Temperature-driven selection on metabolic traits increases the strength of an algal-grazer interaction in naturally warmed streams

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ABSTRACT

Trophic interactions are important determinants of the structure and functioning of ecosystems. As the metabolism and consumption rates of ectotherms increase sharply with temperature, there are major concerns that global warming will increase the strength of trophic interactions, destabilizing food webs, and altering ecosystem structure and function. We used geothermally warmed streams that span a ~10ºC temperature gradient to investigate the interplay between temperature-driven selection on traits related to metabolism and resource acquisition, and the interaction strength between the keystone gastropod grazer, the wandering snail *Radix balthica*, and a common algal resource. Populations from a warm stream (~28ºC) had higher maximal metabolic rates and optimal temperatures than their counterparts from a cold stream (~17ºC). We found that metabolic rates of the population originating from a warmer stream were higher across all measurement temperatures. A reciprocal transplant experiment demonstrated that the interaction strengths between the grazer and its algal resource were highest for both populations when transplanted into the warm stream. In line with the thermal dependence of respiration, interaction strengths of grazers from the warm stream were always higher than those of grazers from the cold stream. These findings suggest that warming can increase the strength of algal-grazer interactions through the thermodynamic effects of higher temperatures on physiological rates as well as through correlated increases in *per capita* metabolism and consumption.

Keywords: Consumer-resource interactions, global warming, metabolism, thermal adaptation, interaction strength

INTRODUCTION

The strength of consumer-resource interactions (e.g. the effect of a consumer on the population density of its prey) plays a critical role in shaping the stability of food webs (May, 1973; Paine, 1980; McCann *et al.*, 1998; Otto *et al.*, 2007). Grazing is an important class of consumer-resource interaction, determining the flux of energy and materials from autotrophs to heterotrophs. There are currently major concerns that global warming will increase the impact of grazers on algal or plant communities because the ingestion and respiration rates of heterotrophs tend to be more sensitive to rising temperatures than rates of photosynthesis and growth in autotrophs (O'Connor, 2009; Gilbert *et al.*, 2014; West & Post, 2016). Stronger interactions have the potential to destabilise food webs and consequently, warming induced increases in interaction strengths could have fundamental implications for ecosystem structure and function. For example, elevated grazing rates in aquatic ecosystems, driven by the mismatch in thermal sensitivity between autotrophs and heterotrophs, are a key driver of projected declines in aquatic primary production over the 21st century in models of ocean biogeochemistry (Laufkötter *et al.*, 2015).

The effects of temperature on metabolic rates and traits associated with consumer-resource interactions (e.g. attack rates, handling times) often follow characteristic unimodal thermal response curves, in which rates increase exponentially to an optimum and decline rapidly thereafter (Dell *et al.*, 2011; Englund *et al.*, 2011; Rall *et al.*, 2012; Dell *et al.*, 2014; Gilbert *et al.*, 2014). Integrating thermal responses for metabolism and interaction-traits with dynamical models of consumer-resource interactions offers a promising framework for predicting food web responses to global warming (Vasseur & McCann, 2005; Shurin *et al.*, 2012; Binzer *et al.*, 2015). However, thermal response curves are often flexible, and can shift when organisms are exposed to novel thermal environments, both via phenotypic plasticity and adaptive evolution (Angilletta *et al.*, 2003; Kingsolver *et al.*, 2004; Deutsch *et al.*, 2008;

90 Kingsolver & Huey, 2008). Consequently, plasticity and evolution have the potential to
91 modulate the effects of rising temperatures on the strength of species interactions (Sentis *et*
92 *al.*, 2015). For example, if metabolic rates are down-regulated after long-term exposure to
93 higher temperatures (Addo-Bediako *et al.*, 2000), then compensatory metabolic responses to
94 warming could mitigate predicted increases in consumer-resource interaction strength. How
95 these long-term responses to warming affect rates of metabolism and in turn, the strength of
96 consumer-resource interactions, are largely unknown, limiting our ability to predict how
97 trophic interactions will change in response to warming in the long-term.

98 There is evidence from studies across naturally occurring thermal gradients over large
99 spatial scales, that local thermal adaptation can play an important role in shaping the strength
100 of species interactions (Barton, 2011; De Block *et al.*, 2012). While these studies provide
101 important insights into how consumer-resource interactions are shaped by evolution across
102 thermal gradients (Fukami & Wardle, 2005), their usefulness for understanding responses to
103 rapid climate warming might be limited, because other factors, such as day length, light
104 intensity and precipitation, tend to be confounded with temperature along such broad scale
105 spatial gradients. Furthermore, the timescales over which local adaptation has occurred in
106 such broad scale studies could be much longer than the rapid evolutionary change required to
107 keep pace with climate warming (Loarie *et al.*, 2009; Hoffmann & Sgrò, 2011). Here, we
108 investigate how temperature-driven selection on traits that determine the thermal responses of
109 metabolism and resource acquisition affect the strength of a keystone grazing interaction (the
110 gastropod *Radix balthica,* which grazes algal biofilms in streams) in naturally warmed
111 Icelandic geothermal streams spanning a gradient of 11ºC. Critically, temperature is the main
112 abiotic factor that varies among streams in the catchment and is not correlated with pH,
113 conductivity or inorganic nutrient concentrations (see Table 1). These streams are thought to
114 have been subject to geothermal heating for at least the last century (OGorman *et al.*, 2012).

This system therefore provides the opportunity to investigate how long-term differences in temperature between otherwise similar sites shape the expression of metabolic traits and the subsequent impact of any temperature-driven selection on species interactions in a natural system. Specifically, we ask: can long-term differences in temperature drive selection for metabolic traits that attenuate the direct effects of warming on the strength of consumer-resource interactions?

METHODS

Study site

The streams are located North of the Hveragerði valley, in the south east of the Hengil high temperature geothermal field, Iceland (N64° 0' 2.944" W21° 11' 17.451") and consist of a catchment of 11 streams spanning a temperature gradient of approximately 20 ºC (see Figure 1 and SI Figure 1). Two streams, stream 5 (17.5 ºC ± 4.5 ºC, hereafter 'cold stream') and stream 11A (28.3 ºC ± 1.3 ºC, hereafter 'warm stream', see Table 1 for a comparison of other chemical and physical parameters), were chosen for experiments due to their close proximity to each other, the large temperature differential and similar abundances of the keystone grazer, *Radix balthica*. The grazer plays an important functional role in geothermal stream ecosystems, where grazer biomass as well as grazing rates are strongly influenced by temperature (OGorman *et al.*, 2012). The two streams are similar in all other measured physical and chemical characteristics but differ in average temperature by 11 ºC (see Table 1), and hence present an opportunity to investigate how the effects long-term differences in temperature shape consumer-resource interactions.

Grazer metabolism

To quantify whether the different thermal regimes in the two adjacent streams resulted in divergence in metabolic traits of *R. balthica* we measured the acute responses of respiration

142 to a broad gradient in temperature. We collected 33 individuals of similar weight and length
143 from each stream, which were cleaned from any algal debris to avoid carry-over of a food
144 source into the tank or subsequent respiratory measurements on the oxygen electrode. The
145 snails were kept overnight in aerated tanks at the average stream temperature of origin and in
146 the absence of a food source to minimise any potential effects of differences in food quantity
147 or quality between streams. Respiration was quantified as the rate of oxygen consumption in
148 a Clark-Type oxygen electrode, measured between 4 – 44 ºC in 4 ºC increments (11
149 temperatures in total). At each temperature, respiration was measured for 3 individuals, and a
150 different set of individuals was measured at each temperature (i.e. each animal was only
151 subjected to a single assay). Individuals were allowed 15 minutes at the assay temperature
152 prior to the measurements. The subsequent thermal responses of respiration were quantified
153 using a modification of the Sharpe-Schoolfield equation (see (Schoolfield *et al.*, 1981) and
154 (Sharpe & DeMichele, 1977)for the original equation):

155 $$\ln\big(b(T)\big) = E_a\left(\frac{1}{kT_c} - \frac{1}{kT}\right) + \ln\big(b(T_c)\big) + \alpha\ln(M_i) - \ln\left(1 + e^{E_h\left(\frac{1}{kT_h} - \frac{1}{kT}\right)}\right) \quad (1)$$

156 where $b(T)$, is the *per capita* metabolic rate (µmol O_2 L^{-1} h^{-1}) at temperature T in Kelvin (K),
157 k is Boltzmann's constant (8.62×10^{-5} eV K^{-1}), E_a is an apparent activation energy (in eV) for
158 the metabolic process, ln ($b(T_c)$) is the rate of metabolism normalised to an arbitrary
159 reference temperature, T_c = 18 ºC, where no low or high temperature inactivation is
160 experienced. M_i is the mass (g) of an individual i, α is the allometric scaling exponent that
161 characterises the power-law relation of mass and metabolic rate (Brown *et al.*, 2004). E_h
162 characterizes temperature-induced inactivation of enzyme kinetics above T_h where half the
163 enzymes are rendered non-functional. Differentiating equation (1) and solving for the global
164 maxima yields an expression for the optimum temperature

165 $$T_{opt} = \frac{E_h T_h}{E_h + kT_h \ln\left(\frac{E_h}{E_a} - 1\right)} \quad (2)$$

166 Equation (1) differs from the Sharpe-Schoolfield equation (Sharpe & DeMichele, 1977;
167 Schoolfield *et al.*, 1981) in a number of ways. First, we account for the power law relation
168 between body mass and metabolic rate, M^{α} (Brown *et al.*, 2004). Second, we exclude
169 parameters from Eq. (1) used to characterize low-temperature inactivation due to insufficient
170 data to quantify this phenomenon in our analysis. Third, rather than characterize temperature
171 effects below T_{opt} using the Eyring (1935) relation, $\left(\frac{T}{T_c}\right) e^{E_a(\frac{1}{kT_c}-\frac{1}{kT})}$, we instead use the
172 simpler Boltzmann factor, $e^{E_a(\frac{1}{kT_c}-\frac{1}{kT})}$. This simplification enables an explicit solution for T_{opt}
173 (Eq. 2) and facilitates more direct comparison with previous work on the temperature
174 dependence of metabolism using metabolic theory e.g. (Allen *et al.*,2005, Gillooly, 2001;
175 Brown *et al.*, 2004; Van M Savage *et al.*, 2015).
176 The parameters, ln $b(T_c)$, α, E_a, E_h, T_h, and T_{opt}, in Eqs. (1) & (2) represent traits
177 characterising the metabolic thermal response that we expect to be under selection in *R.*
178 *balthica* inhabiting the hot and cold streams. We tested for differences in each of the
179 parameters between the populations of *R. balthica* by fitting the respiration data to Eq. (1)
180 using generalised non-linear least squares regression (within the 'gnls' function in the 'nlme'
181 package for R, package version 3.1-128) and including 'origin' as a two level factor (i.e.
182 'cold' and 'warm' stream). We tested for differences between populations for each parameter
183 by sequentially removing the effect of 'origin' on each parameter and comparing the Akaike
184 information criterion for small sample sizes (AICc) for all possible models (see SI Table 1
185 and SI Table 2) using the 'aictab' and 'modavg' functions from the AICcmodavg package
186 (package version 2.1-0). The model chosen for further exploration was that with the lowest
187 (AICc) value. Model averaging was carried out when models fell within 2 AICc units of each
188 other, and the conditional averages of the parameters were used for curve fitting and
189 interpretation (see also Table 2). The relative importance of the fixed factors in the averaged
190 model was determined using the sum of their relative weights.

191

192 **Reciprocal transplant experiment**

193 We carried out a reciprocal transplant experiment to determine how long-term differences in
194 temperature and the resultant impacts on metabolic traits affect the strength of algal-grazer
195 interactions. We achieved this by placing snails from each population in microcosms
196 consisting of a tissue culture flask on which diatom biofilms had been established. Diatoms
197 of the genera*, Acnanthes, Nitzschia, Navicula*, and *Gomphonema* are common in streams
198 across the Hengill volcanic area (Gudmundsdottir *et al.* 2013)and were ordered from culture
199 collections (Culture collection of algae and protozoa and Sciento) and grown in the
200 laboratory in mixed assemblages to yield common resource for testing the effects of
201 temperature and local adaptation on grazing . The diatom assemblages were inoculated into
202 Corning plastic translucent flasks (maximum volume 1L) with 20 mL COMBO medium
203 (Kilham *et al.*, 1998), and brought to a salinity of 5-10 (equivalent to approximately 5-10 g
204 salts/kg water) to match the slightly elevated salinity and conductivity found in these thermal
205 stream environments (Gudmundsdottir et al. 2013). The flasks were turned onto their sides to
206 allow for a larger area of biofilm growth on the base (~ 60 cm^2 in total per flask) and the algal
207 communities were left to grow for 14 days prior to the experiment. After 14 days, all flasks
208 had substantial biofilm development on the base and were used as microcosms for the *in situ*
209 reciprocal transplant experiment. Analysis of control flasks (no grazer) showed that growth of
210 the diatom lawn *per se* did not differ significantly for flasks placed in hot or cold streams (SI
211 Figure 2, one-way ANOVA $F_{1,10} = 1.28$, $P = 0.26$). Thus, any changes to the biofilm biomass
212 in the experiment can be attributed to the per capita effects of the grazer.

213 The experiment consisted of 3 treatments (each with 6 replicate microcosms placed in
214 each of the 2 streams): (i) a control microcosm in which a biofilm was present and no *R.*
215 *balthica* were added, (ii) an 'origin' treatment in which *R. balthica* that were resident in the

216 stream were added to microcosms, and (iii) a 'transplanted' treatment in which *R. balthica*
217 that were from the adjacent stream were added to microcosms. *R. balthica* individuals were
218 collected from the 2 streams the day before the experiment and were starved for 24h in the
219 laboratory in aerated tanks at the average temperature of the stream of origin. There was no
220 significant difference in average snail weight between the two streams (see SI Figure 3, one-
221 way ANOVA: $F_{1,408} = 0.15$, $P = 0.7$). Microcosms were assembled by adding 3 snails of
222 similar body dimensions (0.35 ± 0.03 g of *R. balthica* weight reported as blotted fresh weight
223 throughout) and 100 mL of 0.4 µm filtered water from the stream in which the microcosm
224 was to be placed. This resulted in a grazer density of 5 individuals m^{-2}, which was
225 comparable to the average *in situ* density in the streams (see SI Figure 4, no significant
226 difference in in situ density between the two-streams: one-way ANOVA: $F_{1,66}$, P = 0.54).
227 This design was preferred to a set-up with each microcosm holding a single grazer, which
228 attempt to exclude the effects of mutual interference on feeding behaviour e.g. (Skalski &
229 Gilliam, 2001; Rall *et al.*, 2009; Lang *et al.*, 2011; Vucic-Pestic *et al.*, 2011), because (i) the
230 experimental densities are representative of natural conditions; and (ii) the consumption rates
231 of a single individual were insufficient to detect a significant change in algal biomass. The
232 microcosms were submerged in each stream and the snails were left to graze for 48 hours.
233 We observed no grazer mortality over the experimental period.
234
235 **Interaction strength**
236 At the end of the experiment, algal biomass in each of the microcosms was quantified via
237 methanol chlorophyll extraction modified from (Holm-Hansen & Riemann, 1978). Here, the
238 walls of the microcosms were scrubbed until all biofilm particles were in suspension. The
239 solution was filtered onto a 0.4µm GF/F filter, which was then ground in methanol for 5
240 minutes. The samples were centrifuged at 3500 rpm for 15 minutes and the absorbance of the

241 supernatant was measured at 632nm, 665nm, and 750nm. Total chlorophyll content in µg
242 mL^{-1} was then calculated as described in Holm-Hansen & Riemann (1978). The *per capita*
243 interaction strength in each microcosm was then estimated by calculating the dynamic index
244 (DI, see also (Berlow *et al.*, 2004)for a technically similar set-up):

245 $$DI = \frac{\ln\left(\frac{N}{D}\right)}{Yt} \quad (3)$$

246 where DI is the dynamic index (g C^{-1} h^{-1}), *N* is total chlorophyll (sum of Chl *a* + Chl *c*)
247 content of control, *D* total chlorophyll in the grazed microcosm, *Y* is the grazer biomass (g
248 C), and *t* is time in hours. Snail blotted wet weight was converted to carbon mass (in grams)
249 using conversion factors that assume dry weight to be 7.5% of the blotted wet weight
250 (Ricciardi & Bourget, 1998) and a carbon content of 22% dry weight (Burgmer *et al.*, 2010).
251 We carried out two analyses using the data from the reciprocal transplant experiment.
252 The first analysis, used a generalised linear model (GLM), with 'interaction strength' as the
253 response variable and 'origin' ('cold' or 'warm' stream) and 'transplant temperature' (17.5
254 and 28.3 ºC) as potentially interacting factors. We used this analysis to determine (i) whether
255 interaction strengths differed between snails that originated from the warm or cold streams
256 (e.g. a main effect of 'origin'); (ii) whether interaction strengths were temperature dependent
257 (e.g. a main effect of 'temperature'); and (iii) whether the temperature dependence of
258 interaction strength differed between the snails from the cold and warm streams (e.g.
259 interaction between 'origin' and 'temperature').
260 The design of the reciprocal transplant experiment also enabled us to disentangle
261 short-term temperature responses attributable to acclimation (e.g. responses to the
262 temperature in the 'transplanted' stream) from those reflecting processes operating over
263 longer, time scales (e.g. adaptation to the stream of 'origin'). Note that these 'long-term'
264 effects, which we call 'adaptation', could reflect strict genetic microevolution (e.g. resulting
265 in divergent genotypes among populations) or they could represent non-genetic effects of the

266 different temperature regimes that manifest over ontogenetic development, but are
267 nevertheless adaptive (Bonduriansky *et al.*, 2011) . In the second GLM we included
268 'interaction strength' as the response variable and 'timescale' ('short' or 'long') and
269 'transplant temperature' (17.5 and 28.3 ºC) as potentially interacting factors. Here, 'short-
270 term' temperature responses were characterised as the change in interaction strength between
271 the stream of origin and the transplant stream. By contrast, the 'long-term' temperature
272 response was characterised as the change in interaction strength comparing measurements
273 made only when the snails were in their stream of origin. For better comparison of the
274 steepness of the respiration reaction norms, we re-express the transplant temperature data as
275 Boltzmann temperatures $\left(\frac{1}{kT_c} - \frac{1}{kT}\right)$ so that the coefficients of the model yield activation
276 energies in units of eV (see Eq. (1)). In this analysis, a significant interaction between
277 'transplant temperature' and 'timescale' would demonstrate that the temperature dependence
278 of interaction strength differs between the 'short-term' (E_{short}, change in interaction strength
279 between the stream of origin and the transplant stream, see also Fig. 3), and 'long-term'
280 (E_{long}, i.e. change in interaction strength comparing measurements made only when the snails
281 were in their stream of origin, see also Fig. 3). We assume that E_{short} captures rapid
282 physiological plasticity (e.g. acclimation) in interaction strength in response to a change in
283 temperature and E_{long} captures processes operating over longer timescales – e.g. genetic
284 microevolution and non-genetic developmental effects. Consequently, the component of the
285 temperature sensitivity attributable to 'adaptation' (recognising that this might be genetically
286 and/or developmentally determined) is given by $E_{\text{adapt}} = E_{\text{long}} - E_{\text{short}}$.
287
288 **RESULTS**
289 **Metabolic thermal response curves**

290 The allometric scaling coefficient, α, and the apparent activation energy, E_a, were consistent
291 between the populations of *R. balthica* from the cold and warm streams (see Table 2 for
292 model comparison and estimated parameter values). The temperature normalised rate of
293 respiration, ln $b(T_c)$, and T_h (the temperature at which respiration was 50% inactivated) were
294 both higher in the population of *R. balthica* from the warm stream. Because the optimum
295 temperature, T_{opt}, depends strongly on T_h (see Eq. (2)), T_{opt} was higher in *R. balthica* from
296 the warmer stream (T_{opt} warm = 38.25 ± 0.6 ºC; T_{opt} cold = 33.05 ± 1.5 ºC). As ln $b(T_c)$ and
297 T_{opt} were both higher, the warm populations of *R. balthica* had elevated metabolic rates
298 across the full range of measurement temperatures (Fig. 2).
299
300 **Local adaptation of interaction strength**
301 Interaction strength increased with elevated transplant temperature for the populations of *R.*
302 *balthica* from both the warm and the cold streams (Fig. 3A; main effect of 'transplant
303 temperature' GLM $t_{1,21} = 2.56$; $P < 0.01$). Furthermore, interaction strengths were
304 consistently higher for the populations of *R. balthica* from the warm stream in both transplant
305 temperatures (Fig. 3A; GLM main effect of 'origin' $t_{1,121} = 2.92$; $P < 0.005$). These findings
306 are consistent with the higher respiration rates observed in the warm population (Fig. 2) and
307 highlight the association between metabolism and interaction strength.
308
309 **Disentangling the short- and long-term effects of warming on interaction strengths**
310 Our experimental design enabled us to compare temperature sensitivities that capture short-
311 term thermal acclimation (e.g. changes in interaction strength in response to the reciprocal
312 transplant) as well as the long-term temperature sensitivity, which also includes effects of
313 local adaptation (e.g. changes in rates between warm and cold populations quantified in the
314 stream of origin). We found that interaction strength increased with temperature in both the

315 short- and the long-term (Fig. 3). However, the magnitude of the temperature response was
316 significantly larger in the long-term (Fig. 3; interaction between 'transplant temperature' and
317 'timescale' on interaction strength; $GLM_{1,18} = -2.91$; $p < 0.05$), where, the average E_{short} was
318 0.46 eV, while E_{long} was significantly higher at 0.99 eV. This divergence between the short-
319 and long-term temperature sensitivities implies a non-trivial contribution of adaptation in
320 amplifying the effects of temperature on interaction strength *in situ*, with the contribution of
321 E_{adapt} of 0.51 and 0.53 eV in the cold and warm adapted populations respectively.
322
323 **DISCUSSION**
324 Understanding how global warming will affect the strength of consumer-resource interactions
325 and the stability of aquatic food webs is a fundamental challenge in evolutionary ecology that
326 requires insight on the short-term effects of temperature on metabolism and interaction traits
327 and they are modulated by evolutionary and developmental processes over longer time scales.
328 There is evidence from terrestrial (Rall *et al.*, 2009; Barton, 2011; Vucic-Pestic *et al.*, 2011;
329 Brose *et al.*, 2012), freshwater (Kratina *et al.*, 2012) and marine ecosystems (Sanford, 1999),
330 that warming is likely to increase the strength of consumer-resource interactions, at least in
331 the short-term, owing to the exponential effects of temperature on the consumption rates of
332 mobile ectothermic consumers (Dell *et al.*, 2014; Gilbert *et al.*, 2014). What is less clear
333 however, is how long-term responses to rising temperatures will modulate the direct effects
334 of warming on species interactions. Space-for-time substitutions across broad spatial scales
335 indicate that local adaptation to different thermal regimes can play an important role in
336 shaping species interactions, often compensating for the direct effects of temperature on
337 interaction traits (Barton, 2011; De Block *et al.*, 2012). Here, we build on this work by
338 investigating the effects of temperature and local adaptation on the interaction between the
339 gastropod grazer, *R. balthica*, and its algal resource. Our study contributes novel insights in a

340 number of ways. First, we explore patterns of local adaptation over a relatively small spatial
341 scale (m as opposed to km). The two streams in our experiment are separated by
342 approximately 500 m but differ in temperature by 11 ºC. Because dispersal, gene flow and
343 genetic divergence among populations in this species are strongly related to geographic
344 distance (Johansson *et al.*, 2016), our study over a relatively small spatial scale, provides
345 insight into how metabolic and resource acquisition traits in closely related natural
346 populations have divergeed in response to warming and is therefore directly relevant for
347 understanding the effects of rapid climate change (Richter-Boix *et al.*, 2010; Keller *et al.*,
348 2013; Merilä & Hendry, 2014). Second, we quantified the effects of temperature on both
349 metabolic and consumption rates to determine how temperature-driven selection on key traits
350 shape the effects of long-term warming on the strength of consumer-resource interactions.
351 We found significant variation in the thermal response curves for respiration between
352 the populations of *R. balthica* from the warm and cold streams. The optimum temperature
353 (T_{opt}) for respiration was higher in the warm population (i.e. metabolic rates peaked at higher
354 temperatures). Furthermore, the inactivation energy (E_h) was lower in the warm population,
355 indicating that declines in the rate of respiration after the optimum (i.e. at high temperatures)
356 were less pronounced than in grazers from the cold stream, where metabolic rates peaked at
357 lower temperatures and declined markedly at temperatures above T_{opt}. These divergences
358 indicate that the different thermal regimes in these streams have selected for different
359 metabolic traits in warm and cold populations of *R. balthica*. Whilst the higher T_{opt} and lower
360 E_h in the warm population were in line with expectations assuming local thermal adaptation,
361 we found no evidence that metabolic performance at high temperature was traded-off against
362 performance at low temperature. Instead, metabolic rates were higher for *R. balthica* from the
363 warm stream across all measurement temperatures. These results are in broad agreement with
364 the "hotter is better" hypothesis, which proposes that maximal performance of organisms

365 with higher optimal temperatures should be greater than those with lower optimum
366 temperatures because of the thermodynamic constraints imposed by high temperatures on
367 enzyme kinetics (Huey & Kingsolver, 1993; Kingsolver *et al.*, 2004; Angilletta *et al.*, 2010).
368 Indeed maximal respiration rates in the population from the warm stream were greater than
369 those from the cool (ln(R) warm stream: 3.39 ± 0.14 μmol O_2 L^{-1} h^{-1}, and cool stream: 2.54 ±
370 0.26 μmol O_2 L^{-1} h^{-1}, both ± 1.s.e.m.). The lower E_h, (i.e. the steepness of the decline of the
371 thermal reaction norm past the optimum), and higher ln $b(T_c)$, i.e. the rate of respiration
372 normalised to 18 ºC, in the warm population also meant that the thermal response curve for *R.*
373 *balthica* from the warm stream was broader. In agreement with previous work (e.g. on
374 bacteriophages, (Knies *et al.*, 2009), our data for the gastropod *R. balthica* indicate that
375 adaptation to higher temperatures resulted in both greater maximal metabolic performance
376 and a broader metabolic thermal reaction norm.
377 The general patterns observed in the metabolic traits were also reflected in the effects
378 of temperature on interaction strength. Interaction strength was higher for individuals placed
379 in the warm stream, irrespective of their stream of origin. These findings suggest that
380 elevated temperatures increase consumption rates though the effects of temperature on
381 respiratory physiology, but local adaptation to warmer environments also results in a
382 correlated increase in metabolism and interaction strength at low temperature. This may have
383 important wider implications for the effects of warming on the structure, functioning and
384 stability of aquatic food webs (Rall *et al.*, 2009; O'Connor *et al.*, 2011; Vucic-Pestic *et al.*,
385 2011; Dell *et al.*, 2014; Fussmann *et al.*, 2014; Gilbert *et al.*, 2014). If long-term responses to
386 increasing temperature give rise to higher maximal rates of metabolism and consumption as
387 well as elevating rates at lower temperatures, then the effects of warming on the strength of
388 consumer-resource interactions in the long-term could be greater than previously anticipated
389 (Gilbert *et al.*, 2014). Indeed, work on experimental warming of aquatic ecosystems has

390 shown that increases in the strength of top-down control can have profound effects on
391 community structure and ecosystem processes (Burgmer & Hillebrand, 2011; Kratina *et al.*,
392 2012; Yvon-Durocher *et al.*, 2015). Elevated grazing rates at warmer temperatures can have a
393 wide range of impacts in aquatic systems, with evidence for both increases (Yvon-Durocher
394 *et al.*, 2015) and decreases (Burgmer & Hillebrand, 2011) in algal species richness, biomass
395 and productivity.

396 In our experiments, the thermal sensitivities of metabolic rates were much larger than
397 those of interaction strengths in the short-term (e.g. 0.96 and 0.45 eV respectively), in line
398 with findings in other invertebrate systems (Rall *et al.*, 2009; Vucic-Pestic *et al.*, 2011;
399 Fussmann *et al.*, 2014). These findings suggest that rates of grazing and metabolism were
400 clearly linked, but became decoupled when individuals experience rapid changes in
401 temperature that depart substantially from those in their local environment. In the short-term,
402 if increases in metabolic demands with temperature are greater than those of consumption
403 rates (as found here), then less energy will be transferred from the resource to the consumer,
404 i.e. more is lost through respiration, see also (Rall *et al.*, 2009). If such imbalances are
405 maintained over long periods of time then starvation of the consumer can ultimately result in
406 a decline in top-down control on the resource (Fussmann *et al.*, 2014; Binzer *et al.*, 2015).
407 However, when consumers' feeding rates are more sensitive to temperature than metabolic
408 rates, interaction strengths can become amplified in warmer environments, leading to faster
409 resource depletion and eventually driving either the resource or the consumer to extinction
410 (Vasseur & McCann, 2005). Long-term effects of temperature on interaction strengths have
411 so far only been explored using food web models, parameterised using temperature
412 sensitivities derived from short-term experiments (Vasseur & McCann, 2005; Rall *et al.*,
413 2012; Fussmann *et al.*, 2014). Consequently, such analyses don't capture the evolutionary
414 and developmental effects which can modulate the short-term effects of temperature on *per*

415 *capita* rates. Our results highlight substantial differences between the short- and long-term
416 effects of temperature on interaction strength; implying that longer term processes plays an
417 important role in maintaining the balance between metabolic and consumption rates.
418 We quantified the short- and long-term effects of temperature in the reciprocal
419 transplant experiment. The short-term temperature response (E_{short}) captures the effects of
420 physiological plasticity over the 48h experiment. Conversely, the long-term response (E_{long})
421 also accounts for processes operating over longer timescales, including genetic micro
422 evolution and non-genetic developmental effects of temperature. In our experiment, E_{long} was
423 higher than E_{short}, implying a significant role for long-term proceses in shaping the effects of
424 temperature on *in situ* interaction strengths. Notably, the higher E_{long} was driven both by
425 elevated grazing rates in the warm populations in the warm stream and lower rates in the cold
426 populations in the cold stream. These results diverge from our expectations based on the
427 metabolic cold adaptation hypothesis (Addo-Bediako *et al.*, 2000) which would predict
428 populations from warmer environments should dampen the acute effects of temperature on
429 metabolic rates. On the contrary, our results suggest that adaptation to warming amplified the
430 effects of temperature on metabolic as well as grazing rates. The lower interaction strengths
431 in the population of *R. balthica* from the colder stream highlight unexpected long-term
432 effects of temperature on species interactions. Maintenance of lower than anticipated grazing
433 rates in the cold stream could be selected for since lower grazing rates might result in greater
434 food chain stability and/or stoichiometric homeostasis (Cross *et al.*, 2005; 2014) under the
435 prevailing temperature regime. Thus, understanding the impacts of environmental change on
436 the strength of consumer-resource interactions over timescales that are relevant to the rate of
437 climate change (e.g. gradual warming over decades) will require an appreciation both of the
438 direct effects of rising temperatures on species interactions and the reciprocal feedback
439 between ecological and evolutionary dynamics (Fussmann *et al.*, 2007; Gravel *et al.*, 2010;

Loeuille, 2010; Urban, 2013; Barraclough, 2015)

Conclusions

We used a natural geothermal temperature gradient to investigate how warming influences the strength of algal-grazer interactions via the direct effects of temperature on metabolism and consumption, and indirect feedbacks through adaptation. Metabolic rates and interaction strength increased with temperature in the same way for both the warm and cold populations of *R. balthica*, suggesting that rapid changes in temperature have a consistent effect on interactions between mobile consumers and sessile resources, mediated by the effects of temperature on consumer metabolic rates (Dell *et al.*, 2014). However, the warm populations had higher metabolic and grazing rates across all measurement temperatures compared to their colder counterparts. These findings are consistent with the 'hotter is better and broader' hypothesis (Huey & Kingsolver, 1993; Knies *et al.*, 2009; Angilletta *et al.*, 2010) (e.g. adaptation to warming gives rise to higher maximal metabolic rates and broader thermal reaction norms). In consequence, our results suggest that warming could increase the strength of algal-grazer interactions, which are often 'keystone' interactions in aquatic systems, both via the thermodynamic effects of higher temperatures on enzyme kinetics and through correlated increases in *per capita* metabolism and consumption as organisms adapt to warmer temperatures.

Conflict of interest

The authors declare no conflict of interest

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622

623

624 **Tables**

625 **Table 1. Physical and chemical characteristics of the streams**. Temperature data were
626 collected over a 3-day period. All other parameters were collected on the first day of the day
627 of the experiment. Temperature data are displayed as means ± 1SD. All other data were
628 originally collected for correlation with temperature across the catchment area (all 11
629 streams), so that replication was on the level of stream identity.
630

Parameter	Stream 5	Stream 11
Average temperature (ºC, 5 days)	17.5 ± 4.5	28.3 ± 1.3
pH	7.63	7.17
Conductivity	273.6	235.7
NO_2	0.22	0.24
NO_3	0.57	0.29
NH_4	0.17	0.19
PO_4	0.27	0.35

631

632

633

Table 2. Parameter estimates and output from the best fitting gnls model to the thermal response curves of respiration rates. Differences in treatments are given in **bold.** Parameter estimates are taken from the averaged generalised linear models along with their standard errors (± 1 s.e.m). C = cold stream. W = warm stream. See Supporting Information for details on model selection and information on AICsc scores for all possible models. Here, the model average of the conditional average output for the four best models (within 2 AICc units of each other) is displayed.

Non-linear mixed model output for respiration rates (R)		
Treatment effect on	Estimate	± 1 s.e.m.
E_a	C: 0.96	0.05
ln $R(T_c)$	**C: 1.77**	**0.15**
	W: 2.04	**0.12**
E_h	**C: 5.01**	**0.97**
	W: 3.16	**0.96**
T_h **[K]**	**C: 307.16 (34.01)**	**0.94**
(ºC)	**W: 314.15**	
	(41.00)	**1.69**
		0.78
α	0.36	0.03

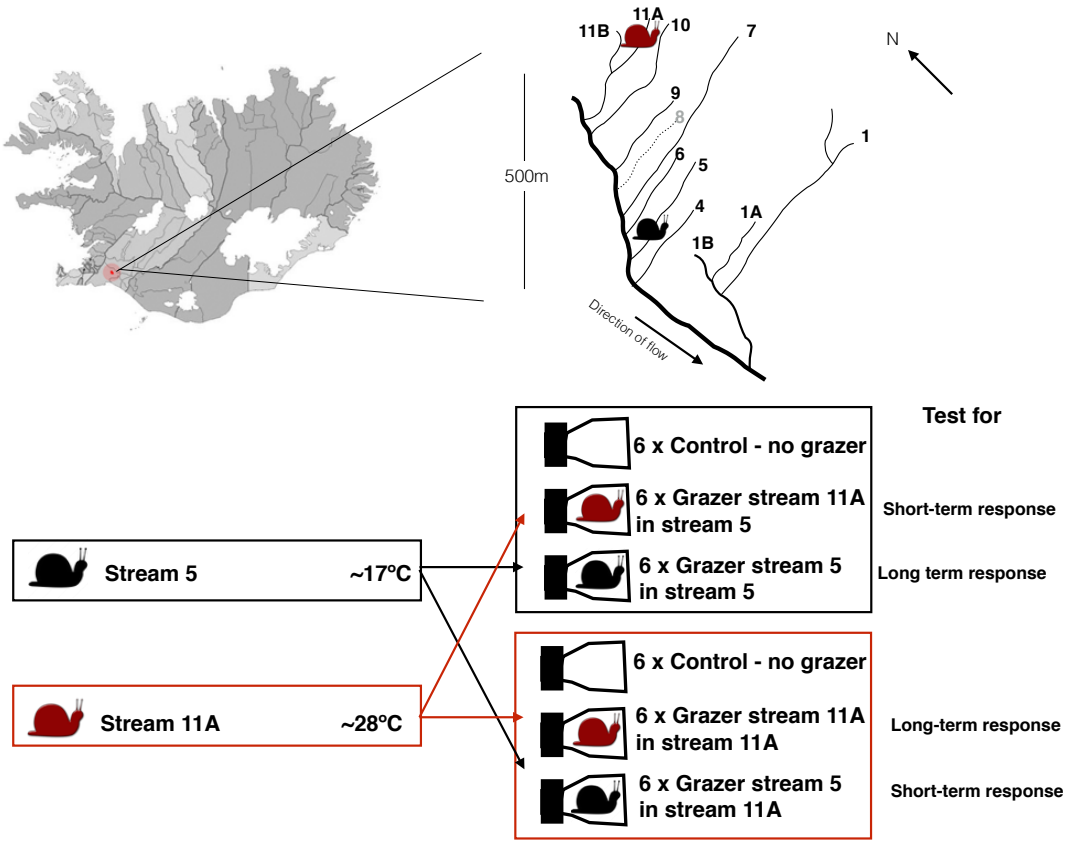
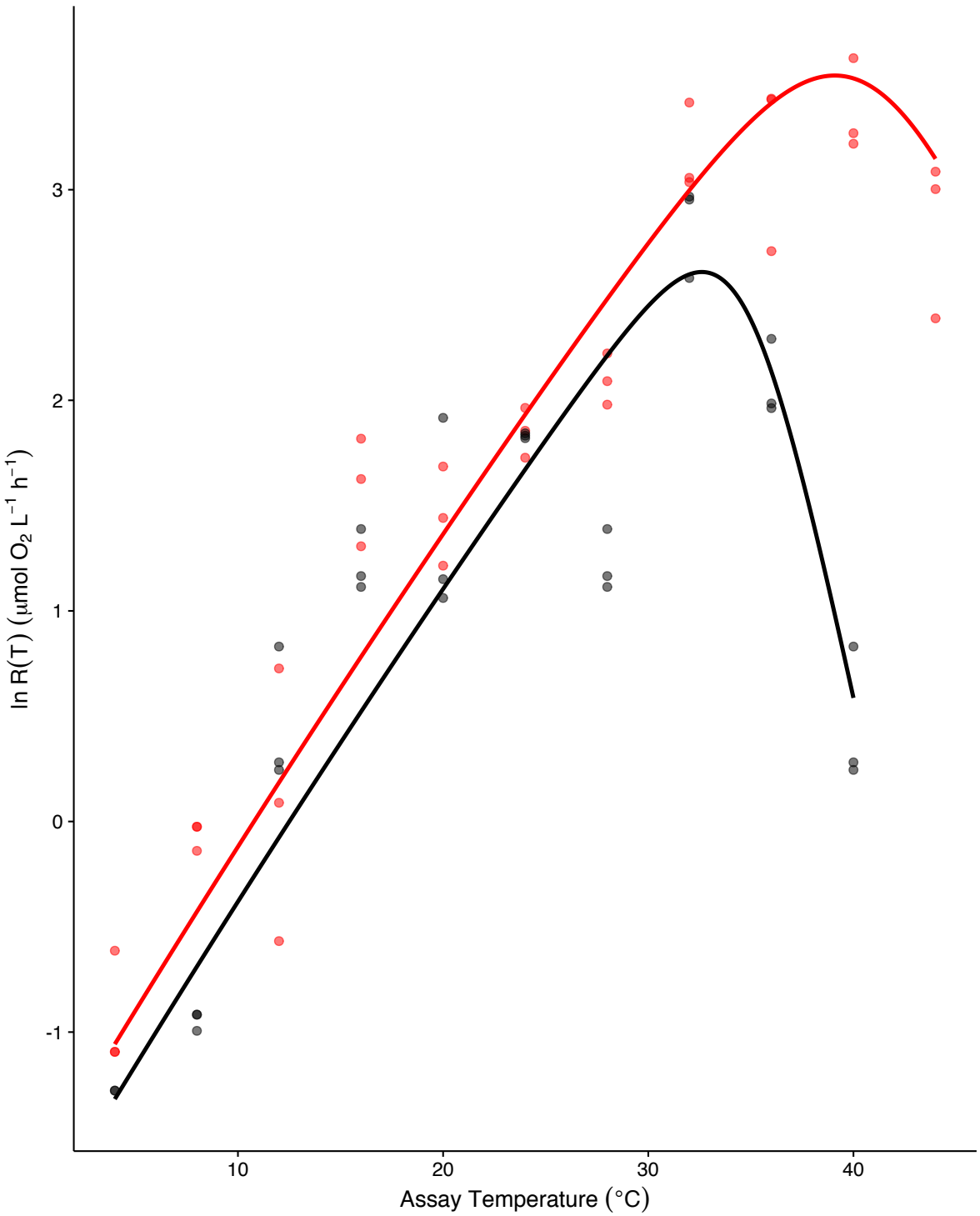
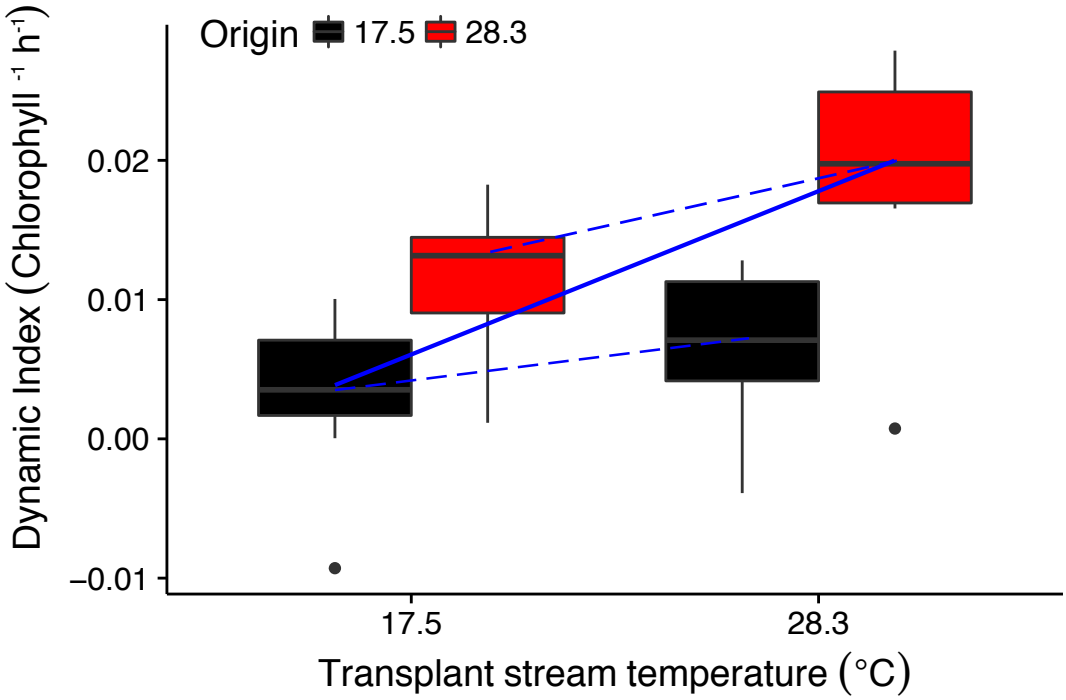


FIGURE 1 Map and experimental set-up. Top panel: The catchment area, with streams used in this experiment indicated by black (for the colder stream 5 with 17.5 ºC ± 4.5 ºC) and red (for the warmer stream 11A with 28.3 ºC ± 1.3 ºC) snail icons. **Lower panel**: Schematic overview of experimental set-up for the grazing experiment.



646
647 **FIGURE 2 Thermal response curves for respiration.** Thermal response curves of respiration rates in µmol
648 O2 L^{-1} h^{-1} as a function of increasing temperature for populations of the snail *Radix balthica* from the cold
649 (black) and warm (red) stream. Lines are derived from fitting a modified Sharpe-Schoolfied equation (see
650 methods) to the rate data. Snails from the warm stream have higher temperature normalised metabolic rates (ln
651 $R(T_c)$, with T_c=18ºC) at all measurement temperatures and have higher optimal temperatures (T_{opt}), than snails
652 from the cold stream. The inactivation energy (E_h) is lower in snails from the warm stream, resulting in a curve
653 that is both broader and elevated in comparison to the thermal response curve of respiration for snails from the
654 cold stream.
655



656

657 **FIGURE 3 Long-term and short-term effects of stream temperature on interaction strength** Long-term
658 and short-term effects of temperature in interaction strength measured via the dynamic index in units of
659 chlorophyll consumed per hour. Populations originating from the warm stream have stronger interaction
660 strength indices in all environments and the highest dynamic index overall was found for snails from the warm
661 stream in their original environment. Interaction strength increased with temperature both in the short-term
662 (E_{short}, dashed blue lines) and in the long-term (E_{long}, solid blue line), with E_{long} significantly greater than E_{short}.
663

Supporting Information

for

Temperature-driven selection on metabolic traits increases the strength of an algal-grazer interaction in naturally warmed streams

Authors

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List of SI Items:

SI Figure 1: The catchment area and experimental set-up.
SI Figure 2: Chlorophyll content in control microcosms.
SI Figure 3: Snail weight for 205 randomly selected snails from each stream
SI Figure 4: Grazer density (snails/m$^{2)}$ in stream 5 (17.5ºC) and stream 11 (28.3ºC)

SI Table 1: AICc comparison details for model simplification of the gnls models fitted to the thermal response data for respiration rates.

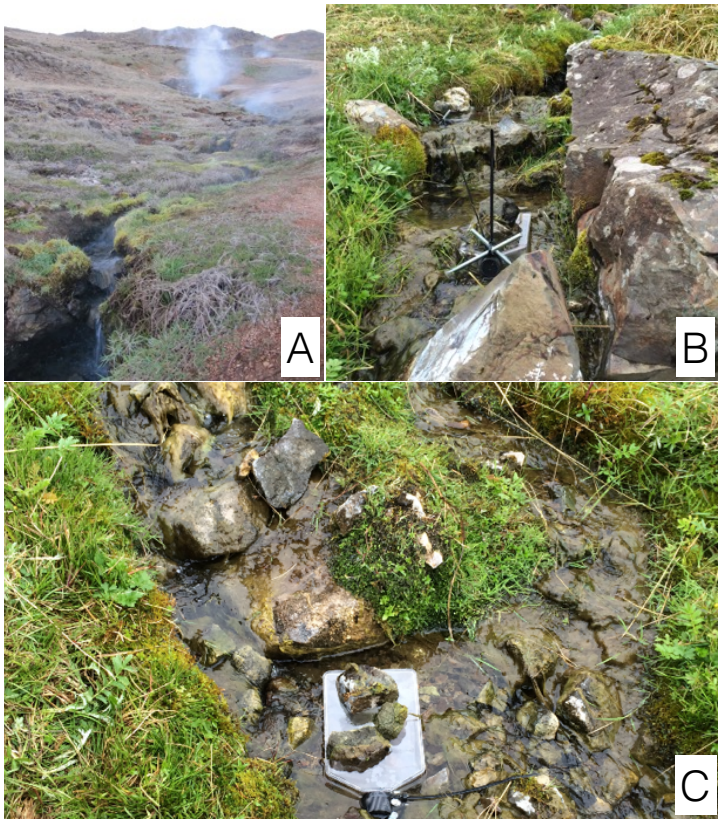

Figure S1: the catchment area and experimental set-up. A: View of representative stream in the Hveragerði catchment area. B and C: Microcosms fixed in stream 5 (Microcosm pictures courtesy of B. Flello).

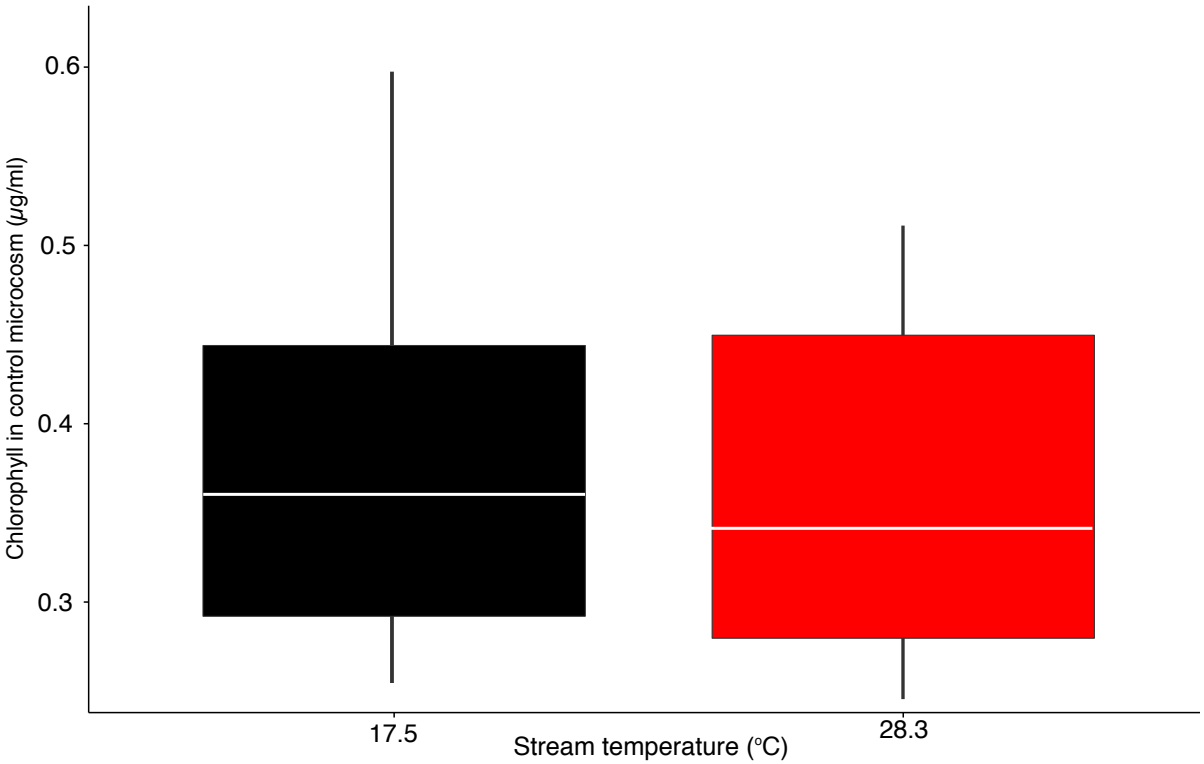

Figure S2: Chlorophyll content in control microcosms. Chlorophyll content at the end of the experiment in control microcosms did not differ significantly between cold and warm streams (one-way ANOVA $F_{1,10}$=1.28, p=0.26) implying growth and mortality of the biofilms was consistent in both treatments.

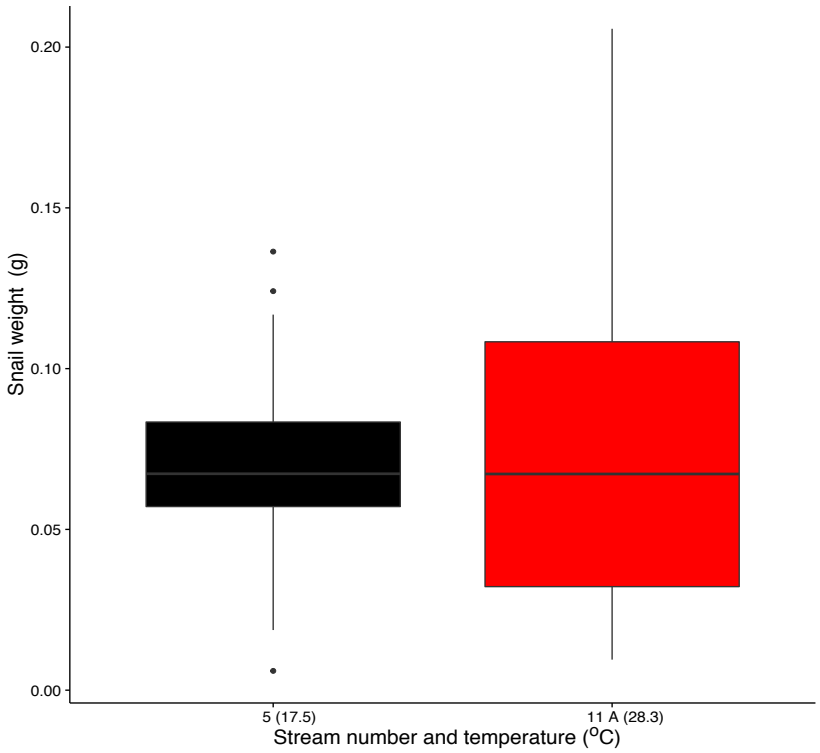

Figure S3: Snail weight for 205 randomly selected snails from each stream. Snail wet weight did not vary significantly between the cold (black) and warm (red) streams (one-way ANOVA: $F_{1,408} = 0.15$, $p = 0.7$).

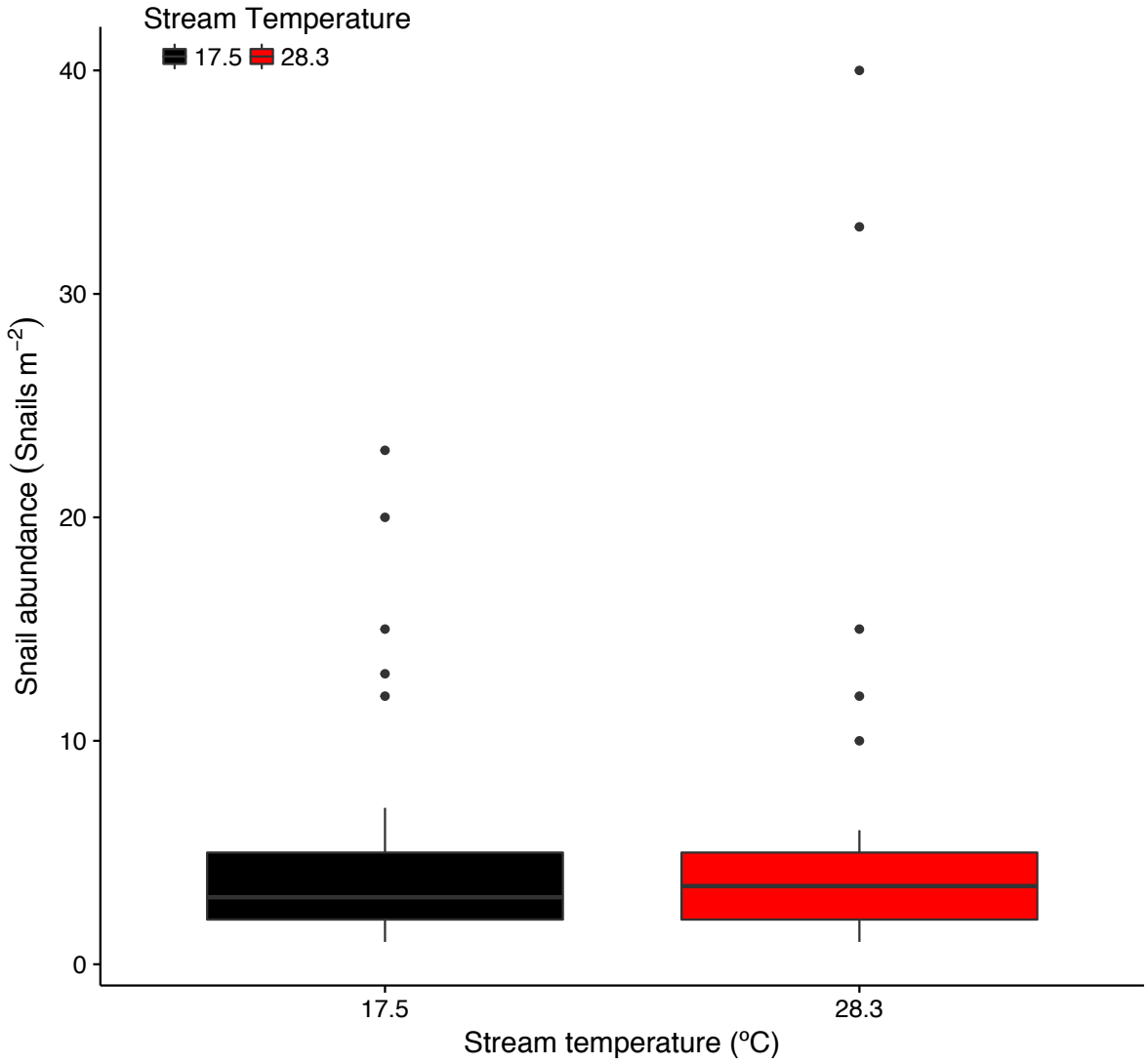

Figure S4: *In-situ* grazer density in the experimental streams. Snails were counted in 34 randomly distributed 1m^2 quadrats. There was no significant difference in snail abundance between streams (one-way ANOVA: $F_{1,66}$, $p = 0.54$). The mean abundance of 5.5 individuals per square meter was comparable to the densities established in the experimetal microcosms. Snails used for the experiments were sampled randomly from these patches.

Table S1. AICc comparison details for model simplification of the gnls models fitted to the thermal response data for respiration rates. Equation 1 was used to account for effects of mass (alpha in the model) on metabolic rate, and fitted to data for respiration (ln R) to characterize acute thermal responses. Model selection was carried out by fist fitting the most complex model to the data and then calculating AICc scores for all possible models with all possible treatment effects, and comparing the models through the aictab function within the AICmodav package (version 2.1-0) in R. The most parsimonious model included differences between warm and cold populations in ln $R(T_c)$, T_h, and E_h, while E_a and α were consistent between populations. For the four models with a difference in AICc of less than two, models were averaged using the modelavg function in the same package. **Lower part of table: Model averaged parameter estimates, with 95% confidence intervals (95%CI) and relative importance as sum of AICc based relative weights (SW), for models where Δ AICc <2.**

Model name	Model details	K	AICC	Dellta AICC	AICc weights	cumulative weights	LogLik
r.gnls19	**alpha+Ea+Eh~ 1, ln.b0+Th~1 + StreamT**	**8**	**84.98**	**0**	**0.26**	**0.26**	**-33.13**
r.gnls7	**ln.b0 +Ea~ 1, alpha+ Eh + Th ~ 1 + StreamT**	**9**	**86.15**	**1.17**	**0.14**	**0.4**	**-32.34**
r.gnls10	**Ea +alpha~ 1,ln.b0+ Eh + Th ~ 1 + StreamT**	**9**	**86.37**	**1.39**	**0.13**	**0.53**	**-32.45**
r.gnls22	**ln.b0+alpha+Ea+Eh~ 1, Th~1 + StreamT**	**7**	**86.51**	**1.53**	**0.12**	**0.65**	**-35.22**
r.gnls13	alpha+Eh~ 1,ln.b0+ Ea+ Th ~ 1 + StreamT	9	87.68	2.7	0.07	0.72	-33.11
r.gnls9	alpha+ Ea + Eh ~ 1 + StreamT,ln.b0 +Th~ 1	9	88.24	3.26	0.05	0.77	-33.39
r.gnls16	ln.b0+alpha+Ea~ 1,Eh+Th~ 1 + StreamT	8	88.81	3.83	0.04	0.8	-35.05
r.gnls1	Ea ~ 1, ln.b0+alpha + Eh + Th ~ 1 + StreamT	10	88.97	3.99	0.04	0.84	-32.33
r.gnls2	ln.b0 ~ 1, alpha +Ea+ Eh + Th ~ 1 + StreamT	10	89.01	4.03	0.03	0.87	-32.35
r.gnls17	ln.b0+alpha+Eh~ 1,Ea+Th~ 1 + StreamT	8	89.12	4.14	0.03	0.91	-35.2
r.gnls3	alpha ~ 1, ln.b0+Ea+ Eh + Th ~ 1 + StreamT	10	89.21	4.23	0.03	0.94	-32.45
r.gnls4	Eh ~ 1, ln.b0+alpha+Ea + Th ~ 1 + StreamT	10	90.02	5.04	0.02	0.96	-32.85
r.gnls5	ln.b0+alpha+Ea+Eh ~ 1 + StreamT,Th ~ 1	10	91.04	6.06	0.01	0.97	-33.36
r.gnls12	ln.b0+ alpha+ Eh ~ 1 + StreamT,Ea +Th~ 1	9	91.24	6.25	0.01	0.98	-34.89
r.gnls6	ln.b0 +alpha~ 1, Ea+ Eh + Th ~ 1 + StreamT	9	91.6	6.61	0.01	0.99	-35.07
r.gnls	Ful model	11	91.97	6.99	0.01	1	-32.34
r.gnls23	Eh~1 + StreamT,ln.b0+alpha+Ea+Th~ 1	7	99.12	14.14	0	1	-41.52

r.gnls18	Ea+Eh~ 1 + StreamT,ln.b0+alpha+Th~ 1	8	102.36	17.38	0	1	-41.82
r.gnls20	ln.b0+Ea~1 + StreamT,alpha+Eh+Th~ 1	8	105.68	20.69	0	1	-43.48
r.gnls8	ln.b0 +Eh~ 1, alpha+ Ea + Th ~ 1 + StreamT	9	106.1	21.12	0	1	-42.32
r.gnls15	ln.b0+ alpha+Ea ~ 1 + StreamT,Eh+Th~ 1	9	107.84	22.86	0	1	-43.19
r.gnls26	Ea~1 + StreamT,ln.b0+alpha+Eh+Th~ 1	7	113.1	28.12	0	1	-48.51
r.gnls25	alpha~1 + StreamT,ln.b0+Ea+Eh+Th~ 1	7	115.95	30.97	0	1	-49.94
r.gnls24	ln.b0~1 + StreamT,alpha+Ea+Eh+Th~ 1	7	116.79	31.81	0	1	-50.36
r.gnls21	ln.b0+alpha~1 + StreamT,Ea+Eh+Th~ 1	8	118.31	33.33	0	1	-49.8
r.gnls27	ln.b0+alpha+Ea+Eh+Th~ 1	6	131.06	46.08	0	1	-58.77
r.gnls11	Ea +Eh~ 1,ln.b0+ alpha+ Th ~ 1 + StreamT	no convergence					
r.gnls14	ln.b0+ Ea+ Eh ~ 1 + StreamT,alpha+Th~ 1	no convergence					

For AICc <2 (bold in upper part of table)

Parameter	lnR(Tc)		Alpha	Ea	Eh		Th [K]	
Stream temperature (ºC)	17.5	28.3	No treatment effect	No treatment effect	17.5	28.3	17.5	28.3
SW (sum of weights)	0.6		0.22	0	0.42		1.00	
Parameter Estimate	1.77	2.04	0.36	0.96	5.01	3.16	307.16	314.15
95% CI [lower,upper]	[0.98-2.77]	[1.09-3.28]	[0.34-0.76]	[0.85-1.07]	[3.07-6.96]	[2.96-5.11]	[305.74-309.99]	[309.04-319.13]