

Size and position (sometimes) matter: small-scale patterns of heat stress associated with two co-occurring mussels with different thermoregulatory behaviour

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Received: 28 April 2016 / Accepted: 8 August 2016
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Abstract Heat-related mass mortalities and local extinctions are expected to rise as the frequency, duration, and intensity of extreme heat events increase due to climate change, particularly in the case of sessile or sedentary species that cannot relocate. Little is known, however, of how biotic factors, such as the size and non-motile behaviour of individuals, contribute to small-scale variation in susceptibility to heat-related mortality during such events. We used infrared thermography to investigate how manipulated mussel bed size and the size structure of individuals within beds influence small-scale variability in the body temperatures of two intertidal mussel species with different thermoregulatory behaviours (gaping vs. non-gaping) during simulated extreme heat conditions. At times, body temperatures of small individuals reached higher temperatures than

large individuals, irrespective of mussel bed size, though this was more apparent for the non-gaping species. Average body temperatures and heating rates of individuals within large mussel beds were generally greater than for individuals within small mussel beds, irrespective of species or individual size. This pattern seems to reflect an effect of the greater circumference/area ratio for small mussel beds as individuals on the windward side of all beds displayed convective cooling and body temperatures 3–5 °C cooler than those on the leeward side. Such high levels of inter-individual variability in body temperatures at small spatial scales suggest the need for a greater appreciation and inclusion of biotic factors in assessing susceptibility of populations to climate change.

Responsible Editor: S. Connell.

Reviewed by A. Smith and an undisclosed expert.

Electronic supplementary material The online version of this article (doi:10.1007/s00227-016-2966-z) contains supplementary material, which is available to authorized users.

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Introduction

Global climate change is expected to increase the frequency, intensity, and duration of extreme heat events, which may lead to increased population mortality and local extinctions (Perkins and Alexander 2013; Oswald and Rood 2014). Indeed, many mass mortalities have already been reported within both the marine and terrestrial environment as populations succumb to prolonged periods of higher than normal heat stress (Hughes et al. 2003; Allen et al. 2010; McKechnie and Wolf 2010; Wernberg et al. 2013). In many instances, however, small-scale patterns of mortality following heat-waves are highly variable, and individuals separated by only a few centimetres apart may experience significantly different probabilities of mortality (Mislán and Wethey 2015). This is particularly true for sessile benthic invertebrates living on rocky intertidal shores where temperature variability amongst microhabitats can vary as much as, if not more than, amongst sites separated by

thousands of kilometres (Harley 2008; Denny et al. 2011). Indeed, recent studies using infrared thermography have revealed that body temperatures of intertidal invertebrates can vary by 4–5 °C between individuals separated by only a few centimetres within the same microhabitat (Lathlean et al. 2012, 2013) and by 8–12 °C for individuals found amongst contrasting microhabitats (Seabra et al. 2011). Such differences amongst microhabitats and individuals can be important when populations and species try to adapt to rapid changes in global temperatures and extreme heat stress as cooler microhabitats may act as thermal refugia (Bell 2013; Carlson et al. 2014; Mislán and Wethey 2015).

Rocky shores and their associated biological communities have emerged as excellent study systems to investigate how microhabitat variability within populations affects the capacity of species' to adapt to climate change (Helmuth et al. 2006; Jimenez et al. 2015). Indeed, with many intertidal invertebrates and algae already living at or close to their upper thermal limits, intertidal communities may act as a bellwether for global climate change (Somero 2002; Denny et al. 2011). Like many other ectothermic organisms, rocky intertidal invertebrates can modulate their body temperatures by altering their behaviour. Such thermoregulatory behaviours include: mushrooming in limpets (Williams et al. 2005), posturing (Marshall and Chua 2012; Seuront and Ng 2016), aggregating (Helmuth 1998; Chaperon and Seuront 2012), or regional heterothermy within the body (Pincebourde et al. 2013). These thermoregulatory behaviours enable intertidal ectotherms to modify their thermal environment within a microhabitat and hence maintain physiological functioning when temperatures rise above a particular threshold. It remains largely uncertain, however, whether such behavioural thermoregulation contributes to the high variability in body temperatures amongst individuals during extreme heat events.

Intertidal communities on the south coast of South Africa are dominated by two intertidal mussels: the indigenous mussel *Perna perna* and the invasive mussel *Mytilus galloprovincialis*, each with different behavioural responses to air exposure. The two coexist on shores with partial habitat segregation, *M. galloprovincialis* generally occurring higher on the shore, but with a region of overlap where the two occur in mixed beds (Bownes and McQuaid 2006). During emersion, *P. perna* exhibits periodic closure and opening of the shell (gaping). This behaviour allows the maintenance of aerobic respiration, but increases levels of water loss and the risk of desiccation due to both evaporation and expulsion of water during valve closure. In contrast, *M. galloprovincialis* keeps its valves closed when exposed to air. This reduces the risk of desiccation, but forces mussels to rely on less efficient anaerobic metabolism (Nicastro et al. 2010). These different responses to aerial exposure, along with different byssal attachment

strengths, are thought to contribute to the different vertical distributions of the two species (Branch and Steffani 2004; Bownes and McQuaid 2006; Zardi et al. 2006; McQuaid et al. 2015).

Recent field and laboratory experiments reveal that the gaping behaviour of *P. perna* is ineffective at reducing body temperatures of solitary mussels and that individuals must also aggregate with conspecifics for evaporative cooling effects to emerge (Nicastro et al. 2012). It remains unclear, however, whether the beneficial properties of aggregating only manifest once mussel beds reach a certain size and whether such evaporative cooling depends on the size of individual mussels or their position within the bed. If so, this may be particularly concerning for populations of *P. perna* along the east coast of South Africa, which has undergone dramatic declines in abundance due to unsustainable subsistence fishing (Tomalin and Kyle 1998), and are experiencing particularly rapid rates of ocean warming in response to the observed and predicted strengthening and warming of the Agulhas Current (Swart and Fyfe 2012; Wu et al. 2012; Biastoch and Böning 2013).

The aim of this study was to investigate the causes of small-scale microhabitat variability in the body temperatures of both *M. galloprovincialis* and *P. perna* during extreme heat events. This was achieved through manipulations of the size and structure of mussel beds and subsequent measurements of small-scale patterns of temperature variability during periods of elevated heat stress using infrared (IR) thermography. Large mussel beds comprised of large individuals were expected to be most effective at ameliorating the effects of extreme heat stress due to their greater thermal inertia whilst aggregations of *P. perna* were expected to maintain lower body temperatures than similar sized aggregations of *M. galloprovincialis* due to their different behaviours during air exposure.

Materials and methods

Experimental design

The study was undertaken on a moderately exposed rocky intertidal shore at Jongensfontein (34°25'12.26"S, 21°21'28.27"E) on the south coast of South Africa where both *M. galloprovincialis* and *P. perna* are abundant in the mid-shore region and display similar size class distributions (Fig. S1). Artificial mussel beds of each species were used to test experimentally whether the heat stress experienced by individual mussels differed depending on (1) the size of a mussel bed, (2) the size of individuals within a mussel bed, and (3) the position of an individual within a bed. Artificial beds were constructed by removing individuals from the mid-shore region, cleaning the outer surface of

encrusting organisms and placing them inside either large (20 cm diameter) or small (10 cm diameter) open circular cages made of coarse plastic mesh (Fig. 1; adapted from Nicastro et al. 2012). Cages were filled with either large (6–7.5 cm shell length) or small (4–5.5 cm in length) individuals to test whether the effect of mussel bed size on the thermal properties of mussels differed depending on the size of individuals. This produced four treatments or individual size/bed size combinations for each species and allowed the investigation of whether the effect of the thermoregulatory behaviour of each species differed with the size and structure of mussel beds.

Once assembled, all cages ($n = 3$ per treatment) were submerged in a large open rock pool for 90 min before simultaneously being removed at midday and interspersed across a large flat section of rocky substratum high on the shore, where they remained for up to 80 min. This rock pool was chosen because it was large and permanently open to the nearshore environment. Thus, the water temperature experienced by mussels during this incubation period would have been equivalent to that experienced by submerged mussels on the rocky shore during high tide. This experiment (hereafter referred to as Experiment I) was replicated across two consecutive low tides on the 22 and 23 January 2015 (austral summer) when daytime low tides fell between 11 a.m. and 1 p.m. This maximised the likelihood of extreme heat stress. The same mussels were used for both replicate experiments with artificial beds being positioned within the exact same designated areas on the rocky platform used the previous day. Individuals within each artificial mussel bed, however, were redistributed amongst cages before the start of the second replicate experiment to ensure no carry over effects from the previous experimental treatments. Hourly air temperature and wind speed data recorded from a coastal weather station located in Stillbaai (10 km east of the field site) were obtained from the South Africa Weather Service. Unfortunately, hourly solar irradiance was not measured at this or any other nearby weather station.

Infrared thermography was used to measure the temporal and spatial variability in mussel body temperatures amongst treatments. Starting shortly after the cages were removed from the water, IR images of each artificial bed were taken with a Fluke Ti25 thermal imaging camera (thermal resolution: <0.09 °C; accuracy ± 2 °C or 2 % of reading, whichever is greater) every 5 min to assess temporal and spatial variability in the body temperatures of multiple individuals within the same bed. Comparisons between shell surface temperatures of mussels measured by IR imagery and internal body temperatures measured with digital thermocouples (4 channel data logging thermometer 800024, SPER SCIENTIFIC Ltd.) revealed a highly

significant positive correlation when assessed in the field ($r^2 = 0.96$, $n = 122$; Lathlean et al. 2016). For the purposes of this study, shell temperatures measured by IR imagery are subsequently referred to as body temperatures hereafter. At each 5-min sampling interval, body temperatures of 10 randomly selected mussels within each cage were selected using the single-point measurement tool in the FLUKE SmartView 3.7.23 software package. Five of these individuals were randomly selected from the windward side of the mussel bed (i.e. the side exposed to the prevailing wind), whilst the other five were selected from the leeward or downwind side of the mussel bed. These five measurements were averaged to give a single mean estimate of body temperature for mussels situated on different sides of the same bed. Mussels surrounding the perimeter of each bed were excluded from analysis to limit potential edge effects on estimates of body temperature.

An additional experiment (hereafter referred to as Experiment II) was carried out during the midday low tide on 24 January 2015 using only the large (20 cm diameter) cages (insufficient numbers of large *M. galloprovincialis* on the shore limited us to one cage size). These cages were filled with either 100 % large individual mussels, 100 % small individual mussels, or a mixture of 50 % small individuals evenly interspersed amongst 50 % large individuals. This was done separately for *P. perna* and *M. galloprovincialis*. Again, all cages ($n = 3$ per treatment) were submerged in the large open rock pool for 90 min before simultaneously being removed at midday and interspersed across a large flat section of rocky substratum high on the shore, where they remained for 80 min. IR images of each artificial bed were taken every 5 min and body temperatures of 10 randomly selected mussels within each cage were measured as above. In cages that contained both large and small individuals, five of these measurements were taken for small individuals within the bed, and five were taken for large individuals. These measurements were then averaged to give a single mean estimate of body temperature for large and/or small mussels situated within the same bed.

Data analysis

Three-way ANOVA was used to test whether body temperatures differed depending on (1) the size of mussel beds (orthogonal, fixed, 2 levels: large or small), (2) the size of mussels within the bed (orthogonal, fixed, 2 levels: large or small), and (3) species (orthogonal, fixed, 2 levels: *P. perna* or *M. galloprovincialis*). Similar analyses were used to test for possible effects on mean body temperatures of position of mussels within a bed by including position (orthogonal, fixed, nested within bed, 2 levels: windward or leeward) as a fourth factor. Both three-way and

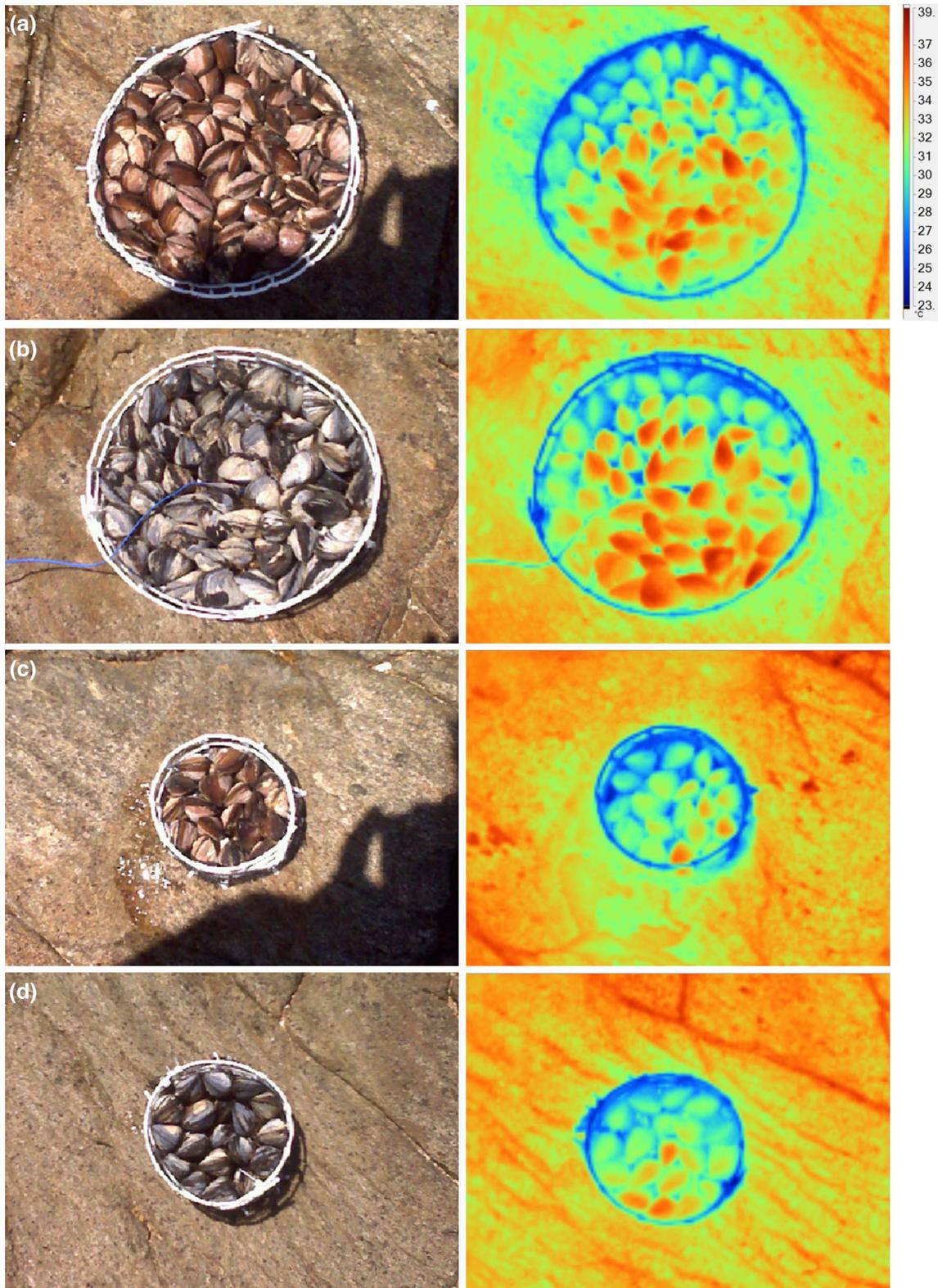


Fig. 1 Illustrative photos (*left*) and infrared images (*right*) of large (**a**, **b**) and small (**c**, **d**) artificial mussel beds filled with either large *P. perna* (**a**, **c**) or large *M. galloprovincialis* (**b**, **d**)

Table 1 Four-way nested ANOVA of the effect of bed size, position within bed (nested), mussel size, and species on mussel body temperatures at 40 and 80 min post-emersion during periods of simulated aerial exposure on 22 and 23 January 2015

Source	22 January 2015				23 January 2015			
	df	MS	F-ratio	p value	df	MS	F-ratio	p value
Body temperature								
40 min								
Bed size	1	33.91	33.85	<0.001	1	8.43	13.14	0.001
Position	1	39.40	39.33	<0.001	1	14.91	23.24	<0.001
Mussel size	1	6.49	6.48	0.016	1	2.47	3.86	0.061
Species	1	8.22	8.20	0.007	1	0.11	0.17	0.682
Mussel size × species	1	13.97	13.94	<0.001	1	0.39	0.60	0.445
Error	32	1			24	0.64		
80 min								
Bed size	1	21.99	23.67	<0.001	1	1.15	1.02	0.323
Position	1	22.81	24.54	<0.001	1	8.02	7.11	0.014
Mussel size	1	10.88	11.70	0.002	1	0.27	0.24	0.630
Species	1	25.25	27.18	<0.001	1	2.36	2.10	0.161
Bed size × species	1	4.33	4.66	0.039	1	0.78	0.69	0.414
Mussel size × species	1	3.98	4.28	0.047	1	0.07	0.06	0.810
Error	32	0.93			24	1.13		

Only factors which returned significant ($p < 0.05$), or marginally insignificant (p close to 0.05) effects for one of the two sampling dates are presented

Bold values represent significant values

four-way nested ANOVAs were undertaken for two time-points (i.e. 40 and 80 min post-emersion, representing the mid and endpoints of the experiment, respectively) during each of the experiments undertaken on 22 and 23 January 2015. Similarly, four-way nested ANOVA was used to test whether body temperature heating rates differed amongst treatments. Heating rates were calculated by subtracting the initial mean body temperatures of mussels from the mean 40 min post-emersion (Rate 1: 0–40 min) and by subtracting the mean body temperature of mussels at 40 min post-emersion from the final mean body temperature 80 min after emersion (Rate 2: 40–80 min). Unfortunately, due to low densities of large (6–7.5 cm) *M. galloprovincialis* individuals at the study site, two treatments were omitted from the experiment carried out on 23 January (i.e. small beds filled with large individuals). For Experiment II undertaken on 24 January 2015, two-way ANOVA was used to test whether mean body temperatures (at 40 and 80 min post-emersion) differed depending on (1) size structure of mussel beds (orthogonal, fixed, 4 levels: all small, all large, small within mixed, large within mixed) and (2) species composition (orthogonal, fixed, 2 levels: *P. perna* or *M. galloprovincialis*). Data were normally distributed and generally showed equal variances (tested using the Shapiro–Wilks tests for normality and Cochran’s tests, respectively). When normality or homoscedasticity assumptions were not met, log transformations were used in some cases to satisfy the assumptions of ANOVA. Tukey–Kramer post hoc tests were used to explore significant effects further.

Results

Experiment I: Effect of mussel bed size, species, size class, and position within bed

Body temperatures of mussels within artificial beds constructed on the 22 January differed significantly depending on: (1) the size of mussel beds, (2) the size of individuals within beds, (3) the position of individuals within beds, and (4) the species of mussel (Table 1). Irrespective of their shell length, individuals within large mussel beds experienced significantly higher (1.5–2 °C) body temperatures than those within small beds (Fig. 2; Table 1). Irrespective of bed size, beds comprised of small individuals displayed higher (1.5–2 °C) body temperatures than those constructed of large individuals, though these differences were greater for beds comprised of non-gaping *M. galloprovincialis* (Fig. 2; Table 1). In addition, individuals located on the windward side of mussel beds experienced body temperatures 2–4 °C lower than those on the leeward side (Fig. 2, Table 1). After 80 min of emersion, differences between large and small mussel beds also varied depending on species composition with differences between large and small *M. galloprovincialis* beds being greater than between large and small *P. perna* beds (Fig. 2, Table 1). In contrast to experiments undertaken on the 22 January, body temperatures recorded on the 23 January did not differ amongst beds comprised of small or large individuals irrespective of species (Fig. 3, Table 1). On both

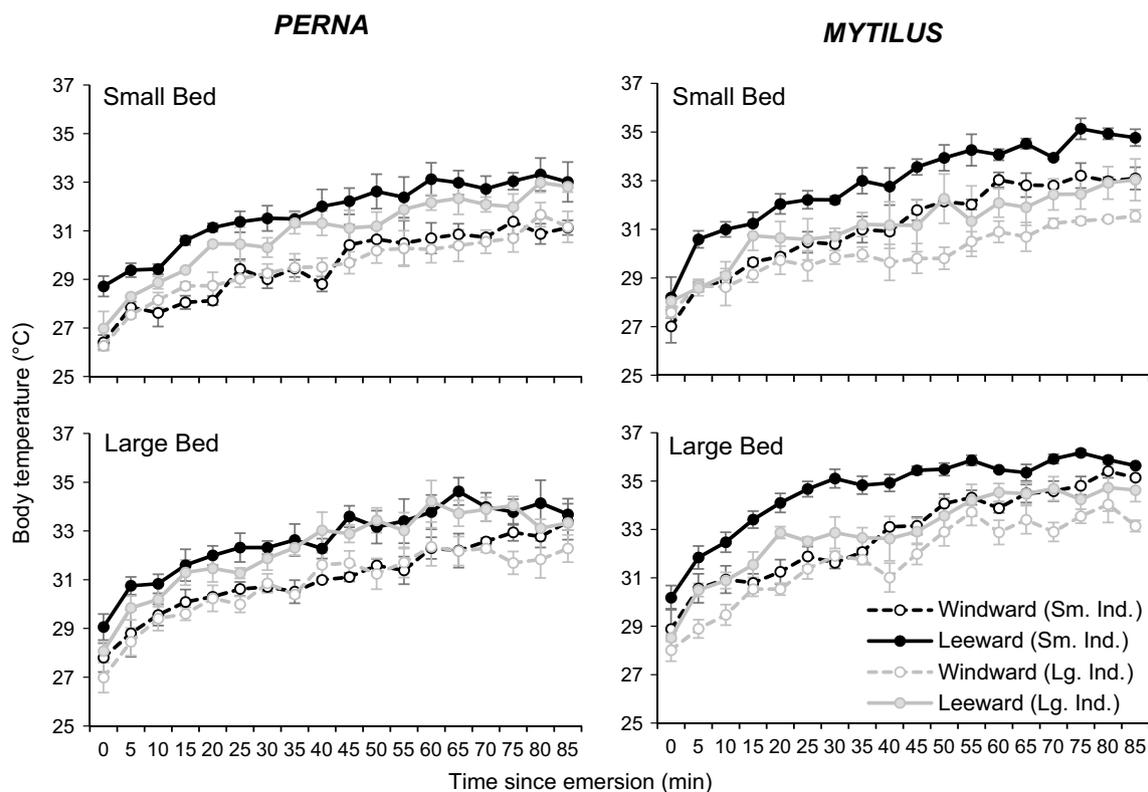


Fig. 2 Changes in mean (\pm SE) body temperatures of individual mussels within large and small artificial mussel beds after being emersed from water (i.e. time zero) on the 22nd January 2015 ($n = 3$

plots). *Left panels* represent mussel beds comprised of large and small *P. perna*; *right panels* represent mussel beds comprised of large and small *M. galloprovincialis*

the 22 and 23 January, however, body temperatures were significantly greater for individuals on the leeward side of mussel beds and for large mussel beds 40 min after emersion (Fig. 3, Table 1).

Heating rates of mussel body temperatures during the first 40 min of the experiment undertaken on the 22 January were significantly faster for individuals on the leeward side of beds, irrespective of species or whether individuals were large or small or situated within large or small beds (Fig. 2; Table 2). Size of individual mussels did, however, influence the heating rates of *M. galloprovincialis* during the first 40 min, with body temperatures of smaller mussels rising faster than those of larger mussels, irrespective of whether individuals were situated within large or small beds or on the leeward or windward side of beds (Fig. 2; Table 2). By contrast, size of bed, size of individuals, and position of individuals within bed did not affect heating rates during the latter half of the experimental period. Instead, heating rates differed only between species, with small *M. galloprovincialis* within large beds displaying significantly higher heating rates than all other treatments (Fig. 2; Table 2). The replicate experiment carried out on the 23 January only reported significant differences in the heating rates of mussel body temperatures between large

and small beds during the first 40 min and between the two different species during the latter 40 min of the experiment (Fig. 3; Table 2).

Experiment II: Effect of size structure and species

At the end of the emersion period, small *P. perna* and *M. galloprovincialis* interspersed amongst larger conspecifics displayed significantly lower body temperatures than small mussels surrounded only by other small conspecifics (Fig. 4; Table 3). The opposite was not true, however, with the body temperatures of large *P. perna* and *M. galloprovincialis* interspersed amongst small conspecifics being equivalent to the body temperatures of large mussels surrounded by other large individuals (Fig. 4; Table 3). As in Experiment I, body temperatures of *M. galloprovincialis* were significantly greater than those experienced by *P. perna*, irrespective of size class structure.

Heating rates did not differ amongst cages filled with large or small *P. perna* (Fig. 4; Table 3). By contrast, heating rates of small *M. galloprovincialis* were significantly lower than larger neighbouring conspecifics within the same bed as well as the heating rates of larger conspecifics entirely surrounded by other large individuals (Table 3).

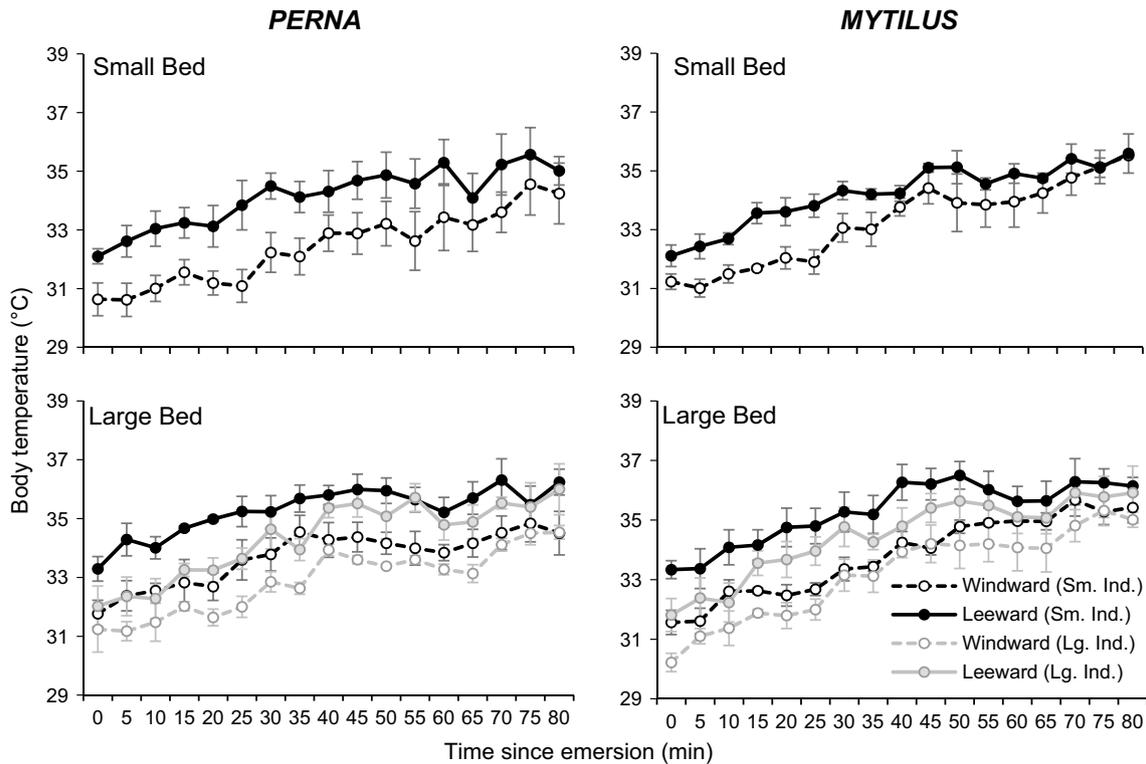


Fig. 3 Changes in mean (\pm SE) body temperatures of individual mussels within large and small artificial mussel beds after being emersed from water (i.e. time zero) on the 23rd January 2015 ($n = 3$

plots). *Left panels* represent mussel beds comprised of large and small *P. perna*; *right panels* represent mussel beds comprised of large and small *M. galloprovincialis*

Table 2 Four-way nested ANOVA of the effect of bed size, position within bed (nested), mussel size and species on the heating rate of mussel body temperatures at 40 and 80 min post-emersion during periods of simulated aerial exposure on 22 and 23 January 2015

Source	22 January 2015				23 January 2015			
	df	MS	F-ratio	p value	df	MS	F-ratio	p value
Body temperature heating rate								
0–40 min								
Bed size	1	4.98	3.86	0.058	1	3.30	4.36	0.048
Position	1	6.07	4.71	0.038	1	0.02	0.03	0.865
Mussel size \times species	1	19.51	15.14	<0.001	1	0.01	0.01	0.972
Error	32	1.29			24	0.76		
40–80 min								
Species	1	4.66	5.84	0.022	1	3.36	6.28	0.019
Bed size \times species	1	2.83	3.56	0.068	1	0.07	0.13	0.717
Mussel size \times species	1	3.04	3.82	0.060	1	0.13	0.25	0.624
Bed size \times position \times species	1	2.31	2.89	0.099	1	0.50	0.94	0.343
Bed size \times mussel size \times species	1	7.62	9.56	0.004	1	0.86	1.60	0.218
Error	32				24	0.54		

Only factors which returned significant ($p < 0.05$) or marginally insignificant (p close to 0.05) effects for one of the two sampling dates are presented

Bold values represent significant values

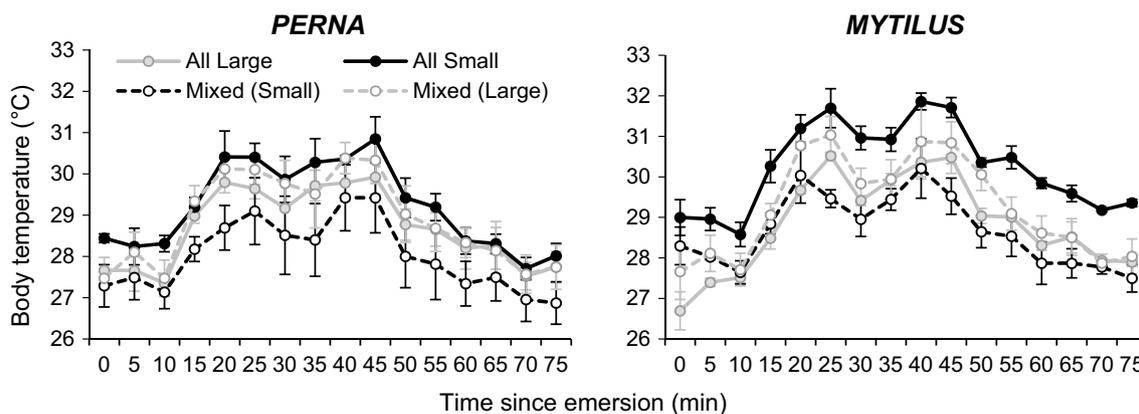


Fig. 4 Changes in mean (\pm SE) body temperatures of individual mussels within large artificial mussel beds comprised of either large, small, or mixed size classes after being emersed from water (i.e. time

zero) on the 24th January 2015 ($n = 3$ plots). *Left panel* represents mussel beds comprised of *P. perna*; *right panel* represents mussel beds comprised of *M. galloprovincialis*

Table 3 Two-way ANOVA of the effect of mussel bed size structure and species on (1) mussel body temperatures at 40 min and 80 min post-emersion and (2) initial heating rates of body temperatures during periods of simulated aerial exposure on 24 January 2015

Source	24 January 2015			
	df	MS	F-ratio	p value
Body temperature				
40 min				
Size structure	3	2.02	2.16	0.133
Species	1	4.22	4.52	0.049
Size structure \times species	3	0.31	0.33	0.801
Error	16	0.94		
Post hoc: <i>Mytilus</i> > <i>Perna</i>				
80 min				
Size structure	3	2.26	5.44	0.009
Species	1	2.20	5.30	0.035
Size structure \times species	3	0.42	1.01	0.413
Error	16	0.42		
Post hoc: all small > mixed small; <i>Mytilus</i> > <i>Perna</i>				
Body temperature heating rate				
0–40 min				
Size structure	3	1.35	4.99	0.012
Species	1	2.47	9.18	0.008
Size structure \times species	3	0.89	3.29	0.048
Error	16	0.27		
Post hoc: PERNA—all non-significant				
MYTILUS—all large and mixed large > mixed small				

Post hoc represents the results of Tukey–Kramer comparisons

Bold values represent significant values

Discussion

The results of our manipulative experiments suggest that the amount of heat stress experienced by mussels during aerial exposure can sometimes be influenced by multiple small-scale biotic factors including: (1) the size of aggregations, (2) the position of individual mussels within aggregations, and (3) the size of the individuals and of their neighbours comprising the aggregation. Our results show that small-scale patterns in thermal variability within mussel beds can vary significantly from 1 day to another even when abiotic conditions differ little. Differences detected between experiments undertaken on the 22nd and 23rd of January were most likely due to slight differences in solar radiation since air temperatures, wind speed, and direction were similar. In fact, heat generated through solar irradiance and re-radiation (emitted by the rock surface) rather than air temperature may determine the heating rate and the highest daily maximum body temperatures of intertidal ectotherms (Helmuth et al. 2010; Marshall et al. 2010). We also demonstrated that small-scale differences in the thermal properties of mussels can vary between two species with different thermoregulatory behaviours. Differences in the body temperatures of small and large mussels, for example, were more pronounced for *M. galloprovincialis*, which does not gape during aerial exposure. Thus, evaporative cooling employed by *P. perna* appears to, at times, override small-scale variability in heat stress. This supports previous research that shows aggregations of gaping *P. perna* are more effective at maintaining lower body

temperatures during aerial exposure than aggregations of the non-gaping *M. galloprovincialis* (Nicastro et al. 2012). Combined with previous studies which show that *M. galloprovincialis* benefits from the evaporative cooling properties of aggregated *P. perna* (Lathlean et al. 2016), we may expect abundances of *M. galloprovincialis* within the mid-shore region to increase if temperatures continue to rise due to global climate change. Indeed, our results indicate that smaller individuals surrounded by larger conspecifics experience cooler body temperatures than those surrounded by similarly sized individuals. Again, by extension, we might expect juvenile *M. galloprovincialis* to benefit the most from the evaporative cooling properties of *P. perna* since they would not suffer the same degree of water loss or desiccation as than their *P. perna* counterparts. However, the lower vertical distributional limit of *M. galloprovincialis* would still be limited by their weaker byssal attachment strength so the relative abundances of the two species within the mid-shore region may not change (Zardi et al. 2006; McQuaid et al. 2015).

Contrary to our expectations, the results suggest that large mussel beds do not always provide the greatest amelioration of heat stress. Indeed, individuals within small mussel beds were generally cooler and at times displayed slower heating rates than those in large mussel beds. This is surprising since the greater biomass of large mussel beds was expected to provide greater thermal inertia than small mussel beds because their larger size supposedly buffers them against rapid changes in environmental conditions (Helmuth 1998). However, this expectation does not take convective cooling into account, and its ability to counteract increases in temperature. With frequent gusts of onshore winds blowing across the study site, small mussel beds would have experienced greater convective cooling than large mussel beds due to their greater surface to volume ratio. This would also explain why, irrespective of mussel bed size, individuals located at the windward side of mussel beds experienced significantly lower body temperatures than those located on the leeward side. These findings indirectly support a heat-budget model developed for the intertidal mussel *Mytilus californianus*, which shows that even moderate increases in mean wind speed counteract the effects of increases in air temperature on body temperatures (Helmuth et al. 2011).

In contrast to differences between large and small mussel beds, larger individual mussels generally maintained lower body temperatures and experienced lower heating rates than smaller mussels, irrespective of species and their thermoregulatory behaviour. This supports our expectations and current physiological principles that suggest the greater body mass of large individuals provides a buffer against changes in abiotic conditions and helps maintain relatively stable body temperatures (Angilletta et al. 2002).

Consequently, individuals that experienced the greatest amount of heat stress were small mussels in large beds comprised of small conspecifics. Our results also suggest that small mussels can reduce their susceptibility to thermal stress by associating with larger conspecifics. This pattern was observed in natural aggregations of mussel beds with the mid-shore region at the study site during aerial exposure (Lathlean et al. 2016) and may help explain the findings of numerous other studies that report higher rates of recruitment and survival of juvenile mussels within than outside mussel beds (e.g. Harris et al. 1998).

Surprisingly little research has been undertaken on the relationship between the size and thermal dynamics of mussel beds, or indeed other autogenic ecosystem engineers. Numerous studies have shown that body temperatures of solitary mussels are generally greater than those within the centre of mussel beds (e.g. Helmuth 1998). Mussels around the edges of beds also experience greater heat stress than those within the centre (Nicastro et al. 2012). It remains unclear whether a certain mussel bed size or threshold is necessary before beneficial cooling properties start to emerge. This was the case for the intertidal gastropod *Nerita atramentosa* where a minimum aggregate size of approximately 216 individuals was necessary for individual body temperatures to differ between those found in the centre of the aggregation to those found around the edge (Chapponon and Seuront 2012). Similarly, aggregation size affected the abundance and diversity of epifaunal communities associated with mussel beds along the southeast coast of South Africa (Jungerstam et al. 2014). By contrast, species richness and abundance of epifaunal assemblages associated with the blue mussel *Mytilus edulis* in Denmark did not vary depending on the size of bed (Svane and Setyobudiandi 1996), though *M. edulis* growth and recruitment were greater within small (<30 cm diameter) isolated patches than within larger mussel beds in the mid-shore region (Svane and Ompi 1993). Such relationships between epifaunal diversity and abundance, as well as mussel growth and recruitment, may be influenced by small-scale differences in the thermal properties of large and small mussel beds as detected in the present study.

The high level of microhabitat variability in body temperatures, even within standardised artificial mussel beds, detected across such small spatial scales may have significant implications for understanding and predicting how these habitat-forming species, and their associated communities, will respond to future extreme heat events. Previous research has shown, for example, that microhabitat selection plays a key role in buffering the effects of extreme heat events on populations of intertidal organisms (Harley 2008; Denny et al. 2011). For instance, mass mortalities of the limpet *Lottia scabra* and the mussel *M. californianus* were closely associated with small-scale variability in

temperature caused by variation in substratum orientation (Harley 2008). Observations taken during the present study, however, might also suggest that inter-individual variability in mussel body temperatures, and consequently differential mortality rates, may occur even within equivalent microhabitats. For example, adjacent individuals within the same mussel bed, but with slightly different orientations towards the sun, displayed differences in body temperatures of 4–5 °C (J.A. Lathlean pers. obs). Similarly, using a biophysical model, Mislán and Wethey (2015) predict that differences in body temperatures of mussels attached either directly to the substratum or on top of other mussels could account for small-scale patterns of mortality following extreme heat events. Fine-scale thermal variability within equivalent microhabitats has also been shown to influence the growth and survival of newly settled barnacles on rocky intertidal shores of southeast Australia (Lathlean et al. 2012, 2013). Consequently, both within and amongst microhabitat variability in the body temperatures of intertidal organisms may play an important role in facilitating the adaptation and survival of these populations and communities to climate change.

Conclusion

This study demonstrates how biotic factors such as behaviour, body size, and position within aggregations all have the ability to influence the small-scale variability of mussel body temperatures within different regions of a mussel bed as well as between mussel beds of different size and structure. Consequently, biotic aspects, such as thermoregulatory behaviour and body size, may influence the body temperatures of intertidal organisms as much as large-scale and microhabitat thermal variability and should therefore receive greater inclusion within climate change adaptation models.

Acknowledgments We would like to thank Jaqui Trassiera for assistance in the field, Gray Williams for contributing to early discussions of the project and Elizabeth Lathlean for constructive comments on earlier versions of the manuscript. This work is based upon research supported by the South African Research Chairs Initiative of the Department of Science and Technology and the National Research Foundation. Funding, an Australian Research Council's Discovery Project (project number DP0988554), and by Fundação para a Ciência e a Tecnologia (FCT; IF/01413/2014/CP1217/CT0004). School of Biological Sciences, The University of Hong Kong is also thanked for the travel support to T.P.T.N.

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