1   **Within- and transgenerational plasticity of a temperate salmonid in response to thermal**

2   **acclimation and acute temperature stress**

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4   **Running title**: Within- and transgenerational plasticity of a temperate salmonid in response to

5   thermal acclimation and acute temperature stress

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21   **Summary:** Brook trout (*Salvelinus fontinalis*) exhibit within-generation and transgenerational

22   plasticity for thermal performance, although neither response appears sufficient to cope with

23   long-term climate change effects.

24

25   **Keywords**: Climate change, brook trout, metabolic rate, thermal tolerance, transgenerational

26   acclimation, plasticity.

ABSTRACT

Environmental temperatures associated with climate change are rising too rapidly for many species to adapt, threatening the persistence of taxa with limited capacities for thermal acclimation. We investigated the capacity for within- and transgenerational responses to increasing environmental temperatures in brook trout (*Salvelinus fontinalis*), a cold-adapted salmonid. Adult fish were acclimated to temperatures within (10□) and above (21□) their thermal optimum for six months before spawning, then mated in a full factorial breeding design to produce offspring from cold- and warm-acclimated parents as well as bidirectional crosses between parents from both temperature treatments. Offspring families were subdivided and reared at two acclimation temperatures (15□ and 19□) representing their current environment and a projected climate change scenario. Offspring thermal physiology was measured as the rate of oxygen consumption (MO_2) during an acute change in temperature ($+2□\ h^{-1}$) to observe their MO_2-temperature relationship. We also recorded resting MO_2, the highest achieved (peak) MO_2, and critical thermal maximum (CTM) as performance metrics. Within-generation plasticity was greater than transgenerational plasticity, with offspring acclimation temperature having demonstrable effects on peak MO_2 and CTM. Transgenerational plasticity was evident as an elevated resting MO_2 and the MO_2-temperature relationship in offspring from warm-acclimated parents. Both parents contributed to offspring thermal responses, although the paternal effect was stronger. Although brook trout exhibit both within- and transgenerational plasticity for thermal physiology, it is unlikely that these will be sufficient for coping with long-term changes to environmental temperatures resulting from climate change.

50 **INTRODUCTION**

51   Environmental warming due to climate change is adversely affecting the physiology and

52 persistence of species and populations globally (Moritz & Agudo, 2013; Whitney *et al*., 2016).

53 With rising temperatures, thermal acclimation (within-generation plasticity, or WGP) allows for

54 some short-term buffering of temperature effects through physiological adjustment but surviving

55 climate change will likely depend on a population's ability to move to more suitable habitats or

56 adapt to warmer conditions (Habary *et al*., 2017). Species or populations that cannot migrate, or

57 are restricted to localized habitats, are particularly vulnerable because they would be left to face

58 temperatures higher than those to which they are physiologically capable of withstanding long-

59 term (Somero, 2010). Although evolutionary change may provide the best option for long-term

60 persistence (Munday *et al.*, 2013), the accelerated rate of climate change is likely too rapid for

61 most organisms to respond (Comte & Olden, 2017). This is especially true for those with long

62 generation times or limited standing genetic variation (Willi *et al*., 2006; Munday *et al*., 2013;

63 Meier *et al*., 2014).

64   One mechanism by which populations may compensate for long-term environmental

65 change is via transgenerational plasticity (TGP; Jablonka *et al*., 1992; Bonduriansky *et al*., 2012;

66 Norouzitallab *et al*., 2019) or epigenetic inheritance. TGP occurs when the inheritance of non-

67 genetic factors and parental effects influence the offspring's phenotype and physiological

68 response to their environment (Deans & Maggert, 2015; Hanson & Skinner, 2016; Charlesworth

69 *et al*., 2017). Environmental changes can likewise induce epigenetic changes (Deans & Maggert,

70 2015; Hanson & Skinner, 2016; Charlesworth *et al*., 2017) that can also be transmitted to the

71 next generation and influence offspring phenotypes and physiology (Guillaume *et al*., 2016;

72 Munday *et al*., 2017). By pre-conditioning their offspring for future environmental conditions,

73 parents may buffer the impacts of environmental stressors and grant more time for the evolution

74 of adaptive responses to occur (Bernatchez, 2016; Smith *et al*., 2016). Because TGP may provide

75 populations with the ability to cope with chronic warming, TGP may represent an adaptive

76 response to climate change (Greenspoon & Spencer, 2018; Yin *et al*., 2019; but see

77 Sánchez☐Tójar *et al*., 2020).

78   Observations of TGP have been documented in a growing number of organisms

79 (reviewed by Donelson *et al*., 2017; Yin *et al*., 2019). In fruit flies (*Drosophila melanogaster*),

80 for example, thermal performance measured as climbing speed improved in offspring from

81   parents reared in thermally variable conditions (Cavieres *et al*., 2019). Developmental rate

82   increased in the marine polychaete (*Ophryotrocha labronica*) following multi-generational

83   exposure to warming (Gibbin *et al*., 2017). In fish at warm ambient temperature, offspring from

84   parents that had been acclimated to warm temperatures had a lower standard metabolic rate or

85   increased maximum metabolic rate compared with offspring from cold acclimated parents

86   (Donelson *et al*., 2012; Shama *et al*., 2014; Donelson *et al*., 2017). To date, most studies of TGP

87   of aquatic vertebrates have focused on temperate or tropical species (Donelson *et al*., 2012;

88   Salinas *et al*., 2012; Shama *et al*., 2014), however, high-latitude species are predicted to be most

89   negatively impacted by climatic warming (Beitinger & Bennett, 2000).

90   Plasticity is thought to evolve in response to environmental variation (Somero, 2010,

91   Colicchio & Herman, 2020), however, the extent to which WGP and TGP operate or interact in

92   organisms that experience variable habitats remains unclear (Leimar & McNamara, 2015). For

93   example, lake trout (*Salvelinus namaycush*) inhabit a thermally stable environment (Martin &

94   Olver, 1980; Wilson & Mandrak, 2004) and exhibit little variation for thermal acclimation

95   (WGP) across populations (McDermid et al., 2013; Kelly et al., 2014). In one of the few studies

96   to investigate TGP in a cold-water species, Penney *et al*. (in press) showed that TGP was also

97   limited in lake trout. In contrast to lake trout, brook trout (*S. fontinalis*) occupy different thermal

98   habitats at different life stages (Biro *et al*., 2008; Smith & Ridgway, 2019), experience relatively

99   high levels of environmental variation (Smith *et al*., 2020) and exhibit within generation thermal

100   acclimation (McCormick *et al*., 1972; Stitt *et al*., 2014; Morrison *et al*., 2020). It is not yet

101   known whether organisms like brook trout that display some variation in WGP for thermal

102   tolerance are more or less capable of TGP.

103   The brook trout is a cold-adapted salmonid native to eastern North America found in cold

104   (10-16☐), well-oxygenated, freshwater habitats such as streams and lakes (Power, 1980; Smith

105   & Ridgway, 2019). Brook trout also have a poor tolerance for warm temperatures (Beitinger &

106   Bennett, 2000) making them highly vulnerable to climate change as temperatures become

107   warmer and suitable habitat is lost (McKenna Jr, 2019). Thermal refugia in lakes are also being

108   reduced as epilimnetic temperatures rise and the metalimnion shrinks (King *et al*., 1999); in

109   some smaller lakes brook trout populations already encounter temperatures that push them to

110   their physiological limits (21-23☐; Smith *et al*., 2020) or prevent reproduction (Warren *et al.*,

111   2012). The vulnerability of salmonids, including brook trout, to increases in temperature

112   associated with climate change has resulted in a wealth of research on this taxon, yet their

113   adaptive capacity, including transgenerational responses, to climate change remains largely

114   unexplored.

115   In this study, we acclimated adult brook trout and their offspring to elevated temperatures

116   to examine within-generation and transgenerational plasticity responses of offspring to a

117   warming environment. We hypothesized that brook trout are capable of WGP and TGP responses

118   to environmental temperatures, and that TGP would enhance upper thermal tolerance. To date,

119   parental contributions to TGP have largely focused on the maternal environment which is

120   thought to have a larger effect on offspring than the paternal environment (Shama *et al*., 2014;

121   Best *et al*., 2018); however, paternal contributions are increasingly being reported across taxa

122   (Hellman *et al*., 2020; Rutkowska *et al*., 2020). Our experimental design provided us with the

123   opportunity to assess both maternal and paternal contributions to offspring thermal responses.

124   We measured offspring thermal physiology as the rate of oxygen consumption (MO_2)

125   during an acute change in temperature ($+2\square$ h^{-1}), to observe their MO_2-temperature relationship.

126   We also recorded resting MO_2, the highest achieved (peak) MO_2, and critical thermal maximum

127   (CTM) as performance metrics. We first predicted that with acclimation to a warmer

128   temperature, offspring peak MO_2 and CTM would increase (demonstrating WGP; Morrison *et*

129   *al*., 2020; Mackey *et al*., 2020), and offspring resting MO_2 will be elevated due to the energetic

130   cost of maintenance at a warmer temperature (Hartman & Cox, 2008; Norin & Metcalfe, 2019).

131   Second, we predicted that offspring with warm-acclimated parents will have a lower resting

132   MO_2, a higher peak MO_2, and a higher CTM compared with offspring with cold-acclimated

133   parents, because TGP would improve thermal tolerance at elevated temperatures (Donelson *et*

134   *al*., 2012). Alternatively, resting MO_2 could be higher in offspring from warm-acclimated

135   parents, following the results of Penney *et al*. (in press). Lastly, we predicted that offspring and

136   parental warm acclimation will interact (i.e., parents anticipate their offspring's environment) to

137   strengthen the effect of TGP on CTM, resting and peak MO_2, and the MO_2-temperature

138   relationship.

139

140   **METHODS**

141   All experiments were approved by the Trent University Animal Care Committee

142   (Protocol # 24794) and the Ontario Ministry of Natural Resources and Forestry (OMNRF)

143   Aquatic Animal Care Committee (Protocol FACC 136) and conducted according to the

144   guidelines outlined by the Canadian Council on Animal Care.

145   The brook trout used for this study originated from wild spawn collections from a native

146   population in Dickson Lake, Algonquin Provincial Park in south-central Ontario, Canada (45°47'

147   N ,78°12' W), and have been kept in the OMNRF hatchery system since 2002 (OMNRF Fish

148   Culture Stocks Catalogue 2005).

149

150   **Experimental design: Adult trout acclimation and breeding**

151   Adult brook trout (age 5; 0.3-0.9 kg) from the Dickson Lake hatchery broodstock were

152   transported to the OMNRF White Lake Fish Culture Station (Sharbot Lake, Ontario, Canada) in

153   the spring of 2015 and implanted with PIT tags. A small caudal finclip (<0.25 cm^2) was taken

154   from each individual and separately stored in 95% ethanol to enable subsequent genetic

155   parentage analysis of offspring families (described in supplemental material). In May 2015,

156   adults were divided into two groups (n = 8 and 9, mixed sex), acclimated to one of two

157   temperatures (10 ± 0.5 and 21 ± 0.5°C, respectively), and held until fall reproduction. The lower

158   temperature was based on the temperature requirements for brook trout spawning, while the

159   warmer temperature was selected to induce thermal stress without exceeding their physiological

160   limits or compromising reproductive success (Hokanson *et al*., 1973; Blanchfield & Ridgway,

161   1997). Each group was kept in a 6000L flow-through tank covered with opaque acrylic lids to

162   reduce stress to the fish, with light allowed in at the inflow and outflow to provide natural

163   photoperiod cues. The tanks received water from White Lake (44°46' N ,78°45' W), and the

164   desired temperatures were achieved by mixing inflows from above and below the lake's

165   thermocline. Fish were acclimated to these temperatures for the duration of the summer, after

166   which the temperature of each tank followed the lake's seasonal cooling from September to

167   December. Tank water temperature and oxygen levels were checked daily, with temperature also

168   logged every hour using two HOBO Tidbit loggers (Onset Computer Corporation, MA, USA)

169   per tank for the duration of the adult acclimation period. The temperature data were collected

170   from the loggers with a HOBO USB optic reader and HOBOware Pro (v. 2.3.0; Onset Computer

171   Corporation, MA, USA) after spawning to track acclimation temperatures throughout the

172   duration of the experiment.

173   Beginning in early October, the reproductive status of the trout was checked weekly by

174   visual inspection following mild anesthesia (0.1 g L^{-1} MS-222; Aqua Life, Syndel Laboratories

175   Ltd., B.C., Canada), and all adults were reproductive by December. As males and females came

176   into reproductive condition, fish were dry-spawned by collecting gametes from anaesthetized

177   fish, subdividing eggs from individual ripe females into two glass jars and fertilizing them with

178   milt from separate males in a factorial cross mating design. In total, we used two males and two

179   females from each of the two temperature treatments (8 adult fish in total) in two 2 x 2 factorial

180   crosses, resulting in 16 families where the offspring were from parents of matched or

181   mismatched thermal histories: C♀xC♂, C♀xW♂, W♀xC♂ and W♀xW♂ where C = cold and W =

182   warm. Egg numbers for all families were equalized so that 140 mL of eggs from each female

183   were sired by each male within each 2 x 2 factorial cross. Fertilized egg families were

184   transported in insulated jars packed inside a cooler with ice packs to the OMNRF Codrington

185   Fish Research Facility (Codrington, Ontario, Canada) where they were transferred to Heath trays

186   receiving freshwater at ambient temperature (5-6□) under constant dim light for development.

187

188   **Experimental design: Offspring temperature acclimation**

189   When fry reached the exogenous feeding stage, we randomly chose 20 offspring from

190   each of the 16 families and divided them into two groups for acclimation to two different

191   temperatures (15 and 19□). We chose the cooler acclimation temperature based on the optimal

192   growth temperature reported for brook trout (McCormick *et al*., 1972), whereas the warm

193   temperature simulated the potential warming due to climate change in the Great Lakes region by

194   the end of the century (Hayhoe *et al*., 2010). Each group of 10 was moved into one of four larger

195   (200 L) tanks: two tanks were designated for 15°C and the other two for 19°C so that each

196   family had 10 representatives acclimated to each temperature. Each tank was separated into four

197   sections to keep the families separate, but due to space constraints two families were kept in each

198   tank section where the families sharing a section had a father in common. Individuals were

199   identified to family after measurement trials by microsatellite genotyping (described in

200   supplementary material).

201   Temperature acclimation began after the offspring were transferred to the larger tanks. We

202   increased the water temperature at a rate of 1□ per day using titanium heaters (500W, model TH-

203   0500, Finnex, IL, USA) with digital temperature controllers (model 192 HC 810M, Finnex, IL,

204   USA) until the water in each tank reached its designated temperature (15 or 19°C). The

205   temperatures were checked and recorded twice daily. During this time the fish were fed 5-6 times

206   a day at 2-3% their body weight. The experiments began after the fish had been acclimated for at

207   least 3-4 weeks.

208

209   **Respirometry set up**

210   We explored the influence of parental thermal history on the MO_2-temperature

211   relationship in the offspring, the resting metabolic rate, peak metabolic rate and upper thermal

212   tolerance of the offspring. The MO_2-temperature relationship was measured as the metabolic rate

213   of the offspring during an acute temperature increase of 2□ per hour, where metabolic rate was

214   measured as the rate of oxygen consumption (MO_2) with closed respirometry. Using this MO_2

215   dataset, we determined the resting and peak metabolic rate of each individual offspring. Resting

216   metabolic rate was recorded as the MO_2 at the individual's acclimation temperature before

217   temperature increased with the acute temperature challenge (Chabot *et al*., 2016), and the peak

218   MO_2 as the individual's highest MO_2 during the trial. Finally, each individual's upper thermal

219   tolerance was measured as the critical thermal maximum (CTM) and was recorded as the

220   temperature at which the fish could no longer maintain equilibrium following acute temperature

221   increase.

222   Each experimental trial used eight custom built respirometers. Respirometers were made

223   from a 8 cm diameter glass tube that was cut at a 4.5 cm length and sealed at one end (i.e. the

224   floor of the chamber). The respirometer lids were made of acrylic. Each lid contained a fitting in

225   the center for an O_2 probe, and valves on opposite sides of the probe fitting to allow water to

226   circulate through the respirometer chamber. Two respirometers were placed in each of four

227   transparent plastic tubs and the tubs were seated on top of two, side-by-side stir plates (one plate

228   per respirometer). The plates were used to spin a magnetic stir bar in each respirometer at

229   approximately 60 RPM to prevent the establishment O_2 gradients in the chambers and to keep

230   water moving past an O_2 probe (model DO-BTA, Vernier Software and Technology, OR, USA)

231   that was inserted into the lid of each respirometer. The O_2 probes were connected to a Lab Pro

232   interface (Vernier Software and Technology) and O_2 concentration within the respirometers was

233   recorded using LoggerPro software (version 3.8.6; Vernier Software and Technology). Each

234   respirometer also contained a perforated steel grid to separate the fish from the stir bar. Water

235   from the tub was circulated through the respirometer at 4.5 L per minute using a submersible

236   pump (universal type 1005, EHEIM GmbH & Co., Deizisau, Germany), and the water in each

237   tub was also circulated with aerated, temperature-controlled freshwater from a source tank.

238

239   **Respirometry and critical thermal maximum protocol**

240   Respirometry trials were conducted from August 9 to September 15, 2016. The night

241   before a trial, eight fish were individually transferred into respirometers where they received a

242   continuous flow of fresh water maintained at their acclimation temperature. They were left to

243   adjust to the experimental apparatus overnight, and a thin sheet of black plastic covered each tub

244   to minimize visual disturbance to the fish during the adjustment period and experimental trial.

245   Fish were fasted for at least 12 hours prior to each trial to eliminate the physiological effects of

246   digestion on the experimental results (Millidine *et al.*, 2009).

247   We began measuring MO_2 in each individual fish the next morning at 7:00. To measure

248   MO_2, the respirometer chambers were sealed by manually closing the respirometer valves and

249   switching off the pumps that circulated water through the chambers. After a 30 second wait

250   period, the reduction in chamber O_2 concentration was recorded for 10 minutes. Afterwards, the

251   flow valves were reopened to restore water circulation. Water temperature was then increased at

252   a rate of 2☐ per hour and the MO_2 of each fish measured at each 1☐ increase with 30 minutes

253   between the repeated MO_2 measurements. The rate of oxygen consumption (MO_2) was

254   calculated as,

$$MO_2 = \frac{(Rate\,of\,decline \in [O_2])(V_R - V_F)x60}{h}$$

255   where *(Rate of decline in [O₂])* is the decline in water oxygen concentration (mg O_2 L^{-1} min^{-1})

256   during the 10-minute measurement period, V_R is the volume (L) of the respirometers, V_F is the

257   volume of the fish (L) and h is the time in hours. The rate of decline was determined with

258   LoggerPro, and we measured the linear fit of the drop in respirometer O_2 concentration over

259   time. If the linear correlation coefficient (r) was below 0.8, the datapoint was excluded from the

260   analysis. This resulted in the exclusion of 225 out of 2,845 total datapoints collected. Some of

261   these excluded values were measures of resting and peak MO2 (of 230 individuals, 43 resting

262   MO_2 and 5 peak MO_2 were not included in the analysis).

263   The critical thermal maximum (CTM) for each fish was recorded as the temperature

264   when it lost its righting response (i.e. equilibrium) and this was recognized as the point at which

265   the fish could no longer maintain an upright position within the respirometer. All fish were

266   closely monitored as temperature increased, and when a fish lost equilibrium it was quickly

267   removed from the respirometer and euthanized with 0.3 g L^{-1} of tricaine methanesulfonate (MS-

268   222; Aqua Life, Syndel Laboratories Ltd., B.C., Canada). Euthanized fish were immediately

269   blotted dry on paper towels, measured for mass to the nearest 0.1 g and fork length (mm) using

270   digital balance and calipers, respectively. Measurements of mass and length were used to

271   calculate condition factor using the following formula,

$$Condition factor = \frac{mass}{(forklength)^3} x 100$$

272   A tissue sample (caudal finclip) was taken from the euthanized fish and individually stored in

273   95% ethanol for microsatellite genotyping to identify each offspring to their respective family

274   (described in supplementary material). Twelve of the 230 fish used for this experiment died at or

275   just prior to collecting CTM, so were not included in the analysis of CTM.

276   To ensure that O_2 would not become limiting at warmer temperatures, we monitored O_2

277   saturation of the water throughout each trial. The source tank O_2 concentration was kept at 6.0-

278   7.0 mg L^{-1} and continuously checked with a YSI Pro probe (Hoskin Scientific, ON, Canada). If

279   saturation levels lowered at high temperatures, O_2 was supplemented to the source tank water

280   using airstones and a tank of compressed O_2 while ensuring that hyperoxia did not occur.

281   Because the fish consumed O_2 with increased rates at higher temperatures, we shortened the

282   measurement period (<10 minutes) as necessary to avoid the O_2 concentration from reaching the

283   critical limit of 3.5 mg O_2 L^{-1} during the MO_2 measurement to avoid inducing a hypoxia

284   response in the fish (Graham, 1949; Doudoroff & Shumway, 1970).

285

286   **Calculations and statistical analysis**

287   The MO_2 measured at the fish's acclimation temperature before temperature began to rise

288   with the acute temperature challenge was considered as the fish's resting MO_2. We report peak

289   MO_2 as the highest MO_2 achieved during the respirometry trial. We do not report aerobic scope

290   here because our measurement of peak MO_2 may not necessarily represent the absolute

291   maximum MO_2 achievable by the offspring; max. MO_2 is typically obtained using exhaustive

292   exercise protocols which we did not use in this study. We analyze whole animal rates of O_2

293   consumption with mass as a covariate rather than perform the analysis on mass-specific values

294   because the former is statistically more appropriate (Hayes & Shonkwiler, 1996). The mean

295 values reported from these models are referred to as mass-adjusted MO_2. The raw data plotted as

296 mass-specific MO_2 is included in the supplementary material (Fig. S1).

297   The effect of parent and offspring acclimation temperatures on mass and condition factor

298 was assessed using a general linear mixed effects model (GLMM). The models for mass and

299 condition factor included offspring acclimation temperature (T_O: cold or warm) and parental

300 acclimation temperature (both parents combined into a single parental group: $C_♀xC_♂$, $C_♀xW_♂$,

301 $W_♀xC_♂$ or $W_♀xW_♂$) as fixed effect predictors. An interaction term between offspring and parent

302 acclimation temperature was also included as a fixed effect predictor to determine if parental

303 acclimation temperature had differential effects on offspring mass and condition depending on

304 whether the offspring were acclimated to a cold or warm temperature. Degree days was included

305 as a random intercept to account for the potential effects of age on mass and condition factor.

306 Degree days were calculated for each fish as the cumulative temperature experienced above $0°C$

307 (Chezik *et al.*, 2013; Cook *et al.*, 2018) until the beginning of the experimental trial. A Tukey's

308 HSD post-hoc analysis was performed if the test determined a significant effect of fixed

309 predictors on mass or condition to uncover where differences occurred among pairwise

310 comparisons.

311   To identify factors contributing to variation in resting MO_2, peak MO_2 and CTM, we

312 evaluated competing statistical models using an Akaike Information Criterion corrected for small

313 sample size (AICc). The possible model terms included offspring acclimation temperature (T_O),

314 maternal acclimation temperature (T_M) and paternal acclimation temperature (T_P) as fixed effect

315 predictors, with interactions between all factors. Models also included offspring *Mass* as a

316 covariate because the warm-acclimated offspring grew heavier than cold-acclimated ones and

317 because metabolic rate scales with mass. The effects of maternal ID (ID_M) and paternal ID (ID_P)

318 were included as random intercepts to control for statistical non-independence of offspring

319 relatedness as some were full- or half-siblings based on the 2x2 factorial mating design. From the

320 model AIC values, we calculated the ΔAIC, evidence ratio (ER) and Akaike weight (Wi) for each

321 model and considered the best models as those with a ΔAIC $\leq$ 2 (Burnham & Anderson, 2002).

322 We used the calculated metrics to compare the models and identify common parameters among

323 the models that explained variation in resting MO_2, peak MO_2 and CTM.

324   The relationships between MO_2 and temperature was curvilinear and could not be fit with

325 a simple polynomial function. Instead, we fitted the relationship using a general additive model

326   (GAM) as described in Penney *et al.* (in press). The GAM was generated by capturing the shape

327   of the MO_2 and acute challenge temperature relationship using all individuals with a cubic

328   regression spline (i.e. GAM), and using just 7 knots to avoid over-fitting. From the GAM, we

329   extracted the predicted MO_2 of each offspring at each challenge temperature to use as a predictor

330   in a general linear mixed model (GLMM). This method provided a workaround for the limitation

331   of GAMs in accommodating complex interaction terms by accounting for the variation in the

332   observed MO_2 due to acute challenge temperature, allowing us to test whether the remaining

333   variation in MO_2 (i.e., that not due to acute challenge temperature) can be explained by offspring

334   and parental acclimation temperature, and their interactions. To account for heterogeneity of

335   variance in MO_2 across acute challenge temperature we weighted the model by acute challenge

336   temperature and included a type I autoregressive correlation structure ($\rho = 0.221$) to correct for

337   autocorrelation between MO_2 measurements as they occur at adjacent time-points during the

338   acute temperature challenge.

339   To detect within-generation plasticity and trans-generational plasticity in the metabolic

340   response of the offspring to an acute temperature challenge, we used a GLMM to test the effect

341   of offspring, maternal and paternal acclimation temperature on the MO_2-temperature

342   relationship. We constructed a global model with the fixed effect terms of acute challenge

343   temperature (T_a, continuous variable), offspring acclimation temperature (T_O; cold and warm),

344   maternal acclimation temperature (T_M; cold and warm), and paternal acclimation temperature

345   (T_P; cold and warm). All possible 2-way, 3-way and 4-way interactions between these terms were

346   also included as fixed terms. As with our previously described models, the GLMM included

347   *Mass* as a continuous predictor and the random intercepts of maternal and paternal ID (ID_M and

348   ID_P). Offspring ID was also included as a random intercept because repeated measurements of

349   MO_2 were collected from each individual at every 1$\square$ increase in temperature.

350   All statistical analyses were conducted in JMP 13 (v. 18.1) or R (v. 3.5.2) with the level

351   of significance set to 0.05. Linearity, homogeneity of variance, sample independence and

352   residual normality were confirmed visually, and with the Shapiro-Wilk W, Levene's and Brown-

353   Forsythe tests. The factors that contributed to variation in body mass and condition factor were

354   investigated using JMP 13. Statistical analyses of the resting and peak MO_2, CTM, and MO_2

355   during the temperature challenge were conducted using R with the 'MuMIn' (Barton, 2019),

356   'lme4' (Bates *et al.*, 2015), 'nlme' (Pinheiro *et al.*, 2019) and 'mgcv' (Wood, 2011) packages. We

357   discovered that one of the peak MO_2 datapoints was five standard deviations below the mean,

358   therefore, this datapoint was not included in the final analysis.

359

360   **RESULTS**

361   **Mass and condition factor**

362   Warm-acclimated offspring were heavier overall compared with the cold-acclimated

363   offspring (19□ offspring: 3.31 ± 0.08 g; 15□ offspring: 2.73 ± 0.08 g, GLMM: $F_{1,24.60} = 25.22$, p

364   < 0.01). Parental acclimation temperature had a significant effect on offspring mass (GLMM:

365     $F_{3,52.71} = 13.78$, $p < 0.01$). Offspring from parental groups $C_♀xC_♂$ and $W_♀xC_♂$ (3.50 ± 0.11 vs.

366   3.22 ± 0.11 g) were significantly heavier ($p < 0.05$) than those from the $C_♀xW_♂$ and $W_♀xW_♂$

367   parental groups (2.66 ± 0.12 vs. 2.69 ± 0.11 g), indicating that offspring with cold-acclimated

368   fathers ($C_♀xC_♂$ and $W_♀xC_♂$) were heavier than those with warm-acclimated fathers. No other

369   parental group comparisons were significantly different. There was no interaction between

370   offspring acclimation and parental acclimation group (GLMM: $F_{3,52.71} = 0.34$, $p = 0.80$).

371   Warm-acclimated offspring had higher condition factor than cold-acclimated offspring

372   (1.0 ± 0.01 vs. 0.96 ± 0.01; GLMM: $F_{1,23.25} = 27.16$, $p < 0.01$) and the condition factor of

373   offspring was significantly affected by parental acclimation temperature (GLMM: $F_{3,53.89} = 6.10$,

374     $p < 0.01$). Offspring from parents that were both cold-acclimated ($C_♀xC_♂$) were in significantly

375   better condition than offspring from parents that were both warm-acclimated ($W_♀xW_♂$; $1.0 \pm$

376   0.01 vs. 0.95 ± 0.01, respectively; $p < 0.05$). No other parental groups differed significantly from

377   each other. There was no interaction between offspring acclimation temperature and parental

378   acclimation group (GLMM: $F_{3,53.89} = 0.13$, $p = 0.94$).

379

380   **Critical thermal maximum**

381   Critical thermal maximum was influenced by offspring acclimation temperature (WGP)

382   but not transgenerational (i.e. parental) acclimation. Offspring acclimation temperature (T_O)

383   along with maternal and paternal ID (random effects: ID_M and ID_P) best explained the variation

384   in offspring CTM ($\Delta AIC \leq 2$, Table 1), no other model was within a $\Delta AIC \leq 2$. The effect of

385   offspring acclimation temperature resulted in an approximately $0.4°C$ higher average CTM in

386   warm-acclimated offspring versus cold-acclimated offspring (1.0 ± 0.01 vs. 0.96 ± 0.01; Fig. 1A

387   and B).

388

Resting and peak metabolic rate

Resting MO_2 was affected by offspring and parental acclimation temperature. Four models best explained variation in resting MO_2, and each included *Mass* and maternal acclimation temperature (T_M), with maternal and paternal ID (ID_M and ID_P) as random effects (Table 1). The first model contained only these factors, while offspring acclimation temperature (T_O) appeared in models 2 and 3 with an interaction occurring between offspring and maternal acclimation temperature in model 3 (Table 1). Paternal acclimation temperature (T_P) appeared only once in the top four models and occurred in model 4, which was 2.11 (ER) less likely to best explain variation in the data when compared with model 1 (Table 1). Plotting the resting MO_2 residuals showed that, in general, warm-acclimated offspring tended to have residual resting MO_2 values slightly below the population mean (Fig. 2 A & B). With regard to parental acclimation temperature, the residual resting MO_2 of cold-acclimated offspring from warm-acclimated mothers (Fig 2A) and warm-acclimated fathers (Fig 2B) was higher than the population mean. In contrast, when offspring from warm-acclimated mothers (Fig 2A) and warm-acclimated fathers (Fig 2B) were warm-acclimated, offspring resting MO_2 was lower than the population mean. Together this suggests a transgenerational effect of lowering resting MO_2 when parents and offspring each experience warming. The interaction between maternal and offspring acclimation temperature ($T_O \cdot T_M$) was evident in the residual plot (Fig. 2A): when mothers were cold-acclimated the residual resting MO_2 of their cold-acclimated offspring was slightly lower than that of their warm-acclimated offspring, this trend reversed when the mothers were warm-acclimated.

Peak MO_2 was also affected by offspring and parental acclimation temperature. Three models best explained variation in peak MO_2 ($\Delta AIC \leq 2$), each with maternal and paternal ID (ID_M and ID_P) as random effects (Table 1). *Mass* and offspring acclimation temperature (T_O) were the best predictors of peak MO_2 as these two fixed effects factors occurred in all three models and were the only fixed parameters in the first model. Paternal (T_P, model 2) and maternal (T_M, model 3) acclimation temperature each appeared only once among the three models. Plots of the residual peak MO_2 showed only slight differences due to offspring acclimation temperature, being marginally lower in warm-acclimated offspring (Fig. 2C & D).

418 Warm-acclimated mothers and warm-acclimated fathers slightly elevated the peak MO_2 in their

419 offspring (Fig. 2 C & D).

420

421 **Metabolic response of offspring to an acute temperature challenge**

422 Offspring mass had a significant effect on oxygen consumption, as rates were higher in

423 heavier fish (*Mass*: p < 0.001; Table 2). Offspring MO_2 also increased with challenge

424 temperature (T_a: p < 0.001; Table 2) and the MO_2-temperature relationship was similar between

425 offspring of cold and warm acclimation temperatures (T_O: p = 0.880; Table 2).

426 There was a transgenerational effect of parental acclimation temperature on offspring

427 MO_2 responses to an acute temperature challenge. The acclimation temperature of the mothers

428 and fathers (Fig. 3A & B, respectively) each significantly affected the offspring's metabolic

429 response to the challenge temperature. That is, the MO_2 of the offspring from warm-acclimated

430 parents was elevated compared with offspring from cold-acclimated parents (Fig. 3A & B).

431 While the effect was significant for both parents, a stronger effect occurred on the paternal side

432 (T_M: p = 0.042; T_P: p = 0.010; Table 2).

433 There was a significant statistical interaction between challenge temperature and paternal

434 acclimation temperature ($T_a \cdot T_P$: p = 0.007; Table 2). Offspring mass-adjusted MO_2 was lower in

435 offspring from cold-acclimated fathers compared to those from warm-acclimated fathers when

436 challenge temperatures were below 25□, but the groups tended to converge at temperatures

437 above 25□ (Fig. 3B). No other interaction terms were significant.

438

439 **DISCUSSION**

440 We saw evidence of WGP and TGP in brook trout upper thermal tolerance and metabolic

441 rate with greater WGP and TGP effects. We found partial support for the prediction that CTM,

442 resting MO_2 and peak MO_2 would all increase when offspring were acclimated to a warmer

443 temperature. As predicted, acclimating offspring to a warmer temperature resulted in slightly

444 (0.4□) higher mean CTM. In contrast, warm-acclimation of the offspring had a modest effect of

445 lowering resting MO_2, with offspring acclimation temperature appearing in half of the top AIC

446 models for resting MO_2. Similarly, peak MO_2 was slightly lower in warm-acclimated offspring.

447 Taken together, these results demonstrate within-generation thermal plasticity in the juvenile

448 brook trout, however, plasticity was more limited than expected based on previous studies (e.g.,

449 McCormick *et al*., 1972; Stitt *et al*., 2014; Morrison *et al*., 2020). Limited within-generation

450 plasticity was further supported by the results of the acute temperature challenge, where the

451 MO_2-temperature relationship did not differ between the two offspring acclimation temperatures

452 (T_O).

453   Our results partially supported the prediction that TGP would improve thermal tolerance

454 at elevated temperatures (Table 1). Parental acclimation temperature had no effect on offspring

455 CTM, but both maternal and paternal acclimation temperature influenced offspring resting MO_2,

456 and to a lesser extent peak MO_2. Maternal and paternal acclimation temperature each contributed

457 to the offspring's response to the acute temperature challenge, with an overall upward shift in the

458 curve for offspring from warm-acclimated parents. To our surprise, paternal TGP contribution to

459 offspring thermal performance (MO_2-temperature relationship) was larger than the maternal

460 contribution.

461

Within-generation plasticity

463   Thermal plasticity is thought to have evolved when organisms experience temperature

464 fluctuations within their lifetimes (Beaman *et al*., 2016). We expected brook trout to show some

465 within-generation plasticity for thermal tolerance based on their use of different habitats with

466 varying thermal conditions throughout their life (Biro *et al*., 2008; Smith and Ridgway, 2019).

467 Additionally, there is evidence that different populations have differing capacities for thermal

468 acclimation (McDermid *et al*., 2012; Stitt *et al*., 2014).

469   Within-generation plasticity was observed in CTM; CTM increased with offspring

470 acclimation temperature. CTM was in the expected range for brook trout acclimated to our

471 temperatures (Wehrly *et al*., 2007; O'Donnell *et al*., 2020), however, plasticity in their upper

472 thermal tolerance appears limited. The CTM only increased by ~0.4□ in warm-acclimated

473 offspring despite the 4□ difference in acclimation temperature between the two groups of

474 offspring. Though the rate of heating used in our experiment (+2 □/hr) has been deemed

475 appropriate rate for measuring CTM in brook trout (Galbreath *et al*., 2004), it is unclear whether

476 the CTM of warm-acclimated offspring would be higher with a slower rate of heating. For

477 example, Morrison *et al*. (2020) found that brook trout acclimated to 15□ and 20□ had

478 significantly different CTM when heating the fish at a rate of 0.3□ per minute (i.e, +18 □/hr).

479   We detected an effect of thermal acclimation on offspring resting MO_2, however, the

480   effect was small (Table 1). Resting metabolic rate typically increases with acclimation

481   temperatures until the individual reaches its *pejus* temperature (approximately 20□ for brook

482   trout: Hartman & Cox, 2008). Thus, we anticipated that resting MO_2 would be higher in warm-

483   acclimated offspring compared to cold-acclimated offspring, largely because they were being

484   measured at a warmer temperature, but we did not see this trend. It is not immediately clear why

485   resting MO_2 was not more elevated in warm-acclimated offspring compared with cold-

486   acclimated offspring. A previous study on variation in upper thermal tolerance and metabolic rate

487   of brook trout found that individuals originating from Dickson Lake (the same lake from which

488   our brook trout originated) had a higher standard metabolic rate (SMR) following acclimation to

489   16□ compared to 20□, though the authors did not suggest the lower SMR at 20□ was due to the

490   fish reaching their *pejus* temperature (Stitt *et al*., 2014). Stitt *et al.*'s (2014) experimental

491   temperatures were comparable to ours (15-16□ and 19-20□), however, the fish tested were of

492   different life stages (yearling vs. adult). The factors influencing resting MO_2 (or SMR) of brook

493   trout warrants further investigation.

494   Offspring acclimation temperature had a modest effect on peak MO_2, demonstrating

495   some within-generation plasticity in this parameter. Some fish species are capable of extending

496   the upper limit of MO_2 (i.e. peak MO_2) when acclimated to warmer temperatures (reviewed by

497   Schulte, 2015). For example, exercise-induced maximum metabolic rate increased by 20-30% in

498   lake trout acclimated from 8 to 15□ (Kelly *et al*., 2014). It is important to note that our

499   measurement of peak MO_2 is not equivalent to maximum MO_2 which is typically measured in

500   the fish after exhaustive exercise or a chase protocol (as in Kelly *et al*., 2014). Our results

501   suggest that brook trout peak MO_2 is capped within the values we observed in our study. This

502   generally agrees with the idea that metabolic ceilings, like peak MO_2 or CTM, are relatively

503   thermally (acclimation) insensitive (Sandblom *et al*., 2016; Norin & Metcalfe, 2019; Morrison *et*

504   *al*., 2020).

505   Offspring acclimation temperature did not influence the offspring's MO_2 response to an

506   acute temperature challenge (+2□ h^{-1}). Interestingly, offspring MO_2 did not begin to rise until the

507   challenge temperature exceeded 23°C. Although the reason for the sudden increase in MO_2 at

508   ~23□ is not immediately clear, it could be due to a physiological stress response(s) being

509   initiated at this temperature, especially considering that 23□ is near the upper incipient lethal

510 temperature recorded for these fish (24□; Fry *et al.*, 1946; Wehrly *et al.*, 2007). Identifying the

511 physiological processes that result in the increased MO_2 at ~23□ in brook trout would require

512 further study, although previous works have reported on correlated mechanisms during an acute

513 thermal challenge. Sandblom & Axelsson (2007) demonstrated an increase in hematocrit, likely

514 resulting from splenic contraction, in rainbow trout when challenged with an acute temperature

515 increase. Splenic contraction would increase the number of red blood cells available to meet the

516 increased oxygen delivery demands associated with acute warming (Clark *et al.*, 2008).

517 Chadwick *et al.* (2015) saw that levels of HSP70 and glucose increased in juvenile brook trout

518 when challenge temperatures reached approximately 21□. It is possible that stress responses,

519 such as induction of molecular chaperones or mobilization of energetic resources were initiated

520 at 23□ in the juvenile brook trout in our study, thus increasing metabolic rate at this temperature.

521

Transgenerational plasticity

523 Both maternal and paternal acclimation temperature affected the offspring's MO_2-

524 temperature relationship, with an overall upward shift for offspring from warm-acclimated

525 parents. This was also reflected in offspring resting MO_2 and it implies that offspring will incur a

526 higher cost of living (Norin & Metcalfe, 2019) when parents are warm-acclimated. It is thought

527 that TGP is adaptive, thus selected for, when the environment changes predictably across

528 generations (Jablonka *et al.*, 1992; Bonduriansky *et al.*, 2012; Norouzitallab *et al.*, 2019; but see

529 Uller *et al.*, 2013). Based on this idea, TGP would be predicted to be weak in stenothermal

530 organisms that have adapted to relatively cold, thermally stable habitats. The limited available

531 evidence supports this: in lake trout (*S. namaycush*), a cold-adapted stenothermal congener of

532 brook trout, TGP was limited, and most evident as elevated MO_2 in warm-acclimated offspring

533 from warm-acclimated parents (Penney *et al.* in press). In contrast, in eurythermal or warm-

534 adapted fish species, metabolic rates are *reduced* in warm-acclimated offspring from warm-

535 acclimated parents compared to cold-acclimated parents (Donelson *et al.*, 2012; Shama *et al.*,

536 2014; Donelson *et al.*, 2017).

537 In our study, peak MO_2 varied only slightly with maternal or paternal acclimation

538 temperature, and no transgenerational effect on offspring CTM was detected. Our results agree

539 with the limited number of studies on the transgenerational effect of warming on maximum

540 metabolic rates and CTM. Sandblom *et al.* (2016) found that European perch (*Perca fluviatilis*)

541   held at higher temperatures for multiple generations displayed little change in their maximum

542   rate of O_2 consumption and CTM. Similarly, the CTM of largemouth bass (*Micropterus*

543   *salmoides*) did not differ after multi-generational exposure to warmer temperature (White &

544   Wahl 2020). Further, CTM and peak MO_2 of lake trout offspring were also not affected by

545   parental exposure to a warmer environment (Penney *et al*., in press), which is perhaps

546   unsurprising given that there was limited WGP in lake trout peak MO_2 and CTM with offspring

547   acclimation in the same study. Together, these studies suggest TGP is unlikely to significantly

548   alter CTM or peak MO_2 in response to increased environmental temperatures over relatively

549   short multi-generation timespans, reinforcing evidence that these metabolic ceilings are likely to

550   be exceeded in ecological timeframes (Sandblom *et al*., 2016; Norin & Metcalfe, 2019; Morrison

551   *et al.*, 2020).

552   We sought to examine the extent to which WGP and TGP operate or interact in brook

553   trout. While WGP was evident in peak MO_2 and CTM, it was through TGP that warm-acclimated

554   parents elevated resting MO_2 and affected the MO_2-temperature relationship in offspring. The

555   importance of TGP relative to WGP may depend on life stage and variation in the habitat

556   experienced at each life stage. It is possible that transgenerational effects are strongest in early-

557   juvenile life stages (Yin *et al*., 2019), but only when the environment is stable. In contrast, WGP

558   may be the favoured strategy when temperatures are more variable (Leimar & McNamara,

559   2015). In fact, in situations where environmental temperature variation exists, TGP effects may

560   be overridden by WGP, as found in stickleback (Shama, 2017). Our study examined juvenile

561   brook trout 5-6 months after hatching, at a time when they would be feeding in shallow depths

562   near shore and near the surface in warmer water (Biro *et al*., 2008). It would be interesting to

563   examine the relative influences of WGP and TGP at earlier or subsequent life stages. We are not

564   aware of any such studies to date, although experiments examining WGP and TGP at multiple

565   life stages could be very informative.

566

Relative parental contributions

568   Although both maternal and paternal thermal history (temperature acclimation) each

569   contributed to offspring thermal physiology, we did not find strong evidence that

570   transgenerational effects were additive (i.e., stronger when the offspring had a warm mother *and*

571   a warm father). Maternal and paternal acclimation temperature ($T_M + T_P$) appeared in the same

572   model only once for resting MO_2 (model 4, Table 1), but not for peak MO_2 or CTM. Similarly,

573   each parent contributed to their offspring's MO_2 response to an acute temperature increase.

574   Paternal effects have received less attention relative to maternal effects (Rutkowska *et al.*,

575   2020) and the size of the epigenetic paternal contribution to such changes relative to the maternal

576   contribution is still debated (reviewed by Best *et al.*, 2018). In the few studies that have tested

577   relative parental contributions to TGP in metabolic traits in fish, the paternal contribution is

578   either less than (Shama *et al.*, 2014) or comparable to the maternal contribution (Penney *et al.*, in

579   press). In this study, fathers surprisingly appeared to have greater contributions to TGP than did

580   mothers. Paternal effects are complex, can depend on the sex of the offspring, and can vary

581   depending on the environment experienced by paternal grandparents (Crean & Bonduriansky,

582   2014; Hellman *et al.*, 2020). Environmentally-mediated epigenetic changes do occur in sperm

583   (Ord *et al.*, 2020; Immler, 2020) and these along with cytoplasmic components can influence

584   offspring phenotypes (summarized by Donkin & Barrès, 2018). Parents can also have opposing

585   effects on gene expression in their offspring despite both parents having received the same

586   treatment, in that a gene may be maternally downregulated but paternally upregulated in the

587   offspring (Bautista *et al.*, 2020). While epigenetic regulation of gene expression may be an

588   underlying factor in the paternal contribution we observed in our study, we are not aware of

589   another study showing such a large paternally-mediated TGP contribution to thermal responses

590   relative to the maternal contribution.

591

592   **Summary**

593   The brook trout in our study displayed a limited transgenerational response to warmer

594   temperature. Transgenerational effects on offspring phenotypes depend on genotype or ecotype

595   (Verhoeven & van Gurp, 2012; Vayda *et al.*, 2018), and TGP is predicted to arise in populations

596   that experience variation in temperature over multiple generations (Beaman *et al.*, 2016; Yin *et*

597   *al.*, 2019). Because different populations of brook trout display variation in thermal tolerance and

598   capacity for acclimation (McDermid *et al.*, 2012; Stitt *et al.*, 2014) it would be informative to

599   assess whether transgenerational responses to warming vary among stream and lake populations,

600   and across the species' range. One might predict, for example, that daily as well as seasonal

601   thermal variation in stream environments (Chadwick & McCormick, 2017) would select for

602   increased TGP compared with lake habitats. Like many other cold-adapted organisms, brook

603   trout are being threatened by climate change-induced habitat impacts (King *et al*., 1999;

604   Robinson *et al*, 2010; Bassar *et al*., 2017). Although previous studies have suggested TGP may

605   buffer the impact of environmental stressors and facilitate an evolutionary response to climate

606   change (Bonduriansky *et al*., 2012; Bernatchez, 2016; Smith *et al*., 2016), the limited response

607   observed in our study suggests that TGP may not sufficiently enable brook trout to keep pace

608   with anticipated changes in environmental temperatures.

609

620

621   **Competing interests**

622   No competing interests declared.

623

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Tables

Table 1: Summary of the top models determined with AIC_C to explain variation in brook trout offspring (age: 5 months) resting rate of oxygen consumption (MO_2), peak MO_2 and critical thermal maximum (CTM) with transgenerational acclimation.

Measure	Model	ΔAIC	ER	W_i	R^2	Model
CTM	1	0	1	1	0.51	$T_O + ID_M + ID_p$
Resting MO_2	1	0	1	0.39	0.19	$Mass + T_M + ID_M + ID_P$
	2	1.10	1.73	0.22	0.21	$Mass + T_O + T_M + ID_M + ID_P$
	3	1.26	1.88	0.21	0.22	$Mass + T_O + T_M + (T_O \cdot T_M) + ID_M + ID_P$
	4	1.49	2.11	0.18	0.17	$Mass + T_M + T_P + ID_M + ID_P$
Peak MO_2	1	0	1	0.48	0.52	$Mass + T_O + ID_M + ID_P$
	2	1.02	1.67	0.29	0.51	$Mass + T_O + T_P + ID_M + ID_P$
	3	1.53	2.15	0.23	0.53	$Mass + T_O + T_M + ID_M + ID_P$

Offspring were from parents acclimated to either a cold or warm temperature and were similarly acclimated to cold or warm temperature. T_P, T_M, T_O are the paternal, maternal and offspring acclimation temperatures, respectively, and ID_M and ID_P are the maternal and paternal individual identification (random effects).

957   **Table 2:** Factors contributing to variation in brook trout rate of oxygen consumption (MO_2).

Parameter	Coefficient	S. E.	DF	t-value	p-value
Intercept	-0.84	0.11	17.87	7.48	<0.001
Mass	0.20	0.02	182.14	12.27	<0.001
T_a	1.19	0.09	2338.40	13.03	<0.001
T_O	0.02	0.12	483.13	0.15	0.880
T_M	0.22	0.11	180.89	2.05	0.042
T_P	0.44	0.14	11.08	3.08	0.010
$T_O \cdot T_M$	0.011	0.15	545.40	0.07	0.941
$T_O \cdot T_P$	-0.05	0.16	869.28	0.30	0.766
$T_M \cdot T_P$	-0.22	0.16	943.48	1.38	0.168
$T_a \cdot T_O$	-0.003	0.12	2339.95	0.02	0.981
$T_a \cdot T_M$	-0.17	0.12	2337.07	1.43	0.152
$T_a \cdot T_P$	-0.36	0.13	2338.77	2.70	0.007
$T_O \cdot T_M \cdot T_P$	-0.15	0.22	646.15	0.70	0.487
$T_a \cdot T_O \cdot T_M$	-0.13	0.17	2345.00	0.78	0.435
$T_a \cdot T_O \cdot T_P$	-0.05	0.18	2343.07	0.27	0.784
$T_a \cdot T_M \cdot T_P$	0.17	0.18	2338.35	0.94	0.348
$T_a \cdot T_O \cdot T_M \cdot T_P$	0.30	0.24	2344.12	1.27	0.205

958   Offspring (age: 5 months) from parents acclimated to either a cold or warm temperature were
959   similarly acclimated to cold or warm temperature. T_P, T_M, T_O are the paternal, maternal and
960   offspring acclimation temperatures, respectively, and T_a represents the acute temperature
961   challenge (the cubic regression spline).

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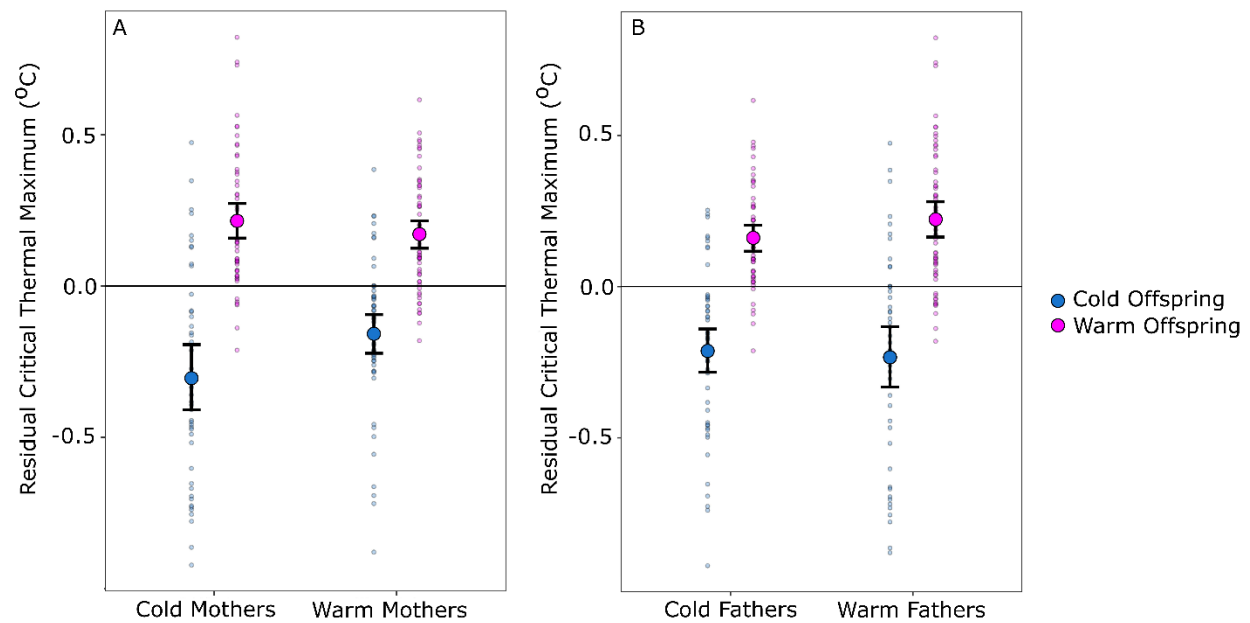963
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967 **Figures**

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970 **Fig. 1:** The effect maternal (A) and paternal (B) acclimation temperature on the critical thermal
971 maximum (CTM) of brook trout offspring (age: 5 months) acclimated to a cold (15□, n = 100) or
972 warm (19□, n = 116) temperature. Values represent the residuals (± confidence intervals) from
973 the population mean, controlling for the effect of mass and family.
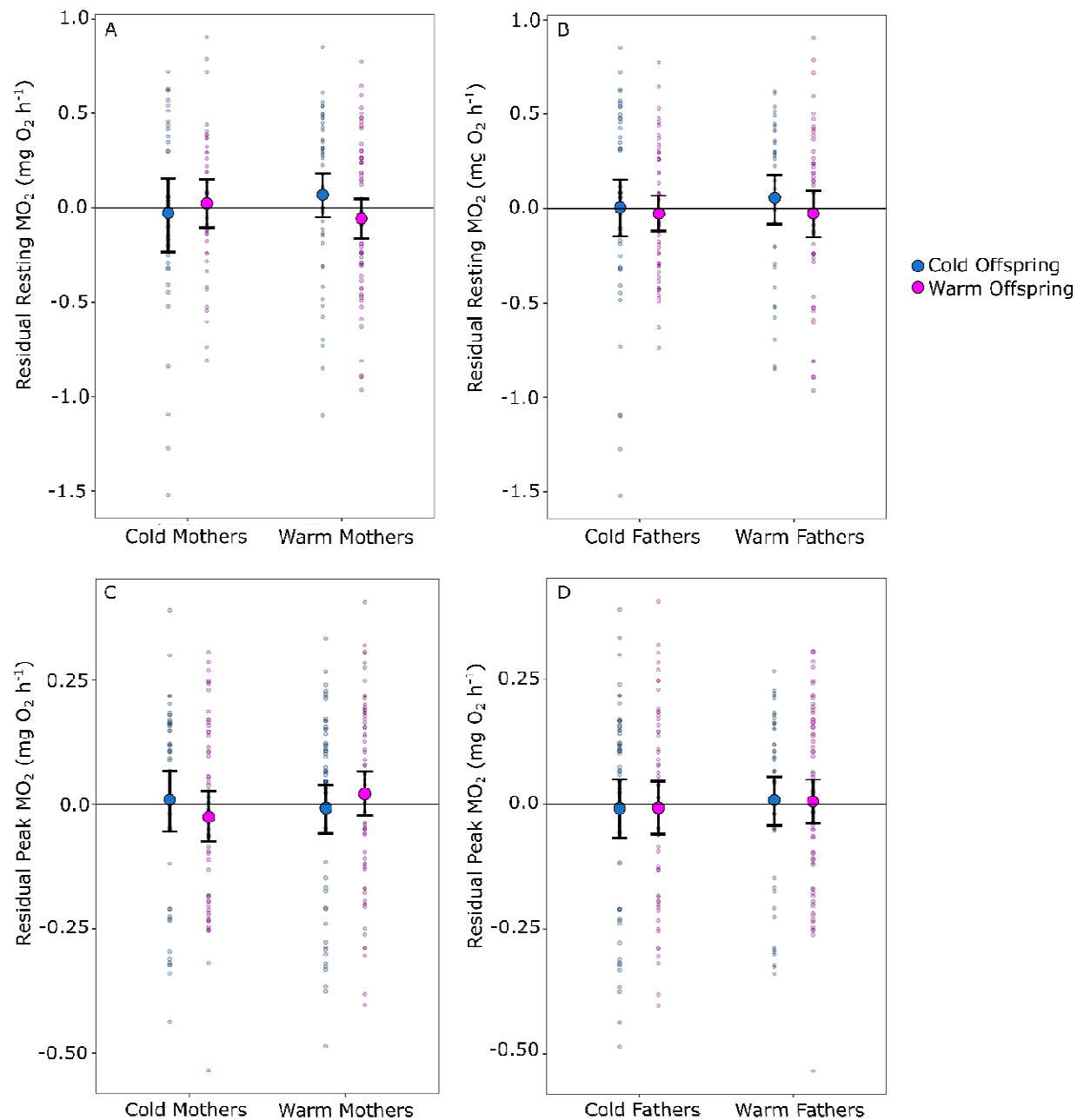
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Fig. 2: The effect of maternal (A and C) and paternal (B and D) acclimation temperature on the resting rate of oxygen consumption (MO_2), and peak MO_2 of brook trout offspring (age: 5 months) acclimated to a cold (15□) or warm (19□) temperature (n = 85-122). Values represent the residuals (± confidence intervals) from the population mean, controlling for the effect of mass and family.
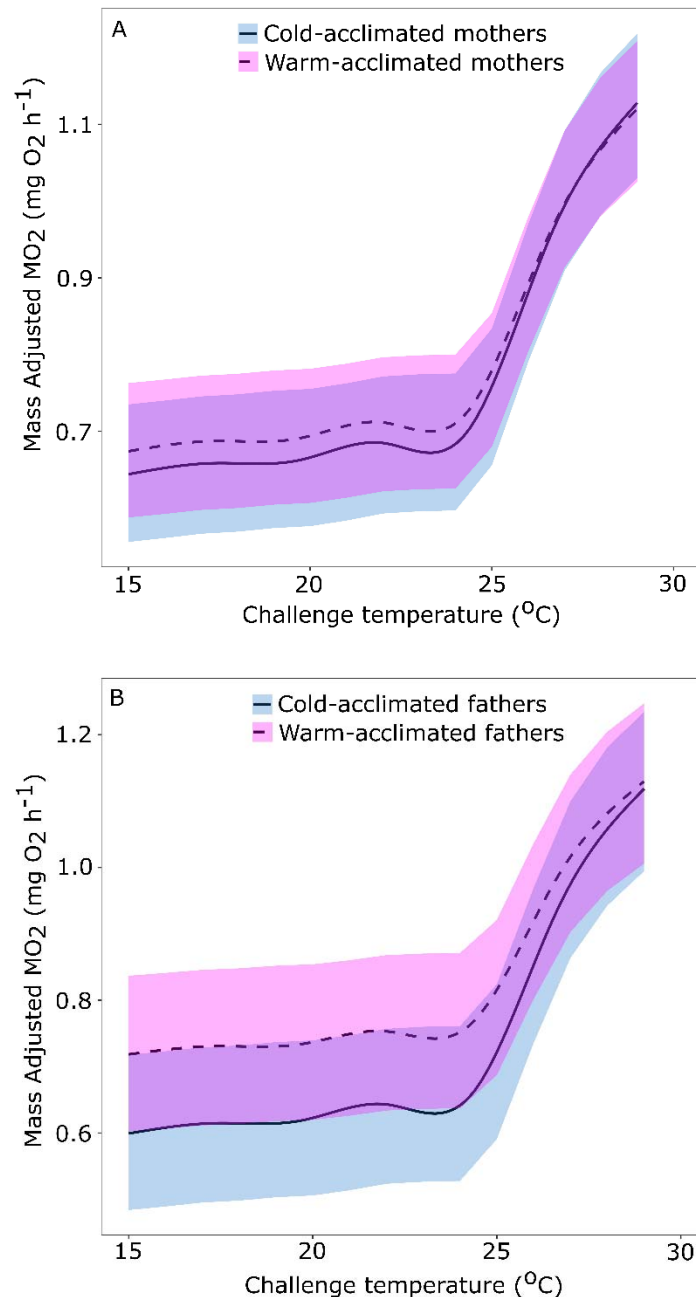
Fig. 3: The influence of A) maternal and B) paternal acclimation temperature on the change in the rate of oxygen consumption (MO$_2$) of cold- (15□, n = 105) and warm- (19□, n = 125) acclimated brook trout offspring (age: 5 months) given an acute temperature challenge of +2°C·h^{-1}. Plots show means and 95% confidence intervals for cold- and warm-acclimated offspring shown in blue and red respectively as estimated from the GLMM where challenge temperature corresponds to a spline. Rates of oxygen consumption (MO$_2$) were statistically-adjusted for effects of body mass (see text).