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Plasticity of thermal performance curves in a narrow range endemic water beetle

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1 **Abstract**

2 Thermal history can plastically alter the response of ectotherms to temperature, and thermal
3 performance curves (TPCs) are powerful tools for exploring how organismal-level performance
4 varies with temperature. Plasticity in TPCs may be favoured in thermally variable habitats,
5 where it can result in fitness benefits. However, thermal physiology remains insufficiently
6 studied for freshwater insects despite freshwater biodiversity being at great risk under
7 global change. Here, we assess how acclimation at either summer or winter average
8 temperatures changes TPCs for locomotion activity and metabolism in *Enochrus*
9 *jesusarribasi* (Hydrophilidae), a water beetle endemic to shallow saline streams in SE
10 Spain. This beetle is a bimodal gas exchanger and so we also assessed how aerial and
11 aquatic gas exchange varied across temperatures for both acclimation treatments.
12 Responses of locomotory TPCs to thermal acclimation were relatively weak, but high
13 temperature acclimated beetles tended to exhibit higher maximum locomotor activity and
14 reduced TPC breadth than those acclimated at low temperature. High temperature
15 acclimation increased the thermal sensitivity of metabolic rates, contrary to the response
16 generally found in aquatic organisms. Higher metabolic rates upon high temperature
17 acclimation were achieved by increasing aerial, rather than aquatic oxygen uptake. Such
18 plastic respiratory behaviour likely contributed to enhanced locomotor performance at
19 temperatures around the optimum and thermal plasticity could thus be an important
20 component in the response of aquatic insects to climate change. However, high
21 temperature acclimation appeared to be detrimental for locomotion in subsequent
22 exposure at upper sublethal temperatures, suggesting that this narrow range endemic may
23 be vulnerable to future climate warming. This study demonstrates that TPCs are context-
24 specific, differing with performance metric as well as thermal history. Such context

25 dependency must be considered when using TPCs to predict organismal responses to
26 climate change.

27 **Keywords**

28 acclimation, metabolism, locomotion, climate change, freshwaters, insects

29

30 **1. Introduction**

31 Ectotherms are able to remodel their physiology to reduce the extent to which
32 physiological rates change in response to temperature (compensation via thermal
33 acclimation) (Angilletta, 2009). Such physiological plasticity is crucial for coping with
34 variable thermal regimes and with the increasing temperatures resulting from ongoing
35 climate change (Huey et al., 2012; Sgrò et al., 2016; Arribas et al., 2017; Morley et al.,
36 2019). The way in which components of organismal-level performance vary with
37 temperature can be captured in thermal performance curves (TPCs - Huey and Stevenson,
38 1979; Schulte et al., 2011; Sinclair et al., 2016). Thermal performance curves can be
39 modified by thermal acclimation in multiple ways (da Silva et al., 2019). Thermal
40 acclimation is here defined as a physiological response following exposure to a new
41 temperature for some period of time (ranging from minutes to days or months), or
42 exposure to a temperature during development (a form of acclimation sometimes referred
43 to as developmental acclimation). Such thermal acclimation may alter the position, slope,
44 height, breadth, optimum or shape of TPCs for performance metrics such as metabolic
45 rate, locomotion, feeding rate, and growth rate (e.g. Johnson and Bennet, 1995;
46 Lachenicht et al., 2010; Seebacher and Grigaltchik, 2014; Bozinovic et al., 2016) (Fig.
47 1).

48 Amongst ectotherms, thermal acclimation has been shown to generally buffer the effects
49 of temperature in freshwater and marine animals. For example, although physiological
50 rates tend to increase with increasing temperature, the increase tends to be less
51 pronounced in aquatic organisms following thermal acclimation, i.e. they exhibit a
52 reduced thermal sensitivity (e.g. Seebacher et al., 2015b). Similarly, warm acclimation
53 may increase an individual's ability to cope with heat stress (e.g. Gunderson and Stillman,
54 2015; Semsar-kazerouni and Verberk, 2018). However, acclimation responses may

55 depend on body size, sex, feeding status of the animals tested and experimental conditions
56 (e.g. the duration of the experimental trial), and may show a high context-dependency
57 across taxonomic groups, geographical regions and realms (e.g. Rohr et al., 2018;
58 Semsar-kazerouni and Verberk, 2018; Gunderson and Stillman, 2015). Furthermore,
59 thermal physiology still remains insufficiently studied for some groups of organisms,
60 such as aquatic insects, meaning that it is unclear whether they conform to the general
61 patterns seen in other aquatic taxa. Indeed, it has been recently demonstrated that the
62 thermal sensitivity of metabolic rates shows substantial variation between groups of
63 aquatic insects (Shah et al., 2020). With freshwater biodiversity being at greater risk than
64 that in any other ecosystem type (Allan and Flecker, 1993; Master, et al., 1998; Albert et
65 al., 2021), it is therefore critical that we improve our understanding of how aquatic insects
66 respond to increasing temperatures.

67 Thermal plasticity may be favoured in thermally variable habitats, as it can result in
68 fitness benefits in such environments (Gabriel et al., 2005; Angilletta, 2009; da Silva et
69 al., 2019). However, terrestrial animals appear to have in general weaker acclimation
70 abilities than aquatic ones (Seebacher et al., 2015), despite generally greater temperature
71 variability in terrestrial habitats compared to aquatic ones. Insects that inhabit
72 Mediterranean inland waters represent ideal study models for exploring the effects of
73 acclimation on thermal performance in variable aquatic systems. The Mediterranean
74 climate is typically defined by large daily and seasonal thermal fluctuations, with hot, dry
75 summers, and cool, wet winters (Paskoff, 1973; Hertig and Jacobeit, 2011; Bonada and
76 Resh, 2013). Some species from these habitats possess significant physiological plasticity
77 in traits related to salinity and desiccation stress tolerance (Pallarés et al., 2017; Botella-
78 Cruz et al., 2019), as well as broad thermal tolerance ranges and the capacity for
79 acclimation of critical thermal limits (e.g. Sánchez-Fernández et al., 2010; Arribas et al.,

80 2012; Botella-Cruz et al., 2016; Carbonell et al., 2017). However, the thermal tolerance
81 of these species has been typically estimated by environmentally unrealistic approaches,
82 employing fast heating rates, that tend to overestimate tolerance limits (Terblanche et al.,
83 2007; Rezende et al., 2014). Thermal stress depends on both heat intensity and the
84 duration of exposure (Rezende et al., 2014) and consequently trials employing fast
85 heating rates might underestimate acclimation capacity compared to slower heating rates
86 or static methods (e.g.: Moyano et al., 2017, Semsar-kazerouni and Verberk, 2018;
87 Salachan et al., 2019). Furthermore, other responses such as shifts in energy metabolism
88 or locomotion, which are major components of an organism's ability to cope with
89 changing environments (Domenici et al., 2007; Dillon et al., 2010; Bahrndoff et al.,
90 2016), have not been fully explored in the context of thermal plasticity (but see Carbonell
91 et al., 2017).

92 Insect locomotion is highly temperature dependent and is progressively impaired outside
93 optimal temperature ranges (Berrigan and Partridge, 1997), long before effects are
94 observed on survival (Kjærsgaard et al., 2015). Locomotion has been shown to be a plastic
95 behavioural trait, influenced by developmental and adult temperature in insects
96 (Angilletta et al., 2002; Barnhdoff et al., 2016). Acclimation may affect different, non-
97 mutually exclusive components of the insect locomotory TPC (Huey and Kingsolver,
98 1993). For example, high temperature acclimation might: i) shift thermal optima (T_{opt})
99 towards higher temperatures (e.g.: Gilchrist et al., 1997), improving performance at
100 higher temperatures, but reducing it at lower ones (Fig. 1a); ii) increase maximum
101 performance (i.e. shift TPC height, Fig. 1b) (Bozinovic et al., 2013); iii) increase critical
102 thermal maximum (CT_{max}) and TPC breadth (Jurriaans and Hoogenboom, 2020) (Fig. 1c)
103 or iv) boost performance at high temperatures at the expense of reducing maximum
104 performance (Seebacher et al., 2015a) (Fig. 1d). Whilst higher locomotor activity is often

105 interpreted as indicating better performance (Angilletta et al., 2002), the interpretation of
106 respiratory reaction norms may be more complex. Higher oxygen consumption rates
107 provide more energy for fitness enhancing processes, but may also imply elevated
108 baseline energetic costs (Pörtner, 2001; Pörtner and Knust, 2007; Magozzi and Calosi,
109 2015; Verberk et al., 2016). In aquatic ectotherms, the exponential increase in oxygen
110 demand with increasing temperature can cause a progressive mismatch between supply
111 and demand (Verberk et al., 2011), which may decrease organismal performance
112 (Pörtner, 2010; Verberk and Bilton, 2013). In freshwater organisms, warm acclimation
113 has shown to decrease oxygen demand at high temperatures and reduce the thermal
114 sensitivity of metabolism (Seebacher et al., 2015b; Semsar-kazerouni and Verberk, 2018).
115 Alternatively, aquatic ectotherms can enhance oxygen uptake and by doing so meet
116 increased demand; for example, Verberk and Bilton (2015) found that a bimodal gas
117 exchanging water bug (i.e. one using both a physical gill and surface exchange) relied
118 increasingly on aerial gas exchange with warming. Aerial gas exchange was likewise
119 argued to be important for high heat tolerance in tropical decapods (Giomi et al., 2014;
120 Fusi et al., 2015). However, no study so far has explored whether reliance on aerial gas
121 exchange is affected by acclimation temperature.

122 Our aim here was to assess the extent to which thermal acclimation alters i) the TPCs for
123 locomotion activity, ii) the thermal sensitivity of metabolic rate and iii) the dependency
124 on aerial gas exchange in a bimodal breathing aquatic insect from a thermally variable
125 habitat. For this, we used the water beetle *Enochrus jeusarribasi* Arribas and Millán,
126 2013 (Coleoptera: Hydrophilidae), which is endemic to intermittent saline streams in a
127 semiarid Mediterranean region (Southeast Spain). We expect this species to be capable
128 of beneficially adjusting performance to acclimation conditions (Fig. 1), as an adaptation
129 to life under the thermally variable conditions experienced in its habitat. However,

130 regional climatic models for southern Europe stress that the Mediterranean is likely to be
131 an especially vulnerable region to global change (Sánchez et al., 2004; Giorgi and
132 Lionello, 2008). Higher, more variable and unpredictable temperatures are anticipated,
133 resulting in novel environmental conditions in the aquatic ecosystems of this area. In such
134 a context, endemic saline species, which often occur as highly isolated populations in
135 these fragmented habitats (e.g. Abellán et al., 2007), may be particularly vulnerable. This
136 may be especially true for lotic species (such as our study beetle), which have low
137 dispersal capacity (Ribera, 2008) and therefore limited potential for range shifts under
138 climate change (Arribas et al., 2017). A better knowledge of thermal performance and its
139 plasticity in such species is important if we are to gain insights into their scope for
140 resilience to climate change via physiological buffering.

141 **2. Material and methods**

142 *2.1. Study species, collection and housing*

143 *Enochrus jeusarribasi* inhabits meso and hypersaline streams in the south and southeast
144 of Spain, where, despite its fragmented distribution, it is often highly abundant within
145 suitable localities. Adults are bimodal breathers that maintain an air store under the elytra
146 into which the functional spiracles open. This air store connects with an air film supported
147 by hydrofuge hairs over a large part of the ventral surface, which acts as a compressible
148 gas gill so that oxygen can be extracted from the water (ref). For gas exchange at the
149 water surface, they break the surface tension with their antennae, forming an air channel
150 between the atmosphere and the ventral air store (Yee and Kehl, 2015).

151 Adult specimens of *E. jeusarribasi* were collected in June 2020 in Rambla Salada, an
152 intermittent hypersaline stream located in Murcia (SE Spain). This area is characterized
153 by high daily and seasonal thermal variation (e.g: water temperature records in Rambla

154 Salada showed a daily thermal variation of 10°C in summer and 5°C in winter, and
155 seasonal variation ranging between 15-22°C; Velasco et al., 2006; Velasco, unpublished
156 data). Water conductivity and temperature at the time of collection, measured with a
157 conductivity-meter (HACH/Hq40d, Hach®, US), were 70 mScm⁻¹ and 24.3°C,
158 respectively. *Enochrus jesusarribasi* has an extensive osmoregulation capacity, resulting
159 in high survival across a wide range of salinities in the laboratory (Pallarés et al., 2015).
160 Specimens were kept in the laboratory in aerated tanks (25 x 20 x 15 cm) in rooms with
161 controlled temperature (20 ± 1°C) and a 12:12 h photoperiod at 35 g l⁻¹ (approx. 50 mScm⁻¹
162 at 20°C, made up using Instant Ocean® salt) for 5 days before the experiments. A
163 maximum of 15 specimens were placed in each tank. During this period and for the entire
164 duration of the experiments, food was provided *ad libitum* (algae and macrophytes
165 collected in Rambla Salada: *Cladophora* sp. and *Ruppia* sp) and the water was renewed
166 every 2-3 days.

167 2.2. Estimating Thermal Performance Curves and their plasticity

168 To assess whether *E. jesusarribasi* had the capacity to shift its TPC following acclimation,
169 specimens were exposed to different acclimation temperatures in rooms with controlled
170 conditions, simulating typical summer (average temperature of 25°C) or winter conditions
171 (average temperature of 10°C) in its collection locality (Velasco et al., 2006), for 5 days.
172 After this acclimation phase, we measured locomotor performance and routine metabolic
173 rate at seven test temperatures, from 5 to 35°C. Independent groups of beetles were used
174 for the measurement of locomotor performance and metabolic rate (N=8 and 10
175 individuals per acclimation treatment and test temperature, respectively). To avoid
176 exposing specimens to an abrupt thermal shift from the acclimation to the test
177 temperature, it was gradually increased or decreased the day before the trials, at a rate of
178 1°C h⁻¹, in a programmable incubation chamber (Sanyo MIR253, Sanyo Electric, Co. Ltd,

179 Japan). Once the corresponding test temperature was reached, it was maintained constant
180 for 2h before starting measurements.

181 *2.2.1. Locomotor performance*

182 To measure locomotor parameters, four specimens were placed individually in four
183 identical circular open tanks of 9.5 cm diameter (arenas hereafter) containing saline water
184 (35 g l^{-1} , 1.5 cm depth). A piece of foam stuck with aquarium safe silicon (Betta Aquatic
185 Products, UK) in the bottom and partially emergent provided structure for either resting
186 underwater or climbing out of the water (a typical behavioural stress-avoidance response
187 in water beetles, see Pallarés et al., 2012). After 30 min for habituation to the arenas, the
188 animals were filmed from above with a GoPro Hero7 Silver camera (GoPro Inc., USA)
189 for 30 min. The procedure was then repeated with another set of four specimens. Videos
190 were analysed with EthoVision XT 14 (Noldus, Netherlands) tracking software, wherein
191 the arenas were divided into resting and active zones (Fig. S1). We obtained different
192 parameters of locomotor performance (distance, mean and maximum velocity, activity
193 and mobility state and time spent in the sponge; see Table S1 for details).

194 *2.2.2. Metabolic rate*

195 Routine metabolic rate was measured using closed respirometry and a similar procedure
196 to that described by Verberk and Bilton (2015) and Scholten et al. (2018) to estimate
197 aerial and aquatic respiration in bimodally breathing insects. We used 2 ml respiratory
198 chambers initially fully filled with sterilized saline water (35 g l^{-1}). Immediately after an
199 animal was inserted, we injected 0.6 ml of air saturated with water vapour and
200 temperature equilibrated. This left an air bubble at the top of the chamber (“air
201 compartment”) that provided sufficient space for the animal (body size: 4.4 – 6 mm) to
202 perform aerial gas exchange. A piece of mesh towards the bottom of the water

203 compartment was provided for the beetles to rest on. Oxygen measurements were made
204 at each test temperature by immersing the chambers in a programmable recirculating
205 water bath (Grant R5 TXF200, Grant Instruments Ltd, UK). The beetles were left
206 undisturbed for 20 min before the actual measurements commenced, for habituation and
207 to reach thermal equilibrium. Oxygen consumption in the air compartment was measured
208 at 5 min intervals using micro-optodes connected to a Fibox 4 fiber optic oxygen meter
209 (PreSens instruments, Germany). Oxygen tensions in the water compartment were
210 measured at 20 min intervals by carefully inverting the respiratory chamber to displace
211 the air bubble and allow the optode to come into contact with the water. Linear regressions
212 were fitted to calculate oxygen consumption rates in each compartment. Rates were
213 corrected for background respiration, which was measured by triplicate blanks at each
214 test temperature.

215 Each experiment (metabolism or locomotion performance) lasted 16 days. Mortality was
216 checked daily in the acclimation tanks and also in three control groups at 20°C (N=15
217 individuals each), being comparably low in both cases (< 15%).

218 *2.3. Data analyses*

219 To evaluate the effect of acclimation (previous exposure to low or high temperatures) on
220 locomotor performance, we used the R package *rTPC* following the method described by
221 Padfield et al. (2021). For simplicity and because some of the locomotor parameters were
222 highly correlated (e.g. distance and mean velocity, see Fig. S2), these analyses were made
223 on three parameters that represented different locomotor responses: distance, maximum
224 velocity and mobility. For each variable, we fitted 13 different TPC models (Table S2)
225 using non-linear least squares (NLLS) regression. Data were log transformed to improve
226 normality. Previous analyses (Gaussian GLMs) were made including sex and wet mass
227 as covariates, but these were excluded from subsequent models as they were found to

228 have no significant effects. Models were ranked by Akaike's Information Criterion,
229 corrected for small sample size (AICc) and we selected the model that on average had the
230 lowest AICc values for each of the three locomotor variables considered, in order to have
231 comparable model parameters for all locomotor traits. Besides the specific model
232 parameters (see results), we obtained the following derived TPC parameters: maximum
233 rate (r_{\max}); optimum temperature (T_{opt}), that is, the temperature where maximum rate is
234 achieved and thermal breadth (T_{br}), the range of temperatures over which the curve's rate
235 is at least 0.8 of peak rates. Uncertainty in the model fit and parameter estimates was
236 assessed by bootstrapping. To avoid having some resampled datasets lacking points
237 beyond the T_{opt} , we used residual bootstrapping (i.e. new datasets were created from the
238 mean centred residuals of the original model fit). Some signs of severe stress (e.g. total
239 immobility, or abrupt random swimming and escape attempts) were observed in
240 locomotor trials at the highest tested temperature (35°C). Consequently, models were
241 fitted excluding this treatment to allow a consistent interpretation of locomotor patterns
242 across temperatures (i.e. higher locomotor activity equates to better performance), and
243 significant differences in locomotor performance at 35°C were independently assessed by
244 Mann-Whitney tests.

245 We analyzed the effect of acclimation temperature on total metabolic rates using linear
246 regressions with test temperature, medium (aerial or aquatic compartment), acclimation
247 temperature and their interactions as predictors, and wet mass as a covariate. We used the
248 Arrhenius transformation of metabolic rates, which presents log-transformed rates as a
249 function of inverse temperature, $(kT)^{-1}$, where k is the Boltzmann constant ($eV K^{-1}$) and
250 T is absolute temperature (K). The slope of this relationship is determined by activation
251 energy (E_a) and reflects the sensitivity of metabolism to changes in temperature. A
252 significant interaction between acclimation and test temperature would then denote a

253 difference in thermal sensitivity between low and high temperature acclimated groups.
254 Linear regression was also used to assess the effect of acclimation on the proportion of
255 aerial respiration over the total respiration. Because acclimation effects on metabolism
256 might differ under colder, suboptimal and warmer, supraoptimal temperatures, we also
257 compared metabolic rates and activation energies between acclimation treatments
258 separately during the ascending and descending phase of the locomotor TPCs.
259 Considering the range of T_{opt} obtained from locomotor TPCs (see results), the
260 temperatures 5-25°C (suboptimum) and 20-35°C (supraoptimum) were used,
261 respectively.

262 All the analyses were performed in R version 4.0.4 (R Core Team, 2019).

263 **3. Results**

264 *3.1. Locomotor performance*

265 Locomotor parameters in general showed the typical unimodal response of TPCs in the
266 temperature range between 5 and 30°C, and a relatively high inter-individual variation
267 within test temperatures (Fig. 2; see also Fig. S2). The different TPC models fitted for
268 distance, maximum velocity and mobility are shown in Figs. S3-S5. The *flinn* model was
269 selected according to AICc values (Table S3).

270 The parameter values estimated from the selected TPC model did not differ between
271 acclimation treatments (95% CIs overlapped) except from mobility r_{max} , which was
272 significantly higher in high than low temperature acclimated individuals (Table 1, Fig.
273 2c). Despite no significant differences, high temperature acclimated individuals tended to
274 show higher increases and decreases of the locomotor TPCs (steeper slopes) in the
275 ascending and descending phase of the curve, respectively, and a lower thermal breadth,

276 especially for distance and mobility (Table 1, Fig. 2). TPCs peaked between 20.9-23.8°C
277 across the different locomotor variables and acclimation treatments (Table 1).

278 At 35°C, some specimens showed signs of severe stress (e.g.: total immobility, abrupt
279 movements or escape attempts), which coincided with a breakpoint in the TPC for some
280 locomotor parameters (Fig. S2). At this highest test temperature, high temperature
281 acclimated individuals showed lower activity than low temperature acclimated ones (they
282 were less mobile and travelled less distance; see Fig. 3a, c) but had a tendency to display
283 more rapid and abrupt swimming (maximum velocity was close to being significantly
284 higher in this group) (Fig. 3b).

285 *3.2. Metabolic rate*

286 Total metabolic rates increased with temperature ($F_{1,243}=375.3$, $P<0.001$; Figure 4a) and
287 body mass ($F_{1,243}=3016.5$, $P<0.001$). High temperature acclimated beetles displayed
288 lower metabolic rates at lower test temperatures than those acclimated at low temperature,
289 whereas at higher temperatures, the opposite was true, with the higher thermal sensitivity
290 in high temperature acclimated beetles resulting in higher metabolic rates (Fig. 4a). The
291 increase in oxygen uptake with temperature was context dependent, differing between
292 acclimation treatments and between aerial and aquatic oxygen uptake rates as well as their
293 interaction (Test temperature x Medium x Acclimation temperature: $F_{1,243}=5.7$, $P=0.017$).
294 Most oxygen was taken up from the air compartment with aerial oxygen uptake rates
295 being on average 18-fold higher than aquatic oxygen uptake rates. The proportion of
296 oxygen taken from the air compartment increased with temperature ($F_{1,117}=23.3$,
297 $P<0.001$), especially in the high temperature acclimated beetles (Test temperature x
298 Acclimation temperature: $F_{1,117}=4.3$, $P=0.041$; Fig. 4b).

299 At suboptimum temperatures (i.e. temperature range 5-25°C), high temperature
300 acclimated beetles showed a significantly higher thermal sensitivity of aerial metabolic
301 rates than low temperature acclimated ones (Test temperature x Acclimation temperature:
302 $F_{1,80}=5.0$, $P=0.028$; $E_a=0.32$ and 0.56 eV, respectively); whilst activation energies of
303 aquatic respiration were similar between both acclimated groups ($E_a=0.30$ and 0.28 eV
304 for warm and cold-acclimated groups, respectively) (Fig. 5). At supraoptimum
305 temperatures (20-35°C), aerial respiration rates were higher in beetles from the high
306 temperature treatment (Acclimation temperature: $F_{1,73}=6.2$, $P=0.015$) but activation
307 energies were similar between cold (0.38 eV) and warm-acclimated beetles (0.42 eV).
308 For aquatic respiration, high temperature acclimated beetles showed a tendency for higher
309 thermal sensitivity ($E_a=0.39$ eV) than low temperature acclimated ones ($E_a=0.19$ eV)
310 (Test temperature x Acclimation temperature: $F_{1,73}=3.3$, $P=0.072$) (Fig. 5).

311 **4. Discussion**

312 Climates characterized by high thermal variability are expected to select for organisms
313 with high physiological plasticity. Accordingly, for *E. jesuarribasi*, acclimation at high
314 temperature was expected to alter locomotor TPCs so that performance is optimized under
315 warmer temperatures (Fig. 1), and to decrease the thermal sensitivity of metabolic rates
316 (e.g. Seebacher et al., 2015b; Semsar-kazerouni and Verberk, 2018). However, our results
317 did not fully conform to such predictions and suggest a more complex picture of the
318 effects of thermal acclimation on locomotion and metabolism in this endemic water
319 beetle.

320 We found relatively weak responses of locomotion TPCs to temperature acclimation,
321 which may in part be due to the high inter-individual variation in locomotor activity
322 within treatment groups, typical of insect locomotor tests (e.g. Lachenicht et al., 2010).
323 Nevertheless, beetles acclimated at high temperature tended to exhibit higher locomotor

324 performance at optimum temperature ranges (20-25°C) but a reduced breadth of
325 locomotor TPCs, suggesting a cost of acclimation under sub and supraoptimum exposure
326 temperature (Hoffmann et al., 2003, Barhndoff et al., 2016). At the higher test temperature
327 (35°C), low temperature acclimated beetles showed higher locomotor activity (distance
328 travelled and mobility) than those from the high temperature treatment. The interpretation
329 of locomotor activity in terms of organismal performance at sublethal temperatures is not
330 straightforward. Some (unsuccessful) escape attempts by flight were observed in both
331 acclimation treatments; so higher mobility could be related with such escape behaviour
332 and then it would mean that beetles acclimated at low temperature were more stressed.
333 However, some specimens, especially high temperature acclimated ones, also showed
334 total immobility for the whole duration of the trial. The incapacity to perform coordinated
335 movement is also a typical sign of thermal stress in insects (Vannier, 1994; Lutterschmidt
336 and Hutchison, 1997; Gallego et al., 2016). Measurement of other stress-related traits
337 (e.g. molecular stress biomarkers) at sublethal temperatures may help elucidate the
338 responses observed here.

339 High temperature acclimation increased the thermal sensitivity of metabolic rates in *E.*
340 *jesusarribasi*. Seebacher et al. (2015b) found the opposite pattern in ectotherms, including
341 freshwater organisms, using a meta-analysis on thermal plasticity of metabolic rate and
342 other physiological rates. However, their dataset only included one aquatic insect species,
343 for which no effect of acclimation was found (Ferris and Wilson, 2012). Neither did
344 acclimation temperature affect the metabolic rate of a Mediterranean population of the
345 saline corixid *Sigara selecta* (Carbonell et al., 2017), but its effects on thermal sensitivity
346 were not measured. Furthermore, specimens of *Sigara selecta*, a bimodal breather, did
347 not have access to aerial gas exchange in their study, which might mask the effect of
348 acclimation on metabolic rates (see below). Lack of comparative studies makes it

349 impossible to assess the extent to which the response of metabolism to acclimation in *E.*
350 *jesusarribasi* reported here is common to other aquatic insects. However, in view of the
351 variation in metabolic rate reaction norms found between and within insect groups (Shah
352 et al., 2020), disparity in how acclimation affects metabolic TPCs could be also expected
353 among them.

354 Differences in total metabolic rates between differently acclimated beetles were mainly
355 associated with changes in either the magnitude or thermal sensitivity of aerial respiration
356 rates, which were much higher than aquatic rates. Compared to air, gas exchange is more
357 difficult under water due to lower rates of oxygen diffusion (Verberk et al., 2011) and
358 higher costs of ventilation owing to the higher viscosity and density of water (Verberk &
359 Atkinson, 2013). Accordingly, with warming-induced increases in metabolic rate, beetles
360 relied increasingly on aerial oxygen uptake. Similar results were obtained in the unrelated
361 bimodal gas exchanging hemipteran *Ilyocoris cimicoides* (Verberk and Bilton, 2015),
362 and freshwater gastropods (Jones, 1961). Interestingly, in our study, such behaviour was
363 more pronounced in individuals acclimated at high temperature. The mode of respiration
364 of aquatic insects has been linked to the extent to which oxygen limitation affects heat
365 tolerance (Verberk and Bilton, 2015). In light of the effect of thermal acclimation in
366 respiratory behaviour observed here, it would be interesting to explore whether the effect
367 of acclimation temperature on performance and heat tolerance might differ amongst
368 aquatic insects with different respiratory modes. For dytiscid beetles this appeared indeed
369 to be the case (ref).

370 The increased oxygen uptake, and the increased reliance on aerial gas exchange in high
371 temperature acclimated beetles is consistent with their tendency of enhanced maximum
372 locomotor performance compared to low temperature acclimated ones. Acclimation
373 temperature was also found to increase the upper lethal limits of *E. jesusarribasi* in a

374 previous study (Arribas et al., 2012). However, as stated above, the lower locomotion
375 activity of high temperature acclimated beetles at 35°C observed in our experiment could
376 be an indicator of sublethal stress. Impaired locomotion might be a consequence of
377 sustained elevated metabolic rates both during acclimation and the subsequent exposure
378 to 35°C, which could have negatively impacted the energy budget by increasing baseline
379 energetic costs (Magozzi and Calosi, 2015; Shah et al., 2020). Such potential sub-critical
380 effects of acclimation temperature might not have been captured by Arribas et al.
381 (2012) as they employed a rapid heating rate to estimate CT_{max} . Alternatively, it is
382 possible that physiological (heat tolerance) and behavioural (locomotion performance)
383 thermal limits show uncorrelated responses to acclimation, as has been recently found in
384 response to artificial selection in *Drosophila suboscuro* (Mesas et al., 2021). Then,
385 despite the overall beneficial effect of acclimation temperature on metabolism and
386 locomotion observed in *E. jesusarribasi*, and its capacity to enhance acute heat tolerance
387 upon acclimation (Arribas et al., 2012), it should be evaluated whether exposure to
388 sublethal high temperatures compromises performance and fitness in the longer term.

389 Given the unprecedented rates of climate change, organisms will have to rely partly on
390 plastic responses, because adaptation via evolutionary changes might be too slow,
391 phylogenetically constrained or limited by low genetic variation (Kellermann et al., 2012;
392 Kelly et al., 2013; Mesas et al., 2021). In such a context, the investigation of TPC for
393 multiple physiological traits and their plasticity is a powerful approach to provide more
394 accurate predictions of species vulnerability (Sinclair et al., 2016; Kellermann et al.,
395 2019). However, thermal plasticity may not be sufficient to keep pace with climate
396 warming (van Heerwaarden et al., 2016). *Enochrus jesusarribasi* shows some degree of
397 plasticity in its locomotion TPCs, and a much greater plasticity of metabolic rates, through
398 the capacity to increase aerial oxygen uptake in response to thermal acclimation.

399 However, the extent to which such acclimation capacity could improve performance at
400 high temperatures is unclear. In the locality used for this study, water temperatures can
401 exceed 35°C in summer (Velasco et al., 2006). The maximum summer air temperature is
402 32.6°C and it is predicted to increase between 1.6 and 5.9°C by 2070, considering the
403 Representative Concentration Pathway (RCP) 4.5 (source: average maximum daily
404 temperature of the warmest month from Worldclim v. 1.4 database;
405 <http://www.worldclim.org>). This species could, therefore, already experience potentially
406 stressful temperatures in nature and would have a narrow thermal safety margin under
407 future climate change scenarios. Such thermal safety margins could be wider if other life-
408 stages show higher thermal tolerance and plasticity than adults, but that seems unlikely
409 for this (and related) species, given the higher stress sensitivity of larvae compared to
410 adults of the studied species (Botella-Cruz et al., 2017) and other aquatic Coleoptera
411 (Pallarés et al., 2020). Theoretically at least, aquatic insects could to some extent mitigate
412 the effects of warming through microhabitat selection, but most shallow aquatic habitats
413 have relatively low spatial variability in thermal conditions, likely limiting the
414 possibilities for behavioural thermoregulation (Gunderson and Stillman, 2015).

415 **5. Conclusions**

416 The narrow range, endemic water beetle *E. jesusarrabasi* shows significant plasticity in
417 metabolic TPCs, as expected for an organism from a thermally variable habitat. However,
418 acclimation effects differed from the general patterns seen in other freshwater organisms,
419 revealing the need for further research on comparative thermal physiology in aquatic
420 insects. This bimodal gas-exchanging beetle exhibited higher metabolic rates following
421 high temperature acclimation, when it increased its aerial, and overall, oxygen uptake, a
422 behaviour that likely contributed to its enhanced locomotor performance.

423 Our results suggest that thermal plasticity could be an important component in the
424 response to climate change in aquatic insects living in thermally variable environments,
425 but also strikes a note of caution, revealing that these responses may be complex and
426 context specific. Thermal responses depend on thermal history and likely differ between
427 performance metrics. Such context dependency needs to be taken into account when
428 predicting organismal responses to a warming world.

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438 **Competing interests**

439 The authors have no competing interest to declare.

440 **Data availability**

441 Data supporting this article will be available online at Figshare upon publication (private
442 link: <https://figshare.com/s/a707ee09231b9d2d4aa5>)

443 **Author contributions**

444 All authors contributed to the study. Susana Pallarés: conceptualization, funding
445 acquisition, methodology, investigation, formal analysis, writing- original draft, project
446 administration. Wilco CEP Verberk: methodology, formal analysis, validation and

447 writing- review and editing. David T Bilton: conceptualization, funding acquisition,
448 resources, validation and writing- review and editing.

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692

693 **Tables**

694 Table 1. Parameter estimates with 95% confidence intervals from locomotor TPC
 695 models. a: parameter that controls the height of the TPC; b: parameter that controls the
 696 slope of the initial increase of the TPC; c: parameter that controls the position and
 697 steepness of the decline of the TPC; r_{max} : maximum rate; T_{opt} : optimum temperature
 698 (°C); T_{br} : thermal breadth (°C). Parameters that differ significantly between acclimation
 699 treatments are shown in bold.

Variable	Parameter	Acclimation temperature (°C)	
		10	25
Distance	<i>a</i>	-0.328 (-0.472, 0.146)	0.012 (-0.192, 0.274)
	<i>b</i>	-0.027 (-0.045, -0.010)	-0.059 (-0.085, -0.038)
	<i>c</i>	0.00064 (0.00021, 0.00111)	0.00135 (0.00082, 0.00199)
	r_{max}	2.56 (2.39, 2.74)	2.76 (2.58, 2.99)
	T_{opt}	20.9 (18.2, 26.6)	21.9 (20.4, 24.0)
	T_{br}	21.4 (17.6, 25)	16.3 (13.4, 19.2)
Maximum velocity	<i>a</i>	0.474 (-0.032, 1.229)	0.665 (0.002, 1.651)
	<i>b</i>	-0.060 (-0.132, -0.012)	-0.086 (-0.176, -0.015)
	<i>c</i>	0.00108 (0, 0.00273)	0.0018 (0.00003, 0.00404)
	r_{max}	1.58 (1.38, 1.80)	1.55 (1.21, 1.76)
	T_{opt}	27.9 (22.5, 30)	23.8 (19.8, 30)
	T_{br}	14.2 (7.4, 21.3)	15.7 (11.9, 25)
Mobility	<i>a</i>	2.660 (2.124, 3.130)	3.340 (2.221, 4.001)
	<i>b</i>	-0.062 (-0.122, -0.009)	-0.159 (-0.238, -0.055)
	<i>c</i>	0.00143 (0, 0.00303)	0.00389 (0.00133, 0.00598)
	r_{max}	0.336 (0.324, 0.350)	0.368 (0.353, 0.396)
	T_{opt}	22 (18.5, 30)	20.4 (18.6, 25.1)
	T_{br}	25 (23.1, 25)	22.8 (19.2, 25)

701 **Figure legends**

702 Figure 1. Possible responses of thermal performance curve (TPC) parameters upon
703 warm acclimation (red): a) thermal optima (T_{opt}) displaced towards high temperatures,
704 b) increase in maximum performance, c) increase in critical thermal maximum (CT_{max})
705 and wider TPC breadth and d) wider TPC breadth at the expense of performance
706 reduction.

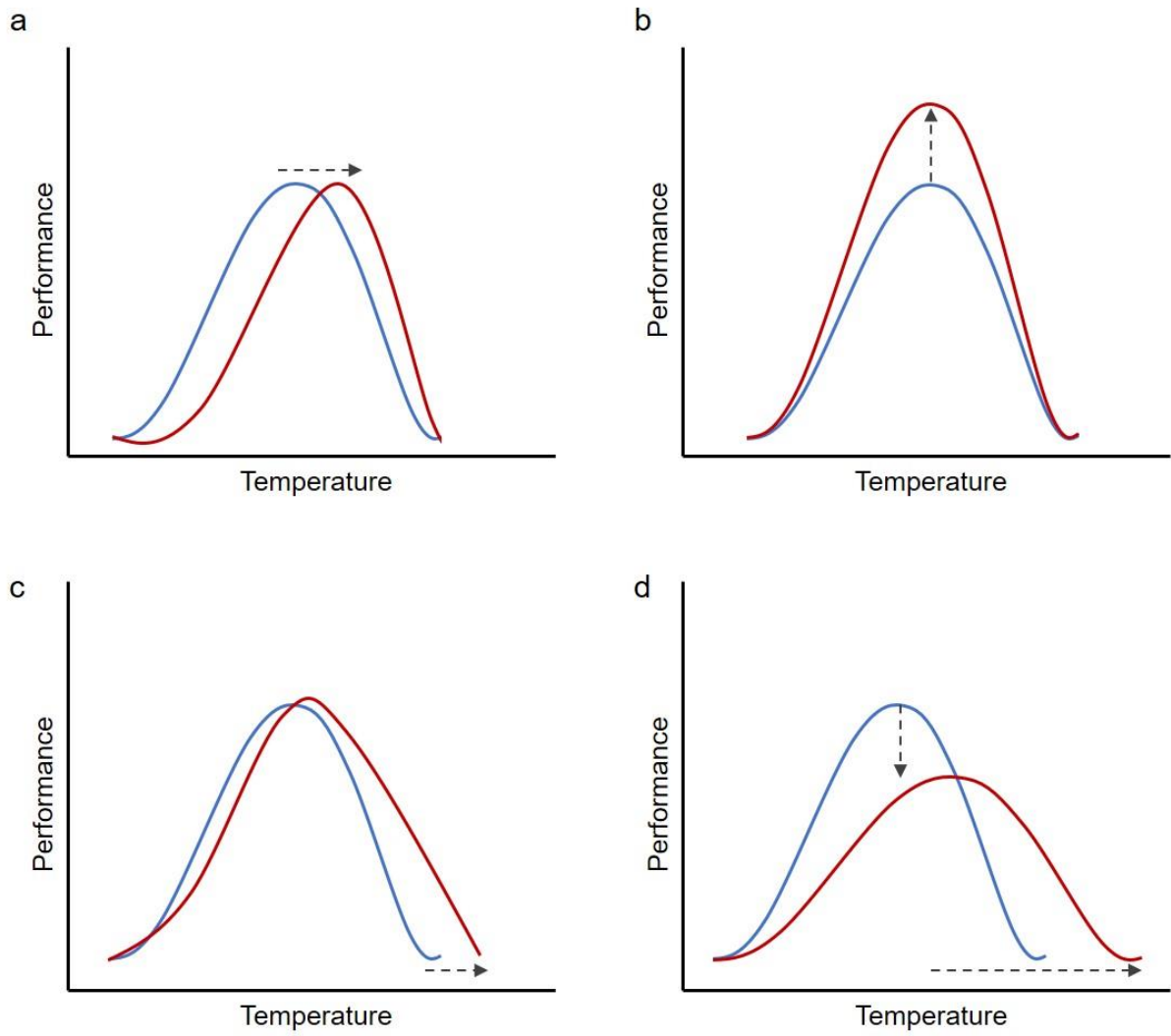
707 Figure 2. Thermal performance curves for locomotor parameters of *Enochrus*
708 *jesusarribasi* adults previously acclimated at different temperatures. Mean \pm se are
709 shown for each test temperature. Shading areas reflect the 95% confidence intervals of
710 the fitted models for each acclimation treatment.

711 Figure 3. Locomotor parameters (mean \pm se) measured at 35°C in *Enochrus*
712 *jesusarribasi* adults previously acclimated at different temperatures. The W-statistic and
713 P-values from Mann-Whitney tests are shown.

714 Figure 4. Total metabolic rate (a) and proportion of aerial respiration (b) in *Enochrus*
715 *jesusarribasi* adults previously acclimated at different temperatures. Shading areas
716 reflect the 95% confidence intervals of the fitted models. Points indicate raw values for
717 each individual tested.

718 Figure 5. Aquatic and aerial metabolic rates (below and above the dashed line,
719 respectively) in *Enochrus jesusarribasi* adults previously acclimated at different
720 temperatures. Shading areas reflect the 95% confidence intervals of the fitted models for
721 suboptimum (5-25°C) and supraoptimum temperatures (25-35°C) for locomotion. Points
722 indicate raw values for each individual tested.

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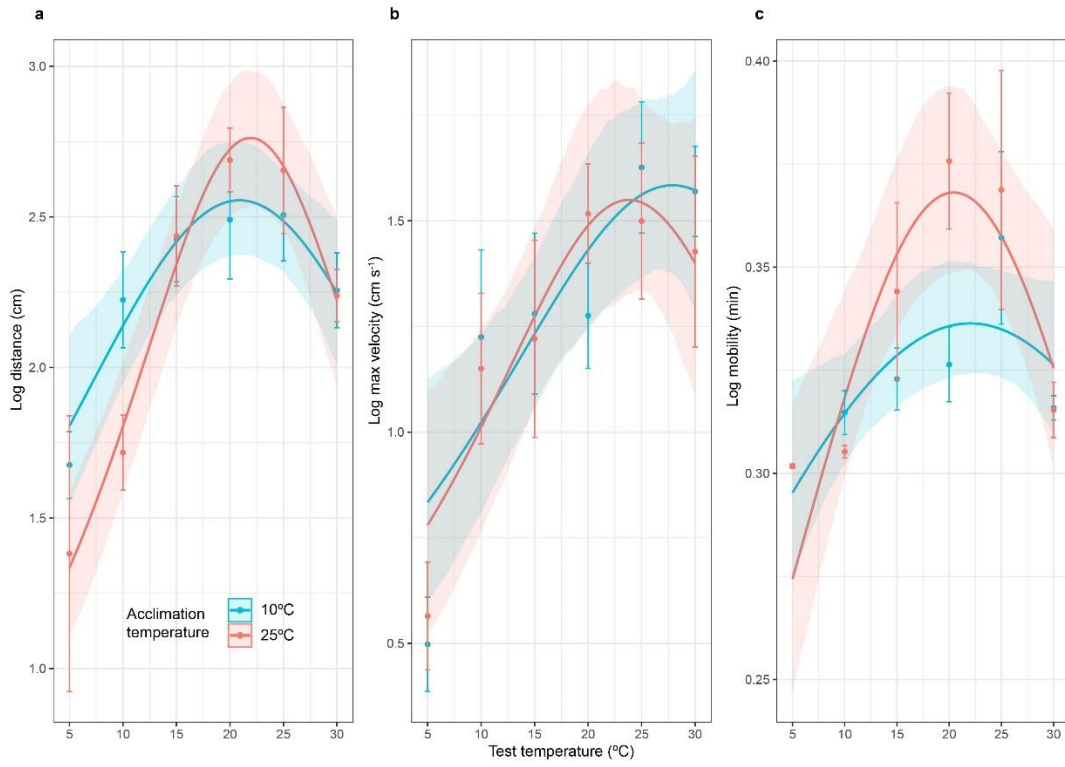


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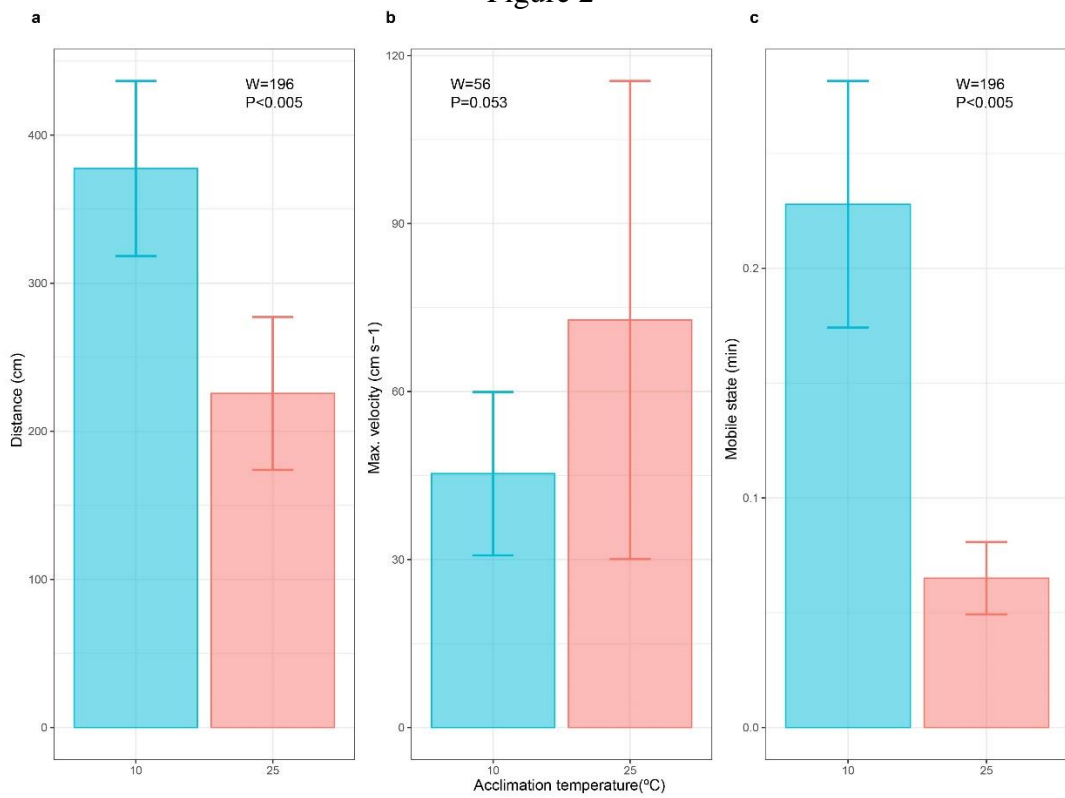
Figure 1



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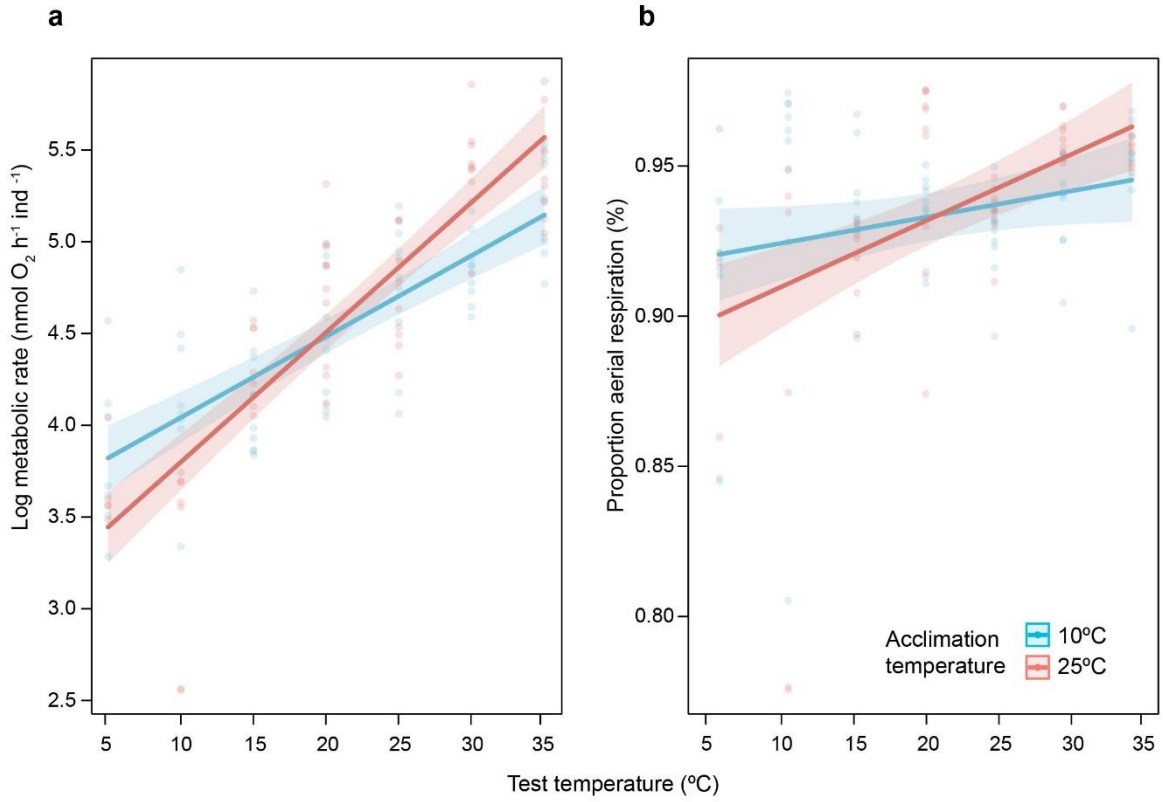
Figure 2



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Figure 3

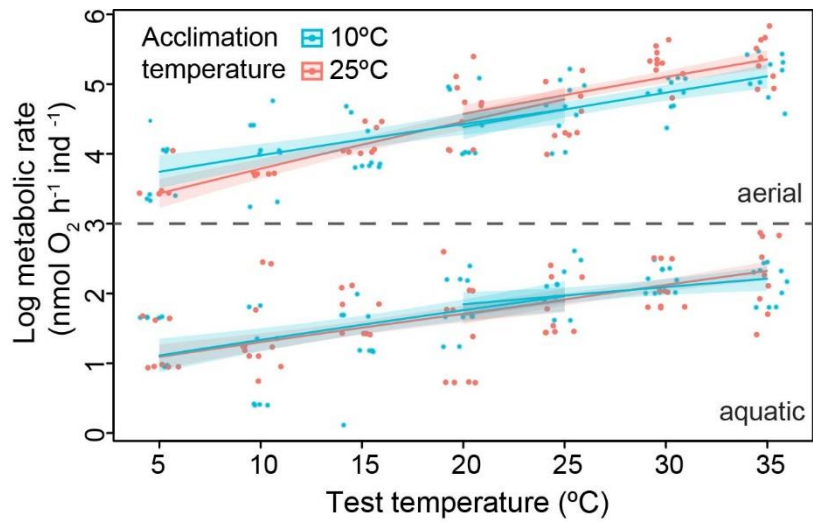


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Figure 4

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Figure 5

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