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

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ABSTRACT

Thermal history can plasticly alter the response of ectotherms to temperature, and thermal performance curves (TPCs) are powerful tools for exploring how organismal-level performance varies with temperature. Plasticity in TPCs may be favoured in thermally variable habitats, where it can result in fitness benefits. However, thermal physiology remains insufficiently studied for freshwater insects despite freshwater biodiversity being at great risk under global change. Here, we assess how acclimation at either summer or winter average temperatures changes TPCs for locomotion activity and metabolism in Enochrus jesusarribasi (Hydrophilidae), a water beetle endemic to shallow saline streams in SE Spain. This beetle is a bimodal gas exchanger and so we also assessed how aerial and aquatic gas exchange varied across temperatures for both acclimation treatments. Responses of locomotory TPCs to thermal acclimation were relatively weak, but high temperature acclimated beetles tended to exhibit higher locomotory performance at temperatures around the optimum and thermal plasticity could thus be an important component in the response of aquatic insects to climate change. However, high temperature acclimation appeared to be detrimental for locomotion in subsequent exposure at upper sublethal temperatures, suggesting that this narrow range endemic may be vulnerable to future climate warming. This study demonstrates that TPCs are context-specific, differing with performance metric as well as thermal history. Such context dependency must be considered when using TPCs to predict organismal responses to climate change.

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; Sgro 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. Exposure to a new temperature for some period of time (ranging from minutes to days or months), or exposure to a temperature during development (developmental acclimation) may alter the position, slope, height, breadth, optimum or shape of TPCs for performance metrics such as metabolic, locomotion, feeding and growth rates (e.g. Johnson and Bennett, 1995; Lachenicht et al., 2010; Seebacher and Grigaltchik, 2014; Bozinovic et al., 2016; da Silva et al., 2019) (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 rising 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, high temperature 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., 2021). 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., 2015b), despite the 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 Jacobite, 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., 2012; Botella-Cruz et al., 2016; Carbonell et al., 2017). However, the thermal tolerance of these species has been typically estimated by environmentally unrealistic approaches, employing fast heating rates, that tend to overestimate tolerance limits (Terblanche et al., 2007; Rezende et al., 2014). Thermal stress depends on both heat intensity and the duration of exposure (Rezende et al., 2014) and consequently trials employing fast heating rates might underestimate acclimation capacity compared to slower heating rates or static methods (e.g.: Moyano et al., 2017; Semsar-Kazerouni and Verberk, 2018; Salachan et al., 2019). Furthermore, other responses such as shifts in energy metabolism or locomotion, which are major components of an organism’s ability to cope with changing environments (Domenici et al., 2007; Dillon et al., 2010; Bahrsdorff et al., 2016), have not been fully explored in the context of thermal plasticity (but see Carbonell et al., 2017).

Insect locomotion is highly temperature dependent and is progressively impaired outside optimal temperature ranges (Berrigan and Partridge, 1997), long before effects are observed on survival (Kjaersgaard et al., 2015). Locomotion has been shown to be a plastic behavioural trait, influenced by developmental and adult temperature in insects.

Fig. 1. Possible responses of thermal performance curve (TPC) parameters upon high temperature acclimation (red): a) thermal optima ($T_{opt}$) displaced towards high temperatures, b) increase in maximum performance, c) increase in critical thermal maximum ($CT_{max}$) and wider TPC breadth and d) wider TPC breadth at the expense of performance reduction. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
TPC (Huey and Kingsolver, 1993). For example, high temperature acclimation might: i) shift thermal optima (T_{opt}) towards higher temperatures (e.g.: Gilchrist et al., 1997), improving performance at higher temperatures, but reducing it at lower ones (Fig. 1a); ii) increase maximum performance (i.e. shift TPC height, Fig. 1b) (Bozinovic et al., 2013); iii) increase critical thermal maximum (CT_{max}) and TPC breadth (Jurriaans and Hoogenboom, 2020) (Fig. 1c) or iv) boost performance at high temperatures at the expense of reducing maximum performance (Seebacher et al., 2015a) (Fig. 1d).

Whilst higher locomotor activity is often interpreted as indicating better performance (Angilletta et al., 2002), the interpretation of respiratory reaction norms may be more complex. Higher oxygen consumption rates provide more energy for fitness enhancing processes, but may also imply elevated baseline energetic costs (Pörtner, 2001; Pörtner and Knust, 2007; Magozzi and Calosi, 2015; Verberk et al., 2016). In aquatic ectotherms, the exponential increase in oxygen demand with increasing temperature can cause a progressive mismatch between supply and demand (Verberk et al., 2013), which may decrease organismal performance (Pörtner, 2010; Verberk and Bilton, 2013). In freshwater organisms, high temperature acclimation might decrease oxygen demand at high temperatures (Seebacher et al., 2015b; Sem-sar-Kazerouni and Verberk, 2018). Alternatively, aquatic ectotherms can enhance oxygen uptake and by doing so meet increased demand; for example, Verberk and Bilton (2015) found that a bimodal gas exchanging water bug (i.e. one using both a physical gill and surface exchange) relied increasingly on aerial gas exchange with warming. Aerial gas exchange was likewise argued to be important for high heat tolerance in tropical decapods (Giomi et al., 2014; Fusi et al., 2015). However, no study so far has explored whether reliance on aerial gas exchange is affected by acclimation temperature.

Our aim here was to assess the extent to which thermal acclimation alters i) the TPCs for locomotion activity, ii) the thermal sensitivity of metabolic rate and iii) the dependency on aerial gas exchange in a bimodal breathing aquatic insect from a thermally variable habitat. For this, we used the water beetle Enochrus jesusarribasi Arribas and Millan, 2013 (Coleoptera: Hydrophilidae), which is endemic to intermittent saline streams in a semiarid Mediterranean region (Southeast Spain). We expect this species to be capable of beneficially adjusting performance to acclimation conditions (Fig. 1), as an adaptation to life under the thermally variable conditions experienced in its habitat. However, regional climatic models for southern Europe stress that the Mediterranean is likely to be an especially vulnerable region to global change (Sánchez et al., 2004; Giorgi and Lionello, 2008). Higher, more variable and unpredictable temperatures are anticipated, resulting in novel environmental conditions in the aquatic ecosystems of this area. In such a context, endemic saline species, which often occur as highly isolated populations in these fragmented habitats (e.g. Abellán et al., 2007), may be particularly vulnerable. This may be especially true for lotic species (such as our study beetle), which have low dispersal capacity (Ribera, 2008) and therefore limited potential for range shifts under climate change (Arribas et al., 2017). A better knowledge of thermal performance and its plasticity in such species is important if we are to gain insights into their scope for resilience to climate change via physiological buffering.

2. Material and methods

2.1. Study species, collection and housing

Enochrus jesusarribasi inhabits meso- and hypersaline streams in the south and southeast of Spain, where, despite its fragmented distribution, it is often highly abundant within suitable localities. Adults are bimodal breathers that maintain an air store under the elytra into which the functional spiracles open. This air store connects with an air film supported by hydrofuge hairs over a large part of the ventral surface, which acts as a compressible gas gill so that oxygen can be extracted from the water (Buchwalter et al., 2019). For gas exchange at the water surface, they break the surface tension with their antennae, forming an air channel between the atmosphere and the ventral air store (Yee and Kehl, 2015).

Adult specimens of E. jesusarribasi were collected in June 2020 in Rambla Salada, an intermittent hypersaline stream located in Murcia (SE Spain). This area is characterized by high daily and seasonal thermal variation (e.g.: water temperature records in Rambla Salada showed a daily thermal variation of 10°C in summer and 5°C in winter, and seasonal variation ranging between 15 and 22°C; Velasco et al., 2006; Velasco, unpublished data). Water conductivity and temperature at the time of collection, measured with a conductivity-meter (HACH/Hq40d, Hach®, US), were 70 mScm⁻¹ and 24.3°C, respectively. Enochrus jesusarribasi has an extensive osmoregulation capacity, resulting in high survival across a wide range of salinities in the laboratory (Pallares et al., 2015). Specimens were kept in the laboratory in aerated tanks (25 x 20 x 15 cm) in rooms with controlled temperature (20 ± 1°C) and a 12:12 h photoperiod at 35 g l⁻³ (approx. 50 mScm⁻¹ at 20°C, made up using Instant Ocean® salt) for 5 days before the experiments. A maximum of 15 specimens were placed in each tank.

2.2. Estimating thermal performance curves and their plasticity

To assess whether E. jesusarribasi had the capacity to shift its TPC following acclimation, specimens were exposed to 25°C (high temperature, HT hereafter) or 10°C (low temperature, LT hereafter), which represent the average temperatures in summer and winter in its collection locality, respectively (Velasco et al., 2006), for 5 days. Acclimation temperatures were kept constant in laboratory rooms with controlled conditions. During housing and acclimation (i.e., 10 days), food was provided ad libitum (algae and macrophytes collected in Rambla Salada: Cladophora sp. and Ruppia sp) and the water was renewed every 3 days. Mortality was checked daily in the acclimation tanks and also in three control groups at 20°C (N = 15 individuals each), being comparably low in both cases (<15%).

After this acclimation phase, we measured locomotor performance and routine metabolic rate at seven test temperatures, from 5 to 35°C. Independent groups of beetles were used for the measurement of locomotor performance and metabolic rate (N = 8 and 10 individuals per acclimation treatment and test temperature, respectively). To avoid exposing specimens to an abrupt thermal shift from the acclimation to the test temperature, it was gradually increased or decreased the day before the trials, at a rate of 1°C h⁻¹, in a programmable incubation chamber (Sanyo MIR253, Sanyo Electric, Co. Ltd, Japan). Once the corresponding test temperature was reached, it was maintained constant for 2h before starting measurements.

2.2.1. Locomotor performance

To measure locomotor parameters, four specimens were placed individually in four identical circular open tanks of 9.5 cm diameter (arenas hereafter) containing saline water (35 g l⁻¹, 1.5 cm depth). A piece of foam stuck with aquarium safe silicon (Betta Aquatic Products, UK) in the bottom and partially emergent provided structure for either resting underwater or climbing out of the water (a typical behavioural stress-avoidance response in water beetles, see Pallares et al., 2012). After 30 min for habituation to the arenas, the animals were filmed from above with a GoPro Hero 7 Silver camera (GoPro Inc., USA) for 30 min. The procedure was then repeated with another set of four specimens. Videos were analyzed with EthoVision XT 14 (Noldus, Netherlands) tracking software, wherein the arenas were divided into resting and active zones (Fig. S1). We obtained different parameters of locomotor performance (distance, mean and maximum velocity, activity and mobility state and time spent in the sponge; see Table S1 for details).
2.2.2. Metabolic rate

Routine metabolic rate was measured using closed respirometry and a similar procedure to that described by Verberk and Bilton (2015) and Scholten et al. (2018) to estimate aerial and aquatic respiration in bimodally breathing insects. We used 2 ml respiratory chambers initially fully filled with sterilized saline water (35 g l⁻¹). Immediately after an animal was inserted, we injected 0.6 ml of air saturated with water vapour and temperature equilibrated. This left an air bubble at the top of the chamber (“air compartment”) that provided sufficient space for the animal (body size: 4.4–6.6 mm) to perform aerial gas exchange. A piece of mesh towards the bottom of the water compartment was provided for the beetles to rest on. Oxygen measurements were made at each test temperature by immersing the chambers in a programmable recirculating water bath (Grant R5 TXF200, Grant Instruments Ltd, UK). The beetles were left undisturbed for 20 min before the actual measurements commenced, for habituation and to reach thermal equilibrium. Oxygen consumption in the air compartment was measured at 5 min intervals using micro-optodes connected to a Fibox 4 fiber optic oxygen meter (PreSens instruments, Germany). Oxygen tensions in the water compartment were measured at 20 min intervals by carefully inverting the respiratory chamber to displace the air bubble and allow the optode to come into contact with the water. Linear regressions were fitted to calculate oxygen consumption rates in each compartment. Rates were corrected for background respiration, which was measured by triplicate blanks at each test temperature.

2.3. Data analyses

To evaluate the effect of acclimation temperature on locomotor performance, we used the R package rTPC following the method described by Padfield et al. (2021). For simplicity and because some of the locomotor parameters were highly correlated (e.g. distance and mean velocity, see Fig. S2), these analyses were made on three parameters that represented different locomotor responses: distance, maximum velocity and mobility. For each variable, we fitted 13 different TPC models (Table S2) using non-linear least squares (NLLS) regression. Data were log transformed to improve normality. Previous analyses (Gaussian GLMs) were made including sex and wet mass as covariates, but these were excluded from subsequent models as they were found to have no significant effects. Models were ranked by Akaike’s Information Criterion, corrected for small sample size (AICc) and we selected the model that on average had the lowest AICc values for each of the three locomotor variables considered, in order to have comparable model parameters for all locomotor traits. Besides the specific model parameters (see results), we obtained the following derived TPC parameters: maximum rate (r_{max}), optimum temperature (T_{opt}), that is, the temperature where maximum rate is achieved and thermal breadth (T_b), the range of temperatures over which the curve’s rate is at least 0.8 of peak rates. Uncertainty in the model fit and parameter estimations was assessed by bootstrapping. To avoid having some resampled datasets lacking points beyond the T_{opt}, we used residual bootstrapping (i.e. new datasets were created from the mean centred residuals of the original model fit). Some signs of severe stress (e.g. total immobility, or abrupt random swimming and escape attempts) were observed in locomotor trials at the highest tested temperature (35°C). Consequently, models were fitted excluding this treatment to allow a consistent interpretation of locomotor patterns across temperatures (i.e. higher locomotor activity equates to better performance), and significant differences in locomotor performance at 35°C were independently assessed by Mann-Whitney tests.

We analyzed the effect of acclimation temperature on total metabolic rates using linear regressions with test temperature, medium (aerial or aquatic compartment), acclimation temperature and their interactions as predictors, and wet mass as a covariate. We used the Arrhenius transformation of metabolic rates, which presents log-transformed rates as a function of inverse temperature, (kT)⁻¹, where k is the Boltzmann constant (eV K⁻¹) and T is absolute temperature (K). The slope of this relationship is determined by activation energy (E_a) and reflects the sensitivity of metabolism to changes in temperature. A significant interaction between acclimation and test temperature would then denote a difference in thermal sensitivity between LT and HT acclimated groups. Linear regression was also used to assess the effect of acclimation on the proportion of aerial respiration over the total respiration. Because acclimation effects on metabolism might differ under lower, suboptimal and higher, supraoptimal temperatures, we also compared metabolic rates and activation energies between acclimation treatments separately during the ascending and descending phase of the locomotor TPCs. Considering the range of T_{opt} obtained from locomotor TPCs (see results), the temperatures 5-25°C (suboptimum) and 20-35°C (supraoptimum) were used, respectively.

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

3. Results

3.1. Locomotor performance

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

The parameter values estimated from the selected TPC model did not differ between acclimation treatments (95% CIs overlapped) except from mobility r_{max}, which was significantly higher in HT than LT acclimated individuals (Table 1, Fig. 2c). Despite no significant differences, HT acclimated individuals tended to show higher increases and decreases of the locomotor TPCs (steeper slopes) in the ascending and descending phase of the curve, respectively, and a lower thermal breadth, especially for distance and mobility (Table 1, Fig. 2). TPCs peaked between 20.9 and 23.8°C across the different locomotor variables and acclimation treatments (Table 1).

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

3.2. Metabolic rate

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

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

4. Discussion

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

We found relatively weak responses of locomotion TPCs to temperature acclimation, which may in part be due to the high inter-individual variation in locomotor activity within treatment groups, typical of insect locomotor tests (e.g. Lachenicht et al., 2016). Nevertheless, beetles acclimated at higher temperature tended to exhibit higher locomotor performance at optimum temperature ranges (20-25 $^\circ$C) but a reduced breadth of locomotor TPCs, suggesting a cost of acclimation under sub and supraoptimum exposure temperature (Hoffmann et al., 2003, Barndoff et al., 2016). At the higher test temperature (35 $^\circ$C), LT acclimated beetles showed higher locomotor activity (distance travelled...
and mobility) than those from the HT treatment. The interpretation of locomotor activity in terms of organismal performance at sublethal temperatures is not straightforward. Some (unsuccessful) escape attempts by flight were observed in both acclimation treatments; so higher mobility could be related with such escape behaviour and then it would mean that beetles acclimated at lower temperature were more stressed. However, some specimens, especially HT acclimated ones, also showed total immobility for the whole duration of the trial. The incapacity to perform coordinated movement is also a typical sign of thermal stress in insects (Vannier, 1994; Lutterschmidt and Hutchison, 1997; Gallego et al., 2016). Measurement of other stress-related traits (e.g. molecular stress biomarkers) at sublethal temperatures may help elucidate the responses observed here.

High temperature acclimation increased the thermal sensitivity of
In our study, such behaviour was more pronounced in individuals acclimated at different temperatures. Shading areas reflect the 95% confidence intervals of the fitted models for suboptimum (5-25°C) and supraoptimum temperatures (25-35°C) for locomotion. Points indicate raw values for each individual tested.

metabolic rates in E. jesusarrabai. Seebacher et al. (2015b) found the opposite pattern in ectotherms, including freshwater organisms, using a meta-analysis on thermal plasticity of metabolic rate and other physiological rates. However, their dataset only included one aquatic insect species, for which no effect of acclimation was found (Ferris and Wilson, 2012). Neither did acclimation temperature affect the metabolic rate of a Mediterranean population of the saline corixid Sigara selecta (Carbonell et al., 2017), but its effects on thermal sensitivity were not measured. Furthermore, specimens of Sigara selecta, a bimodal breather, did not have access to aerial gas exchange in their study, which might mask the effect of acclimation on metabolic rates (see below). Lack of comparative studies makes it impossible to assess the extent to which the response of metabolism to acclimation in E. jesusarrabai reported here is common to other aquatic insects. However, in view of the variation in metabolic rate reaction norms found between and within insect groups (Shah et al., 2021), disparity in how acclimation affects metabolic TPCs could be also expected among them.

Differences in total metabolic rates between differently acclimated beetles were mainly associated with changes in either the magnitude or thermal sensitivity of aerial respiration rates, which were much higher than aquatic rates. Compared to air, gas exchange is more difficult under water due to lower rates of oxygen diffusion (Verberk et al., 2011) and higher costs of ventilation owing to the higher viscosity and density of water (Verberk and Atkinson, 2013). Accordingly, with warming-induced increases in metabolic rate, beetles relied increasingly on aerial oxygen uptake. Similar results were obtained in the unrelated bimodal gas exchanging hemipteran Ilyiocoris cimicoides (Verberk and Bilton, 2015), and freshwater gastropods (Jones, 1961). Interestingly, in our study, such behaviour was more pronounced in individuals acclimated at the higher temperature. The mode of respiration of aquatic insects has been linked to the extent to which oxygen limitation affects performance and heat tolerance might differ amongst aquatic insects with different respiratory modes. For dytiscid beetles this appears indeed to be the case (Verberk et al., 2018).

The increased oxygen uptake, and the increased reliance on aerial gas exchange in HT acclimated beetles is consistent with their tendency of enhanced maximum locomotor performance compared to LT acclimated ones. Acclimation temperature was also found to increase the upper lethal limits of E. jesusarrabai in a previous study (Arribas et al., 2012). However, as stated above, the lower locomotion activity of HT acclimated beetles at 35°C observed in our experiment could be an indicator of sublethal stress. Impaired locomotion might be a consequence of sustained elevated metabolic rates both during acclimation and the subsequent exposure to 35°C, which could have negatively impacted the energy budget by increasing baseline energetic costs (Magozzi and Calosi, 2015; Shah et al., 2021). Such potential sub-critical effects of acclimation temperature might not have been captured by Arribas et al. (2012) as they employed a rapid heating rate to estimate \( C^\text{max} \). Alternatively, it is possible that physiological (heat tolerance) and behavioural (locomotion performance) thermal limits show uncorrelated responses to acclimation, as has been recently found in response to artificial selection in Drosophila subrosa (Mesas et al., 2021). Then, despite the overall beneficial effect of high acclimation temperature on metabolism and locomotion observed in E. jesusarrabai, and its capacity to enhance acute heat tolerance upon acclimation (Arribas et al., 2012), it should be evaluated whether exposure to sublethal high temperatures compromises performance and fitness in the longer term.

Given the unprecedented rates of climate change, organisms will have to rely partly on plastic responses, because adaptation via evolutionary changes might be too slow, phylogenetically constrained or limited by low genetic variation (Kellermann et al., 2012; Kelly et al., 2015; Mesas et al., 2021). In such a context, the investigation of TPC for multiple physiological traits and their plasticity is a powerful approach to provide more accurate predictions of species vulnerability (Sinclair et al., 2016; Kellermann et al., 2019). However, thermal plasticity may not be sufficient to keep pace with climate warming (van Heerwaarden et al., 2016). Enochrus jesusarrabai shows some degree of plasticity in its locomotion TPCs, and a much greater plasticity of metabolic rates, through the capacity to increase aerial oxygen uptake in response to thermal acclimation. However, the extent to which such acclimation capacity could improve performance at high temperatures is unclear. In the locality used for this study, water temperatures can often exceed 35°C in summer (Velasco et al., 2006). The maximum summer air temperature is 32.6°C (average for 1970–2000; source: Worldclim v. 1.4 database; http://www.worldclim.org) and it is predicted to increase between 1.6 and 5.9°C by 2070, considering the Representative Concentration Pathway (RCP) 4.5. This species could, therefore, already experience potentially stressful temperatures in nature and would have a narrow thermal safety margin under future climate change scenarios. Such thermal safety margins could be wider if other life-stages show higher thermal tolerance and plasticity than adults, but that seems unlikely for this (and related) species, given the higher stress sensitivity of larvae compared to adults of the studied species (Botella-Cruz et al., 2017) and other aquatic Coleoptera (Pallarés et al., 2020). Theoretically at least, aquatic insects could to some extent mitigate the effects of warming through microhabitat selection, but most shallow aquatic habitats have relatively low spatial variability in thermal conditions, likely limiting the possibilities for behavioural thermodregulation (Gunderson and Stillman, 2015).

5. Conclusions

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

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

The authors have no competing interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2021.103113.

Author statement

All authors contributed to the study. Susana Pallarés: conceptualization, funding acquisition, methodology, investigation, formal analysis, writing- original draft, project administration. Wilco CEP Verberk: methodology, formal analysis, validation and writing-review and editing. David T Bilton: conceptualization, funding acquisition, resources, validation and writing-review and editing.

Data availability

Data supporting this article are available online at Figshare upon publication (private link: https://figshare.com/s/a707ee09231b9d2d4aa5)

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