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.

29

1. Introduction

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

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

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

Thermal plasticity may be favoured in thermally variable habitats, as it can result in fitness benefits in such environments (Gabriel et al., 2005; Angilletta, 2009; da Silva et al., 2019). However, terrestrial animals appear to have in general weaker acclimation abilities than aquatic ones (Seebacher et al., 2015), despite generally greater temperature variability in terrestrial habitats compared to aquatic ones. Insects that inhabit Mediterranean inland waters represent ideal study models for exploring the effects of acclimation on thermal performance in variable aquatic systems. The Mediterranean climate is typically defined by large daily and seasonal thermal fluctuations, with hot, dry summers, and cool, wet winters (Paskoff, 1973; Hertig and Jacobeit, 2011; Bonada and Resh, 2013). Some species from these habitats possess significant physiological plasticity in traits related to salinity and desiccation stress tolerance (Pallarés et al., 2017; Botella-Cruz et al., 2019), as well as broad thermal tolerance ranges and the capacity for 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 jesusarribasi* 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 jesusarribasi* 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. jesusarribasi* 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 10ºC in summer and 5ºC in winter, and

155   seasonal variation ranging between 15-22ºC; 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 24.3ºC,

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 ± 1ºC) and a 12:12 h photoperiod at 35 gl^{-1} (approx. 50 mScm^{-}

162   ^{1} at 20ºC, 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 25ºC) or winter conditions

171   (average temperature of 10ºC) 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   1ºC 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 gl⁻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 20ºC (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 (35ºC). 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 35ºC were independently assessed by

244   Mann-Whitney tests.

245   We analyzed the effect of acclimation temperature on total metabolic rates using linear

246   regresions 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 5-25ºC (suboptimum) and 20-35ºC (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 30ºC, 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 20.9-23.8ºC

277 across the different locomotor variables and acclimation treatments (Table 1).

278 At 35ºC, 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 5-25ºC), 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 (20-35ºC), 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. jesusarribasi*, 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 (20-25ºC) 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 (35ºC), 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 *Ilyiocoris 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 appeard 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 35ºC 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 35ºC, 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 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 suboscura* (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 35ºC in summer (Velasco et al., 2006). The maximum summer air temperature is

402 32.6ºC and it is predicted to increase between 1.6 and 5.9ºC 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.

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 heigth 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 (ºC); T_{br}: thermal breadth (ºC). Parameters that differ significantly between acclimation

699 treatments are shown in bold.

Variable	Parameter	Acclimation temperature (ºC)	
		10	**25**
Distance	a	-0.328 (-0.472, 0.146)	0.012 (-0.192, 0.274)
	b	-0.027 (-0.045, -0.010)	-0.059 (-0.085, -0.038)
	c	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	a	0.474 (-0.032, 1.229)	0.665 (0.002, 1.651)
	b	-0.060 (-0.132, -0.012)	-0.086 (-0.176, -0.015)
	c	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	a	2.660 (2.124, 3.130)	3.340 (2.221, 4.001)
	b	-0.062 (-0.122, -0.009)	-0.159 (-0.238, -0.055)
	c	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)

700

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
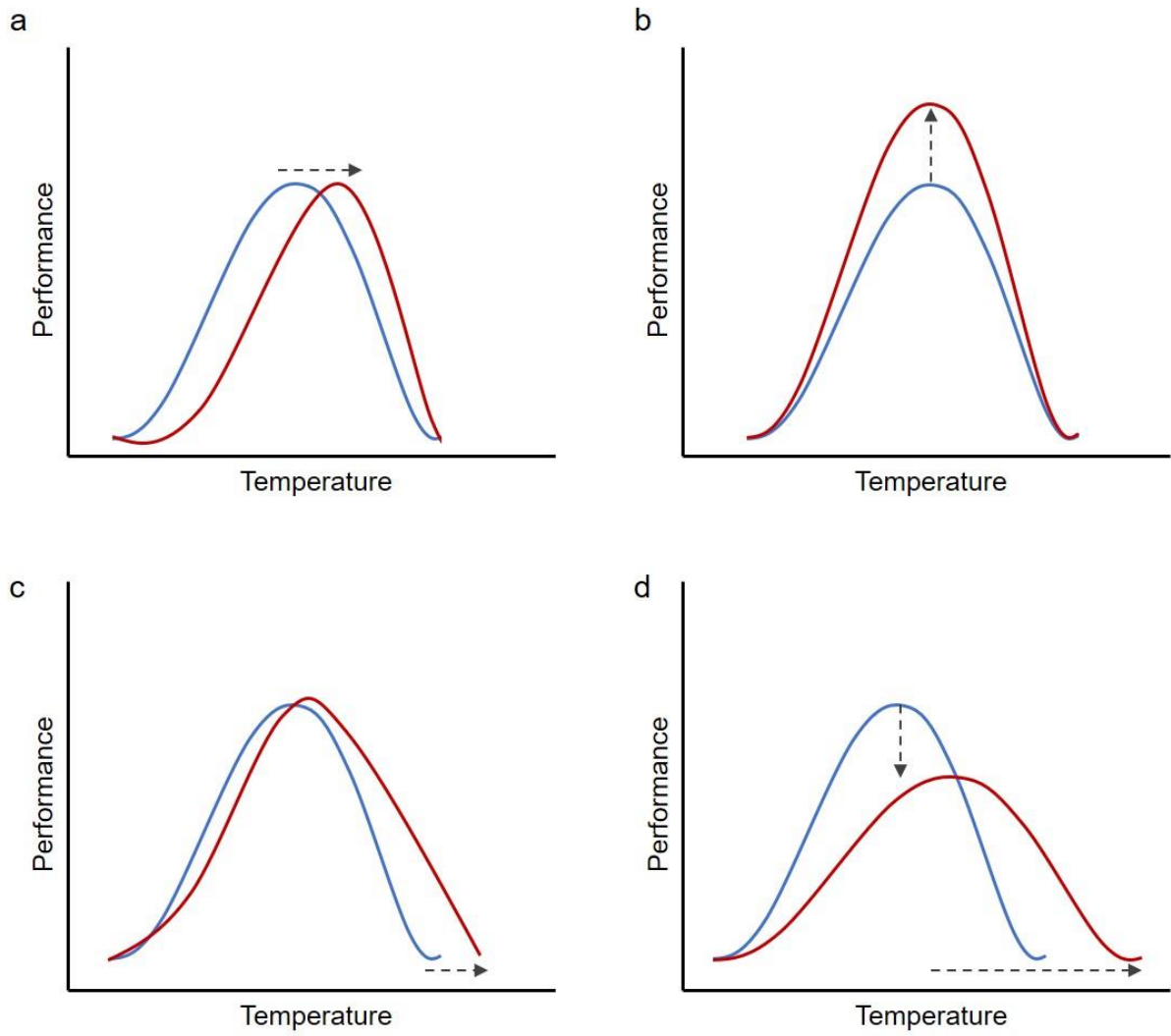
706 reduction.

707 Figure 2. Thermal performance curves for locomotor parameters of *Enochrus*

708 *jesusarribasi* adults previously acclimated at different temperatures. Mean ± 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 ± se) measured at 35ºC 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 (5-25ºC) and supraoptimum temperatures (25-35ºC) for locomotion. Points

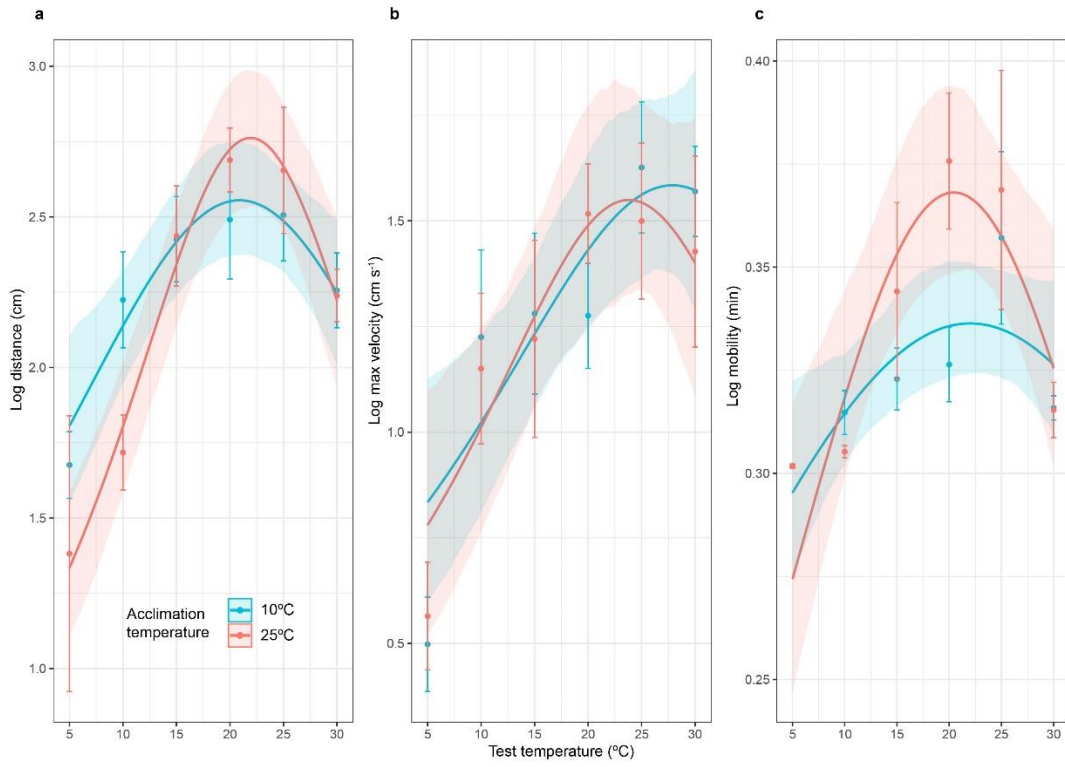722 indicate raw values for each individual tested.

723

Figures
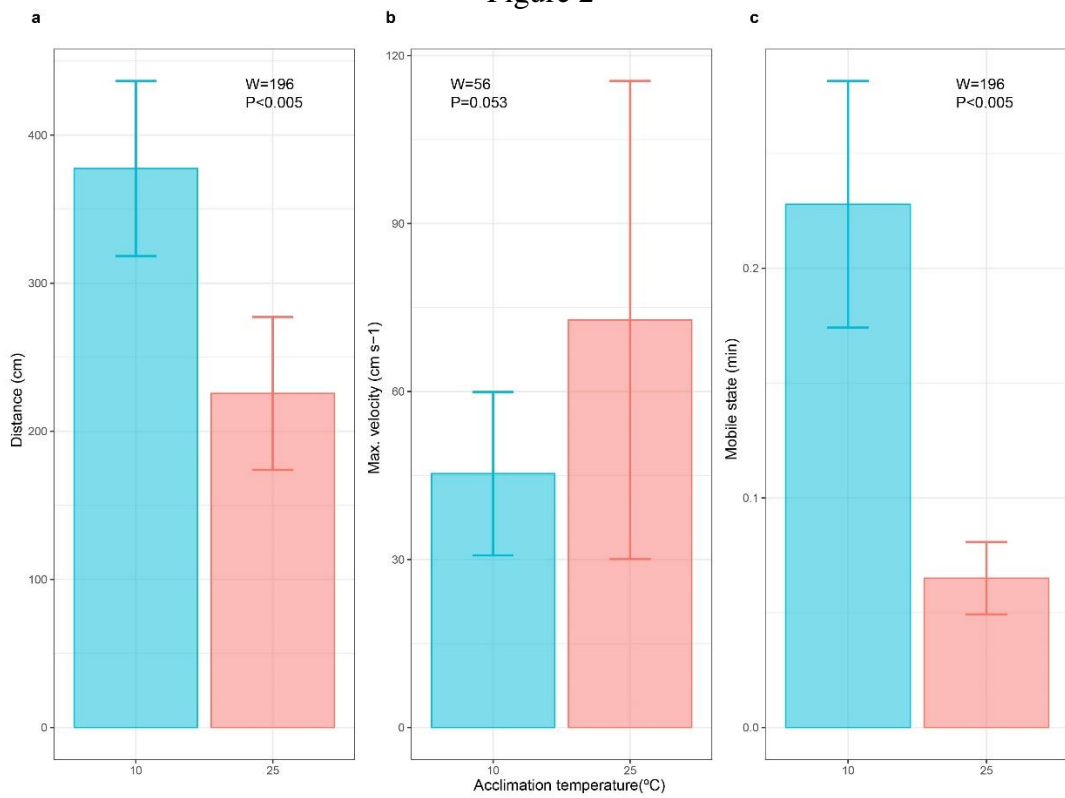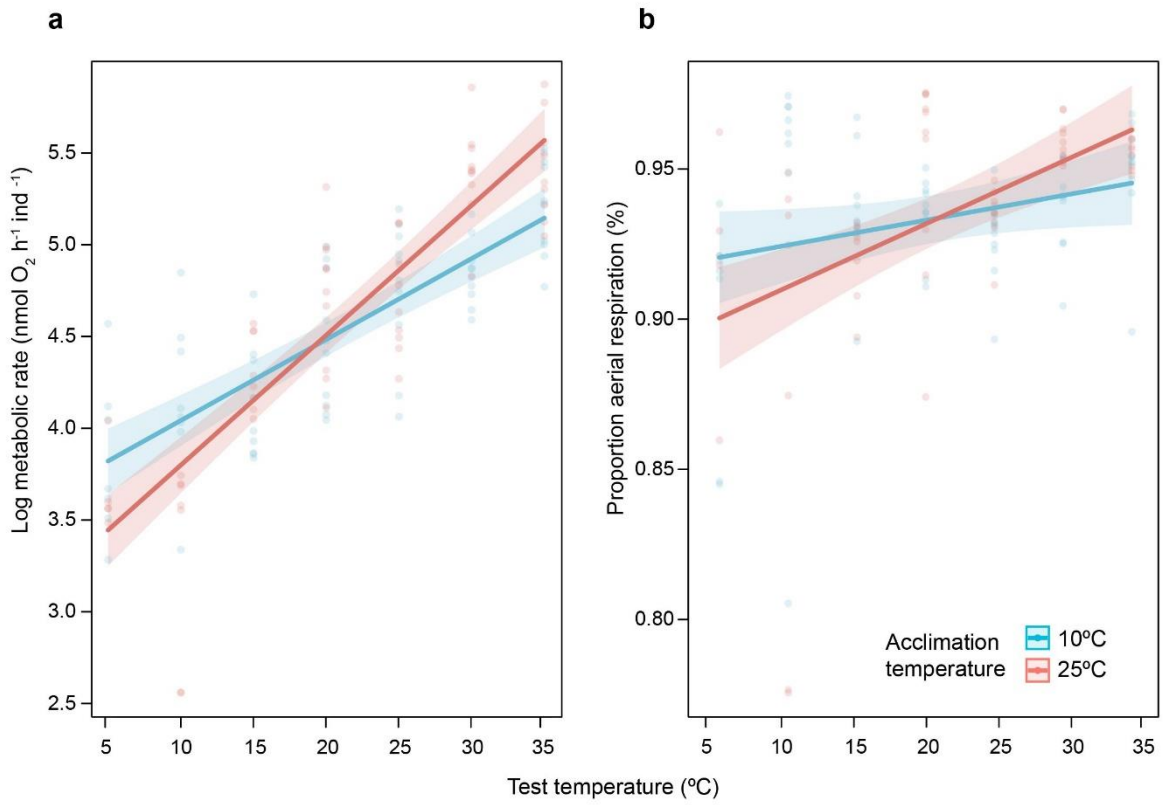
a

b

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726   Figure 1
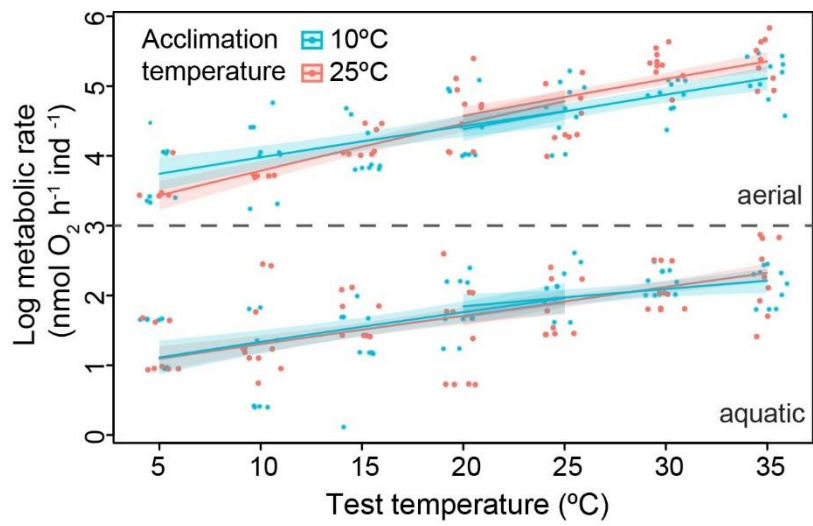727

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Figure 2

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Figure 3

Figure 4

Figure 5

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