A QUANTITATIVE ECOLOGICAL STUDY OF AQUATIC FUNGI FROM BROADCOVE RIVER WITH EMPHASIS ON REPRESENTATIVES OF THE GENUS SAPROLEGNIA

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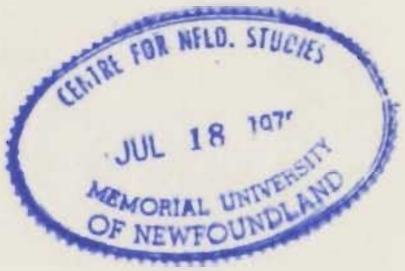
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A QUANTITATIVE ECOLOGICAL STUDY OF AQUATIC FUNGI FROM BROADCOVE RIVER WITH EMPHASIS ON REPRESENTATIVES OF THE GENUS SAPROLEGNIA

by

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ABSTRACT

The fluctuation in the level of representatives of species belonging to the fungal Order Saprolegniales was monitored in Broadcove River over a one year period. Some physical and chemical parameters of the stream were also monitored.

The technique used was designed to provide a quantitative estimate of the number of fungal propagules suspended in the water. Attempts were made to minimize the loss of the viable propagules during processing of the sample. The number of these viable propagules was assumed to give an indication of the level of fungal growth or reproductive activity in the stream just prior to or at the time of collecting the water sample.

Centrifugation, resuspension and plating of the sample on a medium selective for species of the genus Saprolegnia Nees v. Esenbeck were followed by the rapid isolation and subculturing of the colonies as they appeared. Further processing was carried out to obtain sexual structures of the fungi in pure culture for purposes of identification.

Temperature, level of trace elements, sodium, phosphates, dissolved oxygen and total carbon content of the water (chemical oxidation demand) were found to have an effect on the total number of propagules, and on the

number of individual species, in some cases. However, at the lowest recorded temperature, the total number of propagules was reduced regardless of other environmental conditions.

The species fell into three main groups with relation to the temperature of the stream at which their maximum abundance occurred. A small number of species, the winter species, was most frequent when the stream temperature was from 1.5° to 2.5°C. The most numerous category was made up by the intermediate temperature species, or those species which were recorded most frequently at 13°C. Another small group of species, the warm weather species, was most abundant when the stream temperature was 17°C.

Occurrence of heavy rains in spring (March) and autumn (September) resulted in an increase in the level of trace elements in the stream and coincided with the isolation of species not otherwise recorded. Resting propagules of these species washed in from the surrounding soil or present in the water may have been stimulated to germinate and grow by the increased level of micronutrients.

ACKNOWLEDGMENTS

I should like to take this opportunity to thank Dr. Nolan, my supervisor, for his encouraging attitude and faith throughout the study, and for his help in the preparation of this thesis. I am also grateful to Dr. Moskovits and Dr. Brassard, of my supervisory committee, for their interest and advice on the research, and for their comments on the thesis progress reports.

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INTRODUCTION

A. Literature Review

The genus Saprolegnia Nees v. Esenbeck, together with other closely related genera, has been classified by Alexopoulos (1962) as belonging to the Sub-division Eumycotina, Class Oomycetes, Family Saprolegniaceae, Order Saprolegniales. However, the existence of these water moulds has been known for a long time. The earliest description of a species of the Order Saprolegniales was made in 1821 (Coker 1923).

Since that time, numerous accounts describing new genera and species, as well as first records of their occurrence in different localities, have been published (for example, Petersen 1910, Denmark; Coker 1923, 1927, North Carolina, USA; Nagai 1931, Japan; Forbes 1935, Britain; Chaudhuri and Banerjee 1942, India; Beverwijk 1948, The Netherlands; Goldsmith 1948, South Africa; Moreau and Moreau 1948, France; Ziegler 1958, Florida, USA; Howard et al. 1970, Iceland).

Developmental research was carried out at the same time as the taxonomic studies in order to determine the life cycles of the organisms. By growing the fungi on several substrates, various parts of the life cycle could be observed, and the sexual and asexual forms correlated for each isolate to form generic and species concepts (Coker 1923). Changes in the classification of these organisms have occurred as new facts come to light and as techniques

become more refined allowing researchers to use additional characteristics, such as ultrastructural features, for taxonomic purposes (Dick 1969a).

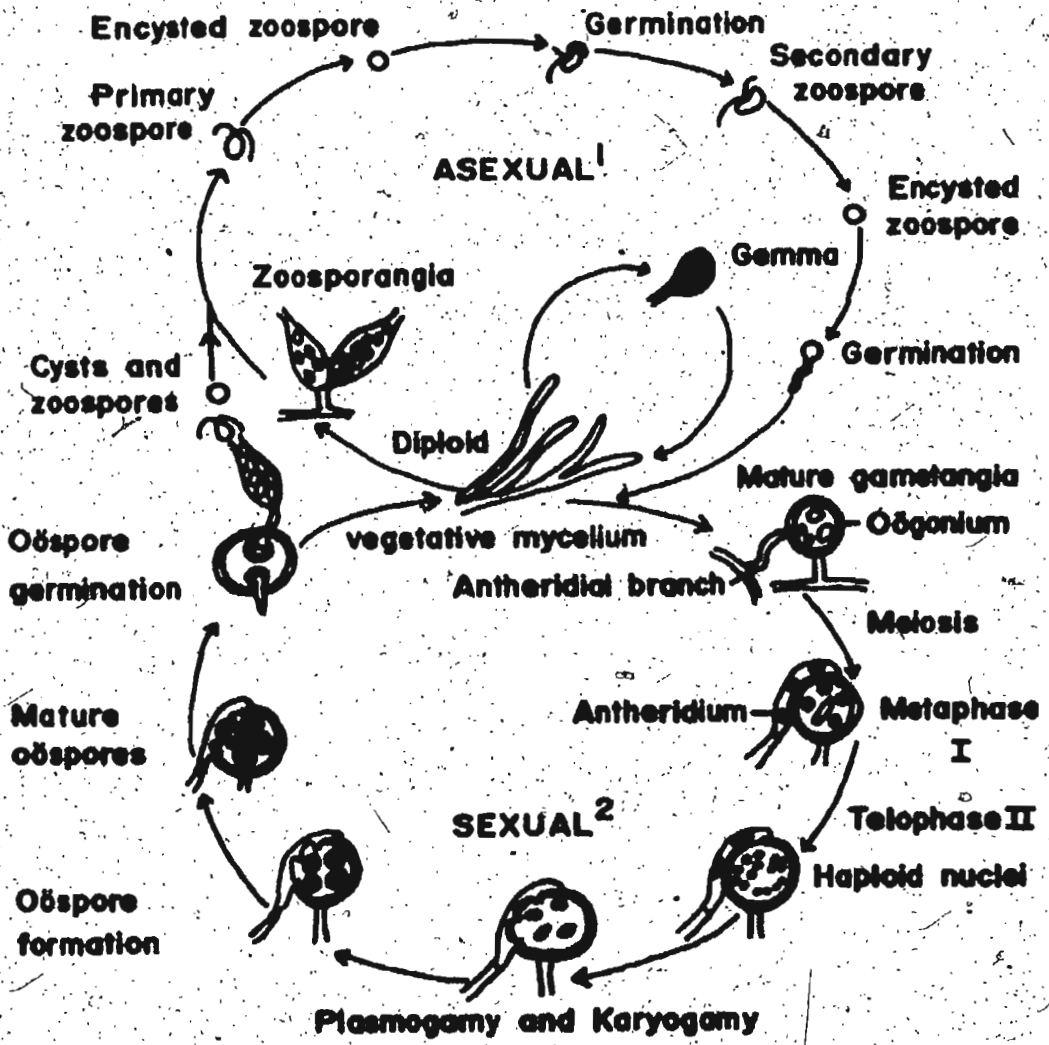
Klebs pioneered physiological studies within this group working with an isolate of Saprolegnia ferax (Gruith) Thuret in 1899. The results of his work, summarized by Coker (1923), give the procedures necessary to induce any part of the life cycle of S. ferax. His techniques have been adapted for use with other species of the aquatic fungi. Thus, these species can be manipulated into an active sexual, asexual or vegetative phase. The generalized life cycle of a species of Saprolegnia is given in Fig. 1.

Most of the species of the Order Saprolegniales are saprophytic on aquatic animal and plant debris, and some are parasitic (Sparrow 1960). In an aquatic system they are, therefore, present on plant substrata such as fallen twigs (Lund 1934) as well as on dead animals including fish, toads, frogs, leeches, earthworms and crayfish (Petersen 1910). There is also ample evidence of their parasitism on fresh water animals. Frog eggs, spawn of toads, fish, fish wounds and fish eggs are attacked by representatives of the Saprolegniaceae (Petersen 1910, Sparrow 1960). Salmon, trout, char (Willoughby 1969), perch (Willoughby 1970), eels and lampreys (Willoughby 1971) are also subject to infection by the aquatic fungi. Infected blackfly pupae (Nolan and Lewis 1974) and nematodes (Nolan 1975) have been collected

Figure 1. Generalized life cycle of a Saprolegnia sp.

[1]From Alexopoulos (1962).

[2]From Flanagan (1970).


Encysted zoospore
Germination
Secondary zoospore
Primary zoospore
ASEXUAL¹
Encysted zoospore
Gemma
Zoosporangia
Germination
Cysts and zoospores
Diploid
Mature gametangia
Oögonium
Oöspore germination
vegetative mycelium
Antheridial branch
Meiosis
Antheridium
Metaphase I
Mature oöspores
SEXUAL²
Telophase II
Haploid nuclei
Oöspore formation
Plasmogamy and Karyogamy

from streams in eastern Newfoundland. Larvae of insects with visible fungal growth have been found in Broadcove River, the sampling site for this study. The members of the Saprolegniales are, therefore, active in a body of water as members of the decomposer cycle. There is an exception to this, in that the species of Saprolegnia and Achlya Nees v. Esenbeck are absent from heavily polluted water and are rarely found in partially polluted areas (Harvey 1952). Of the true aquatic fungi, only representatives of the genus Aphanomyces de Bary show any affinity for polluted water (Harvey 1952).

The above hosts or substrates are direct sources from which aquatic fungi can be obtained for subsequent study. These substrates are colonized by the zoospores of water moulds (Roberts 1963). Consequently, the presence of a fungus on the material indicates that the zoospores of that fungus were present in the water, and that it had recently gone through an asexual stage (see Fig. 1). Gemmae, mycelial fragments or even oospores might also conceivably be involved in colonizing these substrates, and therefore in the propagation of a fungus. Investigators interested in learning about the occurrence of these fungi have either collected them directly from an already infected source in the water (for example, Lund 1934), or used baits (i.e. suitable substrates) as a means of "trapping" these water moulds. The use of a baiting technique was mentioned as early as 1887 by Zopf (Sparrow 1960). Suitable baits

include dead insects and insect larvae, dead fish, twigs, flowers, fruits and hemp seeds (Sparrow 1960). Baiting or "trapping" has been the method used by researchers up to the present time to record the presence of, and give relative frequencies for, fresh-water fungi in general.

Petersen (1910) correlated for the first time seasonal periodicity, environmental factors, and the occurrence of fresh-water fungi. He found spring to be the season in which most aquatic fungi could be collected; but he observed that summer, even with an abundance of vegetation and animal life, was not favourable for fungal growth on submerged vegetation. Coker (1923) conducted a two year study during which 593 collections were made. He also found that, for the great majority of species collected, spring yielded the higher number of isolates.

Waterhouse (1942) detected a seasonal rhythm in moulds collected from a tributary of the Thames River in England. Using baits, she found that the fungi appeared in September, increased to a maximum in December, January and February, then disappeared in the summer. The autumnal rise in numbers was attributed to increased rainfall, abundance of suitable substrates falling from trees and a decline in temperature. The scarcity of water moulds in the summer was ascribed to higher temperatures and falling water level.

This seasonal periodicity, with maxima in spring and autumn, has been noted by many subsequent investigators.

Perrott (1960) attributed it to water temperature, suggesting that the latter is of importance in inducing asexual reproduction and vigorous vegetative growth. A fungus thus affected by the seasonal temperature would be producing numerous zoospores and would, therefore, be recorded as being present in higher numbers.

Dick and Newby (1961) also found seasonal fluctuation, with spring and autumn maxima and lows in June and July, for members of the Saprolegniaceae from the soil. Examples of fluctuation were also seen for individual species. Pythiopsis cymosa de Bary was found to have a minor autumn peak, a large spring maximum and almost total absence in the summer months.

The results of the above investigators agreed with those of Roberts (1963) in that the number of species found during the summer was low, with the numbers again increasing in autumn and reaching a peak in spring. The periodicity of the members of the Saprolegniales was said to depend on the production of zoospores, a part of development influenced by the temperature of the different seasons (Roberts 1963).

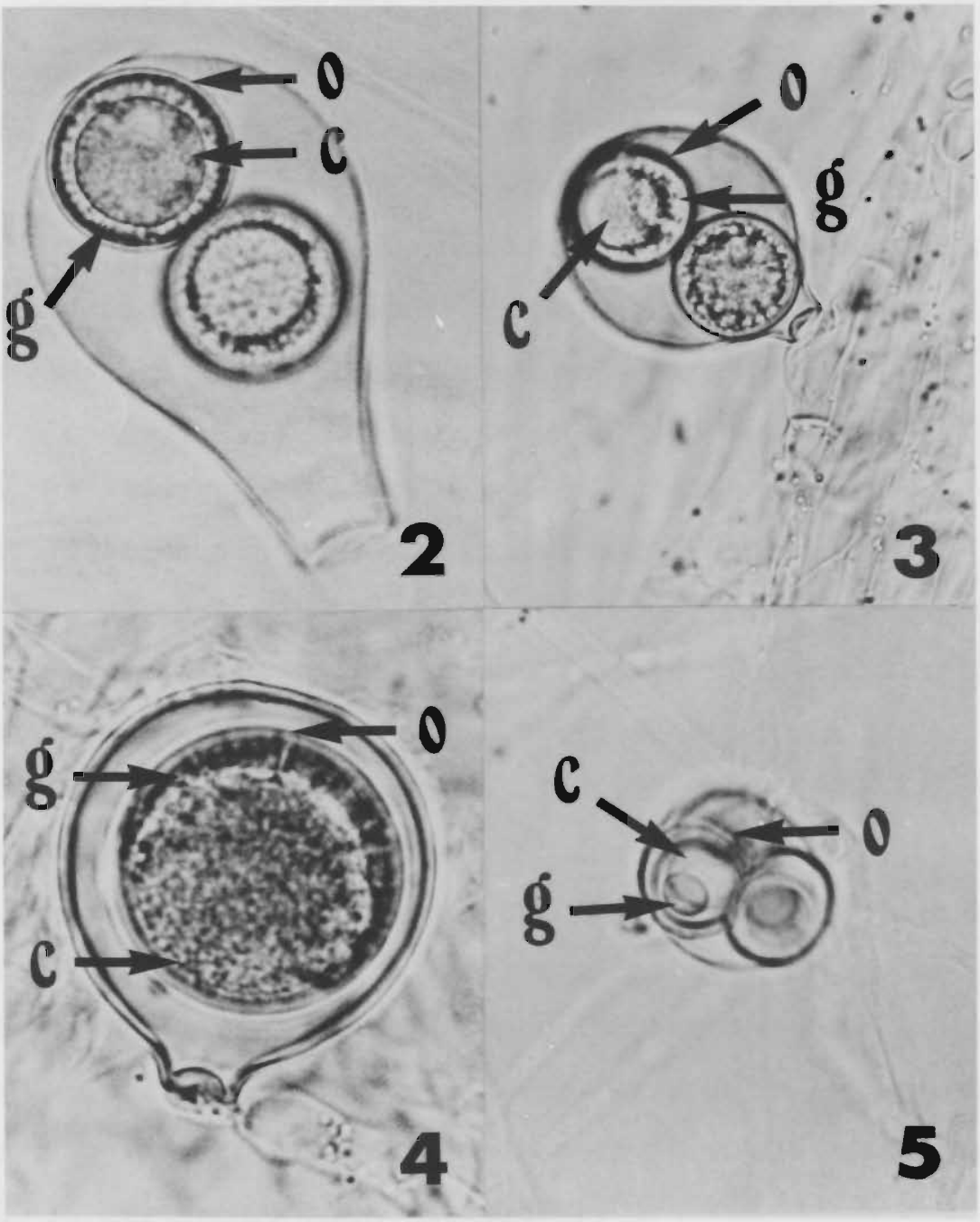
Hughes (1962) suggested dividing the Family Saprolegniaceae into two groups with regard to morphological type and yearly occurrence. One group with centric or subcentric oospores (Fig. 2, 3, 4) had a noticeable seasonal periodicity, whereas some species which form eccentric oospores (Fig. 5) had no discernible periodicity pattern. Species of

Figure 2. Centric oospore. The oospore (o) has one or more layers of oil globules (g) which completely surround the central protoplasm (c). x 1150.

Figure 3. Subcentric oospore, type I. All the oil globule layers (g) in this oospore (o) do not completely surround the central protoplasm (c). x 550.

Figure 4. Subcentric oospore, type II. The layers of oil globules (g) in this oospore (o) do not go completely around the central protoplasm (c). x 1180.

Figure 5. Eccentric oospore. The oil is in one large drop (g) and lies outside of the central protoplasm (c) in this type of oospore (o). x 690.


o
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Saprolegnia which are mainly of the centric or subcentric type were most commonly isolated when the water temperature fell between 7° to 21°C. The temperature ranges into which the highest percentage of a specific oospore type fell were: 7° to 21°C, centric; 7° to 31°C, subcentric; 7° to 37°, eccentric. In a separate set of investigations, Toma (1973) was also able to divide the members of the Saprolegniaceae which he isolated into three natural groups, correlating temperature at which the collection was made with the most prominent oospore type. In cold temperatures (0° to 10°C), most of the species had centric or subcentric oospores. Temperatures from 15° to 30°C yielded mainly species with eccentric oospores. A third group consisting of both centric and eccentric oospore types was found to be apparently indifferent to temperature. These results seem to be supported by a general survey of this type of work being carried out in a wide variety of locations. Hughes (1962) listed the results of many researchers from Latvia (northern latitude 57°) to Puerto Rico (northern latitude 18°). The percentage of centric and subcentric species found appeared to decrease as one went further south, whereas the percentage of eccentric species increased, reaching 98.9% in Puerto Rico and 100% in Haiti (northern latitude 19°).

The findings of Alabi (1971a) corroborate the above. Members of the Saprolegniaceae with eccentric oospores were found all year round in Nigeria. In contrast, species with

subcentric and centric types occurred only during the rainy season. This may suggest seasonal fluctuation of the latter.

The pH in lakes, streams and rivers is a normally varying factor. It depends on the surrounding and underlying soil, rock composition of the area, vegetation and rainfall (Alabi 1971b). Lund (1934) divided aquatic habitats into five categories according to their pH, ranging from highly acid to constantly alkaline. He then collected aquatic fungi from all the localities selected in order to determine whether pH had any effect on the occurrence of the individual species. Many of the fresh-water fungi, such as Saprolegnia ferax, were found to occur in all of the samples. Some species, however, were found preferentially in either acid waters (pH 3.5 to 6.8), for example, Saprolegnia diclina Humphrey, or in alkaline waters (pH 6.5 to 8.4), for example, Saprolegnia asterophora de Bary.

Perrott (1960) found that the species composition of fungal populations was different at each of four collecting stations of different pH. Roberts (1963) was able to divide the aquatic fungi which he had collected into three groups: acid, alkaline and neutral. The fungi fell into these groups as species, with no correlation at the generic level. He found the greatest number of species between pH 5.6 to 7.4. Dick (1963) observed that moderately acid soils had a greater number of species than highly acidic or slightly alkaline soils. His results agree with those of Roberts.

(1963) in that some species are found in characteristic pH ranges. However, pH alone is not sufficient to determine the occurrence of members of the Saprolegniaceae (Dick 1963, Roberts 1963).

The previously mentioned studies all used baiting techniques to determine the presence of zoospores or other fungal propagules in fresh water. The practice of baiting, though, does not give a true picture of the numbers present, but rather of the colonizing ability of the zoospores (Cooke 1961). Species which were present but had no zoosporangia at the time would go unrecorded. The type of bait used also gives a differing impression of the species present (Dick 1966). One major weakness of the hemp seed baiting technique, and presumably true of other baits as well, is that in order for hyphae of the saprolegniaceous fungi to become firmly established on the hemp seed, the two must be in contact for several hours. During this time, resting propagules such as encysted zoospores or gemmae of the Saprolegniaceae could produce sporangia, which in turn might germinate and release zoospores. This would give an indeterminate rise in inoculum potential (Dick 1966). Another disadvantage of this method is that baits attractive to the zoospores of aquatic fungi are also suitable for the growth of protozoans and bacteria. This may result in the profuse but undesirable growth of the latter two types of organisms on the bait (Johnson 1956).

The first attempts to produce a more accurate indication of the population size of water moulds was made by Willoughby in England (Willoughby 1962). He incorporated water samples into agar and submerged the sections of the solidified agar in sterile water. This would increase the possibility of obtaining growth of mainly aquatic fungi. By noting the nature of hyphal branching and type of dehiscence, if present, of the growth fringes, he could assign the resultant growth to a genus. Oogonium production was very infrequent, so species level identifications were impossible. This method gave a numerical indication of the genera present in the aquatic habitat studied. Ji and Dayal (1966) used the same technique in India, and came up with slightly different results in that the genus Achlya was the most numerous. Willoughby (1962) had found Saprolegnia to be the most abundant genus. He suggested that the number of growth fringes was a direct reflection of the number of zoospores, rather than gemmae, oogonia, or mycelial fragments, present in the water (see Fig. 1).

B. Purpose

This study was aimed at obtaining an accurate, quantitative estimate of the number of propagules of representatives of the genus Saprolegnia, and perhaps other closely related genera, present in Broadcove River.

The procedure was designed such that every propagule

present in a given volume of water would have an adequate opportunity to germinate, be isolated and identified. Loss of propagules in the interval between collection and processing was minimized by collecting a larger volume of water than was needed so as to maintain the water sample at stream temperature during that time. The sample was processed in as short an elapsed time as possible, as a decrease in the recorded number of members of the Saprolegniales occurs two hours after the time of collection (Willoughby 1962). The water was centrifuged to concentrate the fungal contents (zoospores, hyphal fragments, oogonia, etc.), then resuspended in a volume of sterile water to give an appropriate dilution of the propagules.

The water sample was not incorporated into warm agar, but was spread on a prepoured, solidified agar surface with a cooled, alcohol flamed glass rod (Klein and Wu 1974). This ensured that any heat sensitive propagules would not be eliminated. Fungi particularly sensitive to lack of oxygen would not encounter any problems on the surface of the agar. Streak plates have been shown to yield a higher count of viable colonies than pour plates, even with samples taken from the same suspension of bacteria (Soestbergen and Lee 1969).

The agar medium chosen was especially selective for species of Saprolegnia (Ho 1975). An antibiotic mixture

retarded the growth of bacteria; the chemical pentachloronitrobenzene (PCNB) delayed the growth of terrestrial fungi. Thus, spores of terrestrial fungi (considered alien, but nevertheless present in an aquatic environment) were inhibited in their germination and potential growth. This decreased the possibility of terrestrial fungi inhibiting the growth of the less vigorous aquatic fungi. The incubation temperature was also a selective force in favour of the water moulds. It was chosen as a result of physiological studies done on aquatic fungi isolated from streams in eastern Newfoundland. Pythiopsis cymosa was found to grow well within the range of temperatures from 12° to 17°C (Nolan and Lewis 1974). Saprolegnia megasperma Coker grew equally well at 13°, 16° and 19°C (Nolan 1975). Saprolegnia ferax, though having an optimum temperature for growth near 24°C, still showed adequate growth at 13°C (Nolan 1976). This latter temperature is lower than the optimum for most terrestrial fungi and would, therefore, favour aquatic fungi. It would also allow for the growth of water moulds regardless of the time of year at which the collection was made.

The interval from plating of the sample to growth of bacteria and terrestrial fungi was lengthened by preventing excess contamination throughout collection and processing. For a given sample size on a given collection date, the lower

the number of spores of terrestrial fungi and bacteria present in each plate, the more effective the PCNB and antibiotics were felt to have been. Once these were used up, undesirable growth could proliferate over the agar surface. Thus, efforts were directed at allowing all the aquatic propagules to germinate and be isolated before this occurred.

Through this method, it is hoped that any saprolegniaceous propagule present in the water will form a single colony. Daily observations of the inoculated plates indicated the development of new colonies, and these were immediately isolated for further processing and identification.

At the time of each collection, temperature, pH and several other chemical parameters of the water were measured in order to determine whether fluctuations in these were reflected in the occurrence of species of Saprolegnia, and of other closely related genera. There is one difference in the manner of recording the influence of pH on occurrence, as carried out in this study, from that commonly used. Instead of choosing different localities with varying pH and studying the flora present at these sites (Lund 1934, Perrott 1960, Dick 1963, Roberts 1963), the method used entailed sampling the same site a total of eight times throughout the year.

MATERIALS AND METHODS

A. Collecting of samples

A collection was made every 56 days (± two or three days) from 1 December, 1975, to 31 December, 1976 (see Table I for collection dates). A twenty litre plastic container (Fig. 6) was first rinsed with seventy percent ethanol in order to remove accumulated spores of bacteria and terrestrial fungi. It was then washed and rinsed several times with water from Broadcove River immediately before sample collection. This was done downstream from the collection site. The container was opened under water at the collection site with its large opening towards the flow of water. The spigot was also opened, allowing water to flow through. When twenty litres had been collected, the cap and spigot were tightly shut under the water before transporting to the laboratory. Meanwhile, the temperature of the stream was recorded and water samples were obtained for chemical analyses. Five Whirl-Pak plastic bags were also filled with water and sealed for pH determinations. The parameters of the stream measured are listed in Table II.

Preliminary investigations indicated that the propagule level in the water was too low to be determined by direct plating of the water sample. Centrifugation of a large volume was, therefore, indicated. Twenty litres gave an overwhelming number of isolates, so that the sample

TABLE I

COLLECTION DATES

Collection number	Date
1	1 December, 1975
2	26 January, 1976
3	22 March, 1976
4	17 May, 1976
5	14 July, 1976
6	7 September, 1976
7	1 November, 1976
8	31 December, 1976

TABLE II

PARAMETERS OF BROADCOVE RIVER MEASURED[a]

Hardness
Kjeldhal nitrogen
Nitrate (NO_3)
Total phosphorus ($T\text{-}PO_4$)
Chemical oxidation demand (COD)
Calcium (Ca)
Magnesium (Mg)
Iron (Fe)
Manganese (Mn)
Copper (Cu)
Zinc (Zn)
Cadmium (Cd)
Sodium (Na)
Potassium (K)
Silicate (SiO_2)
Dissolved oxygen (DO)
Sulphide (S)
pH
Temperature

[a]All parameters excepting temperature and pH were analysed by the Water Analysis Facility, Chemistry Department, Memorial University of Newfoundland.

volume was set at five litres. The actual amount of water brought back to the laboratory was fifteen to twenty litres in order to maintain the sample at stream temperature until centrifugation was carried out.

B. Processing of the water samples

1. Biological analysis

A continuous flow plankton centrifuge (Foerst Mechanical Co., Chicago, Ill.) with a detachable cup and cover was used (Fig. 7, 8). The removable parts, including the screw, were wrapped in Dennisonwrap and autoclaved. They were stored in the freezer compartment of a refrigerator at -24°C for several days prior to use. The centrifuge was set up as shown in Fig. 9 in a cold room (4°C). These precautions were taken to prevent the heating up of the metal parts of the centrifuge during centrifugation, thus protecting the heat sensitive propagules in the water. Five litres were processed at a flow rate of 140 millilitres per minute, a rate achieved by adjusting the flow from the container to as small a volume as possible without stopping the flow altogether.

The centrifugal force of the centrifuge was estimated with the use of a Cenco Xenon stroboscope (Central Scientific Co., Mississauga, Ont.), which gave the number of revolutions per minute of the cup. The following formula for

Figure 6. Collection container (capacity: twenty litres).

Figure 7. Continuous flow plankton centrifuge (thirty-five centimetres high).

Figure 8. Continuous flow plankton centrifuge with removable cover (c).

Figure 9. Equipment as set up for processing of a water sample.

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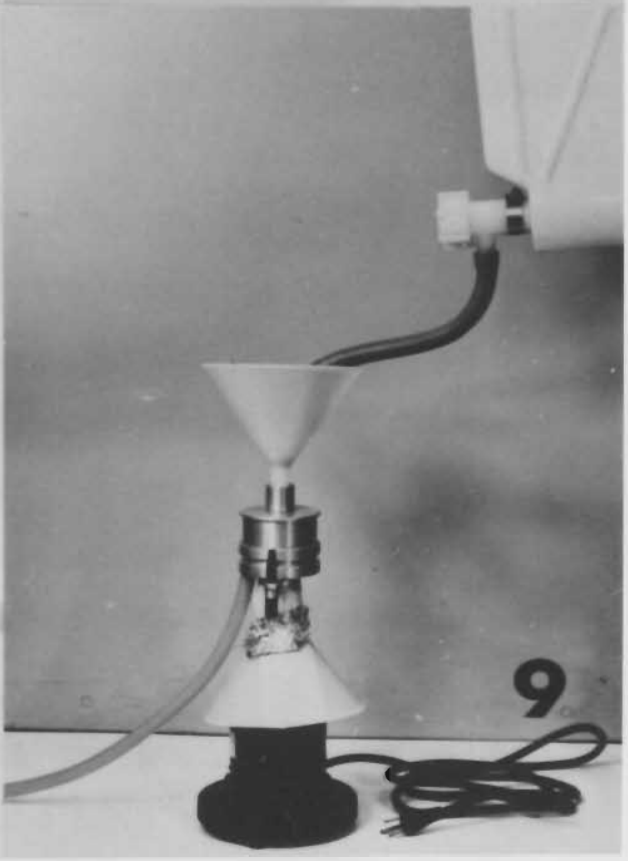

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centrifugal force (RCF) was used:

$$RCF = 0.0000284 \times R \times N^2$$

where R is the radius in inches from the centre of the cup; N is the number of revolutions per minute (an average of three values in this case). Therefore,

$$RCF = 0.0000284 \times 0.95 \times 13,287$$

giving a centrifugal force of 3,585 x g.

It should be noted that this value is higher than that which was in effect during the actual centrifugation of the sample. It was technically not possible to determine the revolutions per minute of the motor while the water was running through, although the motor audibly decreased in speed once processing commenced.

The condensed sample from the five litres was taken to a transfer hood and resuspended with one hundred millilitres of sterile, distilled water. Plates poured with the solidified, selective medium for isolation of species of Saprolegnia (Ho 1975), as listed in Table III, were each inoculated with one millilitre of the resuspended sample. Glass rods flamed with seventy percent ethanol and allowed to cool (Klein and Wu 1974) were used to spread the water evenly over the surface of the agar. The plates were incubated at 13°C. They were kept in the right side up position for about two days until the surface was dry, and then inverted. A wet surface would have been conducive to

TABLE III

A SELECTIVE MEDIUM FOR ISOLATION OF

SAPROLEGNIA SPP. FROM FRESH-WATER

Emerson's yeast-extract, soluble-starch agar (EYPSS)	40.5	g
[a]Penicillin	200,000	I.U.
[a]Streptomycin	200,000	ug
Pentachloronitrobenzene (PCNB)	400	mg
Distilled water	1000	ml

[a]As a lyophilized penicillin-streptomycin mixture (Grand Island Biological Company, Grand Island, New York).

the spread of bacterial colonies and spores.

By holding these plates up to the light, it was possible to detect colonies as they appeared. These minute colonies were cut out along with a small block of surrounding agar. The area of the block was kept as small as possible to avoid a secondary growth later if another colony as yet invisible to the naked eye, or an ungerminated propagule, was nearby. Isolated colonies were transferred individually to plates of Emerson's yeast-extract, soluble-starch agar medium or EYPSS (Difco) containing a streptomycin-penicillin mixture (same concentration as the isolation medium listed in Table III). When the growth had covered approximately three-quarters of the plate, a single hyphal tip was removed and subcultured to another petri dish containing the same medium. This yielded unifungal and often pure cultures, as the antibiotic mixture slowed down the growth of bacteria, leaving some of the hyphal tips bacteria free. When bacterial contamination persisted through several hyphal tip transfers, Raper's ring method was used (Raper 1937), usually with success. If the latter method was not successful, 0.5 g. of potassium tellurite (Difco) were incorporated into one litre of EYPSS medium as an alternate means of removing bacteria. When a pure culture was obtained, a hyphal tip was transferred to a culture tube containing EYPSS agar. These tubes were stored at 10°C, awaiting further processing and

identification.

2. Physical and chemical analyses

While the water sample was being centrifuged, the pH of the five water samples in the Whirl-Paks was measured using a Corning pH meter (Model 7). An average value was obtained which would cover individual variation of each sample.

The water samples obtained for chemical analyses were taken to the Water Analysis Facility (Chemistry Department, Memorial University of Newfoundland), where the required tests were performed.

C. Identification

In order to identify the isolates, the formation of sexual structures had to be induced using the techniques basically devised by Klebs (Coker 1923). The fungi were grown on EYPSS plates and a hyphal tip was transferred from each to a 125 ml Bellco flask containing 50 ml of sterilized "Vittatum A" medium (Vit. A). The composition of this medium (Nolan 1975) is given in Table IV. The inoculated flasks were incubated on a horizontal, gyratory shaker (Psychrotherm, model G-26, New Brunswick Scientific Co., New Brunswick, New Jersey) at 150 rpm and 13°C, in darkness. They were left there for three days, or until a suitable mycelial ball had grown. This ball was transferred to a

TABLE IV

THE COMPOSITION OF "VITTATUM A" MEDIUM[a]

Glucose (g/l)	5
Lactalbumin hydrolysate (g/l)	2
Yeast extract (g/l)	0.5
KH_2PO_4 (ml/l)	19.8[c]
K_2HPO_4 (ml/l)	0.2[c]
Micronutrient solution	
Mn (as $MnCl_2.4H_2O$)	0.009
Zn (as $ZnSO_4.7H_2O$)	0.001
Cu (as $CuSO_4.5H_2O$)	0.001
Fe (as $FeCl_3.6H_2O$)	0.018
Mo (as $Na_2MoO_4.2H_2O$)	0.002
Co (as $CoCl_2.6H_2O$)	0.003
B (as H_3BO_3)	0.046
EDTA[b] (as $Na_2EDTA.2H_2O$)	0.032

[a]Final pH 5.6. All concentrations in mM unless otherwise stated.

[b]EDTA: Ethylenediaminetetraacetic acid.

[c]Total phosphate concentration was 20 mM.

crystallizing dish containing 225 ml of sterile, distilled water and incubated at 13°C.

The fungi were examined microscopically after three weeks to determine whether the sexual cycle had begun. Water was used in making the slides, so that any zoospore discharge or other processes occurring might be observed. The identification of the species of the Saprolegniaceae is still dependent on the size, number and physical characteristics of their sexual structures (Seymour 1970). Classification was accomplished using Seymour's key for the species of Saprolegnia (Seymour 1970). When an isolate was observed to be of a different genus through the nature of its sporangia or hyphae, then other sources were consulted (Coker 1923, Johnson 1956, Sparrow 1960, Scott 1961, and others).

The diameters of fifty to eighty oogonia and of their oospores were measured; type and number of oospores as well as the nature of the oogonial wall, antheridial branch origin and type of antheridial cell were recorded for each oogonium. Size and type of sporangia, if present, and the size of the encysted zoospores were also noted for each isolate. When familiarity with the isolates increased, then the number of oogonia measured was lowered to from ten to twenty. Additional characters used were the diameter of hyphae and any peculiarities which might aid in identifying the

isolate.

Computer analysis of the measurements was carried out to determine whether isolates relegated to a given species differed significantly from one another. The programme used gave confidence intervals (Natrella 1972) about the means for the diameters of the oogonia and oospores. These aided in deciding whether two isolates with similar morphological characteristics could be placed in the same taxonomic group. A combination of the physical characteristics and their sizes was used in making the final decision.

If no sexual or asexual structures had been formed in two months of incubation in the sterile, distilled water, the fungus was considered sterile. When the fungus failed to enter the sexual phase of the life cycle in a similar time period, the isolate was classed as sexually sterile. The waiting period was lessened to from one to one and a half months because of the amount of time available in which to complete the study, coupled with a limited number of crystallizing dishes available for use.

Although non-aquatic fungi were initially delayed in their germination and development by the PCNB on the isolation plates, the chemical was presumably changed to a non-inhibitory form after a few days. Colonies of terrestrial fungi would then start to appear in ever increasing numbers. These colonies had to be isolated on

appearance, being too small to be distinguished as non-aquatic. All colonies were thus isolated until the plate became overgrown with terrestrial fungi, which sporulate rapidly and so increase their numbers, or with aquatic fungi. To distinguish between true and non-aquatic fungi, septation of the hyphae was used as the main dividing feature. Members of the Saprolegniales do not form septa except to delimit reproductive organs (Sparrow 1960). Any septate fungi were recorded as such and discarded. By definition, then, members of the higher Families and Orders of the aquatic fungi which do form regularly spaced and abundant septa were not included in this study.

RESULTS

A. Seasonal fluctuation of certain parameters of water from Broadcove River

1. Chemical parameters

The data resulting from the water analyses (Table V) indicate the different concentrations recorded for each parameter at each collection.

Hardness, magnesium and potassium levels showed a similar fluctuation pattern. The level of hardness varied from 7.86 to 9.89 mg $CaCO_3$/l for most of the samples, with the exception of three peaks. These were observed in the 22 March sample (10.86 mg $CaCO_3$/l), in the 7 September sample (11.21 mg $CaCO_3$/l) and in the 31 December sample (12.51 mg $CaCO_3$/l). Magnesium concentrations fluctuated between 0.838 and 0.920 mg/l for most of the samples with three peaks also being observed. The peaks occurred in the March sample (1.058 mg/l), in the September sample (0.946 mg/l) and the final December sample (0.963 mg/l). Potassium levels were found to range from 0.443 to 0.546 mg/l in all samples excepting those collected in March, September and December, 1976. These latter collections yielded peaks of 0.656 mg/l, 0.691 mg/l and 0.623 mg/l, respectively.

Calcium values ranged from 1.699 to 1.992 mg/l except in the samples collected in May (2.531 mg/l), in

September (2.502 mg/l), and in December, 1976 (2.474 mg/l).

The values recorded for manganese increased from a value of < 0.01 mg/l in the 1 December sample to a minor peak in March (0.103 mg/l), then decreased to a value of < 0.01 mg/l in the July sample. A secondary peak of 0.299 mg/l was recorded in September, with the values dropping to < 0.01 mg/l in November and reaching the highest recorded concentration (0.573 mg/l) in the 31 December sample.

The level of iron fluctuated slightly from 0.06 mg/l in the December sample to 0.058 mg/l in the May sample, with a minor peak occurring in January (0.072 mg/l). The value dropped to < 0.01 mg/l in July. High levels were recorded in September (0.295 mg/l) and December, 1976, (0.3 mg/l), with a low value of 0.029 mg/l occurring in the November sample.

A peak was recorded for the concentration of zinc in the initial December water sample (0.22 mg/l), after which the level decreased and remained low for the rest of the collections. Minor peaks occurred in May (0.041 mg/l) and September (0.038 mg/l); lower values were obtained in July (0.029 mg/l) and in November (< 0.01 mg/l).

Silicate concentrations were found to be highest in the winter months, reaching 1.654 mg/l in the January sample and 1.026 mg/l in the final December collection.

The level fell to its lowest values in the July and November samples. A minor peak of 0.732 mg/l was recorded in September.

Analyses for copper showed < 0.01 mg/l for all samples. Cadmium also appeared in concentrations of < 0.01 mg/l with the exception of the May and November water samples, when the value reached 0.01 mg/l.

A high level was recorded for sodium in the initial December collection (24.58 mg/l), with a drop in January and a subsequent rise in March (9.15 mg/l). The level then decreased, reaching a low in September (7.586 mg/l). By the time of the final December collection, the recorded value had reached 8.6 mg/l.

The level of phosphates increased from 0.037 mg/l in the 1 December sample to 0.05 mg/l in March, dropped slightly in May, then reached a peak of 0.098 mg/l in July. A decrease was recorded in November (0.038 mg/l). The value again reached 0.098 mg/l in December, 1976.

The nitrate level of the stream was highest in the December, 1975, collection. This level fell below 0.005 mg N/l at the time of the March, September and final December collections. Minor peaks were recorded in July (0.066 mg N/l) and November (0.54 mg N/l).

The concentration of Kjeldhal nitrogen increased from 0.169 mg N/l in January to a maximum of 0.827 mg N/l in

September. The lowest concentration was recorded in December, 1976 (< 0.01 mg N/l).

Sulphide levels were low in all the samples. They ranged from 0.004 mg S/l in the December, 1975, sample to 0.005 mg S/l in the January and March collections, and decreased to 0.0 mg S/l in the May and September samples. A peak was recorded in the November sample (0.017 mg S/l).

The amount of dissolved oxygen was highest in the January sample at 9.8 ml/l. It decreased throughout the following collections to 6.3 ml/l in the September sample, then increased to 8.8 ml/l in the December, 1976, sample.

The chemical oxidation demand of the water samples decreased from 11.9 mg C/l in the December, 1975, sample to 8.6 mg C/l in the March sample. The lowest value was recorded in July (5.1 mg C/l). High values were obtained in May (68.6 mg C/l), September (33.9 mg C/l) and December, 1976 (83.2 mg C/l). Organic particles were visibly detectable in the latter water sample.

2. Temperature and pH

The lowest temperature recorded for the stream (1.5°C) occurred in the December, 1975, collection. The water temperature values rose gradually until March (2.5°C). After March, the stream temperature increased perceptibly, reaching 17°C in September. A comparatively rapid drop had occurred by the December, 1976, collection, at which point

TABLE V

WATER ANALYSES DATA[a]

PARAMETER	DATE Dec 1975	Jan 1976	Mar 1976	May 1976	July 1976	Sept 1976	Nov 1976	Dec 1976
Har.[b]	8.86	7.86	10.86	9.89	8.606	11.21	8.51	12.51
Kj-N[c]	0.34	0.169	0.263	0.323	0.429	0.827	0.068	<0.01
NO_3[d]	0.12	0.041	<0.002	0.009	0.066	<0.004	0.054	<0.005
T-PO_4	0.037	0.042	0.05	0.047	0.098	0.074	0.038	0.098
COD[e]	11.9	9.4	8.06	68.6	5.1	33.9	9.05	83.2
Ca	1.916	1.699	2.482	2.531	1.992	2.502	1.958	2.474
Mg	0.92	0.84	1.058	0.838	0.874	0.946	0.868	0.963
Fe	0.06	0.072	0.069	0.058	<0.01	0.295	0.029	0.3
Mn	<0.01	0.015	0.103	0.012	<0.01	0.299	<0.01	0.573
Cu	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Zn	0.22	0.034	0.036	0.041	0.029	0.038	<0.01	0.031
Cd	-	<0.01	<0.01	0.01	<0.01	<0.01	0.01	<0.01
Na	24.58	7.932	9.15	8.532	7.84	7.568	8.629	8.354
K	0.452	0.443	0.656	0.537	0.485	0.691	0.564	0.623
SiO_2[f]	0.962	1.654	0.977	0.646	0.426	0.732	0.481	1.026
DO[g]	-	9.8	9.0	8.0	7.3	6.3	8.3	8.8
S[h]	0.004	0.005	0.005	0.0	0.003	0.0	0.017	0.008

[a]All concentrations in mg/l unless otherwise stated.

[b]Hardness, mg $CaCO_3$/l; [c]Kjeldhal-N; [d]mg N/l; [e]mg C/l; [f]mg Si/l; [g]ml O_2/l; [h]mg S/l.

the water temperature was again at 1.5°C (Fig. 10).

The seasonal pH recorded in Broadcove River ranged from 6.6 at the time of the initial December and September collections to a low of 5.5 in March (Fig. 10). A pH increase was recorded for May, July and September. The pH of the November and final December water samples did not show much variation from the September value.

3. Number of fungal propagules and species

The fluctuation in the number of fungal propagules is shown in Fig. 11. A low number of propagules (or isolates) was present in the first water sample. An estimated 51 propagules per five litres were present in the water. This concentration reached a small maximum of 341 propagules per five litres in the March collection. The propagule level decreased in May and July, reaching a low of 178 fungal propagules per five litres in September. A large increase was recorded at the time of the November collection. The propagule content of the water was 1042 in five litres. By the December, 1976, collection, the total number of propagules had decreased, yielding 91 isolates from five litres.

It should be noted that Fig. 11 reflects the number of aquatic fungi obtained, and does not include isolates of terrestrial nature (see Appendix A). Any mention of fungal propagules refers to aquatic fungi unless otherwise stated.

Figure 10. Temperature (o—o) and pH (□—□) of Broadcove River at the time of each collection.

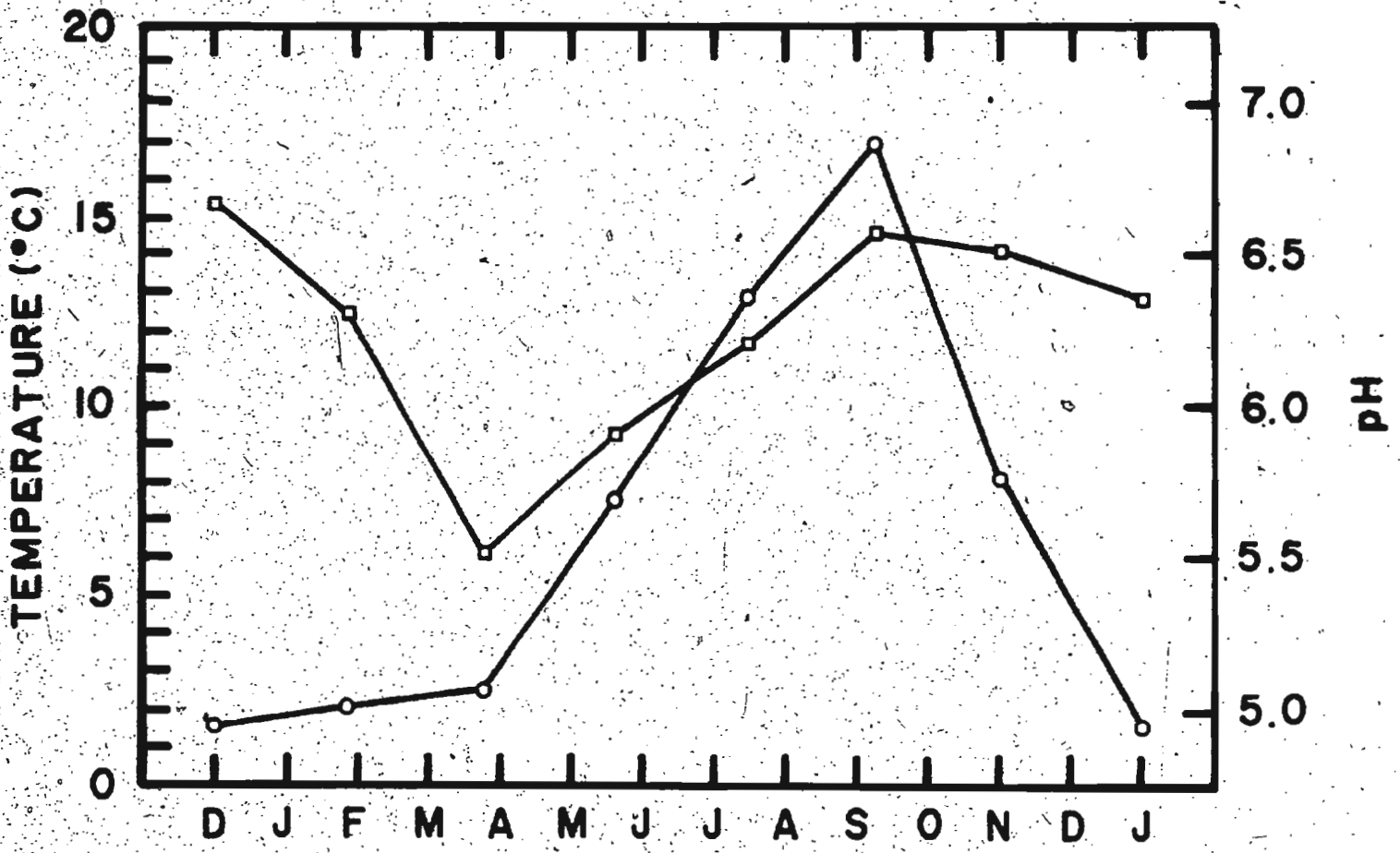

20
15
10
5
0
TEMPERATURE (°C)
7.0
6.5
6.0
5.5
5.0
pH
D J F M A M J J A S O N D J

Figure 11. Seasonal variation of the total number of fungal propagules.

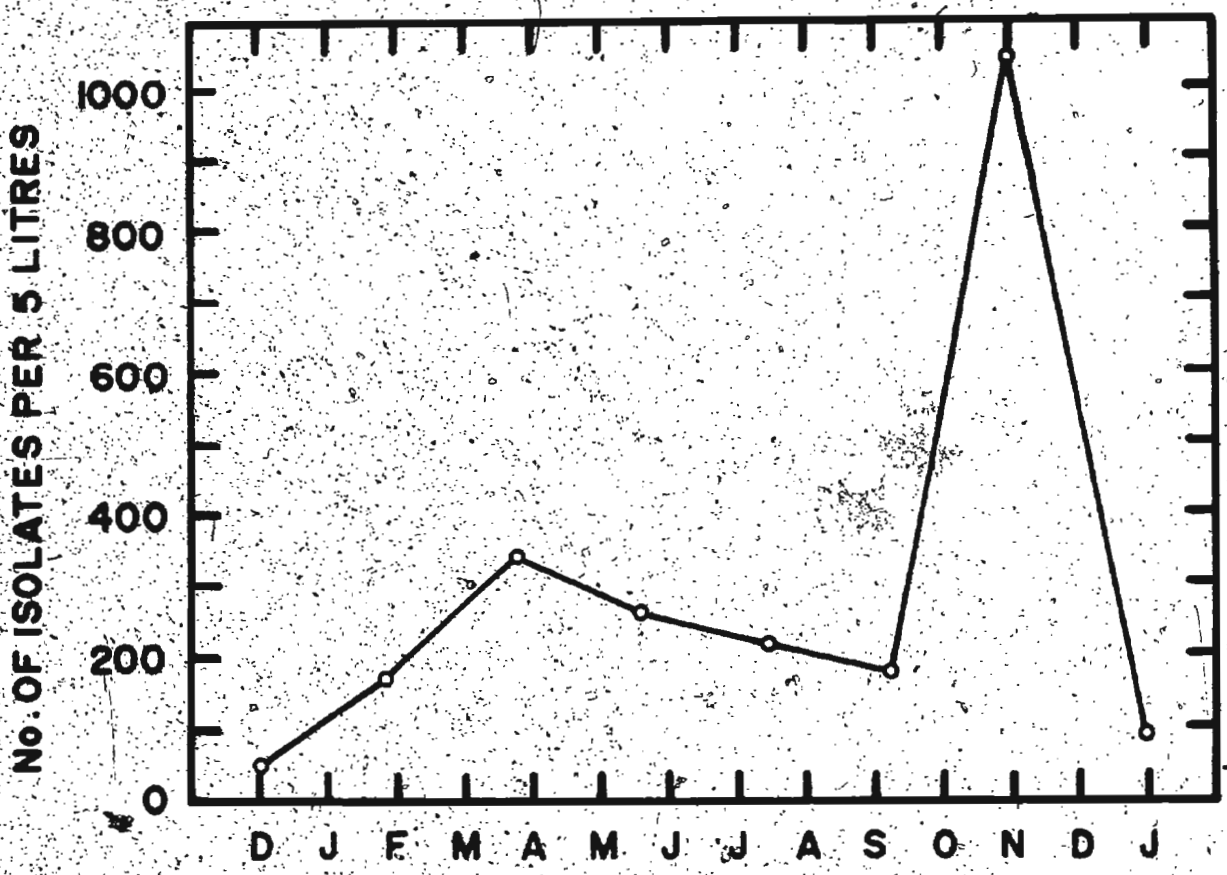

No. OF ISOLATES PER 5 LITRES
1000
800
600
400
200
0
D J F M A M J J A S O N D J

Figure 12 shows the number of species recorded for each collection. A greater variety was obtained in the March, July, September and November collections (ten, thirteen, twelve and twelve, respectively). The January and December, 1976, collections both yielded the least number of species (three). An intermediate number was obtained in the December 1975, and May collections; seven in the former and six in the latter.

B. Species composition of each collection

Collection 1 (1 December, 1975) yielded representatives of seven species from four genera of the Order Saprolegniales (Fig. 13). Saprolegnia monilifera de Bary (Fig. 14, 15, 16, 17, 18, 19, 20) was the most abundant species, comprising 48.57% of the isolates. Saprolegnia australis Elliott (Fig. 21, 22, 23) was the next most numerous at 22.86%, followed by Pythiopsis cymosa (Fig. 24, 25, 26) at 14.28%. A low percentage of the isolates was made up of Saprolegnia diclina (Fig. 27), Achlya oblongata de Bary (Fig. 28, 29, 30), Aphanomyces stellatus de Bary (Fig. 31, 32, 33, 34) and an isolate designated as Sterile I (Fig. 35, 36, 37, 38, 39, 40, 41, 42). The latter was an aquatic, non-septate fungus with very slender, sometimes irregular hyphae, believed to belong to the genus Aphanomyces. This fungus failed to produce sexual and asexual structures in two months of incubation in sterile, distilled

Figure 12. Number of species per sample.

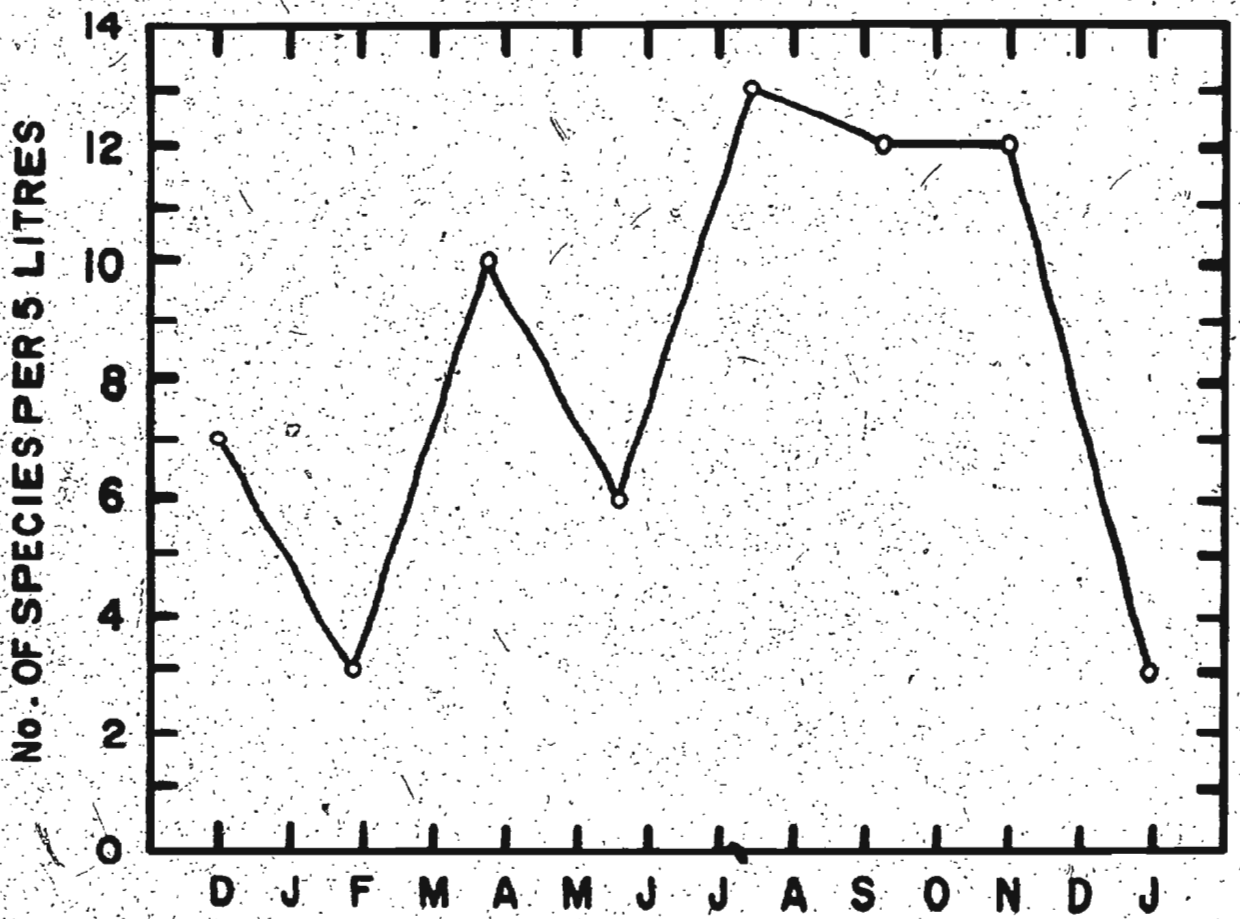

No. OF SPECIES PER 5 LITRES
0
2
4
6
8
10
12
14
D J F M A M J J A S O N D J

Figure 13. Frequency distribution of species in the 1 December, 1975, water sample. (Collection 1).

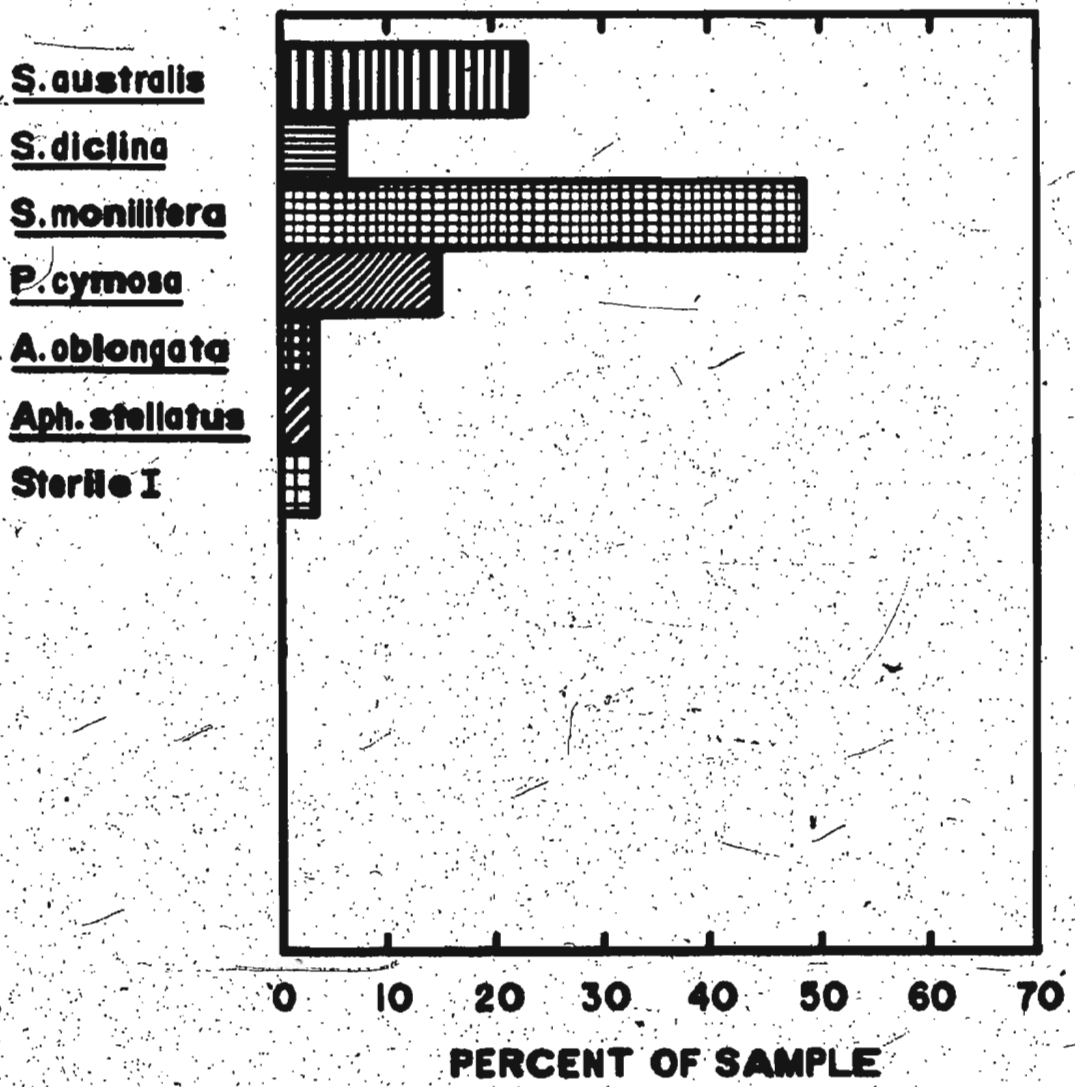

S. australis
S. diclina
S. monilifera
P. cymosa
A. oblongata
Aph. stellatus
Sterile I
0
10
20
30
40
50
60
70
PERCENT OF SAMPLE

Figure 14. Saprolegnia monilifera: Internal proliferation of a zoosporangium. The new zoosporangium (z) grew through the base of the empty zoosporangium (e). x 400.

Figure 15. S. monilifera: Lateral proliferation of zoosporangia. New growth (n) and gemmae (g) growing through the empty walls (e) of two zoosporangia which had proliferated laterally. x 280.

Figure 16. S. monilifera: Catenulate oogonia (arrow). x 660.

Figure 17. S. monilifera: Undeveloped oospores. Oospores (arrow) from an isolate with a high incidence of parasitism. x 730.

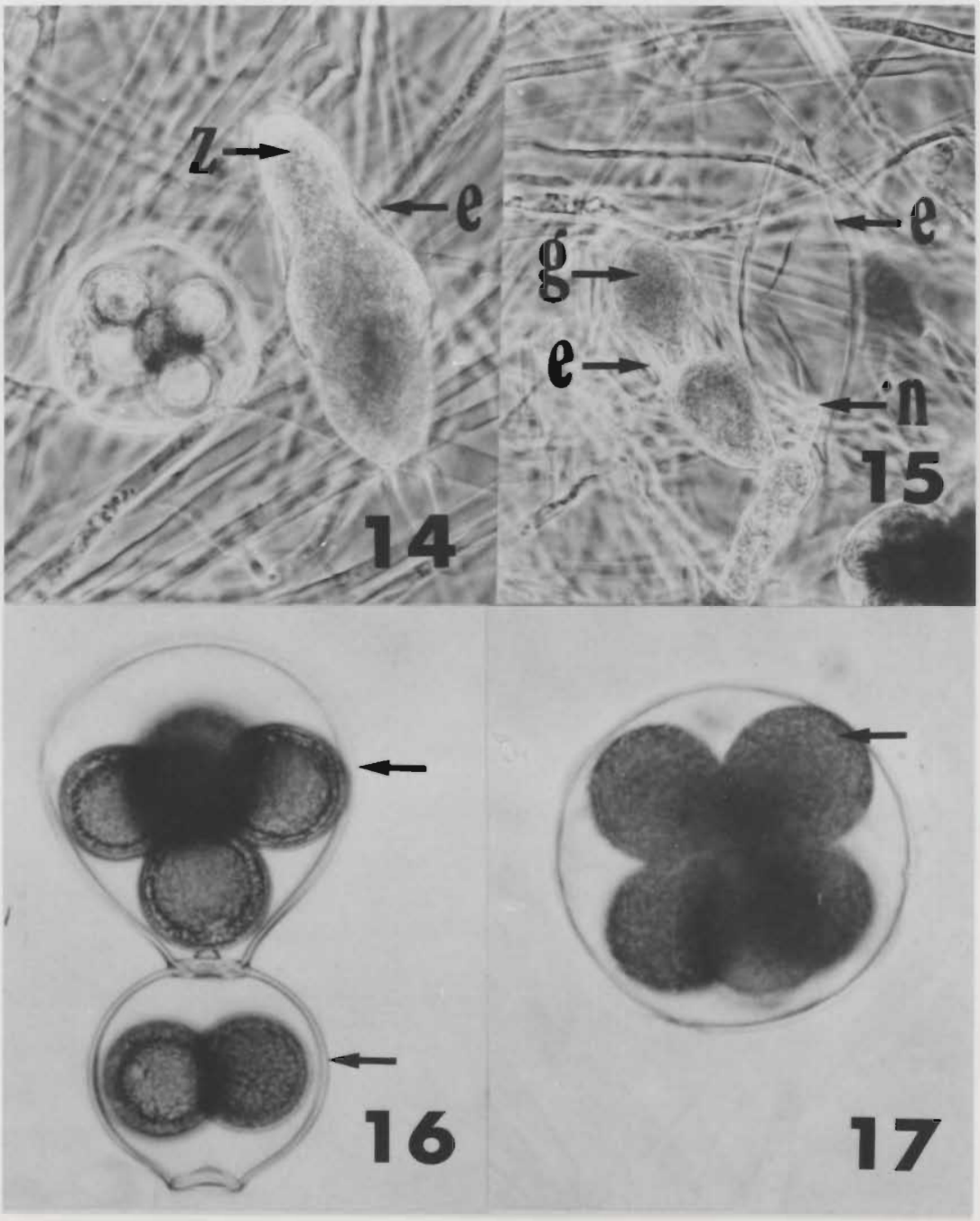
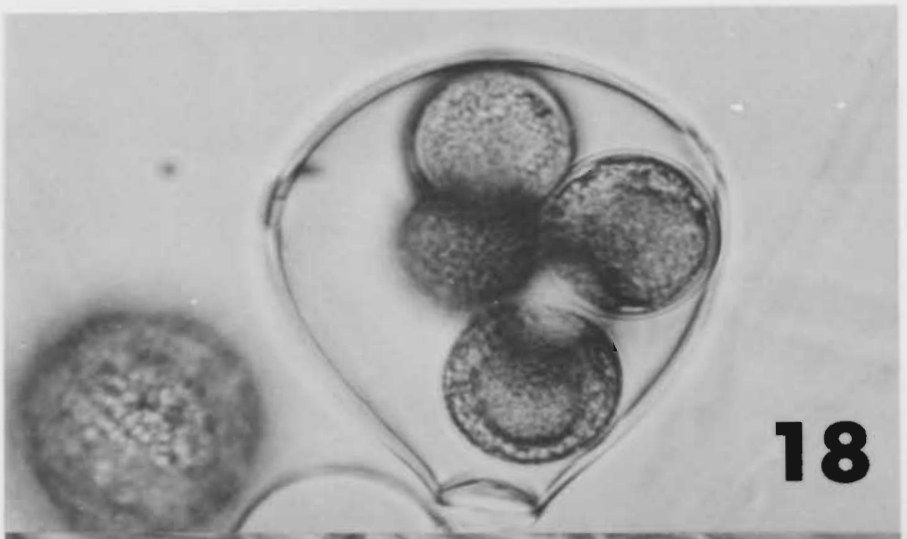
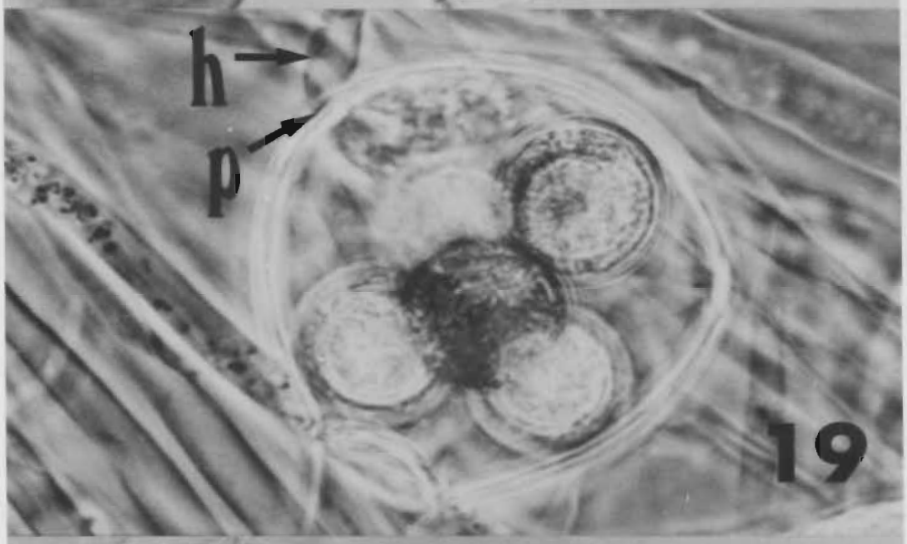

z
e
14
e
g
e
n
15
16
17

Figure 18. S. monilifera: Normal oogonium. x 800.

Figure 19. S. monilifera: Infected oogonium. A parasitic hypha (h) entered the oogonium through a pit (p). x 790.

Figure 20. S. monilifera: Parasitized oogonium. A parasitic hypha (h) had destroyed the oospores (o). x 710.


18


h
p
19

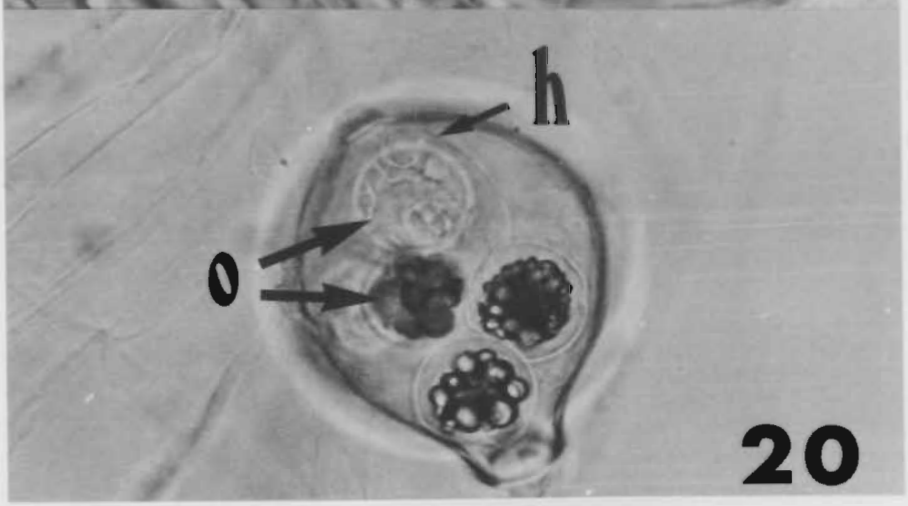

h
o
20

Figure 21. Saprolegnia australis: Laterally appressed antheridial cell (arrow). x 470.

Figure 22. S. australis: Diclinous antheridium (arrow). x 690.

Figure 23. S. australis: Typical oogonium. Oogonium with pits (p) and subcentric oospores (o). x 790.

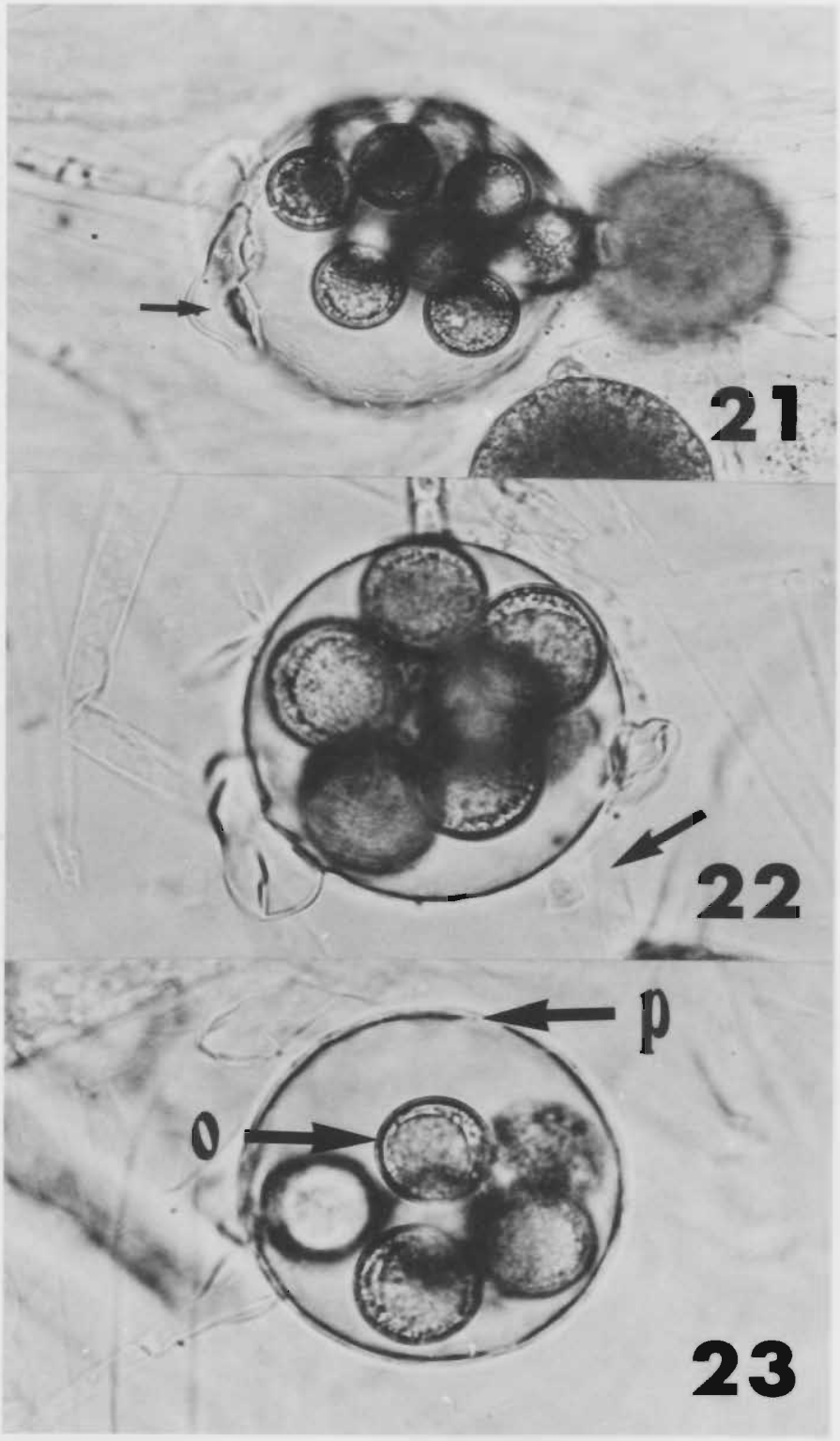

21
22
p
o
23

Figure 24. Pythiopsis cymosa: Oogonium. The characteristic subeccentric arrangement of the oil globules (g) is shown, together with the semihypogynous position of the antheridium (a). x 1110.

Figure 25. P. cymosa: Intercalary oogonium. x 1070.

Figure 26. P. cymosa: Oogonial papilla (arrow). x 1060.

Figure 27. Saprolegnia diclina: Oogonium. The centric oospore (o) and diclinous antheridial branch (a) characteristic of this species are shown here. x 580.

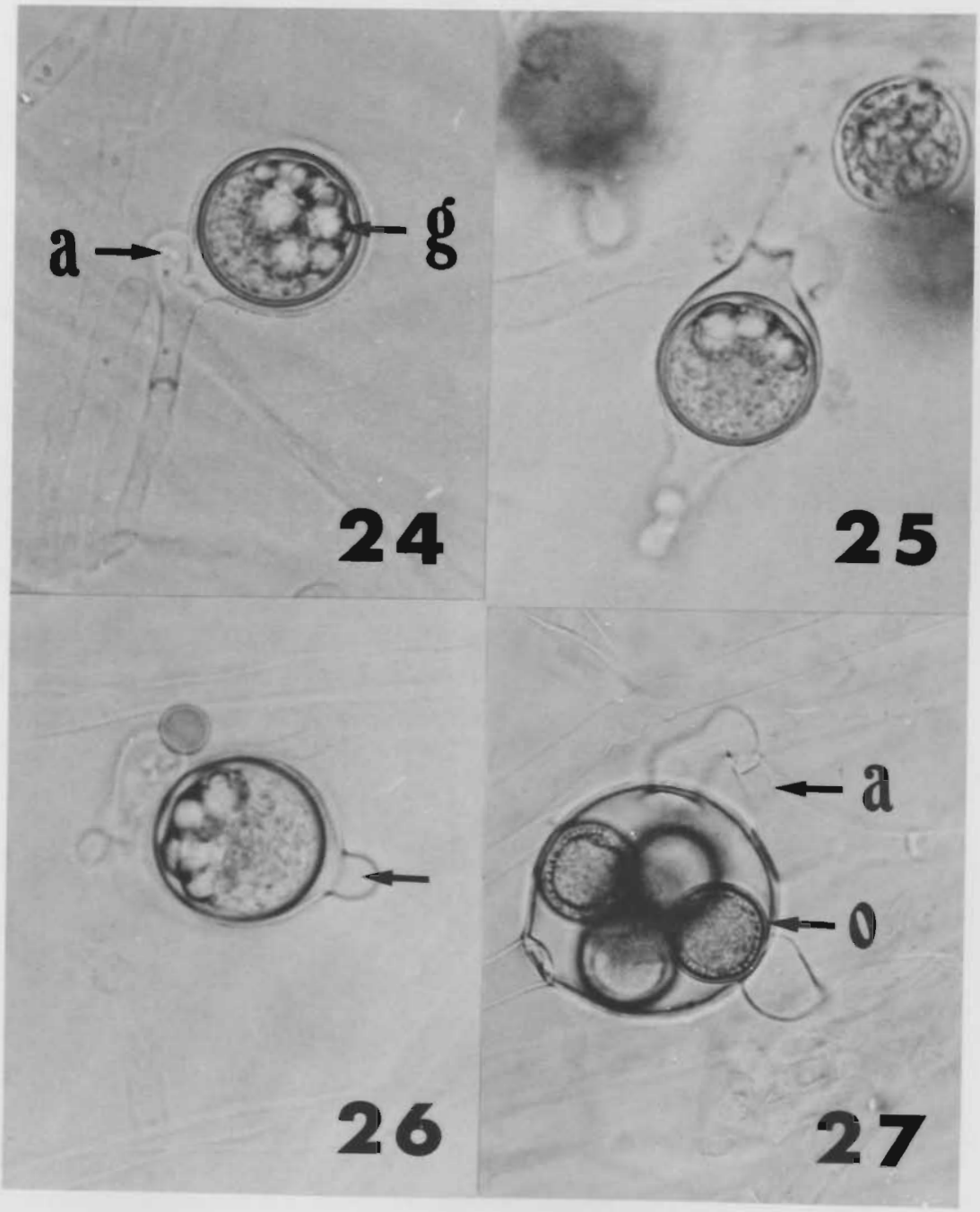
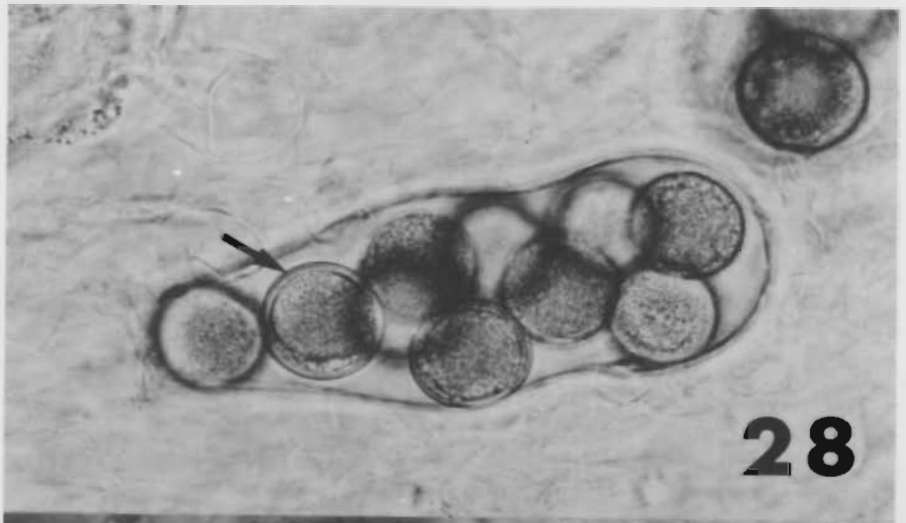

a
g
24
25
26
a
o
27

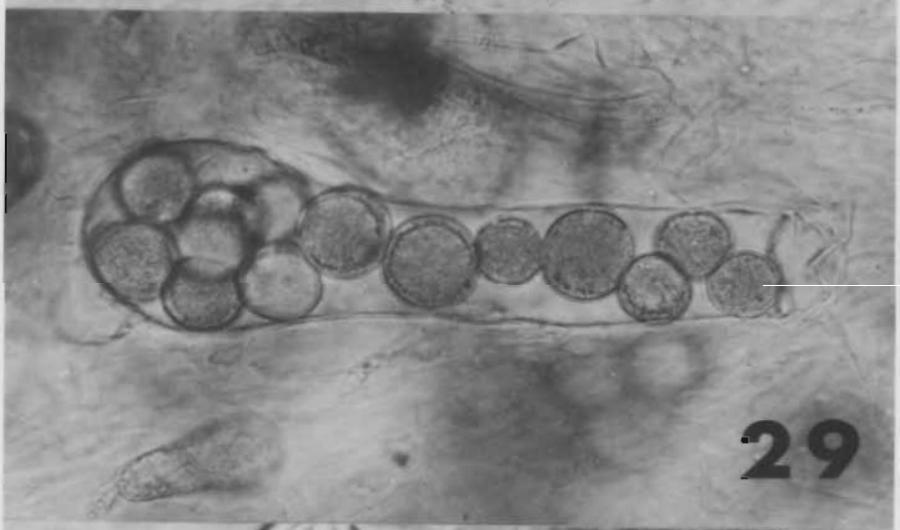
Figure 28. Achlya oblongata: Subcentric type II oospore (arrow), x 560.

Figure 29. A. oblongata: Oogonium from older culture. x 380.

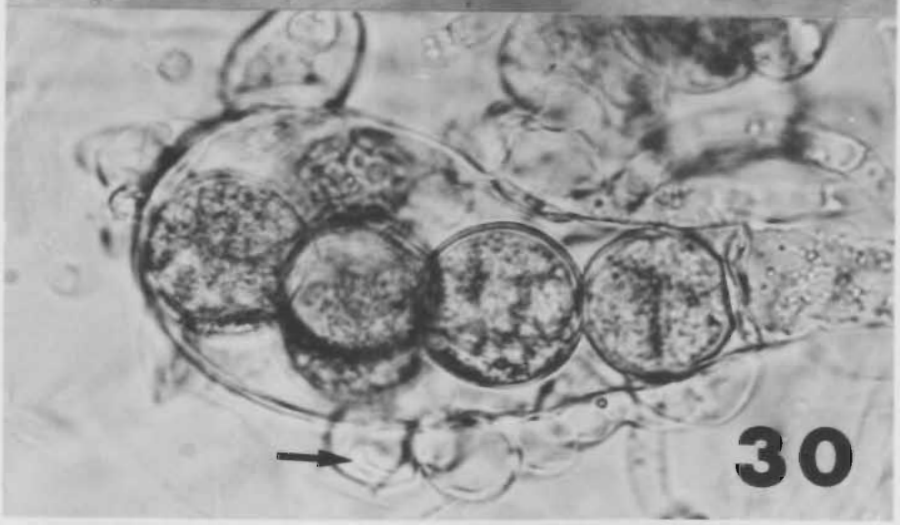
Figure 30. A. oblongata: Oogonium from younger culture. Many laterally appressed antheridial cells (a) are in evidence. x 790.



28



29



30

Figure 31. Aphanomyces stellatus: Oogonium. The fertilzation tube (t) leads to a subcentric type I oospore (o). x 1050.

Figure 32. A. stellatus: Alate oogonium. The fertilization tube (t) leads to a subcentric type II oospore (o). x 1050.

Figure 33. A. stellatus: Papillate oogonium. The oogonial wall shows papillate type projections (p). A pellucid spot (ps) can be seen in the oospore. x 960.

Figure 34. A. stellatus: Tuberculate oogonium. The oogonial wall shows tuberculate projections (arrow). x 960.

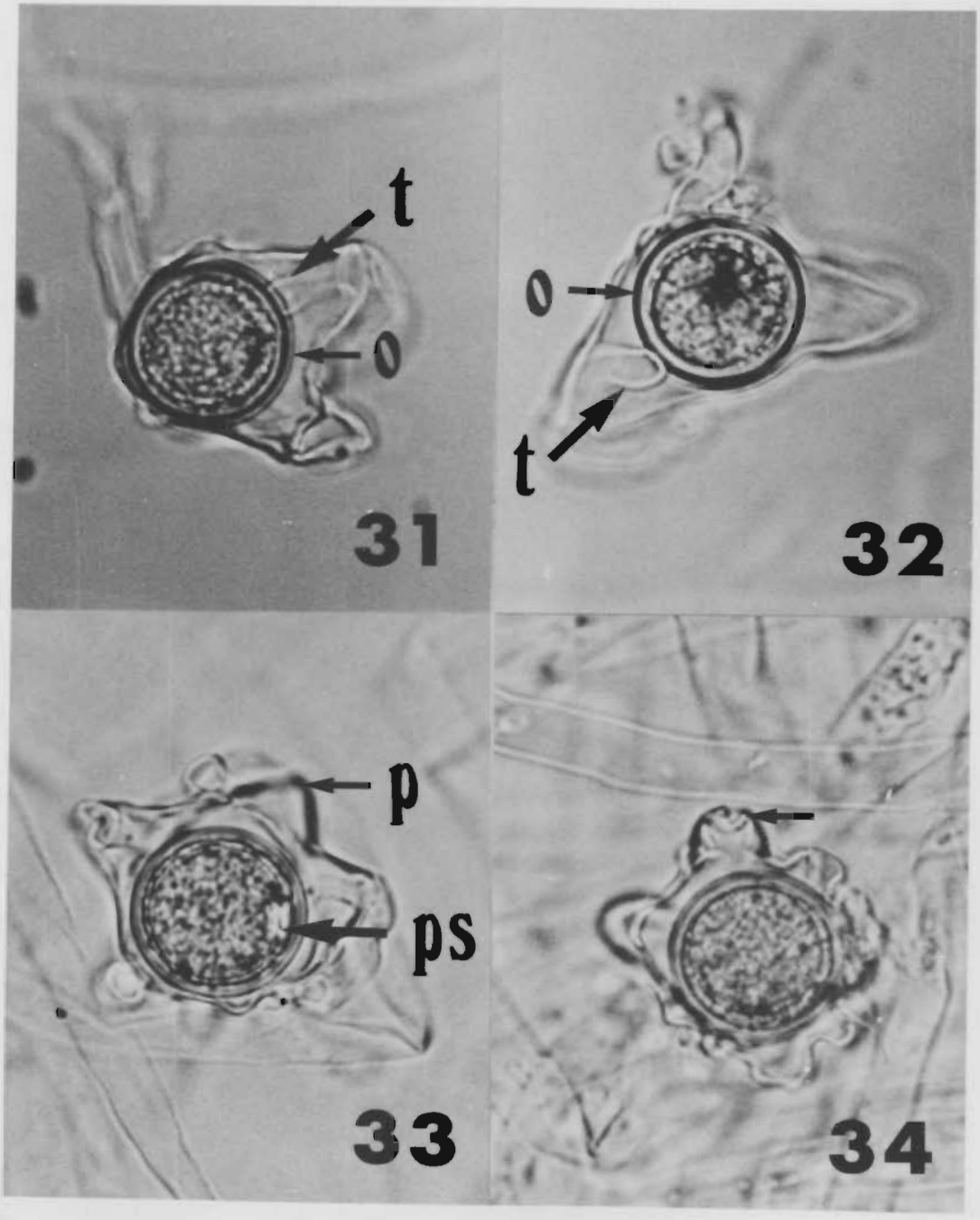

t
o
31
o
t
32
p
ps
33
34

Figure 35. Sterile I: Typical hypha (arrow). x 500.

Figure 36. Sterile I: Coiling hypha (arrow). x 300.

Figure 37. Sterile I: Hyphal coil (arrow). x 340.

Figure 38. Sterile I: Hyphal knot (arrow). x 300.

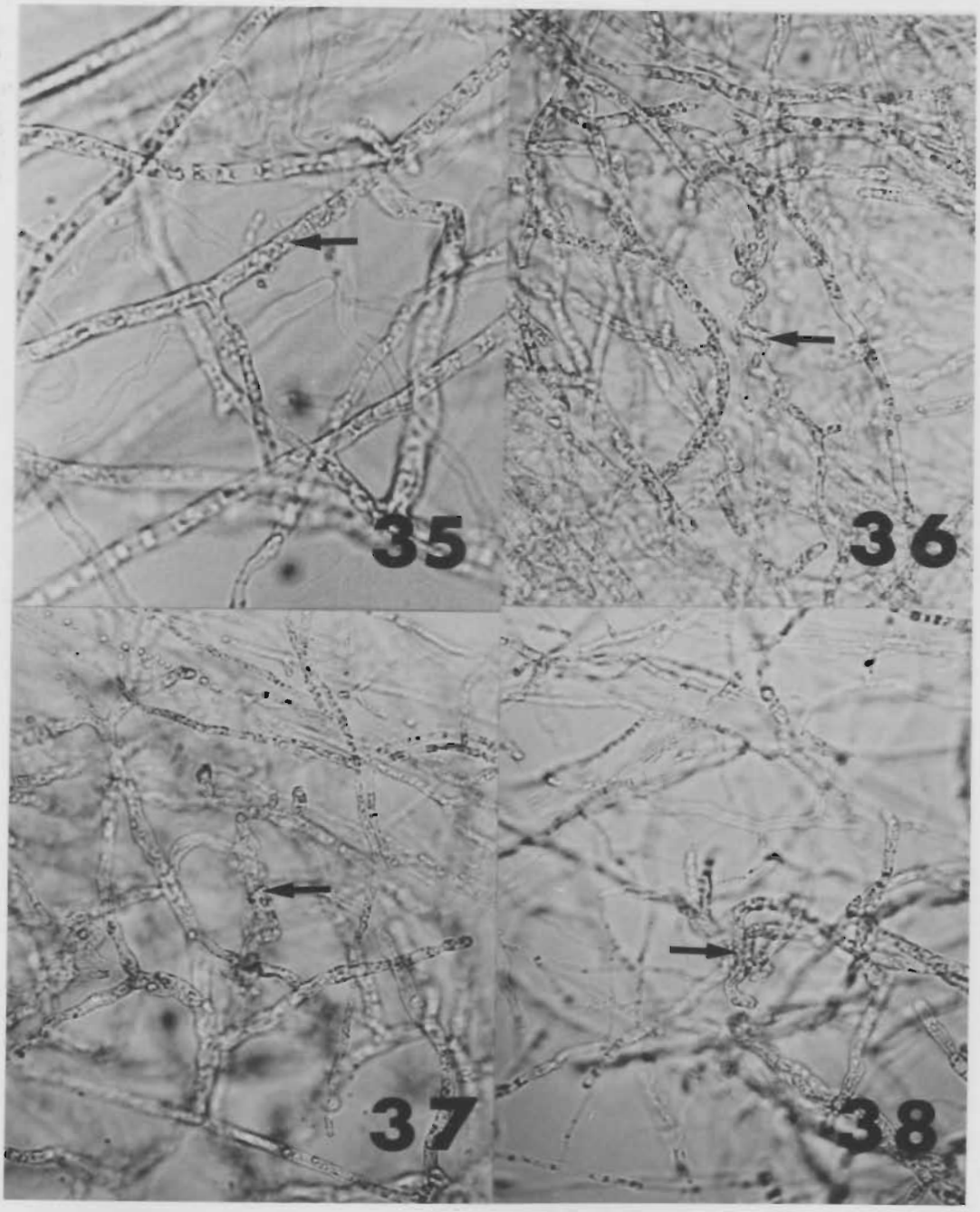
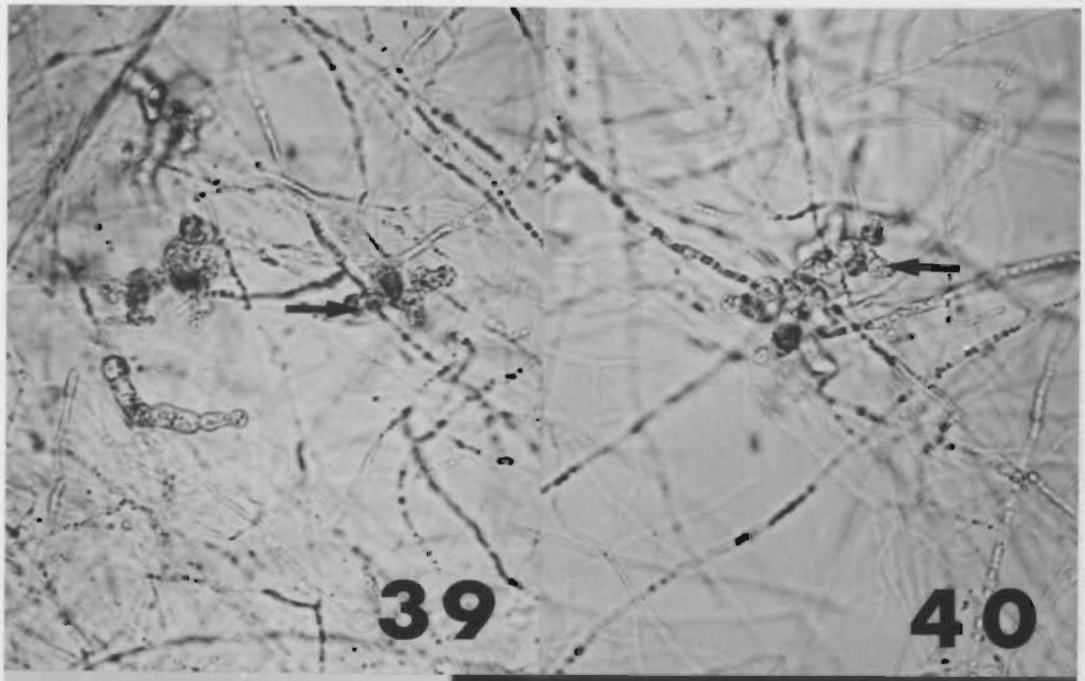

35
36
37
38

Figure 39. Sterile I: Distorted gemma (arrow). x 350.

Figure 40. Sterile I: Irregularly shaped gemma (arrow). x 320.

Figure 41. Sterile I: Parasitic hypha (arrow). x 800.

Figure 42. Sterile I: Arachnoid mycelium (diameter of culture dish = nine centimetres).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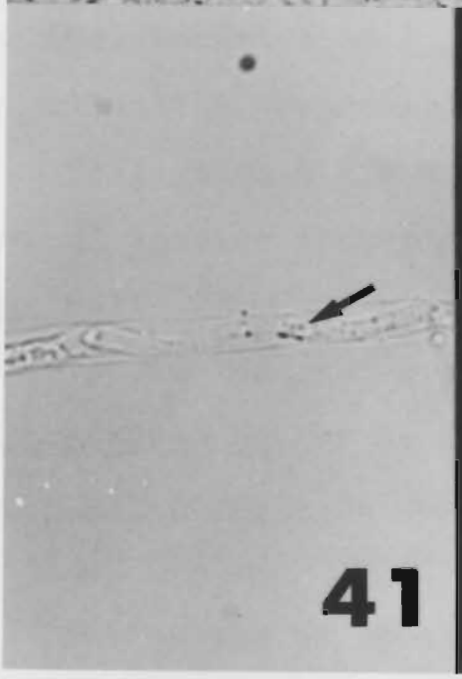
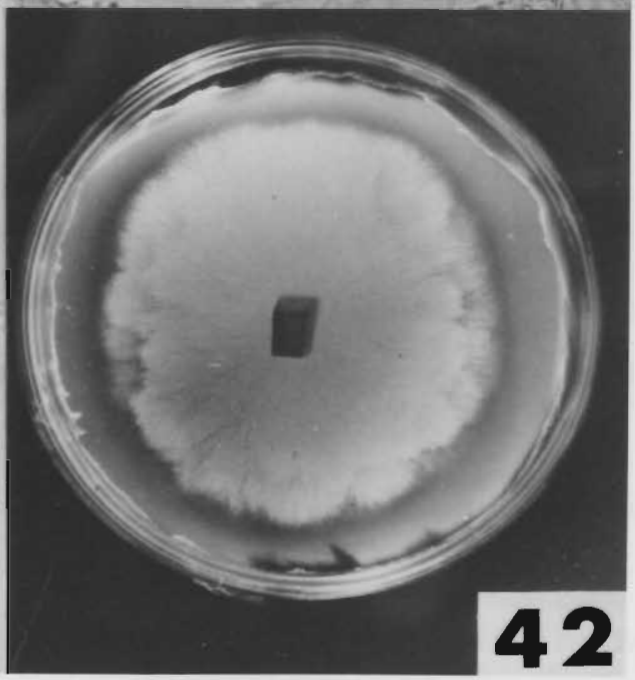
40


41


42

water.

Collection 2 (26 January, 1976) again showed S. monilifera as the most abundant species, making up 66.67% of the isolates. P. cymosa (11.11%) and Aph. stellatus (22.22%) were also present (Fig. 43).

An increase in the number of species present in the water sample was recorded for the third collection, made on 22 March, 1976 (Fig. 44). Saprolegnia ferax (Fig. 45), Saprolegnia terrestris Cookson and Saprolegnia eccentrica Coker appeared for the first time. Members of other genera which were also recorded for the first time included Scoliolegnia asterophora (de Bary) Dick (Fig. 46, 47) and Apodachlyella completa Humphrey of the Order Leptomitales (Fig. 48). Isolates of P. cymosa were the most numerous in this collection, representing 59.57% of the total.

Collection 4 (17 May, 1976) showed a decrease in the number of species present (Fig. 49), with Sterile I and S. australis increasing in frequency. The former increased to 55.17% and the latter to 31.03%. The first appearance of Aphanomyces scaber de Bary (Fig. 50, 51, 52, 53, 54) and Leptolegnia caudata de Bary (Fig. 56, 57) was observed at this time.

The number of species increased to thirteen in the fifth collection (14 July, 1976, Fig. 55). Saprolegnia anisospora de Bary, Saprolegnia parasitica Coker (Fig. 58) and Aphanomyces laevis de Bary were obtained for the first

Figure 43. Frequency distribution of species in the 26 January, 1976, water sample. (Collection 2).

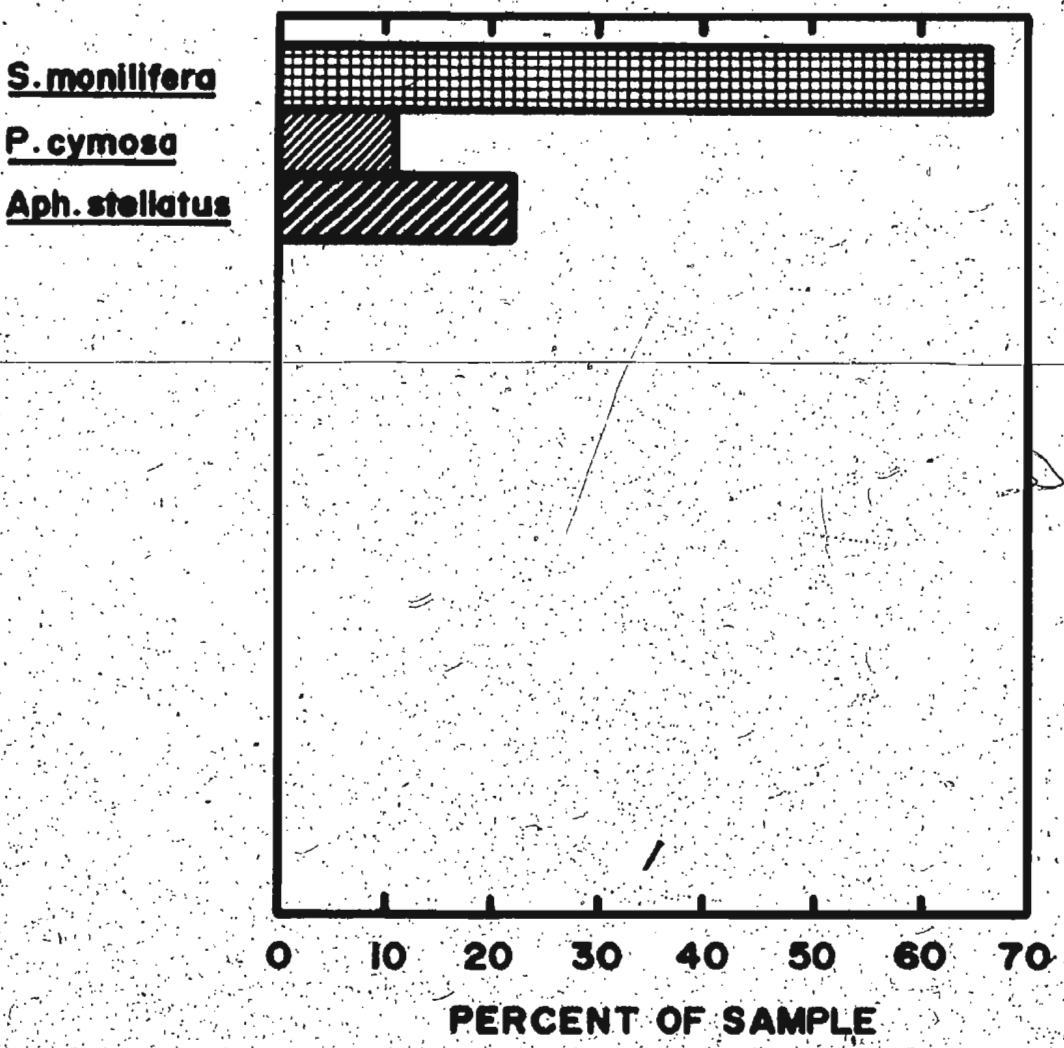

S. monilifera
P. cymosa
Aph. stellatus
0
10
20
30
40
50
60
70
PERCENT OF SAMPLE

Figure 44. Frequency distribution of species in the 22 March, 1976, water sample. (Collection 3).

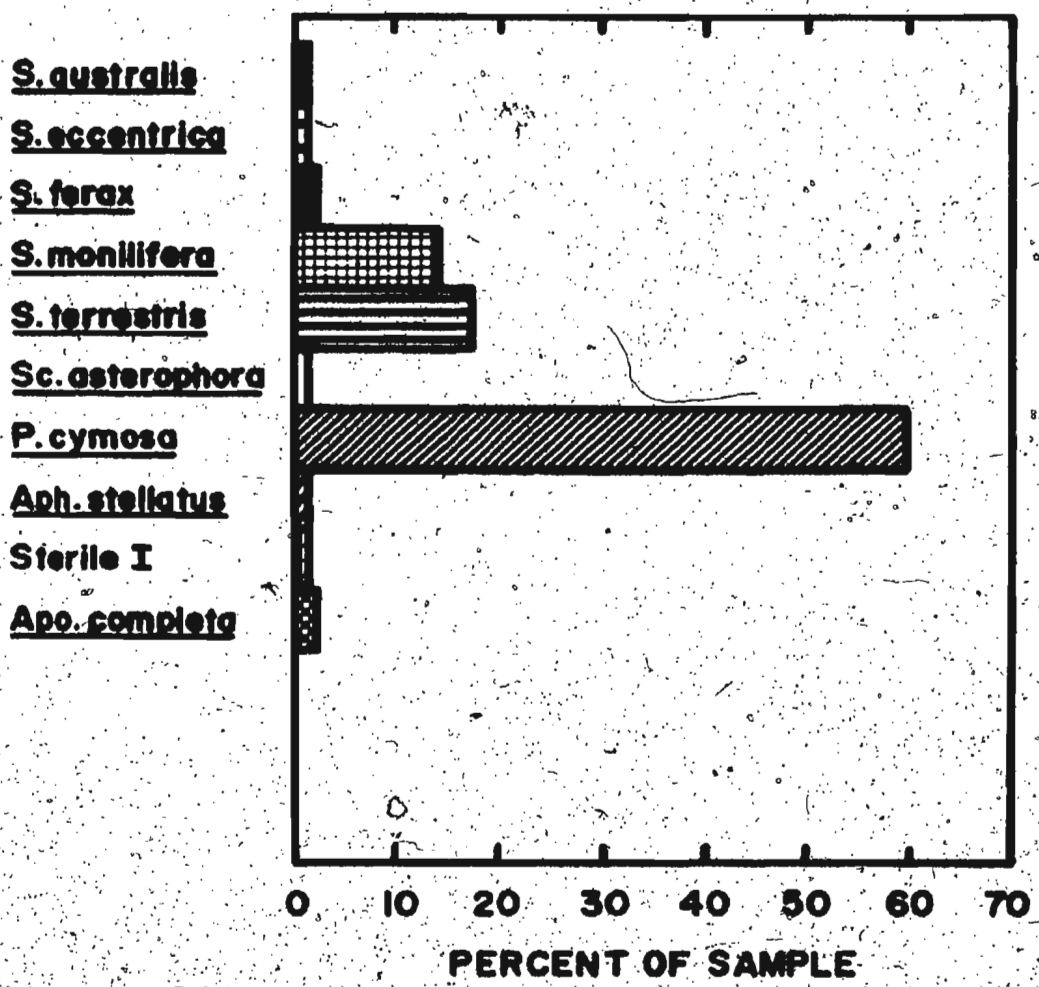
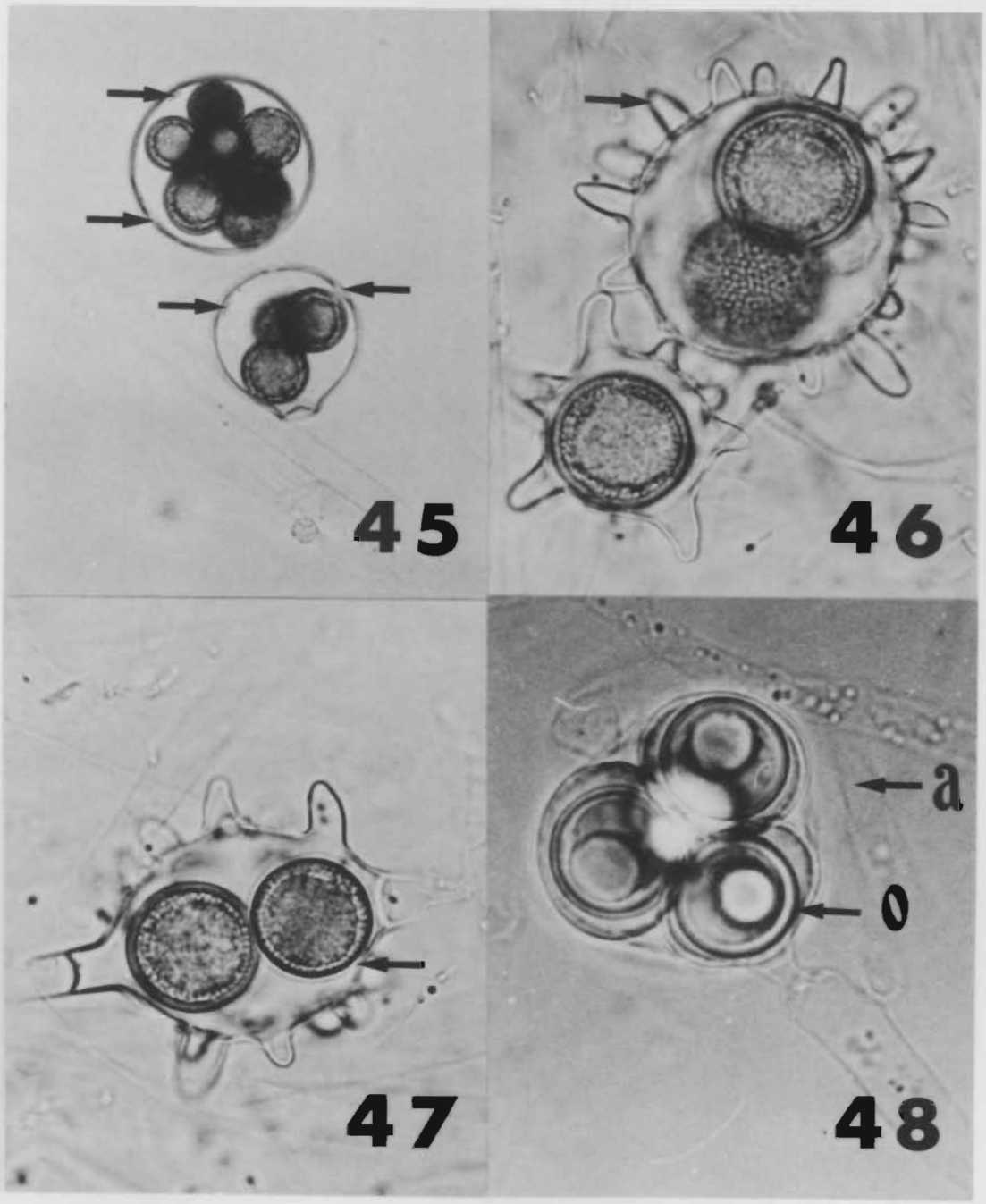

S. australis
S. eccentrica
S. ferax
S. monilifera
S. terrestris
Sc. asterophora
P. cymosa
Aph. stellatus
Sterile I
Apo. complete
0
10
20
30
40
50
60
70
PERCENT OF SAMPLE

Figure 45. Saprolegnia ferax: Oogonia. Arrows indicate the numerous pits. x 560.

Figure 46. Scoliolegnia asterophora: Oogonia. Arrow indicates one of the many papillae. x 680.

Figure 47. S. asterophora: Intercalary oogonium. Subcentric type I oospores (arrow) are evident. x 720.

Figure 48. Apodachlyella completa: Oogonium. Characteristic eccentric oospores (o) and androgynous antheridia (a) are shown. x 730.


45
46
47
a
o
48

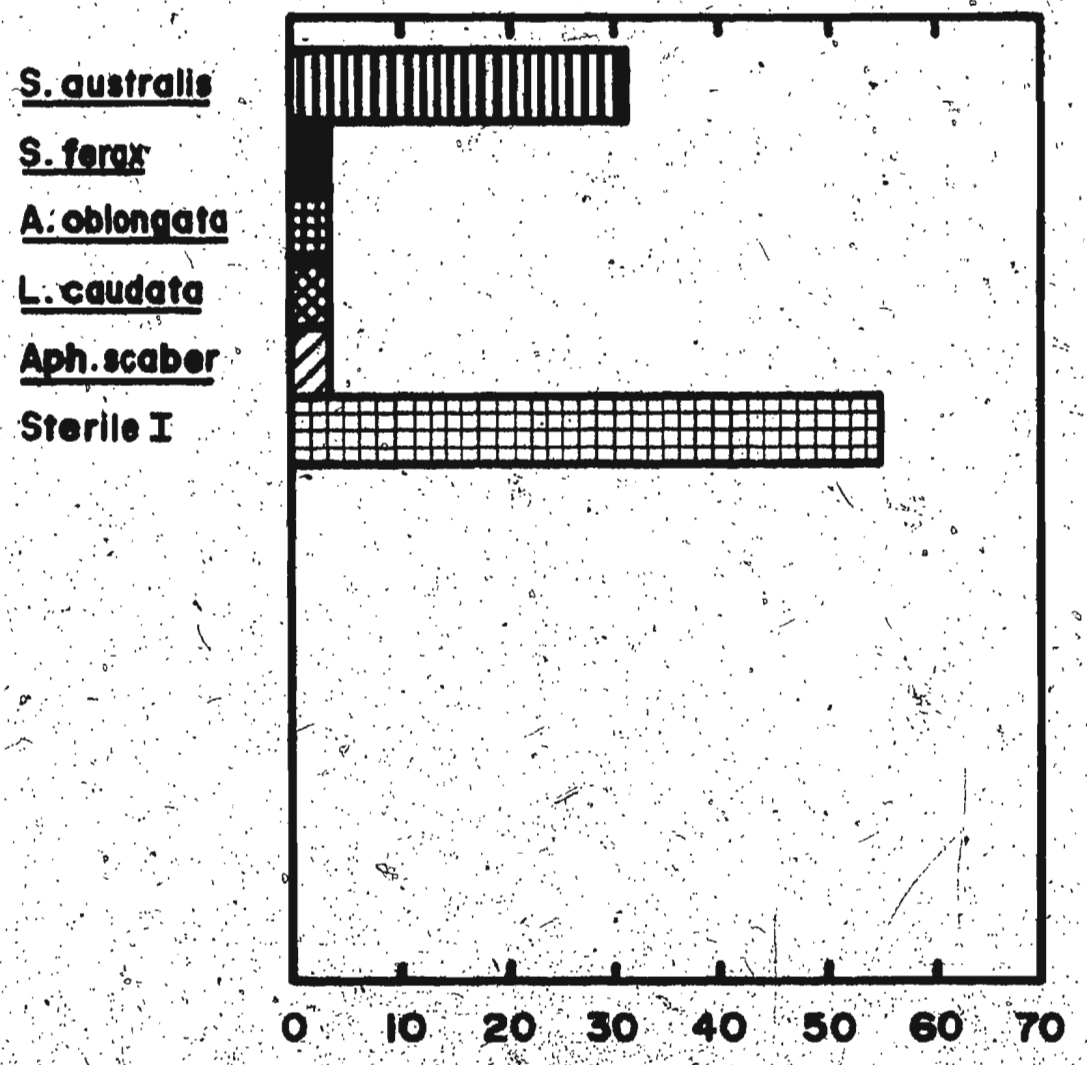

S. australis
S. ferax
A. oblongata
L. caudata
Aph. scaber
Sterile I
0
10
20
30
40
50
60
70
PERCENT OF SAMPLE

Figure 49. Frequency distribution of species in the 17 May, 1976, water sample. (Collection 4).

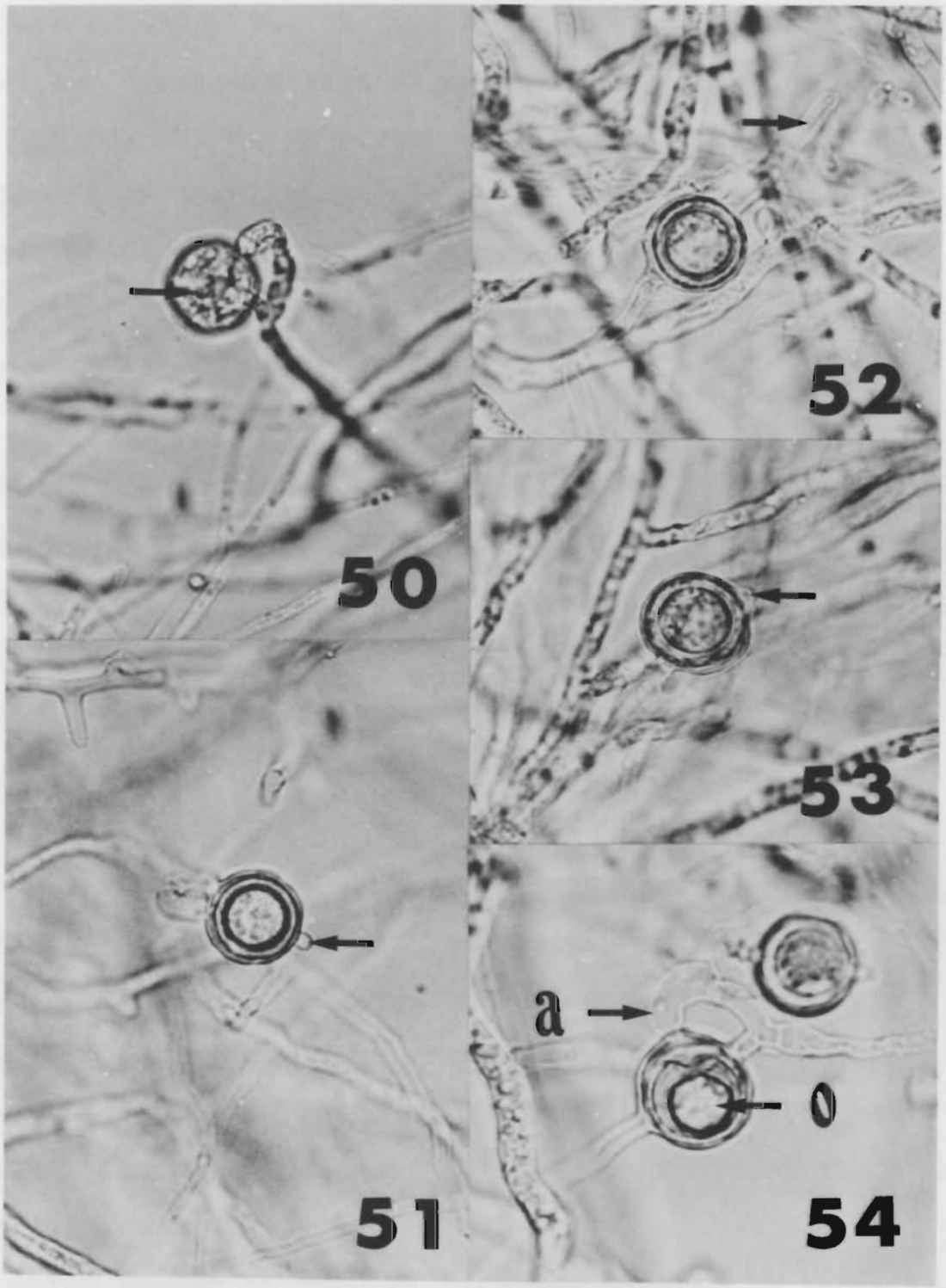
Figure 50. Aphanomyces scaber: Undeveloped oogonium (arrow). x 760.

Figure 51. A. scaber: Short tuberculate oogonial wall projection (arrow). x 800.

Figure 52. A. scaber: Long tubercule (arrow). x 830.

Figure 53. A. scaber: Papilla (arrow). x 710.

Figure 54. A. scaber: Oogonia. A typical eccentric oospore (o) and an androgynous antheridial branch (a) are shown. x 810.


50
52
53
51
a
0
54

time. A second type of unknown, designated Sterile II, was encountered (Fig. 59, 61, 62, 63). All fungi which had gemmae, hyphae and sporangia typical of the genus Saprolegnia, or even Achlya, but which produced no sexual structures during two months of incubation in sterile, distilled water, were placed in this category. S. australis, comprising 39.65% of the isolates, was the most numerous species, followed by L. caudata (21.2%).

Collection 6 (7 September, 1976) revealed the presence of additional species (Fig. 60). These were Saprolegnia unispora Coker et Couch (Fig. 64) and Protoachlya paradoxa (Coker) Coker, which together with Sc. asterophora and S. terrestris made up a small percentage of the total. Sterile I was the most abundant isolate (31.95%) followed by S. australis (20.7%).

Collection 7 (1 November, 1976) did not show any of the occasional species such as S. unispora, Sc. asterophora and Pr. paradoxa (Fig. 65). The reappearance of S. monilifera, P. cymosa and Aph. ste[illegible] was observed. S. australis, S. diclina, S. ferax, S. [illegible]tris, L. caudata and Sterile II decreased in abu[illegible]nce, while the number of Sterile I isolates increased to make up 82.49% of the total number of isolates.

The number of species recorded dropped to three in collection 8 (31 December, 1976). S. monilifera made up

Figure 55. Frequency distribution of species in the 14 July, 1976, water sample. (Collection 5).

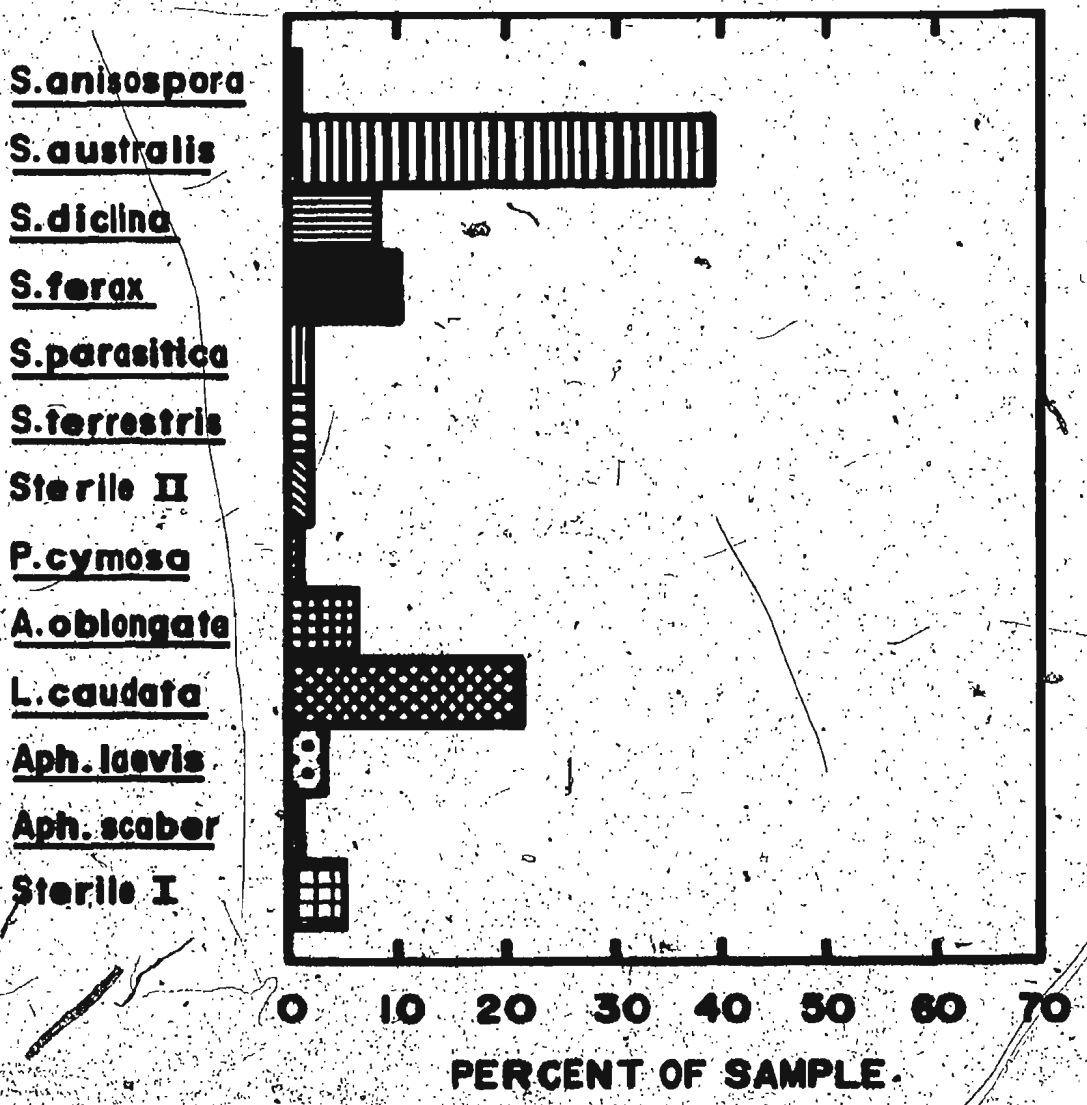

S.anisospora
S.australis
S.diclina
S.ferax
S.parasitica
S.terrestris
Sterile II
P.cymosa
A.oblongata
L.caudata
Aph.laevis
Aph.scaber
Sterile I
0
10
20
30
40
50
60
70
PERCENT OF SAMPLE.

Figure 56. Leptolegnia caudata: Zoospores in a single row. The zoospores (zp) lie in an undifferentiated hypha. x 930.

Figure 57. L. caudata: Oogonium. The subcentric arrangement of the oil globules (g) is shown. x 940.

Figure 58. Saprolegnia parasitica: Oogonia. x 340.

Figure 59. Sterile II: Pyriform gemma (arrow). x 370.

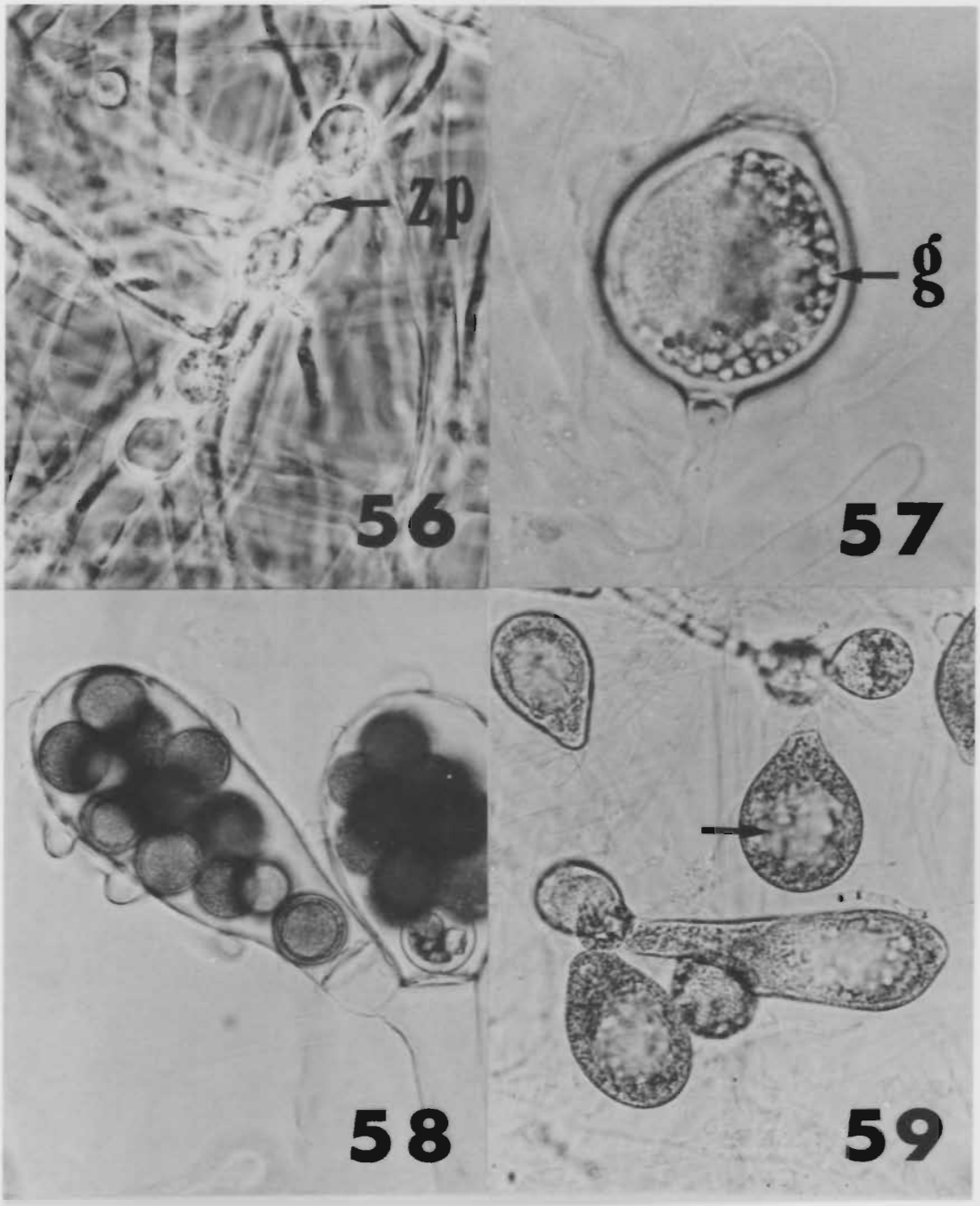

zp
56
g
57
58
59

Figure 60. Frequency distribution of species in the 7 September, 1976, water sample. (Collection 6).

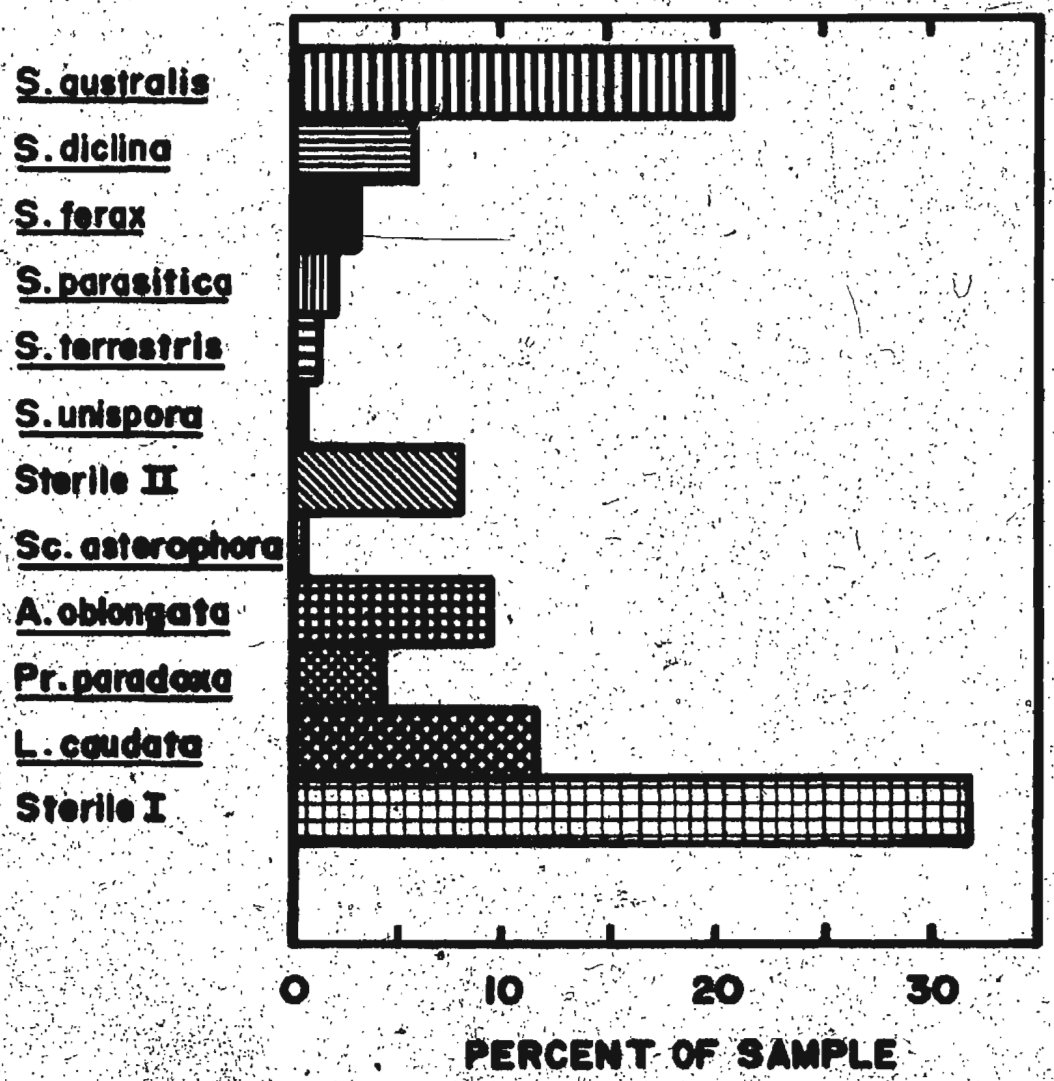

S. australis
S. diclina
S. ferax
S. parasitica
S. terrestris
S. unispora
Sterile II
Sc. asterophora
A. oblongata
Pr. paradoxa
L. caudata
Sterile I
0
10
20
30
PERCENT OF SAMPLE

Figure 61. Sterile II: Filiform gemma (arrow). x 110.

Figure 62. Sterile II: Catenulate gemmae (arrow). Germination of the end gemma can be observed. x 120.

Figure 63. Sterile II: Dictyoid sporangium (arrow). x 150.

Figure 64. Saprolegnia unispora: Subcentric oospore (arrow). x 460.

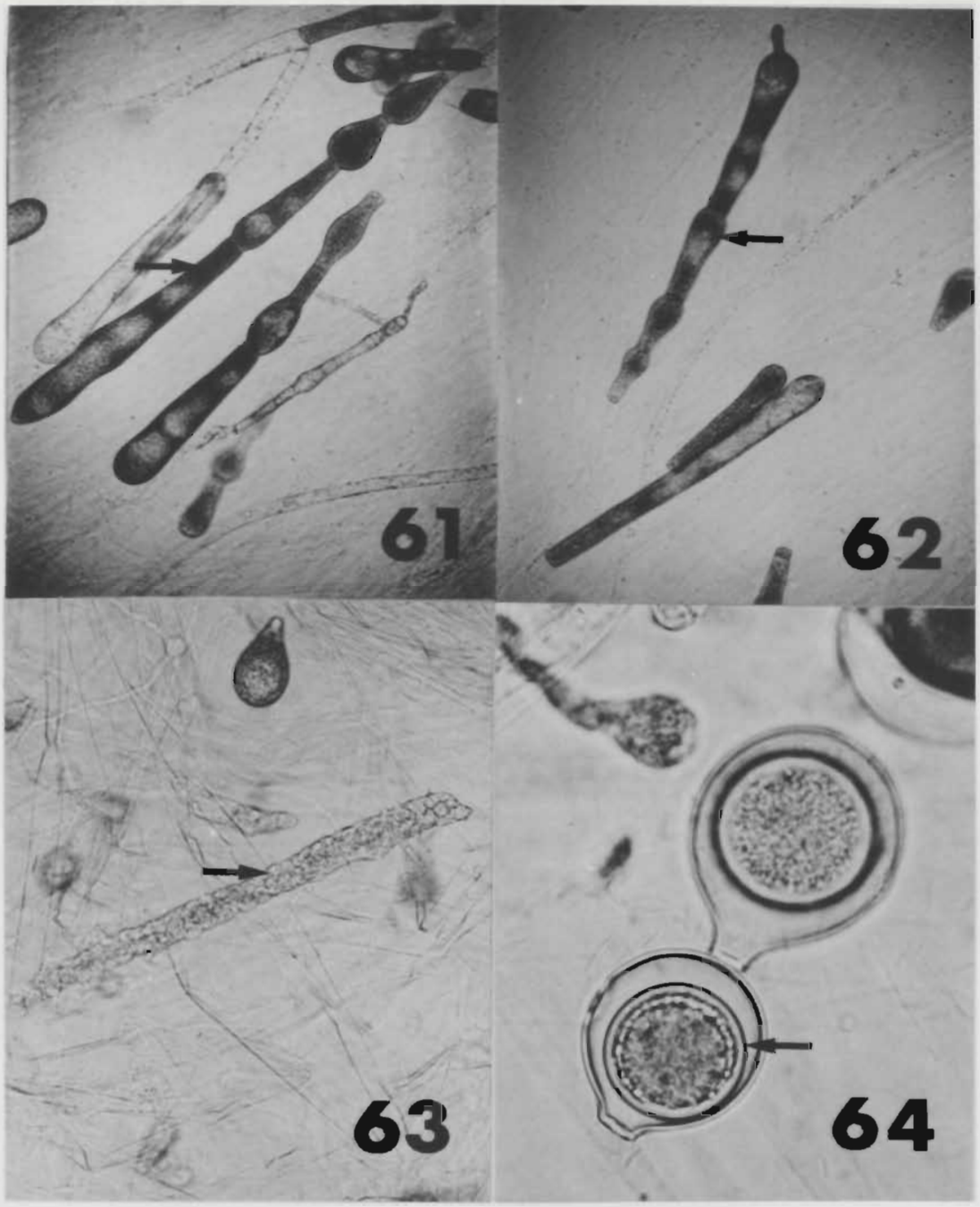
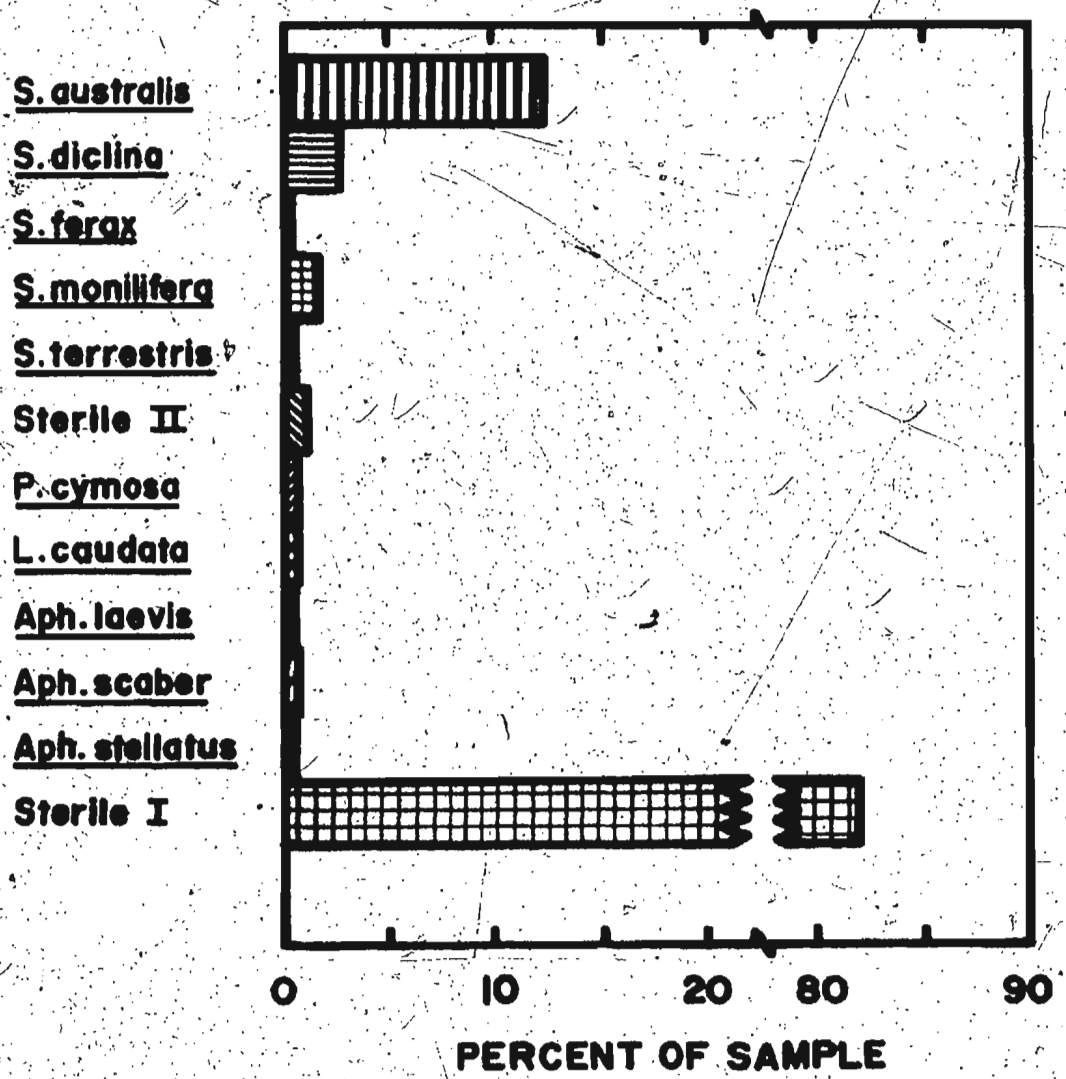

61
62
63
64

Figure 65. Frequency distribution of species in the 1 November, 1976, water sample. (Collection 7).


S. australis
S. diclina
S. ferax
S. moniliferg
S. terrestris
Sterile II
P. cymosa
L. caudata
Aph. laevis
Aph. scaber
Aph. stellatus
Sterile I
0
10
20
80
90
PERCENT OF SAMPLE

89.47% of the isolates, with P. cymosa and Sterile I each composing 5.26% of the total number of aquatic isolates (Fig. 66).

C. Individual fluctuations and trends

The seasonal occurrence of species with maxima in the winter months (December to March), almost total absence from May to September and reappearance in November is shown in Fig. 67. These included S. monilifera, P. cymosa and Aph. stellatus.

Figure 68 shows the fluctuation of species which were either present at low levels or absent from December to March, and which occurred with greatest frequency in July. S. australis, S. diclina, S. ferax and L. caudata fell into this category.

The patterns of occurrence of species which were most abundant in the September sample, but were absent from December to March (with the exception of A. oblongata), are shown in Fig. 69. These species included S. parasitica, A. oblongata and Sterile II.

Further examples of fluctuation of individual species can be seen in Fig. 70.

Some species occurred in only one or two collections. These included S. eccentrica, Sc. asterophora and Apo. completa in March; S. anisospora in July; S. unispora, Sc. asterophora and Pr. paradoxa in September. Their

Figure 66. Frequency distribution of species in the 31 December, 1976, water sample. (Collection 8).

S. monilifera

P. cymosa

Sterile I

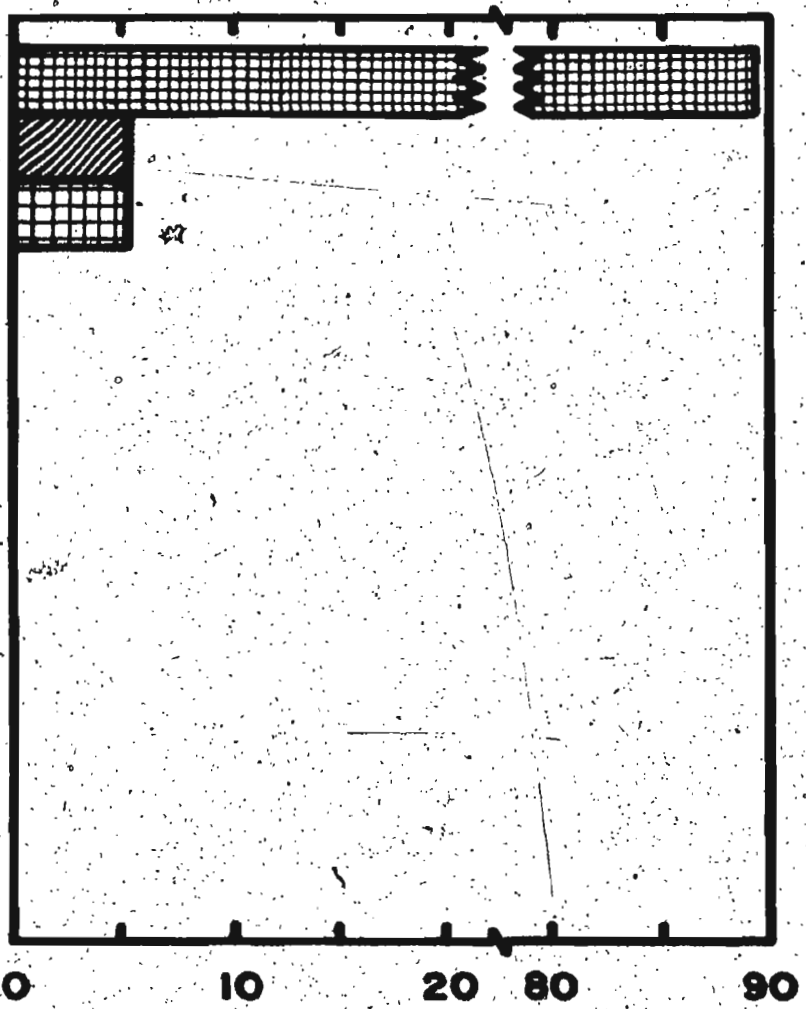


Figure 67. Seasonal fluctuation of the winter species. They are S. monilifera (■—■), P. cymosa (o—o) and Aph. stellatus (●—●).

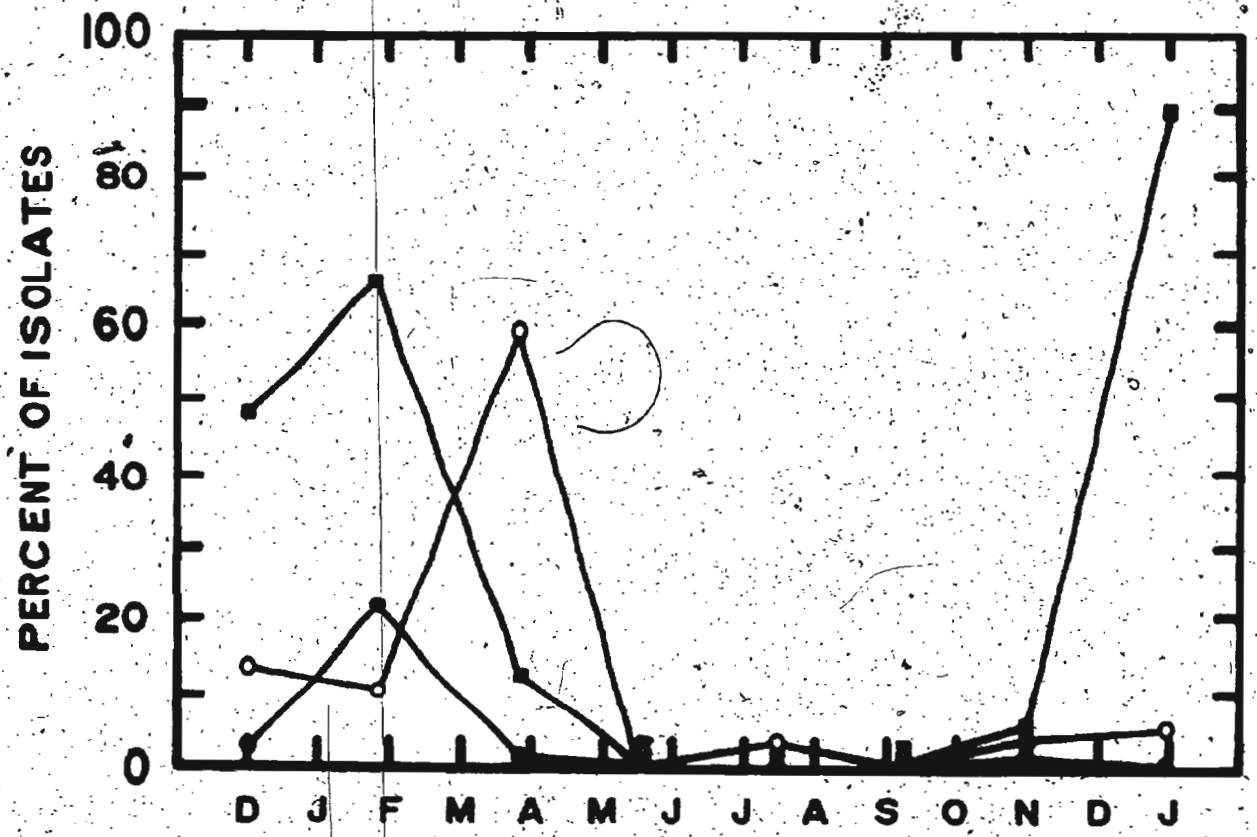

100
80
60
40
20
0
PERCENT OF ISOLATES
D J F M A M J J A S O N D J

Figure 68. Seasonal fluctuation of the intermediate temperature species. They are S. australis (□—□), S. diclina (○—○), S. ferax (■—■) and L. caudata (●—●).

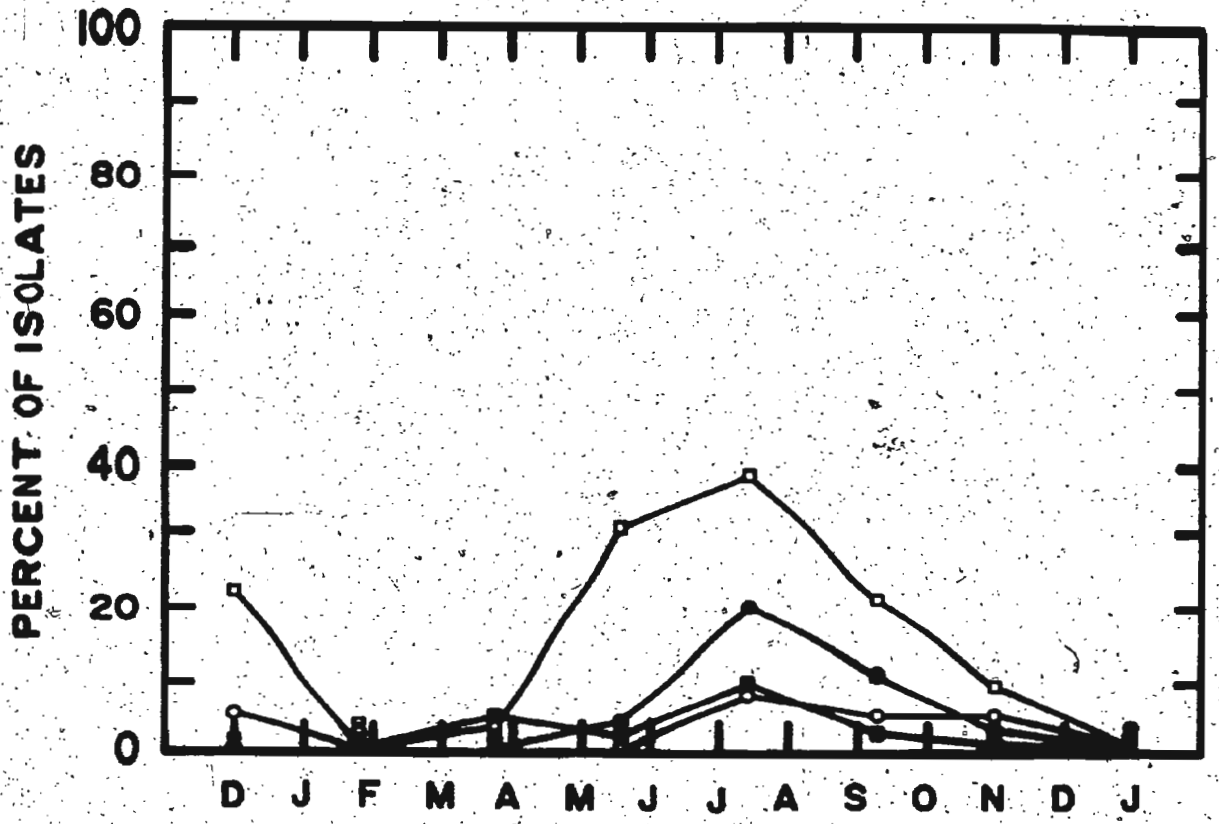

PERCENT OF ISOLATES
100
80
60
40
20
0
D J F M A M J J A S O N D J

Figure 69. Seasonal fluctuation of the warm weather species. They are S. parasitica (▲—▲), A. oblongata (■—■) and Sterile II (●—●).

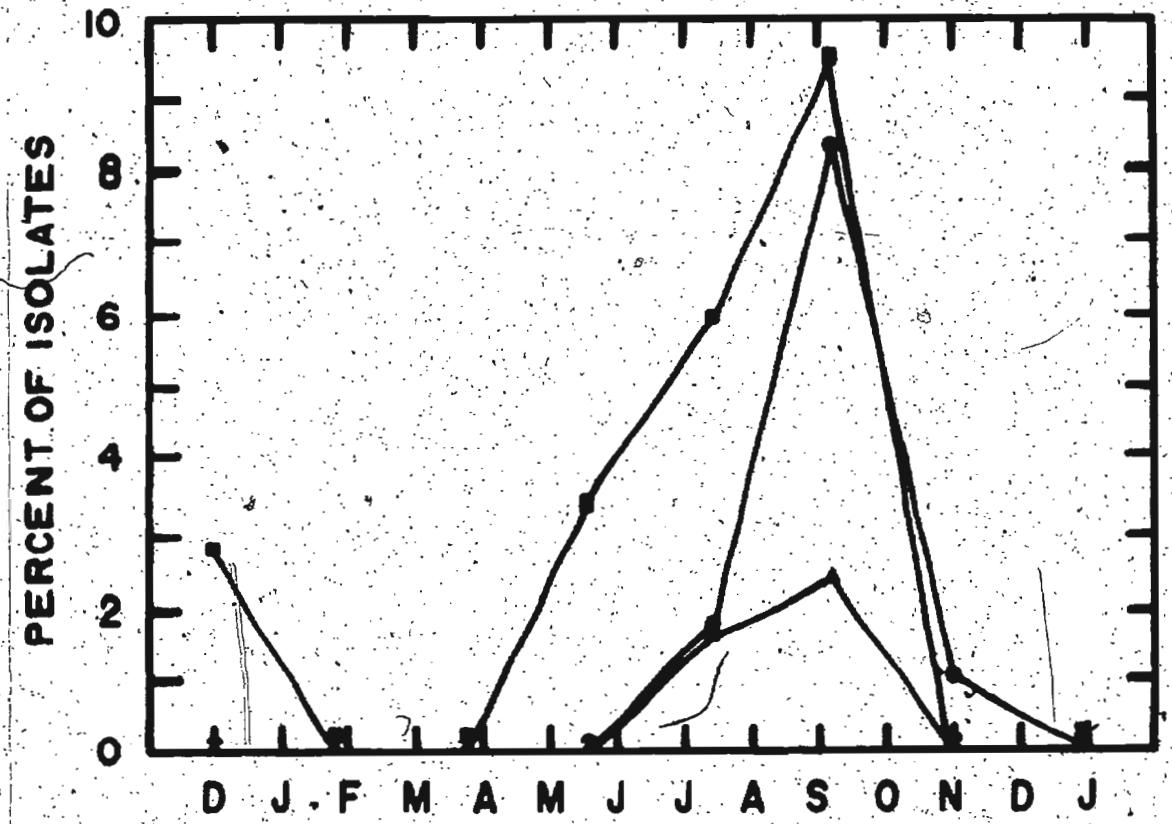
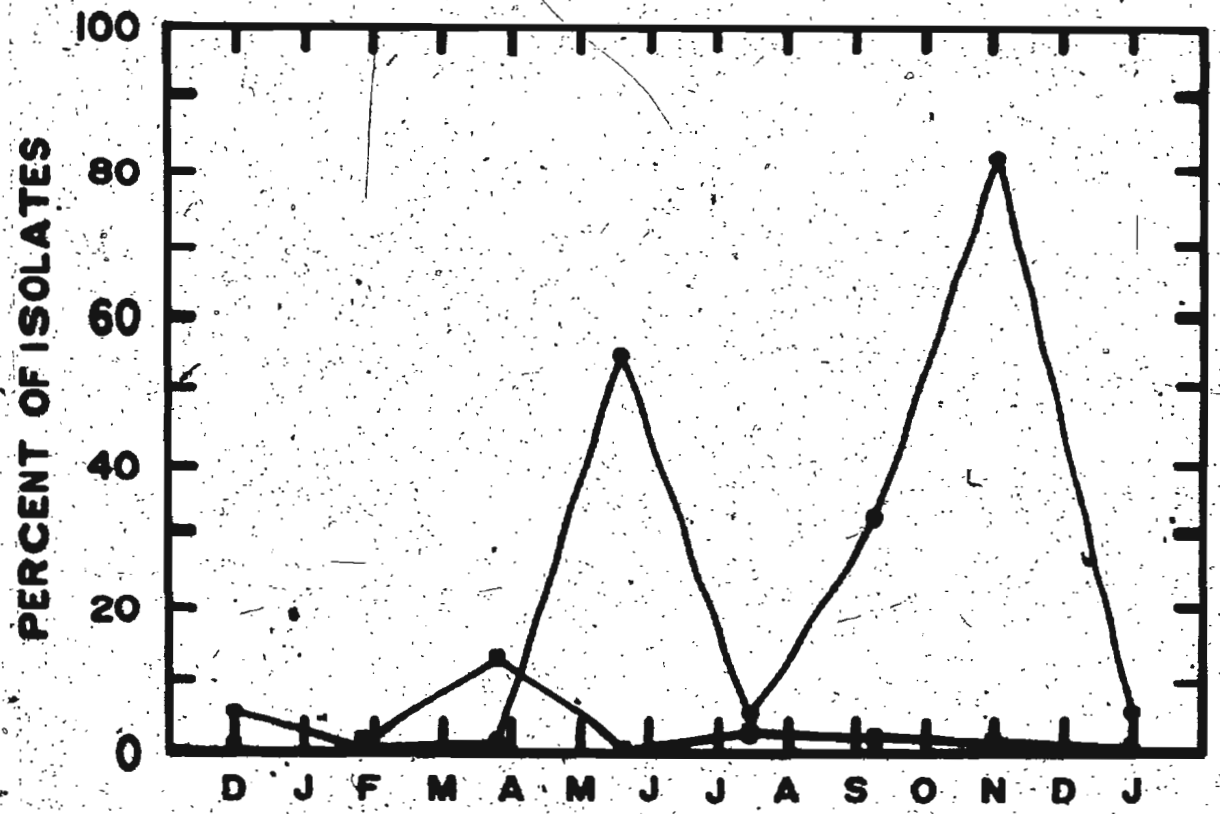

PERCENT OF ISOLATES
0
2
4
6
8
10
D
J
F
M
A
M
J
J
A
S
O
N
D
J

Figure 70. Seasonal fluctuation of S. terrestris (■—■) and Sterile I (●—●).

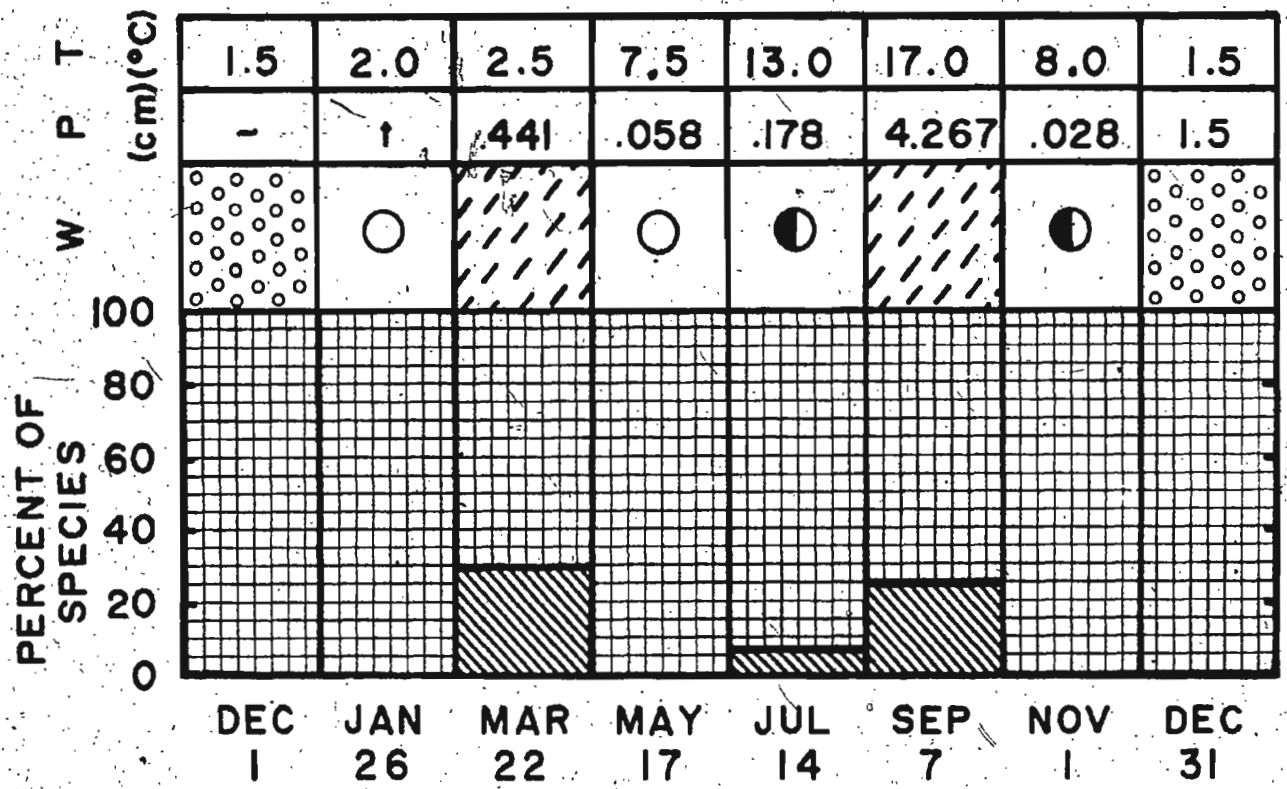

PERCENT OF ISOLATES
100
80
60
40
20
0
D J F M A M J J A S O N D J

occurrence with relation to the weather prevalent at the time of collection is shown in Fig. 71.

D. New records of occurrence for Newfoundland

Of all the species recorded, only two had been previously noted in Newfoundland. These included S. ferax (Nolan 1976) and P. cymosa (Nolan and Lewis 1974). The following species of the Order Saprolegniales were first records: S. anisospora, S. australis, S. diclina, S. eccentrica, S. monilifera, S. parasitica, S. terrestris, S. unispora, Sc. asterophora, Pr. paradoxa, A. oblongata, L. caudata, Aph. laevis, Aph. scaber and Aph. stellatus. Of the Order Leptomitales, Apodachlyella completa was the only representative isolated. It was also a first record for Newfoundland.

Figure 71. Occurrence of the occasional species with relation to the weather prevalent at the time of collection. Occasional species  ; more frequent species  ; weather (W) as follows: Snow  , sun  , rain , overcast  ; temperature (T); precipitation for the date of collection (P) recorded by the St. John's Airport Meteorological Station.


T (°C)
1.5
2.0
2.5
7.5
13.0
17.0
8.0
1.5
P (cm)
-
↑
.441
.058
.178
4.267
.028
1.5
W
PERCENT OF SPECIES
100
80
60
40
20
0
DEC 1
JAN 26
MAR 22
MAY 17
JUL 14
SEP 7
NOV 1
DEC 31

DISCUSSION

The seasonal occurrence of and variation in the total number of aquatic fungi and individual species recorded in Broadcove River was dependent on and closely interrelated with many different factors. Environmental conditions such as temperature, pH and nutrient content of the water must be taken into consideration together with the inherent characteristics of the fungi themselves. Periods of dormancy before germination of the oospores, for example, were not determined by temperature (Perrott 1960), but rather they seemed to be governed by an internal timing mechanism.

The influence of these factors on the fungal population of the stream tended to override one another, so that if one factor reached an extreme (such as the lowest temperature recorded, 1.5°C), its effect was the main one noted regardless of other environmental conditions.

Each aspect of Broadcove River will be discussed individually then synthesized to give some insight into the ecology of the Saprolegniaceae in Broadcove River.

A. Effect of temperature

1. Number of isolates recorded

At very low temperatures, such as 1.5°C recorded at the time of the two December collections, the number of isolates was very low, less than one hundred

propagules in five litres. There was an increase in the number of propagules from 51 to 341 in the first three collections. The corresponding increase in temperature of 1°C between the time of the first and third collections was not thought to be the major cause for this large increase.

The temperature increased from 2.5°C in March to 17°C in September, yet the total number of isolates of aquatic fungi decreased to 178 in five litres. The greatest number of fungal propagules was recorded in November, when the stream temperature had dropped to 8°C.

There appeared to be no direct correlation between the temperature of the stream and the fluctuation in the number of propagules recorded, although a generally higher number was obtained above 1.5°C. It is not known, however, what proportion of the total number of isolates was made up by zoospores released from zoosporangia, as opposed to hyphal fragments resulting from mycelial growth. The species recorded from the cold water (1.5° to 2.5°C) were not the same as those obtained from warmer water. If asexual reproduction were taking place at or before the time of collecting the sample, the number of zoospores released per zoosporangia characteristic for each species would tend to influence the number of propagules recorded for that species, regardless of scant or profuse mycelial growth.

It is not unreasonable to assume that the fungal

growth rates were decreased to a minimum at 1.5°C. Resistant propagules such as ungerminated oospores, gemmae (Dick 1962) and maybe primary and secondary zoospore cysts would perhaps be more common than vegetative hyphae. It seems unlikely that the formation and liberation of zoospores would occur at 1.5°C, as this temperature lies below the ranges observed for production of zoospores in some species of the Saprolegniales (Cotner 1930, Couch 1932, Salvin 1941). However, similar studies have not been carried out with fungi isolated from constantly cold waters such as those of Broadcove River. The possibility exists that species common here may have adapted to the low temperature, with sexual and asexual reproduction occurring in a lower temperature range than that observed for their counterparts isolated from a more temperate region. In this case, even slight mycelial growth may have sporulated, as long as the hyphae were not deficient in nutrient reserves (Dick 1968).

2. Number of species recorded

Low numbers of species were recorded from the water samples obtained when the stream temperature ranged from 1.5° to 7.5°C. An exception occurred in the March collection when the number of species increased to ten, although the temperature of the stream was at 2.5°C. In this instance, other factors must have stimulated fungal activity, and perhaps the temperature was not low enough to curtail or

inhibit this activity.

The largest number of species, thirteen, occurred at 13°C, a temperature favourable for growth of aquatic fungi (Nolan and Lewis 1974, Nolan 1975, Nolan 1976), as well as for the production of abundant zoosporangia in some species of the Saprolegniales (Newby 1948, Powell et al. 1972).

Twelve species were isolated from water samples collected when the stream temperature was at 8° and 17°C. The fact that at 7.5°C (May) the water yielded only six species should not be regarded as being incompatible with a temperature of only 0.5° higher (November) yielding twelve species. We see that other aspects were influencing the activity level of the fungal population of the stream. For example, a stream in November has a higher abundance of suitable substrates such as twigs and leaves (Waterhouse 1942) than that available in May, at the end of winter.

The temperature of the water may be seen to have had an effect on the number of species collected. Collections in which the most species were obtained were made when the water was at the three warmest recorded temperatures. However, the maximum number occurred when the stream was at 13°C in July rather than when it was at 17°C in September. Temperature may have influenced the results in two ways. Directly, by allowing the formation of zoosporangia and liberation of zoospores, thereby increasing the inoculum

potential of species so affected. Indirectly, by increasing the amount of food available, for example, leaves, grasses, insects and insect exuviae, which would increase the mycelial growth of the fungi (Kanouse 1932, Dick 1970). In a stream with rapidly flowing water such as Broadcove River, bits of hyphae would presumably be constantly broken off by the action of the water. If luxuriant mycelial growth was present upstream from the collection site, the amount of hyphal fragments suspended in the water should reflect this increased level of activity.

3. Temperature of the stream and the individual species observed

The species observed could be divided into three groups with respect to the temperature at which they were collected with greatest frequency.

The cold temperature species included S. eccentrica, S. monilifera, S. terrestris, P. cymosa, Aph. stellatus and Apo. completa. They occurred when the stream temperature ranged from 1.5° to 8°C, with the exception of S. terrestris, which also appeared in low numbers up to 17°C, and P. cymosa, which was recorded with a 0.5% frequency at 13°C.

Coker (1923) isolated S. eccentrica at only one time (December) during extensive sampling carried out over a one year period in North Carolina, USA. The present study also found S. eccentrica to be a rare species, as it was only

isolated once in March when the water was at 2.5°C.

S. monilifera occurred most frequently at 1.5°and 2°C. Coker (1923) isolated S. monilifera (= Isoachlya monilifera (de Bary) Kauffman) in January, February and March. These occurrence records may indicate the adaptation of this species to cold temperatures.

S. terrestris had a maximum occurrence in March, though this may have been due to other environmental factors. Sexual structures were produced by this species at 15° and 25°C (Elliott 1968), as well as at 13°C in the present investigation. Sexual reproduction of S. terrestris at these temperatures may account for this species' occurrence in warmer waters (8.0° to 17°C).

P. cymosa occurred most frequently at 2.5°C (March). Previous studies have shown that the optimum temperature for vegetative growth of this species ranges from 12° to 17°C, with scant growth occurring at 3°C (the lowest temperature tested; Nolan and Lewis 1974). This would indicate that a factor other than temperature was directly responsible for the increase in numbers of P. cymosa isolates recorded in March. Both Coker (1923) and Roberts(1963) considered P. cymosa to be a winter species.

The maximum recorded frequency of Aph. stellatus occurred in January (2°C). This species was not recorded at the two warmest water temperatures, contrary to obser-

vations made on British water moulds, where Aph. stellatus was recorded as a constant, year round species (Roberts 1963). SS plates inoculated with Aph. stellatus and incubated at various temperatures from 4° to 30°C yielded growth from 4° to 22°C, with maximum growth occurring at 17° and 22°C (see Appendix E). This would indicate that the absence of this species when the water was warmer was not due to the direct effect of temperature on growth. Rather, the slender mycelium of Aph. stellatus may have been crowded out on available substrates by the stouter, more vigorous mycelium of species of Saprolegnia (Dick 1970).

Other species fell into the second category, that of isolates mainly recorded from water with temperatures ranging from 7.5°C to 17°C with maximum occurrence at 13°C. These intermediate temperature species included S. anisospora, S. australis, S. diclina, L. caudata and Aph. laevis.

Both Coker (1923) and Roberts (1963) reported S. anisospora as a winter species, though they gave no temperature range. It is quite possible that a 13°C water temperature, though occurring in a Newfoundland stream in July, falls within the range of winter or spring temperatures in the regions studied by these two investigators. Certainly, the range of temperatures recorded in Broadcove River throughout the year fell into Hughes' winter range of 7° to 21°C for his collection sites in the south-eastern

United States (Hughes 1962). Under laboratory conditions, S. anisospora grew best at 10°C, producing abundant zoosporangia; growth at 17° to 20°C was reduced and few zoosporangia were produced (Newby 1948). Isolation of an uncommon species probably occurs when that species is at the peak of its activity (Roberts 1963). This seemed to be the case here, as it is possible that S. anisospora was producing zoospores at the time of collection.

The optimum temperature for mycelial growth of S. ferax is 24°C with good growth occurring from 19° to 26°C (Nolan 1976). However, S. ferax was found with greatest frequency at 13°C. Because zoosporangia and oogonia were produced at this temperature in sterile, distilled water, it is possible that the fungus may have been actively sporulating when the July sample was collected. Roberts (1963) considered S. ferax to be a summer species.

Oogonium production for S. diclina has been observed from 5° to 21°C with 18° to 20°C being the optimum (Szanislo 1965). Gemmae production was observed from 5° to 32°C (Szanislo 1965) which may account for this species being recorded in the winter.

Rapid growth was recorded for L. caudata in the range of 25° to 30°C, with a decrease in growth rate occurring below 18°C (Couch 1932). In the same study sexual structures were observed to appear from 18° to 22°C, though some strains

produced sexual structures at 14°C. Coker (1923) recorded this species with greatest frequency in the summer.

Aph. laevis and Aph. scaber were recovered when the stream temperature was at 7.5°, 8° and 13°C but not at 17°C. Scott (1961) stated that Aphanomyces species grew well from 15° to 20°C and produced mature sporangia and sex organs at 15°C. Roberts (1963) reported Aph. laevis as a winter species, which is in keeping with the findings of this study. The absence of these two species from the sample collected when the stream temperature was at 17°C may again be due to competition for substrates with the more vigorous, and also more abundant, Saprolegnia species.

For all the above species except Aph. laevis and Aph. scaber, good growth on EYPSS agar media and sexual and asexual reproduction in sterile distilled water were observed to occur at 13°C. The latter type of propagation may account for the peak in the number of species recorded in the July collection. Alternately, if adequate nutrition was present in the water, then increased mycelial growth may have caused an increase in the fungal material suspended in the stream.

The third category consisted of species whose maximum occurrence coincided with the highest recorded temperature (17°C). These species were not recorded below 7.5°C with the exception of A. oblongata, S. parasitica, S. unispora, A. oblongata, Pr. paradoxa and Sterile II belonged in this

category.

Rapid, heavy growth of the mycelium of S. parasitica occurred from 15° to 30°C, with 25°C being the optimum temperature (Powell et al. 1972). The maximum number of zoospores was produced at 15°C (see Powell et al. 1972). This indicates that S. parasitica would be more likely to be recorded from warmer waters.

Vigorous growth without the production of zoospores has been recorded for S. unispora at 30°C, with many zoospores being produced in an icebox culture (Coker 1923). The isolation of S. unispora from the stream when the water was at 17°C may mean that the growth or sporulation of the fungus was stimulated by the higher temperature.

The optimum temperature for the production and liberation of zoospores by Pr. paradoxa ranged from 23° to 25°C. Zoospore formation and liberation also occurred from 16° to 18°C, though at a slower rate (Cotner 1930). The above information, together with previous records of occurrence (Beverwijk 1948, in July; Coker 1923, predominantly in May) indicates that this is a warm weather species.

Previous investigators have recorded A. oblongata in February (Coker 1923) and from September to March (Forbes 1935). Although this species was recorded here in December, it occurred with highest frequency when the stream

temperature was at 17°C.

The absence of sexual organs in Sterile II when incubated in sterile, distilled water may suggest that the incubation temperature (13°C) was outside the range within which oogonia and antheridia would be produced. Sterile II isolates seemed to prefer warm temperatures.

The warm temperature species appeared with low frequency (below ten percent in any sample). It would seem that the temperature conditions in Broadcove River were not optimal for maximal mycelial growth and reproductive activity of these species. This observation is corroborated by the temperature ranges for abundant vegetative growth of S. parasitica (Powell et al.1972) and Pr. paradoxa (Cotner 1930). The temperature range given for the latter species was above the highest temperature recorded in Broadcove River.

Sterile I occurred with maximum frequency in May (7.5°C) and November (8°C). The numbers decreased during the winter months (1.5° to 2.5°C) and also in July (13°C). The absence of both sexual and asexual organs in these isolates may again mean that the incubation temperature did not lie within the required limits. However, a direct correlation with temperature is not evident other than that a higher number of propagules was present at relatively low temperatures (i.e. 7.5° and 8°C).

B. Effect of pH

The pH of Broadcove River varied from a minimum of 5.5 to a maximum of 6.6. These values lie within the range of slightly acid (5.3 to 6.8) and neutrally acid (5.2 to 7.5) waters according to Lund's classification (Lund 1934).

Many species, because they occurred in most collections, also occurred at all the pH levels recorded. Those species which were recorded in the water when its pH was from 5.5 to 6.6 and with greatest frequency from water measuring 6.2, were S. australis and S. ferax. Also occurring within this pH range were Aph. stellatus (most abundant at pH 6.3), P. cymosa and S. terrestris (most frequent at pH 5.5).

These species, with the exception of S. australis have been reported from varying pH ranges by other investigators. S. ferax has an optimum pH for growth of 5.6 (Nolan 1976). It has been found in soils of pH 4.9 to 6.0 (Dick 1963) and in waters from 7.0 to 8.4 (Lund 1934, Perrott 1960). Aph. stellatus has been found in waters from pH 3.0 to 7.0 (Roberts 1963) and from pH 7.0 to 8.5 (Lund 1934). P. cymosa, with an optimum pH of 6.9 for vegetative growth (Nolan and Lewis 1974), has been isolated from soils of pH 4.2 to 6.0 (Dick 1963) and in waters from pH 5.2 to 7.6 (Roberts 1963). The pH of the water in which maximum occurrence of S. ferax and P. cymosa was observed did not

correspond with the optimum pH for vegetative growth.

A. oblongata, L. caudata and Sterile I were recorded from water with pH 5.9 to 6.6, these species being isolated with greatest frequency when the pH was 6.6, 6.2 and 6.5, respectively. L. caudata grew very well from pH 3.0 to 9.0, forming oogonia at pH 5.0 to 7.0 (Couch 1932). The recording of L. caudata may, therefore, have been due in part to the production of oogonia in the stream. A. oblongata has been isolated from waters with pH 5.0 to 7.2 (Roberts 1963). Records of Aphanomyces sp. (to which genus Sterile I is thought to belong) have been obtained from waters ranging from pH 3.3 to 10.8 (Goldsmith 1948).

The isolation of S. diclina and S. parasitica from Broadcove River occurred when the water ranged from pH 6.2 to 6.6. S. diclina was most abundant when the pH was at 6.2, and S. parasitica when it was at 6.6. Records of S. diclina have been obtained from waters with pH 3.5 to 7.7 (Lund 1934) and pH 7.0 to 8.4 (Perrott 1960). The best mycelial growth for S. parasitica occured at pH 6.0 to 7.0 (Powell et al. 1972)

Aph. laevis was isolated from the water when it was at pH 6.2 and 6.5, with greatest abundance at the former pH. Aph. laevis had been previously found in waters with pH 4.6 to 7.6 (Roberts 1963) and 7.0 to 8.5 (Lund 1934).

Some species which occurred in only one or two

samples were recorded from water with a pH that had previously been reported to yield such a species. For example, S. anisospora occurred when the water was at pH 6.2. Dick (1963) had recorded this species at pH values of over 6.0 and Roberts (1963) had reported it from waters of pH 5.0 and 6.2. Pr. paradoxa, isolated from the sample collected when the stream was at pH 6.6, had previously been recorded at pH 7.0 (Beverwijk 1948). Sc. asterophora, recorded when the pH of Broadcove River was at 5.5 and 6.6, was found to occur in soils with a pH of less than 4.2 (Dick 1963), and in waters ranging from pH 5.2 to 8.4 (Perrott 1960).

The pH of Broadcove River was not a determining factor as to whether a species would be recorded. It seems that other factors affected the level of activity of the fungi in the stream. If these other factors were favourable (for example, abundant nutrients and reasonable temperature) then the species would be present as long as the pH was not prohibitive to metabolic activity and growth of the fungi. The pH range of Broadcove River was a narrow one, and furthermore not extremely alkaline or acidic. It would, therefore, not be inhibitory to any of the species recorded, for the pH range of 5.5 to 6.6 lies well within the wider ranges in which these species had already been reported to occur.

However, the pH of the stream might be influential

in allowing the fungus to go through the various phases of its life cycle. Though vegetative growth can occur over a wide pH range, asexual and sexual reproduction occurs over a narrower range, as in the case of L. caudata (Couch 1932). Germination of oospores is inhibited by low pH in some species of the Saprolegniaceae such as Saprolegnia delica Coker (= S. diclina) and S. ferax (Ziegler 1948). A pH of 4.9 and 5.9 tended to have such an effect, or, if germination did occur, the zoosporangium normally formed by the germ tube (see Fig. 1) was not produced. In this manner pH would affect the recording of a species, for if germination of the oospore was inhibited there would be a resultant decrease in the potential activity of the species so affected in the stream. When the pH again became favourable for germination, the zoosporangia would be formed and liberate zoospores which in turn would encyst, germinate and produce mycelia. This heightened activity could then be picked up during sampling time by the investigator.

It appears, therefore, that the pH of the water in Broadcove River may affect the population of aquatic fungi already present by being favourable or unfavourable for sexual and asexual reproduction, and for germination of the oospores. However, in the presence of abundant nutrients, a fungus thriving on this supply would remain vegetative regardless of whether the pH of the water was favourable

for sexual and/or asexual reproduction.

C. Effect of nutritional and chemical factors of the stream

The monitoring of elements and other chemical parameters of Broadcove River made it possible to determine if, and how, environmental and geochemical activities in the area surrounding the stream affected the fungal population present. The production of sex organs in aquatic fungi is determined by environmental conditions and most characteristics can be varied by nutritional changes (Kauffman 1908). Therefore, the level of elements and compounds in the stream may have been influential in determining whether the fungal propagules present were mycelial, sexual or asexual.

1. Trace elements

The fluctuation of the level of trace elements in Broadcove River was related to general weather conditions throughout the year. The levels of hardness (calcium carbonate), magnesium, manganese and potassium all reached a peak in March (although this was not necessarily the highest value recorded in all collections). Silicates and iron were also high at this time. Increasing air temperatures together with the thawing of the snow and frozen soil, and abundant rain in the days prior to the March collection, combined to give this increase. During the winter, a buildup of trace elements (such as zinc, copper, cadmium and

manganese) occurs in the frozen soil, and also in the snow as a result of ionic diffusion of these metals from the soil (Jonasson and Allan 1973). When the thaw occurs, these metals and their associated anions (bicarbonates, sulphates and chlorides, for example) are flushed into the stream (Jonasson and Allan 1973) thereby increasing the trace nutrient content of the stream. Calcium and zinc had also increased their concentrations in March, reaching peaks in May (although zinc never increased to the original high level measured in the initial December sample). Because Jonasson and Allan (1973) reported that the effects of the spring run-off may persist for several weeks, these high May levels were not inconsistent with the general trend.

A secondary peak in the number of propagules was recorded in March, and the number of species obtained also increased to ten (from three in January). This indicates that fungal activity in Broadcove River had been stimulated by the increased level of micronutrients. Nutritional experiments using Achlya klebsiana Pieters showed that no growth occurred in the absence of, or in the presence of insufficient amounts of calcium, zinc, manganese and iron (Reischer 1951). Steinberg (1948) demonstrated the importance of calcium as a micronutrient in the mycelial growth of Rhizoctonia solani Kühn. Reduced growth of the aquatic phycomycete Catenaria anguillulae Sorokin occurred

when iron, zinc, calcium and magnesium were omitted from the medium (Nolan 1970a). These results indicate that an increase in the level of micronutrients would be favourable for growth of aquatic fungi in the stream.

It is possible that the increase in the level of micronutrients also resulted in the germination of encysted zoospores (Dick 1968) and other resistant propagules such as gemmae. Species thus present in the stream throughout the December and January collections, but which were not recorded due to lack of vigorous activity (or growth), would be picked up on stimulation of germination and subsequent growth. The reappearance or appearance of S. australis, S. ferax, S. terrestris and Sterile I may be explained by the above. Some species such as S. eccentrica, Sc. asterophora and Apo. completa were recorded only in the March collection (and also in the September collection in the case of Sc. asterophora). The increase in the level of micronutrients may have been responsible for their heightened activity and subsequent recovery. The maxima for P. cymosa and S. terrestris attained in this collection may also have been due to the above factors.

A decrease in the level of hardness, magnesium, iron, manganese, potassium and silicates in May, and a further decrease of the above together with calcium and zinc levels in July would appear to have some relation to the decrease

in the total number of fungal propagules observed during these two months. Lack of abundant rain over this period accounted for the decrease in the level of micronutrients. The number of species, however, rose to a maximum in July, with the only record of S. anisospora being obtained in that collection. It has already been mentioned that formation of zoosporangia and oogonia, as well as liberation of zoospores, had been observed in cultures maintained in distilled water at 13°C. It is possible that the temperature of the stream (13°C) and low level of micronutrients recorded in July combined to provide conditions conducive for asexual and sexual reproduction in the stream. Many species might thus have been at a peak of reproductive activity, and so recorded as present, even though the total volume of their mycelial growth may have been reduced.

The micronutrient level peaked again in September, and although the number of species recorded remained high, the total number of propagules was at its lowest point for the warm weather period. The increase in micronutrients was again due to rain water run-off, which carried ions that had built up in the soil over the preceding dry period (Jonasson and Allan 1973).

An increase in the number of species related to the increase in the level of micronutrients did not occur in September as it did in March. The period preceding the March

collection was one of cold temperatures, so one would expect that many species would be present in low numbers at a level too low to be recorded. Although a temperature of 2.5°C by itself may not have been enough to stimulate the resistant propagules of those species to germinate and grow vegetatively, the increase in micronutrients probably had this effect. The period preceding the September collection, on the other hand, was one of warmer temperatures which lay within the ranges for good vegetative growth of most of the species present. The effect of an increase in the level of nutrients would not be as pronounced, as conditions for growth were already favourable, and peak activity had already been recorded for many species. However, the increase in numbers observed for S. parasitica, A. oblongata and Sterile II together with the appearance of S. unispora, Sc. asterophora and Pr. paradoxa may have been partly caused by the higher level of nutrients.

The decrease in numbers of the members of the Saprolegniaceae which occurs during the warmest temperatures has been reported by all the investigators known to have studied seasonal periodicity (for instance, Petersen 1910, Waterhouse 1942, Dick and Newby 1961, Roberts 1963). It may be due to a number of reasons such as the following: Increased abundance of other saprophytic aquatic life (bacteria, for example), as well as increased mobility

of possibly living substrates, making it more difficult for zoospores and other propagules to become established; the nature of the growth present (sexual, asexual or vegetative); dormancy periods of the oospores of the type observed by Perrott (1960) in the aquatic fungus Monoblepharis macandra (Lagerheim) Woronin.

There was another peak in the level of trace elements on 31 December, 1976, but a low water temperature (1.5°C) may have inhibited luxuriant and abundant vegetative growth. However, a higher number of propagules (91) was recorded in this last collection than in the first collection (51), also with a low water temperature (1.5°C). This higher number may have been related to the increased level of micronutrients.

The levels of cadmium and copper were mostly below 0.01 mg/l, never rising above this value. Copper was not found to be stimulatory or necessary for growth in Achlya klebsiana (Reischer 1951), so that the low level of this element in the stream was probably not deleterious to the fungal population.

In a study undertaken where the temperature of the water was more constant (varying only 2° to 7°C), Alabi (1971b) found a direct correlation between the number of species of the Saprolegniaceae collected and the total ion content of the water. Such a clear relationship was

difficult to establish in a stream where the temperature range was from 1.5° to 17°C, as many temperature related variations possibly occur, masking the effect of ion content.

2. Sodium

The primary source of this element in the winter is "road salt" liberally used on the roads of this area. It was, therefore, highest in the winter (at the abnormally high level of 24.58 mg/l in the first collection). A secondary peak was recorded in March, attributable to flushing of the salt accumulated on the frozen ground, by rain. The level of sodium fell over the next collections reaching a low in September, then increased again at the on-set of winter.

The pattern of fluctuation of the total number of propagules followed that of the sodium level, with coincident (but not equal) peaks in March and November. Sodium chloride at low concentrations was found to be stimulatory to the growth of the fresh-water fungus C. anguillulae (Nolan 1970b) and the marine fungus Dendryphiella salina Pugh (Jones and Jennings 1965). Some species of Saprolegnia (such as S. parasitica) were shown to have a high degree of tolerance to salinity (Harrison and Jones 1971). There may thus have been some correlation between the level of sodium and fungal growth in the stream.

The work of Jones and Jennings (1965) showed that sodium at high concentrations inhibited the growth of D. salina, but that the presence of calcium, magnesium, strontium and barium removed this inhibition. Eighty times less calcium than sodium was effective in this respect.

It is possible that the lower number of fungal propagules recorded in the first collection (1.5°C; 24.58 mg Na/l) was in part due to a sodium-related inhibition of vegetative growth of the aquatic fungi present. A higher number of propagules was obtained in the final December sample (1.5°C; 8.354 mg Na/l). If the effects of calcium and magnesium as noted by Jones and Jennings (1965) also apply to fresh-water fungi, then the proportions of these two elements to sodium in the last sample would have prevented an inhibition of growth (by the sodium) from occurring.

3. Phosphates

The level of total phosphates increased from December to March, as did the total number of propagules and species. There was a decrease in May, mirrored by a decrease in the number of propagules and species, and a peak in July. This peak coincided with the maximum number of species, although from this point onwards the level of phosphates and the total number of propagules did not show any correlation. A drop in both the species number and

phosphate level was measured in September. A further drop in the phosphate level occurred in November, yet the number of species remained the same and the number of propagules greatly increased. Perhaps at this point the increase in the propagule level of the stream was due to saprophytic activity and, therefore, the fungi would not be dependent on the soluble phosphate level. The increase in phosphate in the final December collection did not cause a related increase in fungal activity. It is possible that with the more extreme temperature condition prevalent at that time, the ability of the fungi to utilize phosphates was reduced.

4. Nitrates and Kjeldhal nitrogen

The representatives of the Saprolegniales have lost the ability to utilize nitrate-N but can use ammonium-N for growth; the Leptomitales, on the other hand, cannot use either of these nitrogen sources (Cantino and Turian 1959). Willoughby and Redhead (1973) demonstrated that aquatic fungi could withdraw nitrogenous compounds from river water and incorporate them into protein.

The level of nitrate should not affect the presence of the members of the Saprolegniales and Leptomitales, unless indirectly. Other aquatic inhabitants which did utilize the nitrates, then served as a source of nutrients for the Saprolegniales and Leptomitales, would thus affect the results of this study. This aspect was not studied,

however, and cannot be discussed without adding undue speculation.

When the number of propagules and species peaked in March, the nitrate level of the water was less than 0.002 mg N/l. The highest recorded level occurred in the first collection (0.12 mg N/l) when the number of propagules was at its lowest. No correlation appeared to exist.

The level of Kjeldhal nitrogen (nitrogen from ammonia, proteins, amino acids and peptides) coincided in its highest concentration with the maximum occurrence of the warm weather species. The presence of nitrogen was not directly reflected in the trends shown by the number of propagules, species, or individual species present.

5. Dissolved oxygen

Broadcove River is a cascading stream of shallow, rapidly flowing water, and is, therefore, constantly being aerated. Members of the Saprolegniales grow best in shaken cultures, indicating that they are highly aerobic (Powell et al. 1972, Nolan and Lewis 1974). Formation and liberation of zoospores occur twice as fast in aerated cultures as in non-aerated cultures (Cotner 1930). The stream studied provided an ideal habitat for these fungi as far as the availability of oxygen was concerned.

A direct relationship can be seen between the amount

of dissolved oxygen and the temperature of the water. At low temperatures more oxygen was dissolved per unit volume than at high temperatures, so that the least volume of dissolved oxygen, 6.3 ml/l, was recorded at 17°C. In contrast, 9.8 ml/l were recorded when the water was at 2°C.

The decrease in the amount of dissolved oxygen coincided with the decrease in fungal propagules observed from March to September. This may indicate a relationship between the level of dissolved oxygen and the fungal activity in the stream. At colder temperatures, when the dissolved oxygen was highest, the correlation was no longer observed. Presumably, it is the limiting (or most extreme) environmental condition which has the most effect on the fungal population.

The fluctuation of species number and occurrence of individual species could not be directly correlated with the amount of oxygen present in the water. The cold weather species enjoyed the most dissolved oxygen. A clear relationship between dissolved oxygen and number of species could not be established by Alabi (1971b) either, so that it would seem that on a local basis, factors other than the amount of dissolved oxygen determine the species population of a stream. However, if streams of different oxygen content were sampled, an effect might be noted. For

example, Alabi (1971b) did record a low number of species, (from one to six) which may have been due in part to the low level of dissolved oxygen present in the waters he studied (mainly from 2.0 to 6.0 ml/l). This low oxygen content may in turn have been due to the high temperatures of the streams he sampled (from 24° to 33°C). Broadcove River, with a higher amount of dissolved oxygen and lower temperatures, yielded from three to thirteen species.

6. Sulphide

The level of sulphide was found to be 0.005 mg S/l or less in all samples except the November and final December samples, when it increased to 0.17 and 0.008 mg S/l, respectively.

The increase in November coincided with the maximum occurrence of the total number of propagules, and the maximum occurrence of Sterile I. However, 82.5% of the November collection was made up of Sterile I isolates, so these accounted for the high number of propagules. The secondary peak for Sterile I isolates occurred in May, when the sulphide concentration was 0.0 mg S/l. Therefore, the level of sulphide appeared not to be a determining factor on the level of the fungal population in the stream.

7. Chemical oxidation demand

This parameter represents all of the carbon

present which can be chemically oxidized. The level recorded for chemical oxidation demand is higher than the level of biologically degradable organic matter.

Alabi (1971b) observed a direct relationship between the occurrence of fungi and the organic content of the water. The same could not be said for this study, as the three highest values (occurring in May, September and December) were not coincidental with a corresponding increase in fungal activity. The inclusion of small organic particles in the water sample (as noted in the final December collection) may have caused these high values, which would not reflect the actual amount of suspended organic content of the water. These organic particles were probably the result of disturbing the bottom mud at the time of collection.

If the three abnormally high values are disregarded, a better correlation can be observed. A decrease in the carbon content occurred from December, 1975, to July, 1976. The decrease was probably due to a lack of abundant animal and plant life during the cold months. The number of fungal propagules also fell from March to September, indicating a possible relation between the carbon content and number of propagules.

8. Macronutrients

The macro-organic content of the stream

(macro-organic referring to large sources of food such as living and dead insects, nematodes, fish and insect exuviae) was not recorded at the time of collection. Yet at any given time, the number of isolates obtained may have included species which were growing saprophytically or parasitically on these larger food sources.

Insect exuviae are colonized by Aphanomyces, Saprolegnia, Achlya and Leptolegnia species (Dick 1970). Information on the abundance of these and similar substrates present would have helped to explain the occurrence of these fungi in Broadcove River.

D. Individual fluctuations and trends

The overall picture showed an increase in the number of fungal propagules in March, a gradual decrease from May to September and a sharp increase in November (see Fig.11). Each species had an individual fluctuation pattern, however. The isolates fell into three groups with respect to the time of year at which they were recorded, and at which their highest numbers occurred. These were the winter species group (for example, S. monilifera), the intermediate temperature species group (L. caudata, for instance) and the warm temperature species group (such as S. parasitica).

Sterile I isolates did not fall into these categories, having a secondary peak in May, a large primary

peak in November and lows in the winter months and in July. It is possible that the recording of this isolate was related to oospore germination in May, September and November, with dormancy periods in July and from December to March (see Perrott 1960).

Some species occurred in only one or two collections, making up a small percentage of the total isolates for that day. These species could not be said to show any seasonal trend other than that they were recorded from the water when abundant rain was falling. The species in question were S. eccentrica, Sc. asterophora and Apo. completa in March (2.5 cm of rain); S. anisospora in July (0.178 cm of rain); Sc. asterophora, S. unispora and Pr. paradoxa in September (4.3 cm of rain). These species were picked up presumably because their germination and growth increased to the point where they were recordable by the method used. There are two factors, both related to rainfall which may have increased the presence of these species in the stream. One, already discussed, deals with the resultant increase in the level of micronutrients stimulating the germination of resting propagules already present in the stream. The other has to do with the resting propagules present in the surrounding soil being washed into the water. All these species have been recorded in the soil (Dick and Newby 1961, Dick 1966,

Seymour 1970). In studies involving the sampling of water, Saprolegnia (= Scoliolegnia Dick) asterophora has been reported as being an uncommon species (Coker 1923, Beverwijk 1948), as in the present study. Dick (1962), however, commonly recorded it from damp soils.

Dick (1968) stated that resting propagules were responsible for almost all the isolations of aquatic fungi from the soil. These propagules were present as a result of brief periods of activity correlated with favourable conditions. In our case this might be the warming up of the soil on a sunny, winter day, with the melting of the snow and ice in small patches. It seems not unlikely that rains will wash these propagules into the stream, where adequate aeration and a high level of micronutrients will stimulate germination. This might also account for the high frequency of S. terrestris in the spring.

The data obtained indicates propagules washed from the soil by rain may have been the source of species recorded only occasionally in Broadcove River. Further support for this lies in the fact that these species were not recorded in subsequent collections unless, as in the case of Sc. asterophora, heavy rains occurred. Whether these occasional species were already present in the stream or whether they were washed in from the soil cannot be proved conclusively by this study.

E. First records of occurrence for Newfoundland

The majority of the species isolated in this study were new records for Newfoundland, as extensive sampling for aquatic fungi had not been carried out in this area. As is to be expected of species found in a new location, some varied in minor detail from already published descriptions (see Appendix D).

S. monilifera isolates showed a high incidence of parasitism by a non-septate hypha, which at times appeared to originate from the oogonial stalk. The oospores of S. monilifera were destroyed by this parasitic hypha, which entered the oogonium through its pits (Fig. 18, 19, 20). In highly parasitized isolates, the oospheres did not develop into oospores with typical subcentric and centric oil droplets but rather remained dark and granular (Fig. 17). Coker (1923) also mentioned this phenomenon.

The oogonial wall of Aph. stellatus did not always have bluntly conical tubercules, but was sometimes irregularly shaped (Fig. 31, 32). Coker (1923) stated that antheridial branches may be formed early, reaching the oogonium before the papillae are formed. Although many previous investigators have described the oospore of Aph. stellatus as being eccentric (Coker 1923, Nagai 1931, Scott 1961), the Newfoundland isolates clearly had subcentric type I and II oospores. Dick (1971) also

reported subcentric oospores in this species.

Aph. scaber was found to have characteristics of both Aphanomyces irregulare Scott and Aph. scaber. The Newfoundland isolates had smooth to irregular walls (Fig. 52, 53, 54) with a single papilla or tubercule of varying length (Fig. 51, 52, 53). Howard et al. (1970) reduced the two species to synonymy under Aph. scaber.

The isolates classed under Sterile I were believed to belong to the genus Aphanomyces. The growth of these isolates on agar was submerged with no aerial growth; the edges of the colonies advanced in pointed formation (Fig. 42). Scott (1961) described the growth of Aphanomyces species on agar media as "sparse, arachnoid growth with little or no aerial development". Comparison of the hyphae of Sterile I (Fig. 35) with that of Aph. scaber (Fig. 52, 53, 54) shows a resemblance, both being rather thin, vacuolate and much branched. Hyphal coiling (Fig. 36, 37) and hyphal knots (Fig. 38) were often observed. These hyphal knots have been observed in Aph. laevis (Howard et al. 1970). Structures believed to be gemmae were also observed (Fig. 39, 40).

CONCLUSIONS

The fluctuation in the number of propagules and species recorded indicated the level of fungal activity in Broadcove River.

A temperature of 1.5°C seemed inhibitory to this activity, a low number of species and propagules being obtained at this temperature. At warmer temperatures, growth and reproductive activity were not inhibited but were influenced by other factors as well. A direct correlation between the number of fungal isolates and the temperature of the stream could, therefore, not be established.

The secondary spring maximum in both the number of propagules and species was possibly the result of (1) increased level of micronutrients making the stream favourable for germination and growth, (2) a washing in by rain and melting snow of the resting propagules of aquatic fungi present in the surrounding soil. These would also be induced to germinate in the aerated water containing a high micronutrient level.

The number of propagules decreased after March, reaching a low in September. This could be correlated with a general fall in the level of micronutrients, carbon content, sodium and dissolved oxygen in the water. The decrease in the level of micronutrients resulted from a

reduced influx of trace elements and other compounds due to decreased rainfall; reduced carbon content was perhaps due to lack of organic input during the cold weather; dissolved oxygen fell in relation to the warming up of the water.

The species number was highest in July, September and November. The peak in July was attributed to the temperature of the stream, which was favourable for asexual and sexual reproduction. This temperature, together with the low level of micronutrients present at the time, may have caused the mycelia of the species present to produce zoosporangia, zoospores and oogonia.

September rains, with the resultant rise in micronutrients, did not bring about the increase observed in the spring under similar conditions. It is thought that activity was already at a maximum due to the favourable temperature, so that a noticeable increase could not be observed. However, the presence of some species recorded only when abundant rain fell indicated that an influx of soil residents and stimulation of germination and growth may have occurred.

Although most species decreased in frequency by November, a large number of Sterile I., or Aphanomyces, isolates gave a total rise in propagules. This genus is known to flourish on living and dead insects and their

discarded exuviae. Perhaps the abundance of these substrates had increased in November, with the lower temperature becoming unfavourable for other Saprolegnia species.

The increase in the micronutrient level in the final December sample did not result in an increase of fungal activity. The low temperature of the stream was believed to have been inhibitory for this.

The amount of dissolved oxygen in the stream did not show a direct effect on the fluctuation in the number of species. It is possible that the total amount of dissolved oxygen generally present in a stream throughout the year may influence the number of species which can be supported in that aquatic system.

The pH of the stream was important mainly in that it was not too extreme to inhibit the growth of aquatic fungi. Its fluctuation may have been a determining factor on the nature of the propagules collected (whether sexual, asexual or vegetative).

The species obtained fell into three groups: winter, intermediate temperature and warm weather species. Thus, the saprophytic fungi, an important part of any ecosystem, were present throughout the year.

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APPENDIX

APPENDIX A

FINAL DISTRIBUTION OF COLONIES ISOLATED FROM EACH FIVE LITRE SAMPLE

Collection	No. aquatic isolates	No. of species	Terrestrial isolates	Total isolates
1	51	7	802	853
2	170	3	551	721
3	341	10	453	794
4	260	6	582	842
5	218	13	495	713
6	178	12	1556	1734
7	1042	12	473	1515
8	91	3	2048	2139

APPENDIX B

TEMPERATURE AND PH OF BROADCOVE RIVER

Date	Temperature (°C)	pH
1 December, 1975	1.5	6.6
26 January, 1976	2.0	6.3
22 March, 1976	2.5	5.5
17 May, 1976	7.5	5.9
14 July, 1976	13.0	6.2
7 September, 1976	17.0	6.6
1 November, 1976	8.0	6.5
31 December, 1976	1.5	6.4

APPENDIX C

PERCENT[a] OCCURRENCE OF THE SPECIES COLLECTED IN THE PERIOD FROM 1 DECEMBER, 1975, TO 31 DECEMBER, 1976

Species	Dec	Jan	Mar	May	July	Sept	Nov	Dec
S. anisospora	-	-	-	-	0.5	-	-	-
S. australis	22.9	-	1.1	31.0	40.2	20.7	10.6	-
S. diclina	5.7	-	-	-	8.7	5.9	2.4	-
S. eccentrica	-	-	1.1	-	-	-	-	-
S. ferax	-	-	2.1	3.4	10.3	3.6	0.7	-
S. monilifera	48.6	66.7	13.8	-	-	-	1.8	89.5
S. parasitica	-	-	-	-	1.6	2.4	-	-
S. terrestris	-	-	17.0	-	1.6	1.2	0.1	-
S. unispora	-	-	-	-	-	0.6	-	-
Sc. asterophora	-	-	1.1	-	-	0.6	-	-
P. cymosa	14.3	11.1	59.6	-	0.5	-	0.2	5.3
A. oblongata	2.9	-	-	3.4	5.4	9.5	-	-
Pr. paradoxa	-	-	-	-	-	4.7	-	-
Sterile II	-	-	-	-	1.6	8.3	1.0	-
L. caudata	-	-	-	3.4	21.2	11.8	0.2	-
Aph. laevis	-	-	-	-	2.7	-	0.1	-
Aph. scaber	-	-	-	3.4	0.5	-	0.2	-
Aph. stellatus	2.9	22.2	1.1	-	-	-	0.1	-
Sterile I	2.9	-	1.1	55.2	4.9	29.6	82.5	5.3
Apo. completa	-	-	2.1	-	-	-	-	-

[a]Numbers rounded off from two significant figures.

APPENDIX D

COMPARISON OF THE CHARACTERS OF THE NEWFOUNDLAND ISOLATES WITH PREVIOUS DESCRIPTIONS[a]

Isolate and characters	Authors		Newfoundland isolates
S. anisospora	Seymour (1970)		
Oogonia			
Size (diam)	(38-)46-60(-87)		(38-)59(-88)
Oospore			
Size (diam)	(16-)18-24(-34)		(21-)26(-29)
Number/oogonium	(1-)4-6(-11)		(1-)4-6(-11)
Type	Eccentric		Eccentric
Antheridial branch origin	Diclinous		Diclinous
Sporangial renewal	Internal proliferation		Internal proliferation
S. australis	Elliott (1968)	Padgett (1976)	
Oogonia			
Size	(48-)60-80(-110)	(35-)59-74(-96)	(48-)59-80(-112)
Wall	Pitted	Pitted	Pitted

[a]Diameter and length measurements in micrometers and rounded off from two significant figures. For the Newfoundland isolates, brackets indicate the largest and smallest measurement made. One central value is the mean of forty to fifty measurements; a range indicates the range of from 2 to 222 means (of ten to eighty measurements) into which the majority of the isolates fell.

APPENDIX D (CONTINUED)

Isolate and characters	Authors		Newfoundland isolates
S. australis (continued)			
Oospore			
Size	(10-)22-24(-27)	(18-)22-26(-31)	(19-)22-27(-36)
Number/oogonium	(1-)6-12(-30)	(1-)4-8(-23)	(1-)5-10(-18)
Type	Subcentric type I or II often not maturing	Subcentric type I mostly not maturing or soon aborting	Subcentric type I or II sometimes not maturing
Antheridial branch origin	Variable, usually diclinous	Variable, usually diclinous	Diclinous, rarely androgynous
Primary zoosporangial length x width	(20-)40-80(-100)	(84-)189-253(-460) x (15-)20-24(-31)	(95-)218(-418) x (19-)26(-33)
Zoospore discharge	-	Occasionally dictyoid	Occasionally dictyoid
Encysted zoospore (average diam)	10.5	11.7	10.7
S. diclina	Seymour (1970)		
Oogonia			
Size	(32-)52-65(-110)		(46-)54-68(-109)
Oospore			
Size	(12-)23-26(-36)		(21-)22-26(-29)
Number/oogonium	(1-)8-12(-28)		(1-)7-10(-14)
Type	Centric, rarely subcentric		Centric

APPENDIX D (CONTINUED)

Isolate and characters	Authors	Newfoundland isolates
S. diclina (continued)		
Antheridial branch origin	Diclinous	Diclinous
Primary zoosporangia	110-400 x 25-45	135-221 x 26-42
Encysted zoospore (range)	10-12	10.7-11.9
S. eccentrica	Seymour (1970)	
Oogonia Size	(15-)30-35(-42)	(21-)26-36(-39)
Oospore, Size	(12-)24-28(-32)	(15-)24-28(-34)
Number/oogonium	(1-)1(-4)	1(-2)
Type	Eccentric	Eccentric
Antheridial branch origin	Absent	Absent
S. ferax	Seymour (1970)	
Oogonia Size	(31-)60-70(-179)	(43-)60-70(-119)

APPENDIX D (CONTINUED)

Isolate and characters	Authors		Newfoundland isolates
S. ferax (continued)			
Oospore			
Size	(13-)22-25(-44)		(18-)21-24(-27)
Number/oogonium	(1-)10-18(>40)		(1-)10-18(-21)
Type	Centric, rarely subcentric		Centric
Antheridial branch origin	Absent, monoclinous or androgynous		Absent, rarely androgynous
Encysted zoospore	9-12		11.9
S. monilifera	Coker (1923)	Nagai (1931)	
Oogonia			
Size	(40-)50-65(-93)		(45-)51-68(-112)
Oospore			
Size	(17.7)23-25(-33.5)		(18-)25-27(-32)
Number/oogonium	(1-)2-6(-12)		(1-)2-6(-12)
Type	Centric or subcentric		Centric or subcentric
Antheridia	None		None
Primary zoosporangia		155-331 x 38.4-62.4	(51-)175(-559) x (21-)36(-52)
Encysted zoospore	11-11.8		9.7-12.2

APPENDIX D (CONTINUED)

Isolate and characters	Authors		Newfoundland isolates
S. parasitica	Seymour (1970)		
Oogonia			
Size	18-72 x 54-146; 62-75		38-88 x 57-155; 64-78
Oospore			
Size	(16-)18-24(-28)		(17-)21-25(-26)
Number/oogonium	(2-)14-23(-40)		(2-)10-25
Type	Subcentric		Subcentric
Antheridial branch origin	Diclinous, rarely androgynous		Diclinous, rarely androgynous
Encysted zoospores	9-11		10.7-11.9
S. terrestris	Seymour (1970)	Howard *et al.* (1970)	
Oogonia			
Size	(35-)60-65(-91)		(31-)59-65(-76)
Oospore			
Size	(20-)29-32(-41)	Average 24	(18-)25-29(-37)
Number/oogonium	(1-)3-6(-12)	6-11	(1-)4-9(-11)
Type	Subcentric, rarely centric	Subcentric I and II	Subcentric
Antheridial branch origin	Androgynous, 1-3 per oogonium	Androgynous, 4-5 per oogonium	Androgynous, 1-4 per oogonium

APPENDIX D (CONTINUED)

Isolate and characters	Authors	Newfoundland isolates
S. terrestris (continued)		
Encysted zoospore	6-11	9.2-11.5
S. unispora	Seymour (1970)	
Oogonia		
Size	(18-)45-52(-68)	(33-)49(-57)
Oospore		
Size	(18-)32-38(-43)	(24-)33(-43)
Number/oogonium	(1-)1-2(-4)	1
Type	Subcentric or centric	Subcentric or centric
Antheridia	None	None.
P. cymosa	Nolan and Lewis (1974)	
Oogonia		
Size	(17.1-)20.9-34.2(-38.0)	(17-)19-35(-40)
Wall	Smooth	Occasionally papillate
Oospore		
Size	17.1-26.6(-31.35)	17-27(-37)
Number/oogonium	1	1
Type	Subeccentric	Subeccentric
Antheridial branch origin	Monoclinous, androgynous or semihypogynous	Androgynous or semihypogynous

APPENDIX D (CONTINUED)

Isolate and characters	Authors		Newfoundland isolates
Sc. asterophora	Dick (1969b)	Coker (1923)	
Oogonia			
Size	(25-)42-56(-66)	(30-)37-45(-57)	(34-)44-54(-60)
Oospore			
Size	(17-)26-33(-44)	18-35	(23-)29-30(-39)
Number/oogonium	(1-)1-2(-6)	1-2(3-5)	(1-)1-2(-4)
Type	Subcentric	Subcentric	Subcentric
Antheridial branch origin	From cluster of hyphae below the oogonia; present in more than half the oogonia	Androgynous or diclinous, often absent at low temperature	Absent
Pr. paradoxa	Coker (1923)	Beverwijk (1948)	
Oogonia			
Size	32-100	42-75	(45-)56-79(-119)
Oospore			
Size	(22-)30(-37)	(24-)27(-30)	(26-)27-30(-33)
Number/oogonium	(1-)2-6(-12)	(2-)4-5(-10)	(1-)2-6(-11)
Type	Centric	Centric or subcentric	Centric or subcentric
Antheridial branch origin	Diclinous, rarely androgynous	Diclinous	Diclinous, sometimes androgynous

APPENDIX D (CONTINUED)

Isolate and characters	Authors		Newfoundland isolates
A. oblongata	Johnson (1956)	Forbes (1935)	
Oogonia			
Size	(58-)80-120(-183) x (49-)65-90(-124)	75-150 x 45-100	66-262 x 41-85
Oospore			
Size	(19-)24-28(-50)	(22-)27(-31)	(18-)24-28(-34)
Number/oogonium	(1-)8-16(-40)	(2-)5-10(-15)	(1-)5-15(-26)
Type	Infrequently maturing, subcentric I or II	Subcentric	Sometimes not maturing, subcentric I or II
Antheridial branch origin	Diclinous	Diclinous	Diclinous
Sporangia	160-202 x 18-26	350-600 x 30-70	119-607 x 21-38
Encysted zoospore	8-10	10-11.5	9.5-13.1
L. caudata	Coker (1923)	Nagai (1931)	
Oogonia			
Size	30-40	(33-)40.8-45.6(-55)	(33-)37-50(-67)
Oospore			
Size	-	-	(31-)36-47(-58)
Number/oogonium	1	1	1
Type	More or less centric oil droplets	Small oil droplets	Small oil droplets, centric, subcentric or scattered

APPENDIX D (CONTINUED)

Isolate and characters	Authors		Newfoundland isolates
L. caudata (continued)			
Antheridial branch origin	Diclinous	Diclinous	Diclinous
Zoosporangial width	5-18	-	9.5-21.4
Aph. laevis	Coker (1923)	Scott (1961)	
Oogonia Size	18-23	18-35	(17-)22-25(-33)
Oospore Size	(16.5-)19-22(-26)	14-29	(14-)18-21(-31)
Number/oogonium	1	1	1
Type	Eccentric	Eccentric	Eccentric
Antheridial branch origin	Androgynous or diclinous	Diclinous, monoclinous or androgynous	Diclinous
Encysted zoospore	7.3-11	7-11	9.5-10.7
Hyphal diameter	5-7.5	3-10	2.4
Aph. scaber		Scott (1961)	
	Aph. scaber	*Aph. irregulare*	
Oogonia Size	18-24	15-28	(15-)16-18(-21)

APPENDIX D (CONTINUED)

Isolate and characters	Authors		Newfoundland isolates
Aph. scaber (continued)			
Oogonia			
Wall	Numerous short spines	Irregular wall	Irregular wall with occasional short or long tubercules or papillae
Oospore			
Size	12-20	12-20	(12-)14-18(-20)
Number/oogonium	1	1	1
Type	Eccentric	Eccentric	Eccentric
Antheridial branch origin	Diclinous or rarely androgynous	Diclinous, monoclinous, androgynous or sometimes absent	Androgynous, monoclinous or absent
Encysted zoospore	8.5-9.5	8.5-9.5	9.5-10.7
Hyphal diameter	5-7.5	5-8	2-9.5
Aph. stellatus	Nagai (1931)	Scott (1961)	
Oogonia			
Size	19.2-31.2	23.5-32.2	(18-)25-35(-47)
Wall	Tuberculate	Papillate	Papillate, or smooth but irregular
Oospore			
Size	16.8-22.8	15-26	(13-)17-25(-28)

APPENDIX D (CONTINUED)

Isolate and characters	Authors		Newfoundland isolates
Aph. stellatus (continued)			
Oospore			
Number/oogonium	1, rarely 2	1, rarely 2	1
Type	Eccentric	Eccentric	Subcentric I or II
Antheridial branch origin	Androgynous or diclinous	Diclinous, monoclinous, rarely androgynous	Androgynous, monoclinous, sometimes diclinous
Encysted zoospore	8.4-9.6; 11-12	8-10	9.2-11.5
Hyphal diameter	4.8-6	4-7	6.1-11.5
Apo. completa	Humphrey (from Sparrow 1960)		
Oogonia			
Size	23-48		(18-)33-35(-54)
Oospore			
Size	16-24		(12-)18-20(-28)
Number/oogonium	(2-)4-7(-12)		(1-)3-7(-13)
Type	Eccentric		Eccentric
Antheridial branch origin	Androgynous		Androgynous
Antheridial cells	Arranged in a single row		Arranged in a single row

APPENDIX E

COLONY DIAMETERS OF *APHANOMYCES STELLATUS* AFTER FIVE DAYS INCUBATION[a]

Incubation temperature (°C)	Diameter (cm)
4	2.1 x 1.75[b]
10	4.5
13	5.15
17	6.5
22	6.35
24	No growth
30	No growth

[a] Using EYPSS agar media.

[b] All measurements were the result of a single determination made at each temperature.