STUDIES OF INFECTIONS OF FISH BY CERTAIN SAPROLEGNIACEOUS FUNGI

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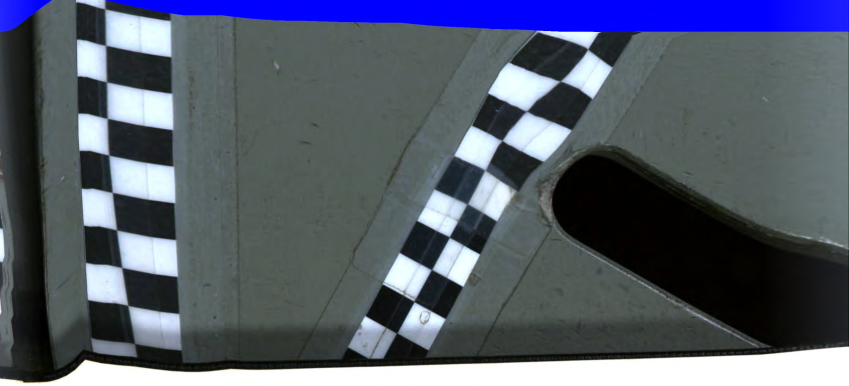

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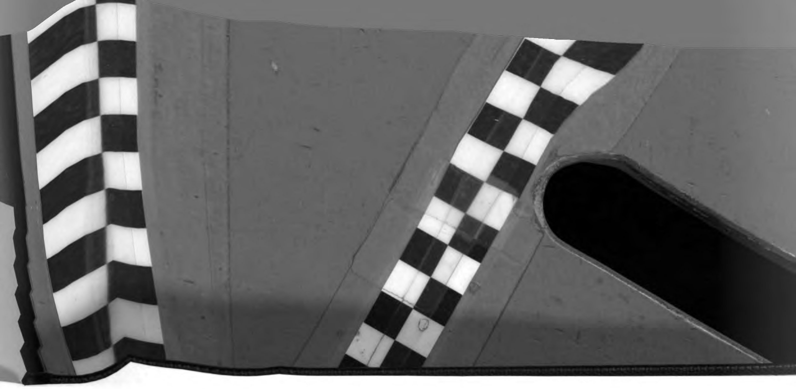

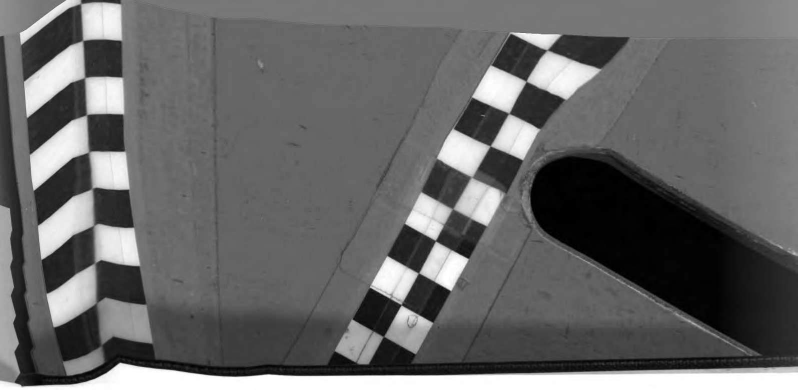

ABSTRACT

STUDIES OF INFECTIONS OF FISH BY CERTAIN SAPROLEGNIACEOUS FUNGI

by Norris Allen Edney, I.

It was shown that mycelial homogenates of *S. parasitica* and *S. ferax* were infectious to both the bluntnose minnow and rosyface shiner. The data indicated that *S. parasitica* and *S. ferax* were highly infectious to the injured fish. A study of mycelial homogenates of *S. parasitica* indicated that an exposure time of 12 hours was necessary to infect the injured bluntnose minnows and 18 hours of exposure to infect the uninjured fish. Death generally resulted in about 24 hours. The uninjured bluntnose minnow was more affected by the fungi than was the uninjured rosyface shiner. It is noteworthy that the use of partitions in the experimental tanks greatly reduced the percentage of infection. These data indicated that physical contact among fish has some effect on the spreading of infectious material.

Mycelial homogenates of *A. flagellata* were found to be slightly infectious to only the injured bluntnose minnows. *Dictyuchus monosporous* did not infect either the injured or uninjured fish.

Of the 8 species of saprolegniaceous fungi used, it was shown that the zoospores of *S. parasitica* were the most pathogenic of the fungi and were primarily wound invaders. Zoospores of *Saprolegnia sp.*, *S. delica* and *A. flagellata* were the only other fungi to be infectious. These fungi were only infectious to injured green sunfish. Partitioned tanks were used to prevent the fish from having physical contact with the fungal mats or with each other.

The fungicidal abilities of 5 experimental chemicals (TD 439, TD 753, TD 235, Potassium Endothal and Dexon) were tested by exposing various species of fungi and the green sunfish to different concentrations of each chemical. It was shown that TD 439 and Dexon were effective in certain concentrations when used continuously, but none was effective as a dip. No chemical was able to stop the growth of all test fungi at a concentration that the green sunfish could tolerate.

Histochemical data from sections of infected fish showed that the fungal growth spread through the skin and then down into the deeper tissue. The skin was completely destroyed by the infiltration of the fungal mycelia. The fungi were reproducing asexually by zoospores.

STUDIES OF INFECTIONS OF FISH BY CERTAIN SAPROLEGNIACEOUS FUNGI

By

Norris Allen Edney, I.

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To My Family

Lillian, Norris II,
Mornita and Albert

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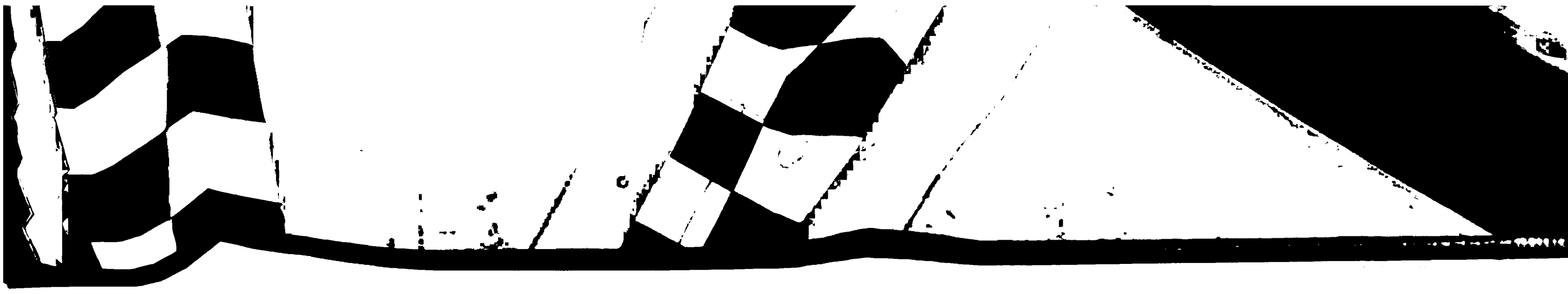

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INTRODUCTION

The study of the pathology of fish is a relatively new area of research. In view of this, investigation of many aspects of it have been inadequate. One such aspect is that of mycoses of fish. Practically all species of fresh water fish appear to be susceptible to fungal infections under certain conditions.

The infectious ability of certain members of the family Saprolegniaceae has been reported by many investigators during the last century. It is generally accepted that whenever fresh water fish are injured or placed under some type of stress, fungal infections often become very evident and mortality very high. However, infections may well occur when no detectable injury or stress is evident. These infections occur in nature and in fish hatcheries. Due to fungal infections, two hatcheries in Michigan were observed to be losing a combined total of 125 of the 3- to 5-year-old brown trout and rainbow trout brood stock each day. Even with treatments and diet changes, this is still an annual occurrence. It is also common to observe fungal infected fish, of many species, as they move into certain rivers and streams to spawn each year. Not only the fish, but also the fish eggs, are highly susceptible to infection by saprolegniaceous fungi. Millions of eggs are destroyed each year by one or more of these fungi. It is difficult to assess the damage done by water molds in open waters. The Michigan Department of Natural Resources reports that a relatively high percentage of trout coming into the weirs of Michigan each year are infected.

infectious. These latter three fungi were only infectious to injured green sunfish. Partitioned tanks were used to prevent the fish from having physical contact with the fungal mats or with each other.

The fungicidal abilities of 5 experimental chemicals (TD 439, TD 753, TD 235, Potassium Endothal and Dexon) were tested by exposing various species of fungi and the green sunfish to different concentrations of each chemical. It was shown that TD 439 and Dexon were effective in certain concentrations when used continuously, but none was effective as a dip. No chemical was able to stop the growth of all test fungi at a concentration that the green sunfish could tolerate.

Histochemical data from sections of infected fish showed that the fungal growth spread through the skin and then down into the deeper tissue. The skin was completely destroyed by the infiltration of the fungal mycelia. The fungi were reproducing asexually by zoospores.

LITERATURE REVIEW

Investigators have for some time been concerned with the problem of mycotic infections of fish. One of the first studies was made by Huxley (1882) of the fungus associated with an epidemic among salmon. Huxley concluded that the fungus involved was *Saprolegnia ferax*, even though his cultures were not pure. He gave no definite proof of the identification. *Saprolegnia ferax* was a well known species that produced sexual reproductive structures on fish and some other media. This species was assumed to be the only fish infectant until Coker (1923) noted that the fungus on fish did not produce sexual reproductive structures. On this basis, he concluded that this was a distinct species and should be called *S. parasitica*. Kanouse (1932) verified the identification of this species as a legitimate one by obtaining the sexual organs of *S. parasitica* on specific media. Tiffney (1939a) demonstrated that *S. parasitica* had the ability to parasitize a very wide range of fish and amphibious species. It was also evident in his work that certain host fish had some type of resistance to the mycoses. The assumption that a single fungal species was responsible for all mycoses of fish seemed to be too simple a solution to the problem. Tiffney (1939b) was able to isolate and identify certain members of the genera *Achlya* and *Dictyuchus* from fish, amphibians, and reptiles. Such data led to the idea that genera of the Saprolegniaceae, other than *Saprolegnia*, were involved in the mycoses of fish and other aquatic animals.

Davis and Lazar (1940) isolated and identified a new species of *Saprolegnia*, *S. invaderis*, from fingerlings of rainbow trout (*Salmo iridens*). It was unique in that infection seemed to take place through the lumen of the stomach rather than the surface of the body. It was also very infectious to healthy fish. Further work with this fungus has not been reported.

The results of the work of Vishniac and Nigrelli (1957) suggested that any member of Saprolegniaceae may infect wounded fish. Injured and uninjured Mexican platyfish, *Xiphophorus maculatus*, were exposed to 19 isolates of saprolegniaceous fungi in pyrex kitchen trays filled with 1.5 liters of tap water. The results indicated that the fungi were not infectious to uninjured fish but would attach and usually kill injured fish. Their investigation was also one of the very few that contributed significant histological data concerning the effect of the infection on the tissue of the host. Paraffin sections of the disease peduncle region of the fish were prepared and stained with hematoxylin-eosin and with Masson's trichrome stain. Histological examination indicated that the destruction of the tissue of the host was due almost exclusively to the penetration of the fungal mycelia.

More recently, Scott and O'Bier (1962) collected 64 isolates of aquatic fungi from 14 states. They found 5 members of Saprolegniaceae that heretofore had not been associated with fish mycoses. For the first time 6 saprolegniaceous species were isolated from eggs of fish. This was primarily a taxonomic investigation. However, evidence was presented to show that certain members of *Saprolegnia* and *Achlya* were infectious to wounded platyfish. The genera *Aphanomyces*, *Pythium*, *Allomyces*, and *Heptomitus* could not be induced to grow on fish under laboratory conditions. They pointed out that infection of wounded fish

with a fungus does not necessarily mean that parasitism is established for that species, nor do negative results prove that the fungus is strictly saprophytic. Positive results only demonstrate that the fungus is capable of growing on at least one species of fish under certain conditions and that in some cases the fungus can cause death to the host. Scott and Warren (1964) studied fungi associated with diseased tropical fish. They included in their work host range studies, infection studies, *in vitro* chemical control studies and tolerance tests. Three genera of fungi, *Saprolegnia, Achlya* and *Pythium*, were isolated from the tropical fish. It was concluded that these fungi appeared to function primarily as wound parasites. Members of the genera *Saprolegnia* and *Achlya* were found to be more vigorous pathogens than those of *Pythium*.

In 1968 Martin demonstrated that 8 of the fungal species studied by Scott and O'Bier (1962) would parasitize the blacknose dace (*Rhinichthip atratulus*), redbelly dace (*Chrosomus erythrogaster*) and goldfish (*Carassius aurutus*). His test indicated that *A. oblongata, A. flagellata, A. racemosa* and both forms of *A. ambisexualis* are equally pathogenic. However, they were less pathogenic than *S. parasitica* and *S. ferax*.

The above studies were basically taxonomic in nature. However, in the last 30 years interest has been directed toward finding an effective control for mycoses of fish. A large number of chemicals have been tested with questionable success, depending on whether the results were evaluated on a short term or a long term basis.

Malachite green was one of the first widely used chemicals. Foster and Woodbury (1936) found that dipping fungus-infected trout in a 1:10,000 solution of malachite green for 5 minutes would rid the fish of the mycoses. O'Donnell (1941) used malachite green on 18 species of

sport fish. He concluded that this dye had fungicidal and therapeutic effects when the fish were dipped in a 1:15,000 solution for 10 to 30 seconds. Malachite green is usually effective at relatively low concentrations. Scott and Warren (1964) demonstrated that this chemical was an effective fungicide *in vitro* at concentrations as low as 2 parts per million (ppm). Tolerance tests indicated that *Lebistes reticulatus* (guppy) and *Helostoma temmineki* (kissing Gourami) were able to retain consciousness for as long as 2 to 3 hours in the lowest effective concentrations. The effectiveness of malachite green at low concentrations was later confirmed by Martin (1968). He exposed 8 species of saprolegniaceous fungi to different concentrations of the dye in petri dishes. He found that it was effective in as little as 1 ppm on *S. parasitica* when exposed for 5 minutes. The growth of *S. ferax* was prevented by exposing it for 5 minutes at a concentration of 5 ppm. *Achlya oblongata* was found to be the most resistant to malachite green. Fifteen ppm were needed to stop the propagation of this fungus. This same report indicated that acriflavine was relatively ineffective as a fungicide.

There are seemingly conflicting reports on the effectiveness of potassium permanganate as a fungicide. Foster and Woodbury (1936) found that a 1:10,000 solution of potassium permanganate was ineffective on diseased trout. However, Hoshina and Ookuba (1956) reported that a 1:10,000 solution of potassium permanganate would eradicate *S. parasitica* in a petri dish culture. It should be noted that Foster and Woodbury tested the chemical on an unidentified fungus or fungi on the body of the fish. It has been pointed out that *S. parasitica* is affected by fungicides at much lower concentrations than other fungi associated with mycoses of fish. It could possibly be that the continued growth of the fungi on the trout was due to fungi other than *S. parasitica*.

The work of Estes (1957) appears to add some credence to the conclusion that potassium permanganate is an effective fungicide on some saprolegniaceous fungi. He conducted a 24-hour exposure treatment on infected goldfish. His results indicated that it was an effective fungicide at concentrations of 2 and 3 ppm. Also, he found that the compound was effective at 8 ppm when exposed for 60 minutes.

Copper sulfate is another very common and widely used fungicide. Foster and Woodbury (1936) found it to be toxic to fish when used at a concentration of 1:2,000 but effective against the fungus at a 1:1,000 concentration. This suggested that copper sulfate should be an excellent chemical agent to use against mycoses of fish. It was concluded by O'Donnell (1947) and verified by Burrows (1949) that copper sulfate was an effective fungicide. However, the effective concentration changed with varying calcium content of different waters.

About 1951 a new group of chemicals became widely studied. Rankin (1952, 1953) found that Phenoxetol (β-phenoxyethylalcohol) was effective on mycoses of goldfish at a concentration of 0.01%. Data supplied by Boehm (1969) indicate that lowest effective concentration of Phenoxetol against *Saprolegnia sp.*, *A. americana* and *Pythium sp.* is 0.5%. He suggested that a possible synergistic effect might be realized by using 1 ppm malachite green plus 0.2% Phenoxetol. It would be well to note that the data of Boehm were taken from fungi exposed in petri dishes and not growing on fish. A related chemical, Para-chlorophenoxetol, was reported by Rankin (1952, 1953) to be an effective fungicide at a concentration of 0.005%. Rankin performed toxicity tests on fish; however, these tests did not yield conclusive data.

Most of the investigations with chemicals did not involve extensive taxonomic descriptions. Some were so general as to refer to the fungus as "white fungus" and gave no taxonomic data (Rankin, 1953). O'Donnell (1941) made a very extensive investigation on the use of malachite green as a fungicide. However, he included little data concerning the taxonomy of the fungi studied. He stated that the fungi were members of Saprolegniaceae and that the most common one was *S. parasitica*. In view of this, it was not always exactly clear which saprolegniaceous species were affected by the chemicals studied. Moreover, none of these investigations involved a long-term study of the effects of the fungicide on the fungi or fishes.

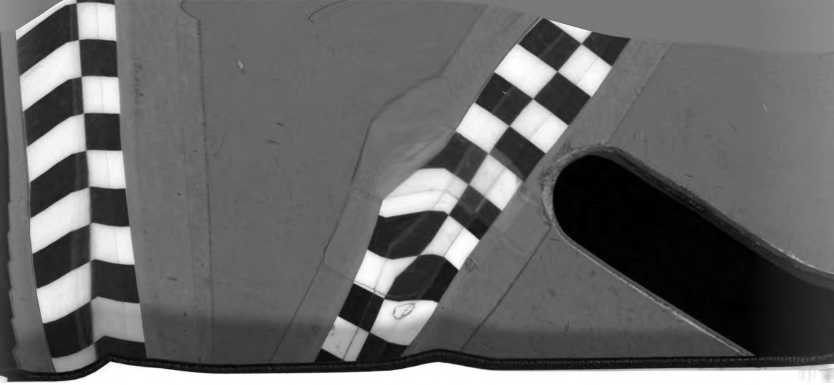
No record was found in the literature of studies made on infections of fish caused by mycelial contact with saprolegniaceous fungi. There are, however, numerous investigations that incorporated a limited study of mycoses caused by zoospore infections. Reports such as those of Tiffney (1939a) and Hoffman (1949) clearly indicated that exposure of fishes to zoospores of certain water molds would cause infections. They also noted that some water molds were more infectious than others. *Saprolegnia parasitica* is considered the most infectious of the members of Saprolegniaceae. It appears conclusive from the works of Tiffney (1939a, 1939b), Vishniac and Nigrelli (1957), and Scott and O'Bier (1962) that zoospores of several genera of Saprolegniaceae primarily attack wounded fish. These fungi are lethal pathogens of a number of fish species.

MATERIALS AND METHODS

General: Genera and species of the Saprolegniaceae used in the experiments were: *Achlya flagellata, Achlya racemosa, Achlya klebsiana, Thraustotheca primoachlya, Dictyuchus monosporous, Saprolegnia parasitica, Saprolegnia delica, Saprolegnia ferax* and *Saprolegnia sp*. The initial stocks were obtained from cultures of Dr. William G. Fields and Dr. E. S. Beneke of Michigan State University. With the help of Dr. L. Allison, Fish Pathologist, Department of Natural Resources, State of Michigan, other cultures were isolated from infected fish taken from fish hatcheries in Paris and Harietta, Michigan, and the Grayling Research Station. The fungi were maintained continuously in petri dishes in distilled water on sterile hemp seeds.

The fish species used were *Pimephales notatus* (bluntnose minnow), *Notropis rubellus* (rosyface shiner), *Lepomis cyanellus* (green sunfish), *Salmo trutta* (brown trout) and *Salmo gairdneri* (rainbow trout). The bluntnose minnows and the rosyface shiners were obtained from Beck Brothers Sport Shop and Nelson Homer Cutstone and Hardware Stores, both of Lansing, Michigan. They were maintained in 10- and 20-gallon tanks in charcoal filtered water from the East Lansing water supply. Because of the frequency of use and the availability of the fish, they were not maintained over extended periods.

The green sunfish were initially obtained from a pond in Barry County owned by Dr. George Lauff (Director, Kellogg Biological Station). Additional sunfish were obtained from Dr. Carl Latta, Biologist-In-Charge,



Institute for Fisheries Research, Ann Arbor, Michigan. The sunfish were maintained in a 189-gallon tank filled with charcoal filtered water which was aerated and filtered with 6 Halvin bottom filters. The tank was cleaned and refilled once each week and fish were fed pellets every other day.

The brown trout were never maintained in the laboratory. Infected and non-infected tissues were taken from the fish at the Grayling Research Station.

Mycelial Infection: Initially, only the water molds *S. parasitica* and *S. ferax* were used. The bluntnose minnow and the rosyface shiner were host fish. Mycelia of the fungi were transferred to petri dishes containing distilled water and oatmeal flakes. The fungi were allowed to grow until the oatmeal flakes were covered. Before sporangial formation oatmeal flake cultures from each fungus were placed in 15 ml of distilled water and homogenized for 5 seconds. These homogenates were taken from the blender and placed in vials until used. A 1-ml sample of each homogenate was placed in a petri dish containing distilled water and hemp seeds to check the viability of each fungus. Ten-ml aliquots of each homogenate were placed in separate tanks containing 2 liters of charcoal filtered water. Additional tanks of water were designated as controls. No mycelia were added to these tanks.

Twenty fish of each species were selected from the stock tanks. Five uninjured fish were placed in each of the control and experimental tanks. The remaining 10 were anesthetized with MS222 (aqueous tricaine methane sulphonate solution). The fish were then injured by removing a few scales and a small portion of skin on the caudal peduncle region. Five of these were placed in each of the two additional control and

experimental tanks. Observations were made daily for 3 days and recorded. At the end of this time or at death mycelia were isolated from the infected fish, grown on hemp seed, and identified. Samples of the water and debris were taken from the stock tanks and baited with hemp seeds.

In order to determine whether infection was caused by contact of one fish with another, each tank was divided into 2 parts with aluminum wire. One fish was placed in each division and allowed to adjust to the tanks for 24 hours before adding mycelial homogenates. Samples of the water from each tank were taken and baited with hemp seeds before the mycelial homogenates were added. Two additional fungi, *D. monosporous* and *A. flagellata*, were incorporated in these experiments.

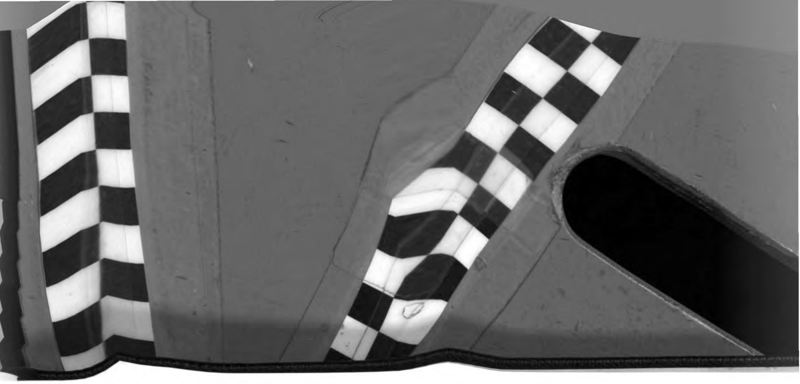
Other experiments were designed to determine more precisely the exposure time needed for mycelia to infect the fish. *Saprolegnia parasitica* and *Pimephales notatus* were used as experimental organisms. The procedures were the same as above, except that 3 fish were removed from both the experimental and control tanks at 6-hour intervals. These fish were transferred to clean tanks and observations made after 24 hours.

Zoospore Infection: Several fungi were used to investigate the infectious capabilities of zoospores. *Lepomis cyanellus* (green sunfish) was used as the host fish. The fish ranged from 5 cm to 9 cm in length. The procedure was modified from Tiffney (1939a) as follows: The water molds were grown in petri dishes on hemp seeds until mats of mycelia were produced. They were allowed to mature and produce sporangia. When numerous sporangia were observed, the mycelial mats were transferred to the bottom of experimental tanks. Fish food pellets were

allowed to dissolve and settle over these mats and served as added media on which the molds grew. Aluminum wire was placed over the mycelial mat in order to prevent physical contact with the fish. The fish were placed in the control and experimental tanks and observations were made over a period of 3 weeks. As soon as an animal died from the disease it was removed from the tank; the fungus was then isolated and identified. In order to determine the role of injury in susceptibility to infections, experiments were performed on both injured and uninjured fish. The fishes were injured in the same manner as described above.

Test of Fungicides: Five fungicides were selected for use in these experiments: Potassium Endothal, TD 753, TD 439, TD 235 and P-(Dimethylamino)benzenediazo sodium sulfonate (Dexon). The first 4 were obtained from Pennsalt Chemical Corporation and the Dexon from Chemagro Corporation. TD 753, TD 439 and TD 235 are experimental quaternary ammonium compounds whose chemical structures have not been released by Pennsalt Chemical Corporation. Solutions of all chemicals were used in varying concentrations.

The fungi used were *S. delica, S. parasitica, D. monosporous, T. primoachlya, A. flagellata,* and *A. klebsiana*. These molds were exposed in petri dishes to different concentrations of the fungicides for various periods of time. Each trial consisted of 3 young developing hemp seed cultures and 3 old mature hemp seed cultures. Several unexposed sterile hemp seeds were added to each petri dish. Observations were made on cultures sustained in the different concentrations of the fungicide after 48 hours and periodically thereafter. With all fungicides, except Potassium Endothal, time exposure experiments were made. The fungi were exposed to different concentrations of the chemical for various intervals of time. They were then washed in distilled water and placed in



a petri dish with distilled water and sterile hemp seeds. The number of hemp seed cultures used for each trial was the same as above. The first observations were made after 24 hours and daily thereafter for 6 days.

The green sunfish was used in experiments similar to those just described. The fishes were exposed in tanks to different concentrations of the fungicides. Sustained and interval exposure tests were performed. At the end of the exposure time the fish were transferred to clean tanks filled with charcoal filtered water. Observations were made at various intervals.

Histochemistry: Tissue sections were taken from infected and non-infected brown trout at the Grayling Research Station. These pieces of tissue were fixed in 10% formalin, mounted in paraffin and cut in 8- to 10-μ sections. The sections were made so that both skin and muscle tissue were included. Several histochemical stains were then employed to differentiate between the fungal structures and fish tissue.

The following histochemical procedures were employed:

1. Delafield's Hematoxylin: The procedures used were the same as Johansen, 1940, as recorded by Jensen (1962). Both eosin and Orange G were used as counter stains.

2. Ehrlich's (1886) Hematoxylin: The procedures followed were as given by Drury, Wallington and Cameron (1967). Ten, 30 and 40 minute staining times were used. Eosin was used as a counter stain.

3. Periodic Acid-Schiff Technique (PAS): The procedure was generally the same as recorded by Beneke (1966). The PAS stain was preceded by the use of 5,5 dimethylcyclohexane 1,3-dione (dimedone)

as an aldehyde blocking agent. A second group of slides (tissue) were stained in Delafield's hematoxylin, counter stained with eosin, rehydrated and placed in 1% periodic acid for 10 minutes. The steps to follow were the same as those outlined by Beneke.

4. Pianese III stain: The methods used were those of Pianese (1896) as recorded by Davenport.

5. Phloxine: Tissue was deparaffinized in xylene and hydrated in the usual manner. A drop of the cytoplasmic stain, phloxine, was placed on the slide and allowed to remain for one minute and removed.

Photomicrographs of selected slides representing control and infected tissues were taken with a 35mm Pentax Spotmatic mounted on a Leitz Ortholux microscope.

Observations of Infected Fish: Two infected rainbow trout were taken on each of 3 occasions from the Grayling Research Station. These fish were infected with *S. parasitica* and *Saprolegnia sp*. Photographs were taken of the fish using a 35mm Voigtlander. The fish were then brought to the laboratory at Michigan State University and observed until death.

RESULTS

In order to determine the infectious ability of mycelia of certain saprolegniaceous fungi, various genera and species were added to tanks containing either the rosyface shiner or bluntnose minnow. The data presented in Table 1 show the infectious ability of mycelial homogenates of *S. parasitica* and *S. ferax* over a period of 3 days. The data indicate that both fungi were highly infectious to injured bluntnose minnows and rosyface shiners. A 24-hour period was necessary to realize 100% infection in all experiments. *Saprolegnia parasitica* infected the uninjured bluntnose minnow within 48 hours but did not infect the rosyface shiner. *Saprolegnia ferax* was only slightly infectious to the uninjured bluntnose minnow and did not infect the rosyface shiner in any experiment. These data indicate that mycelia of some fungi are highly infectious to injured fish. In the case of uninjured fish it seems that there is a difference in the ability of the fungi to cause mycoses. Uninjured rosyface shiners were never infected by the experimental water molds. *Saprolegnia parasitica*, which seemed otherwise so infectious, did not infect these fish.

The above experiments were performed with fish in physical contact with one another. Experiments were designed to determine whether this contact aided the spreading of infectious materials from one fish to another. All tanks were divided with wire screens to separate the fish. The results are shown in Table 2. The fish in these experiments were allowed time to adjust to the experimental tanks before mycelial

Table 1. Mycelial (Homogenate) Infection of Certain Fish

Fungus	Fish and Treatment	No. of Fish	Observations			Infection (%)	Reisolation of fungus from infected fish	Presence of fungus in stock tank
			1 day	2 days	3 days			
Saprolegnia parasitica	*Pimephales notatus* (Bluntnose minnow)							
	Control (without fungus)							
	Injured	5	uninfected	uninfected	uninfected	0		
	Uninjured	5	uninfected	uninfected	uninfected	0		
	Experimental (with fungus)							
	Injured	5	infected	infected		100	Checked out as *S. parasitica*	Not detected in water or bottom samples*
	Uninjured	5	uninfected	infected	infected all dead			
Saprolegnia parasitica	*Notropis rubellus* (Rosyface Shiner)							
	Control (without fungus)							
	Injured	5	uninfected	uninfected	uninfected	0		
	Uninjured	5	uninfected	uninfected	uninfected	0		
	Experimental (with fungus)							
	Injured	5	infected	infected	infected	100	Checked out as *S. parasitica*	Not detected in water or bottom samples*
	Uninjured	5	uninfected	uninfected	uninfected	0		

Table 1 (cont'd.)

Table 1 (cont'd.)

Fungus	Fish and Treatment	No. of Fish	Observations			Infection (%)	Reisolation of fungus from infected fish	Presence of fungus in stock tank
			1 day	2 days	3 days			
Saprolegnia ferax	*Notropis rubellus* (Rosyface Shiner)							
	Control (without fungus)							
	Injured	5	uninfected	uninfected	uninfected	0		
	Uninjured	5	uninfected	uninfected	uninfected	0		
	Experimental (with fungus)							
	Injured	5	infected	infected	infected	100	Checked out as *S. ferax*	Not detected in water or bottom samples*
	Uninjured	5	uninfected	infected	infected	0		
Saprolegnia ferax	*Pimephales notatus* (Bluntnose minnow)							
	Control (without fungus)							
	Injured	5	uninfected	uninfected	uninfected	0		Not detected in water samples*
	Uninjured	5	uninfected	uninfected	uninfected	0		
	Experimental (with fungus)							
	Injured	5	infected	infected all dead		100	Checked out as *S. ferax*	Growth detected from plants and bottom debris
	Uninjured	5	uninfected	1 infected	1 dead infected	20		

*Samples "baited" with hemp seeds.

Table 2. Mycelial (Homogenate) Infection of Certain Fish in Tanks With Aluminum Septa

Fungus	Fish and Treatment	No. of Fish	Observations 1 day	Observations 2 days	Observations 3 days	Infection (%)	Reisolation of fungus from infected fish	Presence of fungus in stock tank
Saprolegnia parasitica	*Pimephales notatus*							
	Control (without fungus)							
	Injured	6	uninfected	uninfected	uninfected	0		
	Uninjured	6	uninfected	uninfected	uninfected	0		
	Experimental (with fungus)							
	Injured	6	infected	all dead		100	Checked out as *S. parasitica*	Not detected in water or bottom samples*
	Uninjured	6	infected	infected	2 dead			
Saprolegnia parasitica	*Notropis rubellus*							
	Control (without fungus)							
	Injured	6	uninfected	uninfected	uninfected	0		
	Uninjured	6	uninfected	uninfected	uninfected	0		
	Experimental (with fungus)							
	Injured	6	uninfected	1 infected	2 infected 1 dead infected	50	Checked out as *S. parasitica*	Not detected in water or bottom samples*
	Uninjured	6	uninfected	uninfected	uninfected	0		
Saprolegnia ferax	*Pimephales notatus*							
	Control (without fungus)							
	Injured	6	uninfected	uninfected	uninfected	0		
	Uninjured	6	uninfected	uninfected	uninfected	0		
	Experimental (with fungus)							
	Injured	6	infected	infected	all dead	100	Checked out as *S. ferax*	Not detected in water or bottom samples*
	Uninjured	6	uninfected	uninfected	uninfected	0		

ble 2 (cont'd.)

Table 2 (cont'd.)

Fungus	Fish and Treatment	No. of Fish	Observations			Infection (%)	Reisolation of fungus from infected fish	Presence of fungus in stock tank
			1 day	2 days	3 days			
Saprolegnia ferax	*Notropis rubellus*							
	Control (without fungus)							
	Injured	6	uninfected	uninfected	uninfected	0		
	Uninjured	6	uninfected	uninfected	uninfected	0		
	Experimental (with fungus)							
	Injured	6	infected	infected	infected	66	Checked out as *S. ferax*	Not detected in water or bottom samples*
	Uninjured	6	uninfected	uninfected	uninfected	0		
Achlya flagellata	*Pimephales notatus*							
	Control (without fungus)							
	Injured	6	uninfected	uninfected	uninfected	0		
	Uninjured	6	uninfected	uninfected	uninfected	0		
	Experimental (with fungus)							
	Injured	6	uninfected	2 infected	2 dead infected	33	Checked out as *A. flagellata*	Not detected in water or bottom samples*
	Uninjured	6	uninfected	uninfected	uninfected	0		
Achlya flagellata	*Notropis rubellus*							
	Control (without fungus)							
	Injured	6	uninfected	uninfected	uninfected	0		
	Uninjured	6	uninfected	uninfected	uninfected	0		
	Experimental (with fungus)							
	Injured	6	uninfected	uninfected	uninfected	0		Not detected in water or bottom samples*
	Uninjured	6	uninfected	uninfected	uninfected	0		

Table 2 (cont'd.)

Fungus	Fish and Treatment	No. of Fish	Observations 1 day	 2 days	 3 days	Infection (%)	Reisolation of fungus from infected fish	Presence of fungus in stock tank
Dictyuchus monosporous	*Pimephales notatus*							
	Control (without fungus)							
	Injured	6	uninfected	uninfected	uninfected	0		
	Uninjured	6	uninfected	uninfected	uninfected	0		
	Experimental (with fungus)							
	Injured	6	uninfected	uninfected	uninfected	0		Not detected in water or bottom samples*
	Uninjured	6	uninfected	uninfected	uninfected	0		
Dictyuchus monosporous	*Notropis rubellus*							
	Control (without fungus)							
	Injured	6	uninfected	uninfected	uninfected	0		
	Uninjured	6	uninfected	uninfected	uninfected	0		
	Experimental (with fungus)							
	Injured	6	uninfected	uninfected	uninfected	0		Not detected in water or bottom samples*
	Uninjured	6	uninfected	uninfected	uninfected	0		

*Samples "baited" with hemp seeds.

homogenates were added. The water in experimental tanks was baited with hemp seeds before the mycelial homogenates were added in order to reasonbly ensure that no fungal spores or mycelia were brought over on the fish from the stock tanks. These checks were all negative.

The data in Table 2 demonstrated that *S. parasitica* infected both the injured bluntnose minnow and rosyface shiners under these conditions. It is noteworthy that there was only 50% infection of the rosyface shiner compared to the 100% infection where the fish were in contact. Moreover, *S. parasitica* infected only 33% of the uninjured bluntnose minnows in the divided tanks, whereas it had infected 100% when all the fish were together. This general decrease in percent infection can be further noted with *S. ferax*. The results suggest that physical contact among fish has some effect on the spreading of infectious material.

Dictyuchus monosporous did not infect either the injured or uninjured fish. This may indicate that *D. monosporous* is either not infectious to fish or is a secondary invader.

Achlya flagellata was only slightly infectious to the injured bluntnose minnow: 33% of these fish were infected, but it was not infectious to the uninjured bluntnose minnow. Neither the injured nor uninjured rosyface shiners were infected by the mycelia of *A. flagellata*. It appears that the role of *A. flagellata* as an infectious saprolegniaceous fungus is similar to that of *D. monosporous*.

These results suggest that there may be a difference in susceptibility of the fish to fungal infections (Tables 1 and 2). The bluntnose minnow appeared to be more susceptible to the mycoses than the rosyface shiner. This was evident with both the injured and uninjured fish. However, it should be pointed out that the mycelial homogenate was

often more concentrated in the lower portion of the tanks and that the bluntnose minnow prefers swimming in this area. Also, the bluntnose minnows were considerably less active than the rosyface shiners. This difference in the behavior of the two species may have had some effect on the results.

All 1 ml samples of mycelial homogenates proved to be viable on hemp seeds. From these data, it appears that all of the homogenates were capable of growth if conditions were favorable.

The fungi from infected fish were reisolated and proved to be the same as the mycelial homogenates in all experiments.

The stock tanks were also checked for the presence of water molds. Except in one instance, these checks were all negative (Tables 1 and 2). Table 1 records the only positive test. No fish in any stock tank were infected during the experiments. This may not be conclusive evidence of the absence of fungi in the stock tank, but it appears that the infectious molds were not present or conditions were unfavorable for their growth.

The above data indicated that the mycelial homogenates were infectious to fish but did not specify the exposure time necessary for infection. Experiments were made to determine the exposure time needed for these infections to occur. Table 3 shows the results of a single species of fish exposed to a single fungal species. The bluntnose minnow was chosen because of its high susceptibility to fungal infections and *S. parasitica* because it was highly infectious. An exposure time of 12 hours was necessary for *S. parasitica* to infect injured bluntnose minnows. However, 18 hours were necessary for the infection to become established on uninjured bluntnose minnows.

Table 3. Varied Exposure Times of *Pimephales notatus* to Mycelial Homogenate of *Saprolegnia parasitica*

Time exposed to control or experimental conditions before being transferred to clean tanks*	Controls Without Fungus						Experimental With Fungus					
	Injured	No. of Fish	Infection (%)	Uninjured	No. of Fish	Infection (%)	Injured	No. of Fish	Infection (%)	Uninjured	No. of Fish	Infection (%)
6 hours	uninfected	3	0	uninfected	3	0	uninfected	3	0	uninfected	3	0
12 hours	uninfected	3	0	uninfected	3	0	infected	3	100	uninfected	3	0
18 hours	uninfected	3	0	uninfected	3	0	infected	3	100	infected	3	66.6
24 hours	uninfected	3	0	uninfected	3	0				infected	3	100
30 hours	uninfected	3	0	uninfected	3	0				infected	3	100
36 hours	uninfected	3	0	uninfected	3	0						

*All observations made after the fish had been exposed and transferred to clean tank for 24 hours.

The ability of zoospores of certain saprolegniaceous fungi to infect different species of fish is recorded frequently in the literature. Experiments were designed to collect specific data concerning the infectious nature of certain fungi to the green sunfish. The results of these experiments are presented in Table 4. In the initial experiments the fish were exposed separately according to size. The data showed that size, within the range used, had no effect on the results. The marked difference in infection of injured fish compared to uninjured fish is noteworthy. The zoospores of *S. parasitica* were highly infectious to injured fish (90%) and only slightly infectious to uninjured fish (20%). *Saprolegnia sp.* showed similar results. *Achlya flagellata* and *S. delica* were the only other fungi to cause infections and these were infectious only to injured fish. *Achlya flagellata* infected 30% of the injured fish exposed to the zoospores, whereas *S. delica* caused mycoses to 40% of the injured fish. *Saprolegnia parasitica* and *Saprolegnia sp.* were slightly infectious to uninjured fish.

A number of chemicals have been used to control or eradicate mycoses of fish. None of these widely used fungicides has been effective over long periods of time. There are vast numbers of chemicals that might be effective fungicides but which have not been tested. Five such chemicals have been considered in this work. Fungi and fish species were exposed to different concentrations of each experimental fungicide. The results of exposing only the fungi to certain fungicides are shown in Tables 5 through 9. These tables record the results of both sustained and interval exposure tests, except for Potassium Endothal (Table 8). The maximum concentration of a given chemical was established by the tolerance of the green sunfish to the chemical as

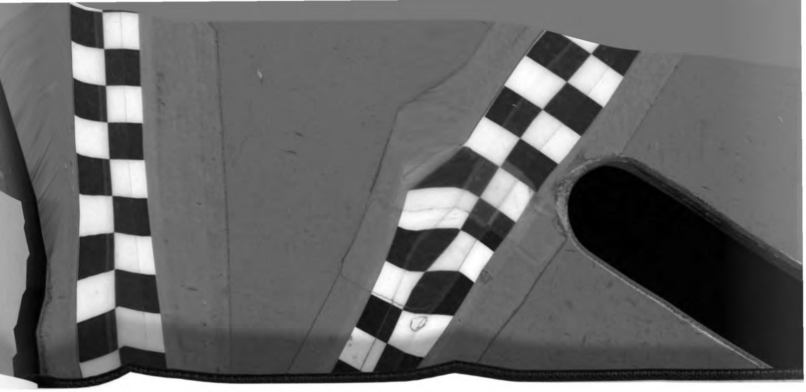
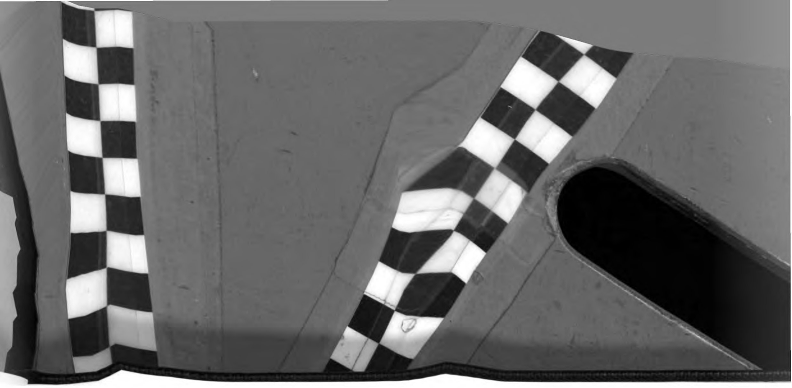


Table 4. Infection of *Lepomis cyanellus* by Zoospores of Various Water Molds

Organism and Treatment	No. of Trials	Total No. of Fish Used	Infected	Uninfected	Percent Infected (of total)
Achlya flagellata					
Injured	5	10	3	7	30
Uninjured	5	10	0	10	0
Achlya racemosa					
Injured	2	4	0	4	0
Uninjured	2	4	0	4	0
Achlya klebsiana					
Injured	3	6	0	6	0
Uninjured	3	6	0	6	0
Dictyuchus monosporous					
Injured	3	6	0	6	0
Uninjured	3	6	0	6	0
Thraustotheca primoachlya					
Injured	3	6	0	6	0
Uninjured	3	6	0	6	0
Saprolegnia parasitica					
Injured	5	10	9	1	90
Uninjured	5	10	2	8	20
Saprolegnia delica					
Injured	5	10	4	6	40
Uninjured	5	10	0	10	0
Saprolegnia sp.					
Injured	3	6	4	2	66.6
Uninjured	3	6	2	4	33.3



explained below in fish sustained exposure test. The length of exposure was also relative to this factor. The results were recorded by using the terms growth (+), no growth (-), inhibited (X) and very inhibited (X̲). These relative terms were used to describe the growth of the fungi in the experimental situation compared to that of the control. Growh is defined as the production of new mycelia and the development of sporangia.

Initially *A. klebsiana* was used in all experiments. It was excluded from some tests because of the failure of stock cultures to grow as rapidly as the other fungi.

TD 439 proved to be effective on fungi sustained in petri dishes for 48 hours (Table 5). The effectiveness of the chemical at a given concentration varied with the species of fungi. The variation in resistance of fungal species to fungicides is not as evident with TD 439 as with other chemicals tested. A concentration as low as 5 ppm was effective in preventing the growth of *S. parasitica* in sustained exposure experiments. For *S. delica* a concentration of 10 ppm was necessary to stop growth. The results with *A. flagellata* and *A. klebsiana* were similar to those of *S. delica.* The most tolerant species were *D. monosporous* and *T. primoachlya*. Twenty ppm were successful in stopping the growth of the former and 30 ppm were needed for the latter. It is noteworthy that all concentrations of TD 439 either stopped or inhibited the growth of all fungi used in these experiments.

TD 439 did not prove to be an effective fungicide in the time exposure tests. The fungi grew in all concentrations of TD 439 when exposed for 10 minutes. Twenty-minute exposures were also ineffective except when used in very high concentrations for certain fungi. *Saprolegnia parasitica* and *S. delica* were inhibited by 90 and 100 ppm,

Table 5. Exposure of Fungi to Different Concentrations of TD 439

Fungus Used	No. of Trials per Concentration	Control	Observations after 48 hours on cultures sustained in concentrations (ppm) 5	10	20	30	40	50	60	70	80	90	100	120	Time Exposed (min)	Observation of fungus after being exposed to fungicide for various intervals (ppm) 5	10	20	30	40	50	60	70	80	90	100
Saprolegnia delica	3	+	X	-	-	-	-	-							10	+	+	+	+	+	+	+	+	+	+	+
															20	+	+	+	+	+	+	+	+	+	X	X
															30	+	+	+	+	+	X	X	X	X	X	X
Saprolegnia parasitica	3	+	-	-	-	-	-								10	+	+	+	+	+	+	+	+	+	+	+
															20	+	+	+	+	+	+	+	+	X	X	X
															30	+	+	+	+	X	X	X	X	X	X	X
Dictyuchus monosporous	3	+	X̲	X̲	-	-	-	-	-	-	-	-	-	-	10	+	+	+	+	+	+	+	+	+	+	+
															20	+	+	+	+	+	+	+	+	+	+	+
															30	+	+	+	+	+	+	+	X	X	X	X
Achlya flagellata	3	+	X	-	-	-	-	-	-						10	+	+	+	+	+	+	+	+	+	+	+
															20	+	+	+	+	+	+	+	+	+	+	+
															30	+	+	+	+	+	+	+	+	X	X	X
Achlya klebsiana	3	+	X	-	-	-																				
Thraustotheca primoachlya															10	+	+	+	+	+	+	+	+	+	+	+
															20	+	+	+	+	+	+	+	+	+	+	+
															30	+	+	+	+	+	+	+	X	X	X	X

+ growth (new mycelia and sporangia development)
X inhibited or very slow growth

- no growth
X̲ very inhibited

*Each trial consisted of 3 young developing hemp seed growths and 3 old mature hemp seed growths.

respectively. Concentrations from 80 to 100 ppm inhibited the growth of all fungi tested when exposed for 30 minutes. The concentrations needed to inhibit *S. parasitica* and *S. delica* were again lower than those needed for other fungi.

Table 6 shows the results of tests made with TD 753. In 48-hour sustained exposure the growth of *S. parasitica* was stopped by a concentration of 20 ppm. *Saprolegnia delica* was inhibited by 20 and 30 ppm, and growth stopped with 40 ppm. Concentrations up to 40 ppm were unable to affect the growth of the other fungi. The growth of *T. primoachlya* was stopped at concentrations of 80 to 100 ppm. The other fungi grew in concentrations up to 100 ppm.

The time exposure test with TD 753 did not show any effective fungicidal concentration when exposed for intervals up to 30 minutes. The growth of *S. parasitica* and *S. delica* was inhibited by 100 ppm.

The last quaternary ammonium compound used was TD 235. The results are shown in Table 7. *Thraustotheca primoachlya* and *S. parasitica* were the only fungi whose growth was stopped by the chemical at a concentration below 100 ppm. A concentration of 70 ppm was effective as a fungicide on both fungi. *Saprolegnia delica* did not grow in concentrations of 100 and 120 ppm. The growth of the other fungi was not affected by the concentrations used. All fungi grew in the time exposure experiments at all concentrations.

As shown in Table 8, Potassium Endothal was the most ineffective fungicide tested. The experimental fungi were able to grow well in concentrations up to 70 ppm. Two fungi, *T. primoachlya* and *S. parasitica*, were inhibited at concentrations of 80 ppm, but all other fungi were able to grow in concentrations up to 100 ppm. In view of the ineffectiveness of Potassium Endothal, no further experiments were performed.

Table 6. Exposure of Fungi to Different Concentrations of TD 753

Fungus Used	No. of Trials per Concentration	Control	Observations, after 48 hours, on culture sustained in concentrations (ppm)											Time Exposed (min)	Observation of fungus exposed to different concentration (ppm) for various intervals					
			5	10	20	30	40	50	60	70	80	90	100		30	40	50	60	80	100
Saprolegnia delica	3	+	+	+	X	X̲	-	-	-	-	-	-	-	10	+	+	+	+	+	X
														20	+	+	+	+	X	X
														30	+	+	+	+	X	X̲
Saprolegnia parasitica	3	+	X	X̲	-	-	-	-	-	-	-	-	-	10	+	+	+	+	+	+
														20	+	+	+	+	+	X
														30	+	+	+	+	+	X
Dictyuchus monosporous	3	+	+	+	+	+	+	+	X	X	X	X̲	X̲	10	+	+	+	+	+	+
														20	+	+	+	+	+	+
														30	+	+	+	+	+	+
Achlya flagellata	3	+	+	X	X	X	X̲	-	X̲	X̲	X̲	X̲	X̲	10	+	+	+	+	+	+
														20	+	+	+	+	+	+
														30	+	+	+	+	+	+
Achlya klebsiana	3	+	+	+	X	X														
Thraustotheca primoachlya	3	+	+	+	+	+	+	+	X	X	-	-	-	10	+	+	+	+	+	+
														20	+	+	+	+	+	+
														30	+	+	+	+	+	+

\+ growth (new mycelia and sporangia development)
X inhibited or very slow growth

\- no growth
X̲ very inhibited

*Each trial consisted of 3 young developing hemp seed growths and 3 old mature hemp seed growths.

Table 7. Exposure of Fungi to Different Concentrations of TD 235

Fungus Used	No. of Trials per Concentration	Control	Observations, after 48 hours, on cultures sustained in concentrations (ppm)												Time Exposed (min)	Observation of fungus exposed to different concentrations (ppm) of fungicide for various intervals									
			5	10	20	30	40	50	60	70	80	90	100	120		5	10	20	30	40	50	60	80	100	120
Saprolegnia delica	3	+	+	+	+	X	X	X	X	X	X̲	X̲	-	-	10	+	+	+	+	+	+	+	+	+	+
															20	+	+	+	+	+	+	+	+	+	+
															30	+	+	+	+	+	+	+	+	+	+
Saprolegnia parasitica	3	+	+	+	X	X	X	X̲	X̲	-	-	-	-	-	10	+	+	+	+	+	+	+	+	+	+
															20	+	+	+	+	+	+	+	+	+	+
															30	+	+	+	+	+	+	+	+	+	+
Dictyuchus monosporous	3	+	+	+	+	+	+	+	+	+	X	X	X	X̲	10		+	+	+	+	+	+	+	+	+
															20		+	+	+	+	+	+	+	+	+
															30		+	+	+	+	+	+	+	+	+
Achlya flagellata	3	+	+	+	X	X	X	X	X	X	X	X	X̲	X̲	10		+	+	+	+	+	+	+	+	+
															20		+	+	+	+	+	+	+	+	+
															30		+	+	+	+	+	+	+	+	+
Achlya klebsiana	3	+	+	X	X	X	X	X	X̲	X̲															
Thraustotheca primoachlya	3	+	+	+	+	+	X	X	-	-	-	-	-	-	10			+	+	+	+	+	+	+	+
															20			+	+	+	+	+	+	+	+
															30			+	+	+	+	+	+	+	+

+ growth (new mycelia and sporangia development) - no growth
X inhibited or very slow growth X̲ very inhibited

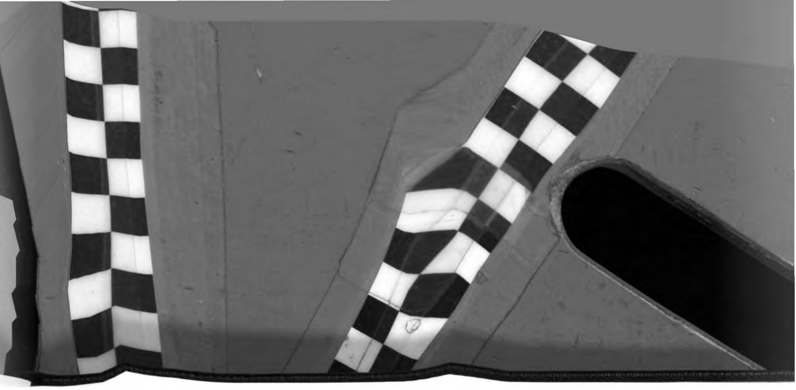
*Each trial consisted of 3 young developing hemp seed growths and 3 old mature hemp seed growths.

Table 8. Exposure of Fungi to Different Concentrations of Potassium Endothal

Fungus Used	No. of Trials per Concentration	Control	Observations, after 48 hours, on cultures sustained in concentrations (ppm)										
			10	20	30	40	50	60	70	80	90	100	120
Saprolegnia delica	3	+	+	+	+	+	+	+	+	+	+	X	X
Saprolegnia parasitica	3	+	+	+	+	+	+	+	+	X	X	X	X
Dictyuchus monosporous	3	+	+	+	+	+	+	+	+	+	+	+	+
Achlya flagellata	3	+	+	+	+	+	+	+	+	+	+	+	+
Achlya klebsiana	3	+	+	+	+	+	+	+	+				
Thraustotheca primoachlya	3	+	+	+	+	+	+	+	+	X	X	X̲	X̲

\+ growth (new mycelia and sporangia development) - no growth
X inhibited or very slow growth X̲ very inhibited

*Each trial consisted of 3 young developing hemp seed growths and 3 old mature hemp seed growths.



Dexon appeared to be a relatively effective fungicide only when used at very high concentrations over a period of 48 hours. Table 9 presents the results of the sustained and time exposure tests with this chemical. Preliminary tests with fish indicated that they were very tolerant to high concentrations of Dexon. In view of this, concentrations as high as 1 x 10^6 ppm were used. In the sustained tests all species of fungi were able to grow in concentrations up to 1200 ppm. *Thraustotheca primoachlya* showed inhibited growth when sustained in a concentration of 1200 ppm. No species of fungi were able to grow when sustained for 48 hours in concentrations of 5 x 10^5 ppm and 1 x 10^6 ppm. These were the only concentrations of any chemical tested that stopped all fungal growth. The time exposure tests with Dexon were not so productive. The data show that the fungicide was effective when the fungi were exposed to a concentration of 1 x 10^6 ppm. The most consistently effective exposure time was 30 minutes.

The value of a given chemical as a fungicide is determined not only by its ability to stop the growth of infectious fungi but also by the tolerance of the host fish. The green sunfish were exposed to the same chemicals and concentrations as were the fungi. Special attention should be given to the chemical tolerance of the fish when compared to the effectiveness of the fungicide on the experimental fungi at a specific concentration and exposure time.

The tolerance of the green sunfish to TD 439 was very low, as shown in Table 10. In sustained tests, 20 ppm were needed to stop the growth of most of the fungi tested for 48 hours. The green sunfish did not tolerate 4 ppm for 48 hours. It sustained a 24-hour exposure in concentrations up to 8 ppm. In concentrations from 40 ppm to 60 ppm the sunfish tolerated a maximum exposure time of 10 minutes. The green

Table 9. Exposure of Fungi to Different Concentrations of Dexon

Fungus Used	No. of Trials per Concentration	Controls	Observations after 48 hours on cultures sustained in conc. (ppm)							Time Exposed (min)	Observations of fungus exposed to different conc. of fungicide for various time intervals						
			400	600	800	1000	1200	$5x10^5$	$1x10^6$		400	600	800	1000	1200	$5x10^5$	$1x10^6$
Achlya flagellata	5	+	+	+	+	+	+	-	-	1	+	+	+	+	+	+	+
										5	+	+	+	+	+	+	X
										10	+	+	+	+	+	+	-
										30	+	+	+	+	+	+	-
Dictyuchus monosporous	5	+	+	+	+	+	+	-	-	1	+	+	+	+	+	+	-
										5	+	+	+	+	+	+	-
										10	+	+	+	+	+	+	X
										30	+	+	+	+	+	X	-
Saprolegnia parasitica	5	+	+	+	+	+	+	-	-	1	+	+	+	+	+	+	+
										5	+	+	+	+	+	+	+
										10	+	+	+	+	+	+	+
										30	+	+	+	+	+	-	-
Saprolegnia delica	5	+	+	+	+	+	+	-	-	1	+	+	+	+	+	+	+
										5	+	+	+	+	+	+	+
										10	+	+	+	+	+	+	+
										30	+	+	+	+	X	+	X

Table 9 (cont'd.)

Fungus Used	No. of Trials per Concentration	Controls	Observations after 48 hours on cultures sustained in conc. (ppm)							Time Exposed (min)	Observations of fungus exposed to different conc. of fungicide for various time intervals						
			400	600	800	1000	1200	$5x10^5$	$1x10^6$		400	600	800	1000	1200	$5x10^5$	$1x10^6$
Achlya klebsiana	5	+	+	+	+	+	+	-	-	1	+	+	+	+	+	+	+
										5	+	+	+	+	+	+	+
										10	+	+	+	+	+	+	+
										30	+	+	+	+	+	+	X
Thraustotheca primoachlya	5	+	+	+	+	+	X	-	-	1	+	+	+	+	+	+	+
										5	+	+	+	+	+	+	+
										10	+	+	+	+	+	+	X
										30	+	+	+	+	X	X	-

Table 10. Exposure of *Lepomis cyanellus* to TD 439

Concentration of Fungicide (ppm)	No. of Trials	No. of Fish/ Trial	Survival when sustained in fungicide										No. of Trials at Each Interval	No. of Fish/ Trial	Survival when removed from fungicide after exposure for time indicated						
			10 min	20 min	30 min	1 hr	2 hr	3 hr	6 hr	12 hr	24 hr	2 days			10 min	20 min	30 min	1 hr	2 hr	3 hr	6 hr
4	4	2	+	+	+	+	+	+	+	+	+	-	2	2	+	+	+	+	+	+	+
8	4	2	+	+	+	+	+	+	+	+	+	-	2	2	+	+	+	+	+	+	+
16	4	2	+	+	+	+	+	+	+	±	-		2	2	+	+	+	-			
23	4	2	+	+	+	+	+	+	±	±	-		2	2	+	+	+	-			
30	4	2	+	+	+	±	±	±	-				2	2	+	-					
40	4	2	+	±	±	-							2	2	+	-	-				
50	4	2	+	±	-	-							2	2	+	-					
60	4	2	+	±	-	-							2	2	+	-	-				
70	4	2	±	±	±	-							2	2	+	-	-				
80	4	2	±	±	-								2	2	-	-	-				
90	4	2	±	-									2	2	-	-					

\+ alive and well
± sick
\- dead

sunfish did not tolerate concentrations of 80 ppm and 90 ppm for 10 minutes. These data demonstrate that TD 439 is not very useful in treating or preventing mycoses of fish.

The results of tests with TD 753, as shown in Table 11, are very similar to those of TD 439. This chemical was somewhat effective against the experimental fungi. However, the effective concentrations and exposure times were well above the tolerance of the green sunfish. Here again it is evident that TD 753 would not be effective in treating the mycoses of fish.

Table 12 shows the results of the exposure of the green sunfish to TD 235. The green sunfish had a relatively high tolerance for this chemical. The fish were able to survive for at least 4 days when sustained in concentrations up to 60 ppm and 6 hours at concentrations up to 80 ppm. One-hour exposures were the maximum that the fish could safely tolerate at concentrations of 90 and 100 ppm. These data indicate that TD 235 might be a useful fungicide. However, as shown in Table 7, *D. monosporous* and *A. flagellata* can grow in concentrations up to 120 ppm. The growth of other fungi was stopped when sustained for 48 hours in concentrations of 100 ppm and 120 ppm.

The green sunfish were very tolerant to Potassium Endothal. The results of these tests are shown in Table 13. None of the concentrations tested was lethal to the green sunfish in either the sustained or time exposure experiments. It is also noteworthy that these same concentrations and exposure times were ineffective against the growth of the experimental fungi.

The results of the experiments with Dexon indicate that it might be effective against mycoses of fish. Table 14 shows that the green sunfish were able to tolerate sustained exposure for 4 hours at a

Table 11. Exposure of *Lepomis cyanellus* to TD 753

Concentration of Fungicide (ppm)	No. of Trials	No. of Fish/ Trial	Survival when sustained in fungicide							No. of Trials at Each Interval	No. of Fish/ Trial	Survival when removed from fungicide after exposure for time indicated							
			1 hr	2 hr	4 hr	6 hr	8 hr	12 hr	24 hr			5 min	10 min	20 min	30 min	1 hr	2 hr	4 hr	6 hr
5	4	2	+	+	+	+	+	+	-	2	2	+	+	+	+	+	+	+	+
10	4	2	+	+	+	+	+	±	-	2	2	+	+	+	+	+	+	-	-
15	4	2	+	+	+	±	-			2	2	+	+	+	+	+	+	-	-
20	4	2	+	±	±	±	-			2	2	+	+	+	-	-	-	-	
25	4	2	+	+	±	-				2	2	+	+	-	-	-	-		
30	4	2	+	±	-					2	2	+	-	-	-	-	-		
40	4	2	+	±	-					2	2	+	-	-	-	-	-		
50	4	2	+	-						2	2	+	-	-	-	-	-		
80	4	2	-							2	2	-	-	-	-				

+ alive and well
± sick
- dead

Table 12. Exposure of *Lepomis cyanellus* to TD 235 (6216)

Concentration of Fungicide (ppm)	No. of Trials	No. of Fish/ Trial	Survival when sustained in fungicide									No. of Trials at Each Interval	No. of Fish/ Trial	Survival when removed from fungicide after being exposed for time indicated					
			1 hr	2 hr	4 hr	6 hr	12 hr	24 hr	2 days	3 days	4 days			1 hr	2 hr	4 hr	6 hr	12 hr	24 hr
5	4	2	+	+	+	+	+	+	+	+	+	2	2	+	+	+	+	+	+
10	4	2	+	+	+	+	+	+	+	+	+	2	2	+	+	+	+	+	+
15	4	2	+	+	+	+	+	+	+	+	+	2	2	+	+	+	+	+	+
20	4	2	+	+	+	+	+	+	+	+	+	2	2	+	+	+	+	+	+
30	4	2	+	+	+	+	+	+	+	+	+	2	2	+	+	+	+	+	+
40	4	2	+	+	+	+	+	+	+	+	+	2	2	+	+	+	+	+	+
50	4	2	+	+	+	+	+	+	+	+	+	2	2	+	+	+	+	+	+
60	4	2	+	+	+	+	+	+	+	+	+	2	2	+	+	+	+	+	+
70	4	2	+	+	+	+	+	+	±	±	-	2	2	+	+	+	+	-	-
80	4	2	+	+	+	+	±	±	-	-	-	2	2	+	+	+	+	-	-
90	4	2	+	+	+	±	-	-	-			2	2	+	+	-	-	-	
100	4	2	+	±	±	±	±	±	-			2	2	+	-	-	-		

+ alive and well
± sick
- dead

Table 13. Exposure of *Lepomis cyanellus* to Potassium Endothal

Concentration of Fungicide (ppm)	No. of Trials	No. of Fish/ Trial	Survival when sustained in fungicide											No. of Trials at Each Interval	No. of Fish/ Trial	Survival when removed from fungicide after being exposed for time indicated						
			1 hr	2 hr	4 hr	6 hr	8 hr	1 day	2 days	3 days	4 days	5 days	6 days			1 hr	2 hr	4 hr	6 hr	8 hr	12 hr	24 hr
10	4	2	+	+	+	+	+	+	+	+	+	+	+	2	2	+	+	+	+	+	+	+
20	4	2	+	+	+	+	+	+	+	+	+	+	+	2	2	+	+	+	+	+	+	+
30	4	2	+	+	+	+	+	+	+	+	+	+	+	2	2		+	+	+	+	+	+
40	4	2	+	+	+	+	+	+	+	+	+	+	+	2	2		+	+	+	+	+	+
50	4	2	+	+	+	+	+	+	+	+	+	+	+	2	2		+	+	+	+	+	+
60	4	2	+	+	+	+	+	+	+	+	+	+	+	2	2		+	+	+	+	+	+
70	4	2	+	+	+	+	+	+	+	+	+	+	+	2	2		+	+	+	+	+	+
80	4	2	+	+	+	+	+	+	+	+	+	+	+	2	2		+	+	+	+	+	+
90	4	2	+	+	+	+	+	+	+	+	+	+	+	2	2		+	+	+	+	+	+
100	4	2	+	+	+	+	+	+	+	+	+	+	+	2	2		+	+	+	+	+	+
120	4	2	+	+	+	+	+	+	+	+	+	+	+	2	2		+	+	+	+	+	+

+ alive

Table 14. Exposure of *Lepomis cyanellus* to Dexon

Concentration of Fungicide (ppm)	No. of Trials	No. of Fish/Trial	Survival when sustained in fungicide									No. of Trials at Each Interval	No. of Fish/Trial	Survival when removed from fungicide after exposure for time indicated										
			10 min	20 min	30 min	1 hr	2 hr	4 hr	6 hr	12 hr	1 day			10 min	20 min	30 min	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr	8 hr
100	4	2	+	+	+	+	+	+	+	+	+	2	2	+	+	+	+	+	+	+	+	+	+	+
200	4	2	+	+	+	+	+	+	+	+	+	2	2	+	+	+	+	+	+	+	+	+	+	+
300	4	2	+	+	+	+	+	+	+	+	+	2	2	+	+	+	+	+	+	+	+	+	+	+
400	4	2	+	+	+	+	+	+	+	+	+	2	2	+	+	+	+	+	+	+	+	+	+	+
600	4	2	+	+	+	+	+	+	+	+	+	2	2	+	+	+	+	+	+	+	+	+	+	+
800	4	2	+	+	+	+	+	+	+	+	+	2	2	+	+	+	+	+	+	+	+	+	+	+
1000	4	2	+	+	+	+	+	+	+	+	+	2	2	+	+	+	+	+	+	+	+	+	+	+
1200	4	2	+	+	+	+	+	+	+	+	±	2	2	+	+	+	+	+	+	+	+	+	+	+
$5x10^5$	4	2	+	+	+	+	+	+	±	-		2	2	+	+	+	+	+	+	+	±	±	±	-
$1x10^6$	4	2	+	+	+	+	+	±	-			2	2	+	+	+	±	±	±	-	-			

+ alive and well
± sick
- dead

concentration of 5 x 10^5 ppm and 2 hours at a concentration of 6 x 10^6 ppm with no adverse effects. However, the time exposure tests indicated that fish exposed for 30 minutes were not affected but that greater exposure time would ultimately kill the fish. Table 9 shows that the growth of the fungi could be stopped or inhibited by exposing them to a concentration of 1 x 10^6 ppm for 30 minutes.

Fungal infections were noted on all species of fish used in this project. The first signs of infection of injured fish always appeared at the site of injury. Tufts of hyphae formed on the caudal peduncle and spread over this region. In fatal infections, the fungal mycelia would spread over the entire body of the fish, covering it with a fuzzy mycelial growth. The time necessary for the fungi to kill the host fish depended on the size and species of the fish. It was noted that 24 hours was enough time for the fungal growth of certain species to cover and kill the bluntnose minnow; however, more than 3 days were usually required for the fungal mat to entirely cover and kill the rosyface shiner. No specific time was established for the fungi to be lethal to the green sunfish. Preliminary experiments indicated that it took much longer for the infection to become established on the fish and to spread over the body. The green sunfish was also relatively active for 24 hours after the body was entirely covered by a mycelial mat. The lesions caused by fungal infections of uninjured fish were similar to those of injured fish except that the initial regions of infection were not always the same. The head, the area around the dorsal fins, and the caudal peduncle were the most susceptible regions to fungal infections. Inflammatory reactions usually occurred in the area immediately adjacent to the spot of infection. As with the infected injured fish, there was no mucus covering the infected areas.

Figure 1 shows the rainbow trout with mycelial mats growing on the regions cited above. It is also evident that the fungus is invading the gill area (opercle) of this fish and spreading along the ventral surface to the pectoral and anal fins. Within 10 days after this photograph was taken, the body was almost entirely covered with the fungal mycelia. The skin on the infected area sloughed off in a sequential manner, with the area of earliest infection sloughing off first. The scales, fins and fin rays were softened or completely lost and/or destroyed. The adipose fin was usually the first to be destroyed.

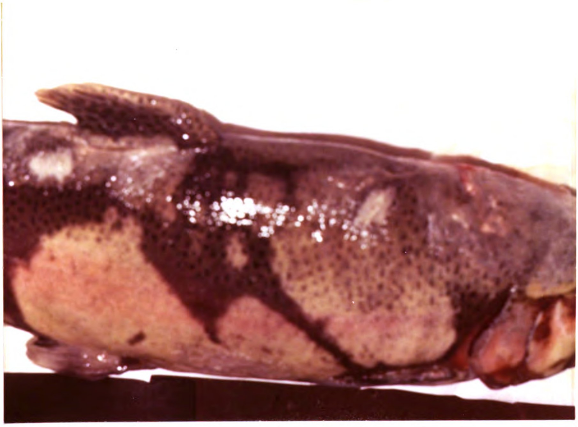
The rainbow trout was also very active with most of the body covered with fungal mycelia. This fish lived for 14 days after the photograph was taken. By this time even the eye orbitals were covered with fungal mycelia, and several small open lesions were on the body.

The time required by the fungi to kill uninjured fish could not be specifically established. It was clear, however, that when the initial infection was in a vital area the time required was much less than when nonvital areas were initially infected.

In order to further ascertain the extent and effect of the growth of the fungi on the fish, tissue blocks were taken from infected and uninfected brown trout. Specific histochemical procedures were performed on these pieces of tissue. The photomicrographs of Figures 2 through 4 show the results.

Figures 2A and 2B are photomicrographs of uninfected tissue of the brown trout stained with Ehrlich's (1886) hematoxylin and counter stained with eosin. Figure 2A illustrates that the skin consists of a dermis (A,2) in which the scales are embedded and a very distinct epidermis (A,1). Fibrous connective tissue underlies the dermis and is separated

Figure 1. Photomicrograph of fungus-infected rainbow trout (approximately 1/4X) showing mycelial growth on dorsal and ventral surface of body.



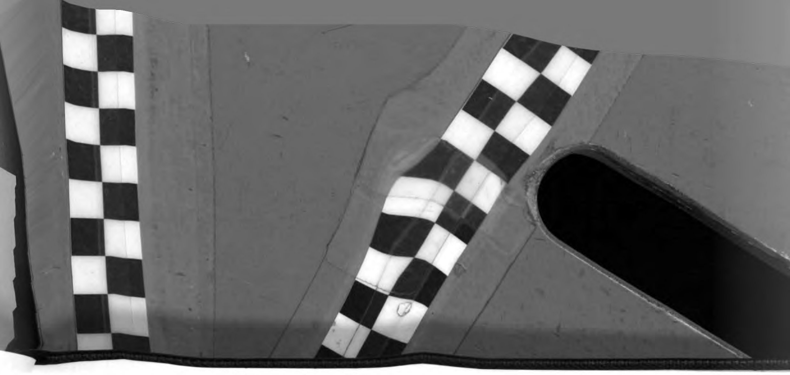
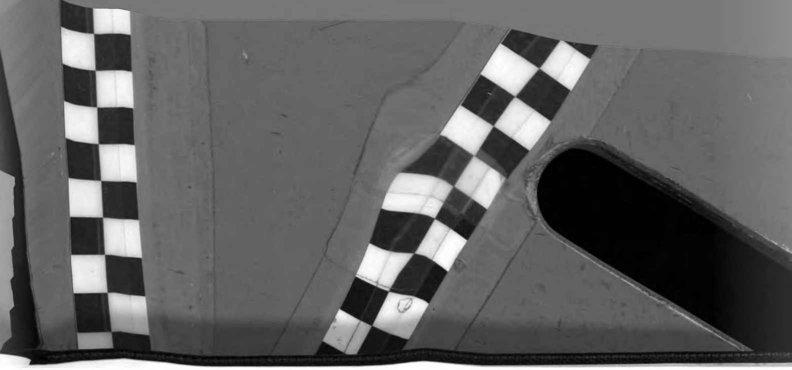
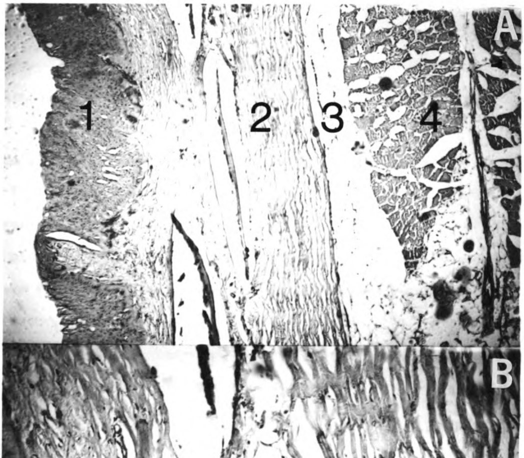


Figure 2. Photomicrograph of a section of uninfected tissue of a brown trout stained with Ehrlich's hematoxylin. Figure 2A shows 400X magnification of skin and muscle; A,1 - epidermis, A,2 - dermis, A,3 - subcutis, A,4 - muscle bundles. Figure 2B shows an enlargement (850X) of the dermis with a small portion of the epidermis and subcutis.




A
1
2
3
4
B

from the muscle (A,4) by a loosely packed subcutis (A,3). The muscle bundles are shown below the subcutis and are separated by myosepta. Figure 2B shows an enlargement of the dermis.

A portion of the dermis and all of the epidermis of an uninfected brown trout is shown in Figure 3. The tissue was stained using the PAS procedure. This photomicrograph shows the numerous mucous glands that are located in the epidermis and the somewhat fibrous nature of the dermis.

The photomicrographs of infected tissue illustrate that the growth of the fungus completely disintegrated the epidermal layer. Figure 4A and Figure 4C further show the infiltration of these mycelia into the dermis of the brown trout. The dermal infiltration is best illustrated by Figure 4C. The orderly fibrous dermis, as seen in Figures 2 and 3, is now disorganized by the growth of fungal mycelia. At this stage of development, no fungal mycelia were evident in the areas of the subcutis or muscle bundles; however, it was noted that the skin was not firmly bound to the muscle tissue, as with the uninfected fish.

The fungus growing on the brown trout was definitely reproducing. Numerous sporangia were present in the mycelial mat on the body of the fish as illustrated by Figures 4A and 4B. No sexual reproductive structures were observed on any of the sections.

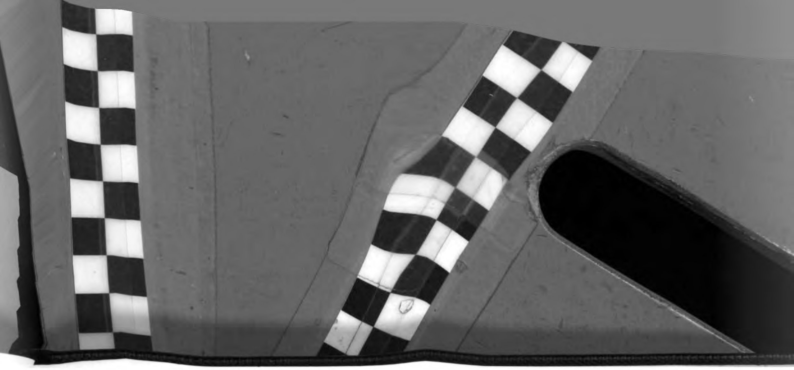
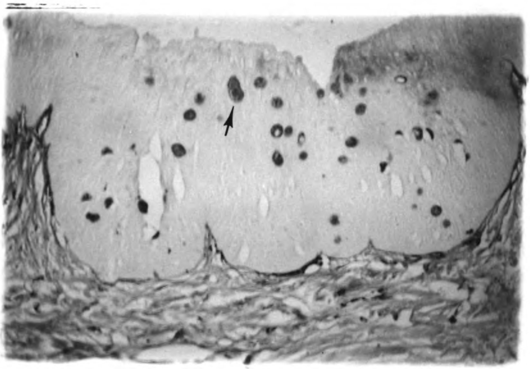


Figure 3. Photomicrograph of a section of epidermis and a portion of the dermis of an uninfected brown trout (1200X). The tissue was stained by the PAS technique. The arrow points to one of the numerous gland cells of the epidermis.



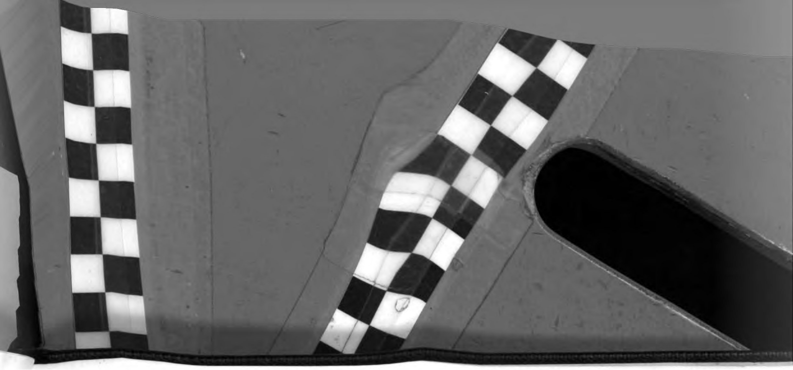


Figure 4. Photomicrographs of a section of fungal infected tissue of a brown trout stained with Ehrlich's hematoxylin (Figures 4A and 4B) and Pianese IIIB stain (Figure 4C). Figure 4A shows the dermis (1200X) with fungal mycelia that have disintegrated the epidermis. Figure 4B (950X) is a view of the fungal mycelia and sporangia growing on the tissue shown in Figure 4A. The arrow points to a sporangium of the fungus. Figure 4C shows (1300X) the infiltration of the dermis by fungal mycelia.

ue

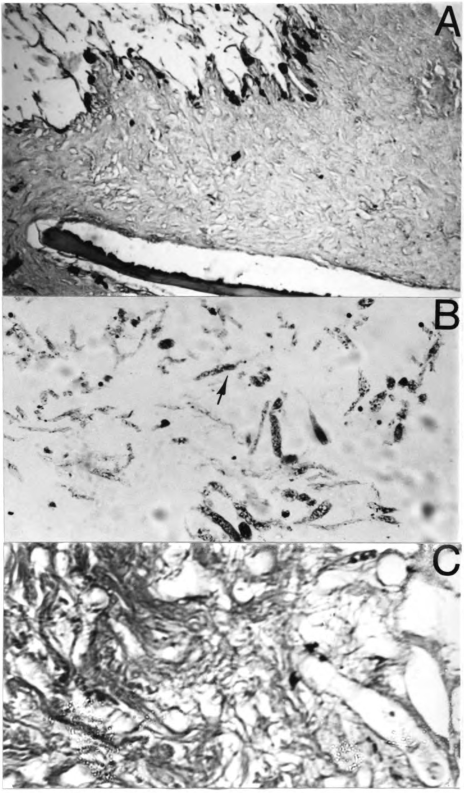
B)

X)

3

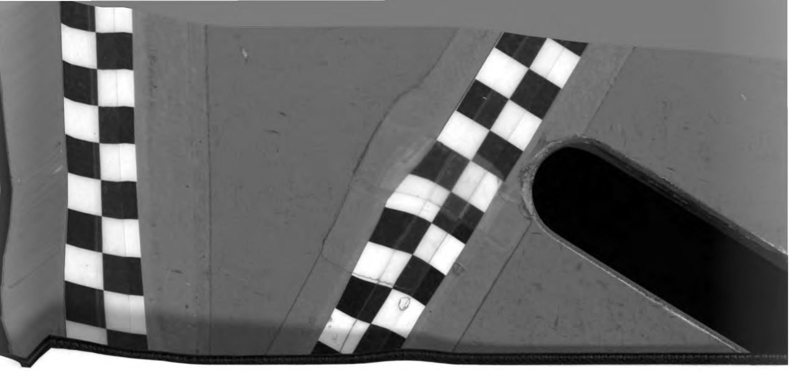
is-

us.



DISCUSSION

The infectious nature of some saprolegniaceous fungi has been known for some time. Several studies in recent years have investigated some phases of the mode of infection of these fungi and the mycoses they cause. None of these studies includes data concerning the ability of mycelia to cause mycotic infections of fish. The results obtained in this study clearly indicate that exposure of fish to homogenates of certain saprolegniaceous fungi will result in mycoses of these fish. Mycelial homogenates of *S. parasitica* and *S. ferax* were very infectious to both the injured bluntnose minnow and the rosyface shiner. They were the most infectious of all the fungi used. *Saprolegnia parasitica* is considered to be the most common and most infectious pathogenic water mold found in nature. *Saprolegnia ferax* is considered next in pathogenicity and frequency of occurrence. This is verified by the works of Agersborg (1933), Scott and O'Bier (1922) and Martin (1968). The results, however, of the present study of mycelial homogenates indicate that *S. parasitica* and *S. ferax* are almost equal in pathogenicity of injured fish. It is also evident that *S. parasitica* is more infectious to uninjured fish than is *S. ferax*, viz., the mycelia of *S. parasitica* were able to infect and kill both injured and uninjured bluntnose minnows within 24 hours, whereas the injured rosyface shiners were only infected by the *S. parasitica* and *S. ferax* during the same time period.

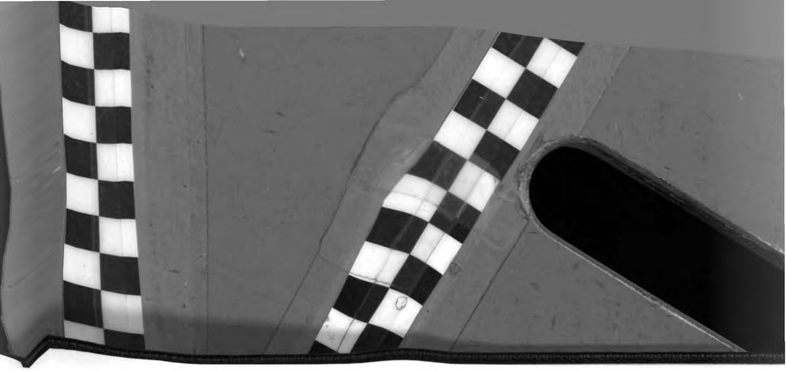


The mycelia of *S. ferax* infected only 5% of the uninjured fish. It appears that the mycelia of *S. ferax* are incapable of being highly infectious except when the fish is injured.

The mycelia of *A. flagellata* proved to be slightly infectious to the injured bluntnose minnow, although the mycelia were not infectious to the uninjured bluntnose minnow nor to any of the rosyface shiners. These data indicate that the mycelia of *A. flagellata* are not nearly as pathogenic as *S. parasitica* or *S. ferax*. Tiffney and Wolf (1937) reported that *A. flagellata* was a naturally occurring pathogen of fish. It may then be a secondary invader or principally infectious in another form.

Dictyuchus monosporous was also reported as being a natural pathogen of fish. Tiffney (1939b) identified several species of saprolegniaceous fungi associated with mycoses of fish; *D. monosporous* was one of the fungi. The mycelia of *D. monosporous* did not prove to be infectious to either the injured or uninjured fish of either species. These data may indicate that *D. monosporous* is not infectious to fish or that it is a secondary invader; however, in making preliminary collections, *D. monosporous* was isolated from an infected sunfish taken from a pond. No other fungi could be isolated from this fish. This finding, therefore, questions whether *D. monosporous* is a primary or secondary invader, or simply host specific. The data may only demonstrate that infections of *D. monosporous* cannot be induced.

Results of the present study have demonstrated that not only are the mycelia of certain fungi infectious but also that contact among fish increased the frequency of infection. It appears that once the fish is infected it becomes a vector by which the infection spreads to other fish. As stated above (Table 2), only 33% of the uninjured bluntnose minnows



and 50% of the injured rosyface shiners were infected when placed in tanks with septa. This represented a decrease of 67% and 50%, respectively, contrasted with the situation when they were exposed in tanks without septa. This could possibly have some effect on results obtained in other studies on infections by exposure to zoospores of these fungi. In studies such as those of Tiffney (1939a, 1939b), Hoffman (1949) and Scott and O'Bier (1962) the fish were allowed to be in physical contact. It is highly possible that the fish that were infected first helped to spread the infection to others by mycelial contact.

The data obtained from the present study of mycelial homogenates of *S. parasitica* indicate that an exposure time of 12 hours was necessary to infect the injured bluntnose minnows and 18 hours of exposure time to infect the uninjured fish. After these exposure times the fish could be removed from the mycelial homogenate and the infection would continue, resulting in death to the fish in about 24 hours.

Thirty-three percent of the uninjured fish exposed to the mycelial homogenate for 18 hours were not infected. Several of the uninjured fish were able to withstand exposures of 24 and 30 hours. Nevertheless, both the 24 and 30 hour exposures were ultimately 100% lethal to the fish.

No experiments were made using the other fungi or the rosyface shiner. Therefore, the exposure needed for these infections to occur is not known.

The data obtained from the study of the infection of the green sunfish by zoospores of various water molds concurred generally with that frequently found in the literature. *Saprolegnia parasitica* was found to be highly infectious to injured green sunfish and only slightly infectious to uninjured fish. Tiffney (1939a, 1939b), Coker (1923), and

Kanouse (1932) reported that *S. parasitica* was a naturally occurring parasite of fish. *Saprolegnia parasitica* was isolated 14 times from localities in 5 states by Scott and O'Bier (1962). They, along with other investigators, were able to induce infections by zoospores using several saprolegniaceous fungi. It was shown that the zoospores of *S. parasitica* were the most pathogenic of the fungi and were primarily a wound invader.

The experiments performed in this work differ from those of Scott and O'Bier and other previous investigators in that the fish were not allowed to come into physical contact with the fungi or with each other. This was accomplished by the use of screen wire over the mycelial mats and wire septa between the fish.

Saprolegnia sp. gave very similar results to those of *S. parasitica*. The species of this fungus could not be determined because of the lack of sexual fruiting structures. It is highly possible that it was also *S. parasitica*. The numerous gemmae formed and the general pattern of growth on hemp seed added credence to the supposition that the two were of the same species.

Scott and O'Bier (1962) recorded that zoospores of *S. delica* were able to infect the platyfish within 24 hours. In their work some of the fish recovered from the infection. The data in Table 4 illustrate that induced infections of green sunfish by zoospores of *S. delica* are possible without mycelial or fish contact. However, none of the infected fish recovered. No specific time was established for the infection to occur or for this fungus to kill the green sunfish. *Saprolegnia delica* proved to be the second most pathogenic species tested if we consider *S. parasitica* and *Saprolegnia sp.* as being of the same species.

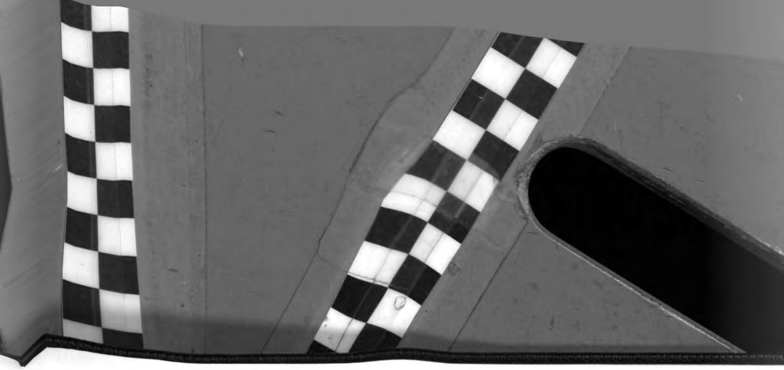
Hoffman (1949) exposed two species of sunfish, three species of minnows and one species of suckers to zoospores of *S. parasitica*, *S. ferax* and *A. racemosa*. His method of exposure was generally the same as that of Klebs (1899), Pieters (1915) and Tiffney (1939a). The fish were held in the experimental tanks from 12 to 24 days. Of the 39 fish exposed, 6 injured and 1 uninjured fish became infected with *S. parasitica* and *S. ferax*. *Achlya racemosa* was not infectious to any of the fish. The results of this work on *S. parasitica* have been discussed above. They do, however, concur with those of Hoffman (1949). The zoospores of *S. ferax* were not used to induce infections of the green sunfish. The zoospores of *A. racemosa* were used and also found not to infect either the injured or uninjured green sunfish. *Achlya racemosa* is recorded by Humphrey (1893) as being a naturally occurring fish parasite.

The zoospores of *A. flagellata* and *A. klebsiana* are also recorded as being pathogenic to fish. Induced infections by zoospores of both fungi have been reported. Vishniac and Nigrelli (1957) were able to induce infections of injured platyfish with zoospores of *A. klebsiana*. The work of Tiffney and Wolf (1937), Scott and O'Bier (1962) and Martin (1968) documented that induced infection by zoospores of *A. flagellata* were possible. In the course of the present study, the zoospores of *A. klebsiana* did not infect either the injured or uninjured green sunfish. *Achlya flagellata* proved to be only slightly infectious to injured sunfish. It appears that these fungi are not primary infectants of the green sunfish.

The zoospores of *D. monosporous* and *T. primoachlya* did not infect any of the sunfish. Vishniac and Nigrelli (1957) induced infections of injured platyfish with zoospores of *T. primoachlya*. The work of Tiffney

(1939b) illustrated that *D. monosporous* was a naturally occurring fish parasite. The failure of the zoospores of these fungi to infect the green sunfish is impossible to explain at this time. These fungi may be host specific to some extent or the green sunfish may be resistant to infection by them. It is also possible that the experimental conditions were not conducive to their development. It should be noted that these fungi did not infect any of the experimental fish, neither by mycelial contact nor by zoospores.

It appears conclusive from this and other works that certain members of Saprolegniaceae are primary wound invaders and very lethal pathogens to certain species of fish. Very little is known about the actual host-parasite relationship. It is not at all clear whether it is truly a host-parasite relationship. The saprolegniaceous fungi are certainly not obligatory parasites. Table 1 records that fungi were isolated from baiting a sample of plants and bottom debris from the bluntnose minnow stock tank. No fish in this stock tank were infected by the fungus during the experiments. It appears that the fungi are capable of living on plant and other materials. It is also widely known and accepted that these fungi will grow well on artificial media such as hemp seeds, sesame seed, special agars and oatmeal flakes. These fungi will not usually produce sexual reproductive structures unless special fruiting media is used. This is particularly true with respect to the genus *Saprolegnia*, the most important genus. The vegetative structures will develop and the complete asexual reproductive cycle will occur. Special fruiting media were reported by Kanouse (1932) for *S. parasitica* but were found not to always work by Scott and O'Bier (1962). There is very little information in the literature concerning the physiology of formation of sexual reproductive structures in fungi.



Fish culturists have for many years been concerned about the problem of control and prevention of mycoses of fish. Several investigators have reported the results of experiments performed on the fungicidal ability of various chemicals. A number of the earlier works, such as O'Donnell (1941), Rankin (1952), and Estes (1957) have been strongly criticised for the lack of conclusive taxonomic data concerning the fungi. It is impossible to assess the value of a chemical as a fungicide without determining the particular species of fungi causing the infection. More recently Scott and Warren (1964) and Martin (1968) demonstrated the fungicidal effect of certain chemicals on specific species of saprolegniaceous fungi.

The results of the present study have indicated that several of the fungicides used were effective in sustained tests. Such results can be noted on Tables 5 and 9, which record the results of TD 439 and Dexon, respectively. None of the chemicals used was effective as a fungicide in the time (interval) exposure tests.

Most of the previous investigations were performed by dipping infected fish into certain chemicals for various lengths of time. The present work was conducted in a similar manner as Martin (1968). The procedure differs in concentrations and chemicals used, the time intervals and the use of hemp seeds as culture media rather than sesame seeds. Moreover, Martin (1968) did not investigate the tolerance of any host fish to the chemicals. The results of such tests cannot be compared directly with those derived from dip treatments of fish infected with fungi. It is a test of the fungicidal abilities of a certain chemical on fungi growing on media suitable for their growth. These tests are very valuable in establishing the worth of a potential fungicide.

It is just as important to test the tolerance of the fish to the chemical as to test its fungicidal effectiveness. In this study it was quite often the case that a given concentration of a chemical was effective against the growth of the fungi but was above the tolerance limits of the host fish. This is to say that a chemical must be an effective fungicide at a concentration that fish can tolerate in order to be considered a good agent for controlling and preventing mycoses of fish.

TD 439 either stopped or inhibited the growth of all fungi used in the sustained experiments. However, the effective concentrations and exposure times were well above the tolerance of the green sunfish. The same is generally true for TD 753. Neither of these chemicals could be considered as an effective fungicide in treating infection caused by certain saprolegniaceous fungi.

The green sunfish had a relatively high tolerance for TD 235 and Potassium Endothal. The concentrations and exposure times needed for TD 235 to stop the growth of the fungi tested was also well above the tolerance limits of the green sunfish.

None of the concentrations of Potassium Endothal tested were lethal to the green sunfish in either the sustained or time exposure experiments. However, all of the fungi used were able to grow in all concentrations of this chemical when sustained for 48-72 hours. There may be a concentration above the tested ones that is effective against the fungi and not lethal to the host fish. Based on the data, as collected, Potassium Endothal did not prove to be a valuable fungicide.

The results of tests using Dexon indicate that it might be effective against fungal infections of fish. The green sunfish was very tolerant to the chemical at high concentrations over long periods of exposure. If it is concluded that a chemical is effective when it is functional

at low concentrations, then Dexon would not be a good fungicide. The only effective concentrations were 5×10^5 ppm and 6×10^6 ppm when in contact with the fungi for a period greater than 30 minutes. Green sunfish exposed for periods greater than 30 minutes ultimately died.

Dexon is a compound that is ordinarily only slightly soluble in water. The chemical is available as a 70% wettable powder. The 70% wettable form was used in these experiments to solve the problem of relative insolubility. All forms of the compound decompose slowly in water. This decomposition is greatly accelerated by light. The decomposition problem appears to be more serious with dilute than with concentrated solutions.

The above statements are based on the over-all effectiveness of the chemicals on the test fungi collectively. It is interesting that the fungus *S. parasitica*, which is reported to be the most pathogenic, was controlled by all test chemicals except Potassium Endothal. This concurs generally with the findings of Scott and Warren (1964) and Martin (1968), even though the chemicals tested were not the same. The other fungi exposed to the chemicals were not highly infectious in either the mycelial or zoospore experiments. It may be possible to use some of the test chemicals to stop the growth of *S. parasitica* on infected fish. However, this would not solve the problem of mycoses of fish. Certain other fungi proved to be highly resistant to the fungicides used and were shown to be infectious to certain fish.

There is little or no histological data in the literature concerning the effect of the infection on the tissue of the host fish. Vishniac and Nigrelli (1957) reported that histological examinations of stained sections of infected platyfish indicated the destruction of the tissue

of the fish was due almost exclusively to the penetration of fungal mycelia. The results of the present study concur with those of Vishniac and Nigrelli (1957).

The photomicrographs of infected tissue illustrated that the growth of the fungus completely disintegrated the epidermal layer. Figure 3 shows a portion of the epidermis with numerous mucous glands. They are very common and widely distributed in this area. These glands produce mucin, a glycoprotein, which with water forms mucus. The thick, slimy lubricating secretion serves to protect the fish and reduce the friction. It is generally accepted that this mucus has no fungicidal ability. However, it does offer a coating for the body that is constantly changing and thus decreasing the possibility of fungal infections. The destruction of the epidermis by the infiltration of fungal mycelia therefore destroys the gland cells as well (Figure 4A).

Figure 4C shows an enlargement of the dermis area of Figure 4A. The orderly dermis, as seen in Figures 2 and 3, was infiltrated by the fungal mycelia. It appears that initially the mycoses tend to spread through the skin very rapidly covering the body of the fish with a fuzzy mycelial growth. Figure 4B illustrates that the fungus is reproducing asexually. Therefore, the spreading of the fungus may be due to both mycelial growth through the skin and germination and growth of zoospores. It was evident at this stage of development that no mycelia had penetrated into the subcutis and muscle bundle areas. The lack of penetration of the fungal hyphae into the deeper tissues of the host fish at this stage of development tends to further support the growth pattern discussed above. This may account for the rainbow trout shown in Figure 1 and other experimental fish being active for relatively long periods after the body was almost completely covered with a mycelial mat. These

data may further explain why the smaller fish were killed so rapidly. Vishniac and Nigrelli (1957) reported that the penetration of the hyphae into deep tissues such as the muscle bundles resulted in hyalinization or complete destruction. However, this occurred after the epithelium was sloughed off and the scales and fin rays were softened or destroyed.

In the present study several lesions were observed on the body of the fish as the infection progressed. The lesions were quite often observed in the areas of initial infection. One such lesion is illustrated on the dorsal anterior portion of the rainbow trout shown on Figure 1. It appears that these lesions result from the exposure of muscle tissue after the skin sloughs off. This is not to imply that all lesions on infected fish result in this manner. Some lesions are simply the results of injury to the fish.

SUMMARY

Mycelial homogenates of various species of saprolegniaceous fungi were found to be infectious to both the bluntnose minnow and rosyface shiner. The data indicated that *S. parasitica* and *S. ferax* were highly infectious to the injured fish. A study of mycelial homogenates of *S. parasitica* indicated that an exposure time of 12 hours was necessary to infect the injured bluntnose minnows and 18 hours of exposure to infect the uninjured fish. Death generally resulted in about 24 hours. The uninjured bluntnose minnow was more affected by the fungi than was the uninjured rosyface shiner. It is noteworthy that the use of partitions in the experimental tanks greatly reduced the percentage of infection. These data indicated that physical contact among fish has some effect on the spreading of infectious material.

Mycelial homogenates of *A. flagellata* were found to be slightly infectious to only the injured bluntnose minnows. *Dictyuchus monosporous* did not infect either the injured or uninjured fish.

The data obtained from the study of infections by zoospores of certain saprolegniaceous fungi concurred generally with that frequently found in the literature. The experiments performed in this work differ with previous studies in that the fish were not allowed to come into physical contact with the fungi or with each other.

It was shown that the zoospores of *S. parasitica* were the most pathogenic of the fungi and were primarily a wound invader. Zoospores of *Saprolegnia sp.*, *S. delica* and *A. flagellata* were the only other fungi

to be infectious. These fungi were only infectious to injured green sunfish.

In the present study various species of saprolegniaceous fungi were exposed to different concentrations of five experimental chemicals. The results indicated that TD 439 and Dexon were effective in sustained exposure tests but none was effective as a dip. TD 439, TD 753 and Dexon were able to stop the growth of common infectants such as *S. parasitica*. No chemical tested was able to stop the growth of all test fungi at a concentration that the green sunfish could tolerate.

Observations made on infected fish indicated that the fish were often very active with the body almost covered by the growth of the fungus. It appears from histochemical data that the growth spread through the skin and then down into the deeper tissue. The skin was completely destroyed by the infiltration of the fungal mycelia. The fungi were reproducing asexually by zoospores. Therefore, the spreading of the fungus may be due to both mycelial growth through the skin and germination and growth of zoospores.

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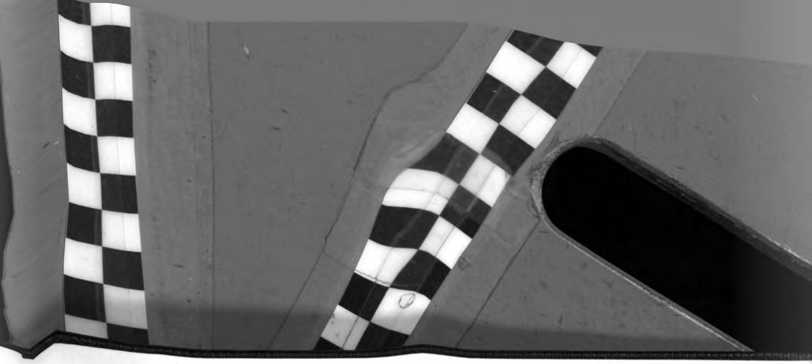

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