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ORIGINAL ARTICLE

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Thermally mediated body temperature, water content and aggregation behaviour in the intertidal gastropod *Nerita atramentosa*

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Abstract Intertidal organisms are vulnerable to global warming as they already live at, or near to, the upper limit of their thermal tolerance window. The behaviour of ectotherms could, however, dampen their limited physiological abilities to respond to climate change (e.g. drier and warmer environmental conditions) which could substantially increase their survival rates. The behaviour of ectotherms is still mostly overlooked in climate change studies. Here, we investigate the potential of aggregation behaviour to compensate for climate change in an intertidal gastropod species (*Nerita atramentosa*) in South Australia. We used thermal imaging to investigate (1) the heterogeneity in individual snail water content and body temperature and surrounding substratum temperature on two topographically different habitats (i.e. rock platform and boulders) separated by 250 m at both day- and night-times, (2) the potential relationship between environment temperature (air and substratum) and snail water content and body temperature, and (3) the potential buffering effect of aggregation behaviour on snail water content and body temperature. Both substratum and snail temperature were more heterogeneous at small spatial scales (a few centimetres to a few metres) than between habitats. This reinforces the evidence that mobile intertidal ectotherms could survive locally under warmer conditions if they can locate and move behaviourally in local thermal refuges. *N. atramentosa* behaviour, water content and body temperature during emersion seem to be related to the thermal stability and local conditions of the habitat occupied. Aggregation behaviour reduces both desiccation and heat stresses but only on the boulder field. Further investigations are required to identify the different behavioural strategies used by ectothermic species to adapt to heat and dehydrating conditions at the habitat level. Ultimately, this information constitutes a fundamental prerequisite to implement conservation management plans for ectothermic species identified as vulnerable in the warming climate.

Keywords Body temperature · Water content · Aggregation · Snail · Thermal imaging · Rocky shore · Ectotherms · Desiccation stress · Thermal stress · Conservation management

Introduction

Understanding how ectotherms adapt physiologically to heat stress and thermoregulate in response to climate warming is essential to determining the limits of their thermal tolerance window (Hofmann and Todgham 2010). This is critical since temperature has a paramount effect on physiological processes cascading from genes to organisms (Somero 2002). This impacts individual performance and fitness (Huey and Berrigan 2001), and ultimately sets species biogeographic ranges (Hofmann and Todgham 2010). The physiological thermoregulatory abilities of intertidal ectotherms are, however, limited since they already live at, or near to, their upper thermal tolerance limit (Somero 2002). It is thus critical to examine non-physiological means of thermoregulation that could maintain the fitness of intertidal species, hence to some extent the balance of the intertidal ecosystem, in the changing climate.

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Ectothermic species can maintain body temperatures within their thermal tolerance window by responding to heat stress over a range of (1) temporal scales spanning from minutes to generations, hence involving processes such as behavioural thermoregulation (Dubois et al. 2009; Munoz et al. 2005), acclimatisation (Sinclair et al. 2006) and evolutionary adaptation (Somero 2010); and (2) spatial scales ranging from small-scale habitats (Chapperon and Seuront 2011a; Denny et al. 2011) to large-scale geographic distances (Osovitz and Hofmann 2007). In particular, the survival of ectothermic species in the warming climate is linked strongly to the dispersal abilities of both organisms and populations within and across heterogeneous environments in order to select thermally favourable habitats (Sorte et al. 2011). For instance, some intertidal invertebrates thermoregulate through microhabitat selection (e.g. Garrity 1984) while some terrestrial vertebrates such as reptiles shuttle between sun and shade in order to take advantage or avoid solar radiation (e.g. Díaz and Cabezas- Díaz 2004; Dubois et al. 2009). Intertidal invertebrates also display a range of thermoregulatory behaviours such as shell position adjustment (Munoz et al. 2005) or mushrooming behaviour (Williams et al. 2005). On intertidal rocky shores, the frequent formation of aggregates that contain up to hundreds of individuals (Chapman and Underwood 1996) has typically been considered to reduce both thermal and desiccation stresses (Garrity 1984; Chapman and Underwood 1992; Raffaelli and Hawkins 1996; Stafford and Davies 2005, 2011, 2012). Aggregations may indeed maintain moisture and reduce evaporation rate through the minimisation of the surface-to-volume ratio in contact with the atmosphere (Chase et al. 1980). For instance, aggregated *Nodilittorina peruviana* maintain water content and thermoregulate better than solitary individuals (Rojas et al. 2000). Similarly, aggregated mussels exhibit lower body temperatures (4–5 °C) than solitary mussels (Helmuth 1998).

The main objective of this study was to examine whether the aggregation behaviour widespread among intertidal snails provide benefits in terms of body temperature and water content in dry and hot environmental conditions and to discuss its potential implication in the warming climate. In this context, this study assessed (1) the heterogeneity in snail temperature, water content and environment temperature; (2) the relationship between snail water content, body temperature and environment temperature; and (3) the potential buffering effect of aggregation behaviour on snail body temperature and water content in different environmental conditions. The mosaic patterns of environmental/animal temperature and the benefits of aggregation behaviour in relation to snail temperature and water content identified under specific conditions provide insight into potential conservation and management plans that may be implemented to protect ectothermic animals and their habitats from heat and desiccation stresses.

Materials and methods

Study organisms and sampling strategy

In this study, we investigated the potential links between aggregation, heat and desiccation stresses in the intertidal prosobranch gastropod *Nerita atramentosa* (Reeve 1855). *N. atramentosa* was specifically chosen since it is typically found in large aggregations (up to several hundreds of individuals) on temperate South Australian rocky shores (C.C. and L.S., personal observation), and plays a strategic role on intertidal rocky shores (Underwood 1984). *N. atramentosa* appears to be particularly vulnerable to extreme heat, and hence the warming climate, due to its (1) geographic position, (2) shell colour and (3) limited thermoregulatory abilities. First, South Australia is the driest state in Australia and is predicted to become both drier and warmer with an increase in the frequency of extreme maximum temperature (CSIRO 2010). South Australian oceanic waters have also warmed up more rapidly than anticipated and poleward range extension and decline of reef organisms have already been observed (Wernberg et al. 2011). Secondly, *N. atramentosa* has a black-pigmented shell that enhances both the absorption and retention of solar radiation during emersion more than the surrounding substratum (McMahon 1990). Finally, *N. atramentosa* individuals remain attached to the substratum even above temperatures inducing heat coma (i.e. 38.9 °C) and some specimens exhibit a lack or limited evaporative cooling ability (McMahon 1990). *N. atramentosa* successful dominance on South Australian rocky shores nevertheless suggests the existence of some behavioural adjustment of its body temperature and water content to cope with South Australian summer conditions, such as the formation of aggregates.

This study was conducted during the austral summer (February 2010) on a moderately exposed rocky shore located in South Australia (Marino Rocks). This site is characterised by an alongshore gradient in substratum topographic complexity that includes rock platforms, rocks and boulders, and abounds with intertidal snails (e.g. *Bembicium* sp., *Austrocochlea* sp.), especially *Nerita atramentosa* (Reeve 1855) found at each tidal height.

In order to assess the potential effect of aggregation behaviour on both snail body temperature and water content, we considered solitary and aggregated individuals. An individual was considered aggregated when there was a direct shell contact with the shell of at least another conspecific (Chapperon and Seuront 2011a, b). However, all aggregated individuals were collected randomly from different aggregations containing at least five individuals. Individuals were collected only from the centre of aggregations. Overall, body temperature and water content were measured from 90 to 100 adult individuals (17.3 ± 2.71 mm, $\bar{x} \pm \mathrm{SD}$) during each sampling session (i.e. daytime and nighttime low tides). All individuals were chosen randomly 1 m away from each side of a 30 m transect running parallel to the sea at mid-shore level. Note that the body size of *N. atramentosa* individuals did not differ significantly between habitats and sampling days ($P > 0.05$); hence this rules out the bias related by the potential effect of body size on thermoregulatory performance and behaviour (e.g. Helmuth 1998; Williams et al. 2005).

To assess the potential spatial variability in snail temperature, water content and environment temperature and in the effects of aggregation behaviour on snail body temperature and water content, we selected two habitats located 250 m apart and characterised by distinct topographical complexity, and related shelter availability. The first habitat was a horizontal rock platform (35°2′32.93″S, 138°3′35.01″E) composed by a flat rock substratum punctuated by small pits and crevices and the presence of sparse rocks. *N. atramentosa* was found mainly under rocks within aggregations at daytime low tides (Chapperon and Seuront 2011a). Individuals situated under rocks were hence studied in this habitat. The second habitat was a boulder field (35°2′43.11″S, 138°3′28.30″E) characterized by a complex topography formed by the presence of large rocks, crevices (i.e. depression that fits at least an individual) and deeper depressions such as pools (a few centimetres up to 80 cm deep). *N. atramentosa* individuals occupied mainly crevices within aggregations at daytime low tides (Chapperon and Seuront 2011a).

The potential temporal variability in the effects of aggregation behaviour on snail body temperature and water content was investigated during emersion at day- and night-times. Daytime field work was conducted at midday low tide (i.e. 12.00 a.m. to 2.00 p.m.) during 4 successive days on the rock platform and the boulder field. Midday low tides were chosen as they set the most severe episodes of thermal stress that select for heat tolerance (Somero 2010). Nighttime field work was carried out 3 h after sunset over two successive nights in both habitats. Days and nights of samplings were different between habitats (4 days and 2 nights per habitat). Field work always started when the tide was at its lowest level.

Environment and snail mantle temperature

The air temperatures measured at the regional scale (AT_{BOM}) during the sampling times in both habitats were collected from the Bureau of Meteorology of Australia (BOM) from a weather station situated 10 km away from the study site in order to ensure that the regional scale meteorological forcing typically considered in intertidal studies (Lathlean et al. 2011) was similar between sampling times in both habitats. The air temperature was also measured under each studied rock (AT_{UR}) using a digital thermometer on the rock platform.

Substratum and snail body temperatures (ST and BT, respectively) were measured with a thermal imaging camera (Fluke® Ti20, Fluke Corporation, Everett, WA). Infrared thermography is a non-contact and non-invasive technique to measure temperature through the creation of a picture of infrared energy emissions from any surface in the visible spectrum (Helmuth 2002). Thermal imaging has been used recently to measure the temperature of intertidal invertebrates (Cox and Smith 2011) such as snails (e.g. *Nerita atramentosa* on rocky shores, Caddy-Retalic et al. 2011; Chapperon and Seuront 2011a; *Littoraria scabra* in mangroves, Chapperon and Seuront 2011b). The thermal sensitivity of the thermal camera is ≤ 0.2 °C at 30 °C and the temperature measurement accuracy is 2 % or 2 °C, whichever is greater. Emissivity values of both shell snails and substrata were set at 0.95; see Chapperon and Seuront (2011a) for further explanations. A thermal picture of each organism and surrounding substratum were taken and further analysed with the InsideIR 4.0 software, ver. 2006 (Fluke Corporation) to assess snail body and substratum temperatures (Chapperon and Seuront 2011a). For each thermal picture, a digital picture (Olympus J1 Tough-60, Olympus Imaging Corporation, Centre Valley, PA) was taken jointly to analyze the individual BT in regards to behavioural properties (i.e. solitary or aggregated). Mantle temperature (MT) of each individual was subsequently estimated from the body temperature, BT (Caddy-Retalic et al. 2011). The ratio, MST_{ratio}, between MT and ST was further calculated for each individual to identify the temperature deviation between organism and surrounding substratum. A total of 248 solitary and 322 aggregated individuals were studied on the boulder field ($N_{total} = 570$), and 304 solitary and 261 aggregated individuals on the rock platform ($N_{total} = 565$). Snails were considered within two microhabitats on the boulder field, i.e. flat rocks and crevices both exposed to solar radiation during daytimes. *N. atramentosa* temperature does not differ significantly between these two microhabitats (Chapperon and Seuront 2011a). On the rock platform, studied individuals were found under rocks where they consistently aggregate and shelter from thermal stress (Chapperon and Seuront 2011a).

Snail water content

Individual water content ($N = 1{,}135$) was measured in order to evaluate the impact of aggregation behaviour on the dehydration state of individuals under different desiccation stress conditions (midday and nighttime) and in two different habitats. More specifically, each photographed organism was removed from its substratum and stored in a sealed pre-weighted tube. Individual fresh weight (W_f) was measured immediately upon return to the laboratory. Organism dry weight (W_d) was assessed after 24 h spent at 60 °C (Edgar 1990). Absolute water content of each organism (g) was further calculated as $W_f - W_d$, and normalised to the volume of each organism which was approximated by the product of its length (L), width (w) and height (h). The water content WC of each organism (g cm^{-3}) was finally calculated as $v_{H_2O} = \frac{W_f - W_d}{L \times w \times h} \times 1000$. Note that no significant relationship ($P > 0.05$) was found between the water content of individuals and the time of sampling (i.e. from the beginning to the end of samplings). While this suggests that the duration of emersion does not impact our results, it is possible that the measured individual water contents are not only representative of the desiccation experienced by individual at low tide since it also depends on the duration of foraging activities undertaken during the previous high tide (Stafford et al. 2012), i.e. an individual that moves for a longer period will lose more water. However, this is unlikely to affect our water content measurements as a recent study has shown that *N. atramentosa* individuals consistently move over similar distances from one low tide to the next (C.C. and L.S., unpublished data).

Statistical analysis

Because the distributions of the variables WC, MT, ST and MST$_{ratio}$ diverged significantly from the normality assumption (Kolmogorov–Smirnov test, $P < 0.05$), non-parametric statistics were used throughout this work. As not temporal differences were detected ($P > 0.05$) in the studied variables between the four daytime sampling nor between the two nighttime sampling, the data were pooled into two categories, "night" and "day", within each habitat. The potential relationships between AT$_{BOM}$, AT$_{UR}$, WC, MT, ST were investigated using the Spearman's rank correlation ρ. The relationship between AT$_{BOM}$ and AT$_{UR}$ was specifically investigated on the rock platform to identify whether environmental temperatures measured at the regional scale are representative of local scale thermal conditions. The potential difference between MT and ST in both habitats was further examined with the Mann–Whitney U test. The effect of sampling location (rock platform vs. boulders), time (day vs. night) and individual status (aggregated vs. solitary individuals) on WC, MT, ST and MST$_{ratio}$ was investigated with the Mann–Whitney U test. We did not use Bonferroni correction because this correction is conservative and results in greatly diminished power to detect differentiation among pairs of sample collections. Instead, we used the more robust modified false discovery rate procedure (Benjamini and Yekutieli 2001; Narum 2006), with the critical value α_c estimated from the predetermined value of α ($\alpha = 0.05$) and the number of hypothesis tests (i.e. the number of pairwise tests) k as $\alpha_c = \alpha / \sum_{i=1}^{k} (1/i)$. Analysis of variance (three factors, ANOVA) could not be performed since the data failed normality and homoscedasticity even after log transformation. All statistical analyses were performed with PASW Statistics 18 (SPSS, 2009, Chicago, IL).

Results

Space–time variability in air temperature

The mean air temperature measured at the regional scale i.e. AT$_{BOM}$ was on average 22.3 ± 2.4 °C, ($\bar{x} \pm$ SD) and 25.3 ± 3.6 °C at day- and night-time, respectively. On the boulder field, AT$_{BOM}$ was on average 25.2 ± 3.0 °C during the day and 22.1 ± 5.4 °C during the night. AT$_{BOM}$ did not significantly differ (Mann–Whitney U test, $P > 0.05$) between habitats at day- and night-times. On the rock platform, the air temperature measured under rocks (AT$_{UR}$) varied between 24 and 39 °C averaging at 30.1 ± 3.1 °C at daytime, and between 20 and 28 °C averaging at 23.1 ± 1.6 °C at nighttime. AT$_{BOM}$ and AT$_{UR}$ were not significantly correlated ($P > 0.05$).

Space–time variability in substratum and snail temperatures

Mantle and substratum temperatures were not correlated significantly to AT$_{BOM}$ in both habitats ($P > 0.05$). However, individual MT was positively and significantly correlated to ST in both habitats ($P < 0.001$, Fig. 1). Also note that while MT was significantly higher than ST in both habitats ($P < 0.001$), MT increased significantly faster than ST ($P < 0.05$) in both habitats. On the rock platform, individual MT and ST were also positively and significantly correlated to AT$_{UR}$ ($P < 0.001$). In both habitats, MT, ST and MST$_{ratio}$ were significantly greater during the day than at night (Tables 1, 2; Figs. 1, 2a).

During daytime, both MT and ST were significantly cooler on the rock platform than on the boulder field (Tables 1, 2). Nevertheless, no significant difference in MST$_{ratio}$ was found between habitats during the day (Tables 1, 2; Fig. 2a). At nighttime, MT was not significantly different between habitats (Tables 1, 2). ST was significantly warmer on the rock platform than on the boulder field (Tables 1, 2) during the night. MST$_{ratio}$ was significantly greater on boulders than on the rock platform at nighttime (Tables 1, 2; Fig. 2a).

Space–time variability in *N. atramentosa* water content

No significant correlation was found between the mean water content of individuals and AT$_{BOM}$, AT$_{UR}$, MT and ST in both habitats during either the daytime and nighttime field experiments ($P > 0.05$). On the rock platform, WC did not significantly differ between day and night (Tables 1, 2; Fig. 2b). In contrast, on the boulder field, WC was significantly greater at night than during the day (Tables 1, 2; Fig. 2b). At daytime, the water content of individuals did not significantly differ between the two habitats (Tables 1, 2; Fig. 2b). At nighttime, however, the water content was significantly

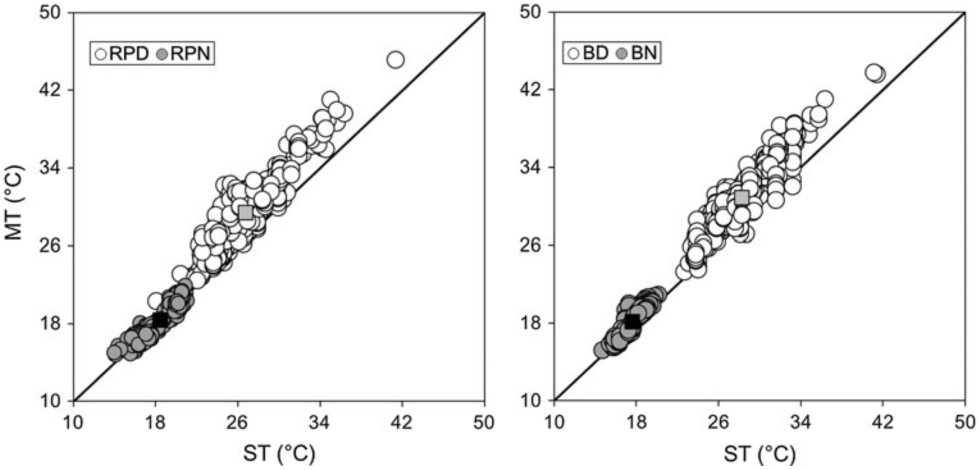

Fig. 1 Mantle temperature (MT) of *Nerita atramentosa* individuals versus surrounding substratum temperature (ST) in both habitats (*RP* rock platform; *B* boulders) during daytime (*D white dots*) and nighttime experiments (*N grey dots*). Mean MT and mean ST in both habitats are represented by *grey* and *black squares* during daytime and at night, respectively. The *black line* represents the first bissectrix, i.e. MT = ST. RP-day: *N* = 378, RP-night: *N* = 187, B-day: *N* = 380, B-night: *N* = 190

Table 1 Mean (± SD), minimum and maximum observed values of water content (WC), mantle temperature (MT), substratum temperature (ST), mantle to substratum temperature ratio (MST_{ratio}) in aggregated (A) and solitary (S) individuals in both habitats (rock platform and boulder field) during daytime and nighttime experiments

		Rock platform		Boulder field	
		Day *N*(A) = 224 *N*(S) = 154	Night *N*(A) = 37 *N*(S) = 150	Day *N*(A) = 212 *N*(S) = 168	Night *N*(A) = 110 *N*(S) = 80
A-WC (g cm^{-3})	$\bar{x}$ (SD)	0.112 (0.022)	0.115 (0.016)	0.115 (0.019)	0.130 (0.012)
	Min	0.031	0.089	0.049	0.083
	Max	0.314	0.160	0.187	0.161
S-WC (g cm^{-3})	$\bar{x}$ (SD)	0.119 (0.048)	0.113 (0.014)	0.109 (0.020)	0.133 (0.011)
	Min	0.057	0.079	0.041	0.103
	Max	0.443	0.162	0.188	0.161
A-MT (°C)	$\bar{x}$ (SD)	29.096 (3.123)	18.827 (1.727)	30.898 (3.251)	17.977 (1.357)
	Min	22.414	15.717	23.502	15.695
	Max	36.630	21.356	39.446	20.203
S-MT (°C)	$\bar{x}$ (SD)	29.663 (4.703)	18.185 (1.701)	30.669 (3.755)	18.219 (1.736)
	Min	20.257	14.737	23.282	15.145
	Max	45.092	21.895	43.735	20.863
A-ST (°C)	$\bar{x}$ (SD)	26.750 (2.567)	19.150 (1.426)	28.641 (2.658)	17.640 (1.109)
	Min	22.131	16.438	23.846	15.773
	Max	32.046	20.652	35.789	19.626
S-ST (°C)	$\bar{x}$ (SD)	27.074 (3.929)	18.419 (1.721)	27.951 (3.181)	17.650 (1.231)
	Min	18.185	14.110	22.704	15.775
	Max	41.444	21.151	41.443	20.209
A-MST_{ratio}	$\bar{x}$ (SD)	1.088 (0.056)	0.982 (0.031)	1.078 (0.048)	1.019 (0.028)
	Min	0.987	0.926	0.944	0.9664
	Max	1.223	1.051	1.191	1.153
S-MST_{ratio}	$\bar{x}$ (SD)	1.095 (0.057)	0.988 (0.035)	1.097 (0.038)	1.031 (0.041)
	Min	0.982	0.929	1.018	0.965
	Max	1.298	1.086	1.209	1.135

greater on the boulder field than on the rock platform (Tables 1, 2; Fig. 2b).

Aggregation and thermal stress

During the day, on the boulder field, MT did not differ significantly between aggregated and solitary snails (Table 3). Solitary individuals occupied cooler substratum than aggregated individuals (Table 3). Aggregation behaviour significantly reduced the temperature deviation between snails and surrounding substratum (i.e. MST_{ratio}, Table 3; Fig. 2a). During the day, on the rock platform, no significant difference in MT, ST and MST_{ratio} was observed between solitary and aggregated individuals (Table 3; Fig. 2a). At nighttime, on the

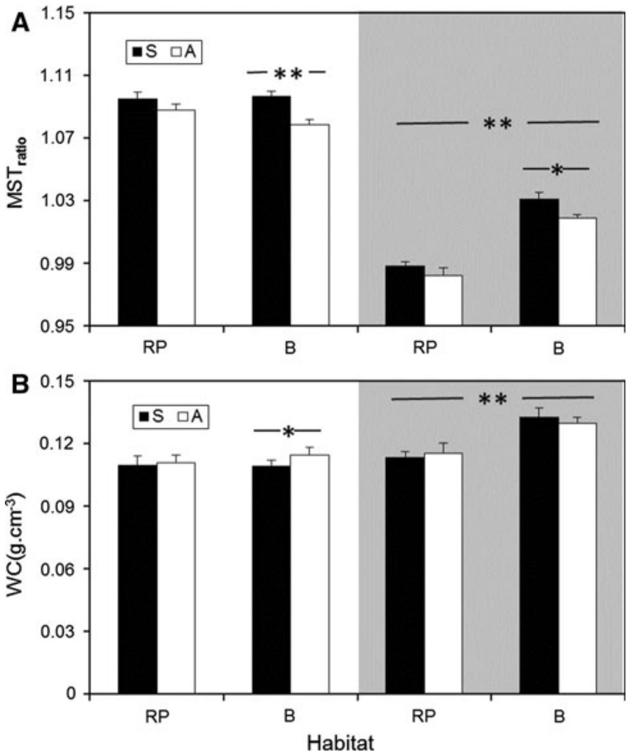

Fig. 2 Water content (WC) and mantle to substratum temperature ratio (MST_{ratio}) of solitary (S, *black bars*) and aggregated (A, *white bars*) *Nerita atramentosa* individuals in both habitats (RP, rock platform; B, boulders) at both times (day: *white* background, night: *grey* background). Values are means and *error bars* represent standard deviations. Significance of the differences in WC and MST_{ratio} between aggregated and solitary individuals in both habitats at both times, and between habitats at daytime and at nighttime are indicated by *asterisks*, $*P < 0.05$, $**P < 0.01$. Note that significant differences ($P < 0.01$) between day- and nighttime in MST_{ratio} in both habitats and in WC in the boulder field are not represented in this figure to improve clarity. RP-day solitary: $N = 108$ for WC, $N = 154$ for MST_{ratio}, RP-day aggregated: $N = 175$ for WC, $N = 224$ for MST_{ratio}, RP-night solitary: $N = 150$, RP-night aggregated: $N = 37$, boulders-day solitary: $N = 168$, boulders-day aggregated: $N = 212$, boulders-night solitary: $N = 80$, boulders-night aggregated: $N = 110$

boulder field, MT and ST were not significantly different between the two categories of organisms (Table 3). MST_{ratio} was, however, significantly greater among solitary than aggregated snails (Table 3; Fig. 2a). During the night, on the rock platform, aggregated individuals were significantly warmer and occupied significantly warmer substratum than solitary organisms (Table 3). MST_{ratio} did not differ between snail categories (Table 3; Fig. 2a).

Aggregation and desiccation stress

During the day, on the boulder field, solitary individuals had significantly lower water content than aggregated ones (Table 3; Fig. 2b). In contrast, daytime aggregation behaviour on the rock platform did not increase the water content of snails significantly (Table 3; Fig. 2b). At nighttime, no significant difference between aggregated and solitary organisms was observed in both habitats (Table 3; Fig. 2b).

Discussion

Habitat temperature and thermal stability control snail body temperature, water content and behaviour

As previously observed in others invertebrates (e.g. Bertness 1989), *N. atramentosa* body temperature was significantly and positively correlated to the local temperature (ST and/or AT_{UR}), but was not coupled to air temperature measured at the regional scale (AT_{BOM}). This reinforces the growing evidence that climate change models used to predict future biogeographic species ranges should integrate environmental variables such as ST (e.g. this study, Chapperon and Seuront 2011a, b) but also solar radiations (e.g. Marshall et al. 2010) and meteorological conditions measured at scales relevant to individual organisms rather than variables measured at larger spatial scales, e.g. regional air temperature (Helmuth et al. 2011). This is particularly relevant since both circatidal and intra-habitat variability in ST, hence in MT (e.g. up to 18 °C on the boulder field on a single day) was greater than the temperature variability (i.e. 1.5 °C) observed between habitats separated by ca. 250 m. These results are consistent with recent studies suggesting that micro-site thermal variations in both terrestrial (Scherrer and Körner 2010) and marine environments (Helmuth et al. 2006; Denny et al. 2011; Meager et al. 2011) largely exceed those observed between latitudes. We also emphasise that short-term variations in ST and in BT (Table 1) surpass seasonal variations; see also Seabra et al. (2011). Here, the maximal ST difference on the boulder field (20.5 °C, Table 1) was greater than the mean seasonal ST variations (i.e. 10.25 °C between autumn and summer) previously recorded on this habitat (Chapperon and Seuront 2011a).

In contrast, the water content of *N. atramentosa* was not correlated significantly to any measured environmental temperature nor to snail temperature in both habitats during both day- and night-times. Therefore, the water content and the small spatial scale variations in ST, hence in MT, likely stem from the local interaction between multiple environmental factors, e.g. degree of exposure to solar radiation, intensity of local wind (Helmuth 1998), substratum orientation (Seabra et al. 2011), and mass (Gedan et al. 2011), that create niche-specific micro-climatic conditions that vary over time. In the present study, individuals were exposed directly to the surrounding environmental conditions on the boulder field (e.g. solar irradiance, wind), hence these abiotic factors likely drove the heat fluxes in and out snail bodies (Helmuth et al. 2011). This may explain both the

Table 2 Mann–Whitney *U* test to investigate differences in WC, MT, ST, $\mathrm{MST}_{\mathrm{ratio}}$ in aggregated and solitary snails and in all individuals between day time (D) and night time (N) in both habitats (RP, rock platform; B, boulders), and between habitats (RP, B) at both times (D, N)

Individual status	RP vs. B	Days			Night		
		Z	*P*	Sig.	*Z*	*P*	Sig.
Aggregated	WC	−3.007	0.003	B > RP	−5.494	< 0.001	B > RP
	MT	−5.745	< 0.001	B > RP	−3.067	0.002	B < RP
	ST	−6.653	< 0.001	B > RP	−5.352	< 0.001	B < RP
	$\mathrm{MST}_{\mathrm{ratio}}$	−1.540	0.124	NS[a]	−5.633	< 0.001	B > RP
Solitary	WC	−0.373	0.709	NS	−9.436	< 0.001	B > RP
	MT	−2.713	0.007	B > RP	−0.067	0.947	NS
	ST	−3.198	0.001	B > RP	−3.589	< 0.001	B < RP
	$\mathrm{MST}_{\mathrm{ratio}}$	−0.651	0.515	NS	−7.053	< 0.001	B > RP
All individuals	WC	−1.678	0.093	NS	−11.672	< 0.001	B > RP
	MT	−5.974	< 0.001	B > RP	−1.561	0.119	NS
	ST	−7.050	< 0.001	B > RP	−6.003	< 0.001	B < RP
	$\mathrm{MST}_{\mathrm{ratio}}$	−0.635	0.525	NS	−9.322	< 0.001	B > RP
Individual status	D vs. N	Rock platform			Boulder field		
		Z	*P*	Sig.	*Z*	*P*	Sig.
Aggregated	WC	−1.093	0.274	NS	−7.310	< 0.001	D < N
	MT	−9.743	< 0.001	D > N	−14.718	< 0.001	D > N
	ST	−9.744	< 0.001	D > N	−14.719	< 0.001	D > N
	$\mathrm{MST}_{\mathrm{ratio}}$	−8.877	< 0.001	D > N	−10.363	< 0.001	D > N
Solitary	WC	−1.722	0.085	NS	−9.560	< 0.001	D < N
	MT	−15.026	< 0.001	D > N	−12.726	< 0.001	D > N
	ST	−14.928	< 0.001	D > N	−12.725	< 0.001	D > N
	$\mathrm{MST}_{\mathrm{ratio}}$	−13.568	< 0.001	D > N	−9.502	< 0.001	D > N
All individuals	WC	−1.377	0.168	NS	−11.972	< 0.001	D < N
	MT	−19.327	< 0.001	D > N	−19.477	< 0.001	D > N
	ST	−19.276	< 0.001	D > N	−19.477	< 0.001	D > N
	$\mathrm{MST}_{\mathrm{ratio}}$	−17.206	< 0.001	D > N	−14.222	< 0.001	D > N

[a] Non significant

Table 3 Mann–Whitney *U* test to investigate differences in WC, MT, ST, $\mathrm{MST}_{\mathrm{ratio}}$ between aggregated (A) and solitary individuals (S) at both times (D, day time; N night time) in both habitats (*B* boulders, *RP* rock platform)

S vs. A	Days			Night		
	Z	*P*	Sig.	*Z*	*P*	Sig.
Boulder field						
WC	−3.254	0.001	S < A	−1.833	0.067	NS[a]
MT	−1.188	0.235	NS	−1.478	0.139	NS
ST	−2.996	0.003	S < A	−0.176	0.860	NS
$\mathrm{MST}_{\mathrm{ratio}}$	−3.283	0.001	S > A	−2.025	0.043	S > A
Rock platform						
WC	−0.034	0.973	NS	−0.593	0.553	NS
MT	−0.154	0.877	NS	−1.978	0.048	S < A
ST	−0.242	0.808	NS	−2.516	0.012	S < A
$\mathrm{MST}_{\mathrm{ratio}}$	−0.933	0.351	NS	−0.743	0.458	NS

[a] Non significant

greater substratum and individual body temperatures (i.e. up to 1.5 °C, Table 1) observed during daytime on the boulder field and also the significant individual water loss from night- to daytime (Table 1). Further work is, however, required to quantify the contribution of others abiotic factors (e.g. wind speed, which was not measured in this study) in the determinism of *N. atramentosa* body temperature and water content. Nevertheless, the boulder field did not seem to be as thermally stressful as anticipated since $\mathrm{MST}_{\mathrm{ratio}}$ were not significantly different from those found under rocks. This is likely related to the large substratum mass of boulders (i.e. > 256 mm), which is expected to buffer heat and desiccation stresses more than crevices, flat and smaller rocks such as cobbles (64–256 mm; Bertness 1989; Chapperon and Seuront 2011a; Gedan et al. 2011). Besides, rock bottoms shelter snails from the dry and hot conditions that prevail on the rock platform during the day

(Chapperon and Seuront 2011a). This microhabitat protected from both the sun and the wind, is likely to maintain a micro-climate with constant temperature and humidity as suggested by the steady water content of individuals between day- and nighttime (Table 1).

Some ectotherms such as limpets (Sinclair et al. 2006) or snakes (Huey et al. 1989) thermoregulate by retreating under rocks. Likewise, *N. atramentosa* takes advantage of the less stressful environmental conditions found under rocks on rock platforms under summer conditions (Chapperon and Seuront 2011a). Besides, *N. atramentosa* also seem to behaviourally adjust its water content to the local microclimate of the habitat it occupies. The significantly greater water content observed at nighttime on the boulder field indeed suggests that individuals stored more water than on the rock platform where no dehydration stress seemed to occur during the day. Individuals might have anticipated the risk of dehydration stress that likely took place on boulders in summer by increasing their water storage in order to maintain water levels even after exposure to dehydrating conditions.

Aggregation behaviour buffers local environmental conditions

Aggregation behaviour significantly increased the water content and decreased the body temperature of *N. atramentosa* in comparison to the surrounding substratum, but only in one of the two habitats considered (i.e. the boulder field), and at both day- and nighttimes. This is in agreement with the spatial variability in the role of aggregation behaviour of the barnacle *Semibalanus balanoides* (Bertness 1989) and the littorinid snail *Nodilittorina peruviana* (Rojas et al. 2000) in buffering the effects of heat and desiccation conditions. Similarly, the inter-habitat variability in the buffering effect of *N. atramentosa* aggregation behaviour was likely governed by the thermal stability of habitats or local climatic conditions (Bertness 1989; Rojas et al. 2000). Although more data collected on different species, and in a range of habitats and climates are needed to generalise these results, they nevertheless suggest a great flexibility in the ability of *N. atramentosa* to behaviourally buffer and respond to local environmental cues by adopting an adequate strategy, such as the aggregation behaviour (this study) or microhabitat selection behaviour reported in previous work on *N. atramentosa* (Chapperon and Seuront 2011a).

N. atramentosa aggregations observed under rocks on the rock platform were induced neither by temperature nor by desiccation conditions. Aggregations of intertidal invertebrates have been shown to be advantageous in regards to a range of biological (e.g. reproduction, Cudney-Bueno and Prescott 2008; predation risk, Coleman et al. 2004) and environmental factors (e.g. hydrodynamism, Moran 1985). It is thus possible that *N. atramentosa* formed aggregations to reduce predation by the reef crab *Ozius truncatus* (Chilton and Bull 1984) and/or to breed, as observed in other invertebrates (Cudney-Bueno and Prescott 2008). Aggregations may also result from the following of conspecific mucous trails (e.g. Chelazzi et al. 1985; Stafford and Davies 2005; Chapperon and Seuront 2011b) and food, home or conspecific source odour trails (Chelazzi et al. 1985; Chapperon and Seuront 2009). Since individuals preferentially select rock bottoms in summer to thermoregulate (Chapperon and Seuront 2011a), snails may naturally aggregate as the number of shelters (i.e. rocks) is limited. Further investigations are, however, required to clearly determine the factors underlying *N. atramentosa* aggregation behaviour on rock platforms.

Conclusion

Although further temporal investigations from a range of environments and species would ensure the generality of the present results, this study contributes to the growing evidence that mobile invertebrates may be less vulnerable than previously thought to heat extremes, desiccation conditions and the increase in mean temperature predicted in the warming climate. Intertidal mobile, slow moving and sessile ectotherms whose body temperature is intrinsically related to the substratum temperature might locally survive warmer, drier and extreme heat conditions as a combined effect of (1) the heterogeneity in environment thermal properties identified at the centimetre scale (e.g. this study; Chapperon and Seuront 2011a, b; Denny et al. 2011) that creates potential thermal refuges under heat stress (e.g. rock bottoms in this study), (2) the thermal stability status of habitats (i.e. boulder field) that may naturally buffer heat and desiccation stresses (e.g. this study; Gedan et al. 2011), (3) the behavioural ability of mobile ectotherms to explore the environmental heterogeneity at the niche level and to select a thermally favourable microhabitat or substratum (Chapperon and Seuront 2011a, b; Miller and Denny 2011) and (4) the intraspecific flexibility in the behaviour of mobile ectotherms that display different strategies (e.g. aggregation, microhabitat selection) specific to local conditions in order to buffer heat and desiccation stresses (e.g. this study; Chapperon and Seuront 2011a, b).

This study particularly stresses the need to further investigate the potential effects of various behavioural strategies identified in different species (Garrity 1984; Munoz et al. 2005; Chapperon and Seuront 2011a, b; Miller and Denny 2011) on body temperatures and water contents as they could be essential for survival under more stressful environmental conditions. After identifying these thermoregulatory behaviours at species and habitat levels, some regulations to protect organisms from e.g. being disturbed and stressed (e.g. non removal or dislodgement of organisms) could ultimately be implemented within local conservation and management plans (e.g. installation of artificial thermal refuges) in order to decrease their vulnerability under new environmental conditions.

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