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1 Consequences of thermal plasticity for hypoxic performance in coastal amphipods

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7
8 Keywords

9 acclimation, adaptive, plasticity, multistressor, temperature, hypoxia

10
11 Abstract

12 Physiological plasticity may confer an ability to deal with the effect of rapid climate
13 change on aquatic ectotherms. However, plasticity induced by one stressor may only
14 be adaptive *in situ* if it generates cross-tolerance to other stressors. Understanding
15 the consequences of thermal acclimation on hypoxia thresholds is vital to
16 understanding future climate-driven hypoxia. We tested if thermal acclimation
17 benefits hypoxic performance in four closely-related amphipod species. The effects
18 of thermal acclimation (7 days at 10 or 20 °C) on routine metabolic rate (RMR) and
19 critical oxygen tensions (P_{crit}) were determined at a standardised test temperature
20 (20 °C). *Gammarus chevreuxi* and *Echinogammarus marinus* displayed increased
21 P_{crit} with acute warming but warm acclimation negated this increase. P_{crit} of
22 *Gammarus duebeni* was thermally insensitive. *Gammarus zaddachi* displayed
23 increased P_{crit} upon acute warming but little change *via* acclimation. Cross-tolerance
24 between thermal plasticity and hypoxia may improve performance for some, but not
25 all, species under future environmental change.

35 1. Introduction

36 Physiological plasticity (or acclimation) has been suggested as a key mechanism by
37 which organisms buffer the effects of future anthropogenic climate change
38 (Seebacher et al., 2015). Acclimation can be thought of as long-term remodelling of
39 physiological systems in response to single abiotic factors, and is related, but distinct
40 from acclimatisation, a response to multiple environmental variables (Prosser, 1973).
41 Acclimation effects were historically interpreted as always being adaptive (Huey and
42 Berrigan, 1996), an assumption tested formally in the form of the “beneficial
43 acclimation hypothesis (BAH)” (Leroi et al., 1994). A prediction from the BAH was
44 that an organism acclimated to a particular environment should always show greater
45 fitness compared to one that has not had the chance to acclimate (Leroi et al., 1994).
46 An evolutionary framework exists for testing the BAH (Huey and Berrigan, 1996;
47 Precht et al., 1973) stipulating that organisms acclimated to different conditions must
48 be directly competed post-acclimation under standardised test conditions to
49 determine fitness (or a proxy, e.g. metabolic performance, MO_2) (Huey et al., 1999).
50 To date, tests of the BAH provide some support (Angiletta, 2009) but often fail to
51 demonstrate a fitness benefit (Hildebrandt et al., 2018; Wilson and Franklin, 2002;
52 Woods and Harrison, 2002). This could be because the tests sometimes are neither
53 direct nor complete (Wilson and Franklin, 2002; Woods and Harrison, 2002).
54 However, it could also be that the hypothesis does not generalise and may represent
55 only one of several potential outcomes of acclimation (Huey et al., 1999). Perhaps as
56 a result, current studies now rarely explicitly test the original definition of the BAH
57 proposed by Leroi et al., (1994). The notion of “beneficial acclimation” is now
58 considered somewhat less strictly (i.e. acclimation may improve performance in
59 some, but not all, cases as the BAH proposed), and is now more commonly referred
60 to as “adaptive plasticity” (Hildebrandt et al., 2018; Seebacher et al., 2015).

61

62 Tests of adaptive plasticity are largely restricted to the effects of single stressors
63 (Hildebrandt et al., 2018; Seebacher et al., 2015). Less is known about whether
64 acclimation to one stressor is beneficial or detrimental to performance under a
65 second stressor, known as cross-tolerance or cross-susceptibility respectively
66 (Todgham and Stillman, 2013). Understanding the interplay between stressors is
67 critical to better understand and predict the ability of species to modulate their
68 physiology and maintain performance in altered multistressor environments (Orr et

69 al., 2020; Todgham and Stillman, 2013). Within marine ecosystems, the threat posed
70 by spreading hypoxia and rising sea temperature is a global concern (Breitburg et
71 al., 2018). Despite this, relatively little is known of the responses of marine species to
72 hypoxia and chronic warming with a bias towards studies of their acute interaction
73 (McBryan et al., 2013). Studies predict that marine species will experience
74 reductions in hypoxic performance, namely compromised oxyregulation, as indicated
75 by an increase in the critical oxygen tension (P_{crit}) (Herreid, 1980; Rogers et al.,
76 2016). More recent studies have integrated P_{crit} with other key metabolic traits such
77 as maximum metabolic rate (MMR) and aerobic scope (i.e. the difference between
78 maximum and standard metabolic rate (SMR)) (Seibel and Deutsch, 2020; Seibel et
79 al., 2021). Results from meta-analyses point to the need to maintain MMR two to
80 three times higher than SMR to meet the aerobic requirements of living processes
81 (i.e. a factorial aerobic scope (FAS) of 2-3, $FAS=MMR/SMR$) (Deutsch et al., 2015).
82 A temperature-induced increase in P_{crit} is accounted for by the fact that oxygen
83 supply capacity (α , α) increases to meet maximum demand, which increases
84 more slowly with temperature than does resting demand. Therefore, P_{crit} is re-
85 interpreted as an indicator of aerobic scope and oxygen supply, not tolerance of low
86 oxygen, *per se* (Seibel and Deutsch, 2020; Seibel et al., 2021). This is important as
87 raised P_{crit} , associated with reduced FAS could have detrimental fitness
88 consequences (Deutsch et al., 2015).

89
90 In any case, changes to physiological performance based solely upon the acute
91 interaction between stressors exclude the possibility of physiological plasticity and it
92 is not known if thermal acclimation could offset the acute reduction in hypoxic
93 performance observed in unacclimated individuals (McBryan et al., 2013; McBryan et
94 al., 2016). The methodological approach testing for the adaptive value of plasticity
95 (Huey and Berrigan, 1996) extends to measuring performance differences under an
96 additional stressor. Few studies have taken this approach, which involves comparing
97 performance under hypoxia at standardised acute test temperatures (T_t) after
98 exposure to different acclimation temperatures (T_{acc}) (Collins et al., 2021a), a
99 method long used to test for thermal acclimation effects under normoxic conditions
100 (Precht et al., 1973; Seebacher et al., 2015). Studies have instead largely opted to
101 test hypoxic responses solely at the individuals' respective acclimation temperature
102 ($T_{acc} = T_t$). While this approach to investigating acclimation has merit in keeping

103 studies focussed upon physiological function and moves toward ecological realism
104 (Schulte et al., 2011), it does not enable testing of whether thermal plasticity
105 explicitly benefits hypoxic performance. There is currently limited evidence that
106 thermal acclimation explicitly benefits hypoxic performance across aquatic species
107 (Collins et al., 2021a) but predictions have been made from theoretical models. For
108 example, the OCLTT hypothesis predicts that warm acclimation could be beneficial
109 by induction of longer-term mechanisms to alleviate internal oxygen limitation
110 (Pörtner, 2010). Also, an increase to MO_2 caused by acute warming (associated with
111 raised P_{crit} (Herreid, 1980)) can, in some species, be offset by acclimation (Prosser,
112 1973) which could be predicted to lower P_{crit} . So, empirical studies are required to
113 directly test if thermal acclimation can improve hypoxic performance using
114 appropriate experimental designs that compete individuals under hypoxia at
115 standardised T_t post-acclimation.

116

117 Therefore, we tested if thermal acclimation is beneficial for hypoxic performance.
118 Individuals of closely-related amphipod species were acclimated for at least one
119 week to one of two different acclimation temperatures ($T_{acc} = 10$ or 20 °C) before
120 MO_2 and hypoxic performance, measured as P_{crit} , were determined at a
121 standardised warm test temperature ($T_t = 20$ °C). The responses of three
122 congeneric, and one very closely-related genus of gammaridean amphipod species
123 were compared: *Gammarus zaddachi* (Sexton, 1912), *Gammarus duebeni*
124 (Lilljeborg, 1852), *Gammarus chevreuxi* (Sexton, 1913) and *Echinogammarus*
125 *marinus* (Leach, 1913). These species while closely-related, and possessing similar
126 morphologies, experience very different environmental conditions, with congeners
127 occurring from freshwater to marine habitats (Lincoln, 1979).

128

129 2 Materials and Methods

130 2.1 Animal collection and maintenance

131 Amphipods were collected using a hand-net from various locations on the Plym and
132 Tamar estuaries, Plymouth, UK. *G. zaddachi* was obtained from a freshwater stream
133 in Budshead Woods, Plymouth (50.418965N, -4.164803W). Shading by woodland
134 results in fairly mild river temperatures ($T = 11.5 - 16$ °C, Collins, unpubl.). *G.*
135 *duebeni* inhabits a brackishwater stream feeding the River Tamar, Saltash

136 (50.426192 N, -4.215582 W) and is subject to tidal influence (T = min. 4 °C (winter) =
137 max. 20 °C in summer) (Uncles and Stephens, 2001). *E. marinus* inhabits an
138 intertidal mudflat, Tamar Estuary, Saltash (50.415392 N, -4.210981 W), where it is
139 found in shallow water (few centimetres depth) below boulders and decaying algae.
140 Temperatures are relatively mild (T = 8-12 °C) outside of summer months where
141 rapid diurnal temperature changes occur (T=12 - 32°C) (Uncles and Stephens, 2001;
142 Collins 2021). *G. chevreuxi* inhabits a brackishwater stream feeding the Plym
143 Estuary, Plymouth (50.391290 N, -4.084795 W) which can experience variable
144 temperature (T = 13 - 20.5 °C, Collins unpubl) accompanied by hypoxia (down to
145 ~13% a.s.) (Collins, 2019). All individuals were transferred to the laboratory within 2
146 h of collection and kept in small aquaria (Vol.= 10 L, T = 10 °C, 12 h L:12h D regime)
147 containing water at the appropriate salinity: S = 3 for the freshwater population of *G.*
148 *zaddachi*, S = 15 for the brackishwater population of *G. duebeni* and *G. chevreuxi*,
149 and S = 32 for *E. marinus* which inhabits the mouth of estuaries. Amphipods are
150 “shredders” of organic material which is often abundant where they occur (Lincoln,
151 1979). Therefore, all individuals were fed carrot *ad libitum*. Only adult males
152 identified using morphological criteria (Lincoln, 1979) (wet mass (mean ± SE): *G.*
153 *zaddachi* = 40.9 ± 1.0 mg, *G. duebeni* = 60.8 ± 2.0 mg, *G. chevreuxi* = 14.2 ± 0.3
154 mg, *E. marinus* = 94.0 ± 2.9 mg) were used in the experiments described below.

155

156

157 2.2 Experimental design and exposure conditions

158 Individuals were acclimated for at least 7 days to one of two different temperatures:
159 $T_{acc} = 10\text{ °C}$ (control) or 20 °C . *G. zaddachi*, *G. duebeni*, and *E. marinus* were
160 acclimated for 7 days, which, has been shown to be sufficient for full thermal
161 acclimation of most gammarids (Bulnheim, 1979). However, *G. chevreuxi* took longer
162 to acclimate and therefore we utilised an acclimation period of 3 weeks for this
163 species. All acclimations were carried out using the mesocosm system, described in
164 Collins et al., (2021b), which consisted of sealed aquaria (Vol. = 1.4 L) that were
165 partially-immersed in plastic trays filled with deionised water to buffer the effects of
166 any small air temperature fluctuations. The entire mesocosm was located within a
167 controlled temperature environment (T = 15 °C). Water in the control treatment (T_{acc}
168 = 10 °C, 10.1 ± 0.7) was maintained using a water chiller (L-350, Guangdong Boyu

169 Group Ltd., China). Water for the elevated temperature treatment ($T_{acc} = 20\text{ }^{\circ}\text{C}$, 20.4
170 ± 0.9) was supplied from an adjustable water heater (100 W aquarium heater,
171 EHEIM GmbH & Co KG, Germany). Water was circulated around the tray using a
172 pump (Koralia Nano Evolution 900 Circulation Pump, Hydor, Italy). To minimise the
173 possibility of hypercapnia, air supplied by an air pump (Mistral 2000, Aqua Medic
174 GmbH, Germany) was scrubbed for CO_2 by passing it through a trap with NaOH (1
175 M), which was replaced every 2 – 3 d. Complete water changes were carried out
176 every 3 - 4 d. Other environmental factors were kept constant ($\text{O}_2 = 90.7 \pm 1.9\%$ a.s.,
177 $S = 32.0 \pm 1.5$ for *E. marinus* and $S = 14.2 \pm 1.3$ for the brackish species, $\text{pH} = 8.1 \pm$
178 0.2). Water temperature and PO_2 were measured daily using an oxygen microsensor
179 (Pm-Pst7, Presens, Germany) and temperature probe (Pst 100, Presens, Germany)
180 with a dissolved oxygen meter (Microx 4, Presens, Germany). Salinity and pH were
181 measured every 1 - 2 d using a refractometer (HI96822 Digital Refractometer, Hanna
182 Instruments, USA) and bench top pH meter (S400 SevenExcellence pH/mV meter,
183 Mettler-Toledo International Inc., USA).

184

185

186 2.3 Effects of thermal acclimation on routine MO_2 and P_{crit}

187 Changes in routine MO_2 (RMR) for $T_{acc} = 10\text{ }^{\circ}\text{C}$ acclimated individuals during
188 exposure to acutely declining PO_2 were measured for individuals at one of two T_t (T_t
189 $= 10\text{ }^{\circ}\text{C}$ (control) or $20\text{ }^{\circ}\text{C}$ (acutely warmed)). For individuals kept at $T_{acc} = 20\text{ }^{\circ}\text{C}$,
190 responses were tested at $T_t = 20\text{ }^{\circ}\text{C}$ (acclimated). RMR and P_{crit} were measured
191 using a closed bottle respirometry technique (Collins et al., 2021b; Truebano et al.,
192 2018). Individuals were starved for 12 h prior to MO_2 measurements. After
193 acclimation, individuals were transferred to a controlled temperature environment (T_t
194 $= 10\text{ }^{\circ}\text{C}$ or $20\text{ }^{\circ}\text{C}$) and allowed to settle for 1 h following the thermal shift (Dorgelo,
195 1973) before any measurements of RMR were obtained. An individual was carefully
196 introduced into a submerged respirometer (Vol. = 1.85 mL for *G. zaddachi* and *G.*
197 *duebeni*, reduced to 1.1 mL with a perspex insert for *G. chevreuxi*, and 5 mL for *E.*
198 *marinus* because of its larger size). Each chamber was fitted with a non-invasive
199 oxygen sensor spot (SP-PSt3-NAU-D3-YOP, PreSens, Germany). For the 1.85 mL
200 bottles, amphipod ventilatory activity was sufficient to ensure mixing within the
201 chamber but, for the 5 mL chambers, a magnetic flea rotated by a magnetic stir plate

202 was used (MIX 15 eco; 2mag AG, Germany). Amphipods were separated from the
203 magnetic flea by a square of plastic mesh (mesh size = 2 mm), which also served as
204 a substrate for the amphipod to attach to. Bottles were sealed using a gas-tight lid,
205 and individuals allowed to acutely reduce the water oxygen content. The PO₂ within
206 the chamber was monitored by applying a single-channel optical sensor (POF-L2.5-
207 1SMA, Presens, Germany) coupled to an oxygen meter (Fibox 4, Presens,
208 Germany) to each of the sensor spots within the chambers at regular intervals over
209 the course of several hours dependent upon temperature and species, Appendix A
210 for details). Individuals were allowed to deplete the oxygen until P_{crit} had been
211 reached (end PO₂ = ~3 - 8% a.s. in most cases except for *E. marinus* at T_t = 20 °C
212 where end PO₂ = ~10% a.s. due to a much greater P_{crit}) before the bottle was
213 opened, the individual removed and gently blotted dry using tissue paper. Wet mass
214 was determined using a microbalance (MSA225P-000-DA, Göttingen Sartorius AG,
215 Germany, ± 0.01 mg). Two empty chambers were included to control for background
216 microbial respiration. Separate individuals were tested in each experimental
217 condition to minimise any confounding effects and stress resulting from repeat
218 exposure to hypoxia and different temperatures.

219

220 2.4 Statistical analysis

221 All statistical analyses were performed using R statistical software (V4.1.2, R Core
222 Team, 2016). The package 'respirometry' (Seibel et al., 2021) was utilised to
223 calculate RMR (*calc_MO2()* function). RMR is expressed as μL O₂ g⁻¹ h⁻¹. Data
224 collected in the first 10-30 min were omitted from formal analysis to reduce the
225 effects of handling stress on initial rates of O₂ uptake as found for other gammarids
226 (Hervant et al., 1999; Semsar-Kazerooni and Verberk, 2018). Data were considered
227 equivalent to RMR as we sampled the mean MO₂ over the range of environmental
228 PO₂s individuals were seen to oxyregulate. P_{crit} was determined (*calc_pcrit()*
229 function) using the alpha method (Seibel et al., 2021). A small number of points were
230 removed to allow a good fit of the segmented regression (removed points are
231 indicated in Appendix A). Oxygen supply capacity (α) was calculated by dividing
232 RMR by P_{crit}. For *E. marinus* at T_t = 10 °C, data were collected from three repeats of
233 the same experiment and were pooled as responses were not significantly different
234 (P > 0.05). Levene's tests were used to test for variance homogeneity (P > 0.05). For

235 each species, one-way ANOVA and *post-hoc* Tukey tests were used to detect
236 differences ($P < 0.05$) in (1) RMR (2) oxygen supply capacity (alpha, RMR/P_{crit}) (3)
237 $P_{crit-\alpha}$ between temperature treatments (“control” ($T_{acc} = 10\text{ °C}$ and $T_t = 10\text{ °C}$);
238 “acutely warmed” ($T_{acc} = 10\text{ °C}$ and $T_t = 20\text{ °C}$); and “acclimated” ($T_{acc} = 20\text{ °C}$ and T_t
239 $=20\text{ °C}$)). Mass was included as a covariate where it had a significant effect ($P <$
240 0.05). Data were \log_{10} transformed where required to meet assumptions of normality
241 of model residuals. Thermal sensitivity of the aforementioned metabolic parameters
242 was quantified by calculating mean Q_{10} values using the equation:

$$(1) Q_{10} = R_2/R_1^{(10/T_2-T_1)}.$$

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244
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246 where R_2 is the measured rate at temperature T_2 and R_1 is the measured rate at
247 temperature T_1 .

248

249 3. Results

250 *Gammarus zaddachi* displayed a significant increase in RMR with temperature ($F_{2,34}$
251 $= 25.8$, $P < 0.001$, Fig. 1A) with RMR greater upon acute warming compared to
252 controls ($P < 0.001$). RMR of acclimated individuals did not differ from those acutely
253 warmed ($P = 0.227$). Oxygen supply capacity was similarly affected by treatment
254 ($F_{2,33} = 6.4$, $P = 0.005$, Fig. 1B), increasing with acute warming ($P = 0.014$) but not
255 further changed by acclimation ($P = 0.96$). Mass significantly affected oxygen supply
256 capacity ($F_{1,33} = 6.2$, $P = 0.018$, Fig. 1B), but with no interaction with treatment. P_{crit}
257 was thermally sensitive across treatments ($F_{2,34} = 7.0$, $P = 0.003$, Fig. 1C) increasing
258 with acute warming compared to controls ($P = 0.002$). P_{crit} values of warm acclimated
259 individuals displayed an intermediate response but were marginally higher than P_{crit}
260 of control individuals ($P = 0.068$). In both acutely warmed and acclimated individuals,
261 Q_{10} -RMR exceeded $Q_{10-\alpha}$, consistent with predictions of raised P_{crit} (Table 1).

262 *Gammarus duebeni* displayed a significant difference in RMR with treatment ($F_{2,17} =$
263 5.1 , $P = 0.018$, Fig. 1D) . RMR was raised with acute warming compared to control
264 temperature ($P = 0.014$) with RMR of acclimated animals displaying an intermediate
265 response. Alpha was significantly affected by treatment ($F_{2,16} = 12.2$, $P < 0.001$, Fig.
266 1E) and mass ($F_{1,16} = 8.6$, $P = 0.010$) but not their interaction. α increased under
267 acute warming compared to controls ($P < 0.001$) and was significantly different

268 following acclimation ($P = 0.042$). $Q_{10-\alpha}$ was greater than Q_{10-RMR} (Table 1)
269 associated with the observed thermally insensitive P_{crit} ($F_{2,17} = 0.19$, $P = 0.827$, Fig.
270 1F). *E. marinus* displayed significantly altered RMR ($F_{2,27} = 53.7$, $P < 0.001$, Fig. 1G)
271 with increased RMR upon acute warming compared to controls ($P < 0.001$) which
272 was partially offset by acclimation ($P = 0.012$). Significant differences in alpha was
273 observed with mass ($F_{1,26} = 15.4$, $P < 0.001$) and treatment ($F_{2,26} = 6.2$, $P = 0.006$,
274 Fig. 1H) but with no interaction. Alpha declined under acute warming compared to
275 control temperature ($P = 0.004$) associated with high thermal sensitivity of P_{crit}
276 relative to RMR (Table 1) which partially returned to control levels following
277 acclimation ($P > 0.05$). *E. marinus* displayed significant differences in P_{crit} with
278 treatment ($F_{2,26} = 68.0$, $P < 0.001$, Fig. 1I) and mass ($F_{1,26} = 6.6$, $P = 0.016$) but not
279 their interaction. Increased P_{crit} was observed upon acute warming compared to
280 controls ($P < 0.001$) which was partially offset by acclimation ($P < 0.001$).

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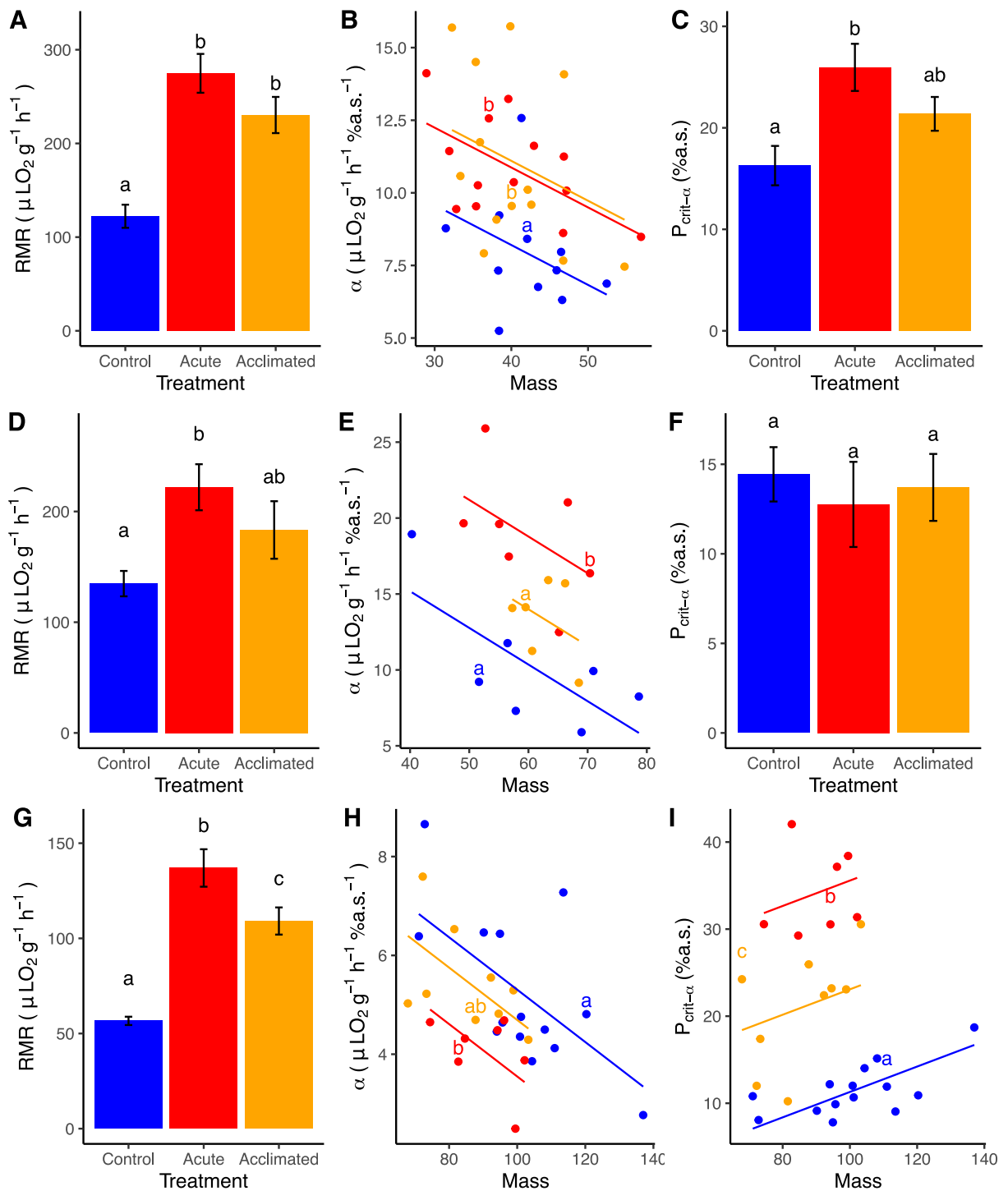
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305 **Fig. 1. Effect of acute warming and thermal acclimation on RMR, α and P_{crit} in**306 **gammarid amphipods.** Responses were measured following exposure to control307 temperature ($T_{\text{acc}} = 10\text{ }^\circ\text{C}$, $T_t = 10\text{ }^\circ\text{C}$), acute warming ($T_{\text{acc}} = 10\text{ }^\circ\text{C}$, $T_t = 20\text{ }^\circ\text{C}$) or308 warm acclimation ($T_{\text{acc}} = 20\text{ }^\circ\text{C}$, $T_t = 20\text{ }^\circ\text{C}$) in (A-C) *G. zaddachi* ($n = 11 - 13$

309 individuals per treatment), (D-F) *G. duebeni* (n = 6-7 individuals per treatment), (G-I)
310 *E. marinus* (n = 7 - 14) individuals per treatment) (supporting data in Appendix B).

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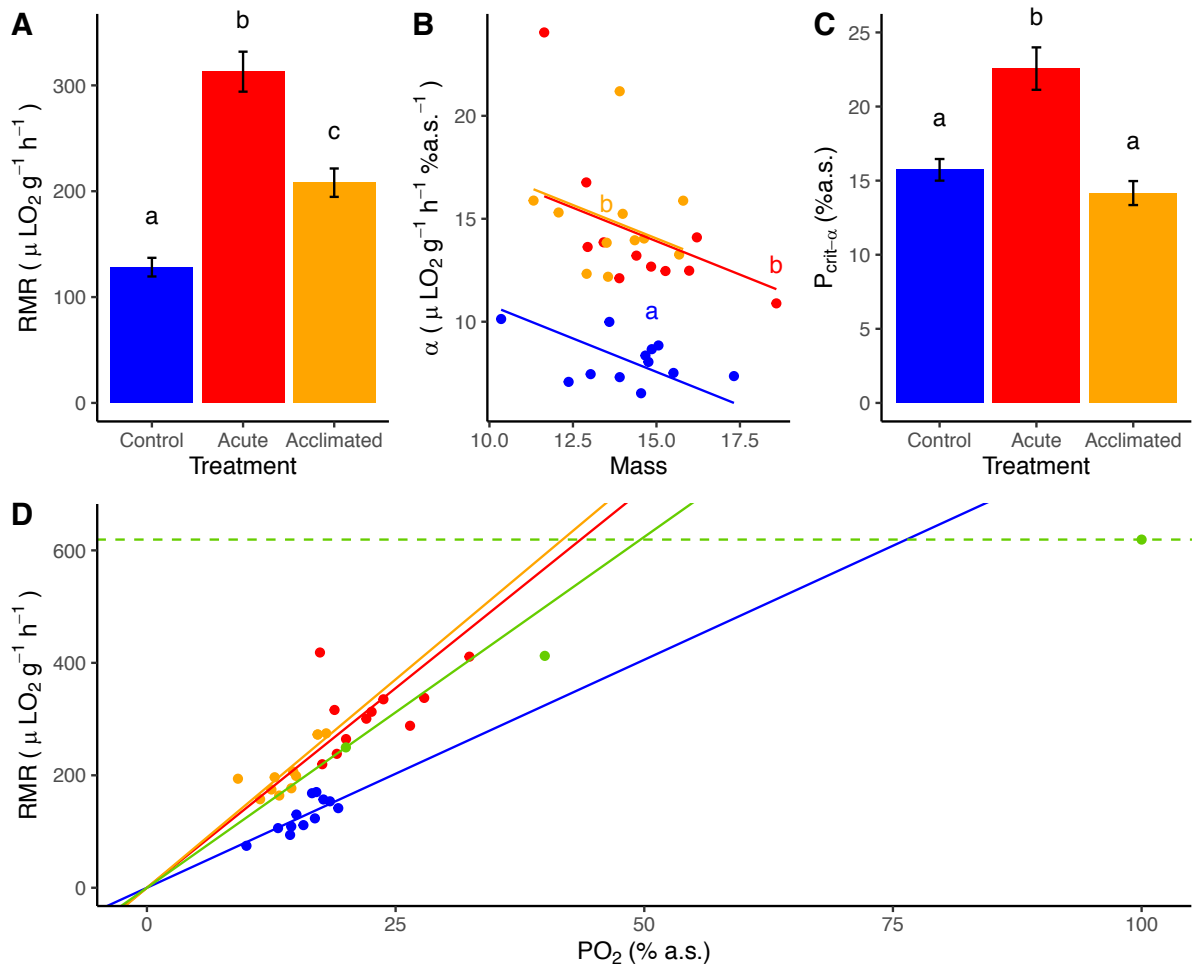
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313 For *G.chevreuxi*, RMR was significantly affected by treatment ($F_{2,31} = 43.9$, $P <$
314 0.001 , Fig. 2A). RMR increased under acute warming compared to controls ($P <$
315 0.001). Warm acclimation partially offset increased RMR compared to the acutely
316 warmed group ($P < 0.001$). Alpha was significantly affected by treatment ($F_{2,30} =$
317 65.4 , $P < 0.001$, Fig. 2B) and mass ($F_{1,30} = 8.3$, $P = 0.007$, Fig. 2B) but not their
318 interaction. Alpha increased under acute warming compared to controls ($P < 0.001$)
319 but was not further affected by acclimation at warm test temperature ($P = 0.94$). P_{crit}
320 was significantly affected by treatment ($F_{2,31} = 18.6$, $P < 0.001$, Fig. 2C) showing an
321 increase under acute warming compared to controls ($P < 0.001$). Following
322 acclimation, P_{crit} was significantly lower than under acute warming ($P < 0.001$) and
323 had returned to the control level ($P = 0.527$). Under acute warming, $Q_{10}\text{-RMR} > Q_{10}\text{-}$
324 α associated with raised P_{crit} (Table 1). Warm acclimated reductions in RMR resulted
325 in $Q_{10}\text{-RMR} < Q_{10}\text{-}\alpha$ associated with a full recovery of P_{crit} . Additionally, mean α at
326 $10\text{ }^{\circ}\text{C}$ and $20\text{ }^{\circ}\text{C}$ in the current study lay either side of mean α measured at $T=15\text{ }^{\circ}\text{C}$
327 from our previous measurements of *G. chevreuxi* (Collins et al., 2019) validating the
328 current RMR and P_{crit} results (Fig. 1D).

329

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332

333 **Fig. 2. Effect of acute warming and thermal acclimation on hypoxic**
 334 **performance of *Gammarus chevreuxi*.** Effects on (A) RMR (B) oxygen supply
 335 capacity (RMR/ P_{crit}) and (C) P_{crit} (n=11-12 individuals per treatment). (D)
 336 Comparison of the results from current study with previous data for *Gammarus*
 337 *chevreuxi* at 15 °C with MMR measured under reduced PO_2 (100, 40 and 20% a.s.,
 338 green circles) (Collins, 2019). Alpha for 10 °C (blue line) and 20 °C (red, orange
 339 lines) falls either side of alpha for 15 °C (green solid line, alpha estimated at 20%
 340 a.s. by MMR/ PO_2). P_{crit} estimated to be ~20% a.s. at 15 °C as MMR=SMR and
 341 AS=0. Extrapolation of α against the MMR observed at 100% a.s. (dashed green
 342 line) gave an estimated $P_{crit-max}$ = 46% a.s. consistent with the experimentally
 343 measured reduction in MMR at 40% a.s.

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347

348 Table 1. Mean thermal sensitivity (Q_{10}) of oxygen demand (RMR), oxygen supply
 349 capacity (α) and P_{crit} in acutely warmed and acclimated gammarid amphipods.
 350

Species	Treatment	Q_{10} -RMR	Q_{10} - α	Q_{10} - P_{crit}
<i>G. chevreuxi</i>	Acute	2.43	1.75	1.43
	Acclimated	1.62	1.82	0.90
<i>G. zaddachi</i>	Acute	2.24	1.37	1.59
	Acclimated	1.88	1.40	1.31
<i>G. duebeni</i>	Acute	1.65	1.86	0.88
	Acclimated	1.36	1.31	0.95
<i>E. marinus</i>	Acute	2.42	0.77	2.99
	Acclimated	1.93	1.04	1.83

351

352

353 4. Discussion

354 Tests of improved hypoxic performance following thermal acclimation are limited,
 355 and mostly single species studies (e.g. McBryan et al., 2016). Comparative studies
 356 of cross-tolerance between warming and hypoxia are rare with only one comparable
 357 study on fish which demonstrated that thermal acclimation effects tended to be
 358 neutral or detrimental to hypoxic performance (Jung et al., 2020). For the gammarid
 359 species tested here, there was cross-tolerance between warm acclimation and
 360 hypoxia in two of the four species, *Gammarus chevreuxi* and *Echinogammarus*
 361 *marinus*. Hypoxic performance of *G. duebeni* was thermally insensitive but *G.*
 362 *zaddachi* displayed sensitivity to acute warming with limited modifying effects of
 363 acclimation. Warm acclimation had no direct negative effects on hypoxic
 364 performance across the tested species. This could be interpreted as chronic
 365 warming may not further exacerbate the detrimental effects of acute thermal
 366 conditions on hypoxic performance for this ecologically-important invertebrate group.
 367

368 4.1 Hypoxic performance under acute warming scenarios

369 Acute multistressor exposure typically leads to reduced performance but precludes
 370 the possibility of cross-tolerance via longer-term plasticity (Gunderson et al., 2016).

371 Under acute warming, raised P_{crit} is common amongst aquatic ectotherms (Herreid,
372 1980; McBryan et al., 2013; Seibel and Deutsch, 2020). *G. chevreuxi*, *G. zaddachi*
373 and *E. marinus* all displayed increased P_{crit} with acute warming due to thermal
374 sensitivity of RMR exceeding that of oxygen supply capacity consistent with model
375 predictions (Seibel et al., 2021). Despite inhabiting a thermally variable mud flat,
376 hypoxic performance of *E. marinus* was surprisingly sensitive to acute warming, with
377 thermal sensitivity of P_{crit} being greater than RMR leading to a reduction in oxygen
378 supply capacity. A similar response was observed in tropical fish (Nilsson et al.
379 2011). As oxygen supply capacity tends to increase with warming (Seibel and
380 Deutsch, 2020), the response could be interpreted as the physiology of *E. marinus*
381 failing under the combination of acute warming and hypoxia. *E. marinus* is the
382 largest and least active of the tested species (Collins, unpubl. obs.). Intraspecifically,
383 *E. marinus* showed increased P_{crit} with size but no significant interaction between
384 warming and mass. This might suggest that, all else being equal, larger organisms
385 are not more vulnerable to future deoxygenation than smaller conspecifics.
386 Conversely, *G. duebeni* did not display raised P_{crit} with acute warming which may be
387 attributable to the temperature induced rise in oxygen supply capacity exceeding the
388 rise in demand. This is consistent with previous studies showing *G. duebeni* is a
389 stress tolerant species with relatively low thermal sensitivity of MO_2 (Sutcliffe, 1984).
390 It is possible that test temperatures exceeding 20 °C may increase P_{crit} in this
391 species. However, 20 °C represents the upper range of temperatures experienced in
392 local tributaries (Uncles and Stephens, 2001) so *G. duebeni* is well equipped to deal
393 with hypoxia experienced *in situ*. In contrast, the increase in P_{crit} with acute warming
394 in the other species suggests that, apart from *via* acclimation, most amphipods could
395 be predicted to display impaired hypoxic performance in a warming environment.

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398 4.2 Cross-tolerance between thermal acclimation and hypoxia?

399 The experimental design adopted here enabled explicit identification of
400 beneficial/detrimental effects of acclimation on hypoxic performance. This approach
401 has not been applied to crustacean species previously, with evidence for aquatic
402 species being restricted to fish and molluscs (Collins et al., 2021a). Different effects
403 of chronic warming on RMR and P_{crit} were observed between the species. *G.*

404 *zaddachi* displayed no ability to acclimate its MO_2 . There was no clear acclimation
405 response of P_{crit} which displayed a non-significant intermediate response between
406 control individuals and those which were acutely warmed. This may reflect marked
407 interindividual variability in their ability to acclimate to warm temperature, and could
408 result from the severity of the acclimation temperature used. A temperature of 20 °C
409 was selected to facilitate direct comparisons in performance between the tested
410 gammarid species. However, *G. zaddachi* typically only experiences a maximum
411 temperature of 16 °C *in situ* so it is possible that 20 °C is physiologically detrimental
412 and overrides acclimation responses (Woods and Harrison, 2002). Future work could
413 examine whether acclimation to ecologically relevant temperatures improve hypoxic
414 performance in this species.

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416 *G. duebeni*, *G. chevreuxi* and *E. marinus* displayed a partial acclimation of MO_2
417 consistent with other studies of gammarid species (Bulnheim, 1979; Whiteley et al.,
418 2011). Evidence from normoxic individuals suggests physiological alterations to
419 reduce oxygen demand may be beneficial to reduce costs of living at warm
420 temperature (Seebacher et al., 2015). Environmental data show hypoxia and high
421 temperature (~20 °C) occur within both the Plym and Tamar estuaries (Collins et al.,
422 2019; Collins et al., 2021b; Uncles and Stephens, 2001). Our data show that the
423 ability to reduce metabolic costs under chronic warming may additionally have
424 beneficial consequences for hypoxic performance. Acclimated reductions in MO_2
425 could be predicted to confer lowered P_{crit} , although has rarely been demonstrated
426 experimentally for marine organisms (Collins et al., 2021a). The few species tested
427 so far have display limited capacity to acclimate MO_2 upon chronic warming (Collins
428 et al., 2021a). *Echinogammarus marinus* and *G. chevreuxi* displayed cross-tolerance
429 between warm acclimation and hypoxia, evidenced by a reduction in P_{crit} following
430 acclimation compared to acutely warmed individuals. For *E. marinus*, a small
431 reduction in P_{crit} was associated with a partial reduction in MO_2 . Additionally, there
432 may be effects of thermal acclimation on mechanisms that enhance oxygen delivery
433 for this species. Transcriptome data for this species indicates acclimation to 20 °C
434 induces upregulation of hemocyanin genes that may be associated with increasing
435 oxygen supply (Collins et al., 2021b), although this requires confirmation at the
436 protein level. Acclimation of P_{crit} was not complete suggesting overall reduced

437 hypoxic performance in a chronically warming world. Interestingly, *G. chevreuxi*
438 displayed a partial reduction in MO_2 associated with a full compensation of P_{crit} ,
439 which may be explained by the oxygen supply capacity being able to meet the
440 reduced oxygen demand. *G. duebeni* displayed thermally insensitive P_{crit}
441 irrespectively of whether warming was acute or chronic, possibly suggesting some
442 insensitivity to future chronic climate-driven deoxygenation.

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445 5. Conclusions

446 Overall, interspecific variation in the beneficial/detrimental consequences of thermal
447 acclimation on single stressor (thermal) performance has been well documented
448 (Angiletta, 2009; Huey and Berrigan, 1996) and variation in species acclimation
449 capacity is predicted to determine “winners” and “losers” under future environmental
450 change (Somero, 2010; Stillman, 2003). We suggest that this notion may be
451 extended to include the consequences of thermal acclimation for cross-tolerance to
452 other stressors, such as hypoxia, which was also subject to considerable
453 interspecific variation. Across the species we tested, any effects of thermal plasticity
454 on P_{crit} seemed to be associated with thermal plasticity of MO_2 , i.e. some species
455 displayed a warm-acclimated reduction in MO_2 associated with reduced P_{crit} . Species
456 unable to thermally acclimate MO_2 and P_{crit} may be vulnerable to future change
457 (Nilsson et al., 2010) while those able to improve hypoxic performance may be more
458 resilient (McBryan et al., 2016). Whether acclimation can fully offset acute thermal
459 effects on hypoxic performance remains to be determined for most marine taxa
460 (Collins et al., 2021a), yet a greater understanding of the interplay of plasticity
461 between these interacting stressors is essential to accurately assess the ecological
462 threat posed to marine ecosystems by multiple anthropogenic drivers (Breitburg et
463 al., 2018; Crain et al., 2008; McBryan et al., 2013).

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475

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482

483 Data availability

484 Supporting datasets are found in accompanying supplementary materials.

485

486 Authors' contributions

487 MC carried out the physiological measurements, data analysis and drafted the
488 manuscript; MT and JIS participated in the design of the study, data analysis and
489 preparing the draft manuscript. All authors gave final approval for publication.

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