

1 **Reduction in standard metabolic rate after multigenerational exposure to**
2 **elevated temperatures in the wild**

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18

19 **Abstract**

20 In light of global climate change, there is a pressing need to understand and predict the
21 capacity of populations to respond to rising temperatures. Metabolic rate is a key trait that is
22 likely to influence the ability to cope with climate change. Yet, empirical and theoretical
23 work on metabolic rate responses to temperature changes has so far produced mixed results
24 and conflicting predictions. Our study addresses this issue using a novel approach of
25 comparing fish populations in geothermally warmed lakes and adjacent ambient-
26 temperature lakes in Iceland. This unique ‘natural experiment’ provides repeated and
27 independent examples of populations experiencing contrasting thermal environments for
28 many generations over a small geographic scale, thereby avoiding the confounding factors
29 associated with latitudinal or elevational comparisons. Using Icelandic sticklebacks from
30 three warm and three cold habitats, we measured individual metabolic rates across a range
31 of acclimation temperatures to obtain reaction norms for each population. We found a
32 general pattern for a lower standard metabolic rate in sticklebacks from warm habitats, as
33 predicted by Krogh’s rule. Metabolic rate differences between warm- and cold-habitat
34 sticklebacks were more pronounced at more extreme acclimation temperatures, suggesting
35 the release of cryptic genetic variation upon exposure to novel conditions, which can reveal
36 hidden evolutionary potential. Lastly, we found a stronger divergence in metabolic rate
37 between thermal habitats in allopatry than sympatry, indicating that gene flow may
38 constrain physiological adaptation when dispersal between warm and cold habitats is
39 possible. In sum, our study suggests that fish may diverge toward a lower standard
40 metabolic rate in a warming world, but this might depend on connectivity and gene flow
41 between different thermal habitats.

42 **Introduction**

43 Climate change poses a substantial threat to biodiversity, as rising temperatures are altering
44 abiotic and biotic environmental conditions and imposing novel selection pressures on
45 organisms (Crozier & Hutchings 2014). In light of this, there is now a pressing need to
46 understand the capacity of populations to respond and adapt to increasing temperatures.
47 This knowledge is critical for our ability to predict the impacts of climate change and to
48 inform management and conservation efforts (Donelson et al. 2011, Sinclair et al. 2016,
49 Campbell et al. 2017). Ectothermic animals are particularly vulnerable to changes in
50 ambient temperature, because this directly influences their body temperature (Zuo et al.
51 2012). Ectotherms are therefore expected to adapt to climate change through plastic and/or
52 evolutionary changes in a wide range of physiological, morphological, and behavioural
53 traits (Crozier & Hutchings 2014).

54 A key trait that is likely to influence the capacity of ectothermic animals to cope with
55 increasing temperatures is metabolic rate (Donelson et al. 2012). Standard and maximum
56 metabolic rates are among the most commonly measured physiological traits and can have
57 important fitness implications for organisms (White and Kearney 2013, Pettersen et al.
58 2018). Standard metabolic rate (SMR) refers to the minimum rate of energy throughput
59 needed to sustain an animal at rest, whereas maximum metabolic rate (MMR) sets the
60 upper limit for the capacity to perform oxygen-consuming physiological activities (Killen et
61 al. 2016). Another important physiological variable is absolute aerobic scope (AS), which is
62 calculated as the difference between an individual's SMR and MMR. AS represents an
63 individual's total capacity for simultaneous oxygen-consuming tasks above maintenance
64 requirements (e.g., digestion, activity, growth; Pörtner & Farrell 2008). In ectotherms, AS

65 may increase with temperature up to an optimum, after which it declines with further
66 temperature increases. AS has therefore been suggested to play a key role in the response of
67 fish populations to changing temperatures (Farrell 2016, Sandblom et al. 2016). Notably,
68 however, many fish species appear to show little or no change in AS with changes in
69 temperature (Lefevre 2016, Nati et al. 2016, Jutfelt et al. 2018), so the degree to which AS
70 limits adaptive responses to warming remains an open question.

71 It is also still unclear how SMR and MMR will evolve in response to climate change.
72 According to Krogh's rule (also known as metabolic cold adaptation), ectotherms living in
73 cold environments should have higher metabolic rates than those in warm environments,
74 when the two are observed at the same temperature (Krogh 1916, Gaston et al. 2009). This
75 view is in line with the concept of countergradient variation, which is a result of stabilising
76 selection favouring similar phenotypes in different environments (Marcil et al. 2006) and
77 can arise when genetic differences in a trait counteract environmental effects (Conover &
78 Schultz 1995). An alternative hypothesis is that natural selection will favour a reduction in
79 metabolic rate in animals living at low temperatures if the energetic cost of cellular
80 respiration exceeds the benefit of additional ATP production (Clarke 1980, Clarke 1991,
81 Clark 1993, Chown and Gaston 1999). These opposing predictions have led to much
82 controversy and debate about the effects of temperature changes on metabolic rate
83 adaptation (Huey and Berrigan 1996, Chown and Gaston 1999, Addo-Bediako et al. 2002).

84 Until now, empirical studies aiming to address this issue have focused on interspecific
85 and intraspecific variation in metabolic rate across contrasting thermal habitats, such as
86 populations from different latitudes or elevations (e.g., Tsuji 1988, White et al. 2012,
87 Gaitán-Espitia and Nespolo 2014). However, there are many confounding factors associated

88 with these comparisons. For example, high-latitude habitats are not only colder on average
89 but also experience more extreme temporal fluctuations in temperature and photoperiod
90 than low-latitude habitats. These confounding factors may partly explain the mixed results
91 of previous research on the relationship between thermal environment and metabolic rate
92 (Clarke 2003, White and Kearney 2013, Alton et al. 2017).

93 Here, we use a novel approach by comparing Icelandic populations of threespine
94 sticklebacks (*Gasterosteus aculeatus*) found in geothermally warmed lakes and adjacent
95 ambient-temperature lakes. This unique ‘natural experiment’ provides repeated and
96 independent examples of populations experiencing contrasting thermal environments for
97 many generations over a small geographic scale, thereby avoiding the confounding factors
98 associated with latitudinal or elevational comparisons. In addition, there are no substantial
99 differences in water chemistry between geothermally warmed and ambient-temperature
100 lakes, allowing us to isolate the effects of temperature. We have previously shown that there
101 is strong morphological divergence between sticklebacks from these warm and cold habitats,
102 suggesting local adaptation (Pilakouta et al. 2019).

103 Using sticklebacks from three warm and three cold habitats, we measured individual
104 metabolic rates across a range of acclimation temperatures (10°C, 15°C, and 20°C) to obtain
105 reaction norms for each population. We examined whether living in a warm environment
106 over several generations results in a suppressed metabolic rate, providing a powerful test of
107 the controversial Krogh’s rule. If metabolic rate responds in a similar way to thermal habitat
108 across multiple populations, this would suggest that metabolic rate evolution in response to
109 climate change may be predictable (Bolnick et al. 2018). We also investigated whether the
110 steepness of metabolic rate reaction norms differs between populations from warm vs cold

111 environments. Given the potential importance of physiological adaptation for population
112 persistence (Donelson et al. 2011, Sinclair et al. 2016), our study could provide valuable
113 insights into the capacity of ectotherms to cope with climate change.

114

115 **Methods**

116 *Study animals*

117 Using unbaited minnow traps, we collected adult threespine sticklebacks from six freshwater
118 populations in Iceland in May–June 2016 (Table 1). Two of these populations were
119 allopatric, meaning that the warm and cold habitats were in neighbouring but separate
120 water bodies with no potential for gene flow (Figure 1). We also sampled two sympatric
121 warm-cold population pairs, where the warm and cold habitats were in the same water
122 body, with no physical barriers between them (Figure 1).

123 The cold habitats have all existed for thousands of years, since the last glacial period
124 (Einarsson et al. 2004), but there is some variation in the age of the warm habitats (Table 1).
125 The ‘Mývatn warm’ and Grettislaug sites have been naturally heated by geothermal activity
126 for over 2000 years (Hight 1965, Einarsson 1982). In contrast, the ‘Áshildarholtsvatn warm’
127 habitat originated only 50–70 years ago, fed by excess hot water runoff from nearby
128 residences using geothermal heating.

129

130 *Animal husbandry*

131 After being transported to the University of Glasgow, these fish were kept at densities of 10-
132 15 individuals per 10-litre tank in a common recirculation system at 15°C. The tanks
133 contained plastic plants as shelter. Fish were fed *ad libitum* twice a day with a mixture of

134 frozen bloodworms, *Mysis* shrimp, and *Daphnia*. Two months before the experiments, all
135 fish were anaesthetised using benzocaine and marked with visible implant elastomer tags
136 (Northwest Marine Technology Inc) to allow individual identification. They were kept at a
137 12h light:12h dark photoperiod throughout the experiment.

138 Fish were acclimated to 10°C, 15°C, or 20°C for at least one month before measuring
139 their metabolic rate. Different individuals were used at each of these temperatures. The
140 intermediate temperature (15°C) is close to the maximum temperature experienced by fish
141 in cold habitats in the summer and the minimum temperature experienced by fish in warm
142 habitats in the winter (Table 1). The lowest temperature in this range (10°C) is not generally
143 experienced by warm-habitat fish in the wild, and the highest temperature in this range
144 (20°C) is not experienced by cold-habitat fish (Table 1). Exposing fish to these unfamiliar
145 temperatures allowed us to examine the release of cryptic genetic variation in metabolic rate
146 and thus hidden evolutionary potential to respond to thermal changes (Paaby & Rockman
147 2014, Shama 2017).

148

149 *Metabolic rate measurements*

150 We used intermittent flow-through respirometry to estimate individual metabolic rates by
151 measuring oxygen uptake. Sixteen cylindrical, glass respirometry chambers (83 ml) were
152 submerged in a 93-L experimental tank (780 mm × 570 mm × 210 mm) containing air-
153 saturated water. The water temperature within the experimental tank was maintained at
154 10°C, 15°C, or 20°C depending on the treatment. This was done using a thermostated
155 reservoir connected to the experimental tank by a thermoregulator (TMP-REG system,
156 Loligo Systems, Denmark), which allowed us to maintain the water temperature within

157 0.2°C of our target temperature for the entire 24-h trial period. To maintain good water
158 mixing and avoid an oxygen gradient in the respirometry chambers, we used a peristaltic
159 pump (Masterflex, Cole-Parmer), which moved water through the chambers and around an
160 external circuit of gas-impermeable tubing (Masterflex, Cole-Parmer). Oxygen
161 concentration in the chambers was measured every two seconds using four Firesting
162 channel oxygen meters with sixteen associated sensors (PyroScience GmbH, Aachen,
163 Germany). To account for bacterial respiration during the trials, background bacterial
164 oxygen consumption was measured before and after each trial in the 16 respirometry
165 chambers (see *Data analysis*). A UV filter connected to the experimental tank was also used
166 to sterilise the water and minimise bacterial respiration.

167 We fasted fish for 48 h before the trials because metabolic rate increases during
168 digestion (Killen 2014). Trials started around 14:00 each day. Immediately before being
169 placed into a respirometry chamber, each fish was subjected to exhaustive exercise by being
170 chased in a circular tank; this allowed us to measure their maximum metabolic capacity
171 (Killen et al. 2012, Clark et al. 2013, Killen et al. 2017). After complete exhaustion, which
172 always occurred within 2–3 min of chasing, fish were placed into individual respirometers.
173 Rates of oxygen uptake were then measured in 3-min intervals over a 15-min period, during
174 which the respirometers were sealed and the decrease in oxygen content was used to
175 calculate rate of oxygen uptake (see *Data analysis*). The maximum rate of oxygen uptake
176 measured during these five 3-min intervals was used as a proxy for the maximum metabolic
177 rate (MMR).

178 The fish were left in the respirometers undisturbed until around 14:00 the following
179 day. Every 9 min, an automated water pump (Eheim GmbH & Co. KG, Germany) would

180 switch on for 2 min flushing the respirometers with aerated water. Based on the decrease in
181 oxygen concentration during the 7-min off-cycle of the pumps, we calculated the rate of
182 oxygen uptake. Standard metabolic rate (SMR) was estimated as the lowest 10th percentile
183 of measurements taken throughout the measurement period (Dupont-Prinet et al. 2010,
184 Killen 2014), excluding the first 5 h during which oxygen consumption was elevated due to
185 handling stress (Killen 2014). Absolute aerobic scope (AAS) was calculated as the difference
186 between SMR and MMR. Factorial aerobic scope (FAS) was calculated as the ratio of
187 MMR and SMR.

188 During the trial period, the experimental bath was covered with black plastic to avoid
189 external disturbances. We also covered the sides of the respirometry chambers with opaque
190 material to prevent visual stimuli from other individuals in the same trial. Fish were
191 removed from the respirometer after 24 h, at which point we weighed them and returned
192 them to their initial holding tank. Our sample sizes ranged between 15 and 33 per
193 population per temperature (Supplementary Tables 1 & 2). Due to equipment failure during
194 a small proportion of our trials, we have slightly more measurements of MMR than SMR
195 and AS (Supplementary Tables 1 & 2).

196

197 *Data analysis*

198 Oxygen concentration data derived from Firesting software were analysed in LabChart 7
199 (ADI Instruments Pty Ltd, Australia). To calculate oxygen consumption rates (MO_2), we used
200 the average slope of each 7-min off-cycle measurement period derived from the linear
201 regressions between oxygen consumption over time ($\text{mg O}_2 \text{ h}^{-1}$). All MO_2 data were
202 corrected for the volume of the respirometry chamber and body mass of the fish. Using the

203 bacterial respiration data collected for all chambers before and after trial measurements, we
204 assumed a linear increase in bacterial oxygen consumption over time. The estimated
205 bacterial background respiration at any given timepoint was then subtracted from the MO_2
206 data.

207 Statistical analyses were performed using R version 3.5.1 (R Core Team 2017), and
208 figures were generated using the ggplot2 package (Wickham 2009). SMR, MMR, AAS,
209 FAS, and body mass were log-transformed for use in models because of a non-linear
210 relationship between metabolic rate and mass. For each response variable (SMR, MMR,
211 AAS, and FAS), we used general linear models (GLM) with the following explanatory
212 variables: thermal habitat (warm or cold), population pair (allopatric, sympatric 1, or
213 sympatric 2), acclimation temperature (10°C, 15°C, or 20°C), and all possible interactions
214 between these three factors. A significant effect of the three-way interaction between thermal
215 habitat, population pair, and acclimation temperature would indicate non-parallel
216 divergence of metabolic rate reaction norms across population pairs (Bolnick et al. 2018).
217 After checking for homogeneity of slopes, body mass was also included as a covariate in all
218 models to correct for the effects of mass on metabolic rate. All model assumptions were
219 tested and verified, and the statistical results reported below are the values from the full
220 models including all interactions. Our results for AAS (Table 2, Figure 2C) versus FAS are
221 largely similar (Supplementary Table 3, Supplementary Figure 1), and we only present the
222 results for AAS, which is considered to be more informative and robust (Halsey et al. 2018).

223

224 **Results**

225 As expected, larger fish had higher absolute metabolic rates, and metabolic rate tended to
226 increase with acclimation temperature (Table 2). Acclimation temperature explained over
227 30% of the variation in SMR but only 7% and 3% of the variation in MMR and AAS,
228 respectively (Table 2). SMR was therefore more variable than MMR and AAS in response
229 to acclimation temperature. Fish from warm and cold habitats also differed in the steepness
230 of their metabolic rate reaction norms (Figure 2). In terms of SMR, warm-habitat fish had a
231 steeper metabolic rate reaction norm than cold-habitat fish in the allopatric population pair
232 but a less steep reaction norm in sympatric population 1 (Figure 2A).

233 We found a statistically significant three-way interaction between thermal habitat,
234 population pair, and acclimation temperature on SMR and AAS (Table 2). There was a
235 marginally non-significant effect of this three-way interaction on MMR (Table 2). This
236 interaction indicates that the divergence in metabolic rate reaction norms between warm-
237 and cold-habitat fish varied across the three population pairs (Figure 2). For example, there
238 was a stronger divergence in SMR and MMR between the warm- and cold-habitat fish in
239 the allopatric populations than in the sympatric populations (Figure 2A and 2B). Similarly,
240 the strong effect of population pair and acclimation temperature \times population pair on SMR
241 (Table 2) indicates that local adaptation may be driving variation in this trait across different
242 sites.

243 Differences in SMR between fish from warm and cold habitats tended to be more
244 pronounced at more extreme acclimation temperatures: in the allopatric population pair,
245 thermal divergence in SMR decreased with increasing acclimation temperature, and in
246 sympatric population 1, thermal divergence in SMR increased with increasing acclimation
247 temperature (Figure 2A). However, in sympatric population 2, there was no divergence in

248 SMR between warm- and cold-habitat fish at any of the three acclimation temperatures
249 (Figure 2A).

250 Similar to SMR, differences in MMR between thermal habitats tended to be more
251 pronounced at more extreme acclimation temperatures. Warm-habitat fish from sympatric
252 population 2 had a lower MMR and AAS than cold-habitat fish when both were acclimated
253 to 20°C (Figure 2B and 2C). In the other two population pairs, warm-habitat fish had a
254 lower MMR and AAS than cold-habitat fish at 10°C (Figures 2B and 2C).

255

256 **Discussion**

257 We have taken advantage of a unique study system of geothermally heated and ambient-
258 temperature populations to test whether fish in warm environments show a suppressed or
259 elevated metabolic rate compared to those in cold environments. We found a general
260 pattern for a lower SMR in sticklebacks originating from warm habitats, although the extent
261 of this effect varied depending on the population pair and acclimation temperature.
262 Interestingly, the SMR of warm-habitat sticklebacks at their naturally experienced
263 temperatures (15-20°C) was similar to the SMR of cold-habitat sticklebacks at their naturally
264 experienced temperature (10-15°C). This results in similar metabolic costs in these
265 contrasting thermal environments and is consistent with the predictions of countergradient
266 variation and Krogh's rule. Our findings therefore suggest that fish may evolve a lower
267 metabolic rate as global temperatures increase in response to climate change.

268 In our study system, some populations of sticklebacks living in warm or cold habitats
269 are in separate water bodies (allopatry), while others are found in different parts of the same
270 water body (sympatry). Given the potential for gene flow between sympatric but not

271 allopatric population pairs, we might expect sympatric pairs to be less phenotypically
272 divergent than allopatric pairs (Hendry and Taylor 2004, Pinho and Hey 2010).
273 Alternatively, sympatric pairs might be more divergent because of character displacement,
274 whereby differences between morphs are more pronounced in areas where they co-occur
275 and minimised in areas where their distributions do not overlap (Brown and Wilson 1956,
276 Losos 2011). Our findings show a stronger divergence in metabolic rate between
277 sticklebacks from warm and cold habitats in allopatry than in sympatry. This suggests that
278 gene flow may constrain physiological adaptation in natural populations where physical
279 dispersal between thermal habitats is possible (Lenormand 2002, Hendry and Taylor 2004).
280 It also indicates that metabolic responses to thermal habitat vary across populations, making
281 it difficult to predict metabolic rate evolution in response to climate change.

282 The two sympatric populations differed in the extent of thermal divergence in their
283 metabolic rate reaction norms: sympatric population 1 showed a greater degree of
284 divergence than sympatric population 2. It is possible that this variation is related to
285 differences in the age of these warm habitats (Table 1). In populations that have been
286 diverging for longer, there is more scope for natural selection and genetic drift to introduce
287 adaptive or stochastic phenotypic differences (Ord and Summers 2015). In our study system,
288 we might thus expect the younger population pair (i.e., sympatric 1) to be less divergent
289 than the older populations pair (i.e., sympatric 2). However, we found the opposite pattern,
290 whereby the young sympatric population showed a greater degree of divergence in
291 metabolism between fish from warm and cold habitats. In this young sympatric population,
292 there was thermal divergence in both SMR and MMR reaction norms, whereas in the older
293 sympatric population, there was no divergence in SMR and only a small divergence in

294 MMR. These results are even more surprising considering that the warm and cold habitats
295 of the younger sympatric population are only tens of meters apart, whereas they are a few
296 kilometres apart for the older sympatric population (Table 1). One possible explanation is
297 that there are differences in food availability in the two sympatric populations (White and
298 Kearney 2013). Low food availability in the warm habitat of sympatric population 1, along
299 with elevated temperatures, may favour a lower SMR to allow organisms to resist starvation
300 (Alton et al. 2017). Correspondingly, if there is high food availability in the warm habitat of
301 sympatric population 2 (O’Gorman et al. 2016), selection due to temperature and food
302 availability might be operating in opposite directions, resulting in a similar SMR in the cold
303 and warm habitats (Figure 2A).

304 Another interesting finding was a higher degree of variability in SMR than MMR and
305 AS in response to acclimation temperature. Across the three warm-cold population pairs,
306 acclimation temperature explained over 30% of the variation in SMR but only 7% and 3%
307 of the variation in MMR and AS, respectively. If rising ambient temperatures cause a
308 greater increase in SMR relative to MMR, this will lead to a decrease in AS (Donelson et al.
309 2011). Our results support this prediction, as warm-habitat sticklebacks tended to have a
310 lower AS than cold-habitat sticklebacks at a high temperature (20°C). It has been proposed
311 that the capacity to meet increased oxygen demands at elevated temperatures may
312 determine the persistence of fish populations in a warming climate (Donelson et al. 2011;
313 but see Lefevre 2016, Jutfelt et al. 2018), so it is important to better understand and predict
314 changes in AS in response to temperature changes (Sinclair et al. 2016).

315 By measuring metabolic rates across a range of temperatures, rather than a single
316 temperature as is typically done (Bruneaux et al. 2014), we were able to compare the

317 steepness of the metabolic reaction norms of warm versus cold populations. In terms of
318 SMR, warm-habitat sticklebacks had a steeper metabolic rate reaction norm than cold-
319 habitat sticklebacks in the allopatric population pair but a less steep reaction norm in
320 sympatric population 1. Despite these contrasting trends, a common pattern emerging from
321 our results was that differences between warm- and cold-habitat sticklebacks tended to be
322 more pronounced at more extreme temperatures (10 °C or 20°C). This suggests that cryptic
323 genetic variation was released upon exposure to these novel conditions, thus revealing
324 hidden evolutionary potential (Paaby & Rockman 2014, Shama 2017). If we had only
325 measured metabolic rates at an intermediate temperature, we would have detected little or
326 no differences between warm- and cold-habitat sticklebacks. Our findings thus demonstrate
327 that when studying individuals originating from different thermal environments in a
328 common garden setting, it is much more informative to compare their reaction norms rather
329 than use a single acclimation temperature (Bruneaux et al. 2014).

330 Moreover, using populations exposed to contrasting thermal habitats for many
331 generations allowed us to examine long-term responses to elevated temperatures (Huss et al.
332 2019). This avoids the limitations of short-term laboratory experiments that expose animals
333 to high temperature for only one or a few generations. Nevertheless, the relative
334 contribution of genetic change and plasticity in driving metabolic rate differences in this
335 study system is still unknown. A recent review suggested that metabolic rate is generally
336 highly heritable, although active metabolic rate tends to be more heritable than resting
337 metabolic rate (Pettersen et al. 2018). Further work on the heritability of metabolic traits in
338 this system would allow us to assess their potential to respond to selection.

339 In summary, we have shown a general pattern for a lower metabolic rate in fish from
340 warm habitats, which provides a powerful test of and support for the controversial Krogh's
341 rule. We also found evidence for a stronger divergence in metabolic rate between warm and
342 cold habitats in allopatry than sympatry, suggesting that gene flow may constrain
343 physiological adaptation when dispersal between thermal habitats is possible. By focusing
344 on natural populations living in contrasting thermal environments over a small geographic
345 scale, our study offers valuable insights into how fishes and other ectotherms might
346 physiologically adapt to global climate change.

347

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352

353 **Ethical statement**

354 Our study adheres to the ASAB/ABS Guidelines for the Use of Animals in Research, the
355 institutional guidelines at University of Glasgow, and the legal requirements of the UK
356 Home Office (Project License P89482164).

357

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361

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Table 1. Sampling locations of warm- and cold-habitat threespine sticklebacks collected in May–June 2016 in Iceland. Distance refers to how far apart the warm-habitat and cold-habitat sample sites are for each warm-cold pair. All cold habitats have existed since the last glacial period and are therefore approximately 10,000 years old, whereas warm habitats can be classified as either young (<80 years old) or old (>2,000 years old). The summer and winter temperatures listed are the average water temperatures recorded at each sampling location during the corresponding seasons.

Population pair	Water body	Thermal habitat	Age of warm habitat (years)	Distance (km)	Summer temperature (°C)	Winter temperature (°C)
allopatric populations	Grettislaug	warm	>2,000	21.04	24.9	13.5
	Garðsvatn	cold			14.6	2.2
sympatric population 1	Áshildarholtsvatn	warm	50–70	0.05	24.1	12.5
		cold			12.2	3.4
sympatric population 2	Mývatn	warm	>2,000	3.18	22.8	22.0
		cold			11.5	1.0

Table 2. Results of general linear models testing the effects of thermal habitat (warm or cold), population pair (allopatric, sympatric 1, or sympatric 2), acclimation temperature (10°C, 15°C, or 20°C), and their interactions on SMR, MMR, and AS in threespine stickleback from six populations in Iceland. Df denotes degrees of freedom. Eta-squared (η^2) represents the percent variance explained by each factor, which was calculated by dividing the sum of squares for each factor by the total sum of squares and multiplying by 100.

	Standard metabolic rate (SMR)				Maximum metabolic rate (MMR)				Aerobic scope (AS)			
	η^2	df	<i>F</i>	<i>P</i>	η^2	Df	<i>F</i>	<i>P</i>	η^2	df	<i>F</i>	<i>P</i>
Thermal habitat	0.82	1	9.77	0.002	1.38	1	10.4	0.001	0.99	1	6.26	0.013
Population pair	1.38	2	8.25	<0.001	0.81	2	3.27	0.039	2.20	2	7.09	<0.001
Acclimation temperature	31.7	2	192	<0.001	6.92	2	26.9	<0.001	2.97	2	9.56	<0.001
Mass	29.3	1	355	<0.001	35.9	1	281	<0.001	28.6	1	186	<0.001
Thermal habitat × Population pair	0.94	2	5.65	0.004	0.35	2	1.56	0.21	0.22	2	0.89	0.41
Thermal habitat × Acclimation temperature	0.03	2	0.14	0.87	1.15	2	4.29	0.014	1.43	2	4.74	0.009
Population pair × Acclimation temperature	3.32	2	10.0	<0.001	0.46	2	0.98	0.42	1.21	2	2.05	0.087
Thermal habitat × Population pair × Acclimation temperature	0.82	4	2.49	0.043	1.15	4	2.25	0.063	2.97	4	4.79	<0.001
Error	31.7	385			51.9	406			59.4	385		

allopatric populations Grettislaug & Garðsvatn

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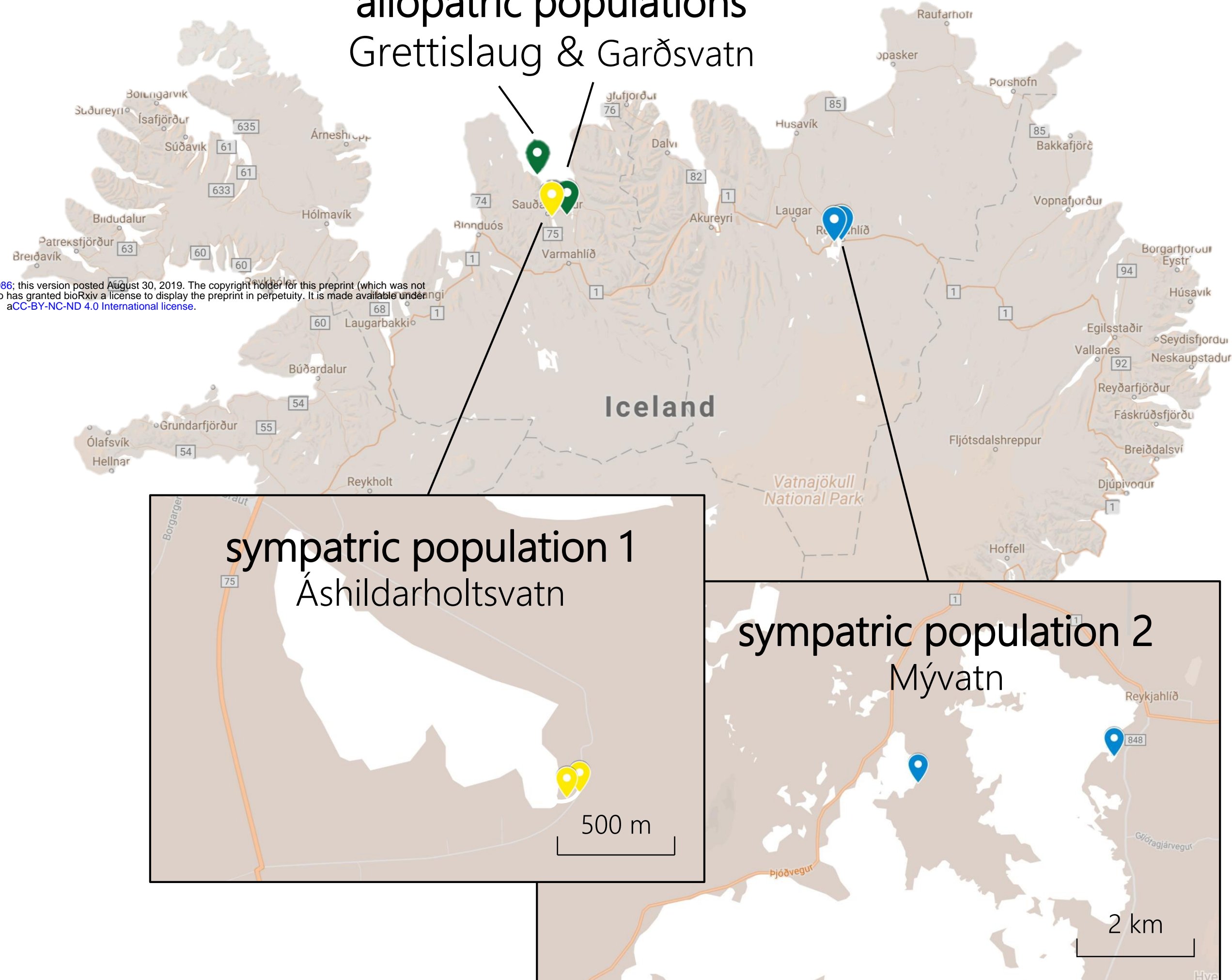


Figure 1. Map of Iceland showing the sampling locations of warm- and cold-habitat sticklebacks we collected for this study. Each of the three population pairs is indicated by a different colour.

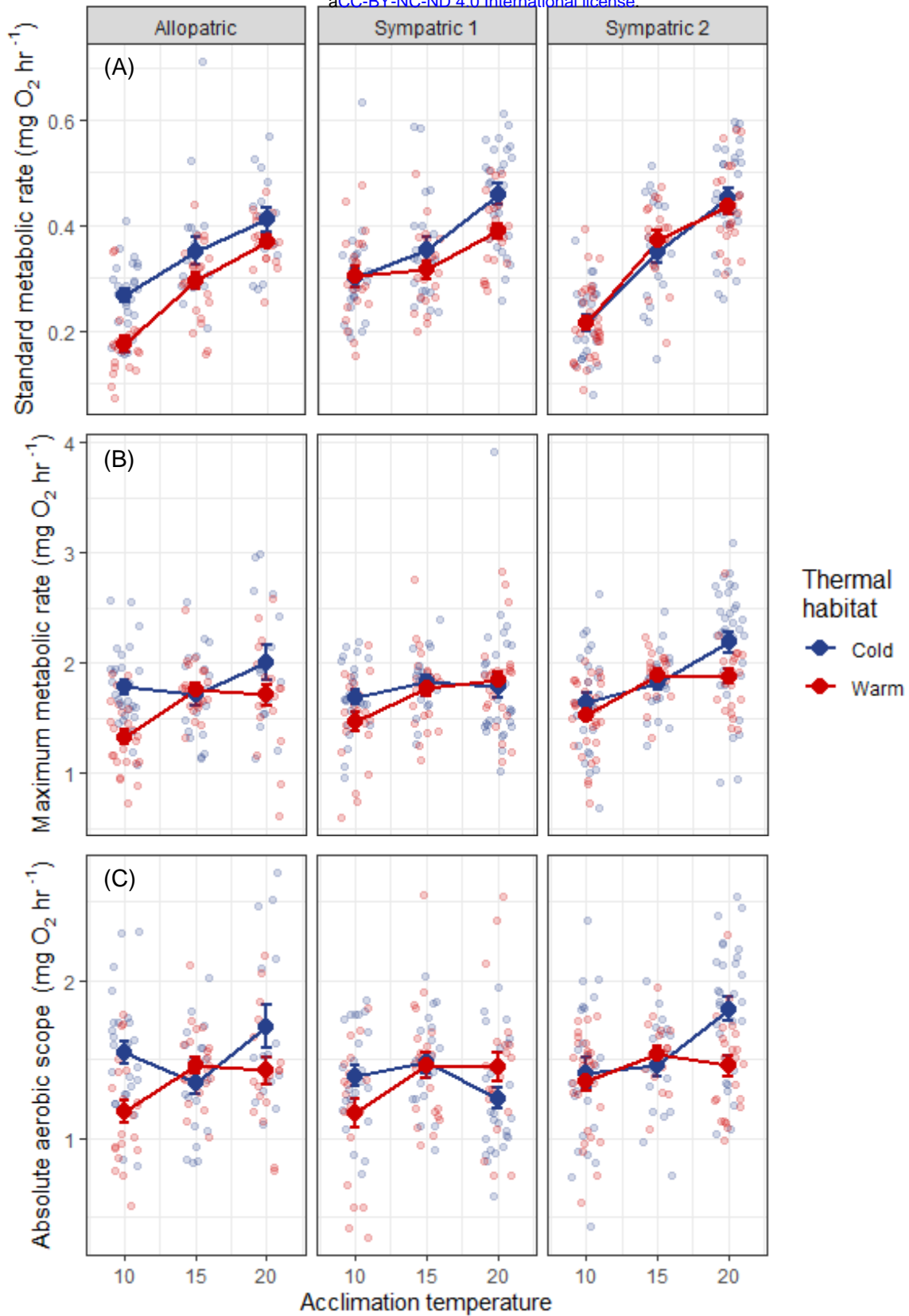


Figure 1. Metabolic rate (mg O₂ hr⁻¹) of threespine sticklebacks from cold and warm habitats in Iceland that were acclimated to 10°C, 15°C, or 20°C. We show mass-corrected standard metabolic rate (A), maximum metabolic rate (B), and aerobic scope

(C). Error bars indicate standard errors, and small circles represent individual data points (blue=cold thermal habitat, red=warm thermal habitat). ‘Allopatric’ refers to Grettislaug and Garðsvatn, ‘sympatric 1’ refers to Áshildarholtsvatn, and ‘sympatric 2’ refers to Mývatn.