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Publisher: Taylor & Francis

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North American Journal of Fisheries Management

Publication details, including instructions for authors and subscription information:

<http://www.tandfonline.com/loi/ujfm20>

Effects of Rotenone on Columbia Spotted Frogs *Rana luteiventris* during Field Applications in Lentic Habitats of Southwestern Montana

Hilary G. Billman^a, Carter G. Kruse^b, Sophie St-Hilaire^a, Todd M. Koel^c, Jeffrey L. Arnold^c & Charles R. Peterson^a

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

^b Turner Enterprises, Incorporated, 1123 Research Drive, Bozeman, Montana, 59718, USA

^c Yellowstone Center for Resources, Fisheries and Aquatic Sciences Section, Post Office Box 168, Yellowstone National Park, Wyoming, 82190, USA

Version of record first published: 27 Jul 2012

To cite this article: Hilary G. Billman, Carter G. Kruse, Sophie St-Hilaire, Todd M. Koel, Jeffrey L. Arnold & Charles 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*, 32:4, 781-789

To link to this article: <http://dx.doi.org/10.1080/02755947.2012.692349>

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ARTICLE

Effects of Rotenone on Columbia Spotted Frogs *Rana luteiventris* during Field Applications in Lentic Habitats of Southwestern Montana

Hilary G. Billman*

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

Carter G. Kruse

Turner Enterprises, Incorporated, 1123 Research Drive, Bozeman, Montana 59718, USA

Sophie St-Hilaire

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

Todd M. Koel and Jeffrey L. Arnold

Yellowstone Center for Resources, Fisheries and Aquatic Sciences Section, Post Office Box 168, Yellowstone National Park, Wyoming 82190, USA

Charles R. Peterson

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

Abstract

Fisheries managers are restoring native populations by removing nonnative fishes worldwide. Increasingly, the piscicide rotenone is used to accomplish this. Fish introductions and removals change the aquatic environment, and it is important to consider the impacts of these actions on nontarget species, including amphibians. Laboratory experiments have shown that rotenone can negatively affect tadpoles. We therefore assessed the effects of rotenone used on two wild amphibian populations. The commercial piscicide formulation CFT Legumine (5% rotenone) was applied at 1 mg/L (50 µg/L rotenone) to a lake containing nonnative trout in Yellowstone National Park (YNP) in 2006 and two fishless wetlands on private lands in southwestern Montana in 2008. Amphibian surveys were conducted immediately prior to and after the rotenone treatments to obtain tadpole population estimates. Follow-up surveys were conducted 1 year posttreatment to estimate tadpole recovery. In YNP, additional surveys were conducted 2 and 3 years postapplication to observe longer-term effects of fish removal and the subsequent introduction of native fish. Within 24 h following application of rotenone, there was 100% mortality in gill-breathing tadpoles, but nongill-breathing metamorphs, juveniles, and adults were apparently unaffected. In the years following, tadpoles repopulated all waters and population levels were similar to, or, in the case of YNP because of concurrent fish removal, higher than pretreatment levels. In YNP, tadpole abundance and distribution decreased after westslope cutthroat trout *Oncorhynchus clarkii lewisi* were stocked in the treated lake.

*Corresponding author: hilary.billman@montana.edu

Received October 22, 2011; accepted May 4, 2012

As a result of significant declines in native fish populations, often caused by interactions with nonnative fishes, federal and state agencies and nongovernmental organizations are increasingly implementing nonnative fish eradication projects to conserve and restore populations of native species. Because of its proven efficacy (Shepard et al. 2002), chemical removal has become a common technique for accomplishing this goal (Mangum and Madrigal 1999; Finlayson et al. 2000; Ling 2003; McClay 2005; Hamilton et al. 2009). In particular, application of rotenone products has increased globally because of its success and reliability in removing unwanted fish (McClay 2005; Finlayson et al. 2010). While the effects of rotenone on fish are better understood (Meadows 1973; Amey 1984; Finlayson 2000; Britton and Brazier 2006; Grisak et al. 2007a), less is known about the impacts of rotenone applications on nontarget species, like amphibians.

Understanding the impacts of a chemical application to remove fish on amphibians is important because of the role amphibians play in aquatic and terrestrial ecosystems. Amphibians are both prey and predators and are therefore an integral component of ecological communities. As predators, adult amphibians can reduce populations of invertebrate species. For example, in one recent study, larval mosquitoes, a common disease vector, were denser in ponds that had fewer insect and amphibian predators (Chase and Shulman 2009). As prey, amphibians are a key dietary component for a variety of organisms, including mammals, fish, reptiles, and birds (Blaustein et al. 1994; Young et al. 2004). Thus, actions that impact amphibian communities could have cascading effects on an aquatic ecosystem.

The introduction of rotenone can have immediate, negative impacts on nontarget, gill-breathing aquatic organisms, such as amphibians (Fontenot et al. 1994; McCoid and Bettoli 1996; Maxell 2000; Patla 2005). Laboratory studies have demonstrated that CFT Legumine (5% rotenone) can cause significant mortality in boreal toad *Anaxyrus boreas* and Columbia spotted frog *Rana luteiventris* tadpoles at dosages commonly used in field applications (Billman et al. 2011). Similarly, Grisak et al. (2007b) documented mortality in a variety of native Montana amphibian larvae after exposure to rotenone products. The longer-term consequences of this mortality (i.e., localized population reduction, loss of a larval year-class, or impacts on future reproduction), if any, are not well understood.

The removal of introduced fish, whether permanent or temporary, can permit the recovery or expansion of local amphibian populations by returning ecological communities to a more native composition (Pilliod and Peterson 2001; Knapp 2005; Boone et al. 2007; Walston and Mullin 2007). The removal of introduced, predatory rainbow trout *Oncorhynchus mykiss* and brook trout *Salvelinus fontinalis* in the Sierra Nevada, California, enabled significant, localized recovery of declining yellow-legged frogs *Rana muscosa* (Vredenburg and Wake 2004). Not only a direct predator, introduced fishes can indirectly, negatively influence the distribution and foraging time of

amphibians (Binckley and Resetarits 2003; Orizaola and Braña 2003; Benard 2006; Barr and Babbitt 2007).

To maintain and preserve extant populations of amphibians during fish eradication projects, it is important to measure the effects of rotenone under field conditions, thereby building upon knowledge gained from recent laboratory experiments on amphibians (Grisak et al. 2007b; Billman et al. 2011). The overall goal of this research was to document some of the short- and longer-term impacts of rotenone exposure on amphibians in a field setting. Specifically, our objectives were to (1) determine the effects of a commonly used rotenone product, CFT Legumine (5% rotenone), on amphibian populations under field conditions and (2) describe the changes in a tadpole population before fish removal and after subsequent fish introduction. In doing so, we hope to provide information that will facilitate amphibian conservation during fish eradication projects.

METHODS

Study Site

Yellowstone National Park.—In Yellowstone National Park (YNP), fisheries management was historically guided by the need to provide a high quality angling experience for visitors, and many of YNP's waters were stocked with nonnative fish species (Varley and Schullery 1998). In YNP waters, native populations have been completely extirpated, reduced in abundance, or compromised because of hybridization or competition with nonnative fish. Under a new management paradigm, YNP fisheries managers seek to reverse this trend and populations of westslope cutthroat trout (WCT) *Oncorhynchus clarkii lewisi* are a current focus of YNP's native fish restoration goals (Koel et al. 2006). The East Fork Specimen Creek drainage, beginning at its headwater lake, High Lake, was chosen to be the initial focus of WCT restoration in YNP (Koel and York 2006). Though historically fishless, High Lake was stocked in 1937 with Yellowstone cutthroat trout (YCT) *Oncorhynchus clarkii bouvieri*, which are not native to the watershed.

High Lake has a surface area of approximately 3.2 ha and is located in Montana in the northwestern corner of YNP at an elevation of 2638 m. The lake and an associated wetland complex form the headwaters of the East Fork Specimen Creek, a tributary to the Gallatin River in the upper Missouri River drainage (Figure 1). High Lake was treated twice with rotenone in early and mid-August 2006 to remove introduced YCT prior to the introduction of WCT, the native trout of the upper Missouri River drainage (Shepard et al. 2002). Two nearby, fishless wetlands (North [0.17 ha] and South [0.13 ha]) were designated as untreated sites (controls). The South and North wetlands were located approximately 190 m and 620 m from High Lake, respectively, and were separated by approximately 760 m (Figure 1). The primary vegetation in all three bodies of water was aquatic

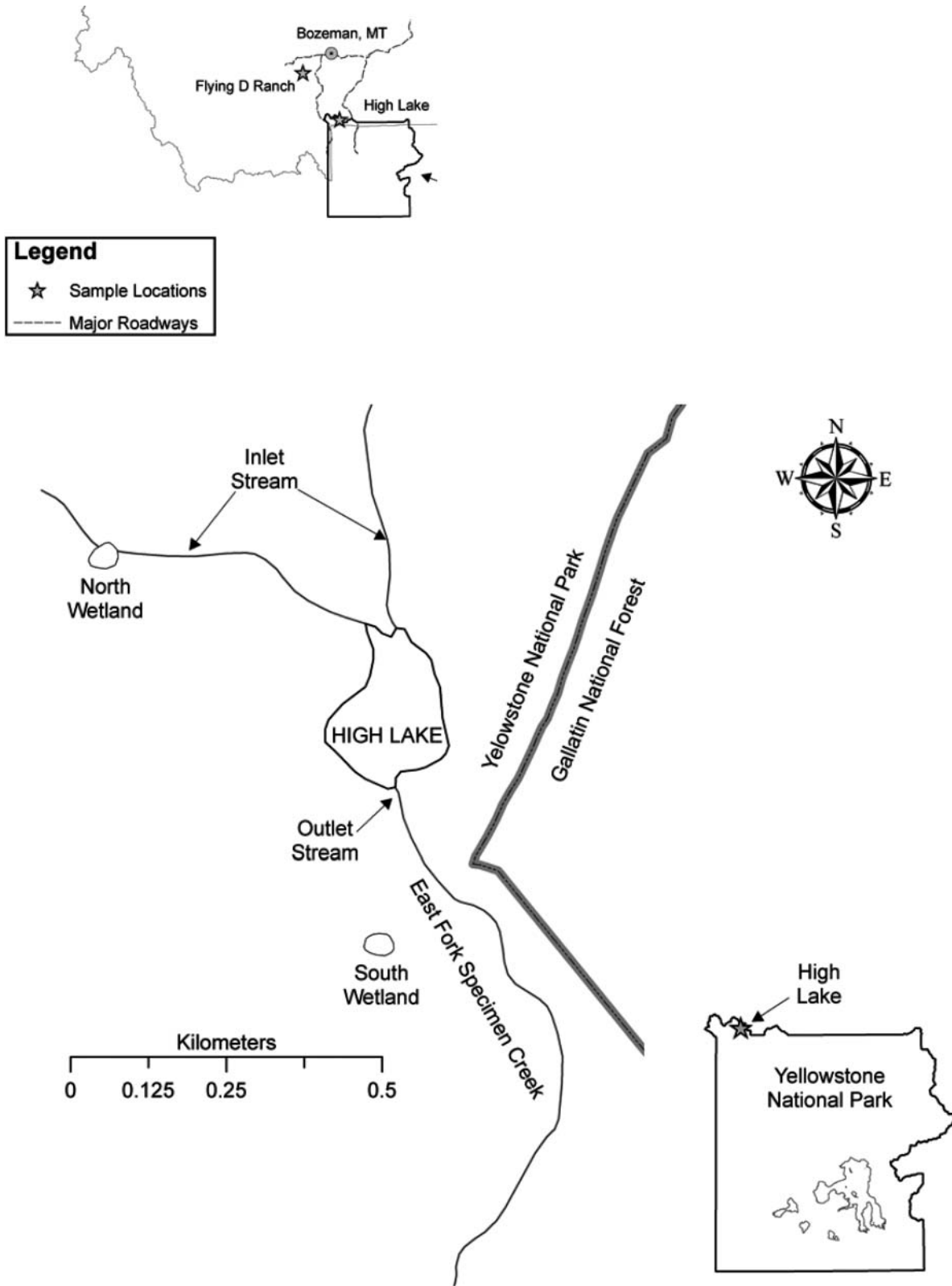


FIGURE 1. Locations of the High Lake and Flying D Ranch study areas. The High Lake area included the outlet channel (amphibian breeding site), North wetland and South wetland.

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sedges (genus *Scirpus*), and the sites retained water throughout the summer season.

Flying D Ranch.—Four small fishless wetlands on the Flying D Ranch in the Cherry Creek drainage were also studied (Figure 1). Cherry Creek is a tributary to the Madison River drainage (upper Missouri River system) where a native WCT restoration project is currently underway. The four wetlands were located between 1463 and 1830 m in elevation and were known amphibian breeding sites. Wetlands #1, #2, and #3 were characterized as vegetated primarily by aquatic sedges (genus *Scirpus*) across 76–100% of the wetland, while Wetland #4 was vegetated by cattails (genus *Typha*) across 26–50%. All four wetlands varied in average depth and diameter but were less than 0.10 ha and 1 m in depth. The wetlands retain water throughout the summer. Wetlands #1 and #3 were treated with rotenone while Wetlands #2 and #4 served as study controls.

The High Lake and Flying D Ranch sites were in relatively close geographic proximity and were similar in repopulation potential (i.e., immediacy to other wetlands containing amphibians). However, they differed in important ways. High Lake contained an established population of fish prior to rotenone application, while the Flying D Ranch wetlands did not. High Lake was a high elevation lake whereas the Flying D Ranch sites were small, mid to low elevation wetlands.

Yellowstone National Park

Amphibian surveys were conducted at High Lake and the two adjacent wetlands using U.S. Geological Survey Amphibian Research & Monitoring Initiative protocols (Corn et al. 2005). The initial pretreatment survey at High Lake took place midday in early August 2006, while the North and South wetlands were surveyed the following day, with approximately equal time spent at each wetland. These surveys consisted of walking the entire margin of the wetland to both randomly and strategically (i.e., when tadpoles were observed) capture tadpoles by dip net to determine the presence and distribution of larvae. The captured tadpoles were identified to species and aged according to the Gosner staging system (Gosner 1960). Mark–recapture population estimates (Thompson et al. 1998) were obtained by marking each captured tadpole with a 2–3 mm tail clip during the initial survey, releasing them at or near the point of capture, and then collecting tadpoles during a second recapture survey within 48 h to count the number of marked and unmarked individuals. High Lake was surveyed a second time on August 6, while the recapture surveys at the North and South wetlands took place on August 8. During these surveys, the general distribution of juvenile and adult frogs and toads was assessed visually.

Approximately 15 h prior to the rotenone application in High Lake, three mesh sentinel cages holding captured individuals were placed at different locations around High Lake to ensure that any observed effects were a result of rotenone application and not other environmental factors. One cage, containing 18 tadpoles, was placed at the midway point along the east side of the outlet channel; a second cage with 18 tadpoles was

placed along the margin at the north end of High Lake; and a third cage with three adult frogs was placed at the midway point along the west side of the outlet channel. A control cage containing 19 tadpoles was placed in the untreated South wetland. All captured individuals, both adults and tadpoles, were Columbia spotted frogs, and all tadpoles placed in the sentinel cages were captured in the High Lake outlet channel. Sentinel cages were checked the following morning immediately prior to the rotenone treatment to record any overnight mortality. During the treatment, a fifth sentinel cage containing 18 tadpoles was placed in the East Fork Specimen Creek (above the detoxification station) to determine if moving water impacted tadpole survival differently than suspension in the outlet or lake.

The CFT Legumine was applied to High Lake at an estimated concentration of 1 mg/L. Inflatable rafts with outboard motors were used to distribute the piscicide within the lake, and backpack sprayers were used to apply rotenone to the lake margins and the outlet channel. A total of 17.5 gal of piscicide were applied to the lake, outlet channel, inlet streams, and spring seeps. In order to detoxify rotenone leaving High Lake, potassium permanganate was applied to the East Fork Specimen Creek at the end of the outflow channel. The North and South wetlands had no connectivity with High Lake and were not treated. The following day, 24 h after rotenone was first applied to the lake, a second tadpole survey of the outflow channel was conducted and sentinel cages were removed. Surviving individuals in sentinel cages were released.

The only fish killed during the rotenone treatment were YCT. Crews searched for and collected all visible fish and adult amphibian carcasses during the 24 h period following treatment. Total length (mm) and weight (g) were measured on a subset of dead fish collected.

In July 2007, approximately 1 year after rotenone treatment, amphibian breeding and mark–recapture surveys were conducted at High Lake and the two adjacent wetlands, as previously described. We assessed tadpole distribution in the outlet channel and lake margin in the absence of fish. Immediately following these surveys, WCT embryos (via remote streamside incubators) and mixed-age individuals were stocked in High Lake. Similar mark–recapture amphibian surveys and fish stocking events were conducted in 2008 and 2009 at High Lake.

Data from the mark–recapture surveys were used to obtain a tadpole population estimate at each of the three sites for each of the 4 years sampling took place. Specifically, because High Lake and the two control wetlands met the basic assumptions of a closed system (i.e., no deaths, births, immigration, or emigration between the mark and recapture events), Chapman's modification of the Lincoln-Petersen equation (Thompson et al. 1998) was used to calculate the population estimates and 95% confidence intervals.

Flying D Ranch

In May 2008, several small, fishless wetlands in the Cherry Creek drainage on the Flying D Ranch were assessed for

suitability as experimental rotenone treatment sites. Four of these wetlands were strategically chosen for inclusion in this study. Previous surveys of wetlands in this area documented breeding by Columbia spotted frogs and boreal toads, but we attempted to choose wetlands that contained only Columbia spotted frogs to reduce the potential negative impacts of rotenone application to boreal toads, a regionally sensitive species. The sites were similar in size, configuration, habitat, and opportunity for Columbia spotted frog tadpole repopulation the following breeding season. Two wetlands (#1 and #3) were treated with rotenone and the other two wetlands (#2 and #4) were designated as controls.

In July 2008, pretreatment amphibian surveys were conducted at each wetland, as described previously for High Lake, to confirm the species present in the four wetlands and to obtain a pretreatment population estimate of tadpoles. An age estimate of the tadpole population at each wetland was obtained by staging captured individuals according to the Gosner staging system (Gosner 1960). The initial surveys (i.e., marking tadpoles) were conducted midday in July at all four wetlands. Recapture surveys were conducted within 48 h for all four wetlands.

Following the completion of the recapture surveys, Wetlands #1 and #3 were treated at approximately 1 mg/L CFT Legumine (5% active rotenone). The CFT Legumine was applied as evenly as possible by pumping pond water with a small gasoline pump and injecting rotenone via siphon into the pump discharge, which was distributed across the wetland surface and into the water column. The dosage of 1 mg/L CFT Legumine was selected as a commonly used field dose in pond and stream treatments and it matched the concentration used at High Lake.

We assessed the effects of treatment over two temporal windows. First, surveys were conducted 24 h posttreatment to determine the short-term impacts of rotenone application on amphibians. Second, in July of 2009, 1 year after the initial rotenone application, amphibian surveys were again conducted at each of the four ponds to obtain a 1-year posttreatment tadpole population estimate. Pre- and posttreatment population estimates were calculated using Chapman's modification of the Lincoln-Petersen equation (Thompson et al. 1998) and 95% confidence intervals.

RESULTS

Yellowstone National Park

At High Lake, only Columbia spotted frogs were documented to be breeding (i.e., there were larvae present). Both Columbia spotted frogs and boreal chorus frogs *Pseudacris maculata* were sampled in the adjacent North and South wetlands (control wetlands). A single adult boreal toad was documented at High Lake from 2006 to 2008, but no egg masses or larvae of this species were ever observed or sampled. The age estimate for tadpoles at High Lake and the two control wetlands sampled during all 4 years was Gosner stage 40–43. Tadpoles were present in High Lake and both wetlands immediately prior to treatment;

however, tadpoles at High Lake were limited to the outlet channel during pretreatment surveys and were not found in the main lake body or around the lake margins.

No live tadpoles were found after the rotenone treatment, but nongill-breathing juveniles and adult-stage frogs were present at multiple points throughout the lake. There was 100% tadpole mortality in the two sentinel cages suspended in the lake and outlet, while the three adults held in a single sentinel cage survived the treatment. No adult or juvenile mortalities were observed in or along the shoreline of High Lake, and posttreatment distribution and abundance appeared similar to pretreatment observations. Comparatively, there was no tadpole mortality in the sentinel cage at the South wetland, and no tadpole or adult mortality was observed in either of the two untreated wetlands adjacent to the lake. There was 11% (2 out of 18) tadpole survival in the fifth sentinel cage which was placed in flowing water at the end of the High Lake outlet channel during treatment.

Pretreatment tadpole population estimates varied from 54 ± 19 (95% confidence interval) in the South wetland to 115 ± 19 at High Lake (Figure 2). In 2007, 1 year after rotenone treatment, the tadpole population estimate at High Lake was six times greater (705 ± 38) than the pretreatment estimate. Following fish introductions at High Lake in 2007, tadpole population estimates declined each year from the 2007 posttreatment high to 541 ± 15 in 2009 but remained higher than the estimate obtained in 2006 before rotenone application. In contrast, the tadpole population estimates in both control wetlands remained similar across years and ranged from 50 to 100 individuals (Figure 2).

In 2006, 793 YCT mortalities were collected from High Lake during and after the rotenone application. Given that High Lake is a relatively small, shallow lake, this was estimated to be a large proportion of the existing pretreatment population. Lengths on 301 individual YCT ranged from 72 mm to 400 mm. Each year from 2007 to 2009, approximately 1,000 mixed-age WCT and 1,800 eyed embryos were introduced into High Lake via remote stream side incubators placed in inlet channels resulting in the introduction of over 8,300 WCT. Sizes of WCT introduced

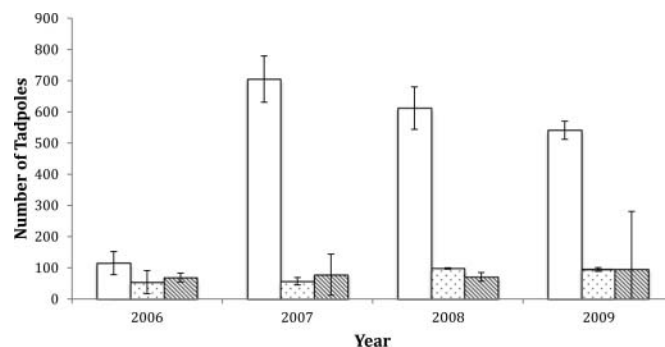


FIGURE 2. Lincoln-Petersen tadpole population estimates (whiskers show 95% confidence intervals) obtained at High Lake (open bars), North wetland (dotted bars), and South wetland (striped bars) in each of 2006–2009.

were similar across years (58–289 mm in 2007; 42–247 mm in 2008; 49–255 mm in 2009). Westslope cutthroat trout have been observed (rising fish and angler catch) in High Lake since the initial stocking in 2007, but posttreatment population estimates of WCT have not been conducted.

Observational data detailing tadpole distribution and behavior at High Lake indicated changes in habitat use in the years following rotenone application. In 2006, tadpole distribution at High Lake was restricted to the margins of the outlet channel, while adults were documented throughout the lake. Tadpoles were skittish and cryptic, remaining predominantly in the sedge-protected portions of the outlet margin. By contrast, tadpoles were observed in 2007 (prior to WCT introduction) throughout the outlet and in the margins around the main lake body. In 2008 and 2009, tadpole distribution became increasingly limited and, by the 2009 survey, was again restricted to the outlet margins, albeit in larger numbers than in 2006.

Flying D Ranch

July surveys at three of the four wetlands selected for our experiment documented breeding by only the Columbia spotted frog, while one wetland (#4), subsequently designated as a control, had both Columbia spotted frog and boreal toad tadpoles present. The age estimate for tadpoles at all four wetlands in both surveys was Gosner stage 40–43. Columbia spotted frog juveniles and adults were documented at all four wetlands before and after the rotenone treatment application.

Since Columbia spotted frog and boreal toad tadpoles were documented in both pre- and posttreatment surveys at Wetland #4, the population estimates for this site are composed of both species. The tadpole population estimates at the four wetlands ranged between 1,869 and 2,266 (Figure 3). Tadpole surveys conducted 24 h after rotenone treatment (1 mg/L CFT Legumine) revealed 100% mortality at treatment ponds, but treatment did not significantly affect tadpole population size in the following breeding season. There was no observed mortality among nongill-breathing juvenile and adult Columbia spotted frogs at either treated wetland, and their distribution and abundance were

similar to pretreatment observations. As expected, no obvious tadpole mortality or die-off was observed at either control ponds, and, although no immediate posttreatment population estimates were collected, numerous tadpoles were present. In 2009, 1 year posttreatment, tadpoles were again present at similar abundance levels at all four wetlands (Figure 3).

DISCUSSION

The results of this study indicated that a summer rotenone application at a common field dose of 1 mg/L product (50 µg/L rotenone) can cause complete mortality in Columbia spotted frog tadpoles but did not appear to cause mortality in metamorphosed juveniles and adults. Tadpole populations rebounded within one breeding season to previous levels in fishless wetlands (Flying D Ranch; Figure 3). However, where fish had been removed as a consequence of the rotenone treatment, tadpole populations appeared to increase to levels greater than before rotenone application (High Lake; Figure 2).

Other field and laboratory research supports our findings that rotenone can have significant, immediate effects on larval-stage amphibians, but not on older, lung-breathing stages. For example, application of rotenone at an unknown concentration to several ponds in the upper Santa Clara River drainage in California resulted in complete mortality in tadpoles of the African clawed frog *Xenopus laevis*, while adults were not affected and successfully bred after the treatment (McCoid and Bettoli 1996). In a controlled laboratory setting, Grisak et al. (2007b) found that rotenone exposure at a range of doses caused 100% mortality in tadpoles of two Rocky Mountain amphibian species, while adults of these same species showed no observable effects at concentrations well above those typically used in pond and stream treatments.

Somewhat contradictory to our field observations, the laboratory studies of Billman et al. (2011) found low mortality (6%) in Gosner stage 44–45 Columbia spotted frog tadpoles exposed to CFT Legumine at 1 mg/L. However, there may be a plausible explanation for this discrepancy. First, the age of Columbia spotted frog tadpoles appears to play a significant role in an individual's susceptibility to the lethal effects of rotenone—very late age tadpoles are more resistant than younger individuals (Billman et al. 2011). Tadpoles exposed to CFT Legumine as part of the Billman et al. (2011) laboratory trials were aged at Gosner stage 44–45, while tadpoles in the field experiments described here were younger. Second, tadpoles in the laboratory challenge received exactly 1 mg/L CFT Legumine. While the treatment sites in this research were treated at an average (across the site) of 1 mg/L CFT Legumine, the effective concentration in the margins of the treated sites—where tadpoles were found—could have exceeded 1 mg/L for a period of time until complete water column mixing occurred. Findings from the laboratory challenge revealed 100% tadpole mortality at 2 mg/L of rotenone product, and it would not be unexpected for initial, localized concentrations (i.e., before complete mixing occurs)

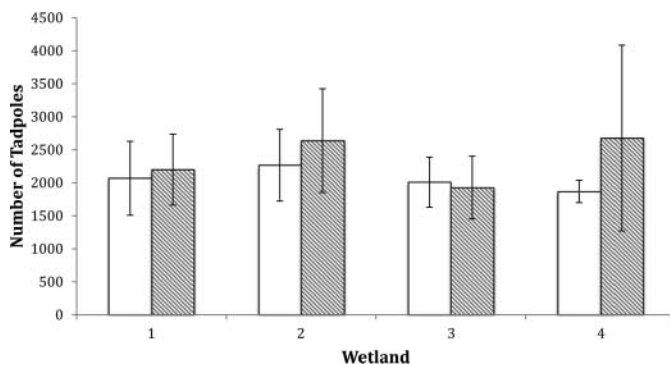


FIGURE 3. Lincoln-Petersen tadpole population estimates (whiskers show 95% confidence intervals) obtained at the control (#2 and #4) and treated (#1 and #3) wetlands on the Flying D Ranch before (July 2008; open bars) and after (July 2009; striped bars) the rotenone treatment (1 mg/L product).

to reach this level. Finally, differences seen between laboratory and field results could also be attributed to a multitude of environmental factors (i.e., water temperature, sunlight, water pH, substrate) that can affect the toxicity of rotenone products in the field.

It is important to recognize the potentially negative impacts of piscicide-induced larval mortality on the aquatic system. In the short-term (i.e., immediately posttreatment), loss of an entire tadpole cohort removes an important link in the food webs of aquatic and terrestrial systems. Longer-term effects of removing an entire tadpole generation from a wetland on the amphibian population in later years when the removed individuals would have been sexually mature are unknown; however, in this study, the short-term effects on population abundance did not persist past 1 year (Figures 2, 3). It should be noted that some amphibian species have highly variable recruitment across years and substantial recruitment events (i.e., >100 metamorphs at a given site) may occur infrequently (Trenham et al. 2003; Greenberg and Tanner 2005). If rotenone was applied during a substantial recruitment event and an entire, large tadpole cohort was lost, it could impact the overall population in future years. Because of these potentially significant, negative consequences, efforts to mitigate tadpole mortality should be an important component of planning a piscicide treatment.

Piscicide applicators might consider a number of strategies for minimizing tadpole mortality in the field. Timing treatments when tadpoles are no longer present may be one option. Mortality may be reduced by waiting until tadpoles are at a very late age stage or by treating at lower dosages (e.g., 0.5 ppm). Tadpole mortality can also be mitigated by collecting tadpoles, either before or during treatment, and holding them until the rotenone is no longer active in the treated water. This technique may prove to be most applicable for fisheries managers in the Rocky Mountains, in particular, where rotenone applications coincide with the larval period (Grisak et al. 2007b; Billman et al. 2011). Further, if fish restoration project guidelines call for multiple rotenone applications and capture–hold mitigation for tadpoles is not practical, we suggest conducting treatments within the same year instead of across consecutive years to avoid loss of multiple tadpole cohorts.

When addressing the impacts of tadpole mortality as a result of rotenone treatment, practitioners should consider general factors, including (1) the conservation status of the amphibian species in the restoration area, (2) the proximity of colonizing amphibian populations to the restoration site, and (3) the life history characteristics of amphibians found at the treatment site. Species prevalence, both locally and regionally, can guide mitigation efforts. Rare or sensitive species may warrant more planning and effort to salvage or avoid treating them than more common amphibian species. Knapp et al. (2001) suggests that amphibian recovery in high alpine lakes, where fish had been removed, was augmented by breeding adults from neighboring source ponds. High Lake and the treated sites on the Flying D Ranch were characterized as having at least one wetland

containing breeding adult amphibians within 500 m. We do not know whether any adults from neighboring ponds bred at any of our treatment sites in the years after treatment. The repopulation we witnessed could have been accomplished by established, resident adults. However, we caution that in isolated habitats it may be important to preserve tadpoles to protect against the unknown, extended effects of losing an entire age-class that cannot be readily replaced by immigrating individuals. The degree of isolation is not constant but rather is dependant on the mobility of the species in question. Some species of Rocky Mountain amphibians have documented movements to breeding sites of up to 3 km (Pilliod and Peterson 2000; Pilliod and Peterson 2001). Generally speaking, amphibians at lower elevations have a shorter life span, longer activity period, and can reach sexual maturity earlier than individuals at high elevation, where conditions and resource availability limit activity period and increase time to sexual maturity (Cvetković et al. 2009). Loss of a tadpole cohort at high elevation could pose a greater risk because it can take longer to replace their breeding contribution.

Piscicide application can also impact amphibian distribution and abundance through the removal or reduction of the fish population. At High Lake in 2007, tadpole abundance increased significantly in the absence of fish, but, as WCT were stocked into the lake from 2007 to 2009, tadpole distribution and abundance at the lake decreased (Figure 2). Columbia spotted frog tadpoles were documented throughout the lake in 2007 after fish removal but, similar to pretreatment conditions, were restricted to the sedge-protected margins of the outlet channel by 2009. Predation can affect tadpole distribution by reducing or eliminating them from a particular area. Predators have been shown to influence the behavior, morphology, and habitat choices of tadpoles of multiple amphibian species (Relyea and Auld 2005; Richter-Boix et al. 2007). Specifically, tadpoles can chemically detect the presence of fish, thereby negatively affecting distribution and feeding (Binckley and Resetarits 2003; Orizaola and Braña 2003; Benard 2006; Barr and Babbitt 2007). The diminished tadpole distribution at High Lake in the presence of fish in 2006, 2008, and 2009 was consistent with this concept of fish avoidance.

Our research provides important information on the effects of rotenone on amphibian populations and confirms research findings on the effects of fish removal and introduction on tadpoles. Environmental factors, such as substrate, water depth, and vegetation, or application patterns could have significant impacts on the toxicity of rotenone to tadpoles and should be more thoroughly investigated. This research cannot address the impacts of removal of an entire tadpole cohort on future adult population size or breeding success. At High Lake, we obtained population estimates for 3 years after tadpole removal but were unable to determine whether the loss of the 2006 cohort of breeding individuals will significantly impact the High Lake Columbia spotted frog population since Columbia spotted frogs in the Rocky Mountains typically reach sexual maturity 4–5 years after their first summer (Koch and Peterson 1995).

It is clear from this study and other similar research (Grisak et al. 2007(b); Billman et al. 2011) that rotenone will negatively impact tadpoles when applied at typical field application doses, but nongill-breathing individuals appear unaffected. As a result, repopulation of treated bodies of water will occur in the breeding season following application. Using these results and considering other factors discussed above can enable practitioners to develop effective methods for mitigating the impacts of piscicide-based fish removal on amphibians and conserve amphibian populations at treated sites.

ACKNOWLEDGMENTS

This research was made possible by the Fisheries and Aquatic Sciences Section in Yellowstone National Park, Idaho State University, and by grants from Turner Enterprises, the Montana Chapter of the American Fisheries Society, and the Fish Fund Initiative of the Yellowstone Park Foundation. Mike Ruhl provided logistical support for portions of the study and Drs. Colden Baxter and Patrick Lang reviewed and improved the manuscript. This work was approved by the Idaho State University Animal Welfare Committee.

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