Consequences of thermal plasticity for hypoxic performance in coastal amphipods

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Abstract

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

1. Introduction

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

Tests of adaptive plasticity are largely restricted to the effects of single stressors (Hildebrandt et al., 2018; Seebacher et al., 2015). Less is known about whether acclimation to one stressor is beneficial or detrimental to performance under a second stressor, known as cross-tolerance or cross-susceptibility respectively (Todgham and Stillman, 2013). Understanding the interplay between stressors is critical to better understand and predict the ability of species to modulate their physiology and maintain performance in altered multistressor environments (Orr et al., 2020; Todgham and Stillman, 2013). Within marine ecosystems, the threat posed by spreading hypoxia and rising sea temperature is a global concern (Breitburg et al., 2018). Despite this, relatively little is known of the responses of marine species to hypoxia and chronic warming with a bias towards studies of their acute interaction (McBryan et al., 2013). Studies predict that marine species will experience reductions in hypoxic performance, namely compromised oxyregulation, as indicated by an increase in the critical oxygen tension (P_{crit}) (Herreid, 1980; Rogers et al., 2016). More recent studies have integrated P_{crit} with other key metabolic traits such as maximum metabolic rate (MMR) and aerobic scope (i.e. the difference between maximum and standard metabolic rate (SMR)) (Seibel and Deutsch, 2020; Seibel et al., 2021). Results from meta-analyses point to the need to maintain MMR two to three times higher than SMR to meet the aerobic requirements of living processes (i.e. a factorial aerobic scope (FAS) of 2-3, FAS=MMR/SMR) (Deutsch et al., 2015). A temperature-induced increase in P_{crit} is accounted for by the fact that oxygen supply capacity (alpha, α) increases to meet maximum demand, which increases more slowly with temperature than does resting demand. Therefore, P_{crit} is re-interpreted as an indicator of aerobic scope and oxygen supply, not tolerance of low oxygen, *per se* (Seibel and Deutsch, 2020; Seibel et al., 2021). This is important as raised P_{crit}, associated with reduced FAS could have detrimental fitness consequences (Deutsch et al., 2015).

In any case, changes to physiological performance based solely upon the acute interaction between stressors exclude the possibility of physiological plasticity and it is not known if thermal acclimation could offset the acute reduction in hypoxic performance observed in unacclimated individuals (McBryan et al., 2013; McBryan et al., 2016). The methodological approach testing for the adaptive value of plasticity (Huey and Berrigan, 1996) extends to measuring performance differences under an additional stressor. Few studies have taken this approach, which involves comparing performance under hypoxia at standardised acute test temperatures (T_t) after exposure to different acclimation temperatures (T_{acc}) (Collins et al., 2021a), a method long used to test for thermal acclimation effects under normoxic conditions (Precht et al., 1973; Seebacher et al., 2015). Studies have instead largely opted to test hypoxic responses solely at the individuals' respective acclimation temperature ($T_{acc} = T_t$). While this approach to investigating acclimation has merit in keeping studies focussed upon physiological function and moves toward ecological realism (Schulte et al., 2011), it does not enable testing of whether thermal plasticity explicitly benefits hypoxic performance. There is currently limited evidence that thermal acclimation explicitly benefits hypoxic performance across aquatic species (Collins et al., 2021a) but predictions have been made from theoretical models. For example, the OCLTT hypothesis predicts that warm acclimation could be beneficial by induction of longer-term mechanisms to alleviate internal oxygen limitation (Pörtner, 2010). Also, an increase to MO_2 caused by acute warming (associated with raised P_{crit} (Herreid, 1980)) can, in some species, be offset by acclimation (Prosser, 1973) which could be predicted to lower P_{crit}. So, empirical studies are required to directly test if thermal acclimation can improve hypoxic performance using appropriate experimental designs that compete individuals under hypoxia at standardised T_t post-acclimation.

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

2 Materials and Methods

2.1 Animal collection and maintenance

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

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

2.2 Experimental design and exposure conditions

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

Group Ltd., China). Water for the elevated temperature treatment (T_{acc} = 20 °C, 20.4 ± 0.9) was supplied from an adjustable water heater (100 W aquarium heater, EHEIM GmbH & Co KG, Germany). Water was circulated around the tray using a pump (Koralia Nano Evolution 900 Circulation Pump, Hydor, Italy). To minimise the possibility of hypercapnia, air supplied by an air pump (Mistral 2000, Aqua Medic GmbH, Germany) was scrubbed for CO_2 by passing it through a trap with NaOH (1 M), which was replaced every 2 – 3 d. Complete water changes were carried out every 3 - 4 d. Other environmental factors were kept constant (O_2 = 90.7 ± 1.9% a.s., S = 32.0 ± 1.5 for *E. marinus* and S = 14.2 ± 1.3 for the brackish species, pH = 8.1 ± 0.2). Water temperature and PO_2 were measured daily using an oxygen microsensor (Pm-Pst7, Presens, Germany) and temperature probe (Pst 100, Presens, Germany) with a dissolved oxygen meter (Microx 4, Presens, Germany). Salinity and pH were measured every 1 - 2 d using a refractometer (HI96822 Digital Refractometer, Hanna Instruments, USA) and bench top pH meter (S400 SevenExcellence pH/mV meter, Mettler-Toledo International Inc., USA).

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

Changes in routine MO_2 (RMR) for T_{acc} = 10 °C acclimated individuals during exposure to acutely declining PO_2 were measured for individuals at one of two T_t (T_t = 10 °C (control) or 20 °C (acutely warmed)). For individuals kept at T_{acc} = 20 °C, responses were tested at T_t = 20°C (acclimated). RMR and P_{crit} were measured using a closed bottle respirometry technique (Collins et al., 2021b; Truebano et al., 2018). Individuals were starved for 12 h prior to MO_2 measurements. After acclimation, individuals were transferred to a controlled temperature environment (T_t = 10 °C or 20 °C) and allowed to settle for 1 h following the thermal shift (Dorgelo, 1973) before any measurements of RMR were obtained. An individual was carefully introduced into a submerged respirometer (Vol. = 1.85 mL for *G. zaddachi* and *G. duebeni*, reduced to 1.1mL with a perspex insert for *G.* chevreuxi, and 5mL for *E. marinus* because of its larger size). Each chamber was fitted with a non-invasive oxygen sensor spot (SP-PSt3-NAU-D3-YOP, PreSens, Germany). For the 1.85 mL bottles, amphipod ventilatory activity was sufficient to ensure mixing within the chamber but, for the 5 mL chambers, a magnetic flea rotated by a magnetic stir plate was used (MIX 15 eco; 2mag AG, Germany). Amphipods were separated from the magnetic flea by a square of plastic mesh (mesh size = 2 mm), which also served as a substrate for the amphipod to attach to. Bottles were sealed using a gas-tight lid, and individuals allowed to acutely reduce the water oxygen content. The PO_2 within the chamber was monitored by applying a single-channel optical sensor (POF-L2.5-1SMA, Presens, Germany) coupled to an oxygen meter (Fibox 4, Presens, Germany) to each of the sensor spots within the chambers at regular intervals over the course of several hours dependent upon temperature and species, Appendix A for details). Individuals were allowed to deplete the oxygen until P_{crit} had been reached (end PO_2 = ~3 - 8% a.s. in most cases except for *E. marinus* at T_t = 20 °C where end PO_2 = ~10% a.s. due to a much greater P_{crit}) before the bottle was opened, the individual removed and gently blotted dry using tissue paper. Wet mass was determined using a microbalance (MSA225P-000-DA, Göttingen Sartorius AG, Germany, ± 0.01 mg). Two empty chambers were included to control for background microbial respiration. Separate individuals were tested in each experimental condition to minimise any confounding effects and stress resulting from repeat exposure to hypoxia and different temperatures.

2.4 Statistical analysis

All statistical analyses were performed using R statistical software (V4.1.2, R Core Team, 2016). The package 'respirometry' (Seibel et al., 2021) was utilised to calculate RMR (*calc_MO2()* function). RMR is expressed as $\mu L\ O_2\ g^{-1}\ h^{-1}$. Data collected in the first 10-30 min were omitted from formal analysis to reduce the effects of handling stress on initial rates of O_2 uptake as found for other gammarids (Hervant et al., 1999; Semsar-Kazerooni and Verberk, 2018). Data were considered equivalent to RMR as we sampled the mean MO_2 over the range of environmental PO_2s individuals were seen to oxyregulate. P_{crit} was determined (*calc_pcrit()* function) using the alpha method (Seibel et al., 2021). A small number of points were removed to allow a good fit of the segmented regression (removed points are indicated in Appendix A). Oxygen supply capacity (α) was calculated by dividing RMR by P_{crit}. For *E. marinus* at T_t = 10 °C, data were collected from three repeats of the same experiment and were pooled as responses were not significantly different ($P > 0.05$). Levene's tests were used to test for variance homogeneity ($P > 0.05$). For each species, one-way ANOVA and *post-hoc* Tukey tests were used to detect differences ($P < 0.05$) in (1) RMR (2) oxygen supply capacity (alpha, RMR/P_{crit}) (3) $P_{crit\text{-}\alpha}$ between temperature treatments (“control” (T_{acc} = 10 °C and T_t = 10 °C); “acutely warmed” (T_{acc} = 10 °C and T_t = 20 °C); and “acclimated” (T_{acc} = 20 °C and T_t =20 °C)). Mass was included as a covariate where it had a significant effect ($P < 0.05$). Data were $\log_{10}$ transformed where required to meet assumptions of normality of model residuals. Thermal sensitivity of the aforementioned metabolic parameters was quantified by calculating mean Q_{10} values using the equation:

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

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

3. Results

Gammarus zaddachi displayed a significant increase in RMR with temperature ($F_{2,34}$ = 25.8, $P < 0.001$, Fig. 1A) with RMR greater upon acute warming compared to controls ($P < 0.001$). RMR of acclimated individuals did not differ from those acutely warmed ($P = 0.227$). Oxygen supply capacity was similarly affected by treatment ($F_{2,33}$ = 6.4, $P = 0.005$, Fig. 1B), increasing with acute warming ($P = 0.014$) but not further changed by acclimation ($P = 0.96$). Mass significantly affected oxygen supply capacity ($F_{1,33}$ = 6.2, $P = 0.018$, Fig. 1B), but with no interaction with treatment. P_{crit} was thermally sensitive across treatments ($F_{2,34}$ = 7.0, $P = 0.003$, Fig. 1C) increasing with acute warming compared to controls ($P = 0.002$). P_{crit} values of warm acclimated individuals displayed an intermediate response but were marginally higher than P_{crit} of control individuals ($P = 0.068$). In both acutely warmed and acclimated individuals, Q_{10}-RMR exceeded Q_{10}-α, consistent with predictions of raised P_{crit} (Table 1). *Gammarus duebeni* displayed a significant difference in RMR with treatment ($F_{2,17}$ = 5.1, $P = 0.018$, Fig. 1D) . RMR was raised with acute warming compared to control temperature ($P = 0.014$) with RMR of acclimated animals displaying an intermediate response. Alpha was significantly affected by treatment ($F_{2,16}$ = 12.2, $P < 0.001$, Fig. 1E) and mass ($F_{1,16}$ = 8.6, $P = 0.010$) but not their interaction. α increased under acute warming compared to controls ($P < 0.001$) and was significantly different following acclimation ($P = 0.042$). Q_{10}-α was greater than Q_{10}-RMR (Table 1) associated with the observed thermally insensitive P_{crit} ($F_{2,17} = 0.19$, $P = 0.827$, Fig. 1F). *E. marinus* displayed significantly altered RMR ($F_{2,27} = 53.7$, $P < 0.001$, Fig. 1G) with increased RMR upon acute warming compared to controls ($P < 0.001$) which was partially offset by acclimation ($P = 0.012$). Significant differences in alpha was observed with mass ($F_{1,26} = 15.4$, $P < 0.001$) and treatment ($F_{2,26} = 6.2$, $P = 0.006$, Fig. 1H) but with no interaction. Alpha declined under acute warming compared to control temperature ($P = 0.004$) associated with high thermal sensitivity of P_{crit} relative to RMR (Table 1) which partially returned to control levels following acclimation ($P > 0.05$). *E. marinus* displayed significant differences in P_{crit} with treatment ($F_{2,26} = 68.0$, $P < 0.001$, Fig. 1I) and mass ($F_{1,26} = 6.6$, $P = 0.016$) but not their interaction. Increased P_{crit} was observed upon acute warming compared to controls ($P < 0.001$) which was partially offset by acclimation ($P < 0.001$).

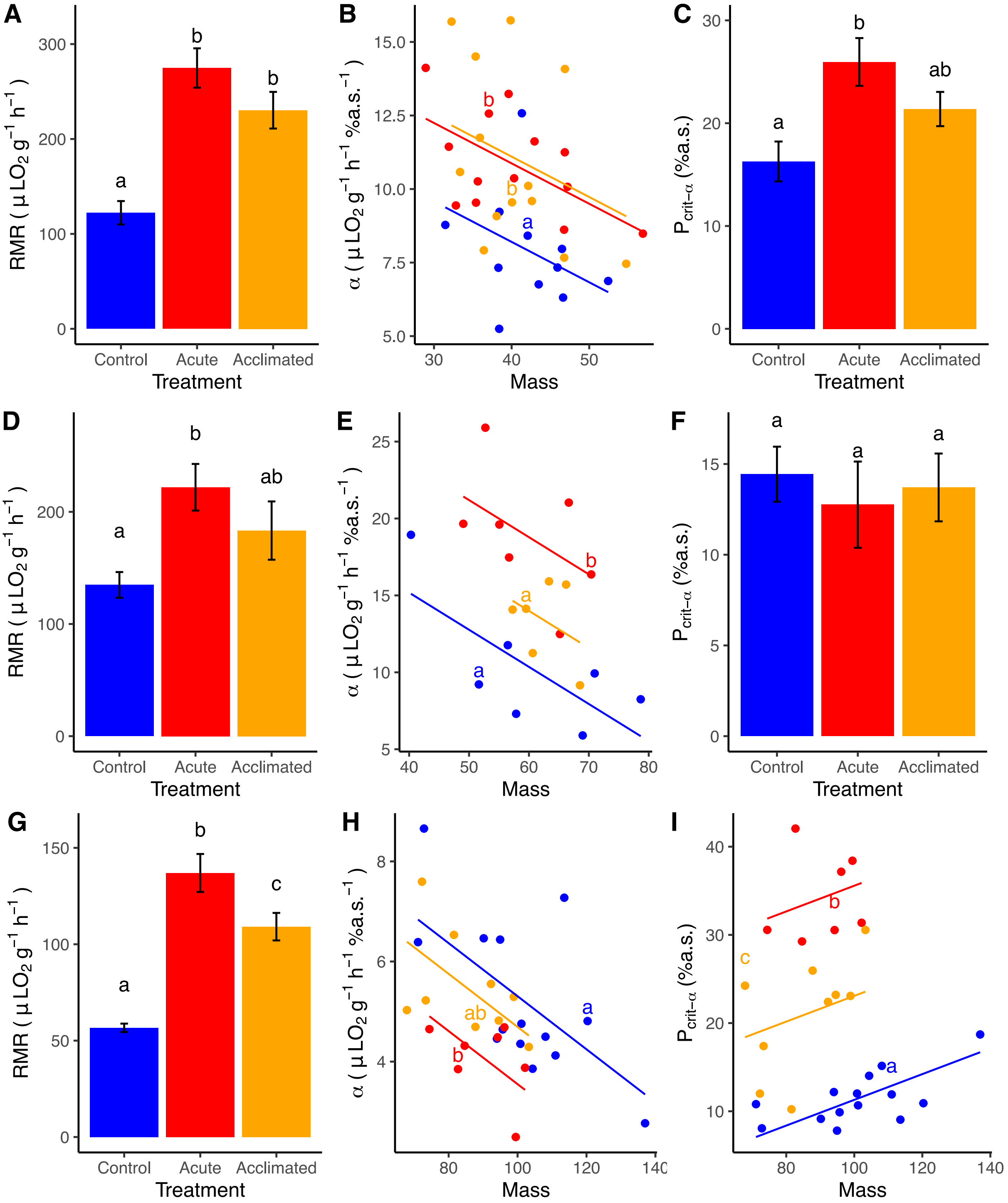

Fig. 1. Effect of acute warming and thermal acclimation on RMR, α and P_{crit} in gammarid amphipods. Responses were measured following exposure to control temperature (T_{acc} = 10 °C, T_t = 10 °C), acute warming (T_{acc} = 10 °C, T_t = 20 °C) or warm acclimation (T_{acc} = 20 °C, T_t = 20 °C) in (A-C) *G. zaddachi* (n = 11 – 13

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

For *G.chevreuxi*, RMR was significantly affected by treatment ($F_{2,31}$ = 43.9, P < 0.001, Fig. 2A). RMR increased under acute warming compared to controls (P < 0.001). Warm acclimation partially offset increased RMR compared to the acutely warmed group (P < 0.001). Alpha was significantly affected by treatment ($F_{2,30}$ = 65.4, P < 0.001, Fig. 2B) and mass ($F_{1,30}$ = 8.3, P = 0.007, Fig. 2B) but not their interaction. Alpha increased under acute warming compared to controls (P < 0.001) but was not further affected by acclimation at warm test temperature (P = 0.94). P_{crit} was significantly affected by treatment ($F_{2,31}$ = 18.6, P < 0.001, Fig. 2C) showing an increase under acute warming compared to controls (P < 0.001). Following acclimation, P_{crit} was significantly lower than under acute warming (P < 0.001) and had returned to the control level (P = 0.527). Under acute warming, Q_{10}-RMR > Q_{10}-α associated with raised P_{crit} (Table 1). Warm acclimated reductions in RMR resulted in Q_{10}-RMR < Q10-α associated with a full recovery of P_{crit}. Additionally, mean α at 10 °C and 20 °C in the current study lay either side of mean α measured at T=15 °C from our previous measurements of *G. chevreuxi* (Collins et al., 2019) validating the current RMR and P_{crit} results (Fig. 1D).

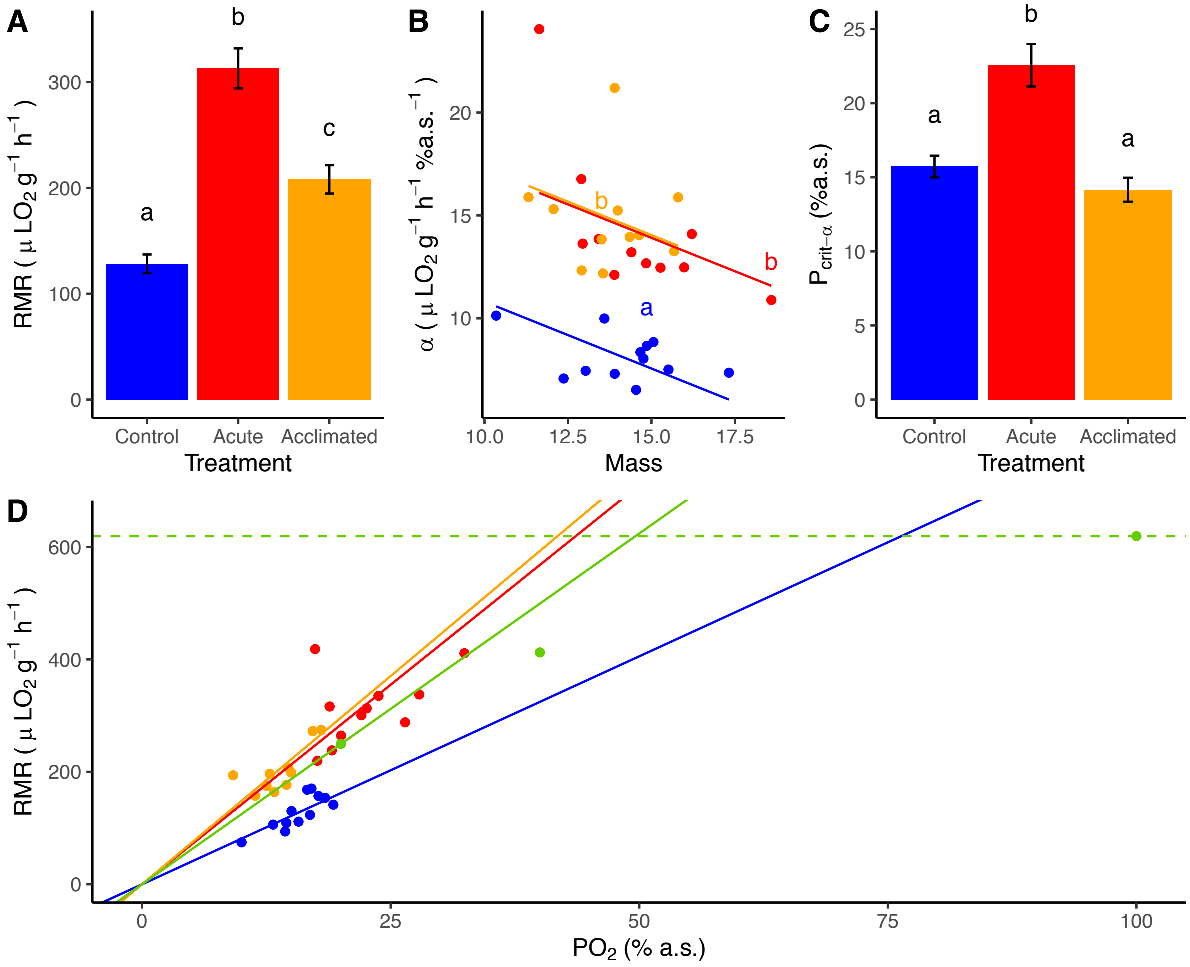

Fig. 2. Effect of acute warming and thermal acclimation on hypoxic performance of *Gammarus chevreuxi*. Effects on (A) RMR (B) oxygen supply capacity (RMR/P$_{crit}$) and (C) P$_{crit}$ (n=11-12 individuals per treatment). (D) Comparison of the results from current study with previous data for *Gammarus chevreuxi* at 15 °C with MMR measured under reduced PO$_2$ (100, 40 and 20% a.s., green circles) (Collins, 2019). Alpha for 10 °C (blue line) and 20 °C (red, orange lines) falls either side of alpha for 15 °C (green solid line, alpha estimated at 20% a.s. by MMR/PO$_2$). P$_{crit}$ estimated to be ~20% a.s. at 15 °C as MMR=SMR and AS=0. Extrapolation of α against the MMR observed at 100% a.s. (dashed green line) gave an estimated P$_{crit-max}$= 46% a.s. consistent with the experimentally measured reduction in MMR at 40% a.s.

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

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

4. Discussion

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

4.1 Hypoxic performance under acute warming scenarios

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

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

4.2 Cross-tolerance between thermal acclimation and hypoxia?

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

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

G. duebeni, *G. chevreuxi* and *E. marinus* displayed a partial acclimation of MO_2 consistent with other studies of gammarid species (Bulnheim, 1979; Whiteley et al., 2011). Evidence from normoxic individuals suggests physiological alterations to reduce oxygen demand may be beneficial to reduce costs of living at warm temperature (Seebacher et al., 2015). Environmental data show hypoxia and high temperature (~20 °C) occur within both the Plym and Tamar estuaries (Collins et al., 2019; Collins et al., 2021b; Uncles and Stephens, 2001). Our data show that the ability to reduce metabolic costs under chronic warming may additionally have beneficial consequences for hypoxic performance. Acclimated reductions in MO_2 could be predicted to confer lowered P_{crit}, although has rarely been demonstrated experimentally for marine organisms (Collins et al., 2021a). The few species tested so far have display limited capacity to acclimate MO_2 upon chronic warming (Collins et al., 2021a). *Echinogammarus marinus* and *G. chevreuxi* displayed cross-tolerance between warm acclimation and hypoxia, evidenced by a reduction in P_{crit} following acclimation compared to acutely warmed individuals. For *E. marinus*, a small reduction in P_{crit} was associated with a partial reduction in MO_2. Additionally, there may be effects of thermal acclimation on mechanisms that enhance oxygen delivery for this species. Transcriptome data for this species indicates acclimation to 20 °C induces upregulation of hemocyanin genes that may be associated with increasing oxygen supply (Collins et al., 2021b), although this requires confirmation at the protein level. Acclimation of P_{crit} was not complete suggesting overall reduced hypoxic performance in a chronically warming world. Interestingly, *G. chevreuxi* displayed a partial reduction in MO_2 associated with a full compensation of P_{crit}, which may be explained by the oxygen supply capacity being able to meet the reduced oxygen demand. *G. duebeni* displayed thermally insensitive P_{crit} irrespectively of whether warming was acute or chronic, possibly suggesting some insensitivity to future chronic climate-driven deoxygenation.

5. Conclusions

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

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Competing Interests

No competing interests declared

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Data availability

Supporting datasets are found in accompanying supplementary materials.

Authors' contributions

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

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