Cutthroat Trout Studies

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The results of the research investigations contained in this report represent work of the authors and may or may not have been implemented as Colorado Parks & Wildlife policy by the Director or the Wildlife Commission.

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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 and Rio Grande Cutthroat Trout (RGCT) Conservation Team processed in 2024 are presented here.

METHODS

Molecular tests were conducted on 172 samples obtained from seven Cutthroat Trout populations distributed across Colorado (Table 1). Six populations came from native range of CRCT, including the Colorado headwaters, Yampa and San Juan rivers. The last population came from the RGCT native range in Colorado. 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) 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 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 2024.

Stream	Water Code	Date	Sample size
Colorado			
Fryingpan, S Fk	23468	7/12/2023	5
McCoy Creek	19421	9/5/2023	20
Rio Grande			
Cat Creek	44242	8/21/2023	30
San Juan			
Bear Creek	46119	7/24/2023	30
Mill Creek	44228	9/14/2023	30
Yampa			
Bear River	21236	10/18/2023	36
Mill Creek	23096	8/21/2023	21

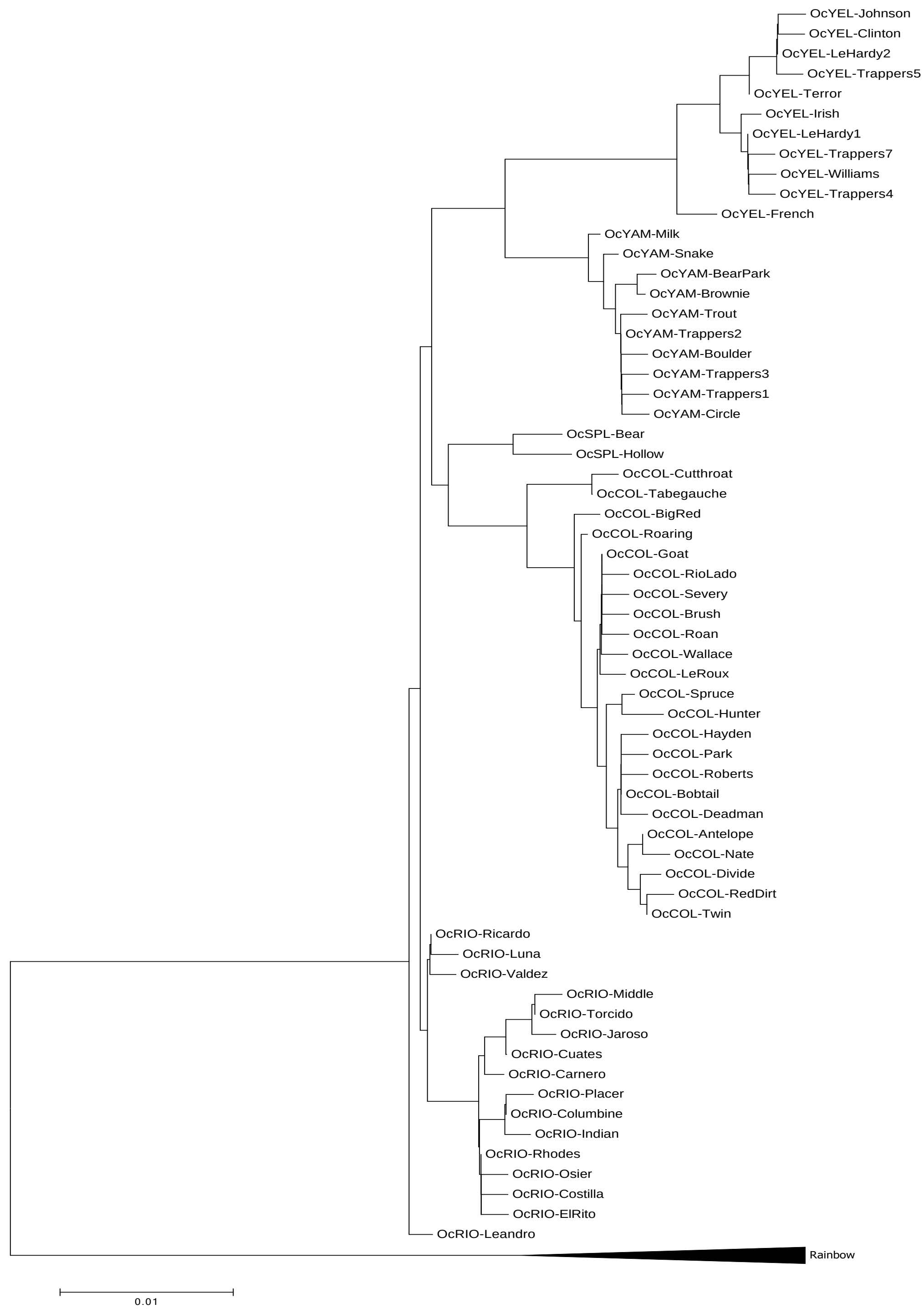

Figure 1. Phylogenetic relationships inferred from 648 base pairs of the mitochondrial NADH dehydrogenase subunit 2 gene for Cutthroat Trout from Colorado. The evolutionary history was developed with the neighbor-joining method in MEGA7, with evolutionary distance units representing the number of base 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 five Cutthroat Trout collections analyzed in 2024, along with the number of samples analyzed, organized by major drainage basin. Percent admixture is given by lineage, including blue and green lineages of Colorado River Cutthroat Trout (bCRCT, gCRCT), Rio Grande Cutthroat Trout (RGCT), Yellowstone Cutthroat Trout (YSCT), and Rainbow Trout (RBT).

Stream	**# Analyzed**	**Lineage**				
		bCRCT	gCRCT	RGCT	YSCT	RBT
Colorado						
McCoy Creek	20	-	100	-	-	-
Rio Grande						
Cat Creek	30	-	-	100	-	-
Cat Creek[1]	30	-	-	100	-	-
San Juan						
Bear Creek	30	99	-	-	1	-
Mill Creek	30	99	-	-	-	-
Yampa						
Mill Creek	21	100	-	-	-	-

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

Table 3. ND2 results from seven Cutthroat Trout collections analyzed in 2024, along with the number of samples analyzed, organized by major drainage basin. ND2 haplotype is given by lineage, including blue and green lineages of Colorado River Cutthroat Trout (bCRCT, gCRCT), Rio Grande Cutthroat Trout (RGCT), Yellowstone Cutthroat Trout (YSCT), and Rainbow Trout (RBT).

Stream	**# Analyzed**	**Lineage**				
		bCRCT	gCRCT	RGCT	YSCT	RBT
Colorado						
Fryingpan, S Fk	5	4	-	-	1	-
McCoy Creek	15	-	15	-	-	-
Rio Grande						
Cat Creek	30	10	-	20	-	-
San Juan						
Bear Creek	30	-	-	-	30	-
Mill Creek	30	30	-	-	-	-
Yampa						
Bear River	36	36	-	-	-	-
Mill Creek	18	18	-	-	-	-

Colorado River basin

Fryingpan, S Fk (WC#23468)— This stream reach above the diversion barrier (13S 363068m E, 4342115m N) had never been sampled before, and it was hoped that some native gCRCT were able to persist. The drainage has received numerous pack plants over the years, though no stocking records appear for this section of stream. Although a small sample size, with four fish displaying the OcYAM-Trappers2 haplotype and the remainder being OcYEL-Fryingpan suggests this stream was likely stocked with Trappers Lake progeny sometime after 1954 and that those fish became established. This is the first detection of the OcYEL-Fryingpan haplotype in Colorado, but with almost a million YSCT stocked in Trappers Lake from 1943 to 1950 (Martinez 1988; Leary and Allendorf 1991), it is perhaps not too surprising that additional diversity continues to crop up.

McCoy Creek (WC# 19421)— While reviewing a culvert replacement project on McCoy Creek, near Edwards, Colorado, CPW biologist Kendall Bakich discovered an old record of a cutthroat trout being captured low in the system on private property. Forest Service crews had found no fish in surveys up high on public lands that often goes dry during drought years, but when Kendall returned on 9/5/2023 to survey the private water again, she was able to collect 20 fin clips that looked to be gCRCT as measured with AFLPs. Only 15 yielded useable mitochondrial sequence data, which all displayed the common OcCOL-Goat haplotype. While it is uncertain if the culvert serves as a complete barrier at this time, the ponds on the Country Club of the

Rockies golf course in the gated community of Arrowhead appear to prevent immigration of nonnative trout from the Eagle River into the system. There is a good chance that this section of stream has not been stocked (no evidence of bCRCT alleles), and perhaps represents a relict population.

Rio Grande basin

Cat Creek (WC#44242)— It is a bit of a mystery how this small conservation population persists given that the stream dries up often. With recent surveys coming up empty handed in the historical sites, this survey focused on the BLM lands downstream below private ownership where a 30 fish sample was obtained. While the AFLP results suggest the population is pure RGCT, the mitochondrial DNA does not. A third of the samples harbored OcYAM-Trappers2 haplotype suggesting bCRCT were stocked on top of this population at some point. Of the remaining 20 samples, three were OcRIO-Rhodes, two were OcRIO-Cuates, and 15 were a new RGCT haplotype now named OcRIO-Cat. This unique bit of genetic diversity warrants additional investigation to determine if the population is severely bottlenecked (given the populations small size and repeated desiccation events), and whether genetic rescue is warranted. Additional work should be performed on the nuclear genome to see if more robust tests reveal any admixture with bCRCT. Exploring options to enhance the population through habitat improvements or flow augmentation should also be considered.

San Juan basin

Bear Creek (WC# 46119)— This stream was surveyed in 2004 by Mike Japhet and his 20 fish sample showed predominantly bCRCT with 2% YSCT alleles by AFLP. Interestingly, 80% of the ND2 haplotypes came back as OcYEL at that time (remainder were OcYAM-Trappers2). Since those tissues were degraded, 30 new fins were collected in July of 2023 in hopes of finding alleles representing the native San Juan Cutthroat Trout. These new results were even more skewed, with AFLPs showing only 1% YSCT admixture and ND2 sequencing suggesting all 30 fish were YSCT, with no evidence of San Juan haplotypes. There is no record of fish being stocked in Bear Creek, but retired DWM Knisley remembers packing unknown trout in there prior to 1990.

Mill Creek (WC# 44228)— Jim White had hoped this population would be another San Juan lineage CRCT, but all 30 fish display the common OcYAM-Trappers2 mitochondrial haplotype. More robust tools are available to search for any relict San Juan alleles hidden in the nuclear genome, but it seems unlikely given the ND2 signature.

Yampa River basin

Bear River (WC# 21236)— This collection effort occurred between Bear Lake and Stillwater Reservoir in the headwaters of the Yampa River. These fish were stocked in the fall of 2023 as part of Morgan Spark's post doc work to look at which genotypes prevail when hatchery stocks are released into the wild. The ND2 sequence data was a bonus consequence and reveals that indeed the NAN broodstock used shows no admixture in the mitochondrial DNA despite a large sample size.

Mill Creek (WC# 23096)— This recently discovered population in the Elkhead Creek drainage occupies just 1.25 miles of stream with perhaps as few as 100 adults. Gaps in age classes suggest that recruitment may not occur annually. The entire population is located on the Babson Carpenter Trust lands. Both AFLP and ND2 suggest that this population represents pure bCRCT, with all 21 fish displaying the common OcYAM-Trappers2 haplotype.

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

OBJECTIVE
Briefly summarize what we know about Cutthroat Trout broodstocks used by Colorado Parks and Wildlife

Colorado Parks and Wildlife Cutthroat Trout broodstocks: populations behind the letters

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INTRODUCTION

A quick survey of the Colorado Parks and Wildlife (CPW) stocking database (T6) reveals names and three letter codes for 34 Cutthroat Trout broodstocks. Most of these have been developed in the last three decades (Table 1) creating considerable confusion. If one were to consider all undocumented wild spawn operations and fish transfers over the entire 125+ year history of the agency, there are no doubt others. Given that pervasive undocumented stocking in the early 20th century has already obscured the native distribution of Colorado's Cutthroat Trout subspecies once (Metcalf et al. 2007, 2012; Rogers et al. 2018a; Bestgen et al. 2019), it is important to document where all these new stocks are going so that managers who follow do not suffer the same fate. While stocking databases do a good job tracking numbers, size, and destinations for these fish, it is important to also provide some context for all these stocks – particularly when future molecular studies uncover odd allele combinations that require explanation. This chapter is also a bit of a plea. If you have additional information on the stocks discussed here (or on others that are not represented), please bring them to my attention so that we can continue to flesh out the history associated with these important captive populations of our native fish.

METHODS & RESULTS

Information presented in this document represent a compilation of information derived from old stocking databases, archived files, personal notes, and oral accounts from current and former CPW staff. Where stocks have been subjected to some form of molecular assessment, that is noted as well. A quick reference summary can be found in Table 1, with more detailed explanations provided in the paragraphs that follow. We use the following abbreviations for the different Cutthroat Trout subspecies:

CRCT - Colorado River Cutthroat Trout
GBCT - Greenback Cutthroat Trout
RGCT - Rio Grande Cutthroat Trout
YSCT - Yellowstone Cutthroat Trout

Table 1. Cutthroat Trout stock name and three letter code are given along with the lineage they represent and years when fish were stocked out. Evidence of admixture with nonnative alleles is indicated in the last column.

Code	Name	Lineage	Stocked		Admixture
BAC	Bear Creek	Greenback	2014 - Present		
CAR	Carr Creek	Green Lineage	2004 - 2020		Slight YSN
CCC	Cunningham Creek	Green Lineage			
CR1	Colorado River A strain	Blue Lineage	1992	2019	
CRN	Colorado River Native	Blue Lineage	1903	2001	Hybrid swarm
GBA	Arkansas Greenbacks	Blue Lineage	2004	2008	
GBN	Greenback Native	Blue Lineage	1977	2022	Slight YSN
GBS	South Platte Greenbacks	Blue Lineage	2002	2012	
GRC	Graneros Creek	Blue Lineage	2004	2006	
HCC	Hayden Creek	Green Lineage	2018	Present	
HCR	Hunters Creek	Blue Lineage			
KCN	Kelso Creek	Green Lineage	2013	Present	
LSR	Little Snake River	Blue Lineage	2006	2008	
NAN	Lake Nanita	Blue Lineage	2003	Present	
NAT	Native	Various	1995	1998	
NAV	Navajo River	Blue Lineage	2006	2018	
PPN	Pikes Peak Native	Blue Lineage	1973	1999	Slight YSN
RGH	Haypress Lake	Rio Grande	2005	Present	
RGN	Rio Grande Native	Rio Grande	1977	Present	
RIO	Rio Blanco Creek	Unknown			
ROA	Roaring Creek	Blue Lineage			
RON	Roan Creek	Green Lineage	2015	2015	
SAC	South Apache Creek	Blue Lineage			
SEV	Severy Creek	Blue Lineage			
SJN	San Juan Native	San Juan	2018	2023	
SR1	Wyoming Snake River	Yellowstone	2012	2012	
SR2	SR1 x SRN	Yellowstone	2012	2015	
SRN	Snake River Native	Yellowstone	1979	Present	
TAC	Taylor Creek	Blue Lineage	2007	2007	Hint YSN?
TRP	Trapper Creek	Blue Lineage	2004	2021	
WAC	West Antelope Creek	Green Lineage			
WEM	Weminuche	Blue Lineage	2005	2021	
WXT	Weminuche x Trapper	Blue Lineage	2016	Present	
YSN	Yellowstone Native	Yellowstone	1905	2001	

BAC – Bear Creek (Greenback Cutthroat Trout)

This broodstock now housed at the Leadville National Fish Hatchery was derived from 66 fish taken from Bear Creek west of Colorado Springs in 2008 (Rogers et al. 2022a). That initial stock has been supplemented annually with wild milt and occasionally with wild spawns. Recent work (Rogers 2023) suggests that genetic diversity in the wild has been captured in the broodstock, but these fish remain extremely challenging to raise with very poor survival to stocking. Efforts to mitigate those shortcomings are ongoing (see other chapters in this volume).

Interestingly, Bear Creek lies outside of the native range of GBCT, flowing off the north side of Pikes Peak in the Arkansas River basin. It appears that these fish were inadvertently stocked in a fishless Bear Creek from a South Platte source by an enterprising hotelier looking to provide angling opportunity to his guests in Jones Park (Kennedy 2010). These fish apparently escaped the pond and established in Bear Creek above a waterfall barrier that protected them from invading Brook Trout. These fish have persisted in isolation in just four miles of stream for over 140 years (Rogers et al. 2022a).

CAR – Carr Creek (green lineage CRCT)
Bill Elmblad (CPW aquatic biologist) reclaimed Carr Creek with rotenone over three consecutive summers (1996-1998), then translocated trout from Roan Creek into Carr Creek in 1998 from lower in Roan Creek, then moving some additional fish in 2000 captured near the upper exclosure. In 2002 he conducted a streamside wild spawn operation on Carr Creek and brought 14,000 fertilized eggs into captivity (representing 54 females) to serve as a broodstock for future recovery projects. Unfortunately, the stock shows signs of YSCT admixture and hints of Rainbow Trout alleles (Rogers 2008; P. Evans, unpublished data) as did the lower portion of the donor population in Roan Creek. The broodstock performed well, moving from Glenwood to Rifle, to the Poudre Rearing Unit, then ending up at the Leadville National Fish Hatchery before being retired in 2020 when demand for these admixed fish dwindled.

CCC – Cunningham Creek (green lineage CRCT)
A new barrier was built on Cunningham Creek in 1997 to prepare for a reclamation project that would allow the reversal of the Brook Trout invasion into the system. Around that time, Alan Czenkush brought Cunningham Creek trout into the Glenwood hatchery presumably to develop a broodstock with the primary goal of expanding the population downstream to the constructed barrier in addition to using them for other reclamation projects in the basin. Unfortunately, those wild fish did very poorly in the hatchery and many died. In an effort to save the remainder, Alan apparently stocked them back into Cunningham Creek. Brook Trout have since invaded the upper reaches of the creek, essentially replacing the entire native Cutthroat Trout population. Developing a broodstock now would be very difficult.

CR1 – Colorado River A strain (blue lineage CRCT)
This code appears to have been developed in concert with the first wild egg take at Lake Nanita in Rocky Mountain National Park. That effort started in 1990, producing eggs that were sent to the Glenwood Springs Hatchery to create a broodstock that would allow the eventual phasing out of the CRN broodstock below. Lake Nanita was founded from Trappers Lake fry taken in 1931, prior to the stocking of YSCT in that lake in the 1940s. They represent a purer form of CRCT, and were used in a number of reclamation projects. Stocking records indicate CR1 have been stocked from 1992 through 2019, though this brood continues to produce eggs for stocking – we

simply refer to them as NAN now (see below). A wild egg take is conducted up at Lake Nanita every three years to provide new genetic material to the broodstock.

CRN – Colorado River Native (blue lineage CRCT)
This represents the oldest Cutthroat Trout broodstock to receive a code. In 1903, game and fish biologists began to take eggs at Trappers Lake in the headwaters of the White River. Between 1903 and 1938, over 80 million were produced and spread across the state in waters that could support trout (Rogers et al. 2018a). A population crash at Trappers Lake assumed to have been caused by an eye fluke forced operations to be suspended for a time. The population rebounded and anglers began to complain about the small size of the prolific fish. Managers felt that stocking readily available YSCT would help improve the size structure as YSCT are typically larger at age. Close to a million YSCT were stocked into Trappers Lake from 1943 to 1950 (Martinez 1988; Leary and Allendorf 1991). They were successful in improving the size structure, but unfortunately, also in producing a hybrid swarm with lower conservation value than the original CRCT. Wild spawn operation resumed at Trappers Lake in the early 1950s and proceeded until 2001 when demand for they admixed fish dwindled in favor of the pure CRCT being produced from Lake Nanita stocks (CR1 or NAN).

Note – Old stocking records show Trappers Lake progeny as "N" with the three letter codes not being invoked until 1973 when more rigorous recording of fish stocking events began.

GBA – Arkansas Greenbacks (blue lineage CRCT)
Roughly 300 adult fish were pulled from North Taylor, South Apache, and Graneros creeks in 2002 to create a blended broodstock of what were thought to be Arkansas Basin GBCT. All progeny produced in 2003 went back into the broodstock, with the first fish being stocked out in 2004. By 2008 it became apparent that these fish were not the native trout of the Arkansas Basin (Metcalf et al. 2012; Rogers et al. 2018a; Bestgen et al. 2019), and stocking was discontinued. New molecular evidence suggests these populations were likely founded from Trappers Lake progeny prior to 1938. As such, the broodstock was blended with the GBN "recreational greenback" brood at the Poudre River State Fish Hatchery to produce "sparkies" (South Platte and Arkansas recreational "greenbacks").

GBN – Greenback Native (blue lineage CRCT)
This is one of the longest running and well-traveled CPW captive Cutthroat Trout broodstocks, having started at the Bellvue Watson and Fish Research hatcheries in 1977, then moving to the Salida Isolation Unit in the 2000s, then ending up at the Poudre River Fish Hatchery. The composition of the stock mirrors that for GBS below, with the addition of Boehmer Reservoir in 2001 that inadvertently added more blue lineage alleles and "essence of Yellowstone" (Rogers and Kennedy 2008). More recently, Rio Grande alleles have also turned up in the stock, but the source is still under investigation.

GBS – South Platte Greenbacks (blue lineage CRCT)
The development of this blended broodstock of putative South Platte native GBCT was initiated in 2000. Progeny derived from the South Fork Poudre and Roaring Creek in the Cache la Poudre basin, and Hunter Creek in the St. Vrain drainage were combined, and their progeny stocked out

from 2002-2012. All three donor populations have since been determined to be feral populations of blue lineage CRCT, likely descended from Trappers Lake. As such, GBS were blended with GBA to form the "sparkies" which were rolled into the GBN broodstock for recreational stocking efforts.

GRC – Graneros Creek (blue lineage CRCT)
This stock was developed to provide progeny for the Arkansas basin blended broodstock (GBA). It was discontinued once it became apparent that these were likely descended from Trappers Lake CRCT (Metcalf et al. 2007; Bestgen et al. 2019)

HCC – Hayden Creek (east slope green lineage CRCT)
These fish represent the only extant population of Cutthroat Trout sharing the mitochondrial haplotype with museum specimens David Starr Jordan collected from Twin Lakes in 1889 (Rogers 2021). A July 2016 wildfire in the headwaters of the Hayden Creek drainage southeast of Salida, Colorado 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). A rescue effort aimed at securing this population was implemented, using five crews with backpack electrofishers to remove roughly half of the resident population. Smaller fish were targeted in an effort to leave enough mature adults to repopulate the stream if ash flows did not materialize (Rogers 2020). One hundred fifty-eight trout were taken into the Roaring Judy Hatchery isolation facility to mature and provide future progeny for repatriation efforts.

The rescue effort proved prescient, as a late season storm hit the headwaters of South Hayden Creek in late September of 2016. As predicted in the BAER report (USDA 2016), the resulting debris flows were catastrophic. Electrofishing surveys in 2017 recovered no fish above the barrier that protected the Cutthroat Trout in South Hayden Creek from downstream nonnative invaders. Without the salvage effort we would have lost this unique piece of Cutthroat Trout diversity.

Eighteen of the females brought into the Roaring Judy State Fish Hatchery produced viable eggs in 2017 that were used to develop the new broodstock (Rogers 2021). Survival to hatch in 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 affecting survival. To that end, we genotyped the 133 individuals that remained in 2018 and compared survival of families created from either closely related or unrelated parents (Rogers 2020). Differences in survival based on relatedness did not materialize, but rather egg quality was the primary driver of survival. Though the South Hayden Creek broodstock has recently been moved from CPWs Roaring Judy Hatchery to a dedicated building at the federal USFWS hatchery near Leadville, Colorado, the former facility still plays a key role in conserving these fish, as eyed embryos are transferred back to Roaring Judy Hatchery and its more favorable temperatures for growth.

HCR – Hunters Creek (blue lineage CRCT)

Hunters Creek lies east of the Continental Divide in Rocky Mountain National Park, and drains into the North Fork of the Saint Vrain. This population was discovered in 1985, and determined to be pure using meristic traits (Dwyer and Rosenlund 1988). Milt derived from this population in 1986-1988 was used to add genetic diversity to the GBCT broodstock housed at the Bozeman Fish Technology Center and used in the Greenback Recovery Program (Dwyer and Rosenlund 1988; Kennedy 2014). Eight wild spawn takes were performed on Hunters Creek from 1989-2006 to provide fish for the developing South Platte GBCT Trout broodstock (GBS; Kennedy 2014). Additional genetic testing confirmed that this population was free of nonnative Rainbow Trout and YSCT alleles (Kennedy 2014). In 2007, molecular studies began to reveal the link between blue lineage CRCT east of the Divide and west slope sources, specifically Trappers Lake in the headwaters of the White River (Metcalf et al. 2007, 2012; Rogers et al. 2018a; Bestgen et al. 2019), effectively ending the use of these fish conservation efforts.

KCN – Kelso Creek (green lineage CRCT)

These Gunnison basin fish were first translocated into Woods Lake west of Telluride in 2012 to form the basis of a green lineage CRCT wild brood lake. From 2013-2018, wild spawn operations were conducted at Kelso Creek to provide additional genetic diversity to the developing Woods Lake stock. Annual spawn operations are now conducted at Woods Lake, with an additional wild spawn operation at Kelso Creek in 2021.

LSR – Battle Creek (blue lineage CRCT)

Aquatic biologist Bill Elmblad set about developing a brood source of Cutthroat Trout to use for reclamation projects in the Little Snake River drainage of northwest Colorado. A suitable source population was identified in Battle Creek, just north of the Colorado border in Wyoming. Fish were sourced by Steve Sharon of Wyoming Game and Fish via request from Rich Kolecki. Fish were stocked annually into the two small Three Forks Ranch Ponds from 2006-2008. The current status of these fish is uncertain.

NAN – Lake Nanita (blue lineage CRCT)

Broodstock development was initiated in 1990 with progeny of wild spawn operations at Lake Nanita in Rocky Mountain National Park conducted from 1990-present, generally on a three-year rotation. This population was likely founded from a single stocking of Trappers Lake progeny in 1931, prior to the introduction of YSCT and the resulting hybrid swarm that has developed in Trappers Lake since (see CRN above). The Glenwood captive broodstock was eliminated in 2016 to clear the hatchery of bacterial kidney disease. This stock was then reestablished over the next three summers with wild spawn operations conducted at Lake Nanita. As before, these operations used to infuse wild alleles into the broodstock, are now back on a three-year cycle.

NAT – Native

This three-letter code appears to have been generated more for field survey work (particularly for SciColl permitees who might have difficulty recognizing the different strains of Cutthroat Trout). While only four stocking events allege to have stocked NAT into the wild (Wolford Reservoir, Horsetooth Reservoir, Cowdrey Reservoir, and the Air Force Academy Ponds in the late 1990s), several thousand survey records that can be found in the ADAMAS database that include NAT.

These date back to 1971 suggesting that NAT became the convenient alternative to N when the switch to three-letter codes was made.

NAV – Navajo River (blue lineage CRCT))

The headwaters of the Navajo River in southwest Colorado lie on the Banded Peak Ranch above a Bridal Veil Falls that protects the resident Cutthroat Trout population from invading nonnative trout. The population was tested for evidence of nonnative alleles in the past (Evans and Shiozawa 2004), and none were found. Mike Japhet conducted wild spawn operations above the falls to create a captive broodstock at the Durango Fish Hatchery for future conservation efforts. Eggs were produced from the captive brood from 2006 to 2018 at which point demand for these fish withered following the discovery that they were not the native trout of the San Juan basin (Rogers 2008; Metcalf et al. 2012; Rogers et al. 2018a). It was thought that the founding population might have been stocked above the falls in the early 1970's by former WCO Judd Cooney, who also formerly worked as Banded Peak Ranch manager (M. Japhet, personal communication). As WCO/DWM, Judd would certainly have had access to Trappers Lake CRCT at the hatchery in Durango. The absence of Yellowstone alleles however suggests a much earlier founding since almost a million YSCT were stocked into Trappers Lake in the 1940s (see CRN above). Evidence of YSCT admixture remains on all fish produced at Trappers Lake after that time. Rather than using limited hatchery resources to maintain this broodstock, they were moved to George's Lake in the same basin.

PPN – Pikes Peak Native (blue lineage CRCT)

Although T6 suggests this code has been in use since 1973, these lakes on the south slope of Pikes Peak have been used as a Cutthroat Trout egg source as far back as 1911, and projeny were distributed widely. A thorough discussion on the origin and history of these fish can be found in Rogers and Kennedy (2008).

RGH – Haypress Lake (Rio Grande Cutthroat Trout)

This stock represents those fertilized eggs that come from Haypress Lake directly (wild spawn operations). From 2005-2011 these fish would have been derived from West Indian Creek, Placer Creek, Osier Creek and North Carnero Creek (Rogers 2012). The lake was depopulated at that point to rid the water of bacterial kidney disease and some nonnative trout alleles. Following reclamation, the population was reestablished with fish from Osier and Medano creeks. Their progeny have been produced at Haypress Lake since 2020.

RGN – Rio Grande Native (Rio Grande Cutthroat Trout)

Another well-traveled and long-time broodstock, production of RGN started in 1977 by biologists from the "region" (SW Region) who presumably obtained eggs from Haypress Lake. In 1992, production was shifted to the Fish Research Hatchery (FRH), with the last eggs coming from Haypress Lake in 1994 prior to an outbreak of bacterial kidney disease. The first record of brood fish being transferred to the Poudre River Hatchery from FRH was in 1997. The Pitkin Fish Hatchery began to develop the RGN broodstock in 1999, with the first eggs produced in 2001. They continue to produce RGCT for the state today.

RIO – Rio Blanco "Creek"
This code is a bit enigmatic as the only Rio Blanco in Colorado lies in the San Juan drainage. Although it does harbor an admixed population (with Rainbow Trout) of San Juan CRCT, that was not discovered until 2020, yet the code was established by Terry Robinson in 2006. Terry has no recollection of what this code was created for, and nor does John Alves or Mike Japhet the longtime CPW biologist for the basin who promoted to SW Senior Biologist in 2005. Jim White, the local biologist from 2005-2024, grew up on Rio Blanco, and would certainly have known about a broodstock developed in that drainage. It appears Ace Riverman updated the RIO record in December of 2018 to add length-weight regressions, but RIO still does not show up in ADAMAS or T6 records.

ROA – Roaring Creek (blue lineage CRCT)
Roaring Creek lies in the headwaters of the Cache la Poudre drainage east of the Continental Divide. These fish were thought to be pure GBCT until the advent of new molecular testing which deemed them blue lineage CRCT descended from Trappers Lake stock prior to 1938 (Bestgen et al. 2019). These fish were never stocked out from a hatchery, rather, the code was established to track movements of fish into Zimmerman Lake and the developing GBN wild brood source there.

RON – Roan Creek (green lineage CRCT)
Roan Creek lies tucked away in the Escalante Breaks west of the Roan Plateau in northwest Colorado. The stream and harbors a genetically and phenotypically unique population of Cutthroat Trout native to the Colorado River basin. A failing barrier has allowed nonnative trout to invade the system. In an effort to reduce nonnative Rainbow Trout and YSCT admixture in this population, eggs from wild females were fertilized with either milt from two or three males. These individual families were raised in individual egg cups and tanks at the Fish Research Hatchery in Bellvue, Colorado. Fin clips were obtained from every parent and subjected to four different molecular tests looking for evidence of nonnative alleles. Admixed families were culled, leaving only families free of nonnative alleles to be used in the development of the new broodstock. Over the four-year period (2012-2015), we created 33 families with gametes from 108 parents. Unfortunately, only 65 parents were represented in the final broodstock as many families had to be eliminated because of a single admixed parent.

This broodstock began producing progeny in 2015 which were stocked into the headwaters of the newly reclaimed East Fork of Parachute Creek. That winter, bacterial kidney disease (BKD) was detected in the RON broodstock at the Glenwood State Fish Hatchery requiring the entire facility be depopulated. Given the resources put into developing this stock and the importance of preserving it, we explored releasing the broodstock in East Fork Parachute Creek (the only suitable vacant habitat that was already positive for *Renibacterium salmoninarum*, the causative agent of BKD). The remaining stock was helicoptered into the East Fork Parachute Creek on April 7, 2016, and continues to thrive at the time of this writing.

SAC – South Apache Creek (blue lineage CRCT)
South Apache Creek fish were collected to form part of the blended Arkansas basin GBCT broodstock (GBA). While early molecular testing confirmed the absence of Rainbow Trout and

YSCT alleles, this appears to be another population east of the Continental Divide that was founded from Trappers Lake progeny in the headwaters of the White River prior to 1938 (Metcalf et al. 2007; Bestgen et al. 2019).

SEV – Severy Creek (east slope green lineage CRCT)

Severy Creek is one of a handful of pure green lineage populations east of the Continental Divide. Ongoing research seeks to determine if they made it there on their own or whether they were stocked from west slope sources. No progeny from this broodstock was ever stocked out, and it is not entirely clear if it ever existed as a formal broodstock. It was more likely established to simply track a proposed translocation of Severy Creek Cutthroat Trout into Newlin Creek. Efforts to determine whether that translocation actually took place are still underway.

SJN – San Juan Native (San Juan lineage CRCT)

Study of Cutthroat Trout specimens housed in our nation's oldest museums revealed that the San Juan River basin in Colorado historically harbored a discrete lineage of CRCT thought to be extinct (Metcalf et al. 2012). Extensive molecular surveys across the basin documented the presence of eight relict populations (Rogers et al. 2018b). A broodstock was needed to meet conservation goals in the drainage, a process initiated by Jim White with fish from Big Bend and Clear creeks in the headwaters of the Animas River basin. Biologist Mike Japhet founded the Clear Creek population by translocating 204 individuals from Big Bend Creek in 1987.

SR1 – Wyoming Snake River (Snake River Cutthroat Trout)

In an effort to improve the genetic diversity of the SRN broodstock, additional Snake River Cutthroat Trout were solicited from the Auburn Hatchery in Wyoming circa 2010. These fish were out of phase with the fall spawning SRN at the Crystal River Fish Hatchery, requiring cryopreservation of milt to accomplish fertilization (R. Streater, personal communication). Progeny of this stock were blended with SRN and only the original brood culls were stocked out.

SR2 – SR1 x SRN (Snake River Cutthroat Trout)

This hybrid stock resulted from the crossing of SR1 with SRN to diversify the SRN broodstock. This process repeated itself over several generations until a fall spawning, more genetically diverse broodstock was the result.

SRN – Snake River Native (Snake River Cutthroat Trout)

Though not native to the state, Snake River Cutthroat Trout are a popular sportfish due to their rapid growth in our mid-elevation reservoirs. The fall spawning nature of this broodstock makes them logistically practical for the production and stocking of recreational fish. This longstanding broodstock resides at the Crystal River Fish Hatchery which produces up to a million eyed SRN eggs annually. Substantial deformities in progeny from this stock that was derived from just 16 individuals prompted the diversification of the broodstock starting in the 2010s (see SR1 and SR2 above). That effort and the switch to premium feeds has largely eliminated past problems with this stock.

TAC – Taylor Creek (blue lineage CRCT)
Forty North Taylor Creek fish were brought into captivity in September 2002 to form the GBA blended Arkansas basin GBCT. The only ones ever to be stocked out as TAC were released into a few waters near the Mount Shavano State Fish Hatchery in 2007 (e.g., Frantz Lake). Like the other populations used in developing the GBA broodstock, North Taylor Creek trout appear to be descended from Trappers Lake progeny moved east of the Continental Divide prior to 1938 (Rogers et al. 2018a; Bestgen et al. 2019).

TRP – Trapper Creek (blue lineage CRCT)
Not to be confused with Trappers Lake, Trapper Creek lies on top of the Roan Plateau. Bill Elmblad brought this population into the Glenwood Hatchery around the time of the 2002 drought to develop a brood source for reclamation projects in the Colorado River basin. Nuclear and mitochondrial DNA testing suggests this drainage was likely fishless historically, and that the CRCT population became established prior to 1938 by undocumented stocking of progeny derived from Trappers Lake.

WAC – West Antelope Creek (green lineage CRCT)
Located in the Gunnison basin, this small population was another casualty of the 2002 drought. When it became evident that this stream might dry up entirely, CPW biologist Dan Brauch sought to bring a portion of the population into captivity. With hatchery isolation space already full, he found a home for them in a "living stream" at Western State University. These fish performed poorly there and ended up contracting bacterial kidney disease which meant they could not be repatriated into the wild. A small population remains in West Antelope Creek at the time of this writing. They display a relatively uncommon gCRCT mitochondrial haplotype only found in a handful of other populations in the Gunnison basin. With luck, perhaps a renewed effort to replicate this valuable population will allow us to redeploy this three-letter code.

WEM – Weminuche (blue lineage CRCT)
This broodstock was developed by CPW biologist Mike Japhet who took spawn from the Cutthroat Trout in the East Fork of the Piedra River during the historic drought of 2001-2002. This was the only time flows were low enough to capture spawners by electrofishing. He and his crew held the fish in net pens for a week or so until they were ripe enough to spawn streamside. There were no official (or unofficial) stocking records for these streams so they were assumed to be native trout of the San Juan basin. Recent molecular testing has showed that they were likely established from Trappers Lake progeny prior to 1938 (Metcalf et al. 2007; Bestgen et al. 2019). Progeny from this brood were stocked out from 2005 through 2021, at which point they were blended with the Trapper Creek strain to produce a more robust and genetically diverse fish (see below)

WXT – Weminuche x Trapper Creek (blue lineage CRCT)
Molecular testing confirmed that both Weminuche and Trapper Creek populations were likely founded from Trappers Lake progeny prior to 1938 (Rogers 2008; Bestgen et al. 2019), and do not represent the native trout of the San Juan basin. Given ongoing issues with low survival to stocking, these two broods were blended at the Durango State Fish Hatchery in hopes that additional genetic diversity would improve fitness. Indeed, both survival and growth of progeny

improved significantly (T. Mourning, Durango State Fish Hatchery, personal communication), and those fitness benefits have continued well beyond the F1 generation suggesting a genetic basis rather than simply hybrid vigor.

YSN – Yellowstone Native (Yellowstone Cutthroat Trout)

While CPW's digital stocking database suggests that YSCT were stocked in the state from 1981 to 2001, that only represents a small fraction of these fish coming into Colorado from the extensive spawning operations conducted near LeHardy Rapids on the Yellowstone River below the lake in Yellowstone National Park. Long-time CPW researcher and fish historian Bill Wiltizius maintained that the first YSN would have gone to the Leadville National Fish Hatchery in 1905 when they stopped claiming to raise native Yellowfin Cutthroat Trout (B. Wiltzius, unpublished data). If that was not the first instance, then they were certainly brought to the fish hatchery in Estes Park in 1910 (Wiltzius 1985). From 1912 to 1953 an additional 70 million YSCT were distributed across Colorado (Varley 1979)

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RESEARCH PRIORITY
Culture of native Cutthroat Trout

OBJECTIVE
Attempt to mitigate effects of Blue Sac Disease on survival of Greenback Cutthroat Trout with nutritional supplementation.

Nutritional supplementation to mitigate effects of Blue Sac Disease on survival of Greenback Cutthroat Trout

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INTRODUCTION

Culture of native Cutthroat Trout (*Oncorhynchus clarkii*) is always more challenging than domestic strains of Rainbow Trout (*O. mykiss*), but some are particularly difficult. Unfortunately, this is especially true for some of our most valuable stocks (Rogers et al. 2022a, 2022b). For example, Greenback Cutthroat Trout (GBCT; *Oncorhynchus clarkii ssp*.) derived from Bear Creek on the north slope of Pikes Peak, Colorado, are plagued by poor growth (Rogers et al. 2022a), low genetic diversity (Rogers et al. 2022b), and display numerous physical deformities. Survival to stocking with these fish can be as low as 5% in a production setting, compared to domestic Rainbow Trout strains that may exceed 90% (B. Johnson, Mt. Shavano Fish Hatchery, personal communication). This necessitates the acquisition of hundreds of thousands of fertilized eggs annually simply to meet modest recovery goals. The majority of mortalities appear to be the result of Blue Sac Disease (Wolf 1954, 1957; Bauder et al. 2005; Eriksson et al. 2022; Ban et al. 2023). Symptomatic fish eventually perish, unable to accept feed.

We continue to search for ways to make our captive and wild spawn operations more efficient, as even a modest doubling of survival would greatly reduce the burden on our hatchery system (each mortality must be removed by hand). Interestingly, we do see improved survival in eggs derived from our stocked GBCT population in Zimmerman Lake, with survival to stocking averaging 23% over the last eight years (B. Johnson, unpublished data), and up to 43% in intensive lab culture of individual families (Rogers et al. 2022a). Since the captive broodstock at Leadville and the stocked population in Zimmerman Lake come from the same source, the genetic bottleneck likely only explains part of the problem. We suspect some micronutrients afforded fish in the wild benefits developing eggs over those fed a hatchery diet at Leadville and Mount Shavano, suggesting that diet supplements might be beneficial.

To guide our selection of supplements to investigate, we examined a study of the GBCT proteome comparing 10 BSD fish with 10 normal fish (Brown 2021). In that data, we observed a downregulation of a number of proteins associated with protein synthesis. Particularly striking was the 0.2-fold downregulation of nicotinamide nucleotide transhydrogenase (NNT), a protein that plays a central role in cellular energy production and combats oxidative stress. Interestingly, evidence from an earlier heat shock transcriptome study (D. Petcoff, unpublished data) suggests GBCT are under greater oxidative stress than a robust population of Colorado River Cutthroat Trout (CRCT; *O. c. pleuriticus*) and subsequently are subjected to up-regulation of pathways that produce NAD+. Tryptophan is an essential amino acid in both mammals and fish, and is metabolized through the kynurenine pathway (KP). It is required for protein synthesis, and is the ultimate source of NAD+. In addition, several of its metabolites participate in a broad array of cellular functions (Petcoff and Rogers 2022), but some can cause cellular damage (Reyes-Ocampo et al. 2015). Since Niacin can enter the KP pathway closer to the NAD+ end product, it produces fewer potentially toxic metabolites. If GBCT have an enzyme with full or partial loss of function somewhere along the KP, looking at ratios of KP metabolites from both tryptophan- and niacin-supplemented alevins should help pinpoint where important genetic alterations lie. As such, we elected to explore both tryptophan and niacin along with a more broad-spectrum vitamin supplement popular in fish culture, and their potential to improve survival over a control group in GBCT.

METHODS

Brood conditioning

Five hundred Bear Creek Greenback Cutthroat Trout brood fish representing the 2020 year-class were divided into in four 40-in round tanks (500 L), with treatment randomly assigned to each tank. Although we intended to house only females in the treatment tanks, these trout were not yet sorted by sex which remains fairly cryptic in three-year old trout. As such, each tank was populated with 76 trout to ensure that our target of 12 spawns from each treatment was met on a single spawn day. Trout were reared with BioBrood feed (Bio-Oregon, Longview, Washington) as per the manufacturer's recommendations, and weighed 2.8 fish/lb when the feed trials were initiated in January 2023. Each supplement comprised one of four treatment groups, and were assigned randomly to each of the four round tanks (Table 1). Each group was hand fed to help ensure that all feed was consumed and that water-soluble supplements made it into the fish. Water temperature sensors (HOBO U22 Pro v2; Onset Computer Corp., Bourne, Massachusetts) were deployed in each tank to record temperature every 20 min.

Table 1. Supplements used in this study and their sources. Treatments were assigned randomly to tanks

Treatment	Tank #	Supplement	Application
Control	2	NA	BioBrood[1] feed only
Niacin	4	Nicotinic acid powder[2]	Mixed in a slurry with vegetable oil, then coated onto feed at a rate of 100 mg/kg
Tryptophan	1	Tryptophan powder[3]	Mixed in a slurry with vegetable oil, then coated onto feed at a rate of 2g/kg
Vitamin boost	3	Vitamarin F[4]	Coated on feed weekly to ensure that Vitamin C remained stable at a rate of 1 oz/kg feed

[1]Bio-Oregon, Longview, Washington
[2]Nicotinic acid powder (Catalog #481918-100GM), MilliporeSigma, Burlington, Massachusetts)
[3]Reagent-grade tryptophan powder (Catalog #T0254-100G), MilliporeSigma, Burlington, Massachusetts
[4]Vitamarin F, Brightwell Aquatics, Fort Payne, Alabama

Spawning and rearing

On July 6, 2023, milt from twelve ripe Bear Creek males was extruded into a dry glass bowl from which 200 ul from each male was pooled into 48 ml of extender, then split between two culture vials (Rogers 2010), oxygenated and stored on ice. Female trout from each treatment group were stripped of their eggs, with 12 eggs from each spawn flash frozen in two 4.5 ml cryotubes dry ice to preserve metabolites for future study on egg quality. Each spawn was fertilized with 1.0 ml of the extended milt, and placed in 14 x 18 x 5 cm rectangular screen egg baskets with lids to keep individual spawns separate while incubating in the Heath trays (four per tray). Eggs were water-hardened for 30 min in 50 ppm iodine (Ovadine; Syndel, Ferndale, Washington). One basket from each treatment group was placed in each Heath tray with position assigned randomly using custom LabVIEW code (National Instruments, Austin, Texas). Twelve trays were set to incubation in two Heath stacks. Water temperature data loggers were used to record water temperatures every 15 min (HOBO Pendent MX; Onset Computer Corp., Bourne, Massachusetts). At 39 d post fertilization, eggs were bumped and transferred to trays with 6 bin dividers and each treatment was combined into two Heath trays each there were then randomly placed, one in each Heath stack to facilitate picking dead eggs. Dead eggs (or fry) were removed twice weekly and the number recorded so that survival to hatch and swim-up could be determined. At 56 days post fertilization (just before the fry were ready to swim up and accept feed), the experiment was terminated. We used three visual characteristics to indicate which alevins were afflicted with BSD. The most prominent was the loss of a sharp margin around the yolk sac as it becomes more rounded. This was usually followed by pallid skin tone, and mouths that remained agape. Ten alevins from each group (5 BSD and 5 normal phenotype) per family were flash frozen in individual 4.5 ml cryotubes on dry ice and stored at -70°C to ensure longterm metabolite preservation. An additional eight samples (4 BSD and 4 normal phenotype) from each family were preserved in RNA-Later (Fisher Scientific, Waltham, Massachusetts) and also stored at -70°C both for subsequent DNA, RNA, and protein work. Survival between groups was evaluated on transformed ($\sqrt{\sin^{-1}}$) data with an ANOVA and Tukey's HSD post hoc test to examine differences among treatments. Statistical analyses were performed in R (R Core Team 2021) at $\alpha = 0.05$.

RESULTS & DISCUSSION

Overall, we saw robust survival to the alevin stage for this GBCT broodstock, averaging 54% for all families. Survival varied substantially by family even within treatments (Figure 1), ranging from 0 - 79%. With this extreme variation, none of the mean values by treatment were significantly different even when survival rates were arcsin transformed (ANOVA p=0.299). Only when the highest and lowest extremes were trimmed out of each group was a modest difference revealed (ANOVA p=0.008). With that filtering, only survival in the Tryptophan group registered higher than Niacin and Vitamarin at $p<0.05$. It should be noted that the Niacin group only consisted of six families, as a broken lid on one of the Heath trays allowed healthy fish to escape in the other six families preventing us from calculating accurate survival estimates for them.

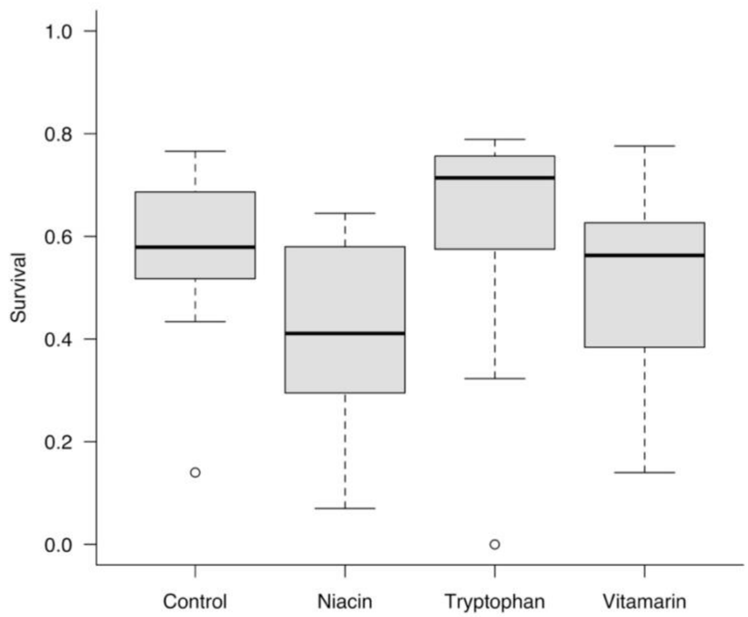

Figure 1. Survival to the alevin stage for each of four diet treatments was evaluated. Thick horizontal black bars represent population-specific medians (°C). Boxes span the interquartile range and whiskers extend 1.5x past that range

Survival to the alevin stage was extremely variable between families even within groups. As the same extended pooled milt was used to fertilize all families, this variation was the result of variation in female egg quality. This phenomenon is often observed when families are cultured separately (Rogers 2020; Rogers et al. 2022a), and makes detecting significant differences between groups challenging. With luck, subsequent metabolite work on the alevins with and without signs of BSD will be more revealing.

ACKNOWLEDGMENTS

We thank the crew at the Leadville National Fish Hatchery for raising these challenging fish.

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RESEARCH PRIORITY
Culture of native Cutthroat Trout

OBJECTIVE
To improve early life survival in native Greenback Cutthroat Trout

Water hardening embryos in a thiamine supplemented bath fails to improve survival in Greenback Cutthroat Trout

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INTRODUCTION

Colorado's state fish, the Greenback Cutthroat Trout (GBCT; *Oncorhynchus clarkii ssp*.), has been held as an example of what is possible with focused conservation efforts afforded by the Endangered Species Act (ESA; Rogers et al. 2018). One of the original fish taxa listed under the Act when passed in 1973, they were thought to have been extirpated in 1937 (Trotter 2008). Several putative relict populations were discovered in the 1960s that were then used to found a recovery effort that spanned several decades (Dwyer and Rosenlund 1988; Behnke 2002). This concerted conservation effort resulted in first a downlisting to "Threatened" status in 1978, then ultimately the development of a management plan that would allow for full delisting from the ESA, as all recovery goals had been met (USFWS 1998).

The delisting effort was upended by the discovery that the putative GBCT used in the recovery effort were not in fact the native GBCT of the South Platte basin, but were instead descendants of feral populations of Colorado River Cutthroat Trout (*O. clarkii pleuriticus*) established outside their native range east of the Continental Divide through undocumented stocking in the very early 1900s (Metcalf et al. 2012). Fortuitously, that study also revealed that GBCT were not extirpated, and that a relict population matching museum specimens collected from the South Platte basin in the 1800s persisted in the headwaters of a little stream outside of Colorado Springs on the northern flank of Pikes Peak (Metcalf et al. 2012; Rogers et al. 2018; Bestgen et al. 2019). This small population was likely founded from just a handful of individuals and has gone through at least several large bottlenecks resulting in a population that exhibits little genetic diversity today (Rogers et al. 2022). Despite the attendant challenges associated with managing a genetically depauperate fish (Allendorf and Luikart 2007; Whiteley et al. 2015), this population now forms the foundation of the Greenback Cutthroat Trout recovery effort.

Unfortunately, this fish remains extremely difficult to raise in captivity, with extremely poor survival to fingerling stage (~5%), which requires that hundreds of thousands of fertilized eggs be taken each year just to meet recovery needs. This places a heavy burden on hatchery resources that could be greatly reduced if even nominal improvements in survival could be realized. Elevated survival rates in embryos taken from a feral broodstock in Zimmerman Lake raised side by side with captive brood progeny at Mt Shavano, suggests that something in the hatchery diet of parents is deficient. Though a genetic bottleneck will likely prevent us from ever obtaining the higher survival rates typically enjoyed for native Cutthroat Trout stocks, this does suggest that some diet supplements might improve survival rates.

Poor survival has been documented in other salmonids, and attributed to thiamine deficiencies (Wooster et al. 2000; Balk et al. 2016; Harder et al. 2018), sometimes precipitated by diets rich in thiaminases (Honeyfield et al. 2005). These deficiencies can be remedied often as simply as water hardening the fertilized eggs in a thiamine bath (Brown et al. 2005; Reed et al. 2023). For example, a bath of 1000 ppm thiamine was successful in doubling survival in a blended broodstock of Colorado River Cutthroat Trout at CPW's Durango Hatchery (T. Mourning, personal communication). Here we use the captive GBCT broodstock (Rogers et al. 2022) at Leadville National Fish hatchery to explore whether survival in the progeny can similarly be improved by water hardening in a 1000 ppm thiamine bath.

METHODS

Three water hardening treatments were used in this study (Table 1). Eggs from this experiment were derived from the "Bear Creek" GBCT broodstock housed at the Leadville National Fish Hatchery (LNFH) in June of 2024. On June 18th, we paired each age-3 female with an age-4 male, generating a total of 96 families. Fertilized eggs from three pairings were combined and mixed, then split into thirds, with one third water hardened in 3 L of 1000 ppm thiamine solution in water from the Mt Shavano State Fish Hatchery (MSSFH), one third in 3 L of 1000 ppm thiamine in LNFH water), and a third into 3 L of LNFH water with no thiamine added to serve as a control. This was repeated four times such that each 3 L bucket contained a third of the embryos from 12 spawns at which point eggs were water hardened for an additional 60 min. Temperatures were maintained at 43 F throughout the hardening process by setting the buckets in a bath circulated with LNFH hatchery water. This process was repeated seven more times so that all 96 families were exposed to the same combination of treatments. Hatchery staff at both hatcheries were blind to treatments, with buckets tracked only by color codes (Table 1).

Table 1. Water samples were collected from each treatment to characterize total alkalinity and water hardness. Blind color codes are also revealed in this table.

Treatment	Color	Spawn day	Total alkalinity (mg/L)	Hardness (mg/L)
MSSFH + T	Red	6/18/24	306	210
LNFH + T	Yellow	6/18/24	120	10
LNFH	Green	6/18/24	10	8
MSSFH + T	Red	6/26/24[1]	-	-
LNFH + T	Yellow	6/26/24	102	14
LNFH	Green	6/26/24	13	14

[1]MSSFH water not retested on second day

Once water hardened, eggs were rinsed with LNFH water, then set in 5-gallon round drink coolers for transport to MSSFH. Upon arrival at MSSFH, eggs were bathed in 100 ppm iodine (Ovadine; Syndel, Ferndale, Washington) for 10 min, then set to incubation at 46 F in three Heath Trays. Temperatures were raised at the eyed stage to 52 F until fry were stocked out. Survival to eye, hatch, swim-up, and fingerling size just prior to stocking was recorded. This entire process was repeated again on June 26th with age-3 females paired with age-4 males (n=60 spawns) and age-4 females paired with age-3 males (n=30 spawns) so that an additional three troughs representing one of each treatment could be evaluated (n=2 for each treatment for the study).

RESULTS & DISCUSSION

Overall, mean GBCT survival in this experiment to fingerling size (3 months post-fertilization) was very high relative to years past (20% vs ~5 %). This was true for production fish not associated with this study as well, with all LNFH lots spawned without wild alleles registering mean survival of 17% when raised at MSSFH. As in previous years culturing Bear Creek GBCT, the largest drops in survival are realized from hatch to swim-up (Figure 1).

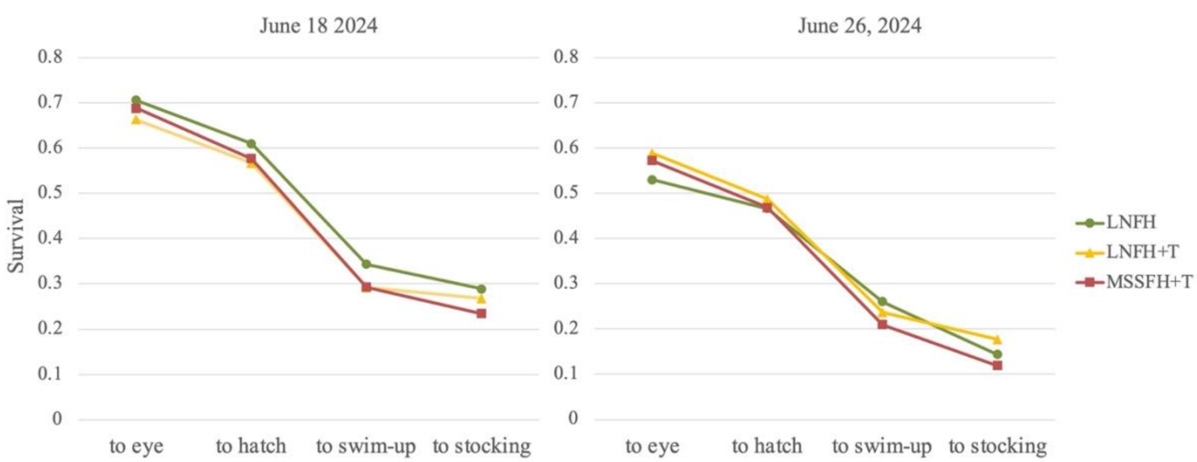

Figure 1. Survival of fertilized eggs taken either on June 18 or 26, 2024 to eye, hatch, swim-up, and stocking (three months post fertilization). Treatments represent a control group bathed in Leadville National Fish Hatchery water (LNFH), with 1000 ppm thiamine added to the water hardening bath (LNFH+T), and using water from Mount Shavano State Fish Hatchery (MSSFH+T).

We did not detect meaningful differences in survival with water hardening in a thiamine bath with the Bear Creek GBCT. The lack of success could not be attributed to water hardness, as the same outcome was obtained both with hard water from MSSFH and soft water from LNFH. This is consistent with Brown et al. (2005) who also showed no effect of water hardness on survival.

Though large numbers of families were generated in the study, true replication is limited. Other Cutthroat Trout culture studies often maintain families only through hatch or alevin life stages (see pages 21-25 in this volume or Rogers 2020). This allows for easy replication of individual families in egg cups or other small containers, but limits the ability to assess survival to stocking. Unfortunately, Bear Creek GBCT suffer extremely high mortality through swim-up, and even as fingerlings. To capture those last critical life stages, we elected to pool samples and rear them in large troughs as we do with production fish used in the conservation of GBCT. While this only allowed for two replicates for each treatment, each included 96 families per trough on two different occasions. We hope the large sample sizes provide compelling evidence that differences would not be observed even if additional replicates were possible.

Replicates were not combined because reduced overall survival in second day may simply have been the result of eggs being mildly overripe. The average number of eggs per female alludes to this (604 on 6/18 and only 530 on 6/26), despite a third of the females on the second day being from older females (we should have expected more eggs per female from them). In addition, older females typically ripen earlier in the spawning run, suggesting that egg quality on the second day might have been reduced.

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We thank Toby Mourning for encouraging the study along with Jesse Lepak for discussions on thiamine deficiency. Paige Moran and the crew at the Leadville National Fish Hatchery are thanked for rearing the brood fish used in this study. We are also indebted to Megan McConville for retrieving and running the water samples in Table 1, and Morgan Wadsworth and Andy Claus for assisting with the spawn operation and fry rearing along with others at the Mount Shavano State Fish Hatchery.

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RESEARCH PRIORITY
Greenback Cutthroat Trout Conservation

OBJECTIVE
Explore whether low dissolved oxygen could be responsible for poor recruitment of stocked Greenback Cutthroat Trout fingerlings at Zimmerman Lake, Colorado.

Continuous monitoring reveals critically low dissolved oxygen levels in a consequential Cutthroat Trout brood lake

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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 subsequent recovery effort (Rogers et al. 2022). Following reclamation in 2013, Zimmerman Lake 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 recent years has been poor. Earlier studies ruled out large-scale emigration as a probable cause (Rogers 2020), leading some to suspect predation (both avian and cannibalism). Stocking densities were increased in hopes of allowing more fish to escape predation. The number of yearling Cutthroat Trout stocked in the lake was doubled in 2021 and 2022, then again in 2023 and 2024 with the addition of Age 0 fry, yet the standing crop remains low.

With few large fish remaining in the lake, the predation hypothesis became less viable, leading CPW biologists to continue exploring other causes. On April 26, 2023, dissolved oxygen (DO) levels were measured with a Hach multi-meter (Model HQ40d; Loveland, Colorado) under winter ice at 10 stations across the lake (Figure 1). Most stations detected critically low DO levels (B. Wright, unpublished data), but increasing levels were measured with proximity to the inlet. Recognizing that a time series would be more beneficial, we deployed two logger arrays to examine DO levels continuously during the late winter season at a deep-water site and near the inlet where higher DO levels were observed. Results from those two arrays are presented here.

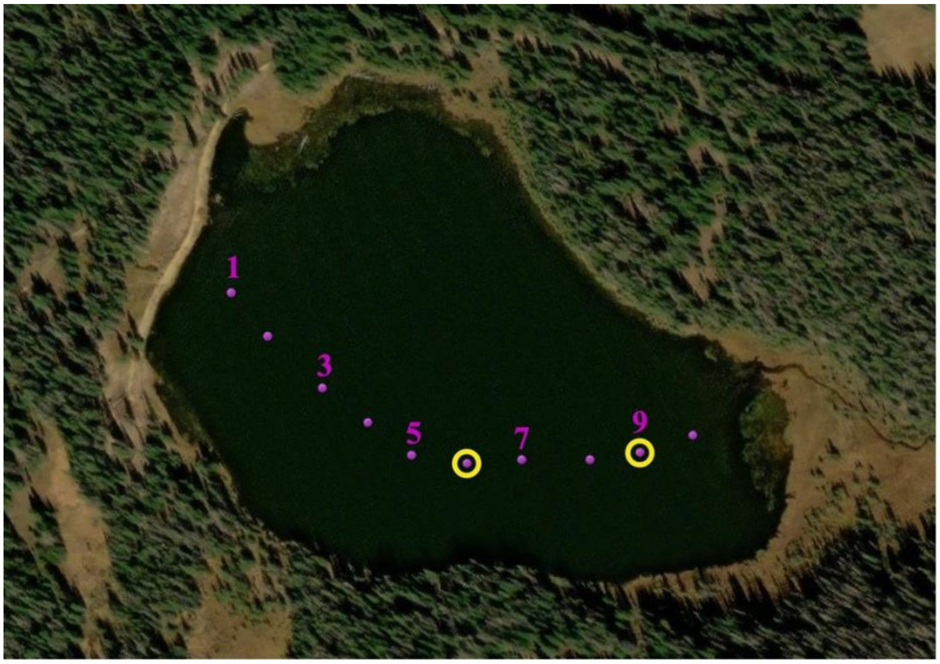

Figure 1. Location of April 26, 2023 mobile dissolved oxygen sampling stations on Zimmerman Lake, Colorado. Fixed array locations in this study are highlighted with yellow rings.

METHODS

Zimmerman Lake sits at an elevation of 3,199 m (10,495 ft) above sea level, and covers an area of 5.4 ha (13.4 acres). The maximum depth of 7.7 m (25.3 ft) can be found near Station 6 (Figure 1), while the mean depth is 2.7 m (9.0 ft) at full pool (both measured on June 26, 2023). We monitored DO every 10 min from March 21st through June 24th 2024 using five miniDOT loggers (Precision Measurement Engineering, Inc.) arranged on two arrays (Figure 2). Three were deployed on a deep-water array set at 13T 426459m E, 4488163m N (Station 6). A second array was deployed near the inlet at 13T 426541m E, 4488170m N (Station 9; Figure 1). The upper logger in each array was set with the sensing surface 0.6 m below the ice so that drifting ice floes during spring melt would not move the arrays.

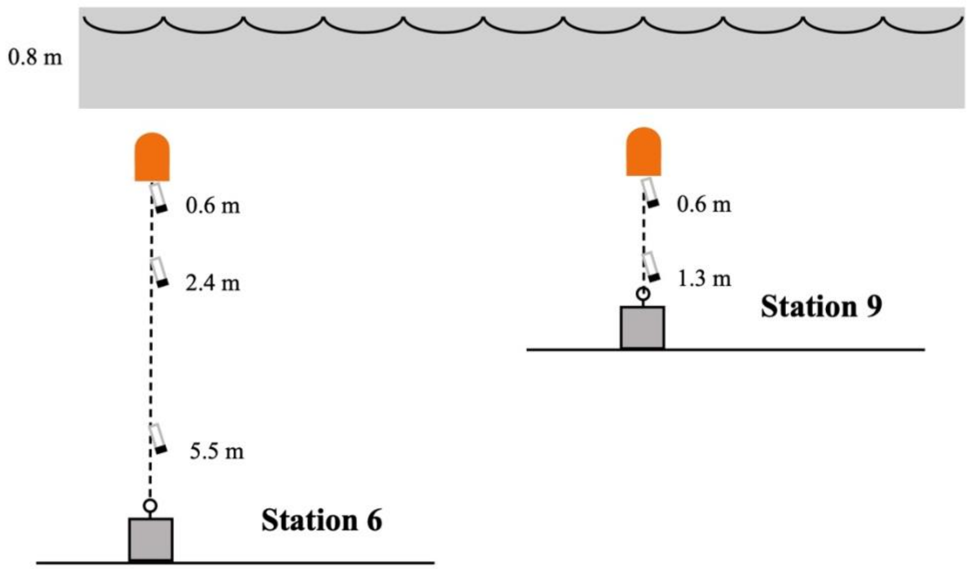

Figure 2. Dissolved oxygen sensors were deployed on chain link arrays anchored to a concrete weight but floated with a foam buoy designed to keep the chain taught under the ice (gray rectangle). Sensors were fastened to the chain with plastic zip ties with sensor face depth indicated to the right of each logger. Arrays were deployed just below the ice layer such that the sensor surface was 0.6 m below the bottom of the ice shelf. Melting ice and surrounding snowpack raised the June 25, surface elevation when the loggers were recovered by an additional 0.8 m (2.7 ft) over the former basal surface of the ice shelf, leaving loggers deeper in the water column after ice-out.

Sensor arrays were recovered on June 25, 2024 and the 145 daily records for each day for each logger were downloaded. Custom code was written in LabVIEW (National Instruments, Austin, Texas) to calculate average daily means for each of the 95 days each of the five loggers were deployed. Influence of snow cover on DO levels was explored using Snow Water Equivalents (SWE; m) and Snow Depth (m) obtained from the nearest snow telemetry (SNOTEL) site at Joe Wright Creek (3,085 m (10,120 ft); https://wcc.sc.egov.usda.gov/nwcc/site?sitenum=551). Average daily ambient air temperatures from that same site were later downloaded as well.

RESULTS & DISCUSSION

Sensors deployed just under the ice shelf registered the highest DO levels, with very little oxygen detected below 1.2 m (4 ft) for most of the winter (Figure 3, 4). Water quality did not improve until late May when heavy snow melt was underway, contributing large volumes of oxygenated water to the system (Figure 5). Recovery of DO deeper in the water column did not occur until June 10th (Figure 3). The steep increase in available oxygen on that day suggests it coincided with ice-out and wind-driven mixing with the inlet area opening up perhaps four days prior (Figure 4). The influx of water from melting snow and ice added 0.8 m (2.7 ft) to the summer surface elevation over the basal ice surface, leaving our DO loggers deeper in the water column once the ice melted off.

Water quality was improved throughout the winter near the inlet, though even here, levels dipped below 4 mg/L in late April (Figure 4). The lowest recording of 1.2 mg/L was registered on May 10th. Cutthroat Trout avoid DO levels below 4 mg/L and fail to thrive under those conditions (Hickman and Raleigh 1982). Progeny of Zimmerman Lake Greenback Cutthroat Trout were able to survive very short exposures to DO levels as low as 1.2 mg/L (Rogers et al. 2022), but 2.3 mg/L was too low to support half of the Cutthroat Trout in a 24-hour laboratory study (Wagner et al. 2001).

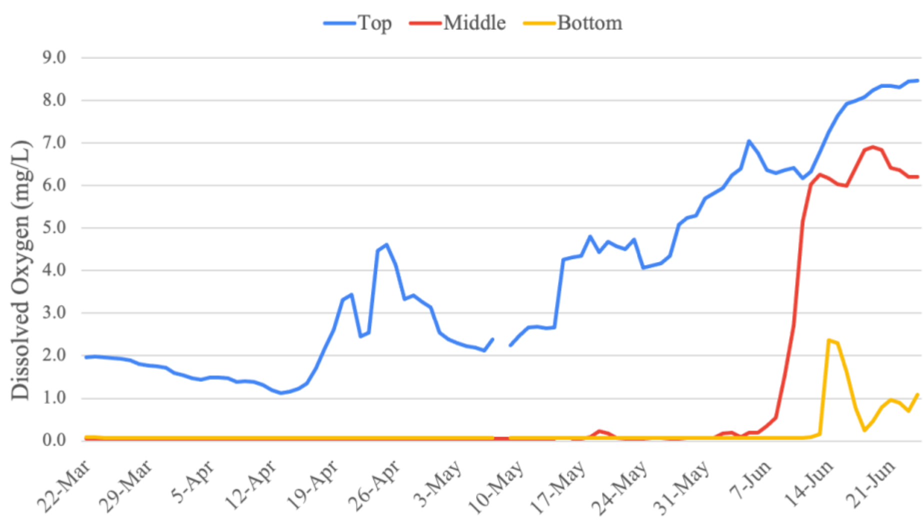

Figure 3. Station 6 (deep water) dissolved oxygen levels recorded every 10 min at 0.6 m (Top), 2.4 m (Middle), and 5.5 m (Bottom) below the basal ice surface.

Figure 4. Station 9 (shallow water) dissolved oxygen levels recorded every 10 min at 0.6 m (Top) and 1.2 m (Bottom) below the basal ice surface.

Snow water equivalents (SWE; Figure 5) and snow depths (Figure 6) obtained from the nearby Joe Wright SNOTEL site did not reveal any sharp increases in early May when water quality just under the basal surface of the ice deteriorated markedly (Figure 7). In fact, there seemed to be little correlation between snow depths and water quality until mid-May when rapid and consistent declines in both SWE and snow depth commenced with the onset of spring runoff. Mean daily ambient air temperatures did a better job explaining the subtle variations seen in DO levels just under the ice. At both sites, increases in water quality arrive shortly after sustained mean daily temperature spikes above freezing (Figure 8). For example, melting snow in late April may have begun to improve water quality before the return of cold temperatures allowed DO levels to deteriorate again before mid-May when mean daily temperatures rose above freezing for the remainder of the summer.

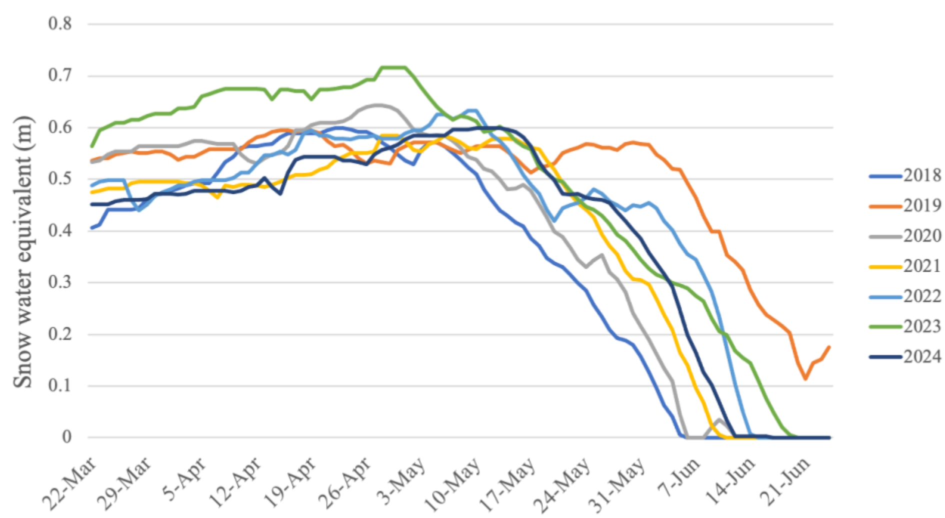

Figure 5. Time series of Snow Water Equivalents (m) for the Joe Wright SNOTEL site by year

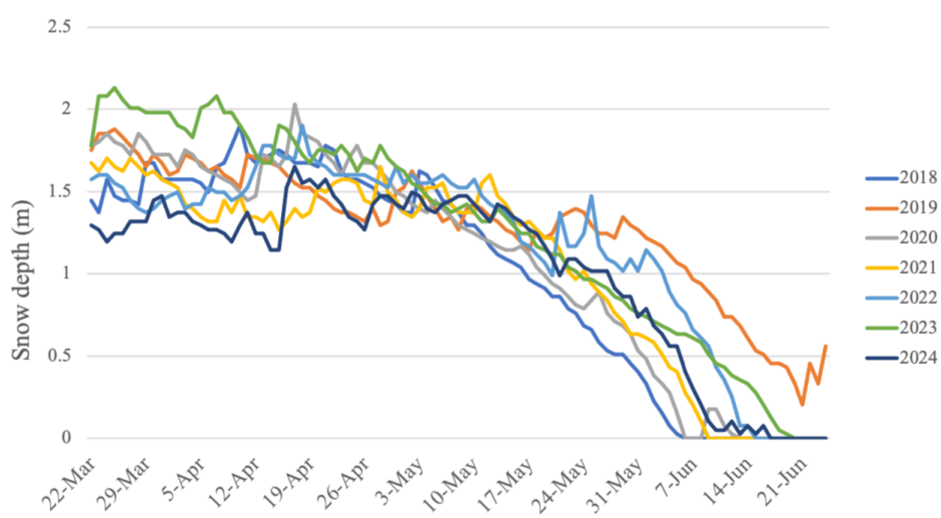

Figure 6. Time series of Snow Depth (m) for the Joe Wright SNOTEL site by year

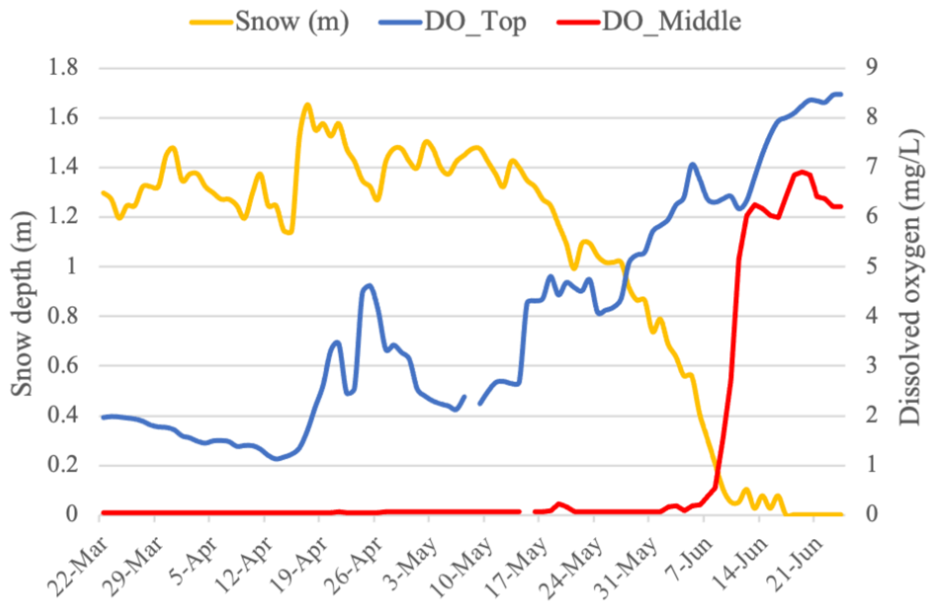

Figure 7. Time series of 2024 Snow Depth (m) registered at the Joe Wright SNOTEL site overlayed on dissolved oxygen levels obtained at the deep-water site (Station 6) in Zimmerman Lake. Values recovered from the top logger (0.6 m below the ice surface) are shown in blue, while the red line indicates values from the logger set 2.4 m below the ice surface.

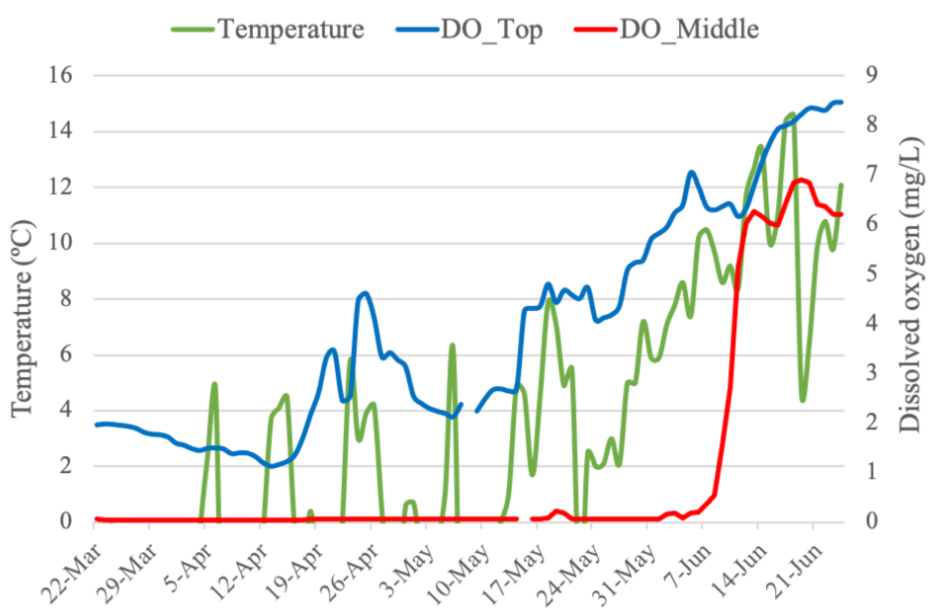

Figure 8. Mean daily ambient temperatures (green) registered at the Joe Wright SNOTEL site overlayed on dissolved oxygen levels obtained at the deep-water site (Station 6) in Zimmerman Lake. Values recovered from the top logger (0.6 m below the ice surface) are shown in blue, while the red line indicates values from the logger set 1.2 m below the ice surface.

Earlier mobile surveys coupled with the data derived from this study reveal that only a very small portion of the lake harbors DO levels suitable for trout in late winter. If much of the lake is inhospitable during that time, then the entire trout population will be concentrated just under the surface near the inlet. Concentrating large adults with younger year-classes would certainly facilitate cannibalism, allowing even a low density of larger predatory fish to have an outsized influence on recruitment. Further research will be necessary to determine if that or direct mortality from poor water quality is responsible for the recent bleak recruitment seen in Zimmerman Lake.

ACKNOWLEDGMENTS

We thank Andrew Treble for assisting with the winter deployment of the dissolved oxygen sensor arrays

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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

Rogers K. B., L. M. Martin, G. Langer, B. Atkinson, F. B. Wright, H. Crockett, C. Kennedy, and D. Krieger. 2024. In Memoriam: Bruce D. Rosenlund. Fisheries 49:91-92.

Abstract.— NA

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. *In press*. 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

Abstract.— Background. Blue lineage Colorado River Cutthroat Trout (CRCTb; *Oncorhynchus clarkii pleuriticus*) conservation populations may be at risk from wildfire and post-fire debris flows.
Aim. Predict burn severity and potential post-fire debris flow hazard classifications to CRCTb conservation populations at risk 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, ecosystem, and weather variables (mean adjusted R2 = 0.54). Predictions of high or moderate burn severity covered 1.1 (15%) and 1.5 (19%) 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 nodes.

Conclusions. Predicted burn severity and potential post-fire debris flow indicated moderate to high risk for remaining CRCTb conservation populations.
Implications. Future management actions can incorporate predicted burn severity and potential post-fire debris flow to mitigate impacts to CRCTb and other at-risk resource values.

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. *In press*. Cyclical multi-trophic-level responses to a volatile, introduced forage fish: learning from four decades of food web observation to inform management. Fisheries

Abstract.— Species introductions can have significant effects on recipient ecosystems. 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 was observed as well. Additionally, opossum shrimp *Mysis diluviana* became absent from both predator diets and intermittent surveys, and Daphnia community composition shifted, and densities declined significantly. These patterns were repeated during two different time periods of increased Rainbow Smelt abundance in Horsetooth Reservoir, 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. Several predictors (i.e., high estimated Rainbow Smelt abundance, high catch rates of large Walleye, and low Daphnia densities) were associated with poor Walleye recruitment. Indicators like these could inform timely management decisions to maximize the benefits that influential introduced species (like Rainbow Smelt) offer, while minimizing or overcoming their undesirable effects.

Presentations *(chronological)*

Rogers, K. B. December 7, 2023. Some thoughts on the recent AFS decision to recognize four Cutthroat Trout species. Colorado River Cutthroat Trout Conservation Team meeting, Grand Junction, Colorado.

Rogers, K. B. February 7, 2024. The fish behind the letters: a summary of consequential native trout broodstocks. CPW Aquatic Section meeting, Mt. Princeton Hot Springs, Colorado.

Rogers, K. B., A. Whiteley, S. Amish, C. Kennedy, C. Noble. February 28, 2024. Annual infusions of wild milt fail to improve genetic diversity in a consequential broodstock of Cutthroat Trout. Colorado Wyoming American Fisheries Society meeting, Laramie, Wyoming.

Evans, R. P., A. Kokkonen, P. Searle, K. B. Rogers, D. Shiozawa. April 9, 2024. Taxonomic (r)evolutions in *Oncorhynchus clarkii* (Cutthroat Trout): the future starts now. Coastal Cutthroat Trout Symposium, Newport, Oregon.