# Cutthroat Trout Studies

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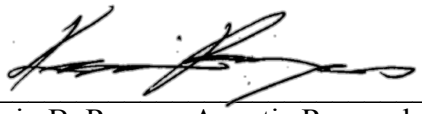
Prepared by: 

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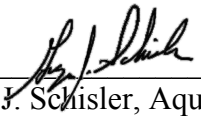
Approved by: 

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Date: 11/30/2020

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# CUTTHROAT TROUT INVESTIGATIONS

Period Covered: December 1, 2019 to November 30, 2020

## 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

## INTRODUCTION

Pervasive undocumented stocking in the early 20$^{th}$ 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 a candidate population should be considered a Conservation Population (sensu UDWR 2000; Hirsch et al. 2006; 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 Colorado River Cutthroat Trout (CRCT) Conservation Team, Rio Grande Cutthroat Trout (RGCT) Conservation Team, and Greenback Cutthroat Trout (GBCT) Recovery Team processed in 2020 are presented here.

## METHODS

Four hundred and fifty one Cutthroat Trout were collected from 22 populations distributed across Colorado (Table 1). Nineteen came from the CRCT range, one from the RGCT range, and two from the South Platte and Arkansas River drainages. 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 80% reagent grade ethanol (Rogers 2007). 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 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 of the lineage represented. These include 1) Blue Lineage CRCT native to the Yampa, White, and Green River basins (YAM), 2) Green Lineage CRCT 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 MEGA7 (Kumar et al. 2016).

**Table 1.** Stream names organized by major drainage basin, water codes, collection dates, and number of fin clips collected for molecular tests conducted in 2020

| Stream | Water Code | Date | Sample size |
|---|---|---|---|
| *Colorado* | | | |
| Derby Creek, M | 19984 | 8/14/2019 | 11 |
| Hunter Creek | 23230 | 8/1/2019 | 20 |
| Lake Creek, E | 27234 | 8/28/2019 | 33 |
| *Dolores* | | | |
| Coal Creek | 39138 | 8/25/2019 | 30 |
| Disappointment Creek | 39758 | 10/9/2019 | 30 |
| Tenderfoot Creek | 43567 | 7/25/2019 | 7 |
| *Gunnison* | | | |
| Basin Creek | 44626 | 8/29/2018 | 20 |
| Rock Creek | 45870 | 8/27/2019 | 20 |
| Second Creek | 45921 | 8/30/2018 | 20 |
| Smith Fork, N Fk | 40535 | 7/12/2017 | 20 |
| Twin Creek, N | 46238 | 8/23/2017 | 20 |
| Twin Creek, S | 46240 | 8/23/2017 | 20 |
| *Rio Grande* | | | |
| Willow Creek | 39831 | 10/17/2018 | 30 |
| *San Juan* | | | |
| Fall Creek | 38117 | 8/5/2019 | 19 |
| Fall Creek | 38117 | 7/13/2020 | 9 |

| | | | |
|---|---|---|---|
| Hermosa Creek | 45802 | 6/23/2020 | 12 |
| Hermosa Creek, E Fk | 47628 | 9/5/2019 | 2 |
| Rio Blanco #2 | 38439 | 10/1/2019 | 4 |
| Rito Blanco | 38441 | 8/22/2019 | 30 |
| Sheep Creek | 39716 | 8/1/2019 | 1 |
| South Platte | | | |
| Clear Creek, W Fk | 10582 | 11/2/2018 | 10 |
| Rock Creek | 30661 | 2017 | 38 |

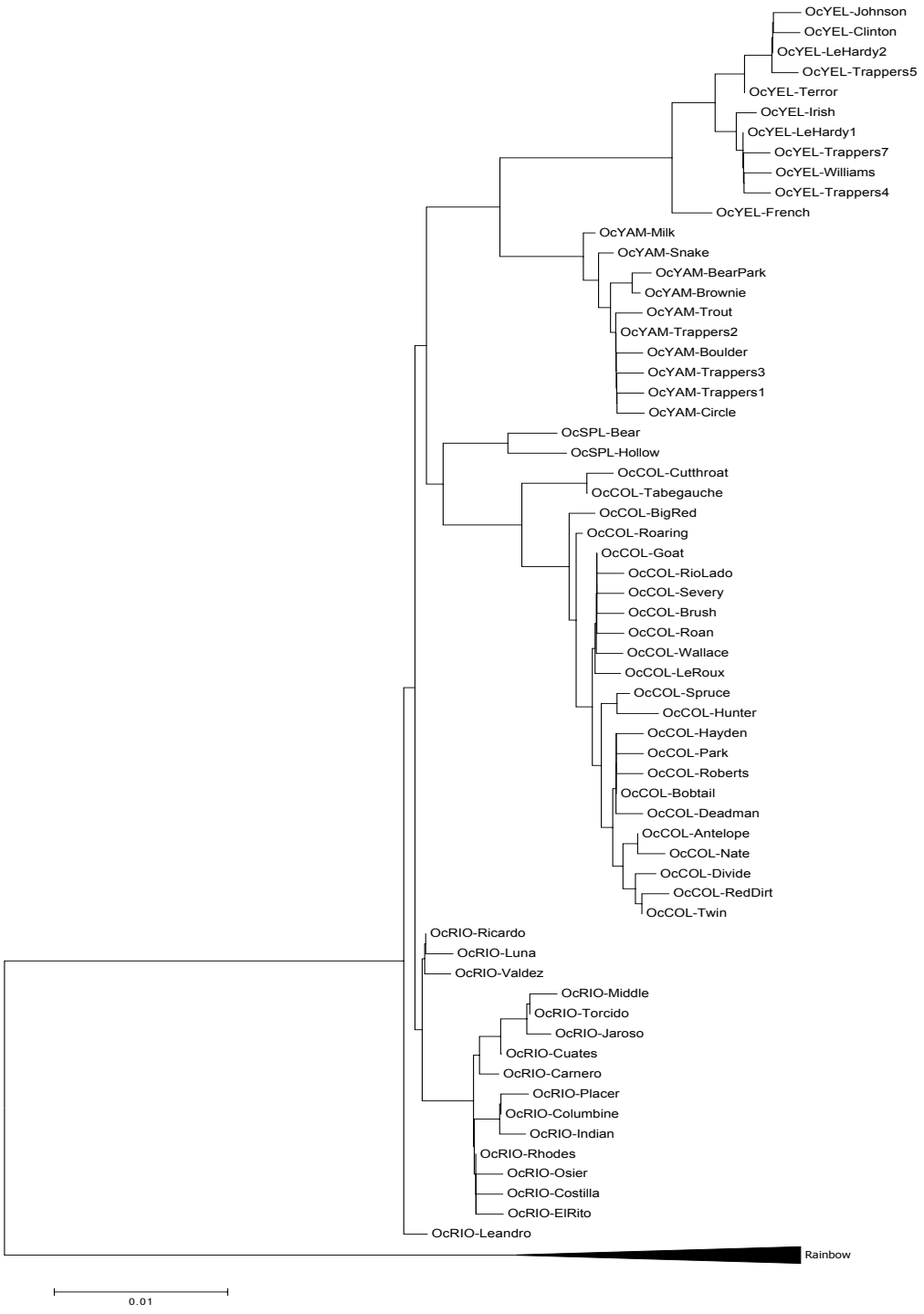


**Figure 1.** Phylogenetic relationships inferred from 648 base pairs of the mitochondrial NADH dehydrogenase subunit 2 gene for Cutthroat Trout from Colorado. The evolutionary history was developed with the neighbor-joining method in MEGA7, with evolutionary distance units representing the number of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

## RESULTS & DISCUSSION

### *Headwaters of the Colorado River*

Collections from three Cutthroat Trout populations in the headwaters of the Colorado River suggest they are better than 90% pure (Table 2) using AFLP nuclear markers, and are therefore considered Conservation Populations. The Middle Fork of Derby Creek represents a newly discovered population of Blue Lineage CRCT established upstream of a very steep cascade section, presumably from historical stocking of headwater lakes. The slight evidence of Yellowstone Cutthroat Trout (YSCT) admixture, and mitochondrial haplotypes that match those found in Trappers Lake (Table 3), suggests this population was founded by stocking some time after the 1950s when YSCT were introduced into Trappers Lake (Rogers et al. 2018). Fins collected from Hunter Creek confirmed the presence of a pure Green Lineage CRCT population there both by AFLP (Table 2), and ND2 (Table 3). Samples from the East Fork of Lake Creek appear to be pure Blue Lineage CRCT by AFLPs (Table 2). Sequence data from the ND2 gene should be obtained to confirm the heritage of this population

**Table 2.** AFLP results from 11 Cutthroat Trout populations analyzed in 2020, along with the number of samples analyzed, organized by major drainage basin. Percent admixture is given by lineage, including Blue and Green Lineage (CRCTb, CRCTg), Rio Grande Cutthroat Trout (RGCT), Yellowstone Cutthroat Trout (YSCT), and Rainbow Trout (RBT).

| Stream | # Analyzed | Lineage | | | | |
|---|---|---|---|---|---|---|
| | | CRCTb | CRCTg | RGCT | YSCT | RBT |
| *Colorado* | | | | | | |
| Derby Creek, M | 11 | 98 | - | - | 2 | - |
| Hunter Creek | 20 | - | 99 | - | - | - |
| Lake Creek, E | 33 | 100 | - | - | - | - |
| *Dolores* | | | | | | |
| Coal Creek | 30 | 98 | - | - | - | 1 |
| Disappointment Creek | 30 | 100 | - | - | - | - |
| *San Juan* | | | | | | |
| Fall Creek | 19 | 87 | - | - | - | 13 |
| Fall Creek | 9 | 94 | 2 | - | - | 3 |
| Hermosa Creek | 12 | 27 | 5 | 2 | 1 | 65 |
| Hermosa Creek, E Fk | 2 | 12 | - | 6 | - | 82 |
| Rio Blanco #2 | 6 | 65 | 1 | 23 | - | 11 |
| Rito Blanco | 30 | 99 | - | - | - | 1 |

**Table 3.** ND2 results from 19 Cutthroat Trout populations analyzed in 2020, along with the number of samples analyzed, organized by major drainage basin. ND2 haplotype is given by lineage, including Blue and Green Lineage (CRCTb, CRCTg), Rio Grande Cutthroat Trout (RGCT), Yellowstone Cutthroat Trout (YSCT), and Rainbow Trout (RBT).

| Stream | # Analyzed | Lineage | | | | |
|---|---|---|---|---|---|---|
| | | CRCTb | CRCTg | RGCT | YSCT | RBT |
| *Colorado* | | | | | | |
| Derby Creek, M | 11 | 10 | - | - | 1 | - |
| Hunter Creek | 20 | - | 20 | - | - | - |
| *Dolores* | | | | | | |
| Coal Creek | 30 | 1 | 26 | - | 3 | 0 |
| Disappointment Creek | 30 | - | 30 | - | - | - |
| Tenderfoot Creek | 7 | - | 7 | - | - | - |
| *Gunnison* | | | | | | |
| Basin Creek | 20 | - | 20 | - | - | - |
| Rock Creek | 20 | - | 20 | - | - | - |
| Second Creek | 20 | - | 20 | - | - | - |
| Smith Fork, N Fk | 20 | 3 | 12 | - | 5 | - |
| Twin Creek, N | 20 | - | 20 | - | - | - |
| Twin Creek, S | 20 | - | 20 | - | - | - |
| *Rio Grande* | | | | | | |
| Willow Creek | 30 | - | - | 14 | - | 16 |
| *San Juan* | | | | | | |
| Fall Creek | 19 | - | 15 | - | - | 4 |
| Fall Creek | 9 | - | 8 | - | - | 1 |
| Hermosa Creek, E Fk | 2 | - | - | - | - | 2 |
| Rio Blanco #2 | 4 | - | 3 | - | - | 1 |
| Rito Blanco | 30 | 8 | 21 | - | - | 1 |
| Sheep Creek | 1 | - | 1 | - | - | - |
| South Platte | | | | | | |
| Clear Creek W Fk | 6 | 1 | 5 | - | - | - |
| Rock Creek | 34 | | | | | |

### *Dolores River basin*

The search in the Dolores Basin for additional pure Green Lineage CRCT populations continues, but Coal Creek does not appear to one of them. Evidence of past stocking is evident, with clear rainbow admixture in one fish (Table 2), and both Blue Lineage CRCT and YSCT haplotypes in four fish (Table 3) – likely again the result of mid-20$^{th}$ century stocking of progeny from Trappers Lake. Inconsistent lineage assignments between ND2 and AFLP tests in both Coal Creek and Disappointment Creek is concerning, and should be explored further with alternative assays, particularly since earlier AFLP work on Disappointment Creek suggested that these fish were Green Lineage CRCT (as the current ND2 data suggests). Although only seven fish were collected from Tenderfoot Creek, the mitochondrial DNA sequence data looks promising with all seven fish displaying Green Lineage CRCT haplotypes (OcCOL-Goat). A full complement of 30 fins from this population would allow a more robust assessment of purity in this population.

### *Gunnison River basin*

Basin, Rock, Second, and both Twin Creeks are all tributaries of the Clear Fork of Muddy Creek. Since previous AFLP assays suggested that all are Green Lineage CRCT, we wanted to confirm the absence of nonnative haplotypes and explore what mitochondrial haplotype diversity remains. All samples displayed one of four Green Lineage CRCT haplotypes, with all four (OcCOL-Goat, OcCOL-Bobtail, OcCOL-Antelope, and OcCOL-Twin) present in the South Twin Creek population alone. The North Fork of the Smith Fork of the Gunnison River northeast of Crawford appears to have been stocked with Trappers Lake progeny (Blue Lineage CRCT) at some point after the mid 1950s. While the majority of fish in the sample display the OcCOL-Bobtail haplotype, both the common OcYAM-Trappers2 and OcYEL-LeHardy1 haplotypes are also present.

### *Rio Grande basin*

Willow Creek flows through a private ranch, whose owners would like to reclaim the stream to return native fish to the drainage. While AFLP tests on these same samples in 2019 suggested some evidence of mild rainbow admixture, ND2 sequence data suggests that admixture in the current population is a substantial problem. Over half of the samples displayed Rainbow Trout haplotypes (Figure 3).

### *San Juan River basin*

The recent discovery of extant San Juan Lineage CRCT populations (Rogers et al. 2018b) spurred intense interest in discovering more. Although the standard AFLP test (Rogers 2008) does not screen for San Juan CRCT specifically, it does provide a useful assay for detecting admixture with Rainbow Trout or YSCT. Some evidence of Rainbow Trout admixture was present in the nuclear genome of each collection (Table 2), and was validated by the presence of

Rainbow Trout haplotypes (Table 3), with the exception of Sheep Creek. That single fish was captured below a natural waterfall barrier amongst a number of Brown Trout, and displayed the less common OcCOL-Cutthroat San Juan Lineage haplotype (Figure 1).

The presence of Rainbow Trout alleles in Fall Creek was expected since these collections were made below the Highway 160 culvert barrier where Rainbow Trout and Brook Trout are sympatric with Cutthroat Trout. Cutthroat Trout over 100 mm were PIT tagged and screened with AFLPs to assess purity. A portion of the ND2 gene was sequenced to verify that fish from both collections also contained a San Juan Lineage haplotype. Five of the 28 Cutthroat Trout captured did not (Table 3). Trout with Rainbow Trout phenotypes were discovered in Hermosa Creek following reclamation. Fin clips were acquired to determine if they were wild origin (admixed with Cutthroat Trout) or stocked illegally. The AFLP data (Figure 2) suggests they were wild. Slight apparent Rainbow Trout admixture in a couple of Cutthroat Trout from Rito Blanco was verified by the presence of one Rainbow Trout haplotype (OmClearwater) and 8 OcYAM-Trappers2 (Blue Lineage CRCT) haplotypes suggesting not only past stocking of Rainbow Trout, but also Blue Lineage CRCT likely from Trappers Lake on top of what once was a San Juan Lineage CRCT population (remaining fish displayed the OcCOL-Tabeguache haplotype). The few samples from Rio Blanco #2 also suggest past stocking of Rainbow Trout.

### *South Platte River basin*

Ten samples were collected from the West Fork of Clear Creek near the Henderson Mine to determine the likely origin of these fish. DNAs from four fish were too degraded to obtain reliable sequence data, while 5 of the remaining 6 displayed the OcCOL-Bobtail haplotype (Figure 1) – the sole haplotype found in Bobtail Creek west of the Continental Divide. This confirms the suspected origin of these fish, as emigrants entrained in the Gumlick Tunnel, and delivered east to the Clear Creek basin. Interestingly, one fish displayed a OcYAM-Milk haplotype that would have come from a different source. Perhaps this fish emigrated from nearby Cone (Blue) Lake, as it is stocked regularly with recreational Cutthroat Trout (GBN) that may contain the OcYAM-Milk haplotype.

Thirty-eight fish were collected from Rock Creek in 2017 following reclamation to confirm that only stocked Bear Creek progeny (BAC) remained. Our standard 648 bp of sequence data from the ND2 gene was obtained from 33 fish, while 4 samples were too degraded to assign to obtain reliable sequence data. One sample (Pisces #153730) yielded 618 bp of mtDNA sequence that was consistent with the common OcSPL-Bear haplotype (Figure 1), as were the 33 full samples, confirming that the reclamation effort appeared to be successful.

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## RESEARCH PRIORITY

Status and conservation of an undescribed lineage of Cutthroat Trout native to the southern Rocky Mountains

## OBJECTIVE

Identify and characterize conservation populations of the native trout of the Colorado, Gunnison, and Dolores basin headwaters

## INTRODUCTION

Colorado River Cutthroat Trout *Oncoryhnchus clarkii pleuriticus* (CRCT) represent one of the southern-most of the 14 recognized native Cutthroat Trout subspecies of the Rocky Mountains (Behnke 1992; Behnke 2002; Trotter 2008). Like other inland Cutthroat Trout, CRCT now occupy a fraction of their historic range (11%; Hirsch et al. 2013), primarily in isolated headwater habitats protected from invading nonnative salmonids (Behnke 2002; Fausch et al. 2009; Hirsch et al. 2013; Penaluna et al. 2016). The 361 CRCT Conservation Populations (those wild self-sustaining populations that display less than 10% admixture with nonnative alleles; UDWR 2000; Shepard et al. 2005; Ziegler et al. 2019), identified in Hirsch et al. (2013), are distributed from the San Juan basin in southern Colorado, to the headwaters of the Green River in northern Wyoming, and from the Continental Divide west to central Utah. Three broad lineages of Cutthroat Trout have been identified within this range, with the "Blue" Lineage indigenous to the Green, White, and Yampa rivers, the "Green" Lineage native to the headwaters of the Colorado, Gunnison, and Dolores rivers, and the San Juan Lineage native to its namesake basin (Metcalf et al. 2012; Rogers et al. 2018; Bestgen et al. 2019). Our objectives for this study were to 1) characterize those CRCT populations that represent the Green Lineage, and 2) describe the purity and genetic diversity captured in those remaining populations.

## METHODS

### *Identifying conservation populations*

In response to the listing petition in 1999, the CRCT Conservation Team launched an ambitious effort to catalogue what was known of remaining CRCT populations. Since that time, over 80 biologists have provided input into a living database that acts as a repository for information relevant for evaluating the status of CRCT (Hirsch et al. 2006, 2013). This database is linked directly to a geographic information system where barriers and population extents could be mapped onto stream data layers from the National Hydrography Dataset Plus version 2 (NHDPlus v2; 1:100,000; www.horizon-systems.com/nhdplus/NHDPlusV2_home.php) in real time. This CRCT database tracks the location of all known self-sustaining relatively pure CRCT populations, delineates what habitats would have been historically occupied, and acts as a repository for numerous population attributes and genetic purity assessments relevant for

conservation efforts. Populations are organized by 4-digit hydrologic unit codes that serve as geographic management units (GMUs) delineating major drainage basins (Hirsch et al. 2006, 2013). Since stocked populations of CRCT have become established outside their native range east of the Continental Divide (Metcalf et al. 2007, 2012; Rogers et al. 2018), a similar database for GBCT (unpublished) was merged with the CRCT database for this study. Once the genetic information was paired with the merged ICP databases, those populations meeting "conservation population" criteria (UDWR 2000; Shepard et al. 2005; Muhlfeld et al. 2015, 2016) were identified. Conservation populations (CP) are populations that link adjacent occupied habitats (current distributions; CD) with fish that are better than 90% pure as measured with a variety of molecular methods (Hirsch et al. 2013; Rogers et al. 2018; Bestgen et al. 2019).

***Molecular screening***

Following the development of the merged database, CRCT Conservation Team members launched a massive survey effort to assess purity in CRCT populations using a consistent approach. Tissue samples representing 9,319 trout from 297 populations collected across the range of CRCT from 1998-2019 were collected in addition to 1,452 samples from 42 populations from what was considered historic GBCT habitat east of the Continental Divide outside the native range of CRCT where pure populations founded by stocking efforts a century ago may persist (Metcalf et al. 2007, 2012; Rogers et al. 2018). Trout DNA was extracted from caudal fin clips, then amplified using a polymerase chain reaction (PCR) to produce amplified fragment length polymorphism marker fragments (AFLP) as in earlier studies (Metcalf et al. 2007; Rogers 2010; Bestgen et al. 2019). Where Green Lineage CRCT were identified, a 648 bp portion of the NADH dehydrogenase subunit 2 (ND2) mitochondrial gene was sequenced and analyzed using methods employed in Bestgen et al. (2019).

## RESULTS

***Molecular findings***

Fin clips from 10,352 Cutthroat Trout were genotyped representing populations from 339 sites across the native range of CRCT and GBCT. Seventy seven sites harbored CPs representing the Green Lineage form with better than 90% purity, of which 68 lotic CPs and a single small lentic CP are scattered across their putative native range west of the Continental Divide (Rogers 2010; Metcalf et al. 2012; Rogers et al. 2018) in the Colorado (28 CPs), Gunnison (28 CPs), and Dolores (13 CPs) river basins (Figure 1). These populations occupy 465 stream km, or just 3% of what historically would have been occupied in those three drainages (Hirsch et al. 2013). Like other inland Cutthroat Trout, these populations are largely restricted to headwater reaches within their native range. Interestingly, despite a robust history of culture and stocking (Metcalf et al. 2012), the Green Lineage has not been detected outside their putative native range west of the Continental Divide. Only east of the Divide do several populations persist in pure form in the South Platte (three populations) and the Arkansas (two streams) river basins.

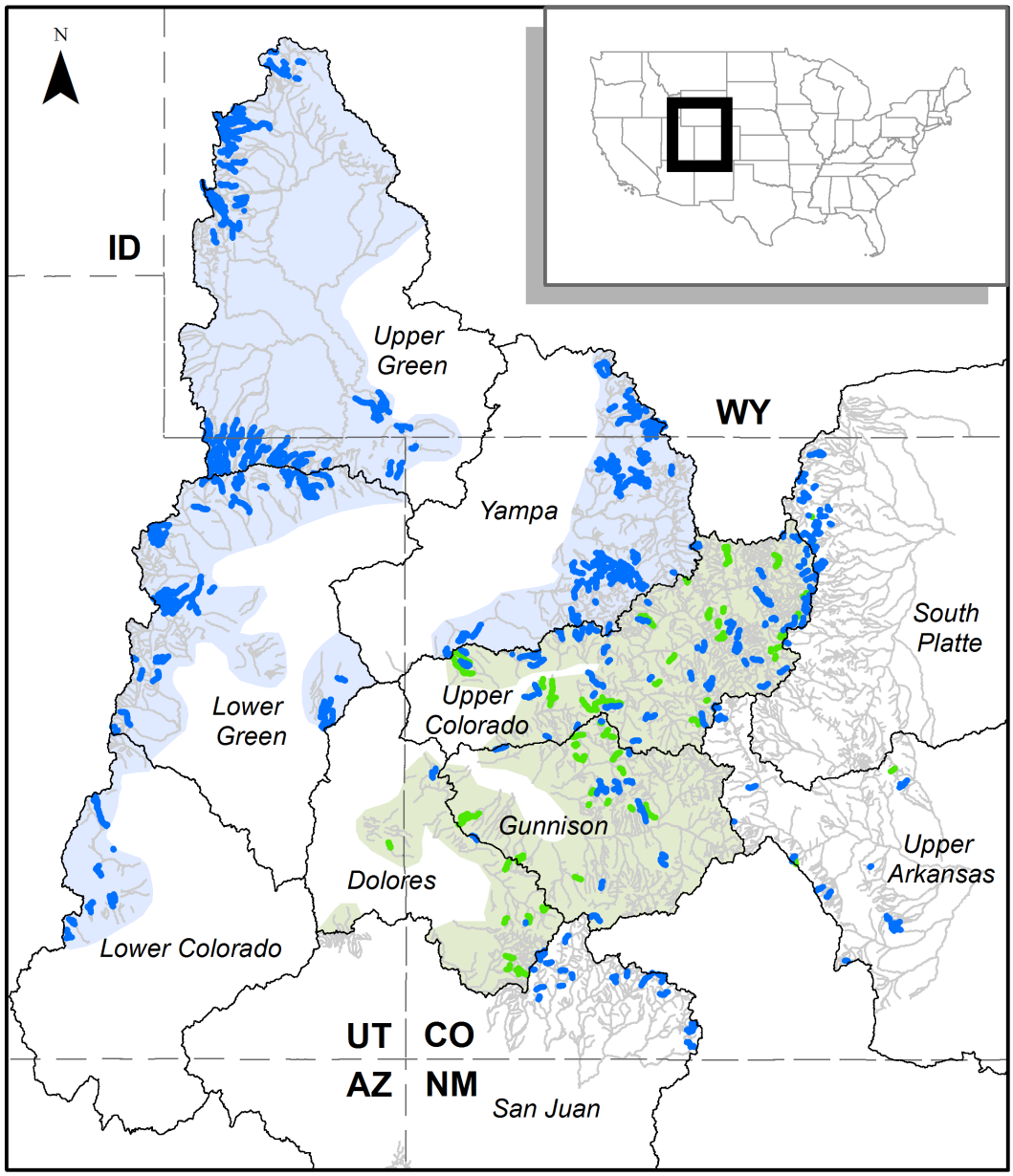


**Figure 1.** Geographic location of Green Lineage CRCT conservation populations shown in green overlaid on top of their putative historic range (green shading). Blue Lineage CRCT conservation populations are shown in blue, and their putative historic range shown with blue shading. Populations are organized by second level (4 digit) hydrologic unit codes (HUCs) from the National Hydrography Dataset (NHD) that are also presented.

Of the 69 Pacific slope Green Lineage CPs, 43 appear to be genetically unaltered (purity by AFLP ≥99%), while 26 show some mild (<10%) admixture with YSCT or Rainbow Trout alleles. As such, 250 of the 465 stream km occupied by Green Lineage CPs look to be genetically unaltered, and are therefore considered core conservation populations (sensu UDWR

2000; Shepard et al. 2005; Muhlfeld et al. 2015, 2016). Nineteen of these are found in the Colorado Headwaters GMU, 18 in the Gunnison GMU and 6 in the Dolores GMU. Only the Hague Creek CP in the South Platte basin and South Hayden Creek in the Arkansas basin appear to meet core conservation criteria east of the Continental Divide.

We recovered 21 mitochondrial haplotypes from all 77 Green Lineage CPs. While the majority of populations harbored only a single haplotype, some contained as many as four. The two most common haplotypes were found in every drainage basin, but rare haplotypes were usually restricted to a single basin. For instance, two haplotypes were found only east of the Continental Divide but not on the West Slope. Interestingly, despite sequencing the ND2 fragment on roughly equal numbers of fish (1553 Blue Lineage and 1764 Green Lineage), we recovered twice as many Green Lineage haplotypes (21 vs. only 11 that fell in the Blue Lineage clade) even though the Blue Lineage CRCT samples were acquired across a native range that is 36% larger (18,625 vs 13,703 stream km; Hirsch et al. 2013). Percent sequence divergence (D) between Green Lineage CRCT ND2 mtDNA and other Cutthroat Trout taxa ranged from 1.1% to 3.9%. They appeared to be most closely related to the San Juan CRCT (D = 1.1%) and to Cutthroat Trout native to the South Platte and Rio Grande rivers (D = 1.8%), but less closely aligned with the Blue Lineage CRCT to which they share a subspecies designation (D = 2.4%). As expected, they also appear to be less related to more distant subspecies such as YSCT, Westslope Cutthroat Trout, Lahonton Cutthroat Trout (*O. c. henshawi*) and Coastal Cutthroat Trout (*O. c. clarkii*; D > 3.0%).

***Occupied stream reaches***

Within the native CRCT range west of the Continental Divide, Green Lineage trout occupied habitat patches that were smaller on average than the remaining Blue Lineage CPs (6.8 vs 10.6 km; t=1.969, p=0.001), with Green Lineage populations ranging from 0.22 to 26.0 km and Blue Lineage ones from 0.03 to 101.7 km. Median values for Green Lineage CPs occupied stream lengths were 86% of Blue Lineage populations (4.9 vs. 5.7 km, respectively). Eighty-one percent (56 of 69) Green Lineage CPs were listed as isolated while only 67% (196 of 294) Blue Lineage CPs received that designation. One quarter (n=17) of the 69 Green Lineage CPs west of the Continental Divide are already invaded by nonnative competing salmonids. Thirteen of these now harbor Brook Trout (*Salvelinus fontinalis*), while Brown Trout (*Salmo trutta*) are found in two more. Both Brook and Brown trout are found in the East Fork of Deep Creek and Schaefer Creek. Two more (Carter and Roan Creek) have been invaded by hybridizing Rainbow Trout, while the remainder live in allopatry. Robust population size estimation was made possible with 142 multi-pass population surveys conducted in the last two decades on 54 of the 69 CPs. Densities ranged from 10-1314 trout ≥ 75mm/km, with mean values significantly higher in allopatric populations (293 trout ≥75mm/km) than in those sympatric with Brook or Brown Trout (104 trout ≥ 75mm/km).

## DISCUSSION

The Green Lineage CRCT appears to be a diagnosable unit of biodiversity. In addition to the mitochondrial sequence data presented here and elsewhere (Metcalf et al. 2012; Rogers et al.

2014; Rogers et al. 2018), nuclear markers such as microsatellites (Metcalf et al. 2007), AFLPs (Metcalf et al. 2007, Rogers 2010), and sequence data (Love Stowell 2011; Rogers et al. 2018) all suggest a clear separation between these two groups that may date back 1.2-1.7 Ma (Shiozawa et al. 2018). In addition, Green Lineage trout can be distinguished from the Blue Lineage with morphological and meristic traits (Bestgen et al.2019) and, in hindsight, with protein electrophoresis (Kanda et al. 2000) as well, though not recognized by those authors. The distinction between groups was more subtle in a study of SNPs spread throughout the nuclear genome (Houston et al. 2012), and was not reflected in a limited sample of CRCT using a Y-chromosome marker near the sex determining gene (Brunelli et al. 2013). This latter discovery is not surprising given that the gene region explored evolves 3-13x more slowly than mitochondrial DNA (Brunelli et al. 2013).

The taxonomy of Cutthroat Trout is complex, and in need of systematic revision (Markle 2018). Molecular results presented here further complicate classification of Blue and Green lineages of CRCT, as this is not simply a case of two forms being present within a broader taxon. While nuclear DNA sequence data suggests the two clades are sister taxa (Love Stowell 2011; Houston et al. 2012; Rogers et al. 2018), the ND2 mitochondrial DNA suggests that Green Lineage CRCT are more closely related to RGCT and GBCT native to the South Platte basin, than the Blue Lineage. If additional work on the nuclear genome consistently reveals the same pattern, nomenclature for Cutthroat Trout of the southern Rocky Mountains should either recognize 1) all as a single taxon with distinct population segments of management significance – perhaps harkening back to the early days of settlement when they were all referred to as "black-spotted natives" (Wiltzius 1985; Behnke 2002; Trotter 2008), or 2), recognize each as a distinct subspecies as has been the case, but now recognizing Green and Blue lineages as discrete, and assign them type specimens and trinomial names.

While these two lineages can be separated with morphological treatments (Bestgen et al. 2019), molecular methods remain the primary tool for diagnosis. As such, we are fortunate to have isolated DNA from every documented Green Lineage population, which provides rare insight not typically enjoyed by other fish taxa. Although Green Lineage CRCT comprise only 69 of the 361 recognized CRCT populations across their native range and are only found in 3% of the habitat they would have historically occupied, they still harbor 66% of the ND2 mtDNA haplotype diversity recovered from CRCT. Bestgen et al. (2019) observed substantial geographic structuring among RGCT and Blue Lineage CRCT haplotypes, but not as much among Green Lineage CRCT, presumably because the three major drainages covering their putative native range are linked by coldwater confluences (Figure 1). However, with the additional samples collected in this study, a clearer pattern has emerged. While several haplotypes are indeed very common and widespread, the majority (76%) are restricted to a single GMU. Eight of these are found in the Upper Colorado GMU alone, which would have covered over half (53%) of historically occupied habitat in the putative native range of Green Lineage CRCT.

Rare haplotypes recovered by intensive molecular canvassing helps identify peripheral populations that can be otherwise challenging to discriminate in aquatic systems (Bunnell et al. 2004; Haak et al. 2010). Many Green Lineage CRCT populations persist in often-marginal

habitats around the periphery of the range, particularly in places without headwater lakes that might have encouraged past stocking events. Peripheral populations are often subject to different selection pressures than those faced in the center of the range, and may contain genetic diversity that could allow adaptation to harsher environmental conditions (Lesica and Allendorf 1995; Haak et al. 2010; Hodge et al. 2017). Focusing conservation efforts in these areas should be a priority so that the genetic diversity they display (and perhaps undiscovered local adaptations) can be protected (Muhlfeld et al. 2016).

We identified 68 lotic and a single lentic CP of Green Lineage CRCT distributed across 15 counties in Colorado and one in southeast Utah across their putative native range in the Upper Colorado, Gunnison, and Dolores River basins (Metcalf et al. 2012; Rogers et al. 2018). These populations are small, with median occupied stream lengths of 4.9 km, most of which are isolated. While other interior Cutthroat Trout taxa can still be found in >10% of their native ranges (e. g. 11% for CRCT, Hirsch et al. 2013; 12% for RGCT, Alves et al. 2008; 35% for Bonneville Cutthroat Trout, *O. c. utah*, Budy et al. 2019; 42% for YSCT, Gresswell 2011), the Green Lineage of CRCT occupies just 3% of its native range, making them a priority for conservation efforts.

***Green Lineage trout east of the Continental Divide***

In addition, eight CPs representing this clade can be found east of the Continental Divide in the South Platte and Arkansas basins respectively. Evidence of a single Green Lineage CRCT mtDNA haplotype (OcCOL-Severy) can be found in many more populations east of the Continental Divide descending from Como Creek source stock used in the GBCT recovery effort from 1981-1992 (Dwyer and Rosenlund 1988; USFWS 1998). Curiously, the nuclear genome of these fish appear to align more closely with Blue Lineage CRCT, perhaps evidence of stocking induced admixture in those fish.

Given the extensive history of anthropogenic stocking starting at the turn of the 19th century (Wiltzius 1985; Metcalf et al. 2007, 2012; Love Stowell et al. 2015), it should not be a surprise that Green Lineage CRCT can be found east of the Continental Divide outside of their putative native range (Rogers et al. 2018). Cutthroat Trout eggs were collected from at least ten wild spawn operations conducted within the Colorado and Gunnison River basins prior to 1940. Few of these operations produced enough eggs to satisfy the needs of the collecting agencies, and as such, these operations generally ceased after a few years. This was not the case for the Alexander Lake complex atop the south side of the Grand Mesa (Gunnison River drainage) which was essentially the exclusive source of Cutthroat Trout eggs for the Leadville National Fish Hatchery (LNFH) in the early 1900s. Between 1899 and 1909 the LNFH collected 47 million fertilized eggs from ten lakes and streams in two separate basins draining the Alexander Lakes. Progeny were distributed to 16 states and two foreign countries during that time, with 29 million distributed to waters across the state of Colorado that could support trout (Metcalf et al. 2012). The State of Colorado took over egg collecting operations at these lakes on the Grand Mesa in 1913 and continued collecting eggs until 1931. However, by 1916, nonnative Rainbow Trout had been stocked into all of them, making it unlikely that pure Green Lineage CRCT were still being produced after that point.

Despite a clear mechanism for establishing CRCT east of the Continental Divide, there are a few curiosities that should prevent us from discounting the possibility that these fish invaded across the Divide on their own, perhaps at the end of the Pleistocene: 1) Green Lineage CRCT populations east of the Continental Divide are morphologically distinct from their cousins west of the Divide with Bestgen et al. (2019) able to assign specimens back to their basin of origin using just morphological traits and spotting patterns (but consider Hickman and Behnke [1979] assertion that small founding populations can yield morphomeristic traits that skew towards extreme rather than modal values). 2) Of the 21 mitochondrial haplotypes recovered in the Green Lineage CRCT clade, 19 are found only west of the Continental Divide while two are found only east of the Divide (with minor exceptions in Rocky Mountain National Park where stocking was particularly intense; Love Stowell et al. 2015). 3) Museum collections gathered in 1889 allegedly from Twin Lakes in the headwaters of the Arkansas River basin contained fish displaying mitochondrial haplotypes from both Yellowfin Cutthroat Trout (*O. c. macdonaldii*), and Green Lineage CRCT (Metcalf et al. 2012), though it should be noted that by that time Rainbow Trout, Lake Trout (*Salvelinus namaycush*), Brook Trout, and Atlantic Salmon (*Salmo salar*) had all been stocked in the lakes as well (Wiltzius 1985). 4) The Green Lineage CRCT haplotype recovered from two 1889 Twin Lake area samples match those from only a single extant population – those in South Hayden Creek, also in the Arkansas basin. It is conceivable that the Ewing Ditch built in 1880 to bring water from the west side of the Continental Divide (Eagle River basin) to Leadville might also have brought Green Lineage CRCT with it, but it is difficult to reconcile how in just nine years they could have moved 36 km down the Arkansas River, and several more up Lake Creek into Twin Lakes, then proliferated enough to make up a substantial portion of Jordan's catch. Whether Green Lineage CRCT became established east of the Divide on their own, or through anthropogenic support remains a mystery. Their somewhat unique genetic and phenotypic attributes however suggest these fish should continue to be a focus of conservation efforts.

***Admixed populations***

For this assessment, we have used 90% purity as the metric for recognizing populations with conservation value as have many others managing interior Cutthroat Trout (UDWR 2000; Shepard et al. 2005; Alves et al. 2008; Muhlfeld et al. 2015; Zeigler et al. 2019) and CRCT in particular (Hirsch et al. 2006, 2013; Williams et al. 2009; Roberts et al. 2013, 2017). The USFWS considered any population with fewer than 20% nonnative alleles when evaluating Westslope Cutthroat Trout for listing under the ESA (USFWS 2003), as those with less than 20% admixture with still appear phenotypically to be good representatives of the subspecies (Campton and Kaeding 2005; but see Allendorf et al. 2004, 2005). We do not advocate relaxing the 90% standard, particularly since admixed populations may act as sources for nonnative alleles to other populations, but do wish to acknowledge that we recovered a private haplotype in a very admixed population in Wallace Creek in the Upper Colorado GMU. Though obscured by nonnative alleles that keep them from being recognized here as a CP, they appear to harbor original Green Lineage CRCT diversity that is unaccounted for elsewhere. Others have suggested that genetic diversity occluded by nonnative alleles should also be preserved where possible (Peacock and Kirchoff 2004). Since the populations are isolated, and no shortage of potential repatriation sites exist in the Upper Colorado GMU, we also advocate looking elsewhere to implement reclamation projects, allowing this population to persist as hybrid

swarm.

In addition, many CRCT populations contain YSCT alleles (Bestgen et al. 2019). Large scale stocking across the western United States of over 800 million YSCT produced from Yellowstone Lake between the years of 1889 and 1957 (Varley 1979) brought that taxon into proximity with CRCT with which they will readily hybridize (Varley and Gresswell 1988). While extensive introgressive hybridization with YSCT (or Rainbow Trout) precludes them from being considered as a CP, mild YSCT admixture (<10%) appears to be present in 15 of the 69 Green Lineage CPs in their native range, affecting their conservation value. While these populations are still phenotypically good representatives of Green Lineage CRCT, the YSCT alleles they harbor preclude them from being used as brood stock to found new populations.

***Management recommendations***

Green Lineage CRCT share many of the same challenges facing subspecies of Cutthroat Trout across the western United States. Though only recently recognized as a discrete taxonomic entity (Metcalf et al. 2012; Rogers et al. 2018; Bestgen et al. 2019), several decades of conservation efforts for CRCT prevented populations from slipping through the cracks. Now that we recognize that this lineage represents a unique piece of Cutthroat Trout diversity in the southern Rocky Mountains, it is imperative that CRCT conservation efforts continue their recent focus on preserving and replicating Green Lineage CRCT populations in the near-term with projects occurring in their native range, so they can enjoy the fruits of conservation programs that have helped other southwestern trout become more secure.

Regardless of how these fish are recognized taxonomically, enlightened conservation should include preserving all pieces of Cutthroat Trout diversity that remains. It is clear that although these fish only occupy three percent of their native range, substantial historical diversity remains across the landscape. Providing redundancy among those pieces should be the focus of conservation efforts going forward. Where possible, direct translocations should be considered to minimize domesticating selection and potential disease transfer when introduced into scarce hatchery resources, while allowing greater flexibility and efficiency in replicating pockets of genetic diversity that still exist (George et al. 2009; Fitzpatrick et al. 2014). In recognition of the broad genetic diversity that still remains and the geographic structuring it reveals, sources for repatriation efforts should be matched to major drainage basins (Fitzpatrick et al. 2014). Consistent with the current CRCT Conservation Strategy (CRCTCT 2006) we also believe it is important to establish several additional Green Lineage CRCT metapopulations to provide resiliency (Haak and Williams 2012), and mitigate some of the risk associated with small population sizes.

The CRCT conservation team has recognized the urgency for preserving this lineage, and has already completed numerous projects over the last five years that secure existing diversity identified by unique mitochondrial haplotypes such as the Deadman Gulch CP where a reclamation project following barrier installation has doubled the amount of available habitat. On the Roan Plateau, managers have reclaimed 12 km of some of the most productive headwater trout stream habitat in Colorado and repopulated with progeny derived from four years of wild spawn operations on Roan Creek – a population with a unique haplotype currently being invaded

by Brook and Rainbow Trout. The Kelso Creek CP has been replicated in 8 ha Woods Lake to serve as a brood source for other reclamation projects in the basin, while another creative manager was able to raise US$ 1.2 M to fund construction of a more efficient irrigation system that allows 40% more water to flow back into Abrams Creek, securing that unique population and enhancing occupied habitat 5 km downstream. We are encouraged by the CRCT Conservation Team's active conservation program, and their robust track record of securing existing populations and replicating them in newly reclaimed habitats. Their efforts provide optimism that these precious pieces of native Cutthroat Trout diversity will persist well into the future.

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## RESEARCH PRIORITY

Spawn matrixing fails to improve survival in a unique Cutthroat Trout population following fire-mediated extirpation in the wild

## OBJECTIVE

To evaluate if genotyping parents then spawning the most distantly related ones in a small trout population can mitigate the effects of a genetic bottleneck in the progeny and improve their survival.

## INTRODUCTION

The presence of two broad clades within Colorado River Cutthroat Trout *Oncoryhnchus clarkii pleuriticus* has been well documented (Metcalf et al. 2007, Rogers et al. 2010, Metcalf et al. 2012, Rogers et al. 2018, Bestgen et al. 2019), with a fairly common one occupying the headwaters of the Green, Yampa, and White River basins (Blue Lineage), and a less common form that historically called the headwaters of the Colorado, Gunnison, and Dolores Rivers home (Green Lineage). Both were stocked widely on both sides of the Continental Divide in Colorado in the early 1900s (Metcalf et al. 2012), which served to occlude their native ranges. Curiously, specimens of the Green Lineage form were collected east of the Continental Divide in the Arkansas River basin by David Starr Jordan from Twin Lakes in 1889, prior to the advent of large-scale trans-basin stocking efforts. As such, we are unable to rule out the possibility that they migrated across the Divide perhaps after the last ice age and persisted in Twin Lakes in sympatry with the now extinct Yellowfin Cutthroat Trout (*O. c. macdonaldi*). These specimens harbor a mitochondrial ND2 haplotype found nowhere in the putative native range of this lineage west of the Divide, but in a single extant population also in the Arkansas River basin in the South Prong of Hayden Creek (Rogers et al. 2018).

A July 2016 wildfire in the headwaters of the Hayden Creek drainage scorched 6,685 ha of mixed conifer forest. Post-fire erosion potential was significant, making it likely that lethal debris flows would materialize during the late summer monsoon season (USDA 2016). Given the unique nature of this population, a rescue effort aimed at securing this population was conducted. Five crews used backpack electrofishers to remove roughly half of the resident population (194 fish). Smaller fish were targeted in an effort to leave enough mature adults to repopulate the stream if ash flows did not materialize. Thirty-six were translocated to the upstream reaches of Newlin Creek that also lies in the Arkansas River basin, while the remainder (158 trout) were taken into the Roaring Judy Hatchery isolation facility to mature and provide future progeny for repatriation efforts. One hundred and fifty two persisted a year later, 18 of which produced viable eggs in 2017. These were used to produce 2,700 ten month-old progeny for future broodstock development. Survival to hatch of these 18 families was highly variable (S. Firestone, unpublished data) ranging from 6% to 85%. The small size of the founding population coupled with variable survival led us to explore the possibility that genetic factors

were partially responsible. To that end, we set out to genotype the 133 individuals that remained in 2018 and compare survival of families created from either closely related or unrelated parents.

## METHODS

Each of the remaining 133 Cutthroat Trout housed at the Roaring Judy Hatchery in Colorado received a PIT tag (Biomark, Boise, Idaho) injected either into the body cavity or the dorsal musculature (Hodge et al. 2015; Mamer et al. 2016), as well as an alphanumeric visual implant tag (Northwest Marine Technology, Shaw Island, Washington) injected into the postorbital adipose tissue behind their left eye so that individuals could be rapidly identified (Kincaid and Calkins 1992; McMahon et al. 1996; Hughes et al. 2000; Davis et al. 2014). A one $cm^2$ piece of the caudal fin was removed from each fish and stored in 95% ethanol for subsequent genotyping.

Nuclear DNA was isolated using standard protocols as in (Bestgen et al. 2019). We examined nuclear genetic variation at 30 microsatellite loci generated at either the Montana Conservation Genetics Lab (Missoula, Montana; 18 loci) or at Pisces Molecular (Boulder, Colorado, 12 loci) as in Pritchard et al. (2007). Fourteen of these were polymorphic and used in subsequent analyses. We used the R package "related" (Frasier 2018) to estimate relatedness values of all pairwise individuals. We used the "compare estimators" function to simulate individuals with known relatedness (full-sibs, half-sibs, parent-offspring, and unrelated) and to compare four commonly used relatedness estimators: 1) Wang estimator (Wang 2002), 2) Li et al. estimator (Li et al. 1993), 3) Lynch and Ritland estimator (Lynch and Ritland 1999), and 4) Queller and Goodnight estimator (Queller and Goodnight 1989). The simulations were parameterized with the observed allele frequencies at the variable microsatellite loci. We used the estimator that produced the highest correlation between observed and expected values to estimate relatedness between all possible trout pairings.

Once measures of relatedness were established for all pairings, we used custom code (LabVIEW, National Instruments, Austin, Texas) to quickly interrogate both PIT and VIalpha databases to identify both an unrelated and a related male for a target ripe female. We also identified another ripe female that was unrelated to our target female. Once these four fish were isolated, eggs from each female were stripped into two bowls, each which was then fertilized with equal volumes of milt from each male as in Figure 1. This paired design allowed for both the viability of the eggs as well as the milt to be assessed independent of their relatedness. These four families were then deposited into adjacent isolated lanes in a Heath tray (MariSource, Burlington, Washington). This process was repeated seven times, to produce 28 paired families held in a single Heath stack, incubated in 7.8 °C water. Dead eggs were removed daily, and survival to hatch and swim-up was recorded. We used a mixed model in R to evaluate whether a fish lived or died was a function of relatedness, with sex as a random effect (R Core Team 2017).

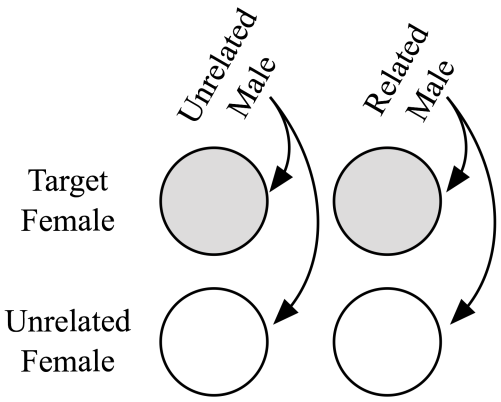


**Figure 1.** Once a target female was selected, equal volumes of her eggs were stripped into two bowls that were then fertilized by an unrelated and related male. Those same males were used to fertilize the eggs of a female unrelated to the target female as well, so that egg and milt viability could be evaluated

## RESULTS & DISCUSSION

### *Microsatellite genotyping*

Fourteen of the 30 microsatellite loci we examined were polymorphic. The number of alleles per locus ranged from 2 – 11 (mean = 3.4), while expected heterozygosity ranged from 0.196 to 0.825 (mean = 0.456). Estimators of relatedness were highly correlated, but the highest correlation between observed and expected values came from the Lynch and Ritland estimator, so we used that to judge relatedness between all possible pairings among the remaining trout rescued from South Hayden Creek (Figure 2).

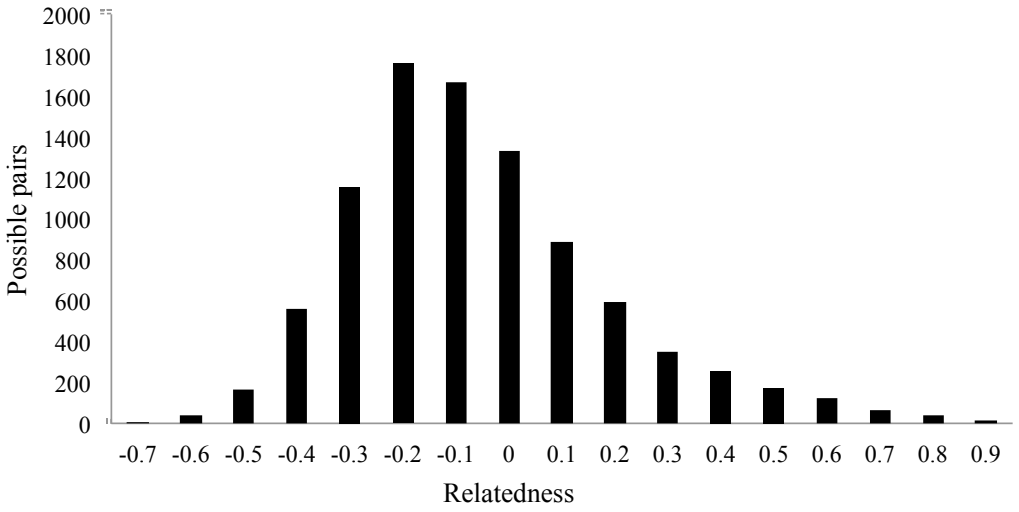


**Figure 2.** Relatedness values between all possible 9,200 pairings of the 133 rescued trout from South Hayden Creek. Less related pairs show decreasing negative values, while positive values suggest increasing relatedness.

***Post swim-up survival***

Survival to swim-up was highly variable, ranging from 0% to 81% (Table 1) despite consistent rearing conditions. Although a broad range of relatedness values were used in the experiment (-0.335 to 0.607; see Figure 2), relatedness did not correlate with survival (p=0.703). However, there was a strong sex effect, with females accounting for 98% of the variance registered. This was a bit surprising, as managers often use additional males to fertilize pans of eggs if the milt appears watery or otherwise suspect. In practice, it appears that milt is consistently viable across males. Egg quality produced by females, however, is extremely variable and drives eventual survival of fry past the alevin stage.

**Table 1.** Parents, relatedness and survival in 28 families of South Hayden Creek Cutthroat Trout progeny.

| Family | Female ID | Male ID | Relatedness | Survival |
|---|---|---|---|---|
| 1 | A1 | H1 | -0.253 | 0.00 |
| 2 | A1 | H2 | 0.294 | 0.00 |
| 3 | A2 | H1 | 0.386 | 0.41 |
| 4 | A2 | H2 | 0.239 | 0.46 |
| 5 | B1 | I1 | -0.335 | 0.21 |
| 6 | B1 | I2 | 0.607 | 0.58 |
| 7 | B2 | I1 | -0.105 | 0.00 |
| 8 | B2 | I2 | -0.28 | 0.00 |

| | | | | |
|---|---|---|---|---|
| 9 | C1 | J1 | -0.221 | 0.67 |
| 10 | C1 | J2 | 0.226 | 0.78 |
| 11 | C2 | J1 | -0.024 | 0.13 |
| 12 | C2 | J2 | -0.133 | 0.17 |
| | | | | |
| 13 | D1 | K1 | -0.3 | 0.21 |
| 14 | D1 | K2 | 0.322 | 0.15 |
| 15 | D2 | K1 | 0.374 | 0.81 |
| 16 | D2 | K2 | -0.172 | 0.75 |
| | | | | |
| 17 | E1 | L1 | -0.299 | 0.81 |
| 18 | E1 | L2 | 0.446 | 0.79 |
| 19 | E2 | L1 | -0.16 | 0.48 |
| 20 | E2 | L2 | 0.027 | 0.48 |
| | | | | |
| 21 | F1 | M1 | -0.265 | 0.26 |
| 22 | F1 | M2 | 0.422 | 0.28 |
| 23 | F2 | M1 | -0.093 | 0.72 |
| 24 | F2 | M2 | 0.085 | 0.74 |
| | | | | |
| 25 | G1 | N1 | -0.324 | 0.00 |
| 26 | G1 | N2 | 0.224 | 0.00 |
| 27 | G2 | N1 | 0.152 | 0.62 |
| 28 | G2 | N2 | -0.069 | 0.75 |

That spawn matrixing failed to yield a boost in survival, was unexpected given the litany of studies have demonstrated the utility of genetic rescue (Hogg et al. 2006; Fredrickson et al. 2007; Johnson et al. 2010). However, similar results have been seen in other Cutthroat Trout broodstocks developed from small founding populations (e. g. Bear Creek Greenback Cutthroat Trout; C. Kennedy, U.S Fish and Wildlife Service, personal communication). Although we demonstrated that the microsatellite markers were sufficient to characterize relatedness in this experiment, they remain a proxy for genes that confer fitness (i.e. survival to hatch). The Cutthroat Trout population in South Hayden Creek is small (less than 1000 individuals; G. Policky, CPW, unpublished data), and isolated above a man made barrier. Low genetic diversity is perhaps the result of a small founding population. If deleterious alleles that drive egg survival were not present in that founding population or have since been purged through a genetic sweep, we might not expect to see differences in survival (Swindell and Bouzat 2006; Facon et al. 2011; Hedrick et al. 2016).

Spawn matrixing as a culture strategy is becoming more common in the conservation of native salmonids (e.g. Gila Trout and Lahonton Cutthroat Trout), but our results underscore the importance of conducting studies to rigorously assess the benefits of such a program, as the costs

can be substantial. Not only is marking and genotyping large broodstocks expensive and time consuming, matrixing requires substantial handling of fish during a time when males are particularly vulnerable to infection (Richards and Pickering 1978; Pickering and Christie 1980). In rescue efforts such as South Hayden Creek where only 133 fish remain from the original population, that is particularly consequential. In addition, when matrixing adults in the hatchery, generally fewer adults are used to create the next generation than would be incorporated in a typical production spawn operation (E. Stege, Leadville National Fish Hatchery, personal communication). More importantly, all matrix projects to date have been performed on hatchery brood stocks which are subject to domesticating selection pressures. Our preference would be to rely instead on wild brood stocks so that domestication is mitigated and natural selection continues to operate (Hedrick et al. 2016). Culture of the rare Bear Creek Greenback Cutthroat Trout (Metcalf et al. 2012; Rogers et al. 2018) suggests that eggs from wild females perform much better anyway, with egg to fingerling survival rates being 6-fold greater when eggs were derived from the wild broodstock (29% at Zimmerman Lake) rather than eggs from that same stock reared in captivity at the Leadville National Fish Hatchery (5%; Bryan Johnson, CPW, unpublished data). Clearly, additional factors drive egg quality that may play a greater roll than spawn matrixing can overcome.

## ACKNOWLEDGMENTS

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## RESEARCH PRIORITY

Using stage-structured matrix models to inform rescue efforts for rare native trout

## OBJECTIVE

Maximize resiliency of resident Cutthroat Trout populations when performing salvage operations following wildfire

## INTRODUCTION

Extreme drought conditions over much of Colorado in 2020 (https://droughtmonitor.unl.edu/) produced a record number of large fires in the state. The two largest in Colorado history continue to smolder at the time of this writing. More broadly, Colorado's top 20 largest wildfires have all occurred in the last two decades (Table 1), precipitated by extreme drought conditions (Figure 1) as a result of a warming climate (Seager et al. 2007; Litschert et al. 2012). Colorado's first mega-fire (those that burn over 100,000 acres or 40,468 ha) occurred in 2002, yet five more have been added to the list in the last seven years.

**Table 1.** Ignition dates of the twenty largest wildfires in Colorado history arranged by decreasing burn area.

| Fire | Year | Date | Burn area ac (ha) |
|---|---|---|---|
| Cameron Peak | 2020 | Aug 13 | 208,913 (84,554) |
| East Troublesome | 2020 | Oct 14 | 193,812 (78,433) |
| Pine Gulch | 2020 | Jul 31 | 139,007 (56,254) |
| Hayman | 2002 | Jun 8 | 138114 (55893) |
| West Fork Complex | 2013 | Jun 5 | 109615 (44360) |
| Spring Creek | 2018 | Jun 27 | 108045 (43724) |
| High Park | 2012 | Jun 9 | 87284 (35323) |
| Missionary Ridge | 2002 | Jun 9 | 71739 (29032) |
| 416 | 2018 | Jun 1 | 52778 (21358) |
| Bridger | 2008 | Jun 8 | 46612 (18863) |
| Last Chance | 2012 | Jun 25 | 44000 (17806) |
| Milemarker 117 | 2018 | Apr 17 | 42795 (17319) |
| Beaver Creek | 2016 | Jun 19 | 38380 (15532) |
| Bull Draw | 2018 | Jul 29 | 36520 (14779) |
| Badger Hole | 2018 | Apr 17 | 33609 (13601) |
| Grizzly Creek | 2020 | Aug 10 | 32631 (13205) |
| Logan | 2017 | Mar 6 | 32564 (13178) |
| Mt Zirkel Complex | 2002 | Jul 12 | 31016 (12552) |

| | | | |
|---|---|---|---|
| Burn Canyon | 2002 | Jul 14 | 30573 (12372) |
| Trinidad Complex | 2002 | Jun 3 | 27084 (10961) |

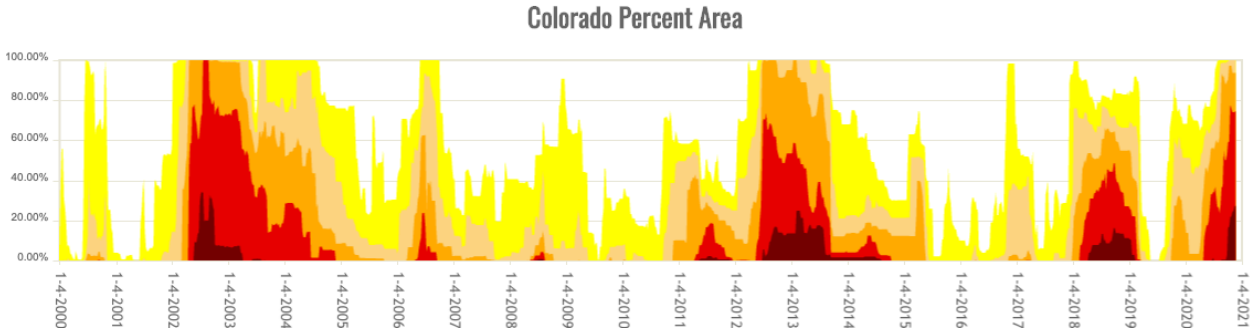


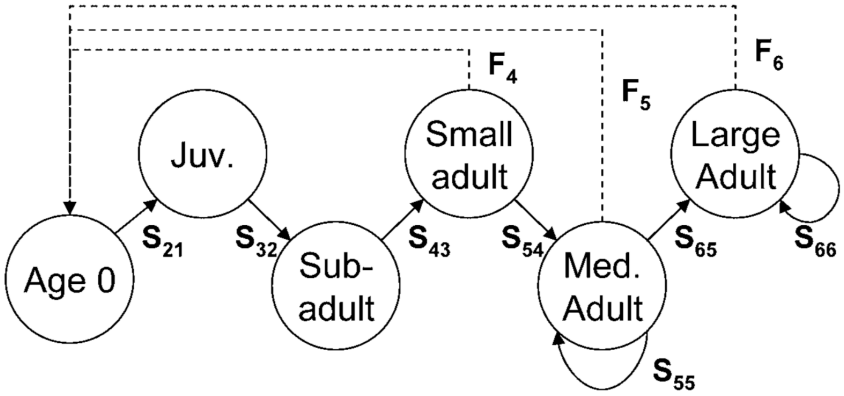

**Figure 1.** Palmer Drought Severity index data downloaded from the United States Drought monitor site (https://droughtmonitor.unl.edu/) reveal the percent of Colorado experiencing "abnormally dry" conditions (yellow) to "exceptional drought" (brown) by year over the last two decades.

Though the increase in wildfire intensity, scale, and fire season duration is not unexpected (McKenzie et al. 2004; Westerling et al. 2006; Westerling 2016), it is clear that forests previously deemed resilient to wildfire may in fact not be. With increasingly large fires, more Cutthroat Trout Conservation Populations (CPs; sensu UDWR 2000; Hirsch et al. 2013; Zeigler et al. 2019) will be vulnerable, and informed rescue efforts will need to be mobilized quickly before debris flows materialize and decimate populations (Rinne 1996; Howell 2006; Burton 2005; Sedell et al. 2015). In 2020 alone, over 20 CPs were jeopardized by fire and debris flows, with rescue plans initiated for Roan Creek, Bobtail Creek, Steelman Creek, and Trail Creek. To inform these rescue efforts, I used a stage structured matrix model to project the consequences of removing variable numbers of trout from each age class following a fire so that resiliency of the source population can be maximized should lethal debris flows not materialize.

## METHODS

I coded the age-structured matrix model for Cutthroat Trout presented in Peterson et al. (2008; Figure 2) in the LabVIEW programming environment (National Instruments, Austin, Texas). Vital rates derived from four CRCT streams in that same paper and Peterson et al. (2004) were used to populate the realization matrix (Figure 2). For the purposes of this exercise, I assumed Cutthroat Trout populations were living in allopatry, with densities mirroring those found across 121 RGCT conservation populations with median values of 312 trout >75mm/km (Zeigler et al. 2019). With a median stream length of 6.3 km (Zeigler et al. 2019), this suggests an average total population size of just under 2000 fish >75mm, and a simulated stable age structure array of [1200, 480, 160, 80, 20] representing the number of [Juvenile, Sub-adult, Small adult, Medium adult, Large adult] trout in the population (Figure 2). Since fish were being rescued (removed)

from the burned watershed, I assumed the remaining population was left below carrying capacity, and that density dependence therefore would not apply. Currently, the model is deterministic, but stochasticity could be incorporated in the future, which would be beneficial – particularly when exploring persistence and recovery in small populations.




$$A_{CT} = \begin{pmatrix} 0 & 0 & 0 & F_4 & F_5 & F_6 \\ S_{21} & 0 & 0 & 0 & 0 & 0 \\ 0 & S_{32} & 0 & 0 & 0 & 0 \\ 0 & 0 & S_{43} & 0 & 0 & 0 \\ 0 & 0 & 0 & S_{54} & S_{55} & 0 \\ 0 & 0 & 0 & 0 & S_{65} & S_{66} \end{pmatrix}$$

**Figure 2.** Life cycle and associated matrix representation of the stage-based population model for stream-resident Cutthroat Trout. Figure and vital rates (Survival [S] and Fecundity [F]) taken from Peterson et al. (2008).

Using the initial conditions above, I simulated removals of 80% (essentially everything you can capture electrofishing in a stream with habitat conducive to high capture probabilities), 40% (retaining every other fish captured), as well as entire year-classes to assess the relative influence of removing an entire cohort on population stability. The return of the adult (age 3-5) population numbers to pre-salvage levels was used as a metric to assess recovery. In addition, I simulated removal of 60 fish for lethal disease samples, and 120 fish which was the average number used to found the South Hayden broodstock in 2016 (158 as above), the Bear Creek broodstock in 2008 (66 fish; B. Johnson, CPW, personal communication), and the original Carr Creek repatriation effort translocated from Roan Creek in 1998 (150 fish; B. Elmblad, retired CPW, personal communication).

## RESULTS & DISCUSSION

In general, rescue efforts that focused on younger age classes tended to be more benign. Even when 80% of the age 0 cohort was removed, only mild dips in the eventual adult population were

detected, with full recovery achieved within four years (Figure 3). Conversely, when only half of all fish captured (including adults) were removed, those adult populations took over twice as long to rebound. This confirms that removing younger fish is preferred when trying to preserve resiliency in donor populations. High fecundity in large females allows them to compensate for substantial reductions particularly in the young-of-year age group that has not yet recruited to the population. This is precisely why the recovery plan for Greenback Cutthroat Trout only requires recruitment in at least two out of every five-year period for a population to be considered stable (USFWS 1998). In addition, estimates derived here are somewhat conservative, as Cutthroat Trout (particularly from higher elevation populations) can live longer than six years (Behnke 2002; Trotter 2008), allowing more adults to be stockpiled in a population. However, it should be noted that egg quality begins to deteriorate in older females (B. Johnson, CPW, personal communication).

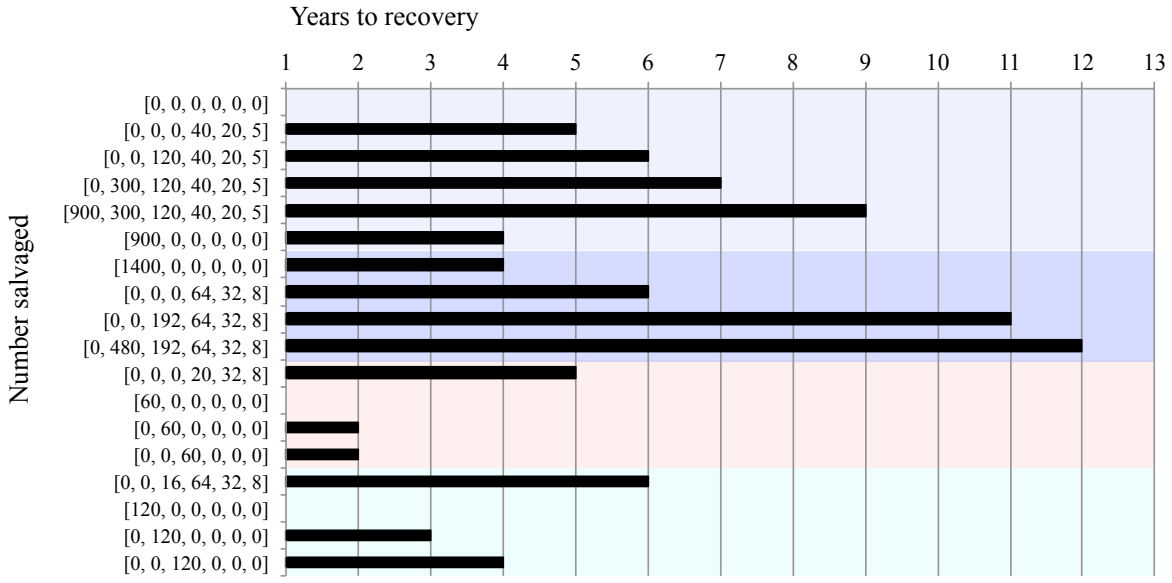



**Figure 3.** Source population projected years to recovery (return of adults to baseline values) based on numbers of fish rescued in each stage ([Age-0, Juvenile, Sub-adult, Small adult, Medium adult, Large adult]). Lighter blue shading represent scenarios where numbers of adults, sub-adults, juveniles, or age-0s were halved following rescue efforts. Darker blue numbers reflect 80% of the target stages being removed. Red shading highlights the consequences of taking a 60 fish sample for disease testing, while green represents removing 120 fish for broodstock development

***Timing***

In cold high elevation streams, it is possible that young-of-year Cutthroat Trout have not accumulated enough degree-days to emerge from the gravels during the traditional peak of the fire season. This was likely the case for the August Williams Fork fire where typical emergence in Bobtail Creek (3230 m) does not occur until September (KBR, unpublished data). However,

future fire seasons are expected to last further into fall (Rocca et al. 2014; Balch et al. 2017) which was certainly the case with the East Troublesome fire that burned over 87,000 acres (35,200 ha) in a single night on October 21st. This precipitated the planning of a rescue effort for the CP in Trail Creek, but electrofishing surveys on November 5th, 2020 suggested there was nothing left of this population to salvage (J. Ewert, CPW, personal communication).

***Establishing broodstocks***

The same approach used here can also be used to evaluate the resiliency of a donor population when individuals are collected to establish either a new population by direct transfer, or by establishing a captive brood stock so that large numbers of progeny can be produced for repatriation efforts. Here too, if the initial broodstock can be developed from younger age classes rather than adults, recovery time of the donor population can cut in half or more (Figure 3). If captive broodstocks are to be developed, some consideration should be given to aligning age structures so that an adequate number of females are available to provide a robust year-class when they mature. Collection of age-0 or age-1 fish also provides a two-year delay before eggs will be available which can be beneficial if reclamation projects need to be implemented before repatriation can occur.

***Disease sampling***

The collection of 60 trout (lethal samples) is commonplace when screening for common pathogens in the wild (USFWS and AFS-FHS 2014). This number ensures that one can have 95% confidence that a pathogen will be detected when prevalence is as low as 5%. During the widespread screening of Cutthroat Trout populations for the whirling disease parasite (*Myxobolus cerebralis*) in the late 1990s, managers were concerned whether small populations could tolerate that level of harvest. Clearly sampling younger cohorts in the population is more benign than harvesting mature fish (Figure 3).

***Other considerations***

This model assumes there is no penalty for driving source populations to precariously low numbers, since numbers eventually rebound. In small populations however, extreme care should be taken to prevent forcing a genetic bottleneck (Allendorf and Luikart 2007; Whiteley et al. 2013; Robinson et al. 2017) on either the rescued fish that will serve as future brood stock (see pages 21-27 above), or the population that is left behind to rebound should ash flows not materialize.

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## RESEARCH PRIORITY

Greenback Cutthroat Trout escapement from Zimmerman Lake, Colorado

## OBJECTIVE

Explore whether Greenback Cutthroat Trout escapement from Zimmerman Lake is responsible for poor recruitment of stocked fingerlings in some years.

## INTRODUCTION

The discovery of a single remaining population of the native trout of the South Platte River basin in Bear Creek, Colorado (Metcalf et al. 2012; Rogers et al. 2018; Bestgen et al. 2019), spurred the rapid development of both captive and wild brood sources to produce progeny needed for the recovery effort. Following reclamation in 2013, Zimmerman Lake (10,495 ft) became the first repatriated population, receiving 1,000 yearling Greenback Cutthroat Trout (*Oncorhynchus clarkii ssp*.) in August of 2014. Similar numbers have since been stocked annually each July, yet recruitment in some years has been poor. The outlet pipe is not screened, and though not a problem initially, we wondered if a significant number of stocked fish were leaving the system, as fish are unable to return to the lake once entrained in the outlet works. Emigrating fish must therefore take up residence in the stream below the outlet, further downstream in Joe Wright Creek, or 0.7 miles downstream in Joe Wright Reservoir. All Cutthroat Trout stocked into Zimmerman Lake receive PIT tags so that individual growth and survival can be evaluated. In this study, we took advantage of those unique tags to readily monitor escapement by deploying antenna readers downstream of the outlet works during the summer of 2020.

## METHODS

Fertilized Greenback Cutthroat Trout eggs produced from broodstock at the Leadville National Fish Hatchery or Zimmerman Lake were hatched at the Salida Isolation Unit in 50°F (10°C) water, then reared for 12 months in 55°F (13°C) water. In the first week of June each year (three weeks prior to stocking) we isolated 1,000 fingerlings (mean total length 2015-2020 = 4.2 in (122 mm). Each fish was weighed and measured, received a unique PIT tag (HDX12 or HPT9, Biomark, Boise, Idaho) in the dorsal musculature (Daugherty and Buckmeier 2009; Younk et al. 2010; Bodine and Fleming 2014; Mamer and Meyer 2016), and given a visual implant elastomer tag (VIE; Northwest Marine Technology, Shaw Island, Washington) so that relatedness could be easily identified during wild spawn operations. A tissue sample and photograph of each fish were also obtained for subsequent meristic and molecular studies. Fish were released in Zimmerman Lake a month later, after wild spawn operations on the lake were complete.

Three submersible PIT tag readers (Biomark, Boise, Idaho) were deployed in the outlet pool below the earthen dam on Zimmerman Lake, arranged as indicated in Figure 1 so that

escapement through the outlet works could be monitored. Readers were configured so that a PIT tag was only recorded if five minutes had elapsed since it was last recorded, or if a different PIT tag pinged the antenna. Batteries were replaced every two weeks at which time archived data were downloaded from the readers. Flows were not measured directly at the outlet of Zimmerman Lake, but correlations with movement were explored with surrogate data from Joe Wright Creek downstream, obtained from https://waterdata.usgs.gov/co/nwis/inventory/?site_no=06746095. Similarly, we calculated average daily Snow Water Equivalents (SWE; in) from a nearby snow telemetry (SNOTEL) site (10,120 ft; https://wcc.sc.egov.usda.gov/nwcc/site?sitenum=551).


**Figure 1.** Antenna locations in the outlet pool below Zimmerman Lake. The outlet works are housed in the culvert in the bottom left of the photograph

## RESULTS

All three antennae were successfully deployed on July 2, 2020 following the last spawn take effort. Unfortunately, the entire monitoring effort only lasted 36 days, as the Cameron Peak Fire erupted on August 13th, shutting down access to the field site. Antennae batteries appeared to fail around August 6th. Special access permission was granted on October 6th to retrieve gear before winter. In all, 21,810 pings from 13 unique fish were recorded on the three antennae over

the 36-day period. The majority (67%) were recorded by Antenna A located in the deepest part of the outlet channel, while only 8% of the pings came from Antenna B, and 25% on Antenna C. Every fish visited each antennae at least once, and were recorded anywhere from 4-6660 times by the array. Each fish spent from 1 min - 36 days in the outlet pool area (median = 6.5 days), and all but one pinged Antenna C last, indicating movement downstream out of the study area (Table 1).

**Table 1.** Arrival dates in the outlet pool for each fish are provided, along with the number of days fish remained in the pool, and the year they were stocked.

| PIT tag | Arrival | Duration (d) | Stocked |
|---|---|---|---|
| 3DD.003BDD3DB1 | 7/2/20 | 1 | 2015 |
| 3DD.003BF14EFE | 7/2/20 | 7 | 2017 |
| 3D6.1D59583027 | 7/2/20 | 1 | 2018 |
| 3D6.1D595830FC | 7/2/20 | 6 | 2018 |
| 3D6.1D595832A5 | 7/2/20 | 36 | 2018 |
| 3D6.1D5958311A | 7/3/20 | 2 | 2018 |
| 3D6.1D59729DD8 | 7/4/20 | 2 | 2018 |
| 3D6.1D59BDFBE9 | 7/3/20 | 33 | 2020 |
| 3D6.1D59BD94AD | 7/4/20 | 34 | 2020 |
| 3D6.1D59B284C7 | 7/8/20 | 12 | 2020 |
| 3D6.1D59BDFCDE | 7/10/20 | 27 | 2020 |
| 3D6.1D59BDA8CD | 7/28/20 | 8 | 2020 |
| 3D6.1D59BDFB24 | 8/5/20 | 1 | 2020 |

One tag (3D3.0E19BD8CAD) was not present in the tagging database, nor did it exit the system via downstream Antenna C as was typical. We suspect that this tag was carried by a boreal toad that visited the outlet pool in the predawn hours of August 6, 2020.

The majority of escapement occurred in the first week following submersible reader deployment (Figure 2). Interestingly, all adult escapement occurred during this time, while recently stocked fish (2019 year-class) continued to become entrained in the outlet works throughout the remainder of the study period. To explore the influence of flow on entrainment, we plotted the seasonal hydrograph for the nearest USGS flow gage downstream on Joe Wright Creek. Flows here dropped from 15 cubic feet per second (CFS) at the start of our study to just 6 CFS at the end on August 6$^{th}$ (Figure 3). Unfortunately, flows in Joe Wright Creek are influenced by the Michigan Ditch, which provides snowmelt from basins at higher elevations than feed Zimmerman Lake. To find a more representative surrogate for flows in the outlet of Zimmerman Lake, we examined SWE in the nearest SNOTEL site located 0.7 mi to the southwest at slightly lower elevation (Figure 4). Rapid declines in June SWE at this site appear to consistently predict timing of the spawn run. Peak spawn (defined as the date at which more females are ripe than

green) varies by year, and is strongly correlated (r2 = 0.99) with the date at which SWE dips below 0.5 ft (Figure 5).

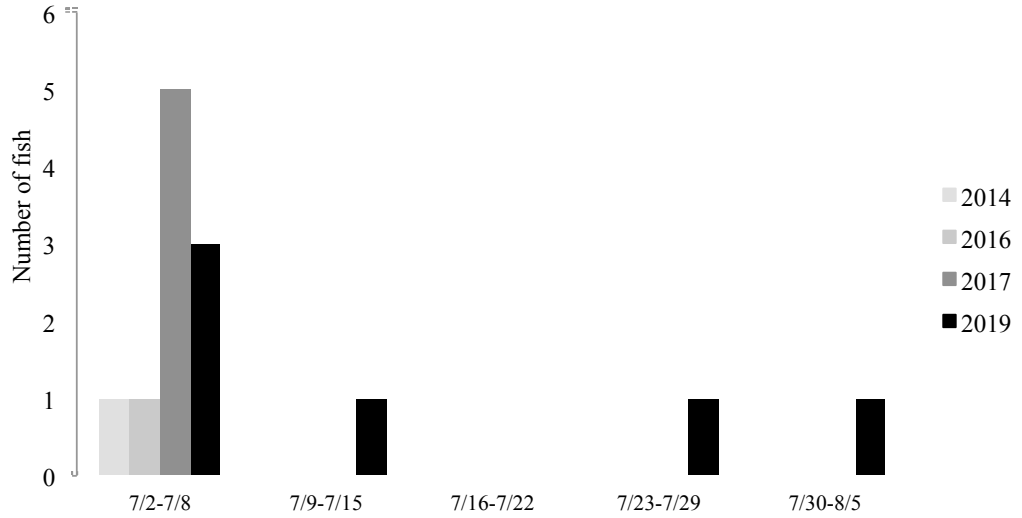


**Figure 2.** Number of weekly new Cutthroat Trout arrivals to the outlet pool below Zimmerman Lake. Bar shading represent the year in which the emigrating Cutthroat Trout hatched.

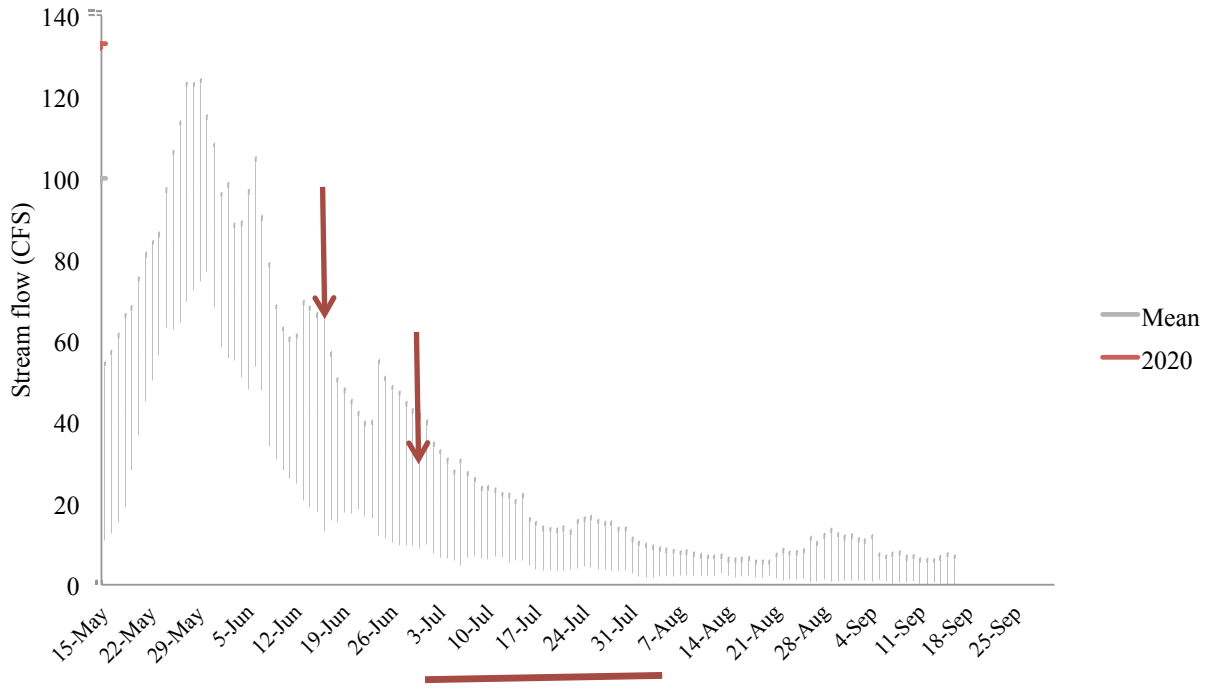


**Figure 3.** Mean stream flows recorded in Joe Wright Creek (gray line) upstream of Joe Wright Reservoir from 2013-2019, along with associated 95% confidence intervals. The red line

represents values from 2020. Submersible PIT readers were deployed on the dates covered by the heavy bar below the x-axis. Arrows represent the beginning and end of the spawning run.

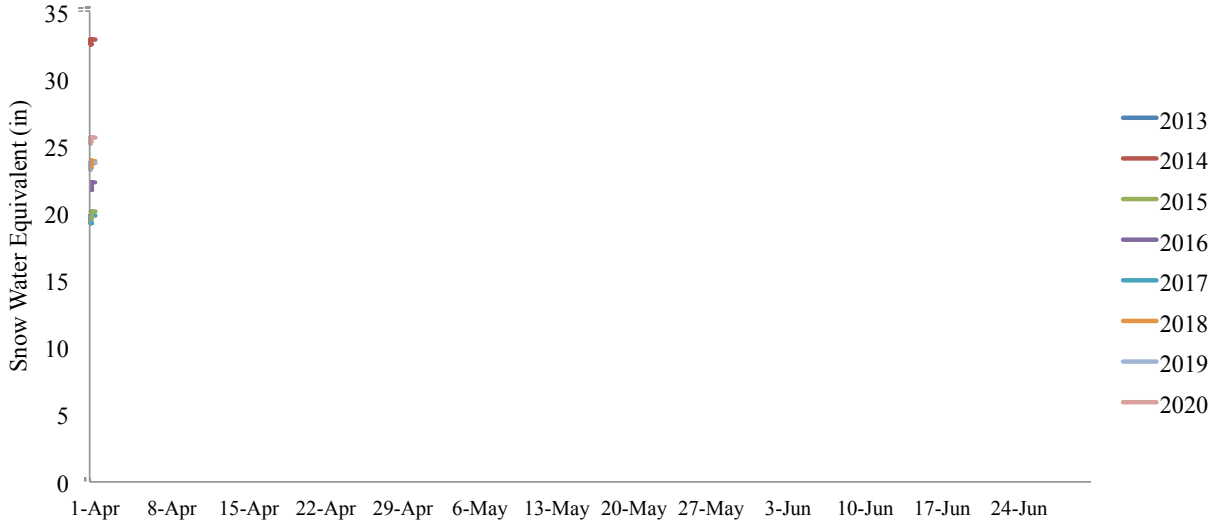


**Figure 4.** Time series of Snow Water Equivalents (in) for the Joe Wright SNOTEL site by year

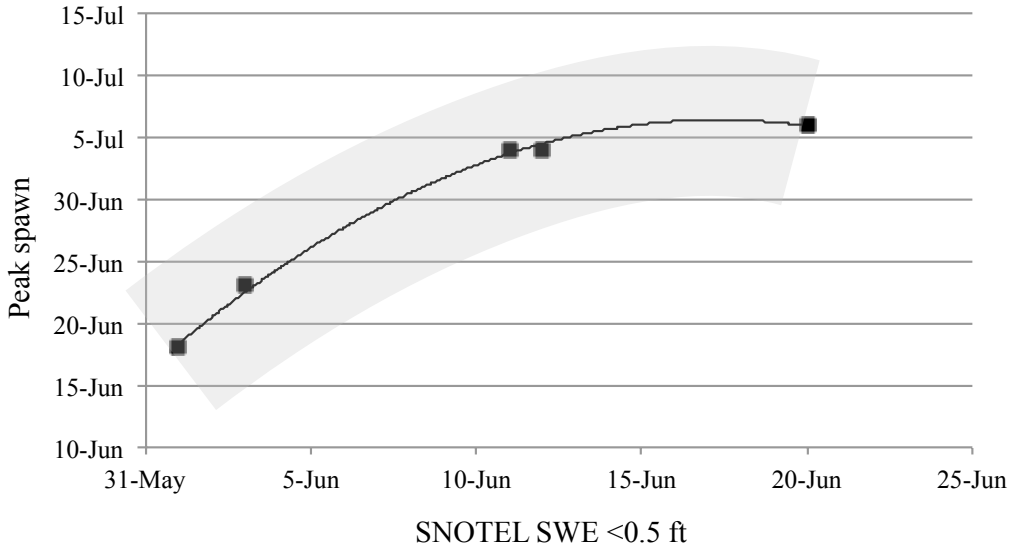


**Figure 5.** Relationship between peak spawn (the day the number of ripe females begins to exceed the number of green females in the run) as a function of SWE <6 in. Gray shading covers the approximate range of the spawn run, from the point at which females begin to ripen, to when most are done spawning and green fish are no longer present.

## DISCUSSION

Despite a short residence time in the outlet pool by some fish (<1 min), we were pleased to see that all fish pinged every antenna while moving through. This gives us confidence that while read ranges on the submersible readers can be less than a foot with the PIT tags we used, it was unlikely that any fish made it through our gauntlet of readers undetected. Though it is unfortunate that access precluded running the antennae readers for the entire summer, our results hint at the timing of escapement, and provide some insight into the magnitude of the problem. While tagged Cutthroat Trout continued to emigrate from Zimmerman Lake over the duration of the study, all adult fish emigration occurred in the week following our spawn operation (Figure 2). This finding is consistent with other studies on Cutthroat Trout in Colorado that documented increased movement associated with spawning (Hodge et al. 2017, 2017b). Escapement did not appear to be induced by handling stress during the spawn operation, as only one of the seven adult emigrants was handled during the two-week spawn operation (a 14 in ripe male stocked in 2017). Emigration rates later in the summer were much lower, and consisted entirely of recently stocked yearling Cutthroat Trout. As such, it is unlikely that population declines could be attributed to fall or winter emigration when flows were even lower. However, since monitoring was not initiated until the spawn was essentially over, the possibility of substantial emigration during high flows associated with snow melt and spawning activities can not be ruled out. We advocate repeating this monitoring effort in 2021 with an attempt to deploy the submersible readers before the ice comes off Zimmerman Lake.

## ACKNOWLEDGMENTS

I wish to acknowledge my collaborators Boyd Wright and Alex Jouney (Northeast Aquatics, Colorado Parks and Wildlife, Fort Collins). We thank Kevin Thompson and Jenn Logan for loaning us the wheel antennae and batteries needed for this effort. Kevin is also thanked for coming up to Zimmerman Lake to oversee proper deployment of the antennae and software training. We also wish to acknowledge Andrew Martin and the 32 students and technicians who have helped tag all the fish stocked into Zimmerman Lake since 2013, and Bryan Johnson and the crew at CPWs Mount Shavano Hatchery for raising these fish and indulging our annual tagging operations.

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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, and archiving information for the future.

## ACCOMPLISHMENTS

### *Peer-reviewed publications*

Bestgen, K. R., **K. B. Rogers**, R. Granger. 2019. Distinct phenotypes of native Cutthroat Trout emerge under a molecular model of lineage distributions. Transactions of the American Fisheries Society 148:442-463. *Awarded the Phelps Memorial Award given to the best genetics paper published in an AFS journal in 2019*.

*Abstract*.— Recent molecular investigations using contemporary and century-old museum specimens questioned the traditional four subspecies taxonomic arrangement of Cutthroat Trout *Oncorhynchus clarkii* in the southern Rocky Mountains and revealed six lineages, including two that are likely extinct. We examined extant lineage specimens to determine whether morpho-meristic taxonomic approaches better classified Cutthroat Trout under (1) the traditional Geographic Model, which recognizes different subspecies east and west of the Continental Divide and in the Rio Grande basin; or (2) the Molecular Model, which uses genotypes to assign populations to four lineages. Classification success of the Molecular Model was higher than that of the Geographic Model, whether comparisons involved single-trait, principal components, or discriminant function analyses. Native east slope South Platte River trout (putative Greenback Cutthroat Trout *O. clarkii stomias*) were distinct and correctly classified, as were 83% of Rio Grande Cutthroat Trout *O. clarkii virginalis* populations. In all, 100% of the Blue Lineage populations of Colorado River Cutthroat Trout *O. clarkii pleuriticus* (putative west slope native of the White, Yampa, Green, and downstream Colorado River drainages) and 71% of the Green Lineage populations of Colorado River Cutthroat Trout (native to west slope Gunnison and Dolores River drainages and Colorado River headwaters) were correctly classified (89% overall)

under the Molecular Model. Green Lineage misclassifications were mainly from morphologically and genetically distinct populations located east of the Continental Divide, whose native status remains unknown. In contrast, only 63% of those east slope and west slope Cutthroat Trout populations were correctly classified under the Geographic Model. Cohesion of distinct phenotypes and genotypes of present-day native Cutthroat Trout lineages was remarkable given widespread and massive early stocking of various lineages outside of their native ranges. Strong congruence of morphological and molecular patterns demonstrated the power of joint morphological and molecular analyses. We encourage management that preserves diversity of these rare Cutthroat Trout lineages that evolved in concert with their environment.

Zeigler, M. P., **K. B. Rogers**, J. J. Roberts, A. S. Todd, and K. D. Fausch. 2019. Predicting persistence of Rio Grande Cutthroat Trout populations in an uncertain future. North American Journal of Fisheries Management 39:819-848. *Awarded best publication in the North American Journal of Fisheries Management for 2019*.

*Abstract*.— The Rio Grande Cutthroat Trout *Oncorhynchus clarkii virginalis* (RGCT) occupies just 12% of its ancestral range. As the southernmost subspecies of Cutthroat Trout, we expect a warming climate to bring additional stressors to RGCT populations, such as increased stream temperatures, reduced streamflows, and increased incidence of wildfire. We developed a Bayesian network (BN) model using site-specific data, empirical research, and expert knowledge to estimate the probability of persistence for each of the 121 remaining RGCT conservation populations and to rank the severity of the threats they face. These inputs quantified the genetic risks (e.g., inbreeding risk and hybridization risk), population demographics (disease risk, habitat suitability, and survival), and probability of stochastic disturbances (stream drying risk and wildfire risk) in an uncertain future. We also created stream temperature and base flow discharge models coupled with regionally downscaled climate projections to predict future abiotic conditions at short-term (2040s) and long-term (2080s) time horizons. In the absence of active management, we predicted a decrease in the average probability of population persistence from 0.53 (current) to 0.31 (2040s) and 0.26 (2080s). Only 11% of these populations were predicted to have a greater than 75% chance of persisting to the 2080s. Threat of invasion by nonnative trout had the strongest effect on population persistence. Of the 78 populations that are already invaded or lacking complete barriers, 60% were estimated to be extirpated by 2080 and the remainder averaged only a 10% chance of persistence. In contrast, the effects of increased stream temperatures were predicted to affect the future persistence of only 9% of the 121 RGCT populations remaining, as most have been restricted to high-elevation habitats.

Budy, P., **K. B. Rogers**, Y. Kanno, B. Penaluna, N. P. Hitt, G. P. Thiede, J. Dunham, C. Mellison, W. L. Somer. 2019. Distribution and status of trouts and chars in North America. Pages 193-250 in J. L. Kershner, J. E. Williams, R. E. Gresswell, and J. Lobon-Cervia, editors. Trout and char of the world. American Fisheries Society, Bethesda, Maryland.

*Summary*.— Trout and char span the continent of North America, hugging its coasts and occupying many catchments throughout the interior of the continent. They have endured and persisted as North America has changed through time, including advances and retreats of glaciers, volcanic eruptions, enormous floods, and the formation of mountain ranges and plateaus. Most trout and char are found in mountainous catchments, requiring specific combinations of flow, temperature, velocity, depth, and cover to thrive. Recent research has led to the revision of the origin and diversification of trout and char. Although common fish names still refer to some of these fishes and others as trout, char, or salmon, recent realignments show that in North America, trout are Pacific trout *Oncorhynchus spp*. and char encompass all fishes in the genus of *Salvelinus*. Fishes in the genus of *Salmo* are also referred to as salmon or trout, and trout species of *Salmo spp*. are native to catchments that drain into the North Atlantic, mainly in Europe and the Atlantic Isles. The taxonomy of North American trout is complicated, changes frequently, and is often under debate. This manuscript represents the best current understanding of distribution and status but is likely to continue to evolve as the science improves.

Herrmann, S. J., D. W. R. Nimmo, J. S. Carsella, I. V. Melnykov, C. M. Kennedy, **K. B. Rogers**, and L. M. Hermann-Hoesing. 2020. Differential bioaccumulation of mercury and selenium in stomach contents and tissues of three Colorado, USA, Cutthroat Trout populations. Bulletin of Environmental Contamination and Toxicology 104:595-601.

*Abstract*.—Total mercury (THg) and selenium (TSe) levels were measured in stomach contents (SC) and twelve tissues of cutthroat trout (*Oncorhynchus clarkii*) occurring in three high-elevation lakes of Colorado, USA, inhabiting watersheds absent past and cur- rent mining activities. For 32 of 36 tissues, including muscle, mean THg wet weight (ww) concentrations were greater than in the diet (SC) for all sites, indicating biomagnification. Ranges of THg (µg/kg ww) for SC and stomach tissue (ST) were 1.23–73.54 and 14.55–61.35, respectively. Selenium concentrations in fish muscle were not greater than in the SC indicating a trophic transfer factor < 1.0. However, in several other tissues, mean Se dry weight (dw) levels were greater than in SC for all three lakes. Ranges of TSe for SC and ST were 166–7544 and 797–7523 (µg/kg dw), respectively. The muscle to egg/ ovary ratio for Se averaged 2.30, 4.60, and 2.68 for the three populations. The variability of SC (planktonic vs. benthic) and differential distributions of THg and TSe in SC and organ-tissues generated questions focusing on the seasonal, physiological, and genetic drivers of these organometal(loid)s in subalpine trout.

***Presentations***

Rogers, K. B., S. Firestone, A. Whiteley, and P. Lukacs. December 4, 2019. Spawn matrixing fails to improve survival in a unique Cutthroat Trout population following fire-mediated extirpation in the wild. Colorado River Cutthroat Trout Conservation Team Meeting, Grand Junction, Colorado.

Rogers, K. B., J. J. Roberts, S. E. Albeke, C. M. Kennedy, J. S. Wood. February 27, 2020.

Status and conservation of an undescribed lineage of Cutthroat Trout native to the southern Rocky Mountains. Colorado/Wyoming Chapter AFS meeting, Laramie, Wyoming. *Awarded best professional paper.*

Roberts, J., C. Kennedy, K. B. Rogers, K. D. Fausch, and F. B. Wright . February 27, 2020. Influence of changing lake and stream thermal regimes in the Southern Rocky Mountains on Greenback Cutthroat Trout: Insights from dense sensor networks and past conservation efforts

Rogers, K. B., J. Roberts, S. Albeke, C. Kennedy, J. Wood. March 13, 2020. Status and conservation of an undescribed lineage of Cutthroat Trout native to the southern Rocky Mountains. Meeker, Colorado [cancelled]

Rogers, K. B. August 10, 2020. Cutthroats, climate and CoViD: why ignoring science is a bad idea. DWM pack training. Bellaire, Colorado

Rogers, K. B. October 17, 2020. Conserving Cutthroat Trout in Colorado. Trout Unlimited Rendevouz [Virtual].

Rogers, K. B., A. Jouney, and F. B. Wright. November 13, 2020. Escapement of Greenback Cutthroat Trout from Zimmerman Lake, Colorado. Greenback Recovery Team Meeting [Virtual]