Cutthroat Trout Studies

Kevin B. Rogers
Aquatic Research Scientist

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Tracy Davis, Hatchery Technician, Fish Research Hatchery
Troy Meyer, Hatchery Technician, Fish Research Hatchery

Karen Hertel, Librarian

Prepared by: _____
Kevin B. Rogers, Aquatic Research Scientist

Approved by: _____
George J. Schisler, Aquatic Wildlife Research Chief

Date: 12/4/23

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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CUTTHROAT TROUT INVESTIGATIONS

Period Covered: December 1, 2022 to November 30, 2023

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 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 Colorado River Cutthroat Trout (CRCT) Conservation Team, Rio Grande Cutthroat Trout (RGCT) Conservation Team, and Greenback Cutthroat Trout (GBCT) Recovery Team processed in 2023 are presented here.

METHODS

Molecular tests were conducted on 347 samples obtained from 15 Cutthroat Trout populations distributed across Colorado (Table 1). Four populations came from the Arkansas headwaters, seven from the CRCT native range, four from the Platte River headwaters, and one from the RGCT native range. A small piece of the top of the caudal fin from each fish was clipped off and stored in 3.5 mL cryogenic vials filled with 95% reagent grade ethanol. Fin tissues were delivered to Pisces Molecular (Boulder, Colorado) for subsequent genetic analyses. Isolation of DNA, the production of amplified fragment length polymorphism (AFLPs), sequencing of 648 bp of the NADH dehydrogenase subunit 2 (ND2) mitochondrial gene, and subsequent molecular

analyses are detailed elsewhere (Rogers 2010; Rogers et al. 2014; Bestgen et al. 2019). Rather than assigning numbers or letters to each mitochondrial haplotype recovered, I use the name of the body of water where the haplotype was first discovered, preceded by Oc (the native trout, *Oncorhynchus clarkii*) and three letters that describe the major drainage basin where the lineage is native. These include 1) 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 2023.

Stream	Water Code	Date	Sample size
Arkansas			
Chalk Creek	31879	8/26/04	31
Lake Creek, N Fk	31536	7/18/22	35
Low Pass Gulch	29896	6/22/22	20
Colorado			
Bennett Gulch	25963	9/14/22	20
Cache Creek	22543	7/24/07	30
Caribou Lake	65690	7/13/22	12
Castle Creek	25658	8/17/22	16
Dolores			
Snow Spur Creek	46688	10/7/22	30
Gunnison			
Rock Creek	45870	8/8/2022	26
Platte			
Herman Gulch	14287	10/4/2022	18
Newcomb Creek	11659	9/23/2022	30
Platte Gulch	30677	9/27/2022	10
Wheeler Lakes	81454	7/15/2022	10
Rio Grande			
Squirrel Canyon	39768	6/9/2022	30
Yampa			
Sunbutter Creek	60256	9/27/2017	29

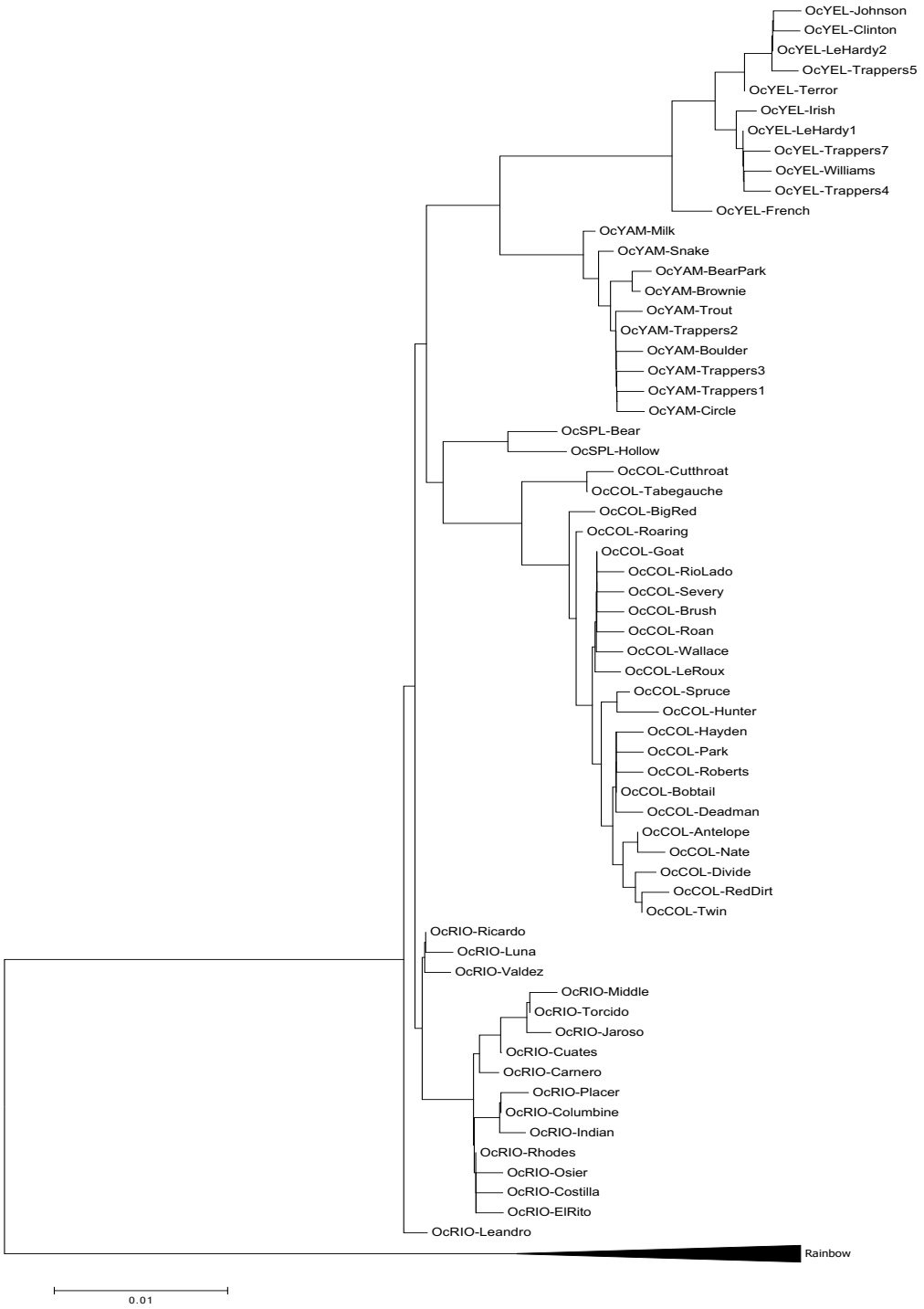

Figure 1. Phylogenetic relationships inferred from 648 base pairs of the mitochondrial NADH dehydrogenase subunit 2 gene for Cutthroat Trout from Colorado. The evolutionary history was developed with the neighbor-joining method in MEGA7, with evolutionary distance units

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.

Arkansas River basin

The search for relict Yellowfin Cutthroat Trout (YFCT) alleles in the Arkansas River basin continues. No evidence of the extinct YFCT was found in the Cutthroat Trout collections in 2022.

Chalk Creek (WC#31879)— These fins were collected in 2004 by now retired biologist G. Policky in hopes of finding some native trout diversity. Inspection at the time with AFLPs suggested they were pure YSN. With renewed interest in searching for relict mtDNA of the extinct YFCT, we sequenced the ND2 gene on all 30 samples he collected. Not surprisingly, all were represented by two YSN haploytypes. What is noteworthy is that while 18 were the common OcYEL-LeHardy2 haplotype, 12 were a new haplotype (OcYEL-Chalk) not seen anywhere else in the state.

Lake Creek, N Fk (WC#31536)— Like the South Fork of Lake Creek that was analyzed previously (Rogers 2022), AFLP data (Table 2) suggests these fish are essentially Yellowstone Cutthroat Trout (YSCT), while the mtDNA revealed a little bit of Blue Lineage CRCT (bCRCT) influence (Table 3). Although both Yellowstone and bCRCT alleles would have been present in the known stocking record of Pikes Peak Natives introduced somewhere in the creek in the late 1970s, we would have expected to see predominantly bCRCT alleles if they were the founding trout (Rogers and Kennedy 2008). The preponderance of YSCT alleles suggests they are either adaptive in this system, or that undocumented stocking of pure YSCT occurred in the decades prior. These fish are isolated above chemical barriers, but unfortunately show no evidence of the extinct YFCT.

Low Pass Gulch (WC#29896)— This collection followed reports of from local anglers of two phenotypically distinct forms of Cutthroat Trout residing isolated above a series of beaver ponds that have kept invading Brook Trout at bay. Curiously, although nine documented stocking events occurred somewhere in the stream over the past 50 years (PPN, CRN, and GBN), they would have introduced predominantly bCRCT alleles into the system (Rogers and Kennedy 2008), yet this collection appears to be pure YSCT by AFLP (Table 2) and ND2 sequence data (19 OcYEL-LeHardy1 and 1 OcYEL-LeHardy2). Sample number LPG-168301 failed to sequence in the forward direction, but the reverse sequence was high quality and aligned with OcYEL-LeHardy1 to which it was then assigned. Phenotypically, these Cutthroat Trout do not display the spotting pattern typical of the Snake River form of YSCT (Figure 2), suggesting perhaps an older founding population when large numbers of pure YSCT derived from

Yellowstone Lake were introduced into the state (Varley 1979). No evidence of YFCT was detected in this sample.

Figure 2. Typical phenotype obtained from Low Pass Gulch trout on 6/22/2022 by A. Townsend.

Table 2. AFLP results from 9 Cutthroat Trout collections analyzed in 2023, along with the number of samples analyzed, organized by major drainage basin. Percent admixture is given by lineage, including Blue and Green Lineage (bCRCT, gCRCT), Rio Grande Cutthroat Trout (RGCT), Yellowstone Cutthroat Trout (YSCT), and Rainbow Trout (RBT).

Stream	# Analyzed	Lineage				
		bCRCT	gCRCT	RGCT	YSCT	RBT
Arkansas						
Lake Creek, N Fk	35	-	-	-	100	-
Low Pass Gulch	20	-	-	-	100	-
Colorado						
Bennett Gulch	20	5	95	-	-	-
Caribou Lake	12	1	-	2	97	-
Castle Creek	16	-	100	-	-	-
Dolores						
Snow Spur Creek	30	-	99	-	-	-
Gunnison						
Rock Creek	26	-	100	-	-	-
Platte						
Newcomb Creek	30	100	-	-	-	-
Platte Gulch	10	-	-	8	91	-

Table 3. ND2 results from 14 Cutthroat Trout collections analyzed in 2023, along with the number of samples analyzed, organized by major drainage basin. ND2 haplotype is given by lineage, including Blue and Green Lineage 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
Arkansas						
Chalk Creek	30	-	-	-	30	-
Lake Creek, N Fk	35	3	-	-	32	-
Low Pass Gulch	20	-	-	-	20	-
Colorado						
Bennett Gulch	20	1	19	-	-	-
Cache Creek	20	1	19	-	-	-
Caribou Lake	12	1	-	-	11	-
Castle Creek	16	-	16	-	-	-
Dolores						
Snow Spur Creek	20	2	18	-	-	-
Platte						
Herman Gulch[1]	18	4	-	-	1	-
Newcomb Creek	30	26	3	-	1	-
Platte Gulch	10	9	-	-	1	-
Wheeler Lakes	10	4	-	1	2	-
Rio Grande						
Squirrel Canyon	30	-	-	30	-	-
Yampa						
Sunbutter Creek	29	23	-	-	6	-

[1]Thirteen remaining fish harbored the OcSPL-Bear haplotype

Colorado River basin

Bennett Gulch (WC#25963)— This isolated stream has been stocked repeatedly (from 2000-2020) with pack fish from Lake Nanita below a diversion structure that sweeps the stream, so we expected it to be bCRCT. But when tested 11 samples in 2021, both AFLP and ND2 sequence data suggested it is a pure gCRCT population displaying the common OcCOL-Goat haplotype. Samples sizes were boosted in 2022 with 20 additional samples collected from upstream of the water diversion where Cutthroat Trout are allopatric. All but one of these fish appeared to be pure gCRCT with the OcCOL-Goat ND2 haplotype while sample BEG1-167186 registered bCRCT both by AFLP and ND2 (OcYAM-Trappers2). This is an interesting system where a Brown Trout population resides upstream of Brook Trout perhaps because of increased tolerance to cadmium.

Cache Creek (WC#22543)— Hints of bCRCT and YSCT admixture were detected in this gCRCT population by AFLP when originally analyzed in 2008. Sequence data on 10 of the original fins however only recovered gCRCT ND2 haplotypes. As a gCRCT conservation population, a more robust examination of the mtDNA was warranted, so the remaining 20 samples were also sequenced. While 19 harbored the OcCOL-Goat haplotype, OcYAM-Trappers1 was detected in a single fish suggesting Trappers Lake progeny were stocked on top of this population sometime after the early 1950s.

Caribou Lake (WC# 65690)— This lake in the Indian Peaks Wilderness was last stocked in 1993 with CRCT from our recreational broodstock and features a robust and prolific self-sustaining Cutthroat Trout fishery with a wide range of fish sizes. The hope was that it might now meet Conservation Population criteria. Surprisingly, it would indeed meet the 90% purity goal for CP consideration – if we were managing for YSCT, with the population appearing to be 97% YSCT by AFLP (Table 2) with 11 fish displaying a common Yellowstone haplotype (OcYEL-LeHardy1) and one the common blue lineage haplotype (OcYAM-Trappers2), likely of Trappers Lake descent. Based only on molecular data, this population was likely founded from a wild spawn operation at Trappers Lake after 1954. The samples size is admittedly small, but suggests YSCT alleles may be adaptive in Caribou Lake.

Castle Creek (WC# 25658)— After discovering a single Cutthroat Trout in Castle Creek in 2015, biologist K. Bakich returned in 2022 to search for additional fish further upstream, and was able to secure 16 specimens with some difficulty as this is not a robust population. This tributary of Big Alkali Creek that drains into the Colorado River near Catamount contains a naturally erosive geology which when combined with the high gradient, may limit this population. Although barriers that protect this population have not yet been identified, dewatered sections downstream may preclude invasion of nonnative trout. Not only do these fish appear to be a Core Conservation Population of green lineage Colorado River Cutthroat Trout, but all harbor a unique haplotype henceforth referred to as OcCOL-Castle.

Dolores River basin

Snow Spur Creek (WC#46688)— This stream flows right along Highway 145 between Telluride and Dolores near the top of Lizardhead Pass. The population occupies a mile and a half of stream and is protected by a new wide box culvert velocity barrier that replaced an old culvert barrier that presumably served the same function. Only brown trout are found below the barrier, and only cutthroat trout above. Although AFLP results suggest the population is 99% pure gCRCT, mtDNA suggest potentially more admixture with two of 20 samples displaying the common OcYAM-Trappers2 haplotype while the remainder were all OcCOL-Goat. It is likely that trout spawned at Trappers Lake were stocked on top of this population at some point. Although we cannot be sure that the OcCOL fish were native, no natural barriers would have precluded fish from moving up from the Dolores River into Snow Spur Creek historically. Chemical reclamation of this population would be challenging as there is a lot of wet meadow habitat and it is a popular and highly visible fishery.

Gunnison River basin

Rock Creek (WC#45870)— The upstream reaches of this population were historically protected by a log barrier. Previous fin collections were obtained between this barrier and more robust natural barriers further upstream and found to be pure gCRCT (Rogers 2020). Upon returning in 2022, biologist E. Gardunio noted that the log barrier was compromised, with Brook Trout invading above. This presumably allowed the passage of admixed Cutthroat Trout from below to also invade, requiring the treatment reach for the pending reclamation effort to be expanded upstream. This sample was acquired from above the more robust natural barriers to confirm that those fish remained a pure allopatric population of gCRCT, which they appear to be (Table 2).

Platte River basin

Herman Gulch (WC# 14287)— In April of 2022 we were alerted to the presence of several fish caught by an angler above the I-70 barrier culvert that protects the Herman Gulch conservation population of Greenback Cutthroat Trout that did not appear to display the typical Bear Creek phenotype (Figure 3). Biologist B. Wright surveyed the "Cascade Reach" just above the I-70 culvert and captured 14 trout, one of which (the downstream most specimen) appeared to look like the nonnative hybrid Cutthroat Trout that reside below the I-70 culvert where he collected 4 more. Sequence data (Table 3) revealed that indeed, the suspect trout above the culvert was an imposter (OcYAM-Trappers2), with mtDNA matching the four below the culvert (OcYAM-Trappers2 and OcYEL-LeHardy1) likely of Trappers Lake descent. The cascades represent an even more formidable barrier to upstream trout invasion than even the I-70 culvert (B. Wright, personal communication), so the upstream conservation population does not appear to be in jeopardy. We have no way of knowing whether the hybrid fish invaded through the culvert or whether they survived the 2015 reclamation, but future mechanical removal efforts could help answer that question.

Figure 3. Typical phenotype obtained from Low Pass Gulch trout on 6/22/2022 by CPW biologist A. Townsend.

Newcomb Creek (WC# 11659)— These samples were collected northeast of Buffalo Pass off of the Newcomb Creek Trail with a goal of confirming their likely bCRCT heritage in the absence of any stocking records. Because this sample had matching photographs, an AFLP test was conducted as well, which confirmed their blue lineage origin (Table 2). Results of the ND2 sequencing suggested a more complicated history (Table 3) with a gCRCT haplotype (OcCOL-Goat) showing up in 3 of the 30 fish sampled. This stimulated further investigation of old stocking archives that revealed indeed "cutthroat trout" were stocked in Newcomb Creek in 1915 and 1928, and then again in 1951. This latter event is noteworthy in that a wild spawn operation was not conducted at Trappers Lake that year, and might help explain the presence of gCRCT alleles. No salmonids are native to the North Platte (Wiltzius 1985, Behnke 2002), so this diversity had a stocking origin

Platte Gulch (WC# 30677)— Although this population has never been stocked directly, it is clear that the population was founded from repeated stocking events in Upper and Lower Wheeler lakes. The outlet of these lakes bisects Platte Gulch, and fish escaping those systems could easily have established a feral population below. Although the AFLP data suggested predominantly YSCT alleles (albeit with a small sample size of 10 fish), the ND2 sequence data recovered the OcYAM-Trappers2 haplotype in 9 fish, while only one harbored the OcYEL-Lehardy1 haplotype, consistent with a PPN founding origin (Rogers and Kennedy 2008). Interestingly, no Rainbow Trout alleles were detected in this stream despite thousands of them being stocked every year in downstream Montgomery Reservoir suggesting a barrier to migration out of the reservoir. Photographs of each fish collected on this sampling effort were taken, and provide a good reference for variation in phenotype that arises from admixture.

Wheeler Lakes (WC# 81454)— These alpine lakes are stocked regularly with a variety of Cutthroat Trout stocks (PPN, GBN, CAR, NAN, and YSN) on top of a feral Brook Trout population. Brook Trout however have not established in Platte Gulch downstream however, leading to speculation that gene flow out of the reservoir was perhaps not occurring. Three of the 10 samples failed to sequence in both the forward and reverse direction, but the remainder produced bCRCT and YSCT haplotypes consistent with the stocking history. Interestingly, a Rio Grande haplotype (OcRIO-Indian) was also detected, a discovery that warrants further investigation.

Rio Grande basin

Squirrel Canyon (WC#39768)— This newly discovered trout population in a small tributary stream to the North Fork of Trinchera Creek has no stocking records associated with it and appears to be pure RGCT by both the standard and RG-CR AFLP tests (Rogers 2022). Because these fish are to be used in upcoming reclamation projects on the Trinchera Ranch, a thorough inspection of the mitochondrial DNA was warranted. We sequenced the ND2 gene from all 30 fish from the August 17, 2021 collection, and only recovered a single haplotype (OcRIO-Rhodes). While genetic diversity may be low in this population, they appear to be pure Rio Grande Cutthroat Trout.

Yampa River basin

Sunbutter Creek (WC#60256)— This recently discovered trout population in a small tributary stream to the Williams Fork of the Yampa appeared to be pure bCRCT when analyzed with AFLPs in 2018. We sequenced the ND2 gene from this population in hopes of finding blue lineage haplotypes that might have been native to the upper Yampa (e.g. OcYAM-Trout), but unfortunately instead recovered YSN haplotypes in 6 of the 29 fish sampled, implying that AFLP data likely overestimated purity. Of the 23 samples that yielded bCRCT haplotypes, all were the common OcYAM-Trappers2 variety.

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REFERENCES

Behnke, R. J. 2002. Trout and salmon of North America. The Free Press. New York, New York.

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.

Hirsch, C. L., M. R. Dare, and S. E. Albeke. 2013. Range-wide status of Colorado River Cutthroat Trout (*Oncorhynchus clarkii pleuriticus*): 2010. Colorado River Cutthroat Trout Conservation Team Report. Colorado Parks and Wildlife, Fort Collins, Colorado. Available online at http://cpw.state.co.us/cutthroat-trout.

Metcalf, J. L., V. L. Pritchard, S. M. Silvestri, J. B. Jenkins, J. S. Wood, D. E. Cowley, R. P. Evans, D. K. Shiozawa, and A. P. Martin. 2007. Across the great divide: genetic forensics reveals misidentification of endangered cutthroat trout populations. Molecular Ecology 16:4445-4454.

Metcalf, J. L., S. L. Stowell, C. M. Kennedy, K. B. Rogers, D. McDonald, J. Epp, K. Keepers, A. Cooper, J. J. Austin, and A. P. Martin. 2012. Historical stocking data and 19th century DNA reveal human- induced changes to native diversity and distribution of Cutthroat Trout. Molecular Ecology 21:5194–5207.

Rogers, K. B. 2010. Cutthroat trout taxonomy: exploring the heritage of Colorado's state fish. Pages 152-157 *in* R. F. Carline and C. LoSapio, editors. Wild Trout X: Sustaining wild trout in a changing world. Wild Trout Symposium, Bozeman, Montana. Available: http://www.wildtroutsymposium.com/proceedings.php

Rogers, K. B. 2020. Cutthroat Trout investigations. Colorado Parks and Wildlife Progress Report, Fort Collins. Available online at: https://cpw.state.co.us/learn/Pages/ResearchAquaticPublications.aspx (November 2021).

Rogers, K. B. 2022. Cutthroat Trout investigations. Colorado Parks and Wildlife Progress Report, Fort Collins. Available online at: https://cpw.state.co.us/learn/Pages/ResearchAquaticPublications.aspx.

Rogers, K. B., K. R. Bestgen, and J. Epp. 2014. Using genetic diversity to inform conservation efforts for native Cutthroat Trout of the southern Rocky Mountains. Pages 218-228 *in* R. F. Carline and C. LoSapio, editors. Wild Trout XI: Looking back and moving forward. Wild Trout Symposium, West Yellowstone, Montana. Available online at http://www.wildtroutsymposium.com/proceedings.php

Rogers, K. B., K. R. Bestgen, S. M. Love Stowell, and A. P. Martin. 2018. Cutthroat Trout diversity in the southern Rocky Mountains. Pages 323-341 *in* P. Trotter, P. Bisson, B. Roper, and L. Schultz, editors. Evolutionary

biology and taxonomy of Cutthroat Trout (*Oncorhynchus clarkii*), American Fisheries Society Special Publication 36, Bethesda, Maryland.

Rogers, K. B., and C. M. Kennedy. 2008. Seven Lakes and the Pike's Peak native (PPN): history and current disposition of a critical cutthroat trout brood stock. Colorado Division of Wildlife report, Fort Collins. Available online at http://cpw.state.co.us/learn/Pages/ResearchGreenbackCutthroatTrout.aspx.

Tamura K., G. Stecher, and S. Kumar. 2021. MEGA11: Molecular Evolutionary Genetics Analysis version 11. Molecular Biology and Evolution 38:3022-3027.

UDWR. 2000. Genetic considerations associated with cutthroat trout management: a position paper. Publication Number 00-26, Utah Division of Wildlife Resources, Salt Lake City, Utah. Available: https://cpw.state.co.us/cutthroat-trout. (November 2020).

USFWS (U.S. Fish and Wildlife Service). 2014. 12-month finding on a petition to list Rio Grande Cutthroat Trout as an endangered or threatened species. Federal Register 79:190(October 1, 2014):59140– 59150.

Wiltzius, W. J. 1985. Fish culture and stocking in Colorado, 1872-1978. Colorado Division of Wildlife, Division Report 12.

Varley, J. D. 1979. Record of egg shipments from Yellowstone fishes, 1914-1955. U. S. National Park Service, Information Paper 36, Yellowstone National Park, Wyoming.

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.

RESEARCH PRIORITY

Fitness in small Cutthroat Trout populations

OBJECTIVE

Annual infusions of wild milt fail to improve genetic diversity in a consequential broodstock of Cutthroat Trout

INTRODUCTION

Loss of genetic diversity can pose a serious threat to small populations (Vucetich and Waite 1999; Hedrick and Kalinowski 2000), and is an important component of extinction risk (Frankham 1998; Frankham and Ralls 1998). When populations lose alleles, increases in individual homozygosity can reduce fitness (Markert et al. 2010), often manifested in lower survival rates (Westemeier et al. 1998; Slate et al. 2000; Fritzsche et al. 2006). This problem is particularly relevant to the conservation of native Cutthroat Trout *Oncorhynchus clarkii* in the southern Rocky Mountains. Remaining populations only occupy a small fraction of their historic ranges (Alves et al. 2008; Hirsch et al. 2013; Penaluna et al. 2016), usually in small isolated headwater habitats protected from nonnative invasions by impassable barriers to fish movement (Fausch et al. 2009). Many of these populations simply do not occupy large enough stream reaches to support large populations (Hilderbrand and Kershner 2000; Young et al. 2005) needed to maintain robust effective population (Ne) sizes and adaptive potential (Franklin 1980) leaving them more vulnerable to inbreeding depression (Rieman and Allendorf 2001; Allendorf and Luikart 2007).

Nowhere is this problem more pronounced than in the recently rediscovered Greenback Cutthroat Trout *O. clarkii ssp.* (GBCT), Colorado's state fish. The subspecies has persisted in one locality as a single isolated population outside its native range for the last 130 years and is both genetically (Metcalf et al. 2012; Rogers et al. 2018), and phenotypically distinct (Bestgen et al. 2019). Apparently founded from stocked trout escaping a constructed headwater pond in the Bear Creek drainage (Kennedy 2010), this population occupies just 7 km of first-order stream habitat protected from invasion by downstream nonnative Brook Trout (*Salvelinus fontinalis*) by a natural waterfall barrier. Recognizing the precarious nature of this population, the GBCT Recovery Program (a consortium of state and federal partner managers) set about developing a broodstock from which subsequent wild populations could be founded. In 2008, 66 indivduals were captured and brought into captivity. Two years later, 16 females produced ripe eggs that were fertilized with milt from 37 captive males to develop the initial broodstock

This stock remains extremely difficult to raise and maintain in captivity, plagued with deformities, and challenged by poor survival and growth even when cultured in small lots (Rogers et al. 2022b), presumably a result of low genetic diversity (Rogers et al. 2022a). In an effort to maximize this diversity with the least cost to the source population, the broodstock has

been supplemented annually with milt obtained from wild males, and fertilized eggs from an occasional wild ripe female (Table 1). The goal of this study was to determine if those efforts have indeed boosted genetic diversity in the broodstock, and if the suite of diversity remaining in Bear Creek (above and below Josephine Falls which bisects the population) has been incorporate into the broodstock.

Table 1: Number of ripe male and female GBCT captured during annual collection efforts on Bear Creek. Milt was stripped from males and used to fertilize eggs from the captive broodstock housed at the Leadville National Fish Hatchery. Eggs from ripe females incidentally captured during these same collections were fertilized with the same milt (from Rogers et al. 2022a).

Year	Male	Female
2013	9	1
2014	10	1
2015	15	0
2016	17	6
2017	4	3
2018	11	2
2019	28	3
2020	17	0
2021	0	0
2022	32	1
Totals	143	17

METHODS

Caudal fin clips were obtained from fish collected in Bear Creek both above and below Josephine Falls, from early (2013) and recent (2020) broodstocks of those same fish housed at the Leadville National Fish Hatchery, and those in Zimmerman Lake (Table 2). Fin clips were stored in 95% ethanol as in Rogers (2007). A proteinase K tissue lysis and spin-column purification protocol following manufacturer specifications (Qiagen DNeasy Kit) was used to isolate DNA from the fin clip samples at Pisces Molecular (Boulder, Colorado), then delivered to the Conservation Genomics Lab at the University of Montana, Missoula for genotyping.

Genotyping

We used restriction site-associated DNA sequencing (RADseq; Miller et al. 2007; Baird et al. 2008; Davey and Blaxter 2011) as an alternative to whole genome sequencing for single nucleotide polymorphism (SNP) detection to evaluate diversity in fish between all groups (Table 2). Variable SNPs were filtered such that a minimum of 3 copies of each allele were required, with a read depth >10 fragments per fish. We removed individuals missing >75% of the loci, as well as loci that were missing in >25% of the fish. We also removed those that failed to meet Hardy-Weinberg equilibrium or showed evidence of linkage disequilibrium. In addition, we compared the RADseq data to an existing SNP panel containing 1,026 diagnostic markers for Rainbow Trout to confirm that no admixture was present. All filtering and subsequent analyses were used to parse SNPs and calculate heterozygosity conducted in R (R Core Team 2021).

RESULTS

A total of 164 fish representing five groups were analyzed as part of this study (Table 2). Within these fish, 1,902,988 variable SNP sites were identified, that were distilled down to 1,242 variable sites following the described filtering protocols. The proportion of heterozygous loci at

these sites was significantly greater in the collection from below Josephine Falls where the Leadville NFH broodstock was derived and where current annual milt supplementation collections are made compared to the population above the falls (Figure 1 and 2). The most heterozygosity was measured in the Leadville broodstocks and at Zimmerman Lake. Genetic differentiation measured with Fst was also greatest between the Upper and Lower sites on Bear Creek than any other comparison between groups (Table 3).

Table 2: Number of fin clips from Bear Creek Greenback Cutthroat Trout collected for each group in this study, along with the number actually used in the analyses.

Group	Collected	Date	Analyzed
Bear Creek-Lower	43	9/24/2020	42
Bear Creek-Upper	22	9/23/2020	22
Leadville NFH 2013	30	3/18/2021	28
Leadville NFH 2020	30	3/18/2021	29
Zimmerman Lake 2020	60	4/5/2020	43

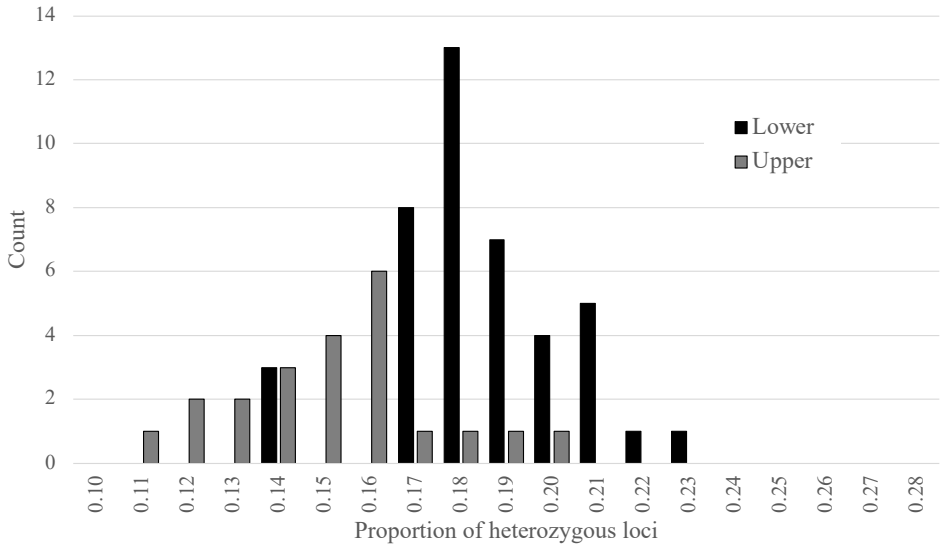

Figure 1: Proportion of heterozygous loci found in fish collected from Bear Creek above (Upper) and below (Lower) Josephine Falls.

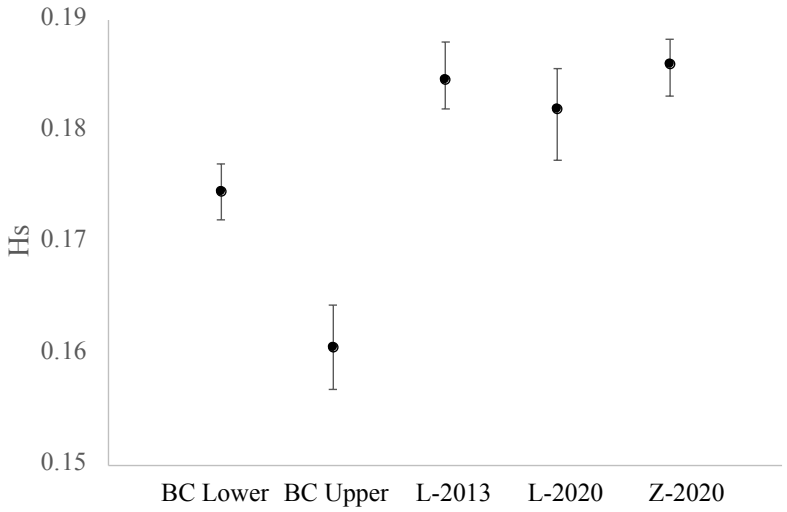

Figure 2: Mean heterozygosity (Hs) from trout collected from Bear Creek below above (Upper) and below (Lower) Josephine Falls compared to those from early (L-2013) and recent (L-2020) Leadville National Fish Hatchery broodstock as well as those from Zimmerman Lake (Z-2020). Error bars represent 95% confidence intervals bootstrapped across individuals (to characterize the more relevant uncertainty associated with sampling rather than among-locus variation).

Table 3: Genetic differentiation between Bear Creek (BC Upper and BC Lower), 2013 and 2020 Leadville National Fish Hatchery stock (L-2013 and L-2020), and 2020 Zimmerman Lake stock (Z-2020), and as measured with Fst.

	BC Lower	**BC Upper**	**L-2013**	**L-2020**	**Z-2020**
BC Lower	-				
BC Upper	0.054	-			
L-2013	0.042	0.084	-		
L-2020	0.047	0.083	0.018	-	
Z-2020	0.045	0.079	0.023	0.015	-

Closer inspection of the individual allele calls for the 1,242 loci revealed additional interesting observations. Of these loci, 270 were fixed above the falls but heterozygous below. These low frequency alleles (median frequency = 3.2%) were lost from above presumably due to genetic drift. This suggests that 22% of alleles found in this population are now fixed above the falls. In 84 cases, loci were homozygous below the falls but heterozygous above albeit at low frequency (median = 5.0%).

DISCUSSION

Phenotypic markers used in Bestgen et al. (2019) documented traits in the Bear Creek trout such

as high spot counts and low numbers of basibranchial teeth that are usually associated with Rainbow Trout admixture (Behnke 2002). Perhaps the most consequential finding of this study came when the RADseq data was compared to a SNP panel of 1,026 diagnostic markers for Rainbow Trout, and found none in the Bear Creek population, or in the broodstocks. Although we have inspected this population with a whole suite of molecular tests (AFLPs, microsatellites, mtDNA, 6 nuclear genes, the Y-chromosome, and an earlier SNP panel with 32 markers), it is nonetheless very encouraging that no Rainbow Trout admixture was detected here with this new much higher resolution panel.

While seven years of milt infusion have failed to increase the genetic diversity of the Leadville NFH broodstock, we can take solace in the fact that the current broodstock appears to represent the population in Bear Creek very well. We had hoped that more uncaptured diversity in the wild would have provided an opportunity to add more diversity to the broodstock with targeted milt acquisition that would improve growth and survival of progeny. With little diversity in the donor population however, the 66 fish collected in 2008 used to establish the broodstock appear to have captured what diversity remains of this rare lineage of native trout. We continue to advocate for wild milt collections however, as those supplemental infusions have kept the broodstocks from drifting apart from the donor source in Bear Creek, and Fst values have not grown larger.

The GBCT Recovery Team and partners continue to establish new populations of GBCT with progeny from the same broodstocks examined here in reclaimed waters across their putative native range in the headwaters of the South Platte River (Metcalf et al. 2012), with a half-dozen populations repatriated to date. The RADseq work presented here serves as a valuable baseline for comparing future accumulation of new mutations and genetic drift in these new wild populations that are currently being established.

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REFERENCES

Allendorf, F. W. and G. Luikart. 2007. Conservation and the genetics of populations. Blackwell Publishing, Malden, Massachusetts, USA.

Alves, J. E., K. A. Patten, D. E. Brauch, and P. M. Jones. 2008. Range-wide status of Rio Grande Cutthroat Trout (*Oncorhynchus clarki virginalis*): 2008. Colorado Division of Wildlife, Fort Collins. Available online at http://cpw.state.co.us/cutthroat-trout

Baird, N. A., P. D. Etter, T. S. Atwood, M. C. Curry, A L. Shiver. Z. A. Lewis, E. U. Selker, W. A. Cresko, and E. A. Johnson. 2008. Rapid SNP discovery and genetic mapping using sequenced RAD markers. PLoS ONE

3(10): e3376.
Behnke, R.J. 2002. Trout and salmon of North America. The Free Press, New York, New York.
Bestgen, K. R., K. B. Rogers, and 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.
Davey, J. W. and M. L. Blaxter. 2011. RADSeq: next-generation population genetics. Briefings in Functional Genomics 9:416-423.
Fausch, K. D., B. E. Rieman, J. B. Dunham, M. K. Young, and D. P. Peterson. 2009. Invasion versus isolation: trade-offs in managing native salmonids with barriers to upstream movement. Conservation Biology 23:859-870.
Frankham, R. 1998. Inbreeding and extinction: island populations. Conservation Biology 12: 665–675.
Frankham, R., and K. Ralls. 1998. Inbreeding leads to extinction. Nature 392:441–442.
Franklin, I. R. 1980. Evolutionary changes in small populations. Pages 135-150 *in* Soulé, M. E., and B. A. Wilcox, editors. Conservation biology: an evolutionary-ecological perspective. Sinauer Associates, Sunderland, Massachusetts, USA.
Fritzsche, P., K. Neumann, K. Nasdal, and R. Gattermann. 2006. Differences in reproductive success between laboratory and wild-derived golden hamsters (*Mesocricetus auratus*) as a consequence of inbreeding. Behavioral Ecology and Sociobiology 60:220-226.
Hedrick, P. W., and S. T. Kalinowski. 2000. Inbreeding depression in conservation biology. Annual Review of Ecology and Systematics 31:139-162.
Hilderbrand, R. H., and J. L. Kershner. 2000. Conserving inland Cutthroat Trout in small streams: how much stream is enough? North American Journal of Fisheries Management 20:513-520.
Hirsch, C. L., M. R. Dare, and S. E. Albeke. 2013. Range-wide status of Colorado River Cutthroat Trout (*Oncorhynchus clarkii pleuriticus*): 2010. Colorado River Cutthroat Trout Conservation Team Report. Colorado Parks and Wildlife, Fort Collins, Colorado. Available online at http://cpw.state.co.us/cutthroat-trout
Kennedy, C. M. 2010. Weird Bear Creek: A history of a unique Cutthroat Trout population. Technical Report USFWS, 1-9.
Markert, J. A., D. M. Champlin, R. Gutjahr-Gobell, J. S. Grear, A. Kuhn, T. J. McGreevy Jr., A. Roth, M. J. Bagley, and D. E. Naci. 2010. Population genetic diversity and fitness in multiple environments BMC Evolutionary Biology 10:205.
Metcalf J. L., S. L. Stowell, C. M. Kennedy, K. B. Rogers, D. McDonald, J. Epp, K. Keepers, A. Cooper, J. J. Austin, and A. P. Martin. 2012. Historical stocking data and 19th century DNA reveal human-induced changes to native diversity and distribution of cutthroat trout. Molecular Ecology 21:5194-5207.
Miller, M. R., J. P. Dunham, A. Amores, W. A. Cresko, and E. A. Johnson. 2007. Rapid and cost-effective polymorphism identification and genotyping using restriction site associated DNA (RAD) markers. Genome Research 127:240-248.
Penaluna, B. E., A. Abad'a-Cardoso, J. B. Dunham, F. J. Garcia de Leon, R. E. Gresswell, A. Ruiz-Luna, E. B. Taylor, B. B. Shepard, R. Al-Chokhachy, C. C. Muhlfeld, K. R. Bestgen, K. B. Rogers, M. A. Escalante, E. R. Keeley, G. M. Temple, J. E. Williams, K. R. Matthews, R. Pierce, R. L. Mayden, R. P. Kovach, J. C. Garza, and K. D. Fausch. 2016. Conservation of native Pacific trout diversity in western North America. Fisheries 41:287-300.
R Core Team. 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.
Rieman, B. E., and F. W. Allendorf. 2001. Effective population size and genetic conservation criteria for Bull Trout. North American Journal of Fisheries Management 21:756-764.
Rogers, K. B. 2007. A suggested protocol for collecting cutthroat trout tissues for subsequent genetic analysis. Colorado Division of Wildlife, Fort Collins. Available: https://cpw.state.co.us/learn/Pages/ResearchCutthroatTrout.aspx
Rogers, K. B., K. R. Bestgen, S. M. Love Stowell, and A. P. Martin. 2018. Cutthroat Trout diversity in the southern Rocky Mountains. Pages 323-341 *in* P. Trotter, P. Bisson, L. Schultz, and B. Roper, editors. Cutthroat Trout: evolutionary biology and taxonomy, American Fisheries Society, Special Publication 36, Bethesda, Maryland.
Rogers, K. B., J. R. Anderson, S. F. Brinkman, and A. P. Martin. 2022a. Inbreeding depression reduces fitness in Colorado's last remaining Greenback Cutthroat Trout: consequences for management. Pages 185-194 *in* J. S. Gregory, editor. Proceedings of Wild Trout XIII Symposium: Reducing the gap between science and

public opinion. West Yellowstone, Montana. Available https://www.wildtroutsymposium.com/proceedings.php

Rogers, K. B., B. J. Sucher, B. W. Hodge, and C. A. Myrick. 2022b. Thermal tolerance in Cutthroat Trout of the southern Rocky Mountains. Canadian Journal of Fisheries and Aquatic Sciences 79:2043-2055.

Slate, J., L. E. B. Kruuk, T. C. Marshall, J. M. Pemberton, and T. H. Clutton-Brock. 2000. Inbreeding depression influences lifetime breeding success in a wild population of red deer (*Cervus elaphus*). Proceedings of the Royal Society of London, Series B 267:1657-1662.

Vucetich, J. A., and T. A. Waite. 1999. Erosion of heterozygosity in fluctuating populations. Conservation Biology 13:860–868.

Westemeier, R., J. Brawn, S. Simpson, T. Esker, R. Jansen, J. Walk, E. Kershner, J. Bouzat, and K. Paige. 1998. Tracking the long-term decline and recovery of an isolated population. Science 282:1695-1698.

Young, M. K., P. M. Guenther-Gloss, and A. D. Ficke. 2005. P redicting Cutthroat Trout (*Oncorhynchus clarkii*) abundance in high-elevation streams: revisiting a model of translocation success. Canadian Journal of Fisheries and Aquatic Sciences 62:2399-2408.

RESEARCH PRIORITY

Culture of native Cutthroat Trout

OBJECTIVE

Assess suitability of commercial milt and egg extenders for enhancing trout survival

INTRODUCTION

Culture of rare native Cutthroat Trout continues to be a challenge, with hatcheries reporting very low survival rates, particularly for some of our most valuable stocks (B. Johnson, Mt. Shavano Fish Hatchery, personal communication). As such, we continue to search for ways to make our captive and wild spawn operations more efficient with more positive outcomes. The company IMV Technologies (Maple Grove, Minnesota) produces products including egg and milt extenders that aim to help achieve those goals. While CPW managers continue to use extended milt with good success (Rogers 2010; Rogers et al. 2022), we have not explored storage of unfertilized eggs for extended periods of time prior to fertilization. Storage might allow for logistical benefits such as are currently enjoyed by the Greenback Cutthroat Trout recovery effort that uses extended wild milt to introduce more genetic diversity to the Bear Creek Greenback Cutthroat Trout broodstock housed at the Leadville National Fish Hatchery (GCTRT 2019).

The egg extender, OvaFish (available from Syndel, Ferndale, Washington), is purported to allow treated eggs to remain viable for up to 7 days (following manufacturer's instructions for use). Hatchery managers have also noticed that collecting eggs directly into an OvaFish bath can boost survival of eggs to hatch over the traditional dry method of spawning (R. Streater, personal communication). The ease and efficiency of this approach allows smaller spawning crews to collect the same number of viable eggs over a shorter period of time if large batches of eggs are collected, then fertilized at once with extended milt from a similar number of males. Broodstock managers in the past have cautioned against approaches that expose pans of unfertilized eggs from multiple females with milt from pooled malse, as that can reduce genetic diversity by allowing that male to sire a disproportionate number of offspring (Withler 1988; Campton 2004). This is particularly true if milt stripped from males is added sequentially to the pans of unfertilized eggs, as most will be fertilized by the first milt they are exposed to. It was hypothesized that extended milt might ameliorate this concern somewhat in that the milt too is mixed and diluted prior to being exposed to the unfertilized eggs, potentially giving individual sperm an equal opportunity at fertilization when they are exposed to the extended eggs.

Here we sought to address three questions as part of the same study: 1) Does fertilizing eggs in OvaFish enhance survival of fertilized eggs over traditional "dry spawn" approaches in ovarian fluid? 2) Can viable eggs be stored in OvaFish for extended periods of time (1-7 days), and 3) If milt from multiple males is pooled in StorFish milt extender, are all males well represented in the

progeny? While this last question has historically been challenging to prove, modern molecular tools now allow us to do just that with assurance.

METHODS

We used Hofer x Harrison strain broodstock of Rainbow Trout (*O. mykiss*) housed at the Crystal River Hatchery as a readily available surrogate sister taxon for Cutthroat Trout in this experiment. On January 31, 2023, ten Age 2 adult males were stripped of their milt into a StorFish bath following manufacturers protocols (Syndel, Ferndale, Washington). A fin clip was obtained from each male (Rogers 2007) and stored in 95% ethanol so that parentage analyses could be completed following the experiment. Eggs from ten Age 3 adult females (approximately 3000 eggs each) were also stripped on January 31, and well mixed in a large bowl. Roughly 20% were split into two smaller bowls and fertilized with 20 ml of the extended milt in ovarian fluid while the remainder were diluted into 180 ml of OvaFish, then split into eight bowls and either fertilized with extended milt or stored at 4°C for subsequent fertilization on Day 1, 3, and 7 with extended milt from ten new males each day (Figure 1).

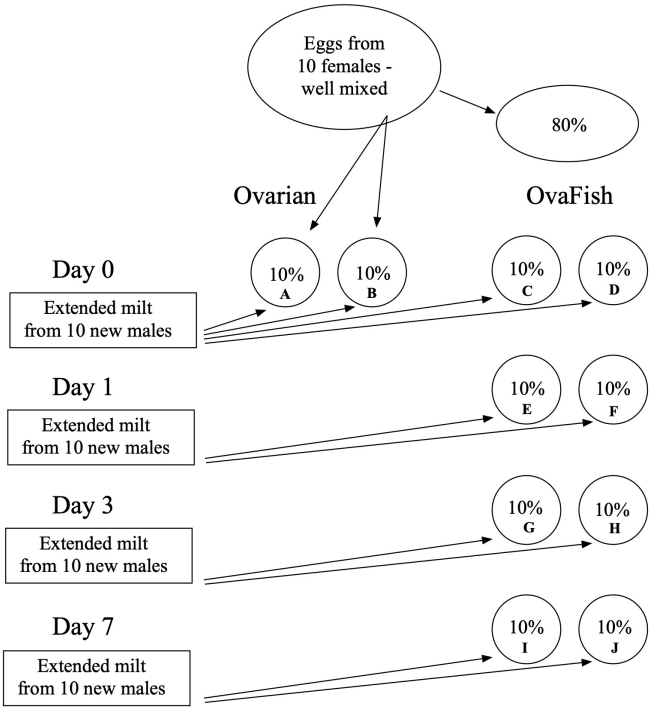

Figure 1. Eggs were stripped from 10 female trout, well mixed, then fertilized with extended pooled milt from 10 males either in the presence of ovarian fluid or OvaFish. Unfertilized eggs were stored at 4°C in OvaFish for 1, 3, or 7 days, then fertilized with pooled extended milt from 10 new males, and survival to hatch was monitored.

Rearing
The ten bowls of embryos (Figure 1) were set to incubation in two Heath stacks percolated with 10.6 °C (51°F) well water. Since it was important that position in the Heath stack did not influence outcomes, we randomly assigned egg group to tray position (excluding the top position) with custom randomization software (LabVIEW, National Instruments, Austin, Texas). Positions were stratified by stack such that Bowls A, C, E, G, and I were placed in one stack and Bowls B, D, F, H, and J were set in the other. Once the contents of each bowl was spread on a Heath tray, a photograph was taken, and the number of eggs counted using DotDotGoose (Ersts 2023).

Viability of the embryos was assessed (dead eggs picked) prior to bump, after bumping (21 days after fertilization), through hatch, and at the alevin stage. On March 15, 2023, 100 alevins were sacrificed from Bowls A, B, C, D and stored in individual 4.5 ml cryotubes in 95% ethanol for subsequent parentage testing. An additional 100 alevins from Bowls E, F, G, and H were sacrificed and stored in four 120 ml ethanol filled specimen cups should further parentage work be warranted.

Parentage testing
Genomic DNA was extracted from fin clips using the Nexttec Genomic DNA Isolation Kit (XpressBio, Thurmont, Maryland) following manufacturer's protocol. Samples were screened with a panel of single nucleotide polymorphic (SNP) loci (GTseq v5.0 368) optimized for genetic stock identification and parentage studies involving *O. mykiss*. Genotyping of the SNP panel followed Genotyping in Thousands Sequencing (GTseq) protocols developed by Campbell et al (2015). Data summaries and formatting for specific genetic software programs was completed in R version 3.4.1 (R Core Team 2017, Vienna, Austria).

Parentage assignments were performed using the program SNPPIT (Anderson 2010, 2012). We required a 90% complete genotype for a sample to be included in the analyses, and used an estimated SNP genotyping error rate of 1% or a per-allele rate of 0.5%. The SNPPIT program uses a maximum likelihood algorithm for parentage assignments (Anderson 2010). The confidence of parentage assignments can be evaluated using the logarithm of odds (LOD) score reported in SNPPIT. The LOD score is the natural logarithm of the likelihood of the parental trio hypothesis divided by the likelihood of the nonparental hypothesis for a trio. We used an LOD cutoff of ≥14 to minimize false positive and false negative assignments, as this criterion has been shown to produce robust and accurate assignments that concur with steelhead spawning hatchery records (Hess et al. 2016).

RESULTS

Results were unexpected (Figure 2), with significantly better survival to the alevin life stage occurring in the groups of eggs fertilized in ovarian (96%) fluid rather than those fertilized in OvaFish (79%). In addition, although Syndel maintains that viable eggs can be stored in Ovafish for up to seven days, that was not consistent with our results. In this study, significant declines in survival to the alevin stage were documented even for those eggs stored in OvaFish for just 24

hours (58%), with numbers dropping to 20% after three days and just 0.2% after seven days in storage.

Parentage

Of the 400 juveniles examined, all samples genotyped at a >90% completeness. Sex ratios were near even with 208 males in the progeny and 192 females. We successfully assigned parentage to 397/400 samples analyzed in SNPPIT. All assigned samples exhibited LOD scores >17 indicating that they were high confident assignments. No consistent trends in parentage were detected between those eggs bathed in ovarian fluid vs those in OvaFish, so all four replicates were combined in this analysis (Figure 3). Variation in egg quality was detected among females, with the ten mothers represented from 2-15% of the progeny (Figure 3). Milt viability was even more variable, with the ten fathers represented in 2-30% of the progeny.

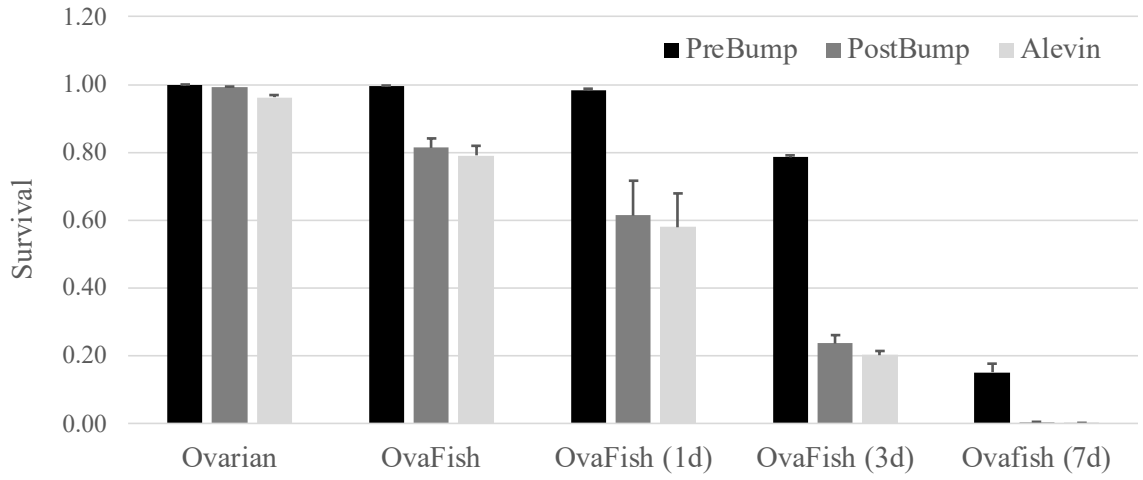

Figure 2. Embryo survival to before and after eggs were bumped (21 d after fertilization) was recorded along with survival of alevins to swim-up under each of the egg treatments. Error bars represent 95% confidence intervals.

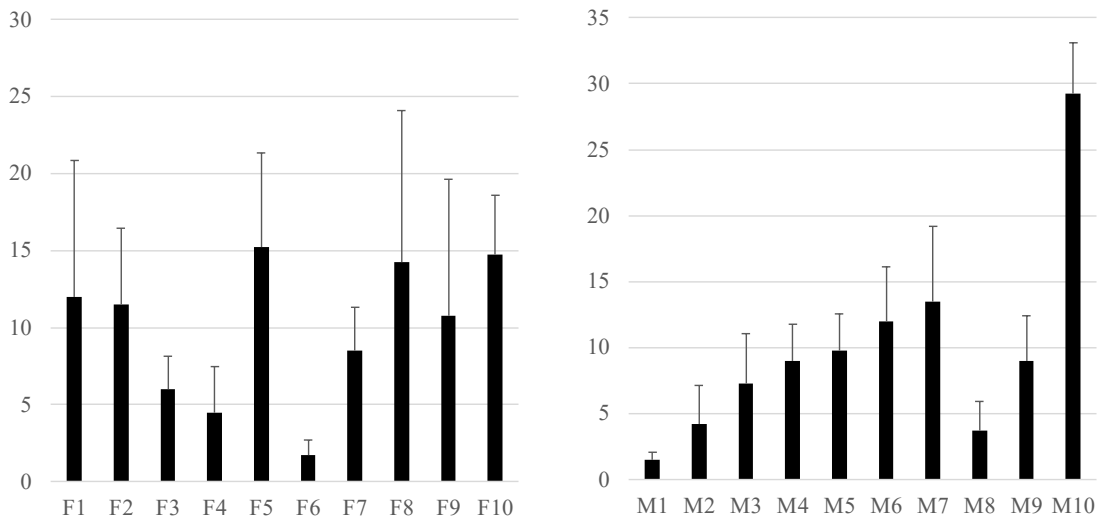

Figure 3. Average percent of alevins derived from each mother (Female; left panel) or father (Male; right panel) in the four Day 0 treatments (A, B, C, and D). Error bars represent 95% confidence intervals.

DISCUSSION

Despite anecdotal evidence suggesting bathing eggs in OvaFish prior to fertilization could enhance survival in trout embryos, our results suggested otherwise. Traditional "dry" spawning methods where eggs are fertilized in the ovarian fluids that come with them while stripping gravid females into bowls saw significantly higher survival to the alevin stage (96%) than those eggs treated with Ovafish (79%). Using OvaFish as an egg "extender" to store green eggs until milt is available to fertilize them also does not appear to be a practical. After just 24 hours in storage, mean survival to the alevin stage was only 58%, dropping to 20% after three days in storage, and virtually no survival if eggs were stored for seven days prior to fertilization. The ability to extend and store milt has provided us flexibility when adding genetic diversity from the wild to hatchery broodstocks of our native trout (GCTRT 2019). We had hoped that the ability to store eggs would grant us additional flexibility, but it appears that will not be the case.

Parentage

We were able to successfully assign parentage in 397 of the 400 alevins tested in this study. Although we cannot pinpoint why 3 failed, there is possibility that those fish were triploid, a condition that can complicate parental assignment. Similar to other studies on trout, we detected substantial variation in female egg quality (Rogers et al. 2020), with individual mothers contributing anywhere from 2-15% of the progeny

A common concern in native Cutthroat Trout conservation is the use of a single male to fertilize a full pan of eggs during wild or hatchery spawn operations. These concerns are elevated when working with high-value genetically depauperate populations (Rogers et al. 2022) where milt from some males can appear watery or otherwise deficient. Earlier work suggests that even in

these instances, there is no such thing as "bad milt" (Love-Stowell 2016; Rogers et al. 2020). This study corroborated those findings (albeit from a more genetically diverse broodstock), in that all males were represented in the progeny. However, it is clear that some milt is much more competitive than others. A single male in this study sired almost a third of the progeny – more than the combined contribution of half the remaining males. These results emphasize the need to continue with 1:1 spawns when working with sensitive valuable brood stocks.

ACKNOWLEDGMENTS

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REFERENCES

Anderson, E. C. 2010. Computational algorithms and user-friendly software for parentage-based tagging of Pacific salmonids. Final report submitted to the Pacific Salmon Commission's Chinook Technical Committee (US Section).

Anderson, E. C. 2012. Large-scale parentage inference with SNPs: an efficient algorithm for statistical confidence of parent pair allocations. Statistical Applications in Genetics and Molecular Biology 11:1544-6115.

Campbell, N. R., S. A. Harmon, and S. R. Narum. 2015. Genotyping-in-Thousands by sequencing (GT-seq): a cost effective SNP genotyping method based on custom amplicon sequencing. Molecular Ecology Resources 15:855-867.

Campton, D. E. 2004. Sperm competition in salmon hatcheries: the need to institutionalize genetically benign spawning protocols. Transactions of the American Fisheries Society 133:1277–1289.

Ersts, P. J. 2023. DotDotGoose (version 1.6.0). American Museum of Natural History, Center for Biodiversity and Conservation. Available from https://biodiversityinformatics.amnh.org/open_source/dotdotgoose (Accessed November 2023).

Maureen A. Hess, M. A., J. E. Hess, A. P. Matala, R. A. French, C. A. Steele, J. C. Lovtang, and S. R. Narum, 2016. Migrating adult steelhead utilize a thermal refuge during summer periods with high water temperatures. ICES Journal of Marine Science 73:2616-2624.

GCTRT (Greenback Cutthroat Trout Recovery Team). 2019. Recovery outline for the Greenback Cutthroat Trout (*Oncorhynchus clarkii stomias*). U. S. Fish and Wildlife Service, Lakewood, Colorado.

Love-Stowell, S. M. 2016. Conservation of the Greenback Cutthroat Trout: from genomics and controlled crosses to educating scientists and stakeholders. Doctoral dissertation. University of Colorado, Boulder.

Rogers, K. B. 2007. A suggested protocol for collecting Cutthroat Trout tissues for subsequent genetic analysis. Colorado Division of Wildlife, Fort Collins.

Rogers, K. B. 2010. A suggested protocol for extending milt from male salmonids for use in wild spawn operations. Colorado Division of Wildlife, Fort Collins. Available: https://cpw.state.co.us/Documents/Research/Aquatic/CutthroatTrout/ExtendMiltProtocol.pdf (Accessed November 2023).

Rogers, K. B., Firestone, S., A. Whiteley, and P. M. Lukacs. 2020. Spawn matrixing fails to improve survival in a unique Cutthroat Trout population following fire-mediated extirpation in the wild. Pages 21-27 in K. Rogers, editor. Cutthroat Trout Studies, Colorado Parks and Wildlife Annual Research Report, Steamboat Springs, Colorado.

Rogers, K. B., J. R. Anderson, S. F. Brinkman, and A. P. Martin. 2022. Inbreeding depression reduces fitness in Colorado's last remaining Greenback Cutthroat Trout: consequences for management. Pages 185-194 in J. S. Gregory, editor. Proceedings of Wild Trout XIII Symposium: Reducing the gap between science and public opinion. West Yellowstone, Montana.

Withler, R. E. 1988. Genetic consequences of fertilizing Chinook Salmon (*Oncorhynchus tshawytscha*) eggs with pooled milt. Aquaculture 68:15– 25.

RESEARCH PRIORITY

Cutthroat Trout habitat conservation

OBJECTIVE

Preparing for reclamation: mapping the bathymetry of Lower Winchell Lake

INTRODUCTION

In an effort to reestablish native Rio Grande Cutthroat Trout (*Oncorhynchus clarkii virginalis*) across their native range (Alves et al. 2008), Colorado Parks and Wildlife (CPW) is assisting the Trinchera Ranch with reclamation efforts. One water that has attracted particular attention is Lower Winchell Lake for its ability to sustain a naturally reproducing population of introgressed nonnative Cutthroat Trout despite its high elevation (12,350 ft). Although not necessarily comprehensive, the stocking records show that 2,000 Pikes Peak Natives (Rogers and Kennedy 2008) were stocked in Lower Winchell Lake in 1978. An additional stocking event was scheduled nine years later to introduce 420 Snake River Cutthroat Trout (*O. clarkii behnkei*), though the Trinchera Ranch apparently cancelled that request (M. Japhet, personal communication).

Of particular concern is the presence of these now feral nonnative trout upstream of a core conservation population of Rio Grande Cutthroat Trout in Little Ute Creek. To protect the purity of this population from invasion from above, CPW would like to replace the population in Lower Winchell Lake with pure Rio Grande Cutthroat Trout, necessitating the removal of the current residents with the piscicide rotenone. Determining how much rotenone would be required for a successful reclamation is the first step in that process. The goal of this effort was therefore to map the bathymetry of Lower Winchell Lake and calculate surface acreage and volume at any surface elevation.

METHODS

We used a Humminbird model 597ci HD (Humminbird, Eufaula, Alabama) depth finder with a transducer deployed off the bow of an 8.5 lb pontoon boat (Model ODC 420 ULT, Creek Company, Steamboat Springs, Colorado) to acquire position and depth measurements every several seconds while using fins to propel the boat around Lower Winchell Lake at about 0.8 kmph on July 25, 2023. This unit draws 550 mA, and was powered with a lightweight 12v lithium iron phosphate 9.6 AH battery (K2 Energy Solutions, Inc, Henderson, Nevada) that provided adequate power for the four-hour survey. The transducer face was set 18 cm below the water surface, so all depth readings were increased by that amount. Surface elevation was recorded on prominent landmarks and a stake deployed with the stake surface aligning with the water surface on the survey date (Figure 2) so that volumes could be calculated for any future surface elevation. A perimeter

was established by walking a Forerunner 935 GPS watch (Garmin, Olathe, Kansas) around the shoreline recording waypoints every second, resulting in a single long track to define the wetted perimeter.

Figure 2: Surface elevation of Lower Winchell Lake during the survey on July 25, 2023 was flush with the top of a piece of rebar ring-stake pounded into the substrate next to a large boulder on the east shore of the lake near UTMx= 458324, UTMy= 4157981 (Zone 13).

All transect data was then exported to SD flash media then imported to HumminbirdPC software so that transect locations could be visualized in GoogleEarth to aid in culling erroneous data. Individual transects were copied from HumminbirdPC using the "Select all" and "copy" commands then pasted into Excel where false depths or those with erroneous position information (due to poor satellite coverage) were eliminated. The Excel depth and perimeter files were then converted to ASCII tab delimited text for download into custom code written in LabVIEW software titled DecimalMinutesToDecimalDegrees.vi that converts decimal minutes recorded in the Humminbird unit to decimal degrees. Decimal degrees were converted to UTMs with the "Dutch formulae" (T. Neebling, Wyoming Game and Fish), then pasted into the depth and perimeter spreadsheets for each lake. Individual depth readings were converted to a raster map with 2 m grid cells using additional custom code (GenerateRasterHumminbird.vi) that calculates the average depth recorded in each cell. GPS error was listed as 0.6 m during much of the survey, though values as high as 30m were documented at some points due to poor satellite orientation relative to the steep cliffs that circle the lake. Portions of transects affected by these anomalies were removed. Transducer face depth was set 18 cm (0.59 ft) below the lake surface which was added back to each depth reading, and the delta lake level was set to 0 m. Corners of the map for Lower Winchell Lake were set at WestUTMx= 458000, EastUTMx= 458450, SouthUTMy= 4157550 and NorthUTMy= 4157900. Two files were created: a perimeter file with depth = 0 feet, and a concatenated depth file with all remaining transects with depths rounded to the nearest foot increment (MapII GIS software can only handle integers). Output files were opened back up in Excel and converted to SYLK files (.slk) that were then opened in MapII. All maps were inverted

and rectified with the "Flip <map> vertically" operation. Command-I was used to get info on each layer and convert units to 2 m (adding the m is essential, as is selecting the m radio button). This revealed that the corners selected from the perimeter file obtained by the Garmin watch did not align with the UTM coordinates derived from the depth finder. To bring them into alignment, 52 m were added to every depth Easting, and 218 m were subtracted from every depth Northing. The perimeter point map defining the lake boundary was made continuous manually in Map II (this is important to keep deep water readings from invading shore when interpolated) and the perimeter value (obtained while walking the shoreline) was set to 0 in the legend with the Recode operation. The depth layer was covered with the perimeter layer, and the resulting map color reset to multichrome (Color → Color Sequence → Multichrome). This new map was interpolated by octants with a mask of the lake perimeter, weighting the nearest point within an octant by its distance from the target depth to be estimated. In order for the perimeter mask to function, it had to be filled in manually so that all points within the lake were non-void cells. The Cover command was used to join the perimeter file with the depth transects rather than Combine, as Combine will simply compress the legend to eliminate depths that have no values. I used the Page Setup function in MapII to adjust the size of the map, to include a scale and vertical legend (small), then exported to PICT Version2. This file was ungrouped in Aldus Superpaint so that the position of the legend and scale could be changed, then regrouped and resaved as a PICT file. The PICT file was saved to a ZIP drive and imported into Microsoft Word as a picture. The map legend including raster counts for each depth strata was exported from MapII to an Excel file where surface area, mean and maximum depths, and volumetric measurements were calculated.

RESULTS & DISCUSSION

A total of 1185 depth measurements were recorded while running 15 transacts on Lower Winchell Lake on July 25, 2023 with transect placement indicated in Figure 3. The steep granite walls surrounding the lake reduced the number of satellites available to the GPS resulting in poor position information in a number of the transects. Two transects had to be discarded, as were portions of four more. Subsequent analysis was restricted to just 928 points from 13 transects.

Figure 3: Fifteen depth transects were run on Lower Winchell Lake and projected on Google Earth.

The perimeter mask revealed that the lake covers a surface area of 6.79 ha (16.77 acres). Lake mean depth is 6.92 m (22.7 ft), with a measured maximum depth of 17.80 m (58.4 ft). Volumetric estimates were generated from the interpolated map (Figure 4), displaying both metric and imperial units to facilitate reclamation effort planning. On July 25, 2023, Lower Winchell Lake held 469,746 m^3 (380.8 AF) of water. A formula that describes lake volume as a function of surface elevation was developed (Figure 5) to allow rapid estimation of lake volume at any elevation.

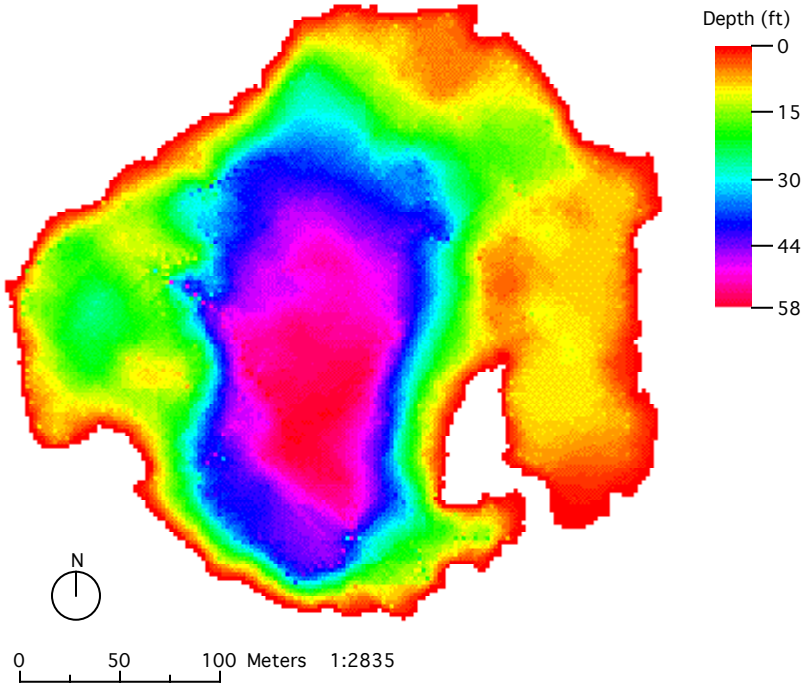

Figure 4: Thirteen depth transects obtained on Lower Winchell Lake were combined into a single file which was then covered by a file containing all perimeter points, then interpolated by

octants with a mask of the lake perimeter. The resulting bathymetric profile shows the average depth for each 2 m raster.

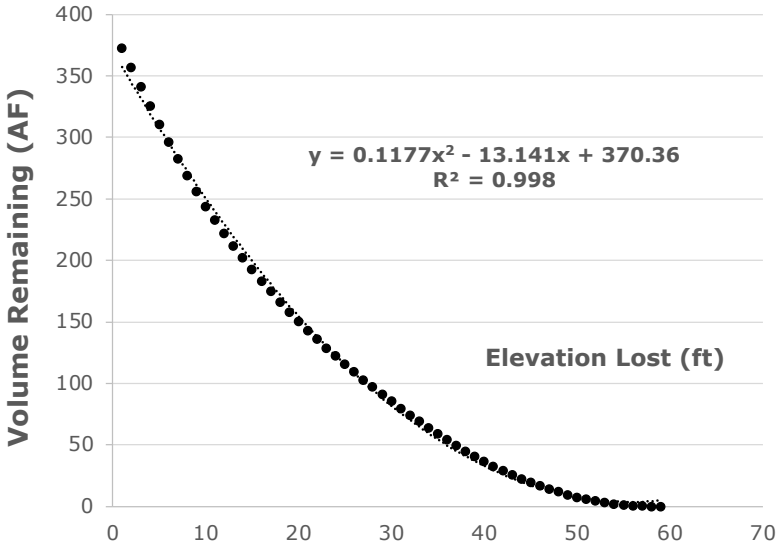

Figure 5: Total acre feet (AF) for every foot drop in lake elevation from our survey surface elevation was calculated, and fit with the second order polynomial shown. Results are expressed in imperial units to facilitate reclamation effort planning.

ACKNOWLEDGMENTS

I wish to acknowledge my collaborator Reece Samuelson, Trinchera Ranch biologist

REFERENCES

Alves, J. E., K. A. Patten, D. E. Brauch, and P. M. Jones. 2008. Range-wide status assessment of Rio Grande Cutthroat Trout (*Oncorhynchus clarkii virginalis*): 2008. Colorado Division of Wildlife, Fort Collins. Available: http://cpw.state.co.us/learn/Pages/ResearchRioGrandeCutthroatTrout.aspx (accessed November 2023)

Rogers, K. B., and C. M. Kennedy. 2008. Seven Lakes and the Pike's Peak native (PPN): history and current disposition of a critical cutthroat trout brood stock. Colorado Division of Wildlife report, Fort Collins. Available online at http://cpw.state.co.us/learn/Pages/ResearchGreenbackCutthroatTrout.aspx (accessed November 2023)

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

K. B. Rogers, J. R. Anderson, S. F. Brinkman, and A. P. Martin. 2022. Inbreeding depression reduces fitness in Colorado's last remaining Greenback Cutthroat Trout: consequences for management. Pages 185-194 *in* J. S. Gregory, editor. Proceedings of Wild Trout XIII Symposium: Reducing the gap between science and public opinion. West Yellowstone, Montana.

Abstract.— Recent molecular studies of historical and modern trout specimens from the southern Rocky Mountains revealed Colorado's state fish, the Greenback Cutthroat Trout *Oncorhynchus clarkii ssp.* (GBCT), was only represented by a single relict population. That revelation spawned an intense recovery effort to conserve the taxon. However, hatchery propagation has been complicated by poor survival of offspring. High prevalence of physical deformities and very low heterozygosity suggests what remains of the taxon may carry a high inbreeding load. Outcrosses with individuals from a sister subspecies in a common garden experiment showed a strong effect of genotype on fitness, doubling both survival and growth. Such pronounced hybrid vigor suggests genetic restoration by outcrossing may be a viable strategy for improving individual fitness against a background of inbreeding depression. This presents an interesting dilemma for managers, where the conservation focus has rightly been on repatriating pure individuals that best represent the trout that occupied these streams prior to European settlement. Unfortunately, this can mean the removal of extant robust populations of Cutthroat Trout in the South Platte basin that are native to western Colorado, and replacing them with the native trout that now appears compromised by reduced genetic diversity. With only a single source population of GBCT remaining, any efforts at genetic

rescue would necessarily involve outcrossing with a different taxon, intentionally introducing nonnative admixture which then fails to preserve what little remains of this lineage (extinction through hybridization). We advocate a multi-pronged strategy where the pure population is replicated in disparate locations to mitigate loss from stochastic events, but also promote genetic rescue for other populations using a closely related sister taxon to help reset the evolutionary trajectory of these fish should fitness deficiencies make establishing robust wild populations impossible.

K. B. Rogers, and B. W. Hodge. 2023. An inexpensive method for reliable recovery of stream temperature data. North American Journal of Fisheries Management 43:1349-1358.

Abstract.— Objective: Water temperature is perhaps the single most important environmental driver of fish populations. The strong relationship between fish and water temperature allows fisheries managers to make predictions about the influence of temperature on fishes under both current and future climatic conditions. These predictions are more robust if based on year-round and long-term data. However, water temperature data are commonly compromised or lost altogether when data-logging temperature sensors are damaged or go missing. In recognition of the need for reliable ways to collect long-term, year-round temperature data, we designed, implemented, and tested a durable but cryptic logger deployment and retrieval system.
Methods: We used metal housings and stakes to protect and anchor temperature loggers on the streambed and, when necessary, used a metal detector to assist with logger recovery. We then evaluated logger recovery rates across 12 years and 312 deployments at 85 sites in first- to ninth-order Rocky Mountain streams and rivers. Result: Although we recovered only 73% of loggers with traditional means of retrieval (e.g., GPS or photo), presumably owing to the inconspicuous nature of our metal housings and streambed anchor stakes, we recovered 96% of loggers when a metal detector was also used. Ordinal and binary logistic regression revealed that a metal detector was especially beneficial when trying to recover loggers from unfamiliar monitoring sites or those deployed for long periods of time (years).
Conclusion: Our methods could be replicated for a reliable and inexpensive approach to acquiring year-round stream temperature data.

Presentations *(chronological)*

Rogers, K. B. January 23, 2023. New molecular tools for managers: beyond purity and heritage assessments. Rio Grande Cutthroat Trout Conservation Team meeting, Trinchera Ranch, Colorado.

Henderson, R., B. Atkinson, B. Hodge, K. B. Rogers. March 2, 2023. Mechanical eradication of Brook Trout from a small, urban Rocky Mountain stream. Colorado Wyoming chapter of the American Fisheries Society, Fort Collins, Colorado.

Evans, R. P., K. B. Rogers, D. Shiozawa, A. Kokkonen. T. Van Orden. June 28, 2023. Cutthroat Trout - chromosome-level genome assembly, transcriptomes, and low coverage whole

genome sequencing. Coastal Salmonid Genetics Symposium, Boise, Idaho.

In Memoriam

Bruce D. Rosenlund
6/18/1946 - 8/31/2023

We lost an undisputed champion for native trout recovery this fall with the passing of Bruce Rosenlund following his battle with cancer. Born and raised in Salt Lake City, Utah, Bruce was first exposed to fish by his father and grandfather who took him fishing every Saturday. Those trips instilled a passion in him that never perished. After earning two Bachelor degrees in Fish and Wildlife Biology and Botany from the University of Utah in 1969, he was hired by the U. S. Fish and Wildlife Service (USFWS) as a biologist for Lake Mohave in their Bureau of Sport Fisheries and Wildlife program. His next post included a Certified Pathologist at the then-Leetown Science Center in Kearneysville, West Virginia. After spending a few years diagnosing fish diseases, Bruce moved back west to serve as a member of the Gila Trout Recovery Team while working as a Biologist at the Alchesay National Fish Hatchery in Whiteriver, Arizona. The prospect of more alpine skiing led him to Colorado in 1975 after securing the Assistant Hatchery Manager position at the Leadville National Fish Hatchery. In 1977, Bruce was promoted to Project Leader for the USFWS Colorado Fish and Wildlife Conservation Office, eventually supervising 30 employees in that position. This office works cooperatively with the U.S. Department of Defense, U.S. Geological Survey, U.S. Forest Service, National Park Service, National Wildlife Refuge System, and various State agencies to provide conservation, protection, and management of fish, wildlife and forest resources for the Mountain-Prairie Region.

It was here that Bruce's passion for native trout flourished, coinciding with the initial recovery effort for Greenback Cutthroat Trout – Colorado's state fish. Bruce completed dozens of piscicide reclamations that benefited Cutthroat Trout in Rocky Mountain National Park, pioneering new approaches using rotenone and antimycin to eradicate nonnative fish and accommodate the return of native taxa. His extensive experience recovering native species

spurred others around the country to request his services or seek his council when executing projects. Bruce's guidance implementing recovery efforts for a number of different fish taxa, bolstered his recognition as the world's leading expert on conducting aquatic reclamation projects. Over time, administrative duties and serving on (or leading) both Greenback Cutthroat Trout and the Preble's Meadow Jumping Mouse recovery teams pulled him further away from the field until his retirement in 2011, though that did not preclude him from leading the effort to secure a new broodstock of Bear Creek trout in 2008.

Fortunately, the State of Colorado was able to lure Bruce out of retirement in 2012 as a mentor supporting a new generation of biologists planning and implementing complex reclamation projects to benefit native species. Bruce would often quip that his 40-year career with the USFWS prepared him to be an Aquatic Technician for Colorado Parks and Wildlife (CPW). The advantage of an experienced veteran working alongside you is immeasurable. Thanks in part to Bruce, over the next decade, CPW biologists would advance Cutthroat Trout conservation efforts by conducting over 44 reclamation projects, a rarity for most state fish and wildlife agencies.

Bruce's legacy in aquatic conservation can be measured in the litany of publications and achievement awards he accumulated over his long and storied career including those from the U.S. Fish and Wildlife Service, Colorado Wildlife Society, Trout Unlimited, and Department of Interior. Most impressive perhaps is that he remains the only individual to receive the Award of Excellence from the Colorado/Wyoming chapter of AFS twice – a testament to his influence on multiple generations of biologists.

Bruce leaves behind his wife Karil Frohboese of Evergreen, Colorado and son Rob Rosenlund and family of Salt Lake City, Utah, along with an army of biologists he mentored who now possess the skills to carry on his life's work. All who cherish our native aquatic species owe a debt of gratitude to Bruce and his tireless efforts to preserve these priceless gems for future generations.

Kevin B. Rogers
kevin.rogers@state.co.us
Lori M. Martin
Greg Langer
Bill Atkinson
F. Boyd Wright
Harry Crockett
Chris Kennedy
Doug Krieger