INVESTIGATIONS IN FISH CONTROL

58. Acute Toxicity of the Lampricide 3-Trifluoromethyl-4-nitrophenol (TFM) to Nymphs of Mayflies (Hexagenia sp.)

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ACUTE TOXICITY OF THE LAMPRICIDE 3-TRIFLUOROMETHYL-4-NITROPHENOL (TFM) TO NYMPHS OF MAYFLIES (HEXAGENIA SP.)

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ABSTRACT

A recycling test apparatus and burrow-containing artificial substrates were used to determine the toxicity of the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) against Hexagenia mayfly nymphs. Toxicity was relatively independent of temperature, but was greater in soft water than in hard water, and much greater at low than at high pH's; 12-h LC50's were 4.0 at pH 6.5 and 270.0 at pH 9.5.

INTRODUCTION

Hexagenia mayflies play a vital ecological role in lakes, rivers, and streams (Needham et al. 1935, Hunt 1953, Britt 1955, Fremling 1960, Swanson 1967). Nymphs of Hexagenia are important to fish because they convert organic detritus, algae, and bacteria into high quality fish food. The detritus-mayfly-fish food chain is short and efficient. Inasmuch as the life cycle of Hexagenia lasts at least a year in the Great Lakes and in most tributary streams, nymphs are available to fish in all seasons. Because the nymphs pass through many molts, sizes are available to suit the needs of most fish species.

Since Hexagenia nymphs prefer silt bottoms where they can construct burrows, they usually inhabit the same areas used by lamprey ammocoetes. Silted streams, for example, provide suitable habitat for both. It is important to determine the effect of lampricides on Hexagenia nymphs because they may be eradicated in lamprey control areas if the lampricides are toxic to them.

Hexagenia nymphs are good test organisms because they are easily collected and cultured and their large size makes them easy to handle and observe. Their tendency to abandon their burrows when stressed makes it possible to accurately assess early effects of toxicants.

METHODS

Either reconstituted water supplied by the Fish Control Laboratory, La Crosse, Wis., or water from a 12-m deep sand point well was used in the tests. The reconstituted water contained 48 mg/l of $NaHCO_3$, 30 mg/l of $CaSO_4$, 30 mg/l of $MgSO_4$, and 2 mg/l of KCl; was slightly alkaline (pH 7.2-7.6); and was soft (hardness 40-48 and alkalinity 30-35 as mg/l of $CaCO_3$).

The well water was hard, having a total alkalinity of 331, total hardness of 384, and calcium hardness of 260 (all as mg/l of $CaCO_3$). Resistivity at 25 C was 1,277 ohms and pH was 7.42. Chemical constitutents (mg/l) included ammonia nitrogen, 0.38; nitrite, 0.005; nitrate, 0.05; sulfate, 42.5; orthophosphate, 0.05; total iron, 0.28; manganese, <0.05; sodium, 32.5; calcium, 59.0; magnesium, 18.3; and potassium, 3.7.

Hexagenia nymphs were used as experimental animals, and cultures were maintained in the laboratory according to methods described by Fremling (1967). Relatively large nymphs (20-22 mm) were used in all experiments because they were easy to handle and observe. Last instar nymphs were not knowingly used because physiological stresses involved in transformation to the adult stage are atypical and emergence during the experiments was not desired. Although most test nymphs were H. bilineata from laboratory

cultures, their number was supplemented in all experiments by nymphs of H. bilineata and H. limbata collected from the Mississippi River. Species collected from the river were not separated because the nymphs were not in their last instar and because undue handling was undesirable. Nymphs collected from the river were placed in laboratory rearing tanks to acclimate for at least 1 wk before they were used in tests.

Nymphs were collected from the rearing tanks by gently sifting mud through a coarse screen; they were then transferred to fresh water where they acclimated for 6 h before being used in tests. A large syringe, filled with test water from the appropriate vessel, was used to flush the remaining nymphs from their burrows to determine if any were dead. Each nymph was classified as normal, dead, or stressed (as indicated by active swimming, rapid gill movements, or loss of equilibrium). All tests were conducted in a basement laboratory which had no windows. Overhead incandescent lamps provided constant light.

Special glass toxicity test vessels (Fig. 1) were assembled with silicone glue. Each

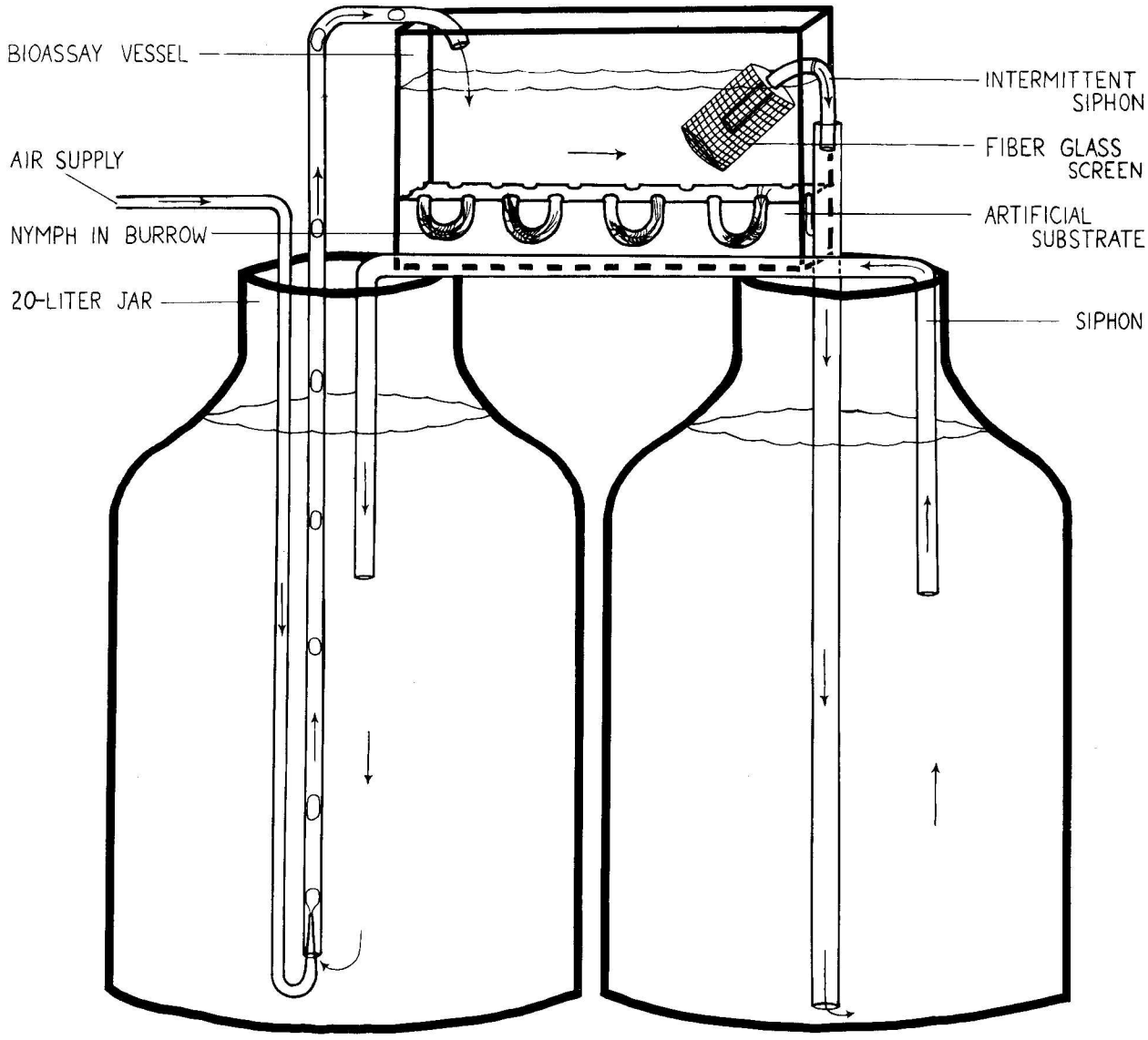

Fig. 1. Recycling bioassay apparatus with a burrow-containing epoxy substrate for use with Hexagenia mayfly nymphs and nonvolatile test chemicals.

vessel contained an epoxy resin substrate, 23 cm long, 5 cm wide, and 5 cm deep. Each substrate contained 10 burrows constructed as described by Fremling and Schoening (1973). Constant recirculation of test water through each vessel was assured by pumping water via an air lift (made of 4 and 6 mm I.D. glass tubing) from a 20-liter glass jar through the test vessel to another 20-liter glass jar via an intermittent siphon. A fiber glass screen prevented the escape of test animals. Water was constantly returned by a siphon (10 mm I.D. glass tubing) from the second jar to the first. Recirculation of the test water, which was permissible because TFM is relatively nonvolatile, made it possible to maintain precise control over toxicant concentrations. Accumulation of inhibitory concentrations of degradation and metabolic products was unlikely because of the large volume of water used (38 liters).

Six two-jar units were placed in each of two large water baths so that temperature could be accurately controlled. Each series of six units included one control unit in which no TFM was used and five units which contained various concentrations of TFM.

Stock solutions of field grade TFM (35.7%) and purified TFM (95%) were made by dissolving the chemical in acetone and diluting it with water. Stock solution was added in equal amounts to both jars of each unit, stirred thoroughly, and allowed to circulate between the jars until mixing was complete. Concentrations of TFM were monitored before nymphs were added and periodically throughout each experiment with a Beckman DB spectrophotometer (Olson and Marking 1973).

Waters of various hardnesses and pH's were used, as described by Marking (1969) and Marking and Dawson (1972), respectively. LC50 values and 95% confidence limits for each test were determined according to methods described by Litchfield and Wilcoxon (1949).

RESULTS

Purified and field grade TFM were toxic to mayfly nymphs at all temperatures tested (17.0 - 26.5 C--see Table 1). The change in toxicity was usually insignificant ($P>0.05$) for single temperature increments; an exception being the increment between 23.5 and 24.4 C in hard water at 24-h exposure. The exception indicates that TFM is more toxic at the higher of the two temperatures. Since the temperature difference is small, however, the data may reflect biological variation among groups of organisms rather than an influence of temperature. Although TFM is toxic in short

Table 1. Toxicity of TFM (based on active ingredient) to Hexagenia mayfly nymphs in waters of different hardness and temperature. LC50 values and 95% confidence intervals (in parentheses) are listed as µl/l for 35.7% TFM and as mg/l for 95% TFM.

Hardness (mg/l)	Temp. (°C)	Formulation of TFM (%)	Hours of exposure			
			6	12	24	96
384	17	35.7	-- --	10.5 (8.47-13.0)	6.50 (5.23-8.07)	3.90 (3.0-5.07)
384	21.8	95	-- --	-- --	6.00 (5.18-6.95)	4.30 (3.45-5.36)
384	23.5	95	--	10.5 (9.38-11.8)	7.00 (5.79-8.47)	4.20 (3.41-5.17)
384	24.4	95	11.2 (10.0-12.5)	6.50 (5.21-8.11)	3.50 (2.28-5.36)	-- --
44	18.2	35.7	-- --	-- --	4.75 (4.20-5.37)	2.50 (1.80-3.46)
44	26.5	35.7	-- --	4.70 (3.96-5.58)	3.50 (2.96-4.14)	2.18 (1.73-2.74)

exposures (12-h or less), LC50's at these exposures are not much greater than those for 24-h exposures. Comparisons made over a wider temperature range might show greater significance.

Water hardness influenced the toxicity of TFM to Hexagenia nymphs. After 24-h exposures, TFM was considerably more toxic in soft water than in hard water of similar pH (7.1-7.6) and temperature (Table 1). The 24-h LC50's were 6.50 and 4.75 μl/l of TFM in hard and soft water, respectively, at the lower temperatures (17.0 and 18.2 C). At the higher temperatures (24.4 and 26.5 C), TFM was more toxic in soft than in hard water at the 12-h exposure but the difference was nil at 24-h.

The toxicity of TFM to Hexagenia mayfly nymphs was influenced drastically by the pH of water (Table 2). The 24-h LC50 at pH 6.5 (2.50) was significantly greater ($P<0.05$) than that at pH 7.5 (3.35), and the LC50 at pH 8.5 (18.8) was more than 5 times the value at pH 7.5 (3.35). The toxicity of TFM to the nymphs was lowest at pH 9.5 and the 24-h LC50 was almost 70 times greater than that value at pH 6.5.

DISCUSSION

TFM is apparently less toxic to some invertebrate animals than it is to lampreys. Experiments by Erkkila (1962) revealed that concentrations of TFM below 20 μl/l caused insignificant mortality to isopods, gammarids, crayfish, dragonflies, water boatmen, and case-building caddisflies; that concentrations below 10 μl/l were harmless to glossiphonid leeches, stoneflies, bloodworms and snails; and that mortality was significant in Hexagenia at 6 μl/l. Smith (1967) showed that mortality of hydras, turbellarians, blackflies, and Hexagenia mayflies was almost complete in the laboratory when these animals were exposed to TFM at 10 μl/l for prolonged periods.

In the present study, TFM was toxic to Hexagenia mayflies in ranges similar to those reported above. Except at pH of 8.5 or over, the 24-h LC50 of TFM was always less than 10 μl/l. At low pH in soft water the material was especially toxic. At pH 6.5, for example, the 96-h LC50 was 1.18 (Table 2).

Table 2. Toxicity of TFM (35.7%) to Hexagenia mayfly nymphs in soft water (40-48 mg/l total hardness as $CaCO_3$) at temperatures of 22-23 C and at selected pH values. LC50 values and 95% confidence intervals (in parentheses) are listed as μl/l TFM.

pH	Hours of exposure				
	12	24	48	72	96
6.5	4.00 (3.47-4.61)	2.50 (2.16-2.90)	1.31 (1.04-1.65)	-- --	1.18 (0.91-1.53)
7.5	-- --	3.35 (2.99-3.76)	2.50 (2.17-2.87)	2.00 (1.63-2.46)	-- --
8.5	27.3 (22.4-33.0)	18.8 (16.6-21.4)	13.0[1]	-- --	5.00 (3.68-6.80)
9.5	270 (221-329)	174 (156-194)	100 (87.2-115)	64.2 (49.5-83.3)	60.0 (45.7-78.8)

[1]No confidence interval reported because of insufficient data.

Hexagenia nymphs are less sensitive than larval lampreys to TFM when both species are tested in standard laboratory water. Dawson et al. (in press) found the 24-h LC99 for ammocoetes to be 0.90 at pH 6.5, 3.25 at pH 7.5, and 12.0 at pH 8.5. In the present study, 24-h LC50 values for Hexagenia nymphs were 2.50 at pH 6.5, 3.35 at pH 7.5, and 18.8 at pH 8.5.

In all tests there was a marked tendency for treated nymphs to abandon their burrows for varying lengths of time before they actually succumbed. In nature, this behavior would, on one hand, increase the vulnerability of nymphs to predation; on the other hand, however, free-swimming nymphs might swim, or be swept by the current, out of the zone of lethal TFM concentrations. There is no assurance, however, that nymphs would find suitable substrate in an open lake or that they would recover from the effects of the toxicant.

Hexagenia mayflies are able to recolonize denuded areas by downstream drift of nymphs and by upstream flight of ovipositing adults (Fremling 1973). It is likely that Hexagenia populations killed by TFM applications would become reestablished. Complete reestablishment would probably require a year or more, however.

The artificial substrate apparatus used in this study proved very satisfactory as indicated by the fact that in 10 96-h tests the controls showed no mortality in three tests, 10% in five tests, 20% in one test, and 30% in one test. In the test in which mortality was 30%, two of the dead were nymphs which died during transformation to the subimaginal stage. Nymphs frequently molted to the next nymphal instar in the bioassay vessels.

Artificial substrates such as those used in this study provide semidarkness, thigmotactic surfaces, and seclusion for test nymphs. Consequently, the nymphs swim less and their susceptibility to toxicants is not enhanced by fatigue as it is in standard test vessels.

CONCLUSIONS

1. A recycling toxicity test apparatus with artificial substrates was suitable for tests of TFM against Hexagenia nymphs.

2. Toxicity of TFM to Hexagenia nymphs is relatively independent of temperature.

3. Toxicity of TFM to Hexagenia nymphs is greater in soft than in hard water.

4. Toxicity of TFM to Hexagenia nymphs is much greater at low than at high pH's.

5. Although TFM is more toxic to ammocetes than to Hexagenia nymphs in soft water, applications of TFM that exceed the minimum effective concentrations for lamprey larvae may kill the nymphs.

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