



# Exposure to fluctuating temperature increases thermal sensitivity in two lineages of the intertidal mussel *Perna perna*

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**ABSTRACT:** Studies of climate change effects on individuals and populations are mostly couched in terms of lethal temperature limits, but 2 other elements make predictions difficult: the effects of sublethal stress and intraspecific variability. Sublethal stress, such as exposure to highly variable temperatures, can cause carryover effects that influence how an animal responds to subsequent events and its long-term fitness. Additionally, intra-specific genetic variability can allow more complex species responses to climate change. We used 2 genetically distinct lineages of the intertidal mussel *Perna perna* to investigate the effects of sublethal temperature variability and genetic identity on thermal sensitivity estimates derived from standard metabolic rate measurements. Mussels were exposed to one of 3 acclimation treatments: measurements done immediately after collection (i.e. field fresh), or exposure to 2 wk of either constant temperature or fluctuating temperatures. The mussels were then exposed to rising temperatures within the range normally experienced in the field while oxygen consumption was recorded. We found no effect of lineage or treatment on the aerobic standard metabolic rate of mussels, but  $Q_{10}$ , a proxy for thermal sensitivity, was influenced by treatment. While  $Q_{10}$  values under constant temperature did not differ significantly from those for field-fresh mussels, both were significantly lower than for fluctuating temperatures. Thus, temperature variability increased thermal sensitivity, but our results indicate that this was independent of genetic identity. Chronic increased temperature variability and repeated exposure to high (but sublethal) temperatures under climate change are likely to increase mussel temperature sensitivity and decrease performance. Such alterations to fitness are expected to occur before animals are exposed to lethal temperatures.

**KEY WORDS:** Acclimation · Marine intertidal zone · Thermal ecology · Stress physiology ·  $Q_{10}$

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## 1. INTRODUCTION

The effects of climate change are often studied in the context of the lethal temperature limits of animals, but not the less obvious effects of sublethal stress. Exposure to temperatures that are above those normally experienced or to highly variable temperatures can cause carryover effects that can influence the response of an animal to subsequent events. The term carryover effects is used in 2 broad senses: trans-generational effects by which the performance of offspring is affected by parental experiences (e.g. Rivest et al. 2017, Donelson et al. 2018) and within-

individual effects such as effects in one season influencing performance in subsequent seasons. The term is often used in the within-individual sense in the case of migratory species such as turtles (e.g. Ceriani et al. 2015) or birds (e.g. Inger et al. 2010, Harrison et al. 2011, Szostek & Becker 2015) but also for the persistence of negative effects after a stressor has been removed, such as the exposure of birch saplings to elevated ozone effects (e.g. Oksanen & Saleem 1999). O'Connor et al. (2014, p. 2) defined carryover effects as occurring when 'an individual's previous history and experience explains their current performance'. We use the term in this latter sense, and the birch ex-

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ample also introduces the importance of intraspecific variability. Anticipating the response of species to the effects of thermal variability is complicated by the possibility of intraspecific variability based not only on acclimation and phenotypic responses, but also on the existence of genetically distinct populations or lineages or, in the case of the birch example, clones with different tolerances of ozone.

Temperature is a primary environmental driver of animal physiology (Harley & Helmuth 2003, Szathmary et al. 2009, Kordas et al. 2011, Rojas et al. 2014), and, globally, shallow coastal waters and ocean surface waters to a depth of 70 m have increased in temperature by approximately  $0.11^{\circ}\text{C decade}^{-1}$  from 1971–2010 (Byrne & Przeslawski 2013, IPCC 2014, Vinagre et al. 2018). There are 2 components to thermal stress: intensity and duration (McCue 2004, Williams et al. 2016). High-intensity temperature stress of short duration can result in an acute response including phenotypically plastic adaptive strategies, while prolonged duration of stressful temperatures or even a marginal but protracted increase in temperature above normal can lead to a variety of adaptive strategies that may not be reversible (Semsar-kazerouni & Verberk 2018, Sorte et al. 2019).

The underlying physiology of an animal can change as it acclimates to thermal changes (da Silva et al. 2019); tissue composition and enzyme expression can rearrange in a timeframe of hours to weeks (Sorte et al. 2019). This implies that animals exposed to ongoing changes in climate will be able to acclimate to their new thermal regimes (Nilsson-Örtman & Johansson 2017). However, when animals are exposed to prolonged changes in temperature, or increases in temperature variability, their physiological capacity to acclimate and maintain homeostasis can be compromised, often resulting in increased oxygen demand, making them vulnerable to future changes in temperature through carryover effects (Vasseur et al. 2014, Schulte 2015, Semsar-kazerouni & Verberk 2018). Thermal sensitivity to a change in temperature regime can be described using  $Q_{10}$  values measured over short time periods, with high values indicating a high stress response to a given stimulus and low values indicating that the animal was under very little stress during exposure (Newell 1969, Vaquer-Sunyer & Duarte 2011, Havird et al. 2020).

Investigations of exposure to chronic extreme temperatures and increases in mean temperature (e.g. Kennedy & Mihursky 1972, Hoffmann 2010, Clusella-Trullas et al. 2011, Denny et al. 2011, Stevens & Gobler 2018) indicate that episodic extreme temperatures that are not lethal but fall outside the opti-

mal temperature range (termed sublethal temperatures here) have a greater effect on the evolution of thermal performance curves than lethal temperatures (see review by Williams et al. 2016). Exposure to stressful sublethal temperatures (i.e. temperatures that increase stress and possibly cause injury but do not cause death) often have physiological consequences, such as constraints on aerobic metabolism, shifts in energy utilisation or the induction of heat-shock protein synthesis (Jansen et al. 2009, Vasseur et al. 2014). Exposure to sublethal temperatures can induce carryover effects that may be the result of damage during acclimation or acclimatisation (O'Connor et al. 2014, Williams et al. 2016). By studying carryover effects in an active acclimation context, one can use the results to forecast the responses of animals to future climate change (Inger et al. 2010). To understand responses to long-term changes in mean temperatures or temperature variability, however, it is important to determine if sublethal temperatures can be generalised across, or even within, taxa. For example, do conspecifics from different climatic zones experience similar temperatures as stressful and exhibit similar carryover effects when exposed to the same temperatures (Williams et al. 2016)?

Intertidal animals are exposed to daily immersion and emersion (Stillman 2002, Crickenberger et al. 2015, Tagliarolo & McQuaid 2015, Sorte et al. 2019) and are expected to experience rapid increases in temperature variability because of both exposure to air and the low thermal inertia of such shallow-water systems (Vinagre et al. 2018), making this habitat especially sensitive to climate change. Particularly important will be the responses of autogenic ecological engineering organisms that ameliorate environmental stress, as these will have knock-on effects for the species that depend on them as habitat. In this case, we addressed our questions using mussels, as they are important in the maintenance of biodiversity (Seed 1996, Arribas et al. 2014). We used 2 genetically distinct lineages (termed East and West) of the intertidal mussel *Perna perna* to investigate intraspecific variability in the effects of temperature variability. The species has Indo-Pacific origins, and the 2 lineages diverged recently, the West lineage having passed through the Tethys seaway into the Atlantic and down the west coast of Africa before making secondary contact with the East lineage (Cunha et al. 2014). At present, the West lineage is absent from the upwelling dominated west coast of South Africa, where temperatures are too low to permit reproduction (Tagliarolo et al. 2016; see our Fig. 1). The 2 lineages are morphologically indistinguishable but dif-

fer in the strength of their byssal attachment and gaping behaviour (Zardi et al. 2015), and now overlap across approximately 200 km of the south coast of South Africa (Zardi et al. 2007).

Although thermal limits generally correlate with tidal height in intertidal species (Stillman 2002), finer scale aspects of their physiology can be better predictors of distribution. For example, the East and West lineages of *P. perna* do not differ in their upper thermal limits (30°C), but significant differences in their Arrhenius activation energies correlate with their distributions along the cool/west to warm/east gradient of the South African coastline, indicating that sub-specific differences in physiology can be important in determining species' distributions (Tagliarolo & McQuaid 2015). Here, we used  $Q_{10}$  estimates on standard metabolic rate to evaluate the effects of acclimation history on sensitivity to an acute rise in temperatures in the 2 lineages of *P. perna*. Because we have no information on possible alterations to anaerobic metabolism, as used here, the term standard metabolic rate refers specifically to aerobic metabolism. The metabolic machinery includes a repertoire of molecular responses that can signal both positive (e.g. an adaptive heat-shock response) and negative (e.g. high energetic costs) effects on fitness (Somero 2005), which complicates our ability to accurately quantify the performance of intertidal organisms. Within the sublethal temperature range, the stress-response capacity of an organism is not necessarily limited by a passive heat-shock response, but by its ability to maintain physiological homeostasis (Pörtner 2010). Thus, by measuring the mussels' thermal sensitivity under an acute temperature ramping, we effectively tested their capacity to prevent further excess damage (Havird et al. 2020). We hypothesised (1) that animals exposed to fluctuating temperatures would exhibit a carryover effect, being more sensitive to temperature changes as shown by higher  $Q_{10}$  values and, given differences in their activation energies, that (2) such carryover effects would be stronger in the eastern lineage.

## 2. MATERIALS AND METHODS

### 2.1. Sampling sites and field body temperature

Because the 2 lineages of *Perna perna* are morphologically indistinguishable, we were forced to collect mussels from sites located on either side of the region of distributional overlap described by Zardi et al. (2007). The East and West lineages were therefore

collected from Port Edward (31° 03' 28.9" S, 30° 13' 37.2" E) and Brenton-on-Sea (34° 04' 32.8" S, 23° 01' 41.4" E), respectively (Fig. 1). At each site, we collected 40 individuals of similar size (mean  $\pm$  SD shell length: 4.03  $\pm$  0.2 cm); 12 individuals were needed per treatment per lineage and additional individuals were collected in case of mortality due to the stress of transport. Mortality during transport was nevertheless negligible (ca. 2.5%). Mussels were taken to the laboratory at Rhodes University, while kept moist and cool in insulated boxes, with travel times of 7–8 h. Collections were done within the same spring-tide period in April 2018. We removed epibionts and allowed mussels to acclimate in water at 20°C for 24 h before the treatments began. The mussels were housed in aquaria in a climate-controlled room with 20 ind. aquarium<sup>-1</sup>. The lineages were kept separate from each other.

To characterise their natural habitats, field body temperatures were estimated using biomimetic temperature loggers (i.e. 'robomussels'; Lathlean & McQuaid 2017). We built robomussels by placing loggers (iButton DS1922, Maxim Integrated) in empty shells (4 cm length) of *P. perna* filled with waterproofing electrical resin (3M Scotchcast 48FR). Temperature data were recorded every 30 min at 0.5°C resolution between April 2015 and April 2017. At each site, we used Z-spar Splash Compound (Kopper's Co.) to attach 3 robomussels in the mid-intertidal zone, amongst live individuals.

### 2.2. Acclimation treatments

Prior to estimating the thermal sensitivity of mussels from the 2 lineages, we exposed individuals to

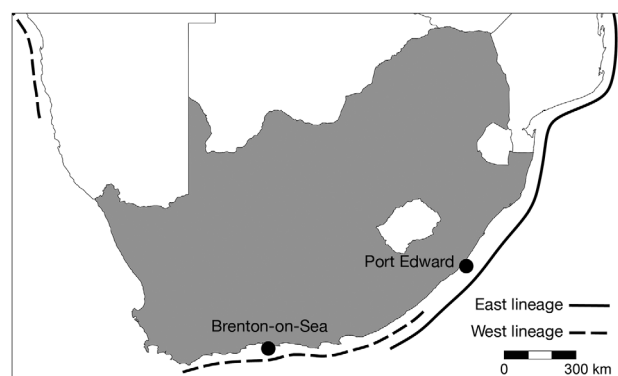


Fig. 1. Sampling sites for collection of West and East lineage *Perna perna* along the coast of South Africa. Brenton-on-Sea (34° 04' 32.8" S, 23° 01' 41.4" E); Port Edward (31° 03' 28.9" S, 30° 13' 37.2" E)

either (1) a short, 13 h period of recovery in seawater at 20°C (field-fresh condition); (2) a 2 wk treatment period at constant temperature; or (3) a 2 wk treatment period of fluctuating temperatures. A 2 wk acclimation period was based on previous studies and reflects the 2 wk tidal changes influenced by the lunar cycle (Hicks & McMahon 2002b, Tagliarolo & McQuaid 2015). Both the constant and fluctuating treatments were subjected to daily submergence and aerial-exposure periods of 21 and 3 h, respectively, mimicking the natural conditions experienced by this species in the intertidal zone. To reach the target level of variability in body temperatures, we conducted the experiments in climate-controlled rooms. Under the constant temperature regime, animals were maintained at 20°C in air and water. Under the fluctuating temperature regime, mussels were kept at 20°C while submersed, and exposed to alternating days of increased (24°C) and decreased (16°C) temperatures while in air (Fig. 2). The upper and lower temperatures were chosen as these were the maximum and minimum temperatures common to both sites. Animals were maintained in unfiltered, natural seawater that was completely renewed every 2 d with natural seawater collected from a local beach to maintain water quality and avoid the depletion of food. The seawater was approximately 20°C when collected. The aquariums were also cleaned of fouling and pseudofaeces accumulated by the mussels.

### 2.3. Respiration rate and thermal sensitivity

To determine the thermal sensitivity of mussels, we exposed them to a temperature ramping protocol while measuring respiration rates. Prior to the temperature ramping protocol, we starved the mussels for 13 h in filtered seawater (0.7 µm pore size, glass-fibre filter, Whatman). Individuals ( $n = 12$  lineage<sup>-1</sup> treatment<sup>-1</sup>) were then placed in individual 200 ml closed chambers filled with oxygenated, filtered seawater which was homogenised using magnetic stirrers. We ran this experiment in a climate-controlled room that allowed the manipulation of air temperature. By increasing the ambient temperature, we raised the seawater temperature in the chambers from 20 to 30°C at a

rate of  $\sim 0.14^\circ\text{C min}^{-1}$ , consistent with field observations (Tagliarolo & McQuaid 2015). Some individuals were seen gaping during the ramping protocol, but this behaviour stopped once the water temperature passed 25°C. Every 5 min, we measured the seawater oxygen concentration in duplicate using non-invasive sensor spots attached to the inside of each chamber, and a fibre-optic sensor (Fibox 3, Pro-Sense). Sensor spots were calibrated before measurements based on 2 points: 0% O<sub>2</sub> concentration reached using nitrogen gas, and 20.9%, using ambient air. The exact temperature change was measured in a separate chamber using a temperature logger (iButton, DS1922L, Dallas Maxim) and the Fibox 3 RTD temperature sensor. At the end of the experiments, animals were dissected, their soft tissues were dried for 24 h at 60°C and weighed to the nearest 0.001 g. We also ran the protocol without the mussels to control for any changes in oxygen not caused by their presence. Respiration rates were calculated as a mean for each 5 min interval as the difference between the final and initial oxygen concentrations and normalised to individual dry weight as  $\mu\text{mol O}_2 \text{ g}^{-1} \text{ h}^{-1}$ .

For each mussel, we estimated  $Q_{10}$  using the equation  $Q_{10} = (R_2/R_1)^{(10/(T_2-T_1))}$  (Newell 1969), where  $R_1$  and  $R_2$  are the respiration rates measured at temperatures  $T_1$  (21°C) and  $T_2$  (26°C), respectively. We excluded measurements taken outside the 21–26°C range due to high variability in the data. Below this range, the high variability was likely related to the initial accli-

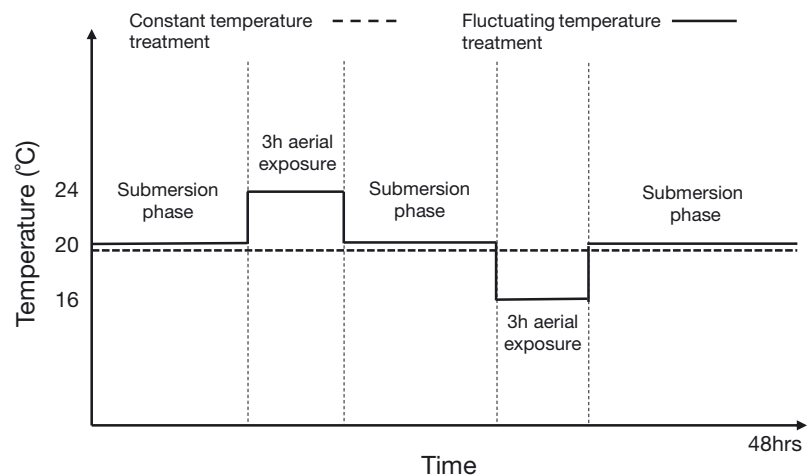


Fig. 2. Schematic representation of the treatments used during this investigation. Water temperature was maintained at 20°C for all treatments. The constant-temperature treatment was set consistently at 20°C during emersion. Air temperature in the fluctuating temperature treatment was cycled over 2 d. The first day, individuals were exposed to an increase in temperature (24°C) and the following day exposed to a reduced temperature (16°C)

mation of mussels to their chambers, and above the range, individuals were approaching an upper thermal threshold. The standard metabolic rates at those temperatures were derived from linear regressions fitted between respiration rate ( $\mu\text{mol O}_2 \text{ g}^{-1} \text{ h}^{-1}$ ) and the mean temperature experienced during each 5 min recording interval.

#### 2.4. Statistical analyses

To test for the effects of acclimation treatment (3 levels) and lineage (2 levels) on the thermal sensitivity of *P. perna* (i.e.  $Q_{10}$ ), we used general linear models with Gaussian error distributions, computed in R v.3.6 (R Core Team 2019). We determined the relative support for models including different combinations of predictors following the information-theoretic paradigm (Burnham & Anderson 2002). We calculated Akaike's information criterion corrected for small sample sizes ( $\text{AIC}_C$ ) and their weights ( $w\text{AIC}_C$ ) to provide a relative probability of model 'truth', assuming that the true model is included in the model set (Link & Barker 2006). For the top-ranked model, we evaluated the significance of the effects using likelihood-ratio tests using the package 'car' (Fox & Weisberg 2019). We examined the assumptions of normality and homogeneity of variances visually, based on Normal  $Q-Q$  and Scale-Location plots, respectively. These plots suggested that models performed on the raw data were non-parametric (Fig. A1 in Appendix 1), so we re-ran them using  $\log_{10}$ -transformed values, which better fit the parametric error assumptions. To identify differences among factor levels, we did simultaneous tests for general linear hypotheses using the package 'multcomp' (Hothorn et al. 2008).

We also examined the effects of lineage and acclimation treatment on the metabolic rates of *P. perna*. To control for the positive effect of the covariate temperature, we worked with the residuals of the linear model between respiration rate and temperature (i.e. temperature covariate-adjusted residuals) (Jakob et al. 1996). This was computed as a linear-mixed effect model using the package 'lme4' (Bates et al. 2015), with individual mussels as a random variable to account for non-independence of measurements taken at different temperatures. The fixed effects of lineage and acclimation treatment were then assessed via general linear models (assuming Gaussian error distribution) and using the information-theoretic paradigm and likelihood-ratio tests, as above.

### 3. RESULTS

#### 3.1. Field body temperature

Using robomussel temperatures as a proxy for body temperature, we found that the temperatures experienced in the field by East and West lineage *P. perna* differed markedly, in both mean and variance (Fig. 3). The mean body temperature of the East lineage at Port Edward was warmer ( $21.82^\circ\text{C}$ ) than that of the West lineage at Brenton-on-sea ( $17.87^\circ\text{C}$ ). However, the range and variability were higher for the West (min. =  $8.41$ , 1<sup>st</sup> quartile =  $15.79$ , 3<sup>rd</sup> quartile =  $19.62$ , max. =  $41.60^\circ\text{C}$ ) than the East lineage (min. =  $15.36$ , 1<sup>st</sup> quartile =  $20.62$ , 3<sup>rd</sup> quartile =  $22.86$ , max. =  $36.78^\circ\text{C}$ ) (Fig. 3).

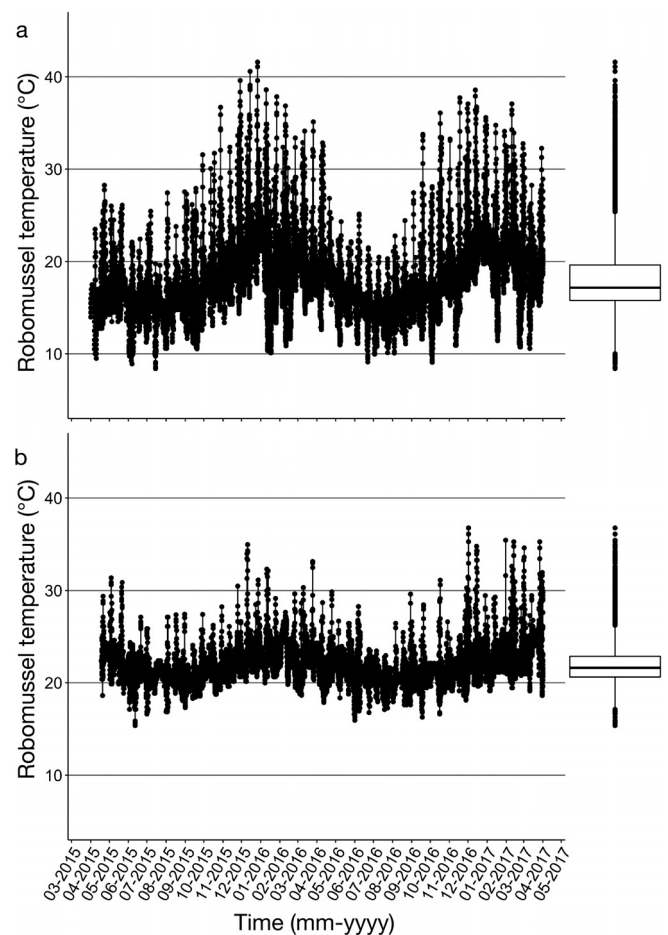


Fig. 3. Body temperatures experienced by (a) West and (b) East lineage *Perna perna* at Brenton-on-sea and Port Edward, respectively (see Fig. 1). Temperature data were recorded using robomussels deployed on the mid-shore level between April 2015 and April 2017. Box-plot parameters: boxes: 25<sup>th</sup> and 75<sup>th</sup> percentiles; horizontal lines: medians; whiskers: extreme values within 1.5 times the interquartile range; points: outliers



### 3.2. Thermal sensitivity and respiration rate

The top-ranked model included only acclimation treatment as a predictor variable (Table 1). The relative support for this model was substantially higher ( $wAIC_C = 0.650$ ) than for the one containing lineage ( $wAIC_C = 0.249$ ). Using the top-ranked model, we confirmed the strong effect of treatment based on a likelihood-ratio test ( $\chi^2 = 14.387$ ,  $df = 2$ ,  $p = 0.0008$ ). The post hoc test showed that  $Q_{10}$  was higher for the fluctuating-temperature treatment than the field-fresh ( $p = 0.0059$ ) and constant temperature ( $p = 0.0047$ ) treatments, with no differences detected between the latter 2 ( $p = 0.9884$ ) (Fig. 4).

Table 1. Model rankings to examine the combined effects of acclimation treatment ( $T$ ) and lineage ( $L$ ) on the thermal sensitivity ( $Q_{10}$ ,  $\log_2$  transformed) of *Perna perna*. LL: log likelihood; df: model degrees of freedom;  $\Delta AIC_C$ : difference in Akaike's information criterion (corrected for small sample size) between the current and top-ranked models;  $wAIC_C$ :  $AIC_C$  weight ( $\sim$ model relative probability)

Model	df	LL	$AIC_C$	$\Delta AIC_C$	$wAIC_C$
$Q_{10} \sim T$	4	-39.009	86.7	0	0.650
$Q_{10} \sim T + L$	5	-38.791	88.6	1.92	0.249
$Q_{10} \sim \text{Null}$	2	-43.777	91.8	5.05	0.052
$Q_{10} \sim L$	3	-43.275	93.0	6.25	0.028
$Q_{10} \sim T + L + T \times L$	7	-38.770	93.5	6.84	0.021

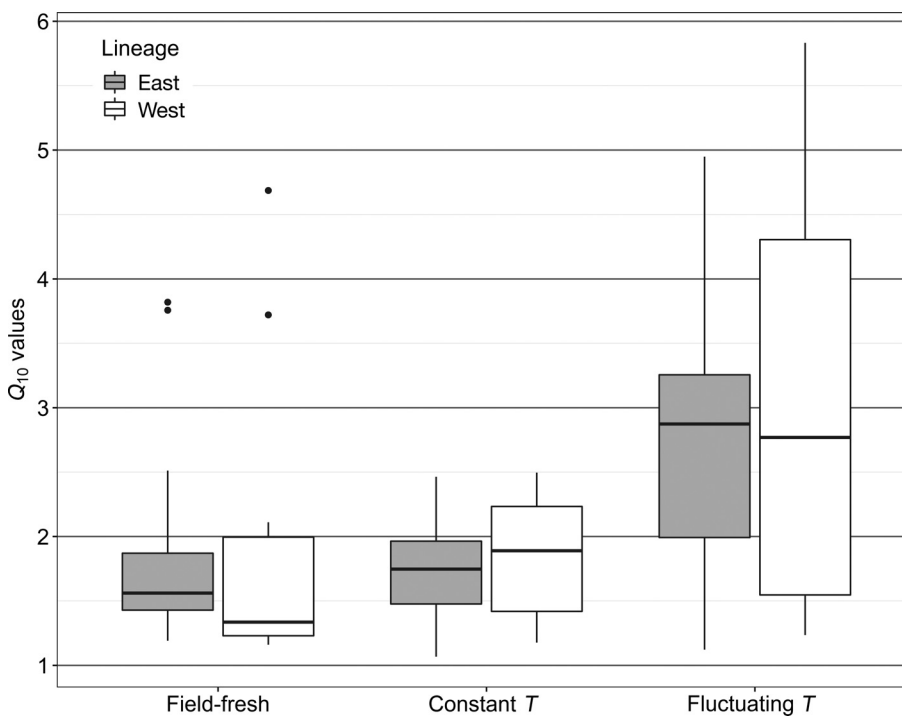


Fig. 4. Thermal sensitivity,  $Q_{10}$  values of East and West lineages of *Perna perna* estimated for individuals that were field fresh, or acclimated to either constant or fluctuating temperatures for 2 wk. The definitions of the box-plot elements are given in Fig. 3

After accounting for the linear effect of temperature on the respiration rate of *P. perna*, we found no support for significant effects of acclimation treatment or lineage (Fig. 5, Table 2) on the standard metabolic rate.

## 4. DISCUSSION

Early research on the responses of organisms to climate change was necessarily done at a relatively coarse level, notably by documenting species-level range shifts. There is increasing evidence, however, that more nuanced effects—such as interactions among environmental drivers or among species, the effects of adaptation and the level of biodiversity—may all lead to quite different end results (Davis et al. 1998, Reusch et al. 2005, Poloczanska et al. 2013, Rastelli et al. 2020). Given ongoing increases in global temperatures, there is a tendency to focus on critical thermal maxima, with species often treated as relatively homogenous entities for modelling purposes. This assumes that species respond to changing conditions in a uniform fashion, but there is increasing evidence from organisms as diverse as macroalgae and insects that different within-species lineages may respond quite differently (e.g. Nicastro et al. 2013, Meynard et al. 2017). In contrast to inter-specific assessments (e.g. Wedemeyer 1973,

Table 2. Model rankings to examine the combined effects of acclimation treatment ( $T$ ) and lineage ( $L$ ) on the temperature covariate-adjusted residuals of respiration rates in *Perna perna*. LL: log likelihood; df: model degrees of freedom;  $\Delta AIC_C$ : difference in Akaike's information criterion (corrected for small sample size) between the current and top-ranked models;  $wAIC_C$ :  $AIC_C$  weight ( $\sim$ model relative probability)

Model	df	LL	$AIC_C$	$\Delta AIC_C$	$wAIC_C$
Residuals $\sim$ null	2	365.312	-726.6	0	0.414
Residuals $\sim T$	4	367.053	-726.0	0.58	0.309
Residuals $\sim L$	3	365.323	-724.6	2.01	0.152
Residuals $\sim L + T$	5	367.056	-724.0	2.62	0.111
Residuals $\sim T + L + T \times L$	7	367.086	-719.9	6.69	0.015

Das et al. 2002), few large-scale studies incorporate within-species variation in physiological tolerances or address the implications of chronic exposure to the stress induced by sublethal but variable temperatures. Such stress can induce carryover effects that lead to a reduction in fitness (Harrison et al. 2011) that will manifest before temperatures reach lethal levels. Because they are almost all of marine origin and adapted to regular exposure to air, intertidal organisms are interesting models to test for carryover effects that result from thermally variable environments. Our experiments revealed the effects of repeated exposure to sublethal stress on the metabolism of mussels, with no effect of intra-specific genetic variability.

#### 4.1. Lineage effect

Because the 2 lineages we studied are indistinguishable externally, we necessarily confounded lineage and collection site. The range of temperatures experienced at our 2 sites are clearly different (Fig. 3), and yet we found no significant differences between field-fresh individuals of the 2 lineages in either standard metabolic rate or  $Q_{10}$  (Tables 1 & 2).

Generally, warm-acclimated animals exhibit a lower absolute metabolic rate than cool-acclimated animals at the same temperature (Pickens 1965, McCue 2004). In our case, however, field-fresh animals from our 2 sites showed no difference in standard metabolic rate, despite marked differences in their field body temperatures (Port Edward: 21.82°C; Brenton-on-Sea: 17.87°C). Nor did they differ in their reactions to the 3 treatments, resulting in no significant effect of lineage or its interaction with treatment on  $Q_{10}$  or respiration rates (Tables 1 & 2), despite the previously demonstrated genetic differences and evolutionary histories of the 2 lineages (Zardi et al. 2007, Cunha et al. 2014). This similarity in physiological responses was also unexpected because Brenton-on-Sea in the west is subject to occasional upwelling and exhibits greater temperature

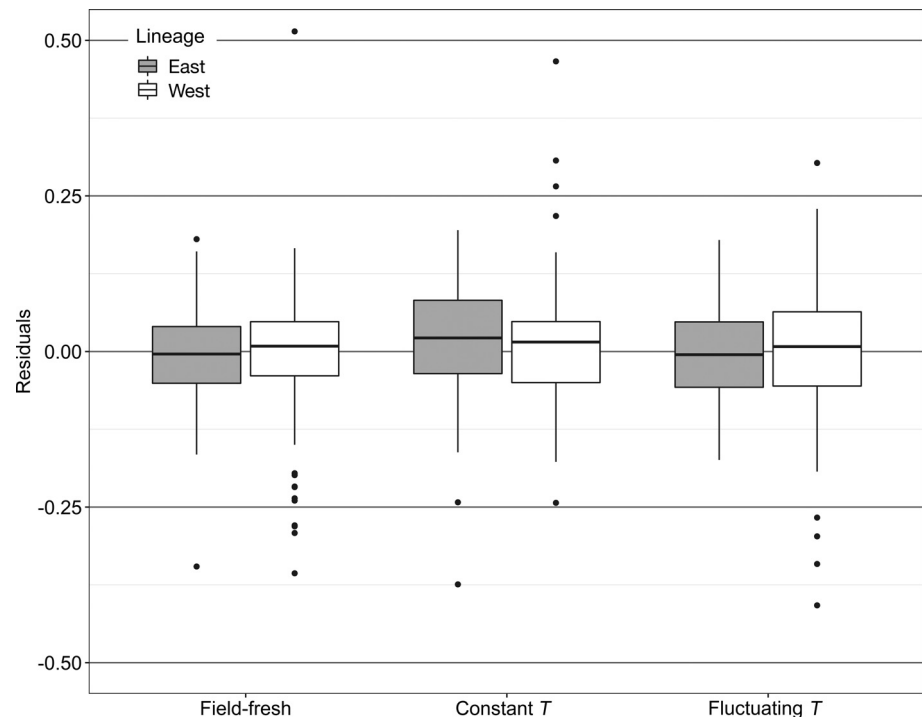


Fig. 5. Residuals of the linear model relating standard metabolic rate to temperature as a function of acclimation treatment (field fresh, constant temperature, and fluctuating temperature) and lineage (East and West). The definitions of the box-plot elements are given in Fig. 3

variability than Port Edward in the east. Although the difference between lineages was non-significant for field-fresh animals, the higher  $Q_{10}$  values for Brenton-on-Sea suggest that mussels there may have already exhibited some degree of carryover effect, reflecting the stress of more variable temperatures. This was surprising given the higher activation energy of the eastern lineage (Tagliarolo & McQuaid 2015), a characteristic of warm-acclimated species (van Dijk et al. 1999, Somero 2005), and the fact that species living under more variable conditions are generally expected to have a lower  $Q_{10}$  (Newell & Northcroft 1967, Huey & Kingsolver 1993, Angilletta et al. 2002). Our findings likely differ from those of these earlier studies due to differences in experimental designs.

While earlier studies have focussed mainly on metabolic responses to temperatures experienced over prolonged periods, we were interested in quantifying a stress response to fluctuating temperature conditions based on measurements of metabolic rate during an acute temperature ramping. In a meta-study of acclimation and  $Q_{10}$  effects, Havird et al. (2020) linked greater stress directly with elevated  $Q_{10}$  measured over short time periods, and we interpret the non-significantly higher  $Q_{10}$  of field-fresh animals from the thermally variable site as a suggestion of higher stress levels caused by higher variability in temperature.

#### 4.2. Carryover effect

Jansen et al. (2009) showed that intertidal mussels held under constant temperature and then exposed to a rapid increase in temperature exhibited high levels of stress due to heat shock, but we found no difference between field-fresh and constant-temperature treatments; both showing  $Q_{10}$  values (close to 2) that indicate minimal stress (Zuo et al. 2012). This finding indicates that there was no experimental artefact and that the conditions experienced in the field due to tidal emersion/submersion prior to collection were well within tolerance limits, imposing stress below the levels at which carryover effects become detectable and allowing our experimental animals to cope with a sudden temperature rise without a significant increase in  $Q_{10}$ . In contrast, experimental exposure to fluctuating temperatures led to increased thermal sensitivity, with a mean  $Q_{10}$  close to 3 for both lineages (Fig. 4), even though the temperatures experienced were well below lethal levels (Segnini de Bravo et al. 1998, Hicks & McMahon 2002a, Tagliarolo & McQuaid 2015).

Although there was no significant difference between the field-fresh and constant-temperature treatments, it was striking that, for both lineages, mean  $Q_{10}$  values decreased in the sequence fluctuating temperature > field fresh > constant temperature. Clearly, levels of stress lie on a gradient from non-existent to severe, and as the experiment was run in April when temperature variability was relatively low, this ranking suggests that carryover effects may be detectable in summer (e.g. December–February) when temperature variability is much greater, particularly in the west (Fig. 3a).

It was also notable that within-treatment variability was minimal under constant temperatures. High variability in field-fresh animals, particularly the West lineage (Fig. 4), could reflect the nature of very small-scale variability in the temperatures that individuals experience under natural conditions (Helmuth 1998, Denny et al. 2011, Lathlean et al. 2016). Lathlean et al. (2016) noted that the place a mussel occupies on the rocky shore can affect its internal temperature significantly compared to that of its neighbours. Particularly important are its position with respect to the edge or centre of the mussel bed and the direction of air movement.

Jimenez et al. (2015) found that laboratory acclimation reduced the variability in antioxidant capacity in the mussel *Mytilus californianus* by 30% compared to field-fresh animals. Interestingly, this effect only occurred for mussels collected from a cool site, with no effect for those from a warmer site. In their case, the sites represented different aspects of a rocky outcrop, so that the difference in temperature regime was due to shading at one site but not the other. In our case, the difference between sites reflected differences in both air and water temperatures. Jimenez et al. (2015) ascribed the difference in response of their populations to context-dependent genotype–environment interactions and hypothesised that the population from the cool site harboured greater genetic diversity, leading to greater physiological variation. There is no evidence of differences in genetic diversity between our lineages (Zardi et al. 2015), which might explain the lack of differences in  $Q_{10}$  between field-fresh East and West individuals of *Perna perna*.

Tagliarolo & McQuaid (2015) suggested that sublethal effects may be better predictors of the distribution of mussels than their thermal limits. Our results provide a possible mechanism for this phenomenon through the increased sensitivity to temperature and its associated energy costs induced by higher ther-



mal variability (Somero 2005). For intertidal species, climate change implies not only rising average temperatures, but also exposure to greater thermal variability through alternating exposure to air and sea temperatures and more frequent exposure to stressful, sublethal temperatures. Chronic exposure to sublethal temperatures is likely to increase carryover effects by increasing thermal sensitivity. Consequently, animals could experience significant reductions in fitness before being exposed to their upper lethal limits. Because of the ecosystem engineer services that intertidal mussels provide, such a reduction in fitness will also degrade the structure of the communities they maintain.

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### Appendix 1

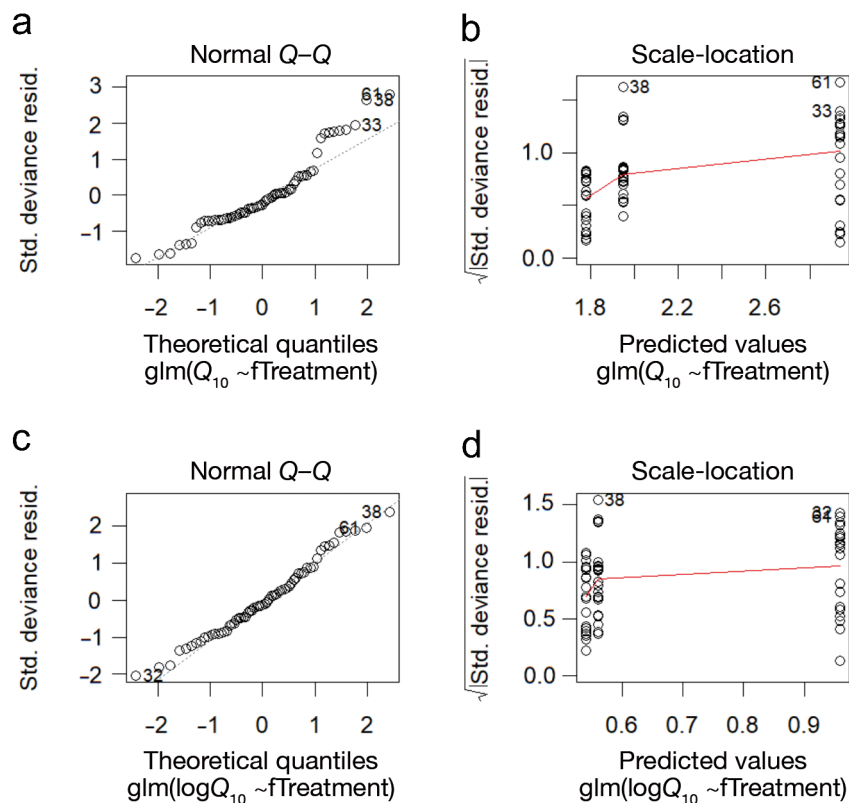


Fig. A1. Diagnostic plots to examine assumptions of normality in the distribution of residuals (Normal Q–Q plots) and homogeneity of variances (Scale–Location plots) for models done using (a,b) raw  $Q_{10}$  data and (c,d)  $\log_{10}$ -transformed  $Q_{10}$  data

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