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Great Lakes Fishery Commission

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by

PATRICK J. MANION
U.S. Fish and Wildlife Service
Federal Building, Fort Snelling
Twin Cities, Minnesota 55111

LEE H. HANSON
U.S. Fish and Wildlife Service
Hammond Bay Biological Station
Millersburg, Michigan 49759

MICHAEL F. FODALE
U.S. Fish and Wildlife Service
Marquette Biological Station
Marquette, Michigan 49855

RELATION OF pH TO TOXICITY OF LAMPRICIDE TFM IN THE LABORATORY

by

T. D. BILLS, L. L. MARKING, G. E. HOWE, and J. J. RACH
U.S. Fish and Wildlife Service
National Fisheries Research Center
P.O. Box 818
La Crosse, Wisconsin 54602-08 18

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STERILIZING EFFECT OF CESIUM-137 IRRADIATION ON MALE SEA LAMPREYS RELEASED IN THE BIG GARLIC RIVER, MICHIGAN [1]

by Patrick J. Manion, Lee H. Hanson, and Michael F. Fodale

ABSTRACT

A total of 300 spawning-run sea lampreys *(Petromyzon marinus)* were released in the Big Garlic River, Marquette County, Michigan, June 6, 1984, to determine the effect of cesium-137 irradiation on the nesting and spawning behavior of males and on the viability of eggs. We released 100 irradiated males, 100 normal males, and 100 normal females. The lampreys constructed 63 nests in which they spawned successfully. Irradiated males showed no abnormal nest building or spawning behavior and competed effectively with normal males for females. The survival of eggs in nests in which irradiated males spawned with females was higher than expected (38% alive). Survival was lower (20%) in nests of early spawners, than in those nests of late spawners (62%); early spawners may have been more mature than late spawners at the time of irradiation. We concluded that gonads in spawning-run sea lampreys must be in advanced stages of development if irradiation is to be an effective sterilant.

INTRODUCTION

The use of selective toxicants in the Great Lakes (Applegate et al. 1961; Howell et al. 1964; Manion 1969) has resulted in the successful control of the sea lamprey *(Petromyzon marinus).* Populations in Lake Superior have been reduced by about 90% from precontrol levels (Smith et al. 1974), and reductions are believed to have been similar in Lakes Michigan, Huron, and Ontario. The sea lamprey populations can be reduced further, however, only by treating major sea lamprey producing streams more frequently, as suggested by Smith et al. (1974), or by developing other methods of control to remove remnant sea lamprey populations that survive chemical treatments.

Hanson and Manion (1978, 1980) demonstrated that it would be feasible to use the technique of releasing sterile males to control sea lampreys. They used the chemosterilant P, P-bis (1-aziridinyl)-N-methylphosphinothioic amide (bisazir) to sterilize males and reduce the number of viable larvae produced in a stream. Although bisazir effectively sterilizes males and has no noticeable effect on their nest building activities, spawning behavior, or mating competitiveness, it is also a mutagenic compound and care must be taken during treatment of the lampreys to prevent accidental exposure of personnel to the chemical.

[1]This study was part of a program conducted by the U.S. Fish and Wildlife Service under contract with the Great Lakes Fishery Commission.

Laboratory studies conducted at the Hammond Bay (Michigan) Biological Station indicated that irradiation has potential for sterilizing male sea lampreys (Annual Report of Great Lakes Fishery Commission 1984). On the basis of these findings, we conducted a field study in which normal and irradiated lampreys were released in a known sea lamprey producing stream, and observed them under natural conditions to determine the effect of irradiation on the nest building and spawning behavior of males and on the viability of eggs.

STUDY SITE

The Big Garlic River, Marquette County, Michigan, was used previously as a site for studies of sea lampreys (Manion and McLain 1971; Hanson and Manion 1978, 1980). The stream is small (average flow 0.4 $m^3/_s$), has clear water that facilitates the observation of spawning lampreys, and has excellent spawning and larval habitats.

The section of river used in this study extends from Mac's Falls 2.5 km upstream to Kreigs Falls. A temporary downstream trap was constructed at the lower end of the study area. Two fyke nets (132 cm wide by 66 cm high) placed below the trap fished about 90% of the stream volume during periods of normal flow. The trap and fyke nets allowed us to monitor downstream movement of stocked adult lampreys and prevented most of them from leaving the study area. A thermograph and staff gauge in the stream monitored water temperature and level.

COLLECTION AND RELEASE OF LAMPREYS

Spawning-run sea lampreys, collected from a trap in the Cheboygan River (a tributary of Lake Huron) on May 18, 1984, were transferred to the Hammond Bay Biological Station, where they were weighed, sexed according to external characteristics (Vladykov 1949), and marked with a V-shaped notch in the dorsal fin. Males to be irradiated were marked on the posterior dorsal and normal males on the anterior dorsal. Females were not marked. Average weights were 218 g (range 134-352 g) for normal males, 218 g (range 118-318 g) for irradiated males, and 213 g (range 98-330 g) for females. Males to be irradiated were transported to Wayne State University, Detroit, Michigan, where they were exposed to cesium-137 irradiation from a Gammacell- irradiator on May 22. A plastic bag was placed in a sample tray and about three liters of water were added to the bag. Ten lampreys were placed in the water and water depth was adjusted to 7.6 cm to cover the lampreys. Water temperature was 11°C; no aeration was provided during exposure. Lampreys were irradiated at a rate of 115 rads/min (total irradiation time 17.4 min) to provide a total dose of 2000 rads. After irradiation, the lampreys were returned to the Hammond Bay Biological Station and placed in a concrete tank supplied with running water. On June 6, 300 adult lampreys-100 each of irradiated males, normal males, and normal

females-were transported to the Big Garlic River (which was known to be free of other sea lampreys) and released at the head of the study area.

DOWNSTREAM MOVEMENT

The trap and fyke nets were usually checked daily. Unspawned lampreys captured alive were returned upstream to the original release point until July 1; thereafter they were released in the middle of the study area. None were returned upstream after July 6 because many were spent, dying, or dead. Downstream movement was greatest during the first week after release (June 7-13), when 91 were captured in the trap (64 on the first day), and 1 was taken in a fyke net. After the first week, downstream movement virtually ceased until spawning began and only six adults were captured from June 14 to 22. No escapement from the trap was noted until June 27, when it was breeched during a flash flood. Examination of areas below the study site revealed one sterile male and one small nest, which contained no eggs. A permanent inclined-plane trap (McLain and Manion 1967), 3.9 km below the study area, captured eight adults (six after July 5). All were dead and none were spent.

The numbers of irradiated males and normal males and females that were captured downstream before the onset of spawning were similar: 35 irradiated males, 34 normal males, and 29 females. During and after the spawning period 21 irradiated males, 13 normal males, and 3 1 females were taken. The higher catch of females was consistent with previous observations that females move about actively during this period, perhaps in search of males (Hanson and Manion 1978).

NEST CONSTRUCTION AND SPAWNING

After the release of lampreys, the stream was surveyed daily and each occupied nest was marked with a stone painted an aluminum color and bearing a red number. Occupied nests were checked each day and the mark, sex, nest building activity, and spawning behavior of the occupants were recorded. Spawning began on June 22 and continued through July 7.

The nest building behavior of irradiated and normal males appeared to be identical, and similar to that observed by Manion and McLain (197 1) and Hanson and Manion (1978, 1980). We detected no differences in the size or shape of nests constructed by irradiated and normal lampreys.

Spawning acts of irradiated and normal males were similar and normal as judged by published descriptions by Gage (1928) and Applegate (1950), and previous observations by Manion and McLain (1971) and Hanson and Manion (1978, 1980).

During the nest building and spawning period (June 22-July 7), 73 observations made of nesting lampreys showed irradiated males with females on 37 nests and normal males with females on 36 nests. Counts of random lampreys

(not on nests) on July 3-6 showed 36 irradiated and 37 normal males. The ratio of irradiated to normal males on nests and moving at random showed the two equal groups were undifferentiated as to sexual performance. Although the percentage of irradiated and normal males seen on nests was almost identical, irradiated males occupied previously constructed nests about twice as often as normal males (19% and 9%, respectively).

Of the 73 observations of nesting lampreys, 66 (90%) were monogamous and 7 (10%) were polygamous (1 male with 2 females on six nests and 1 male with 3 females on one nest). The rate of polygamous nesting was almost identical to that found by Manion and McLain (1971), who released 39% females, compared with 33% in the present study. No polyandrous nesting was seen. Males were seen fighting for nests on five occasions. Irradiated and normal males replaced each other about equally. Water temperatures during the first occupancy by a male through the spawning period ranged from 9-19°C (average 15°C). When spawning began on June 22, water temperature had reached 17°C.

DEVELOPMENT OF EMBRYOS AND EVACUATION OF NESTS

On July 9 and 10 after spawning was completed, all nests were examined for the presence or absence of eggs and to determine embryonic development. Eggs were found in 63 nests and small samples were taken according to the method described by Hanson and Manion (1978) and preserved in 5% formalin. Embryos were examined microscopically and assigned to the developmental stages described by Piavis (1961). This information, along with observations on spawning, was used to determine when the nests were to be evacuated.

The device used to collect eggs and prolarvae from the nests and the method of dismantling nests were similar to that described by Manion (1968). Nests containing eggs were dismantled and eggs and prolarvae were removed about 18 days after deposition. This period allowed full development of the embryos, but was shorter than the time required for the prolarvae to develop to a stage at which they would be able to leave the nest. About 85% of the live embryos or prolarvae collected were in stages 13 (prehatching), 14 (hatching) or 15 (pigmentation)-Piavis (1961) - indicating that eggs were removed from most nests at the appropriate time. Many nests were so close together that they overlapped and were termed multiple nests. Of the 63 successful nests, 30 were single, 11 were multiple, and the status of the remaining 22 was uncertain. Because multiple nests could not be separated with any accuracy, each was evaluated as a single unit.

Eggs collected from each nest were preserved in 5% formalin and later subsampled with a Folsom plankton splitter. Each sample was divided successively in half until 1/16th of the eggs remained. To determine the numerical accuracy of the splitter, two 1/16th subsamples were compared from each of 24 nests. The difference between these paired subsamples averaged 5.8% (range 1-14%). The 1/16th subsample from each nest was examined microscopically to

TABLE 1. Survival of eggs found in 63 nests in the Big Garlic River where spawning lampreys were released June 6, 1984.

Type of occupancy	Number of nests	Eggs			
		Dead	Alive	Total	% Alive
Normal males	21	4 670	9 240	13 910	66
Irradiated males	16	6 135	3 668	9 803	38
Both normal and irradiated males	4	2 392	2446	4 838	51
No spawning observed	22	5 109	11031	16 140	68
Total	63	18 306	26 385	44 691	59

determine mortality and assign the living eggs and prolarvae to a developmental stage.

NESTING OBSERVATIONS AND ESTIMATED PRODUCTION OF EGGS AND PROLARVAE

Spawning lampreys were observed daily. Among the 30 single nests that were successful, normal males were seen spawning on 20 and irradiated males on 10. Among the 11 multiple nests that were successful, 1 was spawned by a normal male, 6 by irradiated males, 2 by one normal and one irradiated male, and 2 by one normal and two irradiated males. Among the successful nests not known to be single or multiple, either no lampreys or single lampreys were observed.

We examined 44,691 eggs from the 63 successful nests (Table 1). Of the 13,910 eggs from 21 nests (20 single, 1 multiple) where only normal males were seen spawning, 9,240 or 66% (range 4-93%) were alive. The survival of eggs was somewhat lower in 16 nests (10 single, 6 multiple) where only irradiated males were seen spawning. Of the 9,803 eggs examined, 3,668 or 38% (range 0-93%) were alive.

Our observations of irradiated males on nests in the Big Garlic River differed widely from those reported by Hanson and Manion (1980), who studied the results of pairings of chemosterilized males with normal females on 44 nests: 38 (86%) of their nests contained only dead eggs and the other 6 contained only a few developing embryos. The degree of sterilization in the present study was obviously far less. For the 16 nests in which only irradiated males were seen spawning, all eggs were dead in only 1, and more than 80% were dead in only 10 (63%). Survival was lower in nests occupied by irradiated males during the first half of the spawning season than during the second half. Based on nests dismantled from July 9-17th (early spawners) and nests from July 18-27th (later spawners) survival was 20 and 62% respectively. Since the most mature lampreys spawn first, the effectiveness of cesium-137 irradiation may depend on the maturity of a lamprey at the time of treatment.

DISCUSSION

Inasmuch as irradiated and normal males were released in the study area at a ratio of 1:1, nearly 50% of the spawning observed should have involved irradiated males, and the total mortality of eggs should have been about 50% if irradiated males were completely sterile and no natural mortality occurred.

Nest samplings showed that 18,306 (41%) of the 44,691 eggs examined were dead. Although normal natural mortality is unknown, it is believed to be about 15% (Hanson and Manion 1980). The application of a 15% mortality (6,704) would reduce the number killed by irradiation to 11,602 eggs and the reduction in reproductive potential due to irradiation would be about 3 1% rather than the expected 50%.

The higher than expected survival is attributed to the probability that not all irradiated males were effectively sterilized. This study indicated that male sea lampreys irradiated in early stages of maturity were not as effectively sterilized as were the more mature animals. This shortcoming may limit the use of irradiation unless the more immature males can be held for a period of time before treatment to allow their testes to develop to a more sensitive stage.

The field study showed that irradiation had no noticeable effect on nest building or spawning behavior of males and that irradiated males competed effectively with normal males for nesting sites and for females. It also demonstrated that the release of irradiated males into a stream reduced the number of prolarvae produced.

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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.

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., AND P. J. MANION. 1978. Chemosterilization of the sea lamprey *(Petromyzon marinus).* Great Lakes Fish. Comm. Tech. Rep. 29. *15* PP.

HANSON, L. H., AND P. J. MANION. 1980. Sterility method of pest control and

its potential role in an integrated sea lamprey *(Petromyzon marinus)* control program. Can. J. Fish. Aquat. Sci. 37: 2108-2117.

HOWELL, J. H., E. L. KING, JR., A. J. SMITH, AND L. H. HANSON. 1964. Synergism of 5,2'-dichloro-4'-nitro-salicylanilide and 3-trifluormethyl-4-nitrophenol in a selective lamprey larvicide. Great Lakes Fish. Comm. Tech. Rep. 8. 21 pp.

MANION, P. J. 1968. Production of sea lamprey larvae from nests in *two* Lake Superior streams. Trans. Amer. Fish. Soc. 97:484-486.

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-l 17.

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.

RELATION OF pH TO TOXICITY OF LAMPRICIDE TFM IN THE LABORATORY

by T. D. Bills, L. L. Marking, G. E. Howe, and J. J. Rach

ABSTRACT

In the control of larval sea lamprey with 3-trifluoromethyl-4-nitrophenol (TFM) in tributaries of the Great Lakes, occasional kills of other fishes have caused concern about the effects of the chemical on non-target organisms. Stream treatment rates have been based on previous application rates, alkalinity measurements, results of on-site toxicity tests, or combinations of these. However, our laboratory studies in 1987 showed that pH is the primary factor that affects the toxicity of TFM (the lower the pH, the greater the toxicity): even small changes in pH alter the toxicity, whereas substantial changes in alkalinity have little effect. In 12-h exposures, the 96-h LC50 for TFM to rainbow trout (Salmo gairdneri) ranged from about 0.9 mg/L at pH 6.5 to > 100 mg/L at pH 9.5, but (at pH 7.5) the LC50's differed little at total alkalinities of about 18 mg/L and 207 mg/L. Decreases in pH as small as 0.5 pH unit caused nontoxic solutions to become toxic to rainbow trout. Some kills of non-target fish during stream treatments were reportedly caused by decreases in pH, and (conversely) that some stream treatments for sea lampreys were ineffective because pH increased.

INTRODUCTION

The lampricide 3-trifluoromethyl-4-nitrophenol (TFM) is used extensively in tributaries of the Great Lakes to selectively kill larval sea lampreys (Petromyzon marinus) in the presence of other fishes (Applegate et al. 1958). The toxicity of TFM has long been known to be influenced by chemical and physical properties of water; as pH, conductivity, and alkalinity of the water increased, additional amounts of TFM were required to kill the larvae (Applegate et al. 1961). Consequently, the required amount of TFM varied from stream to stream and season to season. Howell and Marquette (1962) reported that stream side bioassays were useful for predicting the concentration that was lethal to larval sea lamprey and safe for nontarget fishes. Kanayama (1963) initially correlated the toxicity of TFM with the alkalinity and conductivity of stream waters and projected a curve for estimating effective treatment rates for field applications. Seelye et al. (1988) also developed a regression equation for estimating concentrations of TFM and Bayer 73 for stream treatments under a broad range of alkalinities. The primary emphasis of both studies relies on alkalinity as the primary factor that influences the toxicity of TFM to aquatic **organisms**

Laboratory studies showed that residues of TFM in fish tissues are

negatively correlated with the dissociation of the molecule in water at different pH's. For example, when fish were exposed to a 1 mg/L solution of TFM for 12 h at pH's 6, 7, 8, or 9, the TFM residues in muscle were 3.21, 1.5, 0.33, and 0.03 μg/g, respectively (Hunn and Allen 1974). The dissociation constant of the TFM molecule is 6.07; above that pH the molecule becomes increasingly ionized and consequently transfers less readily across the gill membrane. Since the pH of water solutions governs the uptake rate, changes in pH would be expected to alter the toxicity.

Marking and Olson (1975), who conducted laboratory tests to determine the effects of temperature, water hardness, and pH on the toxicity of TFM to fish, reported that toxicity increased only slightly at high temperatures and in soft water, but increased markedly at low pH. The toxicity of TFM to fish exposed at pH 9.5 and 6.5 differed by factors of 50 x for salmonid species and by more than 20 X for warmwater fishes. These results supported the ionization theory of Hunn and Allen (1974) and identified pH as the chemical factor with the greatest influence on toxicity.

Weise (1984) reported that the pH of a stream may vary significantly during a 24-h period and at different locations on a stream during a TFM application. As an example, during a chemical treatment of South Sandy Creek, New York, the pH values varied from 8.0 to 8.7 at the primary application point but were about 7.9 to 9.1 when the chemical reached a point 10.4 km downstream. Alkalinities at the primary application site did not change during the interim. Also, a treatment of the Little Salmon River in New York, the diurnal pH ranged from about 7.7 to 8.7, but the alkalinity did not change.

Methods currently used to determine treatment rates are based on previous application rates, alkalinity measurements, results of on-site toxicity tests, or a combination of these (Smith et al. 1974). Even though pH has been shown to be the primary factor that influences the toxicity of TFM, it is not generally considered to be a reliable parameter for the selection of rates for field applications. In the past, alkalinity was the only water chemistry characteristic generally used to establish TFM treatment levels. However, recently the effect of pH is being taken into account during stream treatments by the Canadian Agent for sea lamprey control in the Great Lakes. Canadian treatment crews monitor pH every 4 hours and in some cases raise TFM concentrations or lengthen the lampricide bank to counteract increased pH if the kill of sea lamprey is not being achieved. In the U.S., treatment crews (U. S. Agent) monitor pH daily but make no allowances for shifts in pH (personal communication, David A. Johnson, Marquette Biological Station, Marquette, Michigan).

The purpose of this study was to separate the effects of pH and alkalinity on the toxicity of TFM to rainbow trout (Salmo *gairdneri)* to demonstrate that changes in pH can be responsible for changing toxicity, and to show that changes in alkalinity have little effect on toxicity of the lampricide.

MATERIALS AND METHODS

Static test procedures used in this study closely followed those outlined by the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975)

and ASTM (1980). Test waters were prepared according to standardized procedures, except that the alkalinity of the waters was adjusted by the addition of selected quantities of sodium bicarbonate. After the test vessels were filled with the desired test water, the pH of the water was adjusted by the addition of sodium hydroxide or hydrochloric acid. Exposures were continuous for 96 h.

The TFM (39.9% active ingredient; obtained from American Hoechst Chemical Company, Somerville, New Jersey) was weighed on an electrobalance and dissolved in water for a stock solution. Enough of the stock solution was delivered to the test chambers to yield the desired concentrations (expressed here as milligrams of active ingredient per liter). Glass jars containing 15 L of oxygen-saturated water were used for all tests. Temperatures were regulated by immersing the test jars in a constant temperature water bath at 12°C. We exposed 20 fish to each concentration; all tests were conducted in duplicate.

Rainbow trout used in all tests were cultured at the National Fisheries Research Center-La Crosse, Wisconsin, and maintained according to the standard procedures for handling bioassay fish (Hunn et al. 1968). Fish were acclimated to test conditions for 24 h before each test. Mortalities were recorded at 1, 3, 6, 9, 12, and 24 h on the first day and daily thereafter during the remainder of the 96-h test.

Alkalinity, pH, temperature, and dissolved oxygen were monitored throughout the test. To maintain a constant pH for the first 24 h, we checked and adjusted (if necessary) solutions every 1 to 2 h. After 24 h, the pH in each of the test solutions was allowed to equilibrate, and changes in pH and mortality were then recorded daily. The toxicity of TFM was determined in water of four pH's, ranging from 6.5 to 9.5 at alkalinities of 17.8 to 296.6 mg/L (expressed as $CaCO_3$).

The methods of Litchfield and Wilcoxon (1949) were used for computation of LC50's and 95% confidence intervals. All data fulfilled the chi-square test requirement for acceptability.

RESULTS

The toxicity of TFM to rainbow trout during the first 24 h of exposure was about equal at different alkalinity levels at any given pH (Table 1). For instance, at pH 6.5, the 12-h LC50's were from 1.20 mg/L at low alkalinity and 0.88 mg/L at the high alkalinity. The difference was significant only at the extreme (highest and lowest) alkalinities. At pH 9.5, 100 mg/L of TFM was not toxic to rainbow trout at any of the six alkalinities tested.

During the first 24 h of exposure, the toxicity of TFM to rainbow trout decreased as the pH increased. The 12-h LC50's at the low alkalinity level were 1.20 mg/L at pH 6.5, 5.10 at pH 7.5, 10.5 at pH 8.5, and > 100 at pH 9.5 (Table 1). Toxicities differed by factors of about 5 X from pH 6.5 to 7.5, and about 100 X from pH 6.5 to 9.5. The toxicity of TFM at other exposure periods was also significantly different at the four pH levels.

After 24-h the pH of test solutions changed because pH adjustments were

TABLE I. Toxicity of TFM (LC50 and 95% confidence interval, mg/L) to rainbow trout in water of selected pH's and alkalinities at 12°C.

pH and alkalinity (mg/L as $CaCO_3$)	Duration of exposure (hours)				
	3	6	9	12	24
6.5					
22.2	1.28	1.20	1.20	1.20	1.20
	1.11-1.48	1.08-1.33	1.08-1.33	1.08-1.33	1.08-1.33
35.6	1.10	1.08	1.08	1.08	1.05
	0.980-1.24	0.952-1.22	0.952-1.22	0.952-1.22	0.824-1.34
77.2	1.05	0.980	0.980	0.980	0.980
	0.924-I 19	0.884-1.08	0.884-1.08	0.884-1.08	0.884-1.08
103.5	0.960	0.880	0.880	0.880	0.880
	0,868-1.06	0.815-0.950	0.815-0.950	0.815-0.950	0.815-0.950
7.5					
17.8	5.35	5.10	5.10	5.10	5.10
	4.95-5.78	4.59-5.66	4.59-5.66	4.59-5.66	4.59-5.66
31.8	6.20	6.00	6.00	6.00	4.90
	5.64-6.82	5.51-6.53	5.51-6.53	5.51-6.53	4.26-5.63
58.7	6.40	6.18	6.00	6.00	5.60
	5.76-7.11	5.85-6.53	5.45-6.60	5.45-6.60	5.03-6.24
90.0	7.00	6.80	6.50	6.40	5.80
	6.40-7.66	6.25-7.39	5.91-7.14	5.70-7.18	5.26-6.40
154.2	4.90	4.40	4.40	4.40	4.39
	4.36-5.50	4.12-4.69	4.12-4.69	4.12-4.69	4.15-4.65
207.1	5.42	4.65	4.36	4.36	4.29
	4.81-6.11	4.25-5.09	4.08-4.71	4.08-4.71	3.95-4.66
8.5					
21.3	11.6	11.4	11.2	10.5	10.5
	10.2-13.2	9.70-13.4	9.38-13.4	8.54-12.9	9.07-12.1
35.4	17.0	12.6	12.4	12.3	12.2
	14.7-19.6	10.4-15.3	10.6-14.4	10.8-14.0	10.7-13.9
63.8	28.0	14.9	14.7	14.7	14.7
	22.7-34.4	13.2-16.8	12.9-16.8	12.9-16.8	12.9-16.8
92.6	23.0	14.2	14.2	14.2	14.2
	21.1-25.0	12.3-16.3	11.6-17.3	11.6-17.3	11.6-17.3
171.9	35.7	34.8	34.8	34.8	34.0
	31.9-39.9	30.9-39.2	30.9-39.2	30.9-39.2	30.6-37.8
257.7	38.9	38.9	38.9	38.9	35.9
	34.8-43.5	34.8-43.5	34.8-43.5	34.8-43.5	32.0-40.2
9.5"					

[a]At pH 9.5 and alkalinities of 35.3-296.6, all LC50's were > 100

discontinued. The pH of the 6.5 solution changed little and, correspondingly, the toxicity remained consistent in the 24- to 96-h exposures (Table 2). In solutions at pH 7.5 and alkalinities of 90.0 mg/L or less, the pH decreased. This decrease increased the toxicity of TFM; however, at alkalinities of 154.2 and 207.1 mg/L, the pH increased rather than decreased, but toxicity did not change. In tests at pH 8.5 and 9.5, the pH in all test solutions decreased progressively after 24 h and was accompanied by an increase in toxicity, which was more pronounced at pH

TABLE 2. Toxicity of TFM (LC50 95% confidence interval, and pH in parentheses) to rainbow trout in water of different alkalinities and various pH's at 12°C.

Initial pH and alkalinity (mg/L as $CaCO_3$)	Duration of exposure (hours)			
	24	48	72	96
6.5				
22.2	1.20	1.12	1.12	1.12
	1.08-1.33	1.00-1.25	1.00-1.25	1.00-1.25
	(6.57)	(6.55)	(6.53)	(6.52)
35.6	1.05	1.05	1.05	1.05
	0.824-1.34	0.824-1.34	0.824-1.34	0,824-1.34
	(6.61)	(6.64)		(6.65)
77.2	0.980	0.980	(6.64)	0.980
	0.884-1.08	0.884-1.08	0.884-1.08	0.X84-1.08
	(6.62)	(6.73)	(6.73)	(6.75)
103.5	0.880	0.880	0.880	0.880
	0.815-0.950	0.815-0.950	0.815-0.950	0.815-0.950
	(6.65)	(6.78)	(6.79)	(6.84)
7.5				
17.8	5.10	2.80	2.20	2.00
	4.59-5.66	2.41-3.24	1.83-2.64	1.74-2.30
	(7.41)	(6.96)	(6.91)	(6.86)
31.8	4.90	3.60	2.75	2.45
	4.26-5.63	3.12-4.15	2.40-3.14	2.24-2.68
	(7.48)	(7.38)	(7.36)	(7.06)
58.7	5.60'	4.90	4.00	3.63
	5.03-6.24	4.44-5.40	3.61-4.44	3.23-4.07
	(7.47)	(7.33)	(7.29)	(7.30)
90.0	5.80'	5.00	4.40	4.20
	5.26-6.40	4.60-5.43	4.03-4.81	3.82-4.62
	(7.49)	(7.46)	(7.46)	(7.44)
154.2	4.39	4.39	4.39	4.26
	4.15-4.65	4.15-4.65	4.15-4.65	3.94-4.60
	(7.52)	(7.73)	(7.78)	(7.75)
207.1	4.29	4.29	4.29	4.29
	3.95-4.66	3.95-4.66	3.95-4.66	3.95-4.66
	(7.56)	(7.87)	(7.94)	(7.96)
8.5				
21.3	10.5	3.45	2.43	2.30
	9.07-12.1	3.10-3.84	2.03-2.91	1.90-2.78
	(8.75)	(7.30)	(7.11)	(7.12)
35.4	12.2	3.35	3.48	2.80
	10.7-13.9	2.53-4.43	3.11-3.89	2.41-3.25
	(8.67)	(7.51)	(7.33)	(7.35)
63.8	14.7	5.40	4.40	3.60
	12.9-16.8	4.59-6.35	3.72-5.20	2.97-4.36
	(8.60)	(7.74)	(7.57)	(7.51)
92.6	14.2	7.60	6.00	5.60
	11.6-17.3	6.37-9.06	5.07-7.09	5.20-6.03
	(8.54)	(7.92)	(7.72)	(7.66)
171.9	34.0	18.2	14.3	12.3
	30.6-37.8	15.3-21.6	12.6-16.3	10.7-14.1
	(8.51)	(8.12)	(8.01)	(7.96)

TABLE 2. Continued

Initial pH and alkalinity (mg/L as $CaCO_3$)	Duration of exposure (hours)			
	24	48	72	96
257.7	35.9	27.3	19.4	14.6
	32.0-40.2	23.5-31.6	17.3-21.8	12.8-16.7
	(8.51)	(8.24)	(8.15)	(8.11)
9.5				
35.3	> 100	32.1	2.50	< 200
	(9.53)	(8.49)	1.65-3.79	-
			(7.23)	(7.18)
51.8	> 100	42.0	5.80	3.37
	(9.47)	(8.87)	4.52-7.44	2.36-4.81
			(8.01)	(7.58)
88.4	> 100	64.0	15.4	8.70
	(9.42)	57.7-70.9	14.5-18.5	7.36-10.3
		(9.00)	(8.49)	(7.80)
124.6	> 100	98.0	62.0	20.0
	(9.51)	92.5-104	56.4-68.2	16.6-24.0
		(9.21)	(8.96)	(8.55)
198.1	> 100	88.0	49.0	21.9
		81.6-94.8	45.5-52.8	18.6-25.8
	(9.43)	(9.05)	(8.78)	(8.46)
296.6	> 100	> 100	77.9	60.0
			71.1-85.3	51.4-70.0
	(9.45)	(9.17)	(9.04)	(8.88)

9.5 than that at lower pH's. Toxicity increased by a factor of 50 x at low alkalinity (35.3 mg/L) when the pH decreased by 2.3 units. Toxicity increased by a factor of about 2 x at the high alkalinity (296.6 mg/L) when the pH decreased by 0.6 unit.

In test solutions adjusted to pH 8.5 at various alkalinities, the LC50's ranged from 10.5 to 35.9 mg/L in 24-h exposures and were 2.30 to 14.6 mg/L after 96-h exposures (Table 2). A decrease in pH of about 0.5 pH unit doubled the toxicity of the chemical. For example, in water of pH 8.54 and a total alkalinity of 92.6 mg/L, the 24-h LC50 was 14.2 mg/L. Between 24 and 48 h, the pH of the test solution decreased to 7.92 and the resulting LC50 was 7.60 mg/L.

The toxicity of TFM was about equal at the different alkalinity levels at pH 6.5 and 7.5 at exposures of 24 to 96 h (Table 1). In the tests at pH 8.5 and 9.5, the toxicity differed at the six alkalinity levels; however, the pH decrease was greater in these solutions than at lower pH's and presumably increased the toxicity accordingly.

DISCUSSION

Lethal concentrations of the lampricide TFM kills fish rapidly, usually in 1 to 6 h, and minimal lethal concentrations are nearly equal for short and longer

term exposures. For example, Marking et al. (1975) found that the 24-h LC50 (6.03 mg/L) was identical to the 30-day LC50 for lake trout (Salvelinus namaycush). Dawson et al. (1975) demonstrated that there were. no significant differences between the 24-and 96-h LC50's for sea lamprey larvae at five stages of development (stage 14, hatching; through stage 18, 7-cm larvae).

Although the toxicity of TFM to fish and sea lamprey has previously been shown to be affected by pH (Dawson et al. 1975; Marking and Olson 1975), pH has not been considered as a factor in determining concentrations selected for field use. Instead, treatment concentrations have been based on previous application rates, alkalinity measurements, the results of on-site toxicity tests, or a combination of these (Smith et al. 1974). Seelye et al. (1988) demonstrated the correlation of alkalinity and the toxicity of TFM. They provided a set of guidelines for field personnel to select safe treatment concentrations of TFM and Bayer 73 in waters with alkalinities ranging from 40 to 200 mg/L. In most instances, the treatment zones presented should yield safe, successful treatments for control of sea lamprey with minimal impacts on nontarget organisms. However, in some waters, even though the alkalinities remain the same, there are significant diurnal cycles of oxygen and pH caused by external factors such as photosynthesis and respiration. These changes in pH also change the amount of TFM in the unionized form, which in turn increases or decreases the amount available to produce toxicosis (Hunn and Allen 1974). Although the Canadian treatment crews do not use pH as a determining factor for application rates, they are now modifying their treatment regimes to counteract increases in pH during stream treatments (personal communication, David A. Johnson, Marquette Biological Station, Marquette, Michigan).

The cyclic changes in pH and oxygen must be accounted for when treatment concentrations of TFM are being selected. Although measurements are relatively easy to make, they must be monitored at several points over time because they are highly susceptible to change, especially in soft water, in which the pH sometimes varies more than one unit during the course of day (Frey 1963; Hynes 1970). Frey (1963) noted that, in slow moving waters, the pH was substantially higher at the surface than in the water column. Hynes (1970) lists two factors that are responsible for diurnal changes in the pH of water: rain (because of the high content of CO_2 and the free acid form of sulfates); and photosynthesis and respiration, which cause changes in CO_2 and O_2, especially in soft water streams. These changes are most pronounced in shallow streams with relatively sluggish flows. During daylight, photosynthesis often increases the O_2 levels and decreases CO_2 levels, and pH increases rapidly; at night, the increased respiration of plants and animals increases the CO_2 level and causes a decrease in pH.

Decreases in pH caused by these factors can change the toxicity of TFM enough to kill nontarget organisms in some sections of streams. Conversely, increases in pH may reduce the level of toxicity of TFM and result in incomplete kills of sea lamprey larvae. For example, treatment of Mayhew Creek, Ontario, with a concentration of TFM based on alkalinity levels, failed to kill most of the sea lamprey larvae (Schleen 1979). The pH values recorded during the treatment

ranged from 9.24 to 9.89. A retreatment of the same stream with a similar concentration of TFM, but at a pH of 7.7 to 7.8, resulted in a successful kill of the remaining sea lamprey larvae. Another example of the effect of pH on the toxicity of the lampricide was demonstrated by Bills and Johnson (1988). During a treatment of the Millicoquins River, Michigan, on May 15, 1988, they pumped water from the river containing 4.2 mg/L of TFM into a continuous flow bioassay unit and raised or lowered the pH by approximately 1.0 unit from that of the river (pH 8.35) to determine what, if any, effect this would have on target and nontarget mortality. They exposed brook trout *(Salvelinus fontinalis),* rainbow trout, fathead minnow *(Pimephales promelas),* mayfly nymphs *(Hexagenia* sp.), and sea lamprey ammocoetes in cages in the river and in the bioassay water with altered pH. In the river exposures, all ammocoetes and mayfly nymphs were killed during the 12-hour treatment. No mortality occurred among any nontarget fishes. In contrast, in the raised pH exposures, only 55% of the ammocoetes were killed, and there were no mortalities among any nontarget organisms. In the lowered pH test, all ammocoetes and all nontarget organisms were killed during the exposure and most were killed in the first 3 h of exposure. This study showed that a decreased pH during a treatment could have a deleterious effect on nontarget organisms, and conversely, an increased pH could produce an incomplete kill of sea lamprey.

Past treatment rates, on-site toxicity test results, and alkalinity measurements are useful tools in selecting TFM concentrations; however, if the pH is different from past treatments or if changes in pH are occurring daily then pH must be considered when treatment rates are being determined. If pH values in the stream differ significantly from those observed in the past, or if there is a substantial diurnal shift in pH, the treatments may be unsuccessful or result in the kill of non-target organisms, depending on the direction of the pH shift. Diurnal fluctuations are influenced seasonally and treatment of streams with significant changes in pH during the summer period may be scheduled for spring or fall treatments when pH shifts are at a minimum.

REFERENCES

APPLEGATE, V. C., J. H. HOWELL, AND M. A. SMITH. 1958. Use of mononitrophenols containing halogens as selective sea lamprey larvicides. Science 127(3294):336-338.

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.

ASTM COMMITTEE E-35 ON PESTICIDES. 1980. Standard practice for conducting toxicity tests with fishes, macroinvertebrates, and amphibians, E729-80. Pages l-25 *in* Annual Book of ASTM Standards, Part 4b. End use and consumer products. American Society for Testing and Materials, Philadelphia, PA.

BILLS, T. D., AND D. A. JOHNSON. 1988. Effects of raised or lowered pH on the

toxicity of TFM to sea lamprey larvae and nontarget species in a stream treatment. U.S. Fish Wildl. Serv., Special Report. National Fisheries Research Center-La Crosse, Wisconsin, 54602. 19 pp.

COMMITTEE ON METHODS FOR TOXICITY TESTS WITH AQUATIC ORGANISMS. 1975. Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians, Ecological Research Seminars, EPA (Environmental Protection Agency). 660/3/75-009. 6 pp.

DAWSON, V. K., K. B. CUMMINGS, AND P. A. GILDERHUS. 1975. Laboratory efficacy of 3-trifluoromethyl-4-nitrophenol (TFM) as a lampricide. U.S. Fish Wildl. Serv., Invest. Fish Control 63. 13 pp.

FREY, D. J. 1963. Wisconsin: The Birge-Juday Era. In D. G. Frey (ed.) Limnology in North America. Univ. Wisc., Madison, WI. 54 pp.

HOWELL, J. H., AND W. M. MARQUETTE. 1962. Use of mobile bioassay equipment in the chemical control of sea lamprey. U.S. Fish Wildl. Serv., Spec. Sci. Rep., Fish. No. 418. 9 pp.

HUNN, J. B., R. A. SCHOETTGER, AND E. W. WHEALDON. 1968. Observations on the handling and maintenance of bioassay fish. Prog. Fish-Cult. 30:164-167.

HUNN, J. B., AND J. L. ALLEN. 1974. Movement of drugs across the gills of fishes. Annu. Rev. Pharmacol. Vol. 14. p. 47-55.

HYNES, H. B. N. 1970. The ecology of running waters. Univ. Toronto Press. The Chaucer Press Ltd. Bungay, Suffolk, Great Britain. pp. 36-52.

KANAYAMA, R. K. 1963. The use of alkalinity and conductivity measurements to estimate concentrations of 3-trifluormethyl-4-nitrophenol required for treating lamprey streams. Great Lakes Fish. Comm. Tech. Rep. 7. 10 pp.

LITCHFIELD, J. T., JR., AND F. WILCOXON. 1949. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. 96:99-113.

MARKING, L. L., T. D. BILLS, AND J. H. CHANDLER. 1975. Toxicity of the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) to nontarget fish in flow-through tests. U.S. Fish Wildl. Serv., Invest. Fish Control 61. 9 pp.

MARKING, L. L., AND L. E. OLSON. 1975. Toxicity of the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) to non-target fish in static tests. U.S. Fish Wildl. Serv., Invest. Fish Control 60. 27 pp.

SCHLEEN, L. P. 1979. Effects of high pH's on a lampricide (TFM) treatment of Mayhew Creek, 1979. Sea Lamprey Control Technical Report No. 3, Sea Lamprey Control Centre, Sault Ste. Marie, Ontario. 7 pp.

SEELYE, J. G., D. A. JOHNSON, J. G. WEISE, AND F. L. KING. 1988. Guide for determining application rates of lampricides for control of sea lamprey ammocoetes. Great Lakes Fish. Comm., Tech. Rep. 52. 23 pp.

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.

WEISE, J. G. 1984. Summary of chemical use to manage sea lamprey *(Petromyzon marinus)* populations in Canada and New York State, 1958-84. Sea Lamprey Control Technical Report No. 4, Sea Lamprey Control Centre, Sault Ste. Marie, Ontario. 84 pp.

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