Cutthroat Trout Studies

Kevin B. Rogers
Aquatic Research Scientist

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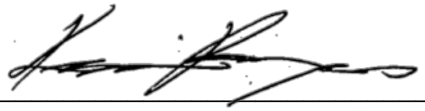
Prepared by: 

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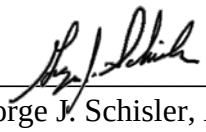
Approved by: 

George J. Schisler, Aquatic Wildlife Research Chief

Date: 11/30/2021

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CUTTHROAT TROUT INVESTIGATIONS

Period Covered: December 1, 2020 to November 30, 2021

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 2021 are presented here.

METHODS

Three hundred and one Cutthroat Trout were collected from 17 populations distributed across Colorado (Table 1). Fourteen came from the CRCT range, one from the RGCT range, and two from the South Platte 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 80% reagent grade ethanol (Rogers 2007). Fin tissues were delivered to Pisces Molecular (Boulder, Colorado) for subsequent genetic analyses. Isolation of DNA, the production of amplified fragment length polymorphism (AFLPs), sequencing of 648 bp of the NADH dehydrogenase subunit 2 (ND2)

mitochondrial gene, and subsequent molecular analyses are detailed elsewhere (Rogers 2010; Rogers et al. 2014; Bestgen et al. 2019). Rather than assigning numbers or letters to each haplotype recovered, I use the name of the body of water where the haplotype was first discovered, preceded by Oc (the native trout, *Oncorhynchus clarkii*) and three letters that describe the major drainage basin of the lineage represented. These include 1) Blue Lineage CRCT native to the Yampa, White, and Green River basins (YAM), 2) Green Lineage CRCT native to the Colorado, Gunnison, and Dolores River basins (COL), 3) RGCT native to the Rio Grande basin (RIO), 4) the native trout of the South Platte River basin (SPL), and 5) the nonnative Yellowstone Cutthroat Trout (YEL) stocked widely across Colorado in the middle of the last century. This approach allows for easy inclusion of newly discovered haplotypes and facilitates communication toward management and conservation goals. Mitochondrial haplotypes were compared to a reference set derived from Cutthroat Trout samples collected across Colorado over the last two decades (Figure 1) using MEGA7 (Kumar et al. 2016).

Table 1. Stream names organized by major drainage basin, water codes, collection dates, and number of fin clips collected for molecular tests conducted in 2021.

Stream	Water Code	Date	Sample size
Dolores			
Cold Creek	39227	7/30/2020	22
Deep Creek	39671	8/17/2020	30
Marguerite Creek	41412	7/28/2020	22
Gunnison			
Broke Leg Slough	47023	7/15/2020	30
Coal Creek, Little	46993	8/24/2020	28
Cunningham Creek	38519	8/13/2020	24
Dyke Creek	39885	7/30/2020	16
Lone Pine Creek	45591	8/25/2020	23
Spring Creek, W Fk	43339	6/23/2020	7
Steuben Creek, W	46137	9/22/2020	19
Rio Grande			
Jim Creek	44254	6/2/2020	30
San Juan			
Fall Creek	38117	8/26/2020	4
Fall Creek	38117	7/26/2021	5
Himes Creek	39502	6/14/2021	31
Rincon La Vaca Creek	43852	9/29/2020	1
Wolf Creek, S Fk	66649	8/26/2020	2
South Platte			

Platte Gulch	30677	10/22/2020	7
Rock Creek	30661	8/13/2020	1

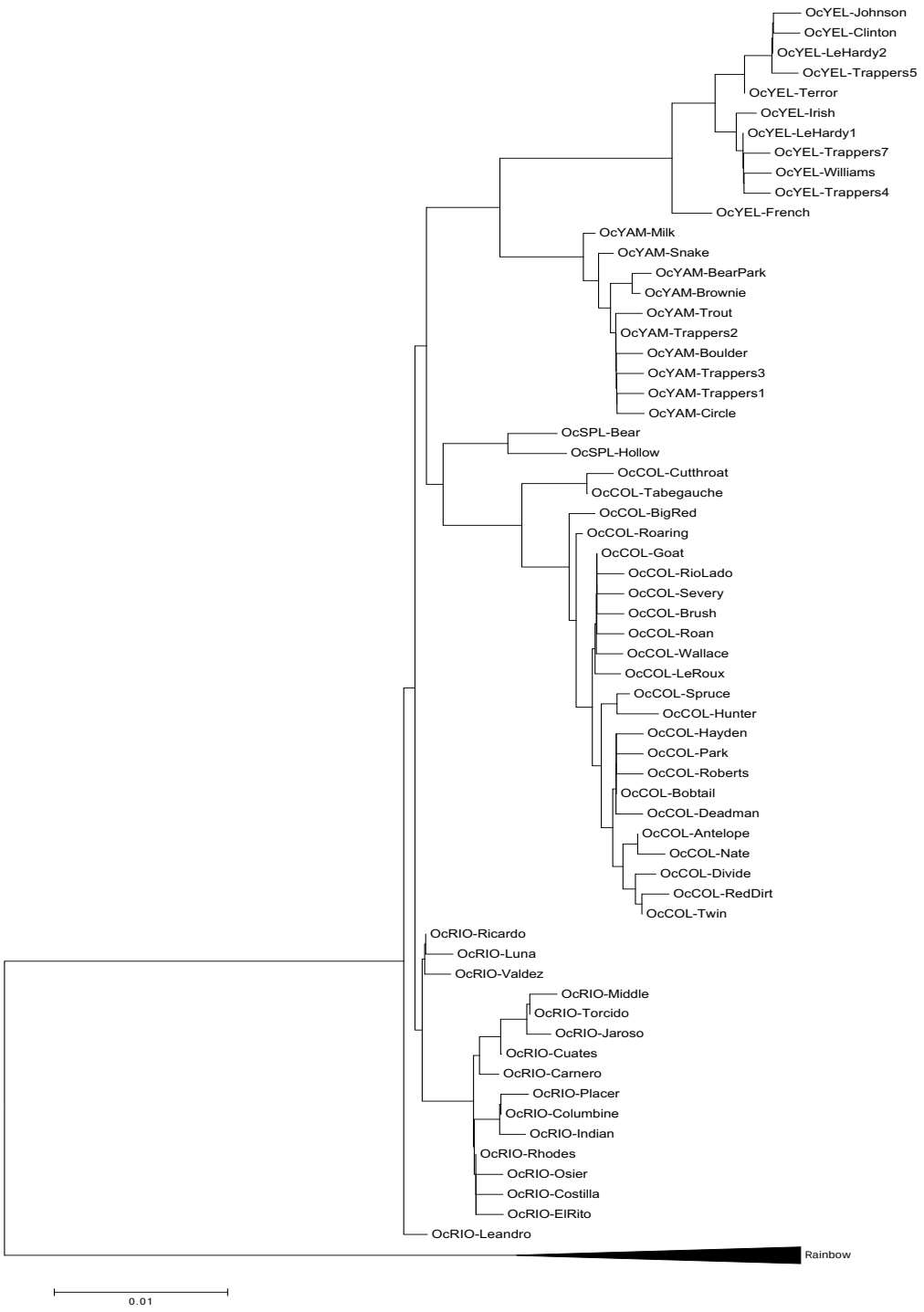

Figure 1. Phylogenetic relationships inferred from 648 base pairs of the mitochondrial NADH dehydrogenase subunit 2 gene for Cutthroat Trout from Colorado. The evolutionary history was developed with the neighbor-joining method in MEGA7, with evolutionary distance units representing the number of base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

Dolores River basin

The search for new Green Lineage CRCT (gCRCT) populations in the Dolores basin continues. All three collections of Cutthroat Trout in 2020 appear to be better than 90% pure (Table 2) using AFLP nuclear markers, and are therefore considered as CPs.

Cold Creek (WC#39227)— Unfortunately, AFLP data suggests that Cold Creek trout appear to be Yellowstone Cutthroat Trout (YSCT) rather than the native gCRCT. This contrasts sharply with the mtDNA results (Table 3) that suggested majority gCRCT haplotypes with a few Blue Lineage CRCT (bCRCT). Curiously, no YSCT haplotypes were recovered.

Deep Creek (WC#39671)— Cutthroat Trout from the Deep Creek drainage have been tested previously, and were even considered a potential brood source for Woods Lake prior to a 2008 flood that nearly eradicated them. The population has since rebounded (E. Gardunio, personal communication), but odd phenotypic traits (white fin tips and odd spotting patterns) led to additional testing. Evidence of nonnative trout admixture was not documented by AFLP or mtDNA sequencing, but further study is recommended if these fish are to be considered for future brood stock.

Marguerite Creek (WC#41412)— The discovery of pure gCRCT in Marguerite Creek was particularly exciting. Not only do they appear to be pure, but the two haplotypes recovered in the population (OcCOL- Rio Lado and OcCOL-Roaring), are not common and are only found in the Dolores Basin, suggesting these fish are indeed indigenous.

Table 2. AFLP results from 16 Cutthroat Trout collections analyzed in 2021, 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
Dolores						
Cold Creek	22	-	-	-	100	-
Deep Creek	30	-	100	-	-	-
Marguerite Creek	22	-	100	-	-	-

Gunnison						
Broke Leg Slough	30	1	-	-	99	-
Coal Creek, Little	28	-	100	-	-	-
Cunningham Creek	24	-	100	-	-	-
Dyke Creek	16	-	100	-	-	-
Lone Pine Creek	23	-	99	-	-	-
Spring Creek, W Fk	7	1	-	-	99	-
Steuben Creek, W	19	-	100	-	-	-
Rio Grande						
Jim Creek	30	-	-	100	-	-
Jim Creek[1]	30	-	-	100	-	-
San Juan						
Fall Creek	4	100	-	-	-	-
Fall Creek	5	93	1	1	-	5
Himes Creek	31	98	-	-	-	1
Wolf Creek, S Fk	2	100	-	-	-	-

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

Table 3. ND2 results from 15 Cutthroat Trout collections analyzed in 2021, 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
Dolores						
Cold Creek	22	4	18	-	-	-
Deep Creek	30	-	30	-	-	-
Marguerite Creek	22	-	22	-	-	-
Gunnison						
Broke Leg Slough	29	21	-	-	8	-
Coal Creek, Little	28	-	28	-	-	-
Dyke Creek	16	-	16	-	-	-
Lone Pine Creek	22	-	22	-	-	-
Spring Creek, W Fk	7	-	-	-	7	-
Rio Grande						
Jim Creek	30	2	-	28	-	-

San Juan						
Fall Creek	4	-	4[1]	-	-	-
Fall Creek	5	-	5[1]	-	-	-
Rincon La Vaca Creek	1	-	-	-	1	-
Wolf Creek, S Fk	2	-	2[1]	-	-	-
South Platte						
Platte Gulch	7	7	-	-	-	-
Rock Creek	1[2]	-	-	-	-	-

[1]All are the OcCOL-Tabeguache haplotype typical for San Juan Lineage CRCT
[2]This fish displayed the OcSPL-Bear haplotype, the same as found in the Bear Creek broodstock, native trout of the South Platte basin.

Gunnison River basin

Broke Leg Slough (WC#47023)— This isolated water would likely have been fishless historically, but if Yellowfin Cutthroat Trout were indeed cultured on the Grand Mesa (Behnke and Wiltzius 1982), this might have been an undetected repository for them. Unfortunately, both AFLP and ND2 data suggest heavy influence of YSCT. The presence of the common OcYAM-Trappers2 haplotype in 21 fish suggests this population was likely founded from Trappers Lake progeny sometime in the last 70 years (Rogers et al. 2018).

Coal Creek, Little (WC#46993)— The Cutthroat Trout in Little Coal Creek northeast of Crawford near the Smith Fork of the Gunnison, are protected from downstream Brook Trout by a major beaver pond complex. Both nuclear and mtDNA genetic data suggest this is a pure gCRCT population with 11 fish displaying the rare OcCOL-Twin haplotype only found in the Gunnison basin.

Cunningham Creek (WC#38519)— This stream has been sampled previously, but concern with the Overland Ditch potentially bringing Rainbow Trout into the system precipitated another survey. Fish were collected above and below the ditch crossing, and Rainbow Trout admixture was not detected with AFLPs.

Dyke Creek (WC#39885)— This gCRCT population is currently being invaded by nonnative Brook Trout, now occupying habitat 1 km above the pack trail crossing. Mechanical removal of nonnatives has occurred, but capture probabilities are poor making it unlikely that this population can persist on its own. To inform whether a rescue effort is warranted, an additional molecular survey was conducted. Subtle evidence of bCRCT found in a 2007 collection by AFLP, but that was not the case in this smaller 16 fish sample 13 years later. Fourteen of the samples displayed the rare OcCOL-LeRoux found only in four other streams, all in the Gunnison Basin.

Lone Pine Creek (WC#45591)— This population is listed as a "current distribution" in the CRCT

Conservation Team's ICP database (Hirsch et al. 2013), but with no genetic data to support that designation. There is a stocking record for this stream which is perhaps why fin clips have not been collected in the past. It is possible that stocked fish were placed downstream near the bridge where currently Brook, Brown, and Rainbow Trout are found. A steep cascade section appears to keep these nonnative fish from invading the upstream Cutthroat Trout population. The resident Cutthroat Trout appear pure by AFLP, with perhaps a few questionable fish. Nineteen displayed the common OcCOL-Bobtail haplotype with three harboring the rare OcCOL-Twin. One sample failed to sequence, and was not included.

Spring Creek, W Fk (WC#43339)—This is a very small population southwest of Montrose, Colorado, and is only listed as a "current distribution" in the ICP database. While the other forks appear to have hybrid Cutthroat Trout or Brook Trout, these looked pure. Although only a seven fish sample, nuclear AFLP data suggested these were almost pure YSCT. All seven harbored a YSCT haplotype (OcYEL-LeHardy2), no doubt a relict population founded in the mid-1900s when Colorado imported millions of Cutthroat Trout eggs from Yellowstone National Park (Varley and Gresswell 1988).

Steuben Creek, W (WC#46137)— Cutthroat Trout occur just downstream of the barrier that protects the CP from invasion by Brook Trout. Since this entire drainage is isolated, it is likely that these fish originate from the upstream CP. If pure, and Brook Trout suppression were implemented, a sizeable number of fish could be reared here on down to a pond on private ground that could then be used to serve as a brood source for founding additional populations. These fish indeed appear to herald from the upstream CP, as nuclear AFLP data shows no signs of nonnative trout admixture.

Rio Grande basin

Jim Creek (WC#44254)— This stream is listed as a CP (Bakevich et al. 2019), but there was some question as to whether the population actually met those criteria. Although reclaimed in the 1980s, the barrier protecting the population failed shortly thereafter. Samples were collected from five different locations along the stream, and all appear to be pure by AFLP using both the standard AFLP test and the more sensitive RG-CR test (Rogers et al. 2011). Unfortunately, 2 of the 30 fish sampled harbored OcYAM-Trappers2 haplotype, perhaps reflecting their West Indian Creek heritage from which they were founded (Rogers 2012).

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 Rainbow Trout or YSCT. Only two mitochondrial haplotypes have been detected in extant San Juan lineage CRCT, the common OcCOL-Tabeguache, and the more rare OcCOL-Cutthroat.

Fall Creek (WC#38117)— The first collection of four fish in August 2020 did not suggest any Rainbow Trout admixture in either the nuclear or mitochondrial genomes, while the second collection in July 2021 did. This was entirely due to one fish (Pisces #FAL5-160500). All nine fish sampled in these two collections contained the OcCOL-Tabeguache haplotype, consistent with those in the CP upstream of the highway. The presence of Rainbow Trout alleles in some Fall Creek trout was expected since these collections were made below the Highway 160 culvert barrier where Rainbow Trout and Brook Trout are sympatric with Cutthroat Trout.

Himes Creek (WC#39502)— Cutthroat Trout collected previously in 2007 from this population suggested that it was pure CRCT (Bestgen et al. 2019). Several fish in a recent survey however displayed evidence of Rainbow Trout admixture (Figure 2), precipitating the additional molecular tests presented here. These subsequent tests did reveal that at least one fish (Pisces# HIC1-160466) carried a significant number of Rainbow Trout alleles, accounting for all of the admixture seen (Table 2). Additional testing of the nuclear genome is recommended prior to using these fish to found additional populations. In addition, these same 31 samples should be screened for evidence of Rainbow Trout mtDNA.

Figure 2. Caudal region of a Cutthroat Trout (HIC1-160465) collected on 6/14/2021 from Himes Creek, Colorado. Note the white tipped anal fin on the anal (Photo credit – J. White).

Rincon La Vaca Creek (WC#43852)— Only one fish was collected from this small population above the Raber Lohr Ditch diversion near Weminuche Pass making purity assessments difficult. As such, only the ND2 mtDNA gene was sequenced, revealing a new haplotype of YSCT not previously seen in Colorado (OcYEL-LaVaca). This brings the total number of YSCT ND2 haplotypes registered in the state to thirteen.

Wolf Creek S Fk (WC#44254)— These two fish were originally moved from below the Fall Creek Conservation Population into reclaimed water in the South Fork of Wolf Creek before being delivered to the isolation unit at CPW's Durango Hatchery. Testing with AFLP did not show evidence of Rainbow Trout admixture, but with only two fish, these results should be treated as suspect. Both fish carried the San Juan lineage CRCT haplotype found in the Fall Creek population (OcCOL-Tabeguache).

South Platte River basin

Platte Gulch (WC#30677)— As part of continued efforts to search for evidence of the native trout of the South Platte basin (Metcalf et al. 2012; Rogers et al. 2018), samples obtained from Platte Gulch in October of 2020 were screened for evidence of native haplotypes. Only bCRCT haplotypes were recovered (OcYAM-Trappers1 and OcYAM-Trappers2).

Rock Creek (WC#30661)— This fish was recovered following Phase 4 of the Rock Creek reclamation project. The OcSPL-Bear haplotype it harbored confirms that it came from the original release into Phase 1 upstream, consistent with a successful reclamation in this drainage (Rogers 2020).

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REFERENCES

Bakevich, B. D., R. J. Paggen, and B. W. Felt. 2019. Range-wide status of Rio Grande cutthroat trout (*Oncorhynchus clarkii virginalis*): 2016. Rio Grande Cutthroat Trout Conservation Team report. New Mexico Department of Game and Fish, Santa Fe, New Mexico. Available onine at https://cpw.state.co.us/learn/Pages/ResearchRioGrandeCutthroatTrout.aspx (November 2021)

Behnke, R. J., and W. J. Wiltzius. 1982. The enigma of the Yellowfin Trout. Proceedings of the Annual Meeting, Colorado-Wyoming chapter of the American Fisheries Society 17:8-15.

Bestgen, K. R., K. B. Rogers, R. Granger. 2019. Distinct phenotypes of native Cutthroat Trout emerge under a molecular model of lineage distributions. Transactions of the American Fisheries Society 148:442-463.

Hirsch, C. L., M. R. Dare, and S. E. Albeke. 2013. Range-wide status of Colorado River Cutthroat Trout (*Oncorhynchus clarkii pleuriticus*): 2010. Colorado River Cutthroat Trout Conservation Team Report. Colorado Parks and Wildlife, Fort Collins. Available: https://cpw.state.co.us/learn/Pages/ResearchColoradoRiverCutthroatTrout.aspx. (November 2021).

Kumar S., Stecher G., and Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33:1870-1874.

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

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

Rogers, K. B. 2007. A suggested protocol for collecting cutthroat trout tissues for subsequent genetic analysis. Colorado Divison of Wildlife. Fort Collins, Colorado.

Rogers, K. B. 2008. Using amplified fragment length polymorphisms to characterize purity of cutthroat trout in Colorado: results from 2007. Colorado Division of Wildlife. Fort Collins, Colorado.

Rogers, K. B. 2010. Cutthroat trout taxonomy: exploring the heritage of Colorado's state fish. Pages 152-157 *in* R.

F. Carline and C. LoSapio, editors. Wild Trout X: Sustaining wild trout in a changing world. Wild Trout Symposium, Bozeman, Montana. Available: http://www.wildtroutsymposium.com/proceedings.php

Rogers, K. B. 2012. Summary of recent molecular work conducted on cutthroat trout tissue samples from Haypress Lake, Colorado. Colorado Parks and Wildlife. Fort Collins, Colorado

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

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

Rogers, K. B., K. R. Bestgen, S. M. Love Stowell, and A. P. Martin. 2018. Cutthroat Trout diversity in the southern Rocky Mountains. Pages 323-341 *in* P. Trotter, P. Bisson, B. Roper, and L. Schultz, editors. Evolutionary biology and taxonomy of Cutthroat Trout (*Oncorhynchus clarkii*), American Fisheries Society Special Publication 36, Bethesda, Maryland.

Rogers, K. B., J. Epp, and J. Wood. 2011. Development of an amplified fragment length polymorphism (AFLP) test to distinguish Colorado River from Rio Grande Cutthroat Trout. Colorado Parks and Wildlife report, Fort Collins. Available online at https://cpw.state.co.us/learn/Pages/ResearchCutthroatTrout.aspx (November 2021).

Rogers, K. B., J. White, and M. Japhet. 2018b. Rediscovery of a lost Cutthroat Trout lineage in the San Juan Mountains of southwest Colorado. Colorado Parks and Wildlife, Fort Collins, Colorado.

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

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

Varley, J. D., and R. E. Gresswell. 1988. Ecology, status, and management of the Yellowstone Cutthroat Trout. Pages 13–24 *in* R. E. Gresswell, editor. Status and management of interior stocks of Cutthroat Trout. American Fisheries Society, Symposium 4, Bethesda, Maryland.

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

RESEARCH PRIORITY

DNA recovery from archived trout tissues

OBJECTIVE

To ensure the long-term persistence of useable DNA from archived trout fin clips for future genomic studies

INTRODUCTION

Archived tissue samples have played a critical role in resolving the convoluted taxonomy of Cutthroat Trout (Metcalf et al. 2012), but improperly stored specimens resulting in DNA degradation make subsequent molecular analyses more difficult (Nagy 2010; Oosting et al. 2020). This is especially true for new molecular whole-genome approaches (e. g. RADseq, whole-genome sequencing, and long-range sequencing.) that require high concentrations of high-molecular-weight DNA (Graham et al. 2015; Oosting et al. 2020). While archiving isolated DNAs in -80°C freezers is ideal, and has been used with good success in reevaluating collections from decades ago (Nagy 2010), it is not always practical. In addition, residual fin tissues remain after the initial DNA extractions, and may serve useful down the road and should be archived for future molecular work. While 80% ethanol (Rogers 2007) has been used with good success for preserving fin tissues in the short-term, DNA quality extracted from fins stored in this medium for more than several years is highly variable (KBR, unpublished data). Although 95% ethanol tends to desiccate tissues making DNA recovery more difficult, it does appear to be a better long-term storage medium (Flournoy et al. 1996; Nagy 2010; Stein et al. 2013). Others have stored tissues dry with good success (Anchordoquy and Molina 2007; Nagy 2010), and more specifically with Cutthroat Trout fin clips (M. Peacock, University of Nevada, personal communication). This approach has numerous benefits for long-term storage since individual collection information can be recorded on coin envelopes that can be efficiently stored in relatively small spaces. Here we explore the long-term viability of DNA stored dry compared to conventional ethanol storage.

METHODS

Caudal fin clips were obtained from 32 Colorado River Cutthroat Trout from Circle Creek on October 17, 2012 following a translocation effort to establish a new population in Fish Creek in Steamboat Springs, Colorado. Each fin was split in two, with one half being place in 3.5 ml vials containing 80% ethanol (Rogers 2007), while the other half was placed in wax paper and slipped into a coin envelope (Item #S11485, Uline, Waukegan, Illinois). Both samples were stored in the same cardboard box at room temperature.

In July 2015 (three years after collection), DNA from eight paired samples were isolated using

DNA a proteinase K tissue lysis and spin-column DNA purification protocol following the manufacturer's specifications (DNeasy Kit; Qiagen, Hilden, Germany), as in Bestgen et al. (2019). Five ul from each sample were electrophoresed on a 2% agarose gel. The DNA was visualized and photographed on a 254 nm UV trans-illuminator after staining for 45 minutes in a 0.0004% ethidium bromide (4 µg EtBr/ml) solution. We included a known concentration of size standards on each gel so that a qualitative assessment of DNA quantity and quality could be made. In addition, DNA fragment size can be used as a proxy for DNA quality because it is a key metric for sequencing providers wishing to anticipate that likelihood that representative genomic datasets can be generated on any sequencing platform (Oosting et al. 2020).

These same DNAs from eight paired samples were then stored at -20°C for six more years before being run again in 2021 on a gel using the same protocol. Finally, DNA from the remaining 24 paired fins that remained stored in ethanol or dry at room temperature was isolated in October 2021 (nine years after collection). These too were subjected to the same protocol. Six categories were used to rank the quantity and quality of DNA in each sample indicated from the gels (Figure 1). Very high-quality DNA (VH) yielded a bright visible DNA smear with total DNA concentration equal to the binding capacity of the spin column extraction procedure and a very bright band at ~2 kbp. High quality (H) samples had a substantial amount of DNA present marked by an easily visible smear, particularly at ~2 kbp. Good quality samples (G) provided a visible DNA smear indicating a significant amount of DNA present, with a band at ~2 kbp. That band was only faintly visible in marginal samples (M) and the stain intensity of the DNA smear was greatly reduced. In low quality samples (L), only a faint DNA smear was visible and/or average molecular weight was low (<500 bp). If no DNA was detected on the gel (ND), then less than 5 ng were present and the quality could not be determined.

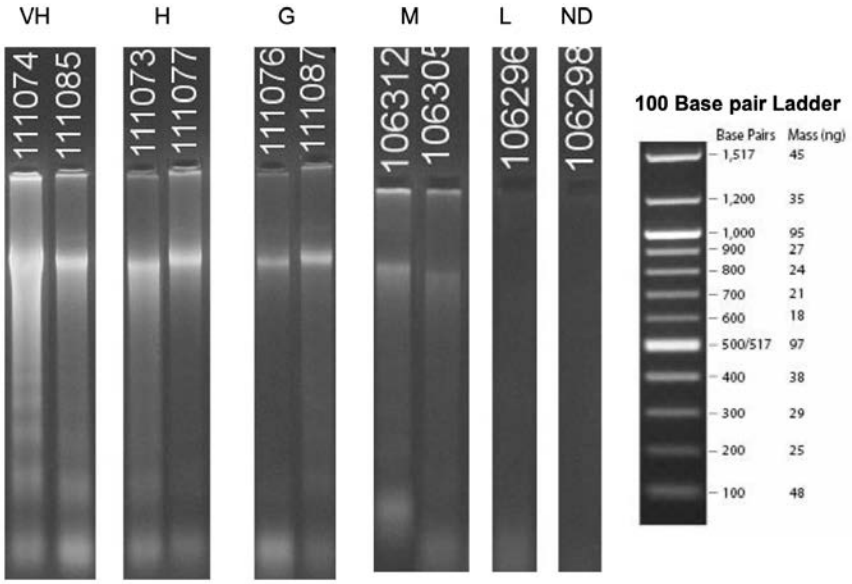

Figure 1. Six categories were used to rank the quantity and quality of DNA in each sample. Very high quality DNA (VH) yielded a bright visible DNA smear with total DNA concentration equal to the binding capacity of the spin column extraction procedure and a very bright band at

~2 kbp. High quality (H) samples had a substantial amount of DNA present marked by an easily visible smear, particularly at ~2 kbp. Good quality samples (G) provided a visible DNA smear indicating a significant amount of DNA present, with a band at ~2 kbp. That band was only faintly visible in marginal samples (M) and the stain intensity of the DNA smear was greatly reduced. In low quality samples (L), only a faint DNA smear was visible and/or average molecular weight was low (<500 bp). If no DNA was detected on the gel (ND), then less than 5 ng were present and the quality could not be determined.

The quantity of DNA is estimated by comparing the intensity of the total amount of EtBr staining material visible as an intense band, with known concentrations of DNA in the size standards included on each gel. A band that is barely visible on a gel photo contains approximately 5 to 10ng of DNA, while a very bright band contains approximately 200 to 300ng. DNA quality was evaluated by assessing the size and appearance of EtBr staining material (both lower molecular weight smear and the largest "band" visible). Pipetting DNA through a standard micro-pipettor tip shears DNA to approximately 2,000 base pairs (2 kbp) in size. Intact DNA therefore appears as a sharp band at ~2 kbp with a diffuse smear of smaller fragments. Slightly degraded DNA shows a smear beginning at ~2 kbp, but the band is diffuse, and the smaller fragment smear increases in intensity relative to the 2 kbp band. As DNA degradation increases, the smear decreases in size and becomes more diffuse, indicating increased variation in fragment sizes. When the average size of the DNA fragments drops below 500 bp, the chances of PCR primers binding to a fragment that contains the full-length target sequence is greatly reduced.

RESULTS

Useable DNA was recovered from all 32 paired samples collected from Circle Creek in 2012, and for all samples, storage in ethanol was superior to dry storage. Substantial high molecular weight DNA persisted in the dry storage samples three years post collection (Figures 2 and 3), with all samples registering good quality (G). After nine years however, those same fin clips produced DNAs that were too poorly degraded to be useful in many applications (Figure 4, medium and low quality). Isolating DNA and storing them at -20°C appeared to halt degradation, with DNAs from the first eight samples processed in 2015 yielding the same DNA quality in 2021 when they were electrophoresed a second time (Figure 3). Samples stored in 80% ethanol produced very high-quality DNA in all cases.

3 years

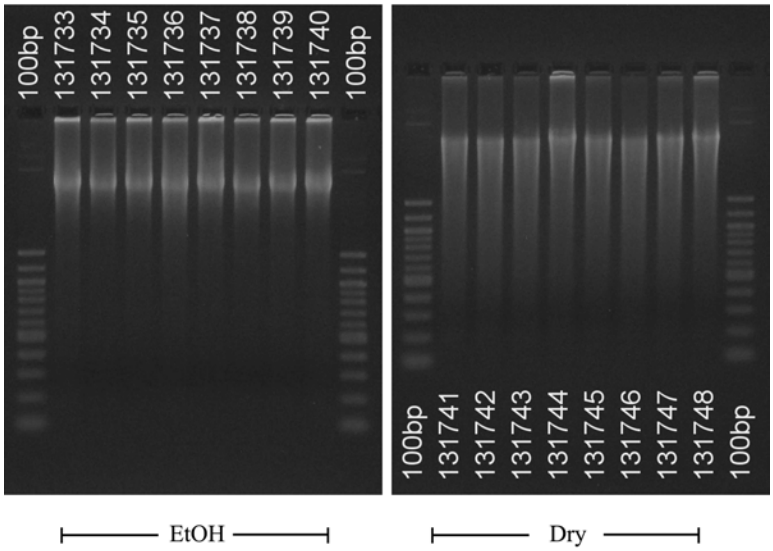

Figure 2. Eight fin clips collected on October 15, 2012 from Circle Creek were each split with half stored in 80% ethanol (EtOH) while the other half was stored dry in a coin envelope. DNA was isolated in July 2015 and electrophoresed on an agarose gel to assess quality.

3 years (plus 6)

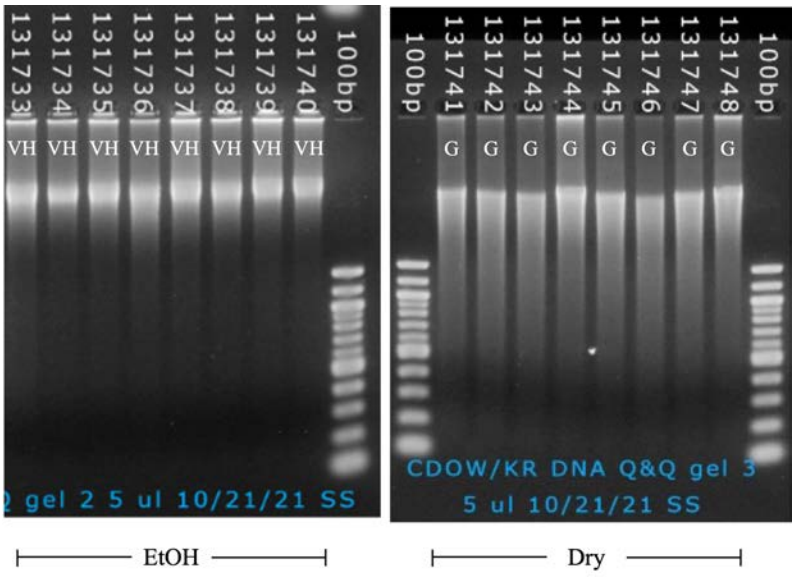

Figure 3. Eight fin clips collected on October 15, 2012 from Circle Creek were each split with half stored in 80% ethanol (EtOH) while the other half was stored dry in a coin envelope. DNA was isolated in July 2015 and stored at -20°C for six years before thawed and electrophoresed on an agarose gel to assess quality which is presented at the top of each lane (very high = VH, good

= G). Pisces accession numbers are also listed above each lane with base pair size ladders down the sides of each gel.

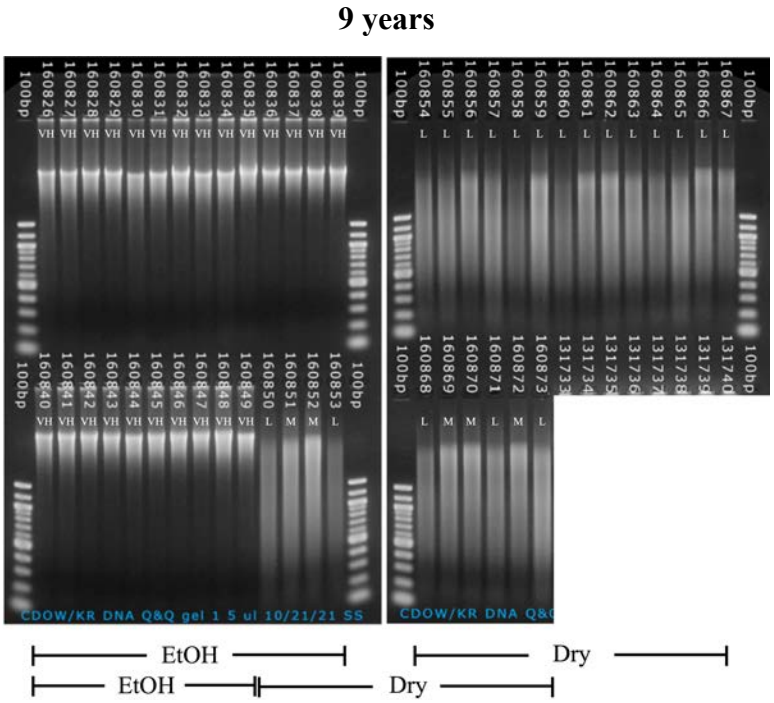

Figure 4. 24 fin clips collected on October 15, 2012 from Circle Creek were each split with half stored in 80% ethanol (EtOH) while the other half was stored dry in a coin envelope. DNA was isolated in October 2021 and electrophoresed on an agarose gel to assess quality which is presented at the top of each lane (very high = VH, medium = M, and low = L). Pisces accession numbers are also listed above each lane with base pair size ladders down the sides of each gel.

DISCUSSION

Not only is low quality DNA problematic for high-throughput applications (Graham et al. 2015; Oosting et al. 2020) and eDNA experiments (Mauvisseau et al. 2021), but even simple sequencing tasks risk failing to amplify in PCR experiments. This is especially concerning if long target sequences are used, potentially resulting in false negative results. We should continue to explore efficient and inexpensive long-term storage options for preserving fin clips, particularly those that have already provided DNA for current analyses. With new molecular methods emerging every year, it is clear that we may wish to revisit these samples in the perhaps distant future. Over time, many of our extant Cutthroat Trout populations may be extirpated, making repeated sampling efforts sometimes impossible. While long-term storage in ethanol is possible, it is much less efficient than dry storage. Several researchers have followed up initial preservation in ethanol with silica desiccation and dry storage with good results (Nsubuga et al. 2004; Roeder et al. 2004). Like ethanol, heat treatments have also been used to kill enzymes that

degrade DNA, allowing for better yields in dry stored samples (Oosting et al. 2020). This may be a productive avenue for future research along with exploring other alternative storage media (Kilpatrick 2002; Michaud and Foran 2011; Gray et al. 2013; Ivanova et al. 2013), so that tissues collected now can serve as long-term archives for future studies.

Management recommendations

Although good yields were recovered from samples stored in 80% ethanol here, we recommend switching to preserving samples in 95% lab grade ethanol instead. This recommendation is contrary to Rogers (2007), but should be superior for long term storage by ensuring anhydrous conditions (Dean and Ballard 2001; Nagy 2010). In addition, special care should be taken to preserve tissues quickly, then store them in cool and dark location protected from ultraviolet light that can severely damage DNA (Nagy 2010). Minimizing the delay between collection and extraction (Roon et al. 2003), and then freezing isolated DNA will provide the best long-term yields for resolving future unanticipated questions.

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REFERENCES

Anchordoquy, T. J., and M. C. Molina. 2007. Preservation of DNA. Cell Preservation Technology 5:180–188.

Bestgen, K. R., K. B. Rogers, R. Granger. 2019. Distinct phenotypes of native Cutthroat Trout emerge under a molecular model of lineage distributions. Transactions of the American Fisheries Society 148:442-463.

Dean, M. D. and J. W. O. Ballard. 2001. Factors affecting mitochondrial DNA quality from museum preserved *Drosophila simulans*. Entomologia Experimentalis et Applicata 98: 279–283.

Flournoy L. E., R. P. Adams, and R. N. Pandy. 1996. Interim and archival preservation of plant specimens in alcohols for DNA studies. Biotechniques 20: 657–660.

Graham, C. F., T. C. Glenn, A. G. McArthur, D. R. Boreham, T. Kieran, S. Lance, R. G. Manzon, J. A. Martino, T. Pierson, S. M. Rogers, J. Y. Wilson, and C. M. Somers. 2015. Impacts of degraded DNA on restriction enzyme associated DNA sequencing (RADSeq). Molecular Ecology Resources 15:1304–1315.

Gray, M. A., Z. A. Pratte, and C. A. Kellogg. 2013. Comparison of DNA preservation methods for environmental bacterial community samples. Microbiology Ecology 83:468–477.

Ivanova, N. V., and M. L. Kuzmina, M. L. 2013. Protocols for dry DNA storage and shipment at room temperature. Molecular Ecology Resources, 13:890–898.

Kilpatrick, C. W. 2002. Noncryogenic preservation of mammalian tissues for DNA extraction: an assessment of storage methods. Biochemical Genetics 40:53–62.

Mauvisseau, Q., D. Halfmaerten, S. Neyrinck, A. Burian, and R. Brys. 2021. Effects of preservation strategies on environmental DNA detection and quantification using ddPCR. Environmental DNA. 2021;3:815–822.

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

Michaud, C. L., D. R. Foran. 2011. Simplified field preservation of tissues for subsequent DNA analyses. Journal of Forensic Sciences 56:846–852.

Nagy, Z. T. 2010. A hands-on overview of tissue preservation methods for molecular genetic analyses. Organisms

Diversity and Evolution 10: 91–105.
Nsubuga, A. N., M. M. Robbins, D. Roeder, P. A. Morin, C. Boesch, and L. Vigilant. 2004. Factors affecting the amount of genomic DNA extracted from ape faeces and the identification of an improved sample storage method. Molecular Ecology 13:2089-2094.
Oosting, T., E. Hilario, M. Wellenreuther, and P. A. Ritchie. 2020. DNA degradation in fish: Practical solutions and guidelines to improve DNA preservation for genomic research. Ecology and Evolution 10:8643–8651.
Roeder, A. D., F. I Archer, H. N. Poinar, and P. A. Morin. 2004. A novel method for collection and preservation of faeces for genetic studies. Molecular Ecology Notes 4:761-764
Rogers, K. B. 2007. A suggested protocol for collecting cutthroat trout tissues for subsequent genetic analysis. Colorado Division of Wildlife, Fort Collins.
Roon, D. A., L. P. Waits, and K. C. Kendall. 2003. A quantitative evaluation of two methods for preserving hair samples. Molecular Ecology Notes 3:163-166.
Stein, E. D., B. P. White, R. D. Mazor, P. E. Miller and E. M. Pilgrim. 2013. Evaluating ethanol-based sample preservation to facilitate use of DNA barcoding in routine freshwater biomonitoring programs using benthic macroinvertebrates. PloS ONE 8(1): e51273. Doi:10.1371/journal.pone.0051273.

RESEARCH PRIORITY

Resolving the heritage of Colorado's native Cutthroat Trout

OBJECTIVE

Document what is known about the Cutthroat Trout population in South Hayden Creek to inform future management actions

INTRODUCTION

Despite extensive taxonomic work on Colorado's native Cutthroat Trout *Oncoryhnchus clarkii* over the last decade (Metcalf et al. 2012; Bestgen et al. 2019), several unanswered questions remain (Rogers et al 2018). One that is particularly relevant to management involves the heritage of the Cutthroat Trout population in the South Fork of Hayden Creek in the Arkansas River basin southeast of Salida, Colorado. This population continues to appear in the popular media because it covers a compelling story including a July 2016 fire that forced an evacuation of the trout population, and subsequent repatriation efforts from those salvaged fish. In an effort to inform those media stories and document what we know about the Cutthroat Trout that resided in South Hayden Creek prior to the Hayden Pass Fire, I have summarized what we know and don't know about the evolutionary history of this population, as well as recent events and management actions that may be important for future managers.

EVOLUTIONARY HISTORY

While Cutthroat Trout were long thought to have split from Rainbow Trout (*O. mykiss*) some two million years before present (Ma; Behnke 1992, 2002), recent work suggests that they evolutionary past spans a much longer period. Cutthroat Trout fossils collected from the Truckee Basin in modern day western Nevada suggest the split from Rainbow Trout occurred over 10.2 Ma (Stearly and Smith 2016; Smith and Stearly 2018). This corroborates information from molecular clocks (Shiozawa et al. 2018) that also support a split between Cutthroat Trout and Rainbow Trout occurring 9.2 to 10.7 Ma. In Colorado, additional diversity arose 1.3-1.6 Ma (Shiozawa et al. 2018) spread among six lineages. Three appear east of the Continental Divide in the South Platte (Greenback Cutthroat Trout), Arkansas (Yellowfin Cutthroat Trout, YFCT, now extinct), and Rio Grande river basins (Rio Grande Cutthroat Trout). West of the Continental Divide, three lineages appear under the umbrella of Colorado River Cutthroat Trout (CRCT; Metcalf et al. 2012, Rogers et al. 2018): A "blue" lineage indigenous to the Green, White, and Yampa rivers, a "green" lineage native to the headwaters of the Colorado, Gunnison, and Dolores rivers (gCRCT), and a San Juan lineage native to its namesake basin (Metcalf et al. 2012; Rogers et al. 2018; Bestgen et al. 2019).

We have identified 69 extant gCRCT conservations populations to date west of the Continental

Divide (Rogers 2020). In addition, a handful of populations from this same clade (see Figure 1, page 4) are found east of the Divide in the South Platte and Arkansas basins (Rogers 2020), and are distinct from the native Greenback Cutthroat Trout native to the South Platte basin (Metcalf et al. 2012; Bestgen et al. 2019). Given the extensive history of anthropogenic stocking starting at the turn of the 19th century (Wiltzius 1985; Metcalf et al. 2007, 2012; Love Stowell et al. 2015), it is not surprising that evidence of gCRCT can be found east of the Continental Divide outside of their putative native range (Rogers et al. 2018). Cutthroat Trout eggs were collected from at least ten wild spawn operations conducted within the Colorado and Gunnison River basins prior to 1940. Few of these operations produced enough eggs to satisfy the needs of the collecting agencies, and as such, these operations generally ceased after a few years. This was not the case for the Alexander Lake complex on the south side of the Grand Mesa (Gunnison River drainage) which was essentially the exclusive source of Cutthroat Trout eggs for the Leadville National Fish Hatchery (LNFH) in the early 1900s. Between 1899 and 1909 the LNFH collected 47 million fertilized eggs from ten lakes and streams in two separate basins draining the Alexander Lakes. Progeny were distributed to 16 states and two foreign countries during that time, with 29 million distributed to waters across the state of Colorado that could support trout (Metcalf et al. 2012). The State of Colorado took over egg collecting operations at these lakes on the Grand Mesa in 1913 and continued collecting eggs until 1931. However, by 1916, nonnative Rainbow Trout had been stocked into all of them, making it unlikely that pure gCRCT were still being produced after that point.

Despite a clear mechanism for establishing gCRCT east of the Continental Divide, we cannot dismiss the possibility that these fish invaded across this barrier on their own, perhaps at the end of the Pleistocene. Of the 21 mitochondrial haplotypes recovered in the gCRCT clade, 19 are found only west of the Continental Divide while two are found only on the east side (Rogers 2020). Populations harboring these two haplotypes are also morphologically distinct from their cousins west of the divide (Bestgen et al. 2019) though small founding populations can yield morphomeristic traits that skew towards extreme rather than modal values (Hickman and Behnke 1979).

Particularly curious are the museum specimens collected by David Starr Jordan 1889 from Twin Lakes in the headwaters of the Arkansas River basin. He maintained that there were two kinds of trout native to the lake – the Yellowfin or "Salmon Trout" and the smaller "Greenback Trout" (Jordan and Everman 1890). George Fisher, an "enthusiastic" angler from Leadville alerted Jordan to the presence of Yellowfin Trout in Twin Lakes and helped Jordan capture "about ten specimens of this species… with the fly in the lower Twin Lakes" (Jordan and Everman 1890). These specimens are stored at the National Museum of Natural History (Smithsonian) in Washington D. C., and at the California Academy of Science (Behnke and Wiltzius 1982). Ancient DNA methods (Metcalf et al. 2012) were used to recover mitochondrial haplotypes from Jordan's collection housed at both museums (Table 1). Indeed, the type specimen of Yellowfin Cutthroat Trout (*O. c. macdonaldii*) harbored a unique haplotype representing a distinct clade of Cutthroat Trout (Metcalf et al. 2012). This corroborated Robert Behnke's assertion that they were a discrete subspecies, with "the samples being drawn from two distinct, and reproductively isolated populations" (Behnke and Wiltzius 1982).

Table 1. Disposition of the fish David Starr Jordan collected in 1889 from the Twin Lakes area split between the National Museum of Natural History (NMNH) and the California Academy of Sciences (CAS). The putative Cutthroat Trout subspecies listed by Jordan is followed by the museum collection number. The number of fish in each jar (Fish#) followed by the subset sampled by J. Metcalf in 2012 (Sampled), and the number she was able to obtain useable DNA sequence from (Amplified) are listed, along with the clade they belong to based on their mitochondrial haplotype

Water	Subspecies	Collection #	Fish #	Sampled	Amplified	Clade
NMNH						
Twin Lakes[1]	*macdonaldi*	41641	4	4	4	YFCT
Lake Creek[2]	*stomias*	41690	1	1	1	gCRCT
Arkansas River[3]	*stomias*	41702	6	2	1	YFCT
Twin Lakes[4]	*macdonaldi*	41730	1	1	1	YFCT
Twin Lakes	*stomias*	126839	2	1	0	-
Outlet Lower[5]	*stomias*	63760	10	2	0	-
CAS						
Twin Lakes	*stomias*	90205	4	1	1	gCRCT
Twin Lakes	*stomias*	209435	3	1	1	YFCT

[1]These are YFCT co-types (para types) as per R. R. Miller 1945
[2]Listed as Lake Creek by Granite, just downstream of Twin Lakes
[3]Near Leadville, Colorado; 5 of 6 fish were Age 1, only the largest fish amplified
[4]This is the YFCT type specimen; presumably from the same collection as NMNH 41641. This specimen was thought to be lost, but found by R. R. Miller in 1945
[5]These collected from the outlet of Lower Twin Lake by Juday in 1902

If indeed these two populations were reproductively isolated, then mitochondrial haplotypes should reflect the subspecies they came from. Table 1 reveals that although all putative YFCT displayed YFCT haplotypes, the same was not true for Jordan's putative *O. c. stomias* samples. Of those four specimens where useable DNA sequence was obtained (Metcalf et al. 2012), only one from Twin Lakes and one immediately downstream in Lake Creek had a gCRCT haplotype, while putative *O. c. stomias* from the Arkansas River near Leadville and another from Twin Lakes, each harbored a YFCT haplotype. This is either evidence of hybridization, or, of difficulty distinguishing YFCT from gCRCT using visual characteristics in smaller specimens. Either way, it provides evidence that gCRCT were established in Twin Lakes in 1889, prior to the advent of large-scale stocking throughout Colorado (though it should be noted that Rainbow Trout, Lake Trout (*Salvelinus namaycush*), Brook Trout (*Salvelinus fontinalis*), and Atlantic Salmon (*Salmo salar*) had all been stocked in Twin Lakes by then (Wiltzius 1985). The only extant population of trout that harbor mitochondrial haplotypes matching the two "Greenback Trout" collected by Jordan from Twin Lakes (gCRCT) were those in South Hayden Creek, further downstream in the Arkansas basin, making them a high priority for conservation.

Although we can't rule out the possibility of a natural invasion and the evolution of two sympatric Cutthroat Trout lineages, stocking remains the most parsimonious explanation for the presence of gCRCT east of the Continental Divide. Other alternative mechanisms might also explain the pattern of lineage distribution:

Water diversions

It is conceivable that early water diversions from west of the Continental Divide into the Arkansas River basin might have brought fish in with them. The Ewing Ditch built in 1880 was the first documented such diversion, bringing water across the Divide from the Eagle River basin to Leadville. The Eagle River basin is found in the putative native range of gCRCT (Metcalf et al 2012; Rogers et al. 2018). However, it is difficult to reconcile how in just nine years gCRCT could have moved 36 km down the Arkansas River, and several more up Lake Creek into Twin Lakes, then proliferated enough to make up a substantial portion of Jordan's catch. Whether additional undocumented diversions from the Pacific slope occurred prior to the construction of the Ewing Ditch is unknown.

Inaccurate labeling

It is also possible that over the 130-year history of the museum specimens, the labels may no longer accurately reflect the contents of a sample jar. Either confusion during the initial curation such as occurred with the type specimens of Greenback Cutthroat Trout that were actually Rio Grande Cutthroat Trout (Metcalf et al. 2012; Rogers 2012), or perhaps something as simple as a shattered specimen jar that required an alternative home for the preserved contents. Perhaps that would explain the presence of a Rio Grande Chub (*Gila pandora*) specimen in the jar that contains the San Juan Cutthroat Trout (NMNH #17071) collected by Aiken in 1872 from Pagosa Springs. These species live on opposite sides of the Continental Divide, and could not have been part of the same collection, despite being in the same jar. The fact that putative *O. c. stomias* specimens harbored YFCT haplotypes at both NMNH and CAS however, argues against mislabeling subsequent to the original collection.

Whether gCRCT became established east of the Divide on their own, or through anthropogenic means (e. g. stocking from Twin Lakes or west slope sources) remains a mystery. Their somewhat unique genetic and phenotypic attributes however suggest these fish should continue to be a focus of conservation efforts. They represent a unique piece of the evolutionary puzzle that comprise our native trout.

HAYDEN PASS FIRE

A July 2016 wildfire in the headwaters of the Hayden Creek drainage scorched 6,685 ha of mixed conifer forest. Post-fire erosion potential was significant, making it likely that lethal debris flows would materialize during the late summer monsoon season (USDA 2016). Given the unique nature of this population, a rescue effort aimed at securing this population was conducted. Five crews used backpack electrofishers to remove roughly half of the resident population (194 fish). Smaller fish were targeted in an effort to leave enough mature adults to

repopulate the stream if ash flows did not materialize (Rogers 2020). Thirty-six were translocated to the upstream reaches of Newlin Creek that also lies in the Arkansas River basin, while the remainder (158 trout) were taken into the Roaring Judy Hatchery isolation facility to mature and provide future progeny for repatriation efforts.

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

BROOD DEVELOPMENT

Of the 158 trout evacuated from South Hayden Creek, 152 persisted a year later, 18 of which produced viable eggs in 2017. These were used to produce 2,700 ten-month-old progeny for future broodstock development. Survival to hatch of these 18 families was highly variable (S. Firestone, unpublished data) ranging from 6% to 85%. The small size of the founding population coupled with variable survival led us to explore the possibility that genetic factors were partially responsible. To that end, we genotyped the 133 individuals that remained in 2018 and compared survival of families created from either closely related or unrelated parents (Rogers 2020). Differences in survival based on relatedness did not materialize, but rather egg quality was the primary driver of survival. As such, rather than continue with spawn matrixing, standard practices for maintaining genetic diversity in broodstocks were followed such as crossing across yearclasses, and fertilizing each female with a single male. In addition, a representative sample of the eggs produced on each spawn day are retained to minimize the influence of individual females on future broodstock development. Though the South Hayden Creek broodstock has recently been moved from CPWs Roaring Judy Hatchery to a dedicated building at the federal USFWS hatchery near Leadville, Colorado, the former facility still plays a key role in conserving these fish, as eyed embryos are transferred back to Roaring Judy Hatchery to take advantage of more favorable temperatures for growth there. Although the original trout salvaged prior to the Hayden Pass fire have now been retired, and reside in the Leadville Hatchery's "show" pond, their progeny continue to produce enough fertilized eggs to satisfy conservation efforts aimed at reestablishing these fish in the wild as well as broodstock replacement (Table 2).

Table 2. Disposition of progeny from the South Hayden Creek broodstock released into the wild in 2021.

Water	Water Code	Age	Number
Roaring Judy Hatchery			
Cottonwood Creek	32742	0	3200
French Creek, N Fk	29828	0	3000
Hardscrabble Creek, S	29985	0, 1	2971
Iowa Gulch	33198	1	702
Middle Creek, S	30372	0, 1	2500
Newlin Creek	30514	0	3025
Ruxton Creek, S	31095	0	2000
Leadville Hatchery			
McNasser Gulch	30512	2	1042

MANAGEMENT RECOMMENDATIONS

With new molecular tools emerging every year, those who follow may be in a better position to resolve the mysteries outlined here. Short-read high throughput sequencing should allow us to begin to examine the nuclear genome even in these highly degraded specimens putting us in a position to answer whether YFCT haplotypes in putative *O. c. stomias* specimens reflect hybridization or misidentification. In addition, 22 specimens (Table 1) remain unsampled or did not amplify in the Metcalf et al. study (2012). Revisiting those specimens would allow us to gain more resolution into what exactly existed in the Twin Lakes area in 1889.

Establishing multiple wild naturally reproducing populations would help secure this piece of Cutthroat Trout diversity into the future. If in addition, one or two naturally reproducing populations could be established in habitat that includes a connected lake, that would allow for subsequent wild spawn operations which would obviate the need to maintain this broodstock further in CPW and USFWS's hatchery systems. Not only would this free up space for other critical stocks that might face similar challenges in the future, but it would provide the additional benefit of natural selection pressures on this stock should additional populations need to be established in the wild.

ACKNOWLEDGMENTS

I wish to thank Seth Firestone and his staff at CPW's Roaring Judy Hatchery for diligently maintaining this brood stock since the fire, and for tracking the fate of all spawns from these fish.

REFERENCES

Behnke, R. J. 1992. Native trout of western North America. American Fisheries Society Monograph 6. American Fisheries Society, Bethesda, Maryland.

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

Behnke, R. J., and W. J. Wiltzius. 1982. The eniga of the yellowfin trout. Proceedings of the Annual Meeting, Colorado-Wyoming chapter of the American Fisheries Society 17:8-15.

Bestgen, K. R., K. B. Rogers, R. Granger. 2019. Distinct phenotypes of native Cutthroat Trout emerge under a molecular model of lineage distributions. Transactions of the American Fisheries Society 148:442-463.

Hickman, T. J. and R. J. Behnke. 1979. Probable discovery of the original Pyramid Lake Cutthroat Trout. The Progressive Fish-Culturist, 41:135-137.

Jordan, D. S., and B. W. Evermann. 1890. Description of the yellow-finned trout of Twin Lakes, Colorado. Proceedings of the National Museum 12:453-454.

Love Stowell, S. M., C. M. Kennedy, S. C. Beals, J. L. Metcalf, and A. P. Martin. 2015. The genetic legacy of more than a century of stocking trout: a case study in Rocky Mountain National Park, Colorado, USA. Canadian Journal of Fisheries and Aquatic Sciences 72:1565-1574.

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

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

Rogers, K. B. 2020. Cutthroat Trout studies. Colorado Parks and Wildlife Progress Report, Fort Collins.

Rogers, K. B., K. R. Bestgen, S. M. Love Stowell, and A. P. Martin. 2018. Cutthroat Trout diversity in the southern Rocky Mountains. Pages 323-341 *in* P. Trotter, P. Bisson, L. Schultz, and B. Roper, editors. Cutthroat Trout: evolutionary biology and taxonomy. American Fisheries Society, Special Publication 36, Bethesda, Maryland.

Shiozawa, D. K. , R. P. Evans, D. D. Houston, and P. J. Unmack. 2018. Geographic variation, isolation, and evolution of Cutthroat Trout with comments on future directions for management and research. Pages 129-172 *in* P. Trotter, P. Bisson, L. Schultz, and B. Roper, editors. Cutthroat Trout: evolutionary biology and taxonomy. American Fisheries Society, Special Publication 36, Bethesda, Maryland.

Smith, G. R., and R. F. Stearley. 2018. The fossil record of Cutthroat Trout: implications for evolution and conservation. Pages 77-101 *in* P. Trotter, P. Bisson, L. Schultz, and B. Roper, editors. Cutthroat Trout: evolutionary biology and taxonomy. American Fisheries Society, Special Publication 36, Bethesda, Maryland.

Stearley, R. F. and G. R. Smith. 2016. Salmonid fishes from Mio-Pliocene lake sediments in the western Snake River Plain and the Great Basin. Pages 1-43 *in* Fishes of the Mio-Pliocene western Snake River Plain and vicinity. Miscellaneous Publications of the Museum of Zoology, University of Michigan, No. 204.

USDA (United States Department of Agriculture - Forest Service). 2016. Hayden Pass Fire: burned area report. FS-2500-8 (6/06). Canon City, Colorado.

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

RESEARCH PRIORITY

Adaptive capacity in native Cutthroat Trout: conservation in a warming climate

OBJECTIVE

Characterize thermal tolerance in five consequential stocks of Cutthroat Trout

INTRODUCTION

Native Cutthroat Trout (*Oncorhynchus clarkii*) of the southern Rocky Mountains face a variety of threats that have reduced occupied habitat roughly tenfold since European settlement (Penaluna et al. 2016; Budy et al. 2019). These declines are primarily driven by the invasion of nonnative trout (Peterson et al. 2004; Fausch 2008; Meredith et al. 2017; Al-Chokhachy and Sepulveda 2019; Zeigler et al. 2019), but the advent of a warming climate (Cook et al. 2004; Saunders et al. 2008; Hansen et al. 2012) will bring additional challenges for indigenous salmonids (Rahel et al. 1996; Williams et al. 2009; Wenger et al. 2011; Isaak et al. 2012; Roberts et al. 2017). The distribution of trout is predicated by thermal requirements (Dunham et al. 2003; Al-Chokhachy et al. 2013; Isaak et al. 2017), and some scientists have predicted substantial range contractions as a result of increasing temperature (Williams et al. 2009; Wenger et al. 2011; Isaak et al. 2012; but see Roberts et al. 2013; Zeigler et al. 2019).

The potentially dire consequences of a radically altered future climate have become key considerations in the management of Cutthroat Trout. For example, the U. S. Fish and Wildlife Service (USFWS) now considers the effects of climate change on future species persistence when evaluating listing species under the Endangered Species Act of 1973 (USFWS 2008). Meanwhile, a flurry of models have been developed to predict how remaining Cutthroat Trout populations will fare in the future (Roberts et al. 2013, 2017; Zeigler et al. 2019). These models focus on both acute and chronic thermal consequences of climate warming to Cutthroat Trout (Todd et al. 2008). Acute effects influence short-term survival, and a number of associated thresholds have been derived from lab-based studies (Becker and Genoway 1979; Johnstone and Rahel 2003; Bear et al. 2007; McDermid et al. 2012; Recsetar et al. 2012; Zeigler et al. 2013). Chronic effects influence the long-term growth and recruitment potential of trout exposed to a given thermal regime and are also characterized by a variety of approaches (Harig and Fausch 2002; Coleman and Fausch 2007a; Roberts et al. 2013; Isaak et al. 2017).

Models that assess rangewide persistence of Cutthroat Trout use thermal vital rates from rigorous lab studies conducted on a handful of Cutthroat Trout stocks (Bear et al. 2007; Coleman and Fausch 2007b; Zeigler et al 2013), applied uniformly to all populations within a subspecies (e.g., Roberts et al. 2013; Zeigler et al. 2019). However, some researchers have demonstrated that temperature tolerance can vary within subspecies (Wagner et al. 2001; Eliason et al. 2011; Drinan et al. 2012; Underwood et al. 2012; Narum et al. 2013), and that even feral populations of the same stock established in warmer thermal regimes can develop higher thermal tolerance over

time (K. Rogers, unpublished). Salmonids occupy variable and dynamic environments that foster adaptation on a local scale in response to temperature (Kaeding 1996; Kavanagh et al. 2010; Narum et al. 2013; Pearse and Campbell 2018), and that adaptation can occur quickly (e.g., 9-14 generations in Sockeye Salmon, O. nerka; Hendry et al. 1998). Acknowledging and characterizing this variation in thermal tolerance will improve predictive ability of models forecasting future persistence. More importantly, it could identify thermally tolerant stocks that could be used to repatriate habitats that are predicted to become less thermally suitable in the future, leading to more successful reclamation projects. A better understanding of the range of thermal tolerance within subspecies will also inform setting water quality standards that protect habitat (Todd et al. 2008; Mandeville et al. 2019).

We explored acute and chronic measures of thermal tolerance in five consequential populations of native Cutthroat Trout. To bracket a range of potential variation in local adaptation, we included populations exposed to a broad spectrum of temperature regimes. We included Colorado River Cutthroat Trout *O. c. pleuriticus* (CRCT) from Milk Creek, a low-elevation population that exhibits a unique ability to persist in an unusually warm environment (Hodge et al. 2017). Greenback Cutthroat Trout *O. c. stomias* (GBCT) from Zimmerman Lake were included because they appear to thrive in hatcheries with very cold water (B. Johnson, Colorado Parks and Wildlife, personal communication). This population is particularly important as it was founded from Bear Creek progeny, the last representatives of the native trout of the Platte River basin (Metcalf et al. 2012; Rogers et al. 2018; Bestgen et al. 2019). The pure CRCT from Lake Nanita were included because they represent the widely stocked indigenous trout of Trappers Lake, following a 1931 introduction into this historically fishless lake in Rocky Mountain National Park (Kennedy 2014). These oft studied fish serve as a useful baseline to compare this work to existing research on growth and thermal tolerance (e.g., Coleman and Fausch 2007b; Brandt 2009; Underwood et al. 2012). We included Yellowstone Cutthroat Trout *O. c. bouvieri* (YSCT) from LeHardy Rapids below Yellowstone Lake, as they too were widely stocked around the Rocky Mountain region, with 70 million YSCT distributed across Colorado from 1912-1953 (Varley 1979). Finally, we included the current population in Trappers Lake, the most prolific Cutthroat Trout broodstock in the southern Rocky Mountains, and source of many robust extant feral CRCT populations (Rogers et al. 2018). Close to a million YSCT were stocked into Trappers Lake from 1943-1950 resulting in a hybrid swarm today (Martinez 1988; Leary and Allendorf 1991).

METHODS

We collected fertilized Cutthroat Trout eggs for this study using a variety of methods. We conducted wild spawn operations on the four Cutthroat Trout populations native to Colorado (Table 1) during peak spawning activity in June of 2018. Adult males and females were collected from each population by electrofishing (Milk Creek, Trappers Lake), trapping (Zimmerman Lake), or seining (Lake Nanita). Each female was stripped into a dry bowl, then fertilized with milt from a single male. Five families were produced from each of the populations, with the exception of Milk Creek, where one of five females produced an insufficient number of eggs. In addition, five females from the Yellowstone Lake broodstock

housed at the Story Hatchery in Story, Wyoming were fertilized with milt from wild male YSCT captured above LeHardy Rapids in the Yellowstone River, Yellowstone National Park. Fertilized eggs were immediately water hardened for 10 minutes in a 100-ppm buffered iodine bath (Argentyne, Argent Aquaculture LLC, Redmond, Washington), then transported to the Colorado State University Foothills Fisheries Laboratory in Fort Collins, Colorado.

Table 1. Colorado River Cutthroat Trout (CRCT), Greenback Cutthroat Trout (GBCT), and Yellowstone Cutthroat Trout (YSCT) populations examined in this study, including their location (decimal degrees) and spawn date for wild egg collections in 2018.

Population	Subspecies	Latitude (ºN)	Longitude (ºW)	Spawn Date
Milk Creek[1]	CRCT	40.170	107.660	June 1
Zimmerman Lake[2]	GBCT	40.541	105.869	June 22
Lake Nanita[3]	CRCT	40.256	105.716	June 28
Yellowstone River[4]	YSCT	44.573	110.372	May 17
Trappers Lake[5]	CRCT	39.986	107.232	June 9

[1]Putative aboriginal population native to the lower Yampa River basin
[2]Population founded in 2014 with progeny from Bear Creek derived broodstock; native Cutthroat Trout of the South Platte River basin
[3]Founded from pure Trappers Lake stock in 1931
[4]Female YSCT from the Story Hatchery were fertilized with wild males captured from above LeHardy Rapids on the Yellowstone River, source of Colorado introductions
[5]CRCT now hybridized with Yellowstone Cutthroat Trout stocked in the lake from 1943-

All eggs were handled and incubated under common garden conditions in a flow-through laboratory system. Upon arrival at the lab, eggs were bathed in a 50 ppm iodine solution for 30 min. Families were then then split between two 10–cm diameter egg cups (Brinkman et al. 2013) and suspended in one of 24 randomly assigned 74-L round polyethylene tanks. Each tank was fitted with a center stand pipe drain to facilitate daily cleaning, and all tanks received water from a single head tank where water temperatures were regulated using a solenoid valve regulated by a digital temperature controller (Love model 16B AC; Dwyer Instruments, Michigan City, Indiana). Water temperatures were recorded every 10 min by four temperature thermagraphs distributed throughout the system (HOBO U22 Pro v2; Onset Computer Corp., Bourne, Massachusetts). Each cup received 50 mL/s of 10°C water dripped over the top of a single layer of eggs incubated over a mesh screen (mean = 297 eggs/cup, 95% CI = ± 36 eggs). When over 90% of the embryos in an egg cup had hatched, the contents were decanted into the round tank in which the cups were bathed, and the date recorded as the hatch date for that family. Degree days (the cumulative sum of mean daily temperatures) from fertilization to hatch were calculated for each tank in WaTSS (Rogers 2015).

Fry from all populations were reared under common conditions. Input flows for each tank were set to 600 mL/s and rearing temperatures to 13°C. Emergent trout fry were fed five times per

day with BioVita mash (BioOregon, Longview, Washington) and were supplemented with daily infusions of brine shrimp Artemia spp. nauplii. At three weeks post swim-up, the diet was switched over to BioVita starter feed exclusively, which was distributed five times per by day by automatic feeders (FishMate F14, Chewy.com, Dania Beach, Florida). Feed rates were adjusted per manufacturers specifications. Tanks were cleaned twice daily to remove uneaten food and waste. Lids were placed over the tanks to simulate overhead cover and reduce disturbance to the fish (Bear et al. 2007), and photoperiod was matched to ambient conditions over the course of the study. Survival of fry was monitored several times per day. We transformed survival data (sin^{-1}) and used ANOVA to test for differences among populations. All statistical analyses were performed in R (R Core Team 2020) at $\alpha = 0.05$, unless otherwise specified.

Critical Thermal Maxima

The critical thermal maximum (CTM) of each population was determined using 20 fry from each of the five families acclimated at 13°C. At 72 d post-hatch, individual fry were loaded into a cylindrical tolerance chamber (180 mm long x 38 mm diameter; working volume: 204 ml) following the design of Crocker and Cech (1997). Each chamber was fitted with an upstream flow diffuser to provide uniform distribution of water arriving at 450 ml/min. Water temperatures were regulated with a microprocessor-based temperature controller (Love C-series, Dwyer Instruments, Michigan City, Indiana) that mixed warm and cold water to achieve target temperatures. Fish were acclimated in the chambers for 60 min at 13°C, after which temperatures were increased 1°C every three minutes (Becker and Genoway 1979; Underwood et al. 2012; Brinkman et al. 2013). This increase was gradual enough to allow body temperatures to match ambient conditions (Brinkman et al. 2013), but rapid enough to prevent thermal acclimation (Smith and Fausch 1997). Temperatures were monitored to the nearest 0.1°C with a 12-channel scanning thermocouple thermometer (Model 69200, Eutech Instruments, Singapore), logging temperature in each chamber every 10 s. On final loss of equilibrium, temperature was reduced back to 13°C by removing the warm water line to the chamber. Fish were transferred to screened plastic cups and held in the 13°C rearing tanks for 24 hours to ensure full recovery from the CTM exposure. We tested for differences in critical thermal maxima using ANOVA and Tukey's HSD post-hoc test.

Growth and Ultimate Upper Incipient Lethal Temperature

Growth and UUILT trials were conducted simultaneously by testing each population with three replicates at six different temperature treatments. Because of logistical constraints, we were only able to run these experiments for 21 days. For each population, 18 lots of 15 fish, each representing an even mix of each of the remaining families, were isolated at 126 d post hatch. Each fish was weighed and measured, then lots were randomly assigned to 1 of 18 9.6-L grow-out tanks (Model ZT950, Aquaneering Inc., San Diego, California). Tanks were plumbed into six different semi-closed recirculating grow-out systems, each with a working volume of 306 L. Target temperatures for each system were 11, 14, 17, 20, 23, and 26°C, maintained with either 800 W or 1500 W submersible titanium aquarium heaters with automatic temperature controllers (Finnex, Chicago, Illinois). Three replicate grow-out tanks each receiving 500 ml/min at each temperature for each population were set in holding baths of the same temperature (also regulated with submersible heaters), to insulate against temperature swings. Any mortalities during the growth experiment were weighed and measured, then preserved. After 21 d, the fish

were euthanized with MS-222 (10 min exposure in 250 mg/L buffered with sodium bicarbonate), and each was measured and weighed.

With different populations reaching the 126 d post-hatch start threshold at different times, it was impossible to acclimate fish by increasing 1°C each day until target temperatures were reached, as in other studies (Bear et al. 2007; Brinkman et al. 2013; Zeigler et al. 2013). Accordingly, we acclimated fish by moving their tanks through the temperature ladder provided by the grow-out system baths. For example, fish that were destined to spend 21 d at 23°C were moved from the 13°C round rearing tanks to three 14°C chambers three days prior to the experiment, then to the 17°C bath two days prior, followed by the 20°C bath the day before, reaching the target temperature of 23°C on Day 0. With each transition, the volume of water in the tank was replaced with new (3°C warmer) water over an 18 min period, which served as the tempering phase each day.

We used established analytical methods to calculate the UUILT and optimal growth temperature (OGT) of each population, with one notable exception. We estimated the UUILT as the median lethal temperature survived by 50% of the population for 7 days and 21 days using the trimmed Spearman–Karber technique (Hamilton et al. 1977) in the ecotoxicology package (EPA 2015) for R. Relative daily growth rate was calculated as in Bear et al. (2007) for each tank and plotted against mean temperature for that tank, then fitted with a second-order (quadratic) polynomial regression, as is customary (Eaton et al. 1995; Lyytikainen and Jobling 1998; Bear et al. 2007; Zeigler et al. 2013; Brinkman et al. 2013). Because several of the data sets illustrated an asymmetric response in growth to temperature, we also fit a third-order (cubic) regression model for each population (Bevelhimer et al. 1985). We then estimated OGT from predicted response curves, and used a boostrap approach (e. g. Manly 1991) to calculate 95% confidence intervals around each estimated optimum. We resampled growth data for each population at each temperature step with replacement, then refit the quadratic and cubic curves 1,000 times to generate a distribution around the test statistic (observed OGT). Relative support for quadratic and cubic models was compared using Akaike's information criterion adjusted for small sample sizes (AICc; Burnham and Anderson 2002). A cubic model was considered better-supported than the nominal quadratic model if adding the third-order term reduced the AICc by more than two units.

RESULTS

Survival from spawn to the onset of temperature trials ranged from 39% to 61% and was comparable among the five Cutthroat Trout populations ($F_{4,19} = 0.958$, $P = 0.453$). Mean survival from spawn to hatch ranged from 53% in the Yellowstone eggs to 89% in the Trappers Lake eggs (overall mean = 70%), and mean survival from hatch to 72 d post hatch ranged from 61% in GBCT from Zimmerman Lake to 75% in CRCT from Nanita Lake (overall mean = 68%). Neither survival from spawn to hatch, nor survival from hatch to 72 post hatch differed among populations ($F_{4,19} \leq 2.251$, $P \geq 0.102$). The range in survival rates was as large or larger within some populations as it was across populations. For example, survival from spawn to 72 d post hatch ranged from 34% to 61% among GBCT families from Zimmerman Lake. Measured

degree-days (from 0°C) to hatch averaged 310°C-days across all families, but ranged from 268 - 370°C-days. The number of degree-days required for fertilized eggs to hatch was also variable between populations, with mean values ranging from 277°C-days in trout from Trappers Lake to 341°C-days in CRCT from Lake Nanita.

Acute effects - CTM and UUILT

The two acute temperature metrics of CTM and UUILT offered contradictory outcomes. Critical thermal maxima differed between populations ($P < 0.05$; Figure 1), with Milk Creek and Lake Nanita CRCT both tolerating significantly lower (27.5°C) temperatures than Yellowstone River YSCT and Zimmerman Lake GBCT (28.2°C and 28.3°C respectively). Average CTM for the admixed progeny from Trappers Lake (27.9°C) was intermediate between the ancestral sources, and not significantly different from either (Figure 1). All but two of the 462 fish subjected to this thermal stress test recovered within 24 hrs of the CTM trial. Ultimate upper incipient lethal temperatures did not differ among populations, during either the 7-day or 21- day trial (Table 2). Population metrics were comparable between trials and the difference between 7-day and 21-day estimates of the UUILT never exceeded 0.09°C.

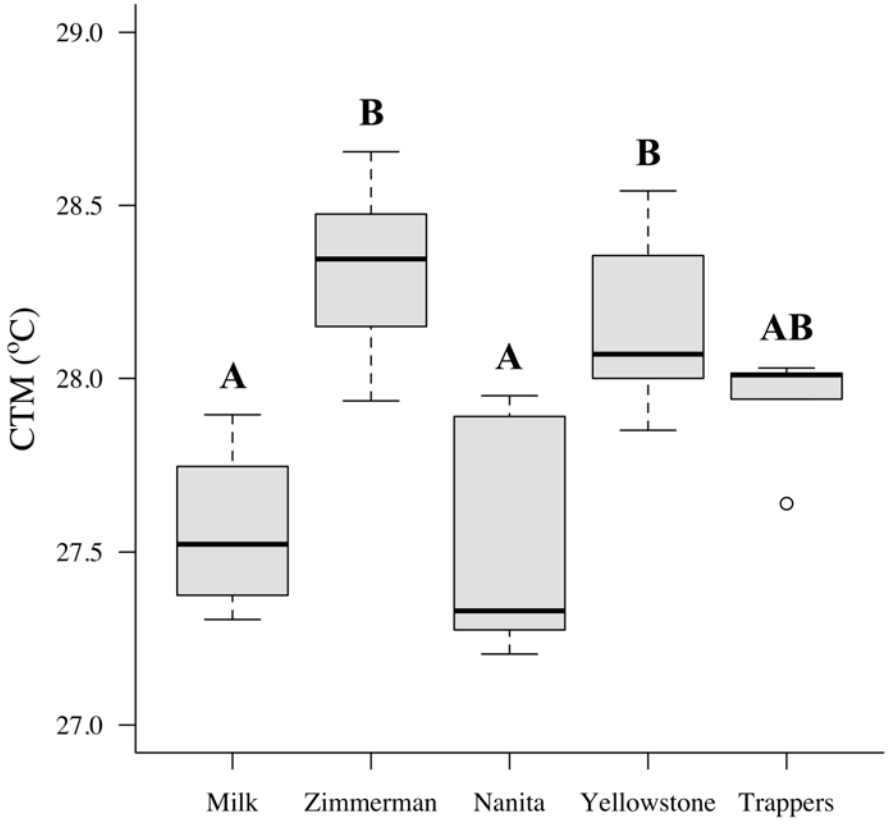

Figure 1. Comparison of critical thermal maxima (CTM) among five populations of Cutthroat Trout. Thick horizontal black bars represent population-specific medians (°C). Boxes span the interquartile range and whiskers extend 1.5x past that range. Populations that are not

significantly different from each other (P > 0.05) share the same bold letter.

Table 2. Ultimate Upper Incipient Lethal Temperatures (UUILT; °C) and 95% confidence limits (CL) for Cutthroat Trout populations examined during 7-day and 21-day trials.

Population (sub-species)	7-day (95% CL)	21-day (95% CL)
Milk Creek	24.34 (24.20, 24.47)	24.34 (24.20, 24.47)
Zimmerman Lake	24.45 (24.45, 24.45)	24.44 (24.39, 24.48)
Lake Nanita	24.45 (24.45, 24.45)	24.40 (24.32, 24.49)
Yellowstone River	24.42 (24.35, 24.49)	24.42 (24.35, 24.49)
Trappers Lake	24.51 (24.41, 24.62)	24.42 (24.35, 24.49)

Chronic effects – OGT

Growth rates differed among populations and temperatures (Figure 2). All populations grew at the 14°C and 17°C treatments (though not necessarily at the same rates); all but Zimmerman Lake fish grew consistently at 20°C; and only one grow-out tank (a Trappers Lake lot) accumulated weight at 23°C. All populations perished at the 26°C treatment. The maximum relative growth rate of hybrid Cutthroat Trout from Trappers Lake (mean maximum = 3.70% at 20°C) was 6-7x higher than the maximum growth rate of GBCT from Zimmerman Lake (mean maximum = 0.57% at 14°C).

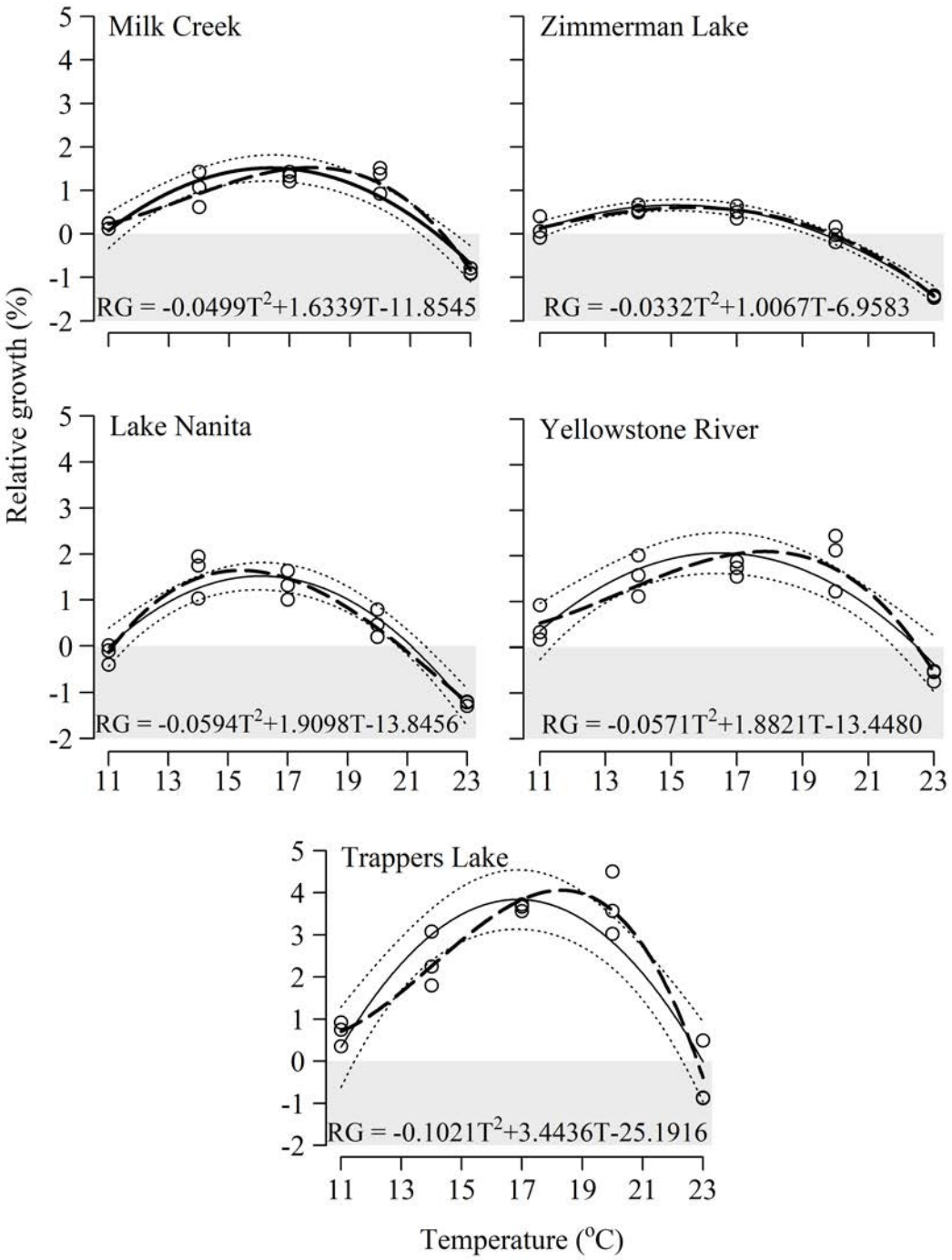

Figure 2. Relative daily growth as a function of temperature in five populations of Cutthroat Trout fit with a standard quadratic least squared regression (solid line; equation above the x-axis) and associated 95% confidence interval (dotted lines). The heavy dashed line represents the fitted cubic regression.

When predicting growth as a function of temperature, cubic models performed as well or better than quadratic models (Table 3). With cubic equations, temperature and its two higher order terms explained from 81% to 96% of the variation in relative growth (mean = 90%); whereas, with quadratic equations, temperature and its second order term explained from 76% to 96% of the variation in relative growth (mean = 85%). For three of five populations, the cubic model was better supported than the quadratic model (ΔAICc > 2). For these same populations, estimates of optima differed significantly between second- and third-order equations. Because cubic models performed as well (2 of 5) or better (3 of 5) than quadratic models, we focused on the former for comparisons between populations.

Table 3. Comparison between second-order (quadratic) and third-order (cubic) models used to predict optimal growth temperatures with associated 95% confidence intervals in five populations of Cutthroat Trout (AICc = Akaike's Information Criterion corrected for small sample size).

	Quadratic			Cubic		
Population	Optimum (°C)	R^2	AICc	Optimum (°C)	R^2	AICc
Milk Creek	16.36 (16.19, 16.53)	0.85	19.485	17.74 (16.78, 18.43)	0.92	13.987
Zimmerman Lake	15.14 (14.60, 15.52)	0.96	-3.833	15.59 (14.94, 16.27)	0.96	-1.683
Lake Nanita	16.09 (15.86, 16.29)	0.90	19.210	15.42 (14.97, 16.16)	0.92	19.376
Yellowstone River	16.48 (16.07, 16.78)	0.76	31.356	17.85 (16.48, 18.60)	0.81	30.682
Trappers Lake	16.86 (16.61, 17.18)	0.79	44.690	18.30 (17.55, 18.78)	0.90	37.738

Based on the results of cubic temperature-growth models, OGT differed among populations (Table 3). Two groups emerged with more than 2°C of separation between them. Optimal growth temperatures were 15.4-15.6°C in GBCT from Zimmerman Lake and CRCT from Lake Nanita, versus 17.7-18.3°C in CRCT from Milk Creek, YSCT from Yellowstone River, and introgressed hybrids of the two (CRCT x YSCT) from Trappers Lake.

DISCUSSION

Survival from spawn to experiment was surprisingly similar and high among wild Cutthroat Trout populations used in this study. On average, 70% of eggs spawned and fertilized in the wild hatched, with 99% hatching in some families. Even the Zimmerman Lake eggs that are especially challenging to raise (B. Johnson, Colorado Parks and Wildlife, unpublished data), saw average survival to hatch of 71% and survival from spawn to 72 d post hatch of 43%. We attribute the high rates of survival to intensive husbandry practices only possible with small lots of fertilized eggs. Keeping individual families separate and eggs distributed in a single layer resting in the bottom of the egg cups allowed early detection of fungal infections that can ravage developing embryos (Arndt et al. 2001). Even with these intensive culture practices, we still saw extreme variation in survival to hatch among families consistent with other studies that suggest individual female egg quality is strongly correlated with embryo survival (Wipf and Barnes 2012). Because survival prior to the temperature experiments did not differ among populations, we can assume that differences in thermal trial performance were indeed the result of variability in thermal tolerance.

Although CTMs in this study differed among populations, our observed values of 27.5 – 28.3°C fell within the range of 26.7 - 29.1°C documented in a half dozen other salmonid taxa reviewed by Brinkman et al. (2013). Interestingly, our mean CTM for the Lake Nanita fish acclimated to 13°C (27.5°C) was slightly higher than that reported by Underwood et al. (2012) for age 1+ year fish of the same strain acclimated to 15°C (26.9°C), but consistent with their finding of reduced thermal tolerance with age also seen with size in other trout studies (Galbreath et al. 2006; Brinkman et al. 2013). Our CTM values did not appear to reflect the thermal regimes of the source waters very well, however (Figure A.1). Cold Lake Nanita and warm Milk Creek shared the same CTM value (27.5°C), while GBCT from Zimmerman Lake that appear to thrive in cold water displayed the highest CTM (28.3°C). The lower value for Milk Creek fish was unexpected given the extreme thermal conditions they face in their natal waters (Hodge et al. 2017), however behavioral plasticity coupled with extreme variation in diel temperatures can allow fish to persist in otherwise unforgiving environments (Schrank et al. 2003; McCullough et al. 2009; Hodge et al. 2017).

Our findings regarding UUILTs both aligned with and differed from other observations of Cutthroat Trout. During our experiment, the traditional 7-d test (e.g., Brett 1952; Dickerson and Vinyard 1999; Johnstone and Rahel 2003) yielded a mean UUILT of 24.4°C (range = 24.3°C – 24.5°C). Zeigler et al. (2013) obtained a 7-d UUILT value of 24.7°C for Rio Grande Cutthroat Trout fry and Bear et al. (2007) documented 24.2°C for Westslope Cutthroat Trout. Bear et al. (2007) suggested that sharp declines in survival could occur beyond the 7-d time interval, noting that in their 60-d trial, UUILT was 1.8°C lower for Rainbow Trout and 4.6°C lower for Westslope Cutthroat Trout. Our 21-d trials did not suggest a similar drop, with the mean UUILT value remaining at 24.4°C, though with near 100% survival in the 23°C trial, and 0% survival in the 26°C group, we were not able to resolve fine scale differences in UUILT that might have materialized if more temperature treatments were used.

Differences in acute effects between subspecies of Cutthroat Trout studied here were either

relatively small (CTM) or nonexistent (UUILT) similar to some other studies on salmonids (McCullough 1999; McCullough et al. 2009). Given variation seen in other CRCT however (Underwood et al. 2012), we were surprised to not see elevated lethal threshold thermal tolerance among the Milk Creek fish given the fairly hostile environment in which they evolved. Even more surprising was the apparent lack of correlation between the thermal regimes experienced by the host populations and CTM and UUILT values (Figure A.1). This suggests that perhaps upper thermal tolerance limits are governed by molecular pathways that are not very plastic (Logan and Buckley 2015; Ooman and Hutchings 2017), and that even with an evolutionary history in warm thermal environments, these subspecies do not gain much additional lethal threshold tolerance. This is concerning to those involved with conservation efforts because it might be evidence that these fish are already operating at close to the maximum attainable level of thermal tolerance. Continued increases in environmental temperatures, even when those acclimation temperatures approach those of putative optimal growth, may not derive further thermal acclimation benefits.

A different message was delivered by examination of OGT. Our observed optima in CRCT from Lake Nanita and GBCT from Zimmerman Lake (15.4-15.6°C) are similar to those observed during other laboratory-based experiments. For example, Zeigler et al. (2013) showed peak growth in a sister taxon, the Rio Grande Cutthroat Trout (*O. c. virginalis*, at 15.3°C, while Brandt (2009) demonstrated optimal growth in CRCT at 15.3–16.4°C. Bear et al. (2007) found that Westslope Cutthroat Trout (WSCT; *O. c. lewisi*) growth peaked at a slightly colder 13.6°C. Our observed optima in CRCT from Milk Creek, YSCT from Yellowstone Lake, and CRCT-YSCT hybrids from Trappers Lake (17.7-18.3°C) however, are more similar to the OGT of Brown Trout Salmo Trutta and Rainbow Trout O. mykiss than to those of other Cutthroat Trout taxa (Brinkman et al. 2013, and sources therein). The seemingly anomalous temperature optima are at least in part due to fitting differences between quadratic and cubic models. Based on fitted quadric curves, the OGT of the two populations with Yellowstone Cutthroat Trout alleles are only 16.5-16.9°C.

Although researchers typically estimate OGT from the fitted curve of a quadratic regression model (e.g., Bear et al. 2007; Zeigler et al. 2013; Brinkman et al. 2013), our findings suggest that optima might be better identified from the curve of a cubic model. The cubic function was more parsimonious than the quadratic function on three of five counts (i.e., $\Delta AIC > 2$), and equally supported on the other two. In four of five cases, adding a cubic term increased the explanatory power (R^2) of the temperature-growth model. Comparison of quadratic and cubic models for the Trappers Lake population illustrates the potential limitation of applying an implicitly symmetrical relationship to asymmetrical data. Whereas the fitted curve from the quadratic function ran below all data points at the 20°C temperature step, the treatment at which Trappers Lake fish exhibited the highest mean growth rate, the fitted curve for the cubic function ran through the middle of those data points. With the improvement in fit came a significant and seemingly appropriate increase in the estimated OGT (from 16.9°C to 18.3°C).

The accuracy of estimated growth optima could be especially relevant when evaluating the fundamental thermal niche these fish occupy. Defined as the range from 3°C lower to 1°C higher than the OGT (Christie and Regier 1988), this niche would range from 13.4°C -17.4°C for the Milk Creek trout fit with a quadratic function. Yet our own results suggest that these fish

grow as well or better at 20°C – outside the range of that niche. One expects that as temperature exceeds limits defined by the fundamental thermal niche, a decrease in individual growth and a reduction in population viability should occur (Zeigler et al. 2013). More importantly, laboratory based studies such as these are used to establish thermal habitat protection standards (Armour 1991; Todd et al. 2008), yet these very standards would serve to disqualify habitats for future reclamation efforts in waters where the fish could exhibit their fastest growth. Addition of a third order term to the polynomial used to fit the data would help mitigate that risk, and allow continued use of the optimum growth temperature to characterize the upper range of suitable thermal habitat for the long-term persistence of salmonids (McCullough 1999; Selong et al. 2001; Dunham et al. 2003). However, we should recognize that selecting suitable habitat based only on growth optima may ignore the ability for local food resources (quality and quantity) to keep up with increased metabolic demand needed for trout to thrive in warmer water.

We expected to see support for local thermal adaptation in growth within the Cutthroat Trout populations given the findings of others (McCullough et al. 2009; Drinan et al. 2012; Underwood et al. 2012). Unlike the acute metrics, the sublethal measure of growth did follow our expectation of warmer host waters producing fish that displayed improved growth at warmer temperatures, with OGT occurring for Milk Creek fish at 17.7ºC. Optimal growth temperatures in YSCT were also high (17.8ºC), perhaps because these trout likely occupied many waters historically that exceeded 26ºC (Varley and Gresswell 1988). This trait appears to be heritable as it is also manifested in the current progeny from Trappers Lake that now contain many YSCT alleles (Martinez 1988; Leary and Allendorf 1991; Rogers et al. 2018), despite the original inhabitants of that lake (now found in Lake Nanita) displaying much lower growth at higher temperatures (Figure 2).

One of our more interesting findings was that Trappers Lake fish exhibited the highest growth rate. Although this population of mixed (CRCT x YSCT) origin displayed a CTM and OGT that was intermediate to its two ancestral stocks, it grew faster at temperature than both ancestral stocks (estimated peak growth of 4.1% vs 1.6-2.1% per day). Hybrid vigor could play a role in boosting growth (Donaldson et al. 1957; Rosenfield et al. 2004). However, we should not necessarily be persuaded into equating strong growth with population performance. While growth in GBCT from Zimmerman Lake was startlingly poor, slow growth could be advantageous in the small, pool-limited stream where this wild brood stock was sourced (Bear Creek; J. Valladares, U. S. Forest Service, unpublished data).

Management implications

Here we demonstrated that Cutthroat Trout stocks respond differently to variation in thermal regime, and that models used to predict future persistence should account for this variation. Clearly, some stocks of trout are more tolerant of warm temperatures than others, and therefore more capable of persisting in the face of a warming climate. Consideration of stock-specific attributes of thermal tolerance can be used to help guide which stocks would be best suited for lower elevation waters that are facing the immediate consequences of climate change. Similarly, knowledge of stock-specific limitations might inform the choice of streams or habitats for repatriation. For example, results of this study revealed that the thermal niche for remaining GBCT appears to be quite narrow and thus the candidate pool of potential recipient waters

relatively small. Thermal regimes in these waters should be studied carefully before embarking on costly and labor-intensive reclamation projects.

Although we observed considerable variation between stocks in temperature-growth relationships, we found relatively little variation in acute temperature measures such as CTM and UUILT. This suggests differences are found at the margin, and that the capacity to adapt could be limited at the upper bounds. If habitat is variable enough to allow for quick behavioral shifts in habitat use (e.g., Kaeding 1996; Hodge et al. 2017), then persistence is likely. However, if a warming climate eliminates those refugia, the ability of these fish to persist will be challenged.

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REFERENCES

Al-Chokhachy et al. 2013. Characterizing the thermal suitability of instream habitat for salmonids: a cautionary example from the Rocky Mountains. Transactions of the American Fisheries Society 142:793-801.

Al-Chokhachy, R., and A. J. Sepulveda. 2019. Impacts of nonnative Brown Trout on Yellowstone Cutthroat Trout in a tributary stream. North American Journal of Fisheries Management 39:17-28.

Armour, C. L. 1991. Guidance for evaluating and recommending temperature regimes to protect fish. U.S. Fish and Wildlife Service Biological Report 90(22).

Arndt, R. E., E. J. Wagner, and M. Routledge. 2001. Reducing or withholding hydrogen peroxide treatment during a critical stage of rainbow trout development: effects on eyed eggs, hatch, deformities, and fungal control. North American Journal of Aquaculture 63:161-166.

Bear, E. A., T. E. McMahon, and A. V. Zale. 2007. Comparative thermal requirements of Westslope Cutthroat Trout: implications for species interactions and development of thermal protection standards. Transactions of the American Fisheries Society 136:1113-1121.

Becker, C. D., and R. G. Genoway. 1979. Evaluation of the critical thermal maximum for determining thermal tolerance of freshwater fish. Environmental Biology of Fishes 4:245-256.

Bestgen, K. R., K. B. Rogers, and R. Granger. 2019. Distinct phenotypes of native Cutthroat Trout emerge under a molecular model of lineage distributions. Transactions of the American Fisheries Society 148:442-463.

Bevelhimer, M. S., R. A. Stein, and R. F. Carline. 1985. Assessing significance of physiological differences among three *Esocids* with a bioenergetics model. Canadian Journal of Fisheries and Aquatic Sciences 42:57:69.

Brett, J. R. 1952. Temperature tolerance in young Pacific salmon, genus *Oncorhynchus*. Journal of the Fisheries Research Board of Canada 9:265–322.

Brinkman, S. F., H. J. Crockett, and K. B. Rogers. 2013. Upper thermal tolerance of mountain whitefish (*Prosopium williamsoni*) eggs and fry. Transactions of the American Fisheries Society 142:824-831.

Brandt, M. M. 2009. Optimal starter diets and culture conditions for Colorado River Cutthroat Trout (*Oncorhynchus clarkii pleuriticus*). Master's thesis. Colorado State University, Fort Collins, Colorado.

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

Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodel inference: a practical information theoretic approach. 2nd edition. Springer Verlag, New York, New York, USA.

Christie, G. C., and H. A. Regier. 1988. Measures of optimal thermal habitat and their relationship to yields for four commercial fish species. Canadian Journal of Fisheries and Aquatic Sciences 45:301–314.

Cleveland, W. S. 1979. Robust locally weighted regression and smoothing scatterplots. Journal of the American Statistical Association 74:829-836.

Cleveland, W. S., and S. J. Devlin. 1988. Locally-weighted regression: an approach to regression analysis by local fitting. Journal of the American Statistical Association 83:596-610.

Coleman, M. A., and K. D. Fausch. 2007a. Cold summer temperature limits recruitment of age-0 Cutthroat Trout in high-elevation Colorado streams. Transactions of the American Fisheries Society 136:1231-1244.

Coleman, M. A., and K. D. Fausch. 2007b. Cold summer temperature regimes cause a recruitment bottleneck in age-0 Colorado River Cutthroat Trout reared in laboratory streams. Transactions of the American Fisheries Society 136:639-654.

Cook, E. R, C. A. Woodhouse, C. M. Eakin, D. M. Meko, and D. W. Stahle. 2004. Long-term aridity changes in the western United States. Science 306:1015-1018.

Crocker, C. E., and J. J. Cech, Jr. 1997. Effects of environmental hypoxia on oxygen consumption rate and swimming activity in juvenile White Sturgeon, *Acipenser transmontanus*, in relation to temperature and life intervals. Environmental Biology of Fishes 50:383-389.

Dickerson, B. R., and G. L. Vinyard. 1999. Effects of high chronic temperatures and diel temperature cycles on the survival and growth of Lahontan Cutthroat Trout. Transactions of the American Fisheries Society 128:516–521.

Donaldson, L. R, D. D. Hansler, and T. N. Buckridge. 1957. Interracial hybridization of Cutthroat Trout, Salmo clarkii, and its Use in fisheries management. Transactions of the American Fisheries Society 86:350-360.

Drinan, D. P., A. V. Zale, M. A. H. Webb, M. L. Taper, B. B. Shepard, and S. T. Kalinowski. 2012. Evidence of local adaptation in Westslope Cutthroat Trout. Transactions of the American Fisheries Society 141:872–880.

Dunham, J., R. Schroeter, and B. Rieman. 2003. Influence of maximum water temperature on occurrence of Lahontan Cutthroat Trout within streams. North American Journal of Fisheries Management 23 1042-1049.

Eaton, J. G., J. H. McCormick, B. E. Goodno, D. G. O'Brien, H. G. Stefany, M. Hondzo, and R. M. Scheller. 1995. A field information-based system for estimating fish temperature tolerances. Fisheries 20:10 –18.

Eliason, E. J., T. D. Clark, M. J. Hague, L. M. Hanson, Z. S. Gallagher, K. M. Jeffries, M. K. Gale, D. A. Patterson, S. G. Hinch, A. P. Farrell. 2011. Differences in thermal tolerance among Sockeye Salmon populations. Science 332:109-112.

Fausch, K. D. 2008. A paradox of trout invasions in North America. Biological Invasions 10:685-701.

Fröhlicher, T. L., M. Winton, J. L. Sarmiento. 2014. Continued global warming after CO2 emissions stoppage. Nature Climate Change 4:40-44.

Galbreath, P. F., N. D. Adams, L. W. Sherrill, and T. H. Martin. 2006. Thermal tolerance of diploid versus triploid Rainbow Trout and Brook Trout assessed by time to chronic lethal maximum. Environmental Biology of Fishes 75:183–193.

Hamilton, M. A., R. C. Russo, and R. V. Thurston. 1977. Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. Environmental Science and Technology 11:714–719.

Hansen, J., M. Sato, and R. Ruedy. 2012. Perception of climate change. Proceedings of the National Academy of Sciences 109:E2415-E2423.
Harig, A. L., and K. D. Fausch. 2002. Minimum habitat requirements for establishing translocated Cutthroat Trout populations. Ecological Applications 12:535-551.
Hendry, A. P., J. E. Hensleigh, and R. R. Reisenbichler. 1998. Incubation temperature, developmental biology, and the divergence of Sockeye Salmon (*Oncorhynchus nerka*) within Lake Washington. Canadian Journal of Fisheries and Aquatic Sciences 55:1387-1394.
Hodge, B. W., K. D. Battige, and K. B. Rogers. 2017. Seasonal and temperature-related movement of Colorado River cutthroat trout in a low-elevation, Rocky Mountain stream. Ecology and Evolution 7:2346-2356.
Isaak, D. J., C. H. Luce, B. E. Rieman, D. E. Nagel, E. E. Peterson, D. L. Horan, S. Parkes, and G. L. Chandler. 2010. Effects of climate change and wildfire on stream temperatures and salmonid thermal habitat in a mountain river network. Ecological Applications 20:1350–1371.
Isaak, D. J., C. C. Muhlfeld, A. S. Todd, R. Al-Chokhachy, J. J. Roberts, J. L. Kershner, K. D. Fausch, S. W. Hostetler. 2012. The past as prelude to the future for understanding 21st century climate effects on Rocky Mountain trout. Fisheries 37:542-556.
Isaak, D. J., S. J. Wenger, and M. K. Young. 2017. Big biology meets microclimatology: defining thermal niches of ectotherms at landscape scales for conservation planning. Ecological Applications 27:977-990.
Johnstone, H. C., and F. J. Rahel. 2003. Assessing temperature tolerance of Bonneville Cutthroat Trout based on constant and cycling thermal regimes. Transactions of the American Fisheries Society 132:92-99.
Kaeding, L. R. 1996. Use of coolwater tributaries of a geothermally heated stream by Rainbow and Brown Trout, *Oncorhynchus mykiss* and *Salmo trutta*. American Midland Naturalist 135:283-292.
Kavanagh, K. D., T. O. Haugen, F. Gregersen, J. Jernvall, and L, A. Vollestad. 2010. Contemporary temperature-driven divergence in a Nordic freshwater fish under conditions commonly thought to hinder adaptation. BMC Evolutionary Biology 10:350
Kennedy, C. M. 2014. Rocky Mountain National Park fisheries and aquatic management. U. S. Fish and Wildlife Service, Lakewood, Colorado.
Leary, R. F., and F. W. Allendorf. 1991. Genetic and meristic analysis of Colorado River Cutthroat Trout. Wild Trout and Salmon Genetics Laboratory report 91/1. University of Montana, Bozeman.
Logan, C. A., and B. A. Buckley. 2015. Transcriptomic responses to environmental temperature in eurythermal and stenothermal fishes. Journal of Experimental Biology 218:1915-1924.
Lyytikainen, T., and M. Jobling. 1998. The effects of temperature, temperature shift, and temperature fluctuation on daily feed intake, growth, and proximate composition of underyearling Lake Inari Arctic charr (*Salvelinus alpinus* (L.)). Nordic Journal of Freshwater Research 74:87–94.
Mandeville, C. P., F. J. Rahel, L. S. Patterson, A. W. Walters. 2019. Integrating fish assemblage data, modeled stream temperatures, and thermal tolerance metrics to develop thermal guilds for water temperature regulation: Wyoming case study. Transactions of the American Fisheries Society 148:739-754.
Manly, B. F. J. 1991. Randomization and Monte Carlo methods in biology. Chapman and Hall, London.
Martinez, A. M. 1988. Identification and status of Colorado River Cutthroat Trout in Colorado. Pages 81-89 *in* R. E. Gresswell, editor. Status and management of interior stocks of Cutthroat Trout. American Fisheries Society Symposium 4, Bethesda, Maryland.
McCullough, D. A. 1999. A review and synthesis of effects of alterations to the water temperature regime on freshwater life stages of salmonids, with special reference to Chinook Salmon. U.S. Environmental Protection Agency Report EPA 910-R-99-010, Seattle.
McCullough, D. A., J. M. Bartholow, H. I. Jager, R. L. Beschta, E. F. Cheslak, M. L. Deas, J. L. Ebersole, J. S. Foott, S. L. Johnson, K. R. Marine, M. G. Mesa, J. H. Petersen, Y. Souchon, K. F. Tiffan, and W. A. Wurtsbaugh. 2009. Research in thermal biology: burning questions for coldwater stream fishes. Reviews in Fisheries Science 17:90–115.
McDermid, J. L., F. A. Fischer, M. Al-Shamlih, W. N. Sloan, N. E. Jones, and C. C. Wilson. 2012. Transactions of the American Fisheries Society 141:1230-1235.
Meredith, C. S., P. Budy, and M. Hooten. 2017. Assessing abiotic conditions influencing the longitudinal distribution of exotic Brown Trout in a mountain stream: a spatially-explicit modeling approach. Biological Invasions 19:503-519.
Metcalf, J. L., S. L. Stowell, C. M. Kennedy, K. B. Rogers, D. McDonald, J. Epp, K. Keepers, A. Cooper, J. J. Austin, and A. P. Martin. 2012. Historical stocking data and 19th century DNA reveal human-induced

changes to native diversity and distribution of cutthroat trout. Molecular Ecology 21:5194-5207.
Narum, S. R., N. R. Campbell, K. A. Meyer, M. R. Miller, and R. W. Hardy. 2013. Thermal adaptation and acclimation of ectotherms from differing aquatic climates. Molecular Ecology 22:3090-3097.
Ooman, R. A., and J. A. Hutchings. 2017. Transcriptomic responses to environmental change in fishes: insights from RNA sequencing. Facets 2:610-641.
Pearse, D. E., and M. A. Campbell. 2018. Ancestry and adaptation of Rainbow Trout in Yosemite National Park. Fisheries 43:472-484.
Penaluna, B. E., A. Abadía-Cardoso, J. B. Dunham, F. J. García de León, R. E. Gresswell, A. Ruiz Luna, E. B. Taylor, B. B. Shepard, R. Al-Chokhachy, C. C. Muhlfeld, K. R. Bestgen, K. B. Rogers, M. A. Escalante, E. R. Keeley, G. Temple, J. E. Williams, K. Matthews, R. Pierce, R. L. Mayden, R. P. Kovach, J. C. Garza, and K. D. Fausch. 2016. Conservation of Native Pacific Trout Diversity in Western North America. Fisheries 41:286-300.
Peterson, D. P., K. D. Fausch and G. C. White. 2004. Population ecology of an invasion: Effects of Brook Trout on native Cutthroat Trout. Ecological Applications 14:754-772.
Rahel, F. J., C. J. Keleher, and J. L. Anderson. 1996. Potential habitat loss and population fragmentation for cold water sh in the North Platte River drainage of the Rocky Mountains: response to climate warming. Limnology and Oceanography 41:1116–1123.
Recsetar, M. S., M. P. Zeigler, D. L. Ward, S. A. Bonar, and C. A. Caldwell. 2012. Relationship between fish size and upper thermal tolerance. Transactions of the American Fisheries Society 141:1433-1438.
Ricker, W. E. 1979. Growth rates and models. Pages 677–743 *in* W. S. Hoar, D. J. Randall, and J. R. Brett, editors. Fish physiology, volume 8. Academic Press, New York.
Roberts, J. J., K. D. Fausch, D. P. Peterson, and M. B. Hooten. 2013. Fragmentation and thermal risks from climate change interact to affect persistence of native trout in the Colorado River basin. Global Change Biology 19:1383-1398.
Roberts, J. J., K. D. Fausch, M. B. Hooten, and D. P. Peterson. 2017. Nonnative trout invasions combined with climate change threaten persistence of isolated Cutthroat Trout populations in the southern Rocky Mountains. North American Journal of Fisheries Management 37:2, 314-325.
Rogers, K. B. 2015. User manual for WaTSS 3.0 (Water Temperature Summary Software). Colorado Parks and Wildlife, Steamboat Springs, Colorado. Available: http://cpw.state.co.us/learn/Pages/ ResearchAquaticSoftware.aspx (January 2021)
Rogers, K. B., K. R. Bestgen, S. M. Love Stowell, and A. P. Martin. 2018. Cutthroat Trout diversity in the southern Rocky Mountains. Pages 323-341 *in* P. Trotter, P. Bisson, L. Schultz, and B. Roper, editors. Cutthroat Trout: evolutionary biology and taxonomy. American Fisheries Society, Special Publication 36, Bethesda, Maryland.
Rosenfield, J. A., S. Nolasco, S. Lindauer, C. Sandoval, A. Kodric-Brown. 2004. The role of hybrid vigor in the replacement of Pecos Pupfish by Its hybrids with Sheepshead Minnow. Conservation Biology 18:1589-1598.
Selong, J. H., T. E. McMahon, A. V. Zale, and F. T. Barrows. 2001. Effect of temperature on growth and survival of bull trout, with application of an improved method for determining thermal tolerance in fishes. Transactions of the American Fisheries Society 130:1026–1037.
Smith, R. K., and K. D. Fausch. 1997. Thermal tolerance and vegetation preference of Arkansas Darter and Johnny Darter from Colorado plains streams. Transactions of the American Fisheries Society 126:676–686.
Todd, A. S., M. A. Coleman, A. M. Konowal, M. K. May, S. Johnson, N. K. M. Vieira and J. F. Saunders. 2008. Development of new water temperature criteria to protect Colorado's fisheries. Fisheries 33:9, 433-443.
Underwood, Z. E., C. A. Myrick, and K. B. Rogers. 2012. Effect of acclimation temperature and the upper thermal tolerance of Colorado River cutthroat trout *Oncorhynchus clarkii pleuriticus*. Journal of Fish Biology 80:2420-2433.
USFWS (U. S. Fish and Wildlife Service). 2008. Endangered and threatened wildlife and plants: determination of threatened status for the Polar Bear (*Ursus maritimus*) throughout its range. Federal Register 73(95):28212– 28303.
Varley, J. D. 1979. Record of egg shipments from Yellowstone fishes, 1914-1955. U. S. National Park Service, Information Paper 36, Yellowstone National Park, Wyoming.
Wagner, E. J., R. E. Arndt, and M. Brough. 2001. Comparative tolerance of four stocks of Cutthroat Trout to extremes in temperature, salinity, and hypoxia. Western North American Naturalist 61:434-444.

Wenger, S. J., D. J. Isaak, C. H. Luce, H. M. Neville, K. D. Fausch, J. B. Dunham, D. C. Dauwalter, M. K. Young, M. M. Elsner, B. E. Rieman, A. F. Hamlet, and J. E. Williams. 2011. Flow regime, temperature, and biotic interactions drive differential declines of trout species under climate change. Proceedings of the National Academy of Sciences 108:14175–14180.

Williams, J. E., A. L. Haak, H. M. Neville, and W. T. Coyler. 2009. Potential consequences of climate change to persistence of Cutthroat Trout populations. North American Journal of Fisheries Management 29:533-548.

Wipf, M. W., and M. E. Barnes. 2012. Parental male effects on landlocked fall Chinook Salmon progeny survival. North American Journal of Aquaculture 74:443-448.

Zeigler, M. P., S. F. Brinkman, C. A. Caldwell, A. S. Todd, M. S. Recsetar, and S. A. Bonar. 2013. Upper thermal tolerances of Rio Grande Cutthroat Trout under constant and fluctuating temperatures. Transactions of the American Fisheries Society 142:1395-1405.

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

APPENDIX

Summary temperature metrics for source population waters

Long-term water temperature monitoring provided summary statistics useful for characterizing the thermal regimes experienced by each population. Digital thermagraphs (HOBO Water Temp Pro v2, Onset Computer Corp, Bourne, Massachusetts) were deployed in metal housings anchored to the streambed in areas likely to scour. Units were set to acquire temperatures every 30 or 60 min, and thermagraphs were swapped out every 1-4 years. Data from the Yellowstone River above LeHardy Rapids were obtained from Yellowstone National Park (T. Koel, National Park Service, unpublished). We used WaTSS v. 3.0 (Rogers 2015) to summarize temperature data (Table A.1; Todd et al. 2008, Isaak et al. 2010, Roberts et al. 2013), then explored the relationship of source population elevation (m), MWMT (ºC), and M30AT (ºC) against either critical thermal maxima (ºC; CTM) or the optimum temperature for growth (ºC; OGT)

Elevation was only loosely correlated with thermal regime in the populations used here, reflecting the combination of lake and stream sources from disparate latitudes. As a consequence, it was also poorly correlated with CTM or OGT ($R^2 < 0.17$; Figure A.1). Critical thermal maxima for these diverse populations also did not appear to be tied to the thermal regime these trout evolved in. Only for OGT was there a strong relationship (particularly for M30AT, $R^2 < 0.87$), where fish derived from warmer thermal regimes exhibited better growth in warmer water. This suggests that adaptive capacity for OGT might be more responsive to environmental conditions than CTM which is more likely to be constrained by molecular properties (Logan and Buckley 2015; Ooman and Hutchings 2017).

Table A.1. Annual maximum 30 d average temperature (M30AT), the maximum weekly maximum temperature (MWMT), and daily maximum temperature (2-hr rolling average maximum) were calculated for each study population.

Population	Year	MWMT (ºC)	M30AT (ºC)	Daily Max (ºC)
Milk Creek[1]				
	2012	25.0	18.6	
	2013	25.3	18.6	
	2015	22.5	16.4	23.3
	2016	23.6	17.1	24.7
	2017	25.0	18.3	25.2
	2018	25.8	18.9	27.0
	2019	23.3	16.8	24.4
Mean		24.4	17.8	25.7

Zimmerman Lake[2]				
	2012	15.8	13.1	16.4
	2013	14.5	12.3	15.1
	2014	13.7	11.6	14.6
	2015	13.6	11.8	13.9
	2016	14.1	12.5	14.6
	2017	14.5	12.3	15.2
	2018	15.1	12.8	15.9
	2019	14.4	12.7	15.3
	2020	14.6	12.7	15.2
Mean		14.5	12.4	15.1
Lake Nanita[3]				
	2002	19.2	15.7	20.0
	2003	16.4	14.1	17.5
	2004	16.1	12.8	17.0
	2005	15.8	13.0	16.6
	2013	17.6	14.3	18.1
	2014	14.4	12.4	14.9
	2015	16.2	13.6	17.2
	2017	15.3	12.5	16.2
	2018	18.1	14.6	18.7
	2019	15.6	12.8	16.1
	2020	17.2	14.2	17.7
Mean		16.5	13.6	17.3
Yellowstone River[4]				
	2001	21.2	16.9	22.2
	2002	19.8	17.0	20.6
Mean		20.5	17.0	21.4
Trappers Lake[5]				
	2012	19.0	17.6	20.4
	2013	19.1	17.5	20.1
	2014	18.8	16.6	19.8
	2015	17.6	16.0	18.6
	2016	18.2	16.5	19.3
	2017	18.8	17.1	20.8

	2018	18.9	17.4	19.7
	2019	18.2	16.6	19.5
	2020	18.4	16.6	19.2
Mean		18.6	16.9	19.7

[1]Thermagraph located at 40.18419° N 107.66350° W

[2]These data describe the thermal regime of the founding population in Bear Creek at 38.81768° N 104.89614° W

[3]Thermagraph located in the outlet of Lake Nanita at 40.25889° N 105.71621° W

[4]Thermagraph located near the outlet of Yellowstone Lake at Fishing Bridge

[5]Thermagraph located in the outlet of Trappers Lake at 39.99698° N 107.23090° W

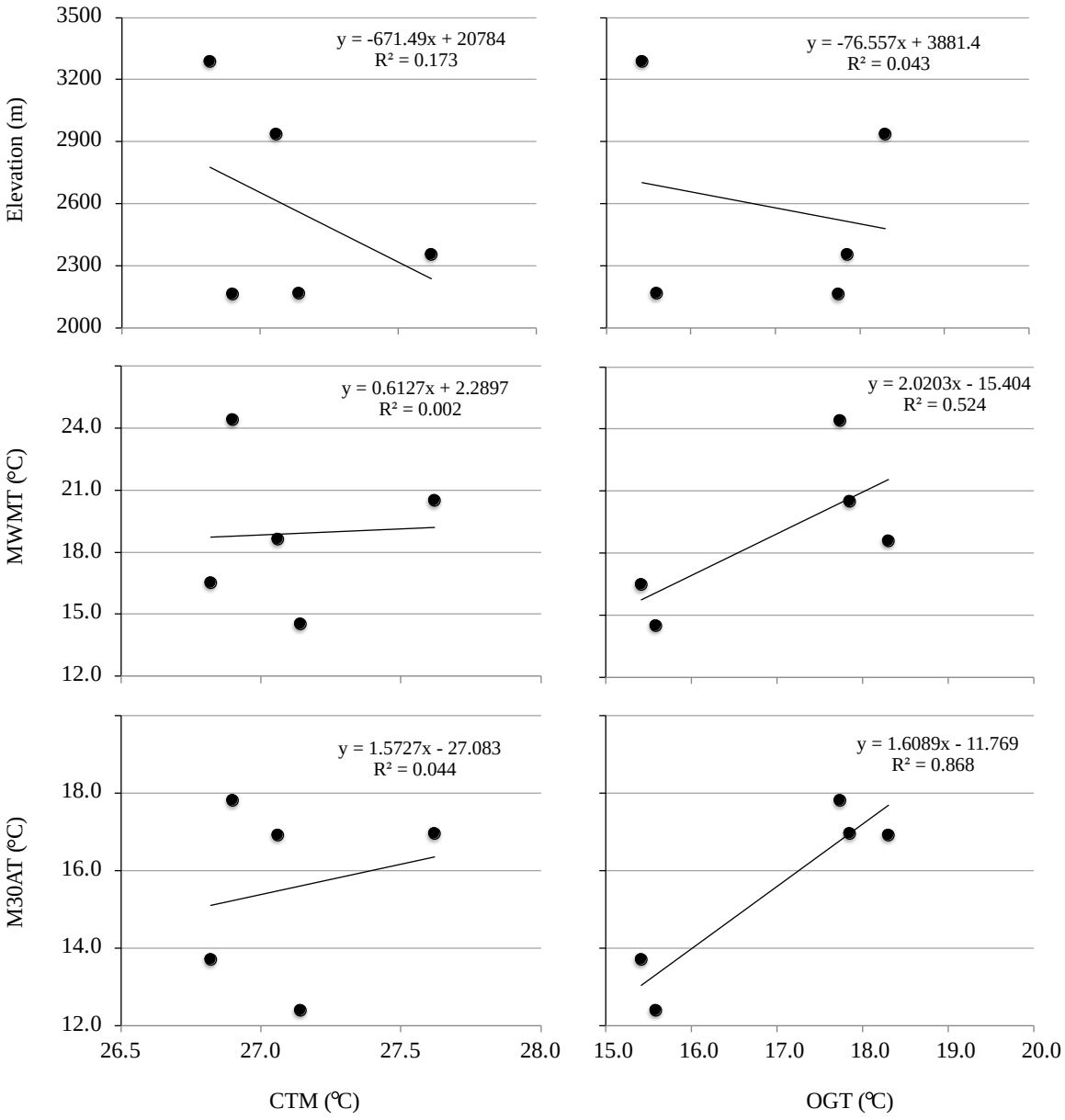

Figure A.1. Critical thermal maxima (CTM) and optimal growth temperatures (OGT) for each population were plotted against elevation, maximum weekly maximum temperature (MWMT), and maximum 30-d average temperature (M30AT) experienced by each population.

RESEARCH PRIORITY

Cutthroat Trout habitat conservation

OBJECTIVE

Characterize the bathymetry and water volume in Hack Lake, Colorado

INTRODUCTION

The potential for commercial development of water in the headwaters of the Hack Creek watershed could jeopardize Hack Lake and the springs feeding it. To protect these waters for native trout and angling opportunity, the BLM intends to file for a natural lake level water right protection with the Colorado Water Conservation Board. Accurate bathymetric maps are needed in conjunction with spring source discharge (collected by BLM staff on the same day) for petitions to protect natural lake levels to be successful. Although Hack Lake currently does not support natural recruitment of Cutthroat Trout, robust spring inflows could allow for it if a suitable spawning channel were built. That combined with a reclamation project informed by the bathymetry developed here would provide for a wild naturally reproducing population. To facilitate potential future efforts, I maintained imperial units in all calculations.

METHODS

All mapping gear was packed 3.5 miles into Hack Lake, Garfield County, Colorado from the Hack Lake trailhead on September 22, 2021. Gear included a Humminbird model 597ci HD (Humminbird, Eufaula, Alabama) depth finder with a transducer deployed off the bow of an 8.5 lb pontoon boat (Figure 1; Model ODC 420 ULT, Creek Company, Steamboat Springs, Colorado) to acquire position and depth measurements every several seconds while using fins to propel the boat around the lake at about 0.8 kmph. This unit draws 550 mA, and was powered with a lightweight 12v lithium iron phosphate 9.6 AH battery (K2 Energy Solutions, Inc, Henderson, Nevada) that provided adequate power for the three-hour survey. The transducer face was set 10 in below the water surface, so all depth readings were increased by that amount. Two perimeters were was established by walking the Humminbird unit (without the transducer attached) around the shoreline with the sonar feature disabled (Setup Menu tab → Sonar → Off) and depth values defaulting to 0 ft. The first perimeter traced the existing waters edge at time of survey while the second approximated the full pool elevation, clearly visible during the time of the survey by changes in vegetation ringing the lake (Figure 1). BLM staff used standard survey procedures reveal that the lake elevation during the time of the survey was 2.9 feet lower than full pool levels on which the water rights should be filed. As such, this too was added to recorded depths, so that bathymetry and volumetric measurements reflect full pool conditions.

Figure 1. Hack Lake bathymetry was surveyed on September 22, 2021 with a surface elevation 2.9 ft below full pool elevation estimated by shoreline vegetation.

All transect data was then exported to SD flash media then imported to HumminbirdPC software so that transect locations could be visualized in GoogleEarth to aid in culling erroneous data. Individual transects were copied from HumminbirdPC using the “Select all” and “copy” commands then pasted into Excel where false depths (first record or shallow water) were eliminated. The Excel depth and perimeter files were then converted to ASCII tab delimited text for download into custom code written in LabVIEW software titled DecimalMinutesToDecimalDegrees.vi that converts decimal minutes recorded in the Humminbird unit to decimal degrees. Decimal degrees were converted to UTMs with the “Dutch formulae” (T. Neebling, Wyoming Game and Fish), then pasted into the depth and perimeter spreadsheets for each lake. Individual depth readings were converted to a raster map with 1 m grid cells using additional custom code (GenerateRasterHumminbird.vi) that calculates the average depth recorded in each cell. GPS error was listed as 0.6 m during the time of the survey. Transducer face depth was set 25 cm below the lake surface which was added back to each depth reading along with 2.9 ft to reflect the surface elevation drop from full pool conditions. Full pool perimeter coordinates were set to 0 ft depth while the actual surface perimeter was set to 2.9 ft. Corners of the map for Hack Lake were set at WestUTMx= 316760, EastUTMx= 316920, SouthUTMy= 4409950, and NorthUTMy= 4410060. Three files were created: a full pool perimeter file with depth = 0 feet, an actual perimeter file with depth = 2.9 ft, and a concatenated depth file with all remaining transects with depths rounded to the nearest foot increment (MapII GIS software can only handle integers). Output files were opened back up in Excel and converted to SYLK files (.slk) that were then opened in MapII (John Wiley & Sons, New York). All maps were inverted and rectified with the "Flip <map> vertically" operation. Command-I was used to get info on each layer and convert units to 1 m (adding the m is essential, as is selecting the m radio button).

A similar procedure was used to generate a map/mask of the lake perimeter coordinates. This

provided a point map defining the lake boundary that was made continuous manually in Map II (this is important to keep deep water readings from invading shore when interpolated) and the perimeter value was set to 0 in the legend (obtained while walking the shoreline). The depth layer was covered with the actual perimeter layer, and full pool perimeter layer, and the resulting map color reset to multichrome (Color → Color Sequence → Multichrome). The Cover command was used to join the perimeter file with the depth transects rather than Combine, as the latter will simply compress the legend to eliminate depths that have no values. Prior to interpolation, the map was smoothed using the Scan <map> Average function in MapII to reduce the influence of any erroneous GPS readings that might have remained undetected in the data. This new map was then interpolated by octants with a mask of the lake perimeter, weighting the nearest point within an octant by its distance from the target depth to be estimated. In order for the perimeter mask to function, it had to be filled in manually so that all points within the lake were non-void cells. I used the Page Setup function in MapII to adjust the size of the map, to include a scale and vertical legend (small), then exported to PICT Version2. This file was ungrouped in Superpaint (Aldus Corporation, Seattle, Washington) so that the position of the legend and scale could be changed, then regrouped and resaved as a PICT file. The PICT file was saved to a ZIP drive and imported into Microsoft Word as a picture. The map legend including raster counts for each depth strata was exported from MapII to an Excel file where surface area, mean and maximum depths, and volumetric measurements were calculated.

RESULTS & DISCUSSION

A total of 403 depth measurements were recorded while running 13 transacts on Hack Lake on September 22, 2021. Survey track placements are shown (Figure 2), but only represent 9 of the 13 tracks as 3.5 tracks had to be culled due to poor GPS coverage during approximately 45 min of the survey.

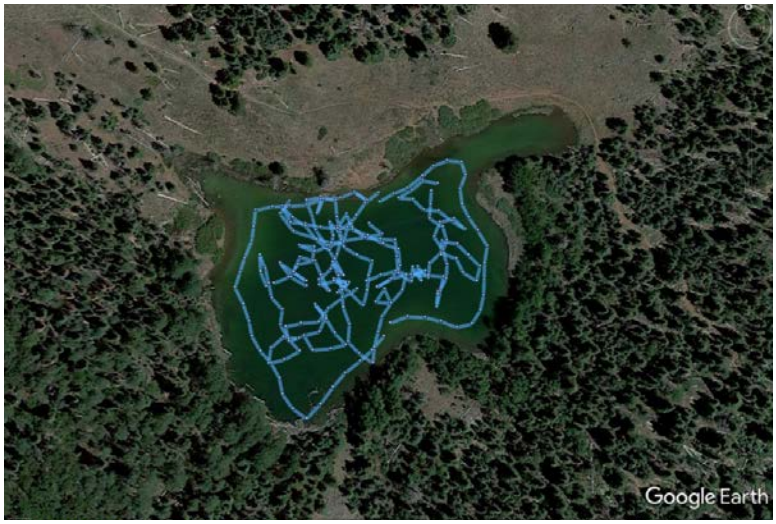

Figure 2. Nine depth tracks were run on Hack Lake and combined with a track of the wetted perimeter on September 22, 2021, projected in Google Earth.

The full pool perimeter mask revealed that the lake covers a surface area of 0.52 ha (1.29 acres). Because the lake elevation was down at the time of the survey, nearshore depths above the September 22nd 2021 elevation and full pool were inferred by interpolation. Lake mean depth at full pool is 1.96 m (6.4 ft), with a max depth of 3.72 m (12.2 ft). Volumetric estimates were generated from the interpolated map (Figure 3), displaying both metric and imperial units to facilitate reclamation effort planning. At full pool, Hack Lake is estimated to hold 11,005 m^3 (8.92 AF) of water. A formula that describes lake volume as a function of surface elevation was developed (Figure 4) to allow rapid estimation of lake volume at any elevation.

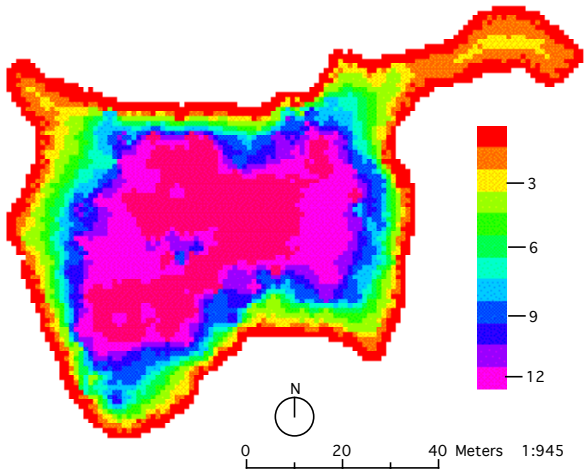

Figure 3. Nine depth transects obtained on Hack Lake were combined into a single file which was then covered by files containing all perimeter points at full pool and at time of survey, then interpolated by octants with a mask of the lake perimeter. The resulting bathymetric profile shows the average depth for each 1 m raster.

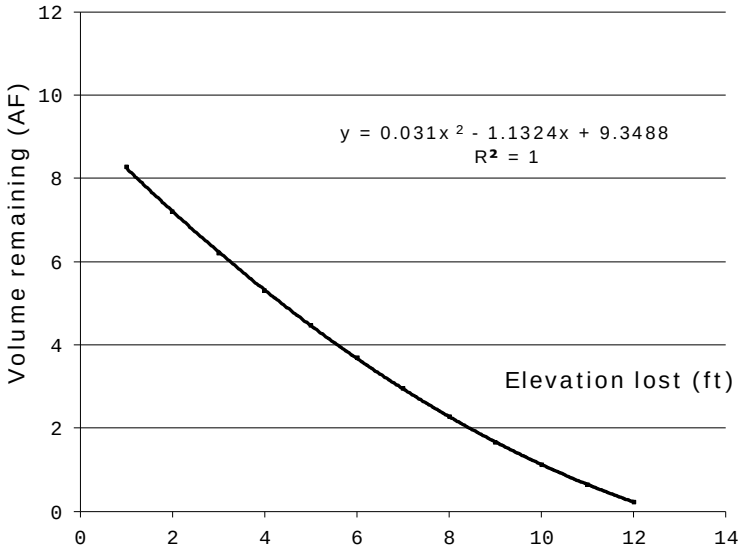

Figure 4. Total acre feet (AF) for every foot drop in lake elevation from our survey surface elevation was calculated, and fit with the second order polynomial shown. Results are expressed in imperial units to facilitate reclamation effort planning.

ACKNOWLEDGMENTS

I wish to acknowledge my collaborators Tom Fresques and Roy Smith from the BLM, and CPW Area Biologist Kendall Bakich.

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. *In review*. Thermal tolerance in Cutthroat Trout of the southern Rocky Mountains. Canadian Journal of Fisheries and Aquatic Sciences.

Abstract.— With stream temperatures expected to rise across the southern Rocky Mountains, the ability of native fishes to tolerate warming temperatures has become a critical concern for those tasked with preserving and managing coldwater species. We used common garden experiments to evaluate the thermal tolerance of Cutthroat Trout *Oncorhynchus clarkii* fry from five populations representing three sub-species: Colorado River Cutthroat Trout (CRCT, *O. c. pleuriticus*) from Milk Creek and Lake Nanita, Greenback Cutthroat Trout (GBCT, *O. c. stomias*) from Zimmerman Lake, Yellowstone Cutthroat Trout (YSCT, *O. c. bouvieri*) from the Yellowstone River, and a CRCT-YSCT hybrid from Trappers Lake. Critical thermal maxima (CTM) were evaluated through traditional exposure trials following acclimation at 13°C, while optimal growth and ultimate upper incipient lethal temperatures (UUILT) were examined over the course of 21-day trials at six static treatments (11, 14, 17, 20, 23, and 26ºC). 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 the fit of models to data. Based on fitted curves from cubic models, optimal growth temperatures were significantly lower among CRCT from Lake Nanita and GBCT from Zimmerman Lake (15.4-15.6°C) than among CRCT from Milk Creek, YSCT from the Yellowstone River, and CRCT-YSCT hybrids

from Trappers Lake (17.7-18.3°C). Peak relative growth ranged from <1% per day in the Zimmerman Lake GBCT to > 4% per day in the Trappers Lake trout. 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

Rogers, K. B., B. J. Sucher, B. W. Hodge, and C. A. Myrick. May 11, 2021. Thermal tolerance in Cutthroat Trout of the southern Rocky Mountains. Western Division American Fisheries Society annual meeting (virtual).

Petcoff, D. W., K. B. Rogers, B. J. Sucher, G. Webster, and C. A. Myrick. May 11, 2021. Thermal tolerance and inbreeding depression: transcriptomic responses of three subspecies of Cutthroat Trout to acute heat challenge. Western Division American Fisheries Society annual meeting (virtual).

Rogers, K. B. June 10, 2021. Colorado Parks and Wildlife aquatics research: a brief overview. Colorado Wildlife Commission Meeting, Trinidad, Colorado.

Rogers, K. B. August 2, 2021. Should we have mosquitos? CRISPR and Cutthroat Trout. DWM pack training. Bel-Aire, Colorado.