Vol. 535: 145–159, 2015
doi: 10.3354/meps11434

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published September 15

Sub-lethal and sub-specific temperature effects are better predictors of mussel distribution than thermal tolerance

Morgana Tagliarolo*, Christopher D. McQuaid

Department of Zoology and Entomology, Rhodes University, Grahamstown 6140, South Africa

ABSTRACT: Three classes of mussel, the invasive *Mytilus galloprovincialis* and 2 genetic lineages of the indigenous *Perna perna*, show partially overlapping distributions along a large-scale temperature gradient in South Africa. We asked whether their physiological tolerances explain their distributional limits, investigating the effects of acute temperature change on heart rate, oxygen consumption and anaerobic end-product accumulation in air and water in the laboratory. For all 3 classes, heart rate and oxygen consumption were significantly reduced in air and were not correlated with temperature. During immersion, temperature had powerful effects on metabolic rate, but the ranking of heart rates reversed between heat and cold stress. The eastern *P. perna* lineage had higher heart rates with rising temperatures, while *M. galloprovincialis* showed higher heart rates during cooling. Despite no difference in upper thermal limits among mussel classes, their Arrhenius activation energies differed significantly, in parallel with their warm to cool distributions in the order *P. perna* east > *P. perna* west > *M. galloprovincialis*. The results indicate that the eastern lineage of *P. perna* is better adapted to warm temperatures and *M. galloprovincialis* is better adapted to high shore conditions. Upper thermal limits gave only a crude approximation of the effects of temperature stress. Although the thermal limits of these populations were similar, their overall responses to stress differed markedly, reflecting their distributions and potentially affecting their competitive interactions. We suggest that thermal limits offer poor explanations for species distributions, and highlight the critical importance of both sub-lethal effects and sub-specific differences in physiology.

KEY WORDS: Thermal limits · Mussel · Physiology · Metabolism

INTRODUCTION

Physiological adaptation to temperature plays an important role in establishing the biogeographic distributions of both aquatic and terrestrial species (Somero 2005, Braby & Somero 2006). The identification of thermal limits can help us to understand biogeographic patterns and explain the dynamics governing the distributions of native and invasive species (Lockwood & Somero 2011). Physiological adaptations are particularly important for intertidal organisms, which are subject to strong thermal and desiccation stresses during low tide periods (Connell 1972). Intertidal organisms experience important variations in body temperature between immersion and emersion, and evidence suggests that both air and seawater temperature can affect the physiological functions and biogeographic distributions of such species (Helmuth et al. 2006).

In South Africa, intertidal mussel populations exist on the lower shore and are dominated by the invasive *Mytilus galloprovincialis* Lmk. on the cool-temperate west coast and the indigenous *Perna perna* L. on the subtropical east coast (Zardi et al. 2007). The 2 species coexist on the warm-temperate south coast where they compete with one another (Rius &

*Corresponding author: morgana.tagliarolo@gmail.com

McQuaid 2006, 2009) and show clear vertical separation. *M. galloprovincialis* and *P. perna* dominate the upper and lower mussel zones, respectively, with an area of overlap and mixed species beds in the mid-mussel zone (Bownes & McQuaid 2006).

The geographic expansion of *M. galloprovincialis* along the coast from its point of introduction on the west coast appears to have reached at least temporary limits (Robinson et al. 2005), and it is present from Lüderitz in southern Namibia to East London on the south coast of South Africa (Zardi et al. 2007). *P. perna* is abundant along the entire east and south coasts of South Africa, but has never been present along about 1000 km of the west coast (Fig. 1), which is strongly affected by the Benguela upwelling system, where *M. galloprovincialis* has replaced other indigenous species as the dominant mussel (Branch & Steffani 2004). Analysis of mitochondrial DNA reveals that *P. perna* comprises 2 genetic lineages with a non-sister relationship along the South African coast (Cunha et al. 2014). Divergence of the 2 lineages appears to be very recent (310 kyr) and there is no clear morphological differentiation between them (Cunha et al. 2014). The western lineage includes individuals from both Namibia and the south coast of South Africa. The eastern lineage includes individuals from the southeast and east coasts of South Africa. The 2 lineages overlap for ca. 200 km on the southeast coast in the region where *M. galloprovincialis* reaches its eastern limit in southern Africa. For convenience, we refer to *Mytilus* and the 2 lineages of *Perna* as 'classes'.

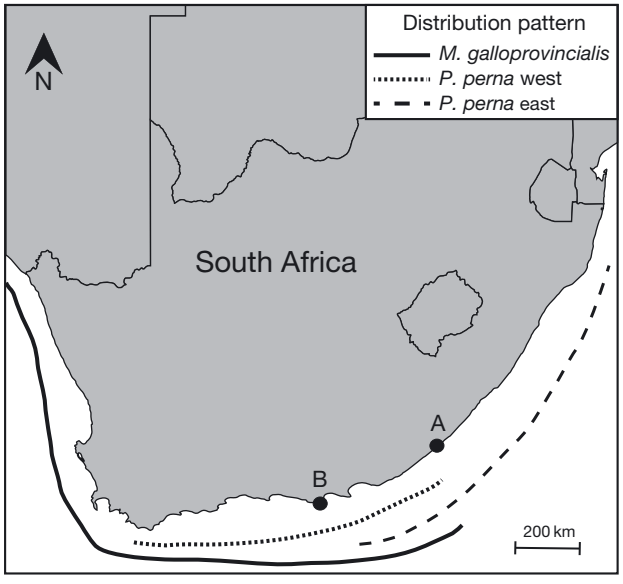

Fig. 1. Sampling sites in South Africa for *Perna perna* east (A, Kei Mouth) and for *Mytilus galloprovincialis* and *P. perna* west (B, Saint Francis Bay) and species geographic distribution pattern

Despite the ability of mussels to disperse through a planktonic larval stage, geographic differences in the species and genetic composition of communities are common (Ladoukakis et al. 2002). Previous studies investigated several possible mechanisms that may explain the geographic distribution of *M. galloprovincialis* and the 2 *P. perna* lineages, and hydrodynamic conditions are shown to be a possible barrier to larval dispersion on different parts of the South African coast (McQuaid & Phillips 2000, Zardi et al. 2011). Laboratory experiments reveal that the 2 lineages of *P. perna* have different physiological responses to high temperatures and different tolerances to sand stress (Zardi et al. 2011). Although the invasive *M. galloprovincialis* has weaker attachment strength, it can maintain higher recruitment and growth rates than *P. perna* (van Erkom Schurink & Griffiths 1993, Nicastro et al. 2010a), and these characteristics can help explain the great capacity of the invasive species to compete for space (Erlandsson et al. 2006). The study of thermal limits of the 2 species and the 2 lineages living on the South African coasts is limited to the analysis of mortality rates (Zardi et al. 2011). Data from studies performed in several other countries (Hicks & McMahon 2002) suggest that *M. galloprovincialis* is more adapted to cold conditions than *P. perna* but, unfortunately, few details of thermal tolerance or metabolic responses are available for low temperature and emersion conditions.

We tested the hypothesis that the distributions of *M. galloprovincialis* and the 2 *P. perna* lineages are driven by their physiological and metabolic thermal adaptations. To do this, the effects of warming and cooling stress were studied in the laboratory under conditions of both emersion and immersion using different temperature ramping protocols. The thermal limits of *Mytilus* and the 2 *Perna* lineages were studied by applying 2 different ramping protocols and monitoring the heart rate responses. A third protocol focused on acute temperature changes to allow a better understanding of the metabolic mechanisms involved in response to thermal stress by quantifying 3 physiological parameters: heart rate, oxygen consumption and anaerobic end-product production. We hypothesized that the invasive species, *M. galloprovincialis*, has a greater ability to tolerate cold stress and that *P. perna* is better adapted to warm conditions, with the eastern lineage being more tolerant of high temperatures than the western lineage.

MATERIALS AND METHODS

Sampling

As the 2 genetic lineages of *Perna perna* do not show morphological differences, we selected sites where each lineage exists in isolation from the other (Fig. 1), based on their distributions and that of *Mytilus galloprovincialis* as described by Zardi et al. (2007). The heart rate of *P. perna* belonging to the eastern lineage (hereafter *P. perna* east) was measured for mussels sampled at Kei Mouth (Site A in Fig. 1; 32° 42′ 17″ S, 28° 22′ 23″ E). Mussels from the western lineage (*P. perna* west) and *M. galloprovincialis* were collected at Saint Francis Bay (Site B in Fig. 1; 34° 10′ 15″ S, 24° 50′ 05″ E). Both sites are semi-protected shores with a similar semi-diurnal tidal regime and with a spring-tide amplitude of about 2 m. The annual average air and water temperatures at the 2 sites are, respectively, 18.0 and 18.6°C in Kei Mouth, and 17.6 and 17.9°C in Saint Francis Bay (unpubl. data from *in situ* temperature loggers).

Mussels of similar size (3 to 4 cm) were collected and transported to the laboratory within 4 h, in air while being kept damp. In the laboratory, individuals were cleaned of epibionts and allowed to recover from sampling stress for at least 48 h in a constant temperature room at 18°C, in seawater tanks with a simulated natural tidal cycle. Seawater was continuously aerated using an aquarium air pump and mussels were fed daily with a commercial phytoplankton mix (PhytoGreen-M, Brightwell Aquatics). Mussels were kept in 20 l tanks, each containing about 50 individuals at any given time, for a maximum of 7 d. A preliminary test of stability of pH, temperature and oxygen conditions indicated that cleaning the tanks and replacing a quarter of the seawater every third day avoided significant pH changes. Temperature was checked daily and oxygen level in the seawater was checked every 2 to 3 d. Each individual was subjected to only 1 treatment.

Temperature ramping

The thermal tolerance limits of *Mytilus* and the 2 *Perna* lineages were estimated using 2 ramping protocols (A and B) in order to test the effects of different treatments on their heart rates. Previous studies of the thermal limits of intertidal organisms have used ramping rates varying from 0.02 to 0.3°C min^{-1} (Walther et al. 2009, Fangue et al. 2011), while studies on mussels have used ramping rates between 0.10 and 0.13°C min^{-1} (Braby & Somero 2006, Logan et al. 2012). Field estimations of body temperatures, using biomimetics or robomussels, indicate that on hot days mussel body temperatures can rise at approximately 10°C h^{-1} (data used as the basis for Zardi et al. 2011); consequently, we used a ramping rate of 5°C per 30 min (0.17°C min^{-1}). The ramping rate was the same for the 2 ramping protocols, in order to allow comparison of the results, and mussels were exposed to ramping temperatures using a programmable water bath (Grant GP200, Grant Instruments), in which the individuals were kept in aerated seawater or in air at 100% humidity.

For the first protocol (Protocol A), mussels were collected between August and September 2013, and the ramping protocol was performed under both emersed and immersed conditions. Temperature in the water bath was increased from 18 to 45°C or decreased from 18 to 0°C while heart rates were recorded continuously.

The second ramping protocol (Protocol B) subjected mussels to a more extreme treatment. Individuals were collected between July and August 2014 and, in order to perform a continuous ramping protocol from 7 to 45°C on the same individuals, they were gradually cooled from 18 to 7°C over the course of 6 d. After collection, mussels were kept at 18°C for the first day and subsequently transferred to a second room at 15°C for a further 2 d, before being transferred to a water bath programmed for cooling from 15 to 7°C at a rate of 2°C d^{-1}, while being kept in aerated natural seawater and fed daily.

Heart rate was monitored using a non-invasive technique introduced by Depledge et al. (1996) and modified by Burnett et al. (2013). A sensor (CNY-70), consisting of an infrared emitter and phototransistor, was permanently glued next to the mid-dorsal posterior hinge area that corresponds to the position of the heart. Preliminary tests showed that the heart rate signals stabilized 10 to 15 min after handling, so the mussels were left undisturbed for about 15 min after attachment of the sensor, to recover before the start of recording. The signal was amplified, digitized using a data acquisition system (PowerLab 4/30, ADInstruments) and recorded with the associated software (LabChart 7.0, ADInstruments) at a rate of 200 samples per second. The temperature in the water bath was recorded using a T-type thermocouple probe connected to the data acquisition system. Aerial and underwater experiments were alternated during the day, following the natural tidal regime. As mussels were subjected to high levels of stress during the ramping protocol, each individual

was used for only 1 treatment (cooling or heating). Previous studies found that the automated counting of heartbeats can introduce errors, as heartbeat signals differ among individuals (Burnett et al. 2013). At the same time, manual counting is time consuming and can also be questionable in terms of reliability (Kaufmann et al. 2011). For these reasons, we used a software package able to combine both automatic peak detection and manual detection of artefacts (ARTiiFACT) (Kaufmann et al. 2011). Manual correction was applied after automatic detection in order to eliminate miscounting where small sections of the signal were clearly aberrant, reflecting animal movement rather than heartbeats. Heart rate was monitored continuously and the individual heartbeat (*HR*, beats per minute) was estimated using the following 3 steps: first, a global threshold criterion was applied to the raw data to automatically calculate the interbeat interval; second, errors in the automatic calculation of beats due, for example, to body movements or equipment noise were corrected manually; third, an instantaneous heartbeat was calculated and averaged per minute.

Acute temperature exposure

An acute temperature exposure protocol (Protocol C) was applied to compare the physiological responses of mussels exposed to different temperatures for the same amount of time under emersed and submersed conditions. For this third protocol, *HR*, oxygen consumption and anaerobic end-product concentration were measured in mussels that were collected between August and September 2013 under 4 temperature conditions (9, 18, 27 and 36°C) both in air (100% humidity) and in natural seawater.

This protocol involved 2 steps. First, temperature was increased or decreased at different ramping rates from 18°C (average ambient temperature at the 2 sites during the sampling period) to reach 9, 18, 27 or 36°C within 30 min. Second, temperature was kept stable at the target value (each of the above 4 temperatures) for 30 min, after which heart rate and oxygen consumption were measured simultaneously for the same individuals.

Heart rate was measured using an infrared sensor integrated with the respiratory chamber as described. Respirometry was performed in polyethylene terephthalate (PET) plastic jars in which the seawater or air was circulated using a micropump (D200S, TCS Micropumps) in a closed circuit. Oxygen concentration was measured at the beginning and end of the 30 min incubation using a FIBOX 3 (Presens) with a sensor spot attached to the inside wall of the plastic jar. At the end of the experiment, shell length and shell-free dry weight were obtained by drying the mussel flesh at 60°C for 24 h. Oxygen consumption was calculated as the difference between the beginning and the end concentrations (average of 1 min recording with a sampling rate of 1 value per second) and the results were expressed per gram of dry biomass (μmol O_2 h^{-1} [g dry wt]$^{-1}$).

The same acute temperature exposure protocol was applied to different individuals in order to perform an analysis of succinic acid accumulation. Succinic acid is the most significant end-product of anaerobiosis in mussels and exhibits a strong rhythmic pattern correlated with emergence and immersion (Connor & Gracey 2012). Previous studies on intertidal bivalves suggest that the adductor muscle accumulates succinic acid more rapidly than other tissues and is an appropriate model for the study of metabolites (de Zwaan et al. 1982, Michaelidis et al. 2005). After incubation under emersed or immersed conditions, mussels were quickly dissected on a bed of broken ice and the posterior adductor muscle was excised, closed in an Eppendorf tube and immediately shock-frozen in liquid nitrogen. Samples of muscle were preserved at –80°C before being lyophilized for 10 h. The dry tissue was then ground to powder, weighed and a sample of approximately 20 to 40 mg was deproteinized by the addition of 1 M ice-cold perchloric acid. The homogenate was centrifuged for 10 min with a Spectrafuge Mini (Labnet International). The supernatant from the perchloric acid extraction was neutralized to a pH of about 8 with 1 M potassium hydroxide (measured by dropping a few microlitres of sample onto pH paper; Hydrion, Sigma-Aldrich), kept on ice for 30 min and then centrifuged again as above. The extract was stored at –80°C before analysis. The concentration of succinic acid was assayed using a commercial kit (Megazime, International Ireland) according to the manufacturer's instructions. The succinic acid assay procedure applies the method of succinyl-CoA synthetase, pyruvate kinase and lactate dehydrogenase, according to Beutler (1989).

Data treatment

Arrhenius plots were utilized to evaluate the effect of temperature on heart rates following this equation:

$$\ln HR = \ln a - \frac{E_a}{R} \times \frac{1}{T}$$

where *HR* is the individual heartbeat (beats min^{-1}), *a* is a normalization constant, E_a is the activation energy (J mol^{-1}), *R* is the ideal gas constant (8.31 J K^{-1} mol^{-1}) and *T* is the absolute temperature (K).

The Arrhenius breakpoint temperature (ABT) was determined only for the warming protocol (no clear breakpoints were found for the cooling protocol) using piecewise regression with a Quasi-Newton estimation method (STATISTICA v. 12, Statsoft). Differences in breakpoints among *Mytilus* and the 2 *Perna* lineages were tested by 1-way ANOVA (n = 5 individuals per mussel class).

Linear regressions were calculated from the Arrhenius plot, excluding *HR* data higher than the breakpoint, and the R^2 values for each class or treatment are expressed as a mean ± SE.

Inter-individual variability in the linearized Arrhenius plots was tested for each experimental population using ANCOVA. Differences among *Mytilus* and the 2 *Perna* lineages and among treatments for Protocols A and B (again excluding *HR* data higher than the breakpoint) were tested by applying a generalized linear model (GLM) using a type III regression for unbalanced data.

Differences among mussel classes, temperatures and immersion/emersion were tested using ANOVA followed by the post hoc Tukey's HSD test on the data obtained with Protocol C. All statistical tests were performed with STATISTICA v. 12 (Statsoft).

RESULTS

Ramping protocol

Ramping Protocol A revealed obvious and important differences in heart rates between immersion and emersion (Fig. 2). Arrhenius plots from Protocol A (Fig. 3) showed that, in air, heart rate was poorly correlated with temperature when the temperature was increased from 18 to 45°C ($R^2 < 0.5$ for all studied individuals). When the temperature was decreased from 18 to 0°C in air, the correlation between heart rate and temperature was slightly stronger, particularly for *Mytilus galloprovincialis* and *Perna perna* west (mean of 5 individuals, $R^2 = 0.70 \pm 0.32$ for *M. galloprovincialis*, 0.80 ± 0.12 for *P. perna* west and 0.36 ± 0.36 for *P. perna* east). In contrast, when heart rate was measured in water, there was a strong correlation with temperature for all 3 classes of mussel in both heating and cooling ramping experiments (Tables 1–3). As the Arrhenius plots calculated for immersion revealed significant inter-individual differences for all classes (ANCOVA, $p < 0.001$), a GLM considering individual variability was used to compare *Mytilus* and the 2 *Perna* lineages. The heating experiment revealed significant differences in slope, with *P. perna* east having a higher slope than the other 2 mussels ($F = 30.6$, $p < 0.0001$; Tukey's HSD, $p < 0.001$) (Table 1), while during the cooling treatment the only differences were between *P. perna* west and *M. galloprovincialis*, with the slope being greater for *P. perna* west ($F = 4.5$, $p < 0.05$; Tukey's HSD, $p < 0.05$) (Table 2). Under constant heating (Protocol A), the limits of heart rate performance were similar among both *P. perna* lineages and *M. galloprovincialis*, and the temperature eliciting a sharp drop in cardiac performance (ABT) did not differ significantly among them (1-way ANOVA, $p > 0.5$) (Table 1). No breakpoint was detectable for the cooling treatment, but no heart rate was detectable at temperatures below 5°C, even though the mussels were alive and able to recover when subsequently warmed (Arrhenius parameters in Table 2).

As heart rate was not correlated with temperature during emersion, only data from immersion were analysed for ramping Protocol B (Fig. 4). The cardiac activity of mussels exposed to an increasing ramp from 7 to 45°C was strongly correlated with temperature (Table 3). The ABT was not significantly different between *M. galloprovincialis* and either lineage of *P. perna* (1-way ANOVA, $p > 0.5$), but the GLM showed significant differences among *Mytilus* and the 2 *Perna* lineages in the slopes of the Arrhenius plots ($F = 139.9$, $p < 0.0001$; Tukey's HSD, $p < 0.001$) (Table 3).

Acute temperature exposure

Cardiac response to acute temperature exposure strongly reflected temperature and medium, with *Mytilus* and the 2 *Perna* lineages reacting in a similar way to these factors (Table 4). The post hoc Tukey test revealed that, during aerial exposure, there were no significant differences among mean *HR* values at the 4 tested temperatures (Fig. 5). Conversely, the temperature effect was strong during immersion. *Mytilus* and the 2 *Perna* lineages showed a similar pattern of increasing *HR* from 9 to 27°C and a decline at 36°C. Although the effects of temperature and emersion on *HR* were similar for *M. galloprovincialis* and both lineages of *P. perna*, the heart rate of *M. galloprovincialis* was significantly lower than those of the 2 *P. perna* lineages, which did not differ (post hoc tests, $\alpha = 0.05$).

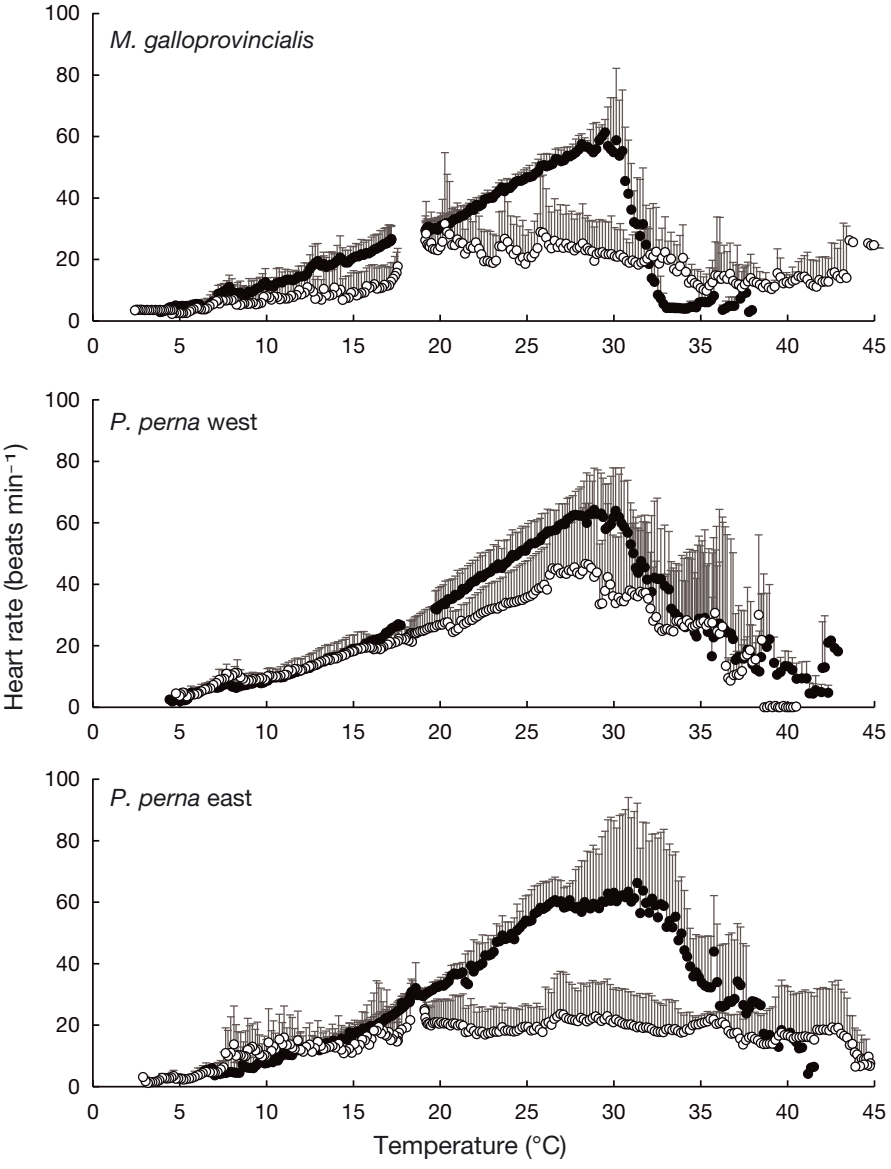

Fig. 2. Effect of mean temperature increase and decrease from 18°C (ramping Protocol A) in air (○) and underwater (●) on heart rate of *Mytilus galloprovincialis*, *Perna perna* west and *P. perna* east (mean ± SD; n = 5 for each group)

Oxygen consumption during acute temperature exposure was similarly strongly affected by emersion with higher average rates in water than air (Table 4). During emersion, temperature had no significant effect on the oxygen consumption for any of the studied mussel classes (Fig. 6). In contrast, post hoc Tukey

Table 1. Mean (± SE) values for Arrhenius plot regression parameters from the results from the immersion heating Protocol A (*a*: normalization constant; E_a: activation energy, J mol^{-1}; *R*: ideal gas constant, 8.31 J K^{-1} mol^{-1}), R^2 for the linear regression of ln heart rate on the inverse of absolute temperature and Arrhenius break point temperature (ABT). Post hoc results indicate homogenous groups identified by GLM analysis of slopes of Arrhenius plots. n = 5 individuals per treatment

	Mean ln(*a*)	Mean E_a/R	Mean R^2	Mean ABT	Post hoc
Mytilus galloprovincialis	25.38 ± 2.90	6.43 ± 0.86	0.95 ± 0.05	30.25 ± 1.10	a
Perna perna west	28.63 ± 2.34	7.37 ± 0.71	0.98 ± 0.01	30.21 ± 1.85	a
Perna perna east	31.74 ± 4.98	8.28 ± 1.45	0.94 ± 0.06	30.51 ± 3.10	b

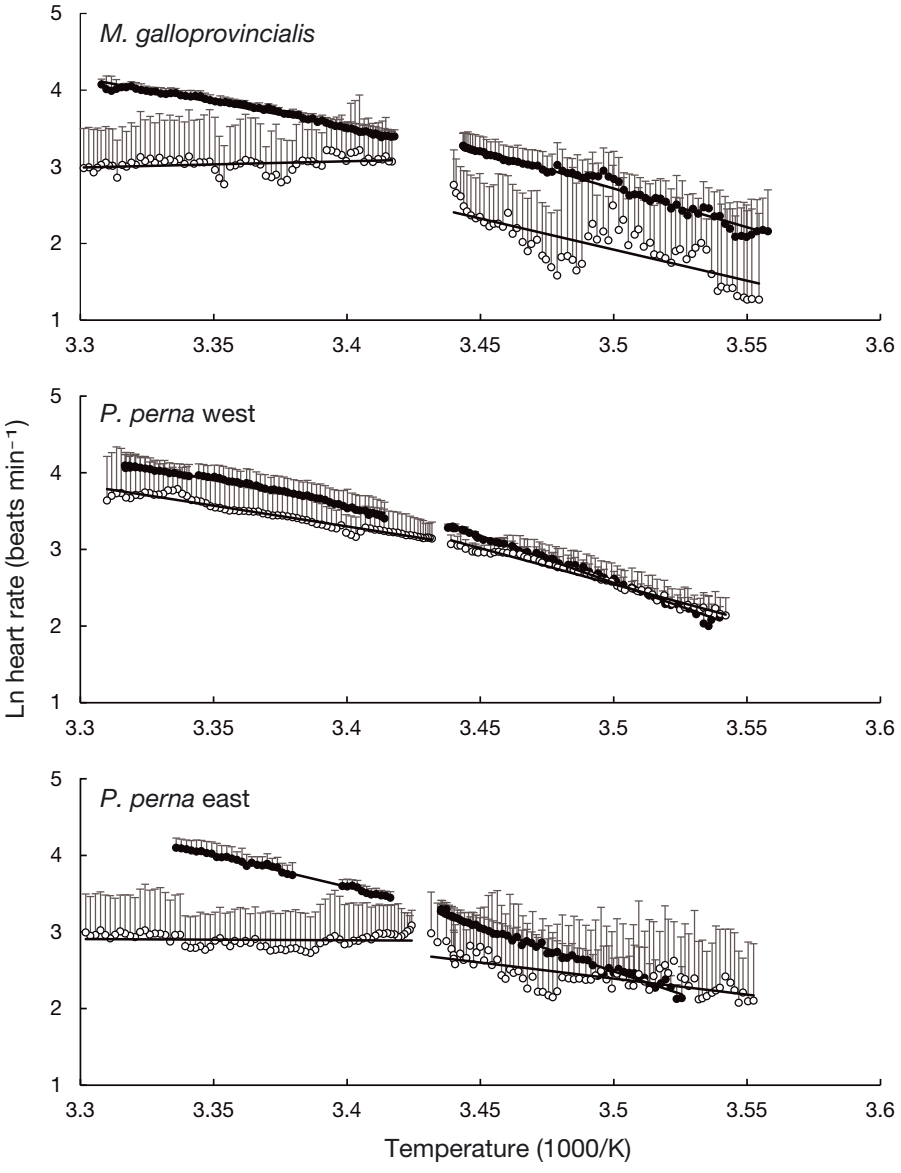

Fig. 3. Arrhenius plot for the heart rate against mean temperature for *Mytilus galloprovincialis*, *Perna perna* west and *P. perna* east (mean ± SD; n = 5 for each group). Temperature has been increased and decreased from 18°C (ramping Protocol A) in air (○) and underwater (●). Average regression parameters are presented in Tables 1 & 2

tests indicated that, during immersion, the oxygen consumption of *M. galloprovincialis* and *P. perna* east increased significantly at high temperatures (Fig. 6). ANOVA indicated that values for the 3 classes differed, with significantly higher values for *M. galloprovincialis* than the *Perna* lineages (Table 4). The in-

Table 2. Mean (± SE) values for Arrhenius plot regression parameters from the results from the immersion cooling Protocol A, R^2 for the linear regression of ln heart rate on the inverse of absolute temperature. Post hoc results indicate homogenous groups identified by GLM analysis of slopes of Arrhenius plots. n = 5 individuals per treatment

	Mean ln (*a*)	Mean E_a/R	Mean R^2	Post hoc
Mytilus galloprovincialis	36.63 ± 5.6	9.68 ± 1.62	0.87 ± 0.07	a
Perna perna west	45.46 ± 6.91	12.26 ± 2.01	0.97 ± 0.02	b
Perna perna east	43.63 ± 13.74	11.75 ± 3.96	0.85 ± 0.19	ab

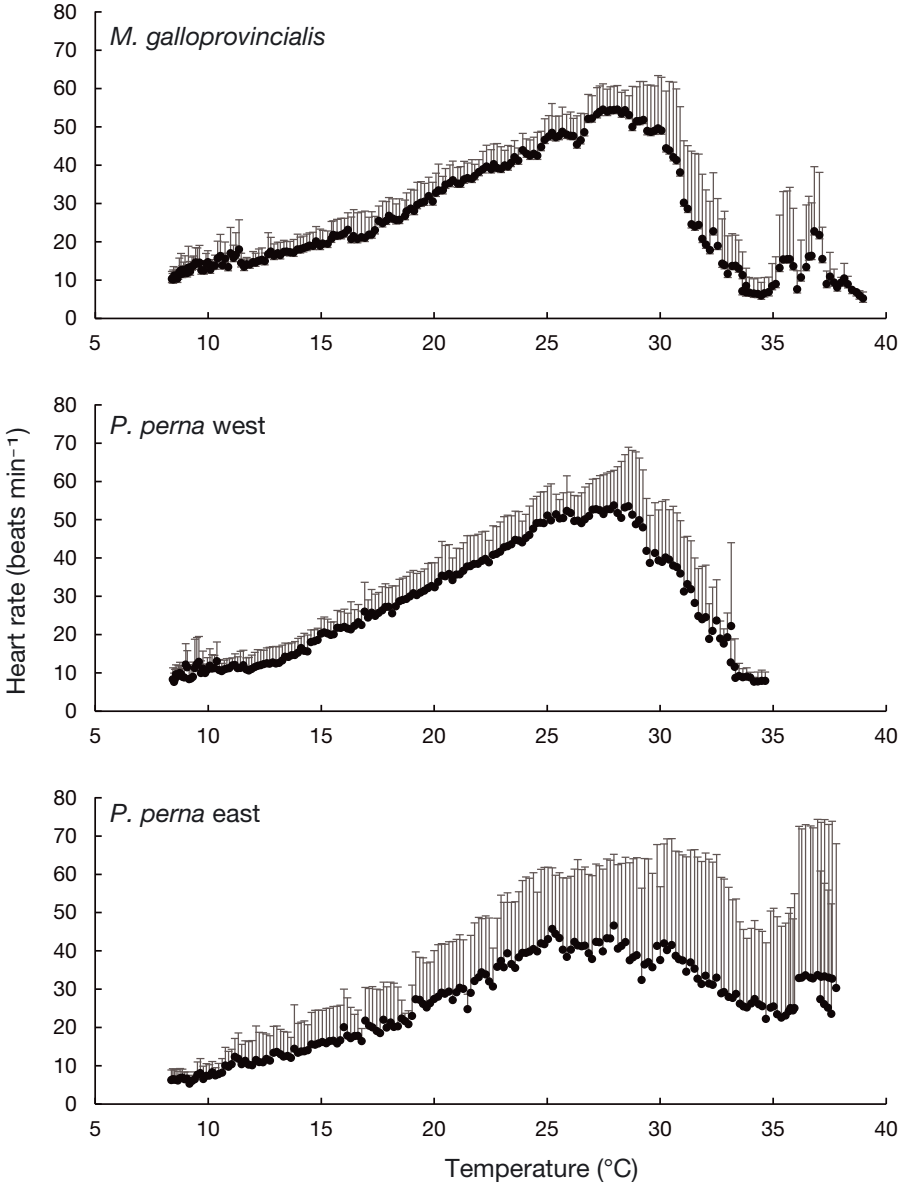

Fig. 4. Relationship between mean temperature and heart rate during continuous heating (ramping Protocol B) for *Mytilus galloprovincialis*, *Perna perna* west and *P. perna* east (mean ± SD, n = 5 for each group)

teraction between mussel class and the factor immersion/emersion was statistically significant, suggesting that the oxygen metabolism of *Mytilus* and the 2 *Perna* lineages reacted differently to aerial exposure. Post hoc Tukey tests revealed that oxygen consumption during immersion was significantly higher than

Table 3. Mean (± SE) values for Arrhenius plot regression parameters from the results from the immersion Protocol B, R^2 for the linear regression of ln heart rate on the inverse of absolute temperature and ABT. Post hoc results indicate homogenous groups identified by GLM analysis of slopes of Arrhenius plots. n = 5 individuals per treatment

	Mean ln(*a*)	Mean E_a/R	Mean R^2	Mean ABT	Post hoc
Mytilus galloprovincialis	27.58 ± 3.26	7.09 ± 0.96	0.96 ± 0.03	29.08 ± 0.77	a
Perna perna west	31.91 ± 3.84	8.34 ± 1.14	0.97 ± 0.03	27.91 ± 2.48	b
Perna perna east	36.61 ± 5.36	9.79 ± 1.50	0.84 ± 0.10	28.95 ± 4.12	c

Table 4. Results of GLM model applied to heart rate, oxygen consumption and succinate content data (Protocol C) with class, temperature and immersion/emersion as factors. ***p < 0.0005, **p < 0.005, *p < 0.05; ns: not significant

	Heart rate			Oxygen consumption			Succinate content		
	F	df	p	*F*	df	p	*F*	df	p
Class	215	2	*	25	2	***	1	2	ns
Temperature	47	3	***	13	3	***	56	3	***
Immersion/emersion	223	1	***	138	1	***	24	1	***
Species × temperature	1	6	ns	1	6	ns	5	6	***
Species × immersion/emersion	1	2	ns	16	2	***	5	2	**
Temperature × immersion/emersion	23	3	***	16	3	***	3	3	ns
Species × temperature × immersion/emersion	0	6	ns	1	6	ns	2	6	ns

during emersion at 27°C for all 3 classes and at 36°C for both *M. galloprovincialis* and *P. perna* east (Fig. 6).

ANOVA of succinic acid concentrations showed that the factors temperature and immersion/emersion strongly affected anaerobic metabolism (Table 4). There was no effect of mussel class as a main factor, but class interacted significantly with both other factors, indicating different reactions to the same stresses (Table 4). While *M. galloprovincialis* and *P. perna* west had similar responses to temperature, *P. perna* east showed the lowest succinic content at 9°C and statistically higher values at 27 and 36°C (Tukey's HSD, $p < 0.0005$; Fig. 7). Post hoc tests on the interaction between class and immersion/emersion revealed no differences between immersion and emersion for *M. galloprovincialis* (Tukey's HSD, $p > 0.5$), while succinic acid concentrations were significantly different between emersion and immersion for both *P. perna* lineages (Tukey's HSD on interaction, $p < 0.0005$).

DISCUSSION

The obvious interpretation of correlations between species distributions and temperature regimes is that distribution is set by the limits of thermal tolerance, but here we show that sub-lethal effects may be better predictors. Additionally, we show that sub-lethal effects are linked not only to species distributions, but that they can differ at the sub-specific level among genetic lineages of the same species.

Aerial exposure

Thermal stress and desiccation are particularly acute problems for marine species living an intertidal existence and strongly affect species distributions and survival in rocky intertidal habitats (Somero 2002). Some intertidal molluscs are able to maintain similar metabolic performances during low and high tide (McMahon 1990, Stillman & Somero 1996, Huang et al. 2015), but this seems not to be a generalized physiological adaptation to intertidal life (McMahon et al. 1991, Sokolova & Pörtner 2001, Tagliarolo et al. 2012), and our species showed the more typical responses. Both *Perna perna* and *Mytilus galloprovincialis* showed a dramatic reduction of heart rate and oxygen consumption combined with an increase of anaerobic end-product concentration during emersion. In air, cardiac activity and oxygen consumption were poorly correlated with temperature, especially at high temperatures, for both the ramping and acute temperature exposure protocols. Ramping Protocol A showed that *HR* was strongly affected by aerial exposure and the reduction noticed after 2 h in air indicates that physiological responses during low tide may be more strongly affected by desiccation.

During emersion, *P. perna* and *M. galloprovincialis* have different behavioural responses; *P. perna* periodically opens and closes the shell valves (gaping), while *M. galloprovincialis* is a non-gaping species (Nicastro et al. 2010b). Valve closure in bivalves restricts gas exchange, resulting in hypoxia, hypercapnia and an increase in the acidity of the mantle cavity water (Burnett 1988). Previous studies suggest that gaping behaviour allows *P. perna* to avoid accumulating metabolites during hypoxia and facilitates gas exchange so that aerobic respiration can be maintained during aerial exposure (Coleman & Trueman 1971, McMahon 1988, Marshall & McQuaid 1993). *P. perna* individuals exhibited gaping behaviour during our experiments as soon as they were exposed to air, as observed by Nicastro et al. (2010b), and earlier studies on these species show that gaping can increase humidity and decrease temperatures in

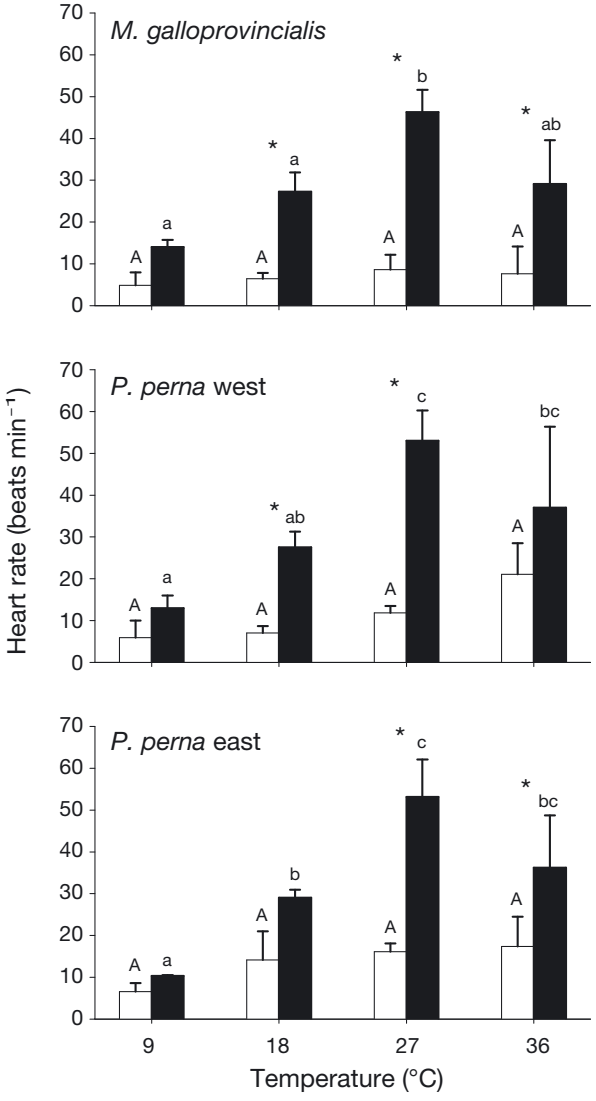

Fig. 5. Heart rate of *Mytilus galloprovincialis*, *Perna perna* west and *P. perna* east submitted to acute temperature stress for 30 min (Protocol C) in air (white bar) and water (black bar) conditions. Data represent mean + SE for n = 4 mussels per treatment. Capital letters indicate significant differences among temperatures in air; lowercase letters indicate significant differences among temperatures in water (Tukey's HSD, $p < 0.05$). *Significant differences (Tukey's HSD, $p < 0.05$) between emersed and immersed values measured at a specific temperature

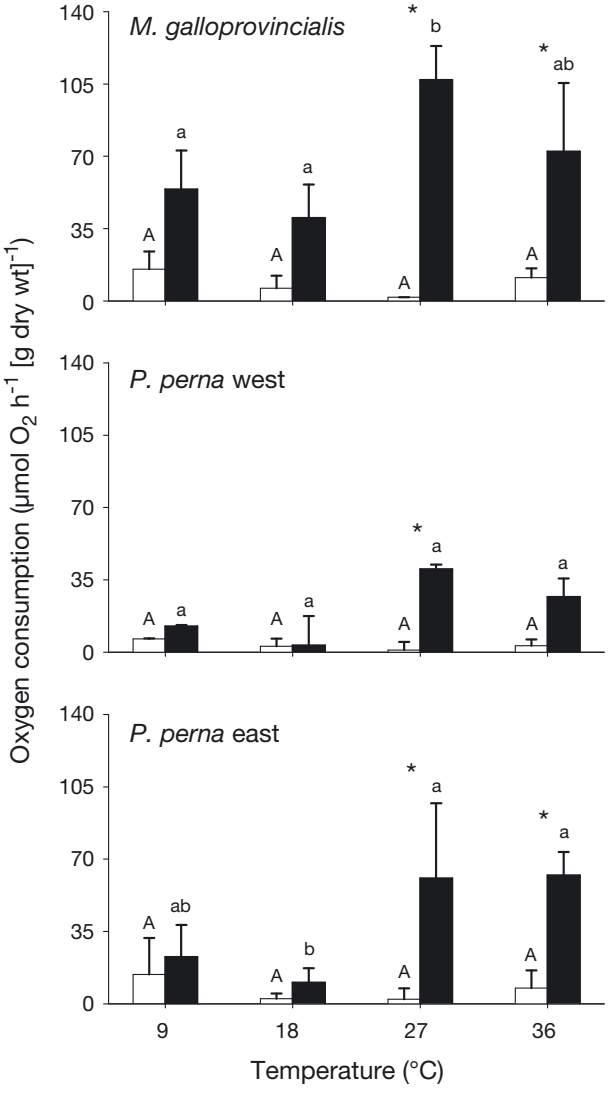

Fig. 6. Oxygen consumption of *Mytilus galloprovincialis*, *Perna perna* west and *P. perna* east submitted to acute temperature stress for 30 min (Protocol C) in air (white bar) and water (black bar) conditions. Data represent mean + SE for n = 4 mussels per treatment. Capital letters indicate significant differences among temperatures in air; lowercase letters indicate significant differences among temperatures in water (Tukey's HSD, $p < 0.05$). *Significant differences (Tukey's HSD, $p < 0.05$) between emersed and immersed values measured at a specific temperature

dense groups, though not solitary individuals (Nicastro et al. 2012). On the other hand, gaping increases water losses and the risk of desiccation, and can be considered an adaptation only for species living in the low intertidal (Nicastro et al. 2010b). Our study measured oxygen consumption and succinate concentration in air and water, and the results suggest that gaping by *P. perna* does not increase aerobic respiration during emersion, as oxygen consumption in air did not differ among *Mytilus* and the 2 *Perna* lineages; indeed, values were slightly (but not significantly) higher for *M. galloprovincialis*.

Succinate is an intermediate end-product of the glucose and aspartate pathways, and is found in different marine bivalve species (Michaelidis et al. 2005, Babarro et al. 2007, Connor & Gracey 2012). Our study focused only on succinate production, as it is recognized as a good indicator of anaerobic meta-

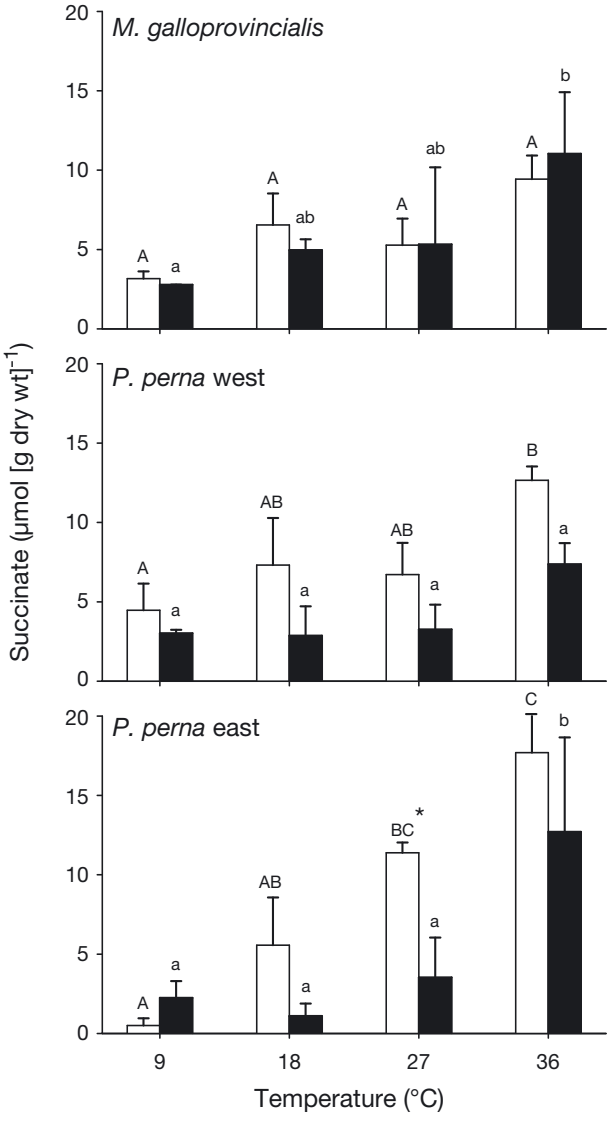

Fig. 7. Succinic acid concentration in adductor muscle of *Mytilus galloprovincialis*, *Perna perna* west and *P. perna* east submitted to acute temperature stress for 30 min (Protocol C) in air (white bar) and water (black bar) conditions. Data represent mean + SE for n = 4 mussels per treatment. Capital letters indicate significant differences among temperatures in air; lowercase letters indicate significant differences among temperatures in water (Tukey's HSD, $p < 0.05$). *Significant differences (Tukey's HSD, $p < 0.05$) between emersed and immersed values measured at a specific temperature

bolism during the first hours following emergence (Connor & Gracey 2012). Bivalves possess muscle fibres that can remain contracted for prolonged periods with minimal energy expenditure (Mellon & Mpitsos 1967, Livingstone & Bayne 1977), but vigorous muscle activity can still require instantaneous energy supply through anaerobiosis (Lee & Lee 2011). The presence of higher succinate concentrations in the adductor muscles of *P. perna* could also be due to muscular activity associated with gaping behaviour, and further studies analysing different tissues and other possible anaerobic end-products such as alanine, malate and propionate would clarify the anaerobic pathways used by these species.

Our experiments performed during emersion suggest that *Mytilus* and the 2 *Perna* lineages were able to modulate their metabolism through metabolic depression to survive during low tide aerial exposure. *M. galloprovincialis* was the only class that did not show significant differences in succinate production between emersion and immersion, indicating that 1 h of air exposure produced minimal metabolic stress on this species. The fact that *M. galloprovincialis* exhibited the lowest cardiac activity and no succinic acid accumulation at all studied temperatures suggests a greater capacity for thermally insensitive metabolism and possibly metabolic depression, which would increase survival during prolonged exposure to air (Sokolova & Pörtner 2001, Marshall et al. 2011). These metabolic characteristics, coupled with high heart rates during immersion, indicate that *M. galloprovincialis* is better adapted than *P. perna* to grow and survive at higher shore levels. Low metabolic rates during emersion would allow *M. galloprovincialis* to withstand longer aerial exposure and its higher metabolism in water would allow it to compensate for shorter submersion periods, when feeding, calcification and elimination of the anaerobic end-products accumulated during low tide occur (Bourget & Crisp 1975, Connor & Gracey 2012). This offers a physiological basis for the observed vertical segregation of these populations, with *M. galloprovincialis* extending higher up the shore than *P. perna* and showing better survival and stronger competitive abilities in the upper mussel zone in translocation experiments (Bownes & McQuaid 2006, Rius & McQuaid 2009).

The experiments performed in air revealed important differences between the 2 *P. perna* lineages. Exposure to air affected *P. perna* east more strongly than *P. perna* west, as seen in greater anaerobic end-product accumulation at high temperatures and slightly, though not significantly, higher heart rates. *P. perna* east seemed to show poor modulation of its metabolism as reductions of oxygen consumption in air were compensated for by a higher anaerobic contribution. The east and west coasts are characterized by different tidal conditions; the mean range of neap tides is greater on the west coast and the mean range of spring tides is higher on the east coast (South African Navy Hydrographic Office 2014). Those tidal

conditions could potentially cause more frequent exposure to air for individuals living on the west coast, which could explain why the western lineage seems better adapted for survival in air. Alternatively, different vertical positioning on the shore could be correlated with the fact that *P. perna* east is less well adapted to aerial conditions. Indeed, recent studies indicate that the upper limit for *P. perna* east occurs significantly lower on the shore than that of the western lineage (G. I. Zardi & C. D. McQuaid unpubl. data).

Immersion

The experiment performed underwater indicated that the thermal limits for our populations were very similar and ranged between around 5 and 30°C. The use of a ramping protocol to estimate thermal limits in ectotherms is shown to be strongly dependent on the experimental protocol used (Rezende et al. 2011). To evaluate the effect of different ramping protocols on the calculated thermal limits, we compared the heart rates obtained using 2 ramping protocols and an acute temperature exposure experiment to derive a robust estimation of the physiological limits of *M. galloprovincialis* and *P. perna*. The critical thermal maxima calculated showed similar results, which indicated that the upper thermal limits of *M. galloprovincialis* and both *P. perna* lineages were between 27 and 31°C.

Several studies emphasize the importance of thermal and physiological limits in explaining the biogeographical distributions of closely related species, but laboratory experiments are unfortunately strongly dependent on the speed of the ramping and can generally be considered only an approximation of the real thermal limits experienced by the individuals under natural conditions (Stillman 2002, Braby & Somero 2006, Jones et al. 2009, Kuo & Sanford 2009). An alternative way to compare species' thermal sensitivities is to use the Arrhenius activation energy (E_a). Previous studies show that warm-acclimated species show higher E_a values, indicating that the metabolic rate increases more strongly with rising temperature (van Dijk et al. 1999, Somero 2004). Our study demonstrated that, even though *M. galloprovincialis* and the 2 *P. perna* lineages showed very similar upper thermal limits, their metabolic responses to temperature variation and the stress of aerial exposure differed. During immersion, the heart rates of the 2 *P. perna* lineages were generally similar, but *P. perna* east showed a significantly higher E_a than the other 2 species during heating experiments. *P. perna* east is found only on the southeast and east coasts of South Africa, that is, broadly within a subtropical biogeographic province characterized by seawater temperatures ranging between 16 and 22°C in winter and 18 and 27°C in summer (Smit et al. 2013). The difference in metabolism between the 2 lineages indicates that *P. perna* east can maintain higher metabolic rates and probably grow faster than the western lineage under conditions of warm water. A common garden experiment assessing genetically controlled differences in growth rate of the 2 lineages could validate this hypothesis (Chiba et al. 2007).

Our results from experiments on acute temperature stress in water revealed cardiorespiratory failure above 27°C for *Mytilus* and both *Perna* lineages, coupled with an increase in anaerobic end-products. This pattern is consistent with the recent theories of oxygen and capacity limited thermal tolerance (OCLTT) that emphasize the collapse of aerobic scope and the onset of anaerobiosis in the pessimum temperature range due to the limited capacity of oxygen uptake and transport (Pörtner 2012, Sokolova et al. 2012). In our study, the individuals immersed in 9°C seawater also showed a decrease in cardiac rate but the contribution of the anaerobic pathway was very limited, suggesting a temporary limitation and suppression of the metabolism. Moreover, during emersion heart rates were thermally insensitive and only *P. perna* showed temperature-dependent succinic acid production. These findings indicate that *M. galloprovincialis* seems to compensate for the loss of aerobic energy by exploiting metabolic depression and passive tolerance more effectively than *P. perna*, which seems to compensate for the loss through anaerobiosis. *M. galloprovincialis* showed the highest oxygen consumption rates and had generally higher heart rates during immersion, especially during cooling from 18 to 5°C. *P. perna* was absent from the upwelling-dominated Benguela system of the west coast of South Africa even before the arrival of *M. galloprovincialis*, and previous studies suggest that this reflects the lower water temperatures there (Zardi et al. 2007, 2011). Our results show that, even if *M. galloprovincialis* seems better adapted to cold conditions, both *P. perna* lineages are able to survive and maintain efficient metabolism in cold seawater, as their oxygen consumption and heart rates at 9°C were similar to those of *M. galloprovincialis*. Other mechanisms such as larval intolerance of low temperatures or post-settlement mortality may be better explanations for the absence of *P. perna* from the

cold-water Benguela system of the west coast. Although feeding rates of *Mytilus edulis* larvae increase with temperature (Bayne 1965), the reproduction and larval dispersal of *P. perna* from South America are mostly affected by high temperatures (Romero & Moreira 1980) so that the upper thermal limits for embryonic development and the survival of veliger larvae (25 and 30°C, respectively) are likely to prevent colonization where temperatures approach these values (Romero & Moreira 1980, Vélez & Epifanio 1981, Hicks & McMahon 2005). No information is available on cold limitations for the larvae of *P. perna*, and Romero & Moreira (1980) suggest that the species is quite cold tolerant. Nevertheless, it is possible that at the low temperatures prevalent on the west coast, the larvae of *P. perna* are simply unable to sustain themselves.

CONCLUSIONS

The different responses of heart rate, oxygen consumption and succinic acid concentration that we found among *Mytilus galloprovincialis* and the 2 *Perna perna* lineages do not only demonstrate how physiological adaptation determines spatial distributions; our results also show that species (or different lineages of the same species) may respond similarly to some forms of stress, but not others. *M. galloprovincialis* is shown to be the better competitor on the high shore (Rius & McQuaid 2009), while *P. perna* east is the least adapted to aerial conditions. *M. galloprovincialis* proved better adapted to cold conditions as its cardiac activity was highest and its E_a the lowest at low temperatures, while *P. perna* east had the highest E_a values. Despite this, the thermal limits of *Mytilus* and the 2 *Perna* lineages were very similar and lethal temperatures cannot be considered to be the main factor affecting their biogeographical distributions. Rather, as they have similar niche requirements, the effects of repeated exposure to sub-lethal effects of temperature on their competitive abilities may be critical (Fly & Hilbish 2013).

Our results indicate that physiological responses to thermal stress are correlated with the thermal regimes experienced by different species, and even by different genetic lineages within a species. However, recognizing this requires information that goes beyond simple upper thermal limits and addresses sub-lethal effects, indicating that the most important effects of thermal stress on species distributions may be manifested through its influence on competitive abilities.

Acknowledgements. This work is based on research supported by the South African Research Chairs Initiative of the Department of Science and Technology and the National Research Foundation, and was supported by a Rhodes University post-doctoral fellowship. We thank Prof. Anthony Sullivan for help with the heart rate probes and Prof. Adrienne Edkins for support with succinic acid analysis. Many thanks also to Jaqueline Trassierra, Eleonora Puccinelli and Francesca Porri for assistance in the field.

LITERATURE CITED

Babarro JMF, Labarta U, Reiriz MJF (2007) Energy metabolism and performance of *Mytilus galloprovincialis* under anaerobiosis. J Mar Biol Assoc UK 87:941–946

Bayne BL (1965) Growth and the delay of metamorphosis of the larvae of *Mytilus edulis* (L.). Ophelia 2:1–47

Beutler HO (1989) Succinate. In: Bergmeyer HU (ed) Methods of enzymatic analysis. VCH Publishers, Cambridge, p 25–33

Bourget E, Crisp DJ (1975) Factors affecting deposition of the shell in *Balanus balanoides* (L.). J Mar Biol Assoc UK 55:231–249

Bownes SJ, McQuaid CD (2006) Will the invasive mussel *Mytilus galloprovincialis* Lamarck replace the indigenous *Perna perna* L. on the south coast of South Africa? J Exp Mar Biol Ecol 338:140–151

Braby CE, Somero GN (2006) Following the heart: temperature and salinity effects on heart rate in native and invasive species of blue mussels (genus *Mytilus*). J Exp Biol 209:2554–2566

Branch GM, Steffani NC (2004) Can we predict the effects of alien species? A case-history of the invasion of South Africa by *Mytilus galloprovincialis* (Lamarck). J Exp Mar Biol Ecol 300:189–215

Burnett LE (1988) Physiological responses to air exposure: acid-base balance and the role of branchial water stores. Am Zool 28:125–135

Burnett NP, Seabra R, de Pirro M, Wethey DS and others (2013) An improved noninvasive method for measuring heartbeat of intertidal animals. Limnol Oceanogr Methods 11:91–100

Chiba S, Arnott SA, Conover DO (2007) Coevolution of foraging behavior with intrinsic growth rate: risk-taking in naturally and artificially selected growth genotypes of *Menidia menidia.* Oecologia 154:237–246

Coleman N, Trueman ER (1971) The effect of aerial exposure on the activity of the mussels *Mytilus edulis* (L.) and *Modiolus modiolus* (L.). J Exp Mar Biol Ecol 7:295–304

Connell JH (1972) Community interactions on marine rocky intertidal shores. Annu Rev Ecol Syst 3:169–192

Connor KM, Gracey AY (2012) High resolution analysis of metabolic cycles in the intertidal mussel *Mytilus californianus.* Am J Physiol-Reg I 302:R103–R111

Cunha RL, Nicastro KR, Costa J, McQuaid CD, Serrão EA, Zardi GI (2014) Wider sampling reveals a non-sister relationship for geographically contiguous lineages of a marine mussel. Ecol Evol 4:2070–2081

de Zwaan A, de Bont AMT, Verhoeven A (1982) Anaerobic energy metabolism in isolated adductor muscle of the sea mussel *Mytilus edulis* L. J Comp Physiol 149:137–143

Depledge MH, Lundebye AK, Curtis T, Aagaard A, Andersen BB (1996) Automated interpulse-duration assessment (AIDA): a new technique for detecting disturbances

in cardiac activity in selected macroinvertebrates. Mar Biol 126:313–319

Erlandsson J, Pal P, McQuaid CD (2006) Re-colonisation rate differs between co-existing indigenous and invasive intertidal mussels following major disturbance. Mar Ecol Prog Ser 320:169–176

Fangue NA, Osborne EJ, Todgham AE, Schulte PM (2011) The onset temperature of the heat-shock response and whole-organism thermal tolerance are tightly correlated in both laboratory-acclimated and field-acclimatized tidepool sculpins (*Oligocottus maculosus*). Physiol Biochem Zool 84:341–352

Fly EK, Hilbish TJ (2013) Physiological energetics and biogeographic range limits of three congeneric mussel species. Oecologia 172:35–46

Helmuth B, Broitman BR, Blanchette CA, Gilman S and others (2006) Mosaic patterns of thermal stress in the rocky intertidal zone: implications for climate change. Ecol Monogr 76:461–479

Hicks D, McMahon R (2002) Temperature acclimation of upper and lower thermal limits and freeze resistance in the nonindigenous brown mussel, *Perna perna* (L.), from the Gulf of Mexico. Mar Biol 140:1167–1179

Hicks DW, McMahon RF (2005) Effects of temperature on chronic hypoxia tolerance in the non-indigenous brown mussel, *Perna perna* (Bivalvia: Mytilidae) from the Texas Gulf of Mexico. J. Molluscan Stud. 71:401–408

Huang X, Wang T, Ye Z, Han G, Dong Y (2015) Temperature relations of aerial and aquatic physiological performance in a mid-intertidal limpet *Cellana toreuma*: adaptation to rapid changes in thermal stress during emersion. Integr Zool 10:159–170

Jones SJ, Mieszkowska N, Wethey DS (2009) Linking thermal tolerances and biogeography: *Mytilus edulis* (L.) at its southern limit on the east coast of the United States. Biol Bull 217:73–85

Kaufmann T, Sütterlin S, Schulz SM, Vögele C (2011) ARTiiFACT: a tool for heart rate artifact processing and heart rate variability analysis. Behav Res Methods 43: 1161–1170

Kuo ESL, Sanford E (2009) Geographic variation in the upper thermal limits of an intertidal snail: implications for climate envelope models. Mar Ecol Prog Ser 388: 137–146

Ladoukakis ED, Saavedra C, Magoulas A, Zouros E (2002) Mitochondrial DNA variation in a species with two mitochondrial genomes: the case of *Mytilus galloprovincialis* from the Atlantic, the Mediterranean and the Black Sea. Mol Ecol 11:755–769

Lee AC, Lee KT (2011) The enzyme activities of opine and lactate dehydrogenase in the gills, mantle, foot, and adductor of the hard clam *Meretrix lusoria*. J Mar Sci Technol 19:361–367

Livingstone DR, Bayne BL (1977) Responses of *Mytilus edulis* L. to low oxygen tension: anaerobic metabolism of the posterior adductor muscle and mantle tissues. J Comp Physiol 114:143–155

Lockwood BL, Somero GN (2011) Invasive and native blue mussels (genus *Mytilus*) on the California coast: the role of physiology in a biological invasion. J Exp Mar Biol Ecol 400:167–174

Logan CA, Kost LE, Somero GN (2012) Latitudinal differences in *Mytilus californianus* thermal physiology. Mar Ecol Prog Ser 450:93–105

Marshall DJ, McQuaid CD (1993) Differential physiological and behavioural responses of the intertidal mussels, *Choromytilus meridionalis* (Kr.) and *Perna perna* (L.), to exposure to hypoxia and air: a basis for spatial separation. J Exp Mar Biol Ecol 171:225–237

Marshall DJ, Dong Y, McQuaid CD, Williams GA (2011) Thermal adaptation in the intertidal snail *Echinolittorina malaccana* contradicts current theory by revealing the crucial roles of resting metabolism. J Exp Biol 214: 3649–3657

McMahon RF (1988) Respiratory response to periodic emergence in intertidal molluscs. Integr Comp Biol 28:97–114

McMahon RF (1990) Thermal tolerance, evaporative water loss, air-water oxygen consumption and zonation of intertidal prosobranchs: a new synthesis. Hydrobiologia 193:241–260

McMahon BR, Burggren WW, Pinder AW, Wheatly MG (1991) Air exposure and physiological compensation in a tropical intertidal chiton, *Chiton stokesii* (Mollusca: Polyplacophora). Physiol Zool 64:728–747

McQuaid CD, Phillips TE (2000) Limited wind-driven dispersal of intertidal mussel larvae: *in situ* evidence from the plankton and the spread of the invasive species *Mytilus galloprovincialis* in South Africa. Mar Ecol Prog Ser 201:211–220

Mellon D, Mpitsos GJ (1967) Response heterogeneity in adductor muscle efferents of the surf clam. J Exp Biol 46: 585–597

Michaelidis B, Haas D, Grieshaber MK (2005) Extracellular and intracellular acid base status with regard to the energy metabolism in the oyster *Crassostrea gigas* during exposure to air. Physiol Biochem Zool 78:373–383

Nicastro K, Zardi G, McQuaid C (2010a) Differential reproductive investment, attachment strength and mortality of invasive and indigenous mussels across heterogeneous environments. Biol Invasions 12:2165–2177

Nicastro KR, Zardi GI, McQuaid CD, Stephens L, Radloff S, Blatch GL (2010b) The role of gaping behaviour in habitat partitioning between coexisting intertidal mussels. BMC Ecol 10:1–11

Nicastro KR, Zardi GI, McQuaid CD, Pearson GA, Serrao EA (2012) Love thy neighbour: group properties of gaping behaviour in mussel aggregation. PLoS ONE 7:e47382

Pörtner HO (2012) Integrating climate-related stressor effects on marine organisms: unifying principles linking molecule to ecosystem-level changes. Mar Ecol Prog Ser 470:273–290

Rezende EL, Tejedo M, Santos M (2011) Estimating the adaptive potential of critical thermal limits: methodological problems and evolutionary implications. Funct Ecol 25:111–121

Rius M, McQuaid CD (2006) Wave action and competitive interaction between the invasive mussel *Mytilus galloprovincialis* and the indigenous *Perna perna* in South Africa. Mar Biol 150:69–78

Rius M, McQuaid CD (2009) Facilitation and competition between invasive and indigenous mussels over a gradient of physical stress. Basic Appl Ecol 10:607–613

Robinson T, Griffiths C, McQuaid C, Rius M (2005) Marine alien species of South Africa — status and impacts. Afr J Mar Sci 27:297–306

Romero SMB, Moreira GS (1980) The combined effects of salinity and temperature on the survival of embryos and veliger larvae of *Perna perna* (Linne, 1758) (Mollusca–Bivalvia). Bol Fisiol Anim Univ Sao Paulo 5:45–58

Smit AJ, Roberts M, Anderson RJ, Dufois F and others (2013)

A coastal seawater temperature dataset for biogeographical studies: large biases between *in situ* and remotely-sensed data sets around the coast of South Africa. PLoS ONE 8:e81944

Sokolova IM, Pörtner HO (2001) Physiological adaptations to high intertidal life involve improved water conservation abilities and metabolic rate depression in *Littorina saxatilis.* Mar Ecol Prog Ser 224:171–186

Sokolova IM, Frederich M, Bagwe R, Lannig G, Sukhotin AA (2012) Energy homeostasis as an integrative tool for assessing limits of environmental stress tolerance in aquatic invertebrates. Mar Environ Res 79:1–15

Somero GN (2002) Thermal physiology and vertical zonation of intertidal animals: optima, limits, and costs of living. Integr Comp Biol 42:780–789

Somero GN (2004) Adaptation of enzymes to temperature: searching for basic 'strategies.'. Comp Biochem Physiol B 139:321–333

Somero GN (2005) Linking biogeography to physiology: evolutionary and acclimatory adjustments of thermal limits. Front Zool 2:1

South African Navy Hydrographic Office (2014) South African tide tables. Available at http://www.sanho.co.za/tides/tide_index.htm

Stillman JH (2002) Causes and consequences of thermal tolerance limits in rocky intertidal porcelain crabs, genus *Petrolisthes.* Integr Comp Biol 42:790–796

Stillman J, Somero G (1996) Adaptation to temperature stress and aerial exposure in congeneric species of intertidal porcelain crabs (genus *Petrolisthes*): correlation of physiology, biochemistry and morphology with vertical distribution. J Exp Biol 199:1845–1855

Tagliarolo M, Clavier J, Chauvaud L, Koken M, Grall J (2012) Metabolism in blue mussel: intertidal and subtidal beds compared. Aquat Biol 17:167–180

van Dijk PL, Tesch C, Hardewig I, Pörtner HO (1999) Physiological disturbances at critically high temperatures: a comparison between stenothermal Antarctic and eurythermal temperate eelpouts (*Zoarcidae*). J Exp Biol 202: 3611–3621

van Erkom Schurink C, Griffiths CL (1993) Factors affecting relative rates of growth in four South African mussel species. Aquaculture 109:257–273

Vélez A, Epifanio CE (1981) Effects of temperature and ration on gametogenesis and growth in the tropical mussel *Perna perna* (L.). Aquaculture 22:21–26

Walther K, Sartoris FJ, Bock C, Pörtner HO (2009) Impact of anthropogenic ocean acidification on thermal tolerance of the spider crab *Hyas araneus.* Biogeosci Discuss 6: 2837–2861

Zardi GI, McQuaid CD, Teske PR, Barker NP (2007) Unexpected genetic structure of mussel populations in South Africa: indigenous *Perna perna* and invasive *Mytilus galloprovincialis.* Mar Ecol Prog Ser 337:135–144

Zardi GI, Nicastro KR, McQuaid CD, Hancke L, Helmuth B (2011) The combination of selection and dispersal helps explain genetic structure in intertidal mussels. Oecologia 165:947–958

Editorial responsibility: Inna Sokolova, Charlotte, North Carolina, USA

Submitted: November 3, 2014; Accepted: July 20, 2015
Proofs received from author(s): September 2, 2015