Transactions of the American Fisheries Society

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/utaf20

Toxicity of the Piscicide Rotenone to Columbia Spotted Frog and Boreal Toad Tadpoles

Hilary G. Billman [a b], Sophie St-Hilaire [a], Carter G. Kruse [c], Teri S. Peterson [d] & Charles R. Peterson [a]

[a] Department of Biological Sciences, Idaho State University, 921 South 8th Avenue, Pocatello, Idaho, 83209, USA

[b] Bozeman Fish Technology Center, 4050 Bridger Canyon Road, Bozeman, Montana, 59715, USA

[c] Turner Enterprises, Inc., 1123 Research Drive, Bozeman, Montana, 59718, USA

[d] College of Business, Idaho State University, 921 South 8th Avenue, Pocatello, Idaho, 83209, USA

Available online: 27 Jul 2011

To cite this article: Hilary G. Billman, Sophie St-Hilaire, Carter G. Kruse, Teri S. Peterson & Charles R. Peterson (2011): Toxicity of the Piscicide Rotenone to Columbia Spotted Frog and Boreal Toad Tadpoles, Transactions of the American Fisheries Society, 140:4, 919-927

To link to this article: http://dx.doi.org/10.1080/00028487.2011.599260

Transactions of the American Fisheries Society 140:919–927, 2011

ISSN: 0002-8487 print / 1548-8659 online
DOI: 10.1080/00028487.2011.599260

ARTICLE

Toxicity of the Piscicide Rotenone to Columbia Spotted Frog and Boreal Toad Tadpoles

Hilary G. Billman*[1] and Sophie St-Hilaire
Department of Biological Sciences, Idaho State University, 921 South 8th Avenue, Pocatello, Idaho 83209, USA

Carter G. Kruse
Turner Enterprises, Inc., 1123 Research Drive, Bozeman, Montana 59718, USA

Teri S. Peterson
College of Business, Idaho State University, 921 South 8th Avenue, Pocatello, Idaho 83209, USA

Charles R. Peterson
Department of Biological Sciences, Idaho State University, 921 South 8th Avenue, Pocatello, Idaho 83209, USA

Abstract

The piscicide rotenone is commonly used to remove nonnative fishes from natural aquatic systems. While the effects of rotenone on fish are well documented, the effects of this chemical on amphibians are less well known. We determined the toxicity of the rotenone formulation CFT Legumine (5% rotenone) to three ages—Gosner age ranges 21–25, 30–35, and 40–45—of tadpoles of the Columbia spotted frog *Rana luteiventris* and the boreal toad *Anaxyrus boreas* under laboratory conditions. Tadpoles of both species were exposed to 0.1, 0.5, and 1.0 mg/L CFT Legumine (0.005, 0.025, and 0.050 mg/L rotenone, respectively) in static, 96-h exposure trials; surviving individuals were placed in rotenone-free water and raised until metamorphosis. In an additional experiment, Columbia spotted frog tadpoles were exposed to 1.0 mg/L CFT Legumine for 1, 2, 3, or 4 h before being placed in rotenone-free water for the duration of a 96-h exposure period. Tadpole mortality increased with increases in CFT Legumine concentration and exposure period. Individuals exposed to 1.0 mg/L of product experienced significantly greater mortality than did control tadpoles ($P < 0.001$), with 99–100% mortality occurring in the youngest age-group (Gosner 21–25) in both species. In Columbia spotted frog tadpoles, mortality decreased as age increased, while age did not affect mortality in boreal toad tadpoles. Rotenone produced no biologically significant effects on growth or metamorphosis. Our findings suggest that the use of 1.0 mg/L CFT Legumine to remove nonnative fish may cause significant mortality to larval amphibians if they are exposed for 96 h; exposures to lower dosages (0.5 mg/L of product) or for shorter durations (≤4 h), however, resulted in less mortality. Fisheries managers can use these results to improve amphibian conservation in fish restoration areas and reduce the impacts on larval amphibian populations.

Native salmonid species are experiencing significant declines throughout much of the United States. In some cases, this decline has been attributed to the presence of nonnative fish species (Behnke 1992; Finlayson et al. 2005; Hamilton et al. 2009). In an effort to reverse this trend, a growing number of fisheries managers are adopting programs to remove nonnative fish. Fish removal can be accomplished by a variety of techniques, but an effective method for large-scale, complete

*Corresponding author: hilary.billman@montana.edu
[1]Present address: Bozeman Fish Technology Center, 4050 Bridger Canyon Road, Bozeman, Montana 59715, USA.
Received March 18, 2010; accepted February 18, 2011

removal has been the use of the piscicides rotenone and antimycin (Finlayson et al. 2005, 2010a; Moore et al. 2008), which are approved by the U.S. Environmental Protection Agency (EPA). Chemical removal, especially in larger systems with complex habitat, is often more cost and time effective, and has a higher probability of success than traditional methods, like gillnetting or electrofishing (Shepard et al. 2002). As a result, the use of piscicides in fisheries management is increasing (Mangum and Madrigal 1999; Finlayson et al. 2000; Ling 2002; McClay 2005; Hamilton et al. 2009). Rotenone products, in particular, have a proven record of consistent and efficacious removal of undesirable fish (McClay 2005; Finlayson et al. 2010a).

Rotenone is an organic compound made from the roots of tropical legumes (Fontenot et al. 1994; Bettoli and Maceina 1996). In water, it is readily absorbed across the gill epithelium layer of aquatic species, and induces mortality by acting as a phosphorylation inhibitor (Hollingworth 2001). Rotenone's effects on fish are well documented (Meadows 1973; Amey 1984; Finlayson et al. 2000; Britton 2006; Grisak et al. 2007a), but effects on nontarget aquatic organisms, like amphibians, are not well understood. Because rotenone enters readily across the gill membrane, larval amphibians have the potential to be negatively affected by the use of this piscicide (Fontenot et al. 1994; McCoid and Bettoli 1996; Patla 2005). As a result, some rotenone applications to aquatic systems have been timed to avoid amphibian breeding (Mullin et al. 2004; Walston and Mullin 2007). However, in regions with extended winters and late springs, like the Rocky Mountain West in the United States, application of rotenone is often concurrent with the breeding season or larval period of amphibians. This may have negative consequences for larval amphibian populations.

Amphibians are an important component of aquatic ecosystems and, in the western United States, fish removal projects present an interesting amphibian conservation dilemma. Introduced fishes can often reduce amphibian populations through predation (Knapp et al. 2007), and removal of nonnative fish is likely to have an overall positive effect on amphibian populations (Bradford et al. 1993; Mullin et al. 2004; Knapp 2005; Walston and Mullin 2007). However, the use of chemicals, like rotenone, to accomplish this removal has the potential to negatively affect nontarget larval amphibian populations by causing mortality during their application. Given the increased use of rotenone and its indiscriminate effects on gill-breathing organisms, it is important to improve our understanding of its effects on amphibians to conserve local populations.

Rotenone products (5% active ingredient) are typically applied to streams and ponds at a formulation concentration range of 0.5–1.0 mg/L (0.025–0.050 mg/L rotenone) for the removal of nonnative trout (Finlayson et al. 2000, 2010a, 2010b; Grisak et al. 2007b). Allowable application concentrations can be as high as 4 mg/L (0.20 mg/L rotenone) and as low as 0.1 mg/L (0.005 mg/L rotenone). Rotenone exposure in lentic systems may last for several weeks, but rotenone exposure in lotic systems typically lasts for 4–8 h (Prentiss, Inc., Alpharetta, Georgia).

Early research provides an initial understanding of the general effects of rotenone at a range of doses on both adult and larval frogs. Farringer (1972) reported LC50 values of greater than 3.2 mg/L (product) exposure (Noxfish formulation; produced by Prentiss, Inc., Alpharetta, Georgia) for adult northern leopard frogs *Rana pipiens*, indicating high tolerance of the piscicide in adults as nongill breathers. The 24-h LC50 values for tadpoles of various species in another study, however, were below or within the range of doses used for fish removal, and demonstrated that rotenone applications could have negative effects on larval amphibians (Fontenot et al. 1994). Interestingly, Hamilton (1941), in one of the earliest studies addressing the effects of rotenone products on larval amphibians, recorded LC50 values of 0.1 mg/L of product (powdered derris in water [5% or 0.005 mg/L rotenone]) (Fontenot et al. 1994). This perhaps indicates that, for this particular formulation, there is no concentration low enough to be tolerated by tadpoles that could still potentially negatively affect fish.

These studies begin to provide important information on the response of tadpoles to rotenone exposure, but they do not explain the seemingly different effects of rotenone on multi-aged, multispecies tadpole cohorts observed during field treatments (C. Kruse, Turner Enterprises, personal observation). Among other things, the effects of treatment dose, age, and species on rotenone's toxicity to amphibian larvae need further investigation because these factors could potentially affect the toxicity of rotenone to tadpoles. Additionally, it is not known whether rotenone exposure results in sublethal effects or if all durations of exposure to rotenone are lethal to tadpoles. The objectives of this study were to determine (1) sensitivities of different ages of tadpoles of the Columbia spotted frog *R. luteiventris* and the boreal toad *Anaxyrus boreas* to multiple rotenone concentrations bracketing standard levels used for trout removal, (2) possible sublethal, chronic effects of rotenone exposure on tadpole growth and metamorphosis, and (3) whether the duration of rotenone exposure affects the probability of tadpole mortality. By selecting two species that frequently coexist with fish in the intermountain region of the western United States, the overarching goal of this study was to provide fisheries managers with information that would enable amphibian conservation in conjunction with native fish restoration.

METHODS

Approximately 1,500 early-age (Gosner stage 21–25, Gosner 1960) tadpoles of the Columbia spotted frog and the boreal toad were collected from a single pond in southwestern Montana in early May 2008 and 2009. Tadpoles were collected by dip net from multiple clutches and transported 40 km to a private laboratory in Bozeman, Montana, where they were evenly distributed among four 379-L (100 gal) outdoor holding tanks containing well water (pH 7.5–7.6). Water temperatures in the holding tanks fluctuated with ambient outdoor temperatures and a portion of each tank was shaded. Well water, as opposed to municipal tap water, was used because it was not chlorinated.

Tadpoles were fed a mixed diet of algae wafers and Mazuri dry animal meal (National Aquatic Species Restoration Facility, Alamosa, Colorado) as needed to maintain a constantly available food source. Ammonia and nitrate levels in the holding tanks were monitored daily with a LaMotte ammonia nitrogen test kit (LaMotte, Chestertown, Maryland). To prevent complications from poor water quality (i.e., high ammonia), holding-tank water was changed every other day or when ammonia levels were greater than 0.25 mg/L (McDiarmid and Altig 1999).

Exposure trials.—Exposure trials addressing treatment dose, age, and species effects were conducted as a 3 × 4 factorial experiment (three tadpole ages tested at four treatment levels). Tadpoles of both species at three different ages—early (Gosner age range, 21–25), middle (Gosner age range, 30–35), and late (Gosner age range, 40–45)—were exposed to four treatments: a control treatment and three rotenone treatments. Rotenone treatments used CFT Legumine (5% active rotenone; produced by Prentiss) at 0.1, 0.5, and 1.0 mg/L, where 1.0 mg/L represents a commonly recommended field dose for streams and ponds (Grisak et al. 2007b; Prentiss, Inc., Alpharetta, Georgia, CFT Legumine label). Glass fish tanks (7.6 L) with 6 L of the nonchlorinated well water were used in the experiments with eight replicate tanks per treatment level. Water used in the test tanks was held at ambient room temperature (19–21°C) and at a pH of 7.5. Before beginning a trial, tadpoles were removed from the outdoor holding tanks and held indoors in a 38-L tank for 24 h.

Because of laboratory constraints, the exposure trials for Columbia spotted frog tadpoles were conducted in 2008 while those for boreal toad tadpoles were completed in 2009. However, to test for species effects within the same year, an exposure trial at the 1.0-mg/L treatment level (all age-groups) for the Columbia spotted frog was repeated alongside the boreal toad trials in 2009. For the first two Columbia spotted frog age-groups, 10 tadpoles were randomly assigned to each of 32 experimental tanks (four dosages × eight replicates). In the late-age trial for this species, the number per tank was decreased to five to protect against maintenance-related mortalities. For the trial of the youngest boreal toad tadpoles, 10 tadpoles were randomly assigned to each of the 32 experimental tanks (four dosages × eight replicates). As these tadpoles increased in age and size, the number per tank decreased to five tadpoles (Gosner stage, 30–35) and three tadpoles (Gosner stage, 40–45). Tanks were randomly assigned to treatment (dosage) levels. Tadpoles were given approximately 1 h of acclimation time in the actual test tanks and fed a portion of an algae wafer before the treatment application. The temperature in the laboratory always ranged from 19°C to 21°C.

A treatment solution was made by combining 0.25 mL of well-agitated CFT Legumine from a recently opened stock barrel and 625 mL of nonchlorinated well water. The amount of the solution delivered to each tank depended on the treatment level (0.1, 0.5, or 1.0 mg/L). Control tanks received 15 mL of nonchlorinated well water, the same volume of water as rotenone product dispensed to the 1.0-mg/L treatment level. Different pipettes were used for each treatment group and tank water was mixed after receiving the treatment for even distribution. Because a large number of the oldest (Gosner 40–45) Columbia spotted frog tadpoles survived the highest treatment level (1.0 mg/L) in 2008, an additional trial at a treatment level of 2.0 mg/L was conducted to determine whether these older tadpoles were affected by a higher concentration that could be considered to be at the upper end of dosages used in the intermountain west. All methods for this trial were as described above. Tadpole mortality was assessed every 2 h for the first 10 h after treatment and then twice a day for three subsequent days, as per American Society for Testing and Materials (ASTM) standards (ASTM 2002). Mortality was determined visually (tail curling) and physically (lack of response to gentle prodding).

Sublethal effects trials.—After the 96-h exposure to 1.0 mg/L CFT Legumine, survivors at each age-group were pooled into tanks containing rotenone-free water and monitored until metamorphosis; an equal number of control tadpoles were kept separately under similar conditions. The number of replicate tanks and tadpoles per tank varied, depending on the number of survivors and laboratory space, but tadpoles were allocated to minimize density-related effects on growth and survival. The number of tadpoles per tank was the same between treatments, and food was dispensed as evenly as possible across tanks. Delayed mortalities were documented and visual assessments of development (changes in size, body shape, and tail absorption) were monitored. Metamorphosis was determined visually by the loss of the tail. Upon reaching this stage, these individuals were weighed, measured from snout to urostyle (snout–urostyle length, SUL), and then euthanized with an overdose of tricaine methanesulfonate (MS-222). Time to metamorphosis was recorded.

Length of exposure trials.—In 2009, all three age-groups of Columbia spotted frog tadpoles, collected and housed as described above, were exposed to CFT Legumine for one of five treatment times—1.0 mg/L for 1, 2, 3, 4, or 96 h—and then placed in rotenone-free water. There were six replicate tanks per treatment. For the youngest age trial, 10 tadpoles were randomly assigned to each of the tanks. As tadpoles increased in size, the number per tank decreased to five tadpoles (middle age-group) and three tadpoles (late age-group). Untreated tanks were used as controls during each set of treatments. Tadpole mortality was assessed for all treatment groups every 2 h for the first 10 h after treatment and then twice a day for three subsequent days, as per ASTM standards (ASTM 2002).

Data analyses.—Data from the 2008 and 2009 exposure trials were used in separate analyses in the following ways. Dosage effects for Columbia spotted frog tadpoles were assessed by plotting 2008 mortality data by treatment level against time for each age-group separately, while 2009 mortality data were used to determine dosage effects for boreal toad tadpoles. Age and species effects in both boreal toads and Columbia spotted frogs were determined separately at the 1.0-mg/L treatment level with 2009 mortality data only. To assess age effects, mortality at the field application dose of 1.0 mg/L was compared across age-groups by means of logistic regression, in which tadpole age

was a predictor of mortality, the binomial response. Tank effects were tested for by nesting tanks within treatment. The interaction between age and species at the 1.0-mg/L treatment level was tested with a cross-product test in the logistic regression analysis.

Weight, SUL, and time to metamorphosis between exposed and unexposed tadpoles at each age-group for which there were data were compared statistically for Columbia spotted frogs by means of an analysis of variance (ANOVA) and a Kruskal–Wallace test. Delayed mortality was defined as mortality that occurred after the initial 96-h exposure trial was complete. Delayed mortalities of control and treated tadpoles were compared for Columbia spotted frogs by means of a Fisher's exact test and for boreal toads with logistic regression.

The effects of exposure at 1.0 mg/L for 4 h were compared with those of exposure to 1.0 mg/L for 96 h by means of a Fisher's exact test. This analysis was run for each age-group. All statistical analyses were run in Minitab version 16 (Minitab 2010).

RESULTS

There were no tank effects detected in any of the exposure or exposure–duration trials (all P-values ≥ 0.07). We could not determine tank effect in all of the sublethal trials because in some instances we only had one tank for each treatment group.

Exposure Trials

Rotenone exposure for 96 h caused mortality in tadpoles in both species at all ages tested, but mortality was not uniform across doses. Mortality generally decreased as age increased for the Columbia spotted frog tadpoles (Figure 1; Table 1). The youngest tadpole age-group of both species experienced mortality sooner (within 2–4 h) than did older individuals (8–10 h). Overall, Columbia spotted frog tadpoles appeared less sensitive to rotenone than did boreal toad tadpoles (Figures 1, 2; Table 2).

In 2008, early-age Columbia spotted frog tadpoles treated at 1.0 mg/L for 96 h experienced average mortality of 100%. Average mortality at this treatment level declined for the middle and older age-groups (Figure 1; Table 1). Mortality of Columbia spotted frog tadpoles treated at 0.5 mg/L for 96 h did not occur as quickly and with the exception of the youngest age was, on average, less than that observed at the 1.0-mg/L treatment (Figure 1; Table 2). Mortality at the 0.1-mg/L treatment was low for all age-groups. The 2.0-mg/L treatment caused 100% mortality after 96 h in late-age Columbia spotted frog tadpoles (Figure 1; Table 2).

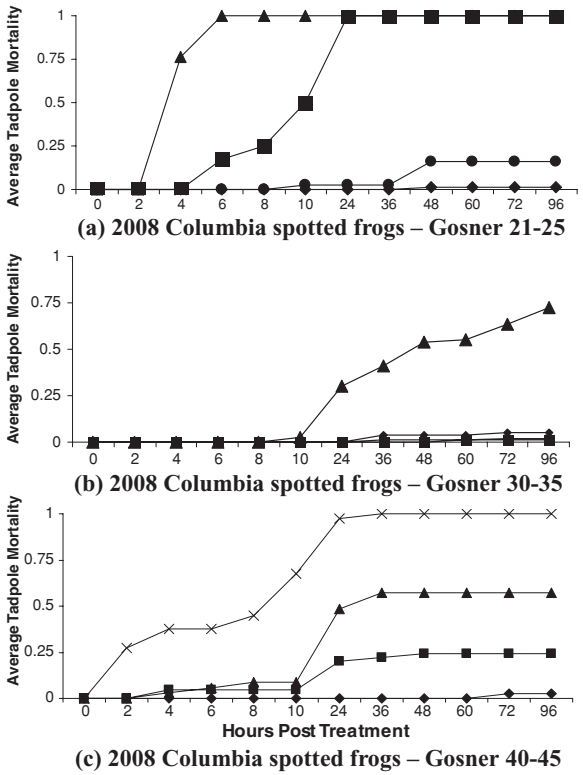

FIGURE 1. Cumulative mortality curves for Columbia spotted frog tadpoles at **(a)** Gosner age 21–25, **(b)** Gosner age 30–35, and **(c)** Gosner age 40–45 exposed to different concentrations of rotenone in 2008. Triangles denote the 1.0-mg/L treatment, squares the 0.5-mg/L treatment, circles the 0.1-mg/L treatment, and diamonds the control group. The stars in panel (c) represent a 2.0-mg/L treatment. Average mortality was standardized by the number of tadpoles per tank.

TABLE 1. Average ± SD mortality in 2008 and 2009 of Columbia spotted frog tadpoles at three ages after 96 h of exposure to CFT Legumine at 1.0 mg/L. Exposed tadpoles were significantly more likely to die than controls (z). Analysis of 2009 mortality data indicated a significant difference in mortality between exposed late-age tadpoles and both early- and middle-age individuals (y).

Gosner age-group	2008 mortality	2009 mortality
21–25	1.0 z	1.0 zy
30–35	0.73 ± 0.24 z	1.0 zy
40–45	0.57 ± 0.33 z	0.06 ± 0.05 z

In 2009, we documented complete mortality in early- and middle-age Columbia spotted frog tadpoles after 96 h of exposure to CFT Legumine at 1.0 mg/L, while late-age tadpoles that were similarly exposed experienced very low average mortality (Table 1). The difference between the mortality rates of late and early or middle age-group Columbia spotted frog tadpoles in 2009 was significant (late versus early age: $Z = -5.20$, $P < 0.001$; late versus middle age: $Z = -4.51$, $P < 0.001$). Exposed boreal toad tadpoles experienced high average mortality after 96 h of exposure to 1.0 mg/L in the early, middle, and late ages (Table 2). Age did not significantly affect mortality in boreal toad tadpoles at this treatment level (Figure 2; Table 2; P-values ≥ 0.07). Similar to what was observed in Columbia spotted frogs, mortality in boreal toad tadpoles treated at 0.5 mg/L for 96 h occurred later and declined as tadpole age increased (Figure 2; Table 2).

TABLE 2. Average ± SD mortality of Columbia spotted frog (SF) and boreal toad (BT) tadpoles at three ages after 96 h of exposure to CFT Legumine at all treatment doses. In some cases, exposed tadpoles were significantly more likely to die than were controls (z). Late-age boreal toad tadpoles experienced significantly higher mortality than did frog tadpoles at the same age (y).

	Treatment dose			
Species (Gosner age-group)	0.1 mg/L	0.5 mg/L	1.0 mg/L	2.0 mg/L (2008 only)
SF (21–25)	0.01 ± 0.31	1.0 z	1.0 z	
BT (21–25)	0	0.48 ± 0.20 z	0.99 ± 0.04 z	
SF (30–35)	0.02 ± 0.05	0.02 ± 0.03	1.0 z	
BT (30–35)	0.25 ± 0.32 z	0.38 ± 0.47 z	0.83 ± 0.20 z	
SF (40–45)		0.25 ± 0.31 z	0.06 ± 0.14	1.0 z
BT (40–45)	0	0.17 ± 0.25	0.96 ± 0.12 zy	

No statistically significant effect of species on mortality in tadpoles exposed to CFT Legumine at 1.0 mg/L in 2009 at the early and middle ages was evident ($Z = -0.65$, $P = 0.51$). However, there was a significant effect of species in the late age-group. Columbia spotted frog tadpoles at this age appeared more resistant to the effects of rotenone than did boreal toad tadpoles ($Z = -5.47$, $P < 0.001$).

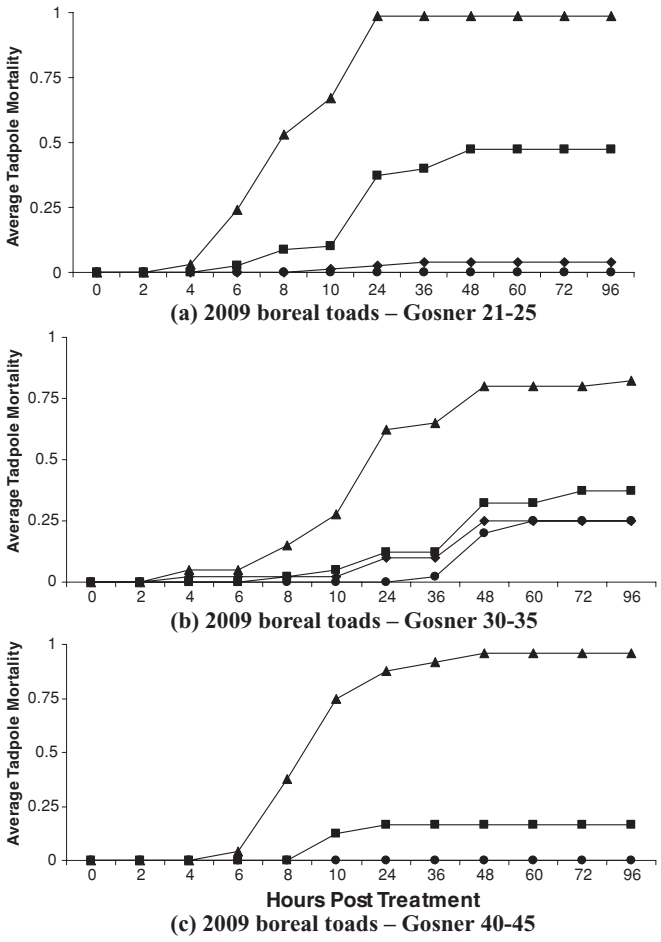

FIGURE 2. Cumulative mortality curves for boreal toad tadpoles at **(a)** Gosner age 21–25, **(b)** Gosner age 30–35, and **(c)** Gosner age 40–45 exposed to different concentrations of rotenone in 2009. See Figure 1 for additional details.

Sublethal Effects Trials

The Columbia spotted frog survivors used for these trials included exposed (1.0 mg/L) and unexposed tadpoles from the middle and late age-groups treated in 2008. We had too few survivors in the early stage to include in the sublethal effects analysis. There were no significant differences in measured traits between exposed and control tadpoles in the middle age-group. Among late-age Columbia spotted frog tadpoles, individuals exposed at 1.0 mg/L were 0.38 g heavier than their negative control counterparts ($F_{1, 25} = 5.19$, $P = 0.031$). In 2009, there were an insufficient number of boreal toad tadpole survivors at the early and late ages, so the only individuals included in this portion of the research were exposed (1.0 mg/L) and unexposed middle-age individuals. We did not document significant differences in measured traits observed between exposed and control middle-age boreal toad tadpoles (all P-values > 0.07).

There was no significant difference in delayed mortality between control and exposed middle-age boreal toad tadpoles, though there was a substantial difference in delayed mortality between exposed and control Columbia spotted frog tadpoles in the middle age-group. In the middle-age range, treated (1.0 mg/L) Columbia spotted frog tadpoles had a total delayed mortality of 0.59 ($N = 13$ out of 22) compared with only 0.09 for the control tadpoles. When this total delayed mortality is added to the mortality observed during the actual exposure trial, cumulative mortality of the middle-age Columbia spotted frog tadpoles treated at 1.0 mg/L in 2008 was 0.80, which was closer to the complete mortality seen in 2009. There were no observed delayed mortalities in either control or treated surviving Columbia spotted frog tadpoles in the late-age-range trial.

Length of Exposure Trials

Columbia spotted frog tadpoles exposed to 1.0 mg/L CFT Legumine for less than 4 h and then revived in freshwater experienced very low mortality, so only results comparing 4 h and 96 h of exposure are reported here. There was significantly less mortality between both early and middle-age Columbia spotted

TABLE 3. Average ± SD mortality in 2009 of Columbia spotted frog tadpoles at three ages after 4 and 96 h of exposure to CFT Legumine at 1.0 mg/L. Tadpoles exposed for 4 h were significantly less likely to die than those exposed for 96 h in both the early- and middle-age trials, while there was no difference in mortality at the late age.

Gosner age-group	Average mortality (4-h treatment)	Average mortality (96-h treatment)	Fisher's exact test
21–25	0.83 ± 0.20	1.0	$P < 0.001$
30–35	0.20 ± 0.25	1.0	$P < 0.001$
40–45	0.17 ± 0.28	0.06 ± 0.14	$P = 0.602$

frog tadpoles exposed at 1.0 mg/L for 4 h and those exposed for 96 h ($P < 0.001$ at both age-groups) (Table 3), but not in the oldest age-group. Survival of individuals exposed for 4 h and then revived was, on average, 0.92, 0.80, and 0.83 for early-, middle-, and late-age tadpoles, respectively. By comparison, there was no survival of early- and middle-age tadpoles exposed at 1.0 mg/L for 96 h in 2009, while the oldest-age tadpoles exposed for the full 96 h experienced high survival (Table 3).

DISCUSSION

This study suggests that rotenone can be lethal to Columbia spotted frog and boreal toad tadpoles at different dose levels, including one commonly used in fish removal projects (1.0 mg/L of product), especially in lentic systems where larval amphibians can be exposed to rotenone treatments for much longer than the typical stream treatment of 4–8 h. These results are similar to those found in two recent studies showing that rotenone is lethal to larvae of multiple amphibian species, often at doses well below levels regularly used in field application (Grisak et al. 2007b; E. E. Little and R. D. Calfee, U.S. Geological Survey, unpublished data). Our results demonstrate to some extent, a dose–response relationship, indicating that lower doses of, or shorter exposure to, CFT Legumine resulted in fewer mortalities. Treating at 0.5 mg/L in a field setting would probably result in fewer tadpole mortalities than would treating at 1.0 mg/L. A 4-h treatment would also be expected to cause less mortality than an 8-h treatment.

Mortality of Columbia spotted frog tadpoles decreased with age, but no age effects were seen with boreal toad tadpoles. Regardless of the species, if tadpoles were younger than Gosner age 35, there was very high mortality when exposed to 1.0 mg/L CFT Legumine for 96 h. In the youngest age-group tested, even treatment levels of 0.5 mg/L caused at least 50% mortality for both species. At the oldest age, Columbia spotted frog tadpoles were significantly less likely to die than were boreal toad tadpoles when exposed at 1.0 mg/L product for 96 h in this experiment.

The significant effect of age and species at the late-age stage range seen in this portion of the research may have been a result of the physiological changes occurring in ranid (frog) but not in anaxyrid (toad) tadpoles. Throughout the tadpole phase, members of the ranid family (e.g., Columbia spotted frogs) undergo lung development to supplement oxygen intake and, by the end of the late stage, rely very little on breathing through the gills. Anaxyrid tadpoles, by contrast, are fundamentally lungless and remain gill-breathers throughout this life stage (McDiarmid and Altig 1999). Rotenone is absorbed across the gill membrane, and both the age and species effects seen in late-stage Columbia spotted frog tadpoles may be attributed to the shift from gill to lung breathing. Alternatively, the age and species effects observed in the late-age Columbia spotted frog tadpole trials could be attributed to differences in tolerance to rotenone. Differences in the abilities of particular fish species to metabolize rotenone exist, making certain fish more sensitive to the chemical than others (Willis and Ling 2000; Grisak et al. 2007b). Late-age Columbia spotted frog tadpoles may have a greater ability to metabolize rotenone than do boreal toad tadpoles or younger Columbia spotted frog tadpoles, potentially accounting for the significant differences in mortality seen in these trials.

The variation seen in late-age Columbia spotted frog tadpole mortality at 1.0 mg/L between 2008 and 2009 may also be a function of lung development in ranid tadpoles. Tadpoles used in the 2008 trial were within the assigned age range (Gosner 40–45), but were, on average, on the younger side of the range. There was 57% mortality in this trial. Tadpoles in the 2009 trial, however, were purposefully tested on the older side of the late-age range, and experienced only 6% mortality. This difference in mortality could be a result of the quickening shift to lung breathing at the end of this life phase. Random variation may account for differences seen in mortality between years, but this seems less plausible given the number of replicates used per treatment (e.g., eight tanks).

The pattern of mortality observed in our trials gives some indication as to timing rotenone treatments in order to minimize tadpole death. Timing rotenone applications to avoid the larval stage altogether would be ideal, but in areas like the Rocky Mountain West, accessibility and flow conditions can make treatment difficult to impossible during times in which larval amphibians are not present. Rotenone applications generally occur during a 3-month-long window of time in mid to late summer through early fall (Grisak et al. 2007b), which is typically the breeding and larval period for Rocky Mountain amphibian populations. Columbia spotted frogs and boreal toads, for example, begin breeding and egg-laying in May and June, often while ice and snow still cover portions of a lake or pond; tadpoles of both species typically take 2 to 3 months to metamorphose (Hovingh 1993; Koch and Peterson 1995; Werner et al. 2004). Given the overlap of the tadpole stage of many Rocky Mountain amphibian species and the optimal period for rotenone applications, fisheries managers could time applications to minimize tadpole mortality. Our results demonstrate that, at least in Columbia spotted frogs, tadpoles at a very late age experience lower rates of mortality, indicating that timing rotenone applications to coincide with this larval age period could result in lower Columbia spotted frog tadpole death.

The conclusions of this study should be interpreted with caution, as they were derived from controlled laboratory experiments that used precisely measured amounts of rotenone. During field treatments, it may be difficult to treat an entire water body at exactly 1.0 mg/L, for instance, because of irregularities in water body shapes (leading to estimated, rather than actually measured, water volumes), overlap in drip-station treatment in flowing water, or, initially, incomplete and uneven mixing of rotenone in the water column. Additionally, to obtain 100% fish mortality, dosing at levels higher than 1.0 mg/L may be necessary to overcome field conditions, such as high pH, turbidity, or solar radiation. Limited evidence from our study suggests that treating at higher concentrations, such as 2.0 mg/L, can result in very high tadpole mortality, even at a late age. On the other hand, if field conditions allow for an application of rotenone at a treatment level lower than 1.0 mg/L or a shorter application period (e.g., 4 h), lower tadpole mortality could be expected.

In the sublethal-effects portion of this study, because the majority of tadpoles of either species at Gosner age 35 or younger died when exposed to this dosage of CFT Legumine, we focused on the effects of rotenone on metamorphosis in late-age Columbia spotted frog tadpoles. Although this component was limited because of the lack of tank replication and the inability to test for tank effects, rotenone did not appear to have negative effects on the size or timing of metamorphosis of late-age Columbia spotted frog tadpoles, and there was no delayed mortality. In fact, exposed late age-group tadpoles were heavier than controls; this could have potentially resulted from slight differences in feeding, tadpole densities, or both. Because we did not find consistent results for the variables tested in this portion of the study suggests that feeding and tadpole density did not have an effect. The few surviving middle-age Columbia spotted frog tadpoles ($n = 7$) at this treatment level (1.0 mg/L of product) did experience higher delayed mortality than observed in their control counterparts. Despite that findings from the sublethal effects portion of the study appeared biologically insignificant and support the conclusions of Finlayson et al. (2010a) that rotenone is an acute toxin with little potential for chronic toxicity, the importance of potential effects on characteristics like weight, SUL, and time to metamorphosis cannot be understated given their influence on future survival of these amphibians as juveniles and adults (Bridges 2002; Buckley et al. 2005; Capellan and Nicieza 2007). Other pesticides, like atrazine, can cause significant sublethal effects in amphibians (Cowman and Mazanti 2000).

The length of exposure to rotenone also affected the level of mortality in Columbia spotted frog tadpoles, with shorter exposure periods resulting in fewer mortalities. This was particularly evident in the early and middle age-groups. At these two age ranges, Columbia spotted frog tadpoles exposed to CFT Legumine at 1.0 mg/L for 4 h experienced significantly lower mortality than those exposed for 96 h (early age: $P < 0.001$; late age: $P < 0.001$; Figure 3). We did not run comparative

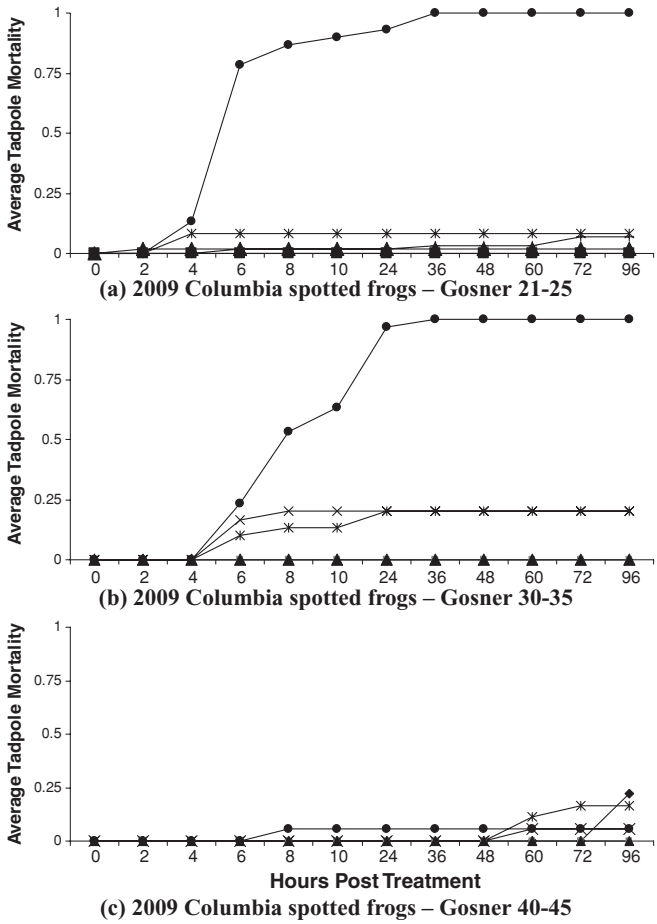

FIGURE 3. Average mortality over the 96-h posttreatment period of Columbia spotted frog tadpoles at **(a)** Gosner age 21–25, **(b)** Gosner age 30–35, and **(c)** Gosner age 40–45 exposed to 1.0 mg/L of rotenone, by treatment group (exposure length). Circles denote the 96-h exposure treatment, stars the 4-h exposure treatment, crosses the 3-h exposure treatment, triangles the 2-h exposure treatment, squares the 1-h exposure treatment, and diamonds the control group.

analyses at each treatment level (e.g., exposure length), but the trends seen in Figure 3 give clear insight into the negative effects of exposure length on mortality and, more importantly, the point at which significant mortality begins to occur. At the two youngest ages, significant mortality began at 6 h. There was no discernable trend among late-age tadpoles; mortality in exposed tadpoles in this age-group was never higher than 20%.

These findings provide options for tadpole conservation that should be considered while planning a rotenone treatment. If rotenone application must coincide with early- or middle-age Columbia spotted frog tadpoles or boreal toad tadpoles of any age, our results suggest that fisheries managers can reduce mortality in any of three ways. Given available resources, larval amphibians in restoration areas could be conserved by removing them before rotenone application and reintroducing them when the rotenone is no longer active. According to our research, if tadpoles cannot be removed before rotenone application, they can be collected in the first 4 h after treatment before significant

mortality occurs and allowed to recover in fresh, untreated water. Exposure to rotenone causes lethargy in tadpoles, making them easy to capture. Finally, if applications of less than 1.0 mg/L in concentration or less than 8 h of exposure will still accomplish fish removal goals, managers could use lower dosages of rotenone and shorter exposure periods to reduce negative effects on larval amphibians. These recommendations might allow fisheries managers to salvage the bulk of a tadpole population during a rotenone application instead of potentially losing the entire cohort.

This study provides information on the effects of age, species, and exposure duration on the toxicity of rotenone to tadpoles, but much remains to be done. Continued field research and laboratory experiments addressing, among other things, the effects of the environment on rotenone's toxicity to larval amphibians are needed. Rotenone decomposition is affected by several environmental factors, such as sunlight, water temperature, organic debris, and water pH (Fontenot et al. 1994), and effects on amphibians in the field may ultimately be influenced by the chemical's interactions with these environmental factors. Overall, it is clear that rotenone applications could result in widespread larval mortality, but the present results suggest that this mortality can be avoided or partially mitigated. Native fish restoration and amphibian conservation are not mutually exclusive, and the results of this research provide insight into ways in which both can be accomplished.

ACKNOWLEDGMENTS

This research was made possible by grants from Turner Enterprises, Inc. (TEI) in Bozeman, Montana, and the Montana Chapter of the American Fisheries Society. Equipment and laboratory facilities necessary for the experiments were provided by TEI. We also thank Idaho State University (ISU) for funding and the use of facilities in the Department of Biological Sciences. This research was highly labor intensive and would not have succeeded without the dedication of TEI seasonal technicians. Special thanks also go to John Treanor and Kate Olsen for volunteering their time. The methodology for the laboratory trials was approved by the Idaho State University Animal Welfare Committee.

REFERENCES

ASTM (American Society for Testing and Materials International). 2002. Standard guide for conducting toxicity tests on test materials with fishes, macroinvertebrates and amphibians. ASTM, E 727-796, Conshohocken, Pennsylvania.

Amey, M. J. 1984. The application of liquid derris (5% Rotenone) to a spring-fed upland pond to eradicate perch (*Perca fluviatilis*): 3 year post-application monitoring. Fisheries Management 15:75–76.

Behnke, R. J. 1992. Native trout of western North America. American Fisheries Society, Monograph 6, Bethesda, Maryland.

Bettoli, P. W., and M. J. Maceina. 1996. Sampling with toxicants. Pages 303–333 *in* B. R. Murphy and D. W. Willis, editors. Fisheries techniques, 2nd edition. American Fisheries Society, Bethesda, Maryland.

Bradford, D. F., F. Tabatabai, and D. M. Graber. 1993. Isolation of remaining populations of the native frog, *Rana muscosa*, by introduced fishes in Sequoia and Kings Canyon national parks, California. Conservation Biology 7:882–888.

Bridges, C. M. 2002. Tadpoles balance foraging and predator avoidance: effects of predation, pond drying, and hunger. Journal of Herpetology 36:627–634.

Britton, J. R. 2006. Eradicating the invasive topmouth gudgeon, *Pseudorasbora parva*, from a recreational fishery in northern England. Fisheries Management and Ecology 13:329–335.

Buckley, C. R., S. F. Michael, and D. J. Irschick. 2005. Early hatching decreases jumping performance in a direct-developing frog, *Eleutherodactylus coqui*. Functional Ecology 19:67–72.

Capellan, E., and A. G. Nicieza. 2007. Trade-offs across life stages: does predator-induced hatching plasticity reduce anuran post-metamorphic performance? Evolutionary Ecology 21:445–458.

Cowman, D. F., and L. E. Mazanti. 2000. Ecotoxicology of "new generation" pesticides to amphibians. Pages 233–268 *in* D. W. Sparling, G. Linder, and C. A. Bishop, editors. Ecotoxicology of amphibians and reptiles. Society of Environmental Toxicology and Chemistry, Columbia, Missouri.

Farringer, J. E. 1972. The determination of the acute toxicity of rotenone and bayer 73 to selected aquatic organisms. Master's thesis. University of Wisconsin, La Crosse.

Finlayson, B. J., R. A. Schnick, R. L. Cailteux, L. DeMong, W. D. Horton, W. McClay, C. W. Thompson, and G. J. Tichacek. 2000. Rotenone use in fisheries management: administrative and technical guidelines manual. American Fisheries Society, Bethesda, Maryland.

Finlayson, B. J., R. A. Schnick, D. Skaar, J. Anderson, L. Demong, D. Duffield, W. Horton, and J. Steinkjer. 2010a. Planning and standard operating procedures for the use of rotenone in fish management: rotenone SOP manual. American Fisheries Society, Bethesda, Maryland.

Finlayson, B. J., W. Somar, D. Duffield, D. Propst, C. Mellison, T. Pettengill, H. Sexauer, T. Nesler, S. Gurtin, J. Elliot, F. Partridge, and D. Skaar. 2005. Native inland trout restoration on national forests in the western United States: time for improvement? Fisheries 30:10–19.

Finlayson, B., W. Somer, and M. Vinson. 2010b. Rotenone toxicity to rainbow trout and several mountain stream insects. North American Journal of Fisheries Management 30:102–111.

Fontenot, L. W., G. P. Noblet, and S. G. Platt. 1994. Rotenone hazards to amphibians and reptiles. Herpetological Review 25:150–156.

Gosner, K. L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica 16:183–190.

Grisak, G. G., M. E. Schnee, G. L. Michael, D. R. Skaar. 2007a. Toxicity of fintrol (antimycin) and prenfish (rotenone) to slimy sculpin. Intermountain Journal of Sciences 13:9–12.

Grisak, G., D. R. Skaar, G. L. Michael, M. E. Schnee, B. L. Marotz. 2007b. Toxicity of fintrol (antimycin) and prenfish (rotenone) to three amphibian species. Intermountain Journal of Sciences 13:1–8.

Hamilton, B. T., S. E. Moore, T. B. Williams, N. Darby, M. R. Vinson. 2009. Comparative effects of rotenone and antimycin on macroinvertebrate diversity in two streams in Great Basin National Park, Nevada. North American Journal of Fisheries Management 29:1620–1635.

Hamilton, H. L. 1941. The biological action of rotenone on freshwater animals. Proceedings of the Iowa Academy of Sciences 48:467–479.

Hollingworth, R. 2001. Inhibitors and uncouplers of mitochondrial oxidative phosphorylation. Pages 1169–1263 *in* R. Krieger, editor. Handbook of pesticide toxicology, 2nd edition. Academic Press, New York.

Hovingh, P. 1993. Aquatic habitats, life-history observations, and zoogeographic considerations of the spotted frog (*Rana pretiosa*) in Tule Valley, Utah. Great Basin Naturalist 53:168–179.

Knapp, R. A. 2005. Effects of nonnative fish and habitat characteristics on lentic herpetofauna in Yosemite National Park, USA. Biological Conservation 121:265–279.

Knapp, R. A., D. M. Boiano, and V. T. Vredenburg. 2007. Removal of nonnative fish results in population expansion of a declining amphibian (mountain yellow-legged frog, *Rana muscosa*). Biological Conservation 135:11–20.

Koch, E. D., and C. R. Peterson. 1995. Amphibians and reptiles of Yellowstone and Grand Teton national parks. University of Utah Press, Salt Lake City.

Ling, N. 2002. Rotenone: a review of its toxicity and use for fisheries management. Science for Conservation 211:1–40.

Mangum, F. A., and J. L. Madrigal. 1999. Rotenone effects on aquatic macroinvertebrates of the Strawberry River, Utah: a five-year summary. Journal of Freshwater Ecology 14:125–135.

Meadows, B. S. 1973. The use of rotenone in fisheries management. Journal of Institutional Fisheries Management 4:12–17.

McClay, W. 2005. Rotenone use in North America (1988–2002). Fisheries 30:29–31.

McCoid, M. J., and P. W. Bettoli. 1996. Additional evidence for rotenone hazards to turtles andamphibians. Herpetological Review 27:70–71.

McDiarmid, R. W., and R. Altig. 1999. Tadpoles: the biology of anuran larvae. University of Chicago Press, Chicago.

Minitab. 2010. Minitab 16 statistical software. Minitab, State College, Pennsylvania.

Moore, S., M. Kulp, B. Rosenlund, J. Brooks, and D. Propst. 2008. A field manual for the use of antimycin A for restoration of native fish populations. U.S. National Park Service, Natural Resource Report NPS/NRPC/NRR-2008/033, Fort Collins, Colorado.

Mullin, S. J., J. B. Towey, and R. E. Szafoni. 2004. Using rotenone to enhance native amphibian breeding habitat in ponds (Illinois). Ecological Restoration 22:305–306.

Patla, D. A. 2005. Protecting amphibians while restoring fish populations. Pages 275–276 *in* M. Lannoo, editor. Amphibian declines: the conservation status of United States species. University of California Press, Berkeley.

Shepard, B. B., R. Spoon, and L. Nelson. 2002. A native westslope cutthroat trout population responds positively after brook trout removal and habitat restoration. Intermountain Journal of Sciences 8:191–211.

Walston, L. J., and S. J. Mullin. 2007. Responses of a pond-breeding amphibian community to the experimental removal of predatory fish. American Midland Naturalist 157:63–73.

Werner, J. K., B. A. Maxell, P. Hendricks, and D. L. Flath. 2004. Amphibians and reptiles of Montana. Mountain Press, Missoula, Montana.

Willis, K., and N. Ling. 2000. Sensitivities of mosquitofish and black mudfish to a piscicide: could rotenone be used to control mosquitofish in New Zealand wetlands? New Zealand Journal of Zoology 27:85–91.