

This is a self-archived version of an original article. This version may differ from the original in pagination and typographic details.

Author(s): Pisano, Olivia M.; Kuparinen, Anna; Hutchings, Jeffrey A.

Title: Cyclical and stochastic thermal variability affects survival and growth in brook trout

Year: 2019

Version: Accepted version (Final draft)

Copyright: © 2019 Elsevier Ltd.

Rights: CC BY-NC-ND 4.0

Rights url: <https://creativecommons.org/licenses/by-nc-nd/4.0/>

Please cite the original version:

Pisano, O. M., Kuparinen, A., & Hutchings, J. A. (2019). Cyclical and stochastic thermal variability affects survival and growth in brook trout. *Journal of Thermal Biology*, 84, 221-227.

<https://doi.org/10.1016/j.jtherbio.2019.07.012>

1 Cyclical and Stochastic Thermal Variability
2 Affects Survival and Growth in Brook Trout

3
4 Olivia M. Pisano ^a

5 Anna Kuparinen ^b

6 Jeffrey A. Hutchings ^{a, c}

7
8 ^a Department of Biology, Dalhousie University, 1355 Oxford Street, Halifax, NS B3H4R2,
9 Canada; email: oliviapisano@icloud.com

10
11 ^b Dept Biological and Environmental Science, University of Jyväskylä, PO Box 35, FI-40014
12 Jyväskylä, Finland; anna.k.kuparinen@jyu.fi

13
14 ^c Institute of Marine Research, Flødevigen Marine Research Station, N-4817 His, Norway;
15 jhutch@dal.ca

16
17 Corresponding author: Jeffrey A. Hutchings, Department of Biology, Dalhousie University, 1355
18 Oxford Street, Halifax, NS B3H4R2, Canada; jhutch@dal.ca; tel: +1 902 494 2687

19
20 **Keywords:** temperature; common-garden; fluctuation; constancy; fitness; stochasticity

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: 10⁰ C; range: 7-13⁰ 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.

37 **1. Introduction**

38 Temperature affects ectotherm physiology (Angilletta et al., 2004; Pörtner and Farrell,
39 2008; Farrell, 2009) with consequences for individual fitness and population viability,
40 particularly under forecasted changes in climate (Rieman et al., 2007; Wenger et al., 2011a,b). In
41 addition to directional shifts, increased thermal variability is predicted to be a concomitant of
42 climate change (Hanson et al., 2012; Wang and Dillon, 2014). However, considerably less
43 attention has been directed to how variability in temperature affects fitness-related traits
44 independently of changes to the mean (Vasseur et al., 2014; Dowd et al., 2015). This represents
45 an important knowledge gap, given that thermal variability can represent a central determinant of
46 ectotherm responses to environmental change (Colinet et al., 2015; Sinclair et al., 2016).

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

57 Predicted responses to thermal fluctuations will also depend on the degree to which the
58 temperature variations encompass the thermal optimum for the species, or population, under
59 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 $9.59 \pm 2.41^\circ\text{C}$. 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 $\pm 3^\circ$ 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⁻¹
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^o and 17^o 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 ($CV = \sigma/\text{mean}$) 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 ($\pm \sigma$) did not differ among
212 treatments (constant: $10.89 \pm 0.35^{\circ}\text{C}$; cyclical: $10.53 \pm 2.16^{\circ}\text{C}$; low-stochasticity: $10.52 \pm$
213 2.47°C ; high-stochasticity: $10.08 \pm 2.29^{\circ}\text{C}$; $F_{[1,122]} = 2.649$; $p=0.106$). 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 ($\pm 3^{\circ}\text{C}$) around a mean of 23°
257 when compared to eggs incubated at a constant temperature of 23°C (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 (7⁰ to
284 13⁰ 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 (~10⁰ 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

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

357

358 **Acknowledgements**

359 We are grateful to Dylan Fraser for securing the eggs for our use, Jim Eddington for fish-
360 husbandry logistics, and Melanie Massey and Bror Jonsson for discussion. We thank four
361 anonymous referees for their very helpful comments and criticism. Funding: This work was
362 supported by the Natural Sciences and Engineering Research Council of Canada (170146-2013);
363 the Academy of Finland; and the European Research Council (COMPLEX-FISH 400820).

364

365 **References**

- 366 Angilletta, M., Steury, T., Sears, M., 2004. Temperature, growth rate, and body size in
367 ectotherms: fitting pieces of a life-history puzzle. *Integr. Comp. Biol.* 44, 498-509.
- 368 Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using
369 lme4. *J. Stat. Softw.* 67, 1-48.
- 370 Belmar-Lucero, S., Wood, J.L.A., Scott, S., Harbicht, A.B., Hutchings, J.A., Fraser, D.J., 2012.
371 Concurrent habitat and life history influences on effective/census population size ratios in
372 stream-dwelling trout. *Ecol. Evol.* 2, 562-573.
- 373 Bernhardt, J.R., Sunday, J.M., Thompson, P.L., O'Connor, M.I., 2018. Nonlinear averaging of
374 thermal experience predicts population growth rates in a thermally variable environment.
375 *Proc. R. Soc. B.* 285, 20181076.

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

511

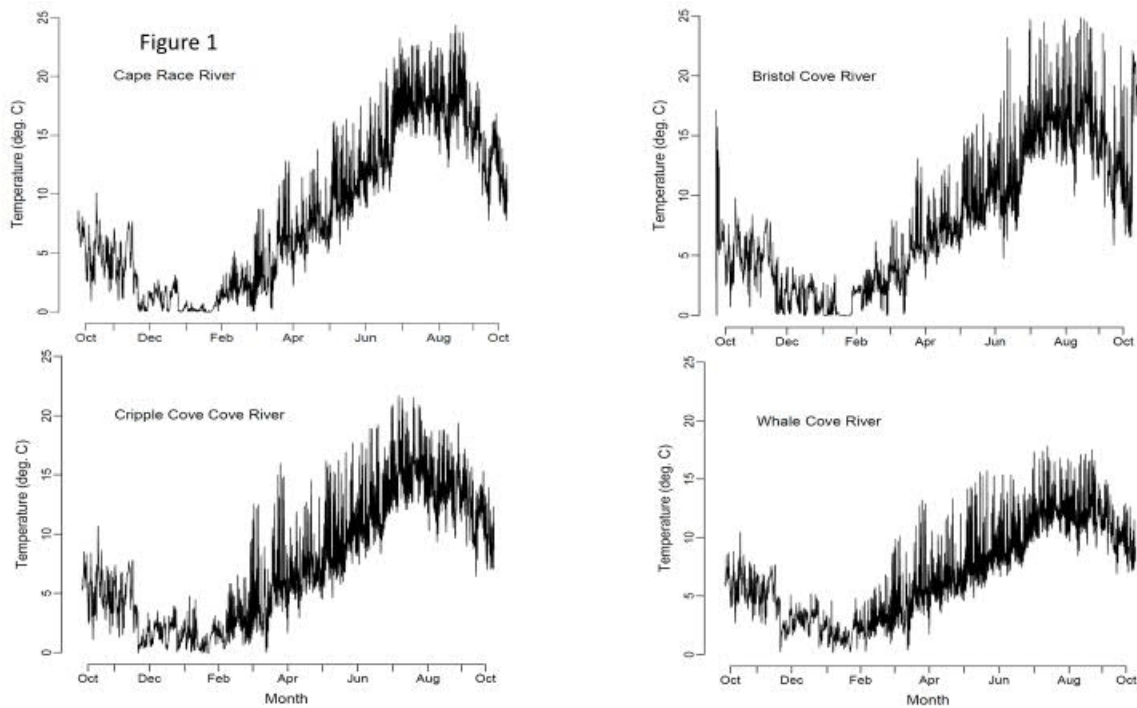
512

513

514

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

517



518

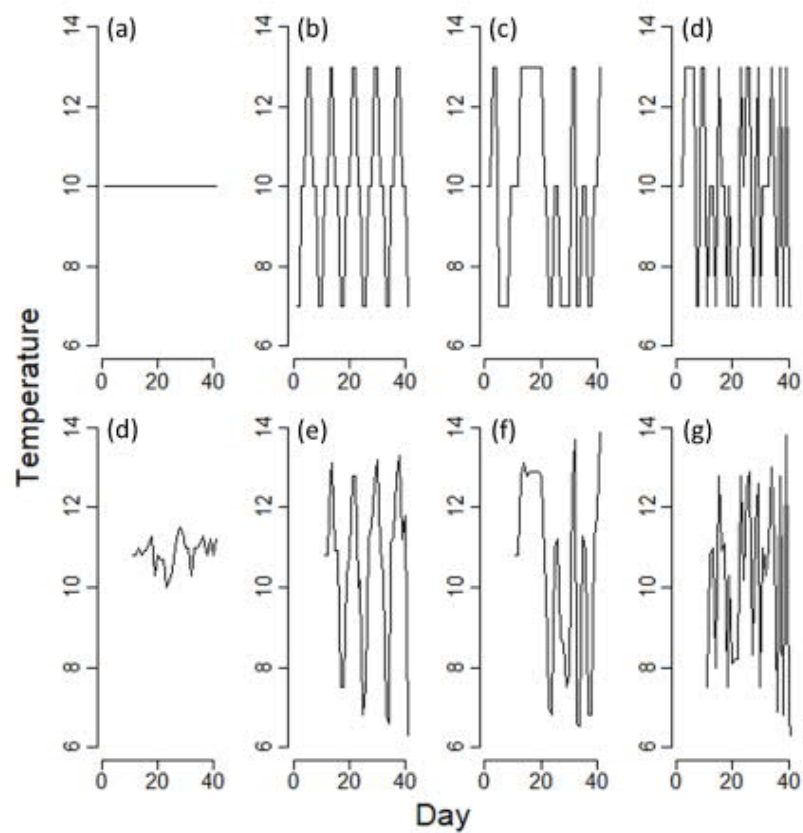
519

520

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.

526

Figure 2



527

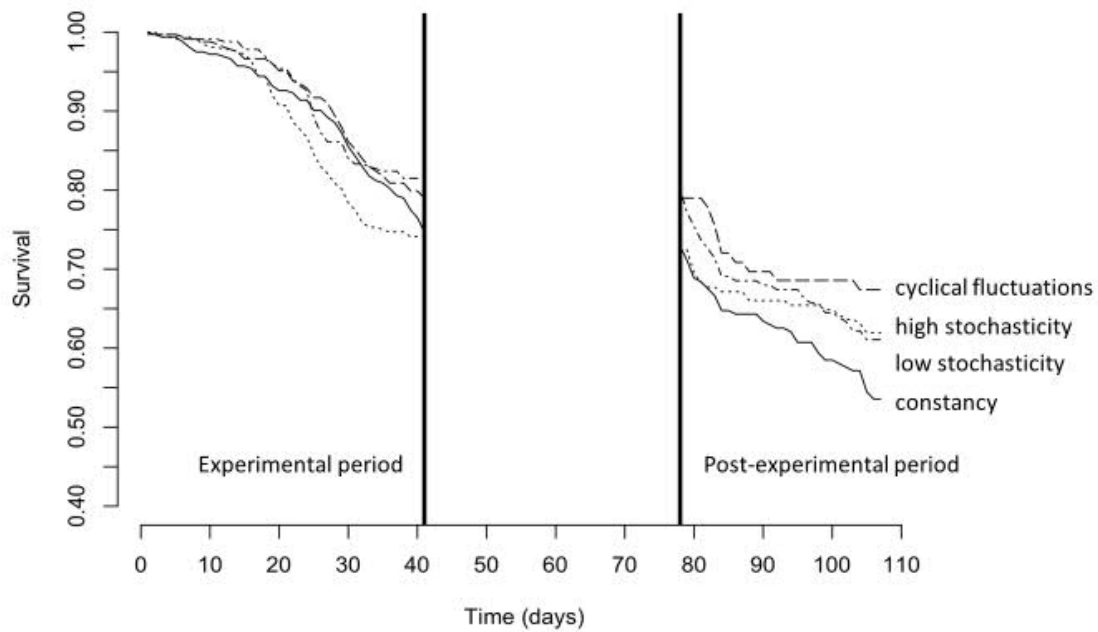
528

529

530 Figure 3. Kaplan-Meier survival patterns for brook trout subjected to four thermal variability
531 treatments during and after the experimental period.

532

Figure 3



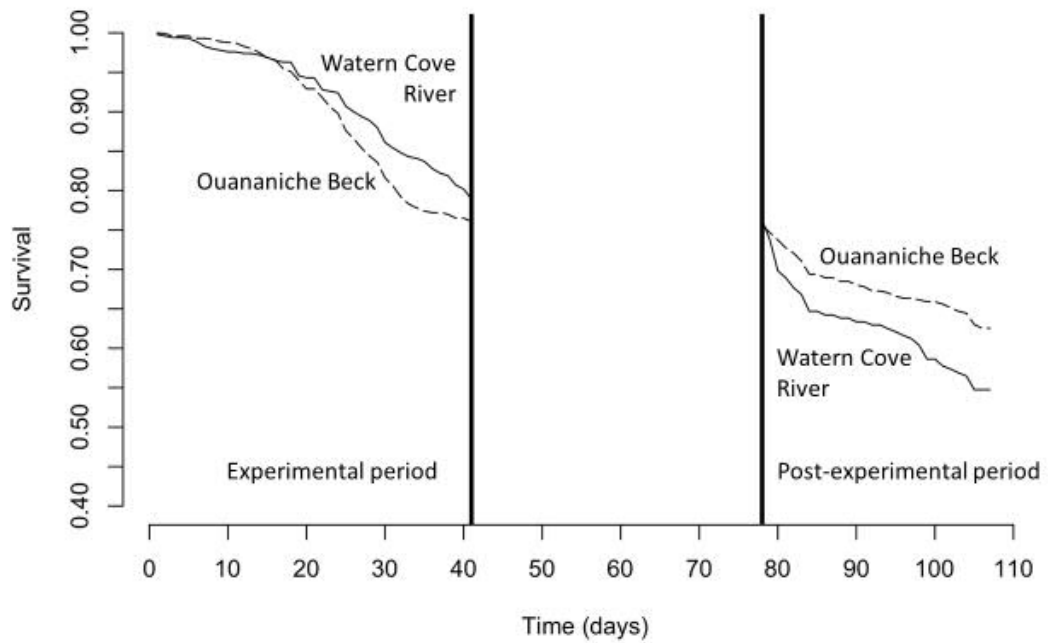
533

534

535

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

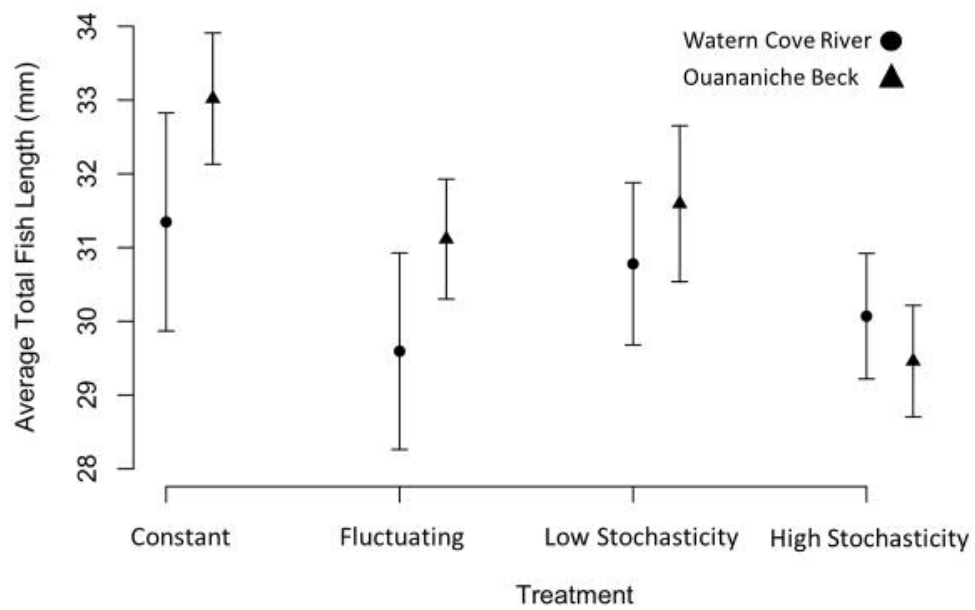


539

540

541 Figure 5. Average total length (mm; \pm 95% CI) of brook trout from two populations (Water
542 Cove River, Ouananiche Beck) subjected to four thermal variability treatments, at the
543 termination of the experimental period.

Figure 5



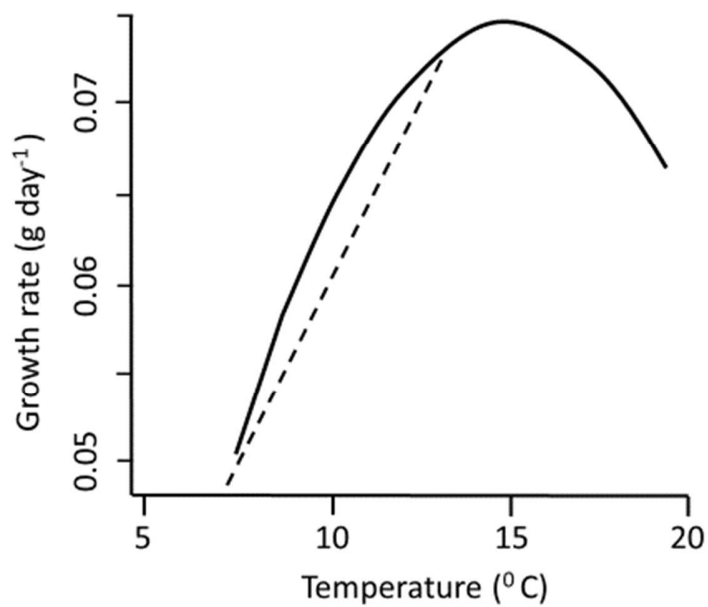
544

545

546

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^o and 13^oC, the
549 average growth rate (dashed line) is less than the growth rate at the average temperature
550 of 10^oC (solid line).

Figure 6



551