CHEMOSTERILIZATION OF THE SEA LAMPREY (*PETROMYZON MARINUS*)

Great Lakes Fishery Commission

TECHNICAL REPORT No. 29

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ABSTRACT

The chemical, P,P-bis(1-aziridinyl)-N-methylphosphinothioic amide (bisazir), was found in laboratory studies to be an effective sterilant for both sexes of adult sea lampreys *(Petromyzon* marinus) when given intraperitoneally at a dosage of 100 mg per kilogram of body weight. A total of 300 normal spawning-run sea lampreys and 300 injected with bisazir were released into the Big Garlic River, Marquette County, Mich&n, (a small stream divided into five sections by natural barriers), to determine the effect of bisazir on the nesting and spawning behavior of the adults and on the production of larvae. The lampreys constructed and spawned in 95 nests. Sterile adults showed no abnormal nest building or spawning behavior. Sterile males competed effectively with normal males for females. Egg samples taken from nests indicated that eggs in nests where sterile males spawned with sterile or normal females did not hatch, although some embryonic development occurred. Extensive surveys with electric shockers produced no larvae in stream sections where sterile males spawned, but yielded numerous larvae in sections where normal males spawned with normal females. These findings suggest that the release of sterile males may be an effective tool in an integrated approach to control of sea lampreys in the Great Lakes.

INTRODUCTION

The use of selective toxicants to control the sea lamprey *(Petromyzon marinus)* in the Great Lakes (Applegate et al. 1961; Howell et al. 1964; Manion 1969) has resulted in a drastic reduction in the lamprey population. In a 13-year period (1958-70), 323 treatments on 115 Lake Superior tributaries reduced sea lamprey populations in the lake by about 90% from precontrol levels (Smith et al. 1974). Similar reductions in lamprey populations are believed to have occurred in Lakes Michigan and Huron. The lamprey population can be reduced further, however, only by treating major lamprey-producing streams more frequently, as suggested by Smith et al. (1974) or by developing other methods of control to attack remnant lamprey

1 Contribution 534 of the U.S. Fish and Wildlife Service, Great Lakes Fishery Laboratory, Ann Arbor, Michigan 48105. This study was part of a program conducted by the U.S. Fish and Wildlife Service under contract with the Great Lakes Fishery Commission.

[2]This paper reflects the results of research only. Mention of a pesticide does not constitute recommendation or endorsement by the U.S. Government.

populations not now being killed by the chemical toxicants. One method listed by Hanson (1970) for possible use in an integrated sea lamprey control program was the sterile-animal-release technique. This technique was developed and used successfully to eliminate the screw-worm fly *(Callitroga hominivorax),* a serious pest of livestock in the southeastern United States (Knipling 1960). A basic consideration for employing the technique is the development of a method that induces sterility but does not affect the mating competitiveness and behavior of the pest to be controlled (Knipling 1964).

Primarily on the basis of Knipling's findings, we initiated a study to develop a chemosterilant for adult sea lampreys. It consisted of two parts: (1) laboratory studies to identify a chemical, that would produce sterility in sea lampreys, and (2) a field study in which both normal and sterilized lampreys were released into a known sea lamprey producing stream and observed under natural conditions throughout their spawning period to determine the effect of the chemosterilant on the nest building and spawning behavior of lampreys of either sex and on the mating competitiveness of the males. Also essential was the verification under field conditions that lampreys injected with the chemosterilant were sterile. The laboratory studies were conducted in 1971-73 by the first author, and the field study in 1974 by both authors.

LABORATORY STUDY

The artificial stream

In years before 1971, spawning-run lampreys held at the Hammond Bay Biological Station, in a concrete tank supplied with flowing water from Lake Huron, underwent normal gonadal development. They did not spawn, however, unless they were held under more natural stream-like conditions, including suitable flow velocities and a suitable substrate. Therefore we constructed an artificial stream in the laboratory in which lampreys would spawn and could be easily observed (Fig. 1). The stream was built in a concrete raceway 6.6 m long, 1.2 m wide, and 0.5 m deep. Seven wooden baffles (1.4 m long, 4 cm wide, and 30 cm high) were placed diagonally in alternate directions on the floor of the raceway to create areas of high and low water velocity in the stream. Gravel (diameter range, l-100 mm; average, 30 mm) taken from lamprey spawning grounds of a local stream was spread on the floor of the raceway to a depth of about 12 cm.

Water from Lake Huron was delivered to the head of the raceway at a rate of 374 liters/min. Water depth varied from 18 cm at the head of the raceway to 12 cm at the lower end. The water flowed around the baffles and passed through the opening (about 8 cm wide) between the downstream end of each baffle and the wall of the raceway. Water velocity averaged about 0.3 m/s along the upstream face of each baffle and was almost nil on the downstream side.

The stream was illuminated with four 100-W incandescent light bulbs during normal working hours (0800-1630) and with daylight from windows. Water temperatures were continuously recorded on a thermograph.

Lamprey stock, treatment, and egg collection

Lampreys used in the laboratory study were captured in a trap at an electrical weir in the Ocqueoc River, Presque Isle County, Michigan, as they migrated upstream to spawn and were transferred to the concrete holding tank that had been used in earlier years. Lampreys were removed from the tank as needed, injected with one of the chemicals to be tested (Tables 1 and 2) tagged to permit individual recognition (Petersen tags 9.5 mm in diameter), and placed in the artificial stream. Most chemicals injected into the lampreys were dissolved in a saline solution (9 g NaCl/1000 ml distilled water), but Sudan black B was suspended in propylene glycol and 5-fluoroorotic acid was dissolved in sodium bicarbonate solution. All injections were made intraperitoneally just posterior to the liver with a tuberculin syringe and a 25-gauge, 16-mm hypodermic needle. Two groups of uninjected lampreys-a group of 5 that were tagged and a group of 10 that were untagged-served as controls. Since no effects due to tagging were observed, the data from the two control groups were combined (Table 1).

Lampreys in the artificial stream were observed periodically during normal working hours. No spawning was observed during May or June. The lampreys moved little during this time and usually remained in large groups in corners behind the baffles, attached to stones, the baffle, or the wall of the raceway. As water temperatures increased and sexual maturity approached during early July, swimming activity gradually increased. Lampreys were first observed spawning on July 12, and last on August 1. Water temperatures during the spawning period fluctuated between 15 and 20 C.

Lampreys observed in the spawning act (Fig. 2) were removed from the artificial stream and artificially spawned with normal spawning lampreys of the opposite sex which were taken off nests in nearby natural streams. To facilitate care and handling of the embryos, we kept only a small portion of the total number of eggs from each female. Eggs were placed in lo-liter glass battery jars (250 mm in diameter) that contained 6 liters of Lake Huron water. The jars were partly immersed in a constant temperature water bath held at 18.3 C, the temperature that Piavis (1961) determined to be optimum for the development of sea lamprey embryos. We changed the water frequently, removed dead eggs and embryos when detected, and maintained oxygen levels near saturation by aerating the water with stone air breakers.

Selection of a chemosterilant for field testing

Of the 14 chemicals tested in 1971-73, 7 (ethyl methanesulfonate; 1,3-propanediol dimethanesulfonate; hexamethylmelamine hydrochloride; hexamethylphosphoric triamide; 3-chloro-1,2-propanediol; colchicine; and Sudan black B) were found to have no sterilizing action on adult lampreys, at

Table 1. Effects of intraperitoneal injection of various chemicals (bisazir excluded; see Table 2) on spawning behavior and sterility of sea lampreys held in an artificial stream in the laboratory. Injected lampreys that displayed spawning behavior were removed and artificially spawned with normal sea lampreys.

Chemical[a] and dose rate (mg/kg) if not limited to a single rate of 100 mg/kg	Number of sea lampreys in artificial stream and (in parentheses) number observed spawning and spawned artificially		Percentage of artificially spawned individuals sterilized[b]	
	Males	Females	Males	Females
None (control)	5 (5)	10 (7)	0	0
Alkylating agents				
Alkanesulfonates				
Ethyl methanesulfonate				
100	2 (1)	3 (0)	0	
300	2 (1)	2 (0)	0	
Methyl methanesulfonate				
50	2 (0)	2 (2)	-	0
100	2 (0)	3 (2)		50
200	3 (1)	2 (2)	100	0
1,3-Propanediol dimethanesulfonate (A13-51904)	5 (2)	5 (3)	0	0
Aziridines				
N,N'-hexamethylenebis(1-aziridinecarboxamide) (A13-50172)	6 (1)	4 (0)	100	
Tris(1-aziridinyl)phosphine oxide (tepa, A13-24915)	5 (1)	5 (0)	100	
Tris(1-aziridinyl)phosphine sulfide (thiotepa, A13-24916)	5 (2)	5 (0)	100	--
Nonalkylating dimethylamino compounds[c]				
3,5-Bis(dimethylamino)-1,2,4-dithiazolium chloride (A13-51160)	4 (1)	6 (0)	100	
Hexamethylmelamine hydrochloride (hemel, A13-50905)	3(3)	7 (3)	33	0
Hexamethylphosphoric triamide (hempa, A13-50882	3(3)	7 (3)	0	0
Other compounds				
3,Chloro-1,2-propanediol (A13-11200)	8 (6)	2 (0)	17	-
Colchicine				
100	0 (-)	5 (0)	-	-
300	2 (0)	3 (0)		-
Sudan black B	5 (4)	5 (1)	0	0
5-Fluoroorotic acid (A13-26398)	7 (3)	3 (1)	0	100

a Numbers in parentheses indicate the USDA code numbers. These compounds were obtained from Dr. A. B. Borkovec, U.S. Department of Agriculture,
b Insect Chemosterilants Laboratory, Beltsville, Maryland 20705. Where not otherwise indicated, dose rate was 100 mg/kg.
c An individual was considered sterile if eggs produced or fertilized by that individual failed to develop to burrowing prolarval stage (stage 17 of Piavis 1961).

Table 2. Effects of intraperitoneal injection of bisazir-P,P-bis(l-aziridinyl)-N-methylphosphinothioic amide (A13-61585)[a]-on sea lampreys held in an artificial stream in the laboratory. [Each injected lamprey that exhibited spawning behavior was artificially spawned with a normal lamprey.]

Dose rate (mg/kg), and sex	Number observed spawning and spawned artificially	Average number of eggs in sample (range in parentheses)	Percentage survival to stage 17 (range in parentheses)	Highest stage reached in individual sample [b]
10 Male	4	1330 (600-2072)	1.9 (0.0-3.5)	16-17
Female	2	3348 (2862-3869)	52.5 (25.9-88.9)	17
25 Male	2	1784 (1553-2014)	0.1 (0.1-0.1)	17
50. Male	2	1501 (1002-2000)	0.3 (0.0-0.7)	4,17
Female	2	2129 (1148-3109)	0.0 (0.0-0.1)	11,17
100 Male	6	1720 (958-2000)	0.0 (0.0-0.1)	5-17[c]
Female	4	1610 (135-2000)	0.0 (0.0-0.0)	l-11

[a] USDA code number. This compound supplied by Dr. A. B. Borkovec, U.S. Department of Agriculture, Insect Chemosterilants Laboratory, Beltsville, Maryland 20705.

[b] Stages of development after Piavis (1961). Stages 1-13 describe the development of the embryos from zygotes to hatching. Stages 14 (hatching), 15 (pigmentation), and 16 (gill-cleft) describe their development until they are able to leave the nest and burrow in the bottom mud (stage 17).

[c] One abnormal stage 17 produced.

the dose rates tested (Table 1). Compounds were considered to have exhibited sterilizing activity only if 100% of the artificially spawned individuals of either sex produced no offspring. Because of the small numbers of lampreys tested (one or two) for six of the compounds that exhibited sterilizing action, these findings should be considered tentative. Of the seven chemicals with potential sterilizing action, only P,P-bis(1-aziridinyl)-N-methylphosphinothioic amide (bisazir) was effective against both male and female lampreys. At doses of 10-100 mg per kilogram of body weight, bisazir caused complete or nearly complete sterility in males (Table 2). At 10 mg/kg, survival to stage 17 (burrowing prolarvae; Piavis 1961) of embryos from injected females averaged 52.5%; at higher doses, survival to stage 17 was 0.1% or less. Thus, bisazir appeared to be a promising sterilant for field testing.

Much work with insect sterilization has centered on the use of aziridines (Borkovec 1962). The compound bisazir, an alkylating agent of the aziridine group, was first synthesized in 1968 at the Insect Chemosterilants Laboratory, Beltsville, Maryland (A. B. Borkovec, personal communication), and has

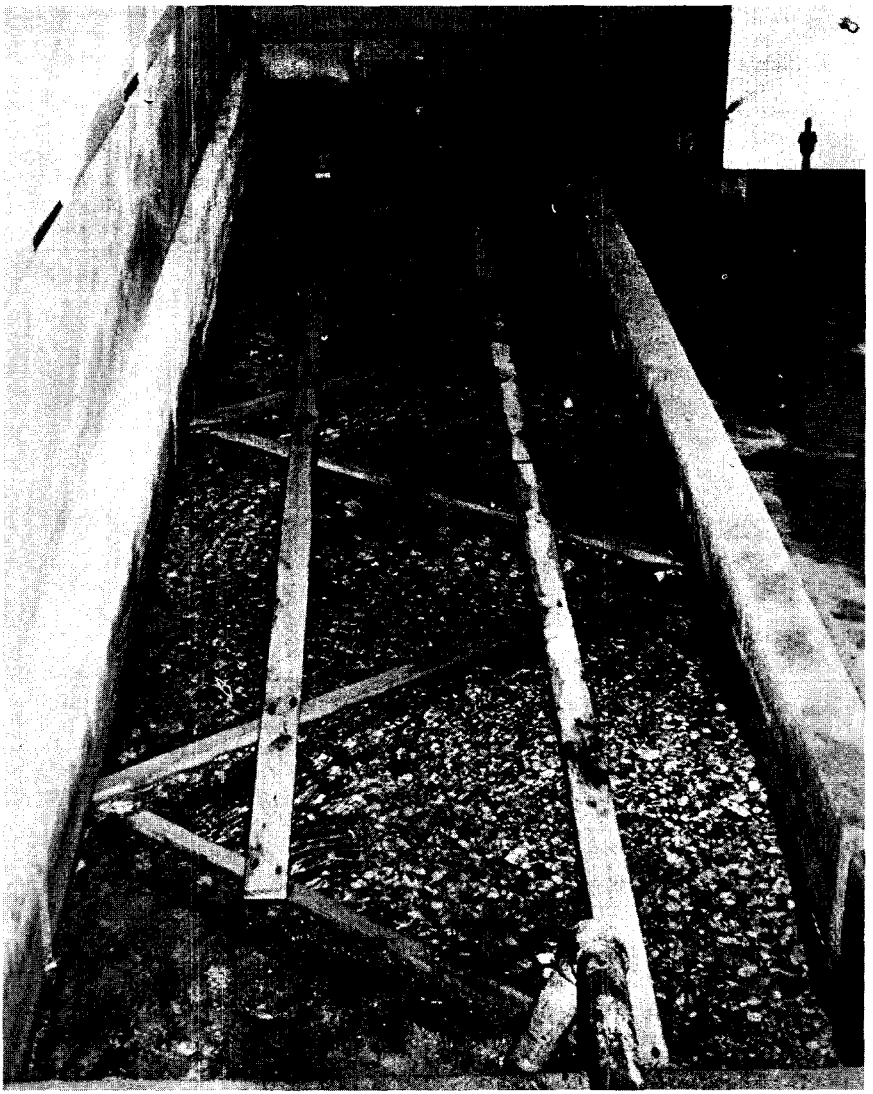

Figure 1. Artificial stream used for screening of potential chemosterilants at Hammond Bay Biological Station.

sterilizing activity against male houseflies, *Musca domestica* (Chang et al. 1970), and male pupae of the mosquito, *Anopheles albimanus* (Lofgren et al. 1973). The present study is the first known demonstration of the effectiveness of bisazir as a sterilant for fish.

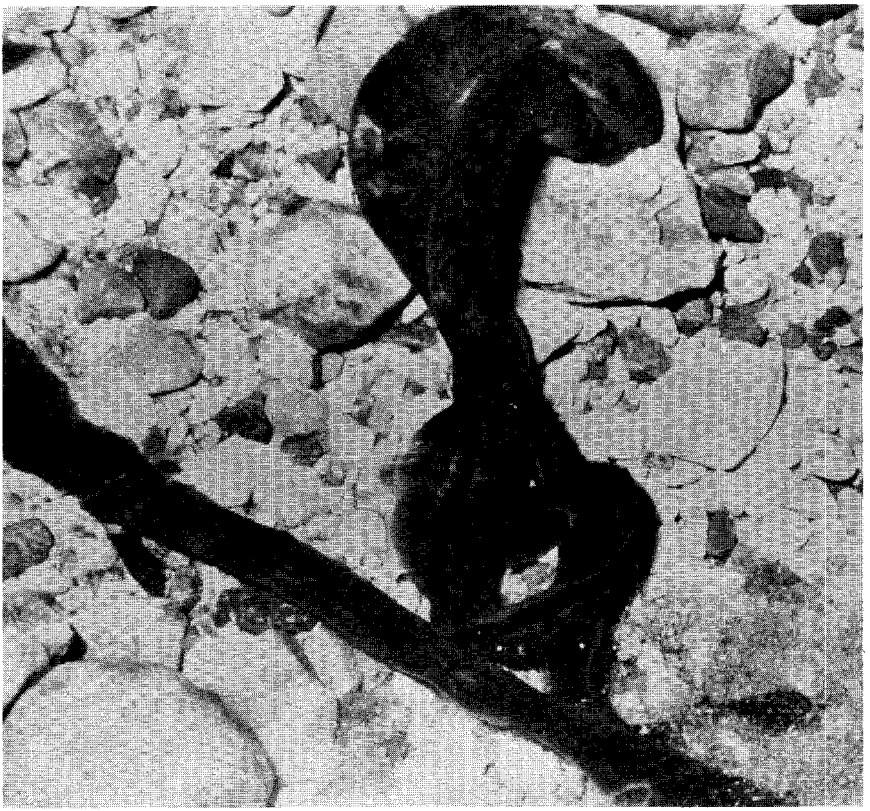

Figure 2. Sea lampreys spawning in the artificial stream shown in Fig. 1.

FIELD STUDY

The study site

The 8.9-km long stretch of the Big Garlic River (Marquette County, Michigan) selected as the site for the field study extended from a concrete dam 1 km above the mouth, upstream to Pat's Falls (Fig. 3). The dam blocked the upstream movement of fish and was the site of a modified inclined-plane trap that was installed to collect lampreys moving downstream (McLain and Manion 1967). The study area was especially well suited to our needs because it contains five natural waterfalls, each of which is a barrier to the upstream movement of lampreys and other fish. These natural barriers, and the man-made barrier at the lower end of the area, divided the study area into five sections where different experimental conditions could be established and tested. The sections were numbered from I (farthest downstream) to V

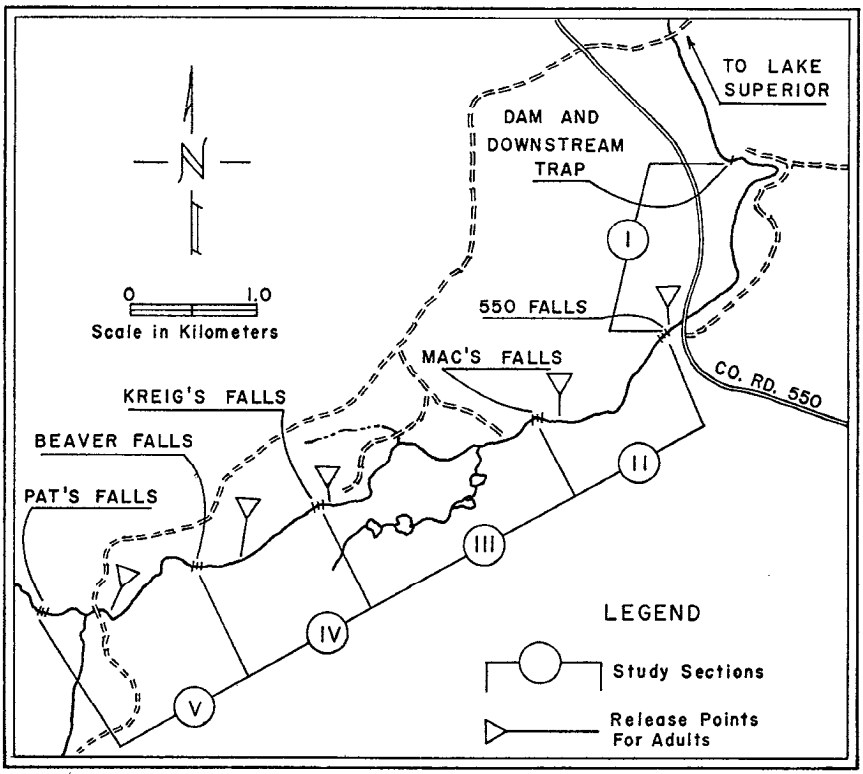

Figure 3. Study areas in the Big Garlic River, Marquette County, Michigan.

(farthest upstream). The limits, lengths, gradients, and bottom types of these sections were described by Manion and McLain (1971). Good spawning habitat is present in sections II-V, but is limited to the upper 200 m in section I. All sections have good larval habitat and are otherwise suitable for sea lamprey production (Manion and McLain 1971). To further segregate the lampreys in the five sections, we installed two fyke nets (132 cm wide by 66 cm high) near the downstream end of each of sections II, III, IV and V. These nets and the trap at the lower end of section I allowed us to monitor the downstream movement of adult lampreys and prevented many of them from leaving their area of release. Tagged, unspawned lampreys captured in the fyke nets and trap were returned upstream to their original release sites. The fyke nets were installed on June 1 and removed on July 15, 1974, after spawning had ceased; the inclined-plane trap was operated throughout the year.

A thermograph was installed at the downstream end of the study area. Water temperatures were also taken with a pocket thermometer throughout sections II to V during the spawning season.

Collection and release of lampreys

On May 21-23, 1974, about 1,000 spawning-run lampreys were collected by hand below a dam on the Manistique River, a tributary of Lake Michigan, and transferred to live cages in the Chocolay River near Marquette, Michigan.

On May 29-31, over 600 lampreys in the live cages were sexed according to their external characteristics (Vladykov 1949); 300 males and 300 females were then weighed individually to the nearest gram and tagged to permit individual identification. Average weights were 263 g (range 142400 g) for the females and 259 g (range 128396 g) for the males. Later observations of 270 tagged adults during spawning, when secondary sex characteristics were fully developed and unmistakable, showed that the original sex determinations were completely accurate. A Petersen tag, consisting of a white, numbered disc and a colored, unnumbered disc, was attached with a nickel pin, 45-mm long, inserted through the dorsal musculature just anterior to the dorsal fin. The color of the unnumbered disc identified the date and place of release, while its location on the body identified the sex of the individual (the colored disc was placed on the right side of the males and the left side of the females:). It also indicated whether the lamprey was to be sterilized with bisazir. A total of 300 tagged individuals (150 males and 150 females) were then sterilized with an intraperitoneal injection of bisazir (100 mg/kg) which had been dissolved in saline (9 g NaCl/1000 ml distilled water) to produce a 10,000 mg/l stock solution. A second group of tagged individuals (50 males and 50 females) was injected with saline (10 ml/kg) to determine mortality associated with injection and to serve as controls. The 600 tagged lampreys were held in the live cages for 72 hours to allow the sterilized lampreys sufficient time to recover (lampreys injected with bisazir appeared anesthetized for about 20 hours), and to determine tagging or injection mortality (none occurred).

On June 1-3, the tagged lampreys were transported to the Big Garlic River and released in the study area. The lampreys were released in groups at the head of each of the five sections as follows: section 1,50 normal males and 50 normal females, injected with saline only; section II, 50 sterilized females and 50 normal males; section III, 50 sterilized and 50 normal males and 50 sterilized and 50 normal females; section IV, 50 sterilized males and 50 normal females; and section V, 50 sterilized males and 50 sterilized females. The lampreys in section I served as a control group. The population in section III was chosen primarily to determine the effect of bisazir on the nest building activity, spawning behavior, and mating competitiveness of sterile animals. The populations in sections II, IV, and V were chosen to test the laboratory findings (that lampreys injected with bisazir are sterile) and to provide us with behavioral information as well.

Downstream movement

The lampreys moved about for nearly a week after they were released, as indicated by the fyke net and trap catches (28 lampreys were captured by June 8). Movement then ceased for 18 days and no lampreys were captured or observed anywhere in the stream until spawning began on June 26. However,

a flash flood on June 16 inundated the trap for 24 hours and prevented us from fishing the fyke nets for 3 days. We do not know how much movement between sections occurred during this period, but believe it was insignificant. During the spawning period (June 26 to July 8), 46 females and 20 males were captured. The greater catch of females is consistent with the observation that they moved about much more than the males during this period (the male usually started building the nest and was joined by a female after the nest was partly built). Lampreys collected toward the end of the spawning period or later were spent and dying or dead.

On the basis of fyke net catches, the number of sterile and normal lampreys that moved downstream from sections II, III, and IV (where both sterile and normal lampreys were present) was nearly identical. Before the onset of spawning (June 26), 8 sterilized lampreys (5 males, 3 females) and 8 normal lampreys (3 males, 5 females) were captured; thereafter, 14 sterile lampreys (2 males, 12 females) and 15 normal lampreys (6 males, 9 females) were captured. Thus, the compound had no apparent effect on lamprey movement. Information on the downstream movement of lampreys from sections I and V is not included, primarily because sterile and normal lampreys were not both present in each section, and therefore the information is not directly comparable. Sightings of lampreys in the stream were not used because many had lost their tags, and it was difficult to determine the tag numbers of the others without disturbing them.

Nest construction and spawning

After the lampreys were released, we surveyed the stream daily and marked each newly constructed nest with an aluminum-painted stone bearing a red number. Occupied nests were observed for 20 minutes each day and the tag color (and number, if possible), sex, physical condition, nest building activity, and spawning behavior of the nest occupants were recorded. Spawning began on June 26 and continued for 13 days, through July 8. Live adults were seen in the river until July 13. A spent, live female was captured in the dowstream trap on July 3 1.

The nest construction behavior of sterilized and normal lampreys appeared to be identical. Sterilized and normal males selected nest sites in typical spawning areas (riffles and gravel) and began construction of the nest. Usually within 24 hours, the male was joined by one or more females who assisted in the nest construction. Once spawning began, further nest building continued in the intervals between spawning acts. We observed no difference in the size or shape of nests constructed by sterile or normal lampreys. All nests were similar in appearance to those described by Applegate (1950).

The spawning acts of sterilized and normal lampreys observed during this study were judged to be identical and normal on the basis of descriptions by Gage (1928), Applegate (1950), and Manion and McLain (1971), and the experience of one of us (Manion) who had observed the spawning act performed on several hundred nests in the Big Garlic, Traverse, and Little Garlic rivers, Lake Superior from 1960 to 1973. Observations on 50 nests

revealed that the average spawning act lasted about 5 seconds (range 3-6 seconds) and was repeated an average of every 4-5 minutes among actively spawning pairs. The duration of spawning on a nest varied from 1 to 3 days and was similar to that observed in 1960 by Manion and McLain (1971).

Of the 102 observations of nesting lampreys, 57 were of monogamous pairings and 45 were of polygamous groups (one male and two females spawned in 32 nests, one male and three females in 8 nests, and one male each spawned with four, five, and six females in 3, 1, and 1 nests, respectively). No polyandrous nesting was observed, although we did observe males fighting for nests on numerous occasions (sterile males fought as vigorously as normal males and succeeded in driving off normal males as often as not). The 44% incidence of polygamous nesting in the Big Garlic River was much higher than the 9% observed by Manion and McLain (1971), or the 16% observed in the Ocqueoc River, Lake Huron, by Applegate (1950). The higher incidence in the present study was probably due to the high percentage (50) of females in the spawning population. This percentage was only 39 in the study reported by Manion and McLain (1971). (The sex ratio in the Ocqueoc River study by Applegate was not reported.) The apparent absence of polyandrous nesting in 1974 is not surprising; it is relatively rare among sea lampreys-usually from 1 to 5% (Applegate 1950; Manion and McLain 1971).

Autopsies of spent females indicated that sterilized females had laid a full complement of eggs. Unspawned eggs in 7 sterilized and 10 normal, spent lampreys averaged 0.7% and 1.2% respectively, as estimated from length-fecundity estimates for spawning females (Applegate 1950). These percentages (present study) are similar to the 2.2% average found in postspawning females by Manion and McLain (1971).

The relationship between water temperature and nest construction and spawning by the sea lamprey is not completely understood. In the present study, water temperature first reached 15 C on June 26, when spawning was first observed. From June 26 through July 8 (when spawning ended), water temperatures varied from 10.0 to 22.8 C (average 15.7 C) and were 1.7 C higher in the lower end of the study area then at the upper end.

Viability of eggs and development of embryos

After spawning was completed, we sampled the nests to determine the viability of the eggs and to follow the survival and development of the embryos. Sampling was scheduled on the basis of the time required for embryos to reach certain developmental stages in the laboratory (Piavis 1961) and also on the basis of the information (Manion and McLain 1971) that most larvae left the nests in the Big Garlic River within 22 days after fertilization. We sampled most of the nests three times (July 6-9, 13-15, and 23-25). Not all nests were sampled during each sampling period because some were not built until after the first sampling period, and some were overlooked. Larger samples were taken from the nests during the final sampling period than during the two earlier ones.

The net used to collect eggs and embryos from the nests consisted of a double layer of cheesecloth stretched between two lengths of 10-mm diameter

dowling. The net was placed below the downstream lip of the nest, and eggs and embryos were dislodged by inserting a spatula into the nest just anterior to the crest and thrusting upward. Most eggs and embryos were in that part of the nest, at a depth of 7 to 15 cm. Eggs and embryos carried into the net by the current were preserved in 4% formalin. They were later examined microscopically, and developing embryos were assigned developmental stages on the basis of the description by Piavis (1961). Disintegration of the embryo, the most obvious indication of death, was used to separate live from dead embryos.

Lampreys spawned in 95 nests: 4 in section I, 13 in II, 36 in III, 23 in IV, and 19 in V. In 1960, when 722 adults (282 females, 430 males) were released into sections II, III, and IV, lampreys spawned in 161 nests (Manion and McLain 1971). The smaller number of nests in 1974 than in 1960 was probably due to the smaller number of lampreys released (600 vs. 722), a much higher incidence of polygamous nesting (44% vs. 9%), and the poor spawning habitat in section I (which was not used in the 1960 study). Analysis of the samples of eggs and embryos taken from nests in each of these sections follows, and is summarized in Table 3.

Section I (50 males and 50 females, injected with saline only).-We found and sampled two nests on July 9, three on July 15, and four on July 25. The

Table 3. Development and survival of sea lamprey embryos in nests in sections I-V of the Big Garlic River, where groups of spawning sea lampreys were released June 1-3, 1974. Stages of development were described by Piavis (1961).

Section and date (July)		Total number nests sampled	Number of nests with live embryos	Eggs and embryos (all nests)		
				Average number per sample	Percentage live embryos per sample	Ranges in stages of development
I	9	2	2	66.0	81.8	10-13
	15	3	2	120.7	63.8	13-14
	25	4	4	319.5	88.0	13-16
II	9	10	9	90.8	61.9	5-13
	15	11	8	104.0	38.3	Y-15
	25	10	6	286.7	22.0	P-16
III	8	36	17	155.0	18.0	3-13
	14	34	14	98.2	17.6	Y-14
	24	36	15	223.7	19.8	12-17
IV	6	22	14	90.1	25.7	5-Y
	13	22	2	100.0	0.1	9
	23	21	1	237.2	< 0.1	12
V	6	12	5	86.3	34.0	2-10
	13	17	2	87.5	1.3	8-Y
	23	19	0	235.8	0.0	

average percentage of live embryos in all nests was 81.8, 63.8, and 88.0, respectively, during the three sampling periods. The sample from one nest on July 15 consisted of only dead embryos, suggesting (in view of our findings in other sections) that it had been taken from a nest in which a sterile individual from an upstream section had spawned. The final samples taken on July 25 showed that all our nests were highly successful-embryo development had progressed normally and hatching (stage 14) had occurred. The relative scarcity of nests in section I was probably due to the limited amount of suitable spawning habitat in the section (about 200 m of stream), as mentioned earlier.

Section II (50 normal males and 50 sterile females).-We found 13 nests in this section. The downstream movement of normal females and sterile males into this section prevented us from determining which nests were occupied solely by sterile females and normal males and confirming whether injected females were sterile. At least one normal pair of lampreys was observed in five of the nests at one time or another. Young lampreys were produced in these nests and three others. The average percentage of live embryos for each sampling period declined from 61.9% on July 9, to 22.0% on July 25.

Section III (50 sterile and 50 normal males, and 50 sterile and 50 normal females).-We found 36 nests in this section. No live embryos were found in 21 nests when last sampled on July 24. In several of the nests that had live embryos, production was much lower than would normally be expected. This was most likely caused by the spawning of either a normal male with both normal and sterilized females or by the replacement of a normal male by a sterilized male in the nest. The percentage of live embryos per sampling period ranged from 17.6% to 19.8%.

Section IV (50 sterile males and 50 normal females).-We located 23 nests in this section. Of 1,982 embryos examined from the July 6 samples, 510 (25.7%) were alive, and all live embryos were in developmental stages 5-9. During the second sampling period, only 3 of 2,199 embryos (0.1%) examined were alive. All three were at stage 9 (gastrula). During the third sampling period, only 1 of 4,981 embryos (< 0.1%) examined was alive. This embryo had reached stage 12 (head formation).

Section V (50 sterile males and 50 sterile females).-We found 19 nests in this section. Of 1,035 embryos examined during the first sampling period, 352 (34.0%) were alive (in developmental stages 2-10); of 1,487 examined during the second sampling period, only 19 (1.3%) were alive. These were in developmental stages 8 and 9. None of the 4,480 embryos examined during the third sampling period was alive.

Production of larvae

Final determinations for the presence or absence of young-of-the-year larvae in the study area were made on September 23-27 with a back-pack shocker similar to that used by Braem and Ebel (1961). Spot surveys for presence or absence were made in sections I-III, and most of the available

larval habitat (about 371 m") was surveyed in sections IV and V. Larvae were numerous near nesting sites in sections I, II, and III, where nesting between normal adults had taken place and where we had found nests containing viable embryos during the last sampling period. No larvae were found in section IV, where sterile males and normal females had been released and where sterile males and sterile females from section V were also found, nor in section V where only sterile males and sterile females were present.

SUMMARY AND CONCLUSIONS

The laboratory studies showed that bisazir sterilized both sexes when injected at the rate of 100 mg/kg into the body cavity of adult sea lampreys. Sterility was caused by a dominant lethal mutation in both the sperm and the egg, since fertilization took place and some development of the embryos occurred before death.

The field study confirmed that males injected with bisazir were sterilized. Sterility of the females could not be confirmed because normal females and sterile males moved downstream into section II of the study area. However, according to E. F. Knipling (personal communication), the effect obtained by destroying the females and sterilizing and releasing only the males is the same as that obtained by sterilizing and releasing both sexes. Because sex can be distinguished accurately, only males need to be sterilized to effect control of reproduction. This approach would reduce the number of lampreys to be injected and released.

The field study also showed that the injected chemosterilant had no noticeable effect on the nest building and spawning behavior of lampreys of either sex and did not destroy the mating competitiveness of the males. This is a basic requirement for successful use of the sterile-male-release technique.

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REFERENCES

APPLEGATE, V. C.
1950. Natural history of the sea lamprey *(Petromyzon marinus)* in Michigan. U.S. Fish Wildl. Serv., Spec. Sci. Rep. Fish. 55. 237 pp.

APPLEGATE, V. C., J. H. HOWELL, J. W. MOFFETT, B. G. H. JOHNSON, and M. A. SMITH
1961. Use of 3-trifluormethyl-4-nitrophenol as a selective sea lamprey larvicide. Great Lakes Fish. Comm., Tech. Rep. 1. 35 pp.

BORKOVEC, A. B.
1962. Sexual sterilization of insects by chemicals. Science 137 (3535):1034-1037.

BRAEM, R. A., and W. J. EBEL
1961. A back-pack shocker for collecting lamprey ammocetes. Prog. Fish-Cult. 23:87-91.
CHANG, S. C., C. W. WOODS, and A. B. BORKOVEC
1970. Sterilizing activity of bis(l-aziridinyl)phosphine oxides and sulfides in male house flies. J. Econ. Entomol. 63: 1744-1746.
GAGE, S. H.
1928. The lampreys of New York State-life history and economics. Pages 158-191 *in* Biological survey of the Oswego River system. N.Y. Conserv. Dep. Suppl. 17th Annu. Rep. (1927).
HANSON, L. H.
1970. Prospects for the integrated control of the sea lamprey. Report of Interim Meeting, December 1970. Great Lakes Fish. Comm., Ann Arbor, Appendix VI, 23 pp. (Mimeo.)
HOWELL, J. H., E. L. KING, JR., A. J. SMITH, and L. H. HANSON
1964. Synergism of 5,2'-dichloro-4'-nitrosalicylanilide and 3-trifluormethyl-4-nitrophenol in a selective lamprey larvicide. Great Lakes Fish. Comm., Tech. Rep. 8. 21 pp.
KNIPLING, E. F.
1960. The eradication of the screw-worm fly. Sci. Am. 203:54-61.
1964. The potential role of the sterility method for insect population control with special reference to combining this method with conventional methods. U.S. Dep. Agric. Res. Serv. ARS-33-98. 54 pp.
LOFGREN. C. S.. M. D. BOSTON. and A. B. BORKOVEC
1973. Aziridmylphosphine oxides and sulfides as chemosterilants in male pupae of *Anopheles albimanus* Wiedemann. Mosq. News 33: 187-189.
MANION, P. J.
1969. Evaluation of lamprey larvicides in the Big Garlic River and Saux Head Lake. J. Fish. Res. Board Can. 26:3077-3082.
MANION, P. J., and A. L. McLain
1971. Biology of larval sea lampreys *(Petromyzon marinus)* of the 1960 year class, isolated in the Big Garlic River, Michigan, 1960-65. Great Lakes Fish. Comm., Tech. Rep. 16. 35 pp.
MCLAIN, A. L. and P. J. MANION
1967. An all-season trap for downstream-migrating fish and other aquatic organisms. Prog. Fish-Cult. 29:114-117.
PIAVIS, G. W.
1961. Embryological stages in the sea lamprey and effects of temperature on development. U.S. Fish Wildl. Serv., Fish. Bull. 61:111-143.
SMITH, B. R., J. J. TIBBLES, and B. G. H. JOHNSON
1974. Control of the sea lamprey *(Petromyzon marinus)* in Lake Superior, 1953-70. Great Lakes Fish. Comm., Tech. Rep. 26. 60 pp.
VLADYKOV, V. D.
1949. Quebec lampreys. 1.-List of species and their economical importance. Dep. Fish., Province of Quebec, Contrib. 26. 67 pp.

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