# Thermal tolerance in Cutthroat Trout of the southern Rocky Mountains

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<th>Journal:</th>
<th><em>Canadian Journal of Fisheries and Aquatic Sciences</em></th>
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<td>cjfas-2021-0352.R1</td>
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<td>Article</td>
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<td>Date Submitted by the Author:</td>
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| Keyword: | Cutthroat Trout, Adaptive capacity, Thermal tolerance, Critical thermal maxima |
| Is the invited manuscript for consideration in a Special Issue? : | Not applicable (regular submission) |
Thermal tolerance in Cutthroat Trout of the southern Rocky Mountains

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Competing interests: The authors declare there are no competing interests.
Abstract.—With temperatures expected to rise across the southern Rocky Mountains, the ability of native fishes to tolerate stream warming has become a critical concern for those tasked with preserving coldwater species. We used common garden experiments to evaluate the thermal tolerance of Cutthroat Trout *Oncorhynchus clarkii* fry from five populations important to managers representing three sub-species. Critical thermal maxima (CTM) were evaluated through traditional exposure trials, while optimal growth and ultimate upper incipient lethal temperatures (UUILT) were examined over the course of 21-day trials at six static temperature treatments. Whereas CTMs differed among populations (mean = 27.91°C, SD = 0.35°C), UUILTs did not (mean = 24.40°C, SD = 0.04°C). Comparison of cubic temperature-growth functions to the traditional quadratic functions showed that adding a third-order term for temperature can improve model fit, and revealed substantial differences in optimal growth temperatures (15.4-18.3°C). Knowledge of these thermal tolerance thresholds will help to predict the consequences of a warming climate, identify suitable habitats for repatriation, and inform water quality temperature standards established to protect these fish into the future.

Introduction

A radically altered future climate (Cook et al. 2004; Hansen et al. 2012; Hansen et al. 2013) is predicted to have dire consequences for the conservation of native coldwater
fishes (Rahel et al. 1996; Ficke et al. 2007; Paukert et al. 2021). In addition to challenges that accompany warming such as wildfire and drought (Brown et al. 2001; Howell 2006, Seager et al. 2007, 2013), temperature itself is a master abiotic driver affecting all physiological processes in ectotherms (Fry 1971, Whitney 2012), and Cutthroat Trout (Oncorhynchus clarkii) are no exception. An iconic species already suffering tenfold reductions in range in the southern Rocky Mountains since European settlement (Penaluna et al. 2016; Budy et al. 2019), Cutthroat Trout have long been the focus of intense conservation efforts (Gresswell 1988; Trotter 2008; Penaluna et al. 2016). 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 will bring additional challenges (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; Eby et al. 2014). 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 

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 wild populations established from the same stock 
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; Jensen et al. 2008; 
Kavanagh et al. 2010; Narum et al. 2013; Whitney 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 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 the founding source of many robust extant wild 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.

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 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 (\( \sqrt[\text{sin}^{-1}] \)) 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 bootstrap 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 four units, as that would indicate little to no support for the quadratic fit (Burnham and Anderson 2002).
Ethics approval: Research was conducted in accordance with all applicable laws, guidelines and regulations, and was approved by the Colorado State University Institutional Animal Care and Use Committee (protocol #16-6670A).

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.

**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).

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%).
of five populations, the quadratic model performed as well as the cubic model, but in the remaining two, the cubic fit was superior ($\Delta$AIC$_c > 4$). For those two populations, estimates of optima differed significantly between second- and third-order equations. Because cubic models performed as well (3 of 5) or better (2 of 5) than quadratic models, we focused on the former for comparisons between populations.

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

Exposure of five Cutthroat Trout stocks to thermal challenges in a common garden framework showed that thermal tolerance (particularly for chronic measures like OGT) are variable and hereditary. In addition, these traits may reflect adaptation to natal water temperature conditions. Acute challenges yielded more unexpected results either not differing between stocks (UUILT) or not reflecting environmental conditions in the founding source waters (CTM). This may suggest that while adaptation for improved growth in a warming climate can occur, adaptation toward thermal tolerance during acute challenges may be more difficult to achieve, complicating conservation of these iconic fish.
Survival

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 embryos 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.

Acute effects - CTM and UUILT

While 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 stock 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 are more successfully cultured 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 could have been 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 (Table A.1) and CTM and UUILT values (Figure A.1). This suggests that perhaps upper thermal tolerance limits are governed by molecular pathways that may not be very plastic (Chown et al. 2010; Logan and Buckley 2015; Ooman and Hutchings 2017), 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.

Chronic effects – OGT

A different message was delivered by examination of OGT, consistent with other sub-lethal temperature response studies on salmonids (Steel et al. 2012). 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 in some cases, optima are better isolated from the curve of a cubic model. While the quadratic function performed as well as the cubic function in three of five cases, the cubic function was clearly superior in the remaining two (\(\Delta \text{AIC} > 4\)). Importantly, for these remaining two, both quadratic and cubic functions arrived at the same OGT values (Table 3), suggesting that the cubic approach worked in all cases while the quadratic only in some (60%). In addition, 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 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; Whitney et al. 2013). 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 (Figure A.1), with OGT occurring for Milk Creek fish at 17.7°C. Optimal growth temperatures in YSCT were also high (17.8°C), perhaps because these trout likely occupied many waters historically that exceeded 26°C (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, that differences are heritable, 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 will therefore display unequal suitability to future environmental conditions (Whitney et al. 2013). Field experiments should be conducted to determine if these apparent fitness benefits translate into improved population viability 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.
Acknowledgements

The authors wish to thank Brad Neuschwanger for his expert culture advice. Biologists Guy Campbell (Yellowstone Cutthroat Trout), Jon Ewert (Lake Nanita), Tory Eyre (Milk Creek), and Boyd Wright (Zimmerman Lake) are thanked for coordinating wild spawn operations from which these eggs were derived. We thank Sarah McCollum, Tyler Swarr, and the CSU Fish Culture students for designing and assembling the 24-tank rearing system used in this study, as well as Joe Baer, Kevin Fitzgerald, Chase Garvey, Lita Hernandez, Katie Rohwer, Chloe Schaub, and Brent Wells for providing critical lab support maintaining fish used in this study and assisting with the thermal tolerance trials. We thank Paul Lukacs for insight on bootstrapping confidence intervals around growth optima, Todd Koel for temperature data from Fishing Bridge on the Yellowstone River, and Doug Petcoff for providing a friendly review of an earlier version of this manuscript.

Author contribution statement:

KBR: Conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, software, supervision, validation, visualization, writing (original).

BJS: Data curation, investigation, methodology, validation, writing (review and editing).

BWH: Conceptualization, formal analysis, methodology, visualization, writing (review and editing).

CAM: Conceptualization, funding acquisition, methodology, project administration, resources, supervision, validation, writing (review and editing).
Funding: This project was funded by a grant awarded by Colorado’s Species Conservation Trust Fund, Grant SCTFW805.

Data availability: Data generated or analyzed during this study are available from the corresponding author upon reasonable request.

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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.

<table>
<thead>
<tr>
<th>Population</th>
<th>Subspecies</th>
<th>Latitude (ºN)</th>
<th>Longitude (ºW)</th>
<th>Spawn Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk Creek(^a)</td>
<td>CRCT</td>
<td>40.170</td>
<td>107.660</td>
<td>June 1</td>
</tr>
<tr>
<td>Zimmerman Lake(^b)</td>
<td>GBCT</td>
<td>40.541</td>
<td>105.869</td>
<td>June 22</td>
</tr>
<tr>
<td>Lake Nanita(^c)</td>
<td>CRCT</td>
<td>40.256</td>
<td>105.716</td>
<td>June 28</td>
</tr>
<tr>
<td>Yellowstone River(^d)</td>
<td>YSCT</td>
<td>44.573</td>
<td>110.372</td>
<td>May 17</td>
</tr>
<tr>
<td>Trappers Lake(^e)</td>
<td>CRCT</td>
<td>39.986</td>
<td>107.232</td>
<td>June 9</td>
</tr>
</tbody>
</table>

\(^a\)Putative aboriginal population native to the lower Yampa River basin

\(^b\)Population founded in 2014 with progeny from Bear Creek derived broodstock; native Cutthroat Trout of the South Platte River basin

\(^c\)Founded from pure Trappers Lake stock in 1931

\(^d\)Female YSCT from the Story Hatchery were fertilized with wild males captured from above LeHardy Rapids on the Yellowstone River, source of Colorado introductions

\(^e\)CRCT now hybridized with Yellowstone Cutthroat Trout stocked in the lake from 1943-1950
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.

<table>
<thead>
<tr>
<th>Population (sub-species)</th>
<th>7-day (95% CL)</th>
<th>21-day (95% CL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk Creek</td>
<td>24.34 (24.20, 24.47)</td>
<td>24.34 (24.20, 24.47)</td>
</tr>
<tr>
<td>Lake Nanita</td>
<td>24.45 (24.45, 24.45)</td>
<td>24.40 (24.32, 24.49)</td>
</tr>
<tr>
<td>Yellowstone River</td>
<td>24.42 (24.35, 24.49)</td>
<td>24.42 (24.35, 24.49)</td>
</tr>
<tr>
<td>Trappers Lake</td>
<td>24.51 (24.41, 24.62)</td>
<td>24.42 (24.35, 24.49)</td>
</tr>
</tbody>
</table>
Table 3.— Optimal growth temperatures, associated 95% confidence limits, and model fits (R²) for five populations of Cutthroat Trout. We evaluated support for second-order (quadratic) or third-order (cubic) temperature-growth models within each population using AICc (Akaike’s Information Criterion corrected for small sample size).

<table>
<thead>
<tr>
<th>Population</th>
<th>Quadratic</th>
<th>Cubic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Optimum (°C)</td>
<td>R²</td>
</tr>
<tr>
<td>Milk Creek</td>
<td>16.36 (16.19, 16.53)</td>
<td>0.85</td>
</tr>
<tr>
<td>Zimmerman Lake</td>
<td>15.14 (14.60, 15.52)</td>
<td>0.96</td>
</tr>
<tr>
<td>Lake Nanita</td>
<td>16.09 (15.86, 16.29)</td>
<td>0.90</td>
</tr>
<tr>
<td>Yellowstone River</td>
<td>16.48 (16.07, 16.78)</td>
<td>0.76</td>
</tr>
<tr>
<td>Trappers Lake</td>
<td>16.86 (16.61, 17.18)</td>
<td>0.79</td>
</tr>
</tbody>
</table>
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.
Figure 2

Milk Creek

\[ RG = 9.03 - 0.01T^3 + 0.19T^2 - 2.34T \]

Zimmerman Lake

\[ RG = -1.58 - 0.01T^3 + 0.03T^2 - 0.02T \]

Lake Nanita

\[ RG = -28.86 + 0.01T^3 - 0.23T^2 + 4.77T \]

Yellowstone River

\[ RG = 10.76 - 0.01T^3 + 0.22T^2 - 2.73T \]

Trappers Lake

\[ RG = 25.45 - 0.01T^3 + 0.49T^2 - 6.20T \]
Figure 2.— Relative daily growth as a function of temperature in five populations of Cutthroat Trout fit with a standard cubic 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.

Appendix: Summary temperature metrics for source population waters

Table A.1.— Long-term water temperature monitoring was achieved by deploying digital thermographs\textsuperscript{a} in metal housings anchored to the streambed in areas likely to scour. Units were set to acquire temperatures every 30 or 60 min, and thermographs were replaced every 1-4 years. Annual maximum 30 d average temperature (M30AT\textsuperscript{b}), the maximum weekly maximum temperature (MWMT\textsuperscript{c}), and daily maximum temperature (2-hr rolling average maximum\textsuperscript{d}) were calculated\textsuperscript{e} for each study population.

<table>
<thead>
<tr>
<th>Population</th>
<th>Year</th>
<th>MWMT (°C)</th>
<th>M30AT (°C)</th>
<th>Daily Max (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk Creek\textsuperscript{f}</td>
<td>2012</td>
<td>25.0</td>
<td>18.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>25.3</td>
<td>18.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2015</td>
<td>22.5</td>
<td>16.4</td>
<td>23.3</td>
</tr>
<tr>
<td></td>
<td>2016</td>
<td>23.6</td>
<td>17.1</td>
<td>24.7</td>
</tr>
<tr>
<td></td>
<td>2017</td>
<td>25.0</td>
<td>18.3</td>
<td>25.2</td>
</tr>
<tr>
<td></td>
<td>2018</td>
<td>25.8</td>
<td>18.9</td>
<td>27.0</td>
</tr>
<tr>
<td></td>
<td>2019</td>
<td>23.3</td>
<td>16.8</td>
<td>24.4</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>24.4</td>
<td>17.8</td>
<td>25.7</td>
</tr>
<tr>
<td>Year</td>
<td>Zimmerman Lake</td>
<td>Lake Nanita</td>
<td>Yellowstone River</td>
<td>Trappers Lake</td>
</tr>
<tr>
<td>------</td>
<td>----------------</td>
<td>-------------</td>
<td>-------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>2012</td>
<td>15.8</td>
<td>19.2</td>
<td>21.2</td>
<td>19.0</td>
</tr>
<tr>
<td>2013</td>
<td>14.5</td>
<td>16.4</td>
<td>19.8</td>
<td>19.1</td>
</tr>
<tr>
<td>2014</td>
<td>13.7</td>
<td>16.1</td>
<td>18.8</td>
<td>18.8</td>
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<tr>
<td>2015</td>
<td>13.6</td>
<td>15.8</td>
<td>17.6</td>
<td>17.6</td>
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<tr>
<td>2016</td>
<td>14.1</td>
<td>17.6</td>
<td>18.2</td>
<td>18.2</td>
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<tr>
<td>2017</td>
<td>14.5</td>
<td>15.3</td>
<td>18.9</td>
<td>18.8</td>
</tr>
<tr>
<td>2018</td>
<td>14.5</td>
<td>15.6</td>
<td>19.5</td>
<td>19.0</td>
</tr>
<tr>
<td>2019</td>
<td>14.4</td>
<td>16.2</td>
<td>19.7</td>
<td>19.1</td>
</tr>
<tr>
<td>2020</td>
<td>14.6</td>
<td>17.2</td>
<td>19.8</td>
<td>18.9</td>
</tr>
<tr>
<td>Mean</td>
<td>14.5</td>
<td>16.5</td>
<td>20.5</td>
<td>20.5</td>
</tr>
</tbody>
</table>

Mean  

© The Author(s) or their Institution(s)
<table>
<thead>
<tr>
<th>Year</th>
<th>2019</th>
<th>2020</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>18.2</td>
<td>18.4</td>
<td>18.6</td>
</tr>
<tr>
<td></td>
<td>16.6</td>
<td>16.6</td>
<td>16.9</td>
</tr>
<tr>
<td></td>
<td>19.5</td>
<td>19.2</td>
<td>19.7</td>
</tr>
</tbody>
</table>

aHOBO Water Temp Pro v2 (Onset Computer Corp, Bourne, Massachusetts)
bChronic metric used for characterizing growth conditions (Roberts et al. 2013)
cAcute metric used for characterizing upper bound for persistence (Isaak et al. 2010)
dAcute metric used for setting water quality standards (Todd et al. 2008)
eTemperature summary metrics calculated using WaTSS v. 3.0 (Rogers 2015)
fThermagraph located at 40.18419° N 107.66350° W
gThese data describe the thermal regime of the founding population in Bear Creek at 38.81768° N 104.89614° W
hThermagraph located in the outlet of Lake Nanita at 40.25889° N 105.71621° W
iThermagraph located below the outlet of Yellowstone Lake near Fishing Bridge; temperature data obtained from Yellowstone National Park (T. Koel, National Park Service, unpublished)
jjThermagraph 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.