Montana Chapter of the American Fisheries Society
Resource Action Fund Grant Request

The ExCom will review funding requests for the MCAFS Resource Action Fund. For more details please see the MCAFS web page at www.fisheries.org/AFSmontana, or contact an ExCom member.

I. APPLICANT INFORMATION

a. Applicant Name____Turner Enterprises, Inc.____________________________

Mailing Address____1123 Research Drive__________________________________

City or Town_____Bozeman___________________**State**___MT____**Zip**_59718_

Telephone______406-556-8500____

b. Contact person if different than above_ Carter Kruse and Hilary Billman __________

Address if different than applicant____

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Telephone if different than applicant_______________________________________

II. PROJECT INFORMATION (attach additional pages as needed)

a. Project Title__ Investigating the effects of piscicides on larval amphibians___________

b. Purpose of Project (goals and objectives):

1) To determine the effect of varying doses of rotenone (CFT Legumine formulation) on the survival of larval amphibians (spotted frog and boreal toad) at different age stages under laboratory conditions.

2) To determine the effect of varying doses of rotenone on weight, SVL, and time to metamorphosis of larval amphibians at different age stages (spotted frog and boreal toad) under laboratory conditions (if survival occurs).

3) To determine if the effects of rotenone are reversible in spotted frogs and boreal toad tadpoles at field level doses at specific life stages (to be addressed as time permits).

4) To determine the population level effects of rotenone on amphibians in the field.

The objectives of this study meet the larger goal of determining ways to mitigate potential negative impacts of piscicides on amphibian communities. A more focused understanding of reaction to piscicide exposure, if any, will help fisheries manager's better plan fish removals to minimize impact on amphibians.

c. Brief Project Description:

This premise of this project is rooted in two assertions: the first by scientists and biologists that if fish restoration projects utilizing rotenone are conducted according to label directions little potential harm is caused to non-target organisms such as amphibians and aquatic invertebrates; the second by opponents to piscicide based restoration projects that these chemicals cause significant and long-lasting effects to non-target organisms. We propose to conduct laboratory investigations that will look at dosage and age related responses to rotenone by two amphibian species in a laboratory setting. Columbia spotted frogs and boreal toad larvae would be exposed to 5 different dosages at 5 maturation stages to determine likelihood of mortality at a given dosage and age. A certain portion of these exposed individuals would also be monitored for sub-lethal effects such as slowed maturation, weight loss or reduction in weight gain, size, etc. (see attached draft study plan). Potential population effects would also be studied in the field under more natural conditions by conducting pre- and post rotenone treatment amphibian surveys on small wetland habitats. These studies would be conducted alongside regularly scheduled rotenone treatments, as well as in small wetlands that would be treated solely to understand potential effects on amphibians. Some of this work would be conducted on the Flying D Ranch owned by Turner Enterprises, Inc., as well as in other locations as opportunity arises. The field component will be dependent on the issuance of permits and the actual implementation of rotenone treatment. The goal is to better understand the potential effects of rotenone treatment on these species and to provide important guidance to those conducting rotenone treatments for fisheries management and conservation work on how to minimize or mitigate effects on amphibians.

d. Project Starting Date_____ April 15, 2008**_________________________________**

e. Project Completion Date___ December 31, 2009**_____________________________**

III. PROJECT BUDGET

a. RAF Grant Request (dollars)________$2,000**____________________________________**

b. Contribution from Other Sources:

Turner Enterprises, Inc.	$20,000
Yellowstone National Park	$10,000
Idaho State University	$10,000

b. Total Project Cost___________________ $42,000 **_________________________________**

c. Have Other Funding Sources Been Secured?_____________________________________

____________________Yes, those mentioned above. ________________________________

d. Names and Amounts of Other Funding Sources___________________________________

_____________________See above. ____

e. Is MCAFS the Only Source of Funding for this Project?______ No. **_________________**

Why or Why not?

The primary support for this graduate project will come from the organizations listed above. Turner Enterprises, Inc. will provide laboratory space, tuition support and a research assistantship for the 2008-2009 school year, and a part-time technician to assist with laboratory and field work. Yellowstone National Park will employ the graduate student during summers 2008 and 2009 and allow her to work on the research project on an as needed basis. Idaho State University provided a teaching assistantship during the 2007-2008 school year. We are seeking these additional funds from MT AFS to cover some of the laboratory expenses, such as small aquaria in which to conduct trials, rotenone chemical, safety equipment, office supplies, and similar materials.

IV. PROJECT BENEFITS (attach additional pages as needed)

a. Benefits to Native Species

This project has clear and obvious benefits to native species. Piscicides are primarily used in Montana as a tool for native species restoration via removal of competing and hybridizing non-native species. These types of restoration projects are coming under increasing scrutiny, primarily from opponents who argue that effects on non-target organisms are significant and long-lasting. This project proposes to look closely at the effects of one of two EPA approved piscicides, rotenone, on two amphibian species, which are often discussed as species that are unintentionally affected by piscicide treatments. The results from this work could provide several outcomes: a) if rotenone is shown to be relatively benign to these amphibian species at field level dosages, then opponents of these projects have one less argument, improving the likelihood that piscicide based native fish restoration projects will be allowed to move forward; b) if rotenone is shown to effect certain life stages more than others then managers can better plan piscicide treatments to avoid harming amphibian populations (such as in timing of treatment); c) if rotenone is shown to be harmful to all age classes of larvae then we will better understand the risk these chemicals pose to native amphibian populations, allowing us to plan these types of projects better to avoid or minimize effects, or to use alternative methods of achieving a similar goal. Any information that will improve our understanding of how piscicides affect non-target organisms will allow us to plan these types of treatments more strategically to the benefit of all native aquatic species.

b. Short Term Benefits to the Fisheries Resource

c. Long Term Benefits to the Fisheries Resource

We believe that the short and long term benefits of this work are similar and fall under five broad categories (see also attached study plan for more explanation).

1. Understanding effects of rotenone on non-target amphibian species. The results of this work will supplement the current rotenone-amphibian literature, most of which is dated, and provide much better resolution regarding rotenone effects on at least these two amphibian species. Most rotenone-amphibian work has been done on adult or sub-adult frogs (air breathing) or larger, more mature larvae (tadpoles). There is some indication that younger aged tadpoles may be more susceptible to piscicides, but how much so and at what ages is relatively unknown. This work should begin to elucidate some of these questions and provide us a recent assessment of what impact rotenone based projects might be having on amphibians, both directly (immediate and delayed mortality) and indirectly (sub-lethal effects). Similar to the native fishes that rotenone is often employed to help, many amphibian species are declining as well and these data will all better assessment of whether a piscicide project designed to help one native species is (or is not) harming another. Further, information such as provided by this project may be used in non-native amphibian removals as well by demonstrating when unwanted species may be most susceptible to rotenone – bullfrogs for example.

2. Better planning and management of piscicide projects. Any information that will improve our planning and deployment of a piscicide such as rotenone will be beneficial for both non-target amphibians and fish. This project may show that later stage tadpoles are less susceptible to rotenone, thus advocating for late summer deployment of piscicides (or showing that late summer applications, as is typically done, are relatively harmless to amphibians). Similarly, better information will allow us to answer permitting and critical questions more frankly and potentially improve our ability to move projects forward, regardless of what this project ultimately shows in terms of effects to amphibians.

3. Fine tuning field techniques. The results from this work may allow us to fine tune our application of rotenone and improve efficiency. For example if it is shown that 1 ppm harms mid-stage larvae, but lower dosages do not, we may be able to treat sensitive areas with a lower dosage (less than label instruction) that still kills fish, but does not harm non-target organisms. Similarly, two low dosage treatments may eliminate the unwanted fish, but not harm amphibians.

4. Guiding future research. This work will not answer all the questions regarding rotenone and non-target organisms – far from it. However, it could provide valuable instruction to future research projects on methodologies, starting points, potential effects, additional questions, maximum and minimum experimental dosages, and other similar variables. In other words, this work will provide a platform from which additional work can be conducted.

5. Additional information for managers in the public arena. As mentioned in topic #2, any information that can fine tune or add to what we know about effects on non-target organisms will help managers respond to criticism or opposition to piscicide projects, especially in a public forum. Clear and defensible data will allow project proponents to provide concise, professional, understandable responses to questions regarding unintended effects.

d. Does this Project Have the Support of an Appropriate MCAFS Committee?_________

If so, which Committee?___ This proposal will be simultaneously submitted to the EXCOM and Species of Special Concern Committee.

DRAFT

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Investigating the Effects of Piscicides on Larval Amphibians

Introduction

Amphibian populations are currently in a state of significant decline and disruption world-wide (Knapp et. al 2007). These changes in abundance have been attributed to a variety of factors, such as disease, climate change, and habitat destruction, and, because amphibians play a key role in structuring ecological communities, the importance of understanding these rapid declines cannot be overstated (Alford & Richards 1999; Gibbs & Breisch 2001; Collins & Storfer 2003; Corn 2003; Blaustein & Bancroft 2007). Similarly, changes in the abiotic environment – climate change, disease outbreaks, and habitat alteration – may be reflected in amphibian populations, making them useful indicators of ecosystem health both in Yellowstone and world-wide (Young et al. 2004; Whiles et al. 2006; Patla et al. 2007).

In Yellowstone National Park (YNP) and the Greater Yellowstone Area (GYA), current changes to local environments include fish restoration activities. Return of some aquatic systems in YNP to historically fishless status may have significantly positive results for resident frog, toad, and salamander populations; the impacts of non-native fish, and their subsequent removal, on amphibians have been well documented (Bradford et al. 1993; Pilliod & Peterson 2000; Pilliod & Peterson 2001; Mullin et al. 2004; Vredenburg & Wake 2004; Knapp 2005; Welsh et al. 2006; Walston & Mullin 2007). Case in point, removal of introduced rainbow trout *O. mykiss* and brook trout *Salvelinus fontinalis* in the Sierra Nevada enabled significant recovery of declining yellow-legged frogs *Rana muscosa* (Vredenburg & Wake 2004; Knapp et al. 2007). Fish restoration's use of toxic substances, however, has the potential to negatively impact amphibians further and has not been adequately researched.

Westslope Cutthroat Trout Restoration in Yellowstone

Fisheries management in Yellowstone National Park (YNP) was historically guided by the need to provide high quality fishing experiences for visitors. While approximately 40% of the park's waters were originally fishless, Yellowstone's managers stocked many of them, with both native and nonnative fish species, so as to turn the park into a haven for anglers (Varley and Schullery 1998). In many park waters, the native cutthroat trout *Oncorhynchus clarki* and Arctic grayling *Thymallus arcticus* populations have been completely extirpated, reduced in abundance, or compromised because of hybridization with nonnative fish. YNP fisheries managers currently seek to reverse this trend by removing non-native and hybridized fishes and restoring native cutthroat trout to historic habitat (Koel et al. 2006).

Westslope cutthroat trout *O. c. lewisi* populations are a current focus of the park's native fish restoration goals. Beginning with the East Fork Specimen Creek and High Lake, located at the headwaters of the watershed, Yellowstone National Park is attempting to restore westslope cutthroat trout by first removing all introduced nonnative and hybridized fish from these areas, and, subsequently, restocking genetically pure westslope cutthroat trout (Koel and York 2006).[1] Fish removal can be accomplished by a variety of techniques, but the most promising and effective has, thus far, been use of the approved piscicides, rotenone and antimycin (Finlayson 2000). These chemicals have, however, the potential to harm aquatic, non-target species, especially amphibians (Fontenot et al. 1994; McCoid & Bettoli 1996; Maxell 2000; Patla 2005) and this potential has not been adequately researched. YNP has successfully used rotenone and antimycin in previous restoration efforts and intends to continue using either one or both in future restoration activities (Koel et al. 2006). To best manage amphibian populations that may be affected by this aspect of restoration, a more complete, laboratory-based understanding of how these chemicals affect amphibian communities is warranted (Figure 1).

[1] High Lake was historically fishless until 1937 when the NPS stocked it with Yellowstone cutthroat trout. The East Fork Specimen Creek contained westslope cutthroat trout, though they are currently hybridized. Technically, current initiatives at High Lake are not "restoration" since westslopes have never existed in the lake. Future work in the creek and remainder of the drainage will, however, restore genetically pure westslope cutthroat trout.

The proposed laboratory and field studies were developed in light of the discovery that rotenone had immediate impacts on larval amphibian communities (mortality) at High Lake.[2]

The objectives of the laboratory study are:

4) **To determine the effect of varying doses of rotenone (CFT Legumine formulation) on the survival of larval amphibians (spotted frog and boreal toad) at different age stages under laboratory conditions.**

5) **To determine the effect of varying doses of rotenone on weight, SVL, and time to metamorphosis of larval amphibians at different age stages (spotted frog and boreal toad) under laboratory conditions (if survival occurs).**

6) **To determine if the effects of rotenone are reversible in spotted frogs and boreal toad tadpoles at field level doses at specific life stages (to be addressed as time permits).**

The objective of the field study is:

To determine the population level effects of rotenone on amphibians in the field.

The objectives of this study meet the larger goal of determining ways to mitigate the negative impacts of piscicides on amphibian communities. A more focused understanding of the exact traits affected by piscicide exposure will help fisheries managers better plan fish removals to minimize impact on amphibians.

Methods, Design, and Analysis

Turner Enterprises – Laboratory Component

Research Question 1: Is there a measurable difference between survival of larval amphibians (spotted frog, boreal toad) at different age stages after exposure to different levels of rotenone?

→ *Prediction: Tadpole survival will increase with age and decrease at high doses.*

Around the 3rd week in April (approximate time of tadpole availability), 500 Columbia spotted frog tadpoles will be collected from Turner Enterprises lands and held in a stock tank. Boreal toad tadpoles will be collected subsequently and held in a separate tank. Tadpoles will be removed from the stock tank every 5 days to obtain different life stages. American Society for Testing and Materials (ASTM 2002) standards for toxicity testing on amphibians will be followed, with minimal exceptions.

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[2] Results of surveys conducted before and after piscicide application at High Lake revealed that rotenone was fatal to gill-breathing, larval spotted frogs. Recently metamorphosed, juvenile, and adult spotted frogs and chorus frogs survived treatment. In 2007, spotted frogs significantly re-populated High Lake. Tadpoles were observed throughout the lake – both in historic and new places in the lake – and adults/juveniles were seen in numbers comparable to 2006. Comparatively, spotted frog larvae increased in number at High Lake as a result of fish removal (Figure 2; Table 1), while the larval populations at two nearby “control” wetlands remained relatively constant. Surveys in 2008 & 2009 will demonstrate how the population responds to the restoration of native westslope cutthroat trout to High Lake.

For each of the two species, 15 tadpoles at each of 5 stages – Gosner stages 22, 25, 30, 35, and 40 (Gosner 1960) – will be randomly assigned to each of five treatments: Rotenone (CFT Legumine formulation) at 4 different concentrations and a negative control.* After a period of tadpole acclimation (1-2 days), rotenone will be applied to 5 replicate tanks at each of 5 doses per life stage: 0 mg/L (water control), 0.1mg/L, 0.5mg/L, 1mg/L, and 2 mg/L. Rotenone will be held in tanks for 96 hours in accordance with ASTM standards. Previously determined water chemistry factors – pH, temperature, conductivity, etc. – will be held constant; well-water (pH of 7.5-8) will be the water source used. Behavior and mortality of the animals will be monitored every three hours for the first 24 hours and twice a day thereafter until metamorphosis. Survival analysis will be done to compare the effect of treatments on survivability.

Research Question 2: Are there measurable, sub-lethal effects on tadpoles as a result of piscicide exposure?

→ *Prediction: Rotenone exposure will result in decreased weight and SVL but will increase time to metamorphosis.*

Tadpoles that survive the treatments from the initial exposure will be maintained for the remaining time until metamorphosis (approximately 4 weeks). Weight will be monitored weekly. Snout-vent length (SVL) will be measured once the tadpoles have metamorphosed at the end of the study. Time to metamorphosis will be recorded for all tadpoles. Differences in weight, SVL, and average time to metamorphosis between treatments will be compared using an ANOVA controlling for tank effects.*

* Tadpole development at the source ponds will be monitored simultaneously to provide additional control for tank effects.

Turner Enterprises – Field Component

Research Question #1: Is there a measurable difference in amphibian adult & larval distribution and abundance before and after rotenone application?

→ *Prediction: Rotenone application will have a negative, immediate effect on larval distribution and abundance but will not affect adult distribution and abundance.*

Research Question #2: Will treated ponds have tadpoles the year after rotenone treatment?

→ *Prediction: Treated ponds will have reproducing adults in them the year following application as made evident by the presence of tadpoles.*

To answer the afore-mentioned research questions, amphibian populations at rotenone treated and untreated ponds will be montitored before, during, and one year after the treatment. Treatment and control ponds to be included in this study will be chosen based on similarity of water chemistry and habitat characteristics (to be determined once this information is obtained). Final choices on both the number and type of sites used will be made in April after site visits.

Pre-Treatment

Immediately pre-treatment, water chemistry data will be collected at control and treatment ponds and mark-recapture surveys will be conducted at all ponds. Mark-recapture data will be used to obtain population estimates (Research Question #1). Tail clips of randomly chosen tadpoles and mouth swabs from all adults caught will be collected for the genetic analysis (Research Question #2; it may be possible to determine the origin of the amphibian populations during the course of the study, which would help determine whether animals are re-colonizing from other areas after the rotenone treatment). These pre-treatment surveys will occur between 10 A.M. and 2 P.M. on successive days. Sentinel cages will be set-up at treatment and control ponds. Mouth swabs from adults will also be collected from ponds at specific distances away from treated ponds (Research Question #2).

During Treatment

Visual surveys of treated water bodies will be conducted during rotenone application to determine immediate effects of treatment (Research Question #1).

Post-Treatment (2009)

During the breeding season after rotenone application (2009), mark-recapture surveys will be conducted at the same time of year at the same treated and control ponds from the previous year to obtain population estimates (Research Question #1). Tail clips and mouth swabs will be collected as before (Research Question #2). Mouth swabs from adults will also be collected from ponds at specific distances away from treated ponds (Research Question #2).

Basic Time Schedule for Proposed Work with TEI

Summer 2008:
- → Collect field data at TEI
- → Run tank experiments at TEI

Fall 2008:
- → Course work at Montana State University
- → Run tank experiments at TEI lab

Spring 2009:
- → Finish course work at Idaho State University

Summer 2009:
- → Complete field experiments at TEI

Budget Table

Description	Cost
Tank experiment equipment, materials, and supplies for exposure study	TEI lab
RA Stipend (September 2008-May 2009)	$10,000.00
Tuition Costs Spring 2009 (includes 1 credit for Fall 2008 to maintain enrollment & 9 credits Spring 2009) (ISU)	$3,487.20
SubTotal	**$13,487.20**
	10% of Total
ISU Overhead Charges	$1,348.72
Total	**$14,835.92**

Figures & Tables

Table 1. Minimum Number of Tadpoles Alive in 2006 & 2007.

	Minimum Number Alive 2006	Minimum Number Alive 2007
Lake	68	529
South Wetland	54	45
North Wetland	69	78

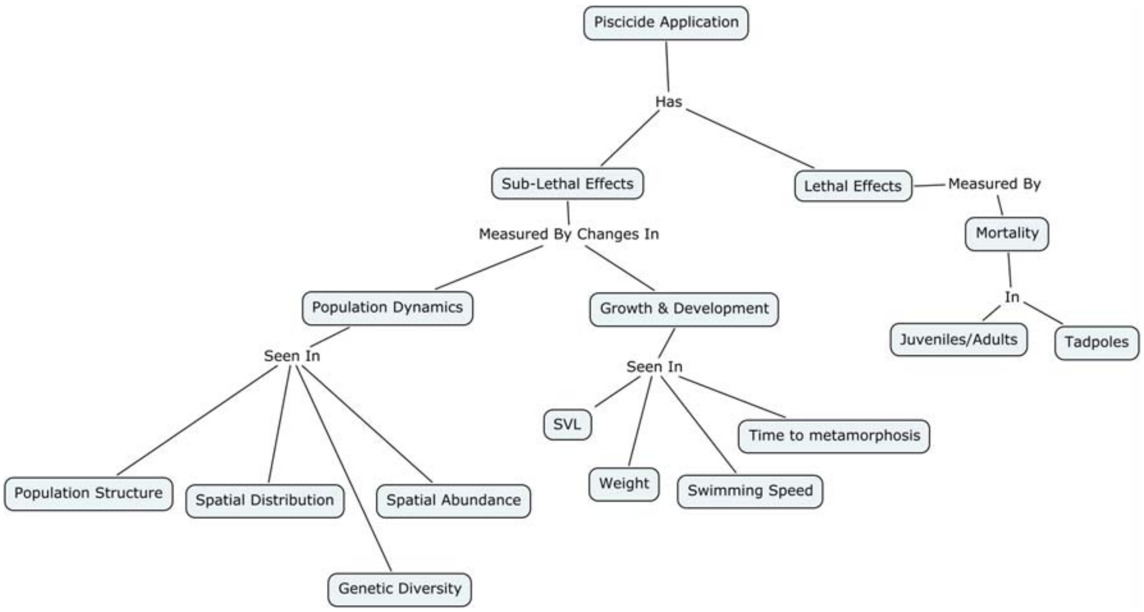

Figure 1. Concept map detailing schematic for how piscicide application could potentially affect amphibian populations.

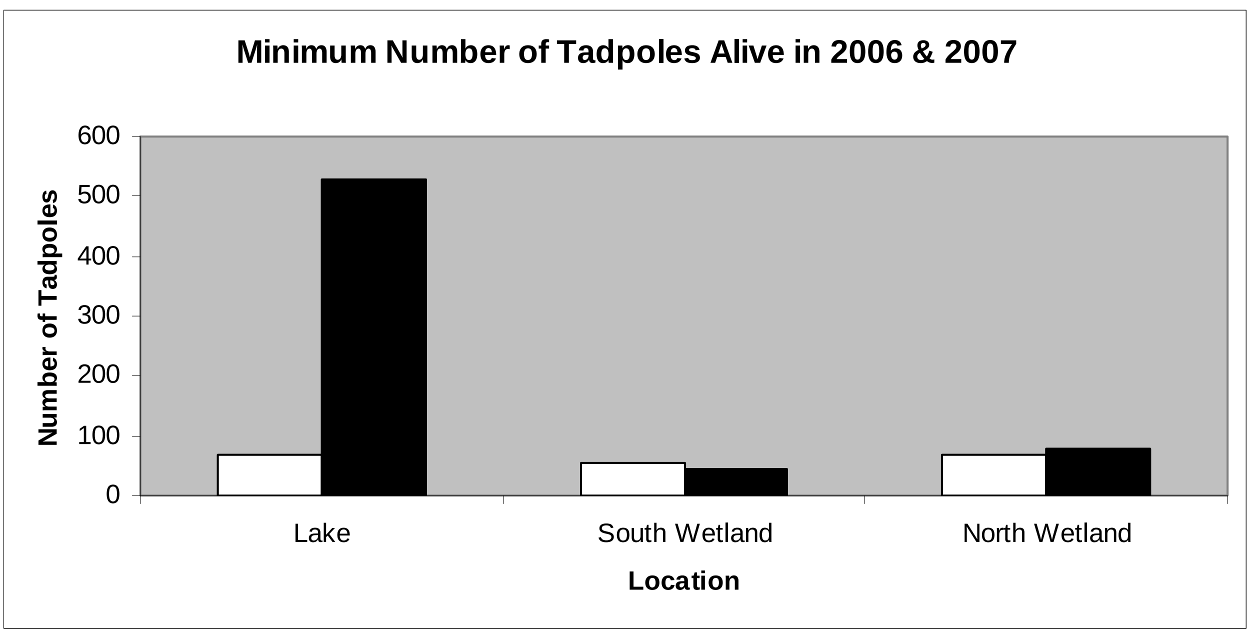

Figure 2. Minimum Number of Tadpoles Alive: 2006 vs. 2007. Open bars represent number of tadpoles in 2006, solid bars represent number of tadpoles in 2007.

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