i>	+	+	+	+	+	(+)	(+)
<i>Nitzschia palea</i>	+	+	+	a	+		
<i>Euglena viridis</i>	+						
<i>E. mutabilis</i>	+	+					
<i>Oscillatoria tenuis</i>	+	+	+	a	a	a	a

Table 17.

Ditto, Stanmore Common pond sample.

Table 17.

% moisture	85	75		35		15	12
<i>Chlamydomonas</i> sp.	+	(+)	(+)	(+)	(+)		
<i>Ulothrix subtilis</i>	+	+	+				
<i>Pinnularia viridis</i>	+	+	+	+	+	(+)	(+)
<i>Euglena mutabilis</i>	+						

Table 18.

Ditto, Rowley Green "normal" pond sample.

Table 18.

% moisture	92	88		73		13	10
<i>Microspora stagnorum</i>	+	+	+				
<i>Cosmarium praemorsum</i>	+	+	+				
<i>Pinnularia viridis</i>	+	+	+	+	+	(+)	(+)
<i>Nitzschia palea</i>	+	+	+	+	+	+	+
<i>Anabaena</i> sp.	+	+	+				

Table 19.

Percentage moisture estimates of, and algae observed in, litter and mud from the Rowley Green "acid" pond during drying in the laboratory. The sample was collected in July 1953.

Table 19.

Date	July				August		
	14	20	23	28	1	5	10
Days of drying	0	6	9	14	18	22	27
% moisture	87	74		11		11	9
<i>Chlorococcum infusionum</i>	+	+	+	+	+	+	+
<i>Ulothrix subtilis</i>	+	+	+	+	(+)		
<i>Microspora floccosa</i>	+						
<i>Mougeotia parvula</i>	+	+	+				
<i>Pinnularia viridis</i>	+	+	+	(+)	(+)	(+)	
<i>Euglena mutabilis</i>	+						

Table 20.

Algae appearing in liquid culture (Beijerinck (1895) 0.05% solution) of dried litter and mud.

Table 20.

Pond No.	I		III	IV	V
	22	27	27	27	27
<i>Chlamydomonas</i> sp.	+	+	+	+	+
<i>Chlorococcum infusionum</i>		+	+	+	
<i>Scenedesmus quadricauda</i>	+		+		
<i>Ulothrix subtilis</i>		+	+		+
<i>Microspora stagnorum</i>	+	+	+		
<i>Microthamnion Kützingerianum</i>					+
<i>Spirogyra cylindrica</i>			+		
<i>Closterium acerosum</i>		+			
<i>Oedogonium</i> sp.		+			
<i>Cryptomonas ovata</i>					+
<i>Anabaena</i> sp.		+		+	
<i>Oscillatoria tenuis</i>	+	+			

Table 21.

Effect of artificial drought upon *Pinnularia viridis*. The sample was collected from the Rowley Green "normal" pond in July 1953.

Table 21.

Days of drying	0	6	9	14	18	22	27
% moisture	92	88		75		13	10
Number of cells counted in 50 high-power fields		236	200	200	207	205	200
% of the cells motile		8	7	3	1	0	0
" " " " non-motile		27	26	27	20	17	10
" " " " dead		65	67	70	79	83	90

Table 22.

The effect of further drying upon algae exposed in nature. The original sample consisted of green, surface scum collected from the margin of the Englefield Green pond in September 1953. At the time of the collection sample a was saturated with moisture while sample b had already begun to dry. The figures are counts of cells in 50 high-power microscope fields.

Table 22.

Date Days of drying in the laboratory	a, "wet" patch.			b, "dry" patch.		
	September			September		
	15	18	24	15	18	24
	0	3	9	0	3	9
Chlamydomonas sp.	2			1		
Pendorina morum				1		
Volvox aureus	1					
Scenedesmus quadricauda	1			1	2	
Oedogonium sp.	10			24	10	
Cosmarium preemorsum	1			1		
Closterium eboracense				1		
Nitzschia palea	6			2		
Euglena deses	6			2		
E. viridis	1			1		
Phacus caudata	1					
Trachelomonas volvocina	1					
Oscillatoria tenuis	306	744	1080	78	138	300

Table 23.

Algae appearing in liquid culture (sterile, distilled water) of the laboratory-dried scum (sample b) after 9 and 15 days of drying.

N.B. In liquid culture of the dried scum from the "wet" patch, sample a, only Anabaena sp. and Oscillatoria tenuis appeared.

Table 23.

Number of days drying before liquid culture	9	15
Scenedesmus quadricauda	+	+
Microspora stagnorum	+	+
Stigeoclonium tenue	+	
Oedogonium sp.	+	
Cryptomonas ovata	+	
Anabaena sp.		+
Oscillatoria tenuis		+

Moisture estimations of, and frequency counts of algae in, the large-scale drying experiments. (Tables 24 to 27, pages 108 to 112).

In the tables 24 to 27, the figures for moisture contents are as follows:-

- Phase A. means of 4 separate estimates.
- " B. " " 2 " " "
- " C. estimates of the upper (0 - 0.5cm.) and lower (0.5 - 3.0cm.) layers of litter and/or mud. N.B. Only one figure is given where the estimates for upper and lower layers were the same.

and the frequency counts of algae as follows:-

- Phase A. total numbers of cells counted in a total of 200 high-power microscope fields.
- " B. total numbers of cells counted in a total of 100 high-power microscope fields.
- " C. total numbers of cells counted in 50 high-power microscope fields.

Table 24. Fond I (Englefield Green).

Sample number	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18		
Date	1953 (Phase A)						1954 (Phase B)						1954 (Phase C)						
Days of drying	15	18	21	24	27	31	14	20	27	5	20	17	15	16	16	12	11		
% moisture	3	8	9	12	15	19	33	37	48	54	69	97	125	188	217	244	273		
<i>Chlamydomonas</i> spp.	2	4			7	1											7	15	
<i>Ulothrix subtilis</i>																		35	28
<i>Microspora stagnorum</i>	7	8	5	4	4	4	10				8								
<i>M. floccosa</i>		6		6															
<i>Microthamnion Kützianium</i>																			12
<i>Hougetia</i> sp.A.		4																	
<i>Spirogyra</i> sp.A.		4	3																
<i>Cosmarium praecorsum</i>		4	1		3														
<i>C. cucurbitinum</i>			1								1								
<i>Pleurotaenium trabecula</i>					1														
<i>Oedogonium</i> sp.		5	24		7								10						
<i>Pinnularia viridis</i>		4	2	5	4	1				1									
<i>Nitzschia palea</i>		5	11	23	13	9	1												
<i>Chromulina ovalis</i>																			8
<i>Euglena viridis</i>		3	3	1	2	1													
<i>E. deses</i>		4		4	2	1													
<i>E. mutabilis</i>		3		2	1														
<i>E. minima</i>					2														
<i>Phacus caudata</i>					1														
<i>Trachelomonas hispida</i>					1														
<i>Anabaena</i> spp.		40		11	8														14
<i>Oscillatoria tenuis</i>		96	150	20	280	40	37				20	20							10

Table 25. Pond III (Stanmore Common).
Large-scale drying experiments (see page 109).

Table 25. Pond III (Stanmore Common).

Sample number	1953 August (Phase A)							1954 Sept. Oct. Nov. Dec. Feb. Mar. (Phase B)							1954 Apr. May Oct. (Phase C)			
	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	21
Date	15	18	21	24	27	31	14	20	27	5	20	17	15	15	16	18	11	22
Days of drying	3	6	9	12	15	19	33	37	46	54	69	97	125	188	217	244	273	434
% moisture	81	78	71	77	68	53	13	16	14	13	11	24	28	61	48	9/40	5	18
Chlamydomonas spp.	7	2		5	10	19						2						
Chlorococcum infusionum				3	3	23			1	2	2	1						1
Ulothrix subtilis	56	10	36	36	94	72			8				17	46	100			(16)
Microthamnion Pützingerianum			8		13													
Mougeotia parvula	4			10														
Spirogyra cylindrica	7																	
Pinnularia viridis	5	10	6	3	5	1							1					
Nitzschia palea	2																	
Euglena viridis	2	1	1	5	5	2												
E. deses	1																	
E. mutabilis	22		3	4	4	10												
Trachelomonas caudata	1																	
Lepocinclis teres			9	4	3	4												
Glenodinium pulvisculus																		2

Table 26. Pond IV (Rowley Green "normal" pond).
Large-scale drying experiments (see page 109).

Sample number	1953 (Phase A)							1954 (Phase B)							1954 (Phase C)			
	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
Date	August							Sept.			Oct.		Nov.		Dec.		1954	
	15	18	21	24	27	31	14	20	27	5	20	17	15	15	16	12	11	
Days of drying	3	6	9	12	15	19	33	37	46	54	69	97	125	188	217	244	273	
% moisture	71	68	67	73	65	58	19	15	14	12	13	19	14	32	20	44	8	
Chlamydomonas sp.																8		
Chlorococcum infusionum										2	3	2					2	
Ulothrix subtilis	4	6	4											12	6			
Microspora stagnorum	4	6																
Pinnularia viridis	5	1	2	3	2	1												
Nitzschia palea	1	1	1	1		7												
naviculoid diatoms															4			
Euglena viridis	1																	
E. deses	1																	
E. mutabilis	1																	
Anabaena sp.						29												
Oscillatoria tenuis																	14	

Tables 30 and 31.

Re-wetting of litter and mud (see page 113).

Table 30. Pond III (Stanmore Common).

Sample number	22	23	24	25	26	27	28	29	30	31	32			
Date	1954							1955						
	26	27	5	15	23	4	28	3	4	4	3			
Days of exposure to weather	4	5	14	24	32	43	57	84	123	154	183			
% moisture 0-0.5cm.	58	40	65	65	70	79	83				72			
% moisture 0.5-3.0cm.	27	21	54	25	45	35	86		F L O O D E D.					
Chlamydomonas spp.						3	1	7	8	3	1	22	11	
Chlorococcum infusionum	1	1				10				1				
Ulothrix subtilis									91	20		30		
Microspora stagnorum										10		23		
M. floccosa												30	30	
Stigeoclonium tenue											60	90	60	
Microthamnion Kützianum											20	37		
Spirogyra cylindrica									3					
Glenodinium pulvisculus							1	10	2	10	1	16	2	6

Table 31. Pond V (Rowley Green "acid" pond).

% moisture	0-0.5cm.	19	17	59	74	59	85	88	L	W	L	W	L	W
0-0.5cm.	36	12	61	28	75	85	81							
0.5-3.0cm.	19	17	59	74	59	85	88							
Chlamydomonas spp.						1		1	2	5	13	5		
Chlorogonium suchlorum								1		326		67		
Chlorococcum infusionum									1					
Ulothrix subtilis								6	8	10	17			
Microspora stagnorum										10	10	10		
M. floccosa										75		10		
Mougeotia parvula										5				
Microthamnion Kützianum											20	22		
Glenodinium pulvisculus								1		2		7		

L = litter samples.
W = water samples.

Tables 32 to 35 (pages 115 to 117).

Algae appearing in soil solution cultures of the litter and/or mud of the large-scale drying experiments.

N.B. Samples number 26 and 29 were taken after exposure (in October 1954) of the litter and mud to the weather.

During phase C (i.e. from sample number 17 onwards) samples of litter and/or mud for starting liquid cultures were taken from the upper (0 - 0.5cm.) and lower (0.5 - 3.0cm) layers. In the tables, the symbol of presence in brackets, thus: (+), indicates the appearance of that species in liquid culture of the lower layer sample only.

Table 32. Fond I (Englefield Green).

Sample number	9	11	12	17	20	26	29
	1953		1954				
Date of sampling	Sep. 20	Oct. 5	20	April 12	Aug. 16	Nov. 23	Feb. 2
<i>Chlamydomonas</i> spp.	+	+	+	+	+	+	+
<i>Gonium pectorale</i>	+		+	+	(+)		
<i>G. sociale</i>		+					(+)
<i>Eudorina elegans</i>		+					
<i>Chlorococcum infusionum</i>	+				+		+
<i>Scenedesmus obliquus</i>	+		+				
<i>S. quadricauda</i>	+	+	+		(+)	(+)	(+)
<i>Characium Pringsheimii</i>			+	+			
<i>Ulothrix subtilis</i>	+		+	+	+	+	+
<i>Microspora stagnorum</i>	+	+	+				
<i>Stigeoclonium tenue</i>	+	+		+	+	(+)	(+)
<i>Microthamnion Kützingerium</i>	+	+				+	(+)
<i>Aphanochaete repens</i>		+	+				(+)
<i>Protoderma viride</i>	+						
<i>Spirogyra</i> sp.A.	+	+	+	(+)			
<i>Spirogyra</i> sp.B.					+		
<i>Spirogyra</i> sp.C.						(+)	(+)
<i>Cosmarium praemorsum</i>	+	+	+	(+)	+	(+)	+
<i>C. cucurbitinum</i>	+	+	+				
<i>C. botrytis</i>							+
<i>Glosterium leibleinii</i>	+	+					
<i>Cl. acerosum</i>			+				
<i>Cl. attenuatum</i>			+				(+)
<i>Oedogonium</i> sp.	+	+	+	(+)			(+)
<i>Ophiocytium arbuscula</i>						(+)	(+)
<i>Tribonema viride</i>				(+)		+	
<i>Cryptomonas ovata</i>		+		+			
<i>Glenodinium pulvisculus</i>	+						
<i>Anabaena</i> spp.	+	+	+	+	(+)	(+)	
<i>Oscillatoria tenuis</i>	+	+	+			+	+
<i>Nitzschia palea</i>					+	+	
<i>Hantzschia amphioxys</i>						+	
<i>Stauroneis phoenicenteron</i>	+		+				
Total number of species	19	16	17	11	10	13	15

Tables 33 and 34.

Algae appearing in soil solution culture of the litter and/or mud of the large-scale drying experiments (see page 115).

Table 33. Pond III (Stannmore Common).

Sample number	9	11	12	17	20	26	29
Date of sampling	Sep. 20	Oct. 5	20	April 12	1954 Aug. 16	Nov. 23	1955 Feb. 2
Chlamydomonas spp.				(+)	+	+	+
Chlorococcum infusionum	+	+	+	+	(+)		+
Ulothrix subtilis	+	+	+	+	+	+	+
Microspora stagnorum	+	+	+				+
Stigeoclonium tenue						+	+
Mougeotia parvula	+		+	(+)			
Spirogyra cylindrica	+	+	+		+		
Cryptomonas ovata		+	+				
Glenodinium pulvisculus		+					
Total number of species	5	6	6	4	4	3	5

Table 34. Pond V (Rowley Green "acid" pond).

Sample number	9	11	12	17	20	26	29
Chlamydomonas spp.	+	+	+	+	+	+	+
Chlorogonium euchlorum	+			(+)			+
Chlorococcum infusionum	+						
Ulothrix subtilis	+	+	+	+	+	+	+
Microspora floccosa			+				
Stigeoclonium tenue	+		+	+	(+)		
Microthamnion Kützingerianum							+
Mougeotia parvula	+		+	(+)			
Closterium setaceum	+						
Total number of species	7	2	5	5	3	2	4

Table 35.

Algae appearing in soil solution culture of the
litter and/or mud of the large-scale drying experiments.
(see page 115).

Table 35. Pond IV (Rowley Green "normal" pond).

Sample number	9	11	12	17	20	26	29
	1953			1954			
Date of sampling	Sep.	Oct.		April	Aug.	Nov.	Feb.
	20	5	20	12	16	23	2
Chlamydomonas spp.	+	+	+	+	+	+	+
Gonium pectorale	+					(+)	+
G. sociale	+	+					
Chlorococcum infusionum	+	+	+	+	+	+	+
Characium Pringsheimii	+	+		+			
Dactylococcus bicaudatus				+			
Ulothrix subtilis	+	+	+	+	+	+	+
Microspora stagnorum	+	+	+				
Stigeoclonium tenue						(+)	+
Microthamnion Kützingianum	+	+			(+)		+
Mougeotia sp.A.	+		+				
Spirogyra sp.A.	+	+	+				
Spirogyra sp.B.						(+)	
Tribonema viride	+			(+)	(+)	(+)	+
T. subtilissimum						(+)	+
Nitzschia palea	+			+		+	+
Hantzschia amphioxys				+			+
naviculoid diatoms				(+)	+	+	+
Anabaena spp.	+		+	+		+	+
Oscillatoria tenuis	+		+	+	+	+	+
Total number of species	14	8	8	11	7	12	13

Table 36.

A comparison of algal species appearing in soil solution culture of dried litter or mud samples after complete (A) and partial (B) immersion. The samples were collected in June 1955.

Table 35.

Pond No. Culture series	I		II		III		IV		V		
	A	B	A	B	A	B	A	B	A	B	
Chlamydomonas spp.	+	+	+	+		+	+	+	+	+	+
Gonium pectorale	+	+	+	+							+
Eudorina elegans											+
Scenedesmus quadricauda	+	+	+	+							+
Ulothrix subtilis			+	+	+	+					+
Microspora stagnorum					+	+					+
Microthamnion Kützingianum				+							+
M.strictissimum						+					
Spirogyra sp.	+	+									
S.cylindrica					+	+					
S.nitida			+								
Mougeotia parvula											+
Zygnema sp.B.						+					
Closterium Ehrenbergii	+	+									
C.percerosum		+									
C.acerosum var elongatum		+									
C.striolatum		+									
Cosmarium impressulum											+
naviculoid diatoms		+		+		+					
Hantzschia amphioxys							+	+			
Peredinium cinctum											+
Anabaena spp.	+	+		+			+	+			
Oscillatoria tenuis	+	+					+	+			
Total number of species	7	12	4	7	3	7	4	4	6	8	

Table 37.

A comparison of algal species appearing in soil solution culture of pond margin litter or mud; wet samples (=control) (A), samples dried slowly (B) and samples dried rapidly (C). The samples were collected in April 1955.

Table 37.

Pond No. Culture series	I			II			III			IV			V		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Chlamydomonas spp.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pandorina morum										+					
Chlorococcum infusionum	+	+		+	+	+									
Scenedesmus quadricauda	+	+	+												
S.obliquus	+	+													
Dactylococcus bicaudatus							+	+					+	+	+
Ulothrix subtilis	+	+		+	+		+	+	+	+	+	+	+	+	+
Microspora floccosa							+	+							
Stigeoclonium tenue	+	+					+			+	+				
Microthamnion strictissimum							+	+	+				+	+	+
Oedogonium sp.	+														
Netrium oblongum var. cylindricum							+								
Zygnema sp.A.							+	+	+						
Zygnema sp.B.															
Spirogyra spp.	+			+											
Mougeotia sp.A.				+											
Cosmarium cucurbitinum	+	+	+												
C.impressulum	+														
C.botrytis	+														
Tribonema viride										+	+	+			
T.subtilissimum										+	+	+			
naviculoid diatoms	+	+		+			+			+	+		+	+	
Finnularia viridis							+								
Nitzschia palea				+											
N.linearis										+					
Hantzschia amphioxys											+				
Cryptomonas ovata															+
Euglena viridis	+			+											
E.mutabilis	+														
Trachelomonas hispida	+														
Anabaena spp.	+	+								+	+				
Oscillatoria tenuis	+	+	+							+	+				
Total number of species	17	9	5	8	3	2	10	6	4	12	9	5	5	6	4

Section IV. The Stratification of Algae in

Pond Margin Litter and Mud.

A. Introduction.

Attention has been paid in the past to the subterranean soil-algal flora (Bristol-Roach, 1927; Petersen, 1935; John, 1942), but little or no information is available regarding the presence of hydroterrestrial or usually aquatic algae below the surface of litter and/or mud at pond margins. In order to investigate this aspect of the survival of pond algae during dry periods work has been carried out by the methods as described below.

B. Methods and Results.

1) A Buried Slide Technique.

The buried slide technique for the study of soil microflora has been used successfully in Mycological work (Starkey, 1929; Cholodny, 1930; Rossi et al., 1936) and a method has been devised, based upon this technique, for studying the algae present below the surface of pond margin litter and/or mud.

At various times throughout 1954 sets of slides were buried at the margins of the five ponds described in Section II. Exposure of marginal mud and litter did not occur to the same extent at all the ponds, so that more sets of

slides were buried at some ponds than at the others. The slides were buried usually at $\frac{1}{2}$, 1, 2, 3 and 4cm., in duplicate, by digging a pit, making horizontal incisions at the required depth with a sterile knife, and inserting carefully the slides which had previously been cleaned by soaking 24 hours in chromic acid and sterilized by rinsing in 70% alcohol and flaming. A difference here from the Mycological slide technique is the horizontal placing of the slides at various depths. This method has been adopted in view of the low, or completely lacking, activity of algae below the surface, the slides becoming, in effect, traps to catch algae descending passively into the deeper layers of litter and/or mud from above. After two weeks the slides were collected and one of each pair placed in soil solution culture in a petri dish. The other slides, for direct observation, were washed in order to remove particles of mud and litter, dried and mounted in lactophenol. The whole surface of each of the direct observation slides was examined immediately, and the algae present listed. The slides in culture were removed once a week for three or four weeks and examined by placing on a glass plate, which has been previously sterilized. A microphotograph (Figure 13), taken after three weeks soil solution culture, of a slide which had been buried for

two weeks, 3cm. deep in the litter of the Stanmore Common pond during March 1954, shows how an initially apparently bare slide will come to support growths of algae.



naviculoid diatom
-dividing cell.

Dactylococcus
bicaudatus.

200 μ

Figure 13. Surface of a buried slide after 3 weeks soil solution culture.

The direct observation method has revealed the state in which certain algae may be found in the deeper litter layers, and the soil solution culture of parallel slides has yielded information regarding the viability of algae in those layers.

Only from the Stanmore Common pond have sufficient slide sets been taken for any general conclusions to be reached from the results, and in this case it was found that there is a tendency towards a concentration of algae

in the top 1/2 cm. of marginal litter, and a decrease in algae with increasing depth (Table 38). This is in accord with the observations of Petersen (1935) and Tohan (1953) for soil algae. There seemed to be a similar tendency for the Englefield Green pond, but too few samples were taken from the Rowley Green ponds for any such conclusions to be reached. At the Rowley Green ponds, however, the slide sets were placed in position only during some of the relatively dry, warm weather of 1954 (late April, late May and early July). For these ponds, the concentration of algae at the various levels as indicated in Table 38, may reflect their occurrence in nature and not be associated with the small numbers of samples.

Using the slide technique, algal species have been found from certain ponds that were not found by any other sampling or culture methods. These algae include Dactylococcus bicaudatus, from the Englefield Green pond; Scenedesmus quadricauda and Oocystis solitaria from the Rowley Green "normal" pond; the latter species also from the Rowley Green "acid" pond; Anabaena sp. and Tribonema subtilissimum from the Stanmore Common pond; and Hormidium flaccidum from all the ponds investigated.

Some of the results recorded in Table 39, showing the

Table 38. The frequency of appearance of algal species in soil solution cultures of buried slides.

Pond	Stanmore Common				Englefield Green				Rowley "normal"			Rowley "acid"					
	1	2	3	4	1	2	3	4	1	4	1	3	4				
Depth of slide in cm.	14	11	8	10	12	6	5	3	2	4	2	1	1	3	2	1	3
number of cultures	14	11	8	10	12	6	5	3	2	4	2	1	1	3	2	1	3
<i>Chlamydomonas</i> spp.	4	3	2	3	2	3	2		1	1	1	1		1			3
<i>Chlorococcum infusionum</i>	6	4		2	1	1											1
<i>Characium Fringsheimii</i>	1					1	1	1		1							1
<i>Dactylococcus bicaudatus</i>	2	1		1		1	1			1	2	1					3
<i>Oocystis solitaria</i>											2						1
<i>Ankistrodesmus falcatus</i>						2			1			1					
<i>Scenedesmus quadricauda</i>						2		2	1	1							
<i>Chlorosphaera consociata</i>	6	3	1	1	1												
<i>Hormidium flaccidum</i>		1									2	1		1	1	1	1
<i>Ulothrix subtilis</i>	9	6	2	3	2	3	1	1	1	1							3
<i>Microspora stagnorum</i>	6	6	4			4	1	1	1	1							2
<i>M. floccosa</i>	3																
<i>Microthamnion Mitzingianum</i>	7	2	2	1	1	1	1	3	1								
<i>Stigeoclonium tenue</i>	3	2	1	1	1												
<i>Spirogyra</i> sp. A.	1								1								
<i>S. cylindrica</i>	5	6	2	1	1												
<i>Mougeotia</i> sp. A.			1														
<i>M. parvula</i>	4	2		1													
<i>Zygnema</i> sp.			1														
<i>Cosmarium cucurbitinum</i>	1																
<i>Netrium oblongum</i> var. <i>cylindricum</i>	1	1															
<i>Tribonema viride</i>	2	2		1	1	1					1						
<i>T. vulgare</i>	1	1		1	1	1											
<i>T. subtilissimum</i>	2	3	2	1		1								1			
<i>Cryptomonas ovata</i>	2	1															
<i>Finnularia viridis</i>	4	2	1	1	1	3	3	1	1	1							
naviculoid diatoms	7	5	1	4	2	5	3	3	1	2	1	1	1				
<i>Mitsschia palea</i>						5	2	1	1	3	1	1	1				
<i>Bantzschia amphioxys</i>										1				1			
<i>Euglena deses</i>			1						1								
<i>E. mutabilis</i>	1			1													
<i>Dinobryon</i> sp.	1	1															
<i>Synura</i> sp.			1	1													
<i>Anabaena</i> spp.					1	5	2	1		1	1	1	1				1
<i>Oscillatoria tenuis</i>	1					1	1	1									
Total number of species	24	20	13	15	13	17	11	12	9	11	8	4	9	5	4	3	7
" species frequency	80	53	21	23	16	40	18	17	9	14							
Mean " /culture	57	47	35	23	13	67	35	57	45	35							

occurrence of algae on "direct observation" slides, are of interest. The diatoms at the Stanmore Common pond, for instance, were found at progressively greater depths through the year as the water level dropped. The occurrence of the diatoms in parallel slide cultures shows that the cells found were living cells. The same thing may have been happening with *Nitzschia palea* at the Rowley Green "normal" pond and *Mougeotia parvula* at the Stanmore Common pond. It is not definitely suggested that there is an active descent of these algae into the deeper layers of litter and mud, but it is not unreasonable to suppose that cells carried passively into the deeper layers will tend to survive, while those cells remaining on or near the surface will tend to be killed off when water levels drop and drying occurs. For motile algae, however, the possibility of active descent must not be precluded (C.f. pages 130-132). During extremely dry years, species which might otherwise disappear from the pond, may survive in this way, their reappearance in the water at some later date depending upon chance disturbance of the marginal litter.

Some insight into the ways in which certain species may survive drying has been obtained by watching their

Table 39. Direct observation of buried slides.

Algae observed	Pond	Depth	Month(s) (1954)	Growth in culture
<i>Dactylococcus bicaudatus</i>	Rowley "acid"	½cm.	May-June	Yes
<i>Ulothrix subtilis</i>	Englefield	2cm.	February	"
" "	Stanmore Comm.	½cm.	August	"
" "	" "	1cm.	July	"
" "	Rowley "acid"	½cm.	May-June	"
<i>Microspora stagnorum</i>	Stanmore Comm.	4cm.	September	Jan.-April, ½-2cm. August, 1cm.
<i>Mougeotia parvula</i>	" "	½cm.	August	½ and 1cm.
" "	" "	1cm.	July	½cm. only
" "	" "	4cm.	September	½ and 4cm.
<i>Spirogyra cylindrica</i>	" "	½cm.	August	1-4cm., from zygo-spores.
<i>Zygnema</i> sp.	" "	1cm.	May-June	January only, 2cm.
<i>Pinnularia viridis</i>	Englefield	2cm.	January	Yes
" "	Stanmore Comm.	½cm.	Feb.-May	"
" "	" "	1cm.	July	"
" "	" "	4cm.	September	August and September
Navicularoid diatoms	Englefield	½cm.	February	Yes
" "	Stanmore Comm.	½cm.	Feb.-June	January - August.
" "	" "	1cm.	May-August	February - August.
" "	" "	4cm.	September	August and September.
<i>Nitzschia palea</i>	Rowley "normal"	1cm.	April-May	Yes
" "	" "	5cm.	May-June	"
<i>Tribonema viride</i>	Stanmore Comm.	½cm.	May-June	"

N.B. The last column in this table refers to the results obtained for the algae listed upon placing buried slides in soil solution culture.

"Yes" indicates that growth of the algae was observed for the equivalent buried slide placed in culture solution. Other information summarises the occurrence in culture on alternative or additional occasions, and at various depths. E.g. *Microspora stagnorum* grew in all cultures of slides which had been buried at ½-2cm. during January to April and in a culture of a slide which had been buried at 1cm. during August.

development in slide cultures. All the filaments of the Spirogyra species here considered, for example, have been found developing from zygospores. This suggests that, at least in the species of Spirogyra encountered, survival depends entirely upon the existence of zygospores. In the single case where a few cells of Spirogyra cylindrica were found on a "direct observation" slide, they were only found at a depth of $\frac{1}{2}$ cm., and not in the lower layers. Although the early stages of conjugation have been observed in Mougeotia parvula, zygospores have never been found, so that this species would appear to survive by means of vegetative cells.

Filaments of species of Tribonema have always been found in the slide cultures, with an attaching stalk which indicates their zoosporic origin. This suggests that resting cells in the litter, therefore, whether akinetes or hypnospores, germinate initially to form zoospores. A filament of six cells found on a direct observation slide from a depth of $\frac{1}{2}$ cm. in the litter of the Stanmore Common pond broke up very easily, releasing the thick-walled cells when the cover slip was pressed lightly (Figure 14). The filament, which was about 80 microns in total length, had penetrated the litter to a depth of $\frac{1}{2}$ cm. The individual

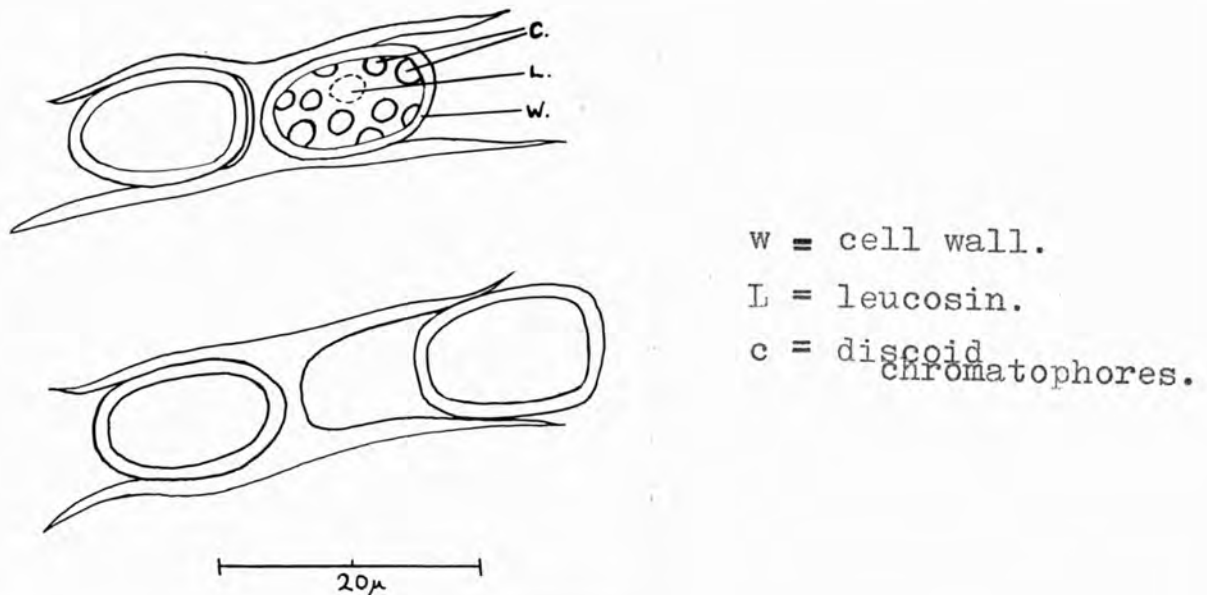


Figure 14. Tribonema viride, as observed on a $\frac{1}{2}$ cm. direct observation slide. N.B. In the figure only one cell is shown with contents; the other cells were similar in appearance.

cells, being far smaller than the filament, would have been able to pass deeper into the litter. This helps to explain the appearance of Tribonema viride in a 4cm. deep slide culture of the Stanmore Common pond marginal litter (Table 38). Separate cells may be carried deeper into the litter and/or mud, and this in itself might assist their survival during a severe drought. Both Tribonema viride and T.vulgare appeared in soil solution cultures of the $\frac{1}{2}$ cm. slides from the Stanmore Common pond throughout the year, appearing also in May in cultures of the 1 and 3cm. slides. Finally, in August, they appeared in culture of the 4cm. slide. This suggests that, as with the diatoms

investigated, viable stages may descend progressively deeper into the litter, through the year, as the water level drops.

Two members of the Chrysophyceae, Synura and Dinobryon sp., appeared in slide cultures from the Stanmore Common pond in late February, nearly two months before they were found in the pond water which occurred when the water rose and flooded the position where the slides had been buried. This position was found to coincide with the water level recorded for the previous year (1953) at the time these species disappeared from the water. This suggests the existence of Chrysophyceae resting spores at a particular zone of the pond margin corresponding to the edge of the water at the time the motile stages disappeared from the water. The non-appearance of these species in earlier slide cultures, the slides for which had been buried nearer to the position of the lowest water level, tends to support this conclusion. But there is an alternative explanation: between the times that the earlier slides were buried and the time that the slides that yielded the Chrysophyceae in culture were buried, there was a very cold spell, during which time several inches of ice formed on the water. The severe cold broke up the dry litter and

may have released the spores, which previously had been held in position, so allowing them to drop onto the buried slides. The non-appearance of Synura on the $\frac{1}{2}$ cm. slide cultures and its appearance in the 1 and 3cm. slide cultures suggests that descent of the spores into the deeper litter layers may assist in the survival of this alga from one season to the next through the dry period of the year.

The appearance of Euglena deses in cultures of slides only from a depth of 2cm. at both the Englefield Green and Stanmore Common ponds (Table 38) is interesting, and suggests that cells at or near the surface lose their viability upon exposure. The species did not appear in cultures of slides buried at 1cm. or 3cm., and there is thus some evidence that perennation takes place in a zone between 1cm. and 3cm. below the surface.

The two species of Microspora investigated, M. stagnorum and M. floccosa, also appear only to a limited depth (Table 38), but these species have both been found to survive exposure for some time on the litter surface (Section II and III) which would tend to obviate the necessity for a survival mechanism involving passive or active descent into the deeper litter layers.

the surface and the other side the bottom of the tube.

2) A micro-sampling Technique.

At the Stanmore Common pond, by early August in 1953 and by early September in 1954, the water level had dropped to such an extent that most of the pond floor was exposed. This was found to consist mainly of layers of dead leaves dropped in previous years from the trees (Betula pendula and Quercus robur) surrounding the pond. The existence of such a substrate suggested a method by which it would be possible to carry out some investigation of the micro-stratification of the algae. Samples of the dead leaves, or leaf fragments, were collected in small specimen tubes (2" x $\frac{1}{2}$ "). Three samples, each being approximately the thickness of a single dead leaf, were taken at each visit to the pond, as follows:-

1. a fragment of leaf from the surface of the leaf litter.
2. " " " " immediately below 1.
3. " " " " " " 2.

Care was taken in sampling to avoid, as far as possible, the contamination of one sample from another. In the laboratory each sample was shaken vigorously with about 5ml. of sterile, distilled water. After allowing to settle, 2 drops were taken from each sample, one from the surface and the other from the bottom of the tube,

and placed on a glass slide. To this was added a scrape taken with a sharp scalpel from the surface of the leaf. The samples were examined microscopically, using a x15 ocular and a 4mm. objective, counting the number of cells of each species in a total of 50 fields. Then a low-power survey of the whole mount was carried out, noting any further species present.

Interesting results were obtained using this technique, and individual species or groups of species are discussed below.

The results for *Pinnularia viridis* in 1953 (Table 40a) suggested that there was a vertical movement of the cells associated with the weather conditions at the time of collection of the samples, the cells moving upwards in damp conditions, and downwards in dry conditions, or during rainfall. The results obtained the following year (Table 40b) and a statistical analysis of the results of both years (See Appendix 2, p. 202), carried out in view of the very small number of cells being dealt with, confirm this conclusion, which is in agreement with Petersen's view (1935) regarding the possible up and down wanderings of motile algae in the top millimeters of soil in response to changes of climate. That all cells considered were of

Table 40a. *Pinnularia viridis*, micro-stratification of cells in leaf-litter (1953).

Collection number	19	20	21	22	23	24	25	26	27	28
Date	August			Sept.			October			
Date	8	13	16	19	26	16	30	7	15	29
Weather	stratum									
Weather	dry	dry	rain	rain	damp	damp	rain	damp	damp	rain
Number of cells	1) 2	2	1	1	3	1	0	1	3	1
in 50 h.p. fields	2) 1	3	2	5	1	1	0	1	1	2
	3) 5	1	3	2	1	1	4	0	1	4

Table 40b. *P. viridis*, micro-stratification of cells in leaf-litter (1954).

Collection number	50	51	52	53	53a	54	55	56
Date	September			October			Nov.	
Date	1	8	16	22	29	7	20	3
Weather	stratum							
Weather	dry	rain	dry	damp	damp	dry	damp	damp
% moisture content of marginal litter				63	55	73	65	
Number of cells	1) 0	1	2	3	3	0	2	4
in 50 h.p. fields	2) 2	0	4	2	1	0	0	1
	3) 0	1	2	0	0	3	0	0

N.B. The terms "rain", "dry" and "damp", as used in the above tables and in table 41 (page 131), have the following significance:-

"rain", rain falling at the time the samples were taken.

"dry", no rainfall during the 24 hours before the time of sampling. For collections number 20 and 52 there was some rainfall 2 days before sampling, while for the other "dry" samples there was no rainfall during 5 days or more before sampling.

"damp", some rainfall no more than 2 hours before the time of sampling.

more or less of the same size, no narrow cells being found, tends to rule out the possibility that this fluctuation in numbers of cells at different levels is merely a reflection of varying rates of cell division. The vertical movements of Pinnularia viridis may play a not unimportant part in the survival of this species during dry periods. That these movements have been found to occur at all, even though over only small distances, suggests that the occurrence of cells at progressively greater depths in the marginal litter as the water level drops (see pages 123 - 124), may be due to an active rather than a passive downward movement.

The counts of total flagellates in the 1954 samples (including Chlamydomonas sp., Cryptomonas ovata, Euglenadeses, E. mutabilis, Lepocinclis ovum and Trachelomonas volvocina) also show some association between the numbers of cells at different micro-layers, and the weather conditions (Table 41).

Table 41. Total flagellates, 1954.

Collection No.	50	51	52	53	53a	54	55	56
Date.	1	8	16	22	29	7	20	3
Weather.	dry	rain	dry	damp	damp	dry	damp	damp
No. of cells	1	1	5	4	5	1	2	5
in 50 h.p.	2	0	5	0	2	0	0	3
fields.	3	0	2	2	0	1	0	1

Statistical analysis of these results has revealed a highly significant difference (approximately at the 1% level - see Appendix 2, p. 204) between the numbers of cells in the uppermost and lowermost layers in optimum conditions (i.e. damp, but rainless days, collection numbers 53, 53a, 55 and 56). Any differences between the numbers of cells in the uppermost and lowermost layers in other conditions have not been found significantly different. From these results it may be concluded that there is possibly an upward movement of flagellates in the microlayers during optimum conditions, and it may reasonably be postulated that a downward movement occurs in conditions other than optimum.

The two most abundant species of green filamentous algae found on the leaf strata in 1953 were Microspora floccosa (Table 42a) and Spirogyra cylindrica (Table 43), the former being found in the vegetative state even after nearly 2 months of exposure (cf. Section II), while the latter disappeared after 3 to 5 weeks of exposure, relying for survival upon earlier-formed zygospores (cf. the buried slide technique and Section II).

In 1954 Microspora floccosa (Table 42b) was more frequent on the leaf strata than in the previous year,

Table 42a. Microspora floccosa, micro-stratification of cells in leaf-litter (1953).

Collection number	19	20	21	22	23	24	25	26	27	28
Date	August			Sept.			October			
	8	13	16	19	26	16	30	7	15	29
stratum										
Number of cells	1) 30	77	15				8			
in 50 h.p. fields	2) 8	17								
	3) 8									

Table 42b. Microspora floccosa, micro-stratification of cells in leaf-litter (1954).

Collection number	50	51	52	53	53a	54	55	56
Date	September			October			Nov.	
	1	8	16	22	29	7	20	3
stratum								
Number of cells	1) 71	22	20	120	50	10	Ak. 18	
in 50 h.p. fields	2) 27	0	4	33	10	8	Ak. Ak.	
	3) 8				10	3		

Ak. = akinetes.

Table 43. Spirogyra cylindrica, micro-stratification of cells in leaf-litter (1953).

Collection number	19	20	21	22	23	24	25	26	27	28
Date	August			Sept.			October			
	8	13	16	19	26	16	30	7	15	29
stratum										
Number of cells	1) 90	67	70	78	38					
in 50 h.p. fields	2) 33	4	4		2	Nil.....				
	3) 8				2					

which is possibly due to the relatively cooler and damper conditions in 1954. Nevertheless, some akinete formation was observed in 1954 after prolonged exposure (nearly 2 months), when the frequency of vegetative cells was decreasing (cf. Section II). Spirogyra cylindrica, so frequent in 1953, was replaced in 1954 largely by Mougeotia parvula (Table 44), vegetative cells of which survived the prolonged exposure though they showed a considerable decrease towards the end of this period.

Table 44. Mougeotia parvula, 1954.

Collection No.	50	51	52	53	53a	54	55	56
Date.	September				October		Nov.	
	1	8	16	22	29	7	20	3
No. of cells in 50 h.p. fields.	1) 196	100	161	133	100	120	53	32
	2) 39	12	59	73	70	8	6	22
	3) 4	4	6	10	7	2	2	6

Although early stages in conjugation of Mougeotia parvula filaments have been observed, zygospores have never been found, and this species would appear to survive drying conditions by means of more or less unmodified vegetative cells. That the filaments fragment more easily and the cells are considerably smaller in this species than in non-motile cells or cysts, found only in the upper two leaf strata. Motile cells of this species had disappeared

Microspora floccosa and Spirogyra cylindrica might help to account for its more frequent occurrence in the lower leaf strata. This, in itself, may be a factor assisting the survival of Mougeotia parvula during very dry conditions.

Other green, filamentous algae found (which included Ulothrix subtilis, Microspora stagnorum and Microthamnion Kutzingianum) occurred too irregularly or too infrequently for the counts made to have any significance. (Figure 25B)

Cells of species of the Euglenineae were never found in the lowermost layer of litter and, for Euglena deses and E. mutabilis, both of which appeared in soil solution culture of buried slides, this suggests that the formation of resting cysts, or death of the cells, occurs on, or very near, the litter surface. None of the Euglenineae encountered in this investigation, including Euglena viridis, Lepocinclis ovum and Trachelomonas volvocina, was found frequently but these species occurred irregularly and sporadically on the leaf strata. I), cysts having

Species of the Dinophyceae were not found on the leaf strata samples in 1953, but in 1954 cells of Peredinium cinctum and Glenodinium pulvisculus were observed from time to time. Peredinium cinctum was represented by non-motile cells or cysts, found only in the upper two leaf strata. Motile cells of this species had disappeared

from the pond in early September 1954 when the water level dropped, and did not re-appear until May 1955. Attempts to induce the appearance of motile cells by soil solution culture of the cysts did not succeed. Non-motile cells or cysts of Glenodinium pulvisculus (Figure 15A) found in the lowermost layer of the leaf strata at collection 53a, were placed in soil solution culture, and motile cells appeared within a week. Motile cells (Figure 15B) were also found in all strata at collection 56 when the weather had become much damper and the water level was rising again. The non-motile cells observed were, then, genuine resting stages and not merely dead or dying cells. Each possessed a thick membrane (Figure 15A) and contained one or more orange-red globules of oil which were also observed initially in the motile cells (Figure 15B). It has been found that Glenodinium pulvisculus forms resting stages (cysts) in the water of the Stanmore Common pond in Spring (cf. Section II), cysts having been observed frequently in samples of water collected during April and May in 1954 and in March and April of 1955. When the water level dropped in late August 1954, these cysts were found deposited on the exposed layer of leaves. G. pulvisculus was not found in any form in the

water samples collected in the Spring and early Summer of 1953, which may help to explain the absence of this species from the leaf strata samples of that year.

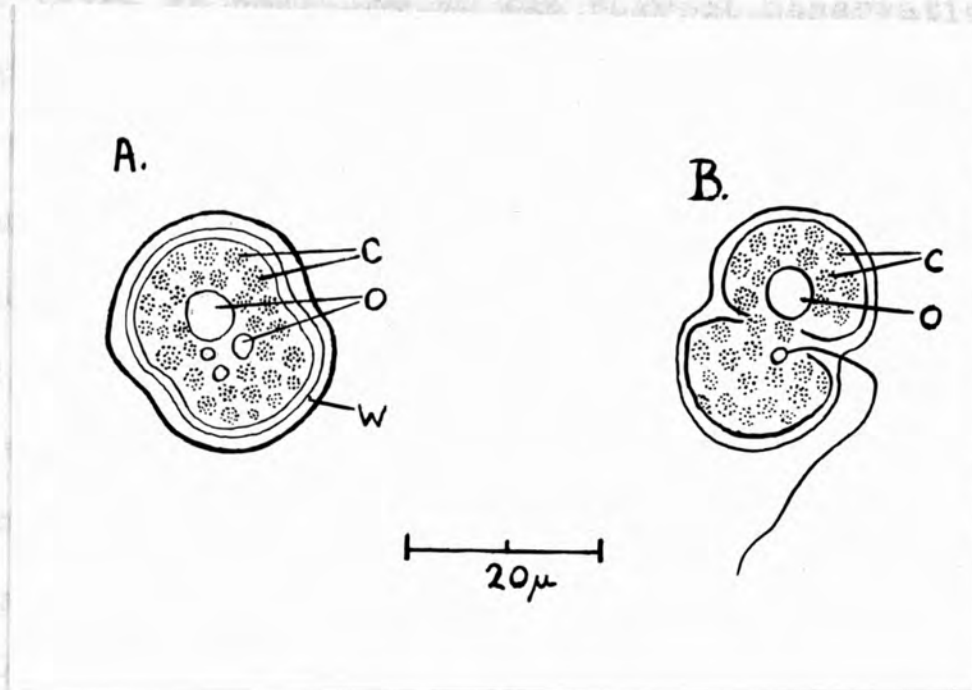


Figure 15. *Glenodinium pulvisculus*. A, cyst; B, motile cell; c, discoid chromatophores; o, red oil globules; w, outer wall of cyst.

C. Summary and Conclusions.

Two methods have been employed in an investigation of the stratification, microstratification and vertical movements, in nature, of certain algae at pond margins, and from the results obtained the following conclusions may be made:-

Pinnularia viridis cells and certain flagellates seem to be able to wander small distances up and down in

a. The buried slide technique may be as successfully used in Algological as in Mycological studies, particularly where the soil solution culture method, as described before, is employed in addition to the "direct observation" only technique.

b. In the litter of the only pond investigated fully (Stanmore Common pond) there has been found to exist a surface concentration of algal "germs" similar to that found for soil algae by Petersen (1935) and Tchan (1953).

c. Some of the algae investigated, mainly diatoms, appear to descend progressively deeper into the marginal litter as the water level drops during the year.

d. Species of Spirogyra here encountered have been found to survive in the litter only as zygospores, whereas Mougeotia parvula survives as vegetative cells, and the species of Tribonema and Microspora as modified vegetative cells or akinetes.

e. Certain species, e.g. Euglena deses, apparently lose their viability below a certain depth (about 2cm.) while other species, e.g. Microspora floccosa, have not been found to descend below a certain depth (about 1cm.).

f. Pinnularia viridis cells and certain flagellates seem to be able to wander small distances up and down in

the surface litter in response to climatic changes.

g. Motile cells of Glenodinium pulvisculus appeared in leaf strata samples at the onset of damper conditions, when the water level began to rise, where previously only thick-walled, oil-containing cysts had been found.

which, although relevant to the whole subject, have not been included in any of the preceding sections. These observations and experiments are best treated for the present as separate entities, and are recorded below:

1) Wall thickening and mucilage formation in certain filamentous algae.

Ulothrix. Filaments have been collected during several months of the year (March, April, August and October) from such different habitats as stream banks, pond water, pond margin litter (all on Stanmore Common) and a sandy heath (Chobham Common) soil surface, and subjected to drying.

Species dealt with include Ulothrix zonata, U. aequalis and a small species, referred to in this investigation as

U. subtilis. Upon slow drying on mud or soil, marked

wall thickening was observed in every experiment. To test whether this apparent wall thickening was merely due to the survival of filaments with already thickened walls and the dying of thin-walled filaments during drying, the

Section V. Various Observations and Experiments
not Included in the Foregoing Sections.

Certain observations have been made, and experiments carried out, in the course of the present investigation which, although relevant to the whole subject, have not been included in any of the preceding sections. These observations and experiments are best treated for the present as separate entities, and are recorded below:

1) Wall thickening and mucilage formation in certain filamentous algae.

Ulothrix. Filaments have been collected during several months of the year (March, April, August and October) from such different habitats as stream banks, pond water, pond margin litter (all on Stanmore Common) and a sandy heath (Chobham Common) soil surface, and subjected to drying. Species dealt with include Ulothrix zonata, U.aequalis and a small species, referred to in this investigation as U.subtilis. Upon slow drying on mud or soil, marked wall thickening was observed in every experiment. To test whether this apparent wall thickening was merely due to the survival of filaments with already thickened walls and the dying of thin-walled filaments during drying, the

following experiments were carried out: a single filament of U.zonata in a hanging-drop was mounted in a Wards tube through which was being drawn a damp air current to allow slow drying of the filament to occur. The filament, which initially possessed a fairly thin sheath (Figure 16A), showed what appeared to be thickening of the sheath after 18 hours drying (Figure 16B).

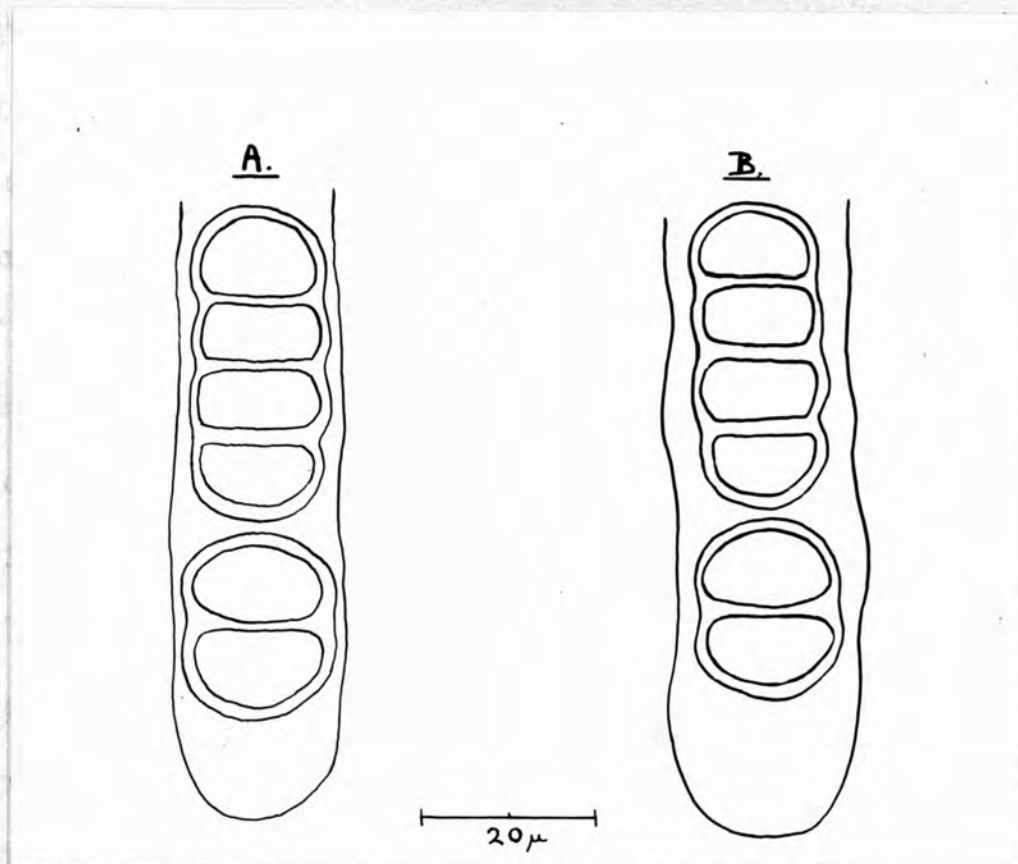


Figure 16. Ulothrix zonata, part of a filament dried experimentally. A. before drying; B. after 18 hours drying.

No microchemical investigation was carried out on this particular filament, but other filaments were investigated. Dried filaments of U.zonata with thickened sheaths (by sheath is meant covering material external to the wall of each individual cell) similar to those produced in the Wards tube experiments were tested with iodine in potassium iodide followed by concentrated sulphuric acid. The major part of the thickened sheath stained, transiently, a bright blue colour indicating that the sheath consists largely of cellulose. A thin peripheral region of the sheath did not appear to stain. Staining with Ruthenium red, however, produced a red coloration in the peripheral part of the sheath of dried filaments, either in patches or as a complete layer; while staining with methylene blue produced a blue-violet colour. These results suggest that the periphery of the sheath is a pectic mucilage layer. When filaments were dry-mounted the outer part of the filament sheath appeared wrinkled, contracted, and in some cases stratified, suggesting that upon drying the mucilage layer contracts around the filament. The mucilage may thus act in a protective fashion as does the shrinking cell wall in the Hormidium stage of Prasiola crispa as described by Fritsch (1922). After prolonged drying (4 months or more)

what may have been a fatty pellicle was detected using sudan black stain. A fatty pellicle has previously been detected surrounding filaments of Ulothrix and Hormidium by Jane and Woodhead (1941) and in Cladophorella calcicola by Fritsch (1944). As found by Jane and Woodhead (1941), there appeared to be a mucilage layer outside the pellicle. This suggests that the pellicle is formed after the outer layers of the wall become mucilaginous.

Ulothrix zonata and the other forms of Ulothrix investigated showed wall thickenings upon every drying experiment carried out. Filaments in liquid culture (Beijerinck 0.05% solution), or kept damp on mud or soil, showed no wall thickenings. Some filaments collected from Chobham Common had formed thick walls in nature and this may have been associated with the extreme droughts experienced at times on a sandy soil of the type shown at Chobham. Upon re-wetting of dried filaments, except where all the cells of a filament had died, it has been observed that the wall-thickening is re-absorbed or lost. In the majority of cases it has been found that growth of the filament eventually continues upon re-wetting. In a drying experiment in which filaments were dried rapidly,

liquid culture after 12 days. These two species, then, showed quite different reactions to drought conditions.

within 2 hours, in drops of liquid, wall thickening was not observed. Filaments dried in this fashion failed to continue growth when replaced in liquid culture.

The results obtained suggest that wall thickening, which appears to be associated with natural or artificial slow drying, may fulfil a protective function. On the other hand, it may be merely a reflection of some metabolic change and have no functional significance.

Filaments referred to U.subtilis have been found to survive severe and prolonged artificial drying (Section III), the drought material showing wall-thickening. This is possibly an indication of the survival value of this thickening. Oedogonium. A few cells with somewhat thickened walls, of a species of Oedogonium (species "a"), were found to have survived rather prolonged drying (69 days) in the large-scale drying experiments (Section III), but another species (species "b") was found never to survive experimental drying of even short periods (2 weeks) in the vegetative state. This latter species, however, produced oospores in a liquid culture which was allowed to evaporate at room temperature in the laboratory over a period of 6 months. These oospores survived a further very severe drought of 4 months, and germinated in fresh 0.05% Beijerinck solution. Sporelings were observed in this liquid culture after 12 days. These two species, then, showed quite different reactions to drought conditions.

Oedogonium sp.a will survive by wall thickening, and possibly by other modifications of the vegetative cells (to form what might be regarded as "akinetes") while the vegetative cells of Oedogonium sp.b are killed off by a drought, the species only surviving if resistant oospores have been produced prior to the drought.

2) Liquid cultures of pond margin litter and mud.

From time to time during the investigation of five small ponds (Section II) liquid cultures were set up by placing small portions (about 0.5gm.) of litter and/or mud scraped from the surface about 6" from the water's edge, in soil solution in crystallising dishes. Some of the results obtained are presented briefly in the tables 45 to 50. Where the species were also found, at the time of the initiation of each culture, by direct observation, they are recorded thus (+), and where they were not found by direct observation, thus (0). Many more species, relatively, not found by direct observation, appeared in cultures of litter and/or mud from the Englefield Green, Stanmore and Rowley Green "normal" ponds, than in cultures of the other two ("acid") ponds. This is probably due mainly to the lower water-holding capacities of the mud and litter of these "normal" ponds (See Section II).

is before

sample before

At a lower moisture content fewer species might be expected to be in the vegetative state. That the number of species found in pond margin samples by direct observation is governed in part by the dampness of the season, is suggested by the results obtained for the Englefield Green pond (Table 45) during the years 1953-54; relatively fewer species, not found initially, appeared in culture during 1954 than in the drier, warmer 1953.

The following species, with the pond number(s) concerned, appeared in culture and were never found initially by direct observation:- Eudorina elegans (I), Chlorococcum infusioenum (I,IV), Ankistrodesmus falcatus (I,II), Stigeoclonium tenue (I,II), Cosmarium impressulum (I,II), C.cucurbitinum (III), Tribonema subtilissimum (I), Oscillatoria limosa (I), Stauroneis phoenicenteron (II), Gomphonema acuminatum (IV) and Ulothrix zonata (III). Some species, e.g. Ulothrix zonata (see Section II), were not found initially as they are usually infrequent at the ponds concerned, while for other species it has been assumed that they were rare at the time of the collection, or were represented only by resting stages. It is unlikely that the normal vegetative stage of Eudorina elegans would be overlooked in the initial

Tables 45 and 46.

Algae appearing in soil solution cultures of pond margin litter and mud.

Collection number	Pond I (Englefield Green)				
	1	2	19	45	48
	1953		1954		
Month:	April	May	Aug.	June	Aug.
<u>Chlamydomonas</u> spp.	+		+	o	+
<u>Eudorina elegans</u>		o	o		
<u>Chlorococcum infusioenum</u>			o		
<u>Scenedesmus quadricauda</u>				o	o
<u>Ankistrodesmus falcatus</u>		o	o		
<u>Ulothrix subtilis</u>	+			+	
<u>Stigeoclonium tenue</u>		o			o
<u>Microspora floccosa</u>		o			
<u>Oedogonium</u> sp.a.	o		+		
<u>Cosmarium impressulum</u>		o	o	o	
naviculoid diatoms				+	+
<u>Tribonema subtilissimum</u>					o
<u>Anabaena</u> spp.				+	+
<u>Oscillatoria tenuis</u>	o		+	+	+
<u>O.limosa</u>	o				
<u>Euglena deses</u>	o		o		
<u>E.mutabilis</u>	o	o	o	+	+
<u>Phacus triqueter</u>		+	o		

Collection number	Pond II (Stammore)		
	41	45	48
Month(1954):	April	June	Aug.
<u>Chlamydomonas</u> spp.	o	+	
<u>Scenedesmus quadricauda</u>		o	+
<u>Ankistrodesmus falcatus</u>			o
<u>Microthamnion Rützingianum</u>	o		
<u>Stigeoclonium tenue</u>			o
<u>Oedogonium</u> sp.			o
<u>Stauroneis phoenicenteron</u>			o
naviculoid diatoms	+	+	+
<u>Nitzschia palea</u>			+
<u>Euglena mutabilis</u>	+	o	+
<u>E.deses</u>			+
<u>Anabaena</u> sp.			+
<u>Oscillatoria tenuis</u>			+

+ = algae observed in the sample before soil solution culture.
o = algae not observed in the sample before soil solution culture.

observation, so in the case of this species it may be concluded that the resting stage was a single cell, possibly a zygote. One species of Cosmarium, C. cucurbitinum, may have survived exposure more or less in the vegetative state. In the course of the large-scale drying experiments (see Section III), a vegetative cell of this species survived 54 days of drying to a moisture content of about 4.5%. It is not known how the other observed species of Cosmarium, C. impressulum, had survived.

For the Stanmore Common pond (Table 47) and to a much lesser extent, the Rowley Green "acid" pond (Table 49), where large areas of litter were exposed even during the rather wet summer of 1954, there is some suggestion in the tables that there is a zonation of algal species at the pond margins. Species appearing in a series of cultures of the Stanmore Common pond litter, set up in September 1954 (Table 50), and results of direct observations (Section II) bear out this conclusion. Three species, Ulothrix subtilis, Zygnema sp.B. and Netrium oblongum var. cylindricum, show definite preference for the upper zones, while two species, Mougeotia parvula and Microspora floccosa, show preference for the lower

Tables 47, 48 and 49.

Algae appearing in soil solution cultures of pond margin litter and/or mud.

Table 47. Pond III (Stanmore Common).

Collection number	1	2	41	45	48	52	
Year:	1953		1954				
Month:	April	May	April	June	May	Aug.	Sep.
Water level in centimetres	0	+1	-4	-4	-14	-20	-30
Distance of water edge from marker in centimetres	0	15	45	45	90	180	210
<i>Chlamydomonas</i> spp.			+	+		+	+
<i>Ulothrix subtilis</i>	+	+	+	+		o	
<i>U. zonata</i>					o		
<i>Microspora floccosa</i>					+	+	+
<i>Microthamnion Kützingianum</i>	o	o					
<i>Spirogyra cylindrica</i>					+	o	o
<i>Mougeotia parvula</i>					+	+	+
<i>Zygnema</i> sp. B.	+						
<i>Netrium oblongum</i> var. <i>cylindricum</i>				+			
<i>Cosmerium cucurbitinum</i>				o			
naviculoid diatoms			+	+	+	+	+
<i>Pinnularia viridis</i>			+		+	+	+
<i>Cryptomonas ovata</i>			+		+		+
<i>Synura</i> sp.			+				
<i>Euglena viridis</i>		o					
<i>E. deesei</i>	o						
<i>E. mutabilis</i>		o	+	+	+		
<i>Peredinium Willei</i>						+	

Table 48. Pond IV (Rowley Green "normal" pond).

Collection number	41	45	48
Month (1954)	April	June	Aug.
<i>Chlamydomonas</i> spp.	o		o
<i>Chlorococcum infusionum</i>			o
<i>Ulothrix subtilis</i>	+		o
naviculoid diatoms			+
<i>Pinnularia viridis</i>	+	+	
<i>Nitzschia peles</i>	+	+	+
<i>Gomphonema scuminatum</i>		o	
<i>Tribonema viride</i>	+	+	
<i>Euglena mutabilis</i>			+
<i>Anabaena</i> spp.		+	o
<i>Oscillatoria tenuis</i>		o	o

Table 49. Pond V (Rowley Green "acid" pond).

Collection number	41	45	48
Water level in centimetres.	-5	0	-18
Distance of water edge from marker in centimetres.	90	80	180
<i>Chlamydomonas</i> spp.	o	o	+
<i>Ulothrix subtilis</i>	o	o	+
<i>Microthamnion Kützingianum</i>			+
<i>Mougeotia parvula</i>			+
<i>Pinnularia viridis</i>			+
naviculoid diatoms		+	+
<i>Euglena mutabilis</i>	+		+

zones. All five of these species are able to withstand some exposure and drought with little, or no, modification of the vegetative cells (see Sections II and III) but, apparently, the first three algae named are able to withstand more severe and prolonged drought than the latter two species.

The apparent preference by *Spirogyra cylindrica* for the lower zone is probably due to the zygospores being formed in 1953 and 1954, during June - July, at which time the water level had already fallen sufficiently to expose the upper zones.

3) Coenobial Volvocales.

Five species, *Gonium pectorale*, *G. sociale*, *Pandorina morum*, *Eudorina elegans* and *Volvox aureus*, have been found during the investigation of five small ponds (Sections II and III) and of these species, two, *Pandorina morum* and *Eudorina elegans*, have been found at times on the litter surface above the water level. Coenobia of both species appear to retain their flagella and their motility while there is very little free water remaining, and have been observed swimming in the water films surrounding mud and litter particles. Coenobia of *Eudorina elegans* have been found to contract in drying

Table 50. Marginal zonation of algae at the Stanmore Common pond.

Zone number	7	5	4	3
Distance above water in cm.	30	15	2.5	0-1.0
" away from " edge "	180	90	15	5
<i>Chlamydomonas</i> spp.	+	+	+	+
<i>Ulothrix subtilis</i>	+	+		
<i>Microspora floccosa</i>		+	a	+
<i>Microthamnion Kützingianum</i>		+	+	+
<i>Mougeotia parvula</i>	+	e	e	a
<i>Spirogyra cylindrica</i>				+
<i>Zygnema</i> sp.E.	a			
<i>Netrium oblongum</i> var. <i>cylindricum</i>	+			
naviculoid diatoms	+	+	+	+
<i>Pinnularia viridis</i>	+	+	+	+
<i>Cryptomonas ovata</i>		+	+	+
<i>Euglena mutabilis</i>		+		+
<i>Anabaena</i> sp.	a			

+ = present in the culture within three months.

a = abundant " " " " " "

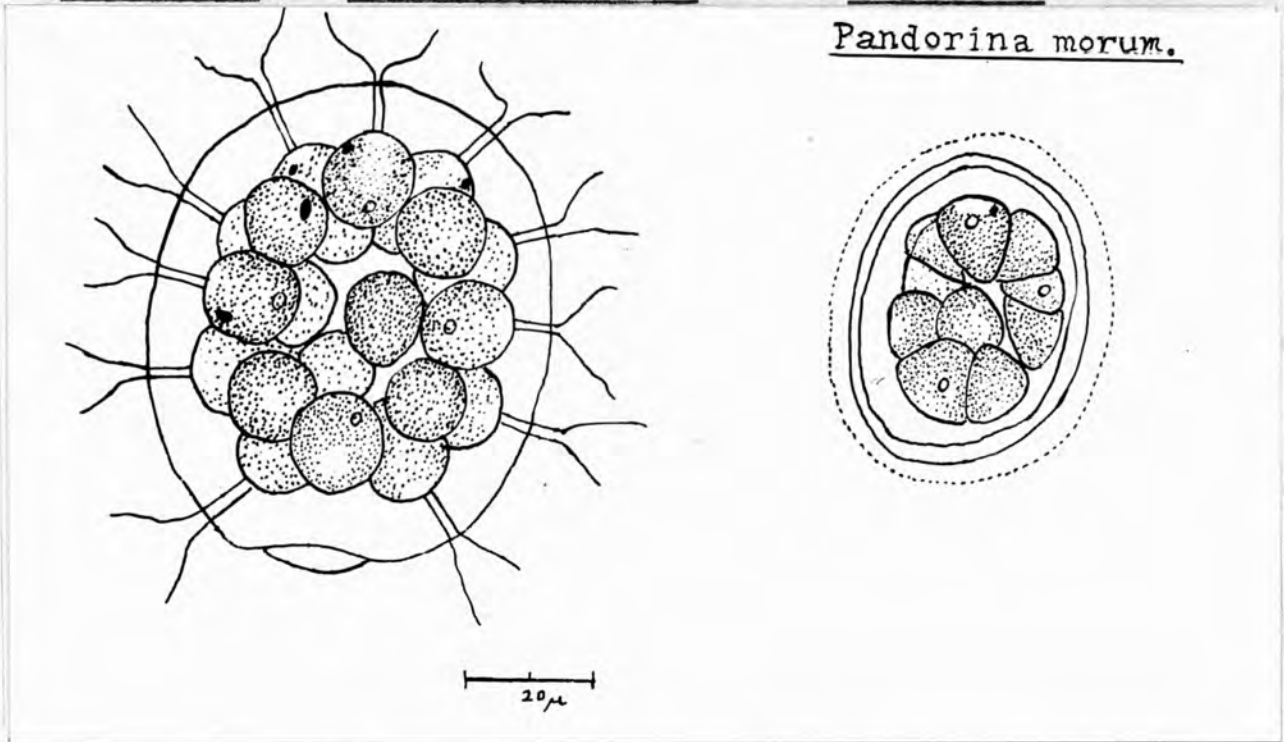
Samples of litter were collected from the margin of the Stanmore Common pond in September 1954 and placed in soil solution culture.

conditions, the usually loosely arranged cells becoming compactly arranged within the mucilage investment (Figure 17). That this occurrence may aid the survival of this species during continued drying is suggested by the results obtained in the course of the drying experiments (Section III), although survival through a very prolonged drought may occur only by means of oospores.

Figure 17. Eudorina elegans.

Figure 18.

Pandorina morum.



The already compact coenobium of Pandorina morum is able to contract very little further, if at all, and in this species some stratification of the mucilage investment occurs subsequent to the loss of the flagella after continued drying (Figure 18). The inner layer of this

investment was found to colour violet-blue with methylene blue stain, and red with ruthenium red, which suggests that it is a pectic-mucilage layer. Pandorina morum appeared in liquid cultures of marginal litter and/or mud previously exposed to short, mild drying in nature or experimentally (see Sections II and III). The species did not appear, however, after more severe or prolonged drying. The investment of the coenobium of this species may afford some protection, but the results obtained suggest that this species is not highly resistant to drying.

Both species of Gonium, G. pectorale and G. sociale, have survived prolonged experimental drying on pond margin litter and mud (Section III). For G. pectorale, the initial reaction to drought is loss of the flagella, followed by a contraction of the coenobium, rather as in Eudorina elegans. The reaction to a concentrated solution (a 1% solution of Tidman's sea salt was used for the experiment) is similar. Complete coenobia, however, have never been found to survive drying for very long, and results suggest (C.f. Section III) that survival is by means of zygospores. That G. sociale has not been found in nature in the course of the present investigation, and that this species appeared in liquid cultures of dried litter and mud (Section III)

suggests that although drought-resistant zygospores may be present, favourable environmental conditions were not realized in the water of the ponds in nature during the course of the present investigation.

The relatively very large coenobia of Volvox aureus soon collapse upon drying and do not appear to survive, this species never appearing in liquid cultures of dried litter and/or mud. But it may be that zygospores were rather rare and, by chance, never placed in liquid culture.

4) Haematococcus pluvialis.

This species was found in the grounds of Royal Holloway College in habitats some of which are comparable with, and somewhat similar to, the ephemeral rain-water pools described by Droop (1953). Three out of four small depressions (each 1" deep x 3" diameter) in concrete slabs were found to contain rather sparse populations of this species accompanying other vegetation including mosses and filamentous algae (mainly Ulothrix), while one depression, free of other vegetation, contained a very dense and almost pure population of Haematococcus pluvialis. Pure populations were also found in four depressions examined in iron drain covers. Changes of environment were expected, and that motile cells appear more quickly in the dilute than in the concentrated solutions, appear in far greater

fall the depressions filled rapidly with water and in dry, warm weather they dried up again almost equally rapidly. On one occasion a depression, filled with water at 6 o'clock in the evening, was found to be about half-full at 10.30 the next morning, and contained no free water one hour later. The temperature also changed rapidly at times, a change from 23°C. to 37°C. being recorded within 4 hours in May 1954. The temperature of the water within which macrozooids were found was 23°C. to 30°C., and it is interesting to compare this range with that reported by Droop (1953) of 17°C. to 25.3°C. for the Baltic strain of this species.

To test the reaction of the cysts to various salt concentrations, a suspension was prepared by shaking dried cysts in sterile distilled water in a test-tube, and inoculating one drop into each of the following concentrations of Tidman's sea salt: 0.05%, 0.1%, 0.2%, 0.4%, 1.0% and 5.0%. At intervals after this, samples were withdrawn from the cultures and examined, the percentage of motile, flagellate cells being estimated in a count of about 500 cells in each case. The results, recorded briefly in Table 51, although rather erratic, show clearly that motile cells appear more quickly in the dilute than in the concentrated solutions, appear in far greater

quantity in the water than in the salt solutions, and persist for longest in the 0.05% solution than in any other. In the 1% and 5% solutions motile cells were never found. The concentration range for swarming is, then, 0.0% to <1.0% Tidman's sea salt with an optimum at 0.0% to 0.05%. These results are interesting in view of the report by Droop (1953) that the salinity range within which swarming occurred in nature, of the Baltic physiological strain, was 0.0% to 2.0% NaCl. with a maximum between 0.1% and 0.4%. To test whether the cysts in the highest concentration (5.0%) of Tidman's sea salt used were still alive, re-inoculations were made into sterile distilled water, and the results obtained (Table 52) suggest that although swarming may occur in distilled water more rapidly after a shorter time in a high concentration, a greater quantity of motile cells will appear after a longer period in a high concentration. Direct observations of water samples collected from the small pools after rain suggests a tendency for the same thing to occur in nature, a higher proportion of motile cells (96% on one occasion) than being found in the relatively warmer, drier summer months (May to August) than in the winter months (October to December) of 1954. This may have been due to temperature

Tables 51 and 52.

Experiments with the cysts of *Haematococcus pluvialis*.

Table 51. Inoculation of cysts into water and into various concentrations of Tidman's sea salt.

Number of days after inoculation.	1	4	9	12	18	32	46
% cells motile in water.	20	39	2.1	1.9	0.2	0	0
" " " " 0.05% sea salt.	0.3	8.3	4.2	2.6	2.1	0.4	0.5
" " " " 0.10% "	0	3.2	0	2.0	0.4	1.8	0
" " " " 0.20% "	0	2.1	0	6.1	1.0	3.2	0
" " " " 0.40% "	0	0	0	3.8	1.4	0	0

Table 52. Re-inoculation of cysts from 5% sea salt into water.

Number of days after re-inoculation.	1	5	14	30	44
% cells motile in water (A).	25	20	15	1	0
" " " " " (B).	0	56	60	44	1

(A) = cysts which had been kept in 5% sea salt for 18 days.
 (B) = " " " " " " " " " " " " " " 32 "

differences.

Cysts are well known to be highly resistant to desiccation, and according to the observations made in the course of the present investigation it would seem that the longer and more severe the drought, or the longer the period of high concentration undergone, the more the growth rate is apparently enhanced subsequent to the dry period. Haematococcus pluvialis is a species, then, that not only will survive despite dry periods, but may thrive because of them.

Elliot (1934) has described haematocysts as possessing "a heavy, resistant cellulose wall separated by an appreciable space from the protoplast". It has been found that this space may be seen immediately when haematocysts are placed in water or relatively dilute solutions (Tidman's sea salt to 10% or alcohol to 70%) but that in high concentrations (20% sea salt or absolute alcohol) the space cannot initially be seen, though it does appear in some cells after a time (48% of the cells in 20% sea salt and 40% of the cells in absolute alcohol after 1 hour). In dry-mounted cells this space cannot be seen, the wall being then in close contact with the protoplast. It seems quite likely that survival of the cysts during extremely

dry conditions is associated with the above described observations, which suggest that cells are capable of round-taking in water when there is very little available and that in completely dry conditions the wall shrinks protectively round the protoplast.

Experiments in which macrozooids were exposed to sudden drying all resulted in death of the cells, suggesting that it is due to the cysts, and perhaps to some extent to the palmella stage, that drought is survived.

The survival and success of populations of Haematococcus pluvialis in such harsh habitats as those described by Droop (1953) and in the above account, is due largely to the existence in the life-cycle of the resistant, encysted stage. The major factor influencing the life cycle appears to be the concentration of the medium.

5) Euglena.

Cysts of species of Euglena have been found at times in nature, and have been induced experimentally by drying in the laboratory. Attempts to revive these cysts in liquid culture (using soil solution, Beijerinck 0.05% solution and sterile distilled water) have all failed.

In one of the large-scale drying experiments (Section III) in a sample with a moisture content of 8% from an area of motile cells present in 50 low-power fields of each

which had varied between 4% and 11% moisture over the previous four weeks, was found a flat plate of about 20 rounded-off Euglena cells surrounded by mucilage in which were imbedded filaments of Oscillatoria tenuis. The outermost Euglena cells showed no movement, but the innermost, more thoroughly protected by the mucilage and possibly also by the non-motile outer cells, possessed prominent eyespots and were actively metabolic. A similar group of cells was found once in nature at the margin of the Englefield Green pond, again with Oscillatoria in the outer mucilage layer. Attempts to induce this condition in the laboratory have failed. Most of the mucilage present in this group of cells was probably that derived from the Euglena. The surrounding filaments of Oscillatoria, however, forming their own mucilage, may provide some additional protection to the Euglena cells during drying.

Dense patches of Euglena found exposed on the surface of pond margin mud and beginning to dry in nature were dried for a further four weeks in the laboratory. Thin slices were then cut from the dried material with a sharp scalpel from four progressively deeper layers, and placed in 0.05% Beijerinck solution in four separate watch glasses. After six days, observations were made counting the number of motile cells present in 50 low-power fields of each

culture. No motile cells appeared in the culture of the outermost layer, 30 motile cells were counted in the next layer, and 50 in each of the two innermost layers. This suggests clearly the protective function performed by the outermost layers of dead and dying cells during dry periods. A further sample of Euglena cells initially very damp, was dried more rapidly (within 7 days) in the laboratory in a watch glass. Then three scrapes were taken from the surface, and one from the underlying layer, and 50 high-power observations made, again counting the number of motile cells present in each sample. In the first three samples, of a total of 120 cells counted, all cells were non-motile, while in the last sample 4 of a total of 48 cells were motile. The species referred to in the foregoing account is E. viridis, but other species, including E. deses and E. mutabilis, have been observed to behave in the same way at times of drought.

Species of Euglena, and other members of the Eugleninae, have consistently failed to appear in laboratory cultures after severe drying (Section III) but this may be due to adverse laboratory conditions.

6) Glenodinium pulvisculus.

In several ponds on Stanmore Common resting cysts of

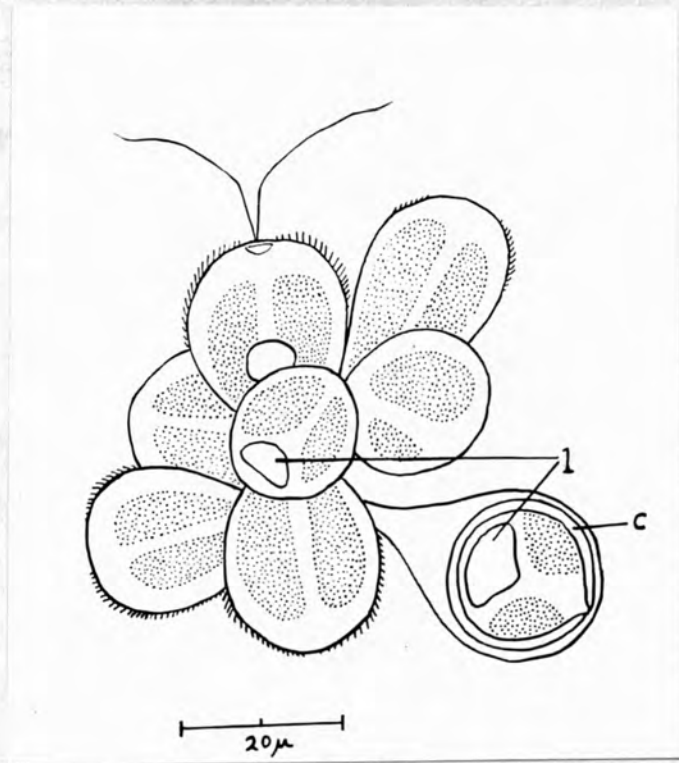
Glenodinium pulvisculus have been found floating on the water surface. That these cysts have been found after the occurrence of many motile cells suggests that cyst formation of this species occurs in the water, in response to conditions other than that of drought. The cysts, however, have been found to survive drying in nature and by experiment (C.f. Sections III and IV). The cyst wall is of two layers, a thin outer layer and a thicker inner layer. The cysts are impermeable to stains such as methylene blue and ruthenium red, and although no positive evidence by using the Sudan stains, has been obtained, there may be a surrounding cuticle. Red or orange-red oil globules are contained in the cysts and these have been found, in some cases, to persist for a while when the cell regains its motility (Section V).

7) Synura agg. (See Section II).

This flagellate was found with the greatest frequency each spring (March to April) of the years 1953 to 1955, in the Stanmore Common and Rowley Green "acid" ponds. Cysts were found on the litter surface at the pond margins when the water level dropped (Section II) and their presence deeper in the litter has been inferred by the appearance

of colonies in the slide-culture experiments (Section V).

In the laboratory, motile colonies were placed on damp litter in watch glasses and also on damp, sterile soil in petri dishes. These were then allowed to undergo slow drying at room temperature. As the free water of the decreased, the movements of the colonies became progressively more sluggish, and eventually the colonies began to break up into separate cells each of which lost its flagella and commenced rounding off (Figure 19).



1 = leucosin.
c = cyst wall.

Figure 19. Synura sp., cyst formation during experimental drying.

Conspicuous white masses found in the cells were assumed to be leucosin. After further drying, cysts only were found. Samples of the dried soil were inoculated into soil solution and more or less normal motile colonies were observed within 8 days. The moisture content of the soil when the colonies began to break up was approximately 60%. Observation of samples indicates that cyst formation also occurs in nature in damp conditions at pond margins, near the waters edge. With this flagellate then, as with Glenodinium pulvisculus, cyst formation may not be a direct reaction to complete drought. The cysts, however, once formed, may resist considerable exposure and drying.

and/or were successfully cultured, to allow of detailed description.

Cryptomonas sp.A. (Figure 20 A and B). This species was found in the greatest abundance in the Stanmore pond during autumn and winter of 1953 and 1954. The cells are similar to those described by Lund (1942), from the plankton of the Clay pit, Richmond Park, and referred to as Cryptomonas A. The cell dimensions are $34-44\mu \times 16-21\mu$, as compared with Lund's measurements of $34.1-54\mu \times 12.4-17.8\mu$ for Cryptomonas A. There is one marked difference from Lund's Cryptomonas A.: two distinct chromatophores, arranged dorso-ventrally

VI. Taxonomic Notes.

Certain algae encountered, and dealt with ecologically in the foregoing account, warrant detailed descriptions.

1) Cryptomonas spp.

It was pointed out by Lund in 1942 that although individuals of Cryptomonas may be abundant in fresh water habitats, knowledge of their morphology and taxonomy is in an unsatisfactory state. This is also largely true for today. Species of Cryptomonas were found at all of the five ponds described in Section II and in other ponds on Harrow Weald Common, near Staines and near Virginia Water. Only three of the forms encountered occurred in sufficient abundance, and/or were successfully cultured, to allow of detailed description.

Cryptomonas sp.A. (Figure 20 A and B). This species was found in the greatest abundance in the Stanmore pond during autumn and winter of 1953 and 1954. The cells are similar to those described by Lund (1942), from the plankton of the Clay pit, Richmond Park, and referred to as Cryptomonas A. The cell dimensions are $34-44\mu \times 16-21\mu$, as compared with Lund's measurements of $34.1-54\mu \times 12.4-17.8\mu$ for Cryptomonas A. There is one marked difference from Lund's Cryptomonas A.: two distinct chromatophores, arranged dorso-ventrally

(Figure 20B), are present in some of the cells, instead of the single chromatophore as described by Lund. In Section II this species has been referred to C. curvata Ehrenb.

Cryptomonas sp. B. (Figure 20D). This species was found on almost every occasion whenever and wherever the foregoing species was found. The dimensions are $25-30\mu \times 15-18\mu$ and the cells are somewhat flattened. There is an anterior, dorsal projection which is considerably less prominent than that in C. curvata (sp.A.). There are two chromatophores, yellow-brown to olive-green in colour, arranged dorso-ventrally. This species is referred to C. ovata.

Cryptomonas sp. C. (Figure 20C) appeared in a soil solution culture of litter collected 6" away from and 2" above the edge of a small pond (about 5 feet in diameter) on Harrow Weald Common in October 1952. Cell size, $13-18\mu \times 8-10\mu$; shape, more or less ovoid but with a tendency for the dorsal side to be convex and the ventral side somewhat flattened; two olive-green chromatophores arranged dorso-ventrally; pyrenoids were found in the cells either as two separate bodies, or as two, merging to form one larger body (Figure 20C). The description suggests a relationship with C. parapyrenoidifera Skuja.

This species was not again encountered either in nature or in culture and would appear to be rare compared with the other two species described.

2) Spirogyra spp.

During the course of the present investigation twelve forms of Spirogyra filaments have been encountered. It has been possible to name only three of these forms, since the remainder have never been found in the fertile condition.

Spirogyra sp.A. Vegetative cells of this species first appeared in each of the years 1953 to 1955, sometime between early May and mid-June (see Section II), in the Stanmore Common pond. Each year conjugation has occurred during June or July. The species was very abundant in 1953, but was largely replaced in 1954 and 1955 by Mougeotia pavula.

Spirogyra sp.A. is described as follows:- Vegetative cells 15-16 μ x 140-200 μ , replicate end walls; one chromatophore in each cell; conjugation scalariform or lateral; fertile cells inflated to 30-40 μ ; zygospores ellipsoid 50-70 μ x 25-30 μ ; median spore wall yellow-brown, smooth. This description could fit either S.cylindrica Czurda 1932 or S.austriaca Czurda 1932, so that agreement is reached with Transeau's view (1951) that "neither the description nor the figure of S.austriaca Czurda 1932 clearly separates

it from this species (S.cylindrica Czurda 1932)". This species has, then, been referred, in Section II, to S.cylindrica Czurda 1932.

Spirogyra sp.B. This species appeared in a soil solution culture of dried litter from the Englefield Green pond (Section III). Vegetative cells were $22-30\mu \times 60-150\mu$ with replicate end walls, one chromatophore in each cell; conjugation was lateral and scalariform and the fertile cells were inflated to $35-42\mu$. The cells became unhealthy in the culture before the zygospores were formed. This form is referred to S.grevilleana (Hassall) Kutzing 1849.

Spirogyra sp.D. (Figure 21). Fertile cells of this species were found in the Englefield Green pond in June 1955. Vegetative cells were $70-80\mu \times 90-150\mu$, with plane end walls, and contained 2-5 chromatophores. Conjugation was scalariform with tubes formed by both gametangia, and the fertile cells were cylindrical or slightly enlarged. The zygospores were ellipsoid or ovoid and measured $60-70\mu \times 90-120\mu$, and the median spore wall was smooth and light-brown. This species is referred to S.nitida (Dillwyn) Link 1833.

3) Euglena variabilis Klebs.

This species was found by Lund (1942) in the clay pit, Richmond Park. Cells of Euglena which fit Lund's description

of E. variabilis were found on one isolated occasion (in May 1953) in the course of the present investigation on the mud surface 1" away from the water edge of the Stanmore pond.

4) Lepocinclis spp.

Six species of Lepocinclis have been encountered in the present investigation, usually in the late summer or autumn, individuals often reaching their maximum frequency during October. They are all aquatic, having been found only very rarely more than 6" from the pond margins, and do not, apparently, survive if exposed to severe drying.

L. ovum var. Globula (Perty) Lemmerman. (Figure 22A).

Individuals of this variety of L. ovum appeared suddenly in the Stanmore Common pond in September 1954, reached a very high maximum (75 cells counted in 50 high-power fields), and then decreased rapidly, although cells were encountered from time to time until May 1955. Cells were also found, though very rarely, at the Rowley Green "acid" pond during the same period. The cells measured (20 or more at each observation) were approximately $27\mu \times 20\mu$, this being almost the maximum size attained by the cells described by Conrad (1934). The only other difference from Conrad's description is that all the cells found had colour-less membranes.

L.teres (Schmitz) France. (Figure 22B). This species occurred at the Stanmore Common and Rowley Green "acid" ponds from August 1953 to early January 1954, with a maximum during October. The species was also found, though less frequently, in the autumn of 1954. The lower frequency may have been due to the duller, cooler and damper weather of that year. There is a prominent eye spot (Figure 22B, L. heterochila Lemmerman. was found only during October 1954, st.) but this is the only departure from the description of Conrad (1934). Cells were found on rare occasions being found in 50 high-power fields. The cells were nearly one metre away from, and 15cm. or more above, the water, and these cells, non-motile and lacking flagella, may have been resting cells. All attempts to induce growth of these cells in liquid culture, however, failed.

L.texta (Dujardin) Lemmerman. This species was found only on one occasion (November 2nd 1954) at the Rowley Green "acid" pond, but it was, at that time, very abundant.

L.heterochila Kufferath. Cells of this species, found at the Stanmore Common pond from May to August 1954, and from February to April 1955, were very occasionally found in the transition zones of the pond margin (5cm. to 15cm. away from the edge of the water) but, again, seemed unable to withstand prolonged exposure and drought.

L. reeuwykiana Conrad. This species, with the distinctive shape and membrane, found previously in Holland by Conrad (1934), was found at the Stanmore Common pond in September 1954, and at the Englefield Green pond in June 1955, but was always rather rare, never more than 4 cells being counted in 50 high-power fields.

L. Butschlii Lemmerman. was found only during October 1954, at the Stanmore pond and was rare, only three or four cells being found in 50 high-power fields. The cells were approximately $35\mu \times 20\mu$ in size.

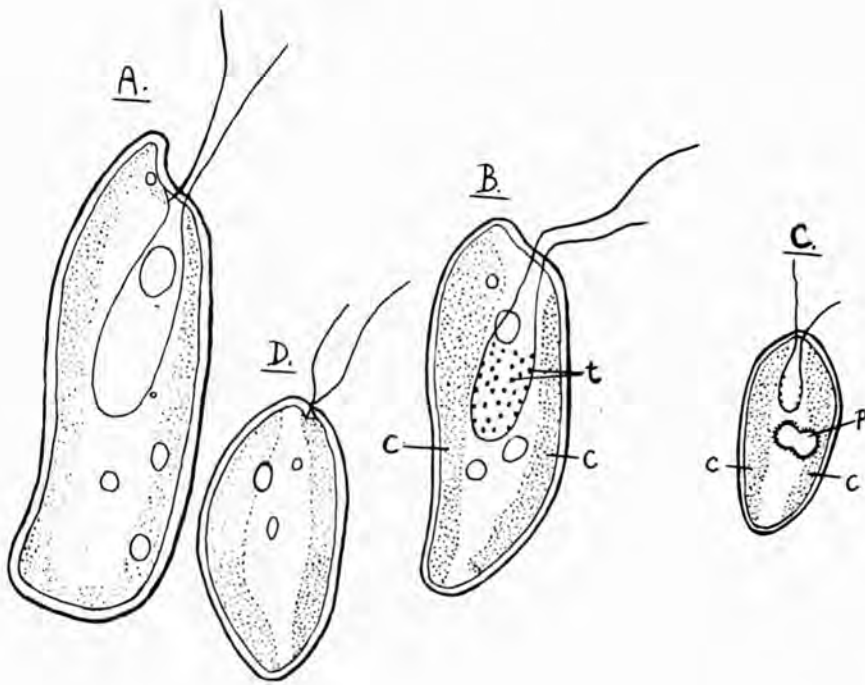
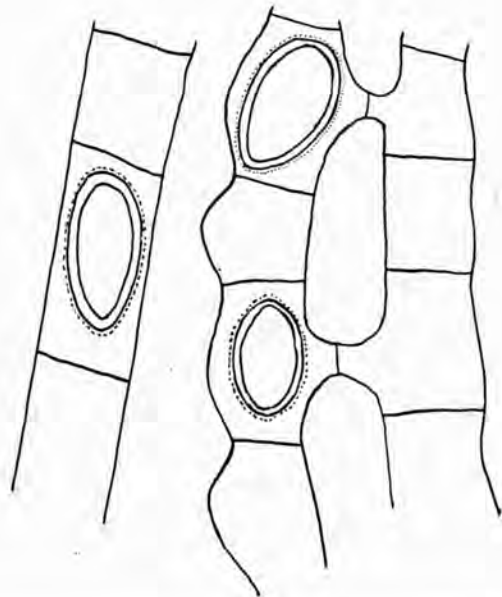


Figure 20. A and B, Cryptomonas sp. A. A, lateral view showing anterior projection and posterior curvature. B, semi-lateral view showing dorso-ventral arrangement of the two chromatophores. C, Cryptomonas sp. C. D, Cryptomonas sp. B. c, chromatophores; t, trichocysts; p, pyrenoids; (A, x1700; B, x1500; C, x2150; D, x1650).

Figure 21.
Spirogyra sp. D. (x190).



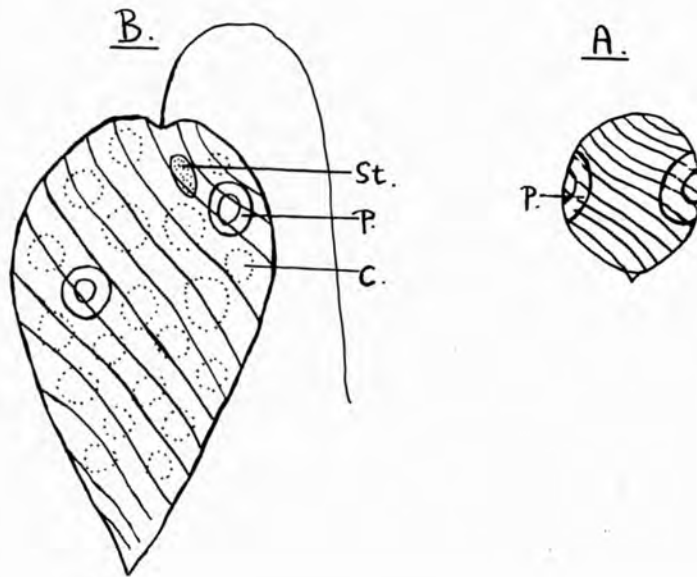


Figure 22. A. Lepocinclis ovum var. globula
(Perty) Lemmerman. B. Lepocinclis teres
(Schmitz) France. st, eyespot; p, paramylon;
C, chromatophore. (A x900, B, x1485).

Investigated by a VII. Summary.

- 1) An investigation has been carried out, from September 1952 to July 1955, on the survival of fresh water algae during dry periods. This investigation has included more than two years observation (April 1953 to June 1955) of the algal flora in the water and upon the marginal mud and litter of five ponds. on as a whole follows:
- 2) Four aspects of the survival of algae during dry periods have been investigated:
 - (i) the survival of algal species during dry periods in nature. In summer months, when water is draining
 - (ii) the survival of algal species during more or less prolonged artificial drought. Drying experiments (small-scale in the laboratory and large-scale outdoor) of varying duration and severity were carried out.
 - (iii) the accumulation of fatty reserve substance in cells of certain algae subjected to drought (See Appendix 1, p.178). to a certain extent, upon some
 - (iv) the descent of cells into the deeper layers of litter and/or mud at pond margins during dry periods. The stratification of algae in exposed litter or mud was investigated by a modification of the Rossi-Cholodny slide technique. Micro-stratification was of

investigated by a micro-sampling method.

These four aspects of the problem, though all are associated in some way with survival during dry periods, have been treated in different ways using various observational and experimental techniques.

3) A general summary of the conclusions arrived at in the investigation as a whole follows:

1. In drying conditions, when precipitation is exceeded by evaporation, the amount of moisture remaining available to the algae becomes a factor of importance. In summer months, when water is draining and/or evaporating away from ponds faster than it is being replaced by rainfall, areas of mud and litter are exposed for days, weeks, or even months at a time, and many algae may be stranded in the drying upper regions of pond margins. The survival of a number of those algae may depend, to a certain extent, upon some retention of the moisture by the surface layers of marginal litter and mud.
2. There is, therefore, a tendency for the presence of certain algae to depend upon two major factors in this respect; the frequency and severity of

2. drying up of a pond, and the nature of the pond margin. These two factors will depend upon a complex of other factors.
3. It may be, then, that certain species of algae occur only in larger bodies of water, as they could not survive from one season to the next through the complete drying up of a smaller pond. An example of this may be Cladophora fracta, which occurs only in the largest of the five ponds investigated, the Stanmore pond.
4. The algal flora of ponds which are liable to dry up completely, or almost so, will depend to some extent upon the nature of the pond margin. Considering only, for the purpose of this discussion, organic matter content, a pond margin with a low content of organic matter will tend to have a lower water-holding capacity than a pond margin with a high content of organic matter. The only algae likely to survive upon such a margin will be those capable of withstanding severe desiccation. If, on the other hand, the pond margin consists largely of litter, with a high organic content, and a high water-holding capacity, then species with less-resistant stages may survive the drought.

5. Populations of usually aquatic algae are reduced drastically upon drying after exposure on marginal litter or mud, and the critical moisture content below which algal populations decrease seems to be about 50% (of the wet weight).
6. Despite this drastic reduction in numbers of living cells, most species encountered were found to survive through drought periods from one season to the next. The ways in which some at least of these algae may do so is outlined below:
- A. Algae which may survive drought as zygospores or oospores:-
Chlamydomonas spp., Gonium pectorale, G. sociale,
Eudorina elegans, Chlorogonium euchlorum,
Oedogonium sp.B., Spirogyra cylindrica,
Mougeotia sp.B., Cosmarium botrytis.
- B. Algae which may survive drought as cysts:-
Synura agg., Dinobryon sp., Glenodinium pulvisculus.
- C. Algae which may survive drought as akinetes:-
Ulothrix subtilis, U. zonata, Microspora floccosa, M. stagnorum, Zygnema sp.B.
- D. Algae which may survive drought as apparently

normal vegetative cells:- Chlorococcum infusionum,

Netrium oblongum var. cylindricum, Mougeotia
parvula.

E. algae which may survive drought as vegetative cells with one or more of the following modifications:-

a. Thickened cell wall:

^XChlorococcum infusionum, ^XUlothrix subtilis,
^XU.zonata, ^XMicrospora floccosa, ^XM.stagnorum,
^XOedogonium sp.A., ^XZygnema sp.B.

b. Mucilaginous sheath or gelatinous envelope:

^XChlorococcum infusion, ^XUlothrix subtilis,
^XU.zonata, ^XMicrospora floccosa, ^XM.stagnorum,
^XNostoc commune.

c. Accumulation of oily matter in the cells:

^XUlothrix subtilis, ^XU.zonata, ^XMicrospora
^Xfloccosa, ^XM.stagnorum, ^XOedogonium sp.A.,
^XZygnema sp.B., ^XCosmarium cucurbitinum,
^XPinnularia viridis, ^XP. appendiculata,
^XStauroneis phoenicenteron, ^XNitzschia palea.

N.B. ^X species with more than one modification.

F. Algae which may be assisted in their survival of dry conditions by descent (active or passive) of cells into deeper litter layers:-

- Chlamydomonas spp., Chlorococcum infusionum,
Dactylococcus bicaudatus, Oocystis solitaria,
Ankistrodesmus falcatus, Scenedesmus quadricauda,
Hormidium flaccidum, Ulothrix subtilis,
Microspora floccosa, M. stagnorum, Microthamnion
Kutzingianum, Stigeoclonium tenue, Mougeotia
parvula, Tribonema viride, T. vulgare,
T. subtilissimum, Synura, Pinnularia viridis,
Navicula spp., Nitzschia palea, Hantzschia
amphioxys, Euglena deses, E. mutabilis, Anabaena
spp., Oscillatoria tenuis.

7. For those species found during the present investigation but not named in the foregoing list, conclusions regarding their survival during drought must necessarily be purely conjectural. The species of Closterium found (see species list), for example, may all be capable of producing drought-resistant zygospores, but a great deal more attention to detail would have to be paid to an investigation of this problem than was possible in the present work.
8. The re-appearance of algae after a dry period may depend upon the degree, rate and nature of

re-wetting. A sudden immersion in water may inhibit or delay the re-appearance of certain species. Re-wetting experiments carried out (Section III, p.84) suggest that some of the species of Naviculoid diatoms encountered in the present investigation may fall into this category. The zygospores of certain of the conjugales, including three species of Closterium and one of Spirogyra, may also be inhibited in their germination by total immersion.

4) Further problems include the following:

1. The occurrence of such a delicate form as Chrysococcus rufescens, of which cysts are unknown, in the Stanmore Common pond which, of all the five ponds investigated, is the most likely to dry up.
2. In the same way it is not exactly known how species of Lepocinclis, Phacus, Trachelomonas and Cryptomonas survive drought, although the occurrence of Cryptomonas ovata to a depth of 1cm. in the litter at the margin of the Stanmore Common pond (Section IV) suggests a way in which this species may survive.
3. Another problem, concerning the stratification of algae in marginal litter or mud, is: how far will

algal cells descend into the litter or mud in response to drought (or passively) yet remain viable and, if motile, capable of ascending to the surface upon the return of damp conditions?

4. Alternatively, if the cells are beyond a depth where an active ascent is possible, or if they are static cells which originally descended passively, what are the chances of a re-appearance of the species at the surface by a disturbance of the litter or mud?

The methods used to estimate apparent changes in oil content were simple, and the results are only of value if used comparatively. It is emphasized that the conclusions arrived at are no more than tentative. They may, however, suggest a line along which the subject might be further investigated.

The Accumulation of Oil During Drought.

Appendix 1.

A. Introduction.

The accumulation of oil during drought.

What algal cells subjected to drought accumulate substances of a fatty nature has been reported many times. In view of the well-known association between dry conditions and the accumulation of oil in the cells of Fritsch (1916) described the cells of *Lyngbya ericetorum* some algae, observations were made on the amount of oil as containing fat globules, particularly in the peripheral part of the protoplast, and observed small globules forming in cells of certain species subjected to drying. Since this work was a divergence from the direct line of enquiry a semi-continuous stratum in some of the skinetes of and did not receive much attention it has been thought best to present the results as a supplement to the thesis rather than to include them in the body of the work.

Closterothrix calcicola (1944). Fiscoy (1917) found that filaments of *Horridium flaccidum* which became stocked with large and abundant granules are capable of much longer resistance to drought than filaments in which the granules content were simple, and the results are only of value if disappeared or became small and scanty. Petersen (1935) used comparatively. It is emphasised that the conclusions arrived at are no more than tentative. They may, however, desiccation, stated that in Diatom cells from dry soil suggest a line along which the subject might be further investigated. and Salah (1952) observed the accumulation of oily matter in Diatoms during drought. Kahn (1949 - unpublished M.Sc. thesis) found that during hot periods there was an increase of the granular inclusions in the cells of the serial algae he investigated. Regarding resistance to conditions other than drought, Strom (1924) stated that algae may survive the cold of winter in the vegetative state surrounded by thick mucilage coats, or with the cellular contents strongly

The Accumulation of Oil During Drought.

A. Introduction.

That algal cells subjected to drought accumulate substances of a fatty nature has been reported many times. Fritsch (1916) described the cells of Zygonium ericetorum as containing fat globules, particularly in the peripheral part of the protoplast, and observed small globules forming a semi-continuous stratum in some of the akinetes of Cladophorella calcicola (1944). Piercy (1917) found that filaments of Hormidium flaccidum which became stocked with large and abundant granules are capable of much longer resistance to drought than filaments in which the granules disappeared or became small and scanty. Petersen (1935) in considering the adaptation of soil algae to resist desiccation, stated that in Diatom cells from dry soil samples, large masses of oily substances are often seen, and Salah (1952) observed the accumulation of oily matter in Diatoms during drought. Kahn (1949 - unpublished M.Sc. thesis) found that during hot periods there was an increase of the granular inclusions in the cells of the aerial algae he investigated. Regarding resistance to conditions other than drought, Strom (1924) stated that algae may survive the cold of winter in the vegetative state surrounded by thick mucilage coats, or with the cellular contents strongly

condensed and often filled with oil. There seems to be more or less general agreement that the presence of oil in algal cells will aid resistance to adverse conditions in general and drought in particular.

Fritsch and Haines (1923) have correlated the granular nature of the cells of a number of terrestrial algae with their resistance to plasmolysis; and Heide (1939), working with the fungus Endomyces vernalis, maintained maximum fat content in the cells by means of sodium sulphate, explaining that the presence of fat in some abundance was due in part to the osmotic effect of the increased sulphate ion.

D. von Denffer (1949) has carried out considerable experimental work with the diatom Nitzschia palea, and although finding that complete desiccation resulted in death of the cells, concluded that resistance to drying is dependent upon the formation of fat. Quantitative estimates of fat were made by Denffer, and presented as the percentage of the dry weight of the cells. It was shown that fat content increased as a population of cells aged, fat eventually almost filling each cell. Denffer also clearly demonstrated the reserve nature of such fat: cells containing fat showed growth when placed in double-distilled water in darkness, while fat-free cells showed no growth under the same conditions. A suggestion put

forward by this author may help to account for the greater resistance to drought of the cells containing fat. The danger of penetration of air into the frustule and the subsequent splitting of the silica shell is, he suggests, diminished by the replacement of the watery vacuoles in the cells by fat. However, his experiments on the revival of Nitzschia palea after experimental drying all gave negative results, and he concluded that this species is extremely sensitive to desiccation. Fogg (1953) noted that, regarding fat accumulation and its association with nitrogen deficiency, quantitative information was available only for Chlorella pyrenoidosa, a reduction in the concentration of ammonia or nitrate nitrogen being followed by fat accumulation. This may also occur in response to high light intensities or low concentration of free oxygen. Fogg's statement that cells storing extremely high proportions of lipide are not degenerate and will resume growth if transferred to a suitable medium agrees with the views of Denffer, as do most of the observations made and experiments carried out in the course of this part of the present investigation. The main purposes in view have been: 1. to determine if there is a direct relationship between fat accumulation and drought conditions in a number of algae; 2. to discover

whether algae which have accumulated considerable fat in their cells survive more or less severe drought; 3. whether they are, or are not, more likely to survive a drought than fat-free cells.

The following species have been investigated regarding the formation of oil in cells: Pinnularia viridis, P. appendiculata, Nitzschia palea, N. frustulum and Microspora stagnorum. With the exception of Nitzschia frustulum, which was obtained from the Cambridge culture collection, the species named above were found at one or more of the ponds described in Section II. Some notes are included on a number of species other than those named above.

B. Experimental Methods and Results and Other Observations.

Several methods for subjecting algae to artificial drought have been employed, the most usual being to allow litter and/or mud samples containing the algae to dry slowly in the air. For some experiments litter collected from pond margins has been autoclaved, re-wetted with sterile, glass-distilled water, inoculated with the algal species being investigated, and then allowed to dry. Drying has also been carried out in glass dishes and watch glasses, on filter paper, on agar and on sterile soil.

For estimating the changes in oil content of the yeast cells a simple counting and measuring technique was employed. This method, although its limitations are both fully realized, has been found to give quite satisfactory results, for comparative purposes, without recourse to elaborate bio-chemical techniques. From the drying, experimental cultures, samples were withdrawn from time to time, stained with Sudan Black, and a number of cells examined, the linear dimensions of cells and the diameter of oil globules being measured in microns. Slightly different methods were used for each species as follows:-

Pinnularia viridis. For each cell, the length and width, in girdle view, and the diameter of each oil globule observed were measured. The oil globules were placed in classes according to their diameter:- 1) 1 - 3 microns; 2) >3 - 6 microns; 3) >6 - 10 microns, and the values 1, 8 and 64, respectively, assigned to these categories to enable the oil globules to be considered on a volume basis. The sum of the values obtained for each cell is referred to as the "oil number". In order that cells of different sizes might be considered, a ratio R was calculated for each cell, this being the ratio of the "oil number" to the cell "area" (i.e. length x width in girdle view).

For more accurate results it would have been necessary to consider cell volumes. These could have been calculated by measuring the length of each cell and the widths, both in girdle and in valve view. This would have involved considerable practical difficulties as the cells observed, with few exceptions, tended to orientate themselves in girdle view. It has been found by measurement, however, that greater variations occur in the dimensions considered (i.e. length and width in girdle view) than occur in the widths of the cells in valve view. In comparing two samples of six cells each (one sample of relatively long cells, 90-172 μ , and the other of relatively short cells, 72-86 μ) it was found that the maximum variation in length was 60% and the maximum variation in width in girdle view was 52%. The maximum variation in width in valve view, however, was only 25% with a mean difference between the two samples of only 10%. So, for the purposes of the present investigation, the dimensions of the transapical axes have been ignored, and the above mentioned "area" has been taken as being related approximately to the volume of each cell by a constant. Cell lengths and widths were

To obtain more convenient figures the value R was multiplied in each case by 1000. For each sample, usually

of 40 cells, the mean cell dimensions, "oil number" and $R \times 1000$ values were calculated.

Pinnularia appendiculata. Cell dimensions were measured as above, but the method for estimating the "oil number" was modified slightly, the oil globules being considered in four categories according to their diameters in microns: 1) 0-2, 2) 2-4, 3) 4-6 and 4) 6-8, and the values 1, 27, 125 and 343 given respectively to these categories. As little variation in size of the cells of P.appendiculata was observed, mean "oil numbers" alone have been taken as the measure of the oil contents of the cells of each sample.

Nitzschia palea and N.frustulum. Cells were measured as above but oil globules were measured within finer limits.

In N.palea globules of 1, 2 and 3 microns diameter were listed and given the values 1, 8 and 27 respectively, and in N.frustulum, in addition to these, globules of 4 and 5 microns diameter were listed and given the values 64 and 125 respectively. The cells of each of these species were more or less uniform in size so, again, mean "oil numbers" only were considered for each sample.

Microspora stagnorum. Cell lengths and widths were measured and oil globules of 1, 2, 3 and 4 microns diameter recorded, giving them the values 1, 8, 27 and 64 respectively.

As cells varied considerably in length a ratio R was calculated for each cell, this being taken as the ratio of the "oil number" to the cell volume (calculated as the product of the length and πr^2). As for Pinnularia viridis the R value was multiplied by 1000. In the drying experiments, occasional estimates were made of the moisture contents of the substrate. The results, mainly in tabular form, are presented and considered below. In almost every case it has been possible to demonstrate an association between drying conditions and oil accumulation. It should be noted that the "oil numbers" and "R values" are merely comparative and have no quantitative significance.

Some of the results have been tested statistically (see Appendix 2B, p.204), and it has been found that the increase in R in the first experiment with Pinnularia viridis (Table 53) from the start of the experiment to the 9th day is highly significant.

Table 53. Pinnularia viridis; Experiment 1.

Time in days	0	3	6	9	12
Cells per sample	36	42	40	40	40
Mean cell "area"	3405	3023	3385	3841	3709 (microns ²)
" " oil numbers"	32.1	19.2	42.2	160.5	170.1
" R value	9.8	6.5	13.8	44.0	51.5

The apparent initial decrease in R during the first 3 days, however, has been found to have no statistical significance.

In the second experiment with P.viridis (Table 54), moisture estimations were made and there is some indication of an association between decreasing moisture and the accumulation of oil. The final decrease in R is not statistically significant. (See Appendix 2B)

Table 54. P.viridis: Experiment 2.

Time in days	0	4	8	11
% moisture	90%	85%	67%	14%
Cells per sample	21	28	40	40
Mean cell "area"	2895	2967	3551	2757
" "oil number"	39.3	35.3	178.4	90.5
" R value	16.5	18.6	44.9	30.1

In addition to making estimates of oil in cells of P.viridis dried artificially in the laboratory, certain samples collected from pond margins were also dealt with, and some interesting results obtained. Samples of litter were collected at various distances from, and heights above the water edge of the Stanmore Common pond in October 1953 and late June 1954, and the cells of P. viridis therein estimated for oil contents (Tables 55 and 56).

Table 55. Oil in P.viridis. October 1953.

Distance from water edge	5cm	15cm	1 metre
Height above water level	1cm	2.5cm	10cm
Cells per sample	4	6	7
Mean cell area	2347	2004	2178
" "oil number"	50	84	155
" R value	21.2	41.8	71.5

The results in table 55, although the samples are very small, suggest that there is an association between the distance of the cells from the free water and their oil contents. This is fully confirmed by the results, obtained the following year, shown in Table 56, the difference between the mean R value 5cm away from the water edge and the mean R value 1 metre away from the edge being highly significant.

Table 56. Oil in P.viridis. June 1954

Distance from water edge	5cm	15cm	60cm	1 met.
Height above water level	0.5cm	1cm	7.5cm	18cm
Cells per sample	23	15	25	4
Mean cell area	3515	3439	3344	2259
" "oil number"	103.3	225.0	308.5	343.0
" R value	31.5	65.5	92.0	142.5

Two weeks after the June 1954 collection was made, a further sample of 20 P.viridis cells from 5cm away from the water edge was estimated for oil, and the mean cell dimensions,

"oil number", and R value, obtained were found to compare closely with those of the earlier collection, being, respectively, 3546, 107.2 and 30.7. These results confirm the earlier figures and suggest that a sample of about 20 cells does give a reasonable estimate of the oil content of a population of P. viridis.

All attempts to induce normal growth after these experiments were unsuccessful with this species, which was probably due largely to the severity of the droughts induced artificially in the laboratory. Two living cells of this species were found in the course of the large-scale drying experiments (Section III) after long periods of exposure and drought. One, found on the mud of the Englefield Green pond when the moisture content was 4.5% had a ratio of "oil number" to "cell area" (R value) of 33, and became motile in water. The other, found on the litter of the Stanmore Common pond after 3 months drying, had an R value of 46 and also became motile in water. But neither of these cells was induced to commence growth (i.e. cell division).

Single cells of Pinnularia appendiculata, obtained from the Stanmore Common pond, were isolated and placed on $1\frac{1}{2}\%$ Beijerinck agar, so producing clone cultures for

experimentation. The mean "oil number" showed a significant increase initially upon drying (Table 57) and, upon continued drying, a significant decrease.

Table 57. Pinnularia appendiculata. Experiment 1.

Cells dried on sterile litter.

% moisture	66%	56%	47%
Cells per sample	40	40	25
Mean "oil number"	106.9	155.9	1.6

D. von Denffer showed (1949) that fat accumulation occurred in populations of Nitzschia palea as they aged, and to allow for this possibility in the drying experiments control samples were taken from the agar plate culture parallel with the experimental samples. No significant change in the mean "oil number" occurred in the cells of P. appendiculata on agar during the duration of the experiment the control range of "oil numbers" being 87.4-119.3.

In the last sample of the drying experiment, 16 of the 25 cells observed contained no visible oil globules, while the remaining cells contained very little oil. These dried cells continued growth upon replacement on agar, all the cells becoming free of oil. This suggests the reserve nature of the accumulated oil.

Cells of Pinnularia appendiculata dried more rapidly on filter paper, on small pieces of agar and on glass slides, showed no accumulation of oil, and failed to continue growth upon re-availability of moisture (i.e. when replaced on agar).

During the course of the oil accumulation experiments some investigation was made of other storage products, and, in the case of P. appendiculata, staining with Methylene Blue often revealed small, but quite prominent, violet-red granules, which are assumed to be volutin. These granules were usually considerably more dense in the polar regions of the cells (Figure 23A) and may be associated with the oil globules, being found in one cell almost completely surrounding an oil globule. (Figure 23B).

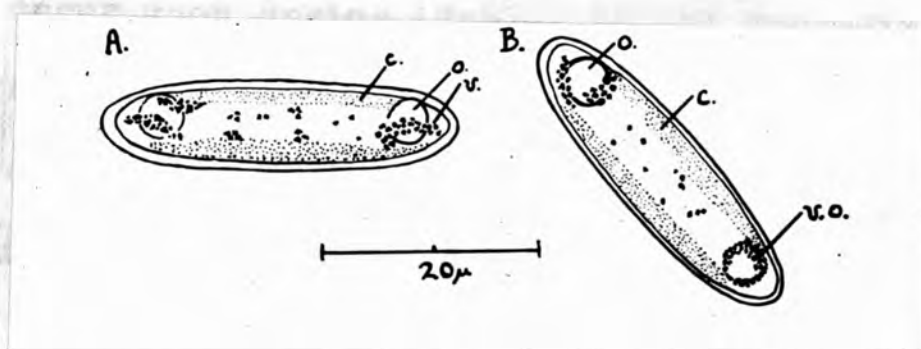


Figure 23. Pinnularia appendiculata, cells stained with methylene blue. A. Cell showing granules, v, which are assumed to be volutin, concentrated at the polar regions of the cell, near the oil globules, o. B. Cell showing, at one pole, granules almost completely surrounding an oil globule, v.o. c = chromatophore.

This may be comparable with the globules described for Navicula oblonga by Kolbe (referred to by Fritsch (1935, page 599). Due to the relatively small quantities of volutin found in the cells it has not been possible to make any quantitative estimates of it, as for the oil. It should, however, be noted that volutin, tested for only by using methylene blue, has usually been found in cells containing relatively moderate quantities of oil, and never in cells with large oil globules (more than 4 microns in diameter). This suggests that, in this species, volutin is used up by the cell as oil is formed.

In one of the two species of Nitzschia investigated, N. frustulum, a significant accumulation of oil has been found to occur upon drying (Tables 58 and Appendix 2B p. 206) and the relationship between decreasing moisture and increasing oil in this species is shown in Figure 24. As for Pinnularia appendiculata, control samples were also estimated for oil during the drying experiment. The minimum "oil number" obtained from the control samples was 26.2 and the maximum 34.8. In Figure 24, the control range has been extended to the left to include the minimum "oil number" obtained from the drying experiment.

Table 58. Nitzschia frustulum: cells dried on litter.

% moisture	71%	67%	64%	61%
Cells per sample	40	40	40	40
Mean "oil number"	14.7	36.8	53.8	69.4

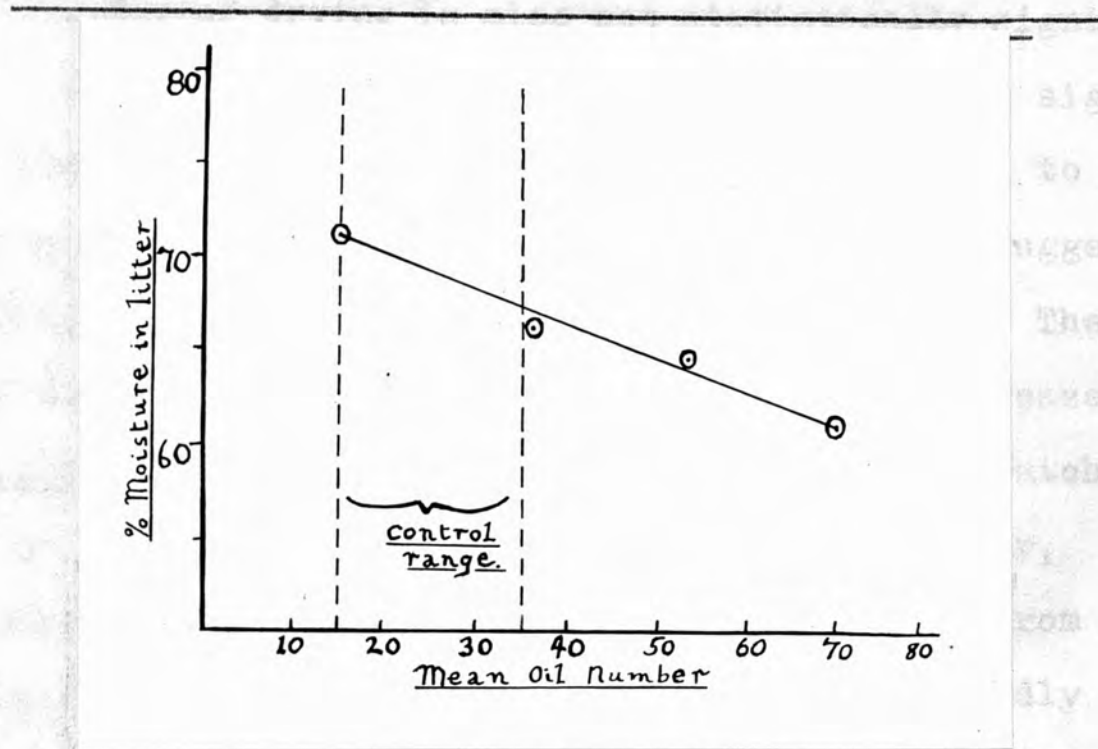


Figure 24. The accumulation of oil in Nitzschia frustulum during drying. The control range was actually 26.2 - 34.8 but this has been extended to the left, in the diagram, to include the minimum (first sample) oil number calculated for the cells dried on litter.

Table 59. Nitzschia palea: cells dried on litter.

Time in days	0	2	5	7	10
% moisture			90%	84%	17%
Cells per sample	40	40	40	40	36
Mean "oil number"	2.25	3.36	5.02	6.02	4.6

In Nitzschia palea, the total increase in the mean "oil number" during the first 7 days of drying (Table 59) has not been found statistically significant.

The decrease in the mean "oil number" from the 7th to the 10th day of drying is also not statistically significant. Upon immersion in soil solution, however, a significant decrease did occur, to 0.97 after two days and to 0.22 after five days (40 cells per sample in each case), suggesting, again, the possible reserve nature of the oil. The quantity of oil present in cells also eventually decreased, in Nitzschia frustulum, upon severe drying in waxed watch glasses or prolonged drying (6 months or more) on agar, oil globules disappearing completely, or almost so, from the cells. In 14 of a sample of 25 cells dried rapidly in a waxed watch glass, and in 28 of a sample of 40 cells dried for a prolonged period on agar, oil globules were completely absent or represented (and then in only two cells of those observed) by two very small globules (no more than 1 micron in diameter). When stained with Sudan Black, a thin peripheral region of the protoplast in these cells showed as a prominent, dark line (Figure 25). If this is a layer of fatty material, then it might provide protection against loss of water by the cells and would suggest that, in Nitzschia frustulum at least, fat plays

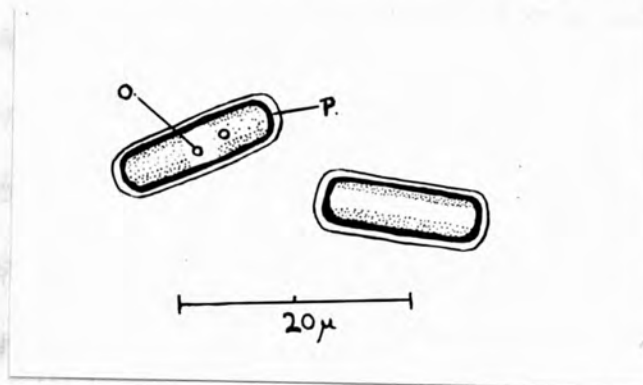


Figure 25. Nitzschia frustulum, cells stained with sudan black. p, dark-stained peripheral region; o, small oil globule.

This is reminiscent of the peripheral layer of fat globules described by Fritsch (1916) in the cells of Zygonium ericetorum subjected to gradual desiccation. It is possible that the peripheral layer observed in Nitzschia frustulum is a layer of fat comparable with that in the cells of Zygonium ericetorum, but despite careful observations it was not possible to detect a granular nature to this layer in the diatom. Alternatively, this peripheral region may have been a layer of cutin. If this is a layer of fatty material, then it might provide protection against loss of water by the cells and would suggest that, in Nitzschia frustulum at least, fat plays

a fundamental part in the survival of cells during very dry conditions. A far more intensive examination of this aspect of the problem than was possible in the course of the present investigation would help, it is felt, to reveal the solutions to many of the questions relating to survival of aquatic algae in general, and diatoms in particular, during dry periods.

As with Pinnularia appendiculata, dried cells of the two Nitzschia species investigated recovered upon re-wetting with soil solution or upon agar. Due, however, to the difficulty of manually isolating, without damaging, such small cells, it was impossible to distinguish between living and possibly moribund cells of N. frustulum. It is not known, therefore, which individual cells survived of those containing much oil and those containing little or no oil but possessing what might have been a peripheral fat layer. Again, cells dried rapidly showed no accumulation of fat and no recovery upon re-wetting.

Table 60 shows the changes of mean cell volumes, mean "oil numbers" and mean R values (ratio, of "oil number" to cell volume, $\times 10^3$) in samples of Microspora stagnorum, with changes of moisture content of the substrate (sterile soil in a petri dish).

The apparent initial decrease of oil is not statistically significant, but the increase from the 8th to the 12th day is significant (see Appendix 2B, P. 206). The marked increase from the 15th to the 94th day, during the severe and prolonged drought, is highly significant, as is the decrease of oil that occurs during the 9 days after re-wetting with soil solution on the 94th day. An interesting feature of the second period, the 103rd to the 119th day, is the more rapid accumulation of oil in the cells during drying which, though possibly due to different laboratory conditions, may have been due to a change in the metabolism of the cells induced by the previous long drought.

A few plasmolysis experiments were carried out with *Microspora stagnorum* similar to those of Fritsch and Haines (1923). It was found that filaments collected in water samples from ponds, or taken from soil solution culture, showed a uniform reaction of the cells to solutions of Tidman's sea salt. Filaments which had undergone prolonged experimental drying, however, did not show a uniform reaction of the cells. They behaved instead as did the terrestrial algae investigated by Fritsch and Haines (1923). Counting approximately 1,000 cells within 10 minutes, in each experiment, it was found that a 1.6% solution of

Table 60.

Microspora stagnorum, changes in mean cell volumes, "oil numbers" and "R values" of the cells of filaments dried, re-wetted with soil solution and dried again on sterile soil.

Sample No.	1	2	3	4	5	6	7	8	9
Total time in days.	6	8	12	15	94	103	109	112	119
% moisture.	54	26	22	22	2	65	33	30	24
Mean cell volume in microns ³ .	633	637	627	536	539	775	772	581	332
Mean "oil No."	1.25	0.70	3.50	1.72	7.05	1.50	3.80	8.80	4.30
Mean "R value"	1.73	1.08	5.70	3.20	13.20	1.88	4.76	14.70	14.88
	First drying.				Re-wet.		Second drying.		

sea salt, which strongly plasmolysed 65-75% of the cells of filaments kept in liquid, slightly plasmolysed only 1.5% of the cells of filaments which had undergone prolonged drought, leaving the remainder unaffected. A 4% solution of sea salt left 50% of the cells of the drought material unaffected, strongly plasmolysing only 16.5% of them, while even an 8% solution left a few cells, always those containing the most oil, unplasmolysed. Within a week, drought material returned to soil solution culture showed once again a more or less uniform reaction of the cells to plasmolysis, 65-75% of a sample of about 1,000 cells, all containing few or no oil globules, becoming strongly plasmolysed within 10 minutes in a 1.6% solution of sea salt. The change in osmotic concentration which appears in this species to be associated with the oil content of the cells, may help to account for the survival of Microspora stagnorum, and possibly also the related species M. floccosa, during prolonged, and at times severe, drought (Section III). It may also be associated with the prolonged existence of vegetative stages on exposed litter deposits in nature (Section II).

Testing for the presence of starch in the cells of Microspora stagnorum, with iodine in potassium iodide,

revealed such small quantities, relative to the amounts of oil present in the cells of drought material, that it was not considered worth while to attempt quantitative estimates. Once only were large starch grains found in the cells which had, at some time, undergone prolonged experimental drought, and these cells, which had been re-wetted with Beijerinck 0.05% solution 3 days before the starch test was made, were found, subsequently, to be dead or dying.

Accumulated oil has been found in the cells of several species of algae in nature (Section II) and in drying experiments (Section III). In nature it has been found to occur in response, at times, to conditions other than those of drought. Filaments of Microspora floccosa, for instance, were found in the water of the Stanmore Common pond, and the Rowley Green "acid" pond (Section II), in mid-May 1955, with cells containing large oil globules. The oil may possibly have been formed in response to the increase in the water temperature that occurred through April and early May after a rather prolonged cold period lasting from early January.

of fat in the cells of Nitzschia frustulum subjected to drought.

5. With the exception of Pinnularia viridis, all the species investigated recovered from drying provided

C. Summary.

1. The following species of algae have been dried in the laboratory and in every case it was possible to demonstrate, using a simple counting and measuring technique, that oil accumulation occurred in the cells in relation to decreasing moisture: Pinnularia viridis, P. appendiculata, Nitzschia frustulum and Microspora stagnorum. That Nitzschia palea accumulated oil during drying can only be concluded indirectly from the observation that oil present in dried cells underwent a significant decrease upon re-immersion in liquid (soil solution).
2. It was found that, in nature, the amount of oil present in cells of Pinnularia viridis corresponded with the distance of the cells away from the edge of the water and the height above it.
3. In Pinnularia appendiculata, there appears to be some association between the oil globules and volutin granules, the quantitative relationship, if such exists, possibly being a reciprocal one (see p.192).
4. There is some evidence to suggest the existence of a thin, peripheral layer of fat in the cells of Nitzschia frustulum subjected to drought.
5. With the exception of Pinnularia viridis, all the species investigated recovered from drying provided the

cells ^{had} ~~has~~ formed oil initially.

6. Prolonged drying may result in the eventual loss of accumulated oil. This suggests the reserve nature of the oil.

7. Rapid drying did not result in any observable accumulation of oil and always resulted in death of the cells.

8. In Microspora stagnorum, a higher osmotic concentration as well as oil accumulation appears to be associated with exposure of cells to drought, and experimental results suggest that both occurrences are reversible.

9. Only relatively small amounts of starch have been found in the dried cells of Microspora stagnorum.

10. Oil accumulation may occur in response to conditions other than drought.

Sample number

Number of
cells per
sample

The mean results were compared with the following table and consulting Table III of Yates, 1943 Table III, the results of significance obtained.

APPENDIX 2.

Statistical Analysis of Results.

A. Micro-sampling Technique.

Pinnularia viridis.

The Tables 40a and 40b (p. 130) were re-arranged as follows:-

Table 40a.

Favourable conditions for cells to be at surface (i.e. damp, cool)								Unfavourable conditions (i.e. dry or raining)									
Sample number	23	24	26	27	Σ	n	\bar{x}	19	20	21	22	25	28	Σ	n	\bar{x}	
Stratum																	
Number of cells per sample	1	3	1	1	3	8	4	2	2	2	1	1	0	1	7	6	1.17
	2	1	1	1	1	4	4	1	1	3	2	5	0	2	13	6	2.2
	3	1	1	0	1	3	4	0.75	5	1	3	2	4	4	19	6	3.2

Table 40b.

Favourable conditions for cells to be at surface (i.e. damp, cool)								Unfavourable conditions (i.e. dry or raining)							
Sample number	53	53a	55	56	Σ	n	\bar{x}	50	51	52	54	Σ	n	\bar{x}	
Stratum															
Number of cells per sample	1	3	3	2	4	12	4	3	0	1	2	0	3	4	0.75
	2	2	1	0	1	4	4	1	2	0	4	0	6	4	1.5
	3	0	0	0	0	0	4	0	0	1	2	3	6	4	1.5

Stratum 1 in favourable and in unfavourable conditions. The mean results were compared, and, by calculating the following terms and consulting Tables of t (Fisher and Yates, 1943 Table III), the levels of significance

obtained. There is an obvious difference between strata 1 and 3 in conditions. The figure given here (2.0%) is the significance level corresponding to the comparison between strata 1 and 2.

$$\bar{x} = \frac{\sum(x)}{n_1}; \quad \bar{x}' = \frac{\sum(x')}{n_2}$$

$$\sigma^2 = \left[\frac{\sum(x'^2)}{n_2} - \frac{(\sum(x'))^2}{n_2} + \frac{\sum(x^2)}{n_1} - \frac{(\sum(x))^2}{n_1} \right] / (n_1 + n_2 - 2)$$

$$t = \frac{\bar{x} - \bar{x}'}{\sigma} \sqrt{\frac{n_1 \times n_2}{n_1 + n_2}}$$

Summarised below are the significance levels corresponding to the calculated values of t obtained in comparing a number of the means in the tables on the previous page. Where a difference is considered to be significant the figure for significance level is underlined.

Microstratification of Pinnularia viridis cells.

<u>Means compared</u>	<u>Level of significance.</u>	
	<u>Table 40a (1953)</u>	<u>Table 40b (1954)</u>
Strata 1 and 3 in favourable conditions	10.0%	* <u>2.0%</u>
Strata 1 and 3 in unfavourable conditions	<u>2.0%</u>	40.0%
Stratum 1 in favourable and in unfavourable conditions.	30.0%	<u>2.0%</u>
Stratum 3 in favourable and in unfavourable conditions.	<u>1.0%</u>	10.0%

* There is an obvious difference between strata 1 and 3 in Table 40b. The figure given here (2.0%) is the significance level corresponding to the comparison between strata 1 and 2.

Levels of Significance.

Total flagellates.

Table 41 (p. 131) was re-arranged as follows:-

Experiment (1)									Experiment (2)						
Favourable conditions for cells to be at surface (i.e. damp, cool)									Unfavourable conditions (i.e. dry or raining)						
Sample number	53	53a	55	56	Σ	n	\bar{x}		50	51	52	54	Σ	n	\bar{x}
Stratum															
Number of cells per sample	1	4	5	2	5	16	4	4	1	1	5	1	8	4	2
	2	0	2	0	3	5	4	1.25	2	0	5	0	7	4	1.75
	3	0	1	0	1	2	4	0.5	0	2	2	0	4	4	1

Analysing these results as described above, it was

found that the level of significance corresponding to the value of t obtained in comparing the means of strata 1 and 3 in favourable conditions is 1.0%. No significant difference could be found between any other means.

B. The Accumulation of oil.

The mean values obtained for each species ("oil numbers" or "R values") during the course of the experiments, were compared with one another, as described above (p. 202). Using the same terms, t values were calculated and, by reference to the table of t, levels of significance obtained. These are tabulated below for each species discussed. The minimum oil number in the control experiment was calculated to be significant only to the 10% level.

Levels of Significance.

Pinnularia viridis.

<u>Experiment (1)</u>				<u>Experiment (2)</u>				
Sample No.		2	3	4	Sample No.		3	4
	Mean R value	6.5	13.8	44.0		Mean R value	44.9	30.1
1	9.8	20%	20%	0.1%	1	16.5	2%	20%
4	44.4	0.1%	1%	-	3	44.9	-	20%

Samples collected from the margin of the Stanmore Common pond,
June 1954.

Distance above the water level		1cm.	10cm.	15cm.
	Mean R value	65.5	92.0	142.5
0	31.5	1%	1%	0.1%
1cm.	65.5	-	5%	0.1%
10cm.	92.0		-	0.1%

P. appendiculata.

Sample No.		2	3	4
	Mean Oil No.	155.9	371.6	1.6
1	106.9	30%	0.1%	0.1%
2	155.9	-	0.1%	0.1%
3	371.6		-	0.1%

N.B. The difference between the samples of maximum and minimum oil number in the control experiment was calculated to be significant only to the 10% level.

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N. frustulum. (1927). 'Determining the moisture equivalent of

Sample No.	Oil No.	1	2	3	4
1	14.7	-	0.1%	0.1%	0.1%
2	36.8	-	-	1.0%	0.1%
3	53.8	-	-	-	10%

In the control experiment, the minimum oil number obtained was 26.2 and the maximum 34.8. The difference between these means was calculated to be significant only to the 30% level.

Nitzschia palea.

Sample No.	Oil No.	2	3	4	5	6	7
1	2.25	60%	30%	10%	-	-	-
4	6.02	-	-	-	50%	0.1%	0.1%
6	0.97	-	-	-	1.0%	-	10%

Microspora stagnorum. (1953).

Sample No.	Mean "R value"	2	3	4	5	6	7	8
1	1.73	30%	5%	-	-	-	-	-
2	1.08	-	2%	-	-	-	-	-
3	5.70	-	-	30%	1.0%	-	-	-
4	3.20	-	-	-	0.1%	-	-	-
5	13.20	-	-	-	-	0.1%	-	-
6	1.88	-	-	-	-	-	1.0%	-
7	4.76	-	-	-	-	-	-	0.1%

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ERRATA

Page 5, line 20: for Figure 16 read Figure 13

Page 23, line 10: for somewhat read somewhere

Page 43, line 17: for 22 read 23

Page 46, line 14: for Twelve read Eleven

Page 55, line 3: for 5 read 4

Page 64, line 18: for x975 read x875

Page 93, line 11: for page 93 read page 109

Page 201, line 1: for has read had