3-Trifluoromethyl-4-nitrophenol (TFM) 1 Control of Tadpoles in Culture Ponds

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Abstract

In definitive 96-hour toxicity tests, the LC50 for TFM to small bullfrog larvae was 1.39 mg/L compared to 5.50 mg/L for fathead minnows. Also, the 96-hour LC90 for TFM to bullfrog tadpoles was 2.31 mg/L and the LC10 for TFM to fathead minnows was 3.40 mg/L. On-site toxicity tests in three ponds using containers filled with pond water demonstrated that large and small bullfrog and green frog (R. clamitans) larvae were more sensitive to TFM than fathead minnows or bluegill (Lepomis macrochirus).

Fathead minnow (Pimephales promelas) production ponds in Ohio have yielded as much as 785 kg/ha (706 pounds/acre) of tadpoles, which represented approximately 78% of the standing crop in the pond (Kendall 1984). In southern states where the growing season is longer and ponds are not usually drained after harvest, tadpole biomass may represent an even greater problem as illustrated by the inadvertent 2,242 kg/ha (2,106 pounds/acre) tadpole production in Alabama minnow ponds (Prather et al. 1953). Incidental frog larvae production has also been noted by the Ohio Division of Wildlife in ponds producing percids and esocids (Clayton Lakes, Ohio Division of Wildlife, personal communication), and in National Hatchery facilities in Texas producing centrachids (Charmichael and Tomasso 1983). Tadpole contamination problems have also been noted in many private facilities in southern states raising catfish, baitfish, and ornamentals (Harry Dupree, U.S. Fish and Wildlife Service, Stuttgart, Arkansas, personal communication).

The presence of tadpoles may cause a variety of problems in fish culture ponds. When fish are harvested, tadpoles must be sorted and removed, which is time consuming and also causes physical injury to the cultured species. The presence of tadpoles in the harvest also makes accurate production quantification difficult. Frog larvae effectively compete for natural food resources (Dickman 1978; Seale 1980). Furthermore, tadpoles also readily consume both pelleted and flaked forms of artificial feeds used in fish production. Tadpoles may also inhibit fish growth as noted in some Illinois (Helms 1967) and central Ohio production ponds because they contribute to the total biomass of the pond, and may introduce interspecific social stress factors on the cultured fish species.

Currently, tadpoles are controlled by costly and often haphazard mechanical means such as removing the eggs by hand, using ducks to eat the eggs and larvae (Woynarovich 1975), killing adult frogs (Prather et al. 1953), or mowing pond banks to reduce frog habitat. As an alternative, a selective amphibicide which could control tadpoles without endangering production of target culture species, or adversely changing the balance of the pond community would be useful. At least two selective poisons have been used to attempt control of tadpoles in fish culture ponds: sodium acetarsenite (Paris green) baited with cottonseed meal (Hutchens and Nord 1955), and formalin (Helms 1967). Helms found that sodium acetarsenite was ineffective, and formalin pro-

1TFM is labeled for use by Great Lakes Fishery Research Personnel for use in controlling sea lamprey (Petromyzon marinus) only, and registration does not extend to tadpole control in static systems.
duced severe oxygen depletion in the ponds and complete tadpole control could not be achieved. Also, the minimum lethal concentration for bullfrog (Rana catesbeiana) larvae was near the maximum safe concentration for fish. Thus, no simple, safe chemical method exists to control frog larvae in culture facilities. Frog larvae (especially bullfrog tadpoles) are generally quite hardy and are usually more resistant to physical and chemical stress than many fish species. However, various chemical compounds may have the potential to be lethal to frog larvae at concentrations that would not impact the cultured species. The objectives of this study were to 1) identify a potentially selective amphibicide through preliminary screening of three selected compounds, 2) determine the relative toxicity of the successful candidate compound (TFM) in the laboratory to young bullfrog larvae and to the fathead minnow, and 3) ascertain comparative results of TFM toxicity under preliminary field conditions using on-site toxicity tests.

**Methods**

**Laboratory Studies**

Three compounds were evaluated for their selective toxicity to frog larvae: endosulphan (Thiodan®) (Mayer 1973; Gopal et al. 1981), tannic acid (George Nace, Amphitech Inc., Ann Arbor, Michigan, personal communication), and 3-trifluoromethyl-4-nitrophenol (TFM) (Chandler and Marking 1975; Marking and Olsen 1975).

Endosulphan (manufactured by Hoechst) is a 50% wetable powder. Stock solutions were prepared by dissolving in ETOH, which was then serially diluted with soft reconstituted water (Dawson et al. 1975) for test solutions. The control organisms exposed to endosulphan were also exposed to the highest ETOH concentrations as in the maximum exposure vessel. Technical grade tannic acid (MCB Manufacturing Chemists) stock solutions were made with double deionized water, which was serially diluted with reconstituted water. Formulated TFM (35.7% active ingredient (A.I.)), obtained from the National Fishery Research Laboratory, La Crosse, WI, was measured gravimetrically and diluted with double deionized water to prepare stock solutions. Stocks were serially diluted with reconstituted water for working solutions. Preliminary trials were conducted in 1 L beakers, using adult fathead minnows and small (first year) bullfrog larvae which were collected from central Ohio ponds, and laboratory acclimated for at least one week before testing.

**Laboratory Tests**

For definitive 96-hour tests (EPA 1975), small bullfrog larvae (50–60mm total length (T.L.)) and adult fathead minnows (45–60mm T.L.) were collected from central Ohio ponds and were acclimated in the laboratory for three weeks prior to testing. Field grade TFM (35.7% A.I.) was measured gravimetrically and diluted with double deionized water to prepare stock solutions for static tests. Fifty liters of soft reconstituted water (with a hardness and alkalinity of 45 and 32 mg/L CaCO₃ respectively and a pH of 7.4) was added to each of seventeen, 75-L, all-glass aquaria, and the appropriate amount of stock solution was pipetted to yield desired concentrations. The solutions were then mixed by stirring prior to introducing test animals. Ten fathead minnows or ten frog larvae were added to each test vessel; loading did not exceed 0.5g/L. Survival/mortality observations were made at 1, 3, 6, 12, 24, 48, 72, and 96 hours and were based on the absence of movement and a lack of response to gentle prodding. Dead organisms were removed after each observation.

**On-Site Toxicity Tests**

Tests were conducted in three ponds (A, B, and C) with different water qualities (Table 1). Hardness ranged from 140–220 mg/L and alkalinity from 70–110 mg/L for the three ponds. Average pH values were consistently above 7.0 and ranged from 7.7–9.2. In field tests, formulated TFM (36.1% A.I.) concentrations were based on active ingredient. In all cases, TFM was added to the test vessels and mixed thoroughly prior to introduction of the test organisms.
Table 1. Average hardness, alkalinity, and pH values for ponds A, B, and C.

<table>
<thead>
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<th>hardness*</th>
<th>alkalinity*</th>
<th>pH</th>
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<tbody>
<tr>
<td>pond A</td>
<td>220</td>
<td>110</td>
<td>7.8</td>
</tr>
<tr>
<td>pond B</td>
<td>150</td>
<td>70</td>
<td>9.2</td>
</tr>
<tr>
<td>pond C</td>
<td>140</td>
<td>80</td>
<td>7.7</td>
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*measured as mg/L CaCO₃

In pond A, 30-gal plastic garbage cans containing 92 L of pond water were suspended in the pond from a floating frame. Two separate tests were performed; in the first test 10 adult fathead minnows and 10 small bullfrog larvae were used, and in the second test 10 fathead minnows, 10 small bullfrog larvae, and 10 large (second year) bullfrog larvae were used. In pond B, polyethylene bags (0.96m x 1.65m x 4 mils) were filled with 214 L of pond water and suspended in the pond from a clothesline apparatus (Burress 1975). Fifteen small green frog larvae, 10 large bullfrog larvae, and 20 adult fathead minnows collected from the pond were introduced into each bag. In pond C, polyethylene bags were also used, exposing 20 small bullfrog larvae and 20 bluegill (Lepomis macrochirus) fingerlings.

TFM concentrations were determined colorimetrically (Olsen and Marking 1973) for all tests. An Orion model 701A pH/mV meter was used for pH measurements. Total hardness was measured in the laboratory by EDTA titration; total alkalinity was measured by potentiometric titration (APHA et al. 1976). For field tests, hardness and alkalinity were measured using Bausch and Lomb Spectrokits™. Laboratory tests were conducted in a Sherr environmental chamber with photoperiod set at 14 hours of daylight and 10 hours darkness; water temperature was maintained at 22°C (71.6°F). The methods of Litchfield and Wilcoxon (1949) were used in computation of the LC₅₀s and 95% confidence intervals.

Results

Laboratory Tests

In the preliminary trials, tannic acid was not selectively toxic to frog larvae, but endosulphan and TFM showed evidence of either similar or selective toxicity (Fig. 1). However, endosulphan was not tested further because of its low water solubility and environmental persistence (McEwen and Stephanson 1979) and high toxicity to mammals and wildfowl (Stetter 1983; Hudson et al. 1984). Therefore, only TFM was used in 96-hour toxicity tests.

Results of the definitive 96-hour bioassays show a definite, non-overlapping difference in confidence intervals for the LC₅₀s for TFM to bullfrog larvae and fathead minnows. The LC₅₀ for TFM to small bullfrog larvae and fathead minnows was 1.39 (95% C.I. = 1.08-1.78) and 5.50 (95% C.I. = 4.54-6.67) mg/L respectively. Thus, fathead minnows were significantly (3.96 times <1.43-10.93>) more resistant to TFM than bullfrog larvae. The 96-hour LC₉₀ for TFM to bullfrog larvae and the 96-hour LC₁₀ for TFM to fathead minnows was 2.31 and 3.40 mg/L respectively (Fig. 2).

Fathead minnows subjected to lethal doses of TFM showed classic signs of anoxia, including irregular opercular movement, gulping at the surface, and erratic swimming movements followed by a loss of balance and finally death with splayed opercula. Initially, tadpoles were hyperactive at the surface of the water and near the sides of the aquaria. Afterwards, they gulped air at the surface and exhibited diffi-
cully maintaining neutral or negative buoyancy, and weakened response to tactile stimuli. Most tadpoles succumbed at the surface of the aquaria. In almost all cases, the tadpoles had small (3–6 mm) (0.12–0.24 in) external necrotic lesions around the operculum.

On-Site Tests

In all trials, frog larvae were more sensitive to TFM than were fathead minnows (ponds A and B) or bluegill fry (Pond C) (Fig. 3). Trials in pond A show that large (second year) bullfrog larvae were more resistant than small (first year) larvae, and in pond B, green frog larvae were more sensitive to TFM than large bullfrog larvae.

Discussion

Data from both laboratory and on-site toxicity tests suggest that bluegill and fathead minnows are more resistant to TFM than large (second year) bullfrog larvae > small (first year) bullfrog larvae > green frog larvae. However, statistical analysis is not possible because of different water qualities at each site. Differences resulting between laboratory results and on-site tests agree with contentions that TFM is most effective in softer, more acidic waters (Marking and Olsen 1975; Schnick 1972).

Necrotic tissue about the operculum of moribund and dead tadpoles, and behavioral responses similar to those of TFM treated lampreys (Applegate et al. 1961) suggest an analogous response to TFM. Lampreys seem unable to conjugate sufficient quantities of TFM to render it harmless (Lech and Statham 1975), and to date, it is not known whether amphibians, like lampreys, also lack this ability. Closer physiological and biochemical examination will be necessary to determine the actual mode of action to tadpoles.

Both laboratory and on-site toxicity data suggest that 100% mortality to frog larvae could be achieved with minimal (or no) effect to the fish exposed. However, the LC100 calculated for TFM to minnows and the LC90 for TFM to tadpoles based on the definitive laboratory tests, indicate that only a narrow margin of safety exists for TFM to be used for selective control of young bullfrog larvae in fathead minnow production facilities. Toxicity data for other cultured fish species (Table 2) suggest that TFM could be used as a selective amphibicide in other production facilities as well. Nevertheless, on-site toxicity tests should be used to determine that safety margin and correct dosage.

TFM costs approximately $16.54/kg A.I. Based on the amount of TFM needed to selectively control young bullfrog larvae in our bluegill production pond (pond C) dictated by the on-site bag test (3.0 mg/L), the approximate cost would be $56.25 per acre-foot. However, in situations with harder, more alkaline water (such as pond A), a greater quantity of TFM would be needed (11.0 mg/L) and the estimated costs here could range as high as $206.25 per acre-foot. This estimated four-fold difference in price to control frog larvae emphasizes the need to conduct a cost-benefit analysis before treatment.

Several additional factors beyond cost-effectiveness should be considered in order to appropriately time a TFM application in a production impoundment. As TFM is known to have an algistatic effect (Maki 1980), it might not be advisable to make an application when plankton growth is being encouraged. However, analysis of collections of plankton and
Fig. 3. Results (% mortality) from on-site toxicity tests with TFM.

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Table 2. Toxicity of field grade TFM (35.4%) to fingerling fish in toxicity tests at 12°C.

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<thead>
<tr>
<th>Fish Species</th>
<th>LC_{50} (mg/L)</th>
<th>95% C.I. (mg/L)</th>
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<tr>
<td>goldfish (Carassius auratus)</td>
<td>5.00</td>
<td>(3.97–6.29)</td>
</tr>
<tr>
<td>golden shiner (Notemogonus crysoleucus)</td>
<td>7.62</td>
<td>(6.29–9.23)</td>
</tr>
<tr>
<td>green sunfish (Lepomis cyanellus)</td>
<td>9.40</td>
<td>(7.88–11.20)</td>
</tr>
<tr>
<td>bluegill (Lepomis macrochirus)</td>
<td>16.2</td>
<td>(13.6–19.3)</td>
</tr>
<tr>
<td>largemouth bass (Micropterus salmoides)</td>
<td>6.04</td>
<td>(4.07–8.97)</td>
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*a*from Marking and Olsen (1975).

benthic organisms in TFM treated ponds in Arkansas indicated that food organisms were not affected by applications at concentrations of 12.0 and 14.0 mg/L (USFWS 1963). On the other hand, where artificial diets are used and natural plankton availability is not essential, early application would be desirable to reduce interspecific competition and permit time for natural photodegradation to occur (Carey and Fox 1981) before draining for harvest. Frogs have an extended breeding season in some latitudes, wherein an early application would not be desirable unless frog egg-laying had ceased, or TFM residuals in the pond would be sufficient to control the eggs or newly hatched larvae. Studies on TFM toxicities to various life stages of frog larvae, as well as in-pond studies could refine the technique and further elucidate the environmental fate of TFM in static systems. Other mononitrophenol compounds should also be screened in the search for other effective control agents.

**Acknowledgments**

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