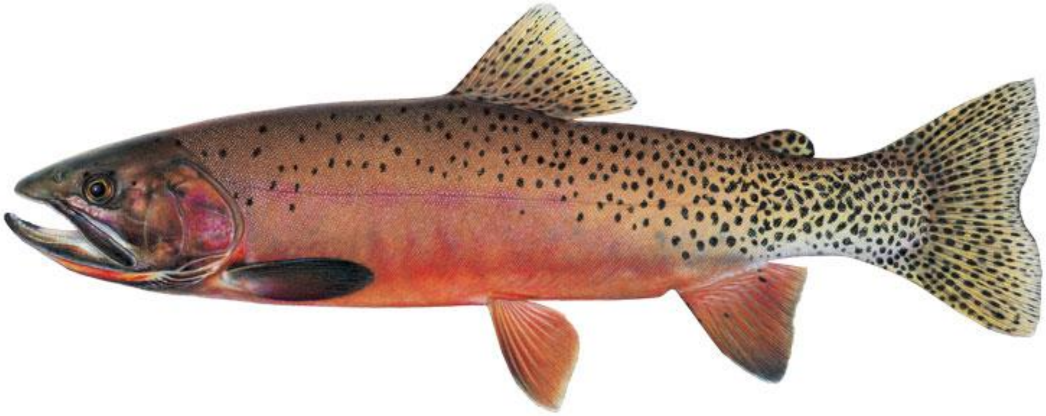
Northcentral Montana Westslope Cutthroat Trout Restoration Project



2020 Cost Share Report

USFS-Challenge Cost Share Agreements

13-CS-11011500-016 and 17-CS-11011500-045

State Wildlife Grant # 3482

MoTAC Project # 2020-5

Prepared By:

Alex Poole

Montana Fish, Wildlife, and Parks

Region 4

Great Falls, MT

December 2020

Table of Contents

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).

Westslope Cutthroat Trout were listed as a “State Species of Concern” by Montana Fish, Wildlife and Parks (MFWP) in 1972 because of the loss of nonhybridized populations and extensive reduction of occupied habitat. A petition was made to list the WCT status as threatened under the Endangered Species Act (ESA) in 1997.

In 1999, a WCT Conservation Agreement (WCA) was developed by Montana with the assistance of a technical committee (formed in 1994) and a steering committee (formed in 1996), and this agreement was signed by state, federal, and non-governmental organizations. In 2000, a plan was implemented to complete the goals of the WCA and restore WCT in northcentral Montana (Tews et al. 2000).

In April of 2000, following an extensive status review, the U.S. Fish and Wildlife Service (USFWS) determined that Westslope Cutthroat Trout were “not warranted” for federal listing. That finding was challenged in federal court, and the court remanded the not warranted finding back to the USFWS for additional review. In 2003, after additional review, the USFWS determined that WCT are not likely to become a threatened or endangered species in the foreseeable future, therefore listing was not warranted.

A Federal Challenge Cost Share Agreement was established in 2001 between MFWP and the United States Forest Service (USFS) to implement and fund WCT restoration (Tews et al. 2000) as outlined by the WCA. Funding for the 2015 WCT restoration project was provided by the Environmental Protection Agency (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 (MFWP) provided additional funding for WCT restoration. At the November 2019 Missouri River Technical Advisory Committee (MoTAC) meeting, MFWP was awarded $13,500 from Northwestern Energy to fund a fisheries technician to work directly with the MFWP native species biologist on the Northcentral Montana WCT Restoration Project. This document specifically addresses work performed under the 2020 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, and the upper Missouri-Dearborn River (Figure 1).

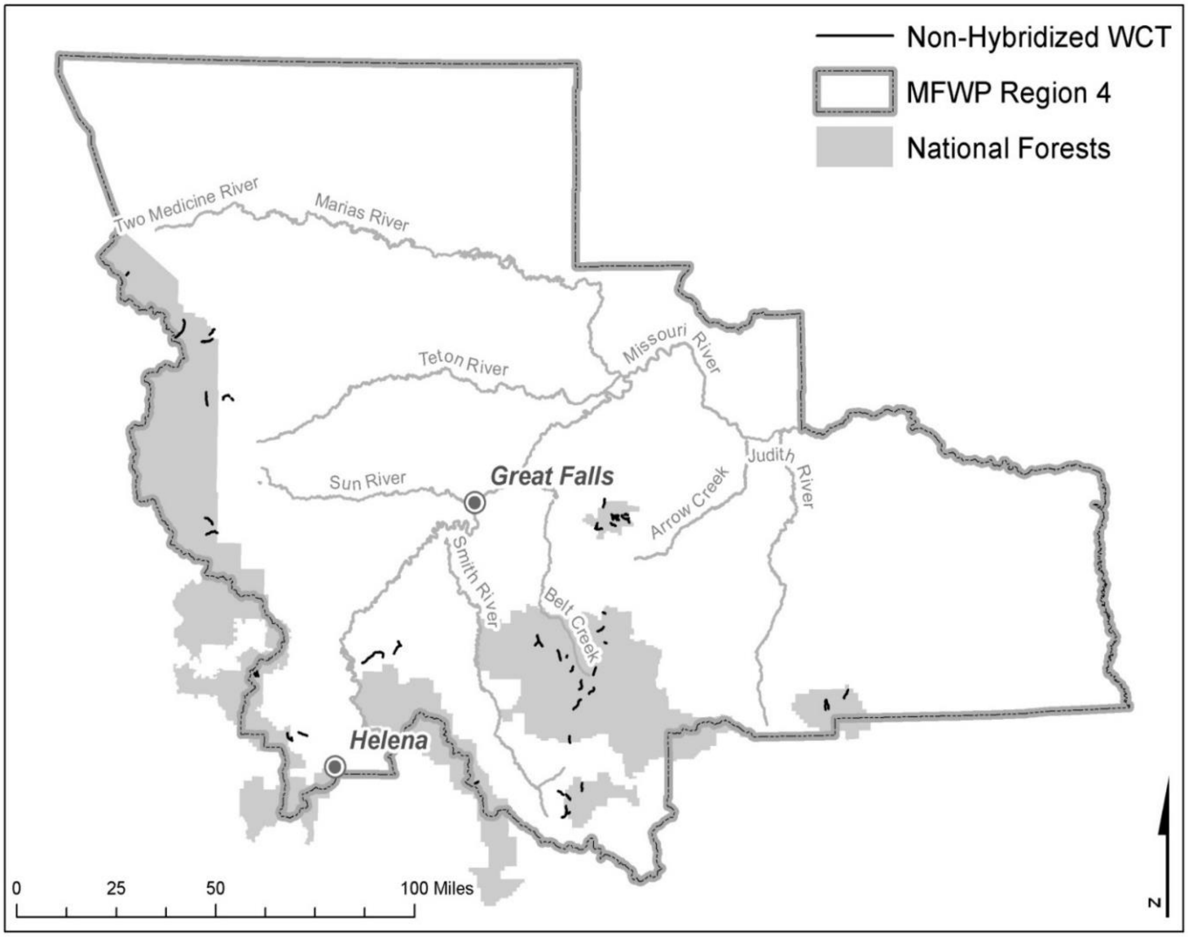


Figure 1. Study area in northcentral Montana with non-hybridized WCT populations (indicated in bold black).

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). Fyke nets were used to sample fish from lotic habitats. If genetic samples were needed tissue 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 (MFWP 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 2020 field season include: barrier maintenance, nonnative species suppression and removal, wild WCT transfers to restored waters and extensive population and genetic monitoring.

Restoration Efforts in Northcentral Montana

The scope of the work completed by MFWP in 2020 is described in the following maps, text, and histograms. The USFS and MFWP 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, and Upper Missouri-Dearborn River.

I. Arrow Creek Subbasin

Cottonwood Creek

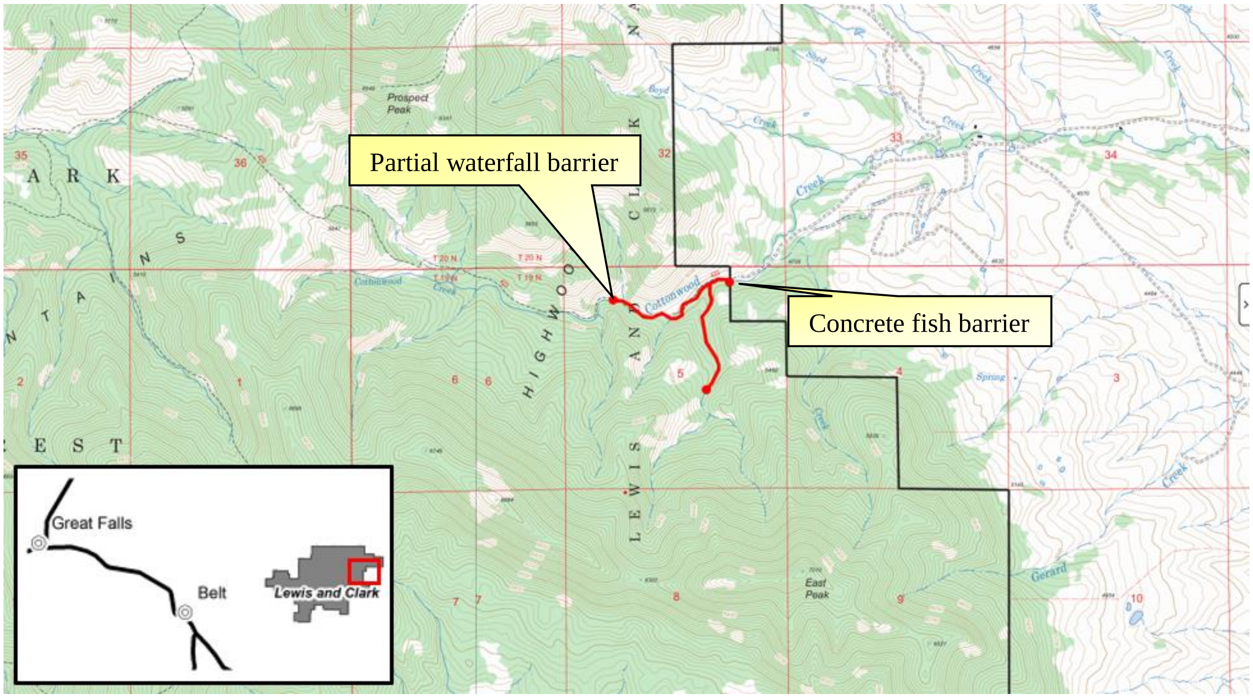


Figure 2. Cottonwood Creek in the Arrow Creek subbasin. Stream segments delineated in red indicate areas sampled in 2020.

Background
Cottonwood Creek contains a nonhybridized population of WCT partially protected by a natural waterfall 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 initially 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. There were no Brook Trout detected above the natural waterfall (47.44354, -110.48680) located approximately one mile upstream of the barrier when it was surveyed in 2016 and spot shocked in 2018 and 2019.

2020 Removal
A single pass electrofishing removal effort was performed on Cottonwood Creek on September 30th and October 1st of 2020. 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 age-1 and older were recorded. A total of 270 WCT were collected in the mainstem of Cottonwood Creek between the barriers and 209 WCT were collected in the 1st tributary. No Brook Trout were detected during the 2020 removal

effort. Multiple age classes of WCT were present throughout the area sampled and age-0 WCT were abundant in the 1st tributary and mainstem near the partial waterfall barrier.

II. Belt Creek Subbasin

Carpenter Creek

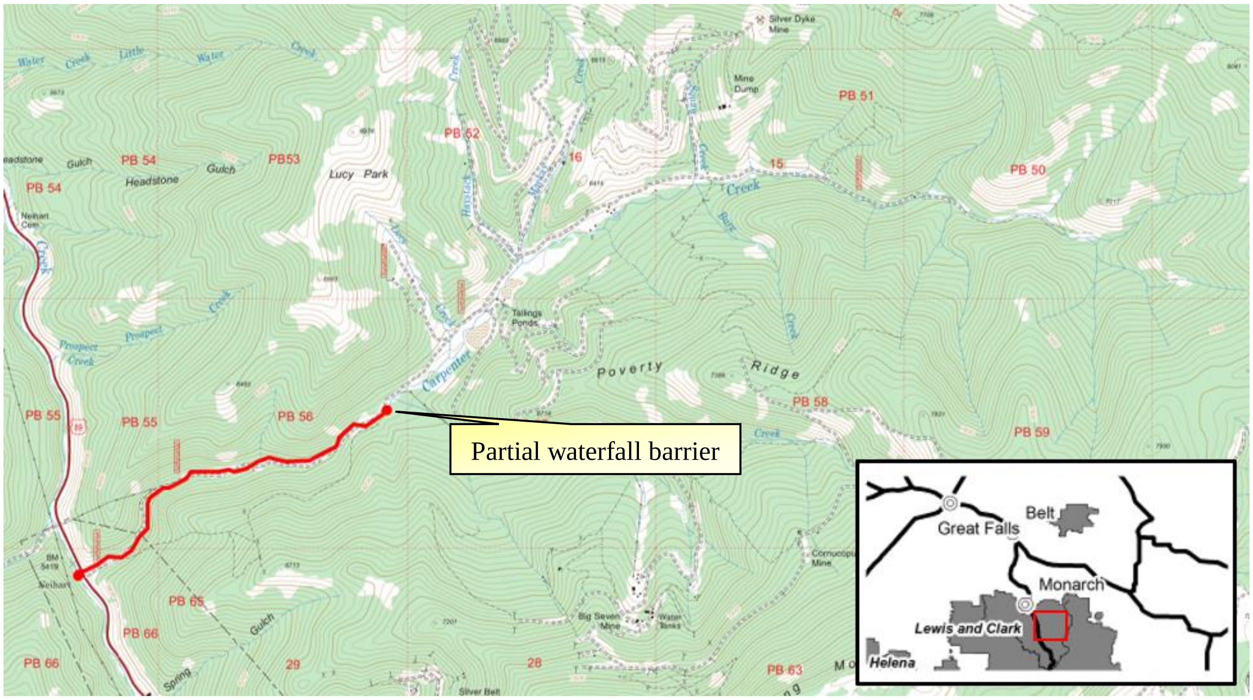
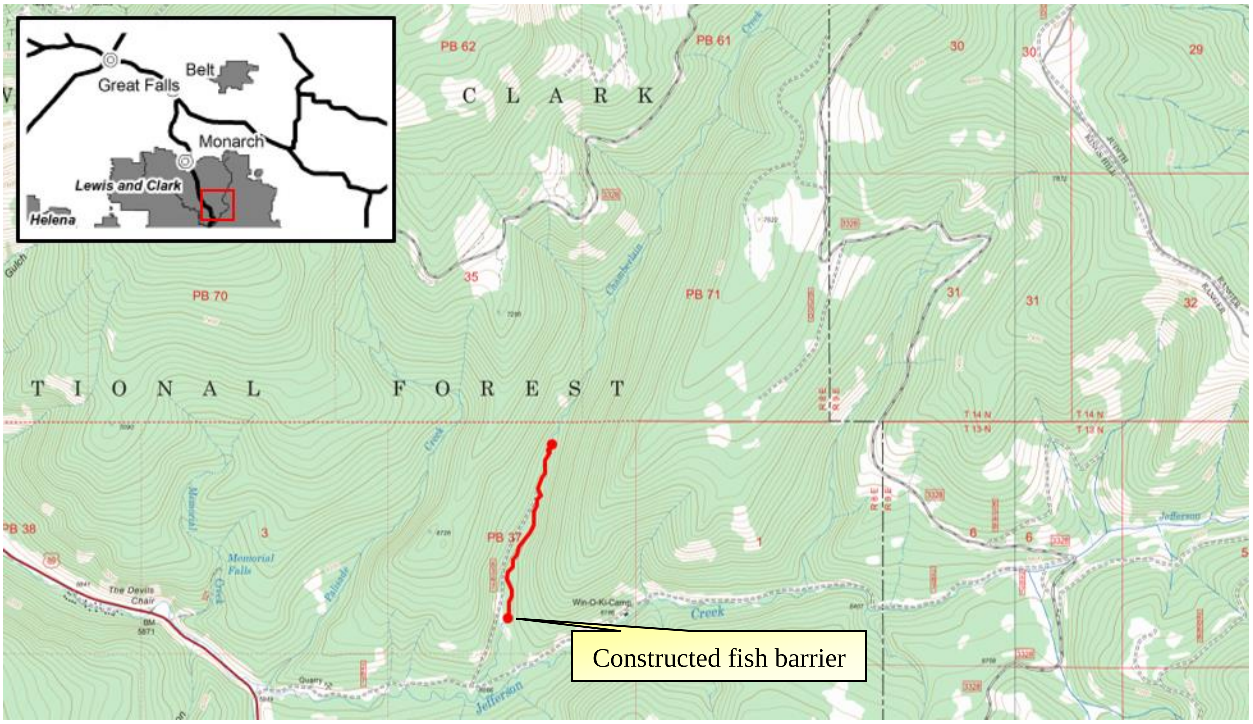


Figure 3. Carpenter Creek in the Belt Creek subbasin. The stream segment delineated in red indicates the area sampled in 2020.

Background
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 3). During past sampling efforts this lower section has always been devoid of fish; 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 removed annually from this section since 2015. In 2016 seven nonnative fish were removed, 4 in 2017, 5 in 2018, and 1 in 2019. Carpenter Creek WCT populations were estimated and genetic samples obtained in 2018. A total of 591 fish 100 mm and greater were estimated in Carpenter Creek over approximately 2.5 kilometers of occupied habitat.

2020 Monitoring
Lower Carpenter Creek was electrofished from the confluence of Belt Creek to the partial waterfall barrier to remove nonnative trout in a single pass removal effort on August 3rd and 4th, 2020. Two fish were removed during this effort: an apparent hybrid WCT located at the US89 highway crossing and a Brook Trout located just downstream from the first FS RD 3323 crossing. No other fish were observed, and it was noted that aquatic macroinvertebrates were rare.

Chamberlain Creek



Constructed fish barrier

Figure 4. Chamberlain Creek in the Belt Creek subbasin. The stream segment delineated in red indicates the area sampled in 2020.

Background

A barrier was built on Chamberlain Creek in 2002 to protect a slightly hybridized population of WCT. The barrier design requires maintenance several times a year to remove build-up of debris on a grate that is designed to block fish from jumping over the edge of the barrier. Large rocks were placed just beyond the base of the concrete splash pad in the fall of 2018 to create a continuation of the pad to reduce the probability of a fish using the pool to jump over the barrier when the grate is blocked in the future. This water continues to be monitored for nonnative fish invasions. A 200-400 m section of stream immediately upstream of the barrier was sampled in 2014, 2015, 2017, and 2018 to monitor the stream for presence of nonnative fish. No Brook Trout have been found above the barrier to date.

2020 Monitoring

Chamberlain Creek was visited on June 16th and October 7th of 2020 to remove debris from the grate on the constructed fish barrier. On August 17th, 2020 Chamberlain Creek was electrofished from the constructed fish barrier to a point approximately 1.26 km upstream to monitor for the presence of nonnative fish (Figure 4). Only WCT and Rocky Mountain Sculpin *Cottus bondi* were observed in this effort. A total of 113 WCT were collected and a subsample of 31 were measured to develop a length frequency histogram (Figure 5). Densities of WCT appeared to be low, especially in proximity to the barrier. However, relative condition was noted to be excellent. Rocky Mountain Sculpin were only observed in the lower drainage.

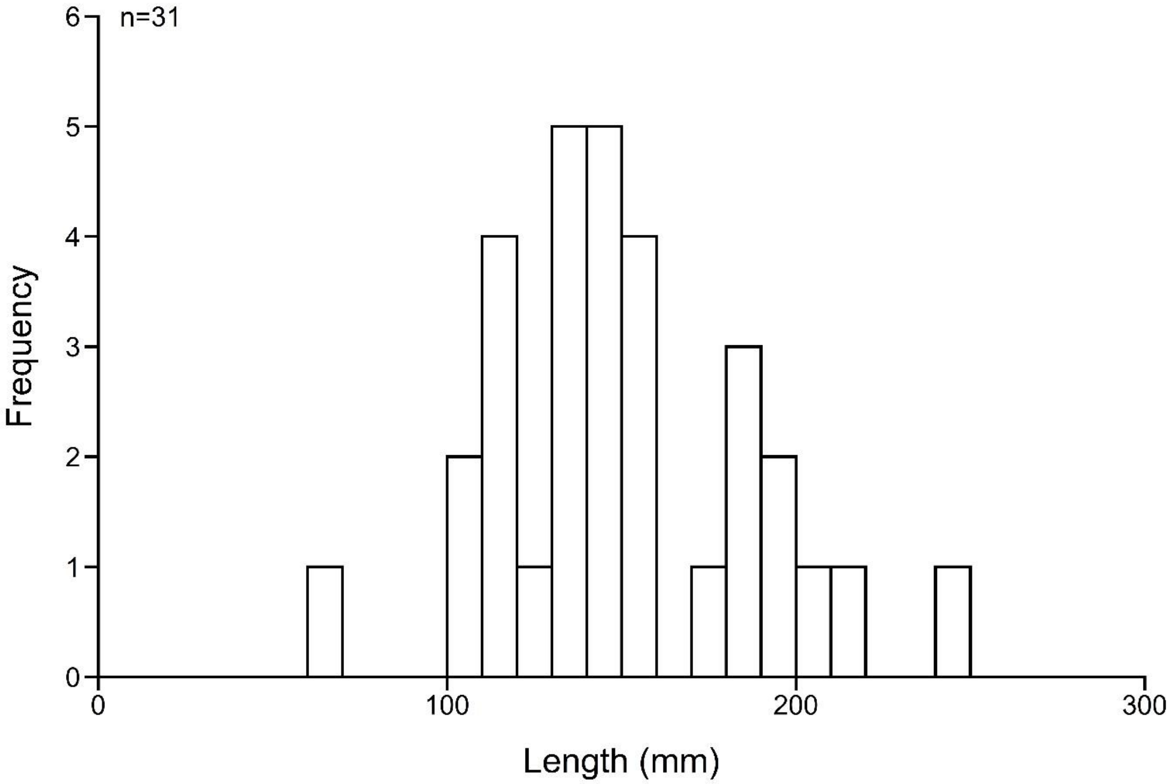


Figure 5. Length-frequency histogram of WCT subsampled from Chamberlain Creek in 2020.

Dry Fork Belt Creek and Tributaries

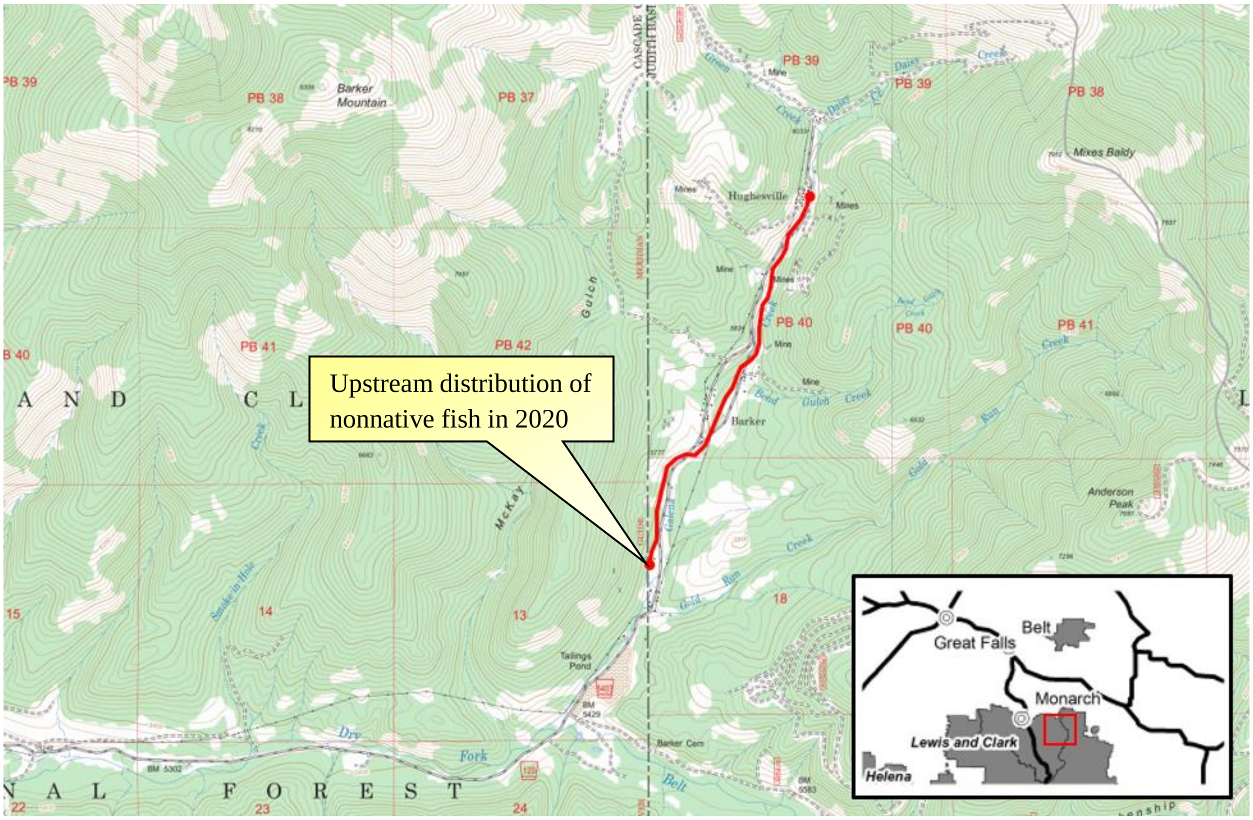


Figure 6. Galena Creek in the Belt Creek subbasin. The stream segment delineated in red indicates the area sampled in 2020.

Background

The Environmental Protection Agency (EPA) listed the Dry Fork Belt Creek area as a federal superfund site in 2000 because of mining wastes left behind after a century of lead silver ore mining. Presently mining effluent is leaching from the mining operations significantly lowering the pH levels creating a toxic environment for aquatic organisms, particularly in the Galena Creek drainage. Annual monitoring of streams in the Barker-Hughesville area has tracked mine restoration effects on adjacent fish populations. The data collected from monitoring shows population numbers appear to be similar from year to year. However, as reclamation continues, water quality is expected to improve allowing nonnative fish to expand their distribution within the drainage.

Several tributaries of Dry Fork Belt Creek contain populations of WCT that are >99% genetically pure including Gold Run Creek, Bender Creek, Spruce Creek, Sawmill Creek, Henn Gulch, Villars Creek, Charcoal Creek and upper Dry Fork Belt Creek. These small populations vary in their level of security to nonnative trout invasion with several currently containing sympatric Brook Trout.

A fish barrier was constructed in the summer of 2015 near the mouth of Dry Fork Belt Creek. The funding for the structure was provided from the Resource Development Grant Program through Cascade Conservation District, Northwestern Energy, Montana Fish, Wildlife and Parks Future Fisheries, and the EPA. Pre-piscicide treatment work began in 2016 and is ongoing.

Mine effluent from the Barker-Hughesville area has historically restricted fish distribution in the Galena Creek drainage. Mine tailings in the headwaters of the drainage are actively being remediated with bentonite clay to filter the contaminated water before it reaches the stream. As remediation continues, water quality is expected to improve facilitating the upstream movement of nonnative trout. Galena Creek has been electrofished annually since 2017 to evaluate the upstream distribution of fish below the acid runoff. In the 2017-2019 period, the uppermost distribution of fish in the drainage appeared to be near the confluence of Bend Gulch Creek.

2020 Monitoring

Galena Creek was monitored for the presence of nonnative fish by a single pass electrofishing effort performed on July 15th, 2020. Galena Creek was sampled from just upstream of the confluence of Gold Run Creek to the top of the Block P Mine Complex site (USEPA 2016; Figure 6). Multiple Brook Trout were collected just upstream of the confluence of Gold Run Creek (47.06227, -110.64470). However, unlike previous years sampling, no other fish were observed upstream of this point.

Graveyard Gulch

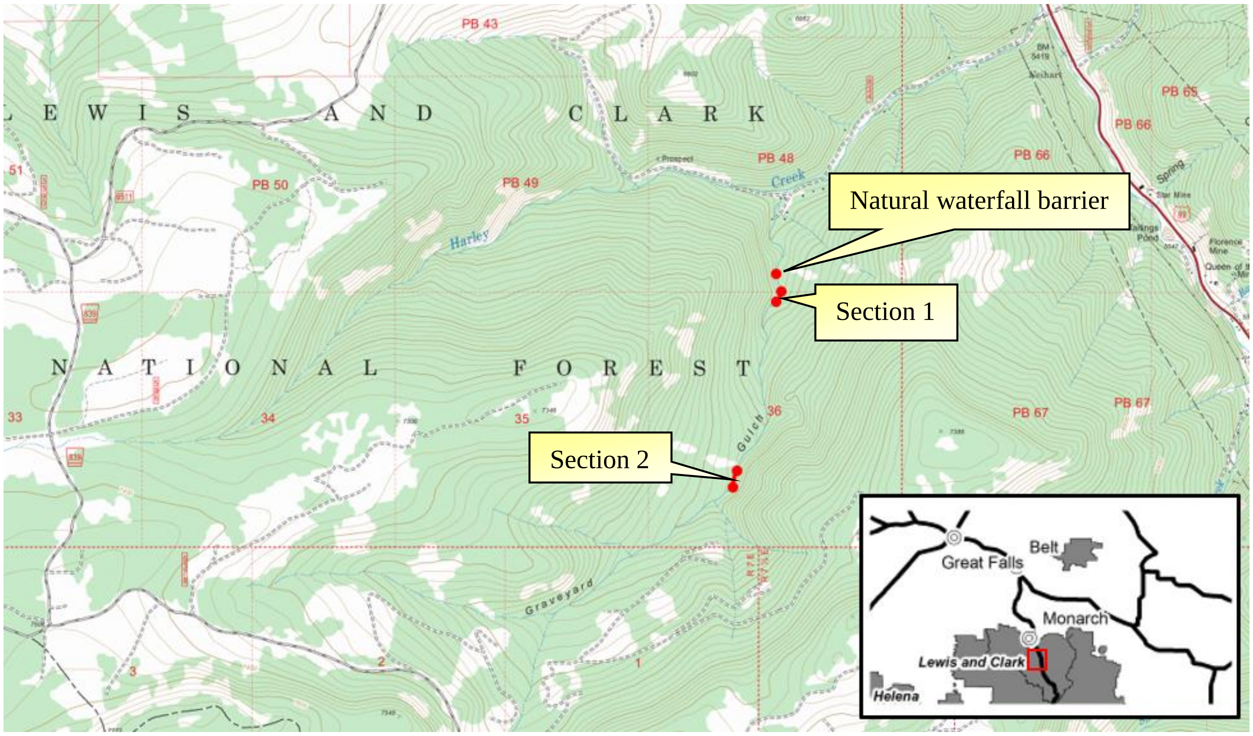


Figure 7. Graveyard Gulch in the Belt Creek subbasin. The stream segments delineated in red indicate the areas sampled in 2020.

Background

Graveyard Gulch contains a nonhybridized population of WCT protected from nonnative trout invasion by a small waterfall barrier located about 0.63 km above its confluence with Harley Creek (46.93949, -110.77791; Figure 7). To date, no Brook Trout have been found upstream of the waterfall during periodic genetics monitoring. However, Brook Trout are present immediately downstream of the waterfall barrier. The drop of the waterfall appears to lack sufficient height to be secure under all flow conditions, yet nonnative trout appear to be precluded from upstream movement. The buildup of sediment and rocks directly downstream of the waterfall were removed in 2016, 2018, and 2019 to reduce the depth and size of the jump pool with intent to further reduce the likelihood of nonnative trout invasion.

2020 Monitoring

The Graveyard Gulch WCT population was monitored on August 18th, 2020 to assess their current status. Two 100 m fish population estimation sections were established; one located just above the natural waterfall barrier and one located about a mile upstream. Multiple pass depletion methods were used estimate population abundance. WCT abundance was similar at both the upper and lower section on Graveyard Gulch with an estimated 300 and 310 fish/km, respectively (Figure 8). WCT biomass was slightly greater at the lower section when compared to the upper section.

On August 25th, 2020 the plunge pool below the Graveyard Gulch waterfall barrier was again reduced by removing the buildup of sediment and rocks downstream to reduce the likelihood of nonnative trout invasion.

Graveyard Gulch —NATIVE TROUT POPULATION SURVEY

1. General Information— Date: **August 18, 2020** Biologist: **A. Poole, E. Archer**
2. Stream Information—
   Name, section, county: **Graveyard Gulch, 36, 48, Cascade**
3. Survey Site Information (see attached map)—
   Upstream range of native trout (general description and GPS): **2.91 km above natural waterfall barrier (estimated; 46.92010, -110.79640)**
   Downstream range of native trout (general description and GPS): **natural waterfall barrier (46.93949, -110.77791)**
   Location (GPS) and description of barriers: **natural waterfall barrier (46.93949, -110.77791)**
   Stream Length—Occupied habitat: **2.91 km (1.81 mi)** Available habitat: **2.91 km (1.81 mi)**
   Survey method & equipment: **backpack battery electrofisher; two-pass depletion**
   Survey sites (general description and UTM)—
   Section 1: **130 m upstream of natural waterfall barrier; 46.93837 -110.77747**
   Section 2: **1400 m upstream of natural waterfall barrier; 46.92821 -110.78116**

Parameter	**Section 1**	**Section 2**
Section length (m)	100 m	100 m
Mean stream width (m) (n)	3.17 m (10)	3.14 m (10)
Section area (hectares)	0.032 ha	0.031 ha
WCT		
Removal Pattern	24 6	19 8
Population estimate	31 (±3)	30 (±8)
Capture probability	0.750	0.579
Mean length (mm) (n)	138 (30)	133 (27)
Mean weight (g) (n)	31 (30)	25 (27)
Mean KTL (n)	1.09 (30)	0.89 (27)
Number fish per km (95 % CI)	310 (±30)	300 (±80)
Number fish per ha (95 % CI)	969 (±94)	967 (±258)
Biomass (kg per ha) (95 % CI)	30 (±3)	24 (±7)

Figure 8. Graveyard Gulch fish population estimation results.

Middle Fork Little Belt Creek

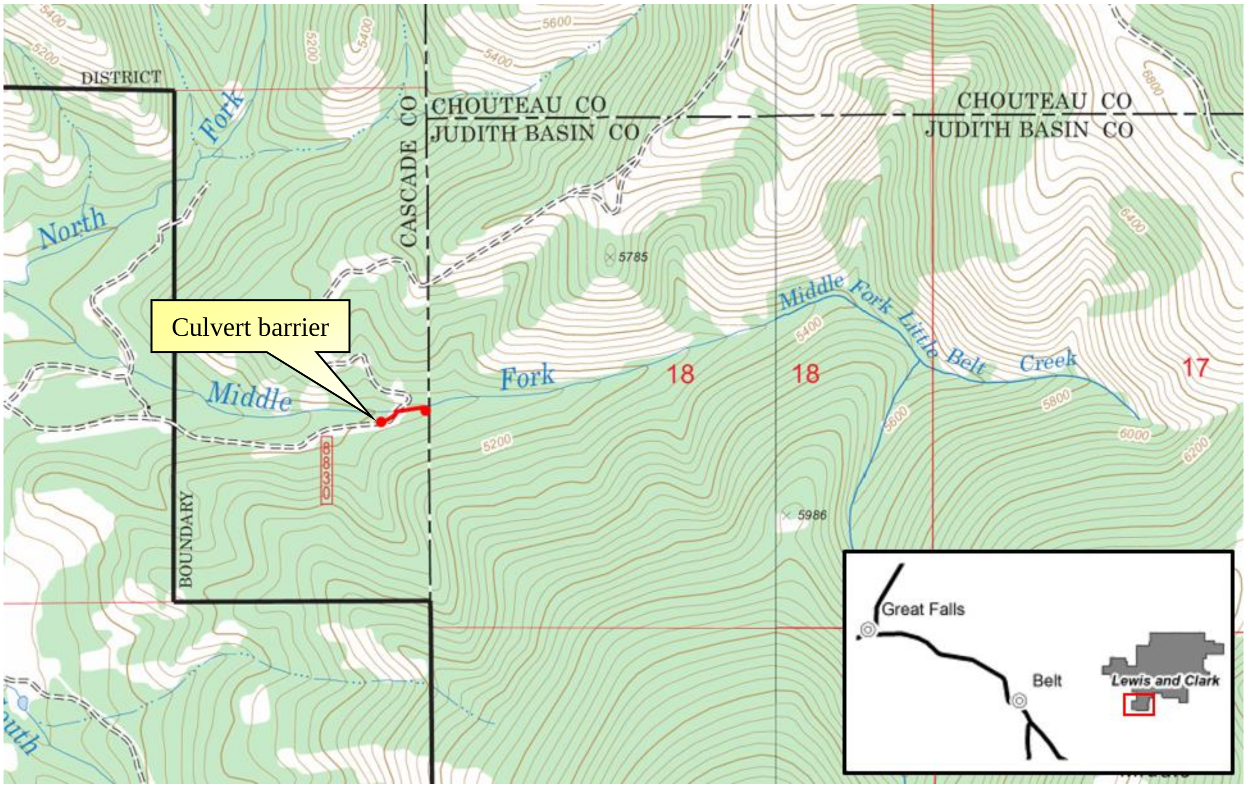


Figure 9. Middle Fork Little Belt Creek in the Belt Creek subbasin. The stream segment delineated in red indicates the area sampled in 2020.

Background

Middle Fork Little Belt Creek contains a slightly hybridized population of WCT protected from nonnative trout invasion by a culvert barrier at the FS RD 8830 crossing. A sympatric Brook Trout population was mechanically removed from 1997 to 2004. Since 2004, no Brook Trout have been detected above the barrier during monitoring efforts.

2020 Monitoring

A 150 m section of Middle Fork Little Belt Creek was electroshocked above the culvert barrier to monitor for nonnative trout presence on June 24th, 2020 (Figure 9). Only WCT were observed (n=14), no Brook Trout were detected.

O’Brien Creek

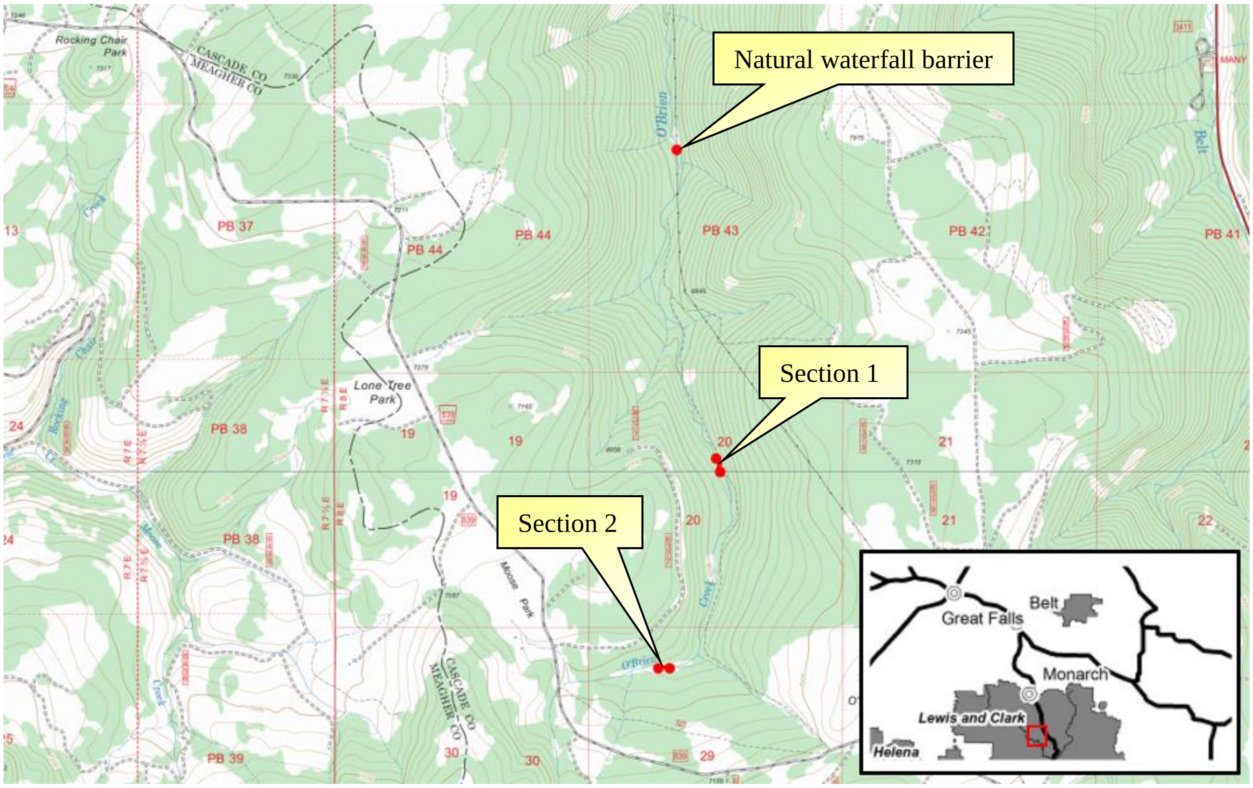


Figure 10. O’Brien Creek in the Belt Creek subbasin. The stream segments delineated in red indicate the areas sampled in 2020.

Background

O’Brien Creek contains a nonhybridized population of WCT above a natural waterfall barrier located upstream of the Neihart municipal water supply reservoir (Figure 10). This populations genetic status has been monitored periodically since 1997. O’Brien Creek has previously served as a source population for WCT restoration efforts in Tyrell Creek (Smith River subbasin).

2020 Monitoring

The O’Brien Creek WCT population was monitored on July 21st and 28th, 2020 to assess their current status. Two 100 m sections were established to conduct fish population estimates; one located approximately 2.15 km above the natural waterfall barrier and one 0.7 km downstream of the FS RD 839 crossing. Multiple pass depletion methods were used to estimate population abundance. WCT abundance was higher at the lower population estimate section when compared to the upper section with an estimated 280 and 190 fish/km, respectively (Figure 11). Tissue samples were collected from 28 WCT collected during fish population estimates for genetic analysis.

O'Brien Creek —NATIVE TROUT POPULATION SURVEY

1. General Information— Date: **July 21, 28, 2020** Biologist: **A. Poole**
2. Stream Information—
   Name, section, county: **O'Brien Creek, 20, 29, Cascade**
3. Survey Site Information (see attached map)—
   Upstream range of native trout (general description and GPS): **FS RD 839 crossing (estimated; 46.86250, -110.74420)**
   Downstream range of native trout (general description and GPS): **natural waterfall barrier (46.89330, -110.73450)**
   Location (GPS) and description of barriers: **natural waterfall barrier (46.89330, -110.73450)**
   Stream Length—Occupied habitat: 4**.54 km (2.82 mi)** Available habitat: 7**.13 km**[1] **(4.43 mi)**
   Survey method & equipment: **backpack battery electrofisher; two-pass depletion**
   Survey sites (general description and UTM)—
   Section 1: **2.24 km upstream of natural waterfall barrier; 46.87580, -110.73130**
   Section 2: **657 m downstream of FS RD 839 crossing; 46.86380, -110.73510**

Parameter	**Section 1**	**Section 2**
Section length (m)	100 m	100 m
Mean stream width (m) (n)	2.75 m (10)	2.02 m (10)
Section area (hectares)	0.028 ha	0.020 ha
WCT		
Removal Pattern	24 4	15 4
Population estimate	28 (±2)	19 (±2)
Capture probability	0.833	0.733
Mean length (mm) (n)	150 (28)	137 (19)
Mean weight (g) (n)	NA	33 (19)
Mean KTL (n)	NA	0.94 (19)
Number fish per km (95 % CI)	280 (±20)	190 (±20)
Number fish per ha (95 % CI)	1,000 (±71)	950 (±100)
Biomass (kg per ha) (95 % CI)	NA	31 (±3)

4. Comments:

[1] – Includes 2.59 km (1.61 mi) of habitat between the Neihart municipal water supply reservoir and the natural waterfall barrier currently occupied by Brook Trout and hybridized WCT

Figure 11. O'Brien Creek fish population estimation results.

Pilgrim Creek

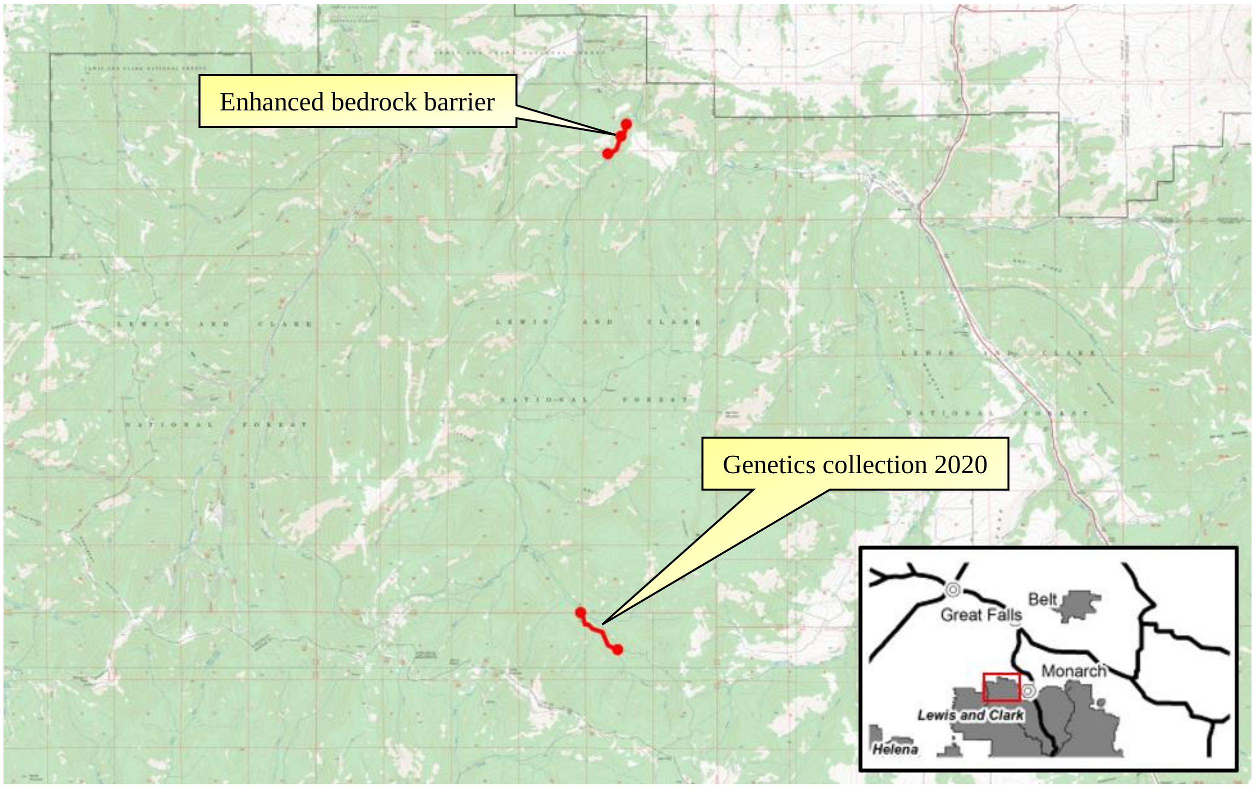


Figure 12. Pilgrim Creek in the Belt Creek subbasin. The stream segments delineated in red indicate the areas sampled in 2020.

Background

Pilgrim Creek contains a WCT population of varying genetic status with nonhybridized fish occupying the headwaters of the creek and slightly hybridized fish becoming more prevalent in downstream reaches. A small natural bedrock barrier located approximately 700 m upstream of the confluence of Belt Creek was enhanced in 2003 with native rock to increase the drop height (Figure 12). WCT were collected from above the barrier, marked with an adipose clip, and released below to test the efficacy of the enhanced barrier in 2014. In 2018, Pilgrim Creek was shocked above the barrier to detect any marked fish. No marked fish were detected during this effort and an additional 16 fish were marked below the barrier.

2020 Monitoring

On August 24th, 2020, tissue samples were collected from 30 WCT in the headwaters of Pilgrim Creek for genetic analysis. Fish were collected from three localities within a 1.41 km reach upstream of the Deer Creek confluence. These samples will be used to determine if hybridization has spread to Pilgrim Creek headwaters since the last genetic analysis was performed in 2009.

On September 14th, 2020, Pilgrim Creek was backpack electrofished in the vicinity of the enhanced bedrock barrier to evaluate barrier effectiveness. A 550 m section of Pilgrim Creek was shocked above the barrier and a 330 m section was shocked below the barrier. A total of 105 fish were collected above the barrier. These fish all phenotypically appeared to be WCT except for one obvious WCTxRB hybrid. The hybrid individual possessed an adipose clip, indicating it had been marked below the barrier in 2014 or 2018. All fish collected above the barrier were marked with an adipose clip and released below the barrier to further evaluate its effectiveness. Additionally, 46 fish were collected from below the Pilgrim Creek barrier. Below the barrier the catch was dominated by Rainbow Trout with a smaller contingent of Brook Trout, WCTxRB hybrids, and WCT also present. These fish were marked with an adipose clip and released below the barrier.

The enhanced bedrock barrier appears to be somewhat effective in restricting the upstream movement of nonnative fish. However, the appearance of a marked WCTxRB hybrid above the barrier indicates that larger fish can negotiate the barrier under certain flow conditions. Blasting of the bedrock at the barrier site would be required to increase the barrier drop height and overall effectiveness.

III. Judith River Subbasin

North Fork Running Wolf Creek

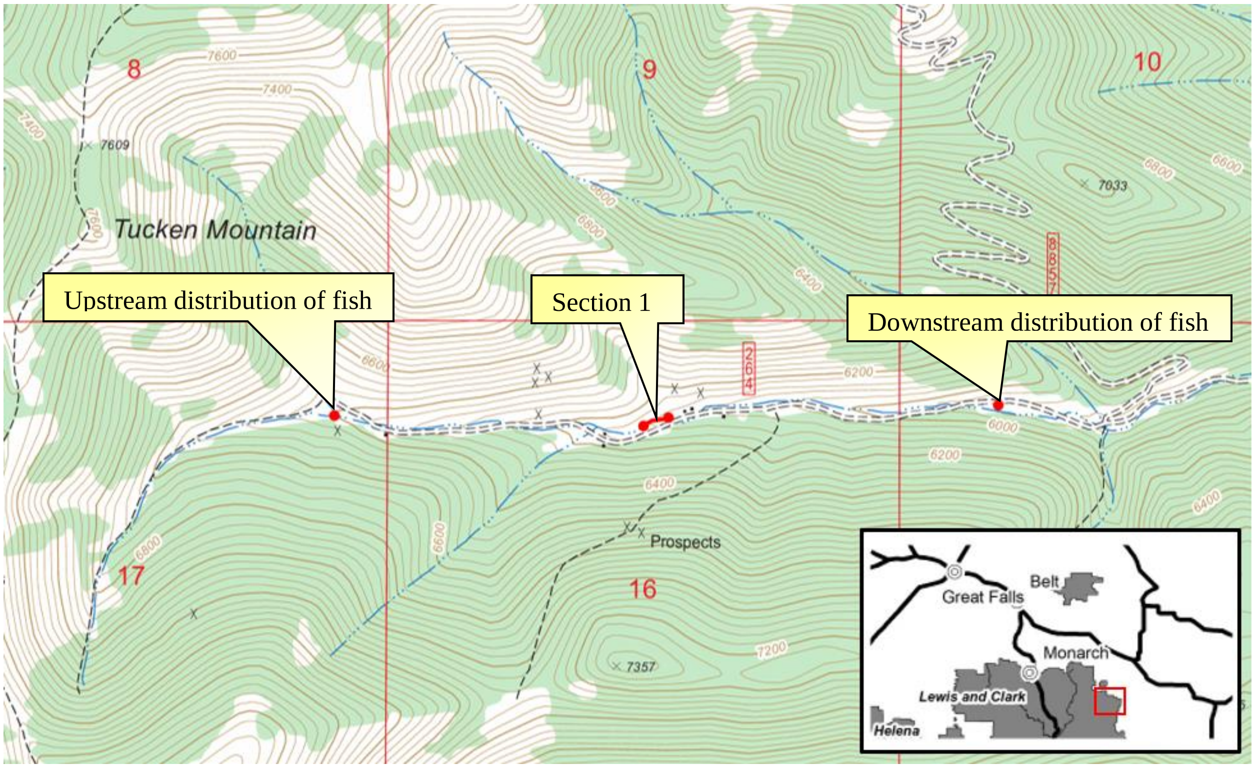


Figure 14. North Fork Running Wolf Creek in the Judith River subbasin. The stream segments delineated in red indicate areas sampled in 2020.

Background

North Fork Running Wolf Creek contains a population of nonhybridized WCT separated from downstream nonnative trout populations by a dry channel. This population inhabits a very small perennial reach of stream approximately 2.12 km in length (Figure 14). The genetic status of this population has been monitored periodically since 1994. No physical barriers separate this population from downstream Brook Trout populations and the potential for invasion during extreme flow events does exist.

2020 Monitoring

The North Fork Running Wolf Creek WCT population was monitored on September 21st, 2020 to assess their current status. A 100 m population estimation section was established near the Forest Service cabins where previous years sampling has been conducted. Multiple pass depletion methods were used to estimate population abundance. WCT abundance was relatively robust compared to other WCT streams within the Little Belt Mountains with an estimated 560 fish/km (Figure 15). Additionally, 30 tissue samples were collected from WCT at three localities for genetic analysis: 10 from the population estimate section, 10 near the headwater springs, and 10 near the downstream extent of water.

North Fork Running Wolf Creek —NATIVE TROUT POPULATION SURVEY

1. General Information— Date: **September 21, 2020** Biologist: **A. Poole**
2. Stream Information—
   Name, section, county: **North Fork Running Wolf Creek, 16, Judith Basin**
3. Survey Site Information (see attached map)—
   Upstream range of native trout (general description and GPS): **850 m above FS cabins (46.97910, -110.47440)**
   Downstream range of native trout (general description and GPS): **360 m above FS RD 8857 junction (46.97930, -110.44690)**
   Location (GPS) and description of barriers: **NA**
   Stream Length—Occupied habitat: **2.12 km (1.32 mi)** Available habitat: **2.12 km (1.32 mi)**
   Survey method & equipment: **backpack battery electrofisher; two-pass depletion**
   Survey sites (general description and UTM)—
   Section 1: **At Forest Service cabins; 46.97900, -110.46060**

Parameter	**Section 1**
Section length (m)	100 m
Mean stream width (m) (n)	1.01 m (10)
Section area (hectares)	0.011 ha
WCT	
Removal Pattern	46 9
Population estimate	56 (±3)
Capture probability	0.804
Mean length (mm) (n)	112 (56)
Mean weight (g) (n)	19 (56)
Mean KTL (n)	0.81 (56)
Number fish per km (95 % CI)	560 (±30)
Number fish per ha (95 % CI)	5,090 (±273)
Biomass (kg per ha) (95 % CI)	97 (±5)

Figure 15. North Fork Running Wolf Creek fish population estimation results.

IV. Smith River Subbasin

Adams Creek

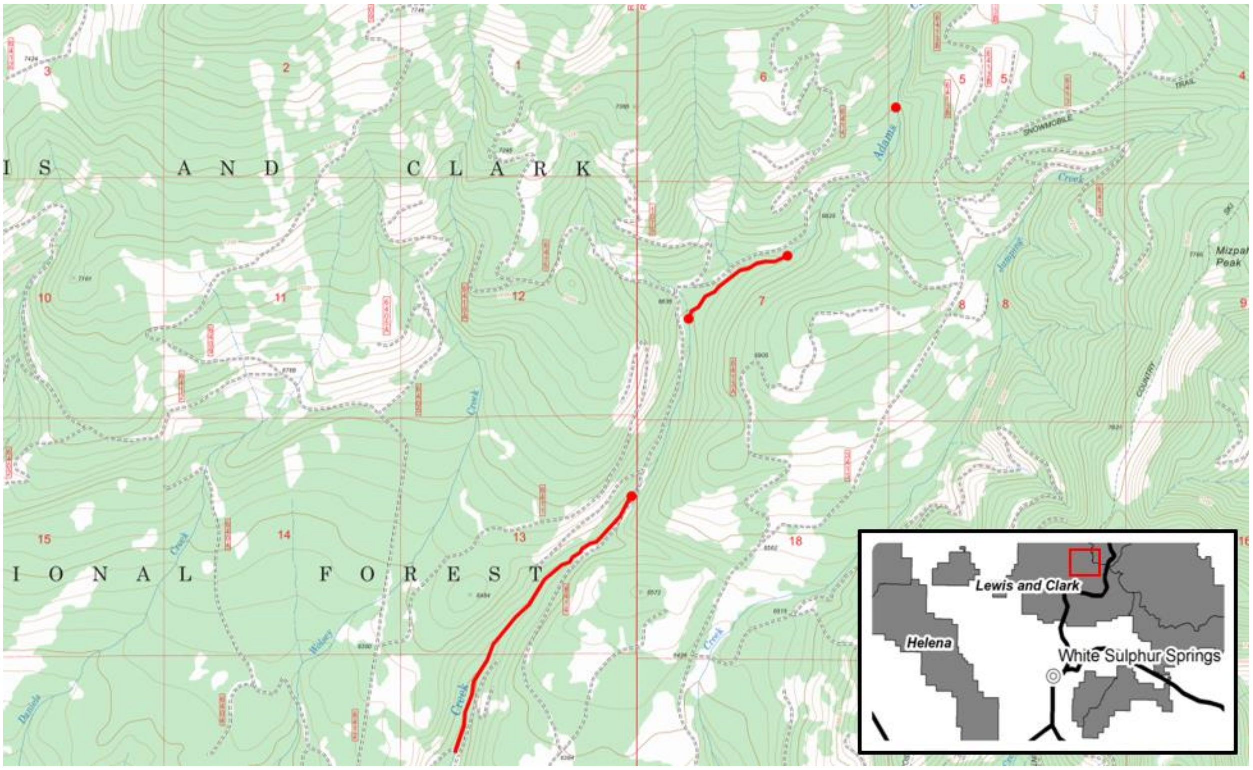


Figure 16. Adams Creek in the Smith River subbasin. The stream segments delineated in red indicate areas sampled in 2020.

Background

Adams Creek is a tributary to Sheep Creek in the Smith River drainage and contains a hybridized population of cutthroat trout and nonnative Brook Trout. Genetic analysis of cutthroat trout collected in 1990 revealed Adams Creek contained a hybrid swarm between Westslope Cutthroat Trout and Rainbow Trout. The percent Rainbow Trout admixture in the sample was 0.40. Adams Creek has been identified as a potential WCT restoration site.

2020 Monitoring

Adams Creek was surveyed on September 3rd, 2020 to locate any potential barriers or barrier sites for future WCT restoration projects (Figure 16). The lower section of the creek was accessed at the FS RD 6412 crossing and visually inspected upstream for approximately 2.17 km. The creek in this section was low gradient throughout and no potential barrier locations were located. The upper watershed was accessed by FS RD 6414. An 800 m section of Adams Creek was surveyed in the upper watershed (section 7) and again no potential barrier locations were located. A final upstream location was evaluated for the presence of a bedrock control feature but no barriers to fish movement were found (section 5). If Adams Creek was selected for WCT restoration, a culvert style barrier at the FS RD 6412 crossing would likely be the only suitable location for a fish barrier. Removal of nonnative trout in Adams Creek would be logistically complex. Several springs, seeps, and off channel water features were noted during visual surveys. Additionally, large amounts of woody debris and macrophytes were observed throughout the stream.

Camas Lake and Big Camas Creek

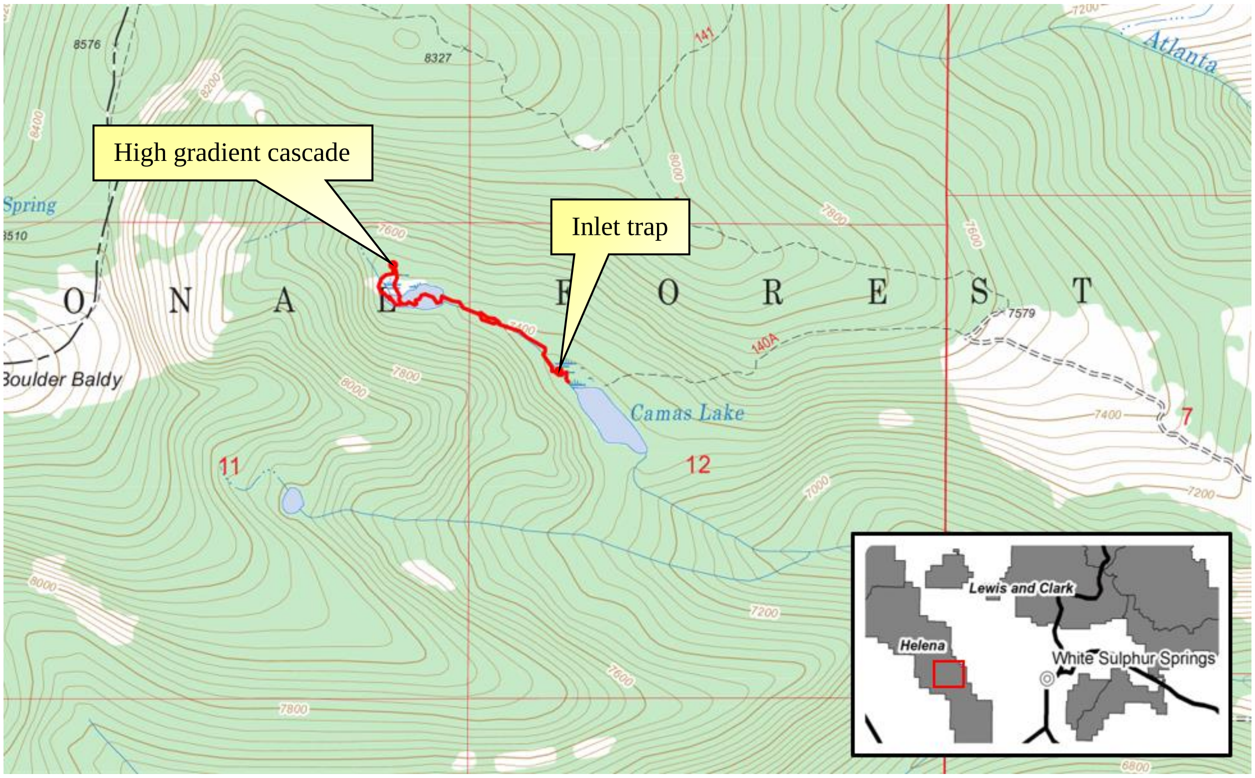


Figure 17. Camas Lake and Big Camas Creek in the Smith River subbasin. The stream segments delineated in red indicate areas sampled in 2020.

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 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 years 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. Starting in 2017, the installation of modified a fyke net in the Camas Lake inlet has been used in conjunction with electrofishing to remove fish entering the stream during the spring spawning season.

2020 Monitoring

The Camas Lake inlet trap was installed May 27th, 2020 (Figure 17). Discharge of Big Camas Creek above Camas Lake did not reach a level suitable for backpack electrofishing until June 12th, 2020. In total, the Camas Lake trap was checked 16 times during the 2020 field season. Big Camas Creek above Camas Lake was electrofished 11 times in 2020. A total of 421 fish were collected in 2020 (Table 1); 230 caught in the inlet trap and 191 caught electrofishing upper Big Camas Creek. Total catch of YCT was lower in 2020 compared to previous years. However, several YCT and unidentified Cutthroat Trout (CT) under 120 mm were also collected indicating successful spawning is occurring despite the presence of the trap and removal efforts (Figure 18). Genetic samples were collected from fish under 120 mm to detect hybridization. The inlet trap was removed on July 30th, 2020.

Table 1. Total catch from Camas Lake and upper Big Camas Creek 2016-2020. Based on visual identification of fish collected. WCT in parenthesis represent fish with intact adipose fins removed from the population.

	2016	**2017**	**2018**	**2019**	**2020**
WCT	549 (255)	636 (417)	169 (62)	175 (54)	335 (121)
YCT	92	108	163	139	83
WCTxYCT	161	62	8		1
CT					2

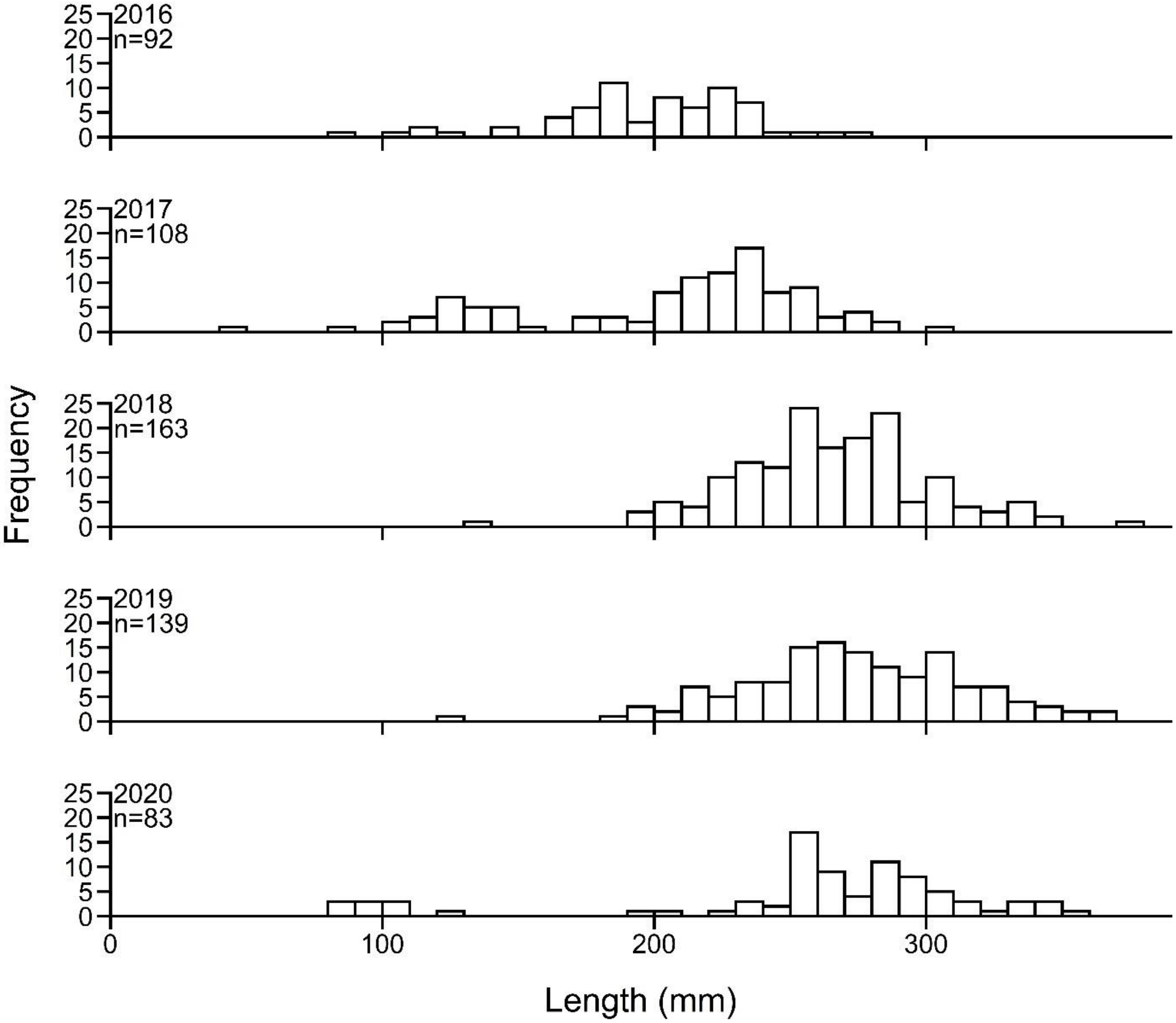


Figure 18. Length-frequency histograms of total YCT catch from Camas Lake and upper Big Camas Creek from 2016-2020.

Coyote Creek

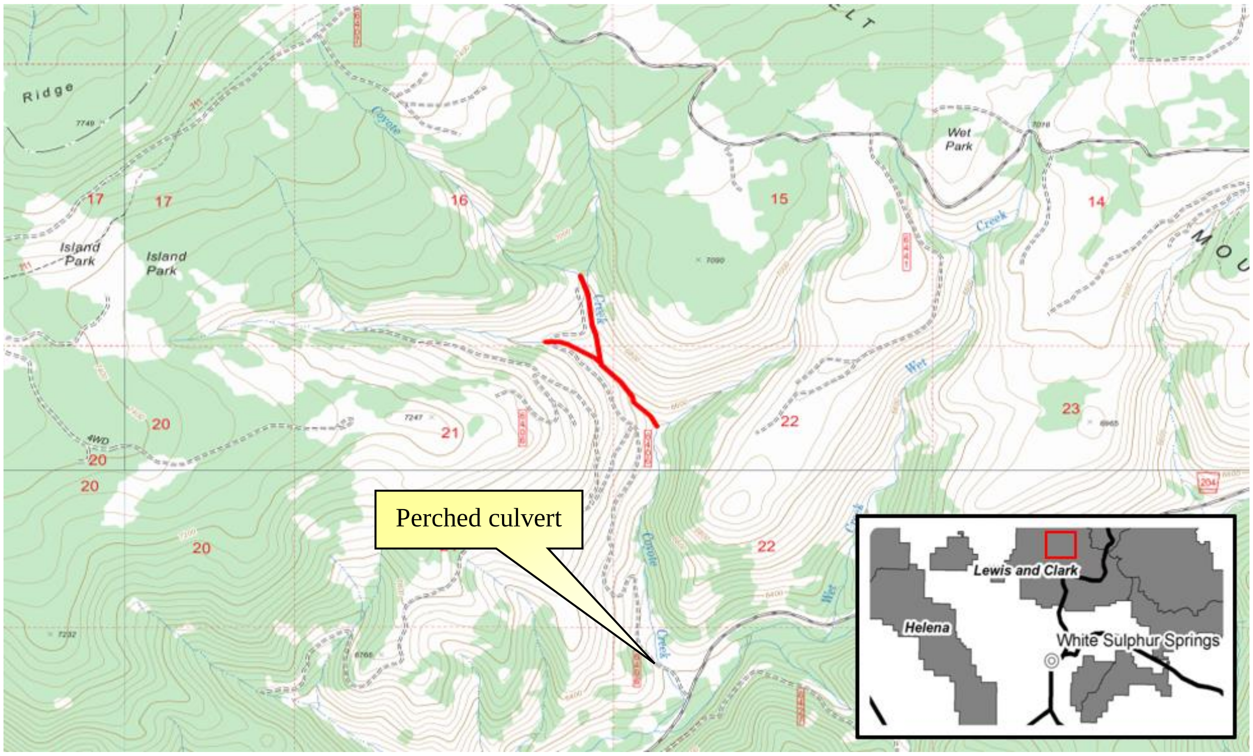


Figure 19. Coyote Creek in the Smith River subbasin. The stream segment delineated in red indicates the area sampled in 2020.

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 potentially a barrier to fish movement.

2020 Monitoring

On October 5th, 2020, Coyote Creek was surveyed to assess its potential for a future WCT restoration project. The creek was accessed by FS RD 6406 by way of FS RD 204 (Moose Creek Road). Where FS RD 6406 crosses Coyote Creek the perched culvert was inspected which could potentially serve as future barrier site (46.8650, -110.8398; Figure 20). The site appeared to be suitable with enough gradient to accommodate a culvert style barrier.

Figure 20. Perched culvert at FS RD 6406 crossing on Coyote Creek (46.8650, -110.8398).

Coyote Creek was hiked and spot shocked from the confluence of the lower tributary upstream to the forks of the mainstem and then up the western tributary to the road crossing. Brook Trout was the only species observed throughout the system. The lower tributary appeared to flow ~1 cfs and was likely fish bearing. Coyote Creek enters a high gradient reach between the tributaries flowing over several bedrock cascade features. However, no barriers to fish movement were found. At the confluence of the western tributary and Coyote Creek it was noted that the western tributary provides the majority of the flow with the mainstem contributing less than half the flow of this tributary. Continuing up the mainstem of Coyote Creek we found that the fork to the north, or upper tributary that appears on the map, was barely flowing and did not appear to be fish bearing as we found no Brook Trout. Coyote Creek was electrofished upstream of the confluence of the forks and Brook Trout were present at this location. Returning downstream to the western tributary, Brook Trout were captured while spot electrofishing from this point up to the road crossing.

Coyote Creek appears to have potential for WCT restoration if a barrier were to be installed at the FS RD 6406 crossing. Based on our preliminary investigation if a barrier was installed at this location it could provide around 4.5 miles of habitat. However, future investigations would be needed to confirm total amount of fish bearing habitat and that habitat may vary dependent upon precipitation patterns.

Fourmile Creek

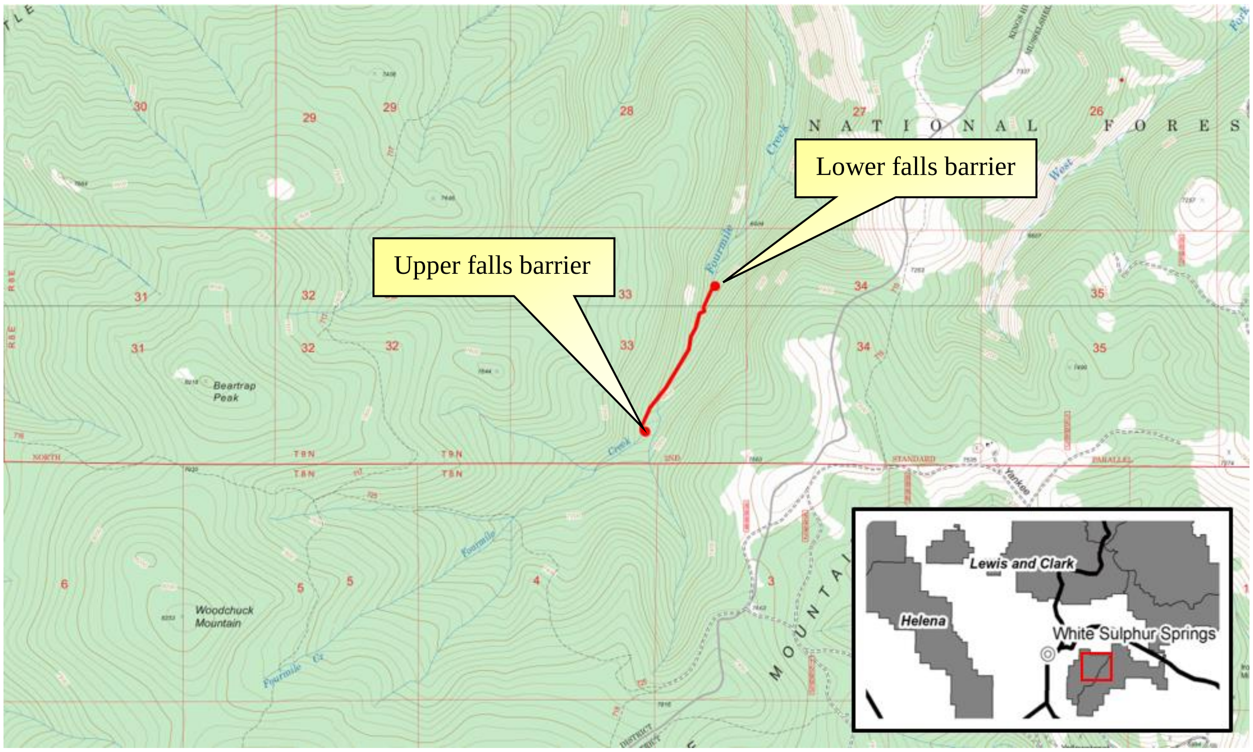


Figure 21. Fourmile Creek in the Smith River subbasin. The stream segment delineated in red indicates the area sampled in 2020.

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 waterfall 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.

2020 Monitoring

Fourmile Creek was surveyed on September 15th, 2020 to determine the location of the natural waterfall barriers and evaluate potential for future WCT translocations. Fourmile Creek was accessed by trail 725 from Yankee Jim Ridge and surveyed downstream. An upper barrier falls was located at 46.49239, -110.72236 (Figure 22). Fish were observed immediately downstream of this barrier. A backpack electrofisher was used to collect 30 fish for genetic analysis. These fish appeared phenotypically to be WCT (Figure 23). Fourmile Creek was spot shocked from this point downstream until a second barrier falls was located at 46.50130, -110.71620 (Figure 24). Only WCT were observed in this reach between the barriers. Downstream of the lower barrier falls Brook Trout and hybridized WCT were observed.

A 1.13 km section of Fourmile Creek appears to support a fairly robust population of WCT isolated between two bedrock falls barriers. Based on our preliminary survey, it appears the 2000 transfer of 50 WCT from Richardson Creek was successful. Genetic analysis of the samples collected in 2020 should help clarify the origin of the putative WCT. Suitable habitat is present upstream of the current occupied reach and upstream expansion would be advisable to ensure the persistence of this small population.

Figure 22. Upper natural falls barrier on Fourmile Creek (46.49239, -110.72236).

Figure 23. WCT collected from Fourmile Creek below the upper natural falls barrier.

Figure 24. Lower natural falls barrier on Fourmile Creek (46.50130, -110.71620).

Iron Mines Creek

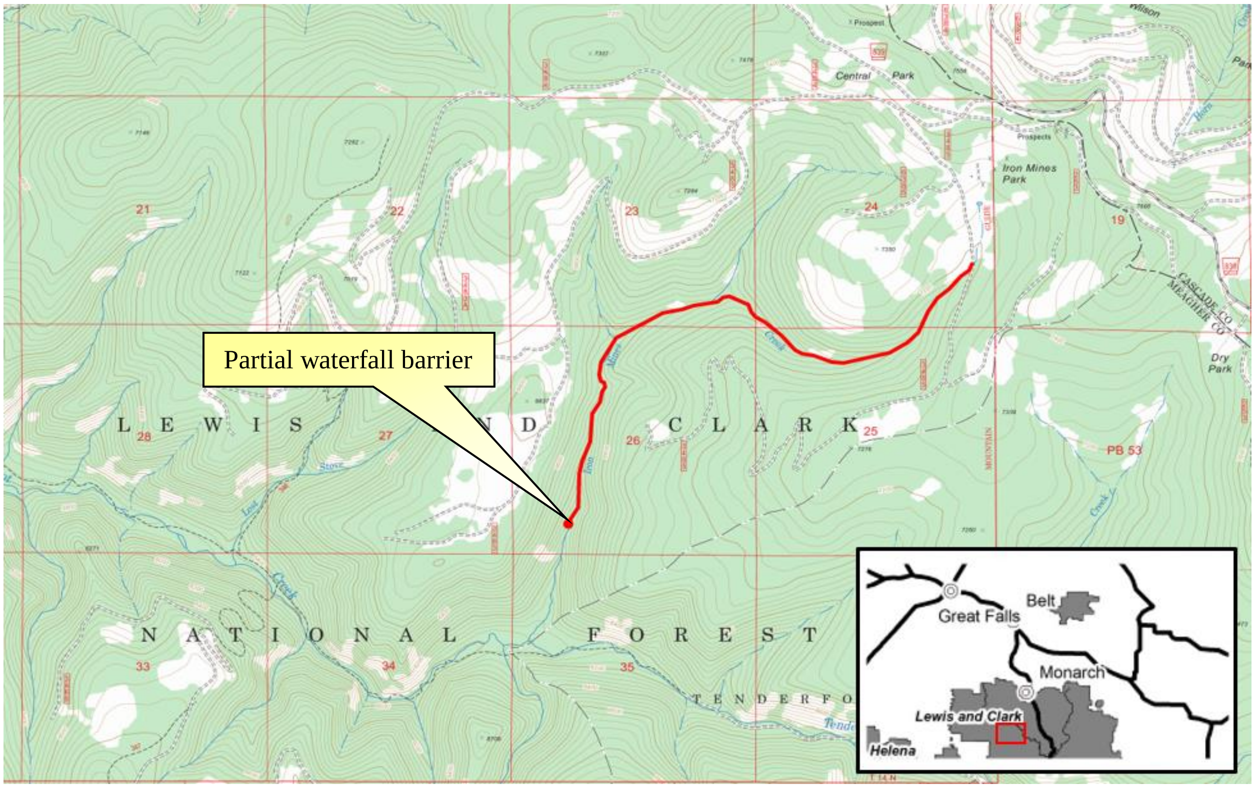


Figure 25. Iron Mines Creek in the Smith River subbasin. The stream segments delineated in red indicate the areas sampled in 2020.

Background

Iron Mines Creek in the Smith River drainage contains a hybridized population of Westslope Cutthroat Trout above a partial waterfall barrier upstream from its confluence with Tenderfoot Creek. Previous sampling performed by the U.S. Forest Service in 1998 collected only cutthroat trout in the headwaters of the drainage. Subsequent genetic analysis of fish collected revealed a hybrid swarm between Westslope Cutthroat Trout and Yellowstone Cutthroat Trout. The percent Yellowstone Cutthroat Trout admixture in the sample was 0.09. Iron Mines Creek was identified as a potential WCT restoration site in previous status reports (Moser et al. 2011).

2020 Monitoring

Iron Mines Creek was surveyed on September 22nd, 2020 to determine the location of the waterfall barrier and evaluate the potential for future WCT restoration. The stream was accessed from the FS RD 3485 crossing of Iron Mines Creek (section 24), just before the locked gate. Immediately upstream of this road crossing both hybrid cutthroat trout as well as Brook Trout were observed. The upper portion of the mainstem creek is small, flowing ~1 cfs with limited pool habitat. However, fish were observed throughout this reach including Brook Trout on redds. The stream flows through mostly open meadows lined with willow; several seeps are present throughout this reach and form off-channel pools. The creek appears to double in size at the confluence of the unnamed upper tributary (Figure 25) and this tributary has similar if not slightly greater flow. From here the stream continues to flow through meadows lined with willows with occasional conifers until reaching the unnamed lower tributary (Figure 25). This tributary contributes ~1 cfs of flow and is likely fish bearing. From here downstream the gradient begins to increase and the stream flows through a mixed conifer forest often with exposed scree slopes on the eastern bank.

The falls barrier was located at Lat/Long: 46.94129, -110.93581 (Figure 26). The total vertical drop of this bedrock feature appeared to be around 5 feet. However, several intermediate rocks precluded a clean drop and resulted in step pools. The plunge pool beneath this falls was significant, around 5 to 6 feet deep. While the velocity at the base flows that we observed this barrier site at appeared to preclude most fish movement, it is likely that this is not a complete barrier as previously indicated. The presence of hybrid cutthroat trout and Brook Trout upstream of this location would also suggest it is not a complete fish passage barrier as originally suspected. However, this site could potentially be modified to create more of a vertical drop by blasting and removing some of the intermediate rocks. Fieldwork would likely be difficult given the remoteness of the site.

Figure 26. Partial falls barrier on Iron Mines Creek (46.94129, -110.93581)

Jumping Creek

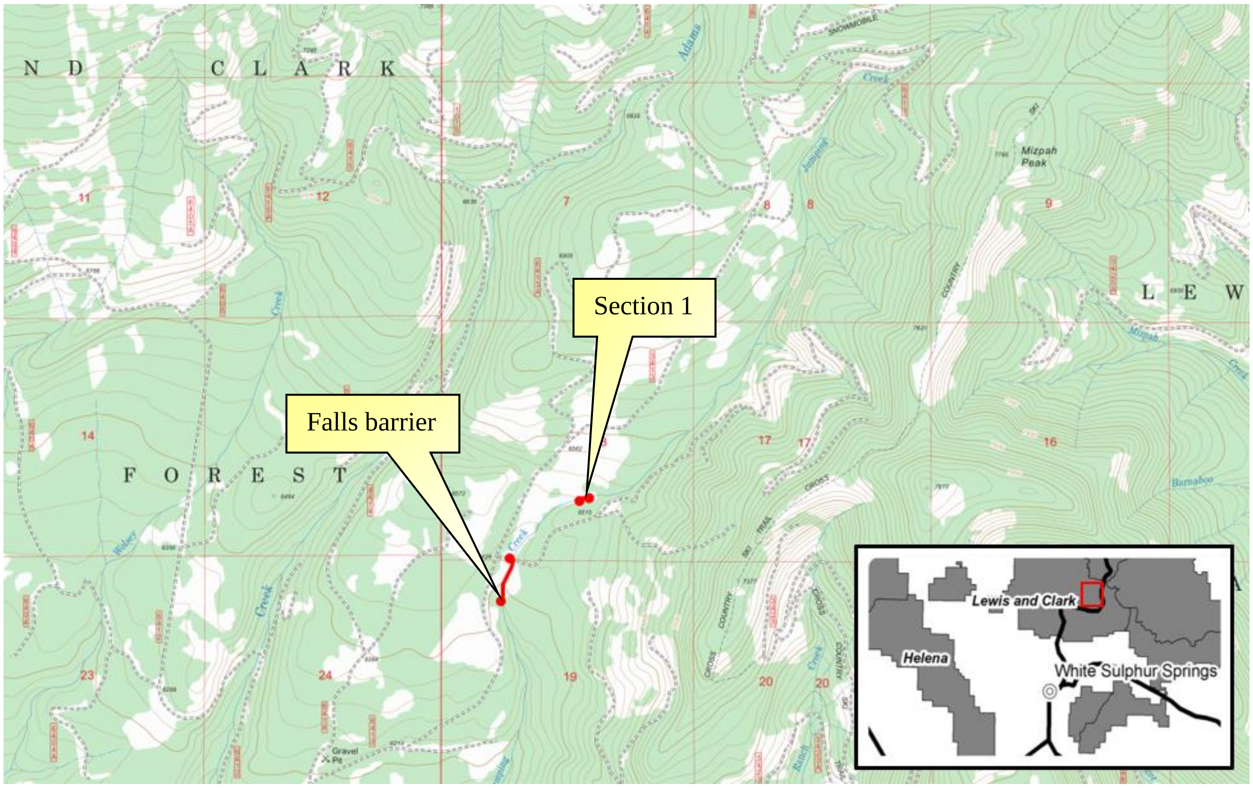


Figure 27. Jumping Creek in the Smith River subbasin. The stream segments delineated in red indicate the areas sampled in 2020.

Background

Genetic analysis of WCT collected in the headwaters of Jumping Creek in the early 2000s revealed a small nonhybridized population persisted alongside nonnative Brook Trout. Mechanical removal of Brook Trout was performed for three years (2005-2007) with little effect. In 2007 and 2008 a natural bedrock cascade was enhanced with blasting to create a suitable barrier falls. In 2008 the WCT population (71 juveniles and adults) was salvaged and transferred to Tyrell Creek (Smith River drainage). Jumping Creek was chemically treated in 2008 and 2009 to remove nonnative Brook Trout. Remote site incubators were used to reestablish a WCT population in Jumping Creek from 2010-2013 using embryos from South Fork Willow, Cottonwood, and Lone Willow Creeks (all Smith River drainage). Presence/absence surveys conducted in 2014, 2015, and 2017 confirmed the establishment of the WCT population.

2020 Monitoring

Jumping Creek was surveyed on September 2nd, 2020. A 340 m section of stream directly above the fish barrier was electrofished to detect the presence of nonnative Brook Trout (Figure 27). Only WCT were collected (81 age-1 and older fish) in this effort. Additionally, a fish population estimate section was established on lower Jumping Creek to assess the population's status. Based on the results of the two-pass depletion, lower Jumping Creek supports an estimated 260 fish/km (Figure 28). An additional fish population estimate section is likely required to characterize upper Jumping Creek as the gradient and habitat differ from the lower creek.

Jumping Creek —NATIVE TROUT POPULATION SURVEY

1. General Information— Date: **September 2, 2020** Biologist: **A. Poole**
2. Stream Information—
   Name, section, county: **Jumping Creek, 18, 19, Meagher**
3. Survey Site Information (see attached map)—
   Upstream range of native trout (general description and GPS): **FS RD 6413 crossing (estimated; 46.82640, -110.73780)**
   Downstream range of native trout (general description and GPS): **Blasted falls barrier (46.79300, -110.77540)**
   Location (GPS) and description of barriers: **Blasted falls barrier (46.79300, -110.77540)**
   Stream Length—Occupied habitat: **4.95 km (3.08 mi)** Available habitat: **8.58 km (5.33 mi)**[1]
   Survey method & equipment: **backpack battery electrofisher; two-pass depletion**
   Survey sites (general description and UTM)—
   Section 1: **Top of meadow section; 46.79923 -110.76841**

Parameter	**Section 1**
Section length (m)	100 m
Mean stream width (m) (n)	2.19 m (10)
Section area (hectares)	0.022 ha
WCT	
Removal Pattern	22 4
Population estimate	26 (±2)
Capture probability	0.818
Mean length (mm) (n)	169 (26)
Mean weight (g) (n)	NA
Mean KTL (n)	NA
Number fish per km (95 % CI)	260 (±20)
Number fish per ha (95 % CI)	1,181 (±91)
Biomass (kg per ha) (95 % CI)	NA

4. Comments:

[1] – Includes 3.3 km (2.05 mi) of habitat between US 89 and the blasted waterfall barrier currently occupied by Brook Trout.

Figure 28. Jumping Creek fish population estimation results.

Middle Fork Big Camas Creek

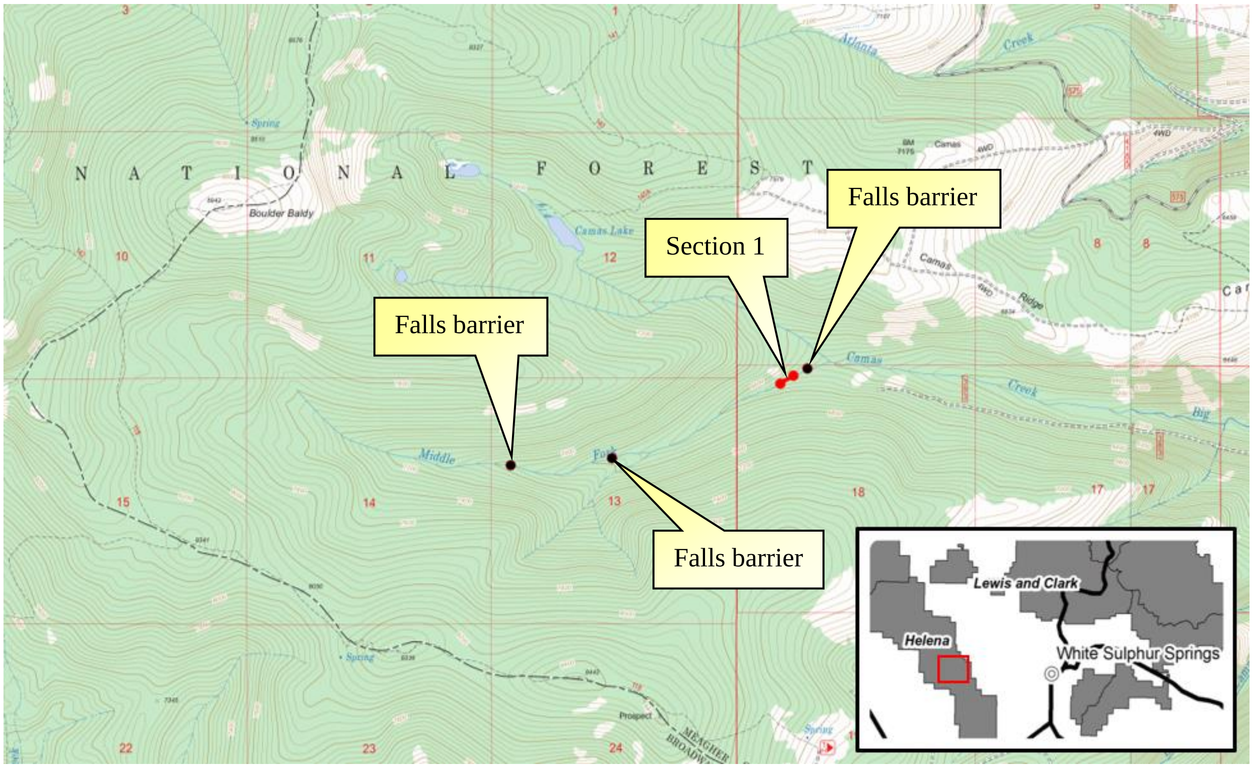


Figure 29. Middle Fork Big Camas Creek in the Smith River subbasin. The stream segment delineated in red indicates the area sampled in 2020.

Background

Surveys performed in 2001 by the Forest Service found about 2.41 km of fishless habitat on Middle Fork Big Camas Creek above a natural waterfall barrier located near the confluence of Big Camas Creek. This habitat is further fragmented by 2 additional barriers located upstream (Figure 29). In 2003 and 2005, 80 and 40 nonhybridized WCT were transferred from Cottonwood Creek (Smith River drainage) to Middle Fork Big Camas Creek upstream of the lower falls barrier. Subsequent sampling of Middle Fork Big Camas Creek confirmed that the transferred WCT survived, successfully reproduced and established.

2020 Monitoring

The Middle Fork Big Camas Creek WCT population was monitored on August 27th, 2020 to assess their current population status. A 100 m population estimate section was established upstream of the lower barrier falls. Multiple pass depletion methods were used to estimate population abundance. Estimated WCT abundance was lower in Middle Fork Big Camas Creek at 130 fish/km when compared to other creeks sampled in the Smith River drainage in 2020 (Figure 30). The WCT catch in the section sampled was dominated by older fish with no fish under 120 mm sampled. Further evaluation of this population is warranted to determine persistence of WCT above the upstream falls barriers.

Middle Fork Big Camas Creek **—**NATIVE TROUT POPULATION SURVEY

1. General Information— Date: **August 27, 2020** Biologist: **A. Poole**
2. Stream Information—
   Name, section, county: **Middle Fork Big Camas Creek, 13, 14, 18, Meagher**
3. Survey Site Information (see attached map)—
   Upstream range of native trout (general description and GPS): **850 m above 3rd falls barrier (46.54160, -111.31360)**
   Downstream range of native trout (general description and GPS): **Lower falls barrier (46.54670, -111.27770)**
   Location (GPS) and description of barriers: **Lower falls barrier (46.54670, -111.27770), 2nd falls barrier (46.54140, -111.29440), 3rd falls barrier (46.54100, -111.30310)**
   Stream Length—Occupied habitat: **3.13 km (1.94 mi)** Available habitat: **3.13 km (1.94 mi)**
   Survey method & equipment: **backpack battery electrofisher; two-pass depletion**
   Survey sites (general description and UTM)—
   Section 1: **Above lower barrier falls; 46.54625 -111.27887**

Parameter	**Section 1**
Section length (m)	100 m
Mean stream width (m) (n)	3.28 m (10)
Section area (hectares)	0.033 ha
WCT	
Removal Pattern	10 3
Population estimate	13 (±2)
Capture probability	0.700
Mean length (mm) (n)	180 (13)
Mean weight (g) (n)	63 (13)
Mean KTL (n)	0.99 (13)
Number fish per km (95 % CI)	130 (±20)
Number fish per ha (95 % CI)	394 (±61)
Biomass (kg per ha) (95 % CI)	25 (±4)

Figure 30. Middle Fork Big Camas Creek fish population estimation results.

Richardson Creek

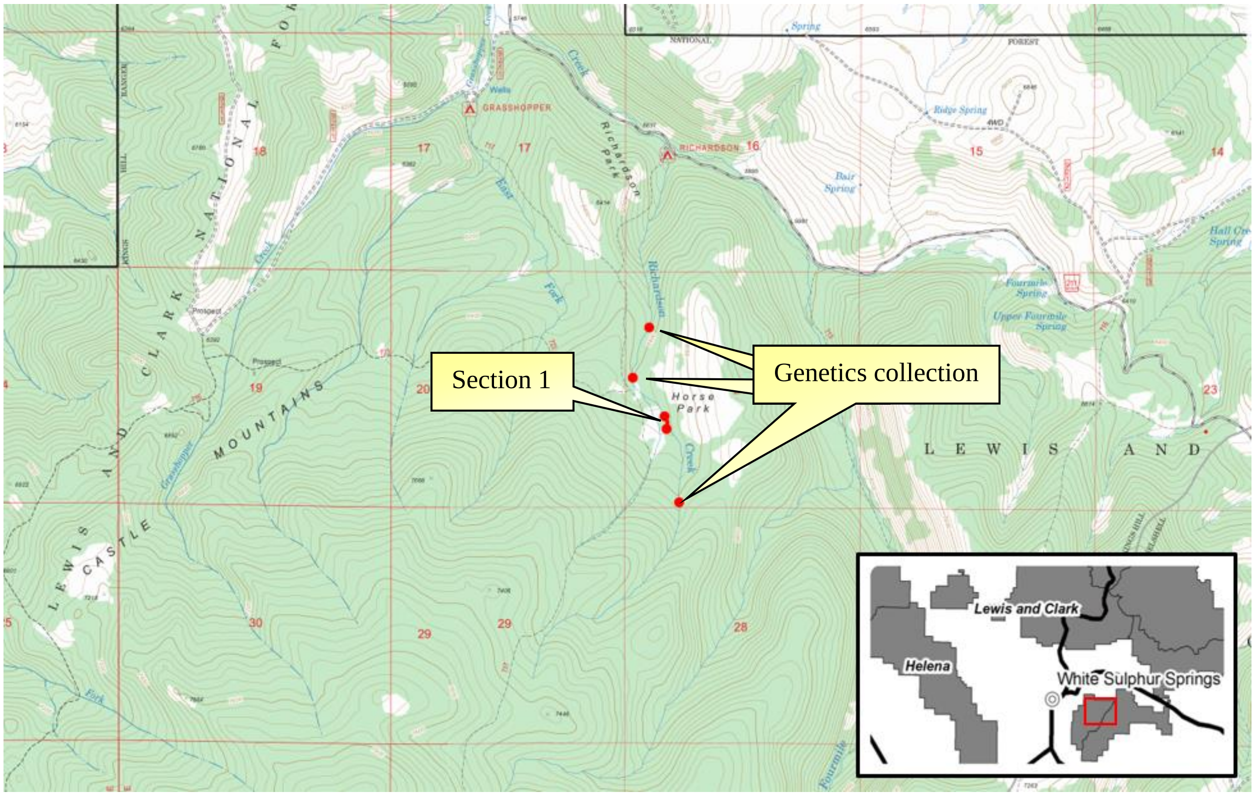


Figure 31. Richardson Creek in the Smith River subbasin. The stream segments delineated in red indicate the areas sampled in 2020.

Background

Richardson Creek contains a slightly hybridized population of WCT marginally protected from downstream nonnative fish by a series of cascades upstream of the Forest Service campground (Figure 31). This population inhabits a small reach approximately 3.51 km in length. Sampling performed by the Forest Service from 1996-1999 found low densities of WCT and estimated the total population size in this 3.51 km section between 200-700 WCT. At that time the risk for extirpation of WCT in Richardson Creek was noted to be very high because of limited habitat, absence of secure downstream barriers, and grazing impacts. Cattle exclusion fencing was installed in two different riparian areas on Richardson Creek in 1997 to improve stream health and function. In 2000, 50 WCT were transferred from Richardson Creek to upper Fourmile Creek (Smith River drainage) to replicate this population.

2020 Monitoring

Richardson Creek was surveyed on September 9th and September 21st, 2020. A 100 m population estimate section was established within the upper riparian exclosure to monitor the status of the WCT population. Multiple pass depletion methods were used to estimate population abundance. A robust population of WCT was found in the estimate section with an estimated 98 WCT age-1 and older occurring in the 100 m section (Figure 32). It was noted during this visit that the gate to the upper riparian exclosure was open and several cattle were observed inside the exclosure area. Tissue samples from 30 WCT were collected for an updated genetics analysis from three locations in Richardson Creek: below the riparian exclosures, within the lower riparian exclosure, and upstream of the exclosures.

Richardson Creek —NATIVE TROUT POPULATION SURVEY

1. General Information— Date: **September 9, 2020** Biologist: **A. Poole**
2. Stream Information—
    Name, section, county: **Richardson Creek, 21, Meagher**
3. Survey Site Information (see attached map)—
    Upstream range of native trout (general description and GPS): **2.11 km above top of upper exclosure (estimated; 46.50900, -110.74240)**
    Downstream range of native trout (general description and GPS): **Top of high gradient cascade (estimated; 46.53420, -110.73310)**
    Location (GPS) and description of barriers: **NA**
    Stream Length—Occupied habitat: **3.51 km (2.18 mi)** Available habitat: **4.38 km (2.72 mi)[1]**
    Survey method & equipment: **backpack battery electrofisher; two-pass depletion**
    Survey sites (general description and UTM)—
    Section 1: **Upper riparian exclosure; 46.52480, -110.73074**

Parameter	**Section 1**
Section length (m)	100 m
Mean stream width (m) (n)	1.14 m (10)
Section area (hectares)	0.011 ha
WCT	
Removal Pattern	74 19
Population estimate	98 (±8)
Capture probability	0.743
Mean length (mm) (n)	103 (93)
Mean weight (g) (n)	13 (93)
Mean KTL (n)	0.75 (93)
Number fish per km (95 % CI)	980 (±80)
Number fish per ha (95 % CI)	8,909 (±727)
Biomass (kg per ha) (95 % CI)	116 (±10)

4. Comments:

[1] – Includes 870 (0.54 mi) of habitat downstream of high gradient reach currently occupied by Brook Trout.

Figure 32. Richardson Creek fish population estimation results.

V. Sun River Subbasin

Lange Creek

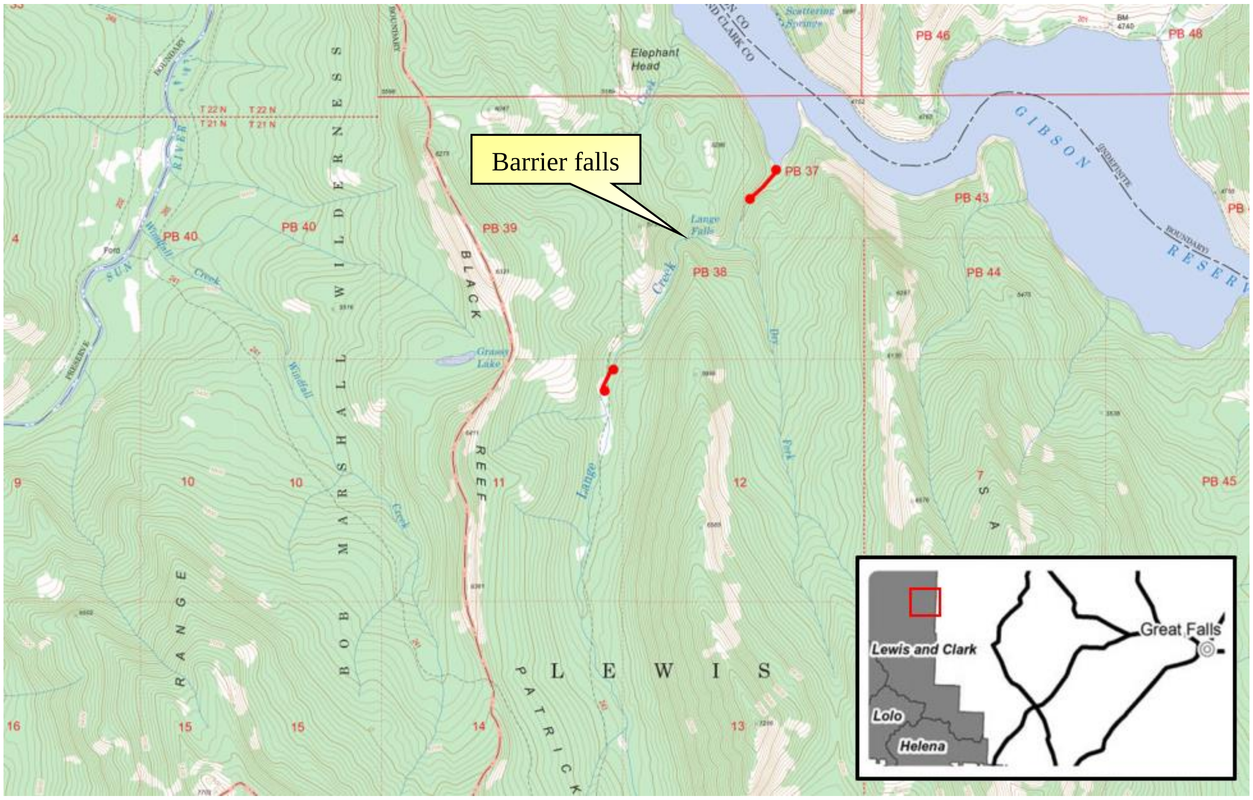


Figure 33. Lange Creek in the Sun River subbasin. The stream segments delineated in red indicate the areas sampled in 2020.

Background

Surveys of Lange Creek in 2005 found no fish above a significant natural waterfall barrier (Lange Falls) located approximately 950 m upstream of Gibson Reservoir (Figure 33). Temperature and invertebrate data indicated that Lange Creek was a suitable candidate for WCT transfer. In 2009, 140 live juvenile and adult WCT were transferred from North Fork Willow Creek (Teton River drainage) by helicopter to Lange Creek. An additional transfer of 125 WCT was completed in 2010. Subsequent sampling in 2012 documented that transferred WCT were naturally reproducing and established.

2020 Monitoring

Lange Creek was identified as a potential source population for WCT restoration efforts underway in Hyde Creek (Two Medicine River drainage). A fish health inspection and genetics collection was performed in preparation for a future wild fish transfer of WCT from Lange Creek to Hyde Creek. An approximately 270 m section of lower Lange Creek was electrofished June 23rd, 2020 to collect fish for the fish health inspection. An additional 150 m section was electrofished above the barrier to obtain more fish for the health inspection and genetic analysis. A total of 30 Rainbow Trout and 15 Brook Trout were collected below the barrier and 15 WCT were collected above the barrier. Whirling disease was detected in 4/6 pools of Rainbow Trout by digest and PCR in the fish health inspection report. However, the 15 WCT collected above Lange Falls all tested negative for whirling disease. An additional 38 WCT were collected above Lange Falls on July 28th, 2020 to complete the fish health inspection and obtain additional genetic samples. No pathogens were detected in samples submitted for testing from WCT collected above Lange Falls.

The genetic analysis of WCT collected above Lange Falls revealed the presence of one Rainbow Trout allele at one Rainbow Trout diagnostic marker and two Yellowstone Cutthroat Trout alleles at one Yellowstone Cutthroat Trout diagnostic marker (Appendix A). The samples from Lange Creek were compared to past genetic results from North Fork Willow Creek, the source population used to found the Lange Creek WCT population. The two Yellowstone Cutthroat Trout alleles were also detected at a marker in a recent sample from North Fork Willow Creek and likely represent a rare WCT genetic variation rather than Yellowstone Cutthroat Trout hybridization. However, the single Rainbow Trout allele detected in the Lange Creek sample was at a different marker than another single Rainbow Trout allele detected in a 2018 North Fork Willow Creek sample. The fact that Rainbow Trout alleles have now been observed at multiple markers greatly increases the probability that there is some Rainbow Trout ancestry in the North Fork Willow Creek population and thus Lange Creek as well. If the observed genetic variation of the Lange Creek WCT is representative of Rainbow Trout hybridization, the proportion Rainbow Trout admixture is ~0.2%. Nonetheless, fish from North Fork Willow Creek and Lange Creek still have high conservation value given the rarity of WCT in the Sun River and Teton Rvier subbasins.

On September 28th, 2020, 80 WCT were collected from Lange Creek (Figure 34) and transferred to a holding area near Gibson Reservoir with the use of stock animals and held overnight. On September 29th, 2020 the 80 WCT were transferred to Hyde Creek by helicopter and released successfully with no observed mortalities.

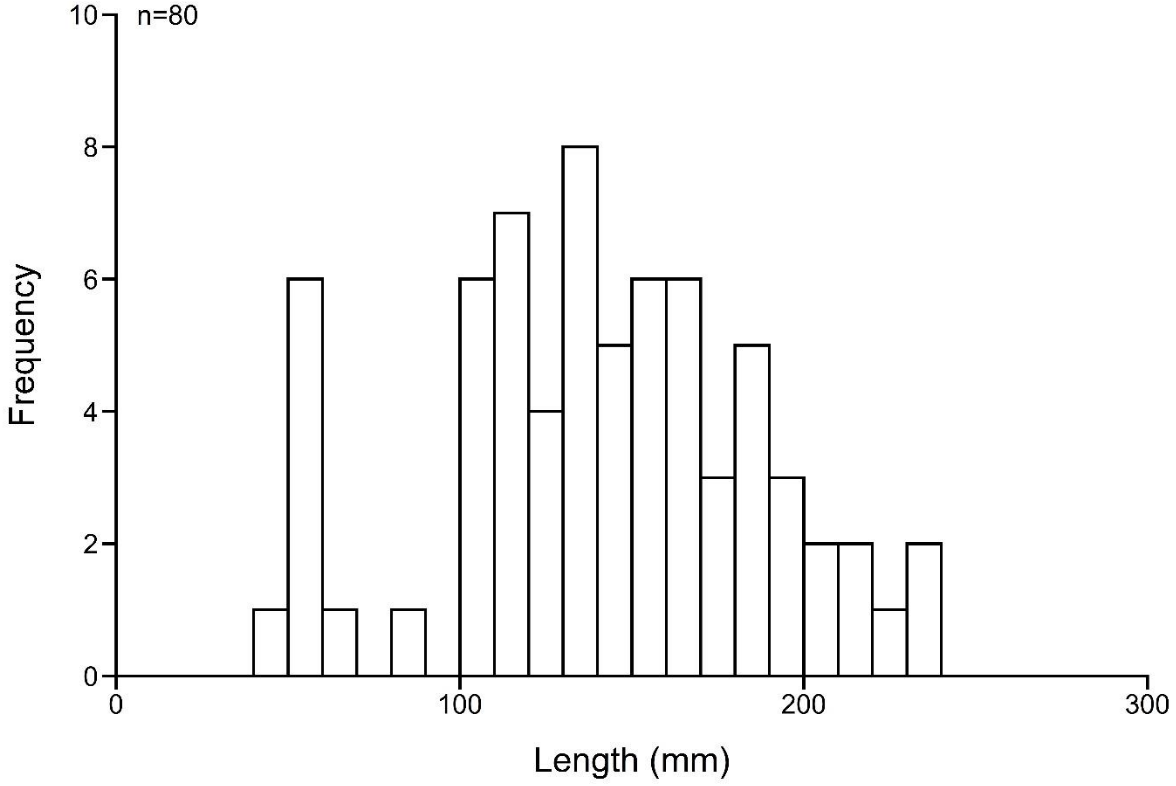


Figure 34. Length-frequency histogram of WCT transferred from Lange Creek to Hyde Creek.

North Fork Ford Creek

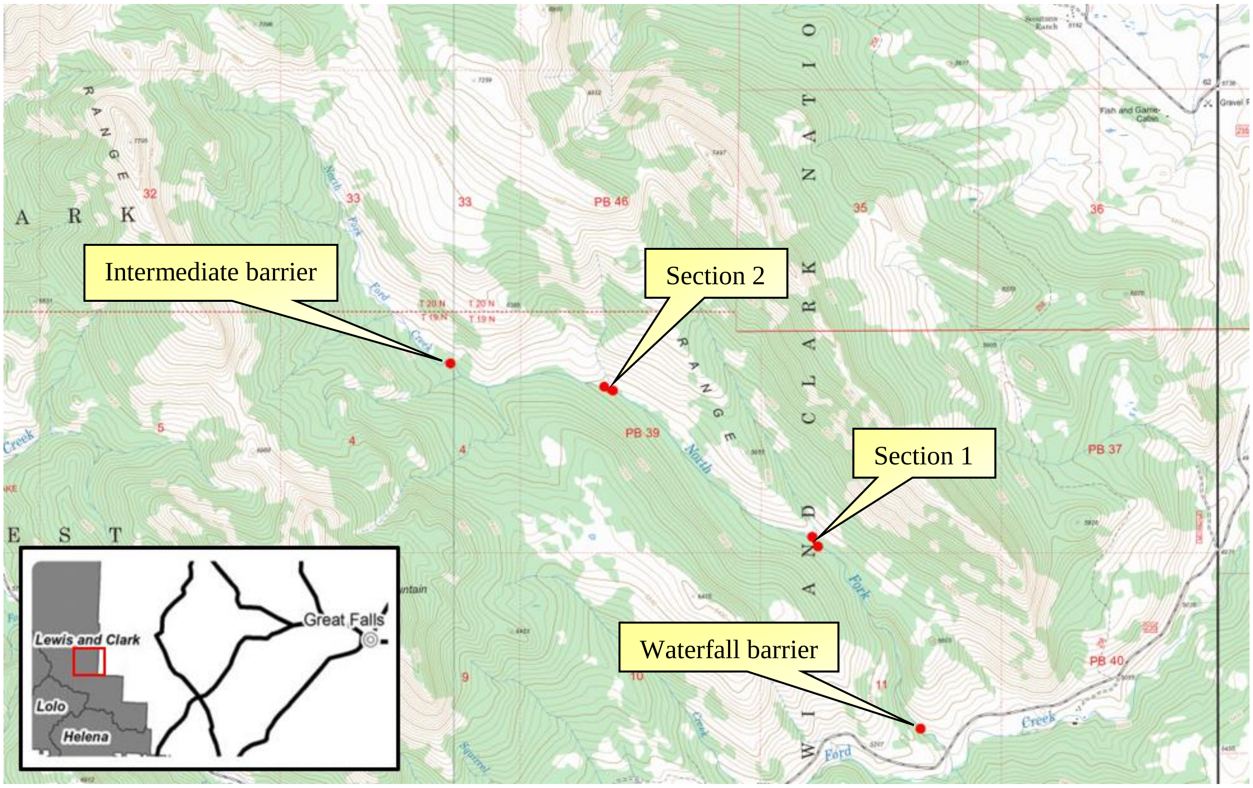


Figure 35. North Fork Ford Creek in the Sun River subbasin. The stream segments delineated in red indicate the areas sampled in 2020.

Background

North Fork Ford Creek contains a nonhybridized population of WCT above a significant waterfall barrier located approximately 1.03 km above FS RD 235 (Figure 35). This population was established following the transfer of 209 WCT from East Fork Big Spring Creek (Judith River drainage) in 2004 and 2005. Subsequent sampling of North Fork Ford Creek from 2007-2015 documented a well established WCT population with high abundance of age-0 and age-1 fish. Heavy grazing and its accompanied deleterious impacts have been noted in North Fork Ford Creek and high levels of fine sediment have been observed throughout the creek.

2020 Monitoring

North Fork Ford Creek was surveyed on August 31st and September 1st, 2020 to assess the current status of the WCT population. Two 100 m fish population estimate sections were established in proximity to previous years sampling locations. Multiple pass depletion methods were used to estimate population abundance. A robust population of WCT was found in both estimate sections with an estimated 71 and 98 age-1 and older fish occurring in section 1 and section 2, respectively. Several age-0 WCT were observed in both sections but were not included in population estimates. Adult and larval tailed frogs were also observed at both sections. Heavy grazing, degraded range conditions, and high sediment loads were again noted throughout the drainage.

North Fork Ford Creek —NATIVE TROUT POPULATION SURVEY

1. General Information— Date: **August 31, September 1, 2020** Biologist: **A. Poole**
2. Stream Information—
   Name, section, county: **North Fork Ford Creek, 38, 39, Lewis and Clark**
3. Survey Site Information (see attached map)—
   Upstream range of native trout (general description and GPS): **intermediate barrier (estimated; 47.43424, -112.74980)**
   Downstream range of native trout (general description and GPS): **natural waterfall barrier (47.41230, -112.70860)**
   Location (GPS) and description of barriers: **natural waterfall barrier (46.89330, -110.73450), intermediate barrier (estimated; 47.43424, -112.74980)**
   Stream Length—Occupied habitat: **3.67 km (2.28 mi)** Available habitat: **6.92 km[1] (4.29 mi)**
   Survey method & equipment: **backpack battery electrofisher; two and three-pass depletion**
   Survey sites (general description and UTM)—
   Section 1: **0.72 km upstream of natural waterfall barrier; 47.42324 -112.71767**
   Section 2: **3.28 km upstream of natural waterfall barrier; 47.43259 -112.73589**

Parameter	**Section 1**	**Section 2**
Section length (m)	100 m	100 m
Mean stream width (m) (n)	3.81 m (10)	3.01 m (10)
Section area (hectares)	0.038 ha	0.030 ha
WCT		
Removal Pattern	44 18 6	61 24
Population estimate	71 (±5)	98 (±17)
Capture probability	0.636	0.607
Mean length (mm) (n)	138 (68)	115 (85)
Mean weight (g) (n)	NA	22 (85)
Mean KTL (n)	NA	0.89 (19)
Number fish per km (95 % CI)	710 (±50)	980 (±170)
Number fish per ha (95 % CI)	1,868 (±132)	3,266 (±567)
Biomass (kg per ha) (95 % CI)	NA	72 (±13)

4. Comments:

[1] **–** Includes 1.03 km (0.64 mi) of habitat between FS RD 235 and the natural waterfall barrier and 2.22 km (1.38 mi) above the intermediate barrier.

Figure 36. North Fork Ford Creek fish population estimation results.

VI. Teton River Subbasin

North Fork Willow Creek

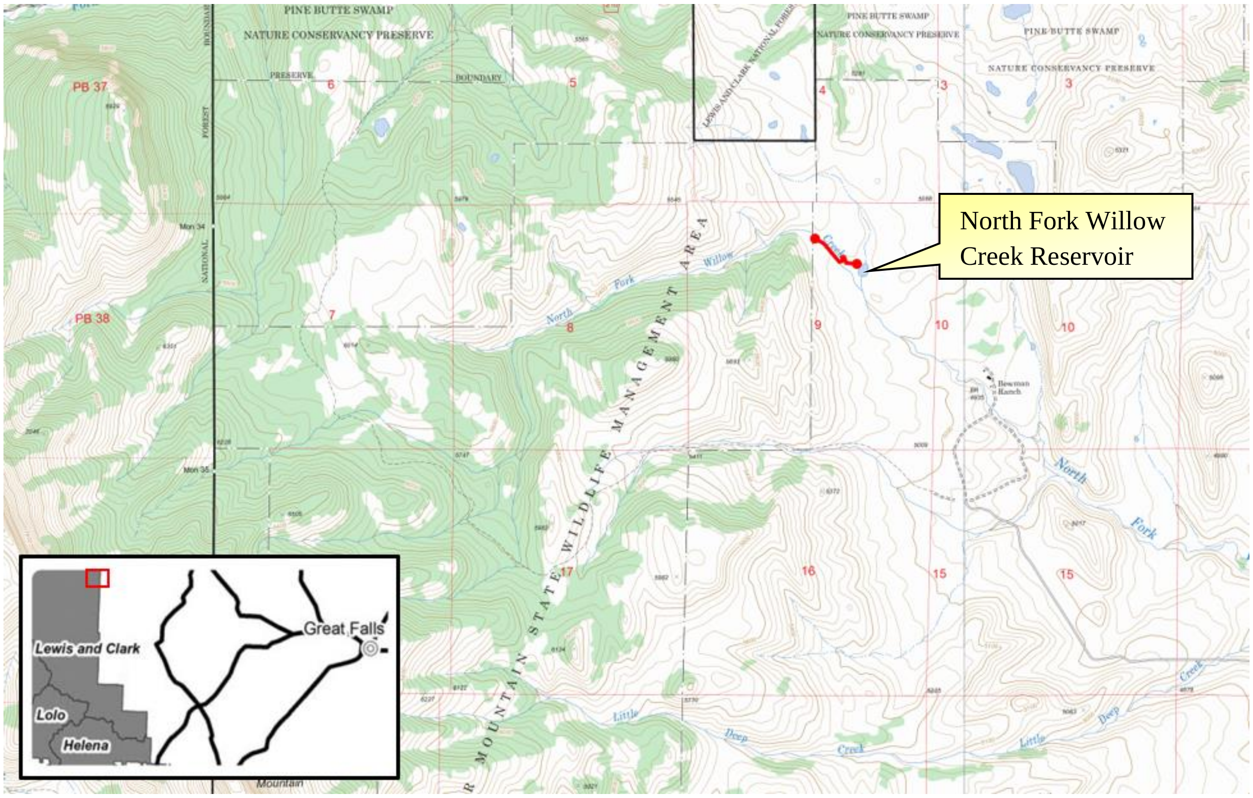


Figure 37. North Fork Willow Creek in the Teton River subbasin. The stream segment delineated in red indicates the area sampled in 2020.

Background

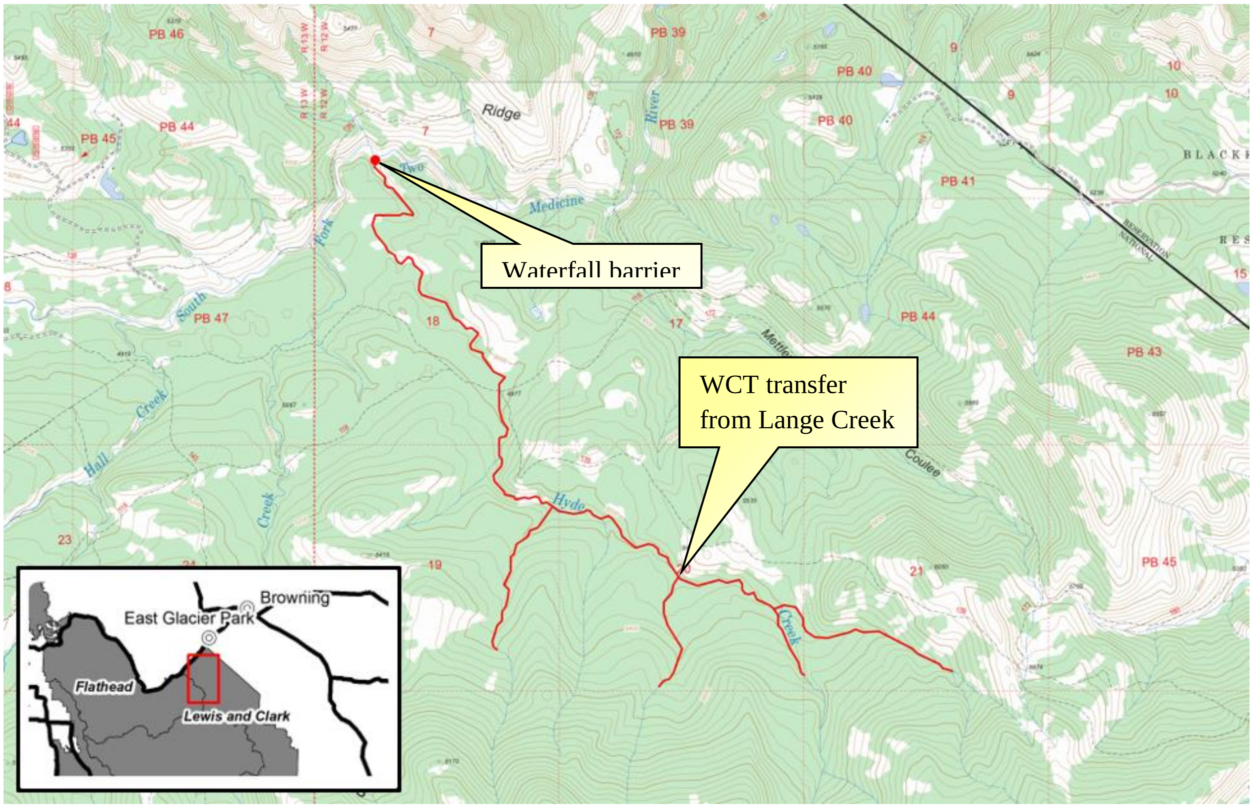
North Fork Willow Creek contains a likely slightly hybridized population of WCT thought to be protected from nonnative fish by several downstream barriers including beaver dams, the Pine Butte Swamp, and a dry channel. North Fork Willow Creek reservoir, located on the Bowman Ranch, is currently occupied by a small adfluvial population of WCT (Figure 37). These WCT represent a small fraction of the fish biomass, which primarily consists of White Suckers *Catostomus commersoni* and Northern Redbelly Dace *Phoxinus eos*. The North Fork Willow Creek WCT population was replicated in Lange Creek (Sun River drainage) after the transfer of 140 and 125 fish in 2009 and 2010, respectively.

2020 Monitoring

North Fork Willow Creek and reservoir were sampled on July 27th, 2020 to assess the potential of the WCT population as a source for transfer to Hyde Creek. Four 1/4” trap nets were used to collect fish in the reservoir and the creek was electrofished upstream to the Ear Mountain Wildlife Management Area boundary. Only White Suckers and Northern Redbelly Dace were collected in the reservoir trap nets. Backpack electrofishing of the creek upstream of the reservoir yielded a catch of 25 WCT in about 225 m of stream. These fish appeared to be primarily age-1 ranging in size from 76-116 mm in length. The North Fork Willow Creek WCT population was removed from consideration as a source for transfer to Hyde Creek based on the low densities observed.

VII. Two Medicine River Subbasin

Hyde Creek


Waterfall barrier

WCT transfer from Lange Creek

Figure 38. Hyde Creek in the Two Medicine River subbasin. The stream segments delineated in red indicate the areas sampled in 2020.

<u>Background</u>

A significant waterfall barrier at the mouth of Hyde Creek prevents upstream movement of nonnative fish from the South Fork Two Medicine River. Approximately 5.5 miles of Hyde Creek and tributaries were chemically treated in 2014 to remove nonnative Brook Trout. Subsequent sampling in 2015 found Brook Trout had persisted in Hyde Creek. Hyde Creek and tributaries were chemically treated again in 2018 following extensive pretreatment work. Posttreatment surveys following the 2018 treatment did not detect the presence of fish. Hyde Creek was again treated in 2019 to ensure a complete removal of nonnative fish. No fish were observed during the course of the 2019 treatment.

<u>2020 Monitoring</u>

Hyde Creek was surveyed on August 5th and 6th, 2020 to detect the presence of nonnative fish following the 2019 chemical treatment. The majority of the system was backpack electrofished over the two-day period (Figure 38). No fish were detected during this effort. The findings of the 2020 electrofishing effort corroborate the 2019 treatment observations indicating a successful removal had been achieved on Hyde Creek. On September 29th, 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.

VIII. Upper Missouri-Dearborn River Subbasin

Big Coulee Creek

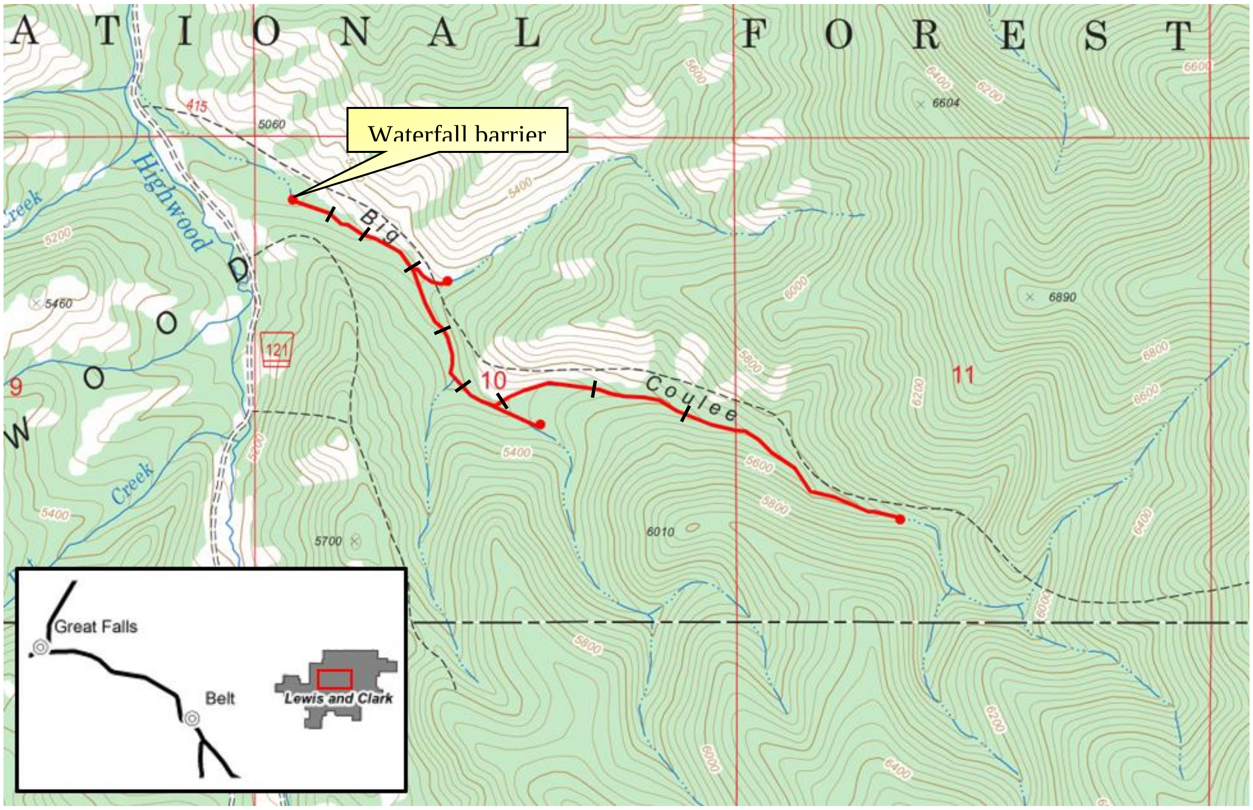


Figure 39. Big Coulee Creek in the Upper Missouri-Dearborn River subbasin. The stream segments delineated in red indicate the areas sampled in 2020. Black lines indicate section breaks.

Background

Big Coulee Creek, 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 Creek 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 2019, approximately 663 Brook Trout were removed including ~200 in 2015, ~330 in 2016, ~110 in 2017, 15 in 2018, and 8 in 2019. Majority of Brook Trout removed prior to 2018 appeared to be age-0 and age-1 fish with very few large adults found during removal efforts.

2020 Monitoring

Brook Trout removal was performed on 2.83 km of Big Coulee Creek from August 10th-13th, 2020. Eight sections of Big Coulee Creek were two pass electrofished and an additional ninth section was electrofished in a single pass effort (Figure 39). A total of 3,711 WCT and 8 Brook Trout were collected in the 2020 removal effort (Table 2). Brook Trout were distributed between the confluence of the lower and upper tributary with a single Brook Trout collected in the lower tributary. All Brook Trout appeared to be age-1 fish of the same year class.

Table 2. Big Coulee Creek electrofishing catch by section.

	Section 1	**Section 2**	**Section 3**	**Section 4**	**Section 5**	**Section 6**	**Section 7**	**Section 8**	**Section 9**
Pass 1	211 WCT	246 WCT	390 WCT	460 WCT 3 EB	346 WCT 3 EB	246 WCT	402 WCT	325 WCT	414 WCT
Pass 2	51 WCT	92 WCT	90 WCT	98 WCT 1 EB	76 WCT	71 WCT 1 EB	89 WCT	88 WCT	

Falls Creek

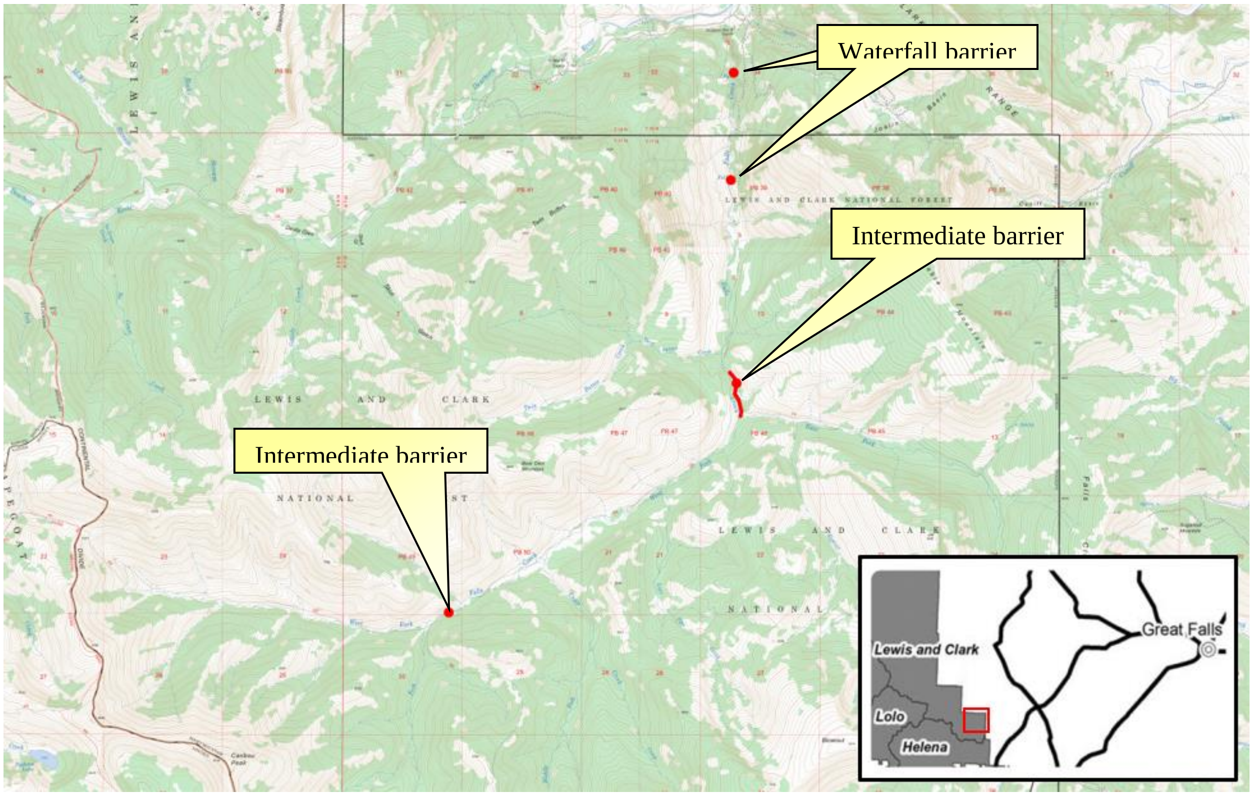


Figure 40. Falls Creek in the Upper Missouri-Dearborn River subbasin. The stream segments delineated in red indicate the areas sampled in 2020.

Background

Falls Creek, a tributary of the Dearborn River, has long been identified as a potential WCT restoration site because of the presence of large natural waterfall barriers. The Falls Creek drainage was historically fishless above the first major waterfall but has been stocked with Brook Trout and Rainbow Trout. A 2019 Forest Service land acquisition at the mouth of Falls Creek has increased accessibility to the drainage. This change in land ownership would simplify logistics of a potential future WCT restoration project.

2020 Monitoring

A site visit was conducted on Falls Creek on August 27th, 2020 to evaluate the potential for a WCT restoration project. Two intermediate barriers were surveyed to evaluate their ability to restrict fish movement thereby breaking up the potential treatment area into smaller sections or phases (Figure 40; Clancey et al. 2019). The lower intermediate barrier is located below the confluence of East and West Fork Falls Creek (Figure 41) and the upper intermediate barrier is located on West Fork Falls Creek (Figure 42). Fish were collected, marked, and released below the lower intermediate barrier to evaluate its ability to restrict upstream movement. A total of 56 fish (Brook Trout and Rainbow Trout) were collected and adipose clipped from a 650 m section of Falls Creek and released immediately below the barrier. Continued monitoring will be completed to evaluate the efficacy of this barrier. The upper intermediate barrier was only visually inspected because of time constraints. Previous sampling conducted by the Forest Service in 1994 and 2004 indicated that this was a complete barrier to fish passage with no fish found upstream of this point. However, sampling above this intermediate barrier should be conducted again based on the visual assessment.

Figure 41. Intermediate barrier located below the confluence of East and West Fork Falls Creek (47.23358, -112.48278).

Figure 42. Intermediate barrier on West Fork Falls Creek (47.20630, -112.53442).

North Fork Highwood Creek

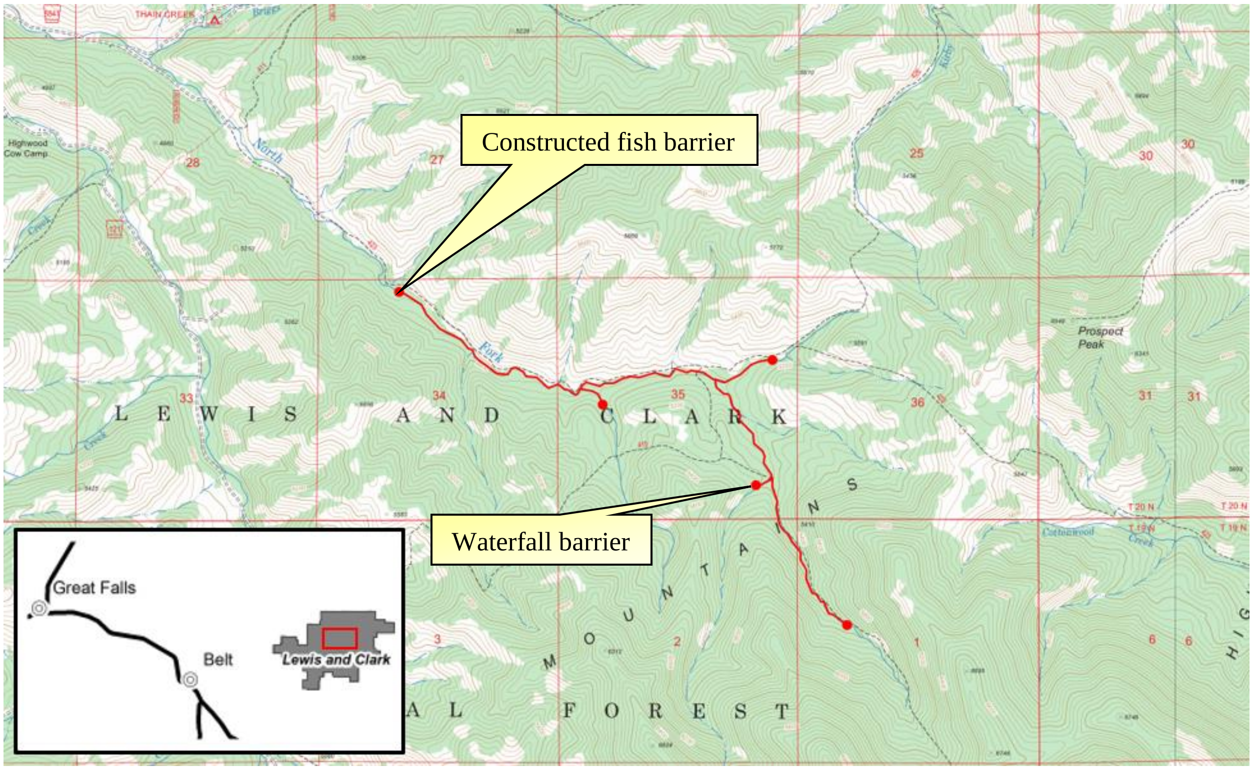


Figure 43. North Fork Highwood Creek in the Upper Missouri-Dearborn River subbasin. The stream segments delineated in red indicate the areas sampled in 2020.

Background

North Fork Highwood Creek contains a nonhybridized population of WCT that is restricted to a small tributary stream above a natural waterfall barrier. WCT restoration efforts began in 2011 with the construction of a fish barrier on the mainstem North Fork Highwood Creek approximately 1.75 km upstream of the Forest Service trailhead. North Fork Highwood Creek was chemically treated three times from 2012 through 2014 to remove nonnative Brook Trout above the constructed fish barrier. However, backpack electrofishing of the creek in 2015 found Brook Trout present just downstream of the natural waterfall barrier that protects the nonhybridized WCT population. Extensive backpack electrofishing of North Fork Highwood Creek in 2015 found 5 Brook Trout in a 100 m reach downstream of the WCT bearing tributary. Subsequent shocking since 2015 has not detected Brook Trout above the constructed barrier. In 2017, 500 adipose clipped triploid WCT were stocked at two locations upstream of the constructed barrier to provide recreational opportunities for anglers as the wild population expands.

2020 Monitoring

North Fork Highwood Creek was surveyed on August 19th and 20th, 2020. A single pass backpack electrofishing effort was conducted on the mainstem portion of the creek from the constructed fish barrier to the upstream end of fish distribution (Figure 43). Several tributaries were also electrofished in this effort. A total of 827 WCT were collected and no Brook Trout were detected. Of the 827 WCT collected, only 60 were found to be adipose clipped triploids located predominately in the lower reach close to the constructed barrier. The wild WCT population appears to be expanding and well established in the headwaters of the drainage. However, densities in the lower 2.12 km of the creek were low with only 70 WCT collected.

Wegner Creek

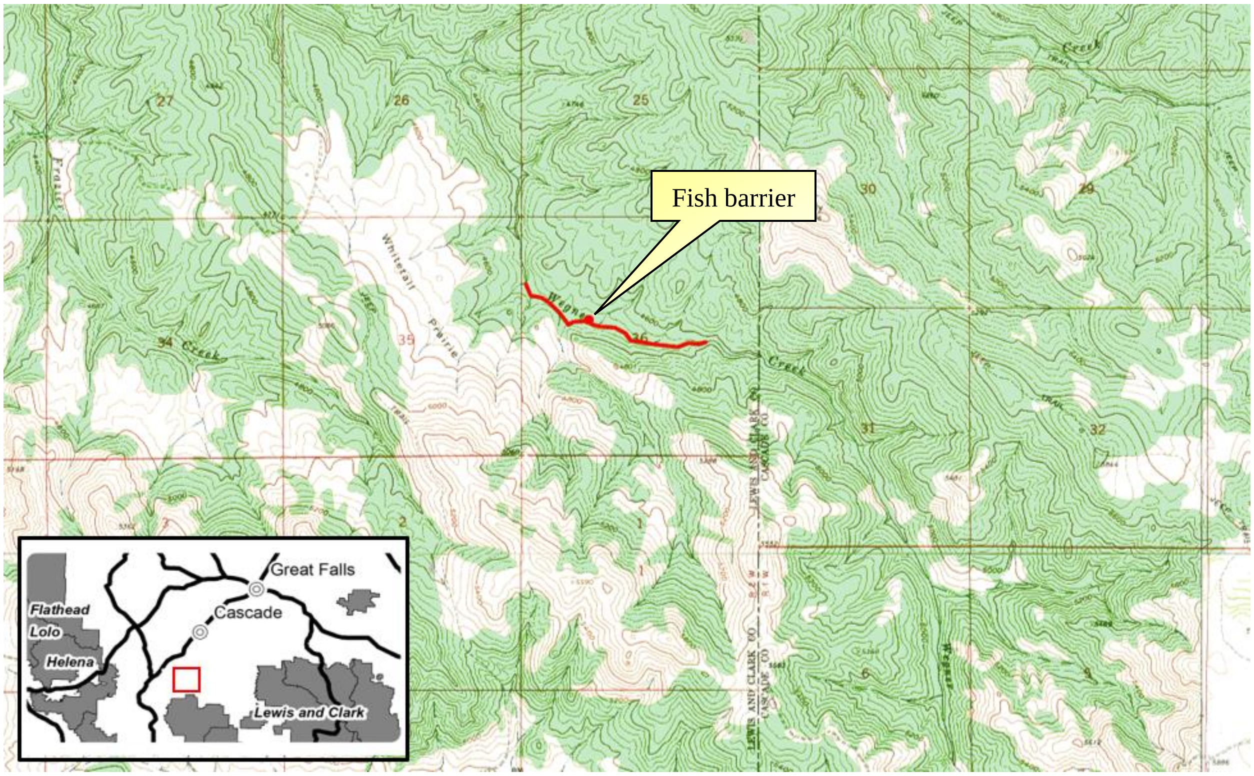


Figure 44. Wegner Creek in the Upper Missouri-Dearborn River subbasin. The stream segment delineated in red indicates the area sampled in 2020.

Background

In 2014, the Beartooth Wildlife Management Area expanded by 2,840-acres and that addition included 1.29 km of Wegner Creek (Figure 44). 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.

2020 Monitoring

Wegner Creek was surveyed on May 5th and July 8th, 2020 to evaluate the efficacy of the barrier. On May 5th, a short section of Wegner Creek was electrofished above the barrier to detect the presence of large Rainbow Trout as observed in 2018. Only age-1 Rainbow Trout (55-77 mm) and Rocky Mountain Sculpin were observed in this effort. A 100 m section of Wegner Creek below the barrier was also electrofished. An additional 25 Brook Trout and 11 Rainbow Trout were adipose clipped and released below the barrier. Wegner Creek was visited again on July 8th, 2020. A 900 m section of stream above the barrier was electrofished to detect the presence of marked fish that may have bypassed the barrier. A total of 97 Rainbow Trout were collected and removed in this effort; no marked fish were detected. All Rainbow Trout collected appeared to be age-1 fish of the same year class (Figure 45). Additionally, a trail camera was installed at the barrier site to document barrier performance under different flow conditions.

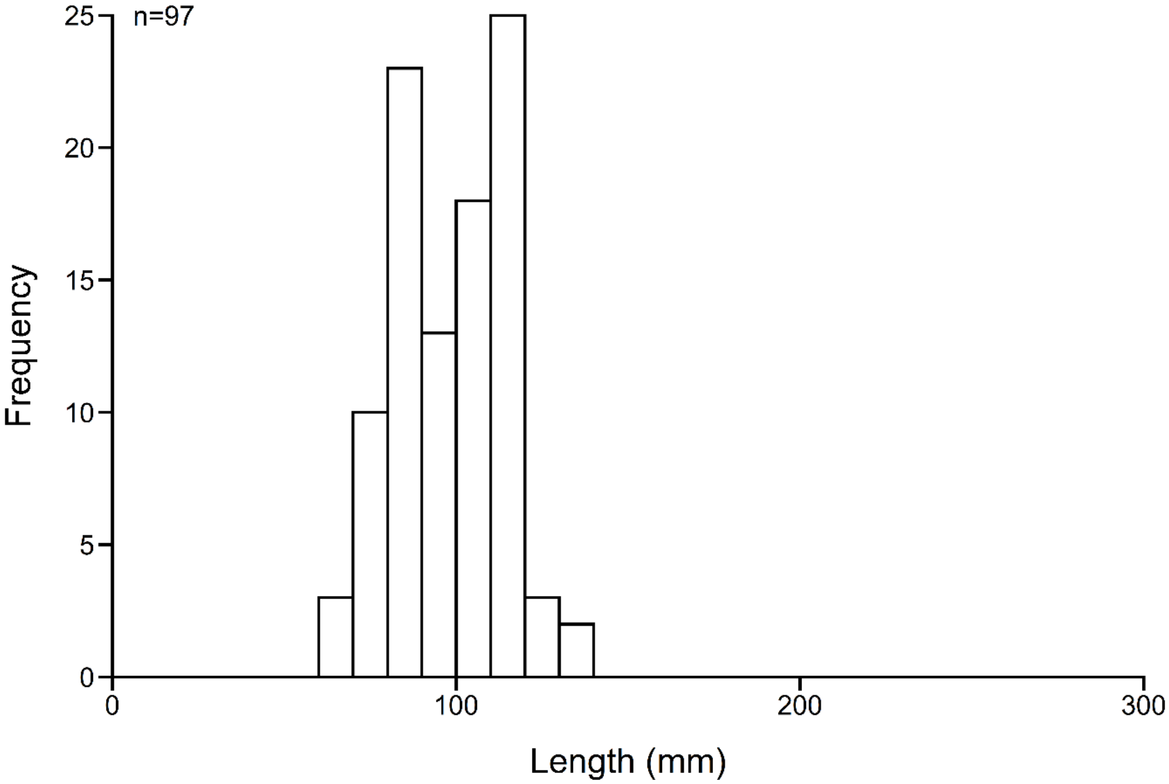


Figure 45. Length-frequency histogram of RB collected above the barrier on Wegner Creek on July 8th, 2020.

Literature Cited

Allendorf, F. W. and R. F. Leary. 1988. Conservation and distribution of genetic variation in a polytypic species, the Cutthroat Trout. Conservation Biology 2:170-184.

Behnke, R. J. 2002. Trout and salmon of North America. The Free Press, New York.

Carle, F. L. and M. R. Strub. 1978. A new method for estimating population size from removal data. Biometrics 34:621-630.

Clancey, P. T., B. B. Shepard, C. G. Kruse, S. A. Barndt, L. Nelson, B. C. Roberts, and R. B. Turner. 2019. Collaboration, commitment, and adaptive learning enable eradication of nonnative trout and establishment of native Westslope Cutthroat Trout into one-hundred kilometers of Cherry Creek, a tributary to the Madison River, Montana. American Fisheries Society, Symposium 91:589-647.

Heckel, J. W., M. C. Quist, C. J. Watkins, and A. M. Dux. 2020. Distribution and abundance of Westslope Cutthroat Trout in relation to habitat characteristics at multiple spatial scales. North American Journal of Fisheries Management 40:893-909.

Muhlfeld, C. C., V. S. D'Angelo, C. Downs, J. Powell, S. Amish, G. Luikart, R. Kovach, M. Boyer, and S. Kalinowski. 2016. Genetic status and conservation of Westslope Cutthroat Trout in Glacier National Park. Transactions of the American Fisheries Society 145:1093-1109.

MFWP (Montana Department of Fish, Wildlife and Parks). 1999. Memorandum of understanding and conservation agreement for Westslope Cutthroat Trout *(Oncorhynchus clarkii lewisi)* in Montana. Montana Department of Fish, Wildlife and Parks.

MFWP (Montana Department of Fish, Wildlife and Parks). 2007. Memorandum of understanding and conservation agreement for Westslope Cutthroat Trout and Yellowstone Cutthroat Trout in Montana. Montana Department of Fish, Wildlife and Parks.

Moser, D., A. Tews, D. Yerk, G. Grisak, G. Liknes, M. Enk, and A. Harper. 2009. Status and conservation needs for Westslope Cutthroat Trout in northcentral Montana. Montana Fish, Wildlife and Parks, Great Falls, MT.

Moser, D., A. Tews, D. Yerk, G. Grisak, G. Liknes, M. Enk, A. Harper, and K. Cikanek. 2011. Status and conservation needs for Westslope Cutthroat Trout in northcentral Montana. Montana Fish, Wildlife and Parks, Great Falls, MT.

Shepard, B. B., B. E. May and W. Urie. 2003. Status of Westslope Cutthroat Trout (*Oncorhynchus clarkii lewisi*) in the United States: 2002. Westslope Cutthroat Interagency Conservation Team. 94 pp.

Shepard, B. B., B. E. May, and W. Urie. 2005. Status and conservation of Westslope Cutthroat Trout within in western United States. North American Journal of Fisheries Management 25:1426-1440.

Shepard, B. B. B. Sanborn, L. Ulmer, and D. C. Lee. 1997. Status and risk extinction for Westslope Cutthroat Trout in the upper Missouri River Basin, Montana. North American Journal of Fisheries Management 17:1158-1172.

Tews, A., M. Enk, W. Hill, S. Dalbey, G. Liknes and S. Leathe. 2000. Westslope Cutthroat Trout (*Oncorhynchus clarkii lewisi*) in northcentral Montana: status and restoration strategies. Montana Fish, Wildlife and Parks in collaboration with the Lewis and Clark National Forest, Great Falls, MT.

USEPA (U.S. Environmental Protection Agency). 2019. Final remedial investigation report, Barker-Hughesville mining district superfund site, Cascade and Judith Basin Counties, Montana. USEPA, Work Assignment No. 3383-325, Helena, Montana.

Zippin, C. 1958. The removal method of population estimation. Journal of Wildlife Management 22:82-90.

Appendix A

Date: July 21, 2020
Biologist(s): Katie Vivian
Location(s) and sampling date:

1. Lange Creek (6/23/2020)

Agency: Montana Fish, Wildlife & Parks
Target species: Westslope cutthroat trout
Authors: Ryan Kovach, Sally Painter, Angela Lodmell

PROJECT SUMMARY: Genetic samples from Lange 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.

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 westlope 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/westlope 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
Lange Creek		16	WCT?			

Lange Creek

We detected one rainbow trout allele at one rainbow diagnostic marker, and two Yellowstone cutthroat trout alleles at one Yellowstone diagnostic marker. None of the westslope diagnostic markers were polymorphic. Lange Creek was historically fishless, but was stocked with fish from North Fork Willow Creek in 2008. Thus, we compared these genetic results to past results from North Fork Willow Creek.

The two Yellowstone alleles were detected at a marker that was also polymorphic in a recent sample (2018) from North Fork Willow Creek (*OclYSD113109_Garza*; see sample #5120). It seems quite likely that the variation we consistently detect at that marker may be rare westslope cutthroat trout genetic variation rather than Yellowstone cutthroat trout hybridization. In contrast, the single rainbow trout allele that we detected was at a different marker (*OmyRD_RAD_30378_Hoh*) than another single rainbow trout allele we detected in the 2018 North Fork Willow Creek sample (*FLU_OmyRD_RAD_5666_Hoh*). Both of those markers typically have very robust diagnostic properties (i.e., westslope cutthroat trout genetic variation is rare at those markers). The fact that we have now observed rainbow trout alleles are multiple diagnostic markers greatly increases the probability that there is some rainbow trout ancestry in the North Fork Willow Creek population and thus Lange Creek as well. If the observed variation is representative of rainbow trout hybridization, the proportion rainbow trout admixture is ~0.2%. It important to note that we did not detect rainbow trout alleles in North Fork Willow Creek in a number of previous samples collected prior to 2018 (#425, #1994), #2139, and #3448/3449), and combined, our power to detect the same amount of hybridization was high (>99% chance of detecting 0.2% rainbow trout ancestry in a hybrid swarm). Thus, it is plausible that the observed variation represents westslope cutthroat trout genetic variation rather rainbow trout hybridization, but we cannot rule out the alternative until additional data are collected (either more samples or use genomic approaches with the existing samples).

From a management perspective, using fish from Lange Creek for translocation does come with some genetic risk. In a best-case scenario, the population of trout in Lange Creek are non-hybridized westslope cutthroat trout. Alternatively, fish in Lange Creek may have a very trivial amount of rainbow trout ancestry. It is probably best to assume the latter scenario is accurate, but this does not necessarily preclude translocation depending on context. In either scenario, the fish from Lange Creek and North Fork Willow Creek still have very high conservation value given the rarity of westslope cutthroat trout in that portion of their historical range.

Methods and Data Analysis

We developed a 'chip' specifically for analysis of supposed westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) samples. 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 (*O. c. bouvieri*) 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, Table 1). 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 (Table 2).

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.

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 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 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.

The chip also contained 34 loci that are generally polymorphic within westslope cutthroat trout populations. Information from these loci can be used to address issues concerning the relative amount of genetic variation within and divergence among westslope cutthroat trout populations.
Finally, the chip contained two mitochondrial DNA (mtDNA) loci that differentiate cutthroat and rainbow trout. Data from these loci were used only if an individual appeared to be an F_1 hybrid. Because mtDNA is inherited only from females (maternal inheritance), in this situation we can determine the taxon of the female, and by default the taxon of the male, that produced the hybrid.

When two or more samples were collected from the same area of a water body in different years or different reaches of a stream in the same or different years, we used the log likelihood G test of Goudet et al. (1996) in GENEPOP version 4.2 (Rousset 2008) to test for genetic (allele frequency) differences among the samples. In instances where multiple loci were compared among samples and some demonstrated significant differences, significance was determined using Rice's (1989) method for correcting for multiple comparisons (modified level of significance). When no differences were detected at the modified level, any observed differences were considered to most likely represent chance departures from homogeneity and the samples were usually combined for further analysis. When evidence of genetic differences was detected among samples they were generally kept separate for analysis and the relative amount of divergence among them was estimated as F_{ST} using the method of Weir and Cockerham (1984) available in GENEPOP version 4.2. Assuming it is at equilibrium, F_{ST} allows one to assess the relative amount of historic gene flow between populations, i.e. the degree of reproductive isolation.

In samples containing 10 or more individuals appearing to be non-hybridized westslope cutthroat trout, we compared the observed genotypic distributions at the polymorphic loci to expected random mating genotypic proportions (Hardy-Weinberg proportions) using *pegas* package (Paradis 2010) in Program R. A deficit of observed heterozygotes can arise in a sample if it contains individuals from two or more genetically divergent populations or the fish in it are experiencing a fair to high amount of inbreeding at the population level. Conversely, fish produced from a very small number of parents may show an excess of heterozygotes compared to expected random mating proportions (Pudovkin et al. 1996, 2010; Luikart and Cornuet 1999). Since multiple comparisons were performed in most cases, significance was again determined at the modified level.

In samples showing significant departures of observed genotypic distributions from expected Hardy-Weinberg genotypic proportions at four or more loci (note four or more loci are required to establish a statistically significant trend), we used chi-square analysis of only these loci to determine if there was a significant trend for these loci to show an excess or deficit of heterozygotes. In each sample we also used all the polymorphic loci regardless of significance to determine if there was a statistically significant trend for a sample to express a slight deficit or excess of heterozygotes compared to random mating proportions. Furthermore, we calculated expected heterozygosity and the proportion of polymorphic loci to quantify genetic variation in each population, and compared observed values to values from other nonhybridized populations in Montana.

Literature Cited

Amish, S. J., P. A. Hohenlohe, S. Painter, R. F. Leary, C. Muhlfeld, F. W. Allendorf, and G. Luikart. 2012. RAD sequencing yields a high success rate for westslope cutthroat and rainbow trout species-diagnostic SNP assays. Molecular Ecology Resources 12:653-660.

Campbell, N. R., S. J. Amish, V. L. Pritchard, K. S. McKelvey, M. K. Young, M. K. Schwartz, J. C. Garza, G. Luikart, and S. R. Narum. 2012. Development and evaluation of 200 novel SNP assays for population genetic studies of westslope cutthroat trout and genetic identification of related taxa. Molecular Ecology Resources 12:942-949.

Frankham, R., J. D. Ballou, M. D. B. Eldridge, et al. 2011. Predicting the probability of outbreeding depression. Conservation Biology 25:465-475.

Finger, J. A., M. R. Stephens, N. W. Clipperton, and B. May. 2009. Six diagnostic single nucleotide polymorphism markers for detecting introgression between cutthroat and rainbow trouts. Molecular Ecology Resources 9:759-763.

Goudet, J., M. Raymond, T. deMeeus, and F. Rousset. 1996. Testing differentiation in diploid populations. Genetics 144:1933-1940.

Harwood, A. S., and R. B. Phillips. 2011. A suite of twelve single nucleotide polymorphism markers for detecting introgression between cutthroat and rainbow trout. Molecular Ecology Resources 11:382-385.

Jombart, T., S. Devillard, and F. Balloux. 2010. Discriminant analysis of principle components: a new method for the analysis of genetically structured populations. BMC Genetics 11:94.

Kalinowski, S. T., B. J. Novak, D. P. Drinan, R. deM Jennings, and N. V. Vu. 2011. Diagnostic single nucleotide polymorphisms identifying westslope cutthroat trout (*Oncorhynchus clarkii lewisi*), Yellowstone cutthroat trout (*Oncorhynchus clarkii bouvieri*) and rainbow trout (*Oncorhynchus mykiss*). Molecular Ecology Resources 11:389-393.

Kalinowski, S. T., A. P. Wagner, and M. L. Taper. 2006. ML-RELATE: a computer program for maximum likelihood estimation of relatedness and relationship. Molecular Ecology Notes 6:576-579.

Luikart, G. and J.-M. Cornuet. 1999. Estimating the effective number of breeders from heterozygote excess in progeny. Genetics 151:1211-1216.

Paradis E. 2010. pegas: an R package for population genetics with an integrated-modular approach. Bioinformatics 26: 419-420.

Pew, J., P. H. Muir, J. Wang, and T. R. Frasier. 2015. Related: an R package for analysing pairwise relatedness from codominant molecular markers. Molecular Ecology Resources 15:557–561.

Pudovkin, A. I., O. L. Zhdanova, and D. Hedgecock. 1996. On the potential for estimating the effective number of breeders from heterozygote excess in progeny. Genetics 144:383-387.

Pudovkin, A. I., O. L. Zhdanova, and D. Hedgecock. 2010. Sampling properties of the heterozygote-excess estimator of the effective number of breeders. Conservation Genetics 11:759-771.

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution 43:223-225.

Rousset, F. 2008. GENEPOP’007: a complete re-implementation of the GENEPOP software for Windows and Linux. Molecular Ecology Resources 8:103-106.

Wang, J. 2002. An estimator for pairwise relatedness using molecular markers. Genetics 160:1203–1215.

Weir, B. S., and C. C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution 28:1358-1370.

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