A STUDY OF THE DISTRIBUTION OF CERTAIN MEMBERS OF THE SAPROLEGNIALES

Abstract

The distribution of members of the Saprolegniales in twenty two natural waters,ranging in pH. from 3.6 to 8.0, throughout the United Kingdom during the period October 1956 to April 1958 is described. The method of isolation and the standardised sampling technique are fully explained. The twenty seven species collected are shown to be distributed according to the hydrogen ion concentration of the waters in which they occur. It is possible to arrange them in three main groups. There is a distinct seasonal variation in occurence of the species in those waters which were sampled at regular intervals and it is possible to distinguish "summer" and "winter" species. The results of a study on growth and reproduction of certain selected species in pure culture and their bearing on the distribution pattern is discussed.

C O N T E N T S

INTRODUCTION

The Saprolegniales are mainly saprophytic fungi able to grow on substrata as widely different as twigs, fruits, seeds, insect cadavera and boiled egg white. Some members of the order are facultative parasites; for example Tiffney (1939) listed Saprolegnia ferax, Saprolegnia parasitica and Achlya flagellata as parasites of fish, amphibians and reptiles. In the genus Aphanomyces there are many obligate parasites; some, like A. raphii, cause root rots of higher plants; others parasitise algae and A.parasiticus is an endoparasite of Achlya. Extrogella is a genus composed entirely of species which are obligate parasites of algae.

Originally it was believed that members of the Saprolegniales were exclusively aquatic, but the work of Harvey (1925) showed that they are also found in soils. They have been isolated in many countries and since the 1920's lists of species found in Great Britain, Bohemia, Bulgaria, Denmark, Germnay, Ukraine, India, China, Japan, Australia and North America have been published.

It is with the predominantly saprophytic members of the order that this investigation is concerned. They are readily isolated because their zoospores will colonise a great variety of bait. They can be "caught" by leaving the bait in the water in which they occur, or by baiting samples of this water. Despite the simplicity of this technique and the fact that it has been known for a considerable time, it is surprising how haphazard the collecting has been and up to the present little ecolgical.has been carried out. This investigation attempts to compare the distribution of saprophytic members of the Saprolegniales in natural waters of different hydrogen ion concentration varying from acid bogs to constantly alkaline lakes. It has not been possible to study the whole of the Saprolegniaceious flora of the habitats selected for the following reasons:-

(1) It was not practicable to use more than one kind of bait.

(2) The method of sampling depends on the colonisation of the hemp seed bait used by the zoospores of the fungus and it does not give any information about the occurrence of the fungus in the vegetative condition. The mycelium may be living in the water but unless the zoospores are produced, its presence may not be detected.

(3) If after colonisation of the bait the fungus does not produce oogonia, identification is usually impossible. Some species may be unrecorded for this reason.

The information obtained using the baiting technique relates only to the water moulds which produce zoospores freely and which form oogonia when growing on hempseeds. However, by standardising the methods of sampling and sampling at regular intervals throughout the year, it is possible to compare the seasonal distribution of these fungi in waters of different hydrogen ion concentrations.

Since the method of sampling depends on the presence of zoospores, some attempt has been made to discover the importance of certain enviromental factors on zoospore production. In Part II of this thesis, work on the growth and reproduction of certain species in pure culture is recorded. The influence of such factors as temperature and pH; and of calcium, phosphate and carbonate ions on these species has been recorded.

Historical Review

A review of the literature shows that since the time of Ledermüller (1760) attention has been paid to the taxonomy, cytology, and latterly, the physiology of the aquatic phycomycetes. From the end of last century species lists for many countries have been published. The first British list was Massee's (1891) and comprised eight species. A year later, Humphrey (1892) in North America, published a monograph on the Saprolegniaceae which included all existing North American records of these fungi. Sparrow (1923), Coker (1927) and Couch (1931) have also been pioneers in the study of the Saprolegniales in North America. Ivimey-Cook (1933,1936), Forbes (1935), Sparrow (1936) and Morgan (1938,1939) have contributed towards knowledge of the group in Great Britain. In other countries, lists of species have been published by various authors.

There is however, little published work on the influence of enviromental factors on these fungi. In particular, there is only one comprehensive paper on the effect of pH. on distribution, viz; that of Lund (1934) which lists the aquatic phycomycetes found in waters of differenct pH. in Denmark. He divided the waters sampled there into five groups:-

1. Highly acid (pH 3.5 to 4.0)
2. Slightly acid (pH 5.3 to 6.8)
3. Neutrally acid (pH 5.2 to 7.5)
4. Neutrally alkaline (pH 6.5 to 7.7)
5. Constantly alkaline (pH 7.6 to 8.4)

He found that some of his species were common to several of the pH. groups, as is indeed to be expected with the wide and loose classification which he adopted for his groups; nevertheless some species do appear to be tolerant to a limited pH. range and some were characteristic of a particular group.

The species characteristic of highly acid and constantly alkaline waters are:-

Highly acid waters (pH. 3.5 to 4.0)	Constantly alkaline waters (pH 7.6 to 8.4)
Saprolegnia diclina	Saprolegnia ferax
S. delica	S. hypogyna
S. monoica var. montana	S. mixta
S. litoralis	Achlya colorata
S. latviaca	A. oligocantha
S. torulosa	A. racemosa
S. variabilis	A. radiosa
Aplanes androgynous	Aphanomyces laevis
Achlya americana	Aph. stellatis
A. caroliniana	Dictyuchus magnusii
A. treasleana	D. monosporus
	Isoachlya parasitica
	Pythiopsis humphreyana

Lund's lists are full and contain a record of the species collected in each habitat by a variety of direct and indirect methods. Although they form a valuable record of the distribution of the species in the various habitats, the collecting was haphazard in character. No attempt was made to sample régularly and he gives no record of seasonal variation.

In 1941, Wolf and Wolf published a list of watermoulds found in California, in a cypress swamp of pH. 7.6. The following eight members of the Saprolegniales were included in this list.

Achlya americana
A. conspicua
A. flagellata
A. proliferoides
Aphanomyces laevis
Dictyuchus monosporus
Saprolegnia delica
S. ferax.

Three of these species, Achlya flagellata, Dictyuchus monosporus and Saprolegnia ferax occur in Lund's list for "neutrally alkaline" waters. Aphanomyces laevis and Achlya americana were found in Lund's "constantly alkaline" waters and three species were not recorded by Lund.

The literature relating to seasonal variation is somewhat more extensive than that concerning distribution in relation to pH. (see Peterson (1910), Coker (1923), Forbes (1935), Morgan (1939) and Goldie-Smith (1948).

Peterson (1910) was the pioneer in the work on seasonal distribution. He showed that in Denmark the Saprolegniales are generally found from Spring until November and not during the Winter. He attributed their absence during the Winter to the fact that the habitats were frozen.

Coker (1923) in the introduction to his monograph gives the results of regular collections taken during 1912 around Chapel Hill, Carolina. He concluded that there was some seasonal variation in distribution since although most of the species he recorded occurred all the year round, some were found more abundantly in the cold season and others in the warm season. The four following species:-

Achlya racemosa

Isoachlya monilifera

Leptomitus lacteus

Pythiopsis cymosa

were found only in the Winter and the Spring. There were no species found SOLELY in the Summer. The greatest number of species were collected in the Winter and the Spring, eighteen species being found during both these seasons. Only eight species were found in the Summer and ten in the Autumn. By calculating the percentage abundance of every species each month, Coker was able to show that most species occurred more frequently in the Spring. One, Saprolegnia monoica was more abundant in the Winter. Four species, Dictyuchus sterile, Leptolegnia caudata, Pythiopsis humphreyana and Saprolegnia diclina were found in greater numbers in the Summer and two species, Achlya hypogyna and Aphanomyces spp. in the Autumn.

Forbes (1935) observed a "marked periodic variation in abundance" in species collected from ponds around Manchester. Four of the species studied, Achlya racemosa, Achlya radiosa, Leptomitus lacteus and Saprolegnia monoica showed their maximum frequency of abundance in the Spring and Saprolegnia ferax in the Autumn. These results agree with Coker's findings (1913) and with Allen's unpublished work in Bristol between 1932 and 1933 (see Forbes 1935). Unfortunately, neither, neither Forbes' nor Allen's work is complete as sampling did not take place during the Summer months. Morgan (1939) also found seasonal variation occurred but gave no details of the species concerned.

Goldie-Smith (M.Sc. thesis, University of London 1948) indicated that although her work was not conclusive it did show that at certain periods of the year some species of the Saprolegniales were more abundant. Her observations on *Saprolegnia ferax* and *Saprolegnia monoica* agree with those of Forbes.

MATERIALS AND METHODS

Methods of Sampling.

Two methods of sampling, one direct and the other indirect, were used. Since the direct method of sampling was unsuitable for routine examinations of the water, the indirect one was devised to enable quantitative results to be obtained.

In the direct method, surface sterilised fruits of hawthorn and tomato were submerged in tubes in some of the sites. This method was tried for only six months because although it proved excellent for collecting Pythium species, the only members of the Saprolegniales caught in this way were those that were extremely abundant in samples collected by the method described below in which many more species were collected.

In the direct method, a water sample was removed from the site and baited with hemp seed. The method of sampling and baiting was standardised so that the results from the various habitats could be compared. Preliminary investigations showed that the number of species "caught" on the bait at any one sampling did not vary according to the volume of water sampled, the same number of species being obtained in sampling jars ranging in capacity from 25 ml. to 950 ml. It was found that the number of species obtained during a sample did vary according to the depth from which the water sample was taken. More species were collected from the bottom than the surface waters of the pond or lake. For example, baiting water from the South Pond in the Royal Holloway College grounds resulted in six species being obtained from a water sample from the surface and eleven from the bottom. This can be regarded as fairly typical of what happened generally.

The standardised procedure adopted at each sampling was as follows:-

The sample was always taken at the same place at the margin of the water where the depth of water was four to five inches. Five similar collecting jars were used at each sampling. These were of 250 ml. capacity and had wide necks and screwtop lids. The jars were inverted into the water until just above the floor of the pond before being filled. Each sample was baited with ten sterile half hemp seeds as soon as possible after this and never more than six hours after being filled. Each half hemp seed was suspended just below the surface of the water on a sterile glass hook. The collecting jars were kept in the dark for twentyfour hours in the laboratory. (It had been found that there was no increase in the number of species colonising the bait after twentytwo hours).

After this period, the colonised seeds were transferred to sterile distilled water in crystalising dishes - five seeds to each dish. The depth of water was about $\frac{1}{4}$ inch and contained crystalline penicillin the concentration 2000 units/litre water to depress bacterial growth. Thus ten dishes, each containing five half hemp seeds were obtained from any one sample. Half of these were placed in an incubator at 22°C and the other half in a refrigerator at 5°C. These two temperatures were selected because earlier experiments had shown that some species develop their oogonia more freely at the lower temperature. The seeds were examined once a week for five weeks, the water being changed after each examination. It was found that some species did not develop oogonia until the fifth week, there being a succession of development on the seeds. In recording the frequency of isolation of any one species, the two series were treated separately so that the maximum number of possible isolations for a species was twentyfive.

Experiments were made using artificial bait in place of hemp seed in the hope that more standard conditions might thereby be introduced. Agar blocks containing different concentrations of a water extract of hemp seeds were substituted for the half hemp seeds but lack of time prevented a perfecting of this technique and it was abandoned in favour of natural bait.

Media

The medium used to obtain species in culture contained:-

1.5 g. maltose
0.4 g. peptone
20.0 g. agar
1000 ml distilled water.

The purified cultures were maintained in the laboratory on potato extract agar except for Achlya polyandra, Achlya treasleana and Saprolegnia monoica which were kept on Quaker Oat agar slopes.

Stock cultures for use as inocula in the physiological experiments were grown in the above maltose - peptone agar at 22°C.

Water analysis

The hydrogen-ion concentration and the temperature of the water were noted each time samples of water were taken. The calcium, magnesium and phosphate concentration and hardness of the water of the habitats sampled regularly was estimated from time to time.

Hydrogen ion concentration

A Lovibond comparameter was used to determine pH. If the site sampled gave an unexpected result with the Lovibond comparameter, such as did the water of the South pond in April 1957, the results were checked with a Cambridge pH. meter.

Hardness

Total hardness was estimated by titrating 20 ml. portions of the water against 1% potassium palmatate solution. It was considered that the titration was complete when the lather on the sample lasted five minutes with the flask lying on its side.

Permament hardness was estimated by titrating 20 ml. portions of 100 ml. water, which had been boiled for one hour and made up to 100 ml. again with distilled mater, against 1% potassium palmatate solution, the end point being judged in the same way as with total hardness.

Temporary hardness was calculated by deducting the permament hardness from the total hardness.

Calcium, Magnesium and Phosphate content

The procedure was carried out as described by Thresh, Beale and Suckling (1949). In the case of magnesium, the amount of precipitate of magnesium phosphate was estimated by means of a diffraction spectrophotometer. In Deniges method of phosphate determination, the degree of blue coloration was also estimated by the diffraction spectrophotometer but relatively few phosphate determinations were made.

DESCRIPTION OF HABITATS

The areas of waters sampled were selected so that they were as widely different as possible. They were situated is various parts of Britain and included lakes like Loch Morlich (Invernesshire), Malham Tarn and the Winterburn reservoir (Yorkshire), Llyn Cwellyn (Caernavonshire) and Virginia Water (Surrey); moorland pools and bogs, lowland heaths as well as an artificial pond in Surrey. The hydrogen ion concentration of these waters ranged from 3.6 to 8.0. Some of these waters, as is indicated below, were sampled at regular and fairly frequent intervals, others less frequently and sometimes sporadically.

Sites sampled regularly

The following five habitats were sampled every two months with the exception of South Pond II, Royal Holloway College grounds and Chobham Common bog which were sampled every month.

Malham Tarn, Yorkshire

Grid ref:	34/894667
Height above sea level:	1250 ft.
pH. range:	7.8 to 8.6
Calcium content:	83 to 102 parts/million
Magnesium content:	13 to 15 parts/million

Malham Tarn is a shallow lake on ordovician shale, roughly half a mile square and up to twelve feet deep. A peat bank, six feet hight, rises from the western side of the tarn, while the other three sides are bordered by grassland. The land on the north side is basic and on the east and south sides acidic. The stream feeding the tarn drains the neighbouring limestone area and its water is alkaline.

The bottom of the tarn is rocky. Apart from two areas of vegetation where silting occurs, the bottom is composed of calcareous boulders of different sizes. Towards the middle of the tarn extensive Chara beds are found. In the shallow areas the boulders are colonised by Fontinalis antipyretica and algae like Cladophora and Chaetophora.

The tarn has been sampled in many places both around the edge and elsewhere at various depths. Site 5a was the only place sampled at regular intervals. Two tree trunks lay in the water here which enabled sampling to take place about a foot out from the water's edge at a depth of five inches.

South Pond II, Royal Holloway College grounds, Englefield Green, Surrey.

Grid ref:	41/998705
Height above sea level:	150 ft.
pH. range:	7.6 to 5.0
Calcium content:	115 to 137 parts/million
Magnesium content:	95 to 100 parts/million

South Pond II is a small artificial pond in the wooded grounds of Royal College, Englefield Green, Surrey. Water drains into this pond from South Pond I. Near the point of entry of the water the pond is shallow. The site sampled monthly was towards the other end and here the water is deeper and even at the edge is eighteen inches in depth. The pond is surrounded by Rhododendrons but around the pond's edge Dryopteris filix-mas and Equisetum sp. grew.

When the pond was first sampled, from October 1956 to April 1957, the pH. was regularly 7.6. In April 1957, the pH. dropped suddenly to 5.6, probably as the result of a change in the drainage. The pH. remained hereafter between 5.0 and 5.8.

Molinetium Pool (Site 1b) Yorkshire

Grid ref:	34/889664
Height above sea level:	1250 ft.
pH. range:	4.6 to 5.8
Calcium content:	26 to 35 parts/million
Magnesium content:	60 to 67 parts/million

The roughly circular pool, five feet in diameter and about a foot deep in the centre, is situated in acid pastureland dominated by Molinia caerulea at the south end of Malham Tarn. The source of water for this pool is mainly rainfall, but a certain amount is surface drainage from the adjacent pastureland.

The bottom of the pool consists of fine silt in which Glyceria fluitans grows. At one end of the pool a clump of Juncus squarrosus is found. The following vegetation grows in the water:-

Glyceria fluitans
Juncus squarrosus
Carex nigra.

Chobhan Common Bog, Surrey

Grid ref:	41/969661
Height above sea level:	150 ft.
pH. range:	4.8 to 5.2
Calcium content:	8 to 22 parts/million
Magnesium content:	11 to 14 parts/million

This valley bog, on Bagshott sands, is shallow and, although keeping its character, is tending to dry up. It lies between two low hills which are which are dominated by Calluna vulgaris and Ulex minor. A stream, formed from the drainage water from the hills, runs through the centre of the bog. A carr, composed of Birch and Willow with occasional saplings of Scots pine, has grown up around the stream. On either side of the carr, a sphagnum bog covers the valley bottom. Sphagnum cuspidatum and Polytrichum commune dominate the wetter parts while Sphagnum magellanicum is very common in the dryer regions further up the hillside. The following species were found among this moss:

Eriophorum angustifolium
Juncus articulatis
Molinea caerulea
Eleocharis palustris
Drosera rotundifolia

The site sampled regularly each month, was a bog pool normally about four inches deep. It was surrounded by Polytricum commune and contained Sphagnum cuspidatum.

Tarn Moss (Site 6b) Yorkshire.

Grid ref:	34/889670
Height above sea level:	1250 ft.
pH. range:	3.6 to 4.8
Calcium content:	35 to 39 parts/million
Magnesium content:	11 to 40 parts/million

Tarn moss is an old raised peat bog on the west side of Malham Tarn, Small pools of very acid water are everywhere on the Callunetum on the peat. One of these pools was sampled regularly.

Site 6b was about four feet long by a foot wide in area and normally about four inches deep. The water level was maintained by rainfall. The vegetation growing in the water is dominated by *Eriophorum angustifolium*. The leafy liverworts, *Calypogeia trichomanes* and *Cephalozia bicuspidata* were found growing among the submerged stems of the cotton grass. At times vast amounts of *Ulothrix sp*. were also found in the water. Bordering the pool is the following vegetation:-

Eriophorum angustifoloum
Eriophorum vaginatum
Molinia caerulea
Calluna vulgaris
Juncus squarrosus
Campylopus pyriformis.

Sites sampled frequently

Three of these habitats, The Winterburn reservoir, Carex nigra swamp and Fountains Fell Tarn were sampled regularly from March to September 1957 and in April 1958. Fountains Fell Tarn was also sampled in October 1956 and the Carex nigra swamp in

November 1957. Virginia water was sampled monthly from November 1957 to November 1958.

Winterburn Reservoir, Yorkshire

Grid ref:	34/946611
Height above sea level:	700 ft.
pH. range;	7.2 to 8.2
Calcium content:	73 parts/million

The Winterburn reservoir, which lies about seven miles southeast from Malham Tarn, is roughly half a mile long, It is subject to very considerable fluctuations in its water level and when low, *Equisetum sp*. grows on the exposed mud.

Virginia Water, Surrey.

Grid ref:	41/979687
Height above sea level:	136 ft.
pH. range:	7.2 to 7.4
Calcium content:	64 to 74 parts/million.

This large ornamental lake extends for over a mile in an east-west direction in Windsor Great Park. The lake, surrounded by trees, is relatively shallow and was drained dry during the 1939/1945 war.

Carex nigra swamp (Site 4b) Yorkshire.

Grid ref:	34/889672
Height above sea level:	1250 ft.
pH. range:	6.0 to 6.8
Calcium content:	35 to 37 parts/million
Magnesium content:	40 parts/million.

This site is in a slightly acid swampy area on the northwest corner of Malham Tarn. It is sheltered on one side by willows and on the other by rhodedendrons. The swamp itself is dominated by <u>Carex nigra</u> and associated with this is:-

Caltha palustris
Cardamine palustria
Eriophorum vaginatum
Polentilla palustris.

<u>Fountains Fell Tarn</u>, Yorkshire

Grid ref:	34/667899
Height above sea level:	2100 ft.
pH. range:	3.8 to 4.6

This small highly acid tarn is situated just below the summit of Fountains Fell. The top of the fell is composed of peaty moorland dominated by <u>Vaccinum myrtilis</u>. The bottom of the tarn, although predominantly sandy, is peaty in places.

Sites sampled occasionally

Apart from Ha Mire and the stream from the tarn, all the habitats in this section were sampled once. Ha Mire was sampled twice and the stream from Malham Tarn, three times.

Stream draining Malham Tarn, Yorkshire

Grid ref:	34/893659
Height above sea level:	1225 ft.
pH. range:	7.4 to 8.2

This stream ran for half a mile before disappearing underground. For about half its length it travelled through acid grassland. The stream's alkaline waters were sampled once in the limestone area and twice in the acid grassland.

The pool in Great Close Mire, Yorkshire.

Grid ref:	34/905663
Height above sea level:	1250 ft.
pH:	7.6

Small Pool near Loch Morlich, Invernesshire

Grid ref:	
Height above sea level:	
pH.	7.4

This pool in the Rothiemurchus forest was sampled by the Royal Holloway College's botanical expedition to Scotland in July 1957, as were the other two waters in this region. The pool, was overgrown with Sparganum ramosum.

Lemna covered pond (S_1), Surrey.

Grid ref:	41/975682
Height above sea level:	200 ft.
pH;	6.7

Loch Morlich, Invernesshire.

pH:	6.1

Ha Mire, Yorkshire

Grid ref: 34/667899
Height above sea level: 1250 ft.
pH. range: 6.2 to 6.8

This pond, containing *Potamogeton* sp. is in the marshy ground to the east of Malham Tarn.

Pond near Valley End, (S_2) Surrey.

Grid ref: 41/967641
Height above sea level: 200 ft.
pH: 6.0

Studland (Littlesea), Dorset.

Grid ref:
Height above sea level: 20 ft.
pH: 5.8

Llyn Cwellyn, Caernavonshire

Grid ref: 23/575551
Height above sea level: 500 ft.
pH; 5.8

Thurzeley Pond, Surrey.

Grid ref: 49/891415
Height above sea level: 200 ft.
pH; 5.6

Pond at edge of Chobham Common, Surrey.

Grid ref: 41/966643
Height above sea level: 200 ft.
pH: 5.4

Berks Tarn, Yorkshire

Grid ref: 34/752928
Height above sea level: 2000 ft.
pH: 3.6

DISTRIBUTION IN NATURAL WATERS.

Distribution in waters of different pH.

General distribution.

During this investigation samples from twenty-two natural waters were studied and twenty-seven species of the Saprolegniales were isolated. Table 1. lists the species hitherto recorded in the British Isles and on it twenty-two of the species found during the present investigations are marked with a cross. The remaining five species have not been previously recorded in Great Britain and are:-

Achlya glomerata
A. hypogyna
A. treasleana
Saprolegnia latviaca
S. litoralis.

Howarth found Achlya treasleana, Saprolegnia latviaca and Saprolegnia litoralis in Chobham bog but did not publish her findings. Achlya glomerata has been recorded from North America and Germany, and Achlya hypogyna from North America, Germany and China.

Distribution and pH.

The distribution of the twenty-seven species in relation to the pH. of the water of the habitat is shown in Table 2. It was found that the greatest number of species were found in the habitats where the pH. of the water was in the range 7.4 to 5.6. Graph 1. gives the data from the sites sampled more than four times. Seventeen species were found in the Carex nigra swamp where the pH(6.4)

Table 1.

Published records of members of the Saprolegniales found in the British Isles - Aquatic species.

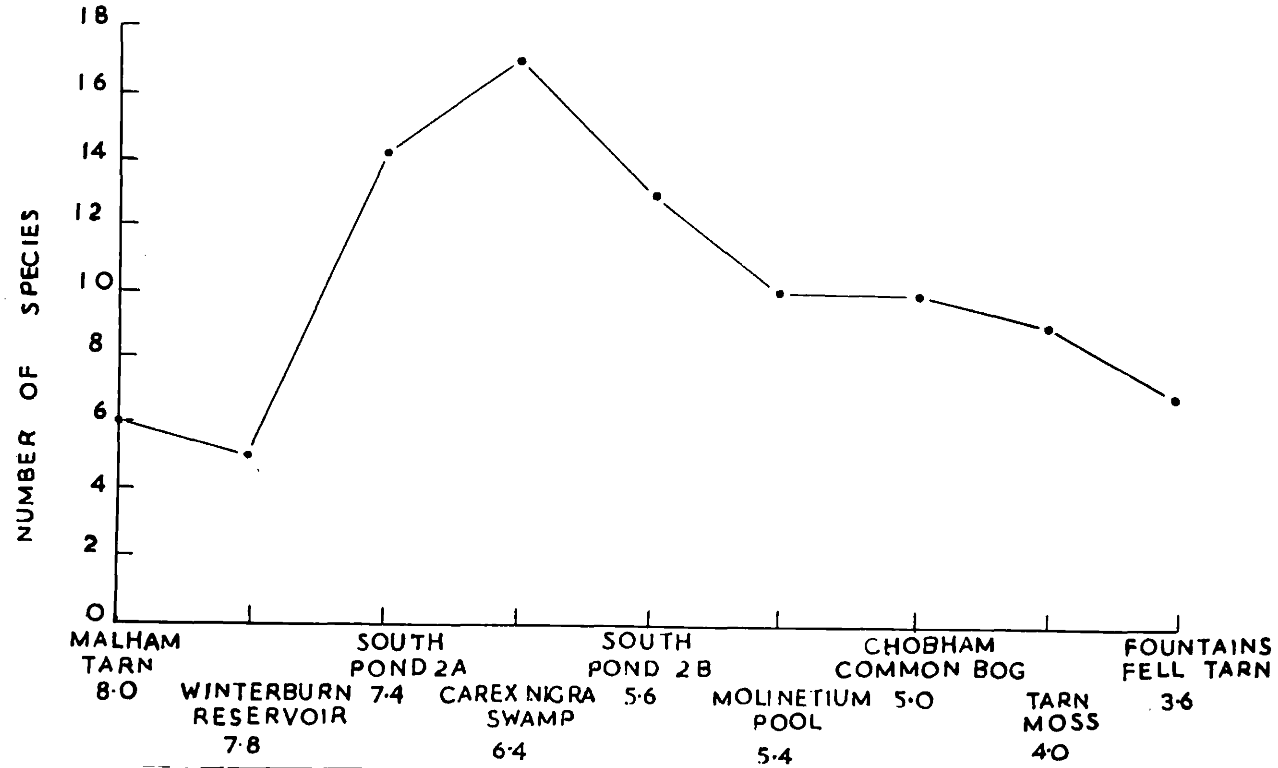
x	Achlya americana	Humphrey	Trow, Ramsbottom, Forbes, Cook & Morgan
x	Achlya apiculata	de Bary	Ramsbottom, Forbes, Cook & Morgan
	Achlya apiculata var. prolifera	Coker & Couch	Forbes
	Achlya caroliniana	Coker	Forbes, Cook & Morgan
x	Achlya colorata	Pringsheim	Ramsbottom, Sparrow, Forbes, Brown
	Achlya conspicua	Coker	Forbes
	Achlya cornuta	Archer	Masee, Ramsbottom
	Achlya crustosa	Coker	Cook & Morgan
	Achlya debaryana	Humphrey	Trow, Forbes
x	Achlya flagellata	Coker	Cook & Morgan
	Achlya Klebsiana	Peters	Forbes
x	Achlya megasperma	Humphrey	Forbes
x	Achlya oblongata	de Bary	Forbes
	Achlya oblongata var. gigantica	Forbes	Forbes
x	Achlya oligocantha	de Bary	Haines
	Achlya orion	Coker & Couch	Forbes
x	Achlya polyandra	Hildebrand	Massee, Trow, Ramsbottom, Forbes, Cook & Morgan
	Achlya prolifera	(Nees), de Bary	Trow, Forbes
x	Achlya racemosa	Hildebrand	Ramsbottom, Forbes, Cook & Morgan, Brown
x	Achlya radiosa	Maurizio	Forbes, Cook & Morgan, Brown
	Achlya recurva	Cornu	Forbes
	Achlya spinosa	de Bary	Ramsbottom
	Aplanes androgynous	(Archer)Humphrey	Massee, Ramsbottom
	Aphanomyces euticFes	Dreschler	Cook & Morgan
	Aphanomyces parasiticus	Coker	Sparrow
x	Aphanomyces laevis	de Bary	Trow, Cook & Morgan
	Aphanomyces scaber	de Bary	Cook & Morgan
x	Aphanomyces stellatis	de Bary	Massee, Trow, Cook & Morgan
	Dictyuchus monosporus	Lietgieb	Massee, Ramsbottom, Barnes & Melville, Forbes
x	Dictyuchus sterile	Coker	Sparrow
	Isoachlya eccentrica	Coker	Cook & Morgan
	Isoachlya monilifera	(de Bary), Coker.	Cook & Morgan
x	Isoachlya toruloides	Kaufman & Coker	Cook & Morgan, Brown
	Isoachlya unisperma	Coker & Couch	Cook & Morgan, Brown
x	Pythiopsis cymosa	de Bary	Cook & Morgan
	Pythiopsis intermedia	Coker	Cook & Morgan
x	Saprolegnia anisospora	Coker	Newby
x	Saprolegnia asterophora	de Bary	Sparrow, Forbes, Cook & Morgan
	Saprolegnia crustosa var 11	Maurizio	Brown
x	Saprolegnia delica	Coker	Brown
x	Saprolegnia diclina	Humphrey	Trow, Cook & Morgan
x	Saprolegnia ferax	(Gruith) Thuret	Massee, Trow, Ramsbottom, Forbes, Cook & Morgan, Brown
x	Saprolegnia mixta	de Bary	Trow, Forbes, Cook & Morgan, Brown
x	Saprolegnia monoica	Pringsheim	Massee, Forbes, Cook & Morgan, Brown
x	Saprolegnia monoica var. glomerata	Tiesenhausen	Cook & Morgan
	Saprolegnia paradoxa	Maurizio	Forbes, Brown, Newby
	Saprolegnia torulosa	de Bary	Ramsbottom
	Calyptrolegnia achyloides	Coker & Couch	Forbes
	Brevilegnia diclina	Harvey	Forbes
	Leptolegnia sp.		Forbes
	Thraustotheca clavata	(de Bary) Humphrey	Cook & Morgan, Sparrow, Barnes & Melville
	Geolegnia sp.		Barnes & Melville

x Species found also in this investigation.

Table 2

Distribution of species in the different waters

Species	8.0 Marlham Tarn	7.8 Winterburn Reservoir	7.87 Great Close Mire	7.8 Stream from Tarn	7.4±0 Pool near Loch Morlich	7.4 Virginia Water	7.4 South Pond IIa	6.7±0 S_1	6.4 4B	6.2 Ha Mire	6.1±0 Loch Morlich	6.0±0 S_2	5.8±0 Studland	5.6 South Pond IIb	5.8±0 Llyn Cwellyn	5.6±0 Thursley Pond	5.4±0 S_3	5.2 Ib	5.0 Chobham Bog	4.0 Tarn Moss	4.0 Fountains Fell Tarn	3.6±0 Berks Tarn
Achlya oligocantha	+	+																				
Saprolegnia mixta	+	+																				
Achlya polyandra	+	+				+			+													
Pythiopsis cymosa	+						+															
Saprolegnia ferax	+	+	+	+	+	+	+	+	+	+		+	+	+				+				
Achlya flagellata			+	+	+		+	+	+		+		+	+								
Achlya radiosa	+						+		+	+				+	+			+				
Aphanomyces laevis	+			+		+	+		+				+					+				
Dictyuchus sterile														+								
Achlya oblongata						+	+						+	+								
Achlya glomerata							+		+					+								
Saprolegnis anisospora										+				+								
Achlya megasperma												+										
Achlya apiculata							+		+				+	+	+		+					
Isoachlya toruloides						+			+									+				
Achlya racemosa							+		+			+	+	+	+			+				
Achlya colorata							+		+			+	+	+		+	+					
Achlya hypogyna						+			+	+			+	+		+	+					
Saprolegnia diclina						+	+		+	+			+	+	+	+			+			
Saprolegnia monoica						+	+		+			+	+	+	+		+	+	+	+		
Aphanomyces stellatis							+		+	+	+		+	+	+		+	+	+	+	+	
Saprolegnia delica						+	+		+		+	+	+	+	+	+	+	+	+	+	+	+
Achlya americana									+	+	+	+					+	+	+	+	+	+
Saprolegnia litoralis									+	+		+	+	+		+	+	+	+	+	+	+
Saprolegnia asterophora									+	+			+		+			+	+	+	+	+
Achlya treasleana													+		+	+		+	+	+	+	+
Saprolegnia latwiaca																			+	+	+	



<u>Graph 1</u> Total number of species found in the sites that have been sampled several times.

came in the middle of this range. Six were found in Malham Tarn (pH. 8.0) and seven in Fountains Fell Tarn (pH.3.6).

It can be seen in Table 2. that the fungi collected fall into three groups according to the pH. of the water in which they occur:-

1. Acid group - Comprising those species found in soft waters, i.e. with a pH. below 5.2.

2. Alkaline group - Containing species found in waters with a pH. above 7.8.

3. Neutral group - Comprising species found over a wide range of pH. between 7.4 and 5.2.

1. Acid group.

Five species are characteristic of waters where the pH. ranges from 5.2 to 3.6. They are:-

Achlya americana
Saprolegnia litoralis
S.asterophora.
Achlya treasleana.
S. latviaca.

Two of these, Achlya treasleana and Saprolegnia latviaca appear to have a restricted range, Saprolegnia latviaca not having been found above pH.5.0, and Achlya treasleana not above pH. 5.8. Achlya americana, Saprolegnia litoralis and Saprolegnia asterophora showed a wider range and extended into waters of pH. 6.4.

Four other species, Saprolegnia diclina, S.delica, S.monoica and Aphanomyces stellatis were also collected from acid waters. They have not been included in this group as they extend over a wider range of pH; having been found in waters of pH. 7.4. As will be shown later, (p.27) most of these species were most frequently isolated in waters where the pH. was above 5.2.

2. Alkaline group.

The following seven species were isolated from waters where the pH. was 7.8 or higher. Of these, four species can be considered as characteristic of alkaline waters:-

Achlya oligocantha
Saprolegnia mixta
Achlya polyandra
Saprolegnia ferax.

Three of these, Achlya oligocantha, A. polyandra and Saprolegnia mixta were not found in waters below pH.6.4. Saprolegnia ferax had a much wider range and was found in waters of pH.5.2 Three of these four species, Achlya polyandra, Saprolegnia ferax and S.mixta were frequently found. Achlya oligocantha was only observed a few times.

The four species:-

Pythiopsis cymosa
Achlya flagellata
A. radiosa
Aphanomyces laevis

although isolated occasionally from alkaline waters, occurred more frequently in the pH. range 7.6 to 5.6 and will be considered as members of the "neutral" group.

3. Neutral group.

The majority of species were found in waters ranging from pH. 5.6 to 7.4. The fungi can be divided into three subgroups:-

Sub group (a) - contains the fungi confined to the range pH. 7.4 to 5.2. These were:-

Dictyuchus sterile
Achlya oblongata
A. glomerata.
Saprolegnia anisospora
Achlya mega-sperma
A. apiculata
Isoachlya toruloides
Achlya racemosa
A. colorata
A. hypogyna.

Sub group (b) - are found in more alkaline of these waters (pH. 8.0 to 5.2). For example:-

Pythiopsis cymosa
Achlya flagellata
A. radiosa
Aphanomyces laevis.

Sub group (c) - Fungi of the "neutral" group which are also found in more acid waters (pH. 5.6 to 4.5). The following four fungi fall into this sub group:-

Saprolegnia diclina
S. monoica
Aphabomyces stellatis
Saprolegnia delica.

-----------oOo------------

It is interesting to note the distribution of species in South Pond 2. From November 1956 to April 1957, when the pH. was 7.4, two species with an alkaline distribution, Aphanomyces laevis and Pythiopsis cymosa were collected. In April 1957, when the pH. shifted markedly to the acid side (pH.5.6) these species were no longer found, but <u>Achlya hypogyna</u>, a species of more acid waters, was isolated frequently.

<u>Frequency of isolation of the species in the three groups.</u>

The number of half hemp seeds on which each fungus occurred in a sampling is referred to as the frequency of isolation of the species. This was used in the investigation as a measure of the abundance of the zoospores in the waters concerned. Thus, 20% means that the fungus under investigation occurred on 5 half hemp seeds out of a maximum of 25. This method of expressing the frequency of isolation may be criticized since each sampling consisted of five dishes each containing five half hemp seeds. It was thought, however, that by the time a fungus infecting one of the half hemp seeds produced its zoospores, the other seeds in the dish would be too crowded with fungi to form a suitable substratum for a fresh zoospore infection. It is in fact known from observation that a species may occur on only one seed of the five in a dish.

The frequency of isolation of each species was found from the seven following habitats:-

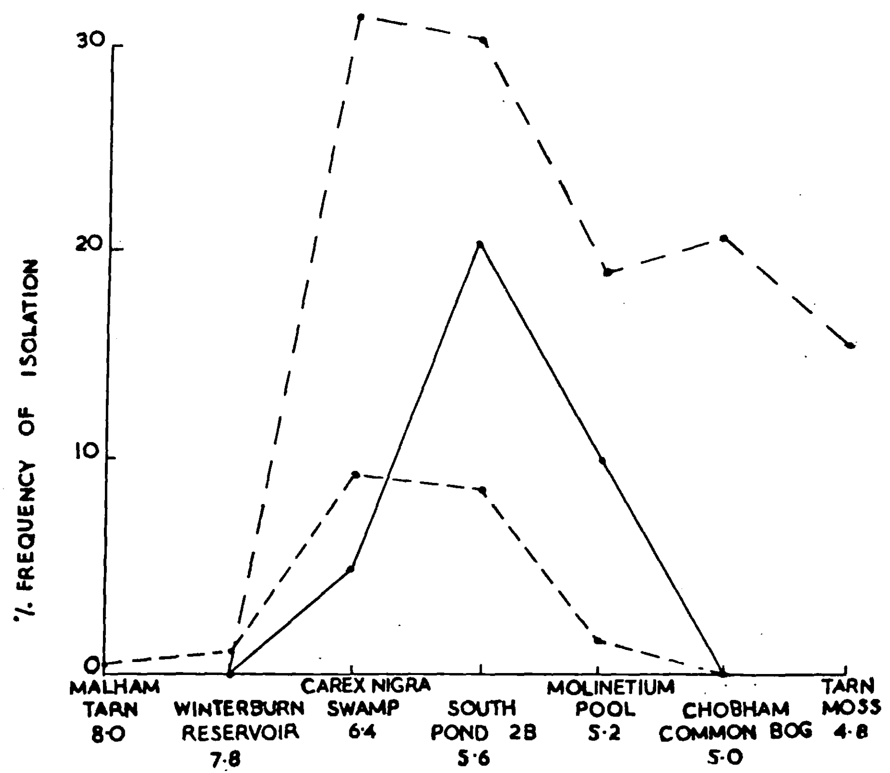
Malham Tarn
Winterburn Reservoir
Carex nigra swamp
South Pond 2
Molinetium swamp
Chobham Common Bog
Tarn Moss.

Seven samples were taken from each habitat during the period

1956 to 1957, so that the total possible number of isolations for each species was 175. For convenience this was reduced to a percentage. The totals for the seven sites are shown in Table 3.

It can be seen from the table, that the species that are here regarded as characteristic acid species have their maximum percentage frequency of isolation in waters of pH. 5.0 to 4.0, and were not found in waters where the pH. was above 6.4. The maximum percentage frequency of isolation for "alkaline" species was in the range 8.0 to 7.8. None of these species was found below pH.5.2.

Species assigned to the "neutral" group were found in greatest numbers between pH. 5.2 and 6.4; although Saprolegnia monoica was exceptional in showing a maximum percentage frequency of isolation at pH.5.0, although its range extends to pH.7.6. The average frequency of isolation of each sub group of the "neutral" species have been drawn out separately in Graph 2. It can be seen from this graph that the species of sub group (c) are of the most frequent occurrence and they are found in greatest numbers between pH 6.4 and 5.6. Those species considered to belong to sub group (b) were found in the smallest numbers but again these species are found most frequently in the range pH 6.4 to 5.6. The graph for sub group (a) differs from these two in that the maximum frequency of isolation was at pH 5.6 and the graph sloped steeply on either side of this point instead of falling off gradually on one side or the other as shown by sub group (b) or (c).



Graph 2 Average percentage fre-quency of isolation of the three subgroups of the "neutral" species occuring in the habitats sampled frequently.

Key ---------------- subgroup a.
-- . -- . -- . -- subgroup b.
---------- -- - Subgroup c.

Table 3

Percentage frequency of isolation of species found in the sites sampled regularly during 1956 and 1957

Group	Species	Site: Malham Tarn	Winter- burn Res.	South Pond 11	Site 4b	Site 1b	Chobham bog	Tarn Moss
	pH.	8.0	7.8	5.6	6.4	5.2	5.0	4.0
"Alkaline"	Achlya oligocantha	16.0	10.0					
"	Saprolegnia mixta	24.4	26.0					
"	Achlya polyandra	65.2	30.0		2.0			
"	Saprolegnia ferax	62.4	65.2	28.0	75.2	26.0		
"Neutral"	Pythiopsis cymosa	0.4		4.0				
"	Achlya flagellata		1.2	17.2	26.0			
"	Achlya radiosa			9.6	3.2			
"	Aphanomyces laevis	0.4	4.0	2.5	1.2	5.2		
"	Achlya oblongata			21.6	1.2			
"	Achlya glomerata			2.4	1.2			
"	Achlya apiculata			17.6	3.2			
"	Achlya racemosa			42.8	15.2	66.0		
"	Achlya colorata			37.6	1.2			
"	Achlya hypogyna			4.4	9.2			
"	Saprolegnia diclina			50.4	8.0	5.2	4.4	
"	Saprolegnia delica			40.4	31.2	33.2	5.2	18.8
"	Aphanomyces stellatis			9.8	44.0	6.8	19.6	2.4
"Acid"	Saprolegnia monoica			20.0	41.4	24.4	54.8	30.8
"	Achlya americana				7.2	0.4	32.0	82.4
"	Saprolegnia litoralis				5.2	23.2	34.4	42.4
"	Saprolegnia asterophora				7.2	1.2	25.2	12.0
"	Achlya treasleana					16.0	35.6	10.8
"	Saprolegnia latviaca							0.4

Distribution according to Depth.

The floor of Malham Tarn was sampled at different depths of water ranging from five inches at the edge to twelve feet in the centre. These samples from the floor of the lake were obtained by means of a modification of Lund's method of sampling algae (1949).

A rubber tube, capable of holding 250 ml. water and stoppered at one end, was lowered to the floor of the lake, the stoppered end first. When the bottom of the lake had been reached, the other end of the tube was gently lowered by means of string, until the tube was lying on its side. When the bubbles of air displaced from the tube by water, had ceased rising to the surface, the tube was hauled to the surface and the sample placed in a collecting jar.

It was found that the water moulds did not appear to be restricted to the waters around the margins of the lake. The frequency of isolation of the species was also similar at all depths investigated. Thus in July 1957, Saprolegnia ferax showed 100% frequency of isolation at five inches, five feet and twelve feet.

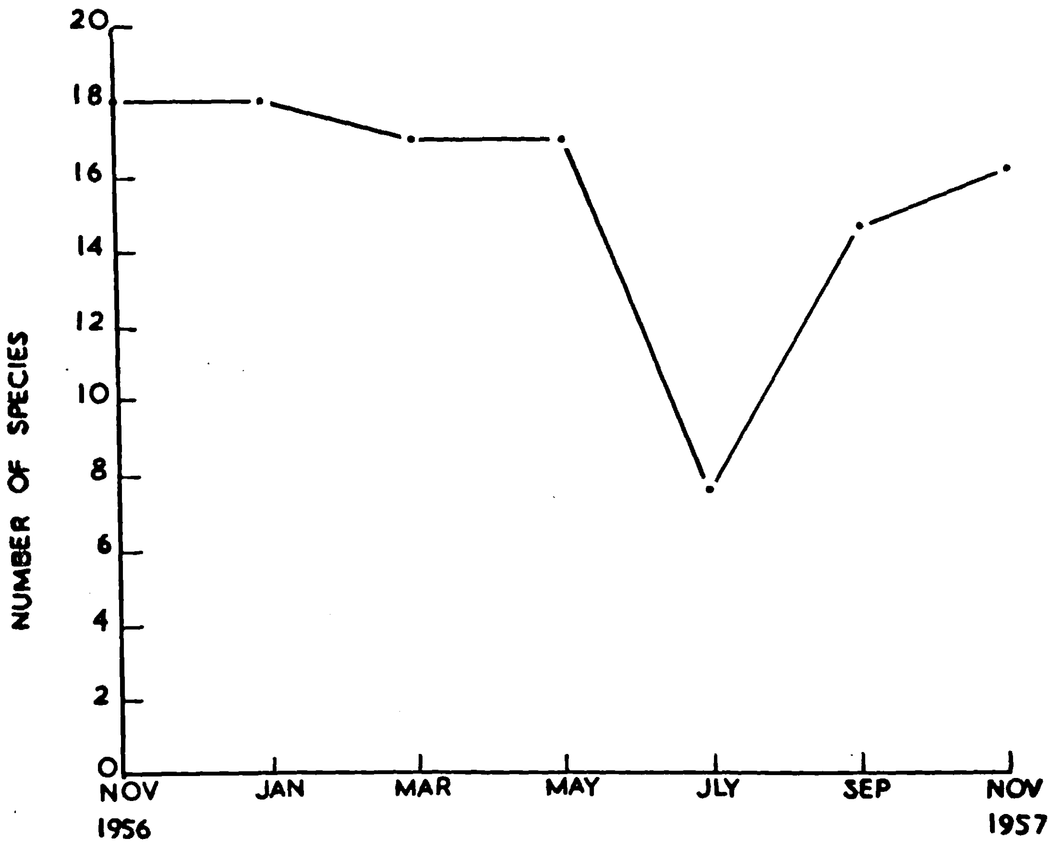
Seasonal Variation.

An analysis of the results obtained from the five places sampled regularly gave evidence that the watermoulds show seasonal fluctuations in their occurrence. Three of these sites, Malham Tarn, Molinetium Pool and Tarn Moss were sampled every two months from November 1956 to November 1957. In the other two sites sampling was continued until summer 1958.

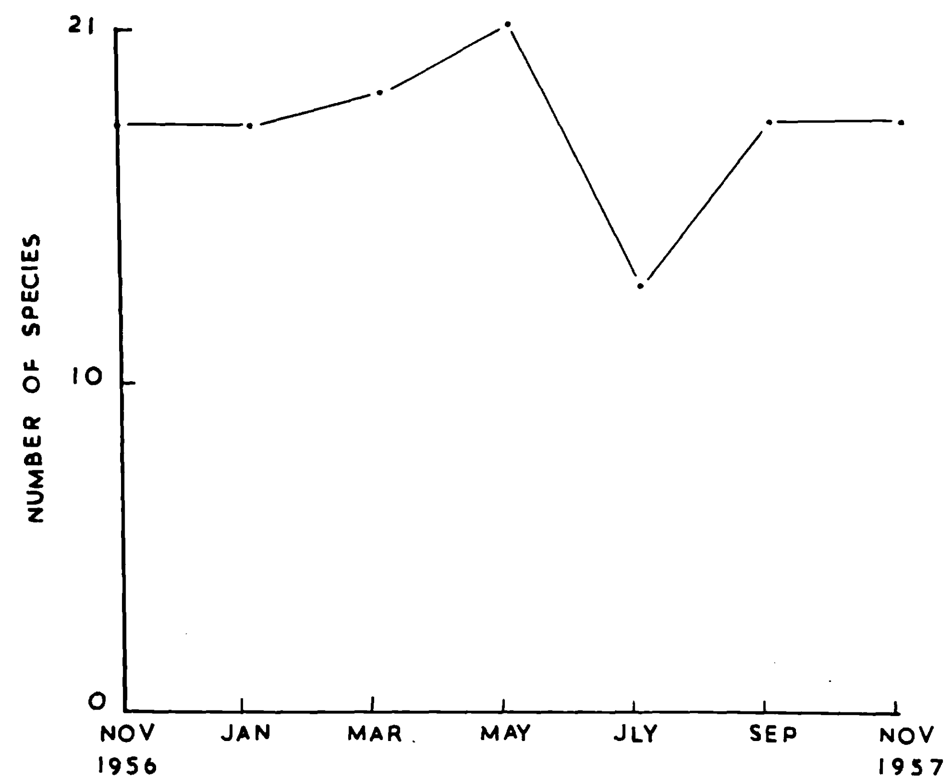
In Graph 3., which shows the total number of species isolated from these five sites throughout twelve months, it can be seen that there was a pronounced drop in the number of species occurring in the summer. Nine species were found in July, as opposed to seventeen in May, and fifteen in September. During the winter monthd the numbers fluctuated between sixteen and eighteen.

Graph 4. shows the total number of species collected from all the habitats studied. It shows a similar pattern of periodicity but it is noteworthy that there is a slight peak in the spring. This will be discussed later.

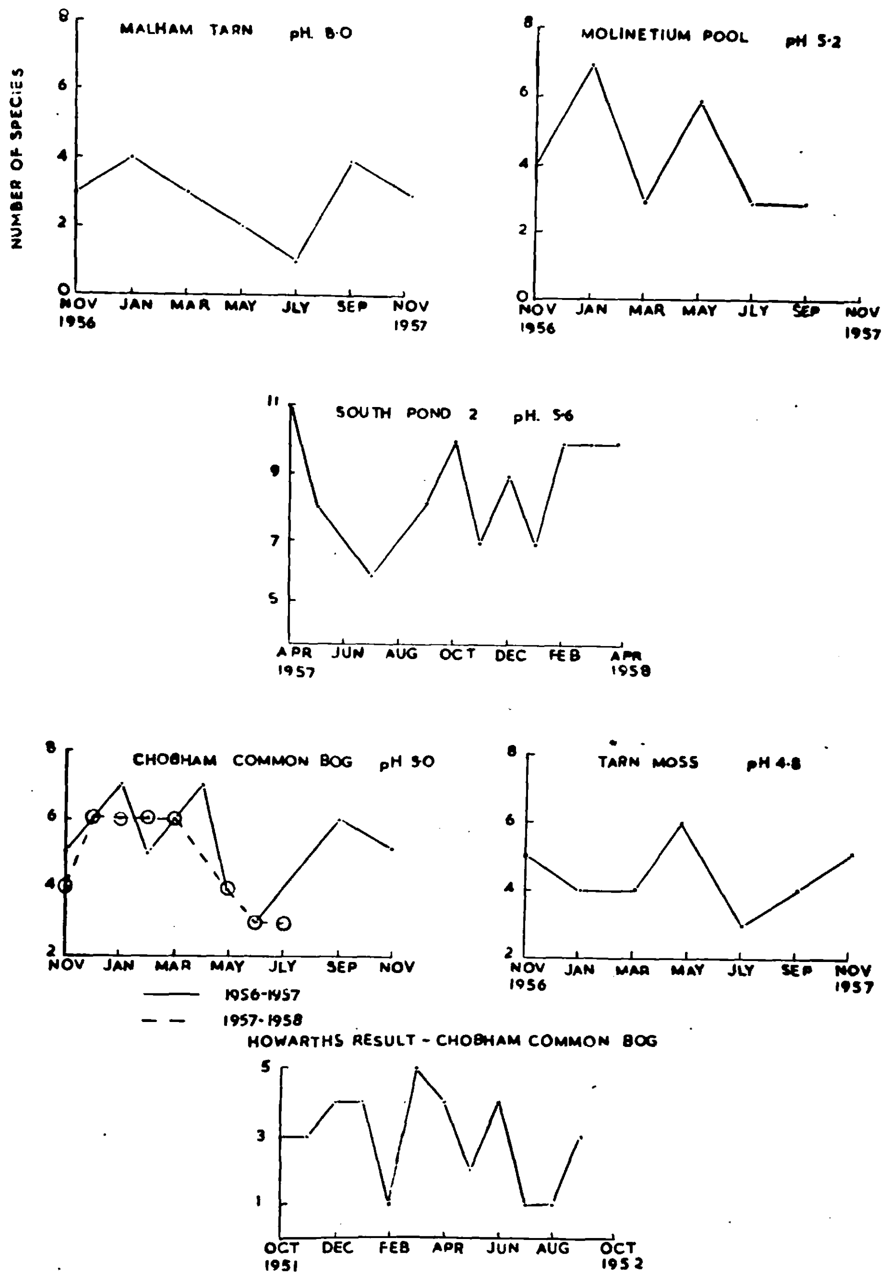
Graph 5. shows the separate results for the five sites which were used in Graph 3. It can be seen that the occurrence of species during the year follows the same overall pattern in both acid and alkaline habitats. The numbers of species from South Pond 2 have been given from April 1957 to April 1958, as the change in pH. in March 1957 may have complicated earlier results. Chobham Common Bog was sampled at monthly intervals for two years. It was



Graph 3 Total number of species from the sites sampled regularly during 1956 and 1957.



Graph 4 Total number of species from all the sites sampled during 1956 and 1957.



Graph 5 The number of species isolated from habitats sampled at regular intervals throughout the year 1956 - 1957.

unfortunate that sampling could not take place between August and September 1957. During 1951 and 1952 the bog was sampled every month by Howarth (unpublished data). She found that in August and September the number isolated remained low. The graph of her results is given for comparison

Graph 5. also illustrates how during certain months there occurred a curious fall in the number and frequency of species isolated. This appeared to be due to the bait being heavily colonised by bacteria which depressed the growth of the fungi. The drop occurred during the period when the changeover from winter to spring conditions might be expected. In most sites the drop occurred during February and March 1957. Chobham Common bog also showed a similar fall in the number of isolations in November 1957. It is probable that the large numbers of bacteria present on the bait may have been due to a large bacterial population in the water at these times.

The low percentgge of isolations obtained by Coker (1912) in February and December of that year may have been due to the same reason.

Further analysis of the results indicated that individual species showed different types of periodicity throughout the year. Table 4 shows the distribution in time of the twentyseven species isolated between 1956 and 1957.

Table 4. Seasonal distribution of the species in all the sites.

Species.	Nov.	Jan.	Mar.	May	Jly.	Sep.	Nov.	
Achlya americana	++	++	++	++	+	++	++	
A. apiculata	++	+	+	+	+	+	+	
A. colorata	++	+	+	++	+	+	++	
A. racemosa	++	++	+	+	+	+	+	"Constant
Aphanomyces stellatis	+	+	+	+	+	+	+	species
Saprolegnia delica	++	+	+	+	+	++	+	
Achlya radiosa	x		x	x	x		x	
Dictyuchus sterile			x		x			
Saprolegnia ferax	++	+	+	++	++	++	++	
S. litoralis	+	+	+	++	++	++	++	
Achlya flagellata	+	+	++	++	++	++	+	"Summer"
Achlya treasleana	++	++	+	+	++	++	++	species
A. hypogyna	+		+	++	+	+	+	
A. oligocantha				x		x	x	
Achlya polyandra	++	++	++	++		++	++	
Saprolegnia asterophora	+	++	++	+		+	+	
S. diclina	++	++	+	+		++	+	
S. mixta	++	+	+	+		+	+	
S. monoica	++	++	++	+			+	
Aphanomyces laevis	+	+	+					"Winter"
Pythiopsis cymosa	+	+	+					species
Isoachlya toruloides	+	+	+					
Achlya glomerata		x	x	x				
A. oblongata	x	x	x					
A. megasperma			x					
Saprolegnia anisospora	x		x					
Saprolegnia latviaca			x					

The data given in Table 4 were obtained from all the habitats.sampled. The more abundant species have been shown by a cross (+). When percentage frequency of isolation of any one of these species was more than 50% two crosses have been used. Species isolated infrequently have been shown with a diagonal cross. The species have been placed in three more or less distinct groups related to their time of occurrence "Winter", Summer," and "Constant" species. These will be considered separately below.

Winter species

There were thirteen species which occurred during the Winter and were not isolated in the Summer. Eight of these species were commonly found. Of these eight species, four were absent from collections made from May to September, one from June to November, and three from June to August. They are:-

Absent May to September

Aphanomyces laevis
Achlya polyandra
Isolachlya toruloides
Pythiopsis cymosa

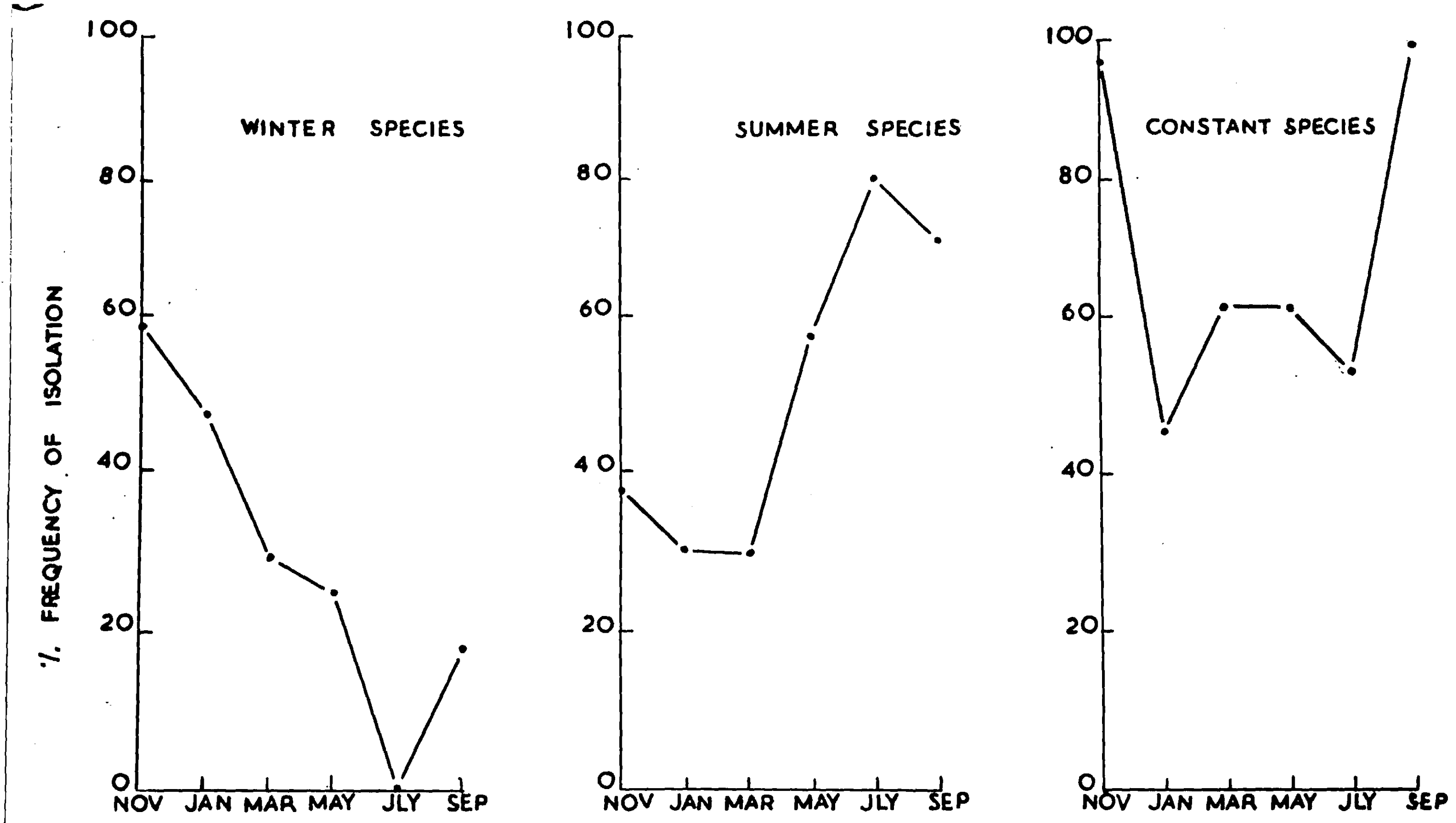
Absent June to November

Saprolegnia monoica

Absent June to August

Saprolegnia diclina
Saprolegnia asterophora
Saprolegnia mixta

Two of the remaining five species Achlya megasperma and Saprolegnia latviaca were rare species and the three others, Achlya glomerata, Achlya oblongata and Saprolegnia anisospora were found only occasionally even in the Winter. As yet, there is not enough evidence to confirm that these five species belong to the "Winter" species group.



Graph 6 Total number of isolations from each site sampled every two months expressed as a percentage of the possible total.

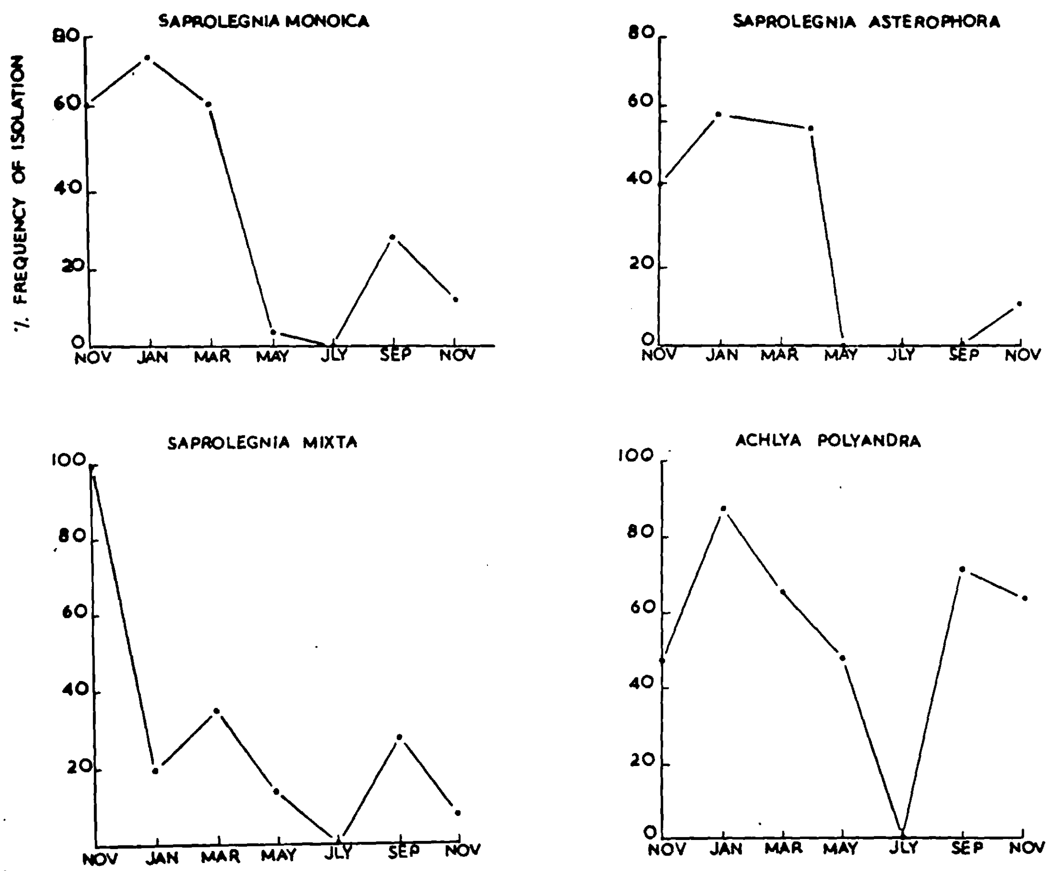
They are included here as they were isolated only during the Winter and Spring. It might be expected that a species found occasionally in an area would be isolated at a time when it was at the peak of its activity.

In Graph 6, the total number of isolations of each group of species found every two months is expressed as a percentage of the possible total. It can be seen that the Graph for the "Winter" species shows that the number of isolations falls off as the Summer advances and begins to build up again in the Autumn. The percentage frequency of isolation of four of the "Winter" species is shown in Graph 7 and it can be observed that these show similar but not identical patterns in their frequency. In November 1957 both Saprolegnia mixta and Saprolegnia monoica show a decrease in the percentage frequency of isolation. This was caused by large numbers of bacteria being present in the water samples and then being transferred to the hemp seeds and there reducing the growth of these fungi.

Summer species

Six species have their highest frequency of occurrence in Summer and a low frequency of occurrence during the coldest months (January to March). These are:-

Achlya flagellata	Achlya treasleana
A. hypogyna	Saprolegnia ferax
A. oligocantha	S. litoralis



Graph 7 Percentage frequency of isolation of "winter" species from November 1956 to November 1957.

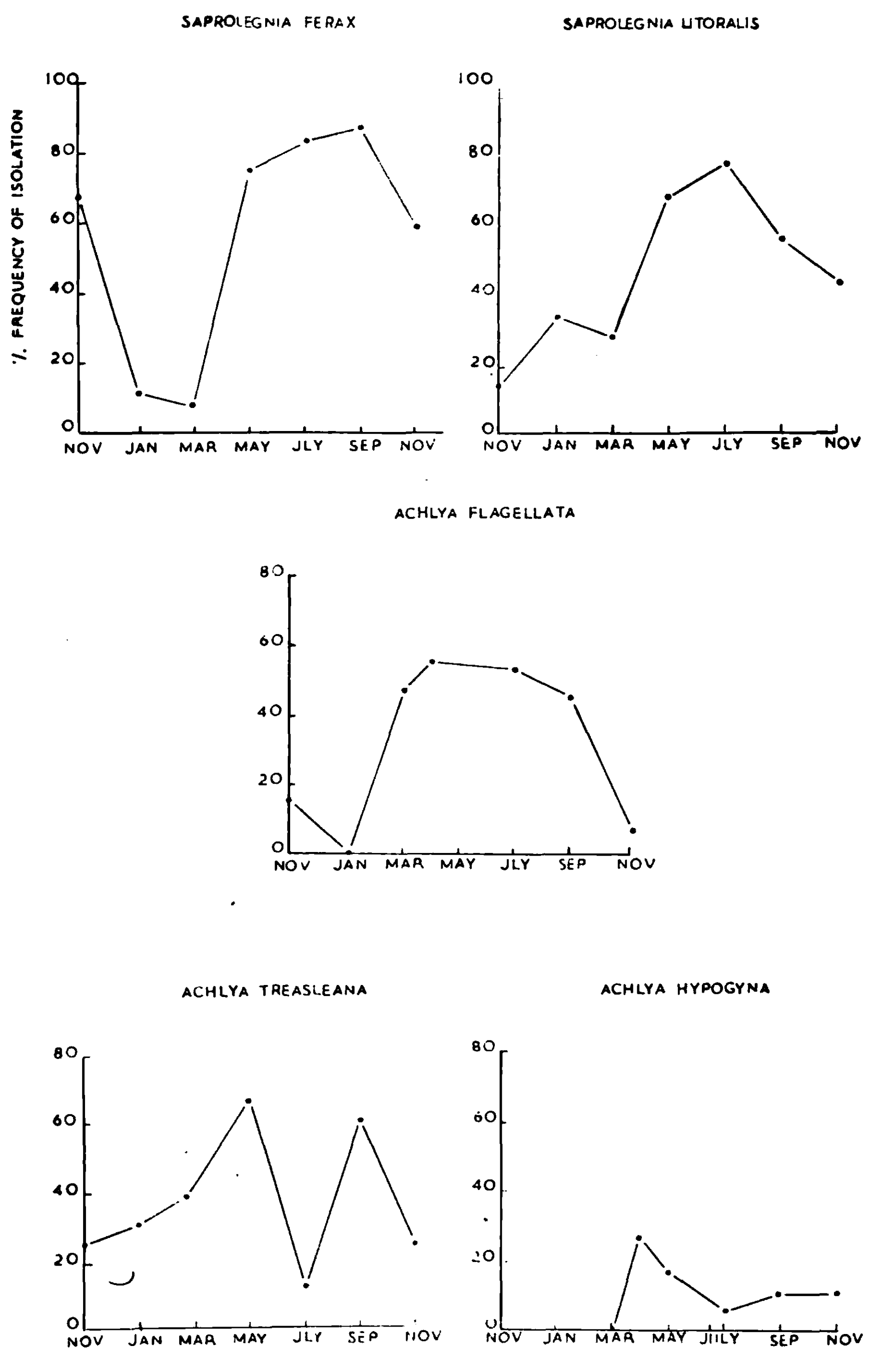
<u>Achlya hypogyna</u> and <u>Achlya oligocantha</u> were found only occasionally and although not present during the Winter; there is not enough evidence to state categorically that they do not occur then. The remaining four species, while occurring throughout the year, had their highest percentage frequency of occurrence during the Summer months, (See Graph 8).

At first sight, <u>Achlya treasleana</u> does not appear to belong to this group. In both the years 1956-1957 and 1957-58, the percentage frequency of isolation was 100% in April and December. However, the number of isolations fell off in January and by February the percentage frequency of isolation was less than 40%. Since the "Summer" species have been defined as those with a low frequency of isolation during the coldest part of the year (January to March) there seem grounds on the slender evidence available for including this species with the "Summer" group.

<u>Constant species</u>

Six of these species shown in Table 4 occurred throughout the year 1956-1957 whereas the other two were found at infrequent intervals. This group contains:-

Achlya americana
Achlya apiculata
Achlya colorata
Achlya racemosa
Achlya radiosa
Aphanomyces stellatis
Dictyuchus sterile
Saprolegnia delica



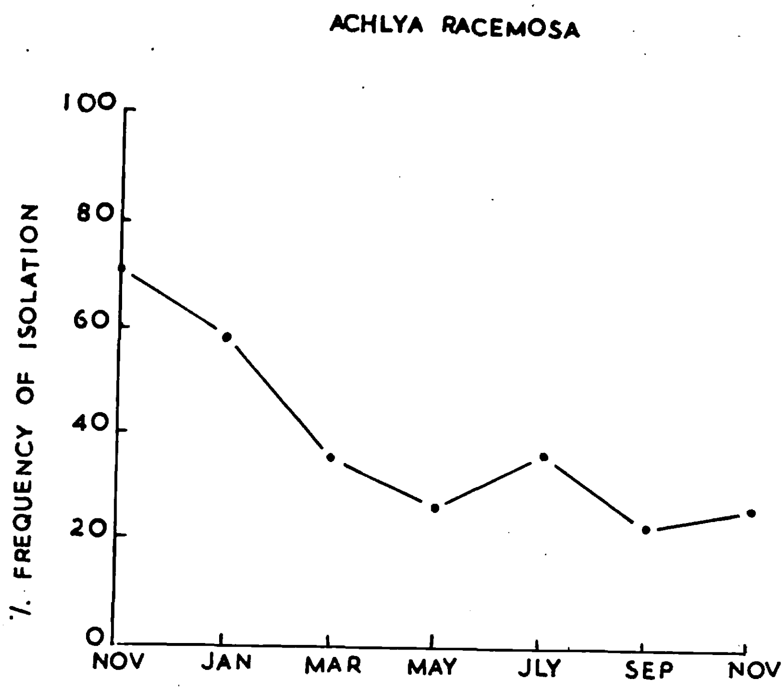
Graph 8 Percentage frequency of isolation of the "summer" species from November 1956 to November 1957.

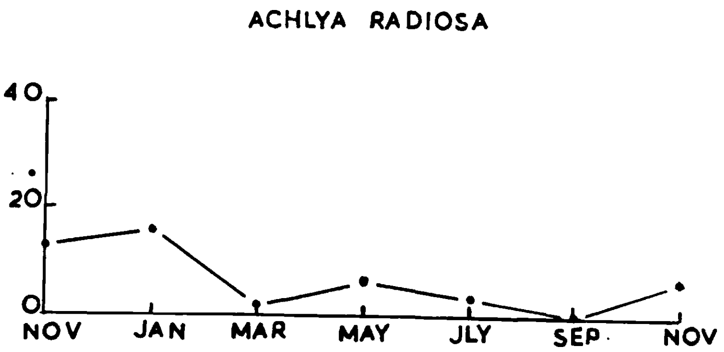
With the exception of Achlya radiosa and Dictyuchus sterile they are all species which occur commonly and have a percentage frequency of isolation of over 10% throughout the year. Graph 6 shows the total percentage frequency of isolation of the six commonly occurring species.

Graph 9 shows the percentage frequency of isolation of seven of the "Constant" species. It can be seen that in this group there are fluctuations in frequency but these do not occur in the same time of the year as in Summer species. It is possible that a wider study would reveal "Autumn" and "Spring" species.

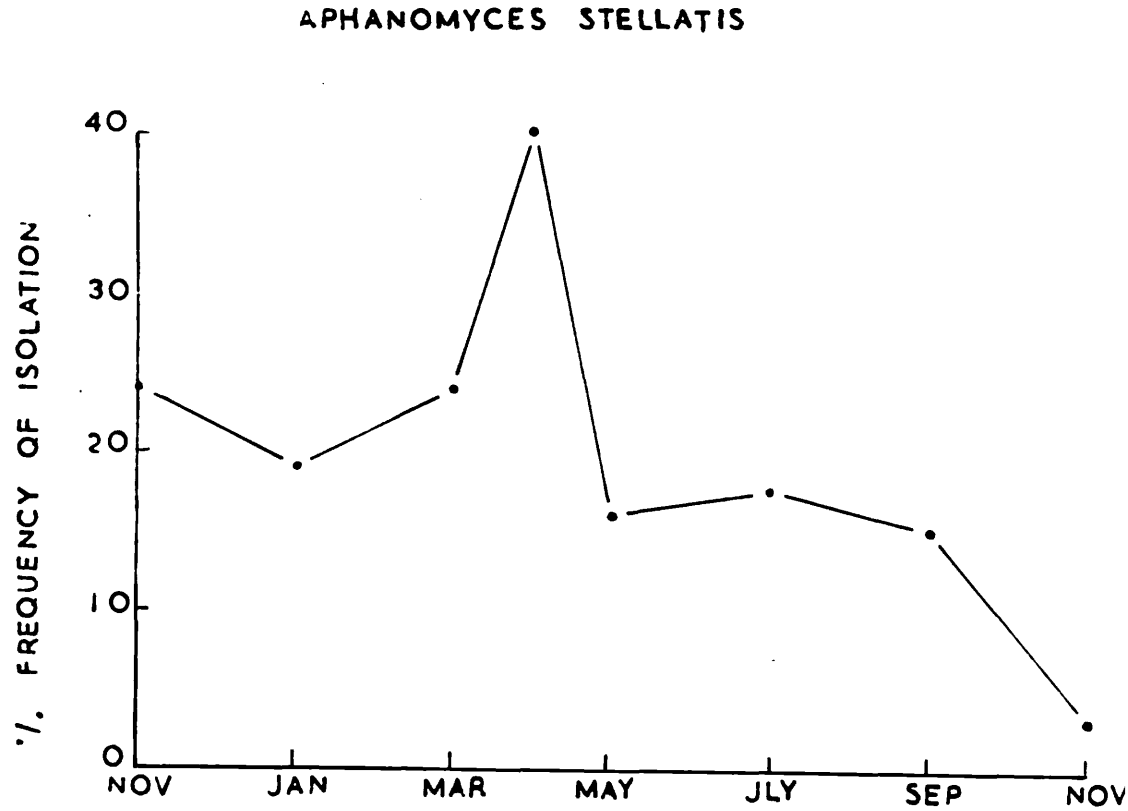
Some species such as Achlya racemosa and Achlya radiosa show a similar percentage frequency of isolation all the year round. Aphanomyces stellatis, although found throughout the year, has a high frequency in Spring. Others, like Saprolegnia delica and Achlya apiculata have a high frequency during the Autumn. Achlya americana and Achlya colorata have a high percentage frequency percentage in the Winter and, although found in the Summer, their percentage frequency was very low. These resemble the "Winter" species in their frequency of isolation except that they are found during the Summer months.

It is probable that the Spring peak in Graph 2 which occurred in May, is due to both "Summer" and "Winter" species being present together. The rise in

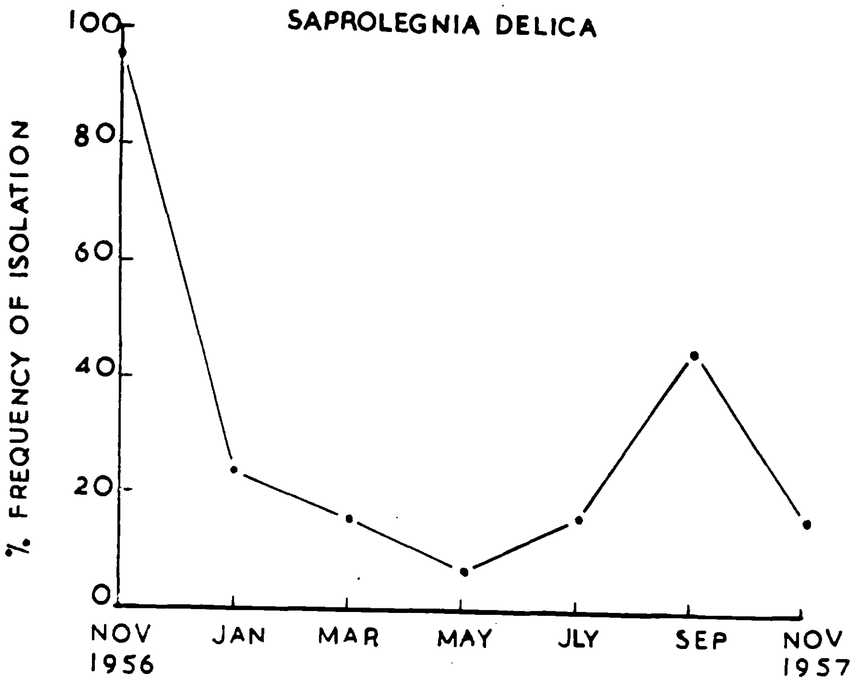


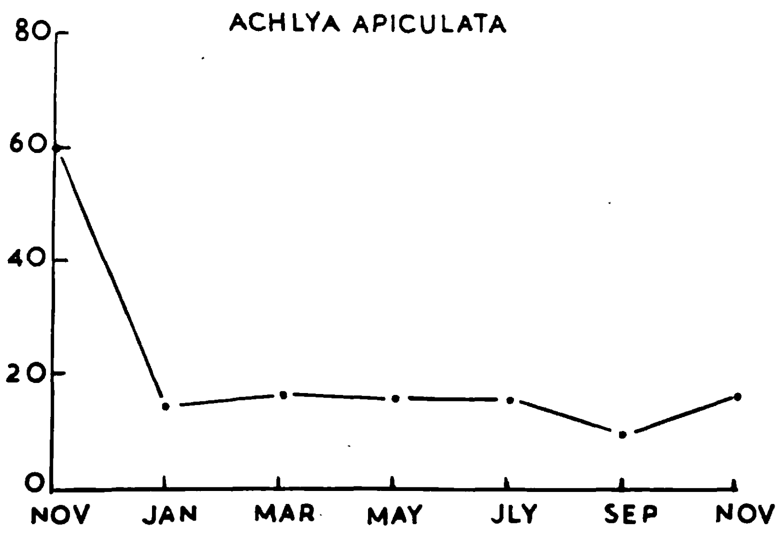


Graph 9a Percentage frequency of isolation of "constant" species from November 1956 to November 1957.

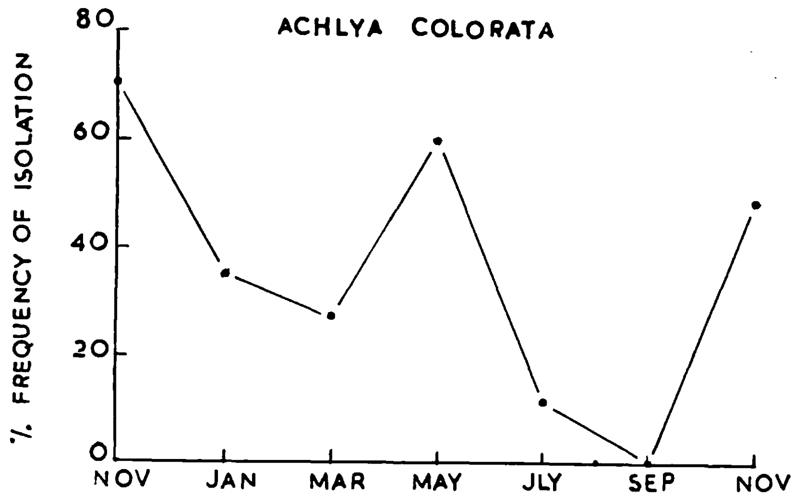


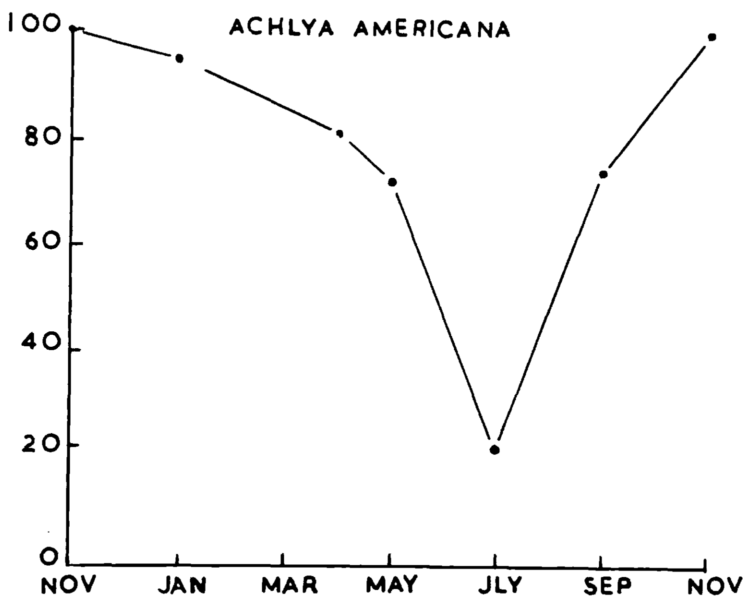
Graph 9b Percentage frequency of isolation of "constant" species from November 1956 to November 1957.





Graph 9c Percentage frequency of isolation of "constant" species from November 1956 to November 1957.





Graph 9d Percentage frequency of isolation of "constant" species from November 1956 to November 1957.

Autumn is probably brought about by the return of the "Winter" species to zoospore activity. The change in temperature, light intensity and release of nutrients in the water may all contribute to the return to activity of these species.

Since these results represent the observations of one season, the evidence for seasonal variation must be interpreted with caution. However, the results of sampling during the second year in Chobham Common bog confirm those obtained the first year.

PHYSIOLOGICAL STUDIES ON CERTAIN WATERMOULDS.

This section has been divided into two parts. In Part I. the effect of temperature on growth and reproduction is described. These experiments were carried out in an attempt to find whether the "winter", "summer" and "constant" species showed different temperature requirements for growth and reproduction. Experiments carried out to investigate any correlation between pH. and the effect of phosphate, carbonate and calcium ions on reproduction are described in Part II.

The following eight species were selected for detailed work. They were originally isolated from hyphal tips:-

	Name	Distribution.
"Winter" species	Achlya polyandra.	"alkaline" group.
	Saprolegnia monoica.	"neutral" group with acid trend.
"Summer" species.	Achlya flagellata.	"neutral" group with alkaline trend.
	Achlya hypogyna.	"neutral" group with acid trend.
	Saprolegnia ferax.	"alkaline" group.
	Saprolegnia litoralis.	"acid" group.
"Constant" species.	Achlya americana.	"acid" group.
	Saprolegnia delica.	"neutral" group with acid trend.

The species were maintained on maltose-peptone agar in petri dishes at 22°C. The standard inoculum for all experiments was taken from these agar plates. It consisted of agar discs 7 mm. in diameter, one of which was added to each petri dish used in the experiments.

Growth was estimated by measuring the average diameter of the colonies daily.

When factors affecting the asexual reproduction of the fungi were being investigated, a haemocytometer slide was used to count the number of zoospores in samples of water taken from petri dishes in which the fungi had been growing for a week. Although accurate, this method was lengthy and a quicker method was devised and more frequently used. This relied on the zoospores, produced by the fungi in liquid culture, swimming away and germinating to produce separate mycelial colonies. By counting the numbers of these new colonies after two days, before the parent mycelium had reached the edge of the plate, a rough estimate of the number of zoospores produced could be made. This method, although crude, was quick and did give a rough indication of zoospore production under different conditions.

Temperature

The effect of temperature on growth, sexual reproduction and zoospore formation may contribute in all cases towards seasonal periodicity. The following experiments have been carried out on the eight selected species in order to investigate the action of temperature on their growth and reproduction.

Vegetative growth

The amount of vegetative growth at 5°, 18°, 22°, 24°, 27°, 30°, and $37^{\circ}C$. was estimated visually after five days. The results have been summarised in Table 5. No measurements have been given and instead the relative growth is shown by crosses. It was not possible from the temperatures used to deduce the optimum temperature for growth. These experiments need to be repeated under condition where growth can be made to occur at constant temperatures between 5° and $18^{\circ}C$. No facilities were available for this when the study was carried out.

Table 5. - Effect of temperature on growth in liquid culture after five days.

Group.	Species.	5°	18°	22°	27°	30°
"Winter"	Achlya polyandra	11	1111	1111	11	1
"	Saprolegnia monoica	11	11111	11111	1	
"Constant"	Achlya americana	11	11111	1111	111	1
"	Saprolegnia delica	11	11111	11111	111	11
"Summer"	Achlya flagellata	1	1111	11111	11111	111
"	A. hypogyna	111	111	111	111	11
"	Saprolegnia ferax	11	11111	11111	1111	111
"	S. litoralis	1	11111	11111	1111	111

Key:- 1 - less than 1 cm. growth;
11111- more than 5 cm. growth.

It can be seen that the "summer, "winter" and "constant" species tend to have different temperature requirements for growth. The "winter" species do not tolerate such high temperatures as the other groups. Growth ceased with Saprolegnia monoica at 27°C., and with Achlya polyandra between 30° and 32°C. The maximum temperature of the "summer" species was higher, that of Achlya flagellata being near 40°C. and the others about 33° to 35°C. The maximum temperature of the "constant" species, although similar to that of the majority of the "summer" species, was slightly lower.

The above experiments were repeated using glucose instead of maltose in the medium and also with hemp seeds. Very similar results were obtained.

Zoospore Production.

The number of zoospore colonies produced in liquid media after two days at temperatures ranging from 5^{o} to $30^{o}C$. are summarised in Table 6.

Table 6. - Effect of temperature on zoospore production in liquid media after two days.

Group.	Species.	5^{o}	18^{o}	22^{o}	25^{o}	27^{o}	30^{o}
"Winter"	Saprolegnia monoica	+++	++	+	-	-	-
"	Achlya polyandra	+	+	+++	++	++	-
"Summer"	Achlya americana	+++	+++	++	+++	++	+++
"	Saprolegnia delica	+	+	++	++	++	+
"Constant"	Achlya hypogyna	-	++	++	+	++	-
"	Saprolegnia litoralis	+	++	+++	++	++	+
"	S. ferax	+	++	++	+++	+++	+++
"	Achlya flagellata	+	++	+++	+++	+++	+++

Key:- - few zoospores
+++ many zoospores

The relative number of zoospore colonies produced at each temperature and not the actual numbers are shown. It was felt that giving the numbers would confuse the issue as some species, such as Achlya americana even at the optimum temperature for zoospore production produced ten colonies. Other species, like Achlya flagellata would produce fifteen or more at the extreme temperatures tried and over seventy at the optimum.

It will be observed that all the species, apart from the "Summer" species, Achlya hypogyna produced zoospores at 5°C. The "Winter" species and Achlya hypogyna did not produce zoospores at 30°C. Saprolegnia monoica ceased zoospore production at temperatures above 22°C and it was this species alone that produced the largest number of zoospores at 5°C. Three species produced the largest number of zoospores at 22°C and others between 18° and 30°C.

The following six species were then selected in order to examine the effect of temperature in a more detailed fashion; a haemocytometer slide being used to count the zoospores produced.

"Winter" species	Achlya polyandra Saprolegnia monoica
"Summer" species	Saprolegnia ferax Saprolegnia litoralis
"Constant" species	Achlya americana Saprolegnia delica

Three hemp seeds, on which grew two day old colonies of the species to be studied, were put in petri dishes containing 20ml sterile distilled water. Three replicas of these were kept each at 5°, 22°, 27° and 37°C for a week. After this time, the contents of each dish were shaken vigourously and the samples were examined using a haemocytometer slide. The Poisson test showed that twenty samples from each petri dish yielded significant results. The results of this experiment are shown in Table 7.

Table 7

Effect of temperature on zoospore production using a haemocytometer slide. The fungi were grown on hemp seed in water. The results, from sixty samples taken at each temperature gives the average number of zoospores 0.1mm water.

Group	Species	5°C	22°C	27°C	37°C
"Winter"	Achlya polyandra	20	27	27	0
	Saprolegnia monoica	22	17	18	0
"Constant"	Achlya americana	14	26	21	0
	Saprolegnia delica	11	20	10	0
"Summer"	Saprolegnia litoralis	17	24	23	0
	Saprolegnia ferax	11	17	15	0

Once again, the species can be divided into three groups. In the "Winter" species group Saprolegnia monoica produced the largest number of zoospores at 5° C. Achlya polyandra, the other example of a "Winter" species resembled the "Summer" species where the maximum number of zoospores were produced between 22° and 27°C. Lack of time prevented more work being done with members of the "Winter" species to see whether Achlya polyandra , the only other "Winter" species in culture, behaved anomalously. Both "Constant" species produced the greatest number of zoospores at 22°C.

---------oOo---------

Oogonial production

The influence of temperature on oogonial production was not examined in great detail.

The species listed in Table 8 were grown on hemp seeds at the two temperatures 5° and 22°C. The presence of oogonia on 350 hemp seeds at each temperature were recorded by counting the number of hemp seeds on which each of the sixteen species were found. The proportions of hemp seeds on which each of the sixteen species were formed at 5° as compared with those at 22°C was calculated.

Table 8

Proportions of hemp seeds on which oogonia had been formed at 5° and 22°C.

Group	Species	Prop. oogonia 5°: 22°C.
"Winter"	Achlya glomerata	8:1
"	Saprolegnia asterophora	2:1
"	Isoachlya toruloides	3:1
"	Achlya polyandra	2:1
"	Saprolegnia monoica	1:1
"Constant"	Aphanomyces stellatis	5:1
"	Saprolegnia delica	2:1
"	Achlya americana	3:5
"	Achlya racemosa	3:5
"	Achlya apiculata	1:5
"Summer"	Achlya oligocantha	1:1
"	Saprolegnia ferax	1:1
"	Saprolegnia litoralis	1:1
"	Achlya treasleana	1:5
"	Achlya flagellata	2:25
"	Achlya hypogyna	3:50

It can be seen that the ratio was greatest for the "Winter" species. The "Summer" species have a low ratio, as would be expected, with the exception of Saprolegnia ferax and Saprolegnia litoralis. These species form their oogonia readily at both temperatures.

It is only the "constant" species which have members in all three sections.

Evidence for the validity of the concept "winter", "constant" and "summer" species is provided by these investigations into the effect of the influence of temperature on certain of these watermoulds. It has been shown that members of each group generally had their growth and reproduction influenced in the same way.

Sexual reproduction, with the production of resistant oospores which carry the species through unfavourable periods, throws light on the difference between "summer" and "winter" species. It has been known since De Bary and Sorokine that the oospores of some species of the Saprolegniales take a long time to germinate. Zeigler (1948) gave the times taken before germination of oospores started for 28 members of the Saprolegniales. He found that under optimum conditions for germination, Achlya glomerata and Brevilegnia linearis had the shortest resting period before germination began. The oospores of both took just over three weeks to start germination. Isoachlya intermedia and Aphanomyces stellatis were the slowest to germinate and had resting periods of over five months.

It is likely that the oospores produced in the late spring by the "winter" species rest during the following warm period when the fungus may not be active vegetatively and germinate in the autumn. The oogonia produced by the "summer"species at the end of the warm season lie dormant over the colder months and germinate when the temperature becomes warmer, so that the population of these species increases during the late spring.

The effect of temperature on growth and zoospore production is somewhat different in the three groups. The temperature for the greatest growth and zoospore production was rather higher than would be expected under natural conditions, being equivalent to the summer maximum in the waters sampled. However, under natural conditions, the interplay of several enviromental factors may cause the optimum for growth or reproduction of a certain factor to be different under natural conditions than under controlled experimental conditions. In laboratory experiments, the effect on the organism of one factor at a time was studied. In Nature, an interplay of enviromental factors occurs which is not considered in laboratory experiments. It is conceivable that in the natural habitat the influence of one factor on another can modify the so called optimum for any factor, so that it would appear that conditions best for growth in nature were not at the optimum found in laboratory experiments. The conditions that promote the best growth in nature could be regarded as the "enviromental optimum".

The fact that the optimum temperature for the growth of the fungi concerned was greater than the expected "enviromental optimum" is probably due to the competition between the fungi and bacteria not being examined under pure culture conditions. The optimum termperature for bacterial growth is higher than that for fungi. At the optimum terperature for growth of the fungus the bacteria are able to multiply more rapidly than at the lower temperature where the fungus can still grow moderately well. "Summer" species may also differ from "Winter" species in their high tolerance to bacteria. This argument assumes, of course, perhaps unjustifiably, that there are not "Winter" and "Summer forms of bacteria.

Ion Concentration

The presence of various ions in the water may account for the distribution range shown by the watermoulds. The effect of the concentration of hydrogen, phosphate, carbonate and calcium ions on the growth and reproduction of the selected fungi were therefore studied.

Vegetative Growth a. Hydrogen ion concentration

The eight selected species with the addition of:-

Achlya treasleana	- "Acid" group
Achlya colorata	- "Neutral" group
Saprolegnia diclina	- "Neutral" group
Aphanomyces laevis	- "Neutral" group with basic trend.

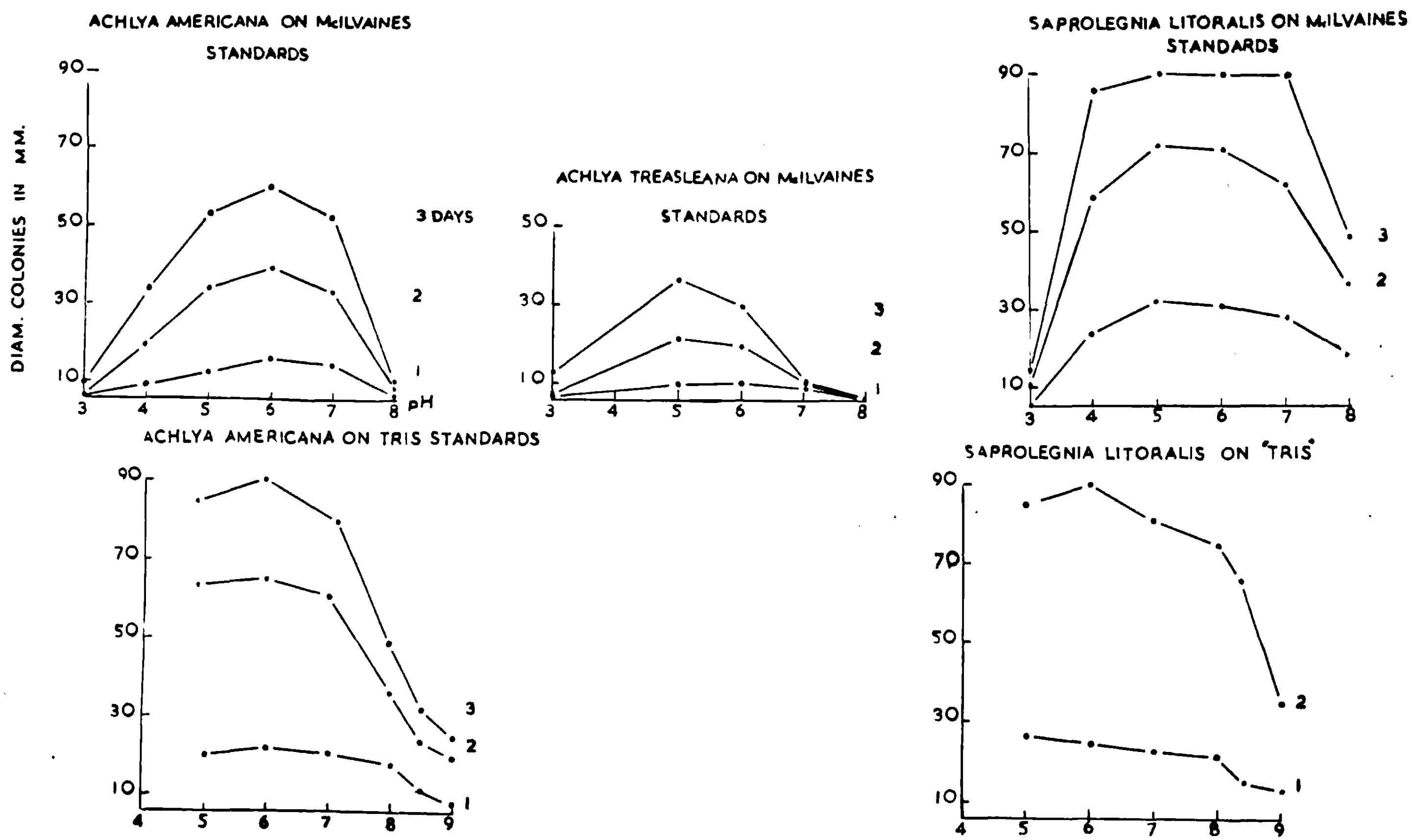
were grown on agar and liquid cultures of maltose - peptone medium buffered with an equal volume of Mc.Ilvaine's standard buffer solutions (phosphate-citric acid series) at pH's ranging from 3.0 to 8.0. Graphs 10, 11, and 12 show the daily increase in the diameters of the colonies.

Acid Group

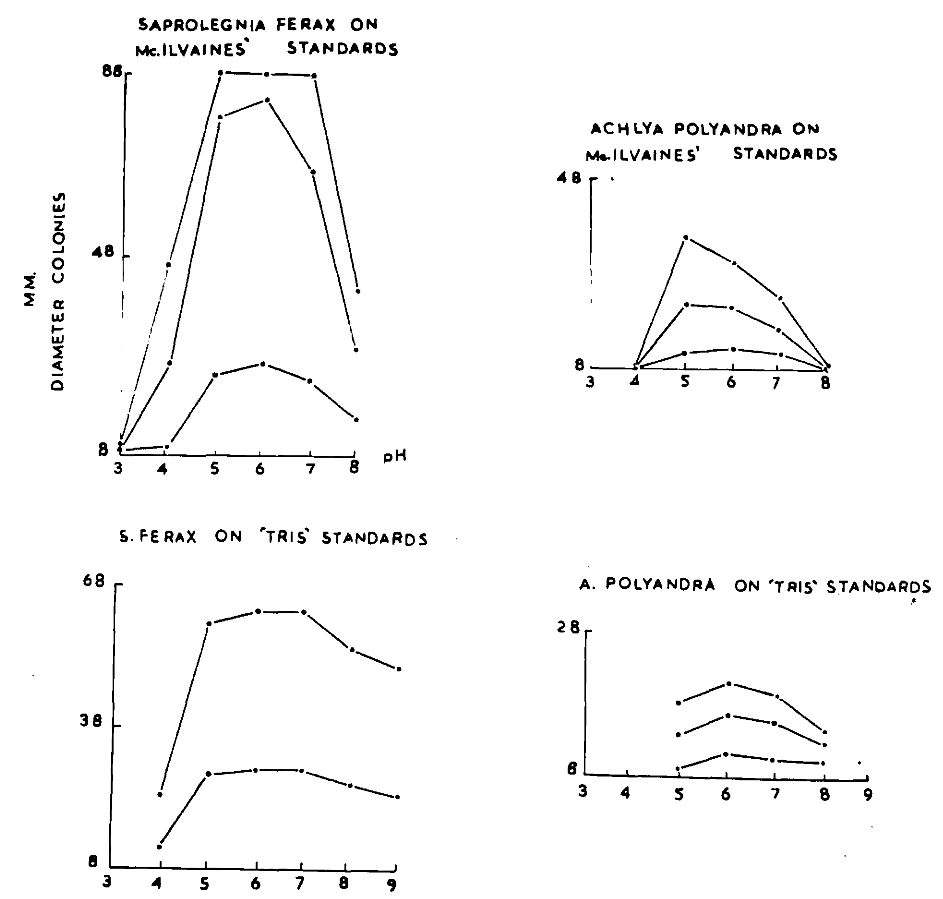
The rate of growth as measured by linear spread was at an optimum between pH. 5.0 and 6.0 for all these species. They were capable of growth at pH. 3.0. Growth fell off more rapidly on the alkaline than on the acid side, Achlya americana and A. treasleana both showing practically no growth at pH. 8.0. Saprolegnia litoralis, however, grew quite well at this pH.

Alkaline Group

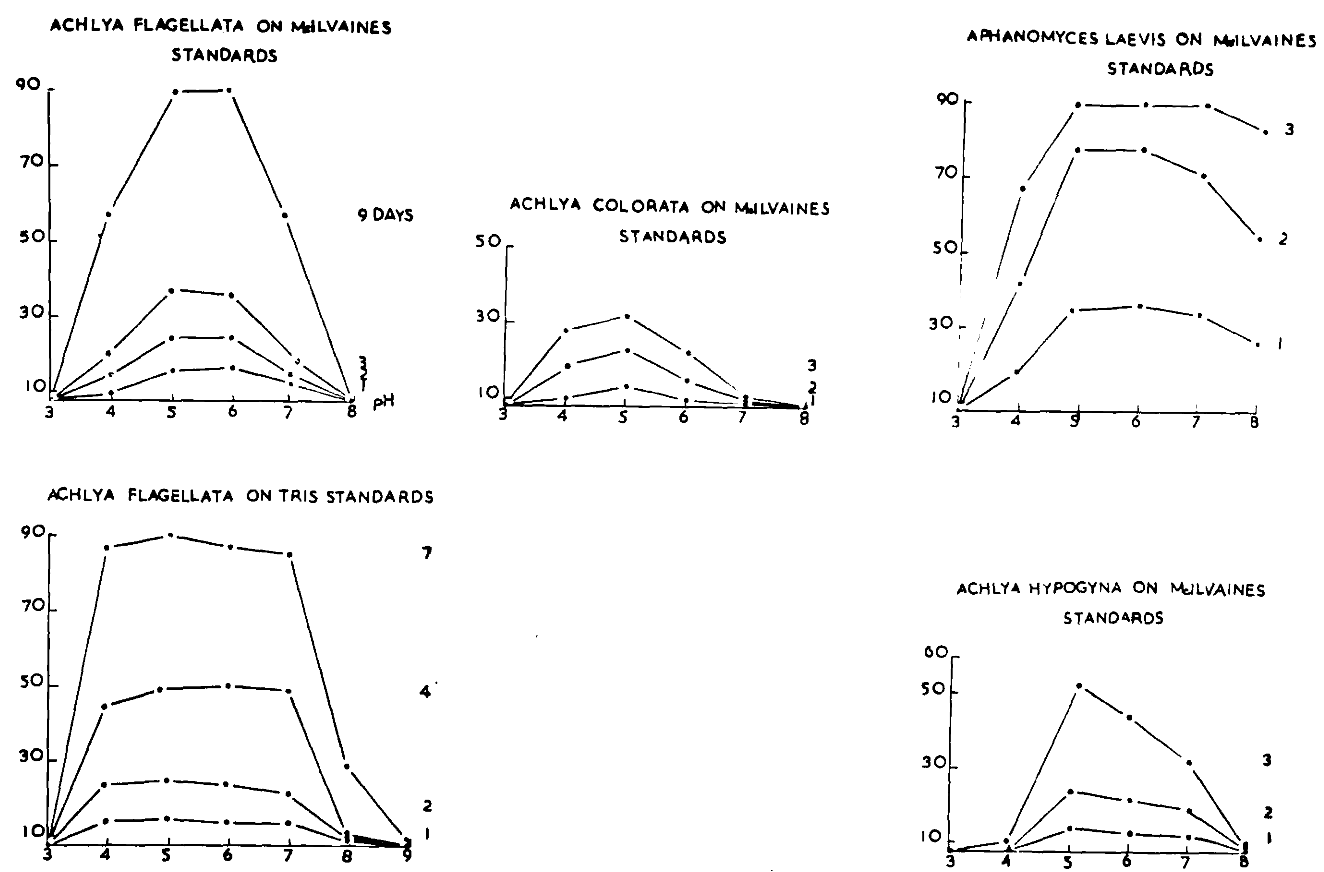
The optimum pH. for growth shown by Achlya polyandra and Saprolegnia ferax was also between pH. 5.0 and 6.0. These two fungi had a narrower range at the acid end. Achlya polyandra grew between pH. 5.0 and 7.0 and Saprolegnia ferax from pH. 8.0 to 4.0. In both cases growth fell off more rapidly on the acid side.



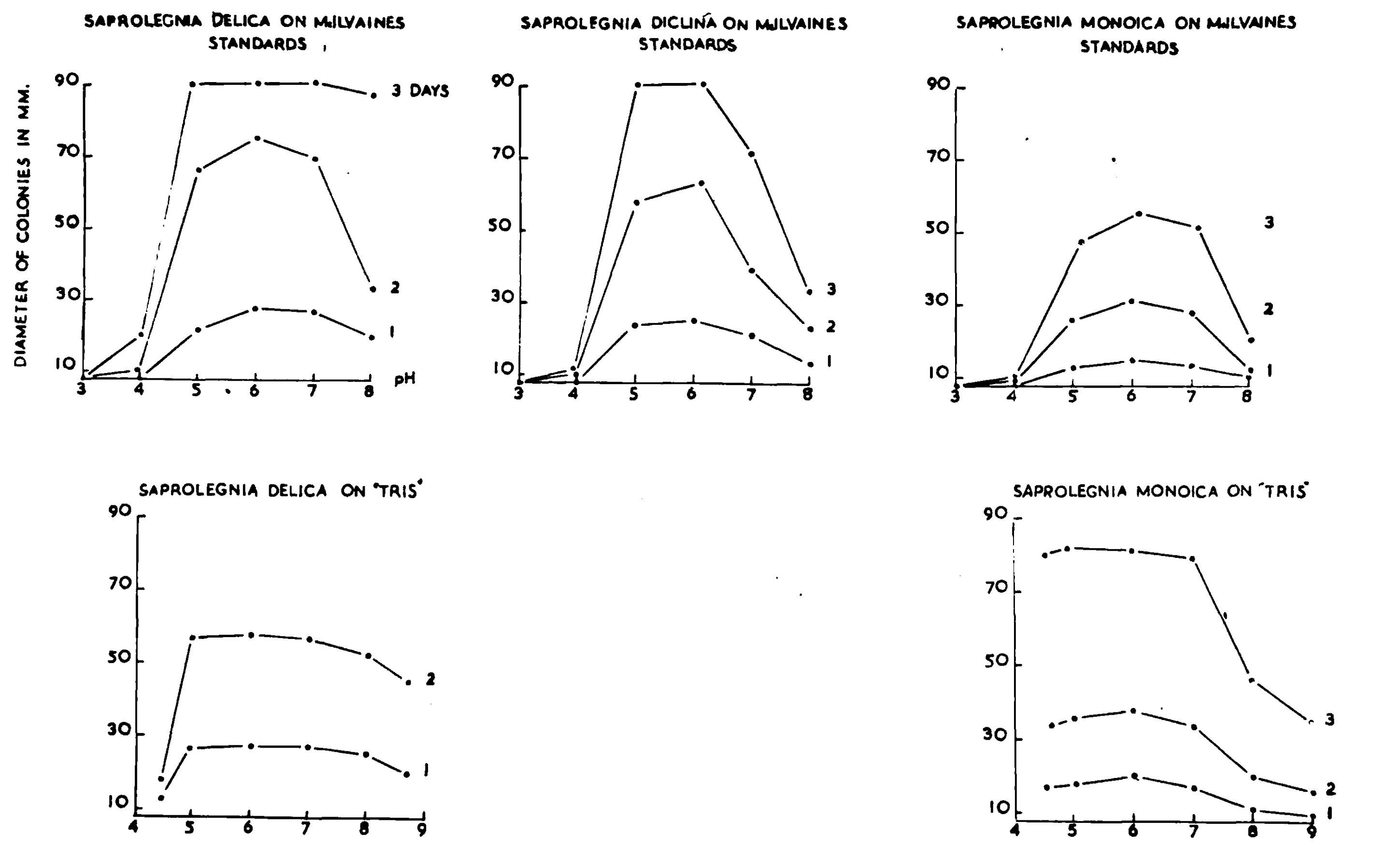
Graph 10   Action of pH. on the growth of the "acid" species on media containing "Tris" or McIlvaines buffer standards.



Graph 11 Action of pH. on the growth of "alkaline" species on media containing "Tris" or McIlvaines buffer standards.



Graph 12a Action of pH. on the growth of "neutral" species on media containing "Tris" or McIlvaines buffer standards.



<u>Graph 12b</u> Action of pH . on the growth of "neutral" species on media containing "Tris" or McIlvaines buffer standards

These results can be correlated with the distribution of the fungi in the acid and alkaline waters except that it was surprising that Achlya polyandra did not grow above pH. 7.0. This result was unexpected and will be discussed later.

Neutral Group

The optimum for growth was again between pH. 5.0 and 6.0. Except for Achlya flagellata the growth range was from pH. 4.0 to 8.0. Three species, Saprolegnia delica, S. diclina and S. monoica, which have a natural distribution in the more acid waters, show greater growth at pH. 8.0 than 4.0. This result is surprising and cannot be explained.

Table 9 gives data of experiments in which the fungi were grown in liquid media. The results, although showing that growth was possible at a lower pH. in liquid than on solid medium, support the above observations. The two "acid" group species and Achlya flagellata grew well at pH. 3.0 although all the other species gave a very slight indication of growth at this pH.

Table 9

Growth on liquid medium in the pH range
3.0 to 8.0 after 4 days.

Series	Species	3.0	4.0	5.0	6.0	7.0	8.0
Alkaline	Achlya polyandra	+	+	+	++	+	
Alkaline	Saprolegnia ferax	+	++++	+++++	++++	++++	+++
Neutral	Saprolegnia delica	+	++++	+++++	+++	+++	+++
Neutral	Saprolegnia monoica	+	++++	+++++	+++	+++	++
Neutral	Achlya flagellata	++	+++	+++	++	+	+
Acid	Achlya americana	+++	+++++	++++	++++	+	
Acid	Saprolegnia litoralis	+++	+++++	+++++	+++++	++++	+++

\+ - less than 1 cm growth; +++++ - more than 5 cm growth.

b. Phosphate Concentration

The effect of the phosphate concentration of the medium was investigated in the hopethat it might be connected with the unexpected small amount of growth of certain members of the "alkaline" group at pH. 8.0. A series of experiments were set up using the McIlvaine's citric acid phosphate buffer standard solution (with phosphate in the concentration of 10.7 grams/litre) and for comparison a similar pH. range having the solutions buffered with "Tris" (containing 15 grams hydroxymethyl amino methane/litre) - hydrochloric acid buffer solutions in which no phosphate was present.

The effect on the growth of the eight fungi, with the exception of Achlya hypogyna, of the two buffer standards at the different pH's is summarised in Graphs 8, 9 and 10. It can be seen that below pH.7.0 the behaviour of all species on the two buffers were similar.

The optimum pH. for growth differed with the buffer used. Thus the "acid" species growing on media containing McIlvaine's buffer standards had an optimum between pH. 5.0 and 6.0 while when "Tris" was used the optimum was at 6.0. On medium containing this buffer, the optimum for the "alkaline" species also shifted and was between 6.0 and 7.0 as opposed to between 5.0 and 6.0 as found when the McIlvaine's standards were used. The optimum for growth in the "neutral" group was similar in both cases although slightly higher when "Tris" was used.

The use of "Tris" also emphasised the different pH. requirements for the growth of the "acid" and "alkaline" species. Above pH. 6.0 the growth of the "acid" species fell off rapidly and there was much less growth at 7.0 than 6.0. There was very little difference in growth rates at pH. 6.0 and 7.0 with the "alkaline" species when "Tris" was used and the retardation of growth at the more "alkaline" pH's as experienced with McIlvaine's buffer standards was not apparent. The "neutral" species resemble the "alkaline" species when grown on "Tris" in that growth is only a little less at pH. 7.0 than 6.0. Saprolegnia delica showed similar growth curves with both buffers. <u>Saprolegnia monica</u> appeared to be able to tolerate more acid conditions when "Tris" was used.

A comparison of the rates of growth of all species on McIlvaine's buffer standards and "Tris" showed that at least above pH. 7.0 growth was greater on "Tris". It can be inferred that the concentration of phosphate in the McIlvaine's standards depressed the growth of all species. The increase in phosphate concentration appears to have affected the growth of species of the "Alkaline" group more markedly than the other species. For example, at pH. 8.0 <u>Saprolegnia ferax</u> (Alkaline group) and <u>Saprolegnia delica</u> (Neutral group) took the following number of days to cover plates using the two buffer solutions:

Buffer	Saprolegnia delica	Saprolegnia ferax
McIlvaine's	4 days	5 days
"Tris"	4 days	4 days

<u>Achlya americana</u>, <u>Achlya flagellata</u> and <u>Achlya polyandra</u> which grew slightly or not at all at pH. 8.0 with McIlvaine's standards were capable of growth at pH. 9.0 with "Tris".

The effect of various concentrations of potassium dihydrogen phosphate from 0 to 0.5 g/litre was now investigated. The highest concentration of this series, 0.5 g/litre was less than the concentration of phosphate in the McIlvaine buffer standard of pH. 3.0 (2.22 g PO_4H/litre). It was found that even at concentrations as low as 0.001g/litre phoaphate depressed the growth rate of three species,

These were:-

Saprolegnia monoica (Neutral species)
Saprolegnia ferax (Alkaline species)
Achlya polyandra (Alkaline species)

A concentration of 0.5 g/litre phosphate tended to stimulate growth in the other species.

It can therefore be seen that the use of buffer standards containing phosphate is not to be recommended unless the effect of phosphate on the organism is known.

The concentration of phosphate in natural waters may tend to limit the distribution of species whose growth is retarded by small quantities of phosphate. It is interesting that the alkaline species, which are found in waters where the phosphate is unavailable, are sensitive to an increase in the phosphate concentration.

c. Calcium concentration

The effect of various concentrations of calcium chloride, ranging from 0 to 37.765 g/litre on the growth of the selected fungi was investigated. It was found that all species, apart from *Saprolegnia ferax* showed a similar amount of growth on all concentrations of calcium chloride up to 7.55 g/litre. With *Saprolegnia ferax*, more growth was obtained at 7.55 g/litre calcium chloride solution than at the weaker concentrations. Five species including those of the acid group, showed growth with 37.765 g/litre calcium chloride solution. This growth was not so vigourous as at the lower concentrations. *Achlya flagellata* and *Achlya hypogyna* (neutral group) and *Achlya polyandra* (alkaline group) did not grow at this concentration .

These results indicate that the concentration of calcium in the water does not generally affect the distribution of the species. This is not surprising since on analysing the calcium content of the various waters sampled regularly it was found that South Pond 2, a water included in the "Neutral" group, contained more calcium than the water at Malham Tarn which was inhabited by the alkaline species.

The greater growth shown by *Saprolegnia ferax* when 7.553 g/litre calcium chloride was added as compared with its growth in weaker concentrations may account for its presence in waters such as South Pond 2 and Malham Tarn. This was the only species which showed greater growth with an increase in the calcium content in culture.

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Zoospore Production

It was thought advisable to investigate the effect of the concentration of hydrogen, phosphate and carbonate ions on zoospore production. unfortunately lack of time prevented using a heamocytometer slide for estimating the number of zoospores produced by species. The quicker method of counting the number of new colonies formed in liquid culture was used instead. The results obtained give a rough estimate of zoospore germination under various conditions.

Hydrogen ion concentration

Six species were grown in liquid maltose-peptone medium buffered with McIlvaine's standards from pH. 3.0 to 8.0. Table 10 gives the number of colonies produced at each pH.

Table 10

Zoospore production in the pH. range 3.0 to 8.0 using McIlvaine's buffer standards. Each result is the average number of colonies from five plates.

Group	Species	3	4	5	6	7	8
Alkaline	Saprolegnia ferax	0	1	12	9	13	24
Neutral with acid trend	Saprolegnia delica	0	40	30	30	25	22
Neutral with acid trend	Saprolegnia monoica	0	18	8	15	7	5
Neutral with alkaline trend	Achlya flagellata	14	20	12	12	0	0
Acid	Achlya americana	1	2	13	8	0	0
Acid	Saprolegnia litoralis	15	15	10	4	11	13

It can be seen that only the acid species and Achlya flagellata produced zoospores colonies at pH. 3.0 Saprolegnia ferax (alkaline group) produces the greatest number of zoospores colonies at pH. 8.0. With Achlya americana (acid group) zoospores colonies were found at the high pH's and the greatest number of zoospores colonies were observed at pH. 5.0.

These results are a little difficult to interpret as the experiment was done before the effect of the phosphate in the McIlvaine's buffer standards on vegetative growth was investigated. It is possible that the phosphate in the solution depressed the number of zoospores colonies produced by the members of the "alkaline" group and further investigations are needed.

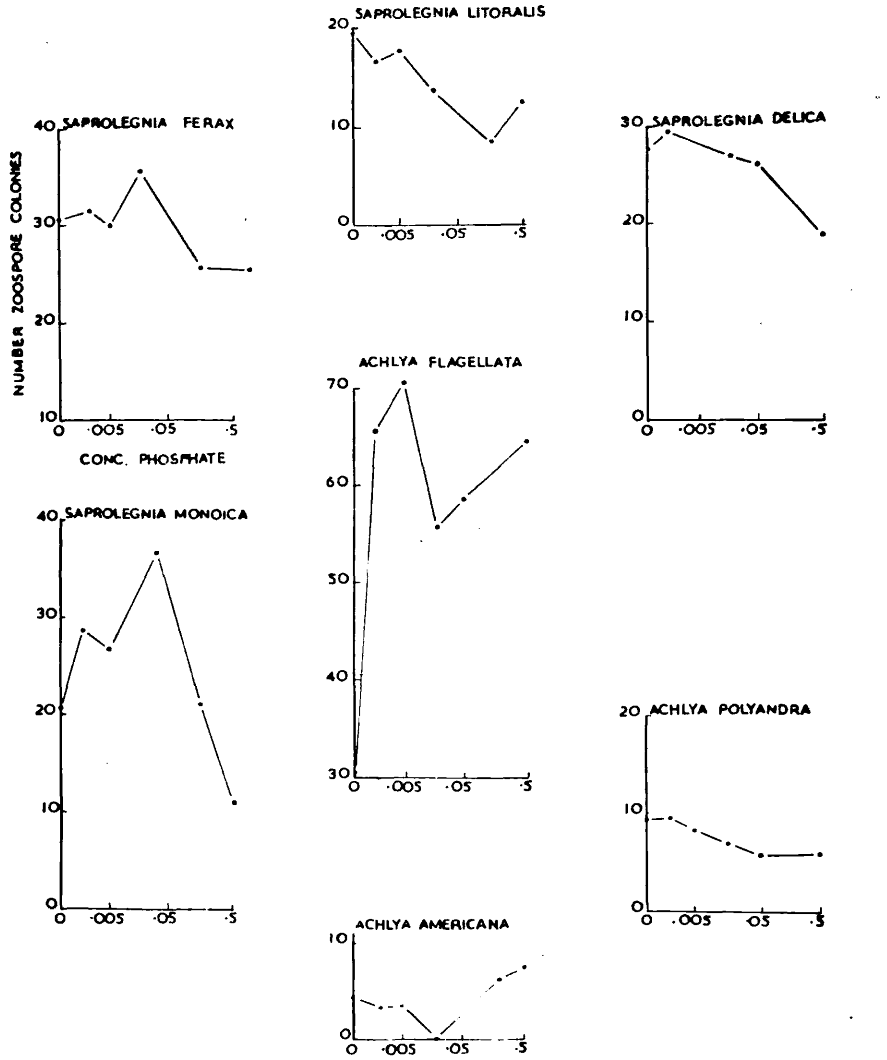
Phosphate ion Concentration

These results, obtained by estimating the numbers of zoospores colonies produced by the species growing in a buffered medium containing various concentrations of potassium dihydrogen phosphate, are not conclusive. The results are presented in Graph 13.

An increase in the phosphate concentration depressed numbers in zoospore colonies of *Saprolegnia monoica*, *Saprolegnia delica* (both "Neutral" species with acid trend) *Saprolegnia ferax* and *Achlya polyandra* ("Alkaline" species). The other three species show a depression in the zoospore output in the range 0.01 to 0.1 g/litre phosphate. Apart from this depression, *Achlya americana* and *Achlya flagellata* both show an increase in zoospore activity at the higher phosphate concentrations. *Saprolegnia ferax* resembles members of the first group.

Carbonate ion concentration

Wills' (1954) work on *Phytophora nicotineae* showed that the addition of carbonate in the form of sodium carbonate increases sporangial formation. At a pH. greater than 8.0 sporangial formation was induced by using this salt alone. It was therefore decided to investigate the effect of sodium carbonate on the zoospores production of the selected species of Saprolegiales,



Graph 13 Effect of phosphate on the production of zoospore colonies.

particularly as the concentration of carbonate in the different waters varied considerably.

The species, apart from *Achlya hypogyna* were grown in maltose-peptone medium containing concentrations of 0.5 and 0.05 g/litre sodium carbonate. The medium was buffered at pH. 8.4 and 7.1 with "Tris".

The results were not very conclusive but apart from the acid species and *Achlya flagellata* the species tended to behave in the same way that Wills found with *Phytophora nicotineae*. The effect of carbonate on *Achlya flagellata* and the two "Acid" species was to reduce the output of zoospores at pH. 8.4.

It is possible that although pH. is the determining factor in zoospore production, the presence of other ions becomes important.

Production of Oogonia.

Little work has been done with regard to the effect of external factors on oogonial production. The following results, on the influence of pH. on oogonial formation were incidentally observed while studying the effect of pH. on growth in solid media.

Only the two acid species, Achlya americana and Saprolegnia litoralis were found to form oogonia on buffered solid medium. Oogonia were produced by Saprolegnia litoralis from pH. 3.0 to 7.0 and Achlya americana from pH. 3.0 to 6.0. It was interesting to observe that oospores produced by Achlya americana at pH. 5.0 and 6.0 soon disintegrated.

It could be seen that both these species did not produce resting stages when the medium became progressively more alkaline. This may be the reason why Saprolegnia litoralis, although capable of producing zoospores at pH. 8.0, is not found in alkaline waters.

DISCUSSION.

Distribution in Natural Waters.

In this investigation, members of the Saprolegniales found in waters of different pH have been listed. This distribution agrees in general with that found by Lund in Denmark and Wolf and Wolf in North America. There are certain differences and in Lund's list six species,

Aphanomyces stellatis
Achlya colorata
A. racemosa
A. radiosa
Saprolegnia ferax
S. monoica

showed a wider range of pH tolerance than their English counterparts. The only species with a marked difference in its distribution was Achlya americana. Lund found this in waters of pH. 8.0 and Wolf and Wolf in waters of pH 7.6. It was not found in England in waters above 6.4. It is puzzlin that this species apparently characteristic of acid waters i England, should tolerate such alkaline waters in Denmark and North America. This could be accounted for by a difference in variety.

Although the different species have been show to occur in waters of certain pH ranges, it is probable that the hydrogen ion concentration determines solely the lower limits of the distribution of each species. Experimental work showed that although most species produced zoospores and were capable of growth at pH 8.0, only the "acid" specie did so at pH 3.0. It is likely that some other factors determine the presence of species in alkaline waters.

Evidence has been accumulating that factors other than the amount of calcium present in soils determines the calcicole and calcifuge habit in plants. Balme (1953) suggested that calcicoles had become adapted to the specialis conditions in calcareous soils such as the high pH; high calcium ion concentration, low basic ratio and deficiency of ions such as potassium, magnesium and phosphate. The extreme calcifuges were adapted to an acid pH; high ferric ion concentration and nitrogen in the form of ammonia. Intermediate between these two groups were those species which were succes ful in less specialised conditions.

Experiments carried out under optimal conditions, and therefore interpreted with caution, have indicat that the Saprolegniales are not affected by the amount of calcium present. Members of the "acid" group were able to tolerate a lower pH than the other species. The "alkaline" group species were found to be sensitive to increasing phosphate concentrations. It is possible that some of the "alkaline" species, although showing a wide range of pH. tolerance, could be confined to waters with low phosphate concentration. In soils containing great amounts of calcium carbonate and which are of an alkaline pH, it is known that phosphate is unavailable.

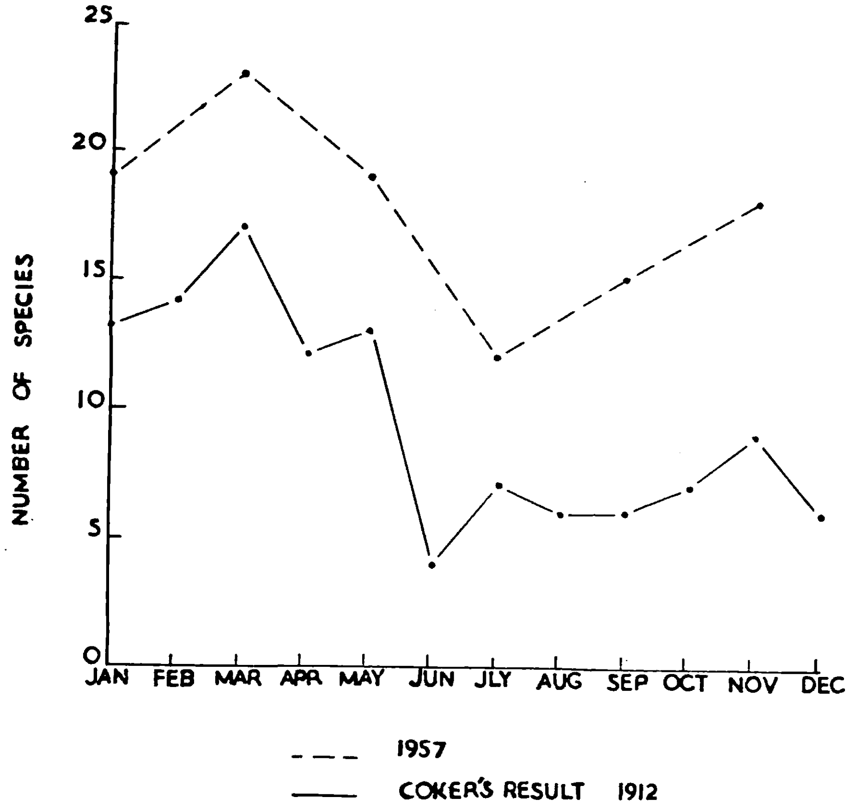
There is a possibility that the content of the water is not the deciding factor in the distribution of these fungi. The fungi live on the debris in the water and the type of decaying vegetation found in acid waters as opposed to that in alkaline waters may be the factor that decides their distribution. Bacteria generally inhabit waters with a basic pH and in alkaline waters these would be responsible for the decomposition of vegetation. In acid conditions the fungi play a more active part in the decay of debris. The acid species may be capable of breaking down debris, while the alkaline species live on secondary products.

Seasonal Variation.

On the whole, the observations on seasonal variation, as described by Coker (1912) and Forbes (1935) agree with the above account. The number of species Coker found in each month throughout the year 1912 in U.S.A. is shown in Graph 14, in which the present results (Graph4) have been superimposed. It can be seen that the two result are comparable, and in both cases the number of species fou during the warm season was low. In the Autumn, the numbers began to build up again, but it was not until the spring that the greatest number of species was found.

On examining the percentage abundance of every species found by Coker each month, it was found that these species could be divided into "summer", "winter" and "constant" groups. With the notable exception of Saprolegnia ferax, his "winter", "summer" and "constant" groups include the same spécies as in this investigation. Forbes' figures for the percentage occurrence for Saprolegnia ferax indicates that this species is a "summer" species in Englar and not a "winter" species as it appears to be in North America.

Forbes' results appear to be in agreement with those found in this investigation. Unfortunately, sampling did not take place after May 1935 and it is unknow whether two of her species Achlya radiosa and Achlya racemo



Graph 14 Total number of species found throughout the year 1912 by Coker. The results obtained from all the habitats sampled in 1957 has been superimposed.

behaved as "constant" species or members of another group. It would have been interesting to compare results for the summer months with those obtained above.

It has been found that the method of sampling used in this investigation and also that of Forbes did not give a good indication of the abundance of species such as Achlya glomerata and Achlya radiosa. These species occur sporadically , probably because their zoospores do not swim far before encysting and so do not become well distributed through the water.

When the Saprolegniales from various habitats are described, it appears important to note the time of year at which the collection is made. The absence of species from a list may be due to the time when the habitat was sampled. A sampling in summer would yield a different fungus flora from one in winter. Ideally, the habitat should be sampled at least four times a year before a list of watermoulds found there is drawn up.

In this work, the effect of temperature on zoospore production was investigated to ascertain if species of the three groups showed any difference in the behaviour of their zoospores. Other workers, studying the effect of temperature on zoospore production, have given temperature ranges for other members of the Saprolegniales.

The optimum temperature for zoospore production in _Saprolegnia anisospora_ given by Coker (1923) is what would be expected for a "winter" species. This result is in close accordance with that found by Newby (1948). Coker's result for _Achlya apiculata_ is also not surprising for a "constant" species. The optimum for zoospore production of other species belonging to the "winter" is higher than expected. Klebs (1899) gives the optimum for _Saprolegnia mixta_ as between 24° and 28° C. Coker shows that _Isoachlya toruloides_ has an optimum at 26°C. The optimum, according to Cotner (1930) for zoospore production in _Saprolegnia monoica var. glomerata_ is also at this temperature. This result is higher than that obtained for the strain of _Saprolegnia monoica_ used in the above experiments. The optimum temperature for _Aphanomyces eutiches_ (26°- 28°C) was also higher than that given by Jones and Drechsler (20°C) in 1925.

It is unlikely that zoospore production depends merely on temperature, although Salvin (1941) did show that first and second stage swarmers were affected differently, and the second stage swarmers were active over a narrower pH. range. This could cause a differentiation between the three groups since different temperatures could affect the number of zoospores that survive once they have left their cysts. Salvin's work (1940) showed that spore germination was also influenced by the amount of oxygen and nutrient present. The problem of zoospore production is likely to be very complex and more work is needed before it would be possible to say how great an effect the production of zoospores has upon seasonal variation.

SUMMARY.

1. Members of the Saprolegniales collected from natural waters in Great Britain between 1950 and 1958, fall into three groups - "acid","alkaline" and "neutral" according to the hydrogen ion concentration of the waters in which they occur. These observations are also in harmony with those obtained by Lund in Denmark (1935)

2. There may be a seasonal distribution and the species collected can be divided into "winter", "summer" and "constant" species. The distribution found in this country agrees generally with that found by Coker in North America and Forbes in Great Britain.

3. A study of the growth of selected species in culture indicated that temperature and hydrogen ion concentration are important factors in controlling growth and zoospore production of these fungi.

4. It has been shown that many species are sensitive to phosphate concentration, hence the use of buffer standards containing phosphate must be avoided unless the effect of phosphate on the species being studied is known.

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