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Fish and Wildlife Service, Clarence F. Pautzke, Commissioner
Bureau of Sport Fisheries and Wildlife, Daniel H. Janzen, Director

Fishery Leaflet 564

Washington, D. C. 20240 December 1963

PARASITES OF FRESHWATER FISH

I. FUNGI

1. Fungi (Saprolegnia and Relatives) of Fish and Fish Eggs

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Species of the genus Saprolegnia are usually implicated in fungus diseases of fish and fish eggs, although Achlya, Aphanomyces, Leptomitus, and Pythium have also been reported (Scott and O'Bier, 1962). These fungus diseases of fish are often considered secondary invaders following injury, but once they start growing on a fish the lesions usually continue to enlarge and may cause death unless medication is provided. Fungi often attack dead fish eggs and soon encompass adjacent live eggs, which are attacked and killed, thus constituting one of the most important egg diseases. These fungi grow on many types of decaying organic matter and are widespread in nature.

MORPHOLOGY, IDENTIFICATION, AND LIFE CYCLE

The presence of fungus on fish or fish eggs is indicated by a white, cottony growth which consists of a mass (mycelium) of nonseptate filaments (hyphae) each of which is about 20 microns in diameter. Under low magnification the filaments of older infections may be seen to terminate in clublike enlargements which contain the flagellated zoospores (fig. 1). These eventually escape and are responsible for infection of other fish or eggs.

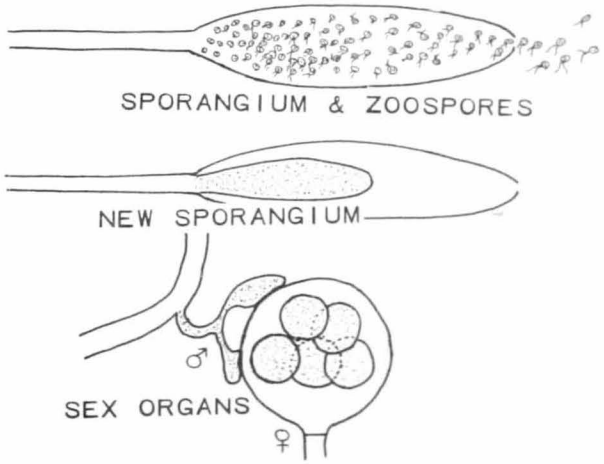

Figure 1.--Saprolegnia
TOP - Sporangium with many flagellated zoospores
MIDDLE - An old sporangium with a new one proliferating into it, a characteristic of the genus
BOTTOM - Sex organs, antheridium on left, oogonium on right. These are usually found only on special media.

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Fish eggs.--During incubation, some eggs die and may soon be invaded by fungus. In time, surrounding eggs are covered by the mycelium, and death is the result. Unless control measures are exercised, the ever-expanding growth will claim virtually every egg. Kanouse (1932) found circumstantial evidence that under some conditions (probably crowding) fungus filaments could penetrate living eggs. Under experimental conditions, living eggs (which were in no way crowded) were not invaded. Davis (1953) states that there is no evidence that Saprolegnia can develop on normal eggs unless foreign organic matter is present.

Fish.--Injuries produced by spawning activity and other trauma, or lesions caused by other infections, are often attacked (Hoffman, 1949; Vishniac and Nigrelli, 1957; Scott and O'Bier, 1962). Holding warm-water fish in cold water during summer and debility caused by other factors probably render fish susceptible to fungus attack.

These fungi belong to the class Phycomycetes and are typified by the nonseptate mycelium. Phycomycetes belonging to the Saprolegnia family (Saprolegniaceae) are characterized by having club-shaped sporangia containing zoospores (fig. 1) and round oogonia (sexual reproductive structures). The latter can be obtained only by culturing on special material (Hoffman, 1949; Scott and O'Bier, 1962; Scott, Powell, and Seymour, 1963). Species identification can be made only after studying these structures. The other genera mentioned above can be likewise identified, but Scott and O'Bier (1962) should be consulted.

Depending upon the temperature, 24 to 48 hours are required to complete a minimum life cycle of reproductive spore to mycelium to reproductive spore.

TRANSMISSION AND PATHOGENICITY

These fungi grow on many types of decomposing organic matter, and the resulting asexual reproductive spores are almost universally present in natural waters.

Susceptibility of dead eggs and fish is generally universal. Under favorable conditio healthy eggs resist fungus invasion, and at tim dead eggs do not succumb to penetration for many days after turning white. Infertile eggs times can be incubated a month or longer in the presence of Saprolegnia without being invaded.

GEOGRAPHICAL RANGE

Saprolegniaceae are almost universally present in natural fresh waters.

There are no known seasonal restrictions to infestation of fish eggs by Saprolegnia but fish are more likely to be affected in early spring (temperate zone) and following spawnin activity.

CONTROL AND TREATMENT

Good sanitation and cleanliness are absolutely essential to effective control of dise and/or parasites among the more or less cro unnatural conditions necessary for efficiency in intensive culture. This applies with great emphasis to incubation and hatching of fish egg and the rearing of fish. Although, as mentior above, the exact classification of fungus on infected eggs or fish is a task for the trained student, it is not necessary for effective contr of the condition.

EGGS

There are two methods of control of fungus: one is mechanical, the other is chemic Mechanical control is effected by removing dead and infected eggs two or three times a week. This is a time-consuming procedure, and some healthy eggs are usually injured in th process. Chemical control is effected with zinc-free malachite-green oxalate, a fungicida analine dye. The chemical method is simpler, cheaper, and certainly more efficient. Early reports of daily flush treatments claimed effective control by adding stock solutions of the dye at heads and at midpoints of the troughs. Based upon average trough capacity, final dilution of the stock solution resulted in from abou one-fourth to one-third of 1 ppm. Burrows

)49) recommends an hour-long treatment with ppm dye given twice weekly. He stated that constant-flow siphon was valuable in maining the desired concentration during the ur period. Cummins (1954) used a flush atment for pikeperch eggs. Johnson et al. 955) effectively used the same concentration d duration in a large-scale mechanized eration. Some hatcheries have found the ice-weekly treatment inadequate, and though perimental results are not available, emical evidence indicates that daily flush-type eatment at about 5 ppm can usually be given thout harm to the eggs.

Small-scale trials should be conducted to termine how often treatment is necessary and ether recommended concentrations prove xic to the eggs. Preparation of stock solutions recommended to minimize staining the person d clothing by the light dye powder. Burrows 949) points out that beyond 1 part of dye in 0 parts of water the dye will recrystalize and curacy will be lost. Hublou (1959) described plexiglass constant-flow siphon for treating gs with malachite green.

Formalin at 1:500 to 1:1,000 for 15 inutes has also been used with good results urrows, 1949; Reddecliff, 1958, 1961; Steffens, 62).

Copper sulfate 1:200,000 for 1 hour has so been used for fungus control.

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here are three methods of applying malachite reen oxalate for treatment and prophylaxis:

1. Dipping fish.-- Immerse fish in 15,000 malachite green for 10 to 30 seconds Foster and Woodbury, 1936; O'Donnell, 1941)

2. One-hour treatment.--Add aqueous olution of chemical to aquarium or pond, allow o remain 1 hour and replace with fresh water. he concentrations recommended are as follows: or trout and salmon 1 to 3.3 ppm (Fish, 1938; ucker and Whipple, 1951; Johnson et al., 1955) ut these concentrations sometimes kill fish, rticularly sick ones in clean aquariums. Hublou (1958) found 7.5 ppm to be lethal to small fish. Continuous-flow methods for salmonids as described by Burrows (1949) and Johnson et al. (1955) could be adapted to any facilities requiring large amounts of running water. Clemens and Sneed (1958) found 0.3 ppm not to be toxic to fingerling catfish for 1 hour but recommended a 1 ppm flush. Jackson 2/ (1962) found 2 ppm at 54° for 1 hour safe for bluegills.

3. Prolonged treatment.--Unfortunately prolonged treatment has not been worked out. As an aid to further testing, however, the following concentrations have been found nontoxic to fish; the effect on external fungi has not been reported: Foster (1936, one third of 1 ppm; Hublou (1958), 1.25 ppm nontoxic to trout in dirt ponds; Amlacher (1961a) and Steffens et al. (1962), 0.15 to 0.2 ppm nontoxic to trout; Clemens and Sneed (1958), 0.1 ppm nontoxic to catfish: Amlacher (1961a), 0.9 ppm nontoxic to carp.

CAUTION: Malachite green is toxic to mammals and fish if used too often, but there is no danger if it is used carefully and only occasionally. It is not stored in fish tissue, but fish that have been treated often should not be eaten (Steffens et al., 1962). Because zinc is very toxic to fish, one should request zinc-free malachite-green oxalate. It can be purchased from many chemical supply houses; the price is about $10 a pound, probably less for large lots.

Other chemicals should be tested. Patterson (1950) used acriflavine, 0.75 mg. per gallon, to control Oodinium and fungus on aquarium fish. Amlacher (1961b) recommends potassium permanganate 1:10,000 for 5 to 10 minutes. Fungistatic antibiotics such as griseofulvin should be tested.

2/ Jackson, H. 1962. Personal communication Fish Control Lab., La Crosse, Wis.

ANNOTATED BIBLIOGRAPHY

(References indicated by an asterisk are of special interest to fish culturists. The most nearly complete bibliography on malachite green can be found in Steffens et al., 1962)

Amlacher, E.

1961a. Die Wirkung des Malachitgrüns auf Fische, Fischparasiten (Ichthyophthirius, Trichodina), Kleinkrebse und Wasserpflanzen. Deutsche Fischerei Zeitung, vol. 8, No. 1, pp. 12-15.

Malachite green at 0.15 ppm for 6 days killed Ichthyophthirius. Trout tolerate 0.2 ppm and carp 0.9 ppm. The effect on daphnia and plants is discussed.

*1961b. Tachenbuch der Fischkrankheiten. (Pocketbook of fish diseases) Gustav Fischer Verlag, Jena, 286 pp.

Pp. 39, 149. Discusses treatment of fungus and other diseases.

Arasaki, S.K., K. Nozawa, and M. Mizaki.

1958. On the pathogenicity of water mold. II. (in Japanese, English summary). Bulletin Japanese Society Fish. Vol. 23, pp. 593-598.

The susceptibility of various strains to malachite green was tested in culture. As little as 0.02 ppm restrained the growth of hyphae and sporangia.

Burrows, Roger E.

*1949. Prophylactic treatment for control of fungus (Saprolegnia parasitica) on salmon eggs. The Progressive Fish-Culturist, vol. 11, No. 2, pp. 97-103, illus.

Eggs were treated with malachite green, formalin, Roccal, and Hyamine. Malachite green was very effective and had the greatest margin of safety in use. The constant-flow siphon is illustrated and described. Treatment technique is described in detail.

Clemens, H.P., and K.E. Sneed.

*1958. The chemical control of some diseases and parasites of channel catfish. The Progressive Fish-Culturist, vol. 20, No. 1, pp. 8-15.

Six chemicals were tested against vario organisms and lethal as well as nonletha concentrations determined. Malachite green at 0.4 ppm for 1 hour and at 0.10 p for 96 hours was not harmful to the catfi but at 0.79 and 0.14 ppm was.

*1959. Lethal doses of several commer chemicals for fingerling channe catfish. USFWS, Special Scien Report--Fisheries, No. 316, 1

Lethal and non-lethal concentrations of chemicals including malachite green we determined. Nonlethal concentrations o formalin were 316 ppm for 1 hour and 50 ppm for 96 hours; of potassium permang nate was 9.1 ppm for 1 hour.

Cummins, R., Jr.

1954. Malachite-green oxalate used t control fungus on yellow pikepe eggs in jar hatchery operations The Progressive Fish-Culturist vol. 16, No. 2, pp. 79-82.

Six grams of malachite green were adde each battery of jars (50 gal. per min. fl and repeated three times. It appeared t rid the eggs of all fungus.

Davis, H.S.

*1953. Culture and diseases of game fi University of California Press, Berkeley and Los Angeles, 332 illus.

Pp. 275-281. There is an excellent sect on fungus disease of fish. Egg infestatio is also discussed in detail. Prophylactic treatments for control of fungus on eggs given, and the information is up to date. (The author inadvertently ommitted the description of the constant-flow siphon, but the interested reader will find it given by Burrows, 1941, listed in this bibliography.)

Fish, F. F.

*1938. Simplified methods for the prolonged treatment of fish disease Transactions American Fisheri Society, vol. 68, pp. 178-187.

Fish found malachite green 3.3 ppm for 60 minutes and 1.25 ppm for 90 minutes adequate for treating salmon fingerlings. Also discusses potassium permanganate, copper sulfate, formalin, salt and boric acid.

ster, Fred. J., and L. Woodbury.

*1936. The use of malachite green as a fish fungicide and antiseptic. The Progressive Fish-Culturist, (U.S. Bureau of Fisheries, Memo I-131) No. 18, pp. 7-9.

This is the first account of use of malachite green in the treatment of fungus infestation of fish. The authors were highly successful in eliminating infestations in fish. Egg treatment was also successfully tried. Concentration and other details are given. Recommended concentration is about one-third of 1 ppm.

ublou, W.F.

*1958. The use of malachite green to control Trichodina. The Progressive Fish-Culturist, vol. 20, No. 3, pp. 129-132.

Malachite green at 1.25 to 5 ppm for 30 minutes controlled Trichodina but 7.5 ppm for 1 hour killed trout fingerlings. The chemical was applied to dirt ponds and allowed to dissipate.

*1959. A plexiglass constant-flow siphon. The Progressive Fish-Culturist, vol. 21, No. 1, pp. 47-48.

The apparatus described was designed to deliver 30 ml. per minute of 1:200 malachite green to the hatchery trough.

hnson, Harlan E., C.D. Adams, and R.J. IcElrath.

*1955. A new method of treating salmon eggs and fry with malachite green. The Progressive Fish-Culturist, vol. 17, No. 2, pp. 76-78, illus.

The article describes and illustrates construction details of pump-driven system of injecting metered volumes of malachite-green stock solution into a hatching-house water system. The device maintained a 5-ppm solution for hour-long periods, fungus was effectively inhibited by semi-weekly treatments, and labor was reduced to one-fifth of premechanized requirements.

Kanouse, Bessie B.

1932. A physiological and morphological study of Saprolegnia parasitica. Mycologia, vol. 24, No. 5, pp. 431-452, illus.

The study of Saprolegnia is briefly reviewed. The organism was cultured on a variety of media, and sexual organs were developed. This is the first demonstration of sexual organs in this organism. Complete morphology is described and illustrated. Observations were made and experiments conducted on parasitism by this fungus. Recommendation for control of Saprolegnia in the hatchery are given: mechanical removal and disposal of infected materials constitute effective control measures.

O'Donnell, D.J.

*1941. A new method of combating fungus infections. The Progressive Fish-Culturist, vol. 56, pp. 18-20.

The dip method of 1:15,000 for 10 to 30 seconds is described.

*1947. The disinfection maintenance of trout hatcheries for the control of disease, with special reference to furunculosis, Transactions American Fisheries Society, vol. 74, pp. 26-34.

Page 34 is devoted to "Treating Eggs for Fungus". Directions are given for using both copper sulphate and malachite green. Malachite green concentration is very light--amounting to about one-quarter of 1 ppm.

Patterson, E.E.

1950. Aquarium Journal, vol. 21, No. 2, p. 36.

Used acriflavine, 0.46 grain to 40 gallons, for control of Oodinium and fungus.

Anon.

1955. Malachite green used to prevent fungus on lake trout eggs. The Progressive Fish-Culturist, vol. 16, No. 1, p. 38, illus.

Malachite green was found to be very effective to inhibit fungus infestation of incubating lake trout eggs. Methods of preparation and application of a stock solution are given. The concentration used was about 10 ppm.

Reddecliff, J.M.

1958, 1961. Formalin as a fungicide in the jar method of egg incubation. Notes for fish culturists from the Benner Spring Fish Research Station, Pennsylvania Fish Commission.

Rucker, R.R., and W.J. Whipple.

*1951. Effect of bactericides on steelhead trout fry. The Progressive Fish-Culturist, vol. 13, No. 1, pp. 43-44.

Malachite green at 5 ppm for 30 minutes was not toxic to steelhead trout fry.

Scott, W.W., and A.H. O'Bier.

1962. Aquatic fungi associated with diseased fish and fish eggs. The Progressive Fish-Culturist, vol. 24, No. 1, pp. 3-15.

Sixty-four samples of fungi from 14 States were collected and studied. Five species of Saprolegnia, 2 of Achlya, 2 of Aphanomyces, 2 of Pythium, and one of Leptomitus were found. Some of these would infect wounded fish, others not.

Scott, W.W., J.R. Powell, and R.L. Seymou

1963. Pure culture techniques applied the growth of Saprolegnia species on a chemically defined medium. Virginia Journal of Science, vol. No. 2, pp. 42-46.

Eight species were established in pure culture on a chemically defined medium and produced antheridia, oogonia, and mature oospores.

Steffens, W.

1962. Verhütung des Saprolegnia-Befal von Forelleneiern durch Formale Deutsche Fishcherei Zeitung, Ban 9, pp. 287-289.

Described a method of treating trout eggs with formalin 1:600 for 15 minutes.

Steffens, W., U. Kieder, D.Nahring, and H. Hattop.

1962. Moglichkeiten und Gefahren der Anwendung von Malachitgrun in de Fischerei. (Advantages and dange of the use of malachite green in fi culture). Zeitschrift fur Fischer 10, pp. 745-771, (in German).

This is a worldwide review and also a report on the authors' own research. The chemistry, toxicity, use and dangers of malachite green are discussed. There is an excellent bibliography.

Vishniac, H.S., and R.F. Nigrelli.

1957. The ability of Saprolegniaceae to parasitize platyfish. Zoologica, vol. 42, part 4, pp. 131-134.

The authors infected platyfish having a standardized wound with 16 species belon ing to 7 genera. Tissue destruction was reported to be due almost exclusively to the fungus.