OBSERVATIONS AND EXPERIMENTS ON SAPROLEGNIA INFESTING FISH.

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The fresh-water fish in the aquarium of the U. S. Fish Commission at the World's Fair having been more or less troubled by a fungous disease, the writer was asked in October to make a study of the same. A glance in the microscope at material taken from a diseased fish was sufficient to determine that a common fish-pest, *Saprolegnia*, was at work; but as it was not present in its mature stage, further study, including culture experiments, was undertaken.

The fresh-water fish occupied twenty-eight tanks, just within the wall of the circular room, and also a number of larger tanks, separated from the former by a wide aisle. In the center of the room a large circular basin was also given up to them. The water was supplied by way of a crib a few miles out in Lake Michigan, and after having its solid matter precipitated by alum and passing through a sand filter it escaped in several small jets into the individual tanks, entering with sufficient force to carry down air bubbles from one to two feet. The water passed off by an overflow at the top, and was not used again, but drained into the lake. The tanks were emptied once or twice a week and cleaned, while the central basin was run down every night and refilled. It was in certain of the smaller tanks that the disease was worst. The fish were brought to the aquarium in the cars of the U. S. Fish Commission, chiefly from the Great Lakes and from streams of the Mississippi Valley or from breeding stations of the Commission. They were carried in water cooled by ice, but the transfer to the aquarium was so managed that the temperature was gradually raised before they were placed in the various tanks.

According to the statement of the assistant in charge, the fish never showed signs of disease when they arrived. There were no means of regulating the temperature of the water in the aquarium, and at the higher degrees (68° to 74° F.) the development of the fungus seemed to be considerably stimulated. The fish were fed corn bread, liver, beef, or minnows, according to species.

The fungus developed on all parts of the fish, though perhaps more abundantly or more frequently on the tail, fins, or head. According to the host or stage of infection, it would be limited to small, definite patches, or spread all over the fish. In general it formed a tuft of white threads that radiated out from the body a distance of about a third to half an inch. While a fish might be abundantly covered with the fungus, so that in water the threads would float out in a radiating mass, when the fish was removed from the water the threads, too weak to support themselves erect, would collapse, and the fish would then present a slimy appearance, and would not seem so badly infested. In those that were but slightly attacked the fungus was, as a rule, more definitely limited to spots on different parts of the body. Usually in such cases it was developed as a bunch of filaments on some bruised place. Occasionally fish could be found on which the fungus had grown as a mass of filaments covering the eyes and causing blindness. Sometimes the tail and fins had become so lacerated, at least in part by the action of this fungus, as to be almost destroyed. If new arrivals were affected, it was usually within a week after they were placed in the aquarium that they began to show the disease plainly. Within three or four days of its beginning the surface would become abnormally slimy, when an examination of the epithelial débris, covering the fish, would reveal the presence of numerous prostrate filaments of the fungus imbedded in this material. From these the erect radiating filaments were rapidly produced, so that in a few days fully developed zoösporangia could be found.

There is no doubt that this fungus was primarily a saprophyte, as are most of its family, and that its parasitic habit is a secondary acquirement. It now acts in either capacity, as opportunity offers. Probably the abundance of slime on some kinds of fish has much to do with the fact that the fungus obtains its first hold on them. If the fish is not protected by well-developed scales, or an unusually thick skin, it is especially at the mercy of its invader. Having gained entrance, the fungus makes its presence manifest by causing local irritation. The blood can sometimes be seen in reddish patches in and about the part affected, and the fish frequently shows a desire to scratch the injured spot by rubbing against some object or scrubbing itself in the sand on the bottom of its tank. In a more advanced stage the fish becomes sluggish and frequently dies, when a vigorous growth of the fungus follows until bacteria put a stop to it by causing putrefaction of the body. Often a badly infested fish will recover, and minor attacks come and go quite frequently among the more hardy species.

None of the marine animals in the aquarium were attacked by this fungous disease. In the fresh-water section a genus closely allied to *Saprolegnia* (*Achlya*) was found developed considerably on the eggs of a crayfish as they were attached to the parent. Some of the turtles were said to be "fungused," but no examination was made of material from this source. The growth on all the fresh-water fish seemed to be the same, and in all cases a *Saprolegnia*. There was a difference as to susceptibility, this apparently depending to some extent on the age of the fish or the species. Young fish seemed comparatively exempt from the disease. If one died and remained in the water it readily became covered with the fungus, but while living they were not much affected. This was true even of the same species in different stages of development. For example, a tank of young black bass showed no signs of disease, while mature fish of that species almost invariably "fungused."

Among the mature species there likewise seemed to be a wide difference in the power of resisting infection. Undoubtedly such factors as bruises, rubbed scales, changed and unusual surroundings, or insufficiently developed epidermal protection had much to do in determining liability to infection. For instance, fish with a tough skin, as the Mississippi catfish, or with a development of prickly and bony plates like the sturgeon, were usually free from the disease, while such as sunfish and black bass were quite badly affected. Certain species, as the goldfish, which have been reported in other places as sometimes becoming infected, escaped here, giving no evidence of the disease. It is also a fact that all those foreign fish that were bred by the Fish Commission, and that are commonly cultivated as aquarium fish, such as the goldfish, already mentioned, golden ide, and tench, escaped entirely, while native fish recently collected from streams, etc., were quite apt to become infected unless well provided with resisting epidermal parts. Daily examination of the aquaria during a period of three weeks showed that at that time sturgeon, burbot, mud catfish, Mississippi catfish, carp (American, scale, mirror, and leather), tench, goldfish, golden ide, *young* trout (brook, Von Behr, and Loch Leven), and *young* black bass were practically free from attack; that small-mouthed black bass, rock-bass, brook trout (adults), yellow catfish, spotted catfish, buffalo, dogfish, paddle-fish, moon-eye, the long-nosed and short-nosed gar, the redhorse, and the common suckers were somewhat affected, often but slightly and usually in bruised places; and that the black bass (*adult*), white bass, yellow bass, calico bass, yellow perch, grayling, pike, pike perch, sheepshead, sand pike, bream, blue sunfish, warmouth, and individual cases of the sucker and redhorse were badly diseased.

It has long been known that dead insects falling into water are quite likely to have developed on them a growth of the species of fungi under discussion,* and botanists have taken advantage of this fact for the development of these fungi, placing insects in water likely to contain such forms, or inoculating insects and then placing them in pure water. In such cases, in a few days, a vigorous growth of fungous filaments radiates from the insect on all sides, and the appearance and development of the fungus can be studied as desired. The only drawback to such cultures is that bacteria are likely to develop on the dead insect, and after a time seriously interfere with the growth of the fungus; but, wishing to make a study of the water, such cultures were undertaken. As the fungus developed abundantly on the liver fed to the fish, if it remained a few days in the water, this was first used for culture experiments, but bacteria were developed in such numbers on the meat that it was found useless, and flies —the only insects to be had—were resorted to with fairly good results. It was desired to test the water immediately before its entrance to the tanks, in order to learn if the germs were then present; to test it in the different tanks, and in various parts of the same tank, to ascertain if the germs were present or absent, or relatively more or less abundant in the different situations; and, lastly, to determine if different fungous forms existed in the water taken from these various places. An examination of water taken directly from the lake and of the hygeia water used for drinking purposes on the Exposition grounds, was also made.

The method pursued was as follows: A small bottle of the water to be tested was taken and a fly or two dropped in it and left for twenty-four hours—a sufficient time for the infection of the fly if fungous germs were present—when the water was replaced by that known to be pure. Twenty-four hours later, if the culture was successful, a slight growth of the fungus could be seen radiating from the insect, and one or two days thereafter the zoösporangia began to form. After the expiration of a week,

*Ledermüller, *Mikroskopische Ergötzungen*, 1760.

further growth in length was not noticed. The filaments usually reached a length of a third to half an inch. In ten days or two weeks further development was usually stopped by bacteria. No cultures being successful in the hygeia water, it was regarded as pure and used to place the flies in after their inoculation.

At first no cultures were obtained from water taken just before its entrance into the various tanks of the aquarium, and it was thought to be free from the fungus, but as more were attempted an occasional growth of *Saprolegnia* appeared, quite similar to that found on the fish. About a third of the tests of water from this source gave cultures—a proportion large enough to indicate that the water supply may have been the original source of infection. The fact that cultures were thus occasionally obtained from the water just before it entered the tanks was probably due to the imperfect working of the filter system, which was sometimes out of order, the water in the tanks then becoming quite turbid. It was said that the clearness of the water was similarly affected when the lake was especially rough. When the fungus had once obtained a foothold in the tanks it became, of course, much more abundant in its spore forms. Cultures obtained from water from the lagoon and from the edge of the lake yielded in every case some form of *Saprolegniaceæ.*

There was some slight difference in the number of successful cultures in water taken from the various tanks. For example, growths were almost invariably obtained from those tanks that contained infected fish, while water from tanks in which the fish were not diseased yielded cultures in scarcely more than half the cases.

Another difference was found in the character of the growths. While on the fish themselves nothing but a species of *Saprolegnia* was found, in water cultures from the different tanks three genera were encountered; viz., *Saprolegnia*, *Achlya*, and *Leptomitus*. The *Leptomitus* was found only as a secondary product in a few impure cultures that were not well developed. Pure cultures of either the *Saprolegnia* or the *Achlya* were usually obtained, but occasionally a growth was found in which they both occurred. Still another distinction was noted: The water cultures from aquaria containing badly "fungused" fish almost invariably gave *Saprolegnia*—commonly a pure growth; those from aquaria in which the fish were healthy, generally gave *Achlya*; while those from tanks in which the fish were slightly affected by the disease yielded either *Achlya* or *Saprolegnia* or an impure growth of both. The *Achlya* and *Saprolegnia* cultures could be readily distinguished even with the naked eye. The former had the longer filaments, and the bunch of zoöspores clustered at the opening of the emptied zoösporangia could easily be seen. In cultures of the *Saprolegnia* the filaments formed a rather dense growth which radiated from the fly for about a third of an inch. When the zoösporangia began to form, the threads became more opaque at their free ends.

The tests of water taken from the tanks at various depths gave us no differential results. It seemed to make no essential difference in the character of the growths whether the water came from the top, the center, or the bottom of a tank.

Numerous experiments were made by placing a little of the fungus taken from diseased fish on flies in hygeia water. These cultures were always successful, and, so far as one could judge from the asexual stage, were of the same species as those obtained from the water cultures which gave growths of *Saprolegnia.*

Altogether, something over 100 cultures with flies were made, but in none of those which yielded the *Saprolegnia*, and in none of the numerous specimens examined from the fish, were there any signs of the mature stage of the fungus. In the *Achlya* cultures, however, this stage was reached occasionally. When the cultures of *Saprolegnia* were about ten days old, further development being prevented by bacteria, the production of the normal zoösporangia was arrested, and what are known as "resting sporangia" were formed. These were produced, in a moniliform manner, by the constriction of the filaments into one or several cells in which the protoplasm was not differentiated into zoöspores, and which were somewhat shorter in comparison to their breadth than were the usual zoösporangia. The results of most of the cultures that were made with flies are given below:

Tabulated results of culture experiments.

Sources of infection.	Successful.	Failed.
Aquarium water	5	10
Hygeia water	....	6
Lake Michigan water	3	....
Lagoon water	2	....
Central Pool water	4	....
Fungous inoculations	15	....
Water from experiment tank	2	2
Water from tank containing badly diseased fish:		
Top	6	1
Center	2	....
Bottom	6	....
Bottom stirred	3	1
Water from tank containing fish but little diseased:		
Top	1	1
Center	1	....
Bottom stirred	1	....
Water from tank containing healthy fish:		
Top	3	2
Center	1	3
Bottom	4	1
Bottom stirred	2	1

The minute structure and life-history of such fungous forms have been so thoroughly made out by eminent specialists that no investigation along this line was made, save to observe those phenomena which might be easily seen with ordinary microscopic manipulations. The fungus consists of branched, hyaline filaments, without septa, except as these are found cutting off the reproductive parts of the threads. It is made up of a root-like or rhizoid part that penetrates the fish, and a vegetative and reproductive part that radiates from the host. The former consists of branched tapering threads which pierce the tissues for a short distance, but are easily pulled out. The function of this part is to obtain nourishment for the growth of the external parts. Prostrate threads are found running through the natural slime covering the fish, and from these are produced the erect radiating hyphæ so plainly seen when in the water. The development of these threads appears to be very rapid when viewed under the microscope, although the growth made under favorable conditions in two days is only about a third of an inch. From actual measurements of filaments of the fungus placed in water and watched under the microscope, it was found that certain threads made a growth of about 300 microns in an hour. Two others, watched for twenty minutes, gave in that time a growth of 90 and 47 microns respectively; and yet another filament, observed during two periods of five minutes each, made a growth of 28 microns each time. In ordinary cultures the rate of growth depends upon the condition of the medium, host, etc.

The first evident sign of a spore stage is when the filaments, having reached a certain length, become more or less clavate at their free ends. It is then noticed that the protoplasm is much more abundant here. A constant circulation of granules in the protoplasm is going on, and the small nuclei are seen gradually moving along the cell wall toward the end of the filament. A band of protoplasm, free from granules, is next formed at the lower end of the clavate part of the thread, and across the lower side of this is rapidly developed a horizontal septum shutting off the club-shaped part of the hypha. Within this cell is now seen a shifting of protoplasm, with an appearance and disappearance of vacuoles. The septum has in the meantime become slightly convex downward, and the hyaline band has again become granular. When this shifting movement has continued for some time, the protoplasm is seen to form gradually into spore-like masses thickly crowded together and lining the cell wall. These become less distinct at times, and at last seem to lose their identity entirely. In the meantime a short papilla has been forming on the end of the cell, and the septum becomes convex upward. The spores again become distinct, though crowded closely together, and suddenly those near the papilla are seen to be forced into it and pressed against the wall, which is quickly ruptured. The zoöspores now rush out through the ruptured place, and soon swim out of sight; but all may not succeed in escaping from the zoösporangium.

Their form is ovate to oblong-ovate, with two cilia on the smaller hyaline end. By the lashing of these they keep up a rapid movement for some five or ten minutes, when, in the majority of cases, this ciliary motion gradually lessens and finally stops. Assuming now a spherical shape, the spore loses its cilia and secretes a cell wall. In some cases, however, those zoöspores which failed to escape from the zoösporangium were seen to move around for nearly an hour before becoming quiet. These spores while motile are without a proper cell wall, and frequently slightly change their shape. In the meantime the septum at the bottom of the zoösporangium becomes greatly convex upward, and a new growth of the filament takes place within the wall of the empty zoösporangium, this in time giving rise to another zoösporangium.

The zoöspores, after a short period of rest, are said to go through a second swarming, and then become stationary, as before. Soon after this second swarming the true germination of the spore takes place. This begins with a slight distention of the spore on one side. This distended part gradually becomes elongated, and is then seen as a germ-tube proceeding from the side of the spore. The growth of this tube is very rapid, although less so than that of the filaments of the fungus, being slowest while the tube is getting a good start and, at the last, when the protoplasm is about exhausted, but otherwise nearly uniform. The germ-tube tends to be bent or flexuous rather than perfectly straight. After it has made a slight growth vacuoles or rifts begin to appear in the protoplasm of the spore. These widen as the contents are gradually drawn into the tube, until the spore is entirely emptied. One germ-tube was observed that had reached a length of 145 microns before the germination had entirely ceased. These germ-tubes are without septa or branches, and each spore forms but one such tube. Spores germinated in water made a growth of the germ-tube equal to about 1 micron each minute during the time observed. The rate of growth of three different spores was respectively as follows: 45 μ in 45 minutes; 15 μ in 20 minutes; and 42 μ in 32 minutes.

It is by the germination of these zoöspores that the fungus chiefly propagates itself. They are asexual and short-lived, but are continually produced in enormous numbers, and are easily carried to their hosts by means of their cilia. The double swarming also favors distribution, and thus tends to render infection more probable. The fungus gains entrance to its host by its germ-tubes, which pierce the tissue.

Besides the zoösporangia with their zoöspores, the *Saprolegniaceæ* have another reproductive stage, consisting of antheridia and oögonia, the mature spore form of these being the oöspore. In the fungus studied these were never found. They are quite apt, however, to be produced less abundantly than the zoösporangia and zoöspores, even rarely, according to some authors,* and at special seasons only. While the generic distinctions of this group of fungi are based mostly on the characters of zoösporangia and zoöspores, the classification of the species depends almost entirely on the character of the antheridia, oögonia, and oöspores. It was impossible, therefore, to determine exactly the species to which this fungus belongs.

Enough was seen, however, to show that it belonged to what was indiscriminately called *Saprolegnia ferax* before De Bary's system of classification was proposed. By this author, however, *S. ferax* is divided into several distinct species, and with one of these, *S. mixta* (as described by Fischer in Rabenhorst's *Kryptogamen-Flora*), the fungus in question seemed most nearly to agree. During November it was said that a decided change in the appearance of the fungus on affected fish took place. Perhaps if material could have been then obtained, the mature form might have been found.

In the cultures made a parasite frequently appeared upon the fungus itself. This was what Cornu first called *Olpidiopsis saprolegniæ*. It belongs to the same great group (*Phycomycetes*) as does the *Saprolegnia*. It consisted of oval sporangia developed in the spherically-enlarged ends of the *Saprolegnia* filaments. The zoösporangium formed a short tube, which pierced the wall of the host filament, and it was through this that the numerous small zoöspores escaped. This parasite so enlarged the ends of the filaments it occupied as to make them visible to the naked eye.

The development of the fungus was of such a character as to render it a serious pest in the aquarium, and various efforts were made by those in charge to lessen its ravages. The tanks were drawn down once or twice a week, but this did not diminish the disease to any great extent. The standard remedy—salt—placed in the bottom of the tanks seemed to lessen the disease, and baths of salt water improved the appearance of the fish, destroying the fungus to some extent. Experiments were tried to determine the effect of salt water of different degrees of density on cultures of the fungus. The ordinary sea water of the aquarium was strong enough to cause a shrinking and collapsing of the protoplasmic lining of the fungous filaments soon after these were placed in it. Cultures on flies also failed to develop when placed in this water even when considerably diluted; but in one part of sea water to five parts of fresh a vigorous growth of the inoculated fungus was once obtained.

No special care had been taken to isolate healthy fish as they were brought in and it was suggested that this measure was worth a trial, notwithstanding the fact that an occasional culture had been obtained from the filtered water just before its entrance into the tanks. A tank was consequently selected, emptied, and thoroughly scrubbed throughout, and washed all over with a four per cent solution of carbolic acid. It was then rinsed with fresh aquarium water and emptied, after which it

*Walpole and Huxley, in Twenty-first Report Inspectors of Fisheries, England and Wales.

was filled with this water which was left running in the ordinary way for about a week, care being taken to dip nothing into the tank. A few days after the tank had been cleaned the filter seemed to work imperfectly, the water in all the tanks becoming cloudy. The fish selected for this special tank was the small-mouthed black bass, a species which had "fungused" somewhat readily. Twenty-three of the best specimens of a car-load were selected and placed in the prepared tank, the remainder of the lot being placed with fish that were more or less infected. None of the fish showed signs of the disease when taken from the car. Cultures made from water taken from the tank just after it was cleaned gave a very slight growth. Water taken from the tank the fifth day after cleaning gave no growth, while other cultures attempted in the filtered water, both before and after its entrance into the tank after the fish were placed in the tank, once gave *Saprolegnia* and once failed. This would seem to indicate that the zoöspores were present in the water, though in comparatively small numbers. As the tank was so thoroughly cleaned, the only possible source of infection in this case was the water supply. Four days after the fish were placed in the tank they began to have the very slimy appearance that accompanies the first stage of the fungous attack, and an examination of this slime showed that fungous threads were present, though not yet abundant. The bass that were placed with diseased fish seemed but little more parasitized, but later both lots became badly diseased, and in a fortnight were practically all gone.

The above experiment and the cultures made from the aquarium water before its entrance into the tanks show clearly that the original source of infection was the water supply—a difficulty it would seem impossible to remedy, at least with an imperfect action of the filter.*

It has long been known that species of *Saprolegnia* are sometimes found as parasites on aquatic animals. They are closely related in their structure to certain algae, and may be regarded as degenerated forms, which, because of their saprophytic or parasitic habits, have lost their chlorophyll. We are indebted to such investigators as Cornu, Pringsheim, and De Bary for much of our knowledge of the life-histories of these forms. Concerning the fungus most frequently found as a parasite on fish, English and Scotch writers seem to have written most, a fact due perhaps to the great outbreaks of the disease among the fish in the rivers of Great Britain in the years 1877 to 1880, during which time the loss was so great as to seriously interfere with the fisheries industry. Many kinds of fish were affected, but the salmon most disastrously. About the same time certain authors note an unusual occurrence of the disease among the fish of some of the rivers of this country.

Our American botanists have done comparatively little with this class of fungi. The most elaborate work is a monograph by Humphrey, treating of American forms, and giving the general life-history and the literature of this group. Hine (1878) published a general article on this class of fungi, and Galloway (1891) has dealt with the life-history of *Saprolegnia monoica*. Gerard (1878) and Lockwood (1890) have published articles treating of the special fungus of fish called by them *S. ferax;* otherwise—if we except the articles copied in American publications from foreign sources—our literature on the subject seems to be limited to mere references to *S. ferax* as a parasite of fish in certain places.

*This filter was heavily overtaxed at the time, usually filtering more than twice the amount of water for which it was intended.

LITERATURE.

Perhaps no class of fungi has been more thoroughly studied than the *Saprolegniaceæ*, owing, no doubt, to the ease with which these forms may be observed through all their various stages of development. This has given rise to a very extended literature of the subject, and I append references to all items accessible to me in which *Saprolegnia* is mentioned as a parasite of fish. The name of the article is not usually given, but the reader is cited directly to the page on which mention of *Saprolegnia* occurs, and for convenience the character of the reference is briefly indicated.

BARON DE LA VALETTE ST. GEORGE, 1879. Circular No. 3, German Fishery Association. (Translated by Jacobsen, in Rep. U. S. Fish Comm. for 1878, p. 509.)
Among enemies of fish mentions *S. ferax* as destructive to eggs and even live fish. Recommends a continuous supply of pure cold water.

BENCKE, 1885. *Feinde der Teichwirtschaft*, in "Die Teichwirtschaft." (Translated by Jacobsen, in Bull. U. S. Fish. Comm., VI, p. 342.)
Refers to a saprolegnious disease of fish in central Europe, and says it may be cured by placing the fish in a 1 per cent. solution of salt water, or by washing them with still salter water.

BENNETT, 1842. Parasitic Fungi in Living Animals. Trans. Roy. Soc. Edinburgh, vol. XV, pt. II, pp. 284, 287; also as separate.
Gives account of "colorless confervæ" found by different authors on fish and their eggs.

BERKELEY, 1864. Intellectual Observer, p. 147.
Mentions certain *Saprolegniaceæ* as destructive to fish and their ova.

BERLESE and DE TONI, 1888. Saccardo's Sylloge Fungorum, vol. VII, pt. I, p. 270.
Mentions *S. ferax* as found on dead flies, fish, salamanders, etc.

BESSEY, 1885. Botany for High Schools and Colleges, p. 257.
Notes *S. ferax* as growing on dead and living fish, and refers to the epidemic in England in 1878.

BOTANISCHE ZEITUNG, 1868, p. 829.
A short note in which Cohn is said to have produced death of goldfish by artificial infection with *Achlya*.

BROOK, 1879. Trans. and Proc. Bot. Soc. Edinburgh, vol. XIII, p. 389.
Gives notes on salmon disease of the Esk and Eden rivers.

BUCKLAND, WALPOLE, and YOUNG, 1880. Report on disease which has recently prevailed among salmon.
Treats of *Saprolegnia*.

CENTURY DICTIONARY, 1891, vol. V, pp. 5315, 5340.
Under "salmon-disease," and under "*Saprolegnia*," mention of *S. ferax* as destructive to fish, especially salmon.

CLARK, 1874. Amer. Nat., vol. VIII, p. 363.
Mentions occurrence of *Achlya prolifera* (?) on live fish in a house aquarium, giving method of treatment, etc.

COOKE, 1880. Grevillea, vol. IX, p. 9. (Copied in Jour. Roy. Micr. Soc. 1880, p. 997.)
States that Rutherford found bacteria present in muscles of diseased salmon, which he thought might cause the disease. Cooke thinks it due to *S. ferax*.

COOPER. Micr. Jour., vol. I, p. 149.
Notes a fungous growth that killed goldfish.

DE BARY, 1888. *Botanische Zeitung*, p. 617.
Mentions *S. mixta* as found on sick fish.

FARLOW AND SEYMOUR, 1891. Host Index U. S. Fungi, p. 179.
Mentions *S. ferax* as reported in United States on several kinds of fish.

FISCHER, 1892. *Raben. Krypt. Flora (Pilze)*, Lief. L., pp. 337, 340.
Mentions occurrence of *S. monoica* and *S. thureti* on dead fish, and *S. mixta* on sick fish.

GERARD, 1879. Proc. Poughkeepsie Soc. Nat. Sci. 1878, p. 25.
Gives an account of an epidemic among fish of Passaic River, N. J., in which species affected in order of mortality were as follows: Sucker, mullet, chub, roach, sunfish, yellow perch, catfish, and a few pickerel.

GOODSIR, 1846. Trans. Bot. Soc. Edinburgh, vol. I. (Article noticed in Bot. Zeit. 1846, p. 479.)
Describes a plant developing on gills and fins of goldfish.

HARKNESS AND MOORE, 1880. Pacific Coast Fungi, p. 32.
S. ferax mentioned as common on dead flies and living salmon.

HINE, 1878. Amer. Quart. Micr. Jour., vol. I, pp. 20, 145.
In a general article on *Saprolegniaceæ* mentions forms parasitic on fish, etc.

HOFFMAN, 1867. *Botanische Zeitung*, p. 345.
In an article on the relation of a *Saprolegnia* and a *Mucor*, mentions the occurrence of the former on fish.

HUXLEY, 1882. Quart. Jour. Micr. Soc., vol. XXII, n. s., p. 311. (Abstract of article in Am. Month. Micr. Jour., III, p. 137.)
Treats of *Saprolegnia* in its relation to epidemic disease among salmon.

KNIGHT, 1885. Bull. Torrey Bot. Club, vol. XII, p. 56.
Notes that a goldfish succumbed to attack of *S. ferax*.
LAMBOTTE, 1880. *Flore Mycologique Belge*, p. 112.
In a description of *S. ferax*, says it occurs on dead fish, salamanders, etc.
LINDSTEDT, 1872. *Synop. der Saprolegniaceen*, pp. 39, 42, 46.
Mentions *Saprolegnia* as being found by old authors on dead and living fish, and gives *S. ferax* as occurring on dead fish, etc.
LOCKWOOD, 1890. Jour. N. Y. Micr. Soc., vol. VI, p. 67.
Gives an extended popular and scientific account of *S. ferax* on sunfish, etc.
MURRAY, 1885. Journal of Botany, vol. XXIII, p. 303.
Gives notes on inoculation of fish with *S. ferax* and says disease was introduced into aquarium through earthworm.
PECK, 1886. Thirty-ninth Rep. N. Y. State Mus. Nat. Hist., p. 49.
Notes *S. ferax* as found on aquarium fish and those in artificial ponds in New York.
ROBSON, 1880. Science Gossip; also Amer. Month. Micr. Jour., vol. I, p. 103.
An article on salmon disease said to be due to *S. ferax*.
RYDER, 1881. Bull. U. S. Fish Com., vol. I, p. 181.
Mentions a saprolegnious growth quite destructive to fish eggs.
———, 1882. Bull. U. S. Fish Com., vol. II, pp. 189, 190.
Gives account of action of *S. ferax* and of attempted preventive experiments
SCHENCK, 1890. *Handbuch der Botanik*, vol. IV, p. 567.
Gives *S. thureti* of De Bary as occurring on dead and living fish, their eggs, etc.
SCHNETZLER, 1888. Mag. Nat. Hist., vol I, p. 162.
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Gives scientific descriptions of *S. monoica* and *S. ferax*, and says they occur on dead insects, organic matter, living fish, etc.
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VALÉRY-MAYET, 1885. Bull. U. S. Fish Comm., vol. V, p. 272.
Extract from letter giving account of trouble with supposed *Saprolegnia* in hatching salmon eggs.
WALPOLE AND HUXLEY, 1881. Twenty-first Ann. Rep. Inspectors Fisheries England and Wales. (Reprinted in Bull. U. S. Fish Comm., I, p. 429.)
Authors give a very comprehensive account of the salmon disease of rivers of England and Wales. Mature state of fungus not being found, absolute determination of species was impossible.

To the above may be added the following references to early writers who note the appearance of this or similar fungi on various aquatic animals, as salamanders, frogs, etc.: *Carus*, Nova Acta, 1823, vol. II, p. 493; *Hannover*, Müller's Archives, 1839, p. 338, and 1842, p. 73; *Stilling*, Müller's Archives, 1841, p. 279; *Valentin*, Repertorium, vol. V, p. 44.