Northcentral Montana Westslope Cutthroat Trout Restoration Project

2022 Annual Report

MoTAC Project # 2022-7

MT Aquatic SWG Management Program # F19AF00704

MT Aquatic SWG Survey & Inventory Program # F19AF00705

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Region 4

Great Falls, MT

December 2022

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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 2021 Missouri River Technical Advisory Committee (MoTAC) meeting, FWP was awarded $16,316 from Northwestern Energy to fund a fisheries technician to work directly with the FWP native species biologist on the Northcentral Montana WCT Restoration Project. This document specifically addresses work performed under the 2022 Federal Challenge Cost Share Agreement for WCT restoration in northcentral Montana.

Study Area

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

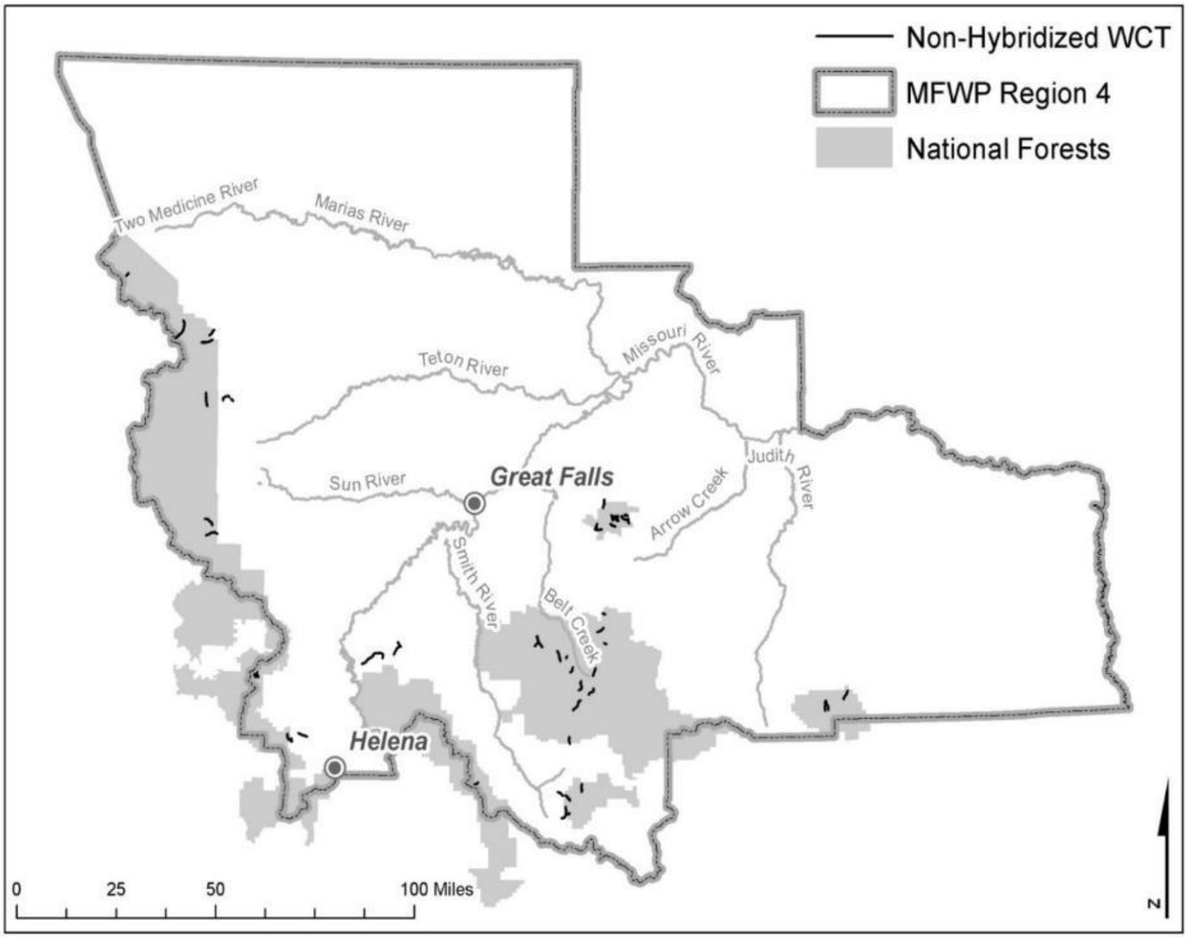

Figure 1. Study area in northcentral Montana with nonhybridized WCT populations (indicated in bold black).

Methods

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

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

Conservation techniques used during the 2022 field season include: fish barrier construction, fish barrier maintenance, mechanical removal of nonnative trout, and WCT population and genetic monitoring.

Restoration Efforts in Northcentral Montana

The scope of the work completed by FWP in 2022 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

Cottonwood Creek

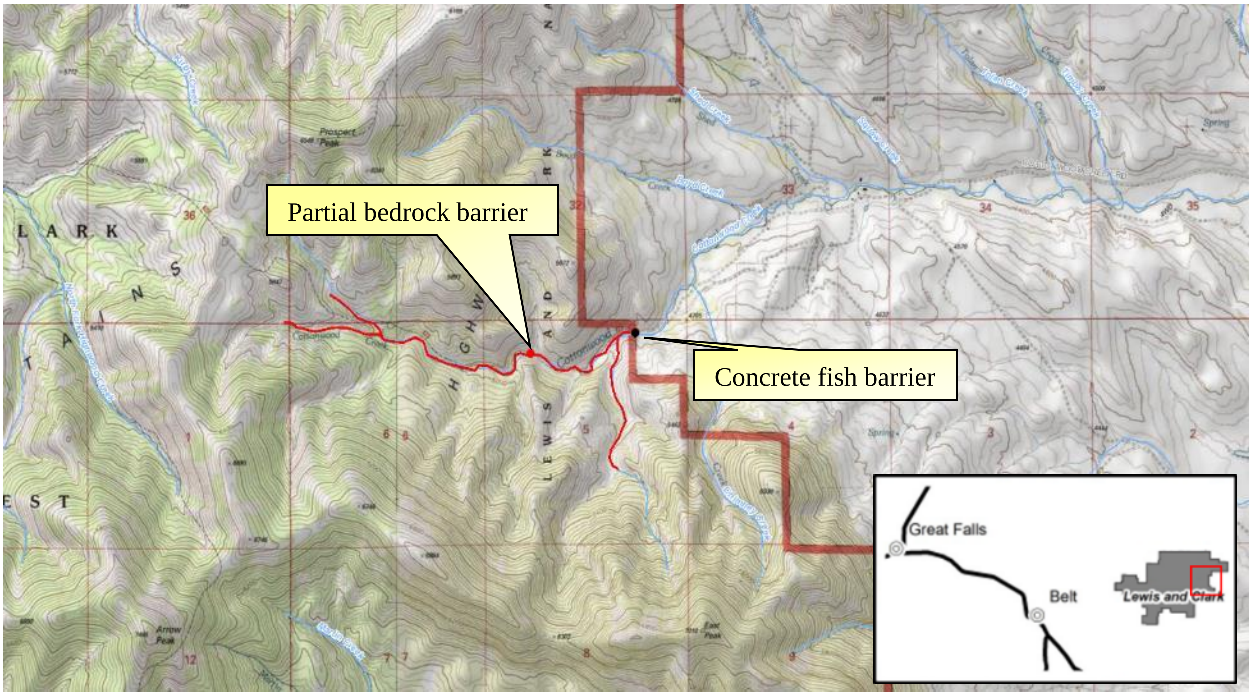

Figure 2. Cottonwood Creek in the Arrow Creek subbasin. Stream segments delineated in red indicate extent of WCT occupied habitat.

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.

2022 Monitoring

A single pass electrofishing monitoring effort was performed on Cottonwood Creek on September 1st and 2nd of 2022. 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 235 WCT were collected in the mainstem of Cottonwood Creek between the barriers and 609 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

Carpenter Creek

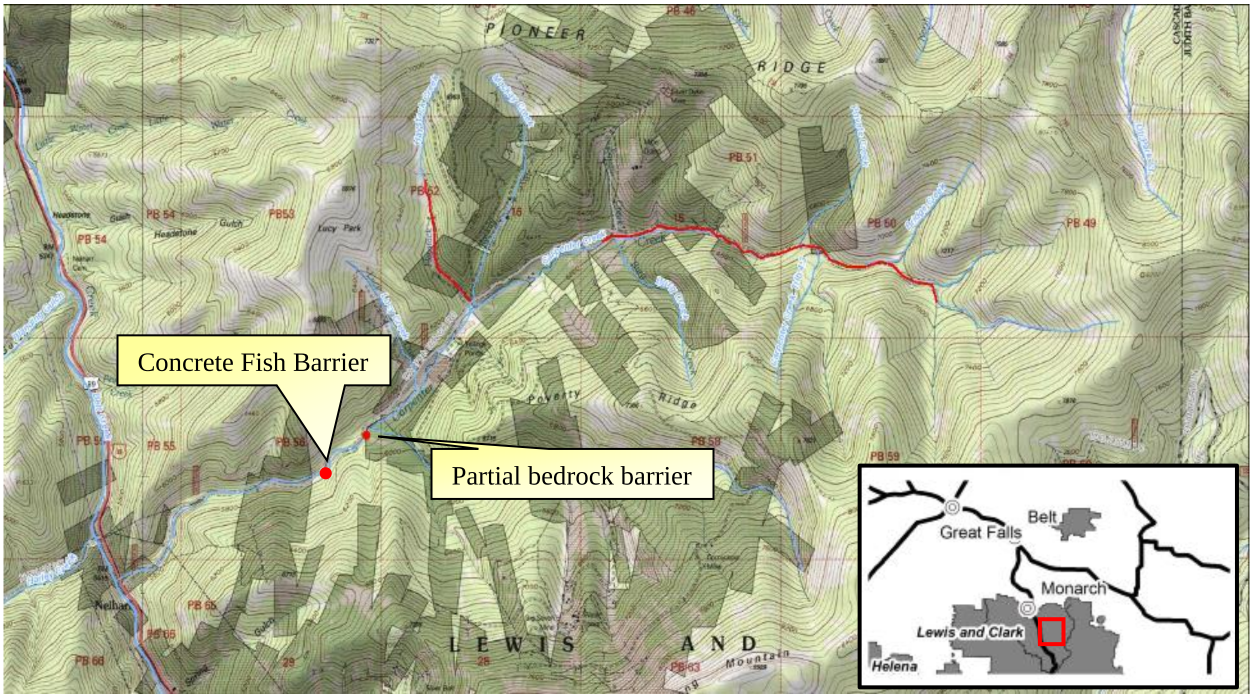

Figure 3. Carpenter Creek in the Belt Creek subbasin. The stream segments delineated in red indicate the areas occupied by nonhybridized WCT.

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 recognized and is currently being pursued. A section of Carpenter Creek near the mouth is shocked annually (from the confluence of Belt Creek to a partial waterfall barrier near the confluence of Snow Creek; Figure 3). During past sampling efforts no fish have been detected in this reach; however, in 2015 two fish were caught near the mouth. The presence of fish was a positive response in improving water quality but provided concern for the potential for nonnative invasion and subsequent risk of WCT loss to hybridization. Nonnative fish have been monitored annually in this section since 2015. In 2016 seven nonnative fish were collected, 4 in 2017, 5 in 2018, 1 in 2019, and 2 in 2020 and 2021. 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

In the summer of 2021, Northwestern Energy funded the survey and design of a concrete fish barrier to protect the nonhybridized WCT populations within the Carpenter Creek drainage. Funding for the construction of the barrier was secured in 2022 from the following sources: Northwestern Energy, U.S. Forest Service, Future Fisheries Improvement Program, and the State Wildlife Grant program. Construction on the barrier began in mid-September 2022. Site preparation, dewatering, and pouring of the concrete footer was accomplished prior to winter shut down of the construction site in early November 2022 (Figure 4). Construction will resume in summer 2023 following spring runoff.

Figure 4. Concrete footer at Carpenter Creek fish barrier construction site.

2022 Monitoring

Lower Carpenter Creek was electrofished from the confluence of Belt Creek to the partial bedrock barrier to monitor nonnative trout presence in a single pass effort on September 12th and 13th, 2022. Six brook trout and three rainbow trout were collected in this effort. Fish were distributed throughout the section sampled with the uppermost fish collected just downstream of the partial bedrock barrier (upstream of the fish barrier construction site). Continued monitoring of lower Carpenter Creek is warranted until the concrete fish barrier construction is completed and no nonnative trout are detected in the reach of stream between the barriers.

Additional electrofishing effort was expended upstream of the partial bedrock barrier in 2022 to determine if nonnative trout were present. A 0.87-mile reach of Carpenter Creek was electrofished starting at the partial bedrock barrier to the Haystack Creek confluence. A 0.33-mile reach of Snow Creek was also electrofished starting at the Carpenter Creek confluence. No fish were detected in these efforts.

Charcoal Creek

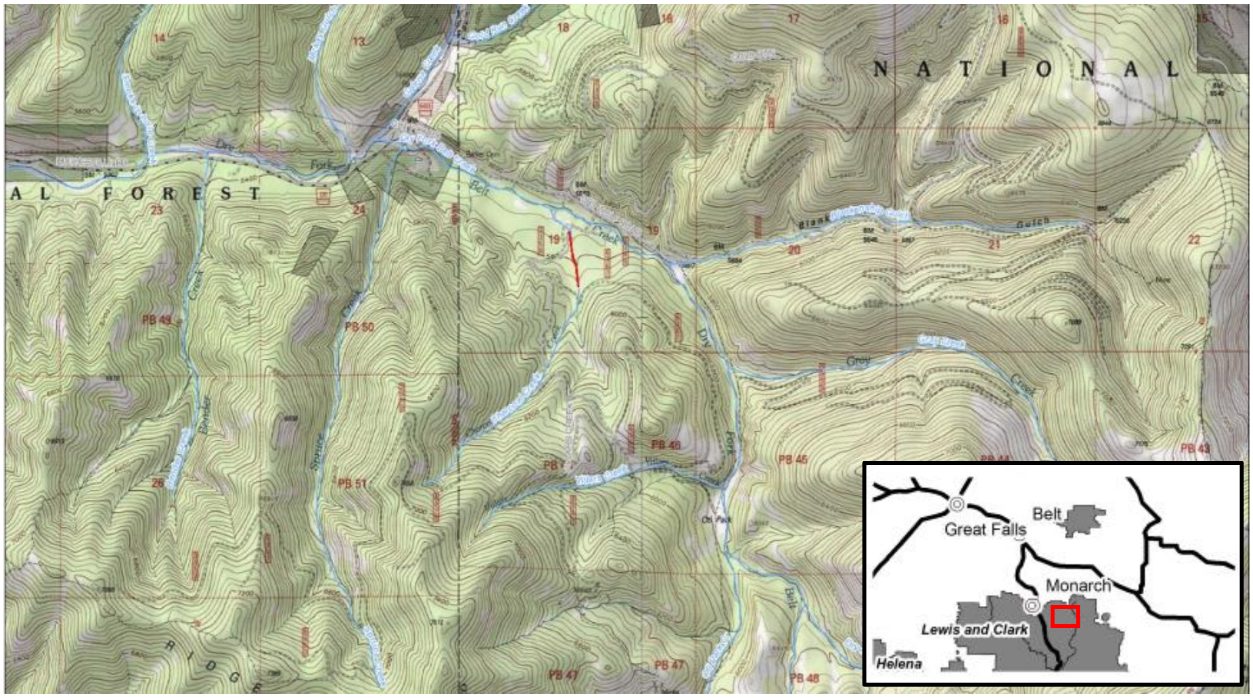

Figure 5. Charcoal Creek in the Belt Creek subbasin. The stream segment delineated in red indicates the area surveyed in 2022.

Background

Charcoal Creek is a tributary of Dry Fork Belt Creek located approximately 10 miles east of the community of Monarch, MT. The stream contains both WCT and brook trout in approximately 0.76 miles of habitat. Genetic analysis (SNPs) of nine WCT collected in 2012 indicated that most of the samples came from nonhybridized WCT but did include one rainbow-cutthroat hybrid.

2022 Monitoring

Updated genetic monitoring of the Charcoal Creek WCT population was performed on August 1st, 2022. A single electrofishing pass was made to collect fish from 0.21 miles of habitat starting at the FS RD 3350 crossing. A total of 84 fish were collected: 24 WCT and 60 brook trout. Genetic samples were collected from 20 WCT for analysis in 2023.

Gold Run Creek

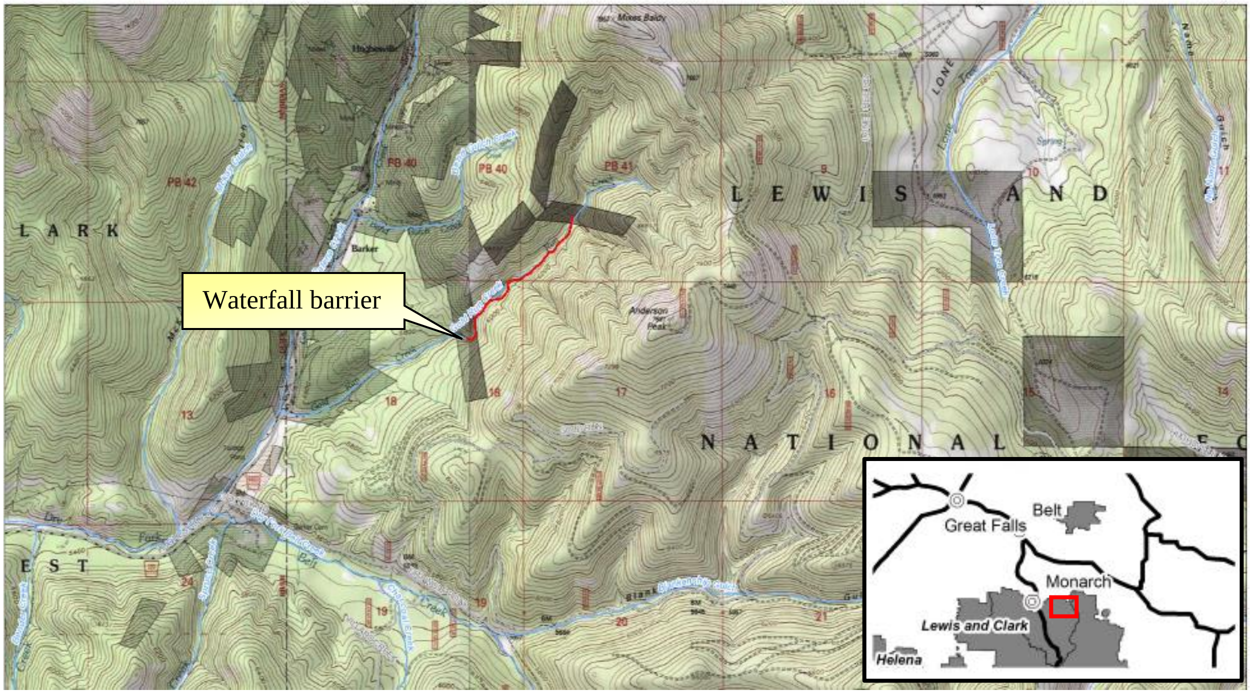

Figure 6. Gold Run Creek in the Belt Creek subbasin. The stream segment delineated in red indicates the area occupied by nonhybridized WCT.

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.

2022 Monitoring

FWP staff assisted UM researchers in their annual sampling of Gold Run Creek as part of the ongoing genetic rescue study. The entire fish bearing reach above the waterfall barrier was sampled over a two-day period on September 7th and 8th. A total of 590 WCT were collected in 2022. Data collected in 2022 will help inform demographic and genetic models for Gold Run Creek allowing for a detailed examination of genetic rescue techniques on isolated WCT populations.

North Fork Little Belt Creek

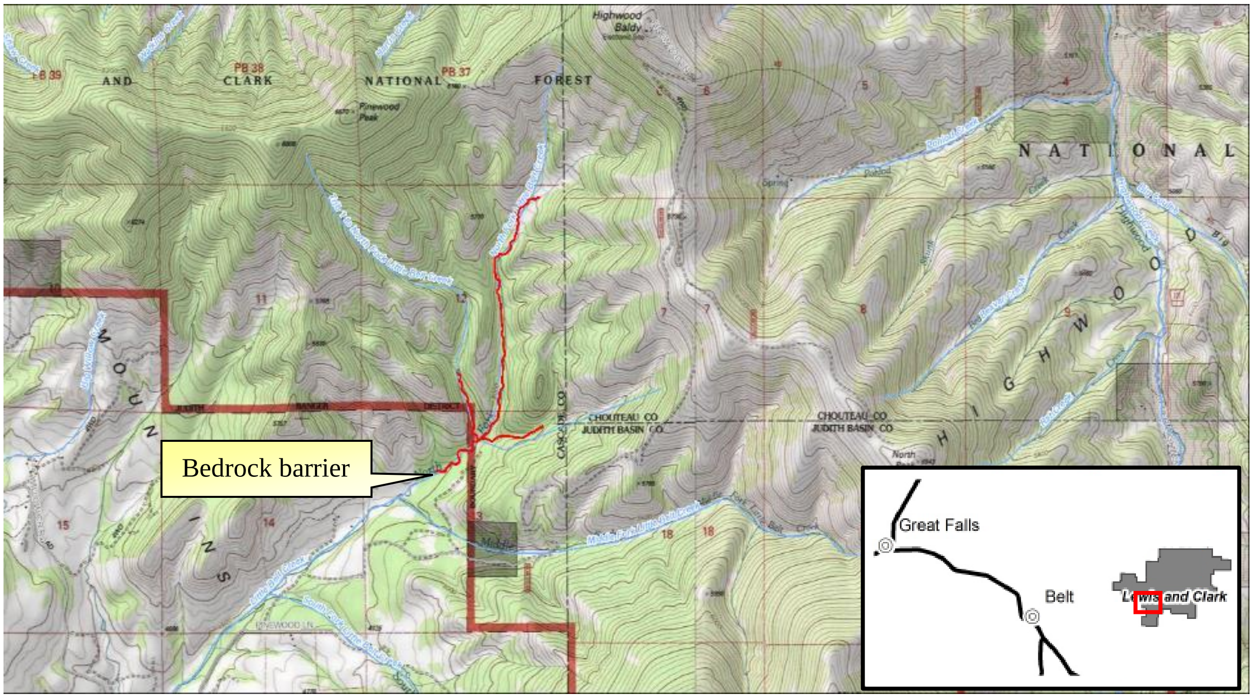

Figure 7. North Fork Little Belt Creek in the Belt Creek subbasin. The stream segments delineated in red indicate the areas occupied by nonhybridized WCT.

Background

North Fork Little Belt Creek is a tributary of Little Belt Creek in the Highwood Mountains located approximately 13 miles east of the community of Belt, MT. A natural bedrock barrier isolates a nonhybridized WCT population in the upper 2.4 miles of the stream. North Fork Little Belt was included as a study site in the UM genetic rescue study. In 2020, UM researchers collected a single brook trout above the bedrock barrier during annual sampling. This was the first collection record of a brook trout above the bedrock barrier.

2022 Monitoring

North Fork Little Belt Creek was sampled on August 16th, 2022, to monitor brook trout presence above the bedrock barrier. Two crews backpack electrofished two reaches of the creek; FWP staff sampled from the barrier to the first tributary confluence and the Lewis and Clark National Forest crew sampled the first unnamed tributary. A total of 222 WCT were collected in the mainstem reach sampled and 84 WCT were collected from the first tributary. No brook trout were detected in either reach sampled above the barrier. The large pool immediately below the barrier was also shocked and 35 brook trout and 5 WCT were collected. Upper North Fork Little Belt Creek should continue to be periodically monitored for brook trout presence.

Palisade Creek

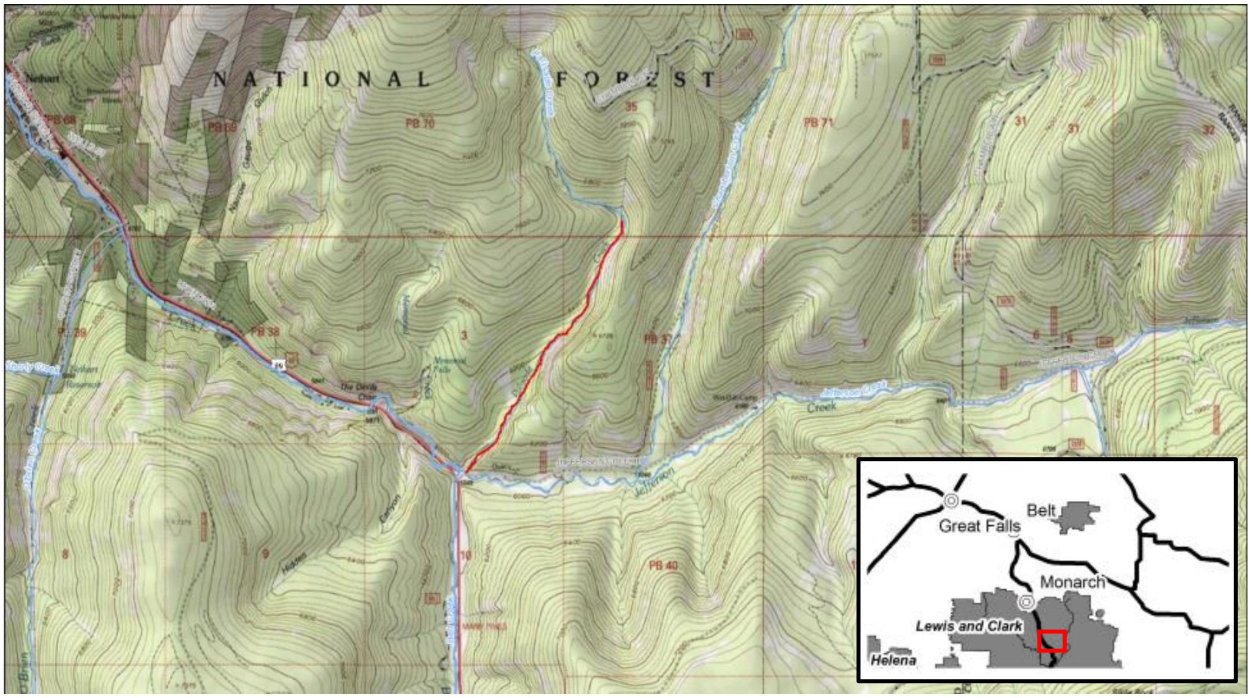

Figure 8. Palisade Creek in the Belt Creek subbasin. The stream segment delineated in red indicates the area occupied by WCT.

Background

Palisade Creek is a tributary of Jefferson Creek in the Belt Creek subbasin located approximately 2 miles southeast of the community of Neihart, MT. A ten fish genetic sample collected in 2003 was determined to be comprised of nonhybridized WCT. No barriers isolate Palisade Creek from Jefferson Creek and the rest of the upper Belt Creek drainage.

2022 Monitoring

Palisade Creek was surveyed on July 28th, 2022, to collect updated genetic samples. A 1.19-mile reach of Palisade Creek was backpack electrofished starting at the confluence with Jefferson Creek. A total of 37 WCT, 32 brook trout, and one rainbow-cutthroat hybrid was collected. Genetic tissue samples were collected from 20 of the WCT sampled for genetic analysis in 2023.

Spruce Creek

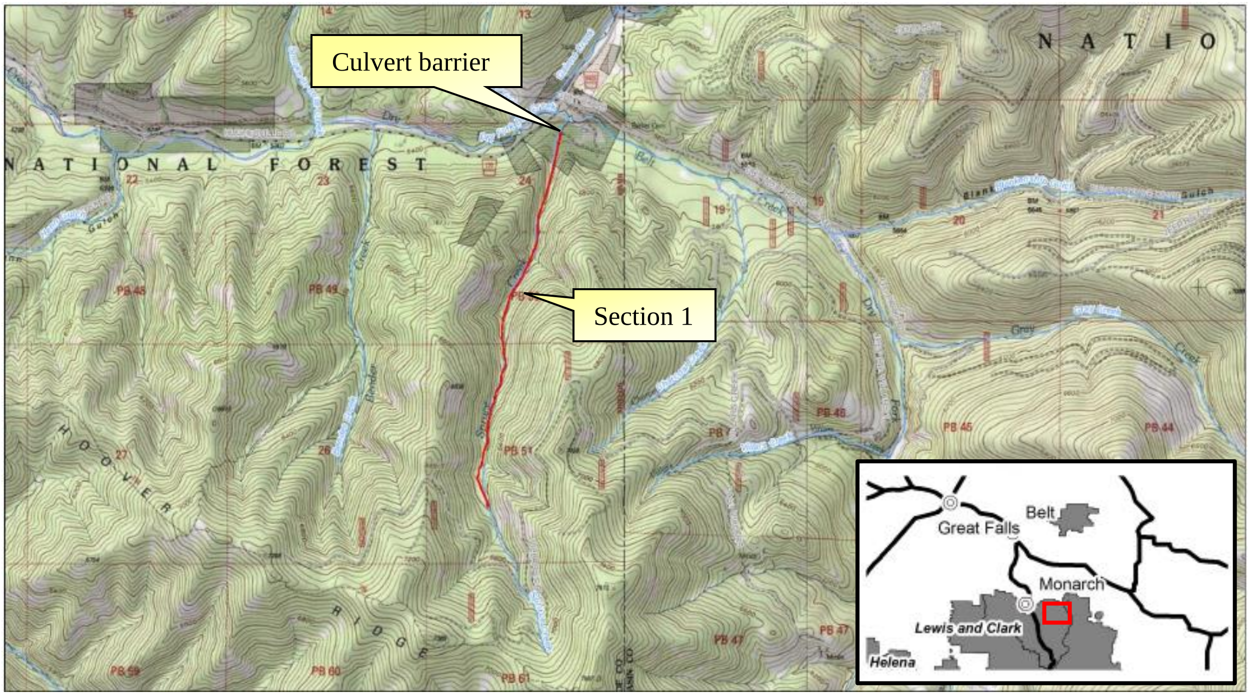

Figure 9. Spruce Creek in the Belt Creek subbasin. The stream segment delineated in red indicates the area occupied by WCT.

Background

Spruce Creek is a tributary of Dry Fork Belt Creek located approximately 9 miles southeast of the community of Monarch, MT. A culvert located on private land just downstream of the Lewis and Clark National Forest boundary prevents brook trout from accessing the upper 1.82 miles of fish habitat. Genetic testing of the WCT population above the culvert from 1997-2012 has indicated a mixed population of slightly hybridized fish found lower in the drainage and nonhybridized WCT in the headwaters. No complete fish barriers are known to exist in the drainage; however, several small partial bedrock barriers may seasonally restrict fish movement. A population estimate was performed in August of 2016 and 626 WCT were estimated to inhabit Spruce Creek above the culvert.

2022 Monitoring

Spruce Creek was surveyed on September 26th and 27th to update demographic and genetic monitoring of the WCT population. A fish population estimate was performed on September 26th, 2022, to estimate the abundance of the Spruce Creek WCT population. A 100 m population estimate section was established 0.98 km upstream of the Lewis and Clark National Forest boundary. Multiple pass depletion methods were used to estimate population abundance. An estimated 780 fish/km were found based on the results of the two-pass depletion, putting the total WCT population at 2,285 (±176) individuals if extrapolated to the entire reach (Figure 10).

Spruce Creek was surveyed again on September 27th, 2022, to collect updated genetic samples and determine the upper distribution of fish. The uppermost fish was located at 47.02685, -110.65937. Two locations were sampled in the headwaters of Spruce Creek to collect fish for genetic samples. A total of 20 samples were collected for updated genetic analysis in 2023.

Spruce Creek —NATIVE TROUT POPULATION SURVEY

1. General Information— Date: **September 26th, 2022** Biologist: **A. Poole**
2. Stream Information—
    Name, section, county: **Spruce Creek, 50, Cascade**
3. Survey Site Information (see attached map)—
    Upstream range of native trout (general description and GPS): **1.82 mi above culvert barrier (47.02685, -110.65937)**
    Downstream range of native trout (general description and GPS): **Culvert barrier (47.04981, -110.65300)**
    Location (GPS) and description of barriers: **Culvert barrier (47.04981, -110.65300)**
    Stream Length—Occupied habitat: **2.93 km (1.82 mi)** Available habitat: **3.06 km (1.9 mi)**
    Survey method & equipment: **backpack battery electrofisher; two-pass depletion**
    Survey sites (general description and UTM)—
    Section 1: **0.98 km upstream of Forest Service boundary; 47.04140, -110.65676**

Parameter	**Section 1**
Section length (m)	100 m
Mean stream width (m) (n)	1.99 m (10)
Section area (hectares)	0.020 ha
WCT	
Removal Pattern	53 17
Population estimate	78 (±6)
Capture probability	0.679
Mean length (mm) (n)	116 (70)
Mean weight (g) (n)	15 (70)
Mean KTL (n)	0.84 (70)
Number fish per km (95 % CI)	780 (±60)
Number fish per ha (95 % CI)	3,900 (±300)
Biomass (kg per ha) (95 % CI)	59 (±5)

Figure 10. Spruce Creek fish population estimate results.

Unnamed Tributary to Harley Creek

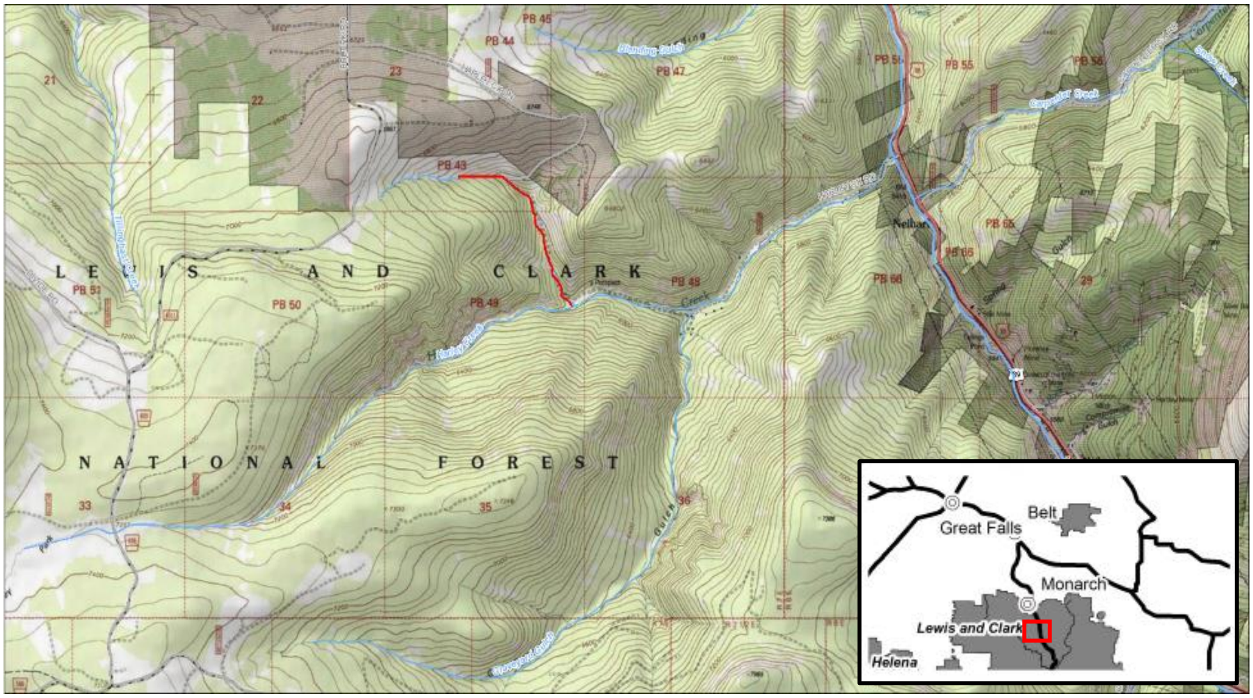

Figure 11. Unnamed tributary to Harley Creek in the Belt Creek subbasin. The stream segment delineated in red indicates the area occupied by WCT.

Background

Harley Creek is a tributary of Belt Creek located just west of the community of Neihart, MT. The Harley Creek drainage contains a slightly hybridized WCT population in the mainstem creek and a nonhybridized WCT population in the principal tributary Graveyard Gulch. Brook trout are also present in the mainstem of Harley Creek. A 14 fish sample collected in 1999 from the unnamed tributary which enters Harley Creek from the north in section 49 indicated a nonhybridized WCT population present when analyzed in 2000.

2022 Monitoring

The unnamed tributary to Harley Creek was sampled on May 11th, 2022, to collect updated genetic samples from the WCT population. A 0.6-mile reach of the tributary was backpack electrofished starting at the confluence with Harley Creek. A total of 42 WCT and 4 brook trout were collected in this effort. Genetic samples were collected from 25 WCT to update the current genetic status of this population. Brook trout appeared to be restricted to the lower reach of the stream below a perched culvert at the 2nd FS RD 834 crossing (46.94728, -110.79221).

Villars Creek

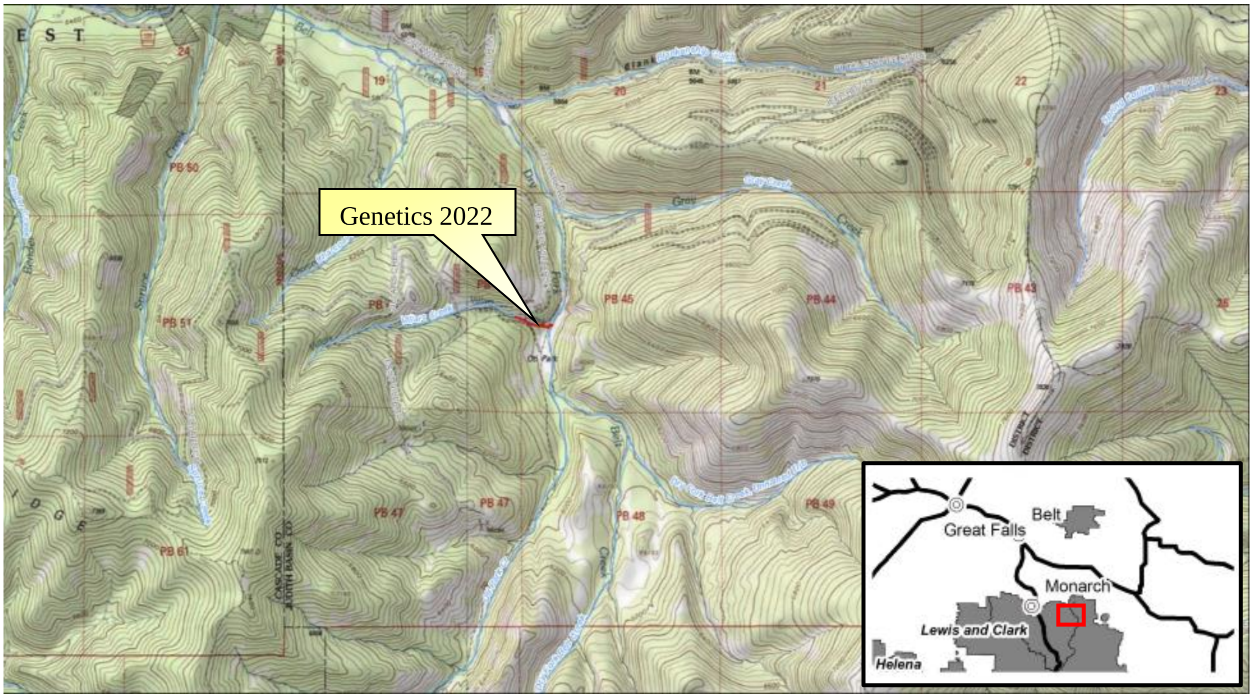

Figure 12. Villars Creek in the Belt Creek subbasin. The stream segment delineated in red indicates the area occupied by WCT.

Background

Villars Creek is a small tributary of Dry Fork Belt Creek in the Belt Creek subbasin located approximately 11 miles southeast of the community of Monarch, MT. The creek contains about 0.21-miles of fish bearing habitat and contains both WCT and brook trout. A 21 fish sample collected in 2012 indicated that the WCT population in Villars Creek was nonhybridized.

2022 Monitoring

Villars Creek was sampled on August 1st, 2022, to collect updated genetic samples from the WCT population. A 0.13-mile reach of Villars Creek was backpack electrofished starting at the Dry Fork Belt Creek confluence. A total of 23 WCT and 8 brook trout were collected in this effort. Genetic samples were collected from 20 WCT to update the current genetic status of this population.

III. Judith River Subbasin

Cleveland Creek

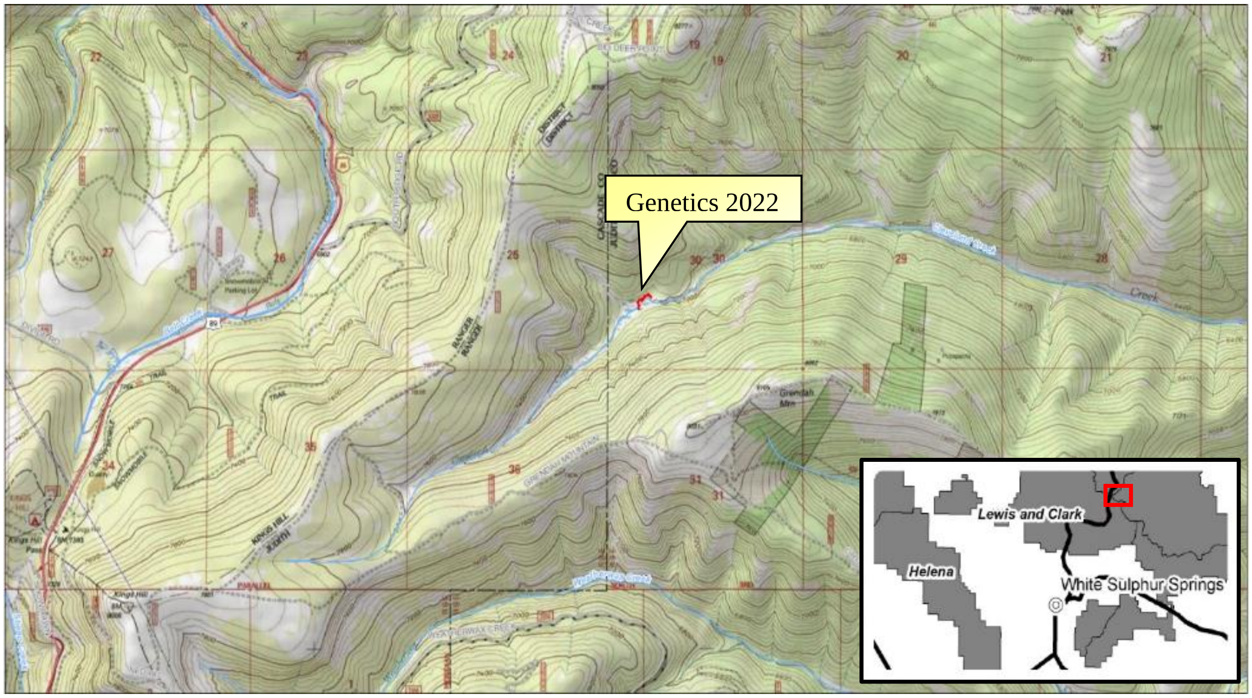

Figure 13. Cleveland Creek in the Judith River subbasin. The stream segment delineated in red indicates the area sampled in 2022.

Background

Cleveland Creek is a headwater tributary of the Middle Fork Judith River in the Judith River subbasin located just east of Kings Hill Pass in the Little Belt Mountains. The creek contains hybridized WCT as well as brook and rainbow Trout in its lower reaches. A ten fish genetic sample collected in 1996 indicated that Cleveland Creek contained a hybridized WCT population with an 85% WCT genetic contribution.

2022 Monitoring

Cleveland Creek was sampled on August 31st, 2022, as part of a basin-wide effort to update genetic status of WCT in the Middle Fork Judith River drainage. The headwater meadow reach of the creek was backpack electrofished in a single pass effort. A total of 30 WCT were collected and sampled for updated genetic analysis.

Hell Creek

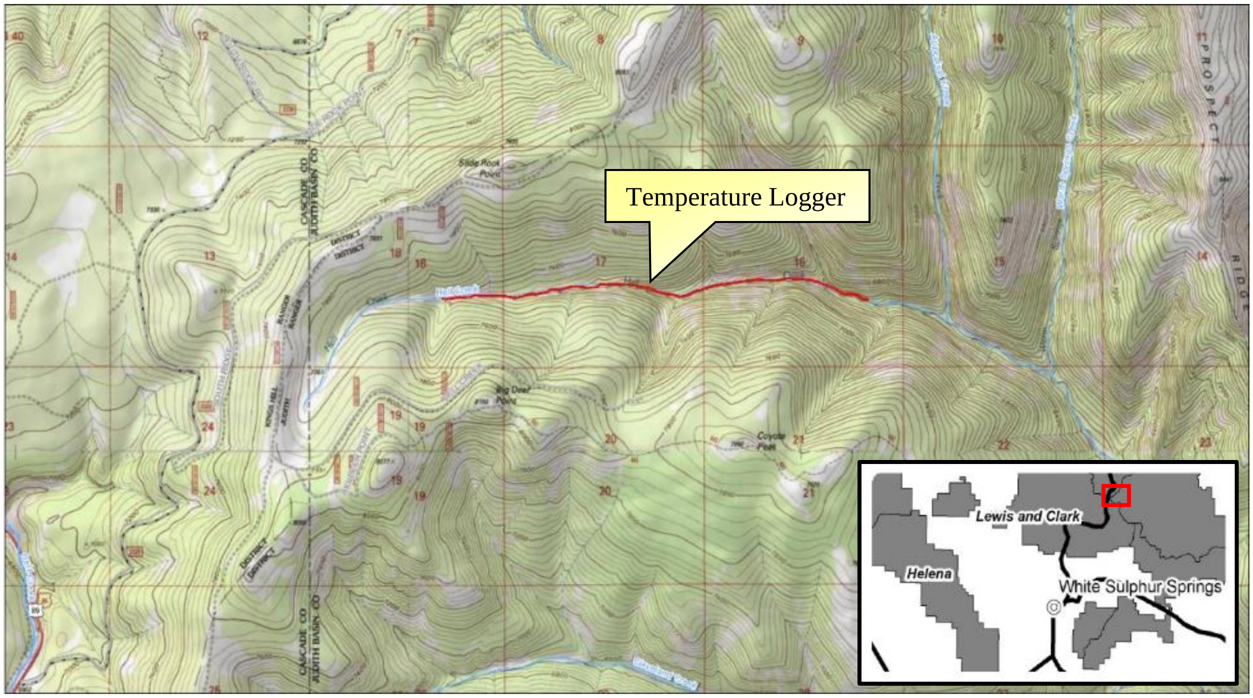

Figure 14. Hell Creek in the Judith River subbasin. The stream segment delineated in red indicates the area electrofished in 2022.

Background

Hell Creek is a headwater tributary of the Middle Fork Judith River in the Judith River subbasin located just east of Kings Hill Pass in the Little Belt Mountains. A visual survey of Hell and Appraisal Creeks River was conducted on July 31, 2008. The survey goal was to locate fish barriers and determine if adequate habitat above the barriers was present to support trout. Fish were not visually observed in Hell Creek or Appraisal Creek. However, WCT were sampled in Warm Springs Creek about 1.2 miles downstream of the Hell Creek confluence in 1996. Habitat adequate to support trout, including spawning substrate, exists above a permanent barrier on Appraisal Creek and a bedrock cascade on Hell Creek. Water temperature monitoring and fish and habitat surveys were recommended to determine if introducing WCT into Hell Creek is feasible and warranted.

2022 Monitoring

Hell Creek was sampled on June 28th, 2022, to determine fish presence, evaluate habitat, investigate potential barriers, and deploy a temperature logger. A 2.19-mile reach of Hell Creek was backpack electrofished and no fish were detected. Habitat appeared excellent with many large pools, woody debris, and spawning substrate observed throughout. The partial bedrock barrier observed in 2008 was relocated and GPS coordinates were collected for this feature (Figure 15; 46.88571, -110.60003). A temperature logger was deployed immediately downstream of the bedrock barrier.

Figure 15. Hell Creek bedrock cascade feature located at 46.88571, -110.60003.

An additional visual survey of Appraisal, Hell, and Warm Springs Creek was conducted on July 19th, 2022, to determine if additional fish barriers were present in the drainage. A 1.51-mile reach of Appraisal, 0.81-mile reach of Hell, and 1.3-mile reach of Warm Springs Creek were surveyed in this effort. No fish were visually observed, and no additional permanent fish barriers were found. A large headcut was found on Warm Springs Creek that likely seasonally restricts fish movement but is not a complete barrier (Figure 16; 46.87256, -110.54961).

Figure 16. Warm Springs Creek headcut located at 46.87256, -110.54961.

The temperature logger was retrieved on October 6th, 2022, after being deployed for 70 days. The mean August stream temperature was 6.23°C. Hell Creek appears too cold to support a WCT population and likely explains the lack of fish collected or observed in 2022.

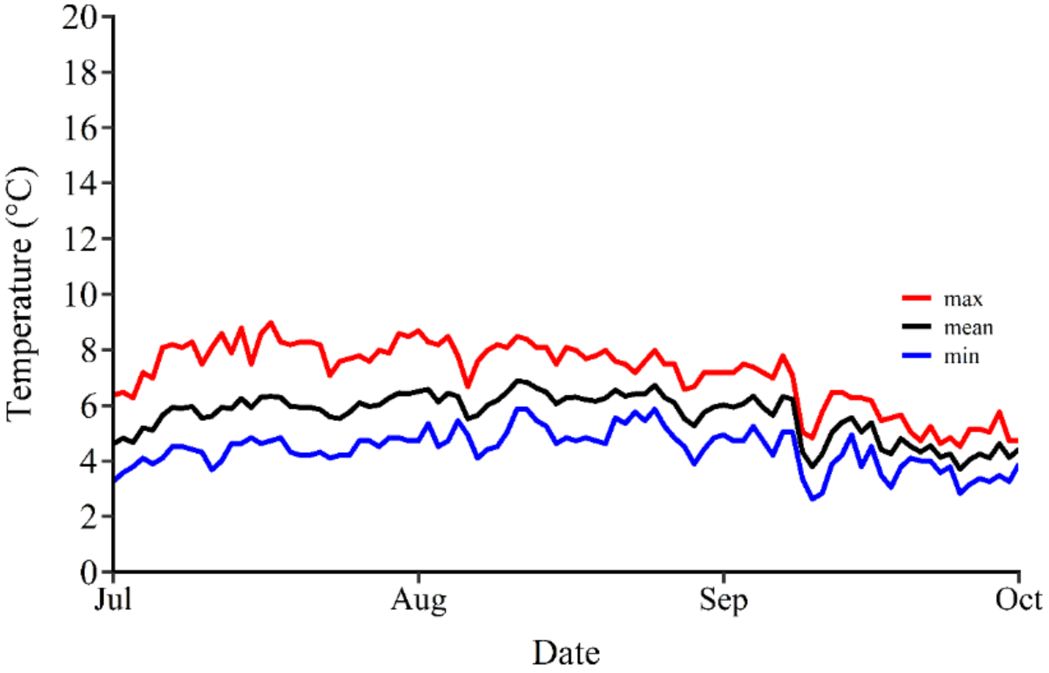

Figure 17. Daily maximum (red line), mean (black line), and minimum (blue line) stream temperatures from Hell Creek. Temperature logger was deployed on June 28, 2022 and collected October 6, 2022.

South Fork Judith River

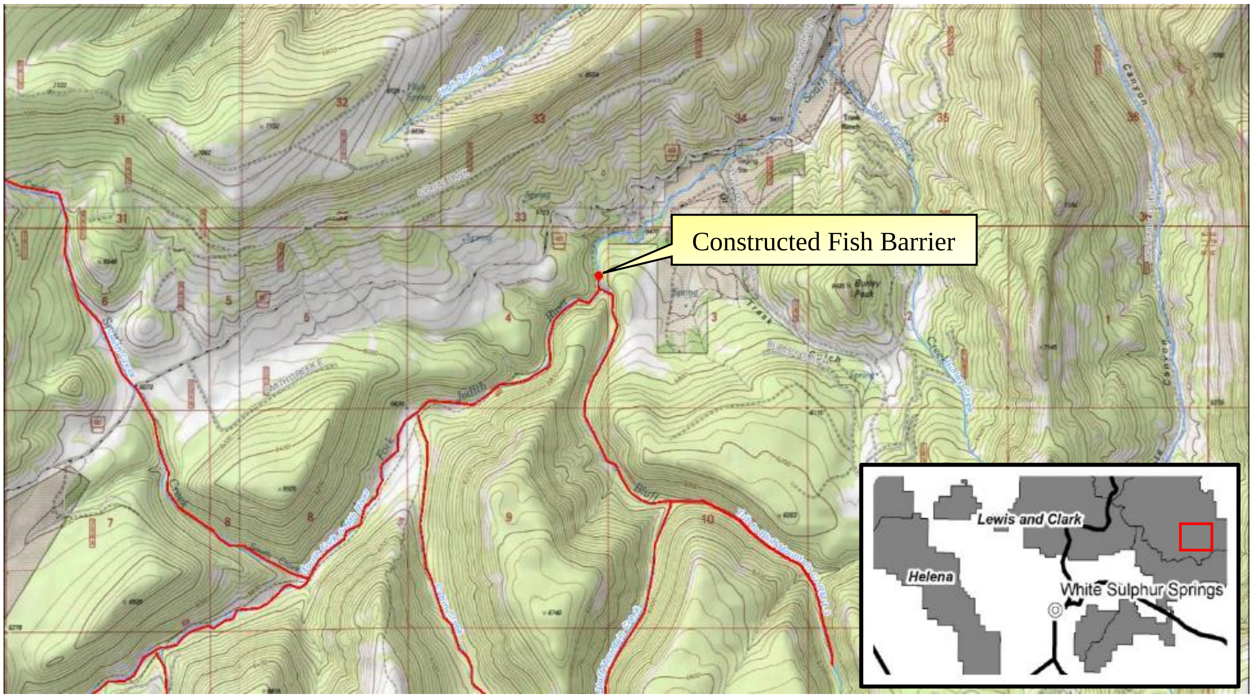

Figure 18. South Fork Judith River in the Judith River subbasin. The stream segments delineated in red indicate the areas occupied by the South Fork Judith River WCT conservation population.

Background

The upper South Fork Judith River and its tributaries contains a large population of WCT and rainbow-cutthroat hybrids of varying levels of hybridization. To prevent continued hybridization and upstream colonization by nonnative fishes, a fish barrier was constructed in 2006. After construction of the barrier, highly hybridized rainbow-cutthroat hybrids and brook trout were removed from 13 miles of the mainstem South Fork Judith and its tributaries from 2006-2008 in an intensive mechanical removal effort. After 2008, nonnative removals occurred in the South Fork Judith and tributaries sporadically. Following the intensive removal effort in 2008, the South Fork Judith River has been stocked annually with 2,000-10,000 two-inch MO12 WCT from the Washoe Park Trout Hatchery. The original goal for the South Fork Judith River was to maintain a WCT metapopulation of > 95% WCT genetic contribution.

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

2022 Monitoring

The South Fork Judith River was sampled on August 17th and 18th, 2022, to update genetic monitoring of the WCT population and determine presence/absence of marked nonnative fish above the barrier. On August 17th, three reaches of the South Fork Judith River were backpack electrofished to collect genetic samples to assess the efficacy of the genetic swamping efforts underway since 2008. A 30 fish sample was collected from the 1st trail crossing above the barrier, at the Cabin Creek confluence, and at the Russian Creek confluence.

On August 18th, 2022, a single pass backpack electrofishing effort was made to detect presence of nonnative fish above the fish barrier. Two reaches were sampled: from the fish barrier to the first trail crossing and starting at the second trail crossing. No marked nonnative trout were detected. A total of 22 nonnative trout (rainbow, brook, and rainbow-cutthroat hybrids) collected above the barrier were marked (adipose clip) and released below the fish barrier.

Weatherwax Creek

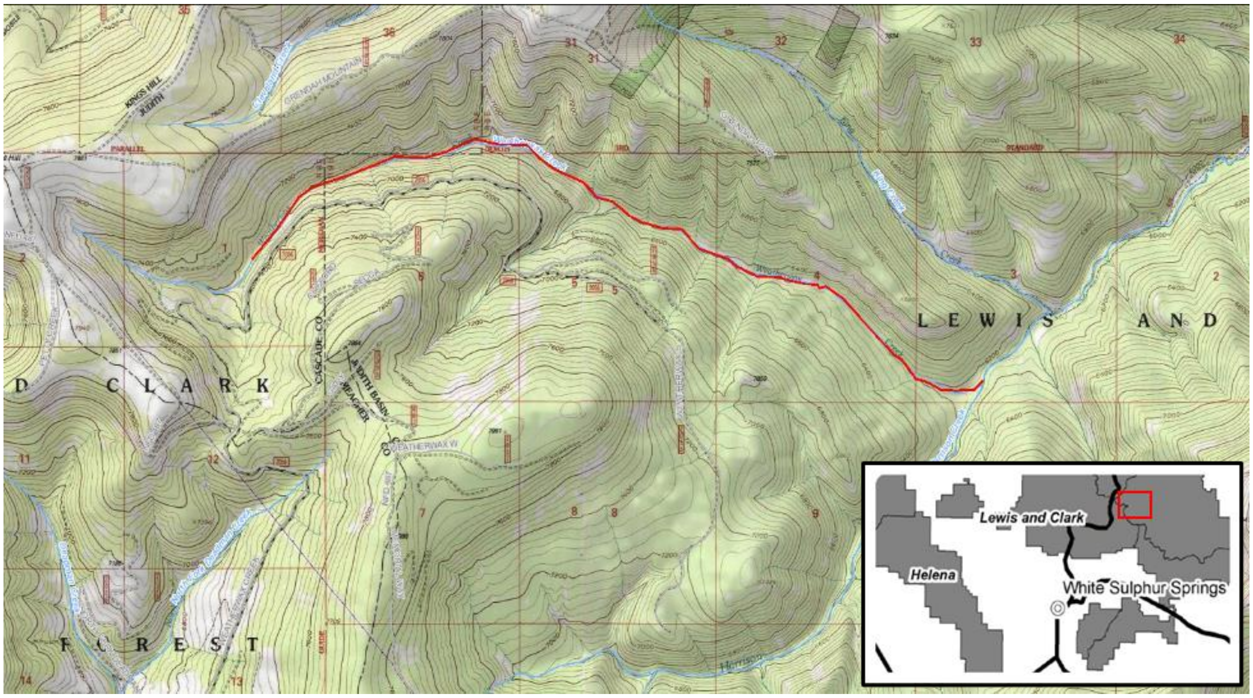

Figure 19. Weatherwax Creek in the Judith River subbasin. The stream segments delineated in red indicate areas occupied by WCT.

Background

Weatherwax Creek is a headwater tributary of the Middle Fork Judith River located just east of Kings Hill Pass southeast of the town of Neihart, MT. The stream contains both WCT and brook trout. Periodic demographic and genetic monitoring has occurred in the drainage since 1996. Genetic samples collected from the lower drainage near the confluence of Harrison Creek in 1996 indicated a 91.3% WCT population (n = 10). Genetic samples were again collected in 2003 from 25 fish approximately 2 miles upstream of the Harrison Creek confluence. This sample indicated a nonhybridized population of WCT still inhabited the headwaters of the stream. However, no known barriers to fish movement exist in the drainage. Updated genetic samples collected in 2021 from the headwaters of Weatherwax Creek found that hybridization has continued to spread upstream (Appendix A). However, there was a substantial excess of fish with little or no rainbow trout ancestry, two individuals with fairly high rainbow trout ancestry (>20%), and the remainder of fish had relatively low rainbow trout ancestry (<10%). The population of WCT in Weatherwax Creek is at high risk of extirpation given the current genetic trajectory of this population. At present it may be possible to “rescue” putatively non-hybridized individuals, otherwise, this population may have little to no conservation value in the very near future.

2022 Monitoring

A 1.28-mile reach of Weatherwax Creek was backpack electrofished on August 24th and 30th, 2022, to PIT tag WCT, collect genetic samples for genomic analysis, and collect brook trout for fish health inspection. This effort is part of a genetic rescue project to prevent the genomic extinction of the Weatherwax Creek WCT population. A total of 98 WCT were tagged in this effort. Additionally, 37 brook trout were submitted for fish health inspection. No pathogens were detected in the submitted sample. With the results of the genomic analysis, plans will be made to transfer nonhybridized WCT from Weatherwax Creek to fishless habitat elsewhere.

IV. Smith River Subbasin

Camas Lake and Big Camas Creek

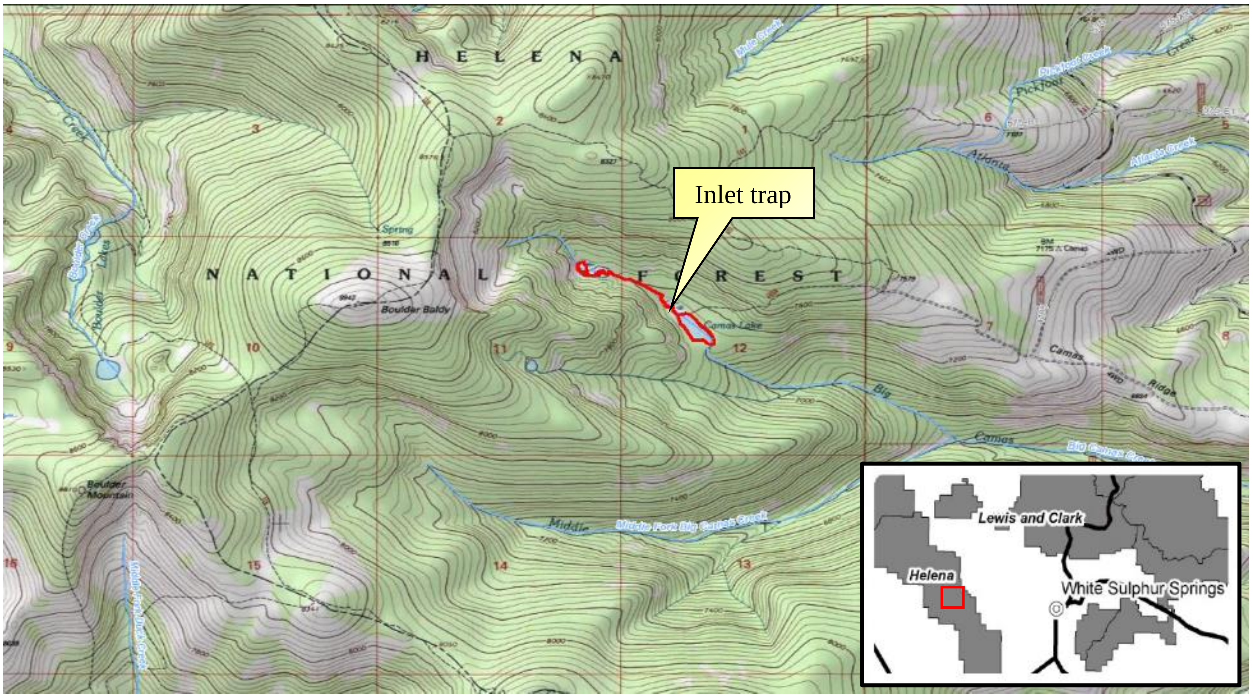

Figure 20. Camas Lake and Big Camas Creek in the Smith River subbasin. The stream segments delineated in red indicate areas sampled in 2021.

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.

2022 Monitoring

The Camas Lake inlet trap was installed June 9th, 2022. Discharge of Big Camas Creek above Camas Lake did not reach a level suitable for backpack electrofishing until June 22nd, 2022. In total, the Camas Lake trap was checked 13 times during the 2022 field season. Big Camas Creek above Camas Lake was electrofished 10 times in 2022. A total of 199 fish were collected in the inlet trap in 2022: 15 YCT were caught and removed and 184 WCT were trapped and passed upstream. An additional two YCT were caught and removed backpack electrofishing upper Big Camas Creek and one YCT was caught and removed by angling on Camas Lake. Total catch of YCT (n=18) was the lowest on record compared to previous years. Unidentified cutthroat trout under 120 mm were also collected and removed while backpack electrofishing (n=274).

Daisy Creek

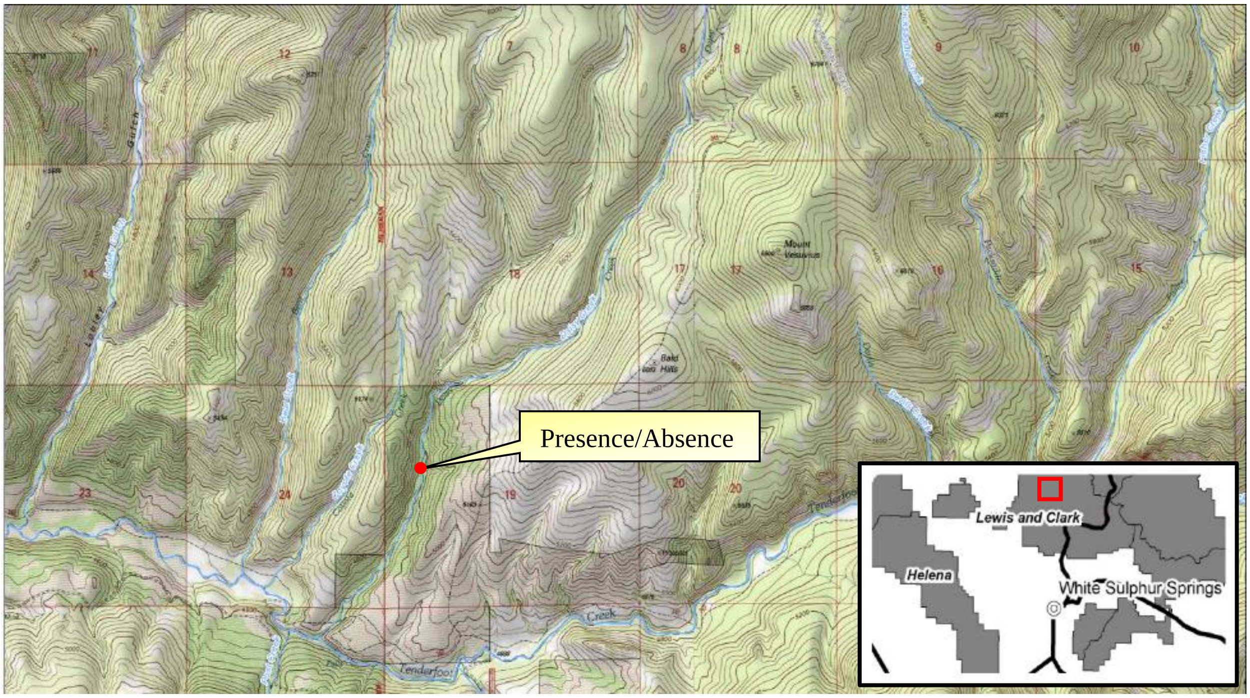

Figure 21. Daisy Creek in the Smith River subbasin. The stream segment delineated in red indicates the area sampled in 2022.

Background

Daisy Creek is a tributary of Tenderfoot Creek in the Smith River drainage located approximately 30 miles north of the community of White Sulphur Springs, MT. No previous sampling records from this location exist; however, brook trout are reported to be present (Ritter 2015).

2022 Monitoring

Daisy Creek was sampled on July 10th, 2022, to determine fish presence/absence above a culvert located approximately 1 mile upstream of the Tenderfoot Creek confluence. A 50 m reach of Daisy Creek was backpack elctrofished and fish were collected both above and below the culvert. Seven rainbow trout and three brook trout were collected in total. Daisy Creek could potentially serve as a site for WCT restoration if a fish barrier were to be installed at the culvert location (46.96269, -111.15462), as channel morphology appears suitable.

Daniels Creek

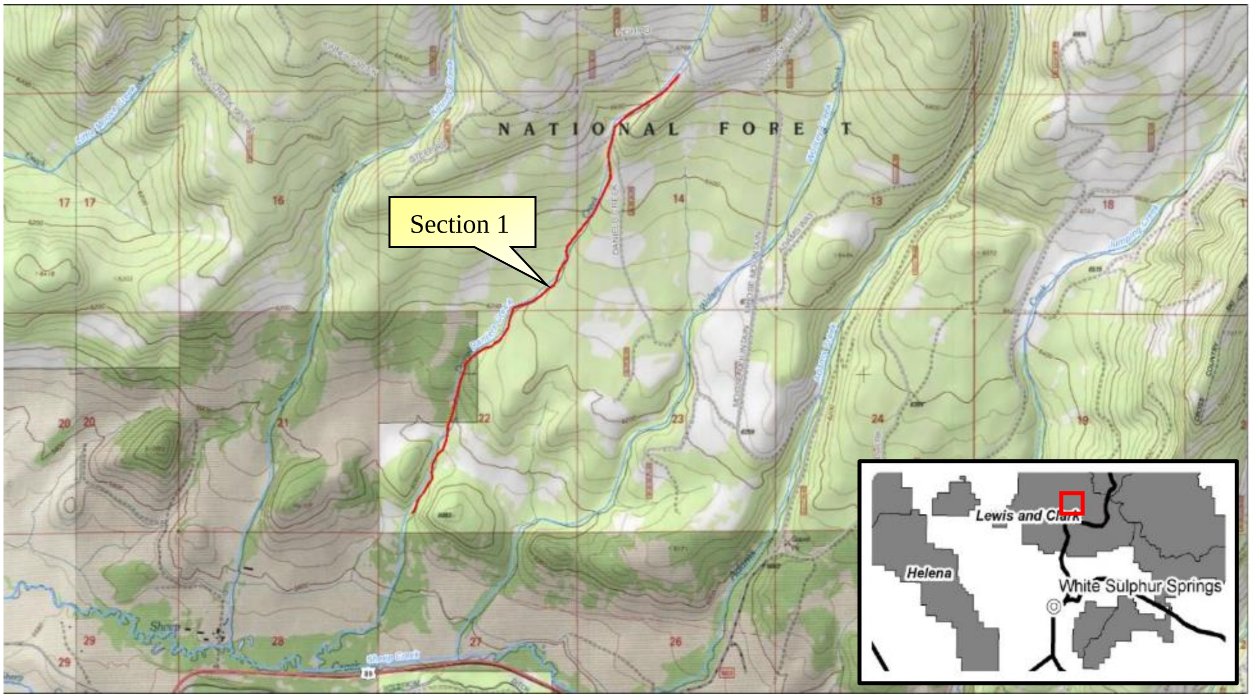

Figure 22. Daniels Creek in the Smith River subbasin. The stream segments delineated in red indicate areas occupied by WCT.

Background

Daniels Creek is a tributary of Sheep Creek in the Smith River drainage located approximately 16 miles north of the community of White Sulphur Springs, MT. The creek contains both WCT, brook trout, and Rocky Mountain sculpin. Genetic monitoring of WCT from 1994-2001 indicated a nonhybridized population was present. However, updated samples collected in 2010 confirmed that rainbow trout hybridization is occurring in the drainage. An irrigation diversion located on private land was thought to protect the stream from upstream migrating nonnative trout but is likely only a partial barrier.

2022 Monitoring

A fish population estimate was performed on July 27th, 2022, to estimate the abundance of the Daniels Creek WCT population. A 100 m population estimate section was established 1.31 miles downstream of the Adams Creek Road (FS RD 6412) crossing. Multiple pass depletion methods were used to estimate population abundance. An estimated 50 fish/km were found based on the results of the two-pass depletion, putting the total WCT population at 212 (±8) individuals if extrapolated to the entire reach (Figure 23). Brook trout dominated the catch in the 100 m section sampled outnumbering WCT four to one. The population of WCT in Daniels Creek is likely at high risk of extirpation given the current density of brook trout.

The Lewis and Clark National Forest is currently working on a Watershed Restoration Action Plan (WRAP) for the upper Sheep Creek subwatershed. Fish barrier construction on Daniels Creek has been identified as a potential project within the WRAP. If approved, funding may become available to retrofit the irrigation diversion into a functional fish barrier.

Daniels Creek —NATIVE TROUT POPULATION SURVEY

1. General Information— Date: **July 27th, 2022** Biologist: **A. Poole**
2. Stream Information—
    - Name, section, county: **Daniels Creek, 15, Meagher**
3. Survey Site Information (see attached map)—
    - Upstream range of native trout (general description and GPS): **Adams Creek Road crossing (46.81212, -110.81093)**
    - Downstream range of native trout (general description and GPS): **Irrigation diversion (46.78234, -110.84017)**
    - Location (GPS) and description of barriers: **Irrigation diversion (46.78234, -110.84017)**
    - Stream Length—Occupied habitat: **4.24 km (2.64 mi)** Available habitat: **6.01 km (3.79 mi)[1]**
    - Survey method & equipment: **backpack battery electrofisher; two-pass depletion**
    - Survey sites (general description and UTM)—
    - Section 1: **2.11 km downstream of Adams Creek Road crossing; 46.79709, -110.82549**

Parameter	**Section 1**
Section length (m)	100 m
Mean stream width (m) (n)	2.35 m (10)
Section area (hectares)	0.023 ha
WCT	
Removal Pattern	3 1
Population estimate	5 (±2)
Capture probability	0.571
Mean length (mm) (n)	170 (4)
Mean weight (g) (n)	53 (4)
Mean KTL (n)	0.93 (4)
Number fish per km (95 % CI)	50 (±20)
Number fish per ha (95 % CI)	217 (±87)
Biomass (kg per ha) (95 % CI)	12 (±7)

4. Comments:

[1] – Includes 1.77 km (1.1 mi) of habitat below the irrigation diversion.

Other species sampled: Brook trout and Rocky Mountain sculpin.

Brook trout density and biomass:

Parameter	**Section 1**
Number fish per km (95 % CI)	200 (±60)
Number fish per ha (95 % CI)	870 (±261)
Biomass (kg per ha) (95 % CI)	31 (±9)

Figure 23. Daniels Creek fish population estimate results.

Fourmile Creek

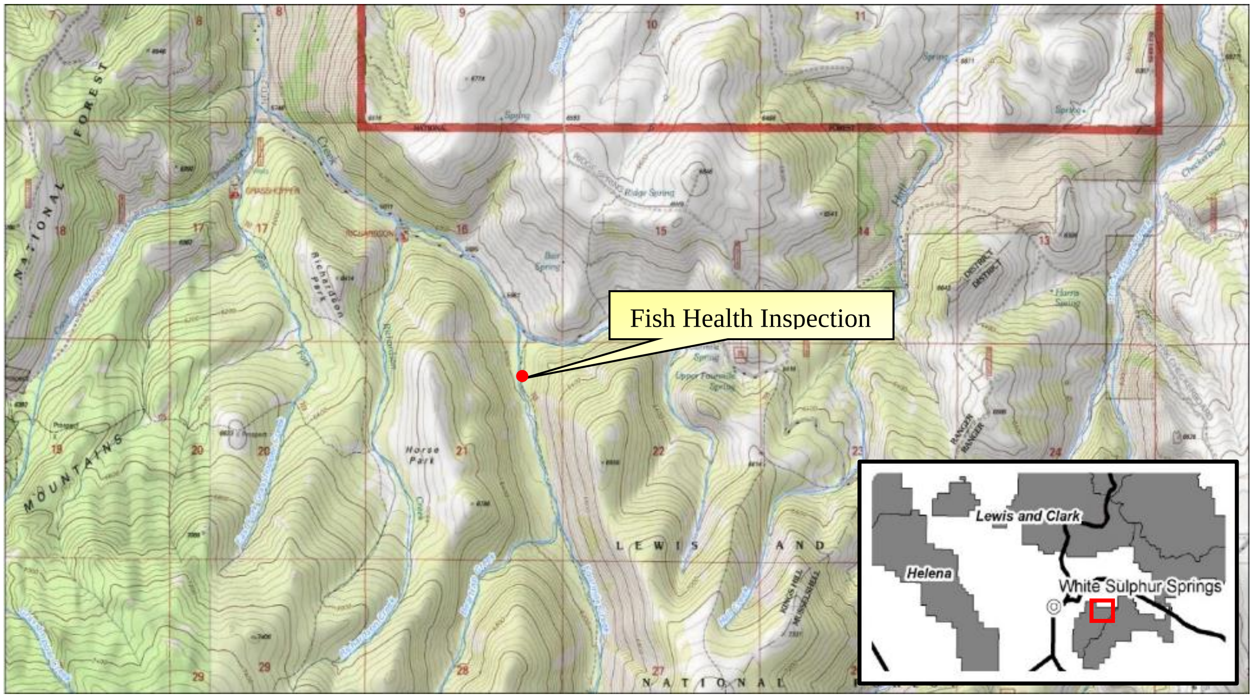

Figure 24. Fourmile Creek in the Smith River subbasin. The stream segment delineated in red indicates the area sampled in 2022.

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 24). 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 0.70-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.

2022 Monitoring

Fourmile Creek was surveyed on July 12th, 2022, to collect brook trout for a fish health inspection report. This is part of an ongoing project to expand the nonhybridized WCT population in the headwaters of Fourmile Creek to occupy all available habitat as proposed by the original EA regarding the transfer of fish from Richardson Creek to Fourmile Creek. A 60 fish sample was collected from Fourmile Creek starting at the #713 trailhead. No pathogens were detected in samples submitted for testing. A wild fish transfer has been submitted to the Aquatic Health Advisory Committee (AHAC) for the upstream transfer of 20-25 WCT annually for a period of three years.

Gipsy Creek

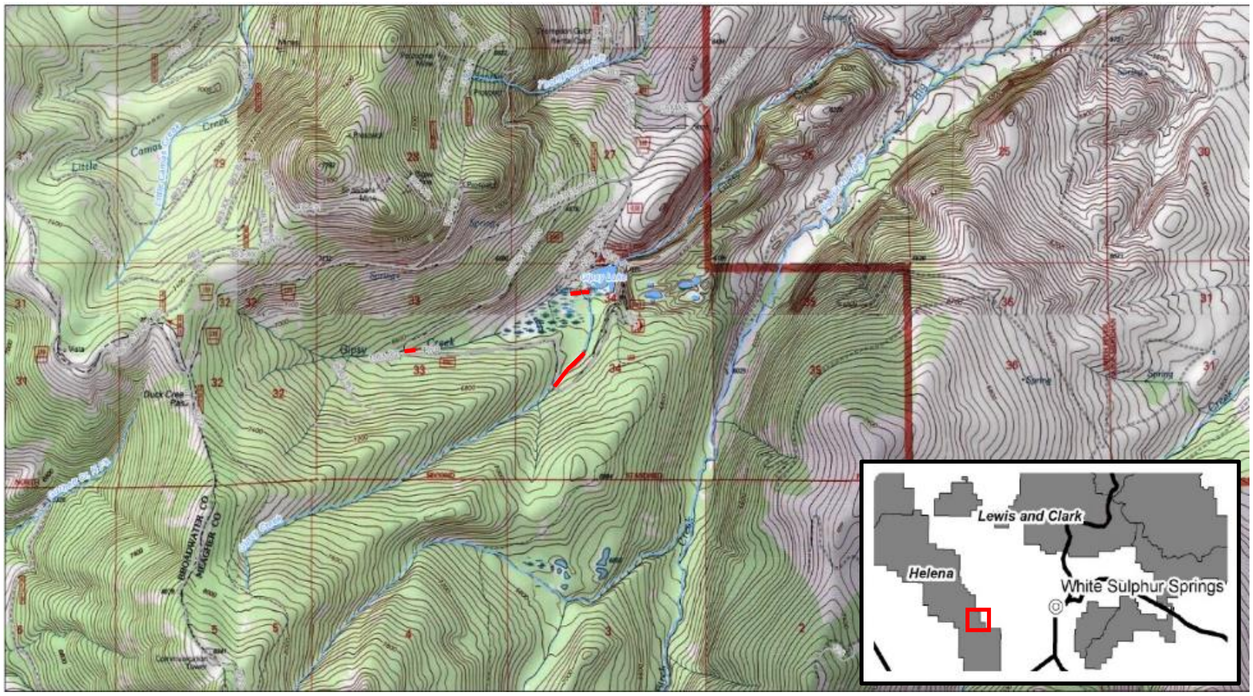

Figure 25. Gipsy Creek in the Smith River subbasin. The stream segments delineated in red indicate the areas sampled in 2022.

Background

Gipsy Creek is a tributary of Big Birch Creek in the Smith River subbasin located approximately 14 miles southeast of the community of White Sulphur Springs, MT. The creek is impounded by an earthen dam that creates Gipsy Lake, a small 7-acre lake that supports a put-and-take rainbow trout fishery maintained through annual stocking. Gipsy Lake also features a developed Forest Service campground and recreation site. Gipsy Lake has been identified in the past as a potential site for WCT restoration. However, no sampling data from the lake or creek exists and habitat evaluations have not been performed.

2022 Monitoring

Gipsy Creek was sampled on July 26th, 2022, to evaluate stream habitat above Gipsy Lake and collect baseline data on the present fish assemblage. Three reaches of Gipsy Creek were backpack electrofished: immediately above Gipsy Lake, below FS RD 8961, and the unnamed tributary below the campground (Figure 25). Fish were only detected in the reach above Gipsy Lake. A total of nine brook trout, one rainbow trout, one rainbow-cutthroat hybrid, and eight Rocky Mountain sculpin were collected. Habitat appeared suitable for WCT, however; only around 1.3 miles of stream habitat is present above Gipsy Lake. Gipsy Lake is shallow and only provides marginal fish habitat. It is unlikely that this area would be able to support a secure conservation population.

Kinney Creek

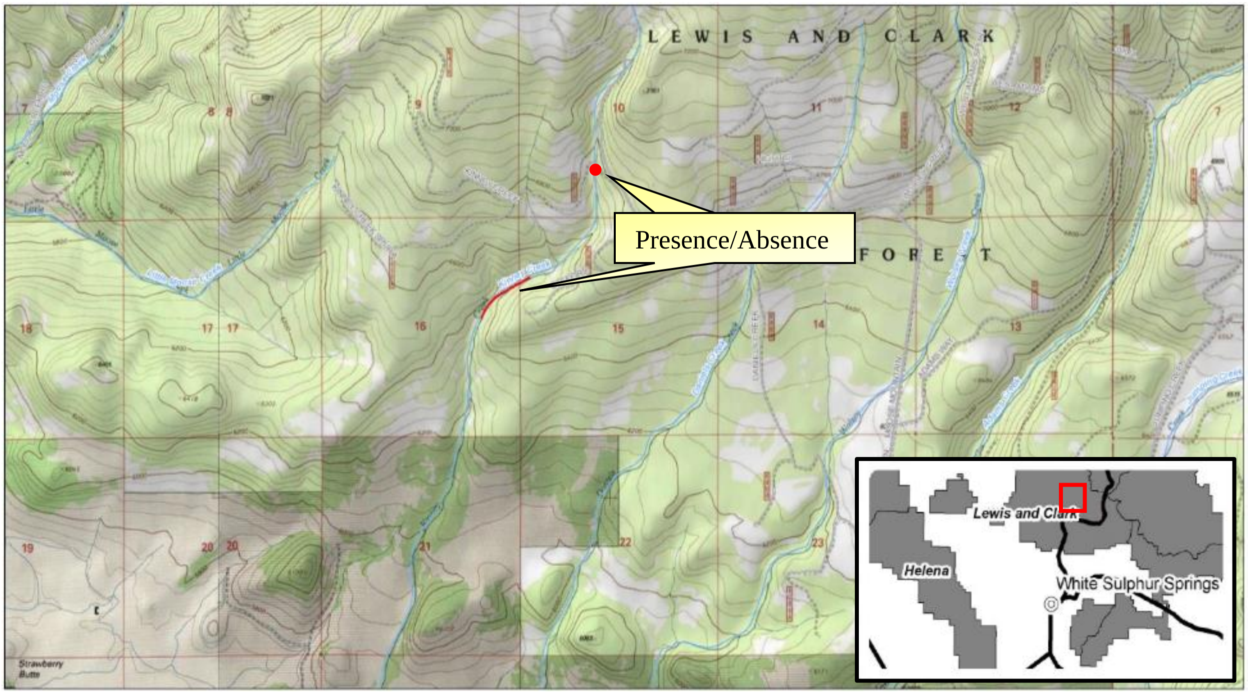

Figure 26. Kinney Creek in the Smith River subbasin. The stream segments delineated in red indicate the areas sampled in 2022.

Background

Kinney Creek is a tributary of Sheep Creek in the Smith River drainage located approximately 16 miles north of the community of White Sulphur Springs, MT. No previous collection records exist from this location. Several neighboring streams support or have historically supported WCT populations in the recent past (Daniels, Deadman, Sheep, and Wolsey).

2022 Monitoring

Kinney Creek was sampled on July 27th and September 15th, 2022, to establish bassline presence/absence data for this locale. Two reaches were backpack electrofished: one below the closed FS RD 6410 and one near the end of FS RD 6402 (Figure 26). No fish were detected at either location. Fish habitat appeared marginal with low base flows and substrate mainly consisting of embedded cobbles and silt. The lower reach sampled contained large quantities of woody debris resulting in an undefined channel. Kinney Creek is unlikely to harbor any remnant WCT population or serve as a potential restoration location in the future.

Wolsey Creek

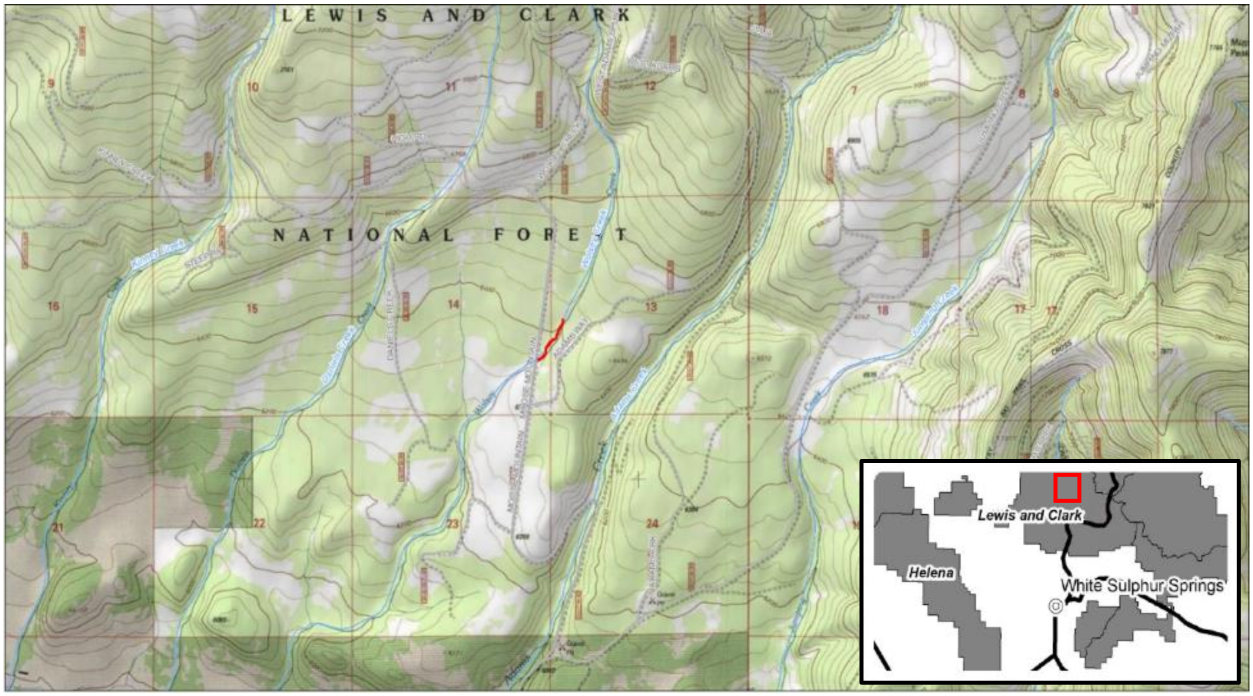

Figure 27. Wolsey Creek in the Smith River subbasin. The stream segment delineated in red indicates the area sampled in 2022.

Background

Wolsey Creek is a tributary of Sheep Creek in the Smith River drainage located approximately 16 miles north of the community of White Sulphur Springs, MT. Historic sampling records from 1973 documented WCT presence in this drainage. Subsequent sampling from 2000-2005 has only detected nonnative brook trout in Wolsey Creek.

2022 Monitoring

Wolsey Creek was sampled on September 15th, 2022, to detect presence/absence of WCT. A 358 m reach of Wolsey Creek was backpack electrofished starting at the FS RD 6412 crossing. A total of 46 brook trout were collected. Rocky Mountain sculpin was also common in this reach. No WCT were collected. If any future attempts are made to detect remnant WCT, effort should be focused on the headwaters of Wolsey Creek.

V. Sun River Subbasin

Little Willow Creek and Lime Gulch

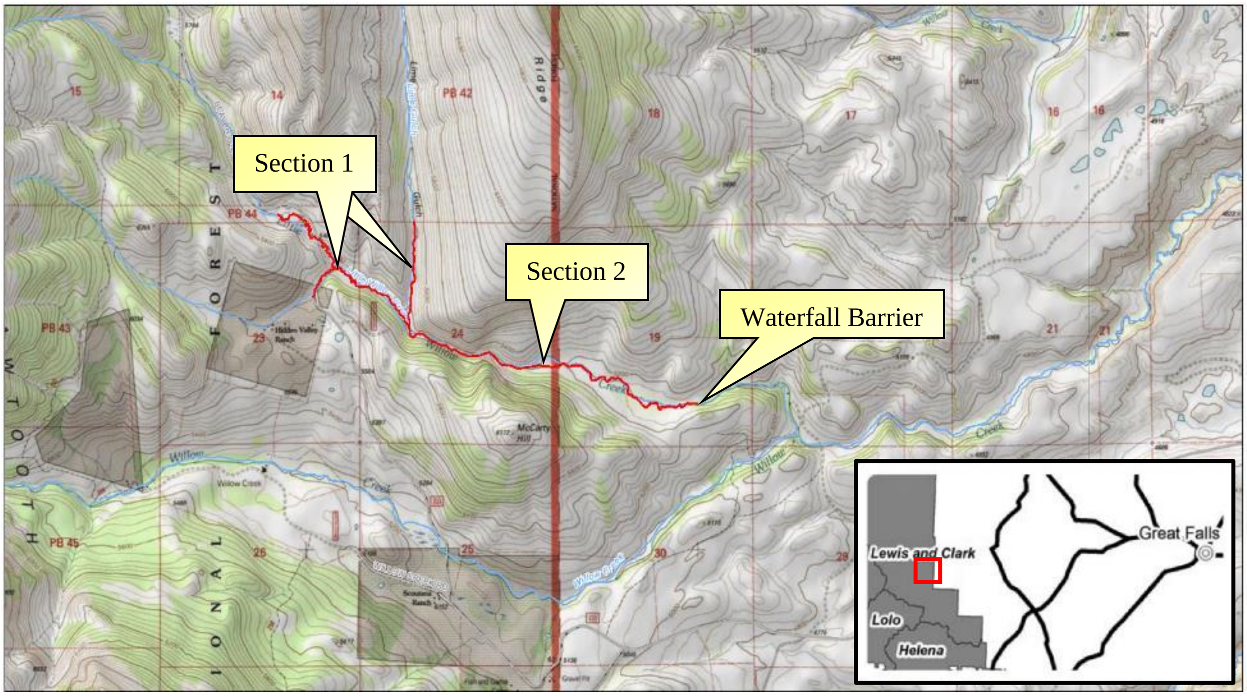

Figure 28. Little Willow Creek and Lime Gulch in the Sun River subbasin. The stream segments delineated in red indicate the areas occupied by WCT.

Background

Little Willow Creek is a tributary of Willow Creek in the Sun River drainage located approximately 12 miles west of the community of Augusta, MT. Little Willow Creek, its principal tributary Lime Gulch, and an unnamed tributary (Hidden Valley Creek) support a slightly hybridized, interconnected WCT conservation population. A large waterfall barrier located on private land isolates the upper 3.64 miles of fish habitat within the drainage. Rocky Mountain sculpin is also present in Little Willow Creek. Genetic monitoring has occurred within the drainage periodically form 1991-1998 with the most recent results coming from Lime Gulch indicating a 98.1% WCT population present.

2022 Monitoring

Updated genetic and demographic monitoring of the Little Willow Creek WCT population was conducted in 2022. Genetic samples were collected from three reaches of Little Willow Creek on May 2nd and 4th, 2022. A total of 25 samples were collected in this effort and will submitted for analysis in 2023.

Fish population estimates were performed September 19th – 21st, 2022, to estimate the abundance of the Little Willow Creek WCT population. Three 100 m population estimate sections were established: two on Little Willow Creek and one on Lime Gulch. Multiple pass depletion methods were used to estimate population abundance. Estimated abundance was high across all three sites sampled resulting in an average of 850 fish/km. A total WCT population of 4981 (±176) is estimated to be present in Little Willow Creek if extrapolated to the entire stream (Figures 29 & 30).

Little Willow Creek —NATIVE TROUT POPULATION SURVEY

1. General Information— Date: **September 19, 21, 2022** Biologist: **A. Poole**
2. Stream Information—
   Name, section, county: **Little Willow Creek, 23 & 24 , Lewis and Clark**
3. Survey Site Information (see attached map)—
   Upstream range of native trout (general description and GPS): **Spring (47.48013, -112.71225)**
   Downstream range of native trout (general description and GPS): **Waterfall barrier (47.46783, -112.66722)**
   Location (GPS) and description of barriers: **Waterfall barrier (47.46783, -112.66722)**
   Stream Length—Occupied habitat: **5.86 km (3.64 mi)** Available habitat: **5.86 km (3.64 mi)**
   Survey method & equipment: **backpack battery electrofisher; two-pass depletion**
   Survey sites (general description and UTM)—
   Section 1: **Downstream of Lime Gulch trailhead; 47.47635, -112.70513**
   Section 2: **Upstream of Forest Service boundary; 47.47010, -112.68679**

Parameter	**Section 1**	**Section 2**
Section length (m)	100 m	100 m
Mean stream width (m) (n)	2.21 m (10)	3.54 m (10)
Section area (hectares)	0.022 ha	0.035 ha
WCT		
Removal Pattern	100 18	42 14
Population estimate	122 (±3)	63 (±6)
Capture probability	0.819	0.666
Mean length (mm) (n)	110 (118)	144 (56)
Mean weight (g) (n)	15 (118)	34 (56)
Mean KTL (n)	0.87 (118)	0.85 (56)
Number fish per km (95 % CI)	1,220 (±10)	630 (±60)
Number fish per ha (95 % CI)	5,545 (±136)	1,800 (±171)
Biomass (kg per ha) (95 % CI)	83 (±2)	61 (±3)

4. Comments:

Other species present: Rocky Mountain sculpin at Section 1.

Figure 29. Little Willow Creek fish population estimate results.

Lime Gulch —NATIVE TROUT POPULATION SURVEY

1. General Information— Date: **September 20th, 2022** Biologist: **A. Poole**
2. Stream Information—
   Name, section, county: **Lime Gulch, 24, Lewis and Clark**
3. Survey Site Information (see attached map)—
   Upstream range of native trout (general description and GPS): **Spring (47.47965, -112.69770)**
   Downstream range of native trout (general description and GPS): **Little Willow Creek confluence (47.47292, -112.69823)**
   Location (GPS) and description of barriers: **none**
   Stream Length—Occupied habitat: **0.797 km (0.50 mi)** Available habitat: **0.797 km (0.50 mi)**
   Survey method & equipment: **backpack battery electrofisher; two-pass depletion**
   Survey sites (general description and UTM)—
   Section 1: **0.365 km above Little Willow Creek confluence; 47.47587, -112.69768**

Parameter	**Section 1**
Section length (m)	100 m
Mean stream width (m) (n)	1.96 m (10)
Section area (hectares)	0.020 ha
WCT	
Removal Pattern	58 9
Population estimate	69 (±2)
Capture probability	0.838
Mean length (mm) (n)	120 (67)
Mean weight (g) (n)	18 (67)
Mean KTL (n)	0.86 (67)
Number fish per km (95 % CI)	690 (±20)
Number fish per ha (95 % CI)	3,450 (±100)
Biomass (kg per ha) (95 % CI)	62 (±2)

Figure 30. Lime Gulch fish population estimate results.

VI. Teton River Subbasin

Olney Creek

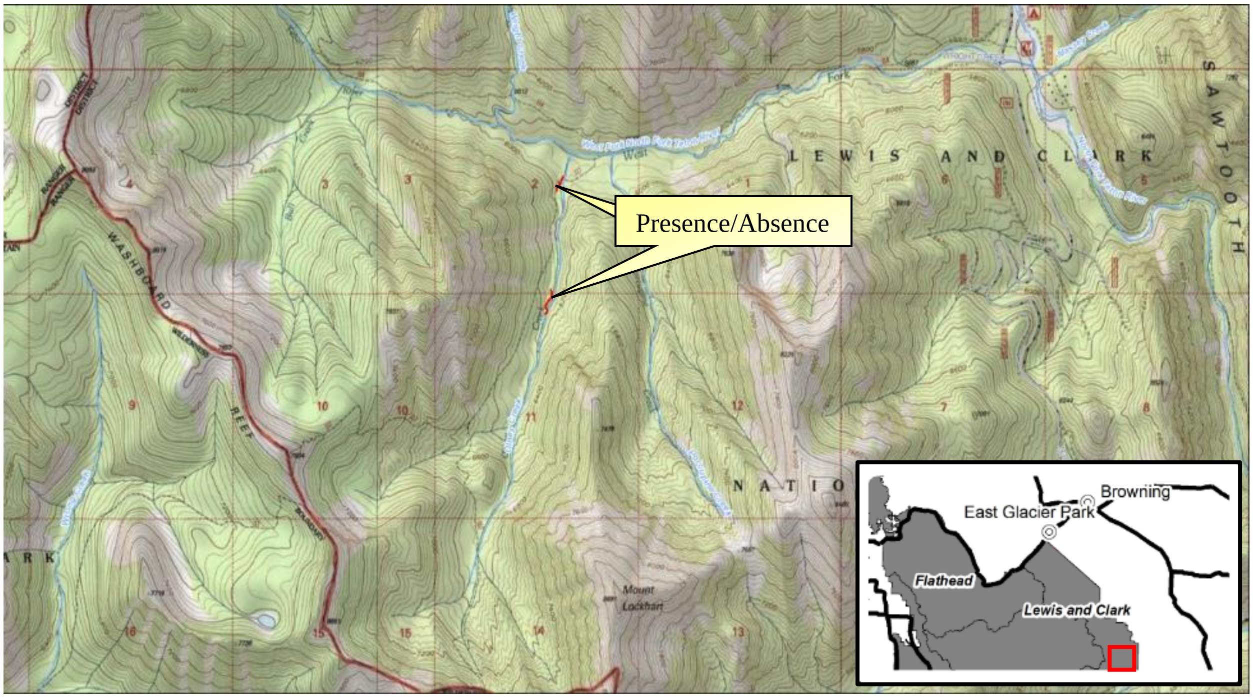

Figure 31. Olney Creek in the Teton River subbasin. The stream segments delineated in red indicate the areas sampled in 2022.

Background

Olney Creek is a tributary of the West Fork Teton River in the Teton River subbasin located approximately 32 miles northwest of the community of Choteau, MT. No previous collection records exist for this locality. However, WCT were collected from the West Fork Teton River in 1996.

2022 Monitoring

Olney Creek was sampled on July 6th, 2022, to determine WCT presence/absence and locate potential fish barriers. Two reaches of Olney Creek were backpack electrofished: one at the trail #117 crossing and another 0.57 miles upstream. Two brook trout were collected in the lower reach sampled and no fish were detected in the upper reach. A large bedrock cascade was located at the head of the upper reach located at 47.94162, -112.85813 (Figure 32) that appears to isolate the upper 1.0 mile of habitat. Physical habitat in Olney Creek appears excellent but for unknow reasons only supports low densities of brook trout.

Figure 32. Olney Creek bedrock barrier located at 47.94162, -112.85813.

VII. Two Medicine River Subbasin

Pike Creek

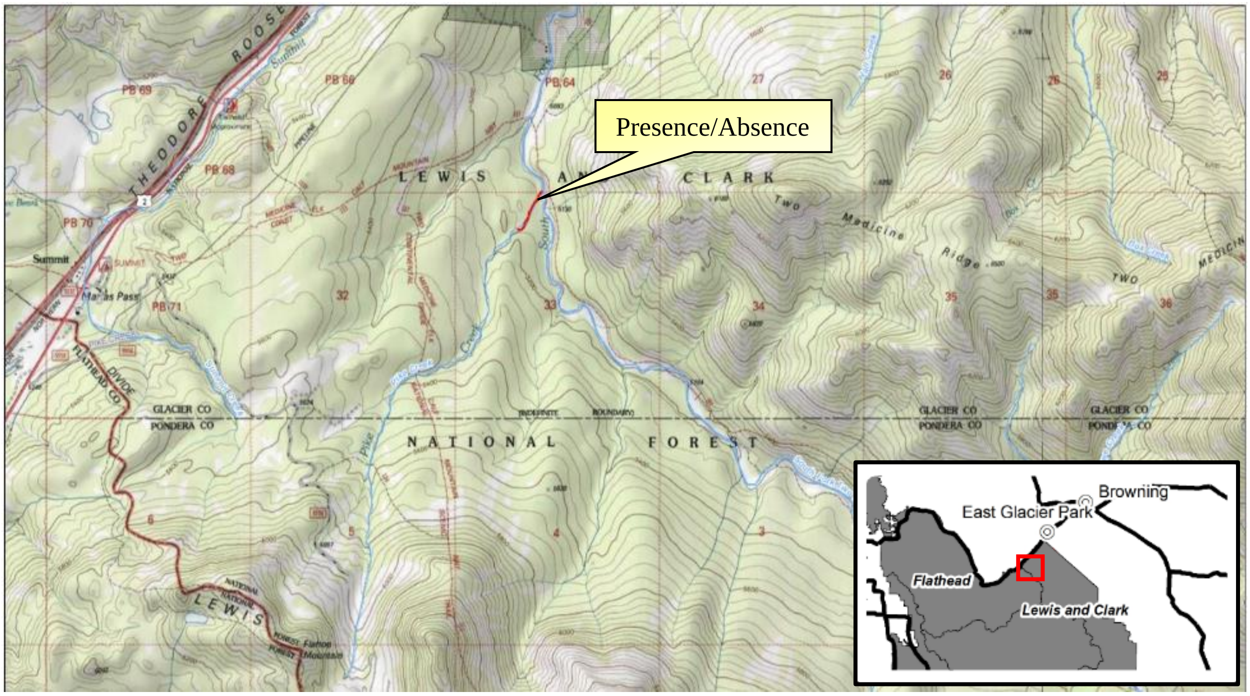

Figure 33. Pike Creek in the Two Medicine River subbasin. The stream segment delineated indicates the area sampled in 2022.

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. No previous collection records exist for this locality. Forest Service records documented the presence of a bedrock barrier at the mouth of Pike Creek that isolates this drainage from the South Fork Two Medicine River.

2022 Monitoring
Pike Creek was sampled on October 3rd, 2022, to determine WCT presence/absence and evaluate habitat above the bedrock barrier. A 350 m reach of Pike Creek was backpack electrofished starting at the confluence with the South Fork Two Medicine River. Rocky Mountain sculpin was the only fish collected and found to be abundant throughout the reach sampled. The Pike Creek watershed was highly impacted by the 2007 Skyland Fire resulting in a channel filled with large woody debris. The stream appears suitable for WCT; however, only around 1.69 miles of habitat would likely be available above the bedrock barrier.

Phillips Creek

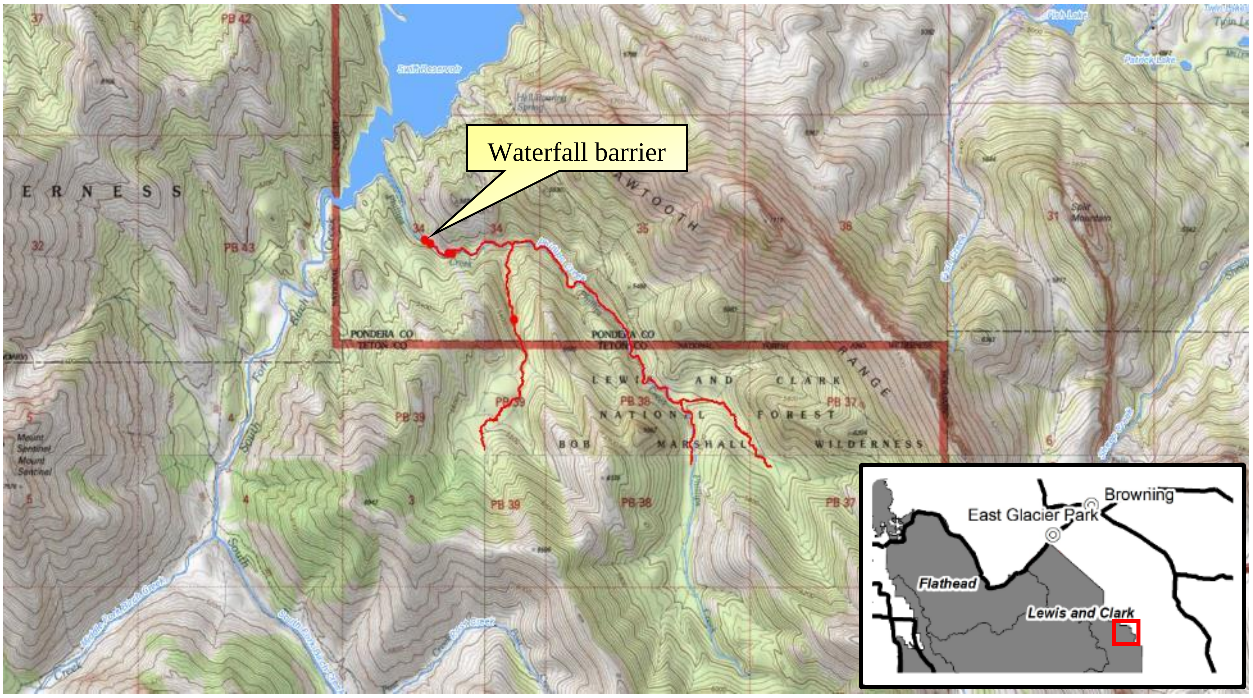

Figure 34. Phillips Creek in the Two Medicine River subbasin. The stream segments delineated in red indicate potential WCT restoration area.

Background

Phillips Creek is a tributary of Swift Reservoir in the Two Medicine River subbasin located west of the community of Dupuyer, MT. Approximately 0.2 miles upstream from the Swift Reservoir Trail #143 crossing, a 30 ft tall waterfall isolates the upper 4.2 miles of habitat in Phillips Creek (48.13874, -112.88255; Figure 34). Presence/absence sampling performed in 2021 confirmed the stream to be fishless above this barrier.

2022 Monitoring

A temperature logger was deployed in Phillips Creek on May 16th, 2022, to further evaluate habitat suitability for WCT. The logger was placed approximately 0.13 miles above the waterfall barrier at 48.13775, -112.87996 and retrieved on November 1st, 2022, after being deployed for a total of 169 days. The mean August stream temperature was 10.85°C, indicating suitable thermal habitat is present in Phillips Creek to support WCT (Figure 35).

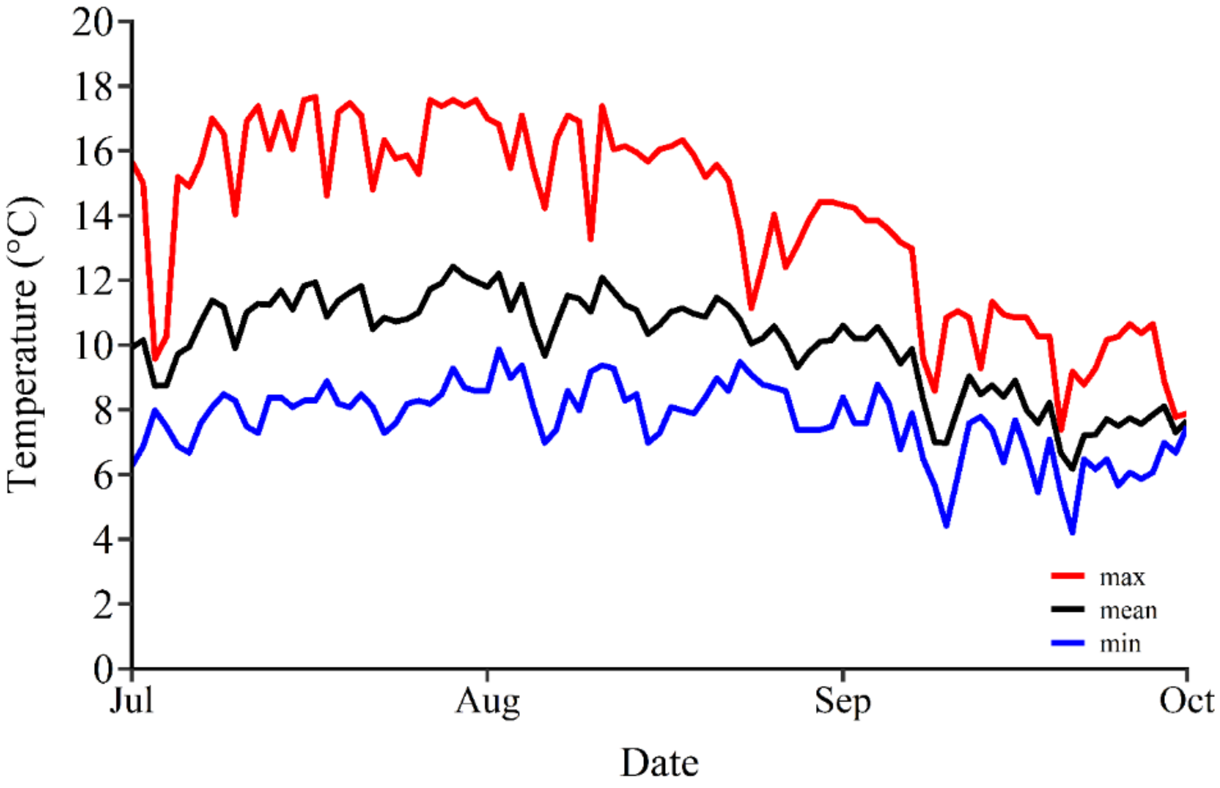

Figure 35. Daily maximum (red line), mean (black line), and minimum (blue line) stream temperatures from Phillips Creek. Temperature logger was deployed on May 16, 2022 and collected November 1, 2022.

South Fork Birch Creek

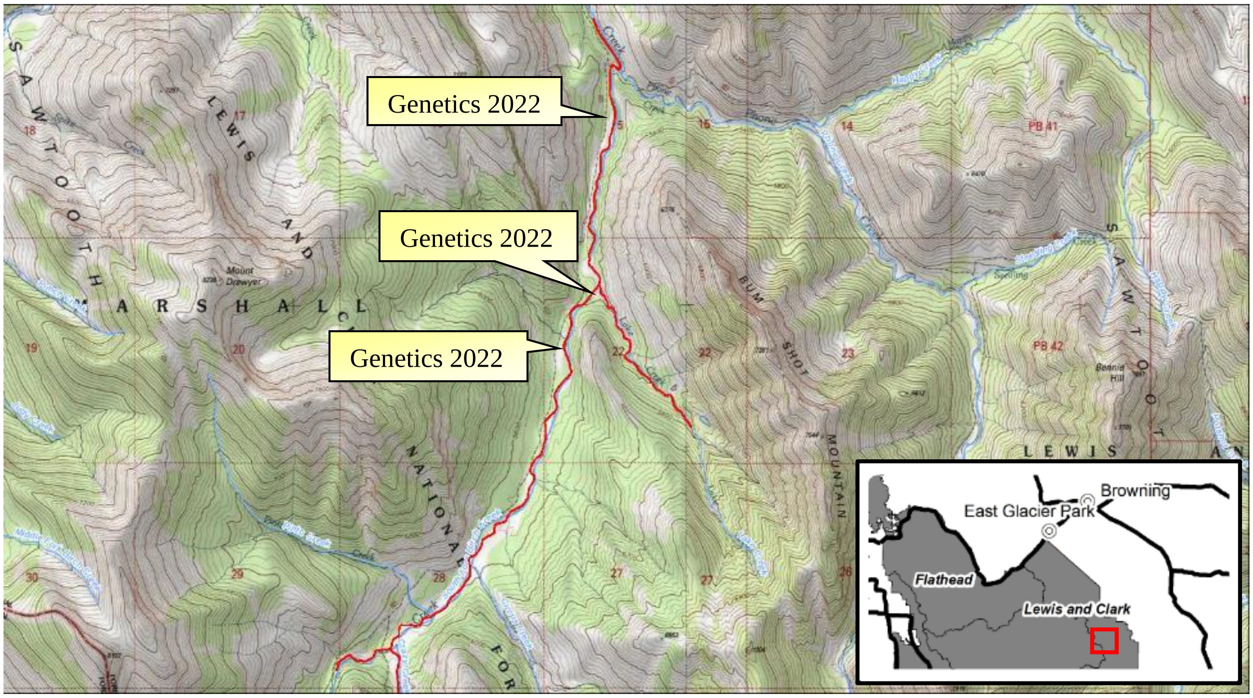

Figure 36. South Fork Birch Creek in the Two Medicine River subbasin. The stream segments delineated in red indicate the area occupied by nonhybridized WCT.

Background

South Fork Birch Creek is a tributary of Swift Reservoir in the Two Medicine River subbasin located west of the community of Dupuyer, MT. Originally fishless, South Fork Birch Creek now contains a nonhybridized population of WCT established by the transfer of 171 fish from Nort Fork Little Belt Creek in 1974. Genetic monitoring of this population has only occurred once with a 9 fish sample collected in 1995.

2022 Monitoring

South Fork Birch Creek was surveyed on August 2nd and 3rd, 2022, to collect updated genetic samples and document the presence of intermediate fish barriers. Three reaches were sampled with hook-and-line by two anglers. A total of 21 WCT were collected in 8.36 hours of combined fishing effort over the two-day period (*C/f* = 2.51). No other fish species were detected. A previously undocumented intermediate fish barrier was located upstream of the Lake Creek confluence at 48.08300, -112.88781 (Figure 37).

Figure 37. Bedrock barrier on South Fork Birch Creek located upstream of Lake Creek confluence at 48.08300, -112.88781.

Summit Creek

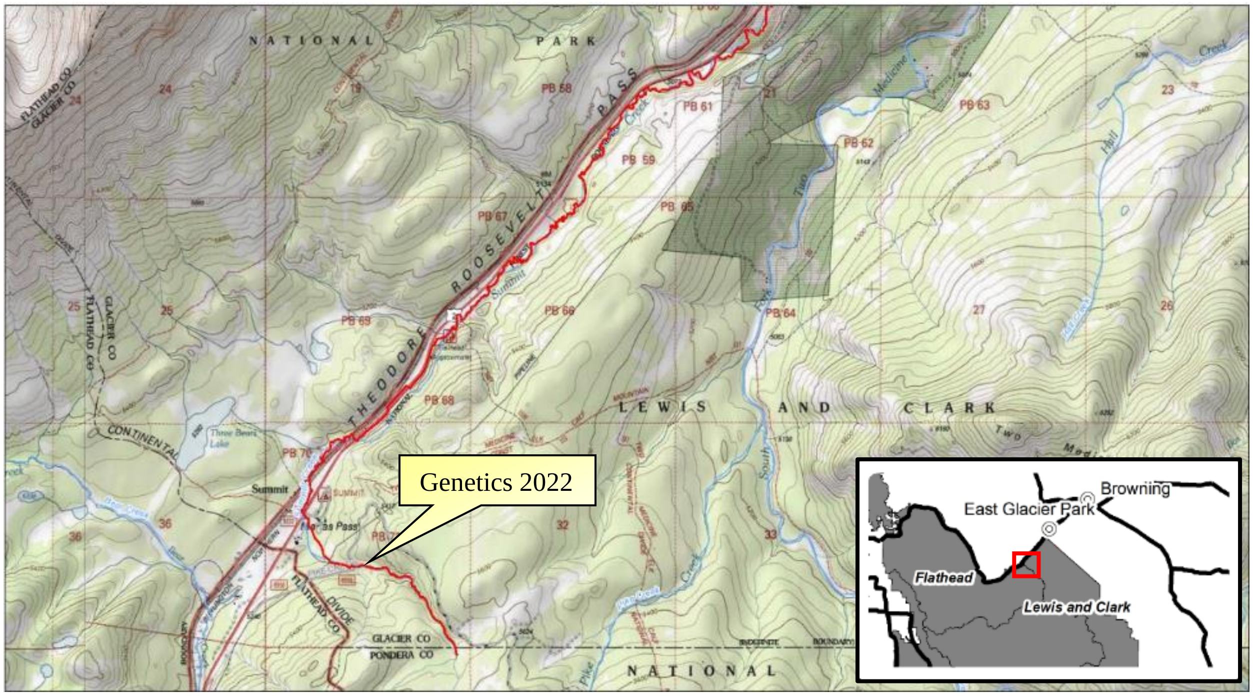

Figure 38. Summit Creek in the Two Medicine River subbasin. The stream segments delineated in red indicate the area occupied by WCT.

<u>Background</u>

Summit Creek is a tributary of the South Fork Two Medicine River located approximately 6 miles southeast of the community of East Glacier Park, MT. Genetic monitoring of the Summit Creek WCT population was performed once in 1992. A 20 fish sample from the middle reach of the creek indicated the population was a hybrid swarm with 91.8% WCT genetic ancestry.

<u>2022 Monitoring</u>

Updated genetic monitoring of Summit Creek WCT was performed in 2022 to determine if the population still holds conservation value. The headwaters of Summit Creek were backpack electrofished on October 3rd, 2022, above the Pike Creek Road (FS RD 8958) crossing. Genetic samples were collected from 20 WCT for analysis in 2023. Rocky Mountain sculpin was the only other fish species observed in this reach.

VIII. Upper Missouri River Subbasin

Beaver Creek

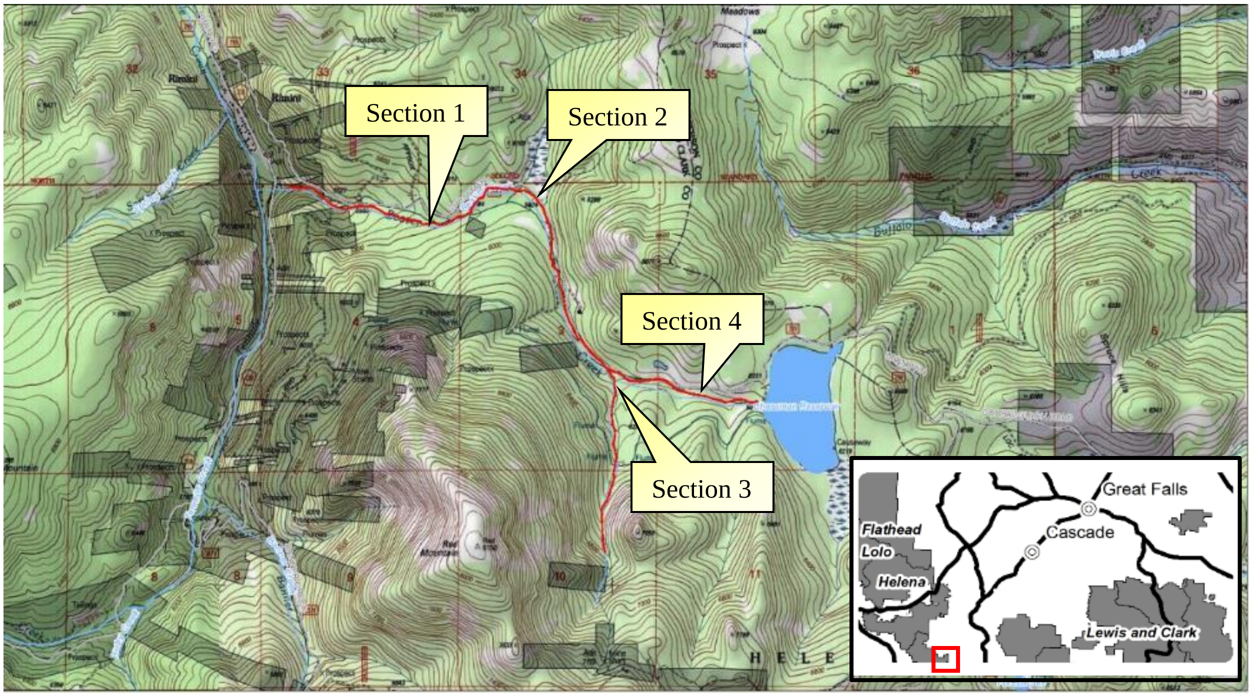

Figure 39. Beaver Creek in the Upper Missouri River subbasin. The stream segments delineated in red indicate potential WCT conservation area.

Background

Beaver Creek is a tributary of Tenmile Creek in the Upper Missouri River subbasin located 11 miles southeast of Helena, MT. Beaver Creek is isolated from Tenmile Creek by a natural waterfall barrier as well as a City of Helena water diversion structure. No previous fish collection records for this locality could be found.

2022 Monitoring

Beaver Creek was sampled on May 18th, 2022, to determine fish presence/absence above the waterfall and diversion barriers. Four reaches of Beaver Creek were backpack electrofished (Figure 39) that averaged 180 m in length. No fish were detected at any of the sites sampled. Fish habitat appeared to be excellent with abundant macroinvertebrates, spawning gravels, and deep plunge pools observed.

A temperature logger was deployed in Beaver Creek on June 17th, 2022, to further evaluate habitat suitability for WCT. The logger was placed within the first electrofishing reach at 46.48302, -112.23042 and retrieved on September 22nd, 2022, after being deployed for a total of 97 days. The mean August stream temperature was 9.54°C, indicating suitable thermal habitat is present in Beaver Creek to support WCT (Figure 40).

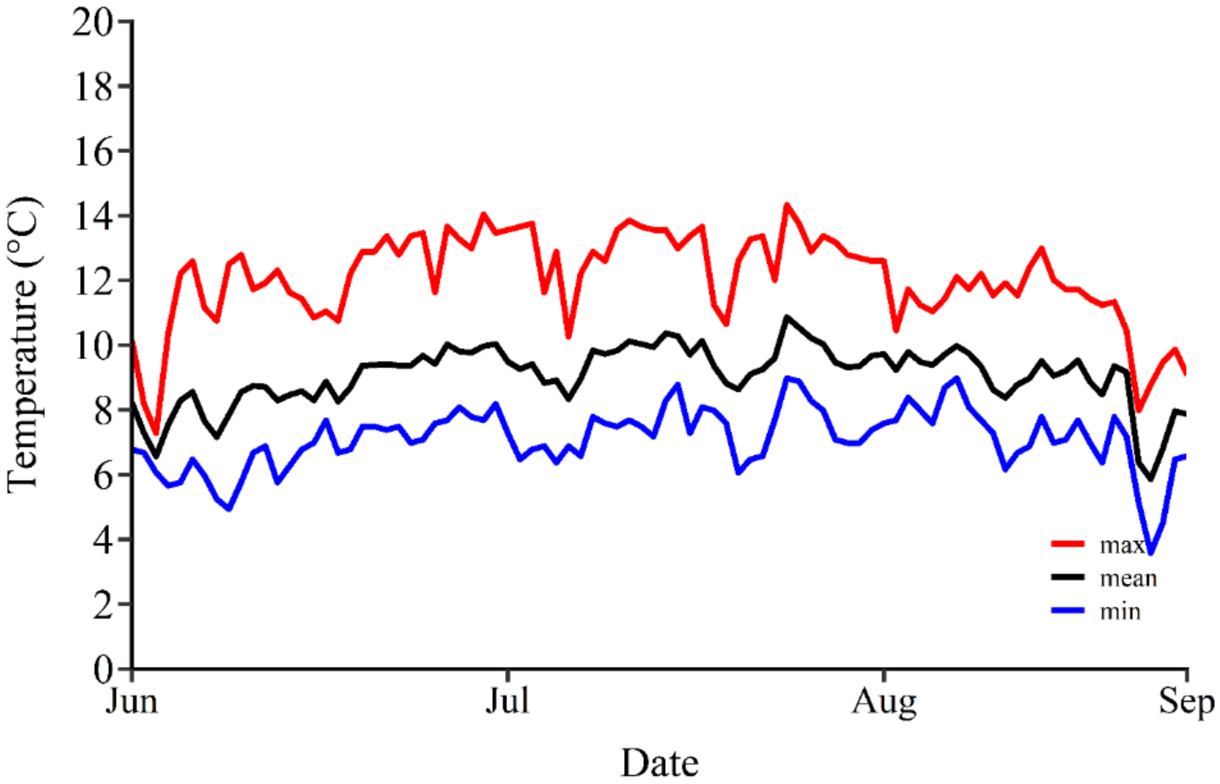

Figure 40. Daily maximum (red line), mean (black line), and minimum (blue line) stream temperatures from Beaver Creek. Temperature logger was deployed on June 17, 2022 and collected September 22, 2022.

Beaver Creek has been identified as a suitable location for establishing a WCT conservation population. This site should be considered as a recipient waterbody for the at-risk Page Gulch WCT population. Transfers from additional source populations, such as Threemile Creek, may be needed to bolster fish numbers.

Cottonwood Creek

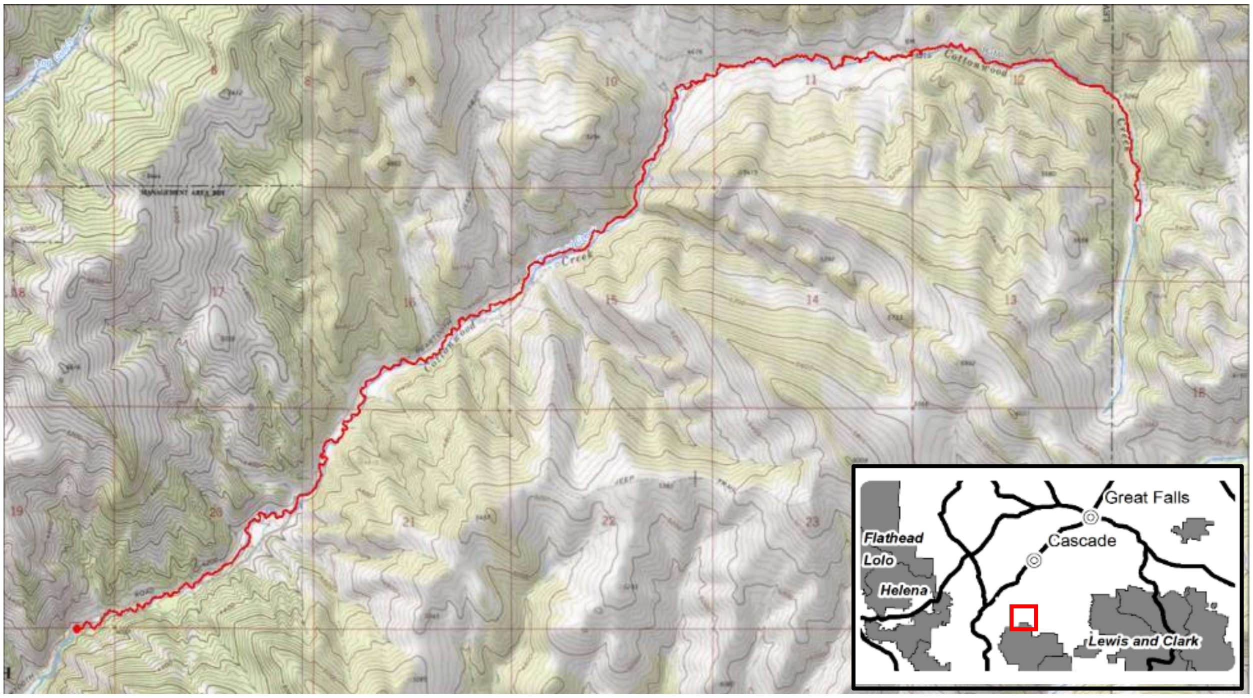

Figure 41. Cottonwood Creek in the Upper Missouri River subbasin. The stream segment delineated in red indicates the area occupied by nonhybridized WCT.

Background

Cottonwood Creek is a tributary to Holter Lake in the Upper Missouri River subbasin located on the Beartooth Wildlife Management Area. In 2010, a pre-cast concrete barrier was constructed on Cottonwood Creek (MoTac Project #753-10) to replace a previously installed undersized fish barrier. Following piscicide treatment, nonhybridized WCT from Threemile Creek and White Creek (Upper Missouri subbasin) were restored by means of RSIs from 2009-2013. However, Yellowstone Cutthroat Trout hybridization was detected in the White Creek population in 2014.

2022 Monitoring

Genetic monitoring of the Cottonwood Creek WCT population was performed on April 21st, 2022. Five sites were backpack electrofished to collect fish from throughout the drainage. A total of 28 WCT were collected and 25 were sampled for updated genetic analysis. Flows were noted to be very low with large portions of the lower drainage dry.

Elkhorn Creek

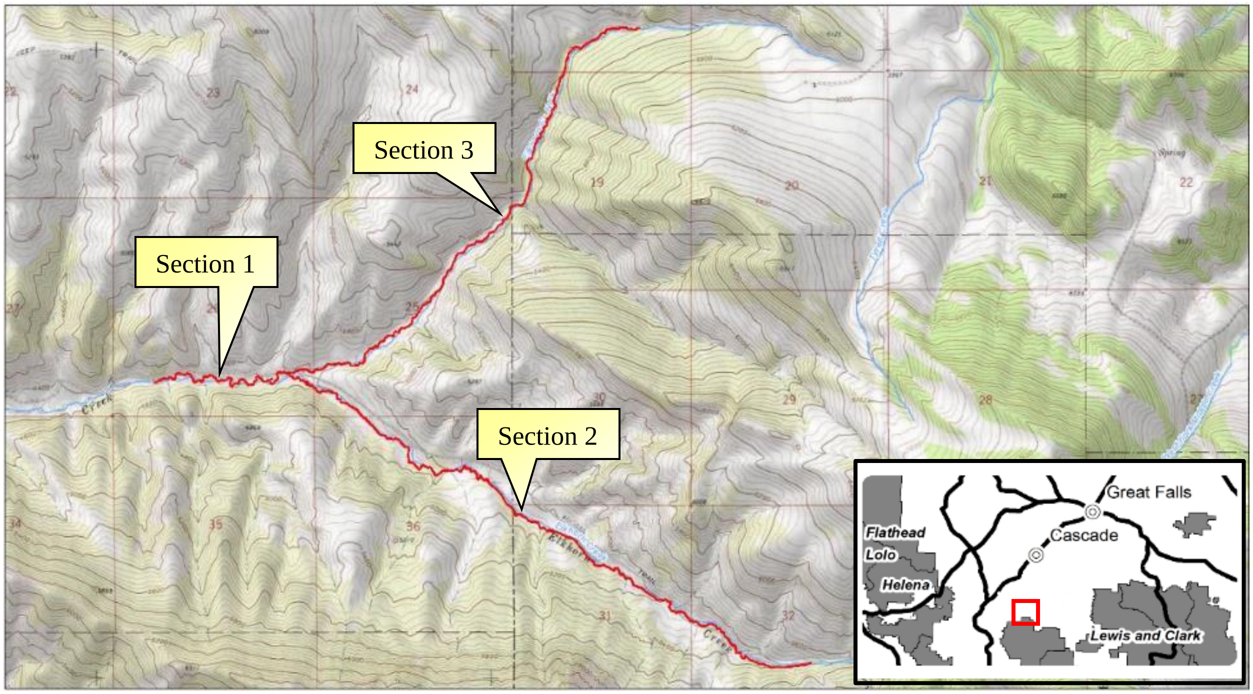

Figure 42. Elkhorn Creek in the Upper Missouri River subbasin. The stream segments delineated in red indicates the area occupied by WCT.

Background

Elkhorn Creek is a tributary of Willow Creek in the Upper Missouri River subbasin located on the Beartooth Wildlife Management Area. In 1972, FWP constructed a gabion fish barrier in the lower reaches of Elkhorn Creek. Rotenone was used to remove rainbow-cutthroat hybrids from approximately three miles of stream above the constructed barrier. The treated reach of stream naturally re-colonized from a source of nonhybridized WCT remaining in the headwaters of Elkhorn Creek. Genetic samples collected in 1996 indicated that the Elkhorn Creek population was still nonhybridized. In 2002, genetic samples indicated a recent hybridization event had occurred, likely because of a failure of the gabion fish barrier.

In 2012, a concrete fish barrier was constructed upstream of the original barrier location in an area with more suitable channel morphology. Highly hybridized WCT were suppressed in the lower reaches of Elkhorn Creek above the new barrier from 2012-2014. Following mechanical removals, nonhybridized WCT from Threemile Creek (Upper Missouri subbasin) were restored by means of RSIs from 2014-2016 to genetically swamp the lower reach of the stream.

2022 Monitoring

Demographic monitoring of the Elkhorn Creek WCT population was performed on October 18th-20th, 2022. Three 100 m population estimate sections were established: two on the mainstem Elkhorn Creek and one on the North Fork Elkhorn Creek. Multiple pass depletion methods were used to estimate population abundance. Estimated abundance averaged 177 fish/km across the three sites sampled. A total WCT population of 2186 (±165) is estimated to be present in Elkhorn Creek if extrapolated to the entire area occupied above the concrete fish barrier (Figure 43).

Elkhorn Creek —NATIVE TROUT POPULATION SURVEY

1. General Information— Date: **October 18th-20th, 2022** Biologist: **A. Poole**
2. Stream Information—
   Name, section, county: **Elkhorn Creek, 25, 26, 31, Lewis and Clark**
3. Survey Site Information (see attached map)—
   Upstream range of native trout (general description and GPS):
   Downstream range of native trout (general description and GPS): **Concrete fish barrier (46.93615, -111.82799)**
   Location (GPS) and description of barriers: **Concrete fish barrier (46.93615, -111.82799)**
   Stream Length—Occupied habitat: **12.35 km (7.67 mi)** Available habitat: **21.97 km[1] (13.65 mi)**
   Survey method & equipment: **backpack battery electrofisher; two-pass depletion**
   Survey sites (general description and UTM)—
   Section 1: **Below forks; 46.93660, -111.82197**
   Section 2: **South fork; 46.92696, -111.78838**
   Section 3: **North fork; 46.94568, -111.79310**

Parameter	**Section 1**	**Section 2**	**Section 3**
Section length (m)	100 m	100 m	100 m
Mean stream width (m) (n)	2.08 m (10)	1.45 m (10)	1.35 m (10)
Section area (hectares)	0.021 ha	0.015 ha	0.013 ha
WCT			
Removal Pattern	12 1	18 11 2	7 1
Population estimate	13 (±1)	32 (±2)	8 (±1)
Capture probability	0.929	0.633	0.857
Mean length (mm) (n)	184 (13)	107 (31)	116 (8)
Mean weight (g) (n)	61 (13)	10 (31)	16 (8)
Mean KTL (n)	0.83 (13)	0.77 (31)	0.83 (8)
Number fish per km (95 % CI)	130 (±10)	320 (±20)	80 (±10)
Number fish per ha (95 % CI)	619 (±48)	2,133 (±134)	615 (±77)
Biomass (kg per ha) (95 % CI)	38 (±3)	21 (±)	10 (±1)

4. Comments:

[1] – Includes 9.62 km (5.98 mi) of habitat between Willow Creek and concrete fish barrier.

Other species present: Rocky Mountain sculpin at sections 1-2.

Figure 43. Elkhorn Creek fish population estimate results.

Gold Creek

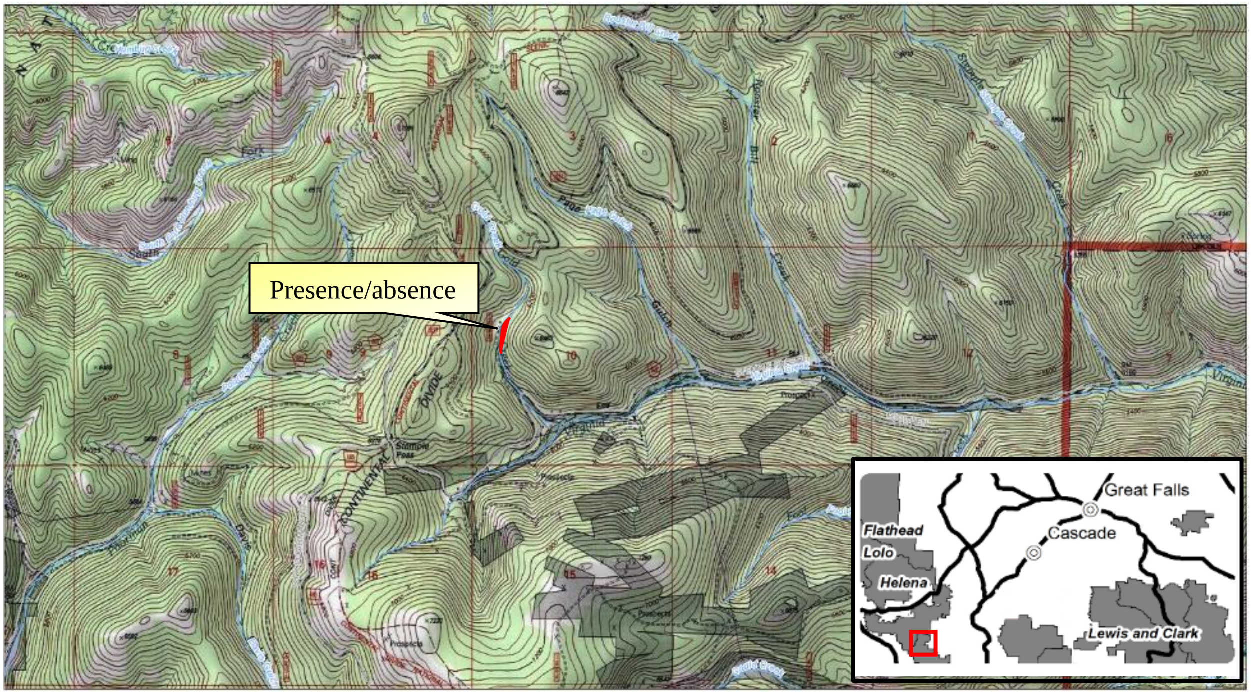

Figure 44. Gold Creek in the Upper Missouri River subbasin. The stream segment delineated in red indicates the area sampled in 2022.

Background

Gold Creek is a tributary of Virginia Creek in the Upper Missouri River subbasin located approximately 24 miles northeast of Helena, MT. No previous collection records for this waterbody could be found. Several nearby streams contain conservation populations of WCT (Trout, Stemple, Rooster Bill, Fool Hen, Page Gulch, and Virginia Creeks).

2022 Monitoring

Gold Creek was surveyed om July 13th, 2022, to determine WCT presence/absence in the drainage. A 143 m reach of Gold Creek was backpack electrofished near of the end of FS RD 1888. Two brook trout were collected in the area sampled and no WCT were detected. Stream flow was very low and fish habitat appeared marginal.

Gould Creek

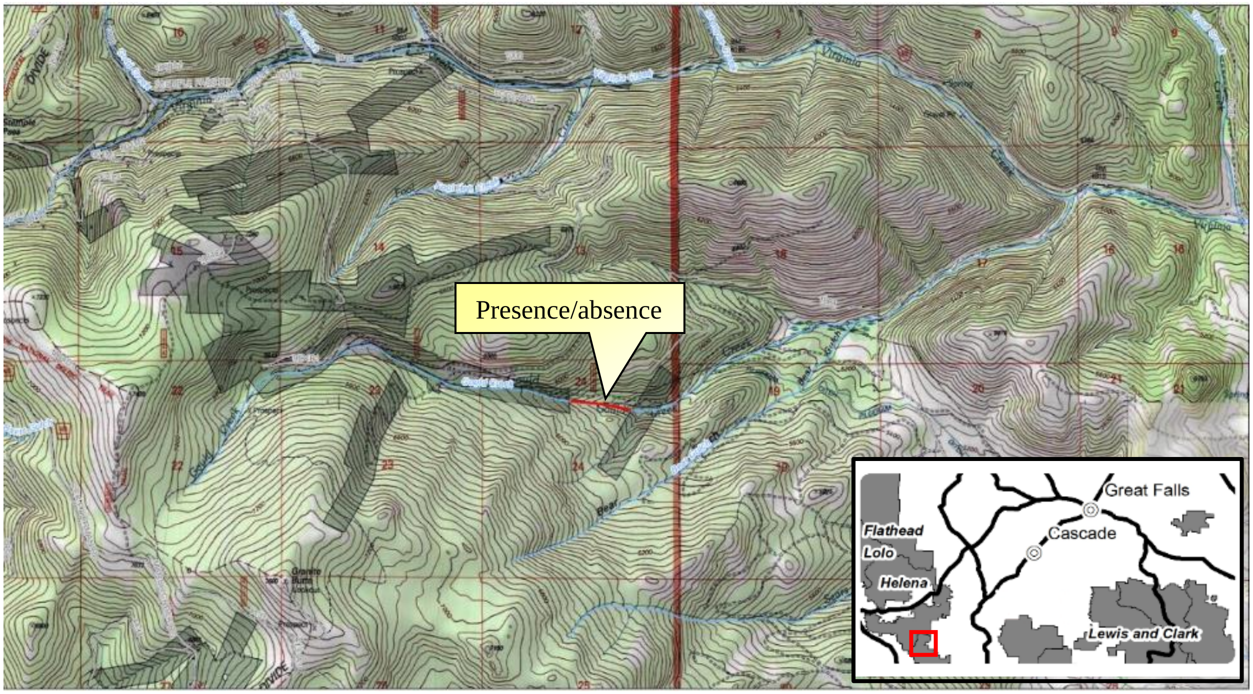

Figure 45. Gould Creek in the Upper Missouri River subbasin. The stream segment delineated in red indicates the area sampled in 2022.

Background

Gould Creek is a tributary of Virginia Creek in the Upper Missouri River subbasin located approximately 24 miles northeast of Helena, MT. No previous collection records for this waterbody could be found. Several nearby streams contain conservation populations of WCT (Trout, Stemple, Rooster Bill, Fool Hen, Page Gulch, and Virginia Creeks).

2022 Monitoring

Gould Creek was surveyed om July 13th, 2022, to determine WCT presence/absence in the drainage. A 362 m reach of Gould Creek was backpack electrofished on the Helena National Forest above the Grady Ranch. A total of 29 brook trout were collected in the area sampled and no WCT were detected. The Gould Creek watershed was impacted by the 2010 Davis Fire resulting in a channel filled with large woody debris in the reach sampled. WCT may persist in downstream reaches below the Forest Service boundary.

Page Gulch

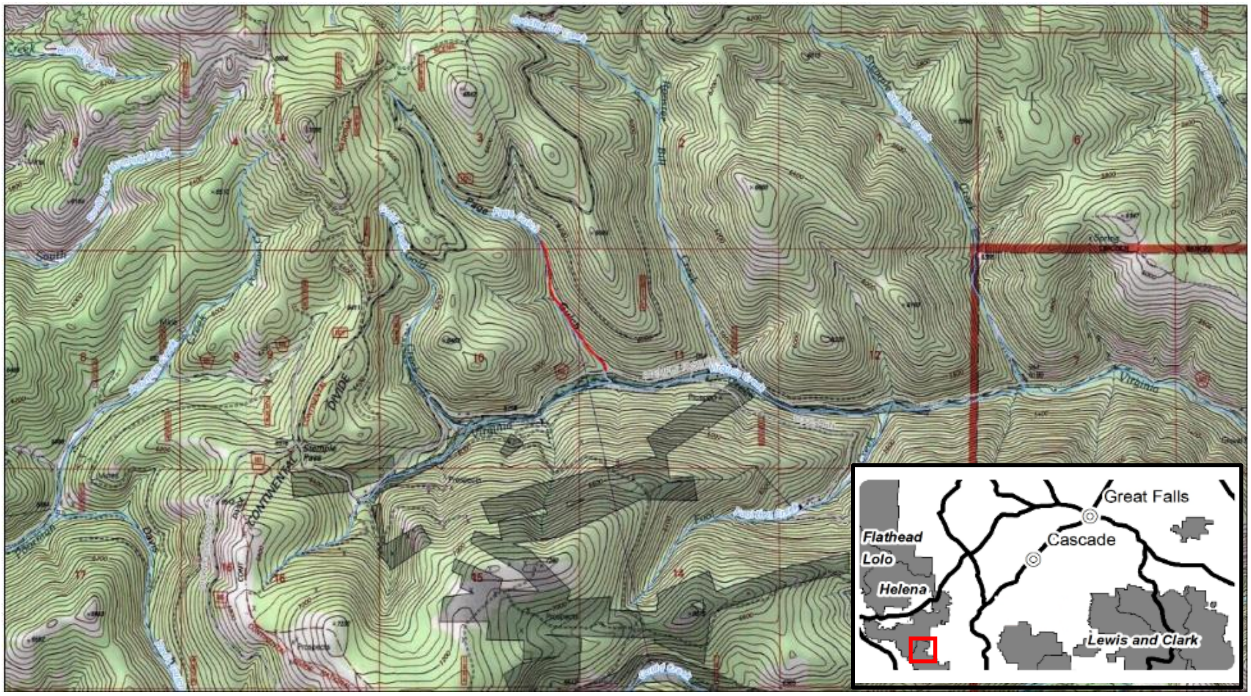

Figure 46. Page Gulch in the Upper Missouri River subbasin. The stream segment delineated in red indicates the area occupied by WCT.

Background

Genetic monitoring of WCT collected in Page Gulch in 1997 indicated a nonhybridized population was present (n=6). Updated genetic samples collected in 2021(n=20) found clear evidence of rainbow trout hybridization (Appendix A). 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); potentially F2 hybrids that backcrossed with WCT. 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, which suggests that Page Gulch is not likely to harbor either a core or conservation population within one or two generations.

2022 Monitoring

A genetic rescue project was initiated on Page Gulch in the summer of 2022. The entire fish bearing reach of the creek was backpack electrofished on August 22-23rd, 2022, in an effort to PIT tag and collect genetic samples from all WCT greater than 70 mm in length. A total of 93 WCT were tagged over the two-day sampling period. Genomic analysis of the 93 fish tagged will inform future efforts on the transfer of nonhybridized individuals to fishless habitat elsewhere. Additionally, 34 brook trout were collected in Page Gulch and submitted for fish health inspection. Results of the fish health inspection detected *Myxobolus cerebralis*, the parasite that causes whirling disease, in the brook trout submitted.

Pike Creek

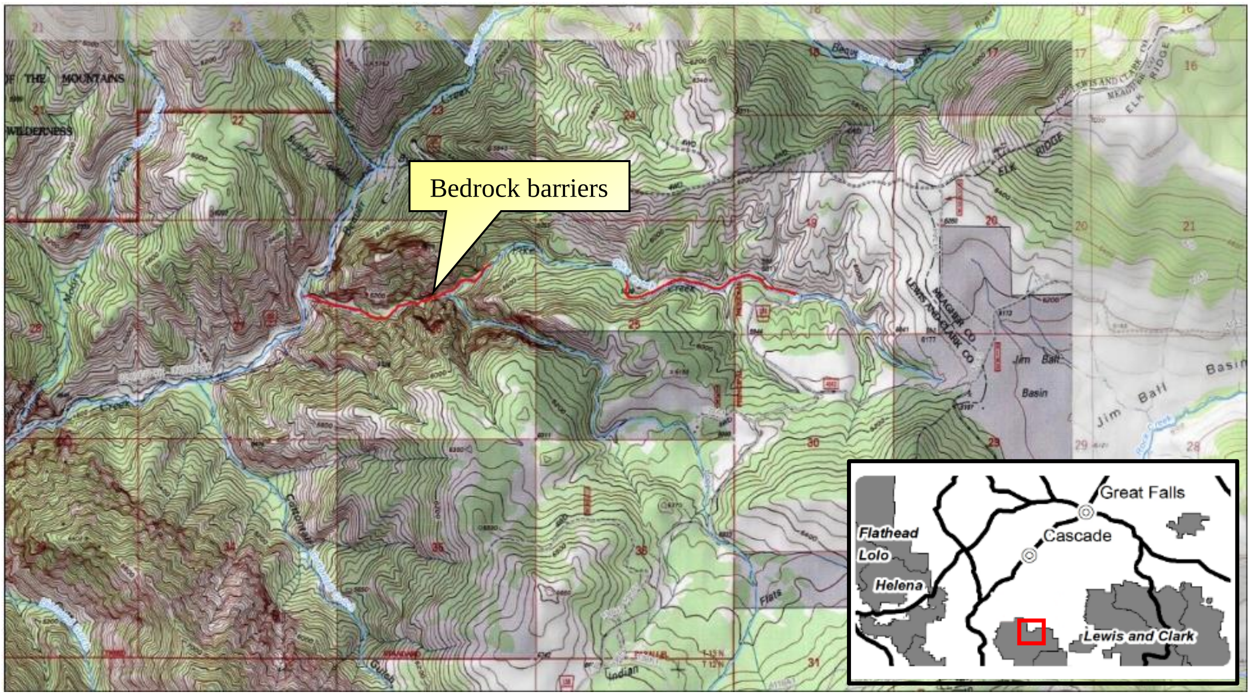

Figure 47. Pike Creek in the Upper Missouri River subbasin. The stream segments delineated in red indicate the areas sampled in 2022.

Background

Pike Creek is a tributary of Beaver Creek in the Upper Missouri River subbasin located approximately 9 miles northeast of the community of York, MT. No previous collection records exist for this locality.

2022 Monitoring

Pike Creek was surveyed on June 21st, 2022, for presence of fish barriers and presence/absence of fish. A 1.1-mile reach of Pike Creek was backpack electrofished and no fish were collected or observed. Three bedrock fish barriers were located in the reach of Pike Creek surveyed. A temperature logger was launched at 46.85898, -111.68928 to determine if Pike Creek could support a WCT population in the future.

Pike Creek was revisited on August 29th, 2022, to further evaluate fish habitat in the upper drainage. Very low flow was observed in the upper 1-mile reach surveyed below the FS RD 138 crossing. The temperature logger was retrieved from the lower reach and was found to be dry. Temperature data revealed that the stream likely went dry around July 21st, 2022. Pike Creek appears to lack suitable habitat to support a WCT population.

Tenmile Creek

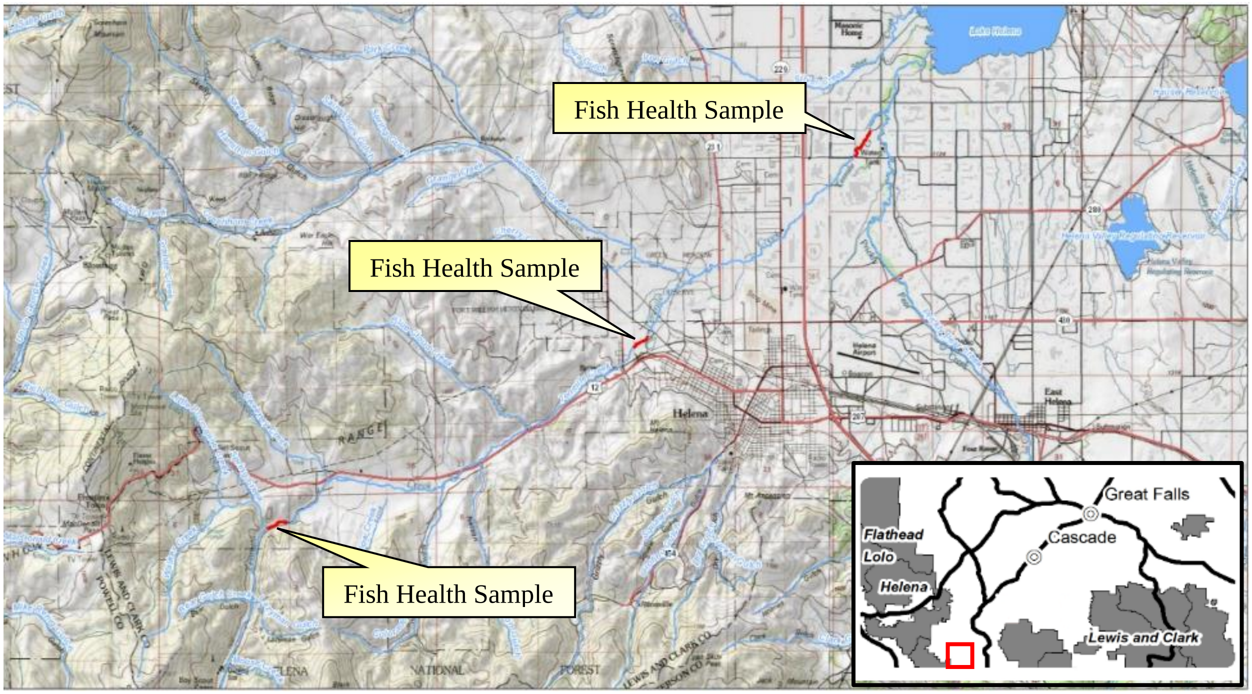

Figure 48. Tenmile Creek in the Upper Missouri River subbasin. The stream segments delineated in red indicate the areas sampled in 2022.

Background

Tenmile Creek is a tributary of Prickly Pear Creek in the Upper Missouri River subbasin. The upper watershed consists of steep, forested mountain slopes while the lower watershed is made up of prairie and residential and retail development in and near the city of Helena, MT. The Tenmile Creek drainage serves as a source of drinking water for the City of Helena. Although impacted by a long history of hard rock mining, Tenmile Creek currently supports a coldwater fishery consisting mainly of rainbow, brook, and brown trout.

2022 Monitoring

Two fish health inspections were performed on Tenmile Creek on October 25th-26th, 2022, to better understand the risks associated with a potential wild fish transfer of WCT from Page Gulch to Beaver Creek. Two 60 fish samples were collected: one from the upper watershed (1st Rimini Road crossing) and one combined sample from two localities in the lower watershed (Sierra Road crossing and Tenmile Creek Park). *M. cerebralis*, the parasite that causes whirling disease, was detected in a both samples.

Virginia Creek

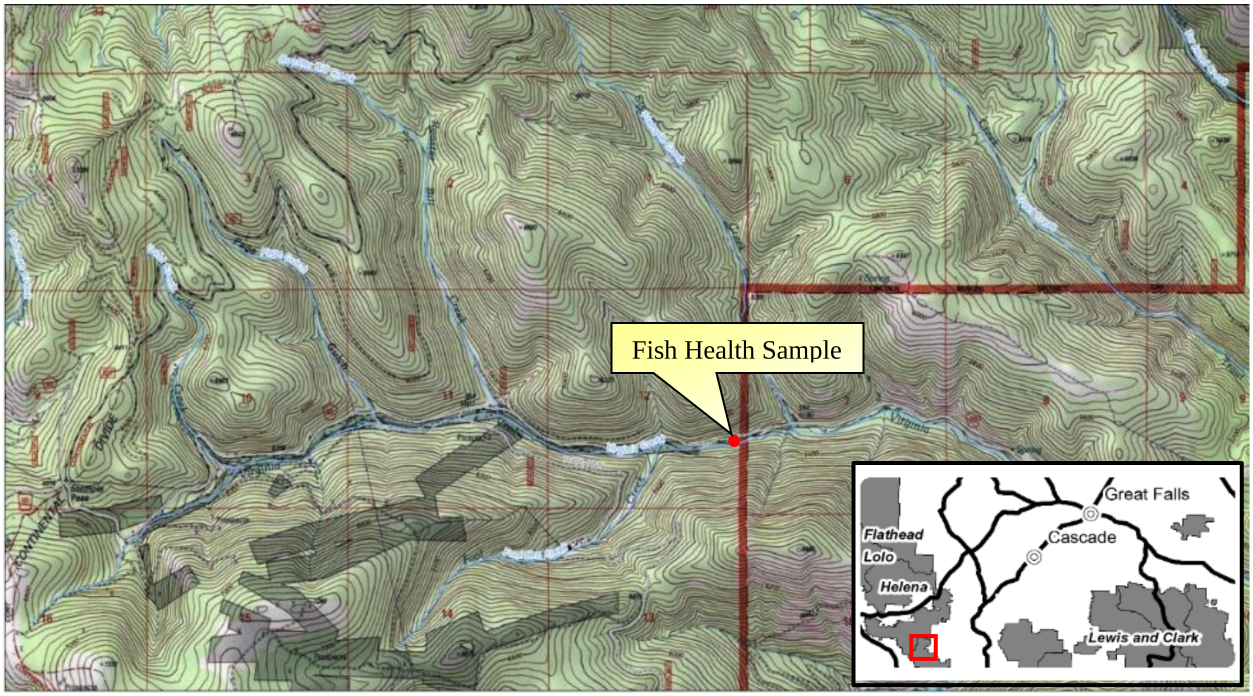

Figure 49. Virginia Creek in the Upper Missouri River subbasin. The stream segment delineated in red indicates the area sampled in 2022.

Background

Virginia Creek is a tributary of Canyon Creek in the Upper Missouri River subbasin located approximately 24 miles northeast of Helena, MT. No previous fish collection records for this locality could be found.

2022 Monitoring

Virginia Creek was sampled on August 22nd, 2022, to collect additional fish for the Page Gulch fish health inspection. A 140 m reach of Virginia Creek was backpack electrofished starting at the Helena National Forest service boundary. A total of 11 brook trout and 14 rainbow and rainbow-cutthroat trout hybrids were collected in this effort. Rocky Mountain sculpin was also observed. Results of the fish health inspection detected *Myxobolus cerebralis*, the parasite that causes whirling disease, in the brook and rainbow trout collected from Virginia Creek.

IX. Upper Missouri-Dearborn River Subbasin

Big Coulee Creek

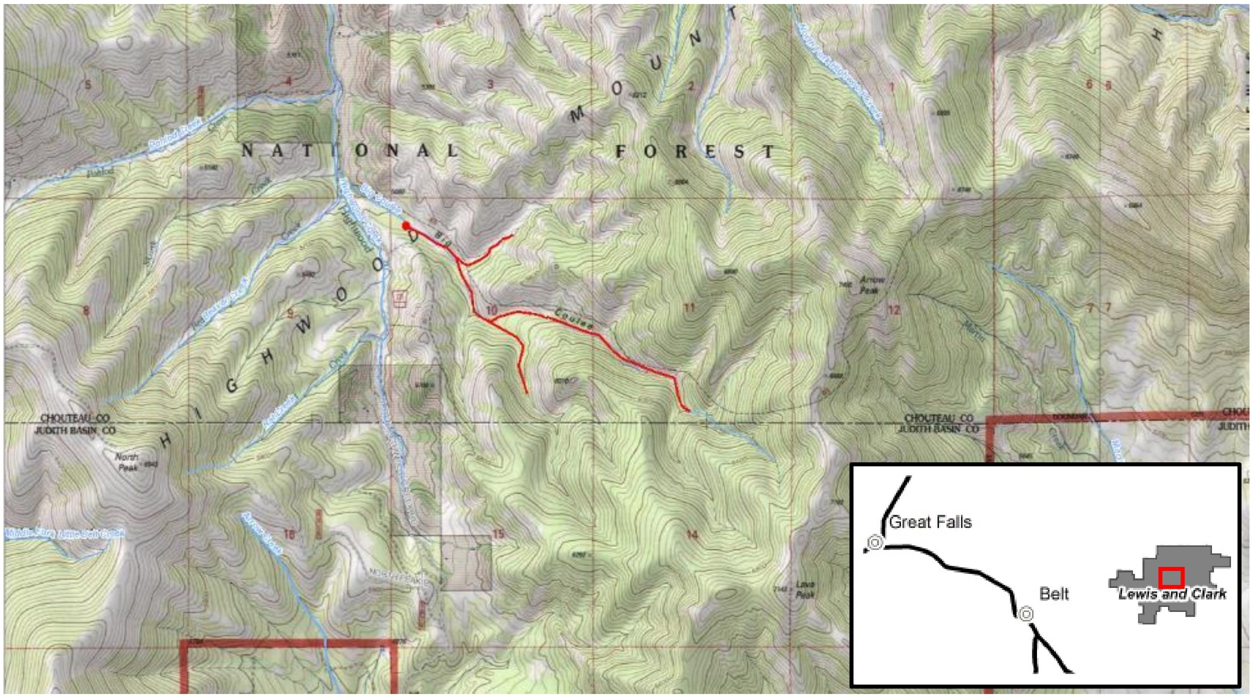

Figure 50. Big Coulee in the Upper Missouri-Dearborn River subbasin. The stream segments delineated in red indicate the areas occupied by nonhybridized WCT.

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 2020, approximately 671 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.

2022 Monitoring

WCT population monitoring was performed on 1.76 miles of Big Coulee from August 8th-15th, 2022. Eight sections of Big Coulee were two-pass backpack electrofished and an additional ninth section was electrofished in a single pass effort. A total of 2,459 WCT were collected in the 2022 monitoring effort

(Table 1). No brook trout were detected in 2022, the first time in seven years. 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	**Section 2**	**Section 3**	**Section 4**	**Section 5**	**Section 6**	**Section 7**	**Section 8**	**Section 9**
Pass 1	142 WCT	154 WCT[1]	349 WCT	325 WCT	102 WCT	201 WCT	208 WCT	261 WCT	303 WCT
Pass 2	67 WCT	42 WCT[1]	42 WCT	40 WCT	41 WCT	40 WCT	59 WCT	83 WCT	

[1] – Total catch reported (n=196). Pass 1 and 2 catch estimated from average capture probability of other sections (p=0.725).

Wegner Creek

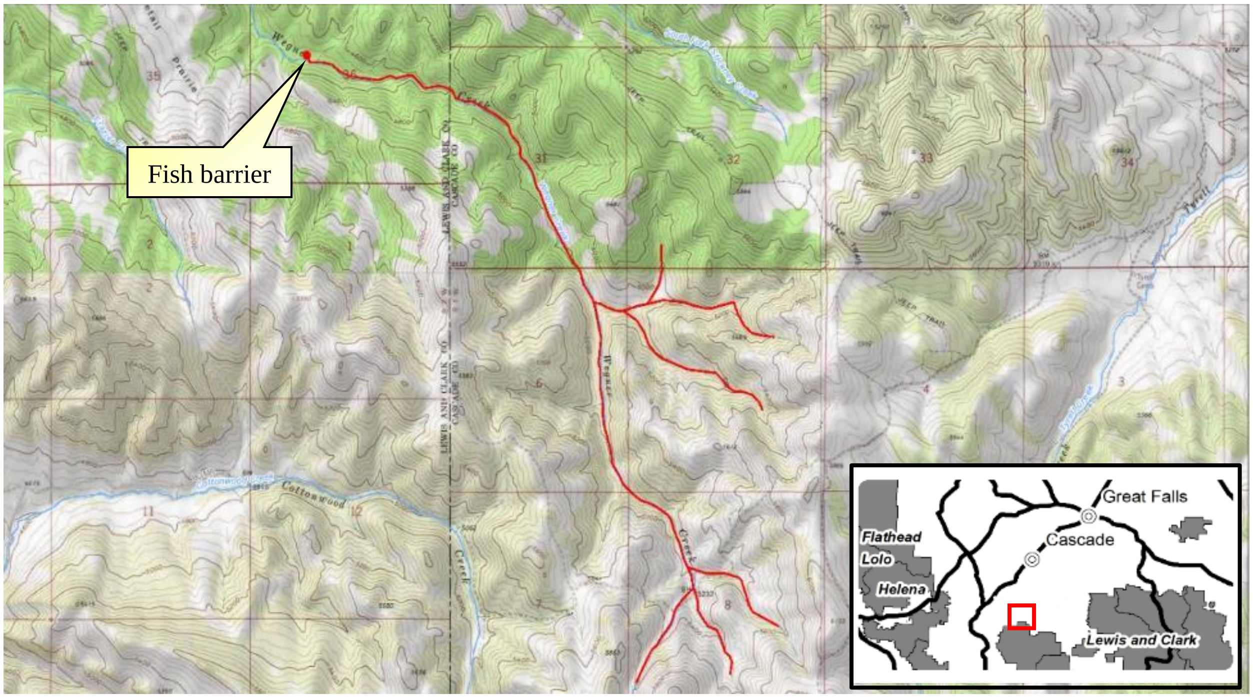

Figure 51. Wegner Creek in the Upper Missouri-Dearborn River subbasin. The stream segments delineated in red indicate potential WCT restoration area.

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.

2022 Monitoring

Wegner Creek was surveyed on May 5th and 9th, 2022 to evaluate the efficacy of the fish barrier. On May 5th, 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 9th, 2022, a 150 m reach below the barrier was electrofished in a marking effort to evaluate barrier efficacy. A total of 68 Brook Trout were adipose clipped and released below the barrier.

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Appendix A

Date: May 20, 2022

Biologist(s): Alex Poole, Adam Strainer, Katie Vivian, Jason Mullen, Clint Smith

Location(s) and sampling date:

1. Page Gulch (46.8987055, -112.46265; 10/19/21)
2. Fool Hen Creek (46.8902, -112.43862; 07/29/20)
3. Rooster Bill Creek (46.90223, -112.45591; 08/18/20)
4. Belt Creek (46.87009, -110.66866; 07/01/21)
5. Logging Creek (47.03336, -111.06470; 07/07/21)
6. Weatherwax Creek (46.83610, -110.64814; 07/22/21)
7. Pickfoot Creek (46.57937, -111.24504; 05/27/21)
8. Moudess Creek (47.36566, -112.69059; 08/03/21)
9. Green Gulch (47.82989, -112.75393; 07/20/21)
10. Threemile Creek (46.69673, -112.18244; 0624/21)

Agency: Montana Fish Wildlife and Parks

Target species: Westslope cutthroat trout

Authors: Ryan Kovach, Sally Painter, Angela Lodmell

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

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

RESULTS, DISCUSSION, AND RECOMMENDATIONS

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

Sample	ID	N	Taxa	% WCT	% RBT	% YCT
Page Gulch	5419	20	WCT x RBT	94.0	6.0	
Fool Hen Creek	5420	20	WCT x RBT x YCT	93.7	6.3	

Rooster Bill Creek	5421	20	WCT x RBT x YCT	90.6	9.2	0.2
Belt Creek	5422	30	WCT x YCT	99.0	1.0	
Logging Creek	5423	20	WCT x RBT x YCT	70.0	28.9	1.1
Weatherwax Creek	5424	25	WCT x RBT x YCT	95.2	4.6	0.2
Pickfoot Creek	5425	25	WCT x RBT x YCT	94.3	0.3	5.4
Moudess Creek	5426	25	WCT x RBT x YCT	95.1	3.8	1.1
Green Gulch	5427	25	WCT x RBT	98.9	1.1	
Threemile Creek	5428	20	WCT	100.0		

Page Gulch

We detected rainbow trout alleles at eight rainbow diagnostic markers and eleven westslope diagnostic markers were polymorphic. There was clearly rainbow trout hybridization present in this sample from Page Gulch. We did not detect any Yellowstone cutthroat trout alleles.

Rainbow trout alleles were non-randomly distributed among individuals in the sample ($P < 0.001$). Instead, there was a clear bimodal pattern where many individuals appeared to be non-hybridized westslope cutthroat trout (~45% of the sample), and a fairly large percentage of individuals that had ~28% rainbow trout ancestry (25% of the sample); those individuals may be F2 hybrids that backcrossed with a westslope cutthroat trout. The remaining individuals had intermediate rainbow trout ancestry (Fig. 1). This pattern of individual rainbow trout ancestry could arise due to non-random mating between westslope cutthroat trout and rainbow trout hybrids, or because of recent immigration of rainbow trout or hybrids into the population. In this case, we strongly favor the alternative explanation because the data are highly consistent with expectations if hybridization were recent, and consistent with that hypothesis, a previous sample from the population (albeit small) failed to detect any rainbow trout ancestry (#1899).

These data have several implications for the westslope population in Page Gulch: (1) it is obviously at extremely high risk of genomic extinction within the very immediate future; and (2) there appears to be high immigration of rainbow trout genes into this population, which suggests that Page Gulch is not likely to harbor either a core or conservation population within one or two generations. The latter finding also suggests that this population is connected to some rainbow (or hybrid) source population. At this stage, it is possible to “rescue” the remaining non-hybridized fish in the population (i.e., tagging them with PIT tags, using genomics to obtain precise estimates of rainbow trout ancestry, re-capturing, and moving them to another location).

If that is not possible, it would be unwise to invest additional resources into this population as it will likely have very little conservation value in the very near future. At present and assuming random mating in the future, the population will be ~94% westslope cutthroat trout, and 6% rainbow trout; however, we expect the percent rainbow trout ancestry to increase rapidly.

Fool Hen Creek

Genetic results in Fool Hen Creek were similar to those in Page Gulch, a result that is not unexpected given their geographic proximity to one another. We detected rainbow trout alleles at ten rainbow trout diagnostic markers, and six westslope markers were polymorphic. We did not detect any Yellowstone alleles. Clearly, there was rainbow trout ancestry present in the sample.

Rainbow trout alleles were non-randomly distributed among individuals in the sample ($P < 0.001$) from Fool Hen Creek. There was a slight excess of what appeared to be non-hybridized westslope cutthroat trout, and some hybrids with slightly higher rainbow trout ancestry than expected (Figure 2). However, there were many individuals with low levels of rainbow trout ancestry, which suggests that hybridization has been occurring for multiple generations. Indeed, genomic data (collected for a separate effort) demonstrate that all fish in this sample have rainbow trout ancestry (i.e., non-hybridized westslope are no longer present in Fool Hen Creek).

Assuming Fool Hen Creek is physically connected to a source of rainbow trout or their hybrids, it is reasonable to assume that non-native ancestry will increase over time, but with no previous data, it is unclear whether hybridization dynamics are stable or changing in this population. At present, the population is composed of 93.7% westslope cutthroat trout ancestry, and 6.3% rainbow trout ancestry.

Rooster Bill Creek

Genetic results in Rooster Bill Creek were like both Page Gulch and Fool Hen Creek (nearby streams). We detected rainbow trout alleles at eighteen rainbow diagnostic markers, and nineteen westslope diagnostic markers were polymorphic. We also detected a single Yellowstone cutthroat trout allele. Genomic data from the same samples (collected for a different purpose) confirmed that there was a very small amount of Yellowstone cutthroat trout ancestry in Rooster Bill Creek (~0.2%), in addition to a relatively large amount of rainbow trout ancestry.

Rainbow trout alleles were non-randomly distributed among individuals in the sample ($P < 0.001$) from Rooster Bill Creek. Relative to an expected distribution of rainbow trout ancestry in a randomly mating population (Fig. 3), there was a substantial excess of fish that appeared to be non-hybridized westslope cutthroat trout, and five individuals with fairly high rainbow trout ancestry (>20%). This strongly suggests there has been a recent immigration of rainbow trout hybrids into Rooster Bill Creek, a hypothesis that is consistent with past sampling in 1994 that failed to detect any rainbow trout alleles (#907). Clearly, the genetic status of Rooster Bill Creek has changed markedly since that time.

Further analysis of the genomic data demonstrated that only one of the fish was a non-hybrid. As such, the population is rapidly approaching or has already achieved genomic extinction; percent rainbow trout ancestry (assuming random mating in the future) is approximately 9.2% and it appears very likely this number will increase in the immediate future.

Belt Creek

We did not detect any rainbow trout alleles, but we did detect Yellowstone alleles at four Yellowstone markers, and three westslope diagnostic markers were polymorphic. These data provide very strong evidence that the fish in Belt Creek have some Yellowstone cutthroat trout ancestry.

Yellowstone cutthroat trout alleles were randomly distributed among individuals in the sample (P = 0.056), suggesting the sample was collected from a hybrid swarm between Yellowstone cutthroat trout and westslope cutthroat trout, with a relatively small percentage of Yellowstone cutthroat trout ancestry (1.0%). Previous samples from upper Belt Creek (#1010, 1317) did not detect any Yellowstone cutthroat trout ancestry. This could suggest that Yellowstone ancestry may have recently increased, or we simply failed to detect Yellowstone cutthroat trout in the previous samples. In this case, we strongly favor the latter interpretation because (1) Yellowstone cutthroat trout alleles are randomly distributed among individuals (this would not be case if hybridization was recent) and (2) we had a fairly high chance of failing to detect <1% Yellowstone cutthroat trout ancestry given previous sample sizes (at least 5% under optimistic scenarios but realistically the percent chance is higher, perhaps considerably so).

Logging Creek

We detected rainbow trout alleles at all rainbow trout diagnostic markers, all westslope diagnostic markers were polymorphic, and we also detected Yellowstone alleles at two Yellowstone diagnostic markers. These data provide definitive evidence that fish in Logging Creek have both rainbow trout and Yellowstone cutthroat trout ancestry.

Rainbow trout alleles were non-randomly distributed among individuals ($P < 0.001$), as individual ancestry varied widely from fish with little to no rainbow trout ancestry, to fish with as much as ~83% rainbow trout ancestry (Fig. 4). Yellowstone cutthroat trout alleles were found at far lower frequency but were randomly distributed among individuals (P = 0.271). Overall, these results are substantially different than previous results from the same section of the Creek (#371); in that sample, collected in 1989, we did not detect any rainbow trout or Yellowstone cutthroat trout alleles. Given that only five samples were collected in 1989, it is unclear if the substantial difference between this sample and the previous sample is due to sampling limitation (power) or a major change in the hybridization status of this population. We suspect that both are true, and on some level, it is a moot point given the genetic status of the population; all individuals in this sample were hybrids, and assuming the population mates randomly moving forward it will be composed of 1.1% Yellowstone cutthroat trout, 28.9% rainbow trout, and 70.0% westslope cutthroat trout ancestry.

Weatherwax Creek

We detected rainbow trout alleles at eighteen rainbow diagnostic markers, and nineteen westslope diagnostic markers were polymorphic. We also detected Yellowstone alleles at two Yellowstone diagnostic markers. Together, the data demonstrate that there are fish in Weatherwax Creek with rainbow trout ancestry, and very likely, a small amount of Yellowstone ancestry. We only detected three Yellowstone alleles, and as such, it is not possible to do any further analysis. With the data in hand, it appears the population may have ~0.2% Yellowstone cutthroat trout ancestry.

Rainbow trout alleles were non-randomly ($P < 0.001$) distributed among the individuals in the sample. Relative to random expectation, there was a substantial excess of fish with little or no rainbow trout ancestry, two individuals with fairly high rainbow trout ancestry (>20%), and the remainder of fish had relatively low rainbow trout ancestry (<10%) (Fig. 5). This pattern suggests there is non-random mating between non-hybridized westslope cutthroat trout and hybrids, or there has been a recent invasion of hybrids into the stream. Historical samples collected in 2003 strongly support the latter interpretation, as we failed to detect any rainbow trout or Yellowstone cutthroat trout alleles at that time (#3035). This was unlikely to be the result of sampling limitations, as we had high power to detect similar amounts of rainbow trout ancestry (>99.9% chance of detecting a similar amount of rainbow ancestry). At present, the genetic makeup of the population (assuming random mating moving forward) is 95.2% westslope cutthroat trout, 4.6% rainbow trout, and 0.2% Yellowstone cutthroat trout.

Overall, the population of westslope cutthroat trout in Weatherwax is very clearly at high risk of extirpation given the current genetic trajectory of this population. At present it may be possible to “rescue” putatively non-hybridized individuals, otherwise, this population may have little to no conservation value in the very near future.

Pickfoot Creek

In the sample from Pickfoot Creek we detected rainbow trout alleles at one rainbow diagnostic marker, Yellowstone cutthroat trout alleles at seven Yellowstone diagnostic markers, and eight westslope markers were polymorphic. Therefore, there is clear evidence of rainbow trout and Yellowstone cutthroat trout hybridization in Pickfoot Creek. We only detected three rainbow trout alleles, and as such, it is not possible to do any further analysis with those data; it appears the population may have ~0.3% rainbow trout ancestry.

Yellowstone cutthroat trout alleles were randomly distributed among the fish in the sample ($P = 0.988$), which indicates that the sample was collected from a hybrid swarm between westslope cutthroat trout and Yellowstone cutthroat trout. Overall, the fish population in Pickfoot Creek has 94.3% westslope cutthroat, 0.3% rainbow trout, and 5. 4% Yellowstone cutthroat trout ancestry. Given that non-native alleles were broadly and randomly distributed among individuals, we suspect that hybridization dynamics are relatively static in this stream, but given that this is the first sample from this location, we have no data to confirm/refute that hypothesis.

Moudess Creek

We detected rainbow trout alleles at six rainbow diagnostic markers, Yellowstone cutthroat trout alleles at two Yellowstone diagnostic markers, and eight westslope diagnostic markers were polymorphic. The fish in Moudess Creek are hybrids between westslope, rainbow trout, and Yellowstone cutthroat trout.

Rainbow ($P = 0.055$) and Yellowstone cutthroat trout ($P = 0.068$) alleles were randomly distributed among individuals, suggesting the samples was collected from a hybrid swarm between westslope, rainbow, and Yellowstone cutthroat trout. Overall, the fish population in Moudess Creek has 95.1% westslope cutthroat, 3.8% rainbow trout, and 1.1% Yellowstone cutthroat trout ancestry. These results are quite consistent with a past sample from the same section of Moudess Creek (#1183). They are, however, different than another sample collected slightly upstream in the Moudess Creek watershed; in that sample (#4691), we did not detect any rainbow or Yellowstone cutthroat trout ancestry. This may suggest that a non-hybridized population does exist in the headwaters of Moudess Creek, but at present, we can say with certainty that there are few if any remaining non-hybridized westslope cutthroat trout in this particular section of Moudess Creek.

Green Gulch

In the sample from Green Gulch, we detected rainbow trout alleles at three rainbow trout diagnostic markers, and five westslope diagnostic markers were polymorphic. We did not detect any Yellowstone cutthroat trout alleles. Therefore, there was evidence of rainbow trout hybridization in Green Gulch.

Rainbow trout alleles were randomly distributed among individuals in the sample ($P = 0.149$), which suggests the sample was collected from a hybrid swarm between rainbow trout and westslope cutthroat trout. The percent rainbow trout ancestry in the hybrid swarm was 1.1. A previously sample from this section of Green Gulch (#804) did not detect any rainbow trout alleles, but rainbow trout hybridization has been detected in lower sections of Green Gulch (#3103). This may suggest there has been an upstream expansion of rainbow trout hybridization, or we failed to detect rainbow trout genetic ancestry in the previous sample (i.e., 804). With such a small sample size ($n = 8$) in the previous sample, we had a fairly high probability (0.36) of failing to detect the same amount of rainbow trout ancestry as observed in this sample. Thus, it seems quite likely that sampling limitations may explain the lack of rainbow trout alleles in the previous sample. However, we cannot exclude the possibility that hybridization is recent. Under the latter scenario, some non-hybridized fish may still be present. If there was interest in identifying and moving those individuals, we could re-run this sample using genomic methods to determine whether there are any non-hybridized fish in this collection, and thus, any future opportunities to salvage non-hybrids in Green Gulch.

Threemile Creek

We did not detect any rainbow trout or Yellowstone cutthroat trout alleles, and none of the westslope diagnostic markers were polymorphic. This is consistent with many previous samples from Threemile Creek (#2737, 3102, 3777, 4368, 4512, and 4664). Together the data provide very strong evidence that Threemile Creek contains a non-hybridized population of westslope cutthroat trout.

We used genotypes at the westslope cutthroat trout polymorphic loci to describe patterns of genetic variation in the sample. Genotypic proportions conformed to Hardy-Weinberg expectations (Fisher's combined $P = 0.999$), suggesting that the sample was collected from a random mating population. The average expected heterozygosity in the sample was 0.098 and the proportion of polymorphic loci was 0.281 (Table 2). These results are very similar to previous estimates of genetic variation in Threemile

Creek based on samples collected in 2013 and 2014; at that time our estimate for the expected heterozygosity was 0.086 and the proportion of polymorphic loci was 0.313. The stability of genetic variation over this temporal window (7-8 years) suggests that the genetic effective population size is not extremely small (i.e., we would have only detected significant changes if the effective size was very small). More broadly, these results are very similar to the mean values observed for other non-hybridized populations of westslope cutthroat trout in in the Missouri River basin; the average expected heterozygosity is 0.104 and the mean proportion of polymorphic loci is 0.340 (Table 2). At present, this population is not an immediate candidate for genetic rescue efforts. However, it may be worth considering human-assisted gene flow at a future date.

TABLES

Table 2. Average expected heterozygosity (He), proportion of polymorphic markers (Poly), watershed, barrier type, and sample size for westslope cutthroat trout populations in the Missouri River drainage.

Population	Watershed	Barrier	N	He	Poly
Alkali	Beaverhead	Natural - waterfall	50	0.124	0.438
Brays Canyon	Beaverhead	Anthro - demographic	105	0.062	0.281
Cottonwood	Beaverhead	Natural - waterfall	111	0.035	0.125
Jake Canyon	Beaverhead	Anthro - demographic	25	0.064	0.156
Left Fork Stone	Beaverhead	Anthro - mine	36	0.121	0.406
Reservoir	Beaverhead	Natural - intermittent	75	0.063	0.250
Upper Buffalo	Beaverhead	Anthro - demographic	25	0.098	0.313
White Creek	Beaverhead		12	0.226	0.625
Carpenter	Belt	Anthro - mine	35	0.175	0.516
Crawford	Belt	Natural - waterfall	54	0.123	0.438
Gold Run	Belt	Anthro - mine	69	0.092	0.313
Graveyard	Belt	Natural - waterfall	24	0.131	0.344
N. Fork Little Belt	Belt	Natural - waterfall	50	0.122	0.438
American	Big Hole	Anthro - dam	29	0.159	0.531
Bear	Big Hole	Anthro - demographic	16	0.121	0.375
Bender	Big Hole	Anthro - demographic	36	0.034	0.125
Blind Canyon	Big Hole	Anthro - demographic	25	0.054	0.125
Hell Roaring	Big Hole	Natural - cascade	18	0.018	0.097
Little American	Big Hole	Anthro - demographic	30	0.056	0.258
Mono	Big Hole	Natural - cascade	15	0.095	0.344
Papoose	Big Hole	Natural - cascade	25	0.037	0.125
Plimpton	Big Hole	Natural - cascade	70	0.107	0.344

Rabbia	Big Hole	Anthro - demographic	37	0.087	0.344
Doolittle	Big Hole	Anthro - demographic	49	0.057	0.156
Sappington	Big Hole		22	0.250	0.750
S. Fork N. Fork Divide	Big Hole	Anthro - dam	9	0.045	0.097
Spruce	Big Hole	Anthro - irrigation	26	0.221	0.594
Squaw	Big Hole	Anthro - demographic	26	0.101	0.500
Squaw Lake	Big Hole	Anthro - demographic	30	0.076	0.250
Twelvemile	Big Hole	Anthro - demographic	41	0.104	0.281
Little Boulder	Boulder	Anthro - mine	25	0.189	0.563
Muskrat	Boulder	Anthro - demographic	13	0.253	0.781
Bostwick	Gallatin		84	0.021	0.092
Garrott Creek	Gallatin		14	0.060	0.156
Wild Horse	Gallatin	Natural - cascade	30	0.036	0.125
E. Fork Big Spring	Judith	Natural - intermittent	30	0.143	0.419
W. Fork Cottonwood	Judith	Anthro - demographic	29	0.112	0.313
Last Chance	Madison	Natural - intermittent	19	0.057	0.188
Big Coulee	Missouri Dearborn	Natural - waterfall	32	0.194	0.531
N. Fork Highwood	Missouri Dearborn	Natural - waterfall	119	0.15	0.531
Bean	Red Rock	Anthro - irrigation	50	0.063	0.188
Bear	Red Rock	Anthro - irrigation	25	0.02	0.063
Craver	Red Rock	Anthro - demographic	25	0.066	0.188
Browns	Red Rock	Anthro - irrigation	158	0.283	0.906
E. Fork Clover	Red Rock	Natural - cascade	25	0.113	0.313
Meadow	Red Rock	Natural - intermittent	130	0.126	0.438
N. Fork Everson	Red Rock	Anthro - culvert	28	0.097	0.323
Simpson	Red Rock	Natural - intermittent	50	0.087	0.250

Painter	Red Rock	Anthro - culvert	111	0.179	0.813
S. Fork Everson	Red Rock	Natural - intermittent	27	0.067	0.281
Dark Hollow	Ruby	Anthro - demographic	50	0.055	0.344
Jack	Ruby	Natural - intermittent	143	0.05	0.156
Meadow Fork Greenhorn	Ruby	Anthro - cascade	25	0.096	0.323
Mill Gulch	Ruby		12	0.046	0.125
Ramshorn	Ruby	Anthro - irrigation	90	0.004	0.031
S. Fork Greenhorn	Ruby	Anthro - demographic	10	0.086	0.281
Lone Willow	Smith	Anthro - dam	65	0.034	0.219
N. Fork Willow	Sun	Natural - intermittent	25	0.149	0.500
Sidney	Two Medicine	Natural - waterfall	65	0.276	0.813
Midvale	Two Medicine	Anthro – dam	28	0.211	0.625
Dutchman	Upper Missouri	Anthro - demographic	50	0.183	0.563
Hall	Upper Missouri	Anthro - culvert	28	0.06	0.188
Skelly Gulch	Upper Missouri	Anthro - culvert	27	0.100	0.406
S. Fork Quartz	Upper Missouri	Anthro - culvert	40	0.027	0.125
Staubach	Upper Missouri	Anthro - demographic	32	0.021	0.094
Threemile	Upper Missouri	Anthro - dam	44	0.086	0.313
Whites	Upper Missouri	Natural - intermittent	24	0.082	0.281

FIGURES

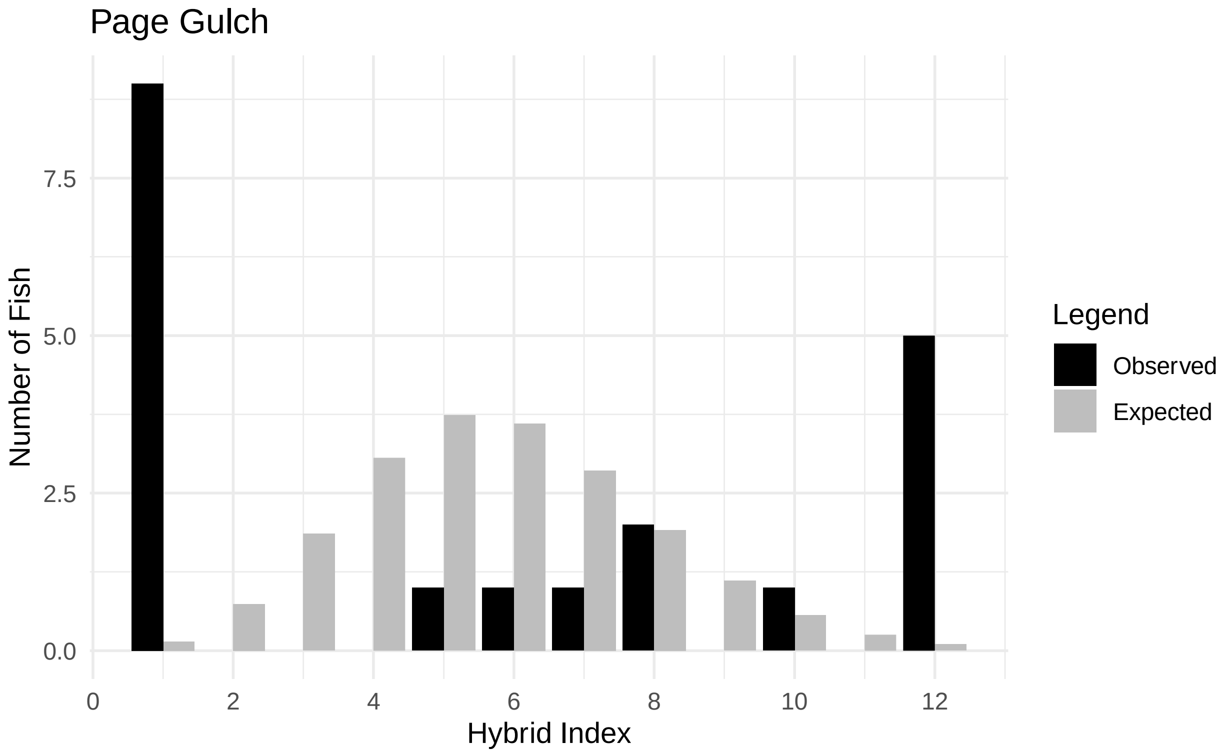

Figure 1. Observed and expected (random) distribution of rainbow trout hybrid indices among fish in a sample from Page Gulch.

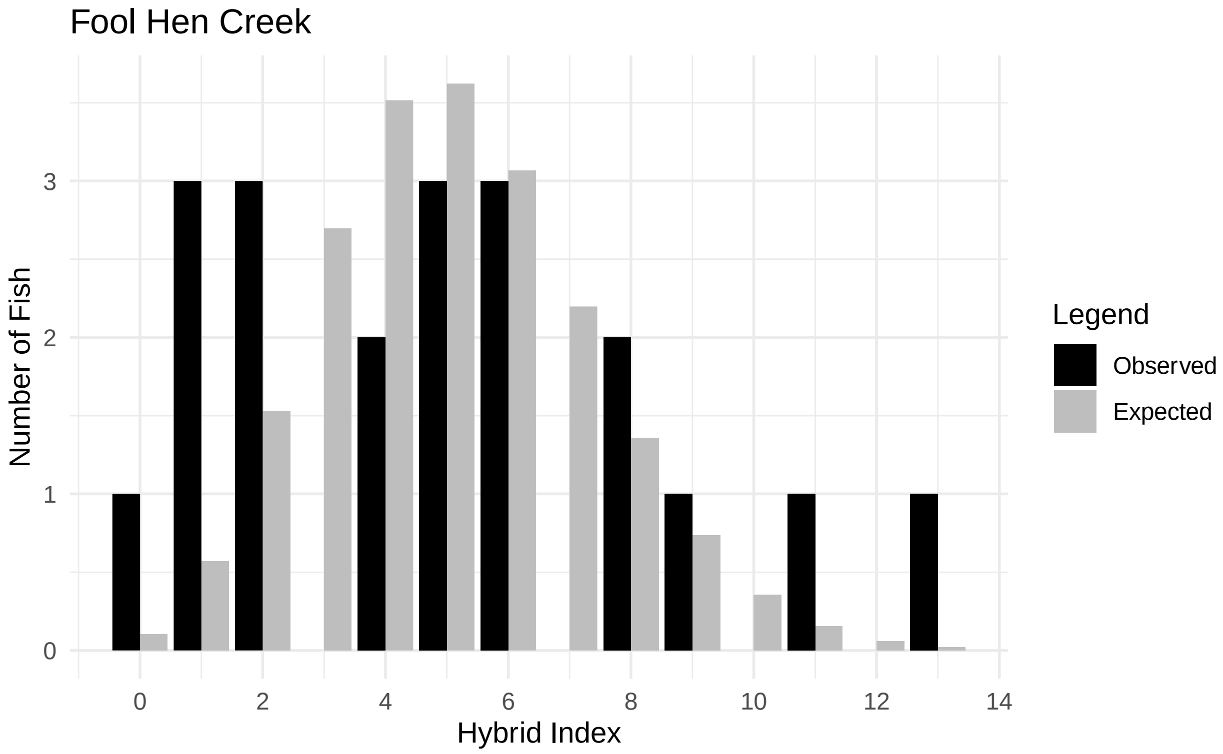

Figure 2. Observed and expected (random) distribution of rainbow trout hybrid indices among fish in a sample from Fool Hen Creek.

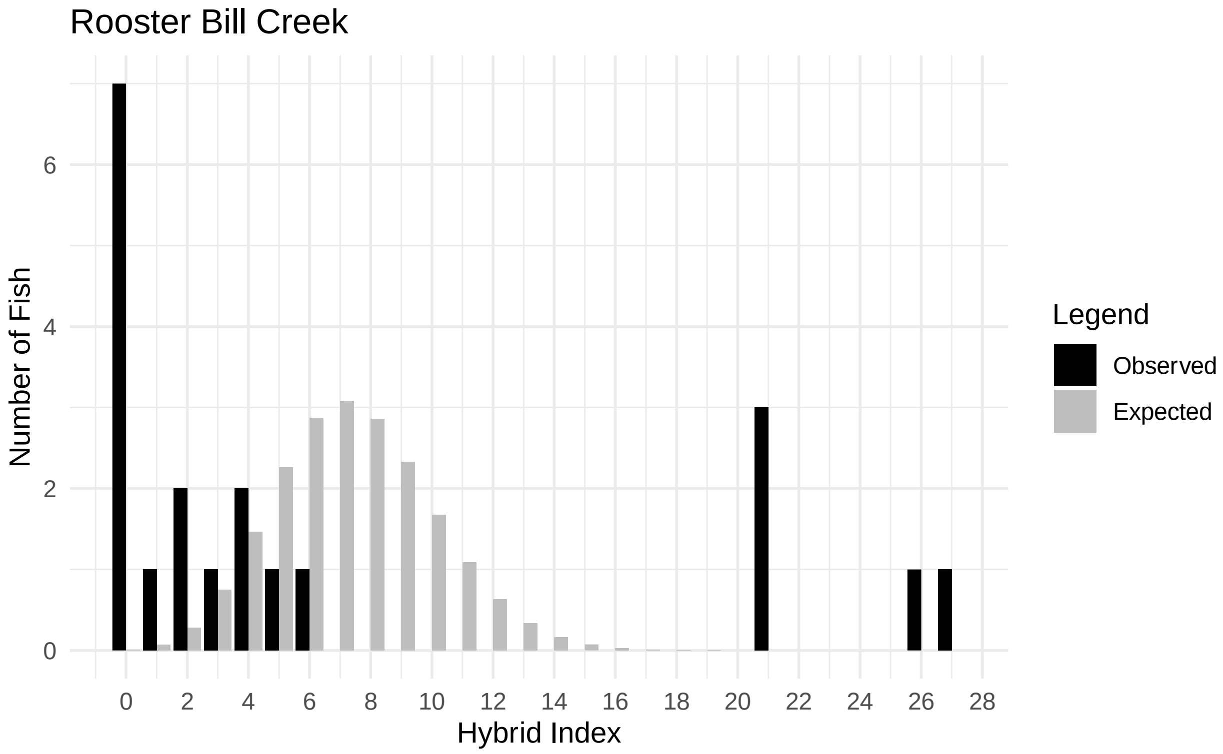

Figure 3. Observed and expected (random) distribution of rainbow trout hybrid indices among fish in a sample from Rooster Bill Creek.

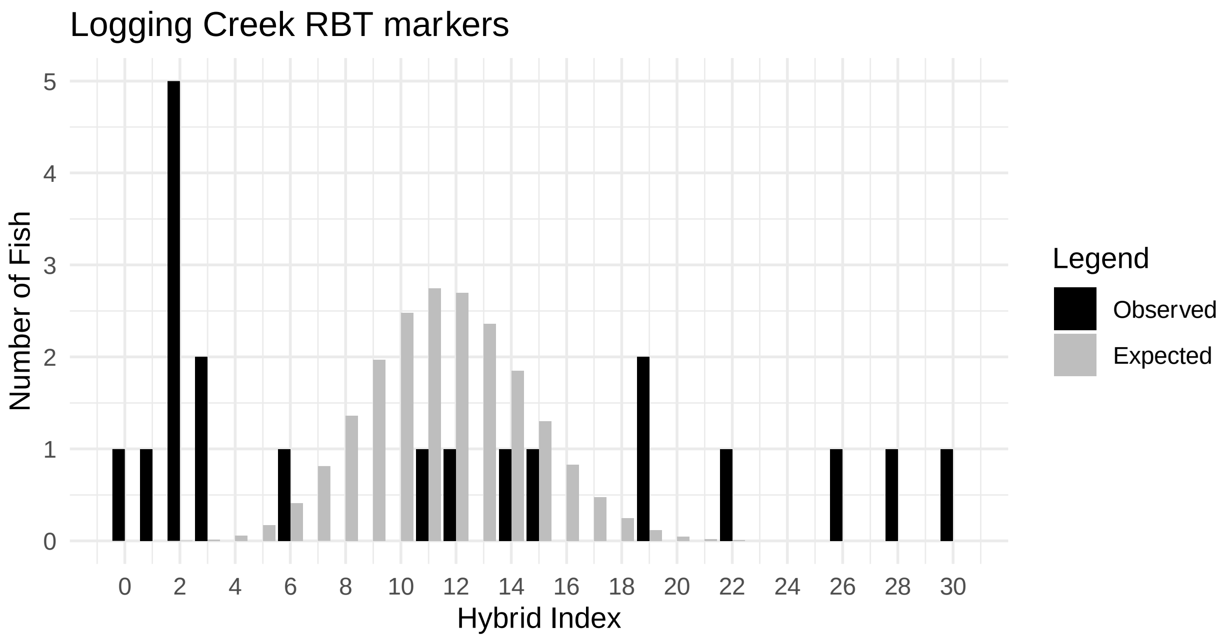

Figure 4. Observed and expected (random) distribution of rainbow trout hybrid indices among fish in a sample from Logging Creek.

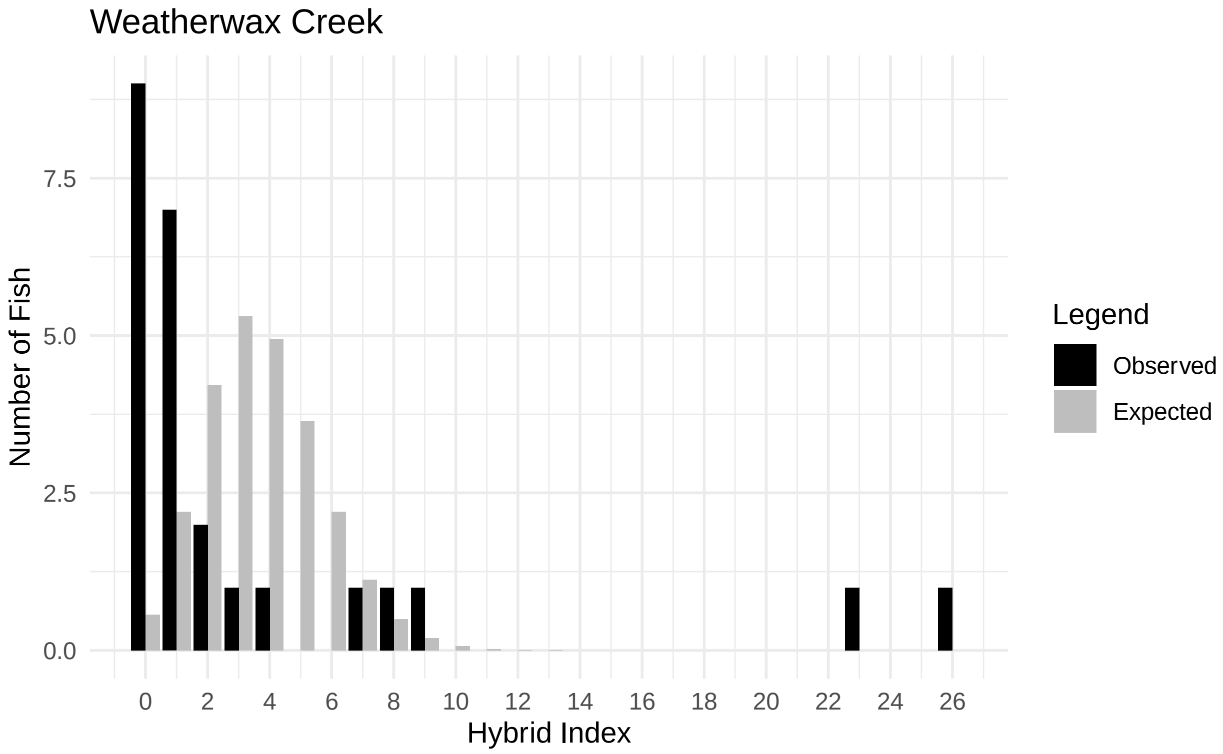

Figure 5. Observed and expected (random) distribution of rainbow trout hybrid indices among fish in a sample from Weatherwax Creek.

Methods and Data Analysis

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

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

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

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

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

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

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

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

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Table1

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

Rainbow Markers

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

Table 1-continued

Westslope Markers

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

Table 1-continued

Yellowstone Markers

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

Table 2

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

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