Cutthroat Trout Studies

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CUTTHROAT TROUT INVESTIGATIONS

Period Covered: December 1, 2021 to November 30, 2022

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. 20013; 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 2022 are presented here.

METHODS

Molecular tests were conducted on 373 samples obtained from 22 Cutthroat Trout populations distributed across Colorado (Table 1). Eighteen came from the CRCT range, three from the RGCT range, and one from the Arkansas River drainage. 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 haplotype recovered, I use the name of the body of water where the haplotype was first discovered, preceded by Oc (the native trout, *Oncorhynchus clarkii*) and three letters that describe the major drainage basin of the lineage represented. These include 1) Blue Lineage CRCT native to the Yampa, White, and Green River basins (YAM), 2) Green Lineage CRCT native to the Colorado, Gunnison, and Dolores River basins (COL), 3) RGCT native to the Rio Grande basin (RIO), 4) the native trout of the South Platte River basin (SPL), and 5) the nonnative Yellowstone Cutthroat Trout (YEL) stocked widely across Colorado in the middle of the last century. This approach allows for easy inclusion of newly discovered haplotypes and facilitates communication toward management and conservation goals. Mitochondrial haplotypes were compared to a reference set derived from Cutthroat Trout samples collected across Colorado over the last two decades (Figure 1) using MEGA7 (Kumar et al. 2016).

Table 1. Stream names organized by major drainage basin, water codes, collection dates, and number of fin clips collected for molecular tests conducted in 2022.

Stream	Water Code	Date	Sample size
Arkansas			
Lake Creek, S Fk	30231	8/4/2021	11
Colorado			
Bennett Gulch	25963	8/30/2018	12
Big Hole Creek	19487	9/16/2021	8
Island Lake Creek	15605	8/5/2021	10
Lincoln Creek	20987	9/8/2021	25
McCullough Gulch	21129	8/17/2021	11
Roan Creek	21701	8/17/2021	30
Salt Creek, E	28147	7/29/2021	11
Spruce Creek (lower)	22133	8/17/2021	16
Spruce Creek (upper)	22133	7/29/2021	21
Stillwater Creek	22171	8/18/2021	3
Yule Creek	26585	8/20/2022	2
Gunnison			
Antelope Creek, W.	48016	7/7/2022	20
Rio Grande			
Canon Bonito	38744	7/20/2021	30
Little Ute Creek	49379	8/15/2021	30
Squirrel Canyon	39768	6/9/2022	30
San Juan			
Himes Creek	39502	6/9/2022	15
Pine River	41284	7/20/2021	9
Rincon LaVaca Creek	43852	7/19/2021	9
Rito Blanco River[1]	38441	8/9/2021	30

White			
Brush Creek	19299	7/28/2021	6
Douglas Creek, E	23127	7/28/2021	34

[1]No molecular tests run; isolated and archived DNA only

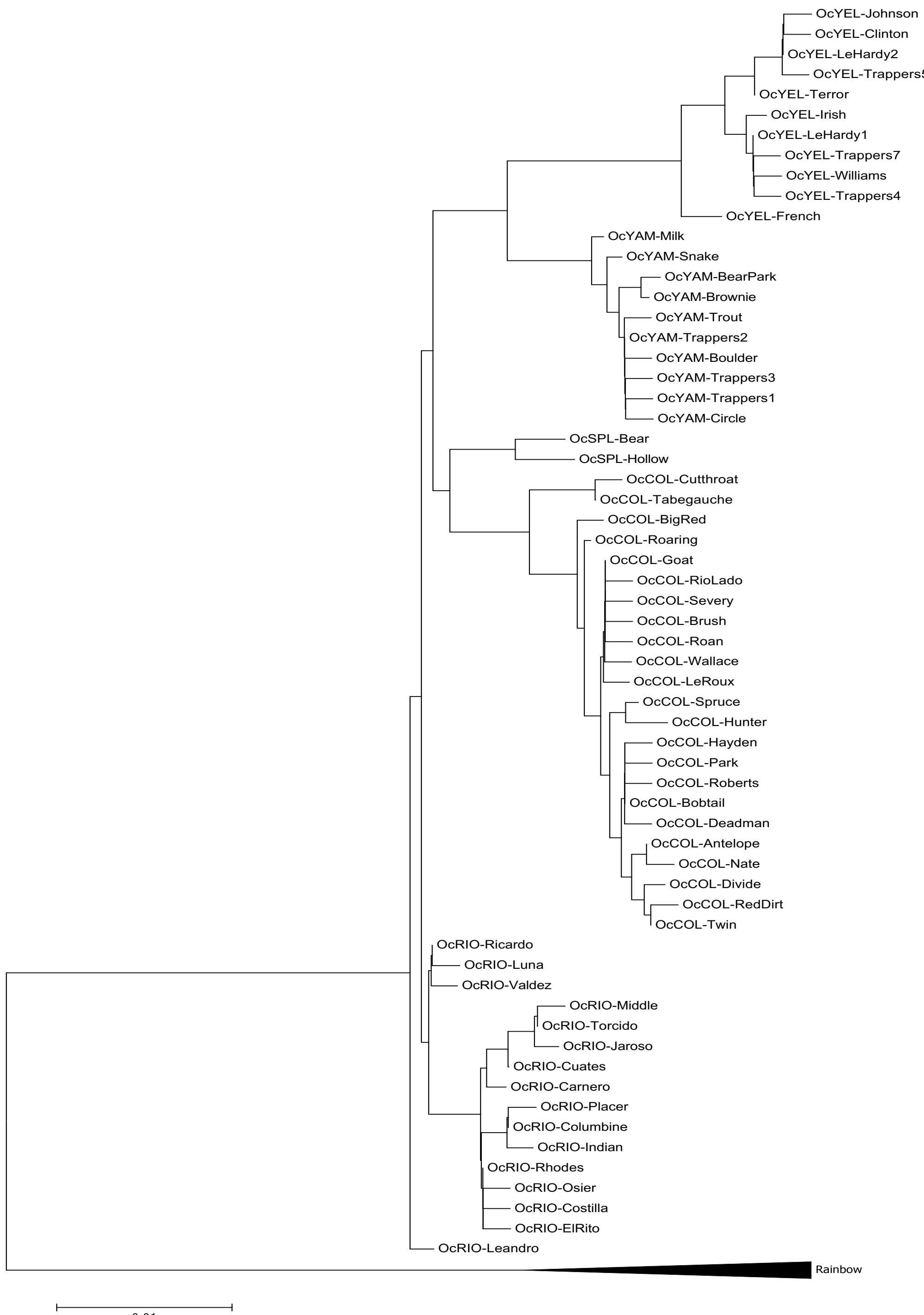

Figure 1. Phylogenetic relationships inferred from 648 base pairs of the mitochondrial NADH dehydrogenase subunit 2 gene for Cutthroat Trout from Colorado. The evolutionary history was developed with the neighbor-joining method in MEGA7, with evolutionary distance units representing the number of base 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 single collection of Cutthroat Trout from 2021.

Lake Creek, S Fk (WC#30231)— Not many Cutthroat Trout remain in this population that is being overrun by Brook Trout. In fact, the 11 fish sampled were all that could be collected in a full day of electrofishing. A candidate for future reclamation, we wanted to make sure no YFCT alleles were present in this population. AFLP data (Table 2) suggests these fish are essentially Yellowstone Cutthroat Trout (YSCT), while the mtDNA suggests more Blue Lineage CRCT (bCRCT) influence (Table 3).

Table 2. AFLP results from 19 Cutthroat Trout collections analyzed in 2022, 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, S Fk	11	1	-	-	99	-
Colorado						
Bennett Gulch	12	-	100	-	-	-
Big Hole Creek	8	-	99	-	-	-
Island Lake Creek	10	85	-	-	14	-
Lincoln Creek	25	99	-	-	-	-
McCullough Gulch	11	97	-	-	3	-
Roan Creek	30	-	99	-	-	-
Salt Creek, E	11	3	91	-	4	2
Spruce Creek (lower)[1]	16	99	-	-	-	-
Spruce Creek (upper)[1]	21	99	-	-	-	-
Gunnison						
Antelope Creek, W	20	-	100	-	-	-
Rio Grande						
Canon Bonito	30	3	-	-	95	2

Canon Bonito[2]	30	99	-	1	-	-
Little Ute Creek	30	-	-	100	-	-
Little Ute Creek[2]	30	-	-	100	-	-
Squirrel Canyon	30	-	-	100	-	-
Squirrel Canyon[2]	30	-	-	100	-	-
San Juan						
Himes Creek	15	99	-	-	-	-
Pine River[3]	9	95	-	-	4	-
Rincon LaVaca[3]	9	99	-	-	1	-
White						
Brush Creek	6	-	-	-	-	100
Douglas Creek, E	34	97	-	-	-	2

[1]Both Spruce Creek collections analyzed in a single STRUCTURE run; upper and lower mean q-values were calculated from that single run
[2]This represents the RGCT – bCRCT specific AFLP test with K=2
[3]Pine River and Rincon LaVaca collections analyzed in a single STRUCTURE run; mean q-values for each were calculated from that single run

Table 3. ND2 results from 16 Cutthroat Trout collections analyzed in 2022, along with the number of samples analyzed, organized by major drainage basin. ND2 haplotype 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, S Fk	10	8	-	-	2	-
Colorado						
Bennett Gulch	11	-	11	-	-	-
Big Hole Creek	8	-	8	-	-	-
Island Lake Creek	4[1]	3	-	-	1	-
Lincoln Creek	24	19		-	5	-
McCullough Gulch	11	6	-	-	5	-
Salt Creek, E	11	2	6	-	2	1
Spruce Creek (lower)	16	8	7	-	1	-
Spruce Creek (upper)	21	13	-	-	8	-
Stillwater Creek	3	1	2	-	-	-
Yule Creek	2	1	-	-	1	-
Rio Grande						
Canon Bonito	30	26	-	-	3	1

Little Ute Creek	20	-	-	20	-	-
San Juan						
Pine River	9	-	-	7	2	-
Rincon LaVaca	9	3	3[2]	-	3	-
White						
Brush Creek	6	-	-	-	-	6

[1]Only 4 of 10 samples amplified – did not rerun given probable Trappers Lake heritage
[2]All are the OcCOL-Tabeguache haplotype typical for San Juan Lineage CRCT

Colorado River basin

Bennett Gulch (WC#25963)— This isolated stream has been stocked repeatedly with pack fish from Lake Nanita, so we expected it to be bCRCT. However, both AFLP and ND2 sequence data (Table 2 and 3) suggest it is a pure gCRCT population displaying the common OcCOL-Goat haplotype. Sequence data was only obtained from 11 of 12 samples as Pisces #161637 failed to amplify in both directions. Acquiring more tissues samples is recommended.

Big Hole Creek (WC#19487)— This small population in the Sheephorn Creek watershed might be linked to the population in Three Licks Creek. It is possible that some admixture with YSN is present (Table 2), but difficult to say for certain with such a small sample size. Both OcCOL-Goat (n=6) and OcCOL-Bobtail (n=2) haplotypes are present (Table 3).

Island Lake Creek (WC#15605)— These samples were collected from a string of paternoster lakes below Island Lake in the Indian Peaks Wilderness. Both AFLP and ND2 results (Tables 2 and 3) suggest this population was likely founded from Trappers Lake progeny sometime in the last 70 years (Rogers et al. 2018). Six of the ND2 sequencing reactions did not amplify in either direction, perhaps because of PCR inhibition.

Lincoln Creek (WC#20987)— This isolated population of Cutthroat Trout is protected from invasion by downstream Brook Trout through a mine waste induced chemical barrier. Although predominantly bCRCT, clear evidence of YSCT is found in the mtDNA (Table 3). As such, this population too was likely founded from Trappers Lake progeny sometime in the latter half of the twentieth century (Rogers et al. 2018).

McCullough Gulch (WC#21129)— This self-sustaining wild population was surveyed as part of the proposed Montgomery Reservoir expansion project. Both the AFLP results and the presence of OcYAM-Trappers2 (n=6) and OcYEL-LeHardy1 (n=5) haplotypes suggest this population was founded from Trappers Lake progeny in the latter half of the twentieth century (Rogers et al. 2018).

Roan Creek (WC#21701)—These fins were collected throughout the conservation population along with UTM and photo covariates. A hint of noise was detected in the AFLP runs (Table 2), but no flagrant RBT admixture was apparent.

Salt Creek, E (WC#28147)— Multiple lineages of Cutthroat Trout detected both by AFLP and ND2 sequence data (Tables 2 and 3). Though primarily gCRCT (91% by AFLP), nuclear and mitochondrial markers are also present for bCRCT, YSCT, and RBT.

Spruce Creek (WC#22133)— Also surveyed as part of a proposal to expand Montgomery Reservoir, this population lies downstream of the Mowhawk Lakes below a substantial waterfall barrier. Fin clips were collected from fish above and below a diversion structure that serves as another barrier to upstream movement. Both sections appear to be primarily bCRCT by AFLP (nuclear markers; Table 2), but 7 of 16 fish in the lower section harbored gCRCT mtDNA haplotypes (Table 3) – including one not seen anywhere else (OcCOL-Mohawk). Only bCRCT (OcYAM-Trappers2; n=13) and YSCT (OcYEL-LeHardy1; n=8) haplotypes were detected in the fish upstream of the diversion structure suggesting that the upstream fish were founded from the stocking of the Mohawk Lakes in the latter half of the twentieth century with progeny from a wild egg-take operation at Trappers Lake (Rogers et al. 2018). Green lineage CRCT mtDNA below the diversion structure may represent relict indigenous haplotypes.

Stillwater Creek (WC#22171)— The Pony Park area was burned severely in the East Troublesome blaze in 2020. The remaining Cutthroat Trout population is extremely sparse, yielding only three fish despite extensive electrofishing. With so few fish, only ND2 sequence data was obtained (Table 3): Two OcCOL-Goat (gCRCT) haplotypes were recovered and one OcYAM-Trappers2 (bCRCT).

Yule Creek (WC#26585)— This stream lies below several headwater lakes that have been stocked with Cutthroat Trout but are not connected to the stream. Though only two fish were collected, we sequenced the ND2 gene to determine if gCRCT haplotypes were present, warranting a subsequent collection. Unfortunately, one fish displayed the OcYAM-Trappers2 haplotype and the other, OcYEL-LeHardy1 (Table 3).

Gunnison River basin

Antelope Creek, W (WC#48016)— This population is one of the original gCRCT reference populations for all our genetic work, and remains free of nonnative alleles as measured by AFLPs (Table 2). Given the demand for DNAs from this population for pending and future research projects, Pisces Molecular conducted two DNA extractions for each fish.

Rio Grande basin

Canon Bonito (WC#38744)— This stream in the S Fk Conejos drainage lies above a large waterfall and would therefore have been historically fishless. The site has been proposed for a future reclamation project to benefit RGCT conservation, but little is known about the current feral population, and what would be lost. The current residents are phenotypically extremely variable, as are their molecular signatures (Tables 2 and 3). Evidence of bCRCT, YSCT, and RBT alleles are found in both nuclear and mitochondrial genomes, suggesting a robust past stocking history.

Little Ute Creek (WC#49379)— These fins were collected by Tom Martin on the Trinchera Ranch over two days. This population had been previously tested with PINEs and BIAMs and determined to be pure RGCT which was confirmed by AFLPs here, both by the standard and RG-CR tests (Table 2; Rogers et al. 2011). Three mitochondrial haplotypes were recovered from a 20 fish sample (Table 3): OcRIO-Cuates (n=8), OcRIO-Rhodes (n=7), and OcRIO-Placer (n=5), suggesting good genetic diversity is present in this population.

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 (Table 2). If these fish are to be used in upcoming reclamation projects on the Trinchera Ranch, a thorough inspection of the mitochondrial DNA would also be warranted.

San Juan River basin

Although the standard AFLP test (Rogers 2008) does not screen for San Juan CRCT specifically (Rogers et al. 2018b), it does provide a useful assay for detecting admixture in the nuclear genome with RBT or YSCT. Only two mitochondrial haplotypes have been detected in extant San Juan lineage CRCT, the common OcCOL-Tabeguache, and the rarer OcCOL-Cutthroat haplotype.

Himes Creek (WC#39502)— Cutthroat Trout collected previously in 2007 from this population suggested that it was pure CRCT (Bestgen et al. 2019). However, at least one fish from a June 2021 survey displayed evidence of RBT admixture (Rogers 2021). A small spawn operation was conducted in June of 2022 to provide eggs (and ultimately fry) to repatriate these fish into the headwaters of Wolf Creek. Unfortunately, one parent showed clear evidence of RBT admixture, putting this effort on hold. DNA was also isolated from 22 fins collected on October 7, 2021 from fish that were captured in Himes Creek and moved above a barrier to fish passage in a fishless section of stream. These DNAs will be archived until this new habitat becomes better established and new molecular approaches can reveal how many of the founding population are actually represented in subsequent generations, and how the genetic bottleneck might manifest itself.

Pine River (WC# 41284)— This reach of the Pine River lies 17 miles upstream of the trailhead and above a large waterfall that would have rendered it fishless historically. Given the proximity to Emerald Lake however, it is likely to have been stocked. In an effort to boost samples sizes, these nine fish were run with nine more from neighboring Rincon LaVaca, but then parsed back out for the purposes of this report (Table 2). With AFLPs, these fish appear to be predominantly bCRCT with YSCT admixture but the standard AFLP test has trouble separating bCRCT from RGCT (Rogers et al. 2011). Interestingly, 7 of the 9 fish harbored OcRIO-Carnero haplotypes, making one wonder if Emerald Lake played a role in spreading RGCT outside their native range in places like Rocky Mountain National Park.

Rincon LaVaca (WC#43852)— An additional nine fish were collected collected from this small population above the Raber Lohr Ditch diversion near Weminuche Pass to go with the single fish collected in 2020 (Rogers 2021). This stream is tributary to the Pine River above the same waterfall barrier as the previous Pine River collection. AFLPs from these fish were run through STRUCTURE with the Pine River fish to boost sample sizes, but only the Rincon LaVaca results are reported here (Table 2). These fish appear to be predominantly bCRCT with mild YSCT admixture as well by AFLPs, but that test does not screen for San Juan CRCT DNA. The mtDNA sequence data (Table 3) confirmed the presence of bCRCT and YSCT haplotypes, but three of the fish displayed the native OcCOL-Tabeguache haplotype.

White River basin

Brush Creek (WC#19299)— These samples were collected from above the falls on Scott Brady's property to determine if RBT admixture was present up there as well. Unfortunately, there is not just admixture – these appear to be pure RBT (albeit with a sample size of only six) both by AFLPs and ND2 (Tables 2 and 3).

Douglas Creek, E (WC#23127)— The goal with this collection was to assess RBT admixture in this high-value conservation population upstream of the culvert at 12S 696887, 4391333. Substantial RBT admixture was detected in 2 of the 34 fish sampled (Table 2).

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RESEARCH PRIORITY

Persistence of native Cutthroat Trout in a warming climate

OBJECTIVE

Predicting population persistence in the native trout of the Colorado, Gunnison, and Dolores basin headwaters

INTRODUCTION

Colorado River Cutthroat Trout *Oncoryhnchus clarkii pleuriticus* (CRCT) represent one of the southern-most of the 14 recognized native Cutthroat Trout subspecies of the Rocky Mountains (Behnke 1992; Behnke 2002; Trotter 2008). The rich diversity of this species reflects the many isolated habitats created by the mountains they inhabit. With one of the broadest ranges of Cutthroat Trout, CRCT occupy suitable habitat in the upper Colorado River basin from the headwaters of the Green River in the north to the San Juan River in the south (Behnke 2002). Recent research has identified three distinct lineages within CRCT (Metcalf et al. 2012; Rogers et al. 2018; Bestgen et al. 2019). From north to south, the first is native to the headwaters of the Green, White, and Yampa River basins, as well as a few tributaries that drain into the Colorado River directly below the confluence with the Green River (Bestgen et al. 2019) in northern Colorado, southern Wyoming, and eastern Utah. This clade is often referred to as the "blue lineage" (sensu Metcalf et al. 2012; hereafter CRCTb). The second, "green lineage" (hereafter CRCTg) hails from the headwaters of the Colorado, Gunnison, and Dolores River basins in western Colorado and eastern Utah (Rogers et al. 2018; Bestgen et al. 2019; Rogers 2020), while the third calls the San Juan River basin home (Metcalf et al. 2012; Rogers et al. 2018)

Like other inland Cutthroat Trout, CRCT now occupy a fraction of their historical range), primarily in isolated headwater habitats protected from invading nonnative salmonids (Behnke 2002; Fausch et al. 2009; Penaluna et al. 2016). Range contractions are particularly acute for the remaining 69 CRCTg lineage Conservation Populations (CP; sensu UDWR 2000; Hirsch et al. 2006, 2013; Muhlfeld et al. 2015; Roberts et al. 2013, 2017; Zeigler et al. 2019) that now just occupy 3% of their former range west of the Continental Divide (Rogers 2020), resulting in shifting conservation objectives to ensure their future security. In an effort to inform subsequent conservation and management actions, the objective of this study was to rank the relative vulnerability of each CRCTg population by predicting their probability of persistence in a warming future out to 2040 and 2080.

METHODS

Extant CRCTg CPs were identified in Rogers (2020), and their vulnerability in a warming climate was evaluated using a Bayesian Network model developed for a neighboring sister taxon

(Zeigler et al. 2019), the Rio Grande Cutthroat Trout (RGCT; *O. c. virginalis*). This modeling framework is ideally suited for synthesizing complex interactions of well-studied attributes with expert opinion for those less well studied in an adaptive management framework (Marcot et al. 2006; Hyberg et al. 2006). As our ecological understanding of the taxon improves, so will the need to revisit the model assumptions and framework (e.g. Roberts et al. 2013; 2017). The RGCT model incorporates threats to persistence such as nonnative invasions, small population size, disease, and a changing climate to arrive at the probability that each remaining population will persist to the 2040s and 2080s. Inputs for each parent node identified in this model were populated with CP-specific measures derived primarily from the ICP database (Hirsch et al. 2013), and included metrics such as occupied stream length (km), proximity of nonnative trout and whirling disease, barrier status, population connectivity, and nonnative control. Management biologists responsible for each CP were consulted to arrive at inputs for nodes covering demographic support, drought refugia availability, evidence of intermittency, and anthropogenic influence following protocols outlined in Zeigler et al. (2019). Wildfire risk for each 12-digit HUC across the native range of CRCT was evaluated by Williams et al. (2009), and those same states were used here. Although we lacked information on effective population sizes for these CPs, we assumed they were a quarter of the estimated population size as this was the mean value across a number of published studies (Allendorf et al. 1997; Rieman and Allendorf 2001; Palm et al. 2003; Jensen et al. 2005). Unfortunately, estimated population sizes recorded in the ICP are based on only adult fish (>150 mm; Hirsch et al. 2013), while the BN model used here requires a 75mm cutoff excluding only age-0 fry that have not recruited to the population (Young et al. 2005; Zeigler et al. 2019). This necessitated reanalyzing the 142 multipass electrofishing surveys conducted on these 69 CPs over the last two decades obtained from Colorado Parks and Wildlife. Two-pass removal data were analyzed using the software application JOM 2.4 (Rogers 2006), whereas three-pass removal data were analyzed using the Huggins estimator (Huggins 1989) as implemented in Program MARK (White and Burnham 1999) using fish total length as a covariate. Site-specific population estimates (fish/km) within a CP were averaged, then extrapolated to the entire occupied reach to estimate the population size of fish ≥75 mm. Cutthroat Trout population densities are suppressed when sympatric with competing nonnative salmonids (Peterson et al. 2004; Benjamin and Baxter 2012; Al-Chokhachy and Sepulveda 2019). Acknowledging this, we calculated the average population density for either allopatric or sympatric populations and used those means when field data was not available (n=13 populations).

Values for the stream temperature input nodes were determined using a combination of existing stream temperature values and modeled stream temperature values. We used observed stream temperature data from 43 streams within the native range of CRCTg (Rogers et al. 2018) to generate linear regression equations to convert mean August stream temperature, which is available for every NHDPlus v2 stream segment using the NorWeST dataset (Isaak et. al. 2017), into the two stream temperature metrics (MWMT and M30AT; °C) used in the BN. The regression equation was then used to calculate the MWMT and M30AT for each NHDPlus v2 segment (n=1186) occupied by CRCTg CPs. These values were calculated for the current time horizon (mean of 2005-2015), 2040s, and 2080s. Future climate scenarios used to create the 2040s and 2080s stream temperature values are detailed in Isaak et. al. (2017). Briefly, future climate predictors (i.e., temperature and streamflow) were determined using a ten-model ensemble of global climate models (GCM) using the A1B emission scenario from the third phase coupled model intercomparison project (CMIP3; Hamlet et al. 2013), which is similar to the most recent IPCC (2013) representative concentration pathway (RCP) 6.0 in CMIP5 (Wright et al. 2015).

Baseflow conditions for the BN were determined using previously published streamflow metrics from the macroscale hydrologic Variable Infiltration Capacity (VIC) model (Wenger et al. 2010) and existing data. Current baseflow (i.e., Mean 30 Day Minimum Discharge; M30MD; m^3/s) values were determined from the NHDPlus v2 cumulative drainage area for each stream segment occupied by CRCTg populations. We used the average cumulative drainage area for each CP and converted this to baseflow using the same linear regression equation developed for RGCT populations (Zeigler et al. 2019). We then used the proportional change in mean August discharge between historical and 2040s or 2080s values from the VIC dataset to determine future baseflow values for each NHDPlus v2 stream segment. The time-specific proportional change was applied to the current baseflow values for each CP to determine the future (2040s and 2080s) baseflow stream discharge. The 2040s and 2080s values from the VIC dataset used the same ensemble climate modeling approach and emission scenario as the NorWeST temperature modeling summarized above.

Once input node information was compiled, we performed a sensitivity analysis using the input data to rank the influence each input node had on the probability of CRCTg persistence. This was accomplished by using the best and worst-case values for each input node while keeping all other input nodes at default values (Marcot 2012; Conroy and Peterson 2013; Zeigler et al. 2019). We then ran the BN model provided by Zeigler et al. (2019) to generate probabilities for persistence for each CRCTg population in their putative native range (west of the Continental Divide; Metcalf et al. 2012; Rogers et al. 2018; Bestgen et al. 2019) to 2040 and 2080. The predicted future stream temperatures and baseflows for each NHDPlus v2 stream segment (n=1186) were averaged over the occupied stream length for each population to generate node inputs for each time period. As in Zeigler et al. (2019), we intended to remove segments predicted to be too warm to support CRCT (MWMT > 25.0°C; Zeigler et al. 2013) in future time periods resulting in the loss of occupied stream length for some populations. In practice however, no currently occupied CRCTg CP stream segments were predicted to be unsuitable by 2080, so stream length node inputs remained constant over each time period. Occupied stream length (km) along with predicted stream temperatures and baseflows, were also used as inputs into the BN model to predict the probability of persistence for each CRCTg population during each time period. All BN development and analysis was performed using Netica software 4.16 (Norsys Software Corp., Vancouver, BC, Canada) following the tutorial developed for RGCT at https://cpw.state.co.us/learn/Pages/ResearchCutthroatTrout.aspx.

RESULTS

Projected probabilities of persistence for CRCTg were extremely variable (ranging from 0.00-0.95). Seven CPs are predicted to be extirpated by the 2080s without additional active management, while only 14 CPs (19%) had a better than 75% chance of persisting to that time frame. Median persistence values declined from an average of 0.63 in the current decade to 0.38 by the 2040s, and 0.30 by the 2080s. Predicted probabilities of persistence were higher on average in the Upper Colorado GMU (Figure 1), but some of that is masked by annual mechanical removal efforts of nonnative salmonids in eight CPs that must continue (or alternate solutions implemented) if these populations are to persist. All three GMUs registered steep declines in predicted persistence by 2040 as nearby populations of nonnative competitors invade CPs protected only by partial barriers. Of the 42 populations lacking complete barriers to protect against subsequent invasion, 45% are predicted to have less than 10% chance of persisting to 2080.

We evaluated the relative influence of the seventeen parent nodes thought to drive persistence using data specific to CRCTg populations. The top four most influential nodes (Figure 2) all deal with mitigating the effects of invasion of competing nonnative salmonids, with the presence of a barrier to invading nonnative salmonids as the single most important metric in ascribing persistence to these CPs. Climate driven shifts in stream temperature ranked as the fifth most influential node, albeit not in the manner expected. Maximum 30 d average temperatures (M30AT) are indeed projected to increase on average by 1.48 °C across the currently occupied CPs to 2080, but that level of warming should actually benefit recruitment and growth (Figure 3) in six currently occupied cold streams. Maximum Weekly Maximum Temperatures (MWMT) is projected to similarly increase by 1.32 °C on average by 2080 (Figure 3), but those increases are not projected to place these populations in jeopardy, as additional warming can be realized before temperature thresholds that might reduce survival are met. Baseflow discharges are expected to decline by 0.0047 m3/s by 2080, dropping eight CPs from the "moderate discharge" state into the "low discharge" state, but those flows do not appear likely to cause substantial reductions in persistence (Figure 2).

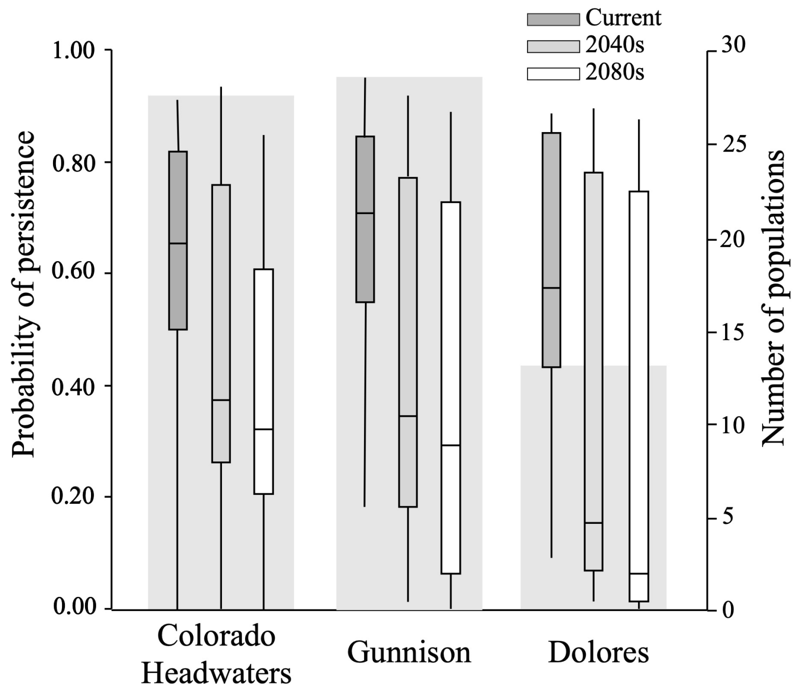

Figure 1. Projected median probabilities of persistence for Colorado River Cutthroat Trout (green lineage) populations during the current time period, 2040s, and 2080s in the three major drainage basins where these fish are putatively native. Each box encompasses the first and third quartiles, while the whiskers cover the range of values predicted for each scenario.

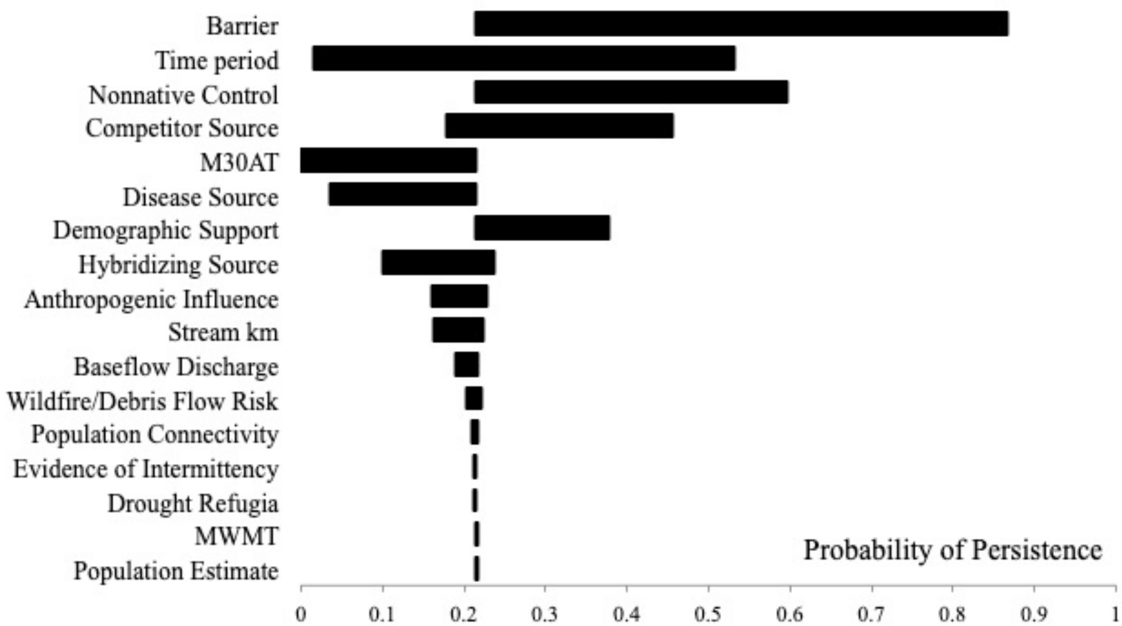

Figure 2. Tornado diagram depicting the sensitivity of probability of persistence in Colorado River Cutthroat Trout (green lineage) populations to the range of parent node inputs observed across all Conservation Populations. The 17 parent node inputs are organized by decreasing relative influence on persistence, and bars show the range of model outputs for the best and worst-case scenarios for each parent node input, while holding all other parent node inputs at their default values.

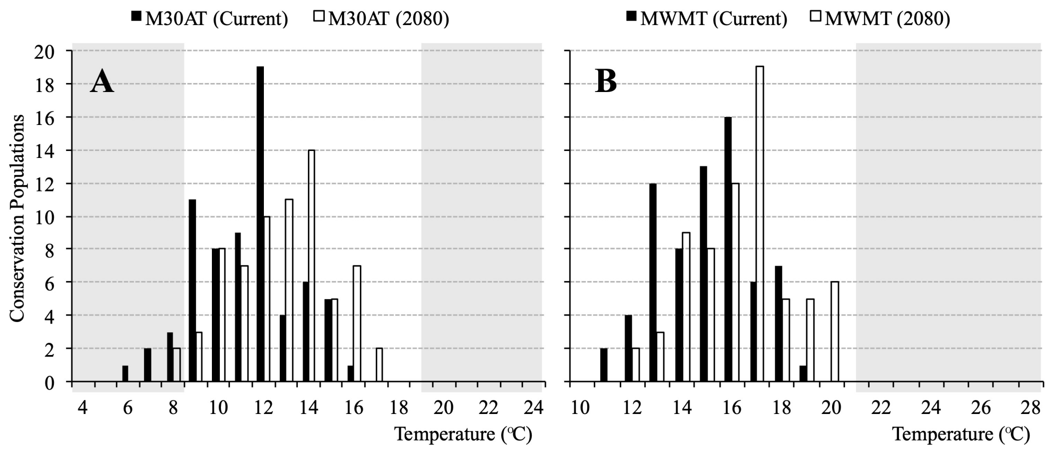

Figure 3. Modeled maximum 30-day average temperatures (M30AT) are shown the left panel (A) for the current time period (black bars) and 2080 (white bars) with the unshaded area representing temperature conditions suitable for good growth and recruitment in Colorado River Cutthroat Trout (green lineage). The right panel (B) shows the same for maximum weekly maximum temperatures (MWMT).

DISCUSSION

While the discovery of this lineage presents some exciting opportunities for preserving the legacy left behind, the future for these fish will likely require some anthropogenic assistance if they are to persist. Like other inland Cutthroat Trout, they face a myriad of challenges (Penaluna et al. 2016; Budy et al. 2019), many of which have already been addressed for CRCT more generally (Hirsch et al. 2006, 2013; Young 2008; Williams et al. 2009; Roberts et al. 2013)

Nonnative invasions

The top four most influential nodes driving persistence of CRCTg in our BN model all relate to the invasion of nonnative salmonids (Figure 2). This is consistent with findings of others that identified invasions of nonnative trout as the greatest threat to persistence of Cutthroat Trout (Behnke 1992; Quist and Hubert 2004; Roberts et al. 2017; Zeigler et al. 2019) either through competition with Brook Trout (Dunham et al. 2002a; Peterson et al. 2004; Fausch 2008) and Brown Trout (Wang and White 1994; McHugh and Budy 2005; Meredith et al. 2017; Al-Chokhachy and Sepulveda 2019), or interbreeding with their sister taxon the Rainbow Trout which can lead to extinction through hybridization (Rhymer and Simberloff 1996; Allendorf et al. 2004; Peacock and Kirchoff 2004; Muhlfeld et el. 2017, but see Young et al. 2017). The threat of nonnative trout invasions into CRCTg populations is immediate, as a quarter of the CPs across their putative native range have already been invaded, primarily by Brook Trout, but four by Brown Trout, and two by Rainbow Trout.

Disease
Perhaps the most proximate disease threat to CRCTg comes from the whirling disease parasite *Myxobolus cerebralis* given the demonstrated vulnerability of Cutthroat Trout to infection (Thompson et al. 1999; Hiner and Moffit 2001; Wagner et al. 2002; DuBey et al. 2007). Vulnerability of a population to whirling disease is mediated by the proximity of the parasite to that population (Schisler and Bergersen 2002) and the presence of a vulnerable form of the intermediate host of the parasite, Tubifex tubifex (Beauchamp et al. 2002; Ayre et al. 2014; Nehring et al. 2014). Though susceptible *T. tubifex* occur at most high elevation streams occupied by native Cutthroat Trout (Nehring et al. 2014), at present, no CRCTg CPs are known to be infected, and only 23% (16 of 69) are catalogued as medium risk (within 10 km of a known infected site; Hirsch et al. 2013). Barriers that prevent immigration of nonnative trout into CRCT populations also help mitigate the spread of the *M. cerebralis* parasite (Ayre et al. 2014), and only 3 of the 16 populations above do not appear to be protected by some sort of barrier.

Small population size
The isolated nature of the fragmented habitats occupied by most remaining CRCTg populations (Rogers 2020) suggests managers should be concerned about genetic considerations associated with small population sizes that can leave them vulnerable to inbreeding depression (Frankham 2005; Neville et al 2006; Allendorf and Luikart 2007; Whiteley et al. 2013). Median occupied reach lengths are just 4.9 km (range 0.2-26.0 km), and only 16 CPs (23%) support estimated numbers of Cutthroat Trout in excess of 2,000 individuals (Ne>500) often deemed necessary to foster robust evolutionary potential, maintain adaptive genetic diversity, avoid genetic drift, and ensure long-term persistence of populations (Franklin 1980; Frankham 1995; Hilderbrand and Kershner 2000; Rieman and Allendorf 2001; Allendorf and Luikart 2007). On the other hand, seven populations (10%) are estimated to contain fewer than 200 individuals (Ne<50), a threshold below which native trout are vulnerable to the immediate effects of inbreeding depression (Rieman and Allendorf 2001; Allendorf and Luikart 2007). Not only are 68% of CRCTg protected by barriers that would slow or preclude refounding or genetic rescue (Wofford et al. 2005), but over 80% of their CPs are listed as isolated (Hirsch et al. 2013), with no nearby CRCT to provide potential immigrants absent human intervention. However, prior to considering or implementing management actions aimed at increasing genetic diversity, managers should rigorously verify that a need exists (e. g. Sato 2006). The loss of genetic diversity is a complex continuous process and not easily explained by simple rules, so although the “50/500” rule (sensu Franklin 1980) has been used widely (Allendorf and Luikart 2007) there is substantial disagreement over its utility in species conservation (Jamieson and Allendorf 2012; Frankham et al. 2013).

Cutthroat Trout have demonstrated incredible tenacity persisting even when population sizes are low. Consider the rediscovery of the GBCT the last population of which was likely founded from just a handful of individuals yet has persisted for over 140 years above a large waterfall barrier (Metcalf et al. 2012; Rogers et al 2018) that prevents any natural genetic rescue. Eight streams isolated by water diversions on the North Fork of the Little Snake River in Wyoming continue to be harbor CRCT despite being isolated by complete barriers for 35-55 years and having estimated census population sizes as low as 15 individuals in one stream and 22 in another (Cook et al. 2010). A number of water diversion structures installed by water utility companies to channel flows from the headwaters of the Colorado River to the metropolitan area on the east side of the Continental Divide inadvertently protected upstream Cutthroat Trout populations for the last 60-80 years including four CRCTg CPs, yet they too persist to this day despite being small and isolated (mean Nc = 325; estimated Ne = 81). While we certainly should aspire to manage for populations with Ne>500, these populations clearly deserve additional study to determine how they are able to persist in isolation over prolonged periods of time.

Genetic concerns notwithstanding, small populations generally occupy small reach lengths. The seven CRCTg CPs estimated to contain fewer than 200 trout each (Ne<50) occupy mean stream reach lengths of 1.58 km, making them extremely vulnerable to catastrophic disturbances (Dunham et al. 2002b; Fausch et al. 2009). Stochastic disturbances such as wildfire (Brown et al. 2001; Howell 2006; Dunham et al. 2007) and subsequent debris/ash flows (Rinne 1996; Burton 2005; Sedell et al. 2015) can result in direct or indirect trout mortality, potentially causing localized extinctions that may encompass entire populations if they occupy small stream fragments (Propst et al. 1992; Rinne 1996; Gresswell 1999). The 2016 wildfire that ravaged the headwaters of Hayden Creek (USDA 2016) should serve as a reminder of the vulnerability of these small populations to subsequent debris flows. Though an extensive salvage effort was mounted to replicate that population in other waters, a subsequent ash flow resulted in local extinction, and rendered habitat in South Hayden Creek unsuitable for years to come. The broad distribution of CRCTg populations will help mitigate some of the risk of stochastic disturbances, as will attentive managers willing to salvage populations when in jeopardy.

A changing climate

As an iconic coldwater fish, the fate of native Cutthroat Trout on a warming planet has garnered significant attention and concern (Williams et al. 2009; Wenger et al. 2011; Isaak et al. 2012; Zeigler et al. 2012). Modeling future abiotic conditions allowed us to gain considerable insight into a future expected to change dramatically (IPCC 2013). Others have used models to predict the effect of rising water temperatures on native trout (Williams et al. 2009; Peterson et al. 2013; Roberts et al. 2013), but those studies focused on specific questions, purposefully not including all the myriad factors known to influence native trout populations. A changing climate will not only bring altered temperature regimes, but also changes in hydrology (Luce and Holden 2009; Williams et al. 2009; Wenger et al. 2011) and stochastic events such as drought (Seager et al. 2007, 2013; Hakala and Hartman 2004), wildfire (McKenzie et al. 2004; Westerling et al. 2006; Litschert et al. 2012; Westerling 2016), and debris flows (Gresswell 1999; Burton 2005) all which can shape the future of cutthroat trout populations (Dunham et al. 2003; Dunham et al. 2007; Williams et al. 2009; Wenger et al. 2011), and were incorporated in this assessment of future persistence.

Although we expected a warming climate to create new challenges for CRCTg as is expected for other inland Cutthroat Trout (Keleher and Rahel 1996; Wenger et al. 2011), our predictions did provide some unexpected results, with six populations benefitting from expected warming as streams that were once too cold now become more hospitable to consistent recruitment and growth while still not experiencing temperatures that might jeopardize survival (Figure 3). Like other CRCT, these populations have been restricted to cold, high-elevation headwater stream fragments where negative consequences of stream warming are reduced (Isaak et al. 2010; Al-

Chokhachy et al. 2013; Roberts et al. 2013), and thermal conditions likely secondary to the challenges presented by invading nonnative salmonids (Cooney et al. 2005; Roberts et al. 2017; Zeigler et al. 2019). Though warming will benefit some current populations, we did not evaluate feasibility of reclaiming lost historically occupied habitats at lower elevation that now may be thermally unsuitable. We encourage managers to use the methods here to evaluate likely future thermal conditions to help identify viable candidate waters for repatriation.

Unfortunately, focusing on just water temperature increases is not sufficient to predict effects of climate change on the stream environment (Jager et al. 1999), as an increase in extreme stochastic events will likely accompany global warming (Easterling et al. 2000) such as stream drying (Cook et al. 2004) and wildfire (McKenzie et al. 2004; Westerling et al. 2006; Morgan et al. 2008; Holden et al. 2018). Although stream flows are predicted to drop only eight CPs from moderate to low discharge states, and those anticipated flows are still adequate to protect trout, we acknowledge that gradual reductions in mean baseflow do not drive extinctions. Rather, rare extreme drought conditions (often regionally synchronous) can dry up streams and their inhabitants with catastrophic long-term consequences, even if they only occur infrequently. While this will certainly pose significant challenges for native trout in the future, it appears to remain secondary to the more proximate threat of invading nonnative salmonids (Figure 2).

Management recommendations

CRCTg share many of the same challenges facing subspecies of Cutthroat Trout across western North America. Though only recently recognized as an identifiable evolutionary unit (Metcalf et al. 2012; Rogers et al. 2018; Bestgen et al. 2019), several decades of conservation efforts for CRCT ensured the persistence of these populations. Now that we recognize that this lineage represents a unique piece of Cutthroat Trout diversity in the southern Rocky Mountains, it is imperative that CRCT conservation efforts continue their recent focus on preserving and replicating CRCTg populations in the near-term with efforts occurring in their native range, so they can enjoy the benefits of conservation programs that have helped other southwestern trout become more secure.

It is clear that although these fish only occupy three percent of their native range, substantial historical diversity remains across the landscape (Rogers 2020). Providing redundancy should be the focus of future conservation efforts. Where possible, direct translocations should be considered to minimize domesticating selection and potential disease transfer when introduced into hatchery settings, while allowing greater flexibility and efficiency in replicating pockets of genetic diversity that still exist (George et al. 2009; Fitzpatrick et al. 2014). In recognition of the broad genetic diversity that still remains and the geographic structuring it reveals, sources for repatriation efforts should be matched to major drainage basins (Fitzpatrick et al. 2014). Consistent with the current CRCT Conservation Strategy (CRCTCT 2006) we also believe it is important to establish several additional CRCTg metapopulations to provide resiliency (Haak and Williams 2012), and mitigate some of the risk associated with small population sizes.

The CRCT Conservation Team has recognized the urgency for preserving the CRCTg, and has already completed numerous projects over the last several years that secure existing diversity (Rogers 2020). We are encouraged by the CRCT Conservation Team's active conservation program, and their robust track record of securing existing populations and replicating them in newly reclaimed habitats. Their efforts provide optimism that our projected probabilities of persistence will prove to be overly pessimistic, and that these precious pieces of native Cutthroat Trout diversity will persist well into the future.

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RESEARCH PRIORITY

Fitness in small Cutthroat Trout populations

OBJECTIVE

Evaluate fitness consequences of inbreeding depression in Colorado's last remaining Greenback 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. Here, 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). With median occupied habitat patch lengths of 6 km (Roberts et al. 2013; Zeigler et al. 2019), many 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). With low Ne, populations can then become more vulnerable to inbreeding depression (Rieman and Allendorf 2001; Allendorf and Luikart 2007).

Nowhere is this problem more pronounced than with the recently rediscovered Greenback Cutthroat Trout *O. clarkii stomias* (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 (Metcalf et al. 2012; Rogers et al. 2018; 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 by a natural waterfall barrier. Their discovery spurred the rapid development of both captive and wild populations as sources for producing progeny as part of a large recovery effort coordinated by the GBCT Recovery Team (USFWS 2019). After the discovery, 66 individuals were brought into captivity in 2008, of which 16 females produced eggs in 2010 that were fertilized with 37 males to develop the initial broodstock. This stock has been supplemented in subsequent years with milt obtained from wild males, and fertilized eggs from an occasional wild ripe female. Hatchery-reared progeny have been introduced into six isolated and geographically distinct reclaimed waters to date, with the goal of establishing multiple viable populations throughout the headwaters of the South Platte basin, the putative native range of GBCT (Metcalf et al. 2012).

While supplemental infusions of milt have helped ensure the broodstock represents the source population well, the source itself has likely faced significant bottlenecks over the past century, first at founding with only a portion escaping their headwater pond confines, and likely subsequently in response to drought or flood events. They currently display the least heterozygosity of any Cutthroat Trout population we have studied (A. Martin, unpublished). In addition, they are extremely challenging to raise in captivity, plagued by poor survival and growth even when cultured in small lots (Rogers et al. in press), and often possess unusual physical deformities.

Large repatriation projects are planned, but apparent inbreeding depression may serve to complicate those efforts (Ralls et al. 1988; Lacy et al. 1996; Hedrick and Kalinowski 2000; but see Visscher et al. 2001; Johnson et al. 2009). Here we explore whether genetic rescue would be a viable method to improve fitness in these last remaining relicts of Cutthroat Trout diversity by comparing performance of the GBCT from Bear Creek with a population representing a sister taxon, the Colorado River Cutthroat Trout (CRCT), as well as their hybrid crosses in a controlled "common garden" setting. We examined four fitness measures on fry: 1) survival to 60-d post-hatch 2) growth of 60-d old fry, along with 3) low dissolved oxygen tolerance, and 4) high temperature tolerance in three-month-old fingerlings, to provide insight into the potential consequences of inbreeding depression on this small population, and whether genetic rescue should be considered (Tallmon et al. 2004; Johnson et al. 2010; Whiteley et al 2015).

METHODS

Spawn timing of the GBCT broodstock derived from Bear Creek was synchronized with broodstock derived from the Carr Creek population of CRCT by raising both stocks on a common water supply at the Leadville National Fish Hatchery. Carr Creek lies on the Roan Plateau, Colorado, and is home to a "green lineage" population of CRCT that appears to be a closely related sister taxon to GBCT (Metcalf et al. 2012; Rogers et al. 2018). We mitigated the chance of an infertile male compromising the experiment by blending stocks of milt from each of two brood sources to fertilize eggs. Milt from each of four ripe Bear Creek males was extruded into a dry glass bowl from which 400 ul was pooled into a flask containing 8 mL of extender (Rogers 2010), oxygenated and stored on ice. This process was then repeated with four Carr Creek males. Each of four gravid Bear Creek females were stripped into two bowls, then fertilized with 1 mL of pooled extended milt from either the four Bear Creek males or the four Carr Creek males. A similar procedure was used with four gravid Carr Creek females to provide a total of 16 distinct families from 16 parents comprising four treatments (Figure 1). Fertilized eggs were water hardened for an hour in 3.8 L drink coolers, randomly assigned a code so that those caring for the eggs would be blind to treatment, then transported to the Colorado Parks and Wildlife Aquatic Toxicology Lab in Fort Collins, Colorado where each family was reared under blind common garden conditions. Upon arrival, eggs were treated with 1600 ppm formalin for 15 minutes (Piper et al. 1982). Two hundred eggs from each clutch were transferred into discrete egg cups and incubated at 10ºC. Egg cups were constructed of 53 mm ID X 75 mm PVC pipe with 1000 µm nylon mesh affixed to the PVC pipe with aquarium-grade silicone adhesive

(Brinkman et al. 2013; Ziegler et al. 2013). Each egg cup was suspended in a 2 L glass tank (18.5 x 9 x 12 cm) and received a flow of 40 mL/min dechlorinated Fort Collins municipal tap water (Brinkman et al. 2013). Egg cups were arranged in a randomized complete block design with four replicates per treatment. Families were randomly assigned numbers 1-16 so identity of parents could be kept blind and then placed in 16 tanks randomized by block.

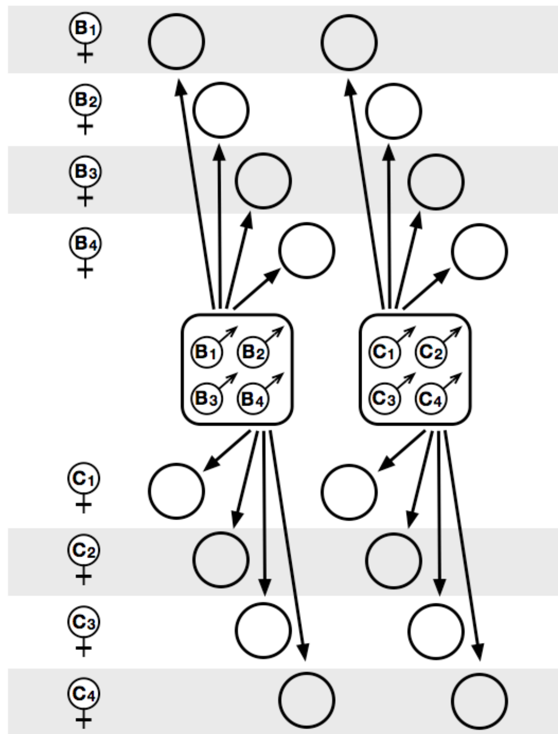

Figure 1. Four gravid females from each of two broodstocks housed at the Leadville National Fish Hatchery were stripped into two bowls each that were then fertilized with either pooled milt from four male Greenback Cutthroat Trout from Bear Creek (B) or four male Colorado River Cutthroat Trout from Carr Creek (C), to generate 16 families.

Survival

Fertilized eggs were monitored daily, with onset of the eyed egg stage, hatch, and swim up being recorded. Hatched fry were carefully removed from each egg cup and released into their respective glass tanks, where they were allowed to develop to swim-up stage. Feeding was initiated once the yolk sacs were absorbed by introducing live *Artemia* nauplii into the tanks and increasing the water temperature to 13.1ºC. Fry were transitioned over to Starter feed (Rangen Inc., Buhl, Idaho) by supplementing with Cyclop-eeze (Argent Chemical Labs, Redmond, Washington). Fry were fed *ad libitum* four times daily with automatic feeders. Feces and uneaten food were siphoned from the tanks daily, along with any egg, larvae, or fry mortalities. Siphoned trout were assigned a sample number and date and preserved in individual vials of 80% ethanol.

Growth

At 60 days post-hatch, ten fish from each family were placed in one of sixteen 2.7 L tanks arranged in a randomized block design where each treatment was represented in each block but blind to those caring for the fish. A water flow rate of 40 mL/min was maintained throughout the study and temperature was maintained at a constant13.1ºC. For the first 8 days, fish were fed 3% of their average weight at Day 0 (60 d post-hatch) based on a batch weight when they were transferred to the test tanks. On Day 8, another batch weight was made and the feeding rate was adjusted to 3% of the average weight from Day 8. The growth experiment ended after 15 days, and another batch weight was obtained.

Hypoxia tolerance

At 14 weeks post-hatch, eight fish from each family were subjected (individually, still blinded) to hypoxia trials to measure when loss of equilibrium occurred with dropping dissolved oxygen levels. Individual fish were placed in 1.75 L glass aquaria fitted with an airstone, a titanium cooling loop, and a temperature probe. The airstone supplied pure nitrogen which served two functions; decreasing the partial pressure of oxygen in the tank in order to remove oxygen from the water, and circulating the water within the tank. Temperature in the tank was maintained at 12.0°C using a temperature probe and temperature controller (Love B-series, Dwyer Instruments, Michigan City, Indiana) which supplied power to a peristaltic pump that supplied ice water through a titanium heat exchanger submerged in the tank. Oxygen levels in the tank were measured using an optical dissolved oxygen probe (ProODO, YSI Inc., Yellow Springs, Ohio). For each trial, fresh 12.0°C water was first added to the experimental chamber, then a fish was introduced and allowed to acclimate for 5 min before the supply of nitrogen was initiated. Oxygen concentrations were monitored continuously while fish were carefully observed to determine when loss of equilibrium (failure to maintain a dorsal-ventral vertical orientation) would occur. The oxygen concentration was recorded when sustained loss of equilibrium exceeded 30 s, at which point the fish was placed in a recovery tank.

Thermal tolerance

Fish used in the hypoxia test were allowed to recover for 7 days before being subjected to a Critical Thermal Maximum (CTM) challenge (Becker and Genoway 1979). These trials occurred in the same 1.75 L tanks described above fitted with the same programmable temperature controller which regulated a submersible aquarium heater to heat the water at a rate of 0.3°C/min, as is standard (Becker and Genoway 1979; Wagner et al. 2001; Underwood et al. 2012). Aeration of the tank maintained saturated dissolved oxygen levels and ensured homogenous temperatures throughout the chamber. Water temperatures were increased until sustained (≥10 s) loss of equilibrium was observed in the fish being tested, at which point the temperature was recorded. Following the test, fish were removed from the experimental tank and allowed to recover.

We used analysis of variance (ANOVA) to test for differences among treatment groups for each fitness measure in R (R Core Team 2020). Treatment means were compared using Tukey's honest significant difference. Genetic diversity of the source populations used to develop the broodstocks was measured using Amplified Fragment Length Polymorphisms (AFLP) on archived DNAs (Rogers 2008; Bestgen et al. 2019) and the program AFLP-SURV (Vekemans et al. 2002). The AFLP selective amplification procedure followed Vos et al. (1995), using the restriction enzymes EcoRI and MseI, with three base selective primers (RI-ACT and MseI-

CAG). Amplified fragments were run on an ABI3130 Genetic Analyzer (36cm array, POP7 polymer) with GeneScan ROX 500 as the size standards. The fragments present in each sample were scored in GeneMapper 4.0 using a binset of 119 fragments previously used for Cutthroat Trout AFLP analyses (Metcalf et al. 2007; Bestgen et al. 2019). Data files were configured for AFLP-SURV and expected heterozygosity (Hj) was calculated using the Bayesian method with non-uniform prior distribution of allele frequencies assuming Hardy-Weinberg equilibrium (inbreeding coefficient Fis = 0.0).

RESULTS

When compared to other native Cutthroat Trout populations across Colorado (Figure 2), our study populations harbored either more genetic diversity (Carr Creek) or much less (Bear Creek). Not surprisingly, expected heterozygosity calculated from AFLP markers was three-fold lower in the Bear Creek source population than the Carr Creek population.

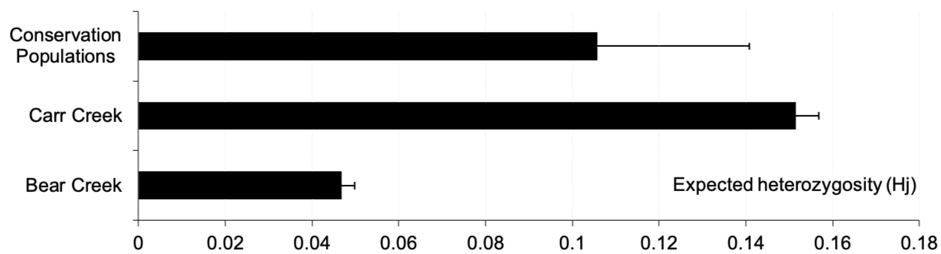

Figure 2: Expected heterozygosity from both Carr Creek and Bear Creek compared to an average Hj value generated from nine conservation populations of native Cutthroat Trout in Colorado. Error bars represent the 95% confidence interval on mean Hj values for the conservation populations (top bar), or around Hj calculated for each population (Carr Creek and Bear Creek).

Survival was monitored daily up to 60-d post-hatch when the growth study was initiated, though the last mortality was recorded at 50 days. Survival was not equal among groups ($P = 0.036$) through 60 d post-hatch. While mean survival for the Carr Creek fish (47.1%) and the hybrids (45.6% and 47.7%) were not different ($P > 0.995$), survival was markedly reduced in the Bear Creek fish (Figure 3a; 20.7%; $P < 0.082$). A similar trend was observed for growth after 60-d post-hatch where again groups were not equal ($P = 0.001$). Average growth of Bear Creek individuals was two-fold lower (0.014 g/g/d) than the Carr Creek and hybrid fish (Figure 3b; 0.028 g/g/d in all cases; $P < 0.003$). Hypoxia tolerance (Figure 3c) and CTM (Figure 3d) were not different among groups ($P = 0.705$, 0.744 respectively).

Some additional observations were made while raising these fish for the fitness challenges discussed above. First, all four families with progeny from two of the Bear Creek mothers contained some albino fry. This occurred regardless of whether eggs were fertilized with Bear or Carr Creek milt, suggesting maternal influence for this trait. In fact, the highest proportion of albino fry were recorded for progeny from Carr Creek milt (Tank 13). Additionally, technicians responsible for raising the fish (and blind to the provenance of the fish in each tank) noted that "tanks 1, 4, 5, 6, 9, 11, and 14 appear to behave differently than the other tanks," and that "the fry seem to avoid light to a greater extent." While these additional comments were unsolicited, indeed these tanks were all progeny from Bear Creek mothers, regardless of whether they were sired by Bear or Carr Creek fathers.

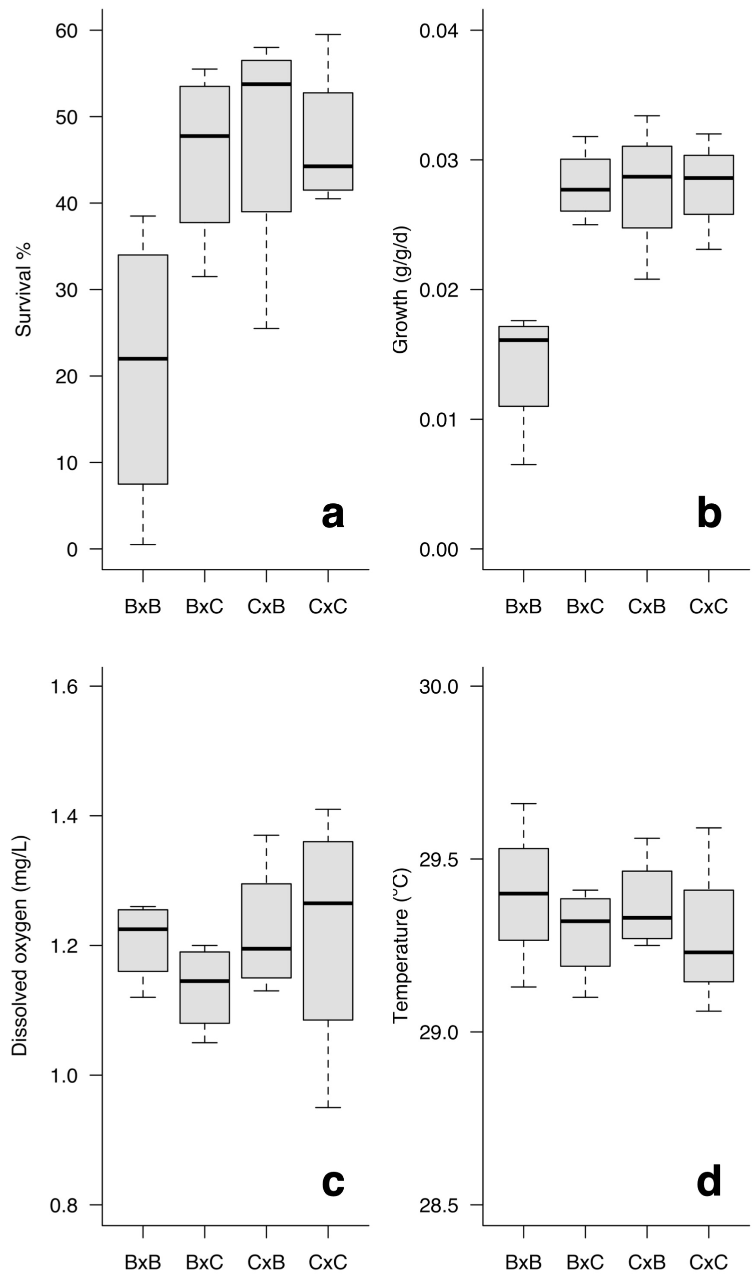

Figure 3: The X-axis labels represent parental fish from Bear Creek (B) or Carr Creek (C) where the first letter represents the female and the second the male in each group of family crosses. Survival to 60-d post-hatch by cross type (a), growth rate at 60-d post-hatch (b), low dissolved oxygen tolerance at 14 weeks (c), and critical thermal maxima at 15 weeks of age (d).

DISCUSSION

Observed survival in Bear Creek fish was only half of what Carr Creek or the hybrid trout were able to achieve. Differences in growth were even more pronounced, with mean values in the Bear Creek trout again being half of what the hybrids and Carr Creek trout displayed. These results provide compelling evidence for an effect of parental relatedness on survival and growth (Figure 3). Offspring survival from the hybrid crosses were identical to that seen in the Carr Creek trout, suggesting that the observed reduction in fitness of the Bear Creek fish is best explained by the effects of recessive, deleterious alleles. Poor survival and growth seen here is consistent with early life history consequences of inbreeding registered in other vertebrate studies on birds (Westemeier et al. 1998; Bensch et al. 1994; Spottiswoode and Møller 2004) and mammals (Slate et al. 2000; Bensch et al. 2006; Johnson et al. 2010).

Heterosis did not increase individual tolerance of hypoxia or CTM, perhaps because the response in parental stocks were also similar (Figure 3). Although others have demonstrated variation in CTM even within subspecies (Underwood et al. 2012), some have suggested that upper thermal tolerance limits are governed by molecular pathways that may not be very plastic (Chown et al. 2010; Logan and Buckley 2015; Ooman and Hutchings 2017). These trout may already be operating at close to the maximum attainable level of thermal and hypoxia tolerance, and increased heterosis may not be able to change that.

Fragmented populations are at high risk for inbreeding depression (Hedrick and Kalinowski 2000) that can lead to local extinction (Lande 1988; Frankham and Ralls 1998; Johnson et al. 2010). In the absence of pedigree data, measures of heterozygosity have long been used as a proxy for inbreeding coefficients in order to identify the costs of inbreeding, and are strongly correlated with fitness measures (Bensch et al. 2006). As anticipated, expected heterozygosity was extremely low in the Bear Creek trout (Figure 2), suggesting that the population has endured at least one substantial bottleneck, and that many more might have been possible. Despite some level of inbreeding depression in the Bear Creek trout, they have been able to persist in their isolated headwater habitat for over 130 years. In stable systems, inbreeding depression-like effects may not manifest themselves, particularly over the near term (Markert et al. 2010). However, the goal for the Bear Creek broodstock is to use their progeny to repatriate GBCT across their former range in the South Platte River basin. How well these fish perform in more challenging environments has yet to be determined, though other studies on inbred organisms suggest this may be problematic (Spielman et al. 2004; Frankham 2015).

Genetic rescue has been proposed as a way to mitigate the negative effects of inbreeding (Allendorf et al. 2001; Tallmon et al. 2004). It can be especially useful for management and conservation because it induces population-level demographic responses with the introduction of new, beneficial alleles (Whiteley et al. 2015; Fitzpatrick et al. 2020). Importantly, one only need introduce a few individuals to a population to see a strong positive response (Mills and Allendorf 1996; Frankham 2015). While genetic rescue remains controversial and is seldom implemented (Tallmon et al. 2004; Whiteley et al. 2015), reticence is usually centered around whether outbreeding depression will make the target population less fit (Edmands 2007; Frankham et al. 2011). Our data provide evidence that this would likely not be the case for GBCT. Rather, the concern here lies squarely on the fact that rescue in this case would necessarily cross alleles from a different subspecies into the population, thereby setting the stage for extinction through hybridization (Rhymer and Simberloff 1996). This scenario is similar to the high-profile dilemma that faced managers trying to save the Florida Panther in the 1990s (Johnson et al. 2010), although the panther subspecies diverged much more recently (Ochoa et al. 2017) than the Cutthroat Trout subspecies (Shiozawa et al. 2018). The stakes are high, as one would not just be resetting the evolutionary trajectory of a population, but also of an entire subspecies as the Bear Creek stock is all that remains of the GBCT.

While recovery and conservation efforts have generally focused on repatriation of indigenous fish free of nonnative alleles (UDWR 2000; Allendorf et al. 2001, 2004), the Bear Creek situation forces us to at least consider alternatives like genetic rescue. Managers should address several questions when contemplating intentional hybridization in this case: 1) How well do Bear Creek fish represent the native trout of the South Platte basin? Backing up the evolutionary trajectory of these fish with genetic rescue might be more palatable if genetic drift following substantial past population bottlenecks has made them poor representatives of the subspecies. 2) Are the detrimental effects of inbreeding depression readily apparent (e.g. reduced viability or an increased proportion of deformed or asymmetric individuals; Allendorf et al. 2001)? 3) Would donor populations offer ecological exchangeability (Crandall et al. 2000), serving the same function in a similar environment?

When deliberating implementation of a rescue program, we believe it is also important to acknowledge that management actions do not have to reflect a binary outcome. The Bear Creek population could be replicated a number of times to secure against further loss, while rescue could be considered in additional populations where environmental conditions are particularly challenging and population persistence would be more likely with additional heterosis (Markert et al. 2010). These two management strategies could even be implemented in the same reclamation project if the system were large enough to accommodate a replicated Bear Creek trout population above barriers to upstream passage, but allow genetic rescue to proceed down below to help foster a more robust population. These scenarios would serve as ideal *in situ* experiments for comparing population growth, individual growth and survival, recruitment, and other key demographic factors. Careful monitoring of these key demographic traits would then help inform whether additional intentional hybridization events should be considered in other reclaimed and repatriated populations of the iconic GBCT.

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RESEARCH PRIORITY

Habitat monitoring in trout streams

OBJECTIVE

Metal housings improve recovery of stream temperature loggers

INTRODUCTION

Water temperature is perhaps the single most important environmental parameter regulating fish populations (Fry 1967; Beitinger and Fitzpatrick 1979; Golovanov 2006). As ectothermic organisms, ambient temperatures influence behavior (Rogers 1998; Elliott 2000; Goniea et al. 2006), growth (Meeuwig et al. 2004; Bear et al. 2007; Rogers et al. 2022), survival (Selong et al. 2001; Bear et al. 2007; Brinkman et al. 2013), competition (DeStaso and Rahel 1994; McMahon et al. 2007), and other physiological processes (Brett 1956; Pörtner & Farrell 2008), as well as defining the range a fish can occupy (de la Hoz Franco and Budy 2005; Isaak et al. 2013a). As such, the fate of coldwater fish in a warming climate has garnered significant attention and concern (Ficke et al. 2007; Rahel and Olden 2008; McCullough et al. 2009; Paukert et al. 2021), resulting in a flurry of modeling efforts to predict future conditions (Loarie et al. 2009; Wenger et al. 2011), particularly surrounding iconic native trout species (Peterson et al. 2013; Roberts et al. 2013, Zeigler et al. 2019). These models require robust field data to parameterize (Isaak et al. 2017; Zeigler et al. 2019), which, in combination with the availability of relatively inexpensive and durable temperature loggers has precipitated an explosion in the use of these devices.

While initially focused on maximum summer temperatures and its role in population persistence (Roberts et al. 2013; Zeigler et al. 2019), or meeting water quality standards (Todd et al. 2008), demands for water temperature monitoring increasingly require understanding the effects of temperature at other times of year (Isaak 2013b). Some of these applications include predicting spawn timing (K. B. Rogers, unpublished) and fry emergence (Crisp 1988; Beacham and Murray 1990, assessing the duration of the growing season and degree day accumulation (Harig and Fausch 2002; Coleman and Fausch 2007; Webb et al. 2008; Isaak et al. 2012). These questions require year-round temperature monitoring, with secure long-term deployments that can further reduce the number of site visits required if they are robust to multiple runoff cycles.

A wide variety of methods have been employed to secure these in stream and lake environments being monitored, with varying degrees of success (Dunham et al. 2005; Isaak and Horan 2011; Olsen 2013; Zeigler et al. 2013). Long-term retention is crucial, as data recorded on lost loggers can never be recovered, representing a missed opportunity to gather what is often critical temperature information. Now that we are asking more questions of the data than simply detecting peak summer temperatures, we need to use robust ways to deploy and recover sensors so that they can collect data year-round without concern of loss during peak runoff, ice flows or human tampering

While commercially available loggers are often designed to be deployed without a protective housing, they are recommended (Dunham et al. 2005), not only to facilitate anchoring the sensor to the stream or lake environment to be monitored, but for shielding the logger from solar radiation (Isaak and Horan 2011), and protecting sensitive electronics (Isaak et al. 2013b; Olsen 2013). Many have used PVC for this purpose as a lightweight material that is easy to work with (Isaak and Horan 2011; Zeigler et al. 2013; Chapin et al. 2014). The logger is then generally attached to some form of anchor ranging from large rocks, logs, concrete blocks, stakes or even fastening loggers to nylon bags filled with rocks to lighten the load on backcountry deployments (Dunham et al. 2005). Isaak and Horan (2011) have advocated for affixing temperature loggers to large boulders with underwater epoxy. While this approach works well in streams with large boulders or other structures to which the loggers can be fastened, such opportunities are not always available. In many situations, researchers wish to monitor stream temperatures in habitats that lack of large boulders on which to attach these sensors – particularly in the deepest pools that may represent the only wetted habitat that remains during drought conditions. In addition, some land use restrictions prevent the use of epoxy in National Parks or Wilderness areas (Olsen 2013).

Regardless of the approach used, the studies referenced above still report losses in excess of 10%, sometimes as high as 30%, despite deployment times that rarely exceeded one year . We argue these losses are still too high given that we only have a single opportunity to acquire this data in a changing climate. Here we evaluated the benefit of using low profile metal housings to mitigate the three main reasons for deployment failure and data logger loss outlined by Dunham et al. (2005): (1) failure to relocate the data logger after initial field deployment; (2) human tampering or vandalism; and (3) natural disturbances. Inconspicuous housings not only protect and secure temperature loggers in the stream environment, but allow the use of a metal detector to aid in their detection and recovery following deployment. These housings were fabricated from inexpensive materials, and only basic tools were required to rapidly assemble and deploy them without need for welding or gluing.

METHODS

Metal housings to protect the temperature loggers used here (HOBO Water Temp Pro v2, Onset Computer Corp, Bourne, Massachusetts) were fabricated from inexpensive materials readily available in most hardware stores. Though prices for individual pieces of hardware are reflected (Table 1), substantial savings can be achieved by purchasing in bulk. The cylindrical temperature loggers (Figure 1) were inserted into housings cut from 1 ¼ in metal electrical conduit, though other sizes of conduit can be substituted to accommodate different logger dimensions. The conduit was cut in 6 in lengths with a chop saw fitted with suitable blade for cutting metal. After filing rough edges, a ¼ in hole was drilled 0.6 in down from the top of the tube. The logger was then held in place by slipping the stainless steel eye-bolt through both holes in the housing after passing through the one on top of the logger, then secured with the lock washer, nut, and stop nut respectively (Figure 1). A stainless-steel carabineer attached the housing assembly to an 18 in ring stake (Item # 78426; http://www.murdochs.com/shop/grip-18-rebar-metal-stake-with-loop/) used to secure the housing to the stream bed.

Table 1. Materials used to fabricate the metal housing and stake for deploying temperature loggers in the field. Stainless steel (SS) hardware was used wherever possible.

Hardware	Dimensions	Cost[a]
Metal conduit (6 in lengths)	1 ¼ in diameter[b]	$1.10
SS eyebolt	¼ in x 3 in	$2.39
SS carabineer	5/16 in x 2 3/8 in	$6.59
SS lock washer	¼ in	$0.19
SS nut	¼ in	$0.18
SS stop nut	¼ in	$0.40
Ring stake (24 inch lengths)	½ in diameter	$3.33
	Total per unit	$14.87

[a]In US$
[b]Electrical conduit actually has an outer diameter of 1 ½ in, but is listed as 1 ¼ in

Figure 1. A cut piece of metal conduit was used to provide the metal housing that protects and shades the temperature logger held in place by a stainless-steel eyebolt. Additional stainless-steel hardware used to fasten the housing to a ring stake which was pounded into the substrate with a metal extension rod.

Deployment
Loggers were deployed in sites that were exposed to primary flow, in areas that would remain underwater even during drought conditions. Sites where the stream scoured naturally were preferred so that sediment did not bury the logger and attenuate the diel fluctuations in recorded temperatures (Rogers 2015). Once a site was selected, the ring-stake was pounded into the substrate with a small sledge hammer such that just the ring remained above the streambed. Rather than attempting to strike the stake underwater, we used a removable pounding extension manufactured from a 5/8 inch zinc-plated coupler ($3.59) connected to a two-foot zinc-plated threaded rod ($6.29) wrapped with duct tape to cover the threads (Figure 1).

Simply obtaining GPS coordinates of the deployment site is generally insufficient to reliably recover deployed loggers (Dunham et al. 2005). As such, we also obtained a photograph of each site, with the pounding extension marking the precise location of the logger. In addition, the date, time, elevation, discharge if available, and a brief description of the deployment site was recorded. Metal housings containing new loggers were swapped out with those in the field on subsequent visits, with temperature data downloaded in the warm confines of the lab rather than in the field. Loggers were generally deployed in the fall to take advantage of base flow conditions, but were allowed to collect data year-round with this approach with minimal risk of logger loss even when exposed to ice and debris flows or high runoff.

Recovery
From 2010-2022, a three-step process was used to relocate and recover deployed temperature loggers. First, a brief written description and UTM coordinates were used to locate the site. If after 5 min search no logger was found, then a digital image of the deployment site was consulted, and the search continued for a minimum of 5 additional minutes. If the logger was still not detected, then a metal detector (Lobo Super Traq, Tesoro Electronics, Prescott, Arizona) with a waterproof removable head was used to continue the search. A minimum search time of 20 minutes was allocated before a logger was considered lost.

RESULTS

Stream temperatures were monitored from September 2010 through November 2022 at 82 stations in trout streams spread across Colorado using the protocols described here. Stations were distributed in first through sixth order streams and rivers ranging in elevation from 5611 - 11110 ft. They were visited anywhere from 19 to 2174 days after deployment (mean days-at-large = 539 d; median = 368 d) to replace the logger with a new one, such that we accumulated data from 312 deployments over that 12-year period. We successfully recovered the temperature logger in 300 cases. In 139 instances (44%), we were able to able to find the logger from memory, armed only with UTM coordinates and a brief description of the site. Some stations were established as early as 2005, prior to the rigorous recovery protocol, and have accumulated as many as 17 repeat visits over that time. An additional 88 deployments (28%) required study of digital photographs in the field to locate the logger for a combined recovery rate of 73%. Even with precise photographs of the pounding extension indicating the logger position during deployment, natural events and shifting substrate concealed an additional 73 loggers (23%) until the metal detector was used, for a combined recovery rate of 96% when all three approaches were available.

Twelve loggers (4%) were never recovered. Seven of these were attributed to intentional human tampering (the ring stake anchoring the housing to the stream bed persisted in four cases). One was inadvertently buried by the construction of new bridge abutment and another was buried under a large barrier built to exclude nonnative salmonid invasions. Only three losses were attributed to natural causes brought about by radically altered stream habitats the result of a rock slide or log jams rearranging stream morphology substantially.

DISCUSSION

Even armed with photographs of deployment locations in addition to descriptions and UTM coordinates, our recovery rates here were lower than typically experienced by other deployment methods. This is not surprising and is no doubt a result of the inconspicuous nature of this protocol. By using a short pin that doesn't extend much above the stream bed, not only is the deployment less vulnerable to high flows and debris that might accumulate on a longer piece of rebar, the subtle low profile makes them less vulnerable to human tampering. This is particularly true if algae growth or invertebrates (e.g. trichoptera cases) camouflage the housing or if it is covered by a large rock when deployed. The benefits of this system are not fully realized until the metal detector is deployed. Not only does the housing provide exceptional durability, but the simultaneous use of a detector greatly enhances recovery rates bumping them from 73% to 96%, and speeds locating deployed temperature sensors when used at the outset.

Because the same individual set and recovered the loggers here, learning and memory was a powerful recovery tool as indicated by the 44% recovery rate essentially from memory, that climbed with repeat deployments. We acknowledge that our recovery results were likely higher than would be experienced by a larger crew where individuals making repeat site visits over time are not possible. It is in these situations, that the metal detector becomes even more valuable tool.

Deployment considerations

In addition to methods described here to facilitate recovery, several other factors should be considered when deploying temperature loggers to ensure that robust data is acquired. For example, during dry years, it is possible that a logger will become exposed and begin recording air rather than stream temperatures. This obviously will compromise the integrity of the data and should be screened for to ensure that the resultant water temperature data are accurate and reliable for decision-making purposes (Chapin et al. 2010). One advantage of year-round deployments is that loggers can be set out during base-flow periods in the fall, when the deepest portions of the stream channel can be readily identified and accessed. In an effort to keep a logger from recording air temperatures, one may be tempted to place them in deep depositional areas where they can become buried. Although this might not change mean temperature values much, it can dramatically attenuate the diel temperature fluctuations experienced by the logger (Rogers 2015) and therefore values like the Daily Max or MWMT. It is important to select sites where the logger will be scoured clean rather than buried.

Since this approach provides a secure method for long-term deployments, one might reconsider how often to acquire temperatures. Rapid recovery made it possible to acquire readings frequently since it is unlikely that storage capacity would be unable to accommodate the additional data. With secure deployment options however, it is realistic to be able to recover loggers 3-4 years after deployment. The biologist is then faced with a tradeoff – higher resolution data or long-term recovery. Colorado state water quality standards require readings every 20 minutes (Todd et al. 2008), yet hourly intervals rarely miss peak temperatures (Dunham et al. 2005). As such, it really depends on the question being asked. Hourly readings are adequate for calculating average daily temperatures or degree day accumulation but if monitoring for peak temperatures in in small streams with large diel temperature fluctuations that will be subjected to intense public scrutiny, then perhaps half hour intervals are warranted. We do not advocate for longer intervals simply because current loggers can store 5 years of hourly temperature readings already, and if the data is not needed during that time then it probably wasn't necessary to begin with.

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RESEARCH PRIORITY

Technical assistance

OBJECTIVE

Develop a standard weight equation for Bluehead Sucker

INTRODUCTION

The Bluehead Sucker *Catostomus discobolus* is native to the Colorado River Basin as well as the Bonneville Basin (primarily the Bear and Weber rivers) in Wyoming, Idaho, and Utah, and the Snake River Basin in Wyoming and Idaho. In the Colorado River Basin, it inhabits both main-stem rivers and tributaries from headwater areas of the Colorado and Green rivers below about 8500 feet to the lower end of the Grand Canyon of the Colorado River. Bluehead Sucker is presently thought to occupy just 50% of historic habitat in the Colorado River Basin (Bezzerides and Bestgen 2002), with reductions in occupied habitat thought to stem from dam emplacement, related habitat modifications (lotic to lentic), flow regime changes (warm and turbid to cool and clear), and alterations in sediment transport dynamics. Within presently occupied habitat, further pressure arises from competition with or predation by introduced nonnative fishes. The species is likely even more imperiled in the Bonneville Basin (Webber et al. 2012; Bangs et al. 2017). Further complicating their future, they are known to hybridize with introduced White Sucker *C. commersoni* (Douglas and Douglas 2007; McDonald et al. 2008) and Longnose Sucker *C. catostomus* (Mandeville et al. 2015; Mandeville et al. 2017), two species whose ranges are likely to increase within the native range of Bluehead Sucker. They also hybridize with Bonneville Basin native Utah Sucker *C. ardens* (Bangs et al. 2017), perhaps partly driven by their reduced numbers compared to Utah Sucker in some sympatric populations.

Bluehead Sucker, along with the often sympatric Flannelmouth Sucker *C. latipinnis* and Roundtail Chub *Gila robusta*, are the subject of a "Range-wide Conservation Agreement and Strategy" (UDWR 2006). This agreement was initiated because of concerns over range contraction coupled with relative paucity of information about the subject species, often collectively referred to as the "three-species". Participants in the agreement include seven states as well as Tribal and Federal entities. The signatory states are obligated to produce state-specific management plans to conserve the subject fishes in order to avoid listing under the Endangered Species Act. Bluehead Sucker is currently listed as a "Tier I species of concern" by Utah and as "special concern" by Wyoming.

In recognition of declining status and the need for more active management around the three-species, Didenko et al. (2004) developed standard weight (W_s) equations for both Flannelmouth Sucker and Roundtail Chub, but unfortunately not Bluehead Sucker. First developed by Wege and Anderson (1978), the W_s equation approach is used as a means to compare fish condition (expressed as relative weight, W_r) among populations of a species across its range, or to examine trends within a population over time, and has served as a useful piece of information for managers charged with monitoring fish populations (Blackwell et al. 2000; Neumann et al. 2012). The W_r concept has gained wide acceptance by fisheries managers – about half of state agencies in the United States reported its use as a standard fishery evaluation technique by the mid-1990s (Blackwell et al. 2000). The use of W_r is now the prevailing method of for evaluating condition using length-weight data from fisheries where insight into population health is desired. As an intuitive way to visualize summarized weight-length information, W_r improved on earlier methods such as Fulton's condition factor (K) and relative condition factor (K_n) by reducing length related bias (Blackwell et al. 2000).

Refinements in the development of standard weight equations sought to reduce bias further for certain species (Willis 1989). Modifications proposed by Murphy et al. (1990) resulted in the regression-line-percentile (RLP) method that was quickly adopted as the standard technique (Blackwell et al. 2000). More recently, Gerow et al. (2004, 2005) demonstrated the likelihood of length-related biases using RLP for development of W_s equations and proposed a new method, termed the empirical data (EmP) method, to avoid such biases. The primary difference between the two is that EmP uses the means of measured weights rather than the means of predicted weights to preclude the introduction of modeling artifacts into the W_s equation (Gerow et al. 2005; Gerow 2010). Ranney et al. (2010) examined both methods using data sets for two species, and concluded that EmP did not alleviate length bias problems in larger fish, and that the differences between W_r calculated by the two methods were inconsequential for their intended usage. A scholarly exchange ensued on the relative merits of the two methods (Gerow 2011; Ranney et al. 2011; Rennie and Verdon 2012), but no consensus was reached. As such, we set out to develop equations using both methods and compare their relative performance for Bluehead Sucker.

Relative weight was used primarily to evaluate condition in game fishes through the early years of implementation, although W_s equations were developed for non-game species early on (Anderson 1980). More recently, fisheries managers have argued that such equations have utility for non-game fishes as well and have developed them for more than 20 species (Bister et al. 2000; Didenko et al. 2004; Richter 2007; Rypel and Richter 2008). As agencies seek to manage Bluehead Sucker, it is important that a full suite of population evaluation tools are available. Therefore, our objective was to develop W_s equations for Bluehead Sucker using both RLP and EmP approaches to complement the work of Didenko et al. (2004), who developed equations for the other two members of the three-species group.

METHODS

Weight-length data for Bluehead Sucker were solicited from biologists and researchers representing the state wildlife agencies managing waters comprising native range for the species, as well as from the U. S. Geological Survey. Generally, we considered data from discrete waters to represent discrete populations. In addition, where large rivers (e.g., Green River or San Juan River) were sampled in multiple states, datasets were maintained separately. Lengths (L) were measured as total length (TL) in mm, and weights (W) in grams. We pooled data from individual populations across available years of data, as did Didenko et al. (2004) for the other members of the three-species complex, which allowed us to meet minimum sample size for some populations.

Ensuring data integrity is critical in the development of W_s equations, as aberrant data can influence model fit (Ranney et al. 2010). Rather than simply scanning raw *W–L* data plots for obvious measurement or data entry errors as has been common practice in the past (e.g., Rogers et al. 1996; Bister et al. 2000; Rennie and Verdon 2008; Rypel and Richter 2008), we sought to make the filtering process more objective by culling fish whose weights fell outside 3.29 SE from the fitted $\log_{10}(W) - \log_{10}(L)$ regression line for each population. Assuming a normal distribution, this tactic excludes one in a thousand samples. Data displaying values outside of this range are very unlikely to legitimately represent the population, and were removed from subsequent analyses. Populations with *W–L* records from fewer than 10 individuals, those with non-significant linear *W–L* regressions on $\log_{10}$-transformed data (P>0.010), or poor correlation coefficients (R^2<0.80) were also excluded (Brown and Murphy 1996; Rogers et al. 1996; Rennie and Verdon 2008).

Custom code was written in the LabVIEW programming environment (National Instruments, Austin, Texas) to generate W_s equations (Rogers et al. 1996; Rogers and Koupal 1997) using both the RLP (Murphy et al. 1990) and EmP (Gerow et al. 2005) approaches. The Blom Method was used for establishing the third quartile for each centimeter increment, as it is particularly well suited for minimizing bias associated with small sample sizes often found with the largest size classes of fish (Gerow 2009). A minimum fish size to incorporate in the analysis was determined by plotting the variance to mean ratio on cm increments for all fish, and determining where that ratio dipped below 0.01 (Guy et al. 1990, Murphy et al. 1990, Didenko et al. 2004). We used a maximum fish size for the RLP equation equal to the largest fish in our dataset, and the largest bin with at least three populations represented to serve as the maximum value for the EmP equation (Gerow et al. 2005). We elected not to split the original dataset, because doing so would have dropped us below the 50-population threshold outlined by others as necessary for the development of a robust W_s equation (Brown and Murphy 1996; Brenden and Murphy 2006). Rather, we used the approach of Bister et al. (2000) and evaluated whether individual population regressions of W_r on L resulted in a consistent bias toward positive or negative regression slopes. We used a 2x2 $\chi 2$ contingency table to test the hypothesis that there was no difference in the number of significant ($p \leq 0.05$) positive and negative regression slopes, using $\alpha = 0.05$.

RESULTS & DISCUSSION

Weight-length data were provided from 110 populations across six states encompassing the native range of Bluehead Sucker in the southern Rocky Mountains. Forty-five of the populations contained fewer than 10 records, and were removed from subsequent analyses. Individual data points from the remaining 65 populations were excluded if measured $\log_{10}(W)$ exceeded 3.29 standard errors distance from the predicted $\log_{10}(W)$ of the population-specific regression, as these likely represented invalid data points. The resulting population data sets were concatenated so that the variance to mean ratio by centimeter group could be determined (Figure 1). The ratio remained below 0.01 for fish ≥ 130 mm, thus establishing our minimum size threshold, and all fish records less than this were removed from the individual population data sets. As a result of these quality-control measures, eight additional population data sets were culled because they fell below the ten fish threshold, yielded non-significant $\log_{10}(W) - \log_{10}(L)$ regressions (P>0.010), or displayed underwhelming correlation coefficients (R^2<0.80).

The final filtered data set comprised 30,713 fish from 57 populations (Table 1); four of these population datasets arose from the Snake River and Bonneville basins, where Unmack et al. (2014) have proposed different species status (*Pantosteus virescens*). We chose to keep them in our dataset because the morphologies of these fish are similar, and the re-classification has not yet been accepted by the American Fisheries Society. Thus, despite a relatively small range for this species and widespread special concern status, we were able to meet the population sample requirement guidelines outlined by Brown and Murphy (1996) and Brenden and Murphy (2006), and the numbers per length class recommended by Gerow et al. (2005) and Ranney et al. (2010). There were only 63 fish over 480 mm in our data set (a very large size for Bluehead Sucker) resulting in fewer than 50 fish per length class for those few largest length bins, as may be expected for studies encompassing the entire length range of a species.

Using a maximum size of 550 mm (the largest fish recorded came from the Weber River in Utah), these data were used to generate the following 75th percentile RLP equation for Bluehead Sucker:

$$\log_{10}(W)_s = -4.987 + 3.012 \log_{10}(L)$$

Where W is weight in grams and L is total length in mm. Using the EmP method, and a maximum size of 520 mm (the maximum length bin represented by three populations), we generated a standard 75th percentile EmP equation of:

$$\log_{10}(W)_s = -4.987 + 3.012 \log_{10}(L)$$

and a quadratic form of:

$$\log_{10}(W)_s = -5.275 + 3.216 \log_{10}(L) + -0.035 (\log_{10}(L))^2$$

Values of W_r from all three equations plotted similarly (Figure 2). The difference between relative weights generated by EmP and RLP equations was < ± 2.2% over the length range 130-480 mm, with EmP estimating higher W_r at the lower end of the length distribution but lower W_r at the upper end (Figure 3). At our maximum length of 550 mm, the EmP method resulted in W_r that was 2.6% higher than that derived with RLP. The difference in W_r between the linear and quadratic forms of EmP was less than 0.9% across the entire length range, with the quadratic form estimating slightly greater W_r throughout. In other sucker species for which both types of equations have been developed, a similar pattern of higher W_r values from EmP compared to RLP in the lower end of the length range was evident for Bridgelip Sucker *C. columbianus* and Largescale Sucker *C. macrocheilus* (Richter 2007) as well as for Blacktail Redhorse *Moxostoma poecilurum* (Rypel and Richter 2008). The EmP-derived W_r values remained higher throughout the length range for Bridgelip Sucker and Blacktail Redhorse, but the difference diminished with increasing fish length. In all cases, the magnitude of the difference seen in the lower length range was greater than we observed for Bluehead Sucker.

Our approach to test for a difference in the prevalence of positive or negative regression slopes that were significantly different than 0 resulted in a $\chi 2$ value of 1.018, df = 1, $p = 0.313$. Thus, there was no evidence in our development data set for a consistent bias in either direction. Our evaluation approach was driven by two considerations. First, Bister et al. (2000) argued that splitting data sets into development and evaluation portions in early RLP W_s equation research and development was actually a test of the RLP approach itself, and its success in fulfilling the purposes for which it was conceived for many prior equations demonstrates that the approach itself is reliable. Second, Bonvechio et al. (2010) argued for a species with very limited distribution, Suwannee Bass *Micropterus notius*, that validation data are less necessary because they are less subject to environmental variation that would drive condition differences across the range. While Bluehead Sucker are not as range-limited as Suwannee Bass, they are far more restricted than many game fish species that have been the subjects of W_s equations. Since our subject species' range is geographically limited to six states in the western United States, and within that range is further limited in the types of waters it inhabits year-round, and furthermore that our development data included populations from the entire range, we opted to use as large a development data set as possible. The number of populations used in the development of our equations exceeded the number used by Didenko et al. (2004) for the other members of the three-species group.

Although either of the linear equations we developed should prove adequate for general management when comparing unexceptional fish, we recommend the use of the RLP-derived W_s equation developed here, given the very minor differences among the three equations and that the W_s equations developed for the other members of the three-species group also used the RLP method (Didenko et al. 2004). Moreover, the W_s equation for White Sucker, widely introduced across the native range of Bluehead Sucker, also employed the RLP technique (Bister et al. 2000).

We do not think length-related bias is of great concern for Bluehead Sucker given the minor differences among the three equations' performance. Moreover, managers are seldom making decisions based on the condition of memorable or larger fish, and the differences in values generated among these three equations are very small when considering the typical mature adult length range of 250 – 420 mm. The equations presented here now allow managers to consistently use W_r for the entire three-species group of fishes.

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RESEARCH PRIORITY

Information transfer

OBJECTIVE

Disseminate results gleaned from applied research efforts

INTRODUCTION

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

ACCOMPLISHMENTS

Peer-reviewed publications

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

Abstract.— With temperatures expected to rise across the southern Rocky Mountains, the ability of native fishes to tolerate stream warming has become a critical concern for those tasked with preserving coldwater species. We used common garden experiments to evaluate the thermal tolerance of Cutthroat Trout *Oncorhynchus clarkii* fry from five populations important to managers representing three sub-species. Critical thermal maxima (CTM) were evaluated through traditional exposure trials, while optimal growth and ultimate upper incipient lethal temperatures (UUILT) were examined over the course of 21-day trials at six static temperature treatments. Whereas CTMs differed among populations (mean = 27.91°C, SD = 0.35ºC), UUILTs did not (mean = 24.40°C, SD = 0.04°C). Comparison of cubic temperature-growth functions to the traditional quadratic functions showed that adding a third-order term for temperature can improve model fit, and revealed substantial differences in optimal growth temperatures (15.4-18.3°C). Knowledge of these thermal tolerance thresholds will help to predict the consequences of a warming climate, identify suitable habitats for repatriation, and inform water quality temperature standards established to protect these fish into the future.

__Presentations__ (chronological)

Rogers, K. B. March 8, 2022. Movement and dispersal by inland trout: how technology has shaped our understanding of these critical attributes. National Trout Unlimited Science Seminar (virtual).

Rogers, K. B., A. Whiteley, and S. Amish. April 26, 2022. Using RADseq to evaluate genetic diversity in Greenback Cutthroat Trout. Greenback Cutthroat Trout Recovery Team meeting, Denver, Colorado.

Rogers, K. B., J. R. Anderson, S. F. Brinkman, A. P. Martin. September 29, 2022. Inbreeding depression reduces fitness in Colorado's last remaining Greenback Cutthroat Trout: Consequences for management. Wild Trout Symposium XIII, West Yellowstone, Montana.

Rogers, K. B. October 26, 2022. Colorado's native trout diversity: insights from molecular tools. BLM statewide biologists meeting, Silt, Colorado.

Evans, R. P., K. B. Rogers, D. Shiozawa. November 18, 2022. A Cutthroat Trout chromosome-level genome assembly. Desert Fishes Council, St. George, Utah (poster presentation).