COMPARISON OF MARK RETENTION AND SURVIVAL OF SEA LAMPREY LARVAE MARKED BY PIGMENT INJECTION AND TAIL CLIPPING

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Great Lakes Fishery Commission

TECHNICAL REPORT 61

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ABSTRACT. Both mark retention and survival were similar for sea lamprey *(Petromyzon marinus)* larvae marked by either a subcutaneous injection of pigment or a tail clip. Survival of all marked and control larvae exceeded 86% alter 14 wk. Use of the vent to speed needle insertion into the caudal sinus did not affect survival. Tail clips are recommended for population estimates where only one mark is needed because they are faster to apply and less visible to predators and collectors than injected pigments. Pigment injection is preferred when more than one mark is required for a stream system because larvae from different treatments can be identified by pigment color or injection site.

INTRODUCTION

Control of sea lampreys *(Petromyzon marinus)* in the Great Lakes relies primarily on the treatment of nursery streams with lampricides (Smith and Tibbles 1980). Mark and recapture techniques are occasionally used to estimate the population of larval sea lampreys in a stream. In one method, larvae are marked by a subcutaneous injection of pigment with a hypodermic needle (Hanson 1972). The injection site varies, but most larvae are marked either near the dorsal fin or in the ventral caudal sinus posterior

to the vent. Injections into the caudal sinus are expedited if the needle is inserted at the vent, but this procedure could damage the digestive tract and increase mortality from infection. Pigment injection is also limited to larvae longer than approximately 50 nun, which precludes its use on young-of-the-year larvae and some yearlings.

Tail clips provide an alternate method for marking all sizes of larvae. The squared-off tail is an easily recognized mark, but little is known of its retention or effects on the survival of larvae. In this study, the following three areas are examined:

1) retention of pigment injections and tail clips and the effects of injection site,

2) injection procedure, and

3) effects of tail clips on the survival of larvae.

METHODS

The study was conducted April 11-July 19, 1989, with 360 sea lamprey larvae ranging in length from 35 to 128 mm. All larvae were anesthetized in a 50 mg/L solution of tricaine methanesulfonate (MS-222) and measured to the nearest 1 mm before marking. Three groups of 60 larvae each were marked by subcutaneous injection with a hypodermic needle (Hanson 1972) of Tracer-glo pigment (Wildlife Supply Co., 301 Cass St., Saginaw, MI, 48602) suspended in carbopol 962 gel (B. F. Goodrich Co., 800 Marble Ave., Cleveland, OH, 44105). A fourth group of 120 larvae was marked with a tail clip.

- Group 1 was injected with red pigment in the caudal sinus just posterior to the vent.

- Group 2 was marked in the same location with green pigment, but the needle was inserted first into the vent and then into the caudal sinus.

- Group 3 was injected with green pigment in the side of the body.

- Group 4 was marked by removing 1-2 mm (approximately 2-4 mm^2, surface area) from the end of the tail with a razor blade.

A control group of 60 larvae was anesthetized and measured but not marked.

After marking, the larvae were held in six 15 1-L aquaria that contained clean beach sand approximately 75 mm deep and supplied with a constant flow of aerated Lake Huron water. The water temperature was not controlled and increased gradually from 1°-20°C as the ambient temperature of Lake Huron increased. Each aquarium contained 60 larvae:

- 10 from each of the three groups marked by injection,
- 20 marked with tail clips, and
- 10 controls.

Larvae were fed once a week with a slurry of baker's yeast (Hanson et al. 1974).

Larvae were removed from the substrate, examined for marks, and allowed to reburrow at 3 wk and 14 wk after marking. Dead larvae on the surface of the substrate were removed and the date of removal, length, and type of mark were recorded. Larval mortality among tanks and marking methods was compared by chi-square contingency tables (Conover 1971).

RESULTS AND DISCUSSION

We pooled the survival data for each group of marked sea lampreys across the six aquaria after analysis revealed no significant differences in survival among aquaria within each group (P ranged from 0.08 to >0.25). Survival through 3 wk was good for all groups and ranged from 100.0% for the controls to 93.3% for those marked in the caudal sinus (Table 1). At least 86.7% of each group of marked or control larvae were alive 14 wk after marking (Table 1). There was no significant difference in survival ($P>0.25$) among the five groups of larvae. Survival was better among larvae longer than 67 mm-only two of the larger larvae (86 mm and 10 1 mm) died during the study.

Table 1. Number alive and percent survival after 3 wk and 14 wk, and initial mean length (range in parentheses) of sea lamprey larvae marked by injection of pigment or tail clip and held April 1 l-July 19, 1989.

Mark location and type	Number alive (week)		Percent survival (week)		Mean length range (mm)
	3	14	3	14	
Caudal sinus (red)	56	52	93.3	86.7	79.8 (47-124)
Vent and caudal sinus (green)	59	55	98.3	91.7	84.4 (53-121)
Side of body (green)	58	54	96.7	90.0	80.4 (49- 113)
Tail clip	119	110	99.2	91.7	73.3 (35-124)
Control	60	56	100.0	93.3	76.6 (53-128)

Marks were readily identifiable on all but one animal after 14 wk or at the time of death. One sea lamprey was consistently misidentified as a control until the end of the study. We probably did not inject enough green pigment into the caudal sinus to produce an adequate mark. Use of the vent to aid injection into the caudal sinus did not cause infection or mortality in the marked larvae (Table 1). Therefore, insertion of the needle at the vent seems acceptable for expediting the marking process.

Ricker (1975) stated that an acceptable marking method should result in marked fish that:

- do not suffer excessive mortality,
- are not more vulnerable, to recapture than unmarked fish,
- retain their marks, and
- are easily recognized when recaptured.

Both pigment injection and tail clipping meet these requirements to varying degrees but each offers its own advantages. Pigment injections are most useful when more than one mark is required. For example, when separate population estimates are conducted in a stream system, a change in pigment color or injection site allows recaptured animals to be identified by location or time of release. However, the highly visible fluorescent pigment could make swimming larvae more vulnerable to predation and more visible to personnel collecting with scap nets particularly if the mark is on the dorsal surface. The positive selection of marked animals during the collections could bias the population estimate.

Tail clips might be preferred in some situations because they:

- can be applied to larvae as small as 35 mm long (Table 1),
- are less visible to predators and collection personnel, and
- are much easier and faster to apply than the pigment injections (20 anesthetized larvae were marked with tail clips in less than 1 min).

Conversely, tail clips provide only a single mark for a stream system and the length of recaptured animals cannot be accurately measured. However, a tail clip does not remove enough skin to affect the ski photosensory system located on the tail of larval lampreys (Young 1935). Observations in the field also indicate that tail clipping does not affect the swimming ability of larvae.

Both methods are superior to the immersion of larvae in stains such as Bismark Brown, which was occasionally used during past field studies. The high visibility of fish marked with Bismark Brown tends to bias the recapture collections and the dye fades within 4 to 14 d (Stott 197 1). Fading of the dye is a problem when a sea lamprey population estimate is made in conjunction with the application of lampricide to a stream, Unexpected delays of a scheduled stream treatment can result in lost marks as the dye fades. For example, 8,031 sea lampreys marked by pigment injection were released in Conneaut Creek (Ohio and Pennsylvania), on August 14, 1986. However, low stream flows delayed treatment for more than 7 wk (T. J. Morse, U.S. Fish and Wildlife Service, Marquette Biological Station, 1924 Industrial Parkway, Marquette, MI, 49855, pers. commun.). If the sea lampreys had been marked by Bismark Brown instead of pigment injections, 10 d of collecting and marking effort by 12 people would have been wasted.

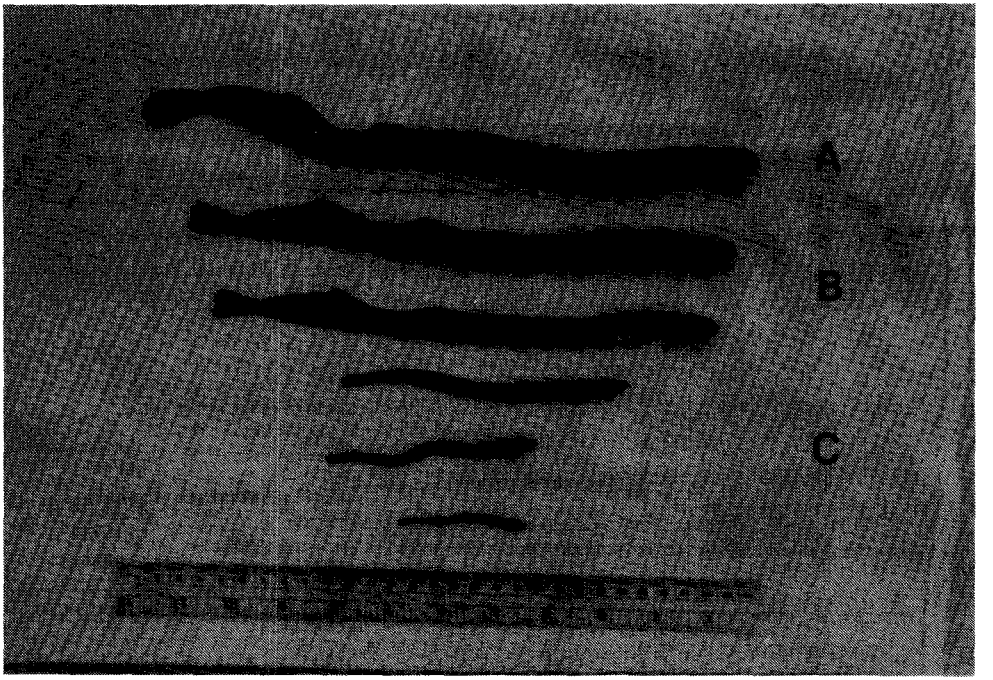

Fig. 1. One unmarked parasitic-phase sea lamprey (A), two parasitic-phase sea lampreys marked with tail clips as larvae (B), and three larvae marked with tail clips and held for 15 mo (C).

Mark retention was good for both pigment injections and tail clips. Pigment injections into newly metamorphosed sea lampreys were readily visible after 9 mo in laboratory studies (Hanson 1972) and after 13 to 17 mo on spawners trapped in tributaries of Lakes Huron and Michigan (L. H. Hanson, U.S. Fish and Wildlife Service, retired, 120 B St., Cheboygan, MI, 4972 1, pers. commun.). Tail clips were visible after 15 mo on larvae marked for the present study (Fig 1). In addition, two sea lampreys (marked as larvae by S. B. Morkert and caged in a stream) retained identifiable tail clips through metamorphosis and 3 mo of parasitic feeding (Fig 1). The longevity of both marks indicates they are suitable for movement and homing studies of parasitic-phase sea lampreys in the Great Lakes. However, a recent study indicates that coded wire tags (CWTs) are probably better suited for marking parasitic-phase sea lampreys because the tags are easy to apply and detect electronically (Bergstedt et al. 1993). Tail clips could still be used in combination with CWTs to simplify the identification of marked animals in some situations. Regardless, low cost and easy application make tail clips and pigment injections excellent choices for marking large numbers of larvae for population estimates in streams.

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COMPARISON OF 3-TRIFLUOROMETHYL-4-NITROPHENOL (TFM) TOXICITIES TO SEA LAMPREYS, RAINBOW TROUT, AND MAYFLY NYMPHS IN CONTINUOUS AND INTERRUPTED 9-H EXPOSURES

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ABSTRACT. Flow-through tests with 12°C Lake Huron water were used to examine the toxicity of 3-trifluoromethyl-4-nitrophenol (TFM) to larval sea lampreys *(Petromyzon marinus), fry* of rainbow trout *(Oncorhynchus mykiss),* and nymphs of burrowing mayfly *(Hexagenia limbata).* The objective of this study was to evaluate and compare the effectiveness of a single, continuous 9-h exposure to TFM with two exposures (totaling 9 h) of TFM that were interrupted by 12 h or 22 h of no exposure. Two exposures to TFM interrupted for 12 h were as effective at killing sea lampreys as a single, continuous 9-h exposure, but the toxicity of TFM to sea lampreys sometimes declined when the interruption period was increased to 22 h. The toxicity of TFM to rainbow trout did not differ significantly among the continuous 9-h exposure and two tests where exposures were interrupted for either 12 h or 22 h. The toxicity of TFM to mayflies was significantly greater in the continuous 9-h exposure than in either of the interrupted (12-h or 22-h) exposures. Mayfly survival increased as the period of interruption increased. A TFM treatment that is interrupted for 12 h or less will still effectively kill larval sea lampreys, could protect nontarget mayflies, and could eliminate rescheduling treatments that are discontinued because of equipment failures or changes in stream conditions.

INTRODUCTION

The lampricide 3-trifluoromethyl-4-nitrophenol (TFM) has been used to kill larval sea lampreys *(Petromyzon marinus)* in tributaries of the Great Lakes since 1958 (Applegate et al. 196 1). To effectively treat an infested stream, TFM is pumped into the stream and maintained at a desired concentration for at least 9 uninterrupted hours. Chemical analyses are conducted routinely during the treatment to monitor the TFM concentration. Stream-treatment application rates of TFM (Smith et al. 1974; Seelye et al. 1988; Lieffers 1990) are determined from:

- previous applications,
- stream alkalinity, and
- on-site TFM toxicity tests using larval sea lampreys and nontarget fish.

The TFM concentrations corresponding to the 9-h LC99.9 value for sea lampreys (minimum lethal concentration) and the LC25 value for nontarget fish (maximum allowable concentration) have been used by sea lamprey control agents for more than 30 yr as the limiting concentrations of TFM used in a stream treatment. However, for most sea lamprey-control stream treatments in recent years, the concentration of TFM corresponding to the LC25 value for nontarget fish has been avoided. TFM concentrations just greater than the corresponding LC99.9 value for sea lampreys have been used.

At least once every season, a lampricide treatment is discontinued (H. J. Lieffers, U.S. Fish and Wildlife Service, retired, Ludington Biological Station, Ludington, MI, 4943 1; W. Westman, Department of Fisheries and Oceans, Sea Lamprey Control Centre, Sault Ste. Marie, Ontario, CANADA, P6A 6W4, pers. commun.) because of equipment failures or changes in physicochemical characteristics of the stream. For example, these changes include:

- sudden changes in flow, pH, or alkalinity;
- decreases in dissolved oxygen (DO); or
- increases in ammonia concentration.

Sea lamprey control agents, from past experience, consider a treatment that is discontinued for more than 2 or 3 h to be ineffective. The agents require that the treatment be started over, or cancelled and rescheduled. Consequently, sea lamprey control agents consider all TFM applied before the interruption to be wasted. For example, assume a can of treatment-grade TFM (36% active) costs $400. Sea lamprey control agents determine a stream (flow 7.6 L/sec) must be treated with 3.0 mg/L TFM for 12 h. Therefore, 100 cans of TFM, or $42,000 of lampricide, are required. After 2 h of applying TFM (17 cans of TFM valued at $6,800), the treatment is stopped because of equipment failure. Currently, if stream treatment is restarted 4 h later, sea lamprey control agents will start over using 100 cans of TFM. As a result, the first 2 h and 17 cans of TFM (valued at $6,800) would be wasted. In 1990, treatment of Cattaraugus Creek was halted after 3 h because of equipment problems. For 1.5 h no TFM was pumped into the stream. Treatment was started over and the TFM concentration was maintained continuously at or above the LC99.9 value for at least 9 h. All TFM pumped during the first 3 h was disregarded and considered wasted.

If the TFM applied before an interruption was considered part of the 9 h of treatment, then resumption of interrupted treatments could result in considerable savings in time, lampricide, and money provided that:

- the mortality of sea lampreys is not decreased, and

- the mortality of nontarget organisms is not increased.

A study by Bills et al. (1985) indicated that the mortality of mayfly nymphs (Hexagenia sp.) decreased with shorter exposures to TFM. As a result, an interrupted TFM treatment may be less harmful to mayfly nymphs than a continuous TFM treatment.

Clark et al. (1987) reported that laboratory toxicity tests must simulate field exposure regimes to adequately predict the effects of a pesticide on the environment. Therefore, to study the effects of interrupted TFM exposures, we conducted a series of continuous and interrupted flow-through TFM toxicity tests on:

- larval sea lampreys,

- fry of rainbow trout *(Oncorhynchus mykiss),* and

- nymphs of burrowing mayfly *(Hexagenia limbata).*

Flow-through acute toxicity tests were conducted to simulate sea lamprey-control stream treatments where the test organisms were exposed to TFM for 9 h. The objective of this study was to evaluate and compare the effect of one continuous 9-h TFM exposure with two TFM exposures (9 h total) that were interrupted for 12 h or 22 h after at least 4 h of exposure.

METHODS

Larval sea lampreys of 49-120 mm (total length) were collected from tributaries of the Great Lakes with electroshockers by Canadian sea lamprey-control personnel, held in flowing Lake Huron water, and maintained with the method of Hanson et al. (1974) for at least 90 d before the toxicity tests. Rainbow trout of 47-88 mm (total length) tested during March and April 1990 were obtained from Northern Trout and Wildlife Farms (10230 E. Sixteen Rd., Manton, MI, 49663). Rainbow trout of 50-76 mm (total length) tested in October 1990 were obtained from the Cedarbrook Trout Farm (1543 Lakeshore Dr., Harrisville, MI, 48740). Both batches of rainbow trout were held in flowing Lake Huron water for at least 45 d before testing. Mayfly nymphs of 27-45 mm (total length) were obtained from Buck's Bait Shop (Route 1, Interlochen, MI, 49643) and maintained with the method of Fremling (1967) for at least 60 d before testing.

Procedures specified by the American Society for Testing and Materials (1985) were used throughout the study except the test tanks were not randomly assigned. Flow-through toxicity tests simulated a stream treatment and included:

- the gradual increase of the TFM concentration from zero to the desired level at the start of the exposure period, and
- the gradual decrease of the concentration to zero at the end of the exposure period.

We used a continuous-flow toxicant delivery system similar to the one described by Bills and Johnson (1992). Modifications included:

- elimination of the baffle plates from the head box,
- an increase in the toxicant water flow to 1.6 L/min in each tank, and
- a reduction in the number of tanks to 6.

One of the 6 tanks (control) received dilution water only. A diaphragm pump (Ecodyne, 1890 Wood Lane, Woodbury, MN, 55 125) delivered TFM to the dilution box at a rate of approximately 25 mL/min.

Toxicity tests were conducted with 12°C Lake Huron water with:

- pH 7.9-8.4,
- total alkalinity 78-93 mg/L as $CaCO_3$,
- total hardness 96 - 110 mg/L as $CaCO_3$, and
- DO 9.8-12.2 mg/L.

Ten f&-swimming larval lampreys, 10 rainbow trout fry, or 20 mayfly nymphs were placed into each tank at least 13 h prior to the start of the toxicity test. Artificial burrows for the mayflies were constructed from glass tubing (Fremling and Mauck 1980) and placed into each mayfly tank.

We conducted 6 groups of 2,3, or 4 tests as described below. Each group of tests consisted of organisms exposed to a single continuous 9-h dose of TFM and organisms exposed to two dosing periods of TFM (totaling 9 h) that were interrupted by 12 h or 22 h between dosing periods. In one test with larval sea lampreys, the test was interrupted for 13 h instead of 12 h. Larval sea lampreys and rainbow trout fry in the interrupted tests were exposed to TFM in two dosage patterns:

1) a 4-h dose of TFM; no TFM for 12 h or 22 h; a 5-h dose of TFM

2) a 6-h dose of TFM; no TFM for 12 h or 22 h; a 3-h dose of TFM

Mayflies were exposed only to the second dosage pattern (a 6-h dose of TFM; no TFM for 12 h or 22 h; a 3-h dose of TFM).

Time-line representations shown in Fig. 1 depict the various dosage patterns described above.

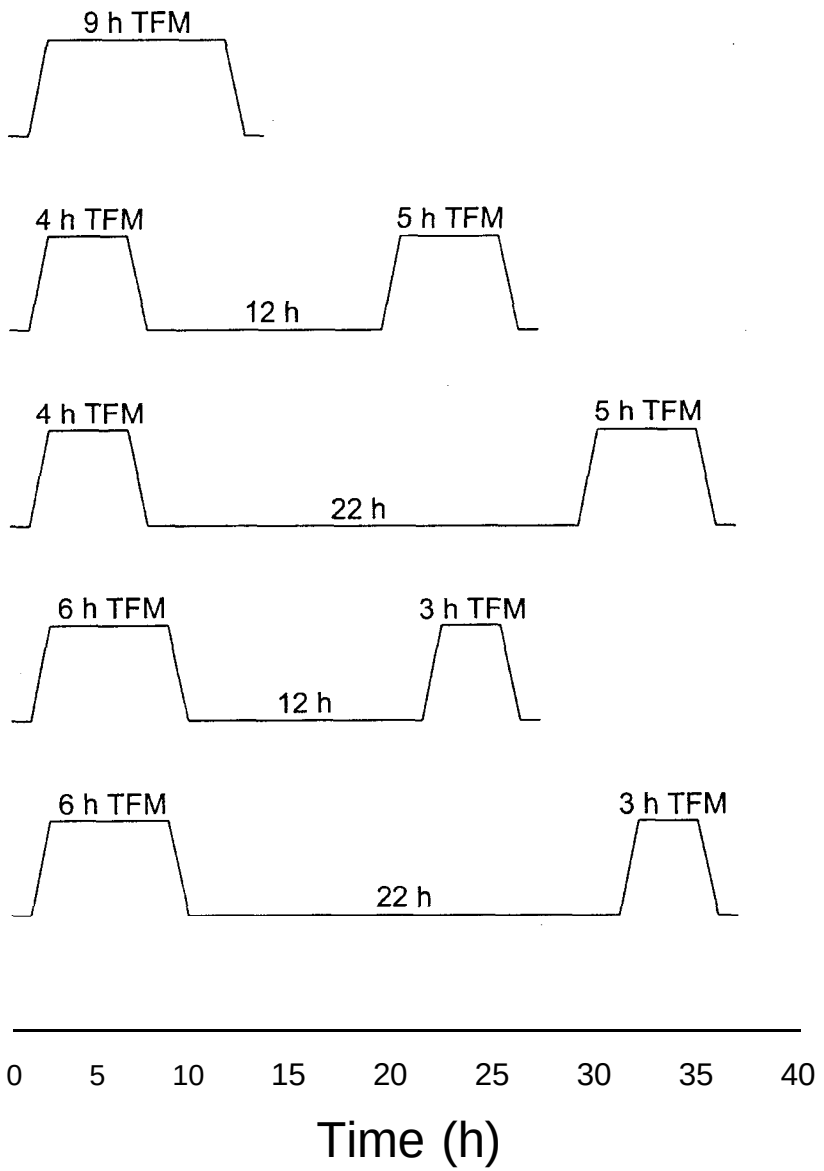

Fig. 1. Time-line representation of TFM dosage patterns in the flow-through toxicity tests. Duration (h) of TFM and no TFM are given above each time line.

Lampricide (TFM, 37.1% active ingredient, isopropanol formulation) from the American Hoechst Chemical Company (P. 0. Box 2500, Somerville, NJ, 08876-1258) was used in all tests. Water samples were collected hourly during TFM exposures, and TFM concentrations were quantified by the spectrophotometric method described by Smith et al. (1960). Concentrations of TFM used in the tests ranged from 0.6 to 5.5 mg/L for larval sea lampreys, 6.4 to 32.4 mg/L for rainbow trout, and 1.7 to 15.6 mg/L for mayflies. Alkalinity, hardness, DO, and pH were determined for the Lake Huron dilution water during each toxicity test. The temperature of the dilution water and mortalities of the aquatic organisms were recorded hourly when pumping the TFM. A final check for mortality was made 22 h after the TFM pump was turned off.

Because this study was intended primarily for use in sea lamprey control, we have reported the LC99.9 values for sea lamprey and the LC25 values for nontarget organisms. The sea lamprey 9-h LC99.9 value was defined by sea lamprey-control personnel as the minimum lethal concentration. A 9-h exposure generally represents the minimum time TFM concentrations are maintained at or above minimum lethal concentration during a stream treatment to kill larval sea lampreys. The LC25 value for nontarget fish generally is the maximum allowable concentration of TFM that could be used during a stream treatment and represents the worst-case situation. Because the LC50 values can be estimated with more precision than either the LC99.9 or LC25 values, we examined the relation between different TFM dosage patterns and LC50 values for all three species. This process helped ensure that no subtle relations were missed between dosage patterns and the toxicity of TFM to any of the three species.

The method of Litchfield and Wilcoxon (1949) was used to calculate the LC99.9, LC50, and LC25 values; compare two toxicity lines for parallelism; and compare the LC50 values from two toxicity tests. Significance was defined as $P \leq 0.05$. Because the Litchfield and Wilcoxon (1949) method only compares LC50 values for significant differences, we used the criterion of nonoverlapping 95% confidence limits to determine significant differences among LC25 values when the toxicity lines were parallel.

RESULTS AND DISCUSSION

Tests with larval sea lampreys indicated that two exposures to TFM (totaling 9 h) separated by 12 h of no exposure were as effective as a single, continuous 9-h exposure. When the interruption time was increased to 22 h, the LC99.9 values for 2 of the 3 22-h interrupted tests were greater than the LC99.9 values for the corresponding continuous and 12- or 13-h interrupted tests (Table 1).

Table 1. The 22-h post exposure LC50 and LC99.9 values, and slope function (toxicity lines) for larval sea lampreys exposed to continuous or interrupted 9-h TFM exposures in 3 groups of tests (95% confidence intervals are in parentheses). Slope-function values with different superscript letters in a group's column are significantly different and the toxicity lines are not parallel. For parallel toxicity lines, LC values with different superscript letters in a group's column are significantly different.

Date	TFM treatment
Group 1	
March 8, 1990	Continuous 9-h TFM
March 27,1990	Continuous 9-h TFM
Mar 21,1990	4-h TFM dose, 12 h no TFM, 5-h TFM dose
April 10,1990	4-h TFM dose, 22 h no TFM, 5-h TFM dose
Group 2	
January 31,1991	Continuous 9-h TFM
February 13,1991	6-h TFM dose, 12 h no TFM, 3-h TFM dose
February 20, 1991	6-h TFM dose, 22 h no TFM, 3-h TFM dose
Group 3	
March 7,1991	Continuous 9-h TFM
March 12,1991	6-h TFM dose, 13 h no TFM, 3-h TFM dose
March 19,1991	6-h TFM dose, 22 h no TFM, 3-h TFM dose

Continued on next page

Table *I, continued*

Slope function	LC50[1] (mg/L TFM)	LC99.9[2] (mg/L TFM)
	Group 1	
1.18 (1.09-1.27)[v]	1.5 (1.3-1.8)	2.5 (1.9-3.3)
1.28 (1.08-1.50)[v, w]	1.3 (1.1-1.6)	2.8 (1.7-4.7)[v, w]
1.17 (1.10-1.24)	1.5 (1.4-1.7)	2.4 (2.0-2.9)
1.29 (1.16-1.43)"	2.0 (1 .7-2.3)[w]	4.3 (3.0-6.1)[w]
	Group 2	
1.23 (1.11-1.36)	1.3 (1.1-1.5)	2.4 (1.6-3.5)
1.12 (1.07-1.18)	1.4 (1.3-1.6)	2.0 (1.7-2.4)
1.13 (1.08-1.19)	1.5 (1.3-1.7)	2.1 (1.8-2.6)
	Group 3	
1.10 (1.07-1.15)[y]	1.2 (1.1-1.3)[y]	1.6 (1.4-1.9)[y]
1.12 (1.07-1.18)[y]	1.3 (1.2-1.5)[y]	1.9 (1.6-2.3)y
1.25 (1.13-1.39)	1.3 (1.0-1.5)	2.5 (1.7-3.7)

[1] LC50 values were compared by the method of Litchfield and Wilcoxon ($P \leq 0.05$, 1949) only if the slope functions were not significantly different (toxicity lines were parallel).
[2] LC99.9 values were compared only if the slope functions were not significantly different. If the 95% confidence limits did not overlap, the LC99.9 values were considered significantly different.

The LC50 value for the 22-h interrupted test in Group 1 was significantly greater than for the continuous 9-h test on March 27, 1990. For half the tests, the slope function of the toxicity lines for the 22-h interrupted tests were significantly different than for the corresponding 9-h continuous tests (Table 1). Tests conducted during January and February 199 1 (Group 2) showed no significant differences for the slope functions of the toxicity lines or LC50 values among the continuous 9-h and the interrupted treatments. We are unsure why 2 of the 3 22-h interrupted treatments were less effective on larval sea lampreys. However, the 22-h interruption period may allow some recovery or healing of the gills and subsequently increase the survival rate of sea lampreys. Mallatt et al. (1987) reported that shorter exposures to TFM produced less damage to the gills of sea lampreys.

When analyzing the TFM exposure vs. time data for larval sea lampreys, we noted that few lampreys died during the first 3 h of exposure (maximum TFM concentration was 3.8 mg/L). Most mortality occurred between 3 and 9 h of exposure; longer exposure hills more larval sea lampreys. During the 22-h post TFM exposure, some lamprey mortality still occurred (Figs. 2,3,4). Also, when comparing treatments and LC50 values (Figs. 2,3,4), the 9-h TFM exposure data and 22-h post-exposure data indicate that the 22-h interrupted treatments tend to be less toxic to larval sea lampreys than the continuous TFM treatments.

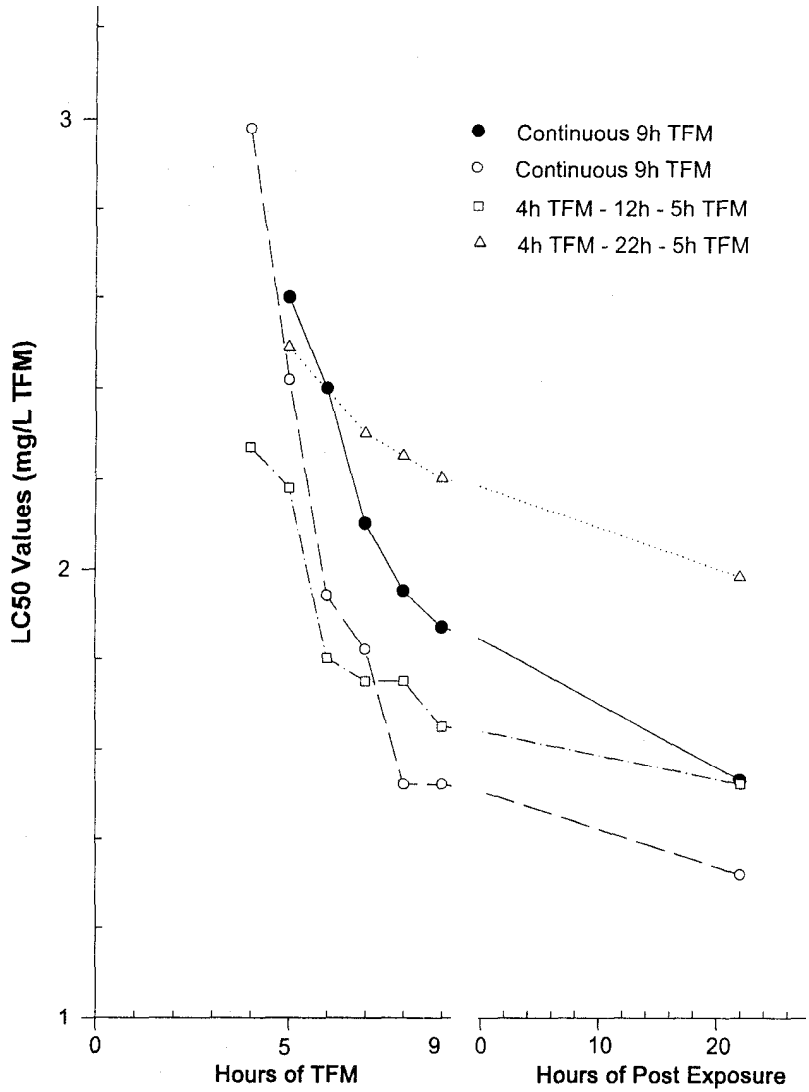

Fig. 2. Changes in Group-l larval sea lamprey LC50 values during 9-h TFM exposures (a continuous 9-h dose of TFM on March 8 and 27, 1990; a 4-h dose of TFM, followed by no TFM for 12 h, followed by a 5-h dose of TFM on March 21, 1990; a 4-h dose of TFM, followed by no TFM for 22 h, followed by a 5-h dose of TFM on April 10,1990).

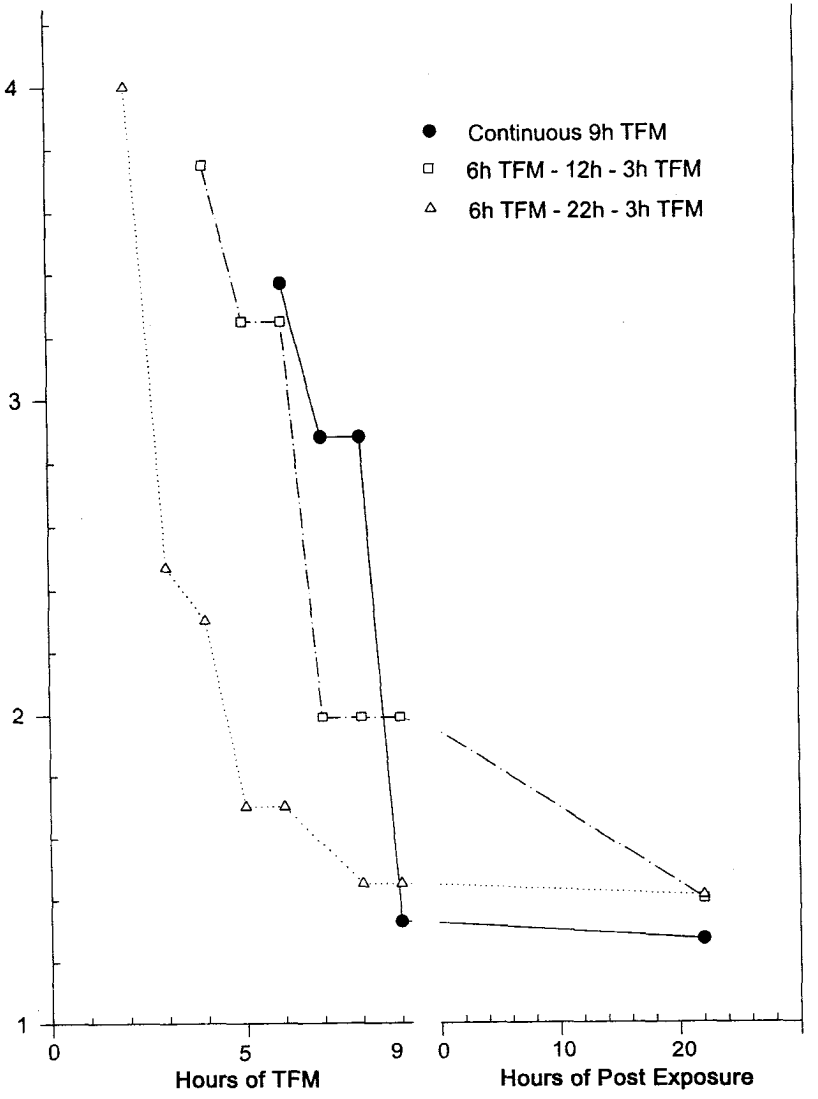

Fig 3. Changes in Group-2 larval sea lamprey LC50 values during 9-h TFM exposures (a continuous 9-h dose of TFM on January 3 1, 199 1; a 6-h dose of TFM, followed by no TFM for 12 h, followed by a 3-h dose of TFM on February 13,199 1; a 6-h dose of TFM, followed by no TFM for 22 h, followed by a 3-h dose of TFM on February 20, 1991).

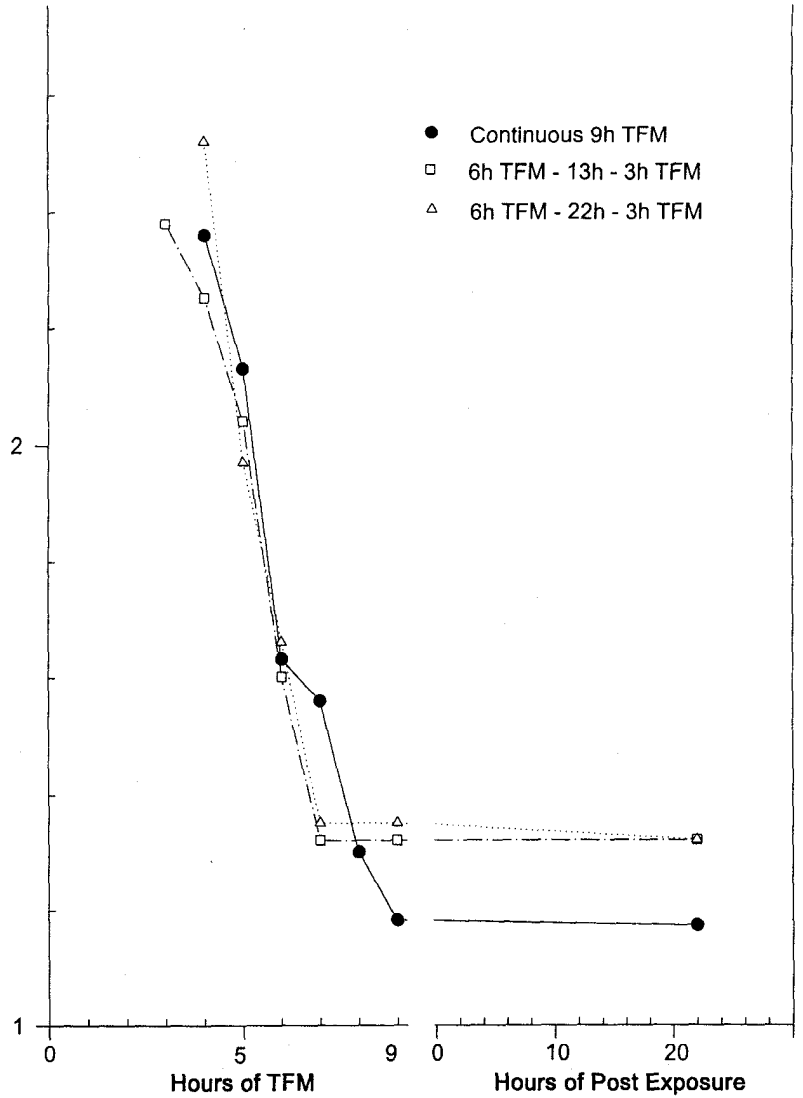

Fig. 4. Changes in Group-3 larval sea lamprey LC50 values during 9-h TFM exposures (a continuous 9-h dose of TFM on March 7, 1991; a 6-h dose of TFM, followed by no TFM for 13 h, followed by a 3-h dose of TFM on March 12, 199 1; a 6-h dose of TFM, followed by no TFM for 22 h, followed by a 3-h dose of TFM on March 19, 1991).

Table 2. Rainbow trout fry LC50 values (mg/L TFM; 95% confidence intervals in parentheses) at specified hours of TFM exposure during continuous or interrupted 9-h TFM exposures in 2 groups of tests. Values with different superscript letters within a group are significantly different (Litchfield and Wilcoxon method', $P \leq 0.05$).

Date	Source of fish	TFM treatment
	Group 4	
April 26, 1990	Northern Trout and Wildlife Farms	Continuous 9-h TFM
May 8, 1990	Northern Trout and Wildlife Farms	4-h TFM dose, 12 h no TFM, 5-h TFM dose
May 15, 1990	Northern Trout and Wildlife Farms	4-h TFM dose, 22 h no TFM 5-h TFM dose
	Group 5	
October 18, 1990	Cedarbrook Trout Farm	Continuous 9-h TFM
October 23, 1990	Cedarbrook Trout Farm	6-h TFM dose, 22 h no TFM, 3-h TFM dose

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Generally, most rainbow trout mortality occurred within 3 h after being exposed to the TFM-after 3 h of exposure, only a few fish died. For example, a 3-h exposure to 32 mg/L, TFM caused 70%-90% mortality. However, in half of the tests, 10%- 20% of the rainbow trout survived the full 9-h exposure to 32 mg/L TFM. Also, no rainbow trout died during the 22-h post-exposure period. Rainbow trout data (Table 2) for continuous and interrupted treatments indicate there were no significant differences among the LC50 values for 6- *or* 9-h TFM exposures and 22-h post exposures. Therefore, the 12- or 22-h interruption did little to affect the time to death or overall mortality for the rainbow trout.

Table 2, *continued*

TFM exposure times			22-h post-exposure time
3 h	6 h	9 h	
	Group 4		
21.1[x] (18.9-23.5)	18.8[x] (15.3-23.2)	18.8[x] (15.3-23.2)	18.8[x] (15.3-23.2)
22.0[x] (19.0-25.4)	21.1[x] (18.9-23.7)	21.1[x] (18.9-23.7)	21.1[x] (18.9-23.7)
	21.0[x] (17.4-25.3)	18.8[x] (16.3-21.7)	18.8[x] (16.3-21.7)
	Group 5		
28.8[y] (25.2-32.9)	24.7[x, y] (21.2-28.8)	21.2 (18.2-24.7)	21.2" (18.2-24.7)
28.3[y, z] (24.4-32.8)	23.2[x, z] (19.6-27.5)	23.2[x, z] (19.6-27.5)	23.2[x, z] (19.6-27.5)

[1]LC50 values were compared with the method of Litchfield and Wilcoxon (1949) only if the slope functions were not significantly different (the toxicity lines must be parallel).

For the 22-h post exposure, we observed no significant difference in the toxicity of TFM to rainbow trout among the continuous 9-h treatments and the treatments interrupted for 12 h or 22 h (Table 3). The rainbow trout LC25 values for the 9-h continuous and interrupted treatments ranged from 16.0 to 19.4 mg/L TFM and were not significantly different. The LC50 values ranged from 18.8 to 23.3 mg/L TFM and were not significantly different. A TFM treatment interrupted for up to 22 h can be resumed and not significantly change rainbow trout mortality.

Analysis of mayfly TFM-exposure vs. time data indicated that longer TFM exposures result in higher mortality. Very few mayfly nymphs died during the first 3 h of TFM exposure. Because of low mortality, LC50 values could not be determined until 6 h of exposure. The mayfly 6-h LC50 values (>11 mg/L, Fig. 5) were considerably greater than the sea lamprey 6-h LC50 values (< 3.2 mg/L, Figs. 2,3,4). At 9 h of exposure, the mayfly LC50 values (11- 14 mg/L, Fig. 5) did not indicate any substantial differences among the treatments. During the 22-h post-exposure period, mayfly mortality was very low for the interrupted treatments. The 22-h post-exposure LC50 values ranged from 10 to 14 mg/L for the interrupted treatments. However, for continuous treatment, considerable mayfly mortality occurred during the 22-h post-exposure period. The mayfly LC50 value declined to 7 mg/L for the continuous 9 h treatments, which was still considerably greater than the sea lamprey 22-h post-exposure LC50 values (<1.6 mg/L, Table 1).

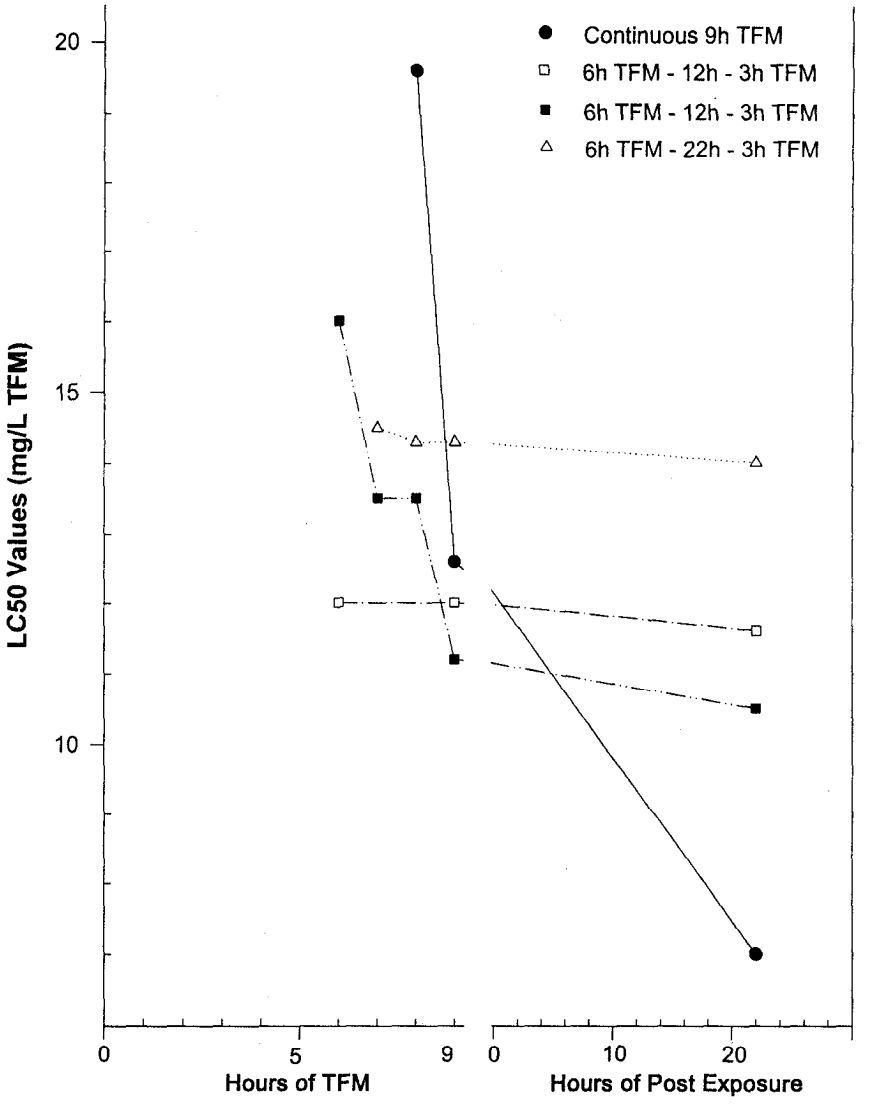

Fig. 5. Changes in mayfly LC50 values during 9-h TFM exposures (a continuous 9-h dose of TFM on January 3, 1991; a 6-h dose of TFM, followed by no TFM for 12 h, followed by a 3-h dose of TFM on January 15 and 23, 1991; a 6-h dose of TFM, followed by no TFM for 22 h, followed by a 3-h dose of TFM on January 8, 199 1).

Table 3. The 22-h post-exposure LC50 and LC25 values and slope function (toxicity lines) for rainbow trout fry exposed to continuous or interrupted 9-h TFM exposures in 2 groups of tests. Confidence intervals (95%) are in parentheses. Values with different superscript letters in a column within a group are significantly different.

Date	Source of fish	TFM treatment
	Group 4	
April 26, 1990	Northern Trout and Wildlife Farms	Continuous 9-h TFM
May 8, 1990	Northern Trout and Wildlife Farms	4-h TFM dose, 12 h no TFM, 5-h TFM dose
May 15, 1990	Northern Trout and Wildlife Farms	4-h TFM dose, 22 h no TFM, 5-h TFM dose
	Group 5	
October 18, 1990	Cedarbrook Trout Farm	Continuous 9-h TFM
October 23, 1990	Cedarbrook Trout Farm	6-h TFM dose, 22 h no TFM, 3 -h TFM dose

Continued on next page

The 22-h post-exposure results indicate that the toxicity of TFM to mayflies was greater in the continuous 9-h TFM treatment than in the interrupted treatments. The 22-h interrupted treatment appears to be the least toxic to mayflies (Table 4, Fig. 5). The LC25 and LC50 values of the 12-h interrupted test (January 15, 1991) could not be compared to the LC25 and LC50 values of the continuous 9-h test (January 3, 199 1) or the other 12-h interrupted test (January 23, 199 1) because the toxicity lines were not parallel (Table 4). The mayfly LC25 values ranged from 5.6 (continuous 9-h treatment) to 9.3 mg/L TFM (22-h interrupted treatment) and were not significantly

Table 3, *continued*

Slope function	LC50[1] (mg/L TFM)	LC25[2] (mg/L TFM)
	Group 4	
1.27 (1.09-1.47)[x]	18.8 (15.2-23.2)[x]	16.0 (12.7-20.2)[x]
1.14 (1.08-1.21)[x]	21.2 (18.9-23.7)[x]	19.4 (17.2-21.9)[x]
1.18 (1.10-1.26)[x]	18.8 (16.3-21.7)[x]	16.8 (14.4-19.6)[x]
	Group 5	
1.28 (1.13-1.45)[x]	21.2 (18.2-24.7)[x]	18.0 (15.2-21.3)[x]
1.31 (1.18-1.45)[x]	23.2 (19.6-27.5)[x]	19.4 (16.1-23.4)[x]

[1] LC50 values were compared with the method of Litchfield and Wilcoxon (1949) ($P \leq 0.05$) only if the slope functions were not significantly different (toxicity lines must be parallel).

[2] LC25 values were compared only if the slope functions were not significantly different. If the 95% confidence limits did not overlap, the LC25 values were considered significantly different.

different among treatments with parallel toxicity lines. The LC50 values ranged from 7.0 (continuous 9-h treatment) to 14.0 mg/L TFM (22-h interrupted treatment). The LC50 values for the continuous 9-h test were significantly different than the LC50 values for the 12-h interrupted test (January 23, 1991) and the 22-h interrupted test (January 8, 1991) (Table 4).

Table 4. The 22-h post-exposure LC50 and LC25 values and slope function (toxicity lines) for mayfly nymphs exposed to continuous or interrupted 9-h TFM exposures. The 95% confidence intervals are in parentheses. Slope-function values with different superscript letters in a column within a group are significantly different and the toxicity lines are not parallel. For parallel toxicity lines, LC values with different superscript letters in a column within a group are significantly different.

Date	TFM treatment	Slope function
	Group 6	
January 3, 1991	Continuous 9 h TFM	1.41 (1.24-1.61)x
January 15, 1991	6-h TFM dose, 12 h no TFM, 3-h TFM dose	2.53 (1.62-3.95)y
January 23, 1991	6-h TFM dose, 12 h no TFM, 3-h TFM dose	1.41 (1.18-1.68)
January 8, 1991	6-h TFM dose, 22 h no TFM, 3 -h TFM dose	1.80 (1.08-3.01)^{x,y}

Date	LC50[1] (mg/L TFM)	LC25[2] (mg/L TFM)
	Group 6	
January 3, 1991	7.0 (6.2-7.9)	5.6 (4.8-6.4)
January 15, 1991	11.6 (8.3-16.2)^{y}	6.2 (4.0-9.7)^{y}
January 23, 1991	10.5 (8.2-13.4)	8.3 (6.4-10.8)
January 8, 199 1	14.0 (10.8-18.1)^{y,z}	9.3 (6.1-14.3)^{y,z}

[1]LC50 values can be compared with the method of Litchfield and Wilcoxon ($P \leq 0.05$, 1949) only if the slope functions were not significantly different (toxicity lines must be parallel).
[2]LC25 values were compared only if the slope functions were not significantly different. If the 95% confidence limits did not overlap, the LC25 values were considered significantly different.

In our study, mortality of mayflies in the continuous 9-h test was greater than in the interrupted tests, and longer exposures resulted in greater mortality. The study of Bills et al. (1985) on mayflies also indicated that length of exposure to TFM was critical to mayfly survival. Of the mayflies exposed to:

- 5.0 mg/L TFM, 70% survived after 6 h but none survived after 9 h;
- 2.5 mg/L TFM, 80% survived after 9 h but only 40% survived after 12 h.

We speculate that if the period of continuous TFM exposure is reduced, mayfly survival might increase. Our study indicated that a continuous 9-h exposure to TFM was more toxic to mayflies than an interrupted 9-h exposure.

Our results (22-h post exposure) indicated mayflies were more resistant to TFM than larval sea lampreys (mayfly LC50 values >7 mg/L and sea lamprey LC50 values <1.4 mg/L) and were similar to those of three previous studies (Smith 1967; Fremling 1975; Maki et al. 1975). The authors of those studies cautioned that TFM concentrations normally used to control sea lampreys might also cause significant mayfly mortality. We suggest that interrupting TFM treatments for 12 h or less might reduce mayfly mortality without decreasing the effectiveness of the treatment for larval sea lampreys.

Historically, a lengthy interruption in lampricide application (because of equipment failure or changes in physicochemical characteristics of the stream) often resulted in treatment cancellation and retreatment at a later date. Our results indicate that short-term interruption of up to 12 h may not require treatment cancellations. The TFM application could be resumed and completed without reducing treatment effectiveness and, as compared to retreating, would result in a savings of lampricide, time, and money.

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