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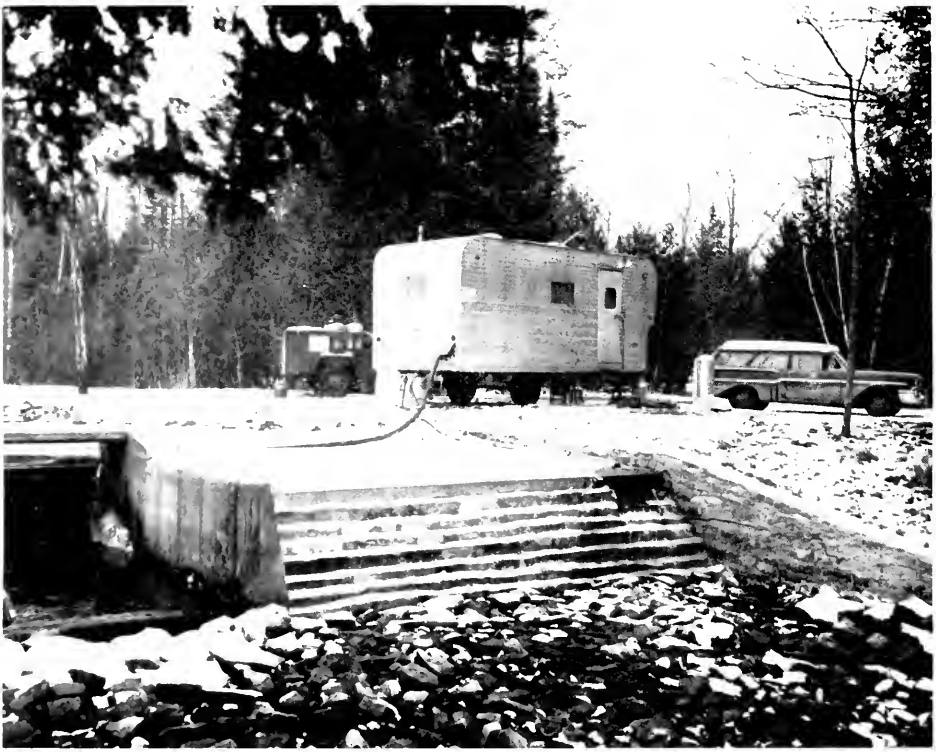

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ABSTRACT

The use of 3-trifluormethyl-4-nitrophenol as a selective toxicant to destroy sea lamprey larvae in streams tributary to the Great Lakes requires that a bioassay be conducted before each application. The wide geographic distribution of the streams requires further that the equipment to conduct the bioassays be mobile. The necessary facilities were installed in an 18-foot housetrailer shell. This equipment is described and the methods for its use are outlined. Selected data from bioassays in water from streams tributary to the U. S. shore of Lake Superior are presented and the significance of these data explained.

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INTRODUCTION

The use of selective larvicides in the control of sea lamprey in the Great Lakes began in 1958 when 11 streams tributary to Lake Superior were treated with 3-trifluormethyl-4-nitrophenol (TFM). This material was one of several discovered by the Bureau of Commercial Fisheries, under contract with the Great Lakes Fishery Commission, at the Hammond Bay Laboratory, Millersburg, Michigan, (Applegate, Howell, and Smith 1958; Applegate et al., 1961).

The early testing and application of TFM revealed that the degree of selectivity between lamprey larvae and stream fishes may vary from stream to stream and also with season in the same water. A determination must be made, therefore, prior to each application, of the minimum concentration of larvicide required to kill all lamprey larvae and the maximum concentration that can be used without causing significant mortality to game fishes of the streams.

The nearest bioassay facility for making these determinations in 1958 was at the Hammond Bay Laboratory, Millersburg, Michigan. Transportation of water from Lake Superior streams to this laboratory was costly and caused delay in obtaining results. Expansion of the chemical-treatment program to all Lake Superior tributaries infested with sea lamprey obviously required a faster method of obtaining results of bioassays. This need was met by installing bioassay equipment in a housetrailer which could be moved from stream to stream. The present report describes the equipment of this mobile laboratory, outlines bioassay procedures, and reviews uses of the results.

BIOASSAY FACILITIES

The equipment was installed in a standard 18-foot housetrailer. Fixtures furnished with the trailer shell were a 30,000-B.T.U. gas furnace, four overhead fluorescent lights, conveniently placed electric outlets, and two 10-inch exhaust fans in the ceiling.

A waterproof power-inlet fixture was mounted on the outside and connected to a fuse box on the inside wall. A voltmeter was placed in the input line to provide a constant check on voltage since a considerable amount of electrical equipment was installed.

Two water troughs (10 by 2 feet and 1 foot deep) of 20-gauge galvanized sheet steel, were framed with 2- by 4-inch boards and fastened to the trailer walls (fig. 1). The troughs and framing were further supported by a table of 2- by 12-inch boards with 4- by 4-inch wooden legs. A 2-inch thickness of fiberglass insulated the sides of the troughs to prevent condensation.

A centrifugal-type, water-circulating pump with 2-inch suction and 1-inch discharge and powered by a 1/2-horsepower electric motor was mounted in the laboratory to pump water directly from the stream to the trailer through a rubber hose. An intake basket covered with fine-mesh screening filtered debris from the incoming water. This basket contained a check valve to prevent loss of water from the hose when the pump was not running.

Water was piped to the troughs by 1/2-inch copper tubing. A shutoff valve was provided for each trough. Standpipes, 2 inches in diameter and 7 inches high, at the end opposite the water source controlled water depth. The standpipes were connected to a drain beneath and at the rear of the trailer. A hose attached to this drain returned overflow water to the stream. Valves were installed at low points in the water system to permit complete drainage when the laboratory was not in use.

The troughs contained space for 22 individual test containers each, or a total of 44 in the two. Each container was provided with a controllable supply of compressed air to maintain oxygen concentrations in the test solutions at or near saturation.

Air for the test jars was furnished by two tank-mounted, air-cooled, single-stage compressors, powered by 1/3-horsepower motors. The primary supply lines from the compressors were interconnected. In the event one compressor failed, the opening of a stopcock allowed the remaining compressor to furnish air to all test jars. Air was distributed to the individual

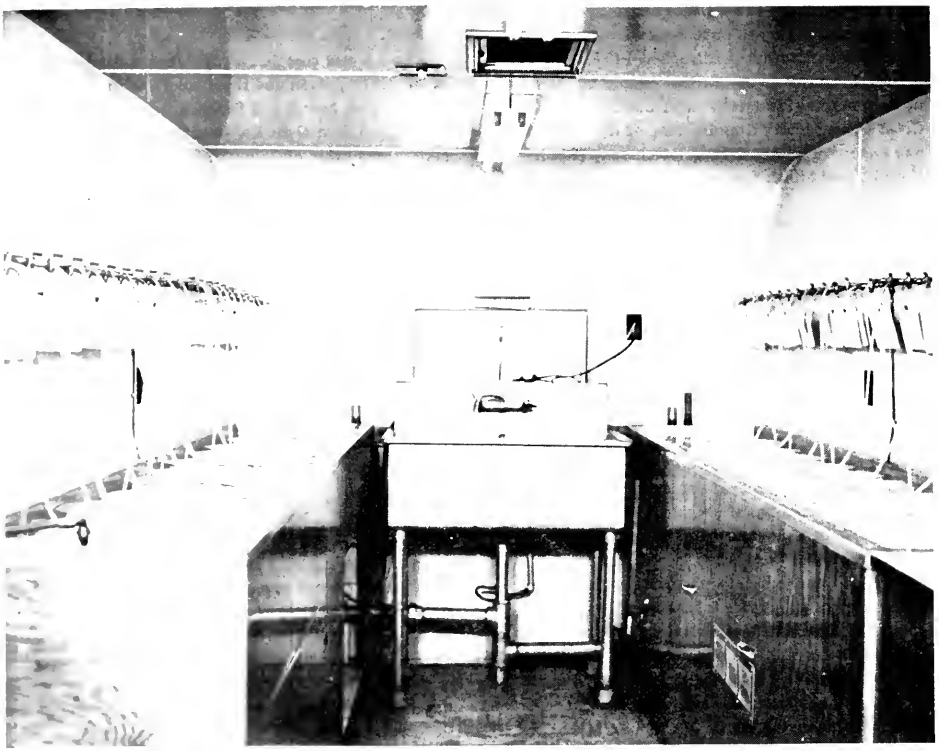

Figure 1. --Interior view of bioassay trailer showing both test troughs.

containers by 1/4-inch copper tubing. Air pressure in the distribution lines was controlled by an adjustable diaphragm-type regulator. An outlet, with a brass stopcock, was provided for each test jar. Vinyl plastic tubing in 34-inch lengths and equipped with stone aerators conveyed air to the test containers.

The electrical power for the bioassay trailer was furnished by a 5-K.V.A., 115-volt generator mounted on the bed of a 4- by 8-foot steel trailer (fig. 2). Six 100-pound tanks of propane carried on the same trailer provided fuel for the generator. The generator trailer also provided transportation for intake and discharge hoses used in the water system and eight 10-gallon milk cans for holding larvae and fish.

BIOASSAY PROCEDURE

Groups of four tests were run at each concentration tested. Thus the 44 places provided for bioassay at 11 different concentrations. Test jars were 7.5-liter polyethylene containers. Use of this type of jar prevented breakage, facilitated storage, and eliminated the problem of keeping glassware clean during field operations. Disposable polyethylene plastic bags were used as liners to prevent chemical contamination of the containers (fig. 3). Five liters of the stream water to be tested were placed in each test jar. The upper portion of the polyethylene bag was then folded over the edge and down the outside. Test concentrations were obtained by adding a 1:200 stock solution of TFM, based on weight of active ingredient, in distilled water. One milliliter of the stock was added to the 5 liters of stream water for each part per million (p.p.m.) desired in the test.

Water circulating through the troughs kept the test solutions near the temperature of the stream. Thus the bioassay information was obtained under the same varying water temperatures that would normally occur during a stream treatment. A single-pen recording thermograph provided a continuous record of water temperatures during a bioassay.

Test positions were numbered consecutively from 1 to 44; the odd-numbered positions were along the near sides of the troughs and the even-numbered positions along the wall. Each bioassay observation card was stamped with the same identifying number as the test container.

TEST ANIMALS

Lamprey larvae, 2 to 4 inches long, and fingerling rainbow trout of approximately the same size proved to be well suited to the test containers and to the volume of test solution. Normally, two trout and two larvae were placed in each jar; since the tests were in quadruplicate, eight animals of each species were subjected to each level of concentration. Occasionally, when supplies of test animals were abundant, three of each species were placed in a container. Conversely, when test specimens were in short supply, only one of each species was used.

Lamprey larvae were obtained from nearby streams with a light portable electric shocker (Braem and Ebel, 1961). Larvae of the various lamprey species in waters tributary to the U. S. shore of Lake Superior, including those in process of transformation into the adult stage, were used in bioassays. We have no evidence that species of lamprey exhibit substantially different susceptability to the larvicide.

Holding facilities for larvae consisted of fine-mesh screen cages with 3 to 6 inches of fine sand or silt in the bottom to permit the larvae to bury themselves. These holding cages were placed in the stream. Larvae could be collected at one time for four or five bioassays and retained in good condition until needed. Lamprey larvae obtained by shocking were allowed to rest at least 24 hours before they were used as test animals.

The rainbow trout were provided by the States of Michigan and Wisconsin. They were usually held in the hatchery until needed for bioassay. Small rainbow trout could be held in a live car in the stream for 4 or 5 days, however, without impairing their value as test animals. The fish were not fed during this time, and they remained vigorous and healthy if not too closely confined.

Test animals that were transferred from one water to another were tempered for one-half hour for each 5° F. difference in water temperatures. The test specimens and air hoses were placed in each test jar prior to the introduction of the chemical. All jars were covered by a sheet of clear glass to prevent the escape of fish and the accidental pollution of test solutions by foreign substances. Fish and larvae were left undisturbed 1 hour to permit them to adapt to the test environment.

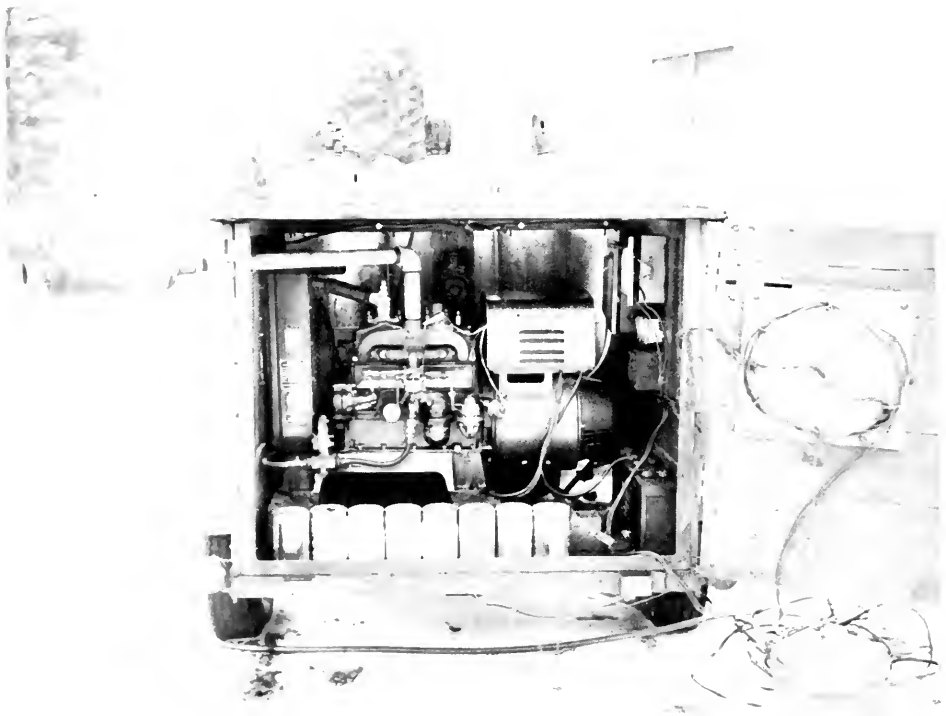

Figure 2. --Power trailer that provides electricity to operate mobile bioassay laboratory.

Figure 3. --Test containers used for bioassay.

TABULATION AND USE OF DATA FROM BIOASSAYS

Hourly observations of test specimens were recorded on the test card for each jar (fig. 4) until the pattern of mortality was well established--usually a period of 5 to 12 hours. Animals still alive were then observed at more extended intervals until they had been exposed to the chemical for approximately 20 hours.

The combined data from the four replications at each concentration were tabulated at the completion of a bioassay to indicate mortality at each observation period. An example of this tabulation for a bioassay in water from the West Branch of the Ontonagon River, Ontonagon County, Michigan, may be found in figure 5, which is adapted from a standard field form. The purpose for which the bioassay information was used was met adequately by records according to arithmetic dosage intervals and observation periods.

Bioassays were designed primarily to provide data from which two levels of concentration--minimum lethal and maximum allowable--could be determined. For our purposes the minimum lethal value was considered to be the lowest concentration in any series producing 100-percent mortality among larval lampreys. The maximum allowable was the highest concentration which did not kill more than 25 percent of the other test species, rainbow trout. These two levels define the range within which concentrations must be maintained in all parts of a stream during treatment.

The difference between the maximum allowable and minimum lethal concentrations is the working range. For a stream to be treated successfully, a working range clearly is required. The most significant figure obtained from a bioassay is not, however, the range itself but the ratio of the working range to the minimum lethal concentration. This ratio indicates the amount by which volume of flow may increase in a stream treated at the maximum allowable concentration before the concentration will reach the minimum lethal level. The importance of the ratio is best illustrated by the examples of table 1 in which various maximum and minimum concentrations are assumed to have been obtained from bioassays. The working ranges are identical in the four hypothetical bioassays, but the practical problems of stream treatment increase progressively from stream A to stream D.

If the original application in stream A is at the maximum allowable concentration of 5 p. p. m., the treated water moving downstream is extremely tolerant of dilution from streambed springs and the inflow of untreated tributaries; additional flow of 4 cubic feet per second (c. f. s.) is permissible for each c. f. s. at the treatment point. Furthermore, treatment in this stream is highly resistant to "washout" by a sudden, unexpected rain.

The increase of the maximum allowable and minimum lethal concentration by a mere 1 p. p. m. (stream B) cuts the permissible additional flow in half--from 4 to 2. This flow is halved once more at concentration limits of 8 and 4 p. p. m. (stream C) and still again at 12 and 8 p. p. m. (stream D).

Table 1. --Hypothetical results of bioassays in four streams to illustrate significance of ratio of working range to minimum lethal concentration

Stream	Concentration (p. p. m.)		Working range (p. p. m.)	Permissible additional flow 1/ (c. f. s.)
	Maximum allowable	Minimum lethal		
A	5	1	4	4.0
B	6	2	4	2.0
C	8	4	4	1.0
D	12	8	4	0.5

1/ Per 1 c. f. s. of flow at point of treatment at the maximum allowable concentration.

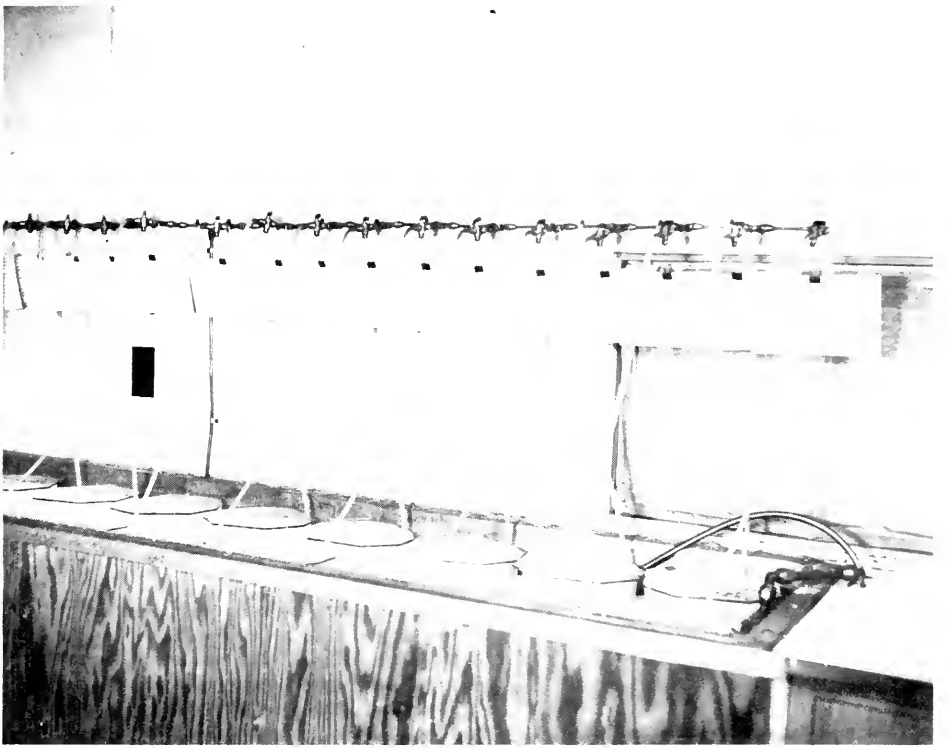

Figure 4. --Test trough in operation, showing air lines and test cards for each container.

Stream: West Branch Ontonagon River Date July 19, 1960

County: Ontonagon State: Michigan

Material Tested: 3-trifluormethyl-4-nitrophenol

Time to death for rainbow trout and lamprey larvae expressed as cumulative totals up to 19 hours

Concentration (p. p. m.)	1st hour	2d hour	3d hour	4th hour	5th hour	6th hour	8th hour	19th hour	Total mortality
				Lamprey larvae					
1.0	...	...	...	...	...	...	...	...	...
1.5	...	...	...	...	...	...	2	3	3
2.0	...	...	1	1	2	3	8	12	12
2.5	...	...	3	8	10	12	...	...	12
3.0	...	...	9	10	12	...	...	...	12
4.0	...	4	11	12	...	...	...	...	12
4.5	...	5	10	12	...	...	...	...	12
5.0	...	7	12	...	...	...	...	...	12
6.0	...	10	12	...	...	...	...	...	12
7.0	...	11	12	...	...	...	...	...	12
8.0	...	12	...	...	...	...	...	...	12
				Rainbow trout					
1.0	...	...	...	...	...	...	...	...	...
1.5	...	...	...	...	...	...	...	...	...
2.0	...	...	...	...	...	...	...	...	...
2.5	...	...	...	...	...	...	...	...	...
3.0	...	...	...	...	...	...	...	1	1
4.0	...	...	...	...	...	...	...	1	1
4.5	...	...	...	...	...	...	...	...	...
5.0	...	...	1	1	1	1	2	3	3
6.0	...	2	2	2	2	2	3	6	6
7.0	1	2	2	2	2	4	4	6	6
8.0	...	4	5	6	7	8	9	10	10

Figure 5. --Method of tabulation for indicating mortality occurring at each observation period.

Table 2. --Results of bioassays in water from the Betsy River (Chippewa County, Michigan) and the Sucker River (Alger County, Michigan)

River and date	Minimum lethal concentration (p. p. m.)	Maximum allowable concentration (p. p. m.)	Working range (p. p. m.)	Permissible additional flow (c. f. s)
Betsy River				
July 24, 1959	2.0	2.0	0.0	0.0
July 31, 1959	2.0	4.0	2.0	1.0
Sept. 9, 1959	1.5	5.0	3.5	2.3
May 29, 1960	1.0	3.0	2.0	2.0
Sucker River				
Oct. 6, 1958	2.0	7.0	5.0	2.5
July 29, 1959	3.0	9.0	6.0	2.0
Aug. 1, 1959	2.0	9.0	7.0	3.5

The treatment of stream B normally would offer no difficult operational problem and treatment of stream C usually could be completed successfully. The permissible additional flow in stream C is, however, the lowest that is normally considered acceptable. If treatment at a value of less than 1.0 c. f. s. of additional permissible flow cannot be avoided, extreme care must be exercised to insure that concentrations in the stream remain within the working range.

The degree to which activity, and with it the working range and permissible additional flow, may vary from time to time in a particular stream is illustrated by the actual records for two Lake Superior tributaries (table 2). The bioassays on the Betsy River produced working ranges from 0 to 3.5 p. p. m. and additional permissible flows of 0.0 to 2.3 c. f. s. This variability of results sometimes makes it necessary to complete a number of bioassays in a stream to determine optimum conditions and time for stream treatment. In the Sucker River (Alger County, Michigan), on the contrary, the bioassay tests consistently indicated favorable conditions for treatment.

ACKNOWLEDGMENTS

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LITERATURE CITED

Applegate, Vernon C., John H. Howell, James W. Moffett, B. G. H. Johnson, and Manning A. Smith
1961. Use of 3-trifluormethyl-4-nitrophenol as a selective sea lamprey larvicide. Great Lakes Fishery Commission, Technical Report No. 1, 35 p.

Applegate, Vernon C., John H. Howell, and Manning A. Smith
1958. Use of mononitrophenols containing halogens as selective sea lamprey larvicides. Science, vol. 127, no. 3294, p. 336-338.

Braem, Robert A., and Wesley J. Ebel
1961. A back-pack shocker for collecting lamprey ammocetes. Progressive Fish-Culturist, vol. 23, no. 2, p. 87-91.

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