Northcentral Montana Westslope Cutthroat Trout Restoration Project

2023 Annual Report

MoTAC Project # 2023-7

MT Aquatic SWG Management Program # F19AF00704

MT Aquatic SWG Survey & Inventory Program # F19AF00705

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Region 4

Great Falls, MT

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Introduction

Westslope cutthroat trout (WCT) *Oncorhynchus clarkii lewisi*, historically the most widely distributed subspecies of cutthroat trout *O. clarkii*, have undergone reductions in distribution and abundance throughout their native range (Behnke 2002; Shepard et al. 2005; Heckel et al. 2020). The upper Missouri River drainage in Montana in particular has experienced marked reductions, with WCT occupying less than 5% of their historical range (Shepard et al. 1997; Shepard et al. 2003). Nonnative species introductions, habitat degradation, fragmentation, and overexploitation have been identified as factors leading to population declines (Shepard et al. 2005; Muhlfeld et al. 2016; Heckel et al. 2020). However, human-induced hybridization with nonnative trout has been especially detrimental causing widespread genomic extinction of WCT populations (Allendorf and Leary 1988; Muhlfeld et al. 2014, Bourret et al. 2022).

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

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

A Federal Challenge Cost Share Agreement was established in 2001 between FWP and the USFS to implement and fund WCT restoration (Tews et al. 2000) as outlined by the MOU. Funding for the 2015 WCT restoration project was provided by the EPA and the State Wildlife Grants (SWG) program. In the 2016-2019 period, Northwestern Energy (formerly PPL Montana), Resource Development Grant Program (RDGP), and the Future Fisheries Program (FWP) provided additional funding for WCT restoration. At the November 2022 Missouri River Technical Advisory Committee (MoTAC) meeting, FWP was awarded $27,889 from Northwestern Energy to fund a fisheries technician to work directly with the FWP native species biologist on the Northcentral Montana WCT Restoration Project. This document specifically addresses work performed under MoTAC Project # 2023-7 for WCT restoration in northcentral Montana.

Study Area

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

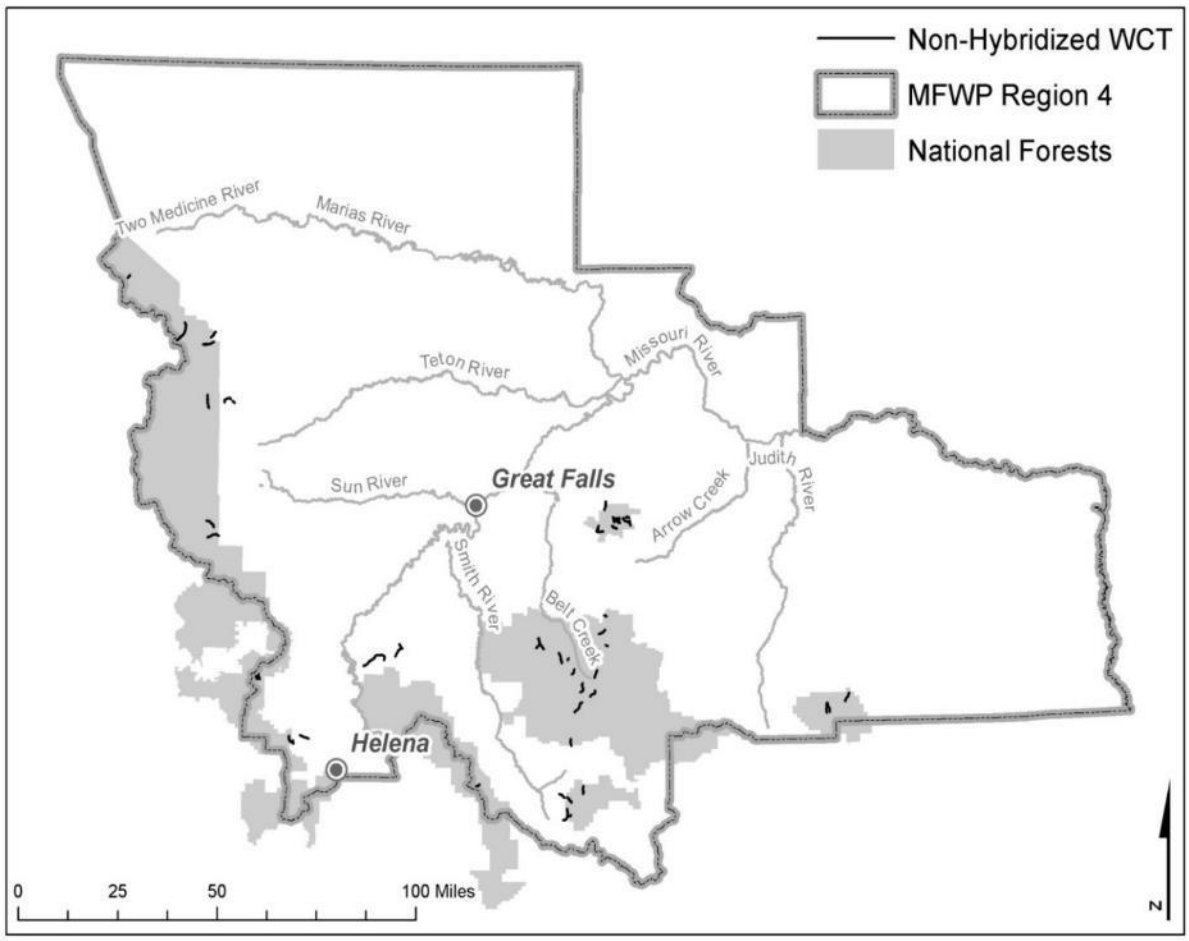

Figure 1. Study area in northcentral Montana with nonhybridized WCT populations (indicated in bold black).

Methods

Sampling of stream fish populations was conducted with a Smith-Root™ model LR-20B and/or model LR-24 battery powered backpack electrofishing unit(s) set to 30 hertz (Hz) at approximately 0.8-1.6 amperes (A) and 300-900 volts (V) dependent on conductivity. Relatively smaller streams were sampled with one backpack electrofishing unit and two backpack electrofishing units were used in tandem in larger streams and rivers. Multiple pass depletion method was typically used to estimate WCT population abundance in sampled streams (Zippin 1958; Carle and Strub 1978). Mean wetted stream width was determined by measuring ten random transects within each survey section. Stream dimensions were

combined with population estimates and mean trout weight to calculate trout density (fish/km, fish/hectare) and biomass (kg/ha). Genetic samples were collected and preserved in 95% ethanol to be sent to the University of Montana Fish Conservation Genetics Lab for genetic analysis. Total length of fish was measured to the nearest millimeter and weight was measured to the nearest gram using an electronic scale. Conductivity in microsiemens (μS) and temperatures in degrees Celsius (°C) was measured and recorded in sampled streams.

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

Conservation techniques applied during the 2023 field season include: fish barrier construction, fish barrier maintenance, fishless habitat evaluation, mechanical removal of nonnative trout, WCT demographic and genetic monitoring, and wild fish transfer.

Restoration Efforts in Northcentral Montana

The scope of the work completed by FWP in 2023 is described in the following maps, text, and histograms. The USFS and FWP worked cooperatively on many of the following projects. This report is organized by USGS hydrological unit code (HUC 8) subbasins where restoration efforts occurred and include: Arrow Creek, Belt Creek, Judith River, Smith River, Sun River, Teton River, Two Medicine River, Upper Missouri River, and Upper Missouri-Dearborn River.

I. Arrow Creek Subbasin

Boyd Creek

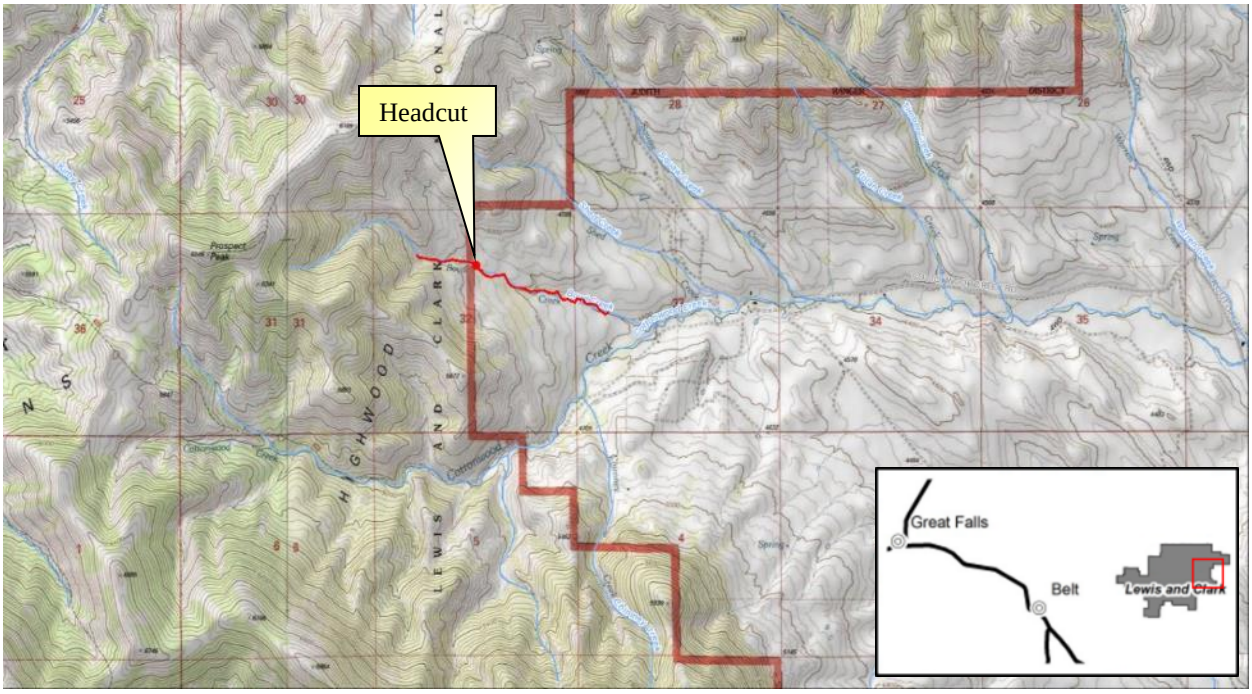

Figure 1. Boyd Creek in the Arrow Creek subbasin. Stream segments delineated in red indicate areas sampled in 2023.

Background

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

2023 Monitoring

Boyd Creek was backpack electrofished on August 8th, 2023, from the confluence of Cottonwood Creek to the headcut at the Forest Service boundary. A total of 107 WCT and 17 brook trout were collected. Young of the year WCT were abundant in the upper reaches of Boyd Creek. Brook trout density declined compared to the previous survey of Boyd Creek in 2021 where 123 brook trout were collected in the same reach.

Cottonwood Creek

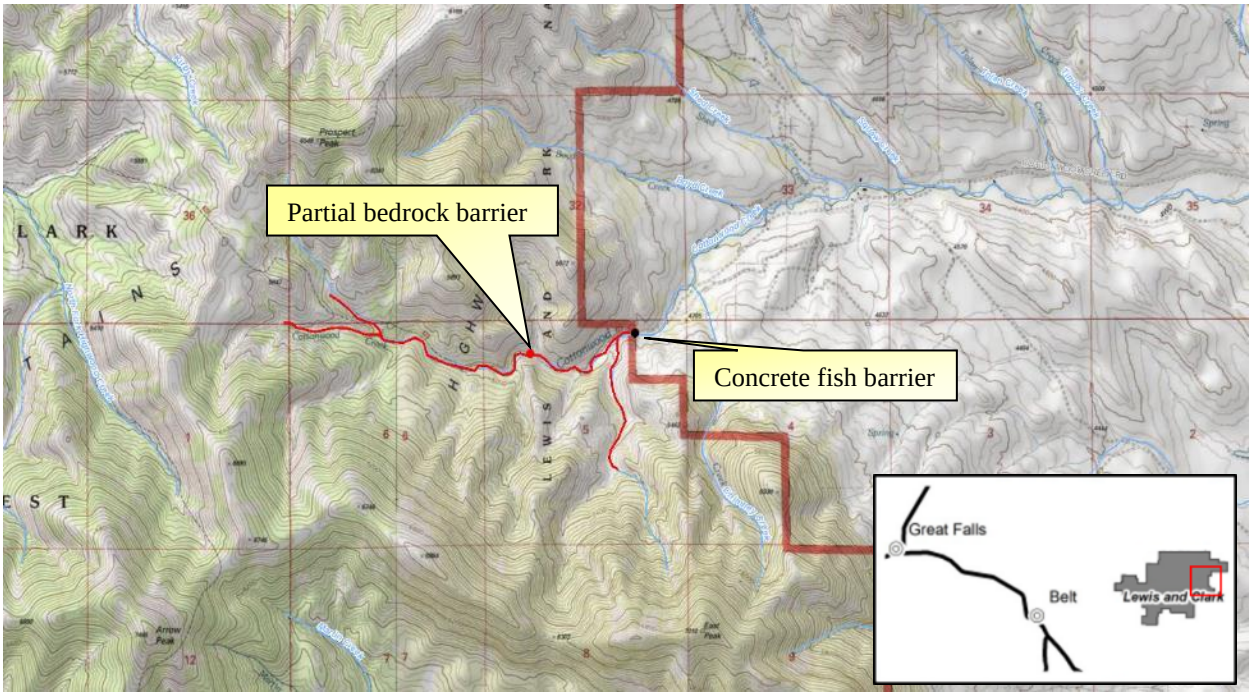

Figure 2. Cottonwood Creek in the Arrow Creek subbasin. Stream segments delineated in red indicate extent of WCT occupied habitat.

Background

Cottonwood Creek contains a nonhybridized population of WCT protected by a natural bedrock barrier. In 2001, a concrete fish barrier was installed at the Lewis and Clark National 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 bedrock barrier from 2000-2005 and appeared effective at removing all brook trout above the constructed fish barrier. Since 2005, monitoring has occurred periodically and in 2015 brook trout were detected upstream of the constructed fish barrier. The origin of these fish is unknown, as the barrier appeared structurally sound and functional during the 2015 sampling. Removals performed in the summer of 2016-2019 resulted in the removal of 34 brook trout. No brook trout were detected above the constructed fish barrier in 2020, a single brook trout was removed in 2021, and no brook trout were detected in 2022.

2023 Monitoring

A single pass electrofishing monitoring effort was performed on Cottonwood Creek on August 7th and 8th of 2023. 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 were recorded. A total of 205 WCT were collected in the mainstem of Cottonwood Creek between the barriers and 589 WCT were collected in the 1st tributary. No brook trout were detected. Annual monitoring of brook trout presence in Cottonwood Creek should continue until three consecutive years of no detections is achieved.

II. Belt Creek Subbasin

Big Timber Gulch

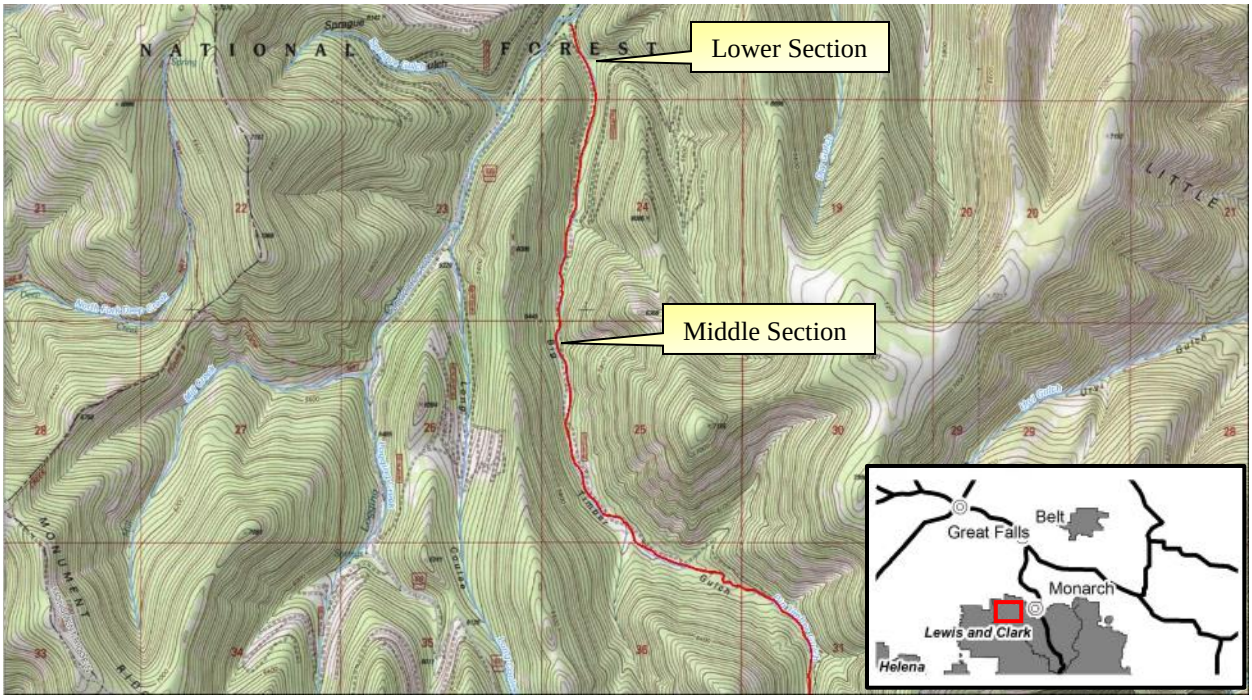

Figure 3. Big Timber Gulch in the Belt Creek subbasin. The two highlighted sections were sampled in 2023.

Background

Big Timber Gulch is a tributary of Logging Creek located approximately 10 miles west of the community of Monarch, MT. Records indicate a single sampling event took place on June 18, 2001, approximately 0.5 miles upstream of the confluence with Logging Creek. No fish were captured during 600 feet of backpack electrofishing.

2023 Monitoring

Big Timber Gulch was revisited on June 27th, 2023, to determine WCT presence. Two reaches were backpack electrofished: one near the Logging Creek confluence and one 1.5 stream miles above the Logging Creek confluence (Figure 3). Fish densities were lower at the lowest site with only eight brook trout and one WCTxRB hybrid collected in approximately 150 m of stream. Fish densities were much higher at the middle site where 29 WCT and six brook trout were collected in 150 m of stream. It is unclear why previous sampling efforts failed to detect fish presence in Big Timber Gulch. Further investigation of Big Timber Gulch is needed to determine the upper limits of fish distribution. Genetic sample collection should be prioritized as this is a previously undocumented WCT containing waterbody.

Carpenter Creek

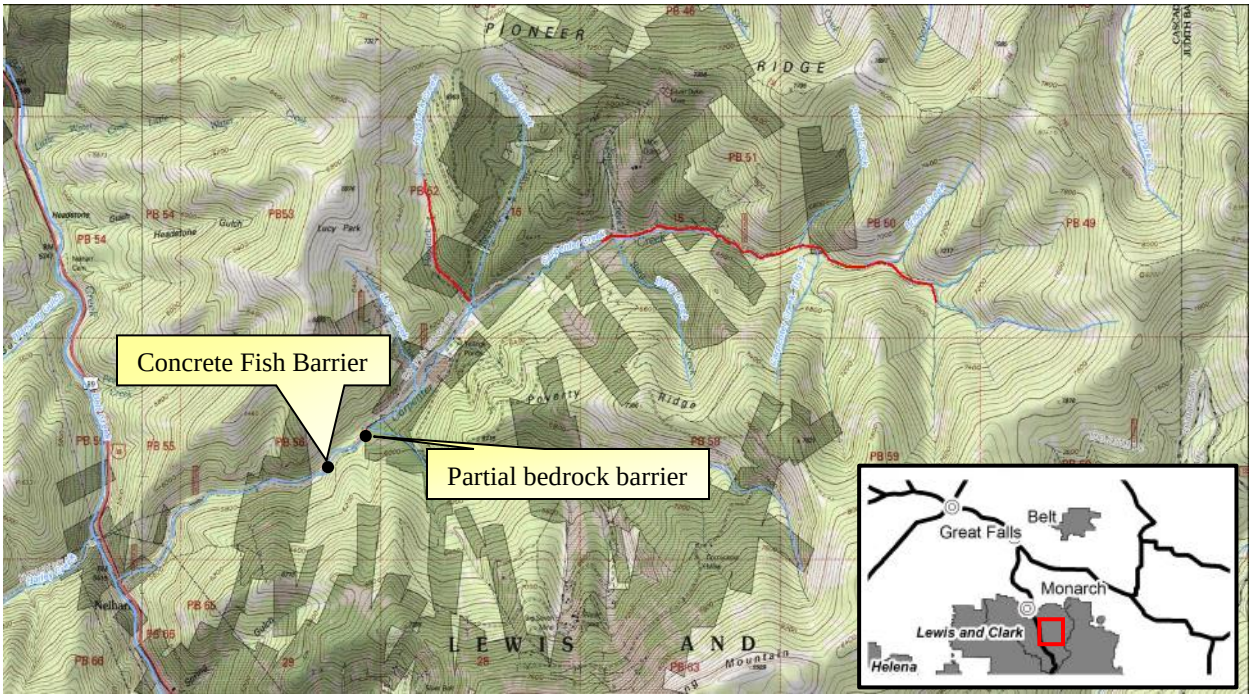

Figure 4. Carpenter Creek in the Belt Creek subbasin. The stream segments delineated in red indicate the areas occupied by nonhybridized WCT.

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 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 4). During past sampling efforts no fish have been detected in this reach; however, in 2015 two fish were caught near the mouth. The presence of fish was a positive response in improving water quality but provided concern for the potential for nonnative invasion and subsequent risk of WCT loss to hybridization. Nonnative fish have been monitored annually in this section since 2015. In 2016 seven nonnative fish were collected, 4 in 2017, 5 in 2018, 1 in 2019, 2 in 2020 and 2021, and 9 in 2022. Demographic and genetic monitoring of Carpenter Creek WCT populations was performed most recently in 2018. A total of 591 fish 100 mm and greater were estimated in Carpenter Creek over approximately 2.5 kilometers of occupied habitat.

Fish Barrier Construction

Construction of the Carpenter Creek fish barrier resumed the week of July 24th, 2023. A considerable amount of sediment and debris had been deposited on the concrete footers following spring runoff. Once the stream was diverted and the construction site cleared, crews completed the forming and pouring of the barrier walls on the week of August 14th. Pouring of the apron was completed the week of August 21st and backfilling of the site began. Backfilling, placing of riprap, removal of stream diversion, and general site grading and cleanup was performed the week of August 28th. Substantial completion of the project was achieved on September 5th.

Figure 5. Completed Carpenter Creek fish barrier.

2023 Monitoring

Upon completion of the fish barrier, Carpenter Creek was backpack electrofished from the newly constructed barrier to the partial bedrock barrier to monitor nonnative trout presence in a single pass effort on September 5th and 11th, 2023. No fish were observed in the reach of Carpenter Creek sampled.

Gold Run Creek

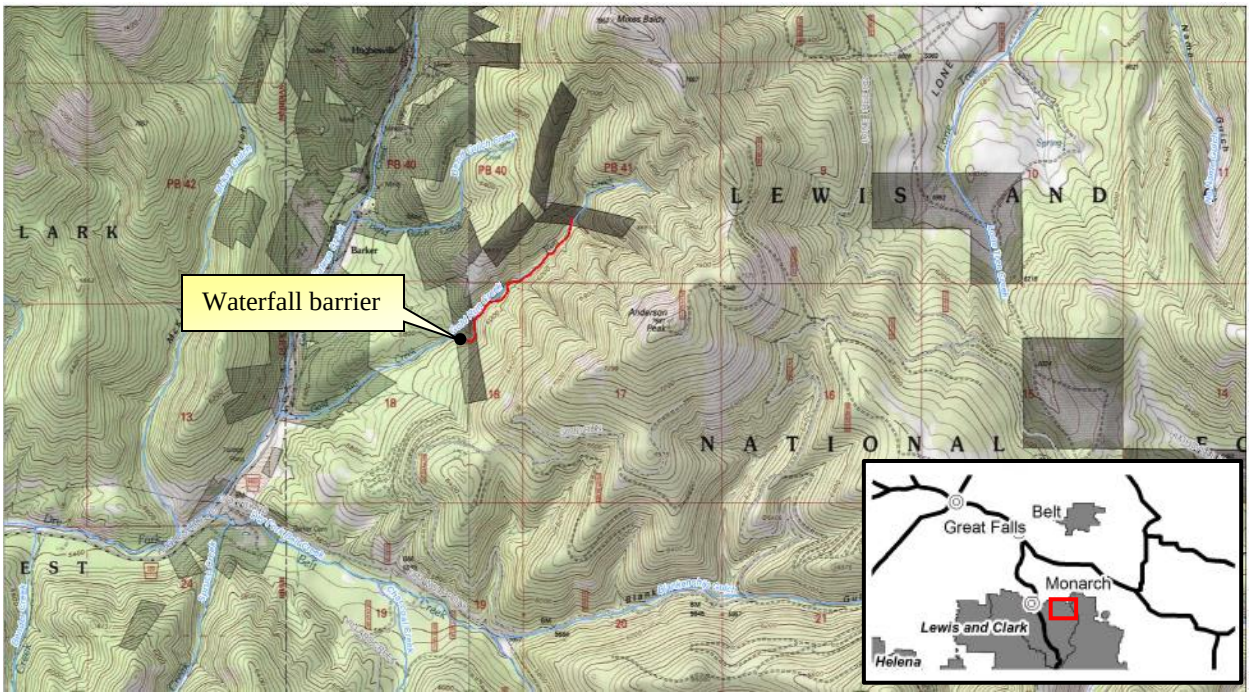

Figure 6. Gold Run Creek in the Belt Creek subbasin. The stream segment delineated in red indicates the area occupied by nonhybridized WCT.

Background

Gold Run Creek is a tributary of Galena Creek in the Dry Fork Belt Creek drainage. A nonhybridized WCT population is present above a 90 ft waterfall barrier located at 47.06454, -110.62597. This population was expanded into upstream fishless habitat from 2001-2006 and now currently occupies approximately 0.88 miles of habitat. Gold Run Creek was included in a University of Montana (UM) genetic rescue study that began in 2017. As part of this study, intensive annual demographic and genetic monitoring of this population has been performed since 2017 by UM researchers.

2023 Monitoring

FWP staff assisted UM researchers in collecting genetic samples from Gold Run Creek as part of the long-term monitoring of the genetic rescue study. Three reaches of Gold Run Creek were sampled (Lower, Middle, and Upper) until 50 previously untagged individual WCT were collected. Genetic tissue samples were collected from untagged WCT and tagged WCT were released after recording tag information. A total of 150 untagged WCT and 109 tagged WCT were collected. Data collected in 2023 will help inform demographic and genetic models for Gold Run Creek allowing for a detailed examination of genetic rescue techniques on isolated WCT populations.

Graveyard Gulch

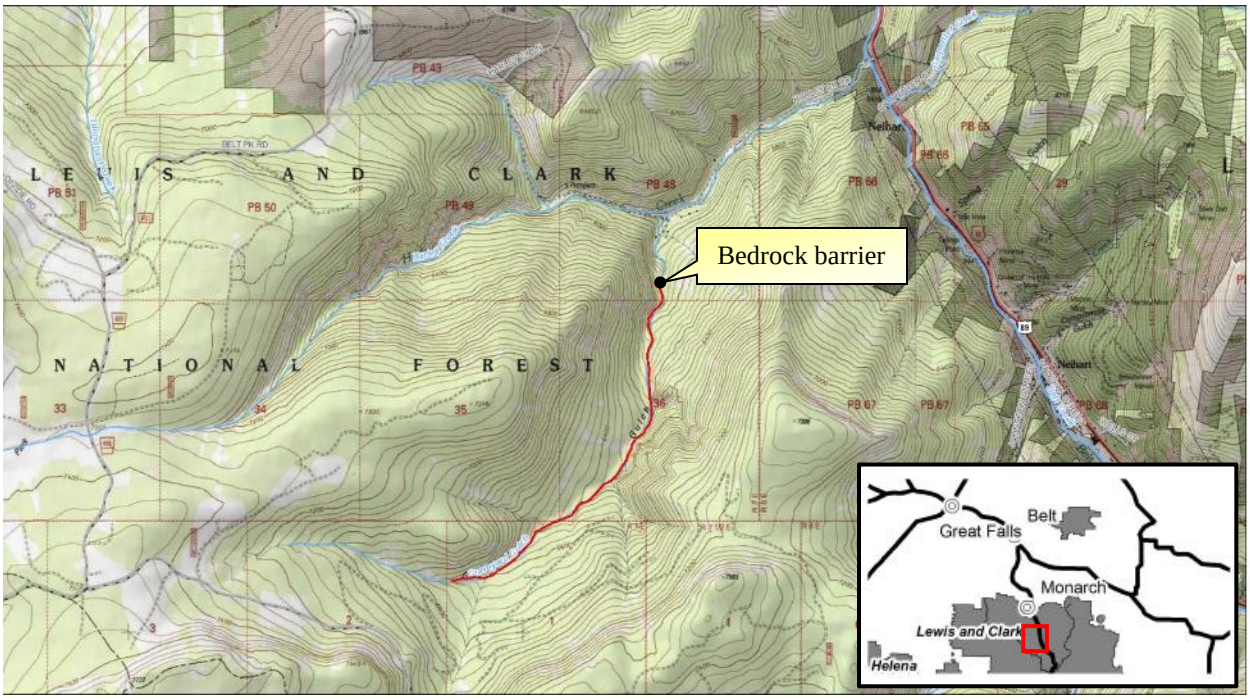

Figure 7. Graveyard Gulch in the Belt Creek subbasin. The stream segment delineated in red indicates the area occupied by nonhybridized WCT.

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 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, 2019, and 2020 to reduce the depth and size of the jump pool with intent to further reduce the likelihood of nonnative trout invasion.

2023 Monitoring

A single pass electrofishing monitoring effort was performed on Graveyard Gulch on September 7th, 2023. A 130 m reach was backpack electrofished starting from the bedrock barrier moving upstream. A total of 24 WCT were collected ranging in size from 86-214 mm in this effort. No brook trout were detected. Following brook trout monitoring, the upper limit of fish distribution was determined by visual observation hiking downstream from FS RD 6378. The uppermost fish was observed at 46.91991, -110.80067. Nonhybridized WCT occupy approximately 2.02 miles above the bedrock barrier in Graveyard Gulch.

Haystack Creek

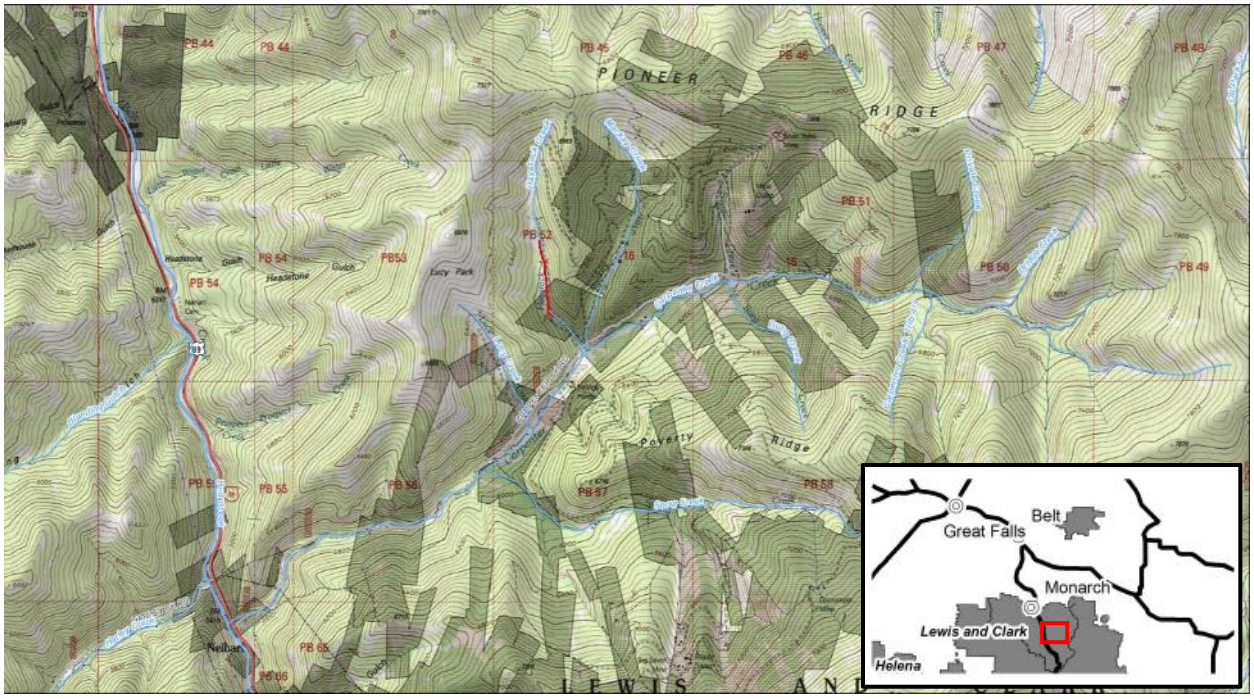

Figure 8. Haystack Creek in the Belt Creek subbasin. The stream segment delineated in red indicates the area occupied by WCT.

Background

Haystack Creek contains a small nonhybridized population of WCT isolated from Carpenter Creek due to poor water quality caused by mining effluent. Demographic and genetic monitoring has been performed periodically from 2010-2019.

2023 Monitoring

Haystack Creek was revisited on August 30th, 2023, to collect updated genetic samples. A single pass backpack electrofishing effort was initiated starting from the Pioneer Lane culvert upstream until 20 WCT were collected. No fish were collected in the lower 350 m of the stream. Fish distribution began immediately above the discharging effluent at the foot of a large tailings pile located at 46.97156, -110.71941. Twenty WCT were collected ranging in size from 69-193 mm. With the completion of the Carpenter Creek fish barrier, Haystack Creek is now protected from nonnative trout invasion. However, it may take several more years until water quality improves to the point where the Haystack and Carpenter Creek WCT populations are reconnected. Updated genetic samples were collected to determine if Haystack Creek is a candidate for genetic rescue in the interim.

Tillinghast Creek

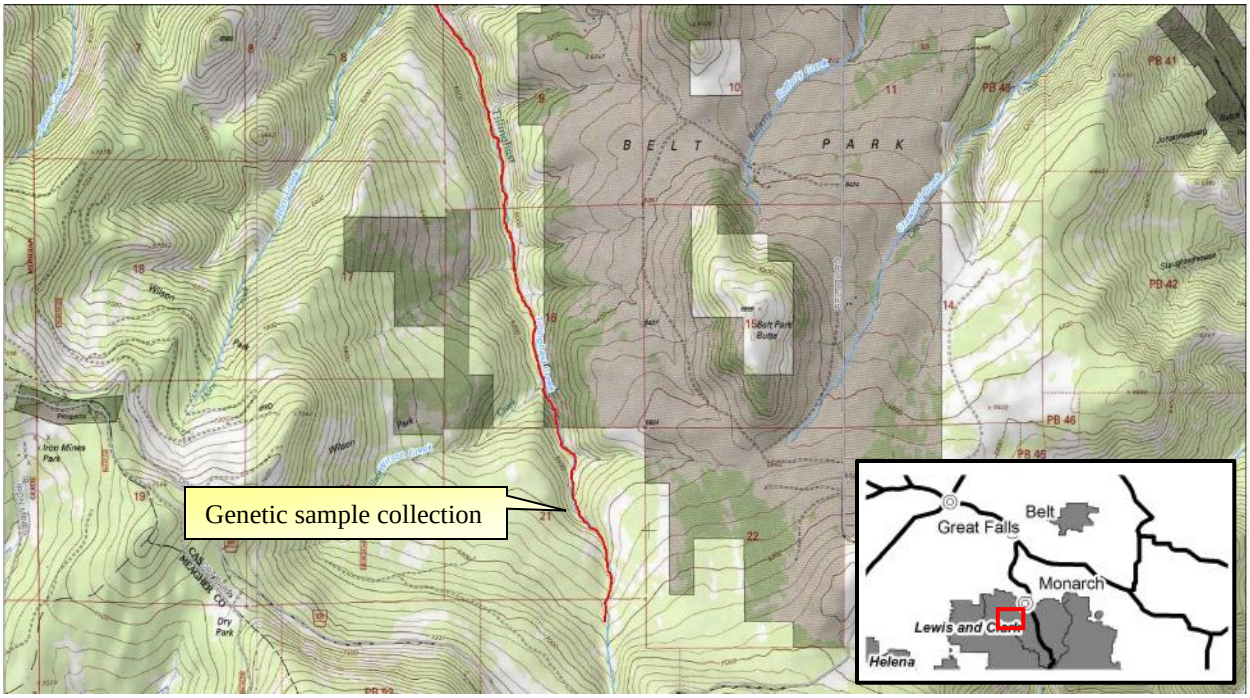

Figure 9. Tillinghast Creek in the Belt Creek subbasin. The stream segment delineated in red indicates the area occupied by WCT.

Background

Tillinghast Creek is a 12-mile-long tributary of Belt Creek located just west of the town of Monarch, MT. Periodic demographic and genetic monitoring has occurred in the upper drainage from 1995-2004. Allozyme analysis of WCT genetic samples collected in 1995 and 1996 indicated that upper Tillinghast Creek could potentially contain nonhybridized WCT. However, a single nonnative YCT and rainbow trout allele were detected at this time.

2023 Monitoring

Tillinghast Creek was sampled on July 25th, 2023, to collect updated genetic samples. The headwaters were sampled in a downstream single pass effort starting at the top of fish distribution (46.95217, -110.83887) until 20 WCT were collected which was approximately 0.7 miles downstream. A total of 56 brook trout and 20 WCT were collected. The fish community appears to have become increasingly brook trout dominated when compared to historical data collected in the 1990s and 2000s.

III. Judith River Subbasin

Big Hill Creek

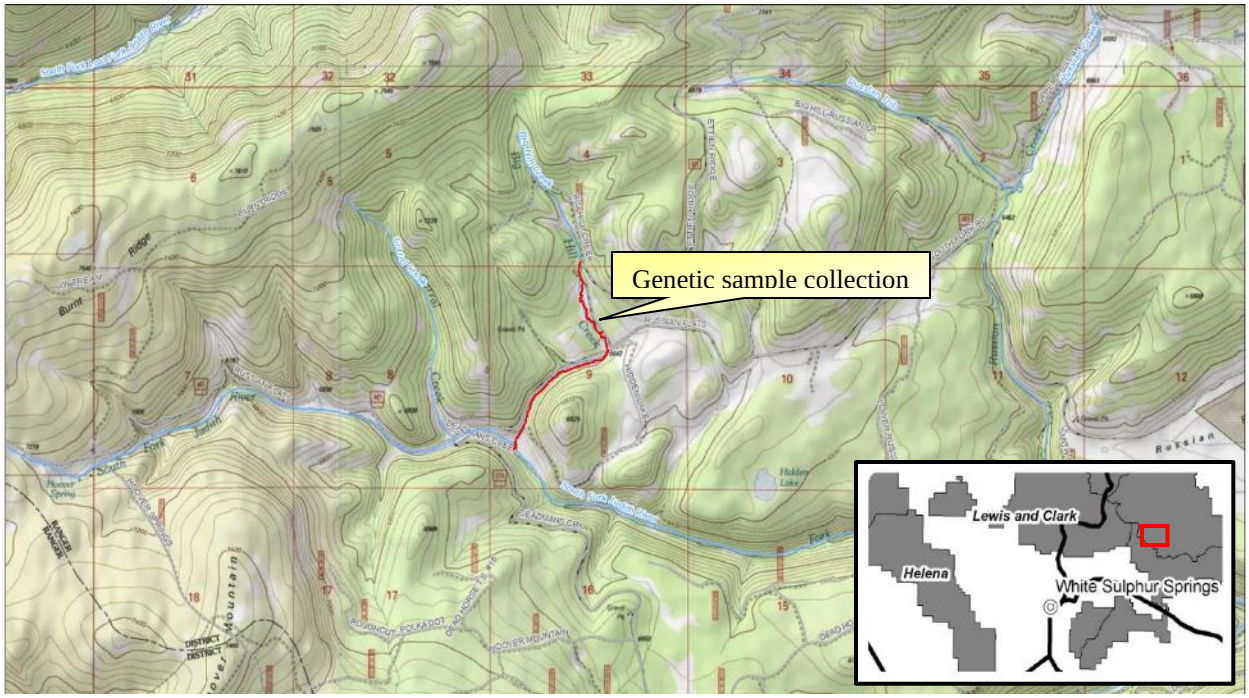

Figure 11. Big Hill Creek in the Judith River subbasin. The stream segment delineated in red indicates the area occupied by WCT.

Background

Big Hill Creek, a tributary of the South Fork Judith River, is located approximately 15 miles southwest of Sapphire Village, MT. Demographic and genetic monitoring of WCT in Big Hill Creek has been performed periodically from 1995-2003. Previous sampling only detected WCT and Rocky Mountain Sculpin. PINEs analysis of 25 WCT collected in 2000 indicated the sample was primarily composed of nonhybridized individuals with one individual fish containing rainbow trout alleles.

2023 Monitoring

Big Hill Creek was sampled on July 24th, 2023, to collect updated genetic samples. A 0.5-mile reach of Big Hill Creek was backpack electrofished above the FS RD 487 culvert. A total of 20 WCT and three brook trout were collected. This is the first-time brook trout have been recorded in Big Hill Creek, potentially indicating an expanding population in the South Fork Judith River headwaters.

Snow Creek

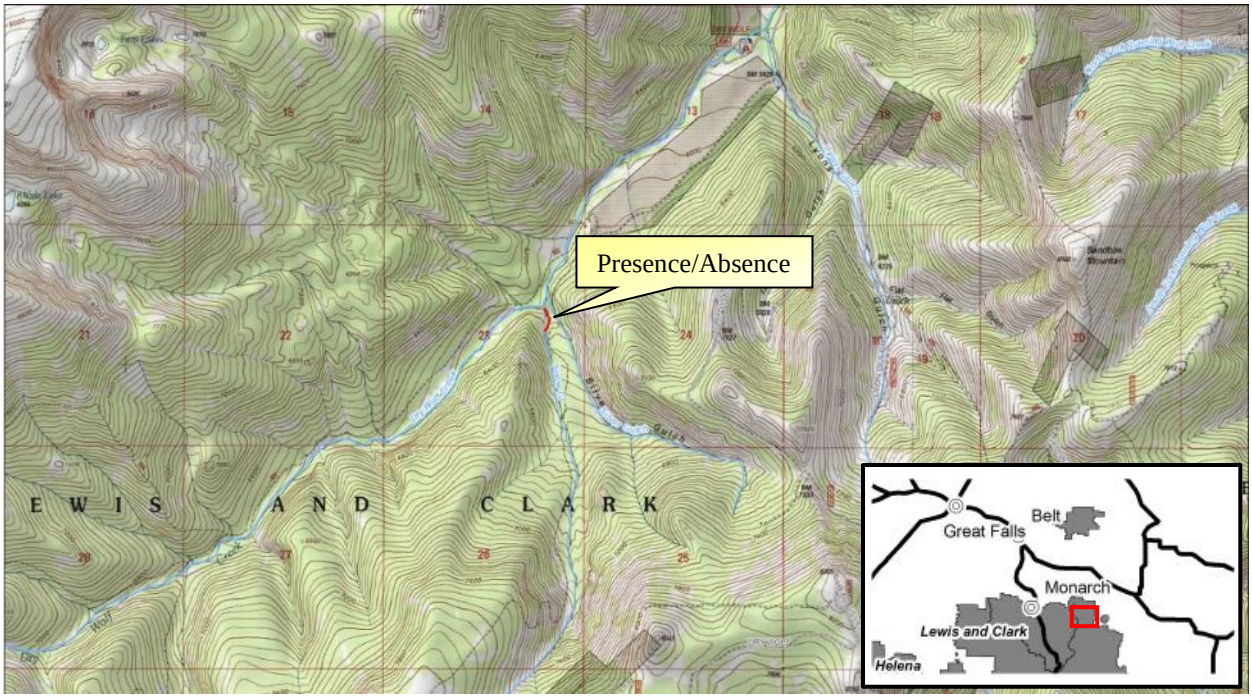

Figure 12. Snow Creek in the Judith River subbasin. The stream segment delineated in red indicates the area sampled in 2023.

Background

Snow Creek, a tributary of Dry Wolf Creek, is located approximately 20 miles southwest of Stanford, MT. A three fish genetic sample collection from Snow Creek in 1994 indicated the creek contained a potentially nonhybridized WCT population. Allozyme analysis found the fish contained mostly WCT alleles at the diagnostic loci analyzed. However, there was evidence of YCT and rainbow trout alleles at two markers. Management recommendations at that time were to consider the population as nonhybridized WCT unless future analysis demonstrated otherwise.

2023 Monitoring

Snow Creek was sampled on September 12th, 2023, to collect additional genetic samples. A single pass electrofishing effort was conducted starting at the Dry Wolf Creek confluence and continuing upstream 200 m. One WCT and three brook trout were collected in the immediate vicinity of the Dry Wolf confluence. No fish were collected further upstream in Snow Creek. It appears that the lower reach of Snow Creek provides minimal habitat for transient fish moving upstream from Dry Wolf Creek and does not support a WCT population of its own.

Unnamed Tributary to Deadhorse Creek

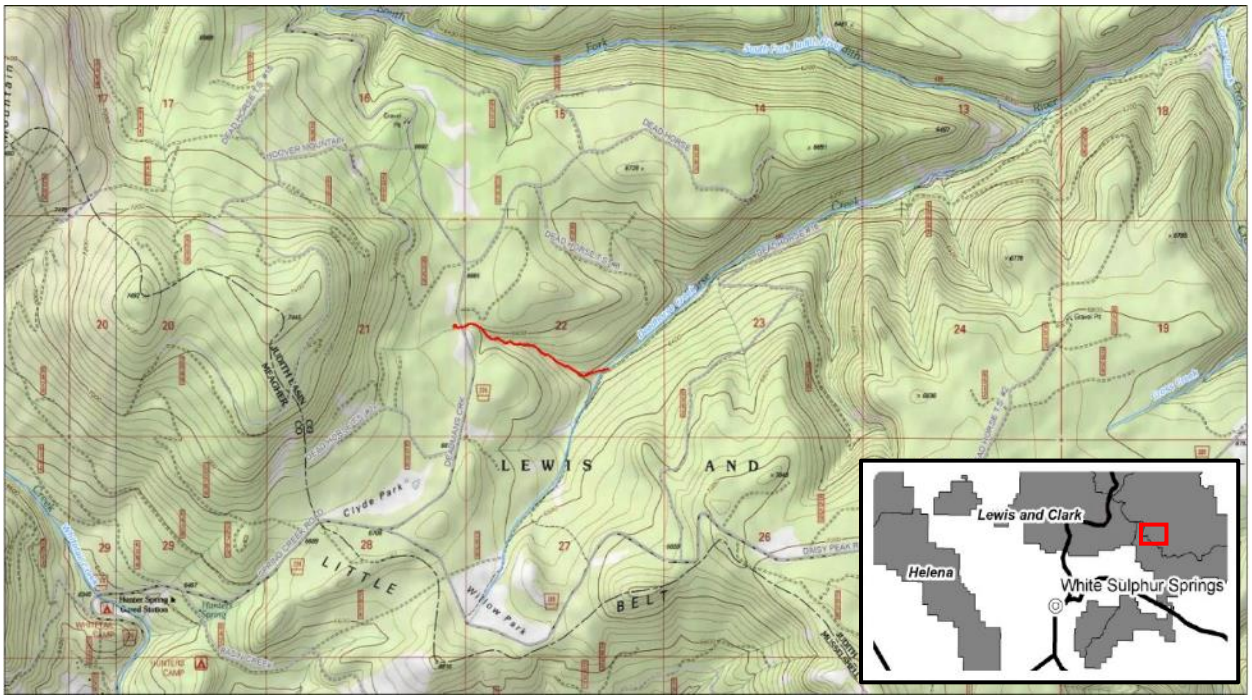

Figure 13. Unnamed Tributary to Deadhorse Creek in the Judith River subbasin. The stream segment delineated in red indicates the area occupied by WCT.

Background

This unnamed tributary enters Deadhorse Creek at river mile 2.5 in Section 22 and can be accessed from FS RD 274 (Spring Creek Road). A previous genetic sample collection in 2003 found a mix of nonhybridized WCT (n=15) and WCTxRB hybrids (n=3). These results suggested the unnamed tributary had recently been invaded by fish from a highly hybridized population.

2023 Monitoring

The unnamed tributary to Deadhorse Creek was sampled on July 24th, 2023, to collect updated genetic samples. The stream was backpack electrofished above and below the FS RD 274 culvert. A total of 20 WCT and nine brook trout were collected. Genetic analysis of these samples will help inform what future conservation actions should be taken for this waterbody.

IV. Smith River Subbasin

Camas Lake and Big Camas Creek

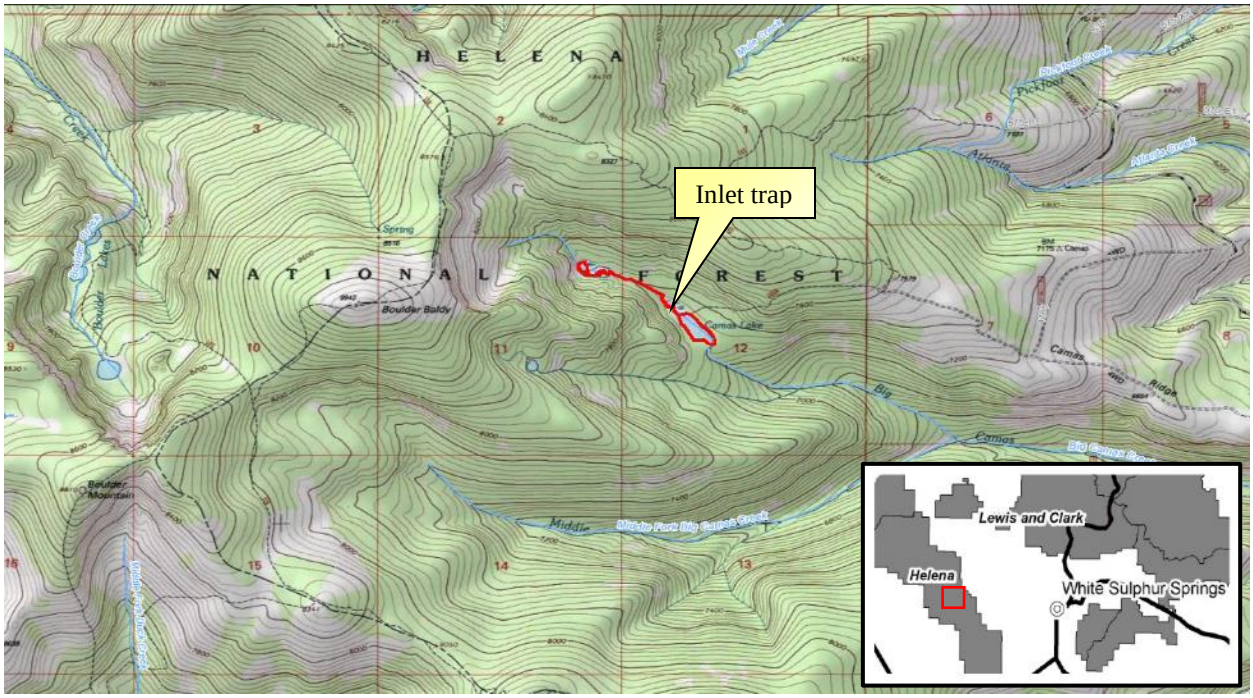

Figure 14. Camas Lake and Big Camas Creek in the Smith River subbasin. The stream segments delineated in red indicate areas sampled in 2021.

Background

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

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

2023 Monitoring

The Camas Lake inlet trap was installed June 7th, 2023. Although winter precipitation in 22-23 was above normal, runoff occurred rapidly, and Big Camas Creek appeared to be past peak discharge upon installation of the trap. In total, the Camas Lake trap was checked 8 times during the 2023 field season. Big Camas Creek above Camas Lake was electrofished 7 times in 2023. A total of 85 fish were collected in the inlet trap in 2023: 17 YCT were caught and removed and 68 WCT were trapped and passed upstream. An additional three YCT were caught and removed backpack electrofishing upper Big Camas Creek. Total catch of YCT (n=20) was similar when compared to 2022 (n=18). Unidentified cutthroat trout under 120 mm were also collected and removed while backpack electrofishing (n=91). The inlet trap was removed on July 6th, 2023, because of low flows.

Cottonwood Creek

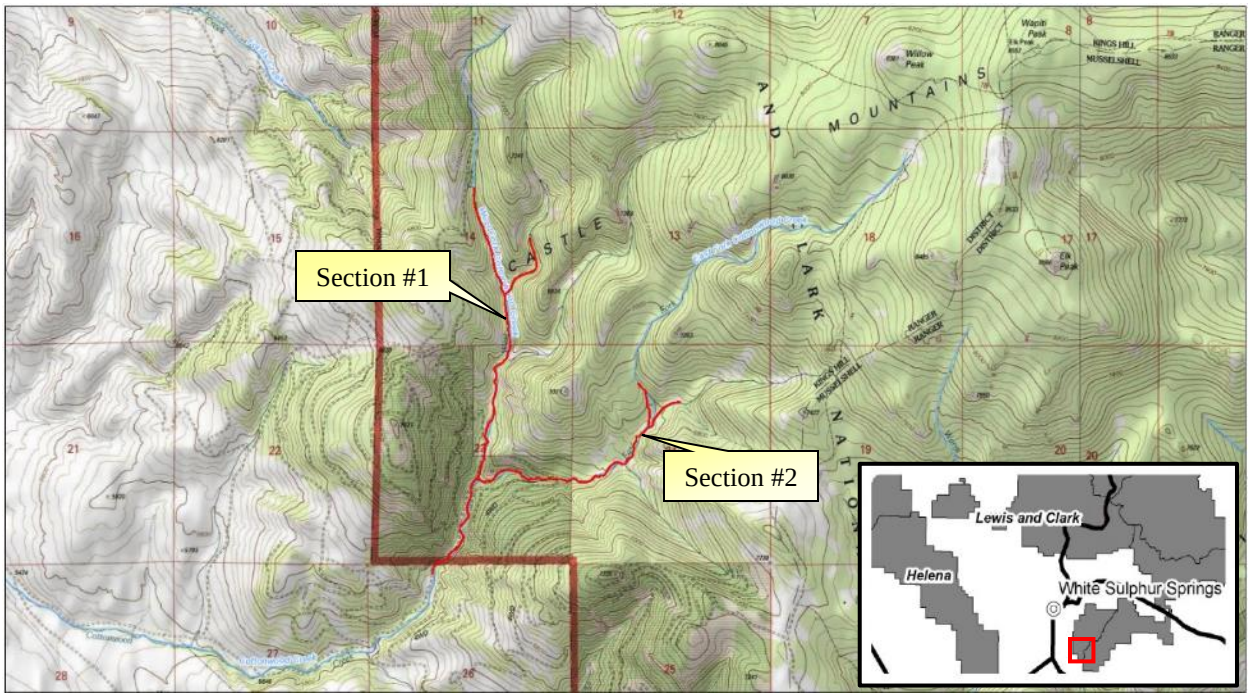

Figure 15. Cottonwood Creek in the Smith River subbasin. The stream segments delineated in red indicate the areas occupied by nonhybridized WCT.

Background

Cottonwood Creek is a tributary of the South Fork Smith River located approximately nine miles southeast of the community of White Sulphur Springs, MT. The stream contains one of four known nonhybridized lineages of native Smith River WCT. A six mile seasonally dry reach of Cottonwood Creek protects this WCT population from nonnative trout invasion. Demographic and genetic monitoring has been performed periodically from 1992-2010. This population has been replicated in Middle Fork Big Camas Creek and used in the mixed source reintroduction of Jumping Creek.

2023 Monitoring

Cottonwood Creek was visited on August 16th and 17th, 2023, to perform population estimates and obtain updated genetic samples. Two 100 m population estimate sections were established: one on West Fork Cottonwood Creek and one on East Fork Cottonwood Creek. Multiple pass depletion methods were used to estimate population abundance. Fish densities were similar at both sites with an estimated 390 fish/km on the West Fork and 380 fish/km on the East Fork (Figure 16). A total population estimate of 2,452 age-1 and older WCT is obtained when these densities are averaged and extrapolated to the entire 6.39 km of occupied habitat. However, uncertainty exists where the upper distribution of fish ends, especially on the East Fork and in the unnamed tributaries. The amount of WCT occupied habitat is likely greater than 6.39 km. Updated genetic samples were collected from 10 WCT from the West Fork and 10 WCT from the East Fork of Cottonwood Creek for future analysis.

Cottonwood Creek —NATIVE TROUT POPULATION SURVEY

1. General Information— Date: **August 16, 17, 2023** Biologist: **A. Poole**
2. Stream Information—
   Name, section, county: **Cottonwood Creek, 14 & 24, Meagher**
3. Survey Site Information (see attached map)—
   Upstream range of native trout (general description and GPS):
   West Fork (46.45209, -110.81300; visual observation)
   East Fork (46.44788, -110.79678; estimated based on historic data)
   Downstream range of native trout (general description and GPS):
   Dry reach (46.43180, -110.81954; varies annually based on precipitation)
   Location (GPS) and description of barriers:
   Stream Length—Occupied habitat: **6.37 km (3.96 mi)** Available habitat: **6.37 km (3.96 mi)**
   Survey method & equipment: **backpack battery electrofisher; two-pass depletion**
   Survey sites (general description and UTM)—
   Section 1: **West Fork Cottonwood Creek – Between unnamed tribs; 46.44881, -110.81171**
   Section 2: **East Fork Cottonwood Creek – Below upper tributary; 46.44102, -110.79756**

Parameter	**Section 1**	**Section 2**
Section length (m)	100 m	100 m
Mean stream width (m) (n)	1.48 m (10)	2.37 m (10)
Section area (hectares)	0.015 ha	0.024 ha
WCT		
Removal Pattern	32 6	33 5
Population estimate	39 (±2)	38 (±1)
Capture probability	0.826	0.844
Mean length (mm) (n)	123 (38)	159 (38)
Mean weight (g) (n)	23 (38)	42 (38)
Mean KTL (n)	0.93 (38)	0.92 (38)
Number fish per km (95 % CI)	390 (±20)	380 (±10)
Number fish per ha (95 % CI)	2,600 (±133)	1,583 (±42)
Biomass (kg per ha) (95 % CI)	60 (±3)	66 (±2)

Figure 16. Cottonwood Creek fish population estimate results.

Fourmile Creek

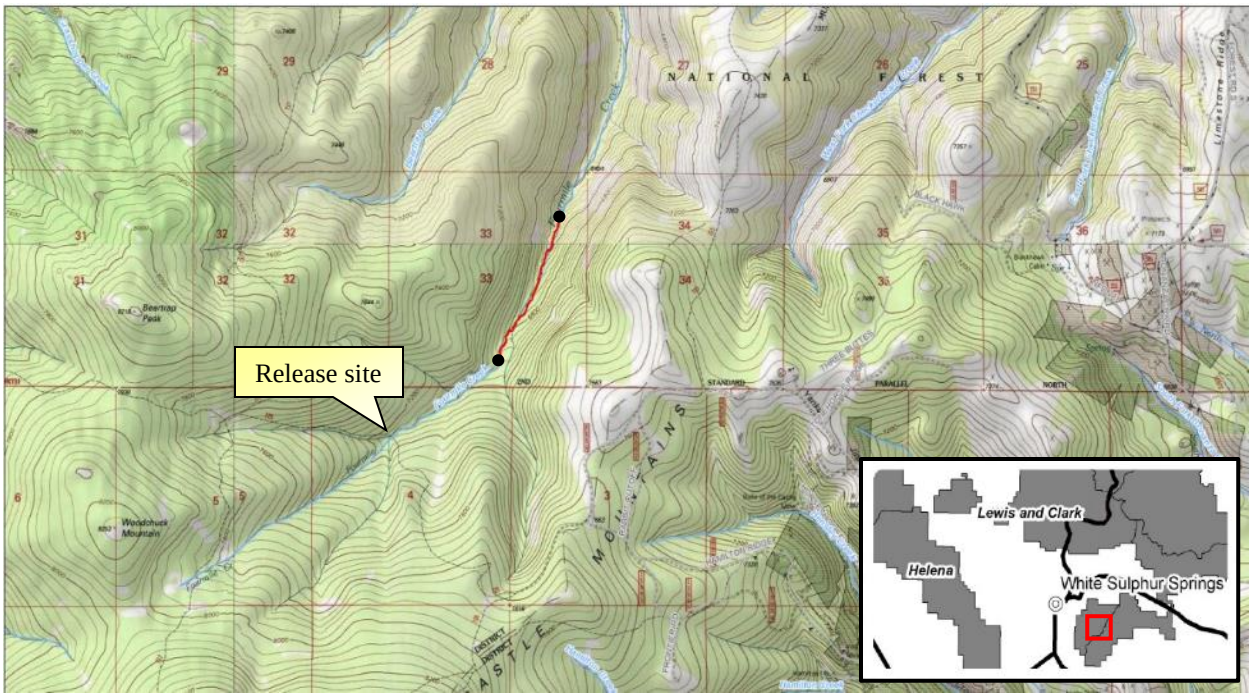

Figure 17. Fourmile Creek in the Smith River subbasin. The stream segment delineated in red indicates the area occupied by nonhybridized WCT. Black circles represent presence of bedrock barriers.

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 17). The perennial reach of Fourmile Creek located upstream of the Lewis and Clark National Forest boundary contains hybridized WCT and brook trout. The headwaters of Fourmile Creek were historically fishless upstream of a series of natural bedrock barriers. In 2000, 50 nonhybridized WCT from nearby Richardson Creek were transferred upstream of the lowest natural waterfall barrier. However, subsequent sampling of upper Fourmile Creek failed to detect the transferred WCT and identified additional upstream barriers. In 2020, upper Fourmile Creek was surveyed again to evaluate habitat for potential WCT transfer opportunities. A 0.75-mile section of Fourmile Creek was found to support a population of nonhybridized WCT isolated between two bedrock barriers. In 2021, an estimated 240 fish/km were found based on the results of a two-pass depletion population estimate, putting the total nonhybridized WCT population at 283 (±12) individuals.

2023 Wild Fish Transfer

A headwater expansion of the nonhybridized WCT population in upper Fourmile Creek was performed on September 26th, 2023. A total of 25 WCT ranging in size from 111-246 mm in total length were collected by backpack electrofishing, placed in fish transfer bags with supplemental oxygen, and hiked upstream into the fishless headwaters of Fourmile Creek. All fish were released in proximity to the Woodchuck Trail crossing (FS TR 725). This represents the first year of a four-year effort to expand the distribution of the nonhybridized WCT population in the headwaters of Fourmile Creek and achieve the goals of the original 2000 environmental assessment.

Beartrap Creek 2023 Monitoring

Stream temperature monitoring continued on the unnamed tributary (Beartrap Creek) of Fourmile Creek in 2023. A temperature logger was launched on June 20th and collected on October 19th. The mean July stream temperature was 8.12°C (Figure 18), which is above the 7.8°C minimum that would likely limit successful cutthroat trout reproduction and recruitment (Harig and Fausch 2002). The battery failed on August 6th, limiting our inference of summer stream temperatures to the month of July. Beartrap Creek should be considered a candidate for translocation of at-risk WCT populations.

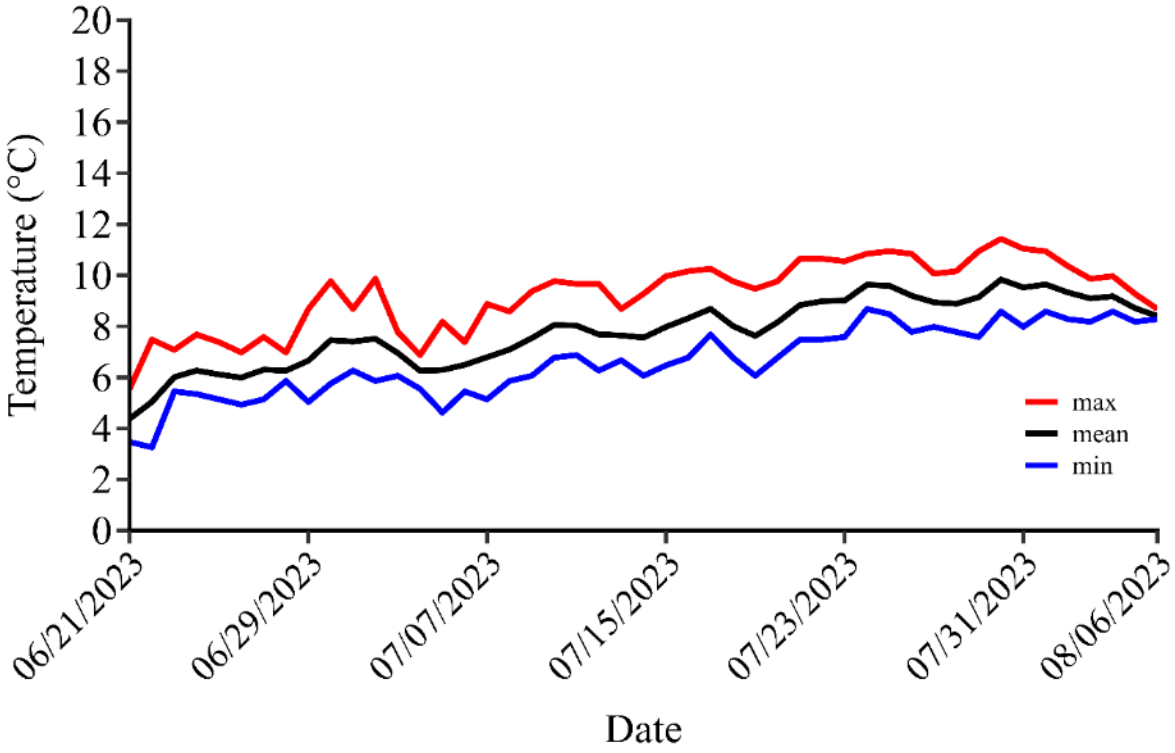

Figure 18. Daily maximum (red line), mean (black line), and minimum (blue line) stream temperatures from Beartrap Creek. Temperature logger was deployed on June 20, 2023 and collected October 19, 2023.

Hound Creek Reservoir and Tyrell Creek

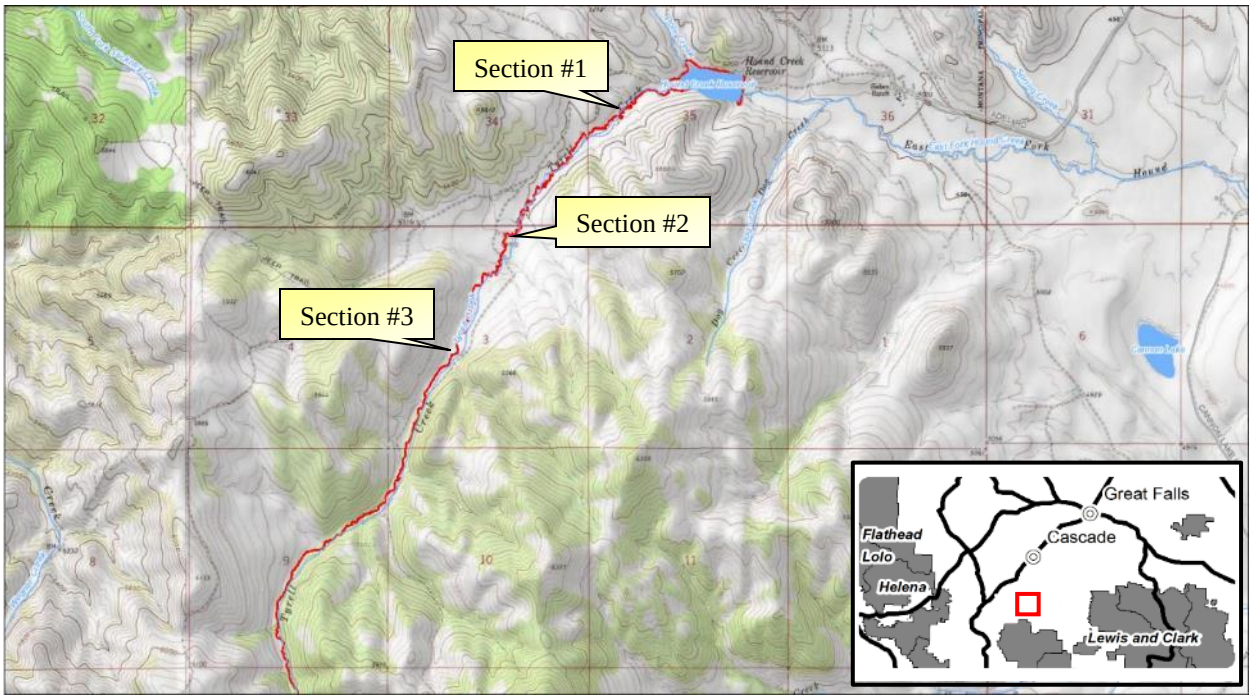

Figure 19. Hound Creek Reservoir and Tyrell Creek in the Smith River subbasin. The stream segments delineated in red indicate areas occupied by WCT.

Background

A large scale piscicide restoration project was initiated in Hound Creek Reservoir and Tyrell Creek in 2001. The piscicide treatment resulted in an incomplete kill of nonnative brook trout. Remaining nonnative fish in Tyrell Creek, Pole Creek and Hound Creek Reservoir were removed with fish traps, gill nets, and backpack electrofishers. Surveys indicated that that these efforts were successful, and the drainage was ready for transfer of nonhybridized WCT. A donor population of WCT was identified in Jumping Creek, also in the Smith River subbasin. In 2008, the entire WCT population in Jumping Creek, 71 juveniles and adults and approximately 1,000 eyed embryos were transferred to Tyrell Creek. To alleviate concerns of a founder effect, additional 49 WCT were moved from upper O'Brien Creek to Tyrell Creek. In 2016, approximately 3,500 eyed embryos were transferred from Lone Willow Creek (Smith Drainage) to remote site incubators in Tyrell Creek. Embryos were originally intended for Big Camas Creek following the piscicide project completed in 2014. However, embryos were diverted to Tyrell Creek after confirmation of YCT persistence in upper Big Camas Creek.

2023 Monitoring

Hound Creek Reservoir and Tyrell Creek were surveyed on August 23rd and 24th, 2023, to determine current population status. Three previously established 100 m long-term monitoring sites were revisited on Tyrell Creek. Hound Creek Reservoir was sampled with a combination of 5 overnight fyke net sets, 2 short set gill nets, and angling. Fish densities were low at all three long-term monitoring sites sampled on Tyrell Creek, ranging from 10 to 70 fish/km (Figure 20). Monitoring performed between 2011 and 2015 found much higher densities of WCT (50-320 fish/km) in sections 1 and 2 while section 3 has supported lower numbers of WCT (0-70 fish/km) since post treatment monitoring began. In Hound Creek Reservoir, a total of 14 WCT were collected by fyke and gill net and an additional 27 WCT were collected by angling. All WCT collected in Hound Creek Reservoir were large adults ranging in size from 14 to 20

inches in length. Several WCT appeared to have eroded/split dorsal and anal fins, potentially remnants of the roughly 12,387 triploid WCT stocked from the Washoe Park Trout Hatchery from 2011-2015 to provide angling opportunity immediately following the piscicide treatment.

It is unclear why fish densities in Tyrell Creek are lower than those recorded in the 2011-2015 period. A series of poor water years in 2021 and 2022 likely have influenced fish numbers in Tyrell Creek. In years with low precipitation, Tyrell Creek may provide marginal WCT habitat, especially in the lower reaches of the stream. However, recruitment was noted by the collection of WCT less than 70 mm in both sections 1 and 2. Future monitoring efforts should attempt to document the upstream distribution of WCT in Tyrell Creek and establish additional long-term monitoring sites further upstream in the system.

Tyrell Creek —NATIVE TROUT POPULATION SURVEY

1. General Information— Date: **August 23, 24, 2023** Biologist: **A. Poole**
2. Stream Information—
   Name, section, county: **Tyrell Creek, 3 & 35, Cascade**
3. Survey Site Information (see attached map)—
   Upstream range of native trout (general description and GPS): **Section 21 (46.95489, -111.74003; estimate, based on satellite imagery)**
   Downstream range of native trout (general description and GPS): **Hound Creek Reservoir (47.00911, -111.69182)**
   Location (GPS) and description of barriers: **Hound Creek Dam (47.00911, -111.69182)**
   Stream Length—Occupied habitat: **10.24 km (6.36 mi)** Available habitat: **10.24 km (6.36 mi)**
   Survey method & equipment: **backpack battery electrofisher; two-pass depletion**
   Survey sites (general description and UTM)—
   Section 1: **Above Beaver Ponds; 47.00808, -111.70348**
   Section 2: **At Barns; 46.99974, -111.71559**
   Section 2: **Below flume; 46.99297, -111.72182**

Parameter	**Section 1**	**Section 2**	**Section 3**
Section length (m)	100 m	100 m	100 m
Mean stream width (m) (n)	2.04 m (10)	1.84 m (10)	1.80 m (10)
Section area (hectares)	0.020 ha	0.018 ha	0.018 ha
WCT			
Removal Pattern	3 1	5 2	1 0
Population estimate	4 (±1)	7 (±1)	1 (NA)
Capture probability	0.571	0.636	1.000
Mean length (mm) (n)	193 (4)	237 (7)	239 (1)
Mean weight (g) (n)	51 (3)	135 (7)	136 (1)
Mean KTL (n)	0.86 (3)	0.99 (7)	1.00 (1)
Number fish per km (95 % CI)	40 (±10)	70 (±10)	10 (NA)
Number fish per ha (95 % CI)	200 (±50)	389 (±56)	56 (NA)
Biomass (kg per ha) (95 % CI)	10 (±3)	53 (±8)	8 (NA)

4. Comments: An additional 9 and 10 WCT less than 70 mm were collected from Sections 1 and 2, respectively. Rocky Mountain Sculpin were abundant at all three survey sites.

Figure 20. Tyrell Creek fish population estimate results.

Middle Fork Big Camas Creek

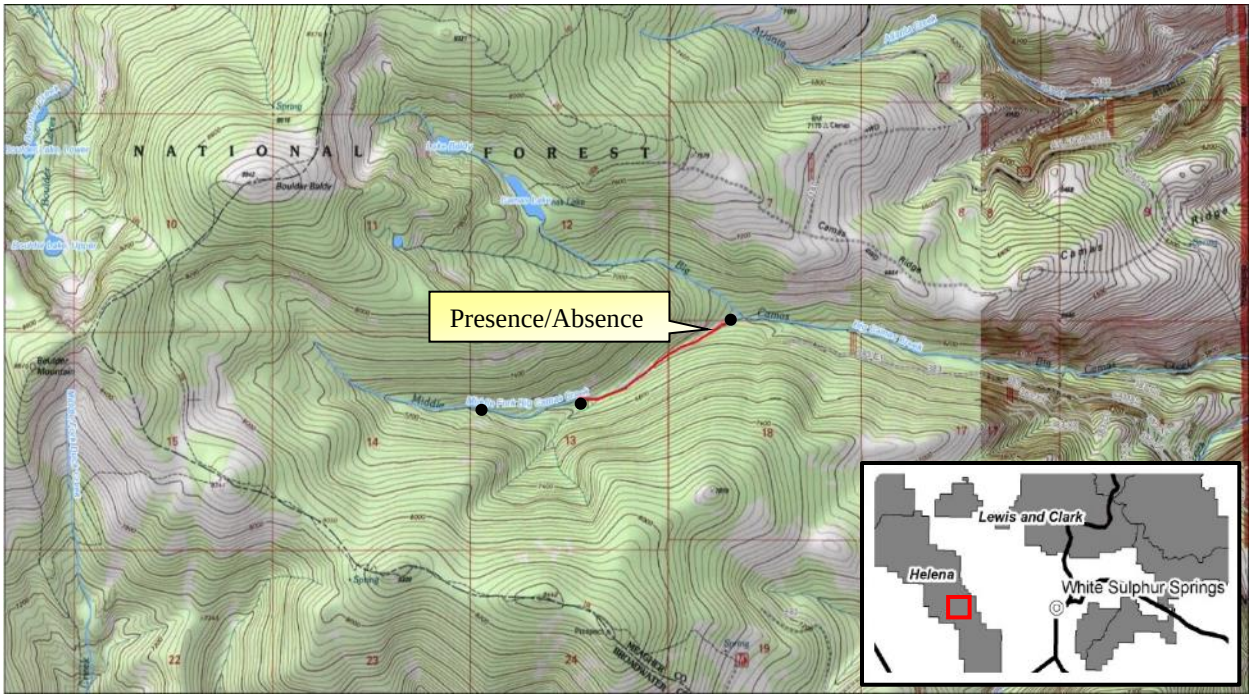

Figure 21. Middle Fork Big Camas Creek in the Smith River subbasin. The stream segment delineated in red indicates the area occupied by WCT. Black circles represent the presence of bedrock fish barriers.

Background

Surveys performed in 2001 by the Forest Service found a 2.41 km reach 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 two additional barriers located upstream (Figure 21). 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. Demographic monitoring performed in 2020 found an estimated WCT density of 130 fish/km in Middle Fork Big Camas Creek.

2023 Monitoring

Presence/absence monitoring was performed on August 10th, 2023, on Middle Fork Big Camas Creek. Most of the watershed burned at high severities in the 2021 Woods Creek Fire and the current status of the population was unknown (Figure 22). An approximately 200 m reach of Middle Fork Big Camas Creek was backpack electrofished from the lower bedrock barrier to the top of the long-term monitoring section established in 2020. A total of 14 WCT were collected ranging in size from 153-200 mm in length. The density of WCT appeared to be similar to pre-fire monitoring levels.

Figure 22. Top of long-term monitoring site on Middle Fork Big Camas Creek pre (2020) and post (2023) Woods Creek Fire.

Nugget Creek

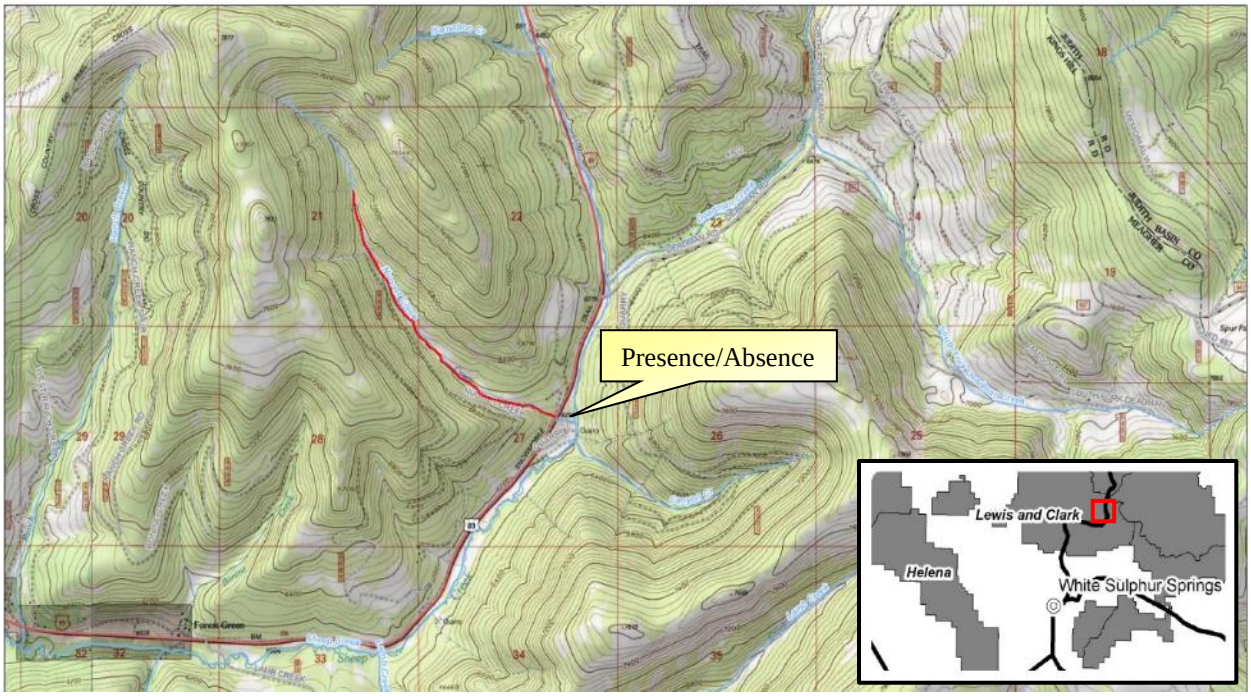

Figure 22. Nugget Creek in the Smith River subbasin. The stream segments delineated in red indicates the fishless habitat upstream of HWY 89.

Background

Nugget Creek is a tributary of Sheep Creek in the Smith River drainage located approximately 18 miles northeast of White Sulphur Springs, MT. Previous sampling in 2011 found that the reach of Nugget Creek above the US 89 was fishless.

2023 Monitoring

Nugget Creek was revisited on September 11th, 2023, to determine fish presence and evaluate habitat. A 140 m reach of Nugget Creek was backpack electrofished from the confluence of Sheep Creek to the US 89 culvert. A total of 17 brook trout were collected ranging in size from 47-199 mm in length. Many additional young of year brook trout were observed but not collected. A 400 m reach of Nugget Creek was spot shocked above the US 89 culvert. Similar to the 2011 shocking results, no fish were collected or observed. Habitat appeared suitable for supporting fish, although the reach sampled lacked deep overwintering pools. Further habitat evaluation should be performed on Nugget Creek to determine if it can support WCT.

Little Camas Creek

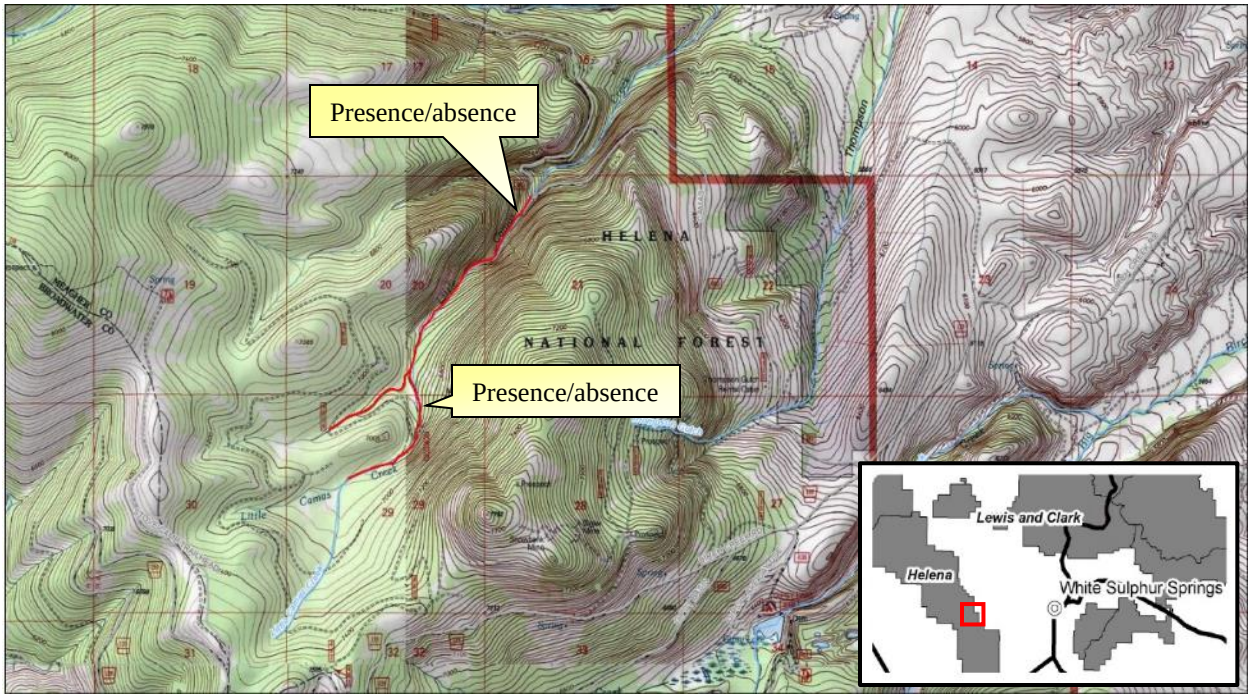

Figure 23. Little Camas Creek in the Smith River subbasin. The stream segment delineated in red indicates the fishless habitat upstream of FS RD 383.

Background

Little Camas Creek is a tributary of Camas Creek in the Smith River Drainage located approximately 15 miles west of White Sulphur Springs, MT. Fisheries surveys performed in the late 1990s and early 2000s found that the upper 2.4 miles of fish habitat located above FS RD 383 (Camas Road) were fishless while brook trout and WCTxRB hybirds were present below this road crossing (Tews et al. 2000). Evidence of historic placer mining exists in this reach and may explain the absence of fish (Phillips and Humphrey 1987).

2023 Monitoring

Little Camas Creek was surveyed on July 7th and 18th, 2023, to determine fish presence/absence, evaluate habitat, and launch a temperature logger. On July 7th, a 550 m reach of Little Camas Creek was backpack electrofished starting at the Camas Road moving upstream. No fish were collected and habitat appeared excellent with many deep overwintering pools, large woody debris, spawning gravels, and abundant macroinvertebrates observed. Little Camas Creek was also spot shocked below the Camas Road crossing to determine fish presence. The uppermost fish was not encountered until 600 m below the FS RD 383 culvert, a single WCTxRB hybrid 225 mm in length. The population appears to be low density, possible impacted by the Woods Creek fire in 2021. On July 18th, a 1.1-mile reach of upper Little Camas Creek was backpack electrofished from the headwaters of the creek downstream to the confluence of the unnamed tributary. No fish were detected, and habitat once again appeared excellent (Figure 24).

Figure 24. Fishless habitat in upper Little Camas Creek.

Stream temperature monitoring of Little Camas Creek occurred in 2023 to assess suitability for WCT. A temperature logger was deployed at 46.53064, -111.23772 just upstream of the Camas Road crossing on July 7th and recovered on October 19th. The mean July stream temperature was 8.77°C and the mean August stream temperature was 9.12°C (Figure 25), which are above the 7.8°C minimum that would likely limit successful cutthroat trout reproduction and recruitment (Harig and Fausch 2002). Little Camas Creek should be considered a candidate for translocation of at-risk WCT populations.

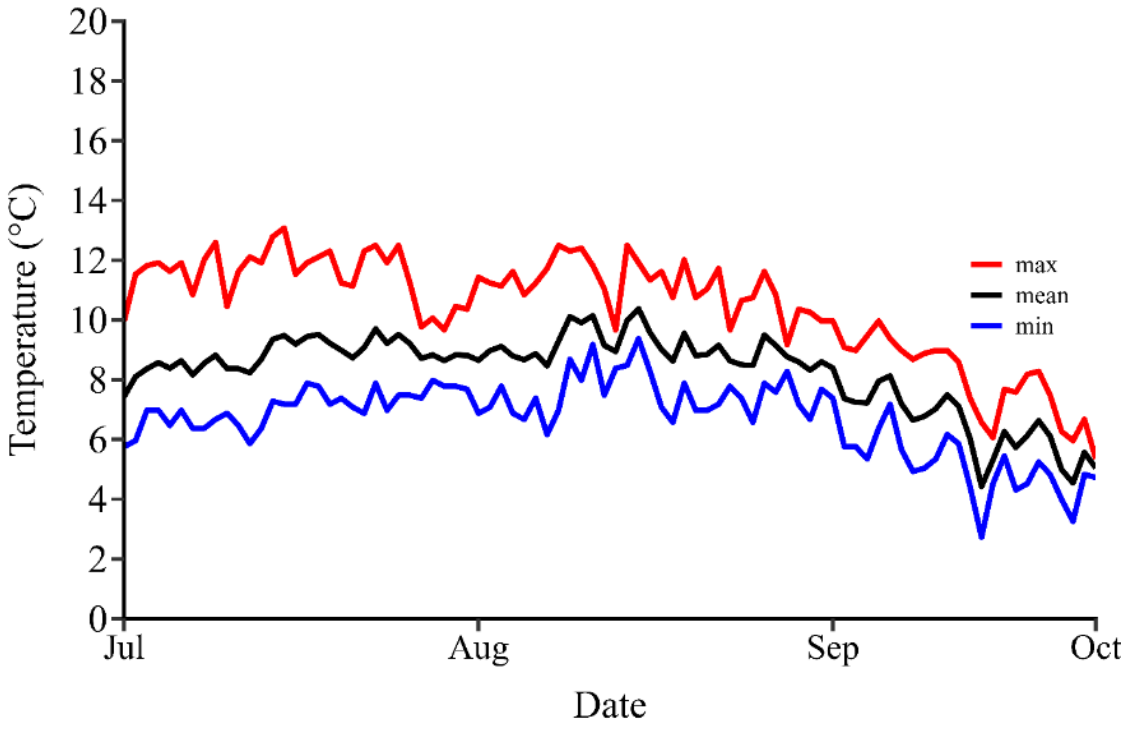

Figure 25. Daily maximum (red line), mean (black line), and minimum (blue line) stream temperatures from Little Camas Creek. Temperature logger was deployed on July 7, 2023 and collected October 19, 2023.

South Fork Deep Creek

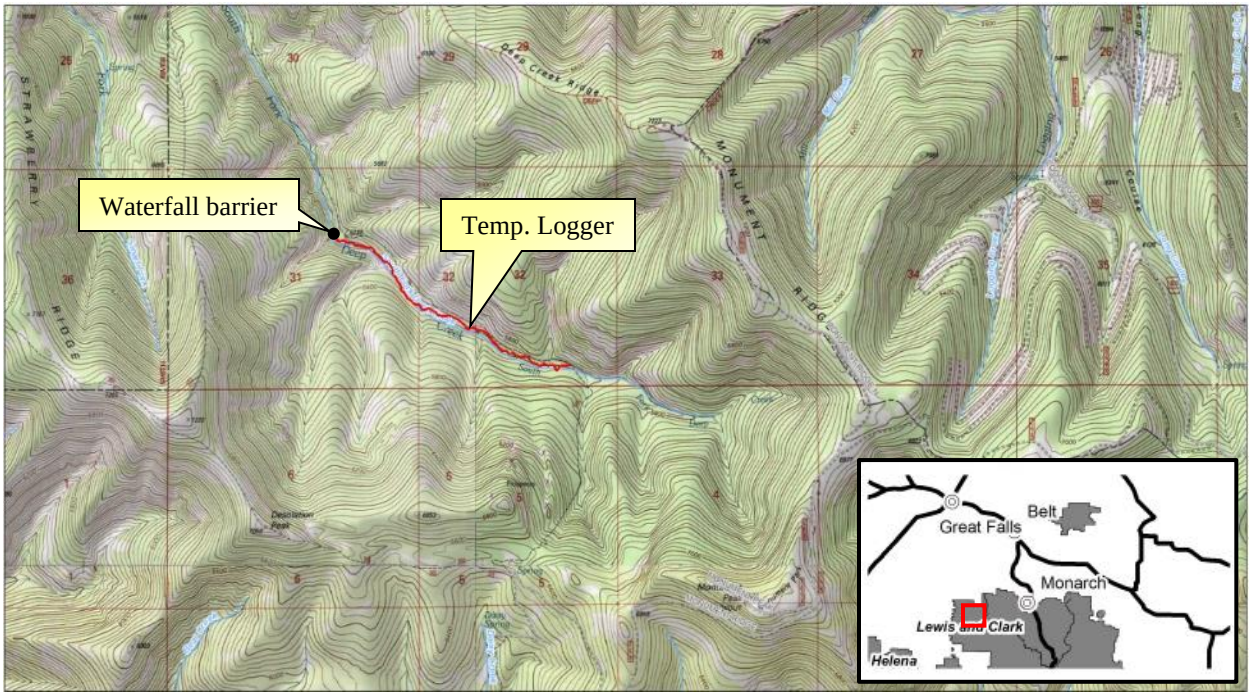

Figure 26. South Fork Deep Creek in the Smith River subbasin. The stream segment delineated in red indicates the fishless habitat upstream of the waterfall barrier.

Background

South Fork Deep Creek, a tributary of Deep Creek in the Smith River drainage, is located approximately 15 miles southwest of Monarch, MT. The lower 2.88 miles of South Fork Deep Creek contains a conservation population of WCT that has periodically been sampled from 1973-2000. The most recent genetic assessment in 2000 indicated this population was most likely a hybrid swarm with a predominant WCT genetic contribution (95.5% WCT 4.5% RB). A waterfall barrier located at 47.02183, -111.14077 limits the upstream distribution of WCT in South Fork Deep Creek (Figure 27).

2023 Monitoring

South Fork Deep Creek was surveyed on June 29th, 2023, to determine fish presence/absence, evaluate habitat, and launch a temperature logger in the fishless headwater reach (Figure 26). South Fork Deep Creek was backpack electrofished from the waterfall barrier to the South Fork Deep Creek Trail #316 crossing where surface water emerged from the dry streambed. No fish were collected, and habitat appeared excellent. A temperature logger was launched at 47.01558, -111.12571 and retrieved on October 5th, 2023. Stream temperatures in South Fork Deep Creek were low and remained stable throughout the summer (Figure 28). The mean July stream temperature was 6.23°C and the mean August stream temperature was 6.80°C, which are well below the 7.8°C minimum that would likely limit successful cutthroat trout reproduction and recruitment (Harig and Fausch 2002). South Fork Deep Creek should not be considered as a candidate for translocation of at-risk WCT populations because of the lack of suitable thermal habitat.

Figure 27. Waterfall barrier located at 47.02183, -111.14077 on South Fork Deep Creek.

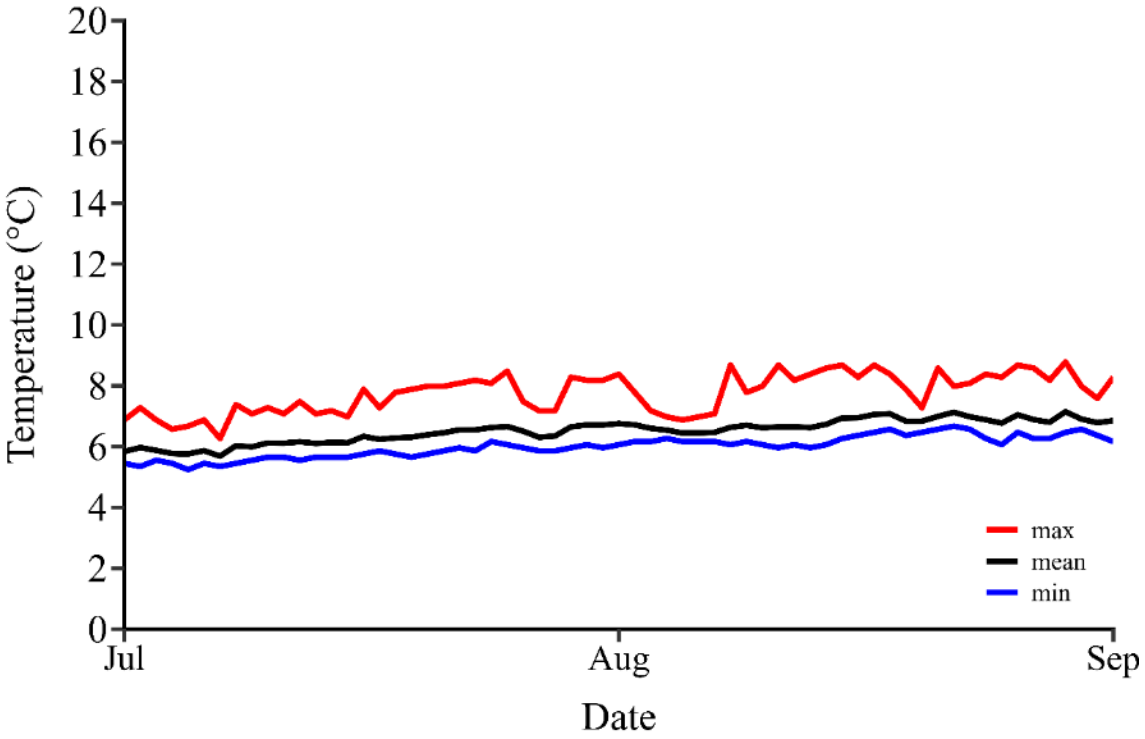

Figure 28. Daily maximum (red line), mean (black line), and minimum (blue line) stream temperatures from South Fork Deep Creek. Temperature logger was deployed on June 29, 2023, and collected October 5, 2023.

South Fork Willow Creek

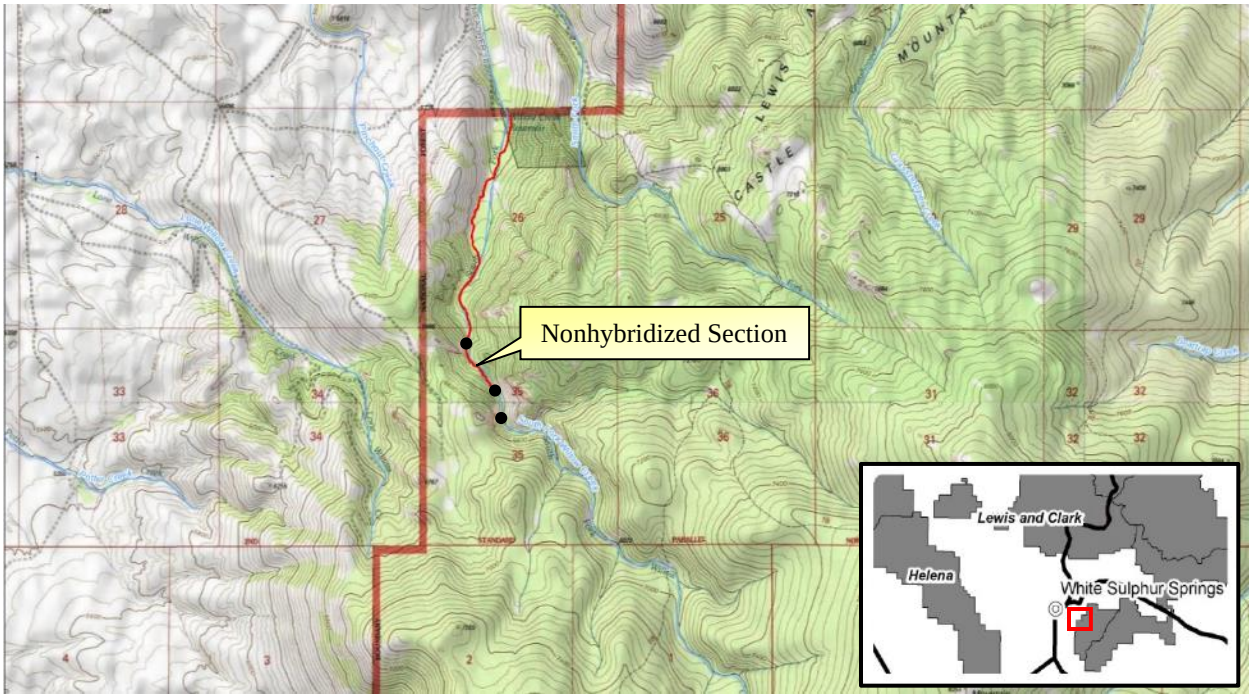

Figure 29. South Fork Willow Creek in the Smith River subbasin. The stream segments delineated in red indicate the areas occupied by WCT. Black circles represent the presence of bedrock fish barriers.

Background

The South Fork Willow Creek WCT population occupies 1.5 miles of habitat located above Willow Creek Reservoir, the municipal water supply reservoir for White Sulphur Springs, MT. The 1.2-mile reach immediately above the reservoir contains nonnative brook trout and slightly hybridized WCT (99.1% WCT 0.9% RB). A natural bedrock barrier isolates an additional 0.3 miles of habitat above this reach occupied by nonhybridized WCT. WCT from South Fork Willow Creek were used as one of three sources in the mixed source reintroduction of Jumping Creek in 2010.

2023 Monitoring

South Fork Willow Creek was visited on August 15th, 2023, to perform updated demographic and genetic monitoring. A 100-m long-term monitoring site was established in the nonhybridized section of South Fork Willow Creek between bedrock barrier #1 and #2. Multiple pass depletion methods were used to estimate population abundance. An estimated density of 260 fish/km were found based on the results of the population estimate (Figure 31). When extrapolated to the entire length of the nonhybridized section (395 m), a total of 103 WCT are estimated to be present. Updated genetic samples (n=20) were also collected from the nonhybridized section to determine present genetic status.

The fishless reach located upstream of the present WCT distribution was evaluated for potential WCT expansion. A 1.4-mile reach was surveyed from bedrock barrier #2 to the Manger Park Trail #719 crossing. Habitat appeared excellent with many deep overwintering pools, large woody debris, spawning gravels, and abundant macroinvertebrates observed (Figure 30). Temperature monitoring should be performed in 2024 to determine thermal suitability for WCT. If suitable, expansion of the nonhybridized WCT in South Fork Willow Creek should be pursued to secure this population in place.

Figure 30. Fishless habitat in headwaters of South Fork Willow Creek.

South Fork Willow Creek —NATIVE TROUT POPULATION SURVEY

1. General Information— Date: **August 15, 2023** Biologist: **A. Poole**
2. Stream Information—
   Name, section, county: **South Fork Willow Creek, 35, Meagher**
3. Survey Site Information (see attached map)—
   Upstream range of native trout (general description and GPS): **Bedrock barrier #2 (46.50071, -110.81304)**
   Downstream range of native trout (general description and GPS): **Willow Creek Reservoir (46.51896, -110.81208)**
   Location (GPS) and description of barriers:
   Bedrock barrier #1 (**46.50331, -110.81603; isolates nonhybridized WCT population)**
   Bedrock barrier #2 (46.50071, -110.81304; limits upstream distribution of WCT)
   Bedrock barrier #3 (46.49954, -110.81248; additional barrier in fishless reach)
   Stream Length—Occupied habitat: **2.35 km (1.46 mi)** Available habitat: **9.71 km[1] (6.03 mi)**
   Survey method & equipment: **backpack battery electrofisher; two-pass depletion**
   Survey sites (general description and UTM)—
   Section 1: **South Fork Willow Creek – Nonhybridized section; 46.50163, -110.81437**

Parameter	**Section 1**
Section length (m)	100 m
Mean stream width (m) (n)	3.58 m (10)
Section area (hectares)	0.036 ha
WCT	
Removal Pattern	24 2
Population estimate	26 (±1)
Capture probability	0.929
Mean length (mm) (n)	173 (26)
Mean weight (g) (n)	56 (26)
Mean KTL (n)	0.92 (26)
Number fish per km (95 % CI)	260 (±10)
Number fish per ha (95 % CI)	722 (±28)
Biomass (kg per ha) (95 % CI)	40 (±2)

4. Comments:

[1] – Includes 7.36 km (4.57 mi) of fishless habitat upstream of bedrock barrier #2 and unnamed tributary.

Figure 31. South Fork Willow Creek fish population estimate results.

Stringer Creek

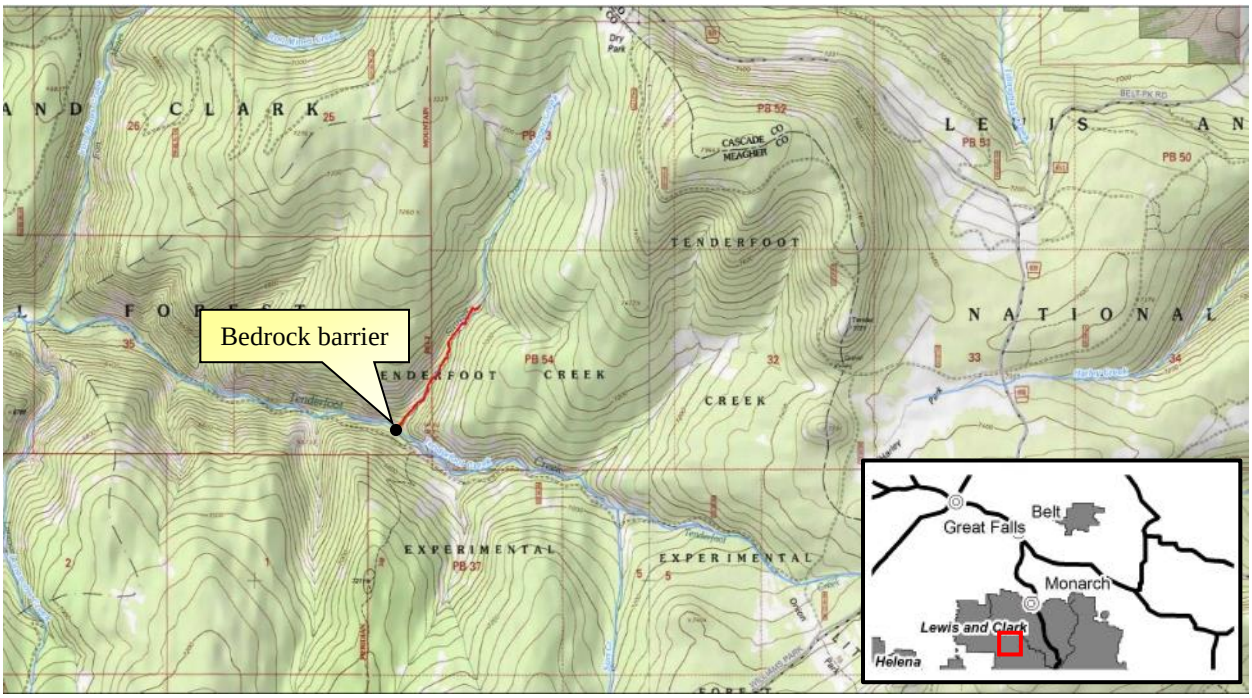

Figure 32. Stringer Creek in the Smith River subbasin. The stream segment delineated in red indicates the fishless area that appears suitable for WCT. The black circle represents presence of bedrock barrier.

Background

Stringer Creek is a tributary of Tenderfoot Creek in the Smith River drainage located approximately 7 miles west of the community of Neihart, MT. Previous sampling conducted in the 1990s found Stringer Creek to be fishless above a bedrock barrier located at the confluence with Tenderfoot Creek (Figure 32). Although habitat was deemed to be marginal, Stringer Creek was suggested as a potential site for the establishment of a WCT conservation population (Shepard et al. 1997).

2023 Monitoring

Stringer Creek was visited on September 27th, 2023, to evaluate habitat and reevaluate fish presence/absence. The length of Stringer Creek was visually surveyed from the headwaters below Dry Park to the confluence with Tenderfoot Creek. The upper portion of the watershed contains minimal fish habitat and is limited by low flows and large cobble substrates. Habitat quality improves below the upper Stringer Creek H flume (46.93473, -110.89355) where the stream increases in discharge, pool habitat frequency increases, and large woody debris and spawning gravels become more common. A 0.77-mile reach of Stringer Creek appears capable of supporting WCT. Temperature data is required to determine if thermal habitat is suitable for WCT introduction. A 70-m reach of Stringer Creek was backpack electrofished from the bedrock barrier to the lower H flume to determine fish presence/absence. No fish were collected or observed in this effort.

Figure 33. Stringer Creek bedrock barrier located at 46.92662, -110.90239.

V. Sun River Subbasin

Bailey Creek

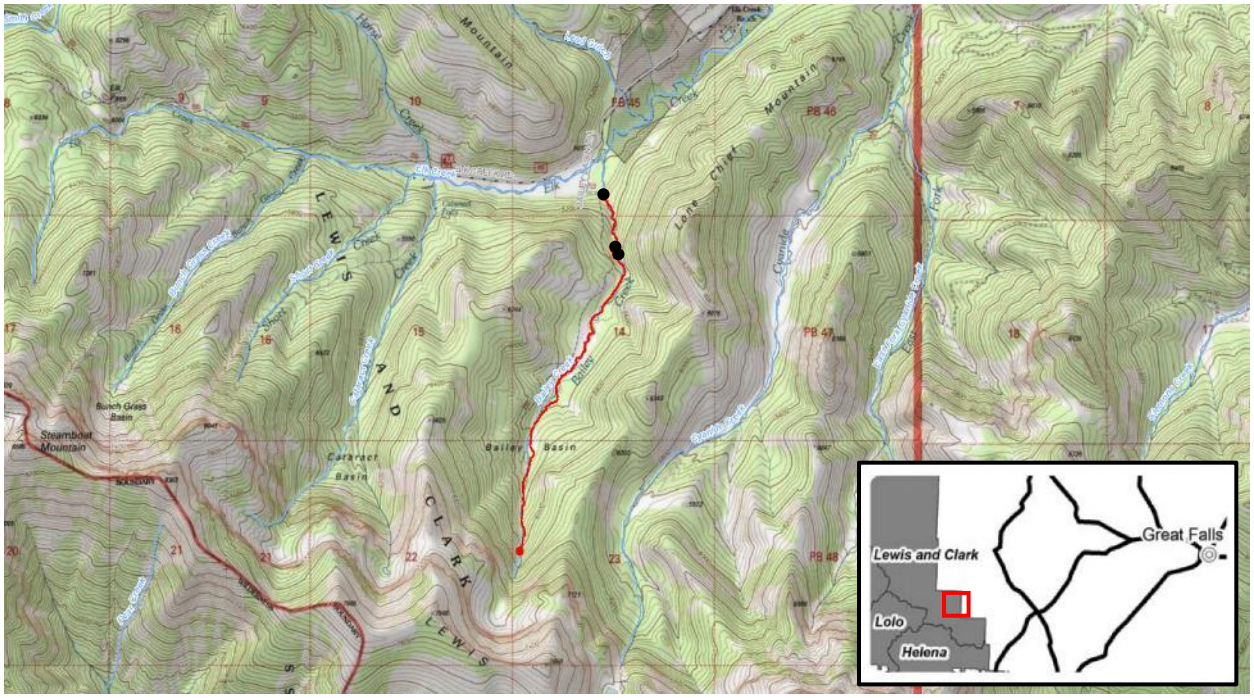

Figure 34. Bailey Creek in the Sun River subbasin. The stream segments delineated in red indicates the fishless habitat surveyed in 2021.

Background

Bailey Creek is a tributary of Elk Creek (Sun River drainage) on the Rocky Mountain Ranger District of the Helena-Lewis and Clark National Forest (Figure 34). Previous survey work conducted in 2021 found Bailey Creek to be fishless above a series of waterfall barriers located upstream of the confluence with Elk Creek. It was estimated that Bailey Creek contained a total of 2.0 miles of habitat that appeared suitable for WCT introduction.

2023 Monitoring

Stream temperature monitoring of Bailey Creek occurred in 2023 to assess suitability for WCT. A temperature logger was deployed at 47.30896, -112.59230 in Bailey Basin on June 13th and recovered on October 4th. The mean July stream temperature was 6.65°C and the mean August stream temperature was 6.57°C (Figure 35), which are below the 7.8°C minimum that would likely limit successful cutthroat trout reproduction and recruitment (Harig and Fausch 2002). Bailey Creek should not be considered a candidate for translocation of at-risk WCT populations because of the lack of suitable thermal habitat.

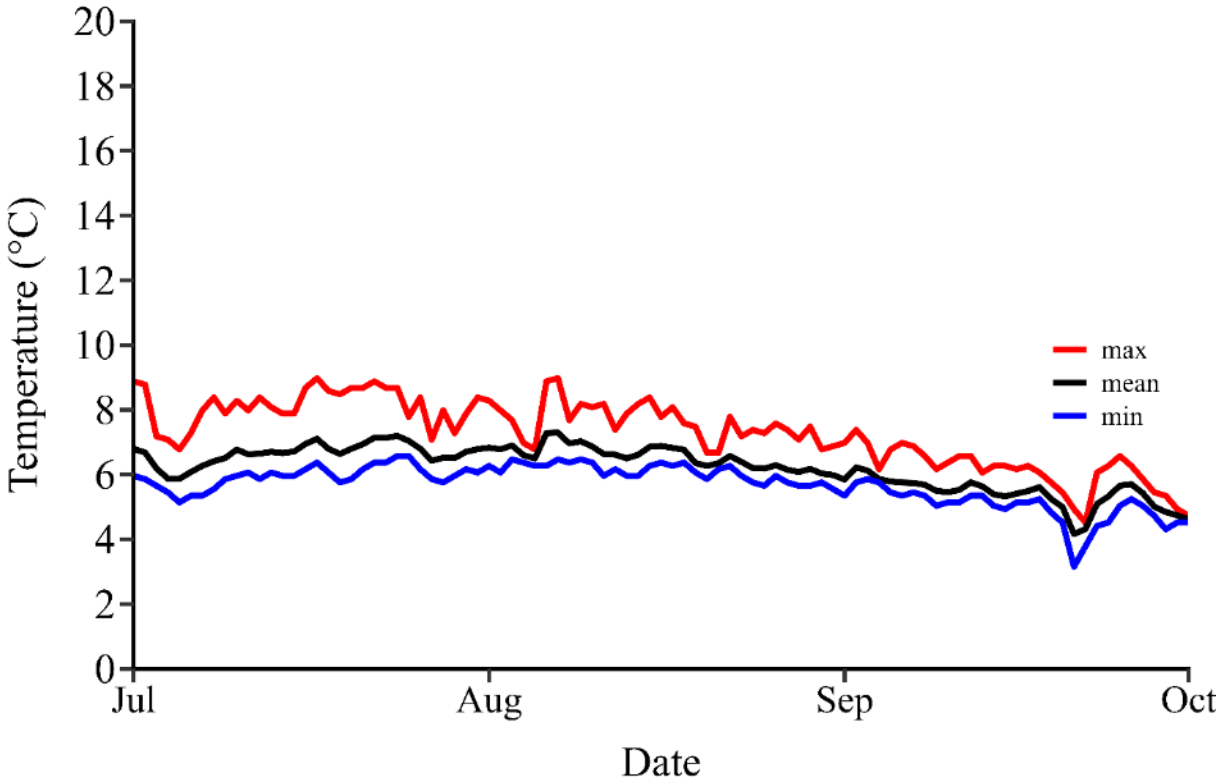

Figure 35. Daily maximum (red line), mean (black line), and minimum (blue line) stream temperatures from Bailey Creek. Temperature logger was deployed on June 13, 2023 and collected October 4, 2023.

VI. Teton River Subbasin

South Fork Teton River

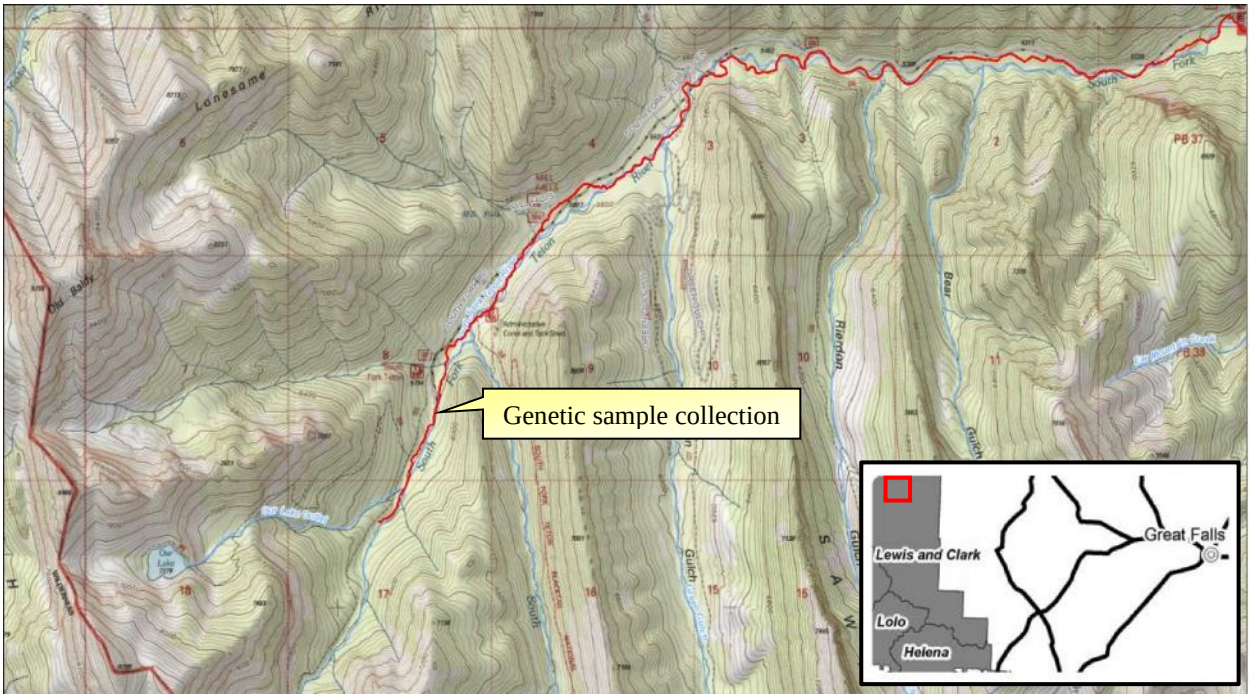

Figure 36. South Fork Teton River in the Teton River subbasin. The stream segments delineated in red indicate the area occupied by WCT.

Background

The South Fork Teton River is a 10-mile-long headwater tributary to the Teton River located approximately 21 miles west of Choteau, MT. This system is seasonally connected but in late summer large portions of the mainstem South Fork Teton go dry and the tributaries become disconnected. Major fish bearing tributaries include Green Gulch and South Fork South Fork Teton River. A single previous genetic sample collection from the mainstem South Fork Teton River in 1994 indicated that the WCT population was nonhybridized at that time.

2023 Monitoring

The South Fork Teton River was sampled on July 20th, 2023, to collect genetic samples as part of an effort to update all at-risk WCT populations that previously were reported as nonhybridized. A 0.6-mile reach was backpack electrofished starting at the South Fork Teton trailhead. A total of 20 WCT were collected ranging in size from 104-233 mm in total length. Fish presence appeared to end at 47.83865, -112.78805. No brook trout were collected in this reach, although they are common in downstream reaches. It was noted that just below the South Fork Teton trailhead streamflow became subsurface.

VII. Two Medicine River Subbasin

Pike Creek

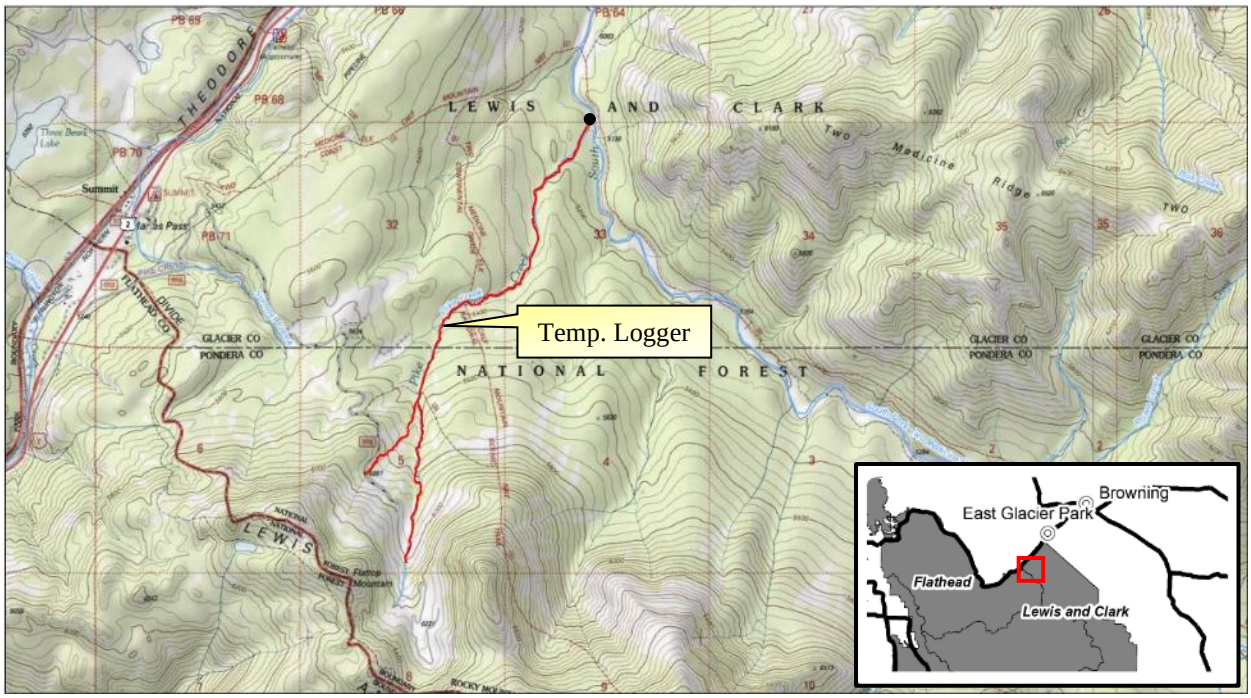

Figure 37. Pike Creek in the Two Medicine River subbasin. The stream segment delineated in red indicates the fishless area that appears suitable for WCT. The black circle represents presence of bedrock barrier.

Background

Pike Creek is a tributary of the South Fork Two Medicine River located approximately 9 miles southeast of the community of East Glacier Park, MT. Previous survey work in 2022 confirmed the presence of a bedrock barrier that isolates this drainage from the South Fork Two Medicine River. Rocky Mountain Sculpin was the only fish species collected above this barrier. The Pike Creek watershed was highly impacted by the 2007 Skyland Fire resulting in a channel filled with large woody debris.

2023 Monitoring

Stream temperature monitoring of Pike Creek occurred in 2023 to assess suitability for WCT. A temperature logger was deployed at 48.31193, -113.31978 above the Forest Service Trail #134 crossing on June 14th and recovered on October 16th. The mean July stream temperature was 10.38°C (Figure 38), which is above the 7.8°C minimum that would likely limit successful cutthroat trout reproduction and recruitment (Harig and Fausch 2002). The battery failed on August 22nd, limiting our inference of summer stream temperatures to the month of July. Pike Creek should be considered as a candidate for translocation of at-risk WCT populations.

A 250 m reach of Pike Creek was backpack electrofished above the Forest Service Trail #134 crossing on June 14th, 2023. No fish were collected or observed in this effort. Habitat was similar to the reach electrofished in 2022 above the bedrock barrier, consisting mainly of large woody debris and thick alders. Tailed frog larvae were abundant as well as aquatic macroinvertebrates.

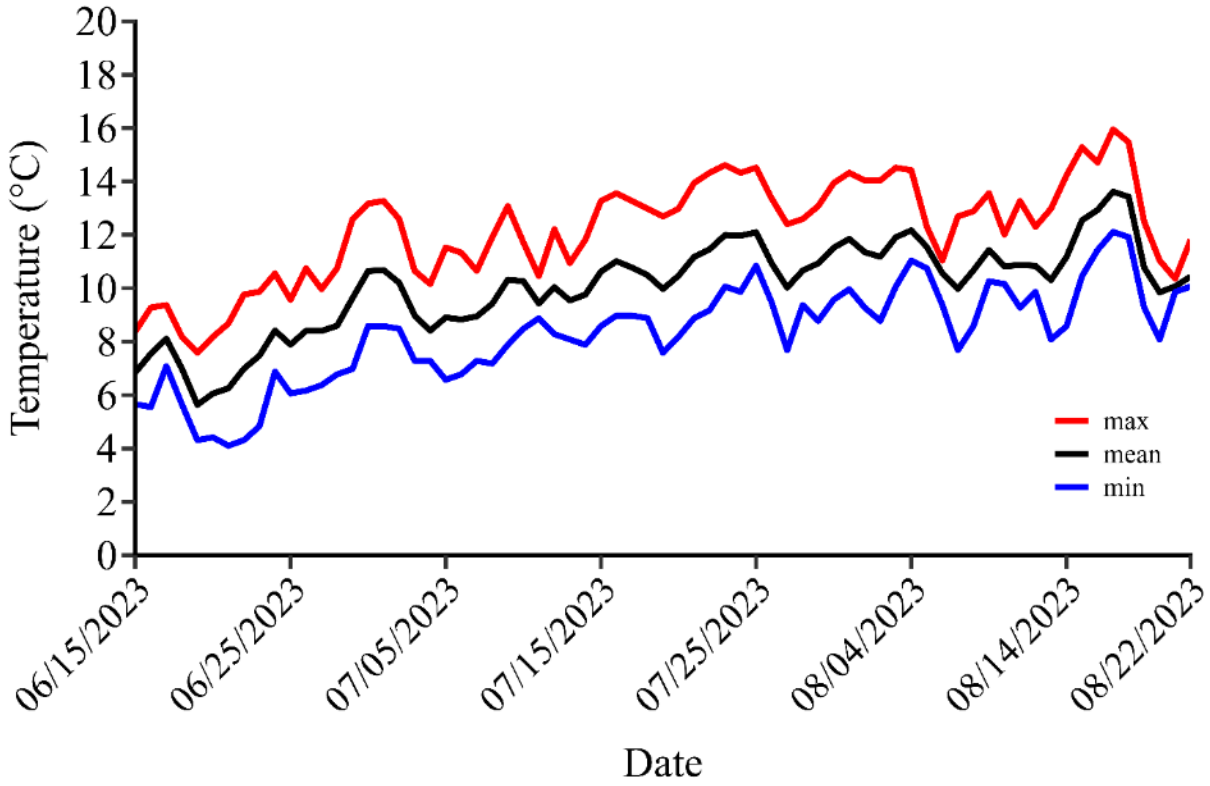

Figure 38. Daily maximum (red line), mean (black line), and minimum (blue line) stream temperatures from Pike Creek. Temperature logger was deployed on June 14, 2023, and collected October 16, 2023. Battery failed on August 22, 2023.

VIII. Upper Missouri River Subbasin

Beartrap Gulch

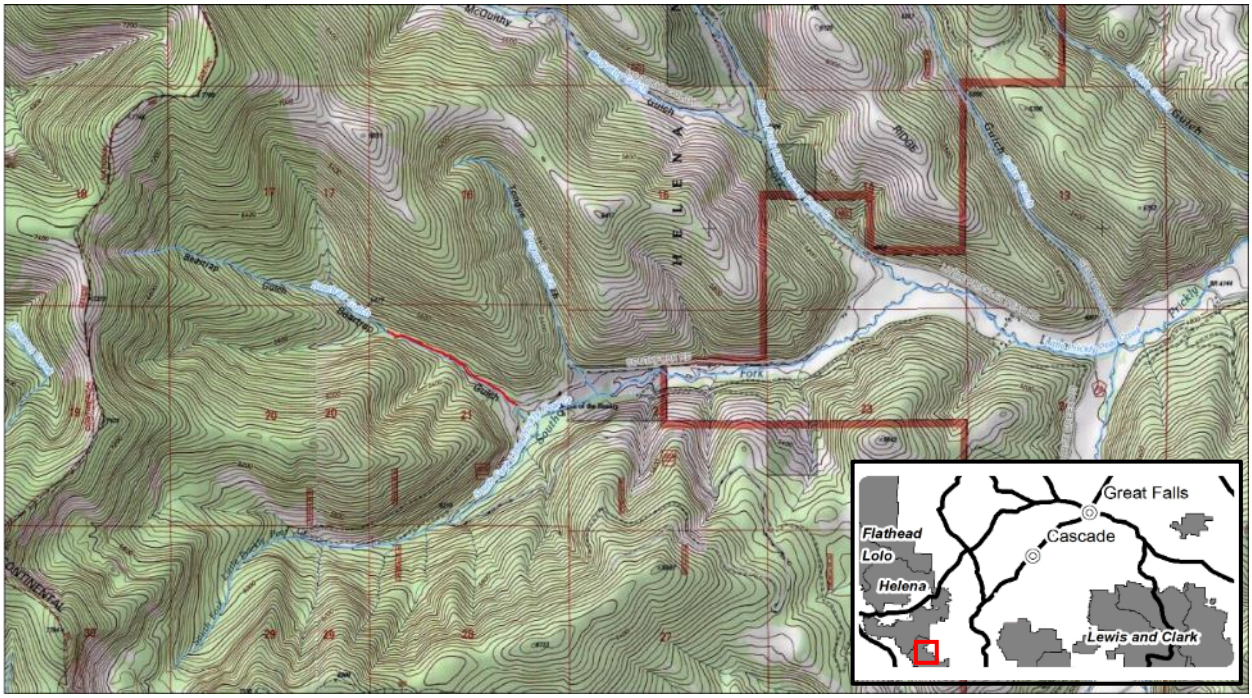

Figure 39. Beartrap Gulch in the Upper Missouri River subbasin. The stream segment delineated in red indicates the area occupied by WCT.

Background

Beartrap Gulch is a tributary of South Fork Prickly Pear Creek in the Upper Missouri River subbasin located approximately 24 miles northwest of Helena, MT. No previous collection records for this waterbody could be found. Genetic samples collected from South Fork Prickly Pear Creek near the Beartrap Creek confluence in 1990 indicated the presence of a WCTxRB hybrid swarm with a significant rainbow trout genetic contribution (65% WCT 35% RB).

2023 Monitoring

Beartrap Gulch was surveyed on September 18th, 2023, to determine WCT presence/absence. A 0.8-mile reach of Beartrap Gulch was backpack electrofished starting at the South Fork Little Prickly Pear Road (FS RD 4038) culvert. A total of 12 fish that visually appeared to be WCT were collected ranging in size from 88-125 mm in length (Figure 40). Two adult brook trout were also collected in this effort. The stream emerged from the streambed near the confluence of the unnamed tributary at 46.78526, -112.49396 above which it was dry. Genetic samples should be collected from the WCT in Beartrap Gulch to determine their genetic status.

The FS RD 4038 culvert was evaluated and appeared to act as a seasonal partial barrier (Figure 41). The culvert was perched at the base flow conditions observed and there was an approximately two-foot drop to the pool below. Two brook trout were collected from the pool below the culvert.

Figure 40. WCT collected in Beartrap Gulch.


Figure 41. Perched culvert at the FS RD 4038 crossing of beartrap Gulch.

Cottonwood Gulch

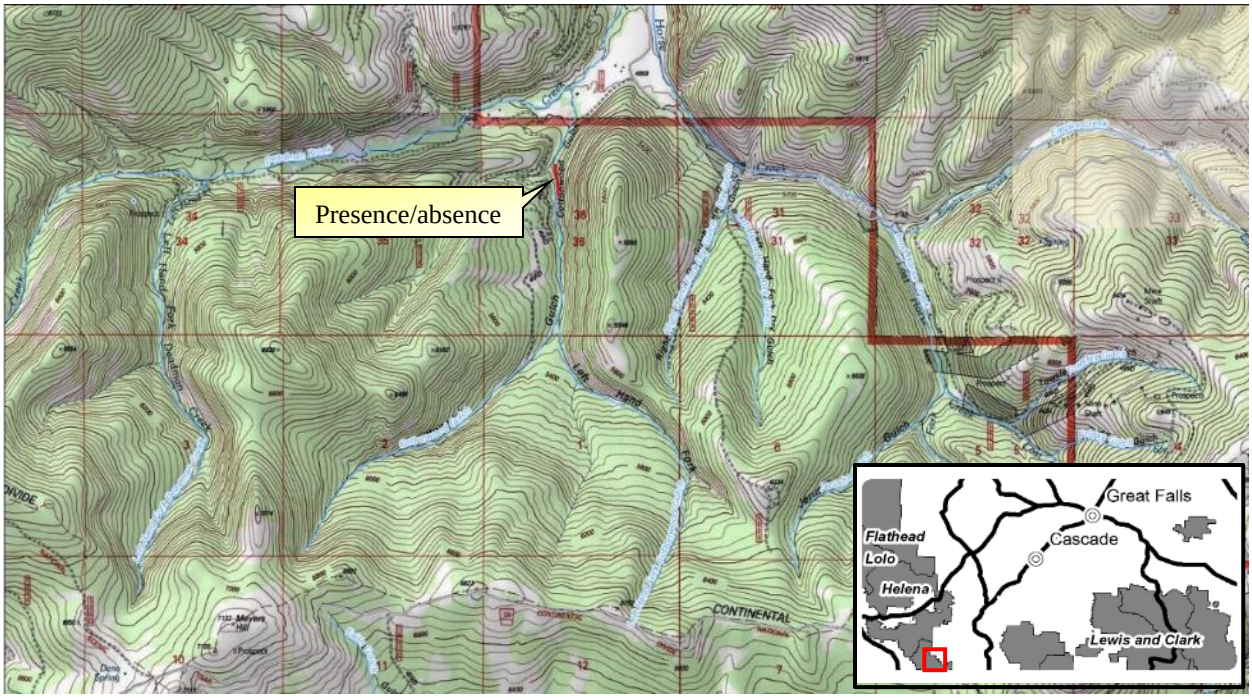

Figure 42. Cottonwood Gulch in the Upper Missouri River subbasin. The stream segment delineated in red indicates the area sampled in 2023.

Background

Cottonwood Gulch is a tributary of Deadman Creek in the Upper Missouri River subbasin located approximately 21 miles northwest of Helena, MT. No previous collection records for this waterbody could be found. Previous sampling of Deadman Creek upstream of the Cottonwood Gulch confluence in 2009 noted only the presence brook trout.

2023 Monitoring

Cottonwood Gulch was surveyed on September 25th, 2023, to determine WCT presence/absence. A 130 m reach of Cottonwood Gulch was backpack electrofished above the dispersed camp sites located off the Cottonwood Gulch Spur Road (FS RD 774). A total of 44 brook trout, 14 rainbow trout, and seven Rocky Mountain Sculpin were collected. No WCT were detected in the area sampled.

Empire Creek

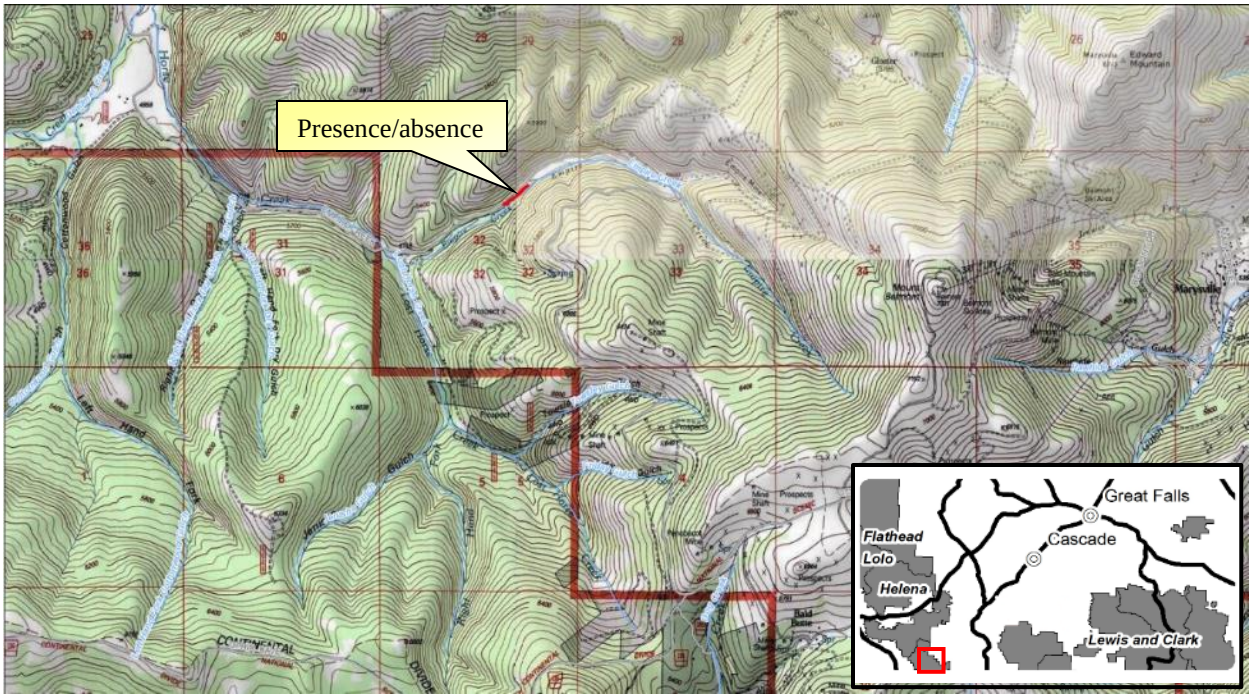

Figure 43. Empire Creek in the Upper Missouri River subbasin. The stream segments delineated in red indicates the area sampled in 2023.

Background

Empire Creek is a tributary of Lost Horse Creek in the Upper Missouri River subbasin located approximately 19 miles northwest of Helena, MT. No previous collection records for this waterbody could be found.

2023 Monitoring

Empire Creek was surveyed on September 25th, 2023, to determine WCT presence/absence. A 250 m reach of Empire Creek was backpack electrofished below the Empire Mine (Figure 43). The reach sampled contains a 0.3 ac instream pond (Figure 44), although the pond itself was not sampled. A total of 27 brook trout and one brown trout were collected. The single brown trout was collated below the pond and only brook trout were collected above the pond. Several brook trout were visually observed in the pond. No WCT were detected in the area sampled.

Figure 44. Instream pond on Empire Creek. Brook trout were visually observed.

Left Hand Fork Deadman Creek

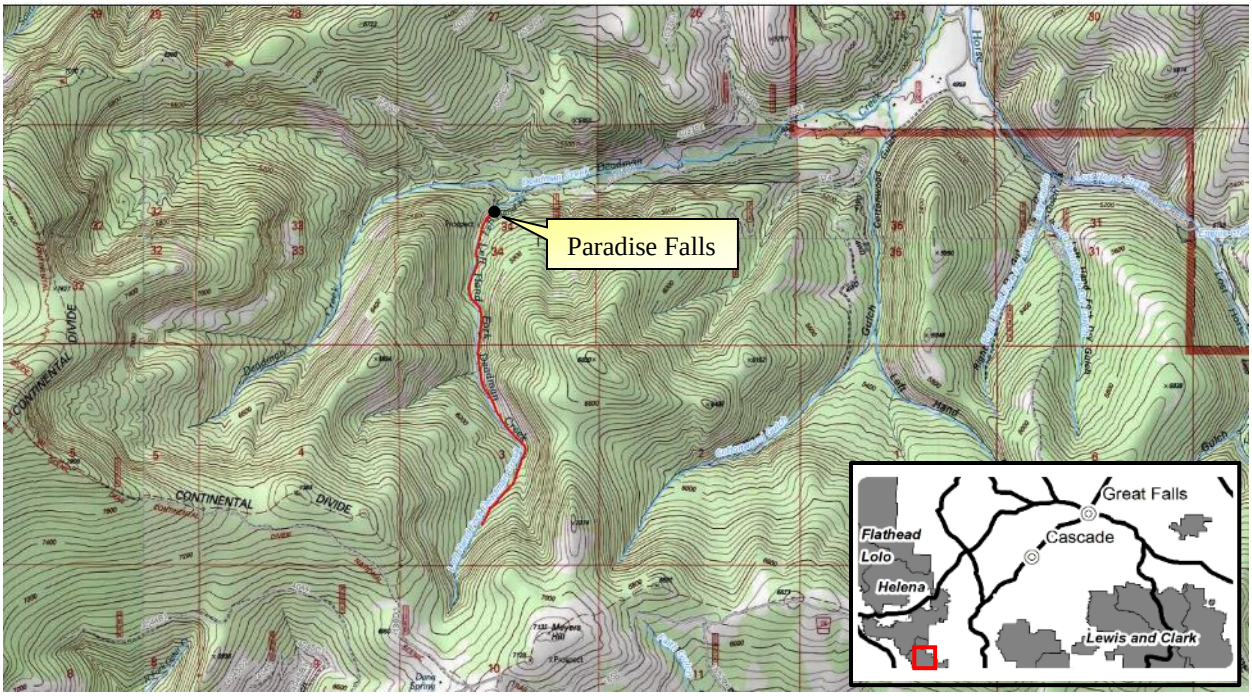

Figure 45. Left Hand Fork Deadman Creek in the Upper Missouri River subbasin. The stream segments delineated in red indicate potential WCT conservation area. The black circle represents presence of waterfall barrier.

Background

Left Hand Fork Deadman Creek is a tributary of Deadman Creek in the Upper Missouri River subbasin located approximately 22 miles northwest of Helena, MT. No previous collection records for this waterbody could be found. Previous sampling of Deadman Creek upstream of the Left Hand Fork Deadman Creek confluence in 2009 noted only the presence brook trout.

2023 Monitoring

Left Hand Fork Deadman Creek surveyed on June 5th and July 19th, 2023, to determine fish/presence absence and evaluate habitat for potential WCT introduction. Paradise Falls, located at 46.75165, -112.46391, was evaluated on June 5th and determined to be a complete barrier to fish movement (Figure 46). The reach immediately below Paradise Falls was backpack electrofished and three brook trout were collected. A 240 m reach above Paradise Falls was backpack electrofished and no fish were collected or observed. Habitat appeared excellent with numerous pools, large woody debris, spawning gravels, and aquatic macroinvertebrates observed. On July 19th, 2023, the headwaters of Left Hand Fork Deadman Creek were backpack electrofished to further evaluate fish presence/absence and continue evaluation of fish habitat. A 1.7-mile reach was surveyed from the spring source downstream to the confluence of the unnamed tributary. No fish were collected or observed. Fish habitat was marginal in the headwaters of Left Hand Fork Deadman Creek and a total of 1.64 miles of suitable habitat was estimated to be present above Paradise Falls.

Stream temperature monitoring of Left Hand Fork Deadman Creek was attempted in 2023 to assess suitability for WCT. A temperature logger was deployed at 46.75029, -112.46526 above Paradise Falls on June 5th and recovered on October 17th. The logger battery failed on June 16th shortly after being launched. Stream temperature monitoring will be performed again in 2024 to determine habitat suitability.

Figure 46. Paradise Falls on Left Hand Fork Deadman Creek located at 46.75165, -112.46391.

Lost Horse Creek

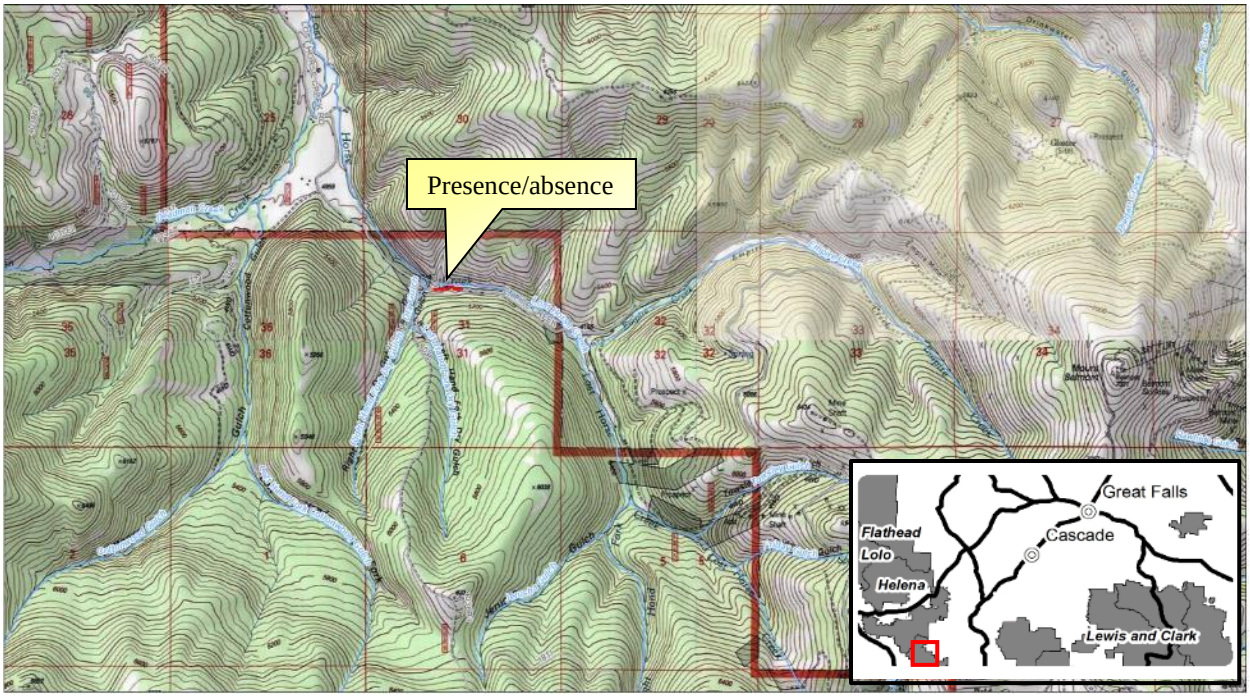

Figure 47. Lost Horse Creek in the Upper Missouri River subbasin. The stream segments delineated in red indicates the area sampled in 2023.

Background

Lost Horse Creek is a tributary of Little Prickly Pear Creek in the Upper Missouri River subbasin located approximately 20 miles northwest of Helena, MT. No previous collection records for this waterbody could be found.

2023 Monitoring

Lost Horse Creek was surveyed on September 25th, 2023, to determine WCT presence/absence. A 200 m reach of Lost Horse Creek was backpack electrofished upstream of the Dry Gulch confluence (Figure 44). A total of seven rainbow trout, five brook trout, and three brown trout were collected. No WCT were detected.

Marsh Creek

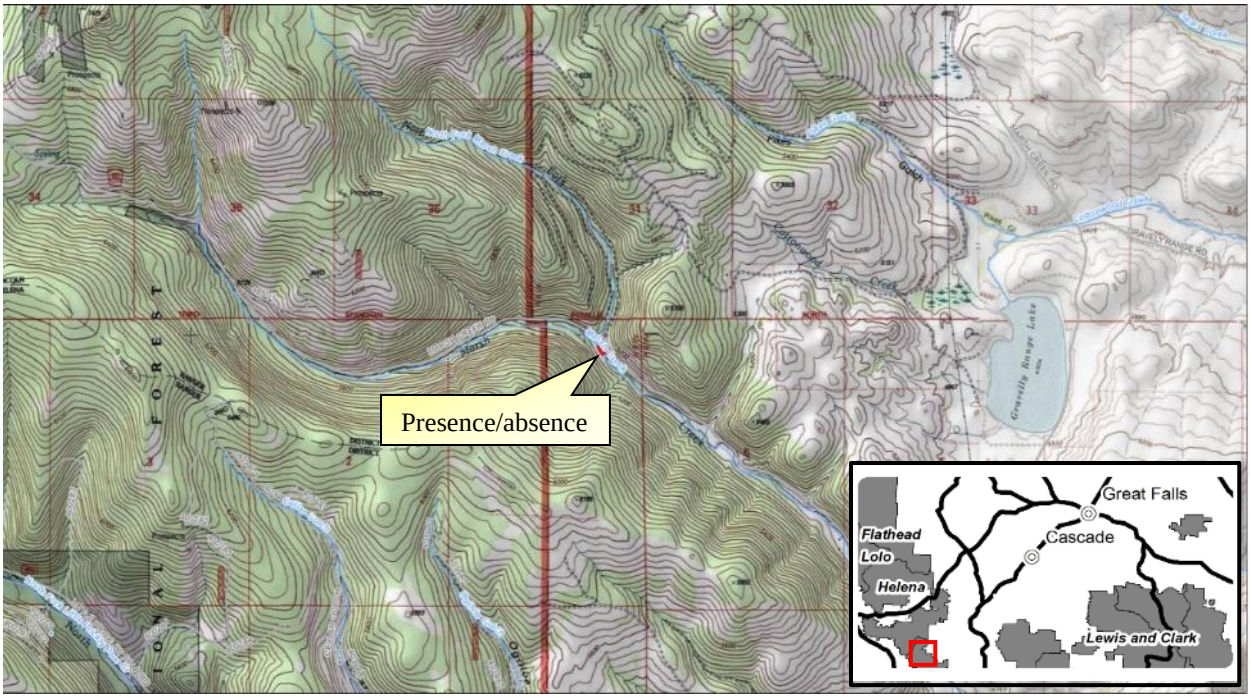

Figure 48. Marsh Creek in the Upper Missouri River subbasin. The stream segment delineated in red indicates the area sampled in 2022.

Background

Marsh Creek is a tributary of Little Prickly Pear Creek in the Upper Missouri River subbasin located approximately 21 miles northwest of Helena, MT. No previous collection records for this waterbody could be found.

2023 Monitoring

Marsh Creek was surveyed on September 18th, 2023, to determine WCT presence/absence. A 75 m reach of Marsh Creek was backpack electrofished below the North Fork Marsh Creek confluence (Figure 45). A total of 33 brook trout were collected. No WCT were detected.

Page Gulch

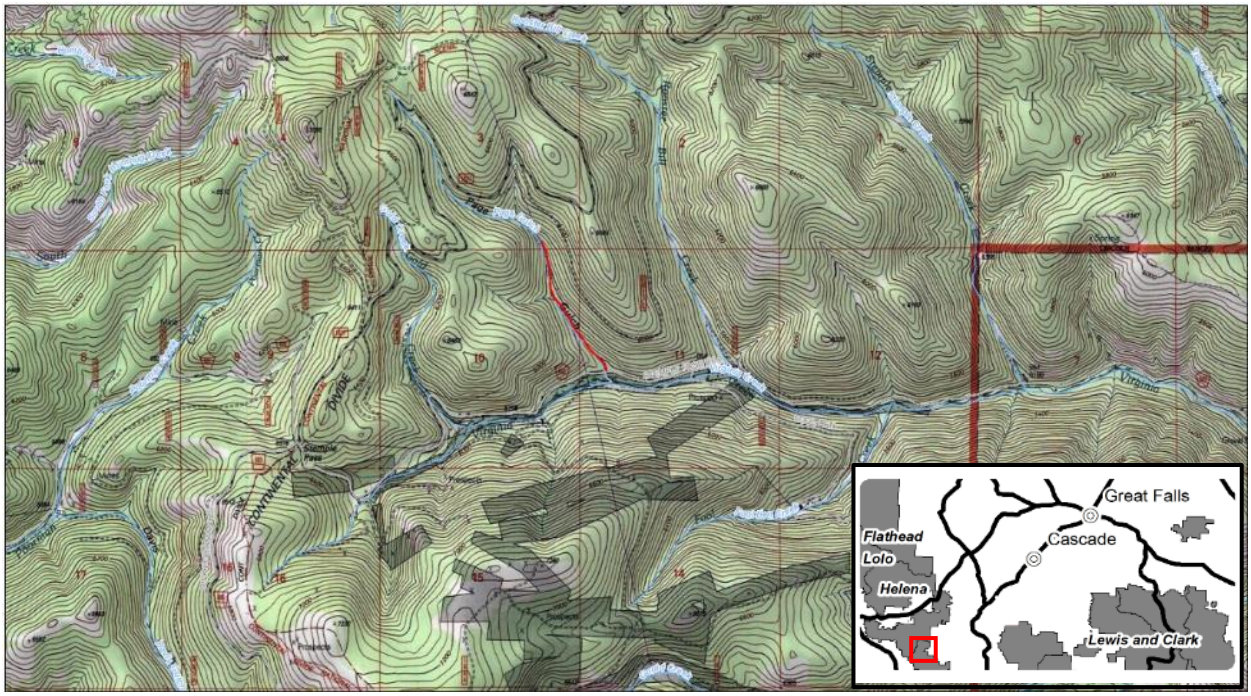

Figure 46. Page Gulch in the Upper Missouri River subbasin. The stream segment delineated in red indicates the area occupied by WCT.

Background

Genetic monitoring of WCT collected in Page Gulch in 1997 indicated a nonhybridized population was present at that time (n=6). Updated genetic samples collected in 2021(n=20) found clear evidence of rainbow trout hybridization. However, rainbow trout alleles were non-randomly distributed among individuals in the sample. A clear bimodal pattern was present where many individuals appeared to be non-hybridized WCT (~45% of the sample), and a fairly large percentage of individuals that had ~28% rainbow trout ancestry (25% of the sample). The WCT population in Page Gulch is at extremely high risk of genomic extinction within the very immediate future as there appears to be high immigration of rainbow trout genes into this population. A genetic triage project was initiated on Page Gulch in the summer of 2022. The entire fish bearing reach of the creek was backpack electrofished to PIT tag and collect genetic samples from all WCT greater than 70 mm in length. A total of 92 WCT were tagged over a two-day sampling period.

2023 Monitoring

Page Gulch was sampled on May 5th and 15th, 2023, to determine if adult WCT from Virginia Creek were accessing the stream for spawning. A 700 m reach was backpack electrofished beginning at the Stemple Pass Road. Discharge was high on both visits resulting in low capture efficiency. On May 5th, only two previously tagged WCT and one brook trout were collected. On May 15th, four previously untagged WCT were collected, PIT tagged, and sampled for genetic analysis. No adult WCT were observed on either visit.

Results from the genomic analysis of the 92 WCT tagged in 2022 were received on July 7th, 2023. Of the 92 individuals tagged, 36 appeared to be nonhybridized WCT (Appendix A). Fourteen individuals possessed a substantial amount of rainbow trout alleles while 32 fish possessed relatively minute amounts of rainbow trout alleles. Ten samples failed to run.

Upper Log Gulch Reservoir and Log Gulch

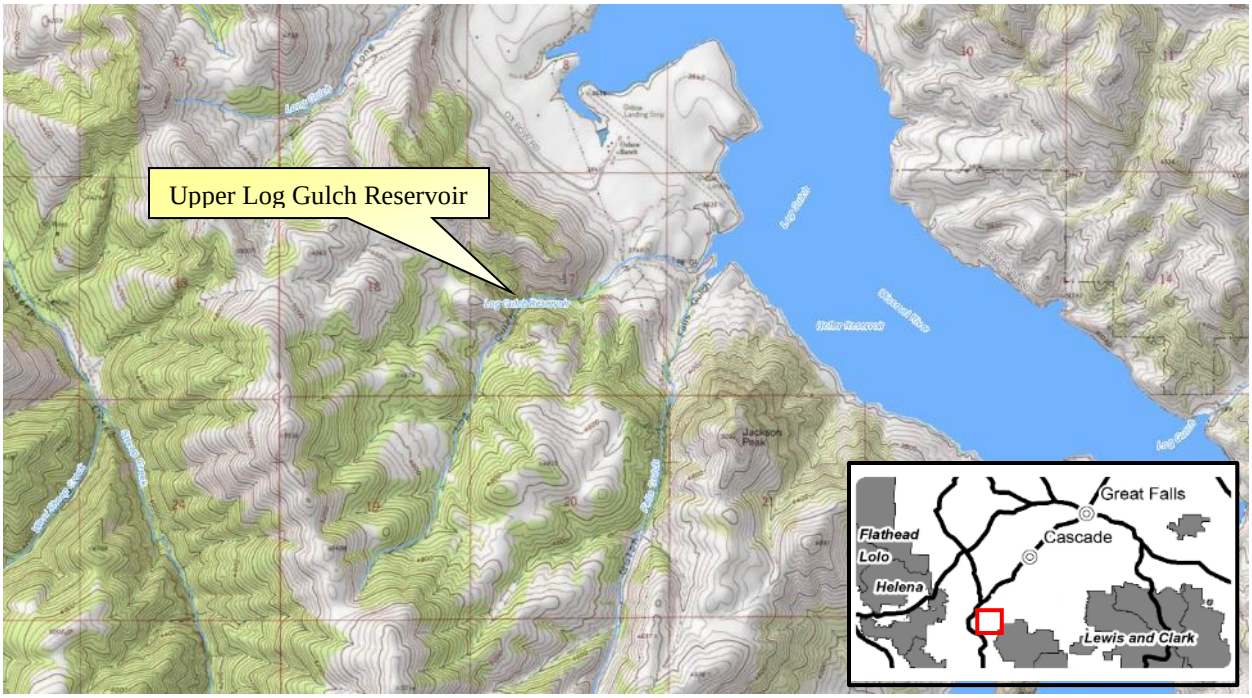

Figure 47. Upper Log Gulch Reservoir in the Upper Missouri River subbasin.

Background

Upper Log Gulch Reservoir is a 0.85-acre impoundment located on the Oxbow Ranch south of the town of Wolf Creek, MT. A Candidate Conservation Agreement with Assurances (CCAA) was secured with the landowners in November 2017 to pursue the establishment of a WCT brood source in the reservoir. Nonnative brown trout were targeted for removal from the pond in 2017-2018 by gill and trap net. A total of 282 brown trout were removed in this effort. In 2019, 86 WCT were transferred from Cottonwood Creek (Upper Missouri River subbasin) and released in Upper Log Gulch Reservoir. An additional 125 WCT were collected from Cottonwood Creek and transferred to Upper Log Gulch Reservoir in 2021.

2023 Monitoring

Upper Log Gulch Reservoir was surveyed on June 21st, 2023, to determine the status of the previously transferred WCT. Two mountain lake gill nets and angling were used to sample the reservoir. Gill nets were checked every half hour to prevent WCT mortality. A total of 9 WCT and 7 brown trout were collected. WCT ranged from 10 to 14 inches in length and brown trout 14 to 23 inches in length (Figure 48). All WCT were released back into Upper Log Gulch Reservoir and brown trout were transferred to Lower Log Gulch Reservoir.

A 375 m reach of Log Gulch above Upper Log Gulch Reservoir was backpack electrofished to determine fish presence/absence. No fish were detected in this effort. A bedrock barrier located just upstream (~20 feet) of the reservoir appears to preclude access to Log Gulch (Figure 49). Evaluation of habitat above this barrier should be performed to determine suitability for WCT introduction. Without access to this reach of Log Gulch for spawning, it is unlikely that WCT will persist in Upper Log Gulch Reservoir.

Figure 48. WCT and brown trout collected from Upper Log Gulch Reservoir in 2023.

Figure 49. Bedrock barrier above Upper Log Gulch Reservoir located at 46.96809, -112.01685.

IX. Upper Missouri-Dearborn River Subbasin

Big Coulee Creek

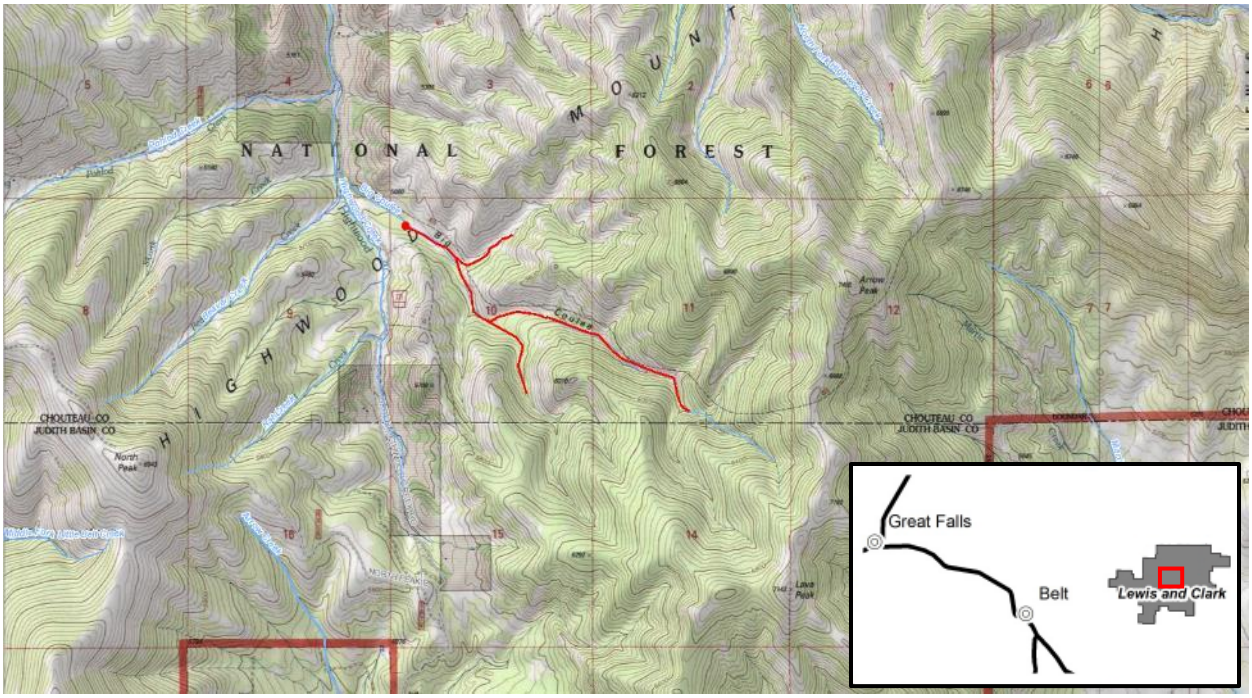

Figure 50. Big Coulee in the Upper Missouri-Dearborn River subbasin. The stream segments delineated in red indicate the areas occupied by nonhybridized WCT.

Background

Big Coulee, a tributary of Highwood Creek, contains a nonhybridized WCT population that has been intensively managed since the late 1990s. A bedrock feature was enhanced on Big Coulee by blasting in 2002 and 2004 to create a barrier to fish movement. From 1997-2008, brook trout were removed to reduce negative impacts on the remaining WCT found above the barrier. 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 WCT in 2016 were classified as nonhybridized.

Nonnative removals were again initiated in 2015 above the barrier. From 2015 to 2021, approximately 674 brook trout were removed including ~200 in 2015, ~330 in 2016, ~110 in 2017, 15 in 2018, 8 in both 2019 and 2020, and 3 in 2021. No brook trout were detected in 2022.

2023 Monitoring

WCT population monitoring was performed on 1.76 miles of Big Coulee from July 31^{stt} - August 3rd, 2023. Eight sections of Big Coulee were two-pass backpack electrofished. A total of 1,789 WCT were collected in the 2023 monitoring effort (Table 1). No brook trout were detected in 2023 for the second consecutive year. Intensive monitoring of brook trout in Big Coulee should continue for at least three consecutive years with no detections.

Table 1. Big Coulee electrofishing catch by section.

	Section 1[1]	**Section 3**	**Section 4**	**Section 5**	**Section 6**	**Section 7**	**Section 8**	**Section 9[2]**
Pass 1	219 WCT	150 WCT	122 WCT	146 WCT	219 WCT	245 WCT	86 WCT	169 WCT
Pass 2	82 WCT	49 WCT	28 WCT	61 WCT	55 WCT	94 WCT	27 WCT	37 WCT

[1] – Sections 1 and 2 were combined in 2023.

[2] – Section 9 was split into three sections that were each two-pass electrofished. Numbers represent combined efforts of three crews.

Wegner Creek

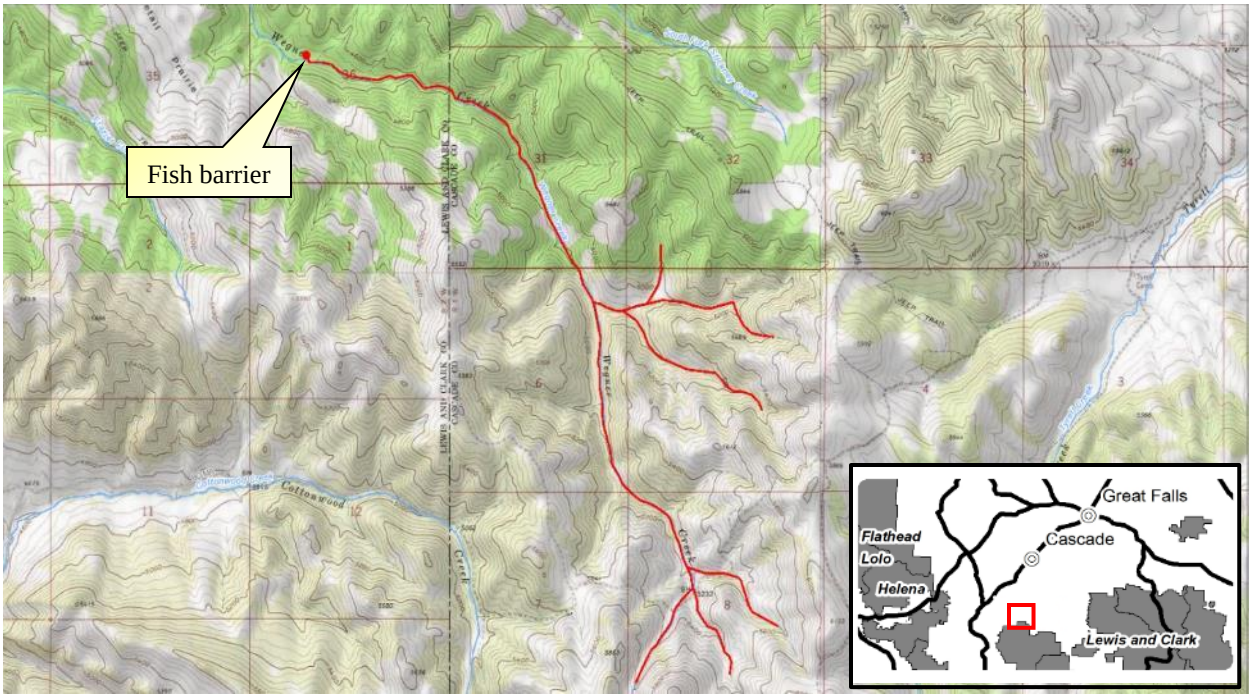

Figure 51. Wegner Creek in the Upper Missouri-Dearborn River subbasin. The stream segments delineated in red indicate potential WCT restoration area.

Background

In 2014, the Beartooth Wildlife Management Area expanded by 2,840-acres and that addition included portions of Wegner Creek (Figure 51). 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 1.5 miles 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 half mile 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 400 m 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. Additional electrofishing above the barrier in 2019 detected brook trout still present in the vicinity of the unnamed tributary, suggesting an incomplete chemical treatment. To test the efficacy of the barrier addition, annual marking of brook and rainbow trout has occurred below the barrier since 2019.

2023 Monitoring

Wegner Creek was surveyed on May 10th and 12th, 2023, to evaluate the efficacy of the fish barrier. On May 10th, a 0.8 mi reach of Wegner Creek was electrofished above the barrier to detect the presence marked fish. No trout were detected in this effort, only Rocky Mountain Sculpin were observed. On May 12th, 2023, a 150 m reach below the barrier was electrofished to detect presence of large migratory

rainbow trout from the Missouri River. A large 444 mm rainbow trout was collected just below the Wegner Creek fish barrier (Figure 52). Spot shocking further downstream resulted in collection of additional large migratory rainbow trout. This marks the first time since 2019 that large rainbow trout were collected in Wegner Creek. The modifications made to the fish barrier in 2019 appear to have prevented rainbow trout from accessing upper Wegner Creek. Monitoring should continue above the barrier in 2024 to determine presence of age-1 fish. Further modifications to the fish barrier should be pursued to enhance functionality under higher flows.

Figure 52. Migratory rainbow trout collected below the Wegner Creek fish barrier in 2023.

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Appendix A
Date: October 16, 2023
Biologist(s): Alex Poole, Katie Vivian
Location(s) and sampling date:

1. Charcoal Creek (47.04694, -110.63353; 08/01/22)
2. Harley Creek Tributary (46.9446, -110.78933; 05/11/22)
3. Palisade Creek (46.90855, -110.68899; 07/28/22)
4. Shorty Creek (46.91490, -110.73287; 09/07/21)
5. Villars Creek (47.03053, -110.61691; 08/01/22)
6. Summit Creek (48.31546, -113.34706; 10/3/22)
7. Cottonwood Creek (46.94874, -111.89869; 04/21/22)
8. South Fork Deep Creek (47.73304, -112.72141; 08/09/22)
9. Page Gulch (46.90344, -112.4682; 08/22/22)

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

PROJECT SUMMARY: Genetic samples from R4 were collected for purposes of describing hybridization status, and for non-hybridized populations, quantifying genetic variation. All Results, Discussion, and Recommendations are described below. Summary statistics for the population samples are in Table 1 (below). Lab and data analysis methods are described in Appendix 1.

RESULTS, DISCUSSION, AND RECOMMENDATIONS

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

Sample	ID	N	Method	Taxa	WCT	RBT	YCT
Charcoal Creek	5584	19	GTseq	WCTxRBT	99.5	0.5	
Harley Creek Tributary	5585	23	GTseq	WCTxRBTxYCT	99.1	0.6	0.3
Palisade Creek	5586	18	GTseq	WCTxRBTxYCT	97.8	1.3	0.9
Shorty Creek	5587	23	GTseq	WCTxRBTxYCT	99.5	0.2	0.3
Villars Creek	5588	20	GTseq	WCTxRBTxYCT			
Summit Creek	5589	18	GTseq	WCTxRBTxYCT	94.6	5.5	0.1
Cottonwood Creek	5590	23	GTseq	WCT	100		
South Fork Deep Creek	5592	23	GTseq	WCT	100		

Page Gulch	5593	84	RADcap	WCT WCTxRBT			

Charcoal Creek
We detected rainbow trout alleles at five rainbow diagnostic markers, and five westslope diagnostic markers were polymorphic. We did not detect any Yellowstone cutthroat trout alleles. The data provide very strong evidence that there is some rainbow trout ancestry in the fish from Charcoal Creek (Fig. 1).

Individual rainbow trout ancestry varied from 0% to 3% (see Appendix 1). We detected non-native ancestry in eleven of the nineteen fish in the sample (58%). Given that the average rainbow trout ancestry across individuals was relatively low (0.5%), and most hybrids had <1% rainbow trout ancestry, it is very likely that we failed to detect non-native ancestry in some of the putatively non-hybridized fish in the sample. Furthermore, genotypic proportions conformed to random mating (Hardy-Weinberg) expectations ($P > 0.999$). As such, it seems reasonable to assume that the sample of fish from Charcoal Creek was collected from a hybrid swarm between westslope cutthroat trout and rainbow trout with approximately 0.5% rainbow trout ancestry. These results are consistent with another sample from Charcoal Creek (#4647).

Harley Creek Tributary
Genetic samples were collected from two reaches of an unnamed tributary to Harley Creek. Allele frequencies were statistically indistinguishable ($P = 0.352$) between the two reaches, suggesting they are genetically connected with one another (i.e., a single population). As such, we combined the samples for analysis.

We detected clear evidence of rainbow trout and Yellowstone cutthroat trout ancestry in the sample from Harley Creek (Fig. 2). Most individuals had small amounts of non-native ancestry (<3%), but one individual had >10% rainbow trout ancestry (see Appendix 1). The presence of a single individual with comparatively higher non-native ancestry may suggest hybrids with higher rainbow trout ancestry are actively invading this stream. Whether this poses a risk worth addressing is somewhat debatable, as the majority of fish in the sample were hybrids (56%), and that value is almost certainly an underestimate given that rainbow trout and Yellowstone cutthroat trout ancestry was relatively low on an individual basis. Furthermore, genotypic proportions conformed to random mating (Hardy-Weinberg) expectations ($P > 0.999$), which together, suggest the sample of fish was collected from a hybrid swarm with approximately 0.6% rainbow trout and 0.3% Yellowstone cutthroat trout ancestry. These results are consistent with a downstream sample in Harley Creek which also detected rainbow trout ancestry (#2917).

Palisade Creek
We detected clear evidence of rainbow trout and Yellowstone cutthroat trout ancestry in the sample from Palisade Creek (Fig. 3). The vast majority of fish in the sample were hybrids (94%), and that value is likely an underestimate given that rainbow trout and Yellowstone

cutthroat trout ancestry was relatively low on an individual basis (see Appendix 1). Furthermore, genotypic proportions conformed to random mating (Hardy-Weinberg) expectations ($P > 0.999$). Palisade Creek appears to harbor a hybrid swarm between westslope cutthroat trout, rainbow trout, and Yellowstone cutthroat trout, with approximately 1.3% rainbow trout and 0.9% Yellowstone cutthroat trout ancestry. A previous sample collected in 2003 (#2919) did not detect any non-native alleles, but the sample was small (n=10), especially given the relatively limited number of diagnostic markers available at that time. Given how widespread non-native ancestry was among fish in this sample, we strongly suspect that hybridization was present, but not detected in the previous sample.

Shorty Creek

In the sample from Shorty Creek we detected rainbow trout alleles at three rainbow diagnostic markers, Yellowstone alleles at two Yellowstone diagnostic markers, and one westslope diagnostic marker was variable. While evidence for non-native ancestry was not overwhelming (i.e., non-native alleles were sparse), the data are fairly convincing that fish in Shorty Creek have both rainbow trout and Yellowstone cutthroat trout ancestry. Given that non-native alleles were relatively rare, further analyses are challenging and potentially misleading. At present it would be best to assume that Shorty Creek harbors a randomly mating ($P = 0.999$) hybrid swarm between westslope cutthroat trout, rainbow trout, and Yellowstone cutthroat trout with a small amount of rainbow (0.2%) and Yellowstone (0.3%) ancestry.

A previous sample collected in 1997 (#1226) did not detect any non-native alleles, but the sample was small (n=5), especially given the relatively limited number of diagnostic markers available at that time. We strongly suspect that non-native ancestry was present, but not detected in the previous sample.

Villars Creek

We clearly detected rainbow trout ancestry in fish from Villars Creek (Fig. 4). We also detected two Yellowstone cutthroat trout alleles at one Yellowstone diagnostic marker, which may suggest there is a very small amount of Yellowstone ancestry, though confirmation of this (putative) Yellowstone ancestry is somewhat meaningless given the obvious rainbow trout ancestry.

Estimates of individual rainbow trout ancestry varied from 0% to 29%, but there was only one individual with >3% rainbow trout ancestry (i.e., the fish with 29% rainbow trout ancestry). That individual clearly had higher rainbow trout ancestry than other individuals in the sample, which strongly suggests it is either an immigrant into the population, or the progeny of a rainbow trout hybrid with substantial rainbow trout ancestry that recently invaded this section of Villars Creek. We detected non-native alleles in half of the fish in the sample (10 out of 20). This is likely an underestimate given that many individuals had relatively small amounts of non-native ancestry (see Appendix 1). Overall, we suspect the sample was collected from a randomly mating ($P > 0.999$) hybrid swarm between westslope cutthroat trout, rainbow trout, and Yellowstone cutthroat trout, that is currently being invaded (or was invaded) by fish with substantially higher rainbow trout ancestry.

However, we cannot rule out the possibility that some of the putatively non-hybridized fish are indeed non-hybridized westslope cutthroat trout. If so, it would be extremely important to salvage them (remove them and translocate elsewhere) as soon as possible. Higher resolution genomic data are needed to resolve this uncertainty; we will move forward with producing that data to more accurately describe the hybridization status of Villars Creek.

Summit Creek

We clearly detected rainbow trout ancestry in fish from Summit Creek (Fig. 5). We also detected two Yellowstone cutthroat trout alleles, which may suggest there is a very small amount of Yellowstone ancestry, though confirmation of this (putative) Yellowstone ancestry is somewhat meaningless given the obvious rainbow trout ancestry.

Individual rainbow trout ancestry ranged from 3% to 11%. All individuals in the sample were hybrids, which clearly demonstrates the sample was collected from a hybrid swarm with a predominant rainbow trout genetic contribution (5.5%) and a very small amount of Yellowstone cutthroat trout ancestry (0.1%). These results are consistent with a previous genetic sample from Summit Creek that was collected lower in the watershed (#623).

Cottonwood Creek

In the sample from Cottonwood Creek we did not detect any rainbow trout or Yellowstone cutthroat trout alleles, and none of the westslope diagnostic markers were polymorphic. The data strongly suggest that Cottonwood Creek harbors non-hybridized westslope cutthroat trout. These results are consistent with recent sampling from Threemile Creek (donor source), which provided strong evidence that Threemile Creek harbored non-hybridized westslope cutthroat trout (#5428). However, the genetic method used here does have higher resolution, so these data provide even greater confidence that Threemile and Cottonwood both harbor non-hybridized westslope.

South Fork Deep Creek

In the sample of 25 fish from South Fork Deep Creek we did not detect any rainbow trout or Yellowstone cutthroat trout alleles, and none of the westslope diagnostic markers were polymorphic. These data provide very strong evidence that South Fork Deep Creek harbors a non-hybridized population of westslope cutthroat trout. These results are consistent with a previous sample from this stream (#4345). However, it should be noted that there are some questions regarding the genetic origination of South Fork Deep Creek (native vs. introduced from source west of the divide). Recent state-wide analyses that were conducted in 2020 strongly suggested that South Fork Deep Creek was potentially derived from a source west of the divide, and this result is consistent with communications that Dave Moser had with Robb Leary. With these data, we have greatly expanded our resolution to address this question, and will soon be able to do so once our westslope genetic baseline is re-established. Until that time, we urge extreme caution when using this population for conservation efforts.

Page Gulch

We obtained quality genetic data from 84 individuals in Page Gulch (see Appendix 1). As expected, we clearly detected rainbow trout ancestry in some individuals. We also detected a very small number of Yellowstone cutthroat trout alleles – at those allele frequencies it is nearly impossible to decipher whether they represent true Yellowstone cutthroat trout ancestry, or are a false positive. Given the conservation situation (genetic salvage), it is likely best to assume the former. Of the 84 individuals, we did not detect any rainbow trout or Yellowstone cutthroat trout alleles in 36 fish. Those fish are suitable for translocation to other water bodies. It should be noted that some of those individuals may have a very small amount of non-native ancestry that we simply failed to detect (<0.1%); that possibility should be taken into consideration when determining a destination for these fish.

FIGURES

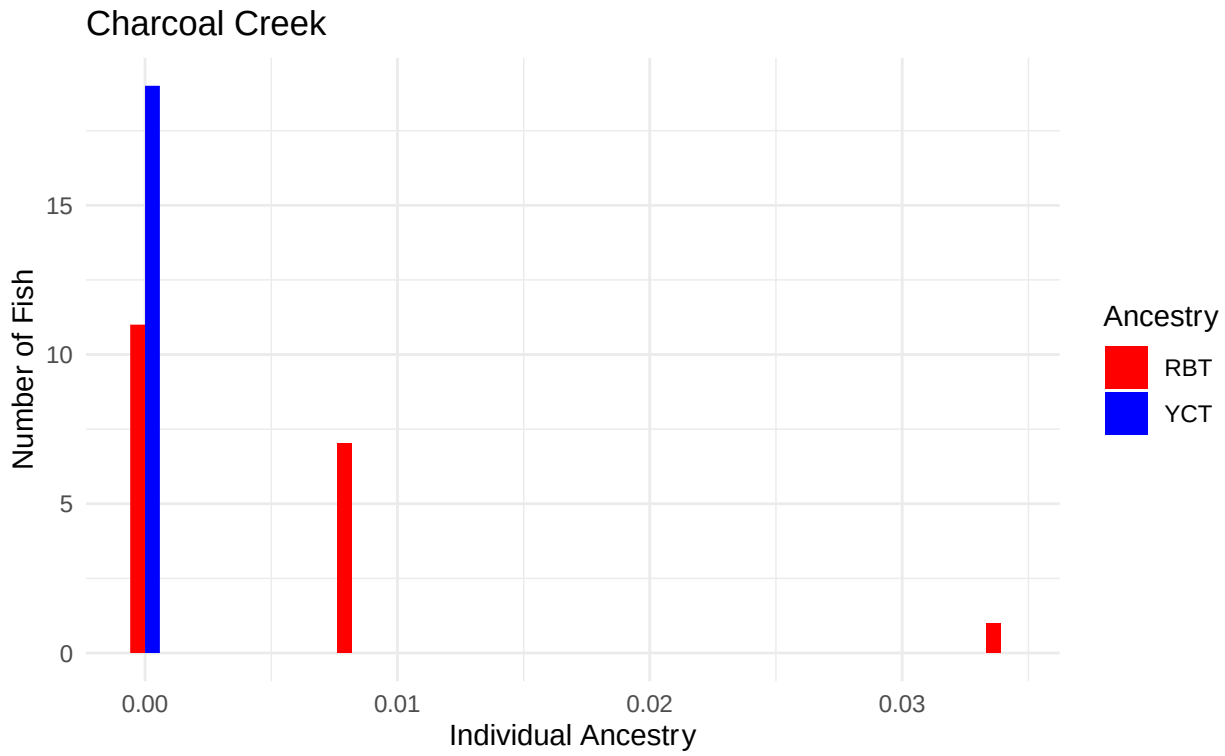

Figure 1. Observed distribution of individual rainbow and Yellowstone cutthroat trout ancestry in a sample of fish from Charley Creek.

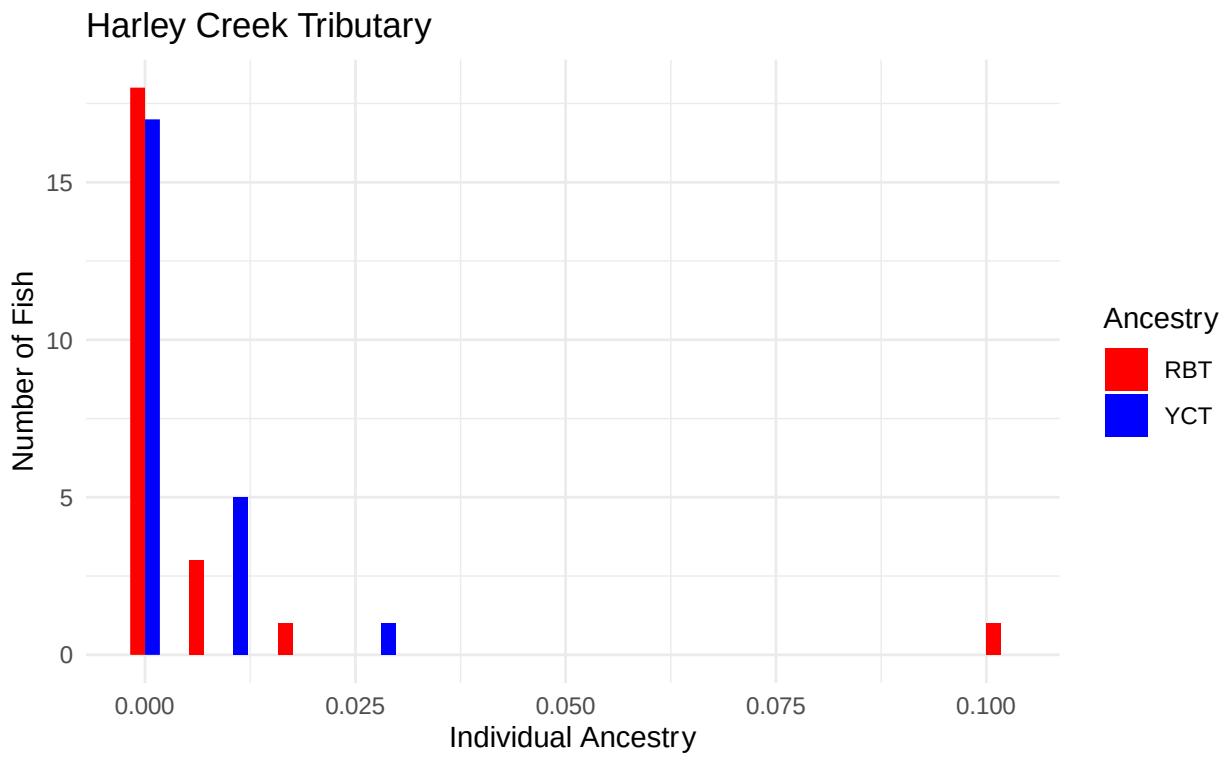

Figure 2. Observed distribution of individual rainbow and Yellowstone cutthroat trout ancestry in a sample of fish from a tributary to Harley Creek.

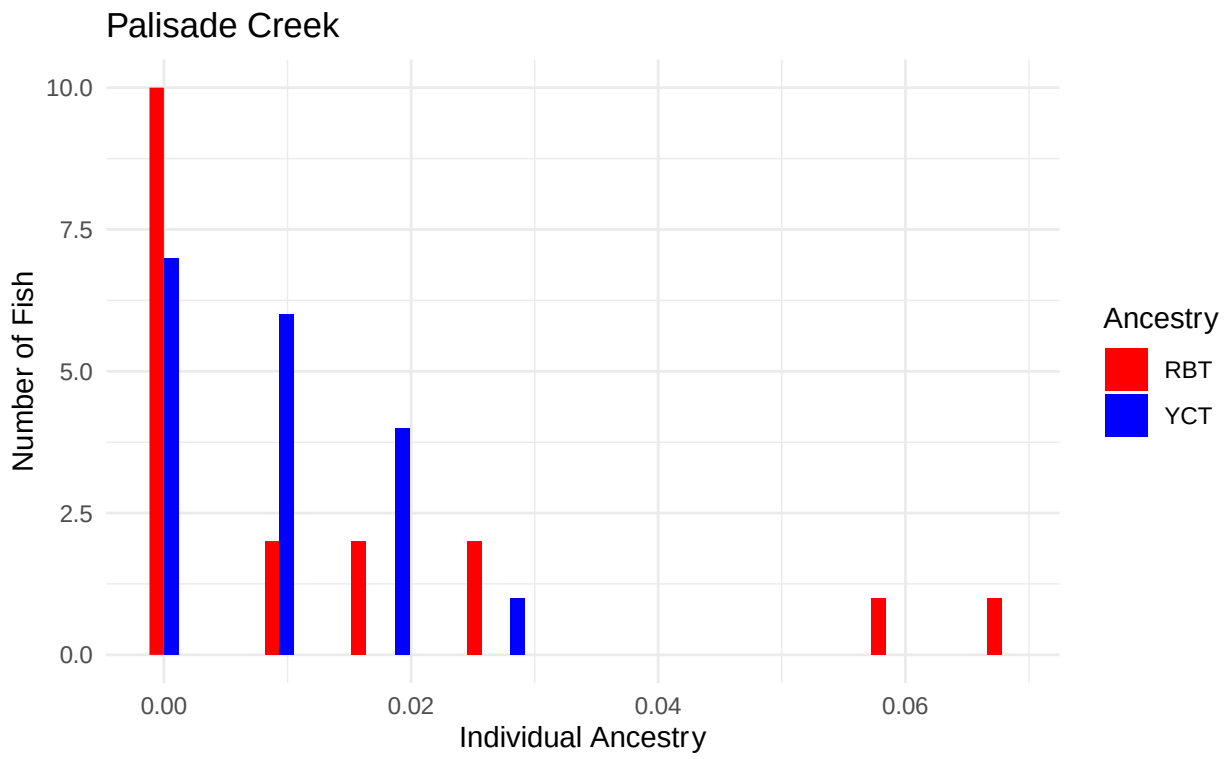

Figure 3. Observed distribution of individual rainbow and Yellowstone cutthroat trout ancestry in a sample of fish from Palisade Creek.

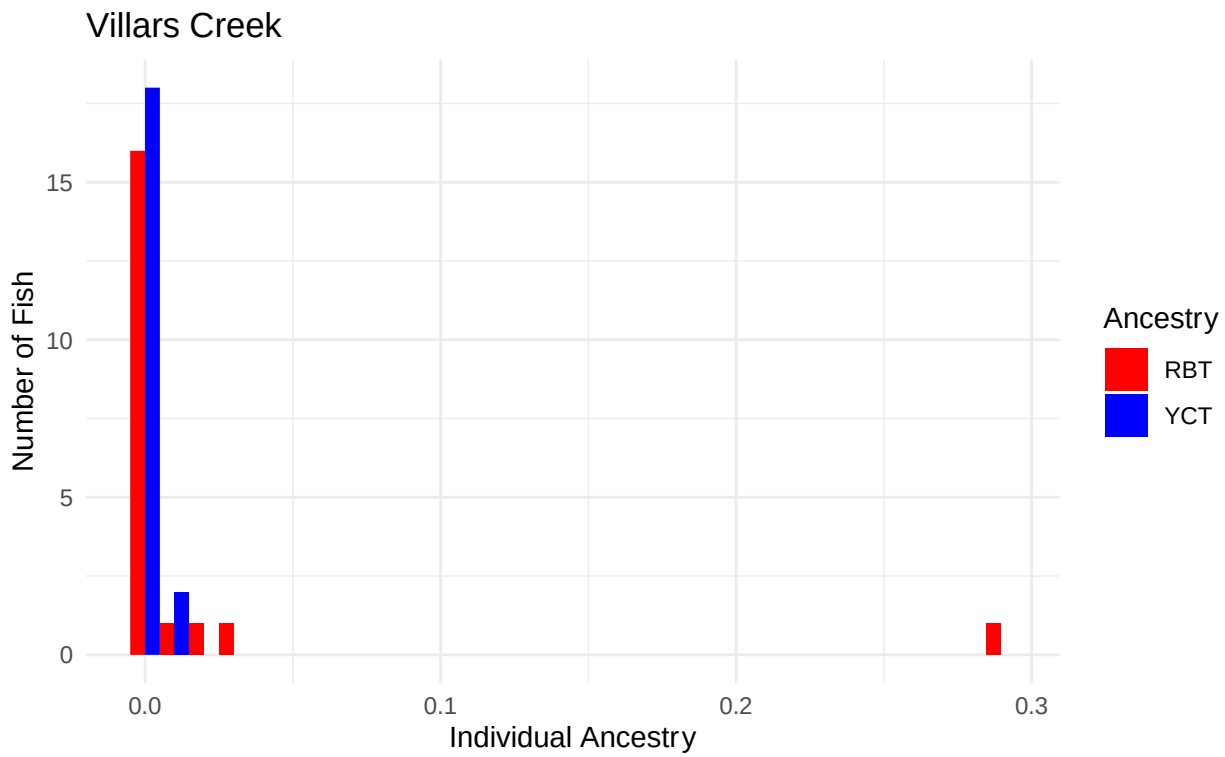

Figure 4. Observed distribution of individual rainbow and Yellowstone cutthroat trout ancestry in a sample of fish from Villars Creek.

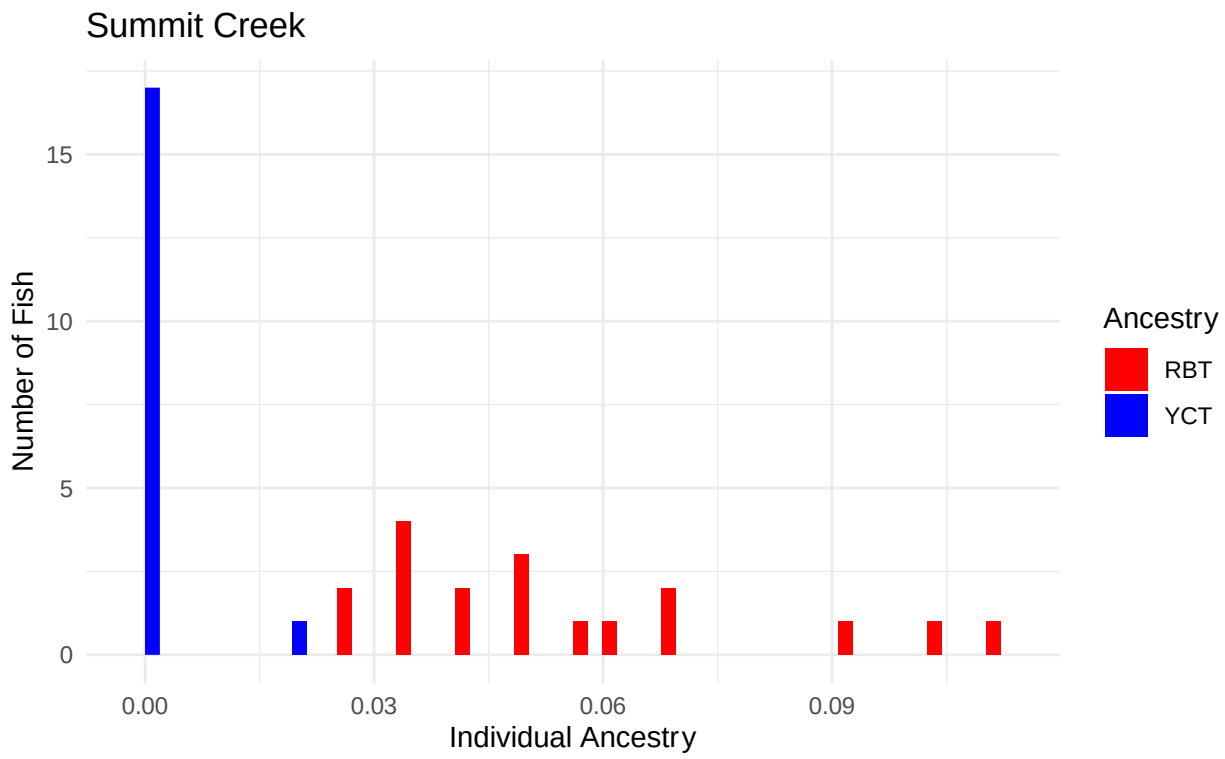

Figure 5. Observed distribution of individual rainbow and Yellowstone cutthroat trout ancestry in a sample of fish from Summit Creek.

Laboratory Methods and Data Analysis

We currently use three different approaches to produce genetic data for westslope cutthroat trout. Each method focuses specifically on single nucleotide polymorphic loci (SNPs) that are variable in westslope cutthroat trout, or differentiate westslope cutthroat trout from rainbow trout or Yellowstone cutthroat trout. In Table 1 we note the genetic method used for each sample, and below we describe the various laboratory approaches and subsequent statistical analyses. Table S1 provides a summary of the various genetic methods.

FWP SNP Chip

We developed a 'chip' specifically for analysis of supposed westslope cutthroat trout (*Oncorhynchus clarkii lewisi*). This chip allows us to simultaneously genotype 91 single nucleotide polymorphic loci (SNPs) in 91 trout using a Fluidigm EP1 Genotyping System. The chip includes 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). The chip also contains 32 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.

WCT GTseq

We developed a genotyping by sequencing in thousands (GTSeq) panel (Campbell et al. 2015) for westslope cutthroat trout. The panel assays (pending) single-nucleotide polymorphisms (SNPs), of which, one acts as a sex marker, (pending) loci differentiate rainbow from westslope cutthroat and Yellowstone cutthroat trout (rainbow markers), (pending) loci distinguish westslope cutthroat from rainbow and Yellowstone cutthroat trout (westslope markers), and (pending) loci that distinguish Yellowstone cutthroat from westslope cutthroat and rainbow trout (Yellowstone markers). Diagnostic markers were strategically located such that they were dispersed across all chromosomes. An additional (pending) loci are polymorphic within westslope cutthroat trout in Montana loci and can be used to address issues concerning the relative amount of genetic variation within and divergence among westslope cutthroat trout populations. Of those, (pending) are shared with (identical to) the polymorphic loci used on the FWP SNP Chip. Data from those markers can be compared directly.

WCT RAD Capture

We developed a RAD-capture (Ali et al. 2016) panel to genotype all samples from westslope cutthroat trout (*Oncorhynchus clarki lewisi*), and their hybrids with rainbow trout (*O. mykiss*) and Yellowstone cutthroat trout (*O. clarki bouvieri*). The RAD-capture "array" (i.e., suite of single-nucleotide polymorphisms (SNPs)) was developed specifically for westslope cutthroat trout and targets 3015 DNA segments (RAD-tags) with known SNPs that (1) differentiate westslope cutthroat trout, rainbow trout, and Yellowstone cutthroat trout, and (2) SNP loci that are polymorphic in westslope cutthroat trout (Amish et al. 2012; Hohenlohe et al 2013; Hand et al. 2015). The total number of loci by marker type is presented in Table S1. All of the SNPs being targeted have been mapped to the existing Arlee rainbow trout (Gao et al. 2021), which means that we know their physical location along each of the 32 chromosome pairs found in the

rainbow genome (Fig. 1). These two aspects of the data play a significant role in data interpretation. It should also be noted that different rainbow trout strains do have structurally different genomes (e.g., Swanson rainbow trout have 29 chromosome pairs but Arlee rainbow have 32 chromosome pairs; see also Pearse et al. 2019), but we generally ignore those differences for analysis and interpretation.

Table S1. The number of SNP loci across laboratory methods for each of several different marker classes.

Method	WCT polymorphic	RBT diagnostic	YCT diagnostic	WCT diagnostic	Sex ID
SNP chip	32	19	20	20	No
GTseq	Pending	Pending	Pending	Pending	Yes
RAD Capture	Pending	Pending	Pending	Pending	No

Data analysis and interpretation

Hybridization

SNP markers that differentiate the three focal species/subspecies from one another are generally called "species-diagnostic", and are used to address questions concerning hybridization across all three lab methods. If, for example, an individual sample possessed alleles characteristic of only westslope cutthroat trout at all westslope diagnostic 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.

In this way, new techniques (RAD-Capture or GTseq) are conceptually identical to the previous MFWP SNP chip that has been used to detect and quantify the extent of hybridization in westslope populations throughout the state, and prior methods that are no longer used in the lab for westslope cutthroat trout (microsatellites and allozymes). However, RAD-Capture and GTseq differ from the previous methods in two fundamental ways. First, the substantial increase in the number of SNPs (hundreds to thousands of diagnostic markers; Table S1) makes it possible to accurately describe ancestry not just within a population, but also within an individual. Moreover, all of the SNPs being targeted have been mapped to the existing rainbow trout genome, which means that we know their physical location along each of the 29 chromosome pairs found in the rainbow genome (Fig. 1). These two aspects of the data play a significant role in data interpretation.

Theoretically, identifying the presence of either rainbow trout or Yellowstone cutthroat trout ancestry in a sample of westslope cutthroat trout is straightforward and mathematically trivial with many diagnostic loci. That is, if we detect any rainbow or Yellowstone alleles in an individual, that individual is a hybrid, and the extent of their rainbow or Yellowstone ancestry can be simply calculated by adding up all the rainbow or Yellowstone alleles divided by the total number of alleles that were genotyped at either the rainbow or Yellowstone diagnostic markers. This same method and formula scales directly to the population level.

In practice, two sources of error, one technical and one biological, make additional interpretation necessary. First, **genotyping errors** – an incorrect call of nucleotides at a single SNP – are nearly impossible to completely eliminate. In the case of a truly non-hybridized population, a genotyping error at a species-diagnostic marker will produce a genotype with either a rainbow or Yellowstone cutthroat trout allele. While error rates at a per-genotype level are remarkably low, generally less than 0.02% (i.e., fewer than two mistakes are made out of approximately every 10000 genotype calls), a simple extrapolation suggests that genotyping errors will almost certainly lead to a few false positive non-native alleles in many population samples.

Second, the species-diagnostic properties of any single marker are rarely error-proof across all westslope cutthroat trout populations. Instead, there can be rare genetic variation in westslope cutthroat trout population that resembles rainbow or Yellowstone or cutthroat trout ancestry (i.e., referred to as **shared polymorphisms**).

Simply stated, with RAD-Capture and GTseq data we actually *expect* to observe a small number of non-native alleles, even in non-hybridized populations. The challenge is determining whether those alleles represent "true" rainbow trout and Yellowstone cutthroat trout admixture. This is where the increased number of diagnostic markers and our understanding of the physical location of each marker in the genome becomes critical. Before describing how those data are used, it is important to note that diagnostic markers (or genes more informally) are "packaged" into chromosomes (Figure S1A), and this physical grouping of markers greatly influences how they are subsequently inherited.

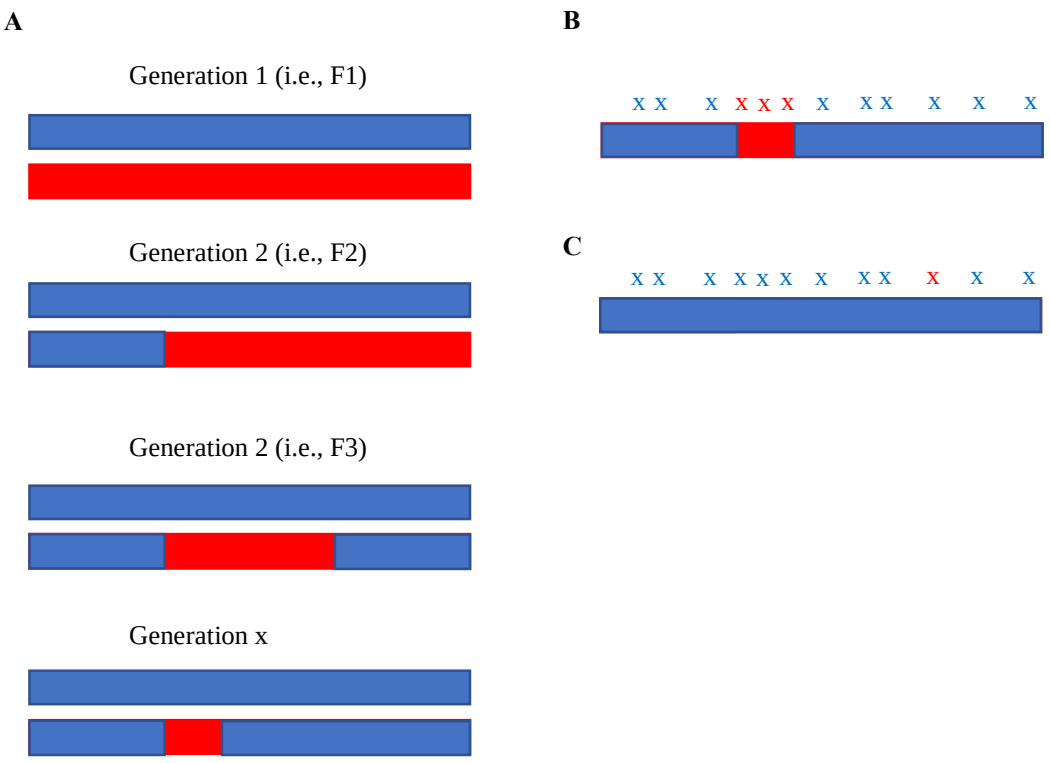

Figure S1. Depiction of rainbow trout ancestry in an individual fish along chromosomes across generations (Panel A), and relative to genetic markers (Panels B and C). A first-generation hybrid contains a complete set of rainbow trout (red) and westslope cutthroat trout (blue) chromosomes (a single pair of chromosomes shown). In a hypothetical hybrid that continues to

backcross with nonhybridized westslope cutthroat trout across multiple generations, the portion of the chromosome that is ancestrally rainbow trout decreases through time, but rainbow trout genes are still "packaged" in a block of genes within the chromosome. With genomic data we can detect those blocks of ancestry because diagnostic markers (x marks) are located across entire chromosomes (Panel B). In cartoon form, diagnostic markers where we detect a westslope allele are colored blue and rainbow trout alleles are colored red. In panel B we would correctly infer that the fish is a hybrid because there is a "run of hybridity" across multiple diagnostic markers ordered along a chromosome. In panel C, we detect a single rainbow trout allele in a fish that is actually a nonhybridized cutthroat trout due to either genotyping error, or westslope cutthroat trout variation.

In the extreme case of an F1 hybrid between rainbow trout and westslope cutthroat trout, that individual will have a complete set of rainbow trout chromosomes from one parent, and a complete set of westslope cutthroat trout chromosomes from the other parent. In that case, any rainbow trout gene on (e.g.,) chromosome 15 will be inherited with all of the other rainbow trout genes on that same chromosome. They are completely non-independent. When hybrids subsequently backcross by mating with one another or each of the parental species, genetic recombination occurs, which essentially "re-arranges" blocks of ancestry along chromosomes. Nevertheless, large "blocks" of ancestry from one species or the other – defined as **runs of hybridity** from here forward – are inherited together along chromosomes. The length of those runs of hybridity decrease over time, meaning that blocks of ancestry become smaller and smaller with each subsequent generation (Figure S1).

Therefore, a hybrid individual contains one or more runs of hybridity, that is, blocks of rainbow trout or Yellowstone cutthroat trout ancestry along chromosomes. In contrast, genotyping errors should occur at random in the genome (i.e., a technical error at one locus). Indeed, the probability of observing two genotyping errors in diagnostic markers that are next to one another on a chromosome is 0.00000008 (very rare). Similarly, shared polymorphisms should be distributed randomly throughout the genome. In other words, when we observe multiple rainbow trout or Yellowstone cutthroat trout alleles in an individual (Figure S1B) and those alleles are proximate to one another on a chromosome it provides extremely strong evidence that the observed alleles are due to hybridization, not genotyping error or westslope cutthroat trout genetic variation. In contrast, a single nonnative allele may or may not be indicative of hybridization (Figure S1C). As such, runs of hybridity are essentially a silver bullet; when we observe non-native alleles in two or more diagnostic markers along a chromosome within an individual, that individual is almost certainly a hybrid. Importantly, marker density is not sufficiently high with GTseq to rely on runs of hybridity alone, but the physical location of diagnostic markers still helps with interpretation of the results when a few non-native alleles are detected in a sample.

To summarize the genetic results when hybridization is present, we simply calculate the proportion of alleles that are from one taxa (e.g., rainbow trout) relative to the total number of alleles sampled in that individual (2 times the number of diagnostic markers) or the entire population (2 * number or diagnostic markers * number of individuals).

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. We define a hybrid swarm as a population in which

the vast majority (>75%) of the individuals are hybrids, and the sample conforms to random mating (i.e., Hardy-Weinberg) expectations.

When samples include hybrids but are not hybrid swarms, 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, historical samples can provide insight into which of the latter two factors appears mainly responsible for the nonrandom distribution of non-native ancestry among individuals in the sample.

In some cases, it can be helpful to determine if a hybrid swarm has conservation value. We define genomic extinction – a population without conservation value – as a population sample in which >90% of individuals are hybrids and we detect non-native alleles at >50% of the diagnostic markers (i.e., non-native ancestry is present across >50% of the genome).

Failure to detect evidence of hybridization in a sample does not necessarily mean the fish are non-hybridized because there is always the possibility that we would not detect evidence of hybridization because of sampling error. That being said, with increasing number of diagnostic markers we have increasingly high resolution to detect very small amounts of non-native ancestry. However, if hybrids are rare in the population itself (e.g., because of recent invasion), we will have relatively low power to detect them, even with larger samples sizes.

Genetic variation and genetic differentiation

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. 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 hierfstat (Goudet 2005). 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).

We used expected heterozygosity (He) and the proportion of polymorphic loci (P) as measures of within population genetic variation (Kovach et al. 2022). As estimates of genetic variation, He and P have strengths and weaknesses. The strength of He is that it is based on the allele frequencies at the loci, is less sensitive than observed heterozygosity (Ho) to sample size, and is a fundamental parameter in population genetic theory (Allendorf et al. 2013). However, He is not

particularly sensitive to the loss of low-frequency alleles (0.01–0.05). Low-frequency alleles have low heterozygosity and, therefore, generally contribute little to the estimate of He. In contrast, P treats the presence or absence of all alleles equally regardless of how variable a locus is within the population, and thus, P is particularly sensitive to the loss of low-frequency alleles through genetic drift and bottlenecks (Allendorf 1986; Luikart et al. 1998). Instead, a major weakness is that P is also sensitive to sample size. Since the weakness of He as an estimate of amounts of genetic variation is a strength of P, and vice versa, we used both statistics to best summarize genetic variation. We estimated He and P for each local population using the R package poppr (Kamvar et al. 2014).

Other analyses may be conducted as helpful on a case-by-case basis.

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