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1 Cyclical and Stochastic Thermal Variability

2 Affects Survival and Growth in Brook Trout

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19

21 **Abstract**

22 Directional changes in temperature have well-documented effects on ectotherms, yet few studies

23 have explored how increased thermal variability (a concomitant of climate change) might affect

24 individual fitness. Using a common-garden experimental protocol, we investigated how

25 bidirectional temperature change can affect survival and growth of brook trout (*Salvelinus*

26 *fontinalis*) and whether the survival and growth responses differ between two populations, using

27 four thermal-variability treatments (mean: 10^0 C; range: 7-13^0 C): (i) constancy; (ii) cyclical

28 fluctuations every two days; (iii) low stochasticity (random changes every 2 days); (iv) high

29 stochasticity (random changes daily). Recently hatched individuals were monitored under

30 thermal variability (6 weeks) and a subsequent one-month period of thermal constancy. We

31 found that variability can positively influence survival, relative to thermal constancy, but

32 negatively affect growth. The observations reported here can be interpreted within the context of

33 Jensen's Inequality (performance at average conditions is unequal to average performance across

34 a range of conditions). Projections of future population viability in the context of climate change

35 would be strengthened by increased experimental attention to the fitness consequences of

36 stochastic and non-stochastic thermal variability.

1. Introduction

Temperature affects ectotherm physiology (Angilletta et al., 2004; Pörtner and Farrell, 2008; Farrell, 2009) with consequences for individual fitness and population viability, particularly under forecasted changes in climate (Rieman et al., 2007; Wenger et al., 2011a,b). In addition to directional shifts, increased thermal variability is predicted to be a concomitant of climate change (Hanson et al., 2012; Wang and Dillon, 2014). However, considerably less attention has been directed to how variability in temperature affects fitness-related traits independently of changes to the mean (Vasseur et al., 2014; Dowd et al., 2015). This represents an important knowledge gap, given that thermal variability can represent a central determinant of ectotherm responses to environmental change (Colinet et al., 2015; Sinclair et al., 2016).

Predictions of how temperature variability might affect individuals and populations depends on how variability is quantified (Dowd et al., 2015; Bozinovic et al., 2016; Sinclair et al., 2016). Thermal variation can be manifest in various ways, e.g., cyclical vs non-cyclical; stochastic vs. non-stochastic; high-amplitude vs. low-amplitude cycles. It can also be manifest at various temporal scales (e.g., days, weeks, months, years), and at levels considered to be extreme in the context of a species' or population's thermal performance curve (Sinclair et al., 2016). This can make it challenging to study the effects of bidirectional changes in temperature under laboratory conditions in a consistent and readily comparable manner both within and among species, which might account for the relative paucity of such studies relative to the amount of research on directional thermal change.

Predicted responses to thermal fluctuations will also depend on the degree to which the temperature variations encompass the thermal optimum for the species, or population, under study (Morash et al., 2018). Here, the application of Jensen's inequality (Jensen, 1909) has

60 proven invaluable in predicting and interpreting changes in metrics of individual 'performance'

61 (e.g., metabolic rate, growth rate) resulting from fluctuating changes in temperature (Ruel and

62 Ayres, 1999; Denny, 2017).

63   Experimental work on thermal variability has largely focused on invertebrates (e.g.,

64 Kingsolver et al., 2009; Williams et al., 2012; Colinet et al., 2015). Among vertebrates, there has

65 been some work on reptiles (Du and Ji, 2006; Les et al., 2009) and amphibians (Niehaus et al.,

66 2012) but comparatively little on fishes (Morash et al., 2018). The effects of thermal variability

67 on per capita population growth rate have been modelled for at least one endotherm (black-faced

68 spoonbill, *Platalea minor*; Pickett et al., 2015) and experimentally explored for the green alga

69 *Tetraselmis tetrahele* (Bernhardt et al., 2018).

70   Here, we examine the effects of thermal variability on two populations of brook trout

71 (*Salvelinus fontinalis*), a fish widely distributed throughout eastern North America. For guidance

72 regarding our laboratory levels of temperature and temperature variability, we examined water

73 temperature data for four rivers in close proximity (<100m to 5km) to our study populations to

74 ensure that our thermal experimental treatments reflected those likely to be experienced under

75 natural conditions. According to Hanson et al. (2012), between the periods of 1951-1980 and

76 1981-2010, the standard deviation (σ) of global surface temperatures increased 16% during

77 summer (June-August) and 7% during winter (December-February). However, more than 20% of

78 the globe experienced an increase of more than 2σ in 2009, 2010, and 2011 (relative to the 1951-

79 1980 baseline; Hanson et al., 2012). Given this information, as discussed in more detail in

80 sections 2.2 and 2.3, our experimental value of 1.24σ can be interpreted as encompassing an

81 empirically defensible increase in thermal variability that trout might be expected to experience

82 under climate change.

83   Our primary objective is to explore how predictably cyclical and stochastic thermal

84   variability might affect survival and growth in the early, post-hatching stage of life. Changes in

85   water temperature are likely to be particularly important in early development, especially for fish

86   such as trout that depend on a yolk sac for nutrition prior to the initiation of exogenous feeding

87   (Jensen et al., 2008). Using a common-garden experimental protocol, we address a secondary

88   objective of determining whether survival and growth responses to thermal variability are likely

89   to differ genetically between populations of the same species.

90

91 **2. Materials and methods**

92   *2.1. Study populations*

93   The two study populations of brook trout inhabit Ouananiche Beck (46° 39.0' N, 53°

94   11.0' W) and Watern Cove River (46° 37.9' N, 53° 9.5' W), small rivers on Cape Race,

95   Newfoundland, Canada (bounded by 53°16' W, 46°45' N, 53°04' E, and 46°38' S). This small,

96   barren, coastal region is traversed by multiple short (0.27-8.10 km), low-order streams most of

97   which contain resident trout populations that are genetically distinct from one another

98   (Hutchings, 1993; Belmar-Lucero et al., 2012; Wood et al., 2014). Life-history differences

99   among populations are thought to represent adaptive responses to environmentally different

100   selective regimes, following habitat fragmentation (Hutchings, 1993, 1996; Wood et al., 2014).

101   Phylogeographic work suggests that the populations originated from a common ancestor and

102   have been isolated since the Wisconsin deglaciation (Danzmann et al., 1998).

103

104   *2.2. Temperature*

105   The experimental protocol subjected trout to either a constant (10°C) or variable

106   temperature (range: 7° to 13°C; section 2.3), based on an empirically defensible suite of values

107   experienced by the two source populations in the wild. The best available temperature data for

108   Cape Race brook trout are those measured hourly over a one-year period (October 2009 to

109   September 2010) in four separate rivers, using HOBO data loggers (Fig. 1; Table 1): Bristol

110   Cove River, Cape Race River, Cripple Cove River, and Whale Cove River. One of our study

111   populations (Ouananiche Beck) is a tributary of Bristol Cove River, and the other (Watern Cove

112   River) is located 2-5 km from these four rivers. Combining data for all four rivers yields a mean

113   of 9.59°C and a σ of 2.41°C for the days between 16 May and 15 June, the approximate time

114   frame originally intended for the experiment.

115   Based on linear quantile-quantile plots for each dataset, the temperature data are

116   distributed normally, meaning that 68.2% of the pooled-temperature values would fall within the

117   range of 9.59 ± 2.41°C. Put another way, at 1σ of the observed average mid-May to mid-June

118   temperatures in 2010, 68.2% of the temperatures experienced by trout would be expected to fall

119   between 7.18 and 12.00°C (a range of 4.82°C). For logistical reasons, the actual dates of our

120   experiment differed slightly from the planned time period, extending from 26 April to 5 June.

121   For these dates, the pooled temperature data for the four Cape Race rivers averaged 8.28°C with

122   a σ of 2.40 (Table 1). Under normality, 68.2% of the temperatures in the wild would fall between

123   5.88 and 10.68°C, a range of 4.80°C. The temperatures to which the experimental trout were

124   exposed ranged between 7 and 13°C. This range (6°C) is 24% greater than that associated with

125   1σ for both the mid-May to Mid-June (4.82°C) and late-April to early-June (4.82°C) periods.

126   Thus, the range in temperatures in our common-garden experiment can be thought of as

127 approximating an anomaly of 1.24σ relative to 2010 conditions, an increase that falls well within

128 the measurable increase in global surface temperatures documented by Hanson et al. (2012).

129

130 *2.3. Experimental design*

131   After one generation in the laboratory, mature adults originating from the two

132 populations were reared and spawned at Concordia University, Montreal, in November 2015. For

133 each population, 5 males were each crossed with 6 different females, resulting in 30 families per

134 population. On 1 February 2016, fertilized eggs and recently hatched individuals were

135 transported to the Aquatron Facility at Dalhousie University where they were acclimated to

136 laboratory conditions in small, 2.8-litre flow-through aquaria at 5°C. On 26 April, trout were

137 subjected to one of four temperature variability treatments: (1) a constant temperature of 10°C;

138 (2) a periodic, cyclical fluctuation of 3°C every two days, with temperatures ranging from 7° to

139 13°C; (3) a stochastic or random fluctuation of $\pm$ 3° or 6°C every two days, with temperatures

140 ranging from 7° to 13°C, i.e., the 'low-stochasticity treatment'; and (4) a treatment analogous to

141 (iii) but with the stochastic temperature change occurring daily, i.e., the 'high-stochasticity

142 treatment'. The temperatures were achieved by cooling or heating ambient water provided to

143 three separate, temperature-controlled header tanks that provided a constant flow (1.5 litres min^{-}

144 ^{1}) of water to each of the experiment tank racks. The temperature of the water in each header

145 tank was measured daily.

146   Fish were randomly selected for each replicate tank from a pool of all available fish in

147 each population. There were 5 and 7 replicates for the Watern Cove and Ouananiche Beck

148 populations, respectively. Twenty-seven individuals were placed in each replicate tank (all tanks

149 were identical) one week before the start of the experiment and subjected to the same

150 photoperiod, light intensity, water flow, and food (fish were fed daily with an identical mixture

151 of live shrimp, *Artemia* spp., and dry Corey Aquafeeds ® 0.7 mm pellets). The periodic

152 treatment followed a cyclical pattern of 7°-10°-13°-10°-7°C. Temperatures associated with the

153 stochastic treatments (either 7°, 10° or 13°) were chosen randomly, using a random number

154 generator (Fig. 2).

155   Three aquarium racks, each supporting sixty 2.8-litre, flow-through tanks, were

156 established at one of the three experimental temperatures. The experimental tanks were separated

157 by rack, or temperature, and randomly allocated to a location within the rack. Temperature

158 changes (i.e., reassignment of tank location among racks) occurred every two days for tanks

159 associated with the constant, cyclical, and low-stochasticity treatments, and every day for those

160 associated with the high-stochasticity treatment. Tanks were randomly allocated to a position on

161 a rack each time a temperature change occurred. Any tank not moved to a different rack on a

162 given day (i.e., staying at the same temperature) was randomly re-allocated to a different position

163 on the same rack. Tanks associated with the high-stochasticity treatment were re-distributed

164 within the same rack if they were subjected to the same temperature for more than two

165 consecutive days.

166   The duration of the experimental period was 41 days, ending on 5 June 2016. The

167 following day, all fish were transferred to the 7°C rack and left undisturbed for 5 weeks. Water

168 temperatures were measured daily for each of the experimental racks to compare the nominal

169 (intended) temperatures with the actual (measured) temperatures. For logistical reasons, actual

170 temperature data were available daily for all three racks from days 11 through 41.

171   On 13 July 2016, a post-experimental monitoring period (31 days) was initiated to

172 examine whether differences in survival and(or) growth between treatments and(or) populations

173   during the experimental period persisted after a period of environmental constancy. Two to three

174   replicates per treatment for each of the populations (twenty, 9-litre, flow-through aquaria in total)

175   were randomly created from the existing tanks. The experimental tanks were randomly allocated

176   to fill one of thirty positions on the single rack. The position of all tanks remained constant until

177   the end of the post-experimental period on 11 August 2016. Each tank experienced the same

178   daily ambient water temperature which ranged between 13^0 and 17^0 C. Fish were fed daily with

179   an identical mixture of dry Corey Aquafeeds 0.7 mm pellets and dry 1.2 mm pellets.

180

181   *2.4. Data collection and analysis*

182   Mortalities were recorded daily for each tank during the experimental and post-

183   experimental periods. Using a Kaplan-Meier survival analysis (Goel et al., 2010), we examined

184   the effect of population, treatment, and replicate on time (in days) to death. A parametric model

185   (*survreg* function in R) with a Weibull distribution and a non-constant hazard (cf. Ergon et al.,

186   2018) with age were used to analyze the survival data.

187   Fish were not anesthetized. A ruler was placed above the fish for reference. Photos were

188   taken with an iPhone 7. Individual lengths were estimated from photographs (using an iPhone 7),

189   using ImageJ 1.50i software (Schneider et al., 2012). Unanesthetized fish were collected from

190   the tanks with a small net and placed on a white, styrofoam plate. A ruler was used as a reference

191   in each photograph, and measurements (to the nearest 0.1 mm) were taken between the two

192   longest points on the body. Once a clear photo had been taken of the entire body, the fish were

193   placed back into the tanks. Length data were analyzed with a linear mixed-effects model. We

194   examined the effect of population (fixed effect), treatment (fixed effect), number of surviving

195 fish in each tank (fixed effect), and replicate (random effect) on total fish length. This analysis

196 was undertaken on the data collected at the end of the experimental period (6 June 2016).

197 Model fitting and stepwise reduction of the models were performed with ANOVA

198 (including all two-way interactions; none of which were significant, $p > 0.05$) and by AIC (*step*

199 function in R). Treatment levels were combined where possible by pooling levels with similar

200 intercept values. This was performed as a means of post-hoc analysis to determine the statistical

201 relationships between treatments. All analyses were conducted with the survival (Therneau and

202 Grambsch, 2000) and lme4 (Bates et al., 2015) packages in R (Version 3.3.2; R Core Team,

203 2016). Differences in average temperature among treatments were tested, using an ANOVA. We

204 used the R package cvequality (Version 0.1.3; Marwick and Krishnamoorthy, 2019) to test for

205 significant differences in the coefficient of variation (CV = σ/mean) between the cyclical, low-

206 stochasticity, and high-stochasticity treatments, using Feltz and Miller's (1996) *D' AD* test

207 statistic.

208

209 **3. Results**

210 During the experimental period, the actual water temperatures differed slightly from the

211 intended nominal temperatures (Fig. 2). Nonetheless, the average ($\pm$ σ) did not differ among

212 treatments (constant: 10.89 ± 0.35^0C; cyclical: 10.53 ± 2.16^0C; low-stochasticity: $10.52 \pm$

213 2.47^0C; high-stochasticity: 10.08 ± 2.29^0C; $F_{[1,122]} = 2.649$; $p=0.106$). Considering only the

214 variable treatments, there were no differences in either the means ($F_{[1,91]} = 0.596$; $p = 0.442$) or

215 the CVs ($D' AD = 0.518$; $p = 0.772$).

216 The number of surviving individuals declined in all four experimental treatments (Fig. 3).

217 The constant, cyclical, and low-stochasticity treatments exhibited similar patterns of decrease

218 during the initial three weeks, with noticeable differences in survival only becoming evident

219 between days 20 and 30, and at the end of the experiment on day 41. A rapid decline in survival

220 was observed among trout in the constant treatment near the end of the experimental period, such

221 that their proportionate survival (0.75) was similar to that of trout in the high-stochasticity

222 treatment (0.74). At the end of the experimental period, the combination of the cyclical and low-

223 stochasticity treatments was associated with significantly higher survivorship than the

224 combination of the constant and high-stochasticity treatments ($p < 0.01$).

225   At the population level, combining all treatments, survival decreased throughout the

226 experimental period (Fig. 4). The Watern Cove population experienced significantly higher

227 survival than the Ouananiche Beck population between days 27 and 38.

228   During the 30-day, post-experimental period, when all trout experienced the same,

229 constant-temperature conditions, survival continued to decline, particularly for fish in the

230 constant-temperature treatment. The treatments were observed to be distinctly grouped into pairs,

231 with the combination of fish in the cyclical and high-stochasticity treatments experiencing

232 significantly higher survival than the combination of the constant and low-stochasticity

233 treatments ($p=0.019$). During the post-experimental period, Watern Cove trout experienced

234 higher mortality than those from Ouananiche Beck $p=0.003$).

235   At the end of the experimental period, fish were significantly longer in the constant and

236 low-stochasticity treatments when compared to the combination of the cyclical and high-

237 stochasticity treatments ($p=<0.001$; Fig. 5). Comparing populations, Watern Cove trout were

238 significantly smaller than those from Ouananiche Beck ($p=0.032$).

239

240

241 **4. Discussion**

242   The present study examined how cyclical and stochastic thermal variability,

243 independently of changes in average temperature, can affect the growth and survival of a

244 widespread freshwater fish. Brook trout survival early in life was lower under constant

245 temperatures than it was under temperatures that fluctuated with regular cycles or with a

246 relatively low level of stochasticity. There was some indication that survival was higher under

247 regularly cyclical temperatures than under temperature regimes characterized by some level of

248 stochasticity. Fish achieved larger sizes under the constant and low-stochasticity treatments. The

249 common-garden experimental protocol provided an opportunity to compare growth and survival

250 responses to thermal variability between two spatially proximate populations. The data suggest

251 that these thermal responses differ genetically at the population level, a finding consistent with a

252 previous thermal-acclimation study on this species (McDermid et al., 2012).

253   Thermal constancy did not yield higher survival probabilities than temperatures that were

254 temporally variable. These results are similar to those reported for some reptiles. In a laboratory

255 study on painted turtles (*Chrysemys picta*) and red-eared sliders (*Trachemys scripta*), Les et al.

256 (2009) found that egg survival was higher at daily fluctuations ($\pm 3^0$C) around a mean of 23^0

257 when compared to eggs incubated at a constant temperature of 23^0C (the lower limit of viable

258 incubation temperatures for the species). For the northern grass lizard (*Takydromus*

259 *septentrionalis*), Du and Ji (2006) reported that fluctuating temperature treatments produced

260 hatchlings with higher locomotor performance, lower mortality, and relatively large body sizes.

261   These findings raise interesting questions concerning the utility of rearing ectotherms at

262 invariant temperatures (Morash et al., 2018), a practice common among laboratory studies of

263 selection, plasticity, and physiological performance. However, from an ecological or

264   evolutionary perspective, our findings of a relatively poor 'performance', as reflected by

265   survival, in a thermally constant environment are not surprising, given that thermal fluctuations

266   are normal for ectotherms in the wild, as amply illustrated in Fig. 1. If, as seems reasonable to

267   assume, ectotherms are locally adapted to naturally occurring variability in temperature, it might

268   not be surprising that they experience higher survival under conditions of fluctuating, rather than

269   constant, temperatures similar to what they would experience in nature (cf. Dowd et al., 2015).

270   This was the conclusion reached by Niehaus et al. (2012) on striped marsh frogs (*Limnodynastes*

271   *peronii*) who found that empirical models based on temperature constancy poorly predicted the

272   performance of this amphibian under fluctuating temperatures.

273   It can also be argued that expectations of higher performance under constant rather than

274   variable temperatures very much depends on the strength of Jensen's inequality (Jensen, 1909)

275   which stipulates that performance under average conditions is unequal to average performance

276   across a range of conditions (Sinclair et al., 2016; Denny, 2017). Although uncommon in the

277   ecological literature (but see Pickett et al. (2015), Bernhardt et al. (2018), and Morash et al.

278   (2018)), consideration of Jensen's Inequality has been prominent in the physiological literature

279   (Martin and Huey, 2008; Denny, 2017).

280   Jensen's inequality pertains to the degree to which the relationship between individual

281   performance and a variable such as temperature departs from linearity (Fig. 6). Non-linear

282   relationships between metrics of performance such as growth have been documented for many

283   fishes, including brook trout (Farrell, 2009). For the range in temperatures examined here (7^0 to

284   13^0 C), the relationship with growth rate represents a decelerating function (sensu Ruel and

285   Ayres, 1999; Fig. 6). According to this function, constancy at the average temperature in our

286   experiment (~10^0 C) is expected to be associated with faster growth than the average growth

287   across the range of temperatures used in the experiment. Thus, the relationship between growth

288   and thermal variability documented in the present study is consistent with expectations based on

289   Jensen's inequality. We also note, however, that the shape and position of a thermal performance

290   curve is likely plastic and may be affected by acclimation temperature range.

291   Regarding survival, Farrell (2009) reported a slightly convex relationship (similar to that

292   for growth; Fig. 6) between aerobic scope and temperature. However, it is unclear whether

293   aerobic scope reliably reflects survival. It has been reported in juvenile Atlantic salmon (*Salmo*

294   *salar*) that thermal fluctuations are associated with both increased (Beauregard et al., 2013;

295   Oligny-Hébert et al., 2015) and reduced (Morash et al., 2018) metabolic rate, but again it is

296   unclear whether metabolic rate is likely to be positively or negatively associated with survival

297   (Burton et al., 2011).

298   Comparatively few studies have examined the effects of temperature variability on

299   metrics of fitness in fishes independently of changes in mean temperature. We are aware of only

300   three studies on survival, two of which also examined growth rate. In an uncontrolled field study

301   on adult brook trout, Xu et al. (2010) reported positive or dome-shaped relationships between

302   survival and temperature variability (as estimated by the CV, which was equal across our three

303   variable-temperature treatments). In another salmonid, Hokanson et al. (1977) showed that

304   temperature variability influenced the survival (and growth) of rainbow trout (*Oncorhynchus*

305   *mykiss*) positively or negatively depending on whether the mean temperature was higher or lower

306   than 16°C, respectively. Carveth et al.'s (2007) work on the effects of thermal change on a

307   southern Arizona fish (spikedace, *Meda fulgida*), while intriguing, confounded changes in

308   temperature fluctuations with changes in mean temperature.

309   Relative to temperature constancy, slower growth has been reported to be associated with

310   thermal fluctuations in several fishes (Cox and Coutant, 1981; Chadwick and McCormick, 2017;

311   Morash et al., 2018). However, as documented by previous researchers (e.g., Morash et al., 2018;

312   Penney et al., 2018), the influence of thermal fluctuations on developmental, physiological, and

313   life-history traits can be conditional on factors such as population origin (Oligny-Hébert et al.,

314   2015; present study), the degree to which thermal fluctuations occur near T_{opt} (the optimum

315   temperature that maximizes performance; Morash et al., 2018), and other abiotic variables

316   (Penney et al., 2018).

317   Notwithstanding some intriguing results and potential avenues for future research, we

318   caution that the creation of thermally variable conditions can take many forms (e.g., cyclical vs

319   non-cyclical; stochastic vs. non-stochastic; high-amplitude vs. low-amplitude cycles) and there

320   can be logistical challenges in appropriately creating the intended variability. For example, upon

321   examination of the actual temperatures experienced by our experimental fish, it was evident that

322   trout in the cyclical treatment experienced more changes in temperature (on 20 of 40 days) than

323   those in the low-stochasticity treatment (15 of 40 days; for comparison, those in the high-

324   stochasticity treatment experienced a change in temperature on 27 of 40 days). As a result, trout

325   exposed to the low-stochasticity treatment may have experienced a greater degree of temperature

326   consistency than originally anticipated. One example of a logistical difficulty we faced was the

327   challenge in creating the high-stochasticity treatment. In addition to experiencing a higher

328   temporal level of thermal stochasticity, the tanks in which these fish resided were shifted every

329   day rather than every two days. This more frequent change in tank position was necessitated by

330   logistical constraints imposed by the temperature-control system in the laboratory. As a

331   consequence, we are unable to conclude whether the differences between the trout in the high-

332 stochasticity treatment and those in the other treatments were associated with differences in

333 thermal stochasticity, frequency of tank relocation, or both (although every effort was made to

334 shift the tanks as carefully as possible and with minimal movement of water within the tanks).

335 Lastly, there can also be non-trivial challenges in replicating levels of thermal variability in the

336 laboratory that are empirically defensible under natural conditions (although we have strived to

337 do so; cf. Fig. 1).

338

339 **5. Conclusions**

340 The present study represents one of few that has explored the effects of thermal

341 variability on metrics of fitness in an aquatic vertebrate. Within the context of our experimental

342 protocol regarding levels of thermal constancy and variability, our results suggest that: (i)

343 temperature variability at some level can positively influence survival relative to thermal

344 constancy; (ii) growth rate is negatively affected by temperature variability; and (iii) common-

345 garden experiments should incorporate empirically defensible measures of thermal variability as

346 the baseline 'treatment' – rather than temperature constancy – for examining the effects of

347 thermal variability on fitness. Given the challenge in determining the appropriate temporal scale

348 at which thermal variability ought to be examined, perhaps an ideal approach would be to

349 compare the effects of thermal variability at multiple temporal scales on fitness-related traits in a

350 single experiment.

351 We conclude that the influence of stochastic and non-stochastic changes in temperature

352 on individual fitness are not readily predictable (in part because thermal performance curves are

353 not static) and that this field of endeavour warrants considerably more attention than it has

354 received to date. Projections of future population viability in the context of climate change would

be strengthened by increased experimental research on the fitness consequences of stochastic and non-stochastic thermal variability.

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References

Angilletta, M., Steury, T., Sears, M., 2004. Temperature, growth rate, and body size in ectotherms: fitting pieces of a life-history puzzle. Integr. Comp. Biol. 44, 498-509.

Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67, 1-48.

Belmar-Lucero, S., Wood, J.L.A., Scott, S., Harbicht, A.B., Hutchings, J.A., Fraser, D.J., 2012. Concurrent habitat and life history influences on effective/census population size ratios in stream-dwelling trout. Ecol. Evol. 2, 562-573.

Bernhardt, J.R., Sunday, J.M., Thompson, P.L., O'Connor, M.I., 2018. Nonlinear averaging of thermal experience predicts population growth rates in a thermally variable environment. Proc. R. Soc. B. 285, 20181076.

376   Beauregard, D., Enders, E., Boisclair, D., Kidd, K., 2013. Consequences of circadian fluctuations

377   in water temperature on the standard metabolic rate of Atlantic salmon parr (*Salmo*

378   *salar*). Can. J. Fish. Aquat. Sci. 70, 1072-1081.

379   Bozinovic, F., Sabat, P., Rezende, E.L., Canals, M., 2016. Temperature variability and thermal

380   performance in ectotherms: acclimation, behaviour, and experimental considerations.

381   Evol. Ecol. Res. 17, 111-124.

382   Burton, T., Killen, S.S., Armstrong, J.D., Metcalfe, N.B., 2011. What causes intraspecific

383   variation in resting metabolic rate and what are its ecological consequences? Proc. R.

384   Soc. B 278, 3465-3473.

385   Carveth, C.J., Widmer, A.M., Bonar, S.A., Simms, J. R., 2007. An examination of the effects of

386   chronic static and fluctuating temperature on the growth and survival of spikedace, *Meda*

387   *fulgida*, with implications for management. J. Therm. Biol. 32, 102-108.

388   Chadwick, J.G., McCormick, S.D., 2017. Upper thermal limits of growth in brook trout and their

389   relationship to stress physiology. J. Exp. Biol. 220, 3976-3987.

390   Colinet, H., Sinclair, B.J., Vernon, P., Renault, D., 2015. Insects in fluctuating thermal

391   environments. Annu. Rev. Entomol. 60, 123-140.

392   Cox, D.K., Coutant, C.C., 1981. Growth dynamics of juvenile striped bass as functions of

393   temperature and ration. Trans. Am. Fish. Soc. 110, 226-238.

394   Danzmann, R.G., Morgan, R.P., Jones, M.W., Bernatchez, L., Ihssen, P.E., 1998. A major sextet

395   of mitochondrial DNA phylogenetic assemblages extant in eastern North American brook

396   charr (*Salvelinus fontinalis*): distribution and postglacial dispersal patterns. Can. J. Zool.

397   76, 1300-1318.

398   Denny, M., 2017. The fallacy of the average: on the ubiquity, utility and continuing novelty of

399   Jensen's inequality. J. Exp. Biol. 220, 139-146.

400   Dowd, W.W., King, F.A., Denny, M.W., 2015. Thermal variation, thermal extremes and the

401   physiological performance of individuals. J. exp. Biol 218, 1956-1967.

402   Du, W.-G., Ji, X., 2006. Effects of constant and fluctuating temperatures on egg survival and

403   hatchling traits in the northern grass lizard (*Takydromus septentrionalis*, Lacertidae). J.

404   Exp. Zool. 305A, 47-54.

405   Ergon, T., Borgan, Ø., Nater, C., Vindenes, Y., 2018. The utility of mortality hazard rates in

406   population analyses. Meth. Ecol. Evol. 9, 2046-2056.

407   Farrell, A.P., 2009. Environment, Antecedents and climate change: lessons from the study of

408   temperature physiology and river migration of salmonids. J. Exp. Biol. 212, 3771-3780.

409   Feltz, C. J., Miller, G. E., 1996. An asymptotic test for the equality of coefficients of variation

410   from k populations. Stat. Med. 15, 647-658.

411   Goel, M.K., Khanna, P., Kishore, J., 2010. Understanding survival analysis: Kaplan-Meier

412   estimate. Int. J. Ayurveda Res. 2010 1, 274-278.

413   Hanson, J., Sato, M., Ruedy, R., 2012. Perception of climate change, Proc. Nat. Acad. Sci. USA

414   109, 14726-14727.

415   Hokanson, K.E.F., Kleiner, C.F., Thorslund, T.W., 1977. Effects of constant temperatures and

416   diel temperature fluctuations on specific growth and mortality rates and yield of juvenile

417   rainbow trout *Salmo gairdneri*. J. Fish. Res. Board Can. 34, 639-648.

418   Hutchings, J.A., 1993. Adaptive life histories effected by age-specific survival and growth rate.

419   Ecology 74, 673-684.

420   Hutchings, J.A., 1996. Adaptive phenotypic plasticity in brook trout, *Salvelinus fontinalis*, life

421   histories. Ecoscience 3, 25-32.

422   Jensen, J.L.W.V., 1909. Sur les fonctions convexes et les inégalités entre les valeurs moyenne.

423   Acta mathematica 30, 175-193.

424   Jensen, L.F., Hansen, M.M., Pertoldi, C., Holdensgaard, G., Mensberg, K.D., Loeschcke, V.,

425   2008. Local adaptation in brown trout early life-history traits: implications for climate

426   change adaptability. Proc. Roy. Soc. Lond. B 275, 2859-2868.

427   Kingsolver, J.G., Ragland, G.J., Diamond, S.E., 2009. Evolution in a constant environment:

428   thermal fluctuations and thermal sensitivity of laboratory and field populations of

429   *Manduca sexta*. Evolution 63, 537-541.

430   Les, H.L., Paitz, R.T., Bowden, R.M., 2009. Living at extremes: development at the edges of

431   viable temperature under constant and fluctuating conditions. Physiol. Biochem. Zool.:

432   Ecol. Evol. Approaches 82, 105–112.

433   Martin, T.L., Huey, R.B., 2008. Why "suboptimal" is optimal: Jensen's inequality and ectotherm

434   thermal preferences. Am. Nat. 171, E102-E118.

435   Marwick, B., Krishnamoorthy, K., 2019. cvequality: Tests for the Equality of Coefficients of

436   Variation from Multiple Groups. R software package version 0.1.3. Retrieved from

437   https://github.com/benmarwick/cvequality, on 18/02/2019.

438   McDermid, J.L., Fischer, F.A., Al-Shamlih, M., Sloan, W.N., Jones, N.E., Wilson, C.C., 2012.

439   Variation in acute thermal tolerance within and among hatchery strains of brook trout.

440   Trans. Am. Fish. Soc. 141, 1230-1235.

441   Morash, A.J., Neufeld, C., MacCormack, T.J., Currie, S., 2018. The importance of incorporating

442   natural thermal variation in when evaluating physiological performance in wild species.

443   J. Exp. Biol. 221, jeb164673. doi:10.1242/jeb.164673

444   Niehaus, A.C., Angilletta, M.J., Sears, M.W., Franklin, C.E., Wilson, R.S., 2012. Predicting the

445   physiological performance of ectotherms in fluctuating thermal environments. J. Exp.

446   Biol. 251, 694-701.

447   Oligny-Hébert, H., Senay, C., Enders, E.C., Boisclair, D., 2015. Effects of diel temperature

448   fluctuation on the standard metabolic rate of juvenile Atlantic salmon (*Salmo salar*):

449   influence of acclimation temperature and provenience. Can. J. Fish. Aquat. Sci. 72, 1306-

450   1315.

451   Penney, H.D., Beirão, J., Purchase, C.F., 2018. Phenotypic plasticity during external embryonic

452   development is affected more by maternal effects than multiple abiotic factors in brook

453   trout. Evol. Ecol. Res. 19, 171-194.

454   Pickett, E.J., Thomson, D.L., Li, T.A., Xing, S., 2015. Jensen's inequality and the impact of

455   short-term environmental variability on long-term population growth rates. PLoS ONE

456   10, e0136072. https://doi.org/10.1371/journal.pone.0136072.

457   Pörtner, H.O., Farrell, A.P., 2008. Physiology and climate change. Science 322, 690-692.

458   R Core Team, 2016. R: A language and environment for statistical computing. Vienna (AT): R

459   Foundation for Statistical Computing; [accessed 29-09-2018]. https://www.r-project.org.

460   Rieman, B.E., Isaak, D.J., Adams, S., Horan, D., Nagel, D., Luce, C., Myers. D., 2007.

461   Anticipated climate warming effects on bull trout habitats and populations across the

462   Interior Columbia River Basin. Trans. Am. Fish. Soc. 136, 1552-1565.

463   Ruel, J.J., Ayres, M.P., 1999. Jensen's inequality predicts effects of environmental variation.

464   Trends Ecol. Evol. 14, 361-366.

465   Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image

466   analysis. Nat. Methods 9, 671-675.

467   Sinclair, B.J., Marshall, K.E., Sewell, M.A., Levesque, D.L., Willett, C.S., Slotsbo, S., Dong, Y.,

468   Harley, C.D.G., Marshall, D.J., Helmuth, B.S., Huey, R.B., 2016. Can we predict

469   ectotherm responses to climate change using thermal performance curves and body

470   temperatures? Ecol. Lett. 19, 1372-1385.

471   Therneau, T.M., Grambsch, P.M., 2000. Modelling Survival Data: Extending the Cox Model.

472   Springer, New York.

473   Vasseur, D.A., DeLong, J.P., Gilbert, B., Greig, H.S., Harlet, C.D., McCann, K.S., Savagae, V.,

474   Tunney, T.D., O'Connor, M.I., 2014. Increased temperature variation poses a greater risk

475   to species that climate warming. Proc. Roy. Soc. B 281, 20132612. doi:

476   10.1098/rspb.2013.2612.

477   Wang, G., Dillon, M.E., 2014. Recent geographic convergence in diurnal and annual temperature

478   cycling patterns flattens global thermal profiles. Nature Climate Change 4, 988-992.

479   Wenger, S.J., Isaak, D.J., Dunham, J.B., Fausch, K.D., Luce, C.H., Neville, H.M., Rieman, B.E.,

480   Young, M.K., Nagel, D.E., Horan, D.L. et al., 2011a. Role of climate and invasive

481   species in structuring trout distributions in the Interior Columbia Basin, USA. Can. J.

482   Fish. Aquat. Sci. 68, 988-1008.

483   Wenger, S.J., Isaak, D.J., Luce, C.H., Neville, H.M., Fausch, K.D., Dunham, J.D., Dauwalter,

484   D.C., Young, M.K., Elsner, M.M., Rieman, B.E. et al., 2011b. Flow regime, temperature,

485   and biotic interactions drive differential declines of trout species under climate change.

486   Proc. Natl. Acad. Sci. USA 108, 14175-14180.

487   Williams, C.M., Marshall, K.E., MacMillan, H.A., Dzurisin, J.D.K., Hellmann, J.J., Sinclair,

488   B.J., 2012. Thermal variability increases the impact of autumnal warming and drives

489   metabolic depression in an overwintering butterfly. PLoS ONE 7, e34470.

490   doi:10.1371/journal.pone.0034470.

491   Wood, J.L.A., Belmar-Lucero, S., Hutchings, J.A., Fraser, D.J., 2014. Relationship of habitat

492   variability to population size in a stream fish. Ecol. Appl. 24, 1085-1100.

493   Xu, C.L., Letcher, B.H., Nislow, K.H., 2010. Size-dependent survival of brook trout *Salvelinus*

494   *fontinalis* in summer: effects of water temperature and stream flow. J. Fish Biol. 76,

495   2342-2369.

496

497 Table 1. Water temperature data (mean; standard deviation, σ), based on hourly measurements

498 recorded in 2010, for four rivers on Cape Race, Newfoundland. Proposed experimental dates: 16

499 May to 15 June; actual experimental dates: 26 April to 5 June.

500

501 502 503 Population	Temperatures during proposed experimental dates		Temperatures during actual experimental dates	
504	mean	σ	mean	σ
505 Bristol Cove River	9.85	2.42	8.30	2.25
506 Cape Race River	10.50	2.29	8.67	2.46
507 Cripple Cove River	9.51	2.64	8.81	2.68
508 Whale Cove River	8.51	2.27	7.34	2.40
509				
510 Pooled data	9.59	2.41	8.28	2.40

511

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515   Figure 1. Water temperature recorded hourly for one year in four rivers on Cape Race,

516   Newfoundland, Canada.

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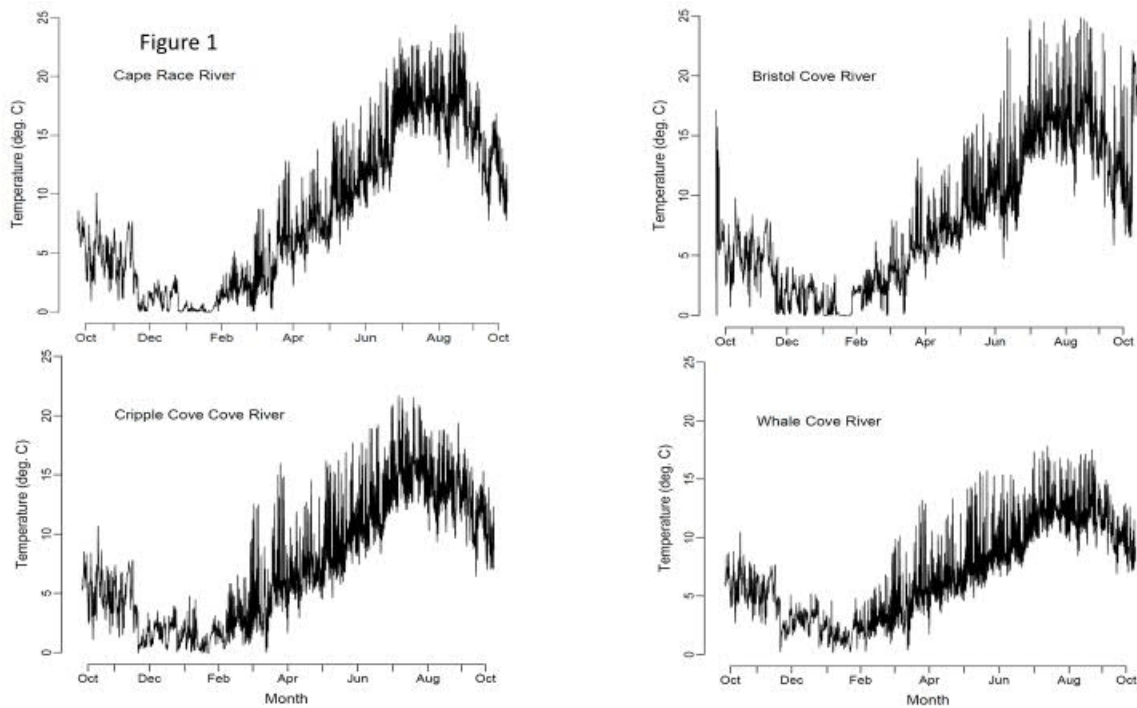
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521   Figure 2. Nominal (intended) daily experimental temperatures under the (a) constant, (b)

522   cyclical, (c) low-stochasticity, and (d) high-stochasticity experimental treatments, and the

523   actual temperatures experienced by brook trout for the (d) constant, (e) cyclical, (f) low-

524   stochasticity, and (g) high-stochasticity treatments during days 11 through 41 of the
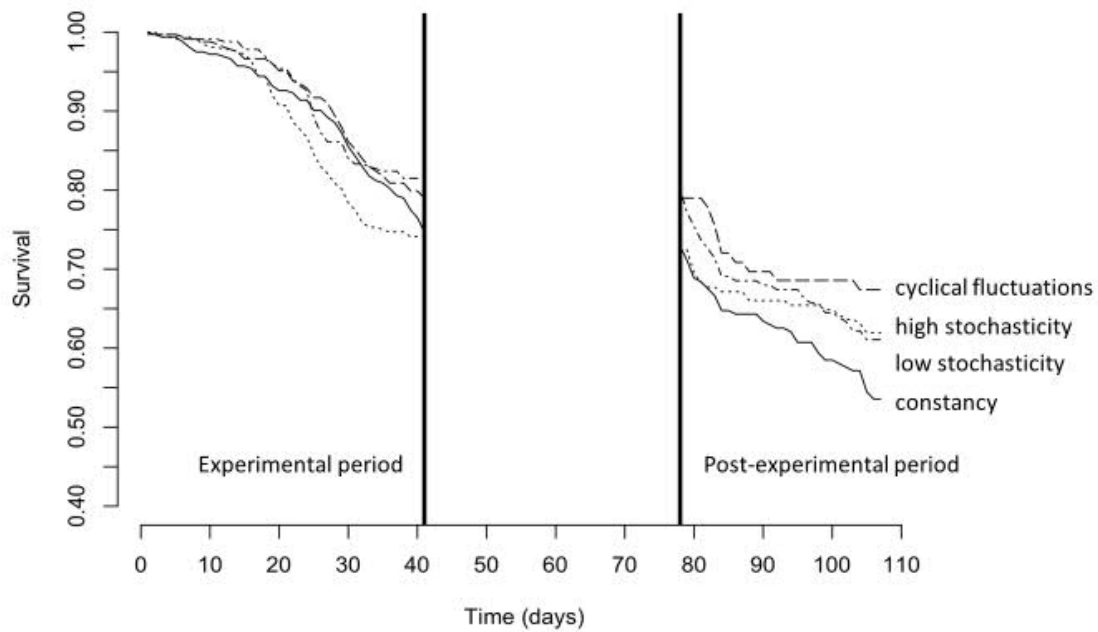
525   experimental period.

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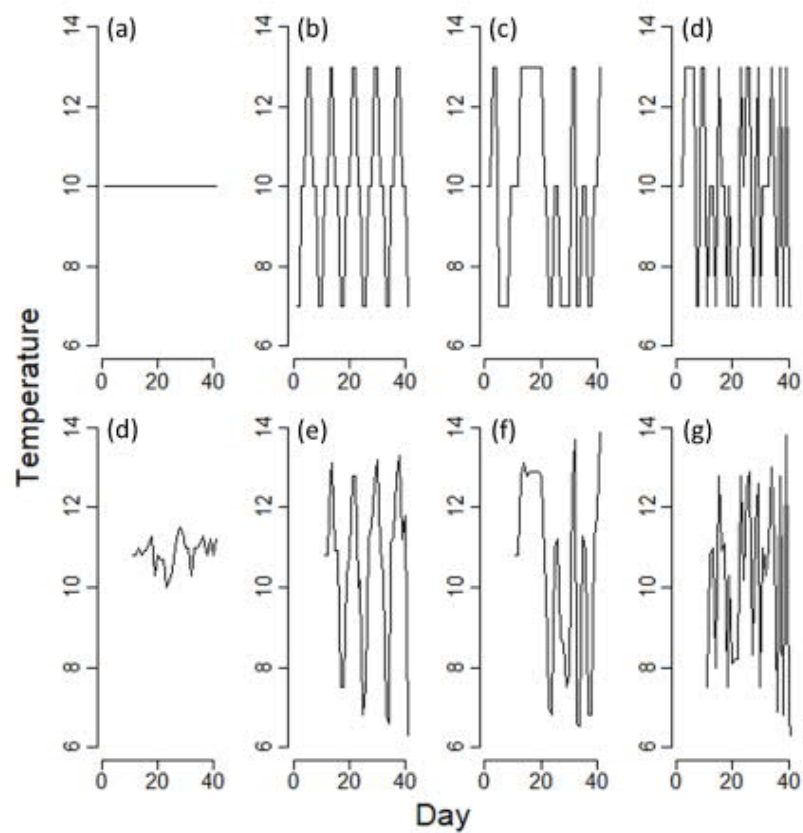
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530   Figure 3. Kaplan-Meier survival patterns for brook trout subjected to four thermal variability

531   treatments during and after the experimental period.
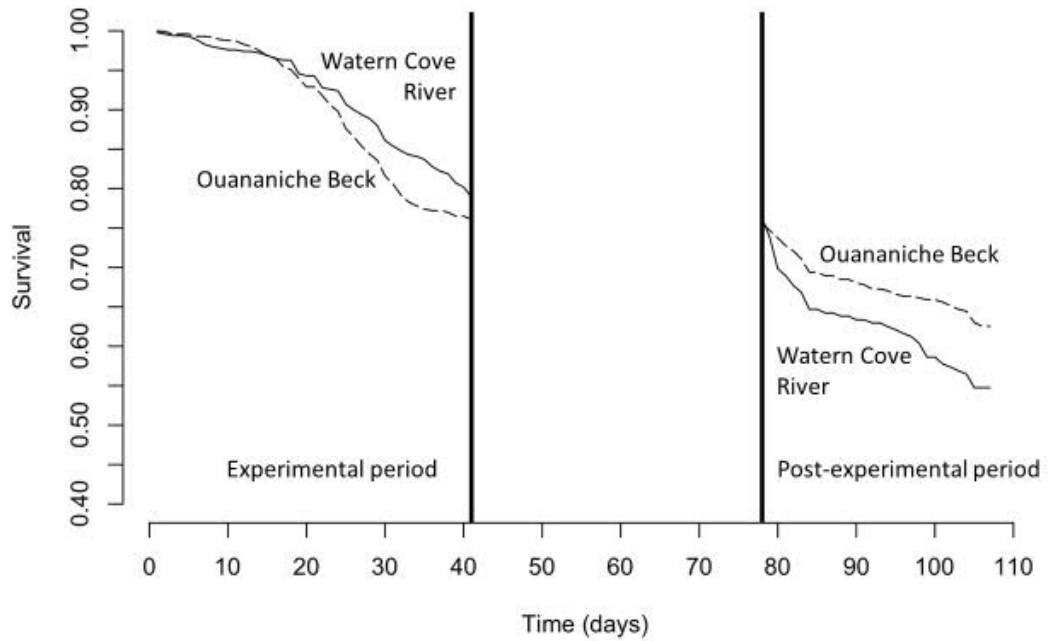
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Figure 3

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536   Figure 4. Kaplan-Meier survival patterns for two populations of brook trout (Ouananiche Beck,

537   Watern Cove River) subjected to four thermal variability treatments during and after the

538   experimental period (data pooled within populations).
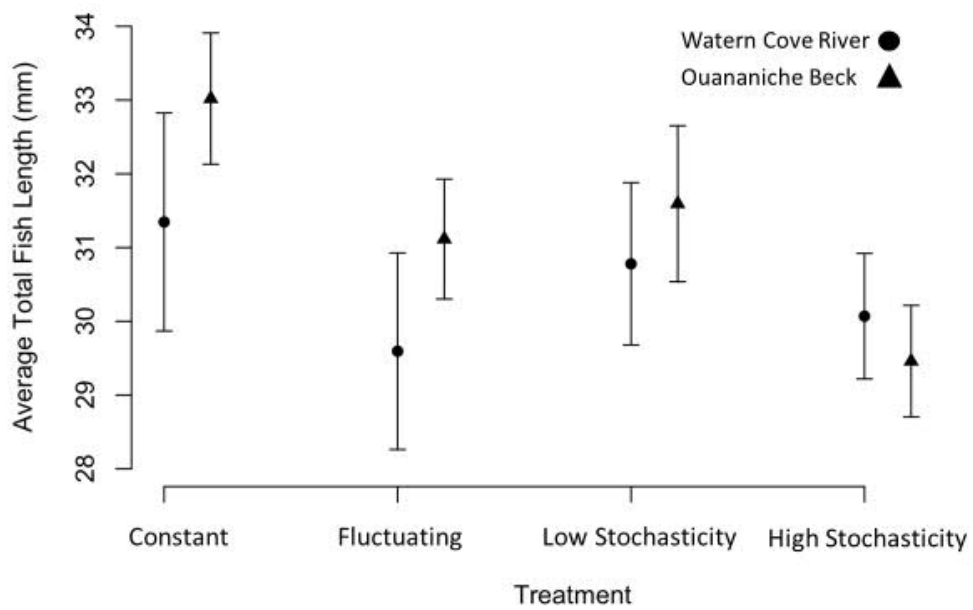

Figure 4

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541   Figure 5. Average total length (mm; ± 95% CI) of brook trout from two populations (Watern

542   Cove River, Ouananiche Beck) subjected to four thermal variability treatments, at the

543   termination of the experimental period.
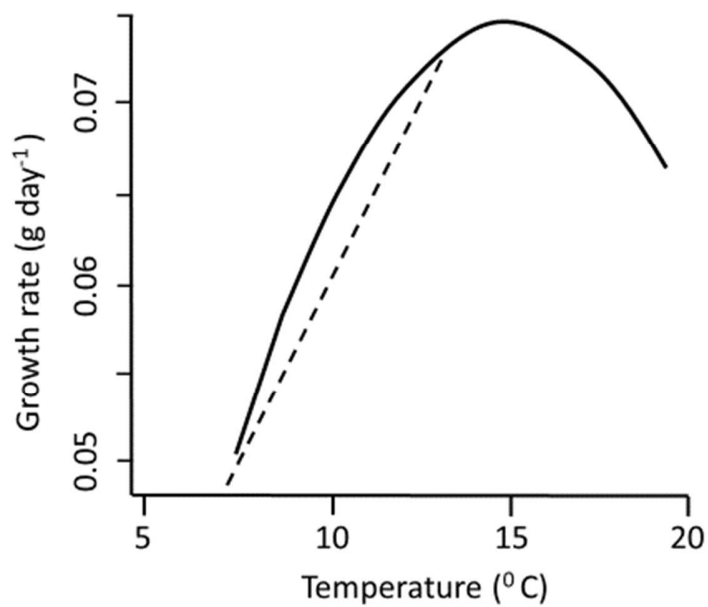
Figure 5

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547   Figure 6. Curvilinear relationship between water temperature and growth rate (solid line) in

548   brook trout, as reported by Farrell (2009). For temperatures between 7^0 and 13^0C, the

549   average growth rate (dashed line) is less than the growth rate at the average temperature

550   of 10^0C (solid line).

Figure 6

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