A STUDY OF THE WATER MOLDS OF THE LYDELL STATE FISH HATCHERY AT COMSTOCK PARK, MICHIGAN

by

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Outline

Introduction

It is a matter of common knowledge among mycologists and ichthyologists that certain water molds are very prevalent in fish hatcheries. Fish and fish eggs are often attacked by such molds which effect the destruction of countless numbers. It is usually assumed that at least one of the species of Saprolegnia, S. parasitica Coker, is parasitic on fish and fish eggs. Although the degree of parasitism is difficult to determine, Kanouse (10) has given some valuable information on this subject in her paper on S. parasitica published in 1932.

Coker (4) in his monograph on the Saprolegniaceae also refers to S. parasitica as being associated with young fish and fish eggs especially in hatcheries, but he also reports the collecting of several other species from fish hatchery ponds.

During the summer of 1934, 252 samples of water were collected from 15 different sources, chiefly from Western Michigan. Eighteen samples of water were collected from ponds of the Lydell State Fish Hatchery at Comstock Park. 12.3% of the samples from Western Michigan produced water molds on hemp seeds, whereas 55.5% of the samples collected from the hatchery ponds yielded molds. This great difference could not escape the attention especially since it was evident that the molds from the fish hatchery

were not all S. parasitica but represented various species. In a conversation with Mr. Claude Lydell, manager of the hatchery, to whom the author is indebted for a great deal of assistance and information, he was informed that these troublesome fungi had increased considerably in the last few years, and that great losses were suffered each year, presumably due to the fungi.

It was evident that a thorough study of the water molds of the fish hatchery, especially with reference to the kinds of molds found, their distribution, the sources of water supply, and soil contamination would not only be interesting and instructive, but might eventually lead to some method of control.

The writer is greatly indebted to Doctor E. A. Bessey, of Michigan State College, under whose direction this work was undertaken, for his helpful advice and encouragement; and to Doctor Ralph Stob, president of Calvin College, who at all times showed a spirit of helpfulness in providing laboratory facilities.

Description of the Hatchery.

The Lydall State Fish Hatchery is located in Comstock Park, two miles north of Grand Rapids on Highway 484. The grounds cover an area of about 32 acres. On this tract 26 ponds of various sizes have been constructed. All but one of these ponds have soil bottoms. Mill Creek, which supplies most of these ponds with water, winds through the hatchery grounds. The hatchery proper receives its water from Strawberry Creek, a tributary of Mill Creek.

Strawberry Creek runs into Mill Creek about three-quarters of a mile north of the hatchery grounds. Three dams have been built, one in Strawberry Creek near its mouth, and the other two in Mill Creek. The accompanying map indicates the position of these dams. From above each dam the water is piped to the various ponds. The pipes are indicated on the map. Each pond receives an inlet pipe and has an overflow pipe which becomes the inlet pipe for another pond. The ponds are thus connected in a series so that the water runs from one into another and then back into Mill Creek. There may be several ponds in a series. Each pond is also supplied with a drainage pipe which, when open, drains the pond and carries its water directly to Mill Creek. The ponds are drained in the fall and the spring of the year. The exact course of the waterflow through the ponds may be traced by following the arrows on the map.

The numbers and letters given on the map correspond to the pond numbers used by the hatchery officials and employees. These numbers and letters will be used in naming the ponds in this paper.

Methods and technique.

In order to study the water mold contamination of this hatchery to the best advantage it was deemed advisable to make collections of both water and soil and to divide these collections into the following main groups:

1. Water from the sources.
2. Water from the ponds.
3. Soil from the bottom of the ponds.
4. Soil from the hatchery ground in the proximity of the ponds.

The water from the sources was collected from four locations as follows:

Location A. Strawberry Creek just above the dam.
Location B. Mill Creek above the point where Strawberry Creek joins it.
Location C. Mill Creek above the first dam.
Location D. Mill Creek above the second dam.

For a more accurate understanding as to the position of these locations the map may be referred to. Ten collections were made from each of the four locations, that is, in all 40 collections from the sources.

Five collections of water were made from each of the 26 ponds, making 130 collections from the ponds.

The soil collections from the bottoms of the ponds had to be taken when the ponds were drained. Since the water is drained out for only a short time the number of collections was necessarily limited. The writer was able to get 4 soil collections from each of the 12 ponds, making 48 in all. In addition to this 19 collections were made from other ponds at random.

For the sake of comparison 48 collections of soil from the hatchery grounds were taken, that is, 4 collections near each of the 12 ponds from which the bottom soil was taken.

For these collections a collecting belt with 18 screw top vials of about 20cc capacity was used. The vials were sterilized each time before taking them out. At the point

where the collection was made the entire vial was submerged and the screw top removed and replaced a few inches below the surface of the water. In this manner the possibility of contamination with mold spores from other sources was eliminated.

Soil collections were made in similar vials. The surface soil was removed at the point where the sample was taken and a soil boring was made with the vial so that nothing but the sterile vial touched the soil that was collected.

In the laboratory, both soil and water samples were transferred to sterile Soyka dishes. To the soil sterile distilled water was added and the two were mixed by agitating the dish. Into the Soyka dishes a couple of sterile hemp seeds and a sterile house fly, sterilized in a pressure cooker under 15 pounds of pressure, were dropped. If mold spores were present the hyphae would usually appear on the hemp seed or fly within one week after the collection was made.

To get these molds into pure culture, single hyphal tips were transferred to potato dextrose agar plates. This isolation of single hyphal tips was performed under a binocular dissecting microscope and by means of two specially constructed needles. The needles were made from the fine platinum wire of a discarded radio tube. A little piece of this wire can easily be fused into the end of an ordinary piece of glass tube, this making it easy to handle. Growth on the agar plates was evident very soon after the isolation of the hyphae. About two days later a piece of

the agar about ½cm square and containing hyphal tips was cut from these plates and transferred to homeopathic vials containing about 10cc of sterile distilled water. For each form isolated at least 4 of these vials were set up. Sterile hemp seeds were dropped into two of these vials, a sterile Dermestid larva into the third, and a sterile house fly into the fourth. The glassware used for these experiments was sterilized in an electric drying oven and the culture media in a pressure cooker at 15 pounds of pressure. During warm weather these cultures were kept in an ice box. During cold weather they were kept in a window box which was specially constructed for this purpose. The box was placed inside of the window. One of the window panes was removed and replaced by galvanized metal which contained an opening for a pipe 4½" in diameter and 14½"long. The other end of the pipe was connected with the box. A damper was placed in the pipe to shut out some of the cold air during very cold weather. The temperature in this box ranged from 10 to 20 degrees centigrade; the average temperature was about 15 degrees centigrade.

In most cases the characteristics needed for the classification of these molds were obtained from these single hyphal cultures. In a few cases, however, single spore cultures were made to determine characteristics more accurately. The technique followed to isolate single spores may be considered as a modified form of the method described by Kauffman (11).

The writer's method is here given somewhat in detail:

1. With fine scissors clip a quantity of mycelium

which is producing zoospores from a culture in one of the vials.

2. Mount this bit of mycelium in a drop of sterile water on a slide.

3. Stir the mycelium in the drop of water by means of fine needles, so as to set free some of the zoospores that may be adhering to the mycelium.

4. Now, with the needles, remove the mycelium, being sure to remove all hyphae and hyphal segments. (In this way only zoospores will remain in the water on the slide.)

5. Invert the slide and place it with the drop downward on potato dextrose agar in a petri dish. (The pressure of the slide will spread the water between the slide and the agar and at the same time disseminate the zoospores.)

6. Leave the petri dish over night.

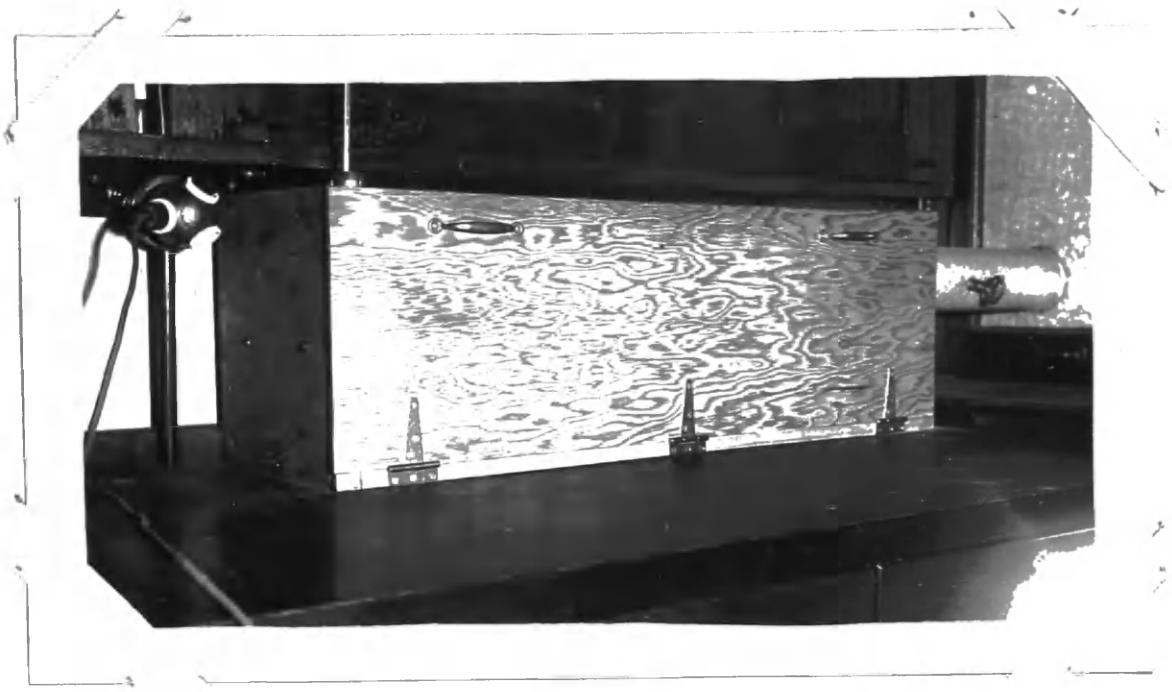
7. Upon microscopic examination the next morning it will be found that many of the zoospores have germinated on the agar.

8. Remove the slide and cut out a bit of agar containing only one germinating zoospore.

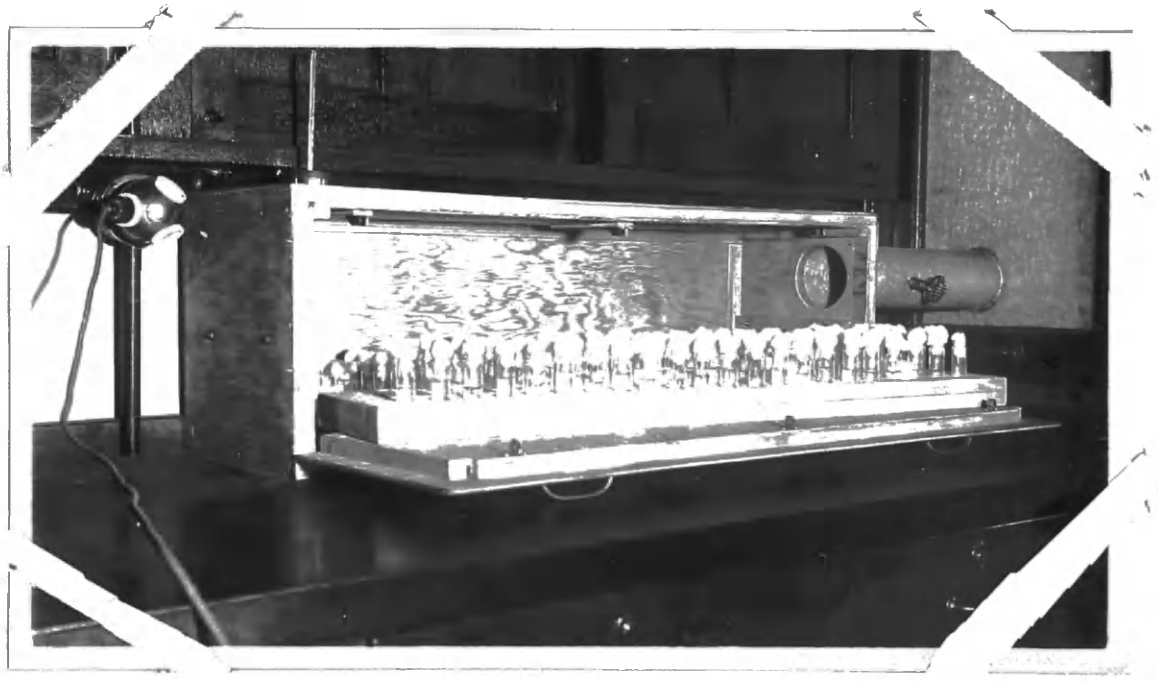
9. Place this in the center of another agar plate and in a few days the plate will be covered with mycelium originating from the single spore.

If the initial culture is relatively free from bacteria and if sterile instruments are used, it was found that this process can be used without danger of contamination, and with considerably more ease and less consumption of time than any other method that was tried.

Of great aid in the study of the details of the developmental processes was the use of moist chamber cultures constructed as suggested by Giltner (6).



Window box used for culturing water molds during cold weather



The open box showing interior construction and one of the two removable shelves with culture vials.

Relative Abundance of Water molds in Sources, Ponds, and Soil.

The degree of water mold contamination of the sources, ponds, and soil as indicated by the molds produced in specimens brought to the laboratory can best be represented by a series of tables and charts.

TABLE I

Showing number of water molds produced in specimens of surface water from sources.

Location of Collection	Number of Specimens Collected	Time of Collection	Number Producing Mold	Percentage Producing Mold
A	10	10/5/34-1/2/35	7	70
B	10	10/5/34 1/2/35	7	70
C	10	10/5/34-1/2/35	6	60
D	10	10/26/34-12/14/34	8	80
Total	40		28	Av. 70

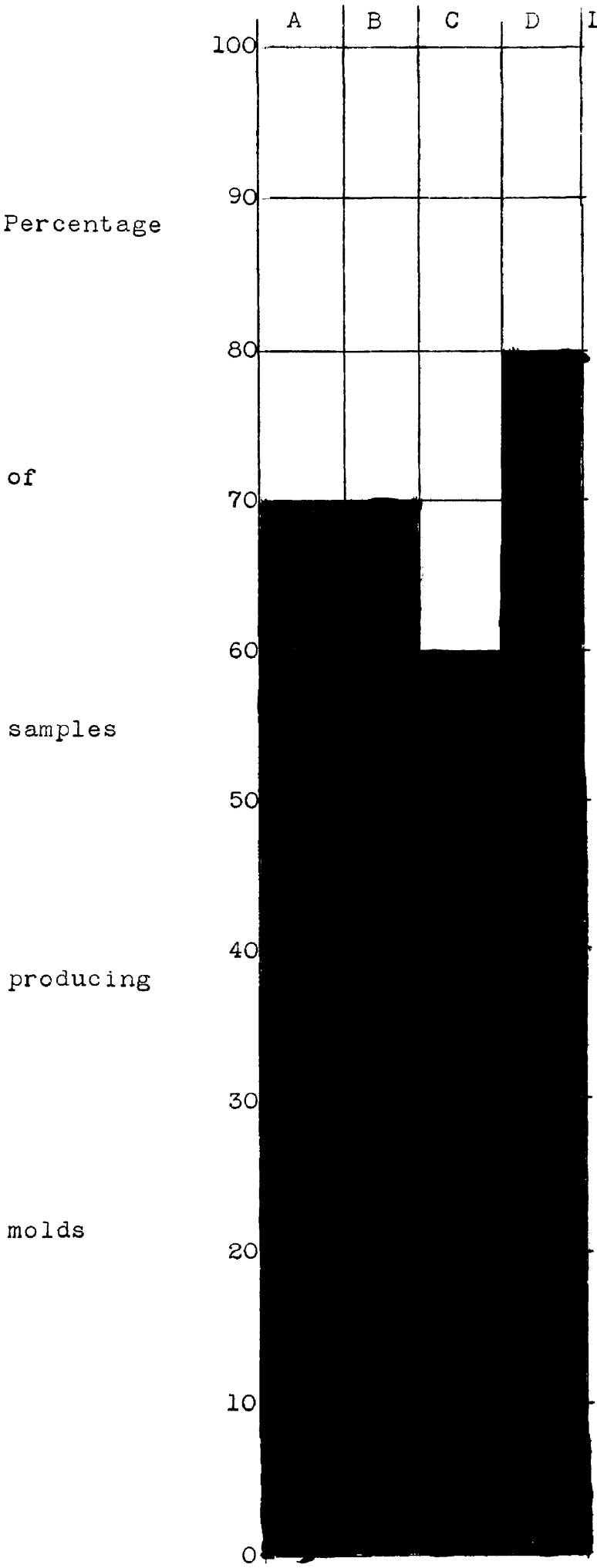


Chart I

Showing graphically the percentage of water samples from the sources that were contaminated with water molds.

70% of all samples contaminated

The table and chart just given show the contamination of surface water from the sources indicated since all the samples were necessarily taken from near the surface. Whereas, the water piped to the ponds, is taken from the deeper layers, a question with regard to the accuracy of the results tabulated above might arise.

In order to determine the contamination of the deeper water hawthorn fruits were suspended in the water in ordinary soap shakers such as are used in kitchens for washing dishes. These hawthorn fruits were left in the water for from one to two weeks and then examined for water molds. The results for this experiment are here tabulated:

TABLE II

Showing the results for the experiment with hawthorn fruits to determine the mold contamination of the deeper layers of water from the sources.

Location	No. of hawthorn fruits suspended	Time of Suspension	No. with water molds	Percentage of fruits with water molds
A	10	10/13/34-10/20/34	6	60
A	11	10/20/34 11/2/34	11	100
A	8	11/2/34 11/16/34	5	62.5
D	8	10/13/34 10/20/34	3	37.5
D	8	10/20/34-11/2/34	6	87.5
D	3	11/2/34-11/16/34	2	66.6
Total	48		34	Av.% 69

These results are strikingly similar to those obtained with the samples of surface water from locations A and D, the latter showing a contamination of 75% whereas these results give an approximate contamination of 69%. The difference is negligible. We conclude, therefore, that the contamination of surface water and deeper water is approximately the same.

The next table and chart give the results obtained for the 130 samples of water collected from the 26 ponds.

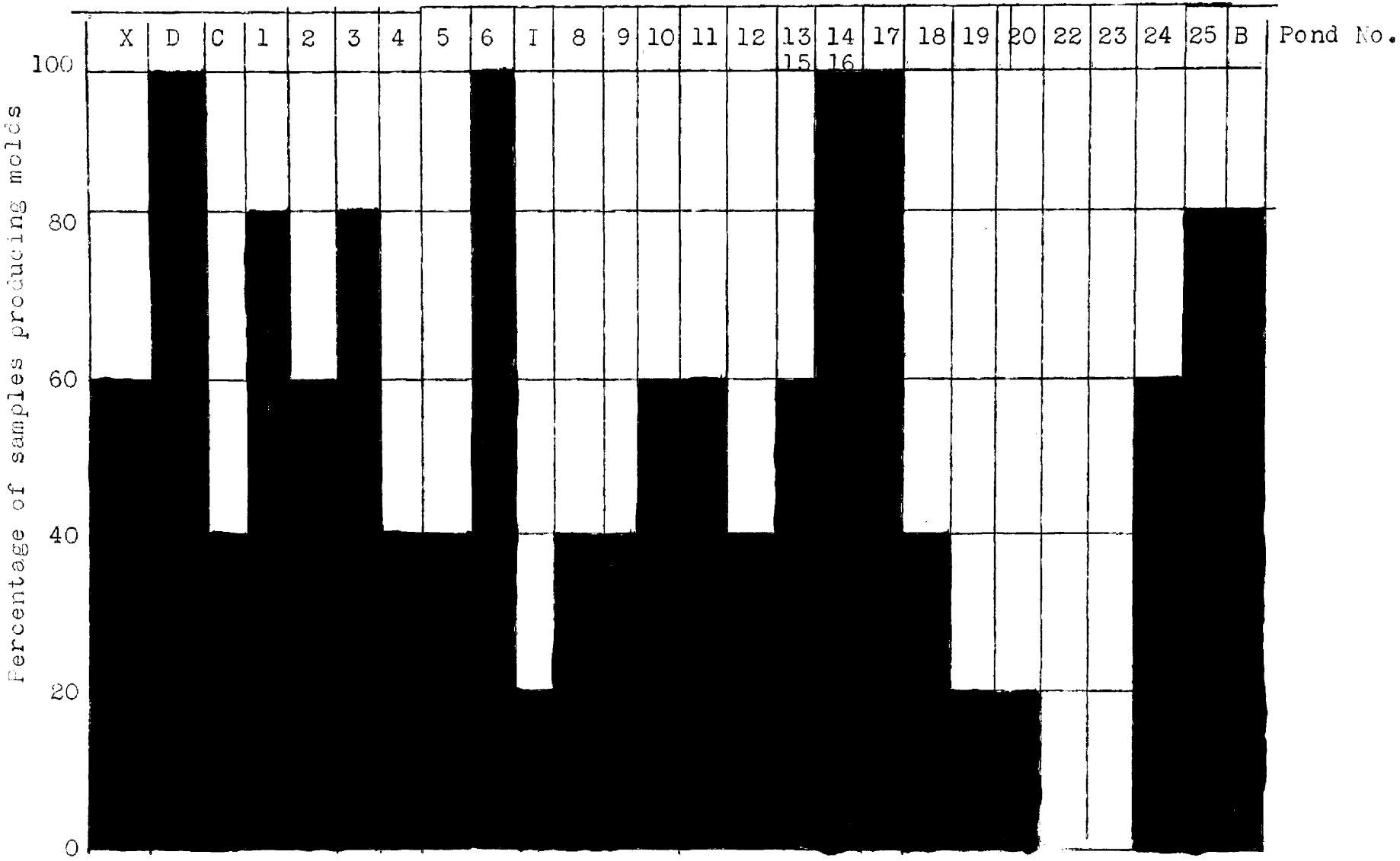
TABLE III

Showing number of molds produced in specimens of water from the ponds.

Pond No.	No. of Collections	Time of Collection From	To	No. Producing molds	% Producing molds
1	5	9/28/34	12/14/34	4	80
2	5	8/14/34	1/2/35	3	60
3	5	9/28/34	12/29/34	4	80
4	5	8/14/34	1/2/35	2	40
5	5	8/14/34	12/29/34	2	40
6	5	8/14/34	12/29/34	5	100
8	5	9/28/34	12/29/34	2	40
9	5	9/28/34	12/29/34	2	40
10	5	9/28/34	12/29/34	3	60
11	5	9/28/34	12/29/34	3	60
12	5	9/28/34	12/29/34	2	40
13	5	12/8/34	12/29/34	3	60
14	5	12/8/34	12/29/34	5	100
17	5	12/14/34	12/14/34	5	100
18	5	12/14/34	12/14/34	2	40
19	5	10/26/34	12/29/34	1	20
20	5	12/29/34	12/29/34	1	20
22	5	10/26/34	1/2/35	0	0
23	5	10/26/34	1/2/35	0	0
25	5	10/26/34	1/2/35	4	80
24	5	1/2/35	1/2/35	3	60
Island	5	9/28/34	12/29/34	1	20
Cement	5	8/14/34	12/1/34	2	40
B	5	8/14/34	12/1/34	4	80
D	5	8/14/34	1/2/34	5	100
X	5	8/14/34	1/2/35	3	60
Total	130			71	Av.% 54.6

CHART II

Showing graphically the percentage of water samples from the ponds that were contaminated with water molds



About 54.6% of all samples contaminated

Table IV and Chart III show the results obtained for the 48 soil collections from the bottom of 12 ponds. About 40% of these collections produced water molds.

Nineteen other soil collections from several different ponds but less than 4 from each pond are not included in this table and chart. The percentage of contamination was somewhat lower for these 19 samples.

TABLE IV

Showing number of water molds produced in 49 samples of soil collected from the bottoms of 12 ponds.

Number of pond	No. of collections	Time of collections From To	No. producing molds	% producing molds.
1	4	11/16/34-11/16/34	0	0
2	4	11/23/34-11/23/34	1	25
3	4	11/9/34 -11/23/34	3	75
5	4	12/1/34 -12/1/34	0	0
6	4	11/16/34-11/16/34	3	75
9	4	11/16/34-11/16/34	0	0
10	4	11/2/34 -12/1/34	2	50
11	4	10/12/34-12/1/34	3	75
12	4	11/9/34 -12/1/34	4	100
13	4	10/12/34-12/1/34	2	50
Island	4	11/16/34-11/16/34	1	25
Cement	4	11/23/34-11/23/34	0	0
Total	48		19	Av.% 39.6

Soil was also collected from the hatchery grounds in the proximity of the ponds listed in Table IV and Chart III. The results obtained for these soil samples are listed in Table V and Chart IV.

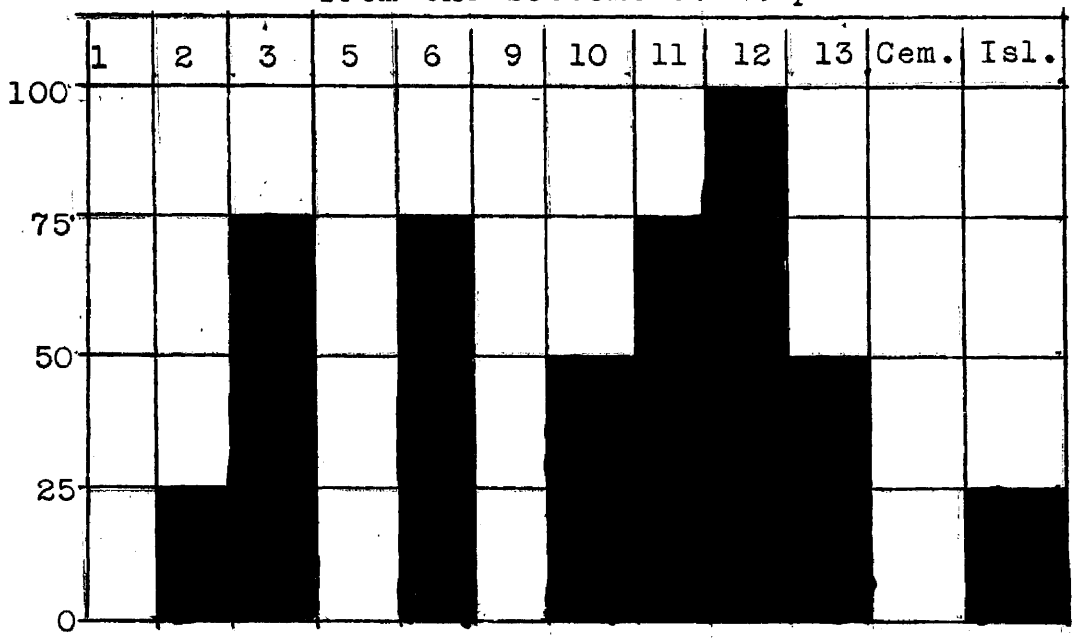
TABLE V

Showing number of water molds produced in 48 specimens of soil collected from the hatchery grounds.

Proximity of Pond No.	No. of Collections	Time of Collection	No. producing molds	% producing molds
1	4	3/22/35/	0	0
2	4	3/22/35	0	0
3	4	3/22/35	0	0
5	4	3/26/35	0	0
6	4	3/26/35	0	0
9	4	4/5/35	1	25
10	4	4/10/35	1	25
11	4	4/10/35	1	25
12	4	4/5/35	2	50
13, 15	4	4/10/35	1	25
Island	4	4/5/35	0	0
Cement	4	3/22/35	1	25
Total	48		7 Av.%	$\leq$15

CHART III

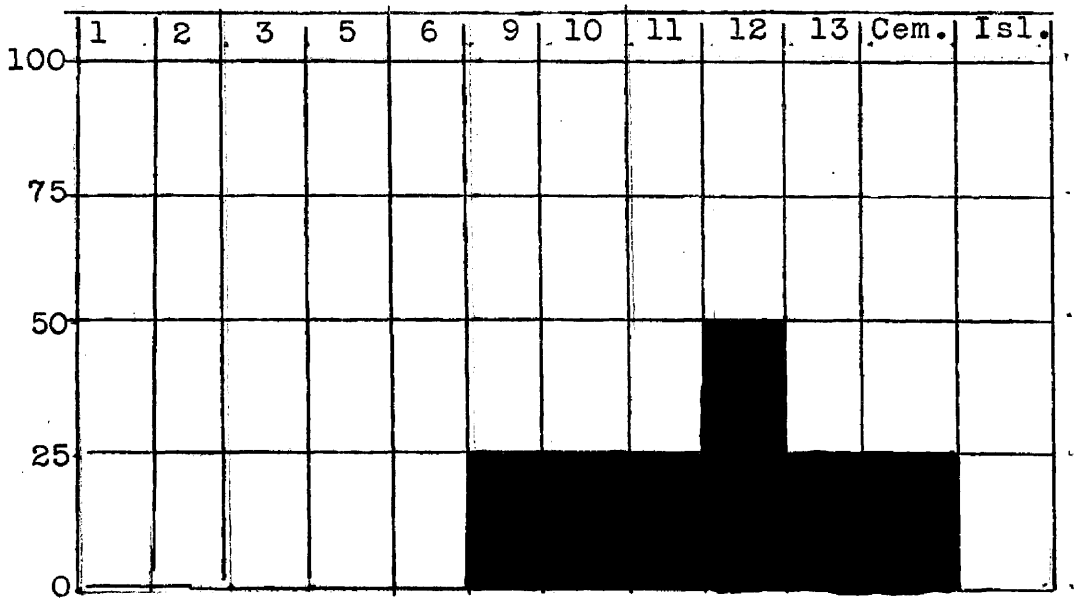
Showing results for 48 soil collections
from the bottoms of 12 ponds



About40% of samples contaminated with water molds

CHART IV

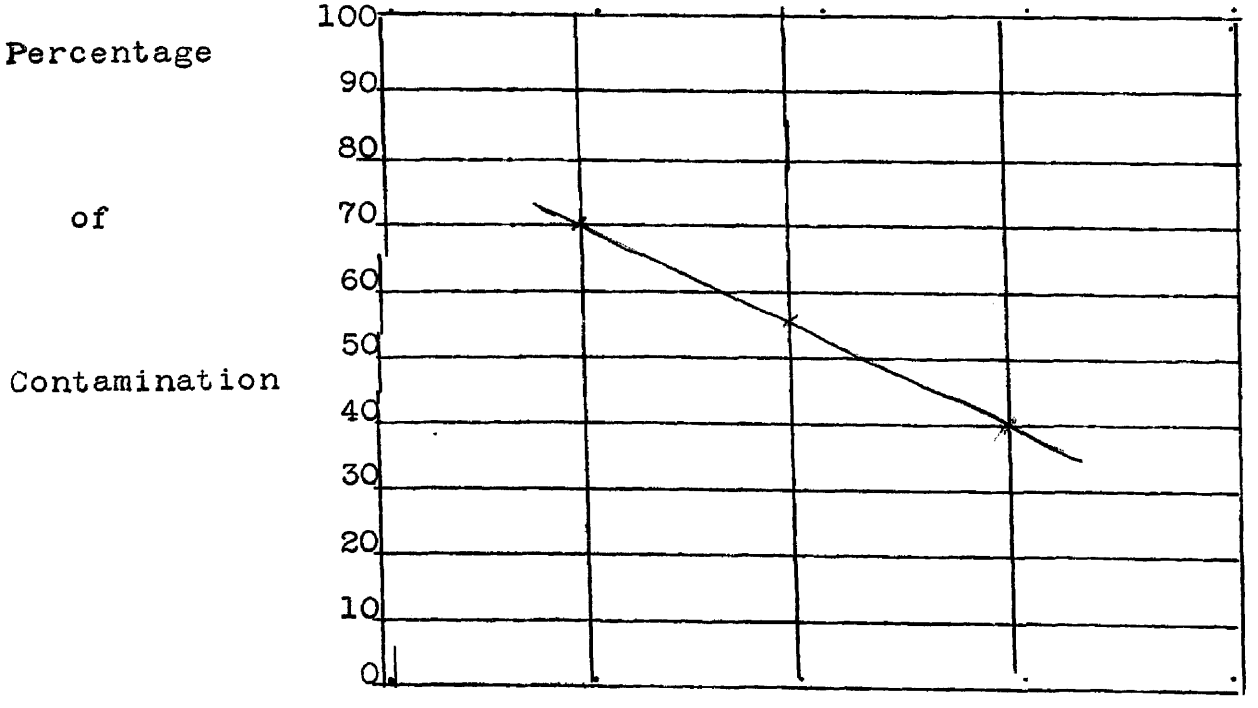
Showing results for 48 soil collections
in proximity of 12 ponds



About 15% of samples contaminated with water molds

A comparison of Tables IV and V and Charts III and IV indicates a striking difference between the contamination of soil samples from the bottom of the ponds and that of the soil from the fish hatchery grounds. It is evident that the relatively high contamination of the soil at the bottoms of the ponds can be traced to the water which is in the ponds for the greater part of the year. Further examination of the data given above indicates a uniform gradation of contamination of the water from the sources to the water in the ponds and again from the water in the ponds to the soil from the bottom of the ponds. In the first the contamination is 15% higher than in the second and in the second the contamination is 15% higher than in the third. The results presented in graphic form give a straight line curve:

Graph representing the relative contamination of sources, water in ponds, and soil from bottom of ponds.



It is doubtful whether the linearity of this curve has any significance. The general direction of the curve is, however, indicative of the general trend of contamination. The high percentage of contamination of the water from sources is no doubt responsible for the lower but nevertheless serious contamination of the water and soil from the ponds.

Since water from all the four sources mentioned above is highly contaminated and since 19 of the 26 ponds receive their water either directly or indirectly from source C, an attempt to correlate the contamination of water from the sources with the contamination of water from the ponds fed by them would hardly be of any value. Suffice it to point out here that whereas water from source C showed a contamination of 60%, the average contamination of the 19 ponds fed by it is 55%. Here again we have the slight drop in contamination from source to ponds as indicated by the graph given above.

Classification and Description of Isolated Molds.

As stated in the section on methods and technique an attempt was made to get the 120 forms produced in the various samples of water and soil into single hyphal-tip or single spore cultures. Ninety-four were successfully isolated and an attempt was made to classify them.

Each form was cultured on hemp seed, a house fly, and a Dermestid larva, in distilled water. From these three cultures the characteristics necessary for classification were in most cases obtained. Eight forms could not be

classified because of lack of information. Three of these forms were definitely Saprolegnia but the species could not be determined. Three others were Achlya but the characteristics for species determination were lacking. The remaining 79 forms were referred to species by the use of the keys given by Coker (4) and Humphrey (8). In each case the descriptions given by Coker and Humphrey were carefully read and compared with the results obtained in the study of the form under consideration. The original descriptions of the species were then consulted. Although in a few cases the writer's form did not agree in all details with the descriptions given for the species in which he placed it, these differences he believes were of no great significance, since they might have been due to differences in environmental conditions. It has repeatedly and clearly been shown by Klebs (13), Kauffman (12), Pieters (15) and others that these fungi are very sensitive to environmental conditions, and show differences in growth and structure dependent upon differences in nutrient media, temperature, and other factors. Taking this into consideration, the writer believes that the differences referred to above were of such a nature that they did not warrant the establishment of any new species. Some of these differences will be mentioned later in connection with the species concerned.

The following is a list of the species found and the number of times each species occurred, followed by a more detailed account for each species:

Name of species	No. of times it occurred
Saprolegnia parasitica	34
Saprolegnia ferax	17
Saprolegnia diclina	4
Saprolegnia hypogyna	4
Saprolegnia anisospora	2
Saprolegnia asterophora	1
Saprolegnia delica	1
Saprolegnia mixta	1
Achlya oblongata var globosa	4
Achlya Klebsiana	3
Achlya racemosa	3
Achlya americana	1
Achlya hypogyna	1
Achlya sp. (sterile)	4

Saprolegnia parasitica Coker

Growth abundant on most media. Hyphae with gemmae abundant, especially on a fly. Gemmae cylindrical, clavate, spherical or subspherical, often in long chains. Proliferation of new hyphae through old zoosporangia common. Zoosporangia abundant in vigorously growing mycelium, cylindrical, averaging 200x26μ, zoospores 10.5-12.25μ. Oogonia few, not occurring in all cultures; their walls smooth, thin, and unpitted. Antheridia diclinous. Diameter of oogonia 40-114μ, mostly 60-80μ. Oospores dark and subcentric, 1 - 20 usually 6 - 8 in each oogonium, their diameters 19-24μ, a few as large as 30μ.

This species occurred in the collections as follows:

Number in writer's collection	Location of Collection	Date of Collection
285	Water from Pond 11	9/28/34
335	Water from Mill Creek, Loc. C	10/26/34
347	Water from Pond 3	11/2/34
348	Water from Pond 4	11/2/34
349	Water from Pond 5	11/2/34
350	Water from Pond 6	11/2/34
351	Water from Cement Pond	11/2/34
379	Soil from Pond 12	11/9/34
381	Water from Mill Creek, Loc. D.	11/9/34
389	Water from Pond 8	11/16/34
403	Soil from Pond 6	11/16/34
417	Soil from Pond 3	11/23/34
424	Water from Mill Creek, Loc. B	11/23/34
425	Water from Mill Creek, Loc. B	11/23/34
426	Soil from Pond 10	12/1/34
428	Soil from Pond 10	12/1/34
435	Soil from Pond 13, 15	12/1/34
437	Water from Mill Creek, Loc. D	12/1/34
447	Water from Pond 14, 16	12/8/34
452	Esyrt gtom Pond 11	12/8/34
453	Water from Pond 10	12/8/34
454	Water from Moll Creek, Loc. C.	12/8/34
456	Water from Mill Creek, Loc. C	12/8/34
462	Water from Pond 17	12/14/34
464	Water from Pond 17	12/14/34
466	Water from Pond 17	12/14/34
476	Water from Mill Creek, Loc. B.	12/14/34
479	Water from Pond 1	12/14/34
483	Water from Pond 12	12/29/34
485	Water from Pond 10	12/29/34
501	Water from Pond X	1/2/35
505	Water from Pond 24	1/2/35
506	Water from Pond 24	1/2/35
509	Water from Pond 25	1/2/35

A glance at the foregoing list will convince one of the general prevalence of Saprolegnia parasitica. This species was collected from all the sources except Location A, Strawberry Creek, and from all but 9 of the 26 ponds. It was found in soil as well as water and it appeared quite consistently from September to January.

Kanouse (10) in 1932 for the first time definitely noted the appearance and described the structure of oogonia in this species. At least 10 of the 34 forms listed above produced oogonia in the writer's cultures. Invariably, however only a few oogonia were found in any one culture. The structure of these agreed on the whole with the description and illustrations of Kanouse. The oogonia observed had diclinous antheridia, their walls were thin, smooth, and unpitted. The oospores were very dark and subcentric. Kanouse states that the diameter of the oogonia is 65-135 x 60-75μ, or 65-95μ. The oogonia here observed measured 40-114μ, mostly 60-80μ. The oospores also ranged larger than the 18-22μ indicated by Kanouse. Their measurements ranged from 19-24μ, and a few exceptionally large ones were 28 and 30 μ in diameter. Notwithstanding these differences, these forms are no doubt Saprolegnia parasitica since the rest of the description and the observations of Kanouse agree perfectly on the oogonia of this species.

In his key to the species of Saprolegnia Coker (4) remarks that S. parasitica probably belongs to the Ferax group. However, since the oogone walls of the species are

thin, and pits, if present, are not conspicuous, and since the antheridia at least in the main are diclinous, this species would naturally fall in the Diclina group.

With reference to an abundance of Saprolegnia parasitica at the fish hatchery the following summary can be made:

> Of the seventeen identified forms yielded by water from the sources 8 or approximately 47% were S. parasitica.
>
> Of the 46 identified forms yielded by water from the ponds 20 or approximately 43% were S. parasitica.
>
> Of the 16 identified forms yielded by soil from the bottoms of the ponds 6 or approximately 40% were S. parasitica.
>
> From these figures it is safe to conclude that from 40-50% of all the molds found at the hatchery are S. parasitica.

Saprolegnia ferax (Gruith) Thuret

Moderately stout hyphae producing a large number of oogonia and relatively few gemmae and zoosporangia. New zoosporangia form in old ones. Zoospores escape separately; zoospores about 10μ in diameter. Oogonia have thick walls with large conspicuous pits, pits 5.25-7:00μ in diameter. Antheridia observed in only a few cases and then appearing to be androgynous, arising from the oogonial stalk. Diameter of oogonia 24-115.5μ, mostly from 60-80μ. Oospores centric, 1 - 20 in an oogonium, mostly less than 10. Diameter of oospores 19-30μ, usually 22-24μ often varying in size in same oogonium.

This species ranks next to S. parasitica so far as frequency of occurrence is concerned. It occurred 17 times as shown in the following list:

Number in writer's collection	Location of Collection	Date of Collection
250	Water from Pond 6	8/14/34
275	Water from Island Pond	9/28/34
286	Water from Pond 11	9/28/34
290	Water from Mill Creek, Loc. B	10/5/34
313	Water from Pond 10	10/12/34
318	Soil from Pond 18	10/12/34
319	Water from Pond 2	10/26/34
352	Water from Pond D	11/2/34
385	Water from Strawberry Creek A	11/9/34
420	Water from Mill Creek C	11/23/34
460	Water from Strawberry Creek A	12/8/34
465	Water from Pond 17	12/14/34
470	Water from Pond 18	12/14/34
471	Water from Pond 18	12/14/34
472	Water from Pond B	12/14/34
474	Water from Pond B	12/14/34
493	Water from Island Pond	12/29/34

With the aid of the keys and descriptions of Coker (4) and Humphrey (8) these forms were rather easily identified. The large number of oogonia and the relatively small number of gemmae were evident in nearly all cultures. With a very few exceptions the measurements of the diameters of oogonia and oospores fell within the extremes given by Coker. An exceptionally small oogonium appearing in a fish hatchery

culture had a diameter of 24μ; an exceptionally large oogonium measuring 115.5μ was found. Coker gives the extremes as 37μ and 97μ, and while Coker gives 14.8μ as a minimum for the diameter of oospores, in the cultures under consideration an oospore measuring 12.25μ was found. The diameter of the pits in the oogonial walls is given as from 4.5μ to 5.5μ. Several measured in these cultures were at least 7μ in diameter. These differences are not deemed to be of sufficient significance for the establishment of a new species or variety. The preponderance of evidence places all of these 17 forms in the species S. ferax. The conclusion, therefore, is that about 21.5% of the identified forms were S. ferax.

Saprolegnia diclina Humphrey

Growth rather abundant. Proliferation of zoosporangia as in Saprolegnia. Many gemmae and oogonia. Oogonia with thin walls, unpitted, diameter 45-80μ. Antheridia on all oogonia diclinous. Oospores mostly 22-26μ, usually 3 - 10 in an oogonium.

This species was found four times:

No. 304 in water from Pond D on 10/12/34

No. 372 in water from Mill Creek, Loc. C on 11/9/34

No. 373 in water from Pond 25 on 11/9/34

No. 486 in water from Pond 19 on 12/29/34

These forms were compared with the descriptions and illustrations of Humphrey (8) and Coker (4) and were found to agree with them. There can be no doubt as to their identity.

Saprolegnia hypogyna Pringsheim.

Hyphae with new sporangia proliferating through older ones. Zoospores 10-12μ in diameter. Many oogonia which are conspicuously pitted. Pits 5-7μ. Most oogonia have a protoplasmic protrusion growing up into them from below. In addition some have androgynous antheridia arising directly below the oogonium. Diameter of oogonia 30-70μ. Oospores 21-26μ; 1 - 15 mostly 3 - 8 in each oogonium.

This species was found four times as follows:

No. 249	in water from Pond 6 on	8/14/34
No. 314	in water from Pond 9 on	10/12/34
No. 410	in soil from Pond 2 on	11/23/34
No. 504	in water from Pond 24 on	1/2/35

From Coker's (4) description of this species it is evident that it is a species with great variation in form. Pringsheim (17) first described the species as a variety of S. ferax. It was recognized as a species by de Bary (2). Maurizio (14) describes at least 5 varieties. In America a form of the species has heretofore been found only by Kauffman in Michigan.

The writer has compared the forms listed above with the original illustrations and descriptions of Pringsheim and de Bary and feels certain that these forms belong to this species. Whether or not they are the same form as the one which Kauffman has found in Michigan has not been determined.

Saprolegnia anisospora de Bary

Dense mycelium growing well on all media used. Hyphae of medium thickness producing zoosporangia whose diameters are

a little greater than the hyphae bearing them. New hyphae readily proliferated through old sporangia. Relatively few zoospores in each zoosporangium. There are at least two distinctly different sizes of zoospores in this species. The smaller ones are from 12-14μ in diameter when encysted, the larger ones up to 17.5μ. Oogonia moderately abundant, 30-60μ in diameter with thin unpitted walls. Antheridia diclinous, branching, oospores 1 - 7 in an oogonium, 17-30μ in diameter, mostly 17-21μ, centric but soon breaking down, some appearing eccentric when old.

This species was encountered twice, both times in water from Pond 14. The record shows:

No. 444 in water from Pond 14 on 12/8/34

No. 480 in water from Pond 14 on 12/29/34

Considerable difficulty was experienced in placing this species. The "durchwachsung" of the zoosporangia clearly showed this form to be a species of Saprolegnia. The oospores however appeared to be eccentric, which is not characteristic for this genus. Single spore cultures were made by the method described in the section on technique. Later a tuft of mycelium originating from a single spore and growing on a Dermestid larva was mounted in a hanging drop. The fungus continued to grow in this drop for at least 48 hours so that a thorough study could be made of the development of zoosporangia and the discharge of zoospores.

Observation soon showed that at least two different sizes of zoospores were discharged from different zoosporangia, a characteristic found in S. anisospora. On the day following

this observation oogonia were starting to form. These bore diclinous antheridia. The oospores were present in these oogonia on the next day and these were centric. Measurements of hyphae, zoospores, oospores, antheridia and zoosporangia were taken and these coincided with those given by Coker for S. anisospora, with the exception that the encysted zoospores varied from 12.25-17.5μ. The smaller ones were few, most of the spores were either 14 or 17.5μ. A single sporangium gave rise either to the larger or the smaller forms. The swimming spores, however, were about 12μ in their smallest diameter which is nearer to the measurements given by Coker (4) who states: "the smallest spores are about 8-9μ in diameter, others from 10.5-11.5μ, the large ones from 13.7-14.8μ." This plant agrees also with the descriptions of de Bary (3) who gives no measurements. The oospores observed in the original culture and which were apparently eccentric were similar to those illustrated by de Bary (3). These oospores are no doubt abnormal and our observations substantiate Coker's suggestion: ".... one is inclined to suspect that de Bary who rarely made a mistake, was in this case wrong in thinking the normal eggs eccentric. His figures clearly show eccentric eggs, but may they not have been breaking down? This seems the more likely as no other Saprolegnia has an eccentric egg."

In connection with the study of this species in the hanging drop some interesting facts may be recorded:

Observations on an immature zoosporangium were started at 4:30 P.M., March 28, 1935. At 8:00 P.M. a spherical knob had formed at the tip of the sporangium. At 9:30 P.M. this knob had taken on an oval shape. Zoospores were not yet

starting to form in the zoosporangium. The next morning, however, at 9:30 the zoospores had been discharged and could be seen encysted a short distance from the mouth of the zoosporangium. These were 17.5μ in diameter.

Because the discharge of zoospores had not actually been observed, another immature sporangium was found for observation. At 11:00 A.M. this zoosporangium appeared at the tip of a hypha 12.25μ in diameter. The sporangium itself was 26.25x140μ. Its protoplasm was dense and was separated from the lighter protoplasm of the hypha by a distinct wall. The sporangial protoplasm, however, showed no signs of breaking up into zoospore initials. At 2:30 P.M. the zoospores had been formed and the characteristic knob had been produced at the apex of the zoosporangium. From 2:30 to 2:35 there was a squirming movement of zoospores within the sporangium. At 2:35 the uppermost zoospore escaped followed immediately by the others, one at a time. At first the escape was very rapid, slowing down as the escape of the final zoospore neared. All the 32 large zoospores had left the zoosporangium in less than a minute and were swimming about independently in quick gyrating motion not far from the mouth of the sporangium. These zoospores were all large, oval-shaped and of equal size, each measuring about 12x21 μ. A pair of long cilia could be seen at one end of many of these zoospores. At 2:45, ten minutes after the escape of the first zoospore, one of them was found to have encysted. After that several encystments were closely watched. In one the process was timed: gyrations stopped and the zoospore lay quivering

or vibrating for 30 seconds, then both ends contracted and the zoospore rounded up into a spherical mass. This also lasted 30 seconds, so that the whole process of encystment lasted one minute. At 2:55, 20 minutes after the escape, all the zoospores had encysted. These encysted forms were 14:00μ in diameter.

The rate of growth of a hyphal tip was observed in this hanging drop culture. The microscope was equipped with an ocular micrometer. It was placed in such a position that a growing hypha lay lengthwise directly underneath the scale. The amount of growth was then observed at intervals for a period of one hour, after which the slide was accidentally moved and the readings could not be continued. The readings showed the following results:

from 1:03 to 1:23 the hypha increased 21:00 microns in length

from 1:23 to 1:43 the hypha increased 19:25 microns in length

from 1:43 to 2:03 the hypha increased 12:25 microns in length.

The total increase for the hour was therefore 52.5 microns. Since the hypha was about 15μ in diameter the increase in one hour was about 3½ times the diameter of the hypha.

Saprolegnia asterophora de Bary

Hyphae with proliferation of new hyphae through old sporangia. On hemp seed and fly numerous small oogonia mostly with papillae, some smooth. Oospores mostly one in an oogonium. Diameter of oospores about 22μ. Diameter of oogonia about 26μ not including papillae.

This form was found only once:

No. 431 in soil from Pond 12 on 12/1/34

The proliferation of new hyphae through old zoosporangia and the oogonia with papillae, having mostly one, seldom two oospores, places this form with S. asterophora. Measurements taken agree with those given by de Bary (1) and Coker (4).

Saprolegnia delica Coker

Mycelium rather delicate with cylindrical sporangia, oogonia with thin walls, pits not conspicuous. All oogonia with delicate diclinous antheridia sometimes nearly surrounding oogonium. Oogonia 50-70μ in diameter. Oospores centric, mostly 5 - 10 in an oogonium. Diameter of oospores mostly 24-26μ.

This form was collected once:

No. 272 in water from Pond 1 on 9/29/34

Its oogonia with thin walls and inconspicuous pits having delicate antheridia which are mostly diclinous places this form with S. delica. Number of oospores per oogonium, size of oogonia and oospores, and the general appearance of the plant all coincide with Coker's original description and illustrations.

Saprolegnia mixta de Bary

Hyphae with numerous oogonia and few gemmae. Oogone walls thick and with conspicuous pits about 7μ in diameter. Many of the oogonia have antheridia some apparently diclinous, chiefly terminals, a few intercalary. Diameter of the oogonis 40-70μ. Number of oospores mostly 1 - 10. Oospores centric, diameter 19-25μ.

This form was collected once:

No. 273 in water from Pond 3 on 9/28/34

This form is very much like S. ferax listed above, but because of the many oogonia with antheridia it must according to keys and descriptions be classed as S. mixta. Measurements agree with those given for that species. Coker (4) calls attention to the similarity of S. ferax and S. mixta.

Achlya oblongata var globosa Humphrey

Mycelium consisting of long, stout hyphae with numerous zoosporangia. New zoosporangia produced below older ones. Zoospores encyst at mouth of zoosporangium. Encysted zoospores 12-15μ. Oogonia abundant, mostly spherical, 50-90μ in diameter, a few larger. Antheridia on nearly all oogonia, diclinous. Oospores numerous, 6 - 20 mostly 12 - 15 in an oogonium, centric, a few apparently subcentric, diameter 22-27μ.

This form was collected under four numbers:

No. 271 in water from Pond 1 on 9/28/34

No. 282 in water from Mill Creek, Loc. D 11/9/34

No. 434 in soil from Pond 13 on 12/1/34

No. 500 in water from Pond D on 1/2/35

The identity of this species cannot very well be mistaken. Since the oogonia were nearly all spherical and oospores averaged more than 10 in an oogonium, these forms are no doubt of the variety globosa, as indicated above, a variety established by Humphrey (8) on the basis of these very characteristics.

When this form was first encountered (No. 271) the

large and long hyphae were very conspicuous on hemp seed. These hyphae were exceptionally broad at the base and tapered to a point at the top. One of the hyphae was found to be 271.25μ in diameter at the base. When the hemp seed was held out of the water the hyphae stood out straight 1.5 cm perpendicular to the surface of the seed.

Achlya racemosa Hildebrand

Short, slender hyphae with terminal zoosporangia of a diameter slightly greater than that of hyphae. Zoospores encyst at mouth of zoosporangium, about 12μ in diameter. Mycelium producing very many oogonia on short racemose stalks. The abundance of oogonia make the mycelium appear as a dense, white fringe aroung the substratum. Oogonia 26.25-50.75μ, with thick, smooth, yellowish walls. Each oogonium bears one or two short, unbranched, tuberous antheridia which arise from the oogonial stalk directly beneath the oogonium. Oospores are 17.5-33.25μ averaging about 24μ in diameter, 1 - 3 sometimes 4 and very rarely 5 in an oogonium.

This species appeared three times:

No. 330	in water from Pond 25 on	10/26/34
No.492	in water from Pond 20 on	12/29/34
No.499	in water from Pond 4 on	1/2/35

This species stands out clearly from the other species of Achlya because of its numerous oogonia on short racemose stalks, and by its short unbranched antheridia arising from the oogonial stalk directly below the oogonium. That

all of the three forms belong to this species is evident since they possess all the characteristics upon which Hildebrand (7) based the species. Later it was described for America by Humphrey (8) and Coker (4). As to details of size in oogonia and oospores and as to number of oospores in an oogonium all of these forms agree. In this respect, however, they differ somewhat from the forms described by Coker (4) and Humphrey (8). Coker states "oogonia rather small 40-70μ". Thirty-two oogonia taken at random from different cultures of our three forms showed that the oogonia varied from 26.25-50.75μ. On the whole, therefore, they are considerably smaller than those described by Coker. Further, Coker (4) states that the oospores are "variable in size 16.6-27.7μ in diameter, most about 22μ centric, 1 -8 in an oogonium, in most cases 2 - 5, centric." Humphrey (8) with reference to number and size of oospores states "1 - 10, commonly 1 - 6, their diameter averaging 25μ." Hildebrand (7) in the original description of the species states, "In den Oogonien haben sich mehrere 3 - 12 Befruchtungskugeln gebildet." As to the size of the oospores, forty oospores picked at random from our cultures varied from 17.50 to 33.25μ with an average diameter of about 24μ. The difference is more marked, however, with respect to number of oospores per oogonium. All of the three investigators cited above placed the oospore number higher than was found to be the case in any of these cultures. The oospores in one hundred oogonia in a mycelium growing on a Dermestid larva were counted. This appeared to be a representative culture so far as this

point is concerned. The result was as follows:

21 oogonia contained 1 oospore
37 oogonia contained 2 oospores
36 oogonia contained 3 oospores
5 oogonia contained 4 oospores
1 oogonium contained 5 oospores

For these forms, therefore, the oospore number is 1 - 3, sometimes 4 and very seldom 5. At no time was an oogonium with more than 5 oospores observed.

One might conclude from these data that a new and distinct variety of A. racemosa has been found. However, here again the differences recorded above may not be essential but may possibly be due to a nutritive factor. We again refer to the work of Pieters (15) on the vegetative vigor and reproduction in Saprolegniaceae. Special reference is made to his Table V (p. 544) where size and number of oogonia, and number of oospores in an oogonium are shown to vary with different percentages of peptone in the medium.

Achlya Klebsiana Pieters

Mycelium consisting of moderately stout hyphae. New hyphae and zoosporangia branching off from older hyphae just below a zoosporangium. Zoospores, about 12μ in diameter, encysting at mouth of zoosporangium. Oogonial walls unpitted. Antheridia diclinous. Diameter of oogonia 63-101μ. Oospores eccentric, 3 - 15 in oogonium, diameter 17.5-24.5μ.

A. Klebsiana was collected on three occasions:

No. 290 in water from Mill Creek Loc. B. on 10/5/34
No. 334 in water from Mill Creek Loc. C on 10/26/34
No. 429 in soil from Pond 11 on 12/1/34

This species was first described from Michigan by Pieters (16). It is no doubt a truly Michigan species. Coker (4) has, however, found it in North Carolina in 1921. The forms listed above agree with the descriptions given by Pieters and Coker in the essential characteristics, such as diclinous antheridia, unpitted oogonial walls, and eccentric oospores. These forms seem to differ, however, from the previously described forms in size of oogonia and number of oospores per oogonium. Pieters does not give the size of the oogonia but in Coker's form they are usually from 48-62μ. In these forms they vary from 63-101μ. Pieters gives 1 - 10 as the number of oospores per oogonium. Coker states 1 - 8 usually 6. In our forms there were from 3 - 15 oospores in an oogonium, averaging 9. The larger average number of oospores is perhaps due to the larger size of the oogonia since the average oospore size is the same as that given by Coker. Notwithstanding the differences given above there can be no doubt as to the identity of the species.

Achlya americana Humphrey

Long tapering hyphae bearing oogonia having eccentric oospores. Walls of oogonia smooth, hyaline, and pitted. Antheridia androgynous arising from the main hypha near the oogonial stalk. Diameter of oogonia 70-90μ. Oospores 7 - 15 in oogonium, 22-26μ in diameter.

Collected as:

No. 422 in water from Strawberry Creek, Loc. A on 11/23/34

This species has not been reported from Michigan before. Since this form agrees with the descriptions and illustrations of both Humphrey (8) and Coker (4) there can be no doubt as to its identity.

Achlya hypogyna Coker and Pemberton

An Achlya with oogonia of various shapes, many with papillae. Diameter of oogonia mostly 50-80μ, oospores 26-30μ, mostly 28μ, 1 - 13 in an oogonium. Antheridia androgynous.

Collected as

No. 432 in soil from Pond 12 on 12/1/34

Except for the number of oospores in an oogonium which is given by Coker as 1 - 7 usually 3 - 5, this form agrees entirely with descriptions and illustrations given for A. hypogyna. In the oogones found in this culture there are from 1 - 13 oospores, the average number being 5. It must without doubt be assigned to this species.

Achlya sp.

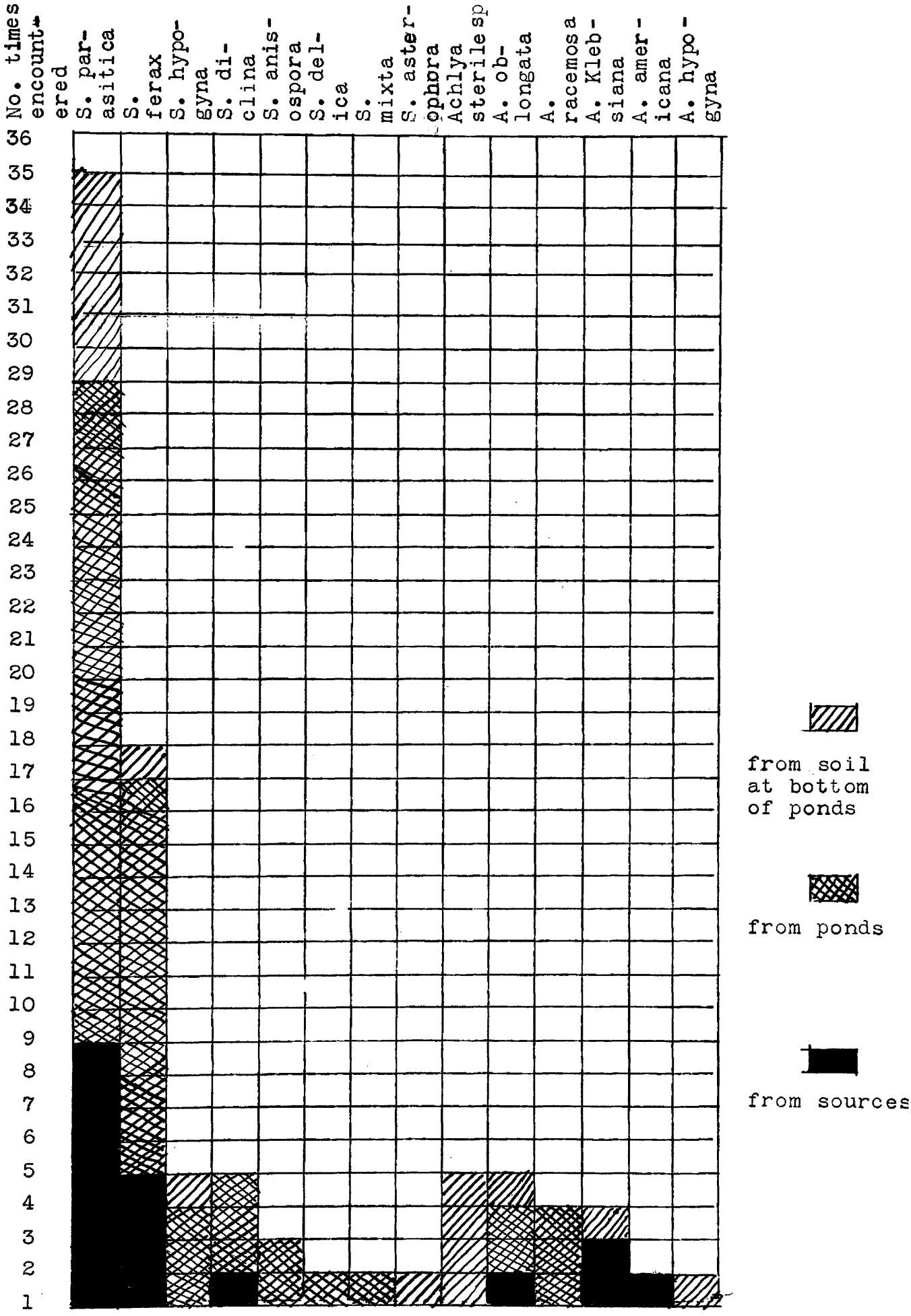
In his monograph on the Saprolegniaceae Coker (4) describes and illustrates an Achlya species which remained sexually sterile for several months and in which one could not induce the formation of oogonia by means of culture media of various compositions. Among our collections were found four forms which agree with the description and illustrations of Coker. They were cultured for from three to four months without producing any sexual organs under conditions in which the other Achlya species produced abundant oogonia. We have come to the conclusion that this

is most likely the same Achlya species as the one referred to by Coker. It occurred in our collections as follows:

No. 384	in soil from Pond 3 on	11/9/34
	cultured until	3/13/35
No. 391	in soil from Island Pond	11/16/34
	cultured until	3/16/35
No. 405	in soil from Pond 6 on	11/16/34
	cultured until	3/15/35
No. 416	in soil from Pond 3 on	11/23/34
	cultured until	3/15/35

The accompanying chart shows graphically the relative abundance of the identified species described on the preceding pages, as well as the relative number collected from sources, water from ponds, and soil from bottom of the ponds:

CHART SHOWING RELATIVE ABUNDANCE OF DIFFERENT SPECIES
(Based on the 79 species identified)



In considering the distribution of any particular species with reference to sources, ponds, and soil, we find that this distribution is very general. That is, any particular species does not confine itself to sources, ponds, or soil. For instance, the most representative species are found in all three situations. Those that were encountered at least four times are likely to come from at least two locations. Seldom is a species found more than once or twice at any particular location.

From water from the sources we obtained: S. parasitica, S. ferax, S. diclina, A. oblongata var globosa, A. Klebsiana, A. americana.

From water from the ponds we collected: S. parasitica, S. ferax, S. diclina, S. hypogyna, S. anisospora, S. delica, S. mixta, A. oblongata var globosa, A. racemosa, A. Klebsiana.

From soil from the bottom of the ponds we collected: S. parasitica, S. ferax, S. hypogyna, S. asterophora, A. hypogyna, Achlya sp. (sterile)

This general distribution of the species indicates a close connection between the contamination of water in the sources, water in the ponds, and soil at the bottom of the ponds.

Experiments to Determine the Extent of Parasitism of Water Molds.

Because of the abundance of these various mold species at the fish hatchery, the problem of their parasitism with reference to fish and fish eggs becomes important. Kanouse (9)

has considered this problem with reference to S. parasitica Coker. Upon the basis of her experiments and observations she concludes that the infection of living eggs and fry by means of zoospores is not likely, but "that the pressure of growing hyphae on the egg membranes when the eggs are held tightly together is sufficient to allow penetration of the mycelium into the living eggs." (p. 449)

Huxley (8) more or less assumed the parasitism of a form of fungus which no doubt was S. parasitica Coker, upon the basis of the numerous infections of living fish in British rivers.

It was deemed advisable, in connection with this problem to determine more definitely the probable parasitism of S. parasitica and other species found in these collections from the hatchery. Accordingly the following experiments were undertaken.

1. Experiment with wall-eyed pike eggs.

Living eggs of the wall-eyed pike, Stizostedion vitreum, were obtained from a shipment received at the hatchery on April 20, 1935. Four apparently living eggs were placed in each of 63 vials, each containing 10cc of sterile distilled water. Into 7 of these vials Dermestid larvae on which the mycelium of S. parasitica was growing, were placed in the water with the eggs. Seven other vials were infected similarly with S. ferax. Still others were infected with S. diclina, S. anisospora, A. oblongata var globosa, and Achlya sp.

(sterile), seven vials for each form. To determine whether the mycelia thus placed in the vials were actually producing zoospores, sterile Dermestid larvae were placed in each of these 42 vials. These larvae floated on top of the water and were not in contact with the mycelia growing on the other larvae. Any subsequent infection of these sterile larvae must therefore be due to zoospores set free by the introduced mycelia. All the molds used were thus shown to be producing zoospores in the water which contained the eggs. Many of the eggs adhered to the sides of the vials and with very few exceptions none of the eggs were in contact with the mycelium at the outset of this experiment. The remaining 21 vials were not infected and were used as controls. This was deemed necessary since as a matter of course the eggs could not be sterilized and some other check was needed in order to determine the likelihood of previous infections. All the vials were placed in an icebox where the temperature ranged from 10 to 15 degrees C., mostly 12 or 13 degrees.

The first careful examination of these vials was made two days, or about 48 hours, after the experiment was started. From then on the vials were examined every day and the observations carefully recorded.

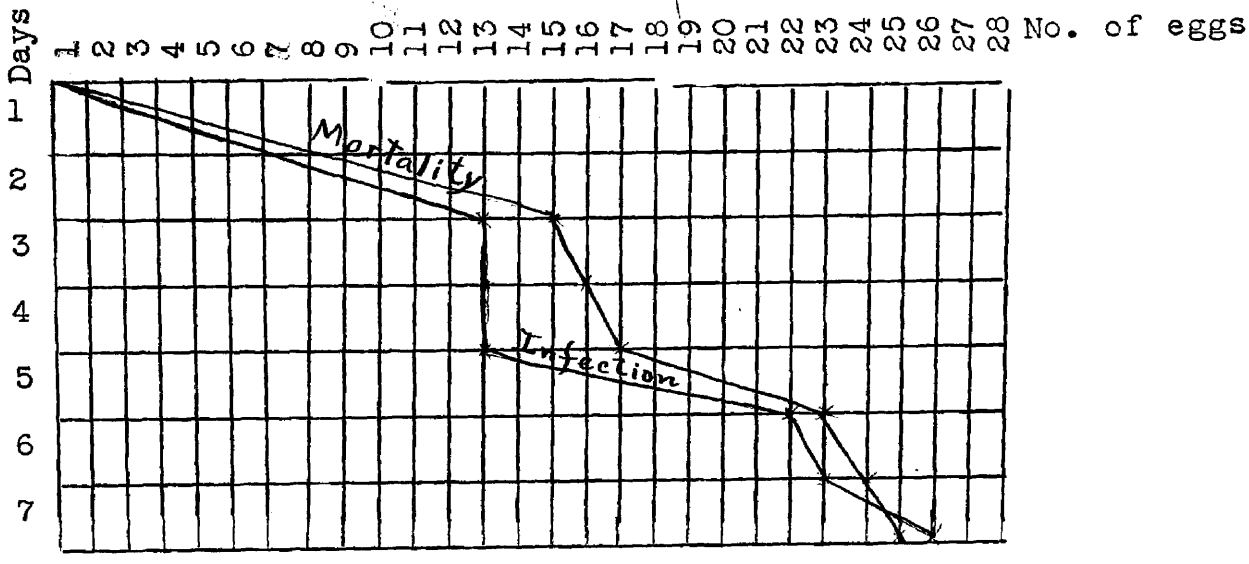
The first examination showed that about 60% of the eggs in the cultures and about 50% of the eggs in the control vials were dead. The high mortality must be regarded as due to factors involved in the sudden changes to which these eggs were subjected when the experiment

was started. It is also possible that many of the eggs were not fertilized and consequently died. This mortality cannot be ascribed to fungus infections since the mortality in the controls was nearly as high as that observed in the cultures. We conclude that only the eggs with the greatest vitality survived the sudden changes. These, therefore, were fit subjects for the object of this experiment.

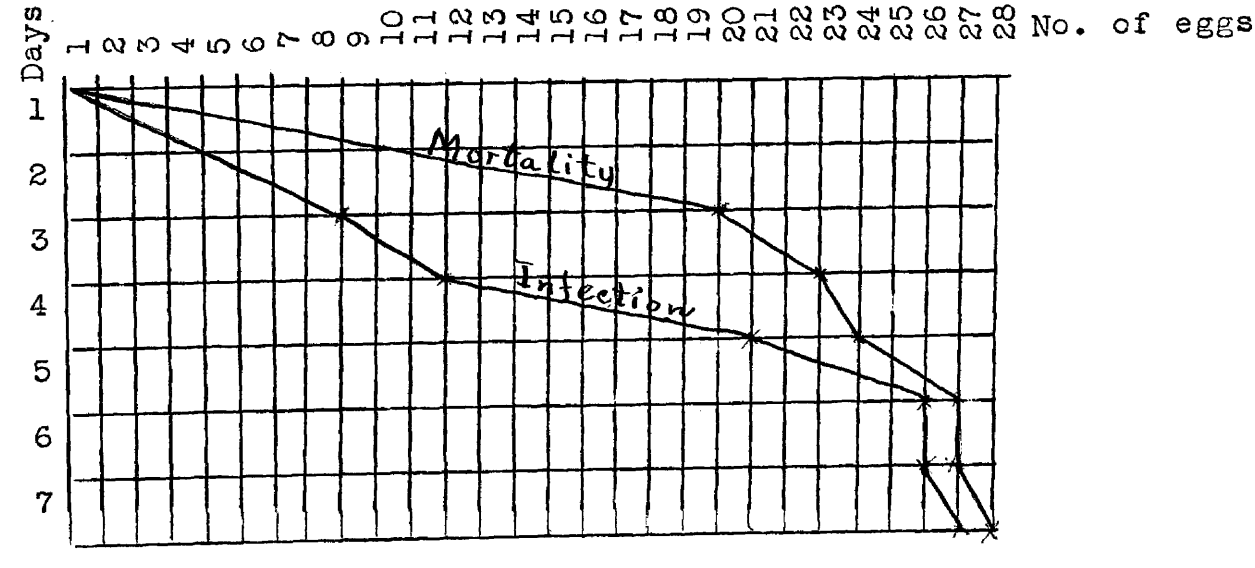
A further general statement may be made with reference to the first observations, namely, that none of the living eggs showed any signs of mold infections, whereas, 49% of the dead eggs in the cultures were definitely infected. None of the dead eggs in the controls were infected.

The observations for each of the mold species were recorded separately and can best be reproduced by a series of graphs. The graphs show the number of eggs that were dead and the number of eggs that were infected on each successive day. A curve for mortality and one for infection were drawn in each case. The proximity of the first curve to the second indicates how readily dead eggs are infected by the particular species of mold. If the infection curve at any point lies above, or to the right of, the mortality curve it indicates that living eggs were found infected.

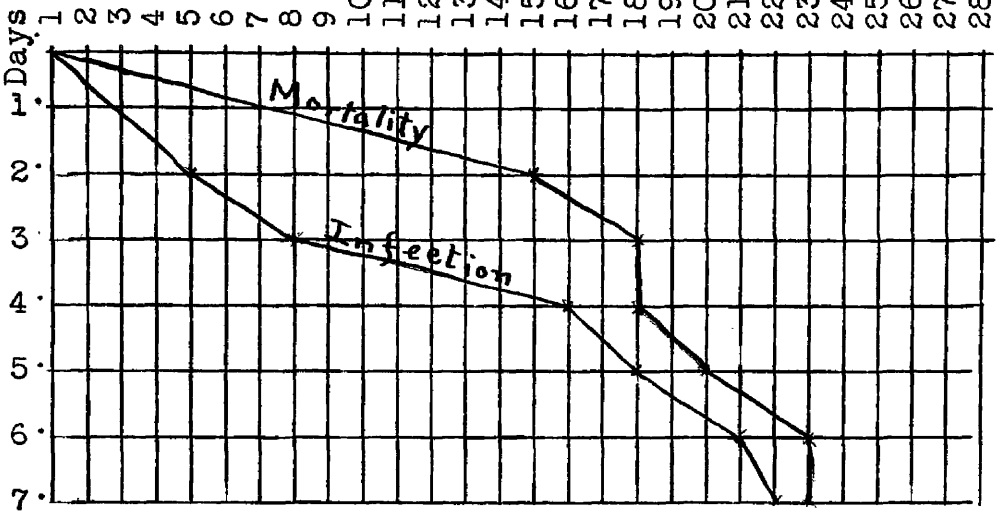
Graph showing Mortality Curve and
Infection Curve for S. parasitica



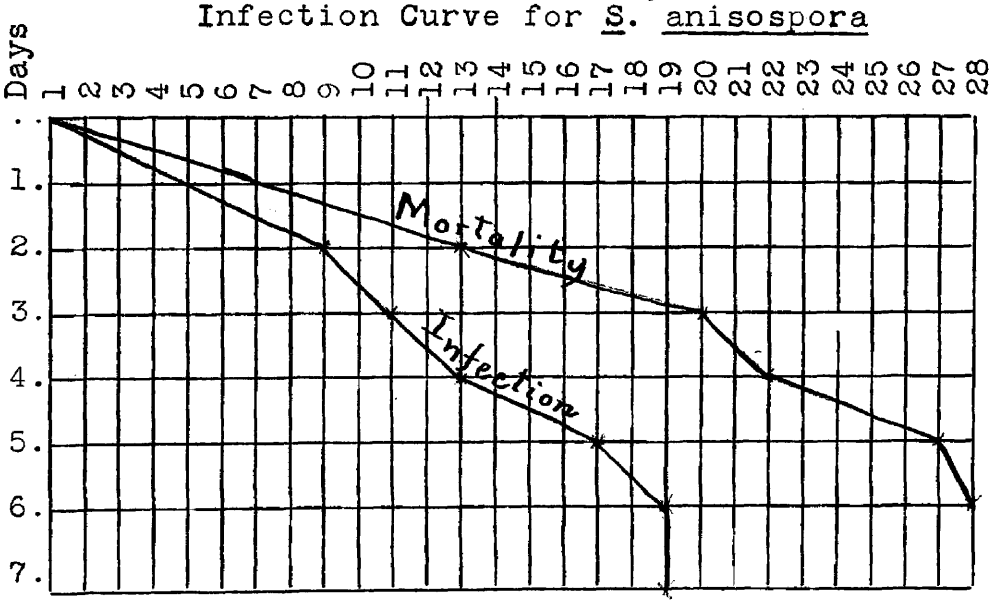
Graph showing Mortality Curve and
Infection Curve for S. ferax



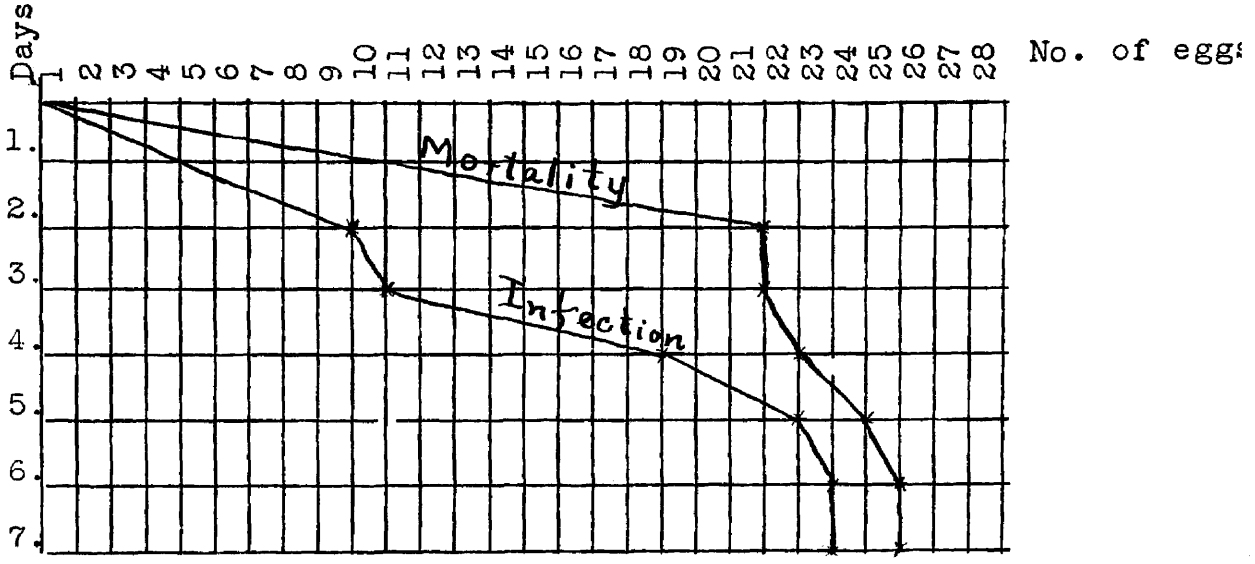
Graph showing Mortality Curve and
Infection Curve for S. diclina



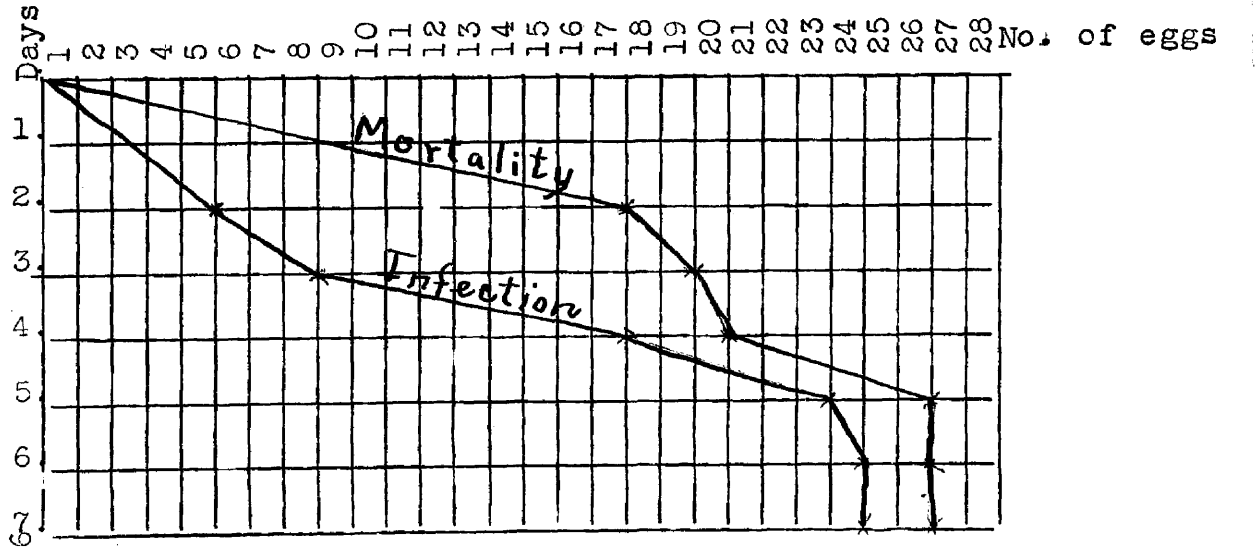
Graph showing Mortality Curve and
Infection Curve for S. anisospora



Graph Showing Mortality Curve and
Infection Curve for A. oblongata var globosa



Graph Showing Mortality Curve and
Infection Curve for Achlya sp.



An examination of the foregoing graphs brings to light certain facts which are here summarized:

1. All of the species used in the experiment infect dead fish eggs more or less readily.

2. S. parasitica appears to infect dead fish eggs more readily than do the other species used in this experiment.

3. S. parasitica is parasitic on fish eggs to a certain extent.

This last statement requires further explanation and amplification. It was observed that in no case was a living egg lying out of contact with the mycelium infected. Such an infection of dead eggs, however, was observed repeatedly in cultures of all of these molds. It appears, therefore, that none of these molds are parasitic on fish eggs so far as infections by isolated zoospores are concerned.

In a few cases living eggs were observed to lie in contact with growing mycelium. In those cases the hyphae enwrapped the eggs so that they could not easily be dislodged from the meshes of the mycelium. These cases were studied carefully with the aid of dissecting and compound microscopes. The mycelium was removed from these eggs in so far as this was possible without injuring the egg. These eggs were then isolated and put in a moist chamber. In this way subsequent development of the mycelium on the egg membranes and of the eggs could be watched. Five eggs were isolated in this manner. Three of these were surrounded by hyphae of S. parasitica, one by hyphae of S. diclina, and one by hyphae of A. oblongata var globosa. In all cases the

hyphae attached themselves to the egg membranes very firmly so that it was hard to remove them. This phenomenon did not seem to be due to any physical factor such as pressure since in all cases the eggs lay close to the mycelium but the hyphae were free to grow around them without becoming attached. Rather, the phenomenon appeared to be due to a positive tropism in the nature of thigmotropism. In no case could hyphae be observed penetrating the egg membranes at this time as was the case in the infected dead eggs. These isolated eggs were kept in the ice box with the other cultures.

Five days after the isolation of the egg surrounded by the hyphae of A. oblongata var globosa, the egg was still alive and developing, containing a living and active embryo. The hyphae showed no further development.

The observations on the egg surrounded by hyphae of S. diclina coincided with those on A. oblongata var globosa. Three days after the isolation this egg was still alive containing a living embryo and showing no infection.

Of the three eggs isolated with hyphae of S. parasitica one was dead the following day and was severely infected, a tuft of new hyphae extending from the focus of infection. The other two eggs were still alive but they showed distinct foci of infection in each case. These eggs were dissected and the foci of infection carefully examined under the compound microscope. It was observed that at these foci of infection small rhizoid-like hyphae were clumped together on the surface of the egg and rhizoids also extended into the egg. Among these clumps of hyphae several germinating

zoospores were also observed. The penetration of the germ tubes, however, could not be determined.

It is due to these observations that in the graph for S. parasitica the infection curve is to the right of the mortality curve on the last day of these observations. These observations also would lead one to conclude that S. parasitica is parasitic on fish eggs at least to some extent. This parasitism is not merely incidental or passive, and due to some external force such as pressure, but it is an active parasitism. It is limited, however, to this extent that living fish eggs do not appear to be readily infected by single zoospores of S. parasitica, but that due to the abundant growth of the hyphae on the surface of the egg the resistance of the egg is broken down and an avenue of entrance for hyphae and perhaps also for germ tubes becomes established.

During the five week period of our observations the eggs in the controls showed no mold infection whatsoever. The mortality rate of the controls was also much lower than that of the cultures. At our first observation two days after the start of the experiment 41 of the 86 control eggs were dead. Seven days after the start of the experiment 44 of the 86 eggs were dead. It may safely be concluded on the basis of these observations that the eggs used for this experiment were not previously infected. The greater rate of mortality in the cultures may be due to the fact that these cultures had actively growing mycelia in them. This, of course, diminished the relative amount of free oxygen in

the culture water. It is possible, therefore that these culture eggs died for lack of oxygen rather than because of any direct effect of the fungi. This conclusion is substantiated by our observations on one of the seven vials containing S. diclina. The vial contained a very weak growth of this fungus as indicated by the shight growth of mycelium. All the eggs in this vial remained alive during the seven day period of observation. That zoospores were present in the water was indicated, however, by the infection of the test larva on the surface of the water.

2. Experiments with eggs of black bass.

In the preceding experiment no difficulty was encountered so far as mold contamination of the experimental eggs was concerned. This was found to be an exceptional condition and yet one which is quite necessary in order to determine with any degree of certainty the parasitism of a particular species of mold. All methods of sterilization are necessarily ruled out. The experimental eggs used in this experiment were taken from a nest found in Pond 2 at the hatchery. They were brought into the laboratory in water with a considerable amount of debris and also with eggs that were already infected with water mold. This mold was very evidently a species of Saprolegnia and most likely S. parasitica. From the material brought into the laboratory 250 eggs were carefully selected. These eggs were picked out one by one, were alive, and were apparently free from any mold contamination. Twenty-five of the eggs were placed

in each of 10 petri dishes containing water from Strawberry Creek which had previously been sterilized, cooled and aerated by agitation. The eggs were spaced so that no two eggs were in contact with each other. Hemp seeds with water molds growing on them were placed in 8 of these 10 petri dishes. Each of the 8 dishes was thus infected with a different species of mold. The remaining 2 dishes were not infected and were used as controls. On the following day, however, the eggs in the control dishes were as badly infected as those in the other dishes. This showed definitely that although apparently free from mold infection, the selected eggs were not actually free from contamination. The experiment as set up could not give any information in regard to the parasitism of any particular species of mold. However, it could still throw light on the general question, "Does any mold zoospore, regardless of species, actually infect living fish eggs?" Here were 250 living eggs of the small-mouthed black bass, Micropterus dolomieu, with no apparent mold infection, all carefully selected, and spaced in water from their natural habitat. Any infection that would subsequently take place must necessarily be due to zoospores set free in the water or to those that were originally clinging to the sticky surface of the egg membrane. These eggs were carefully watched to determine whether mold zoospores would infect living fish eggs. The results of the observations are recorded in the following table.

TABLE VI

Showing the results on observations of 250 small-mouthed bass eggs subjected to the zoospores of various water molds.

Date of observation	No. of living eggs not infected	No. of living eggs infected	No. of dead eggs not infected	No. of dead eggs infected	No. of dead larvae not infected	Total number
6/6/35	250	0	0	0	0	250
6/7/35	98	2	66	84	0	250
6/8/35	22	3	49	176	0	250
6/10/35	0	0	0	239	11	250

Whether in this table an egg was listed as dead or alive depended entirely upon its transparency to light from the substage lamp of the microscope. It is considered likely that some of the eggs recorded as dead and infected may have been alive, since all doubtful cases were placed in this column. The five eggs recorded in Column 2 contained embryos that were definitely alive. These eggs were microscopically examined. It was found in each case that the infection had not penetrated beyond the egg membrane. This membrane could readily be removed with fine needles. The embryo was then set free apparently alive and not at all infected. Several of the dead and infected eggs were also examined. In these cases the egg membranes could not be removed because of the penetration of hyphae into the embryo.

On June 15, 1935, a collection of eggs of the large-mouthed black bass, Micropterus salmoides, was brought into the laboratory from Lake No. 2, of Morgan's chain of lakes

in northern Kent Co. This batch of eggs was also badly contaminated. Only 53 living and apparently non-infected eggs could be isolated. These were put into 4 petri-dishes carefully spaced as in the preceding experiment and observations were made. The results were much as those in the preceding experiment. Twenty-four hours after the start of the experiment, 17 of the eggs were dead and infected, 34 were apparently living and not infected, and 2 eggs were alive and infected. These 2 eggs were again carefully examined, the membranes were removed, and the embryos were without infection. One of these eggs was in an advanced stage of development; the larva was fully formed, its heart was beating, and the blood could be seen circulating through the vessels.

Eleven of the 53 eggs used in this experiment hatched. The larvae were observed for several days. Most of them died in a few days. Five became infected after death. None were infected while alive.

From these experiments it appears that zoospores of at least certain water molds settle upon the sticky membranes of bass eggs and there germinate and penetrate the membrane. The living embryo is not penetrated but due to the thick meshes of hyphae on the membrane the embryo eventually dies and is then penetrated by the hyphae.

3. Experiment with eggs of the bluegill.

Whether one or more species of water molds are capable of infecting living fish eggs was not determined in the

foregoing experiments. An attempt to determine this was made with eggs of the bluegill, Lepomis pallidus. These eggs were taken from Lake No. 2 of Morgan's chain on June 12, 1935. They were taken to the laboratory and experimentation was started the same day. The eggs were found in a bed of fine gravel and shells. None of them showed any mold infection. Four hundred eggs were separated out, cleaned, washed in several changes of sterile water. The eggs were then isolated as in the previous experiments, 25 in a petri-dish, with sterilized water from Strawberry Creek. Of the resulting 16 dishes, 2 were infected with zoospores of S. parasitica by the method described for the experiment with the small-mouthed bass eggs. Similarly, dishes were infected with zoospores of S. ferax, S. diclina, S. anisospora, Achlya oblongata var. globosa, A. americana, and A. Klebsiana, 2 dishes for each mold. The remaining 2 dishes were not infected and were used as controls. Sterile hemp seeds were dropped into each petri-dish to test the presence of zoospores. Although the eggs appeared to be entirely free from molds at the beginning of the experiment and although they were thoroughly washed, it was soon evident from the infection of control eggs that water mold zoospores were carried over into the experiment by the eggs used. This experiment, therefore, again falls short in giving us definite information with respect to the parasitism of any particular species of mold. However, the results do give some valuable information as to the tendency of these species toward infecting the membranes of living fish eggs. The results are tabulated below.

TABLE VII

Showing results for bluegill eggs exposed to zoospores of various water molds.

Name of mold species	Number of infected eggs			Four days after start of experiment			Total
	1 day after start of exp.	2 days after start of exp.	3 days after start of exp.	No. eggs inf.	No. eggs not inf. and hatched	Eggs dead not inf.	
S. parasitica	14	19	20	22	26	2	50
S. ferax	20	22	23	23	25	2	50
S. diclina	11	18	20	20	22	8	50
S. anisospora	0	13	17	17	30	3	50
A. oblongata v gl	1	6	11	11	31	8	50
A. americana	1	12	18	18	29	3	50
A. Klebsiana	3	14	19	24	26	0	50
Control	1	2	12	13	27	10	50

It will be noticed that the preceding table does not record whether the infected eggs were dead or alive. This information was purposely omitted in the table. Since bluegill eggs are small it is difficult to determine whether an egg is dead or alive, even with the use of transmitted light, especially when the egg membrane is covered with small particles of foreign matter. There is reasonable certainty, however, that all or nearly all of the eggs found infected one day after the start of the experiment contained living embryos. Several of the infected egg membranes were removed and living embryos were found inside. One of the 14 eggs infected presumably by S. parasitica hatched a few hours after the infection was observed. All of the infected

eggs were removed from the petri-dishes and placed in other dishes as soon as infection was observed. These infected eggs were kept under observation for some time. In all cases except the one mentioned above, the growth of the mold increased and the embryo was killed. Since after one day the controls were only lightly infected it is evident that the heavy infections found in the dishes inoculated with S. parasitica, S. ferax, and S. diclina were due to the zoospores of these species. In each case the test hemp seed was infected and the eggs were surrounded by active zoospores.

This experiment leads to the conclusion that the outer membranes of living fish eggs are readily infected by zoospores of certain water mold species including S. parasitica and S. ferax, the two species found in such large numbers in the water and soil of the fish hatchery. As a result of such an infection the egg dies, unless the embryo is in an advanced stage of development at the time of infection, in which case the egg will hatch, the larva evidently being unharmed.

4. Experiment with bluegill fry.

In the preceding experiments the parasitism of certain water molds on fish eggs has been definitely shown. Another question to be considered is, "Are living fish fry susceptible to water mold infections?" To obtain an answer to this question the very young bluegill fry produced in the preceding experiment were used. While in the petri-dishes none of the living fry became infected though they were surrounded by an abundance of mold zoospores. In order

to get these fry into conditions for better observations they were transferred from the petri-dishes to vials supplied with fresh, sterile Strawberry Creek water, and hemp seeds with zoospores producing molds of the same species as those used in the preceding experiment. Most of the fry lived for a week or more under these conditions. None of the living fry were infected. The results of the observations are here tabulated:

TABLE VIII

Showing the results of Experiment 4, in which bluegill fry were exposed to various kinds of mold zoospores.

Started June 20

Name of mold species	Date of observation														Totals	
	6/21		6/22		6/24		6/25		6/26		6/27		6/29			
	DEAD		dead		dead		dead		dead		dead		dead			
	I*	ni	i	ni	i	ni	i	ni	i	ni	i	ni	i	ni	i	ni
S. parasitica					1					1	1	1		9	2	11
S. ferax		2										19			0	21
S. diclina		2	2									16			0	20
S. anisospora	1		1	1								12		4	2	17
A. obl. v glob.	2	10		3								11			2	24
A. americana		15		6		1									0	22
A. Klebsiana		6	1	3				2	2	6		1			3	18
Controls				1	1							1	1	7	2	9
* i - infected ni- not infected												Totals			11	142

The results for this experiment show that of the 153 bluegill fry exposed to zoospores of water molds none were infected while alive, and only 11 were found to be infected when dead. The dead fry were removed at each observation. Since so large a majority of the fry were found dead without infection it can be assumed that the few which were found

infected died from reasons other than the mold infection. This experiment, therefore, leads to the conclusion that water molds are not parasitic on fish fry.

5. Experiment with small-mouthed bass fry.

Another experiment to determine the parasitism of water molds on fish fry was conducted at the hatchery with fry of the small mouthed bass. Approximately one hundred fry were put into each of eleven battery jars such as are used in the hatchery for the hatching of eggs. At the bottom of eight of these jars zoospore producing molds were placed. The species used were S. parasitica, S. ferax, S. diclina, S. anisospora, A. oblongata var globosa, A. americana, A. Klebsiana, and A. racemosa. All of these molds were producing zoospores in the laboratory. They were growing on hemp seeds, dermestid larvae, and pieces of boiled fish. Two small dead fish, picked from the hatchery tanks and badly infected with mold, were placed in the ninth jar. The other 2 jars were left as controls. Hatchery water which came directly from Strawberry Creek was used for this experiment. This water was kept running through the jars as it is ordinarily done when eggs are being hatched. The fry were, therefore, under conditions similar to those that obtained when they were hatched.

One day after the start of the experiment all of the fry were alive. About 10% of them, however, were covered with yellowish spots. These were at first thought to be possible foci of mold infection. On the following day the

number of fry having such spots had increased but no mold growth was in evidence. Upon microscopical examination these spots proved to be the cysts of a protozoan parasite, Ichthyophtherius multifilius, an infusorium rather abundant at fish hatcheries and causing a disease known to hatchery men as the "itch". No mold growth was observed on the second day nor was there any dying off of the fry. The start of mold growth was looked for especially at the points of protozoan infection but no such growth appeared even under the microscope. Similar observations obtained for 2 more days. On the fifth day after the start of the experiment many of the fry had died. All the dead specimens were removed and microscopically examined for mold infection. The same was done on the sixth and seventh days. On the seventh day only 108 fry were still alive. The experiment was terminated at this time and the living specimens were also microscopically examined for mold infections. The observations for the 3 days were as follows:

Number of dead specimens infected	411	
Number of dead specimens not infected	406	
Total number of dead specimens examined		817
Number of living specimens examined and found not infected		108
Total number of dead and living specimens examined		925

Infected dead specimens were found in all of the 11 jars during the 3 day period that dead fry were found. This indicates that mold zoospores were present in all of these jars and that the living fry were exposed to them.

Since the control jars were shown to contain mold zoospores by the infection of dead fry, this experiment does not serve to show parasitism for any particular

species of mold. However, since no living specimens were seen with infections in any of the jars, the conclusion that none of the mold species used in this experiment are parasitic on fry of the small mouthed bass is warranted. This conclusion is similar to that reached for the fry of bluegills in the preceding experiment.

6. Experiment with Lake Michigan Shiners

In order to determine the effects of water molds on older fish an experiment with Lake Michigan shiners was undertaken. These fish were chosen because of their convenient size and because they are extensively used at fish hatcheries as food for larger fish. A battery of eleven jars was set up as in the preceding experiment. Into each of these jars 6 shiners were placed, three of which were injured on the side by means of a scalpel. Daily observations for molds were made. These observations were carried on over a period of nearly two weeks. The dead fish were removed as soon as they were found and notes with regard to infection or non-infection were taken. At the close of this observation period, 6 of the 66 fish were still alive and showed no mold infection, 26 had died from the effects of molds, and 34 had died from other causes.

Of the living fish one was an injured specimen. It was taken from the jar supplied with S. ferax. The other 5 had not been injured and were taken as follows:

3 from the jar containing A. oblongata var globosa
1 from the jar containing A. Klebsiana
1 from the jar containing the infected fish.

Of the 34 that died from causes other than mold infection 5 escaped from the top of the aquaria and were found on the floor. The other 27 evidently could not adjust themselves to the confines of aquaria as well as some of their mates. Many of them, however, lived for nearly two weeks.

Special importance is attached to those 26 that died from mold infections. In nearly all cases the infection could be seen on the living fish one or two days before it died. Once the growth got started its spread was very rapid. Not one of the fish lived more than two days after the infection was first observed. In 16 of the 26 cases that died from mold the infection started at the point of injury on the side. In the others the infection started either at the tail or in the gills and head region. This is significant, since it is well known that fish are easily injured during transportation and handling, especially at the anterior and posterior ends. These injuries may be very small and not noticeable to casual observation. It is very likely that not only on the intentionally injured specimens but also on the others the infection started at a point of injury. Some of the infected fish were examined microscopically and the infection was in every case found to be superficial, the hyphae never penetrating deeply into the body tissues. In fact, it appears that the mold establishes itself at the point of injury and from there spreads as a thick mat of mycelium over the rest of the body, but not penetrating the tissues.

Dead and infected fish were found in all the jars used in this experiment, controls as well as the others. This was as expected since in the preceding experiment the water from Strawberry Creek was shown to contain mold zoospores. The results of this experiment, therefore, do not indicate that any particular species of mold is the offender. If, however, we consider the rate of mortality due to water mold in the various jars it does point to the fact that S. parasitica may be the chief if not the only offender. For this reason, the information that has bearing on this point is here given.

TABLE IX

Showing the mortality rate of the fish that died from mold infections

In jar containing:	No. of specimens dying from mold	No. of day on which this mortality occurred	Rate of mold mortality per day
S. parasitica	4	3	1 1/3
S. ferax	1	3	1/3
S. diclina	3	7	3/7
S. anisospora	3	3	1
A. oblongata var. globosa	2	10	1/5
A. Klebsiana	3	6	1/2
A. racemosa	1	8	1/8
Inf. fish	1	6	1/6
Control No. 1	3	9	1/3
Control No. 2	4	12	1/3
	26		

Only the jar containing S. anisospora showed a mortality rate that was at all close to that of S. parasitica as indicated in the column to the right. Since S. anisospora was not found in abundance at the hatchery it is doubtful whether this species contributes toward infections found in the ponds.

The foregoing experiments lead to the following conclusions:

1. One or more species of water molds readily infect the membranes of living fish eggs by means of zoospores as well as by their hyphae. Such an infection nearly always results in the death of the embryo and further penetration of the hyphae.

2. None of the mold species used in these experiments are capable of infecting living fish fry.

3. Saprolegnia parasitica and perhaps also other species of water molds, by means of their zoospores infect living fish at injured surfaces and continue to grow from that point until a large part of the body of the fish becomes covered with a mat of mycelium as a result of which the fish dies.

Discussion of the Necessity and Possibility of Control

Attention has been called in this paper to the general prevalence of several species of water molds in the ponds of the Lydell State Fish Hatchery. It has further been shown that the zoospores of many of these molds infect dead fish eggs readily and that one or more species of water molds will infect the membranes of living fish eggs by means of their zoospores as well as by their hyphae. There is no doubt that the death of many

thousands of fish eggs in these ponds annually is at least in part due to the prevalence of these molds. It is evident that the elimination of water molds from this and similar situations would be of great benefit to fish culture.

The problem of control, however, is a difficult one. It is not sufficient, as is frequently suggested, to keep the ponds free from dead fish and spawn, and other organic debris on which these fungi thrive, so long as the water supply from the sources is highly contaminated with mold zoospores. The problem resolves itself into the purification of the water from the sources and this makes it still more difficult. It is out of the question to keep the streams, which supply the ponds with water, free from the organic matter which supports the life of these Phycomycetes. If, as is the case in this hatchery, the water is conveyed from the sources to the ponds by means of pipes, it would seem that the proper time to rid the water of these dangerous zoospores would be when it passes through the pipes. If at this stage the water were treated with copper sulphate, the zoospores would certainly be eliminated. But this treatment is ruled out for two reasons: first, because the copper ion which kills the spores is also detrimental to the life of fish eggs and young fish, and secondly, because there is a continual flow of water through the pipes and the ponds so that thousands of gallons of water would have to be treated daily. It is doubtful whether the expense of

such an undertaking would warrant its usefulness. If copper ions could be set free in the water at one point and again isolated and taken out of the water at a point further down before it reaches the ponds, the killing of the zoospores might be effected and the problem solved. Whether this would be possible through some method of electrolysis or some chemical process is a question which requires further experimentation and study. The solution of the problem lies, perhaps, along the lines of physics and chemistry and beyond the scope and intentions of this paper. Until such a purification process has been devised the usual precautions to free the ponds from all organic matter supporting the life of fungi should strictly be adhered to. A periodic draining and cleaning of the ponds and the subsoil is at present the best method to keep the fungi under control.

Summary

1. 130 samples of water from 26 ponds, 40 samples of water from sources, and 115 samples of soil were collected from the Lydell Fish Hatchery with the purpose of determining the water mold contamination of this hatchery.

2. A new method for obtaining single-zoospore cultures of water molds is described.

3. Fourteen species of water molds were isolated from the water and soil samples. These were identified and described.

4. It is found the S. parasitica Coker is more

abundant in this situation than any other species, isolated from the samples.

5. The streams which supply the ponds with water were found to be highly contaminated with mold spores of various species, so that the contamination of the ponds and the soil of the hatchery could be traced to these sources.

6. Experiments to determine the extent of parasitism of S. parasitica Coker and other molds were conducted with the result that certain of these molds were found to infect membranes of living fish eggs. They were found not to be parasitic on the fry of fish. One or more of these molds were shown to infect older fish at injured points and to effect the death of such infected fish.

7. The necessity and possibility of water mold control at the hatchery is considered.

LITERATURE CITED

1. Bary, A. de — Einige neue Saprolegnieen. Jahrb. f. wiss. Bot. 2:169-192. Pls 19-21. 1860

2. Bary, A. de — Zu Pringsheim's neuen Beobachtungen über den Befruchtungact der Gattungen Achlya und Saprolegnia. Bot. Zeit. 41:48-54. 1883

3. Bary, A. de — Species der Saprolegnieen. Bot. Zeit. 46:597-645, Pls 9-10. 1888.

4. Coker, W. C. — The Saprolegniaceae, with notes on other water molds. 201 pp. 63 pls. University of North Carolina Press, Chapel Hill, N.C. 1923.

5. Coker, W. C. and Pemberton, J. D. — A New Species of Achlya. Bot. Gaz. 45:194-196. figs 1-6. 1908.

6. Giltner, W. Laboratory Manual in General Microbiology, 472 pp. 72 figs. John Wiley & Sons, Inc. New York. 1926.

7. Hildebrand, F. Mycologische Beitrage I. Ueber einige neue Saprolegnieen. Jahrb. f. wiss. Bot. 6:249-269. Pls 15-16. 1867-68.

8. Humphrey, J. E. The Saprolegniaceae of the United States with notes on other Species. Trans. Am. Phil. Soc. 17:63-148, Pls. 14-20. 1892, 1893.

9. Huxley, T. H. Saprolegnia in Relation to Salmon Disease. Quart. Jour. Mic. Soc. 22:311. 1882.

10. Kanouse, B. B. A Physiological and Morphological Study of Saprolegnia parasitica. Mycologia 27:431-452. Pls 12 and 13. 1932.

11. Kauffman, C. H. A Contribution to the Physiology of the Saprolegniaceae with Special Reference to the Variations of the Sexual Organs. Annals of Botany 23: 362-387. 1908.

12. Kauffman, C. H. Klebs' Theory of the Control of Developmental Processes in Organisms, and its Application to Fungi. Proceedings of the International Congress of Plant Sciences 2:1603-1611. 1929.

13. Klebs, G. Zur Physiologie der Fortpflanzung einiger Pilze II. Jahrb. f. wiss. Bot. 33:513-593. 1899.

14. Maurizio, A. Zur Entwickelungsgeschichte und Systematik der Saprolegnieen. Flora 79:109-158. Pls. 3-5. 1894.

15. Pieters, A. J. The Relation between Vegetative Vigor and the Reproduction in some Saprolegniaceae. Am. Jour. of Bot. 2:529-576. Text fig. 1, 2. 1915.

16. Pieters, A. J. New Species of Achlya and of Saprolegnia. Bot. Gaz. 60:483-490. Pl. 21. 1915.

17. Pringsheim, N. Weitere Nachträge zur Morphologie und Systematik der Saprolegnieen. Jahrb. f. wiss. Bot. 9:191-234. Pls 17-22. 1873.