Rediscovery of a lost Cutthroat Trout lineage in the San Juan Mountains of southwest Colorado.

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Kevin B. Rogers, Aquatic Research Group, Colorado Parks and Wildlife, P.O. Box 775777, Steamboat Springs, CO 80477, kevin.rogers@state.co.us.

Jim White, Colorado Parks and Wildlife, 151 East 16th St, Durango, CO 81301

Mike Japhet, Colorado Parks and Wildlife (retired), 151 East 16th St, Durango, CO 81301

Abstract

The discovery of a distinct lineage of Cutthroat Trout in museum specimens collected from the San Juan basin precipitated an intensive search for any remaining extant populations across the putative native range of this fish. Tissue samples from every known Cutthroat Trout population in the basin were assembled and analyzed with molecular methods. Of these, eight waters harbored Cutthroat Trout with mitochondrial DNA markers that placed them in the San Juan clade (a monophyletic lineage closely aligned with another Colorado River Cutthroat Trout lineage native to the headwaters of the Colorado, Dolores, and Gunnison rivers). Analysis of nuclear DNA amplified fragment length polymorphism markers also suggested they were distinct, with no evidence of introgressive hybridization with Rainbow Trout or Yellowstone Cutthroat Trout. We recommend that morphological studies be conducted on these same fish to evaluate if they can be distinguished with morpho-meristic traits as well. In this report we discuss support for considering these fish as a distinct unit of biodiversity worthy of conservation, as well as the current status of these eight populations.

Introduction

A recent study exploring mitochondrial DNA sequence data acquired from museum specimens of Cutthroat Trout collected in the late 19th century showed that six major monophyletic lineages (clades) occupied the state of Colorado prior to European settlement (Metcalf et al. 2012; Rogers 2012) rather than just three subspecies as previously thought (Behnke 1992, 2002). These clades aligned with major drainage basins that were not linked by coldwater confluences (Figure 1). That study and one that followed examining morphological features (Bestgen et al. 2013) suggested that four clades could still be found on the landscape today. Two are found west of the Continental Divide in what is currently Colorado River Cutthroat Trout (CRCT) habitat in the Green, White, and Yampa River drainages (blue lineage), or the headwaters of the Colorado, Gunnison, and Dolores Rivers (green lineage). The remaining two extant clades are found east of the Divide in the South Platte and Rio Grande River drainages. The Yellowfin Cutthroat Trout that historically occupied the headwaters of the Arkansas River appears to have gone extinct by 1903 just 17 short years after its discovery (Juday 1906). A sixth clade was detected by Metcalf et al. (2012) from a pair of specimens collected by C. E. Aiken from the San Juan River near Pagosa Springs in 1874. Mitochondrial sequence data did not match any of the extant populations examined, and was therefore also presumed extinct by the authors.

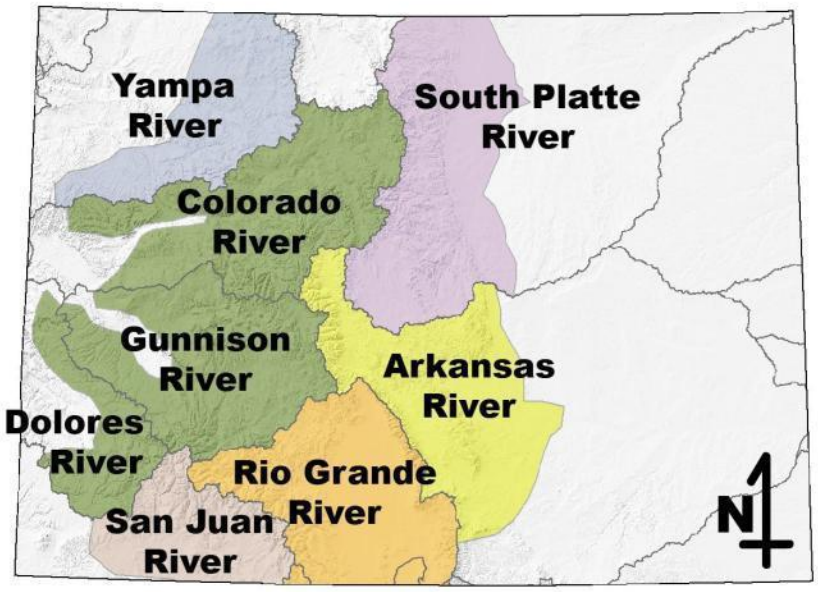

Figure 1: Historical range of six major Cutthroat Trout clades native to Colorado are organized by major drainage basins that do not share coldwater confluences.

An intensive survey effort was launched to confirm that this clade was no longer present on the landscape. Isolated DNA was gathered either from earlier collections in the basin or from new tissue samples (fin clips). Mitochondrial DNA sequence data were obtained, and compared to Aiken's specimens for evidence of haplotypes that would place them in the San Juan clade. Here we report on populations that do share mitochondrial NADH dehydrogenase subunit 2 (ND2) locus haplotypes with Aiken's fish and should therefore be considered for further study. With new samples to examine, we explore the evidence that supports recognizing the San Juan clade as a discrete taxonomic entity worthy of conservation focus, and discuss population characteristics that will help inform management actions designed to secure these remaining small fragmented populations.

Methods

Molecular testing

A survey of Cutthroat Trout conservation waters in the San Juan basin (Hirsch et al. 2013) revealed 20 candidate populations from which fin clips or previously isolated DNAs were obtained. Cutthroat Trout DNA was extracted from fin clips using a proteinase K tissue lysis and spin-column purification protocol following the manufacturers specifications (Qiagen DNeasy, Hilden, Germany). An aliquot of each sample DNA was amplified using primers specific to a region of the ND2 mitochondrial gene in Cutthroat Trout, generating a 648 bp fragment that falls within the same fragment examined in previous studies (Metcalf et al. 2007; Rogers et al. 2011; Loxterman and Keeley 2012; Metcalf et al. 2012). After amplification, residual primers and deoxynucleotides were removed or inactivated. Fluorescently-labeled DNA sequences in the forward and reverse direction for each sample were generated. After sequencing reactions were completed, unincorporated fluorescently labeled nucleotides were removed according to the manufacturer's instructions (Rogers et al. 2011). Samples were run on a capillary sequencer (3130 Genetic Analyzer, Applied Biosystems, Foster City, California). Sequence reads generated from the forward and reverse strands of each sample DNA were assembled using the Contig Express program (Vector NTI 11, Invitrogen, Carlsbad, Caliornia). The assembled contiguous sequence chromatograms were examined for sequence quality and accuracy, and the primer sequences removed from the ends of the fragments. Sequences were aligned in MUSCLE (Edgar 2004) and compared to the suite of genetic diversity found in the NCBI database (Metcalf et al. 2007, 2012; Pritchard et al. 2009; Loxterman and Keeley 2012) and elsewhere (Rogers et al. 2011; Bestgen et al. 2013; Rogers et al. 2014) using MEGA7 (Kumar et al. 2016).

Examination of the nuclear genome was explored with Amplified Fragment Length Polymorphisms (AFLPs; Rogers 2008a; Rogers 2012). AFLP marker fragments were generated using restriction digested DNA (EcoR1 and MseI) and a single pair of +3 PCR primers (ACT for the FAM-labeled forward primer; CAG for the reverse primer). Fragments were separated and sized on an ABI 3130 DNA sequencer (Applied Biosystems, Foster City, California). Using the program Genemapper 4.0 (Applied Biosystems), a genetic fingerprint was produced for each individual by scoring for the

presence or absence of a standardized set of 119 markers between 50 and 450 base pairs in size generated from reference Cutthroat Trout populations (Table 1; Rogers 2008a, 2012). The genetic signature of individuals in the test population were compared to those found in the reference populations using a Bayesian approach for identifying population clusters (Pritchard et al. 2000). Reference populations were selected and grouped by their mtDNA lineage (Metcalf et al. 2007), and not necessarily by geographic or historic subspecies classifications. The similarity or dissimilarity was scored as the admixture proportion, or the probability that each test individual shares a genetic background with each of the cutthroat subspecies reference population groups with the program STRUCTURE 2.3.4 (Falush et al. 2007; Pritchard et al. 2007) and expressed as q values for each subspecies. Average q values from the run with the highest log likelihood (Pritchard et al. 2007) were used to generate the admixture proportions for the unknown population, with confidence intervals generated in program QSTRAP (Rogers 2008b).

Table 1.— Amplified fragment length polymorphisms were used with Program STRUCTURE to assess relatedness and purity of Cutthroat Trout populations in the San Juan basin, Colorado. Reference populations included both lineages of Colorado River Cutthroat Trout (CRCT, blue and green), Rio Grande Cutthroat Trout (RGCT), Yellowstone Cutthroat Trout (YSCT), and Rainbow Trout

Trout lineages	Water	County	Water Code	Collection Date	Sample Size
CRCT - Blue	Williamson Lake (#3)	Inyo	NA	07/31/06	22
	Piedra, E Fk	Hinsdale	42096	02/07/06	20
	Slater Crk, S Fk	Routt	23286	NA	14
	Parachute Crk, E Fk	Garfield	21460	NA	10
CRCT - Green	Severy Creek	El Paso	31312	NA	10
	Antelope Crk, W	Gunnison	48016	02/21/03[e]	21
	Bobtail Creek	Grand	23026	09/03/03	19
RGCT	Canones Creek	Rio Arriba	329	03/29/06	19
	Columbine Creek	Taos	1026	09/17/02	20
	Osier Creek	Conejos	44444	09/22/04	11
	Cuates Creek	Costilla	38141	07/25/05	10
YSCT	Dog Creek	Teton	813220	06/28/01	20

	Willow Creek	Teton	813350	10/26/02	14
	Yellowstone River	Park	TenSleep	03/01/05	12
Rainbow Trout	Colorado River	Grand	21298	NA	10
	Bellaire	Garfield	RifleFalls	03/06/08	9
	Eagle Lake	Garfield	RifleFalls	03/06/08	9
	Erwin	Garfield	RifleFalls	03/06/08	9
	Fish Lake	Garfield	RifleFalls	03/06/08	9
	Kamloops	Garfield	RifleFalls	03/06/08	9
	Tasmanian	Garfield	RifleFalls	01/12/08	9

At this time, the San Juan lineage is defined only from a pair of museum samples whose DNA is so degraded that they cannot serve as a reference population for our standard AFLP test. Instead, we explored AFLP markers from extant candidate populations in program STRUCTURE compared to the reference Cutthroat Trout without using prior population information (no reference populations). We used a burn-in of 10,000 and a MCMC of 20,000, while allowing K to increment from 3 to 7 over 10 iterations each to help isolate genetic structure where it exists. Again, the run with the highest log likelihood was used for subsequent analysis and plotting. The same 119 AFLP loci were examined further with principal coordinate analysis as implemented in GenAlEx 6.502 (Peakall and Smouse 2012) so that major patterns within the multivariate data could be explored. A pairwise Nei's genetic distance matrix was calculated from binary (diploid) allele calls as is appropriate for dominant markers like AFLPs. The resulting table was then plotted over principal coordinate space and the amount of variation explained by the plotted axes was recorded.

Results

We recovered mitochondrial (ND2) haplotypes that matched museum specimens collected by C. E. Aiken from the Pagosa River in 1874 from eight waters in the San Juan River basin (Figure 2; Table 2). Only San Juan clade haplotypes (Figure 3) were recovered from these populations (no evidence of nonnative salmonid admixture in the mitochondrial DNA). Seven of the eight populations shared the same haplotype while the last also harbored a single base pair variant. These haplotypes suggest that the San

Juan lineage is most closely related to the green lineage CRCT (Figure 3), with half the genetic distance between the San Juan and green lineage CRCT as compared to the blue lineage CRCT and the Greenback Cutthroat Trout of the South Platte River basin (Table 3).

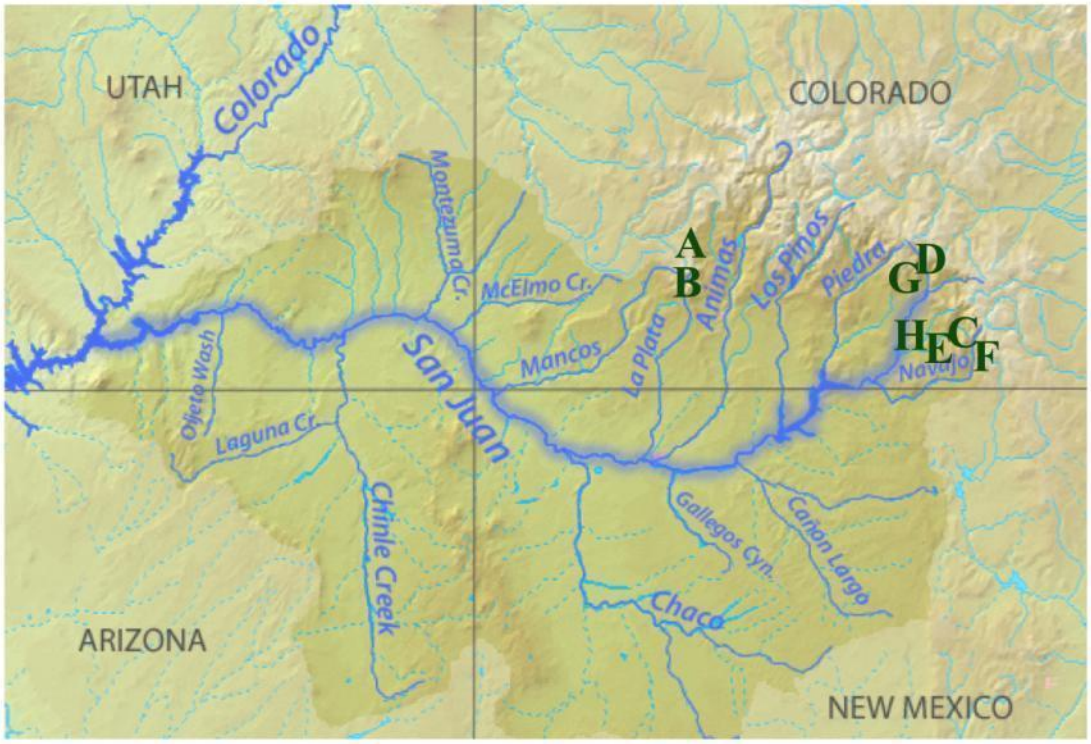

Figure 2: Eight populations of Cutthroat Trout in the San Juan River basin harbor haplotypes characteristic of San Juan basin native. Letters correspond to populations identified in Table 2 below.

Table 2: Eight populations of Cutthroat Trout in the San Juan River basin harbor haplotypes characteristic of San Juan basin native. Elevation (m), of fin collection locations and associated latitude and longitude (decimal degrees) are provided. .

Water	Legend	Water Code	Elevation	Latitude	Longitude
Big Bend Creek	A	47325	2733	37.6	-108.0
Clear Creek*	B	47565	2643	37.5	-107.9
Cutthroat Creek	C	39415	2560	37.1	-106.7
Fall Creek	D	38117	2493	37.4	-106.9
Grayhackle Lake	E	96457	2796	37.1	-106.7
Headache Creek	F	39491	2466	37.1	-106.7
Himes Creek	G	39502	2437	37.4	-106.9
Rio Blanco River	H	38439	2605	37.3	-106.7

*Founded from Big Bend population in 1989

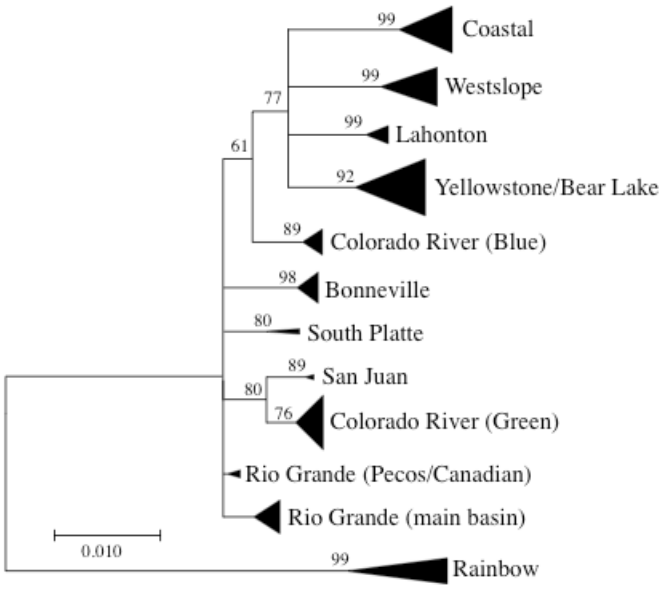

Figure 3: One hundred twenty six nucleotide sequences covering the mitochondrial NADH subunit 2 gene in Cutthroat Trout from across their range were obtained from GenBank and unpublished sources, and compared to 10 Rainbow Trout

sequences. Phylogenetic relationships were inferred with the Minimum Evolution (ME) method as implemented in MEGA7 after all sequences were trimmed to 648 common base pairs. Percent branching support was evaluated with 500 bootstrap replicates and branches with less than 60% were collapsed into polytomies. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. The tree was searched using the Close-Neighbor-Interchange algorithm at a search level of one. The neighbor-joining algorithm was used to generate the initial tree.

Table 3.— Sequence divergence in a portion (648 base pairs) of the NADH subunit 2 gene comparing the San Juan lineage to Rainbow Trout and other Cutthroat Trout clades found in the southern Rocky Mountains using the Maximum Composite Likelihood method.

Clade	**$CRCT_{SJ}$**	$CRCT_G$	$CRCT_B$	GBCT	RGCT	BCT
San Juan ($CRCT_{SJ}$)						
Green ($CRCT_G$)	**0.011**					
Blue ($CRCT_B$)	**0.021**	0.024				
Platte (GBCT)	**0.018**	0.018	0.020			
Rio Grande (RGCT)	**0.013**	0.018	0.018	0.014		
Bonneville (BCT)	**0.021**	0.023	0.022	0.022	0.017	
Rainbow (RBT)	**0.081**	0.083	0.083	0.080	0.074	0.087

Interestingly, results from the standard AFLP tests on the six populations with large enough sample sizes for a reliable test suggest they align with the blue rather than green lineage CRCT (Figure 4) when forced to select between one of five reference

groups (Table 1) despite mitochondrial DNA that suggests a much closer relationship to the green lineage (Figure 3, Table 3).

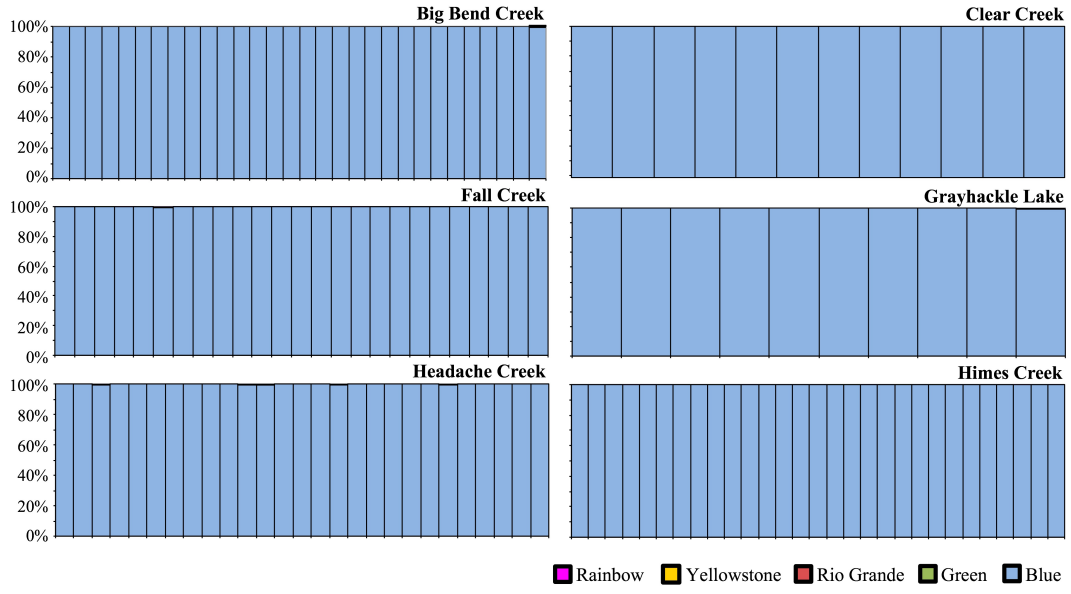

Figure 4: Individual admixture proportions (each bar represents a fish) measured by STRUCTURE with nuclear AFLP markers using K=5 and Rainbow Trout, Yellowstone Cutthroat Trout, Rio Grande Cutthroat Trout, green lineage CRCT and blue lineage CRCT as reference groups (prior information), suggest an alignment with blue lineage CRCT.

With lack of symmetry between nuclear and mitochondrial DNA results, we elected to explore the same 119 AFLP markers further in a principal coordinate framework to see why the San Juan fish might be aligning with blue lineage CRCT. The first two principal coordinates explain 38% of the variation in the data, and clearly suggest why blue lineage was selected over green when STRUCTURE was coerced into choosing between the two (Figure 5). With that information, it became clear that we should run the AFLP marker data through STRUCTURE without using any prior population information, and allowing K to vary from 3 to 7 groups. Surprisingly, San Juan fish appeared to separate from other Cutthroat Trout of the southern Rocky

Mountains at K=3, even before blue lineage CRCT and green lineage CRCT were distinguished (Figure 6). This result is unexpected since the markers used in the development of the standard AFLP test were selected for their ability to distinguish the two. This marker set has already been shown to perform poorly at distinguishing blue lineage CRCT and Rio Grande Cutthroat Trout (Rogers et al. 2011), so it was not unexpected that they did not separate until K=5 (the expected number of groups). Noteworthy was that at K=6 (more than the expected number of groups), STRUCTURE elected to parse out two San Juan clusters, rather than splitting out Rio Grande Cutthroat Trout from the Canadian River basin that have already been identified as unique (Pritchard et al. 2009), and appear to be more distinct than the San Juan fish in principal coordinate space (Figure 5).

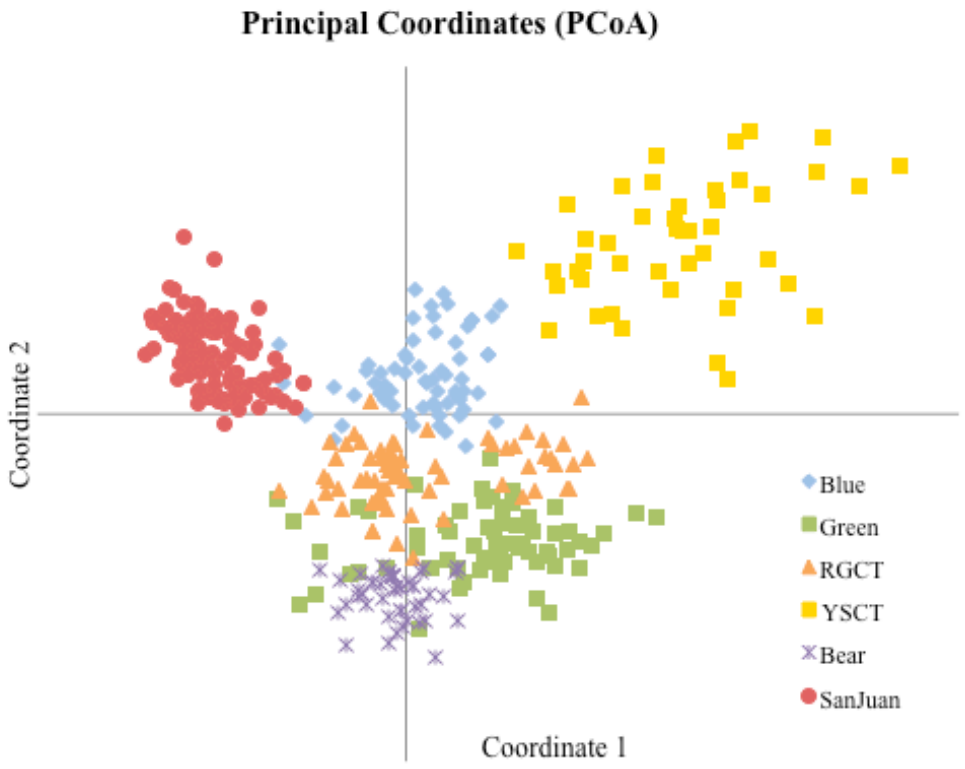

Figure 5: Samples from six putative San Juan lineage populations were included with 50 fish from Bear Creek and the standard AFLP reference fish in a principal

coordinates analysis (GenAlEx 6.5). Results of the first two principal coordinates are plotted with colors representing Cutthroat Trout lineages.

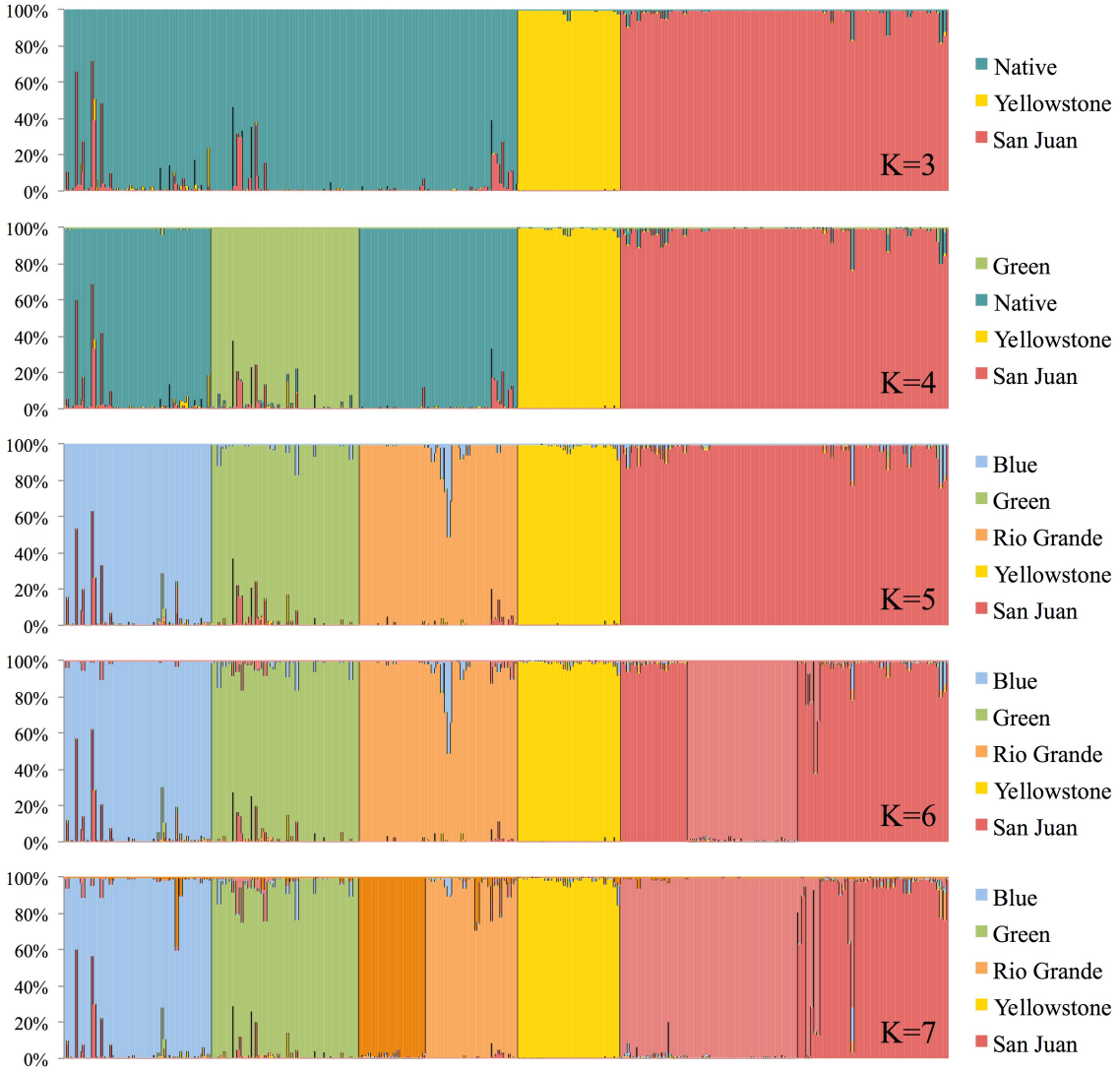

Figure 6: Individual admixture proportions (each bar represents a fish) as measured in STRUCTURE with 119 AFLP markers and no prior population information. Colors correspond to the various Cutthroat Trout lineages considered, and K ranged from 3-7. We use the generic term "Native" to describe clusters that cover more than one lineage.

Discussion

The discovery of admixed fish containing the San Juan mitome allowed for sequencing of the standard 648 bp covered in many other studies of extant Cutthroat Trout populations (Pritchard et al. 2009, Loxterman and Keeley 2012, Bestgen et al. 2013, Rogers et al. 2014) rather than no more than 388 ND2 base pairs explored in the museum study (Metcalf et al. 2012). A close affiliation with green lineage CRCT was revealed when 648 base pairs of the ND2 gene were compared to other native Cutthroat Trout (Figure 3), though the San Juan fish still comprise a monophyletic clade displaying roughly half the genetic distance (1.1%) observed between other Cutthroat Trout lineages across the southern Rocky Mountains (Table 3)

When DNAs were obtained from Cutthroat Trout in the San Juan basin were initially screened with nuclear AFLP markers using our standard reference populations, all fish aligned with blue lineage CRCT. It was assumed that these populations were among the many blue lineage CRCT populations founded across the state of Colorado by early undocumented stocking of pure CRCT produced at Trappers Lake between 1903 and 1938. These populations did not attract further interest until sequencing of their mitochondrial DNA revealed a match with museum specimens collected by Aiken from the San Juan River in Pagosa Springs, Colorado in 1874. This finding precipitated further exploration of the nuclear AFLP data. Since no confirmed reference populations were included in our AFLP analysis, STRUCTURE was coerced into selecting the "best fit" from the existing reference groups. Examination of the principal coordinate plot (Figure 5) not only helps explain why blue lineage CRCT were selected over other lineages, but demonstrated that, at least with the AFLP loci used, nuclear DNA also suggested that the San Juan lineage of Cutthroat Trout are a discrete entity worthy of conservation. To examine if STRUCTURE would also support that assessment, AFLP data were reanalyzed in STRUCTURE without using prior information (reference groups). Even at K=3 the San Juan lineage fish distinguished themselves from other Cutthroat Trout of the southern Rocky Mountains (Figure 6).

Our findings are consistent with earlier studies focused on evaluating admixture with Rainbow Trout and Yellowstone Cutthroat Trout using starch gel protein electrophoresis (Kanda et al. 2000). These authors recorded genetic variation in 16 of 41

loci examined among 24 CRCT populations. They used 10 loci to diagnose admixture with Rainbow Trout and three loci for evaluating admixture with Yellowstone Cutthroat Trout. It is noteworthy that these 16 loci with variable allele frequencies did not separate the one San Juan lineage fish (Headache Creek) from four blue lineage populations (East Fork Piedra River, Lake Nanita, Northwater Creek, Trapper Creek) but did distinguish them from six green lineage populations (Roan Creek, Antelope Creek, Hubbard Creek, Dyke Creek, Little Taylor Creek, and Rio Lado) at the *bGLUA* locus.

Finally, the same DNAs obtained from Headache Creek were shared with the Thorgaard Lab at Washington State University to be included in a Cutthroat Trout rangewide phylogeny developed around the OmyY1 gene near the sex-determining area of the paternally inherited Y-chromosome (Brunelli et al. 2013). Despite 10 CRCT populations being included, only three haplotypes were recovered. This is not an unexpected result since the OmyY1 region evolves 3-13 times more slowly than mitochondrial genes (Brunelli et al. 2013). In fact, several other subspecies (e.g. Lahonton Cutthroat Trout, Coastal Cutthroat Trout, Yellowstone Cutthroat Trout, and Rio Grande Cutthroat Trout) were represented only by a single haplotype (Brunelli et al. 2013). Of the three CRCT haplotypes recovered, one was found across all blue and most green lineage populations, one was found in Roan Creek (a green lineage population), and an additional private haplotype found only in Headache Creek (San Juan lineage). A broader survey of the OmyY1 gene across many more populations is warranted to determine if this marker is diagnostic for the native fish of the San Juan basin.

As genomic resources provide ever-increasing power for detecting finer-scaled genetic structure, we must be leery of equating population structure with species boundaries (Carstens et al. 2013; Sukumaran et al. 2017). If the diagnostic phylogenetic species concept is used to delineate species, great harm could accrue to small isolated populations subjected to inbreeding depression and genetic drift that are no longer considered as candidates for genetic rescue because they are now a putative species (Frankham et al. 2012). Structure revealed by modern molecular methods should only serve as tentative hypothesis of species boundaries (Sukumaran et al. 2017) that should subsequently be tested. Other classes of data (e. g. morphological or ecological) should be used to correctly attribute elements of genetic structure to either species or population-

level processes (Sukumaran et al. 2017). Frankham et al. (2012) conclude that the diagnostic phylogenetic species concept is unsuitable for classifying allopatric populations in particular and for conservation in general.

Whether the San Juan lineage of CRCT represents a discrete taxonomic entity has been debated (Rogers et al. *in press*). Regardless of what we choose to call the native trout of the San Juan River basin, it represents a unit of diversity worthy of conservation (Funk et al. 2012; Rogers et al. 2014; Rogers et al. *in press*) as well as a conservation success story. We are fortunate those who came before adhered to fundamental conservation principles – specifically, preserving all of the pieces, even when they were unaware of the molecular diversity harbored by these fish. While biologists in the 1980s and 1990s had no way of knowing the unique molecular structure hidden in these rare trout, they recognized that with imperfect tools the prudent action was to manage with basin specific stocks, even when morphological trait differences could not obviously be detected. Above all, they focused on securing what populations were left. Now, armed with powerful molecular tools, it is clear how fortunate we are to have these remaining pieces of this evolutionary legacy to manage.

While the rediscovery of what appear to be San Juan lineage Cutthroat Trout presents some exciting opportunities for preserving the legacy of Colorado's native trout, these fish will require some anthropogenic assistance if their future is to be secure. Extant populations are small, with perhaps as few as 1000 fish remaining in aggregate. They occupy just 14.9 km of isolated headwater habitat, with the longest contiguous piece being only 3.8 km. Though protected by natural or man-made barriers, all are vulnerable to drought, fire, and flooding. Fish management histories for each population are detailed in Appendix 2 and include population and genetic surveys, as well as temperature profiles where available. Opportunities for near-term conservation actions include additional survey work, as well as building barriers to protect against nonnative invasions, chemical reclamations, translocations, developing broodstocks, and protecting in-stream flows. The demonstrated track record of successful conservation efforts by the CRCT Conservation Team suggests that all of these actions are reasonable and have been implemented with good success elsewhere across the range of CRCT. We feel fortunate

that this remnant diversity has been identified so that appropriate conservation measures can be enacted to secure the future of these fish in Colorado.

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Appendices

Appendix 1

Sequence data representing 648 base pairs of the mitochondrial NADH subunit 2 gene from extant trout populations in the San Juan drainage that describe the two San Juan haplotypes recovered.

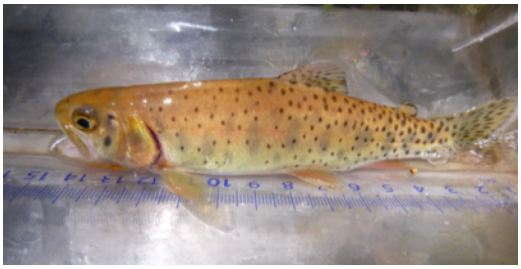
OcCOL-Tabegauche (H-106463)
TAATGTTAGCCCTCGCCCTCAAGCTTGGACTAGCACCCGTTCACTTCTGACTC
CCAGAAGTTCTTCAAGGACTTGAACTCACCACAGGATTAATCCTCTCAACCTG
ACAAAAACTCGCACCCTTCGCACTTATAATTCAAACAGCCCCAACCATCAAC
TCTTCCCTACTTATCGCAATCGGCCTTCTATCAACACTTGTGGGGGGTTGAGG
TGGGCTTAATCAAACCCAACTGCGTAAAATTCTAGCATATTCTTCAATTGCCC
ACCTAGGATGAATAGTACTAATTTTACAATTCGCACCCTCCCTCACACTCCTC
AGCCTATCCCTGTATATTATCATAACATCTTCAGCATTCCTCACATTAAAAAC
CAACAACGCTTTAACCATCAACACTCTCGCGACTTCATGAACTAAATCCCCG
ACCCTTGCCACATTAGCCGCTCTTGTACTGCTGTCCCTTGGGGGTCTCCCCCC
TCTCTCAGGCTTTATACCAAAATGACTTATTTTGCAAGAACTAACAAAGCAAG
GACTCCCGCTATCTGCCACACTAGCTGCTATAACAGCCCTCCTGAGCCTTTAC
TTTTATCTACGACTCTGCTACGCCTTAACCCTCACTATTTATCCCAACACCCTA
ACTGCCACTGCCC

OcCOL-Cutthroat (CUT-126486)
TAATGTTAGCCCTCGCCCTCAAGCTTGGACTAGCACCCGTTCACTTCTGACTC
CCAGAAGTTCTTCAAGGACTTGAACTCACCACAGGATTAATCCTCTCAACCTG
ACAAAAACTCGCACCCTTCGCACTTATAATTCAAACAGCCCCAACCATCAAC
TCTTCCCTACTTATCGCAATCGGCCTTCTATCAACACTTGTGGGGGGTTGAGG
TGGGCTTAATCAAACCCAACTGCGTAAAATTCTAGCATATTCTTCAATTGCCC
ACCTAGGATGAATAGTACTAATTTTACAATTCGCACCCTCCCTCACACTCCTC
AGCCTATCCCTGTATATTATCATAACATCTTCAGCATTCCTCACATTAAAAAC
CAACAACGCTTTAACCATCAACACTCTCGCGACTTCATGAACTAAATCCCCG
ACCCTTGCCACATTAGCCGCTCTTGTACTGCTGTCCCTTGGAGGTCTCCCCCC
TCTCTCAGGCTTTATACCAAAATGACTTATTTTGCAAGAACTAACAAAGCAAG
GACTCCCGCTATCTGCCACACTAGCTGCTATAACAGCCCTCCTGAGCCTTTAC
TTTTATCTACGACTCTGCTACGCCTTAACCCTCACTATTTATCCCAACACCCTA
ACTGCCACTGCCC

Appendix 2 – Populations of interest

A water by water summary of population characteristics relevant to the management of Cutthroat Trout populations in the San Juan River basin that harbor mitochondrial DNA native to the drainage. Colorado Parks and Wildlife (CPW) water codes (WC), CRCT Conservation Population ID's (from Hirsch et al. 2013), and length of occupied habitat in that same database are presented for each water.

Big Bend Creek (WC#47325)
CRCT Conservation Population ID: 14080104cp002
Occupied habitat: 3.2 km



Cutthroat Trout from Big Bend Creek

Big Bend Creek is a tributary to Hermosa Creek in the Animas River Basin. First surveyed in 1987, both hybridized and non-hybridized Cutthroat Trout were noted. In 1989, a 4.5 m bedrock cascading barrier was located, isolating only Cutthroat Trout upstream. With a severe drought that same summer, managers worried that the small population in Big Bend Creek might not persist, so 204 individuals were transplanted from the headwaters of Big Bend Creek to Clear Creek, another tributary of Hermosa Creek. These fish were placed above a 12 m waterfall barrier in the barren headwaters of Clear Creek. It is worth noting that a follow up survey in 1991 (Table A2-1) recovered no fish in Big Bend or Clear Creek. Managers assumed low water conditions over the winter resulted in the extirpation of both populations. Fortunately, subsequent surveys demonstrate these fish are resilient to drought conditions (Table A2-1), and both populations still persist today.

Table A2-1: Survey history and results for the Cutthroat Trout population in Big Bend Creek. Population estimates (adult fish per km) were based on the number of fish captured (n) that exceeded 150 mm over the surveyed station length (m).

Year	Date	n	Length (m)	Fish per km	Comments
1987	Jul 13	51	152	248*	First survey
1989	Jun 13	10	46		Barrier confirmed; fish salvage
1991	Aug 27	0	152		Half mile above barrier; no fish
1995	Oct 19	59	152	119	Half mile above barrier
2000	Aug 24	20	91		Taxonomy collection
2004	Jul 26	22	152	39	Half mile above barrier
2007	Jun 20	68	152	59	At barrier (9 fish >150mm)
2014	Jun 6	6	152	25*	Only single fish>150mm

* Single pass survey

Water temperatures were monitored in the summer of 2014 (May 10 – September 26th). The stream appears to be in no imminent threat of critically warm temperatures, with an MWMT = 14.5 °C with a daily maximum temperature (Todd et al. 2008; Rogers 2015) of 16.1 °C. A M30AT of 11.5 °C suggests that the stream should support robust recruitment (Coleman and Fausch 2007; Roberts et al. 2013), but that growth will be somewhat compromised as an M30AT near 15 °C is usually required for maximizing tissue elaboration in Cutthroat Trout (Bear et al. 2007; Ziegler et al. 2013).

Molecular surveys:

Ten Cutthroat Trout were collected by Mike Japhet on August 24, 2000 from Big Bend Creek and sent to Dr. Robb Leary at the University of Montana for analysis. Although they looked to be pure CRCT by PINES (Paired Interspersed Nuclear Elements), electrophoretic analysis results were less certain. One of three loci allegedly diagnostic for distinguishing CRCT from Yellowstone Cutthroat Trout (Leary 2002) showed Big Bend fish containing alleles characteristic of both taxa. In light of molecular findings from this report, it is certainly possible that the sMEP-1 locus is simply not a diagnostic marker for all CRCT populations.

On June 20, 2007, Jim White collected another 30 fish from three locations above the waterfall barrier for AFLP analysis. These all aligned with blue lineage CRCT when using the standard AFLP reference populations (Rogers 2008a). Twenty of these same DNAs were subsequently sequenced at the ND2 mitochondrial gene and all were found to exhibit the common San Juan lineage haplotype.

Clear Creek (WC#47565)
CRCT Conservation Population ID: 14080104cp001
Occupied habitat: 2.7 km

Cutthroat Trout from Clear Creek (archived)

Clear Creek is a tributary to Hermosa Creek, just south of Big Bend Creek and within the Animas River Basin. Clear Creek was barren of fish above a 12 m waterfall before 204 Cutthroat Trout were transplanted from nearby Big Bend Creek in 1989 with a helicopter. After no fish were captured in 1990 it was thought that the transplanted fish perished because the lower section of Clear Creek went dry. However, a 1996 survey discovered the population above the waterfall was in good condition with moderate densities of fish (140 fish/km). Below the waterfall, it is not unusual in dry years for sections of the creek to go sub-surface only to emerge near the confluence with Hermosa Creek.

A population survey conducted June 17, 2014 yielded 35 Cutthroat Trout ranging in size from 60-240 mm over two removal passes. The estimated density of trout in the 152 m reach of stream was 112 fish/km, with fish displaying robust relative weights despite post-spawn condition (mean Wr =106%). Twelve fish were preserved individually in 10% formalin for phenotypic evaluation and archiving at the Larval Fish Laboratory, Colorado State University, Fort Collins, Colorado.

Water temperatures were recorded hourly in Clear Creek from mid May through September of 2014. The maximum daily temperature (Todd et al. 2008) was 15.8 °C, while the MWMT for that summer was 14.0 °C. Clear Creek is a cold stream with M30AT of only 10.9 °C, well below the optimum for growth (Bear et al. 2007; Ziegler et al. 2013), but enough to register consistent recruitment (Coleman and Fausch 2007; Roberts et al. 2013).

Molecular surveys:

Fin clips from 12 fish from the 2014 survey effort were preserved in 80% ethanol and submitted to Pisces Molecular (Boulder, Colorado) for AFLP analysis. Using our standard reference groups (Rogers 2008a), these fish (like the ones from Big Bend Creek) aligned with blue lineage CRCT. Subsequent sequencing of 648 base pairs from the ND2

mitochondrial gene revealed that they too all harbored the common San Juan lineage CRCT haplotype.

Cutthroat Creek (WC#39415) and Grayhackle Lake (WC#96457)
CRCT Conservation Population ID: 14080101cp005
Occupied habitat: 3.6 km and 1.4 ha

Cutthroat Trout from Grayhackle Lake

Cutthroat Creek is a small tributary to the Navajo River located on the eastern side of the Upper San Juan River Basin within the Banded Peak Ranch. Dolomite and Grayhackle lakes form the headwaters of Cutthroat Creek. The stream and lakes are protected near the confluence with the Navajo River by a 2 m high irrigation diversion structure. The ranch manager reinforced this diversion as a barrier to fish migration in 2016. There is no record of fish being stocked in Grayhackle or Cutthroat Creek, though the lake is accessible via a steep 4-wheel drive logging road. Rick Lapin, the old Banded Peak Ranch manager, reported that he caught "Rio Grande Cutthroat Trout" from Grayhackle Lake. It was later learned that the previous ranch manager, and former Colorado Division of Wildlife officer (Judd Cooney), claimed he stocked Yellowstone Cutthroat Trout in Grayhackle Lake in the late 1970s. In 1998, Mike Japhet discovered an old abandoned automatic fish feeder at the lake and evidence that the spillway was once dammed up with plastic sheeting to deepen the water. Grayhackle Lake has a maximum depth of 8 feet and an average depth of only 5 feet. There is ample spawning habitat in the inlet of the shallow lake and numerous fry were observed during the 1998 fish survey.

Molecular surveys:

The stream was first surveyed on June 20, 1998. Ten trout were collected for meristic and DNA analysis and a population estimate was generated for occupied habitat just above the barrier. Fish density was estimated to be 52 fish/km over 150 mm (total length) with many smaller fish in the survey. If the minimum size threshold is relaxed to 100 mm, the estimate rises to 191 fish/km. The 10 fish samples were sent to Dr. Robb Leary at the University of Montana for electrophoretic analysis. Although rainbow trout alleles were detected at two of ten putatively diagnostic loci (Leary 2002), he suggested they likely represented genetic variation previously unknown in CRCT populations, and therefore not diagnostic. A second collection of 40 fish from the middle and upper

reaches of Cutthroat Creek occurred on July 18 of 2002, and were sent to Dr. Dennis Shiozawa at Brigham Young University for analysis. These samples appeared to be pure CRCT with no evidence of Rainbow Trout or Yellowstone Cutthroat Trout introgression (Evans and Shiozawa 2003). Six more fin clips were collected on June 9th, 2013 and 648 base pairs in the ND2 mitochondrial gene were sequenced. Four of these shared the common San Juan lineage CRCT haplotype while the remaining two harbored a single base pair variant (Appendix 1).

Grayhackle Lake in the headwaters of Cutthroat Creek was surveyed by gillnet on August 20, 1998. An overnight set resulted in the capture of 29 fish ranging from 150-458 mm. Six of these fish were submitted to BYU for purity testing in 1998, while the remainder were too degraded. Results suggest 5 of the 6 fish were pure CRCT but the remaining fish was a Rainbow Trout (Evans and Shiozawa 2000) as measured with both mitochondrial and nuclear markers. An additional 10 fish were collected on June 19th, 2013, none of which appeared to display any Rainbow Trout or Yellowstone Cutthroat Trout admixture as measured with AFLPs (albeit a small sample size). Subsequent sequencing of the ND2 mitochondrial gene showed that all ten fish shared the common San Juan lineage CRCT haplotype.

Fall Creek (WC#38117)
CRCT Conservation Population ID: 14080101cp008
Occupied habitat: 0.3 km

Cutthroat Trout from Fall Creek

Fish were first collected from Fall Creek, located at the base of Wolf Creek Pass and a tributary to the West Fork of the San Juan River, in October 1976. The stream is very small and the Cutthroat Trout only inhabit a reach from Treasure Falls to the Highway 160 road crossing. The highway culvert serves as a barrier to invasion by downstream nonnative trout.

Molecular surveys:

Tissue samples were collected from 10 trout in August 1999 and sent to Dr. Robb Leary at the University of Montana for study. Dr. Leary was unable to extract any high quality nuclear DNA for PINEs testing. Biologist Mike Japhet noted numerous spots on

the heads of the fish suggesting these fish might be introgressed with Rainbow Trout and therefore not worthy of further consideration. As part of the search for the lost San Juan trout, a small sample of fin clips (11 fish) were collected on June 19th, 2014 to at least determine if any remnant San Juan haplotypes were evident. Indeed, all 11 fish harbored the San Juan lineage CRCT haplotype, which precipitated another collection on July 23rd, 2015 of 25 additional fish that would allow for evaluation of Rainbow Trout admixture in the population. Again, all 25 fish displayed the San Juan lineage CRCT haplotype, but more importantly, no evidence of Rainbow or Yellowstone Cutthroat Trout admixture was detected with AFLP markers.

Headache Creek (WC#39491)
CRCT Conservation Population ID: 14080101cp004
Occupied habitat: 1.3 km

Cutthroat Trout from Headache Creek

Headache Creek is a small tributary of the Navajo River within the confines of the Banded Peak Ranch. The fish community was first surveyed on July 22, 1998 and resident Cutthroat Trout were documented despite no stocking history. Headache Creek contains a very small population (85 -152 fish/km) of Cutthroat Trout in an equally small section of the stream (<1 km; Table A2-2). With no natural barrier to protect this population from invasion by nonnative salmonids in the Navajo River, it was not surprising to find Brook Trout also occupying the stream. In 2000, the Banded Peak Ranch managers (Lesli Allison and Anna Jester) contracted Dave Rosgen (Wildland Hydrology Consultants, Fort Collins, Colorado) to build a double drop barrier (Figure A2-1) above the confluence to secure the population from future invasions. Brook Trout were then removed from this reach of Headache Creek during annual single pass electrofishing efforts from 1999 to 2005. The removal effort appears to have been successful, as no brook trout have been detected since 2004. This barrier was fortified in 2017 after high spring flows washed out the downstream drop structure.

Figure A2-1: Double drop fish passage barrier on Headache Creek circa 2009 on left and again in 2017 on right.

Table A2-2: Survey history and results for the Cutthroat Trout population in Headache Creek. Population estimates (adult fish >150 mm per 1.6 km) and associated confidence intervals (95% CI) were generated from two removal passes over 152 m of stream.

Year	Date	n	Density	95% CI	Comments
1998	Jul 22	17			Single pass; 10 samples for genetic analysis
1999	Sep 3	10			Single pass; 10 samples for genetic analysis
2005	Aug 3	15	85	34	High water (Capture P=0.71)[1]
2006	Aug 30	27	152	8	Low water (Capture P=0.91)
2014	Aug 7	17			Single pass[2]
2015	Jun 29	27	85	408	High water/crippled tech (Capture P=0.60)
2017	Jun 5	0			Single pass[3]

[1]Thirty six Cutthroat Trout captured in entire length of occupied habitat
[2]Electrofished around upper headgate and in spawning channel and moved 17 fish to Gramps Ponds
[3]Electrofished spawning channel and adjacent mainstem headgate area looking for spawning Cutthroat Trout but found none

Much of the best habitat in Headache Creek is dewatered by the Virginia Meadow Diversion. The Virginia Meadow irrigation ditch takes about half the water during the irrigation season. However, there is a headgate and ditch downstream near the barrier, that diverts most of the remaining water into a series of 3 reclaimed ponds near the Banded Peak Ranch headquarters that are devoid of nonnative fishes. These basins had been used to contain effluent from oil and gas operations in the 1970s. Although cleaned

and remediated, the liner on the bottom should not be disturbed according to the ranch manager. "Gramps Ponds" average about an acre in size each and were specifically designed to serve as broodstock ponds complete with a small spawning channel entering the ponds and barrier (culvert stand pipe) upon exit. Anecdotal evidence suggests the Cutthroat Trout from Headache Creek have not colonized the lakes in appreciable numbers since they were reclaimed in 2003. Concern over fish mortality precluded setting gill nets for more than a few hours at a time in Gramps Ponds, but a 23 m experimental gill net set for 2 hours near the inlet on the South Pond on May 17, 2014 captured only a single Cutthroat Trout. Two more set on June 2, 2015 (one each in Middle and North ponds) for 3 hours each yielded no fish.

Macroinvertebrate densities and temperatures appear suitable for Cutthroat Trout in the ponds. Freshwater scuds (*Gammarus* sp.) are abundant and a small littoral area has developed around the margins. The headgate from Headache Creek to the spawning channel and ponds remains open during the winter allowing freshly oxygenated water to enter the ponds suggesting winterkill conditions are unlikely. Temperatures did not exceed critical thresholds for Cutthroat Trout from 2015-2017, with near optimal conditions for growth (Table A2-3).

Table A2-3: Maximum daily temperature (MDT), maximum weekly maximum temperature (MWMT), and average 30-day average temperature (M30AT) in ºC were calculated from a temperature logger positioned on the surface of Gramps Pond` #1.

Year	MDT	MWMT	M30AT
2015	16.2	15.7	14.7
2016	16.5	16.1	14.4
2017	14.6	14.0	13.0

Molecular surveys:

Ten trout were collected on July 22, 1998 and sent to Dr. Robb Leary (University of Montana) for analysis with horizontal starch gel protein electrophoresis. Dr. Leary indicated that they were probably pure CRCT fish but noted that they possess a rare allele indistinguishable from Rainbow Trout (Kanda and Leary 1999; Kanda et al. 2000). An additional 10 tissue samples were collected on September 3, 1999 and sent to Brigham Young University for analysis. Both mitochondrial and nuclear DNA suggested this collection did not contain admixture with Rainbow Trout or Yellowstone Cutthroat Trout, and that they were consistent with CRCT (Evans and Shiozawa 2000). Fin clips were collected from two-dozen trout in 2006, which too suggested these fish were pure with no

evidence of nonnative alleles as measured with AFLPs (Rogers 2008a). Subsequent mitochondrial analysis suggested these fish harbor the same San Juan River basin ND2 mitochondrial haplotype found in Aiken's museum specimens collected from Pagosa Springs in 1874.

Himes Creek (WC#39502)
CRCT Conservation Population ID:
14080101cp002+14080101cp003
Occupied habitat: 3.8 km

Cutthroat Trout from Himes Creek

The Cutthroat Trout population in Himes Creek was first discovered in 1994. The adult population (those over 150 mm) has ranged from 46 to 164 fish/km (Table A2-4). Brook Trout are present within the occupied habitat, but occur in very low numbers, mostly within the first quarter mile upstream of the barrier. They appear to have been introduced in the 1930s into a small, shallow headwater beaver pond named Rod and Gun Club Lake. This lake was surveyed with a 75 foot gillnet in 2001 for 1.5 hours. Leeches and larval form Tiger Salamanders were present but no fish were seen or caught. A mechanical removal effort on Brook Trout has been ongoing since 1999. A downstream barrier was constructed in 2001 to protect the population from subsequent invasions of nonnative salmonids, but most of the stream flow is diverted above the barrier and into hay fields during the irrigation season. During that time, the 460 m long channel below the diversion is typically dewatered.

Table A2-4: Survey history and results for the Cutthroat Trout population in Himes Creek. Population estimates (adult fish per km) and associated confidence intervals (95% CI) were generated from two removal passes over 152 m of stream during which time Brook Trout (BRK) were removed

Year	Date	Density	95% CI	BRK	Comments
1994	7/19/94	52	7	1	BRK at Diversion
1998	8/17/98	72	0		
2005	8/01/05	52	7	1	Single 5 inch BRK below diversion
2006	8/29/17			1	BRK at diversion; 137 CRN in reach*
2007	10/1/07	121	34	1	Single 250 mm BRK above diversion

2009	7/15/09			0	83 CRN from barrier to FS diversion tarp
2013	8/15/13	164	58	3	At diversion
2014	8/14/14			3	No pop est; 137 CRN captured
2017	8/02/17	46	8	3	BRK at diversion

*Electrofishing reach starts at USFS/Ranch boundary and ends at the Rod and Gun Club Lake tributary outlet on Himes Creek.

Molecular surveys:

Ten fish from Himes Creek were collected in 1994 (presumably on July 19th while a population estimate was being generated) and preserved in formalin. Don Proebstel (Colorado State University) examined morphological characters on these fish and found them to be consistent with CRCT (meristic counts within range), but did note that they had smaller spots on average than other CRCT (Proebstel et al. 1996). Genetic samples were first collected in 1998 and 1999 from Himes Creek and sent to Drs. Paul Evans and Dennis Shiozawa at BYU. These fish were determined to be pure CRCT and had "Colorado River mtDNA haplotypes". Two unique alleles were identified but not characterized because they were of unknown origin (Evans and Shiozawa 2000). An addition, 30 tissues samples were collected in October of 2007 for AFLP testing. These fish scored 100% CRCT with no evidence of Rainbow Trout or Yellowstone Cutthroat Trout admixture. Subsequent sequencing of the ND2 mitochondrial region from 20 fish showed that all shared the common San Juan lineage CRCT haplotype.

Rio Blanco River (WC#38439)
CRCT Conservation Population ID: NA
Occupied habitat: NA

Cutthroat Trout from Rio Blanco River

The Rio Blanco is a tributary to the Upper San Juan River. It is broken into two management sections by a large diversion dam that services the San Juan-Chama Water Project. The upper section (Rio Blanco #2), flows out of the South San Juan Wilderness then winds primarily through private land down to the diversion structure. There are no barriers to nonnative fishes in this reach, and Rainbow Trout are stocked extensively on the private lands. However, Cutthroat Trout and Brook Trout persist in the high gradient,

unstable upper reaches of the river. An August 13, 1997 electrofishing survey in this area (upstream of Summit Creek confluence) recovered only 2 Brook Trout. No Cutthroat Trout were captured in a brief survey 400 m upstream of the Summit Creek confluence. Periodic floods in the headwaters scour the river channel through a box canyon starting at about Hondo Creek leaving little physical habitat for fishes. Cutthroat Trout are generally found from Hondo Creek upstream. Flash flooding occurred a few days before the August 16, 2013 sampling trip. Within a few hours flows the Rio Blanco rose from a baseflow of 20 cfs to almost 800 cfs then back to baseflow conditions. The scouring effect was obvious (Figure A2-2).

Figure A2-2. Flash flood scoured river channel in the headwaters of the Rio Blanco above Box Canyon.

The Rio Blanco headwaters are steep and no trails pierce the head of this very remote and rugged drainage. There are no headwater lakes and no records of fish being stocked in its two main tributaries (Hondo Creek and Summit Creek). In the fall of 1899, 10,000 fry were stocked somewhere in the drainage but no record of species or location could be found. It is likely that these fish were progeny from the Emerald Lakes Fish Hatchery as were several better-documented earlier stocking events in Archuleta County. Rainbow Trout had already been introduced into Emerald Lakes at that time, but majority of the spawn was likely taken from genetically intact San Juan lineage fish or "native fry". The drainage was stocked again in 1973 with "Pikes Peak natives" (see Rogers and Kennedy 2008), and with Colorado River Cutthroat Trout ("CRN" likely from Trappers Lake sources) in 1986, though we are uncertain as to how far up the drainage these were placed. Author Jim White's family moved to the Rio Blanco basin in 1976 where he grew up fishing the river and surrounding tributaries, but does not ever recall catching Rainbow Trout up in the Blanco Canyon reach in the early 1980s or at any other time.

Molecular surveys:

A return angling trip in 2013 yielded two Cutthroat Trout specimens, both which harbored the common San Juan lineage CRCT ND2 mitochondrial haplotype. This presents the intriguing possibility that San Juan lineage trout remain in this drainage, but a more robust survey effort will be required to evaluate purity in this population.

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