A PRELIMINARY REPORT ON THE CONTROL OF PATHOGENIC FUNGI IN EARTHEN CULTURE PONDS

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Abstract: Copper sulfate was evaluated as a potential control for parasitic fungal infestations on largemouth bass (*Micropterus salmoides*) and striped bass (*Morone saxatilis*). Treatments were planned to reduce the incidence of free-swimming zoospores of the fungi. Largemouth bass fingerlings (15-20 cm T.L.) were stocked in 0.04 ha ponds and given multiple treatments of copper sulfate at 0.5; 1.0 or 2.0 ppm or Dimethylamine salt of 2,4-Dichlorophenoxy (acetic acid) at 2.0 ppm. Chemical treatments were made on the day before stocking and at regular intervals thereafter for 6 treatments. Survival rates of largemouth bass treated with copper sulfate were 96, 91.7 and 100% at concentrations of 0.5; 1.0 and 2.0 ppm respectively; only 10.7% of the controls survived. No significant difference of survival rate between control and 2,4-D treated fish was noted. Striped bass adults were treated with multiple copper sulfate treaments a 1.0 ppm after being captured in gill nets. All striped bass controls were lost, whereas, only 2 of 39 contacted infesetationsof fungi after treatments. Preliminary in-vitro studies showed that Formalin, Malachite Green and Potassium Permanganate are not as effective as copper sulfate.

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Parasitic fungal infestations of fish have been among the most difficult diseases to control. Saprolegniaceae infestations are a widespread problem and almost universally present in natural fresh waters (Hoshina 1963, Tiffney 1939a, 1939b). Heavy chemical concentrations needed to kill established mycelium exceed the levels that can be used in ponds without causing fish mortalities. Recommended treatments for eggs and fish usually involve a high concentration for a short term treatments (Hoffman 1969).

Losses of largemouth bass broodstock to fungal infestations have been encountered on most warm water culture stations. These infestations are generally associated with stress or injury and the mortality rate may be high. In many cases, moving of broodfish between forage ponds during cold weather will trigger an outbreak. In other instances, outbreaks follow infestations of parasitic protozoans or copepods.

At one hatchery, 8,904 largemouth bass fingerlings (15-20 cm T.L.) were harvested from rearing ponds in December and transferred to another station to be marked before stocking. Outbreaks of fungi caused the loss of all but 134 fish despite all control efforts. A similar situation was encountered at Tishomingo National Fish Hatchery when 550 largemouth bass broodstock were moved to another pond in February. A fungal outbreak occurred 10 to 14 days later and despite all treatment efforts, a loss of 60% ensued.

Other fish species that are susceptible to fungal infestations and in which heavy losses are encountered include: striped bass adults, walleye (Stizostedion vitreum), flathead catfish (*Pilodictis olivaris*), and channel catfish (*Ictalurus punctatus*).

The inability to control fungi in culture ponds led to a search for new and effective methods. Most parasitic fungi are transmitted by flagellated zoospores (Kanouse 1932). Removal of viable zoospores in ponds prior to and for a length of time following fish stockings, might keep susceptible fish from contracting infestations until they were able to repair any damage from handling. With this as the objective, a pond test program was developed to treat ponds with multiple treatments of copper sulfate and 2,4-D.

METHODS AND MATERIALS

Largemouth bass fingerlings (15-20 cm T.L.) were obtained from a commercial fish farm (Lot I) and from San Marcos Fish Cultural Development Center (Lot II). Both lots were trained to accept pelleted feed and reared utilizing the technique described by Snow (1971). Lot I was stocked in a .4 surface ha rearing pond and fed a commercial floating trout diet; whereas, Lot II was stocked in a raceway and fed the U.S. Fish & Wildlife Service, Region II open formula sinking trout diet. The feeding rate for Lot I was reduced as water temperatures lowered until feeding ceased (about 9 C) about 30 days before this project was initiated. Lot II was reared in constant temperature water and fed at a maximum rate until just prior to harvest.

Fish from both lots were handled carefully, hauled approximately the same length of time, then transferred to test ponds. Fish in Lot I were marked in the lower portion of the caudal fin with a paper punch, whereas, those in Lot II were marked in the upper portion.

Ponds were selected for tests according to the amount and type of organic matter present. Four ponds had been dry for at least 6 months and contained a thick growth of Bermuda grass, 4 ponds had little or no plant growth, and 5 ponds had been dry about 40 days and contained a heavy growth of dead aquatic plants. Ponds were filled at least one day prior to stocking.

Chemicals tested as possible fungal controls were copper sulfate and the Dimenthylamine salt of 2,4-Dichlorophenoxy (Acetic acid). Rates of chemicals used were 0.5, 1.0 and 2.0 ppm copper sulfate and 2.0 ppm 2,4-D. Three ponds were used as controls, 1 each containing light, medium and heavy amounts of dead vegetation. All chemical treatments were used in 3 ponds except copper sulfate at 2.0 ppm which was tested in only 1 pond containing a light amount of vegetation.

Chemical treatments were applied by the dipper method on the day prior to stocking and on the 1st, 3rd, 6th, 8th, 10th, 13th and 20th day after stocking.

Ponds were observed daily and dead fish removed unless ice cover was present. After 48 days, the ponds were drained and live fish were counted and observed for fungi infestations.

Striped bass

Striped bass ranging from .9 to 12.7 kg were captured using gill nets in Lake Texoma. These fish were transported to the National Fish Hatchery, Tishomingo OK to be used as brood stock. The fish were stocked in 3 .04 surface ha ponds, 1 pond to serve as a control and 2 as test ponds. Test ponds were treated with 1.0 ppm copper sulfate and an equal amount of citric acid on the day preceding stocking and 5 additional treatments applied every other day thereafter.

Adrenalin was injected into the tissue surrounding the eye in about 66 percent of the fish in both treated and control ponds to determine the success of this drug in alleviating stresses of capture and handling. Any fish that succumbed within 5 days after stocking was considered to have died of netting and handling stress and not included in the data tabulated for fungus control. Dead fish were removed daily from the ponds, and the ponds were observed to detect fungal invasions. Thirty-six days after stocking, the ponds were drained and the remaining striped bass were counted.

In-vitro

A culture of fungus was removed from a largemouth bass from a control pond and grown on hemp seed and cornmeal agar. Preliminary in-vitro tests were initiated to test the fungicidal activity against zoospores with formalin, malachite green and potassium permanganate at 0.25, 0.1 and 2.0 ppm respectively. Copper sulfate was tested at 0.25, 0.5 and 1.0 ppm.

Additional fungi were cultured on hemp seed in 40 ml water in 120 mm petri dishes. After zoospore formation, a 0.5 ml aliquot of water containing zoospores was placed in 120 mm petri dishes containing 2 hemp seed halves and 30 ml water. Chemical treatments were added directly to the petri dishes after inoculation. Each chemical and treatment level was tested in triplicate. Each chemical and treatment level (in triplicate) was exposed to 1, 2, and 3 treatments administered at 2 day intervals. The absence of fungal growth was considered an indication of fungicidal activity.

RESULTS

A difference between Lot I and II largemouth bass fingerlings was noted on the morning following stocking in all control and test ponds. Fish from Lot II had turned dark and were resting upright and scattered over the pond bottom; the fish from Lot I were swimming in a loose school. By the second day some of the fish from Lot II were lying on their sides on the pond bottom but were not dead. Dead fish were recovered from ponds on the 5th day after stocking. Within 18 days all fish from Lot II had succumbed.

When dead fish were removed from the control pond and from the ponds treated with 2,4-D they were found to be heavily infested with fungi. Those which received copper sulfate treatments showed no signs of fungi invasion. Two dead fish were left in each pond for over 30 days. When removed, those fish in the copper sulfate treated ponds still had no infestation of fungi, although bacterial decay was well advanced.

Survival rates of fish harvested after being in the ponds 48 days at temperatures ranging from 2 to 9 C are given in Table 1. The ponds were ice covered for about 50 percent of the observation period. No fish infested with fungi were observed in ponds treated with copper sulfate during the study, even though moribund and/or dead fish heavily infested with fungal infestations were observed after the 10th day in control and 2,4-D treated ponds.

Table 1. Survival rate of LMB in culture ponds after multiple chemical treatments to control parasitic fungi.

Pond	*Treatment*	*Survival rate (%)*	*Mean Survival rate (%)*
E-7	C	8	—
F-10	C	10.7	10.7
F-7	C	16	—
E-8	.5 ppm $CuSO_4$	100	—
F-11	.5 ppm $CuSO_4$	96	—
E-6	.5 ppm $CuSO_4$	92	96
E-4	1.0 ppm $CuSO_4$	96	—
E-5	1.0 ppm $CuSO_4$	92	—
F-4	1.0 ppm $CuSO_4$	88	92
F-2	2.0 ppm $CuSO_4$	100	100
E-2	2,4-D	0	—
F-3	2,4-D	5	—
F-1	2,4-D	20	8.3

Striped bass were tested in water temperatures ranging from 13 to 19 C. The presence or absence of fungal growths served as an indicator of the success or absence of control efforts. All fish that succumbed within 5 days after they were stocked were considered to have died of netting and handling stresses and generally had little or no fungi. All fish (17) in the control pond were attacked by fungus and ultimately died. Only 2 of 39 fish in the 2 copper sulfate treated ponds contacted fungi.

Preliminary in-vitro testing substantiated pond observations. Table 2 lists the percentage of hemp seed free of fungal growth. Negative controls had no fungal growth, whereas, all positive controls became infested.

In most instances, at the 0.5 and 1.0 ppm copper sulfate treatment levels, colony size and density were greatly reduced. No control was attained with formalin, potassium permanganate or malachite green at the levels tested, although hyphal abnormalities were noted.

DISCUSSION

The mortality of Lot II largemouth bass fingerlings was unexpected and unexplained. It may have been related to physiological problems associated with an extremely obese body condition. A similar problem was encountered with fish from Lot I before their 30 day starvation period.

The use of copper sulfate for the control of secondary fungal infestations proved effective. The toxicity of copper sulfate in culture ponds is dependent on water hardness and must be used with caution until bioassays show the safe limits to use at each culture station. No variation in activity related to the amount and type of organic material in the ponds was observed.

Additional testing will be required to detremine the minimal number of treatments needed to provide sufficient protection for fish to recover from handling injuries. A fungus control on largemouth bass appears to be needed only when water temperatures are below 15.0 C. Observations of handled largemouth at higher water temperatures have shown that fungal invasions do not cause as high a mortality rate, probably due to higher resistance by the host.

Table 2. Chemical control of fungi on hemp seed in-vitro.

Treatment		*Number of chemical treatments*	*Percent of hemp seed free from fungal growth*
Negative Control			100
Positive Control			0
Malachite green:	0.25 ppm	1	100
$CuSo_4$:	0.1 ppm	1	0
	0.25 ppm	1	0
	0.25	1	16.6
	0.25	3	33.3
	0.5 ppm	1	33.3
	0.5 ppm	2	50.0
	0.5 ppm	3	66.6
	1.0 ppm	1	33.3
	1.0 ppm	2	83.0
	1.0 ppm	3	100
$KMnO_4$:	2.0 ppm	1	0
	2.0 ppm	2	0
	2.0 ppm	3	0
Formalin:	25 ppm	1	0
	25 ppm	2	0
	25 ppm	3	0
Malachite green:	.1 ppm	1	0
	.1 ppm	2	0
	.1 ppm	3	0

The variation between survival rates of control fish and fish treated with 2,4-D is small enough to indicate the chemical had little or no effect on zoospores and did not reduce the intensity of the fungal infestations. The difference in losses between controls and copper sulfate treated ponds is great enough to warrant further investigaion of this chemical when handling largemouth bass in cold water.

Previous work with striped bass adults captured with gill nets had shown that fungal infestations presented a significant threat to survival. The 1977 controlled test showed great differences when compared to a control pond. The loss of all 17 control fish versus only 2 of 39 in 2 test ponds is highly significant. Further work is warranted to refine the use of copper sulfate as a treatment for fungal infestations on striped bass.

The use of formalin, malachite green and potassium permanganate gave no control of fungi on hemp seed in petri dishes, but caused varying abnormalities of the hyphae, mycelium and reproduction processes. Both field and intensive in-vitro testing are planned for these chemicals in the future.

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