White Paper: Chemical and Mechanical Means of Fish Removal

Methods, Effectiveness, and Environmental Effects

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List of Abbreviations

BLM	Bureau of Land Management
CGNF	Custer Gallatin National Forest
DEGEE	diethyl glycol monoethyl ether
DEQ	Montana Department of Environmental Quality
eDNA	Environmental DNA
EPA	Environmental Protection Agency
FWP	Montana Fish, Wildlife & Parks
LD_{50}	lethal dose for 50 % of tested organisms
MSDS	Material data safety sheet
NPS	National Park Service
PEG	Polyethyl glycol
ppb	Parts per billion
ppm	Parts per million
USFS	U. S. Forest Service
USFWS	U. S. Fish and Wildlife Service

1 Introduction

Intentional, widespread introductions of nonnative fishes have provided popular and economically valuable angling opportunities. Illegal introductions and invasion have further increased the distribution of nonnative fishes. Combined, intentional introductions, illegal introductions and invasion have had detrimental effects on native species worldwide. The mechanism by which nonnative fishes displace native species varies with species; however, competition, hybridization, predation, and disease are the primary threats.

The effects of these introductions range from reductions in abundance and distribution of native fishes to extinction. Freshwater fishes had the highest exctinction rate of all vertebrates in the 20th Century, and an estimated 53 to 86 species will go extinct in North America by 2050 (Burkhead 2015). Although many factors can lead to the decline of a given species, species introductions and habitat degradation are the main threats to imperiled freshwater fishes in North America (Jelks et al. 2008). Transglobal introductions of rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*) have earned these species a place on the list of the world's 100 worst invasive alien species (Invasive Species Specialist Group http://www.iucngisd.org/gisd/), with their establishment threatening native fish communities in suitable habitat on most continents.

Rainbow trout, brown trout, and brook trout (*Salvelinus fontinalis*) have been introduced throughout the western U.S. and are popular and economically important game species. Unfortunately, the successful establishment and continued invasion of nonnative salmonids have been exceptionally detrimental to native, freshwater *Oncorhynchus*. This diverse genus of trout and salmon has many species, subspecies and genetically distinct populations of concern within the western U.S., Canada and Mexico. Reductions in the distribution and abundance of freshwater *Oncorhynchus* has resulted in 29 taxa ranking as imperiled, with 1 of those taxa being extinct, and another, the Alvord cutthroat trout, possibly being extinct (Table 1-1; Jelks et al. 2008). All but 1 taxon had habitat degradation listed as a cause for the decline; however, all shared introduction of nonnative species as a factor threatening their existence.

Table 1-1. List of imperiled taxa of *Oncorhynchus* in the western U.S. (from Jelks et al. 2008).

Scientific Name	Common Name	Status
O. chrysogaster	Trucha dorada mexicana	Threatened
O. clarkii alvordensis	Alvord trout	Possibly extinct
O. clarkii bouvieri	Yellowstone cutthroat trout	Threatened
O. clarkii clarkii	Coastal cutthroat trout	Vulnerable
O. clarkii henshawi	Lahontan cutthroat trout	Threatened
O. clarkii lewisi	Westslope cutthroat trout	Threatened
O. clarkii macdonaldi	Yellowfin cutthroat trout	Extinct
O. clarkii pleuriticus	Colorado River cutthroat trout	Vulnerable
O. clarkii stomias	Greenback cutthroat trout	Threatened
O. clarkii virginalis	Rio Grande cutthroat trout	Threatened
O. clarkii ssp.	Humboldt cutthroat trout	Threatened
O. gilae apache	Apache trout	Threatened
O. gilae gilae	Gila trout	Endangered
O. clarkii seleniris	Paiute cutthroat trout	Endangered
O. mykiss aguabonita	South Fork Kern River golden trout	Threatened
O. mykiss aquilarum	Eagle Lake rainbow trout	Threatened
O. mykiss gairdnerii	Redband steelhead trout Owyhee	Vulnerable
O. mykiss nelsoni	Trucha de San Pedro Mártir	Vulnerable
O. mykiss newberrii	Redband trout	
O. mykiss newberrii	Catlow Valley populations	Vulnerable
O. mykiss newberrii	Goose Lake populations	Vulnerable
O. mykiss newberrii	Harney-Malhuer Lake populations	Vulnerable
O. mykiss newberrii	Warner Valley populations	Vulnerable
O. mykiss stonei	McCloud River redband Trout	Vulnerable
O. mykiss whitei	Little Kern River golden Trout	Endangered
O. mykiss ssp.	Truchas de los ríos Acaponeta y Baluarte	Threatened
O. mykiss ssp.	Trucha del Conchos	Threatened
O. mykiss ssp.	Truchas de los ríos Piaxtla, San Lorenzo y Presidio	Threatened
O. mykiss ssp.	Truchas de los ríos Yaqui, Mayo y Guzmán	Threatened

In addition to fishes of the genus *Oncorhynchus*, bull trout (*Salvelinus confluentus*) and Arctic grayling (*Thymallus arcticus*), also salmonids, have decreased substantially in distribution and abundance across their historical range (Liknes and Gould 1987; Quigley and Arbelbide 1997; Rieman et al. 1997; USFWS 1999). Numerous factors have contributed to their declines. Siltation, loss of habitat complexity, passage barriers, warming water temperatures, dewatering and nonnative species have diminished the range and abundance bull trout and Arctic grayling considerably.

States and federal agencies classify organisms based on their relative security and conservation needs. The State of Montana further classifies species based on their security within the state, and throughout their historical distribution, which sometimes differ. The Montana Natural Heritage Program and Montana Fish, Wildlife & Parks (FWP), with input from the Montana Chapter of the American Fisheries Society assign these rankings. Several native salmonids in Montana have 1 or more special status rankings, and these rankings can be complex (Table 1-2). For species of concern in Montana, the complex rankings address polytypic species, where the species is secure, but a subspecies has a different status. Federal rankings are straightforward, and are listed as threatened or sensitive. As the U. S. Fish and Wildlife Service (USFWS) is responsible for deciding whether a species requires protection under the Endangered Species Act (16 U.S.C.A, §1531-155 [Supp. 1996]), a species on the list has a status of listed threatened, or listed endangered. The U.S. Forest Service (USFS) and Bureau of Land Management (BLM) rate salmonid species of concern in Montana as sensitive or threatened.

Table 1-2. Salmonid species of concern in Montana, and assigned status. Definitions of state rankings follow the list.

		State of Montana		Federal Agencies		
Species	Scientific name	Global Rank	State Rank	USFWS	USFS	BLM
Arctic grayling	*Thymallus arcticus*	G5	S1		Sensitive	Sensitive
Bull trout	*Salvelinus confluentus*	G4	S2	Listed Threatened	Threatened	
Columbia River redband Trout	*Oncorhynchus mykiss gairdneri*	G5T4	S1		Sensitive	
Lake trout	*Salvelinus namaycush*	G5	S2			
Pygmy whitefish	*Prosopium coulteri*	G5	S3			
Westslope cutthroat trout	*Oncorhynchus clarkii lewisi*	G4T3	S2		Sensitive	Sensitive
Yellowstone cutthroat trout	*Oncorhynchus clarkii bouvieri*	G4T3	S2		Sensitive	Sensitive

G5 = common, widespread, and abundant (although it may be rare in parts of its range). Not vulnerable in most of its range
G4 = Apparently secure, though it may be quite rare in parts of its range, and/or suspected to be declining.
G5T4 = globally secure; however, subspecies is apparently secure, although it may be quite rare in parts of its range, and/or is suspected to be declining.
G4T3 = apparently secure globally, although the subspecies is potentially at risk because of limited and/or declining numbers, range and/or habitat, even though it may be abundant in some areas.
S1 = At high risk because of extremely limited and/or rapidly declining population numbers, range and/or habitat, making it highly vulnerable to global extinction or extirpation in Montana.
S2 = at risk because of very limited and/or potentially declining population numbers, range and/or habitat, making it vulnerable to global extinction or extirpation in Montana.

Implementation of projects that conserve or restore native species are required by state and federal laws aimed at preventing further loss of distribution, restoring populations when possible, decreasing the need for protection under the Endangered Species Act, and preventing extinction. In accordance with these laws, state and federal agencies have developed policies, and conservation planning documents that provide the framework to conserve native fishes. Several planning documents have been prepared for conservation of westslope cutthroat trout, Yellowstone cutthroat trout, and bull trout in Montana (Table 1-3).

Table 1-3. Planning and strategy documents with relevance to native salmonid conservation in Montana.

Agency	*Citation*	*Website*
Montana cutthroat trout Steering Committee	Memorandum of understanding and conservation agreement for westslope Trout and Yellowstone cutthroat trout in Montana (2007)	http://fwp.mt.gov/fishAndWildlife/management/yellowstoneCT/
U.S. Congress	The Wilderness Act of 1964	https://www.gpo.gov/fdsys/pkg/STATUTE-78/pdf/STATUTE-78-Pg890.pdf
Montana Bull trout Restoration Team	Restoration plan for Bull trout in the Clark Fork River basin and Kootenai River basin, Montana	http://fwp.mt.gov/fishing/license/bullTrout.html
FWP	Wild Fish Transfer Policy (1996)	http://fwp.mt.gov/fishAndWildlife/management/westslopeCT/default.html
USFWS	Columbia Headwaters Recovery Unit Implementation Plan for Bull trout (2015b)	http://www.fws.gov/pacific/bullTrout/pdf/Final_Columbia_Headwaters_RUIP_092915.pdf
FWP	Statewide fisheries management plan (2014)	http://fwp.mt.gov/fishAndWildlife/management/fisheries/statewidePlan/
YNP	Native fish conservation plan environmental assessment (2011)	http://parkplanning.nps.gov/document.cfm?parkID=111&projectID=30504&documentID=37967
USFWS	Recovery plan for the coterminous United States population of Bull trout (2015)	http://www.fws.gov/pacific/bullTrout/pdf/Final_Bull_Trout_Recovery_Plan_092915.pdf

Typically, high elevation waters provide suitable habitat for native salmonids, and constructed or natural barriers protect these waters from reinvasion of nonnative species. The Wilderness Act of 1964 created the National Wilderness Preservation System and designated areas as wilderness, “where the earth and its community of life are untrammeled by man, where man himself is a visitor who does not remain”. Often, designated wilderness occurs at high elevations, and these areas provide habitat that will be resilient to climate change and will be highly suitable for obligate cold-water species, such as cutthroat trout and Bull trout. Climate change models predict a substantial reduction in cold-water habitats in the historical ranges of Bull trout and cutthroat trout in the U.S. over the next 25 years, and designated wilderness has the potential to provide refuge, or a “climate shield”, for native salmonids, and increase the probability of their long-term persistence (Isaak et al. 2015).

The goal of this document is to provide the best scientific evidence to inform decision-making about the preferred method of fish removal for a given project, including those in designated wilderness. Relevant topics include the potential of mechanical and chemical removal to alter nontarget species composition, stream ecology, water quality, stream morphology, and the duration of alterations to these aspects of ecology and stream function. In addition, the effect of fish removal methodologies on wilderness values is a major consideration. This document also identifies conditions that may prevent a method from being ineffective or infeasible.

2 Mechanical Removal

Mechanical removal entails the use of electrofishing, nets, or traps to capture fish. Mechanical removal can be the sole mode of fish removal, or it can be used in conjunction with piscicide. Mechanical removal as the only method can be successful under specific circumstances (Shepard et al. 2014). In some situations, mechanical removal may have greater public acceptance than chemical removal.

Often, angling is suggested as a mechanical means of fish removal, and it can be an adjunct to other methods. Increasing daily catch and possession limits and implementing mandatory kill of nonnatives in fishing regulations, may increase harvest of nonnative fish. For example, in Yellowstone National Park, the National Park Service (NPS) has implemented must kill regulations for rainbow trout caught in Slough Creek and Lake Trout caught in Yellowstone Lake (NPS 2015). Likewise, FWP has liberal daily catch and possession limits for brook trout, and anglers can have 20 brook trout in possession (see State of Montana Fishing Regulations 2017). Nevertheless, angling alone will not meet targets for removal of nonnative fish, which usually require 100% removal, or sufficient reductions of hybridizing species, to meet conservation goals.

Angling is not a viable means of meeting project goals of eradication due to its inefficiency and the difficulty in fishing in remote headwaters. Fry and age-1 fish are invulnerable to fishing and would mature to provide a perpetual source of the targeted species. Moreover, fish targeted for removal often live in high gradient streams covered by deadfall timber. These relatively unfishable reaches would harbor nonnative fish and be a continual source of fish to invade the waters below. Angling may have a role as an addition to other measures; however, because angling would not eliminate all nonnative fish, or appreciably decrease numbers or distribution in many watersheds, it will not be considered further in this document.

2.1 Effectiveness of Mechanical Removal of Fish in Streams

Mechanical removal using electrofishing can be effective under specific conditions (Shepard et al. 2014). Crews of 2 to 3 people removed fish from stream reaches measuring from 1 to nearly 2 miles of stream. Successful removal through electrofishing took as few as 6, or as many as 14 treatments, with each treatment consisting of 2 to 4 electrofishing passes through the reach. Increasing effort from once a year to targeting autumn spawning and winter aggregating behavior also improved efficacy.

Clearing riparian vegetation and woody debris contributed to successful fish removal using electrofishing (Shepard et al. 2014). Before mechanical removal began, field-workers cleared riparian vegetation and woody debris with chain saws. Woody debris and overhanging vegetation are critical components of high quality fish habitat; however, this complex habitat reduces the ability to net fish. Dip nets are easily snagged on branches and twigs, or are too large to reach spaces protected by woody debris. Removal of obstructions to netting fish increases capture efficiency.

Debris removal increased project costs (Shepard et al. 2014). Mechanical removal cost from $3,500 to $5,500 per kilometer. This amount was comparable to the use of piscicide, including labor, chemical, per diem, and travel costs. When clearing vegetation and wood was necessary to eradicate nonnative fish, project costs increased to $8,000 to $9,000 per kilometer.

Mechanical removal has been attempted in several projects in small, headwaters streams with mixed success. Biologists successfully eliminated nonnative rainbow trout from 0.5-miles of stream in Tennessee in 5 treatments (Kulp and Moore 2000). Another effort to remove rainbow trout in streams in the Great Smoky Mountains National Park achieved a great reduction in rainbow trout after 6 years of effort but not eradication (Moore et al. 1986). Mechanical removal efforts in the Rocky Mountains varied in success. Thompson and Rahel (1996) substantially reduced brook trout densities in 3 streams small streams ranging from approximately 2 to 6 miles in length, and no recruitment was observed in the following year. No follow up data were presented to determine if the low numbers of fish that evaded capture were able to reproduce in years after removal efforts stopped. A 3-year mechanical removal effort in a nearly 5-mile long, 2nd order stream in Idaho achieved up to an estimated 88% reduction in brook trout numbers in repeated removal efforts (Meyers et al. 2006). However, 2 years after cessation of brook trout removal, age-0 fish increased by 789%, leaving these researchers to conclude removal on larger streams would be “costly, quixotic enterprises”. Shepard (2010) eradicated brook trout in 4 small streams in Montana less than 2 miles in length, but habitat complexity in the form of dense shrubs, overhanging vegetation, beaver dams and high density of woody debris were effective precluded eradication in 2 other streams.

Native species conservation often happens on a watershed scale, and mechanical removal is infeasible in these situations. For example, over 60 miles of stream and 1 lake were reclaimed for westslope cutthroat trout in the Cherry Creek watershed and 21 alpine lakes and 45 miles of stream habitat were reclaimed for westslope cutthroat trout in the South Fork Flathead River watershed. In the Cherry Creek drainage, piscicide application began in 2003, and westslope cutthroat trout stocking began in 2006, with embryos placed in remote site incubators (RSIs) within streams. By 2015, the watershed supported over 40,000 westslope cutthroat trout (B. B, Shepard, Montana State University, personal communication). Costs associated with debris removal and personnel required for multiple passes on complex watersheds of this size are prohibitive. Moreover, assigning field-workers to a prolonged mechanical removal effort would preclude work on other native species conservations projects for many years.

Soda Butte Creek, a stream that enters Yellowstone National Park near its northeast corner, provides a case study of failure to remove all nonnative fish on a watershed scale, despite substantial effort. Soda Butte Creek is a relatively large watershed with complex habitat, and 21 miles of fish-bearing stream. Brook trout were present in a private pond connected to a headwater tributary, but were prevented from escaping because heavy metals from mine tailings created a chemical barrier. Following remediation of the tailings, brook trout invaded this stronghold for Yellowstone cutthroat trout.

The invasion was alarming due to the extreme threat brook trout pose to the cutthroat trout, especially in headwater streams (Dunham et al. 1997; Petersen et al. 2008; Shepard 2004; Shepard 2010). Moreover, this population of brook trout is poised in the headwaters of the Lamar River watershed, which is a basin-wide stronghold for Yellowstone cutthroat trout. Spread of brook trout downstream would endanger a population of Yellowstone cutthroat trout with immeasurable conservation and recreational value. Total elimination of brook trout was necessary to prevent further invasion and protect the Yellowstone cutthroat trout in Soda Butte Creek.

Removal efforts began in the early 1990s, and a concerted, multi-agency, intensive effort began in 2004, and ended in 2014. FWP, the Custer-Gallatin National Forest (CGNF), and the NPS each sent crews of 10 or more field-workers to conduct intensive yearly electrofishing. This annual event accrued the cost salaries of over 30 field-workers, and their travel and per diem costs. Moreover, the yearly brook trout removal diverted resources from priority actions under the strategy to conserve Yellowstone cutthroat trout in Montana, which include securing imperiled populations, field surveys to evaluate the status of populations that had not been sampled in decades, and searching for previously unidentified populations (Endicott et al. 2012).

The greatest number of brook trout were removed in the first 3 years, with a peak of nearly 11,000 fish in 2005 (Table 2-1). In the remaining years, the overall number of brook trout removed from Soda Butte Creek and its tributaries was relatively static, with efforts yielding approximately 100 to 150 brook trout removed from Soda Butte Creek and its tributaries. The number of brook trout captured decreased in the upper reaches, whereas removal reaches 5 through 7, which extended into Yellowstone National Park remained static, or showed an increasing trend in brook trout numbers.

Table 2-1. Total (and young-of-year) brook trout mechanically removed from Soda Butte Creek within the CGNF, State of Montana, and YNP (see Error! Reference source not found. **for locations of removal reaches).**

Site	***Removal Reach***	***2004***	***2005***	***2006***	***2007***	***2008***	***2009***	***2010***	***2011***	***2012***	***2013***	***2014***
1	HWY 212 to McClaren Mine Tailings	19(1)	3(0)	0(0)	0(0)	0(0)	NS	NS	NS	0(0)	0(0)	1(0)
2	McClaren Mine Tailings to Woody Creek	15(0)	17(0)	3(0)	3(0)	2(0)	NS	NS	NS	NS	NS	0(0)
3	Woody Creek to Sheep Creek	8(2)	43(0)	16(0)	0(0)	1(0)	NS	NS	2(0)	0(0)	0(0)	1(0)
4	Sheep Creek to Silver Gate	251(79)	932(51)	142(6)	45(8)	5(0)	6(0)	NS	30(1)	5(0)	4(0)	2(0)
5	Silver Gate to Yellowstone Park Boundary	9(3)	80(9)	54(2)	48(19)	13(0)	30(2)	16(0)	22(2)	10(0)	2(0)	30(3)
6	Yellowstone Park Boundary to Warm Creek	7(0)	11(0)	0(0)	50(27)	23(2)	56(10)	43(2)	15(0)	29(9)	35(0)	8(0)
7	Warm Creek to Highway X Bridge	0(0)	1(0)	0(0)	0(0)	3(1)	51(12)	68(29)	35(6)	53(10)	54(23)	55(4)
8	Road Bridge I to Road Bridge II	NS	NS	NS	NS	0(0)	1(0)	7(0)	2(0)	11(2)	16(3)	3(0)
9	Road Bridge II to Ice Box Canyon	NS	NS	NS	NS	0(0)	0(0)	NS	0(0)	NS	NS	NS
T	Tributaries	0(0)	17(0)	15(0)	4(0)	1(0)	8(0)	NS	NS	0(0)	54(19)	2(0)
	Total	309	1,104	230	150	48 (3)	152(24)	134(31)	106(10)	108(21)	165(45)	102(7)

*NS= Not Sampled

From 2009 through 2014, the reach from Warm Springs to the next highway bridge downstream consistently yielded about 50 brook trout, and variable but sometimes substantial numbers of brook trout were removed the lower 2 sections. The failure to achieve declines in this reach indicated mechanical removal would not eradicate brook trout, and the downstream reaches of Soda Butte Creek would be a continued source of brook trout to reinvade waters upstream and move downstream into the Lamar River watershed. Habitat complexity in the form of massive debris jams contributed to the inability to achieve full removal (Figure 1).

Figure 1. Example of a debris jam that decreases the ability of removing all brook trout, showing 2-ft diameter log for scale.

The size and number of debris jams also affects feasibility of woody debris removal. For example, in a reach with numerous debris jams of the size and complexity as shown in Figure 1, heavy equipment would be necessary, which would greatly increase project costs, and would be infeasible in remote locations and designated wilderness. Furthermore, disturbance associated with the use of heavy equipment to remove large debris jams would be considerable and may be unacceptable to the public.

The failure of repeated mechanical removal to eradicate brook trout, combined with the threat these fish posed to the Lamar River watershed, led fisheries managers to reevaluate mechanical removal as the preferred option. Removal efforts were costly and other conservation efforts were indefinitely delayed given the amount of effort expended in Soda Butte Creek each year. Moreover, brook trout are capable of explosive resurgence from low densities of fish within a few years (Meyers et al. 2006), which suggested easing up on the expensive and labor intensive yearly mechanical removal efforts would reverse progress within a few years. Consequently, chemical removal emerged as the method with the greatest potential for successful eradication, and would allow the agencies involved to attend to other high priority projects.

Piscicide application occurred in 2015 and 2016, and each treatment was preceded by salvage of resident Yellowstone cutthroat trout in Soda Butte Creek. Sampling for environmental DNA (eDNA) with brook trout markers in summer 2017 found a cluster of brook trout DNA in samples collected downstream of Silver Gate, in reach 5 (Table 2-1). In response to these findings, intensive electrofishing and additional eDNA sampling ensued; however, these efforts did not find evidence of brook trout. Testing for eDNA is sensitive and positive results could be the result of a brook trout carcass stuck in debris still casting off DNA, or transfer of brook trout DNA from waders or piscivorous birds. Monitoring using electrofishing and eDNA sampling will continue to evaluate the effectiveness of piscicide treatment, but no additional treatments are planned, unless monitoring data indicate otherwise (J. Rhoten, FWP, personal communication).

Mechanical removal and chemical removal of nonnative fish usually share the need of a barrier at the downstream end of the project reach. Ideally, a natural barrier, such as a waterfall, is present. Otherwise, one or more barriers must be installed. In streams where mechanical removal is feasible, features such as perched culverts, log cribs, concrete structures, or creation of waterfall by blasting rock are options. If removal approach involves sequential downstream removal efforts, where eradication begins in smaller headwaters, a few to many temporary barriers would be necessary, depending on the spatial scope of the project. The adjacent, downstream reach would later be protected by a barrier, and the upstream, temporary barriers would be removed when the threat of reinvasion from downstream has been eliminated. As mechanical removal has not been successful in larger streams, or streams with complex habitat, large watershed projects would require a prohibitive number of barriers.

In conclusion, mechanical removal can be an effective method of removal of nonnative species under limited circumstances. The length of stream is a major consideration. Mechanical removal has been effective in streams reaches from approximately 1.5- to 2 miles long; however, the level of effort can be considerable, with up to 14 treatments of up to 4 electrofishing passes required (Shepard et al. 2014). Habitat complexity is another concern, with electrofishing being ineffective in complex habitat. Removal of woody debris and riparian vegetation increases probability of removal using mechanical means, but adds considerably to project costs. Moreover, debris removal may not be feasible in large scale projects with remote tributaries and substantial amounts of woody debris. Finally, mechanical removal is not feasible in large, connected watersheds with complex habitat, given limitations in the amount of available labor, the need for numerous barriers, and constraints on capture efficiency. This reality presents a challenge for conservation practitioners since large, complex, interconnected habitats provide the greatest opportunity for long term population persistence.

2.2 Potential Disturbance from Mechanical Removal of Fish in Streams

Disturbance associated with mechanical removal differs substantially from chemical removal in several ways. Both require the presence of field-workers; however, the frequency and duration of human activity is project specific. Unless a waterfall or other existing feature prevents reinvasion of fish from downstream, mechanical and chemical removal require construction of at least 1 barrier, although mechanical removal may require additional barriers as removal proceeds downstream from headwater reaches. Removing vegetation and debris to facilitate capture of fish using electrofishing is a pronounced difference between the methods and has potential for short-term and long-term effects on streams.

2.2.1 Presence of Humans

Mechanical removal requires presence of field-workers performing multiple passes, often for several years. The number of passes and duration of treatment depends on capture efficiency, but crews of 2 or more people would be walking streams, and shocking and netting fish. For small streams, a battery powered backpack electrofisher is the most likely method of removing fish. On larger streams, a boat-mounted electrofishing unit powered by a gas generator would be pulled along the stream. This method typically requires crews of 3 or more people. Gas-powered generators are relatively loud and create noxious exhaust. As removal efforts are effective in short reaches of small streams, backpack electrofishers are more likely to be used than the boat-mounted electrofishers. In wilderness, gear for removal would likely need to be flown in by helicopter, especially if a boat-mounted electrofisher and generator is required.

2.2.2 Barriers

In many cases, mechanical and chemical removal require construction of 1 or more barriers to prevent reinvasion of nonnative fishes. The types of barriers vary, and constructing barriers results in variable amount and type of human activity. In addition, barriers alter sediment and woody debris transport and may require regular maintenance. The type and duration of disturbance varies with the type of barrier. Blasting rock to create a waterfall results in considerable noise, but is brief. Construction of log cribs requires a variety of power tools, field-workers, and transportation of materials and equipment to the site. Installation of perched culverts or concrete barriers requires mobilization of heavy equipment to the site, which includes excavators, concrete trucks, contactor’s vehicles, and materials. In some cases, a road needs to be constructed to provide access to the site, or materials and equipment can be transported by helicopter. The time required to build a barrier varies with size, materials, and equipment required to construct the barrier.

The upper Shields River watershed is an example of an effort to remove brook trout on a watershed scale that is capitalizing on existing or temporarily placed barriers. Periodic sampling beginning in the 1970s found only nonhybridized Yellowstone cutthroat trout, until 2009, when basin-wide sampling found an early invasion of brook trout. Fortunately, this finding coincided with the CGNF's multimillion dollar road improvement project intended to improve water quality, provide fish passage, and improve public access to the forest. Planners made strategic decisions on which fish barriers were to remain, and where to install temporary perched culverts to protect tributaries that had not yet been invaded. A large, permanent barrier was constructed at the downstream end of the project area. FWP, the CGNF and other project partners began mechanical removal from waters not protected by barriers in 2014. Brook trout have apparently been eliminated in a small tributary that was in an extremely early phase of invasion, with only 3 brook trout found over repeated removal efforts. The overall success remains unknown; however, in the event chemical removal becomes the preferred option, not all fish-bearing waters will need to be treated, and the protected streams will provide areas to hold salvaged Yellowstone cutthroat trout during piscicide treatment.

The number of barriers required to prevent reinvasion of nonnative species during a long-term removal effort, in a watershed that did not have heavy equipment already mobilized, would be prohibitive. As fish removal in reaches greater than 2 miles in length are ineffective, barriers would need to be installed at regular, close intervals, and removal would need to proceed in a step-wise fashion. On a watershed scale, and in remote country or designated wilderness, installation of temporary barriers would be costly, result in considerable disturbance, and would potentially be inconsistent with wilderness management objectives.

The influence of the constructed barriers at the downstream end of the treatment reach has potential to affect channel morphology, sediment transport, and conveyance of woody debris. Barriers used in fish removal projects vary with the site. The wood cribs alter bed load and debris transport, and have the potential to fail during floods. Perched culverts need to be installed where road access is available. Moreover, culverts and concrete barriers also have potential to impair transport of bed load and woody debris. Barriers need to be inspected regularly for maintenance, and removal of woody debris and accumulated bed load.

2.2.3 Vegetation and Debris Clearing

Clearing vegetation and debris increases the potential for successful mechanical removal of nonnative fish and can be economically and logistically feasible for small streams less than 2 miles in length (Shepard et al. 2014). Aside from the limited practicality of removing streamside vegetation and debris, these actions have potential for short-term and long-term alterations to stream ecology, benthic invertebrate community composition, water quality, fish habitat, and channel stability.

Removing over-hanging shrubs would have relatively short-term effects on streams, as the functional attributes of riparian vegetation recover quickly in absence of additional disturbance. Nevertheless, these alterations need to be considered, especially given the potential of vegetation removal to affect habitat, forage availability, and water quality for native fish stocked into reclaimed waters, and the influence of vegetation on benthic invertebrate communities, which are highly reliant streamside vegetation as a source of organic input and stream shading.

In forested headwater streams, macroinvertebrate communities depend on terrestrial inputs of organic matter (Vannote et al. 1980). Removing vegetation and increasing primary productivity through greater sun exposure may shift the community composition from invertebrates eating leaf matter, to species that graze algae from rocks and other substrates within the stream. In Montana, cold-water, headwater streams provide habitat for 8 invertebrate species of concern of the genus *Utacapnia* that are cold-water stenotherms and consume leaf litter, (see Montana Natural Heritage Program website). Pre-project planning should include sampling invertebrate communities to evaluate if invertebrate species of concern are present before removing riparian cover.

Removing riparian vegetation can change thermal regime, which would have implications for fish and macroinvertebrates. Canopy density has been found to affect thermal inputs to streams, and warmer water temperatures resulted in reduced salmonid biomass (Platts and Nelson 1989). Increased insolation of the stream surface would be detrimental to Bull trout and cutthroat trout, as these fishes are more sensitive to warmer water than nonnative salmonids (Selong et al. 2001; Sloat et a. 2002; Bear et al. 2011; Dobos et al. 2016). Warmer water temperatures may also alter the community composition of macroinvertebrates, as species vary in their thermal tolerance.

Elimination of these debris jams would increase capture probability; however, it would have longstanding consequences for channel stability and fish habitat. Woody debris promotes channel stability during flood events (Heede 1985). Furthermore, woody debris produces scour that promotes the formation of pools and other habitat features (Heede and Rinne 1990). Recruitment of large woody debris occurs over decades, so woody debris removal would result in long-term alteration of this important component of stream stability and habitat formation.

2.3 Compatibility of Wilderness Values with Mechanical Removal of Fish in Streams

Mechanical removal of nonnative fish brings several potential disturbances that may affect wilderness values, and diminish visitors' appreciation of the wilderness experience. Electrofishing in streams generally entails several crews making multiple electrofishing passes over the course of up to 14 treatments (Shepard et al. 2014), and these are often multiyear projects. The extended and repeated presence of field-workers in the stream increases the human imprint in wilderness. Backpack electrofishers produce a frequent beep when an electrical current is in the water. Larger streams require boat mounted electrofishers equipped with a gasoline powered generator, which produce noise and exhaust. Setting and checking of nets also requires field-workers be present. These disturbances could diminish the peace and solitude of recreationalists visiting designated wilderness.

Transportation of gear into remote areas also has potential to alter wilderness character, increase the human imprint, and diminish the visitor's enjoyment of the peace and tranquility. Personal gear, provisions, and field gear are transported by backpack, horse train, or helicopter. Each mode is a disturbance that increases human presence, causes noise, and results in conditions that may affect enjoyment of wilderness. Constructing barriers in wilderness also entails transporting materials and equipment into wilderness, and the associated noise and human presence is inconsistent with maintaining wilderness character.

Removal of woody debris and streamside vegetation increases the efficiency of electrofishing; however, it brings several short-term and long-term disturbances to the wilderness character. Field-workers removing woody debris would bring more humans into wilderness. The noise and exhaust of power tools is incompatible with wilderness values. Moreover, vegetation and debris removal may alter the ecology of the stream, and remove important components of stream habitat and stability. Regrowth of riparian shrubs would make increased temperatures and reduction of leaf matter a relatively short-term alteration. In contrast, large woody debris may take decades to recruit, which could have long-term effects on fish habitat and channel stability. The long-term alterations with woody debris clearing would be a substantial human imprint and may not be acceptable under the Wilderness Act of 1964.

Clearing debris and vegetation also affects the aesthetics of the stream, and is inconsistent with the concepts of "untrammeled" and "wilderness character". Sawed off stumps, reduced wood in the stream, and significant reduction of the natural riparian overstory are considerable manipulations of the natural environment. Moreover, removal of wood could have long-term effects on recreation, as streams with reduced habitat complexity may have lower carrying capacity for fish. From conservation and recreational angling perspectives, fewer fish is undesirable.

2.4 Methods and Efficacy of Mechanical Removal of Fish in Lakes

Mechanical removal in lakes is typically accomplished through deployment of nets, especially gill nets. Genetic swamping may be used in conjunction with nets. Genetic swamping involves frequent or annual stocking of nonhybridized native fish into a lake, with the goal of decreasing the frequency of nonnative genes within the population.

Not all lakes are candidates for mechanical removal. Connected lakes, those with tributaries and an outlet capable of supporting fish, have a perpetual source of nonnative fish to reinvade. Mechanical removal is an option for isolated high mountain lakes with outfalls that are not connected to fish-bearing streams, or in conjunction with removal efforts in the inlet and outlet streams.

2.5 Potential Disturbance from Mechanical Removal in Lakes

Using netting to eradicate fish may have several negative consequences. The nets may be aesthetically unappealing to people accessing the lake, and they are a relatively long-term disturbance, as they are set for several months. Moreover, non-target species may suffer mortality in nets. Beavers captured in submerged trap nets drown, as do diving birds that become entangled in gill nets.

2.6 Compatibility of Wilderness Values of Mechanical Removal of Fish in Lakes

The primary disturbances associated with mechanical removal of fish in lakes involve increased presence of fieldworkers, and the extended use of nets. Backpackers seeking the tranquility of a mountain lake could have their enjoyment decreased due to presence of other people and gill nets.

3 Chemical Removal

3.1 Background on Rotenone

Piscicides used in fish removal projects include rotenone and antimycin. Rotenone is the focus of this document, as it is currently the most commonly used piscicide in Montana. State and federal agencies tasked with fisheries management have a long history of using rotenone to manage fish populations, spanning as far back as the 1930s. Rotenone is principally applied to improve angling quality and for native fish conservation. Rotenone has been an invaluable tool in restoring native species to waters where they have been extirpated, or are threatened by nonnatives. In cases where nonnative fish have been introduced upstream of natural barriers in waters that was historically fishless, rotenone has been applied to remove nonnative fish, and stock fish in previously unoccupied habitat in its historic range.

Rotenone is a naturally occurring substance derived from the roots of tropical plants in the pea family (Fabaceae), such as the jewel vine (*Derris* spp.) and lacepod (*Lonchocarpus* spp.), which are found in Australia and its surrounding Pacific islands, southern Asia, and South America. Native people have used locally available rotenone for centuries to capture fish for food. Rotenone is also a natural insecticide, and was formerly used in organic gardening and to control parasites such as lice on domestic livestock (Ling 2002).

Rotenone works on the cellular level by disrupting cellular respiration in mitochondria (Hayes 1991), which are the cellular organelles responsible for converting chemicals to energy. Fish are especially vulnerable to low levels of rotenone, as they readily absorb rotenone into the bloodstream through the gill lamellae. Many gilled invertebrates are also vulnerable to rotenone, although many are not nearly as sensitive as fish. In addition, amphibians respire with gills during their earliest life history stage, and are vulnerable to rotenone. Mammals, birds, reptiles and other non-gill breathing organisms lack this rapid absorption route into the bloodstream, and can tolerate exposure to concentrations that are several orders of magnitude higher than levels lethal to fish.

Currently, CFT Legumine™ is a formulation of rotenone most commonly used for nonnative fish removal in Montana. CFT Legumine has the advantage of using nonorganic solvents and dispersants to dissolve and disperse the relatively insoluble rotenone. In contrast, formerly used formulations used organic solvents. These formulations had the disadvantage of being more toxic to field-workers handling and dispensing rotenone, and fish could detect and elude these aromatic compounds.

3.2 Method of Applying Rotenone Treatment in Streams

Rotenone projects begin with a bioassay, or field experiment, to determine the lowest effective concentration of rotenone to kill fish in the receiving water. In practice, lowest effective concentration of rotenone for salmonids in cold-water streams is 25 to 50 parts per billion (ppb), which is roughly equal to ¼ to ½ grains of table salt per liter. The rotenone treatment begins in the headwaters, and tributary streams in the headwaters are treated first. Because rotenone degrades rapidly, drip stations are typically placed at intervals to ensure that chemical from upstream drip stations overlaps with that from downstream drip stations. The spacing between drip stations is also determined with a bioassay that assesses how long rotenone remains lethal to fish during treatment. Sentinel fish in mesh bags allow for determination of the appropriate interval. The treatment proceeds downstream in steps, until all surface waters have been treated.

Wetlands, seeps, and side channels have potential to harbor fish or dilute concentrations of rotenone to sublethal levels. In these areas, powdered rotenone mixed with sand and gelatin is placed at the mouths of small tributaries or seeps, to prevent fish from finding refugia from lethal concentrations of rotenone. Likewise, field-workers with backpack sprayers treat backwaters and isolated pools as toxic concentrations of rotenone may not be achieved in off-channel habitats.

Rotenone treatments need a consistent and sufficient flow of solution to promote a full fish kill. CFT Legumine uses solvents and dispersants to keep the relatively insoluble rotenone in solution, and allow it to spread through the water. These inert ingredients can gel at the colder temperatures occurring during autumn application. Therefore, drip stations require regular monitoring to ensure the diluted rotenone formulation does not clog the aperture dispensing rotenone. To provide a steady supply of rotenone, field-workers stay at each drip station for the duration of the treatment, and monitor flow rate, and unclog the aperture as required. Drip stations may also lose pressure and need to be equilibrated, which requires frequent monitoring. In addition, drip station attendants monitor the sentinel fish upstream of the drip station, to ensure toxic concentrations of rotenone are maintained between drip stations.

Beaver dam impoundments can prevent dispersal of lethal concentrations of rotenone given their depth and complex habitat. Beaver dams are typically breached during the piscicide treatment to foster flow through the area and eliminate potential refugia from rotenone.

Rotenone detoxifies naturally through oxidation, dilution by freshwater and binding with organic sediment. Factors influencing natural oxidation include water temperature, water chemistry, and exposure to organic substances, air, and sunlight (Engstrom-Heg 1972; Gilderhus et al. 1986; Loeb and Engstrom-Heg 1970; Ware 2002). Dilution results from contributions of water from tributaries or upwellings of groundwater.

Establishment of a deactivation station limits the spatial extent of the fish kill by oxidizing rotenone with potassium permanganate. Full neutralization of rotenone requires a short mixing zone, which allows rotenone and potassium permanganate 1/2 -hour of contact time in the stream. Application rates of potassium permanganate are based on stream flow and natural background levels of oxidation. A small handheld colorimeter measures levels of potassium permanganate to guide application rates.

Application of potassium permanganate often requires a power auger that is run soon after piscicide application begins, and continues until sentinel fish show no signs of distress for 4 hours. The amount of potassium permanganate can be considerable, as FWP piscicide policy requires twice the estimated amount of potassium permanganate be on-site during treatment. When the deactivation station is in a remote location, potassium permanganate, the power auger, a generator, and fuel need to be transported to the deactivation station by helicopter or pack stock.

Caged fish allow evaluation of the toxicity and deactivation within the project area, and downstream of the project area. These sentinel fish are placed upstream of drip stations to ensure toxic concentrations of rotenone are maintained between stations. During treatment, the status of sentinel fish downstream of the deactivation station indicates when the water is no longer toxic. The CFT Legumine label specifies that once caged fish show no signs of distress for 4 hours, stream deactivation can cease. Sentinel fish need to be transported to the project area and dispersed throughout the treated area in coolers, which requires helicopter support or pack stock.

Post-treatment monitoring allows evaluation of the efficacy of the rotenone treatment. Traditionally, electrofishing has been the sole method of determining the effectiveness of the piscicide treatment. Electrofishing is labor intensive, time consuming, and has the potential to yield false negative results, especially in areas with complex habitat that decrease capture probability. Environmental DNA (eDNA) is now being used as an adjunct to electrofishing. Water samples collected in the field are tested for the presence of DNA from the fish targeted for removal. This technology requires far less labor and time, and the cost of sample analysis is considerably less than costs associated with employing field crews to cover miles of stream with electrofishers. Furthermore, eDNA results allow for a targeted approach to retreatment, which results in a smaller treatment area, less disturbance to aquatic communities, and less labor.

Once fish are eradicated, native trout are returned or stocked in the project area. In streams where nonhybridized, aboriginal populations of native fish remain, these fish are salvaged before rotenone treatment and returned to the stream the day after rotenone treatment ends. Otherwise, native fish are returned using several approaches. Potential sources of fish include streams in the same watershed, neighboring streams, brood stock acquired from wild fish, or captive brood stock that is regularly infused with wild genes. Imprinting fish on the receiving water prevents fish from leaving. Therefore, raising fertilized eggs to fry in remote site incubators or egg boxes is a primary means of reestablishing the fishery. In waters supporting a recreational fishery, catchable native fish are translocated to the project area, which requires horse trains or helicopter support.

3.3 Methods of Piscicide Treatment in Lakes

Piscicide treatment in lakes differs from stream application, as lakes lack the flow to disperse rotenone. Rotenone is applied to the surface of the lake from a boat, helicopter, or plane. Where incoming tributaries are present, drip stations are placed at their mouths. The treatment concentration follows the same procedure as stream application, with a bioassay determining the lowest effective dose. An electric or gas motor will mix rotenone in the lake, to ensure lethal concentrations occur throughout the lake. Natural deactivation is slower in lakes than in streams, although the same mechanisms contribute to the breakdown of rotenone.

3.4 Toxicity, Persistence, and Fate of CFT Legumine and Its Inert Ingredients in Treated Waters

As CFT Legumine is currently the most commonly used formulation of rotenone in Montana, this is the only formulation addressed. The Environmental Protection Agency (EPA) registered this formula (Reg. No. 75338-2), and approved its use as a piscicide. Information on its chemical composition, persistence in the environment, risks to human health, and ecological risks come from the material data safety sheet (MSDS) and manufacturer's instructions. An MSDS is a form detailing chemical and physical properties of a compound, along with information on safety, exposure limits, protective gear required for safe handling and procedures to clean up spills safely. In addition, Fisher (2007) analyzed the concentrations of major and trace constituents in CFT Legumine, evaluated the toxicity of each, and examined persistence in the environment.

The MSDS for CFT Legumine lists three categories of ingredients for this formula (Table 2). Rotenone comprises 5% of CFT Legumine by weight. Associated resins account for 5%, and the remaining 90% are inert ingredients. The MSDS confirms rotenone's extreme toxicity to fish.

Table 3-1: Composition of CFT Legumine from the material safety data sheet (MSDS)

Chemical Ingredients	*Percentage by Weight*	*CAS. No.*[1]	*TLV*[2] *(units)*
Rotenone	5.00	83-79-4	5 mg/m^3
Other associated resins	5.00		
Inert ingredients including n-methylpyrrolidone	90	872-50-4	Not listed

[1]Chemical abstracts number
[2]A TLV reflects the level of exposure that the typical worker can experience without an unreasonable risk of disease or injury

Analysis of the chemical composition of CFT Legumine found that on average, rotenone comprised 5% of the formula (Table 3-2), consistent with MSDS reporting. Other constituents were solvents or emulsifiers added to assist in the dispersion of the relatively insoluble rotenone. DEGEE, or diethyl glycol monoethyl ether, a water-soluble solvent, was the largest fraction of the CFT Legumine analyzed. Likewise, the solvent n-methylpyrrolidone comprised about 10% of the CFT Legumine. The emulsifier Fennedefo 99 is an inert additive consisting of fatty acids and resin acids (by-products of wood pulp and common constituents of soap formulations), and polyethylene glycols (PEGs). PEGs are common additives in consumer products such as soft drinks, toothpaste, eye drops, and suntan lotions. Trace constituents included exceptionally low concentrations of several forms of benzene, xylene, and naphthalene. These organic compounds were at considerably lower concentrations than measured in Prenfish™, another commercially available formulation of rotenone that uses hydrocarbons to disperse the rotenone. Their presence in trace amounts in CFT Legumine relates to their use as solvents in extracting rotenone from the original plant material.

Table 3-2: Average percent concentrations and ranges of major constituents in CFT Legumine lost (Fisher 2007).

Major CFT LegumineFormula Constituent	*Rotenone*	*Rotenolone*	*n-methylpyrrolidone*	*DEGEE*[1]	*Fennedefo 99*
Average %	5.12	0.718	9.8	61.1	17.1
Range	4.64-5.89	0.43-0.98	8.14-10.8	58.2-63.8	15.8-18.1

[1]diethyl glycol monoethyl ether

Persistence in the environment and toxicity to nontarget organisms are major considerations in determining the potential risks to human health and the environment. Rotenone is a highly reactive molecule; a factor that favors its rapid breakdown in the environment. The molecular constituents of rotenone are carbon, hydrogen, and oxygen and deactivation breaks rotenone into nontoxic compounds of these elements.

The effective rotenone concentration for removal of fish from the family Salmonidae is 25 to 50 ppb, which is roughly equivalent to ¼ to ½ of a grain of table salt per liter, and is well below concentrations found to have lethal or sublethal effects on organisms other than fish or gill-bearing invertebrates and amphibians. The National Academy of Sciences suggested concentrations of 14 ppm (about 8,900 grains of salt per liter) pose no adverse effects to human health from chronic ingestion of water (National Academy of the Sciences 1983). Moreover, concentrations associated with acute toxicity to humans are 300-500 mg per kilogram of body weight (Gleason et al. 1969), which means a 160-pound person would have to drink over 23,000 gallons in one sitting to receive a lethal dose (Finlayson et al. 2000). Similarly, risks to wildlife from ingesting treated water are exceptionally low. For example, ¼-pound bird would have to consume 100 quarts of treated water, or more than 40 pounds of fish and invertebrates, within 24 hours, for a lethal dose (Finlayson et al. 2000). The EPA, in their recent reregistration evaluation of rotenone (EPA 2007), concluded that exposure to rotenone, when applied according to label instructions, did not present unacceptable risks to humans or wildlife. In summary, applying rotenone according to label instructions has no adverse effect on humans or wildlife associated with ingesting water, dead fish, or dead invertebrates.

Several factors influence the persistence of rotenone. Rotenone has a half-life of 14 hours at 24 °C, and 84 hours at 0 °C (Gilderhus et al. 1986, 1988), meaning half of the rotenone is degraded and is no longer toxic in that time. As temperature and sunlight increase, so does degradation of rotenone. Higher alkalinity (>170 mg/L) and pH (>9.0) also increase the rate of degradation. Rotenone tends to bind to and react with organic molecules rendering it ineffective, so nutrient rich waters may need higher concentrations to counteract binding to organic matter. Without deactivation, rotenone degrades to nontoxic levels in one to several days due to its break down and dilution in the aquatic environment.

In streams, mitigative actions further reduce the spatial and temporal extent of rotenone toxicity. A deactivation station releases potassium permanganate up to the effective concentration of 0.5 to 1 ppm. This strong oxidizer rapidly breaks down rotenone into its nontoxic constituents of carbon, oxygen, and hydrogen, with total breakdown occurring within 15 to 30 minutes of exposure, which is typically ¼ to ½-miles stream travel time. Potassium permanganate in turn breaks down into potassium, and manganese dioxide, which are common constituents in surface waters (Finlayson et al. 2000). In addition, potassium permanganate is a commonly used oxidizer in wastewater treatment plants, so its release into streams and rivers is a regular and widespread phenomenon. The result of release of potassium permanganate on water quality is the elimination of toxic concentrations of rotenone, although potassium permanganate can be at lethal concentrations within the deactivation zone. An additional, back up deactivation station provides a safeguard if sentinel fish show signs of rotenone toxicity.

In lakes, the toxicity of rotenone persists longer than in streams. Although deactivation occurs through the same mechanisms, sunlight, natural oxidization, and dilution with inputs of groundwater and streams, inflows are relatively small compared to the volume of the lake.

Binding with organic matter in lake sediments and littoral zones also facilitates the deactivation of rotenone, and is synergistic with temperature. In earthen bottom ponds treated with rotenone, the half-life of rotenone was 2 to 3 times shorter than in concrete lined ponds (Dawson et al.1991). The half-life of rotenone in earthen lined ponds was 1.8 days at 8 °C, 0.7 days at 22 °C, and 1.8 days at 15 °C.

Application of CFT Legumine for 2 days in Lake Davis in California to eradicate invasive northern pike (*Esox lucius*) provided an opportunity to evaluate the persistence and fate of the primary constituents of CFT Legumine in the field (Vasquez et a. 2012). Water temperature, alkalinity, and exposure to sunlight, factors that influence degradation or rotenone were not reported; however, rotenone degradation was within the expected range for a relatively high elevation lake in September. The average maximum lake concentration of rotenone of 58.4 ± 36.6 ppb was attained 2 days post-treatment. This range of concentrations is within the effective concentration for fish eradication efforts. Breakdown of rotenone into rotenolone resulted in the maximum average lake concentration of rotenolone of 174 ± 4 ppb 6 days after treatment. The overall half-life of rotenone and rotenolone in lake water was 5.6 days and 11.1 days respectively. Rotenone had degraded to concentrations lower than analytical reporting limits in 34 days, and rotenolone was below detection limits by 62 days posttreatment. Despite not being able to control for temperature, sunlight or alkalinity, breakdown of rotenone in CFT Legumine was within the predicted range as described by Gilderhus et al. (1986, 1988).

Rotenone can bio-accumulate in the fat tissues of fish that are not exposed to toxic levels (Gingerich and Rach 1985); however, the short duration of exposure, and goal of total fish kill does not allow for accumulation of rotenone in salmonid conservation projects. Field studies of the rotenone-tolerant brown bullhead (*Ameiurus nebulosus*) surviving treatment concentrations of CFT Legumine provided no evidence of prolonged bioaccumulation of rotenone or rotenolone (Vasquez et al. 2012). Rapid degradation of rotenone in treated water was attributed to limiting the opportunity for fish to bioaccumulate rotenone.

Potential toxicity and persistence of the other constituents of the CFT Legumine formulation are additional considerations. Concentrations of n-methylpyrrolidone in treated water (about 2 ppm) have no adverse effects to humans ingesting treated waters. According to the MSDS, ingestion of 1000 ppm per day for three months does not result in harmful effects in humans. In addition, n-methylpyrrolidone does not persist in surface waters given its high biodegradability. In Lake Davis, the average maximum concentration of n- methylpyrrolidone occurred 10 days after treatment and was 156 ± 127 ppb, which is considerably lower than concentrations deemed safe for human consumption in the MSDS. The half-life of n-methylpyrrolidone was 4.6 days, and this chemical degraded to undetectable concentrations by 34 days.

Fisher (2007) examined the toxicity and persistence of other major constituents in CFT Legumine, including DEGEE, fatty acids, PEGs, and trace organic compounds, (benzene, xylene, naphthalene). With proposed application of CFT Legumine, none of these compounds violate water quality standards, nor do they reach concentrations shown to be harmful to wildlife or humans. Furthermore, persistence of these chemicals is not a concern. The trace organics degrade rapidly through photolytic (sunlight) and biological mechanisms. Likewise, the PEGs biodegrade in a few days. The fatty acids also biodegrade, although they would persist longer than the PEGs or benzenes.

Field investigations in Lake Davis (Vasquez 2012) confirmed Fisher's (2007) conclusions that inert constituents of CFT Legumine would degrade rapidly, and be well below concentrations harmful to aquatic organisms or humans. The maximum average lake concentration of DEGEE was 779 ± 632 ppb. Toxicity information from the MSDS for DEGEE indicates bluegill (*Lepomis macrochirus*) survived 96 hours at 10 million ppb. DEGEE degraded relatively rapidly, with a half-life of 7.7 days, and was no longer detectable in treated water after 70 days (Vasquez et al. 2012).

Fennedofo 99 comprises approximately 18% of the CFT Legumine formulation (Fisher 2007). Fennedofo 99 was the most persistent of the main ingredients in CFT Legumine. The maximum average lake concentration was 389 ± 310 ppm at 6 days post-treatment. It had the longest half-life of 13.5 days. Fennedefo 99 dissipated to below reporting limits in 70 days. Despite its longer persistence, this substance is nontoxic, so its persistence did not pose a threat to aquatic life.

Benzene is among the trace compounds in the CFT Legumine formulation. Its treatment concentration in streams would reach 3.44 ppb, whereas the human health standard for chronic exposure to benzene in Montana is 5 ppb. This means the short-term treatment concentrations are less than levels that result in negative health consequences with long-term exposure. Concentrations resulting in acute toxicity, or death of 50% of tested organisms (LD_{50}) for laboratory rats, range from 232,500 ppb to 279,000 ppb. Mice are substantially more tolerant of ingested benzene than rats.

The concentration of naphthalene in treated water is 0.00225 ppm. As a moderately volatile compound, naphthalene does not break down as rapidly as the highly volatile benzene and xylene. Nevertheless, this concentration is exceptionally low, and is undetectable in laboratory analyses. Furthermore, naphthalene concentration of 0.00225 ppm is well below the Montana drinking water standard of 0.1 ppm. The minute concentration of naphthene is treated water is likely inconsequential and short-lived. The lethal dose for 50% of tested organisms ($LD_{50)}$ for rainbow trout is 1.6 ppm, and the LD_{50} for fathead minnow (*Pimephales promelas*) is 6.14 ppm. These concentrations are exorbitantly higher than treatment concentrations of naphthalene.

Trace concentrations of xylene were present in some lots of CFT Legumine (Fisher 2007), but it was not consistently encountered. Like benzene and naphthalene, concentrations of xylene, when present, were orders of magnitude lower than human health standards and acute toxicities for tested organisms. Moreover, dilution in stream application, and its high volatility, means xylene does not present a threat to human health and the environment.

The presence and fate of dead fish is another potential alteration of water quality associated with piscicide treatment. Although removing dead fish is often recommended to avoid conflicts with wildlife, the decay of dead fish does not increase the nutrient budget of the body of water. Decaying fish will return nutrients to the lake or stream, which fertilizes primary producers, and feeds scavenger, which form the base of the trophic pyramid. Therefore, the dead fish do not result in a net increase in the stream or lake’s nutrient budget.

Deactivation at the downstream end of the project area limits the spatial extent of toxic water. Even without deactivation, the rotenone dilutes or breaks down in a matter of days through natural oxidation, binding with organic material or dilution, making the effects on water quality short-term and minor. Effective concentrations of rotenone generally do not travel far, which is why drip station spacing is typically at 1 to 2-mile intervals. The other constituents of the CFT Legumine are not toxic at the concentrations applied, and break down rapidly through hydrolysis, bacterial action, and oxidation (Fisher 2007). Likewise, potassium permanganate degrades rapidly when applied according to the manufacturer’s label. Constituents with longer persistence are nontoxic and do not pose a threat to the environment.

To reduce the potential risks associated with the use of CFT Legumine, the following management practices, mitigation measures, and monitoring efforts are employed.

1. Project personnel are trained in the use of these chemicals including the actions necessary to deal with spills, as prescribed in the MSDS for CFT Legumine.
2. Signs are posted at trailheads and along the stream to warn people not to drink the water, consume dead fish, or have recreational contact with the water.
3. Only the amount of rotenone and potassium permanganate that is needed for immediate use is held near the stream.
4. A deactivation station is set up downstream of the target reach. Potassium permanganate neutralizes the rotenone at this location.
5. Sentinel fish are located below the deactivation station and within the target reach to determine and monitor the effectiveness of both the rotenone and potassium permanganate.
6. An additional deactivation is established downstream from the initial deactivation station as a safeguard.
7. People handling the rotenone wear protective gear as prescribed in the CFT Legumine label.
8. A pretreatment bioassay is conducted to determine the lowest effective concentration and travel time of the chemical in the stream.

9. Rotenone is diluted in water and dripped into the stream at a constant rate using a device that maintains a constant head pressure.

3.5 Effects of Rotenone on Groundwater

Rotenone binds readily to soils and is broken down by soil and in water (Dawson et al. 1991; Skaar 2001; Ware 2002). Because of its strong tendency to bind with soils, its mobility in most soil types is only one inch; although, in sandy soils, rotenone can travel up to three inches (Hisata 2002). Vasquez et al. (2012) reported concentrations of rotenone in lake sediments in units of nanograms per gram, with 1 gram being equal to 1 million nanograms. These exceptionally low concentrations of rotenone and rotenolone in lake sediments suggests leaching of rotenone compounds from the lake bed into groundwater is negligible, as the concentrations of rotenone in lake sediments were minute. Combined, the low mobility, rapid breakdown, and biologically insignificant concentrations of rotenone in lake sediments prevents rotenone from contaminating groundwater.

Groundwater investigations associated with several rotenone projects also indicate application of rotenone, and the inert ingredients, do not threaten groundwater quality. California investigators monitored groundwater in wells adjacent to, and downstream of, rotenone projects, and did not detect rotenone, rotenolone, or any of the other organic compounds in the formulated products (CDFG 1994). Likewise, case studies in Montana have concluded that rotenone movement through groundwater does not occur. For example, FWP monitored a domestic well two weeks and four weeks after applying 90 ppb of rotenone to Lake Tetrault (FWP, unpublished data). This well was down gradient from the lake, and drew water from the same aquifer that drained and fed the lake; however, no rotenone or associated constituents were detectable. FWP has monitored groundwater associated with several other rotenone projects, with wells ranging from 65 to 200 feet from the treated waters. Repeated sampling occurred within periods of up to 21 days, with no detectable concentrations of rotenone or the inert ingredients found.

3.6 Changes in the Diversity or Abundance of Aquatic or Semi-Aquatic Species

As discussed in 3.4 Toxicity, Persistence, and Fate of CFT Legumine and Its Inert Ingredients in Treated Waters, terrestrial species will not be negatively affected by rotenone. The low concentrations used in piscicide projects, rapid breakdown in the environment, exceptionally low toxicity from ingestion, and deactivation at the downstream end of projects makes exposure orders of magnitude lower than toxic levels. Moreover, the duration of exposure would be short, not chronic. Therefore, this section addresses toxicity to organisms with an aquatic life history phase, as these organisms are most likely to be affected by rotenone treatment.

3.6.1 Aquatic Invertebrates

Gilled aquatic invertebrates are nontarget organisms with considerable potential to experience negative effects from rotenone treatment. In streams, benthic populations of true flies, stoneflies, mayflies, and caddisflies are the primary affected taxa. Mayflies, stoneflies, and caddisflies are often grouped as EPTs, which is an abbreviation of the orders Ephemeroptera (E), Plecoptera (P), and Trichoptera (T), and refers to mayflies, stoneflies, and caddisflies respectively. Although individual taxa of EPT vary in tolerance to rotenone, as a group, EPTs are generally more sensitive than non-EPT taxa, such as true flies, aquatic worms, snails, and beetles, and their relative abundance and richness are commonly used measures of stream health.

Drawing general inference on the effects on aquatic invertebrates from the literature is challenging. Treatments in the scientific literature vary in terms of duration and concentration of rotenone. Moreover, investigations often fail to include information such as proximity of treated waters to a recolonization source, such as downstream drift, or dispersal by aerial adults. Sampling methodology often differs among studies, and inconsistency in reporting abundance and taxonomic resolution present other confounding factors.

Although differences in formulation, concentration, and duration of rotenone treatment complicate making robust predictions on the effects of rotenone on macroinvertebrates, the scientific literature allows for some generalizations. Investigations into the effects of rotenone on benthic organisms indicate that rotenone can result in temporary reduction of stream-dwelling invertebrates. In one case, no significant reduction in aquatic invertebrates occurred despite concentrations of rotenone being twice as high as the proposed maximum concentration (Houf and Campbell 1977). In other cases, invertebrates recovered quickly following treatment. For example, following piscicide treatment of a California stream, macroinvertebrates experienced an “explosive resurgence” in numbers, with black fly larvae recovering first, followed by mayflies and caddisflies within six weeks after treatment (Cook and Moore 1969). Stoneflies returned to pretreatment abundances by the following spring.

Another mitigative factor is that invertebrates that were most sensitive to rotenone also tended to have the highest rate of recolonization due to short life cycles (Engstrom-Heg et al. 1978). Although gill-respiring invertebrates are a sensitive group, many are far less sensitive to rotenone than fish (Schnick 1974; Chandler and Marking 1982; Finlayson et al. 2010). Due to their short life cycles (Anderson and Wallace 1984), strong dispersal ability (Pennack 1989), and generally high reproductive potential (Anderson and Wallace 1984), aquatic invertebrates are capable of rapid recovery from disturbance (Boulton et al. 1992; Matthaei et al. 1996).

A study of response of benthic invertebrates in streams in Montana and New Mexico is representative of concentration of CFT Legumine and duration of treatment used in current practice in Montana (Skorupski 2011). Notably, this research included comparisons to nontreated controls, so differences among sampling events resulting from natural variability and temporally consequential conditions, such as weather, could be evaluated statistically. In Cherry Creek and Specimen Creek, both in Montana, rotenone resulted in minimal effects on macroinvertebrates immediately after treatment, although potassium permanganate did influence benthic communities. Rotenone had a greater effect on benthos in streams in New Mexico. Regardless of the initial response, invertebrate communities recovered in all streams within a year.

A native species conservation project in Norway used CFT Legumine to kill all salmon in the watershed to eradicate a parasite that causes high mortality in salmonids (KJærstad et al. 2014). Unlike most piscicide projects in the western U. S., the species of fish targeted for removal was the same species that was intended to benefit from removal. The community was infected with a parasite that causes high mortality in salmonids, and the only way to eliminate the parasite was to remove the fish, as the parasite is short-lived without its salmonid host.

CFT Legumine was applied to maintain a minimum concentration of 0.5 ppm, which is lower than the 1 ppm typical of most piscicide projects in Montana. Duration of treatment was not reported. Like Skorupski (2011), these researchers also sampled untreated areas as controls. CFT Legumine was applied 3 times over a 2-year period, and water temperature varied seasonally with the April and October treatments measuring 4 °C and 8 °C respectively. Water temperature during the August treatment was 20 °C.

After treatment in cool waters in April and October, overall density of invertebrates was slightly depressed, but not significantly. The densities of a few sensitive taxa had decreased; however, these taxa were still present. The response of the macroinvertebrate community to treatment in August was considerably different, with a significant reduction in density, and many taxa remained absent from samples until several months after treatment. Nevertheless, most taxa had recolonized with a year. Warmer water temperature was attributed to the decreased abundance and reduction in richness following the treatment in August. In Montana, treatments usually occur in the fall, when water temperatures are relatively cool, as a mitigative measure to protect amphibians and aquatic invertebrates.

Mangum and Madrigal (1999) is a frequently cited study that evaluated the response of invertebrate population composition following application of rotenone in the Strawberry River watershed in Utah. In contrast to other researchers who reported small reductions in species richness and abundance, followed by rapid recovery of benthic communities (Cooke and Moore 1969; Houf and Campbell 1977; Skorupski 2011; KJærstad et al. 2014), Mangum and Madrigal (1999) reported statistically significant reduction in numbers of select taxa and putative "absence" of up to 8 taxa per sampling station after 5-years of yearly sampling. The disparity of results compared to researchers who found full recovery within a year may be explained by examining the concentration and duration of piscicide application, the validity of key assumptions, and rigor of the study design.

In the Strawberry River project, rotenone application was drastically more excessive than concentrations allowed in FWP's piscicide policy (FWP 2017). Application of rotenone was 150 ppb, which is substantially higher than the 25 to 50 ppb applied in piscicide projects in Montana. Moreover, the duration of treatment was 48 hours, compared to 4 to 8 hours that is required under Montana's piscicide policy (FWP 2017). Furthermore, this high concentration of rotenone, applied at an extremely long duration, was repeated 1 month later. Other studies examined here entailed a single treatment (Cooke and Moore 1969; Houf and Campbell 1977; Skorupski 2011), or evaluated response after subsequent treatments (KJærstad et al. 2014). Combined, these factors make the Strawberry River project profoundly different than piscicide projects implemented under FWP's protocols (FWP 2017) and other studies in the literature, in terms of intensity and frequency of exposure to rotenone.

Mangum and Madrigal's (1999) initial findings were like those reported by other researcher (Cook and Moore 1969; Skorupksi 2011; KJærstad et al. 2014), although reductions in EPT taxa were more pronounced. Invertebrate abundance was decreased in the first sample following treatment, but resurgence of midges, blackflies, crane flies, and aquatic worms, all early colonizers, occurred within 1 to 2 months. Richness of mayflies, stoneflies, and cadissflies was decreased after the first treatment by 45% to 82% for mayflies, 50% to 69% for stoneflies, and 30% to 75% for caddisflies. Mangum and Madrigal (1999) did not report abundance or richness of EPT taxa in subsequent sampling events, so it is not possible to determine if community richness or abundance recovered in the following years.

A limitation of this investigation is its unsupportable assumption that taxa "missing" from samples were missing from the stream. Sampling is not a census, and absence cannot be proven from a sample. The natural variability in distribution, abundance, and species presence in streams confounds assumptions of absence. Streams provide diversity in habitat complexity, and in the number of invertebrate species they support. Rarity of many taxa is common; however, streams can support several hundred species of aquatic invertebrate. Given the substantial potential for rarity, complexity of the habitat, patchiness in distribution, and seasonality of life history stages, no stream has had a census, or complete inventory, of all species present (Entrix 2010). A taxon missing from the sample, is not necessarily absent from the stream.

Mangum and Madrigal (1999) did not account for the natural among month or among year variability of species collected in streams, which is considerable. Monthly sampling of the same location Logan River for 10 years provides a case study of community composition dynamics across time (Vinson et al. 2010). Little variability in numbers of species or genera occurred among sampling events; however, the presence of individual genera or species showed considerable variability. Over 60 genera had been collected at this site; however, the number of individual genera captured regularly was about 40% of the total number of genera found cumulatively. The list of genera continued to grow, with a new one appearing about every 2 months. The genera accumulation curve had been increasing steadily, and showed no sign of flattening out. Mangum and Madrigal (1999) did not report species composition, richness, and abundance in any sampling event, so examination of variability among sampling events is not possible. They also did not report if new taxa appeared in samples, which research suggests is likely (Vinson et al. 2010).

The lack of an untreated control is another limitation in the Mangum and Madrigal (1999) investigation, is inconsistent with the scientific method, and does not allow for prediction based on their data. Without collecting macroinvertebrates in similar reaches that have not been treated, it is not possible to conclude with any certainty that absence of a taxon was the result of piscicide, and not related to natural variation among sampling events or resulting from natural variation in environmental conditions. Macroinvertebrate community composition is naturally stochastic over time. Combined with patchiness in distribution, the naturally random presence of some taxa makes the measure of species presence or absence in a sample an unreliable measure of the effects of rotenone. Current macroinvertebrate assessment protocols evaluate calculated metrics of abundance and richness of categories of invertebrates based on larger taxonomic groups, sensitivity to pollutants, life-span, and trophic function, as natural variability of species composition is considerable (Vinson et al. 2010). This approach controls for random variation in species composition and evaluates stream health on a community level.

Reporting and sampling methodology also confounds the ability to assume absence of a taxa, or draw conclusions with statistical certainty. Mangum and Madrigal (1999) state that the abundance of select taxa of invertebrate were statistically less in the years following piscicide treatment; however, they do not present data in narrative, tabular or graphical form, so critical review of these findings is not possible. Moreover, the assumption that Surber samplers yield a representative sample of invertebrates that captures all the taxa present is not supported by research. A power analysis to estimate the number of invertebrates with a statistical certainty within 5% of the mean, found nearly 450 Surber samples would be required (Chutter 1972). The tremendous variability in biomass among samples suggests similar variability in species collected, and limits the inference that is possible on the presumed absence of a taxon from 3 replicate Surber samples per site. Note that Skorupski (2011) used Surber samplers and traveling kick nets. The traveling kick net method covers the wetted perimeter from bank to bank, and therefore, covers more variability in microhabitats than Surber samplers, which sample discrete patches of streambed.

Given the great natural variability of taxa present among samples, and the highly biased sampling method, Mangum and Madrigal's assumption that absence of a taxon from a sample meant that it was missing from the stream is unsupportable. The Logan River study shows that the great variability among samples limits inference on taxa present. The putative missing taxa accounted for 10% or less of the baseline species present, and Mangum and Madrigal (1999) did not report their abundance in the pre-project samples, so the relative abundance in baseline sampling is unknown. Considering Vinson et al.'s (2010) findings, the presumed absence of 10% of taxa may be attributable to natural variability. Moreover, proving absence is impossible.

The recovery of macroinvertebrate communities reported by most researchers is the result of evolved mechanisms to persist in a disturbance driven ecosystem. Larval drift and reproduction by aerial adults are the primary mechanisms of recovery, and untreated, fishless headwaters provide a source of invertebrates drifting into reclaimed waters. Likewise, aerial adults flying upstream lay eggs and repopulate invertebrate communities. Proximity to adjacent sub-watershed populations further expedites this recovery. Moreover, macroinvertebrates are in a diverse array of life history stages, and recently emerged adults can reproduce soon after treatment. Observations on Lower Deer Creek documented a substantial hatch of caddisflies and midges the day following treatment of an area (C.L. Endicott, FWP, personal communication).

The well-established ability of macroinvertebrates to recover following disturbance, combined with the lower susceptibility of many taxa to rotenone, contributes to rapid recovery of invertebrate populations. Disturbance is a common occurrence in streams, and includes floods, wildfire, and human-caused alterations such as incompatible livestock grazing practices (Mihuc and Minshall 1995; Wohl and Carline 1996; Minshall 2003). These disturbances have greater potential to have long-term effects on stream-dwelling assemblages than rotenone treatments, given longer-term changes in geomorphology, impairment of riparian health and function, and reduced water quality. Rotenone treatment mimics a pulse disturbance, which is common in streams, and macroinvertebrates have evolved under this type of disturbance regime.

In conclusion, the weight of evidence, especially with reference to the more recent investigations that reflect current practice and have a study design with an untreated control (Skorupski 2011; KJærstad et al. 2014) indicates treatment of streams with CFT Legumine results in initial, minor reductions in abundance and richness of benthic invertebrates and recovery of populations within a year. Biomass recovers rapidly as early colonizers exploit available resources and have short-term relief from predation by fish or other invertebrates.

3.6.2 Zooplankton

Rotenone has greater initial effects on abundance and diversity of zooplankton than lotic invertebrates, given the longer period of exposure (Vinson et al. 2010). Recovery of zooplankton varies among taxa, with a dramatic bloom of early colonizers in the first couple of months (Anderson and Beal 1993). Other taxa take longer to recover, but the diversity and abundance can return within 6 months. Leaving dead fish within the lake likely provides the nutrients for recovery of lentic invertebrates, and 70 % of dead fish do not surface (Bradbury 1986).

Biomass of zooplankton recovers rapidly; however, zooplankton community composition can take from 1 week to 3 years to return to pretreatment conditions (Beal and Anderson 1993:

Vinson et al. 2010). Like stream-dwelling invertebrates, zooplankton have life history strategies that aid in rapid recolonization following disturbance (Havel and Shurin 2004). Many taxa are capable of asexual reproduction, which favors rapid recolonization from existing eggs and zooplanktors that survived treatment. Moreover, lakes have a long-term bank of dormant eggs that are resilient to a range of harsh conditions and provide many years of recruitment of zooplankton within a lake. In addition, wind, animals, and humans are primary agents of dispersal of dormant eggs. Numerous fishless lakes are within the project area, and these lakes would provide a nearby source of zooplankton to supplement the existing benthic egg bank.

In a Norwegian lake, the zooplankton were sampled before application of CFT Legumine in 2014, immediately after treatment, and 1-year post-treatment in 2015 (Amekleiv et al. 2015). CFT Legumine had an initial negative effect on zooplankton, with none being detected immediately after treatment. The relative abundance of species of zooplankton changed from pretreatment to 1-year post-treatment with some species comprising a much higher proportion of the zooplankton community. In addition, overall abundance of zooplankton increased considerably in 2015, compared to 2014. Removal of common roach (*Rutilus rutilus*), a species of minnow that preys on zooplankton, was attributed to greater biomass of zoonplankton in 2015, compared with pretreatment abundance in 2014.

3.6.3 Amphibians

Amphibians are closely associated with water, and have potential to be exposed to rotenone during treatment. Montana has several salamanders, toads, and frogs that need to be considered before rotenone application (Table 3-3). Information on reproductive ecology, habitat requirements, and life-history comes from descriptions and literature compiled in the Montana Natural Heritage Program's field guide (MNHP Field Guide: Amphibians) and species descriptions provided by Reichel and Flath (1995).

Table 3-3. Amphibians with potential to be exposed to rotenone in piscicide projects (from Montana Natural Heritage Program).

Order	Common Name	Scientific Name	Gilled Phase Coincide with late summer/early fall piscicide treatment	Status
Caudata/ salamanders	Idaho giant salamander	*Dicamptodon aterrimus*	Yes	G3[1], S2[2]
	Coeur d'Alene salamander	*Plethodon idahoensis*	No	G4[3], S2, sensitive (USFS)
	Long-toed salamander	*Ambystoma macrodactylum*	No	G5[4], S4[5]
	Western tiger salamander	*Ambystoma mavortium*	Yes, neotenic adults	G5, S4
Anura/toads and frogs	Boreal chorus frog	*Pseudacris maculata*	No	G5, S4
	Pacific tree frog	*Pseudacris regilla*	No	G5, S4
	Rocky Mountain tailed frog	*Ascaphus montanus*	Yes	G4, S4
	Columbia spotted frog	*Rana luteiventris*	Yes, at higher elevations	G4, S4
	Western toad	*Anaxyrus boreas*	Yes	G4, S2, sensitive (USFS and BLM)

[1] G3 = Globally the species is potentially at risk because of limited and/or declining numbers, range and/or habitat, even though it may be abundant in some areas.
[2]S2 = In Montana, at risk due to very limited and/or potentially declining population numbers, range and/or habitat, making vulnerable to extirpation.
[3]G4 = Globally, is apparently secure, although it may be rare in parts of its range, and/or suspected to be declining.
[4]G5 = Globally, the species is common, widespread, and abundant, although it may be rare in parts of its range. The species is not vulnerable in most of its range.
[5]S4 = In Montana, the species is apparently secure, although it may be rare in parts of its range, and/or suspected to be declining.

The potential to be exposed to rotenone varies by species. The Idaho giant salamander is present in streams and rivers, and occasionally ponds and lakes, in larval form, or as gill-retaining neotenic adults (Adam Sepulveda, U.S. Geological Service, personal communication) and is the only salamander in Montana that rears in streams. Terrestrial metamorphic adults are rarely seen, and occupy lotic and lentic waters only while breeding. Information on their reproductive ecology and longevity is limited, so evaluating the potential for piscicide projects to have population level effects is difficult to predict. Their vulnerability to rotenone is unknown;

however, as gilled life stages occupy water, rotenone would likely result in mortality. Otherwise, little information is available on fecundity and longevity; however, terrestrial adults would likely be present to replace lost year classes. The Idaho giant salamander has extremely limited distribution in Montana, and piscicide projects in these waters should determine their presence, and pre-project monitoring should determine whether waters slated to be treated are occupied habitat.

Couer d'Alene salamanders are present in western Montana and are restricted to cool, moist environments. Eggs are terrestrial, and larvae do not have an aquatic phase. As adults, these salamanders occupy springs, seeps, waterfall spray zones, stream edges, and talus far from running or standing water. Adults occupying stream edges and treated seeps within a piscicide project area would potentially be exposed to rotenone. Although their vulnerability to rotenone is unknown, other adult amphibians do not experience acute toxicity from exposure to rotenone at concentrations used in fish eradication projects (Grisak et al; 2007; Billman et al. 2011). Moreover, Couer d'Alene salamanders are relatively long-lived, and do not become sexually mature until 3.5 years for males and 4.5 years for females, so other year classes occupying nontreated areas may recolonize treatment areas. Nevertheless, piscicide projects occurring within the range of Couer d'Alene salamanders should determine if this species has potential for exposure. If so, bioassays should be conducted to determine their sensitivity to rotenone, and potential mitigative actions should be evaluated if rotenone exposure is likely to have long-term population level effects on this species.

Long-toed salamanders occupy portions of western Montana east and west of the Continental Divide. This species is unlikely to experience long-term population effects of piscicide treatment. Long-toed salamanders usually lay eggs in fishless ponds or lakes, which would not be treated with rotenone. Even so, larval long-toed salamanders were 5 times more tolerant to Prenfish, a formulation of rotenone using organic solvents and dispersants, than fish, and adult long-toed salamanders survived 96-hour exposure to treatment concentrations of Prenfish used in piscicide projects (Grisak et al. 2007). Adult long-toed salamanders are terrestrial, and breed immediately after snowmelt, they would not be present for fall application of piscicide. The combination of preference for fishless lakes for breeding and terrestrial existence as adults make long-toed salamanders unlikely to be affected by piscicide treatments. In cases where this species breeds in fish-bearing lakes, piscicide treatment may result in the loss or reduction of a year class; however, breeding in following seasons would allow the population to recover.

In mountain lakes, western tiger salamanders are present as gill-bearing adults, or axolotls. At lower elevations, western tiger salamanders exist as terrestrial adults, gilled larvae, and neotenic adults. Little information is available on toxicity of rotenone to western salamanders, although larval salamanders were presumed to be as vulnerable to rotenone as fish (Maxell and Hokit 1999). Nevertheless, observations of substantial numbers of neotenic forms in a reservoir a year after rotenone achieved eradication of fish suggests some resilience to rotenone (Jim Olsen, FWP personal communication). Moreover, western tiger salamanders are resilient to loss of a year class (Bryce Maxell, MNHP, personal communication). Frequently, the older year class of western tiger salamander larvae will cannibalize the newer generation. This strategy ensures the success of the older year class, resulting in staggered year class success.

Clearly, insufficient information is available to draw strong conclusions on the potential for western tiger salamanders to be negatively affected by rotenone treatment. Should native fish conservation projects be considered in waters supporting larval or neotenic western tiger salamanders, bioassays should be performed to evaluate their response to rotenone exposure. Projects should proceed if no long-term population level effects are expected based on tolerance to rotenone, existence of life-history strategies that allow for recovery, or when mitigative actions prevent long-term effects on western tiger salamander populations.

Like gill-bearing aquatic macroinvertebrates, frog and toad larvae are sensitive to rotenone, and exposure to rotenone at levels used to kill fish is acutely toxic to Columbian spotted frog larvae, Rocky Mountain tailed frog larvae, and western toad larvae (Grisak et al. 2007; Billman et al. 2012). Although tadpoles may be vulnerable to rotenone, at least some species may be up to 10 times more tolerant than fish (Chandler and Marking 1982). Treatment in late summer or early fall is a recommended practice to prevent effects on frogs and toads, as many are past the gilled life history stage (Grisak et al. 2007). In the short-term, this practice may not be protective of species that remain as gilled larvae for more than 1 year, or at high elevations, where delay in the breeding season and low temperatures delay metamorphosis. Nevertheless, toads and frogs have considerable potential to recover from this short-term disturbance.

Rocky Mountain tailed frogs are the most tied to water of all the frogs and would likely experience short-term and minor effects from treatment with rotenone. Their reproductive strategy is to mate in August to September, and store the sperm overwinter. Eggs are oviposited the next spring, and metamorphosis occurs up to 4 years later. Therefore, at least 1 year class of tadpoles would be exposed to rotenone, with 2 or more exposures being possible. Nevertheless, their life history strategies make Rocky Mountain tailed frogs resilient to rotenone treatment. Rocky Mountain tailed frogs are a long-lived species, and do not reach reproductive maturity until age 7 or 8. This species would be resilient to rotenone treatment because many older year classes would survive, and treatment concentrations of rotenone do not have an adverse effect on adults (Grisak et al. 2007).

Rocky Mountain tailed frogs are relatively fecund and lay up to 170 eggs. In the spring following rotenone treatment, numerous age classes of adults would be present to oviposit eggs in streams and lakes. When treatments have ceased, Rocky Mountain tailed frogs would have a short-term advantage, as tadpoles would experience little to no predation by fish, until fish populations recover within treated streams. Field observations suggest Rocky Mountain tailed frogs are resilient to rotenone projects. Moreover, this species can disperse downstream, from untreated reaches into treated reaches. A field study in alpine lakes found no significant effect on Rocky Mountain tailed frogs in 2 to 4 years of monitoring following a rotenone treatment (Fried et al. in press), suggesting rotenone treatment would have short-term and minor effects on this species.

Effects on other adult amphibians are insignificant given their low vulnerability to rotenone because of loss of gills, development of lungs, maturation of liver function, mobility, and project timing. Adult Columbian spotted frogs do not suffer an acute response to trout killing concentrations of Prenfish, another commonly used formulation of rotenone that includes organic compounds (Grisak et al. 2007). Piscicide treatments are acutely toxic to gilled tadpoles, but not metamorphs or juveniles (Billman et al. 2011). Columbian spotted frogs breed in mid-April to early June and metamorphose about 60 days after hatching. Vulnerable populations of Columbia spotted frogs are those at tree-line, or elevations above 6,500 to 7,000 feet. These populations are temperature limited, and will remained as gilled tadpoles throughout the winter (Bryce Maxell, Montana Natural Heritage Program, personal communication).

Even if a year class of tadpoles is exposed to lethal concentrations of rotenone, other life-history traits make this species resilient to piscicide projects. Columbian spotted frogs are a relatively long-lived species, and do not reach sexual maturity for 4 to 6 years, depending on sex. The presence of several older year classes means there is a continued source of recruitment. Furthermore, Columbian spotted frogs are relatively fecund and lay egg clusters of 300 to 800 eggs. Field investigation confirms with reproductive capacity of this species, with a substantial rebound of Columbian spotted frog larvae 1 year after piscicide treatment resulted in near total mortality of tadpoles (Billman et al. 2012)

Western toads show the same life stage sensitivity to rotenone, with tadpoles suffering near total mortality to exposure to concentrations of rotenone used in current practice, but resilience to rotenone as metamorphs through adults (Billman et al. 2011). Moreover, adult western toads are likely less sensitive than frogs, given their impermeable skin (Maxell and Hokit 1999). Likewise, adult toads and frogs can leave the aquatic environment, which substantially reduces the potential for exposure (Maxell and Hokit 1999).

Western toads have various characteristics that make them resilient to piscicide projects. Western toads have exceptional fecundity, documentation of egg clutches averaging 5,000 in Colorado, and reaching 16,000 in Montana and 20,000 in the Pacific Northwest. Development from hatching to metamorphosis is related to temperature and can be rapid; however, populations at tree line may fail to metamorphose, and these populations may rely on immigration from lower elevations to persist.

Variability of tolerance to rotenone among species of toad and frog is unknown; however, evidence for resilience to rotenone of other species suggests a general tolerance is possible. A study in Norway examined the response of lake-dwelling amphibians, the common frog (*Rana temoraria*) and common toad (*Bufo bufo*), to treatment with CFT Legumine (Amekleiv et al. 2015). These species were observed before and 1 year after treatment with rotenone, with adults, eggs, and tadpoles being present following treatment. They concluded CFT Legumine had little effect on these species.

3.7 Compatibility of Wilderness Values with Rotenone Treatment in Lakes and Streams

Chemical removal shares some of the same conditions that may affect wilderness values and disturb the wilderness experience with mechanical removal. The presence of field-workers, horses and helicopters transporting gear occurs with both scenarios. However, piscicide is more efficient, and requires fewer treatments, and in most cases, is completed over the course of fewer years. Conditions that increase the number of years of treatment include complex habitat, including beaver ponds and large woody debris jams. Mechanical removal is not feasible under these conditions.

Release of a toxic chemical into wilderness waters, and killing all the existing fish, and some of the invertebrates and amphibians, is a substantial human imprint. Moreover, piscicide is objectionable to some members of the public, not only in designated wilderness, but in principle. Fisheries managers need to weigh all options, and consider the status of declining native fish species, and their potential for long-term persistence within their historical range. Informed decision-making should account for the duration of the disturbance to aquatic communities, as well as the long-term benefits to native fish conservation, especially species of concern, or species listed as threatened or endangered under the Endangered Species Act.

Mortality of nontarget organisms alters wilderness character and is another factor that needs to be considered in planning piscicide projects. Pre-project sampling of invertebrates and amphibians provides a baseline of pretreatment communities, and allows for detection of species of concern. Development of environmental assessments required through the Montana Environmental Policy Act and the National Environmental Policy Act, along with additional evaluations specific to designated wilderness, will assist in the decision-making process, and evaluate whether the negative effects of short-term disturbance outweighs the long-term conservation benefits, especially with federally listed species. Nevertheless, the weight of scientific evidence indicates nontarget aquatic invertebrates recover within 1-year post-treatment, and amphibians are likewise resilient to rotenone treatment. The primary decision is a balance between long-term persistence of an imperiled fish species versus short-term changes in aquatic invertebrate communities.

The presence of dead fish has potential to diminish the enjoyment of designated wilderness and increase probability for conflicts with scavenging bears; however, dead fish would be present for a few days, and most fish would likely never be encountered. Even without scavenging, fish decompose rapidly in lakes and streams, with microbial action being obvious within a few hours. Nevertheless, retrieving dead fish along trails, and sinking dead fish in lakes by puncturing their air bladders would substantially limit the potential for dead fish to reduce the quality of the wilderness experience or increase conflicts with wildlife.

Finally, projects that restore or conserve native fish is consistent with wilderness character, as these would be the species that evolved in the area. Nonnative rainbow trout, brook trout, and brown trout, as well as hybrids, detract from the ecological heritage and potential of designated wilderness.

4 Conclusions

Chemical and mechanical methods to remove nonnative fishes are both viable options in management of native fishes, although mechanical removal is infeasible for removal in streams greater than 2 miles in length, or where complex woody, overhanging vegetation, and beaver dams increase habitat complexity. Each brings a level of disturbance that may negatively affect wilderness values. Conversely, restoring the native species to a stream increases its biological integrity and is consistent with the intent of the Wilderness Act of 1964.

Mechanical removal is feasible in relatively short reaches of stream, or small isolated lakes. Mechanical removal in stream reaches greater than 3 miles has not been attained. Mechanical removal is infeasible in large project areas that encompass many more miles of stream with complex habitat.

Mechanical and chemical removal of fish requires the presence or construction of a barrier to prevent reinvasion of nonnative species. Should mechanical removal be attempted in a larger watershed, temporary barriers would need to be constructed as fish are removed from headwaters

Mechanical removal requires considerably more labor than chemical removal in streams with complex habitat. Mechanical removal is often not effective without removal of streamside vegetation and woody debris, which requires crews using chainsaws. Furthermore, the number of removal events are considerably more numerous, and cover more years than chemical removal. As a result, humans are present in the watershed for more days per year. Moreover, even where feasible, mechanical removal typically takes 4 or more years to achieve eradication.

Removal of streamside vegetation and woody debris results in changes in the trophic functioning of streams, increases water temperature, and decreases channel stability. With recovery of the woody canopy, trophic level composition of macroinvertebrates and water temperatures likely return to pretreatment conditions within a few years. Removal of large woody debris and complex woody debris takes considerably longer, as it requires trees to die and fall across or into streams.

Disturbance associated with chemical removal varies with the size of project area and includes introduction of a toxic substance to surface waters, presence of field-workers attending drip stations, and potentially motorized use for delivery and application of piscicide. Typically, a single treatment lasts 4 to 8 hours, with additional treatments possible in subsequent years, if a full fish kill is not attained in the first year. Field-workers trample streamside vegetation while walking to and from drip stations, although vegetation would recover quickly from this disturbance. Most or all fish, and an unknown proportion of the invertebrate community, die from exposure to rotenone. This disturbance is short-term, as fish are restocked using the best available source. Macroinvertebrate communities recover from invertebrates that are not vulnerable to rotenone, larvae drifting from untreated headwaters reaches and dispersal of aerial adults. With application of best management practices, amphibians are resistant to rotenone, or recover through reproduction or recolonization the following spring.

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