ABSTRACT

AN EVALUATION OF THE SUSCEPTIBILITY OF COHO SALMON, GREEN SUNFISH, AND STEELHEAD TROUT TO PARASITISM BY THE AQUATIC FUNGUS, SAPROLEGNIA PARASITICA

By

Charles William Riley

In recent years the increasing incidence of saprolegniosis in Michigan's wild brown and steelhead trout populations has been reason for concern. The saprolegniacious fungus most commonly reported as a fish parasite is Saprolegnia parasitica. Researchers have shown experimentally that this fungus can act as a lethal primary parasite of healthy fish. There is a great similarity between the fungus disease which occurs in Michigan and a disease known as Ulcerative Dermal Necrosis which has caused widespread mortality in the salmon and trout populations of Great Britain. Various types of unrelated research has provided evidence to confirm that certain environmental stresses serve to increase the susceptibility of fish to these fungus diseases.

The presence of hard pesticides and certain polychlorinated biphenyl's in the aquatic environment has been reported by some observers as rendering fish more susceptible to parasitism by aquatic fungi. The primary objective of this research project was to determine whether the chronic exposure to the pesticide dieldrin would increase the susceptibility of selected fish species to parasitism by S. parasitica. The ancillary objective of this study was to document the pathogenesis of saprolegniosis in fish.

In order to meet the objectives of this study seven tests were conducted, three employing coho salmon, two employing green sunfish,

and two employing steelhead trout. In all tests selected numbers of test fish were exposed to viable forms of S. parasitica. In two tests dieldrin was used as a stress agent. Observations were made to determine if the added stress would effect the susceptibility of the fish to fungal parasitism. Tissue sections were made from fish which contracted saprolegniosis during the study and these were analyzed to determine the development of the disease.

The chronic exposure to dieldrin was not found to significantly enhance the susceptibility of young coho salmon or green sunfish to saprolegniosis under the conditions of this study. However, a small number of infections occurred in apparently healthy fish in tanks containing the pesticide. All other infections were contracted by previously injured fish at the site of injury. Therefore, in the cases of the uninjured fish the dieldrin may have influenced the development of saprolegniosis. A study of the tissue sections made from infected fish showed that the fungus infection normally originates at the site of a superficial injury and spreads into the deep muscular and vascular tissues. The hyphae eventually reach the brain and the disease terminates in the death of the fish. It is quite possible that the undetermined death mechanism of Saprolegnia parasitica is due to the invasion of tissues in the vascular and central nervous system of the parasitized fish.

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INTRODUCTION

Basis for Study

During the last six years the increasing incidence of fungus infections in wild populations of certain salmonid fishes found in Michigan has generated much concern among sportsmen and fishery biologists. It has become apparent that numerous salmon and trout in several Lake Michigan tributary streams are infected with the parasitic aquatic fungus, Saprolegnia parasitica, Coker, 1923. Of all the saprolegniacious fungi known to attack fish, Saprolegnia parasitica is the one most frequently reported as a fish parasite (Stuart and Fuller, 1968).

Members of the genus Saprolegnia are found in nearly all bodies of fresh water in the world. These fungi serve primarily as decomposers of dead organic matter in the aquatic ecosystem. In Michigan, however, S. parasitica is commonly found as a parasite of introduced Pacific salmon (genus Oncorhynchus) which have reached sexual maturity. These fish at the time of spawning are in a state of physical degeneration and are often injured during their migration. These facts partially explain the high incidence of fungus infection among sexually mature members of this particular genus.

At present there appears to be an increasing incidence of saprolegniosis among sexually mature steelhead trout (Salmo gairdneri irideus Gibbons) and brown trout (Salmo trutta fario Linnaeus), which

otherwise appear to be in good health. Little has been done to monitor this disease but it is believed by some to cause significant mortality in Michigan trout populations each year. Researchers in Europe and Japan have shown experimentally that S. parasitica can act as a lethal primary parasite of healthy fish (Stuart and Fuller, 1968).

In Great Britain a disease known as Ulcerative Dermal Necrosis (UDN) has caused widespread mortality in salmon (Salmo salar L.) and native trout populations since it was first recorded in the autumn of 1964 (Willoughby, 1969). There has been and still is much disagreement among observers as to the identity of the etiological agent. Saprolegnia sp. alone has been isolated consistently from the lesions of diseased fish (Stuart and Fuller, 1968). The presence of Saprolegnia sp. is a clear feature of the disease in its advanced stages, when it is firmly established on one or more of the fin bases or in the head region (Willoughby, 1969). Observers in England contend, however, that the fungus is usually an integral part of the lesions from the very outset. Roberts (1972) states, "It would seem that in Ireland, Scotland, and England the same species of aquatic fungus is implicated in UDN and all mycologists who have been involved in this study have stressed that S. parasitica would appear to be intimately involved with the condition and that if it is not in fact the primary etiological agent, it must be considered because of its consistent and specific relationship with the lesions, as much more than a mere opportunist." The factors which initiate UDN may be uncertain but the presence of active Saprolegnia fungus is inevitably lethal (Willoughby, 1969).

To date, very little is known about the factors which contribute to the development of fungus diseases in fish. However, out of research

not specifically designed to study fungus diseases has come the evidence that certain environmental stresses serve to increase the susceptibility of fish to these infections. In writing about UDN, Willoughby (1969) contends that the factors which influence a fish's susceptibility to the disease include species, age, and breeding conditions of the fish, its possible pre-disposition to the disease by chemical or physical factors of the external environment, its immunological barriers, and its exposure to potentially pathogenic organisms, be they algae, bacteria, fungi or viruses.

The presence of certain hard pesticides in the aquatic environment has been suspected of exerting physiological stresses upon certain fish species (Cope, 1965). In working with pesticides and salmonids, Schoenthal (1963), found that these fish were presumably rendered susceptible to fungus diseases by the presence of pesticides in their bodies. Cain (in Johnson, 1968) speculated that pesticides may affect the ability of fish to produce antibodies or interfere in some other way with their natural internal-defense mechanisms. Other reported tests by investigators suggest that conditions may be set up in which the anti-fungal action of the fish's slime covering becomes non-functional or is overwhelmed resulting in fungus development from numerous centers over the whole surface of the fish (Willoughby, 1969). An example of this phenomenon may be Hansen's (1971) report of the development of fungus-like lesions on the body of pinfish (Lagodon rhomboides) after chronic exposure to 5 ppb of the PCB, Aroclor 1254.

Description and Life Cycle of Saprolegnia parasitica

Saprolegnia parasitica is a member of a group of fungi commonly designated as water molds, in the order Saprolegniales, class Oomycetes.

Most species occur abundantly in bodies of freshwater throughout the world. The majority are saprobic, a few, however, can be parasitic.

Coker (1923) originally proposed the name Saprolegnia parasitica for non-fruiting strains (those strains not producing sexual reproductive bodies) of Saprolegnia associated with fish and fish eggs. Seymour (1970) contends that the use of asexual characters and parasitic habit as diagnostic features have made this taxon a convenient repository for all non-fruiting forms of Saprolegnia. Given suitable conditions and sufficient time certain strains of this fungus have been reported to form sexual organs (Stuart and Fuller, 1968). For the positive identification of this species it is now considered necessary to observe the characteristics of these structures.

To meet the needs of this study the following somewhat generalized description of the organism, Saprolegnia parasitica, has been assembled from the works of Seymour (1970) and Alexopoulos (1962). The somatic portion of the thallus is composed of two types of hyphae; the rhizoidal hyphae, which enter the substratum anchoring the organism and absorbing nourishment and the profusely branched hyphae which forms the visible colony of the organism on which the reproductive organs are formed. The mycelium is a variably branched system of tubular, tapering, multinucleated indeterminate hyphae, which, except for reproductive structures, is non-septate (Alexopoulos, 1962). The hyphal walls are composed largely of cellulose.

Under the proper environmental conditions the hyphae give rise to asexual reproductive structures known as sporangia. The sporangia are abundant and typically straight, cylindrical, tapering, structures borne at the tips of somatic hyphae and separated by a septum. They are

densely filled with protoplasm which differentiates at maturity to form zoospores. Renewal of sporangia in this species is commonly by internal proliferation or in basipetalous succession (Seymour, 1970).

Characteristically, the primary zoospores emerge one by one in rapid succession through one exit pore in the tip of the sporangium (saprolegnoid discharge). The primary zoospore has a pyriform body bearing two subapical flagella of nearly equal length. After a period of motility they encyst. The period of encystment may last from a few minutes to several hours at the end of which the primary cyst may germinate directly into new hypha or develop into a reniform, biflagellate secondary zoospore. A short swarming period is followed by encystment and the germination of a germ tube which develops into a hypha and initiates a new colony.

When conditions are favorable for sexual reproduction, the somatic hyphae give rise to oogonia and antheridia. In this species oogonia are sparse and formed only after a prolonged period of time. They usually are formed terminally but may be lateral or intercalary. Their shape may be clavate, irregular (54-146 X 18-72 u) or pyriform (62-75 u in dia.) (Seymour, 1970). The oogonial wall is unpitted, thin and smooth. The oospores are subcentric, spherical, and fill the oogonium. They range from 14 to 23 in number and measure between 18 and 24 u in diameter. The antheridial branches are usually diclinous, simple, delicate and often wrap about the oogonium and its hypha. The antheridial cells are tubular or clavate, simple, occasionally branched and laterally appressed. After fertilization and a prolonged rest period, the oospores are liberated and germinate each producing a germ tube. A sporangium generally develops at the tip of this hypha completing the life cycle (Alexopoulos, 1962).

Under certain conditions resistant bodies known as gemmae or chlamydospores are formed. The gemmae of S. parasitica are abundant, have a clavate, pyriform, or irregular shape, and may be terminal or intercalary. These gemmae function as oogonia or zoosporangia.

The Histological Evolution of Saprolegniosis in Fish

The evoluation of saprolegniosis in fish is not well documented at present. In a work by Nolard-tintigner (1973), however, the evolution of an experimentally induced infection involving Saprolegnia ferax in guppys (Lebistes reticulatus) is described. In the experiment the left flank of each guppy was scarified and the fish were then placed in aquaria containing hemp seed cultures of the fungus. The observations were made during the 25 hour period immediately after the fish were placed in the aquaria. The following is a translation from the original French publication:

> Shortly after the fish were placed in the tanks zoospores became affixed in the area of scarification and encysted. Four hours after the beginning of the experiment, several germ tubes put out by the spores had penetrated the skin and one observed the beginning of penetration of bundles of muscular fibers.
>
> Six hours after scarification mycelial filaments began to surround the muscles. They occupied about half of the thickness of the left flank. The muscular fibers showed an invasion of important zones and of necrosis which give a porous aspect. The fish are entirely normal yet.
>
> The filaments continue their development and after 10 hours, they have almost completely destroyed the dermis. They occupied almost all the inoculated side.
>
> At the end of 12 hours, one observed the first filaments in the spinal marrow.
>
> Between 14 to 16 hours is the appearance of zones of necrosis in the marrow. The blood vessels are also entered, the filaments appearing in the caudal and the aorta vein. From the point of view of their behavior, it is at this moment that the fish begin to present signs of paralysis and hold themselves on one or the other side.
>
> At the end of 18, 20, 22, and 24 hours, the invasion of the various organs is more accentuated; the fungus continues its

> progression at the same rythm. In 18 hours it has spread over most of the inoculated left flank, half of the right flank, and at 22 hours, it was completely spread over the two flanks. The spinal marrow was completely destroyed for several millimeters. The arteries and the veins were blocked by the filaments which stopped the normal flow of blood. The fish were on their sides dying on the bottom of the aquaria. (Nolard-tintigner, 1973).

In her studies, Nolard-tintigner (1973) never observed the germination of spores on the ectoderm of the fish. In reference to the ectoderm she said, "Apparently the spores do not fasten themselves and only germinate on the dermis or on the muscles."

Objectives of the Study

The primary objective of this research project is to determine if the chronic exposure to the hard pesticide dieldrin (HEOD) increases the susceptibility of selected fish species to parasitism by the aquatic fungus Saprolegnia parasitica. The ancillary objective of the study is to document the pathogenesis of saprolegniosis in fish.

GENERAL METHODS AND MATERIALS

Coho salmon (Oncorhynchus kisutch Walbaum) were obtained as fertilized eggs from the egg taking station at the Platte River State Fish Hatchery near Honor, Michigan in October of 1970. Steelhead trout (Salmo gairdneri irideus Gibbons) were also obtained as fertilized eggs in April of 1971 from the same location on the Platte River. After hatching, both the salmon and the trout were reared in tanks located at Michigan State University.

Green sunfish (Lepomis cyanellus Rafinesque) were captured by seining in a flooded gravel pit near the Michigan State University campus. These fish were held in tanks at the university prior to use.

The three species of test fish were maintained on Ewos salmon diet, a dry pelleted food. The fish holding tanks were continuously supplied with dechlorinated Michigan State University tap water. The water was dechlorinated by an activated charcoal filter. Cooling and aeration of the water was accomplished by a Min-O-Cool refrigeration unit located in the 100 gallon head tank which fed the holding tanks and the test tank assembly.

Fungus infected tissue was excised from three living sexually mature coho salmon which were netted at the Platte River State Fish Hatchery. The tissues were transported in sterile containers to Michigan State University where the fungus was isolated and identified as Saprolegnia parasitica. Bacteria-free fungus cultures were established on halved sterile MP5 medium and hemp seeds (Alexopoulos and

Beneke, 1962). From these bacteria-free cultures three cultures of the fungus were established on halved sterile hemp seeds. These hemp seed cultures were placed in small, loosely corked, glass jars half-full of sterile double-distilled water and kept under refrigeration. One additional Saprolegnia parasitica culture was obtained from Dr. A. L. Rogers who isolated the original specimen from a salamander (Pseudolriton sp.). The agar plate cultures and the inoculums employed in the susceptibility tests all originated from these four cultures.

The fungus susceptibility tests were conducted by employing an assembly of from six to eight glass test tanks. Each test tank was constructed by removing the bottom from a twenty liter, round, glass bottle. Eight of these bottomless bottles were placed neck down through separate holes cut in a table top. A rubber stopper, with a glass standpipe inserted, was placed in the neck of each bottle to maintain the selected volume of water and serve as a drain. A larger diameter glass tube was placed over each standpipe to enable each chamber to drain from the bottom.

During continuous flow tests each test tank was supplied continuously with dechlorinated tap water by a serial diluter (Chadwick and Brocksen, 1969). The approximate flow delivered to each test container was 150 ml/min. The volume of each test tank was held at 15 liters during all subsequent tests. The 90 percent replacement time for the water in each test chamber was approximately 3.5 hours (Sprague, 1969). The lowest flow rate of water per gram of test fish used in any one tank was 6.2 liters/gram/day.

In two of the susceptibility tests dieldrin was introduced into the serial diluter from a column of inert material coated with dieldrin (Chadwick and Brocksen, 1969). The pesticide column was constructed in

accordance with the procedure cited by Chadwick and Kugemagi (1968). The column delivered a consistent concentration of dieldrin which was diluted and distributed to selected test tanks in the following mean concentrations: .177, .674, and 2.71 parts per billion (ppb).

One liter grab samples were taken from each test tank at the beginning and end of each test in which dieldrin was employed to determine the mean test concentrations. These water samples were analyzed by gas-liquid chromatography using a Micro-Tek 220 gas chromatograph. The chromatograph was equipped with a ^{63}Ni electron-capture detector and a 1/4 inch by 6 foot glass column packed with 3 percent SE-30 on 60-80 mesh Gas Chrom-Q. The water samples were prepared for analysis by three partition extraction using 100, 50, and 50 ml of redistilled hexane. After drying with anhydrous sodium sulfate, measured volumes of the extracts were injected into the gas chromatograph. Inlet, oven, and detector temperatures were held at 205, 175, and 285°C, respectively.

Weekly grab samples were collected from each test unit and analyzed for dissolved oxygen, total alkalinity, hardness, and residual chlorine. The analyses of these parameters were conducted in accordance with the 13th edition of Standard Methods (American Public Health Assoc. et al., 1971). Temperature and pH were also recorded on a seven day basis. The environmental conditions remained essentially unchanged in all test chambers during most of the experimentation. In all cases these conditions were more than adequate to sustain the particular species of fish being tested. The mean values and the related ranges of each selected chemical and physical parameter are shown in Table 1. Any variance in a specific parameter is noted in the methods of the test in which it occurred. Fifty milliliters of water were taken from each

Table 1. Ranges and means of chemical and physical characteristics of the water used in all test tanks during experimentation.

Parameter	Mean (mg/l)	Range (mg/l)
Dissolved Oxygen	7.6	8.6-6.8
Hardness as $CaCO_3$	334	328-335
Total Alkalinity as $CaCO_3$	319	314-320
Residual Chlorine	< 0.001	---
Temperature ^{o}C	14.2	13.5-15
pH as Standard Units	7.9	7.8-8.0

test tank every five days, placed in a petri fish, and baited with two sterile hemp seed halves. Within two to three days fungal mycelium was seen growing on the cut side of each seed if viable fungal zoospores were present in a particular test tank.

In five tests Saprolegnia parasitica was grown on agar plates and suspended in net bags from the top of selected test chambers. These plate cultures were prepared by placing plugs of MP5 agar medium containing living bacteria-free fungal mycelium on plates of YPSs agar medium (Stevens, 1974). The plates were covered and incubated at room temperature for 48 hours before being placed in the designated test tanks. During the tests each agar plate culture was replaced with a fresh 48 hour old culture every five days.

In five tests a number of the test fish were injured by removing the scales from a small area of skin. All fish used in these tests were anesthetized with MS-222 and the selected animals were injured by scraping the scales from a 1/4 inch square area of skin on the right side of the body just below the dorsal fin. The scale removal was accomplished with a sterile surgical scalpel.

TEST SERIES A - COHO SALMON

Test A_1

Methods and Materials

The purpose of this test was to ascertain whether coho salmon fingerlings would become parasitized by Saprolegnia parasitica when living cultures of the fungus were placed in their tanks. Ten young salmon with a mean weight of 4.1 grams and a mean length of 7.1 centimeters were placed in each of the eight test tanks. One half of the test fish were injured by scale removal to determine if such an injury would enhance the susceptibility of the fish to fungus infection.

The test fish were distributed randomly on the following basis: ten injured fish were placed in tanks 3, 4, and 7; tanks 2 and 6 each received five injured and five uninjured salmon; and tanks 1, 5, and 8 each received ten uninjured fish. Forty-eight hours later agar plate cultures of Saprolegnia parasitica (salmon origin) were placed in tanks 1, 2, 3, 5, 6, and 7. Tanks 4 and 8 contained no fungus and served as controls.

Periodic water samples were collected and analyzed throughout the 23 day test period to monitor the chemical and physical conditions in each test chamber. Samples of water were also collected regularly to confirm the viability of the fungus in the tank by use of hemp seeds as bait in petri dishes.

Results

All water samples which were baited with halved sterile hemp seed were found to contain viable fungal zoospores except those samples collected from tanks 4 and 8.

Three days after the fungus cultures were placed in the test tanks three injured fish in tank 7 had visible signs of fungus growth at the site of scale removal. The following day two injured fish in tank 2 also had evidence of fungus growth in the area where the skin was exposed and reddened. Each of the infected fish died approximately 24 hours after the mycelium on the skins surface became apparent to the naked eye.

The fungus first appeared as a small hair-like patch covering the area from which the scales had been removed. Within 24 hours a ring of fungal mycelium had completely girdled the infected fish. In several cases nearly 25 percent of the body surface was covered by the hair-like mycelium. As the fish became girdled with fungus they began to lose their equilibrium and floated helplessly to the surface of the water. There was a definite loss of mobility and control of that portion of the body posterior to the most forward growth of the fungus.

Discussion

In this test only five fish became parasitized by Saprolegnia parasitica out of the sixty which were exposed to the fungus. This was certainly not a statistically significant number of infections. All five infected salmon had been previously injured and the infections originated at the site of injury in each case. This would tend to

indicate that injury, in this case the removal of scales from the skin of the fish, does enhance a fish's susceptibility to parasitism by Saprolegnia parasitica.

This test revealed that Saprolegnia parasitica will parasitize and kill slightly injured, but otherwise healthy, coho salmon fingerlings. This fact is somewhat contradictory to the common belief that this particular fungus is purely a superficial invader of wounds previously infected by bacteria. In this test none of the injured salmon exhibited any evidence of bacterial infections. By the termination of the test, scale cover had been reestablished on the scraped areas of the 25 surviving test animals which had been injured.

The extraordinarily rapid rate at which the fungus covered the body surface of the fish was somewhat similar to the rate at which it covered the surface of the YPSs agar plate. This particular growth medium is formulated to enhance the growth of fungi. The low temperature of the environment (13.5°C) seem to have little effect upon the growth rate of the fungus on the fish.

Test A_2

Methods and Materials

The purpose of this test was to ascertain whether coho salmon fingerlings would become parasitized by Saprolegnia parasitica when a specially prepared fungal homoginate was added to their tanks. The homogenate was prepared by placing three plugs taken from a bacteria-free Saprolegnia parasitica (salmon origin) culture grown on MP5 agar medium, into each of six 250 ml flasks. Each flask contained 100 mls of liquid YPSs medium. Each flask was stoppered with a cotton plug and placed on

a horizontal shaker table. After incubation on the shaker at 24°C for 72 hours the liquid was decanted from each flask. The cultures were rinsed with sterile double-distilled water. Each culture was then placed in a Waring blender with 100 mls of sterile, double-distilled water and homogenized for 60 seconds. Later the volume of each homogenized culture was brought up to 300 mls by the addition of sterile double-distilled water. Each fungal homogenate was then divided into six 50 ml inocula. The inocula were refrigerated and later used to introduce viable pieces of fungal mycelium and encysted zoospores into the designated test tanks.

Ten salmon fingerlings with a mean weight of 7.9 grams and a mean length of 9.4 centimeters were placed in each of four test tanks. One half of the fish employed in the test were injured by scale removal. Tank 2 received ten injured fish, tanks 3 and 5 received five injured and five uninjured fish, and ten uninjured fish were placed in tank 8.

Forty-eight hours after the test fish were distributed to the test tanks, 50 mls of the fungal homogenate were added to tanks 2, 3, and 8. Tank 5 received no fungus and served as the control.

After the initial and all subsequent inoculations with the fungal homogenate, the test tanks were allowed to drain from the top for two hours. The additional 50 mls of the fungal homogenate were added to tanks 2, 3, and 8 every five days during the fifteen day test. Water samples were collected for fungus baiting in petri dishes 24 hours after each fresh homogenate dose was added to the tank to determine if zoospores were still present.

Results

All water samples which were baited with halved sterile hemp seeds were found to contain viable zoospores except those taken from tank 5.

No evidence of fungus parasitism was apparent in any of the test tanks during this particular test. All of the test animals appeared healthy at the termination of the test.

Discussion

The lack of saprolegniosis among the salmon used in this test seems to indicate that contact with the fungal homogenate did not effectively expose the salmon to the fungus.

Baiting of water samples indicated that all inoculated tanks contained viable hyphae or zoospores of Saprolegnia parasitica for at least 24 hours. It is quite possible that the flow through system eliminated the hyphae and zoospores before the fish could be infected. After each inoculation the pieces of hyphae slowly settled to the bottom of each tank. When the flow of water was resumed most of the mycelium was swept out the drains within 6 hours. For this reason the homogenate was not used in further testing.

Test A_3

Methods and Materials

The purpose of this test was to ascertain whether the presence of dieldrin in tanks containing coho salmon fingerlings would enhance their susceptibility to parasitism by Saprolegnia parasitica. An agar plate culture of Saprolegnia parasitica (salmon origin) was placed in each of eight test tanks containing five injured and five uninjured salmon. The fish employed in this test had a mean weight of 9.22 grams and a mean length of 9.9 centimeters.

Tanks 1 and 2 received a continuous flow of water containing a mean concentration of 0.177 ppb of dieldrin. Tanks 5 and 6 received water containing 0.674 ppb of dieldrin. Tanks 7 and 8 received dieldrin at a concentration of 2.71 ppb. No pesticide was added to tanks 3 and 4 which served as the controls. The duration of the test was thirty-eight days.

Results

Fungus baiting was successful on all water samples taken during this test.

Within the first 18 days of testing all twenty fish had died in tanks seven and eight. The last fish to die in each tank had visible signs of fungus infection prior to death. The infected fish had not been injured prior to beginning the test. In both of these cases of saprolegniosis the infection originated on the gills.

At the end of the third week of testing one fish in tank 5 died after being infected by Saprolegnia parasitica. This fish had not been previously injured. The infection originated on the left side of the caudal peduncle and progressed as the infections described in Test A_1. The brain and a portion of the infected body tissue were removed for tissue sectioning.

On the 30th day of the test one uninjured fish in tank six died after being parasitized by the fungus. The infection originated on top of the fish's head.

Discussion

The salmon exposed to the highest concentration of dieldrin experienced severe stress and massive mortality due to the pesticide

toxicity. Two of the twenty fish exposed to the 2.71 ppb concentration of dieldrin contracted and died of a fungus infection. These fish expired within 24 hours after the mycelid growth became evident to the naked eye. In both cases the infection originated in the gills and did not spread beyond this area. The infected fish experienced a gradual loss of equilibrium and came to rest at the bottom of the tank. The number of fish parasitized in the highest pesticide concentration was not statistically significant. There is, however, some indication that the high concentration of dieldrin may have affected the condition of the fish resulting in the gill tissue of the two surviving fish being more susceptible to invasion by the fungus.

The third fish to die after being parasitized by Saprolegnia parasitica had been exposed to 0.674 ppb of dieldrin for 21 days. This infection like the other two in this test originated in a fish which was healthy prior to its exposure to the pesticide. This fish exhibited the same symptoms as noted in test A_1: loss of muscle control and equilibrium after girdling by the fungus.

After thirty days of exposure to 0.674 ppb of dieldrin a fourth fish died from fungus infection. In this case the fungus infection originated on top of the fish's head and spread across both eyes before death occurred. The salmon gradually lost equilibrium and settled to the bottom of the tank.

The number of fish parasitized by the fungus in the intermediate concentration of dieldrin was not statistically significant. There is, however, an indication that chronic exposure to this concentration of the pesticide does enhance the susceptibility of otherwise healthy young salmon to saprolegniosis.

TEST SERIES B - GREEN SUNFISH

Test B_1

Methods and Materials

The purpose of this test was to ascertain whether young green sunfish would become parasitized by Saprolegnia parasitica when living plate cultures were placed in their tanks. Five fish injured by scale removal and five uninjured fish with a mean weight of 3.9 grams and a mean length of 6.5 centimeters were placed in each of six test tanks.

Tanks 1 and 2 received water at a rate of 150 ml/min. and were maintained at 15°C throughout the test. Tanks 3 and 4 were static and were maintained at 20°C. Tanks 5 and 6 were static and maintained at 12.5°C by means of a cold water bath. A fungus plate culture was placed in tanks 1, 3, and 6. Tanks 2, 4, and 5 contained no fungus and served as the controls.

Results

Fungus baiting was successful on all samples taken from tanks 1, 3, and 6. Baiting was unsuccessful on all samples taken from tanks 2, 4, and 5. There were no apparent fungus infections experienced by any of the fish employed in this 49 day test.

Discussion

There were no discernible differences in the susceptibility of the sunfish to fungus parasitism exhibited during this test. It was

believed that the static environments might enhance susceptibility to infection by prolonging the fish's contact with the fungus. It was also believed that the warm water environment might produce more infections due to an increase in fungal growth and activity. It was apparent that the test fish were not susceptible to saprolegniosis under any of the test conditions.

Test B_2

Methods and Materials

The purpose of this test was to ascertain if young green sunfish would become susceptible to parasitism by Saprolegnia parasitica when stressed by dieldrin in their tank water.

Five sunfish injured by scale removal and five uninjured fish were placed in each of eight test tanks. The test fish had a mean weight of 4.0 grams and a mean length of 6.5 centimeters. Agar plate cultures of the fungus were placed in tanks 1, 4, 5, and 7. Tanks 2, 3, 6, and 8 received no fungus.

The serial diluter equipped with a dieldrin impregnated column delivered one of three different concentrations of the pesticide to the selected test chambers. Each test chamber received the dieldrin solution at the rate of 150 ml/min. In tanks 1 and 2 the mean concentration of dieldrin was 0.177 ppb, tanks 5 and 6 contained a mean concentration of 0.674 ppb, and tanks 7 and 8 contained a mean level of 2.71 ppb. The controls for this test were tanks 3 and 4 which received no dieldrin.

Results

Fungus baiting was successful in all water samples taken from

tanks 1, 4, 5, and 7. Baiting was not successful in any water samples taken from tanks 2, 3, 6, and 8.

Within the first 11 days of the test, 18 of the 20 fish in tanks 7 and 8 died due to dieldrin toxicity. One fish in each of these two tanks survived until the 20th day of the test. None of the sunfish in tanks 7 and 8 were parasitized by the fungus prior to death.

On the 15th day of the test one injured fish in tank 1 died after being infected by the fungus. On the 20th day of the test one injured sunfish died in tank 4 after being infected by Saprolegnia parasitica. Both of these fungus infections originated at the site where the scales were removed. Both infected fish experienced a loss of equilibrium and muscle control prior to death.

During the 24 day test period five sunfish died in tank 5 and six sunfish died in tank 6 due to pesticide toxicity. None of the test fish in either of these tanks was parasitized by the fungus.

Discussion

The dieldrin introduced into the test tanks during this test did not seem to increase the incidence of saprolegniosis among the young green sunfish at any of the concentrations tested. The one case of infection which occurred in the lowest pesticide concentration appeared identical to the infection in the tank which contained no pesticide and to other non-pesticide related infections which occurred in previous tests.

TEST SERIES C - STEELHEAD TROUT

Test C_1

Methods and Materials

The purpose of this test was to ascertain whether steelhead trout would become parasitized by Saprolegnia parasitica when living plate cultures were placed in their tanks or when viable fungul mycelium was inserted beneath the skin. Four trout with a mean weight of 13.4 grams and a mean length of 10.9 centimeters were placed in each of seven test tanks. Before being distributed to tanks 1, 3, 4, 5, and 6, the fish were anesthetized and an incision 1/4 inch in length was made in the skin on the right side just below the dorsal fin. Fish distributed to tanks 2 and 7 were anesthetized but not injured. The fish which were placed in tanks 3 and 5 had a small mass of hyphae with sporangia from a hemp seed culture of Saprolegnia parasitica (salmon origin) implanted with a dissecting needle under the skin at the point of the previously described incision. The fish in tank 4 received a similar mass of hyphae with sporangia previously isolated from a salamander. YPSs agar plate cultures of the salmon origin fungus were placed in tanks 6 and 7. The trout in tanks 1 and 2 were not exposed to either strain of Saprolegnia parasitica and served as controls for this fourteen day test.

Results

Fungus baiting was successful for isolation of the colonies on all water samples taken from tanks 6 and 7. Fungus baiting for isolation

of the colonies was unsuccessful on all water samples collected from the other five tanks during this test. In the later cases no fungal cultures were put into the tanks, however, tanks 3, 4, and 5 had fish inoculated with strains of the fungus.

No apparent incidences of saprolegniosis occurred to the young steelhead during this test. By the termination of testing, the incisions had healed leaving only a slight scar and all the test animals appeared to be healthy.

Discussion

The young trout employed in this test were apparently not susceptible to parasitism by the Saprolegnia parasitica of either origin under the selected test conditions. By incising the skin and placing the fungal mycelium between the skin and the muscle tissue, the fungus was in contact with a suitable growth medium for a prolonged period. This would tend to indicate that more than the contacting of a suitable host is required for the fungus to establish parasitism. The fact that no saprolegniosis occurred in the tanks which contained the plate cultures offers no real conclusions because of the lack of infections in previous tests employing this technique.

Test C_2

Methods and Materials

The purpose of this test was to ascertain whether steelhead trout fingerlings would become parasitized by Saprolegnia parasitica (salmon origin) as a result of subcutaneous and intraperitoneal injections of a solution containing both encysted and motile zoospores. The inoculum

was prepared from three hemp seed fungus cultures. The cultures were incubated at room temperature for 96 hours after which the liquid was drained from each and combined. Before inoculation each water suspension was examined microscopically to confirm the presence of both motile and encysted zoospores.

Four trout with a mean weight of 13.6 grams and a mean length of 10.9 centimeters were placed in each of six test tanks. All fish were anesthetized prior to inoculation. Each of the young trout placed in tank 1 received a .2cc subcutaneous injection of sterile double-distilled water on its right side just below the dorsal fin. These four fish served as the controls. Each fish placed in tanks 2, 3, and 5 received .2cc subcutaneous injections of the water suspension of zoospore in its right side beneath the dorsal fin. The trout in tanks 4 and 6 each received a .2cc intraperitoneal injection of the zoospore suspension just anterior to the anal opening.

Water samples were collected periodically during the ten day test. All test tanks received a continuous flow of dechlorinated water at a rate of 150 ml/min.

Results

Fungus baiting was successful on water samples collected from tanks 2, 3, 4, and 5. Baiting was unsuccessful on all samples collected from tanks 1 and 6.

Six days after inoculation one fish in tank 4 and two fish in tank 5 showed signs of saprolegniosis. Eight days after inoculation two fish in tank 2 showed evidence of fungus parasitism. Two days later one fish in tank 3 also appeared to be infected by the fungus. All six of the fungus infections experienced by the fish in this test

originated in the area where the injection was made. In each case the infected fish gradually lost equilibrium and mobility as its body was girdled by the fungus. In all six cases the disease was terminal within 24 hours after it became apparent to the naked eye.

Discussion

Six of twenty steelhead trout fingerlings injected with a solution containing viable fungal zoospores were parasitized by the fungus and died as a result. Five out of the twelve trout which were inoculated subcutaneously were successfully infected by the fungus. While only one out of eight fish which received the intraperitoneal injections was successfully infected.

The subcutaneous injection of viable Saprolegnia parasitica zoospores in the young steelhead was the most effective method of inducing fungus parasitism employed during this study. This fact indicates that Saprolegnia parasitica has the best chance of parasitizing healthy young fish when viable zoospores are lodged under the skin and allowed to germinate there. It is difficult to relate this method of inoculation to any natural occurrence.

ANALYSES OF SECTIONS MADE FROM TISSUES OF FUNGUS PARASITIZED FISH

Methods and Materials

Tissue sections were made of infected skin and muscular tissue excised from several fish which died after contracting saprolegniosis in tests A_3, B_2, and C_2. The infected tissues along with the whole brain of each individual was placed in a separate vial of Bouin fixative prior to sectioning.

The tissue sectioning was done using the 10 percent formalin and parafin method. Each section was stained with Gomori Methenamine - Silver stain (Emmons, 1970). This stain colors the fish tissue green and the fungal hyphae black. After staining the sections were permanently mounted on glass slides. When microscopic examination of a particular tissue section showed the presence of the fungus, a meticulous examination was made of the associated brain section.

Results

Examination of the skin and muscular tissue sections showed that all five test fish had been parasitized by the fungus. In each case the mycelium caused necrosis of the ectoderm and dermis layers. The hyphae also invaded deeply into the underlying muscles and blood vessels. The study of the associated brain sections revealed that the fungal hyphae had invaded the brain of four of the five infected test fish sectioned.

Discussion

Saprolegniosis is usually considered as a mycosis of the skin and the superficial muscles of the fish. However, tissue sections made from several fish which became infected during this study revealed that Saprolegnia parasitica is also an invader of deep muscle layers and blood vessels. It was also observed that the fungal hyphae invaded the brain of infected individuals prior to their death. It is quite possible that the spinal nervous system is also invaded during the course of the disease. This, along with the invasion of the deep muscle layers, would account for the fish's loss of muscular control and mobility as the body becomes encircled by the mycelium. The route by which the animals' brain was invaded was not determined. It can only be speculated that because neither muscles nor ectodermus were invaded in the head region of the fish sectioned, that the hyphae must have traveled by means of the spinal medulla or the circulatory system. It is also quite possible that the death mechanism of Saprolegnia parasitica may be due to invasion and destruction of tissues in the vascular and central nervous systems by the organism. These findings are very similar to those of Nolard-tintigner (1973) in more sensitive work carried out on Lebistes reticulatus Peters and Xiphophorus helleri Heckel.

CONCLUSION

No statistically significant data was generated during this study to substantiate the contention that the chronic exposure to dieldrin enhances the susceptibility of fishes to parasitism by the fungus Saprolegnia parasitica. There were, however, a small number of infections contracted by fish held in tanks containing the pesticide which tend to indicate the following: chronic exposure to dieldrin may enhance the susceptibility of otherwise healthy young salmon to saprolegniosis and the exposure to lethal concentrations of dieldrin may enhance the susceptibility of surviving salmon to saprolegniosis originating in the gills.

It proved difficult during this study to provide the proper environment for any significant number of the test fish to become parasitized by Saprolegnia parasitica. It was impossible to replicate any number of infections among individuals of the same or different species under the test conditions employed in the study. Therefore, no conclusions were reached in respect to defining the environmental conditions which promote saprolegniosis in fish.

In all cases of saprolegniosis encountered in this study, the infection originated at a site of injury or inoculation except those infections related to dieldrin exposure. It can therefore be concluded, that under normal conditions healthy, young fish are resistant to parasitism by Saprolegnia parasitica. However, when viable fungal

zoospores become lodged in suitable tissue and germinate a terminal disease often results. The 17 cases of saprolegniosis encountered during this study terminated in death approximately 24 hours after each became apparent. In all cases the symptoms were quite similar and could be related to the invasive nature of the fungus as confirmed by the associated tissue sections.

It was established during this study that Saprolegnia parasitica can cause a primary infection in superficial wounds of otherwise healthy young fish. It was demonstrated through tissue sectioning that these superficial infections spread into the deep muscular and vascular tissues of the fish, eventually reaching the brain and terminating in the animals death. It is quite possible that the undetermined death mechanism of Saprolegnia parasitica may be this invasion and destruction of tissues in the vascular and central nervous systems of the parasitized fish.

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