Northcentral Montana Westslope Cutthroat Trout Restoration Project

2021 Annual Report

MoTAC Project # 2021-12

MT Aquatic SWG Management Program # F19AF00704

MT Aquatic SWG Survey & Inventory Program # F19AF00705

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Region 4

Great Falls, MT

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Introduction

Westslope Cutthroat Trout (WCT) *Oncorhynchus clarkii lewisi*, historically the most widely distributed subspecies of Cutthroat Trout *O. clarkii*, have undergone reductions in distribution and abundance throughout their native range (Behnke 2002; Shepard et al. 2005; Heckel et al. 2020). The upper Missouri River drainage in Montana in particular has experienced marked reductions, with WCT occupying less than 5% of their historical range (Shepard et al. 1997; Shepard et al. 2003). Nonnative species introductions, habitat degradation, fragmentation, and overexploitation have been identified as factors leading to population declines (Shepard et al. 2005; Muhlfeld et al. 2016; Heckel et al. 2020). However, human-induced hybridization with nonnative trout has been especially detrimental causing widespread genomic extinction of WCT populations (Allendorf and Leary 1988; Muhlfeld et al. 2014).

The declining status of WCT has led to its designation as a Species of Special Concern by the State of Montana, a Sensitive Species by the U.S. Forest Service (USFS), and a Special Status Species by the U.S. Bureau of Land Management (BLM). In addition, in 1997 a petition was submitted to the U.S. Fish and Wildlife Service (USFWS) to list WCT as “threatened” under the Endangered Species Act (ESA). A 2003 USFWS status reviews found that WCT are “not warranted” for ESA listing; however, this finding was in litigation until 2008 and additional efforts to list WCT under ESA are possible in the future.

In an effort to advance range wide WCT conservation efforts in Montana, a Memorandum of Understanding and Conservation Agreement for Westslope Cutthroat Trout in Montana was developed in 1999 by several federal and state resource agencies (including BLM, Montana Fish, Wildlife & Parks [FWP], USFS, and Yellowstone National Park), non-governmental conservation and industry organizations, tribes, resource users, and private landowners (FWP 1999: MOU). The MOU outlined goals and objectives for WCT conservation in Montana, which if met, would significantly reduce the need for special status designations and listing of WCT under the ESA. The MOU was revised and endorsed by signatories in 2007 (FWP 2007). As outlined in the MOU’s, the primary management goal for WCT in Montana is to ensure the long-term self-sustaining persistence of the subspecies in its historical range. This goal can be achieved by maintaining, protecting, and enhancing all designated WCT “conservation” populations, and by reintroducing WCT to habitats where they have been extirpated.

A Federal Challenge Cost Share Agreement was established in 2001 between FWP and the USFS to implement and fund WCT restoration (Tews et al. 2000) as outlined by the MOU. Funding for the 2015 WCT restoration project was provided by the EPA and the State Wildlife Grants (SWG) program. In the 2016-2019 period, Northwestern Energy (formerly PPL Montana), Resource Development Grant Program (RDGP), and the Future Fisheries Program (FWP) provided additional funding for WCT restoration. At the November 2020 Missouri River Technical Advisory Committee (MoTAC) meeting, FWP was awarded $16,034 from Northwestern Energy to fund a fisheries technician to work directly with the FWP native species biologist on the Northcentral Montana WCT Restoration Project. This document specifically addresses work performed under the 2021 Federal Challenge Cost Share Agreement for WCT restoration in northcentral Montana.

Study Area

The status of WCT in northcentral Montana is described in this document. The following major drainages are included in the general study area: Arrow Creek, Belt Creek, Judith River, Smith River, Sun River, Teton River, Two Medicine River, Upper Missouri River, and the upper Missouri-Dearborn River (Figure 1).

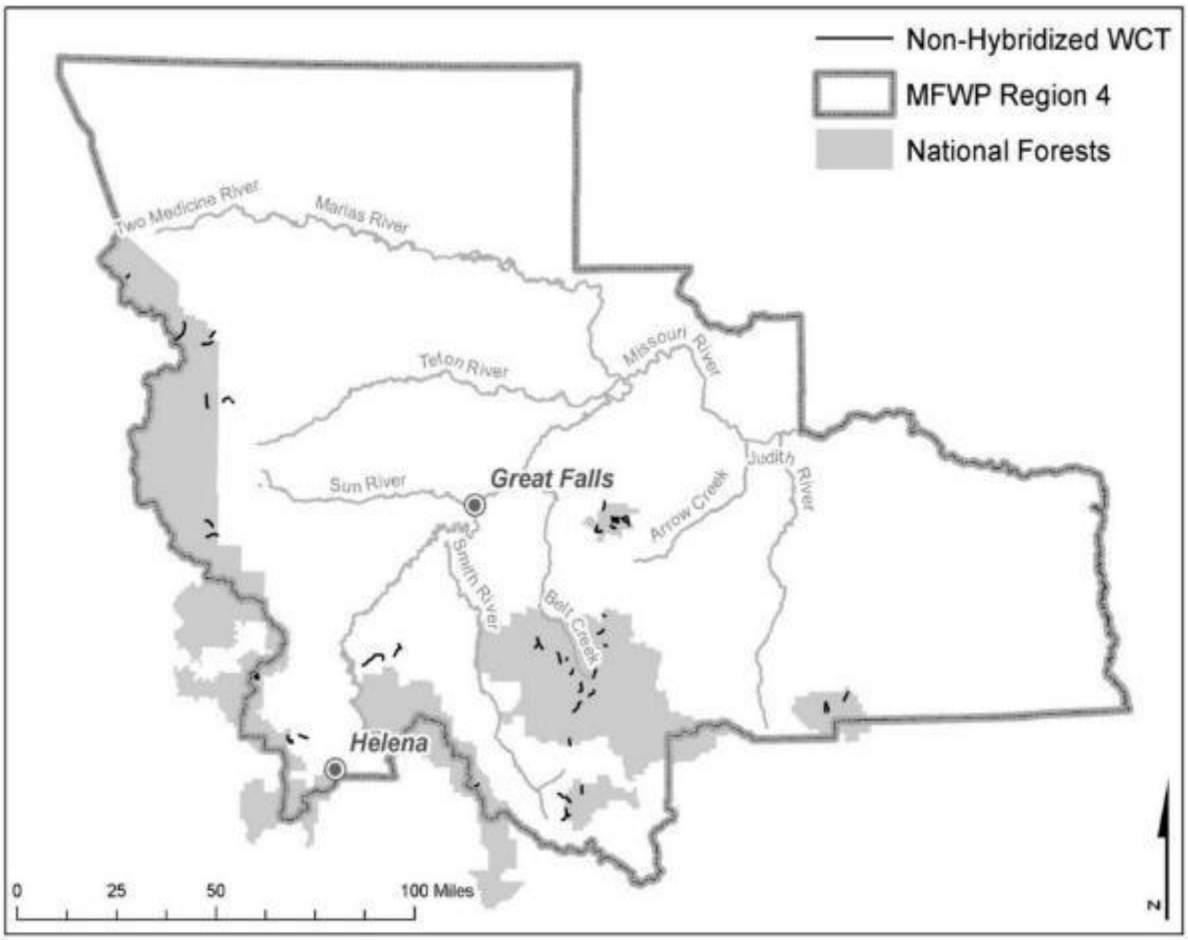

Figure 1. Study area in northcentral Montana with nonhybridized WCT populations (indicated in bold black).

Methods

Sampling of stream fish populations was conducted with a Smith-Root™ model LR-20B and/or model LR-24 battery powered backpack electrofishing unit(s) set to 30 hertz (Hz) at approximately 0.8-1.6 amperes (A) and 300-900 volts (V) dependent on conductivity. Relatively smaller streams were sampled with one backpack electrofishing unit and two backpack electrofishing units were used in tandem in larger streams and rivers. Multiple pass depletion method was typically used to estimate WCT population abundance in sampled streams (Zippin 1958; Carle and Strub 1978). Mean wetted stream width was determined by measuring ten random transects within each survey section. Stream dimensions were combined with population estimates and mean trout weight to calculate trout density (fish/km, fish/hectare) and biomass (kg/ha). Gill nets were used to sample fish from lentic habitats. Genetic samples were collected and preserved in 95% ethanol to be sent to the University of Montana Fish Conservation Genetics Lab for genetic analysis. Total length of fish was measured to the nearest millimeter and weight was measured to the nearest gram using an electronic scale. Conductivity in microsiemens (μS) and temperatures in degrees Celsius (°C) was measured and recorded in sampled streams.

The "Westslope Cutthroat Trout Restoration Plan" (Tews et al. 2000), the 1999 and 2007 Conservation Agreements (FWP 1999, 2007), and the "Status and Conservation Needs Plan" (Moser et al. 2009) are documents that detail the conservation techniques. Efforts include the creation and maintenance of barriers to block upstream movement of nonnative/invasive fish species, decreasing the number of sympatric nonnative fish present through suppression and removal to assist WCT survival, and performing piscicide treatments to create a fishless habitat in which to reestablish WCT. Increasing the range of WCT populations is achieved through transfer of nonhybridized WCT to fishless headwater streams, either in the form of live fish transfers or gametes transferred to remote site incubators (RSIs).

Conservation techniques used during the 2021 field season include: barrier maintenance, wild WCT transfers to restored waters, and WCT population and genetic monitoring.

Restoration Efforts in Northcentral Montana

The scope of the work completed by FWP in 2021 is described in the following maps, text, and histograms. The USFS and FWP worked cooperatively on many of the following projects. This report is organized by USGS hydrological unit code (HUC 8) subbasins where restoration efforts occurred and include: Arrow Creek, Belt Creek, Judith River, Smith River, Sun River, Teton River, Two Medicine River, Upper Missouri River, and Upper Missouri-Dearborn River.

I. Arrow Creek Subbasin

Boyd Creek

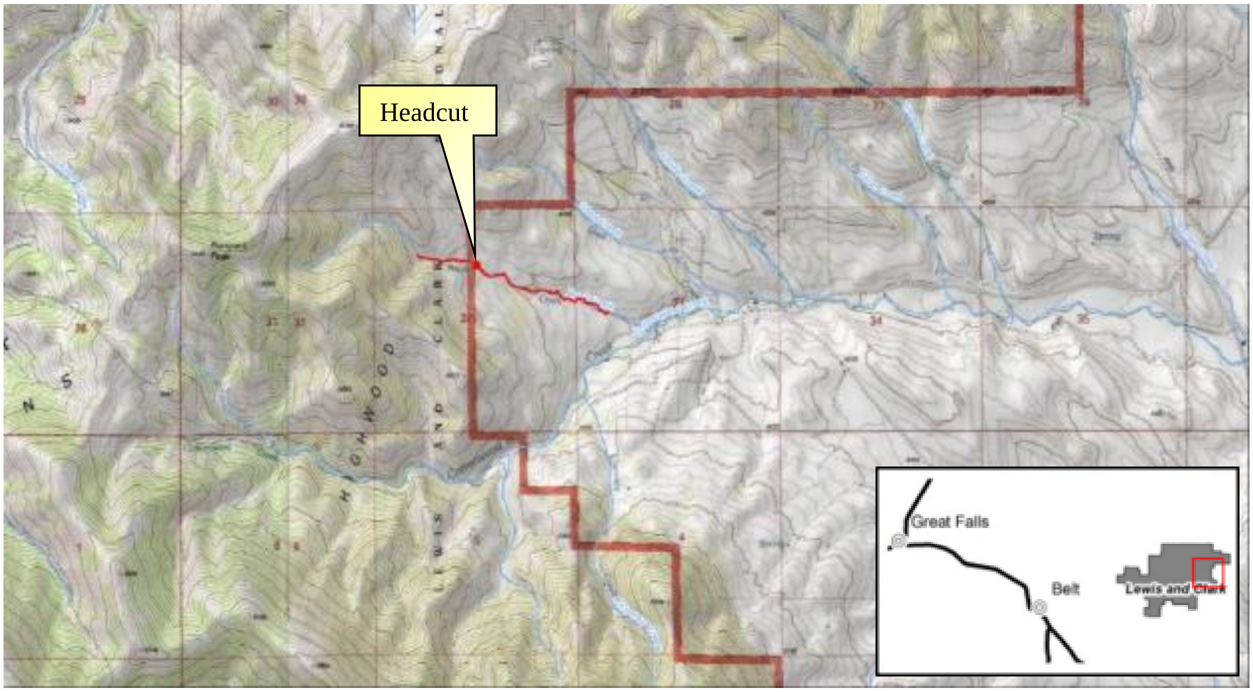

Figure 1. Boyd Creek in the Arrow Creek subbasin. Stream segments delineated in red indicate areas sampled in 2021.

<u>Background</u>
Boyd Creek contains a small nonhybridized population of WCT and a sympatric Brook Trout population. Genetic and demographic monitoring of the Boyd Creek WCT population has been performed periodically from 1996-2017. Genetic samples have been collected and analyzed on four occasions (1996, 2004, 2005, and 2015; n=79) with no detection of nonnative alleles to date. Brook Trout suppression has been performed opportunistically on Boyd Creek, most recently from 2016-2017.

<u>2021 Monitoring</u>
Boyd Creek was sampled on July 29th, 2021. During this low water year Boyd Creek was dry at the confluence of Cottonwood Creek. The entire fish bearing reach was backpack electrofished and a total of 144 WCT and 123 EB were collected. Genetic samples were collected from 30 WCT for updated genetic analysis. A headcut located at 47.45660, -110.48074 just below the Forest Service boundary impeded upstream distribution of EB isolating a small 482 m reach where only WCT occurred.

Cottonwood Creek

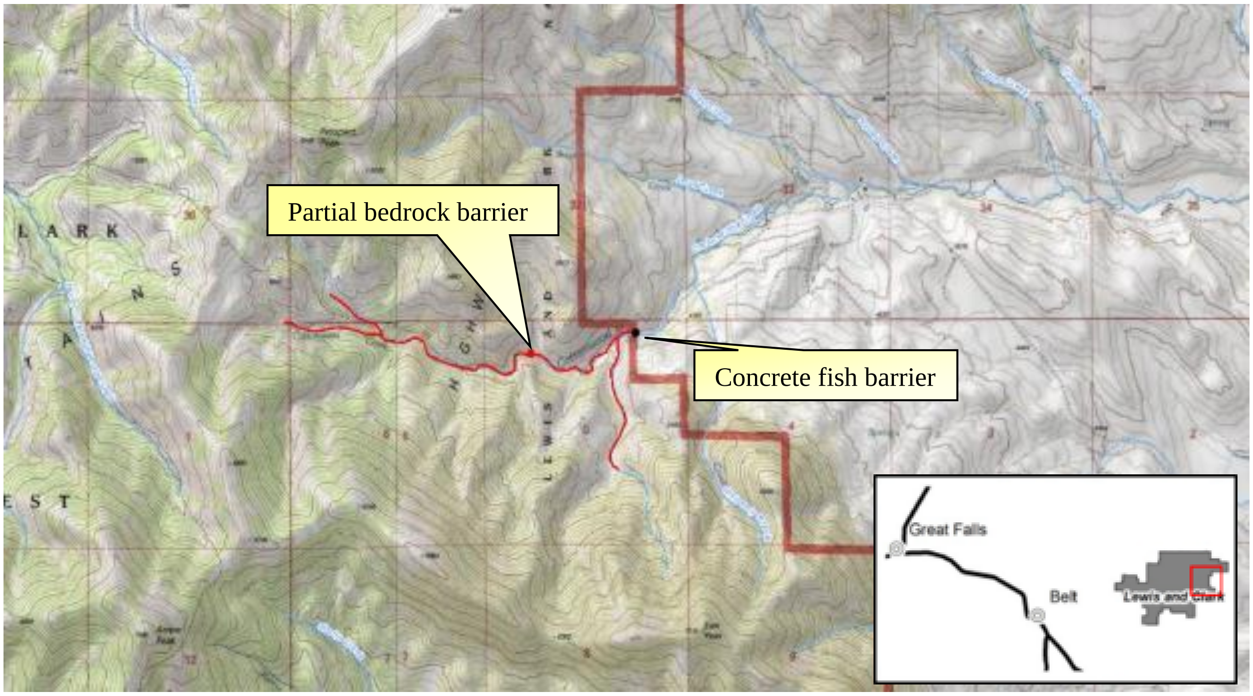

Figure 2. Cottonwood Creek in the Arrow Creek subbasin. Stream segments delineated in red indicate extent of WCT occupied habitat.

Background

Cottonwood Creek contains a nonhybridized population of WCT partially protected by a natural bedrock barrier. In 2001, a concrete fish barrier was installed at the Forest Service boundary (47.44472, -110.47552) to further protect and expand the WCT population (Figure 2). Brook Trout removal was performed between the concrete barrier and partial waterfall barrier from 2000-2005 and appeared effective at removing all Brook Trout above the constructed fish barrier. Since 2005, monitoring has occurred periodically and in 2015 Brook Trout were detected upstream of the constructed fish barrier. The origin of these fish is unknown, as the barrier appeared structurally sound and functional during the 2015 sampling. Removals performed in the summer of 2016-2019 resulted in the removal of 26 Brook Trout in 2016, two in 2017, four in 2018, and two in 2019. No Brook Trout were detected above the constructed fish barrier in 2020.

2021 Monitoring

A single pass electrofishing monitoring effort was performed on Cottonwood Creek on July 28th and 29th of 2021. The mainstem of Cottonwood Creek was shocked from the constructed fish barrier to the partial waterfall barrier and the first tributary was shocked from its confluence with Cottonwood Creek upstream until no fish were detected. Species and total number of fish older were recorded. A total of 423 WCT were collected in the mainstem of Cottonwood Creek between the barriers and 297 WCT were collected in the 1st tributary. A single 239 mm Brook Trout was detected in 2021 located near the constructed fish barrier (47.44488, -112.47604). Annual monitoring of Brook Trout presence in Cottonwood Creek should continue.

II. Belt Creek Subbasin

Belt Creek

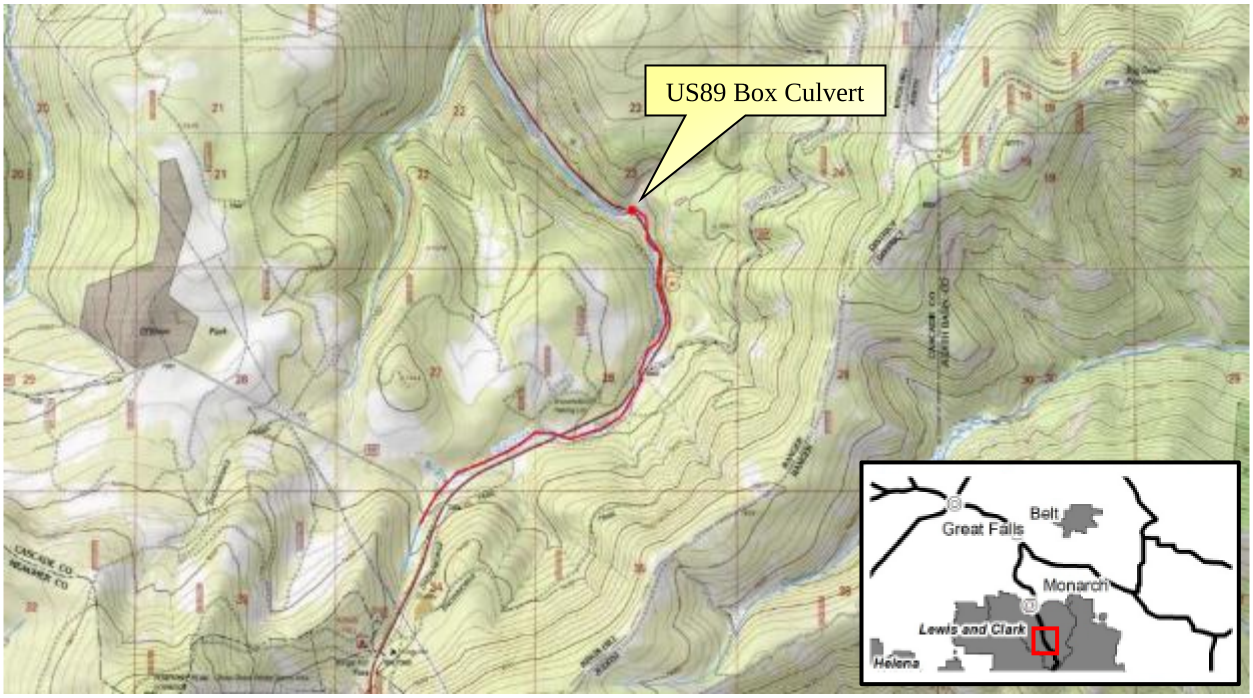

Figure 3. Belt Creek in the Belt Creek subbasin. Stream segments delineated in red indicate extent of nonhybridized WCT occupied habitat.

Background

The Belt Creek subbasin encompasses 798 square miles of the upper Missouri River Drainage. Belt Creek flows approximately 90 miles north to south from its headwaters in the Little Belt Mountains on the Lewis and Clark National Forest near Neihart, Montana to the Missouri River where it enters just downstream of Great Falls, Montana. Historically, the Belt Creek subbasin contained an estimated 249 miles of habitat supporting WCT. Because many headwater streams hold conservation populations of WCT, the mainstem of Belt Creek holds good numbers WCT of varying genetic purity. A large box culvert on US 89 (46.86990, -110.66854) isolates the upper 2.5 miles of Belt Creek from nonnative trout located downstream. Genetic samples collected from WCT in this upper reach from 1994-1998 (n=39) indicated the presence of a nonhybridized population.

2021 Monitoring

The headwaters of Belt Creek were sampled on July 7th, 2021, to collect genetic samples for updated analysis. Three sections of Belt Creek were backpack electrofished to obtain genetic samples in this reach: immediately upstream of the box culvert barrier, above the second culvert upstream of the box culvert, and above the third culvert upstream of the box culvert. Samples were collected from 10 individual WCT from each section for a total sample size of 30. WCT collected ranged in size from 94-243 mm in length. No other fish species were observed.

Bender Creek

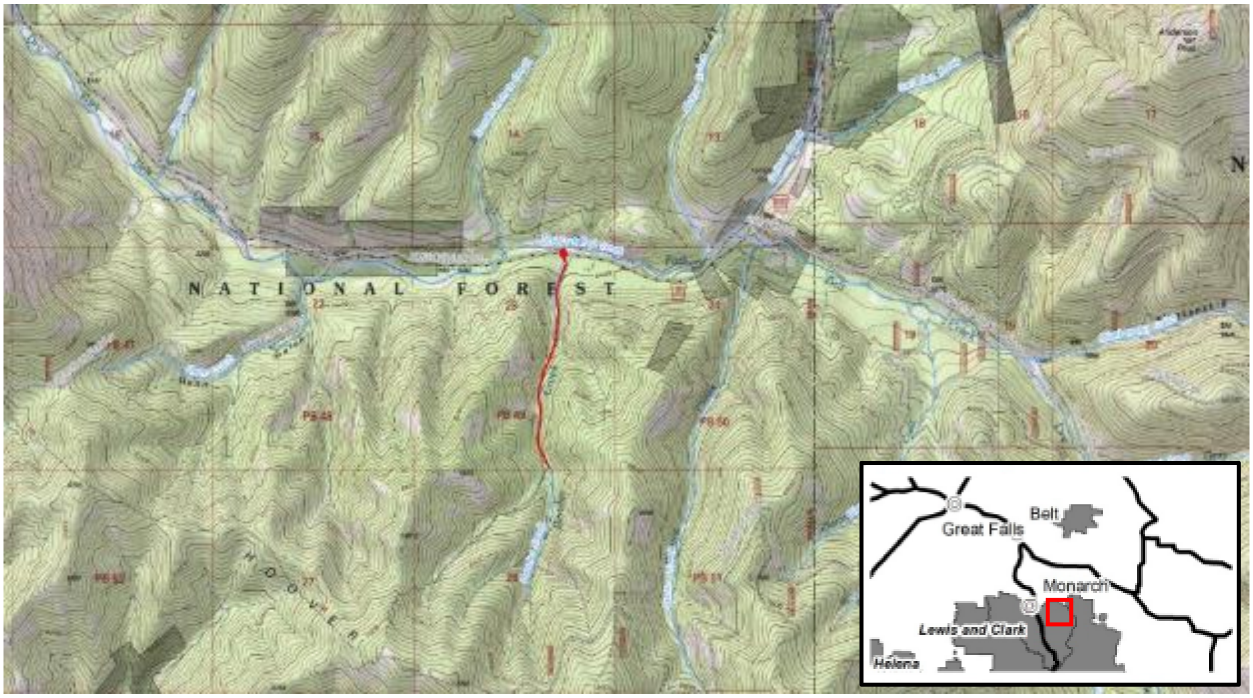

Figure 4. Bender Creek in the Belt Creek subbasin. Stream segments delineated in red indicate extent of WCT occupied habitat.

Background

Bender Creek is a small tributary of Dry Fork Belt Creek located east of the town of Monarch, MT. A perched culvert at the FS RD 120 crossing (47.05192, -110.67141) partially protects Bender Creek from nonnative trout populations located downstream. Upstream of the perched culvert, Bender Creek contains a slightly hybridized (99% WCT, 0.6% RB) population of WCT. Monitoring of Bender Creek has occurred periodically from 1995-2016.

2021 Monitoring

Bender Creek was sampled on June 28th, 2021. A single pass backpack electrofishing effort was made to detect presence/absence of nonnative trout above the perched culvert barrier. A 205 m reach of Bender Creek was sampled and a total of 49 WCT were collected. No other fish species were observed. WCT ranged in size from 42-205 mm in length.

Big Willow Creek

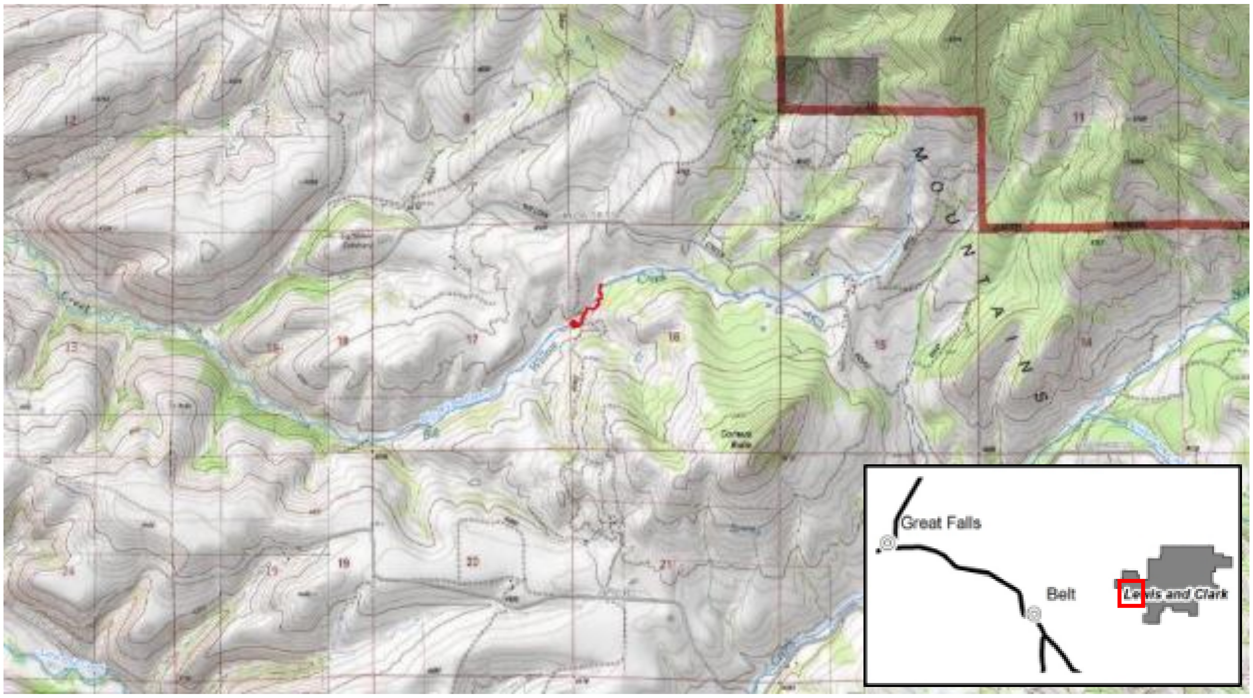

Figure 5. Big Willow Creek in the Belt Creek subbasin. The stream segment delineated in red indicates the area sampled in 2021.

Background

Big Willow Creek is a tributary of Belt Creek that drains the southwest portion of the Highwood Mountains east of the town of Belt, MT. Big Willow Creek has been sampled previously once in 2006 downstream of the East Highwood Road crossing. Seining at this site produced a catch of Lake Chub, Brassy Minnow, White Sucker, Northern Redbelly Dace, Longnose Dace, and Fathead Minnow. Although no salmonids have been documented in Big Willow Creek, nearby tributaries of Belt Creek such as Little Belt Creek support conservation populations of WCT.

2021 Monitoring

The headwaters of Big Willow Creek were sampled on September 28th, 2021. A 400 m reach of Big Willow Creek was backpack electrofished to detect presence/absence of WCT. Longnose Dace, Rocky Mountain Sculpin, and Lake Chub were collected in this effort. No salmonids were detected.

Carpenter Creek

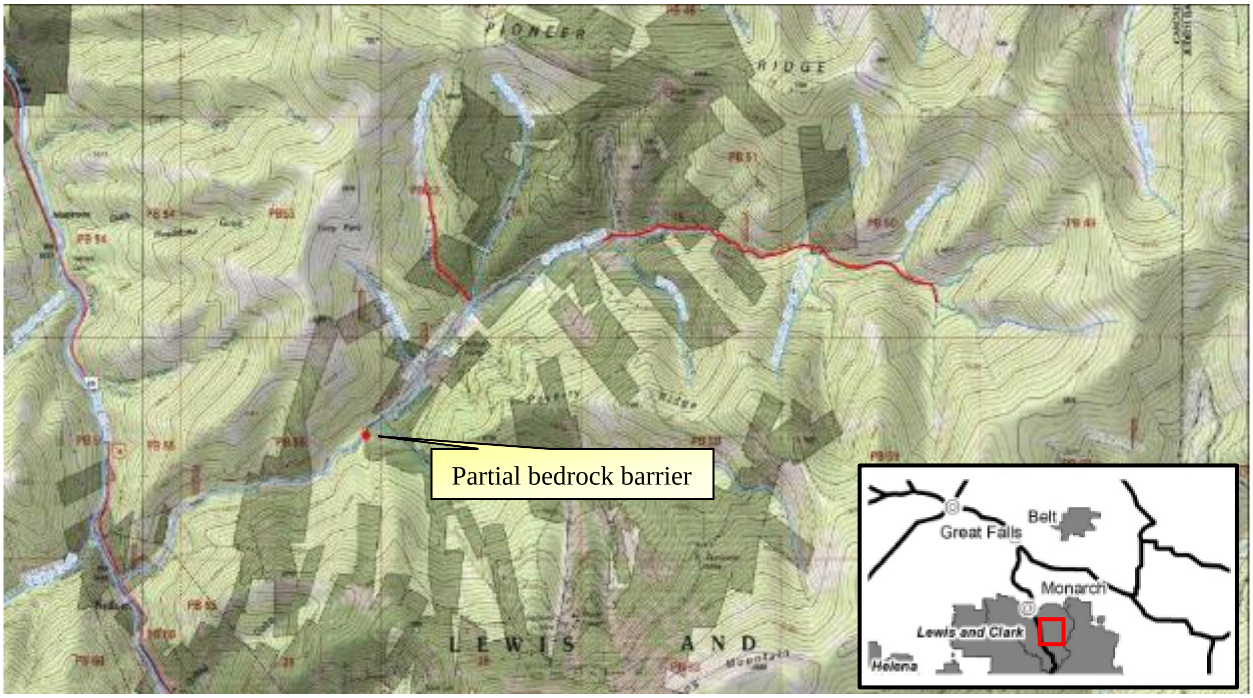

Figure 6. Carpenter Creek in the Belt Creek subbasin. The stream segments delineated in red indicate the areas occupied by nonhybridized WCT.

<u>Background</u>

The Carpenter Creek drainage contains two nonhybridized populations of WCT; one in its headwaters and one in Haystack Creek. Both populations are currently isolated and protected from nonnative species invasions due to poor water quality caused by mining effluent. The area is currently being remediated and it is anticipated that the chemical barrier will eventually dissipate as water quality improves. The need for a physical barrier to preserve the WCT populations is recognized and is currently being pursued. A section of Carpenter Creek near the mouth is shocked annually (from the confluence of Belt Creek to a partial waterfall barrier near the confluence of Snow Creek; Figure 6). During past sampling efforts no fish have been detected in this reach; however, in 2015 two fish were caught near the mouth. The presence of fish was a positive response in improving water quality but provided concern for the potential for nonnative invasion and subsequent risk of WCT loss to hybridization. Nonnative fish have been monitored annually in this section since 2015. In 2016 seven nonnative fish were collected, 4 in 2017, 5 in 2018, 1 in 2019, and 2 in 2020. Demographic and genetic monitoring of Carpenter Creek WCT populations was performed most recently in 2018. A total of 591 fish 100 mm and greater were estimated in Carpenter Creek over approximately 2.5 kilometers of occupied habitat.

<u>2021 Monitoring</u>

Lower Carpenter Creek was electrofished from the confluence of Belt Creek to the partial waterfall barrier to monitor nonnative trout presence in a single pass effort on August 16th and 17th, 2021. Two Brook Trout were detected in this effort located in the first 150 m above the US 89 crossing. No other fish were observed. Continued monitoring of lower Carpenter Creek is warranted until a permanent fish barrier is constructed in the drainage.

Crawford Creek

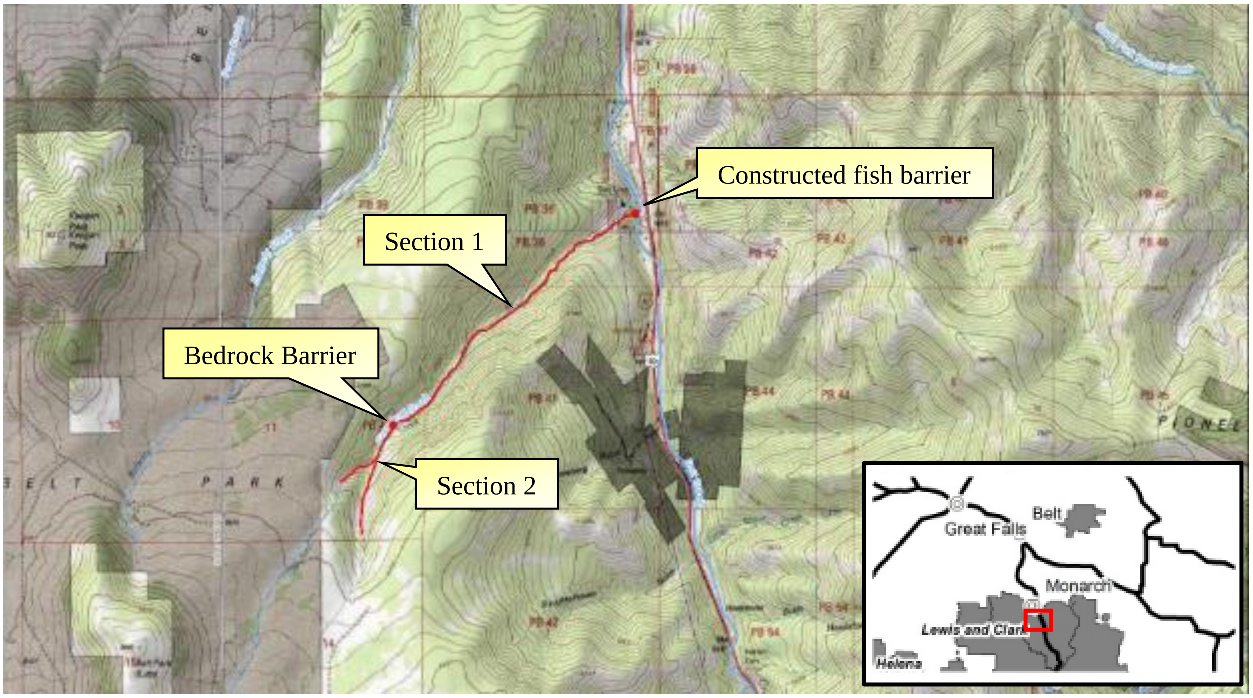

Figure 7. Crawford Creek in the Belt Creek subbasin. The stream segments delineated in red indicate the area occupied by nonhybridized WCT.

Background

Upper Crawford Creek contains a small population of nonhybridized WCT protected by a natural bedrock barrier in the headwaters of the stream (Figure 7). A small concrete barrier was built near the mouth of Crawford Creek in 2005 and piscicide treatments followed in 2006 to expand WCT distribution within the drainage. WCT were transferred from O'Brien Creek to the lower reach of Crawford Creek following the treatment. Failure of the barrier shortly after its completion resulted in non-native fish passage from Belt Creek. In 2013, a culvert barrier was constructed on the FS RD 1122 crossing about 0.1 mile from the confluence with Belt Creek (Figure 7) and a piscicide treatment followed that same year. No nonnative fish have been found upstream of the constructed fish barrier in Crawford Creek following the 2013 piscicide treatment.

2021 Monitoring

Crawford Creek was surveyed on August 11th and September 2nd, 2021. Two 100 m fish population abundance estimate sections were established; one located approximately 1.29 km above the constructed fish barrier (Section 1), and one located 0.16 km above the natural bedrock barrier (Section 2). Multiple pass depletion methods were used estimate population abundance. Estimated WCT density was much higher in Section 2 at 1,110 fish/km compared to 80 fish/km in Section 1. WCT abundance in the lower reach of Crawford Creek below the natural bedrock barrier appears to still be recovering from the 2013 piscicide treatment. Downstream dispersal from the aboriginal population in the headwaters remains slow despite the high densities observed upstream.

Crawford Creek —NATIVE TROUT POPULATION SURVEY

1. General Information— Date: **August 11, 2021 and September 2, 2021** Biologist: **A. Poole**
2. Stream Information—
   Name, section, county: **Crawford Creek, 41, 40, Cascade**
3. Survey Site Information (see attached map)—
   Upstream range of native trout (general description and GPS): **3.22 km above constructed fish barrier (46.98366, -110.80102)**
   Downstream range of native trout (general description and GPS): **constructed fish barrier (47.00104, -110.76982)**
   Location (GPS) and description of barriers: **constructed fish barrier (47.00104, -110.76982); natural bedrock barrier (46.98721, -110.79549)**
   Stream Length—Occupied habitat: **3.78 km (1.81 mi)** Available habitat: **3.78 km (2.35 mi)**
   Survey method & equipment: **backpack battery electrofisher; two-pass depletion**
   Survey sites (general description and UTM)—
   Section 1: **1.28 km upstream of constructed fish barrier; 46.99423, -110.78341**
   Section 2: **0.16 km upstream of natural bedrock barrier; 46.98587, -110.79650**

Parameter	**Section 1**	**Section 2**
Section length (m)	100 m	100 m
Mean stream width (m) (n)	2.82 m (10)	1.81 m (10)
Section area (hectares)	0.028 ha	0.018 ha
WCT		
Removal Pattern	7 1	76 25
Population estimate	8 (±1)	111 (±13)
Capture probability	0.860	0.670
Mean length (mm) (n)	192 (8)	137 (101)
Mean weight (g) (n)	71 (8)	29 (101)
Mean KTL (n)	0.97 (8)	0.85 (101)
Number fish per km (95 % CI)	80 (±10)	1,110 (±130)
Number fish per ha (95 % CI)	285 (±36)	6,166 (±722)
Biomass (kg per ha) (95 % CI)	20 (±3)	178 (±21)

Figure 8. Crawford Creek fish population estimate results.

Logging Creek

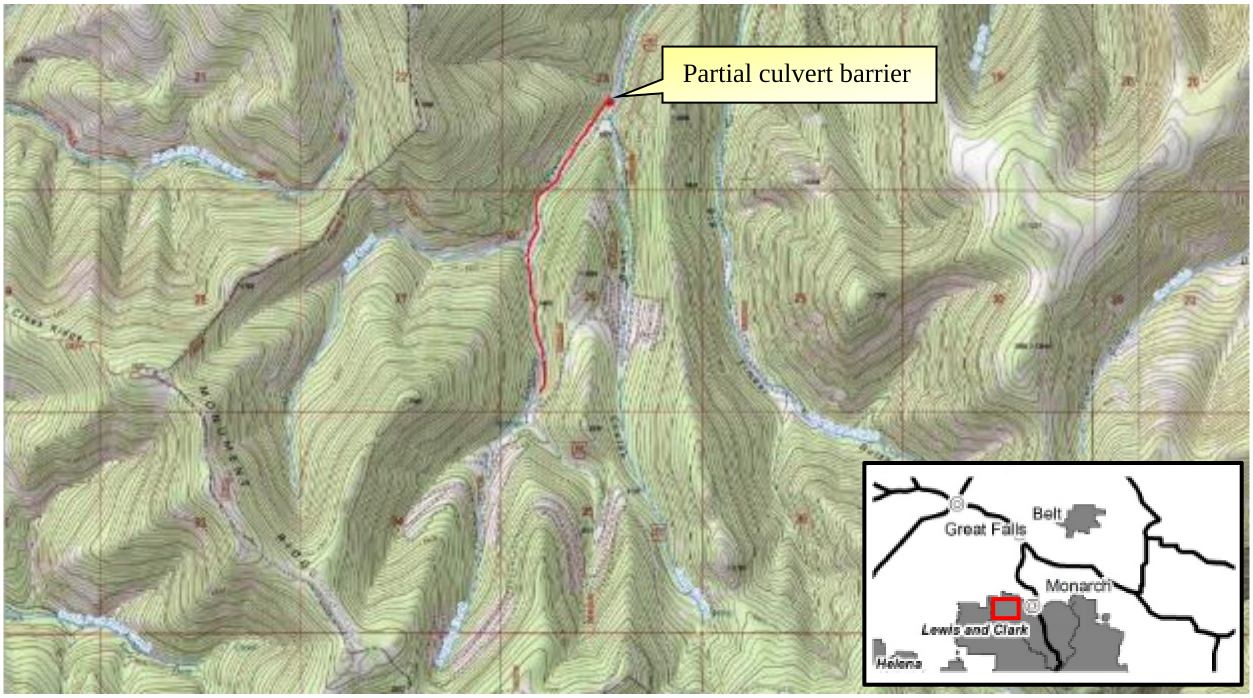

Figure 9. Logging Creek in the Belt Creek subbasin. The stream segment delineated in red indicates the area sampled in 2021.

Background

Logging Creek is an 11.5-mile-long tributary of Belt Creek that drains a 42 square mile area in the Little Belt Mountains. Genetic samples collected by the University of Montana in 1989 indicated a 100% WCT population in the headwaters of Logging Creek. This population is thought to be marginally protected by a culvert located at the FS RD 839 crossing just downstream of the Long Coulee confluence (47.04653, -111.05676).

2021 Monitoring

Logging Creek was sampled on July 7th, 2021, to obtain WCT genetic samples for updated analysis. Two sections of Logging Creek were backpack electrofished above the partial culvert barrier: one starting at the Deep Creek trailhead and another starting at the first culvert above the trailhead. Ten WCT and six Brook Trout were collected in 113 m of stream at the Deep Creek trailhead. In the upper section above the trailhead, ten presumed WCTxRB hybrids were collected and no Brook Trout were detected. A total of 20 genetic samples were collected from the WCT and suspected WCTxRB hybrids and submitted for analysis.

O’Brien Creek

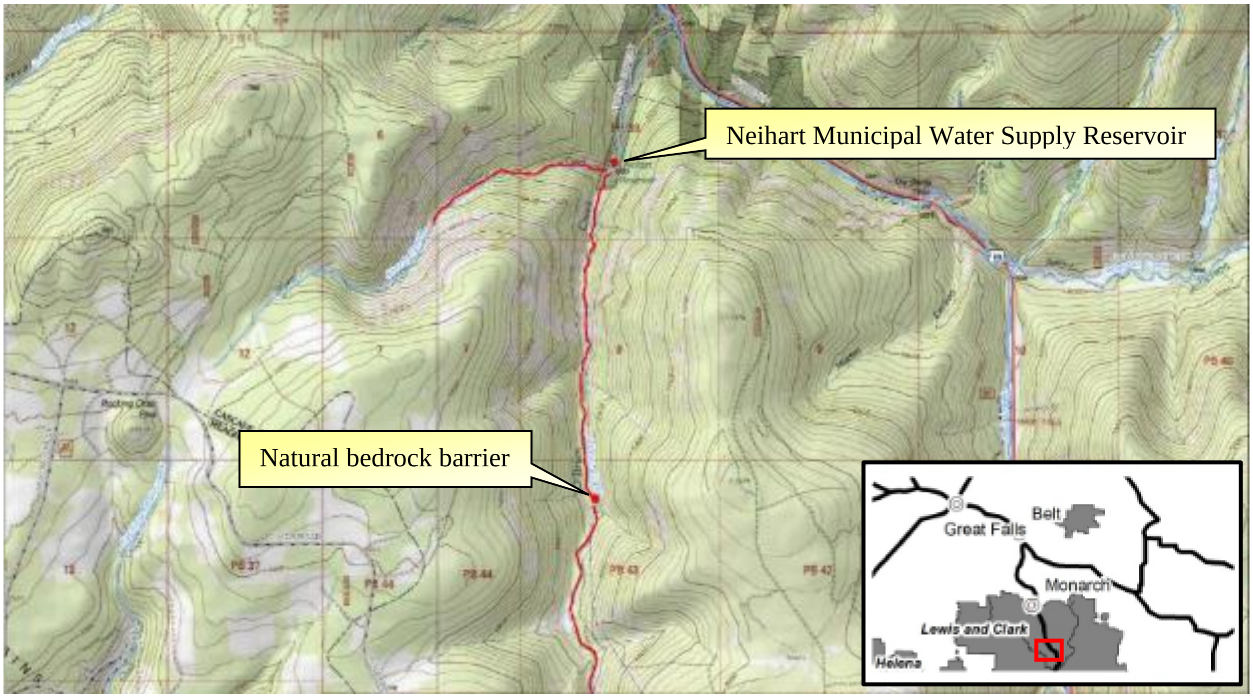

Figure 10. O’Brien Creek in the Belt Creek subbasin. The stream segments delineated in red indicate the areas occupied by WCT.

Background

O’Brien Creek contains a nonhybridized population of WCT above a natural bedrock barrier located upstream of the Neihart municipal water supply reservoir (Figure 10). This populations demographic and genetic status has been monitored periodically since 1997; most recently in 2020. Historic sampling records indicate lower O’brien Creek (between the Neihart Reservoir and the natural bedrock barrier) contains a slightly hybridized WCT population and potentially a sympatric Brook Trout population. A single Brook Trout was recorded from this reach of O’brien Creek in 1997. Subsequent sampling in 2007 detected only WCT in lower O’brien Creek.

2021 Monitoring

Lower O’brien Creek was sampled on September 7th, 2021. A single pass backpack electrofishing effort was made to detect presence/absence of nonnative trout above the Neihart municipal water supply reservoir. A 160 m reach of stream was sampled and 22 WCT were collected in this effort. No Brook Trout were detected. WCT ranged in size from 103-248 mm in total length. Rocky Mountain Sculpin were noted as common. If Brook Trout are not present in lower O’brien Creek, it is likely that this population meets the “secured” criteria as defined in the Montana Statewide Fisheries Management Plan Conservation Goals for Westslope Cutthroat Trout.

Shorty Creek

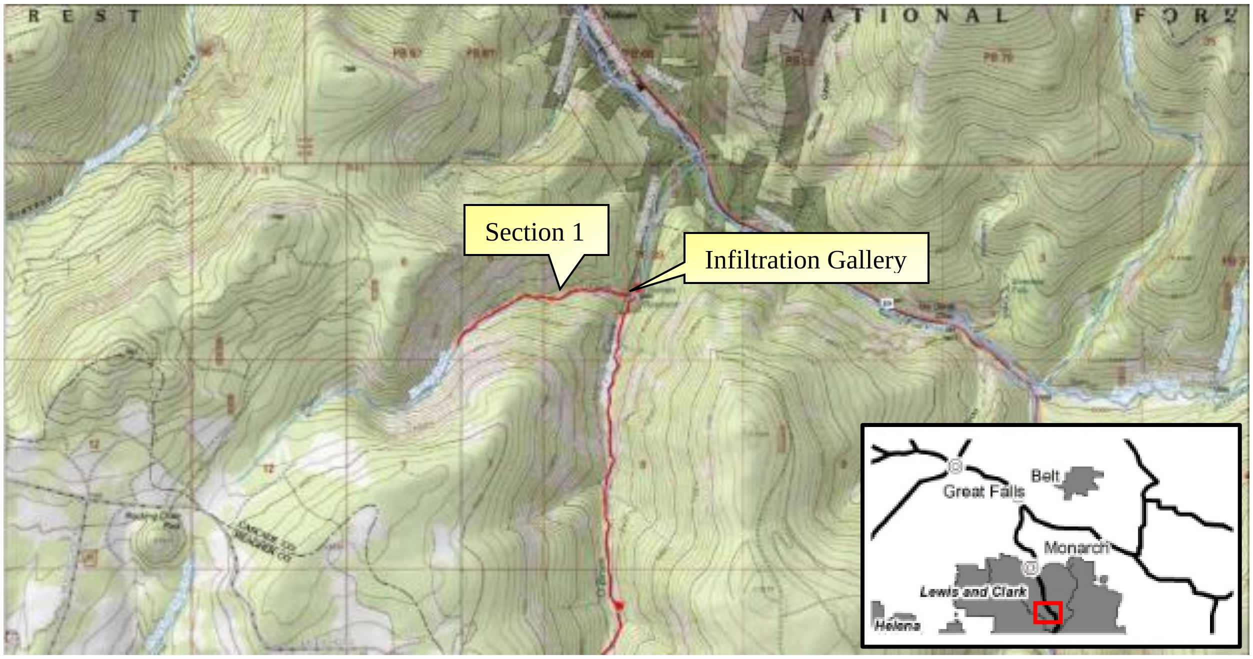

Figure 11. Shorty Creek in the Belt Creek subbasin. The stream segments delineated in red indicate the areas occupied by WCT.

Background

Shorty Creek is a tributary of O'brien Creek that currently serves as the primary drinking water source for the town of Neihart, MT. During high flows Shorty Creek enters O'brien Creek at the Neihart municipal water supply reservoir. For the remainder of the year Shorty Creek is diverted to the Neihart water treatment plant through an infiltration gallery. Previous genetic samples collected from WCT in Shorty Creek indicated a nonhybridized population (1997, n = 5).

2021 Monitoring

Shorty Creek was sampled on September 7th and September 9th, 2021. A single pass backpack electrofishing effort was made to collect 25 WCT from three different sections of Shorty Creek for updated genetic analysis. A 100 m population estimate section was established approximately 500 m upstream from the infiltration gallery in the first meadow. Multiple pass depletion methods were used to estimate population abundance. WCT density in Shorty Creek was similar to other WCT conservation populations located in the Little Belt Mountains at 270 fish/km.

Shorty Creek —NATIVE TROUT POPULATION SURVEY

1. General Information— Date: **September 9th, 2021** Biologist: **A. Poole**
2. Stream Information—
   Name, section, county: **Shorty Creek, 39, Cascade**
3. Survey Site Information (see attached map)—
   Upstream range of native trout (general description and GPS): **1.62 km above infiltration gallery (46.91236, -110.74999)**
   Downstream range of native trout (general description and GPS): **infiltration gallery (46.91490, -110.73287)**
   Location (GPS) and description of barriers: **infiltration gallery (46.91490, -110.73287)**
   Stream Length—Occupied habitat: **1.62 km (1.01 mi)** Available habitat: **1.62 km (1.01 mi)**
   Survey method & equipment: **backpack battery electrofisher; two-pass depletion**
   Survey sites (general description and UTM)—
   Section 1: **0.51 km upstream of infiltration gallery; 46.91489, -110.73950**

Parameter	**Section 1**
Section length (m)	100 m
Mean stream width (m) (n)	1.99 m (10)
Section area (hectares)	0.020 ha
WCT	
Removal Pattern	18 7
Population estimate	27 (±6)
Capture probability	0.610
Mean length (mm) (n)	120 (25)
Mean weight (g) (n)	18 (25)
Mean KTL (n)	0.89 (25)
Number fish per km (95 % CI)	270 (±60)
Number fish per ha (95 % CI)	1,350 (±300)
Biomass (kg per ha) (95 % CI)	24 (±5)

Figure 12. Shorty Creek fish population estimate results.

III. Judith River Subbasin

South Fork Judith River

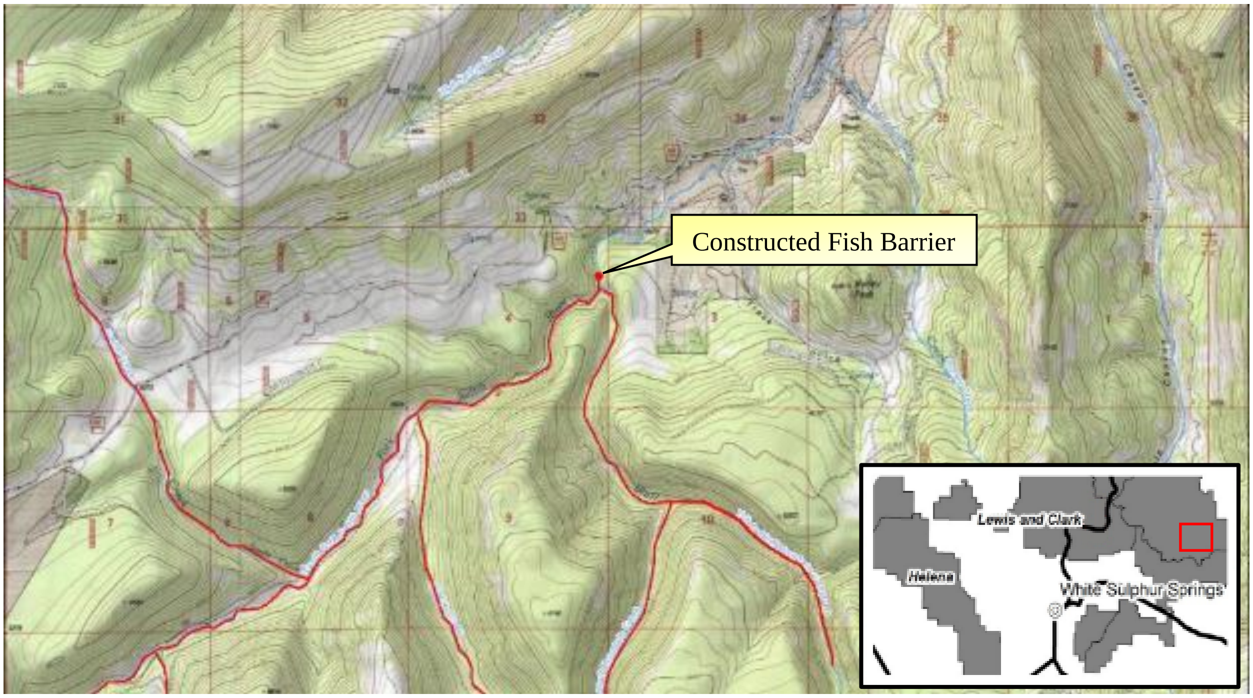

Figure 13. South Fork Judith River in the Judith River subbasin. The stream segments delineated in red indicate the areas occupied by the South Fork Judith River WCT conservation population.

Background

The upper South Fork Judith River and its tributaries contains a large population of WCT and WCTxRB of varying levels of hybridization. To prevent continued hybridization and upstream colonization by nonnative fishes, a conceptual design for a fish barrier near Bluff Mountain Creek was developed in 2002. Design of the barrier and an EA for removal of nonnative fish was completed in 2005 and construction of the barrier was completed in 2006. After construction of the barrier, highly hybridized WCTxRB and Brook Trout were removed from 13 miles of the mainstem South Fork Judith and its tributaries from 2006-2008 in an intensive mechanical removal effort. After 2008, nonnative removals occurred in the South Fork Judith and tributaries periodically. Following the intensive removal effort in 2008, the South Fork Judith River has been stocked annually with 2,000-10,000 two-inch MO12 WCT from the Washoe Park Trout Hatchery. The original goal for the South Fork Judith River was to maintain a WCT metapopulation of > 95% WCT genetic contribution.

In the spring of 2011, a high flow event damaged the riprap surrounding the constructed fish barrier that compromised its integrity. A retrofit was designed and constructed that same year following the high flow event. Since 2015, several barrier evaluations have been performed to determine the effectiveness of the barrier at precluding fish passage. To date, no marked fish have been detected above the barrier.

2021 Monitoring

The South Fork Judith River was sampled on June 22nd and July 26-27th, 2021. A single pass backpack electrofishing effort was made to collect fish for marking to evaluate the effectiveness of the fish barrier. A 450 m reach below the barrier was electrofished on June 22nd and July 26th. A 1.3 km reach above the barrier was electrofished on June 22nd and July 26-27th. All Brook Trout, Rainbow Trout, WCTxRB hybrids collected received an adipose clip and subdermal visual implant elastomer (VIE) mark on their left dentary and were released below the barrier. In total, 262 fish were marked and released below the barrier. Annual monitoring for marked fish above the barrier will continue in 2022.

On November 4th, 2021, 10,000 adipose clipped MO12 WCT were released in the South Fork Judith River at four locations between the constructed fish barrier and the first meadow above the Cabin Creek confluence (2.35 km upstream of barrier).

Weatherwax Creek

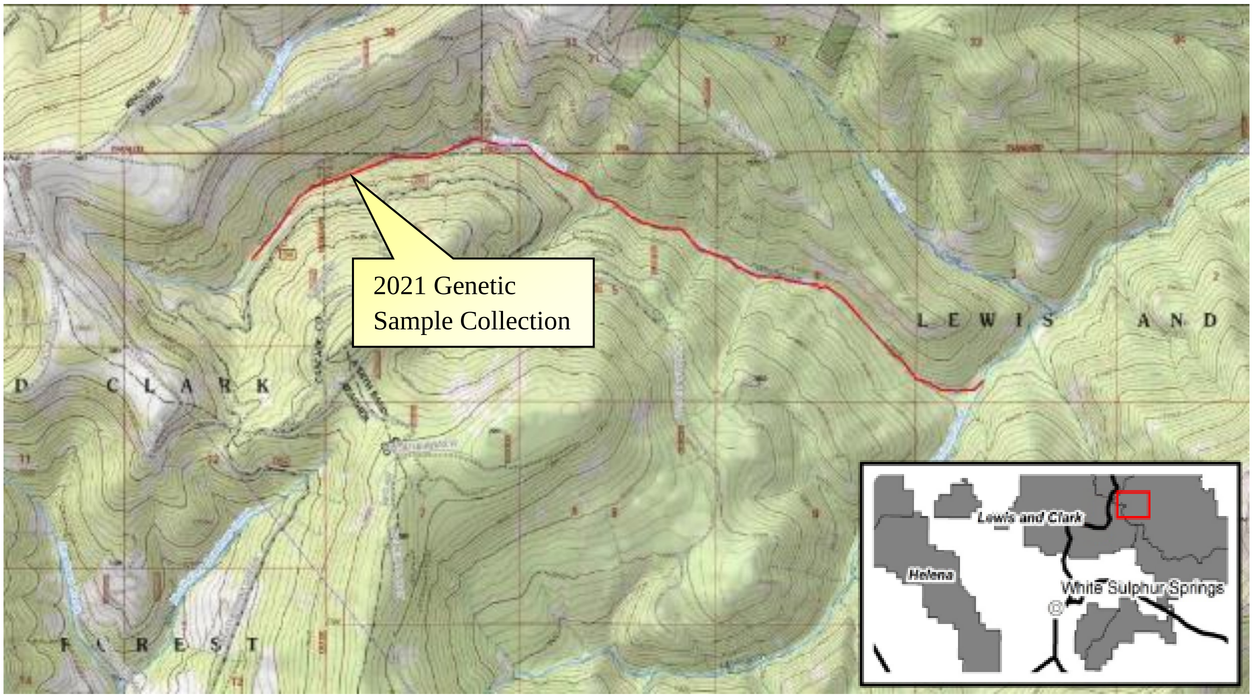

Figure 14. Weatherwax Creek in the Judith River subbasin. The stream segments delineated in red indicate areas occupied by WCT.

<u>Background</u>

Weatherwax Creek is a headwater tributary of the Middle Fork Judith River located just east of Kings Hill Pass southeast of the town of Neihart, MT. The stream contains both WCT and Brook Trout. Periodic demographic and genetic monitoring have occurred in the drainage since 1996. Genetic samples collected from the lower drainage near the confluence of Harrison Creek in 1996 indicated a 91.3% WCT population (n = 10). The most recent genetic analysis of the WCT from Weatherwax Creek in 2003 was collected from 25 fish approximately 2 miles upstream of the Harrison Creek confluence. This sample indicated a nonhybridized population of WCT still inhabited the headwaters of the stream. However, no known barriers to fish movement exist in the drainage.

<u>2021 Monitoring</u>

Weatherwax Creek was sampled on July 22nd, 2021. A headwater reach of stream was targeted for additional genetic analysis approximately 1.5 miles upstream from the 2003 sample. A 965 m reach of stream was backpack electrofished resulting in the collection of 25 WCT and 15 Brook Trout. Genetic samples were collected from all WCT sampled. WCT ranged in size from 103-265 mm in total length.

IV. Smith River Subbasin

Camas Lake and Big Camas Creek

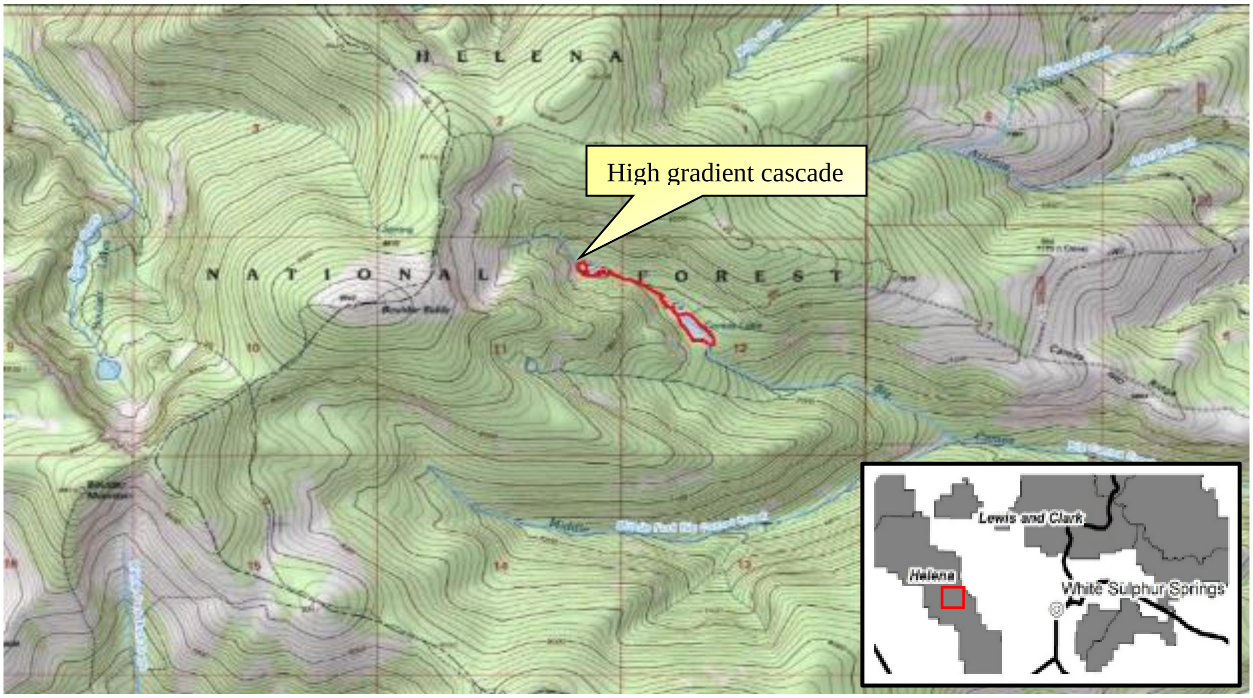

Figure 15. Camas Lake and Big Camas Creek in the Smith River subbasin. The stream segments delineated in red indicate areas sampled in 2021.

Background

Camas Lake and upper Big Camas Creek were likely historically fishless above a series of natural waterfall barriers located upstream of the confluence of Little Camas Creek. Yellowstone Cutthroat Trout *Oncorhynchus clarkii bouvieri* (YCT) were stocked in Camas Lake in 1938 and 1940 and subsequently established a self-sustaining population. Extensive surveys of the Big Camas Creek drainage were conducted in the early 2000's and the area was recognized as a high priority WCT restoration site. In 2014, Camas Lake and Big Camas Creek were chemically treated with rotenone to remove nonnative fish. Approximately 3,600 WCT embryos from Lone Willow Creek (Smith River drainage) were planted in remote site incubators (RSI) in Big Camas Creek in 2015 following the previous year's treatment. Additionally, triploid WCT were planted in Camas Lake to establish a recreational fishery while the wild fish population expanded.

During the 2015 RSI installation in Big Camas Creek, nonnative trout were detected above Camas Lake indicating an incomplete chemical treatment in 2014. Gill netting results from Camas Lake confirmed that YCT had survived the treatment. Backpack electrofishing of the inlet stream was initiated and nonnative trout as well as wild WCT derived from the RSIs were removed to reduce the likelihood of future hybridization. Gill netting was implemented in the summer of 2016 and angling was used 2016-2018 as additional removal methods. The installation of modified a fyke net in the Camas Lake inlet was used from 2017-2020 in conjunction with electrofishing to remove YCT entering the stream during the spring spawning season.

2021 Monitoring

Camas Lake and Big Camas Creek were sampled June 24th, 2021. Two mountain lake gill nets were set in Camas Lake and fished for 5 hours. A total of 21 fish were caught in the gill net sets: 20 WCT and 1 YCT. Big Camas Creek was backpack electrofished from the Camas Lake inlet to the top of fish distribution (high gradient cascade). A total of 103 fish were collected in this effort: 96 WCT and 7 YCT. Of the 96 WCT collected in Big Camas Creek, 44 possessed adipose fins indicating they are potentially 6-year-old fish from the 2015 RSI installation, 7-year-old hatchery triploids from the 2014 fish plant, or wild fish.

Because YCT currently make up a small portion of the fishery in Camas Lake and Big Camas Creek a proposed fish removal project has been submitted to Montana Fish and Wildlife Commission for the 2022 season. The proposed project seeks to mechanically remove YCT from 0.75 miles of Big Camas Creek above Camas Lake using trap nets and backpack electrofishing for a two-week period during the spring spawning season. This mechanical suppression effort will reduce the threat of competition and reduce the number of YCT that can hybridize with the native WCT. The proposed timeline for the action of this mechanical removal project would be 2022-2025. Subsequent years would include continued supplemental stocking of WCT. Ultimately the long-term goal of this project is to establish a WCT conservation population with a >90% WCT genetic contribution. It is anticipated that the proposed suppression effort will decrease the time it takes the population to reach <10% introgression. However, it should be noted that it will take a considerable number of years to reach the long-term genetic goals (estimated 18-36 years) based on the typical generation time of cutthroat trout.

Coyote Creek

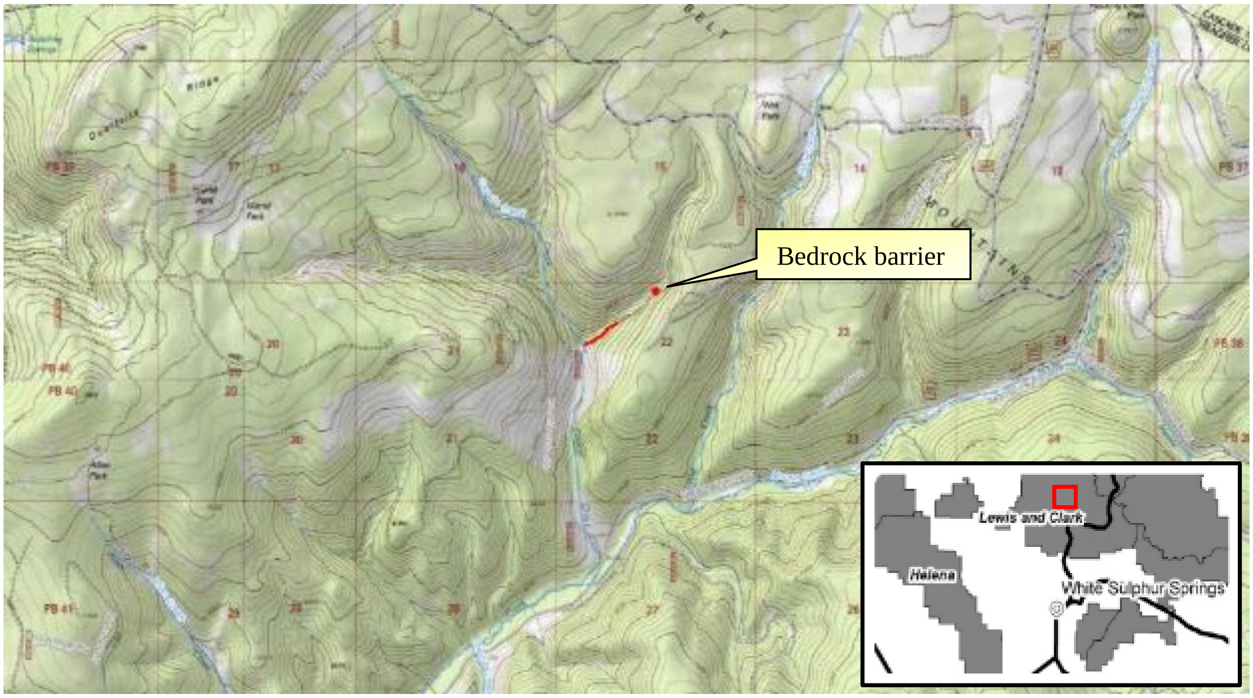

Figure 16. Coyote Creek in the Smith River subbasin. The stream segment delineated in red indicates the area sampled in 2021.

Background

Coyote Creek, a tributary of Moose Creek in the Smith River drainage, contains a population of nonnative Brook Trout (Figure 19). Previous sampling performed by the U.S. Forest Service in 1990 detected only Brook Trout near the confluence of Moose Creek. The culvert at the FS RD 6406 crossing of Coyote Creek has been noted as a potential barrier to fish movement. Coyote Creek was surveyed in 2020 to assess its potential for a future WCT restoration project. Coyote Creek appears to have potential for WCT restoration if a barrier were to be installed at the FS RD 6406 crossing.

2021 Monitoring

On October 10th, 2021, an unnamed tributary of Coyote Creek was surveyed for bedrock fish barriers and WCT presence. The lower 350 m of the unnamed tributary was backpack electrofished (from the confluence of Coyote Creek to its spring source). No fish were detected. A large bedrock barrier was located in the upper drainage at 46.88077, -110.83223. However, the tributary was dry at this location and does not appear to contain perennial water above the bedrock barrier.

Fourmile Creek

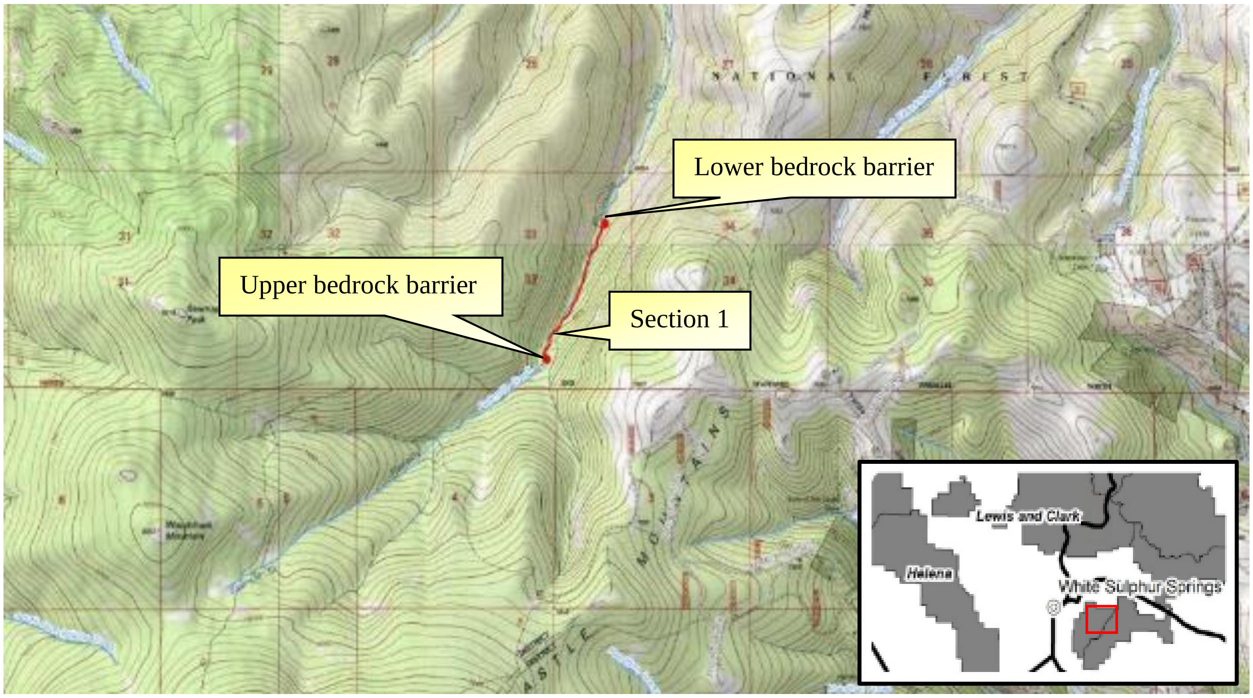

Figure 21. Fourmile Creek in the Smith River subbasin. The stream segment delineated in red indicates the area occupied by nonhybridized WCT.

Background

Fourmile Creek is a tributary of the North Fork Smith River draining the north slope of the Castle Mountains east of the community of White Sulphur Springs (Figure 21). The perennial reach of Fourmile Creek located upstream of the Lewis and Clark National Forest boundary contains hybridized WCT and Brook Trout. The headwaters of Fourmile Creek were historically fishless upstream of a series of natural bedrock barriers. In 2000, 50 nonhybridized WCT from nearby Richardson Creek were transferred upstream of the lowest natural waterfall barrier. However, subsequent sampling of upper Fourmile Creek determined that the transferred WCT did not become established. In 2020, upper Fourmile Creek was surveyed again to evaluate habitat for potential WCT transfer opportunities. A 1.13 km section of Fourmile Creek was found to support a population of WCT isolated between two bedrock falls barriers. Genetic samples were collected from 30 WCT in this reach and submitted for analysis.

2021 Monitoring

In May of 2021, analysis of the 2020 genetic samples was completed and strongly indicated that Fourmile Creek contains nonhybridized WCT likely derived from the 2000 Richardson Creek wild fish transfer (Appendix A).

On June 14th, 2021 a temperature logger was deployed in Fourmile Creek above the upper bedrock barrier in the fishless portion of stream (46.78973, -110.72713) to evaluate potential for future upstream expansion of the WCT population. Mean August stream temperature in upper Fourmile Creek was 9.67° C (Figure 22). On September 14th, 2021, stream discharge measurements and aquatic macroinvertebrates were collected near the temperature logger site. Stream discharge was 0.561 cfs.

A fish population estimate was performed on August 10th, 2021 to estimate the abundance of the Fourmile Creek WCT population. A 100 m population estimate section was established 0.23 km downstream of the upper bedrock barrier. Multiple pass depletion methods were used to estimate population abundance. An estimated 240 fish/km were found based on the results of the two-pass depletion, putting the total nonhybridized WCT population at 283 (±12) individuals if extrapolated to the entire reach (Figure 23).

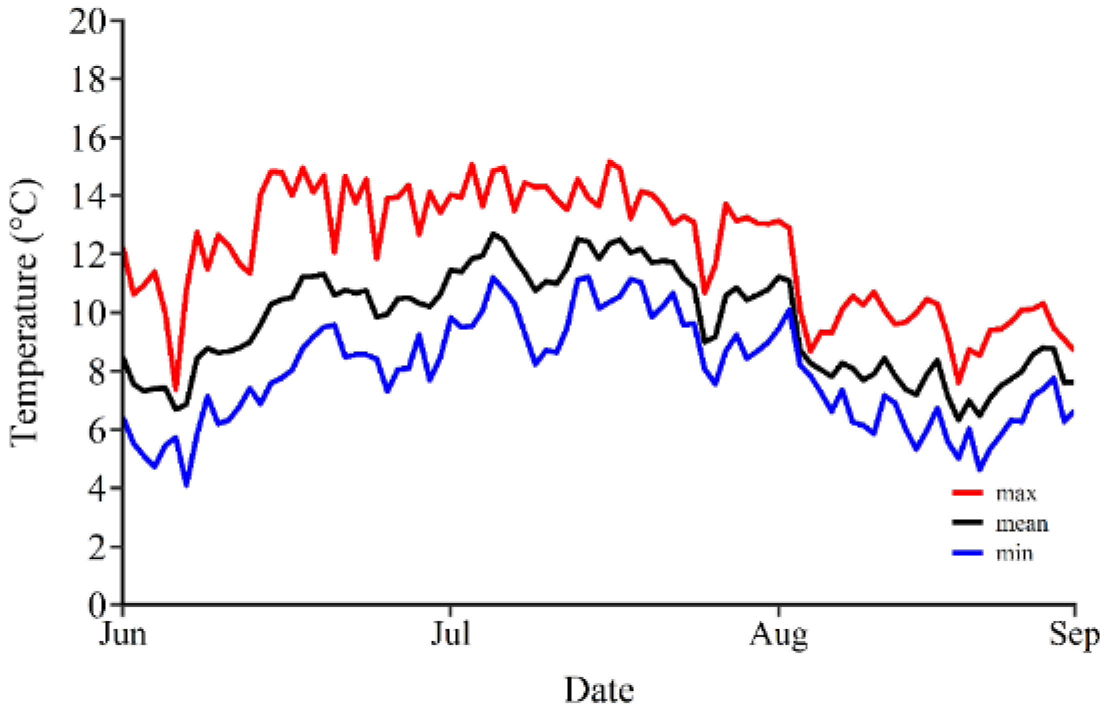

Figure 22. Daily maximum (red line), mean (black line), and minimum (blue line) temperatures from upper Fourmile Creek. The temperature logger was deployed on June 14, 2021 and collected September 14, 2021.

Fourmile Creek **—**NATIVE TROUT POPULATION SURVEY

1. General Information— Date: **August 10th, 2021** Biologist: **A. Poole**
2. Stream Information—
   Name, section, county: **Fourmile Creek, 33, Meagher**
3. Survey Site Information (see attached map)—
   Upstream range of native trout (general description and GPS): **1.18 km above lower bedrock barrier (46.49239, -110.72236)**
   Downstream range of native trout (general description and GPS): **Lower bedrock barrier (46.50130, -110.71620)**
   Location (GPS) and description of barriers: **Lower bedrock barrier (46.50130, -110.71620); Upper bedrock barrier (46.49239, -110.72236)**
   Stream Length—Occupied habitat: **1.18 km (0.74 mi)** Available habitat: **4.33 km (2.69 mi)[1]**
   Survey method & equipment: **backpack battery electrofisher; two-pass depletion**
   Survey sites (general description and UTM)—
   Section 1: **0.23 km downstream of upper bedrock barrier; 46.49422, -110.72154**

Parameter	**Section 1**
Section length (m)	100 m
Mean stream width (m) (n)	2.79 m (10)
Section area (hectares)	0.028 ha
WCT	
Removal Pattern	21 3
Population estimate	24 (±1)
Capture probability	0.860
Mean length (mm) (n)	187 (24)
Mean weight (g) (n)	61 (24)
Mean KTL (n)	0.86 (24)
Number fish per km (95 % CI)	240 (±10)
Number fish per ha (95 % CI)	857 (±35)
Biomass (kg per ha) (95 % CI)	52 (±2)

4. Comments:

[1] – Includes 3.15 km (1.96 mi) of fishless habitat above the upper bedrock barrier.

Figure 23. Fourmile Creek fish population estimate results.

Lake Creek

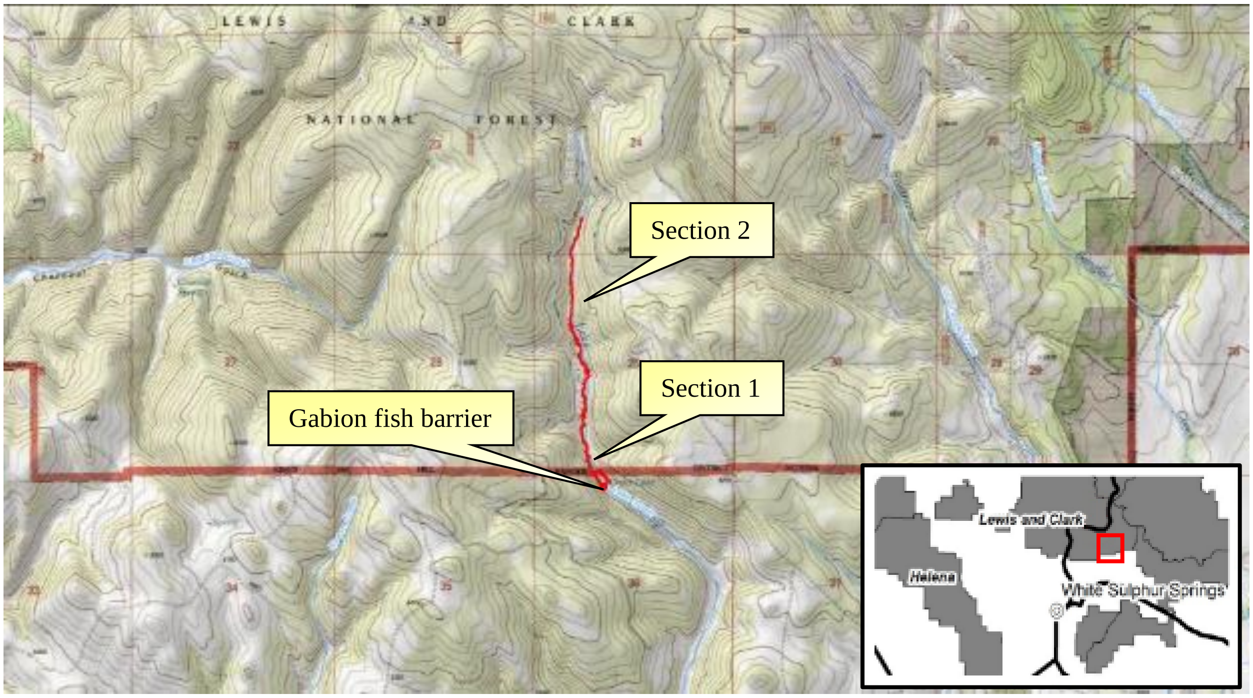

Figure 24. Lake Creek in the Smith River subbasin. The stream segments delineated in red indicate the areas occupied by nonhybridized WCT.

Background

Lake Creek, a tributary of the North Fork Smith River, was surveyed in 2003 and a conceptual design for a fish barrier was drafted. Costs for the project were excessive so a new barrier plan, either pre-cast concrete or gabion baskets was developed. Funding for a new barrier using gabion baskets was obtained in 2005. The gabion barrier was constructed in late summer of 2010. Lake Creek and Crater Lake were treated with piscicides in 2011. Lake Creek was restored with nonhybridized WCT from Lone Willow Creek with the use of remote site incubators from 2012-2014. Demographic monitoring of the Lake Creek WCT population was performed in 2015 and 2017.

2021 Monitoring

Lake Creek was surveyed on August 9th, 2021. Two 100 m fish population estimate sections were established in proximity to previous areas sampled in 2017. Multiple pass depletion methods were used to estimate population abundance. WCT density was high at both sites with an estimated 780 fish/km from the lower section and 480 fish/km from the upper section.

Lake Creek —NATIVE TROUT POPULATION SURVEY

1. General Information— Date: **August 9th, 2021** Biologist: **A. Poole**
2. Stream Information—
   Name, section, county: **Lake Creek, 25, Meagher**
3. Survey Site Information (see attached map)—
   Upstream range of native trout (general description and GPS): **2.67 km above gabion barrier (46.70211, -110.79607)**
   Downstream range of native trout (general description and GPS): **Gabion fish barrier (46.67877, -110.79360)**
   Location (GPS) and description of barriers: **Gabion fish barrier (46.67877, -110.79360)**
   Stream Length—Occupied habitat: **2.67 km (1.66 mi)** Available habitat: **2.67 km (1.66 mi)**
   Survey method & equipment: **backpack battery electrofisher; two-pass depletion**
   Survey sites (general description and UTM)—
   Section 1: **Upstream of Crater Lake; 46.67988, -110.79481**
   Section 2: **Upstream of beaver ponds; 46.68419, -110.79570**

Parameter	**Section 1**	**Section 2**
Section length (m)	100 m	100 m
Mean stream width (m) (n)	1.54 m (10)	1.00 m (10)
Section area (hectares)	0.015 ha	0.010 ha
WCT		
Removal Pattern	46 20	35 10
Population estimate	78 (±18)	48 (±6)
Capture probability	0.570	0.710
Mean length (mm) (n)	113 (66)	108 (45)
Mean weight (g) (n)	15 (66)	15 (45)
Mean KTL (n)	0.85 (66)	0.88 (45)
Number fish per km (95 % CI)	780 (±180)	480 (±60)
Number fish per ha (95 % CI)	5,200 (±1,200)	4,800 (±600)
Biomass (kg per ha) (95 % CI)	78 (±18)	72 (±9)

Figure 25. Lake Creek fish population estimate results.

Lonesome Creek

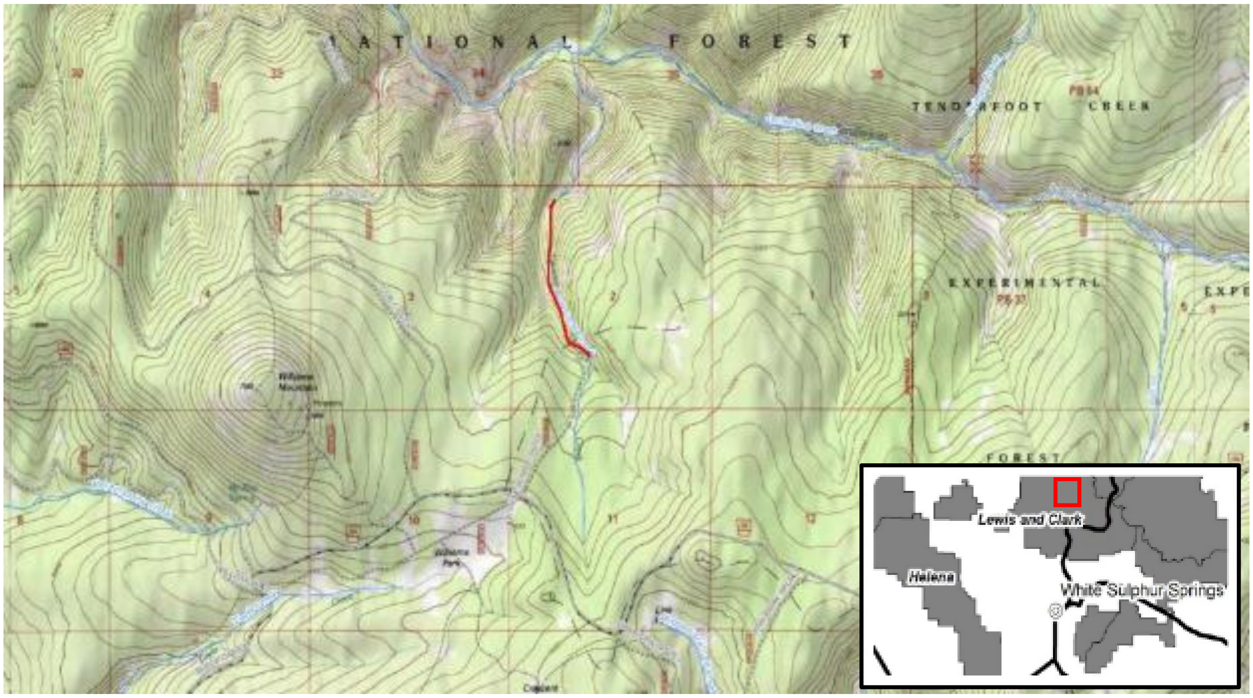

Figure 26. Lonesome Creek in the Smith River subbasin. The stream segment delineated in red indicates the area sampled in 2021.

Background

Lonesome Creek is a tributary of Tenderfoot Creek in the Smith River subbasin. A single collection record exists from Lonesome Creek in the FWP Fisheries Information System indicating 10 WCT were collected in this stream in 2009. However, this collection record is suspect considering no other information could be found relating to this survey and in 2009 monitoring had been performed in Lonesome Creek in the Two Medicine River subbasin.

2021 Monitoring

Lonesome Creek was surveyed on October 18th, 2021, to determine presence/absence of WCT. A 1.29 km reach of stream was sampled in a single pass backpack electrofishing effort. No fish were observed or collected, and flows were noted to be very low. The collection record in the FWP Fisheries Information System is very likely a mislabeled record for the 2009 Lonesome Creek survey in the Two Medicine River subbasin.

Pickfoot Creek

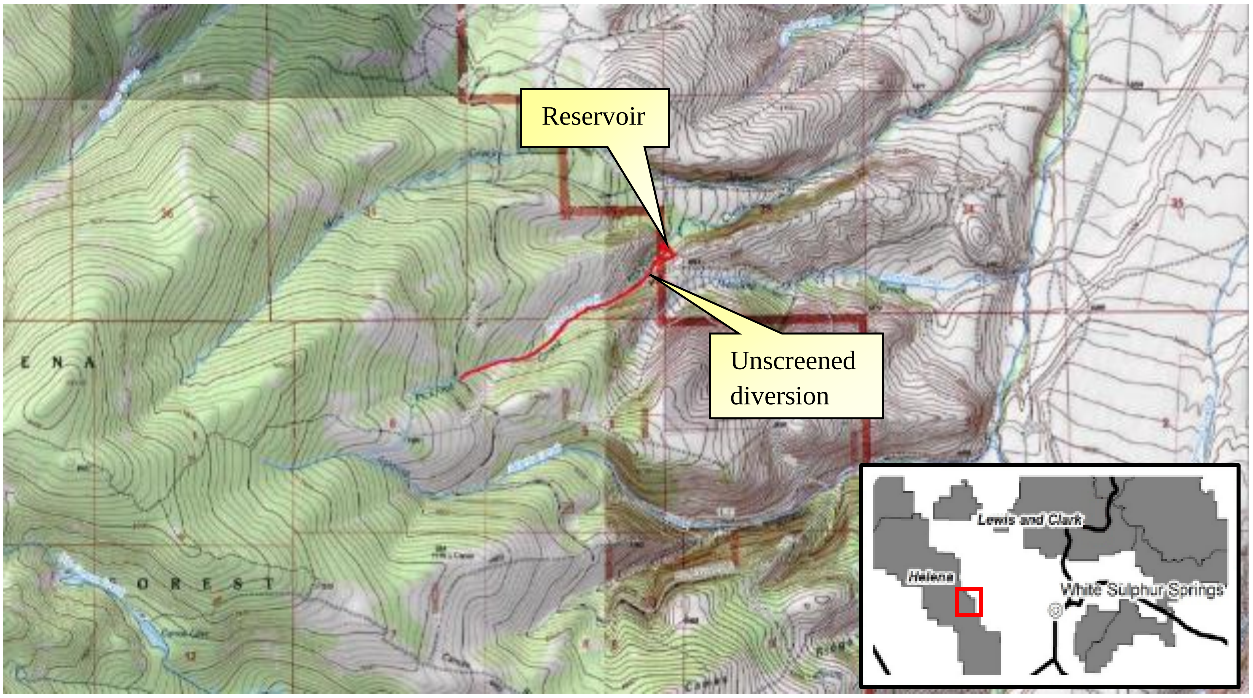

Figure 29. Pickfoot Creek in the Smith River subbasin. The stream segment delineated in red indicates the area sampled in 2021.

Background

Pickfoot Creek is a tributary of Mule Creek in the Smith River subbasin draining the east slope of the Big Belt Mountains west of the community of White Sulphur Springs, MT. No previous collection records exist for this locality.

2021 Monitoring

On May 25th, 2021, Pickfoot Creek was visited to determine fish presence above an in-stream impoundment located at the Lewis and Clark National Forest boundary (Figure 30). A 500 m reach of Pickfoot Creek was backpack electrofished above the impoundment. 37 WCT and a single Brook Trout were collected in the reach sampled. An unscreened diversion was found approximately 300 m upstream from the 2.3-acre impoundment (Figure 31). Roughly half of the streamflow is diverted at this point into a ditch which ultimately drains into the Meadow Creek drainage in section 33. Immediately upstream of the diversion a large concrete slab was found in the stream channel which may act as a partial fish barrier (Figure 32). WCT were found both above and below this slab indicating that adult WCT can pass upstream. The single Brook Trout collected was found below the diversion and may be confined to the lower drainage. Genetic samples were collected from ten WCT. The fish appeared phenotypically representative of WCT (Figure 33).

On May 27th, 2021, Pickfoot Creek was revisited to collect additional WCT genetic samples. Two sections of Pickfoot Creek were electrofished as well as the section of the Meadow Creek ditch from the Forest Service boundary to the unscreened diversion. 30 WCT and 4 Brook Trout were collected in this effort and an additional 9 genetic samples were obtained. Once again, the Brook Trout collected were found immediately upstream of the reservoir, below the diversion.

Pickfoot Creek was revisited on June 4th, 2021, to collect additional genetic samples and determine the upstream distribution of fish. Seven genetic samples were obtained from eight total WCT collected in this effort. The top of fish distribution was found at 46.57270, -111.25978 approximately 515 m below the FS RD 575 crossing. Above this point the stream becomes very high gradient.

In total, 26 genetic samples were collected from Pickfoot Creek in 2021. With the exception of one 204 mm individual, only juvenile WCT were collected (ages 1 and 2). The WCT population in Pickfoot Creek may be primarily adfluvial with adults residing in the reservoir. It appears that the concrete slab precludes Brook Trout from accessing the upper portion of the drainage.

Figure 30. The 2.3-acre reservoir on Pickfoot Creek located at the Lewis and Clark National Forest Boundary.


Figure 31. Unscreened water diversion on Pickfoot Creek located at 46.57757, -111.24736.


Figure 32. Concrete slab in Pickfoot Creek located at 46.57756, -111.24741.

Figure 33. Juvenile WCT collected from Pickfoot Creek.

Tenderfoot Creek

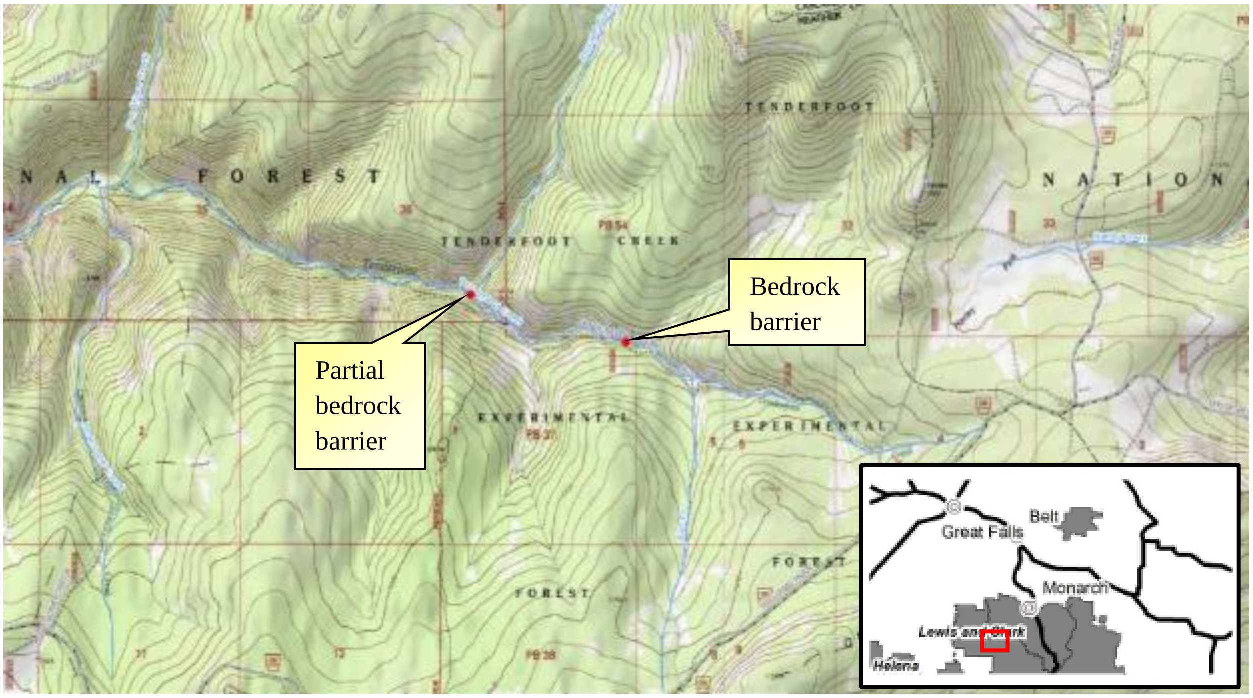

Figure 34. Tenderfoot Creek in the Smith River subbasin.

Background

Tenderfoot Creek is a large tributary of the Smith River draining a significant portion of the Little Belt Mountains. Tenderfoot Creek is protected by a large waterfall barrier just below the confluence of the South Fork of Tenderfoot Creek. Several other falls barriers exist on Tenderfoot Creek in the upper third of the basin. However, no description or photographic documentation of these upper barriers exists. If suitable, upper Tenderfoot Creek may serve as a future WCT restoration site.

2021 Monitoring

Tenderfoot Creek was surveyed on September 20th, 2021. A visual survey was performed to investigate the presence of bedrock barriers that appear in the FWP Fisheries Information System (FIS). Tenderfoot Creek was accessed from Onion Park. Two bedrock features were found but locations differed considerably from those within FIS. The first bedrock barrier encountered is located at 46.92354, -110.88597, below the confluence with Spring Park Creek. This feature is a two-tiered bedrock cascade that appeared to be a complete barrier to fish movement at most flows (Figure 35). A second bedrock feature was encountered at 46.92661, -110.90251 at the Stringer Creek confluence (Figure 36). This bedrock feature is likely only a partial barrier under low flow conditions.


Figure 35. Two-tiered bedrock barrier located at 46.92354, -110.88597.


Figure 36. Partial bedrock barrier located at Stringer Creek confluence (46.92661, -110.90251).

Unnamed Tributary to Fourmile Creek

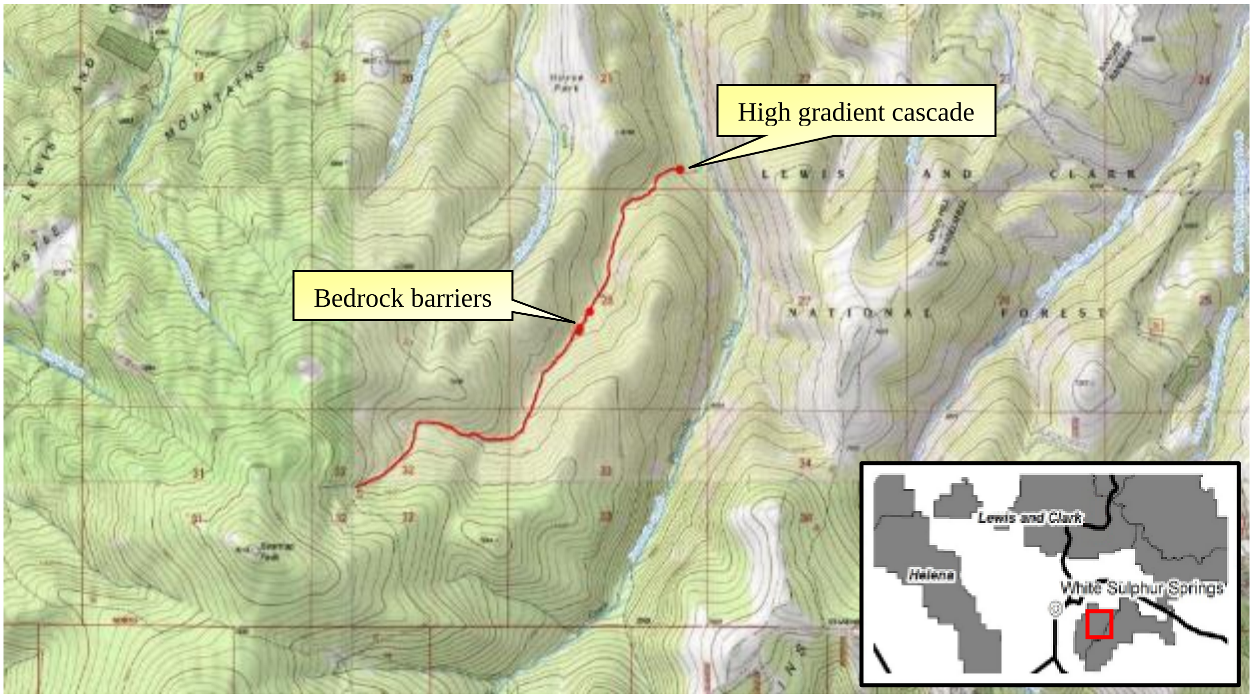

Figure 37. Unnamed tributary to Fourmile Creek in the Smith River subbasin. The stream segment delineated in red indicates the area sampled in 2021.

Background

This unnamed tributary enters Fourmile Creek approximately 1.4 km upstream of the #713 trailhead (Section 21). No previous collection records exist for this locality.

2021 Monitoring

On May 19th, 2021, an unnamed tributary of Fourmile Creek was investigated for the presence of fish and fish barriers. The stream was accessed by Forest Service Trail #713 (Figure 37). A 1.08 km reach of the unnamed tributary was electrofished starting from the confluence with Fourmile Creek. Two Brook Trout and one WCT were collected in the lower 257 m of the unnamed tributary. A 418 m high-gradient reach of the unnamed tributary appeared to limit fish distribution. Although no definitive fish barrier was found in this reach, the cumulative effect of the cascades may preclude upstream fish movement. Habitat appeared excellent containing many pools, spawning gravels, large woody debris, and abundant aquatic macroinvertebrates.

On June 8th, 2021, the unnamed tributary of Fourmile Creek was revisited to further evaluate the presence of fish and fish barriers. A 3.30 km reach of the unnamed tributary was electrofished starting from the point last surveyed on 05/19/21. No fish were collected or observed. Three fish passage barriers were located in a high gradient reach of the unnamed tributary in section 28. The lowermost and largest barrier consisted of a three-tiered bedrock cascade with a total elevation drop of around 6 m (Figure 38). Moving upstream an additional two barriers were located: one approximately 2 m tall and one around 4 m tall.

The majority of the stream flows through a thick lodgepole pine forest with abundant large woody debris and alder common in the riparian zone. The upper 1.42 km of the stream transitions to a montane meadow habitat type where springs and seeps are common in the relatively open riparian zone (Figure 39).

Stoneflies and caddisflies were commonly observed throughout the reach sampled. Numerous deep overwintering pools and suitable spawning gravels were also noted as being widespread. An abundance of large woody debris in the lower reaches of the stream created complex instream habitat. Overall, the fish habitat appears to be excellent and capable of supporting a WCT population. A temperature logger should be deployed to determine mean summer stream temperatures to further evaluate potential to support WCT.

Figure 38. Bedrock barrier located at 46.51103, -110.72583 on the unnamed tributary of Fourmile Creek.


Figure 39. Montane meadow habitat in upper reaches of the unnamed tributary.

V. Sun River Subbasin

<u>Bailey Creek</u>

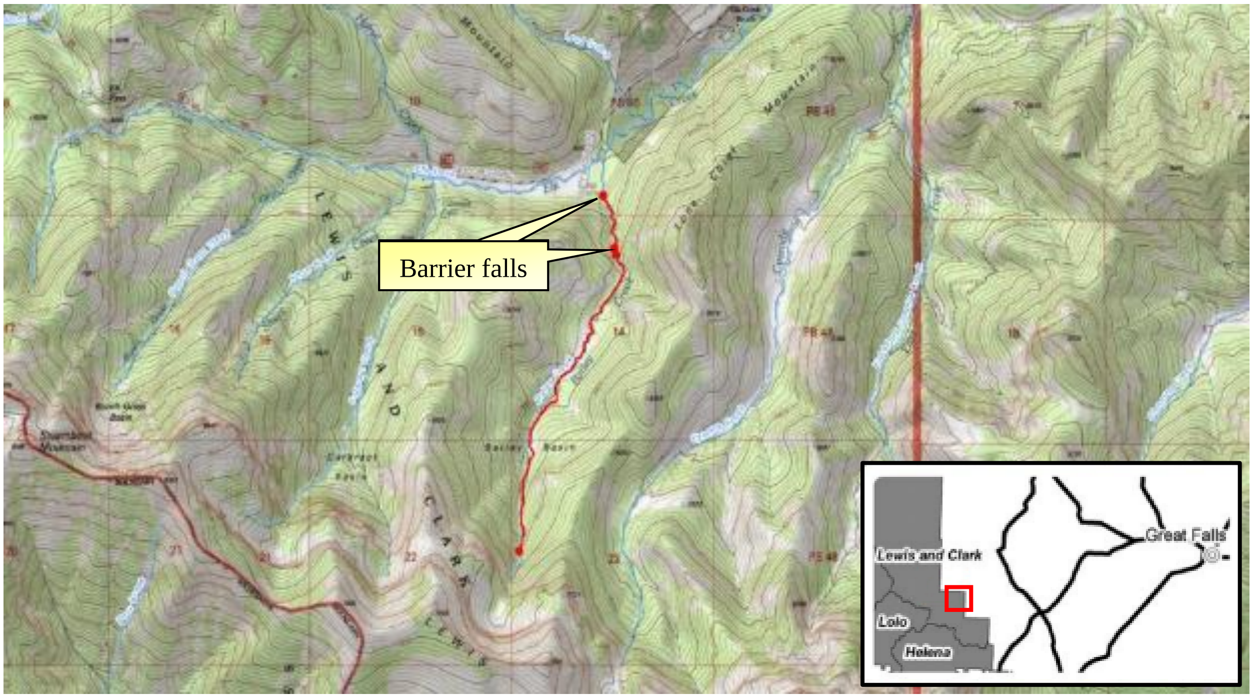

Figure 40. Bailey Creek in the Sun River subbasin. The stream segments delineated in red indicate the areas sampled in 2021.

<u>Background</u>
Bailey Creek is a tributary of Elk Creek (Sun River drainage) on the Rocky Mountain Ranger District of the Helena-Lewis and Clark National Forest (Figure 40). No previous collection records exist for this locality.

<u>2021 Monitoring</u>
On May 3rd, 2021, Bailey Creek was investigated for the presence of natural waterfall barriers and assess potential for future WCT restoration. The stream was accessed by hiking the Bailey Basin Trail #253 from the Bailey Creek dispersed camping area off FS RD 196. The trail parallels Bailey Creek as it steeply climbs to Bailey Basin. Bailey Creek was surveyed downstream from the point where the stream becomes high gradient as it exits Bailey Basin and flows west of Lone Chief Mountain. Several waterfall barriers were located in the high gradient reach and no fish were visually observed (Figure 41). Fish habitat appeared excellent with large overwintering pools and suitable spawning gravels present throughout this reach. The most downstream waterfall barrier was located just upstream of the Bailey Creek dispersed camping area (47.32280, -112.58598; Figure 42). A 124 m section of Bailey Creek was electrofished below this barrier resulting in 3 adult Brook Trout collected (170-215 mm). An additional 250 meters of stream was electrofished above this barrier and no fish were collected.

On June 17th, 2021, Bailey Creek was revisited to further evaluate habitat and fish distribution upstream of the waterfall barriers located in May. A 2.12 km reach of Bailey Creek was electrofished in Bailey Basin. No fish were collected and no fish were visually observed throughout the survey. Fish habitat appeared excellent. Suitable spawning substrate and large overwintering pools were abundant.

Macroinvertebrates were widespread with mayflies, caddisflies, and black fly larvae commonly observed. A small portion of Bailey Creek near the bottom of Bailey Basin may flow subsurface in late summer during low flow years but likely only impacts a short reach of stream as springs add considerable flow downstream of Bailey Basin. Bailey Creek was surveyed upstream until a large 15.2 m waterfall was found at 47.29991, -112.59484. Although fish habitat diminished in the upper 250 m of Bailey Creek, this large waterfall would be the definitive upstream limit of fish habitat. The fishless portion of Bailey Creek that could potentially support an introduced WCT population is approximately 3.20 km in length.

Figure 41. Barrier falls located at 47.31907, -112.58464


Figure 42. Estimated 4.6 m waterfall located at 47.32280, -112.58598.

Big George Gulch

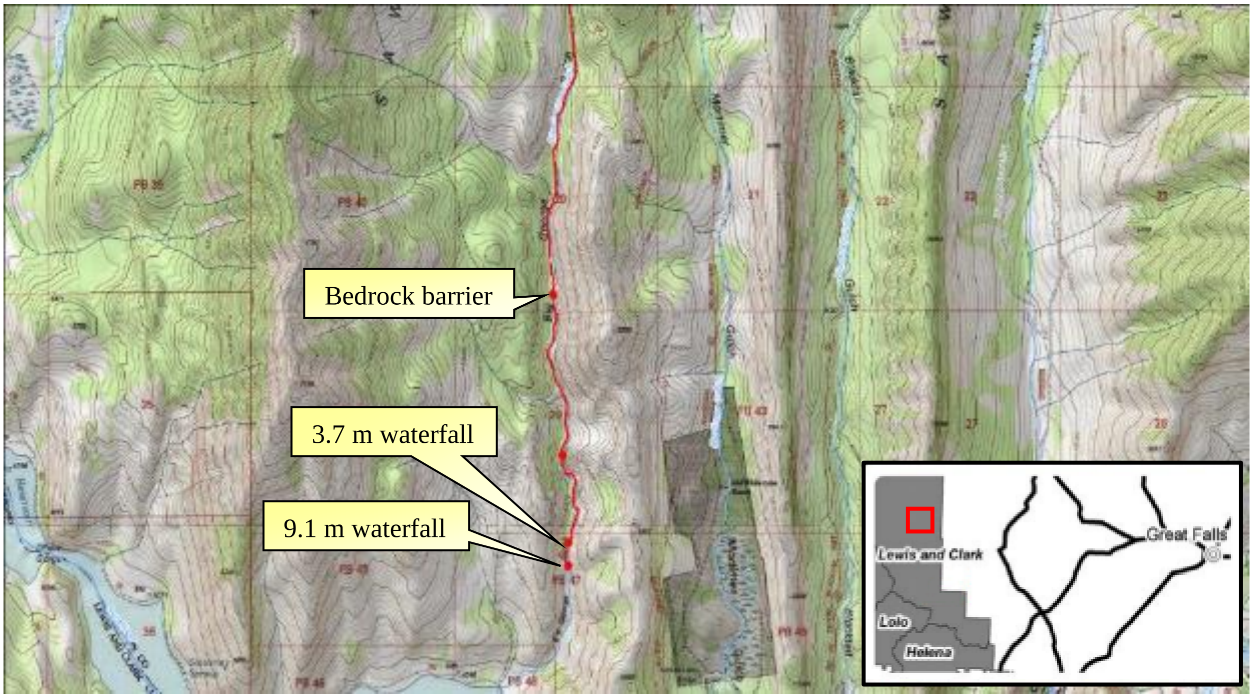

Figure 43. Big George Gulch in the Sun River subbasin. The stream segments delineated in red indicate the areas sampled in 2020.

Background

Big George Gulch is a tributary of Gibson Reservoir (Sun River drainage) on the Rocky Mountain Ranger District of the Helena-Lewis and Clark National Forest (Figure 43). No previous collection records exist for this locality. Historic stocking records indicate 132,560 Rainbow Trout and 36,400 Cutthroat Trout were stocked in Big George Gulch from 1936-1947. However, no information is given on stocking location for these historic plants.

2021 Monitoring

On March 22nd, 2021, Big George Gulch was surveyed for the presence of natural waterfall barriers and to assess potential for future WCT restoration. The stream was accessed by hiking the North Fork Sun River Trail #201 from the Mortimer Gulch Trailhead. The trail crosses Big George Gulch as it exits a narrow canyon and enters a broad floodplain before reaching Gibson Reservoir. Brook Trout and Rainbow Trout were visually observed as being common in the lower 1.21 km of Big George Gulch. Approximately 1.21 km upstream from the trail crossing a large (estimated 9.1 m) waterfall was located (Figure 44). No fish were visually observed above this waterfall for the remainder of the 482 m of stream surveyed. Approximately 150 meters above the lower waterfall, an estimated 3.7 m two-tiered waterfall was located (Figure 45).


Figure 44. Lower waterfall barrier on Big George Gulch located at 47.62232, -112.78571.


Figure 45. 3.7 m waterfall barrier on Big George Gulch located at 47.62376, -112.78576.

On May 13th, 2021, Big George Gulch was revisited to determine fish presence above the waterfall barrier found previously in March. A 1.48 km reach of Big George Gulch was backpack electrofished above the waterfall barrier. No fish were observed or collected in 1655 seconds of effort.

A temperature logger was deployed in Big George Gulch above the lower waterfall barrier from June 15th, 2021, to September 15th, 2021, to assess suitability for WCT restoration. A mean August stream temperature of 11.1° C was recorded indicating suitable stream temperatures to ensure overwinter survival of WCT (Figure 46).

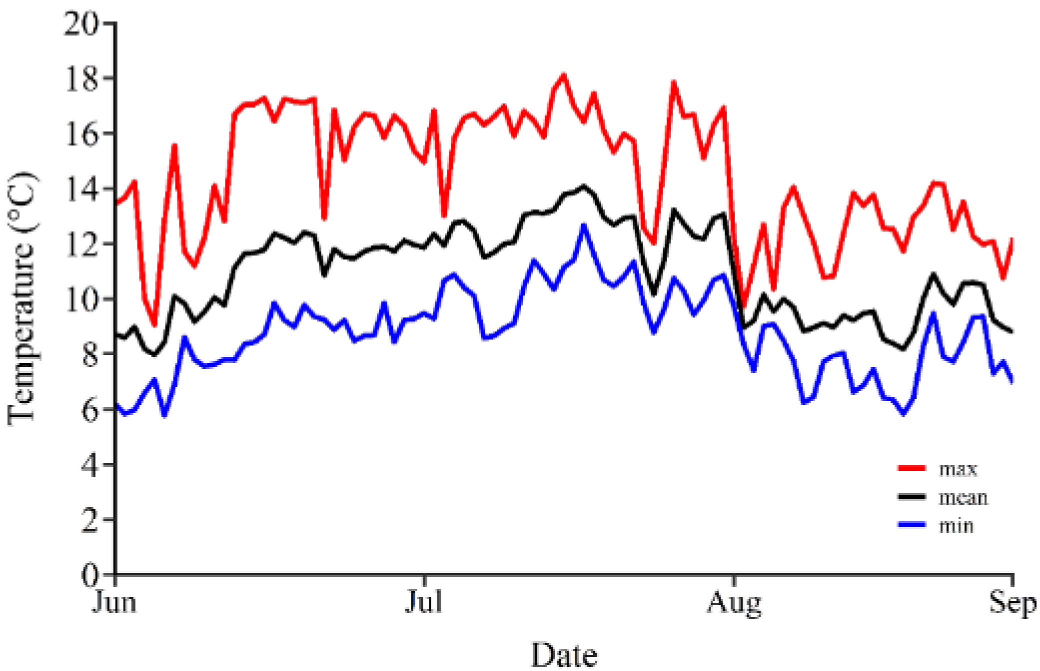

Figure 46. Daily maximum (red line), mean (black line), and minimum (blue line) temperatures from Big George Gulch. The temperature logger was deployed on June 15, 2021 and collected September 15, 2021.

Further habitat evaluation of Big George Gulch was performed on June 29-30th, 2021. A visual survey was performed by hiking the remainder of the creek to the #259 trail crossing. An additional bedrock barrier was located at 47.63990, -112.78733 (Figure 47).


Figure 47. Bedrock barrier on Big George Gulch located at 47.63990, -112.78733.

Stream discharge measurements and aquatic macroinvertebrates were collected from the fishless reach of Big George Gulch on August 5th, 2021. Stream discharge was 1.72 cfs just above the lower waterfall barrier. Three macroinvertebrate kick net samples were collected at 47.62262, -112.78572.

Habitat appears excellent throughout Big George Gulch. Large overwintering pools and suitable spawning gravel are widespread. Large woody debris is common in the upper portion of the drainage. Rocky Mountain Tailed Frog and aquatic macroinvertebrate larvae were noted as common during surveys. Approximately 7.24 km of fishless habitat suitable for WCT is present above the lower waterfall barrier. This stream would likely support a “secured” population of WCT as defined by the Montana Statewide Fisheries Management Plan Conservation Goals for Westslope Cutthroat Trout.

Buttolph Creek

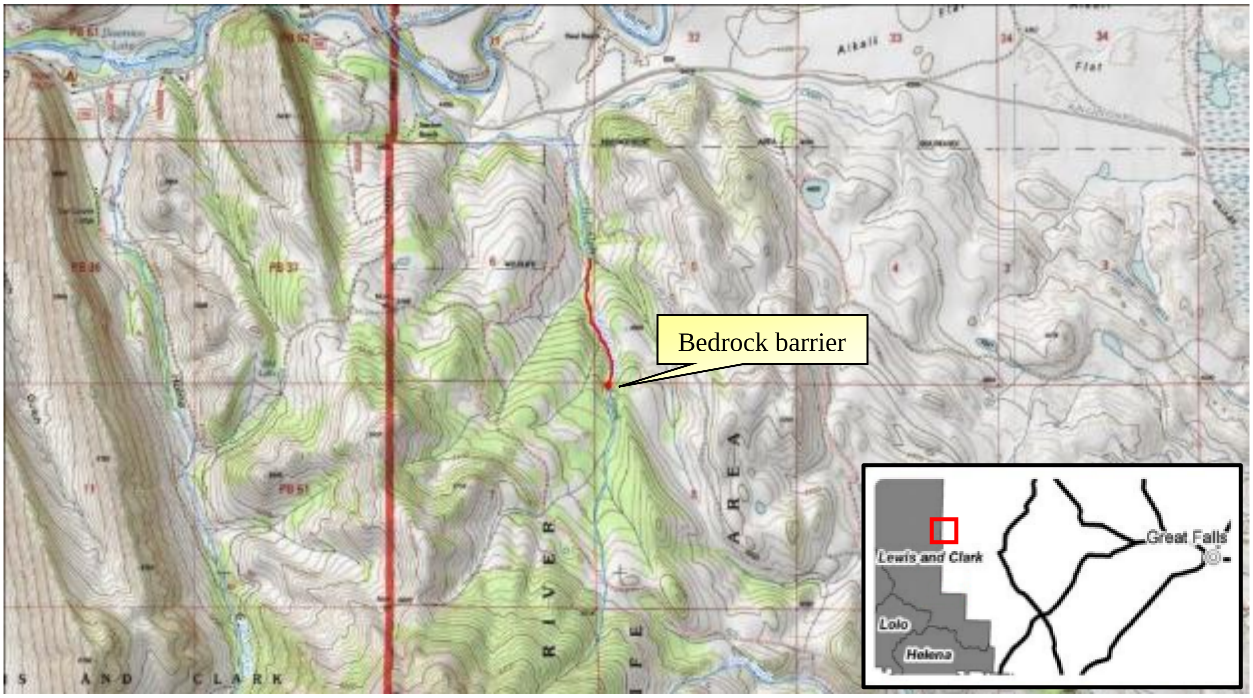

Figure 48. Hoadley Creek in the Sun River subbasin. The stream segment delineated in red indicates potential suitable habitat above the bedrock barrier.

Background

Buttolph Creek is a tributary of the Sun River located on the Sun River Wildlife Management Area west of Augusta, MT. No previous collection records exist for this locality.

2021 Monitoring

Buttolph Creek was surveyed on August 2nd, 2021 for presence of fish barriers and extant populations of WCT. A 0.97 km reach of Buttolph Creek was backpack electrofished and no fish were collected. Rocky Mountain Tailed Frog adults were common. Fish habitat appeared marginal. A bedrock barrier to upstream movement was located at 47.59487, -112.66869.

Hoadley Creek

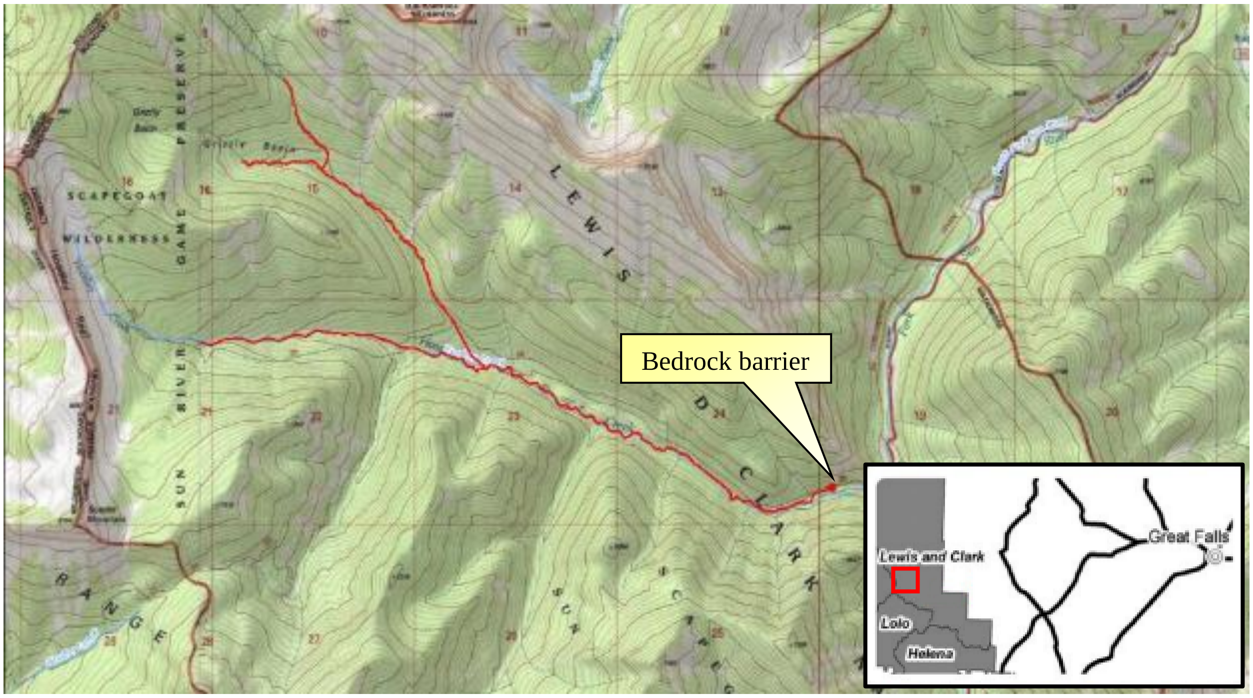

Figure 49. Hoadley Creek in the Sun River subbasin. The stream segment delineated in red indicates potential suitable habitat above the bedrock barrier.

Background
Hoadley Creek is a tributary of the South Fork Sun River located west of the Benchmark trailhead in the Scapegoat Wilderness Area. One previous survey of Hoadley Creek in August 1960 did not observe fish but indicated that fish had been visually observed in October 1959. While no record of a natural fish barrier exists in the FWP Fisheries Information System, maps generated by the Lewis and Clark National Forest indicate the presence of a barrier in lower Hoadley Creek.

2021 Monitoring
Hoadley Creek was surveyed on September 29th, 2021. A large bedrock barrier was located at 47.46859, -112.93388 (Figure 50). The barrier was estimated to be 4.6 m tall and likely a total barrier to fish passage. A 318 m reach of stream was electrofished above the barrier resulting in eight Brook Trout collected. A 219 m reach of Hoadley Creek was electrofished below the barrier resulting in a catch of three Rainbow Trout and 14 Brook Trout.

Further evaluation of Hoadley Creek is warranted given the presence of the large bedrock barrier. Because Rainbow Trout were not detected above the barrier, it may preclude upstream fish movement. The presence of Brook Trout above the barrier is likely a result of historical stocking practices, although no stocking records exists for this locality. Approximately 9.17 km of fish bearing habitat is estimated above this barrier location. Hoadley Creek could likely support a "secured" population of WCT given the amount of habitat protected by the bedrock barrier.


Figure 50. Hoadley Creek bedrock barrier located at 47.46859, -112.93388.

Jakie Creek

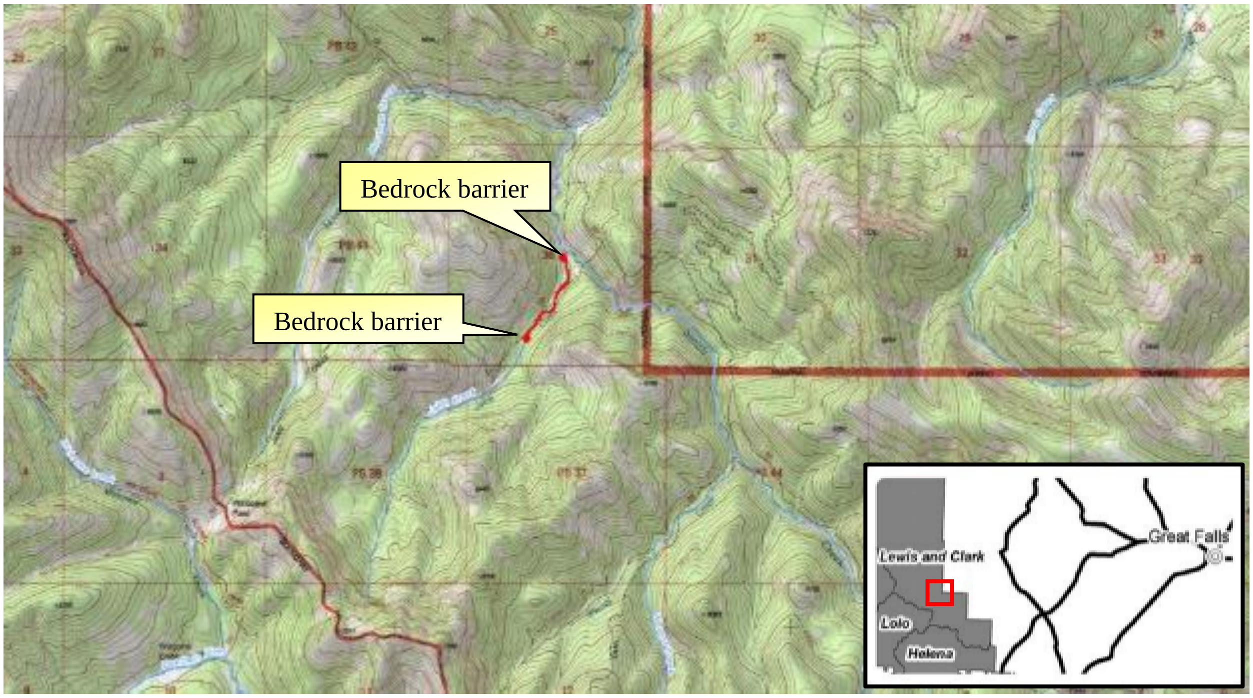

Figure 51. Jakie Creek in the Sun River subbasin. The stream segment delineated in red indicates the area sampled in 2021.

Background

Jakie Creek is a tributary of Smith Creek in the Sun River subbasin located west of Augusta, MT. The upper Smith Creek watershed has been identified as a potential WCT restoration site because of the presence of a large waterfall barrier below the Moudess Creek confluence. However, little is known about current fish distribution within the upper Smith Creek drainage. Jakie Creek was surveyed once in 2013 for the presence of fish barriers and extant populations of WCT. Numerous fish barriers were found in this survey, but their locations and descriptions were not recorded.

2021 Monitoring

Jakie Creek was surveyed on September 22nd, 2021. A 780 m reach was electrofished starting from the confluence with Smith Creek. The first large bedrock barrier was encountered approximately 30 m above the Smith Creek confluence at 47.35713, -112.69146. This bedrock slide was estimated to be about 4.6 m in total height (Figure 52). A second large bedrock barrier was located 750 m upstream at 47.35200, -112.69534. This feature was estimated to be 4-6 m in total height (Figure 53). No fish were collected or observed in Jakie Creek. Rocky Mountain Talied Frog larvae were present.

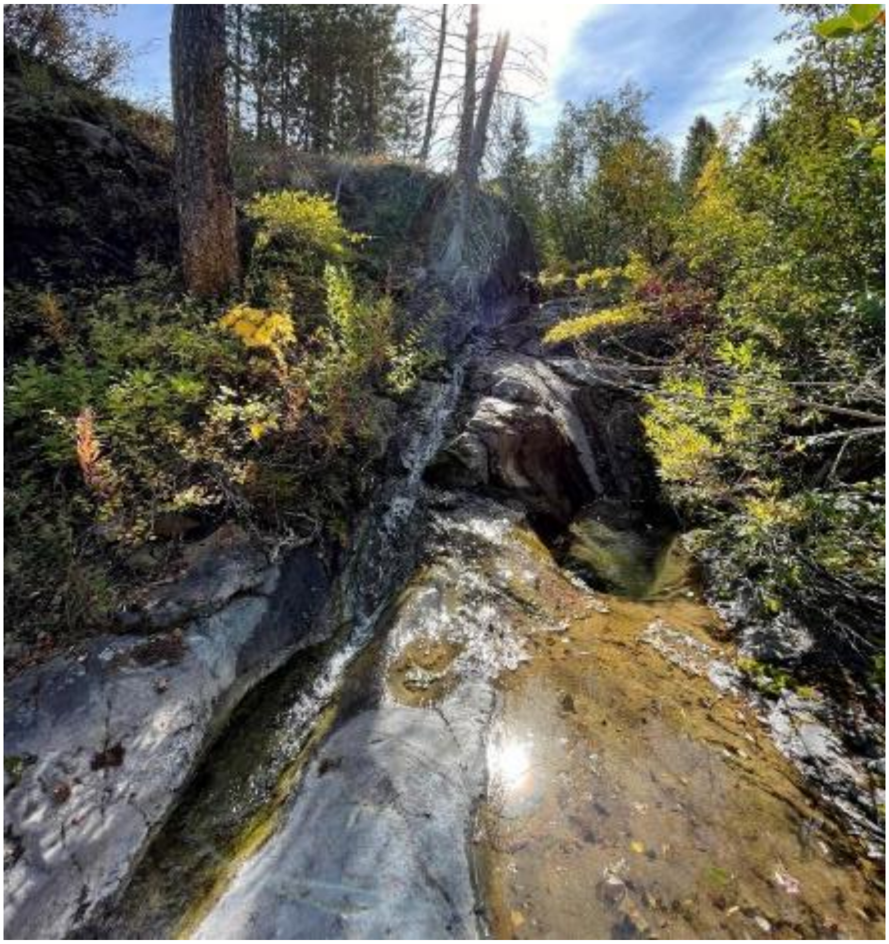

Figure 52. Jakie Creek bedrock barrier located at 47.35713, -112.69146.

Figure 53. Jakie Creek bedrock barrier located at 47.35200, -112.69534.

Moudess Creek

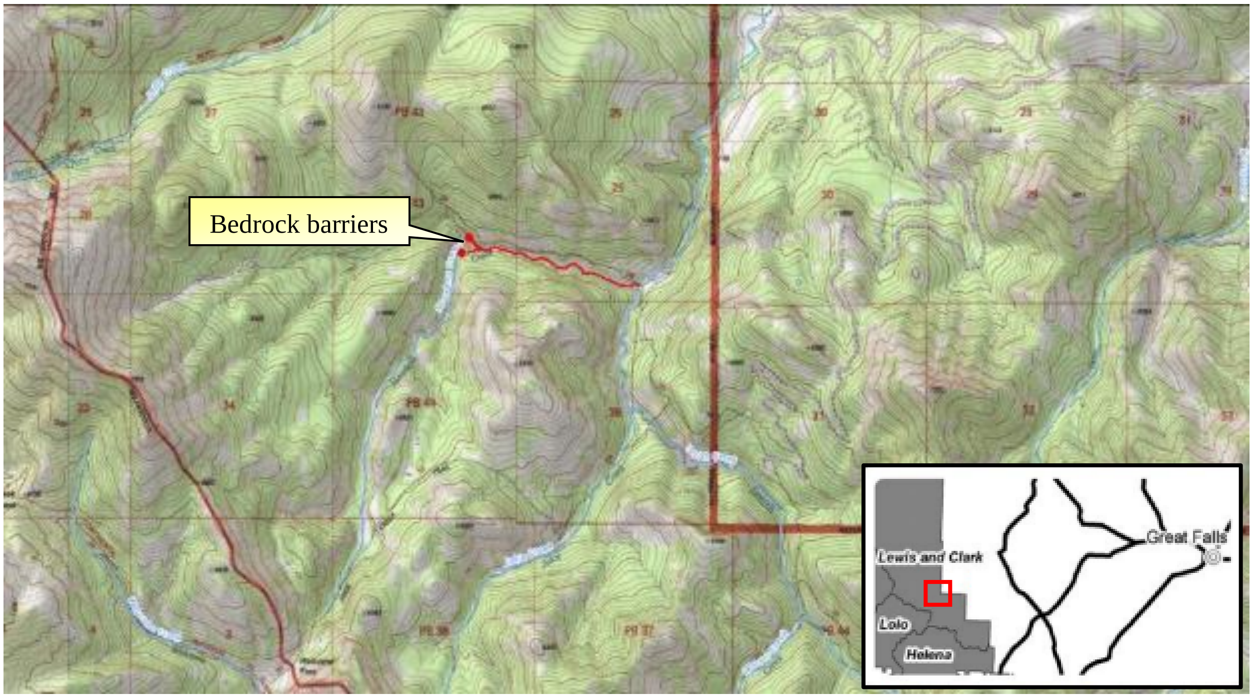

Figure 54. Moudess Creek in the Sun River subbasin. The stream segment delineated in red indicates the area sampled in 2021.

Background

Moudess Creek is a tributary of Smith Creek in the Sun River subbasin located west of Augusta, MT. The upper Smith Creek watershed has been identified as a potential WCT restoration site because of the presence of a large waterfall barrier below the Moudess Creek confluence. However, little is known about current fish distribution within the upper Smith Creek drainage. Genetic analysis of WCT collected in Moudess Creek in 1996 indicated a mixed population of predominantly nonhybridized WCT and one individual possessing RB alleles (n = 5). Genetic samples were again collected from Moudess Creek in 2013 and subsequent analysis failed to detect hybridization (n = 8).

2021 Monitoring

Moudess Creek was surveyed on August 3-4th, 2021 to collect additional WCT genetic samples. A 1.55 km reach of Moudess Creek was backpack electrofished starting from the Smith Creek confluence. A total of 25 WCT and 211 Brook Trout were collected in this effort. A bedrock barrier was located on Moudess Creek at 47.36772, -112.70928 above which no fish were detected (Figure 55). Additionally, an unnamed tributary of Moudess Creek was electrofished and no fish were found. A bedrock barrier was also located on this tributary at 47.36869, -112.70853.


Figure 55. Moudess Creek bedrock barrier located at 47.36772, -112.70928.

Petty Creek

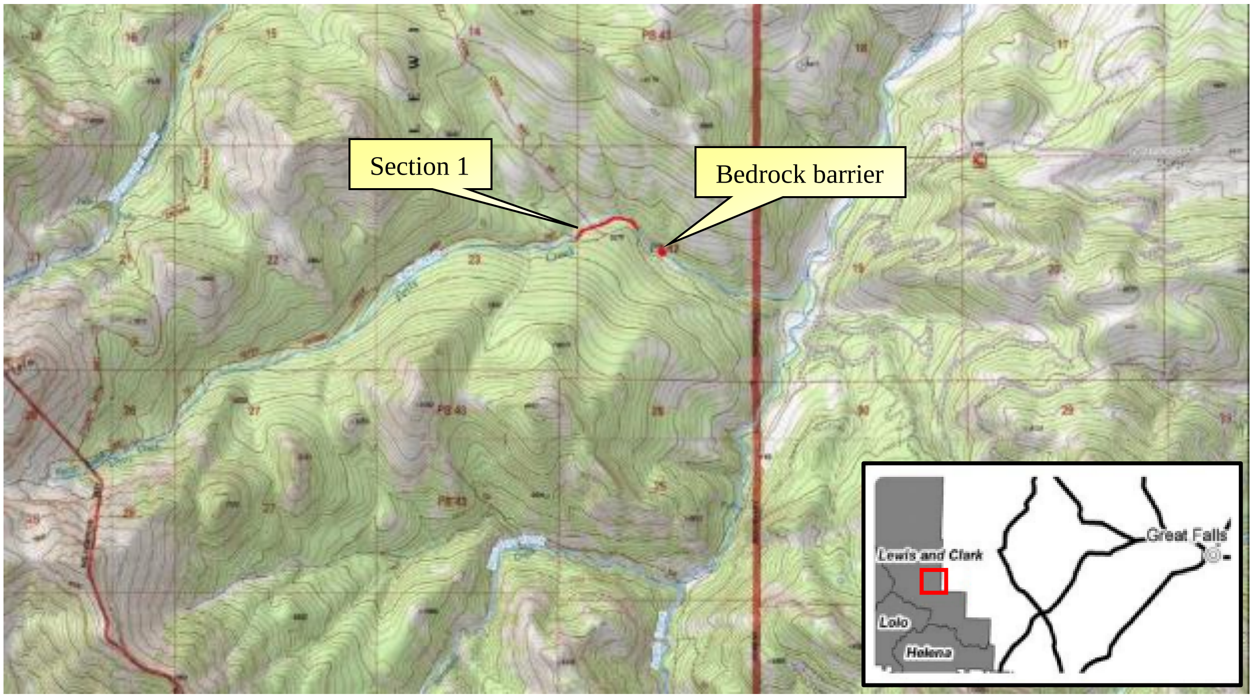

Figure 56. Petty Creek in the Sun River subbasin. The stream segment delineated in red indicates the area sampled in 2021.

Background

Petty Creek is a tributary of Smith Creek in the Sun River subbasin located west of Augusta, MT. In 2002 and 2003, a total of 322 adult and juvenile WCT were transferred from North Fork of Deep Creek (Smith River subbasin) to Petty Creek. Post-transfer demographic monitoring from 2005-2012 revealed limited natural reproduction in Petty Creek. Additionally, genetic analysis of the North Fork Deep Creek WCT population revealed that transferred fish were undoubtedly hybridized (99.3% WCT x 0.7% RBT). The lack of reproduction in Petty Creek was thought to be a result of low summer water temperatures.

2021 Monitoring

Petty Creek was surveyed on September 21st, 2021. A population estimate section was established at the #244 trail crossing, near where previous post-transfer monitoring had been performed. Multiple pass depletion methods were used to estimate population abundance. No fish were collected in the population estimate section (Figure 57). An additional 411 m were shocked downstream of the trail crossing and no fish were detected. The Petty Creek WCT population may have become extirpated sometime between 2012 and 2021. Low water temperatures seem a likely culprit for the failure of this population, the temperature recorded on the September 21st survey was 5.6° C.

Petty Creek —NATIVE TROUT POPULATION SURVEY

1. General Information— Date: **September 21st, 2021** Biologist: **A. Poole**
2. Stream Information—
   Name, section, county: **Petty Creek, 42, Lewis and Clark**
3. Survey Site Information (see attached map)—
   Upstream range of native trout (general description and GPS): **????**
   Downstream range of native trout (general description and GPS): **Bedrock barrier (47.38813, -112.69478)**
   Location (GPS) and description of barriers: **Bedrock barrier (47.38813, -112.69478)**
   Stream Length—Occupied habitat: **????** Available habitat: **5.47 km (3.40 mi)**
   Survey method & equipment: **backpack battery electrofisher; two-pass depletion**
   Survey sites (general description and UTM)—
   Section 1: **Upstream of trail #244 crossing; 47.38888, -112.70019**

Parameter	**Section 1**
Section length (m)	100 m
Mean stream width (m) (n)	3.11 m (10)
Section area (hectares)	0.031 ha
WCT	
Removal Pattern	0
Population estimate	0 (NA)
Capture probability	NA
Mean length (mm) (n)	NA
Mean weight (g) (n)	NA
Mean KTL (n)	NA
Number fish per km (95 % CI)	0 (NA)
Number fish per ha (95 % CI)	0 (NA)
Biomass (kg per ha) (95 % CI)	0 (NA)

Figure 57. Petty Creek fish population estimate results.

Smith Creek

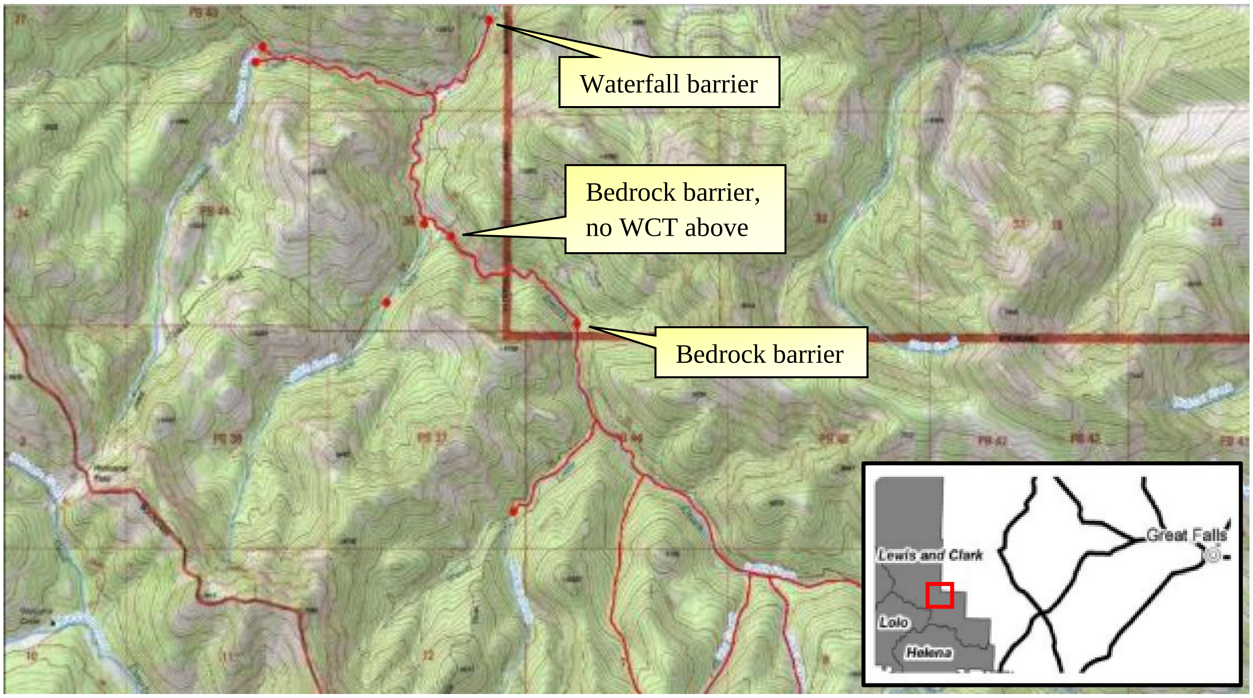

Figure 58. Smith Creek in the Sun River subbasin. The stream segment delineated in red indicates the current fish distribution in upper Smith Creek.

Background

Smith Creek is a tributary of Elk Creek in the Sun River subbasin located southwest of the town of Augusta, MT. The upper Smith Creek watershed has been identified as a potential WCT restoration site because of the presence of a large waterfall barrier below the Moudess Creek confluence. However, little is known about current fish distribution within the upper Smith Creek drainage. Only two previous sampling locations were found in upper Smith Creek from 1996 and 2013. These efforts collected fish immediately above the waterfall barrier and in Moudess Creek. Genetic analysis of WCT collected above the waterfall barrier on Smith Creek in 2013 indicated a hybridized population with an appreciable WCT genetic component (96.4% WCT 3.6% RB).

2021 Monitoring

Demographic surveys of the Smith Creek drainage in 2021 found the distribution of WCT to be restricted to 4.07 km of habitat above the waterfall barrier (2.33 km in Smith Creek, 1.74 km in Moudess Creek). A bedrock barrier on Smith Creek just above the Jakie Creek trail crossing restricts the upstream distribution of WCT (47.35635, -112.68859; Figure 59). Above this point only Brook Trout were collected in Smith Creek. An additional bedrock barrier was located below the Weasel Creek confluence (47.35068, -112.67522; Figure 60). There is an estimated 12.81 km of upper Smith Creek and its tributaries that support only Brook Trout. The presence of the intermediate barriers on Smith Creek would allow the watershed to be split into discrete sections in any future piscicide treatment.


Figure 59. Smith Creek bedrock barrier located at 47.35635, -112.68859 above the Jakie Creek Trail crossing.


Figure 60. Smith Creek bedrock barrier located at 47.35068, -112.67522 below the Weasel Creek confluence.

VI. Teton River Subbasin

Bear Gulch

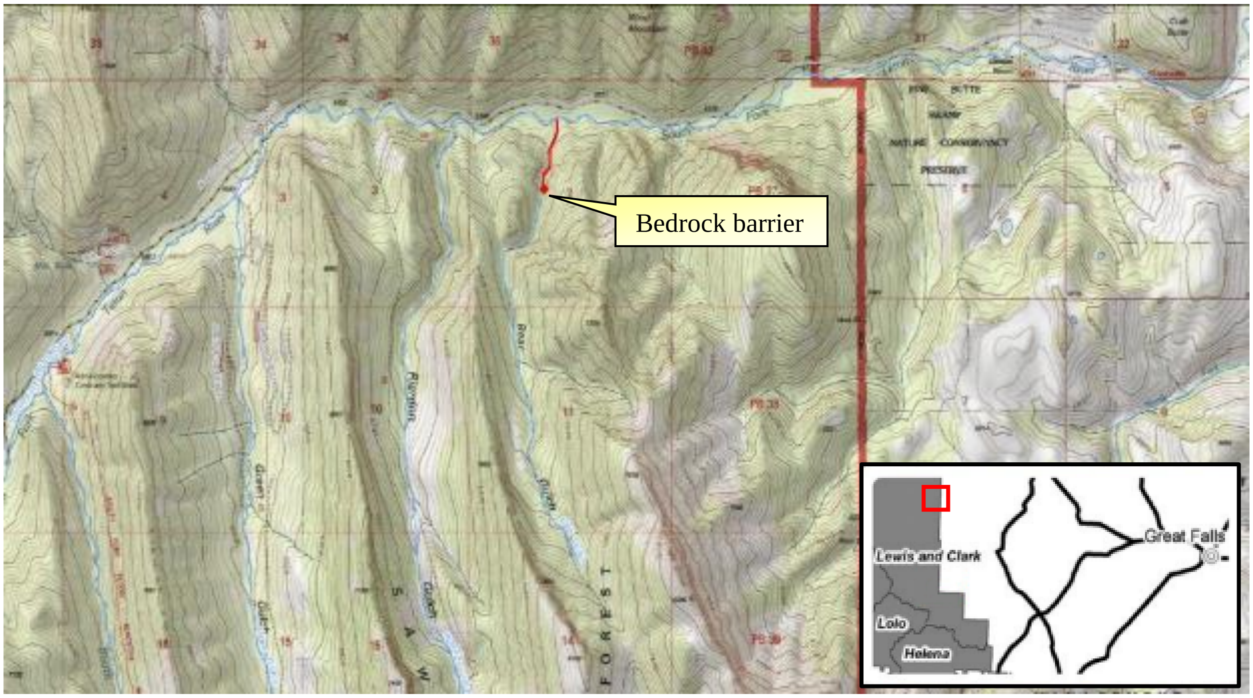

Figure 61. Bear Gulch in the Teton River subbasin. The stream segment delineated in red indicates the area sampled in 2021.

Background

Bear Gulch is a tributary of the South Fork Teton River located west of the town of Choteau, MT. No previous collection records exist for this locality.

2021 Monitoring

Bear Gulch was surveyed on July 8th, 2021, for presence of fish barriers and extant populations of WCT. A 572 m reach of Bear Gulch was visually surveyed beginning at the confluence with the South Fork Teton River. The channel of Bear Gulch was dry for the entire reach surveyed and appears to be ephemeral. The Survey was ended at a large dry bedrock barrier located at 47.86354, -112.72526.

Blackleaf Creek

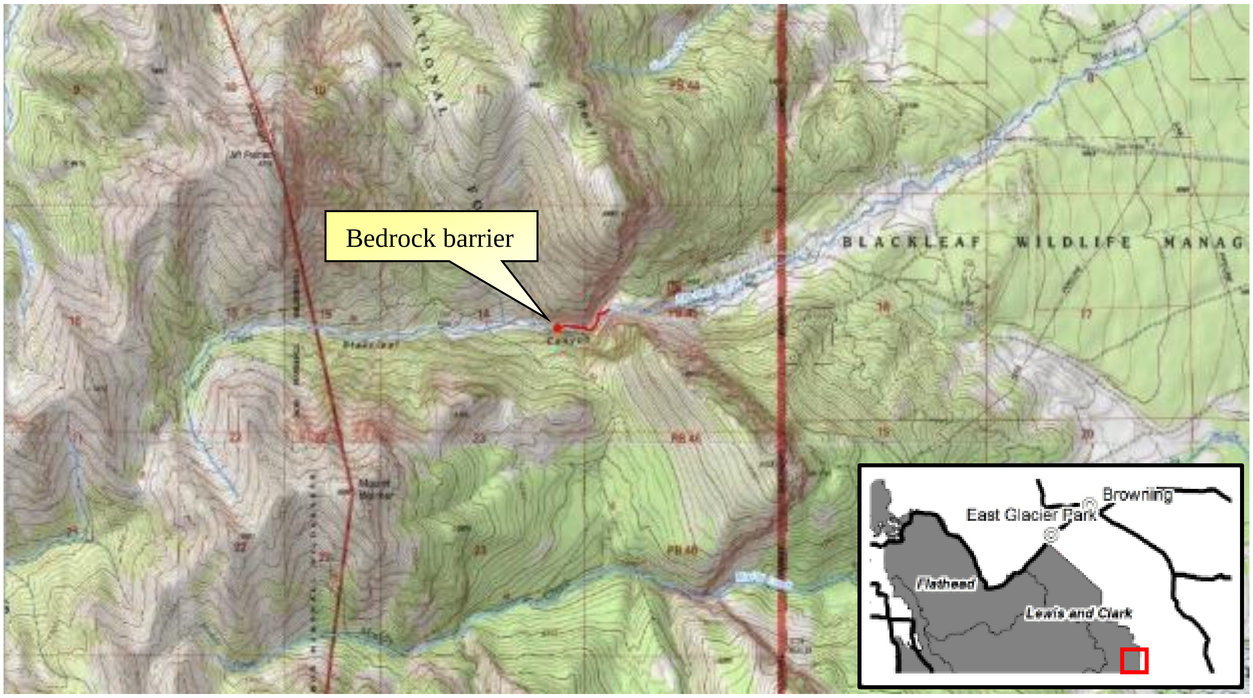

Figure 62. Blackleaf Creek in the Teton River subbasin. The stream segment delineated in red indicates the area sampled in 2021.

Background

Blackleaf Creek is a tributary of Muddy Creek in the Teton River subbasin located on the Rocky Mountain Front west of the town of Bynum, MT. No previous collection records exist for this locality. A tributary of Blackleaf Creek, Cow Creek, contained a nonhybridized WCT population in 1990. However, subsequent sampling of Cow Creek in 1995 and 2003 failed to detect the presence of WCT. Tews et al. (2000) estimated approximately 2.4 km of Cow Creek was inhabited by WCT. Drought and warm water temperatures intensified by the opening of the stream canopy by livestock and the creation of large beaver ponds may have caused the extirpation of the small WCT population in Cow Creek (Moser 2003).

2021 Monitoring

Blackleaf Creek was surveyed on October 19th, 2021, for presence of fish barriers and extant populations of WCT. A 486 m reach of Blackleaf Creek was backpack electrofished upstream of the Blackleaf trailhead. No fish were collected or observed. The survey ended at a 7.6 m waterfall barrier (48.00733, -112.72182; Figure 63).


Figure 63. Waterfall barrier on Blackleaf Creek located at 48.00733, -112.72182.

Green Gulch

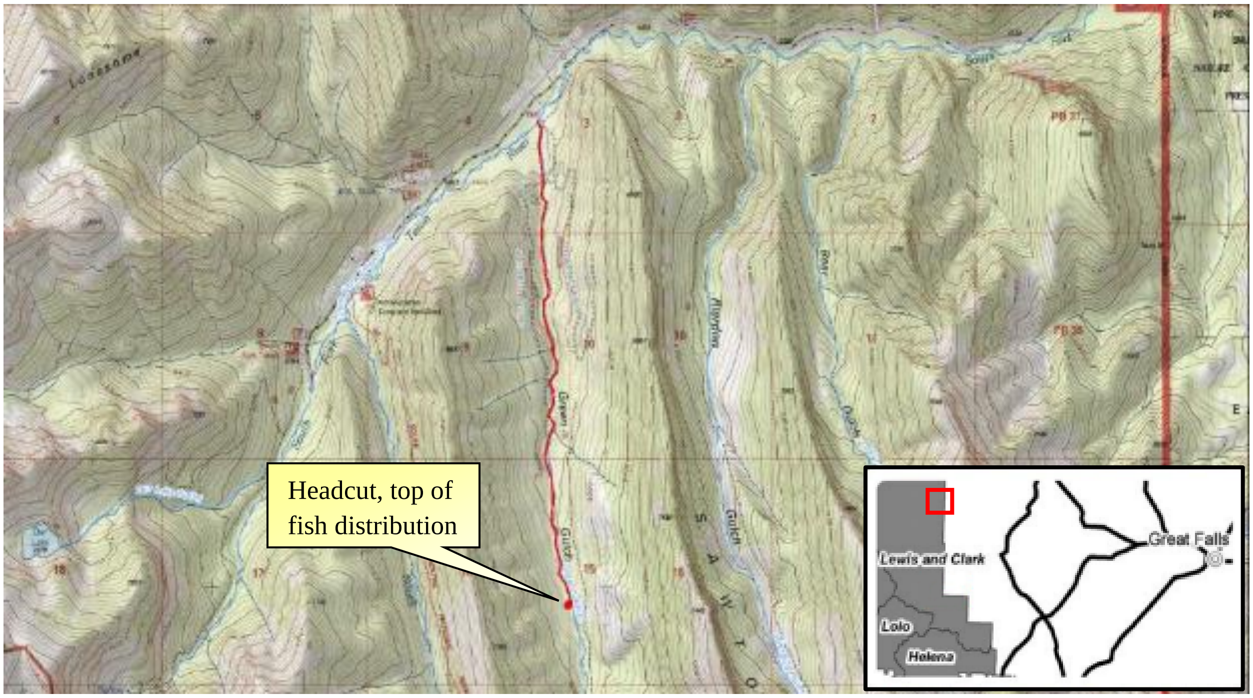

Figure 64. Green Gulch in the Teton River subbasin. The stream segment delineated in red indicate the area occupied by WCT.

Background

Green Gulch is a tributary of the South Fork Teton River located west of the town of Choteau, MT. Genetic samples of WCT collected from Green Gulch in 1992 indicated a hybridized population with a predominant WCT genetic component (94.5% WCT 5.5% RB). Additional samples collected from 1993, 1994, and 2000 found alleles characteristic of RB and YCT that were indistinguishable from WCT polymorphisms. Genetic samples collected from lower and upper Green Gulch in 2003 indicated a hybridized population with a predominant WCT genetic component at the lower site (99% WCT 1% RB) and 100% WCT at the upper site.

2021 Monitoring

Green Gulch was surveyed on July 20th, 2021, to obtain updated WCT genetic samples. A 0.96 km reach of upper Green Gulch was backpack electrofished in which no fish were collected. Flow in this reach was intermittent and habitat marginal. Shocking downstream from the start of the upper reach, the top of fish distribution was found at 47.83198, -112.75484 below a headcut. A 492 m reach of Green Gulch was backpack electrofished starting at the headcut moving downstream. A total of 31 WCT were collected. Rocky Mountain Sculpin and tailed frog larvae were also observed.

Rierdon Gulch

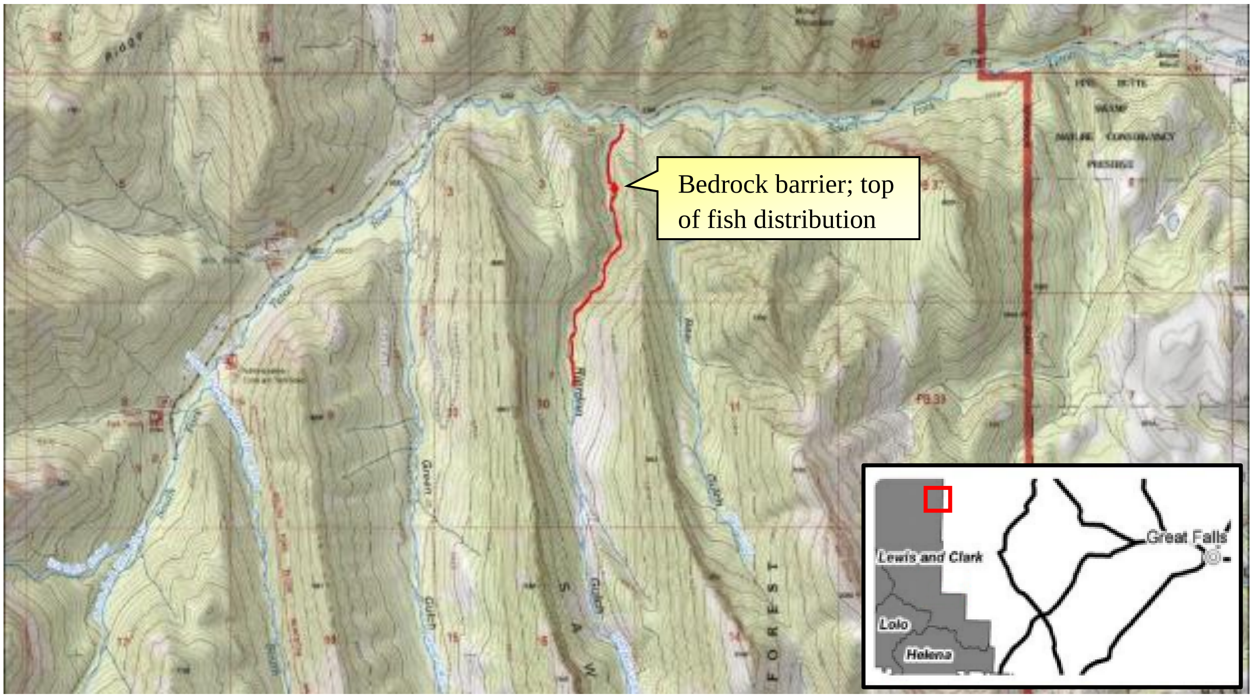

Figure 65. Rierdon Gulch in the Teton River subbasin. The stream segment delineated in red indicates the area sampled in 2021.

Background

Rierdon Gulch is a tributary of the South Fork Teton River located west of the town of Choteau, MT. Genetic samples collected in 1992 from lower Rierdon Gulch indicated a hybridized WCT population with a predominant WCT genetic component (95% WCT 5% RB; n = 15). Additional samples from 1994 collected 0.80 km upstream from the previous collection indicated a 100% WCT population (n = 12).

2021 Monitoring

Rierdon Gulch was surveyed on July 21st, 2021, to obtain updated WCT genetic samples. A 2.25 km reach of Rierdon Gulch was backpack elctrofished downstream starting from the #126 trail crossing. The upper drainage was found to be fishless. A bedrock barrier was located at 47.86322, -112.73541 approximately 531 m upstream from the confluence with the South Fork Teton River (Figure 66). Below this point 52 Brook Trout and six WCT were collected. Genetic samples were obtained from the WCT collected. However, it does not appear that this is a viable WCT population and is comprised of only a few individuals ascending from the South Fork Teton River.


Figure 66. Rierdon Gulch bedrock barrier located at 47.86322, -112.73541.

South Fork South Fork Teton River

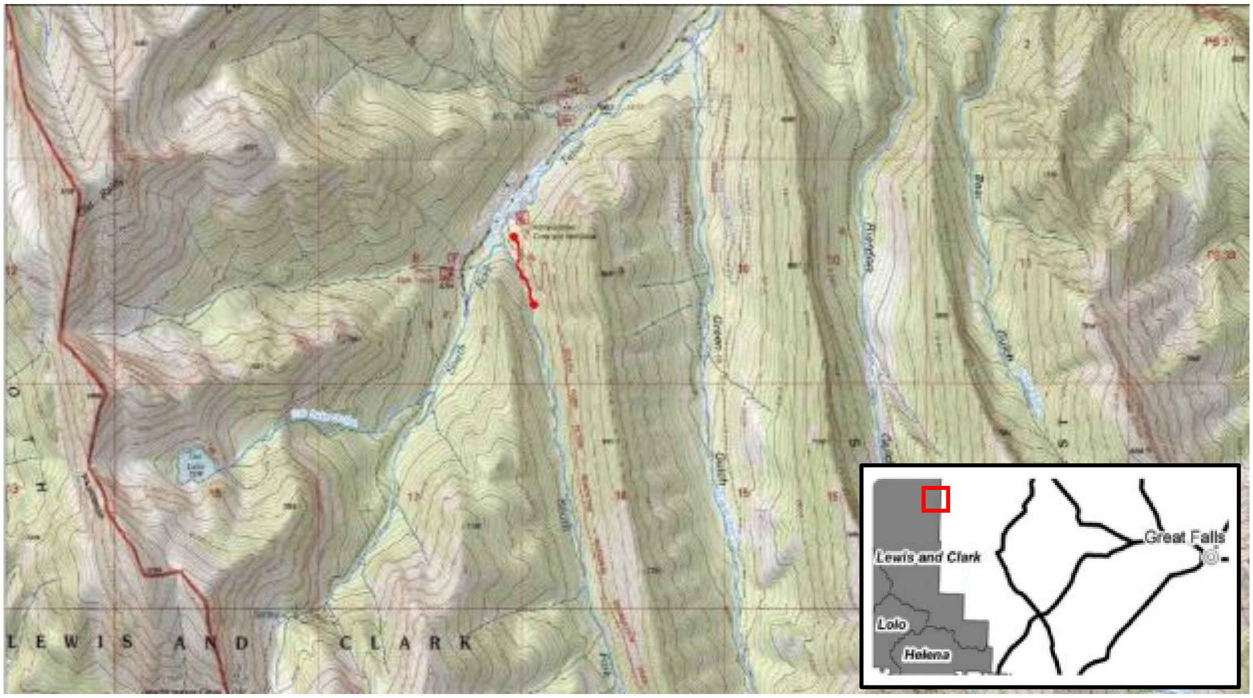

Figure 67. South Fork South Fork Teton River in the Teton River subbasin. The stream segment delineated in red indicates the area sampled in 2021.

Background

The South Fork South Fork Teton River is a tributary of the South Fork Teton River located west of the town of Choteau, MT. No previous collection records exist for this locality. Genetic samples from the South Fork Teton River near the South Fork South Fork confluence in 1998 indicated a nonhybridized WCT population (n = 9). No genetic or demographic monitoring of the upper South Fork Teton River WCT population has occurred since 1999.

2021 Monitoring

The South Fork South Fork Teton River was surveyed on October 19th, 2021, to obtain WCT genetic samples. A 500 m reach was backpack electrofished starting near the South Fork Teton confluence. Both the South Fork South Fork Teton and the South Fork Teton were dry at the confluence of the two drainages. A total of 28 WCT were collected ranging in size from 89-187 mm. Genetic samples were collected from 25 individuals.

Unnamed Tributary to South Fork Teton River

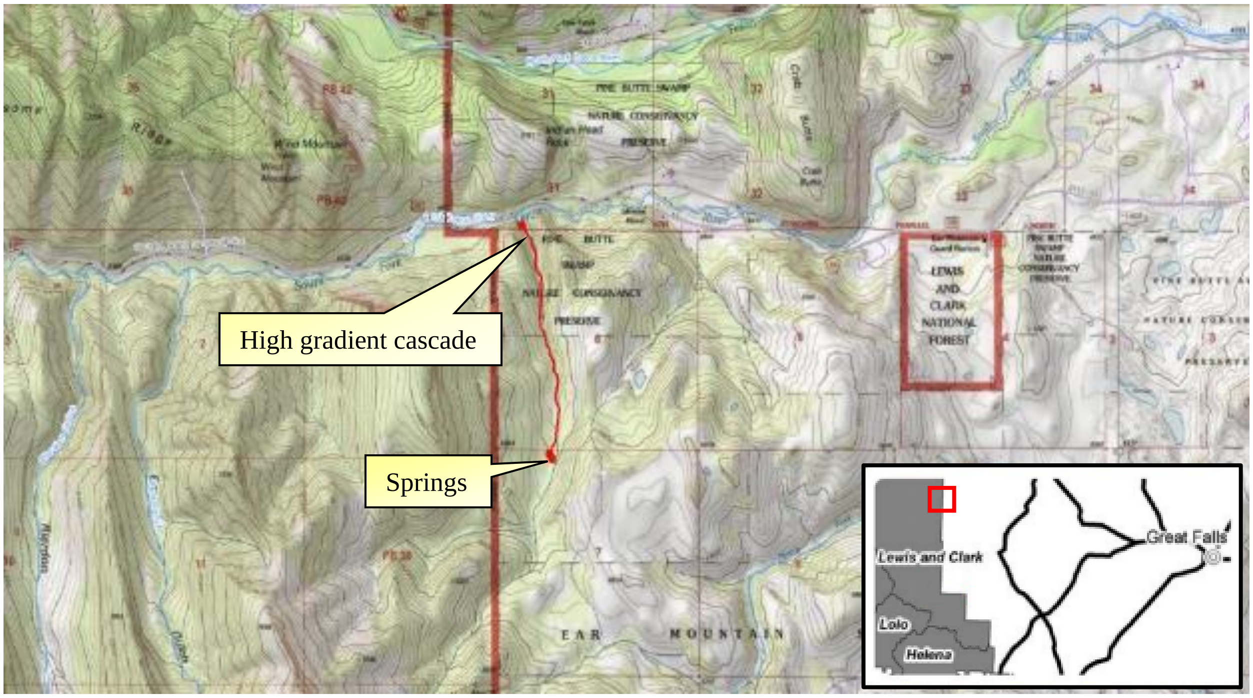

Figure 67. Unnamed Tributary to South Fork Teton River in the Teton River subbasin. The stream segment delineated in red indicates the area sampled in 2021.

Background
This unnamed tributary of the South Fork Teton River drains the eastern slope of Ear Mountain on the Rocky Mountain Front west of the town of Choteau, MT. No previous collection records exist for this locality.

2021 Monitoring
The unnamed tributary was surveyed on July 8th and October 5th, 2021, for presence of fish barriers and extant populations of WCT. A 0.9 km reach of the unnamed tributary was backpack electrofished on July 8th starting at the confluence with the South Fork Teton River. Only the lower 90 m of stream were found to be fish bearing. A total of 13 WCT and eight Brook Trout were collected in this reach. Ten genetic samples were obtained from the collected WCT. No fish were found in 730 m above a small log jam located at 47.87081, -112.68875. Upstream of this point the stream becomes high gradient with numerous bedrock slides that likely preclude upstream fish movement (Figure 68).

On October 5th, 2021 further habitat evaluation was performed in the fishless reach of the unnamed tributary. Two headwater springs were located that supply the creek with flow (Figure 67). A 965 m reach was backpack electrofished downstream of the springs. No fish were collected. High sediment loads were noted in the upper drainage near the spring sources. In total it appears that there is approximately 1.71 km of fishless habitat in the unnamed tributary.

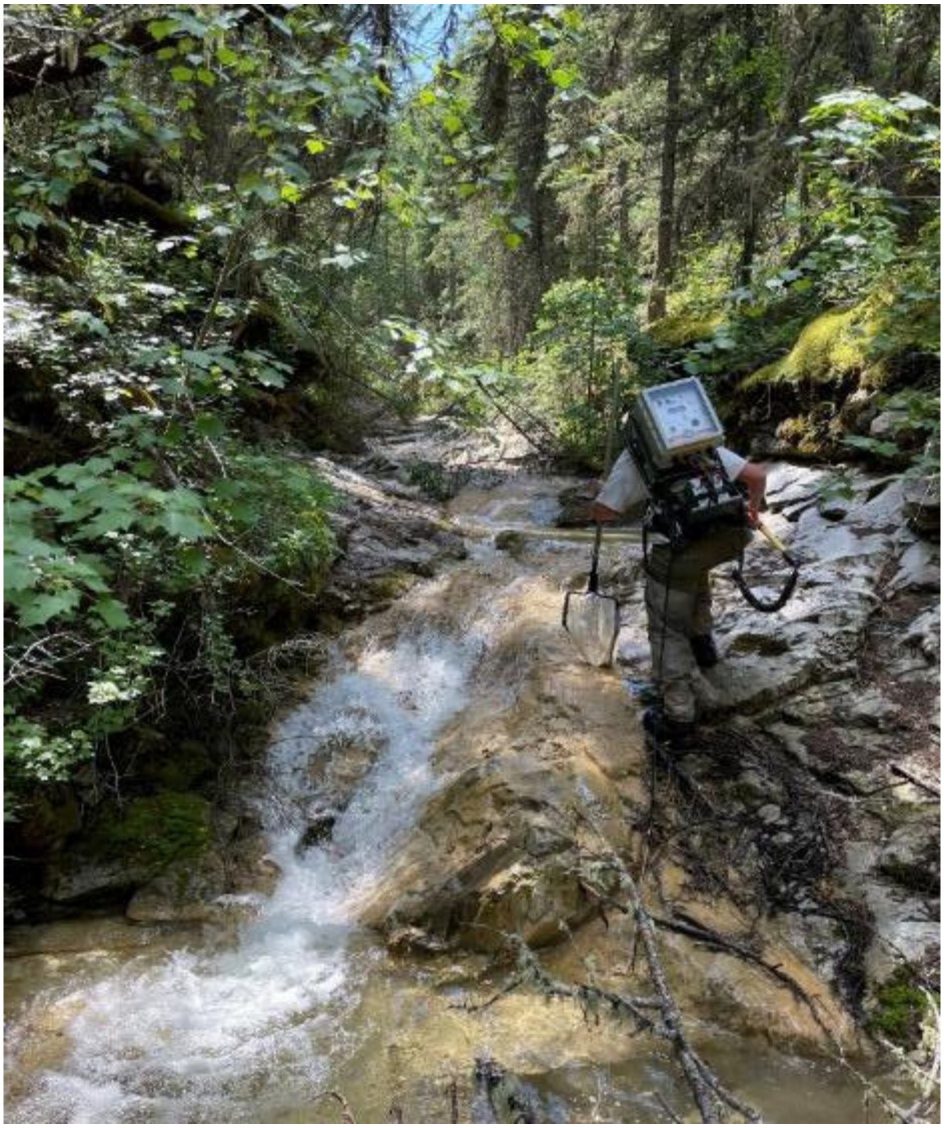
Figure 68. Bedrock feature in the unnamed tributary to South Fork Teton River.

VII. Two Medicine River Subbasin

<u>Box Creek</u>

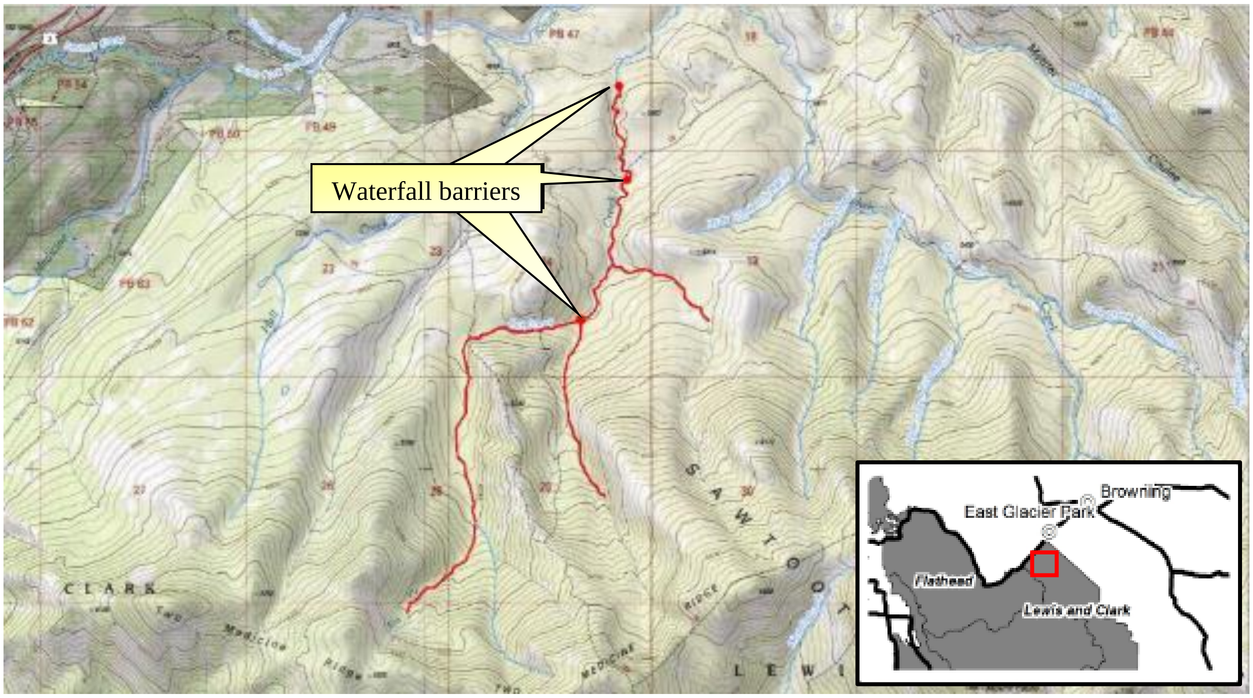

Figure 69. Box Creek in the Two Medicine River subbasin. The stream segments delineated in red indicate potential WCT restoration area.

<u>Background</u>

Box Creek is a tributary of the South Fork Two Medicine River located south of East Glacier, MT. No previous collection records exist for this locality.

<u>2021 Monitoring</u>

Box Creek was surveyed on July 6th, 2021, for presence of fish barriers and extant populations of WCT. Backpack electrofishing was used to determine fish presence and distribution starting at the crossing of the Hyde connecter trail (#159). Approximately 65 m above this trail crossing the first fish barrier was located (48.35777, -113.23029; Figure 70). Below this barrier Rainbow Trout, Rainbow x Cutthroat hybrids, and Brook Trout were collected. Only Brook Trout were collected above this barrier. Moving upstream, another fish barrier was located just upstream of the Jennings trail (#118) crossing (48.35175, -113.22944; Figure 71). A third barrier on the mainstem of Box Creek was located at the confluence of the 2nd unnamed tributary (48.34286, -113.23441; Figure 72). Additionally, a waterfall barrier was also located on the 2nd unnamed tributary at the confluence with Box Creek (48.34282, -113.23443; Figure 72). A short 75 m reach of this tributary was electrofished above the barrier and no fish were collected.

The absence of Rainbow Trout or Rainbow x Cutthroat hybrids in the upper portions of Box Creek indicates that the lower waterfall is likely a complete barrier to fish passage. The presence of Brook Trout throughout the upper drainage is probably a result of historic stocking although no stocking records exist for Box Creek. Further evaluation of the drainage is warranted to determine fish distribution and the extent of fish habitat. Preliminary estimates indicate that there may be up to 7.92 km of habitat suitable for WCT restoration in Box Creek.

Figure 70. Waterfall barrier on Box Creek upstream of Hyde connector trail crossing (48.35777, -113.23029)

Figure 71. Waterfall barrier on Box Creek upstream of Jennings trail crossing (48.35175, -113.22944)

Figure 72. Waterfall barriers on Box Creek (48.34286, -113.23441; right) and the 2nd unnamed tributary (48.34282, -113.23443; left).

Hyde Creek

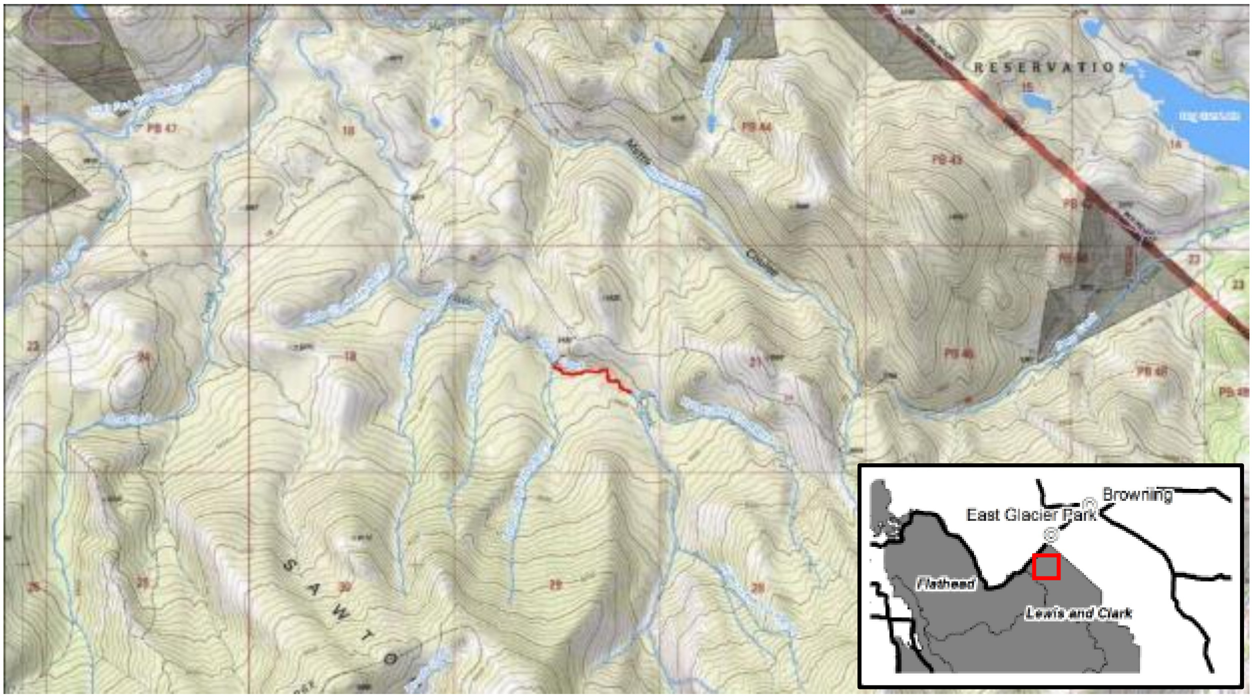

Figure 73. Hyde Creek in the Two Medicine River subbasin. The stream segment delineated indicates the area sampled in 2021.

Background

A significant waterfall barrier at the mouth of Hyde Creek prevents upstream movement of nonnative fish from the South Fork Two Medicine River. Approximately 8.85 km of Hyde Creek and tributaries were chemically treated in 2014, 2018, and 2019 to remove nonnative Brook Trout. In 2020, 80 WCT from Lange Creek (Sun River drainage) were transferred to Hyde Creek by helicopter and released near the mouth of the 4th tributary.

2021 Monitoring

Hyde Creek was surveyed on September 30th, 2021, to determine successful spawning of WCT transferred in 2020. Streamside visual counts were performed according to the methodology described by Bozek and Rahel (1991). A 783 m reach of stream was surveyed beginning at the confluence of the 4th tributary and concluding at the confluence of the north and south forks of Hyde Creek. No fry were visually observed during this survey, however, 16 adult WCT were counted. Conditions were challenging with shadows and deciduous leaf litter obscuring stream margins. Post-transfer monitoring should continue on Hyde Creek to determine establishment of the WCT population.

Phillips Creek

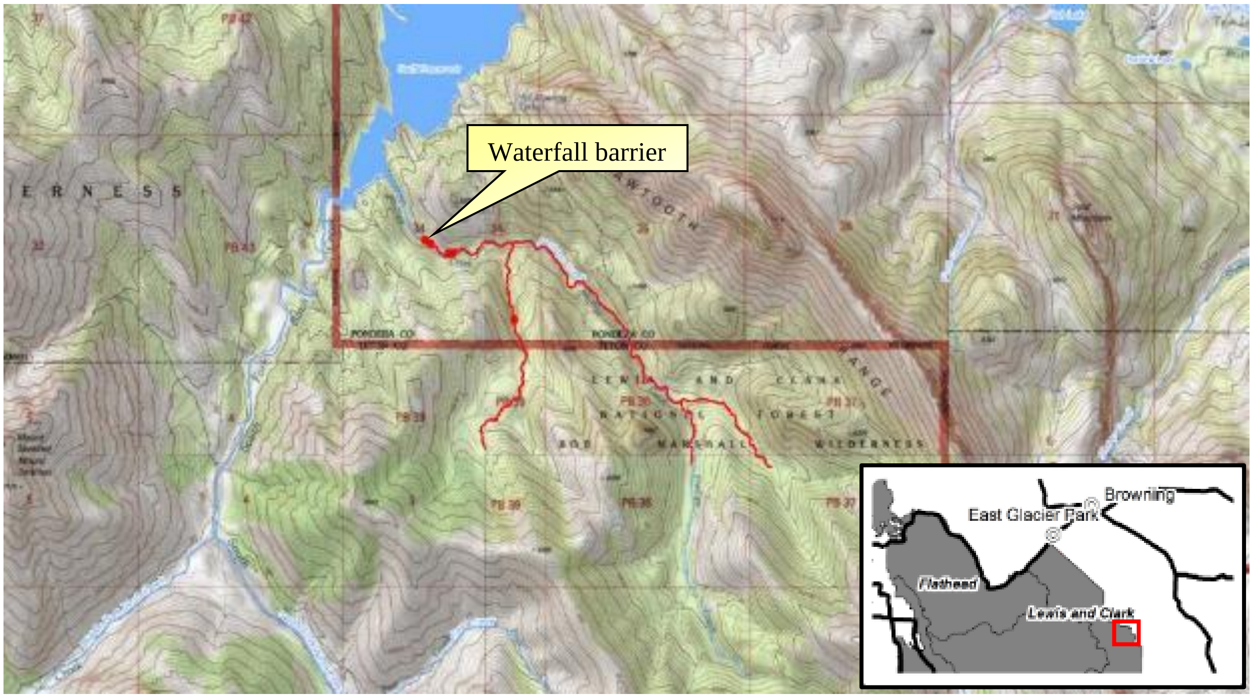

Figure 74. Phillips Creek in the Two Medicine River subbasin. The stream segments delineated in red indicate potential WCT restoration area.

Background

Phillips Creek is a tributary of Swift Reservoir in the Two Medicine River subbasin located west of Dupuyer, MT. No previous collection records exist for this locality.

2021 Monitoring

Phillips Creek was surveyed on May 6th, 2021, for presence of fish barriers. The stream was accessed by hiking the Swift Reservoir Trail #143 from the Swift Dam Trailhead. Approximately 300 m upstream from the trail crossing the first waterfall barrier was encountered. This large two-tiered waterfall in total measures an estimated 15.2 m tall (48.13874, -112.88255; Figure 75). Another three potential bedrock fish barriers were located in the immediate 250 m above the first waterfall barrier. No fish were visually observed in the reach above the 15.2 m waterfall.

On October 4th, 2021, Phillips Creek was surveyed to determine fish presence above the waterfall barrier. A 1.4 km reach of Phillips Creek was backpack electrofished starting at the lower waterfall barrier. An additional 565 m of the lower tributary (Section 34) was also electrofished. No fish were collected in this effort. Fish habitat appeared excellent throughout the area surveyed. Further evaluation of Phillips Creek is warranted. An estimated 6.73 km of fishless habitat above the waterfall barrier could serve as a future WCT restoration site.

Figure 75. Estimated 15.2 m waterfall barrier on Phillips Creek located at 48.13874, -112.88255.

VIII. Upper Missouri River Subbasin

Cottonwood Creek

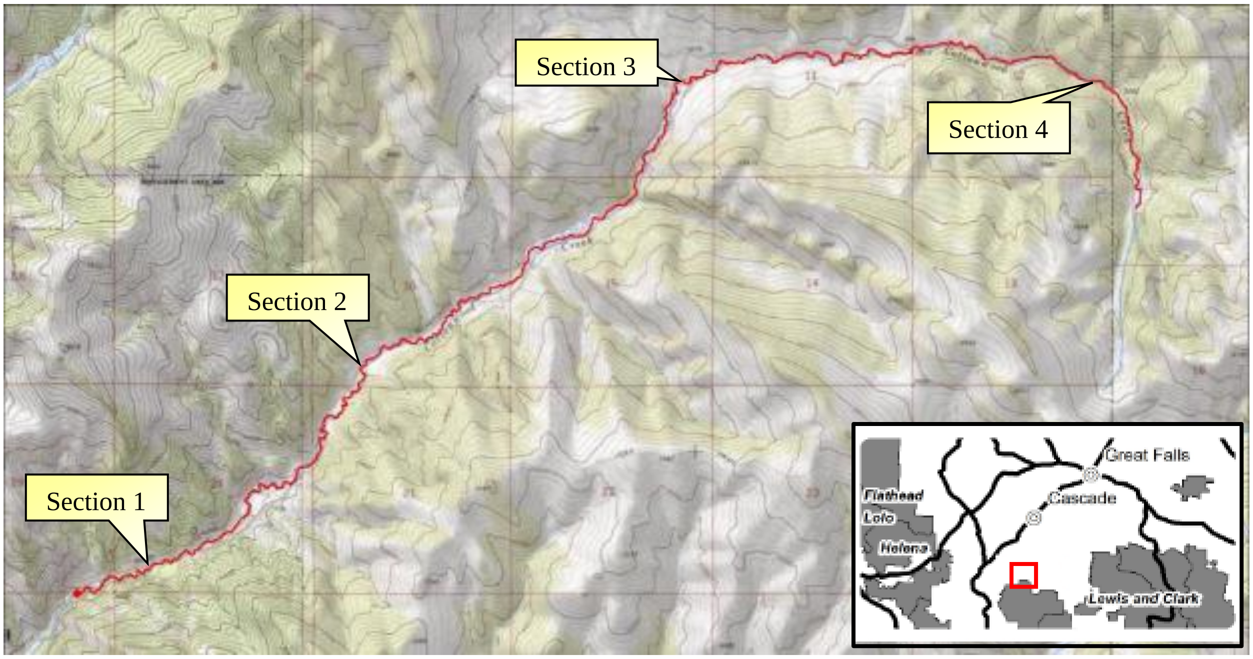

Figure 76. Cottonwood Creek in the Upper Missouri River subbasin. The stream segment delineated in red indicates the area occupied by nonhybridized WCT.

<u>Background</u>

A small concrete fish barrier was installed in Cottonwood Creek in 2000 as part of a WCT restoration project. Two piscicide treatments were performed in 2003 and two more in 2007 to remove nonnative Brook Trout. Two Brook Trout were removed above the barrier post-treatment in 2008, and none were observed in 2009 after extensive shocking. In 2010, a pre-cast concrete barrier was constructed on Cottonwood Creek (MoTac Project #753-10) in 2010 to replace the original undersized fish barrier. Following piscicide treatment, WCT from Threemile Creek and White Creek (Upper Missouri subbasin) were restored by means of RSIs from 2009-2013. Brook Trout were once again observed above the barrier in 2015 and removed. Extensive electrofishing of Cottonwood Creek in 2016 failed to detect any Brook Trout. Cottonwood Creek represents the largest restored WCT population in Region 4 inhabiting approximately 14.32 km of habitat.

<u>2021 Monitoring</u>

Cottonwood Creek was surveyed on August 18-19th, 2021 to obtain updated WCT demographic data. Four 100 m fish population estimate sections were established upstream of the constructed fish barrier. Multiple pass depletion methods were used to estimate population abundance. WCT density was considerably lower at the two lower estimate sections compared to the upper sections (Figure 77). Habitat characteristics differed between the two lower sections and the two upper sections. In the lower sections, stream substrate was dominated by sand/silt and aquatic vegetation was abundant. Substrate in the upper sections was mainly gravel/cobble with little to no aquatic vegetation. Rocky Mountain Sculpin were abundant in Sections 2 and 3 but absent from 1 and 4. Discharge was low in 2021 with at least two reaches of creek (~365 m) with no surface water.

On September 8th, 2021, 125 WCT were collected from Cottonwood Creek and transferred to Upper Log Gulch Reservoir (Figure 78). WCT were collected from the Polloch Meadows reach of Cottonwood Creek and only individuals over 125 mm were selected for transfer. The goal of this transfer is to establish a WCT brood source in Upper Log Gulch Reservoir to aid in future WCT restoration activities.

Cottonwood Creek —NATIVE TROUT POPULATION SURVEY

1. General Information— Date: **August 18-19th, 2021** Biologist: **A. Poole**
2. Stream Information—
   Name, section, county: **Cottonwood Creek, 20, 16, 10, 12, Lewis and Clark**
3. Survey Site Information (see attached map)—
   Upstream range of native trout (general description and GPS): **Sieben Livestock property boundary (46.98341, -111.79022)**
   Downstream range of native trout (general description and GPS): **Constructed fish barrier (46.94839, -111.89972)**
   Location (GPS) and description of barriers: **Constructed fish barrier (46.94839, -111.89972)**
   Stream Length—Occupied habitat: **14.32 km (8.9 mi)** Available habitat: **18.83 km (11.7 mi)**
   Survey method & equipment: **backpack battery electrofisher; two-pass depletion**
   Survey sites (general description and UTM)—
   Section 1: **Above barrier; 46.94982, -111.89278**
   Section 2: **Below Coffee Shop; 46.96393, -111.86956**
   Section 3: **Polloch Meadows; 46.98406, -111.83554**
   Section 4: **Below Sieben; 46.98427, -111.79365**

Parameter	**Section 1**	**Section 2**	**Section 3**	**Section 4**
Section length (m)	100 m	100 m	100 m	100 m
Mean stream width (m) (n)	1.89 m (10)	1.41 m (10)	1.08 m (10)	1.04 m (10)
Section area (hectares)	0.019 ha	0.014 ha	0.011 ha	0.010 ha
WCT				
Removal Pattern	11 1	5 3 1	25 12 2	27 2
Population estimate	12 (±1)	9 (±2)	40 (±3)	29 (±1)
Capture probability	0.910	0.510	0.640	0.930
Mean length (mm) (n)	152 (12)	153 (9)	113 (31)	107 (29)
Mean weight (g) (n)	36 (12)	35 (9)	14 (31)	12 (29)
Mean KTL (n)	0.86 (12)	0.83 (9)	0.83 (31)	0.83 (29)
Number fish per km (95 % CI)	120 (±10)	90 (±20)	400 (±30)	290 (±10)
Number fish per ha (95 % CI)	632 (±53)	643 (±143)	3,636 (±273)	2,900 (±100)
Biomass (kg per ha) (95 % CI)	23 (±2)	23 (±5)	51 (±4)	35 (±1)

Figure 77. Cottonwood Creek fish population estimate results.

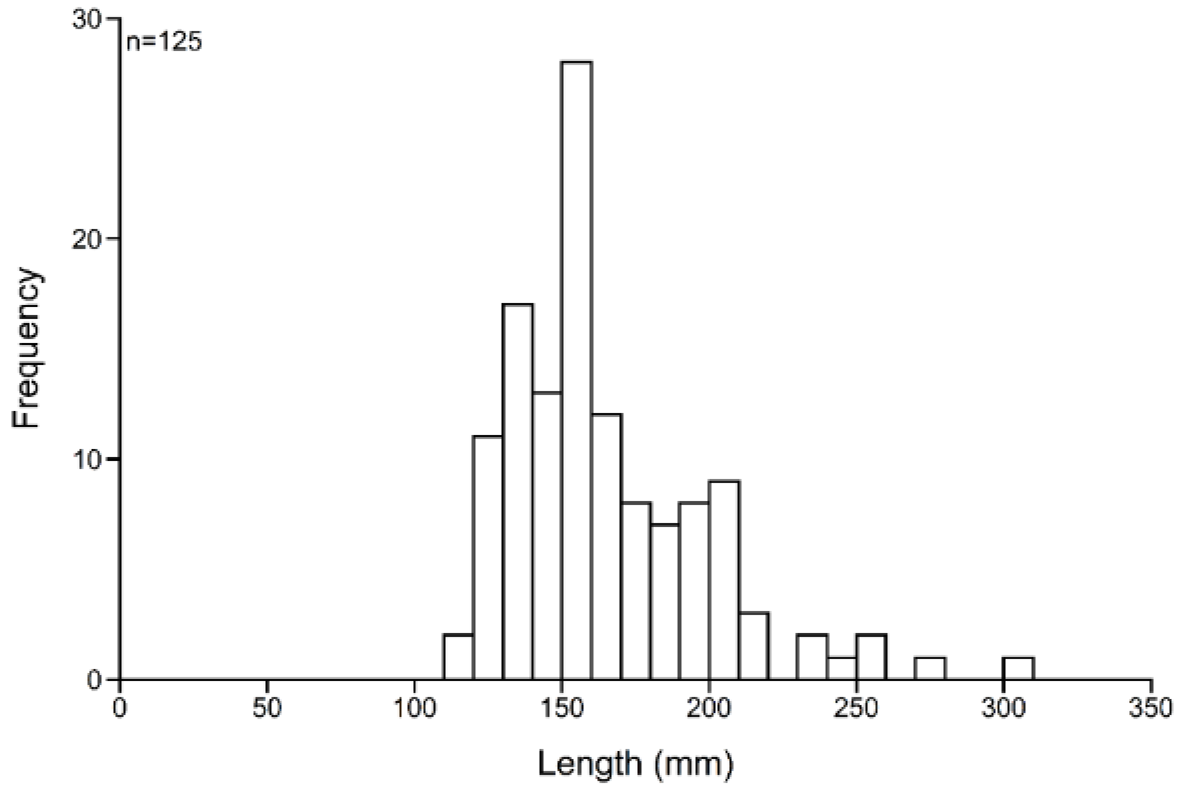

Figure 78. Length frequency histogram of WCT transferred from Cottonwood Creek to Upper Log Gulch Reservoir.

Page Gulch

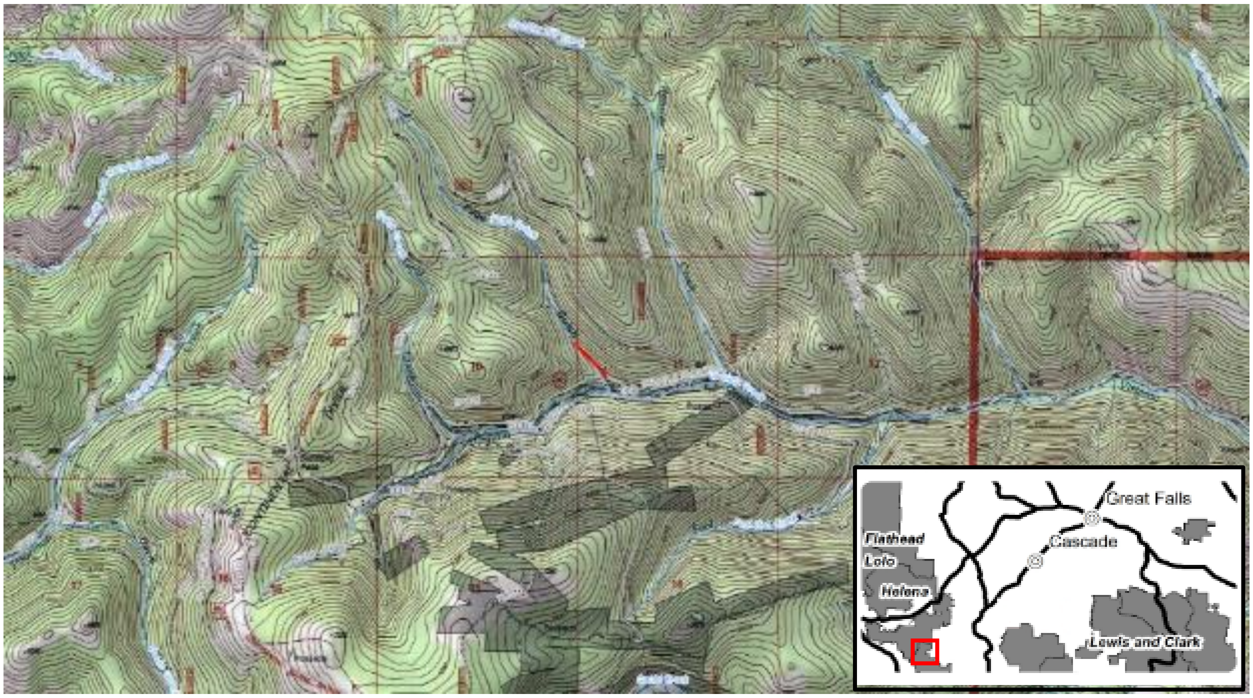

Figure 79. Page Gulch in the Upper Missouri River subbasin. The stream segment delineated in red indicates the area sampled in 2021.

Background

Genetic monitoring of WCT collected in Page Gulch in 1997 indicated alleles characteristic of both Westslope and Yellowstone Cutthroat Trout were present in the population (n = 6). This could indicate a small amount of hybridization, or it could be a rare WCT genetic variation. Updated genetic surveys are required to determine the status of this population.

2021 Monitoring

Page Gulch was surveyed on September 27th, 2021, to obtain updated genetic samples for analysis. A 309 m reach of stream was backpack electrofished starting at the Stemple Pass Road crossing. A total of 46 WCT and 10 Brook Trout were collected. Genetic samples were collected from 25 individual WCT. The majority of WCT captured were around 50 mm in total length and likely age 0. Page Gulch appears to be an important WCT spawning tributary in the upper Virginia Creek drainage.

Skelly Gulch

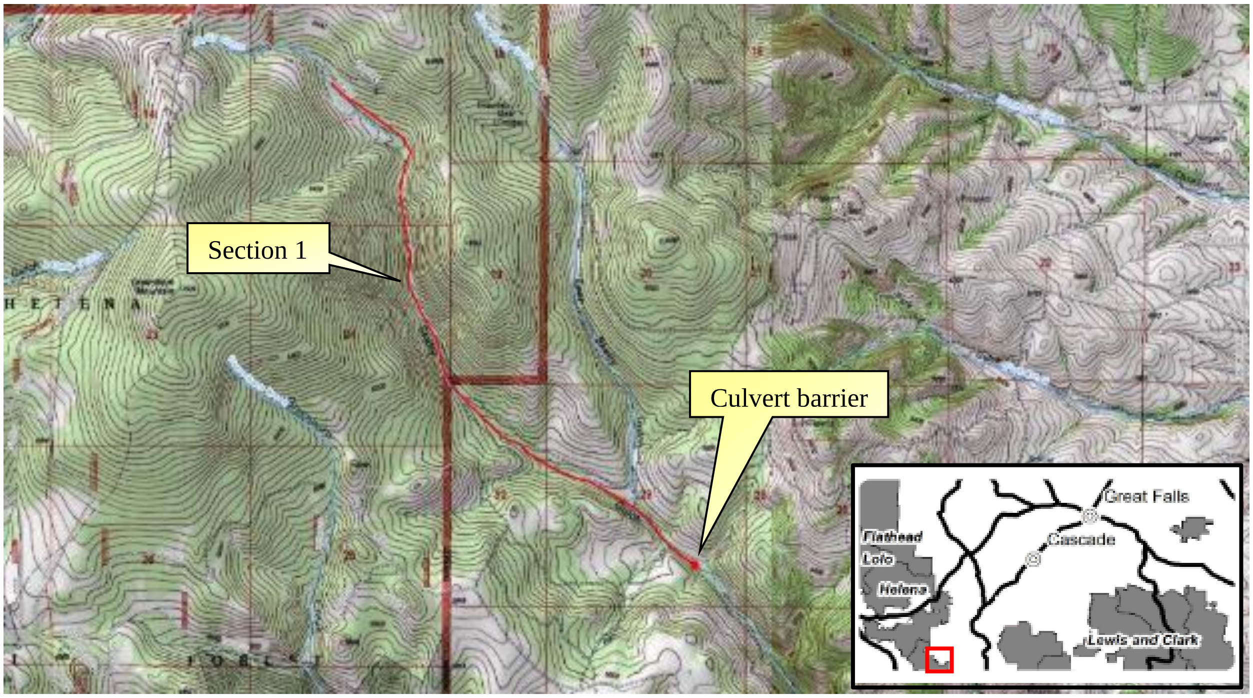

Figure 80. Skelly Gulch in the Upper Missouri River subbasin. The stream segment delineated in red indicates the area occupied by nonhybridized WCT.

Background

Construction of a culvert fish barrier at a road crossing on Skelly Gulch was completed on September 22, 2011. The project involved replacing an existing road crossing, that acted as a partial fish barrier, with a culvert placed at the appropriate slope, length, and drop to be a complete fish barrier. A conservation population of WCT exists above the road crossing, while non-native Brook Trout are prevalent below the road crossing. Genetic monitoring of the Skelly Gulch WCT population has occurred in 1991, 2001, 2002, and 2013. No evidence of hybridization has been detected in this population.

2021 Monitoring

Skelly Gulch was surveyed on August 12th, 2021, to obtain a WCT abundance estimate and updated genetic samples. A single 100 m fish population estimate section was established upstream of the Forest Service boundary. Multiple pass depletion methods were used to estimate population abundance. WCT density was estimated to be 150 fish/km in Skelly Gulch. An additional 263 m of stream was electrofished above the estimate section to collect 10 more WCT for genetic analysis.

Skelly Gulch —NATIVE TROUT POPULATION SURVEY

1. General Information— Date: **August 12th, 2021** Biologist: **A. Poole**
2. Stream Information—
   Name, section, county: **Skelly Gulch, 24, Lewis and Clark**
3. Survey Site Information (see attached map)—
   Upstream range of native trout (general description and GPS): **5.05 km above culvert barrier (46.70876, -112.29794)**
   Downstream range of native trout (general description and GPS): **Culvert barrier (46.67693, -112.25879)**
   Location (GPS) and description of barriers: **Culvert barrier (46.67693, -112.25879)**
   Stream Length—Occupied habitat: **5.05 km (3.14 mi)** Available habitat: **11.10 km (6.90 mi)**
   Survey method & equipment: **backpack battery electrofisher; two-pass depletion**
   Survey sites (general description and UTM)—
   Section 1: **Upstream of Forest Service boundary; 46.69842, -112.28932**

Parameter	**Section 1**
Section length (m)	100 m
Mean stream width (m) (n)	1.75 m (10)
Section area (hectares)	0.017 ha
WCT	
Removal Pattern	12 3
Population estimate	15 (±2)
Capture probability	0.750
Mean length (mm) (n)	115 (15)
Mean weight (g) (n)	14 (15)
Mean KTL (n)	0.88 (15)
Number fish per km (95 % CI)	150 (±20)
Number fish per ha (95 % CI)	882 (±118)
Biomass (kg per ha) (95 % CI)	12 (±2)

Figure 81. Skelly Gulch fish population estimate results.

Upper Log Gulch Reservoir

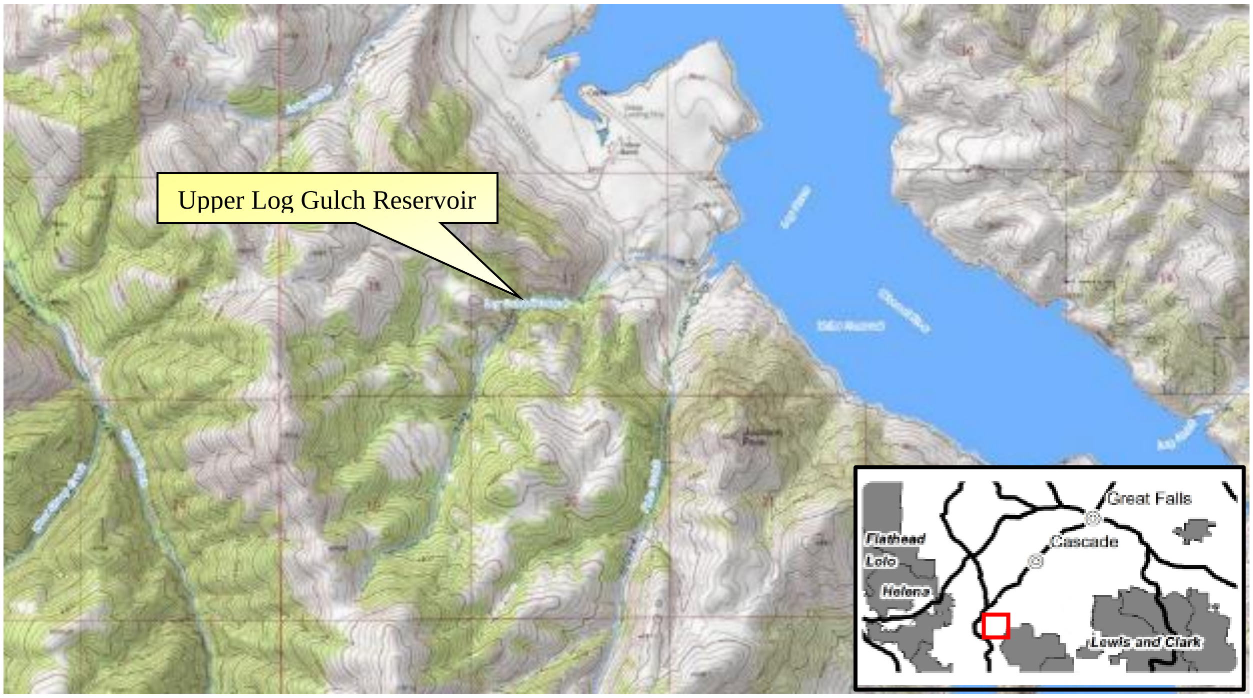

Figure 82. Upper Log Gulch Reservoir in the Upper Missouri River subbasin.

Background

Upper Log Gulch Reservoir is a 0.85-acre impoundment located on the Oxbow Ranch south of the town of Wolf Creek, MT. A Candidate Conservation Agreement with Assurances (CCAA) was secured with the landowners in November 2017 to pursue the establishment of a WCT brood source in the reservoir. Nonnative Brown Trout were targeted for removal from the pond in 2017-2018 by gill and trap net. A total of 282 Brown Trout were removed in this effort. In 2019, 86 WCT were transferred from Cottonwood Creek (Upper Missouri River subbasin) and released in Upper Log Gulch Reservoir.

2021 Monitoring

On September 8th, 2021, 125 WCT were collected from Cottonwood Creek and transferred to Upper Log Gulch Reservoir (Figure 78). WCT were collected from the Polloch Meadows reach of Cottonwood Creek and only individuals over 125 mm were selected for transfer. Five large Brown Trout were visually observed in the reservoir.

IX. Upper Missouri-Dearborn River Subbasin

Big Coulee Creek

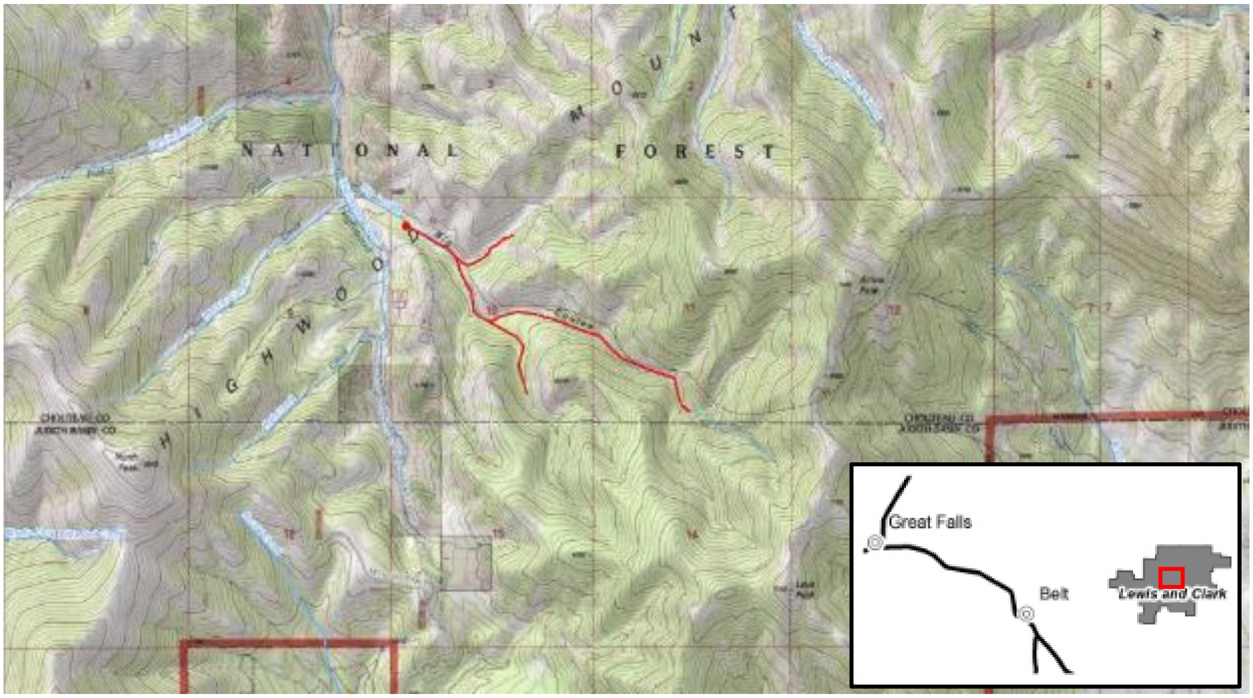

Figure 83. Big Coulee in the Upper Missouri-Dearborn River subbasin. The stream segments delineated in red indicate the areas sampled in 2021

Background

Big Coulee, a tributary to Highwood Creek, contains a nonhybridized WCT population that has been intensively managed since the late 1990s. A natural waterfall was enhanced on Big Coulee by blasting in 2002 and 2004. From 1997-2008, Brook Trout were removed to reduce negative impacts on the remaining WCT found above the barrier. During this time, fencing was installed to reduce grazing pressure and associated impacts and a no fishing regulation was implemented. The reach upstream of the barrier was thought to be devoid of Brook Trout by 2008 and the WCT population was monitored annually from 2009-2015.

In 2015 Brook Trout were discovered above the barrier during annual monitoring efforts. Additionally, a 10-inch fish with Rainbow Trout phenotypic characteristics was found and removed in 2016. Unfortunately, a genetic sample was not collected from this fish to confirm its identity. Genetic samples collected from 32 individual WCT in 2016 were classified as genetically nonhybridized WCT.

Nonnative removals were again initiated in 2015 above the barrier. From 2015 to 2020, approximately 671 Brook Trout were removed including ~200 in 2015, ~330 in 2016, ~110 in 2017, 15 in 2018, and 8 in both 2019 and 2020. The majority of Brook Trout removed prior to 2018 appeared to be age-0 and age-1 fish with few large adults found during removal efforts.

2021 Monitoring

WCT population monitoring was performed on 2.83 km of Big Coulee Creek from August 23rd-30th, 2021. Seven sections of Big Coulee Creek were two pass electrofished and an additional eighth section was electrofished in a single pass effort. A total of 3,639 WCT and 3 Brook Trout were collected in the 2021 monitoring effort (Table 1). Brook Trout were collected in the sections 4 and 5 between the confluence of the lower and upper tributary.

Table 1. Big Coulee electrofishing catch by section.

	Section 1	**Section 2**	**Section 3**	**Section 4**	**Section 5**	**Section 6**	**Section 7**	**Section 8**
Pass 1	202 WCT	229 WCT	405 WCT	386 WCT 1 EB	532 WCT 2 EB	419 WCT	404 WCT	433 WCT
Pass 2	38 WCT	80 WCT	93 WCT	109 WCT	151 WCT[1]	113 WCT	45 WCT[2]	

[1] – Hole found in livewell, 2nd pass count likely includes recaps

[2] – Battery died halfway through 2nd pass

Smith Creek

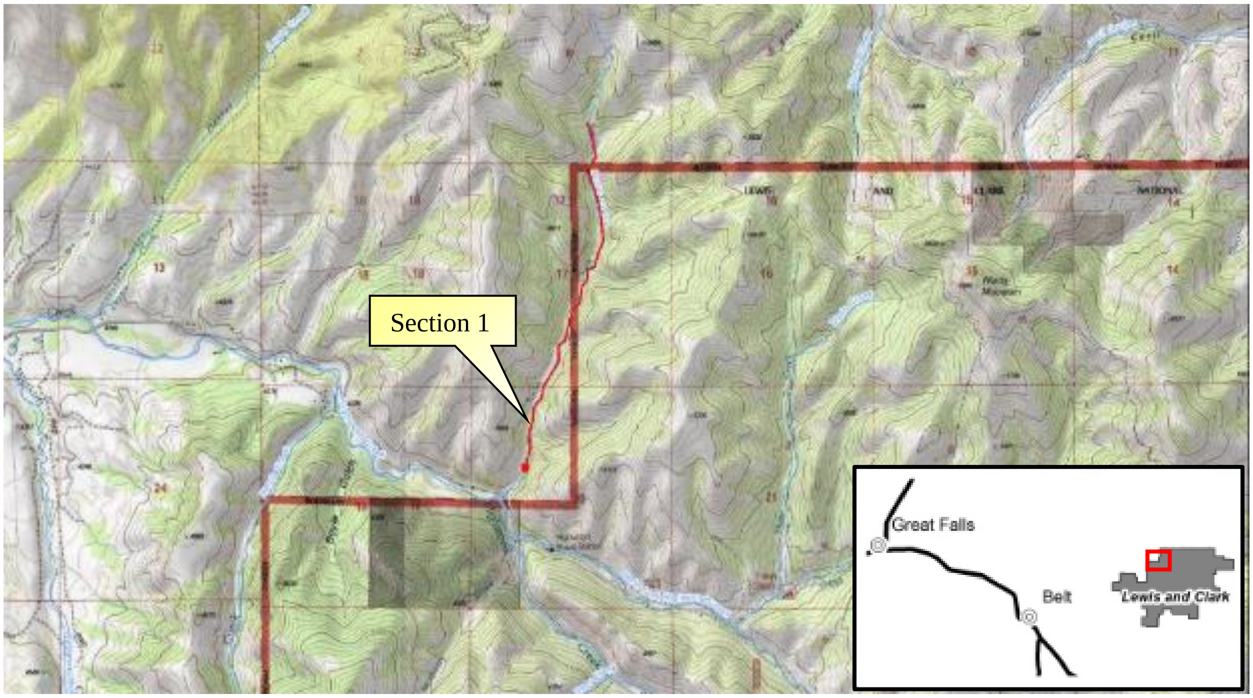

Figure 84. Smith Creek in the Upper Missouri-Dearborn River subbasin. The stream segments delineated in red indicate the area occupied by nonhybridized WCT.

Background
Smith Creek is a tributary of Highwood Creek that contains a restored WCT population. A concrete fish barrier was constructed on Smith Creek in 2010-2011 on private land. Following rotenone treatment in 2011-2012 to remove nonnative Brook Trout, 160 WCT from Big Coulee were transferred from 2012-2013. Post-treatment demographic monitoring has shown a robust WCT population established in Smith Creek.

2021 Monitoring
Smith Creek was surveyed on August 9th, 2021, to obtain an updated WCT abundance estimate. A single 100 m fish population estimate section was established near the site of previous years demographic monitoring. Multiple pass depletion methods were used to estimate population abundance. WCT density was estimated to be 150 fish/km in Smith Creek.

Smith Creek —NATIVE TROUT POPULATION SURVEY

1. General Information— Date: **August 9th, 2021** Biologist: **A. Poole**
2. Stream Information—
   Name, section, county: **Smith Creek, 20, Chouteau**
3. Survey Site Information (see attached map)—
   Upstream range of native trout (general description and GPS): **2.77 km above fish barrier (47.50518, -110.60617)**
   Downstream range of native trout (general description and GPS): **Concrete fish barrier (47.48327, -110.61294)**
   Location (GPS) and description of barriers: **Concrete fish barrier (47.48327, -110.61294)**
   Stream Length—Occupied habitat: **2.77 km (1.72 mi)** Available habitat: **3.06 km (1.90 mi)**
   Survey method & equipment: **backpack battery electrofisher; two-pass depletion**
   Survey sites (general description and UTM)—
   Section 1: **Below house; 47.48568, -110.61242**

Parameter	**Section 1**
Section length (m)	100 m
Mean stream width (m) (n)	2.23 m (10)
Section area (hectares)	0.022 ha
WCT	
Removal Pattern	37 6
Population estimate	43 (±2)
Capture probability	0.840
Mean length (mm) (n)	131 (43)
Mean weight (g) (n)	21 (43)
Mean KTL (n)	0.84 (43)
Number fish per km (95 % CI)	430 (±20)
Number fish per ha (95 % CI)	1,954 (±91)
Biomass (kg per ha) (95 % CI)	41 (±2)

Figure 85. Smith Creek fish population estimate results.

Wegner Creek

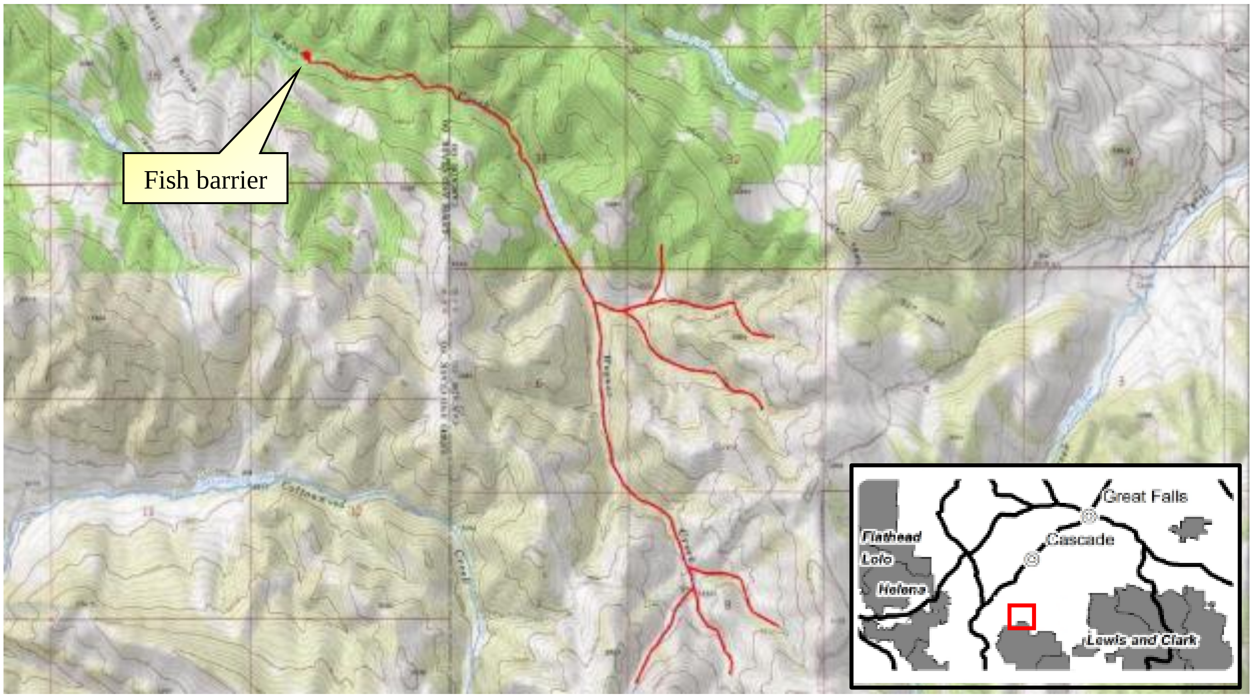

Figure 86. Wegner Creek in the Upper Missouri-Dearborn River subbasin. The stream segments delineated in red indicate potential WCT restoration area.

Background

In 2014, the Beartooth Wildlife Management Area expanded by 2,840-acres and that addition included 1.29 km of Wegner Creek (Figure 86). Wegner Creek, a tributary of the Missouri River, was surveyed in 2015 and found to contain Brook Trout, Rainbow Trout, and Rocky Mountain Sculpin. Based on the high density of trout and sculpin observed, the stream was considered as a potential conservation area for WCT. In 2017, a small concrete barrier was built on a natural bedrock slide to isolate the Wegner Creek headwaters. A piscicide treatment was performed upstream of the barrier on July 10th, 2018. A cursory electrofishing survey of the lower 2.41 km of stream above the barrier was performed in the fall of 2018 to assess the success of the piscicide treatment. About a dozen sculpin were observed in the first 0.8 km of stream above the barrier and no other fish were observed at this time.

In 2019, the stream was sampled upstream of the barrier to further assess the success of the previous year's piscicide treatment. Several large Rainbow Trout were collected in the first 0.4 km above the barrier. After this discovery, the barrier was modified to increase the height by approximately 7 inches and extend the barrier laterally by approximately 6 feet. To test the efficacy of the barrier addition, 133 Brook Trout and 38 Rainbow Trout were shocked below the barrier and given an adipose fin clip before being released. In addition, Wegner Creek was further evaluated above the barrier in 2019 and it was documented that Brook Trout were still present in the system thus suggesting an incomplete chemical treatment. No clipped fish have been detected above the modified Wegner Creek barrier to date.

2021 Monitoring

Wegner Creek was surveyed on May 5th and 11th, 2021 to evaluate the efficacy of the fish barrier. On May 5th, a 1.28 km reach of Wegner Creek was electrofished above the barrier to detect the presence of large Rainbow Trout as observed in 2018. A total of 33 Rainbow Trout (102-183 mm) were collected in this effort, likely offspring of the 2019 Rainbow Trout that bypassed the barrier. On May 11th, 2021, a 150 m reach below the barrier was electrofished in a marking effort to evaluate barrier efficacy. A total of 85 Brook Trout and 8 Rainbow Trout were adipose clipped and released below the barrier. Further evaluation of the Wegner Creek fish barrier is warranted. Stream discharge was very low in 2021 and likely influenced the ability of Rainbow Trout from the Missouri River to access Wegner Creek for spawning.

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Appendix A

Date: May 18, 2021

Biologist(s): Alex Poole

Location(s) and sampling date:

1. Camas Lake (multiple dates summer 2020; 46.55599, -111.30073)
2. Fourmile Creek (09/15/20; 46.49306, -110.72249)
3. North Fork Running Wolf (09/23/20; 46.97899, -110.45136)
4. O'Brien Creek (07/21/20; 46.86377, -110.73514)
5. Pilgrim Creek (08/24/20; 47.01194, -110.94279)
6. Richardson Creek (09/21/20; 46.53027, -110.73213)

Agency: Montana Fish Wildlife and Parks

Target species: Westslope cutthroat trout

Authors: Ryan Kovach, Sally Painter, Angela Lodmell

PROJECT SUMMARY: Genetic samples from Camas Lake, Fourmile Creek, North Fork Running Wolf Creek, O'Brien Creek, Pilgrim Creek and Richardson Creek were analyzed for purposes of describing the presence and extent of non-native genetic admixture from rainbow trout or Yellowstone cutthroat trout. For those locations with previous samples, we compared results to historical data to determine the evolutionary trajectory of the population. Specifically, we describe whether the presence and magnitude of non-native ancestry is static, decreasing, or increasing. Where appropriate, we also compare observed genetic variation to genetic variation in other non-hybridized westslope cutthroat trout populations.

All Results, Discussion, and Recommendations are described below. Summary statistics for the population samples are in Table 1 (below). Lab and data analysis methods are described in Appendix 1.

RESULTS, DISCUSSION, AND RECOMMENDATIONS

Table 1. The presence and extent of rainbow trout and Yellowstone cutthroat trout hybridization from waterbodies within the native range of westslope cutthroat trout. ID refers to the FWP sample ID number and N is the sample size. The Taxa column denotes whether a sample include non-hybridized individuals (WCT), rainbow/westslope hybrids (WCT x RBT), Yellowstone/westslope hybrids (WCT x YCT) or hybrids between all three taxa (WCT x RBT x YCT). The estimate for the percent ancestry of each taxon is presented in the last three columns.

Sample	ID	N	Taxa	% WCT	% RBT	% YCT
Camas Lake	5291	10	YCT			
		1	WCT			
Fourmile Creek	5292	30	WCT			

North Fork Running Wolf	5293	30	WCT
O'Brian Creek	5294	27	WCT
Pilgrim Creek	5295	29	WCT x RBT
Richardson Creek	5296	30	WCT x RBT

Camas Lake

We detected Yellowstone cutthroat trout alleles at all Yellowstone diagnostic markers and all westslope diagnostic markers were polymorphic. Clearly the sample contained fish with Yellowstone cutthroat trout ancestry. We also detected genetic variation at one rainbow diagnostic marker (*OmyRD_RAD_54584_Hoh*), but *OmyRD_RAD_54584_Hoh* is not generally diagnostic in non-hybridized Yellowstone cutthroat trout, which we observed in this sample. As such, we deleted that marker from any further analyses and focus only on patterns of Yellowstone cutthroat trout ancestry.

Interestingly, the sample appeared to be composed of ten non-hybridized Yellowstone cutthroat trout, and one non-hybridized westslope cutthroat trout. We did not detect any hybrids in the sample. Thus, we have no evidence to date of hybridization between the remnant Yellowstone population that survived piscicide treatment and the westslope cutthroat trout that were subsequently translocated into the Camas Lake system (see also report #5049). That being said, with a sample of only eleven fish we had relatively low power to detect hybrids if they are still at low frequency in the population. The single westslope cutthroat trout that we detected appeared to be derived from MO12 based on patterns of genetic variation. As such it was likely a triploid, and not one of the fish moved from Lone Willow Creek.

Fourmile Creek

We failed to detect any rainbow trout alleles at rainbow diagnostic markers, and none of the westslope diagnostic markers were polymorphic. We did detect Yellowstone alleles at one Yellowstone diagnostic marker (*OclYSD106367_Garza*), but that marker is often non-diagnostic in non-hybridized populations of westslope cutthroat trout in Montana (Table 2). We are confident that in this case the observed variation at *OclYSD106367_Garza* is not indicative of Yellowstone hybridization. As such, these data provide strong evidence that this genetic sample was collected from a non-hybridized population of westslope cutthroat trout.

The fish from Fourmile Creek were originally transferred from Richardson Creek, a stream that was previously non-hybridized in 1999 (#1382), but by 2013, rainbow trout hybrids were observed (#4692). Importantly and fortunately, it appears that the transfer occurred prior to recent hybridization in Richardson Creek (see below #5296) and/or, no hybrids were moved.

We further used genotypes at the westslope cutthroat trout polymorphic loci to describe patterns of genetic variation in the sample. Genotypic proportions conformed to Hardy-Weinberg expectations (Fisher's' combined P = 0.649), suggesting that the sample was collected from a single random mating population. The average expected heterozygosity in the sample was (0.023) and the proportion of polymorphic loci was (0.063). In both cases, these values are considerably lower values observed in most other populations of non-hybridized westslope in the Missouri River basin (Table 2); the median expected heterozygosity across populations is 0.094 and the median proportion of polymorphic loci is 0.313. The low genetic variation in this population likely reflects, at least on some level, the bottleneck that occurred during fish transfer. Given the extremely low variation, this population is certainly a candidate stream for future genetic infusion via strategic translocation for genetic rescue.

North Fork Running Wolf

In the genetic sample from North Fork Running Wolf we did not detect any rainbow or Yellowstone alleles, and none of the westslope diagnostic markers were polymorphic. Therefore, it appears that this genetic sample was collected from a non-hybridized population of westslope cutthroat trout. These results are consistent with three previous samples (#995, #1385, and #2268), none of which detected any rainbow trout or Yellowstone cutthroat trout alleles. As such, North Fork Running Wolf almost certainly contains a non-hybridized population of westslope cutthroat trout.

We further used genotypes at the westslope cutthroat trout polymorphic loci to describe patterns of genetic variation in the sample. Genotypic proportions conformed to Hardy-Weinberg expectations (Fisher's' combined $P = 0.497$), suggesting that the sample was collected from a single random mating population. The average expected heterozygosity in the sample was (0.171) and the proportion of polymorphic loci was (0.531). In both cases, these values are considerably higher values observed in most other populations of non-hybridized westslope in the Missouri River basin (Table 2); the median expected heterozygosity across populations is 0.094 and the median proportion of polymorphic loci is 0.313. The high genetic variation in this population, coupled with strong track record of genetic testing (i.e., for hybridization) makes this a useful donor population, at least based on genetic measures, for westslope cutthroat trout conservation work in the Judith and nearby basins.

O'Brien Creek

In the genetic sample from O'Brien Creek we did not detect any rainbow or Yellowstone alleles, and none of the westslope diagnostic markers were polymorphic. Therefore, it appears that this genetic sample was collected from a non-hybridized population of westslope cutthroat trout. These results are consistent with three previous samples from the upper section of O'Brien Creek (#1122, #2016, #2989), none of which detected any rainbow trout or Yellowstone cutthroat trout alleles. As such, O'Brien Creek almost certainly contains a non-hybridized population of westslope cutthroat trout.

We further used genotypes at the westslope cutthroat trout polymorphic loci to describe patterns of genetic variation in the sample. Genotypic proportions conformed to Hardy-Weinberg expectations (Fisher's' combined $P = 0.650$), suggesting that the sample was collected from a single random mating population. The average expected heterozygosity in the sample was (0.203) and the proportion of polymorphic loci was (0.531). These values are considerably higher values observed in most other populations of non-hybridized westslope in the Missouri River basin (Table 2); the median expected heterozygosity across populations is 0.094 and the median proportion of polymorphic loci is 0.313. The high genetic variation in this population, coupled with strong track record of genetic testing (i.e., for hybridization) makes this a useful donor population for westslope cutthroat trout conservation work in the Belt and nearby basins.

Pilgrim Creek

We detected rainbow trout alleles at three rainbow diagnostic markers and three westslope diagnostic markers were polymorphic. We did not detect any Yellowstone cutthroat trout alleles. Therefore, the sample of fish from Pilgrim Creek included fish with rainbow trout ancestry.

Rainbow trout alleles were non-randomly distributed among markers (X^2_{38}=85.89, $P < 0.001$), and among individuals in the sample (X^2_{2}=33.60, $P < 0.001$). The non-random distribution of rainbow alleles among individuals resulted from an excess of individuals with higher than expected rainbow ancestry, and an excess of what appeared to be non-hybridized westslope cutthroat trout (Fig. 1). These results strongly suggest the sample was not collected from an “old” hybrid swarm between westslope cutthroat trout and rainbow trout, which may suggest there is non-random mating among westslope cutthroat trout and hybrids, or there has been recent expansion of hybrids in this stream. In this particular case, we strongly favor the latter hypothesis. Although previous samples failed to detect any rainbow trout alleles in upstream portions of the Pilgrim Creek watershed (#1123, #2017, #3744), rainbow trout hybrids are present in lower portions of this creek (#414, #3946). Unfortunately, it seems extremely likely that hybridization in this sample represents upstream expansion of hybridization from the lower reaches of Pilgrim Creek. The current non-random distribution of rainbow trout alleles suggests there are still some non-hybridized westslope cutthroat trout, and thus, there are still management opportunities for conserving this population. However, given that hybrid have relatively “low” levels of rainbow trout ancestry, genomic methods would be required for any type of fish translocation effort.

Richardson Creek

We detected rainbow trout alleles at six rainbow diagnostic markers and eight westslope diagnostic markers were polymorphic. We did detect Yellowstone alleles at one Yellowstone diagnostic marker (*OclYSD106367_Garza*), but that marker is often non-diagnostic in non-hybridized populations of westslope cutthroat trout in Montana (Table 2). We are confident that in this case the observed variation at *OclYSD106367_Garza* is not indicative of Yellowstone hybridization. Thus, we clearly detected evidence for rainbow trout ancestry in fish from Richardson Creek.

Rainbow trout alleles were non-randomly distributed among markers (X^2_{38}=152.42, $P < 0.001$), and among individuals in the sample (X^2_{5}=100.35, $P < 0.001$). The non-random distribution of rainbow alleles among individuals resulted from an excess of individuals with higher than expected rainbow ancestry, and an excess of what appeared to be non-hybridized westslope cutthroat trout (Fig. 2). These results strongly suggest the sample was not collected from an “old” hybrid swarm between westslope cutthroat trout and rainbow trout, which may suggest there is non-random mating among westslope cutthroat trout and hybrids, or there has been recent expansion of hybrids in this stream. In this particular case, we strongly favor the latter hypothesis. Genetic samples collected in 1999 (#1382) failed to detect any rainbow trout hybrids, but in 2013 we detected two hybrids in a sample of 26 fish (#4692). The sample herein suggests there has been further expansion of hybridization in this stream – we detected rainbow trout alleles in 14 of the 30 fish in this sample.

TABLES

Table 2. Frequency of the allele usually characteristic of Yellowstone cutthroat trout at the Yellowstone diagnostic marker *OclYGD106457_Garza* in samples otherwise appearing to have come from non-hybridized westslope cutthroat trout populations or populations with a slight amount of admixture.

Sample	OclYGD106457_Garza
Blum	0.273
Crevice	0.073
L Mouse	0.167
Blind Canyon	0.022
Danaher Creek	0.005
Morrison Creek	0.050
Starvation	0.087
Wildcat	0.089
Cooper Gulch	0.041
Spruce	0.147
Dirty Ike	0.064
Engle Lake	0.056
Lower Engle Lake	0.136
Upper Nelson Gulch Lake	0.075
Gordon Creek	0.083
Grave Creek	0.032
Little Park Creek	0.136
Cottonwood Creek	0.086
Prospect Creek	0.088
Fourmile Creek	0.733

Table 3. Average expected heterozygosity (He), proportion of polymorphic markers (Poly), watershed, barrier type, and sample size for westslope cutthroat trout populations in the Missouri River drainage.

Population	Watershed	Barrier	N	He	Poly
Alkali	Beaverhead	Natural - waterfall	50	0.124	0.438
Brays Canyon	Beaverhead	Anthro - demographic	105	0.062	0.281
Cottonwood	Beaverhead	Natural - waterfall	111	0.035	0.125
Jake Canyon	Beaverhead	Anthro - demographic	25	0.064	0.156
Left Fork Stone	Beaverhead	Anthro - mine	26	0.129	0.406
Reservoir	Beaverhead	Natural - intermittent	75	0.063	0.250
Upper Buffalo	Beaverhead	Anthro - demographic	25	0.098	0.313
Carpenter	Belt	Anthro - mine	35	0.175	0.516
Crawford	Belt	Natural - waterfall	54	0.123	0.438
Gold Run	Belt	Anthro - mine	69	0.092	0.313
Graveyard	Belt	Natural - waterfall	24	0.131	0.344
N. Fork Little Belt	Belt	Natural - waterfall	50	0.122	0.438
O'Brien Creek	Belt		28	0.203	0.531
American	Big Hole	Anthro - dam	29	0.159	0.531
Bear	Big Hole	Anthro - demographic	16	0.121	0.375
Bender	Big Hole	Anthro - demographic	36	0.034	0.125
Blind Canyon	Big Hole	Anthro - demographic	25	0.054	0.125
Hell Roaring	Big Hole	Natural - cascade	18	0.018	0.097
Little American	Big Hole	Anthro - demographic	30	0.056	0.258
Mono	Big Hole	Natural - cascade	15	0.095	0.344
Papoose	Big Hole	Natural - cascade	25	0.037	0.125
Plimpton	Big Hole	Natural - cascade	70	0.107	0.344
Rabbia	Big Hole	Anthro - demographic	37	0.087	0.344

Doolittle	Big Hole	Anthro - demographic	49	0.057	0.156
S. Fork N. Fork Divide	Big Hole	Anthro - dam	9	0.045	0.097
Spruce	Big Hole	Anthro - irrigation	26	0.221	0.594
Squaw	Big Hole	Anthro - demographic	26	0.101	0.500
Squaw Lake	Big Hole	Anthro - demographic	30	0.076	0.250
Twelvemile	Big Hole	Anthro - demographic	41	0.104	0.281
Little Boulder	Boulder	Anthro - mine	25	0.189	0.563
Muskrat	Boulder	Anthro - demographic	13	0.253	0.781
Wild Horse	Gallatin	Natural - cascade	30	0.036	0.125
E. Fork Big Spring	Judith	Natural - intermittent	30	0.143	0.419
N. Fork Running Wolf	Judith		30	0.171	0.531
W. Fork Cottonwood	Judith	Anthro - demographic	29	0.112	0.313
Last Chance	Madison	Natural - intermittent	19	0.057	0.188
Big Coulee	Missouri Dearborn	Natural - waterfall	32	0.194	0.531
N. Fork Highwood	Missouri Dearborn	Natural - waterfall	119	0.15	0.531
Bean	Red Rock	Anthro - irrigation	50	0.063	0.188
Bear	Red Rock	Anthro - irrigation	25	0.02	0.063
Craver	Red Rock	Anthro - demographic	25	0.066	0.188
Browns	Red Rock	Anthro - irrigation	158	0.283	0.906
E. Fork Clover	Red Rock	Natural - cascade	25	0.113	0.313
Meadow	Red Rock	Natural - intermittent	130	0.126	0.438
N. Fork Everson	Red Rock	Anthro - culvert	28	0.097	0.323
Simpson	Red Rock	Natural - intermittent	50	0.087	0.250
Painter	Red Rock	Anthro - culvert	111	0.179	0.813
S. Fork Everson	Red Rock	Natural - intermittent	27	0.067	0.281
Dark Hollow	Ruby	Anthro - demographic	50	0.055	0.344

Jack	Ruby	Natural - intermittent	143	0.05	0.156
Meadow Fork Greenhorn	Ruby	Anthro - cascade	25	0.096	0.323
Mill Gulch	Ruby		12	0.046	0.125
Ramshorn	Ruby	Anthro - irrigation	90	0.004	0.031
S. Fork Greenhorn	Ruby	Anthro - demographic	10	0.086	0.281
Fourmile	Smith		30	0.023	0.063
Lone Willow	Smith	Anthro - dam	65	0.034	0.219
N. Fork Willow	Sun	Natural - intermittent	25	0.149	0.500
Sidney	Two Medicine	Natural - waterfall	65	0.276	0.813
Midvale	Two Medicine	Anthro – dam	28	0.211	0.625
Dutchman	Upper Missouri	Anthro - demographic	50	0.183	0.563
Hall	Upper Missouri	Anthro - culvert	28	0.06	0.188
Skelly Gulch	Upper Missouri	Anthro - culvert	27	0.100	0.406
S. Fork Quartz	Upper Missouri	Anthro - culvert	40	0.027	0.125
Staubach	Upper Missouri	Anthro - demographic	32	0.021	0.094
Threemile	Upper Missouri	Anthro - dam	44	0.086	0.313
Whites	Upper Missouri	Natural - intermittent	24	0.082	0.281

FIGURES

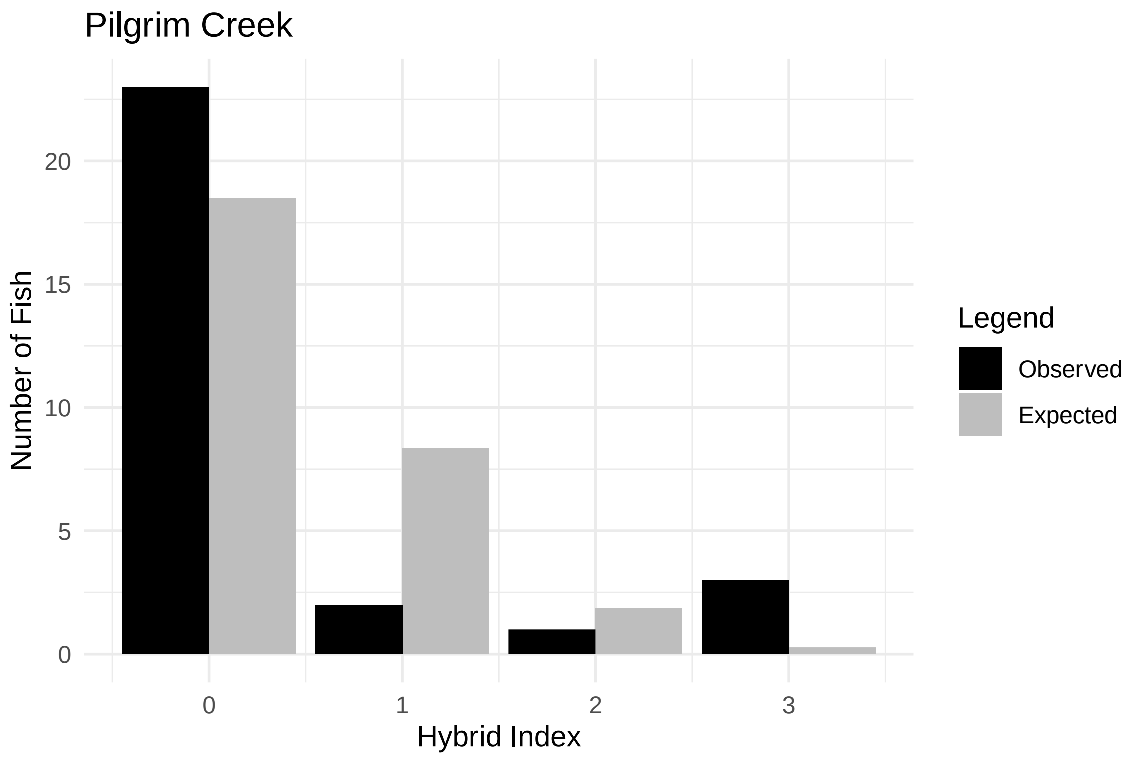

Figure 1. Observed and expected (random) distribution of hybrid indices among fish in a sample from Pilgrim Creek. Note the hybrids with higher individual rainbow trout ancestry relative to random mating expectations, and the excess of individuals that appear to be non-hybridized westslope cutthroat trout.

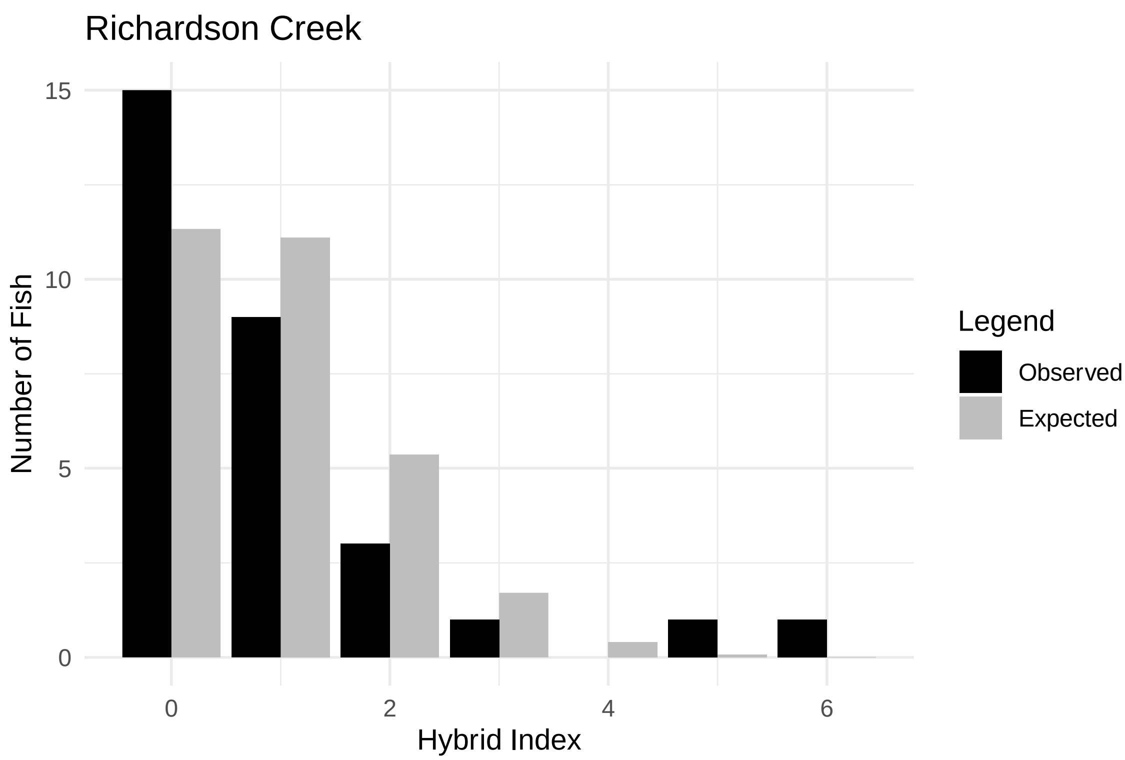

Figure 2. Observed and expected (random) distribution of hybrid indices among fish in a sample from Richardson Creek. Note the hybrids with higher individual rainbow trout ancestry relative to random mating expectations, and the excess of individuals that appear to be non-hybridized westslope cutthroat trout.

Methods and Data Analysis

We developed a 'chip' specifically for analysis of supposed westslope (*Oncorhynchus clarkii lewisi*) and Yellowstone cutthroat trout samples (*O. c. bouvieri*). This chip allows us to simultaneously genotype up to 95 single nucleotide polymorphic loci (SNPs) in 91 trout using a Fluidigm EP1 Genotyping System. Each SNP locus has only two states (alleles). Thus, considering hybridization among rainbow (*O. mykiss*), westslope cutthroat, and Yellowstone cutthroat trout a single locus can only distinguish one of the taxa from the other two. In order to address hybridization issues among these fishes, therefore, each chip contained 19 loci that differentiate rainbow from westslope cutthroat and Yellowstone cutthroat trout (rainbow markers), 20 loci that distinguish westslope cutthroat from rainbow and Yellowstone cutthroat trout (westslope markers), and 20 loci that distinguish Yellowstone cutthroat from westslope cutthroat and rainbow trout (Yellowstone markers). We investigated the diagnostic property of each marker by analyzing them in reference samples that had previously been determined to be non-hybridized westslope cutthroat, Yellowstone cutthroat, or rainbow trout by analysis of allozymes, paired interspersed nuclear elements (PINEs), a combination of insertion/deletion (indel loci) events and microsatellite loci, or two or all of these techniques.

If a sample possessed alleles characteristic of only westslope cutthroat trout at all westslope markers and had no alleles characteristic of rainbow trout at the rainbow markers or Yellowstone cutthroat trout at the Yellowstone markers, then it was considered to only contain non-hybridized westslope cutthroat trout. Evidence for potential hybridization between rainbow and westslope cutthroat trout was generally considered to be present when three criteria were met. First, the sample had to contain alleles characteristic of rainbow trout at, at least, some of the rainbow markers. Next, at least some of the westslope markers also had to be genetically variable (polymorphic). Finally, no Yellowstone cutthroat trout alleles were detected at the Yellowstone markers. In this situation, the alleles at the rainbow markers shared between westslope cutthroat and Yellowstone cutthroat trout can confidently be assigned to having originated from westslope cutthroat trout and the alleles shared between rainbow and Yellowstone cutthroat trout at the westslope markers can confidently be assigned to having originated from rainbow trout. Thus, in terms of hybridization between westslope cutthroat and rainbow trout the data set contains information from 39 diagnostic loci. Likewise, when evidence of hybridization was detected only between westslope and Yellowstone cutthroat trout (no rainbow trout alleles at rainbow markers, at least some westslope markers polymorphic, and Yellowstone cutthroat trout alleles present at, at least, some Yellowstone markers) the data set contains information from 40 diagnostic loci. When all three sets of markers were polymorphic, this generally indicates hybridization among all three taxa. In this situation, the rainbow markers (19) provide information about rainbow trout hybridization and the Yellowstone markers (20) provide information about Yellowstone cutthroat trout hybridization. The same criteria hold when considering hybridization in Yellowstone cutthroat trout, though we focus principally on the Yellowstone and westslope diagnostic markers.

An important aspect of SNPs is that they demonstrate a codominant mode of inheritance. That is, all genotypes are readily distinguishable from each other. Thus, at marker loci the genotype of individuals in a sample can directly be determined. From these data, the proportion of alleles from different taxa in the population sampled can be directly estimated at each marker locus analyzed. These values averaged over all marker loci yields an estimate of the proportion of alleles in the population that can be attributed to one or more taxa (proportion of admixture). In samples showing evidence of hybridization among all three taxa, we estimated the amount of rainbow trout admixture using only the 19 rainbow markers and the amount of Yellowstone cutthroat trout admixture using only the 20 Yellowstone markers. The amount of westslope cutthroat trout admixture was then estimated by subtracting the sum of the former two values from one. We used this procedure so the estimates would sum to one. Because of sampling error, it is unlikely that all three estimates from the marker loci would sum to one.

When evidence of hybridization is detected, the next issue to address is whether or not the sample appears to have come from a hybrid swarm. That is, a random mating population in which the alleles of the hybridizing taxa are randomly distributed among individuals such that essentially all of them are of hybrid origin. A common, but not absolute, attribute of hybrid swarms is that allele frequencies are similar among marker loci because their presence can all be traced to a common origin or origins. Thus, one criterion we used for the assessment of whether or not a sample appeared to have come from a hybrid swarm was whether or not the allele frequencies among diagnostic loci reasonably conformed to homogeneity using contingency table chi-square analysis.

In order to determine whether or not alleles at the marker loci were randomly distributed among the fish in a sample showing evidence of hybridization, we calculated a hybrid index for each fish in the sample. The hybrid index for an individual was calculated as follows. At each marker locus, an allele characteristic of the native taxon was given a value of zero and an allele characteristic of the non-native taxon a value of one. Thus, at a single diagnostic locus the hybrid index for an individual could have a value of zero (only native alleles present, homozygous), one (both native and non-native alleles present, heterozygous), or two (only non-native alleles present, homozygous). These values summed over all diagnostic loci analyzed yields an individual's hybrid index. Considering westslope cutthroat and rainbow trout, therefore, non-hybridized westslope cutthroat trout would have a hybrid index of zero, non-hybridized rainbow trout a hybrid index of 78, F_1 (first generation) hybrids would be heterozygous at all marker loci and have a hybrid index of 39, and post F_1 hybrids could have values ranging from zero to 78. The same patterns holds for Yellowstone cutthroat trout. The distribution of hybrid indices among the fish in a sample was statistically compared to the expected random binomial distribution based on the proportion of admixture estimated from the allele frequencies at the diagnostic loci. If the allele frequencies appeared to be statistically homogeneous among the marker loci and the observed distribution of hybrid indices reasonably conformed to the expected random distribution, then the sample was considered to have come from a hybrid swarm.

In old or hybrid swarms with small effective population size, allele frequencies at marker loci can randomly diverge from homogeneity over time because of genetic drift. In this case, however, the observed distribution of hybrid indices is still expected to reasonably conform to the expected random distribution. Thus, if the allele frequencies were statistically heterogeneous among the marker loci in a sample but, the observed distribution of hybrid indices reasonably conformed to the expected random distribution the sample was also considered to have come from a hybrid swarm.

The strongest evidence that a sample showing evidence of hybridization did not come from a hybrid swarm is failure of the observed distribution of hybrid indices to reasonably conform to the expected random distribution. The most likely reasons for this are that the population has only recently become hybridized or the sample contains individuals from two or more populations with different amounts of admixture. At times, previous samples and the distribution of genotypes at marker loci and the observed distribution of hybrid indices can provide insight into which of the latter two factors appears mainly responsible for the nonrandom distribution of the alleles from the hybridizing taxa among individuals in the sample. At other times, the distribution of genotypes at marker loci and the observed distribution of hybrid indices may provide little or no insight into the cause of the nonrandom distribution of alleles among individuals. The latter situation is expected to be fairly common as the two factors usually responsible for the nonrandom distribution of alleles are not necessarily mutually exclusive. Regardless of the cause, when alleles at the marker loci do not appear to be randomly distributed among individuals in a sample, estimating the amount of admixture often has little if any biological meaning and, therefore, is generally not reported. An exception would be when one is interested in comparing the mean percentage of rainbow trout alleles among the fish in a temporal sequence of samples or when samples were collected from different reaches of a stream.

Failure to detect evidence of hybridization in a sample does not necessarily mean the fish in it are non-hybridized because there is always the possibility that we would not detect evidence of hybridization because of sampling error. When no evidence of hybridization was detected in a sample, we assessed the likelihood the sample contains only non-hybridized westslope or Yellowstone cutthroat trout by determining the chances of not detecting as little as a 0.5 percent genetic contribution of a non-native taxon to a hybrid swarm. This is simply 0.995^{2NX} where N is the number of fish in the sample and X is the number of marker loci analyzed.

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Table1

SNP loci that differentiate rainbow from westslope and Yellowstone cutthroat trout (rainbow markers), westslope cutthroat from rainbow and Yellowstone cutthroat trout (westslope markers), and Yellowstone cutthroat trout from westslope cutthroat and rainbow trout (Yellowstone markers).

Rainbow Markers

	Taxa and characteristic alleles		Reference
	Rainbow	Westslope/Yellowstone	
OmyRD_RAD_29252_Hoh	1	2	Amish et al. 2012
OmyRD_RAD_77157_Hoh	1	2	
OmyRD_RAD_30378_Hoh	1	2	Amish et al. 2012
OclRD_P53T7R1_Har	1	2	Amish et al. 2012
OmyRD_RAD_30423_Hoh	1	2	Harwood and Phillips 2011
OmyRD_RAD_59515_Hoh	1	2	Amish et al. 2012
OclRD_Thymo_320Kal	1	2	Amish et al. 2012
OmyRD_RAD_48301_Hoh	1	2	Kalinowski et al. 2011
OmyRD_RAD_49759_Hoh	1	2	Amish et al. 2012
OclRD_P53T7R2_Har	1	2	Amish et al. 2012
OmyRD_URO_302May	1	2	Harwood and Phillips 2011
OmyRD_RAD_20663_Hoh	1	2	Finger et al. 2009
OmyRD_RAD_51740_Hoh	2	1	Amish et al. 2012
OmyRD_RAD_22111_Hoh	1	2	Amish et al. 2012
OmyRD_RAD_55820_Hoh	2	1	Amish et al. 2012
OmyRD_RAD_5666_Hoh	2	1	Amish et al. 2012
OmyRD_F5_136May	1	2	Amish et al. 2012
OmyRD_RAD_42014_Hoh	2	1	Finger et al. 2009
OmyRD_RAD_54584_Hoh	2	1	Amish et al. 2012

Table 1-continued

Westslope Markers

	Taxa and characteristic alleles		Reference
	Westslope	Rainbow/Yellowstone	
OclWD_CLK3W5_Har	2	1	Harwood and Phillips 2011
OclWD_CLK3W1_Har	2	1	Harwood and Phillips 2011
OclWD101119_Garza	2	1	Campbell et al. 2012
OmyWD_RAD_76689_Hoh	2	1	Amish et al. 2012
OclWD_114315L _Garza	2	1	Campbell et al. 2012
OclWD_Tnsf_387Kal	2	1	Kalinowski et al. 2011
OmyWD_RAD_55391_Hoh	2	1	Amish et al. 2012
OclWD_P53_307Kal	2	1	Kalinowski et al. 2011
OclWD111312_Garza	2	1	Campbell et al. 2012
OclWD_107031L _Garza	2	1	Campbell et al. 2012
OclWD_PrLcW1_Har	2	1	Harwood and Phillips 2011
OmyWD_RAD_54516_Hoh	2	1	Amish et al. 2012
OclWD_105075L_Garza	2	1	Campbell et al. 2012
OmyWD_RAD_52968_Hoh	2	1	Amish et al. 2012
OclWD_114336_Garza	1	2	Campbell et al. 2012
OclWD103713_Garza	2	1	Campbell et al. 2012
OclWD107074_Garza	2	1	Campbell et al. 2012
OclWD109651_Garza	2	1	Campbell et al. 2012
OclWD_129170L _Garza	1	2	Campbell et al. 2012
OclWD_ppie_32NC	1	2	Campbell et al. 2012

Table 1-continued

Yellowstone Markers

	Taxa and characteristic alleles		Reference
	Yellowstone	Westslope/Rainbow	
OclYD_CLK3Y1_Har	2	1	Harwood and Phillips 2011
OclYGD100974_Garza	2	1	Campbell et al. 2012
OclYGD110571_Garza	2	1	Campbell et al. 2012
OclYSD117432_Garza	2	1	Campbell et al. 2012
OclYGD127236_Garza	2	1	Campbell et al. 2012
OclYGD112820_Garza	2	1	Campbell et al. 2012
OclYGD104216_Garza	2	1	Campbell et al. 2012
OclYGD113600_Garza	2	1	Campbell et al. 2012
OclYSD129870_Garza	2	1	Campbell et al. 2012
OclYGD104569_Garza	2	1	Campbell et al. 2012
OclYGD117286_Garza	2	1	Campbell et al. 2012
OclYGD117370_Garza	2	1	Campbell et al. 2012
OclYSD107607_Garza	2	1	Campbell et al. 2012
OclYGD106457_Garza	2	1	Campbell et al. 2012
OclYSD106367_Garza	1	2	Campbell et al. 2012
OclYGD107031_Garza	1	2	Campbell et al. 2012
OclYGD106419_Garza	1	2	Campbell et al. 2012
OclYSD123205_Garza	1	2	Campbell et al. 2012
OclYGD109525_Garza	1	2	Campbell et al. 2012
OclYSD113109_Garza	1	2	Campbell et al. 2012

Table 2

Reference samples used for identification of marker SNPs among westslope cutthroat, rainbow, and Yellowstone cutthroat trout. Taxa: WCT=westslope cutthroat trout, YCT=Yellowstone cutthroat trout, IRT=redband rainbow trout, CRT=coastal rainbow trout. N=sample size.

Sample	Taxa	N	Location
Washoe Park State Trout Hatchery	WCT	12	Anaconda, Montana
Big Foot Creek	WCT	2	Upper Kootenai River, Montana
Runt Creek	WCT	3	Yaak River, Montana
Hawk Creek	WCT	2	North Fork Flathead River, Montana
Werner Creek	WCT	3	North Fork Flathead River, Montana
Morrison Creek	WCT	3	Middle Fork Flathead River, Montana
Sixmile Creek	WCT	3	Swan River, Montana
South Fork Jocko River	WCT	3	Lower Flathead River, Montana
Cottonwood Creek	WCT	3	Upper Clark Fork River, Montana
Copper Creek	WCT	2	Flint-Rock Creek, Montana
Gillispie Creek	WCT	3	Flint-Rock Creek, Montana
Davis Creek	WCT	4	Bitterroot River, Montana
Humbug Creek	WCT	2	Blackfoot River, Montana
Ringeye Creek	WCT	2	Blackfoot River, Montana
Flat Creek	WCT	3	Middle Clark Fork River, Montana
McGinnis Creek	WCT	3	Lower Clark Fork River, Montana
Bear Creek	WCT	1	Red Rock River, Montana
McVey Creek	WCT	1	Big Hole River, Montana
McClellan Creek	WCT	1	Upper Missouri River, Montana
Yellowstone River State Trout Hatchery-Goose Lake	YCT	6	Big Timber, Montana
Slough Creek	YCT	4	Yellowstone River, Montana
Lake Koocanusa	IRT	4	Upper Kootenai River, Montana
North Fork Yahk River	IRT	5	Yahk River, British Columbia
Jocko River State Trout Hatchery Arlee Rainbow	CRT	7	Arlee, Montana