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# Temperature preference does not evolve in sticklebacks despite multigenerational exposure to elevated temperatures

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## ABSTRACT

Given the threat of climate change to biodiversity, a growing number of studies are investigating the potential for organisms to adapt to rising temperatures through changes in their physiology, morphology, and behaviour. Earlier work has predicted that evolutionary adaptation to climate change will be accompanied by a shift in temperature preferences, but empirical evidence for this is limited. Here, we test whether exposure to a warm environment over multiple generations has led to the evolution of higher preferred temperatures in the wild. We have taken advantage of a 'natural experiment' in Iceland, where freshwater populations of threespine sticklebacks (*Gasterosteus aculeatus*) are found in waters warmed by geothermal activity year-round (warm habitats), adjacent to populations in ambient-temperature lakes (cold habitats). We hypothesised that sticklebacks from warm habitats would prefer higher water temperatures than those from cold habitats, but we found no support for this hypothesis. Fish from both warm and cold habitats had an average preferred temperature of 13°C, which is considerably lower than the temperatures experienced by fish in warm habitats for most of the year. Our results therefore challenge the assumption that temperature preferences in ectotherms will readily evolve as a response to climate change. Furthermore, the fact that warm-habitat fish are able to persist at relatively high temperatures despite a lower temperature preference suggests that preferred temperature may be a poor indicator of a population's evolutionary potential and capacity to adapt to a novel thermal environment.

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# INTRODUCTION

Rising temperatures due to climate change pose significant challenges to animal populations, requiring them to move to new habitats or adapt to their existing habitat in order to avoid extinction (Habary et al. 2017). Adaptation is common when relocation is not possible due to geographic barriers, social inertia, or dependence on other species (Habary et al. 2017, Stodola & Ward 2017). Despite accumulating evidence that populations can rapidly adapt to a new thermal environment through changes in their morphology and physiology (Crozier & Hutchings 2014), it is still unclear whether thermoregulatory behaviour also has the capacity to evolve in response to environmental change (Paranjpe et al. 2013, Logan et al. 2018).

It has been suggested that thermoregulatory behaviour should be under strong selection, because it may allow animals to buffer the effects of temperature changes by choosing suitable microhabitats (Fangue et al. 2009, Kearney et al. 2009, Huey et al. 2012). Indeed, animals usually seek temperatures that coincide with their optimal physiological performance and growth, which tends to be determined by their thermal evolutionary history (Jobling 1981, Kellogg & Gift 1983, Diaz et al. 2007, Pörtner & Farrell 2008, Köhler et al. 2011, but see Huey & Bennett 1987). This thermoregulatory behaviour is particularly important in ectotherms, because ambient temperature directly influences their body temperature, making them vulnerable to temperature changes (Zuo et al. 2011). Thus, ectotherms may adapt to increasing environmental temperatures through evolutionary shifts in their temperature preferences that accompany evolutionary changes in their physiology (Kearney et al. 2009, Gilbert & Miles 2017, Logan et al. 2018).

Previous work has examined diel and seasonal changes in temperature preferences (Hesthagen 1979, Røed 1979, Angilletta et al. 1999, Siikavuopio et al. 2014), as well as changes in response to different acclimation temperatures in the laboratory (Røed 1979, Fangue et al. 2009, Habary et al. 2017). However, these experiments can only test individual plasticity in temperature preferences within a single generation. It is therefore

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still unknown whether long-term exposure to a warm environment over multiple generations would lead to the evolution of higher preferred temperatures in the wild.

Our study addresses this gap in our knowledge using a unique 'natural experiment' in Iceland, where freshwater populations of threespine sticklebacks (*Gasterosteus aculeatus*) are found in waters warmed by geothermal activity (warm habitats) adjacent to populations in ambient-temperature lakes (cold habitats). This study system provides repeated and independent examples of populations experiencing long-term contrasting thermal environments over a small geographic scale, thereby avoiding the confounding factors associated with latitudinal or elevational comparisons.

We have previously shown that there is strong divergence in the morphology and physiology of sticklebacks from these warm and cold habitats, suggesting local adaptation (Pilakouta et al. 2019). We therefore hypothesised that fish from warm habitats would show a preference for higher water temperatures than fish from cold habitats. We also predicted that this divergence in temperature preference would be more pronounced in populations that have been exposed to warm water for more generations.

## METHODS

### Study animals

We collected adult threespine sticklebacks from six freshwater populations in Iceland in May–June 2016 (Table 1, Supplementary Figure 1). Two of these populations were allopatric, meaning that the warm and cold habitats were in neighbouring but separate water bodies with no potential for gene flow (Table 1). We also sampled two sympatric warm-cold population pairs, where the warm and cold habitats were in the same water body with no physical barriers between them (Table 1). The cold habitats have all existed for thousands of years, since the last glacial period (Einarsson et al. 2004), but there is some variation in the age of the warm habitats (Table 1). The 'Mývatn warm' and Grettislaug sites have been naturally heated by geothermal activity for over 2000 years (Hight 1965, Einarsson 1982). In contrast, the 'Áshildarholtsvatn warm' population

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originated only 50–100 years ago, fed by excess hot water runoff from nearby residences using geothermal heating. These different timescales make it possible to examine whether populations exposed to higher temperatures for a relatively short time have diverged to the same extent as much older populations.

**Animal husbandry**

We placed 15 individuals from each population in 10-litre tanks in a common recirculation system. They were fed *ad libitum* twice a day with a mixture of frozen bloodworms, *Mysis* shrimp, and *Daphnia*. Before the experiment, all fish were anaesthetised using benzocaine and marked with visible implant elastomer tags (Northwest Marine Technology Inc) to allow individual identification. They were kept at 15 °C and a 12h:12h daylight cycle for at least one month before being used in the experiment. The acclimation temperature of 15 °C was chosen because it is an intermediate temperature for the warm and cold populations: it is close to the maximum temperature experienced by cold populations in the summer and the minimum temperature experienced by warm populations in the winter (Table 1).

**Experimental set up**

We tested each individual's temperature preference using a classical shuttle-box approach in which the animal is allowed to behaviourally adjust the temperature of its surroundings (McCauley 1977, Schurmann & Steffensen 1992, Westhoff & Rosenberger 2016, Macnaughton et al. 2018). Our shuttle-box apparatus (Loligo Systems) consisted of two circular 40-cm diameter choice chambers joined by a 10-cm passage way (Killen 2014). The left chamber was designated as the warm chamber and the right one as the cold chamber. Each choice chamber was filled with water to a depth of 7 cm and was attached to its own external buffer tank. The set-up also included a heating reservoir, kept at 30 °C using aquarium heaters, and a cooling reservoir, kept at 4 °C by an external chilling unit. To adjust the temperature within each buffer tank, water was pumped from

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the buffer tanks through steel coils in the heating and cooling reservoirs. The water temperature in the two choice chambers was continually monitored using in-line temperature probes connected to a computer-driven temperature controller and data acquisition system (DAQ-M, Loligo Systems). In turn, the temperature within each chamber was controlled by software (Shuttlesoft, Loligo Systems), which adjusted the flow from reservoir tanks to change the temperature in the choice chambers as required.

We used two ways of adjusting temperatures in the shuttle-box: a static mode and a dynamic mode. In the static mode, there was a constant temperature in each choice chamber with a 2 °C differential between them. In the dynamic mode, the warm and cold chamber temperatures changed depending on the location of the fish but always maintaining a 2 °C differential. When a fish moved into the warm chamber, the temperature increased at a rate of 2 $^{o}$C $h^{-1}$ in both chambers, whereas when a fish moved into the cold chamber, the temperature decreased at a rate of 2 $^{o}$C $h^{-1}$ in both chambers. Thus, by moving between the warm and cold chambers in response to changing temperatures, fish could regulate the water temperature they experienced (Schurmann & Steffensen 1992, Killen 2014). We tracked fish's movements using a camera (uEye, Imaging Development Systems GmbH) mounted above the shuttle-box.

**Experimental protocol**

All of the fish we tested were nonbreeding adults, and their mass ranged from 0.74 to 3.02 g (mean ± SD = 1.82 ± 0.54). Our sample size was $n$ = 10 for each population (total $n$ = 60). Because feeding history can influence preferred temperature, all fish were fasted for the same amount of time (36 h) before the temperature preference test (Killen 2014).

For the test, a single fish was placed in the shuttle-box at static mode, with the cold chamber at 14 $^{o}$C and the warm chamber at 16 $^{o}$C. The fish was left to acclimate overnight with the lights off. The following morning, we turned on the lights at 9:00 and measured activity (total distance moved) between 9:00-10:00. From 10:00 to 18:00, the

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system was set to dynamic mode, and the fish was allowed to select its preferred temperature.

Core body temperature ($T_b$) was calculated using the following equation (Killen 2014): $T_b = T_a + (T_i - T_a)*e^{-kt}$. Here, $T_a$ is the current water temperature experienced by the fish, $T_i$ is the previous temperature it experienced, $t$ is the time elapsed since experiencing that previous water temperature, and $k$ is the rate coefficient for thermal equilibration. The rate coefficient $k$ varies with body size and is defined as the instantaneous rate of change in body temperature in relation to the difference between $T_a$ and $T_b$ (Pépino et al. 2015). We calculated $k$ using the equation $k = 3.69*m^{-0.574}$, where $m$ denotes mass (Stevens and Fry 1974). The final preferred temperature of each individual was calculated as the mean core body temperature experienced during the final two hours of the test when the system was in dynamic mode (Killen 2014).

### Data analysis

R version 3.4.2 was used for all statistical tests (R Core Team 2017), and the ggplot2 package was used for generating figures (Wickham 2009). To test for differences in temperature preference and activity, we used linear models with the following explanatory variables: body mass, population pair (allopatric, sympatric 1, or sympatric 2, as defined in Table 1), thermal habitat at population of origin (warm or cold), and the interaction between population pair and thermal habitat. Statistical results reported below are the values from the full models for temperature preference and activity.

## RESULTS

There was considerable variation in preferred temperature among individuals, ranging from 8.0 °C to 15.8 °C (Figure 1). However, there was no difference in average preferred temperature between sticklebacks collected from warm and cold habitats ($F_{1,54}$=1.03, $P$=0.31). There was also no evidence for a greater divergence in temperature preference in populations exposed to warm water for more generations (Figure 1). Lastly,

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temperature preference was not influenced by body mass ($F_{1,54}$=1.29, $P$=0.26), population pair ($F_{2,54}$=0.03, $P$=0.97), or the interaction between population pair and thermal habitat ($F_{2,54}$=2.87, $P$=0.07).

Similarly, we found no differences in activity between fish from warm and cold habitats ($F_{1,54}$=0.22, $P$=0.64; Figure 2) or between fish from different population pairs ($F_{2,54}$=0.14, $P$=0.87; Figure 2). Activity also did not depend on body mass ($F_{1,54}$=0.02, $P$=0.89) or the interaction between population pair and thermal habitat ($F_{2,54}$=1.58, $P$=0.21).

## DISCUSSION

Contrary to our hypothesis, fish from populations living in warm habitats did not prefer higher water temperatures than fish from cold habitats. We also found no evidence for a larger difference in temperature preference between warm- and cold-habitat fish from old populations than from young populations. Lastly, average preferred temperature was the same for warm- and cold-habitat fish from both allopatric and sympatric populations, indicating that the lack of a difference in sympatric populations is not necessarily due to gene flow constraining adaptation.

In our study system, populations in the cold habitats can be thought to represent the ancestral populations for those in the warm habitats, so we can consider the average preferred temperature of the cold-habitat fish (13°C) to be the ancestral state. Based on this, our results suggest that temperature preference has not evolved in these populations even after being exposed to elevated temperatures for hundreds to thousands of years. One explanation is that there is a lack of genetic variation in traits associated with temperature preference. Although this may seem surprising, our findings are in line with previous work showing low to no heritability of temperature preferences. For example, in side-blotched lizards (*Uta stansburiana*), maternal effects but not additive genetic variation influence the offspring's temperature preference (Paranjpe et al. 2013). Similarly, a study on brown anole lizards (*Anolis sagrei*) found no differences in and no

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heritability of thermoregulatory behaviour in two populations from contrasting thermal environments (Logan et al. 2018).

Preferred temperature often coincides with optimum temperature for growth, survival, and offspring performance (Jobling 1981, Kellogg & Gift 1983, Diaz et al. 2007, Paranjpe et al. 2013, but see Huey & Bennett 1987), so future work should investigate whether temperature preference reflects optimal physiological performance in this study system (Huey & Bennett 1987, Köhler et al. 2011, Artacho et al. 2015, Gilbert & Miles 2017). However, even if physiological performance peaks at a relatively low temperature, sticklebacks inhabiting warm habitats are clearly able to persist at much higher temperatures during the summer months (Table 1). Hence, we suggest that preferred temperature may be a poor indicator of adaptation and evolutionary potential in relation to the thermal environment.

Our results also raise the question of why warm-habitat fish from sympatric populations are found at non-preferred temperatures in the summer (Table 1), even though they are not restricted by geographic barriers. A plausible explanation is that although only water temperature differed between the two chambers in our experimental set-up, temperature is not the only difference between warm and cold habitats in the wild. Habitat choice is based on many biotic and abiotic factors, which can lead to trade-offs. For example, predation risk may be lower in warm habitats because large piscivorous salmonids are less able to cope with high temperatures (Eliason et al. 2011). Moreover, warm habitats may provide a longer breeding season, leading to a higher reproductive output each annual cycle. Previous work has also found differences in prey type availability between these warm and cold habitats in Iceland (Kreiling et al. 2018). Lastly, it is possible that warm-habitat sticklebacks are found at these sites due to social interactions, such as competition or social inertia, rather than active habitat choice (Stodola & Ward 2017, Jacob et al. 2018).

A potential caveat of our study is that experimental fish were acclimated to 15 °C, which might have predisposed them to display a preferred temperature close to 15 °C.

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However, prior work in many fish species, including sticklebacks, has shown that acclimation temperature does not influence final preferred temperature (Røed 1979, Kelsch & Neill 1990, Pérez et al. 2003, Diaz et al. 2007, Habary et al. 2017, but see Crawshaw 1975). For example, Røed (1979) showed that sticklebacks acclimated to different temperatures for different amounts of time all showed similar preferred temperatures. Similarly, a study on a coral reef fish (*Chromis viridis*) found that preferences for cooler water persist after prolonged acclimation to high temperatures, due to an inability to acclimate at the level of the metabolic rate (Habary et al. 2017).

Here, we report a similar pattern, but on an evolutionary timescale, using populations that have been exposed to high temperatures for many generations in the wild. Our findings highlight the need to re-evaluate the common assumption that temperature preferences in ectotherms will readily evolve as a response to climate change (Kearney et al. 2009, Huey et al. 2012, Gilbert & Miles 2017). Furthermore, the fact that warm-habitat fish are able to persist at high temperatures despite a lower temperature preference suggests that preferred temperature may be a poor indicator of a population's evolutionary potential and capacity to adapt to a novel thermal environment.

## ACKNOWLEDGEMENTS

We thank Iain Hill, Tiffany Armstrong, Anna Persson, and Kári Heiðar Árnason for help with fieldwork in Iceland and Joseph Humble for assistance with animal husbandry. The study was funded by a Natural Environment Research Council Grant (NE/N016734/1) awarded to KJP, NBM, SSK, and JL.

## ETHICAL STATEMENT

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

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## REFERENCES

Angilletta Jr MJ, Montgomery LG, Werner YL. 1999. Temperature preference in geckos: diel variation in juveniles and adults. *Herpetologica* **1**, 212-222.

Artacho P, Saravia J, Ferrandiere BD, Perret S, Galliard JF. 2015. Quantification of correlational selection on thermal physiology, thermoregulatory behavior, and energy metabolism in lizards. *Ecol. Evol.* **5**, 3600– 3609.

Crawshaw LI. 1975. Attainment of the final thermal preferendum in brown bullheads acclimated to different temperatures. *Comparative Biochemistry and Physiology Part A: Physiology* **52**, 171-3.

Crozier LG, Hutchings JA. 2014 Plastic and evolutionary responses to climate change in fish. *Evol. Appl.* **7**, 68–87.

Díaz F, Re AD, González RA, Sánchez LN, Leyva G, Valenzuela F. 2007. Temperature preference and oxygen consumption of the largemouth bass *Micropterus salmoides* (Lacepede) acclimated to different temperatures. *Aquaculture Research* **38**, 1387-94.

Einarsson A. 1982 The palaeolimnology of Lake Mývatn, northern Iceland: plant and animal microfossils in the sediment. *Freshwater Biol.* **12**, 63–82.

Eliason EJ, Clark TD, Hague MJ, Hanson LM, Gallagher ZS, Jeffries KM, Gale MK, Patterson DA, Hinch SG, Farrell AP. 2011. Differences in thermal tolerance among sockeye salmon populations. *Science* **332**, 109–112.

Fangue NA, Podrabsky JE, Crawshaw LI, Schulte PM. 2009. Countergradient variation in temperature preference in populations of killifish *Fundulus heteroclitus*. *Physiol. Biochem. Zool.* **82**, 776–786.

Gilbert AL, Miles DB. 2017. Natural selection on thermal preference, critical thermal maxima and locomotor performance. *Proc. R. Soc. B* **284**, 20170536.

Habary A, Johansen JL, Nay TJ, Steffensen JF, Rummer JL. 2017. Adapt, move or die – how will tropical coral reef fishes cope with ocean warming? *Global Change Biol.* **23**, 566–577.

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Hesthagen IH. 1979. Temperature selection and avoidance in the sand goby, *Pomatoschistus minutus* (Pallas), collected at different seasons. *Env. Biol. Fish.* **4**, 369–377.

Hight GA. 1965. The saga of Grettir the Strong. London, Dent.

Huey RB, Bennett AF. 1987. Phylogenetic studies of coadaptation: preferred temperatures versus optimal performance temperatures of lizards. *Evolution* **41**, 1098-115.

Huey RB, Kearney MR, Krockenberger A, Holtum JA, Jess M, Williams SE. 2012. Predicting organismal vulnerability to climate warming: roles of behaviour, physiology and adaptation. *Phil. Trans. R. Soc. B.* **367**, 1665-79.

Jacob S, Laurent E, Haegeman B, Bertrand R, Prunier JG, Legrand D, Cote J, Chaine AS, Loreau M, Clobert J, Schtickzelle N. 2018. Habitat choice meets thermal specialization: Competition with specialists may drive suboptimal habitat preferences in generalists. *Proceedings of the National Academy of Sciences* **115**, 11988-93.

Jobling M. 1981. Temperature tolerance and the final preferendum rapid methods for the assessment of optimum growth temperatures. *J. Fish Biol*. **19**, 439–455.

Kearney M, Shine R, Porter WP. 2009. The potential for behavioral thermoregulation to buffer "cold-blooded" animals against climate warming. *Proceedings of the National Academy of Sciences* **106**, 3835-40.

Kellogg RL, Gift J. 1983. Relationship between optimum temperatures for growth and preferred temperatures for the young of four fish species. *Tran. Am. Fish Soc.* **112**, 424–430.

Killen SS. 2014. Growth trajectory influences temperature preference in fish through an effect on metabolic rate. *J. Anim. Ecol.* **83**, 1513–1522.

Köhler A, Sadowska J, Olszewska J, Trzeciak P, Berger-Tal O, Tracy CR. 2011. Staying warm or moist? Operative temperature and thermal preferences of common frogs (*Rana temporaria*), and effects on locomotion. *Herpetological Journal* **21**, 17-26.

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Kreiling A-K, Ólafsson J, Pálsson S, Kristjánsson B. 2018. Chironomidae fauna of springs in Iceland: Assessing the ecological relevance behind Tuxen's spring classification. *Journal of Limnology* **77**, 1s.

Logan ML, Curlis JD, Gilbert AL, Miles DB, Chung AK, McGlothlin JW, Cox RM. 2018. Thermal physiology and thermoregulatory behaviour exhibit low heritability despite genetic divergence between lizard populations. *Proc. R. Soc. B* **285**, 20180697.

Macnaughton CJ, Kovachik C, Charles C, Enders EC. 2018. Using the shuttlebox experimental design to determine temperature preference for juvenile Westslope Cutthroat Trout (*Oncorhynchus clarkii lewisi*). *Conservation Physiology* 6, coy018.

McCauley RW. 1977. Laboratory methods for determining temperature preference. *Journal of the Fisheries Board of Canada* **34**, 749-752.

Millet A, Kristjánsson BK, Einarsson Á, Räsänen K. 2013. Spatial phenotypic and genetic structure of threespine stickleback (*Gasterosteus aculeatus*) in a heterogeneous natural system, Laky Mývatn, Iceland. *Ecol. Evol.* **3**, 3219–3232.

Paranjpe DA, Bastiaans E, Patten A, Cooper RD, Sinervo B. 2013. Evidence of maternal effects on temperature preference in side-blotched lizards: implications for evolutionary response to climate change. *Ecol. Evol.* **3**, 1977 – 1991.

Pérez E, Díaz F, Espina S. 2003. Thermoregulatory behavior and critical thermal limits of the angelfish *Pterophyllum scalare* (Pisces: Cichlidae). *Journal of Thermal Biology* 28:531-7.

Pépino M, Goyer K, Magnan P. 2015. Heat transfer in fish: are short excursions between habitats a thermoregulatory behaviour to exploit resources in an unfavourable thermal environment? *Journal of Experimental Biology* 218:3461-3467.

Pilakouta N, Humble J, Hill I, Killen SS, Kristjánsson BK, Skúlason S, Lindström J, Metcalfe NB, Parsons KJ. 2019. Testing the predictability of morphological evolution in naturally warmed stickleback populations. *BioRxiv* [Preprint]. Available at https://doi.org/10.1101/609933.

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Pörtner HO, Farrell AP. 2008. Physiology and Climate Change. *Science* **32**, 690–692.

R Development Core Team. 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. URL: http://www.R-project.org.

Røed KH. 1979. The temperature preference of the three-spined stickleback, *Gasterosteus aculeatus* L. (Pisces), collected at different seasons. *Sarsia* **64**, 137–141.

Schurmann H, Steffensen JF. 1992. Lethal oxygen levels at different temperatures and the preferred temperature during hypoxia of the Atlantic cod, *Gadus morhua* L. *Journal of Fish Biology* **41**, 927-934.

Siikavuopio SI, Saether B-S, Johnsen H, Evensen T, Knudsen R. 2014. Temperature preference of juvenile Arctic charr originating from different thermal environments. *Aquat. Ecol.* **48**, 313–320.

Stevens ED, Fry FEJ. 1974. Heat transfer and body temperatures in non-thermoregulatory teleosts. *Can. J. Zool.* **52**, 1137–1143.

Stodola KW, Ward MP. 2017. The emergent properties of conspecific attraction can limit a species' ability to track environmental change. *Am. Nat.* **189**, 726–733.

Westhoff JT, Rosenberger AE. 2016. A global review of freshwater crayfish temperature tolerance, preference, and optimal growth. *Reviews in Fish Biology and Fisheries* **26**, 329-349.

Wickham H. 2016. ggplot2: elegant graphics for data analysis. New York, NY: Springer.

Zuo W, Moses ME, West GB, Hou C, Brown JH (2011) A general model for effects of temperature on ectotherm ontogenetic growth and development. *Proceedings of the Royal Society B: Biological Sciences* **279**, 1840-1846.

**Table 1.** Sampling locations of warm- and cold-habitat sticklebacks collected in May–June 2016. Distance refers to how far apart the warm-habitat and cold-habitat sampling sites are for each warm-cold population pair. All cold habitats have existed since the last glacial period and are therefore around 10,000 years old, whereas warm habitats can be classified as either young (<100 years old) or old (>1,000 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 | Distance (km) | Summer temperature (ºC) | Winter temperature (ºC) |
|---|---|---|---|---|---|---|
| allopatric populations | Grettislaug | warm | old | 21.04 | 24.9 | 13.5 |
| | Garðsvatn | cold | | | 14.6 | 2.2 |
| sympatric population 1 | Áshildarholtsvatn | warm | young | 0.05 | 24.1 | 12.5 |
| | | cold | | | 12.2 | 3.4 |
| sympatric population 2 | Mývatn | warm | old | 3.18 | 22.8 | 22.0 |
| | | cold | | | 11.5 | 1.0 |

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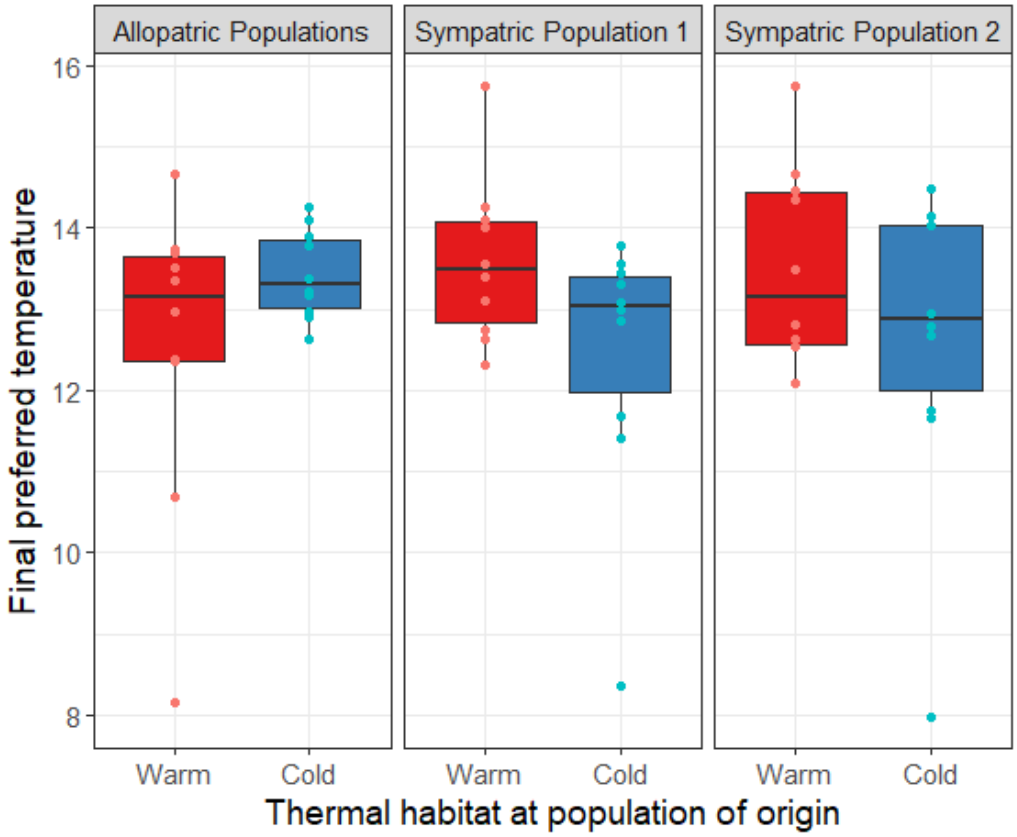


**Figure 1.** Boxplots of preferred temperatures for warm-habitat (red) and cold-habitat (blue) sticklebacks from three population pairs. The filled circles represent individual data points (warm = pink, cold = light blue). 'Allopatric populations' refers to Grettislaug and Garðsvatn, 'sympatric population 1' refers to Áshildarholtsvatn, and 'sympatric population 2' refers to Mývatn.

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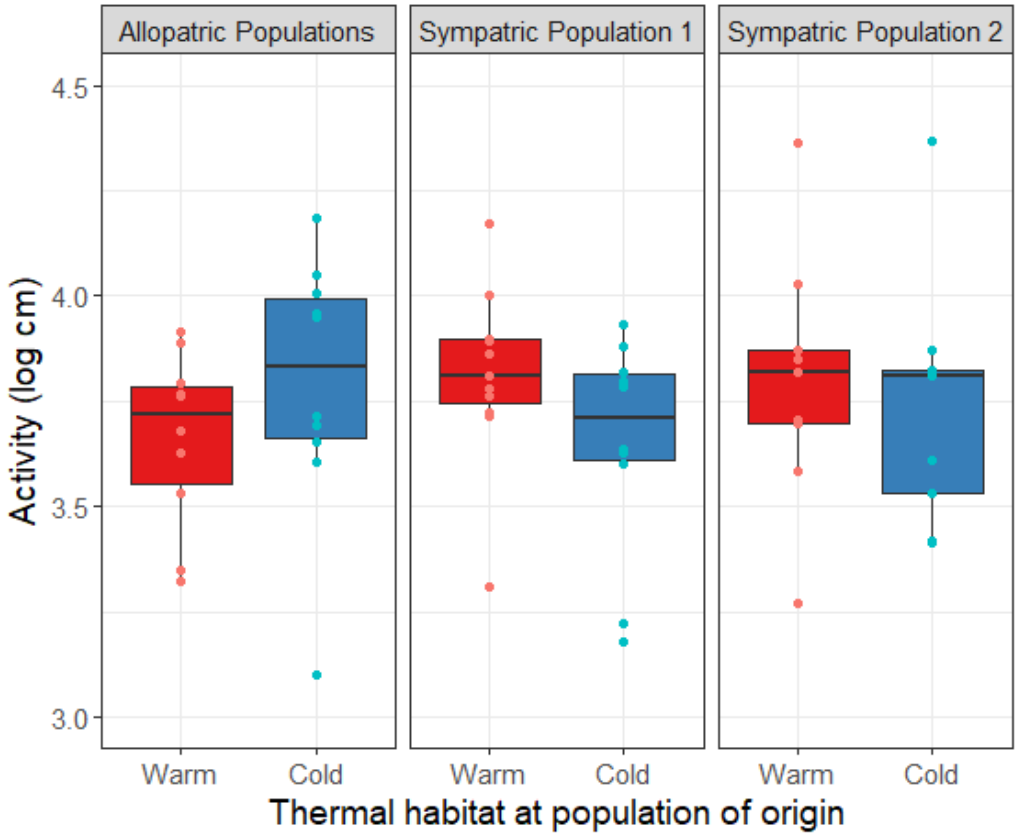


**Figure 2.** Boxplots of activity (log cm travelled in 1 hr) of warm-habitat (red) and cold-habitat (blue) sticklebacks from three population pairs. The filled circles represent individual data points (warm = pink, cold = light blue). 'Allopatric populations' refers to Grettislaug and Garðsvatn, 'sympatric population 1' refers to Áshildarholtsvatn, and 'sympatric population 2' refers to Mývatn.