PRELIMINARY STUDIES ON THE TOLERANCE OF THE WHITE AMUR, *Ctenopharyngodon idella*, TO ROTENONE AND OTHER COMMONLY USED POND TREATMENT CHEMICALS

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ABSTRACT

The White Amur, *Ctenopharyngodon idella*, was found to be able to withstand recommended pond treatment concentrations of potassium permanganate, formalin, copper sulfate, and malachite green. In all instances, 100% of the test fish survived a 96 hour tolerance period at concentrations above the dosages recommended and used by the Arkansas Game and Fish Commission for disease and vegetation control in earthen ponds.

Both rotenone and antimycin were bio-assayed for possible use as a toxicant selective toward the White Amur. Both show promise as 100% mortality was recorded for test fish at extremely low concentrations in aquariums.

INTRODUCTION

The White Amur is being used extensively in Arkansas as a biological control for aquatic vegetation, both by the Arkansas Game and Fish Commission in commission-owned and managed lakes and by commercial fish farmers to control vegetation in rearing ponds with extremely good results. Since the White Amur has not yet become a well known food or game fish, its primary function, other than aquatic vegetation control, is to complement the growth of commercially raised bait and food fishes and the production of game fishes by the Arkansas Game and Fish Commission for the sportsmen of Arkansas. With this fact in mind, it was felt that more information was needed to determine if the White Amur could tolerate the often needed chemical treatments to control disease and poor water quality in the primary species placed in a crowded, artificial pond environment.

The White Amur is quickly proving its value in fish management as a tool for viological control of aquatic vegetation with no ill effects to the existing environment. Many people, however, both in and outside of Arkansas, remain skeptical about the White Amur's use. This is mainly because it is an exotic species and a member of the same family as the German Carp. Most misgivings about the introduction of this fish should be overcome by a quick review of a paper by Mr. Bill Bailey entitled, "Arkansas' Evaluation of the Desirability of Introducing the White Amur (*Ctenopharyngodon idella*, Val.) For Control of Aquatic Weeds". It was felt, however, that a selective toxicant which could effectively control the White Amur would ease the concern of those who remain wary of this "new carp". Thus rotenone and antimycin were added to the list of test chemicals.

ACKNOWLEDGMENTS

I would like to express my appreciation to Mr. Jim Collins for his efforts in schedule shuffling to make the necessary time available, to Mr. Bill Bailey for his very helpful advice, to Mr. Tony Carruth for the use of his pond for the rotenone tests, and to Mr. Joe Hairston for his help in getting the ever necessary work completed.

MATERIALS AND METHODS

All bio-assays were performed at the Joe Hogan State Fish Hatchery in Lonoke, Arkansas, except for two field tests with rotenone which were done in a privately owned pond less than one mile from the hatchery. Since these tests were performed with our own hatchery at Lonoke and area fish farmers in mind, no attempt was made to vary water quality (or chemistry) in any way. The natural water supply was used in all instances with important parameters being monitored. The bio-assays with copper sulfate, potassium permanganate, formalin, and malachite green, were done simultaneously and the rotenone and antimycin tests were done approximately four months later. For the sake of clarity, the tested substances will be divided into two groups. Copper sulfate, potassium permanganate, formalin, and malachite green shall comprise the group referred to as pond treatment chemicals, and the rotenone and antimycin shall be treated separately.

Pond Treatment Chemicals

During these tests 20 gallon aquariums, containing 50 liters of pond water and ten White Amur fingerlings (approximately 5 in.), were used. In an attempt to duplicate actual pond conditions as nearly as possible, water from the supply pond, which services the fish holding facility at the Joe Hogan Hatchery, was used. No aeration of any kind was supplied to the tanks. The water was found to contain 160 ppm calcium hardness with pH ranging from 7.4 to 8.5 over a two week period. Initial water temperatures varied with outside air temperatures, but stabilized for all test tanks at 50-52° F. after standing in the heated building for approximately 24 hours. The dissolved oxygen content of the water varied from day to day as would be expected, but this was somewhat standardized since the water was aerated as it was pumped into the test tanks. Secchi readings were taken each time water was pumped from the pond as an indicator of the organic content to the water. These readings ranged from 19.7 in. to 22.5 in. The control in all cases consisted of 50 liters of pond water with no additives and the ten test fish. While the dissolved oxygen levels dropped dangerously low (Table 1) due to lack of aeration, no mortalities occurred in any of the controls during the allotted 96 hour tolerance period. All parameters reported, i.e., calcium hardness, pH, and DO were determined colorimetrically using HACH chemical kit. All temperatures are recorded in Fahrenheit degrees. All tests were run from January 23 to February 1, 1973.

Table 1. Water Quality Data from Controls Used with Pond Treatment Chemical Tests.
All parameters listed were monitored more frequently than is shown, but for sake of clarity and space only 24 hour values are listed.

CONTROL 1

Time	Temp.	pH	DO	Mortalities
+24 hrs.	52	8.1	3 ppm	0
+48 hrs.	52	7.9	1 ppm	0
+72 hrs.	52	7.4	1 ppm	0
+96 hrs.	52	7.3	1 ppm	0

CONTROL II

Time	Temp.	pH	DO	Mortalities
+24 hrs.	52	8.5	3 ppm	0
+48 hrs.	52	8.1	2 ppm	0
+72 hrs.	52	7.6	1 ppm	0
+96 hrs.	51	7.5	1 ppm	0

CONTROL III

Time	Temp.	pH	DO	Mortalities
+24 hrs.	52	7.3	5 ppm	0
+48 hrs.	54	7.4	3 ppm	0
+72 hrs.	54	7.4	1 ppm	0
+96 hrs.	54	7.4	1 ppm	0

Rotenone and Antimycin

Bio-assays done with these two toxicants were performed in the same manner as those previously discussed. The only exceptions being that a 48 hour tolerance period was allowed for rotenone and a 24 hour tolerance period for antimycin. Toward the end of these tests a DO meter was obtained and DO and temperature were recorded with it instead of the HACH kit.

After initial data had been collected in the aquarium situation, two additional tests were performed using rotenone in a 0.09 acre earthen pond. The pond was drained, allowed to dry for several weeks and then surveyed to compute its volume as accurately as possible. An initial test was performed by stocking the pond with White Amur only. A second test was made with the White Amur and five other species of fish present to test the selectivity of the rotenone for the White Amur.

Formalin

The toxicity of formalin was tested at 50 ppm, 75 ppm, 100 ppm, and 150 ppm. All concentrations were computed by volume using a commercial 37% formaldehyde solution and 100% formalin. The most often recommended dosage of formalin for controlling parasite infections in earthen ponds ranges from 15-25 ppm. At a concentration of 50 ppm no mortalities occurred in 96 hours and all fish were apparently in good condition at the end of this time. At 75 ppm no deaths occurred within the tolerance period, but fish were severely stressed and showed no signs of recovery at the end of 96 hours. One hundred ppm formalin resulted in the first death at 43 hours into the test, another at 51 hours and this continued until there was 100% mortality at 93 hours. At 150 ppm 70% mortality occurred within the first 20 hours (Table 2).

Formalin reacts chemically with free dissolved oxygen in the water forming formic acid thus lowering the amount of oxygen available for the fish. This may have been the cause for some mortalities at 100 ppm and the stress at 75 ppm, but the test indicates that the White Amur can easily withstand recommended dosages of formalin for the treatment of parasitic or fungal infections.

Malachite Green

The toxicity of malachite green was tested at 0.1 ppm, 0.3 ppm, 0.5 ppm, 0.7 ppm, and 1.0 ppm. All concentrations were computed by weight, and a stock solution was prepared at 1000 ppm, using pond water as the solvent. The dosage of malachite green used as a pond treatment at the Joe Hogan Hatchery is 0.1

ppm. At concentrations of 1.0 ppm, 0.7 ppm, and 0.5 ppm 100% mortality occurred in each of the tanks at the end of 20 hours. At concentrations of 0.3 ppm, and 0.1 ppm no mortalities occurred and at no time did the fish show any signs of stress (Table 3).

Apparently then the lethal concentration lies between 0.3 and 0.5 ppm. With this range of tolerance, reasonable care in the use of malachite green should produce safe pond treatments.

Table 2. Results of White Amur Tolerance to Formalin.
In the following table there are instances where the measured parameters are not listed. This is due to either lack of time or because the chemicals themselves masked the end point of the titration method used. In these cases, refer to the controls for the same time interval.

FORMALIN 50 PPM

Time	Temp.	pH	DO	Mortalities
+24 hrs.	53	Control I	2.5 ppm	0
+48 hrs.	53			0
+72 hrs.	53			0
+96 hrs.	51	7.3	1 ppm	0

FORMALIN 75 PPM

Time	Temp.	pH	DO	Mortalities
+24 hrs.	52	Control III		0
+48 hrs.	54			0
+72 hrs.	54			0
+96 hrs.	53		1 ppm	0

FORMALIN 100 PPM

Time	Temp.	pH	DO	Mortalities
+24 hrs.	52	8.3	5 ppm	0
+48 hrs.	51	Control II		2
+72 hrs.	51	7.4	3 ppm	2
+96 hrs.	50	7.3	1 ppm	6

FORMALIN 150 PPM

Time	Temp.	pH	DO	Mortalities
+24 hrs.	52	Control I		7 Tank terminated

Copper Sulfate

The toxicity of copper sulfate was tested at 1 ppm, 3 ppm, and 5 ppm. Copper sulfate penthydrate (CuSO45H2O) was weighed so that a stock solution of 1000 ppm CuSO4 was prepared. The solvent in this case was distilled water to prevent the precipitation of the copper with the carbonate ion in the pond water until the actual test began. The dosage of copper sulfate used as a pond treatment at the Joe Hogan Hatchery is 0.5 ppm. Copper sulfate at a concentration of 5.0 ppm killed 70% of the test fish within 70 hours and the remaining 30% were severely stressed. At a concentration of 3 ppm, 70% of the test fish died within 90 hours and the rest were severely stressed. At 1.0 ppm no mortalities occurred, but the fish at times seemed hyperactive. At all concentrations of copper sulfate the fish remained near the surface as though an oxygen deficiency existed. Periodic checks, however, showed that the dissolved oxygen level remained at least as high as that in the control tanks (Table 4).

Table 3. Results of White Amur Tolerance to Malachite Green.
In the following table there are instances where the measured parameters are not listed. This is due to either lack of time or because the chemicals themselves masked the end point of the titration method used. In these cases, refer to the controls for the same time interval.

MALACHITE GREEN 1.0 PPM

Time	Temp.	pH	DO	Mortalities
+24 hrs.	52	Control I		10 tank terminated

MALACHITE GREEN 0.7 PPM

Time	Temp.	pH	DO	Mortalities
+24 hrs.	52	Control II		10 tank terminated

MALACHITE GREEN 0.5 PPM

Time	Temp.	pH	DO	Mortalities
+24 hrs.	52	Control II		10 tank terminated

MALACHITE GREEN 0.3 and 0.1 PPM

Time	Temp.	pH	DO	Mortalities
+24 hrs.	52	Control II		0
+48 hrs.	51			0
+72 hrs.	51			0
+96 hrs.	50			0

Table 4. Results of White Amur Tolerance to Copper Sulfate.
In the following table there are instances where the measured parameters are not listed. This is due to either lack of time or because the chemicals themselves masked the end point of the titration method used. In these cases, refer to the controls for the same time intervals.

COPPER SULFATE 5 PPM

Time	Temp.	pH	DO	Mortalities
+24 hrs.	52	Control I		0
+48 hrs.	52			0
+72 hrs.	52	7.4	5 ppm	7
				tank terminated

COPPER SULFATE 3 PPM

Time	Temp.	pH	DO	Mortalities
+24 hrs.	51	Control II		0
+48 hrs.	51	7.5	4 ppm	3
+72 hrs.	50	7.5	5 ppm	4
+96 hrs.				3

COPPER SULFATE 1 PPM

Time	Temp.	pH	DO	Mortalities
+24 hrs.	53	Control I		0
+48 hrs.	52			0
+72 hrs.	52			0
+96 hrs.	51	7.1	1 ppm	0

Potassium Permanganate

The toxicity of potassium permanganate was tested at 20 ppm, 15 ppm, 10 ppm, and 5 ppm. A 1000 ppm stock solution was prepared using 97% pure KMnO4 with pond water as the solvent. The recommended pond treatment with potassium permanganate is 2-4 ppm and its value in alleviating oxygen depletions has increased its use at Arkansas' hatcheries. In a concentration of 20 ppm, all ten fish in the tank were dead within five hours, and in a concentration of 15 ppm, all fish were dead within 16 hours. At concentrations of 5 and 10 ppm and initial tests seemed inconclusive. In the initial test of 5 ppm, a slow leak developed in the aquarium which decreased the original 50 liter water level by ten liters by the end of the test period. Even with this lowered water level, all fish survived with no apparent signs of stress. At a concentration of 10 ppm, all fish became badly discolored with the permanaganate and showed signs of severe stress at 15 hours. At 49 hours the test fish began showing signs of recovery, and all survived the 96 hour period. These fish were left in the aquarium, and at approximately 100 hours one fish was dead and the others began showing stress signs again. New tanks were set up and the tests of 5 and 10 ppm were repeated. In the second trial at 10 ppm, there was complete mortality at 16 hours. The fish in the second 5 ppm preparation began showing signs of stress at 24 hours, one death was recorded at +40 hours but by 48 hours the remaining fish began recovering and all survived the tolerance period in good condition (Table 5).

The only explanation offered for this phenomenon is based on the chemical nature of $KMnO_4$ itself. Fish culturists have known for some time that the amount of $KMnO_4$ a fish can withstand is directly affected by the amount of organic matter in the water. Potassium permanganate is a strong oxidizing agent and reacts quite readily with organic materials found in pond water. After a reasonably short time (especially at lower concentrations), no $KMnO_4$ remains in the water but is in the form of potassium hydroxide (KOH) and manganese dioxide (MnO_2). This can be readily seen by the change in color from purple, characteristic of the permanganate, to a brown color characteristic of the MnO_2. The amount of organic matter in the water affects the length of time taken for the $KMnO_4$ to be broken down.

The tests in question were run eight days apart and Secchi readings were taken in the holding pond (water supply) at the beginning of each. The day the water for the first test was taken from the pond the reading was 19.7 inches. These readings indicate a greater amount of organic matter was present in the water used in the first test than in the second and that the $KMnO_4$ dissipated more quickly allowing the test fish to withstand a higher concentration.

Table 5. Results of White Amur Tolerance to Potassium Permanganate. In the following table there are instances where the measured parameters are not listed. This is due to either lack of time or because the chemicals themselves masked the end point of the titration method used. In these cases, refer to the controls for the same time interval.

POTASSIUM PERMANGANATE 20 PPM

Time	Temp.	pH	DO	Mortalities
+24 hrs.	52	Control I		10 tank terminated

POTASSIUM PERMANGANATE 15 PPM

Time	Temp.	pH	DO	Mortalities
+24 hrs.	50	Control III		10 tank terminated

POTASSIUM PERMANGANATE 10 PPM (A)

Time	Temp.	pH	DO	Mortalities
+24 hrs.	52	Control I		0
+48 hrs.	52			0
+72 hrs.	52			0
+96 hrs.	51	7.5	1.5 ppm	0

POTASSIUM PERMANGANATE 10 PPM (B)

Time	Temp.	pH	DO	Mortalities
+24 hrs.	50	Control III		10 tank terminated

POTASSIUM PERMANGANATE 5 PPM (A)

Time	Temp.	pH	DO	Mortalities
+24 hrs.	51	Control II		0
+48 hrs.	52			0
+72 hrs.	53			0
+96 hrs.	51			0

POTASSIUM PERMANGANATE 5 PPM (B)

Time	Temp.	pH	DO	Mortalities
+24 hrs.	52	Control III		0
+48 hrs.	54			0
+72 hrs.	54			0
+96 hrs.	53			0

Antimycin

The toxicity of antimycin was tested at concentrations of 7.0 ppb, 5 ppb, 3 ppb, 2 ppb, 1 ppb, and 0.5 ppb. A concentrated solution of antimycin with acetone as the solvent was obtained from Ayerst Laboratories, manufacturers of Fintrol products. This was used to prepare the desired concentrations in 20 gallon aquariums filled with 50 liters of pond water from the Joe Hogan Hatchery. In test concentrations ranging from 1-7 ppb, 100% mortality occurred within the 24 hour tolerance period. Only at a concentration of 0.5 ppb and in the control did all fish survive. These tests were run in mid-June and water temperatures above 80° F. resulted in low dissolved oxygen levels and prevented longer tests at lower concentrations. This low concentration kill places the White Amur among those fishes most sensitive to antimycin according to Berger (1965). According to Mr. Berger, however, several important species of game fishes are also susceptible to 1.0 ppb concentrations. While antimycin has many characteristics which make it an attractive fish toxicant, it does not appear from these preliminary studies that it would be satisfactory for selectively eradicating the White Amur (Figure 1).

Rotenone

The toxicity of rotenone to the White Amur was tested in 20 gallon aquariums at concentrations of 0.1 ppm, 0.07 ppm, 0.05 ppm, 0.03 ppm, 0.02 ppm, 0.01 ppm, 0.008 ppm, and 0.006 ppm. Tests were run in early May in the same water from the holding pond as previously described with water temperatures remaining a fairly constant 64-66° F. in the fish holding building at the Joe Hogan Hatchery. Powdered bulk rotenone with guaranteed minimum analyses of 5% active ingredient was used in the tests. Analysis by the Arkansas Plant Board Laboratory in Little Rock, Arkansas, showed 5.8% active ingredient and this figure was used in all calculations. Concentrations given are parts per million active ingredient, not of the bulk powder.

All fish in the test tanks with concentrations ranging from 0.02 ppm to 0.1 ppm died within the first 12 hours of the 48 hour tolerance period. The concentration of 0.01 ppm was found to be the most critical for this series of tests and was repeated for more accurate data. Of the 20 fish subjected to this concentration, 14 mortalities were recorded within the 48 hour period and six of the fish survived. At the lower concentrations of 0.008 and 0.006 ppm only one of ten fish succumbed at each concentration (Figure 2).

From the data obtained during the aquarium tests, it appeared that a concentration of 0.01 ppm was the minimum concentration that would appreciably reduce a population of White Amur. With this in mind, and reports from District Fishery Biologists that during rotenone population samples they had observed the White Amur showing stress signs at approximately the same time as the gizzard shad, it was decided that a pond test with rotenone at this concentration would yield valuable information.

A 0.1 acre pond was drained and allowed to stand for several weeks to eradicate the entire fish population. The pond bottom was surveyed so that the volume could be calculated as accurately as possible. Average depth of the pond was 17.73 inches. The pond was pumped full with well water and allowed to stand for 24 hours. Water quality was checked with the following results: temperature = 65° F., calcium hardness = 130 ppm, pH = 7, DO = 9.5 ppm. One hundred White Amur were introduced and allowed to acclimate to the pond for 24 hours. Enough rotenone was added to produce a concentration of 0.01 ppm. At the end of 48 hours the pond was drained and the following results recorded: 28 fish known killed by rotenone, 61 fish were recovered alive, and 11 fish were unaccounted for (assumed to have died and been consumed by scavengers as some of the carcasses recovered were observed to have been partially eaten). This test was conducted in mid-May with water temperatures ranging from a low of 65° F. to a mid-day high of 76° F.

The pond was again filled and a check of water quality showed negligible difference from that listed previously. The pond was stocked as follows: 50 Channel Catfish, *Ictalurus punctatus*; 50 Golden Shiner, *Notemigonus* crysdeucas; 50 White Crappie, *Pomoxis annularis*; 75 Gizzard Shad, *Dorosoma* cepedianum; 50 Green Sunfish, *Lepomis cyanellus*; 50 White Amur. The fish were allowed to acclimate for at least 24 hours. Enough rotenone was added to produce the desired concentration of 0.01 ppm.

This test was performed June 1 - June 5, 1973 and water temperatures ranged from a low of 71° F. to a mid-day high of 86° F. Again, at the end of the 48 hours the pond was drained and assuming that those fish unaccounted for were killed or weakened by rotenone and eaten by scavengers, the following is a list of mortalities: Gizzard Shad - 69, White Amur - 43, Golden Shiner - 39, White Crappie - 29, Green Sunfish - 6, Channel Catfish - 3 (Figure 3).

The 0.01 ppm concentration used is equivalent to approximately 0.2 - 0.3 pounds of bulk (5%) powdered rotenone per acre feet of water. This concentration is the same as that used by Arkansas' District Fisheries Biologists to selectively kill and, therefore, to control the Gizzard Shad population in the state's fishing lakes. While a selective killing of the White Amur in a larger body of water has not been attempted, there is no doubt of the White Amur's sensitivity to rotenone and the fact that it could be easily controlled with rotenone should the need ever arise.

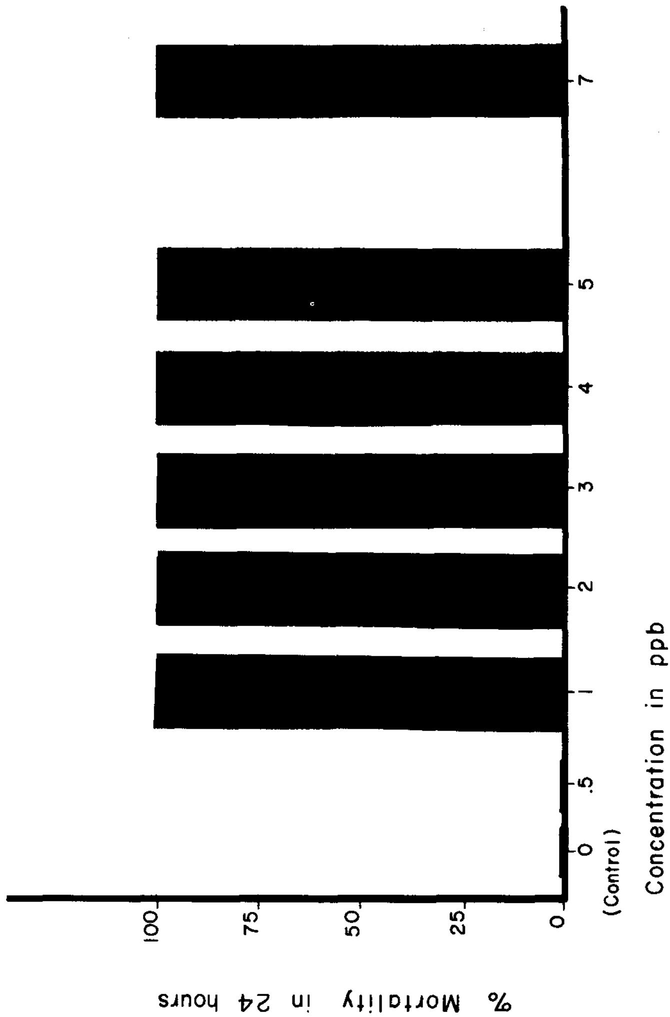

Figure 1. Susceptibility of White Amur to Antimycin in Aquaria.

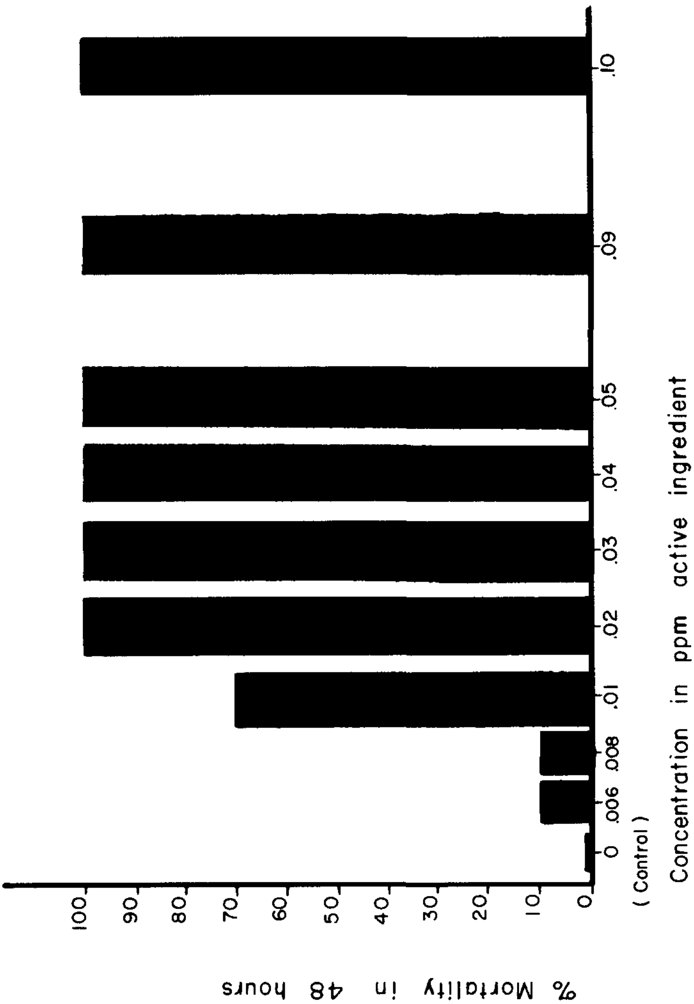

Figure 2. Susceptibility of White Amur to Rotenone in Aquaria.

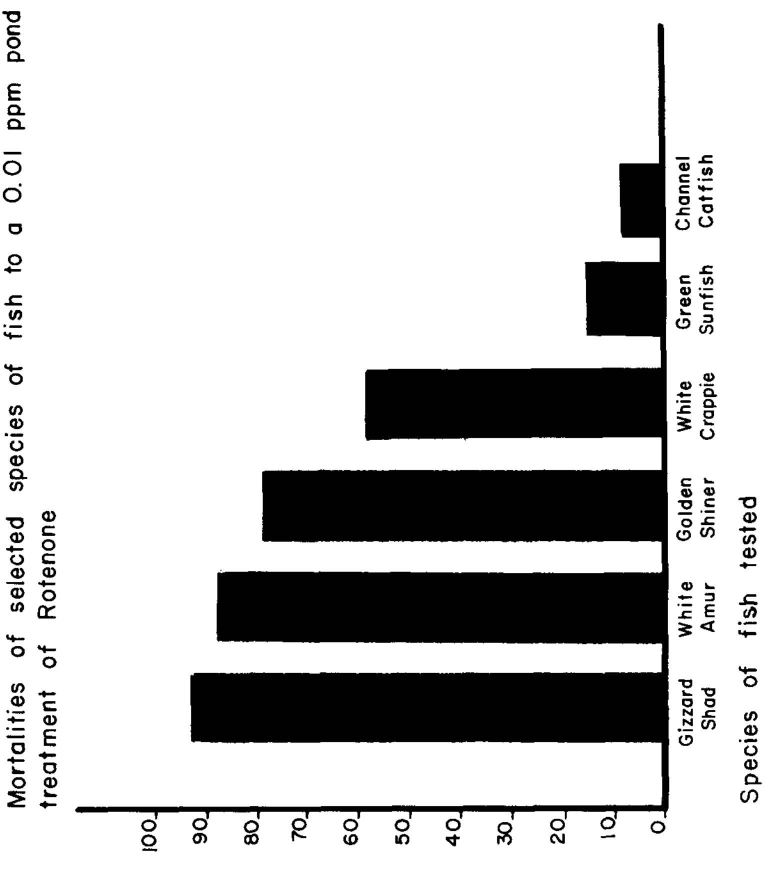

Figure 3. Mortalities of selected species of fish to a 0.01 ppm pond treatment of Rotenone.

SUMMARY

The results of these tests indicate that the White Amur can easily withstand the recommended treatment levels of formalin, malachite green, potassium permanganate, and copper sulfate in the ponds at the Joe Hogan Hatchery. The lethal levels found for copper sulfate, formalin, and malachite green agree very closely with those reported by Clemens and Sneed for the Channel Catfish. The White Amur, however, seems to be more susceptible to potassium permanganate than their findings indicate for the Channel Catfish.

It should be pointed out that the tolerance of any fish to these chemicals may vary with changes in water quality. A progress report by the Southeastern Cooperative Fish Disease Project reports that:

(1) The toxic effects of CuSO4
- a. increase as pH decreases
- b. increase as hardness decreases
- c. increase as iron content increases

(2) The toxic effects of KMnO4
- a. increase as hardness increases
- b. not affected by iron content
- c. increases as temperature increases

(3) The toxic effects of malachite green
- a. increase as pH increases
- b. decrease as hardness increases
- c. not affected by iron content

(4) The toxic effects of formalin are least affected by water chemistry but are accelerated as temperature increases.

Both antimycin and rotenone are effective toxicants with respect to the White Amur. Rotenone, however, holds the greatest appeal because of its greater selectivity for the White Amur.

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