Annual Report of the Hungry Horse Mitigation Program

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I. Executive Summary

In 1991, Montana Fish Wildlife & Parks (FWP) and the Confederated Salish and Kootenai Tribes (CSKT) published the Fisheries Mitigation Plan for Losses Attributable to the Construction and Operation of Hungry Horse Dam (FWP and CSKT 1991). This Mitigation Plan presented fisheries losses, mitigation options, and recommendations to protect, mitigate, and enhance resident fish and aquatic habitat impacted by the construction and operation of Hungry Horse Dam. The Northwest Power and Conservation Council (Council; formerly the Northwest Power Planning Council) approved the loss statement: including annual losses of 250,000 juvenile Bull Trout (*Salvelinus confluentus*) and 65,000 juvenile Westslope Cutthroat Trout (*Oncorhynchus clarkii lewisi*), plus 124 km of critical, low gradient spawning and rearing habitat that was inundated and lost subsequent to the filling of Hungry Horse Reservoir. The Council then directed FWP and CSKT to immediately develop an Implementation Plan, which was adopted for Hungry Horse Dam (FWP and CSKT 1993). On-the-ground mitigation activities began in 1992. This project is one of a few federally funded, Columbia River mitigation projects carrying out a plan to offset a Council-adopted loss statement. Fisheries losses were to be offset by modifying dam operations, restoring or reconnecting habitat, reducing negative non-native species interactions, and implementing hatchery (native fish conservation) technology and offsite mitigation.

The Council subsequently adopted the Flathead Subbasin Plan in 2004 (CSKT and FWP 2004). The federal action agency's 4-H plan is designed to recover Columbia River fish species listed as threatened or endangered under the Endangered Species Act (Federal Caucus 2000). Our mitigation program is directed by a similar scientific framework to offset fisheries losses at various spatial scales, descending from basin-wide mitigation requirements to site-specific actions. Mitigation projects are selected and prioritized based on decision pathways described in the Flathead Subbasin Plan (2004), and specific objectives and tasks are described in detail in the statements of work within contracts 76916 REL 21 and 76916 REL 22. This project will focus on improving conditions for native fish survival and recovery in the upper Flathead River and Lake system. From January through December 2022 our work focused on assessing population level effects of dam operations on native fishes, implementing habitat improvement and fish passage projects, and quantifying and mitigating deleterious effects of non-native aquatic species on native fishes. Specific project descriptions and their objectives are summarized herein.

II. Work Elements / Tasks

A. South Fork Flathead Drainage Westslope Cutthroat Trout Conservation Program

1/1/2023-12/31/2023 76916 REL 22 C, 84064 REL 3 C: Sampling and assays for South Fork WCT project
1/1/2023-12/31/2023 76916 REL 22 D, 84064 REL 3 D: Data analysis for South Fork WCT project
1/1/2023-12/31/2023 76916 REL 22 E, 84064 REL 3 D: Local source WCT collections for Sekokini Springs rearing facility

These Work Elements are associated with Fish Population RM&E, Hydrosystem RM&E, Tributary Habitat RM&E, Tributary Habitat Restoration and Protection, Hatchery Production and Operations, and Predator Control and Management.

Introduction

The South Fork Flathead River drainage comprises greater than half of the remaining interconnected habitat for westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) within this species' historic range. However, long-term persistence of this native species is threatened by hybridization with nonnative rainbow trout (*O. mykiss*) and Yellowstone cutthroat trout (*O. c. bouvieri*) that were stocked in many historically fishless headwater lakes in the South Fork drainage. In an effort to minimize the spread of hybridization, Montana Fish, Wildlife, and Parks developed the South Fork Flathead Drainage Westslope Cutthroat Trout Conservation Program. The objective of this multi-year project is to remove sources of nonnative trout in 21 lakes and reestablish these fisheries with westslope cutthroat trout.

Genetic data from throughout the native range of westslope cutthroat trout indicate that substantial genetic differences exist among populations of this species. The population genetic structure of a species is the result of both random events and natural selection for traits that confer a fitness advantage for individuals in particular environments. Conservation of genetic variation is crucial for long-term persistence of a species and, in the case of westslope cutthroat trout, requires ensuring the continued existence of many populations throughout its range.

Since substantial genetic differences exist among westslope cutthroat trout populations in the South Fork drainage, introduction of westslope cutthroat trout from a single brood source has the potential to homogenize genetic variation and may disrupt important local adaptations within aboriginal populations. From a conservation genetics perspective, the ideal approach would be to use multiple within-drainage stocks for restoration efforts. During the public scoping process for the Environmental Impact Statement (FWP 2005), the development and use of within-drainage stocks of westslope cutthroat trout was identified as a desirable management action to conserve unique and, presumably, locally adapted westslope cutthroat trout populations in the South Fork Flathead drainage. Furthermore, the Memorandum of Understanding and Conservation Agreement for westslope cutthroat trout in Montana recommends that locally adapted, genetically pure populations be maintained (FWP 1999, 2007).

In an effort to conserve genetic variation among westslope cutthroat trout populations, FWP developed the Sekokini Springs Research and Isolation Facility to raise wild westslope cutthroat trout and create short-term within-drainage broodstocks for restoration in the South Fork Flathead drainage. Danaher and Youngs creeks, located in the Bob Marshall Wilderness at the headwaters of the South Fork Flathead River, were selected as the first two donor populations because they contain high densities of non-hybridized, disease-free westslope cutthroat trout. Sullivan and Quintonkon creeks, tributaries to Hungry Horse reservoir, were selected to establish the third donor population.

Methods

FWP aquatic invertebrate sampling
http://www.monitoringmethods.org/Protocol/Details/727

In 2023 staff collected 18 benthic macroinvertebrate (BMI) samples from two sites in Martin creek; the Road 910 culvert site and the Detox site. The sampling is for the ongoing monitoring of the response and recovery of invertebrate communities after fish removal using the piscicide rotenone. Martin creek was treated with rotenone in 2020 and 2021 to remove hybrid trout. Samples were collected two years before treatment and annually post-treatment to monitor the BMI community's recovery to pre-treatment relative abundance (density) and taxa richness (diversity). Sampling was conducted each year in July, August, and September to capture temporal variations in taxa richness and density. Post-treatment monitoring was conducted in 2021, 2022, and 2023. BMI samples were collected from stream riffle habitats using a 0.09 m^2 Surber stream bottom sampler with 500-micron mesh, and each sample consists of 2 Surber plots (0.18 m^2). The samples were fixed with 95% ethyl alcohol and transported back to the lab for sorting and identification. Specimens were identified primarily to Genus (Merrit & Cummins & Berg, 2008; Wiggins, 1996; Stewart & Stark, 1993; Brown, 1976, and Pennak, 1989). Density was calculated as the total number of specimens per 0.18 m^2, and diversity calculated as the total number of taxa per 0.18 m^2.

To evaluate the effects of rotenone on BMI, we analyzed changes in mean density and diversity between sample years for each sample site using a Kruskal–Wallis nonparametric analysis of variance (ANOVA), followed by a post hoc Dunn Test for pairwise multiple comparisons. The criteria used to conclude that the BMI community is recovered is no significant change in mean density and diversity (alpha > 0.05) any year after the second treatment when compared to pre-treatment mean. The data analyses are reported in the results section below. All statistics and figures were generated using R version 4.3.3 (R Core Team 2024).

Results of Aquatic Invertebrate Sampling

The analysis of impacts of rotenone on the BMI community in Martin creek focused on the changes in EPT (Ephemeroptera, Plecoptera, Trichoptera) mean density and diversity. The EPT community is considered the most sensitive to changes in water quality (Mitchell & Stapp 1997), thereby analyzing EPT data to detect changes in density and diversity is a reasonable measure for assessing the effects to the BMI community from rotenone. The number of BMI samples collected at each site and number of years sampled are in table A1a, and ANOVA p-values for changes in mean EPT density and diversity are in table A1b. Boxplots in Figures A1 and A2 illustrate changes in EPT density and diversity pre- and post-treatment at each sample site.

Table A1a. Number of BMI samples collected at each sample site and number of years sampled pre- and post-treatment.

Sample Site	No. Samples Pre-Treatment	No. Years Sampled Pre	No. Samples Post-Treatment	No. Years Sampled Post
Road 910	18	2	27	3
Detox site	9	1	27	3

Table A1b. ANOVA p-values for changes in mean EPT density and diversity pre- post-treatment at each sample site.

Sample Site	EPT Density			EPT Diversity		
	χ^2	*df*	*P*	χ^2	*df*	*P*
Road 910	13.6	4	**0.009**	18.8	4	**0.0009**
Detox site	4.0	3	0.261	15.7	3	**0.001**

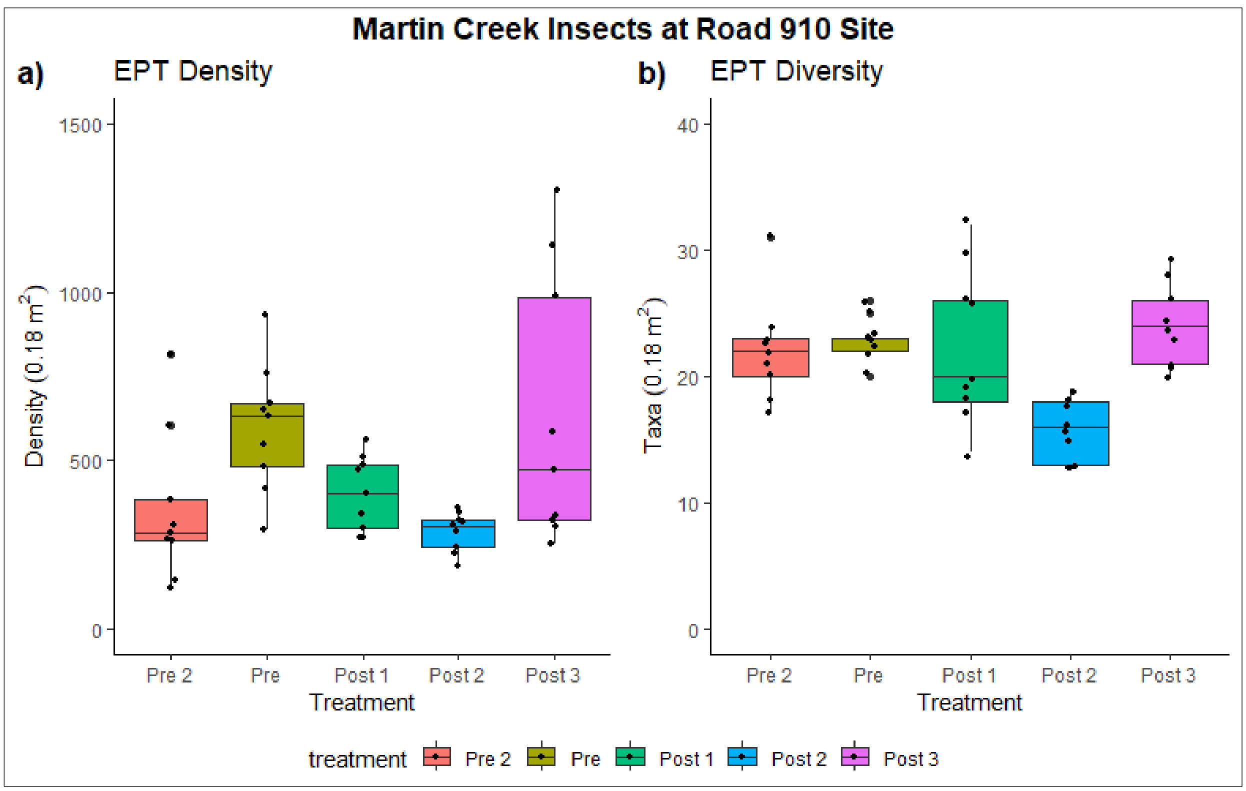

Figure A1. Boxplots of EPT density and diversity pre- and post-treatment for Martin creek at Road 910 sample site.

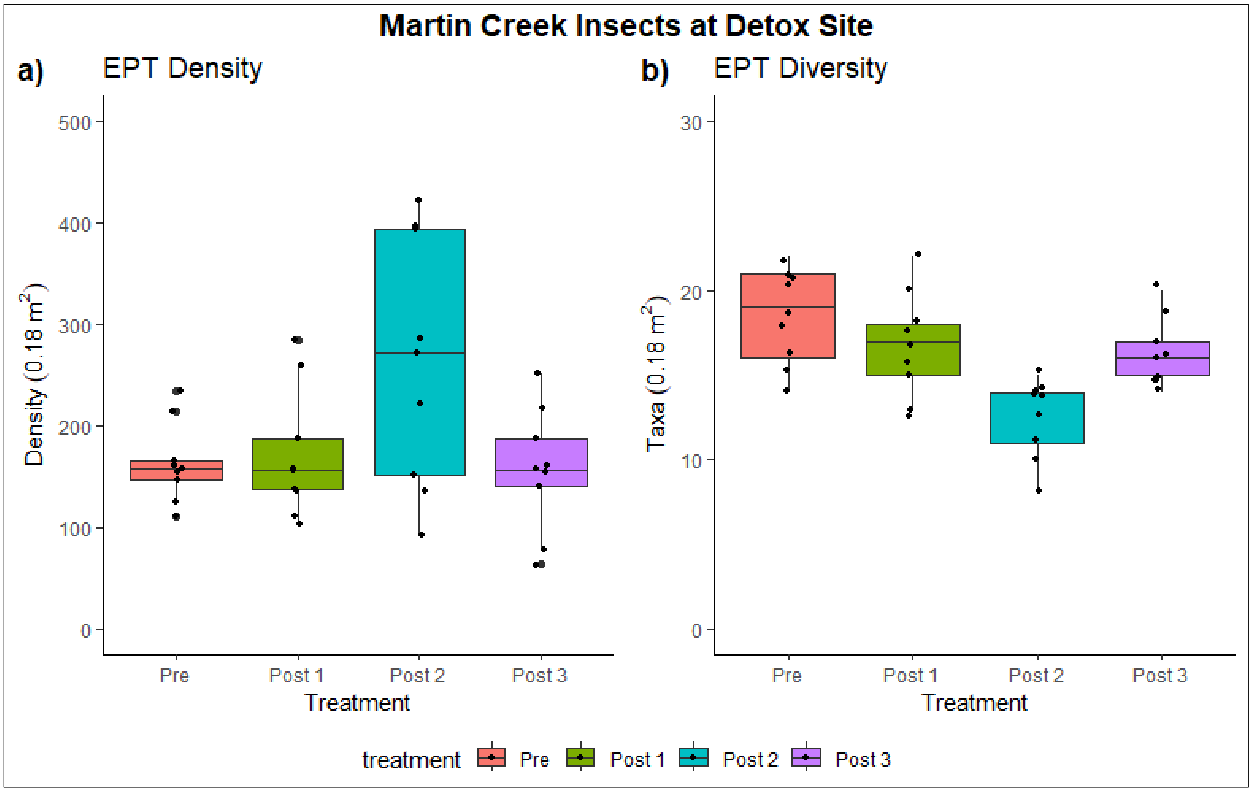

Figure A2. Boxplots of EPT density and diversity pre- and post-treatment for Martin creek at Detox sample site.

Based on the ANOVA results and the post hoc Dunn Test, EPT diversity at both sites 1 year after the second treatment (post 2) showed significant decreases compared to pre-treatment diversity: Road 910 site, figure A1b, ($p = 0.0009$), and Detox site, figure A2b, ($p = 0.001$). EPT density at the Road 910 site, figure A1a, increased between pre-treatment years and then significantly decreased after the second treatment ($p = 0.009$). EPT diversity at both sites increased 2 years after the second treatment (post 3). Based on the Dunn Test, EPT density and diversity at both sites 2 years after the second treatment (post 3) showed no significant change when compared to pretreatment levels. Thus, based on the criteria that there is no significant change in density and diversity any year after the second treatment when compared to pre-treatment mean, we conclude that the BMI community in Martin Creek is recovered.

FWP wild trout collection
http://www.monitoringmethods.org/Protocol/Details/734

Genetic data from throughout the native range of westslope cutthroat trout indicate that substantial genetic differences exist among populations of this species. The population genetic structure of a species is the result of both random events and natural selection for traits that confer a fitness advantage for individuals in particular environments. Conservation of genetic variation is crucial for long-term persistence of a species and, in the case of westslope cutthroat trout, requires ensuring the continued existence of many populations throughout its range. Since substantial genetic differences exist among westslope cutthroat trout populations in the South Fork drainage, introduction of westslope cutthroat trout from a single brood source has the potential to homogenize genetic variation and may disrupt important local adaptations within aboriginal populations. From a conservation genetics perspective, the ideal approach would be to use multiple within-drainage stocks for restoration efforts. During the public scoping process for the Environmental Impact Statement (FWP 2005), the development and use of within-drainage stocks of westslope cutthroat trout was identified as a desirable management action to conserve unique and, presumably, locally adapted westslope cutthroat trout populations in the South Fork Flathead drainage. Furthermore, the Memorandum of Understanding and Conservation Agreement for westslope cutthroat trout in Montana recommends that locally adapted, genetically pure populations be maintained (FWP 1999, 2007).

In 2022, staff collected conservation genetic data from stocks for restocking alpine lakes and new donor populations. See results below.

Sheppard Creek
In the sample from Sheppard Creek we did not detect any rainbow trout or Yellowstone cutthroat trout alleles, and none of the westslope diagnostic markers were polymorphic. These data strongly suggest that Sheppard Creek harbors non-hybridized westslope cutthroat trout and are consistent with past samples that similarly did not detect any non-native ancestry in fish from this section of the stream (#4476, 4961). Previous estimates of genetic variation demonstrate that fish from Sheppard Creek have low to moderate genetic variation relative to other westslope populations in Montana.

Good Creek
We detected two non-westslope alleles at one westslope diagnostic marker in the sample from Good Creek (translocated to Martin Creek). These results are somewhat challenging to interpret; on the one hand it is plausible that the observed variation is actually westslope cutthroat trout genetic variation or genotyping error (lab artifacts), but it is also quite plausible that there is a very small amount (0.1%) of non-native ancestry in fish from Good Creek. The latter scenario is certainly possible given that we used additional diagnostic markers relative to past samples, even those from 2020 (#5279). Additional genomic data would be needed to make any further statements about the genetic status of the fish that were translocated from Good Creek to Martin Creek. Previous estimates of genetic variation demonstrate that fish from Good Creek have high genetic variation.

Chain Lake #1
In the sample from Chain Lake #1 we detected two rainbow trout alleles, and one Yellowstone cutthroat trout alleles, all in one individual. All the other individuals in the sample appeared to be non-hybridized westslope cutthroat trout. With so few non-native alleles it is difficult to determine whether the sample was collected from a hybrid swarm, or alternatively, if there are a few hybrids intermixed with non-hybridized westslope. The data generally suggest the latter, but we cannot exclude the former. In fact, the previous sample from Chain Lake #1 did appear to resemble a hybrid swarm with a very small amount of rainbow trout ancestry (#5270).

Importantly, at least some fish in Chain Lake #1 also appear to have a very small amount of Yellowstone cutthroat trout ancestry. It should be noted that the non-native alleles present in Chain Lake are not surprising given that recent brood sources used to stock the lake also had small amounts of non-native ancestry.

South Fork drainage mountain lakes

The goal of the WCT conservation project is to restore and protect the native trout fishery in the entire South Fork Flathead River drainage. Twenty-one lakes in the headwaters of several drainages in the South Fork were identified as being sources of nonnative and hybridized trout which were dispersing downstream. These lakes and their associated stream networks were scheduled for piscicide treatment to eradicate the current fishery in order to restock with native WCT. Fifteen lakes have been treated with the piscicide rotenone, and six lakes are receiving genetic swamping as an alternative to piscicide treatment (Figure A4).

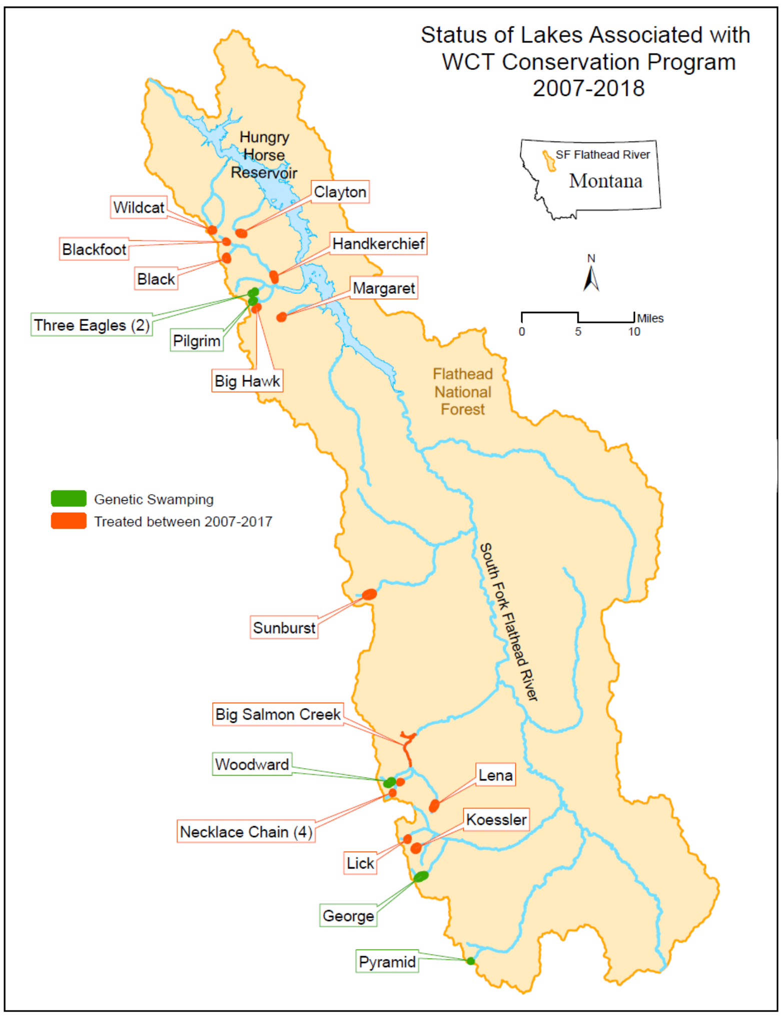

Figure A4. Status of South Fork drainage mountain lakes associated with the WCT Conservation Program.

Investigations into the Pygmy Whitefish in Northwest Montana

Introduction

The genus *Prosopium* contains six species of freshwater whitefish: Bear Lake Whitefish *P. abyssicola*, Bonneville Whitefish *P. spilonotus*, Bonneville Cisco *P. gemmifer,* Mountain Whitefish *P. williamsoni,* Pygmy Whitefish *P. coulterii,* and Round Whitefish *P. cylindraceum*. Of these, the former three are all endemic to Bear Lake on the Idaho-Utah border while Mountain, Pygmy, and Round Whitefish are all found throughout North America. The distribution of the Pygmy Whitefish is associated with glacial refugia (McPhail and Lindsey 1970) and they are primarily found below 100 feet in coldwater lakes (Sullivan and Mackay 2011; MNHP 2020). Pygmy Whitefish are found in the lakes of the Northern Rockies, western Washington, Lake Superior, several southern Alaska basins, the Canadian Arctic, and Ekityki Lake, Russia (Chereshnev and Skopets 1992; Hallock and Mongillo 1998). Such a distribution is likely the consequence of widespread migration during the repeated glaciations of the Pleistocene, approximately 2.6 M – 12,000 years ago (McPhail and Lindsey 1970). Indeed, Alaskan and Pacific (British Columbia, Washington, Idaho, and Montana) populations are highly genetically divergent, a grouping consistent with relict populations being associated with the Beringia and Cascadia glacial refugia (Witt et al. 2011).

The first record of Pygmy Whitefish in Montana was made by Leonard Schultz- University of Washington- in tributaries of Lake McDonald, Glacier National Park (Schulz 1941) and then by George Weisel- University of Montana- from Bull Lake, in the Kootenai drainage (Weisel and Dillon 1954). Since then, 13 more populations have been identified in the state (Table 1). Of these populations, only those in Bull and Flathead Lakes have been examined in any depth; the morphology, reproductive age, and diet of Pygmy Whitefish in both lakes was well described in early investigations (Weisel and Dillon 1954; Hanzel 1970, 1972a, 1972b, 1974; Weisel et al. 1973).

Table 1. Waterbodies with known Pygmy Whitefish observations in MT.

Waterbody Name	Watershed	Approx. Depth
Bull Lake	Middle Kootenai	60 ft
Horseshoe Lake	Fisher	130 ft
Little Bitterroot Lake	Lower Flathead	260 ft
Ashley Lake	Flathead Lake	200 ft
Flathead Lake	Flathead Lake	370 ft
Whitefish Lake	Stillwater	230 ft
Lake McDonald	Middle Fork Flathead	470 ft
Hungry Horse Reservoir	South Fork Flathead	490 ft

Swan Lake	Swan	130 ft
Lindbergh Lake	Swan	120 ft
Holland Lake	Swan	140 ft
Ross Creek	Middle Kootenai	--
Flathead River	Flathead Lake	--
Ashley Creek	Flathead Lake	--

2023 OBJECTIVES: Conduct sampling for Pygmy Whitefish.

Employees with Montana Fish, Wildlife & Parks and the National Park Service placed deep water gill nets in northwestern Montana lakes that may contain Pygmy Whitefish (Table 2). These lakes were chosen due to appropriate maximum depths, being feasible to access during summer/fall, and via consultation with the responsible biologists. In each lake, two to four sinking multi-filament gill nets consisting of 3/8” and 1/2” mesh were set overnight at varying depths and the results are reported in (Table 2).

Table 2. Waterbodies sampled for Pygmy Whitefish in 2023.

Waterbody Name	Watershed	Sample Size
Kintla Lake	North Fork Flathead	12
Bowman Lake	North Fork Flathead	6
St. Mary Lake	Lower Flathead River	12
Lake McDonald	Middle Fork Flathead River	11
Swan Lake	Swan River	30
Lindbergh Lake	Swan River	20
Holland Lake	Swan River	35
Big Salmon Lake	South Fork Flathead River	30
Lake Blaine	Flathead River	0

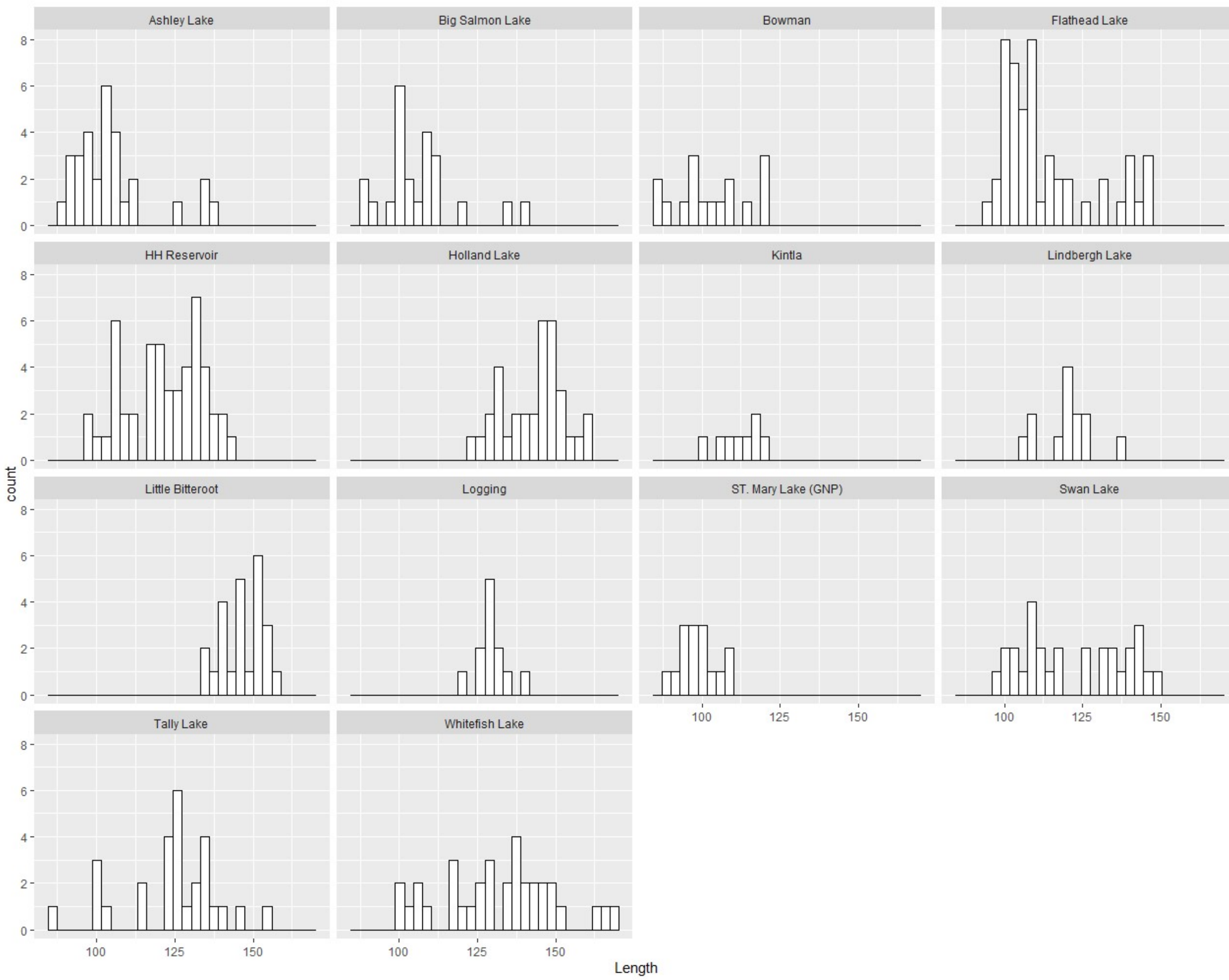

Figure 1. Length frequency results from Pygmy Whitefish gill netting (2021-2023).

Table 3. Indices of genetic diversity per population.

Population	Num	Eff_num	Ho	Hs
Tally Lake	6.333	3.984	0.611	0.595
Whitefish Lake	8.444	5.389	0.707	0.669
Ashley Lake	7.333	4.322	0.658	0.612
Hungry Horse Res.	7.556	3.836	0.567	0.572
Little Bitterroot Lake	7.556	4.942	0.675	0.644
Flathead Lake	9.222	5.215	0.68	0.668
St. Mary Lake_(_GNP)	2	1.265	0.2	0.163

Statistic	Description
Num	Number of alleles
Eff_num	Effective number of alleles
Ho	Observed Heterozygosity
Hs	Heterozygosity Within Populations

Table 4. Results of genetic differentiation among Pygmy Whitefish Populations.

Fst Statistic Table						
Populations	Tally	Whitefish	Ashley	Hungry Horse	Little Bitterroot	Flathead
Whitefish	0.0301					
Ashley	0.1767	0.1558				
Hungry Horse	0.1128	0.0413	0.2026			
Little Bitterroot	0.1117	0.1096	0.0453	0.1171		
Flathead	0.0882	-0.0022	0.1828	0.0378	0.127	
St Mary	0.7081	0.7435	0.8073	0.8189	0.8137	0.7648

2024 Objectives: Montana Fish, Wildlife, and Parks will continue to conduct Pygmy Whitefish sampling until N = 30 individuals are collected from each sampling location. Sampling locations in 2024 include Kintla Lake, Bowman Lake, Lake McDonald, Horseshoe Lake, and Bull Lake.

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B. Hybrid and Rainbow Trout Suppression in the Flathead River System

1/1/2023-12/31/2023 76916 Rel 22 and 84064 Rel 3 I: Investigations of WCT and RBT hybridization in the Flathead River system: data collection
1/1/2023-12/31/2023 76916 Rel 22 and 84064 Rel 3 J: Investigations of WCT and RBT hybridization in the Flathead River system: data analyses
1/1/2023-12/31/2023 76916 Rel 22 and 84064 Rel 3 K: Remove and relocate hybrids and RBT from tributaries in the interconnected Flathead drainage

These Work Elements are associated with Fish Population RM&E, Hydrosystem RM&E, Tributary Habitat RM&E, Tributary Habitat Restoration and Protection, and Predator Control Management.

Introduction and Background

Westslope Cutthroat Trout are one of the handful of native fish species that has adapted over thousands of years to conditions in the Flathead River system. However, the relatively recent introduction of non-native species like Rainbow Trout seriously threaten the persistence of native cutthroat trout through hybridization (interbreeding) and competition for resources. Currently, genetically unaltered Westslope Cutthroat Trout exist in less than 10% of their historic range in the United States and less than 20% of their historic range in Canada. In response to these significant population declines, Montana Fish, Wildlife & Parks (FWP) and the American Fisheries Society classified Westslope Cutthroat Trout as a species of special concern, and the U.S. Forest Service and Bureau of Land Management classified them as a sensitive species. Additionally, a collaborative agreement between resource management agencies, tribes, private organizations, user groups, and landowners was developed to provide guidance on conservation of Westslope Cutthroat Trout throughout its range (FWP 2007).

Within Montana, the South Fork of the Flathead River drainage upstream of Hungry Horse Dam makes up about half of the remaining large, interconnected habitat for genetically unaltered Westslope Cutthroat Trout. The North and Middle forks of the Flathead represent a substantial portion of remaining populations in the state. FWP acknowledges that hybridization will always exist within the mainstem, Middle Fork, and North Fork of the Flathead River, and certain tributaries to these rivers. However, FWP believes that slowing the spread of hybridization and reducing its impacts to remaining genetically unaltered Westslope Cutthroat Trout as well as low-level (less than 10%) hybridized populations is a realistic and important goal in the long-term effort to protect this native species.

To address this goal, FWP identified success measures in the 2013 EA for this work to inform future Westslope Cutthroat Trout conservation strategies in the upper Flathead River system. These measures included slowing the spread of hybridization and reducing hybrid and Rainbow Trout at targeted sources (Figure B1 and B2). In evaluating these metrics, FWP used research that it and its partners have conducted to better understand how hybridization spreads in the affected river system. By tracking fish to their spawning areas using radio telemetry and by studying the genetic structure of fish across the drainage, FWP has learned how to be most efficient and effective in stemming the loss of Westslope Cutthroat Trout populations. Since that research was first conducted in the early 2000s, FWP has removed hybrid and Rainbow Trout by electrofishing and trapping in five key spawning streams that have largely contributed to their spread. These tributaries include Third, Ivy, Rabe, Sekokini, and Abbot creeks. Suppression of hybrid and Rainbow Trout in these targeted locations aims to:

- Mitigate the loss of traits that have evolved locally in Westslope Cutthroat Trout. These traits have helped native cutthroat thrive throughout their native range for thousands of years.
- Retain the ecosystem role served by Westslope Cutthroat Trout, potentially avoiding adverse impacts to other organisms including insects, other fish, birds, and mammals that may result if hybrids and Rainbow Trout replace cutthroat completely.
- Maintain Westslope Cutthroat Trout as a valued sportfish by avoiding unacceptable social and economic impacts associated with losing the opportunity to fish for them.
- Reduce the likelihood of federal Endangered Species Act (ESA) listing and protection of Westslope Cutthroat Trout. ESA listing could limit public opportunity to fish for and otherwise interact with and enjoy this native fish species.
- Protect Montana's state-designated fish, mitigating population impacts and Montana's cultural values associated with the species.

Since 2013, FWP repeated radio telemetry research and updated the genetic information gained during the early 2000s to evaluate progress associated with removing hybrids from source streams (Figure B6). Results of these studies address success measures identified in the 2013 EA for this work, with the following observations in the affected river system as compared to pre-2013:

- A slower rate of hybrid trout expansion from downstream sources (Table B1).
- A reduced number of spawning adults in hybrid source streams (Figures B3 and B4).
- More Westslope Cutthroat Trout captured at targeted hybrid source streams (Figure B5).
- An increase in the proportion of Westslope Cutthroat Trout in targeted hybrid source streams (Tables B2 and B3).
- 53% fewer hybrids and Rainbow Trout spawning in upstream tributaries targeted for suppression – with more fish spawning in the mainstem Flathead River (Figure B6).
- A 19% average increase in angler catch rates for Westslope Cutthroat Trout during 2015 and 2016 as compared to 2002 and 2003 (Figures B8 and B9; Tables B6 and B7).
- A more than 100% increase in the proportion of anglers specifically targeting Westslope Cutthroat Trout upstream of the Stillwater River confluence during 2015 and 2016 as compared to 2002-2003 (Tables B8 and B9).

This effort continues to incorporate lessons learned from past similar actions in FWP's ongoing effort to conserve native Westslope Cutthroat Trout by reducing negative impacts from non-native rainbow and hybrid trout in the affected river system. FWP will continue to monitor the efficacy of suppression by tracking the rate at which hybridization continues to spread in the affected river system, the population genetic structure in streams targeted for suppression, and the relative number of spawning hybrid and Rainbow Trout captured at targeted sources.

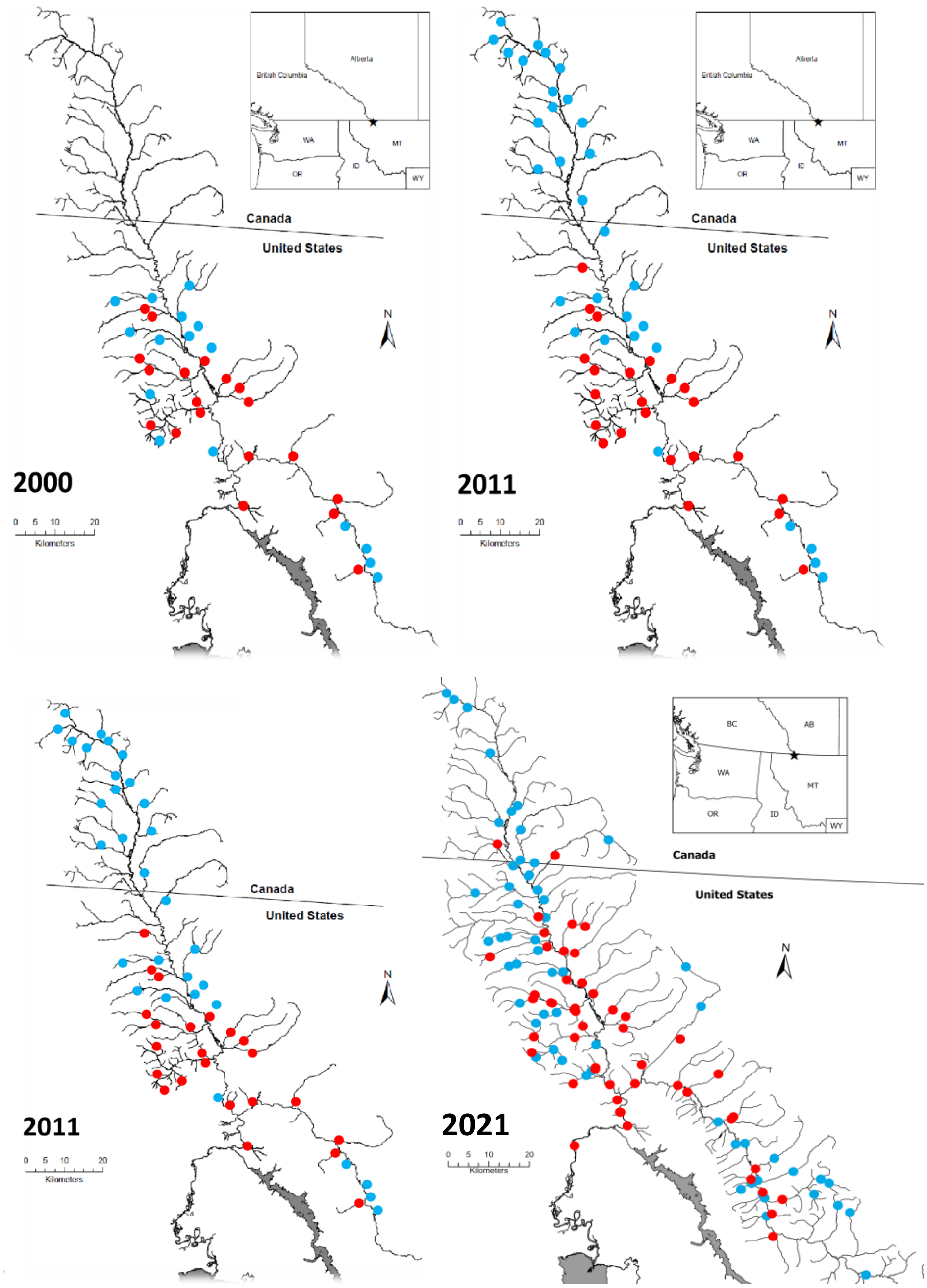


Figure B1.—Temporal spread of hybridization between Westslope Cutthroat Trout and Rainbow Trout in the Flathead River system between 2000 and 2021, based on genetic data (Huston 1984; Hitt et *al*. 2003; Boyer et *al*. 2008; Steed et *al*. 2022). Each dot represents a site sampled, blue indicating genetically pure Westslope Cutthroat Trout and red signifying the presence of Rainbow Trout introgression.

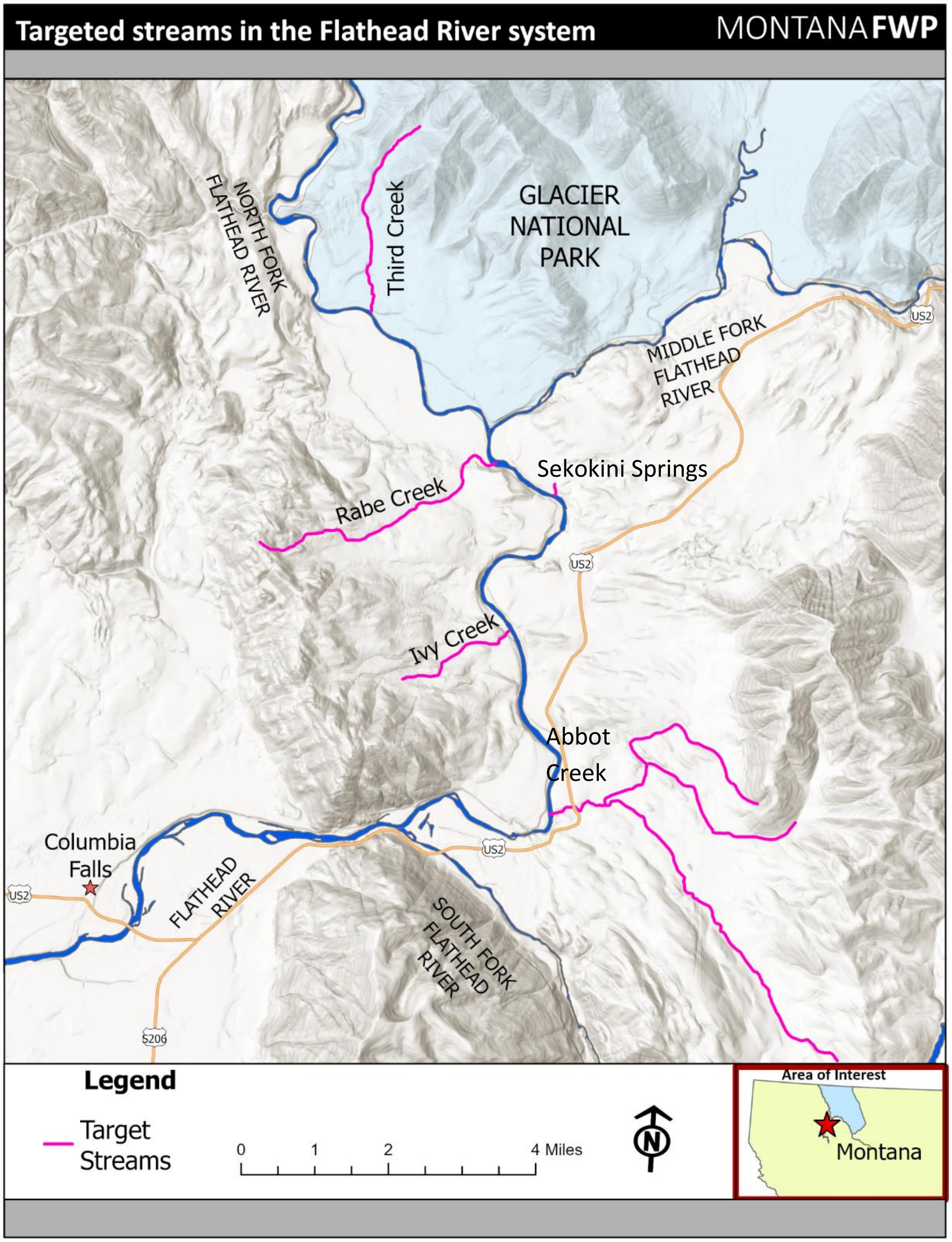


Figure B2.—Locations of tributaries in the Middle and North forks of the Flathead River where hybridization between Westslope Cutthroat Trout and Rainbow Trout has been documented and subsequent rainbow and hybrid trout removal and relocation has been conducted by Montana Fish, Wildlife & Parks.

Methods

Migrant trapping

Migrant traps were deployed in 2022 in three tributary streams of the Flathead to capture upstream migrating trout. One trap was deployed in Abbot Creek downstream of the Highway 2 culvert barrier and about 75 m upstream of the confluence with the Mainstem Flathead River. A second trap was installed about 25 m upstream of the Ivy Creek mouth. Two additional traps (Rabe #1 and #2) were placed in Rabe Creek about 250 m and 75 m upstream of the Mainstem confluence, respectively. Each trap was checked at least once per week and total length (mm), sexual maturity, and genetic samples (fin clips) were taken from all captured fish. The Rabe Creek trap #2 was used to sample fish closer to the mouth during early low water conditions. As spring discharge increased this trap was removed due to inundation and the original upstream trap was operated through the remainder of the spawning season. Rainbow Trout and hybrids were removed and transported to a community fishing pond (Pine Grove Pond) in Kalispell or sacrificed to obtain otoliths for future age validation and microchemical analysis.

Electrofishing

Spawning Rainbow Trout and hybrid fish were also removed from tributary mouths by electrofishing from an 18’ jet boat rigged with fixed-boom anodes during 2022. The Coffelt M22 rectifying unit produced straight DC at 3 to 5 amperes. Effort was concentrated in the Mainstem Flathead River within 50 m of the mouths of Abbot, Rabe, Ivy, Third creeks, and Sekokini Springs. Fish were also collected using backpack electrofishing units within Abbot, Ivy and Third Creeks in 2022. Where fish numbers allowed, a 30 fish sample of fin clips were taken for genetic analysis. Fish that could not be visually identified as hybrid or Rainbow Trout origin were released back into the stream because identification of juvenile fish can be inaccurate. Otherwise, all Rainbow Trout and hybrids captured were treated identically to trap-caught fish. Backpack electrofishing was also used to remove fish and collect genetic samples from targeted tributaries following runoff, treating putative hybrid and Rainbow Trout identically to trap-caught fish.

Results and Discussion

Migrant trapping and electrofishing

Boat electrofishing was conducted between May 4 and June 10 2022 to remove a total of 91 Rainbow Trout and hybrids (Table B4). Traps were operated throughout spring 2022 and removed a total of 45 fish (Table B4). The Abbot Creek trap was installed on March 16 and removed on July 1. The Ivy Creek trap was installed on March 29 and was removed due to a high flow event on June 14. Rabe Creek trap #2 was installed March 22 and removed May 27 and trap #1 was installed on April 25 and removed on July 1. A total of 44 Rainbow Trout and hybrids were removed from within or near Abbot Creek during spring 2022 using boat electrofishing and trapping (Figures B3 and B4; Table B4). A total of 61 Rainbow Trout and hybrids were removed from Rabe Creek and Ivy creeks by boat electrofishing and trapping (37 from Rabe and 24 from Ivy). Boat electrofishing was also used to remove 33 Rainbow Trout and hybrids from the mouths of Third Creek and Sekokini Springs (16 from Third Creek and 17 from Sekokini Springs). Backpack electrofishing was used to remove an additional 39 Oncorhynchus from targeted tributaries during May-July (Table B5).

Additionally, the mouths of Big, Camas, and Anaconda creeks were opportunistically targeted using boat-based electrofishing in the North Fork Flathead River during 2022. Located upstream from historic focal suppression streams, these tributaries have recently exhibited high levels of Rainbow Trout introgression. However, access (discharge limitations), staff, and equipment availability restricted the number of trips. Thus, each stream mouth was sampled once on June 9 2022. Anaconda Creek mouth produced no fish; four hybrids were sampled at the Big Creek mouth, and six hybrids were collected at Camas Creek mouth. Future experimental sites may include other hybridized tributaries in the Middle Fork of the Flathead River.

Migrant trapping

Migrant traps were deployed in 2023 in two tributary streams of the Flathead to capture upstream migrating trout. One trap was deployed in Abbot Creek downstream of the Highway 2 culvert barrier and about 75 m upstream of the confluence with the Mainstem Flathead River. Two additional traps (Rabe #1 and #2) were placed in Rabe Creek about 250 m and 75 m upstream of the Mainstem confluence, respectively. A third stream, Ivy Creek, has typically been trapped in past years. A large rain event in 2022 re-configured much of Ivy Creek's lower stream channel, causing an aggregation of bed load material to deposit at the stream mouth. Due to this build-up of gravel and drought conditions within the basin, Ivy Creek water went sub-surface before reaching the Flathead River. This effectively blocked fish from accessing the stream and the trap was not installed.

Each trap was checked at least once per week and total length (mm), sexual maturity, and genetic samples (fin clips) were taken from all captured fish. The Rabe Creek trap #2 was used to sample fish closer to the mouth during early low water conditions. As spring discharge increased this trap was removed due to inundation and the original upstream trap was operated through the remainder of the spawning season. Rainbow Trout and hybrids were removed and transported to a community fishing pond (Pine Grove Pond) in Kalispell or sacrificed to obtain otoliths for future age validation and microchemical analysis.

Electrofishing

Spawning Rainbow Trout and hybrid fish were also removed from tributary mouths by electrofishing from an 18' jet boat rigged with fixed-boom anodes during 2023. The Coffelt M22 rectifying unit produced straight DC at 3 to 5 amperes. Effort was concentrated in the Mainstem Flathead River within 50 m of the mouths of Abbot, Rabe, Ivy, Third creeks, and Sekokini Springs. In addition to these streams, five other tributary confluences were also sampled at least once in 2023. Anaconda, Camas, and Big Creeks in the North Fork Basin, Rubideau Creek in Middle Fork Basin and an unnamed tributary adjacent to Ivy Creek, dubbed "Aunt Betty Creek," in the Mainstem Flathead River. Fish were also collected using backpack electrofishing units within Abbot, Ivy and Third Creeks in 2023. Where fish numbers allowed, a 30-fish sample of fin clips were taken for genetic analysis. Fish that could not be visually identified as hybrid or Rainbow Trout origin were released back into the stream because identification of juvenile fish can be inaccurate. Otherwise, all Rainbow Trout and hybrids captured were treated identically to trap-caught fish. Backpack electrofishing was also used to remove fish and collect genetic samples from targeted tributaries following runoff, treating putative hybrid and Rainbow Trout identically to trap-caught fish.

Results and Discussion

<u>Migrant trapping and electrofishing</u>

Boat electrofishing was conducted between May 1 and June 1, 2023, and resulted in the removal of 66 Rainbow Trout and hybrids (Table B4). Traps were operated throughout spring 2023 and removed a total of 18 fish (Table B4). The Abbot Creek trap was installed on March 21 and removed on June 9. The Rabe Creek trap #2 was installed April 12 and removed May 12 whereas trap #1 was installed on May 2 and removed on June 9. Backpack electrofishing was used to remove an additional 26 Oncorhynchus from targeted tributaries during May-July (Table B5).

The mouths of Big, Camas, Anaconda, Rubideau and Aunt Betty creeks were opportunistically targeted using boat-based electrofishing in the North and Middle forks of the Flathead River during 2023. Located mostly upstream from historic focal suppression streams, these tributaries contain hybrid trout sources that likely contribute to the spread of introgression in the open Flathead River system. However, access (discharge limitations), staff, and equipment availability restricted the number of trips. For instance, Rubideau Creek was sampled twice (May 8 and 19). The additional stream mouths were sampled once on May 9. Anaconda and Big Creek mouths produced no trout; three hybrids were collected at Camas Creek mouth; five hybrids came from Aunt Betty Creek, and four hybrids were removed from the Rubideau Creek mouth. These additional streams will continue to be targeted in future years as time allows.

<u>Rate of hybrid trout expansion</u>

The rate at which conservation populations of Westslope Cutthroat Trout have become more than 10% genetically altered has slowed since manual removal of hybrids and Rainbow Trout began (Al-Chokhachy et *al*. 2014). “Conservation” populations, as defined by the Memorandum of Understanding and Conservation Agreement for Cutthroat Trout in Montana (FWP 2007) are greater than 90% genetically unaltered Westslope Cutthroat Trout. Based on available data, at least 15 stream-dwelling conservation populations became 10% or more hybridized with Rainbow Trout prior to 2013 as compared to a net of 6 populations since 2013 (Table B1). Further, all fish-sustaining streams targeted for rainbow and hybrid trout removal showed a statistically significant ($p < 0.0001$, $\alpha = 0.01$) increase in the proportion of Westslope Cutthroat Trout since about 2013 (Tables B2 and B3).

Table B1.—Tributaries in the affected Flathead River system in which Westslope Cutthroat Trout became more than 10% genetically altered through hybridization with Rainbow Trout before and after 2013. Note that Rubideau Creek lost its conservation population status prior to 2013 but regained it thereafter, reducing the net number of tributaries lost since 2013.

	Tributary	
Drainage	Pre-2013	Post-2013
North Fork	Anaconda Creek	Big Creek - middle
	Big Creek - lower	Coal Creek - lower
	Camas Creek	McGee Creek
	Cyclone Creek	Meadow Creek - lower
	Dutch Creek	SF Coal Creek -middle
	Langford Creek	Teepee Creek - lower
	Lookout Creek	
	Third Creek	
Middle Fork	Abbot Creek	Pinchot Creek
	Ivy Creek	
	Harrison Creek	
	Lincoln Creek - lower	
	Moccasin Creek	
	Rabe Creek	
	Rubideau Creek - lower*	

*This population became less than 10% genetically altered through hybridization with Rainbow Trout since 2013, restoring its conservation population status.

Table B2.—Changes in the proportion of Westslope Cutthroat Trout (WCT) genetic material observed over time in tributaries targeted for hybrid and Rainbow Trout removal in the affected Flathead River system. All sites demonstrated a statistically significant ($p < 0.0001$, $\alpha = 0.01$) increase in the proportion of cutthroat alleles over time.

		Pre-2013		Post-2013		
Drainage	Tributary	*n*	*p*WCT	*n*	*p*WCT	*p*-value
North Fork	Third Creek	28*	0.083	18	0.466	< 0.0001
Middle Fork	Abbot Creek	30	0.009	47	0.274	< 0.0001
	Ivy Creek - lower	28	0.508	30	0.786	< 0.0001
	Rabe Creek - lower	25	0.443	31	0.577	< 0.0001

*Samples collected in 2015.
$\alpha = 0.01$

Table B3.—Changes in the proportion of genetically-unaltered Westslope Cutthroat Trout (WCT) observed over time in tributaries targeted for hybrid and Rainbow Trout removal in the affected Flathead River system. Two sites demonstrated a statistically significant (p < 0.0001, α = 0.05) increase in the proportion of cutthroat alleles over time.

		Pre-2013		Post-2013		
Drainage	Tributary	n	Proportion WCT	n	Proportion WCT	p-value
North Fork	Third Creek	28*	0	18	0.278	0.0016
Middle Fork	Abbot Creek	30	0	47	0.213	0.0127
	Ivy Creek - lower	28	0	30	0.033	0.1607
	Rabe Creek - lower	25	0	31	0	NA

*Samples collected in 2015.
α = 0.05

Table B4.—Numbers of rainbow and hybrid trout removed from tributaries in the Flathead River system by electrofishing and trapping from 2000 to 2022 (EF = electrofishing the tributary mouth by boat). Values in parentheses indicate the number of fish captured for each day spent electrofishing or trapping (i.e., catch per unit effort).

	Abbot		Ivy		Sekokini		Rabe			Third	
Year	Trap	EF	Trap	EF	Trap	EF	Trap[1]	Trap[2]	EF	EF	Total removed
2000	77(1.2)										77
2001	140(2.1)										140
2002	74(1.4)	114									188
2003	12(0.2)	43									55
2004	158(2.0)	11(5.5)									169
2005	131(1.6)	76(12.7)							8(8.0)		215
2006	77(1.0)	21(7.0)		13(2.2)					14(2.3)	31(5.2)	156
2007	95(1.2)	8(8.0)		5(5.0)		4(4.0)			4(4.0)	4(4.0)	120
2008	45(1.0)	19(4.8)		10(2.5)		1(1.0)			16(4.0)	23(4.6)	114
2009	16(0.2)	10(1.7)		13(2.2)		1(1.0)			19(2.7)	27(3.4)	86
2010	15(0.2)	7(1.8)		11(1.8)		3(1.5)			30(3.8)	21(2.6)	87
2011	20(0.3)	13(0.7)		22(1.1)		14(1.2)	21(0.3)		14(1.1)	20(1.7)	124
2012	44(0.6)	5(0.3)	7(0.1)	10(0.7)	0(0)	5(0.3)	8(0.1)		11(0.7)	12(0.8)	102
2013	8(0.1)	16(0.9)	32(0.4)	24(0.3)	--	12(0.8)	20(0.2)		36(2.0)	40(2.5)	188
2014	16(0.1)	17(0.9)	11(0.1)	19(0.9)	--	15(0.7)	23(0.3)		29(1.3)	40(1.7)	170
2015	10(0.1)	9(0.6)	20(0.2)	18(1.2)	--	6(0.5)	1(<0.1)		33(2.2)	28(2.0)	125
2016	10(0.1)	39(2.6)	5(0.1)	32(2.3)	--	29(1.9)	15(0.2)		42(3.0)	45(3.5)	217
2017	10(0.1)	22(1.3)	2(0.1)	34(2.0)	--	20(1.2)	17(0.2)		33(1.9)	33(1.9)	171
2018	21(0.2)	16(1.5)	16(0.2)	17(1.6)	--	19(2.0)	15(0.2)		22(2.0)	22(2.0)	148
2019	16(0.2)	15(1.4)	4(<0.1)	23(2.1)	--	14(1.3)	2(<0.1)		21(1.9)	21(1.9)	116
2020	4(<0.1)	23(1.5)	1(<0.1)	25(1.7)	--	24(1.6)	2(<0.1)	6(0.3)	17(1.1)	17(1.3)	119
2021	16(0.2)	22(2.8)	14(0.2)	13(1.6)	--	12(1.5)	3(<0.1)	8(0.2)	17(2.1)	16(2.0)	121
2022	20(0.2)	22(2)	10(0.1)	14(1.1)	--	17(1.3)	5(<0.1)	10(0.2)	22(1.5)	16(1.2)	134
2023	12(0.2)	17(1.7)	--	8(0.8)	--	3(0.3)	2(<0.1)	4(0.1)	18(1.8)	20(2.9)	84
Total	1047	546	122	311	--	199	134	28	403	436	3226

Table B5.—Fish removed from targeted hybrid and Rainbow Trout (ONC) suppression streams in the upper Flathead River system during 2023. Westslope Cutthroat Trout (WCT) were returned to the stream following data collection. All length data in mm.

		ONC			WCT		
Stream	Date	n	Length range	Mean length	n	Length range	Mean length
Abbot Creek	6/16/2023	17	75-161	101.8	1	123	-
Ivy Creek	6/06/2023	8	39-414	93.2	23	95-321	151.4
Rabe Creek	-	-	-	-	-	-	-
Third Creek	5/25/2023	2	89-114	101.5	-	-	-

Spawning Rainbow and Hybrid Trout in Targeted Tributaries

The rate at which spawning hybrid and Rainbow Trout have been captured by trapping and electrofishing target stream mouths over time has declined, controlling for effort (Figures B3 and B4). Further, the rate at which Westslope Cutthroat Trout have been encountered during these removal efforts over time has increased (Figure B5).

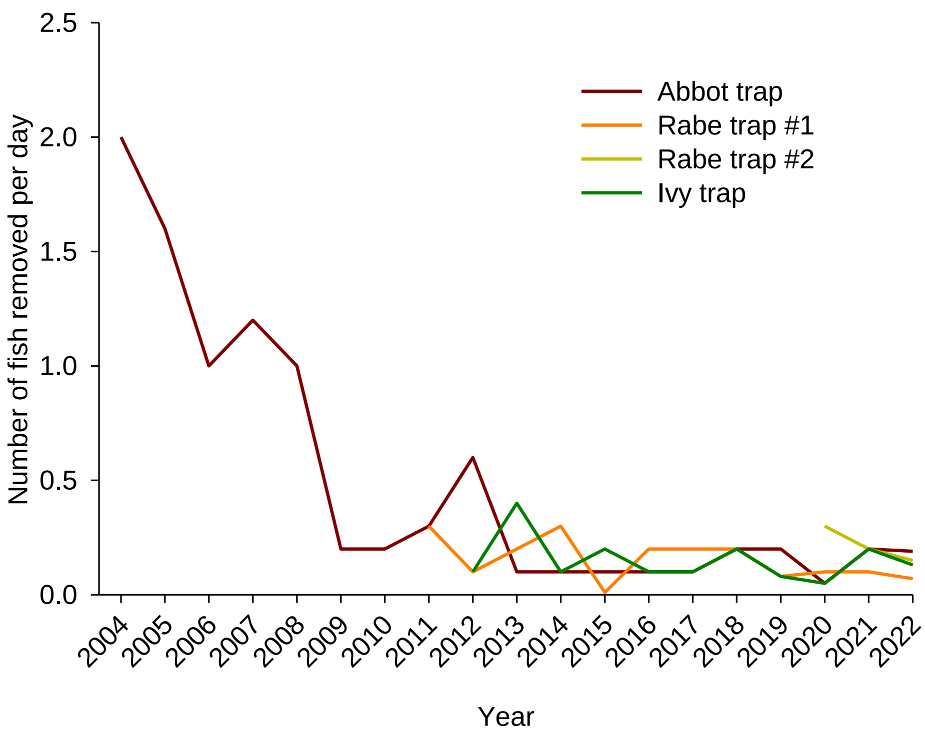


Figure B3.—Catch per unit effort (CPUE) for rainbow and hybrid trout removed during 2000-2023 from seasonal (spring) fish traps installed in Abbot, Ivy, and Rabe Creeks.

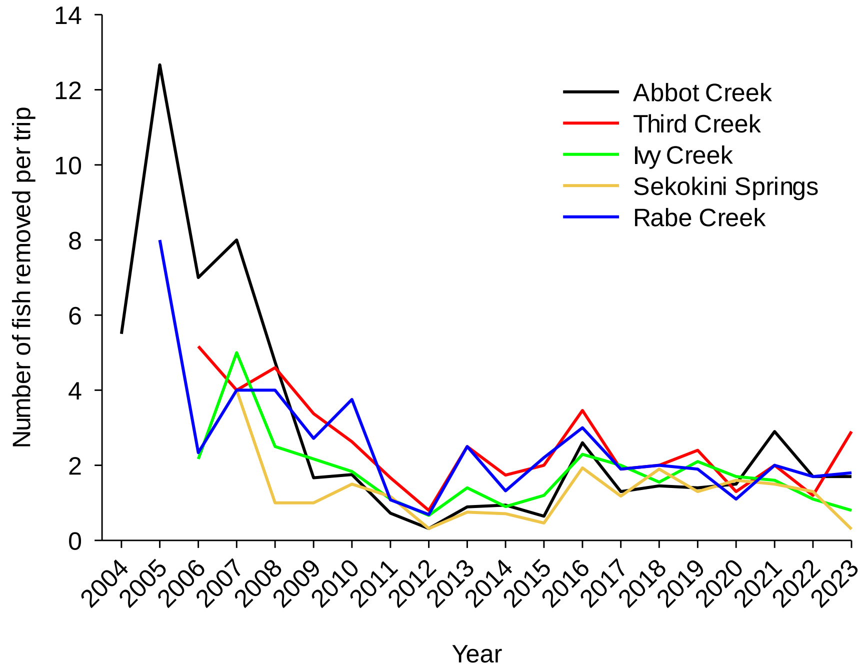


Figure B4.—Catch per unit effort (CPUE) for rainbow and hybrid trout removed during 2000-2023 from the mouths of five tributaries in the Flathead River system by boat electrofishing.

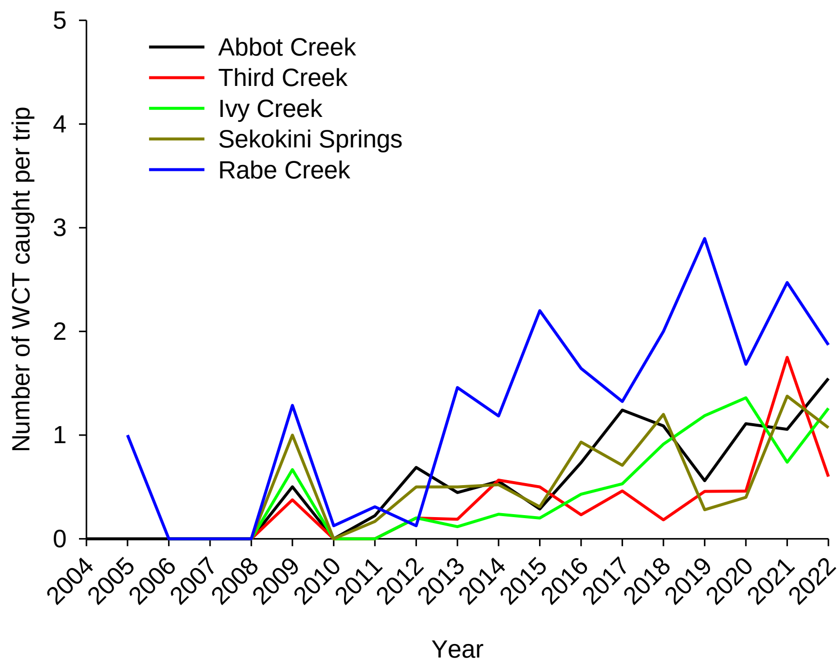


Figure B5.—Catch per unit effort (CPUE) for Westslope Cutthroat Trout (WCT) encountered when removing rainbow and hybrid trout during 2004-2023 from the mouths of five tributaries in the Flathead River system by boat electrofishing.

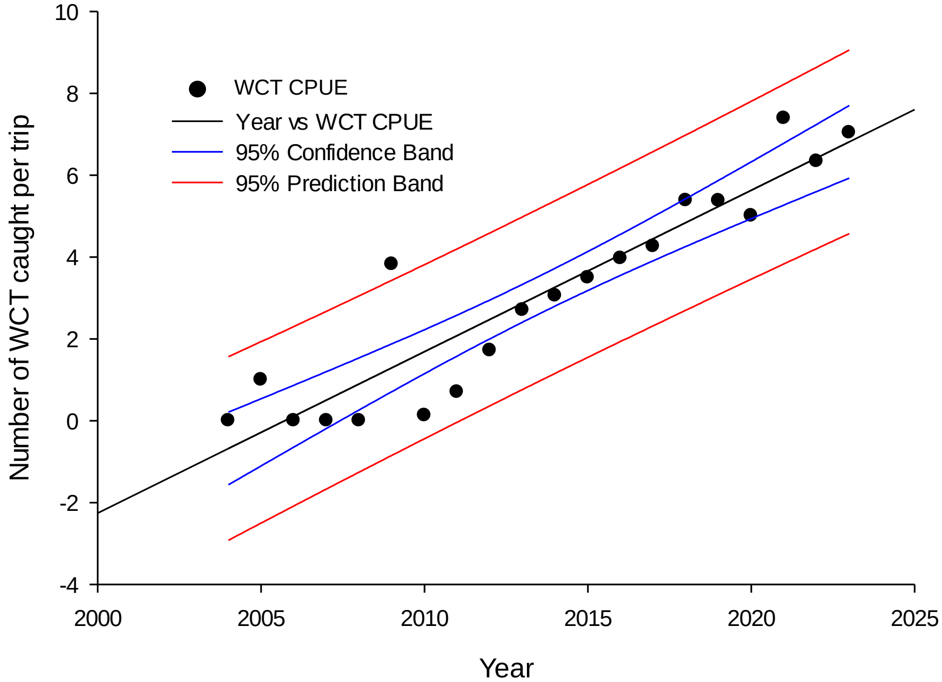


Figure B6.—Linear regression of catch per unit effort (CPUE) by year for Westslope Cutthroat Trout (WCT) encountered at five tributaries targeted for rainbow and hybrid trout suppression in the Flathead River system, r^2 = 0.8568.

Changes in Hybrid and Rainbow Trout Spawning Behavior

Radio telemetry demonstrated that 65% of tagged hybrids and Rainbow Trout likely spawned in the Mainstem, North, Middle, or South forks of the Flathead River and side channels during 2016-2018 as compared to about 12% during 2000-2007 when most spawning occurred in tributaries containing Westslope Cutthroat Trout (Figure B7), suggesting that there may be a shift in the proportional use of spawning habitat by hybrids and Rainbow Trout. Although non-hybridized Westslope Cutthroat Trout continue to be threatened by spreading Rainbow Trout introgression, these results suggest that focused suppression of hybridization sources can be a beneficial strategy for maintaining conservation populations of westslope cutthroat in a large, interconnected river drainage.

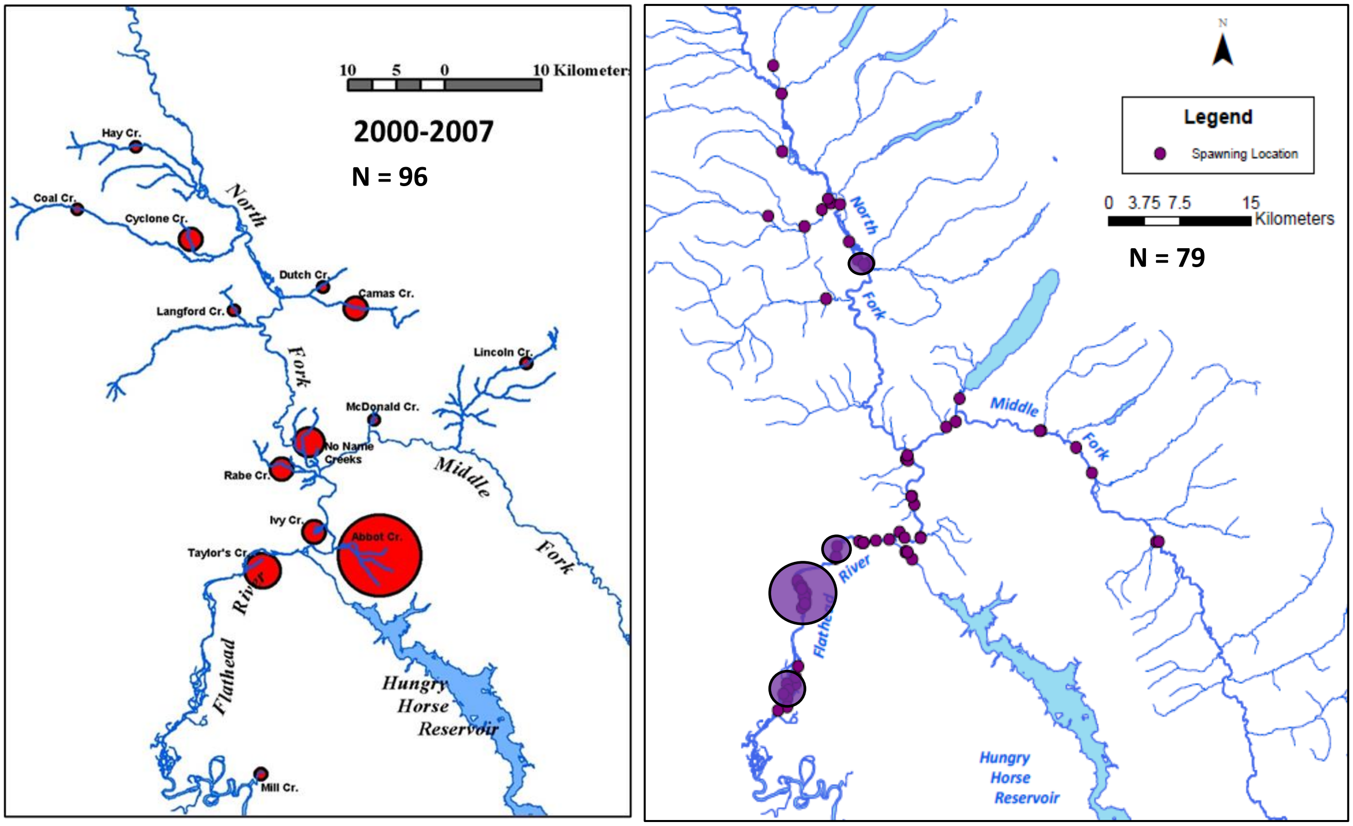


Figure B7.—Spawning locations of hybrid and Rainbow Trout during 2000-2007 (left) (Muhlfeld et *al*. 2009) and 2016-2018 (right) (Steed et *al*. 2020) in the upper Flathead River system. The size of each dot is proportionate to the number of fish presumed to have spawned in each location.

<u>Angling Information</u>

Angler surveys conducted in portions of the affected river system during 2002-2003 and 2015-2016 revealed changes in angler catch rates and preferences over time (Figure B8) (Deleray 2004; Steed and Hunt 2020). Specifically, a 19% average increase in angler catch rates for Westslope Cutthroat Trout was observed during 2015 and 2016 as compared to 2002 and 2003 (Figures B9 and B10; Tables B6 and B7). Further, more than twice the average proportion of anglers explicitly targeted Westslope Cutthroat Trout upstream of the Stillwater River confluence during 2015 and 2016 than they did during 2002-2003 (Tables B8 and B9). These anglers outnumbered those targeting Rainbow Trout by 2-18% during 2002-2003 and by 22-47% during 2015-2016. In other words, over time anglers are catching more Westslope Cutthroat Trout and more of those anglers prefer to target the species over any other trout.

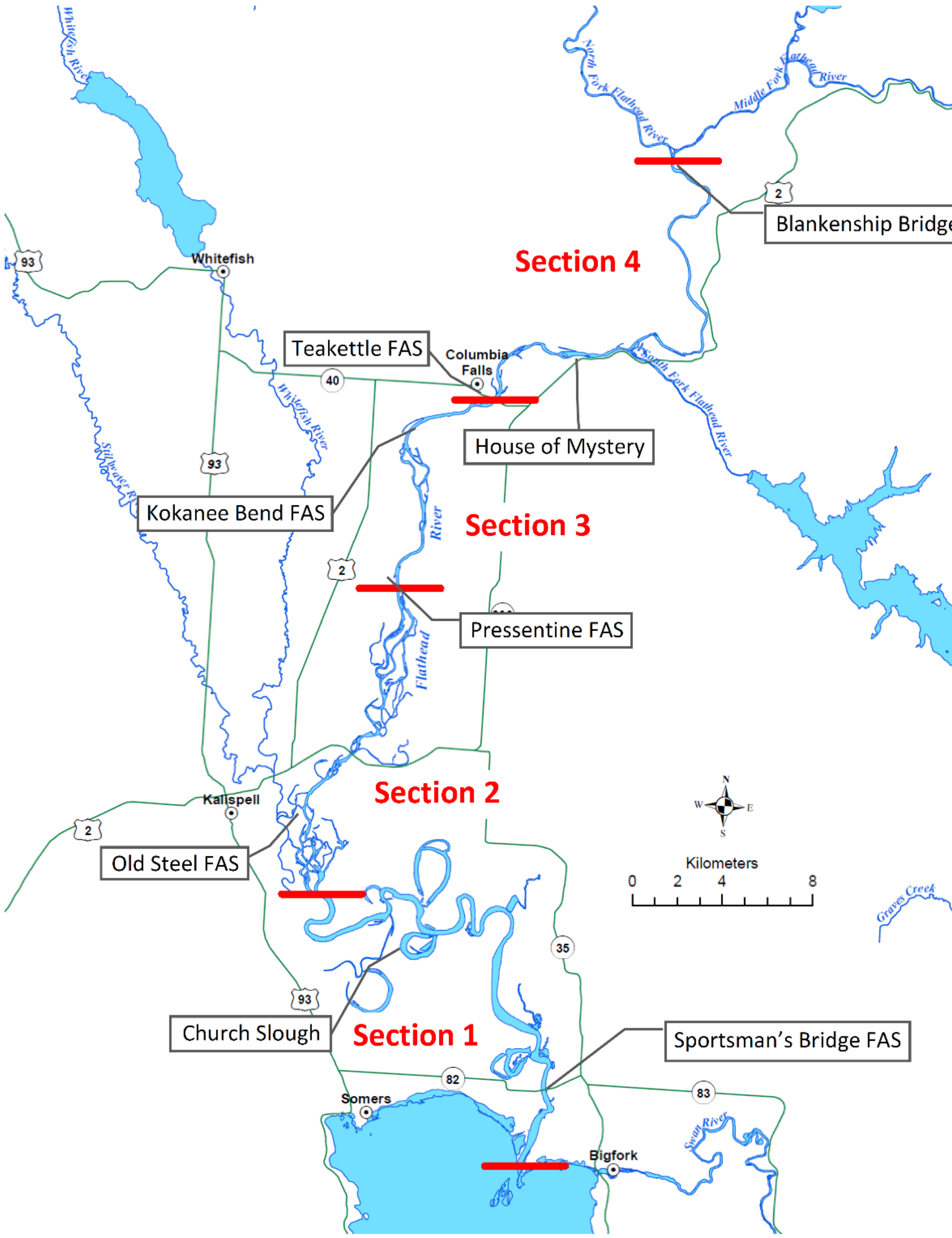


Figure B8.—Study area of the Flathead River system targeted by two separate creel (angler) surveys conducted from June 2002 through May 2003 and March 2015 through February 2016, respectively. An additional Section 5 included the lower Flathead River sloughs, as in Deleray (2004). Angler access points used by creek clerks during 2015-2016 are shown by name and location, where FAS = Fishing Access Site. From Steed and Hunt (2020).

Table B6.—Angler harvest rates (fish per hour), harvest, and catch rates (fish per hour) for all sections combined, Flathead River and associated sloughs 2002-2003. From Deleray (2004).

Species	Harvest Rate	Variance	Number Harvested	Variance	Catch Rate	Variance
Lake Trout	0.05	3.54E-05	1,246	8.41E+04	0.07	5.37E-05
Lake Whitefish	0.70	1.43E-03	21,824	1.11E+07	0.79	7.02E-03
Yellow Perch	0.02	2.36E-05	484	5.74E+04	0.03	4.61E-05
W. Cutthroat Trout	0.00	0.00E+00	0	0.00E+00	0.16	1.10E-04
Rainbow Trout	0.003	2.18E-06	167	1.64E+04	0.03	1.08E-05
Bull Trout	0.00	0.00E+00	0	0.00E+00	0.02	1.01E-05
Pike	0.01	2.39E-06	575	9.35E+03	0.02	5.84E-06
Largemouth Bass	0.0001	6.97E-09	10	1.16E+02	0.02	1.82E-05
Mountain Whitefish	0.00	0.00E+00	0	0.00E+00	0.07	4.61E-05
Other	0.04	1.06E-04	904	1.75E+05	0.06	1.39E-04
Total	0.82	1.60E-03	25,209	1.28E+07	1.25	7.47E-03

Table B7.—Mean angler harvest rates (fish harvested per hour), harvest, and catch rates (fish caught per hour) for all river sections combined in the Flathead River during 2015-2016. From Steed and Hunt (2020).

Species	Harvest rate	Variance	Number harvested	Variance	Catch rate	Variance
Lake Trout	0.0027	1.15E-06	123	1.11E+04	0.01	7.70E-06
Lake Whitefish	0.0581	3.46E-05	1,819	1.31E+05	0.07	4.29E-05
Yellow Perch	0.0050	2.36E-06	427	2.54E+04	0.30	5.30E-04
W. Cutthroat Trout	0.0007	1.13E-07	63*	1.02E+03	0.19	4.75E-05
Rainbow Trout	0.0004	2.13E-08	37	2.87E+02	0.03	2.63E-06
Bull Trout	0.0000	0.0000	0	0.00E+00	0.01	2.31E-06
Northern Pike	0.0098	1.32E-06	1,071	2.50E+04	0.04	7.02E-06
Largemouth Bass	0.0007	4.39E-08	83	1.06E+03	0.03	6.12E-06
Mountain Whitefish	0.0041	1.10E-06	166	3.34E+03	0.02	5.57E-06
Other	0.0111	5.74E-06	1,242	1.67E+05	0.04	3.04E-05
Total	0.0929	4.99E-05	5,031	3.65E+05	0.73	6.82E-04

*Although Westslope Cutthroat Trout could be legally harvested from Section 5 (sloughs) during the creel period, harvest was only observed in Section 2. Thus, these fish were either misidentified, harvested from a slough, or illegally harvested.

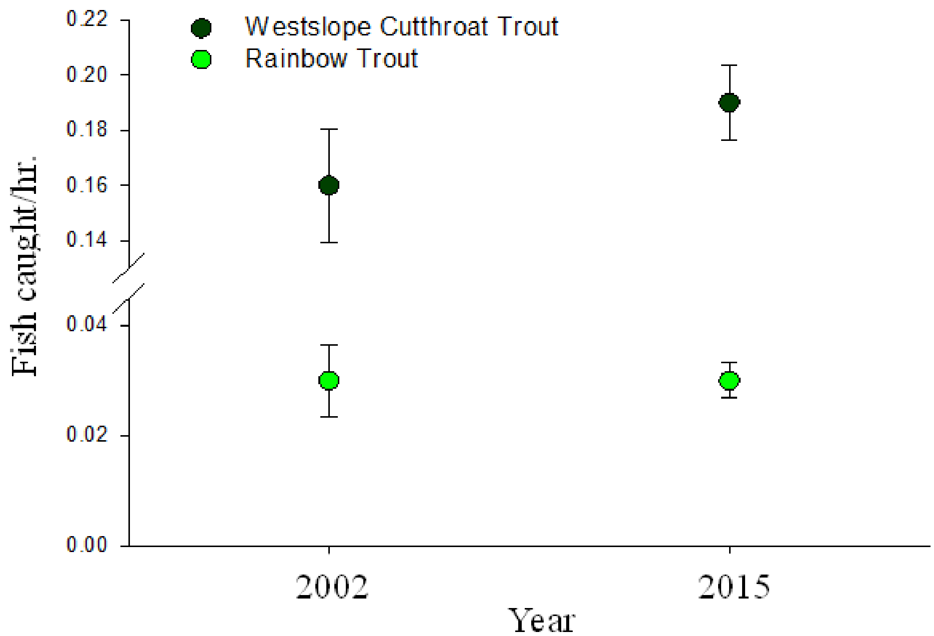


Figure B9.—Catch rates for Westslope Cutthroat Trout and Rainbow Trout in the upper Flathead River system during 2002-2003 (Deleray 2004) and 2015-2016 (Steed and Hunt 2020).

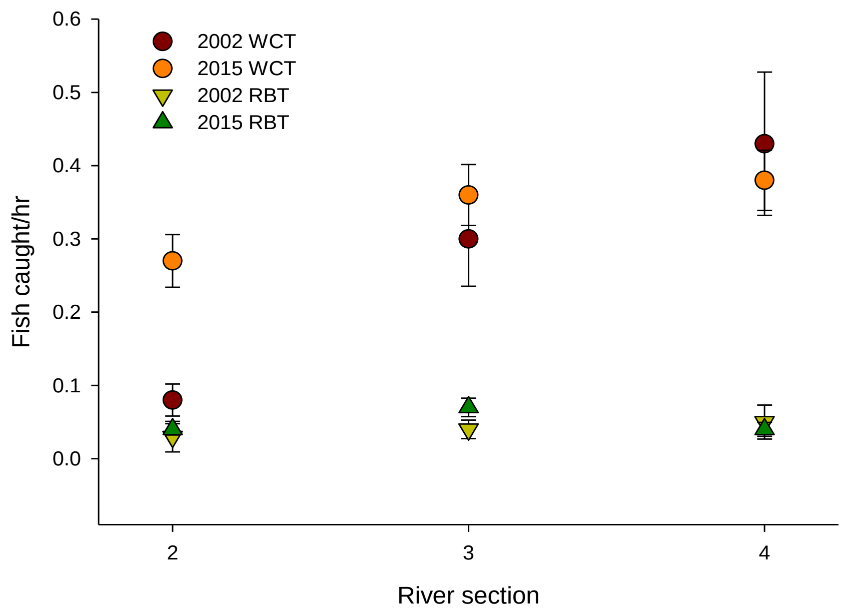


Figure B10.—Catch rates for Westslope Cutthroat Trout and Rainbow Trout in the upper Flathead River system, by survey section, during 2002-2003 (Deleray 2004) and 2015-2016 (Steed and Hunt 2020).

Table B8.—Targeted fish species identified through angler interviews for the five Flathead River sections, 2002-2003. Values are percentages of angler interviews in each section. From Deleray (2004).

	WCT	RBT	No Target	LWF	LT	Pike	Bass	Perch	MWF	Non-Game
Section 4	25.0	9.0	65.4	0.0	0.0	0.0	0.0	0.0	0.6	0.0
Section 3	24.3	6.2	34.6	34.9	0.0	0.0	0.0	0.0	0.0	0.0
Section 2	3.3	1.7	20.7	72.5	0.7	0.1	0.0	0.1	0.6	0.4
Section 1	1.1	0.4	59.3	0.7	15.4	19.6	2.1	0.0	0.0	1.4
Sloughs	0.0	0.0	16.7	0.0	2.1	70.0	7.4	3.8	0.0	0.0

Table B9.— Percentages of anglers who reported targeting specific species during 2015-2016 in the Flathead River system, by river section. WCT = Westslope Cutthroat Trout, RBT = Rainbow Trout, LT = Lake Trout, LWF = Lake Whitefish, NP = Northern Pike, YP = Yellow Perch, Other = non-game and bait species (e.g., Longnose Sucker, Northern Pikeminnow, Peamouth Chub), NT = no specific target species, CR = Black Crappie, LB = Largemouth Bass, and MWF = Mountain Whitefish. From Steed and Hunt (2020).

Section	WCT	RBT	LWF	NP	LT	YP	Other	No Target	CR	LB	MWF
4	34%	8%	3%	0%	1%	0%	0%	53%	0%	0%	1%
3	50%	3%	5%	0%	0%	0%	0%	39%	0%	0%	1%
2	27%	5%	36%	1%	4%	0%	0%	26%	0%	0%	0%
1	1%	2%	3%	9%	10%	1%	0%	63%	1%	10%	1%
5	0%	0%	0%	47%	0%	7%	0%	17%	6%	22%	0%
Weighted mean*	24%	4%	10%	13%	2%	2%	0%	36%	2%	6%	1%

*Using the weighted mean accounts for the differing number of anglers interviewed over time and across river sections, avoiding bias in estimated percentages.

C. Focal Species Monitoring in the Flathead River System

1/1/2023-12/31/2023 76916 REL 22 and 84064 Rel 3 N: Focal species population monitoring in the Mainstem Flathead River system

This Work Element is associated with Fish Population RM&E, Hydrosystem RM&E, Tributary Habitat RM&E, and Tributary Habitat Restoration and Protection

***Oncorhynchus spp.* population monitoring**

Introduction

Population monitoring of focal species in the Flathead River has been conducted for decades, largely centered on *Oncorhynchus spp.* producing long-term trend data informing management and supporting related research and mitigation efforts.

Methods

Catch per unit effort (CPUE) of Rainbow Trout and hybrids was estimated in 2023 in the Mainstem Flathead River to continue a long-term investigation of population trends (Ricker 1975). Methodology of previous surveys was replicated to standardize comparisons (Deleray et *al.* 1999; Steed et *al.* 2008-2023; Boyer et *al.* 2014-2015) (Figure C1).

Marking surveys began at dark on March 1 2023 and continued until two passes were completed by two boats simultaneously surveying each bank. Electrofishing was performed from jet boats rigged with fixed-boom anodes. A Coffelt M22 unit was operated to produce straight DC at 3 to 5 amperes in adherence to FWP electrofishing policy dictating the use of straight DC or pulse rates $\leq$ 30 Hz when sampling waters with native fishes. The recapture survey occurred on March 8 2023, replicating collection protocols used during the marking event.

Passes began at the upstream boundary of each section and progressed downstream along one of the banks to the lower boundary. Shock-time for each pass was recorded to estimate CPUE. All trout were netted, measured for total length (mm), weight (g), and marked with a fin clip during the first sampling event. However, Bull Trout larger than about 400 mm were not consistently netted to minimize impacts on these fish. Passive integrated transponder (PIT) tags were inserted in most Rainbow Trout and hybrids from 2004-2023 to estimate annual growth of recaptured fish. Westslope Cutthroat Trout have also been PIT-tagged in more recent years to better understand interannual movement and growth. Fish were examined upon collection after the recapture event to determine recapture status, with Rainbow Trout and hybrids subsequently relocated to a local community fishing pond, if accessible (Pine Grove Pond, Kalispell).

Catch-per-unit-effort was calculated as the number of a given fish species (Rainbow Trout and hybrids were combined) captured divided by the time (hr) spent electrofishing and the length of the sample section (km) (McMullin and Graham 1981). Abundance estimates were calculated only for adult ($\geq$ 250 mm) and subadult (< 250 mm) Rainbow Trout and hybrid fish. Temperature (°C), discharge (CFS), and conductivity (µS) were also recorded on the sampling nights for comparison to abundance and CPUE estimates.

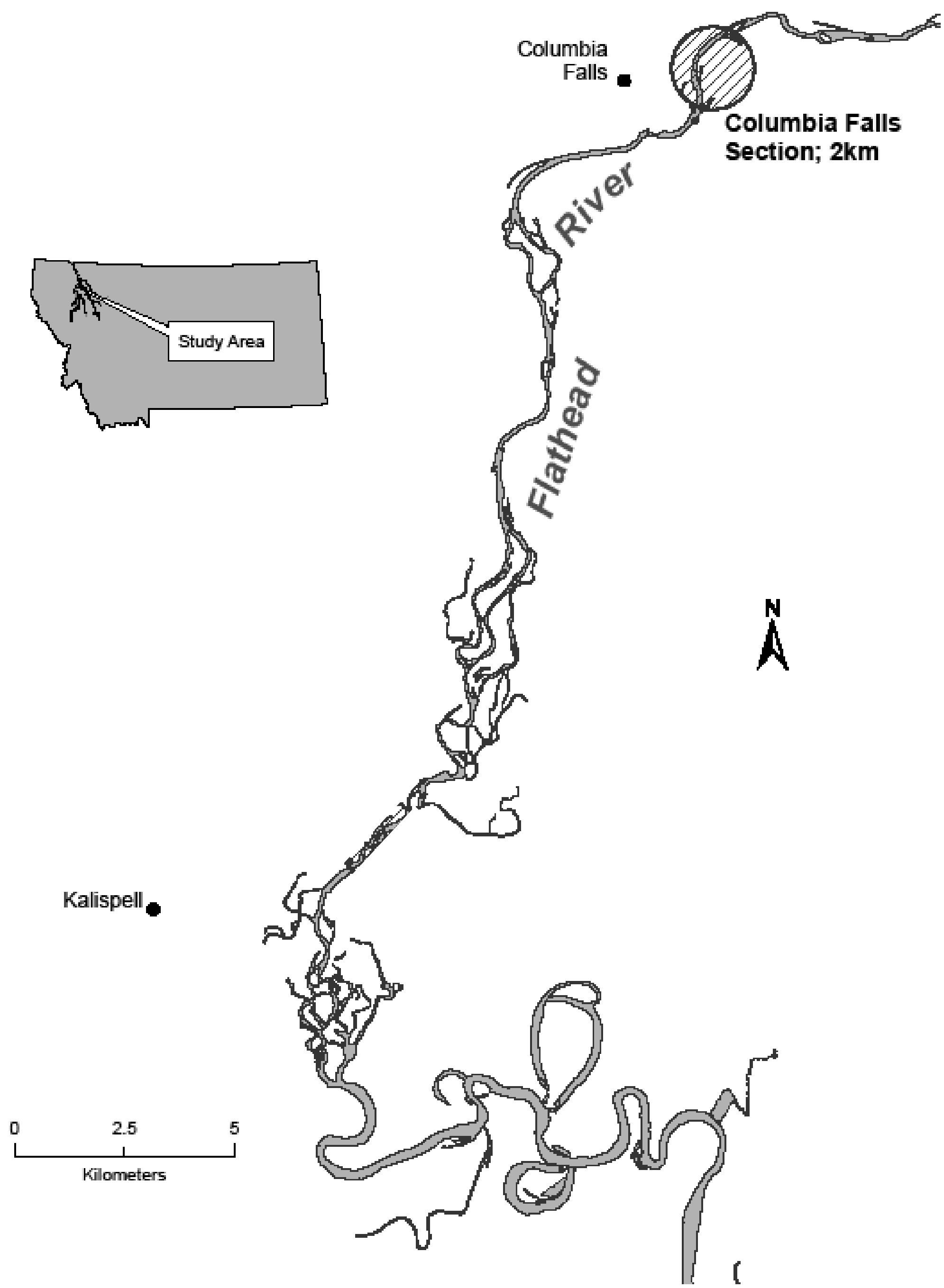


Figure C1.—Spring hybrid and Rainbow Trout electrofishing section in the Mainstem Flathead River.

Results and Discussion

The estimated numbers of hybrid and Rainbow Trout in the section of river surveyed have varied through time but demonstrate no upward or downward trends (Figures C2 and C3). The relatively large differences in estimated abundances from one year to the next suggest that a large change in true numbers of fish would have to occur before FWP would detect it using this method. Hybrid and Rainbow Trout sampled on the last day of the two-day estimate have typically been transported to a community fishing pond since 2009.

Mean total length of fish sampled over both survey nights was 263 mm (range 159-452 mm) for Rainbow Trout and hybrids and 267 mm (range 68-377 mm) for Westslope Cutthroat Trout. Other species netted included six bull trout and two brook trout. Mean daily river discharge was 119 m^3/s during the survey period, water temperature was 1.2^{o}C and conductivity was 87.1 us/cm. The 2022 recapture survey was not completed due to heavy snow squalls limiting visibility. Further, only one marked fish in each size group was captured in the recapture run so an estimate was not calculated for 2023. Subsequently, abundance estimates through 2021 only are shown for context (Figure C2) whereas catch-per-unit-effort (CPUE) was used to describe relative abundance of adult Rainbow Trout and hybrids as well as Westslope Cutthroat Trout in the Flathead River in 2022 and 2023 (Figure C3).

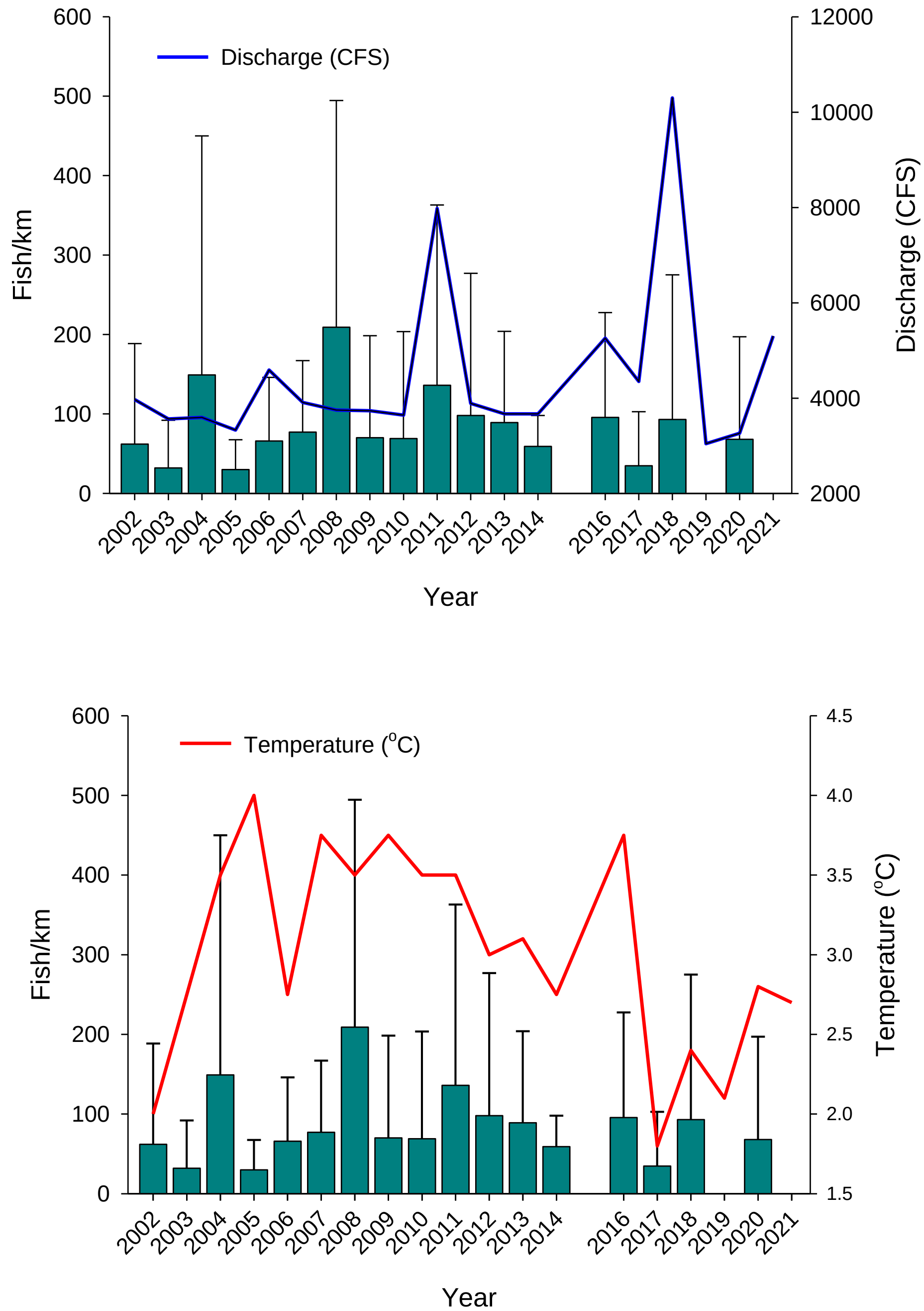
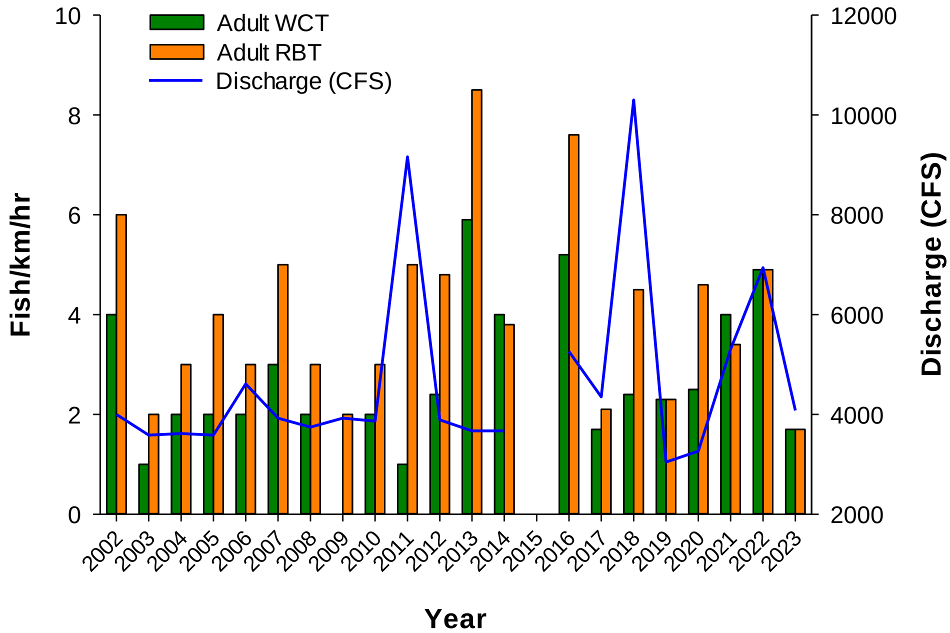


Figure C2.—Estimated number of adult (≥ 250 mm total length) Rainbow and hybrid trout per km, by year, in the mainstem Flathead River near Columbia Falls, Montana during late-winter compared to discharge (CFS) (top) and temperature (bottom). Bars represent 95% confidence limits on estimated abundances. No estimate for trout was produced during 2015 because a Mountain Whitefish estimate was conducted in the same river reach that year. Too few fish were recaptured during 2019, 2021, and 2023 to produce an estimate and a recapture survey was precluded by foul weather during 2022.



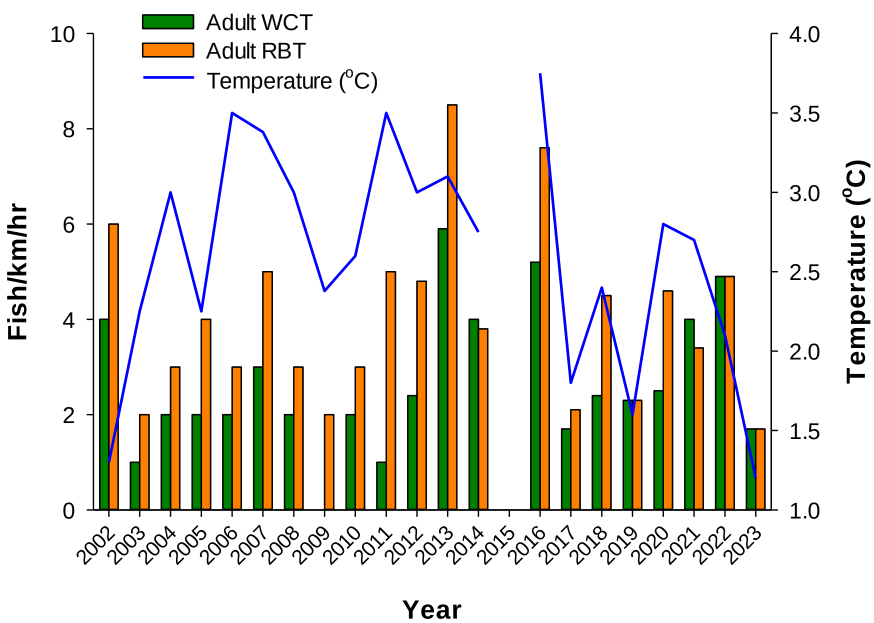


Figure C3.—Catch-per-unit-effort for adult (CPUE) (≥ 250 mm total length) Rainbow Trout and hybrids combined (RBT) alongside adult Westslope Cutthroat Trout (WCT) captured in the Columbia Falls, Montana, electrofishing section of the mainstem Flathead River, by year, during late-winter compared to discharge (CFS) (top) and temperature (bottom).

Genetic monitoring surveys

Methods

In addition to suppression activities described previously, tributaries and lakes in the upper Flathead River system were sampled to provide an updated status of Rb hybridization in the drainage (Figure C4). These efforts were performed during 2015-2023 to evaluate the success of suppression efforts to date and guide future mitigation, research, and monitoring. Collection efforts were collaborative and coordinated among FWP, the U.S. Geological Survey, Glacier National Park, and the University of Montana. During 2018-2023, headwater lakes were incorporated into the sampling effort in the North and Middle forks of the Flathead River (Table C1).

Lakes were sampled July through September 2023 and accessed by foot, bicycle, or helicopter. Angling was the preferred method of capturing fish; however, monofilament sinking gill nets were necessary in some lakes to achieve a larger sample. Nets typically soaked for one to three hours. Fish were held in net pens and released following data collection. Species, length to the nearest mm, and genetic clips were collected from each fish. Genetics samples were analyzed by the Conservation Genetics Laboratory at the University of Montana in Missoula to produce hybrid index scores. These results will inform a strategy for conserving Westslope Cutthroat Trout in the North and Middle forks of the Flathead River. In addition, Bull Trout were sampled identically to WCT where feasible to inform a range-wide genetic assessment of the species (Figure C5 and Table C2).

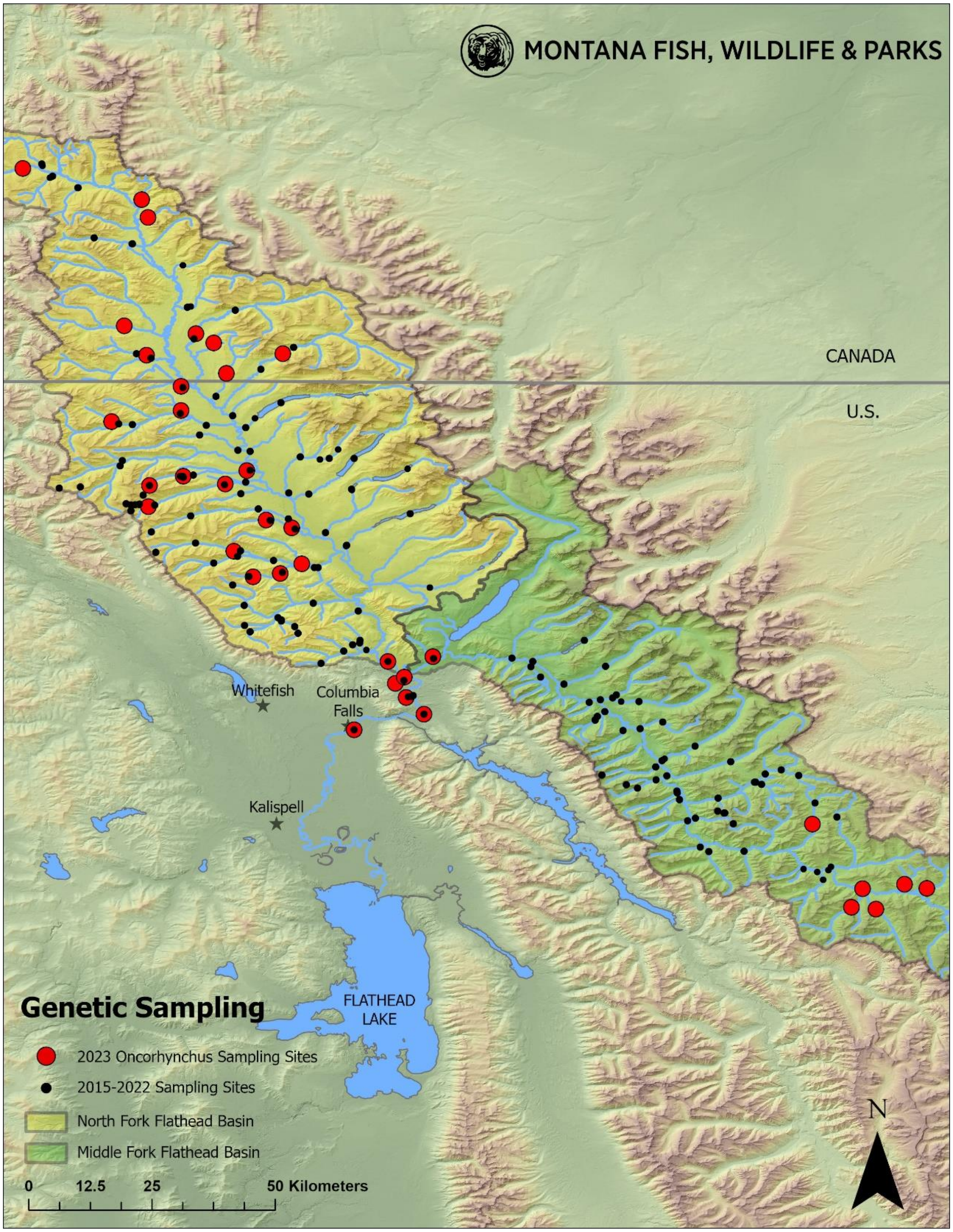


Figure C4.—Locations where fin clips were collected from *Oncorhynchus* during 2015-2023 to evaluate introgression in the Flathead River system, Montana.

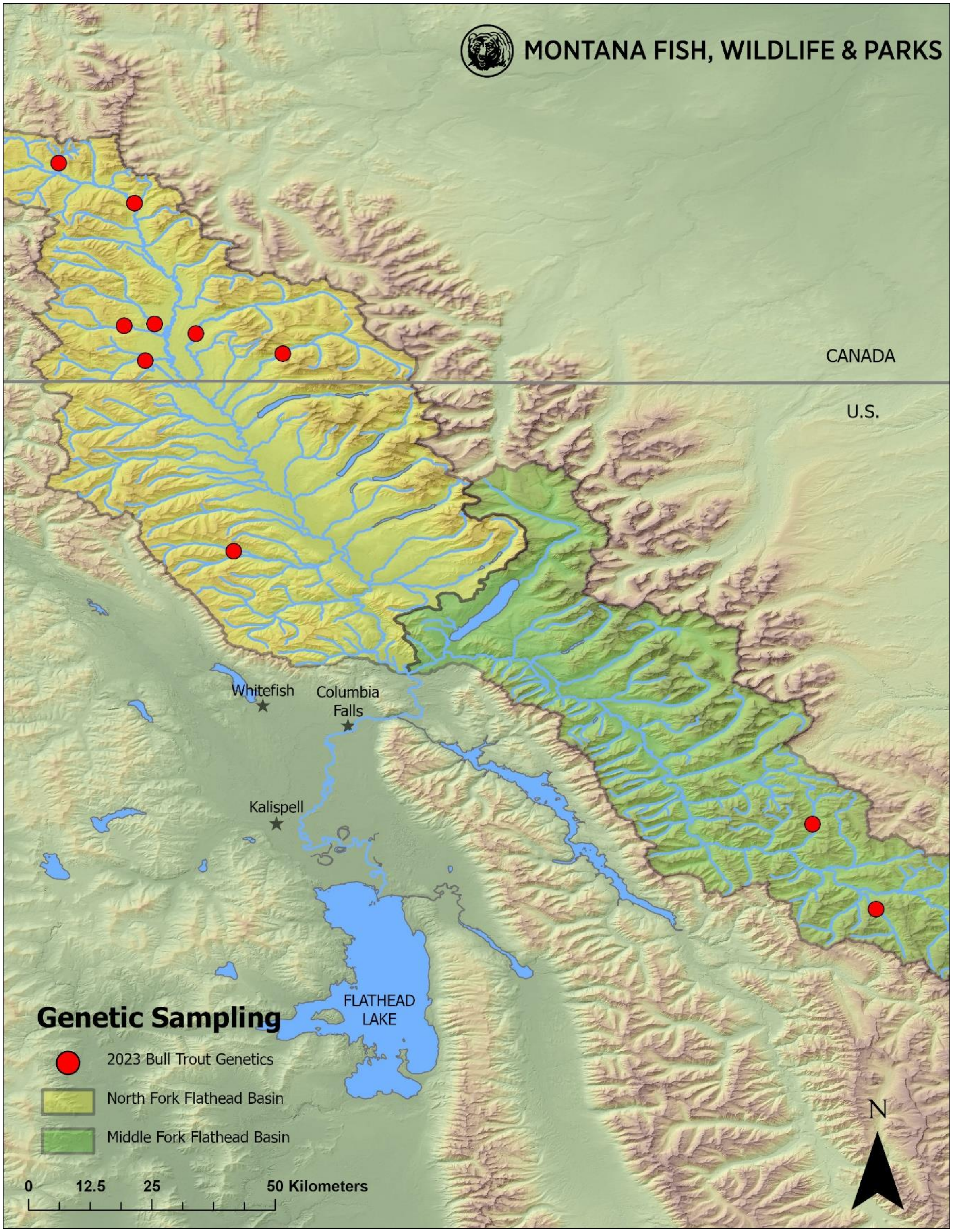


Figure C5.—Locations where fin clips were collected from juvenile bull trout during 2023 to evaluate genetic structure in the Flathead River system, Montana.

Table C1.—Locations surveyed in the North Fork and Middle Fork Flathead drainages during 2023 to collect genetic samples from *Oncorhynchus* for evaluating introgression in the Flathead River system, Montana (n = sample size).

North Fork		Middle Fork	
Location	n	Location	n
Colts Creek	30	Abbot Creek	18*
Cyclone Creek	29	Calbick Creek	31
Deadhorse Creek (Lower)	32	Cox Creek	34
Deadhorse Creek (Upper)	31	Dolly Varden Creek	32
Ketchikan Creek	30	Granite Creek	30
Moose Creek (Lower)	30	Ivy Creek	45
Moose Creek (Middle)	32	Middle Fork River (Schafer)	39
Moose Creek (Upper)	27	Rabe Creek (Lower)	38
Moran Creek	31	Rabe Creek (Middle)	11*
Moran Creek (Lower)	30	Rabe Creek (Upper)	32
North Coal Creek	32	Rubideau Creek	31
Red Meadow Creek (Lower)	30	Schafer Creek	28
Red Meadow Creek (Upper)	31	Third Creek	2*
Tuchuck Creek	32		
Burnham Creek	30		
Cabin Creek	30		
Elder Creek	30		
Kishenehn Creek	33		
Nettie Creek	32		
North Fork Flathead (Upper)	25		
Packhorse Creek	30		
Pollock Creek	33		
Sage Creek	33		

Results and Discussion

Surveys conducted during 2015-2023 to collect genetic samples from *Oncorhynchus* in tributaries of the Flathead River system demonstrated that some drainages continue to remain as genetically unaltered WCT strongholds while others showed increased proportions of RBT introgression since they were sampled last (Figure C6; Tables C3 and C4). These data are critical to informing future management and conservation actions directed toward WCT in the interconnected Flathead River system.

Concurrent with surveys for WCT, genetic samples were collected from 187 Bull Trout across the connected Flathead River system (Table C2). Results of analyses will be reported in future summaries and will inform conservation actions and management of the species.

Table C3.—Hybrid index (HI) scores produced from *Oncorhynchus* sampled in North Fork Flathead River tributaries from 1984 through 2022. Scores range from 0 (genetically unaltered Westslope Cutthroat Trout) to 100 (genetically unaltered Rainbow Trout) and detailed methods can be found in Leary et *al.* 2015.

	Mean HI score								
Site	1984	1994-98	2000-04	2008-11	2015-17	2018-19	2020	2021	2022
Akokala Creek - lower	-	-	-	-	4.7	-	-	-	-
Akokala Creek - mid	-	-	-	-	6.5	-	-	-	-
Akokala Creek - upper	0	-	0	0	1.3	-	-	-	0
Akokala Lake	-	-	-	0	-	-	-	-	0.8
Big Creek - upper	-	-	-	-	-	-	-	0.3	-
Big Creek - middle	-	1.4	-	-	41.9	-	-	-	-
Burnham Creek	-	-	0	0.1	4	-	-	-	-
Camas Creek	-	23.9	21	-	-	-	-	-	53.7
Chain Lake #1	-	-	-	-	-	-	0.2	-	0
Chain Lake #3	-	-	-	-	-	-	51.2*	-	-
Chain Lake #4	0	-	-	-	13.1*	32.2*	-	-	-
Coal Creek	-	-	-	-	28.7	-	-	-	-
Coal Creek - lower	-	3.6	3.6	-	31.9	-	-	-	-
Colts Creek - upper	-	-	0	-	0	-	-	-	-
Commerce Creek	-	-	0	0	0.1	-	-	-	0
Dead Horse Creek - lower	-	-	-	-	0.5	-	-	-	-
Dead Horse Creek - upper	-	-	0	-	0	-	-	-	-
Deep Creek	-	-	0	-	-	-	0	-	-
Depuy Creek	0	-	-	-	-	-	0	-	-
Foisey Creek	-	-	0	0	0.2	-	-	-	0
Ford Creek	-	-	0	-	-	< 1	-	-	-
Harvey Creek	-	-	-	-	0	-	-	-	0
Hawk Creek	-	-	0	-	-	-	0	-	-
Hay Creek - lower	0	1.1	1.4	1.8	0.9	-	-	-	-

Hay Creek - mid	-	-	-	-	0.4	-	-	-	-
Hay Creek - upper	-	-	0	0	0	-	-	-	-
Hay Lake	0	-	-	-	0	-	-	-	-
Ketchikan Creek	-	-	0	-	0.7	-	-	-	-
Kimmerly Creek	-	-	0	-	-	-	0	-	-
Kinnimiki Creek	-	-	-	-	-	-	0	-	-
Kintla Creek	-	-	-	-	0.1	-	-	-	<0.1
Kishenehn Creek	-	-	-	0.5	7.6	-	-	-	-
Logging Creek	-	-	-	-	7.3	-	-	0.8	-
Lower Quartz Lake	-	-	0.2	-	-	-	-	-	0.2
McEvoy Creek - lower	0	-	-	0	0.2	-	-	-	0
McLatchie Creek	0	-	-	0	0.3	-	-	-	0
Meadow Creek - lower	-	-	3.5	3.2	26.4	-	-	-	-
McGinnis Creek – above barrier	0	-	-	-	-	-	65.9*	72.8*	-
McGinnis Creek – below barrier	-	-	9.8	-	-	-	-	-	1.9
Middlepass Creek	-	-	0	0	-	-	-	-	0
Moose Creek - lower	1.5	-	0	-	3.5	-	-	-	-
Moose Creek - mid	-	-	-	-	-	-	0	-	0.2
Moose Creek - upper	0	0	0	0	0	-	0	-	0
Moose Creek - south trib	-	-	-	-	-	-	0	-	-
Moose Lake	-	-	-	-	-	-	-	0.5	-
Moran Creek - lower	-	-	-	-	4.6	-	-	-	-
Moran Creek - upper	-	-	0	-	0	-	-	-	-
Nasukoin Lake	-	0	-	-	-	0.3*	-	-	-
North Fork Coal Creek - lower	-	-	-	-	9.2	-	-	-	-
Quartz Lake	-	-	-	0.1	-	-	-	-	0.1
Red Meadow Creek - lower	0	-	2.2	3.8*	1.4	-	-	-	-
Red Meadow Creek - upper	-	1.4	2.2	0.7	2.2	-	-	-	-
Sage Creek	0	-	-	0	0.4	-	-	-	-

Skookoleel Creek - lower	-	-	0	1.7	2.6	-	0	-	-
Skookoleel Creek - upper	-	-	-	-	0	-	0	-	-
South Fork Canyon Creek	-	-	4*	-	-	-	4.5*	-	-
South Fork Coal Creek - mid	-	6.1	-	-	11.9	-	-	-	-
South Fork Coal Creek - upper	0	-	0.6	-	1	-	-	-	-
South Fork Red Meadow - lower	0	0.9	0.3	-	0.9	-	-	-	-
Spruce Creek	-	-	-	0	0.2	-	-	0.4*	-
Starvation Creek	0	-	-	0	-	< 1	-	-	-
Tepee Creek - lower	-	-	1.3	1.6	10	-	-	-	-
Tepee Creek - upper	-	-	-	-	10.5	-	-	-	-
Third Creek - lower	-	-	65.8	-	91.7	-	-	52.6	-
Tuchuck Creek	-	-	-	-	0.2	-	-	-	-
Werner Creek	-	-	0	-	-	-	-	-	-
Whale Lake	-	90*	-	-	-	0.2*	-	-	-

*Primarily Yellowstone Cutthroat Trout introgression

Table C4.—Hybrid index (HI) scores produced from *Oncorhynchus* sampled in Middle Fork Flathead River tributaries from 1984 through 2022. Scores range from 0 (genetically unaltered Westslope Cutthroat Trout) to 100 (genetically unaltered Rainbow Trout) and detailed methods can be found in Leary et *al.* 2015.

	Mean HI score								
Site	1984	1993-98	2001-04	2010-11	2015-17	2018-19	2020	2021	2022
Abbot Creek - lower	92	97.5	91.6	99.4	68.9	90.4	71.4	-	-
Bear Creek - lower	-	-	-	-	3.3	-	-	-	-
Bear Creek - upper	-	-	-	-	0.2	-	-	-	-
Bradley Creek	-	-	-	-	-	-	-	-	0
Castle Lake	-	-	-	-	-	-	-	1.8*	-
Challenge Creek	-	-	-	-	0	-	-	-	0.1
Charlie Creek	-	-	-	-	-	-	-	1.6*	-
Coal Creek	-	5.3	-	-	-	-	-	-	27.9
Crystal Creek	-	-	-	-	-	-	0	-	-
Dickey Lake	-	-	0	-	-	0	-	-	-
Dirtyface Creek	-	-	-	-	-	-	-	0.4	-
Elk Lake	-	0	-	-	-	0	-	-	-
Essex Creek - mid	-	-	-	-	1	-	-	-	-
Essex Creek - upper	-	1.4	-	-	1	-	-	-	-
Giefer Creek	-	-	-	-	-	0.4*	-	-	-
Ivy Creek - lower	-	-	49.3	41.4	30.1	-	17.3	21.1	-
Ivy Creek - upper	-	-	-	-	11.3	-	-	-	-
Java Creek	-	-	-	-	-	0.9*	-	-	-
Lake Creek	-	-	-	-	-	-	-	-	0
Lincoln Creek - lower	-	-	-	-	91.7	-	-	-	-
Marion Lake	-	81*	-	-	-	72	-	-	-
MF Flathead River - mid	-	0	-	0	-	-	-	-	0.1

Morrison Creek	-	-	-	-	-	< 1*	-	-	0.1
Muir Creek	-	-	-	0.6	0.7	-	-	-	6.2
Park Creek	-	0	-	0.1	2.9	-	-	-	8.4
Pinchot Creek	-	0	-	-	4.7	-	-	10	-
Rabe Creek - lower	-	-	49.1	55.1	43.6	-	-	-	-
Rubideau Creek – lower	-	11.1	-	-	3.5	-	-	-	-
Sheep Creek	-	-	-	-	-	2.7*	-	-	-
Sheep Creek - above barrier	-	-	-	-	-	-	-	0.5	-
Skyland Creek	-	-	-	-	0	0	-	-	-
Spruce Creek	-	-	-	-	-	-	-	6	-
Stanton Creek	-	-	-	-	2	-	-	-	-
Tranquil Basin - east	-	11*	-	-	-	-	-	0.1	-
Tranquil Basin - west	-	43*	-	-	-	-	-	14.3*	-
Tunnel Creek - lower	-	-	-	-	0	-	-	-	-

*Primarily Yellowstone Cutthroat Trout introgression.

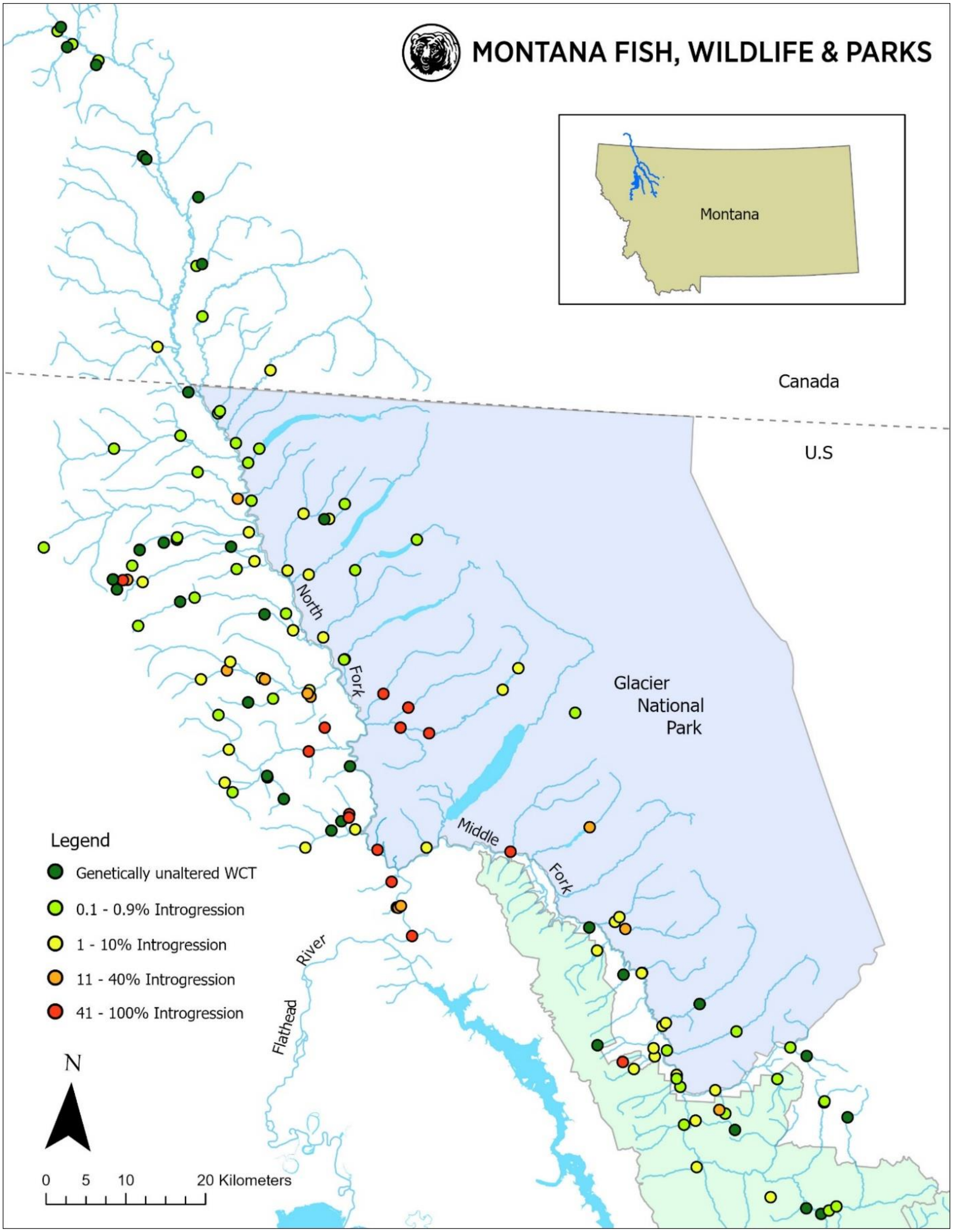


Figure C6.—Status of introgression between Westslope Cutthroat Trout and Rainbow Trout across locations sampled during 2015-2022 in the interconnected Flathead River system.

Fish Passage Barrier Surveys

Introduction

The merits of isolating genetically pure Westslope Cutthroat Trout (WCT) populations upstream of fish passage barriers were considered for application in the Flathead Subbasin beginning in 2019, to both protect existing cutthroat populations and to diversify conservation tools employed in the drainage. While often considered a “last resort” option, the intentional isolation of pure WCT populations from the threat of introgression in the upper Flathead River system may play a key role in the long-term persistence of this iconic species.

A first step included identifying potential candidate streams for barrier construction. We addressed this need by developing a decision framework which weighed opportunities and constraints associated with selecting candidate streams and appropriate sites for barrier construction (Steed et *al.* 2022). We subsequently conducted preliminary surveys of candidate streams during 2018-2021 to characterize site suitability and other decision framework metrics. Data collected identified Moose Creek in the North Fork drainage as a top candidate for barrier construction during autumn 2025, with annual fisheries monitoring conducted since 2020 to establish pre-barrier densities and genetic status. Previously identified candidate streams precluded because introgression was detected, poor barrier site, and/or functional natural isolation. Details of the decision framework, preliminary surveys, and associated conclusions can be found in Steed et *al*. (2022).

Methods

Moose Creek was identified as a top candidate drainage for barrier construction, warranting annual fisheries surveys beginning in 2020. Genetic samples were collected from fish by backpack electrofishing in upper and lower sections upstream of the proposed barrier site (Figure C6). See the Genetics Surveys section for methods used. Two juvenile density estimate reaches were also established upstream from the proposed barrier site during 2020 (Figure C6).

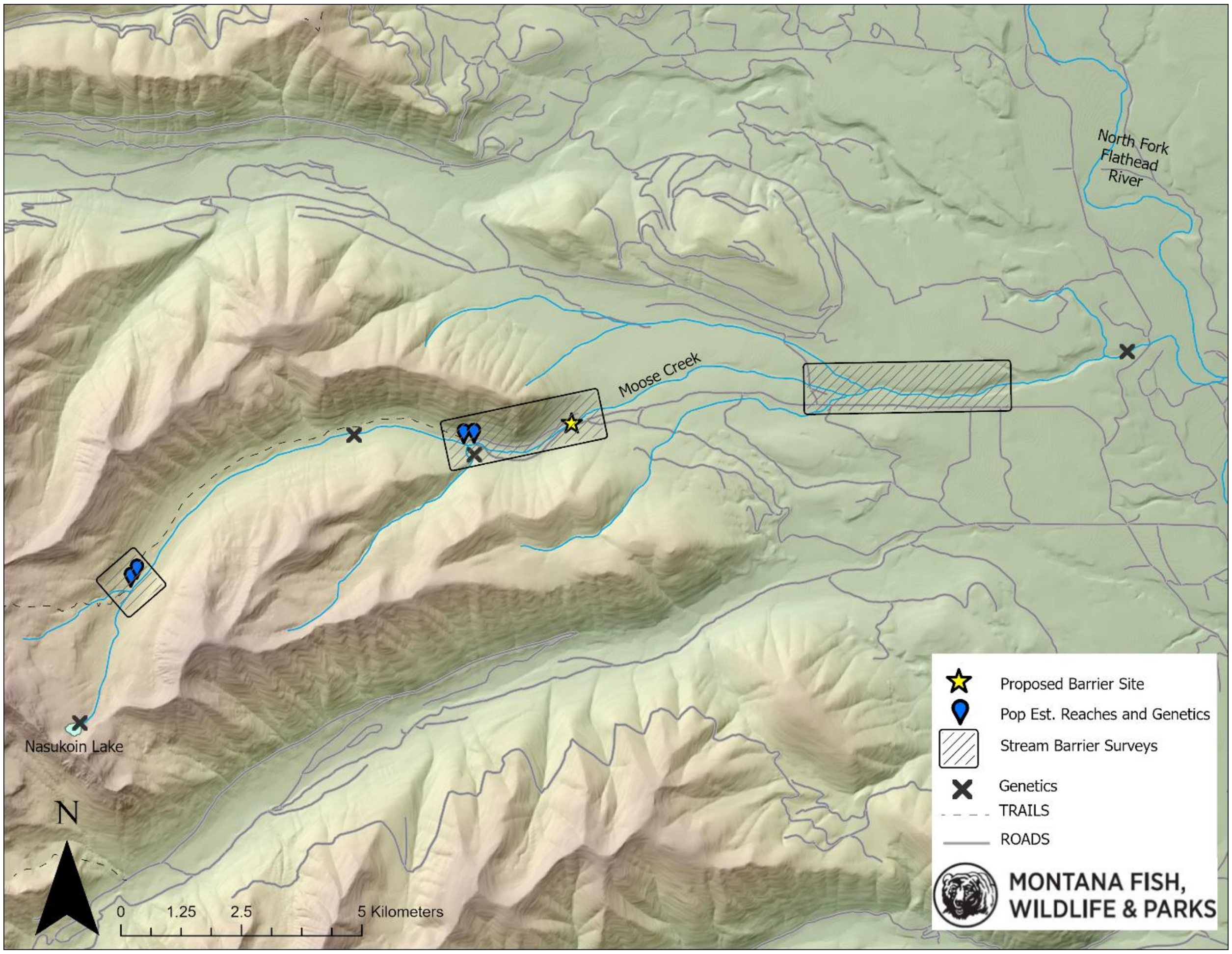


Figure C6.—Moose Creek drainage showing proposed barrier site location and long-term fisheries survey locations.

Results

Juvenile (age-1+) WCT density estimates were conducted at two sites upstream of the proposed barrier location during 2020-2023 (Figure C6; Table C6). Estimated densities were comparable to those reported for other tributaries in the North Fork Flathead drainage and will continue to be monitored annually (Weaver et al. 2005; FWP unpublished data). Genetic results will be available in a subsequent annual report.

Table C6.—Estimated juvenile Westslope Cutthroat Trout densities (age-1+) in 150-m sections established upstream of a proposed fish passage barrier site in Moose Creek, a tributary of the North Fork Flathead River. Capture probability is noted as *p* and 95% confidence intervals are included with estimates.

	2020		2021		2022		2023	
Stream section	Fish/100 m^2	*p*	Fish/100 m^2	*p*	Fish/100 m^2	*p*	Fish/100 m^2	*p*
Upper	7.79 +/- 0.72	0.79	6.64 +/- 0.33	0.88	5.94 +/- 1.26	0.47	4.93 +/- 0.86	0.75
Lower	7.76 +/- 1.17	0.73	6.52 +/- 0.87	0.75	7.73 +/- 2.97	0.61	7.36 +/- 0.88	0.76

Stream and Selective Withdrawal System Temperature Monitoring

Introduction

Monitoring stream temperature is a relatively simple and cost-effective technique for capturing baseline thermal regimes that are important driving factors in the function of aquatic ecosystems (Heck et *al.* 2018). Changes in stream temperature over time can occur by a variety of factors including warming air temperature, changes in riparian habitat (degradation), changes in the shape and complexity of stream channels, decreasing glaciers and snow cover, and changing precipitation patterns (Heck et *al.* 2018). Thermographs in select streams in the Flathead River system have been employed for the last 1-26 years (Table C7) to track long term changes in annual stream temperatures related to these factors.

Monitoring of the thermal releases at Hungry Horse Dam using the Selective Withdrawal System (SWS) will reported herein annually to ensure compliance with FWP guidelines and associated benefits to downstream aquatic resources.

Methods

Streams

We use standard methodology (Dunham et *al.* 2005; Isaak 2011; Heck et *al.* 2018) for monitoring stream temperature using digital data loggers. Stream temperatures are recorded with digital thermographs (Hobo Water Temp Pro v2, accuracy ±0.2 C; Hobo Pendant Mx Water Temp Data Logger, accuracy ±0.5 C; Onset Computer Corporation, Pocasset, Massachusetts, USA) logging daily temperature at hourly intervals. Three long-term locations exist on the Flathead Mainstem just upstream of Flathead Lake, the mainstem of the North Fork and the mainstem of the Middle Fork at USGS gauging sites (Figure C7). Most monitoring locations are in tributaries to the Nork Fork of the Flathead River up to the Canadian border.

Thermographs are fixed into the stream using plastic coated wire attached to a strong stream side anchor point with good protection from high spring flows. Housing for data loggers are made from perforated PVC piping to protect the loggers and provide strong attachment points to instream anchor points. Data loggers are visited ideally once a year for data offloads and to insure data loggers are not lost or damaged for a significant amount of time. Raw temperature data are stored and can be filtered into average daily temperatures, daily min and max, mean monthly temperatures and mean annual temperatures.

Hungry Horse Dam

Data derived from the USGS Waterwatch was used to compare release temperatures to FWP guidelines during the period of SWS operation during 2022 (June-November).

Results and Conclusions

Streams

Stream temperature monitoring databases are continually updated annually and shared openly with other collaborative research projects in the basin (Table C7). Recent thermograph deployments (< 2 years) have been implemented in stream reaches associated with biological data being collected on juvenile Bull Trout and their rearing habitats. Additional thermographs have also been deployed in 2020 on streams being considered for isolating genetically unaltered Westslope Cutthroat Trout (WCT) populations. Two additional sites were installed in Moose and Hay creeks of the North Fork Flathead drainage during 2021. These two sites are located within 200 m of potential fish passage barrier construction sites and continuously record stream temperature, barometric pressure (psi), absolute pressure (psi), differential pressure (psi), and water level (ft). We will use the continuous water level and temperature to better understand flow regimes as it relates to unaltered populations of WCT pre and post fish barrier construction.

Table C7.—Stream locations of long-term thermograph monitoring sites in the Flathead system. Flathead River = FHR, North Fork Flathead River = NF, South Fork Flathead River = SF.

Drainage	Location	Site	Years of record	Daily mean annual temp (°C)
Mainstem	FHR	FHR_01	26	7.9
Middle Fork	MF FHR	FHR_02	26	6.9
	Ivy Creek	FHR_18	18	5.6
	Rabe Creek	FHR_19	15	5.5
	Abbot Creek	FHR_20	15	7.3
	Skyland Creek	FHR_24	4	3.8
	Bear Creek	FHR_25	4	5.2
North Fork	NF FHR	FHR_03	23	6.4
	Langford Creek	FHR_04	20	6.2
	Third Creek	FHR_05	17	6.9
	Cyclone Creek	FHR_06	17	6.7
	Big Creek	FHR_07	12	5.6
	Moran Creek	FHR_08	19	4.0
	Moose Creek	FHR_09	19	3.4
	Tepee Creek	FHR_10	18	5.2
	Red Meadow Creek	FHR_11	17	4.7
	Hay Creek	FHR_12	18	4.2
	Trail Creek	FHR_13	19	4.6
	Colts Creek	FHR_14	20	4.6
	Sage Creek	FHR_15	18	5.4
	Foisey Creek (B.C.)	FHR_16	18	3.5
	NF FHR, McLatchie Br.	FHR_17	13	3.8
	SF Coal Mid	FHR_22	5	3.8
	SF Red Meadow	FHR_23	4	4.2
	Skookoleel Creek	FHR_26	4	3.7
	North Fork Flathead (B.C.)	FHR_27	1	n/a
	Rabe Creek	FHR_28	5	4.9
	Sage Creek	FHR_29	5	5.2
Swan	Goat Creek Upper	SR_01	1	n/a
	Goat Creek Mid	SR_02	1	n/a
	Goat Creek Lower	SR_03	26	7.9
	Squeezer Creek Upper	SR_04	26	6.9
	Squeezer Creek Mid	SR_05	18	5.6
	Squeezer Creek Lower	SR_06	15	5.5
	Gray Wolf Lake Inlet	SR_07	15	7.3
	Gray Wolf Lake Outlet	SR_08	4	3.8

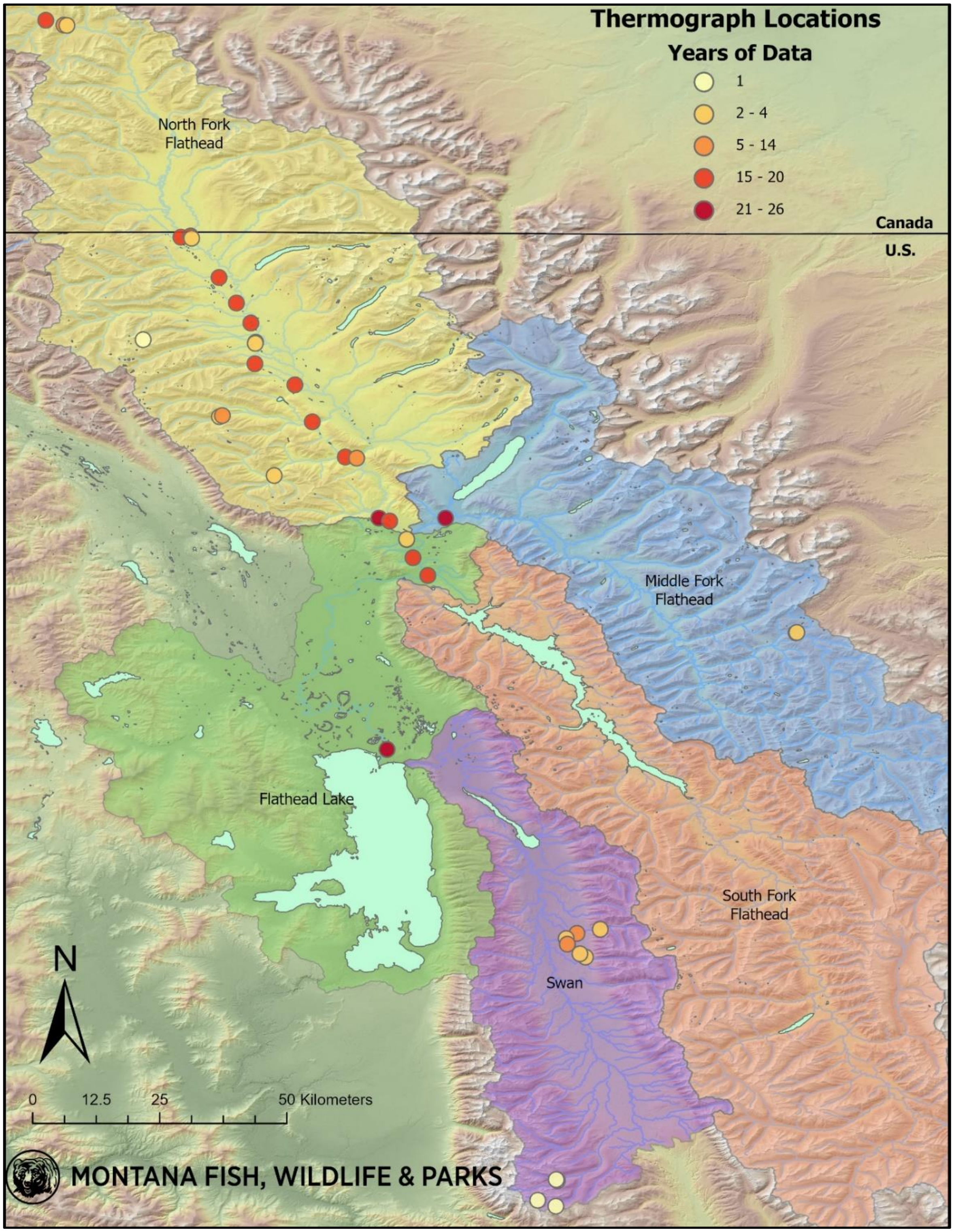


Figure C7.—Thermograph locations in the Flathead River system corresponding to the duration (years) of data continuously recorded.

Hungry Horse Dam

During the period of 2023 that the SWS operated at Hungry Horse Dam, the Bureau of Reclamation exceeded thermal guidelines provided by FWP on 3 separate days (Figure C8; Table C8). This was equivalent to the 3 days it exceeded in 2022 and a decrease from the 9 and 6 days it exceeded in 2021 and 2020, respectively. In contrast, the daily minimum temperature of dam releases fell below minimum recommended values on 54 days during 2023 in comparison to 73 days during 2022, 63 days during 2021, and 56 days during 2020. In most instances, however, this likely reflects reservoir temperatures being too cold to meet guidance values.

Table C8.—Dates in which water temperature (°C) released from Hungry Horse Dam into the South Fork Flathead River exceeded FWP guidelines, as measured at the USGS gauging station 12362500. Data source: USGS Waterwatch.

Date	SF min	SF mean	SF max	FWP min	FWP opt	FWP max	Exceedance
8/19/2020	14.8	15.9	16.9	12.8	14.8	16.8	0.1
8/20/2020	15.1	15.6	16.9	12.8	14.8	16.8	0.1
8/25/2020	14.7	15.8	16.5	12.4	14.4	16.4	0.1
9/13/2020	14.1	14.4	14.8	10.7	12.7	14.7	0.1
9/21/2020	12.7	13.7	14.2	10.1	12.1	14.1	0.1
9/22/2020	13	13.4	14.1	10	12	14	0.1
9/25/2021	13.4	13.6	13.9	9.8	11.8	13.8	0.1
9/26/2021	13.8	13.8	14	9.7	11.7	13.7	0.3
9/27/2021	13.2	13.7	14	9.6	11.6	13.6	0.4
9/28/2021	11.7	12.6	13.7	9.5	11.5	13.5	0.2
10/1/2021	12.8	13.1	13.4	9.3	11.8	13.3	0.1
10/2/2021	12.9	13.1	13.3	9.2	11.7	13.2	0.1
10/3/2021	12.8	13	13.3	9.2	11.6	13.2	0.1
10/5/2021	12.8	13	13.3	9	11.4	13	0.3
10/6/2021	11.9	12.7	13.1	8.9	11.3	12.9	0.2
8/27/2022	14	15.8	17.1	12.2	14.2	16.2	0.9
9/20/2022	10.5	12.1	14.4	10.1	12.1	14.1	0.3
9/21/2022	11.9	13	14.3	10.1	12.1	14.1	0.2
8/18/2023	13.6	15.6	17.4	12.9	14.9	16.9	0.5
9/5/2023	14.1	14.9	15.6	11.5	13.5	15.5	0.1
9/18/2023	11.5	12.8	14.4	10.3	12.3	14.3	0.1

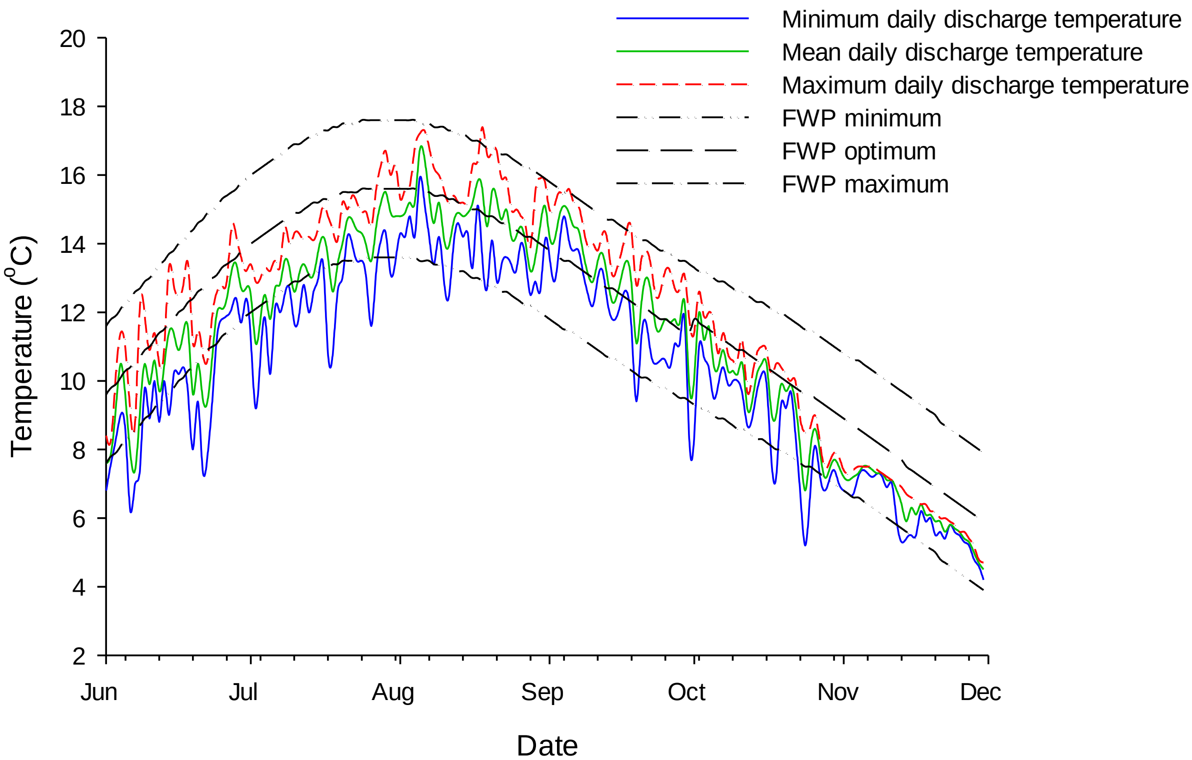


Figure C8.—Thermal discharge from Hungry Horse Dam as reported from USGS gauging station 12362500 during the period of Selective Withdrawal System operation in 2023 compared to FWP's recommended thermal guidelines. Data source: USGS Waterwatch.

Barrier Candidate Stream Discharge Gauges

Introduction

Streamflow and stream temperatures are key influencers of physical and biological processes within stream ecosystems (Heck et *al.* 2018). Recent awareness has pointed to a lack of streamflow data resolution in headwater streams, with the current network of gaging stations in North America focused on at the mainstem river scale (Kovach et *al.* 2019). Existing technology allows for remote continuous discharge stations to better understand relationships between biological, thermal, and hydrologic data in wadable headwater streams (EPA 2014). We aim to use this technology to monitor stream discharges pre and post barrier construction on intentionally isolated genetically unaltered populations of Westslope Cutthroat Trout (WCT).

Methods

We used methodology described by the EPA (2014) for "Best practices for continuous monitoring of temperature and flow in wadable streams". We used Onset Hobo Mx2001 Water Level data logger (Onset Computer Corporation, Pocasset, Massachusetts, USA) to hourly record water temperature, barometric pressure (psi), absolute pressure (psi), differential pressure (psi), and water level (ft) in two stream locations, Moose Creek and Hay Creek. While we aim to isolate upper Moose Creek with a constructed barrier by 2025 to protect genetically unaltered WCT, Hay Creek was removed from initial consideration because introgression was detected upstream of a potential barrier site. However, it may offer opportunity for future piscicide treatment paired with barrier construction and warrants continued maintenance of its level logger. Locations of the continuous discharge stations are within 200 meters downstream of potential constructed barrier sites.

The discharge stations are fixed into the stream with t-posts driven into the stream bed. Cable supports were added with eye-bolt anchors secured into rock boulders to give additional support from downstream water pressures on stations during higher flows. Careful consideration was given to site locations to ensure that the pressure transducer would remain under water during low flows and the stream bank was stable and offered boulder protection during high flows. A WaterMark® Style "A" Stream Gauge (0.00' – 3.33') was used to visually record stage height during site visits and as a calibration reference for the water level recorded by the MX2001 transducer. The stream gauge and MX2001 transducer are all mounted on 2" x 8" material, which is u-bolted to the t-post. The MX2001 transducer is housed within perforated PVC pipe equipped with a well cap for easy access to the transducer.

An elevation survey was conducted to georeference the site immediately following installation of discharge stations. Quantifying initial elevations of the staff gage and pressure transducer establishes a reference point to document changes at the site and ensure that corrections can be made to the stage data if the instruments move due to high flows or ice events (EPA 2014). Elevation surveys will be conducted annually or after major hydrologic events. Each visit to the site consists of recording stream temperature, downloading transducer data via Bluetooth, visually recording stage height (ft), recording water level displayed by transducer and taking a manual cross section flow Q (m^3/s). Recording the observed stage height at every visit is important to detect and correct for drift in transducer water level readings, as gages can be re-calibrated to match the observed stage height. All cross-section discharges are taken using a Hach FH950 handheld flow meter.

Figure C9.—Discharge station on Moose Creek, a tributary of the North Fork Flathead River.

Results and Conclusions

After site installation and elevation survey documentation, sites were visited at least once per month for maintenance, transducer data offloads, and manual cross sectional flow measurements (EPA 2014). We will continue to monitor site conditions and transducer accuracy over time and after high flows and flooding events. Stage data from the transducer and staff gauge will be used with manual discharge measurements to develop a stage-discharge rating curve. Converting stage measurements to streamflow provides valuable flow volume estimates. A minimum of five to ten discharge measurements taken over a variety of stream flows that covers as wide a range as possible is needed to start building a relationship curve (EPA 2014). We currently do not have enough manual flows at varying flow levels to have a strong relationship curve. However, we have run through the exercise of calculating the initial rating curve. We plotted our observed discharges with the observed water level recorded by the transducer using the log-log transformation method (Kennedy 1984; Sauer 2002; EPA 2014; USGS *personal communication*). We also plotted non-transformed data with a power trendline in R (nonlinear least square method). Both methods produced similar results for curve fitting parameters. Additional discharge measurements will be collected during 2023 to build a stronger rating curve. We will also use residual plotting to monitor our rating curves.

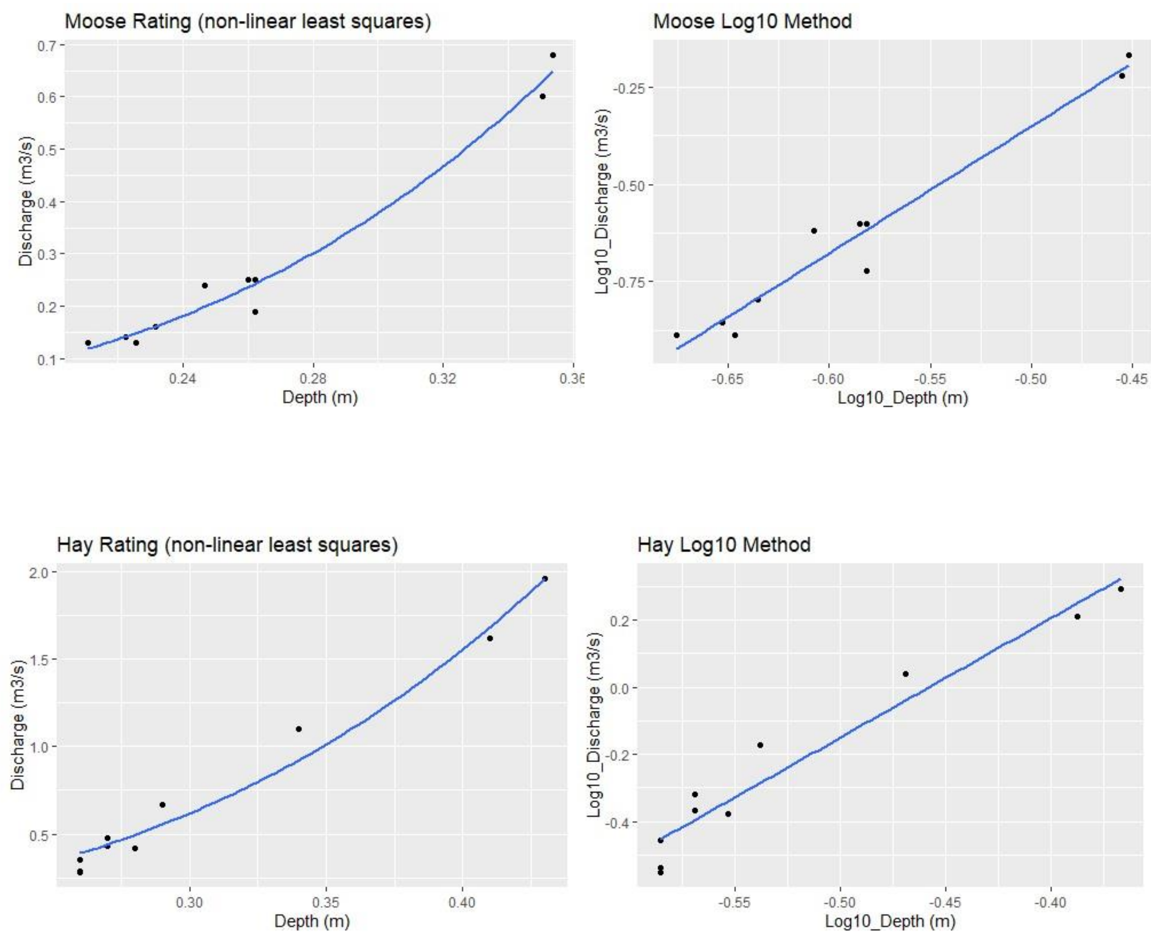


Figure C10.—Rating curves for Hay and Moose creeks. Plots on the left show regular nonlinear least square rating and plots on the right show transformed log-log scale rating. Power function (nonlinear least square) $y=aX^b$ is equivalent to Log10Q=b*(Log10depth) + Log10a.

Table C9.— Discharge sites and manual cross section flows taken during 2021-2023 in tributaries to the North Fork Flathead River.

				Manual flows recorded			
Site Name	Lat.	Long.	Deployed	2021	2022	2023	Total
Moose Creek	48.830233	-114.462286	8/26/2021	4	3	3	10
Hay Creek	48.768528	-114.346607	8/27/2021	5	3	2	10

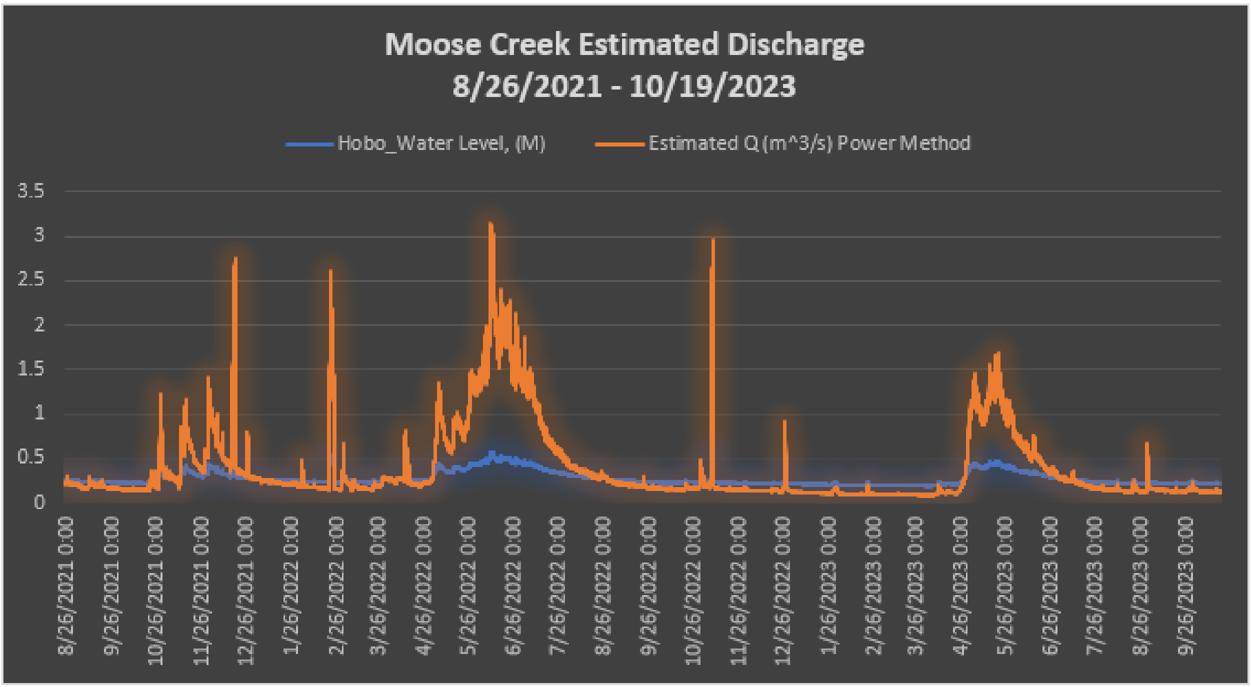


Figure C11.—Estimated discharge Q (m^3/s) hydrograph on Moose Creek at proposed barrier site using power method rating curve from 8/26/2021 to 10/28/2023.

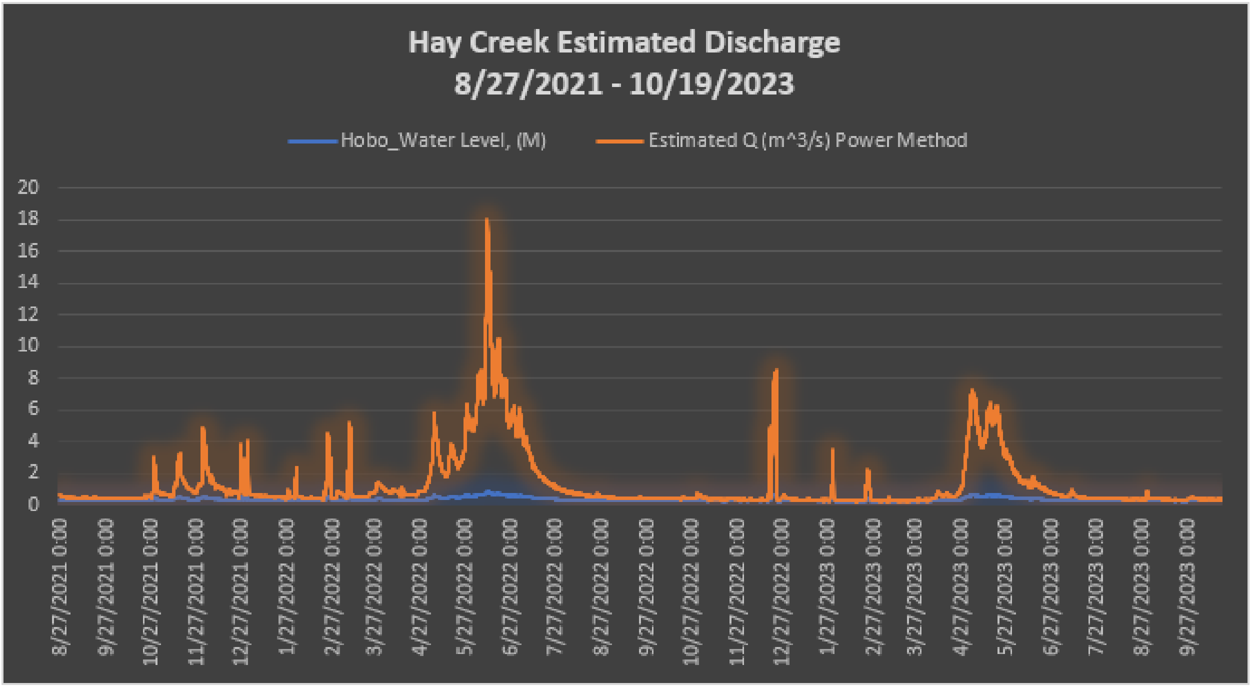


Figure C12.—Estimated discharge Q (m^3/s) hydrograph on Hay Creek at potential barrier site using power method rating curve from 8/27/2021 to 10/28/2023.

Electrofishing Survey of the Whitefish River

Introduction

The Whitefish and Stillwater rivers were surveyed during 2022 to evaluate the potential of establishing long-term fisheries population monitoring reaches using boat-based electrofishing (Steed et *al.* 2023). Access to these drainages is limited because they are primarily bordered by private lands within a semi-urban landscape (Figure C13). A section of the Whitefish River from Pine Grove Fishing Pond to West Reserve Drive was chosen for a single pass electrofishing survey on May 31, 2023 (Figure C14).

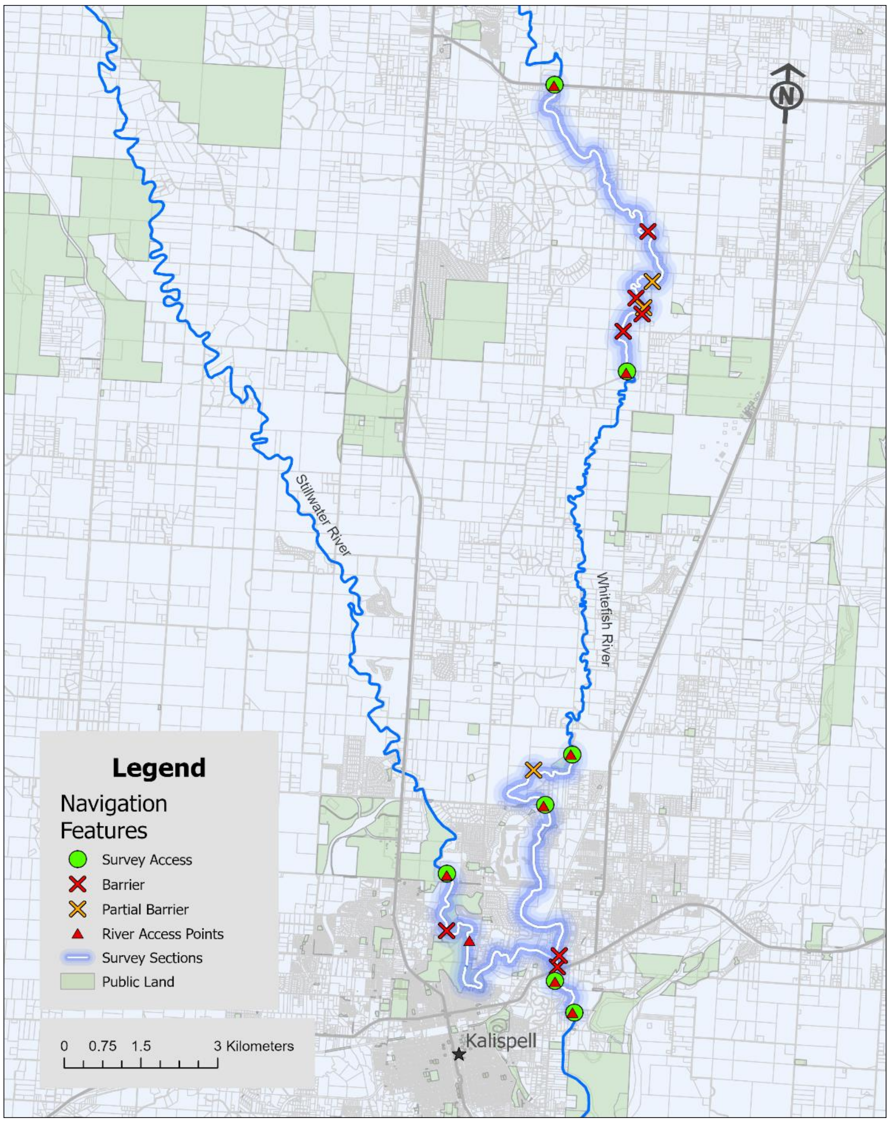


Figure C13.—Locations of reconnaissance surveys performed in the Whitefish and Stillwater drainages during 2022.

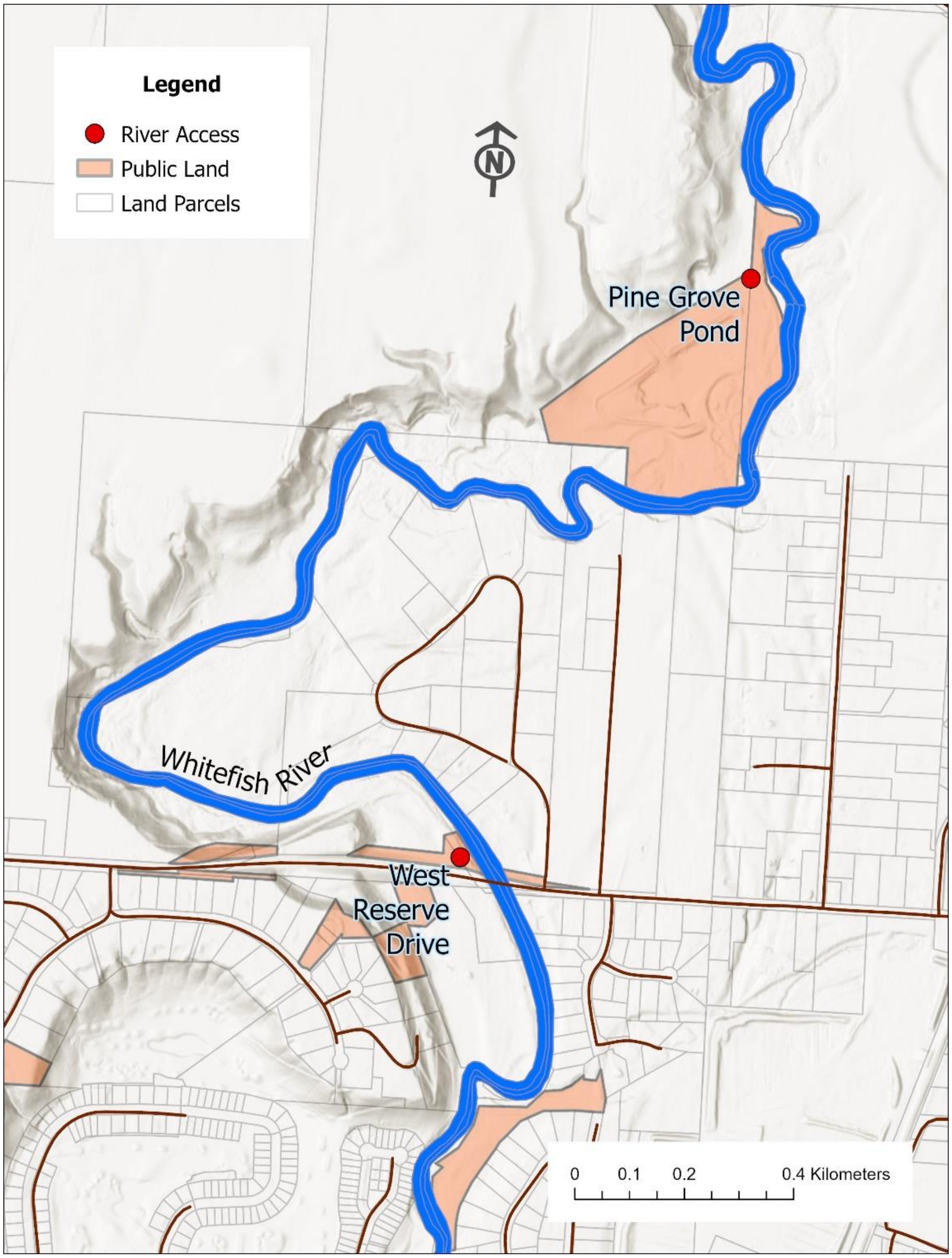


Figure C14.—Location of electrofishing survey performed on the Whitefish River May 31, 2023.

Methods

Electrofishing was performed from a drift boat rigged with fixed-boom anodes. Personnel included one rower/boat operator and one netter. A third person using a small inflatable raft was employed as a safety observer to monitor for potential downstream barriers and explain the survey to any curious landowners. A Coffelt M22 unit was operated to produce straight DC at 3 to 5 amperes in adherence to FWP electrofishing policy dictating the use of straight DC or pulse rates ≤ 30 Hz when sampling waters with native fishes. The survey was conducted during daylight hours and began at Pine Grove Pond and progressed downstream along one of the banks to the lower boundary at West Reserve Drive in Evergreen, MT. Shock-time was recorded to estimate catch-per-unit-effort (CPUE), calculated as the number of a given fish species captured divided by the time (hr) spent electrofishing and the length of the sample section (km) (McMullin and Graham 1981). All fish were netted, identified to species, measured for total length (mm) and released back into the river.

Results

The survey was completed on May 31 2023 on the descending limb of the hydrograph. The discharge was 11.8 m^3/s (USGS gage 12365700) and the reach length was 2.9 km. The water temperature was 16°C and the conductivity was 164 µs/cm. Six species of fish were sampled including Mountain Whitefish, Largescale Sucker, Northern Pikeminnow, Redside Shiner, Westslope Cutthroat Trout and one hybrid trout (Westslope Cutthroat Trout x Rainbow Trout, or *Oncorhynchus sp*.) (Table C10).

Table C10.—Summary of fish sampled in the Whitefish River on May 31 2023.

Species	n	Mean length (mm)	Range (mm)	#/hr	#/km	#/km/hr
Mountain Whitefish	46	233	115-355	41.8	15.9	14.5
Largescale Sucker	14	372	140-450	12.7	4.8	4.4
Northern Pikeminnow	4	291	136-469	3.6	1.4	1.3
Redside Shiner	1	-	131	0.9	0.3	0.3
Westslope Cutthroat Trout	1	-	78	0.9	0.3	0.3
Oncorhynchus sp.	1	-	402	0.9	0.3	0.3

Mountain Whitefish Population Monitoring

Introduction

To broaden our knowledge of native species trends in dam-influenced portions of the Mainstem Flathead River, we initiated a Mountain Whitefish (MWF) population estimation in 2017. Relatively little is known of this native species within the Flathead River system related to its localized life history or population demographics. In 2018, an estimate was performed for MWF in a section of the Mainstem Flathead River near Old Steel Bridge in Kalispell, downstream of where FWP also sampled for trout (Figure C1) to begin monitoring trends in abundance and condition independent of the trout estimate. Methodology used to estimate hybrid and Rainbow Trout abundance upstream (described above) was replicated for standardization (Deleray et *al*. 1999; Steed et *al*. 2008-2023; Boyer et *al*. 2014-2015).

Methods

In the past, both estimates of abundance (Lincoln Peterson) and catch per unit effort (CPUE) of MWF were conducted in the Mainstem Flathead River. However, only a single-pass estimate of CPUE was implemented during 2020 due to concerns with assumption violation during mark and recapture efforts (e.g., immigration, emigration, mortality, etc.). Further, the survey section length was increased from 2 km to 3.3 km. The CPUE survey for MWF began at dark on March 13 2023, completing one pass using two boats simultaneously surveying each bank. Electrofishing was performed from jet boats rigged with fixed-boom anodes. A Coffelt M22 unit was operated to produce straight DC at 3 to 4 amperes in adherence to FWP electrofishing policy dictating the use of straight DC or pulse rates $\leq$ 30 Hz when sampling waters with native fishes. Oxygen was used in onboard live wells to minimize fish mortality.

Passes began at the upstream boundary of each section and progressed downstream along one of the banks to the lower boundary. Shock-time for each pass was recorded to estimate CPUE. All fish were netted, measured for total length (mm) and weight (g). Catch per unit effort was calculated as the number of MWF captured divided by the time (hr) spent electrofishing and the length of the sample section (km) (McMullin and Graham 1981). Temperature (°C), discharge (CFS), and conductivity (μS) were also recorded.

Results

Estimates of relative abundance (CPUE) for adult ($\geq$ 250 mm) and juvenile (<250 mm) MWF were calculated in 2023 (Figures C20 and C21). A total of 578 MWF were sampled, ranging in length from 77 mm to 485 mm (mean = 248 mm). Westslope cutthroat trout, *Oncorhynchus sp*., Bull Trout, Brook Trout and Long-nosed Suckers were also observed during the survey.

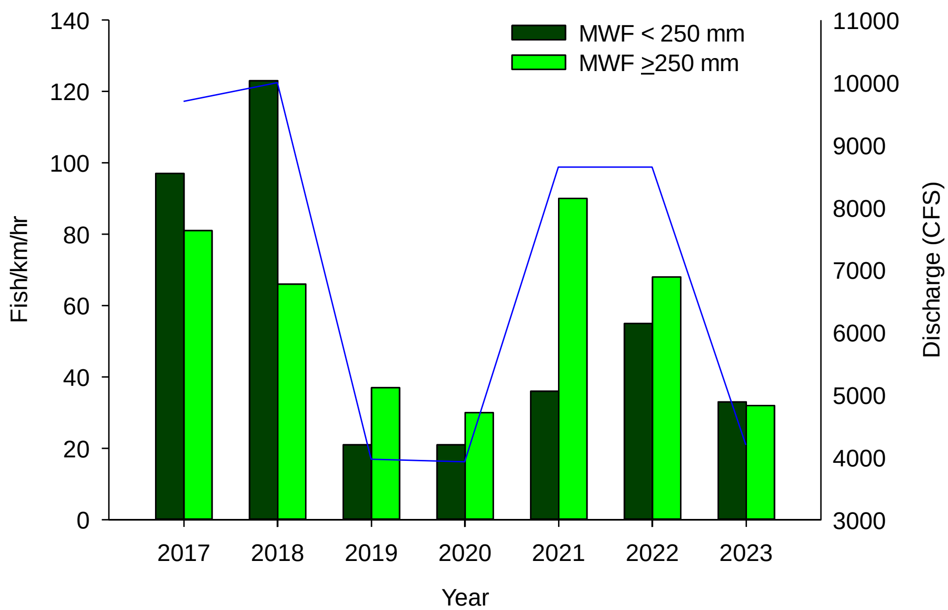


Figure C20.—Catch per unit effort (CPUE), by size class, of Mountain Whitefish relative to discharge at Columbia Falls in the Mainstem Flathead River near Kalispell during March 2017-2023.

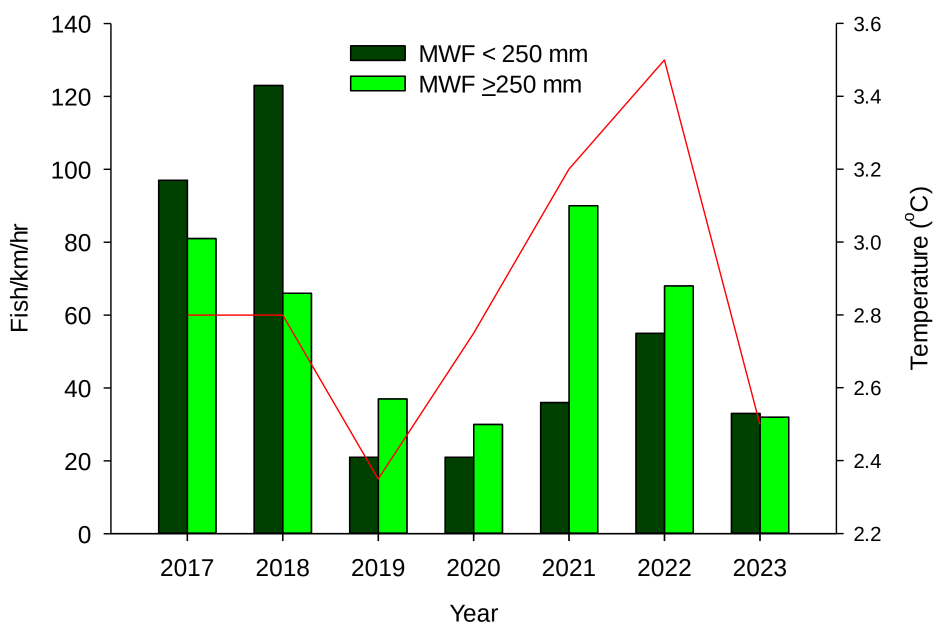


Figure C21.—Catch per unit effort (CPUE), by size class, of Mountain Whitefish relative to temperature at Columbia Falls in the Mainstem Flathead River near Kalispell during March 2017-2023.

D. Bull Trout Warden Patrols

1/1/2023-12/31/2023 76916 REL 22 and 84064 REL 3 O: Expanded Bull Trout patrols/related enforcement actions

This Work Element supports operational costs of additional enforcement patrols and activities intended to provide more aggressive protection of Bull Trout in sensitive project areas and to increase enforcement presence in support of fisheries crews working in remote locations. This additional enforcement work is above and beyond normal capabilities of the enforcement program and helps protect Hungry Horse Mitigation Program investments aimed at promoting Bull Trout recovery.

Summary of Work Element Metrics:

Arrests: 0

Citations issued: 36

Seizure made: 2 Bull Trout, 7 Westslope Cutthroat Tutthroat

E. Bull Trout Spawning Habitat Monitoring and Translocation Opportunities

1/1/2023-12/31/2023 76916 REL 2 and 84064 REL 3 L: Bull trout spawning habitat monitoring: data collection
1/1/2023-12/31/2023 76916 REL 2 and 84064 REL 3 M: Bull trout spawning habitat monitoring: data analysis

Introduction

Successful egg incubation and fry emergence for Bull Trout (*Salvelinus confluentus*) are dependent upon gravel composition, gravel permeability, water temperature and surface flow conditions. Weather, streamflow, and transport of fine sediment and organic material in the stream can change conditions in redds during the incubation period. Redds can be disturbed by other spawning fish, animals, human activities (e.g., logging), or by high flows which displace streambed materials (Chapman 1988). For successful emergence to occur fry need to be able to move within the redd, but high levels of fine sediment can restrict their movements (Bjornn 1969; Phillips et *al.* 1975). In some instances, embryos that incubate and develop successfully can become entombed (trapped by fine sediments). Sediment levels can alter timing of emergence (Alderdice et *al.* 1958; Shumway et *al.* 1964) and affect fry condition at emergence (Silver et *al.* 1963; Koski 1975).

Evaluations of the spawning substrate used by Bull Trout in the Flathead River system have been performed by MFWP in Montana since the early 1980s. Annual substrate coring analyses have provided reliable indicators of embryo survival to emergence, linking landscape level and localized disturbances such as timber harvest to spawning habitat quality. Though extensive monitoring has occurred within the Flathead drainage in Montana, no evaluations of Bull Trout spawning habitat quality has taken place in the headwaters of the North Fork Flathead River (Transboundary Flathead) in British Columbia (B.C.), Canada. The Transboundary Flathead River within Canada typically comprises nearly half of all documented Bull Trout redds across the drainage, and 20% of all interconnected habitat in the entire Flathead River system (North and Middle forks combined). With recent and anticipated logging taking place throughout the drainage, there is urgent need to monitor the quality of Bull Trout spawning habitat through time.

Methods

Sites in immediate proximity to Bull Trout spawning and rearing in the Transboundary Flathead River within B.C. were selected for coring in accordance with methods used in the Flathead drainage in Montana (McNeil and Ahnell 1964; Weaver et *al.* 2006). Sampling was first conducted in March 2014 and was attempted during March 2015 but high flows precluded efforts. Spring and fall sampling was conducted in 2016 and fall sampling was completed in 2017-2022. In 2018, heavy snow accumulations allowed two of the four sites to be sampled. Sampling in 2022-2023 was limited to two of four sites due to spawning habitat shifts.

During the COVID restrictions, all 2020 core sampling was completed by British Columbia Ministry of Forest, Lands, Natural Resource Operations and Rural Development personnel. FWP took possession of these samples for analysis during fall 2022. No core sampling was completed in 2021 due to personnel limitations and COVID restrictions. Typically, samples are partially processed in the field with remaining analyses occurring at FWP headquarters in Kalispell, Montana. Relevant excerpts from Weaver et *al.* 2006 are included below, describing methods employed:

Field crews used a standard 15.2 cm hollow core sampler (McNeil and Ahnell 1964) to collect four samples across each of three transects at each study area (Figure 1). We located actual coring sites on the transects using a stratified random selection process. The total width of stream having suitable depth, velocity and substrate for spawning was visually divided into four equal cells. We randomly took one core sample in each cell. In some study areas we deviated from this procedure due to limited or discontinuous areas of suitable spawning habitat. We selected study areas based on observations of natural spawning. We only sampled in spawning areas used by migratory westslope cutthroat trout and Bull Trout. During the period of study, these fish spawned in the same general areas annually, so sampling locations have remained similar.

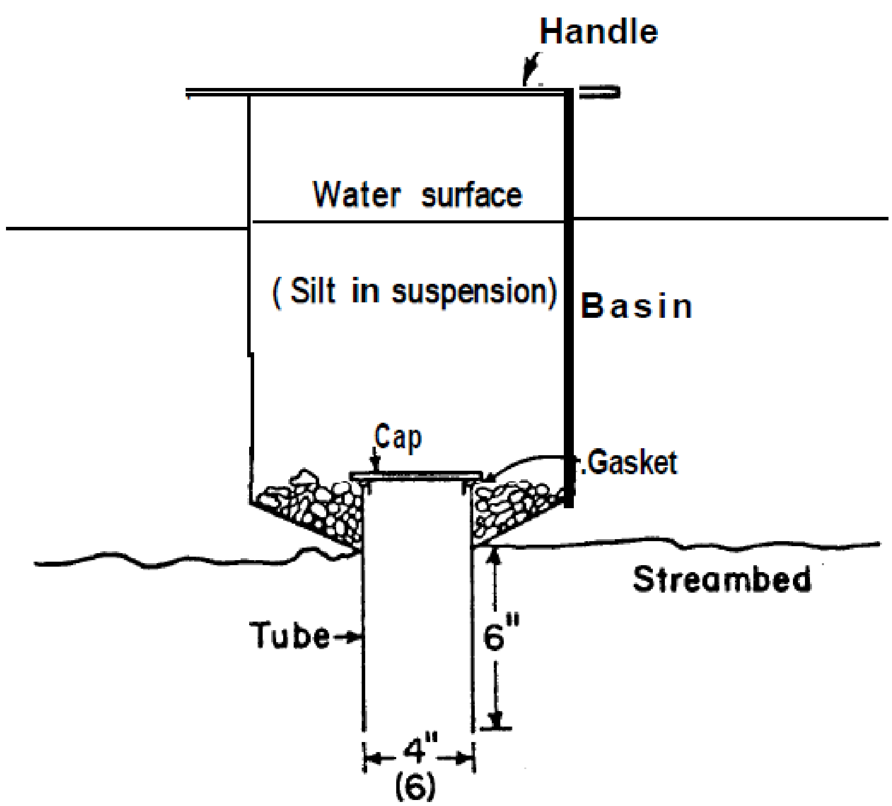


Figure 1. Core sampler used to collect substrate (adapted from McNeil and Ahnell 1964).

Sampling involved working the corer into the streambed to a depth of 15.2 cm. All material inside the sampler is removed and placed in heavy duty plastic bags. We labeled the bags and transported them to the Flathead National Forest Soils Laboratory in Kalispell, Montana, for gravimetric analysis. We sampled the material suspended in water inside the corer using an Imhoff settling cone (Shepard and Graham 1982). Field personnel allowed the cone to settle for 20 minutes before recording the amount of sediment per liter of water. After taking the Imhoff cone sample, they determined total volume of the turbid water inside the corer by measuring the depth and referring to a depth to volume conversion table (Shepard and Graham 1982).

The product of the cone reading (ml of sediment per liter) and the total volume of turbid water inside the corer (liters) yields an approximation of the amount of fine sediment suspended inside the corer after sample remov al. We than applied a wet to dry conversion factor developed for Flathead tributaries by Shepard and Graham (1982), yielding an estimated dry weight (g) for the suspended materi al.

We oven dried the bagged samples and sieve separated them into 13 size classes ranging from >76.1 mm to <0.063 mm in diameter (Table 1). We weighed the material retained on each sieve and calculated the percent dry weight in each size class. The estimated dry weight of the suspended fine material (Imhoff cone results) was added to the weight observed in the pan, to determine the percentage of material <0.063 mm. We summed these percentages, obtaining a cumulative particle size distribution for each sample (Tappel and Bjornn 1983).

Table 1. *Mesh size of sieves used to gravimetrically analyze hollow core (McNeil and Ahnell 1964) streambed substrate samples collected from Flathead River Basin tributaries.*

76.1 mm	*(3.00 inch)*
50.8 mm	*(2.00 inch)*
25.4 mm	*(1.00 inch)*
18.8 mm	*(0.74 inch)*
12.7 mm	*(0.50 inch)*
9.52 mm	*(0.38 inch)*
6.35 mm	*(0.25 inch)*
4.76 mm	*(0.19 inch)*
2.00 mm	*(0.08 inch)*
0.85 mm	*(0.03 inch)*
0.42 mm	*(0.016 inch)*
0.063 mm	*(0.002 inch)*
Pan	*(<0.002 inch)*

We refer to each set of samples by using the median percentage <6.35 mm in diameter. This size class is commonly used to describe spawning gravel quality and it includes the size range typically generated during land management activities. We examined the range of median values for this size class observed throughout the basin. Currently, field crews monitor selected spawning areas utilized by migratory westslope cutthroat and Bull Trout stocks from Flathead Lake.

Results and Conclusions

Results of 2014, spring 2016, and fall 2017-2023 sampling are presented herein. Early run-off conditions precluded coring during 2015. Unfortunately, samples collected during fall 2016 were inadvertently damaged during processing and rendered unusable. Three sites were completed during March 2014 in the Transboundary Flathead (Figures E1 and E2), focused in areas of high Bull Trout redd density. Additional sites were sampled during 2016 (replicated in 2017, 2019, and 2020 and partially replicated in 2018) to improve our understanding of spawning habitat quality in areas of high redd density (Table E1). Conversely, the Howell Creek site was removed from surveys because of accessibility issues. Heavy snow in 2018 restricted sampling to upper and lower sites only. Additionally, two sites were not sampled in 2022 and 2023 due to no redds being observed in the coring site vicinity. Figures E1-E8 illustrate Bull Trout redd locations found during spawning surveys conducted in 2012, 2017-2023.

Results of sieve analyses demonstrate similar values to unimpaired streams in Montana (Figure E9). Sampling will continue to monitor habitat quality through time and inform managers across the region. In the fall of 2022, core samples taken in 2020 were received from British Columbia Ministry of Forest, Lands, Natural Resource Operations and Rural Development personnel. But due to prolonged storage during COVID travel restrictions, labels from 2020 samples were degraded enough to be unreadable and thus not useful for analysis. No samples were collected in 2021 due to short staffing and ongoing COVID restrictions.

Table E1.—Streambed core sampling frequency for sites in the Transboundary Flathead River in British Columbia, Canada during 2014-2023. The Howell site was discontinued due to access challenges.

	Howell	Pincher	Packhorse	Harvey	NF Campsite
2014	Spring	Spring		Spring	
2015	Sampling precluded by high flows				
2016		Spring/Fall	Spring/Fall	Spring/Fall	Spring/Fall
2017		Fall	Fall	Fall	Fall
2018		Fall	Sampling precluded by weather		Fall
2019		Fall	Fall	Fall	Fall
2020		Fall	Fall	Fall	Fall
2021	Sampling precluded by COVID travel restrictions				
2022		Fall		Fall	
2023		Fall		Fall	

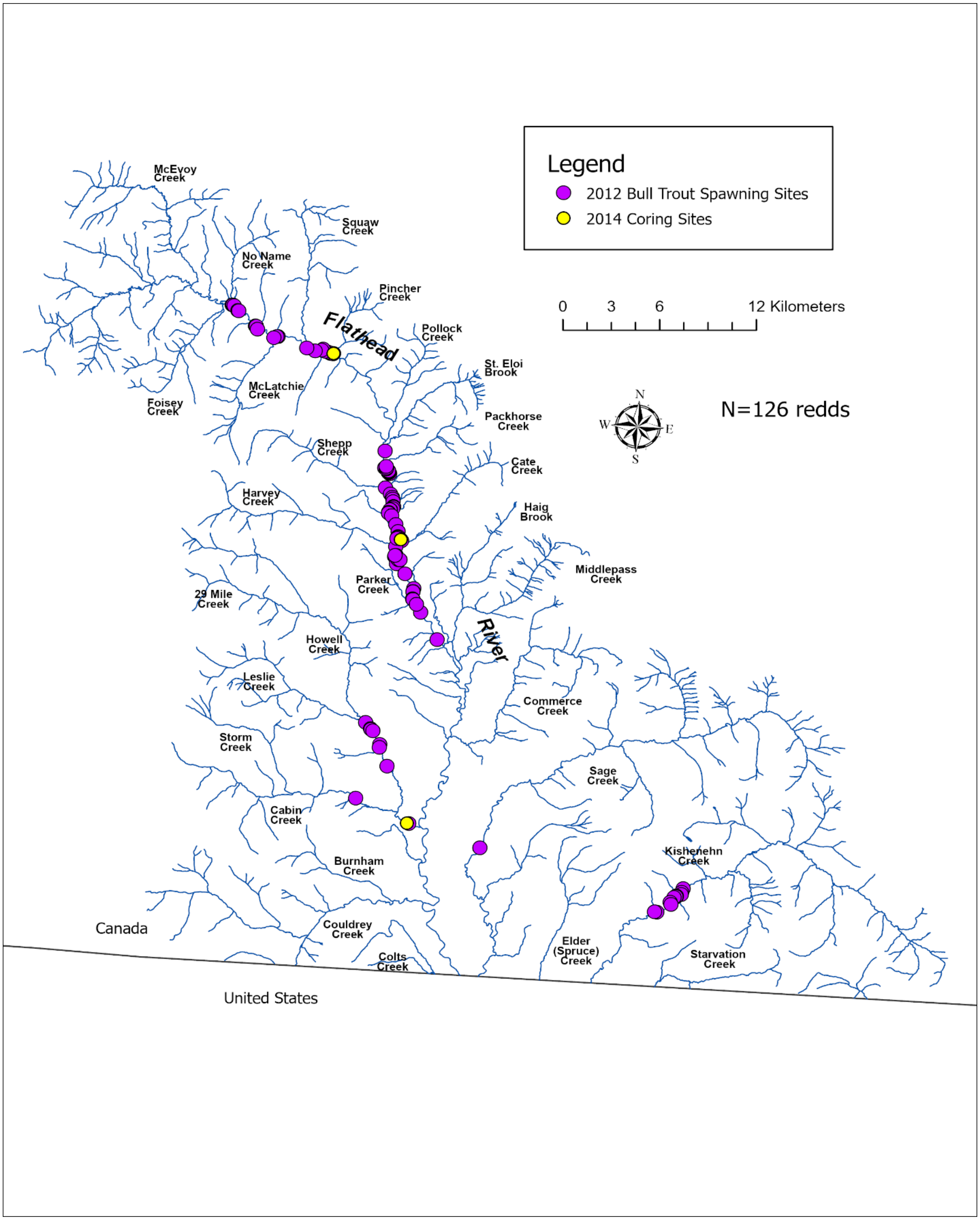


Figure E1.—Locations of substrate core sampling conducted in the Transboundary Flathead River during 2014 in comparison to Bull Trout redd locations during 2012. Note that 2012 was a basinwide redd count year, with all known Bull Trout spawning habitat surveyed for redds.

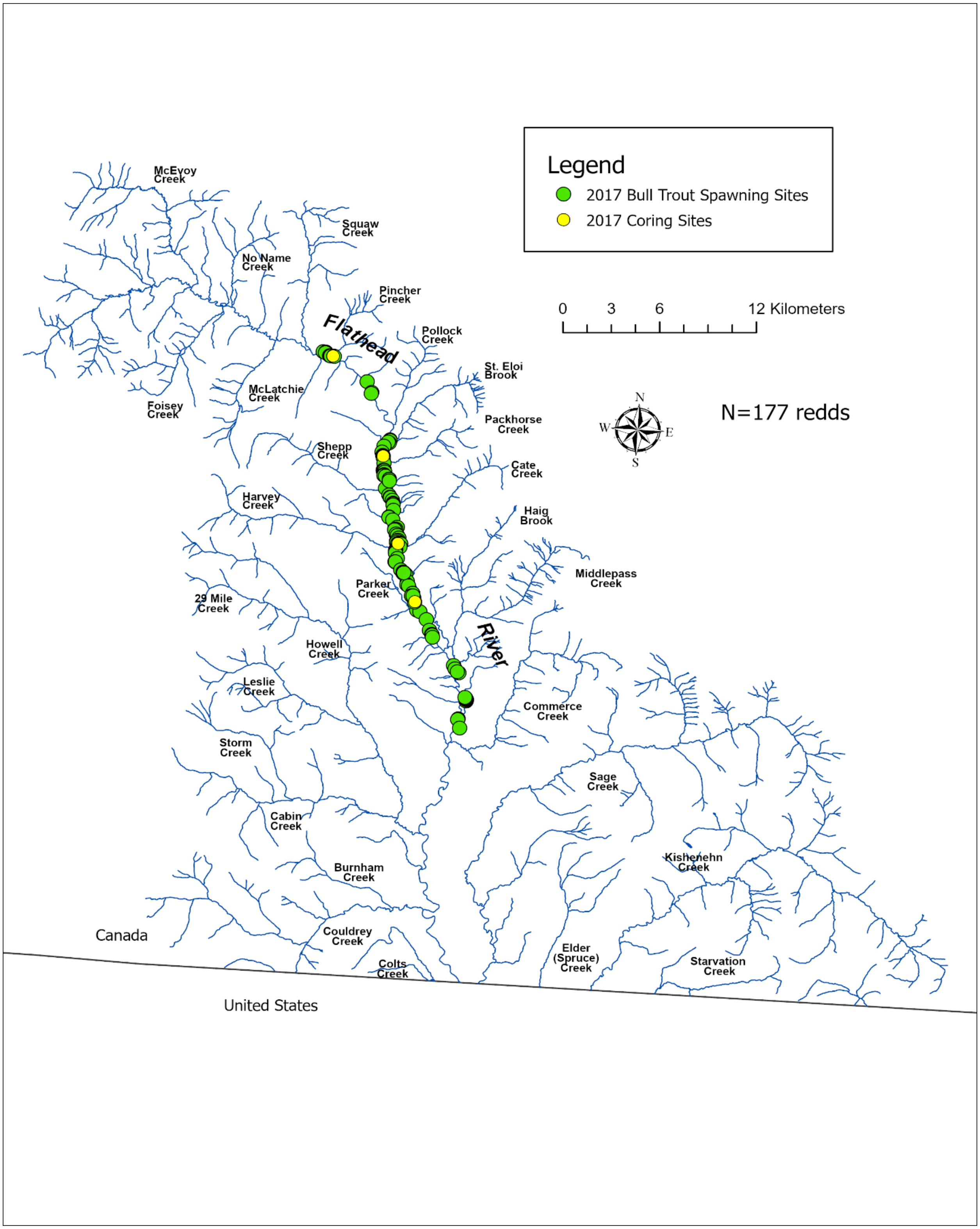


Figure E2.—Locations of substrate core sampling conducted in the Transboundary Flathead River during spring and fall 2016 and fall 2017 relative to Bull Trout redd locations during fall 2017.

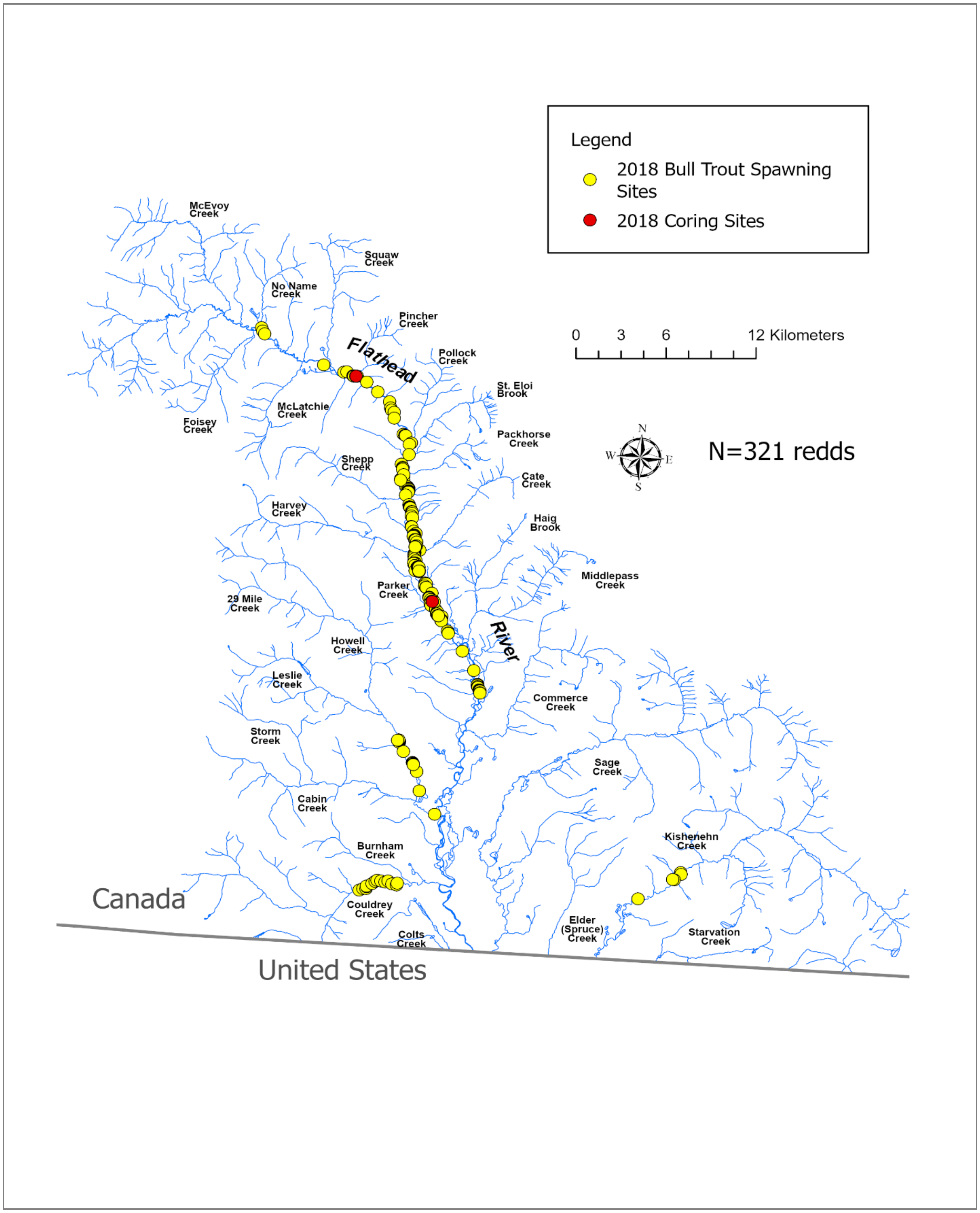


Figure E3.—Locations of substrate core sampling conducted in the Transboundary Flathead River during fall 2018 relative to that year's Bull Trout redd locations. Note that 2018 was a basinwide redd count year, with all known Bull Trout spawning habitat surveyed for redds.

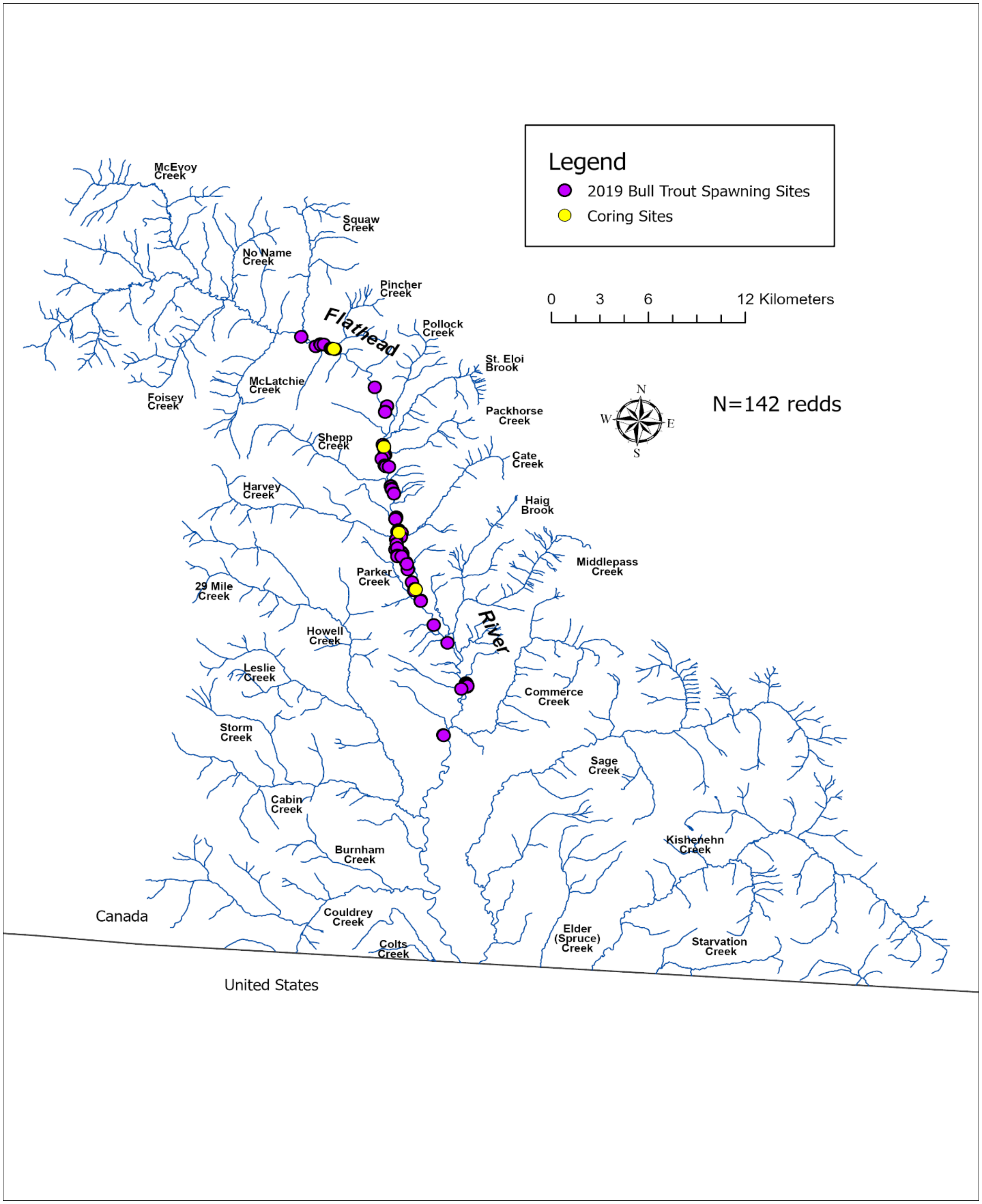


Figure E4. Locations of substrate core sampling conducted in the transboundary Flathead River during fall 2019 and 2020 relative to Bull Trout redd locations during fall 2019.

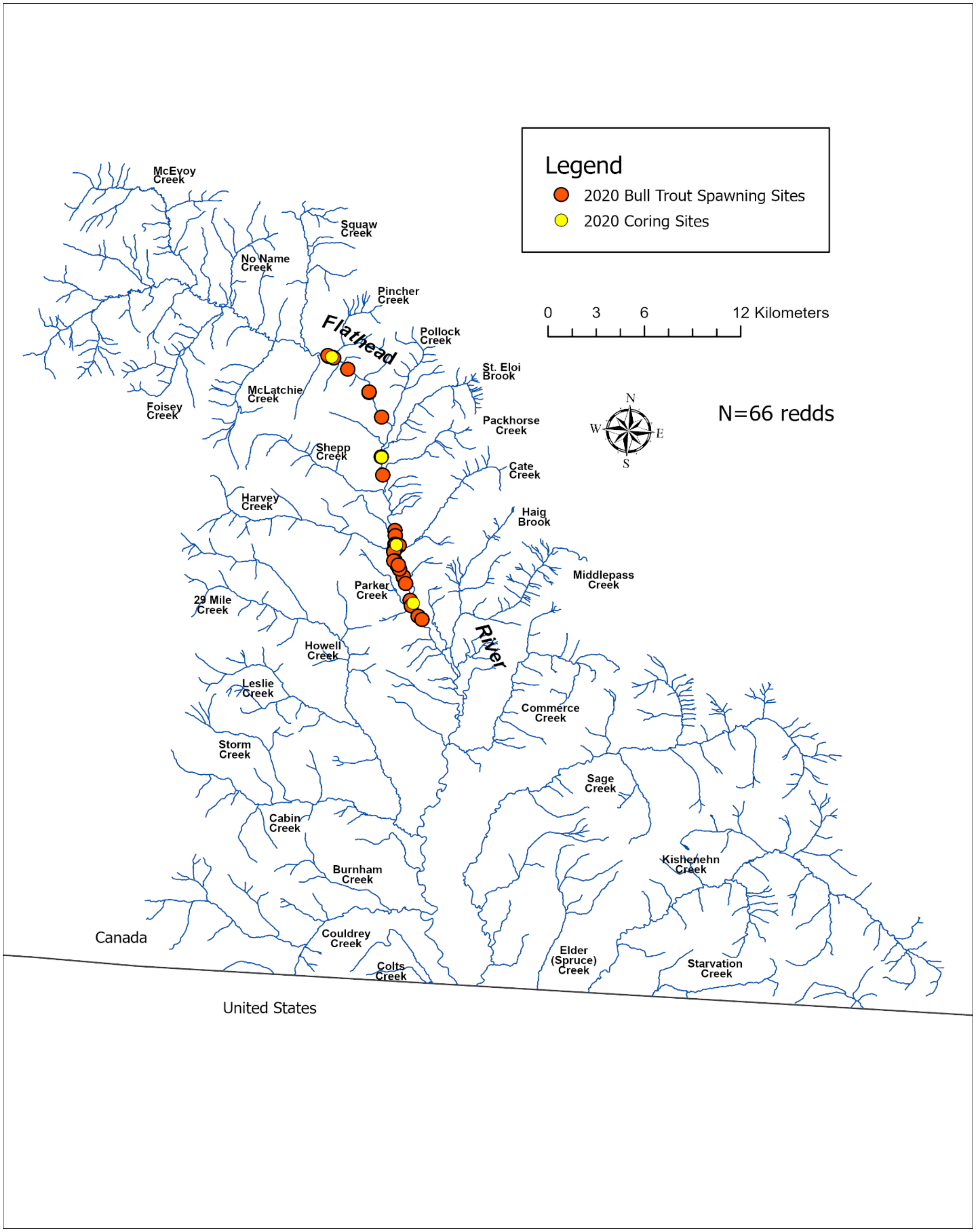


Figure E5. Locations of substrate core sampling conducted in the transboundary Flathead River during fall 2020 relative to Bull Trout redd locations during fall 2020.

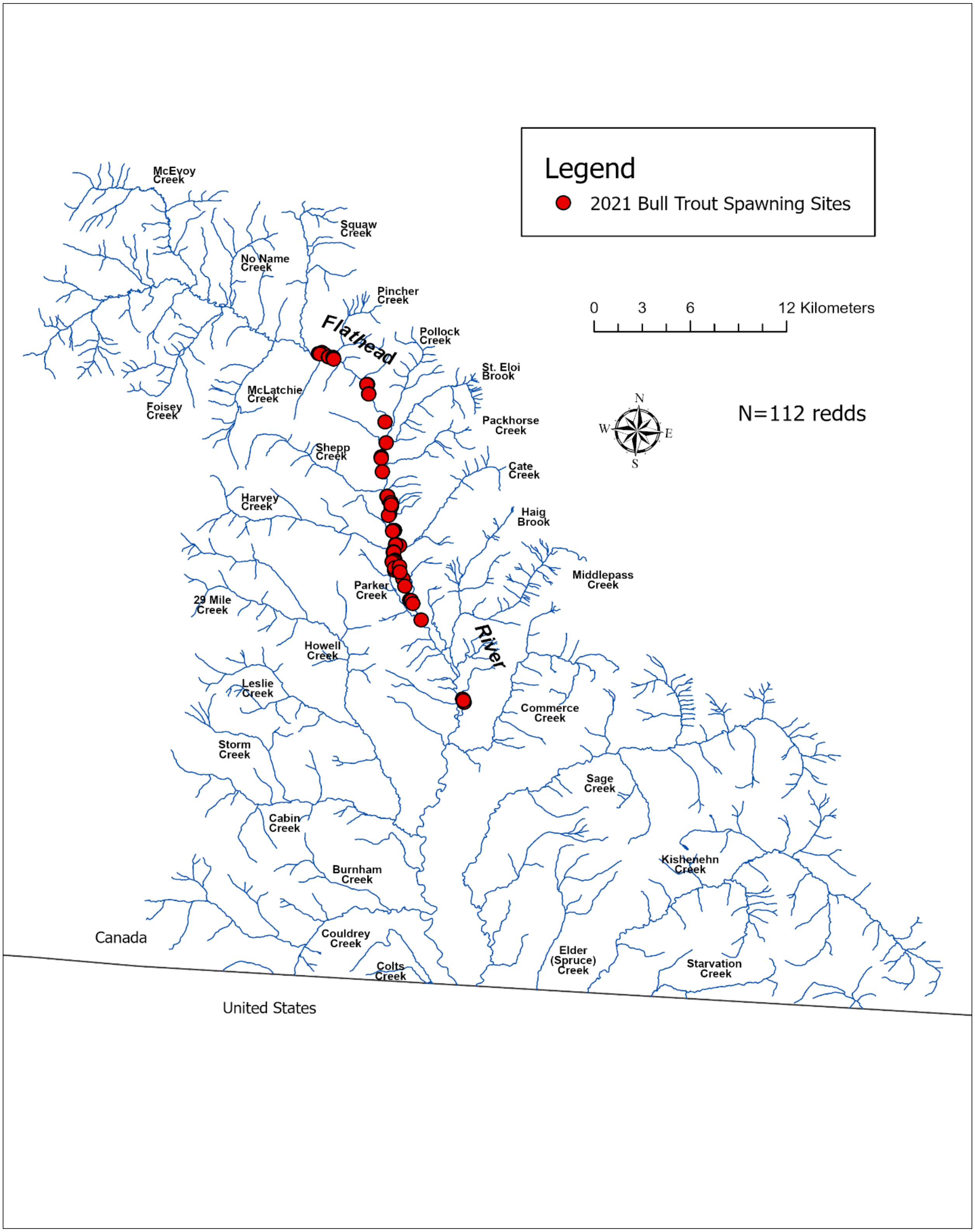


Figure E6. Bull Trout spawning survey redd locations conducted in the transboundary Flathead River during fall 2021.

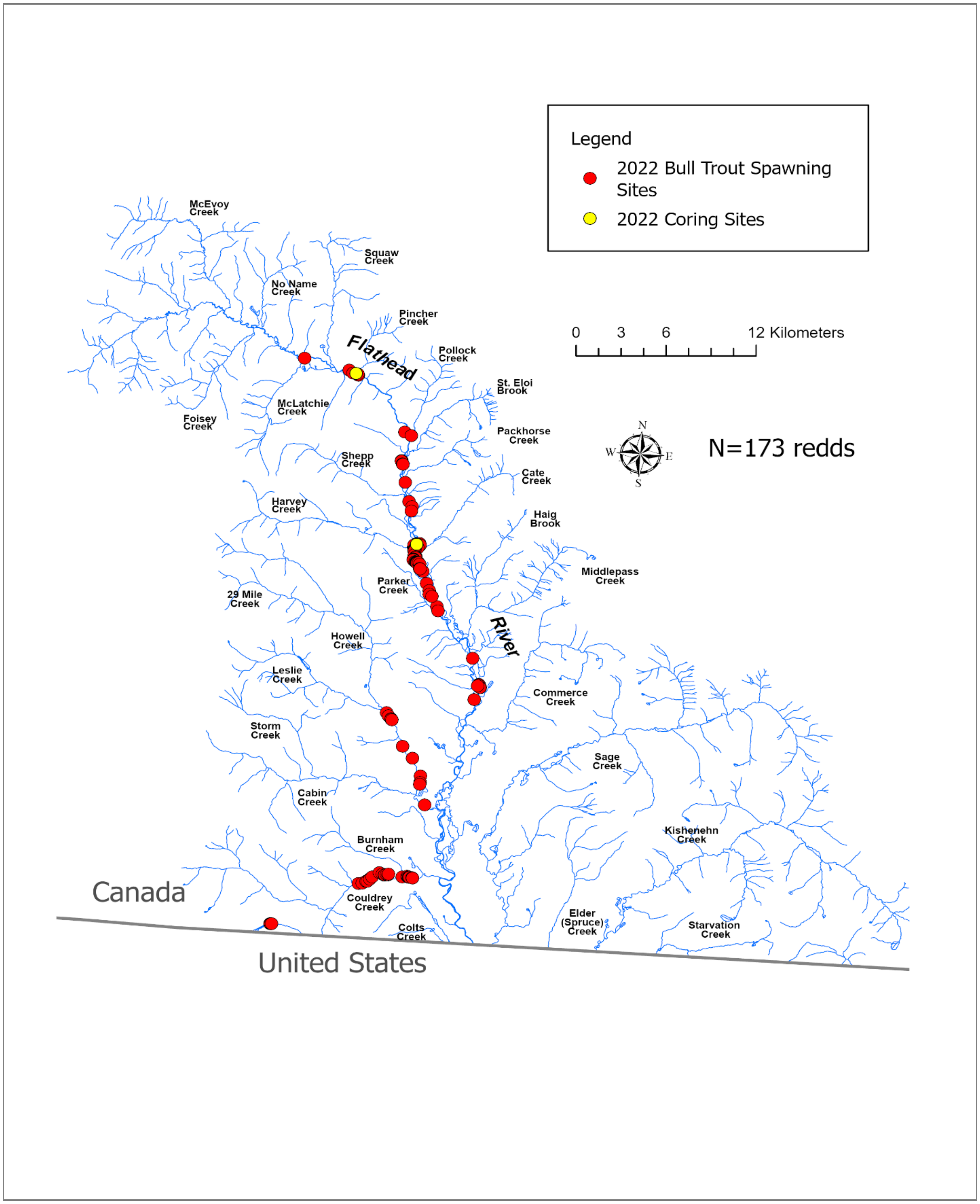


Figure E7. Locations of substrate core sampling conducted in the transboundary Flathead River during fall 2022 relative to Bull Trout redd locations that same year. Note that 2022 was a basinwide redd count year, with all known Bull Trout spawning habitat surveyed for redds.

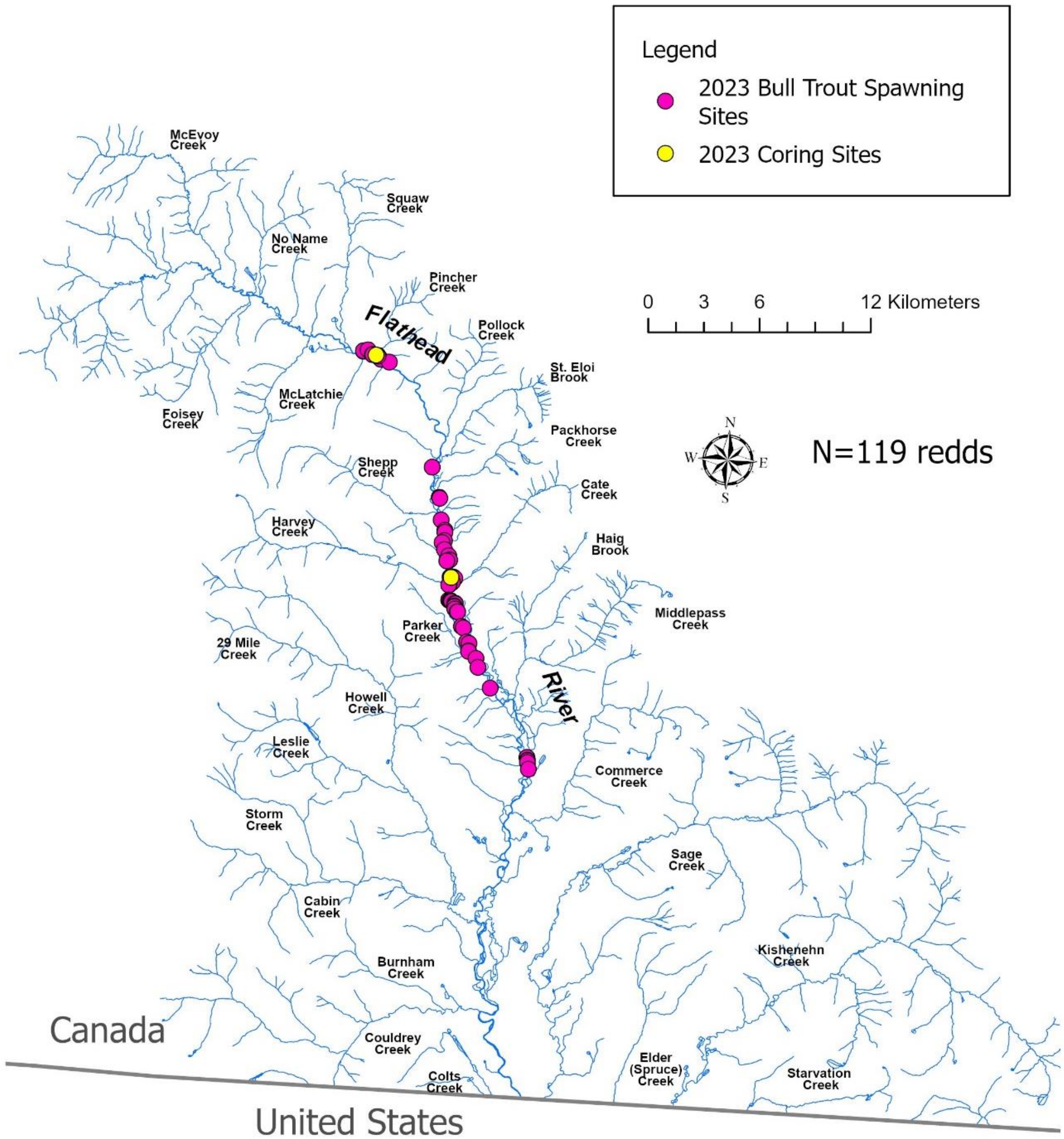


Figure E8. Locations of substrate core sampling conducted in the transboundary Flathead River during fall 2023 relative to Bull Trout redd locations that same year.

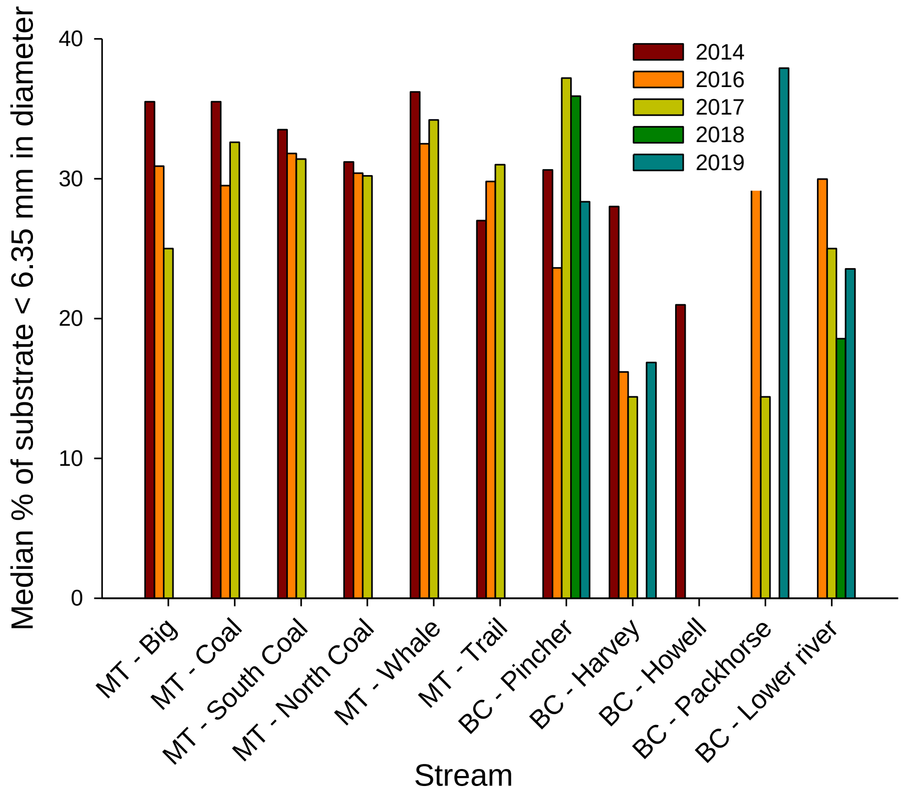


Figure E9.—Streambed coring results for selected streams in the Transboundary (North Fork) Flathead River during 2014, and 2016-2019. Streams are linked to their location with codes (NF = Montana, BC = British Columbia). Spring sampling sites (2014 and 2016 Canadian samples and all Montana samples) document conditions experienced by the offspring of Bull Trout redds created during the prior fall. Conversely, Canadian samples collected during 2017-2019 document conditions for that spawning year because they were collected during fall. Sites reported as 2017 in Montana streams were collected during spring 2018 but reported here as 2017 to standardize comparisons.

Evaluation of Bull Trout Translocation Opportunities in Swan Drainage Headwaters

Introduction

Efforts have increased in recent years to expand the range of habitat occupied by Bull Trout, including within Montana (Hayes and Banish 2017). These opportunities have been facilitated by frameworks developed to inform recipient habitat and donor population suitability (Galloway et *al.* 2016). Building on the momentum and intent to conserve this federally Threatened species, we initiated investigations of habitat suitability within and near lakes at the headwaters of the Swan River drainage beginning in summer 2022. Lakes initially targeted included Gray Wolf (the largest), Lost, High Park, and Crystal. All focal lakes are in the Mission Mountains Wilderness. A summary of surveys conducted in Gray Wolf Lake can be found in Steed et *al.* (2023).

Methods

Crystal Lake and connected stream habitat were targeted during late summer 2023 for habitat suitability surveys modeled after Galloway (2014) (Figure E10). Data were collected during late August to capture base flow conditions that are typically most restrictive to Bull Trout while ensuring our access to the site. Sites required a crew to boat and backpack in.

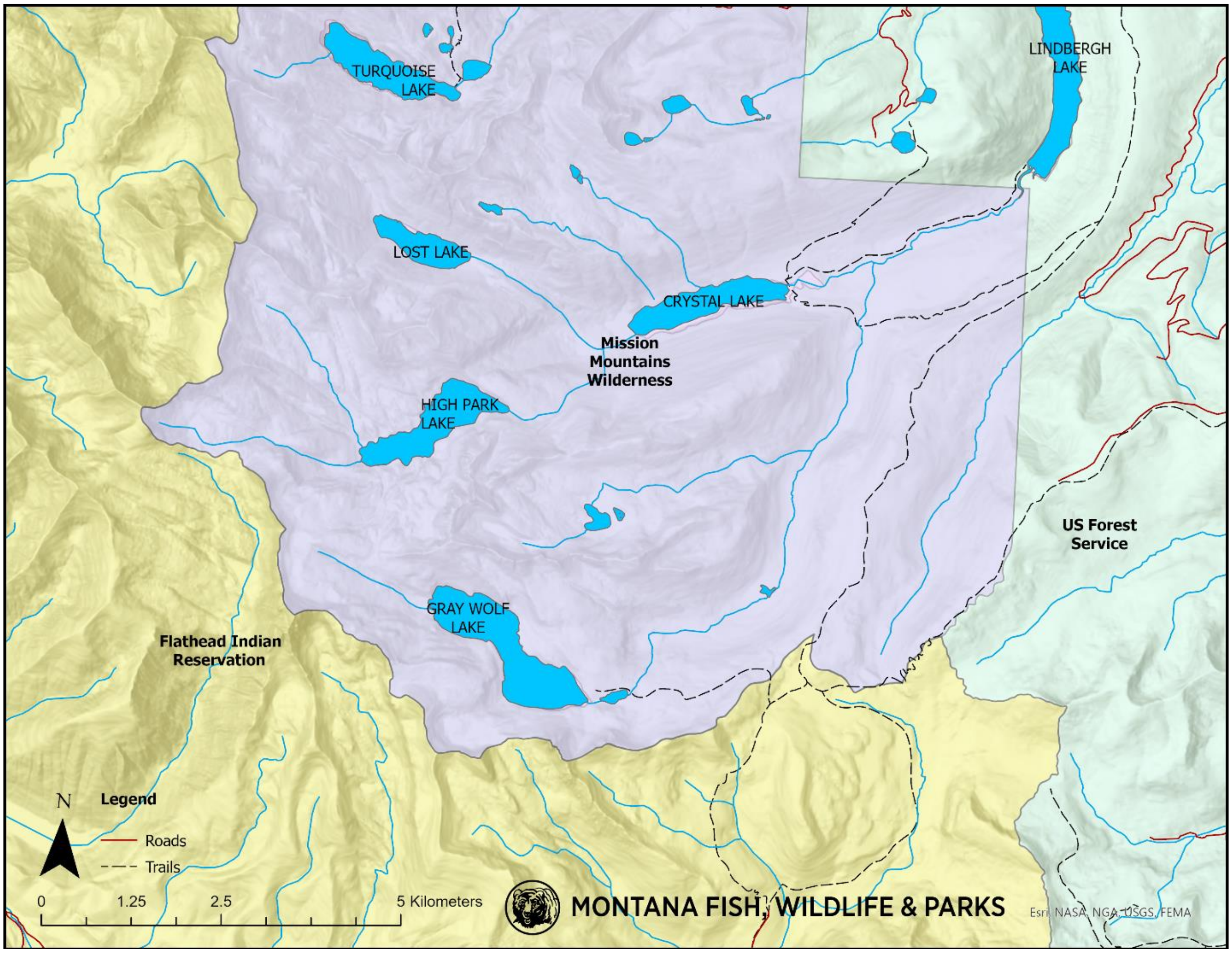


Figure E10.—Headwater lakes of the Swan River drainage, Montana.

Stream Assessment

The two inlets of Crystal Lake (Lost and High Park creeks) were surveyed to characterize biotic and abiotic factors influencing Bull Trout suitability using methods adapted from Galloway (2014). Each stream was surveyed from the mouth upstream to a fish passage barrier, dividing each stream into habitat units. Within each habitat unit, we quantified the percent composition of each habitat type (pool, glide, riffle, cascade, run), substrate type (sand/silt, gravel, cobble, boulder, bedrock), and instream cover (large woody debris, undercut bank, boulder, overhanging vegetation, backwater). We documented fish and amphibian occurrence and collected aquatic macroinvertebrate samples.

Macroinvertebrates were sampled at six sites within each inlet stream to Crystal Lake (12 total), stratified by fast and slow-water mesohabitat using a 250-µm mesh Surber stream bottom sampler (Figure E11) Samples were preserved in 70% ethanol. Thermographs were deployed in each tributary to capture August mean, max, and overwinter temperatures. Barriers to upstream passage were characterized by documenting location with GPS coordinates, height (m), length (m), and type (e.g., waterfall or cascade) when encountered.

The presence of fish and amphibian species in each inlet stream was estimated using single-pass electrofishing surveys (Rieman and McIntyre 1995; Lazorchak et *al.* 1998; Rich et *al*. 2003). While Galloway et *al.* (2016) surveyed every fifth fast and every fifth slow water mesohabitat unit to describe a percentage of total stream length, we surveyed the entire reach within each stream because of the relatively short distances and personnel available. All fish and amphibians captured were identified to species, measured to the nearest mm, and subsequently released. Fin clips were collected from *Oncorhynchus* and preserved in 95% ethyl alcohol for analysis at the University of Montana Conservation Genetics Laboratory in Missoula.

<u>Lake Assessment</u>

Crystal Lake was also evaluated using methods adapted from Galloway (2014) to describe available habitat and existing biotic communities. Gill netting was used to document fish species, size structure, and genetic composition (for *Oncorhynchus*). Gill nets measured 125 ft. by 5 ft. with variable panels (3/4 in., 1.0 in., 1.25 in., 1.5 in., 2.0 in.). Fin clips were collected from *Oncorhynchus* and treated as described for stream sampled fish. Vertical plankton tows were conducted using an 8 in. (203.2 mm) diameter Turtox plankton tow net with an 80 µm mesh size to characterize zooplankton in conjunction with basic bathymetric surveys. Spot electrofishing surveys were conducted over a distance of at least 75 m near the inlets and outlet of the lake. Macroinvertebrates were also sampled at four sites along the shoreline of Crystal Lake using kick-net with a 250-µm mesh using methods modified from Davis et *al.* (2001). We processed samples using methods described for inlet sampling.

Results

<u>Stream Assessment</u>

Stream biotic and abiotic surveys were conducted in the inlets of Crystal Lake during the week of August 28 2023 (Figure E11; Tables E2-E4 and E6). Tailed frogs were documented in both High Park (4) and Lost (2) creeks. Macroinvertebrate samples were collected and are being processed during winter 2023-2024 (Table E4).

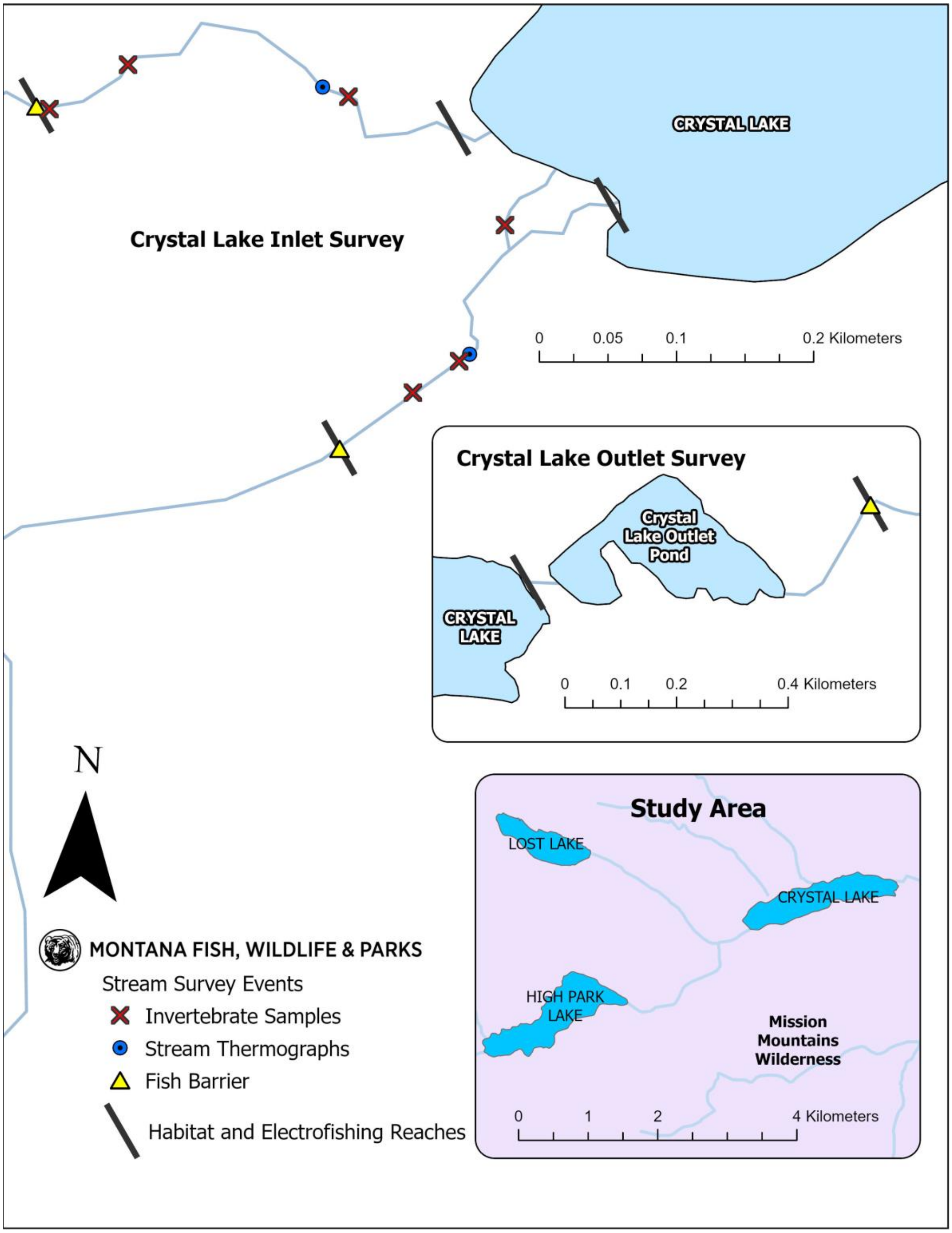


Figure E11.—Lotic habitat surveyed in proximity to Crystal Lake during August 2023. Fish passage barriers were documented, thermographs were installed, and benthic macroinvertebrates were sampled at indicated locations. Habitat and electrofishing surveys were conducted between lines shown.

Figure E12.—Gill net, zooplankton, and macroinvertabrate survey locations in Crystal Lake in the upper Swan drainage, Montana during 2023.

Table E2.—Length and total area surveyed by habitat type deemed free of fish barriers within Crystal Lake inlets during August 2023.

Site	Surveyed stream length (m)	Surveyed stream area (m^2)
High Park Creek	480.1	3239.3
Lost Creek	488.6	4124.0*

* A large beaver complex at the mouth of Lost Creek was not included in the reported area surveyed but accounts for about 1830 m^2 of stream mouth area (Google Earth polygon estimation).

Table E3.—Physical stream characteristics of the two inlets to Crystal Lake. Overhanging vegetation = OHV and large woody debris = LWD.

		High Park Creek		Lost Creek		**Total**	
	Habitat characteristic	Proportion	Area (m^2)	Proportion	Area (m^2)	**Proportion**	**Area (m^2)**
Type	Pool	0.11	370.09	0.03	137.24	**0.14**	**507.33**
	Glide	0.19	616.7	0.66	2706.67	**0.85**	**3323.37**
	Riffle	0.13	429.74	0.13	556.52	**0.26**	**986.26**
	Cascade	0.33	1080.96	0.12	486.47	**0.45**	**1567.43**
	Run	0.23	741.82	0.06	237.06	**0.29**	**978.88**
Substrate	Sand/silt	0.1	311.13	0.28	1165.95	**0.38**	**1477.08**
	Gravel	0.19	625.62	0.46	1889.43	**0.65**	**2515.05**
	Cobble	0.4	1298.31	0.14	576.75	**0.54**	**1875.06**
	Boulder	0.31	1003.27	0.07	269.33	**0.38**	**1272.6**
	Bedrock	0	0.98	0.05	222.49	**0.05**	**223.47**
Instream cover	LWD	0.09	304.25	0.12	497.65	**0.21**	**801.9**
	Undercut bank	0.01	24.75	0.02	93.48	**0.03**	**118.23**
	Boulder	0.31	1002.93	0.07	268.73	**0.38**	**1271.66**
	OHV	0.08	264.57	0.04	174.87	**0.12**	**439.44**
	Backwater	0.02	61.53	0.06	243.73	**0.08**	**305.26**

* A large beaver complex at the mouth of Lost Creek was not included in the reported area surveyed but accounts for about 1830 m^2 of stream mouth area (Google Earth polygon estimation).

Table E4.—Benthic macroinvertebrate samples collected from the two inlets to Crystal Lake and at four sites along the lake shoreline during late August 2023. A 250-µm mesh Surber stream bottom sampler was used at all sites and samples were preserved in 70% ethanol for subsequent identification.

Location	Site	Date	Area sampled (m^2)	Habitat type
High Park Creek	1	8/29/2023	0.09	Slow
	2	8/29/2023	0.09	Slow
	3	8/29/2023	0.09	Slow
	4	8/29/2023	0.09	Fast
	5	8/29/2023	0.09	Fast
	6	8/29/2023	0.09	Fast
Lost Creek	1	8/29/2023	0.09	Slow
	2	8/29/2023	0.09	Slow
	3	8/29/2023	0.09	Slow
	4	8/29/2023	0.09	Fast
	5	8/29/2023	0.09	Fast
	6	8/29/2023	0.09	Fast
Crystal Lake Shoreline (NE)	1	8/28/2023	0.09	Lake Shore
Crystal Lake Shoreline (SE)	2	8/28/2023	0.09	Lake Shore
Crystal Lake Shoreline (SW)	3	8/29/2023	0.09	Lake Shore
Crystal Lake Shoreline (NW)	4	8/29/2023	0.09	Lake Shore

Lake Assessment

Gill netting was conducted on Crystal Lake during late August 2023, capturing a total of 79 *Oncorhynchus* were captured (Figure E12; Table E5). Genetic samples were collected from a random subset of 32 fish for analyses. Vertical plankton tows were conducted at six sites on 8/28/2023 in conjunction with bathymetric surveys (Table E7). Spot electrofishing captured a total of 15 fish. Abundant fry were observed but not susceptible to electrofishing and thus not captured (Table E6).

Table E5.—Gill net and fish data collected from Crystal Lake in the upper Swan drainage, Montana during 2023. All fish captured were *Oncorhynchus*. One net was set targeting Pygmy Whitefish (PWF) but only *Oncorhynchus* were captured, and lengths were not collected from fish.

						Length (mm)		
Net ID	Set date	Soak time (min)	Start depth (m)	End depth (m)	Catch (n)	Range	Mean	S.E.
Net#1 NE	8/28/2023	1230	0	3	33	80-260	196	11
Net#2 SE	8/28/2023	1250	0	6	25	76-297	218	11
Net#3 W	8/29/2023	245	1.5	9.1	5	240-270	255	6
Net#4 W	8/29/2023	255	0	11	7	207-280	247	10
PWF Net#1	8/28/2023	2145	9.1	21.3	9	n/a	n/a	n/a

Table E6.—Electrofishing survey data from the inlets to and shoreline of Crystal Lake in the upper Swan drainage, Montana during August 2023. All fish captured were *Oncorhynchus.*

				Length (mm)		
Reach	Date	Shock time (sec)	Catch (n)	Range	Mean	S.E.
High Park Creek	8/29/2023	1242	3	85-173	142	29
Lost Creek	8/29/2023	1424	3	54-73	63	6
Foot of lake	8/28/2023	631	7	32-51	42	3
Head of lake	8/29/2023	394	8	32-45	38	2

Table E7.—Zooplankton surveys conducted in Crystal Lake in the upper Swan drainage, Montana during August 2023. A 203.2 mm diameter Turox plankton tow net was used for all sampling.

Site	Date	Mesh Size (µm)	Volume Sampled (L)	Sample Depth (m)
Shallow #1	8/28/2023	80	138	4.6
Shallow #2	8/28/2023	80	183	6.1
Shallow #3	8/28/2023	80	273	9.1
Deep #1	8/28/2023	80	732	24.4
Deep #2	8/28/2023	80	732	24.4
Deep #3	8/28/2023	80	732	24.4

Discussion

Aspects of the Crystal Lake system suggest promising potential as a translocation recipient for Bull Trout (Figures E13-E15; Table E9), but further consideration of limiting characteristics is warranted, specifically spawning and overwintering habitat. Relative to most systems evaluated by Galloway et *al.* (2016), the Crystal inlets contained either comparable or a lower quantity of habitat with similar relative complexity. However, a concurrent effort by FWP to develop standard protocols for evaluating Bull Trout translocation protocols for state actions will allow for better evaluation of suitability, with particular focus on minimum rather than relative benchmarks for critical habitat metrics such as total spawning habitat area, minimum water depth necessary for accessing spawning area, overwintering conditions, etc. Once in place, those protocols will be applied to the Crystal system and all subsequently evaluated systems.

Table E9.—Criteria and associated scoring structure used to evaluate habitat suitability of Crystal Lake for Bull Trout translocation, adapted from Galloway et *al*. (2016) and Dunham et *al.* (2011).

Major component	Criterion	Value	Score
Recipient habitat	Mean August stream temperature	TBD 2024	TBD 2024
	Mean August lake temperature	≤ 14°C*	1
Recipient community	Threatened, endangered, or sensitive native species	Not detected	1
	Hybridizing or competing species	Not detected	1
Future threats	Habitat modification	Not likely	1
	Social or economic changes	Not likely	1
	Nonnative species invasions	Not likely	1
	Thermal suitability given climate change**	Likely <13°C**	1
	Disease or parasites	No information	-0.05
	Dispersal potential	Not likely	1
	Establishment potential outside introduction site	Not likely	1
Overall score			8.5***

*Putatively ≤ 14°C due to elevation and aspect
**Value derived from NorWeST projected 2080 stream and lake temperatures
***Score prior to incorporating ground-truthed mean August stream temperature

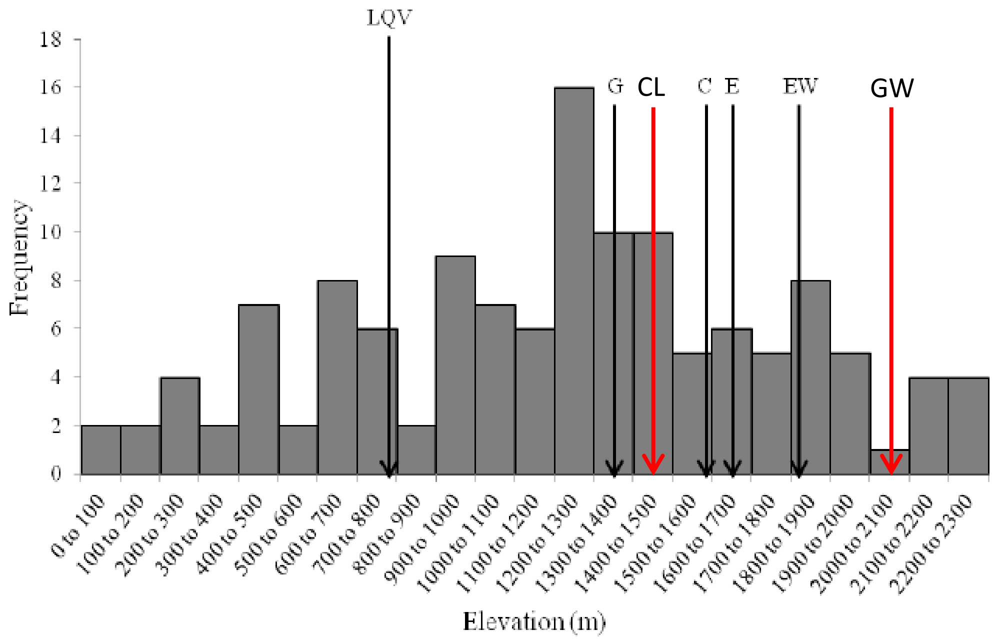


Figure E13.—Elevation of Crystal Lake (CL) and Gray Wolf Lake (GW) compared to elevation distribution of lakes containing Bull Trout within the Columbia River Drainage; C = Camas Lake; G = Grace Lake; E = Lake Evangeline; EW = Lake Ellen Wilson; LQV = Lower quartile value of distribution. Adapted from Galloway (2014).

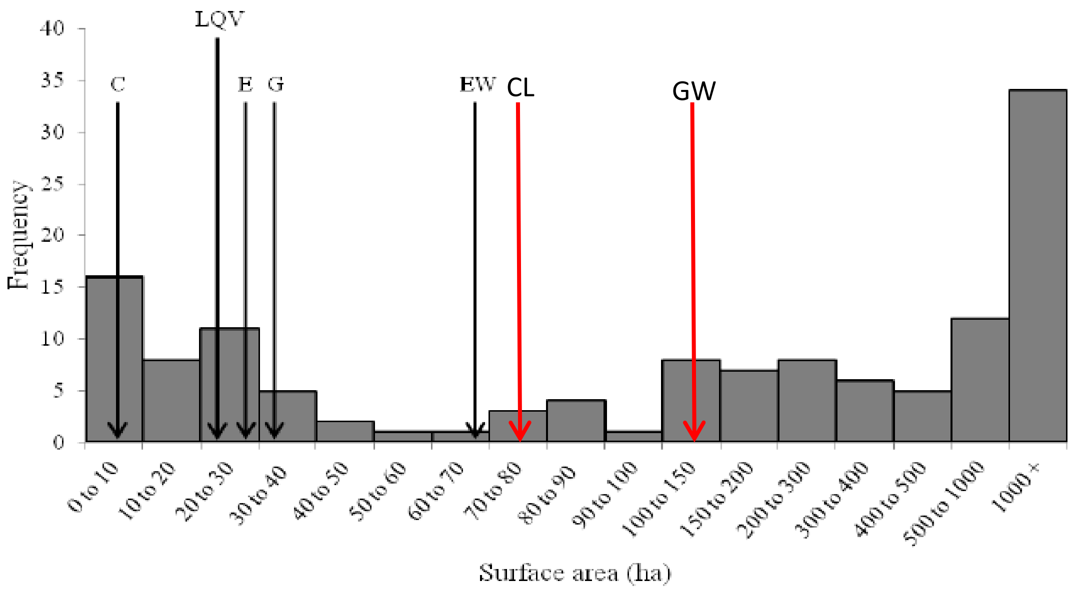


Figure E14.—Surface area of Crystal Lake (CL) and Gray Wolf Lake (GW) compared to elevation distribution of lakes containing Bull Trout within the Columbia River Drainage; C = Camas Lake; G = Grace Lake; E = Lake Evangeline; EW = Lake Ellen Wilson; LQV = Lower quartile value of distribution. Adapted from Galloway (2014).

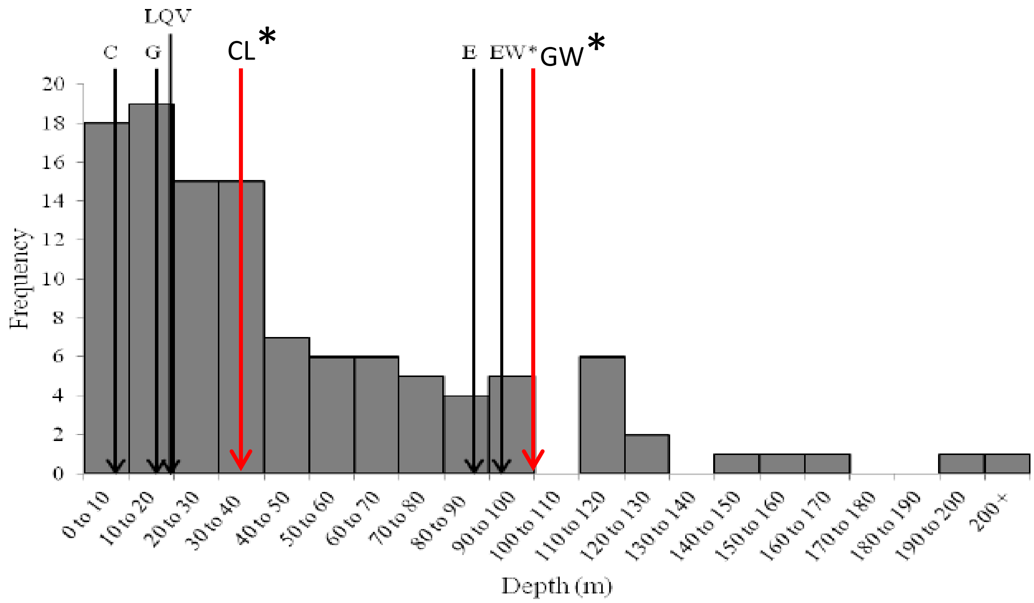


Figure E15.—Depth of Crystal Lake (CL) and Gray Wolf Lake (GW) compared to elevation distribution of lakes containing Bull Trout within the Columbia River Drainage; C = Camas Lake; G = Grace Lake; E = Lake Evangeline; EW = Lake Ellen Wilson; LQV = Lower quartile value of distribution. *Depth was estimated due to equipment limitations. Adapted from Galloway (2014).

F. The Use of Geochemical Markers to Reconstruct Fish Life History

1/1/2023-12/31/2023 76916 REL 11 H, 84064 REL 11 H: Collect/Generate/Validate Field and Lab Data- Geochemical Otolith Analysis to Reconstruct Focal Fish Species Life History

Introduction

Our understanding of the environmental life history of migratory fishes has greatly advanced through the integration of isotopic (e.g. $^{87}Sr/^{86}Sr$, $\delta^{18}O$) and elemental (Sr/Ca) geochemical markers with the chronological properties of fish otoliths (Kennedy et al. 2000, Wurster et al. 2005, Barnett-Johnson et al. 2008, Zimmerman et al. 2013). All bony fishes incorporate elements and respective isotopic markers into the chemical matrix of their otoliths in relation with the water in which they reside (Campana et al. 1999). The naturally occurring isotopic and elemental strontium in water reflects the geological makeup and age of the underlying basin. When large variations in mafic and felsic geology are present in the watershed, otolith microchemistry provides the ability to reconstruct a detailed record of fish life history. The oxygen isotope ($\delta^{18}O$) in water is correlated with temperature, which provides a thermal history of a fish's environment (Wurster et al. 2005). Moreover, when pairing isotopic and elemental geochemical markers with annual rings, otoliths provide a time stamped record of fish movements for their entire life (Muhlfeld et al. 2012). The detailed life history reconstruction is only possible with a heterogeneous underlying geology and temperature regime in the watershed, which can be reflected through water chemistry sampling in select tributary and mainstem habitats (Brennan et al. 2015a).

Previous studies have documented temporal stability in $^{87}Sr/^{86}Sr$ ratios on seasonal (Kennedy et al. 2000, Muhlfeld et al. 2012) and inter-annual (Walther and Thorrold 2009) scales, but the dynamics driving $^{87}Sr/^{86}Sr$ ratio variation in large watersheds are not well understood or predictable (Brennan et al. 2015a). To investigate the thermal histories of migratory species, $\delta^{18}O$ thermometry has been applied to Chinook salmon and Arctic Char in systems with heterogeneous temperature regimes. Kennedy et al. 2000 showed a distinct food signal in the core of hatchery reared Atlantic salmon otoliths.

We sought to understand the relative contribution of local populations to the abundance of Swan Lake Bull Trout. We were interested in the redd abundance proportion compared to the abundance in Swan lake and investigated the age structure in Swan Lake.

Methods

Sagittal otoliths were extracted from collected fish with nonmetallic forceps and stored in dry paper scale envelopes. Otoliths were cleaned with Milli-Q water for 5 minutes and dried overnight and embedded in heat activated Crystal Bond epoxy sulcus side up on pre-cleaned 25 x 75 mm clear glass microscopy slides. Otoliths were polished with 600, 800, and 1200 wet grit silicon carbide adhesive discs in a dorsal plane until the primordium and annual growth rings were visible with reflected light and a compound light microscope.

Otolith sections were assayed for $^{87}Sr/^{86}Sr$ and Sr/Ca with a laser ablation inductively coupled plasma mass spectrometry (ICP-MS). Analysis included a Thermo Finnigan Neptune multiple collector (MC-ICP-MS) coupled to a New Wave 193-nm laser system at the Woods Hole Oceanographic Institution Plasma Mass Spectrometry Laboratory, following the methods reported by Bourret et al. (2014). Sr/Ca was measured in otolith samples by converting 88Sr/48Ca to Sr/Ca mmol/mol based on repeated analysis of MACS3 solid carbonate standard (USGS). $^{87}Sr/^{86}Sr$ and Sr/Ca ratios were quantified with a single ablated transect from otolith core to edge at a perpendicular angle to growth rings.

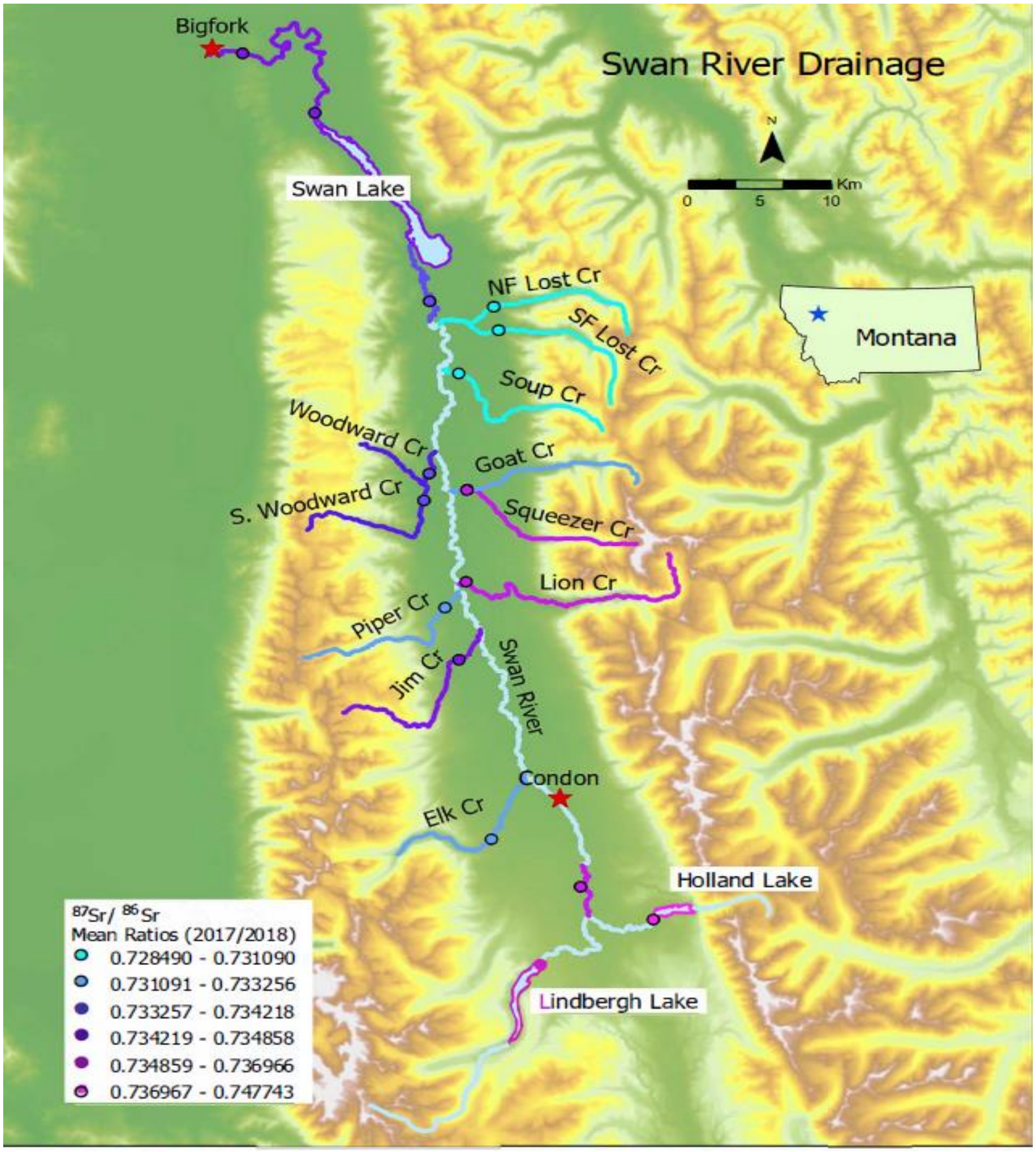


Figure L1. Map of the Swan River drainage showing locations of water and sculpin otolith collections and corresponding $^{87}Sr/^{86}Sr$ values.

Results

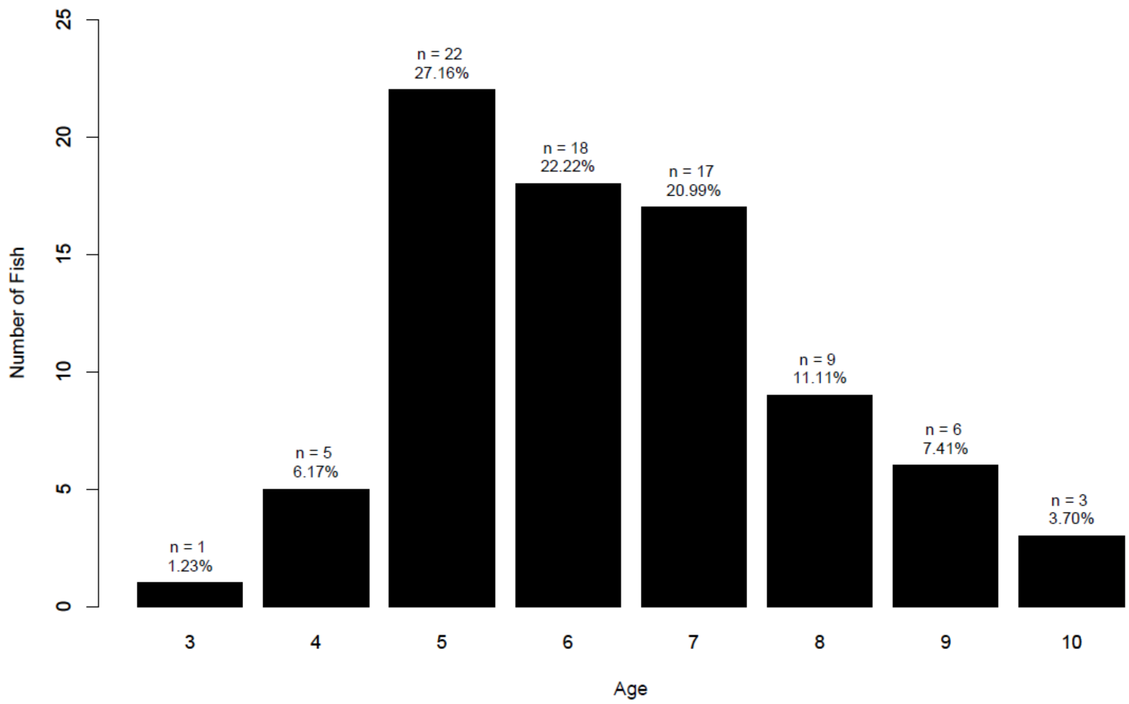


Figure L2. Otolith total age estimates from Bull Trout sampled in Swan Lake, N = 87.

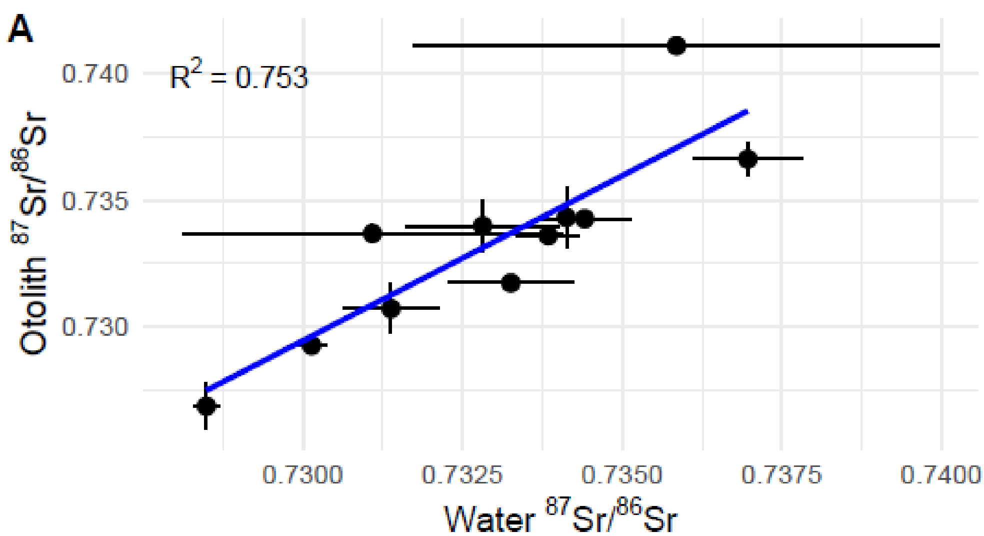


Figure L3. Mean otolith $^{87}Sr/^{86}Sr$ sculpin samples and water$^{87}Sr/^{86}Sr$ samples from all natal locations in the Swan local populations. Confidence intervals represent standard deviations and dots represent and individual local poplulation.

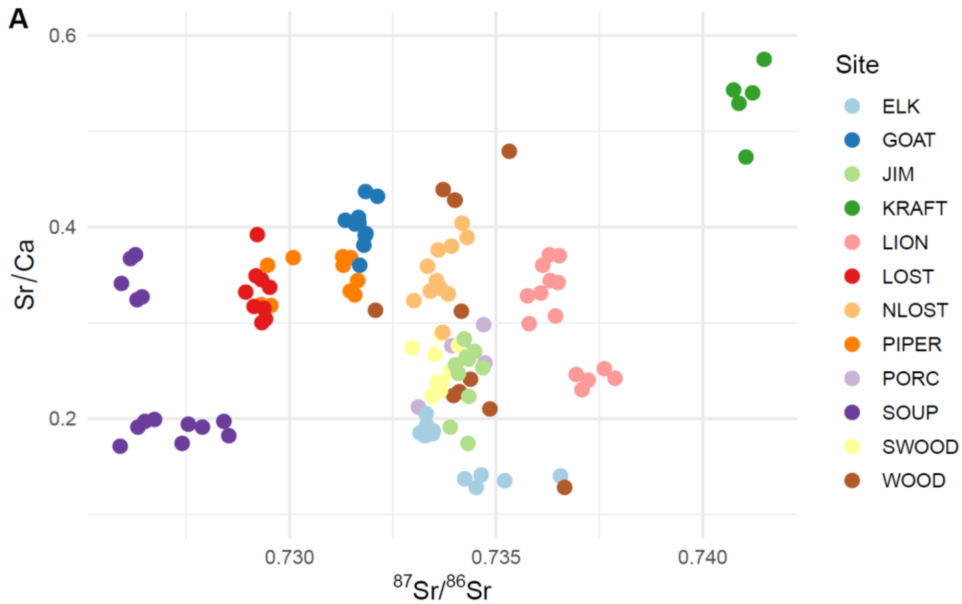


Figure L14. Bivariate otolith plot of Swan Scuplin data with colors representing the local population where the individuals were sampled.

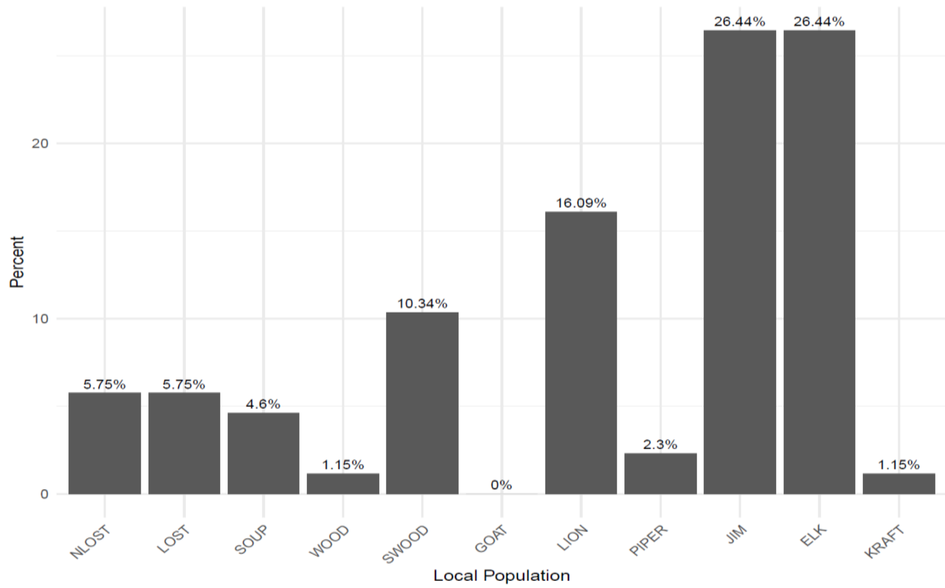


Figure L5. Results from the local population assignment from adult Bull Trout captured in Swan Lake.

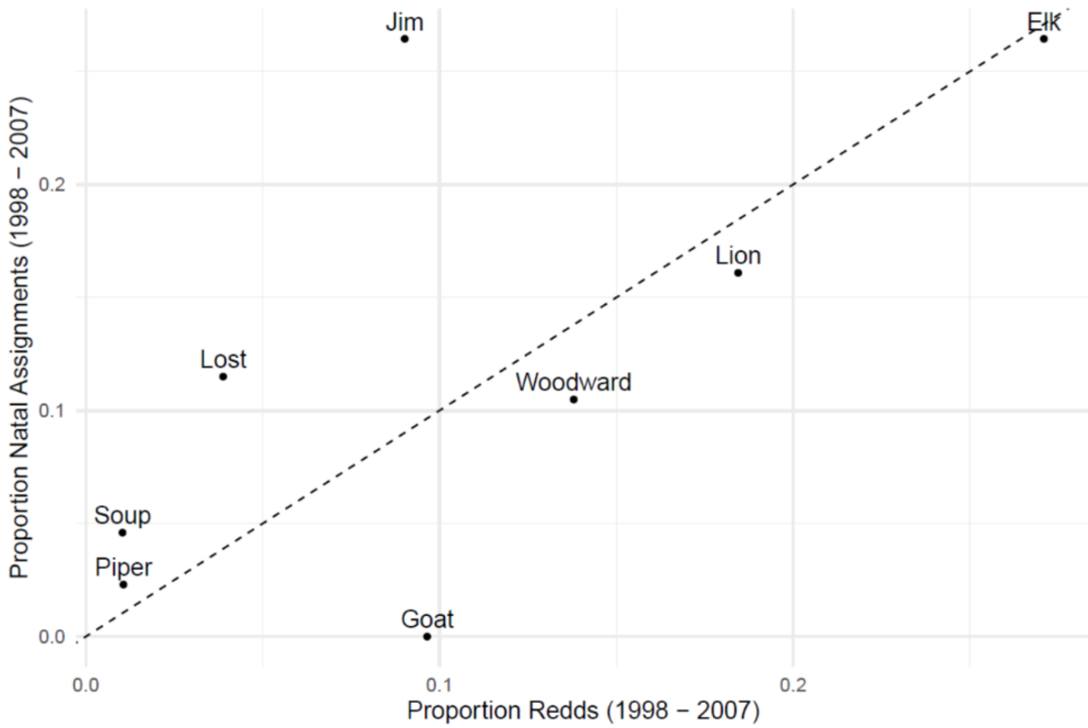


Figure L6. Propoportional analysis comparing Redds with natal assignements from Bull Trout caught in Swan Lake.

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