INVESTIGATIONS IN FISH CONTROL

25. Field Trials of Antimycin as a Selective Toxicant in Channel Catfish Ponds

26. Laboratory Studies on Antimycin A as a Fish Toxicant

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28. Use of Antimycin for Selective Thinning of Sunfish Populations in Ponds

United States Department of the Interior
Fish and Wildlife Service
Bureau of Sport Fisheries and Wildlife

INVESTIGATIONS IN FISH CONTROL

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INVESTIGATIONS IN FISH CONTROL

25. Field Trials of Antimycin as a Selective Toxicant in Channel Catfish Ponds

By Ralph M. Burress, Fishery Biologist and
Charles W. Luhning, Physical Science Technician

United States Department of the Interior
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FIELD TRIALS OF ANTIMYCIN AS A SELECTIVE TOXICANT IN CHANNEL CATFISH PONDS

By Ralph M. Burress, Fishery Biologist
and Charles W. Luhning, Physical Science Technician
Bureau of Sport Fisheries and Wildlife
Southeastern Fish Control Laboratory, Warm Springs, Georgia

ABSTRACT.--Antimycin effectively and economically controlled heavy infestations of green sunfish and golden shiners from selected channel catfish ponds at a Mississippi fish farm. An initial application of 5 p.p.b. of antimycin in two ponds and 7.5 p.p.b. in the third pond eliminated nearly 99 percent of the scalefishes. A followup treatment of 10 p.p.b., 4 days later, further reduced these populations with no apparent effect on yearling catfish. At harvest, three untreated ponds produced 1,474 pounds of scalefishes or an average of 389 pounds per acre, yielded 27.4 percent or 1,155 fewer catfish than the three treated ponds, and contained 1,183 undersize fish or nearly three times as many that were too small for table use. Comparison of the adjusted yields of catfish from treated and untreated ponds, ranging in size from 0.94 to 1.39 acres, indicates that treated ponds produced an additional 1,015 pounds of fish worth $507.50, while antimycin cost only $145.79 -- a net return of $2.48 for each dollar invested in toxicant.

To those engaged in commercial production of channel catfish, the presence of undesirable species of scalefishes in rearing ponds is a potentially serious problem. The Bureau of Commercial Fisheries (1966) reports that about 35 million pounds of catfish are raised annually in the United States, and predicts that commercial production can reach 60 million pounds per year. The rapidly increasing demand has induced numbers of pond owners to undertake catfish production, but many have experienced losses caused by undesirable species of fish in broodponds and rearing ponds. Meyer (1965) discussed the nature of these losses, and reported the results of experiments in which various organophosphate insecticides were tested to determine whether they could be utilized for selective removal of trash fish from ponds used to produce catfish or bigmouth buffalo.

The purpose of this study was to evaluate the effectiveness of antimycin in controlling undesirable scalefishes in soft-water ponds used for commercial production of catfish, and to measure benefits derived from the treatment. The work was done in 6 of 14 dug ponds at a private fish-farm in Columbus, Miss., during the period August 1, 1966, to January 25, 1967.

Our sincere thanks are expressed to the following whose cooperation made this study possible: Mr. Ruben Prescott, owner of the ponds; Wisconsin Alumni Research Foundation, owner of antimycin; and Ayerst Laboratories, New York, producer of antimycin.

METHODS AND MATERIALS

EXPERIMENT DESIGN

The owner of the farm assisted us in selecting three pairs of ponds similar in size, depth, severity of infestation by scalefishes, and average size of channel catfish.

The field work was done in two stages. The first stage, done in August, included (1) sampling of the fish populations in six ponds by seining, (2) applying antimycin in the Fintrol-5 formulation to three ponds, and (3) recovering scalefishes from the treated ponds. The second stage, in October and January, involved (1) draining all ponds, (2) analyzing fish populations, and (3) evaluating the results of the treatment.

DESCRIPTION OF PONDS

The ponds ranged from 0.94 to 1.39 acres in surface area (table 1). They were steep sided and each had a scooped catch basin at the deep end measuring about 15 by 40 by 2 feet. The maximum depth outside the catch basin in the first two pairs of ponds was about 7 feet, while that in the third pair was only 5 feet.

TABLE 1.--Some physical and chemical characteristics of three ponds treated with antimycin

Characteristic	Pond 1	Pond 3	Pond 5
Surface area...............acres.	0.94	1.26	1.17
Average depth..............feet..	5.25	5.20	3.50
Volume................acre-feet..	4.94	6.93	4.10
Secchi disk transparency..inches..	7	12	14
Temperature (celsius) at 7:30 a.m:			
Aug. 1........................	30°	30°	30°
2........................	30°	30°	30°
3........................	28°	28°	28°
pH Aug. 1:			
7:45-8:30 a.m.................	8.2	7.3	7.7
2:30-3:00 p.m.................	10.1	9.4	9.4
5:45-6:30 p.m.................	10.2	--	--
pH Aug. 2:			
7:45-8:30 a.m.................	8.5	7.5	7.1
2:30-3:00 p.m.................	9.9	9.4	9.4
5:45-6:30 p.m.................	9.7	--	9.4
pH Aug. 3:			
7:45-8:30 a.m.................	7.3	7.5	6.9
2:30-3:00 p.m.................	--	9.5	--
5:45-6:30 p.m.................	--	9.2	--

TABLE 2.--Characteristics of water in three treated ponds[1]

Characteristic	Pond 1	Pond 3	Pond 5
Resistivity (26.6° C.)............	11,500	12,500	13,000
pH................................	10.5	10.3	10.8
Alkalinity, total (p.p.m.)........	22.0	19.0	20.0
Total hardness (p.p.m.)...........	20.0	20.0	14.0
Calcium hardness (p.p.m.).........	9.2	10.0	6.0
Sulfate ion (p.p.m.)..............	4.5	7.0	4.0
Total phosphorus (p.p.m.).........	0.1	0.2	0.5
Total iron (p.p.m.)...............	0.9	1.4	1.0
Nitrite nitrogen (p.p.m.).........	Trace[2]	0.02	0.28
Nitrate nitrogen (p.p.m.).........	0.3	0.3	1.1

[1] Samples taken at 4:00 p.m., August 8, refrigerated, and tested at Warm Springs on August 10 by standard methods of analysis.
[2] Trace = less than 0.01 p.p.m.

These ponds contained relatively soft water with total hardness ranging from 14 to 20 p.p.m. (as $CaCO_3$), and pH values were quite high (table 2). The other chemical characteristics indicate that Pond 5 was somewhat more fertile than Ponds 1 and 3. Ponds 1 and 5 received water from a well, while Pond 3 was filled from a deep, open pit which contained scalefishes.

None of the ponds contained growths of filamentous algae or submersed aquatic vegetation. However, each supported a dense bloom of phytoplankton, which caused marked rises in pH values each day. The addition of 25 pounds of commercial catfish feed per day per pond contributed to development of the blooms, and produced noticeable accumulations of organic matter on the bottom in the feeding areas.

Each pond was stocked in 1965 or 1966 with about 2,000 fingerling channel catfish (*Ictalurus punctatus*). In addition, all the ponds contained golden shiners (*Notemigonus crysoleucas*) and green sunfish (*Lepomis cyanellus*). The open-pit water supply was populated by golden shiners, warmouth bass (*Chaenobryttus gulosus*), green sunfish, and bluegills (*Lepomis macrochirus*).

SAMPLING AND HARVESTING

Average lengths and weights of the catfish at the time of treatment were calculated from a sample of about 50 fish from each pond

TABLE 3.--Numbers, weights, and percentages of channel catfish sampled in August in each of three pairs of ponds; the designations (T) and (U) identify treated and untreated ponds

Pond	Date of stocking	Number of fish	Percent of population sampled[1]	Weights (lbs.)	
				Total	Average
1 (T)....	10/23/65	48	2.4	33.40	0.70
2 (U)....	9/06/65	51	3.1	44.00	0.86
3 (T)....	10/23/65	47	2.8	24.25	0.52
4 (U)....	2/09/66	29	3.0	6.25	0.22
5 (T)....	11/09/65	56	3.4	17.50	0.31
6 (U)....	11/09/65	47	2.9	19.25	0.41

[1] Based on number of fish recovered at harvest.

(table 3). When possible, the fish were taken with a 30-foot, 1-inch-mesh seine, which was stretched out just beyond the feeding area and hauled in rapidly while the fish were feeding. This technique worked very well in Ponds 5 and 6, which are comparatively shallow, but in the deeper ponds these samples had to be augmented by catches made with a 125-foot, 1-inch-mesh seine or with a 250-foot, 1-inch-mesh gill net. The size of the sample in Pond 4 was reduced because the water was too deep for effective seining, and gill netting would have killed too many fish. The reduction in sample size was not regarded as serious, since the fish were uniform in length and weight.

Scalefish populations were sampled to obtain an estimate of their abundance in each pond. Two 40-foot hauls were made with a 20-foot, 1/4-inch-mesh seine, and the fish were enumerated by species and discarded (table 4).

Five of the six ponds were harvested in January 1967. All of the catfish were counted and weighed. We also made a fairly accurate measure of the scalefish even though some were lost in the mud.

Pond 2 was drained by the owner of the fish farm on October 3 to meet an urgent need for catfish in his restaurant. The number and dressed weight of the catfish were recorded, and the live weight was computed from these figures. Although scalefishes were not collected at the time of harvest, an estimate of their numbers was made.

TABLE 4.--Number of scalefish of each size group sampled by seining in each of three pairs of ponds (the two size groups represent fish hatched in pond and initial invaders)

Pond	Green sunfish		Golden shiners		Tadpoles
	1-1.5 in.	2-6 in.	1.5-2.25 in.	3-4.5 in.	
1....	321	6	1,510	1	0
2....	338	14	543	0	0
3....	1,410	0	0	0	238
4....	547	50	16	0	0
5....	653	13	25	0	0
6....	scores[1]		hundreds[1]		

[1] Seining data misplaced.

APPLICATION OF TOXICANT

At the time of treatment, the water temperature was above 60° F. and the pH was less than 8.5, hence a concentration of 5 parts per billion (p.p.b.) of antimycin in the Fintrol-5 formulation was selected in accordance with the manufacturer's recommendation. The toxicant was applied from a boat with a hand-operated seed spreader. Treatment of each pond was accomplished in 15-20 minutes between 7:45 and 9:00 a.m. on August 2. We added a supplementary treatment of 2.5 p.p.b. of antimycin to Pond 1 at 9:30 a.m. when we discovered that the pH had risen to 8.5 by 8:30 a.m.

We observed on August 4 that a few scalefish had survived in treated ponds, hence 10 p.p.b. of antimycin were applied to each pond on August 5 in an effort to determine the remnant populations and obtain a better evaluation of the results of the first treatment. These applications were completed between 6:05 and 7:15 a.m., which gave the antimycin almost 2 hours longer to take effect before the diurnal elevation in pH occurred. We recovered all the dead fish within 3 days after each treatment.

DEGRADATION OF TOXICANT

Golden shiners and fingerling green sunfish were seined from Pond 6, and 10 fish of each species were placed in live-boxes in the treated ponds about 24 hours after each application of the toxicant. We considered that degradation of the toxicant was complete when all of the fish survived a 48-hour exposure in the treated water.

RESULTS

FIRST TREATMENT

Small green sunfish and golden shiners were surfacing and gulping air within 15 minutes after we completed the applications of antimycin. Within 3 hours adult fish of both species began surfacing, and some could be taken easily with a dip net. Most of the golden shiners and a substantial proportion of the small green sunfish were recovered on the day of treatment. Larger fish of each species were slower to appear.

We observed several green sunfish in each pond which were too alert and active to be captured by dip net at 6:00 p.m. on the day of treatment. This was the first indication that the treatment was not completely successful. Although collections of fish were good for the next 2 days, the degradation of the toxicant was essentially complete within 24 hours after application as shown by tests with caged fish.

The first application of antimycin was highly effective in removing scalefish populations regardless of their composition (table 5). In Pond 1 we recovered about 187 pounds of scalefish per acre. Golden shiners were dominant, comprising 88.4 percent by number and 78.8 percent by weight. In Pond 3 fingerling green sunfish amounting to 146 pounds per acre constituted more than 99 percent of the scalefish population by number and weight. In Pond 5 the population was comparatively small, amounting to only 44 pounds per acre. Green sunfish of both sizes comprised more than 99 percent of the population by number and weight.

In order to check on the completeness of the kill, we again used the technique of seining at the time of feeding. It was interesting to see that several adult green sunfish which survived exposure to 5.0 and 7.5 p.p.b. concentrations of antimycin just 2 days earlier were not off feed, but responded with characteristic quickness to the familiar sound of food pellets hitting the water.

TABLE 5.--Total numbers and weights in pounds (in parentheses) of scalefish recovered following the first and second applications of antimycin

Species and size groups	Pond 1			Pond 3			Pond 5		
	First (7.5 p.p.b.)	Second (10 p.p.b.)	Total	First (5 p.p.b.)	Second (10 p.p.b.)	Total	First (5 p.p.b.)	Second (10 p.p.b.)	Total
Golden shiner:									
1.5-4.5 inches	31,435 (138.2)	2 (Trace)[1]	31,437 (138.2)	25 (0.3)	12 (0.1)	37 (0.4)	65 (0.3)	0	65 (0.3)
Warmouth:									
4-7 inches	3 (0.4)	7 (1.5)	10 (1.9)	0	0	0	0	0	0
Green sunfish:									
1-1.5 inches	3,858 (20.2)	9 (0.1)	3,867 (20.3)	59,840 (178.0)	1,019 (5.5)	60,859 (183.5)	9,526 (19.8)	3 (Trace)	9,529 (19.8)
2-6 inches	192 (10.4)	52 (4.2)	244 (14.6)	2 (0.3)	3 (0.3)	5 (0.6)	1,133 (31.3)	15 (0.8)	1,148 (32.1)
Bluegill:									
8 inches	1 (0.5)	0	1 (0.5)	0	0	0	0	0	0
TOTAL	35,489 (169.7)	70 (5.8)	35,559 (175.5)	59,867 (178.6)	1,034 (5.9)	60,901 (184.5)	10,724 (51.4)	18 (0.8)	10,742 (52.2)

[1] Trace = less than 0.1 lb.

SECOND TREATMENT

Within 3 hours after the treatment with 10 p.p.b. of antimycin, it was evident that the first treatment had eliminated most of the golden shiners in each pond. Our final count showed that the following percentages of golden shiners had survived: Pond 1, less than 0.01 percent; Pond 3, 32.4 percent; Pond 5, 0.0 percent. More than 99.7 percent of the small green sunfish were eliminated in Ponds 1 and 5; only 9 and 3 fish were recovered in each, respectively. In Pond 3, 1,019 small green sunfish had survived or 1.67 percent of the total number recovered.

Recovery of 52 green sunfish from 2 to 6 inches long in Pond 1 was unexpectedly high, amounting to 21.3 percent of the total number of this size group taken. In Pond 3 we found that 3 of 5 adult green sunfish had survived, while in Pond 5 we recovered only 15 of the 1,148 adult fish originally present.

Seven of 10 adult warmouth taken from Pond 1 had survived the first treatment, whereas the single large bluegill present did not.

Tadpoles began to die in Pond 3 shortly after the second treatment was made, and in 2 days we recovered about 1,990 tadpoles weighing 33.25 pounds. What percentage of the population this represented is not known, but a complete kill did not occur.

Degradation of antimycin was complete in all three ponds within 48 hours after application.

SCALEFISH IN TREATED PONDS AT HARVEST

Antimycin treatments were highly successful in that no golden shiners or warmouth were found in Ponds 1 and 3, and the reduction of green sunfish in each pond was more than 99.99 percent complete (table 6). Since there was no exchange of water between these two ponds and untreated ponds, we assume that the green sunfish had survived the treatment.

Unfortunately the exact results of the treatment of Pond 5 were obscured because an undetermined number of scalefish were carried in subsequently by an overflow from an adjacent pond. Since no golden shiners were recovered in this pond following the second application of antimycin, and since no warmouth were found following either treatment, we are virtually certain that the few individuals of each species which were observed at harvest were post-treatment invaders. The bottom of this pond was exceptionally flat, and contained scores of shallow depressions in the deep, soft mud. Thousands of green sunfish, many of which had hatched following the treatments, were trapped, and we could not make an accurate assessment of

TABLE 6.--Total numbers and total weights in pounds (in parentheses) of scalefish recovered at draining

Species and size groups	Treated ponds			Untreated ponds		
	1	3	5	2	4	6
Golden shiner:						
2.5-5.5 inches......	0	0	Very few[1]	[2] 50,000 (253)	1,428 (50)	31,680 (308)
Green sunfish:						
1.0-3.5 inches......	0	2 (0.2)	[3] 1,000's	[2] 40,000 (125)	39,740 (344)	18,627 (186)
3.5-6.0 inches......	4 (1.5)	7 (1.2)	[3] 150-200	0	2,421 (178)	170 (29)
Bluegill.............	0	1 (0.5)	0	0	0	0
Warmouth.............	0	0	Very few[1]	0	0	3 (1.0)
TOTAL	4 (1.5)	10 (1.9)	--	90,000 (378)	43,589 (572)	50,480 (524)

[1] Probably carried in by overflow from adjacent pond.
[2] Number estimated by pond owner at draining; all sizes included.
[3] Numbers estimated because many small fish were lost in soft mud; some probably carried in by overflow from adjacent pond.

the numbers of green sunfish present. An estimated 150-200 green sunfish from 3.5 to 6.0 inches in length were seen, many of which must have survived the antimycin treatments. Even if we assume that there were 200 and that none had immigrated into the pond, there was a reduction of more than 98.12 percent in the numbers of green sunfish originally present.

SCALEFISH IN UNTREATED PONDS AT HARVEST

Large numbers of scalefish were found when the untreated ponds were drained (table 6). In Pond 4 green sunfish weighing 418 pounds per acre comprised 91.3 percent of the scalefish population by weight, and large golden shiners amounting to about 40 pounds per acre made up the remainder. In Pond 6 golden shiners weighing 268 pounds per acre made up 58.8 percent of the total weight, and green sunfish weighing 187 pounds per acre comprised 41.2 percent of the total weight. The pond owner estimated that there were 40,000 green sunfish and 50,000 golden shiners of several sizes in Pond 2 at harvest. If weight data obtained during our sampling of the population 2 months prior to harvest were applied to this estimate, we can calculate that about 90 pounds of green sunfish and 182 pounds of golden shiners were removed per acre. In all three of these ponds there were hundreds of scalefish large enough to compete directly with the catfish for food.

HARVEST OF CHANNEL CATFISH

Three treated ponds having a combined area of 3.37 acres produced 5,363 catfish weighing 4,855 pounds, and the three untreated ponds having a combined area of 3.79 acres produced 4,208 catfish weighing 3,088 pounds (table 7). The average weight of catfish in the treated and untreated ponds at harvest was 0.91 and 0.73 pounds, respectively. We sorted out the smallest fish from each pond, which weighed from about one-third to one-half pound, and placed them in a single pond for additional feeding. Among the treated ponds, the number of undersize fish ranged from 81 to 226 with a total number of 421, while in the two untreated ponds for which data are available, the numbers of such fish were 235 and 948 for a total of 1,183. The differences are quite pronounced, but not all of them can be attributed solely to the treatment. Some were caused by variations in time of stocking, degree of competition from scalefish populations, and rates of catfish survival which may have been influenced strongly by predation. In the following section, an attempt is made to evaluate the effects produced by these factors and to arrive at a conservative measure of the benefits derived from treatment.

TABLE 7.--Numbers and weights in pounds of channel catfish harvested from treated and untreated ponds

Pond	Area (acres)	Date of stocking[1]	Date of harvest	Harvest per pond				Average weight		Percent gain in weight	Undersize fish		
				Total		Per acre		August sample	At harvest		Number	Av. wt.	Percent
				Number	Weight	Number	Weight						
Treated Ponds:													
1......	0.94	10/23/65	1/12/67	2,036	2,438	2,166	2,594	0.70	1.20	71.1	226	0.35	11.1
3......	1.26	10/23/65	1/04/67	1,701	1,217	1,350	966	0.52	0.72	38.5	81	0.55	4.8
5......	1.17	11/09/65	1/16/67	1,626	1,200	1,389	1,026	0.31	0.74	138.7	114	0.37	7.0
TOTAL	3.37			5,363	4,855	--	--	--	--	--	421	--	--
Untreated Ponds:													
2......	1.39	9/06/65	10/03/66	1,625	[2] 1,527	1,169	[2] 1,099	0.86	0.94	[3] 9.3		No data	
4......	1.25	2/09/66	1/24/67	978	335	782	268	0.22	0.34	54.4	948	0.33	96.9
6......	1.15	11/09/65	1/25/67	1,605	1,226	1,396	1,066	0.41	0.76	85.4	235	0.46	14.6
TOTAL	3.79			4,208	3,088	--	--	--	--	--	1,183	--	--

[1] Fingerling channel catfish 2.5 inches long stocked in all ponds.
[2] When total weight and weight per acre of catfish harvested from Pond 2 were adjusted to compensate for the difference in time of harvest, they became 1,735 and 1,248 pounds, respectively.
[3] Percent gain in weight from sampling on August 1 to harvest on October 3.

DISCUSSION

This field trial demonstrated quite clearly that antimycin can be used to control scalefishes in soft-water ponds without killing catfish, and that the investment in the treatment was well compensated by greater production in treated ponds. The initial treatment was nearly 99 percent effective, and there is good reason to believe that its efficacy would have been enhanced had the application of Fintrol-5 been made at daybreak. This would have allowed a substantially greater amount of exposure time before the rapid rise in pH began to cause degradation of the toxicant. Berger, Lennon, and Hogan (1969) found that the effectiveness of antimycin against fish is influenced substantially by the pH of the medium. Results of their bioassays indicated that the concentration of antimycin required to produce a complete kill of fingerling goldfish in 96 hours at 12^{o} C. was 0.20 p.p.b. at pH 5, 1.10 p.p.b. at pH 8, and 60 p.p.b. at pH 10.

Scalefish populations can be controlled at any time of the year, since water temperature has comparatively little effect on the efficacy of antimycin, and even fingerling catfish are not killed by recommended levels of treatment. Obviously such treatments yield greater benefits if accomplished a few days before ponds are stocked or soon thereafter.

It is thought that more green sunfish survived in Pond 5 because the water was comparatively shallow, and the bottom was completely covered by very soft mud. The sand formulation of antimycin used is designed to release the toxicant at a uniform rate as it sinks through the first 5 feet of water. Since the average depth of the pond was only 3.5 feet, a substantial percentage of the antimycin was not released before the sand sank into the mud. Therefore, we do not recommend lowering hatchery-type ponds prior to treatment if their average depths are not in excess of 5 feet.

The calculations of benefits cannot be based simply on the difference in total weights of catfish harvested from each group of ponds for two reasons: (1) Pond 6 was stocked much later than any of the others, and survival of the fish was by far the poorest in this pond; and (2) Pond 2 was harvested far earlier than the others. Thus, the most conservative approach to assessing the benefits derived from the treatment appears to be that of direct comparison of weight gains made by catfish in each pair of ponds after the time of treatment.

In comparing catfish populations and production in the first pair of ponds, we find that untreated Pond 2 was stocked on September 6, 1965, or about 47 days before Pond 1. Thus, it is not surprising that the average weight of catfish in the untreated pond was 0.16 pounds or 22.9 percent greater at the August sampling. The advantage of earlier stocking, however, was largely negated by the tremendous increase in competition from hundreds of scalefishes which grew large enough to consume food pellets.

Although treated Pond 1 was harvested 101 days later than Pond 2, the following adjustment can be made to permit a meaningful comparison of the gains in weight after the time of treatment. The average weight of catfish in Pond 2 increased by 9.3 percent between sampling time on August 1 and harvest time on October 3, hence the weight gain of the entire catfish population during this 63-day period was about 130 pounds. By subtracting this figure from the harvest figure, we can calculate that their total weight on August 1 was about 1,397 pounds. If we then make the generous assumption that the catfish in Pond 2 would have continued to gain weight at the same rate during the increasingly cold months of October, November, and December, their total gain from August 1 to January 12 would have been 338 pounds, and their adjusted total weight in January would have been 1,735 pounds. By comparison the total weight of catfish in Pond 1 on August 1 was about 1,425 pounds, and their weight at harvest was 2,438 pounds. Thus, their gain amounted to 1,013 pounds, or 675 pounds more than the adjusted weight gain by the catfish in Pond 2.

The comparison of catfish production in the second pair of ponds is complicated by the fact that Pond 3 was stocked on October 23, 1965, and untreated Pond 4 was stocked 109 days later on February 9, 1966. Catfish in the latter pond were further handicapped, because it contained by far the greatest population of adult green sunfish throughout the year as indicated both by seining in August and by the recovery of scalefishes at harvest. Under these circumstances it is not surprising that less than half of the original stock of catfish survived, that their average weight in August was only 0.22 pounds, or that 96.9 percent of them failed to reach marketable size by harvest time. Catfish in treated Pond 3 gained a total of 332 pounds following treatment while those in Pond 4 were able to gain a total of only 120 pounds. Thus, the fish in the treated pond gained 212 pounds more, though the same amounts of feed were offered and there were 723 fewer catfish in the untreated pond. The catfish in Pond 3 gained only 38.5 percent in weight following treatment as compared to 71.4 percent in Pond 1 and 138.7 percent in Pond 5. There was no apparent reason for their comparatively slow growth.

Ponds 5 and 6 were nearly identical in area, were stocked on the same day, were harvested only 9 days apart, and had almost identical rates of catfish survival. Hence, analysis of their production may afford the most clear-cut comparison of the benefits derived from treatment. For some unknown reason, the growth of catfish in Pond 5 prior to treatment was much poorer than that in Pond 6. At the time of August sampling, fish in the untreated pond were 32.3 percent heavier, the average weights of fish in the two ponds being 0.31 and 0.41 pounds, respectively. Following elimination of the scalefish in Pond 5, the fish grew at a much faster rate, and their average weight at harvest was 0.74 pounds as compared to an average of 0.76 pounds in Pond 6. Thus, the percentage difference in average weight was reduced from 32.3 to 2.7 percent. Furthermore, only 114 fish in the treated pond were too small for table use, while 235 of those in the untreated pond were undersize. Catfish in Pond 5 gained 696 pounds following treatment, while those in Pond 6 gained 568 pounds, for a difference of 128 pounds.

At the time of harvest, the local price for pond-reared catfish in the round was $0.50 per pound, hence calculations of the value of the total weight of fish harvested whether of usable or unusable size are based on this figure. Antimycin costs are those of the first treatment only, because it reduced scalefish populations by nearly 99 percent even under the adverse water quality conditions which prevailed when the tests were made.

The greater amount of weight gained by catfish in treated ponds following treatment amounted to a total of 1,015 pounds of fish worth $507.50 (table 8). The cost of antimycin used in the first treatment was $145.79, leaving a net profit of $361.71. Thus, for each dollar invested in antimycin there was a minimum net return of $2.48.

Another rather easily estimated, but comparatively minor, benefit derived from treatment is the saving in the cost of food required to feed out undersize fish from both groups of ponds. Pond 3 yielded the smallest usable size fish, which had an average weight of 0.72 pounds. The 421 undersize fish from the three treated ponds were underweight by 138 pounds, while the 1,183 small fish from the two untreated ponds were underweight by 431 pounds. Thus, if we assume that 1.8 pounds of food are required to produce each additional pound of catfish, and that food cost $125.00 per ton, it would cost $15.52 to feed out fish from the former group of ponds, and $48.47 to feed out those from the latter group, a difference of $32.95. The costs of labor, transportation, pumping of water and other factors attendent upon the greater length of time required to feed out the smaller fish

TABLE 8.--Increase in total yield and market value of channel catfish resulting from use of antimycin to reduce scalefish populations in three ponds

Pond	Differential increase in yield (lbs.)[1]	Value of increased yield	Cost of antimycin	Net profit
1......	675	$337.50	$58.49	$279.01
3......	212	106.00	54.71	51.29
5......	128	64.00	32.59	31.41
TOTAL	1,015	$507.50	$145.79	$361.71

[1] Derived by subtracting adjusted total weight gains in untreated ponds following date of treatment from those in treated ponds.

from the untreated ponds would be difficult to compute, but they undoubtedly would be much greater than the cost of food.

Another interesting approach to evaluation of the economic loss caused by the presence of competing scalefishes is possible if we arbitrarily assume that the amount of food required to produce one pound of scalefish might have produced a pound of catfish instead. On this basis, the untreated ponds could have produced an additional 1,474 pounds of catfish.

We recovered 1,155 fewer catfish from the untreated ponds, a significant factor which alone could spell the difference between operating the ponds at a profit or at a loss. Under the circumstances, we could not determine to what extent survival was reduced by scalefish predation on fingerling catfish, the vastly greater amount of competition of scalefish with catfish in untreated ponds, or factors of disease and accident which unaccountably operated to a much greater extent in the untreated ponds. Whatever the cause, the results were similar to those observed by Swingle (1959), who found that the presence of wild fishes in his experimental ponds reduced channel catfish production.

CONCLUSIONS

1. Nearly 99 percent of the scalefishes in three ponds used for channel catfish production were eliminated by an application of 5.0 p.p.b. of antimycin in two of the ponds and 7.5 p.p.b. in the third pond.

2. A followup treatment with 10 p.p.b. of antimycin further reduced the scalefish populations in all ponds and eliminated 26.4 pounds per acre of tadpoles in Pond 3.

3. The channel catfish were not harmed by the toxicant.

4. Two factors which prevented total eradication of the scalefish were: (a) pH of the water rose to high levels each afternoon and caused rapid detoxification of the antimycin, and (b) average depth of the water in Pond 5 was only 3.5 feet, and full release of the toxicant was not achieved before the sand upon which it was formulated sank into the soft, muddy bottom.

5. The 5.0 and 7.5 p.p.b. concentrations of antimycin degraded to a level harmless to golden shiners and small green sunfish within 24 hours after application, and the 10 p.p.b. concentration degraded within 48 hours.

6. The untreated ponds, which produced an estimated 184,000 scalefish weighing nearly 1,474 pounds, yielded 1,155 fewer catfish than did the treated ponds.

7. If the second pair of ponds (Ponds 3 and 4) in which stocking was done at widely different times is excluded from consideration, the average yield per acre of channel catfish from the treated ponds was greater than the adjusted yield per acre from untreated ponds by about 464 fish weighing 558 pounds.

8. The increase in yield of channel catfish resulting from treatment amounted to 1,015 pounds of fish worth $507.50, while the cost of Fintrol-5 for initial treatment was only $145.79 -- a net return of $2.48 for each dollar invested in toxicant.

9. Selective removal of scalefishes from catfish ponds can be accomplished safely and economically through the use of antimycin.

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INVESTIGATIONS IN FISH CONTROL

26. Laboratory Studies on Antimycin A as a Fish Toxicant

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United States Department of the Interior
Fish and Wildlife Service
Bureau of Sport Fisheries and Wildlife
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LABORATORY STUDIES ON ANTIMYCIN A AS A FISH TOXICANT

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ABSTRACT.--Liquid and sand formulations of antimycin A were tested in laboratory waters of various temperature, hardness, pH, and turbidity against 31 species of fresh-water fish of various sizes and life stages. Each formulation of toxicant was lethal under all water conditions to fish eggs, fry, fingerlings, and adult fish. Trouts are the most sensitive and catfishes the least sensitive. Of the 31 species, 24 succumb to 5 p.p.b. or less of the toxicant; only certain catfishes survive 25 p.p.b. The order of toxicity to various species of fish suggests that antimycin has possibilities for selective or partial control of certain unwanted fish. Although toxic to fish under ice, antimycin is more active in warm water than in cold. It is slightly more active in soft water than in hard; it is more active and persists far longer in water at pH 5 to 8 than at pH 9 or 10. It is active on fish in either clear and turbid waters, and it can be detoxified by potassium permanganate. The results contributed to registration of antimycin A in Fintrol-5 formulation as a fish toxicant.

Antimycin A in a formulation called Fintrol-5 was approved and registered as a fish toxicant in the United States and Canada in 1966. Its early promise as a fishery tool was mentioned by Derse and Strong (1963) and Loeb (1964). It was patented as a piscicide by Strong and Derse in 1964. Concurrently Walker, Lennon, and Berger (1964) made preliminary observations on its toxicity to fish and other aquatic organisms.

The subsequent intensive testing against fish in the laboratory which contributed to approval of antimycin as a fishery tool is discussed in this report. The studies included 31 fishes of various life stages in waters of different qualities and temperatures, indoor and outdoor bioassays, and liquid and sand formulations of the toxicant. The objectives were to define lethal concentrations for fish under a variety of conditions, to assess factors which contribute to degradation of the toxicant, and to find a substance which might be used to detoxify it.

Generous cooperation was afforded throughout the investigation by Wisconsin Alumni Research Foundation, owner of patents on antimycin; Ayerst Laboratories, New York, producer of antimycin; national fish hatcheries; and the Iowa, Minnesota, and Wisconsin Conservation Departments.

METHODS AND MATERIALS

ANTIMYCIN

The toxicant used in these investigations was supplied by the Wisconsin Alumni Research Foundation and by Ayerst Laboratories as

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crystals of antimycin A, 96 to 98 percent pure, or in formulations of antimycin A coated on sand. Stock solutions were prepared by dissolving 10 mg. of crystals in 100 ml. of acetone or ethanol. Although the solutions remain stable for several months if kept in cool, dark storage, fresh stocks were made biweekly.

The sand formulation Fintrol-5 contains 1 percent of antimycin A, 24 percent of Carbowax (polyethylene glycol 6000), and 75 percent of 40-mesh sand by weight. It is designed to release the toxicant into the water within a depth range of 0 to 5 feet as the particles sink to the bottom. Fintrol-15 is an experimental formulation, and the proportions of antimycin, Carbowax, and sand have not been released to us. It is supposed to release the toxicant within the first 15 feet of depth. Other formulations, such as a Fintrol-30 for greater depths, are under consideration.

Stability.--The effect of heat on the stability of antimycin was investigated. Quantities of crystalline and sand-formulated toxicant were subjected to dry heat at 200° C. in a forced-air oven for 15, 30, 60, and 120 minutes. Samples of the heated materials were then tested against fingerling rainbow trout in 96-hour bioassays. Results were tabulated at 0.25, 0.5, 1, 2, 3, 24, and 96 hours.

Detoxification.--Potassium permanganate ($KMnO_4$) was selected for preliminary testing as a detoxifier for antimycin in solution. Five to 500 p.p.b. of $KMnO_4$ were added to solutions which contained 5 p.p.b. of antimycin. Fingerling bluegills were placed in the solutions 6 or 24 hours later and their responses were noted at 6, 12, 24, 48, 72, and 96 hours to detect the extent and rate of detoxification.

BIOASSAY PROCEDURES

Static bioassays of antimycin were conducted as described by Lennon and Walker (1964) and Walker, Lennon, and Berger (1964). Some special tests required innovations.

Column tests.--A plexiglass column 8 feet high and 1 foot in diameter, with walls one-fourth-inch thick, was set up to determine the uniformity and strength of antimycin released by the sand formulation at selected depths. The column was filled with reconstituted water at 12° C., and quantities of Fintrol were applied at the surface and allowed to sink to the bottom. Water from various depths was sampled by siphon and analyzed for the toxicant by 96-hour bioassays with rainbow trout and goldfish.

Simulated field tests.--The availability of greater quantities of antimycin in the spring of 1966 enabled us to initiate trials against communities of small and large fish in 0.01-acre concrete pools. Each pool contained 43,000 liters of pond water at a 3.5-foot depth or 24,668 liters at a 2-foot depth. Selected wild and hatchery-reared fish were stocked in pools 3 to 7 days before treatment with antimycin. Stocking densities ranged from less than 100 to 2,000 pounds per acre. Dead fish were removed daily, and the ponds were drained after each experiment to obtain full tallies of fish.

A series of warm-weather tests was made using 12 species of fish at densities from 1,000 to 2,000 pounds per acre. The concentrations of antimycin applied were 1, 2, 3, 4, 5, 10, and 20 p.p.b. at 11° to 21° C.

Juvenile and adult fish of four species were used in the cold-weather tests in the pools. Tests were made using both clear and turbid water, at 3.4° to 5.5° C.

WATER

Most bioassays were run in reconstituted deionized water (Lennon and Walker, 1964) but well water or pond water was used in some of the outdoor tests.

Temperature.--Heated or chilled water baths were used as needed to control the temperatures of indoor bioassays at 2° to 27° C. Bioassays in outdoor pools were run at ambient temperatures; some under 6 inches of ice and others at temperatures up to 30° C.

Hardness.--Total hardness of bioassay waters ranged from 20 to 400 p.p.m. Most tests took place in our standard, reconstituted water, which has a total hardness of 40 p.p.m.

pH.--The range of pH in bioassay media was 5 to 10. The desired levels were attained by adding buffers to reconstituted water (table 1). Because the contrived levels tended to regress toward neutral, they were restored each 24 hours by adding small amounts of the buffer reagents. Close monitoring of control vessels which contained fish but no toxicant indicated the extent of change and the amount of readjustment necessary.

TABLE 1.--Buffer reagents added to reconstituted water to yield 15 liters of bioassay media of various pH levels

pH	Volume of buffer reagent[1]			(ml.)
	1.0N NaOH	0.1M $KHC_8H_4O_4$	1.0M KH_2PO_4	1.0M H_3BO_3
5 ±0.1....	10	510	--	--
6 ±0.1....	2	--	90	--
7 ±0.1....	10	--	30	--
8 ±0.1....	25	--	30	--
9 ±0.1....	8	--	--	30
10 ±0.1....	20	--	--	21

[1] NaOH = Sodium hydroxide.
$KHC_8H_4O_4$ = Potassium acid phthalate.
KH_2PO_4 = Potassium phosphate (monobasic).
H_3BO_3 = Boric acid.

Turbidity.--The activity of antimycin in the presence of suspended clay was checked in 96-hour bioassays with rainbow trout. Weighed portions of the clay were stirred into the bioassay media to produce turbidities of 1,000 and 5,000 p.p.m. (Secchi disk readings of 10 and 4 inches). The concentrations of antimycin ranged from 0.04 to 0.30 p.p.b. and the temperature was 12° C. All vessels were stirred for 30 seconds during each hour of the first 6 hours of the bioassay and at each 24 hours thereafter to keep the clay in suspension.

The performance of Fintrol-5 was tested against brown trout, goldfish, carp, bluegill, and largemouth bass in turbid water in outdoor, concrete pools at 3.4° to 5.5° C. Each pool contained about 25,000 liters of water at a depth of about 2 feet. Four pools had 2 inches of clay-loam on the bottom and a Secchi disk reading of 6 inches. Four pools had no soil on the bottom, and the water remained clear. Three turbid pools were treated with 5.0, 7.5, and 10.0 p.p.b. of antimycin respectively; the fourth served as a control. Similar concentrations of antimycin were applied in three clear pools, and the fourth pool served as a control. Observations on mortalities of fish were made daily up to 18 days at which time we drained the pools and made a final assessment.

FISH

Most of the fish were obtained from National and State fish hatcheries, but wild specimens were used in certain tests (table 2). All were held under conditions of quarantine and pretest evaluation as described by Lennon and Walker (1964).

Eggs.--We studied the effects of antimycin on fertilized eggs of rainbow trout, northern pike, goldfish, carp, white sucker, and channel catfish. At first, groups of eggs of certain ages, green and eyed, were counted and placed in petri dishes. The dishes were then immersed in solutions of antimycin for certain lengths of time. Later, aluminum wire or saran mesh baskets, approximately 2 by 2 by 2.5 inches were used. Containers of this size were capable of holding 100 rainbow trout eggs.

The baskets of eggs were either exposed for specific periods or remained in the solutions of antimycin until the eggs perished or hatched. Those removed after short exposures were rinsed and placed in hatching jars, troughs, or Heath egg incubators. Groups of control eggs were exposed to solutions of the solvent in water and otherwise handled similarly.

Fry.--Bowfin 21 to 56 days old and rainbow trout 3 to 60 days old were exposed to antimycin in 1- or 5-gallon bioassay vessels. Channel catfish less than 1 day old and largemouth bass 1 to 2 days old were placed in petri dishes containing various concentrations of toxicant. Following exposure, the fish were removed, rinsed, and placed in fresh water for observation.

TABLE 2.--List of 31 fishes exposed to antimycin

Species	Life stage				Source	
	Egg	Fry	Fingerling	Adult[1]	Hatchery	Wild
Shortnose gar, *Lepisosteus platostomus*	--	--	X	X	X	X
Bowfin, *Amia calva*	--	X	X	X	X	X
Rainbow trout, *Salmo gairdneri*	X	X	X	X	X	--
Brown trout, *Salmo trutta*	--	--	X	--	X	--
Brook trout, *Salvelinus fontinalis*	--	--	X	--	X	--
Lake trout, *Salvelinus namaycush*	--	--	X	--	X	--
Northern pike, *Esox lucius*	X	--	X	X	X	X
Goldfish, *Carassius auratus*	X	X	X	X	X	--
Northern redbelly dace, *Chrosomus eos*	--	--	--	X	--	X
Carp, *Cyprinus carpio*	X	--	X	X	X	X
Fathead minnow, *Pimephales promelas*	--	--	--	X	X	--
Quillback, *Carpiodes cyprinus*	--	--	--	X	X	X
White sucker, *Catostomus commersoni*	X	--	X	--	X	--
Bigmouth buffalo, *Ictiobus cyprinellus*	--	--	--	X	X	X
Spotted sucker, *Minytrema melanops*	--	--	--	X	--	X
White catfish, *Ictalurus catus*	--	--	X	--	X	--
Black bullhead, *Ictalurus melas*	--	--	X	X	X	X
Channel catfish, *Ictalurus punctatus*	X	X	X	X	X	--
Flathead catfish, *Pylodictis olivaris*	--	--	X	--	X	--
Brook stickleback, *Eucalia inconstans*	--	--	--	X	X	X
Green sunfish, *Lepomis cyanellus*	--	--	X	X	X	--
Pumpkinseed, *Lepomis gibbosus*	--	--	X	--	X	--
Bluegill, *Lepomis macrochirus*	--	--	X	X	X	--
Longear sunfish, *Lepomis megalotis*	--	--	X	--	X	--
Redear sunfish, *Lepomis microlophus*	--	--	X	--	X	--
Smallmouth bass, *Micropterus dolomieui*	--	--	X	--	X	--
Largemouth bass, *Micropterus salmoides*	--	X	X	X	X	--
Black crappie, *Pomoxis nigromaculatus*	--	--	X	X	X	--
Yellow perch, *Perca flavescens*	--	--	X	--	X	--
Walleye, *Stizostedion v. vitreum*	--	--	X	--	X	--
Freshwater drum, *Aplodinotus grunniens*	--	--	X	--	X	--

[1] May include juvenile as well as adult fish.

Fingerlings.--Routine bioassays were made with small fingerlings which usually range from 0.5 to 2.0 grams each. At least 10 fish of each species were used with each concentration of chemical and in each control. The loading in 5-gallon jars containing 15 liters of test solution or in 1-gallon jars containing 2.5 liters of solution was 1 gram or less of fish per liter. Observations on the responses of the fish to the toxicant were made at 3, 6, 24, 48, 72, and 96 hours.

Adults.--Since it is possible that the responses of adult fish to a toxicant may differ from those of younger fish, we exposed adults of 17 species to antimycin in 500-gallon concrete tanks, in 1,000-gallon vinyl tanks, or in 0.01-acre concrete pools. Most of them were obtained from the wild, and many of the tests were of the community type, involving two or more species at a time. All specimens were held in quarantine for several days to determine whether they were in satisfactory condition for bioassays. They were stocked in the pools 3 to 7 days before exposure to antimycin.

TOXICITY

Effective concentrations.--Results of most of the bioassays of antimycin with fish are expressed as 24-, 48-, 72-, and 96-hour EC_0, EC_{50}, or EC_{100}, that is, the concentrations of toxicant in parts per billion which kill 0, 50, or 100 percent of the fish within 24, 48, 72, or 96 hours. In deriving the expressions, the responses observed in early tests were subjected to probit analyses to estimate the effective concentrations (Litchfield and Wilcoxon, 1949). The estimates were corrected or confirmed by subsequent bioassays with replications. Confidence intervals (C.I.) for EC_{50}'s are given where possible.

Effective contact time (ECT).--Antimycin kills fish slowly, and we suspect that specimens have had lethal exposures well before they showed signs of distress or death. Moreover, EC_{50}'s and EC_{100}'s at 24 through 96 hours in static bioassays do not pinpoint the minimum exposures necessary to kill fish. Nine species of fish were used in trials to define the durations of minimum lethal exposures in given concentrations of antimycin. Following the exposures at 12° C., the fish were transferred to fresh water and observed for at least 96 hours.

Toxicity of injected antimycin.--Six- to 12-inch rainbow trout, carp, and black bullheads were injected intraperitoneally with ethanol solutions of antimycin to determine toxicities which might possibly be compared with the immersional toxicities. The amounts of toxicant in milligrams were selected according to the body weights of the fish in kilograms. The injected volumes ranged from 0.2 to 1 ml. per fish. Control fish were given injections of ethanol in water, with the quantity of solvent equaling the greatest amounts used to dissolve the antimycin. The fish were held at 12° C. for 5 days, except that dead fish were discarded daily.

RESULTS

BIOASSAYS WITH FINGERLINGS

The majority of bioassays of antimycin in the laboratory in waters of various qualities involved small fingerlings. The responses of these fish, therefore, serve as standards by which the responses of other life stages are evaluated.

Temperature.--In general, fish are more susceptible to antimycin at warmer temperatures (table 3). There were twofold to fivefold differences between EC_{100}'s at 7° and 22° C.

Less than 1 p.p.b. of toxicant killed all individuals of 14 species at one or more temperatures. The more sensitive fish are rainbow trout, brown trout, brook trout, lake trout, and walleye. The often undesirable carp, green sunfish, and pumpkinseed are also among the more sensitive fish. In contrast, the catfishes are relatively resistant. At temperatures from 22° to 7° C., 8 to 22 p.p.b. of antimycin are needed to kill channel catfish and 50 to 120 p.p.b. to kill black bullheads.

Water hardness.--Tests of antimycin against rainbow trout in waters of 20, 48, 90, 180, 360, and 400 p.p.m. total hardness demonstrated that the toxicant is slightly less effective in harder water. For example, 0.06 p.p.b. of the antibiotic killed all fish at 20 p.p.m. total hardness but none at 360 p.p.m.; and 0.08 p.p.b. killed less than half the fish at 360 p.p.m. total hardness.

In later tests, the 96-hour EC_{100}'s for rainbow trout, goldfish, and bluegill at 20 and 400 p.p.m. total hardness confirmed that antimycin is a little less effective in hard water.

pH.--The effectiveness of antimycin against fish is influenced substantially by the pH of the medium. Preliminary 96-hour bioassays were conducted with goldfish, at pH 5 to 10. The results indicated that EC_{100}'s increased three fold between pH 5 and 8; six fold between pH 8 and 9; and fourteen fold between pH 9 and 10. More definitive experiments with goldfish in 15-liter solutions of toxicant at pH 5 to 10 at 12° gave 96-hour EC_{100}'s of 0.20 p.p.b. at pH 5; 1.10 p.p.b. at pH 8; and 60 p.p.b. at pH 10 (table 4 and fig. 1).

Subsequently we attempted to conduct experiments with rainbow trout at various levels of pH, but the species were intolerant to the buffers at pH 6 and 10. The results at pH 7, 8, and 9 at 12° indicate that the toxicity of antimycin is reduced at higher pH levels. The 96-hour EC_{100} at pH 7 was 0.04 p.p.b., whereas it was 0.18 at pH 9.

A similar trend was evident in tests with carp at pH 7 through 10. The 96-hour EC_{100}'s of antimycin ranged from 0.6 p.p.b. at pH 7 to 4.0 p.p.b. at pH 9. At pH 10, there was a sharp increase in the EC_{100} to 20.0 p.p.b.

TABLE 3.--Effective concentrations of antimycin on 22 species of fish in 96-hour exposures at selected temperatures

Species	Number of fish	Average size		Temperature (°C.)	Concentrations expressed in p.p.b.			
		Length (inches)	Weight (grams)		EC_0	EC_{100}	EC_{50} and 95-percent C.I.	
Shortnose gar........	120	1.5	0.8	12	0.10	1.00	0.48	0.36- 0.65
Rainbow trout........	180	--	1.5	7	0.06	0.20	0.08	0.07- 0.09
Do.............	180	--	1.5	12	0.02	0.60	0.04	0.03- 0.04
Do.............	204	--	1.6	17	0.01	0.04	0.03	0.02- 0.04
Brook trout..........	180	--	1.5	12	0.02	0.03	0.06	0.06- 0.07
Do.............	148	--	1.5	17	0.01	0.04	0.03	0.03- 0.04
Lake trout...........	60	3.3	4.0	12	0.06	0.10	0.07	0.06- 0.08
Northern pike........	72	--	1.4	7	0.40	>0.60	0.55	0.50- 0.57
Do.............	176	--	0.8	12	0.10	0.40	0.26	0.23- 0.30
Do.............	176	--	2.0	17	0.08	0.20	0.14	0.12- 0.34
Do.............	168	--	1.9	22	<0.10	0.14	0.11	0.08- 0.16
Goldfish.............	192	--	2.3	7	0.20	2.00	1.00	0.83- 1.20
Do.............	200	--	2.0	12	0.20	1.00	0.50	0.42- 0.60
Do.............	192	--	2.0	17	0.10	0.40	0.35	0.25- 0.49
Do.............	216	1.2	0.4	22	0.10	0.40	0.20	0.16- 0.25
Northern redbelly dace	120	1.9	1.1	7	0.10	>0.50	0.52	0.37- 0.73
Do.............	120	1.9	1.1	12	<0.10	0.60	0.18	0.15- 0.21
Do.............	60	1.9	1.1	17	0.04	0.30	0.09	0.07- 0.12
Do.............	60	2.5	2.4	22	0.04	0.10	0.09	0.08- 0.11
Carp.................	120	--	2.0	7	0.20	0.80	0.43	0.36- 0.51
Do.............	240	--	2.0	12	0.10	0.60	0.35	0.30- 0.40
Do.............	120	--	2.0	17	0.10	0.40	0.25	0.21- 0.30
Do.............	96	--	2.0	22	0.04	0.20	0.12	0.09- 0.16
Fathead minnow.......	120	2.1	0.7	7	<0.20	0.40	0.20	0.17- 0.23
Do.............	120	2.1	1.6	12	0.08	0.40	0.21	0.16- 0.28
Do.............	120	2.1	0.7	17	0.06	0.12	0.09	0.08- 0.10
Do.............	120	2.1	1.7	22	0.04	0.08	0.06	0.05- 0.08
Black bullhead.......	120	2.3	2.4	7	40.00	120.00	88.00	73.00-105.00
Do.............	216	2.2	2.1	12	40.00	100.00	45.00	38.80- 52.20
Do.............	156	2.2	2.1	17	20.00	60.00	32.00	27.60- 37.20
Do.............	120	2.4	2.3	22	<20.00	50.00	21.00	15.50- 28.00
Channel catfish......	120	--	1.9	7	6.00	22.00	10.50	9.50- 11.70
Do.............	120	--	1.9	12	4.00	16.00	9.00	7.30- 11.60
Do.............	180	--	1.9	17	2.00	>10.00	7.40	6.60- 8.40
Do.............	240	--	1.9	22	4.00	8.00	5.20	4.90- 5.60
Brook stickleback....	120	2.1	1.1	7	0.40	>0.60	0.55	0.52- 0.58
Do.............	180	2.1	1.1	12	0.10	0.40	0.21	0.18- 0.24
Do.............	120	2.1	1.1	17	0.10	0.25	0.16	0.14- 0.18
Do.............	120	2.1	1.1	22	<0.06	0.08	0.04	0.04- 0.05
Green sunfish........	120	1.4	0.7	7	0.20	0.80	0.50	0.43- 0.59
Do.............	180	1.4	0.7	12	0.10	0.40	0.20	0.15- 0.24
Do.............	216	1.4	0.7	17	0.08	0.25	0.15	0.11- 0.18
Do.............	120	1.3	0.6	22	0.06	0.20	0.11	0.10- 0.12
Pumpkinseed..........	120	1.8	1.3	7	0.10	0.40	0.24	0.20- 0.29
Do.............	180	1.8	1.4	12	0.08	0.20	0.14	0.12- 0.17
Do.............	120	1.8	1.4	17	0.06	0.20	0.09	0.08- 0.10
Do.............	120	1.8	1.3	22	0.04	0.10	0.05	0.05- 0.07
Bluegill.............	120	--	2.5	7	0.20	0.60	0.50	0.45- 0.56
Do.............	120	--	2.5	12	0.08	0.20	0.14	0.11- 0.17
Do.............	180	--	1.3	17	0.06	0.15	0.07	0.06- 0.14
Do.............	180	--	0.8	22	0.04	0.10	0.06	0.05- 0.07
Longear sunfish......	120	2.0	1.0	12	0.05	0.20	0.08	0.07- 0.11
Redear sunfish.......	120	1.8	1.3	17	0.05	0.25	0.09	0.08- 0.11
Smallmouth bass......	120	1.3	0.5	12	0.01	0.10	0.04	0.03- 0.05
Do.............	120	1.3	0.5	17	0.02	0.08	0.04	0.03- 0.04
Do.............	120	1.3	0.5	22	0.01	0.12	0.06	0.04- 0.07
Largemouth bass......	120	1.6	0.8	12	0.08	0.20	0.14	0.09- 0.20
Do.............	120	1.6	0.8	17	0.06	0.20	0.10	0.07- 0.10
Do.............	120	1.6	0.8	22	0.04	0.20	0.09	0.07- 0.11
Yellow perch.........	180	2.0	1.2	7	0.06	0.20	0.12	0.11- 0.14
Do.............	204	--	0.5	12	0.02	0.10	0.05	0.04- 0.06
Do.............	204	2.0	2.0	17	0.04	0.06	0.04	0.04- 0.05
Do.............	208	--	0.5	22	0.02	0.04	0.03	0.03- 0.04
Walleye..............	204	1.5	0.7	12	0.04	0.08	0.04	0.04- 0.05
Do.............	120	1.5	0.7	17	>0.01	0.03	0.02	0.02- 0.03
Freshwater drum......	120	2.9	3.3	7	0.08	0.25	0.14	0.12- 0.17
Do.............	60	2.9	3.3	12	0.04	0.15	0.07	0.06- 0.09
Do.............	60	2.9	3.3	17	0.02	0.06	--	-- --
Do.............	60	2.9	3.3	22	0.01	0.04	0.02	0.01- 0.03

TABLE 4.--Concentrations of antimycin in p.p.b. which caused 0- and 100-percent mortality in fingerling rainbow trout, carp, and goldfish at pH levels 5 through 10 at 12° C.

pH	Number of fish	96-hour	
		EC_0	EC_{100}
Rainbow trout			
7 ±0.1.........	100	0.01	0.04
8 ±0.1.........	100	0.03	0.07
9 ±0.1.........	100	0.06	0.18
Carp			
7 ±0.1.........	100	0.10	0.60
8 ±0.1.........	100	0.10	1.00
9 ±0.1.........	100	1.60	4.00
10 ±0.1.........	100	9.40	20.00
Goldfish			
5 ±0.1.........	110	0.10	0.20
6 ±0.1.........	70	0.10	0.30
7 ±0.1.........	130	0.09	0.40
8 ±0.1.........	70	0.30	1.10
9 ±0.1.........	70	2.00	6.00
10 ±0.1.........	140	20.00	60.00

Turbidity.--The presence of 1,000 and 5,000 p.p.m. of clay in suspension influenced the toxicity of antimycin to 2.5-inch rainbow trout at 12° C. The medium with 1,000 p.p.m. of clay turbidity was only slightly less toxic than the control to the fish at 24, 48, and 96 hours. The high turbidity medium had 24-, 48-, and 96-hour EC_{50}'s of 0.40, 0.14, and 0.10 p.p.b., while those of the control were 0.16, 0.10, and 0.05 p.p.b.

Some additional observations on the performance of antimycin in turbid water are given under Simulated Field Trials.

Effective contact time.--Only brief exposures to antimycin are needed to kill fish (table 5). At 10 p.p.b. of toxicant, the ECT_{100}'s are 1 hour for green sunfish and bluegill, and 4 hours for carp, longear sunfish, and black crappie. In contrast, black bullheads require a minimum exposure of 3 to 4 hours to a concentration of 500 p.p.b. for a complete kill. These data are especially pertinent if the toxicant were to be used in the reclamation of streams or in situations where high pH contributes to rapid degradation.

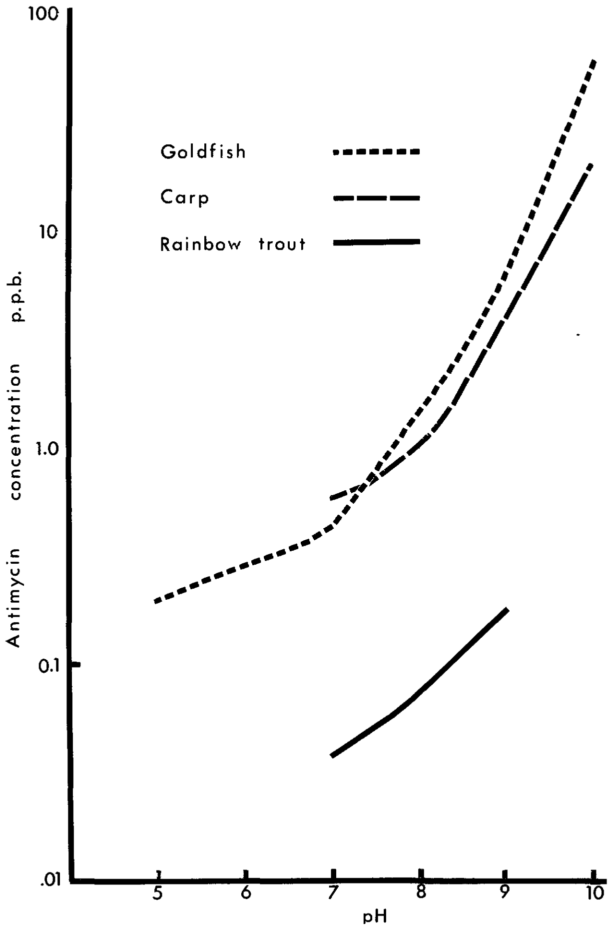

Figure 1.--Relation between various concentrations of antimycin and levels of pH necessary to produce EC_{100}'s for selected fishes at 12° C.

Sand-formulated toxicant.--Preparations of antimycin coated on sand were obtained rather late in the experimental program. Fintrol-5 and Fintrol-15 were compared with crystalline antimycin in acetone solutions for effectiveness against fingerling rainbow trout and carp.

Fintrol-5 contains 10 mg. of antimycin per gram of formulation. Its 96-hour EC_{50}'s for rainbow trout and carp at 12° C. were 0.04 and 0.20 p.p.b. respectively. By comparison, the EC_{50}'s of acetone-antimycin under the same circumstances were rainbow trout 0.03 p.p.b. and carp 0.20 p.p.b.

TABLE 5.--Effective contact time (ECT) of antimycin against selected fishes at 12°C in standard reconstituted water[1]

Species	Number of fish	Average size		Antimycin (p.p.b.)	ECT_0	ECT_{50}	ECT_{100}
		Length (in.)	Weight (g.)				
Goldfish............	50	1.5	1.0	50	< 15 min.	15-30 min.	30-60 min.
Carp................	80	1.6	0.9	1	< 2 hrs.	2-4 hrs.	> 4 hrs.
Do..............	80	1.6	0.9	10	30-60 min.	1-2 hrs.	2-4 hrs.
Do..............	40	1.6	0.9	50	< 7.5 min.	7.5-15 min.	15-30 min.
Do..............	80	1.6	0.9	100	< 4 min.	4-7.5 min.	7.5-15 min.
Redbelly dace........	80	1.9	1.1	1	4 hrs.	7.5 hrs.	12-14 hrs.
Do..............	80	1.9	1.1	10	3.0-7.5 min.	30-45 min.	2 hrs.
Black bullhead.......	80	2.2	2.2	250	< 4 hrs.	> 8 hrs.	> 8 hrs.
Do..............	80	2.2	2.2	500	30-60 min.	1-2 hrs.	3-4 hrs.
Do..............	80	2.2	2.2	1,000	15-30 min.	1-2 hrs.	2-4 hrs.
Green sunfish........	80	1.4	1.0	1	< 6 hrs.	> 6.5 hrs.	22 hrs.
Do..............	80	1.4	1.0	10	< 15 min.	15 min.	30-60 min.
Do..............	80	1.4	1.0	100	< 1 min.	1.5 min.	> 4 min.
Bluegill............	80	1.7	1.1	1	> 15 min.	15-30 min.	30-60 min.
Do..............	80	1.7	1.1	10	> 7.5 min.	7.5-15 min.	15-60 min.
Do..............	80	1.7	1.1	50	1-2 min.	2-4 min.	4-7.5 min.
Do..............	80	1.7	1.1	100	1-2 min.	2-4 min.	4-7.5 min.
Longear sunfish......	60	3.1	7.8	10	15-30 min.	1-2 hrs.	2-4 hrs.
Do..............	60	3.1	7.8	100	1-2 min.	2-7.5 min.	7.5-15 min.
Black crappie........	35	3.6	7.8	1	30-60 min.	> 4 hrs.	> 4 hrs.
Do..............	35	3.6	7.8	10	4-7.5 min.	0.5-2 hrs.	2-4 hrs.
Do..............	35	3.6	7.8	100	1-2 min.	7.5-15 min.	15-30 min.
Yellow perch.........	80	2.2	1.6	1	30 min.	1.0-1.5 hrs.	> 1.5 hrs.
Do..............	80	2.2	1.6	10	≤ 30 min.	30 min.	1-2 hrs.
Do..............	80	2.2	1.6	100	< 15 min.	< 15 min.	< 15 min.

[1] All test animals were observed for a minimum of 96 hours after transfer to fresh water.

Fintrol-15 has 26 mg. of antimycin per gram of formulation. Its EC_{50}'s at 96 hours of 0.07 p.p.b. for rainbow trout and 0.15 p.p.b. for carp compare very favorably with those of acetone-antimycin or Fintrol-5 solutions.

Column tests.--The release of antimycin from Fintrol-5 is apparently uniform but not complete within the first 5 feet of depth in the 8-foot plexiglass column. Samples siphoned at depths of 1 to 5 feet were toxic to all rainbow trout within 24 hours. Four of 16 trout survived in a sample taken at 6 feet, and all trout lived in a sample at 7 feet. There is obviously some further release of toxicant from sand lying on the bottom, because a sample of water drawn at 8 feet killed all trout.

We were unable to test the release of antimycin from Fintrol-15 at depths greater than 8 feet. The bioassays of samples taken at 1 through 8 feet in the column demonstrated a uniform release of toxicant.

Some of the first samples of Fintrol had a tendency to lie on the surface film when sprinkled lightly at the top of the column. This was corrected by the manufacturer, and later samples penetrated the film, sank readily to the bottom, and gave a good dispersion of the toxicant.

BIOASSAYS WITH OTHER LIFE STAGES

Eggs.--Antimycin is toxic to fertilized eggs of rainbow trout, northern pike, goldfish, carp, white sucker, and channel catfish. Recently, Valentine (1966) demonstrated that the antibiotic also kills fertilized eggs of zebra fish (Brachydanio rerio).

The first experiments included newly fertilized, water-hardened eggs of rainbow trout which had developed to about the 32-cell stage during the first 24 hours. Groups of 100 eggs were exposed to 1 and 100 p.p.b. of antimycin for 30, 60, and 120 minutes and then transferred into a Heath egg incubator. Control groups were handled similarly but not exposed to the toxicant. Embryogeny was compared by removing several eggs per day from treated and untreated groups. After 2 weeks, the eggs were sampled each 2 to 4 days. The samples were fixed in 5-percent formalin in 1-percent saline. Chorions were removed, and the embryos were stained in Harris hematoxylin and examined.

Within 24 hours, the eggs exposed to 100 p.p.b. of toxicant for 120 minutes ceased development at about the 64-cell stage. Among control eggs, the blastoderms were well developed. Coagulation of protein was evident in dead eggs on the fourth day after treatment, and all eggs in the group were opaque by the seventh day.

The eggs exposed for only 60 minutes to 100 p.p.b. of toxicant developed somewhat further. Cell division in some continued for 2 days. Protein coagulation in dead eggs continued from the fourth to the 11th day.

Some eggs exposed to 100 p.p.b. of antimycin for 30 minutes showed development comparable to that of controls for 8 to 13 days after treatment. In others, development terminated at the blastoderm stage. The greater number of opaque eggs appeared after 7 days. Microscopic examination of the embryos revealed no gross morphological or anatomical changes attributable to the toxicant.

The eggs exposed to 1 p.p.b. of toxicant and control eggs were "eyed" 15 days after fertilization, and they hatched 16 days later. There was no significant difference in the hatching success of the treated and control groups.

Long-term exposures to small concentrations of antimycin effected eggs of rainbow trout. Acetone solutions or sand formulations of toxicant were added to 1-gallon jars which contained approximately 100 fertilized eggs each. The medium was reconstituted water at 12° C., pH 7.2 to 7.6, and total hardness of 40 p.p.m. The eggs remained in the jars throughout incubation and swim-up.

The eggs became eyed after 15 days and hatched at 29 days, and the sac fry reached swim-up stage at 42 days. Observations continued through 67 days, at which time the control fish were of routine bioassay size, that is, approximately 500 per pound. None of the eggs exposed to 0.5 p.p.b. or more of acetone-antimycin survived to the eyed stage (table 6). Most embryos from eggs exposed to 0.1 p.p.b. lived through the eyed and hatching stages, but mortalities were heavy at swim-up, as the fry seemed unable to take food. Only 7 percent survived through 67 days. All fry from eggs exposed to the sand formulation at 0.1 to 1.0 p.p.b. of antimycin perished within 42 days. Eggs treated with acetone-antimycin solutions survived through 67 days. The control group had a survival of 55 percent at 67 days. Bioassays with fingerling rainbow trout in eggless control solutions of the toxicant demonstrated that both formulations had degraded within the first 14 days of the experiment.

In another series of tests at 12°, the eggs of northern pike exhibited greater sensitivity to antimycin than those of rainbow trout and white sucker (table 7). The green eggs of trout were more sensitive than eyed eggs.

The eggs of goldfish and carp also proved susceptible to antimycin. All goldfish eggs were killed by 2-hour exposures to 7.5 and 10 p.p.b. of toxicant, and only 1 percent survived 5 p.p.b. (table 8). All eggs of goldfish and carp died when exposed to 2.5 p.p.b.

TABLE 6.--Mortality of rainbow trout eggs incubated in solutions of antimycin at 12° C.

Antimycin	Number of eggs	Percent mortality at--					
		3 days	6 days	15 days[1]	29 days[2]	42 days[3]	67 days[4]
In acetone:							
0.10 p.p.b.	107	9	13	30	37	87	93
0.25 p.p.b.	125	10	36	91	93	96	100
0.50 p.p.b.	116	9	100	--	--	--	--
0.75 p.p.b.	105	10	100	--	--	--	--
1.00 p.p.b.	111	12	100	--	--	--	--
On sand:							
0.10 p.p.b.	97	6	7	9	20	100	--
0.25 p.p.b.	90	6	8	18	27	100	--
0.50 p.p.b.	98	5	82	100	--	--	--
0.75 p.p.b.	88	5	76	100	--	--	--
1.00 p.p.b.	101	5	100	--	--	--	--
Control..........	105	4	6	10	35	40	45

[1] Eggs eyed.
[2] Eggs hatched.
[3] Swim-up.
[4] Fingerling stage.

TABLE 7.--Survival of fish eggs after exposure to aqueous solutions of antimycin in acetone at 12° C.

[The postexposure period of observation was 3 to 30 days]

Species and age of eggs	Number of eggs	Length of exposure (min.)	Percentage survival at concentrations (in p.p.b.)		
			1.0	10.0	100.0
Rainbow trout (24 hours after fertilization)	300	30	100	20	0
	300	60	100	0	0
Rainbow trout (3 days before hatching)	300	30	100	100	0
	300	60	100	100	0
	300	120	100	0	0
Northern pike (24 hours after fertilization)[1]	600	30	100	100	0
	600	60	80	50	0
	600	120	25	0	0
White sucker (8 days before hatching)	450	30	100	100	0
	450	60	100	100	0
	450	120	100	0	0

[1] Not feasible to hold 30 days.

TABLE 8.--Survival of 24-hour-old eggs of goldfish after 2-hour exposure to antimycin at 17° C.

Antimycin	Number of eggs	Percentage hatch
1.0 p.p.b.	100	47
2.5 p.p.b.	100	15
5.0 p.p.b.	100	1
7.5 p.p.b.	100	0
10.0 p.p.b.	100	0
Control	100	61

or more throughout the 7-day incubation period. Further experiments at 19° to 23° showed that 10 p.p.b. of antimycin which were allowed to degrade for 96 hours were no longer toxic to goldfish eggs. However, a solution which originally contained 20 p.p.b. of toxicant killed 70 percent of exposed goldfish eggs within 96 hours.

The eggs of channel catfish can be killed with antimycin (table 9), but they are substantially more resistant than those of rainbow trout, northern pike, goldfish, carp, and white sucker. Fertilized eggs about 24 hours old were placed in small baskets of saran mesh. Some were exposed for 2 hours to selected concentrations of antimycin; others were exposed to the toxicant throughout the 6-day incubation period. The solutions of toxicant were aerated, constantly agitated, and temperatures ranged between 25° and 28.5° C. At 100 p.p.b. or less, over 90 percent of the eggs survived 2-hour exposures.

TABLE 9.--Survival of eggs of channel catfish following brief and prolonged exposures to antimycin at 25° to 28.5° C.

Antimycin	Number of eggs	Percentage hatch
Two-hour exposure:		
20 p.p.b.	100	93
40 p.p.b.	100	96
60 p.p.b.	100	93
80 p.p.b.	100	100
100 p.p.b.	200	97
250 p.p.b.	100	19
500 p.p.b.	100	0
750 p.p.b.	100	0
1,000 p.p.b.	100	0
Control	200	95
Six-day exposures:		
10.0 p.p.b.	30	93
12.5 p.p.b.	30	96
15.0 p.p.b.	30	83
17.5 p.p.b.	30	90
20.0 p.p.b.	60	95
25.0 p.p.b.	30	26
27.5 p.p.b.	30	0
30.0 p.p.b.	30	0
40.0 p.p.b.	30	0
50.0 p.p.b.	30	0
Control	60	96

In contrast, all eggs succumbed to prolonged exposures to 27.5 p.p.b. or more of antimycin.

Fry.--Bowfin, rainbow trout, channel catfish, and largemouth bass fry were subjected to antimycin. Because their ages differed, we cannot say which species are the more sensitive at a given age.

Bowfin exhibited increasing resistance to the toxicant with increasing age. The 96-hour EC_{50}'s were 0.13 p.p.b. at 3 weeks of age, 0.24 p.p.b. at 6 weeks, and 0.35 p.p.b. at 8 weeks. A comparable trial with 15 p.p.b. killed 12 adult fish.

The responses of rainbow trout sac fry and advanced fry to antimycin were approximately the same as those of fingerlings (table 10). The 96-hour EC_{50}'s of 5-, 12-, and 18-day-old sac fry at 12° C. were 0.04, 0.03, and 0.05 p.p.b. respectively, whereas the EC_{50} for 44-day-old advanced fry and 60-day-old fingerlings was 0.04 p.p.b.

The fry of channel catfish, only a few hours old, were more sensitive than the eggs to the toxicant. On the other hand, they were more

TABLE 10.--Toxicity of antimycin in p.p.b. to sac and advanced fry of rainbow trout at 12° C.

Age	Number of fish	24-hour				96-hour			
		EC_0	EC_{100}	EC_{50}	C.I.[1]	EC_0	EC_{100}	EC_{50}	C.I.[1]
3 days....	100	0.10	0.40	0.30	.12-.42	--	--	--	--
5 days....	100	0.10	0.60	0.35	.28-.34	0.02	0.08	0.04	.02-.05
11 days....	100	0.06	0.20	0.14	.12-.18	--	--	--	--
12 days....	100	0.06	0.20	0.15	.10-.22	0.02	0.04	0.03	.02-.04
14 days....	100	0.04	0.10	--	--	--	--	--	--
18 days....	100	0.06	0.40	--	--	0.02	0.08	0.05	.03-.06
25 days....	100	0.10	0.40	0.32	.30-.34	0.04	0.08	--	--
28 days....	100	0.04	0.20	0.12	.09-.14	--	--	--	--
44 days....	100	0.08	0.40	0.27	.24-.30	0.02	0.06	0.04	.02-.05
60 days[2]...	100	0.10	0.60	0.45	.36-.46	0.02	0.06	0.04	.03-.05

[1] C.I. = 95-percent confidence intervals for EC_{50}'s
[2] Approximate age of our standard bioassay fish

resistant than the fry of bowfin and rainbow trout. In standard bioassays at 22° C., the 96-hour EC_{50} was 1 p.p.b. For those fry exposed to the toxicant for only 2 hours at 26° C., the EC_{50} was 26.9 p.p.b. and the EC_{100} was 50 p.p.b.

Newly hatched fry of largemouth bass also were exposed to antimycin for a 2-hour period at concentrations of 0.01 to 0.50 p.p.b. Many of the control fry died, however, which makes interpretation of the results difficult. There was a 100-percent mortality of fry exposed to 0.075 p.p.b. within the following 96 hours, but there was a 60-percent mortality during the same period in the control lot of fry. Eighty-two of the 100 fry exposed to the lowest concentration of antimycin, 0.01 p.p.b., died within the following 96 hours.

Juveniles and adults.--Most of the tests of antimycin against larger fish were run in vinyl or concrete pools. An early series in 1,000-gallon, vinyl pools at 15.5° to 17.2° C., pH 8.4 to 8.6, and total hardness of 252 p.p.m. included 10 species of fish. Five p.p.b. or less of toxicant killed all goldfish, carp, fathead minnow, bigmouth buffalo, green sunfish, pumpkinseed, bluegill, and largemouth bass within 144 hours (table 11). Most black bullheads and some channel catfish survived exposures to 10 and 20 p.p.b. Naiads of damsel flies and aquatic plants in the pools were unharmed by the antimycin. Subsequent restocking with small bluegills indicated that the toxicant had degraded within the first 144 hours.

Followup bioassays in vinyl pools determined the concentration of antimycin necessary to kill black bullheads. Included were 40, 80, 120, 160, and 200 p.p.b. of toxicant at 26.6° C., pH 9.0 to 9.6 and five species of fish. The 3.6-inch goldfish, 2.8-inch carp, 2.7-inch green sunfish, and 4.9-inch bluegills were killed within 24 hours by all concentrations. The 5-inch wild black bullheads exhibited stress within 3 hours at 160 and 200 p.p.b., but they recovered fully within 24 hours. At these relatively high levels of pH, the toxicant degraded within 48 hours.

In contrast, 5-inch black bullheads alone in a test at 23° C., pH 7.8 to 8.6, and 287 p.p.m. total hardness, died within 24 hours in 160 and 200 p.p.b. of antimycin. None died at lower concentrations. The toxicant lasted longer at this range of pH, and degradation was not considered complete until 96 hours.

Fifteen-inch white catfish and 6-inch green sunfish were exposed up to 144 hours in 1, 5, and 25 p.p.b. of acetone- or sand-formulated antimycin in vinyl pools at temperatures which ranged between 1° and 16° C., pH 6.2 to 7.0, and total hardness of 18 to 40 p.p.m. All white catfish survived, but the green sunfish died in either formulation at 5 p.p.b. The average degradation time for the acetone formulation was a little more than 7 days; for the sand formulation it was slightly less than that.

A separate series of tests was performed with acetone-antimycin against large fingerling

TABLE 11.--Toxicity of antimycin to various species and sizes of fish in outdoor, vinyl pools at 15.5° to 17.2° C., pH 8.4 to 8.6, and 252 p.p.m. total hardness

Species	Number of fish	Average size		Antimycin (p.p.b.)	Fish mortality at--[1]						Totals		
		Length (in.)	Weight (g.)		6 hours	24 hours	48 hours	72 hours	96 hours	144 hours	Alive	Dead	Lost
Goldfish.............	30	2.5	4.2	Control	0	0	0	0	0	0	10	0	[2] 20
Do.............	30	"	"	0.5	0	0	0	0	0	P	17	1	12
Do.............	30	"	"	1.0	0	0	0	0	0	P	17	1	12
Do.............	30	"	"	5.0	0	0	0	P	P	P	0	20	10
Do.............	30	"	"	10.0	0	P	P	P	P	P	0	16	14
Do.............	30	"	"	20.0	0	P	P	P	P	P	0	17	13
Do.............	30	4.1	24.1	Control	0	0	0	0	0	0	26	0	4
Do.............	30	"	"	0.5	0	0	0	0	0	P	20	1	9
Do.............	30	"	"	1.0	0	0	0	0	0	P	21	1	8
Do.............	30	"	"	5.0	0	0	P	P	P	P	0	26	4
Do.............	30	"	"	10.0	0	P	P	P	P	P	0	29	1
Do.............	30	"	"	20.0	0	P	P	P	P	P	0	27	3
Carp.................	30	2.2	2.5	Control	0	0	0	P	P	P	12	2	16
Do.............	30	"	"	0.5	0	P	P	P	P	P	5	17	8
Do.............	30	"	"	1.0	0	P	P	P	P	T	0	30	0
Do.............	30	"	"	5.0	P	T	T	T	T	T	0	30	0
Do.............	30	"	"	10.0	T	T	T	T	T	T	0	30	0
Do.............	30	"	"	20.0	P	T	T	T	T	T	0	29	1
Do.............	30	6.4	56.4	Control	0	0	0	0	0	0	30	0	0
Do.............	30	"	"	0.5	0	0	0	0	0	0	30	0	0
Do.............	30	"	"	1.0	0	0	0	0	0	P	22	6	2
Do.............	30	"	"	5.0	0	P	P	P	P	T	0	30	0
Do.............	30	"	"	10.0	P	P	P	P	T	T	0	30	0
Do.............	30	"	"	20.0	0	P	T	T	T	T	0	30	0
Fathead minnow.......	30	2.2	4.0	Control	0	0	0	P	P	P	28	2	0
Do.............	30	"	"	0.5	0	P	P	P	P	T	0	30	0
Do.............	30	"	"	1.0	0	P	P	T	T	T	0	30	0
Do.............	30	"	"	5.0	P	T	T	T	T	T	0	30	0
Do.............	30	"	"	10.0	P	T	T	T	T	T	0	30	0
Do.............	30	"	"	20.0	T	T	T	T	T	T	0	30	0
Bigmouth buffalo.....	20	7.2	102.0	Control	0	0	0	0	0	0	18	1	1
Do.............	20	"	"	0.5	0	0	0	0	0	0	19	1	0
Do.............	20	"	"	1.0	0	0	0	0	0	P	12	1	7
Do.............	20	"	"	5.0	0	P	P	P	P	T	0	20	0
Do.............	20	"	"	10.0	0	P	T	T	T	T	0	19	1
Do.............	20	"	"	20.0	0	P	T	T	T	T	0	17	3
Black bullhead.......	30	2.3	2.6	Control	0	0	0	0	0	0	16	0	14
Do.............	30	"	"	0.5	0	0	0	0	0	0	14	0	16
Do.............	30	"	"	1.0	0	0	0	0	0	0	20	0	10
Do.............	30	"	"	5.0	0	0	0	0	0	0	18	1	11
Do.............	30	"	"	10.0	0	0	0	0	0	0	18	1	11
Do.............	30	"	"	20.0	0	0	0	0	0	0	21	0	9
Black bullhead.......	30	5.7	30.1	Control	0	0	0	0	0	0	27	0	3
Do.............	30	"	"	0.5	0	0	0	0	0	0	28	0	2
Do.............	30	"	"	1.0	0	0	0	0	0	0	25	0	5
Do.............	30	"	"	5.0	0	0	0	0	0	0	25	0	5
Do.............	30	"	"	10.0	0	0	0	0	0	0	30	0	0
Do.............	30	"	"	20.0	0	0	0	0	0	0	30	0	0
Channel catfish......	30	2.4	1.8	Control	0	0	P	P	P	P	19	11	0
Do.............	30	"	"	0.5	0	0	0	0	0	P	1	20	9
Do.............	30	"	"	1.0	0	0	0	0	0	P	4	26	0
Do.............	30	"	"	5.0	0	0	0	0	0	0	17	13	0
Do.............	30	"	"	10.0	0	0	0	0	0	0	13	15	2
Do.............	30	"	"	20.0	P	P	P	P	P	P	2	28	0
Green sunfish........	30	1.5	1.0	Control	0	0	0	0	0	0	30	0	0
Do.............	30	"	"	0.5	0	0	P	P	P	T	0	30	0
Do.............	30	"	"	1.0	0	P	P	T	T	T	0	30	0
Do.............	30	"	"	5.0	P	P	T	T	T	T	0	30	0
Do.............	30	"	"	10.0	P	T	T	T	T	T	0	30	0
Do.............	30	"	"	20.0	T	T	T	T	T	T	0	30	0
Green sunfish........	30	2.8	5.4	Control	0	0	0	0	0	0	27	0	3
Do.............	30	"	"	0.5	0	0	0	P	P	P	15	12	3
Do.............	30	"	"	1.0	0	0	P	P	P	P	0	17	13
Do.............	30	"	"	5.0	P	P	P	P	P	P	0	18	12
Do.............	30	"	"	10.0	P	P	P	P	T	T	0	18	12
Do.............	30	"	"	20.0	P	P	P	T	T	T	0	20	10
Pumpkinseed..........	30	3.0	8.4	Control	0	0	0	0	0	0	24	0	6
Do.............	30	"	"	0.5	0	0	0	0	P	P	7	13	10
Do.............	30	"	"	1.0	0	0	0	P	P	P	0	26	4
Do.............	30	"	"	5.0	0	0	P	P	P	P	0	30	0
Do.............	30	"	"	10.0	0	P	P	P	P	T	0	26	4
Do.............	30	"	"	20.0	0	P	P	P	P	T	0	25	5

See footnotes at end of table.

TABLE 11.--Toxicity of antimycin to various species and sizes of fish in outdoor, vinyl pools at 15.5° to 17.2° C., pH 8.4 to 8.6, and 252 p.p.m. total hardness--continued

Species	Number of fish	Average size		Antimycin (p.p.b.)	Fish mortality at--[1]						Totals		
		Length (in.)	Weight (g.)		6 hours	24 hours	48 hours	72 hours	96 hours	144 hours	Alive	Dead	Lost
Bluegill.............	30	1.6	0.8	Control	0	0	0	0	0	0	30	0	0
Do..............	30	"	"	0.5	0	P	T	T	T	T	0	30	0
Do..............	30	"	"	1.0	0	P	T	T	T	T	0	30	0
Do..............	30	"	"	5.0	P	T	T	T	T	T	0	30	0
Do..............	30	"	"	10.0	P	T	T	T	T	T	0	30	0
Do..............	30	"	"	20.0	P	T	T	T	T	T	0	30	0
Bluegill.............	30	5.3	50.0	Control	0	P	P	P	P	P	9	10	11
Do..............	30	"	"	0.5	0	P	P	P	P	P	0	23	7
Do..............	30	"	"	1.0	0	P	P	P	P	P	0	26	4
Do..............	30	"	"	5.0	0	P	P	P	P	T	0	25	5
Do..............	30	"	"	10.0	0	P	P	P	P	T	0	20	10
Do..............	30	"	"	20.0	P	P	P	P	T	T	0	25	5
Largemouth bass......	30	3.0	5.2	Control	0	0	0	0	P	P	0	20	10
Do..............	30	"	"	0.5	0	0	P	P	P	P	11	19	0
Do..............	30	"	"	1.0	P	P	P	P	T	T	0	30	0
Do..............	30	"	"	5.0	P	P	T	T	T	T	0	30	0
Do..............	30	"	"	10.0	P	T	T	T	T	T	0	30	0
Do..............	30	"	"	20.0	T	T	T	T	T	T	0	30	0
Largemouth bass......	10	7.2	81.0	Control	0	0	0	0	0	0	9	0	1
Do..............	10	"	"	0.5	0	0	0	P	P	P	6	4	0
Do..............	10	"	"	1.0	0	0	0	P	P	P	0	8	2
Do..............	10	"	"	5.0	0	0	P	P	P	P	0	10	0
Do..............	10	"	"	10.0	0	0	P	P	P	T	0	10	0
Do..............	10	"	"	20.0	0	P	P	P	P	T	0	8	2

[1] 0 = no mortality, P = partial mortality, T = total mortality.
[2] Unaccountable loss from pools, due in part to predatory birds.

flathead catfish in vinyl pools which contained water of 42 to 48 p.p.m. in total hardness and pH 6.6 to 7.0. One group of 4.5-inch fish survived 96-hour exposures to 50 p.p.b. of antimycin at 21° C. The 96-hour EC_{50} for a group of 5.8-inch flatheads was 54 p.p.b. at 27° C. In a test at 28° C., the 24-, 48-, and 72-hour EC_{50}'s for 5.8-inch fingerlings was 33 p.p.b. Most of the deaths at 27 and 28° C. were within the first 6 hours of the test. In a separate experiment, the 96-hour EC_{50} for adult flathead catfish at 17° C. was 182 p.p.b. (95-percent C.I. = 158-209).

Eight species of fingerling and larger fish were exposed to 0.1, 1.0, and 5.0 p.p.b. of antimycin under ice cover. The sand formulation was applied in vinyl pools containing water at 190 and 282 p.p.m. total hardness and pH 8.5 to 9.0. The activity of the toxicant was influenced far more by the temperature under 5 to 6 inches of ice than by water quality. Concentrations of 0.1 and 1.0 p.p.b. caused no significant mortality of fish. Five p.p.b. of toxicant killed 5.8-inch rainbow trout and 5.9-inch brown trout within 72 hours, and 1.6-inch carp and 3.1-inch longear sunfish within 120 hours (table 12). There were some deaths among 1.4-inch goldfish, 2.1-inch fathead minnow, and 1.4- and 2.8-inch bluegill. No deaths occurred among 3.8-inch goldfish, 7.2-inch carp, and 2.6-inch pumpkinseeds. At the relatively high pH, the toxicant degraded to a point harmless to brown trout within 120 hours after application.

Some tests with adults of resistant species were made indoors in concrete tanks with well water at total hardness of 300 p.p.m., pH 7.6, and 14.5° C. Some of the shortnose gar died at 15 and 20 p.p.b. of sand-formulated antimycin, but 25 p.p.b. were required for complete kills within 96 hours (table 13). All bowfin died at 20 p.p.b. In sharp contrast, black bullheads showed 50-percent mortality at 140 p.p.b. and 100-percent died at 220 p.p.b. Their resistance to the toxicant, therefore, is at least 10 times that of gar and bowfin.

SIMULATED FIELD TRIALS

Community bioassays with juvenile and adult fish in 0.01-acre concrete pools provided further evidence on the concentrations of antimycin needed to kill certain species. The pools were 3.5 feet deep, the water temperatures were 11° to 21° C., pH ranged from 7.9 to 8.6, total hardness from 252 to 290 p.p.m., and total alkalinity from 211 to 250 p.p.m. The concentrations of dissolved oxygen ranged from 8.1 to 11.2 p.p.m. Under these

TABLE 12.--Toxicity of 5 p.p.b. of antimycin in sand formulation to fish in vinyl pools under 5 to 6 inches of ice cover, total hardnesses of 190 and 282 p.p.m. and pH 8.5 to 9.0

Species	Number of fish per pool	Average size		Cumulative kill (hrs.)						Cumulative kill (hrs.)					
				Hardness of 190 p.p.m.						Hardness of 282 p.p.m.					
		Length (in.)	Weight (g.)	6	24	48	72	96	120	6	24	48	72	96	120
Rainbow trout.......	10	5.8	32.0	0	1	9	10	--	--	0	2	10	--	--	--
Brown trout.........	10	5.9	38.0	0	0	3	10	--	--	0	0	5	10	--	--
Goldfish............	10	1.4	0.7	0	2	1	5	5	6	0	0	0	5	9	9
Do..............	10	3.8	22.8	0	0	0	0	0	0	0	0	0	0	0	0
Carp................	10	1.6	0.9	0	0	3	3	9	10	0	0	1	6	10	--
Do..............	10	7.2	82.2	0	0	0	0	0	0	0	0	0	0	0	0
Fathead minnow......	10	2.1	1.6	0	0	0	0	0	0	0	0	0	0	0	1
Bluegill............	20	1.4	0.7	0	0	2	10	15	16	0	0	8	16	20	--
Do..............	10	2.8	5.2	0	0	0	1	1	1	0	0	0	0	1	3
Pumpkinseed.........	10	2.6	6.0	0	0	0	0	0	0	0	0	0	0	0	0
Longear sunfish.....	10	3.1	8.0	0	0	2	3	9	10	0	0	1	5	10	--

TABLE 13.--Toxicity of sand-formulated antimycin to adults of three resistant species in 96 hours at 14.5° C.

Species	Number per concen-tration	Length (in.)		Percent mortality at concentrations of (p.p.b.)[1]								
		Average	Range	10	15	20	25	90	120	140	180	220
Shortnose gar...........	5	23.0	20.0-26.0	0	20	80	100	--	--	--	--	--
Bowfin..................	5	22.0	16.0-24.0	20	60	100	100	--	--	--	--	--
Black bullhead..........	50	6.5	5.5- 9.0	--	--	--	--	0	14	50	74	100

[1] There was no mortality among controls of each species.

conditions, 1 p.p.b. of sand-formulated antimycin killed all rainbow trout and 86 percent of the bluegills but failed to affect the two size groups of goldfish and the carp (table 14). Two p.p.b. killed all spotted suckers and green sunfish as well as the trout and bluegills. Four p.p.b. was the lowest concentration at which all carp perished, but a small number of the larger goldfish survived both 4 and 5 p.p.b. All fish of the 12 species were eradicated by 10 p.p.b.

Caged rainbow trout of fingerling size were placed in the pools each 24 hours. Their survival showed that the toxicant degraded to a safe level for the species within 8 days in every test in all pools.

A cold-weather test in the 0.01-acre pools demonstrated that Fintrol-5 is effective against fish in cold, shallow, clear, or turbid waters. The pools were maintained at 2 feet deep during the 3-week trial. Water temperatures ranged from 3.4° to 5.5° C., pH from 7.4 to 8.2, total hardness from 260 to 320 p.p.m., and alkalinity from 235 to 260 p.p.m. The Secchi disk reading was 6 inches in four turbid pools and to the bottom in four clear pools.

Juvenile or adult brown trout, carp, bluegill, and largemouth bass in clear pools succumbed within 2 to 7 days to 5 p.p.b. of antimycin and within 1 to 3 days at 10 p.p.b. Death was delayed in turbid water; the fish died within 7 to 12 days at 5 p.p.b. and within 3 to 7 days at 10 p.p.b. Large goldfish were the most resistant fish, but all were killed by 5 p.p.b. of antimycin within 15 days in clear water and within 18 days in turbid water; the kill at 10 p.p.b. occurred within 11 days in clear water and within 16 days in turbid water.

Fingerling yellow perch in live cages were used to detect the degradation of Fintrol-5 in the pools. The process was slow in the cold water, but degradation occurred sooner

TABLE 14.--Toxicity of sand-formulated antimycin to juveniles or adults of 12 species of fish in 0.01-acre pools

Species	Number per concentration	Length (in.)		Percent mortality at concentrations of (p.p.b.)							
		Average	Range	1	2	3	4	5	10	20	Control
Rainbow trout	10	9.9	8.8-11.0	100	100	100	100	100	100	100	0
Brown trout	20	7.5	6.5- 8.3	--	--	--	--	--	100	100	0
Lake trout	20	4.7	3.5- 5.2	--	--	--	--	--	100	100	0
Northern pike	10	20.0	12.0-22.5	10	50	100	100	100	100	100	20
Goldfish	30	3.8	3.0- 5.0	0	30	70	--	100	100	100	10
Do.	10	6.9	4.8- 8.1	0	0	30	80	90	100	100	0
Carp	35	12.1	7.4-22.0	0	33	96	100	100	100	100	3
Quillback	8	13.9	8.0-16.0	--	--	100	--	100	--	--	0
Spotted sucker	6	14.8	13.8-15.7	--	100	--	100	--	--	--	0
Green sunfish	40	3.6	2.8- 4.4	--	100	100	--	--	--	--	10
Bluegill	30	6.0	4.2- 7.8	86	100	100	100	100	100	100	5
Largemouth bass	10	7.6	5.4- 8.6	--	50	100	100	100	--	--	0
Black crappie	35	9.8	8.0-11.0	43	--	--	--	--	100	100	20

in clear water than in turbid water. Five and 10 p.p.b. of antimycin disappeared within 8 and 13 days respectively in clear water and within 9 and 17 days in turbid water.

Water temperature has greater influence than turbidity on the response of fish to antimycin, but mortality of the fish is merely retarded rather than reduced in cold and turbid water.

TOXICITY OF INJECTED ANTIMYCIN

Injections of antimycin into the peritoneal cavities of fish are toxic, and the order of toxicity is similar to that observed when fish are immersed in solutions of antimycin. Moreover, the behavior of injected and immersed fish is similar.

Of three species, rainbow trout are the most sensitive to the injected toxicant. Some trout died at 0.01 mg. of antimycin per kg. of body weight, and all died within 24 hours at 0.02 mg./kg. (table 15). Carp are intermediate in sensitivity to injected antimycin, with some dying at 0.14 mg./kg. and all dying at 0.40 mg./kg. or more within 72 hours. In contrast, not less than 20 mg./kg. cause mortalities among black bullheads, and 30 mg./kg. or more are needed for complete kills within 24 hours.

There were no deaths among the control fish of the three species which were injected with the ethanol-water solvent.

STABILITY OF ANTIMYCIN

The shelf life of acetone-antimycin in stock solutions is good in cool, dark storage. Samples held for 2 years were checked periodically for potency against fingerling rainbow trout. The 96-hour EC_{50}'s and confidence intervals at 12° C. exhibited no greater differences in range than expected when using different lots of the same test species. Similarly, the shelf life of the sand formulation appears to be good if it is kept cool and dry, but the shelf life has not been measured specifically.

Antimycin can be destroyed by exposure to high heat for an adequate period of time. Crystalline antimycin which had been subjected to dry heat at 200° C. for 60 minutes or longer, and then dissolved in acetone and bioassayed was not toxic at 5 or 25 p.p.b. to fingerling rainbow trout within 96 hours (table 16). Crystals which were heated at 200° for only 15 or 30 minutes remained as toxic to fish as the unheated control.

TABLE 15.--Toxicity of intraperitoneal injections of antimycin at 12°C.

Species	Weight (grams)		Injection	Fish alive/dead at--				
	Average	Range	mg./kg.	24 hours	48 hours	72 hours	96 hours	120 hours
Rainbow trout..........	33	30-34	0.002	5/0	5/0	5/0	5/0	--
Do..................	33	30-34	0.010	4/1	2/3	2/3	2/3	--
Do..................	33	30-34	0.020	0/5	--	--	--	--
Do..................	33	30-34	Control	5/0	5/0	5/0	5/0	5/0
Carp..................	320	300-330	0.100	5/0	5/0	5/0	5/0	--
Do..................	335	290-390	0.140	--	4/1	2/3	2/3	2/3
Do..................	360	300-430	0.160	--	3/2	3/2	2/3	2/3
Do..................	320	280-360	0.180	--	4/1	2/3	1/4	0/5
Do..................	333	300-370	0.200	5/0	5/0	5/0	4/1	4/1
Do..................	300	280-320	0.400	5/0	2/3	0/5	--	--
Do..................	290	265-310	0.600	5/0	0/5	--	--	--
Do..................	340	310-350	1.000	4/1	0/5	--	--	--
Do..................	325	306-340	Control	5/0	5/0	5/0	5/0	5/0
Black bullhead.........	270	240-300	10.00	4/0	4/0	4/0	4/0	4/0
Do..................	118	100-130	20.00	2/3	2/3	2/3	2/3	2/3
Do..................	121	110-130	25.00	1/4	1/4	1/4	1/4	1/4
Do..................	150	120-170	30.00	0/5	--	--	--	--
Do..................	105	90-140	35.00	0/5	--	--	--	--
Do..................	110	105-120	Control	5/0	5/0	5/0	5/0	5/0

TABLE 16.--Effects of oven-heating at 200° C. on the toxicity of crystalline and sand-formulated antimycin to fingerling rainbow trout at 12°.

[Results presented in numbers of fish alive/dead at selected intervals]

Bioassay time (hours)	Controls	Crystals at 5 p.p.b.				Crystals at 25 p.p.b.				Fintrol-5 at 25 p.p.b.			
		Non-heated	Heated (min.)			Non-heated	Heated (min.)			Non-heated	Heated (min.)		
			15	30	60		30	60	120		30	60	120
0.25.......	30/0	10/0	10/0	10/0	10/0	10/0	10/0	10/0	10/0	10/0	10/0	10/0	10/0
0.50.......	30/0	10/0	10/0	10/0	10/0	10/0	3/7	10/0	10/0	10/0	10/0	10/0	10/0
1.00.......	30/0	10/0	10/0	10/0	10/0	0/10	0/10	10/0	10/0	0/10	8/2	10/0	10/0
2.00.......	30/0	10/0	10/0	10/0	10/0	--	--	10/0	10/0	--	3/9	10/0	10/0
3.00.......	30/0	0/10	0/10	0/10	10/0	--	--	10/0	10/0	--	0/10	10/0	10/0
24.00.......	29/1	--	--	--	10/0	--	--	10/0	10/0	--	--	10/0	10/0
96.00.......	29/1	--	--	--	10/0	--	--	10/0	10/0	--	--	10/0	10/0

DETOXIFICATION WITH POTASSIUM PERMANGANATE

Although antimycin degrades rapidly in water (Derse and Strong, 1963; Walker, Lennon, and Berger, 1964), it may sometimes be desirable to detoxify treated waters. Potassium permanganate was included among candidate detoxifiers because of its activity as a strong oxidizer and the fact that fishery managers are familiar with it.

Crystalline potassium permanganate was dissolved in water and added at concentrations of 5 to 500 p.p.b. to 15-liter bioassay solutions which contained 5 p.p.b. of antimycin at 12° C. Fingerling bluegills were added to the vessels 6 or 24 hours later, and their responses were noted over the following 96 hours. The results reveal that 300 p.p.b. of potassium permanganate detoxifies 5 p.p.b. of antimycin within 6 hours. On the basis of these results, a detailed investigation was initiated on the detoxification of antimycin by potassium permanganate; this will be reported separately.

DISCUSSION

These studies provide abundant evidence that antimycin has a fine potential as a fishery tool. It is a pure compound which fortunately can be formulated simply and with great integrity. The crystals are easily dissolved in quantities of acetone or ethanol which themselves are not harmful to fish. The formulations in Carbowax coated on fine sand also are precise and uniform. Consequently, fish can be exposed easily to exact quantities of active ingredient and with no influence from the carriers.

Because of these advantages, we are using antimycin as a reference compound in routine bioassays of unknown chemicals. Fish of any species may vary from lot to lot and from season to season in their sensitivity to a chemical. Also, fish within a lot may increase or decrease in sensitivity to a chemical depending on stresses involved in their holding and acclimation at the laboratory. These possible variations in sensitivity are not apparent in tests with compounds of unknown activities. We expose all lots of bioassay fish to antimycin, therefore, as a check on their sensitivity to a toxicant.

The order of toxicity of antimycin to 31 species of fish is summarized in table 17. In static 96-hour bioassays, 1 p.p.b. of the toxicant kills the most susceptible fishes, including trouts, walleye, and yellow perch. Five p.p.b. kill fish of intermediate sensitivity, including carp and green sunfish which are often target coarse fish. Of the 31 species, 27 are eliminated by 10 p.p.b., but up to 25 p.p.b. are needed to kill shortnose gar, bowfin, and channel catfish. White catfish, flathead catfish, and black bullheads are comparatively resistant to antimycin and up to 200 p.p.b. is required for eradication.

TABLE 17.--Order of toxicity of antimycin to 31 fishes, including various life stages at different water temperatures and qualities

1.0 p.p.b:	5.0 p.p.b:--Continued
Rainbow trout	Redear sunfish
Brown trout	Pumpkinseed
Brook trout	Green sunfish
Lake trout	Fathead minnow
Walleye	Northern redbelly dace
Yellow perch	Brook stickleback
	Largemouth bass
5.0 p.p.b.:	
White sucker	7.5 - 10.0 p.p.b.:
Smallmouth bass	Goldfish
Freshwater drum	
Black crappie	25 p.p.b.:
Bigmouth buffalo	Shortnose gar
Quillback	Bowfin
Spotted sucker	Channel catfish
Northern pike	200 p.p.b.:
Carp	White catfish
Longear sunfish	Flathead catfish
Bluegill	Black bullhead

In addition to the species above, other fish are known to be killed by antimycin. Walker, Lennon, and Berger (1964) listed gizzard shad (Dorosoma cepedianum), stoneroller (Campostoma anomalum), golden shiner (Notemigonus crysoleucas), yellow bullhead (Ictalurus natalis), brown bullhead (Ictalurus nebulosus), white crappie (Pomoxis annularis), and Iowa darter (Etheostoma exile). Howell[1] found that antimycin at 9 p.p.b. is toxic to larvae of the sea lamprey (Petromyzon marinus) within 21 hours. Lowe (1966) determined that the 48-hour EC_{50} for acetone-antimycin against spot (Leiostomus xanthurus) at 25° C. is 0.23 p.p.b. In his trials of sand-formulated antimycin on juvenile fish, Lowe also found that 10 p.p.b. killed all longnose killifish (Fundulus similis) within 48 hours, and that the 48-hour EC_{50}'s for sea catfish (Galeichthys felis) and sheepshead minnow (Cyprinodon variegatus) at 19° and 23° C. are 10 and 32 p.p.b. respectively. Meyer[2] reported that grass carp (Ctenopharyngoden idellus) are killed by 3 p.p.b. of antimycin and tilapia (Tilapia mossambica) by 5 p.p.b. within 24 hours in aquaria at room temperature.

[1] Letter from John Howell, Supervisory Fishery Biologist, Hammond Bay Biological Station, Bureau of Commercial Fisheries, Millersburg, Mich., 1966.

[2] Letter from Dr. Fred P. Meyer, Chief, Fish Farming Experimental Station, Bureau of Sport Fisheries and Wildlife, Stuttgart, Ark., 1966.

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Antimycin has another advantage as a fish control agent: fish are not repelled or excited by its presence in water. This, coupled with the order of toxicity and effectiveness on various life stages, suggests that the toxicant has possibilities for partial or selective control of fish. The lethal effects on fish eggs, for example, indicate that it might be employed effectively in spawning areas for partial control of unwanted species. Or it might be useful for control of trash fish in catfish production ponds. There are also possibilities worth investigating that the sand formulations, Fintrol-5 and Fintrol-15, might be used in littoral zones or epilimnions of thermally stratified lakes to remove stunted or unwanted fish.

Certain facts disclosed by the studies point to a possible usefulness of antimycin in reclaiming streams. They include the lack of color, odor, or repellency in the toxicant, the relatively brief exposures needed to kill fish, the relatively rapid rate of degradation, and the susceptibility of the antibiotic to detoxification with potassium permanganate.

Also, from the point of view of fishery management, the toxicant is effective in a variety of water qualities. High pH contributes to more rapid degradation of antimycin, but this might be an advantage in certain situations so long as the concentrations applied persist through an effective contact time. Temperature has less effect on the activity of the toxicant than pH, but somewhat greater concentrations may be required in very cold waters and under ice than in waters of moderate temperature. Water hardness has less influence on antimycin than pH or temperature, but toxicity is slightly greater in soft water than in hard water.

A mineral turbidity may retard or reduce the toxicity of antimycin to fish, depending on the amount of material in suspension and whether it remains in suspension or settles out. Similarly, Ferguson et al. (1966) reported that muds reduce the bioactivity of chlorinated hydrocarbon insecticides on fish.

As an outgrowth of these laboratory trials, the development of antimycin as a fishery tool has begun to include comprehensive tests of its toxicity to birds and mammals (Vezina, 1967), and experimental applications against fish in lakes and streams (Lennon, 1966; Berger, 1966). Lennon, Berger, and Gilderhus (1967) adapted seed spreaders for distributing sand-formulated antimycin in field operations. Powers and Bowes (1967) used antimycin to control predaceous fish in a grebe refuge in Guatemala. Hogan (1967) and Burress and Luhning (1969) investigated the utility of antimycin in channel catfish production. Walker (1967) has further defined the amounts of potassium permanganate needed to detoxify various concentrations of antimycin in water.

CONCLUSIONS

1. Antimycin in water or injected intraperitoneally is toxic to fish. Various life stages of some species of fish were included in tests, and the antibiotic was toxic to all, egg through adult.

2. Solutions of antimycin crystals in acetone or ethanol or dry preparations in Carbowax on sand are stable in storage, irreversibly toxic to fish in waters of various qualities and temperatures, and subject to detoxification by potassium permanganate.

3. Natural degradation occurs sooner in hard, alkaline waters than in soft, acid waters. The degradation products are not toxic to fish. The exposure of the antibiotic to dry heat at 200^{0} C. for short periods of time reduces or destroys its toxicity to fish.

4. The purity of the compound, the integrity of its formulations, and its lack of repellency to fish are characteristics which enhance its use for general, partial, or selective control of unwanted fish.

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INVESTIGATIONS IN FISH CONTROL

27. Field Trials of Antimycin A as a Fish Toxicant

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United States Department of the Interior
Fish and Wildlife Service
Bureau of Sport Fisheries and Wildlife
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FIELD TRIALS OF ANTIMYCIN A AS A FISH TOXICANT

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ABSTRACT.--Antimycin A was subjected to field trials as a fish toxicant in 20 ponds and lakes and 5 streams in the East, Midwest, and West of the United States. The formulations of toxicant included three on sand grains which are designed to release antimycin uniformly within certain depths, and one formulation in a liquid. Ten parts per billion or less of the toxicant were effective against most of the 54 species of fish we encountered, including carp, suckers, and green sunfish. Differences in sensitivity among fish suggest possible use of antimycin as a selective toxicant. The efficacy of the toxicant is influenced by pH and water temperature, with slightly higher concentrations necessary at high pH or in cold water. Antimycin does not repel fish, and its toxic action on fish appears to be irreversible. It degrades rapidly, usually within a week. Fish-killing concentrations have little or no effect on other aquatic animals.

The development and registration of a fish toxicant require that the candidate chemical be tested thoroughly in the laboratory and in the field to evaluate its toxicity to different forms of invertebrate and vertebrate life, its efficacy on fish, and its residues in water and fish. The trials in the field must involve at least three ecologically different sites, and target and other organisms typical of those sites. Also, they must be organized, executed, and reported as controlled experiments, and the data from them are reviewed in detail by regulatory agencies at Federal and State levels before the toxicant may be registered for use. Notably, the regulatory authorities warn against submitting the once-common testimonial type of reports on field trials.

The field trials of antimycin A followed intensive testing in the laboratory which demonstrated its potential as a toxicant specific to fish (Walker et al., 1964; Vezina, 1967; Herr et al., 1967; and Berger et al., 1969). The sites were chosen and the trials conducted with the requirements for registration in mind. Thus, waters in the East, Midwest, and West of the United States were included. Soon after each of the early trials, a report was submitted to the Pesticide Regulation Division of the U.S. Department of Agriculture for review and for suggestions on points to consider in subsequent trials.

The objectives of the field trials were:

1. to evaluate the efficacy of antimycin A in several formulations against as many species of fish as possible in cold and warm waters, acid and alkaline waters, and clear and turbid waters,

2. to determine concentrations of antimycin which are effective against such prime target fish as carp, suckers, and green sunfish,

3. to observe any effects of the toxicant on other aquatic animals,

4. to detect whether or not the toxicant repels fish,

5. to find out if the toxic action in fish is irreversible,

6. and to observe the rates of natural and induced degradation of antimycin in water.

The early trials were conducted on very small ponds for two reasons. First, the tests were easier to control and evaluate than in larger waters. Second, the quantities of formulated antimycin were limited because pharmacologists of the producing company were then in the midst of developing and perfecting the Fintrol formulations.

Concurrently, field trials by other investigators bolstered the development of antimycin as a fish toxicant (Loeb, 1964; Lennon, 1966; Lennon et al., 1967; Powers and Bowes, 1967; Powers and Schneberger, 1967; Radonski, 1967; Burress, 1968; and Burress and Luhning, 1969a and 1969b).

The successful accomplishment of field trials was made possible only through the generous cooperation of many people. We are indebted especially to fishery personnel of State conservation departments who provided experimental sites, stocked fish, and manpower; to fishery and wildlife personnel in Bureau hatcheries, refuges, and laboratories who also provided trial sites, fish, and aid; to Wisconsin Alumni Research Foundation, owners of antimycin, for advice and aid; to Ayerst Laboratories, producers of antimycin, for supplying the pure and formulated toxicant at no charge; and to personnel of the Pesticide Regulation Division, U.S. Department of Agriculture, for counsel on the scope and depth of experimental data needed for registration of antimycin as a fishery tool.

MATERIALS AND METHODS

Antimycin A

The early development of antimycin A as a fish toxicant included much consideration and investigation of formulations to meet the requirements of fish managers. A liquid formulation was needed for application in streams and in the very shallow waters of ponds and marshes. Accordingly, an experimental Fintrol-Liquid was prepared, consisting of crystalline antimycin dissolved in acetone or ethanol with a small amount of surfactant added.

We recognized the long-standing problem that fish managers have experienced in getting adequate distribution of liquid or powered toxicants into the deeper waters of ponds and lakes, especially when thermal stratification exists. We sought granular formulations, heavier than water, which would release antimycin within selected depths. Thus, the cumbersome and often imperfect job of pumping a toxicant into the depths of a lake would be avoided. As a result of cooperative efforts among scientists of Ayerst Laboratories, Wisconsin Alumni Research Foundation, and the Bureau of Sport Fisheries and Wildlife, experimental formulations of antimycin on sand were developed.

The sand formulations consist of antimycin incorporated in Carbowax(R) and coated on sand in such a way that the toxicant is released into the water within certain depths as the sand grains sink toward the bottom. Because we expected that most of the early applications of antimycin would be in production ponds, farm ponds, and small lakes, we chose formulations on sand which would release the toxicant evenly within the first 5, 15, and 30 feet of depth. They are called Fintrol-5, Fintrol-15, and Fintrol-30, respectively. Of them, only Fintrol-5 is registered and on the market. Formulations for release of toxicant over greater depths or for delayed release on the bottom are possible and may be developed later.

All of the formulated antimycin used in the field trials was prepared and furnished by Ayerst Laboratories, New York, N.Y.

The liquid formulation of antimycin was applied to streams and shallows of ponds by means of spray apparatus or metering pumps. In most cases, a strong solution of Fintrol-Liquid in water was prepared on site and promptly dispensed. Metering pumps of the type described by Anderson (1962) gave the best performance.

The formulations of antimycin on sand were broadcast by hand, by hand-operated seed spreader, or by powered seed spreaders mounted on boats (Lennon et al., 1967). An attempt was made on each job to achieve as rapid and as even distribution as possible.

Degradation or Detoxification of Antimycin

The degradation of the toxicant in ponds was determined by field bioassays. Fingerling rainbow trout or bluegills were placed in live cages and submerged at selected sites and depths in the ponds at least 24 hours prior to the application of antimycin. Each live cage contained at least 10 specimens of a species and size of fish.

Following application of the toxicant, the fish in live cages were checked frequently during the first 36 to 48 hours to detect the rate and amount of mortality. As soon as all specimens of a species in a live cage were dead, a replacement lot was provided. The survival of all specimens in a live cage for at least 48 hours was indicative that the toxicant had degraded below the lethal concentration or had flowed from the vicinity.

Potassium permanganate was used during two field trials to detoxify the antimycin in effluent waters. Solutions or crystals of $KMnO_4$ were applied to obtain concentrations of 1 to 2 p.p.m. in the water.

Sites of Field Trials

The 13 sites selected for field trials of antimycin included 20 ponds or lakes and 5 streams (tables 1 and 2). The first trials were conducted on Bureau property and involved 14 ponds which are not subject to public fishing. These selections were made not only to obtain good tests of the toxicant, but also as a precaution against undue risks to the public until we knew more about the performance of antimycin in the field.

The ponds and lakes ranged in area from 0.25 to 63 surface acres and in volume from 0.45 to 787 acre-feet (table 2). The streams were small and ranged in volume of flow from 2 to 20 c.f.s. The ranges in water constituents were: pH, 7.0 to 8.8; total hardness, 10 to 350 p.p.m.; and total alkalinity, 13 to 350 p.p.m. (tables 2 and 3). Water temperatures ranged from 5° to 20° C., and all temperatures in this report are expressed in Celsius.

Fifty-four species of freshwater fish were represented in the trials, and they included such problem species as carp, white suckers, and green sunfish (table 4). In the early trials in small ponds, known numbers and sizes of fish were stocked to facilitate evaluations on the performance of the toxicant.

TABLE 1.--Sites of field trials with antimycin A

Town and state	Site	Owned or managed by
	Ponds and lakes	
1. Berlin, N.H........	Berlin NFH[1]	Bureau of Sport Fisheries and Wildlife
2. Cape Vincent, N.Y..	Cape Vincent NFH	Bureau of Sport Fisheries and Wildlife
3. Stuttgart, Ark.....	Warmwater Fish Cultural Laboratory	Bureau of Sport Fisheries and Wildlife
4. Valentine, Neb.....	Valentine NWR[2]	Bureau of Sport Fisheries and Wildlife
5. Saratoga, Wyo......	Saratoga NFH	Bureau of Sport Fisheries and Wildlife
6. West Salem, Wis....	Venterans Memorial Park	La Crosse County, Wisconsin
7. Madison, Wis.......	Lake Katrine	State of Wisconsin
8. Oxford, Wis........	Parker Lake	State of Wisconsin
	Streams	
9. Viroqua, Wis.......	Sidie Hollow Creek	State of Wisconsin
10. Plymouth, Wis......	Mullet River	State of Wisconsin
11. Cataract, Wis......	Rathbone Creek	State of Wisconsin
12. Westfield, Wis.....	Westfield Creek	State of Wisconsin
13. Marshall, Wis......	Waterloo Creek	State of Wisconsin

[1] National Fish Hatchery
[2] National Wildlife Refuge

TABLE 2.--Physical and chemical characteristics of ponds and lakes

Site	Date	Pond or lake	Surface area (acres)	Volume (acre-feet)	Water temp. (°C.)	Chemical characteristics				
						DO (p.p.m.)	CO_2 (p.p.m.)	pH	Total hardness (p.p.m.)	Total alkalinity (p.p.m.)
1. Berlin NFH......	9/64	P1	--	0.45	12	9.6	0.1	7.0	10.0	14.2
		P2	--	1.16	13	9.4	0.1	7.2	10.0	12.8
		P3	--	0.55	12	9.0	0.1	7.2	10.0	14.5
2. Cape Vincent NFH	10/64	P1	1.00	2.93	12	8.9	2.5	8.1	153.0	110.5
		P2	1.00	2.55	12	8.7	3.0	7.9	159.0	118.0
		P3	1.00	2.74	12	8.0	3.2	8.1	155.0	118.0
3. Warmwater Fish Cultural Laboratory.	3/65	P1	0.25	0.58	17	9.6	1.0	8.0	59.0	81.0
		P2	0.25	0.58	17	10.4	1.0	8.1	58.0	78.0
		P3	0.25	0.58	17	9.6	1.0	8.2	55.0	72.0
		P4	0.25	0.58	17	10.4	1.0	8.2	58.0	77.0
		P5	0.25	0.58	17	9.6	1.0	8.1	60.0	79.0
		P6	0.25	0.58	17	10.4	1.0	8.2	52.0	70.0
	12/65	L1	19.40	42.68	12	10.8	--	8.6	112.0	110.0
		L2	0.25	0.58	12	7.4	--	7.9	120.0	148.0
4. Valentine NWR...	5/65	P1	1.80	3.24	19	8.3	0.0	8.4	107.0	155.0
		P2	0.50	1.95	20	--	0.9	7.9	142.0	258.0
5. Saragoga NFH....	10/65	--	1.79	5.84	9	--	--	7.7	237.0[1]	184.0
6. Veterans Memorial Park.	11/65	--	4.78	21.51	13	--	--	7.8	223.0	188.0
7. Lake Katrine....	5/66	--	2.70[2]	10.01	17	--	--	6.8-8.5	32.0	25.0
	10/66	--	30.00	118.00	18	--	--	8.2	--	35.0
8. Parker Lake.....	10/66	--	63.00	787.00	9	--	--	8.2	197.0	--

[1] Total dissolved solids
[2] Partial treatment (small bay)

TABLE 3.--Physical and chemical characteristics of streams

Site	Date	Flow (c.f.s.)	Length of treated section (mi.)	Width (ft.)	Water temp. (°C.)	Chemical characteristics	
						pH	Total hardness (p.p.m.)
9. Sidie Hollow Creek.	8/65	1.76	3.5	4	10 -18	8.3	240
10. Mullet River.......	9/66	10.0	2.5	18	20 -22	8.4	210
11. Rathbone Creek.....	11/66	10.7	6.0	9	5	7.5	24
12. Westfield Creek....	6/67	21.7	3.0	20	20	8.8	170
13. Waterloo Creek.....	7/67	4.3	1.0	30	25	8.6	350

TABLE 4.--Fish exposed to antimycin in field trials

Common name	Scientific name	Sites where found (by number)
American brook lamprey.........	Lampetra lamottei	11, 12
Paddlefish.....................	Polyodon spathula	3
Bowfin.........................	Amia calva	2
Gizzard shad...................	Dorosoma cepedianum	3
Rainbow trout..................	Salmo gairdneri	1, 2, 3, 6, 8, 9, 12
Atlantic salmon................	Salmo salar	1
Brown trout....................	Salmo trutta	2, 6, 9, 10
Brook trout....................	Salvelinus fontinalis	1, 2, 5, 11
Central mudminnow..............	Umbra limi	6, 10, 11
Chain pickerel.................	Esox niger	1
Northern pike..................	Esox lucius	2, 4, 6, 10, 13
Stoneroller....................	Campostoma anomalum	9
Goldfish.......................	Carassius auratus	3
Carp...........................	Cyprinus carpio	2, 3, 4, 6, 7, 8, 10, 12, 13
Golden shiner..................	Notemigonus crysoleucas	1, 2, 3, 6
Common shiner..................	Notropis cornutus	1
Bluntnose minnow...............	Pimephales notatus	12
Fathead minnow.................	Pimephales promelas	4, 5, 6, 7, 12
Blacknose dace.................	Rhinichthys atratulus	1, 9, 12
Longnose dace..................	Rhinichthys cataractae	9
Creek chub.....................	Semotilus atromaculatus	1, 5, 9, 10, 12
Fallfish.......................	Semotilus corporalis	1
White sucker...................	Catostomus commersoni	1, 2, 6, 9, 10, 11, 12, 13
Northern hog sucker............	Hypentelium nigricans	9
Smallmouth buffalo.............	Ictiobus bubalus	3
Bigmouth buffalo...............	Ictiobus cyprinellus	3
Buffalo hybrid.................	Ictiobus sp.	3
White catfish..................	Ictalurus catus	3
Blue catfish...................	Ictalurus furcatus	3
Black bullhead.................	Ictalurus melas	3, 4, 6, 7
Yellow bullhead................	Ictalurus natalis	3
Brown bullhead.................	Ictalurus nebulosus	1, 2
Channel catfish................	Ictalurus punctatus	3, 6
Tadpole madtom.................	Noturus gyrinus	3
Flathead catfish...............	Pylodictus olivaris	3
Mosquitofish...................	Gambusia affinis	3
Brook stickleback..............	Eucalia inconstans	9, 11, 12
White bass.....................	Roccus chrysops	2
Rock bass......................	Ambloplites rupestris	2, 10
Warmouth.......................	Chaenobryttus gulosus	3
Green sunfish..................	Lepomis cyanellus	3, 4, 6, 10
Pumpkinseed....................	Lepomis gibbosus	1, 2, 6, 8, 12
Orangespotted sunfish..........	Lepomis humilis	3
Bluegill.......................	Lepomis macrochirus	1, 2, 3, 4, 6, 8, 10, 12
Smallmouth bass................	Micropterus dolomieui	1, 2
Largemouth bass................	Micropterus salmoides	1, 2, 3, 4, 10, 12
White crappie..................	Pomoxis annularis	6
Black crappie..................	Pomoxis nigromaculatus	2, 3, 4, 10
Iowa darter....................	Etheostoma exile	5
Fantail darter.................	Etheostoma flabellare	12
Johnny darter..................	Etheostoma nigrum	9, 10, 11, 12
Yellow perch...................	Perca flavescens	1, 2, 4, 8
Walleye........................	Stizostedion vitreum vitreum	2
Freshwater drum................	Aplodinotus grunniens	2
Mottled sculpin................	Cottus bairdi	9, 12

Pre- and Post-treatment Surveys

STANDING WATER

The ponds and lakes were mapped carefully, including depths and contours. Their volumes in acre-feet were calculated from measurements of area and depth. Analyses of water chemistry were made on site and correlated with any historical data available. The species, abundance, and sizes of fish were assessed before the trials from stocking records, or by netting or electrofishing. The post-treatment presence of fish was detected by netting, electrofishing, or by draining the ponds.

The populations of bottom fauna and plankton were sampled by standard techniques before and after applications of antimycin, and attempts were made to correlate changes in composition or abundance with causative factors.

FLOWING WATER

The streams were mapped and sounded, and the volumes and velocities were measured with a current meter. Measurements of stretchout and dilution were accomplished in soft water by the salt-resistivity technique (Lennon, 1959; Lennon and Parker, 1959) and in hard water by use of the rhodamine-fluorometer technique (Buchanan, 1964). The losses in concentration of salt or rhodamine dye between selected stations on a stream were assumed to equal the losses of a toxicant in the same flow and distance. On the basis of these data, the sites of fortifying stations were located and the amounts of toxicant to be added at each station were determined.

Analyses of the water were made routinely by standard techniques. The populations of fish were sampled before and after applications of antimycin by electrofishing.

RESULTS

Trials in Ponds and Lakes

BERLIN, NEW HAMPSHIRE

Three, small earth ponds were selected at the Berlin National Fish Hatchery as typical of cold, soft, trout water in northern New England (tables 2, 5, and 6). Sixteen species of wild and hatchery-reared fish were stocked in known numbers at least 1 week prior to the experimental reclamation.

We chose a wide range of concentrations of antimycin because this was the first trial in soft water. One pond was treated with 0.13 p.p.b., another with 1.22 p.p.b., and the third with 12.00 p.p.b. It was also the first field trial of the sand formulation, Fintrol-5.

Nine of the 12 species exposed to 0.13 p.p.b. suffered mortalities. Only common shiners had a complete kill; the other species had partial mortalities ranging from 18 to 75 percent. The fish in this pond, however, received only a 24-hour exposure to the toxicant. There was a large loss of water from the pond because of seepage, and fresh water had to be added each 24 hours. The fish unaffected by the relatively brief exposure to the low concentration were chain pickerel, brown bullheads, and bluegills.

The toxicant at 1.22 p.p.b. caused complete kills of 12 species within 72 hours, and it degraded in 120 hours. A few bluegills and smallmouth bass and all brown bullheads survived.

The 12 p.p.b. of antimycin killed the most sensitive species within 10 hours and all fish except brown bullheads within 48 hours. More than 2 weeks were required for this concentration of toxicant to degrade below fish-killing levels.

TABLE 5.--Applications of antimycin and rates of degradation in field trials

Site	Pond or lake	Formulation (percent active)	Total formu- lation	Antimycin (g.)	Conc. of antimycin in water (p.p.b.)	Degradation time (hrs.)
1. Berlin NFH.........	P1	Fintrol-5, 0.19	38 g.	0.07	0.13	24
	P2	Fintrol-5, 0.19	940 g.	0.76	1.22	120
	P3	Fintrol-5, 0.88	1,000 g.	8.22	12.00	>330
2. Cape Vincent NFH...	P1	Fintrol-5, 0.10	3,790 g.	3.70	1.02	96
	P2	Fintrol-5, 0.29	3,395 g.	9.85	3.12	96
	P3	Fintrol-5, 0.96	3,675 g.	35.40	10.40	96
3. Warmwater Fish Cultural Laboratory March 1965	P1	Fintrol-5, 1.0	360 g.	3.60	5.00	168
	P2	Fintrol-5, 1.0	360 g.	3.60	5.00	168
	P3	Fintrol-5, 1.0	540 g.	5.40	7.50	168
	P4	Fintrol-5, 1.0	540 g.	5.40	7.50	168
	P5	Fintrol-5, 1.0	720 g.	7.20	10.00	168
	P6	Fintrol-5, 1.0	720 g.	7.20	10.00	168
December 1965....	L1	Fintrol-5, 1.0	53 kg.	526.64	10.00	120
	L2	Fintrol-5, 1.0	400 g.	4.00	10.00	120
4. Valentine NWR......	P1	Fintrol-5, 1.0	4,018 g.	40.18	10.00	24
	P2	Fintrol-5, 1.0	2,480 g.	24.80	10.00	120
5. Saratoga NFH.......		Fintrol-5, 1.0	17 kg.	17.20	10.00	--
6. Veterans Memorial Park.		Fintrol-5, 1.0	22 kg.	220.00	10.00	144
7. Lake Katrine						
May 1966.........		Fintrol-5, 1.0	5,000 g.	50.00	4.00	96
Oct. 1966........		Fintrol-5, 1.0	431 kg.	4,313.00	7.50	120
8. Parker Lake........		Fintrol-5, 1.0	288 kg.	2,883.00	10.00	--
		Fintrol-15, 1.5	175 kg.	2,647.00	--	--
		Fintrol-30, 3.2	159 kg.	5,130.00	--	168
		Liquid	--	100.00	--	--
9. Sidie Hollow Creek.		Liquid	--	--	15 for 5 hours	--
10. Mullet River....... (Plymouth Pond)		Liquid, 0.37-0.52	43 l.	206.50	7.5 for 8 hours	--
11. Rathbone Creek.....		Liquid, 10.0	1,540 ml.	154.00	7.5 for 8 hours	--
(Cataract Pond)		Fintrol-5, 1.0	11.6 kg.	115.70	7.5	--
12. Westfield Creek....		Liquid, 10.0	6,250 ml.	625.00	10 for 10 hours	--
13. Waterloo Creek.....		Liquid, 10.0	1,825 ml.	182.00	10 for 5 hours	--

TABLE 6.--Effects of antimycin on fish at Berlin NFH

Species	Size (inches)	Number per pond	Percent killed at		
			0.13 p.p.b.	1.22 p.p.b.	12.0 p.p.b.
Rainbow trout............	5.3-5.9	115-116	18	100	100
Atlantic salmon..........	3.4-3.7	21-22	--	100	100
Brook trout..............	4.0-4.6	111-112	46	100	100
Chain pickerel...........	10.0-12.0	11-12	0	100	100
Golden shiner............	7.7-8.1	4	--	--	100
Common shiner............	4.7-5.0	3-4	100	100	--
Blacknose dace...........	2.0	13-14	--	100	100
Creek chub...............	3.8-4.0	8	--	--	--
Fallfish.................	4.6-4.8	8-9	50	100	100
White sucker.............	9.8-10.6	16	31	100	100
Brown bullhead...........	10.0-10.5	9-10	0	0	0
Pumpkinseed..............	5.5-6.3	12-14	50	100	100
Bluegill.................	7.5-7.9	9	0	44	100
Smallmouth bass..........	4.5-4.8	23-28	38	86	100
Largemouth bass..........	3.6-6.0	8-11	75	100	100
Yellow perch.............	7.0-8.7	12	33	100	100

The results at Berlin NFH were important to subsequent field trials and to further development of the toxicant. Although the 0.13 p.p.b. of antimycin was too small a concentration for a large kill of fish, the no-kill and partial-kill of certain species confirmed laboratory findings that some species are more sensitive than others, and that antimycin has potentials for selective control of sensitive fish. The concentration of 1.22 p.p.b. was also too small for a complete kill of scale fish. On the other hand, the concentration of 12 p.p.b. was too high. We concluded that the optimum concentration for scale fish in the cold, soft water might have been 4 or 5 p.p.b.

The results also confirmed laboratory observations that bullheads are very tolerant of concentrations of antimycin which kill most scale fish. This could be an advantage in some situations, and a disadvantage in others. We concluded, however, that the presence or absence of bullheads could be ignored in most of our field trials because the amounts of toxicant used would be too small to affect them.

This first test of antimycin formulated on sand, Fintrol-5, demonstrated that the preparation is easy and clean to handle and distribute, and the results supported a decision to continue development of sand formulations. We did recommend that a coarser grade of sand be used as the carrier because the fine sand could float on surface film of water or could be blown from the path or distribution by the wind. The manufacturer then changed from 60-mesh sand to 40-mesh.

The pond waters were clear and colorless and afforded good observation of the fish during and after the application of antimycin. The formulation contributed no color or odor to the water, and the fish were not repelled by it. Moreover, it killed the fish slowly and unspectacularly.

CAPE VINCENT, NEW YORK

Three 1-acre ponds at the Cape Vincent NFH were stocked with 20 species of fish and treated with the sand formulation of antimycin. The water was harder and more fertile than at Berlin NFH, and the ponds contained substantial amounts of rooted, aquatic vegetation.

We narrowed the range of test concentrations to 1.02, 3.12, and 10.4 p.p.b. of antimycin (table 7). The lowest concentration killed all trouts, certain minnows, white suckers, and yellow perch. Seven species had partial mortalities ranging from 17 to 97 percent, and seven species had no mortalities. Only bowfin and brown bullheads and some of the pumpkinseeds, smallmouth bass, largemouth bass, and black crappies survived

TABLE 7.--Effects of antimycin on fish at Cape Vincent NFH

Species	Size (inches)	Number per pond	Percent killed at		
			1.02 p.p.b.	3.12 p.p.b	10.4 p.p.b.
Bowfin	16.2-18.8	1	0	0	0
Rainbow trout	7.7-9.0	5-10	100	100	100
Brown trout	9.0-10.3	5-6	100	100	100
Brook trout	9.9-10.7	5-6	100	100	100
Northern pike	14.5-17.1	2-3	0	100	100
Carp, small	2.3-2.5	100	94	100	100
Carp, large	16.8-17.4	1	0	100	--
Minnows	2.4-3.0	70-80	100	100	100
Golden shiner	2.9-3.1	29	97	100	100
White sucker	17.1-19.2	2-8	100	100	100
Brown bullhead	4.2-5.3	29-30	0	0	0
White bass	15.4	0-1	0	--	--
Rock bass	7.0-8.0	7-8	17	100	100
Pumpkinseed	4.5-6.2	10-22	45	88	100
Bluegill	7.6-8.2	9-30	88	100	100
Smallmouth bass	11.5	0-1	0	63	100
Largemouth bass	12.0-14.0	19	0	63	100
Black crappie	6.8-8.0	10-12	70	67	100
Yellow perch	7.2-8.0	9	100	100	100
Walleye	12.1-16.5	4-5	50	100	100
Freshwater drum	16.0-18.0	1-2	0	100	100

exposure to 3.12 p.p.b. Notably, the species eliminated by this concentration included juvenile and adult carp, minnows, and white suckers.

Exposure to 10.4 p.p.b. of antimycin killed 17 of the 19 species, leaving only bowfin and brown bullheads. Comparing the results obtained with 3.12 and 10.4 p.p.b., we concluded that the latter concentration was too high for most of the species involved in the trials.

The sand formulation worked well in the presence of aquatic plants. The sand sank through the submerged weeds, and it sifted nicely through some dense patches of emergent vegetation with no sticking to leaves and stalks. Its advantages over liquid toxicants in weedy water were very apparent.

Among the abundant invertebrates in the ponds, the fresh-water shrimp and aquatic insects were unaffected by the antimycin. Rotifers, copepods, and cladocerans, however, were reduced in numbers in ponds treated with 3.12 and 10.4 p.p.b., but there was question whether the declines of the autumnal pulses were natural or caused wholly by the toxicant.

STUTTQUART, ARKANSAS

Two series of trials were conducted in 1965 at the Bureau's Warmwater Fish Cultural Laboratory. The first, in March, included three pairs of one-quarter acre ponds which were stocked heavily with fish. We narrowed the range of concentrations further, and treated the ponds with 5.0, 7.5, and 10.0 p.p.b. of antimycin in sand formulation. The lowest concentration eliminated 12 of the 14 species, including such important target fish as goldfish, carp, and green sunfish (table 8). There was a partial kill of channel catfish and warmouth. The concentrations of 7.5 and 10.0 p.p.b. removed all species but channel catfish which suffered 3-percent mortalities at each concentration. An examination of the dead catfish disclosed that they were hosts to heavy infestations of Ichthyophthirius.

The second series of trials took place in December in a 20-acre reservoir and a 0.25-acre pond. The fish in the reservoir were well-established populations, some of which were seined and stocked in the small pond. The waters were turbid, and colder and harder than during the March trials. Consequently, we elected to try a concentration of 10 p.p.b. of antimycin in each body of water.

The experimental conditions were heightened because the reservoir had been drawn down to an average depth of about 2 feet, and the bottom was very soft mud. The small pond, on the other hand, ranges from 3 to 4 feet in depth, and the bottom is relatively firm. A comparison of results with Fintrol-5 would indicate whether there was significant loss of antimycin in the reservoir because the sand particles sank into the soft muck before releasing all of the toxicant.

Arrangements also were made to determine the minimum duration of exposure necessary to kill a species of fish. Live cages were

TABLE 8.--Effects of antimycin on fish at the Warmwater Fish Cultural Laboratory, March 1965

Species	Size (inches)	Number per pond	Percent killed at		
			5.0 p.p.b.	7.5 p.p.b.	10.0 p.p.b.
Rainbow trout	4.9-9.1	159-176	100	100	100
Goldfish, small	0.8-1.4	237-321	100	100	100
Goldfish, large	3.0-8.5	643-728	100	100	100
Carp (Israeli), small	3.5-6.0	530-688	100	100	100
Carp (Israeli), large	23.0-26.0	2-6	100	100	100
Golden shiner	2.8-4.5	302-511	100	100	100
Smallmouth buffalo	5.5-13.0	32-72	100	100	100
Bigmouth buffalo	12.0-20.0	76-99	100	100	100
Channel catfish	12.0-18.0	43-99	2	3	3
Warmouth	3.5-5.5	14-26	85	100	100
Green sunfish	2.5-7.0	171-273	100	100	100
Orangespotted sunfish	2.5-3.0	39-59	100	100	100
Bluegill	4.0-6.5	46-56	100	100	100
Largemouth bass	10.0-12.0	28-40	100	100	100

placed in the reservoir and each contained 10 adult bigmouth buffalo. After a 2-, 3-, 4-, 5- or 6-hour exposure to antimycin, the fish were transferred to an untreated pond. Subsequent observations revealed that the fish exposed to antimycin for 2 hours survived. Those fish exposed to the toxicant for 3 or more hours died.

Among the 19 species present in the reservoir, gizzard shad were the most sensitive to the toxicant, and all died in about 24 hours (table 9). The carp, buffalo, and most other species died within 72 hours. Survivors included paddlefish, catfishes, mosquitofish, and a few warmouth.

The toxicant produced a much quicker kill of fish in the 0.25-acre pond, with heavy mortalities occurring within 24 hours. All species except paddlefish and catfishes were dead at 48 hours. All of the paddlefish, however, succumbed in about 72 hours in contrast with no kill of that species in the reservoir.

The trial demonstrated that antimycin works well in hard, cold, turbid water against scalefish without harm to 7 species of catfish. Among the scalefish, mosquitofish and warmouth were relatively tolerant to the toxicant.

The more rapid and complete kill of fish in the deeper, 0.25-acre pond was attributed to a better release of toxicant than occurred in the reservoir. We believe that the sand particles sank into the soft bottom of the reservoir before releasing all of the antimycin. Thus, there is risk of an incomplete kill if the 5-foot-release formulation is used in very shallow water over soft bottom.

VALENTINE, NEBRASKA

The first tests of antimycin in natural ponds occurred at Valentine NWR. Pond 1 has an average depth of only 1.8 feet and a relatively soft bottom. The target fish were wild, yearling carp and green sunfish. In addition, some northern pike and largemouth bass from a nearby lake were stocked prior to the trial (table 10). Pond 2 is an old dugout pond with an average depth of 4.2 feet, and at the time, it had overflowed its banks onto adjacent pasture. This pond receives heavy use by range cattle, and it is highly polluted by manure in the water and along the shore. It contained seven species of stocked fish, including large carp.

Both ponds were treated with the Fintrol-5 formulation although the average depths were less than 5 feet. A concentration of 10 p.p.b. was selected because of the relatively high total alkalinity.

Five species of fish were eliminated from Pond 1 within 48 hours. They included the

TABLE 9.--Effects of antimycin on fish at the Warmwater Fish Cultural Laboratory, December 1965

Species	Size (inches)	Reservoir (10 p.p.b.)		Pond (10 p.p.b.)	
		Abundance	Percent killed	Abundance	Percent killed
Paddlefish.............	43.0-57.0	scarce	0	scarce	100
Gizzard shad...........	5.3-6.6	abundant	100	scarce	100
Goldfish...............	5.0-8.0	scarce	100	--	--
Carp...................	10.4-13.8	common	100	abundant	100
Bigmouth buffalo........	7.2-19.6	common	100	abundant	100
White catfish..........	unknown	scarce	0	scarce	0
Blue catfish...........	unknown	scarce	0	--	--
Black bullhead.........	unknown	common	0	scarce	0
Yellow bullhead.........	unknown	scarce	0	scarce	0
Channel catfish.........	unknown	scarce	0	--	--
Tadpole madtom..........	unknown	common	0	--	--
Flathead catfish........	unknown	scarce	0	--	--
Mosquitofish...........	1.6-2.8	common	99	--	--
Warmouth...............	3.0-6.5	abundant	99	--	--
Green sunfish..........	3.1-7.6	common	100	--	--
Orangespotted sunfish...	1.7-3.5	common	100	--	--
Bluegill...............	2.5-4.2	scarce	100	--	--
Largemouth bass.........	5.3-16.0	common	100	--	--
Black crappie...........	3.2-9.7	scarce	100	--	--

TABLE 10.--Effects of antimycin on fish at Valentine NWR

Species	Size (inches)	Pond 1 (10 p.p.b.)		Pond 2 (10 p.p.b.)	
		Number present	Percent killed	Number present	Percent killed
Northern pike...........	14.0-24.0	6	100	12	33
Carp....................	10.0-26.0	197	100	1,045	100
Fathead minnow..........	1.8-2.9	0	--	32,000	100
Black bullhead..........	-----	42	0	3	0
Green sunfish...........	2.0-8.2	0	--	2,304	100
Bluegill................	5.5-9.2	169	100	27	100
Largemouth bass.........	6.0-15.0	56	100	14	64
Black crappie...........	7.2-8.5	1	100	14	36
Yellow perch............	5.5-9.0	90	100	30	100

fathead minnows, carp, and green sunfish. There were partial kills of northern pike, largemouth bass, and black crappies ranging from 33 to 64 percent. Black bullheads survived.

The kill of fish was more rapid and complete in the deeper water of Pond 2, indicating a better release of the toxicant from the sand formulation. Most of the carp and other species were dead within 24 hours. We were unable to determine what influence the pollution by manure may have had on the activity of the toxicant.

There was no evidence of harm to aquatic insects in the ponds, and daphnia remained abundant, especially in Pond 2, after the treatment.

The principal highlights of the trial at Valentine were that antimycin is effective in alkaline water, in cattle-polluted water, and may eradicate carp and green sunfish at a concentration which permits survival of adult northern pike and largemouth bass.

SARATOGA, WYOMING

The Bureau's Division of Fish Hatcheries requested that we attempt to eradicate fish with antimycin in Lake Creek Lake at the Saratoga NFH. The 1.79-acre lake is spring-fed and serves as the water supply for the hatchery. The average depth is 3.25 feet, the maximum depth is 6 feet, and the water is clear and colorless. Thus, the lake afforded an excellent opportunity to further test the non-repellency of antimycin to fish and the performance of the sand formulation in bottom springs. The hatchery's objective was to rid the lake of fish which might serve as vectors for diseases (table 11). Previous attempts with rotenone and chlorine had failed because the fish were repelled and fled into the springs to avoid contact.

The springs cause a complete exchange of water in the basin of the lake every 27 hours. Because of this, adequate exposure of fish to the toxicant was a critical consideration. Thus, we arranged to maintain at least 10 p.p.b. of antimycin in the cold, relatively hard water for a minimum of 8 hours. This dose was achieved by making an initial application of 10 p.p.b. of Fintrol-5, followed by light applications at 6 and 8 hours later to compensate for spring inflows. Uniform distributions of the sand formulation over the surface were obtained by means of a seed spreader, and the sand grains sank readily into the large, bottom springs.

The water remained clear and colorless during and after the distribution of toxicant. The fish were easy to observe, and they exhibited no alarm or tendency to escape from the treated water. The first evidence of toxic effects was apparent 2 hours after the initial application. The dense schools of large, northern creek chubs began to break up. Otherwise normal in appearance, the chubs

TABLE 11.--Effects of antimycin on fish at Saratoga NFH

Species	Size (inches)	Number present	Percent killed at 10 p.p.b.
Brook trout, small	3.2-8.2	117	100
Brook trout, large	11.2-20.1	35	100
Fathead minnow.....	2.3-3.0	several hundred	100
Creek chub.........	3.4-9.2	1,152	100
Iowa darter........	2.5	10	100

seemed to lose their orientation with respect to schooling. None sought relief in springs. Most of them were dead within 24 hours, and all were dead within 48 hours.

Brook trout in the lake ranged from one-third ounce to 6.6 pounds, and we watched them carefully throughout the operation. They began to die after 3 hours of exposure, and all were dead within 24 hours. None had moved into the springs or tried to escape via the outlet. The responses of the numerous fathead minnows and the small numbers of Iowa darters were similar to those of the creek chubs and brook trout.

The antimycin on sand produced a complete kill of fish in Lake Creek Lake. The toxicant was flushed from the basin by spring flows within 40 hours, and it was detoxified effectively in the 3-c.f.s. outlet stream by a continual application of 1 p.p.m. of potassium permanganate for 40 hours.

WEST SALEM, WISCONSIN

Park Pond is a 4.78-acre, impounded oxbow of the La Crosse River in Veterans Memorial Park, and it is managed for trout on a put-and-take basis. It is fed by bottom springs and an artesian well with cold, very hard water. Occasionally, however, the pond is inundated by the river and becomes contaminated with carp and panfish (table 12). When abundant, the invaders are considered a nuisance, and attempts are made periodically to reduce or eliminate them.

TABLE 12.--Effects of antimycin on fish in Veterans Memorial Park Pond

Species	Size (inches)	Number present	Percent killed at 10 p.p.b.
Rainbow trout	9.6-11.3	6	100
Brown trout	7.8-13.0	54	100
Central mudminnow	4.0 av.	3	100
Northern pike	8.7-12.9	44	100
Carp, small	3.9-10.0	5,968	100
Carp, large	16.0-27.0	61	100
Golden shiner	4.9-6.1	49	100
Fathead minnow	3.3 av.	thousands	100
White sucker	7.8	1	100
Black bullhead	4.1-9.0	100+	0
Channel catfish	7.8 av.	2	0
Green sunfish	3.6	1	100
Pumpkinseed	2.0-4.1	106	100
Bluegill	2.0-3.9	19	100
White crappie	4.6 av.	3	100

We cooperated with the Wisconsin Conservation Department to test antimycin in the Fintrol-5 forumlation in Park Pond against the numerous carp, minnows, ans stunted pan fish. The trial also afforded an opportunity to observe any effects of the toxicant on a large flock of semi-tame mallards on the pond.

The treatment consisted of 10 p.p.b. of antimycin and the sand formulation was distributed by seed spreader from a boat. Rainbow trout, brown trout, and northern pike were the most sensitive, and many were dead within 5 hours. Large numbers of carp and tens of thousands of fathead minnows died within 12 hours. The kill of scale fish was complete within 48 hours, and only catfishes survived.

The mallards gorged themselves on dead fathead minnows and suffered no ill effects from the toxicant or the poisoned fish. The toxicant degraded in 6 days.

Nine days after the application of antimycin, Park Pond was treated with 5 p.p.m. of rotenone to check on the kill of carp and other fish. The treatment confirmed that no scale fish survived the antimycin, and only bullheads and a few channel catfish had remained.

MADISON, WISCONSIN

Lake Katrine, a 30-acre natural lake with warm, soft water, was selected for trials of antimycin against carp eggs in the spring and for total eradication of carp and fathead minnows in the fall.

Two days after the commencement of spawning by carp, a 2.7-acre bay was treated with 4 p.p.b. of antimycin in Fintrol-5. It, and an adjacent bay serving as a control, contained hundreds of thousands of fertilized carp eggs. Within 48 hours, the treated eggs had turned white and opaque, and many were covered with fungus. In contrast, the untreated eggs were alive and reaching the eyed stage. At 96 hours, most of the treated eggs had disintegrated, and extensive sampling failed to disclose any live eggs or fry. The eggs in the control bay, however, were hatching normally and fry were abundant.

During the course of the test, some eggs from the treated and untreated bays were taken to the laboratory. Only 2 percent of the treated eggs hatched, and the fry died within 24 hours. Ninety-seven percent of the untreated eggs hatched, and the fry lived for several weeks until discarded. We concluded that antimycin has potential for killing the spawn of carp in the field.

Several months later, all of Lake Katrine was treated with 7.5 p.p.b. of antimycin in Fintrol-5 to kill carp and fathead minnows. The distribution was made in an hour by seed spreaders mounted on two boats. A heavy kill of fish was apparent within 24 hours, and sampling with large seines two weeks later indicated that the carp and fatheads had been eliminated.

OXFORD, WISCONSIN

We were asked by the Wisconsin Conservation Department to try antimycin on the carp in Parker Lake, a 63-acre natural body of water with a maximum depth of 40 feet. The problem was the abundance of young-of-the-year carp which followed an incomplete reclamation with toxaphene the previous year (table 13).

The cold, hard water was treated with 10 p.p.b. of antimycin in October. Fintrol-5 was applied in the littoral zone, 0 to 10 feet deep. Fintrol-15 and Fintrol-30 were applied to areas of the lake ranging from 11 to 20 feet deep and 21 to 40 feet deep, respectively. Some Fintrol-Liquid was sprayed on very shallow areas along the shore.

The water in the lake was clear and permitted good observation of fish. Thousands of carp and some rainbow trout and bluegills were dead within 24 hours. Although we estimated that 60 percent of the fish were dead within 48 hours, the heavy mortality continued for 2 weeks. One month after treatment, one fingerling bluegill was caught in a fyke net. Intensive netting during the following spring failed to capture any fish, and we concluded that the antimycin had eradicated carp and other species.

TABLE 13.--Effects of antimycin on fish in Parker Lake

Species	Size (inches)	Abundance	Percent killed at 10 p.p.b.
Rainbow trout......	13-18	common	100
Carp..............	2-12	very abundant	100
Pumpkinseed........	3-7	abundant	100
Bluegill...........	2-5	common	100
Yellow perch.......	3-9	rare	100

The good performance of the deeper water formulations, Fintrol-15 and Fintrol-30, justified their further development for management use.

Trials in Streams

VIROQUA, WISCONSIN

The Wisconsin Conservation Department wanted to rid Sidie Hollow Creek of rough fish before filling a new impoundment which is to be managed for trout. The job offered an opportunity to test antimycin against a variety of fish in the cold, hard water of a small trout stream.

We formulated the liquid toxicant at the site by dissolving technical-grade antimycin in ethanol. By means of drip stations, a concentration of 15 p.p.b. of antimycin was maintained for 5 hours at any given site on the stream.

The concentration was too high, and it produced rapid results. Some fish exhibited distress within an hour. Most species were in distress within 3 hours, and mortality of fish was nearly complete in 12 hours (table 14). The bolt of toxicant moving downstream did not repel fish, and there was no evidence of fish fleeing ahead of the bolt. Rather, fish tended to drift involuntarily downstream when they became distressed and unable to maintain their positions in the current.

A post-treatment survey with electrofishing gear demonstrated that the 11 species of fish had been exterminated. This success led to further development and testing of Fintrol-Liquid for field application.

TABLE 14.--Effects of antimycin on fish in streams

Species	Sidie Hollow Creek 15 p.p.b.-5 hrs.		Mullet River 7.5 p.p.b.-8 hrs.		Rathbone Creek 7.5 p.p.b.-8 hrs.		Westfield Creek 10 p.p.b.-10 hrs.		Waterloo Creek 10 p.p.b.-10 hrs.	
	Abundance	Percent killed	Abundance	Percent killed	Abundance	Percent killed	Abundance	Percent killed	Abundance	Percent killed
American brook lamprey..	--	--	--	--	common	100	common	50	--	--
Rainbow trout...........	scarce	100	--	--	--	--	scarce	100	--	--
Brown trout.............	scarce	100	scarce	100	--	--	--	--	--	--
Brook trout.............	--	--	--	--	common	100	--	--	--	--
Central mudminnow.......	--	--	common	100	common	0	--	--	--	--
Northern pike...........	--	--	scarce	100	--	--	--	--	scarce	100
Stoneroller.............	common	100	--	--	--	--	--	--	--	--
Carp....................	--	--	abundant	99+	--	--	abundant	100	abundant	100
Bluntnose minnow........	--	--	--	--	--	--	abundant	100	--	--
Fathead minnow..........	--	--	--	--	--	--	common	100	--	--
Blacknose dace..........	abundant	100	--	--	--	--	abundant	100	--	--
Longnose dace...........	common	100	--	--	--	--	--	--	--	--
Creek chub..............	common	100	common	100	--	--	common	100	--	--
White sucker............	common	100	common	100	common	99+	abundant	100	abundant	100
Northern hog sucker.....	scarce	100	--	--	--	--	--	--	--	--
Brook stickleback.......	common	100	--	--	common	99+	scarce	100	--	--
Rock bass...............	--	--	scarce	100	--	--	--	--	--	--
Green sunfish...........	--	--	scarce	100	--	--	--	--	--	--
Pumpkinseed.............	--	--	--	--	--	--	scarce	100	--	--
Bluegill................	--	--	scarce	100	--	--	common	100	--	--
Largemouth bass.........	--	--	scarce	100	--	--	common	100	--	--
Black crappie...........	--	--	scarce	100	--	--	--	--	--	--
Fantail darter..........	--	--	--	--	--	--	common	100	--	--
Johnny darter...........	common	100	common	100	common	100	common	100	--	--
Mottled sculpin.........	scarce	100	--	--	--	--	common	100	--	--

PLYMOUTH, WISCONSIN

The sport fishery on a portion of the Mullet River had deteriorated because of an abundance of carp, suckers, green sunfish, and other non-game fish. We cooperated with the Wisconsin Conservation Department in reclaiming a 2.5-mile portion of the river, including two small impoundments, with antimycin. Prior to the treatment, one impoundment was drained to stream bed, and the other was lowered to a pool containing 3 acre-feet of water.

The test section was characterized by a slow flow of warm, very hard water. The speed of flow was measured between application stations by use of fluorescein dye. Estimates were made on the stretchout and dilution of a toxicant between stations.

A liquid formulation of antimycin in acetone was prepared at the site. We installed drip stations and tried to maintain a concentration of 7.5 p.p.b. for a minimum of 8 hours at any given point along the stream. The 3 acre-feet of impoundment were treated with Fintrol-5 coincident with the arrival of the flowing bolt of toxicant. When the toxicant reached the downstream dam, both dams were closed. The refilling of the impoundments thus allowed dilution and degradation of the toxicant.

Creek chubs, white suckers, green sunfish, and small black crappies began to die after 2 hours of exposure. Carp were distressed within 2 to 5 hours near sites where the toxicant was introduced. Many adult carp moved into shallow water and took up to 72 hours to die. Nine tons of carp were collected from less than 2 miles of the stream.

An electrofishing survey made 5 days after the reclamation turned up seven carp, two of which appeared to be in distress from antimycin. We estimated that more than 99 percent of the carp and 100 percent of the other fish had been eliminated by the toxicant (table 14).

The relatively slow and imcomplete kill of carp indicated that the concentration of toxicant was marginal. We surmise that the loss of toxicant between fortifying stations was greater than estimated. Thus, the concentration of antimycin was less than 7.5 p.p.b. in places. This error points up the necessity of accurately measuring the stretchout and dilution of a toxicant in streams and fortifying as indicated to maintain an effective concentration along the entire course of target water.

CATARACT, WISCONSIN

Rathbone Creek provided a different experimental situation than the previous streams. It is a small, soft water, trout stream flowing through an infertile sand plain. The object of the reclamation was to reduce or eliminate white suckers to enhance intensive management of the stream for brook trout.

The 6 miles of test water were characterized by sand bottom and boggy shoreline. Two ponds were included; the upper one was drained to stream bed; and the lower pond, at the downstream limit of test water, was partially drained to a pool of 12 acre-feet. The salt-resistivity technique was used to estimate the amounts of toxicant needed at several stations to maintain a concentration of 7.5 p.p.b. of antimycin for a minimum of 8 hours at any given point in the stream. The pool in the lower pond was treated with Fintrol-5 coincident with the arrival of the bolt of toxicant in the stream. The selection of concentration and duration of exposure was based on the fact that the water temperature was only 5^{o} C.

Dead and dying fish appeared in the following order after the treatment commenced: brook trout in 2 to 3 hours, white suckers in 3 to 6 hours, brook sticklebacks and minnows in 5 to 6 hours (table 14). All rainbow trout and fathead minnows in live cages throughout the treatment area were dead within 24 hours. The results, however, were assessed by electrofishing several days later. One fingerling white sucker, two brook sticklebacks, and numerous mudminnows were collected in four sample areas totalling 0.75 miles of stream.

An incomplete distribution of antimycin in the numerous spring seeps and backwaters may have enabled the sucker and sticklebacks to survive. It was apparent, nevertheless, that more than 99 percent of each species succumbed to the toxicant. On the other hand, the mudminnows were unaffected by the treatment. A subsequent bioassay of antimycin against mudminnows from Rathbone Creek showed that an 8-hour exposure to 15 p.p.b. was necessary to kill them. Since mudminnows were eliminated by 10 p.p.b. of antimycin in other field trials, we concluded that the Rathbone strain was relatively resistant.

WESTFIELD, WISCONSIN

The experimental reclamation of a 3-mile portion of Westfield Creek was an attempt to eradicate the abundant carp and suckers (table 14). Power dams are located at the upper and lower ends of the test portion, and the volume of stream flow ranged from 8 c.f.s. at the upper end to 22 c.f.s. at the lower end. The pool of the lower pond was drained to stream channel. Rhodamine-B dye was used to estimate the stretchout and dilution of a toxicant.

Because of the relatively high pH and total hardness of the water, we elected to apply 10 p.p.b. of antimycin in Fintrol-Liquid formulation and to maintain the concentration for at least 10 hours. Most of the toxicant was metered into the stream, but two crews in boats endeavored to spray antimycin on backwaters and pockets off the mainstream.

The target carp and suckers began to exhibit distress in about 8 hours, and many were observed swimming slowly with the current. In the meantime, large numbers of creek chubs, other minnows, bluegills, largemouth bass, and darters were drifting helplessly downstream. We estimated that 98 percent of the fish in the stream were dead at 24 hours, and all were dead at 96 hours. Approximately 23,000 pounds of fish were collected and carp comprised 95 percent by weight.

An electrofishing survey a week after the reclamation resulted in the capture of one brook stickleback and a few small minnows. We believe that these specimens had passed through the upper power dam after the treatment. The target species, carp and suckers, were absent.

MADISON, WISCONSIN

Waterloo Creek was selected to test the effects of a brief exposure to antimycin on carp. A 1-mile portion of the small, very slow stream was blocked off by anchored seines, and observations on flow characteristics were made with rhodamine-B dye. The experimental nature of the reclamation was accented by the fact that the water contained a heavy load of effluent from a municipal, sewage-treatment plant.

A concentration of 10 p.p.b. of antimycin was maintained for 5 hours in the test portion of stream. Fish reacted rapidly, and carp and white suckers began to die within 2 hours. Within 24 hours, thousands of dead carp and suckers and a few northern pike littered the shores, and we found no survivors (table 14). No other species were present.

Although the water at 25° C. was warmer than in any of the previous field trials, we believe that it was not the sole cause for the rapidly toxic action of the antimycin. The sewage effluent may have enhanced the activity of the toxicant or reduced the resistance of the fish.

The dose of antimycin at 10 p.p.b. for 5 hours was considered very effective on carp in this situation.

Effects on Non-target Animals

Every opportunity was taken to observe any effects of antimycin on invertebrate and vertebrate animals. In most situations, the fish-killing concentrations of antimycin had no significant effects on invertebrates (table 15). The first of two exceptions occurred at Cape Vincent NFH when autumn pulses of rotifers, water fleas, and copepods were greatly reduced in ponds treated with 3 and 10 p.p.b. of antimycin. Although we are inclined to believe that the toxicant contributed to the loss of zooplankton, the season's first killing frosts at the time of the experiment may have played some part in the declines.

TABLE 15.--Effects of antimycin on invertebrates

Technical name	Common name	Effect	Concentration of antimycin (p.p.b.)
Rotatoria......	Rotifers	99 percent kill	3.12
Tubificidae....	Aquatic earthworms	no effect	3.12
Cladocera......	Water fleas		
Daphnia		99 percent kill	3.12
		99 percent kill	10.40
Ceriodaphnia		no effect	3.12
		99 percent kill	10.40
Bosmina		no effect	3.12
Copepoda.......	Copepods	99 percent kill	3.12
Ostracoda......	Seed shrimps	no effect	10.40
Amphipoda......	Scuds	Partial mortality	4.0
		no effect	1.04
Decapoda.......	Crawfish	no effect	10.0
	Freshwater shrimp	Partial mortality	10.0
Ephemeroptera..	Mayflies	no effect	10.4
Odonata........	Damselflies	no effect	10,0
Hemiptera			
Corixidae....	Water boatmen	no effect	10.4
Notonectidae.	Backswimmers	no effect	10.4
Diptera			
Tendipedidae.	Midge	no effect	12.0

TABLE 16.--Effect of antimycin on vertebrates exposed to treated water or which fed on fish killed by antimycin

Technical name	Common name	Effect	Concentration of antimycin (p.p.b.)
Ambystomidae.....	Mole salamanders	no effect	10.0
Ranidae..........	Frogs		
Tadpoles		no effect	10.0
Adults		no effect	10.4
Chelydridae......	Turtles	no effect	10.0
Colubridae.......	Water snake	no effect	10.0
Ardeidae.........	Herons	no effect	10.4
Anatinae.........	Surface feeding ducks	no effect	10.0
Aythyinae........	Diving ducks	no effect	10.0
Larinae..........	Gulls	no effect	10.4
Sterninae........	Terns	no effect	10.0

The second exception involved the freshwater shrimp at Saratoga NFH. Most of the shrimp in the pond were exposed to 10 p.p.b. of antimycin without harm. Some, however, were exposed to temporarily higher concentrations in springs, and they perished.

Vertebrates exposed to antimycin during the field trials have included frogs, salamanders, water snakes, turtles, waterfowl, and wading birds (table 16). Many were observed eating antimycin-killed fish. We saw no evidence of mortality or harm among these animals.

DISCUSSION

Antimycin proved to be an effective toxicant for many species of fish in lakes and streams. We endeavored to estimate a minimum effective concentration for the target species in each trial, and as might be expected, we were too high with some concentrations and too low with others. The results, however, permit some generalizations. For example, less toxicant may be required in soft water then in hard water; less is required in warm water than in cold water; and less is required in water with low pH than high pH. Prime target species such as carp and suckers can be eliminated by concentrations of 3 to 10 p.p.b. of antimycin, depending on water quality and temperature. Goldfish, bowfin, and gar are more resistant and may require considerably more than 10 p.p.b. for control. Bullheads and catfish are the most resistant, and they were relatively unharmed by the concentrations used in the trials. The differences in sensitivity of various species to antimycin are leading to tests of the chemical as a selective toxicant.

The reactions of fish to antimycin are slow and unspectacular. The first stages of distress are characterized by the fish losing their orientation and swimming slowly along the shore or near the surface. They have progressively less reaction to stimuli until they can be picked up easily by hand. In streams, they drift involuntarily downstream, feebly trying to maintain their position. They soon lose their equilibrium and turn over on their sides or backs. The final stages are characterized by short alternate periods of quiescence and feeble, erratic swimming. Infrequently, a fish will thrash about on the surface for short periods of time.

The most sensitive species such as trout and yellow perch usually show symptoms of distress in a few hours whereas more resistant ones such as largemouth bass and carp may not be affected for 10 to 24 hours. Mortality of fish is usually complete within 72 to 96 hours after treatment.

Our trials to date have indicated that the effects of antimycin are irreversible. Once fish exhibit symptoms of distress, they die, even if placed in fresh water. In some streams where the chemical moved on past the fish, they lived for many hours in fresh water before dying.

In no case did we see fish exhibit an avoidance reaction to antimycin. They do not seek out springs or untreated areas. The lack of repellency is a significant advantage in spring-fed lakes and in streams.

The formulations of antimycin on sand are effective, easy to apply, and safe to handle. They are almost dust-free when broadcast by hand or by means of mechanical seed spreaders. Fintrol-5 was registered in the United States and Canada, largely as a result of the trials reported here. The formulations for deeper water, Fintrol-15 and Fintrol-30, are to be developed further and registered.

The results in one pond at Stuttgart, Arkansas, and another at Valentine, Nebraska, demonstrated that Fintrol-5 should not be used in water less than 3 feet deep when the bottom is very soft. Under such conditions, the sand grains may sink into the mud before releasing all of the antimycin. The concentration of toxicant in the water may be less, therefore, than desired. A liquid formulation would be better in these circumstances.

The liquid formulation of antimycin performed well in shallow waters of ponds and marshes and in streams. It can be sprayed over the water or dispensed into the water by a metering pump. A surfactant in the formulation aids dispersion in the water. We are confident that Fintrol-Liquid will be registered by the U.S. Department of Agriculture in the near future.

The evaluation of a candidate fish toxicant in the field is difficult because the techniques involved in the reclamation of lakes or streams are primitive, inexact, or unproven. The volumes of lakes are more often estimated than measured. Estimates on the volume of flow and velocity in streams are frequently and seriously inaccurate. The salt-resistivity and dye-fluorometer techniques for detecting the flow characteristics of streams need further development to make them more widely applicable and useful. The influences of water chemistry and temperature on toxicants are not well understood, and additional research is required. The means of dispensing toxicants in various formulations need improvement. Also, more attention must be given to defining the best ways for measuring pre- and post-treatment populations of fish to improve the assessment of reclamations. And, field techniques must be developed for detecting and measuring concentrations of toxicants in lakes and streams.

The deficiencies in the technology of reclamation have caused fishery managers to resort to overdoses of toxicants to obtain satisfactory results. This is a poor and hazardous solution to the problem, and it is unacceptable to authorities who regulate pesticides.

In retrospect, the outstanding lesson derived from the field trails was that lakeside or streamside bioassays of a toxicant should be made against target species immediately prior to a reclamation. Preferrably, the fish and the water in the bioassays are taken from the target lake or stream. Also, the temperatures of the bioassays and the target water should be approximately the same. Moreover, several concentrations of the toxicant which bracket the proposed concentration should be tested. The results of such bioassays should indicate the concentration and formulation of toxicant which are most likely to produce the desired results in the reclamation; the presence of favorable influences of water quality or temperature; and the presence of unusually sensitive or resistant strains of target fish.

The routine use of lakeside or streamside bioassays would benefit efficiency and economy because the number of imperfect reclamations would be reduced or the overdosing of target waters would be decreased. The idea of on site bioassays is not new. The procedure was recommended, for example, in reclaiming streams by Lennon and Parker (1959) and in anesthetizing fish in the field by Schoettger and Julin (1967).

CONCLUSIONS

1. Antimycin is effective as a fish toxicant in standing and flowing waters.

2. The effective concentration for most species, including such prime target species as carp, suckers, and green sunfish, is 10 p.p.b. or less in waters below pH 8.5. Catfishes are not vulnerable, however, to concentrations which are lethal to scale fishes.

3. Fish eggs are killed by the same concentrations used to control free-swimming fish.

4. Antimycin degrades rapidly, especially under alkaline conditions, and most waters can be restocked with fish within 2 weeks after treatment.

5. Antimycin in water can be detoxified by 1 p.p.m. or less of potassium permanganate.

6. The effects of antimycin in fish appear to be irreversible.

7. The toxicant in liquid or sand formulations does not repel fish.

8. The concentration of toxicant to be used in a reclamation should be determined by preliminary bioassays on site against target fish in target water.

9. Antimycin in fish-killing concentrations is largely specific to fish and causes no harm to most of the other aquatic animals.

10. Concentrations greater than 10 p.p.b. are required in waters of high pH and alkalinity.

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INVESTIGATIONS IN FISH CONTROL

28. Use of Antimycin for Selective Thinning of Sunfish Populations in Ponds

By Ralph M. Burress, Fishery Biologist and
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USE OF ANTIMYCIN FOR SELECTIVE THINNING OF SUNFISH POPULATIONS IN PONDS

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ABSTRACT.--Selective removal of bluegills, redear sunfish, and redbreast sunfish was accomplished in six soft-water ponds in west central Georgia by applications of 0.4, 0.6, 0.8, and 1.0 parts per billion (p.p.b.) of antimycin in the Fintrol-5 formulation. Successful treatments were made during winter, spring, and late summer at water temperatures ranging from 46° to 75° F. and under a wide variety of weather conditions. The amount of toxicant applied per treatment was calculated according to total pond volume. The 0.4-p.p.b. treatment made at 75° F. removed 2.9 pounds per acre of largemouth bass less than 6 inches long and 69 pounds per acre of all sizes of sunfishes. The 0.6 p.p.b. applied at 47° F. reduced the numbers of bluegills less than 4 inches in length by about half with very little effect on largemouth bass or larger bluegills. Bass, bluegills, and redear sunfish which survived multiple exposures to antimycin in concentrations up to 1.0 p.p.b. were able to reproduce normally. The toxicant is easy to apply, and costs only $0.64 per acre-foot at the 0.4-p.p.b. concentration.

During the past 15 years, fishery managers have made increasing use of the technique of selectively thinning overcrowded sunfish populations in small impoundments by applying rotenone in shallow areas (Swingle, Prather, and Lawrence, 1953; Hooper and Crance, 1960; Bennett, 1960; Thomaston, 1962). Although the technique is fairly effective under favorable circumstances, its usefulness is rather seriously limited by weather conditions, water temperatures, and other uncontrollable factors which strongly influence fish behavior and distribution within the impoundment. Because of these limitations, there is only a relatively short time each year when the method can be employed. The results of this study indicate that light applications of antimycin can be used to accomplish selective thinning of sunfish populations over a much longer period of time each year and under a much wider range of weather and water conditions than previously has been possible.

The purpose of the tests was threefold: (1) To determine what segments of the fish population would be eliminated by different concentrations of antimycin, (2) to measure the effects of given concentrations of antimycin under different experimental conditions, and (3) to learn whether fish exposed to sublethal concentrations of antimycin would reproduce normally.

The tests were conducted from December 1966 to September 1967 in six privately owned ponds which contained unbalanced fish populations. The ponds are in the vicinity of Warm

Springs, Ga., and they were referred to us by the Work Unit Conservationist, Pine Mountain Soil Conservation District.

Our sincere thanks are expressed to the following whose cooperation made this study possible: Mr. P. A. Gantt, whose help in obtaining the use of ponds for experimental purposes was invaluable; Messrs. W. A. Biggers, Joe Henry, Render Hill, J. F. Reynolds, W. S. Slaughter, and R. A. Todd, owners of the ponds; Wisconsin Alumni Research Foundation, owner of antimycin; and Ayerst Laboratories, New York, producer of antimycin.

METHODS AND MATERIALS

Surface areas of most larger ponds were determined by measuring aerial photographs, and volumes were estimated from soundings. Areas and volumes of the remaining ponds were calculated from field-drawn contour maps. The ponds were treated with one or more of the following concentrations of antimycin: 0.4, 0.6, 0.8, and 1.0 parts per billion (p.p.b.). Not more than three concentrations were used in a single pond, and treatments were made at intervals of not less than 1 month. Intensive efforts to recover dead fish were continued from 4 to 22 days after each application depending upon water temperatures. Surface water temperatures were taken each day, and average values were calculated for the time during which fish were collected. Henry Pond contained a dense growth of cattails and supported such a heavy plankton bloom that it was lowered to about half its original depth prior to the first treatment to enhance recovery of dead fish. All other ponds were overflowing when treated. Final evaluations of the effects of treatment were made in different ways, as explained below.

PONDS AND FISH POPULATIONS

The ponds contained soft water, were spring fed, and were relatively infertile (table 1). They ranged in size from 0.69 to 9.80 acres, were from 3 to 6 feet in average depth, and overflowed at rates from 3.5 to 106.0 gallons per mintue (g.p.m.).

Henry Pond contained a dense stand of cattail (Typha latifolia), and it was lowered about 3 feet prior to the first treatment to facilitate recovery of fish. Only sparse growths of rooted aquatic vegetation were present in the other ponds.

Each pond contained largemouth bass (Micropterus salmoides) and overcrowded populations of one or more of the following species of sunfishes: bluegill (Lepomis macrochirus), redear sunfish (Lepomis microlophus), and white crappie (Pomoxis annularis). Other species encountered included redbreast sunfish (Lepomis auritus), orangespotted sunfish (Lepomis humilis), golden shiner (Notemigonus crysoleucas), brown bullhead (Ictalurus nebulosus), channel catfish (Ictalurus punctatus), and mosquitofish (Gambusia affinis), (table 2). All of the ponds except Reynolds Pond were to be drained eventually and converted to production of channel catfish, hence their fish populations were expendable.

TABLE 1.--Some physical and chemical characteristics of six ponds treated with antimycin

Characteristics	Henry Pond[1]			Todd Pond			Hill Pond		Slaughter Pond	Reynolds Pond	Biggers Pond
Dates of treatment.....	12/05/66	1/13/67	3/08/67	12/08/66	1/09/67	3/07/67	1/31/67	3/07/67	3/13/67	3/27/67	9/11/67
Surface area (acres)...	0.16	0.16	0.77	--	0.69	--	2.25	--	2.24	9.80	2.48
Average depth (feet)...	3.0	3.0	4.0	--	5.0	--	5.0	--	6.0	4.1	4.7
Volume (acre-feet).....	0.48	0.48	3.08	--	3.45	--	11.25	--	13.44	40.18	11.67
Overflow rate (G.P.M.).	5.3	5.3	5.3	--	3.6	--	3.5	--	6.6	5.5	106.0
Secchi disk........... transparency (inches)	10	13	18	60	60	60	36	17	18	10	12
Surface temperature (°F.)	47	50	61	52	46	57	56	59	64	68	75
Total hardness (p.p.m.)	8.0	10.0	10.0	12.0	12.0	15.0	7.0	8.0	8.0	8.0	10.0
Total alkalinity (p.p.m.)	11.0	11.0	9.0	12.0	12.0	12.0	7.5	7.5	8.0	8.0	14.0
pH....................	7.32	6.87	6.90	7.09	7.09	6.90	6.98	6.75	6.55	6.84	7.20

[1] Pond area, depth, and volume increased as pond filled during period of treatment.

TABLE 2.--Species of fish indicated by X were present in six ponds treated with antimycin

Species	Henry Pond	Todd Pond	Hill Pond	Slaughter Pond	Reynolds Pond	Biggers Pond
Largemouth bass........	X	X	X	X	X	X
Bluegill...............	X	X	X	X	X	X
Redear sunfish.........	X	--	X	X	X	X
White crappie..........	--	--	--	--	--	X
Redbreast sunfish......	--	--	--	X	--	X
Orangespotted sunfish..	--	--	--	--	--	X
Golden shiner..........	--	--	--	--	--	X
Brown bullhead.........	--	--	--	--	X	X
Channel catfish........	--	X	--	--	--	X
Mosquitofish...........	--	--	--	--	X	--

APPLICATION OF TOXICANT

In each case, antimycin in the Fintrol-5 formulation was used. The toxicant, which was supplied by Ayerst Laboratories, was a 1-percent formulation of antimycin coated on sand with Carbowax. The amounts of toxicant applied per treatment ranged from 0.8 ounces to 6.56 pounds, and were based on pond volume without regard to maximum depth or amount of overflow. A boat was used to distribute the toxicant from a beaker, a hand-operated seed spreader, or a powered spreader such as described by Lennon, Berger, and Gilderhus (1967), depending upon the size of the area to be treated. We were able to make applications of antimycin without regard to the time of day since diurnal elevations in pH were small in each pond.

TREATMENT EVALUATION

Henry and Todd ponds were drained as much as possible soon after the last application of antimycin, and the remaining fish were recovered by seining and heavier application of toxicant. The percentages of the populations recovered after each application were calculated, and comparisons of relative effectiveness of each treatment made.

Draining of Hill and Slaughter ponds was postponed until September, so that reproductive success of the treated fish could be evaluated. Upon draining, the fish were recovered, processed, and the effect of the treatment was assessed.

Reynolds Pond could not be drained. The effectiveness of its treatment was judged by the numbers and sizes of bass and sunfishes killed and collected within 5 days. Since the pond was large, a representative sample of small fish was picked up along segments of shoreline amounting to about one-fourth of the total. All larger bass and sunfish were picked up around the entire pond margin. The pond was seined 5 months later, in late August and early September, to measure reproductive success of treated fish and to check on the general condition of the fish population.

Biggers Pond will not be drained until further tests are made, hence the results of treatment were evaluated simply by comparing the numbers and sizes of bass and sunfishes recovered.

RESULTS

Because there were such wide variations in pond characteristics, dates and conditions of treatment, and methods of evaluating results, we will discuss each pond separately.

HENRY POND

The first treatment with 0.4 p.p.b. of antimycin killed about 10 pounds per acre of bluegills and redear sunfish, 99.5 percent of which were less than 4 inches long (table 3).

Although 50 percent more antimycin was used in the second treatment, results were virtually nil because of rain which caused the pond to discharge through the overflow pipe which had been lowered to dewater the pond. In addition to diluting the toxicant considerably, the runoff water caused turbidity that hampered recovery of the few fish which were killed.

The final application of 0.8 p.p.b. of antimycin was made under better conditions, and it killed all of the sunfishes except for one redear sunfish. About three-fourths of the small largemouth bass and one-third of the larger bass also died.

TODD POND

The 0.4-p.p.b. application killed no fish other than 30.1 percent of the small bluegills (table 3). Throughout the 2-week-long recovery period many of these fish were observed in distress. Their moribund condition

TABLE 3.--Numbers and weights in pounds per acre and percentages (in parentheses) of various size groups of fishes recovered in six ponds following treatment with antimycin and at draining[1]

Pond	Date	Average temp. (°F.)	Treatment	Combined sunfish species				Largemouth bass				Collection period (days)
				Lengths 1.0-3.9 in.		Lengths 4.0 or more in.		Lengths 1.0-5.9 in.		Lengths 6.0 or more in.		
				Number	Weight	Number	Weight	Number	Weight	Number	Weight	
Henry......	12/05/66	59°	Antimycin (0.4 p.p.b.)	659.0 (8.7)	5.1	3.0 (1.0)	0.2	0 (0.0)	--	0 (0.0)	--	7
	1/13/67	54°	Antimycin (0.6 p.p.b.)	9.0 (0.1)	0.1	0 (0.0)	--	0 (0.0)	--	0 (0.0)	--	11
	3/08/67	69°	Antimycin (0.8 p.p.b.)	6,885.0 (91.2)	61.2	303.0 (98.7)	19.9	5.0 (71.4)	0.6	6.0 (31.6)	5.5	7
	3/20/67		Drained	0 (0.0)	--	1.0 (0.3)		2.0 (28.6)		13.0 (68.4)		
Todd.......	12/08/66	48°	Antimycin (0.4 p.p.b.)	1,380.0 (30.1)	8.4	0 (0.0)	--	0 (0.0)	--	0 (0.0)	--	14
	1/09/67	47°	Antimycin (0.6 p.p.b.)	801.4 (17.5)	5.4	1.4 (2.5)	TR.[2]	2.9 (5.4)	TR.	0 (0.0)	--	22
	3/07/67	62°	Antimycin (0.8 p.p.b.)	2,388.4 (52.1)	15.7	55.1 (97.5)	1.5	46.4 (86.6)	0.2	7.2 (13.1)	8.7	7
	3/20/67		Drained	14.5 (0.3)		0 (0.0)	--	4.3 (8.0)		47.8 (86.9)		
Hill.......	1/31/67	52°	Antimycin (0.8 p.p.b.)	2,827.6 (95.9)	25.5	289.3 (97.0)	12.3	13.3 (78.7)	0.4	1.3 (12.2)	0.3	13
	3/07/67	65°	Antimycin (1.0 p.p.b.)	11.6 (0.4)	TR.	0 (0.0)	--	2.7 (16.0)	0.1	7.1 (67.0)	4.8	6
	9/18/67		Drained	108.9 (3.7)		8.9 (3.0)		0.9 (5.3)		2.2 (20.8)		
Slaughter..	3/13/67	65°	Antimycin (0.6 p.p.b.)	4,333.0 (88.9)	58.8	303.1 (92.6)	21.8	20.1 (34.4)	0.4	3.1 (31.6)	4.0	9
	9/07/67		Drained	542.4 (11.1)		24.1 (7.4)		38.4 (65.6)		6.7 (68.4)		
Biggers....	9/11/67	75°	Antimycin (0.4 p.p.b.)	2,627.0[3]	50.0	[4] 263.7	19.0	264.5	2.7	1.2	0.2	4
Reynolds...	3/27/67	69°	Antimycin (0.6 p.p.b.)	6,326.5	63.3	11.8	1.2	27.4	0.1	2.5	2.2	5

[1] Reynolds and Biggers ponds were not drained following treatment.
[2] TR. = trace, or less than 0.1 pounds per acre.
[3] This group included fish up to 4.9 inches in length.
[4] This group included only fish 5.0 inches or more in length.

was evidenced by blotchy discoloration and by shreds of mucus which hung from their bodies giving some of them a very ragged appearance. Large bass were observed feeding on moribund bluegills on several occasions.

The 0.6-p.p.b. treatment killed an additional 17.5 percent of the small bluegills, 2.5 percent of the sparse population of larger bluegills, and 5.4 percent of the bass less than 6 inches long. Behavior and appearance of the larger bass were normal, but some of the smaller bass which did not die became much darker in color and their eyes became somewhat grayish and opaque in appearance.

The 0.8-p.p.b. treatment eliminated nearly all remaining bluegills, and left only 8.0 percent of the small bass. About 87 percent of the adult bass survived, and more than a third of these exhibited the same darkening of body color and change in eye color previously shown by the smaller bass.

When the pond was drained, several adults were sent to the National Fish Hatchery in

Marion, Alabama, for breeding tests. None of the scores of channel catfish in the pond were harmed by the treatments.

SLAUGHTER POND

We recovered 85 pounds of fish per acre following a 0.6-p.p.b. application of antimycin (table 3). Approximately 90 percent of the bluegills of all sizes were killed, as were about a third of all sizes of bass.

We recovered 71.4 pounds of fish per acre when the pond was drained. Survival of adult redear sunfish was greater than that of adult bluegills, as was true in all ponds in which both species occurred. Reproductive success of largemouth bass and sunfishes had been good.

HILL POND

The first treatment on January 31 with 0.8 p.p.b. of antimycin killed about 96 percent of the sunfishes of all sizes, nearly 79 percent of the bass less than 6 inches in length, and 12 percent of the larger bass. Since the water was cold and deep, the recovery of fish undoubtedly was not as complete as it was following the other tests.

The second treatment amounted to 1.0 p.p.b., and was made on March 7. It killed a few more sunfish, and an additional 16 percent of the small bass and 67 percent of the larger bass.

The few surviving fish reproduced successfully during the summer, and at draining we found a total population amounting to 26.7 pounds per acre. Large bass fingerlings comprised the largest single group by weight in the population, amounting to 11.5 pounds per acre, while fingerling sunfishes amounted to 8.0 pounds per acre.

REYNOLDS POND

We recovered 64.5 pounds of sunfishes and 2.3 pounds of largemouth bass per acre following a single application of 0.6 p.p.b. on March 27 (table 3). A few mosquitofish were killed, but none of the brown bullheads were affected by the treatment.

Reproductive success of bluegills and redear sunfishes was very limited in that only four sunfish fry were taken in six short hauls with a small-mesh seine on August 29. On September 12, four 100-foot hauls made after dark with a 75-foot, 1/2-inch-mesh seine took 131 fingerling bass, 6 bass 5 to 7 inches long, 28 redear sunfish about 5 inches long, 2 bluegills 6 inches long, and 11 brown bullheads 4 to 8 inches long, but no sunfish less than 5 inches long or adult bass were taken.

BIGGERS POND

Following a single application of 0.4 p.p.b. of antimycin to this 2.48-acre pond on September 11, we recovered the following weights of fish per acre: 2.9 pounds of largemouth bass less than 7 inches long; 69 pounds of sunfishes including bluegills, redear sunfish, redbreast sunfish, and orangespotted sunfish; 9.0 pounds per acre of fingerling crappie; and 50.5 pounds of golden shiners from 4 to 9 inches in length. The treatment had no effect on channel catfish or brown bullheads. The surface temperature was 75^{o} F. at time of treatment and conditions for recovery of fish were good, hence the effectiveness of this treatment was considerably greater than that measured in similar trials conducted in winter. Further tests are to be conducted in this pond before it is drained, hence we have no way at present to determine what percentages of the various groups of fishes were removed by the treatment.

DISCUSSION

The results of our exploratory attempts to evaluate the use of antimycin in selective thinning of sunfishes in small impoundments were quite encouraging. Trials made in six pounds under a wide variety of weather conditions and temperatures demonstrated that low concentrations of antimycin are quite selective against populations of panfishes as compared to those of largemouth bass, especially at lower temperatures. Treatments with a concentration of 0.4 p.p.b. of antimycin in December killed up to 8.4 pounds per acre of bluegills less than 4 inches long without killing larger bluegills or bass of any size.

The same concentration applied in September killed 2.9 pounds of small bass and 69 pounds of sunfishes of all sizes per acre. Remedial stocking with fingerling bass can be done easily if it is deemed necessary.

The 0.6-p.p.b. treatments made in small ponds less than 8 feet deep in cold weather removed up to 13.8 pounds per acre of the bluegills less than 4 inches long while killing only 0.01 pounds of the small bass. Similar treatments in warmer weather killed 80.6 pounds per acre of bluegills and only 4.4 pounds of bass in Slaughter Pond, and 64.5 pounds per acre of sunfishes and 2.3 pounds of bass in Reynolds Pond.

The 0.8-p.p.b. treatment proved to be excessively high for use in soft-water ponds in which pH values at time of treatment ranged from 6.75 to 6.98. Sunfish populations were virtually eliminated in three ponds by this concentration, bass less than 6 inches long were reduced by 78.7 to 94.7 percent, and larger bass were reduced by 12.2 to 31.6 percent.

The 1.0-p.p.b. treatment at a temperature of 65^{O} F. was entirely too severe, leaving only 6.7 percent of the sunfish and 26.1 percent of the bass population.

The costs per acre-foot for treatments reported above when based on the list price of $48.00 for an 8.25-pound can of Fintrol-5 were as follows: 0.4 p.p.b., $0.64; 0.6 p.p.b., $0.97; 0.8 p.p.b., $1.28; and 1.0 p.p.b., $1.60. If treatments of soft-water ponds such as ours were made in warm weather, there is a distinct possibility that concentrations even lower than 0.4 p.p.b. would be adequate. A treatment in summer might be especially appropriate in ponds in which neither bass nor sunfish reproduction was adequate, and in which selective removal of a sizeable percentage of the sunfishes could be followed shortly by supplementary stocking of bass fingerlings.

Trials in which fish were held in live boxes in water below the 5-foot level showed that mortality usually occurred more slowly and sometimes was reduced in the deeper water. Thus, the concentration of antimycin should be calculated on the basis of pond volume above the thermocline rather than on total pond volume if lower levels were stratified or uncirculated by wind or other factors. Otherwise, the concentration might be higher than intended, with a correspondingly greater reduction in the fish population. In the case of Biggers Pond, for example, the 578 grams of toxicant used to produce a 0.4-p.p.b. concentration of antimycin in the entire pond volume would have produced a 0.5-p.p.b. concentration if all of the antimycin had been confined to the area above the 5-foot depth at which the thermocline was located. Had this occurred, the concentration would have been 25 percent greater than intended, which would have made a considerable difference in results. In any case, conservative calculation of the volume of water to be treated is preferable to reduce the possibility of overdosage.

Weather and water conditions were regarded as major causes for poor results following the 0.4- and 0.6-p.p.b. treatments of Henry Pond. However, there also is a definite possibility that little benefit was derived from the antimycin which was not released before the sand-formulated material sank into the soft, muddy bottom of the partially dewatered pond. Thus, lowering a pond prior to treatment with a given amount of Fintrol-5 will not necessarily yield better results than would have been achieved if the pond had not been lowered. Furthermore, complete eradication of fish often is easier to achieve in a full pond than in one which has been drained. This is true because pond bottoms usually contain irregularities which may hold enough water to keep fingerling fish alive for several days, and it is difficult to be certain that fish in all pockets of water have been eliminated.

We found conclusive evidence that largemouth bass, bluegills and redear sunfish were able to reproduce successfully after one or more exposures to antimycin. Adult bass which were exposed to successive treatments of 0.4, 0.6, and 0.8 p.p.b. of antimycin during the period December 8, 1966, to March 7, 1967, were able to reproduce normally in broodponds at the National Fish Hatchery, Marion, Alabama in April 1967[1]. Bass, bluegill and redear sunfish which survived exposure to concentrations of 0.8 and 1.0 p.p.b. on January 1 and March 7, 1967, spawned successfully in Hill Pond, as did the same species in Slaughter and Reynolds ponds which were treated with a concentration of 0.6 p.p.b. of antimycin. One interesting observation was made in relation to the effect of antimycin on adult fish. During the period from March 7 to March 28, 30 of 39 adult bass found dead in five ponds were gravid females. Thus, females apparently are more vulnerable than males just prior to the spawning season.

We found no difference in results obtained by the three methods used to apply Fintrol-5 during these trials. The simplest and easiest method was to dribble the material out a small hole in a container held over the bow of the boat. Dispersion of toxicant was adequate in open water of the larger ponds even when successive passes of the boat were about 30 feet apart. However, in shallow, weedy areas where circulation was restricted we applied the material at intervals of not more than 10 feet in an effort to avoid gaps in coverage.

The Fintrol-5 formulation of antimycin gives very good selective control of sunfishes in soft-water ponds at comparatively low cost. The material is easy to apply, and applications can be made over a very wide range of water temperatures and with a minimum of regard for weather conditions. Much remains to be done in working out techniques suited to waters of different qualities and in devising variations in methods for application. Possibly liquid formulations of antimycin could be introduced well below the surface to reduce populations of crappie or other species that inhabit deeper water; manipulation of water flows might be used to attract or to concentrate certain species which then could be treated; concentrations of spawning fishes or nest areas might be treated; shoreline applications of antimycin might be used to cut treatment costs even further.

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1 Personal communication from Jack R. Snow, Station Director, Marion NFH, Marion, Alabama, 1967.

SUMMARY

1. Selective removal of sunfishes was accomplished in six soft-water ponds in west central Georgia by applications of 0.4, 0.6, 0.8, and 1.0 parts per billion (p.p.b.) of antimycin in the Fintrol-5 formulation. The treatments were made during winter, spring, and late summer at water temperatures ranging from 46° to 75° F. and under a variety of weather conditions.

2. Smaller fish of each species are more susceptible than adults; redear sunfish are more resistant than bluegills; and even fingerling bass are more resistant than adult sunfishes.

3. Response to treatment occurred more slowly in colder water, and all species, especially largemouth bass, appeared to be more resistant to antimycin at low temperatures.

4. The 0.8- and 1.0-p.p.b. concentrations were too high even in winter, and there are indications that concentrations less than 0.4 p.p.b. might be adequate when water temperatures are 75° F. or more and the pH is about neutral.

5. The toxicant is easy to apply, and costs only $0.64 per acre-foot at a concentration of 0.4 p.p.b.

6. Largemouth bass, bluegill and redear sunfish which survived multiple exposures to antimycin were able to reproduce normally.

7. Use of antimycin for selective thinning of sunfishes will enable fishery managers to utilize this valuable technique for a longer time each year than previously was possible.

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