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Thermal tolerance in Cutthroat Trout of the southern Rocky Mountains

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Manuscripts

1   **Thermal tolerance in Cutthroat Trout of the southern Rocky Mountains**

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23

24   __*Abstract.*—With temperatures expected to rise across the southern Rocky Mountains,

25   the ability of native fishes to tolerate stream warming has become a critical concern for

26   those tasked with preserving coldwater species. We used common garden experiments to

27   evaluate the thermal tolerance of Cutthroat Trout *Oncorhynchus clarkii* fry from five

28   populations important to managers representing three sub-species. Critical thermal

29   maxima (CTM) were evaluated through traditional exposure trials, while optimal growth

30   and ultimate upper incipient lethal temperatures (UUILT) were examined over the course

31   of 21-day trials at six static temperature treatments. Whereas CTMs differed among

32   populations (mean = 27.91°C, SD = 0.35ºC), UUILTs did not (mean = 24.40°C, SD =

33   0.04°C). Comparison of cubic temperature-growth functions to the traditional quadratic

34   functions showed that adding a third-order term for temperature can improve model fit,

35   and revealed substantial differences in optimal growth temperatures (15.4-18.3°C).

36   Knowledge of these thermal tolerance thresholds will help to predict the consequences of

37   a warming climate, identify suitable habitats for repatriation, and inform water quality

38   temperature standards established to protect these fish into the future.

39

40   Keywords: Cutthroat Trout, adaptive capacity, thermal tolerance, critical thermal

41   maxima

42

43

44   Introduction

45   A radically altered future climate (Cook et al. 2004; Hansen et al. 2012; Hansen et

46   al. 2013) is predicted to have dire consequences for the conservation of native coldwater

47   fishes (Rahel et al. 1996; Ficke et al. 2007; Paukert et al. 2021). In addition to challenges

48   that accompany warming such as wildfire and drought (Brown et al. 2001; Howell 2006,

49   Seager et al. 2007, 2013), temperature itself is a master abiotic driver affecting all

50   physiological processes in ectotherms (Fry 1971, Whitney 2012), and Cutthroat Trout

51   (*Oncorhynchus clarkii*) are no exception. An iconic species already suffering tenfold

52   reductions in range in the southern Rocky Mountains since European settlement

53   (Penaluna et al. 2016; Budy et al. 2019), Cutthroat Trout have long been the focus of

54   intense conservation efforts (Gresswell 1988; Trotter 2008; Penaluna et al. 2016).

55   Declines are primarily driven by the invasion of nonnative trout (Peterson et al. 2004;

56   Fausch 2008; Meredith et al. 2017; Al-Chokhachy and Sepulveda 2019; Zeigler et al.

57   2019), but the advent of a warming climate will bring additional challenges (Williams et

58   al. 2009; Wenger et al. 2011; Isaak et al. 2012; Roberts et al. 2017). The distribution of

59   trout is predicated by thermal requirements (Dunham et al. 2003; Al-Chokhachy et al.

60   2013; Isaak et al. 2017), and some scientists have predicted substantial range contractions

61   as a result of increasing temperature (Williams et al. 2009; Wenger et al. 2011; Isaak et

62   al. 2012; Eby et al. 2014). A flurry of models have been developed to predict how

63   remaining Cutthroat Trout populations will fare in the future (Roberts et al. 2013, 2017;

64   Zeigler et al. 2019). These models focus on both acute and chronic thermal consequences

65   of climate warming to Cutthroat Trout (Todd et al. 2008). Acute effects influence short-

66   term survival, and a number of associated thresholds have been derived from lab-based

67   studies (Becker and Genoway 1979; Johnstone and Rahel 2003; Bear et al. 2007;

68   McDermid et al. 2012; Recsetar et al. 2012; Zeigler et al. 2013). Chronic effects

69   influence the long-term growth and recruitment potential of trout exposed to a given

70   thermal regime and are also characterized by a variety of approaches (Harig and Fausch

71   2002; Coleman and Fausch 2007a; Roberts et al. 2013; Isaak et al. 2017).

72   　　　　Models that assess rangewide persistence of Cutthroat Trout use thermal vital

73   rates from rigorous lab studies conducted on a handful of Cutthroat Trout stocks (Bear et

74   al. 2007; Coleman and Fausch 2007b; Zeigler et al 2013), applied uniformly to all

75   populations within a subspecies (e.g., Roberts et al. 2013; Zeigler et al. 2019). However,

76   some researchers have demonstrated that temperature tolerance can vary within

77   subspecies (Wagner et al. 2001; Eliason et al. 2011; Drinan et al. 2012; Underwood et al.

78   2012; Narum et al. 2013), and that even wild populations established from the same stock

79   in warmer thermal regimes can develop higher thermal tolerance over time (K. Rogers,

80   unpublished). Salmonids occupy variable and dynamic environments that foster

81   adaptation on a local scale in response to temperature (Kaeding 1996; Jensen et al. 2008;

82   Kavanagh et al. 2010; Narum et al. 2013; Whitney et al. 2013; Pearse and Campbell

83   2018), and that adaptation can occur quickly (e.g., 9-14 generations in Sockeye Salmon,

84   *O. nerka*; Hendry et al. 1998). Acknowledging and characterizing this variation in

85   thermal tolerance will improve predictive ability of models forecasting future persistence.

86   More importantly, it could identify thermally tolerant stocks that could be used to

87   repatriate habitats that are predicted to become less thermally suitable in the future,

88   leading to more successful reclamation projects. A better understanding of the range of

89   thermal tolerance within subspecies will also inform setting water quality standards that

90   protect habitat (Todd et al. 2008; Mandeville et al. 2019).

91   　　　　We explored acute and chronic measures of thermal tolerance in five populations

92   of native Cutthroat Trout. To bracket a range of potential variation in local adaptation,

93   we included populations exposed to a broad spectrum of temperature regimes. We

94   included Colorado River Cutthroat Trout *O. c. pleuriticus* (CRCT) from Milk Creek, a

95   low-elevation population that exhibits a unique ability to persist in an unusually warm

96   environment (Hodge et al. 2017). Greenback Cutthroat Trout *O. c. stomias* (GBCT) from

97   Zimmerman Lake were included because they appear to thrive in hatcheries with very

98   cold water (B. Johnson, Colorado Parks and Wildlife, personal communication). This

99   population is particularly important as it was founded from Bear Creek progeny, the last

100   representatives of the native trout of the Platte River basin (Metcalf et al. 2012; Rogers et

101   al. 2018; Bestgen et al. 2019). The pure CRCT from Lake Nanita were included because

102   they represent the widely stocked indigenous trout of Trappers Lake, following a 1931

103   introduction into this historically fishless lake in Rocky Mountain National Park

104   (Kennedy 2014).   These oft studied fish serve as a useful baseline to compare this work

105   to existing research on growth and thermal tolerance (e.g., Coleman and Fausch 2007b;

106   Brandt 2009; Underwood et al. 2012). We included Yellowstone Cutthroat Trout *O. c.*

107   *bouvieri* (YSCT) from LeHardy Rapids below Yellowstone Lake, as they too were

108   widely stocked around the Rocky Mountain region, with 70 million YSCT distributed

109   across Colorado from 1912-1953 (Varley 1979). Finally, we included the current

110   population in Trappers Lake, the most prolific Cutthroat Trout broodstock in the southern

111   Rocky Mountains, and the founding source of many robust extant wild CRCT

112   populations (Rogers et al. 2018). Close to a million YSCT were stocked into Trappers

113   Lake from 1943-1950 resulting in a hybrid swarm today (Martinez 1988; Leary and

114   Allendorf 1991).

115

116   Methods

117   We collected fertilized Cutthroat Trout eggs for this study using a variety of

118   methods. We conducted wild spawn operations on the four Cutthroat Trout populations

119   native to Colorado (Table 1) during peak spawning activity in June of 2018. Adult males

120   and females were collected from each population by electrofishing (Milk Creek, Trappers

121   Lake), trapping (Zimmerman Lake), or seining (Lake Nanita). Each female was stripped

122   into a dry bowl, then fertilized with milt from a single male. Five families were produced

123   from each of the populations, with the exception of Milk Creek, where one of five

124   females produced an insufficient number of eggs. In addition, five females from the

125   Yellowstone Lake broodstock housed at the Story Hatchery in Story, Wyoming were

126   fertilized with milt from wild male YSCT captured above LeHardy Rapids in the

127   Yellowstone River, Yellowstone National Park. Fertilized eggs were immediately water

128   hardened for 10 minutes in a 100-ppm buffered iodine bath (Argentyne, Argent

129   Aquaculture LLC, Redmond, Washington), then transported to the Colorado State

130   University Foothills Fisheries Laboratory in Fort Collins, Colorado.

131   All eggs were handled and incubated under common garden conditions in a flow-

132   through laboratory system. Upon arrival at the lab, eggs were bathed in a 50 ppm iodine

133   solution for 30 min. Families were then then split between two 10–cm diameter egg cups

134   (Brinkman et al. 2013) and suspended in one of 24 randomly assigned 74-L round

135   polyethylene tanks. Each tank was fitted with a center stand pipe drain to facilitate daily

136   cleaning, and all tanks received water from a single head tank where water temperatures

137   were regulated using a solenoid valve regulated by a digital temperature controller (Love

138   model 16B AC; Dwyer Instruments, Michigan City, Indiana). Water temperatures were

139   recorded every 10 min by four temperature thermagraphs distributed throughout the

140   system (HOBO U22 Pro v2; Onset Computer Corp., Bourne, Massachusetts).   Each cup

141   received 50 mL/s of 10°C water dripped over the top of a single layer of eggs incubated

142   over a mesh screen (mean = 297 eggs/cup, 95% CI = ± 36 eggs). When over 90% of the

143   embryos in an egg cup had hatched, the contents were decanted into the round tank in

144   which the cups were bathed, and the date recorded as the hatch date for that family.

145   Degree days (the cumulative sum of mean daily temperatures) from fertilization to hatch

146   were calculated for each tank in WaTSS (Rogers 2015).

147   Fry from all populations were reared under common conditions. Input flows for

148   each tank were set to 600 mL/s and rearing temperatures to 13°C. Emergent trout fry

149   were fed five times per day with BioVita mash (BioOregon, Longview, Washington) and

150   were supplemented with daily infusions of brine shrimp *Artemia spp*. nauplii. At three

151   weeks post swim-up, the diet was switched over to BioVita starter feed exclusively,

152   which was distributed five times per by day by automatic feeders (FishMate F14,

153   Chewy.com, Dania Beach, Florida). Feed rates were adjusted per manufacturers

154   specifications. Tanks were cleaned twice daily to remove uneaten food and waste. Lids

155   were placed over the tanks to simulate overhead cover and reduce disturbance to the fish

156   (Bear et al. 2007), and photoperiod was matched to ambient conditions over the course of

157   the study. Survival of fry was monitored several times per day.   We transformed

158   survival data ($\sqrt{sin^{-1}}$) and used ANOVA to test for differences among populations. All

159   statistical analyses were performed in R (R Core Team 2020) at α = 0.05, unless

160   otherwise specified.

161

162 *Critical Thermal Maxima.*—The critical thermal maximum (CTM) of each population

163 was determined using 20 fry from each of the five families acclimated at 13°C. At 72 d

164 post-hatch, individual fry were loaded into a cylindrical tolerance chamber (180 mm long

165 x 38 mm diameter; working volume: 204 ml) following the design of Crocker and Cech

166 (1997). Each chamber was fitted with an upstream flow diffuser to provide uniform

167 distribution of water arriving at 450 ml/min. Water temperatures were regulated with a

168 microprocessor-based temperature controller (Love C-series, Dwyer Instruments,

169 Michigan City, Indiana) that mixed warm and cold water to achieve target temperatures.

170 Fish were acclimated in the chambers for 60 min at 13°C, after which temperatures were

171 increased 1°C every three minutes (Becker and Genoway 1979; Underwood et al. 2012;

172 Brinkman et al. 2013). This increase was gradual enough to allow body temperatures to

173 match ambient conditions (Brinkman et al. 2013), but rapid enough to prevent thermal

174 acclimation (Smith and Fausch 1997). Temperatures were monitored to the nearest 0.1°C

175 with a 12-channel scanning thermocouple thermometer (Model 69200, Eutech

176 Instruments, Singapore), logging temperature in each chamber every 10 s. On final loss

177 of equilibrium, temperature was reduced back to 13°C by removing the warm water line

178 to the chamber. Fish were transferred to screened plastic cups and held in the 13°C

179 rearing tanks for 24 hours to ensure full recovery from the CTM exposure. We tested for

180 differences in critical thermal maxima using ANOVA and Tukey's HSD *post-hoc* test.

181

182 *Growth and Ultimate Upper Incipient Lethal Temperature.*— Growth and UUILT trials

183 were conducted simultaneously by testing each population with three replicates at six

184 different temperature treatments. Because of logistical constraints, we were only able to

185 run these experiments for 21 days. For each population, 18 lots of 15 fish, each

186 representing an even mix of each of the remaining families, were isolated at 126 d post

187 hatch. Each fish was weighed and measured, then lots were randomly assigned to 1 of 18

188 9.6-L grow-out tanks (Model ZT950, Aquaneering Inc., San Diego, California). Tanks

189 were plumbed into six different semi-closed recirculating grow-out systems, each with a

190 working volume of 306 L. Target temperatures for each system were 11, 14, 17, 20, 23,

191 and 26°C, maintained with either 800 W or 1500 W submersible titanium aquarium

192 heaters with automatic temperature controllers (Finnex, Chicago, Illinois). Three

193 replicate grow-out tanks each receiving 500 ml/min at each temperature for each

194 population were set in holding baths of the same temperature (also regulated with

195 submersible heaters), to insulate against temperature swings. Any mortalities during the

196 growth experiment were weighed and measured, then preserved. After 21 d, the fish

197 were euthanized with MS-222 (10 min exposure in 250 mg/L buffered with sodium

198 bicarbonate), and each was measured and weighed.

199 With different populations reaching the 126 d post-hatch start threshold at

200 different times, it was impossible to acclimate fish by increasing 1°C each day until target

201 temperatures were reached, as in other studies (Bear et al. 2007; Brinkman et al. 2013;

202 Zeigler et al. 2013). Accordingly, we acclimated fish by moving their tanks through the

203 temperature ladder provided by the grow-out system baths. For example, fish that were

204 destined to spend 21 d at 23°C were moved from the 13°C round rearing tanks to three

205 14°C chambers three days prior to the experiment, then to the 17°C bath two days prior,

206 followed by the 20°C bath the day before, reaching the target temperature of 23°C on

207   Day 0. With each transition, the volume of water in the tank was replaced with new (3°C

208   warmer) water over an 18 min period, which served as the tempering phase each day.

209   We used established analytical methods to calculate the UUILT and optimal

210   growth temperature (OGT) of each population, with one notable exception.   We

211   estimated the UUILT as the median lethal temperature survived by 50% of the population

212   for 7 days and 21 days using the trimmed Spearman–Karber technique (Hamilton et al.

213   1977) in the ecotoxicology package (EPA 2015) for R. Relative daily growth rate was

214   calculated as in Bear et al. (2007) for each tank and plotted against mean temperature for

215   that tank, then fitted with a second-order (quadratic) polynomial regression, as is

216   customary (Eaton et al. 1995; Lyytikainen and Jobling 1998; Bear et al. 2007; Zeigler et

217   al. 2013; Brinkman et al. 2013). Because several of the data sets illustrated an

218   asymmetric response in growth to temperature, we also fit a third-order (cubic) regression

219   model for each population (Bevelhimer et al. 1985). We then estimated OGT from

220   predicted response curves, and used a bootstrap approach (e. g. Manly 1991) to calculate

221   95% confidence intervals around each estimated optimum. We resampled growth data

222   for each population at each temperature step with replacement, then refit the quadratic

223   and cubic curves 1,000 times to generate a distribution around the test statistic (observed

224   OGT). Relative support for quadratic and cubic models was compared using Akaike's

225   information criterion adjusted for small sample sizes (AICc; Burnham and Anderson

226   2002). A cubic model was considered better-supported than the nominal quadratic model

227   if adding the third-order term reduced the AICc by more than four units, as that would

228   indicate little to no support for the quadratic fit (Burnham and Anderson 2002).

229

230   Ethics approval: Research was conducted in accordance with all applicable laws,

231   guidelines and regulations, and was approved by the Colorado State University

232   Institutional Animal Care and Use Committee (protocol #16-6670A).

233

234   Results

235   Survival from spawn to the onset of temperature trials ranged from 39% to 61%

236   and was comparable among the five Cutthroat Trout populations ($F_{4,19}$ = 0.958, P =

237   0.453). Mean survival from spawn to hatch ranged from 53% in the Yellowstone eggs to

238   89% in the Trappers Lake eggs (overall mean = 70%), and mean survival from hatch to

239   72 d post hatch ranged from 61% in GBCT from Zimmerman Lake to 75% in CRCT

240   from Nanita Lake (overall mean = 68%). Neither survival from spawn to hatch, nor

241   survival from hatch to 72 post hatch differed among populations ($F_{4,19} \leq 2.251$, P $\geq$

242   0.102). The range in survival rates was as large or larger within some populations as it

243   was across populations. For example, survival from spawn to 72 d post hatch ranged

244   from 34% to 61% among GBCT families from Zimmerman Lake. Measured degree-days

245   (from 0°C) to hatch averaged 310°C-days across all families, but ranged from 268 -

246   370°C-days. The number of degree-days required for fertilized eggs to hatch was also

247   variable between populations, with mean values ranging from 277°C-days in trout from

248   Trappers Lake to 341°C-days in CRCT from Lake Nanita.

249

250   *Acute effects - CTM and UUILT*

251   The two acute temperature metrics of CTM and UUILT offered contradictory

252   outcomes. Critical thermal maxima differed between populations (P < 0.05; Figure 1),

253 with Milk Creek and Lake Nanita CRCT both tolerating significantly lower (27.5°C)

254 temperatures than Yellowstone River YSCT and Zimmerman Lake GBCT (28.2°C and

255 28.3°C respectively). Average CTM for the admixed progeny from Trappers Lake

256 (27.9°C) was intermediate between the ancestral sources, and not significantly different

257 from either (Figure 1). All but two of the 462 fish subjected to this thermal stress test

258 recovered within 24 hrs of the CTM trial. Ultimate upper incipient lethal temperatures

259 did not differ among populations, during either the 7-day or 21- day trial (Table 2).

260 Population metrics were comparable between trials and the difference between 7-day and

261 21-day estimates of the UUILT never exceeded 0.09°C.

262

263 *Chronic effects – OGT*

264 Growth rates differed among populations and temperatures (Figure 2). All

265 populations grew at the 14°C and 17°C treatments (though not necessarily at the same

266 rates); all but Zimmerman Lake fish grew consistently at 20°C; and only one grow-out

267 tank (a Trappers Lake lot) accumulated weight at 23°C. All populations perished at the

268 26°C treatment. The maximum relative growth rate of hybrid Cutthroat Trout from

269 Trappers Lake (mean maximum = 3.70% at 20°C) was 6-7x higher than the maximum

270 growth rate of GBCT from Zimmerman Lake (mean maximum = 0.57% at 14°C).

271 When predicting growth as a function of temperature, cubic models performed as

272 well or better than quadratic models (Table 3). With cubic equations, temperature and its

273 two higher order terms explained from 81% to 96% of the variation in relative growth

274 (mean = 90%); whereas, with quadratic equations, temperature and its second order term

275 explained from 76% to 96% of the variation in relative growth (mean = 85%). For three

276   of five populations, the quadratic model performed as well as the cubic model, but in the

277   remaining two, the cubic fit was superior (ΔAICc > 4). For those two populations,

278   estimates of optima differed significantly between second- and third-order equations.

279   Because cubic models performed as well (3 of 5) or better (2 of 5) than quadratic models,

280   we focused on the former for comparisons between populations.

281   Based on the results of cubic temperature-growth models, OGT differed among

282   populations (Table 3). Two groups emerged with more than 2°C of separation between

283   them. Optimal growth temperatures were 15.4-15.6°C in GBCT from Zimmerman Lake

284   and CRCT from Lake Nanita, versus 17.7-18.3°C in CRCT from Milk Creek, YSCT

285   from Yellowstone River, and introgressed hybrids of the two (CRCT x YSCT) from

286   Trappers Lake.

287

288   Discussion

289   Exposure of five Cutthroat Trout stocks to thermal challenges in a common

290   garden framework showed that thermal tolerance (particularly for chronic measures like

291   OGT) are variable and hereditary. In addition, these traits may reflect adaptation to natal

292   water temperature conditions. Acute challenges yielded more unexpected results either

293   not differing between stocks (UUILT) or not reflecting environmental conditions in the

294   founding source waters (CTM). This may suggest that while adaptation for improved

295   growth in a warming climate can occur, adaptation toward thermal tolerance during acute

296   challenges may be more difficult to achieve, complicating conservation of these iconic

297   fish.

298

299   *Survival*

300   Survival from spawn to experiment was surprisingly similar and high among wild

301   Cutthroat Trout populations used in this study. On average, 70% of eggs spawned and

302   fertilized in the wild hatched, with 99% hatching in some families. Even the Zimmerman

303   Lake embryos that are especially challenging to raise (B. Johnson, Colorado Parks and

304   Wildlife, unpublished data), saw average survival to hatch of 71% and survival from

305   spawn to 72 d post hatch of 43%. We attribute the high rates of survival to intensive

306   husbandry practices only possible with small lots of fertilized eggs. Keeping individual

307   families separate and eggs distributed in a single layer resting in the bottom of the egg

308   cups allowed early detection of fungal infections that can ravage developing embryos

309   (Arndt et al. 2001). Even with these intensive culture practices, we still saw extreme

310   variation in survival to hatch among families consistent with other studies that suggest

311   individual female egg quality is strongly correlated with embryo survival (Wipf and

312   Barnes 2012). Because survival prior to the temperature experiments did not differ

313   among populations, we can assume that differences in thermal trial performance were

314   indeed the result of variability in thermal tolerance.

315

316   *Acute effects - CTM and UUILT*

317   While CTMs in this study differed among populations, our observed values of

318   27.5 – 28.3°C fell within the range of 26.7 - 29.1°C documented in a half dozen other

319   salmonid taxa reviewed by Brinkman et al. (2013). Interestingly, our mean CTM for the

320   Lake Nanita fish acclimated to 13°C (27.5°C) was slightly higher than that reported by

321   Underwood et al. (2012) for age 1+ year fish of the same stock acclimated to 15°C

322   (26.9°C), but consistent with their finding of reduced thermal tolerance with age also

323   seen with size in other trout studies (Galbreath et al. 2006; Brinkman et al. 2013). Our

324   CTM values did not appear to reflect the thermal regimes of the source waters very well,

325   however (Figure A.1). Cold Lake Nanita and warm Milk Creek shared the same CTM

326   value (27.5°C), while GBCT from Zimmerman Lake that are more successfully cultured

327   in cold water displayed the highest CTM (28.3°C). The lower value for Milk Creek fish

328   was unexpected given the extreme thermal conditions they face in their natal waters

329   (Hodge et al. 2017), however behavioral plasticity coupled with extreme variation in diel

330   temperatures can allow fish to persist in otherwise unforgiving environments (Schrank et

331   al. 2003; McCullough et al. 2009; Hodge et al. 2017).

332   Our findings regarding UUILTs both aligned with and differed from other

333   observations of Cutthroat Trout. During our experiment, the traditional 7-d test (e.g.,

334   Brett 1952; Dickerson and Vinyard 1999; Johnstone and Rahel 2003) yielded a mean

335   UUILT of 24.4°C (range = 24.3°C – 24.5°C). Zeigler et al. (2013) obtained a 7-d UUILT

336   value of 24.7°C for Rio Grande Cutthroat Trout fry and Bear et al. (2007) documented

337   24.2°C for Westslope Cutthroat Trout. Bear et al. (2007) suggested that sharp declines in

338   survival could occur beyond the 7-d time interval, noting that in their 60-d trial, UUILT

339   was 1.8°C lower for Rainbow Trout and 4.6°C lower for Westslope Cutthroat Trout. Our

340   21-d trials did not suggest a similar drop, with the mean UUILT value remaining at

341   24.4°C, though with near 100% survival in the 23°C trial, and 0% survival in the 26°C

342   group, we were not able to resolve fine scale differences in UUILT that might have

343   materialized if more temperature treatments could have been used.

344   Differences in acute effects between subspecies of Cutthroat Trout studied here

345   were either relatively small (CTM) or nonexistent (UUILT) similar to some other studies

346   on salmonids (McCullough 1999; McCullough et al. 2009). Given variation seen in other

347   CRCT however (Underwood et al. 2012), we were surprised to not see elevated lethal

348   threshold thermal tolerance among the Milk Creek fish given the fairly hostile

349   environment in which they evolved. Even more surprising was the apparent lack of

350   correlation between the thermal regimes experienced by the host populations (Table A.1)

351   and CTM and UUILT values (Figure A.1). This suggests that perhaps upper thermal

352   tolerance limits are governed by molecular pathways that may not be very plastic (Chown

353   et al. 2010; Logan and Buckley 2015; Ooman and Hutchings 2017), and that even with an

354   evolutionary history in warm thermal environments, these subspecies do not gain much

355   additional lethal threshold tolerance. This is concerning to those involved with

356   conservation efforts because it might be evidence that these fish are already operating at

357   close to the maximum attainable level of thermal tolerance. Continued increases in

358   environmental temperatures, even when those acclimation temperatures approach those

359   of putative optimal growth, may not derive further thermal acclimation benefits.

360

361   *Chronic effects – OGT*

362   A different message was delivered by examination of OGT, consistent with other

363   sub-lethal temperature response studies on salmonids (Steel et al. 2012). Our observed

364   optima in CRCT from Lake Nanita and GBCT from Zimmerman Lake (15.4-15.6°C) are

365   similar to those observed during other laboratory-based experiments. For example,

366   Zeigler et al. (2013) showed peak growth in a sister taxon, the Rio Grande Cutthroat

367   Trout (*O. c. virginalis*, at 15.3°C, while Brandt (2009) demonstrated optimal growth in

368   CRCT at 15.3–16.4°C. Bear et al. (2007) found that Westslope Cutthroat Trout (WSCT;

369   *O. c. lewisi*) growth peaked at a slightly colder 13.6°C. Our observed optima in CRCT

370   from Milk Creek, YSCT from Yellowstone Lake, and CRCT-YSCT hybrids from

371   Trappers Lake (17.7-18.3°C) however, are more similar to the OGT of Brown Trout

372   *Salmo Trutta* and Rainbow Trout *O. mykiss* than to those of other Cutthroat Trout taxa

373   (Brinkman et al. 2013, and sources therein). The seemingly anomalous temperature

374   optima are at least in part due to fitting differences between quadratic and cubic models.

375   Based on fitted quadric curves, the OGT of the two populations with Yellowstone

376   Cutthroat Trout alleles are only 16.5-16.9°C.

377   Although researchers typically estimate OGT from the fitted curve of a quadratic

378   regression model (e.g., Bear et al. 2007; Zeigler et al. 2013; Brinkman et al. 2013), our

379   findings suggest that in some cases, optima are better isolated from the curve of a cubic

380   model. While the quadratic function performed as well as the cubic function in three of

381   five cases, the cubic function was clearly superior in the remaining two (ΔAIC > 4).

382   Importantly, for these remaining two, both quadratic and cubic functions arrived at the

383   same OGT values (Table 3), suggesting that the cubic approach worked in all cases while

384   the quadratic only in some (60%). In addition, in four of five cases, adding a cubic term

385   increased the explanatory power (R^2) of the temperature-growth model. Comparison of

386   quadratic and cubic models for the Trappers Lake population illustrates the potential

387   limitation of applying an implicitly symmetrical relationship to asymmetrical data.

388   Whereas the fitted curve from the quadratic function ran below all data points at the 20°C

389   temperature step (the treatment at which Trappers Lake fish exhibited the highest mean

390 growth rate), the fitted curve for the cubic function ran through the middle of those data

391 points. With the improvement in fit came a significant increase in the estimated OGT

392 (from 16.9°C to 18.3°C).

393   The accuracy of estimated growth optima could be especially relevant when

394 evaluating the fundamental thermal niche these fish occupy. Defined as the range from

395 3°C lower to 1°C higher than the OGT (Christie and Regier 1988), this niche would

396 range from 13.4°C -17.4°C for the Milk Creek trout fit with a quadratic function. Yet our

397 own results suggest that these fish grow as well or better at 20°C – outside the range of

398 that niche. One expects that as temperature exceeds limits defined by the fundamental

399 thermal niche, a decrease in individual growth and a reduction in population viability

400 should occur (Zeigler et al. 2013). More importantly, laboratory based studies such as

401 these are used to establish thermal habitat protection standards (Armour 1991; Todd et al.

402 2008), yet these very standards would serve to disqualify habitats for future reclamation

403 efforts in waters where the fish could exhibit their fastest growth. Addition of a third

404 order term to the polynomial used to fit the data would help mitigate that risk, and allow

405 continued use of the optimum growth temperature to characterize the upper range of

406 suitable thermal habitat for the long-term persistence of salmonids (McCullough 1999;

407 Selong et al. 2001; Dunham et al. 2003). However, we should recognize that selecting

408 suitable habitat based only on growth optima may ignore the ability for local food

409 resources (quality and quantity) to keep up with increased metabolic demand needed for

410 trout to thrive in warmer water.

411   We expected to see support for local thermal adaptation in growth within the

412 Cutthroat Trout populations given the findings of others (McCullough et al. 2009; Drinan

413   et al. 2012; Underwood et al. 2012; Whitney et al. 2013). Unlike the acute metrics, the

414   sublethal measure of growth did follow our expectation of warmer host waters producing

415   fish that displayed improved growth at warmer temperatures (Figure A.1), with OGT

416   occurring for Milk Creek fish at 17.7ºC. Optimal growth temperatures in YSCT were

417   also high (17.8ºC), perhaps because these trout likely occupied many waters historically

418   that exceeded 26ºC (Varley and Gresswell 1988). This trait appears to be heritable as it is

419   also manifested in the current progeny from Trappers Lake that now contain many YSCT

420   alleles (Martinez 1988; Leary and Allendorf 1991; Rogers et al. 2018), despite the

421   original inhabitants of that lake (now found in Lake Nanita) displaying much lower

422   growth at higher temperatures (Figure 2).

423   One of our more interesting findings was that Trappers Lake fish exhibited the

424   highest growth rate. Although this population of mixed (CRCT x YSCT) origin

425   displayed a CTM and OGT that was intermediate to its two ancestral stocks, it grew

426   faster at temperature than both ancestral stocks (estimated peak growth of 4.1% vs 1.6-

427   2.1% per day). Hybrid vigor could play a role in boosting growth (Donaldson et al. 1957;

428   Rosenfield et al. 2004). However, we should not necessarily be persuaded into equating

429   strong growth with population performance. While growth in GBCT from Zimmerman

430   Lake was startlingly poor, slow growth could be advantageous in the small, pool-limited

431   stream where this wild brood stock was sourced (Bear Creek; J. Valladares, U. S. Forest

432   Service, unpublished data).

433

434   Management implications

435   Here we demonstrated that Cutthroat Trout stocks respond differently to variation

436   in thermal regime, that differences are heritable, and that models used to predict future

437   persistence should account for this variation. Clearly, some stocks of trout are more

438   tolerant of warm temperatures than others, and will therefore display unequal suitability

439   to future environmental conditions (Whitney et al. 2013). Field experiments should be

440   conducted to determine if these apparent fitness benefits translate into improved

441   population viability in the face of a warming climate. Consideration of stock-specific

442   attributes of thermal tolerance can be used to help guide which stocks would be best

443   suited for lower elevation waters that are facing the immediate consequences of climate

444   change. Similarly, knowledge of stock-specific limitations might inform the choice of

445   streams or habitats for repatriation. For example, results of this study revealed that the

446   thermal niche for remaining GBCT appears to be quite narrow and thus the candidate

447   pool of potential recipient waters relatively small. Thermal regimes in these waters

448   should be studied carefully before embarking on costly and labor-intensive reclamation

449   projects.

450   Although we observed considerable variation between stocks in temperature-

451   growth relationships, we found relatively little variation in acute temperature measures

452   such as CTM and UUILT. This suggests differences are found at the margin, and that the

453   capacity to adapt could be limited at the upper bounds. If habitat is variable enough to

454   allow for quick behavioral shifts in habitat use (e.g., Kaeding 1996; Hodge et al. 2017),

455   then persistence is likely. However, if a warming climate eliminates those refugia, the

456   ability of these fish to persist will be challenged.

457

471   Author contribution statement:

472   KBR: Conceptualization, data curation, formal analysis, funding acquisition,

473   investigation, methodology, project administration, resources, software, supervision,

474   validation, visualization, writing (original).

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

476   BWH: Conceptualization, formal analysis, methodology, visualization, writing (review

477   and editing).

478   CAM: Conceptualization, funding acquisition, methodology, project administration,

479   resources, supervision, validation, writing (review and editing).

480

483

484 Data availability: Data generated or analyzed during this study are available from the

485 corresponding author upon reasonable request.

486

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808   Table 1.— Colorado River Cutthroat Trout (CRCT), Greenback Cutthroat Trout (GBCT),

809   and Yellowstone Cutthroat Trout (YSCT) populations examined in this study, including

810   their location (decimal degrees) and spawn date for wild egg collections in 2018.

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

819

820   [a]Putative aboriginal population native to the lower Yampa River basin

821   [b]Population founded in 2014 with progeny from Bear Creek derived broodstock; native

822   Cutthroat Trout of the South Platte River basin

823   [c]Founded from pure Trappers Lake stock in 1931

824   [d]Female YSCT from the Story Hatchery were fertilized with wild males captured from

825   above LeHardy Rapids on the Yellowstone River, source of Colorado introductions

826   [e]CRCT now hybridized with Yellowstone Cutthroat Trout stocked in the lake from 1943-

827   1950

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830   Table 2.— Ultimate Upper Incipient Lethal Temperatures (UUILT; °C) and 95%

831   confidence limits (CL) for Cutthroat Trout populations examined during 7-day and 21-

832   day trials.

833

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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)

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843

844 Table 3.— Optimal growth temperatures, associated 95% confidence limits, and model fits (R^2) for five populations of Cutthroat

845 Trout. We evaluated support for second-order (quadratic) or third-order (cubic) temperature-growth models within each population

846 using AICc (Akaike's Information Criterion corrected for small sample size).

847

848

	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

859

860

861   Rogers et al.
862   Figure 1
863
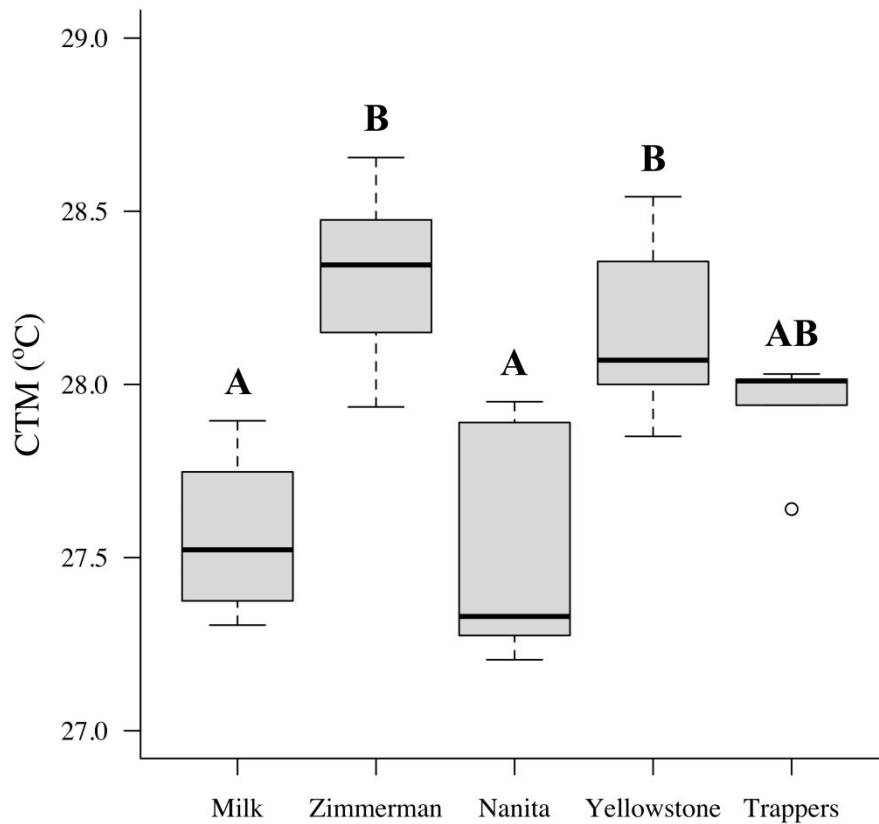
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866   Figure 1.— Comparison of critical thermal maxima (CTM) among five populations of

867   Cutthroat Trout. Thick horizontal black bars represent population-specific medians (°C).
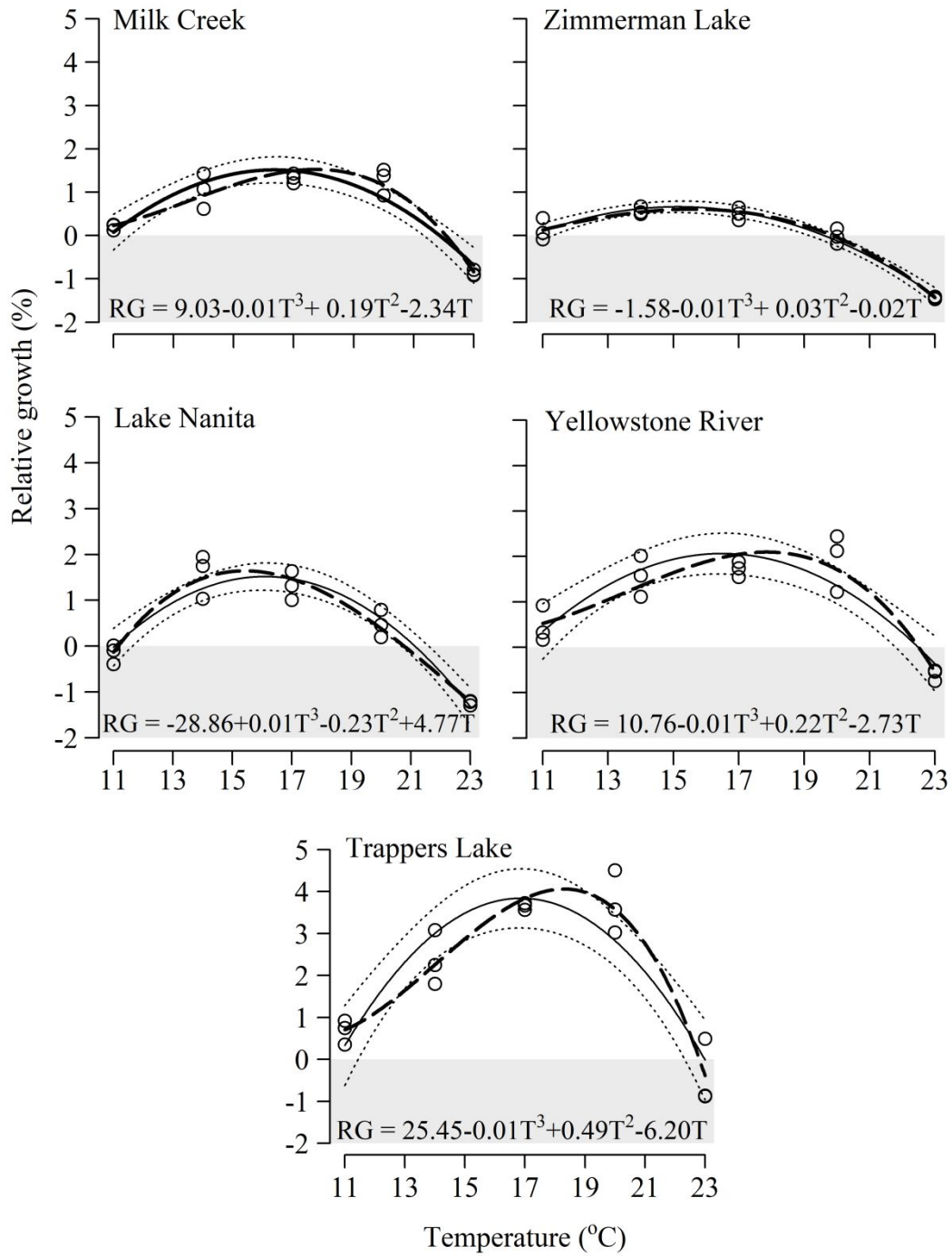
868   Boxes span the interquartile range and whiskers extend 1.5x past that range. Populations

869   that are not significantly different from each other (P > 0.05) share the same bold letter

870

871

872   Rogers et al.
873   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

$RG = 25.45-0.01T^3+0.49T^2-6.20T$

Relative growth (%)

Temperature (^{o}C)

874

875

876

877   Figure 2.— Relative daily growth as a function of temperature in five populations of

878   Cutthroat Trout fit with a standard cubic least squared regression (solid line; equation

879   above the x-axis) and associated 95% confidence interval (dotted lines). The heavy

880   dashed line represents the fitted cubic regression.

881

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883

884   **Appendix: Summary temperature metrics for source population waters**

885

886   Table A.1.— Long-term water temperature monitoring was achieved by deploying digital

887   thermographs[a] in metal housings anchored to the streambed in areas likely to scour.

888   Units were set to acquire temperatures every 30 or 60 min, and thermagraphs were

889   replaced every 1-4 years. Annual maximum 30 d average temperature (M30AT[b]), the

890   maximum weekly maximum temperature (MWMT[c]), and daily maximum temperature

891   (2-hr rolling average maximum[d]) were calculated[e] for each study population.

892

893

Population	Year	MWMT (ºC)	M30AT (ºC)	Daily Max (ºC)
Milk Creek[f]				
	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

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906	Zimmerman Lake[g]			
907		2012	15.8	13.1	16.4
908		2013	14.5	12.3	15.1
909		2014	13.7	11.6	14.6
910		2015	13.6	11.8	13.9
911		2016	14.1	12.5	14.6
912		2017	14.5	12.3	15.2
913		2018	15.1	12.8	15.9
914		2019	14.4	12.7	15.3
915 916		2020	14.6	12.7	15.2
917	Mean		14.5	12.4	15.1
918					
919	Lake Nanita[h]				
920		2002	19.2	15.7	20.0
921		2003	16.4	14.1	17.5
922		2004	16.1	12.8	17.0
923		2005	15.8	13.0	16.6
924		2013	17.6	14.3	18.1
925		2014	14.4	12.4	14.9
926		2015	16.2	13.6	17.2
927		2017	15.3	12.5	16.2
928		2018	18.1	14.6	18.7
929		2019	15.6	12.8	16.1
930 931		2020	17.2	14.2	17.7
932	Mean		16.5	13.6	17.3
933					
934	Yellowstone River[i]				
935		2001	21.2	16.9	22.2
936 937		2002	19.8	17.0	20.6
938	Mean		20.5	17.0	21.4
939					
940	Trappers Lake[j]				
941		2012	19.0	17.6	20.4
942		2013	19.1	17.5	20.1
943		2014	18.8	16.6	19.8
944		2015	17.6	16.0	18.6
945		2016	18.2	16.5	19.3
946		2017	18.8	17.1	20.8
947		2018	18.9	17.4	19.7

948		2019	18.2	16.6	19.5
949		2020	18.4	16.6	19.2
950					
951	Mean		18.6	16.9	19.7
952					

953 [a]HOBO Water Temp Pro v2 (Onset Computer Corp, Bourne, Massachusetts)

954 [b]Chronic metric used for characterizing growth conditions (Roberts et al. 2013)

955 [c]Acute metric used for characterizing upper bound for persistence (Isaak et al. 2010)

956 [d]Acute metric used for setting water quality standards (Todd et al. 2008)

957 [e]Temperature summary metrics calculated using WaTSS v. 3.0 (Rogers 2015)

958 [f]Thermagraph located at 40.18419° N 107.66350° W

959 [g]These data describe the thermal regime of the founding population in Bear Creek at

960 38.81768° N 104.89614° W

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

962 [i]Thermagraph located below the outlet of Yellowstone Lake near Fishing Bridge;

963 temperature data obtained from Yellowstone National Park (T. Koel, National Park

964 Service, unpublished)

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

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968   Elevation (m)   MWMT (℃)   M30AT (℃)
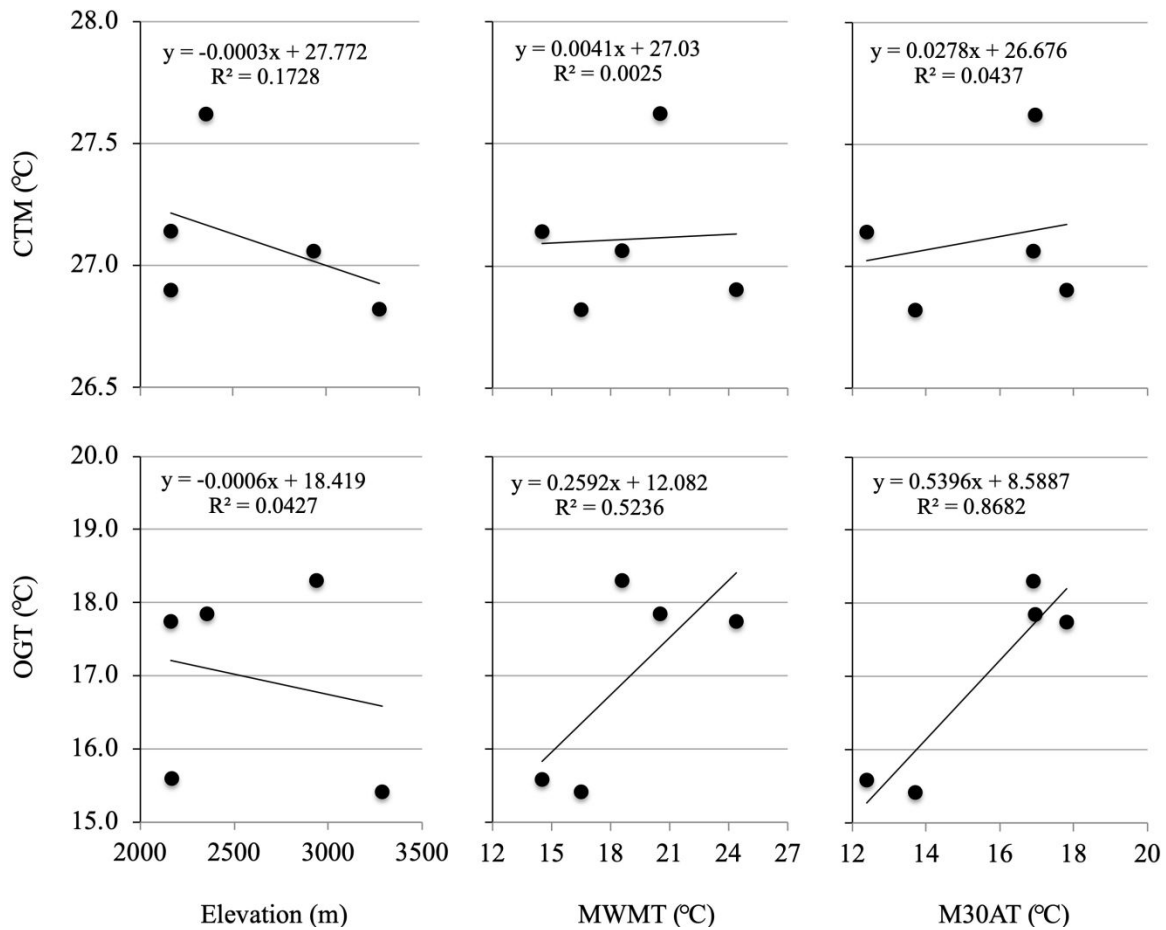
969

970   Figure A.1.—Critical thermal maxima (CTM) and optimal growth temperatures (OGT)

971   for each population were plotted against elevation, maximum weekly maximum

972   temperature (MWMT), and maximum 30-d average temperature (M30AT) experienced

973   by each population.

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