Transactions of the American Fisheries Society

ISSN: 0002-8487 (Print) 1548-8659 (Online) Journal homepage: http://www.tandfonline.com/loi/utaf20

Effects of CFT Legumine (5% Rotenone) on Tadpole Survival and Metamorphosis of Chiricahua Leopard Frogs Lithobates chiricahuensis, Northern Leopard Frogs L. pipiens, and American Bullfrogs L. catesbeianus

Guillermo Alvarez, Colleen A. Caldwell & Carter G. Kruse

To cite this article: Guillermo Alvarez, Colleen A. Caldwell & Carter G. Kruse (2017) Effects of CFT Legumine (5% Rotenone) on Tadpole Survival and Metamorphosis of Chiricahua Leopard Frogs Lithobates chiricahuensis, Northern Leopard Frogs L. pipiens, and American Bullfrogs L. catesbeianus, Transactions of the American Fisheries Society, 146:3, 512-522, DOI: 10.1080/00028487.2017.1285355

To link to this article: http://dx.doi.org/10.1080/00028487.2017.1285355

Published online: 30 Mar 2017.

Transactions of the American Fisheries Society 146:512–522, 2017

ISSN: 0002-8487 print / 1548-8659 online
DOI: 10.1080/00028487.2017.1285355

ARTICLE

Effects of CFT Legumine (5% Rotenone) on Tadpole Survival and Metamorphosis of Chiricahua Leopard Frogs *Lithobates chiricahuensis*, Northern Leopard Frogs *L. pipiens*, and American Bullfrogs *L. catesbeianus*

Guillermo Alvarez
Department of Fish, Wildlife, and Conservation Ecology, New Mexico State University, Box 30003, Mail Stop Code 4901, Las Cruces, New Mexico 88003, USA

Colleen A. Caldwell*
U.S. Geological Survey, New Mexico Cooperative Fish and Wildlife Research Unit, Department of Fish, Wildlife, and Conservation Ecology, New Mexico State University, Box 30003, Mail Stop Code 4901, Las Cruces, New Mexico 88003, USA

Carter G. Kruse
Turner Enterprises, Inc., 901 Technology Boulevard, Bozeman, Montana 59718, USA

Abstract

Amphibians may experience collateral effects if exposed to CFT Legumine (5% rotenone), a piscicide that is used to remove invasive fish. A series of 48-h static toxicity tests assessed the acute effects of CFT Legumine on multi-aged tadpoles of the federally listed Chiricahua leopard frog *Lithobates chiricahuensis*, the widespread northern leopard frog *L. pipiens*, and the increasingly invasive American bullfrog *L. catesbeianus*. At the earliest Gosner stages (GS 21–25), Chiricahua leopard frogs were more sensitive to CFT Legumine (median lethal concentration [LC_{50}] = 0.41–0.58 mg/L) than American bullfrogs (LC_{50} = 0.63–0.69 mg/L) and northern leopard frogs (LC_{50} = 0.91 and 1.17 mg/L). As tadpoles developed (i.e., increase in GS), their sensitivity to rotenone decreased. In a separate series of 48-h static nonrenewal toxicity tests, tadpoles (GS 21–25 and GS 31–36) of all three species were exposed to piscicidal concentrations of CFT Legumine (0.5, 1.0, and 2.0 mg/L) to assess postexposure effects on metamorphosis. In survivors of all three species at both life stages, the time to tail resorption was nearly doubled in comparison with that of controls. For example, mid-age (GS 31–36) Chiricahua leopard frog tadpoles required 210.7 h to complete tail resorption, whereas controls required 108.5 h. However, because tail resorption is a relatively short period in metamorphosis, the total duration of development (days from posthatch to complete metamorphosis) and the final weight did not differ in either age-group surviving nominal concentrations of 0.5-, 1.0-, and 2.0-mg/L CFT Legumine relative to controls. This research demonstrates that the CFT Legumine concentrations commonly used in field applications to remove unwanted fish could result in considerable mortality of the earliest stages of *Lithobates* species. In addition to acute lethality, piscicide treatments may result in delayed tail resorption, which places the tadpoles at risk by increasing their vulnerability to predation and pathogens.

Invasion by exotic species is currently the second leading cause of extinction (Cain et al. 2011) and is an important factor in the decline of native salmonids (Kruse et al. 2013). In aquatic environments, restoration of native fishes often requires the eradication of nonnative fishes by use of rotenone (Finlayson et al. 2000; Ling 2002; Hamilton et al. 2009). A commonly used

*Corresponding author: ccaldwel@usgs.gov
Received October 1, 2016; accepted January 17, 2017

liquid formulation, CFT Legumine (Envincio LLC, Cary, North Carolina), contains 5% rotenone, an organic chemical registered by the Environmental Protection Agency (Registration Number 75338-1; USEPA 2007). As a mitochondrial inhibitor, rotenone's target site of toxic action occurs in areas of the gill lamellae that are high in mitochondria. While the acute and postexposure effects of rotenone in fishes have been more thoroughly reviewed (Ling 2002; McClay 2005), effects on nontarget organisms such as amphibians are not fully understood (Sparling et al. 2000; Knapp et al. 2007). Presumably, amphibians will experience acute toxicity effects of mitochondrial inhibitors when relying on gill lamellae for respiration. Amphibians often co-occur in aquatic habitats slated for native fish restoration and may be affected by rotenone. Native amphibians, however, may benefit from the removal of invasive nonnative fishes and amphibians (e.g., Knapp and Matthews 2000).

CFT Legumine concentrations as high as 4.0 mg/L, although not common, can be used to eliminate nonnative fishes in lakes and ponds (Finlayson et al. 2010). However, field applications of much lower concentrations (1.0 mg/L) are more commonly used and have resulted in local declines of larval Columbia spotted frogs *Rana luteiventris* (Billman et al. 2012). In laboratory conditions, exposure to CFT Legumine at 1.0 mg/L for 96 h resulted in 100% mortality of early age (Gosner stages [GS] 21–25; Gosner 1960) and mid-age (GS 30–35) Columbia spotted frog tadpoles. Older (GS 40–45) tadpoles of the same species experienced 6% mortality when exposed to 1.0-mg/L CFT Legumine (Billman et al. 2011). In the same study, early age (GS 21–25) tadpoles of the boreal toad *Anaxyrus boreas* experienced 99% mortality when exposed to 1.0 mg/L, while older (GS 30–35 and GS 40–45) boreal toad tadpoles experienced 83–96% mortality (Billman et al. 2011).

For amphibians that survive rotenone applications, postexposure effects, such as developmental delay, may cause an untimely migration from natal pools or ponds and increased mortality due to pond desiccation or predation (Bervan 1990). Furthermore, a delay in development can cause anurans to reach maturity at smaller sizes and to have longer pre-reproductive periods and smaller clutch sizes (Semlitsch et al. 1988). Conversely, anurans that reach maturity at larger sizes are less susceptible to predation and reproduce sooner (Semlitsch 1989).

Our primary objectives were to estimate the acute response and postexposure effects of CFT Legumine on tadpoles of three different ranids that could occur within areas slated for piscicide application in New Mexico. An assessment of CFT Legumine's effects on larval amphibians will provide guidance for management agencies on the timing of rotenone application to reduce collateral effects in amphibians. For example, the federally listed and New Mexico state-listed Chiricahua leopard frog *Lithobates chiricahuensis* is critically imperiled due to habitat loss and degradation and the presence of nonnative predators and competitors (Sredl and Howland 1995; USOFR 2002; Hammerson et al. 2004). Chiricahua leopard frogs can breed year-round, representing a longer breeding season compared to other ranids (Scott and Jennings 1985). Tadpoles complete metamorphosis in 3–9 months after hatching and often overwinter before metamorphosing (Jennings 1988). By comparison, the northern leopard frog *L. pipiens*, which is widespread in northern portions of its range but declining and considered a "species of greatest conservation need" in New Mexico (NMDGF 2006), breeds during the spring (April–June) and completes metamorphosis in 3–6 months (Merrell 1977; Hinshaw 1992). The range of the invasive (in New Mexico) American bullfrog *L. catesbeianus* includes well-established populations in 10 states outside of its natural range and in 40 countries around the world (McKercher and Gregorie 2016). Not surprisingly, American bullfrog populations occur throughout low-elevation (900–2,100-m) freshwater habitats in New Mexico (Degenhardt et al. 1996; Stebbins 2003). Across its range, breeding of the American bullfrog occurs from May to July in northern localities, whereas it occurs from February to October in southern localities (e.g., New Mexico; Behler and King 1979). Time to complete metamorphosis may vary from a few months in the species' southern distribution to 3 years in its northern range (Collins 1979). Since American bullfrogs have invaded and negatively impacted native Chiricahua leopard frog and plains leopard frog *L. blairi* populations in New Mexico, a secondary objective of this work was to determine whether American bullfrog larvae are sensitive to and could be removed by an application of rotenone. The current distributions of the three species included in our study overlap with that of a southwestern native trout species, the Rio Grande Cutthroat Trout *Oncorhynchus clarkii virginalis*, which is subject to conservation measures including rotenone treatment for removal of nonnative Rainbow Trout *O. mykiss* that hybridize with the native trout (Kruse et al. 2013). These three frog species were also selected because they represent diversity in body size, habitat use, and reproductive strategy that would provide additional insights on ranid responses to rotenone exposure.

METHODS

Animal husbandry and testing conditions.—The stability of rotenone is highly susceptible to photodegradation and temperature (Cabras et al. 2002; Draper 2002). For example, the half-life of rotenone was 13.9 h at 24°C and 83.9 h at 0°C in ponds (Gilderhus et al. 1986; Finlayson et al. 2000) and 41.8 h at 20–22°C and 84 h at 10–20°C in reservoirs (Finlayson et al. 2000). Thus, environmental variables, such as ambient temperature and natural light, were incorporated into our study design to more accurately assess the rotenone toxicity thresholds for the three ranids. Studies evaluating the effects of pesticides on amphibians are rarely performed under natural conditions of photoperiod and diel temperature range (Boone and James 2003, 2005; Boone et al. 2007). Laboratory studies may overestimate or underestimate toxicological responses

because toxicants in the environment are affected by a variety of physical and chemical factors that are sometimes changed under laboratory settings (Ritter and Bergstrom 2001; Gómez-Mestre and Tejedo 2003; Brown 2010).

Toxicity tests were conducted in a greenhouse (9.1 × 29.3 m) to provide a seasonally relevant light: dark photoperiod. An optimal water temperature range of 18–24°C for pond amphibians (Wright and Whitaker 2001) in holding tanks and testing aquaria was provided by using natural gas heating and evaporative cooling systems, which were controlled by digital sensors located throughout the greenhouse. Experimental conditions in the greenhouse optimized the experiments by providing environmentally relevant diel temperature fluctuations. Temperatures at night were maintained above 18°C, while temperatures during daylight were maintained below 24°C.

The largest remaining metapopulation of Chiricahua leopard frogs in New Mexico resides on the Ladder Ranch in Sierra County. The Ladder Ranch, in cooperation with the U.S. Fish and Wildlife Service, actively conducts conservation activities for the Chiricahua leopard frog, including a captive propagation program (McCaffery and Phillips 2013). From May 2013 to October 2014, six egg clutches from Chiricahua leopard frogs were transported in thermally insulated containers from the Ladder Ranch to the testing facility at the Fabian Garcia Research Facility, New Mexico State University (Doña Ana County). Two egg clutches from northern leopard frogs (NASCO, Fort Atkinson, Wisconsin) and three egg clutches from American bullfrogs (Carolina Biological Supply Company, Burlington, North Carolina) were obtained from commercial suppliers.

Upon arrival at the testing facility, each clutch of eggs was tempered in 30 L of aerated, dechlorinated municipal water and was acclimatized for 24 h prior to transfer to holding tanks. Sodium bisulfite was used to remove chlorine and to neutralize heavy metals (ASTM 2014). Dissolved oxygen concentration (mg/L), conductivity (µS/cm), and pH in the holding tanks and test chambers were monitored daily by using a HACH HQ40d meter (HACH Co., Loveland, Colorado). Ammonia (total N, mg/L; HACH Method 8155) and nitrite (total N, mg/L; HACH Method 8507) concentrations were monitored weekly using a HACH DR2010 spectrophotometer. Temperature (°C) was continuously monitored with HOBO pendant temperature data loggers (Model UA-001; Onset Computer Corp., Bourne, Massachusetts). Dissolved oxygen (6.0–10.0 mg/L), conductivity (400–1,400 µS/cm), pH (7.5–8.3), ammonia (0.12–1.7 mg/L), and nitrite (0.1–0.2 mg/L) levels in the holding tanks were deemed acceptable as optimal water quality conditions for rearing amphibians (Wright and Whitaker 2001; ASTM 2014). Tadpoles were offered commercial flake and algae wafers each day. Water quality conditions in the holding tanks were maintained within the aforementioned ranges by 50% water changes and siphoning of uneaten food and feces every other day.

Acute toxicity tests.—Static 48-h toxicity tests were conducted in accordance with guidelines set forth by the American Society for Testing and Materials (ASTM 2014). A 1.019-g/L stock solution of CFT Legumine (5% rotenone) dissolved in deionized water was diluted serially to obtain a range of nominal test concentrations from 0.125 to 5.0 mg/L. These concentrations included relevant field applications of CFT Legumine (Finlayson et al. 2010). Preliminary tests were conducted using a small number (8–12) of tadpoles of each species within each targeted age-group to determine the range of concentrations that would result in 100% survival and 100% mortality. Thus, an additional test concentration of 5.0-mg/L CFT Legumine was included to ensure that conditions met the ASTM (2014) protocol of 100% lethality. Controls received water with the same characteristics but without the toxicant. Testing containers consisted of 0.946-L, wide-mouth jars containing 900 mL of the CFT Legumine treatment concentration or control water. After test and control solutions were dispensed into pre-labeled jars, the jars were placed in a water bath in a randomized block design. The water bath was circulated via a small submersible pump. Water quality in jars containing larvae was held within acceptable limits by maintaining a density of not more than one tadpole per 100 mL and by withholding feed for 24 h prior to each test (ASTM 2014). Dissolved oxygen (5.0–8.0 mg/L), conductivity (400–1,400 µS/cm), pH (7.5–8.3), ammonia (total N; 0.12–1.7 mg/L), and nitrite (0.1–0.2 mg/L) levels were within ranges deemed acceptable for larval ranids in culture conditions (Wright and Whitaker 2001).

The age of tadpoles selected for testing was confirmed by using Gosner's (1960) identification guidelines of development for larval anurans and included four age-groups: GS 21–25 (complete jaw development), GS 26–30 (hind limb development), GS 31–36 (toe differentiation of the hind limb), and GS 37–40 (forelimb development). For all tests, there were five replicates per treatment (n = 5), five controls, and five tadpoles in each replicate or control jar. Feed was withheld for 24 h prior to testing and during the 48-h testing period; mortalities were removed at 2, 4, 6, 12, 24, and 48 h (ASTM 2014).

Three clutches of Chiricahua leopard frog eggs were included in the acute toxicity assessment, which allowed us to conduct three separate tests for each of the four age-groups. Early age-group (GS 21–25) and hind-limb age-group (GS 26–30) tadpoles were exposed to CFT Legumine concentrations of 0.125, 0.25, 0.5, 1.0, and 2.0 mg/L. Similarly, mid-age (GS 31–36) tadpoles were exposed to CFT Legumine concentrations of 0.5, 1.0, 1.5, and 2.0 mg/L, and late-age (GS 37–40) tadpoles were exposed to CFT Legumine at 1.0, 2.0, 3.0, 4.0, and 5.0 mg/L.

Northern leopard frog tadpoles of the early age-group (GS 21–25) and hind-limb age-group (GS 26–30) were exposed to CFT Legumine concentrations of 0.25, 0.5, 1.0, 1.5, and 2.0 mg/L. Mid-age (GS 31–36) tadpoles were exposed to CFT Legumine at 1.0, 2.0, 3.0, and 4.0 mg/L. Since only two egg clutches were available for this species, two (instead of three)

separate tests were conducted for each of the first three age-groups. Fewer late-age (GS 37–40) tadpoles remained from the two egg clutches; thus, only one test was conducted at CFT Legumine concentrations of 2.0, 3.0, 4.0, and 5.0 mg/L to obtain an LC_{50} relevant to field applications of rotenone (Finlayson et al. 2000).

Due to the seasonality in American bullfrog eggs and the limited number of individuals available for testing upon hatching, only early age (GS 21–25) and mid-age (GS 31–36) larvae were selected for testing. Preliminary range-finding tests indicated that these age-groups were sensitive to environmentally relevant CFT Legumine concentrations, whereas late-age (GS 37–40) tadpoles were not as sensitive. Early age tadpoles of the American bullfrog were exposed to CFT Legumine at 0.5, 1.0, 2.0, and 3.0 mg/L, while mid-age tadpoles were exposed to concentrations of 1.0, 2.0, 3.0, and 4.0 mg/L; three separate tests were conducted for each age-group.

Postexposure effects.—For each species, separate 48-h toxicity tests assessed postexposure effects on surviving tadpoles. Tadpoles of the early age-group (GS 21–25) were exposed to CFT Legumine concentrations of 0.5 and 1.0 mg/L, while mid-age (GS 31–36) tadpoles were exposed to concentrations of 0.5, 1.0, and 2.0 mg/L. Older-age (GS 37–40) tadpoles were not part of the postexposure assessment because their exposures would have occurred during the climax stage of metamorphosis or well beyond the endpoints established in this study. Additionally, treatment concentrations for late-age tadpoles exceeded the field application levels commonly used in rotenone treatments (Finlayson et al. 2000). Each treatment was replicated three times ($n = 3$), with five tadpoles in each replicate.

At the end of the 48-h tests, survivors were individually placed in labeled jars containing 900 mL of control water for postexposure observation until they reached GS 42 (forelimb emergence). Upon reaching forelimb emergence, each premetamorph was isolated in a 19-L aquarium containing 4 L of control water and a small plastic-grass pad to allow the froglet to perch above the water level. Survival and the rate of tail resorption (h) were monitored until the completion of metamorphosis (i.e., when less than 2 mm of tail tissue remained). Metamorphs were euthanized in a lethal concentration of tricaine methanesulfonate (MS-222; Sigma Chemical, St. Louis, Missouri; ASTM 2014). Total duration of development (number of days from hatching to complete tail resorption, or d posthatch [dph]) and wet weight (g) were recorded.

Statistical analysis.—For each acute toxicity test, the trimmed Spearman–Karber method was used to calculate the median lethal concentration (LC_{50}; the concentration that was lethal to 50% of the test population) and the associated 95% confidence intervals (Hamilton et al. 1977). All LC_{50} calculations were performed by using available software (CEE Computer Model Library, Old Dominion University, Norfolk, Virginia). Normality was verified using an *F*-test, where residuals were plotted. One-way ANOVA followed by Fisher's least-significant-difference test was used to determine postexposure effects in tadpoles of all three species. Only tadpoles that completed metamorphosis were included in the analysis, with mass (g), total duration of development (dph), and duration of tail resorption (h) as the response variables. Statistical significance was determined at an α level of 0.05. Except for LC_{50} calculations, statistical analyses were conducted by using R software (R Development Core Team 2007).

RESULTS

Acute Toxicity Tests

Throughout multiple clutches at the earliest stages (GS 21–25), Chiricahua leopard frogs were the most sensitive to CFT Legumine (LC_{50} = 0.41–0.58 mg/L), followed by American bullfrogs (LC_{50} = 0.63–0.69 mg/L) and northern

TABLE 1. CFT Legumine (5% rotenone) 48-h LC_{50} (median lethal concentration; mg/L) values (with 95% confidence intervals in parentheses) obtained from a series of tests conducted on tadpoles at four Gosner stages (GS) for the Chiricahua leopard frog (3 tests per age-group) and northern leopard frog (2 tests per age-group except the late age-group) and at two GSs for the American bullfrog (3 tests per age-group). Dashes indicate that no test was conducted due to the limited number of available tadpoles.

GS	Chiricahua leopard frog	Northern leopard frog	American bullfrog
21–25	0.41 (0.36–0.45)	1.2 (1.10–1.24)	0.65 (0.57–0.75)
	0.58 (0.51–0.66)	0.91 (0.79–1.04)	0.69 (0.50–1.0)
	0.53 (0.29–0.99)	–	0.63 (0.50–1.0)
26–30	1.1 (1.04–1.24)	1.1 (0.90–1.34)	–
	1.2 (1.06–1.30)	1.2 (1.03–1.43)	–
	0.89 (0.79–1.0)	–	–
31–36	1.2 (1.17–1.33)	1.1 (0.78–1.53)	2.2 (1.99–2.42)
	1.3 (1.25–1.41)	1.1 (0.73–1.73)	2.0 (1.74–2.22)
	1.4 (1.30–1.45)	–	2.0 (1.87–2.27)
37–40	3.2 (2.92–3.44)	3.6 (3.33–3.94)	–
	3.1 (2.86–3.27)	–	–
	3.4 (3.11–3.72)	–	–

leopard frogs (LC_{50} = 0.91 and 1.17 mg/L; Table 1). As the tadpoles developed (i.e., an increase in GS), their sensitivity to CFT Legumine decreased. The 48-h LC_{50} values for the mid-age (GS 26–30) tadpoles were 0.89–1.2 mg/L for Chiricahua leopard frogs and 1.1–1.2 mg/L for northern leopard frogs. For tadpoles tested at GS 31–36, Chiricahua leopard frogs (48-h LC_{50} = 1.2–1.4 mg/L) and northern leopard frogs (48-h LC_{50} = 1.1 mg/L) exhibited similar sensitivities to CFT Legumine, and both species were twice as sensitive as American bullfrogs (48-h LC_{50} = 2.0–2.2 mg/L).

Complete mortality of Chiricahua leopard frogs (GS 21–25 and GS 31–36) was observed within 4 h of exposure to 2.0-mg/L CFT Legumine (Figure 1). Likewise, complete mortality in early age (GS 21–25) tadpoles of the northern leopard frog was observed within 4 h of exposure to CFT Legumine at 2.0 mg/L (Figure 2). In contrast, complete mortality in early age (GS 21–25) tadpoles of the American bullfrog was not observed until 48 h after exposure to 2.0-mg/L CFT Legumine (Figure 3).

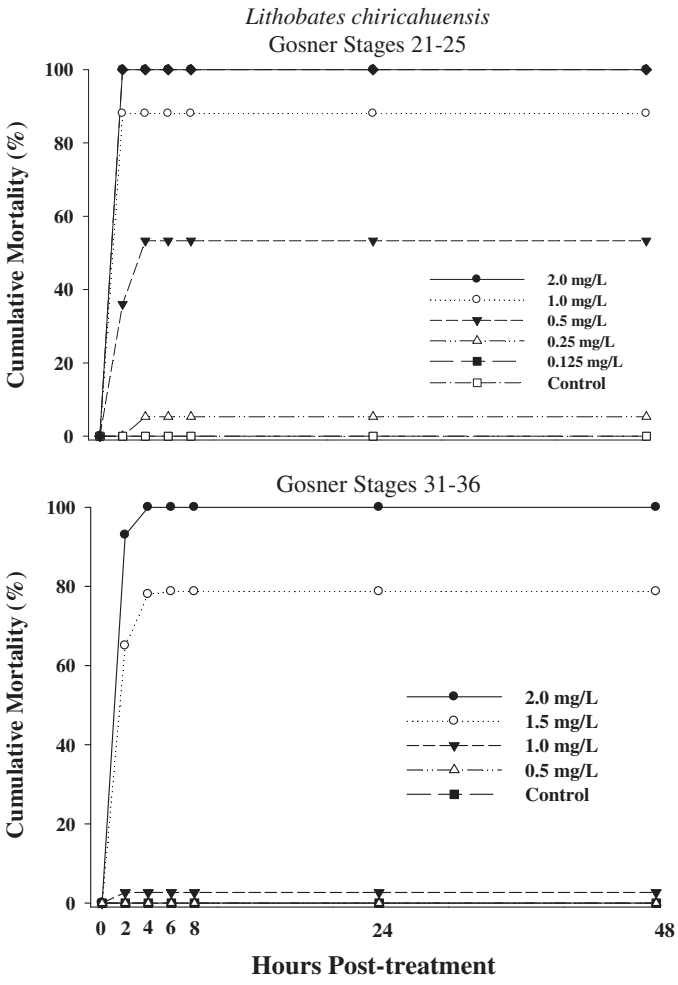

FIGURE 1. Cumulative mortality of two age-groups (Gosner stages 21–25 and 31–36) of Chiricahua leopard frog tadpoles exposed to a range of CFT Legumine (5% rotenone) concentrations (0.125–2.0 mg/L). Data displayed represent the average of the three toxicity tests conducted for each age-group.

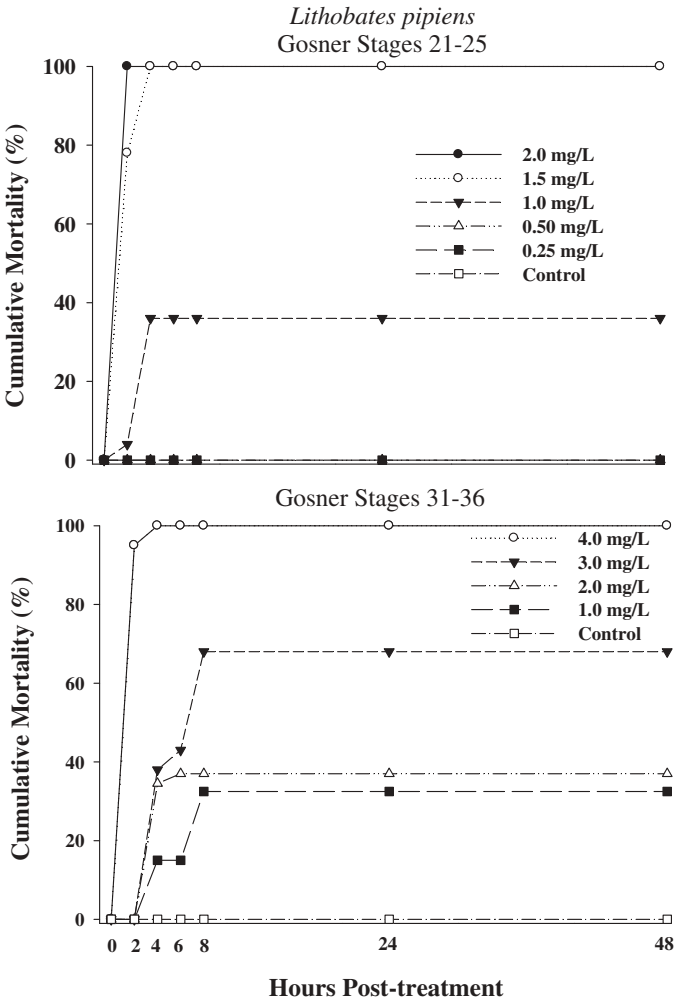

FIGURE 2. Cumulative mortality of two age-groups (Gosner stages 21–25 and 31–36) of northern leopard frog tadpoles exposed to a range of CFT Legumine (5% rotenone) concentrations (0.25–4.0 mg/L). Data displayed represent the average of the two toxicity tests conducted for each age-group.

Postexposure Effects

Chiricahua leopard frog.—Fewer tadpoles of the Chiricahua leopard frog across both age-groups and three egg clutches completed metamorphosis after exposure to a CFT Legumine concentration of 0.5 mg/L (13.0–26.7%) or 1.0 mg/L (0.0–11.7%) compared to controls (36.7–60.0%; Table 2). It is important to note that the postexposure studies were carried out until the last tadpole either completed metamorphosis or died. Thus, the percentage of tadpoles successfully metamorphosing was less than 100% due to mortality, even among the controls. The duration of tail resorption was nearly twice as long in treated tadpoles (120.0–240.0 h) as in controls (54.8–117.9 h) for both age-groups (Table 2). The duration of

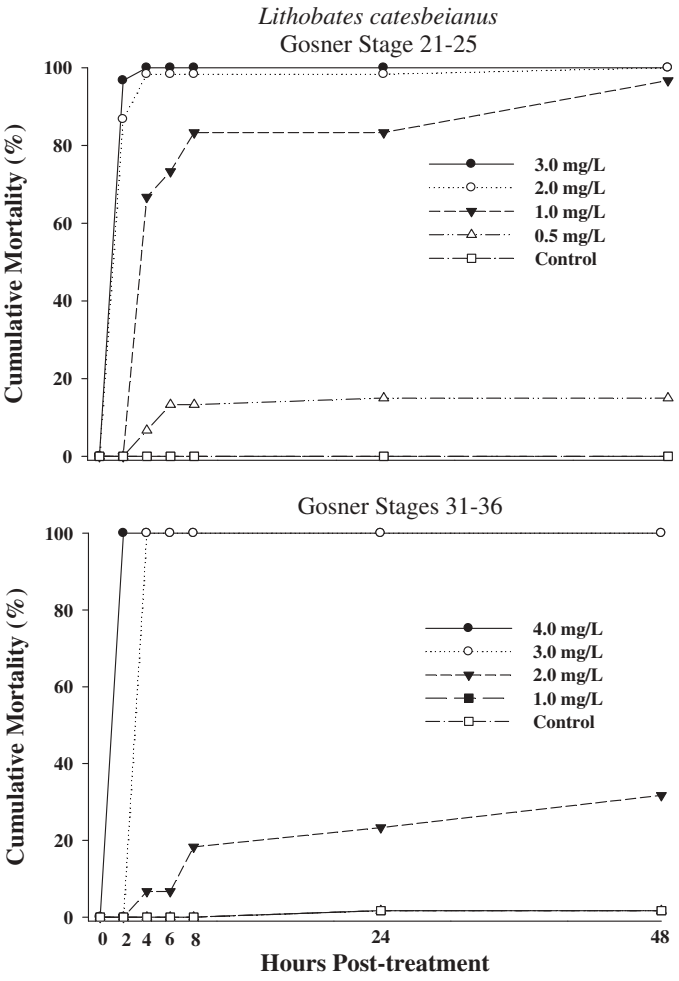

FIGURE 3. Cumulative mortality of two age-groups (Gosner stages 21–25 and 31–36) of American bullfrog tadpoles exposed to a range of CFT Legumine (5% rotenone) concentrations (0.5–4.0 mg/L) during 48-h LC_{50} (median lethal concentration) toxicity tests. Data displayed represent the average of the three toxicity tests conducted for each age-group.

development (dph) and final weight did not differ in either age-group that survived CFT Legumine concentrations of 0.5 and 1.0 mg/L in comparison with the controls.

Northern leopard frog.—Across both age-groups and two egg clutches, fewer northern leopard frog tadpoles completed metamorphosis after exposure to CFT Legumine concentrations of 0.5 mg/L (33%), 1.0 mg/L (20.0–23.3%), and 2.0 mg/L (0.0%) compared with controls (50.0–53.3%; Table 3). The duration of tail resorption was nearly twice as long in treated tadpoles of both age-groups (110.4–185.1 h) as in controls (66.0–99.0 h; Table 3). The duration of development and the final weight did not differ in either age-group surviving 0.5-, 1.0-, and 2.0-mg/L CFT Legumine relative to the controls.

American bullfrog.—Across both age-groups and three egg clutches of American Bullfrogs, fewer tadpoles completed metamorphosis after exposure to CFT Legumine concentrations of 0.5 mg/L (25.0–50.0%), 1.0 mg/L (5.0–58.0%), and 2.0 mg/L (0.0%) compared with controls (50.0–78.0%; Table 4). The duration of tail resorption in treated tadpoles of both age-groups (112.6–192.0 h) was twice that in controls (42.5–90.5 h; Table 4). The duration of development and the final weight did not differ between the earlier age-group (GS 21–25) of tadpoles surviving 0.5- and 1.0-mg/L CFT Legumine and the controls. In contrast, within two of the three egg clutches, American bullfrog tadpoles of the older age-group (GS 31–36) exposed to 1.0-mg/L CFT Legumine experienced a longer duration to complete development (106.6–113.6 d) than did the controls (94.6–104.3 d). These same tadpoles were also larger (1.88–1.91 g) than the controls (1.65–1.73 g) by the end of the study.

DISCUSSION

Acute toxicity was greatest in the earliest larval stages (GS 21–25) of three *Lithobates* species that were exposed to a range of environmentally relevant concentrations of CFT Legumine (5% rotenone). In this study, CFT Legumine LC_{50} values for the earliest larval stages were as low as 0.41 mg/L (21-µg/L rotenone) in Chiricahua leopard frogs, 0.63 mg/L (32-µg/L rotenone) in American bullfrogs, and 0.91 mg/L (46-µg/L rotenone) in northern leopard frogs. Therefore, a CFT Legumine target concentration range of 1.0–2.0 mg/L, which is commonly used in field applications to remove fish, could result in considerable mortality of the earliest stages of these species. Parallel sensitivity of the three species may be due to similarities in their physiology at early life stages, when larval ranids, similar to fish, readily absorb rotenone across their gill lamellae and thereby more effectively deliver rotenone to the site of toxic action in the mitochondria. As development proceeds, the young ranids reduce their reliance on gills for breathing (McDiarmid and Altig 1999), which presumably results in decreased rotenone toxicity due to limited uptake and less toxicant reaching the site of toxic action. Our results agree with those of Chandler and Marking (1982), who identified the gill-breathing larval stage (GS 21) of the southern leopard frog *L. sphenocephalus* (Crother 2012) as the stage most sensitive to Noxfish (5% rotenone). Furthermore, similar research described early age anurans as more sensitive to rotenone than later ages (Grisak et al. 2007; Billman et al. 2011, 2012). It is worth noting that for all life stages of our three study species, most of the mortality occurred within the first 4 h of exposure. This was similar to the work of Billman et al. (2011), who also observed significant mortality of Columbia spotted frogs and boreal toads within 4 h of exposure to a CFT Legumine concentration of 1.0 mg/L. While rotenone's lethality over a short duration (i.e., 4 h) may be due to its short half-life from photodegradation and hydrolysis (Cabras et al. 2002; Draper 2002), our exposure conditions eliminated variation that would be more commonly associated with aquatic environments (see Bettoli and Maceina 1996). Prevailing differences in water chemistry between experimental and environmental settings will alter rotenone's toxicity and

TABLE 2. Average percentage of Chiricahua leopard frog tadpoles that completed metamorphosis at Gosner stage (GS) 47 after being exposed to CFT Legumine (5% rotenone) concentrations of 0.5 and 1.0 mg/L for 48 h at GS 21–25 and GS 31–36 (NA = not available for analysis). For those tadpoles that completed metamorphosis, mean values (±SE) for the time to tail resorption (h), total days of development (d posthatch [dph]), and weight (g) are presented. Results of ANOVA (*F*-statistics and *P*-values) are also presented.

Treatment	Metamorphosis (%)	Tail resorption (h)	Development (dph)	Weight (g)
		Egg clutch 1 (GS 21–25)		
Control	60.0 ± 11.54	82.7 ± 5.81	112.3 ± 1.11	1.03 ± 0.10
0.5 mg/L	13.3 ± 6.66	120.0 ± 24.0	110.0 ± 3.0	1.05 ± 0.15
1.0 mg/L	0.0 ± 0.0	NA	NA	NA
ANOVA	$F_{2, 6} = 16.75$	$F_{1, 9} = 5.72$	$F_{1, 9} = 0.742$	$F_{1, 9} = 0.004$
	$P = 0.003$	$P = 0.040$	$P = 0.411$	$P = 0.945$
		Egg clutch 2 (GS 21–25)		
Control	46.7 ± 6.66	54.8 ± 4.42	114.1 ± 2.09	1.04 ± 0.05
0.5 mg/L	26.7 ± 13.33	144.0 ± 9.79	112.5 ± 2.95	0.87 ± 0.07
1.0 mg/L	0.0 ± 0.00	NA	NA	NA
ANOVA	$F_{2, 6} = 7.4$	$F_{1, 9} = 92.18$	$F_{1, 9} = 0.213$	$F_{1, 9} = 3.49$
	$P = 0.024$	$P < 0.001$	$P = 0.213$	$P = 0.094$
		Egg clutch 3 (GS 21–25)		
Control	46.7 ± 6.66	92.0 ± 7.37	119.2 ± 3.78	1.05 ± 0.09
0.5 mg/L	20.0 ± 0.00	184.0 ± 21.16	120.3 ± 4.48	0.96 ± 0.06
1.0 mg/L	0.0 ± 0.00	NA	NA	NA
ANOVA	$F_{2, 6} = 37$	$F_{1, 7} = 27.42$	$F_{1, 7} = 0.034$	$F_{1, 7} = 0.302$
	$P < 0.001$	$P = 0.001$	$P = 0.857$	$P = 0.599$
		Egg clutch 1 (GS 31–36)		
Control	36.7 ± 3.33	101.4 ± 7.22	116.4 ± 1.37	1.20 ± 0.03
0.5 mg/L	20.0 ± 0.00	178.0 ± 8.0	117.4 ± 1.95	1.00 ± 0.05
1.0 mg/L	3.3 ± 2.24	240.0 ± 24.0	140.0 ± 8.00	1.45 ± 0.15
ANOVA	$F_{2, 33} = 51.56$	$F_{2, 33} = 33.62$	$F_{2, 33} = 11.24$	$F_{2, 33} = 8.45$
	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.001$
		Egg clutch 2 (GS 31–36)		
Control	56.7 ± 3.33	117.9 ± 6.02	117.9 ± 0.93	1.01 ± 0.03
0.5 mg/L	26.7 ± 2.84	174.0 ± 8.89	124.9 ± 1.42	1.16 ± 0.11
1.0 mg/L	3.3 ± 2.24	180.0 ± 12.0	121.5 ± 7.50	1.35 ± 0.15
ANOVA	$F_{2, 33} = 88.45$	$F_{2, 49} = 15.51$	$F_{2, 49} = 8.25$	$F_{2, 49} = 1.94$
	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.154$
		Egg clutch 3 (GS 31–36)		
Control	43.3 ± 4.14	106.2 ± 7.19	181.0 ± 1.70	1.70 ± 0.04
0.5 mg/L	20.0 ± 4.26	184.0 ± 12.29	184.7 ± 2.98	1.69 ± 0.07
1.0 mg/L	11.7 ± 2.97	212.0 ± 31.84	186.7 ± 1.70	1.66 ± 0.04
ANOVA	$F_{2, 33} = 18.29$	$F_{2, 41} = 20.49$	$F_{2, 41} = 1.37$	$F_{2, 41} = 0.067$
	$P < 0.001$	$P < 0.001$	$P = 0.263$	$P = 0.934$

should be carefully considered when assessing toxicity effects on target and nontarget organisms.

Of those tadpoles that survived the acute effects of CFT Legumine, rotenone prolonged the time for tail resorption, which may have important ecological implications related to mobility. A tadpole in mid-resorption of its tail has both a tail and limbs, which results in poor mobility in water and on land in comparison with metamorphosed froglets (Wassersug and Sperry 1977) and increases the tadpole's susceptibility to predation (Dudley et al. 1991). Poor mobility in water would have negative consequences for the tadpole's ability to exhibit burst or fast-start movements. This type of swimming is used

TABLE 3. Average percentage of northern leopard frog tadpoles that completed metamorphosis at Gosner stage (GS) 47 after being exposed to CFT Legumine (5% rotenone) concentrations of 0.5, 1.0, and 2.0 mg/L for 48 h at GS 21–25 and GS 31–36 (NA = not available for analysis). For those tadpoles that completed metamorphosis, mean values (±SE) for the time to tail resorption (h), total days of development (d posthatch [dph]), and weight (g) are presented. Results of ANOVA (*F*-statistics and *P*-values) are also presented.

Treatment	Metamorphosis (%)	Resorption (h)	Development (dph)	Weight (g)
		Egg clutch 1 (GS 21–25)		
Control	53.3 ± 6.66	66.0 ± 9.88	86.9 ± 0.76	1.71 ± 0.04
0.5 mg/L	33.3 ± 6.66	110.4 ± 5.87	85.4 ± 1.63	1.76 ± 0.05
1.0 mg/L	20.0 ± 0.00	112.0 ± 32.0	89.7 ± 1.76	1.86 ± 0.06
ANOVA	$F_{2, 6} = 9.50$ $P = 0.013$	$F_{2, 13} = 4.28$ $P = 0.037$	$F_{2, 13} = 2.12$ $P = 0.159$	$F_{2, 13} = 1.80$ $P = 0.203$
		Egg clutch 1 (GS 31–36)		
Control	50.0 ± 4.47	72.0 ± 6.62	83.1 ± 0.78	1.70 ± 0.04
1.0 mg/L	20.0 ± 0.00	172.0 ± 31.24	84.5 ± 1.36	1.75 ± 0.03
2.0 mg/L	0.0 ± 0.00	NA	NA	NA
ANOVA	$F_{2, 15} = 9.50$ $P < 0.001$	$F_{1, 19} = 21.15$ $P < 0.001$	$F_{1, 19} = 0.897$ $P = 0.355$	$F_{1, 19} = 0.360$ $P = 0.555$
		Egg clutch 2 (GS 31–36)		
Control	53.3 ± 4.21	90.0 ± 6.00	83.7 ± 0.74	1.65 ± 0.05
1.0 mg/L	23.3 ± 3.33	185.1 ± 6.85	84.3 ± 1.20	1.74 ± 0.05
2.0 mg/L	0.0 ± 0.0	NA	NA	NA
ANOVA	$F_{2, 15} = 74.23$ $P < 0.001$	$F_{1, 21} = 87.20$ $P < 0.001$	$F_{1, 21} = 0.189$ $P = 0.667$	$F_{1, 21} = 0.962$ $P = 0.337$

by larval anurans to escape predators and as a behavioral reflex referred to as "bobbing," wherein tadpoles ventilate the lungs by moving quickly from the bottom of the pond to the surface and back down (Wassersug 1992; Wong and Booth 1994). Tail resorption also represents a period of natural immune suppression in tadpoles and thus increases their vulnerability to pathogens (Rollins-Smith 1998). Therefore, prolonged tail resorption due to rotenone exposure not only extends the amount of time the tadpole may experience difficulty in lung ventilation but may also increase its vulnerability to predators and pathogens.

For all three *Lithobates* species, despite the delay in tail resorption, the survivors of nominal CFT Legumine concentrations of 0.5 and 1.0 mg/L experienced only marginal delays in the overall time to complete metamorphosis in comparison with the controls. The total time for tail resorption is a brief, albeit important, benchmark in metamorphosis. Surviving Chiricahua leopard frog tadpoles experienced a marginally longer time to complete metamorphosis and a concomitant increase in weight. For example, control tadpoles weighed 1.23 g on average and completed metamorphosis within an average of 132.6 d ($n = 104$), whereas tadpoles that survived exposure to 1.0-mg/L CFT Legumine were 0.31 g heavier on average than the controls and completed metamorphosis within an average of 164.1 d ($n = 9$). Survivorship was highly reduced and metamorphosis was variable among egg clutches; therefore, the inferences to delay in metamorphosis and weight-related effects should be viewed with caution. However, Billman et al. (2011) also reported an average increase in mass of 0.38 g upon completion of metamorphosis in Columbia spotted frog tadpoles exposed to 1.0-mg/L CFT Legumine compared with controls. Slower or delayed development rates to achieve metamorphosis will increase the time that tadpoles are susceptible to predation and drying events (Semlitsch 1989; Goater et al. 1993; Rose 2005).

In the western United States, American bullfrog invasions have contributed to the decline of the California red-legged frog *Rana draytonii* (Lawler et al. 1999), foothill yellow-legged frog *R. boylii* (Kupferberg 1997), northern red-legged frog *R. aurora* (Kiesecker et al. 2001; Pearl et al. 2004), Oregon spotted frog *R. pretiosa* (Pearl et al. 2004), and several other ranids of the leopard frog complex (Clarkson and Rorabaugh 1989). Global trade and release of the invasive American bullfrog have contributed to the emergence of bullfrog invasions in Canada (Govindarajulu et al. 2006), Uruguay (Laufer et al. 2008), Brazil (Schloegel et al. 2010), Mexico (Luja and Rodríguez-Estrella 2010), and China (Wang et al. 2007). In the current study, concentrations of CFT Legumine (1.0–2.0 mg/L) resulted in complete mortality of the earliest life stage of American bullfrogs. Thus, the same concentrations used to target nonnative fishes could also serve to control invasive

TABLE 4. Average percentage of American bullfrog tadpoles that completed metamorphosis at Gosner stage (GS) 47 after being exposed to CFT Legumine (5% rotenone) concentrations of 0.5, 1.0, and 2.0 mg/L for 48 h at GS 21–25 and GS 31–36 (NA = not available for analysis). For those tadpoles that completed metamorphosis, mean values (±SE) for the time to tail resorption (h), total days of development (d posthatch [dph]), and weight (g) are presented. Results of ANOVA (*F*-statistics and *P*-values) are also presented.

Treatment	Metamorphosis (%)	Resorption (h)	Development (dph)	Weight (g)
		Egg clutch 1 (GS 21–25)		
Control	70.0 ± 5.77	78.4 ± 9.50	107.7 ± 1.06	1.76 ± 0.04
0.5 mg/L	50.0 ± 5.77	129.6 ± 16.47	108.4 ± 0.84	1.69 ± 0.07
1.0 mg/L	5.0 ± 5.00	156.0 ± 12.0	111.5 ± 1.50	2.0 ± 0.05
ANOVA	$F_{2, 9} = 36.27$	$F_{2, 24} = 5.98$	$F_{2, 24} = 1.02$	$F_{2, 24} = 2.64$
	$P < 0.001$	$P = 0.007$	$P = 0.372$	$P = 0.091$
		Egg clutch 2 (GS 21–25)		
Control	58.3 ± 5.27	90.5 ± 5.53	108.9 ± 0.88	1.73 ± 0.06
0.5 mg/L	33.3 ± 5.27	156.0 ± 10.14	112.5 ± 1.90	1.93 ± 0.08
1.0 mg/L	8.3 ± 5.27	132.0 ± 12.0	109.5 ± 0.50	1.90 ± 0.00
ANOVA	$F_{2, 15} = 22.5$	$F_{2, 20} = 20.09$	$F_{2, 20} = 1.98$	$F_{2, 20} = 2.16$
	$P < 0.001$	$P < 0.001$	$P = 0.163$	$P = 0.140$
		Egg clutch 3 (GS 21–25)		
Control	50.0 ± 0.00	90.0 ± 5.22	110.0 ± 0.88	1.85 ± 0.06
0.5 mg/L	25.0 ± 6.45	148.0 ± 7.37	115.5 ± 2.68	2.0 ± 0.08
1.0 mg/L	8.3 ± 5.27	156.0 ± 12.0	110.5 ± 1.50	2.0 ± 0.15
ANOVA	$F_{2, 15} = 19.0$	$F_{2, 17} = 26.75$	$F_{2, 17} = 3.26$	$F_{2, 17} = 1.20$
	$P < 0.001$	$P < 0.001$	$P = 0.063$	$P = 0.325$
		Egg clutch 1 (GS 31–36)		
Control	78.0 ± 2.00	42.5 ± 2.85	94.6 ± 0.52	1.73 ± 0.03
1.0 mg/L	58.0 ± 2.00	112.6 ± 4.77	106.6 ± 0.61	1.88 ± 0.04
2.0 mg/L	0.0 ± 0.00	NA	NA	NA
ANOVA	$F_{2, 27} = 615.5$	$F_{1, 66} = 176.21$	$F_{1, 66} = 218.75$	$F_{1, 66} = 7.76$
	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.006$
		Egg clutch 2 (GS 31–36)		
Control	50.0 ± 6.45	88.0 ± 6.14	104.2 ± 0.83	1.68 ± 0.05
1.0 mg/L	29.2 ± 7.68	192.0 ± 21.9	108.2 ± 2.92	1.81 ± 0.09
2.0 mg/L	0.0 ± 0.00	NA	NA	NA
ANOVA	$F_{2, 15} = 18.79$	$F_{1, 19} = 26.72$	$F_{1, 19} = 2.27$	$F_{1, 19} = 1.50$
	$P < 0.001$	$P < 0.001$	$P = 0.148$	$P = 0.236$
		Egg clutch 3 (GS 31–36)		
Control	54.2 ± 4.16	86.8 ± 5.11	104.3 ± 0.91	1.65 ± 0.05
1.0 mg/L	33.3 ± 5.27	171.0 ± 15.33	113.6 ± 2.72	1.91 ± 0.12
2.0 mg/L	0.0 ± 0.00	NA	NA	NA
ANOVA	$F_{2, 15} = 49.61$	$F_{1, 19} = 38.69$	$F_{1, 19} = 14.94$	$F_{1, 19} = 4.56$
	$P < 0.001$	$P < 0.001$	$P = 0.001$	$P = 0.046$

American bullfrogs by targeting sensitive life stages when other susceptible ranids are not present.

This research documents the sensitivity of three sympatric *Lithobates* species to rotenone and emphasizes the importance of management guidelines that protect the federally listed Chiricahua leopard frog and other amphibian species of conservation need (e.g., the northern leopard frog in New Mexico). These results complement a small but growing body of evidence suggesting that early stage tadpoles of anuran species are susceptible to commonly used field concentrations of rotenone but that toxicity decreases rapidly with tadpole maturation. Billman et al. (2011, 2012) provided useful

management recommendations to reduce collateral effects on species that have been identified in application areas. Typical field applications follow a maximum application frequency that could be offset to consider the timing of important life history events, such as breeding, in nontarget organisms. Geographic origin may contribute to variation in toxicity responses among ranids, emphasizing a continued need for the description of lethal concentrations as well as postexposure effects on these nontarget organisms during fish removal. In addition, overlap in breeding seasons of sympatric *Lithobates* species suggests that comprehensive field surveys should be conducted prior to piscicide applications. These surveys would not only identify the prevalence of nontarget ranid species but also the presence of sensitive life stages. From these surveys, managers would be able to reduce collateral effects of piscicidal applications, which remain an essential tool in the conservation and management of native fishes.

ACKNOWLEDGMENTS

Research was funded through a Share with Wildlife Grant (13-516-0000-00043) from the New Mexico Department of Game and Fish. Additional support was provided by the Turner Endangered Species Fund; the U.S. Fish and Wildlife Service, Ecological Services Field Office; the Department of Fish, Wildlife, and Conservation Ecology, New Mexico State University; and the U.S. Geological Survey, New Mexico Cooperative Fish and Wildlife Research Unit. We are especially grateful to H. Small (Turner Endangered Species Fund) for providing egg clutches of the Chiricahua leopard frog. Approved use of the Chiricahua leopard frog was provided through a federal permit for threatened and endangered species (TE046517-0). Approval of this research was received from the Institutional Animal Care and Use Committee (Protocol Number 2012-025) at New Mexico State University and from the New Mexico Department of Game and Fish (Permit Number 3033). The work was made possible in part due to the help of A. Gandara and personnel at the Fabian Garcia Research Facility. Any use of trade names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

ORCID

Colleen A. Caldwell http://orcid.org/0000-0002-4730-4867

REFERENCES

ASTM (American Society for Testing and Materials). 2014. Standard guide for conducting acute toxicity tests on test materials with fishes, macroinvertebrates, and amphibians. ASTM International, ASTM-E729-96, West Conshohocken, Pennsylvania.

Behler, J. L., and F. W. King, editors. 1979. The Audubon Society field guide to North American reptiles and amphibians. Knopf, New York.

Bervan, K. A. 1990. Factors affecting population fluctuations in larval and adult stages of the wood frog (*Rana sylvatica*). Ecology 71:1599–1608.

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

Billman, H. G., C. G. Kruse, S. St-Hilaire, T. M. Koel, J. F. Arnold, and C. R. Peterson. 2012. Effects of rotenone on Columbia spotted frogs *Rana luteiventris* during field applications in lentic habitats of southwestern Montana. North American Journal of Fisheries Management 32:781–789.

Billman, H. G., S. St-Hilaire, C. G. Kruse, T. S. Peterson, and C. R. Peterson. 2011. Toxicity of the piscicide rotenone to Columbia spotted frog and boreal toad tadpoles. Transactions of the American Fisheries Society 140:919–927.

Boone, M. D., and S. M. James. 2003. Interactions of an insecticide, herbicide, and natural stressors in amphibian community mesocosms. Ecological Applications 13:829–841.

Boone, M. D., and S. M. James. 2005. Aquatic and terrestrial mesocosms in amphibian ecotoxicology. Applied Herpetology 2:231–257.

Boone, M. D., R. D. Semlitsch, E. E. Little, and M. C. Doyle. 2007. Multiple stressors in amphibian communities: effects of chemical contamination, bullfrogs, and fish. Ecological Applications 17:291–301.

Brown, P. J. 2010. Environmental conditions affecting the efficiency and efficacy of piscicides for use in nonnative fish eradication. Doctoral dissertation. Montana State University, Bozeman.

Cabras, P., P. Caboni, M. Cabras, A. Angioni, and M. Russo. 2002. Rotenone residues on olives and in olive oil. Journal of Agricultural and Food Chemistry 50:2576–2580.

Cain, M. L., W. D. Bowman, and S. D. Hacker, editors. 2011. Ecology, 2nd edition. Sinauer, Sunderland, Massachusetts.

Chandler, J. H. Jr., and L. L. Marking. 1982. Toxicity of rotenone to selected aquatic invertebrates and frog larvae. Progressive Fish-Culturist 44:78–80.

Clarkson, R. W., and J. C. Rorabaugh. 1989. Status of leopard frogs (*Rana pipiens* complex: Ranidae) in Arizona and southeastern California. Southwestern Naturalist 34:531–538.

Collins, J. P. 1979. Intrapopulation variation in the body size at metamorphosis and timing of metamorphosis in the bullfrog, *Rana catesbeianus*. Ecology 60:738–749.

Crother, B. I., editor. 2012. Standard common and current scientific names for North American amphibians, turtles, reptiles, and crocodilians. Herpetological Circular 39:1–92.

Degenhardt, W. G., C. W. Painter, and A. H. Price, editors. 1996. The amphibians and reptiles of New Mexico. University of New Mexico Press, Albuquerque.

Draper, W. M. 2002. Near-UV quantum yields for rotenone and piperonyl butoxide. Analyst 127:1370–1374.

Dudley, R., V. A. King, and R. J. Wassersug. 1991. The implications of shape and metamorphosis for drag forces on a generalized pond tadpole (*Rana catesbeiana*). Copeia 1991:252–257.

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. 2010. Planning and standard operating procedures for the use of rotenone in fish management: rotenone SOP manual. American Fisheries Society, Bethesda, Maryland.

Gilderhus, P. A., J. L. Allen, and V. K. Dawson. 1986. Persistence of rotenone in ponds at different temperatures. North American Journal of Fisheries Management 6:129–130.

Goater, C. P., R. D. Semlitsch, and M. V. Bernasconi. 1993. Effects of body size and parasite infection on the locomotory performance of juvenile toads, *Bufo bufo*. Oikos 66:129–136.

Gómez-Mestre, I., and M. Tejedo. 2003. Local adaptation of an anuran amphibian to osmotically stressful environments. Evolution 57:1889–1899.

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

Govindarajulu, P., W. S. Price, and B. R. Anholt. 2006. Introduced bullfrogs (*Rana catesbeiana*) in western Canada: has their ecology diverged? Journal of Herpetology 40:249–260.

Grisak, G., D. R. Skaar, G. L. Michael, M. E. Schnee, and B. L. Marotz. 2007. 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, and 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 9:1620–1635.

Hamilton, M. A., R. C. Russo, and R. V. Thurston. 1977. Trimmed Spearman–Karber method for estimating median lethal concentrations in toxicity bioassays. Environmental Science and Technology 11:714–719.

Hammerson, G., F. Solís, R. Ibáñez, C. Jaramillo, and Q. Fuenmayor. 2004. *Lithobates pipiens*. IUCN Red List of Threatened Species 2004. Available: www.iucnredlist.org. (February 2013).

Hinshaw, S. 1992. Northern leopard frog, *Rana pipiens*. Pages 77–81 *in* M. L. Hunter, J. Albright, and J. Arbuckle, editors. The amphibians and reptiles of Maine. Maine Agricultural Experiment Station, Bulletin 838, Orono.

Jennings, R. D. 1988. Ecological studies of the Chiricahua leopard frog, *Rana chiricahuensis*, in New Mexico. Report to the New Mexico Department of Game and Fish, Share with Wildlife Program, Santa Fe.

Kiesecker, J. M., A. R. Blaustein, and C. L. Miller. 2001. Potential mechanisms underlying the displacement of native red-legged frogs by introduced bullfrogs. Ecology 82:1964–1970.

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.

Knapp, R. A., and K. R. Matthews. 2000. Non-native fish introductions and the decline of the mountain yellow-legged frog from within protected areas. Conservation Biology 14:428–438.

Kruse, C. G., P. Clancey, S. Barndt, K. Patten, and B. Shepard. 2013. Setting the stage for conservation success: large-scale watershed renovation and re-introduction of Cutthroat Trout in the Rocky Mountain region of the USA. Pages 26–32 *in* P. S. Soorae, editor. Global re-introduction perspectives 2013: further case studies from around the globe. International Union for Conservation of Nature, Species Survival Commission, Re-Introduction Specialist Group; and Environment Agency of Abu Dhabi, Abu Dhabi, United Arab Emirates.

Kupferberg, S. J. 1997. Bullfrog (*Rana catesbeiana*) invasion of a California river: the role of larval competition. Ecology 78:1736–1751.

Laufer, G., A. Canavero, D. Nuñez, and R. Maneyro. 2008. Bullfrog (*Lithobates catesbeianus*) invasion in Uruguay. Biological Invasions 10:1183–1189.

Lawler, S. P., D. Dritz, T. Strange, and M. Holyoak. 1999. Effects of introduced mosquitofish and bullfrogs on the threatened California red-legged frog. Conservation Biology 13:613–622.

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

Luja, V. H., and R. Rodríguez-Estrella. 2010. The invasive bullfrog *Lithobates catesbeianus* in oases of Baja California Sur, Mexico: potential effects in a fragile ecosystem. Biological Invasions 12:2979–2983.

McCaffery, M., and M. K. Phillips, editors. 2013. Turner Endangered Species Fund and Turner Biodiversity Divisions annual report, 2013. Turner Endangered Species Fund, Bozeman, Montana.

McClay, W. 2005. Rotenone use in North America (1988–2002). Fisheries 30(4):29–31.

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

McKercher, L., and D. R. Gregorie. 2016. *Lithobates catesbeianus*. U.S. Geological Survey, Nonindigenous Aquatic Species Database, Gainesville, Florida. Available: http://nas.er.usgs.gov/queries/factsheet.aspx?speciesid=71. (September 2011).

Merrell, D. J. 1977. Life history of the leopard frog, *Rana pipiens*, in Minnesota. Bell Museum of Natural History, Occasional Paper 15, Minneapolis, Minnesota.

NMDGF (New Mexico Department of Game and Fish). 2006. Comprehensive wildlife conservation strategy for New Mexico. NMDGF, Santa Fe.

Pearl, C. A., M. J. Adams, R. B. Bury, and B. McCreary. 2004. Asymmetrical effects of introduced bullfrogs (*Rana catesbeiana*) on native ranid frogs in Oregon. Copeia 2004:11–20.

R Development Core Team. 2007. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. Available: www.R-project.org. (Feburary 2017).

Ritter, W. F., and L. Bergstrom. 2001. Nitrogen and water quality. Pages 59–90 *in* W. F. Ritter and A. Shirmohammadi, editors. Agricultural nonpoint source pollution: watershed management and hydrology. Lewis Publishers, Boca Raton, Florida.

Rollins-Smith, L. A. 1998. Metamorphosis and the amphibian immune system. Immunological Reviews 166:221–230.

Rose, C. S. 2005. Integrating ecology and developmental biology to explain the timing of frog metamorphosis. Trends in Ecology and Evolution 20:129–135.

Schloegel, L. M., C. M. Ferreira, T. Y. James, M. Hipolito, J. E. Longcore, A. D. Hyatt, M. Yabsley, A. Martins, R. Mazzoni, A. J. Davies, and P. Daszak. 2010. The North American bullfrog as a reservoir for the spread of *Batrachochytrium dendrobatidis* in Brazil. Animal Conservation 13:53–61.

Scott, N. J. Jr., and R. D. Jennings. 1985. The tadpoles of five species of New Mexican leopard frogs. University of New Mexico Museum of Southwestern Biology Occasional Paper 3.

Semlitsch, R. D. 1989. Effects of body size, sibship, and tail injury on the susceptibility of tadpoles to dragonfly predation. Canadian Journal of Zoology 68:1027–1030.

Semlitsch, R. D., D. C. Scott, and J. H. K. Pechmann. 1988. Time and size at metamorphosis related to adult fitness in *Ambystoma talpoideum*. Ecology 69:184–192.

Sparling, D. W., C. A. Bishop, and G. Linder. 2000. The current status of amphibian and reptile ecotoxicological research. Page 1 *in* D. W. Sparling, G. Linder, and C. A. Bishop, editors. Ecotoxicology of amphibians and reptiles. Society of Environmental Toxicology and Chemistry, Pensacola, Florida.

Sredl, M. J., and J. M. Howland. 1995. Conservation and management of Madrean populations of the Chiricahua leopard frog. U.S. Forest Service General Technical Report RM-GTR-264:379–385.

Stebbins, R. C. 2003. A field guide to western reptiles and amphibians, 3rd edition. Houghton Mifflin, Boston.

USEPA (U.S. Environmental Protection Agency). 2007. Reregistration eligibility decision for rotenone. Prevention, pesticides and toxic substances. USEPA, Report 738-R-07-005, Washington, D.C.

USOFR (U.S. Office of the Federal Register). 2002. Endangered and threatened wildlife and plants; listing and designation of critical habitat for the Chiricahua leopard frog, final rule. Federal Register 77:54(20 March 2012):16324–16424.

Wang, Y., Z. Guo, C. A. Pearl, and Y. Li. 2007. Body size affects the predatory interactions between introduced American bullfrogs (*Rana catesbeiana*) and native anurans in China: an experimental study. Journal of Herpetology 41:514–520.

Wassersug, R. J. 1992. The basic mechanics of ascent and descent by anuran larvae (*Xenopus laevis*). Copeia 1992:890–894.

Wassersug, R. J., and D. G. Sperry. 1977. Relationship of locomotion to differential predation on *Pseudacris triseriata* (Anura, Hylidae). Ecology 58:830–839.

Wong, S., and D. T. Booth. 1994. Hypoxia induces surfacing behavior in brown-striped frog (*Limnodynastes peronii*) larvae. Comparative Biochemistry and Physiology Part A: Physiology 109:437–445.

Wright, K. M., and B. R. Whitaker. 2001. Amphibian medicine and captive husbandry. Krieger Publishing Company, Malabar, Florida.