## EFFECTS OF GRANULAR 2',5-DICHLORO-4'-NITROSALICYLANILIDE (BAYER 73) ON BENTHIC MACROINVERTEBRATES IN A LAKE ENVIRONMENT

EFFICACY OF ANTIMYCIN FOR CONTROL OF LARVAL SEA LAMPREYS (PETROMYZON MARINUS) IN LENTIC HABITATS



**TECHNICAL REPORT No. 34** 

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### EFFECTS OF GRANULAR 2',5-DICHLORO-4'-NITROSALICYLANILIDE (BAYER 73) ON BENTHIC MACROINVERTEBRATES IN A LAKE ENVIRONMENT

## by

## Philip A. Gilderhus

#### ABSTRACT

Benthic organisms were collected before, and during the first 13 days after, treatment of a selected area of a lake with granular Bayer 73 for control of larval sea lamprey (*Petromyzon* marinus) to determine the effects of the treatment on invertebrates. Total numbers of organisms in the treated area dropped 56% in the first 7 days after treatment. Most of the reduction occurred among oligochaetes and midge larvae. Numbers of mayfly nymphs and amphipods were not substantially affected. Total numbers of organisms and numbers of oligochaetes and midge larvae increased between 7 and 13 days *after* treatment. Judging from this study, treatments with Bayer 73 are expected to have only moderate effects of short duration on populations of macroinvertebrates.

#### INTRODUCTION

Larval sea lampreys (Petromyzon marinus) usually burrow in sediments in the bottom of streams where they hatch. Suitable habitat for larvae may also exist in inland lakes or in the Great Lakes near the mouths of streams where lampreys spawn. Significant populations of sea lamprey larvae have been found in these lentic habitats and present a problem in control efforts as they are not usually killed by lampricide treatments made in the streams.

A granular formulation of 2',5-dichloro-4'-nitrosalicylanilide (Bayer 73 or Bayluscide) containing 5% active ingredient is used to assess and control populations of sea lamprey larvae in lentic habitats. This formulation is a valuable survey tool because it is also an irritant and drives larvae from their burrows to the surface of the water where they can be seen and collected. The chemical is not selectively toxic to lampreys, but can act in a selective manner if applied so as to toxify a thin stratum of water at the bottom. Field observations indicate that when treated larvae leave their burrows, most have received a lethal dose of the toxicant (Tibbles 1969). Thus, survey and control can be accomplished in one operation.

This study of the effects of Bayer 73 on benthic invertebrates was conducted to provide information needed for the support of continued registration of Bayer 73 as a sea lamprey larvicide. Sampling was done before and after a routine treatment for assessment and control of sea lampreys in an inland lake.

The test area was in Boardman Lake at the mouth of the Boardman River near Traverse City, Mich. The surface area was 0.83 ha, and depth ranged from 0.6 to 2.0 m. The lake bottom consisted mostly of sand with some silt-sized material intermixed.

Seven sampling stations were established over the study area and marked with anchored floats. A 5% granular formulation of Bayer 73 was applied to the area on 15 July 1975. Application was made with an electric rotary spreader mounted on the stem of a pontoon boat, at a rate of 112 kg/ha (100 lb/a). The concentration of Bayer 73 in the bottom 5 cm of water was computed to be 11.5 mg/l.

A bottom sample was taken with a Ponar dredge of  $0.023 \text{ m}^2 (0.25 \text{ ft}^2)$  within a 1.5-m radius of each station 1 h before treatment and 1,3,7, and 13 days after treatment. Each grab was sieved in No. 30 mesh screen, and material retained in the screen was preserved in a separate jar. Rose-bengal dye, which is absorbed by animal tissue, was added to the preservative to aid in the separation of organisms from detritus. Organisms were separated and enumerated according to the methods of Weber (1973), and each grab was picked in its entirety. Mean numbers of the various taxa per grab, by day after treatment, were compared with pretreatment numbers by the "Student's t" test (P = 0.05). Community structure was assessed by use of the diversity index (Weber 1973) based on taxonomic orders.

#### **RESULTS AND DISCUSSION**

The study area had an adequate richness of taxa and numbers of individual organisms on which to base an assessment of population trends. The number of orders collected varied from 10 to 12 per sampling day.

Four orders of organisms, represented my midges, oligochaetes, amphipods, and mayflies were present in all samples and provided adequate data for determining trends and making statistical analyses. Other orders were present in less than half of the samples.

The estimated total numbers of organisms had declined 56% from  $9,173/m^2$  before treatment to  $4,100/m^2$  7 days after treatment (Table 1). The mean total numbers per sample (Table 2) declined significantly between pretreatment samples (213) and those taken 7 days after treatment (95). The difference in total numbers between pretreatment samples and the 13-day samples was not significant at P = 0.05.

Most of the reduction in total numbers occurred among oligochaetes and midge larvae, the two most abundant groups. Oligochaetes had declined sharply 1 day after treatment and maintained only 20% of their initial abundance by day 7. Although the reduction in numbers of oligochaetes was not significant at the 0.05 level (variability between samples was high), there was a strong trend showing an apparent reduction. A decline in numbers of oligochaetes was expected because these organisms had previ-

		Days after treatment						
Organism	Order	0	1	3	7	13		
Roundworm	Enoplida	67	0	18	141	86		
Oligochaete	Plesiopora Prosopora	4,341 18	1,401 36	1,992 12	861 0	1,371 0		
Leech	Rhynchobdellida Arhynchobdellida	357 0	80 0	18 0	37 0	49 18		
Clam	Eulamellibranchia	105	68	25	37	0		
Snail	Ctenobranchiata Pulmonata	18 6	92 0	37 0	37 0	12 30		
Isopod	Isopoda	6	43	12	49	105		
Amphipod	Amphipoda	209	609	621	965	1,063		
Water boatman	Hemiptera	0	0	0	0	6		
Midge	Diptera	3,554	3,130	2,373	1,654	2,447		
Mayfly	Ephemeroptera	449	639	320	320	314		
Caddis fly	Trichoptera	43	12	18	24	0		
TOTALS	-	9,173	6,110	5,446	4,100	5,494		

Table 1. Estimated total numbers of benthic invertebrates per  $m^2$  in bottom soil samples from Boardman Lake before and after treatment with Bayer 73.

ously been shown to be highly sensitive to Bayer 73 in laboratory tests (Rye 1972).

Midge larvae declined gradually to a maximum reduction of 54% by day 7. The difference between pretreatment and day 7 samples was significant. However, some of their reduction may have been due to natural emergence which normally occurs in early to mid summer. Numbers of oligochaetes and midges increased between day 7 and day 13, although the increase was not significant. Recolonization by organisms from outside the treated area was the most probable source of the increase.

Mayflies decreased by 30% in mean numbers per sample by day 13. However, the pattern showed a rise in numbers at day 1, a decrease at day 7, and essentially no change between days 7 and 13, suggesting that sampling variation or natural emergence was the probable reason for changes in mean numbers. Previous laboratory studies indicated a 24-h LC50 for mayflies of 11.4 mg/l (King 1974), almost the same as the theoretical concentration applied here. It is reasonable to assume that the concentration of chemical present was dispersed into surrounding water in much less than 24 h.

An apparent reduction in the numbers of leeches (order Rhynchobdellida) was also obviously attributable to sampling variation. Ninety-six percent of the individuals in the pretreatment samples were taken in a single grab.

Clams were reduced in numbers after treatment. Although they were never abundant, it was noteworthy that clams were absent from the day 13 samples. The reduction in numbers of clams by day 13 was significant.

		Post-treatment					
	Pretreatment	7	day	13	8 day		
Organism	Mean number	Mean number	Percent reduction	Mean number	Percent reduction		
Roundworm	1.6	3.3 (2.5)	0	2.0	0		
Oligochaete	(1.5) 101.3 (53.2)	20.0 (9.5)	80	(2.0) 31.8 (15.9)	69		
Leech	8.3 (8.1)	0.7 (0.6)	92	1.6 (1.6)	81		
Clam	2.4 (0.9)	0.9 (1.4)	62	<b>0.0</b> <i>a</i> (0.0)	100		
Snail	(0.9) 0.6 (0.4)	0.8 (0.6)	0	(0.0) 1.0 (0.7)	0		
Isopod	(0.4)	(0.0) 1.1 (0.5)	0	2.4 (1.3)	0		
Amphipod	4.8 (1.6)	22.4 (10.3)	0	(1.3) 24.7 (17.0)	0		
Midge	82.6 (18.6)	<b>38.3</b> <i>a</i> (6.5)	54	56.8 (14.3)	31		
Mayfly	10.4 (1.3)	(0.5) 7.4 (1.4)	29	7.3 (1.3)	30		
Caddis fly	1.0 (1.0)	0.6 (0.7)	40	0.0 (0.0)	100		
Total organisms	213.1 (46.7)	<b>95.5</b> <i>a</i> (13.3)	55	127.6 (33.0)	40		

Table 2. Mean numbers of benthic invertebrates per grab, (standard errors in parentheses), and percent reduction from pretreatment numbers after treatment of a lentic habitat with Bayer 73.

a Reduction in numbers from pretreatment samples significant at P = 0.05.

Reduced numbers of both clams and snails were expected, since Bayer 73 is used as a molluscicide in different areas of the world. Various authors have reported LC50's of 0.1 to 0.35 mg/l for snails and 0.3 to 0.4 mg/l for clams (Hamilton 1974) - far below the concentration applied in the treated area.

An apparent increase in the number of amphipods was due, to a large degree, to deliberate biasing in the sampling at two stations where vegetation (Myriophyllum sp.) was present. Because pretreatment samples showed that amphipods were closely associated with the vegetation, subsequent samples were taken on the side of those stations where vegetation was the most dense. However, if data from those two stations are disregarded, there was still an apparent increase in mean numbers per sample from 3.2 before treatment to 7.4 at day 13. This was regarded as ample evidence that numbers of amphipods were not affected by treatment with Bayer 73. Laboratory studies which showed a 96-h LC50 of 3.2 mg/l

		Day	ys after treatr	ment	
Index <sup>a</sup>	0	1	3	7	13
Diversity ( <b>d</b> ) <b>b</b> Equitability (e) <sup>c</sup>	1.77 0.37	2.01 0.59	1.84 0.43	2.25 0.59	2.08 0.55

Table 3. Diversity indices of benthic invertebrates in Boardman Lake before and after treatment with Bayer 73.

**a**Computed as described by Weber (1973). **b**Scale = 0-4.

 $c_{\text{Scale}} = 0-1.$ 

(Sanders 1973) suggested that some reduction of amphipods might have been expected, but none was observe.

The calculated mean diversity (d) of the benthic invertebrates increased slightly after treatment (Table 3). A decrease in d would have indicated an adverse influence on community structure. The apparent increase in d was primarily due to an increase in "equitability" (Table 3) which indicated a more even distribution of numbers of organisms among the various taxa after treatment than before (Weber 1973).

Although there was a reduction in total numbers of organisms immediately after the Bayer 73 treatment, the data do not indicate catastrophic effects and the long term community structure was not adversely affected. Ecological effects should be minor since the use pattern of granular Bayer 73 for control of sea lampreys in lentic habitats generally includes treatment of only a small percentage of the area of any body of water. Furthermore, rapid dilution, dispersion of the chemical, and recolonization of treated areas by organisms from adjacent untreated areas can be expected.

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RYE, R. P., Jr.

## EFFICACY OF ANTIMYCIN FOR CONTROL OF LARVAL SEA LAMPREYS (PETROMYZON MARINUS) IN LENTIC HABITATS

#### by

Philip A. Gilderhus

#### ABSTRACT

Antimycin, in a delayed-release granular formulation, was shown to be effective for control of larval sea lampreys (Petromyzon marinus) in lentic habitats at treatment rates of 7.5 to 150 g of active ingredient per hectare. When the chemical was tested in four inland lakes connected to the Great Lakes and known to harbor larval lampreys, about 90% or more of the larval lampreys present were killed by treatments of 150 g/ha (active ingredient). Treatment at 75 g/ha killed more than 90% in warm water but only 66% in cold water. Some small fish, mostly bottom-dwelling forms, were killed by the treatments.

#### INTRODUCTION

The story of the invasion of the Great Lakes by the sea lamprey (Petromyzon marinus) and the subsequent successful control of this destructive parasite is well known (Applegate 1950; Lawrie 1970). Adult sea lampreys run up tributary streams to spawn. After hatching, the nonparasitic larvae burrow into the stream sediments where they live for several years before they metamorphose into the parasitic form. Although larvae were once assumed to inhabit only streams, extensive surveys of lamprey distribution later revealed populations of larvae to be common in lentic habitats where the spawning streams entered inland lakes or the Great Lakes (Wagner and Stauffer **1962**).

Assessment of lentic populations was difficult until 1966, when it was found that granular 2',5-dichloro-4'-nitrosalicynilide (Bayer 73) drove larvae from their burrows to the surface of the water, where they could be captured (U.S. Bureau of Commercial Fisheries 1966). Later observations indicated that most larvae died soon after being driven from their burrows by Bayer 73 (Tibbles **1967**; Smith et al. 1974). The granular formulation was registered by the U.S. Environmental Protection Agency for use in sea lamprey surveys in 1968. In 1969, 67 deltas and estuaries in U.S. waters were surveyed by use of granular Bayer 73. Thirty-two sites were found to have populations of sea lamprey larvae, and 15 of these populations were large (Smith and King 1970). Treatment with granular Bayer 73 has since become the standard method for surveying lamprey populations in lentic habitat. Continuing effective control of sea lampreys is of critical importance to the fishery resources of the Great Lakes. Thus, the agencies concerned with control of lampreys considered it prudent to seek an alterative chemical for use in lentic habitats in the event that the required review of pesticide registration might reveal problems that would prevent the continued registrations of Bayer 73.

Antimycin was a logical alternative because it was already registered as a fish toxicant, was highly toxic to sea lamprey larvae, and was available in a fast-sinking granular formulation. A series of field trials was conducted in selected lakes with the primary objective being to evaluate the efficacy of antimycin for control of sea lamprey larvae. A secondary objective was to delineate the minimum effective concentration of antimycin. Since the rate of dispersion of the toxicant from the bottom into waters above was not known, the 1973 trials were done at a high concentration of antimycin (calculated as  $300 \mu g/l$  in the bottom 5 cm of water); in the 1974 trials half that concentration was used. One trial (Ocqueoc Lake 1973) included a comparison of the relative efficacies of both antimycin and Bayer 73).

#### MATERIALS AND METHODS

#### Trial Sites

Trial sites were chosen on the basis of four factors: (1) past history of significant lamprey populations, (2) water depth, (3) ease of access, and (4) the degree to which the sites typified the lentic areas inhabited by sea lamprey larvae. Figure 1 shows a schematic diagram of the location of test sites. Adult lampreys typically move upstream from the Great Lakes to spawn, and some move through the inland lakes and spawn upstream from them. Each test site was in an inland lake near the mouth of a stream where lampreys spawned, each had harbored a substantial number of larval lampreys in the past, and each had been treated with Bayer 73 to survey and control the lamprey population. Sites with a gradual drop-off and limited maximum depth were chosen to facilitate the placement of live cages and inspection of the bottom by SCUBA divers.

The perimeter of the area to be treated in each lake was measured with a calibrated cord anchored at one end and stretched to the desired distance from a boat. The comers and perimeter of the treatment areas were marked by floating plastic jugs anchored to the bottom. Significant characteristics of the treatment areas are given in Table 1.

#### Test Cages and Organisms

Although lampreys are primitive fish, the test organisms are here called lampreys and fish, with fish referring to teleosts.

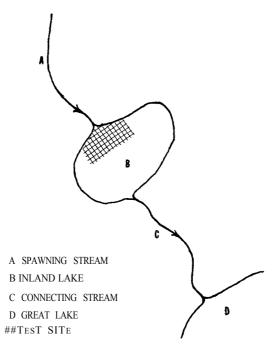


Figure 1. Schematic diagram of sites used in tests of antimycin for control of larval sea lampreys.

Lake	Drainage basin <b>(lake)</b>	Area of plot (ha)	Approximate dimensions (m)	Maximum depth (m)	Total hardness (mg/l)	Water temp (°C)
Charlevoix	Michigan	0.74	76 x 98	7.6	164	22
Ocqueoc (1973)	Huron	1.50	76 x 198	4.6	178	15
Harlow	Superior	0.74	61 x 122	4.0	55	24
Ocqueoc (1974)	Huron	1.95	107 x 183	4.6	168	10

Table 1. Characteristics of sites treated experimentally with antimycin for control of larval sea lampreys.

Sea lamprey larvae and fish were used as test organisms in all trials. Lamprey larvae were confined in cages that consisted of a rectangular metal pan  $17 \times 27 \times 6.5$  cm deep, with a screen cover soldered to the top lip of the pan and extending 9 cm above the hp. The pans were filled with sand from the lake bottom to allow the lamprey larvae to burrow as they do in their natural habitat. Ten larvae were placed in each cage. Cages were spaced so that all areas and depths within the treatment plots were

represented. In the 1973 trials, cages were partly buried by SCUBA divers so the top of the pan was even with the bottom sediments. After the 1973 trials, laboratory studies with antimycin showed no difference in the mortality of lampreys between cages with the pans buried in the bottom sediments and those with the pans merely set on the bottom sediments (E. L. King, Jr., U.S. Fish and Wildlife Service, Hammond Bay Biological Station, personal communication). Consequently, in the 1974 trials the cages were merely lowered to the bottom. Cages were placed about 24 h before treatment in all trials.

Fingerling fish were placed in 6-mm mesh screen cages measuring 23 x 10 x 10 cm. Cages containing five fish each of one or two species were placed 24 h before treatments. Some cages were placed on the bottom and others were suspended at distances of 1.5, 3.0, 4.6, and 6.1 m above the bottom, to check toxic effects at the various levels in the water column throughout the treatment plot.

## Chemicals

Granular antimycin used in the lake trials was formulated for experimental use in sea lamprey control by Ayerst Laboratories. The formulation contained 1% antimycin by weight and had a coating on the granules which permitted them to sink for 2 min before releasing any toxicant. The granules then released 90% of the toxicant in about 10 min. The formulation was designed to toxify a thin stratum of water at the soilwater interface, with the hope of minimizing effects on fish in the water above.

The formulation of Bayer 73 applied was the granular formulation (5% active ingredient) routinely used for sea lamprey surveys.

#### Application Methods

Antimycin and Bayer 73 were applied to the surface of the water with a battery powered rotary spreader on a boat. The experimental plot in each lake was treated on a crisscross pattern to help ensure even distribution.

An exception was Harlow Lake, where sea lamprey larvae were confined to a narrow strip along shore and the edge of the alluvial fan of Bismark Creek. There Bayer 73 was applied with a backpack Solo<sup>1</sup> blower carried by a man walking along the shore.

## Assessment of Results

Cages of lamprey larvae and fish were removed from the water 48 h after all treatments with antimycin and 24 h after the Bayer 73 treatment in Ocqueoc Lake in 1973. Mortalities were assessed immediately upon

<sup>1</sup> Reference to trade names does not imply Government endorsement of commercial products.

retrieval of the cages. The sand in the pans on the lamprey cages was washed through the screen to facilitate the observation of the larvae in the cages.

In addition to observations made on caged specimens, the effects of treatments on wild lamprey larvae and fish were observed by SCUBA divers. Divers inspected the lake bottom 1 to 2 days after antimycin treatments and on the same day that Bayer 73 treatments were made because the activity of Bayer 73 is usually complete within 3 to 4 h. Following each of three antimycin treatments and two Bayer 73 treatments, the two divers swam transects across the treatment plot. They stretched a 1.8-m cord between them and picked up all dead lampreys and fish that the cord passed over. The number of dead specimens per unit of area in the transects was applied to the total area to yield an estimate of the numbers killed in the entire plot.

## LAKE TRIALS

## Lake Charlevoix 1973

The first trial of antimycin was done in Lake Charlevoix, Charlevoix County, Michigan, near the mouth of Horton Creek, a small, lampreyinfested stream. This stream had no alluvial fan where it entered the lake. The bottom consisted of a mixture of sand and clay.

Fifteen cages containing larval lampreys were placed on the bottom with three cages each at depths of 1.5, 3.0, 4.6, 6.0, and 7.6 m (Table 2). Ten cages containing rainbow trout (Salmo gairdneri and 10 cages containing white suckers (*Catostomus commersoni*) were placed at selected distances above the bottom (Table 3).

Lake		Depth (m)						
	O-1.6	1.7-3.2	3.3-4.8	4.9-6.4	6.4+	mortality (%)	Control	
Charlevoix	80 (3)	100 (3)	96 (3)	100 (3)	83 (3)	93	0 (2)	
Ocqueoc 1973	85 (2)	60 (3)	100 (9)	-	-	89	0 (1)	
Harlow	100 (1)	92 (5)	loo (6)	-	-	97	50 (2)	
Ocqueoc 1974	54 (5)	98 (5)	15 (2)	-	-	66	0 (2)	

Table 2. Percent mortality of caged larval lampreys at different depths and (in parentheses) number of **cages<sup>a</sup>** placed at various depths, in areas treated with antimycin for control of sea lampreys.

a All cages (each containing 10 larval lampreys) were placed on the bottom. Control cages were about 75-100 m outside the treated area.

	Dis	Distance above lake bottom (m)				
Lake and species	0	1.5	3.0	4.5	6.1	Controla
Charlevoix						
Rainbow trout	100	75	75	80	50	100
White sucker	(2) 75	(4) 35	(2) 0	(1) 25	(1)	(1) 23
	(3)	(4)	(2)	(1)	-	(1)
Ocqueoc 1973						
Rainbow trout	100	97	loo	-	-	50
	(6)	(6)	(6)			(2)
Harlow			( )			
Brown trout	loo	84	loo	-	-	80
Largemouth bass	100	0	25			0
	(5)	(5)	(4)	-	-	(4)
Ocqueoc 1974						
Largemouth bass	0	6	0	-	-	0
Yellow perch	92	20	25			15
-	(5)	(3)	(4)	-	-	(4)

Table 3. Percent mortality of caged fish, and (in parentheses) number of cages at different levels above the bottom, in areas treated with antimycin for control of sea lampreys.

<sup>a</sup> Cages of control *fish* were placed 75-100 m outside the treated area.

The rainbow trout (5-8 cm long), obtained from a nearby state fish hatchery, suffered high mortalities after being held overnight in an aerated transport tank. Survivors were used in the cages, but were assumed to be highly stressed. The suckers (4.5-6 cm long), obtained from a local minnow dealer, were thin and also appeared to be in marginal physical condition. All cages of lampreys and fish were placed at least 15 m inside the perimeter of the area to be treated so that any dilution of the chemical at the edges of the test area would not significantly influence exposure of the test animals to the toxicant.

The experimental area was treated on 22 August at a rate of 150 g of antimycin per hectare of lake surface, which amounted to a theoretical concentration of 300  $\mu$ g/l in the bottom 5 cm of water.

Mortality of caged lamprey larvae totaled 93%. Eleven of the 15 cages had complete kills, two cages had 1 of 10 survive, and two cages had 4 and 5 survivors, respectively.

Ten of the 11 survivors were in cages near the south edge of the treatment area, indicating a possible movement of water and dilution of the chemical in that area. The mortality of lampreys did not show correlation with depth of water (Table 2).

The results with caged rainbow trout and white suckers are shown in Table 3. While somewhat erratic, the data do show some encouraging trends. Mortality among both species at the bottom was expected. The survival of trout at 1.5 m or more above the bottom was better than the controls; however, the stressed condition of these fish made the results

less than conclusive. The survival of 65% of the suckers at 1.5 m above the bottom was slightly less than controls, but indicated that little antimycin dispersed vertically in the water column. Survival of suckers at 3 m or more above the bottom was better than the controls, indicating no mortality due to the toxicant.

A thorough inspection of the test plot by divers revealed no wild lamprey larvae in the area. Small numbers of johnny darters (*Etheostoma* nigrum) and sculpins (*Cottus* sp.) were found dead on the bottom. Mortality of these bottom-dwelling species was not unexpected. One dead fingerling smallmouth bass (Micropterus *dolomieui*) was also observed.

Clams were very numerous on the bottom, but none were dead when observations were made 2 days after treatment. A few dead mayfly nymphs were seen, but live ones also were present.

## Ocqueoc Lake 1973

The Ocqueoc River, Presque Isle Co., Michigan (volume of flow about 2  $m^3/s$ ), passes through Ocqueoc Lake, and continues several kilometers to Lake Huron. Sea lampreys migrate upstream through Ocqueoc Lake and spawn several kilometers upstream from that lake.

The experimental area in Ocqueoc Lake was located offshore from the alluvial fan at the mouth of the river. The bottom was mostly sand near shore and overlain by silt and detritus beyond the 1.5-m contour.

The experiment in Ocqueoc Lake included treatments and evaluations with both antimycin and Bayer 73 to permit a comparison of the relative effectiveness of the two chemicals.

For the evaluation of antimycin, 14 cages of lamprey larvae were distributed throughout the treatment plot at depths of 1.5 to 4.6 m. Eighteen cages of rainbow trout (five fish per cage) were placed in an arrangement similar to that used for the lampreys. Some cages were placed on the bottom and others suspended at 1.5 and 3.0 m above the bottom. Two cages of control fish were placed 90 m from the treated area, one on the bottom and one 3 m above the bottom.

The experimental plot was treated with antimycin on 27 September at the rate of 150 g of active ingredient per hectare. The antimycin treatment was assessed 1 and 2 days later.

The antimycin treatment resulted in 8% mortality of caged lamprey larvae (Table 2). Only three cages, all within 20 m of the river mouth, contained live lampreys. Presumably, the flow of water from the river diluted the chemical in the area near the mouth. Lampreys picked up by the divers on measured transects indicated a kill of about 1,500 wild larval lampreys in the treated area.

Caged rainbow trout suffered 99% mortality during the observation period. However, the 50% mortality of controls made an assessment of the mortality of caged fish attributable to the antimycin highly questionable. The mortality of caged trout in this trial (Table 3) seems to indicate considerable dispersion of the toxicant throughout the volume of water in the treated area. However, a 1974 trial at this site (discussed later) under similar conditions caused a much lower mortality of caged largemouth bass (*Micropterus salmoides*) and yellow perch (*Perca flavescens*) (Table 3).

The fish recovered on transects covered by the divers indicated that the antimycin treatment killed, about 1,600 wild fish, including common shiners (*Notropis cornutus*), white suckers, logperch (*Percina caprodes*), johnny darters, yellow perch, and sculpins. Most of these were bottomdwelling fishes which would have had little chance to avoid the high concentration of antimycin near the bottom. All of the fish except one yellow perch were less than 7.5 cm long.

Preparation for the Bayer 73 treatment in the same area began on 1 October with the placement of 18 cages of lamprey larvae and 16 cages of rainbow trout. Cages were distributed throughout the treated area in approximately the same pattern as that for the antimycin treatment. The area was treated with Bayer 73 on the morning of 2 October at a rate of 168 kg per hectare of surface. This rate approximates 16 mg/l of active ingredient in the bottom 5 cm of water. Results of the Bayer 73 treatment were assessed that afternoon and on the next day.

The Bayer 73 treatment killed 88% of the caged sea lampreys-a percentage nearly identical to that killed with the antimycin treatment. As with the antimycin treatment, all surviving lamprey larvae were less than 20 m from the mouth of the river. The specimens recovered in the divers' transects indicated that about 10 residual wild larval lampreys were killed in the area by the Bayer 73. The ratio of the number of wild lampreys killed by antimycin to that killed later by Bayer 73 (1,500: 10) further indicated that 90% or more of the lampreys were killed by the antimycin.

A total of 17% of the caged rainbow trout were killed by the Bayer 73, and mortality occurred in 8 of the 18 cages. Observations by the divers indicated a kill of about 130 wild fish, mostly yellow perch. Presumably, most of the bottom-dwelling fish in the treatment area, such as darters and sculpins, had been eliminated by the earlier antimycin treatment.

## Harlow Lake 1974

The trial of antimycin in Harlow Lake was done in an area of the lake near the mouth of Bismark Creek, a small stream with a flow of about  $0.06 \text{ m}^3$ /s. The stream formed an alluvial fan about 30 m wide, beyond which the depth increased to 3 m within 6 m of shore. The bottom was mostly sand inshore and soft organic sediments farther out.

Sea lamprey larvae were confined in 12 cages distributed at different depths throughout the treatment area, Two cages outside the treatment area served as controls. Fourteen cages were used for test fish, and each held five brown trout (*Salmo trutta*) and five largemouth bass.

Some of the cages were checked just before the antimycin treatment. In one lamprey cage and two fish cages on the bottom in deep water offshore, all specimens were dead. Fish in cages 1.5 and 3.0 m above the bottom were alive. Analyses for dissolved oxygen showed 5 mg/l at a depth of 3.6 m and 2.5 mg/l at 4.0 m. The concentration of oxygen was assumed to be critically low for lampreys and fish in the stratum close to the bottom at depths of 4 m or more.

The experimental area was treated on 21 August to supply 74 g of antimycin per hectare or 150  $\mu g/l$  in the bottom 5 cm of water. This was the first trial with the chemical at the reduced concentration. Bayer 73 (treatment method described earlier) was used as a survey tool to determine the number of live lampreys remaining after the antimycin treatment.

Ninety-seven percent of the caged lampreys were dead when the cages were retrieved 2 days after the antimycin treatment. The four survivors were in one cage 6 m from the mouth of the creek. Exclusive of the three cages at depths where dissolved oxygen was critical, the mortality of lamprevs was still 93%. The treatment also killed a large number of wild lamprey larvae: 236 were retrieved by hand in water less than 0.3 m deep along shore, and hundreds more were observed slightly farther from shore. The divers estimated that about 1,000 dead larval lamprevs were on the bottom, all in shallow water and along the face of the delta. The Bayer 73 treatment of the area on 23 August resulted in retrieval of 39 larval lampreys swimming at the surface, 30 of which emerged in a tight corner formed by the edge of the delta and the shoreline, an area that could not be covered by the boat spreading antimycin. No freshly dead larvae were found on the bottom after the Bayer 73 treatment. The number of dead lampreys found after the antimycin treatment (1,000), compared with the number driven to the surface by the Bayer 73 treatment (39); gave further evidence that more than 90% of the sea lamprevs in the treated area were killed by the antimycin treatment.

All fish in cages on the bottom of the lake were dead after 48 h. Their mortality was expected from the concentration of antimycin on the bottom, but the low oxygen concentration there was undoubtedly a contributing factor. Brown trout incurred high mortalities at all levels in the treatment area and in control cages (Table 3), presumably because water temperatures exceeded the upper limits of tolerance for trout. Thus, the results with trout were not regarded as valid. The survival of caged largemouth bass was high; 1.5 and 3.0 m above the bottom (data combined), 89% of the bass survived (Table 3).

Wild fish killed by the antimycin included small (2.5-10 cm) shiners (Notropis sp), white suckers, yellow perch, and one northern pike (Esox lucius), 15 cm long. Total numbers killed were estimated by the divers at about 800.

#### Ocqueoc Lake 1974

The experimental area for the 1974 trial was again located adjacent to the mouth of the Ocqueoc River, and the lake level and volume of flow in the river were approximately the same as during the 1973 trial.

Twelve cages of larval lampreys were placed on the bottom at depths of 1.5 to 4.0 m. Two cages of controls were placed outside the area, at

depths of 1.2 and 6.0 m. Twelve cages of test fish were used in the treatment area, each containing five fingerling largemouth bass and five yellow perch. Four additional cages placed outside the area were used for controls.

The area was treated with antimycin on 27 September at the rate calculated to supply 75 g/ha to the bottom 5 cm of the water column. One hour later an additional 1,360 g of the formulation (13 g of antimycin) were applied to the 0.3 ha which appeared to be most influenced by the current of the river. The cages were checked and the treatment area was inspected by divers on 29 September.

The antimycin treatment killed 66% of the caged lampreys, a somewhat lower percentage than that obtained in the other field trials (Table 2). The mortality pattern did not seem to be closely associated with position of the cages. Specimens placed in cages along the delta and in the areas directly in line with the river current incurred 80% mortality, and those at a depth of 3 to 4 m, 73%. Mortality was complete in 50% of the cages and nil in 25%. These results suggest that the application may not have been uniform. The small amount of the formulation used (7.4 kg/ha or 6.6 lb/a) made it difficult to obtain a uniform application.

The divers who inspected the entire area along the face of the delta found no larvae. On transects from the outer edge of the plot to shore, they found one dead larva on each transect, indicating a maximum estimate of 60 dead wild lampreys in the entire treated plot. On 3 October the area was treated with Bayer 73 to assess the number of wild lampreys not killed by the antimycin. The number driven to the surface and captured alive provided the only measure of the number remaining in the area. The treatment with Bayer 73 resulted in the capture of 54 larval sea lampreys at the surface, indicating that the antimycin probably killed between 50 and 60% (60 of a total of 114) of the wild lampreys.

In addition to the possibility of uneven application, the kill of lampreys in this trial was probably influenced by the water temperature, which was 14 degrees (C) lower than that in the Harlow Lake trial. Earlier studies have established that the effective contact time (ECT) for antimycin is substantially longer at low than at high temperatures (Gilderhus 1972). In some portions of the treatment area in Ocqueoc Lake, dilution of the chemical may have occurred before the lampreys had been exposed to a lethal concentration for an effective period of time. A higher original concentration would have shortened the ECT and probably given control of lampreys comparable with that achieved in the three previous trials.

Largemouth bass in cages were virtually unharmed (only one fish dead) at all depths, including the five cages resting on the bottom. I considered it noteworthy that antimycin showed a considerable degree of selectivity for lampreys over largemouth bass among specimens confined to the bottom. Mortality was 92% in yellow perch confined to the bottom, but mortality of those suspended at 1.5 m or more above the bottom was only 5 to 10 percentage points higher than that of perch in control cages (Table 3).

#### DISCUSSION

Antimycin proved effective for the control of larval sea lampreys in lentic habitats. Three of the four trials resulted in about 90% mortality of the caged and wild larval lampreys present. A treatment rate of 75 g of active ingredient per hectare in the delayed release formulation appeared to be adequate in warm water. The less than adequate control obtained with 75 g/ha in Ocqueoc Lake in 1974 was assumed to be due primarily to the low water temperature (10 C). Antimycin is known to have much longer effective contact times at low temperatures. Treatment rates of 100 to 150 g/ha may be needed at temperatures of perhaps 15 C or lower. Although the trials described here provide broad guidelines for treatment rates, more precise estimates of effective concentration in relation to temperature and water quality remain to be defined.

Treatments with delayed release antimycin killed some test fish and native fishes, especially those confined near the bottom of the treated areas. However, in four trials, no legal-size game fish were observed to have been killed. Although the area near the delta of Bismark Creek was a popular fishing spot for northern pike, and 1.5- to 3.0-kg fish were reported to be common, no catchable-size fish were killed by the antimycin treatment. Ocqueoc Lake is reported to have populations of northern pike, walleyes (Stizostedion vitreum) and other game fish. Although these fishes often frequent areas near the mouths of inlet streams, none were killed by the antimycin treatment. Perhaps because they were not confined, large fish could have moved in and out of the treated areas, or have swum above the bottom, thus avoiding a sufficient continuous exposure to be lethal. The results of these trials suggest that significant kills of game fish should not be expected after treatments with delayed release formulations 'of antimycin. Some mortality of small native fishes is to be expected, but should be of little consequence considering that only a small portion of any lake would be treated for control of larval lampreys.

Antimycin and Bayer 73 each have advantages and disadvantages. Antimycin does not drive lampreys to the surface of the water to provide for estimates of numbers, as Bayer 73 does. However, antimycin is easier to use because the formulation is virtually dust free during application (the Bayer 73 formulation gives off a fine dust of the active ingredient which can be hazardous to the applicator), and the weight of material to be handled is only 1/20 that needed with Bayer 73. At the lower treatment rate of 75 g of antimycin per hectare, a formulation containing 0.5% antimycin rather than 1% would provide twice the material and help ensure more even distribution over the surface area to be treated.

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