Cutthroat Trout Studies

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CUTTHROAT TROUT INVESTIGATIONS

Period Covered: December 1, 2024 to November 30, 2025

PROJECT OBJECTIVE
Conservation of Colorado's native Cutthroat Trout

RESEARCH PRIORITY
Genetic purity and heritage assessments in Colorado's native Cutthroat Trout populations

OBJECTIVE
To assess the genetic purity and heritage of select Cutthroat Trout populations in Colorado

Genetic purity and heritage of select Cutthroat Trout populations in Colorado

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INTRODUCTION

Pervasive undocumented stocking in the early 20th century has obscured the native distribution of Colorado's Cutthroat Trout subspecies (Metcalf et al. 2007, 2012; Rogers et al. 2018; Bestgen et al. 2019). This has necessitated the broad use of molecular testing to unravel the convoluted heritage of each population in the state, and to evaluate purity to determine if each should be considered a Conservation Population (CP; sensu UDWR 2000; Hirsch et al. 2013; Zeigler et al. 2019). Conservation Populations are considered part of the conservation portfolio that is evaluated by the U.S. Fish and Wildlife Service when listing decisions under the Endangered Species Act are made (USFWS 2014). Molecular assay results from samples collected by Colorado Parks and Wildlife (CPW) biologists and others on the Colorado River Cutthroat Trout (CRCT) Conservation Team, Rio Grande Cutthroat Trout (RGCT) Conservation Team, and Greenback Cutthroat Trout Recovery Team processed in 2025 are presented here.

METHODS

Molecular tests were conducted on 279 samples obtained from eleven Cutthroat Trout populations distributed across Colorado (Table 1). Six populations came from the Arkansas basin, four from the native range of CRCT, including the Colorado, Dolores, and Gunnison river basins, and two from the RGCT native range. A small piece of the top of the caudal fin from each fish was clipped off and stored in 3.5 mL cryogenic vials filled with 95% reagent grade ethanol. Fin tissues were delivered to Pisces Molecular (Boulder, Colorado) for subsequent genetic analyses. Isolation of DNA, the production of amplified fragment length polymorphism (AFLPs), sequencing of 648 bp of the NADH dehydrogenase subunit 2 (ND2) mitochondrial

gene, and subsequent molecular analyses are detailed elsewhere (Rogers 2010; Rogers et al. 2014; Bestgen et al. 2019). Rather than assigning numbers or letters to each mitochondrial haplotype recovered, I use the name of the body of water where the haplotype was first discovered, preceded by Oc (the native trout, *Oncorhynchus clarkii*) and three letters that describe the major drainage basin where the lineage is native. These include 1) the Green River lineage of CRCT (GRCT) native to the Yampa, White, and Green River basins (YAM), 2) the Uncompahgre lineage of CRCT (UPCT) native to the Colorado, Gunnison, and Dolores River basins (COL), 3) RGCT native to the Rio Grande basin (RIO), 4) the native trout of the South Platte River basin (SPL), and 5) the nonnative Yellowstone Cutthroat Trout (YEL) stocked widely across Colorado in the middle of the last century. This approach allows for easy inclusion of newly discovered haplotypes and facilitates communication toward management and conservation goals. Mitochondrial haplotypes were compared to a reference set derived from Cutthroat Trout samples collected across Colorado over the last two decades (Figure 1) using MEGA version 11 (Tamura et al. 2021).

Table 1. Stream names organized by major drainage basin, water codes, collection dates, and number of fin clips collected for molecular tests conducted in 2025.

Stream	Water Code	Date	Sample size
Arkansas			
Abeyta Creek, S	28911	9/18/2024	28
Clear Creek, S Fk	31562	9/23/2004	31
Cree Creek	29531	5/29/2024	7
Mineral Creek	32057	9/19/2024	20
Poplar Gulch	30706	8/15/2024	14
Taylor Creek, N	32689	circa 2004	30
Colorado			
Clinton Gulch Reservoir	71679	7/1/2008	30
Dolores			
Big Red Canyon Creek	42452	10/16/2024	30
Gunnison			
Escalante Creek, N (lower)	40080	10/21/2024	30
Escalante Creek, N (upper)	40080	10/21/2024	30
Rio Grande			
Las Cruces Creek	30006	8/14/2014	30
Trinchera Creek, S Fk	48682	6/26/2024	30

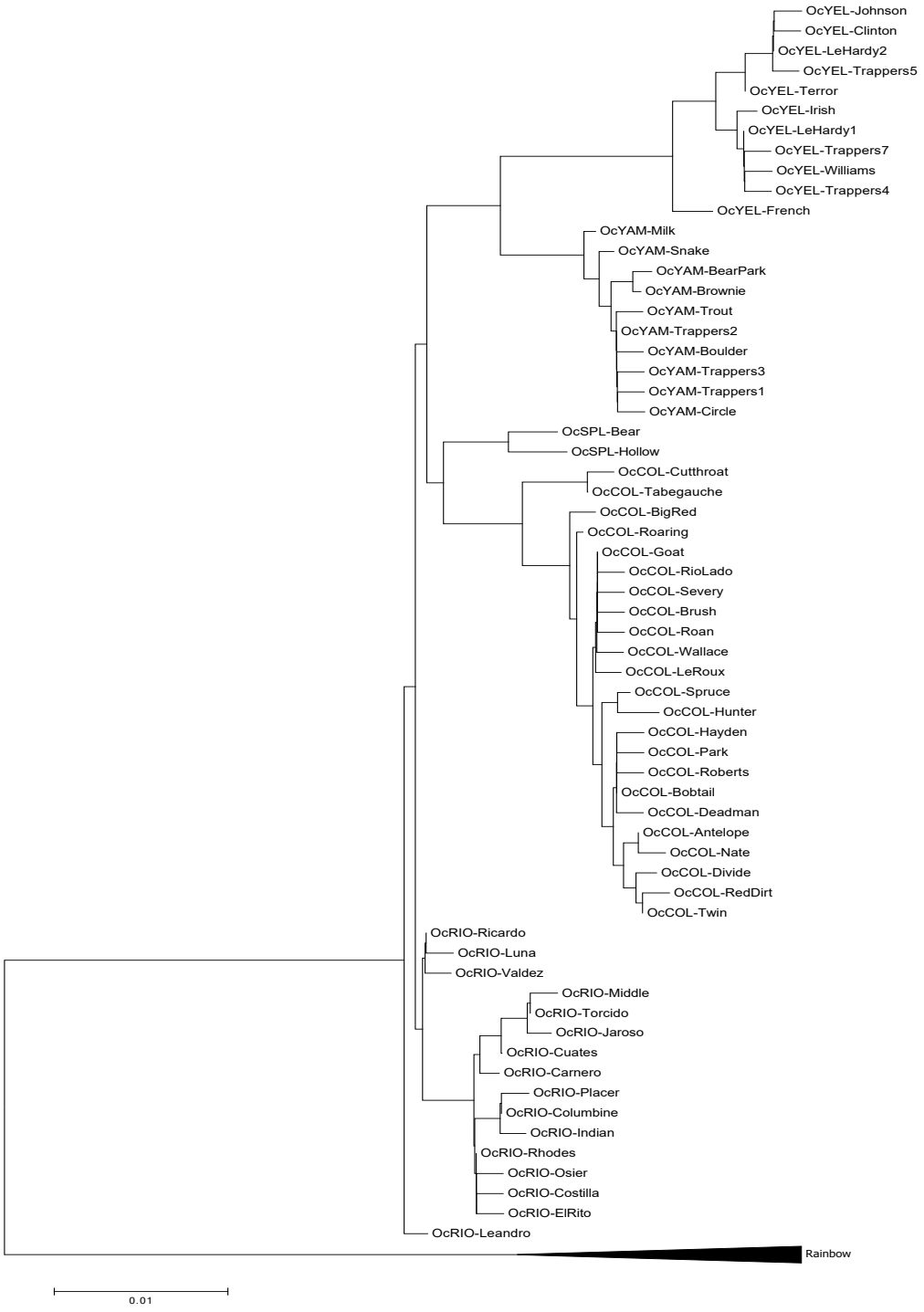

Figure 1. Phylogenetic relationships inferred from 648 base pairs of the mitochondrial NADH dehydrogenase subunit 2 gene for Cutthroat Trout from Colorado. The evolutionary history was developed with the neighbor-joining method in MEGA7, with evolutionary distance units representing the number of base substitutions per site (from Rogers 2020).

RESULTS & DISCUSSION

Results from both nuclear (AFLP; Table 2) and mitochondrial (ND2; Table 3) genetic tests are outlined here for each population, organized by basin. Additional relevant detail about each collection follows the tables.

Table 2. AFLP results from seven Cutthroat Trout collections analyzed in 2025, along with the number of samples analyzed, organized by major drainage basin. Percent admixture is given by lineage, including Green River (blue) and Uncompahgre (green) lineages of Colorado River Cutthroat Trout (GRCT, UPCT), Rio Grande Cutthroat Trout (RGCT), Yellowstone Cutthroat Trout (YSCT), and Rainbow Trout (RBT).

Stream	# Analyzed	Lineage				
		GRCT	UPCT	RGCT	YSCT	RBT
Arkansas						
Cree Creek	7	26	-	1	71	1
Mineral Creek	20	98	-	-	2	-
Poplar Gulch	14	100	-	-	-	-
Dolores						
Big Red Canyon Creek	30	-	98	-	1	-
Gunnison						
Escalante Creek, N (lower)	30	-	97	-	-	2
Escalante Creek, N (upper)	30	1	98	-	-	1
Rio Grande						
Trinchera Creek, S Fk	30	-	-	100	-	-
Trinchera Creek, S Fk[1]	30	-	-	100	-	-

[1]This represents the RGCT – GRCT specific AFLP test with K=2

Table 3. ND2 results from eleven Cutthroat Trout collections analyzed in 2025, including number of samples, organized by major drainage basin. The ND2 haplotype is given by lineage, including Green River (blue) and Uncompahgre (green) lineages of Colorado River Cutthroat Trout (GRCT, UPCT), Rio Grande Cutthroat Trout (RGCT), Yellowstone Cutthroat Trout (YSCT), and Rainbow Trout (RBT).

Stream	# Analyzed	Lineage				
		GRCT	UPCT	RGCT	YSCT	RBT
Arkansas						
Abeyta Creek, S	28	-	-	28	-	-
Clear Creek, S Fk	31	6	-	-	24[1]	-
Cree Creek	7	5	-	-	2	-
Mineral Creek	20	6	5	-	9	-
Poplar Gulch	14	14	-	-	-	-
Taylor Creek, N	30	24[2]	2	-	4	-
Colorado						
Clinton Gulch Reservoir	30	13	2	-	15	-
Dolores						
Big Red Canyon Creek	30	3	-	-	27	-
Gunnison						
Escalante Creek, N (lower)	30	-	30	-	-	-
Escalante Creek, N (upper)	30	-	30	-	-	-
Rio Grande						
Las Cruces	29[3]	-	-	29	-	-
Trinchera Creek, S Fk	30	-	-	30	-	-

[1]Reverse sequence failed on Sample #65376 but forward seq high quality and 100% homologous with #65377, so counted as the same.
[2]Reverse sequence failed on Sample #67241 but forward seq high quality and 100% homologous with #67240, so counted as the same.
[3]Sample #130895 not included as only sequenced 611 bp

Arkansas River basin

Abeyta Creek, S (WC# 28911)— This stream flows down the east side of La Veta Pass right along Highway 160. Despite no stocking history and it's the proximity to the highway, South Abeyta Creek harbors a robust Cutthroat Trout population. A Trout Unlimited volunteer collected 30 samples from this stream on September 18, 2024 for subsequent molecular analysis. Caps on two of the sample vials split and their contents were lost. Of the remaining 28 samples, all displayed the OcRIO-Placer haplotype suggesting evidence of a past stocking event. It is imperative that we analyze the nuclear DNA in these same samples to determine if any

admixture with other lineages of Cutthroat Trout can be found (mitochondrial DNA is a less reliable indicator of past admixture).

Clear Creek, S Fk (WC# 31562)— This collection by Greg Policky in 2004 was subjected to AFLPs at the time, and determined to be a hybrid swarm between GRCT (23%) and YSCT (77%). This is not surprising given the stocking history of PPN in the late 1970s and early 1980s. In addition, Ann Lake in the headwaters continues to get stocked with NAN and historically received PPN and GBN. Renewed interest in the search for relict Yellowfin Cutthroat Trout DNA however, spurred the sequencing of these old samples given proximity to Twin Lakes. Unfortunately, no Yellowfin haplotypes were recovered, and the ND2 results mirrored the AFLP results from two decades prior. Six fish displayed the common OcYAM-Trappers2 haplotype, 23 with OcYEL-LeHardy1, and one with OcYEL-LeHardy2. All three haplotypes would have been common in the PPN and GBN used in past stocking efforts.

Cree Creek (WC# 29531)— The Cree Creek flows above Lake of the Aspens which lies on a private ranch west of Salida that would like CPW to manage the fishery as a refugia for the Hayden Creek Cutthroat Trout (HCC). The ranch has invested in improving the lake habitat, making it an ideal candidate for an HCC brood lake if it could be reclaimed to eliminate the current population of hybrid Cutthroat Trout. The lake is filled with water pulled from Cree Creek, but no molecular surveys have been conducted on that stream because of its checkered stocking history. Pikes Peak Natives (PPN) were stocked in it repeatedly over the years, as well as GBN in 2002, and RBT in 1978. The PPN heritage (Rogers and Kennedy 2008; Rogers 2024) is reflected in both AFLP data and ND2 sequence. A couple of fish even show evidence of RBT admixture from the 1978 stocking event, though no RBT haplotypes were recovered in this small collection (n=7).

Mineral Creek (WC# 32057)— This stream west of Buena Vista in the South Cottonwood drainage has been home to a self-sustaining population of Cutthroat Trout for years, making it a suitable candidate for a reclamation project and future home for HCC. However, no molecular testing has been conducted on the resident population. Alex Townsend collected 20 samples on September 19, 2024 to inform any future reclamation work. AFLP testing suggested this population was predominantly GRCT lineage CRCT (98%) with mild YSCT admixture (2%). Results from ND2 sequence data were more interesting, and a wide variety of haplotypes were recovered. Fifteen fish harbored the common GRCT (n=6) and YSCT (n=9) haplotypes likely introduced from a 1982 PPN stocking event, but the remaining five were UPCT haplotypes (3 OcCOL-Severy and 2 OcCOL-Hayden). This represents the first instance where the OcCOL-Hayden haplotype has been recovered outside of South Prong Hayden Creek or Jordan's Twin Lakes collection from 1889.

Poplar Gulch (WC# 30706)— Flowing south into Chalk Creek west of Nathrop, a series of cascade barriers appear to protect this Cutthroat Trout population from nonnative invaders. There are no stocking records or sampling history for this stream, but AFLP (100% GRCT) and ND2 (all 14 samples harbor OcYAM-Trappers2 haplotype) results suggest it was stocked with descendants from Trappers Lake (GRCT) perhaps prior to 1938 long before YSCT alleles were introduced to that system (Rogers et al. 2018). It would be good to boost the sample size of this

collection from 14 to 30 so that one could feel more confident that no YSCT alleles are present. If future work confirms that only GRCT alleles are present, then this population would have substantially greater conservation value.

Taylor Creek, N (WC# 32689)— North Taylor Creek flows east off the Sangre de Cristo range toward the town of Westcliffe. The resident Cutthroat Trout were sampled around 2004 by Jim Melby and AFLP tests at the time revealed a GRCT population (98%) with slight YSCT admixture (2%) with much of that appearing in one fish (13%). With Megan Lake in the headwaters (no record of stocking record since 1977), and a 1982 stocking record of 1,000 PPN fry released somewhere in the stream, no further molecular work had been done. Recent interest in searching for lost Yellowfin and South Hayden Creek haplotypes in the Arkansas basin allowed sequencing the ND2 gene in these 30 fish.

The presence of YSCT alleles was confirmed in the mitochondrial sequence data with four fish displaying the common OcYEL-LeHardy1 haplotype, while 24 fish harbored common haplotypes found in Trappers Lake (22 OcYAM-Trappers2 and 2 OcYAM-Trappers3). Noteworthy was the presence of 2 OcCOL-Severy haplotypes that we continue to find only east of the Continental Divide, though recent research suggests these fish were likely stocked from west slope sources (Clark 2025).

Colorado River basin

Clinton Gulch Reservoir (WC#71679)— Draining the north slope of Clinton Peak northeast of Leadville, Clinton Creek flows into Clinton Gulch Reservoir that is home to a naturally reproducing population of Cutthroat Trout. Built in 1977 by the Climax Molybdenum Company, the new reservoir was stocked with 455 seven-inch PPN that year (no other stocking events have been reported for the reservoir since). Bruce Rosenlund recalled that a wild spawn operation in 1979 pulled eggs only from "good looking" fish to be used in reestablishing the population in the Timber Creek drainage in Rocky Mountain National Park following a September 1979 reclamation. Does this imply that maybe resident fish already occupied Clinton Creek prior to the formation of the reservoir and the PPN stocking event? A 1999 collection of 14 fish from Clinton Gulch by Sherman Hebein suggests substantial YSCT admixture (29%) into a population of GRCT (61%) with slight evidence of UPCT (6%). This sample appeared somewhat degraded (not processed until 2007), so a new collection of 30 fish was made during the spawning run in 2008 and tested with AFLPs. That collection revealed even more YSCT admixture (55%), but little UPCT admixture (1%). This is consistent with PPN derived from McReynolds Reservoir that also typically show more YSCT admixture, but no UPCT (Rogers and Kennedy 2008).

What is perplexing is that fish in Timber Lake appeared to be essentially UPCT as indicated by ND2 sequence data and microsatellites (Martin 2008). Could the "good looking" fish have been the actual founders of the reservoir population, and native to Clinton Creek prior to the introduction of PPN in 1977? Ted Sedell subsequently obtained 12 fins from Timber Creek in 2008 (Sedell et al. 2015), and AFLPs suggested predominantly GRCT (93%) with mild admixture from UPCT (6%) and YSCT (1%). Mitochondrial ND2 sequence data suggested two

were YSCT (OcYEL-Clinton), one was GRCT, yet nine were UPCT (OcCOL-Goat). DNAs from 20 fish were obtained from Chris Kennedy's circa 2007 collection that was provided to the Martin Lab at the University of Colorado, Boulder. These also suggested predominantly GRCT (99%) with mild UPCT admixture (1%) by AFLP. Here too, substantial discord was recorded with the mitochondrial DNA where all 20 fish had the same UPCT haplotype (OcCOL-Goat) haplotype

The ND2 region from the DNAs of the 30 fish collected in 2008 from Clinton Gulch Reservoir were sequenced here in hopes of shedding some light on this paradox. Thirteen fish harbored the common OcYAM-Trappers2 haplotype and only two fish with the OcCOL-Goat haplotype. Three different OcYEL haplotypes were recovered, one of which is rare, having only been detected in Timber Creek, Clinton Gulch, and a YSCT population in Horn Fork Creek in the Arkansas basin. Perhaps PPN did indeed introduce YSCT alleles into the Timber Creek population? Did any fish remain in Timber Lake and Creek following the 1979 antimycin treatment? Many questions remain, but higher resolution molecular methods will likely be required to resolve the relationship between Clinton Gulch fish and those from the Timber Creek drainage.

Dolores River basin

Big Red Canyon Creek (WC#42452)— While Big Red Canyon Creek northeast of Norwood was sampled in 2009 by Clay Speas (n=27 and 32), and again in 2010 by Dan Kowalski (n=12; Bestgen et al. 2019), none of those collections were obtained as high in the drainage as this collection near the confluence with Kelly Creek. Although the earlier collections detected Rainbow Trout admixture, these specimens from the headwaters look great phenotypically, and if pure might make good candidates for direct transfer into recently reclaimed South Mesa Creek.

Curiously, despite only five fish showing slight evidence of Yellowstone admixture as measured with AFLPs (Table 2), the mitochondrial DNA was overwhelmingly Yellowstone Cutthroat Trout with 27 of 30 fish containing the OcYEL-LaHardy1 haplotype, and only three with the OcCOL-BigRed haplotype. This is in stark contrast to the 2010 collection where 11 of 12 fish were UPGT (8 OcCOL-Goat and 3 OcCOL-BigRed). Future research might include sequencing the ND2 gene in the 2009 the samples to see if the high incidence of Yellowstone haplotypes is a new phenomenon or whether this same contrast was apparent 15 years ago in that much larger sample.

Gunnison River basin

Escalante Creek, N (WC#40080)— Two collections were made on this stream on October 21, 2024 by Eric Gardunio (vials 1-30 from an upstream site at 12S 718693, 4276444, and vials 31-60 collected from a downstream site at 12S 719896, 4276677). Phenotypically, these fish seem to lose some admixture traits as you move upstream, such as reduced incidence of white tipped fins. The downstream collection was made just upstream of a steep cascade that appears to limit

immigration of Rainbow x Cutthroat trout hybrids from below. The upstream collection was made upstream of a double waterfall in a canyon with very few white tipped fins above it, suggesting it might be possible to treat from the falls down and acknowledge that some low-level admixture upstream will persist.

Nuclear DNA results (AFLPs) suggest significant Rainbow Trout admixture in two fish from the downstream section with perhaps mild evidence of Yellowstone Cutthroat Trout and Rainbow Trout in others. The upstream section only harbored one fish with significant Rainbow Trout admixture. Little genetic diversity was recovered in the mitochondrial DNA, with all 60 fish displaying the fairly common OcCOL-Bobtail haplotype (Figure 1)

Rio Grande basin

Las Cruces Creek (WC#30006) —– This tributary of S Fk Trinchera Creek was part of a reclamation project in 1977, and repopulated with fish from West Indian Creek. Ben Felt sampled a 1.2-mile reach in this stream on August 14, 2014, starting in UTM Zone 13 at 480696m E, 4131094m N moving upstream to 482399m E, 4132119m N and collected 30 fin clips. That work indicated this population was a good representative of RGCT with no apparent admixture with nonnative Cutthroat Trout or Rainbow Trout using the standard AFLP test (but not the RG-CR test; Rogers et al. 2011). Those tissues were reanalyzed here to generate ND2 sequence data to determine if anything unexpected remains in this population. This stream is again slated for reclamation to rid the drainage of Brook Trout, though some Cutthroat Trout do remain, and could be moved to the North Fork of Trinchera Creek. The presence of Cutthroat Trout suggests a more recent invasion of Brook Trout – presumably slowed by the road culvert crossing at the bottom and a high stream gradient in the first mile.

Trinchera Creek, S Fk (WC#48682) —– This stream was reclaimed from the headwaters down to Mountain Home Reservoir in 1977. The project was deemed an immediate success with the stream restocked several months later with 2,575 trout electrofished from 1.5 miles of West Indian Creek (Lloyd Hazzard, October 20, 1977 written communication). Of these, 1,545 were over 4 inches. Over time, Brook Trout reappeared and by 2008 had replaced Cutthroat Trout again, though a stronghold of Cutthroat Trout persisted above a beaver pond complex in the South Fork. By 2013, those dams had deteriorated enough that the Brook Trout invasion was near complete. By 2024, only 200-300 cutthroat trout remained in the system, occupying a small tributary (Banana Creek) along with an occasional individual scattered below the newly constructed temporary barrier. Reece Samuelson collected 30 fins from that lower section on June 26, 2024 (spot shocked pools from Las Cruces confluence to Deep Canyon confluence), and those are the subject of this study.

Four mitochondrial haplotypes were recovered in the S Fk of Trinchera Creek, and three in Las Cruces Creek (Figure 2). The presence of the Oc-RIO-Placer haplotype in these two streams (and in nearby Placer Creek) but not in a 2010 collection of 24 fish from the post-reclamation source population in West Indian Creek may suggest that not all of the resident fish were extirpated during the 1977 reclamation and that some of the original stock remained. The higher

incidence of this haplotype in Las Cruces Creek may indicate that this might have been an area where these fish persisted. A more thorough analysis with larger sample sizes from West Indian Creek fish would be needed to gain confidence in this hypothesis (additional fins collected in 2025). Either way, these few remaining Cutthroat Trout appear not to harbor any alleles from other subspecies, and could be translocated to the North Fork of Trinchera Creek.

Note - ND2 sequence from 30 fish out of Squirrel Canyon that drains into the North Fork all had the OcRIO-Rhodes haplotype

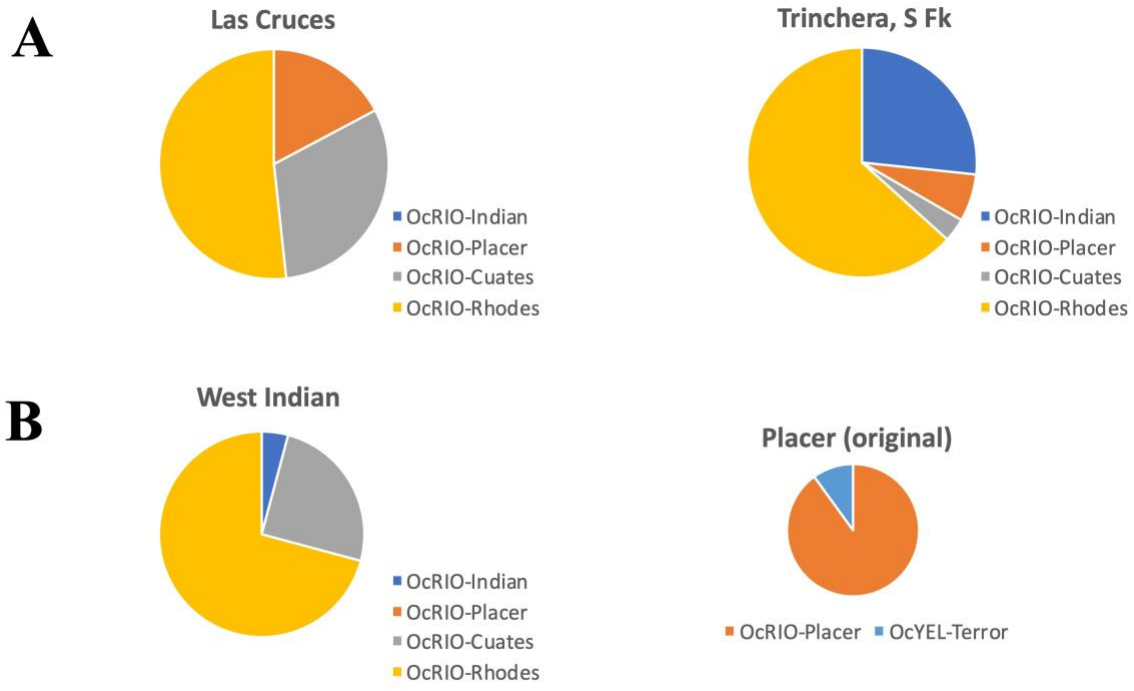

Figure 2: Mitochondrial ND2 haplotypes recovered in A) Las Cruces Creek, tributary of S Fk Trinchera Creek (n=29) and in the South Fork itself (n=30) were compared to B) West Indian Creek (the post-1977 reclamation founding source; n=24), and Placer Creek prior to recent reclamation, a nearby stream thought to contain an aboriginal population (n=10). The area of each pie is proportional to the number of fish in each collection.

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RESEARCH PRIORITY
Cutthroat Trout taxonomy

OBJECTIVE
Determine more stable names for lineages of Colorado River Cutthroat Trout

New common names for lineages of Colorado River Cutthroat Trout

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A BRIEF HISTORY

The nomenclature surrounding inland Cutthroat Trout enjoyed considerable stability over much of the 20th Century, with five subspecies recognized in the Southern Rocky Mountains (SRM; Behnke 1979, 1992, 2002). Bonneville Cutthroat Trout (*Oncorhynchus clarkii utah*) occupied waters in the Bonneville Basin (but see Campbell et al. 2018), while Greenback Cutthroat Trout (GBCT; *O. c. ssp.*) were alleged to be found in the headwaters of the South Platte and Arkansas Rivers, Rio Grande Cutthroat Trout (RGCT; *O. c. virginalis*) inhabited their namesake river basin, and the extinct Yellowfin Cutthroat Trout was historically found in Twin Lakes in the headwaters of the Arkansas River. Native trout west of the Continental Divide in the Colorado River and its tributaries upstream of Lake Powell in eastern Utah, Colorado, and southwest Wyoming were considered Colorado River Cutthroat Trout (CRCT; *O. c. pleuriticus*; Behnke 1979, 1992, 2002). The inability to distinguish these fish consistently from GBCT and RGCT with morphometric and meristic methods spawned a proliferation of molecular studies seeking to find diagnostic markers that would allow managers to reliably identify this subspecies of Cutthroat Trout from others in the SRM region. In 2007, Metcalf et al. were successful in that regard, distinguishing CRCT, GBCT, and RGCT using microsatellite, AFLP, and mitochondrial DNA markers. In fact, distinguishing these subspecies with molecular tools was fairly straightforward, but patterns of natural diversity were occluded by poorly documented large-scale stocking of Cutthroat Trout in the late 19th and early 20th centuries. For example, roughly half of putative GBCT appeared to be CRCT, founded from undocumented stocking east of the Continental Divide (Metcalf et al. 2007) derived from progeny primarily obtained at Trappers Lake in the headwaters of the White River (Rogers et al. 2018). In the Metcalf et al. (2007) study, the authors depicted CRCT fish in blue and the remaining putative GBCT in green. In 2010, Rogers et al. used mitochondrial DNA sequence data and AFLPs to show that the remaining GBCT east of the Divide were very similar to populations of CRCT found in the upper Colorado, Gunnison, and Dolores River basins west of the Divide (Rogers 2010) raising the possibility that the remaining "green" GBCT were not native either. In 2012, Metcalf et al. explored mitochondrial sequence data from museum specimens collected before large scale stocking of trout in Colorado to gain a clearer picture of native trout distributions. They showed that only a single population of putative GBCT were in fact native to the South Platte basin, and that the remainder were also CRCT from west of the Continental Divide, and that CRCT could be divided into two broad clades that displayed genetic distance on par with the other recognized subspecies (Rogers et al. 2018a; Bestgen et al. 2019). In retrospect, this split was also apparent

in early allozyme work (Kanda et al. 2000), and in early mitochondrial and nuclear DNA studies (Evans and Shiozawa 2000, 2001).

Since the discovery of the two broad CRCT clades, managers and biologists continued to refer to them as the "blue" and "green" lineages depicted in Metcalf et al. (2007). The blue lineage represents trout native to the Green, White, and Yampa rivers (including the Escalante and Dirty Devil rivers that ultimately drain into Lake Powell downstream of the confluence with the Green River; Bestgen et al. 2019). The green lineage appears native to the upper Colorado, Gunnison, and Dolores rivers (Metcalf et al. 2012; Rogers et al. 2018a; Bestgen et al. 2019). This practice has led to quite a bit of confusion to those unfamiliar with the original Metcalf et al. 2007 study, as the blue lineage is found in the Green River, and the green lineage is found in the Blue River, and both are quite different than the Greenback Cutthroat Trout native to the South Platte basin. In addition, the consequential type specimen of CRCT is a blue lineage fish from the Green River basin. Since the subspecies epithet *pleuriticus* is tied to that fish then the scientific name for Colorado River Cutthroat Trout would follow the blue lineage. Nonetheless, the color names have become somewhat entrenched over the last decade, showing up repeatedly in the peer-reviewed literature (Metcalf et al. 2012 Rogers et al. 2018a; Bestgen et al. 2019; Kokonnen et al. 2024).

In addition to the blue and green lineages, Metcalf et al. 2012 also identified a third CRCT lineage from two museum specimens of Cutthroat Trout collected from the San Juan River near Pagosa Springs in 1874 as part of the Wheeler Survey charged with mapping the American southwest. With no extant populations sharing mitochondrial haplotypes with these fish, they were presumed extinct (Metcalf et al. 2012). Since that time Rogers et al. (2018b) showed that at least a half dozen populations of those fish persist on the landscape in small isolated populations. These populations are a focal area for CRCT Team conservation efforts. Since its rediscovery, this fish has been referred to as the San Juan Cutthroat Trout (SJCT). As such, confusion induced by the color references are absent, and the name has remained stable since its inception. We recommend continuing to refer to this lineage as SJCT.

Developing more stable common names

Although the blue and green lineage designations made sense to those intimately familiar with their history, they are very confusing for others. To resolve some of this confusion and foster long-term stability in the common name, the CRCT Conservation Team voted in December 2024 on whether to adopt English, Spanish, or Ute names reflecting where the fish are found. Each combination has some benefits and some problems (Table 1).

Table 1. Pros and cons associated with each name pair

Cutthroat Trout	Pro	Con
Blue lineage / Green lineage	None - other than perhaps comfort for those familiar with the history and their use	Blue lineage found in the Green River and Green lineage found in the Blue River; neither is it Greenback Cutthroat Trout; no geographic basis
Green River / Colorado River	Closely tied to geography of native range	Colorado River already means something quite different; changing the usage would require coming up with an alternative name for our Conservation Team
Rio Verde/ Rio Colorado	Still tied to the general geography albeit in Spanish	Provides a triumvirate with Rio Grande Cutthroat Trout; different enough that ensures recognition of a change
Uinta/ Uncompahgre	Honors native people that shared the approximate native range	Geographic link less direct

METHODS & RESULTS

Each attendee was given three dot stickers color coded for the state where the team member resides. Members were then able to distribute their stickers either all for one, or if undecided, among their preferred pairs: 1) blue lineage/green lineage (no change), 2) Green River Cutthroat Trout/Colorado River Cutthroat Trout, 3) Rio Verde Cutthroat Trout/Rio Colorado Cutthroat Trout, or 4) Uinta Cutthroat Trout/Uncompahgre Cutthroat Trout. This initial round of voting was cut short when it became apparent that many team members preferred the name Green River Cutthroat Trout as the common name for the blue lineage and Uncompahgre Cutthroat Trout to represent the green lineage. As such, the structure of the vote was changed so that every member could vote once for any of the proposed blue lineage names and one vote for any of the green lineage names (but not for "Rio Verde" or "Rio Colorado" since those garnered little support in the first round of voting). The results were unequivocal, with two thirds of respondents selecting

Green River Cutthroat Trout to represent the former blue lineage, and two thirds selecting Uncompahgre Cutthroat Trout to represent the former green lineage (Figure 1). The names selected were the most popular in each state as well.

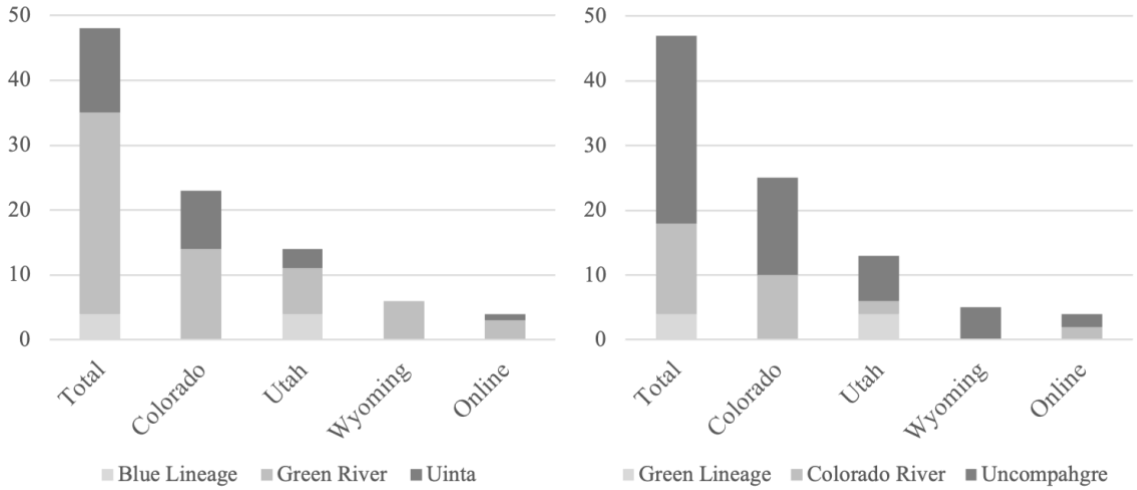

Figure 1. Total votes tallied for blue lineage CRCT names in the left panel, and green lineage CRCT names on the right are also broken down by state where the attendees reside. Online participants were provided access to an anonymous survey that did not include state of origin.

This pair of names does address a number of the shortcomings with other proposed monikers. Green River Cutthroat Trout clearly reflects the bulk of the native range of this taxon, as the White and Yampa rivers are tributary to the Green River. The only exception being the headwaters of the Escalante and Dirty Devil rivers which drain into the Colorado River just downstream of the confluence with the Green River (Bestgen et al. 2019). The link to geography is more subtle with Uncompahgre Cutthroat Trout, but also appropriate as directly translated from the Ute language, this means "where water makes rock red"– a colorful description of the Upper Colorado, Gunnison, and Dolores rivers where this taxon is native. This more subtle tie to the geology of the native range also grants some flexibility as we sort out whether UPCT populations found east of the Continental Divide are native or introduced (Rogers et al. 2018; Bestgen et al. 2019). If native, their range would have included that of the Tabeguache Utes. Should the blue and green lineages be formally redescribed in the future and granted subspecies status, these new common names could serve as subspecies names and remain unchanged. That stability in naming is sought after by the American Fisheries Society's Names of Fishes Committee (Page et al. 2023), particularly for those taxa where the scientific names are likely to change. Though some advocated for using Colorado River Cutthroat Trout to represent the green lineage, names should not be reused to represent something else (Mayr 1942). Fortuitously, by not re-using Colorado River Cutthroat Trout, that name can continue to refer to the three lineages native to the Colorado River basin in aggregate – such as the Colorado River Cutthroat Trout Conservation Team and agreements and strategies that promote the conservation of all three lineages. Confusion will continue in the short-term as users transition to these new

lineage names, but the hope is that in time, no one will remember the blue and green lineage designations.

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RESEARCH PRIORITY
Cutthroat Trout Conservation

OBJECTIVE
Secure rare peripheral populations of native Cutthroat Trout

Roan Creek broodstock development: purging non-native alleles from a rare native Cutthroat Trout population

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INTRODUCTION

The Uncompahgre lineage of Colorado River Cutthroat Trout (formerly green lineage; see pages 12-17, this volume) is the focus of intense conservation efforts as they are only represented by 69 conservation populations. These populations are spread across their putative native range in the Colorado, Gunnison, and Dolores River basins, occupying just 3% of what would have been historically occupied habitat (Rogers et al. 2022). Central to the success of the conservation efforts is the development of a transient broodstock that can be used to repatriate populations following reclamation projects that create vacant habitat (Hirsch et al. 2013; Penaluna et al. 2016). One such population of particular interest is Roan Creek that lies tucked away in the Escalante Breaks west of the Roan Plateau in northwest Colorado. Early interest in replicating this population was due in part to their unique appearance, with very few spots along the trunk in some individuals (Figure 1). More recent research has documented additional reasons to make conserving this stock a priority, as this peripheral population also displays a unique genotype both in the mitochondrial genome (Bestgen et al. 2019; Rogers et al. 2020), and the male sex determining chromosome region Omy1 (Brunelli et al. 2013).

Figure 1. Images of typical Cutthroat Trout from Roan Creek (hand model – Bill Elmblad)

The headwaters of Roan Creek are low in elevation (2000-2250 m; 6600-7400 ft), making this stream unexpected trout habitat, and also one of the first to face challenges associated with a warming climate (Cook et al. 2004; Ficke et al. 2007; Seager et al. 2007; Hansen et al. 2013;

Paukert et al. 2021). The drought of 2002 was the first warning sign, with the entire population forced to occupy a small amount of habitat near spring inflows around 12S 703460m E, 4382398m N due to excessively warm stream temperatures. Exclosures have been constructed to foster growth of riparian cover in hopes of moderating temperatures (and thermal stress), but grazing remains a problem in the remainder of the drainage. A temperature monitoring site was established below the lower BLM exclosure and temperatures are already close to exceeding what Colorado River Cutthroat Trout (CRCT) can tolerate (Table 1; Underwood et al. 2012; Rogers et al. 2022). Roberts et al. (2013) suggest Maximum Weekly Maximum Temperature (MWMT) >26 ºC will result in mortality in CRCT, while Zeigler et al (2019) suggest that threshold is crossed over 25 ºC in Rio Grande Cutthroat Trout, even with diel fluctuations. They suggest MWMT will still have negative effects on growth and survival with MWMT as low as 21 ºC. In addition to the thermal stress now being experienced in Roan Creek, a failing barrier has allowed nonnative trout to invade the headwaters, complicating development of a broodstock needed to replicate this population elsewhere. In an effort to reverse nonnative admixture in this population we attempted to purge alleles introduced by invading Rainbow Trout and Yellowstone Cutthroat Trout (YSCT; *O. clarkii bouvieri*) using an approach outlined below.

Table 1. Maximum Weekly Maximum Temperature (MWMT), Maximum 30-day Average Temperature (M30AT), maximum daily temperature, and accumulated annual degree days calculated over four years from a temperature logger placed at 12S 703768m E 4382093m N below the lower exclosure on Roan Creek.

Year	MWMT (ºC)	M30AT (ºC)	Daily Max (ºC)	Annual Degree Days (ºC)
2012	22.1	12.8	23.2 (73.8 ºF)	2,716
2013	24.7	14.1	26.2 (79.2 ºF)	2,808
2014	21.9	13.7	23.4 (74.1 ºF)	2,773

METHODS

A wild spawn operation (Figure 2) was conducted in the headwaters of Roan Creek from 2012–2015 to secure eggs for a developing broodstock housed at CPWs fish hatchery in Glenwood Springs (GSH). Several visits to the site were made each year to assess the likely timing of peak spawn (when most ripe females would be available). Once a spawn date was selected, likely spawners were collected with backpack electrofishers up and downstream of the spawn operation site near 12S 703794m E, 4382085m N. Adult males and ripe females were sorted out, and held in separate pens in the stream. Eggs were stripped from each female into a dry bowl with ovarian fluids drained off for pathogen testing. Eggs were then split between 2-3 bowls, and fertilized with either two (2014, 2015) or three (2012, 2013) males in an effort to maximize genetic diversity. Milt was activated with filtered (Katadyn BeFree Gravity 10L, Kempttahl, Switzerland) stream water. Fertilized eggs from each family were water hardened with 100 ppm iodine for 10 min then placed in a 3.8 L (1 gal) insulated water cooler and set on ice. A caudal fin clip was obtained from each parent for subsequent genetic testing (Rogers 2007). Egg

coolers were then transported to the Fish Research Hatchery (FRH) in Bellvue, Colorado in plastic Action Packer totes. Each tote held four families, with a fifth cooler holding a temperature logger (Onset Computer Corporation, Bourne, Massachusetts) so that egg temperatures could be monitored during travel on the six hr journey.

Figure 2. Wild spawn operation site at 12S 703794m E, 4382085m N, with adults collected up and downstream of the site.

Culture

Upon arrival at FRH, eggs were treated with 50 ppm iodine for 30 min, then each family was set in a 10-cm egg cup (Brinkman et a. 2013) and suspended in its own 9.5 L (2.5 gal) aquarium (Figure 3). Each cup received 16 mL/s of 11.7°C (53 ºF) water dripped over the top of a single layer of eggs incubated over a mesh screen. When over 90% of the embryos in an egg cup had hatched, the contents were decanted into the aquarium. Emergent trout fry were fed daily infusions of brine shrimp initially, then weaned onto BioVita Size 0 starter feed (BioOregon, Longview, Washington) after one week by feeding a slurry of both. By two weeks post swim-up, fry were hand fed hand fed four times per by day with BioVita starter feed. Feed rates were adjusted per manufacturers specifications. At five months post hatch, a fish health certificate was obtained, and fingerlings were then transferred to GSH for incorporation into the developing broodstock.

Figure 3. Individual families were raised in separate 9.5 L (2.5 gal) aquaria held in an isolation facility at the Fish Research Hatchery so that only those families with no nonnative alleles could be retained and added to the developing broodstock at the Glenwood Springs Hatchery.

Genetic testing

DNAs were isolated from each fin clip from every parent used in the study and screened with four different molecular tests for evidence of nonnative alleles. Tests included 1) the standard AFLP test (Pisces Molecular; Rogers 2008; Bestgen et al. 2019), 2) sequencing 1000+ bp of the ND2 mitochondrial gene (Brigham Young University; BYU), 3) sequencing five nuclear genes that comprised rRNA intervening transcribed region 2 (ITS3), Ikaros (IK), Gonadotropin hormone (GTH), Transferrin (TF), Insulin-like growth factor (IGF) at BYU, and 4) amplification of primer inside the Omy1 sex determining gene followed by a TaqMan assay that identifies a diagnostic SNP for Cutthroat Trout (males only; Metro State University). Families with a parent that appeared to be admixed with either RBT or YSCT by any screening method were culled, leaving only families apparently free of nonnative alleles to be used in the development of the new broodstock.

RESULTS & DISCUSSION

Conducting wild spawn operations on Roan Creek was particularly challenging as the spawning run is extremely short in that stream – likely due to rapid accumulation of degree days in late May. Ripe females were typically available only over a 7-d window which shifted based on spring climatic conditions. Peak spawn was estimated to be on May 19 in 2012, May 25 in 2013, June 1 in 2014, and May 24 in 2015. We spawned every ripe female we were able to capture each year, as surplus males were always available. Over the course of the four-year project, we created 33 families from 114 parents (Table 2a-d). Unfortunately, at most 68 parents were represented in the final broodstock as many families had to be eliminated because of a single admixed parent (12 families), or poor egg quality (two families).

Table 2a. Eight families were created (with 32 parents) at Roan Creek on May 22, 2012 using three males to fertilize each female. Female length (mm) is indicated in the FishID column while admixture as measured with AFLPs is given by qYSCT (Yellowstone Cutthroat Trout admixture) or qRBT (Rainbow Trout admixture). Any evidence of nonnative alleles in five nuclear genes or the Omy1 region are indicated with either YSCT or RBT, otherwise CRCT (Colorado River Cutthroat Trout; these tests do not distinguish CRCT lineages). Families shown in gray were culled because of the fish highlighted in red, leaving five families from 20 parents for inclusion into the broodstock.

Family	FishID	PiscesID	qYSCT	qRBT	ND2 haplotype	Nuclear	Omy1
A	RON-A-215	RON3_116990	0.00	0.00	OcCOLO-Roan	CRCT	
A	RON-A-M1	RON3_116991	0.00	0.00	OcCOLO-Roan	CRCT	CRCT
A	RON-A-M2	RON3_116992	0.00	0.00	OcCOLO-Roan	CRCT	CRCT
A	RON-A-M3	RON3_116993	0.00	0.00[1]	OcCOLO-Roan	CRCT	CRCT
B	RON-B-242	RON3_116994	0.00	0.00	OcCOLO-Roan	CRCT	
B	RON-B-M1	RON3_116995	0.00	0.00	OcCOLO-Roan	CRCT	CRCT
B	RON-B-M2	RON3_116996	0.00	0.00	OcCOLO-Roan	CRCT	CRCT
B	RON-B-M3	RON3_116997	0.00	0.00	OcCOLO-Roan	CRCT	CRCT
C	RON-C-212	RON3_116998	0.00	0.00	OcCOLO-Roan	CRCT	
C	RON-C-M1	RON3_116999	0.00	0.00	OcCOLO-Roan	CRCT	CRCT
C	RON-C-M2	RON3_117000	0.00	0.00	OcCOLO-Roan	CRCT	CRCT
C	RON-C-M3	RON3_117001	0.00	0.00	OcCOLO-Roan	CRCT	CRCT
D	RON-D-216	RON3_117002	0.00	0.00	OcCOLO-Roan	CRCT	
D	RON-D-M1	RON3_117003	0.00	0.00	OcCOLO-Roan	CRCT	CRCT
D	RON-D-M2	RON3_117004	0.00	0.00	OcCOLO-Roan	CRCT	CRCT
D	RON-D-M3	RON3_117005	0.00	0.00	OcCOLO-Roan	CRCT	CRCT
E	RON-E-204	RON3_117006	0.00	0.00	OcCOLO-Roan	CRCT	
E	RON-E-M1	RON3_117007	0.00	0.00	OcCOLO-Roan	CRCT	CRCT
E	RON-E-M2	RON3_117008	0.00	0.00	OcCOLO-Roan	CRCT	CRCT
E	RON-E-M3	RON3_117009	0.00	0.00	OcCOLO-Roan	CRCT	CRCT
F	RON-F-223	RON3_117010	0.00	0.00	OcCOLO-Roan	CRCT	
F	RON-F-M1	RON3_117011	0.00	0.00	OcCOLO-Roan	CRCT	CRCT
F	RON-F-M2	RON3_117012	0.03	0.00	OcCOLO-Roan	CRCT	CRCT
F	RON-F-M3	RON3_117013	0.00	0.00	OcCOLO-Roan	CRCT	CRCT
G	RON-G-192	RON3_117014	0.00	0.00	OcCOLO-Roan	RBT	
G	RON-G-M1	RON3_117015	0.00	0.00	OcCOLO-Roan	CRCT	
G	RON-G-M2	RON3_117016	0.00	0.00	OcCOLO-Roan	CRCT	CRCT
G	RON-G-M3	RON3_117017	0.00	0.00	OcCOLO-Roan	CRCT	CRCT
H	RON-H-225	RON3_117018	0.00	0.00	OcCOLO-Roan	CRCT	
H	RON-H-M1	RON3_117019	0.00	0.00	OcCOLO-Roan	CRCT	CRCT
H	RON-H-M2	RON3_117020	0.00	0.00	OcCOLO-Roan	CRCT	CRCT
H	RON-H-M3	RON3_117021	0.00	0.00	OcCOLO-Private	CRCT	CRCT

[1]Only 0.1% RBT by AFLP3; too conservative in retrospect

Table 2b. Six families were created (with 24 parents) at Roan Creek on May 28, 2013 using three males to fertilize each female. Female length (mm) is indicated in the FishID column along with the number of fry eligible for transfer to GSH. Admixture as measured with AFLPs is given by qYSCT (Yellowstone Cutthroat Trout admixture) or qRBT (Rainbow Trout

admixture). Any evidence of nonnative alleles in five nuclear genes or the Omy1 region are indicated with either YSCT or RBT, otherwise CRCT (Colorado River Cutthroat Trout; where tests do not distinguish CRCT lineages). Families shown in gray were culled because of the fish highlighted in red, leaving three families from 12 parents for inclusion into the broodstock.

Family	FishID	#Fry	PiscesID	qYSCT	qRBT	ND2 haplotype	Nuclear	Omy1
A	RON13-A-M1	31	RON4_122065	0.05	0.01	OcCOLO-Roan	CRCT	CRCT
A	RON13-A-M2		RON4_122066	0.01	0.00	OcCOLO-Roan	CRCT	CRCT
A	RON13-A-M3		RON4_122067	0.00	0.00	OcCOLO-Roan	CRCT	CRCT
B	RON13-B-250	138	RON4_122068	0.00	0.00	OcCOLO-Roan	CRCT	
B	RON13-B-M1		RON4_122069	0.00	0.00	OcCOLO-Roan	CRCT	CRCT
B	RON13-B-M2		RON4_122070	0.01	0.00	OcCOLO-Roan	CRCT	CRCT
B	RON13-B-M3		RON4_122071	0.00	0.00	OcCOLO-Roan	CRCT	CRCT
C	RON13-C-312	397	RON4_122072	0.00	0.00	OcCOLO-Roan	CRCT	
C	RON13-C-M1		RON4_122073	0.07	0.00	OcCOLO-Roan	CRCT	CRCT
C	RON13-C-M2		RON4_122074	0.00	0.00	OcCOLO-Roan	CRCT	CRCT
C	RON13-C-M3		RON4_122075	0.00	0.00	OcCOLO-Roan	CRCT	CRCT
D	RON13-D-184	147	RON4_122076	0.00	0.00	OcCOLO-Roan	CRCT	
D	RON13-D-M1		RON4_122077	0.00	0.00	OcCOLO-Roan	CRCT	CRCT
D	RON13-D-M2		RON4_122078	0.00	0.00	OcCOLO-Roan	CRCT	CRCT
D	RON13-D-M3		RON4_122079	0.00	0.00	OcCOLO-Roan	CRCT	CRCT
E	RON13-E-178	169	RON4_122080	0.00	0.00	OcCOLO-Roan	CRCT	
E	RON13-E-M1		RON4_122081	0.00	0.00	OcCOLO-Roan	CRCT	CRCT
E	RON13-E-M2		RON4_122082	0.00	0.00	OcCOLO-Roan	CRCT	CRCT
E	RON13-E-M3		RON4_122083	0.17	0.00	OcCOLO-Roan	CRCT	CRCT
F	RON13-F-201	0	RON4_122084	0.00	0.00	OcCOLO-Roan	CRCT	
F	RON13-F-M1		RON4_122085	0.00	0.00	OcCOLO-Roan	CRCT	CRCT
F	RON13-F-M2		RON4_122086	0.00	0.00	OcCOLO-Roan	CRCT	CRCT
F	RON13-F-M3		RON4_122087	0.00	0.00	OcCOLO-Roan	CRCT	CRCT

Table 2c. Eight families were created (with 25 parents) at Roan Creek on June 4, 2014 using two males to fertilize each female. Female length (mm) is indicated in the FishID column along with the number of fry eligible for transfer to GSH. Admixture as measured with AFLPs is given by qYSCT (Yellowstone Cutthroat Trout admixture) or qRBT (Rainbow Trout admixture). Any evidence of nonnative alleles in five nuclear genes or the Omy1 region are indicated with either YSCT or RBT, otherwise CRCT (Colorado River Cutthroat Trout; where tests do not distinguish CRCT lineages). Families shown in gray were culled because of the fish highlighted in red, leaving six families from 18 parents for inclusion into broodstock.

Family	FishID	#Fry	PiscesID	qYSCT	qRBT	ND2 haplotype	Nuclear	Omy1
A	RON A-212	82	RON5_127694	0.00	0.00	OcCOLO-Roan	CRCT	
A	RON A-M1		RON5_127695	0.00	0.00	OcCOLO-Roan	CRCT	CRCT
A	RON A-M2		RON5_127696	0.00	0.00	OcCOLO-Roan	CRCT	CRCT
B	RON B-200	27	RON5_127697	0.00	0.00	OcCOLO-Roan	CRCT	
B	RON B-M1		RON5_127698	0.05	0.00	OcCOLO-Roan	CRCT	CRCT
B	RON B-M2		RON5_127699	0.00	0.00	OcCOLO-Roan	CRCT	CRCT

C	RON C-210	159	RON5_127700	0.00	0.00	OcCOLO-Roan	CRCT	
C	RON C-M1		RON5_127701	0.00	0.00	OcCOLO-Roan	CRCT	CRCT
C	RON C-M2		RON5_127702	0.00	0.00	OcCOLO-Roan	CRCT	CRCT
C	RON C-M3		RON5_127703	0.00	0.04	OcCOLO-Roan	CRCT	RBT
D	RON D-204	21	RON5_127704	0.00	0.00	OcCOLO-Roan	CRCT	
D	RON D-M1		RON5_127705	0.00	0.00	OcCOLO-Roan	CRCT	CRCT
D	RON D-M2		RON5_127706	0.00	0.00	OcCOLO-Roan	CRCT	CRCT
E	RON E-212	69	RON5_127707	0.03	0.00	OcCOLO-Roan	CRCT	
E	RON E-M1		RON5_127708	0.00	0.00	OcCOLO-Roan	CRCT	CRCT
E	RON E-M2		RON5_127709	0.00	0.00	OcCOLO-Roan	CRCT	CRCT
F	RON F-240	68	RON5_127710	0.00	0.00	OcCOLO-Roan	CRCT	
F	RON F-M1		RON5_127711	0.00	0.00	OcCOLO-Roan	CRCT	CRCT
F	RON F-M2		RON5_127712	0.00	0.00	OcCOLO-Roan	CRCT	CRCT
G	RON G-170	1	RON5_127713	0.00	0.00	OcCOLO-Roan	CRCT	
G	RON G-M1		RON5_127714	0.00	0.00	OcCOLO-Roan	CRCT	CRCT
G	RON G-M2		RON5_127715	0.00	0.00	OcCOLO-Roan	CRCT	CRCT
H	RON H-174	105	RON5_127716	0.00	0.00	OcCOLO-Roan	CRCT	
H	RON H-M1		RON5_127717	0.01	0.00	OcCOLO-Roan	CRCT	CRCT
H	RON H-M2		RON5_127718	0.00	0.00	OcCOLO-Roan	CRCT	CRCT

Table 2d. Eleven families were created (with 33 parents) at Roan Creek on May 26, 2015 using two males to fertilize each female. Female length (mm) is indicated in the FishID column along with the number of fry eligible for transfer to GSH. Admixture as measured with AFLPs is given by qYSCT (Yellowstone Cutthroat Trout admixture) or qRBT (Rainbow Trout admixture). Any evidence of nonnative alleles in five nuclear genes YSCT or RBT, otherwise CRCT (Colorado River Cutthroat Trout; where tests do not distinguish CRCT lineages). No Omy1 results were available in 2015. Families shown in gray were culled because of the fish highlighted in red, leaving six families from 18 parents for inclusion into the broodstock.

Family	FishID	#Fry	PiscesID	qYSCT	qRBT	ND2 haplotype	Nuclear	Omy1
A	RON-A-190	160	RON6-132804	0.00	0.00	OcCOLO-Roan	CRCT	
A	RON-A-M1		RON6-132805	0.00	0.00	OcCOLO-Roan	CRCT	
A	RON-A-M2		RON6-132806	0.00	0.00	OcCOLO-Roan	CRCT	
B	RON-B-223	180	RON6-132807	0.00	0.00	OcCOLO-Roan	CRCT	
B	RON-B-M1		RON6-132808	0.00	0.00	OcCOLO-Roan	CRCT	
B	RON-B-M2		RON6-132809	0.00	0.00	OcCOLO-Roan	CRCT	
C	RON-C-227	170	RON6-132810	0.00	0.00	OcCOLO-Roan	CRCT	
C	RON-C-M1		RON6-132811	0.00	0.00	OcCOLO-Roan	YSCT	
C	RON-C-M2		RON6-132812	0.00	0.00	OcCOLO-Roan	CRCT	
D	RON-D-200	105	RON6-132813	0.00	0.00	OcCOLO-Roan	CRCT	
D	RON-D-M1		RON6-132814	0.00	0.00	OcCOLO-Roan	CRCT	
D	RON-D-M2		RON6-132815	0.01	0.00	OcCOLO-Roan	CRCT	
E	RON-E-286	384	RON6-132816	0.00	0.00	OcCOLO-Roan	CRCT	
E	RON-E-M1		RON6-132817	0.00	0.00	OcCOLO-Roan	CRCT	
E	RON-E-M2		RON6-132818	0.00	0.00	OcCOLO-Roan	CRCT	
F	RON-F-223	109	RON6-132819	0.00	0.00	OcCOLO-Roan	CRCT	
F	RON-F-M1		RON6-132820	0.00	0.00	OcCOLO-Roan	YSCT	
F	RON-F-M2		RON6-132821	0.01	0.00	OcCOLO-Roan	CRCT	
G	RON-G-229	57	RON6-132822	0.01	0.00	OcCOLO-Roan	CRCT	

G	RON-G-M1		RON6-132823	0.00	0.00	OcCOLO-Roan	CRCT
G	RON-G-M2		RON6-132824	0.00	0.00	OcCOLO-Roan	CRCT
H	RON-H-247	239	RON6-132825	0.00	0.00	OcCOLO-Roan	CRCT
H	RON-H-M1		RON6-132826	0.01	0.17	OcCOLO-Roan	YSCT
H	RON-H-M2		RON6-132827	0.00	0.00	OcCOLO-Roan	YSCT
I	RON-I-273	281	RON6-132828	0.00	0.00	OcCOLO-Roan	CRCT
I	RON-I-M1		RON6-132829	0.00	0.00	OcCOLO-Roan	CRCT
I	RON-I-M2		RON6-132830	0.12	0.00	OcCOLO-Roan	CRCT
J	RON-J-214	202	RON6-132831	0.00	0.00	OcCOLO-Roan	CRCT
J	RON-J-M1		RON6-132832	0.00	0.00	OcCOLO-Roan	CRCT
J	RON-J-M2		RON6-132833	0.00	0.00	OcCOLO-Roan	CRCT
K	RON-K-227	155	RON6-132834	0.00	0.00	OcCOLO-Roan	YSCT
K	RON-K-M1		RON6-132835	0.00	0.00	OcCOLO-Roan	CRCT
K	RON-K-M2		RON6-132836	0.00	0.00	OcCOLO-Roan	CRCT

In 2014, we hoped to reduce the odds of introducing nonnative alleles into each family by only incorporating milt from two males rather than three (as was the case in 2012 and 2013). Future efforts using this purging approach should evaluate prevalence of admixture and run simulations to evaluate the tradeoff between maximizing genetic diversity by adding additional males vs the potential cost of having to cull the family if odds of drawing an admixed male are high. We also acknowledge that the molecular tests used here are likely insufficient to detect all nonnative admixture, and that newer methods (eg. GTseq, RADseq, etc.) would allow much more robust screening. We hope that others will take advantage of these new molecular tools to help purge recently invaded nonnative alleles from similar systems.

Mandatory culling of families coupled with an extremely short window from which to take eggs from the relatively small donor population, we have only been able to represent a handful of adults from each yearclass (20 in 2012, 12 in 2013, 18 in 2014, and 18 in 2015). With relatively few parents represented in the broodstock n=68), inbreeding depression and fitness in populations founded from it will be an ongoing concern (Stockwell et al. 1996; Whiteley et al. 2015). Fortunately, isolated DNAs from every parent used in the development of this broodstock are available. Future monitoring of repatriated populations with more robust molecular tools would allow determining how many of the original 68 parents are actually still represented in the population, and whether a severe bottleneck has likely been imposed.

Note - Given the concern over imposing a genetic bottleneck on this stock, the Glenwood Hatchery was compelled to maintain older brood beyond their third year. Genetic diversity could be maximized by keeping fewer females from each year-class, but maintaining individual year-classes as long as possible. Unfortunately, keeping adult fish around a hatchery too long is also a recipe for encouraging an outbreak of Bacterial Kidney Disease (BKD). In October 2015, older fish began to die unexpectedly and BKD was identified as the proximate cause. Per regulation, the entire hatchery had to be depopulated, and the fish disposed. Thankfully, an exemption was granted to move a good portion of them (potentially containing alleles from all 68 founding parents) to newly reclaimed habitat in East Fork Parachute Creek (pages 28-35,

this volume). It is unfortunate that we could not derive as much benefit from this broodstock as was planned.

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RESEARCH PRIORITY
Cutthroat Trout Conservation

OBJECTIVE
Evaluate the infection status of Renibacterium salmoninarum in a Cutthroat Trout population following removal of Brook Trout.

Renibacterium salmoninarum persists in East Fork Parachute Creek nine years after eradication of Brook Trout

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INTRODUCTION

Flowing west off the Roan Plateau before plunging over a large waterfall on its way to the Colorado River, East Fork of Parachute Creek (EFkP) was historically one of the most prolific Cutthroat Trout (*Oncorhynchus clarkii*) streams in Colorado (Roberts et al. 2013; Zeigler et al. 2019). The stream supports standing populations of age-2 and older Brook and Cutthroat Trout as high as 820 per km (1,320 per mile; Peterson and Fausch 2002). Brook Trout (*Salvelinus fontinalis*) were first detected above the EFkP in 1972, and already comprised over half the trout population (CPW unpublished data). By 2001 over 97% of the fish age-2 and older were Brook Trout (Peterson and Fausch 2002). Surveys conducted in 2010 revealed that the Colorado River Cutthroat Trout were functionally extirpated (Hirsch et al. 2013), completely replaced by Brook Trout. This stream provides particularly compelling trout habitat for its relatively warm temperatures ideal for growth (Table 1; Roberts et al. 2013; Zeigler et al. 2019). The presence of 59 m (195 ft) East Fork Falls that isolates the upper 11.5 km (7.2 miles) of stream would have precluded the presence of trout historically. Though undocumented, the first trout were likely introduced prior to 1938, as molecular tools suggest they were derived from the wild spawn operation at Trappers Lake prior to the introduction of Yellowstone Cutthroat Trout alleles into that system (Evans and Shiozawa 2004; Rogers et al. 2018; Rogers 2024). Because this drainage lies in the headwaters of the Colorado River, the native trout lineage in the immediate area would have been Uncompahgre Cutthroat Trout (sensu Rogers 2025, this volume) rather than Green River Cutthroat Trout that call Trappers Lake home (Metcalf et al. 2012; Rogers et al. 2018; Bestgen et al. 2019).

Table 1. Maximum Weekly Maximum Temperature (MWMT), Maximum 30-day Average Temperature (M30AT), maximum daily temperature, and accumulated annual degree days calculated over four years from a temperature logger placed at 13S 246877m E 4384198m N below the temporary barrier at on East Fork Parachute Creek. The barrier bisects the creek upstream of East Fork Falls.

Year	MWMT (ºC)	M30AT (ºC)	Daily Max (ºC)	Annual Degree Days (ºC)
2019	16.6	12.1	16.9	1,643
2020	16.6	13.3	17.8	1,880
2021	16.7	13.6	17.2	1,882
2022	15.9	12.4	16.8	1,786

With only 69 conservation populations distributed across their native range occupying just 3% of historically occupied habitat (Rogers et al. 2022), Uncompahgre Cutthroat Trout are the focus of intense conservation efforts. Central to those efforts was the development of a broodstock aimed at securing a peripheral population in Roan Creek that harbors a unique genotype (Rogers et al. 2020) and phenotype (Bestgen et al. 2019). Broodstock development was complicated by recent invasions of nonnative trout into Roan Creek which required purging alleles introduced by Rainbow Trout and Yellowstone Cutthroat Trout (detailed on pages 18-27, this volume).

East Fork of Parachute Creek was slated to be the first reintroduction site to receive progeny from the Roan Creek stock. A temporary concrete barrier was installed in 2012 (Figure 1), and planning for a reclamation in the headwaters commenced (Martin and Rosenlund 2014). This upper section was treated with rotenone in August of 2014, and successful eradication of Brook Trout was achieved. The reach downstream of the temporary concrete barrier and upstream of East Fork Falls (Figure 1) was treated in 2018 and 2019, but required several more years of spot treatments, electrofishing, and eDNA sampling to finally eradicate Brook Trout there.

Figure 1. The temporary concrete barrier that bisects the headwaters of E Fk Parachute Creek is shown on the left, while the stream drops over the East Fork Falls from the top left of the right photograph.

Unfortunately, *Renibacterium salmoninarum* (causal agent of Bacterial Kidney Disease; BKD) was detected in the developing brood at the Glenwood Hatchery in October of 2015. This required the hatchery be depopulated and all fish disposed. However, an exemption for the Roan Creek brood was sought for several reasons: 1) nonnative alleles had become well established in Roan Creek, so repeating the complex and expensive process for broodstock development was impossible, 2) Sixty brood culls from 2013 (averaging 9.2 in) and 60 from 2014 (averaging 5.8 in) were already stocked into EFkP in July of 2015 (with a clean health inspection) and 3) Brook Trout below the temporary barrier on East Fork Parachute Creek already tested positive for *R. salmoninarum*. The exemption was granted for some of the fish (Table 2), and on October 28, 2015 some of the Glenwood Hatchery broodstock and their progeny were stocked upstream of the concrete barrier in EFkP. Half of the wild progeny from the 2015 wild spawn operation still at the Fish Research Hatchery (FRH) were stocked five days later. A failed well pump at FRH in April 2016 meant that the remaining 411 fish had to be stocked immediately. Since EFkP is inaccessible by truck or snowmobile at that time of year, the fish had to be flown from a staging area at the base of the Roan Plateau to the top in a helicopter. Fish were distributed upstream of the temporary barrier either on foot or with a firefighting helibucket. In total, 5,262 fish were stocked in the drainage potentially representing at most, 68 parents (Table 2). The objectives for this study were two-fold, 1) we sought to evaluate the population status of these reintroduced fish, and 2) examine whether *R. salmoninarum* still persists in their wild progeny, nine years after stocking in a stream cleared of Brook Trout.

Table 2. Number of parents spawned on Roan Creek by year and the number retained following genetic testing of each parent that contributed to the developing broodstock at Glenwood Springs Hatchery. Progeny of wild parents stocked into E Fk Parachute Creek are indicated as "Stocked Wild", while progeny from the broodstock as "Stocked Hatchery". Average size at stocking is also provided.

Year	Spawned	Retained	Stocked Wild	Stocked Hatchery	Size (mm)
2012	32	20	0	4,000	40 (1.6 in)
2013	24	12	60	-	234 (9.2 in)
2014	19	15	224	-	196 (7.7 in)
2015	33	18	978[1]	-	53 (2.1 in)

[1]411 of these were stocked in April 2016 at 100 mm (4 in)

METHODS

Population size estimation

Cutthroat Trout from the East Fork of Parachute Creek were sampled at Station CR2637 with a three-person crew using two LR-24 electrofishers (Smith-Root, Vancouver, Washington) on July 16, 2024. This was as close to the spawn as practical to coincide with post spawn fish that might be more likely to show signs of BKD if still present in the population. Pulsed DC current (70Hz; 4 ms pulse width) yielding approximately 24 W was used to gather fish over two removal passes. A population estimate was generated using JOM 3.0 (Rogers 2006) on fish age-1 or larger determined from the length frequency of captured fish, as well as using the 150 mm standard cutoff for entry into the Inland Cutthroat Protocol (ICP) database (Hirsch et al. 2013).

BKD testing

Adult Cutthroat Trout collected during the electrofishing survey were supplemented with additional adults gathered by angling to generate a 30 fish sample. Upon collection, all 30 fish were euthanized and immediately dissected. Kidney, liver, and spleen tissues were harvested from each fish for subsequent molecular and immunoassay analysis. Individual tissues were stored in Whirl-Pak bags and frozen at −20°C for later processing.

Quantitative Polymerase Chain Reaction (qPCR) Analysis

For DNA extraction, all frozen tissue samples were thawed and homogenized. Individual spleen, liver, and kidney samples were manually homogenized with a sterile rolling pin while remaining in the original Whirl-Pak bags. Two replicates of approximately 0.25 g of each tissue sample were prepared for DNA extraction. DNA extractions were completed using a Qiagen DNeasy Blood and Tissue Kit (Qiagen, Germantown, Maryland). To improve DNA yield, the AE buffer volume was increased to 400 µL during all DNA extraction protocols to ensure proper rinsing of filters (Elliott et al. 2013).

Quantitative PCR assays were performed on an ABI StepOnePlus System (Applied Biosystems, Foster City, California, USA) to detect a 69-base pair DNA segment of the *msa* gene specific to *R. salmoninarum* (Chase et al. 2006). Each reaction consisted of a final volume of 5 µL of extracted DNA template combined with TaqMan Gene Expression Master Mix (ThermoFisher, Waltham, Massachusetts) and a predetermined primer and probe set. The primer sequences were RS 1238 Forward (5′-GTGACCAACACCCAGATATCCA-3′) and RS 1307 Reverse (5′-TCGCCAGACCACCATTTACC-3′) with MGB probe 1262 (5′-CACCAGATGGAGCAAC-3′) (Chase et al. 2006). DNase-free water was used as a no-template control on each plate. qPCR cycle threshold (Cq) scores were adjusted based on a previously developed standard curve with a cutoff value of 37.75 (Firestone et. al 2025).

Enzyme-linked immunosorbent assay (ELISA)
A double-sandwich ELISA was used to detect soluble antigens of *R. salmoninarum* (Jansson et al. 1996, Elliott et al. 2014). Kidney tissues were prepared to a 1:4 (w/v) dilution in PBS-T20 (phosphate-buffered saline pH 7.4, 0.05% (v/v) Tween-20, and 0.01% (w/v) thimerosal), homogenized, heated at 100°C for 15 minutes, and then stored at −20°C until screening. Affinity-purified *R. salmoninarum* goat antibodies (Kirkegaard and Perry Laboratories, Gaithersburg, Maryland) were used as the coating antibody, and horseradish-peroxidase labeled *R. salmoninarum* goat antibodies served as the antibody conjugate, as previously described (Pascho et al. 1991).

A control microplate was used for each reaction, containing replicates of cell culture water, HRP-conjugate controls, substrate-chromogen controls, and Brook Trout tissue serving as negative controls. Four concentrations of *R. salmoninarum* positive controls (KPL; 1:100, 1:1000, 1:2000, 1:5000) were also processed in replicates for each reaction. Colorimetric development in each sample was measured at 405 nm. Positive samples were determined by optical density (OD) values that were above the average negative control value plus 2 standard deviations (SD). The sensitivity of this ELISA method is approximately between 2 and 20 ng of *R. salmoninarum* (Pascho and Mulcahy 1987).

RESULTS & DISCUSSION

Seventy Cutthroat Trout were collected in two passes (Figure 2) from a necessarily shortened station CR2637 (225 ft), yielding a population estimate of age-1 or larger fish of 1,053 per km (1,696 per mile; capture p=0.82). The estimated adult population size was 306 per km (493 per mile; capture p = 0.76) when using the 150 mm threshold mandated for inclusion into the CRCT online database (Hirsch et al. 2013). Larger adult Cutthroat Trout caught electrofishing were combined with ten angled adults collected immediately above the temporary barrier, and sampled for the presence of *R. salmoninarum.* Mean size was 196 mm, and represented entirely adult fish.

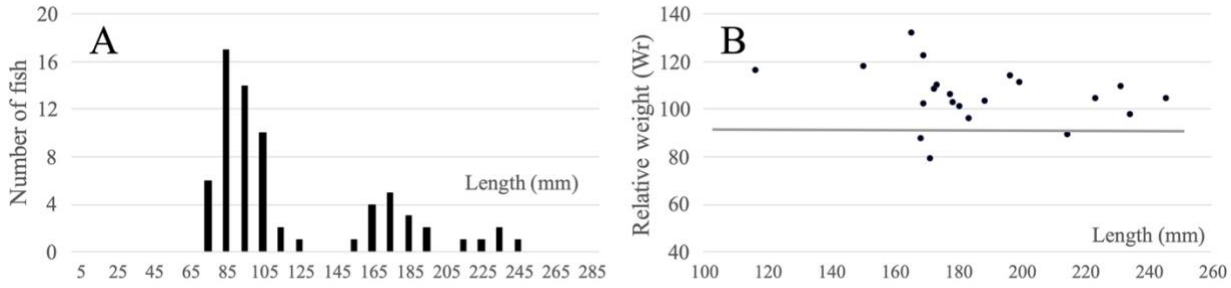

Figure 2. Length frequency of fish captured on July 16, 2024 from the E Fk of Parachute Creek (A), including relative weights on older fish (B). The gray horizontal line represents average condition of around 93%.

Detection of Renibacterium salmoninarum via qPCR
Of the 30 fish sampled for analysis, 17 (57%) were positive for *R. salmoninarum* based on qPCR detection in at least one of the three tissues collected. Detections were distributed across multiple tissues, with 41.2% (7/17) of infected fish having detections in the liver, 29.4% (5/17) in the spleen, and 23.5% (4/17) in the kidney. No fish tested positive in all three tissues. Overall, bacterial loads were low across all tissues. The mean bacterial load was highest in liver tissue (0.3857±0.79 cells per gram of tissue), followed by spleen (0.2431±0.58 cells per gram of tissue) and kidney (0.1589±0.43 cells per gram of tissue).

Detection of Renibacterium salmoninarum via ELISA
In contrast to the qPCR results, ELISA detected *R. salmoninarum* antigen in all 30 (100%) of the sampled fish. While prevalence was high, antigen concentrations were generally low. The majority of positive samples across all tissues fell into the "Low" antigen level category (OD values between 0.074 and 0.199). Specifically, 90.0% of all sampled tissues (27/30) had low antigen concentrations. A smaller proportion of samples showed "Mid" antigen levels (OD values between 0.200 and 0.999), accounting for 10.0% (3/30) of the samples. No samples had "High" antigen levels (OD values of 1.00+). When analyzed by tissue, the proportions of low antigen levels were consistent across kidney, spleen, and liver (all at 83.3%). The highest proportions of mid-level antigen concentrations were found in the spleen and liver tissues (5/30 fish; 16.7%).

Despite the continued presence of *R. salmoninarum*, the introduced fish appear to be doing well, with no clinical signs of disease. Robust natural recruitment was verified, and every year-class was represented. Population estimates are as high as total trout estimates prior to 2001, and fish condition is well above average (Figure 2; mean Wr = 105%). Willow regrowth in the headwaters is exceptional (Figure 3), and if the eradication of Brook Trout continues to be confirmed, then reintroduction of North American beaver *Castor canadensis* should be considered. With DNA isolated and archived for every parent used to found this population, it would be instructive to canvas the current population now and in the future, to determine 1) which parents contributed most to founding the new population, and 2) are those with nonnative alleles disproportionally over or under represented (indicating inbreeding or outbreeding depression).

Figure 3. Negotiating extensive willow growth on E Fk Parachute Creek to obtain a population estimate.

ACKNOWLEDGMENTS

We thank Brad Neuschwanger (Fish Research Hatchery and Dave Davis (Glenwood Hatchery) for raising the Roan Creek brood used to repatriate the East Fork Parachute Creek fishery. Brad Petch is thanked for allowing us to use helicopter time at the last minute to distribute the remaining Roan Creek brood into the headwaters of the stream. We also thank Andrew Held and Alyse Erenberger for assisting with the population surveys and sample collection.

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RESEARCH PRIORITY
Culture of native Cutthroat Trout

OBJECTIVE
To improve early life survival in native Greenback Cutthroat Trout

Milt activator but not extenders enhance embryo survival in Rio Grande Cutthroat Trout (Oncorhynchus clarkii virginalis)

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INTRODUCTION

Fish culture plays a critical role in the conservation and recovery of imperiled species (Froehlich et al. 2017; Osborne et al. 2020; Overton et al. 2024; Bradford et al. 2025; Korman et al. 2025). This is especially true for our native Cutthroat Trout (*Oncorhynchus clarkii*) taxa of the southern Rocky Mountains, where all subspecies face a variety of threats to persistence (Roberts et al. 2013; Penaluna et al. 2016; Zeigler et al. 2019). Teams charged with conservation and recovery of these taxa rely heavily on reclamation projects to restore native trout across their native ranges (Stuber et al. 1988; Alves et al. 2008; Fausch et al. 2009; Hirsch et al. 2013; Penaluna et al. 2016). These projects require large numbers of fish to repatriate newly reclaimed habitats, making hatchery production often the only realistic option for success (Dwyer and Rosenlund 1988).

Unfortunately, native Cutthroat Trout can be difficult to raise in captivity (Rogers et al. 2022a; Rogers et al. 2022b), one of the reasons nonnative salmonids were introduced into the southern Rocky Mountains in the late 1800s (Wiltzius 1985; Behnke 2002). This is especially true for some high-value populations such as the Greenback Cutthroat Trout (*O. clarkii ssp*), where only 5% of the eggs taken survive to fingerling stage (Bryan Johnson, personal communication). This fish is challenged by poor survival and growth even when cultured in small lots (Rogers et al. 2022b), presumably a consequence of low genetic diversity (Rogers et al. 2022a). In such instances, even a 5% increase in survival would translate into a halving of fertilized egg demand (from 800,000 down to 400,000 eggs annually) resulting in huge time and cost savings.

A variety of new culture products are now available that purport to boost fertilization success (Haffrey et al. 2008; Merino et al. 2017). Hatchery managers have cited improved fertilization and survival to hatch with these products, but outcomes are highly variable and their success has not been rigorously tested. Here we use Colorado's single captive Rio Grande Cutthroat Trout (*O. c. virginalis*) broodstock to conduct a robust evaluation that tracks the contributions to survival in progeny from individual parents.

METHODS

Prior to spawning, we prepared stock solutions of the egg extender (Ovafish), milt extender (Storfish), and milt activator (Actifish) sold by Syndel (Ferndale, Washington). These spawning treatments were diluted 10:1 per the manufacturers recommended protocol for salmonids (IMV technologies USA, Brooklyn Park, Minnesota; www.imv-technologies.com)

Gamete collection and fertilization

We collected eggs and milt from a dozen three-year-old female and four-year-old male Rio Grande Cutthroat Trout from CPW's broodstock housed at the Pitkin State Fish Hatchery in Pitkin, Colorado in April of 2024. Eggs stripped from each female were funneled into a large dry measuring cup. Half the eggs were decanted into a smaller measuring cup with 90 ml of Ovafish stock solution, while the remainder were split between two bowls (A and B; Figure 1). Milt was stripped into a dry glass bowl, and 250 ul were pipetted out and used to fertilize bowls A and B, with B then "activated" with 120 ml of Actifish solution while A only received hatchery water as control. Eggs from the Ovafish rinse were strained, then split into bowls C and D, with each receiving 250 ul milt from the same male (Figure 1). Here again, Bowl D received 120 ml of Actifish solution while Bowl C received 120 ml of water. All eggs were given 60 s to fertilize before the excess milt was rinsed off with hatchery water, then water hardened in 100 ppm iodine solution (1% Ovidine, Syndel (Ferndale, Washington) for 10 min.

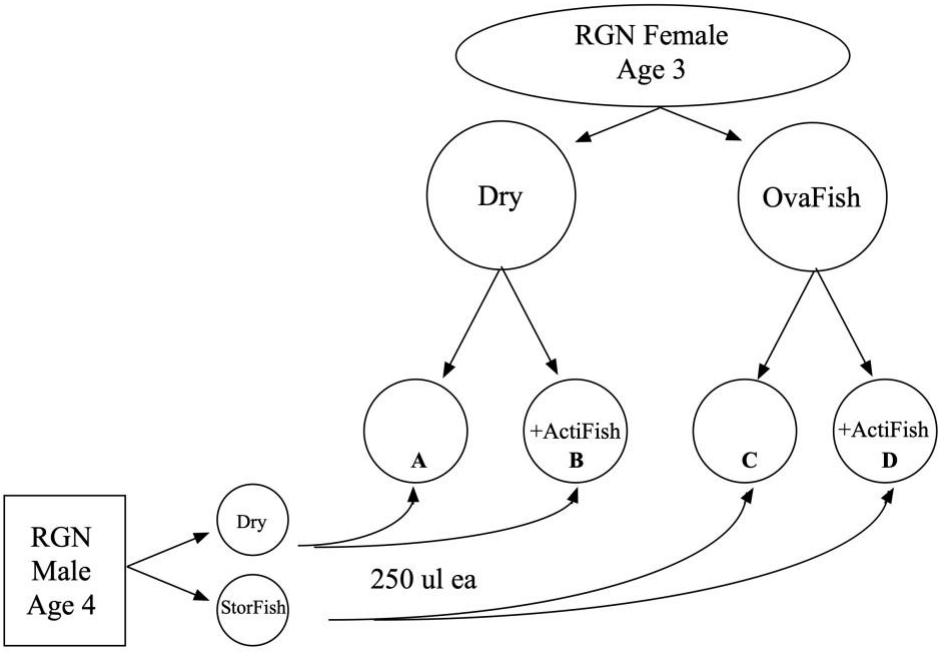

Figure 1. Eggs from a single female were split into two bowls, one that contained egg extender and one that was dry. The contents of each bowl were split into another two bowls labeled A and B (dry) or C, and D (egg extender). Milt (1 ml) was extruded from a single male into a dry bowl. From that bowl, 250 ul were pipetted into to Bowls A through D, followed by activation with water (Bowls A and C), or milt activator (Bowls B and D).

Incubation
Following iodine treatment, eggs were transferred to egg baskets (MariSource, Burlington, Washington) that each occupied a quarter of a Heath tray. Position in the tray was randomly assigned *a priori*, and each basket was labeled with a colored zip tie. Eggs were allowed to complete water hardening for an additional 50 min before being moved. After hardening, each tray was returned to the Heath Stack where it experienced a flow rate of 11.4 l/m [682 l/hr] of first use spring water (pH = 7.5; hardness = 120 ppm). Water temperatures were monitored hourly with a HOBO pendant MX2201 temperature logger (Onset Computer Corporation, Bourne, Massachusetts). Eggs were treated on alternate days with 800 ppm of 35% H_2O_2 administered for 10 min to preclude the development of Saprolegnia fungus.

Embryos were expected to develop a hard eye 22.7 days after fertilization upon accumulating around 234 degree-days (ºC; 420 degree-days ºF), based on hatchery inflow temperatures which averaged 50.5 ºF (10.3 ºC). At 23 days post fertilization, all dead eggs were enumerated and removed prior to bumping (shocking). Dead eggs were again enumerated and removed an hour after bumping, and remaining live embryos were also counted.

Data analyses
Because the same families were used in all four treatments, we were able to interpret the results in a paired-t framework. We examined the difference in survival with the addition of Actifish over controls (Bowl B - Bowl A; henceforth DryA-Dry, Actifish with OvaFish and StorFish (OSA) over OS alone (Bowl D – Bowl C; henceforth OSA-OS), OS over dry control (Bowl C - Bowl A; henceforth OS-Dry), Actifish and OS over Actifish and dry control (Bowl D – Bowl B; henceforth OSA-DryA). With four comparisons, we used a Bonferroni correction (alpha = 0.0125) to assess differences between groups.

RESULTS & DISCUSSION

Measured incubation temperatures averaged 9.5 ºC (49.2 ºF) which allowed the eggs to develop a hard eye at 23 days post fertilization (after accumulating 218 degree-days ºC; 395 degree-days ºF). Logistic constraints precluded rearing the embryos further in isolation, so the experiment was terminated after this stage. We detected extreme variation in survival to the eyed egg stage, with some families displaying robust survival regardless of treatment (Figure 2), some very poor survival regardless of treatment, and some where treatment had a pronounced effect on survival.

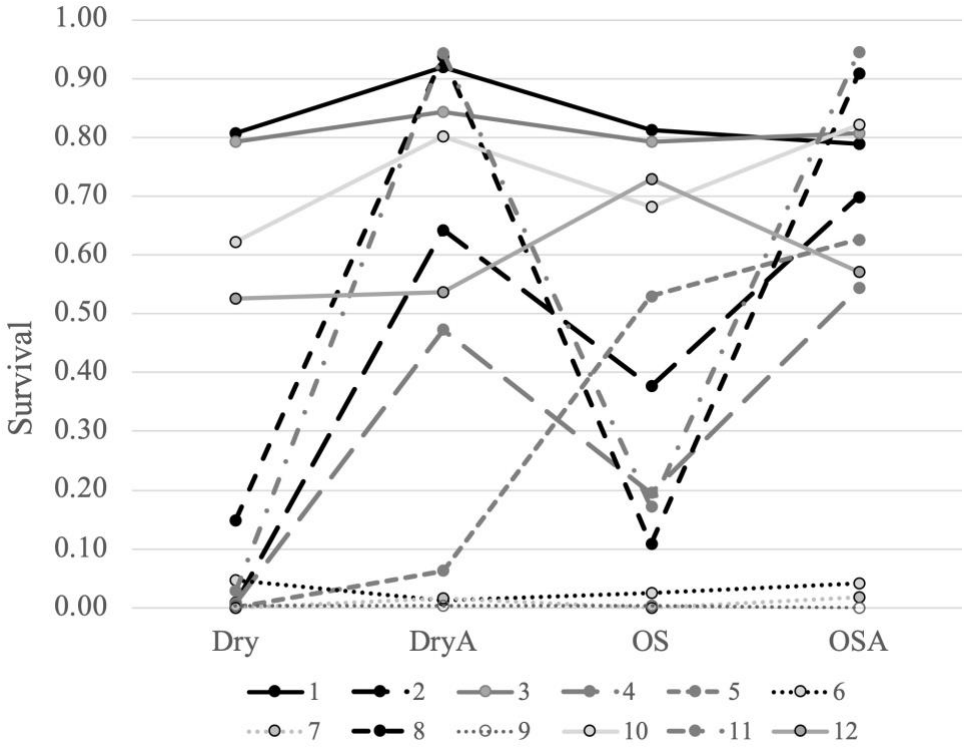

Figure 2. Survival to the eyed egg stage (23 d post fertilization) for each treatment by family. The four treatments were dry method controls (Dry), dry with ActiFish (DryA), OvaFish and StorFish (OS), and OvaFish and StorFish with ActiFish (OSA). Eggs with poor survival across treatments are indicated with dotted lines, while those with very high survival across treatments with solid ones. Dashed lines reflect where treatment had a substantial affect.

Mean survival to eye with the addition of ActiFish was twice that of the control Dry group (0.516 vs 0.249). While eggs treated with OS and ActiFish also survived well to eye (0.564), the influence of Actifish over the the OS alone was not quite as great (0.369). Comparing differences in survival within families across treatments also showed that eggs receiving Actifish displayed over twice the survival on average of their counterparts that received none (Table 1, Figure 3). Variation between families was substantial (Figure 2).

Table 1. Mean differences in survival (S) between the four treatment groups with variance and associated paired t-test one-tailed p-values.

Treatment	Mean Diff S	Variance	P-value
DryA-Dry	0.267	0.116	0.010
OS-Dry	0.120	0.032	0.020
OSA-OS	0.195	0.096	0.026
OSA-DryA	0.048	0.029	0.174

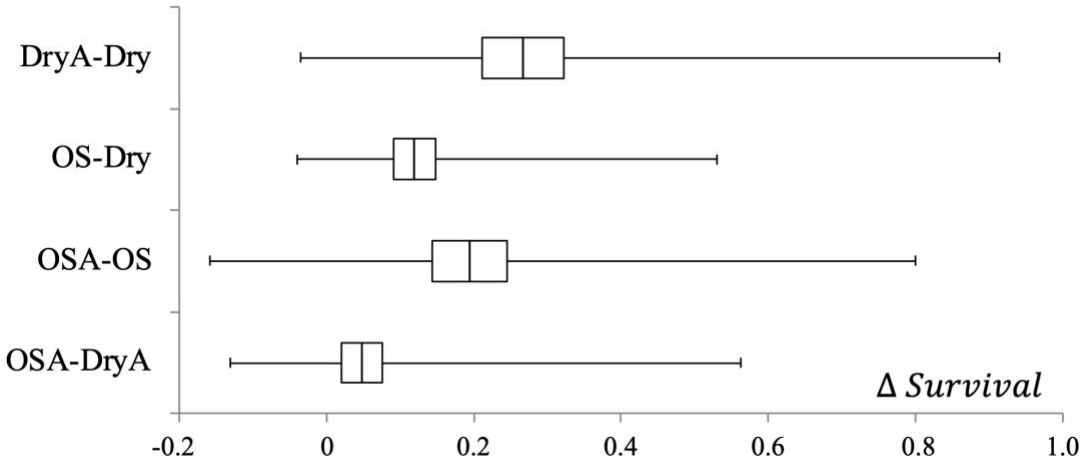

Figure 3. Paired differences between survival to the eyed egg stage with the addition of milt activator alone (DryA-Dry), milt and egg extenders compared to traditional dry method (OS-Dry), milt and egg extenders with and without milt activator (OSA-OS), and milt activator with and without milt and egg extenders (OSA-DryA). Vertical bars represent the mean and 95% confidence intervals around them for each treatment, while whiskers cover the range of values recorded.

Extreme variation in survival to eye by female was detected here as in other studies on Cutthroat Trout (Rogers et al. 2020; Rogers et al. 2022b). Some families did poorly regardless of treatment, while others demonstrated exceptionally high survival across all treatments (Figure 2). The response to treatments was even more substantial in the remaining half of the families, where survival more than doubled by the addition of activated milt. While using ActiFish was responsible for substantial increase in survival to the eyed egg stage, we did not test whether those improvements would also translate to enhanced survival to alevins or stocking. Further study should be conducted to evaluate whether this improvement is sustained through all early life stages, particularly with vulnerable inbred stocks.

ACKNOWLEDGMENTS

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RESEARCH PRIORITY
Taxonomy of Colorado's native Cutthroat Trout

OBJECTIVE
Evaluate genetic distance between Cutthroat Trout lineages for barcoding

Full mitochondrial genome sequence reveals gene bias when delineating subspecies using genetic distance

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INTRODUCTION

Conservation biology relies on clear definitions of what constitutes a species, yet no single species concept works well for all organisms (Wilson 1992; Mayden 1997). Biological species concepts are inadequate for taxa that hybridize freely like Pacific trouts (Campton and Utter 1985; Allendorf et al. 2001), and morphological ones can sometimes fail to reveal discrete evolutionary lineages worthy of protection that might otherwise fall by the wayside (Avise 1989; Moritz 1994; Funk et al. 2012). Conversely, new molecular tools allow phylogenetic species concepts to split groups of populations so finely, that limited conservation resources are spread too thin, threatening the very conservation infrastructure meant to support them (Isaac et al. 2004; Garnett and Christidis 2017). The continued splitting of taxa makes it difficult to enforce legal protections, establish long-term recovery plans, and evaluate priorities for conservation (Mace 2004). Most biologists agree that species are independently evolving lineages of populations or metapopulations, but the debate surrounds the choice of an exact cutoff in the divergence continuum (April et al. 2011)

In an effort to simplify delineation and make it more standardized and objective, practitioners have promoted DNA barcoding as a solution (Hebert et al. 2004; Waugh 2007; Teletchea 2010; April et al. 2011). This approach uses short diagnostic DNA sequences to discern taxa – usually a mitochondrial gene in vertebrates such as cytochrome c oxidase subunit 1 (CO1) or cytochrome b (CytB; Hebert et al. 2003), that circumvents many challenges of traditional taxonomy, particularly with regard to cost. By aligning sequence data to large online reference databases (Ratnasingham and Hebert 2007, 2013), barcoding allows researchers to assign specimens to species with high accuracy, even when DNA from old degraded specimens is used (Metcalf et al. 2012; Oosting et al. 2019). More controversial is the practice of delineating new species with this approach (Rubinoff 2006; Zink and Barrowclough 2008), though many continue to use thresholds of genetic distance to make those discriminations (Ward 2009, April et al 2011; Young et al. .2013). Either way, barcoding's relative ease of use has allowed dramatic improvements in monitoring biodiversity and conservation planning (Hebert et al. 2003; Ji et al. 2013).

Sequence data from a variety of mitochondrial genes have been used to characterize Cutthroat Trout of the southern Rocky Mountains (SRM). Early researchers explored NADH dehydrogenase 4 (ND4), CO1, and CytB (Metcalf et al. 2007; Pritchard et al. 2009; Wilson and Turner 2009). More recently, molecular studies have focused on the NADH dehydrogenase 2 (ND2) gene to discriminate different lineages (Metcalf et al. 2007, 2012; Pritchard et al. 2009; Loxterman and Keeley 2012; Rogers et al. 2018; Bestgen et al. 2019), presumably because the early research showed good separation and subsequent studies found great utility in being consistent. However, single gene phylogenies often yield polytomies, making relationships between lineages sometimes difficult to resolve (Metcalf et al. 2012). To address this issue and characterize the remaining diversity in the mitochondrial genome, we used high-throughput methods to sequence a full mitogenome from a Cutthroat Trout representing five major clades in the SRM. We evaluate genetic distance for all 13 coding genes in the mitochondrial genome along with the transfer RNA genes, two ribosomal genes, and the non-coding D-loop.

METHODS

Archived total genomic DNA isolated using DNeasy tissue kits (Qiagen, Germantown, Maryland) following the manufacturers recommended "mouse tail" protocol was used in this study (Table 1). Total mitochondrial DNA was amplified from each sample in three fragments, using primer pairs as follows: Fragment 1: forward primer in tRNA-Glx, reverse primer in ND3, 5975 bp. Fragment 2: forward primer in tRNA-Gly, reverse primer in CytB, 5972 bp. Fragment 3: Forward primer in CytB, reverse primer in ND2, 5635 bp. These three amplified fragments for each of 8 samples were purified with Qiaquick PCR purification columns (Qiagen, Germantown, Maryland) according to manufacturer's instructions, then sent to the University of Colorado BioFrontiers Institute for library preparation and sequencing. Libraries were prepared by tagmentation and indexed using an Illumina Nextera XT DNA Library Preparation kit, then sequenced with an Illumina MiSeq (Illumina, San Diego, California) using paired end reads (2 x 150 bp).

Table 1. Full Cutthroat Trout mitochondrial genomes analyzed in this study

Lineage	Stream	Sample date	Pisces code
Uncompahgre[1]	Carr Creek	11/16/2006	CRR-68306
Green River[2]	Lake Nanita	7/15/2008	NLK2-86080
Greenback	Bear Creek	6/11/2013	BEA3-127721
Rio Grande	Jaroso Creek	6/27/2012	JAR-120308
Yellowstone	Yellowstone River	3/1/2005	LEH-54434

[1]Formerly referred to as the "green" lineage of Colorado River Cutthroat Trout (sensu Metcalf et al. 2012, Rogers et al. 2018, Bestgen et al. 2019, and see pages 12-17, this volume)
[2]Formerly "blue" lineage of Colorado River Cutthroat Trout

Sequence data was annotated and aligned in Geneious R8 (location) to the Lake Nanita sample as reference. Data was imported into MEGA 5 (Tamura et al. 2011) where a neighbor-joining

tree was generated with a Kimura 2-parameter (K2P) model and pairwise deletion of missing data and 200 bootstrap replicates. The K2P model was used to generate mean sequence divergence between lineages as well (Ward et al. 2005; but see Collins et al. 2012)

RESULTS & DISCUSSION

As a trial run for evaluating this approach for sequencing the full mitochondrial genome, we greatly underestimated the amount of coverage we would achieve from these archived DNAs. While inefficient, this allowed us to be extremely confident in the accuracy of the resulting sequence data. The total mitochondrial genome was 16,658 bp long for three of the samples and 16,657 bp for the remaining two (Table 1). The overall base composition in the Lake Nanita reference sample (NLK2) was asymmetric, with 27.7% A, 26.2% T, 28.9% C, 17.2% G, and an AT content of 53.9%, which is slightly lower than Atlantic Salmon (*Salmo salar*; AT 54.8%), and White-spotted Char (*Salvelinus leucomaenis*; AT 54.7%) (Zhang et al. 2015). The phylogenetic tree (Figure 2) shows expected phylogenetic relationships between all taxa.

Table 1. Assembled length of each mitochondrial genome used in this study along with mean coverage for all positions, and minimum or maximum coverage at any position

Lineage	Sample	Assembled length (bp)	Mean coverage	Min	Max
Uncompahgre	CRR-68306	16,657	6,868	1,178	20,319
Green River	NLK2-86080	16,658	6,222	1,136	20,890
Greenback	BEA3-127721	16,657	5,702	780	17,092
Rio Grande	JAR-120308	16,658	7,793	805	23,767
Yellowstone	LEH-54434	16,658	1,815	171	5,998

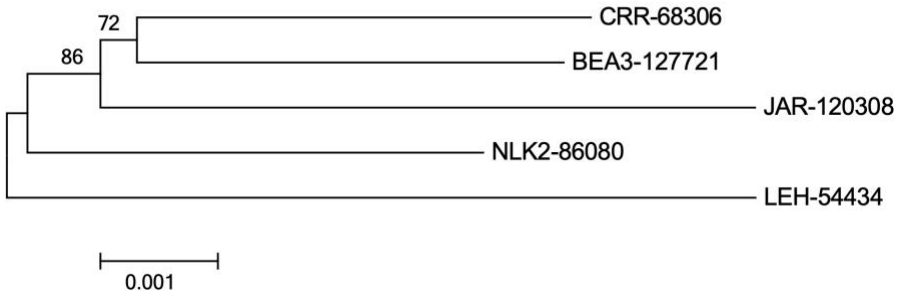

Figure 2. Overall mean genetic distance (sequence divergence) between five lineages of Cutthroat Trout native to the southern Rocky Mountains, indicated in Table 1. The evolutionary history was inferred using a neighbor-joining method with a Kimura 2-parameter (K2P) model and pairwise deletion of missing data and 200 bootstrap replicates. The tree is drawn to scale, with branch lengths measured in the number of base substitutions per site.

Of the 13 protein encoding genes that comprise the trout mitochondrial genome, the ND2 gene was the most variable yielding the highest calculated genetic distance between putative subspecies (Figure 3). Ironically, the cytochrome oxidase I gene that has served as the industry standard for species barcoding (Hebert et al. 2003; April et al. 2011), was one of the least variable. More importantly, when all 16 kilobases in the mitogenome were considered, a phylogeny somewhat similar to what appears in the published literature for ND2 (Metcalf et al. 2012, Rogers et al. 2018) was recovered. However, overall mean genetic distance (sequence divergence) between groups using full mitochondrial genome data was half (1.0%) that registered by the commonly used base pairs from the ND2 gene (2.1%). Genetic distance among lineages ranged from 1.2% between Yellowstone Cutthroat Trout and Rio Grande Cutthroat Trout to as little as 0.7% between Uncompahgre Cutthroat Trout and Greenback Cutthroat Trout. These numbers are important to consider when using thresholds of genetic distance to delineate taxa.

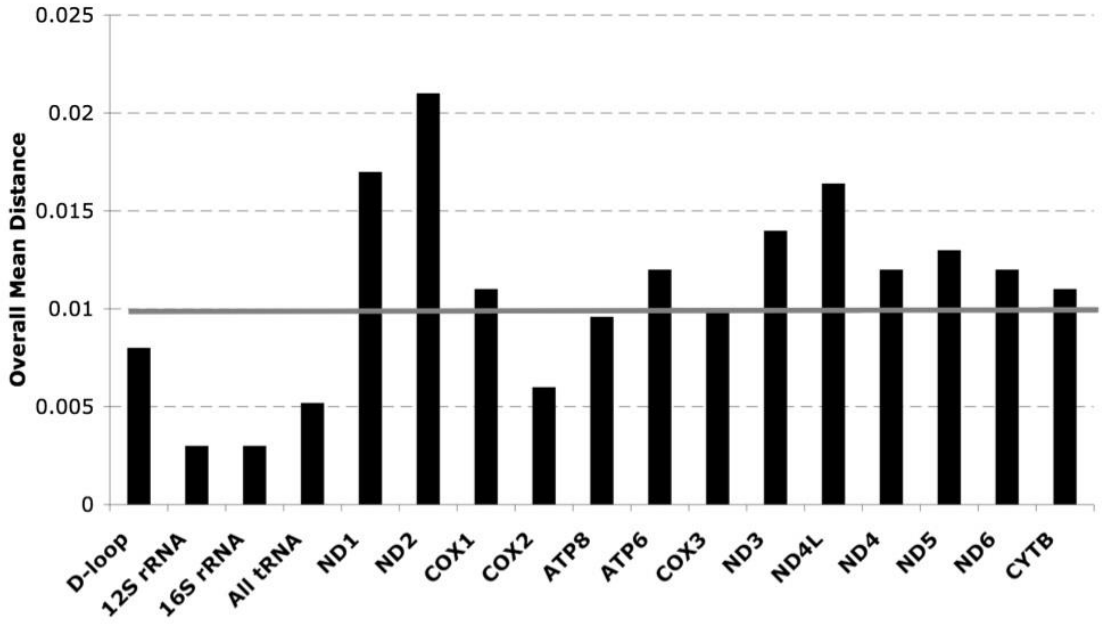

Figure 3. Overall mean genetic distance (sequence divergence) between five lineages of Cutthroat Trout native to the southern Rocky Mountains (Table 1) by gene region across the mitochondrial genome. The overall mean distance is indicated by the gray line.

Several additional observations were noteworthy. While transfer and ribosomal genes were heavily conserved as expected, we anticipated more variation in the mitochondrial D-loop. This region is noncoding, and therefore under weaker selection constraints than coding mtDNA, allowing it to evolve more rapidly. In addition, it also should have higher mutation rates because it is single stranded during replication (Brown et al. 1986; Saccone et al. 2002). Instead, most of the polymorphic SNPs were found in the coding regions of the mitogenome. Importantly, genetic distance was highly variable depending on which genes were used. If "accepted" thresholds for species delineation are made based on genetic distance (Ward 2009; Young et al. 2013), it is imperative that the gene used is specified, and that the relative variation between genes is understood for the taxonomic group in question. For example, if a CO1 standard threshold of 2% is used to apply to ND2 data in salmonids, then that threshold should be

doubled. While mitochondrial DNA provide an important source of data, if used alone or out of context, they offer only a fraction of the information needed to characterize species (Rubinoff 2006).

ACKNOWLEDGMENTS

This study would not have been possible without Janet Epp's diligent lab work that allowed sequencing these full mitogenomes. Her contributions to this field are missed.

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RESEARCH PRIORITY
Information transfer

OBJECTIVE
Disseminate results gleaned from applied research efforts

INTRODUCTION

Management of the aquatic resources of Colorado is facilitated by the close working relationship between researchers and managers, hatchery personnel, and administrators within CPW, as well as extensive collaboration with federal land management partners and outside stakeholders. Dissemination of the results is a critical last step in the applied research effort, so that informed management decisions can be made. While technical assistance is always available from research staff, manuscripts, reports, and presentations are efficient and effective means for communicating results to broader audiences. In addition, these resources serve as valuable archives of information for future generations of biologists and managers.

ACCOMPLISHMENTS

Peer-reviewed publications

Wells, A. G., C. B. Yackulic, J. Kostelnik, A. Bock, R. E. Zuellig, D. M. Carlisle, J. J. Roberts, **K. B. Rogers**, S. M. Munson. 2024. Before the fire: predicting burn severity and potential postfire debris-flow hazards to quantify risk to Colorado River Cutthroat Trout. International Journal of Wildland Fire 33:WF23199.

Abstract.— Background. Colorado River Cutthroat Trout (CRCT; *Oncorhynchus clarkii pleuriticus*) conservation populations may be at risk from wildfire and post-fire debris flows hazards. Aim. To predict burn severity and potential post-fire debris flow hazard classifications to CRCT conservation populations before wildfires occur. Methods. We used remote sensing, spatial analyses, and machine learning to model 28 wildfire incidents (2016–2020) and spatially predict burn severity from pre-wildfire environmental factors to evaluate the likelihood (%) and volume (m3) hazard classification of post-fire debris flow. Key results. Burn severity was best predicted by fuels, followed by topography, physical ecosystem conditions, and weather (mean adjusted R2 = 0.54). Predictions of high or moderate burn severity covered 1.1 (15% of study area) and 1.5 (19% of study area) million ha, respectively, and varied by watershed. Combined high or moderate debris flow hazard classification included 80% of stream reaches with conservation populations and 97% of conservation population point nodes. Conclusions. Predicted burn severity and potential post- fire debris flow indicated moderate to high hazard for CRCT conservation populations native to the Green and Yampa rivers of the Upper Colorado River Basin. Implications. Future management actions can incorporate predicted burn severity and potential post-fire debris flow to mitigate impacts to CRCT and other at-risk resource values before a wildfire occurs.

Lepak, J. M., A. G. Hansen, B. M. Johnson, K. Battige, E. T. Cristan, C. J. Farrell, W. M. Pate, **K. B. Rogers**, A. J. Treble, and T. E. Walsworth. 2025. Cyclical multi-trophic-level responses to a volatile, introduced forage fish: learning from four decades of food web observation to inform management. Fisheries 50: 52-65.

Abstract.— Species introductions can have significant effects on recipient ecosystems. Anticipating potential ecosystem change in response to introduced species based on historical information can help managers prepare for future conditions. Rainbow Smelt *Osmerus mordax* have been introduced widely to improve sport fish growth. As intended, Walleye *Sander vitreus* growth in Horsetooth Reservoir, Colorado increased after Rainbow Smelt introduction, but poor Walleye recruitment occurred as well. Additionally, opossum shrimp *Mysis diluviana* became absent from both predator diets and intermittent surveys, the dominant Daphnia species in Horsetooth Reservoir shifted and Daphnia densities declined significantly. These patterns were repeated during two different time periods of increased Rainbow Smelt abundance, suggesting that Rainbow Smelt have a strong influence on multiple components of the ecosystem. The repetition of responses to Rainbow Smelt offered the opportunity to evaluate indicators to anticipate potential ecosystem regime shifts that restructure predator–prey dynamics across trophic levels. Three predictors (i.e., high estimated Rainbow Smelt abundance, high catch rates of large Walleye, and low Daphnia densities) were associated with poor Walleye recruitment. Simple indicators like these could inform timely management decisions to take advantage of the benefits Rainbow Smelt offer, while lessening their undesirable effects. For example, management decisions could be made, such as preparing for Walleye egg collections, rearing and stocking of Walleye, increasing availability or quality of Walleye spawning habitat, allowing more protective or liberalized adult Walleye harvest to promote natural recruitment, and limiting Rainbow Smelt access to their spawning habitat.

Van Orden, T. S., **K. B. Rogers**, P. Searle, A. L. Kokkonen, D. K. Shiozawa, and R. P. Evans. *In revision*. Full mitochondrial phylogeny of Cutthroat Trout with comments on species delimitation and taxonomy.

Abstract.— Despite the important role that species concepts and taxonomy play in understanding the evolution of organisms, taxonomists have struggled to describe the relationship between distinct lineages of Cutthroat Trout. This struggle inspired a special workshop at the 2015 annual meeting of the American Fisheries Society that emphasized the need for a revised taxonomy of Cutthroat Trout. To further assess the relationship between different Cutthroat Trout lineages, we sequenced and assembled full mitochondrial genomes from 123 Cutthroat Trout from across their native range. We used maximum likelihood and Bayesian phylogenetic approaches to examine the evolutionary relationships between all named Cutthroat Trout subspecies. Through these analyses, we find 11 lineages of Cutthroat Trout that diverged > 2 million years ago, and at least 13 lineages that diverged > 1 million years ago, highlighting the ancient origin of Cutthroat Trout diversity. Despite the ancient split between many Cutthroat Trout lineages, we do not find that Coastal, Westslope, Lahontan, or Yellowstone Cutthroat Trout are more distinct than other lineages, and as such, we continue to recognize a single Cutthroat Trout species (*Oncorhynchus clarkii*) comprised of twelve distinct subspecies, maintaining consistency with conservation efforts, management

plans, ESA-related decisions, while also preserving stability associated with historic nomenclature.

Presentations *(chronological)*

Rogers, K. B. December 5, 2024. Making the impossible possible: clawing rare native trout back from the brink. Area 7 meeting, Grand Junction, Colorado.

Clark, N, **K. B. Rogers**, E. Anderson, Y. Kanno, and C. Wells. December 11, 2024. Whole genome sequencing to characterize Cutthroat Trout populations across the Continental Divide. Colorado River Cutthroat Trout Conservation Team annual meeting, Grand Junction, Colorado.

Lamar, C., and **K. B. Rogers**. January 15, 2025. Milt activator enhances embryo survival in Cutthroat Trout. Great Plains Fishery Workers Association annual meeting, Fort Collins, Colorado.

Rogers, K. B. February 3, 2025. Research in production hatcheries: additional opportunities to advance species conservation. Aquatic Branch Meeting, Nathrop, Colorado.

Clark, N, **K. B. Rogers**, E. Anderson, Y. Kanno, and C. Wells. May 8, 2025. Whole genome sequencing to characterize Cutthroat Trout populations across the Continental Divide. Greenback Cutthroat Trout Recovery Team meeting, Lakewood, Colorado.

Clark, N, **K. B. Rogers**, E. Anderson, Y. Kanno, and C. Wells. May 14, 2025. Characterizing genomic diversity of Colorado's Cutthroat Trout populations across the Continental Divide. Western Division of the American Fisheries Society annual meeting, Westminster, Colorado.

Rogers, K. B. Oct 17, 2025. Requiem for hierarchical species taxonomy: molecular systematics run amok with examples from Cutthroat Trout. Grad-Faculty seminar, Colorado State University, Fort Collins.