On the importance of behavioural adaptations in thermally challenged intertidal ectotherms: implications for climate change studies

Coraline Chapperon

BSc, MSc

Thesis submitted to the Faculty of Sciences and Engineering, School of Biological Sciences of Flinders University in total fulfilment of the requirements of the degree of Doctor of Philosophy of Flinders University

June 2012

Supervisor: Prof. Laurent Seuront
Co-supervisor: Assoc. Prof. Jim Mitchell

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To my mother (Marie), brother (Luc) and grand-mother (Emilienne),
To my son Bastian, the apple of my eye.

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SUMMARY

The recent integration of physiological responses of ectotherms in climate change models has provided a greater insight into the species thermal window of adaptation, hence future distribution ranges. However, there are still fundamental gaps in these climate change models. In the present thesis, intertidal ecosystems were used as outdoor laboratories and valuable models, which used invertebrates as bio-indicators to bridge the gaps between the thermal ecology of ectotherms and climate change models. Intertidal invertebrates already live at or near to the upper edge of their thermal tolerance window, hence have limited physiological abilities to adapt to further warming. Their behavioural adaptations to mean temperature increase and especially to extreme events are then likely to play a major role in the maintenance of individuals' fitness in the warming climate. Nevertheless, the behaviour is still absent from climate change models. In addition, the local environmental context, especially the spatial environmental thermal heterogeneity, is overlooked in climate change models. Yet, this appears critical since mobile ectotherms living in heterogeneous environments such as the intertidal might behaviourally take advantage of thermal mosaics when facing new environmental conditions by, for example, relocating into thermally favourable microhabitats. The large scale approach used in climate change studies also leads to erroneous measurements of ectotherm body temperatures that largely contribute to distribution patterns. Indeed, they usually consider air temperature measured at large spatial scale to be a good proxy for animal body temperatures. Nonetheless, organism thermal properties are determined by multiple non-climatic and biotic variables which interact at the niche level. In this context, the present work aimed to investigate (i) the primary factor(s) that determine body temperature, displacement and distribution patterns of mobile intertidal ectotherms at the individual scale, (ii) the space-time heterogeneity in environmental and invertebrate body temperatures at a range of scales by using thermal imaging, and (iii) the potential adaptive behavioural capacity of intertidal ectotherms to compensate for climate change. The main results show that substratum temperature could be used as a primary determinant for mobile intertidal ectotherms in climate change models, instead of air temperature. I also highlight a high substratum thermal heterogeneity at centimeter scale in different habitats, *i.e.* tropical mangroves and temperate rocky shores. This contributes to the growing evidence that small spatial scale variability in thermal environmental properties can surpass the thermal variability measured at large spatial scales. Besides this evidence for the importance of thermal variability, I have demonstrated that the gastropod species *Littoraria scabra*, in tropical mangroves, and *Nerita atramentosa*, on temperate rocky shores, were able to select thermally favourable microhabitats or substrata to behaviourally thermoregulate. I also emphasise the buffering role of aggregation behaviour under cold, hot and desiccation stresses. In conclusion, the present work shows the need to integrate small spatial scale heterogeneity found in environmental conditions and thermoregulatory behaviours that appeared to be species and habitat-specific into climate change models. This small spatial scale heterogeneity constitutes a fundamental prerequisite to make prediction about ectotherm distribution ranges in the changing climate.

DECLARATION

'I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text’.

Coraline Chapperon

June 2012

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Chapter I. General Introduction

Intertidal habitats constitute one of the harshest and most stressful habitats, and are typified by steep up shore and alongshore environmental gradients created by emersion and exposure to breaking waves (Thompson *et al.*, 2002). Physical conditions such as immersion time (Helmuth, 1998), wave exposure (Helmuth & Denny, 2003; O'Donnell & Denny, 2008) and microclimatic variations (salinity, solar radiations, oxygen concentration; Truchot & Duhamel-Jouve, 1980; McMahon, 2003; Pulgar *et al.*, 2003) are hence extreme and also characterized by high space-time heterogeneity (Helmuth & Denny, 2003). For example, changes in temperatures induced by tidal cycle can be greater than 30°C between low and high tide (Harley & Helmuth, 2003). Intertidal areas, thus, represent convenient outdoor laboratories and valuable models to assess the future consequences of global change. This is particularly relevant since coastal areas from the intertidal zone out to the continental shelf break constitute one of the most ecologically and socio-economically relevant systems (Harley *et al.*, 2006), providing over US$14 trillion worth of ecosystem goods, *i.e.* food and raw materials, and services, *e.g.* disturbance regulation and nutrient cycling, per year (Costanza *et al.*, 1997). Despite this, most research and conservation efforts have been directed towards vertebrates, especially mammals, and terrestrial environments, and have put aside research on invertebrates and marine habitats, except those with economic and/or public interests such as abalone and coral reefs. However, marine invertebrates represent 1 million of the 1.4 million described species, whereas there are only 40,000 vertebrates species (Ponder *et al.*, 2002). Nonetheless, intertidal invertebrates, such as molluscs, contribute substantially to the functioning, structure and productivity of intertidal habitats. They occupy key positions in the trophic web as grazers transfer the matter and energy from the primary producers to the higher consumers (McQuaid & Branch, 1985; Peduzzi & Herndl, 1991). They also control the patterns of distribution, dispersion of others species (Chapman, 2000), hence they determine local biodiversity patterns (Davidson *et al.*, 2004). Their potential extinction, reduction in number or loss of range in the warming climate could disrupt the ecological balance of the whole system and substantially affect economic and tourist activities. Recent studies on coastal marine systems have, however, demonstrated that global warming (Gilman *et al.*, 2008), has already caused mass-mortalities among rocky shore invertebrates (Harley, 2008), changes in trophic interactions (Hawkins *et al.*, 2008; Pincebourde *et al.*, 2009; Kordas *et al.*, 2011; Harley, 2011) and subsequently changes in species abundance, diversity (Harley, 2011) and geographic range (Sagarin *et al.*, 1999; Moore *et al.*, 2007) as well as alterations in community size structure and ecosystem functioning (Dossena *et al.*, 2012). It is hence critical to determine the extent to which intertidal ectotherms that already live at or near to the upper edge of their thermal limits (Somero, 2002) will adapt the future environmental conditions, so that we may anticipate the ecological effects of global warming on intertidal systems and predict future species' geographic ranges.

In the context of global warming, the conceptual approach of climate envelope models used to predict future species distribution and changes in biodiversity are based on the fundamental ecological niche of species (Hutchinson, 1957), which has traditionally been defined as the space or envelop delimited by environmental factors, *e.g.* climatic variables in which a species can survive and grow (Hutchinston, 1957). Hence, bioclimatic envelop models, also called correlative models, spatially map climatic variables, usually temperature, to predict the species geographical range under new climatic conditions using empirical data of the past species distribution in the climate space *i.e.* species realized niche (Pearson & Dawson, 2003; Moore *et al.*, 2007). This modelling approach can be successfully applied to predict species geographic range at large spatial scales (*i.e.* global to regional scale) at which climatic factors dominate (Pearson & Dawson, 2003). However, at smaller scale, the distribution patterns of intertidal molluscs are determined by the interaction of other abiotic and biotic factors such as landscape heterogeneity and trophic interactions including food distribution and predation (Chapman, 2000). Furthermore, due to their limited spatial resolution, climate envelope models consider that habitats and organisms exhibit the same thermal properties, *e.g.* ectotherm body temperatures, to that of water or air temperatures (Broitman *et al.*, 2009). Body temperatures of ectothermic individuals are assumed to be identical to that of the seawater while submerged (Helmuth & Hofmann, 2001). However, they are different from that of the microenvironment in which individuals live and interact during emersion due to the interaction of multiple climate variables such as solar radiation, humidity and wind speed (Helmuth & Hofmann, 2001; Fitzhenry *et al.*, 2004; Broitman *et al.*, 2009). Accordingly, predictions of the future distribution of a species in regards to the air or water temperature in which the species has previously been observed are inaccurate due to the decoupling between habitat climate variables, *e.g.* air temperature, and individual properties, *e.g.* body temperature.

From the organism perspective, physiologically-based mechanistic models such as heat budget models predict ectotherm body temperature, *e.g.* mussels (Helmuth, 1998; Finke *et al.*, 2009) or limpets (Denny & Harley, 2006) using climatic variables such as wind, air temperature, which operate at the individual scale associated to the heat transfer from and out of organisms (Denny & Harley, 2006). These models base their predictions on empirical physiological responses observed at the molecular, cellular or organism levels in different thermal conditions to define individual thermal tolerance and limits that are used to forecast the future biogeographic range limits of a species under different climatic scenario. Coupling those models with global climate change models may provide new insights into the species physiological tolerance to climate variables. They, however, still only draw rough boundaries of the expected species thermal tolerance limits (Helmuth, 2009). Therefore, despite the integration of the physiology in climate change models and the increase focus upon individuals, gaps still persist for accurately predicting future species distribution patterns and potential loss of biodiversity in the warming world. In particular, the space-time heterogeneity at a range of scales in (i) the physiological responses of organisms to environmental parameters and in (ii) the environmental conditions themselves has mostly been overlooked. For instance, recent studies have shown the existence of environmental thermal mosaics at the niche level in intertidal habitats (Jost & Helmuth, 2007) and also in terrestrial habitats, *e.g.* mountains, Scherrer & Körner, 2010. This local thermal variation (*e.g.* 20 cm over a few centimeters, Jost & Helmuth, 2007) may surpass that observed and larger scales (regional and biogeographic scales) and therefore likely to infringe intuitive patterns (Helmuth *et al.*, 2002). It, therefore, appears critical to reach a better understanding of the space-time variability in environmental conditions since it impacts physiological and behavioural responses of individual ectotherms, which ultimately affect their distribution patterns. Another example is that models often consider the mean effect of temperature and neglect the effect of extreme events such as heat waves and cold events, even though they are both predicted to increase in frequency and intensity in the warming climate (Planton *et al.*, 2008) and they have been shown to considerably increase population mortality rates (Harley, 2008; Firth *et al.*, 2011). This is critical for intertidal species, which have limited physiological abilities to adapt to more extreme thermal conditions than those already characterizing intertidal habitats. Even amongst intertidal ectotherms, species are not equal in the warming climate as they do not exhibit similar physiological (acclimatization) capacities to thermal stress due to geographic distances (Dutton & Hofmann, 2009), thermal past history (Meng *et al.*, 2009) and environmental stability (Sinclair *et al.*, 2006; Compton *et al*, 2009). For instance, tropical species appear to be more vulnerable to further climatic fluctuations and thermal extremes since they are specialists over narrower thermal windows than temperate species (Sinclair *et al.*, 2006; Compton *et al.*, 2007; Huey *et al.*, 2012). Since intertidal ectotherms are unlikely to adapt physiologically to future environmental conditions, in order to survive, at least over short periods of time, organisms will either have to thermoregulate locally by behavioural means flee over long distances to select a more thermally favourable habitat.

Intertidal invertebrates have developed a range of behaviours to adjust their body temperatures in their natural fluctuating environment. For example, to reduce the amount of absorbed solar radiation, some snails adapt their resting posture by adjusting the shell orientation towards the sun (Lang *et al.*, 1998; Munoz *et al.*, 2005; Miller & Denny, 2011). Furthermore, mobile individuals have the ability to seek less stressful microhabitats during high tides. They preferentially choose to settle in complex microhabitats characterised by the presence of fissures, crevices, macroalgae or to aggregate with conspecifics rather than resting on exposed platforms (Atkinson & Newbury, 1984; Chapman, 1995; Chapman & Underwood, 1996; Jones & Boulding, 1999; Sinclair *et al.*, 2006). Sessile or slow-moving organisms such as barnacles or limpets cannot select their microhabitat from one low tide to the next and have instead developed other behavioural and morphological adaptive strategies, *e.g.* mushrooming behaviour (Williams *et al.*, 2005) and shell colour (Miller & Denny, 2011). Despite the growing evidence that behavioural adjustments of body temperatures in terrestrial vertebrates (Downs *et al.*, 2008; Kearney *et al.*, 2009, Huey & Tewksbury, 2009) and intertidal invertebrates (Munoz *et al.*, 2005) are critical for the survival of ectotherms in the warming climate at least at local spatial scale (Huey & Tewksbury, 2009), they have so far been excluded from climate change models.

Aims of this thesis.

In this context, the main goals of this thesis were:

(i) to investigate the factors that determine the displacements, distribution and body temperature patterns of intertidal ectotherms, such as snails, at small spatial scales.;

(ii) to examine the variability in environmental temperatures at different spatial and temporal scales and to determine the consequences of this variability on ectotherm body temperatures and behaviour, and;

(iii) to investigate the behavioural adaptations of intertidal organisms to heat, desiccation and cold stress, and the potential for behaviour to compensate for climate change. This information may ultimately change the predictions of current climate change models about future ectotherm distribution patterns and provide a new perspective and guidance in terms of conservation management of intertidal habitats.

This thesis has been prepared in the style of a suite of manuscripts for submission to scientific journals. Since the same method, *i.e.* thermal imaging, has been used to measure ectotherm body temperature and surrounding substratum temperature, there is some redundancy in the Material and Methods sections of each chapter. The studies conducted during this thesis focused upon the intertidal gastropods *Nerita atramentosa* in South Australia and *Littoraria scabra* in Fiji Islands, which appear to be particularly vulnerable to global warming due to their dark pigmented shells, which increase the absorption of solar radiations, and their latitudinal positions. Specifically, (i) ectotherms inhabiting Southern Australian rocky shores have no land southward into which to expand or move their range as an adaptation to local warming (ii) ectotherms in tropical mangroves are predicted to be more vulnerable than temperate species because there would be no optimal temperature ranges to expand into and because the corresponding sea level rises will reduce available habitat. In Chapter II, I investigated the seasonal variability in the displacements and the low tide distribution of *N. atramentosa* at the individual level to identify what environmental and biotic factors determine motion behaviour. This appears critical since the adaptive response of mobile ectotherms to new environmental conditions in the warming climate is likely to be modified by climate-driven changes in a range of variables in their proximate environment, such as change in food abundance. In chapter III, I examined the spatial heterogeneity in the temperature of mangrove roots at centimeter scale and the potential thermoregulatory behaviours of *L. scabra* for microhabitat selection and aggregation behaviour. Similarly, in Chapter IV, I assessed the space-time variability for 2 seasons, 2 habitats and 2 shore levels in the shell temperature of *N. atramentosa* individuals and the surrounding rock substratum with regard to thermoregulatory behaviour through microhabitat selection and aggregation behaviours. In Chapter V and VI, I emphasised aggregation behaviour, which is typically considered to reduce desiccation and thermal stresses in intertidal habitats. Aggregation behaviour could, therefore, occupy a key role in the local survival of snails. For this reason, in Chapter V I investigated the potential buffering effect of aggregation on *N. atramentosa* body temperatures and water content in conditions of high thermal and desiccation stress in two different habitats during the day and the night. Finally, in Chapter VI, I examined the potential thermal benefit of aggregations and the effect of aggregation size and organism spatial position within aggregates on snail thermal benefits. This study was conducted in winter since cold events have been overlooked in climate change studies. Cold events can however cause invertebrate mortalities and hence contribute to the determinism of biogeographic range limits.

The following is a list of references of published and submitted peer-reviewed papers arising during my Doctor of Philosophy studies.

PEER-REVIEWED PUBLICATIONS

Chapperon C., Le Bris C. and Seuront L. (2013) Thermally-mediated body temperature, water content and aggregation behaviour in the intertidal gastropod *Nerita atramentosa, Ecological Research,* accepted, 21/01/2013.

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Chapter II. Temporal shifts in motion behaviour and habitat use in an intertidal gastropod

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ABSTRACT

Animal movements in heterogeneous environments shape most ecological processes from individuals to ecosystems. The identification of the processes underlying animal movements thus has critical implications in a wide range of field. Changes in the motion behaviour of free-ranging species have mainly been reported across different spatial scales but have been less investigated over time. Here the processes potentially triggering temporal changes in movement, microhabitat occupation and distribution patterns of the intertidal herbivorous gastropod Nerita atramentosa *(Reeve 1855) were examined on a South Australian rocky shore during 8 successive daytime low tides considered in four different months. The observed temporal shift in microhabitat occupation and aggregation behaviour was likely an adaptation to both abiotic and biotic stressors such as temperature and food distribution. In contrast, the temporal changes observed in* N. atramentosa *motion behaviour are consistent with optimal foraging strategies driven by the presumed variability in both microalgal density and distribution. Individuals seemed to switch from an extensive foraging strategy in February and May to an intensive strategy in August and October. Specifically,* N. atramentosa *seemed to adopt two different foraging strategies, a Lévy-like foraging strategy in May and a ballistic foraging strategy in February when the resources are expected to be respectively non-depleted and depleted. The Brownian foraging strategy theoretically expected under high food density conditions was however not observed. The potential role of the behavioural flexibility observed in* N. atramentosa *motion behaviour is discussed in relation to the persistence of this species under disrupted environmental conditions.*

Keywords: motion, foraging behaviour, Lévy flight, distribution, herbivorous snails, intertidal environment.

INTRODUCTION

Movements in heterogeneous environments structure many ecological processes ranging from the organism to the ecosystem levels (Bowler & Benton, 2005; Nathan *et al.*, 2008). In particular, understanding the movements of organisms provides insight into the mechanistic links between behaviour and physiology (Matthews & Rodewald, 2010), environment (Barahona & Navarrette, 2010) and biological interactions between trophic levels (Rochette & Dill, 2000). Therefore, the comprehension of the determinism of movements across a range of spatial and temporal scales may critically advance fields such as behavioural ecology (Owen-Smith *et al.,* 2010), population dynamics (Morales *et al.,* 2010), conservation biogeography and biodiversity (Franklin, 2010; Agudo *et al.*, 2011), and climate change (Franklin, 2010; Feder *et al.,* 2010). However, empirical data on individual displacements are still required to reach a complete understanding of what factors and processes lead to the observed dispersion and distribution patterns (Buchanan, 2008; Humphries *et al.;* 2010, Viswanathan, 2010). Ultimately, this information is fundamental to generate dynamic models of movements such as the recently developed space-state models that incorporate both the organism behaviour and environmental variables; see Patterson *et al.* (2008) for a review.

Animals primarily move to find food and mates while avoiding predators in order to maintain individual fitness and potentially ensure the persistence of populations in changing environments (Bowler & Benton, 2005). Organisms thus exhibit innate and acquired motion behavioural strategies to optimize the search for resources (Owen-Smith *et al.*, 2010; Chapperon & Seuront, 2011a), mates (Shane, 2001) and refuges from environmental (*e.g.* thermal stress; Dubois *et al.,* 2009, Chapperon & Seuront, 2011b, c) and biological constraints (*e.g.* predation; Orrock *et al.*, 2010). Specifically, the optimal foraging strategies adopted by a range of terrestrial and aquatic herbivores to maximize resource intake while minimizing energy expenditure (Pyke, 1984; Williams & Little, 2007) are intrinsically defined by (i) their ability to detect the resources and to move accordingly (Nathan *et al.*, 2008), and (ii) the spatial distribution of primary producers as well as both their quality and quantity (Owen-Smith *et al.*, 2010). Besides, others constraints such as the presence of predators and desiccation stress potentially generate trade-offs between the different fitness components of herbivores (Jones & Boulding, 1999; Schmitz *et al.*, 2008). Behavioural changes in the displacements and distribution of individual organisms may thus be required to adapt to new environmental conditions while ensuring basic needs. Movements in spatially structured habitats, especially in the intertidal, are determined by the interaction of multiple abiotic (*e.g.* habitat complexity; Coffin *et al.*, 2008), biotic (*e.g.* food; Seuront *et al.*, 2007) and intrinsic factors (*e.g.* reproductive status; Pardo & Johnson, 2006). The displacements of intertidal organisms are therefore highly variable in space and time as they result from the behavioural responses of organisms to the space-time heterogeneity of the surrounding environment (Chapman, 2000a; Patterson *et al.*, 2008). This specifically implies that intertidal invertebrates must have enough flexibility in their motion behaviour to adjust their movements and their distributions over different spatial (*i.e.* microhabitats to habitats) and temporal scales (*i.e.* minutes to seasons). Recent studies have focused upon the changes in the statistical properties of animal motion behaviour as a response to complex landscapes (Sims *et al.*, 2008) and food density (Humphries *et al.*, 2010) over a wide range of spatial scales. Temporal changes in the statistical properties of animal displacements are, however, still poorly investigated. In this context, this study aimed to (i) quantify the temporal variability in the displacements and the distribution of the gastropod *Nerita atramentosa* on a South Australian temperate rocky shore over four distinct study periods, *i.e.* May (autumn), August (winter) and October (spring) 2009, and in February 2010 (summer), (ii) identify what environmental and/or biotic factors potentially drove the observed patterns and (iii) examine the extent of snail motion behavioural flexibility. This work has been motivated by previous evidence that the motion behaviour of a range of animals is primarily constrained by the distribution and abundance of their resource (*e.g.* Sims *et al.,* 2008, Seuront *et al.*, 2007, Seuront & Vincent, 2008, Humphries *et al.,* 2010), and the assumption that microalgae in South Australian rocky shores distribute and abound similarly to others studied temperate rocky shores, where resources are more abundant at low shore levels than at high shore levels and during winter months than during summer months (Underwood, 1984; Thompson *et al.*, 2004; Jackson *et al.*, 2010). We consequently expected to observe (i) Brownian movements when the food is likely to be the most abundant (*i.e.* august), (ii) ballistic movements when the food is depleted (*i.e.* summer), (iii) Lévy-like movements when the food is anticipated to be scarce but non-depleted (*i.e.* May and October), and (iv) seaward movements especially when the food abundance is predicted to be low (*i.e.* summer).

MATERIALS AND METHODS

Field site and species studied

The displacements and the distribution patterns of 240 *Nerita atramentosa* individuals (Reeve, 1855) were monitored on a temperate intertidal rocky shore in Marino Rocks (South Australia, Australia) during morning and midday low tides (*i.e.* 9 am to 2 pm) at four different periods of the year in May (autumn), August (winter) and October (spring) 2009, and in February 2010 (summer). Seawater and air temperature data during the monitored periods are summarized in Table 1. The study site was located at the upper bound of the intertidal zone of a moderately exposed boulder field (35°2'38.04" S - 138°30'30.13" E) characterised by a variety of microhabitats such as pools and crevices. The rocks of the study site were consistently nearly bare all over the year although macroalgae such as *Ulva* sp. were occasionally observed. This site was chosen due to the presence of herbivorous gastropods such as *Bembicium* sp., *Nodilittorina* sp. and the predominance of the Neritidae *N. atramentosa*. The studied species is a superior competitor for microalgae on intertidal rocky shores (Underwood, 1984), active when immerged and show aggregative tendencies at low tide throughout the year (Underwood, 1976).

Snail collection, tagging and release

For each study period, 60 adult individuals were randomly collected during the low tide using a random number table. The size (*i.e.* shell length and height) of each individual was measured using a calliper and the mean individual size was calculated for each study period (Table 1). Individuals were tagged with glue-on shellfish tags (8×4 mm, Hallprint, Hallprint Pty Ltd, Hindmarsh, South

Australia). Tagging has previously been shown not to affect the motion behaviour of gastropods (*e.g.* Chapman, 2000b; Henry & Jarne, 2007; Seuront *et al.*, 2007). Each individual was subsequently randomly placed within a 25×25 cm area surrounding a release point situated on the top of the centre of a flat boulder (90×40×40 cm). Note that all the individuals used in the present work were collected from flat rocks on the studied boulder field to minimise the behavioural impact of the dislodgement and relocation on the release site. Marked snails were left undisturbed from the release day. The distribution and the displacements of each individual were recorded during the 8 subsequent daytime low tides. Whilst many littoral herbivorous molluscs can remain active during emersion when the rocks are damp (*e.g.* Little, 1989), all *N. atramentosa* individuals were immobile during the monitored low tides. Note that the relative uniformity of boulder size on the release site ensures that snail displacements were not constrained by topographic complexity.

Distribution of *Nerita atramentosa*: microhabitat occupation and status

Microhabitat distribution and individual status (*i.e.* aggregated vs. solitary) of *N. atramentosa* was assessed at each low tide. Microhabitats were classified into 4 categories: flat rock, crevice, rock bottom and pool (Table 2). An individual was considered aggregated when there was a direct shell contact with the shell of at least another conspecific (Chapperon & Seuront, 2011b).

Activity, dispersion and motion behaviour of *Nerita atramentosa*

Activity index

An individual was defined as active when a change in position between two successive daytime low tides was detected. An activity index was calculated as:

$$N_i / N \times 100 \tag{1}$$

where N_i is the total number of mobile individuals between two successive daytime low tides and N is the total number of recorded individuals. Some individuals were, however, found at the exact same location between successive daytime low tides. Since individuals were monitored at daytime low tide, it was impossible to determine whether the lack of movement corresponded to a lack of activity during the high tide or an indication of homing behaviour. However, we never observed any sign of homing behaviour in *N. atramentosa* during our field observations, which to our knowledge has also not been reported in the literature. As a consequence, an individual located at the exact same location over consecutive daytime low tides was considered as a lack of activity throughout this work.

Foraging behaviour

N. atramentosa individual displacements were monitored by measuring the orientation angle θ and the distance displaced *d*, *i.e.* the angle and distance displaced by each individual from the release point to the monitored snail position at each daytime low tide. The orientation angle θ was estimated from the release point using the magnetic North as a reference. The distance displaced *d* was measured by a plastic tape measure laid down the substratum topography from the release point to the snail's position. The x and y coordinates were estimated from the distance displaced d and orientation angle θ as:

$$\begin{cases} x = d \times \cos\theta \\ y = d \times \sin\theta \end{cases} \tag{2}$$

The daily orientation angle θ_i was defined as the angle taken by an individual between two successive daytime low tides and was calculated as:

$$\theta_i = 180 - [(180/\pi)] \times \theta_e \tag{3}$$

with θ_e defined as:

$$\theta_e = arc\cos\ [(p_1p_2)^2 + (p_2p_3)^2 - (p_1p_3)^2]\,/[2(p_1p_2)(p_2p_3)] \tag{4}$$

where (p_1p_2), (p_1p_3) and (p_2p_3) are respectively the distances between the positions p_1 and p_2, p_1 and p_3, and p_2 and p_3 (Jerde & Visscher, 2005). Likewise, the daily distance displaced d_i was defined as the distance displaced by an individual between two successive daytime low tides and was calculated as:

$$d_i = \sqrt{\left(x_{t+1} - x_t\right)^2 + \left(y_{t+1} - y_t\right)^2} \tag{5}$$

Finally, as intermittent motion behaviour has recently been identified in another species of intertidal snail, *Littorina littorea* (Seuront *et al.*, 2007), the potential intermittency arising in the displacements of *N. atramentosa* (*i.e.* a few very large displacements over a wide range of small displacements) was investigated by comparing the probability density function $P(d_i)$ of the daily distance displaced d_i to a power-law distribution of the form:

$$P(d_i) = kd_i^{-\mu} \tag{6}$$

where μ is the slope of the log-log plot of $P(d_i)$ *versus* d_i, and k is a constant. When $1 < \mu \leq 3$, the displacements comprise many small steps connected by longer relocations that characterise Lévy processes; in the special case $\mu = 2$, the movements correspond to a Lévy flight that has been identified in the movement behaviour of a range of organisms ranging from invertebrates to large fish, birds and mammals (*e.g.* Mårell *et al.*, 2002; Bartumeus *et al.*, 2003; Seuront *et al.*, 2007; Sims *et al.*, 2008; Humphries *et al.*, 2010), and considered to be an optimal foraging strategy in places where preys are scarce and randomly distributed (Viswanathan *et al.*, 1999). When $\mu = 1$, the displacements are ballistic (*i.e.* nearly linear displacements), and values $\mu \leq 1$ correspond to probability distribution that cannot be normalised. In contrast, when $\mu \geq 3$, the distribution of daily distance displaced is Gaussian and the movement is equivalent to a Brownian motion (*i.e.* Normally distributed successive displacements occurring in random directions); see Seuront (2010) and Viswanathan *et al.* (2011) for more details. More generally, the smaller the exponent μ , the more intermittent is the distribution of the distances d_i . Because an objective criterion is needed to decide upon an appropriate range of values of the daily distances d_i to include in the regression analysis, we used the values of d_i which satisfied two statistically sound criteria. First, a regression window of a varying width that ranges from a minimum of 5 data points to the entire data set was considered. The smallest window was slid along the entire data set at the smallest available increments, with the whole procedure iterated (n - 4) times, where n is the total number of available data points. Within each window and for each width, the coefficient of determination (r^2) and the sum of the squared residuals for the regression were estimated. Note that parametric regression analysis was used throughout this work instead of non-parametric regression analysis because the residuals were consistently normally distributed (Zar, 2010). The values of d_i (Eq. 6), which maximized the coefficient of determination and minimized the total sum of the squared residuals, a procedure referred to as the R^2-*SSR* criterion (Seuront *et al.*, 2004), were subsequently used to define the scaling range and to estimate the exponent μ.

Dispersion

The ability of *N. atramentosa* individuals to disperse from a release point was investigated using the net travel angle and the net distance travelled, *i.e.* orientation angle and distance displaced between the release point and the last monitored low tide position for each snail.

Statistical analyses

As some individuals were not retrieved between two consecutive daytime low tide, daily distances and daily orientation angles were monitored for a minimum of 3 successive days since a minimum of three coordinates are required to calculate them. Note that this has been done to avoid any bias stemming from the use of observations conducted at different scales; see *e.g.* Seuront (2010). Since the data were not normally distributed (Kolmogorov-Smirnov Test, $p < 0.05$), the Kruskal-Wallis test (KW test hereafter) was run to analyse the temporal variations in individual distribution (aggregation and microhabitat occupation) and displacements (net distance displaced, daily distance displaced d_i). A multiple comparison procedure based on the Tukey test was subsequently used to identify distinct groups of measurements (Zar, 2010). In addition, the non-parametric

Watson's U^2 Test was used to test for temporal differences in the net orientation angles and the daily orientation angles θ_i.

While a range of goodness of fit procedures exists in the literature to evaluate which distribution best fits experimental data (*e.g.* Edwards *et al.,* 2007; Sims *et al.,* 2008; Humphries *et al.,* 2010), we followed Turchin's procedure (Turchin, 1998) to assess the quantitative nature of *N. atramentosa* daily displacement. Specifically, the distributions of daily orientation angles θ_i and net orientation angles were compared to a von Mises distribution using the Watson's U^2 Test, to a Normal distribution using the Kolmogorov-Smirnov Test (Zar, 2010) and to a Uniform distribution by using both tests.

The distributions of daily distances displaced d_i were tested for uniformity with the Kolmogorov-Smirnov test. The autocorrelation functions (ACF) and the Box-Ljung statistic were used to test for autocorrelation in the successive displaced distances for all lags up to 7 moves (Turchin, 1998). All statistical analyses were run in PASW Statistics 18 (SPSS Inc., 2009, IL USA) and ORIANA version 3 (RockWare Inc., 2010, CO, USA).

RESULTS

Temporal variability in *N. atramentosa* distribution pattern

Aggregation behaviour (Table 3, Figure 1A).

The proportions of aggregation were significantly different between the study periods. In particular, the proportions of aggregation in May and August were significantly lower than in October and February. No significant difference was found between August and May, neither between October and February.

Individuals aggregated more frequently within crevices in May (69%) and August (67%) and in pools in October and February (63% of aggregates).

Microhabitat occupation (Table 3, Figure 1B).

No significant difference in the proportions of individuals found under rocks was detectable between the four study periods. In contrast, significant temporal differences were found in the proportions of individuals occupying crevices, pools and flat rocks. More specifically, the proportions of individuals occupying crevices were significantly higher in May than in the other periods. Similarly, higher percentages of organisms were distributed in crevices in August than in October and February. In contrast, the percentages of individuals in pools were significantly greater in February and October than in the two others periods. Likewise, individuals were significantly more frequently observed in pools in August than in May. No significant difference in the percentages of occupation of crevices and pools was found between October and February. Finally, the proportions of individuals resting on flat rocks were significantly lower in February and October, than in August and May. No significant difference in the percentages of individuals on flat rocks was found between May and August and, October and February.

Temporal variability in *N. atramentosa* dispersion and foraging behaviour

Activity index.

In total, 99%, 91% and 45% of individuals were respectively active in May and August, February, and October. The proportions of missing data due to unobserved individuals were higher in the warm months (56% in February, and 41% October) than in the cooler months (32% in August, and 18% in May).

Dispersion (Figure 2).

Net orientation angles θ were 267.56º ± 3.40° ($\bar{x} \pm \mathrm{SE}$), 231.64° ± 13.44°, 216.75° ± 21.05° and 208.04° ± 16.89° in May, February, August and October, respectively. They were significantly higher in May than in the three others study periods ($U^2_{\text{May-Feb}} = 0.248$, $p < 0.05$; $U^2_{\text{May-Aug}} = 0.817$, $p < 0.001$; $U^2_{\text{May-Oct}} = 0.849$, $p < 0.001$). No significant difference was, however, obtained between February, August and October ($U^2_{\text{Feb-Aug}} = 0.052$, $p > 0.05$; $U^2_{\text{Feb-Oct}} = 0.128$, $p > 0.1$; $U^2_{\text{Aug-Oct}} = 0.142$, $p > 0.05$). Net orientation angles distributions were not significantly uniformly distributed ($U^2_{\text{May}} = 2.414$, $p < 0.005$; $U^2_{\text{Aug}} = 0.271$, $p < 0.01$; $U^2_{\text{Oct}} = 0.489$, $p < 0.005$; $U^2_{\text{Feb}} = 0.396$, $p < 0.005$).

The net distance displaced (*d*) by *N. atramentosa* individuals was greater in May (621.50 ± 45.85 cm, $\bar{x} \pm \mathrm{SE}$), than in February (474 ± 51.37 cm), October (263.41 ± 12.74 cm) and August (201. 58 ± 21.58 cm). Significant differences in the net distance displaced were found ($p < 0.001$, Table 3). In particular, the net distances displaced were significantly greater in February and May than in August and October ($Q_{\text{Feb-Aug}} = 3.946 > Q_{0.05,4}$, $Q_{0.05,4} = 2.639$; $Q_{\text{Feb-Oct}} = 3.044 > Q_{0.05,4}$; $Q_{\text{May-Aug}} = 6.446 > Q_{0.05,4}$; $Q_{\text{May-Oct}} = 5.580 > Q_{0.05,4}$). No significant difference in the net distances displaced was observed between February and May ($Q = 0.628 < Q_{0.05,4}$), and August and October ($Q = 1.494 < Q_{0.05,4}$).

Foraging behaviour.

The daily orientation angles θ_i (Table 1) taken by individuals between two successive daytime low tides did not significantly differ between study periods ($U^2_{May-Aug}$= 0.057, p > 0.5; $U^2_{May-Oct}$ = 0.135, 0.2 > p > 0.1; $U^2_{May-Feb}$ = 0.161, 0.1 > p > 0.05; $U^2_{Aug-Oct}$ = 0.147, 0.2 > p > 0.1; $U^2_{Aug-Feb}$= 0.14, 0.2 > p > 0.1; $U^2_{Oct-Feb}$= 0.175, 0.1 > p > 0.05). More specifically, whatever the study period, the distributions of daily orientation angles θ_i did not fit a von Mises distribution (U^2_{May} = 0.619, p < 0.005; U^2_{Aug} = 0.277, p < 0.005; U^2_{Oct} = 0.095, p < 0.05; U^2_{Feb} = 0.317, p < 0.005). Distributions were best fitted by a Uniform distribution in May (Z = 0.785, p = 0.569), August (Z = 0.796, p = 0.550) and October (Z = 1.006, p = 0.264) and by a Normal distribution in February (Z = 1.328, p = 0.059).

Significant differences in the daily distances displaced d_i between successive daytime low tides were observed between the different study periods (Table 3). Specifically, organisms moved over greater distances in February than at the three others periods ($Q_{Feb-May}$ = 3.422 > $Q_{0.05,4}$; $Q_{Feb-Aug}$ = 4.525 > $Q_{0.05,4}$; $Q_{Feb-Oct}$ = 3.550 > $Q_{0.05,4}$). No significant differences in d_i were found between the three others periods ($Q_{May-Aug}$ = 1.735 < $Q_{0.05,4}$; $Q_{May-Oct}$ = 1.136 < $Q_{0.05,4}$; $Q_{Oct-Aug}$= 0.193 < $Q_{0.05,4}$).

The daily distances d_i were not normally (Z_{May} = 3.435, p < 0.001; Z_{Aug}= 1.868, p = 0.002; Z_{Oct} = 1.856, p = 0.002; Z_{Feb} = 1.599, p = 0.012) neither uniformly distributed (Z_{May} = 10.421, p < 0.001; Z_{Aug}= 5.619, p < 0.001; Z_{Oct} = 2.867, p < 0.001; Z_{Feb} = 3.680, p < 0.001). Instead, they were positively skewed for all study periods, leptokurtic in May and August and platykurtic in October and February (Table 1). The log-log linear regressions of the frequency distributions of daily distance displaced were highly significant in May (R^2 = 0.858, df = 8, t = -6.964, $p < 0.001$), August (R^2 = 0.956, df = 5, t = -10.403, $p < 0.001$) and February (R^2 = 0.617, df = 7, t = -3.359, p = 0.012) but not in October (R^2 = 0.439, df = 3, t = -1.533, p = 0.223). More specifically, the distribution of daily distances displaced was close to a Lévy distribution in May ($\mu = 1.90$) which contrasted with the power-law behaviours observed during the others study periods, with $\mu = 1.35$ in August and $\mu = 1.10$ in February. The exponent m observed in May ($m = 1.90$) cannot be statistically distinguished from the theoretical exponent expected for a Lévy flight (*i.e.* $m = 2.00$, $p > 0.05$; modified *t*-test, Zar 2010), while they were significantly lower in August and February ($p < 0.05$). No autocorrelation in distances displaced between successive daytime low tides was found in all periods ($p > 0.05$), except in one individual in May, October and February, and two individuals in August ($p < 0.05$).

DISCUSSION

N. atramentosa distribution patterns as an adaptation to exogenous and endogenous stressors

The clear temporal shift in *N. atramentosa* microhabitat occupation (*i.e.* a gradual increase in pool occupation and decrease in both crevice and flat rock occupation from May to February; Figure 1b) likely results from the behavioural selection of a favourable microhabitat related to the three major environmental stressors found in the intertidal, that are temperature, desiccation and hydrodynamism. In October and February, *N. atramentosa* preferentially selected and aggregated within pools (63% of total aggregation; Figure 1) that reduce both thermal and desiccation stresses (Coffin *et al.,* 2008) more than the underneath of rocks or crevices (Bates & Hicks, 2005). In contrast, in May and August, aggregation within crevices (and pools in August; Figure 1) likely reduced dislodgement risk caused by breaking waves (Miller *et al.*, 2007).

The temporal variability in microhabitat use and aggregation may also be explained by biological factors such as predation, reproduction and feeding. In particular, snails likely aggregated in February as a response to predation risk (as indicated by the consistent observations of crushed and empty shells; Chapperon and Seuront, pers. obs.) also observed in others invertebrates (Coleman, 2008). Breeding aggregation prior to *N. atramentosa* spawning season peak (September to April; Przeslawski, 2008) could also explain the 55% aggregation rate in October (Figure 1a).

The trail and/or odour following behaviour observed in *N. atramentosa* (Chapperon and Seuront, pers. obs.) may also contribute to the formation of aggregates (Stafford *et al.*, 2007; Chapperon & Seuront, 2009, 2011b). Finally, *N. atramentosa* distribution could be primarily determined by the abundance and the small-scale patchiness of microphytobenthos (Seuront & Spilmont, 2002; Murphy *et al.*, 2008). More specifically, individuals may remain aggregated whilst the tide is retreating in the microhabitat that abounds the most with microalgae (*i.e.* crevices in August and May, and rockpools in October and February), and in which grazing activities are undertaken during immersion (Berger & Weaver, 2004). Although no movement was observed in *N. atramentosa* during the monitored daytime low tides, the observed individual distribution could also result from the potential trail following and/or grazing movements of snails on wet rocks during emersion (due to rainfall or awash conditions; Little, 1989).

N. atramentosa dispersion as an adaptation to environmental stress and food availability

N. atramentosa displacements in August and October (Figure 2C-F) suggest maintenance of individuals at their shore level (Williams, 1995). Individuals remained in the vicinity up and mainly down the release point (Figure 2C-F). This limited dispersion indicates that snail activities were restricted to a small area, *i.e.* within a 3 m radius around the release point (Figure 2C-F). Snails might have intensively fed upon microalgae previously identified to be particularly abundant during this period of the year (*i.e.* winter) on others temperate rocky shores in New South Wales and United Kingdom (Underwood, 1984; Thompson *et al.,* 2004; Jackson *et al.,* 2010). This low dispersion is also consistent with the lower seawater temperature (Table 1; Seuffert *et al.,* 2010) and the increase in wave action (Pardo & Johnson, 2006) observed over the study site in August and October (Chapperon & Seuront, pers. obs.) that may have constrained organisms to adhere to the substratum in order to reduce dislodgement risk (Barahona & Navarrete, 2010). Further investigations are, however, required to assess the eventual relationship between wave action force (non-measured in the present study) and *N. atramentosa* movements.

In February and May, *N. atramentosa* moved farther seaward (*i.e.* within a 6 to 9 m radius from the release point; Figure 2A-B, G-H), as observed in others Neritids (Garrity & Levings, 1981). Seaward displacements (Figure 2A-B, G-H) were possibly driven by more suitable feeding and/or environmental conditions prevailing at lower shore levels (Williams, 1994; Gibson, 2003). Particularly, *N. atramentosa* likely foraged extensively to increase the likelihood of encountering microphytobenthos resources that have been demonstrated to be scarce during this period of the year especially at the high shore level on others temperate rocky shores in New South Wales and United Kingdom (Underwood, 1984; Thompson *et al.,* 2004; Jackson *et al.,* 2010). Finally, the increase in seawater temperature (Table 1) may have increased *N. atramentosa* locomotor performance (*e.g.* Barahona & Navarrete, 2010), hence increased their dispersion abilities. In order to validate the hypothesis that snail movements are directed by the space-time variability in microalgal resources, further experiments are however required to investigate the abundance, distribution and availability of microphytobenthos on South Australian rocky shores, that were not measured in the present study. Interpretations of the present results were solely based on the assumption that the space-time variability in the abundance, distribution and availability of South Australian microalgal resources is comparable to that observed on others temperate rocky shores (*i.e.* NSW and UK; Underwood, 1984; Thompson *et al.,* 2004; Jackson *et al.,* 2010).

Intermittency in foraging behaviour: an adaptive response to resource distribution

The foraging behaviour of *N. atramentosa,* further assessed through the probability density function of their successive daily displacements d_i, was found to exhibit intermittent properties, *i.e.* their probability density functions $P(d_i)$ follow a power-law behaviour $P(d_i) = kd_i^{-\mu}$ (see Eq. (6)) in May, August and February. This indicates that the successive displacements of *N. atramentosa* belong to a family of distributions defined according to the value of the exponent μ. This is consistent with an adaptive response of *N. atramentosa* to the Lévy-like patterns previously identified in the spatial distribution of microphytobentos biomass over a range of intertidal environments (Seuront & Spilmont, 2002; Seuront, 2010). A similar response to resource distribution has also been identified in the foraging behaviour of top predators (Sims *et al.,* 2008). In addition, the increase in the exponent μ. observed from $\mu = 1.10$ in February, $\mu = 1.35$ in August, and $m = 1.90$ in May, may be indicative of a concomitant increase in foraging activity as seen from the foraging behaviour of *Littorina littorea* (Seuront *et al.*, 2007).

More specifically, the exponent μ was not significantly distinguished (modified *t*-test, p > 0.05; Zar, 2010) from the value $\mu = 2$ in May, as expected for a Lévy flight. Under the Lévy flights foraging hypothesis, Lévy flights are considered as an optimal foraging strategy where prey is scarce and randomly distributed, while Brownian motion ($\mu = 3$) is expected in environments where prey is abundant (Viswanathan *et al.,* 1999). This is consistent with the low density but non-depleted resource expected in our sampling site in May if microalgal resource abundance follows that observed in others temperate rocky shores of NSW and UK (Underwood, 1984; Thompson *et al.*, 2004, Jackson *et al.*, 2010), and with the Lévy flights identified in the foraging behaviour of *Littorina littorea* on a low food density rocky shore during European winter (Seuront *et al.,* 2007). This contrasts, however, with the nearly ballistic movements (*i.e.* exponent μ close to unity) observed in February and previously found in *N. atramentosa* in the absence of food, *i.e.* depleted resource (Chapperon & Seuront, 2011a). This suggests that under similar conditions of low food density, *N. atramentosa* may behaviourally optimise its foraging behaviour depending on both the spatial patterns and the level of food depletion, *i.e.* depleted vs. non-depleted. The optimal Brownian foraging strategy ($\mu = 3$)

anticipated under conditions of abundant resources (Humphries *et al.,* 2010), as expected in winter and spring months (Underwood, 1984; Thompson *et al.,* 2004; Jackson *et al.,* 2010), was not observed. Instead, organisms were confined to the surroundings of the release point, and exhibited Lévy-like properties in August but not in October. This suggests that the intermittent properties observed in August and October may not be solely related to food foraging, but also impacted by other environmental processes such as hydrodynamism or predation (Bartumeus, 2009).

Technical limitations in movement studies: a challenge for intertidal ecologists

These results were obtained from a mark-release-capture method that provides discontinuous spatial observations of individuals, *i.e.* a "capture history" (Patterson *et al.,* 2008). Snails were thus implicitly, and over-simplistically, assumed to displace linearly during immersion between two successive daytime low tides. *N. atramentosa* individuals, however, have previously exhibited convoluted trajectories and elevated individual speed up to 10.7 cm min^{-1} when observed at high temporal resolution (*i.e.* 15 s; Chapperon & Seuront, 2011a). Mean *N. atramentosa* distance travelled during each study period was therefore likely underestimated. In addition, some individuals were not observed over either short periods (*e.g.* one day) or until the end of the monitoring. This absence of information also constitutes a potential bias in the data set since it is not clear whether those individuals were (i) alive but out of sight hidden in deep depressions, (ii) far beyond the prospection area, (iii) dead due to predation, thermal or desiccation stresses or (iv) dislodged and exported from the studied area by breaking waves. This highlights the difficulties faced by intertidal ecologists in studying movement and distribution patterns in the intertidal that are most often based solely on the interpretations of indirect observations. An increase in the awareness of intertidal ecologists for new technologies, multi-expertise and the development of new biotelemetric devices adapted to small size organisms (Cooke *et al.,* 2004) are hence essential to achieve a more thorough understanding of the patterns of distribution, dispersion and movement in order to implement species conservation and management plans in the changing world.

CONCLUSION

This study highlights that *N. atramentosa* individuals have enough motion behavioural flexibility to adapt (i) their microhabitat occupation and aggregation patterns to the various environmental and biological conditions encountered in different periods of the year, and (ii) their foraging strategy to the predicted stochastic patterns of their landscape (*e.g.* Lévy-like food distribution) based on the hypothesis that South Australian microalgal resources have similar space-time variability in their distribution, abundance and availability that others temperate rocky shores (NSW, UK; Underwood, 1984; Thompson *et al.,* 2004; Jackson *et al.,* 2010). Under this hypothesis, our results also support the fact that the Lévy flights previously observed in *Littorina littorea* (Seuront *et al.,* 2007) might occur among a range of intertidal grazers, but also suggests that Lévy behaviour may not be the universal rule in heterogeneous and fluctuating environments (Humphries *et al.,* 2010). Further investigations are also required to identify the factors triggering the observed switch between different foraging strategies. Although snails are able to detect food sources by chemoreception and to adapt their movements accordingly (Croll, 1983), the presumed switch observed between different searching strategies may also (i) be reminiscent of innate properties in *N. atramentosa* motion behaviour (see also Chapperon & Seuront, 2011a), (ii) resume from an internal clock (*e.g.* endogenous rhythm; Williams & Little, 2007; Gray & Williams, 2010), or (iii) be activated by others environmental factors specific to each season (*e.g.* water temperature). Finally, the observed behavioural flexibility in *N. atramentosa* might constitute a strong evolutionary advantage in stressful and fluctuating environments such as the intertidal which are likely to be heavily impacted by the fast changing world climate.

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Table 1.

Snail body size, meteorological data and metrics used to quantify the motion behaviour of *Nerita atramentosa* in four different periods in 2009-2010. Values are means. Circular statistics run in ORIANA version 3 (RockWare Inc., 2010, CO USA) were used to calculate mean angles and standard deviation of the mean (Zar 2010). Minimum and maximum values are also provided for meteorological data (AT: air temperature, WT: sea water temperature). d_i and θ_i are the daily distances displaced and the orientation angles taken between successive daytime low tides. The skewness (g_1) and the kurtosis (g_2) of the distributions of daily distances displaced d_i are also indicated. All d_i and θ_i values were pooled at each study period. Meteorological data source: Bureau of Meteorology of Australia (Port Stanvac).

		May 2009	August 2009	October 2009	February 2010
Length (cm)	Mean	21	21.61	21.13	17.92
Height (cm)	Mean	13.95	14.66	14.57	14.49
AT (°C)	Mean	11.53	14.59	17.72	21.79
	N	48	240	240	216
	Min	4.9	10.9	11	15.5
	Max	18.4	20.7	29.4	31.2
WT (°C)	Mean	18.73	14.55	16.06	23.01
	N	216	240	240	216
	Min	18.3	14.2	15.3	22.6
	Max	19.6	14.9	18.1	23.6
d_i (cm/day)	Mean	162.03	114.21	131.56	172.46
	N	360	204	100	166
	Min	1	3	1	2
	Max	1055.455	415.21	376.016	490
	$g1$	1.980	1.078	0.277	0.588
	$g2$	5.125	0.714	-1.320	-0.396
θ_i (°)	Mean	91.58	95.28	87.88	109.71
	N	271	94	43	90
	Min	0.55	4.39	1.71×10^{-6}	1.21×10^{-6}
	Max	180	180	180	180

Table 2.

Definitions and roles of the four microhabitats considered in this study.

Microhabitat	Definition	Role
Flat rock	Smooth surface.	Direct exposure to local environmental conditions (*e.g.* solar radiations, breaking waves) and predation.
Crevice	Dry depression deep and wide enough to fit at least one individual from small cracks, pits and fissures to wider depressions.	Shelter from local environmental conditions and predation.
Rock bottom	Bottom of a rock *i.e.* substrate underneath a rock to which individuals attached and underside of a rock from which individuals hung.	Shelter from local environmental conditions and predation.
Pool	Deep depression (a few cm up to 70-80 cm) filled with water during emersion.	Reduction of desiccation and thermal stresses. Potential activation of feeding activities.

Table 3.

Kruskall-Wallis test and subsequent multiple comparisons (post-hoc based on the Tukey test) to investigate the temporal variation (M = May 2009, A = August 2009, O = October 2009, F = February 2010) in the parameters used to quantify *Nerita atramentosa* distribution and movement patterns between the four microhabitats. Results of the tests are indicated in the last column (NS means non-significant).

Parameters		df	χ^2	p	Post-hoc
Distribution patterns	*Aggregation*	3	26.745	<0.001	M=A<O=F
	Under rock occupation	3	0.674	0.879	NS
	Crevice occupation	3	92.108	<0.001	M>A>O=F
	Pool occupation	3	107.568	<0.001	M<A<F=O
	Flat rock occupation	3	83.339	<0.001	M=A>O=F
Movement patterns	*Net distance displaced*	3	54.725	<0.001	A=O<M=F
	Daily distance displaced d_i	3	23.306	<0.001	M=A=O<F

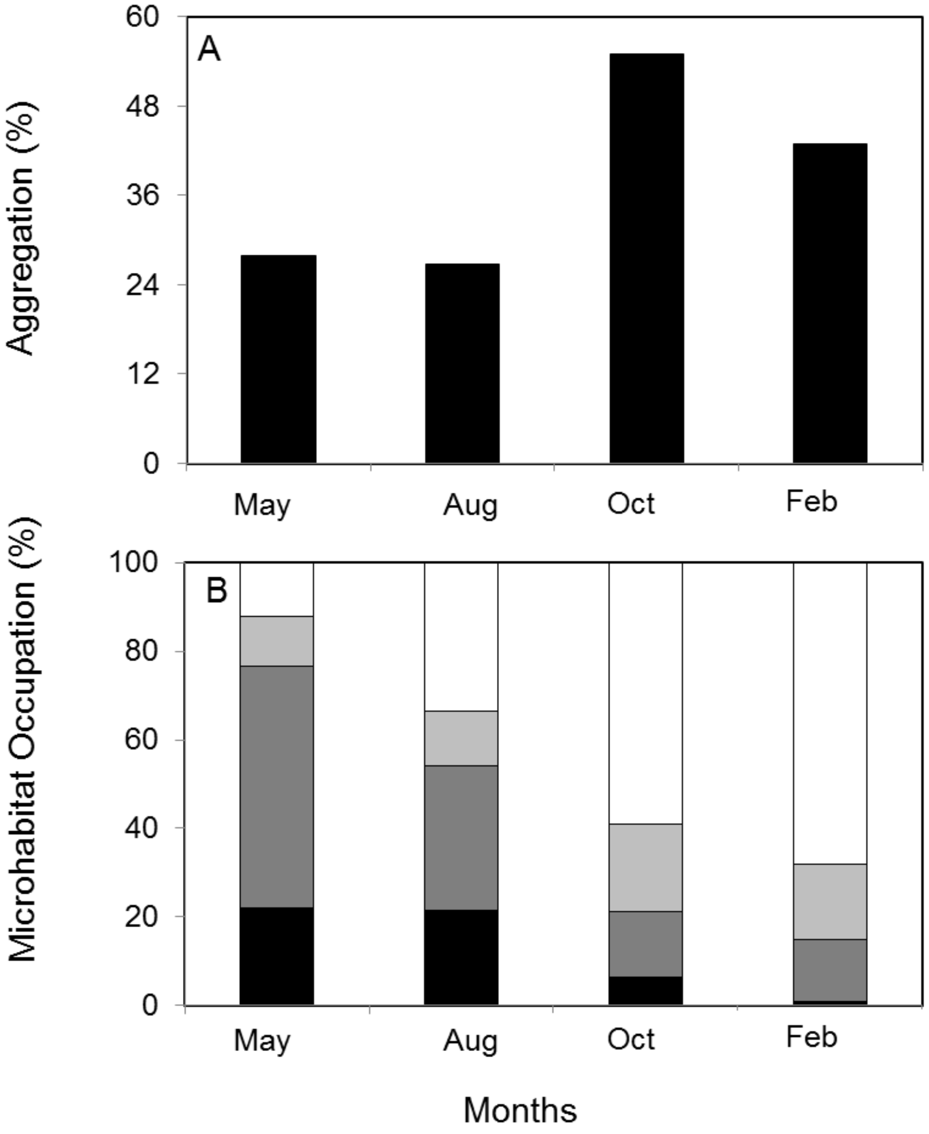

Figure 1.

Proportion of *Nerita atramentosa* individuals aggregated (A) and microhabitat occupation (B) at daytime low tide over 8 days in May 2009 (May), August 2009 (Aug), October 2009 (Oct) and February 2010 (Feb). The four microhabitats considered were pool (white bars), under rock (light grey bars), crevice (dark grey bars) and flat rock (black bars).

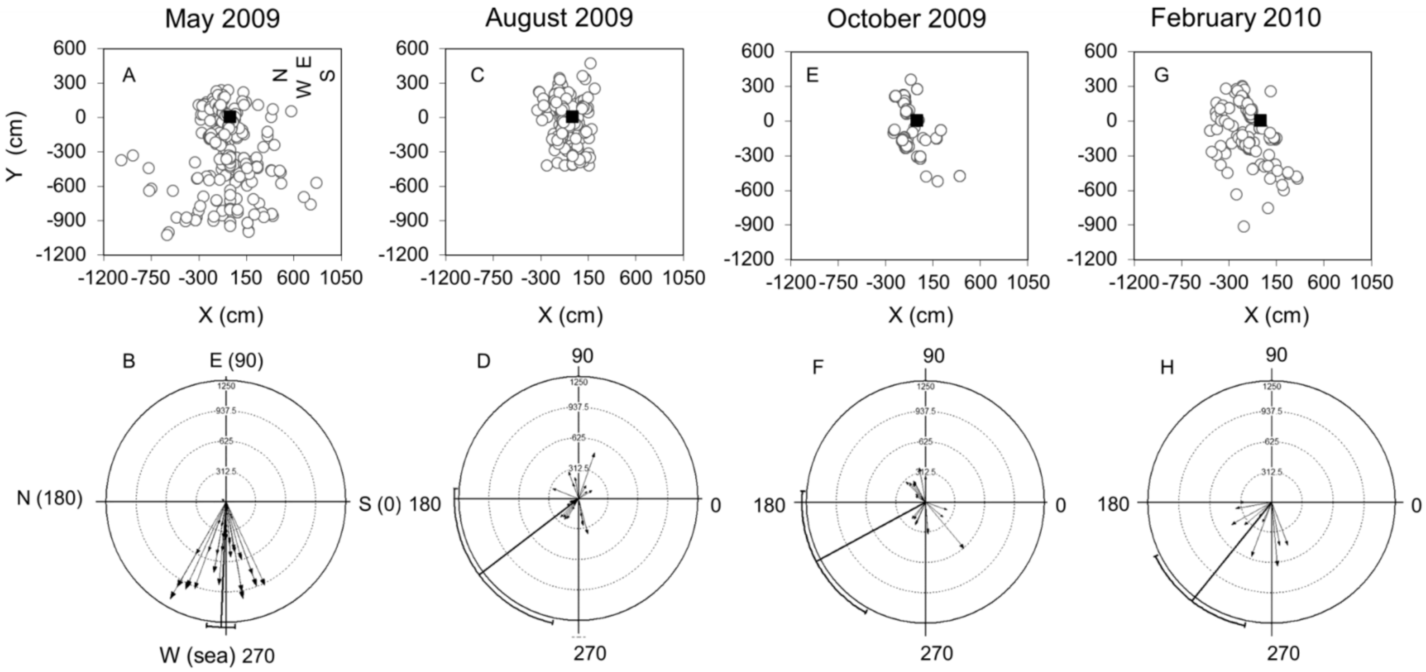

Figure 2.

Two-dimensional positions (cm) of *Nerita atramentosa* individuals (white dots) from the release point (A, C, E, G, black square) recorded at 8 daytime low tides in May 2009 (A, N = 396), August 2009 (C, N = 285), October 2009 (E, N = 285) and February 2010 (G, N = 212). Net directions and distances displaced from the release point (B, D, F, H, centre of circle) in May 2009 (B, N = 42), August 2009 (D, N = 24), October 2009 (F, N = 37) and February 2009 (H, N = 11). Each vector indicates the net direction taken by each individual. The concentric circles represent the distance displaced (increments of 312.5cm from 0 to 1250cm). The length of a vector is indicative of the individual distance travelled from the release point. The solid lines and the arcs are representative of the mean direction and its 95% confidence interval. The bottom of the graphs is oriented seaward.

Chapter III. Behavioural thermoregulation in a tropical gastropod: links to climate change scenarios

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ABSTRACT

Tropical species are vulnerable to global warming because they live at, or near to, their upper thermal threshold limits. Therefore, the predicted increase in the frequency of warming events in the tropics is expected to be critical for the survival of local species. This study explored the major environmental variables which were thought to be correlated with body temperatures of the tropical snail Littoraria scabra *at the niche level. A correlation between body temperature and substrate temperature was detected from field observations which suggests a possible causal relationship between both substrate and body temperatures. In contrast, there was no correlation between body temperature and air temperature. Field observations suggest that 33.4°C may be* L. scabra *upper limit of substrate surface temperature, although further experiments are needed to assess if the upper limit of physiological tolerance is actually different. Since* L. scabra *individuals were free to choose their substrata, the observed distribution pattern at the niche level is related to* L. scabra *behaviour. Additionally, substrate surface temperatures were very heterogeneous at centimeter scale (i.e. from 22.5°C to 53.1°C) and* L. scabra *was shown to select specific substrate temperatures (i.e. between 22.5°C to 33.4°C) rather than microhabitat type. Therefore,* L. scabra *did not seem to behaviourally thermoregulate through microhabitat selection nor aggregation. In contrast, behavioural experiments showed that* L. scabra *has the ability to actively select a thermally favourable site over short temporal scale (i.e. individual average speed of 1.26 cm* min^{-1}*) following exposure to high temperatures above 33.4°C. Hence, this study supports the crucial need to integrate intertidal invertebrate behavioural responses to thermal constraints in climate change studies.*

Keywords: gastropod, *Littoraria scabra*, Fiji Islands, tropical species, temperature, behavioural adaptation, thermal tolerance, thermal stress, extreme events, global change.

INTRODUCTION

Global warming profoundly impacts the structure and function of ecosystems (Walther *et al.*, 2002). At large scales, where forcing variables such as temperature dominate (Pearson & Dawson, 2003), bioclimatic envelope models have shown temperature-induced shifts in the geographic ranges of a variety of marine invertebrates, leading to abundance and biodiversity pattern alteration within coastal marine systems (Hawkins *et al.*, 2008; Cheung *et al.*, 2009). More specifically, shifts in species geographic distribution patterns in response to mean temperature changes result from the colonization of new habitats according to the thermal tolerance of a given species (Pörtner & Knust, 2007; Morley *et al.*, 2009). This implies invertebrate species abilities to disperse over long distances within and across habitats (Kinlan & Gaines, 2003; Williams *et al.*, 2008) and navigate towards more favourable conditions (Chapperon & Seuront, 2009). In addition, species must keep pace with and rapidly respond to fast hydroclimatic fluctuations (Loarie *et al.*, 2009) and also the increase in the frequency and intensity of warming events (IPCC, 2007). This issue is particularly critical in Pacific tropical islands which are among the most vulnerable ecosystems to climate change since the flood and drought cycle is predicted to become more severe under a climate shift towards more sustained El Niño conditions (Terry, 2005). Beyond the need for an evolutionary adaptation in dispersal ability through natural selection (*e.g.* a few years to century; Williams *et al.*, 2008), species must exhibit a flexibility over short temporal scales (*i.e.* minutes to days; Pincebourde *et al.*, 2009; Chapperon & Seuront, 2011) in their behavioural and ecological responses to extreme thermal stress to avoid the risk of extinction.

Particularly, intertidal sessile and slow-moving invertebrates such as mussels and limpets, have recently been confronted to mass mortalities following extreme heat waves (Harley, 2008). Indeed, sessile and slow-moving ectotherms are respectively attached to the substrates permanently and during the low tide. Contrarily to mobile invertebrates, sessile individuals cannot choose to "take flight" to a more thermally suitable microhabitat (Munoz *et al.*, 2005) when the thermal conditions are not favourable. In the specific case of slow-moving organisms, individuals can only take flight during immersion when they are actively foraging (Harley *et al.*, 2009). Therefore, both sessile and slow-moving organisms are restrained to "sit tight" and "fight" the thermal conditions of their surrounding environment during emersion (Munoz *et al.*, 2005). Mobile organisms have the potential to flee non favourable thermal niches. However, moving towards another microhabitat under heat may also indirectly affect their fitness (*e.g.* high cost of mobility; Denny, 1980). This highlights the critical vulnerability of all intertidal organisms to changes in the thermal properties of their environment (Williams *et al.*, 2008). Indeed, they often live at the upper edge of their thermal window (Somero, 2002) within their fundamental ecological niche, *i.e.* the space delimited by environmental factors, which allow species survival and growth (Hutchinston, 1957). However, many ectotherms are able to buffer the effects of the space-time heterogeneity in the thermal stress occurring in the intertidal in relation to *e.g.* diel and tidal cycles, and height shore variations (Helmuth *et al.*, 2005). More specifically, their survival is warranted by an adjustment of their body temperatures through a range of physiological (Somero, 2002), behavioural (Garrity, 1984) and morphological adaptations (Lee & Lim, 2009). Nonetheless, all species do not exhibit the same thermoregulation ability.

Specifically, tropical species that inhabit stable and non-seasonal environments have limited physiological acclimation abilities to thermal stress (Tewksbury *et al.*, 2008) since their thermal windows are narrower than those of temperate species (Compton *et al.*, 2007). In that context, it is critical to determine whether tropical mobile ectotherms have enough behavioural flexibility to compensate for their reduced physiological adaptive abilities, hence to adjust their body temperatures over short temporal scales. This implies a good understanding of the factors involved in the determinism of body temperature changes and an assessment of the impact of behaviour on body temperature patterns.

Body temperatures of intertidal ectotherms during aerial exposure are determined by the heat flux between substrate, atmosphere and organism (Harley *et al.*, 2009). Heat fluxes are driven by the interaction of multiple climate variables, *e.g.* solar radiation, humidity, air temperature (Broitman *et al.*, 2009). In addition, intrinsic properties of ectotherms such as thermal history (Meng *et al.*, 2009) and morphology (Jost & Helmuth, 2007) influence body temperatures. Finally, some ectothermic invertebrates can behaviourally thermoregulate (Pincebourde *et al.*, 2009). Specifically, gastropods may actively flee extreme temperatures, hence reduce their body temperatures, by selecting and moving towards a thermally favourable microhabitat (Garrity, 1984).

In this framework, we investigated the body temperature patterns of the intertidal tropical herbivorous gastropod *Littoraria scabra* at the individual scale in Fiji islands. The motivation of this work was to provide the first insights into the ability of a tropical snail to behaviourally respond to extreme temperatures in an environment where oscillations between high and low periods of drought and a strong variability in the extreme events are expected to occur under climate change scenarios (Mataki *et al.*, 2007). Specifically, the goals of this study were to understand (i) which abiotic factors in the environment surrounding intertidal organisms determine *L. scabra* body temperatures, (ii) if microhabitat selection or aggregation behaviour affect individual body temperatures, and (iii) if *L. scabra* is able to actively select a less stressful microhabitat following a sudden increase in thermal stress.

MATERIALS AND METHODS

Studied area and species

This work was conducted on the roots of a free-standing *Rhizophora* sp. in a mangrove surrounded by a sandy beach, in the province of Tailevu, 70 km northwest of Suva, Viti Levu Island (17º35.471'S-178º29.505'E). The mangrove was characterized by a high abundance (ca. 40 ind m^{-2}) of the generalist herbivorous gastropod *Littoraria scabra* (Linnaeus, 1758). *Littoraria scabra* is a littorinid species commonly found in Indo-Pacific mangrove forests where it inhabits roots and trunks (Reid, 1985). *L. scabra* has separate sexes and its reproduction is characterised by a combination of ovoviviparity and planktrotrophic development. In addition, *L. scabra* is mainly terrestrial and avoid immersion through migration patterns during tidal cycles (Alfaro, 2007). This species maintains its position above the water surface by quickly moving up during the incoming tide and by moving slowly down during the outgoing tide when they actively feed upon replenished resource deposit (Alfaro, 2007). Here, the previously clear migration patterns identified in *L. scabra* (Alfaro, 2007) was not observed during the field observations (non-published data, Chapperon & Seuront). *L. scabra* actively grazes on a wide variety of food items such as plant material, fungal hyphae and spores (Christensen, 1998) with a preference for bacteria and microalgae (Alfaro, 2008). *L. scabra*'s feeding activity is particularly intense at the bottom part of mangrove trees where the food is more abundant and diverse (Alfaro, 2008). At the opposite, the highest parts in the tree do not provide a high variety and quantity of food to feed upon (Alfaro, 2008). In this study, the frequent observation during the field activities of the mangrove crab *Metopograpsus messor* that preys upon *L. scabra* suggests the existence of a high predation risk. This species has also been showed to aggregate as a result of active trail following (Alfaro, 2007). However, there is still no clear understanding of what factors cause *L. scabra* aggregation behaviour. More specifically, the study was undertaken at midday low tides over 4 different days in September 2009, and air temperature (AT) reported to influence the body temperature (*e.g.* Broitman *et al.*, 2009) was consistently recorded on site. Midday low tides were chosen as they pose the most severe episodes of thermal stress which select for heat tolerance (Somero, 2010). In addition, during the low tide, studied roots were emerged which provided snails with a large area of roots to explore.

Substrate and snail body temperature

Thermal imaging was used as a non-contact and non-invasive temperature measurement. Infrared thermography is commonly used in a wide range of fields that includes animal physiology and behaviour (Bulanon *et al.*, 2009; Montanholi *et al.*, 2010). However, thermal imaging has rarely been employed to study ectothermic organisms, particularly in intertidal environments (Helmuth, 2002). Briefly, thermal imaging consists in the transformation of the infrared energy emitted and reflected from an opaque surface into a visible image. The energy radiated by the target is mainly a function of temperature, hence infrared thermography can be considered as a method for temperature measurements (Meola & Carlomagno, 2004). Temperature detection errors using thermal imaging have been related to wetting, time of the day or viewing angles (Meola & Carlomagno, 2004; McCafferty, 2007). Here, the thermal imager lens was never covered with water drops and both individuals and substrates were not wet when photographed. In addition, a recent study (Alchanatis *et al.*, 2010) has demonstrated that the optimal time for the acquisition of canopy temperatures used to map crop water status was midday and that different viewing angles provided a good representation of the leaf water potential. Here, images were consistently taken at midday low tides. Thermal images of haphazardly chosen individuals were obtained with a thermal imaging camera (Fluke Ti20, Fluke Corporation, Australia; Fig. 1a.). The thermal sensitivity of the thermal camera is ≤ 0.02ºC at 30ºC and the temperature measurement accuracy is 2%. Images of 466 *L. scabra* adult individuals (15.9 ± 0.2 cm long and 8.9 ± 0.1 cm high; mean ± SE) were captured on randomly chosen mangrove roots. For each thermal picture, a digital picture (Olympus µ Tough-6000, Olympus Imaging Corporation, USA) was jointly taken to analyze the individual body temperature in regards to behavioural properties (*i.e.* solitary or aggregated; Fig. 1b) and resting microhabitat. An individual was considered aggregated when there was a direct shell-to-shell contact with at least another conspecific. A resting microhabitat was considered as a microhabitat occupied by immobile individuals for a minimum of 15 minutes. *Rhizophora* sp. mangrove trees are characterized by stilt roots that emerge from the lower trunk and move downward to the ground

(Menezes, 2006). Therefore, the studied roots were not parallel to the ground, hence to the sun, but tend towards a perpendicular angle. In this context, a particular attention was given to report microhabitats in which photographed individuals were found to rest. Microhabitats were classified into 3 categories (top, side and under roots) in regards to the exposure to solar radiations occurring when the sun is at its zenith. More specifically, we defined the top of the root as the part directly exposed to the midday sun, *i.e.* 45º on each side of the vertical axis cutting across the root (*i.e.* upper quadrant). Similarly, the bottom of the root corresponded to the part not exposed to the midday sun, 45º apart on each side of the vertical axis and directly opposite the top of the root (*i.e.* lower quadrant). Finally, the sides of the root were the two remaining quadrants, 45º apart on each side of the horizontal axis cutting across the root. Images were subsequently analyzed in the laboratory using InsideIR software version 4.0.1.10 (Fluke Corporation, 2006, USA). For each picture, mean snail body temperature (BT) and mean temperature of the surrounding substrate (ST) were assessed.

Microhabitat selection and aggregation behaviour

As individuals seemed to preferentially occupy the lowest parts of the roots where they form aggregations, we examined whether individuals actively select this microhabitat. More specifically, sets of 6 adult snails (14.3 ± 0.3 cm long and 8.3 ± 0.3 cm high; mean ± SE) were dislodged from a patch situated under the root and randomly placed on top of the same root at midday low tide. Individual displacements were observed to assess the proportion of individuals returning to their previous microhabitats. This translocation experiment was repeated 8 times with a new set of 6 individuals. All individuals involved in this experiment were immobile for at least 15 minutes when dislodged from their substrates.

A previous study has demonstrated that the feeding activity of *L. scabra* in Fiji Islands is particularly intense at the bottom part of mangrove trees (Alfaro, 2008). Indeed, lower levels of roots and trunks provide greater amount and diversity of resources (Alfaro, 2008). In the present study, individuals situated under roots were immobile most of the time (Chapperon & Seuront, pers. obs.). It is hence unlikely that the high frequency of *L. scabra*'s individuals at the bottom part of the roots was related to feeding activity. In this context, the goal of this experiment was to identify whether individuals actively choose to rest under the roots in regards to the thermal properties of the microhabitats. Five thermal profiles were examined from the top to the bottom of 19 haphazardly chosen roots. A similar dislodgement experiment was conducted on thermally homogeneous roots during the night to ensure that the observed displacements were not biased by stimulii such as geotaxis or phototaxis (Petraitis, 1982). In addition, preliminary observations conducted on 60 individuals followed during two consecutive days have shown that both motion and aggregation behaviours of *L. scabra* did not differ between incoming and outgoing tides nor between day and night, hence eliminating a potential impact of circatidal and circadian rhythms on our observations. Finally, as trail following has been reported to impact gastropod aggregation (Chapperon & Seuront, 2009) including in *L. scabra* (Alfaro, 2007), the movements of 27 individuals were video recorded over 70 minutes to assess the frequency of trail following behaviour in *L. scabra* and its potential link with aggregation patterns.

Statistical analyses

A body-to-substrate temperature ratio (BST-ratio hereafter) was calculated to identify whether or not substrate temperature constitutes a good proxy for body temperature. More specifically, BST > 1 means that the body temperature is higher than the substrate temperature. In contrast, when BST < 1, the body temperature is lower than the substrate temperature. Finally, BST = 1 when *L. scabra* body and substrate exhibit the same thermal properties. The distributions of BT, ST and BST-ratio data were not normally distributed (Kolmogorov-Smirnov test, $p < 0.05$). Spearman correlation analyses were then subsequently run to assess the significance of the correlation between BT and ST and the mean values of BT, ST and the climatic variable AT. In addition, comparisons of BT, ST and BST-ratio between solitary and aggregated individuals were conducted using the Mann-Whitney *U*-test, while multiple comparisons between different microhabitats were assessed using the Kruskall Wallis test (KW test hereafter). All statistical analysis was done in PASW Statistics 18 (SPSS Inc., 2009, USA).

RESULTS

Littoraria scabra thermal properties and environment temperature

As no significant differences were found in the proportion of solitary vs. aggregated individuals, nor in BT, ST and BST-ratio between days, the data have been pooled and analyzed globally. On average, BT, ST and BST-ratio were respectively 27.06 ± 0.11°C (mean ± SE), 27.34 ± 0.11°C and 0.99. Overall, BT varied from 22.8°C to 32.6°C and no individual was found on a substrate warmer than 33.4°C (Fig. 2a). Substrate temperatures, however, reached values up to 53.1°C (Fig. 3a). BST-ratio varied from 0.93 to 1.07 and 68% of the BST-ratio values were smaller than 1 (*i.e.* BT < ST). A significant positive correlation (r = 0.98, P = 0.01, n = 466) was found between BT and ST. The slope of the linear regression $BT = 1.01ST - 0.73$ did not differ from a theoretical slope of 1 (Fig. 2a). In contrast, no significant correlation (BT and ST; r = 0.80, P = 0.20, n = 4) was found between mean BT, ST and mean air temperature. On average, BT and ST were about 1.2-fold warmer than air temperature AT. More specifically, BT and ST were on average 4.23 ºC and 4.52ºC warmer than AT over the four days of field observations.

Littoraria scabra thermal properties and behaviour

Of the *L. scabra* individuals studied here, 57% were aggregated on *Rhizophora* sp. roots. Aggregation size varied from 2 to 26 individuals per aggregate (Fig. 4). However, the majority of aggregates were formed by to 2 to 5 individuals (Fig. 4). The temperatures of substrates occupied by solitary and aggregated individuals did not significantly differ (Z = -0.015, P = 0.988). No significant differences were found in BT (Z = -0.129, P = 0.898) and BST-ratio (Z = -0.041, P = 0.967) between solitary and aggregated individuals.

On average, 56, 39 and 5% of *L. scabra* individuals were respectively found under, on the side and on top of roots. More specifically, most individuals (63%) found under roots were aggregated. In contrast, only 52% and 28% of the individuals respectively found on the side and top of roots were aggregated. BT, ST and BST-ratio did not significantly differ between microhabitats (BT: χ^2 = 2.247, df = 2, P = 0.325; ST: χ^2 = 2.693, df = 2, P = 0.260; BST-ratio: χ^2 = 5.464, df = 2, P = 0.065; Fig. 2b). On average, BT was cooler than the substrate under (BST-ratio = 0.99) and on the side of roots (BST-ratio = 0.99) and warmer on top of roots (BST-ratio = 1.02 ± 0.01). Furthermore, 23%, 40% and 48% of BST-ratio were significantly greater than 1 on the side (Z = -8.125, P = 0.01), under (Z = -12.730, P = 0.01) and top of roots (Z = -4.443, P = 0.01), respectively. In addition, mean differences between the maximal and minimal temperatures of the substrates surrounding individuals were 0.67 ± 0.07°C, 0.47 ± 0.02°C and 0.47 ± 0.01°C respectively on top, on the side and at the bottom of the roots.

Thermal profile analysis consistently revealed a significant decrease in temperature (P = 0.001) from the top to the bottom of the roots (Fig. 3) with a mean temperature difference of 6.84 ± 0.46°C. On average, top and bottom root temperatures were 33.86 ± 0.62°C and 27.04 ± 0.28°C, respectively. Maximum temperatures observed on the tops and bottoms of roots were respectively 53.1°C and 33.0°C. All the translocated individuals (N = 48) to the top of the roots returned to their previous microhabitat in 17 ± 2 minutes. The average speed of individuals returning the root bottom was 1.26 ± 0.06 cm min^{-1}.

In contrast, no individual returned to their previous microhabitat during the control experiment conducted during the night on a thermally homogeneous substrate after 30 minutes. In total, 18 individuals over the 27 recorded individuals displayed trail following behaviour (Fig. 5). Snails involved in trail following behaviour joined or created a patch in 83% of the recorded sequences (Fig. 5). Alternatively, only 44% of individuals ended up in aggregation in absence of mucous following.

DISCUSSION

Substrate temperature as a proxy for *L. scabra* body temperatures

Climate envelope models often neglect the variability in thermal stress over a wide range of spatio-temporal scales (Helmuth *et al.*, 2005). Here, the significant decrease in temperature (6.84°C) observed between the top and the bottom of *Rhizophora* sp. roots shows the existence of small-scale heterogeneity in substrate temperatures at the niche level. These observations reinforce previous results showing that the variability in thermal properties at the small-scale may surpass those observed at larger scales (Jost & Helmuth, 2007). Climate envelop models further assume that ectotherms display the same thermal properties as their surrounding environment (Broitman *et al.*, 2009). However, *L. scabra* body temperatures were neither similar nor correlated to air temperature which demonstrates that climate variables and individual body temperatures are decoupled. More specifically, individuals were on average 4.23ºC warmer than air temperature. This result suggests that climate change models using air temperature as a proxy for body temperatures may underestimate the effect of global warming on body temperature patterns of tropical invertebrate species. Likewise, correlated models may also overestimate ectotherms' physiological tolerance, which is already reduced compared to temperate species. Therefore, climate variables gathered at the habitat scale cannot be used for accurate predictions of the future species distribution range (Helmuth *et al.*, 2010).

Besides, molluscs have previously exhibited thermal properties dissimilar to those of the surrounding substrate, *e.g.* the snail *Nodilittorina* sp. and the limpet *Cellana grata* were respectively cooler (Lang *et al.*, 1998) and warmer than their substrates (Williams & Morritt, 1995). Here, *L. scabra* body temperatures were positively correlated to and did not significantly differ from their substrate temperatures (Fig. 2A). This result suggests that substrate temperature may be used as a good proxy for *L. scabra* body temperature. However, it also points out that the variables explaining body temperature patterns are likely to vary between species. It is therefore difficult to define mechanistic warming climate models that apply to all intertidal species. Instead, there is a movement towards the integration in mechanistic models of the ecophysiological responses to environmental changes observed at the population level of multiple species (Peck *et al.*, 2009). Indeed, spatial and temporal variations in body temperature patterns have also been observed within a species caused by the coupling of a few variables (*e.g.* timing of low tides and terrestrial climate; Helmuth *et al.*, 2002). Nevertheless, non-climatic variables such as substrate temperatures that immediately interact with substrate dwelling organisms at the niche level (Szathmary *et al.*, 2009; Marshall *et al.*, 2010) should be taken into consideration in mechanistic climate warming models. In particular, average substrate temperatures could potentially be estimated from average air temperatures; *i.e.* substrate was 4.52ºC warmer than air temperature. However, the relationship between air and substrate temperatures should be investigated at fine spatial scale and site by site. This is particularly necessary since substrate temperatures were heterogeneous at small spatial scale (0.47-0.67ºC to 6.8ºC at the mm and cm scale, respectively) which entails the existence of a thermal mosaic of body temperature patterns at the niche level (Helmuth *et al.*, 2006).

Micro-scale selection of substrate temperatures vs. microhabitat selection

The variability in thermal properties between the three microhabitats investigated here (top, side and bottom of *Rhizophora* sp. roots) is likely to be related to solar radiation exposure. Divergences in substrate temperature have already been referred to the substrate angle (Harley, 2008), solar exposure (Schneider & Helmuth, 2007) and cardinal position (Williams & Morritt, 1995). Therefore, it is possible that the variations in the angle position of the roots to the sun (*i.e.* roots were not parallel to the sun) may have affected the quantity of solar radiations or shading received by the different areas of the roots. Thus, substrate temperatures were also potentially heterogeneous within a microhabitat through for example indirect exposure to solar radiation inferred by reflective surfaces. However, it is unlikely that the small differences in the thermal properties within a microhabitat (*i.e.* < 0.7ºC) surpassed those observed between microhabitats (*i.e.* > 6ºC). Thus, the top of the roots, directly exposed to solar radiation, may be considered as the most thermally stressful microhabitat as shown by the low proportion (5%) of individuals resting on this microhabitat. In contrast, the bottom of the roots was not exposed to midday sun and 56% of observed individuals occupied this microniche. The bottom of roots may hence provide a refuge against thermal stress. However, it is also possible that the potential heterogeneity in the distribution and availability of food resources on mangrove trees contributed to the observed snail distribution pattern. For example, *L. scabra* was previously found to feed more intensely during the low tide at the bottom part of the mangrove trees (roots and trunk) characterized by greater quantity and variety of resources (*e.g.* microalgae, mangrove tissue) than the top area of the trees (Alfaro, 2008). Here, the bottom of the roots, non-exposed to the midday sun, certainly received less light than the top and the bottom of the roots. Therefore, root bottoms potentially provide refuge against thermal and desiccation stresses but also from wind and wave exposure which is favourable for the deposition of epilithic microalgae (*e.g.* Farnsworth & Ellison, 1996). Bottoms of roots as crevices on intertidal rocky shores (Jackson, 2010) may hence abound with a greater quantity and quality of food than other microhabitats. This potentially explains why *L. scabra* was found more frequently under the roots. However, fronts of roots have also been characterized by a greater diversity in algae than back roots, although filamentous algae were more abundant on back of roots (Farnsworth & Ellison, 1996). In addition, only a few individuals were observed to move at the root bottoms during the study (Chapperon & Seuront, pers. obs.), it is hence unlikely that the distribution of individuals was related to feeding activity.

In the present study, our hypothesis suggesting that the distribution of individuals was directed by the thermal properties of microhabitats was supported by the results of translocation experiments since all tested individuals actively returned to the bottom of the roots. Similarly, microhabitats such as crevices in intertidal rocky shores are often more frequently occupied than open surfaces (Judge *et al.*, 2009). The snail preference and choice for a specific location is usually related to the humidity and cooler temperature of the microhabitat as it may protect individuals from desiccation and thermal stresses (Judge *et al.*, 2009). This microselection of thermal niches may be critical for maintaining individual fitness over long term exposure (days to weeks), especially among tropical species which cannot shift further their upper critical thermal tolerance limits (Pörtner, 2002). More specifically, an exposure to temperatures over the pejus temperatures induces a decrease in aerobic scope that implies a decrease in vital activities such as feeding (Pörtner, 2002). These activities completely disappear under anaerobic scope when temperature of exposure is beyond the upper critical temperature threshold (Pörtner, 2002). If the exposure persists, individual growth and reproduction, and finally survival will be affected (Jones & Boulding, 1999; Pörtner, 2002). However, snail resting position at low tide does not always reflect an active microhabitat selection (Crowe & Underwood, 1998), suggesting that active microhabitat selection might be both species-specific and environment-specific.

Here, on substrates of temperatures ranging from 22.5°C to 33.4°C (Fig. 2a), *L. scabra* did not select a specific microhabitat (*i.e.* top, side or bottom; Fig. 2b). Indeed, the three microhabitats displayed similar thermal properties (Fig. 2b) and were not different in terms of humidity (Chapperon & Seuront, pers. obs.). However, the selection of a specific range of substrate temperatures (Fig. 2a) out of an available range reaching 53.1°C suggests a behaviour to keep body temperatures cool as shown for other Littorinids (Rojas *et al.*, 2000). This suggests that 33.4°C may be the upper limit of the substrate thermal tolerance of *L. scabra* although further experiments are needed to assess if this limit is different in terms of physiological tolerance. This issue is, however, well beyond the scope of the present work. Nevertheless, substrate temperature could be used as a good proxy for body temperature of organisms that attach substrates, but only up to a critical temperature threshold, here 33.4°C. Based on substrate temperatures estimated from air temperatures recorded in Fiji in 2009 at noon (Bureau of Meteorology of Fiji, Nausori Airport) and the temperature ratio found in this study between substrate and air (*i.e.* 1.2), *L. scabra* was likely exposed to substrates warmer than 33.4°C for 115 days. It is then critically needed to determine the ability of *L. scabra* to thermoregulate when facing substrates that surpass its substrate thermal tolerance levels.

Escape behaviour as a response to thermal stress

This study provides insight into the behavioural flexibility of *L. scabra* facing extreme changes in environmental conditions at short temporal scales. *L. scabra* is able to rapidly (ca. 17 min) escape from extreme substrate temperatures and actively select a cooler substrate (< 33.4°C). In particular, the observed average speed of 1.26 cm min^{-1} in *L. scabra* appears to be higher than its average speed while avoiding immersion (1 cm min^{-1}; Alfaro, 2007) or feeding (0.6 cm min^{-1}; Alfaro, 2007). This reinforces the advantage of mobile ectotherms that have the ability to take flight from substrate temperatures beyond their critical upper limit temperature (Munoz *et al.*, 2005) At the opposite, in such a situation, sessile or slow moving organisms must sit tight and fight the thermal conditions (Munoz *et al.*, 2005) which may result in mass mortalities following warming events, such as heat wave (Garrabou *et al.*, 2009). In addition, as shown by our control translocation experiment conducted on thermally homogenous roots, the displacements of snails towards their former site were neither biased to geotaxis or phototaxis (Petraitis, 1982) nor migration patterns related to either circatidal or circadian rhythms (Alfaro, 2007). Thus, the return of individuals to the bottom of the roots was most likely activated by the solar radiations reaching the shell and the high conductive heat flux between the substrate and the snail body on top of the roots. This escape behaviour appears essential for *L. scabra* survival since individuals were able to flee sun heat at midday low tide that defines the upper limit of thermal tolerance window (Somero, 2010). Besides, *L. scabra* likely returned to its previous microhabitat by following the mucous trails of conspecifics (Fig. 5) as showed in other Littorinids (Alfaro, 2007; Stafford *et al.*, 2007; Chapperon & Seuront, 2009).

Aggregation behaviour is commonly thought to reduce desiccation and thermal stresses (Rojas *et al.*, 2000). Aggregated *L. scabra* individuals were, however, not cooler than solitary individuals. It is consequently suggested that the moist microhabitat created by the important quantity of mucous present in a patch potentially decreased the desiccation stress (Garrity & Levings, 1984). Therefore, the high production cost of mucous (Denny, 1980) involved in trail following, aggregation, and escape behaviours (Muñoz *et al.*, 2005) may counterbalance the risk of mortality related to desiccation and extreme temperature. Here, *L. scabra* may have aggregated as a result of the individual search for specific substrate temperatures. In addition, the proportion of aggregation in *L. scabra* was observed to be more frequent when the tide was coming up (personal observations). This suggests that aggregation behaviour may potentially reduce the risk of dislodgement due to breaking waves, or the effect of predation by crabs during the high tide. Albeit *L. scabra* aggregation has been showed to be likely related to feeding and/or reproductive activities (Alfaro, 2007), the determinism of its aggregation behaviour is still unclear. Further experiments in laboratory are necessary to identify the factor(s) causing aggregation in *L. scabra* and to confirm that moving towards a cooler substrate is the only efficient survival strategy in the tropical mobile ectotherm *L. scabra* facing extreme temperatures.

CONCLUSION

Climate variables (*e.g.* air temperature) and physiology alone cannot accurately predict the future of organisms in the warming world. Here, we highlight the need to focus on the non-climatic variables (*i.e.* substrate temperatures, solar exposure) that directly interact with mobile individuals at the niche level. This is particularly relevant since behavioural adaptations to non-climatic thermal sources could enhance the tolerance levels of intertidal ectotherms to climatic variables (Marshall *et al.*, 2010). In that context, thermoregulatory behaviours developed by both sessile and mobile ectotherms under high thermal stress, *e.g.* mushrooming behaviours in limpets (Williams & Moritt, 1995; Williams *et al.*, 2008), raised posture and shell orientation in snails (Lang *et al.*, 1998; Munoz *et al.*, 2005), should be integrated in climate change models. Here, the behavioural thermoregulation of *L. scabra* was an escape strategy to substrate temperatures > 33.4ºC. This flight behaviour (Munoz *et al.*, 2005) coupled with the existence of substrate temperature heterogeneity at small spatial scale (*i.e.* a few centimeters), reveal that tropical mobile invertebrates may be less vulnerable to global warming than previously thought.

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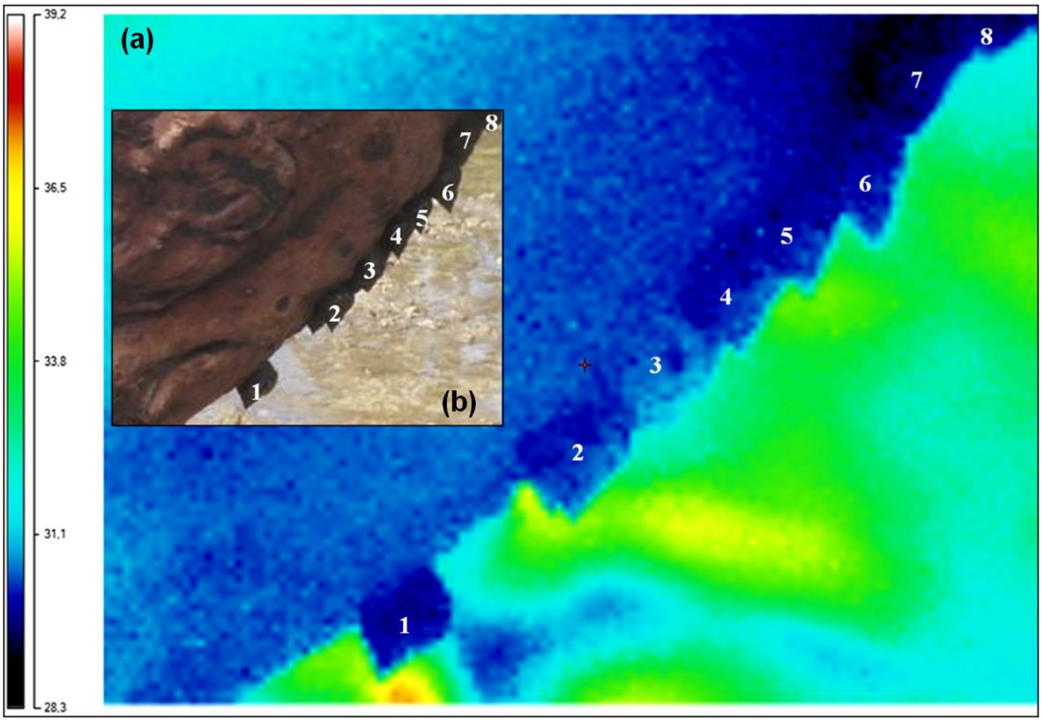

Figure 1.

Thermal (a) and digital (b) pictures of 8 *Littoraria scabra* individuals (30.1 ± 0.1ºC, mean BT ± SE) situated under a *Rhizophora* sp. root (30.0 ± 0.1ºC, mean ± SE). The white numbers indicate the position of each snail. BT: body temperature.

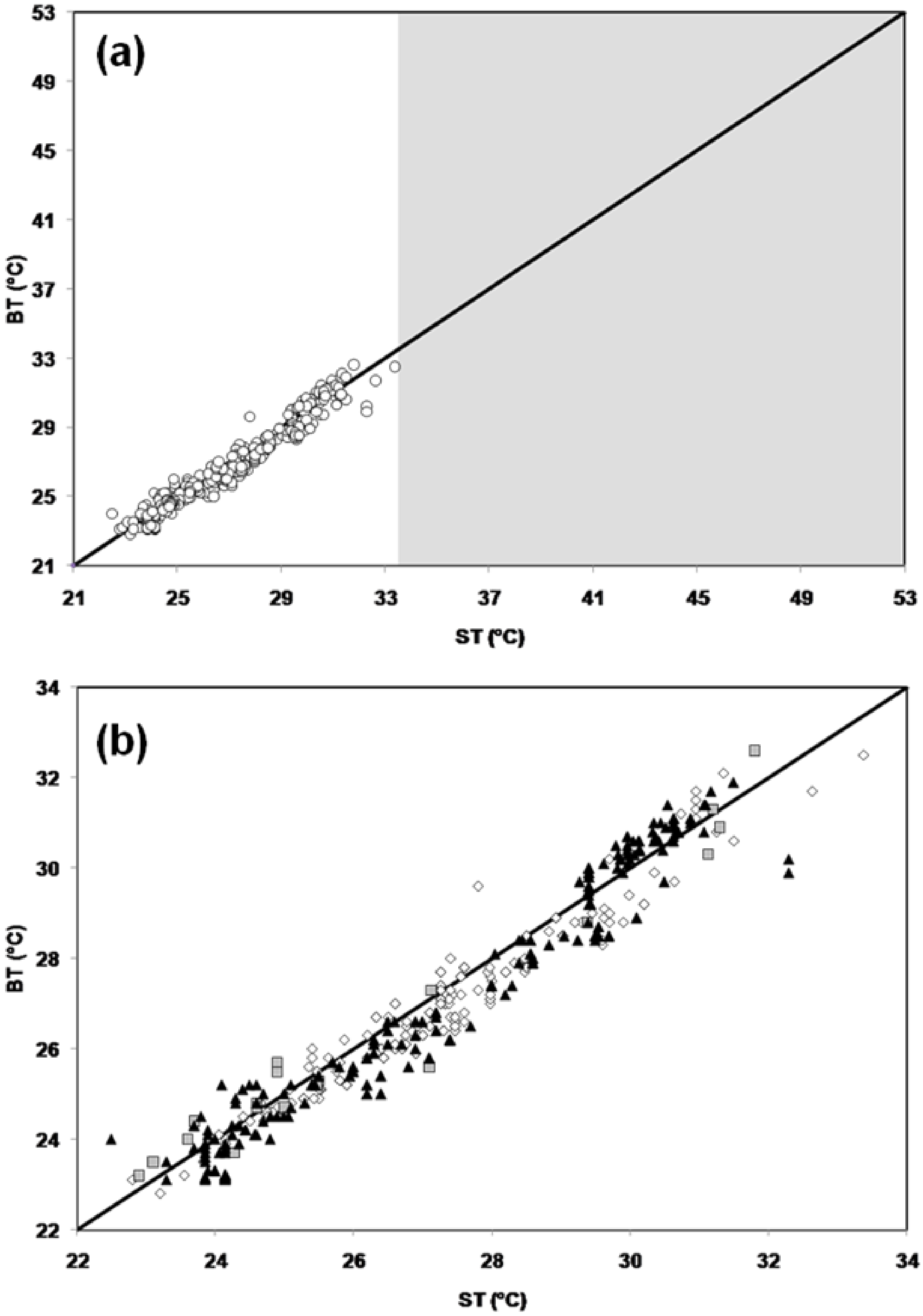

Figure 2.

(a) *Littorina scabra* individual body temperatures (BT; N = 466) versus substrate temperatures (ST), as a function ofthe different *Rhizophora* sp. root microhabitats (b; top: grey squares, side: open dots and under: black triangles). The grey area in (a) corresponds to the range of substrate temperatures where *L. scabra* individuals were never observed, *i.e.* between 33.4ºC and 53.1ºC. The black line represents the first bissectrix, *i.e.* BT = ST.

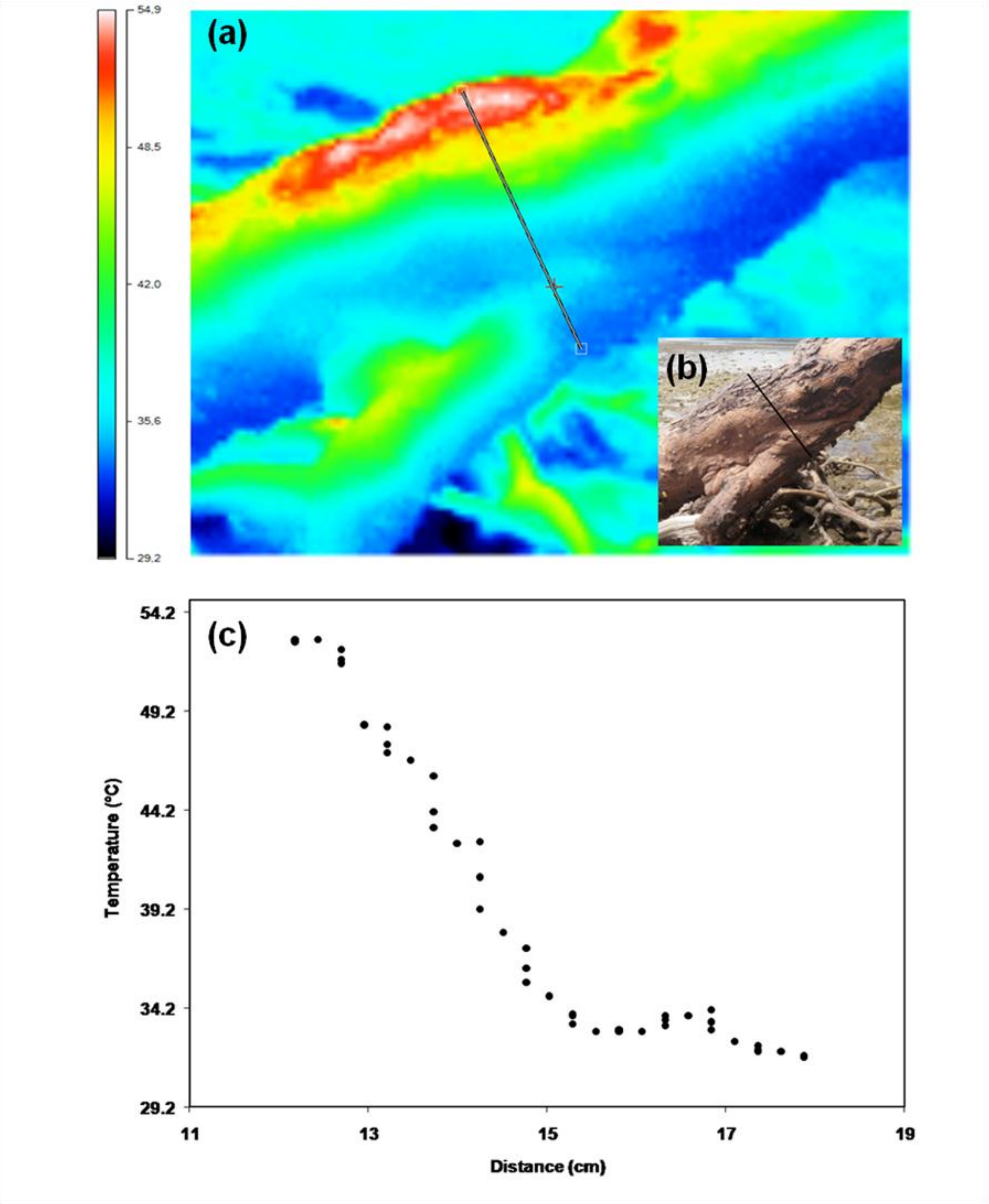

Figure 3.

Thermal (a) and digital (b) pictures of the *Rhizophora* sp. root shown in Fig. 1. Black lines in (a) and (b) correspond to the thermal profile taken from the top (52.8°C) to the bottom (31.7°C) of the root (c). A significant decrease ($p = 0.001$) in surface temperature was found between the top and the bottom of the root.

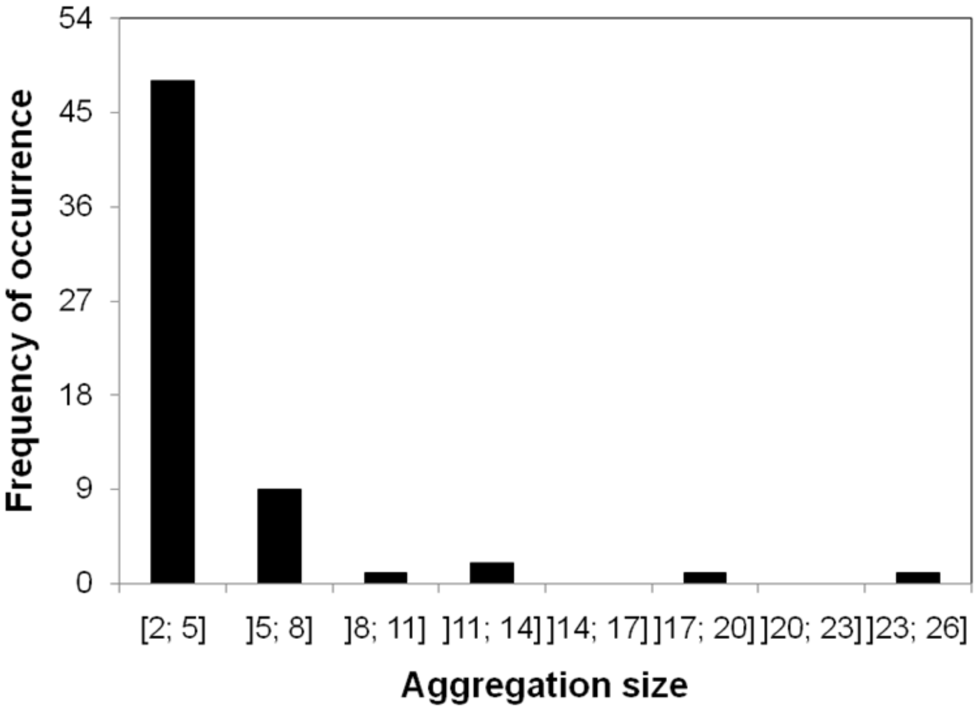

Figure 4.

Frequency of occurrence of *L. scabra* aggregation size (N = 263).

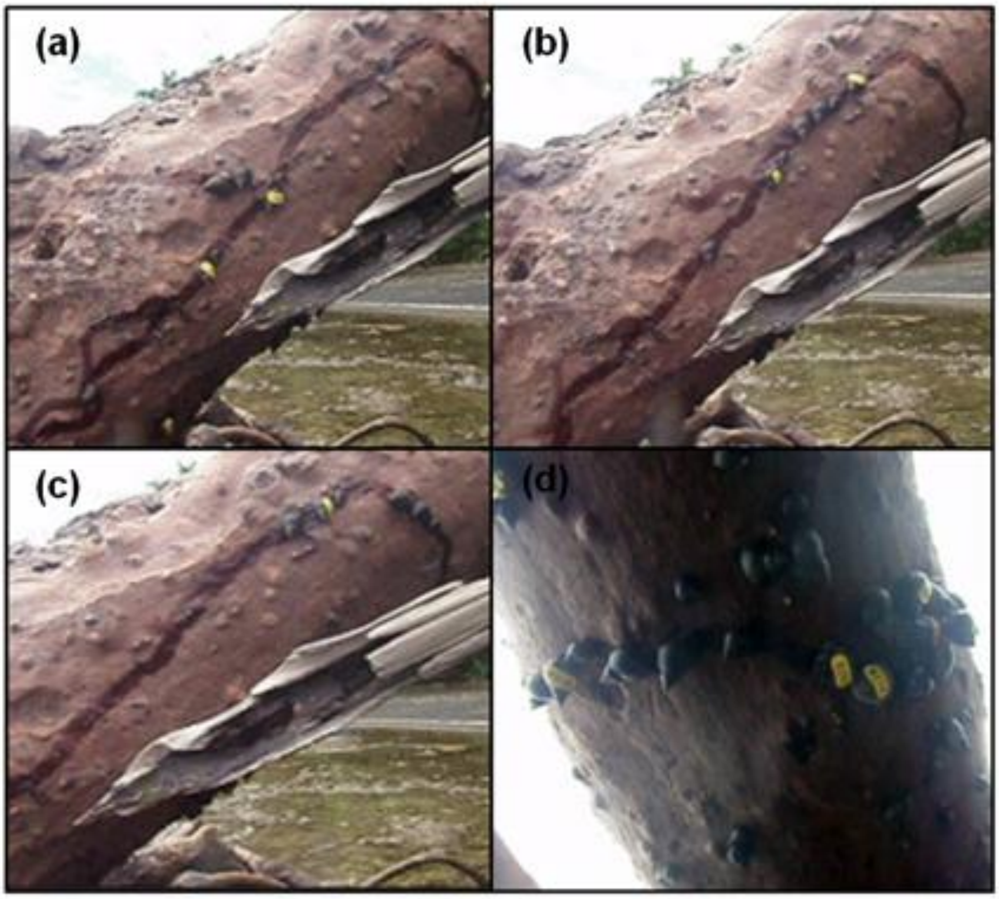

Figure 5.

Sequence of *L. scabra* trail following behaviour recorded on a *Rhizophora* sp. root. Successive images (a, b, c and d) were taken at 1-min intervals. All recorded individuals (N=6) ended up in a patch under the root (d).

Chapter IV. Space-time variability in environmental thermal properties and snail thermoregulatory behaviour

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ABSTRACT

Behavioural adaptations of ectotherms to thermally heterogeneous environments are still overlooked in the literature despite the fact that organismal behaviour could enhance survival in the warming world. This is particularly critical in the intertidal where most ectotherms live at, or near to the upper limit of thermal tolerance. The present study investigated (i) the environmental factors determining the body temperatures of the intertidal gastropod Nerita atramentosa*, (ii) the space-time variability in environmental and individual body temperatures, and (iii) the potential variability in* N. atramentosa *thermoregulatory behaviours, i.e. microhabitat selection and aggregation. Thermal imaging was used to assess the body temperatures of* N. atramentosa *and surrounding substrata over two seasons (autumn and summer), at two shore levels (low vs. high shore levels) within two habitats of different topographic complexity (rock platform and boulders) on the same rocky shore. Snail body and substratum temperatures were significantly and positively correlated within each habitat at both seasons. Substratum temperature may thus be considered as a primary driver of body temperatures of organisms that attach to a substratum. Substratum temperature and others variables such as solar irradiance need to be integrated in climate change models that used single climatic variables (e.g. air temperature) that are not necessarily correlated to individual body temperatures in nature. The high space-time variability in both substratum and body temperatures reinforces the growing evidence that small spatial scale variations may surpass those observed at larger spatial scales.* N. atramentosa *thermoregulatory behaviour under high thermal stress appeared to be habitat-specific. The small spatial scale heterogeneity in environmental and individual temperatures and in* *thermoregulatory behaviours has stressed the need to focus on body temperature patterns at the niche level and to integrate organismal behaviour in climate change models.*

Keywords: behavioural adaptations, body temperature, climate change, gastropod, intertidal habitats, physiology, season, shore height, thermal stress, microhabitat.

INTRODUCTION

Temperature determines a wide range of biological processes that are essential to animal life (Angilletta, 2009). In particular, temperature has an effect on all physiological processes from the molecular to the organismal levels (Pörtner *et al.*, 2006; Kingsolver, 2009). Therefore, changes in temperature affect organism fitness, performance and metabolism (Huey & Berrigan, 2001; Dillon *et al.*, 2010), hence profoundly impact the structure, dynamic and functioning of populations and ecosystems (*e.g.* Morelissen & Harley, 2007). However, the mean temperature and its variability have been predicted to increase in the warming climate (Planton *et al.*, 2008). Nevertheless, levels of thermal tolerance and potential physiological and behavioural abilities of ectotherms to thermoregulate in the future climate, particularly in thermally heterogeneous environments, are still far from being understood.

The mechanistic links between the body temperatures of ectotherms, that control local (Miller *et al.*, 2009) and global distribution patterns (Helmuth *et al.*, 2002), and environmental variables are not as simple as previously anticipated (Helmuth, 2009). More specifically, body temperatures of both terrestrial and marine ectotherms are determined by heat fluxes towards and from an organism (Gates, 1980; Harley *et al.*, 2009) which are subject to variations generated by the interaction between climatic heat sources (heat derived from ambient conditions *i.e.* coarse-scale macroclimatic data such as air and water temperatures, Helmuth, 2002; Vidal *et al.*, 2010), non-climatic heat sources at the niche level (heat originated from the sun *i.e.* solar irradiance, Días & Cabezas-Días, 2004; Marshall *et al.*, 2010), and biotic factors (*e.g.* shell morphology, Harley *et al.*, 2009; Polo-Cavia *et al.*, 2009). Therefore, the variability in a single factor may cause unexpected heterogeneity in body temperatures and leads to counter-intuitive patterns (*e.g.* Helmuth *et al.,* 2002). Body temperatures of snails have also recently been demonstrated to be primarily controlled by non-climatic heat sources at the niche level (*i.e.* solar irradiance) instead of climatic heat sources (i.e air and water temperatures; Marshall *et al.,* 2010). The space-time heterogeneity in organism and environment temperatures and the related physiological and behavioural adaptations require a better understanding in order to predict future species distribution ranges.

This lack of knowledge is particularly critical in intertidal ecosystems that are thermally very heterogeneous over a range of scales, *i.e.* diel, tidal and seasonal variations within latitudinal and vertical clines, and microhabitats (Helmuth *et al.*, 2006; Sinclair *et al.*, 2006). Most intertidal invertebrates are close to the upper limit of their thermal tolerance (Somero, 2002), hence they are critically vulnerable to further changes in temperatures. Intertidal ectotherms have developed a range of physiological (Somero, 2002) and behavioural adaptations (Munoz *et al.*, 2005; Williams *et al.*, 2005) to the natural thermal stress heterogeneity in order to maintain body temperatures within the species thermal tolerance window. Over the last decade, however, most attention has been given to the physiological responses of ectotherms and the development of new thermal sensors (*e.g.* biomimetic loggers, Shine & Kearney, 2001; Schneider & Helmuth, 2007) which have led to the establishment of new physiologically-based mechanistic models, *i.e.* heat budget models. These models have successfully predicted individual body temperature patterns of sessile individuals such as limpets (Denny & Harley, 2006) by integrating the morphology of organisms (*e.g.* shell shape). However, the potential buffering effect of behavioural thermoregulation of mobile ectotherms is still missing within climate change impact models (Kearney *et al.*, 2009).

A few recent studies, though mostly terrestrial, have highlighted the importance of integrating the behaviour of mobile ectotherms (Días & Cabezas-Días, 2004; Kearney *et al.,* 2009) that may increase the survival of mobile organism in a warming world (Huey & Tewksbury, 2009). For example, locomotory abilities allow the exploitation of the ambient heterogeneity, hence the selection of thermally favourable niches (Huey *et al.,* 2002). Due to their intrinsic complex topography, intertidal rocky shores abound with a variety of potential thermal refuges such as crevices, pits, rocks and pools that supply ectotherms with moisture and shade from solar radiations (Jackson, 2010). Therefore, snails may actively select these microhabitats while travelling during the high tide to stabilize their body temperatures following emersion. Besides microhabitat selection behaviour, snails have displayed a range of thermoregulatory behaviours, *e.g.* mucous holdfast, raised posture, shell orientation (Garrity, 1984; Munoz *et al.,* 2005). Particularly, the formation of aggregates, commonly occurring among snails, is typically considered as a behavioural adaptation to desiccation and thermal stresses (*e.g.* Garrity, 1984) although this is not always the case (*e.g.* Coleman, 2010).

In this context, the present study focused on the patterns of body temperatures and the thermoregulatory behaviours of the black snail *Nerita atramentosa* (Fig. 1A, C), a species particularly abundant on South Australian intertidal rocky shores at different spatial scales during cool and hot seasons. More specifically, the main goals of this work were (i) to explore the relationship between body temperatures and substratum surface temperatures at the individual scale, to analyse the space-time variability in (ii) substratum and body temperature patterns over two seasons (autumn vs. summer) in two topographically different habitats along the same rocky shore at small spatial scales (*i.e.* habitat, shore and niche levels), and in (iii) the potential thermoregulatory behaviours (*i.e.* aggregation and selection of thermally favourable niches).

MATERIALS AND METHODS

Studied area and species

This work was conducted on a moderately exposed rocky shore located in Marino Rocks, South Australia (35°02’40S-138°30’30E), characterised by the presence of an alongshore gradient of substratum topographic complexity (*i.e.* rock platform to boulder field). This area supports great abundances of herbivorous gastropod species such as *Bembicium* sp., and *Austrocochlea* sp. and particularly the neritacean *Nerita atramentosa* (Reeve, 1855). Here, we focused on *N. atramentosa* (Fig. 1A, C), which has specifically been chosen as (i) it is the dominant grazer and competitor for microalgae on Australian rocky shores (Underwood & Murphy, 2008), and (ii) it is particularly subject to exposure to high temperatures due to its black pigmented shell and the related high absorption of solar radiation and retention during emersion (McMahon, 1990). This mobile species is able to move between microhabitats during emersion (Chapperon & Seuront, pers. obs.).

The study was undertaken at high and low shore levels (typically between the lower limits reached by the tidal flow at low tide in spring and neap tides, Seuront & Spilmont, 2002) during low tides that occurred in the morning and midday times (*i.e.* between 10 am to 2 pm; Kuo & Sanford, 2009) on four different days in both autumn 2009 (A, May 2009) and summer 2009-2010 (S, December 2009 and January 2010) on two topographically different habitats located 250 m apart. The field work started at the low tide time indicated by the Bureau of Meteorology of Australia and lasted for a minimum period of 2 hours during the incoming tides. The first habitat was a rock platform (RP; 35°2'31.67''-138°30'35.37'') characterised by a flat, smooth rocky substratum with a few shallow pits and crevices at high shore level, and by the presence of pebbles and cobbles at low shore level. The second habitat was a boulder field (B; 35°2'38.04''-138°30'30.13'') mainly characterised by boulders (*i.e.* rock bigger than 256 mm) that provide a range of microhabitats such as pools, pits and crevices.

Snail density and distribution patterns

In each habitat and shore level, 20 quadrats (25 cm × 25 cm) were haphazardly placed within a 25 m^2 area. Digital pictures (digital camera Olympus J1 Tough-60, Olympus lmaging Corporation, Centre Valley, PA, USA) of each quadrat were taken to assess snail density and individual distribution at microscale. Individuals were classified either as being solitary or aggregated. An individual was considered aggregated when there was a direct shell contact with the shell of at least another conspecific. In addition, the microhabitat resting site of each snail was recorded. On both habitats, two microhabitats (flat rock and crevice) were defined in regards to substratum topographic complexity and exposure to solar radiations. Flat rock corresponds to a flat surface bereft of refuge to thermal stress, hence directly exposed to solar radiations. Crevice was defined as a depression wide and deep enough to fit at least one individual that may provide some protection from solar radiation, hence from thermal stress. On the rock platform, an additional microhabitat, under rock, was considered as a sheltered environment which provides entire protection from solar radiations.

Snail body temperature and substratum surface temperature

Tissue temperatures of living animals have mainly been gathered using thermocouples or thermistors (Garrity, 1984; Williams *et al.*, 2005). In this study, we used infrared thermography as a non-contact and non-invasive method of temperature measurement (Helmuth, 2002; Chapperon & Seuront, 2011a). Thermal imaging has recently been shown to be an accurate and reliable tool to measure the mantle tissue temperature of *N. atramentosa* (Caddy-Retalic, 2008). A preliminary approach (Caddy-Retalic, 2008) was undertaken between *N. atramentosa* mantle temperatures measured with a thermistor probe and *N. atramentosa* dorsal shell temperatures assessed with a thermal imager Fluke Ti20 (Fluke Corporation, Everett, Washington, USA). A significant positive correlation (Pearson correlation coefficient, $R^2 = 0.988$, $P < 0.001$) was obtained between the mantle tissue temperatures and the dorsal shell temperatures (Caddy-Retalic, 2008). The significant linear regression between mantle temperatures MTs and dorsal shell temperatures BTs was identified to be $BT = 0.8875 \times MT + 2.7044$ (Caddy-Retalic, 2008).

Here, a thermal image of each individual observed in each quadrat was obtained using a thermal imaging camera Fluke Ti20 (Fluke Corporation, Everett, Washington, USA). The thermal sensitivity of the thermal camera is ≤0.2ºC at 30ºC and the temperature measurement accuracy is 2% or 2°C, whichever is greater. Emissivity value (ε) was calibrated by applying a piece of masking tape characterised by a high emissivity ($\varepsilon = 0.95$) on 10 rocks and 10 snails. Specifically, when the temperature equilibrium was reached between tape and rock, and tape and snail, the emissivity value of the targets (*i.e.* rock, snail) was adjusted in order to obtain a temperature reading similar to that of the electrical tape of known emissivity. Mean emissivity values obtained for rock and body snail were respectively 0.954 ± 0.005 ($\bar{x} \pm \mathrm{SD}$; N = 10) and 0.946 ± 0.009, ranged from 0.94 to 0.99, and 0.91 to 0.98, and cannot be statistically distinguished (Wilcoxon-Mann-Whitney U-test, $p < 0.05$). Note that these emissivity values fall into the range of emissivity values employed for substrata (*i.e.* 0.95 to 1; Campbell & Norman, 1998; Helmuth, 1998; Finke *et al.*, 2006; Bozinovic & Navarrete, 2009) and intertidal invertebrates (*i.e.* 0.96 to 1; Campbell & Norman, 1998; Helmuth, 1998; Denny & Harley, 2006; Finke *et al.*, 2009; Miller *et al.*, 2009). Emissivity value (ε) was consequently assumed to be fairly identical between organism and substratum and was hence set up at 0.95. Pictures of 307 and 203 individuals were collected on the boulder field and rock platform, respectively. Each individual was photographed once. Different individuals were used in the different habitats and shore levels. Images were subsequently analysed using InsideIR software version 4.0.1.10 (Fluke Corporation, 2006, Everett, Washington, USA). For each thermal picture, snail body temperature (*BT*) and temperature of the surrounding substratum (*ST*) were assessed (Fig. 1B, D). A closed curve marker was drawn around each photographed shell in order to calculate the mean value of body (*i.e.* shell) temperature (*BT*). In addition, *ST* was averaged from 4 linear markers drawn on the substratum directly surrounding the individual shell. In particular, the distance between the linear markers and the shell was defined as approximately a quarter of the shell size measured on the picture. In addition, *BT*s and *ST*s were measured during the incoming tides between 10am and 2pm which is the period that selects for heat tolerance (Somero, 2010). It is therefore supposed that the temperatures measured corresponded to the maximal temperatures reached by both individuals and substrates, although this assumption may require further investigations. The mantle temperature *MT* was further calculated from the empirical relationship found between *MT* and *BT* (Caddy-Retalic, 2008). Furthermore, a mantle-to-substratum temperature ratio (MST_{ratio}) was defined to examine whether or not snail mantle temperature (*MT*) was closely related to that of the surrounding substratum (*ST*). The difference between mantle temperature and surrounding substratum (MST_{diff}) was also calculated to quantify the potential difference in temperature between the snail mantle and its substratum.

The distributions of the data *MT*, *ST*, MST_{ratio} and MST_{diff} at both seasons, in both habitats, in the different microhabitats, and the distributions of snail density in both habitats were not all normally distributed (Kolmogorov-Smirnov Test, $P < 0.05$). Non-parametric tests were consequently used throughout the manuscript. Spearman’s correlation coefficients were used to assess the relationship between *MT* and *ST* at both seasons and in both habitats. All pairwise comparisons of *MT*, *ST*, MST_{ratio} and MST_{diff} between habitats, seasons, microhabitats, and aggregated vs. solitary individuals were conducted with the Mann-Whitney *U*-test. Comparisons of *MT*, *ST*, MST_{ratio} and MST_{diff} between the three microhabitats on the rock platform were performed with the Kruskall-Wallis test and subsequent non-parametric post-hoc analyses (based on the Tukey test; Zar, 2010) were done to compare the different groups of measurements. All statistical analysis was done in PASW Statistics 18 (SPSS Inc., 2009, IL, USA).

RESULTS

Space-time dynamic of *N. atramentosa* density and distribution patterns

Boulder field

The density of individuals was significantly higher in summer ($Z = -4.212$, $P < 0.001$; 64 ind m^2) than in autumn (48 ind m^2). The proportion of individuals in crevices was higher at the high shore level in both seasons (Fig. 2A). Instead, individuals rested more often on flat rocks at low shore levels (Fig. 2A). Overall, 58% and 29% of the total number of individuals were aggregated in summer and in autumn, respectively. Aggregation behaviour was more frequent at the high shore level in summer (Fig. 2A). In addition, *N. atramentosa* was found to aggregate more frequently within crevices than on flat rocks (Fig. 2A).

Rock platform

No significant difference in density ($Z = -1.121$, $P = 0.262$) was observed between the two seasons. Overall, *N. atramentosa* was mainly observed on flat rocks (46%) rather than in crevices (35%) or under rocks (19%) over the two seasons. In autumn at low shore level, 96% of individuals were on flat rocks (Fig. 2B). In summer, however, organisms were mainly under rocks (Fig. 2B), particularly at low shore level (Fig. 2B; 63%). Most individuals were solitary in both seasons and at both shore levels (Fig. 2B) with the exception of the high shore level in autumn where 55% of individuals were aggregated. Moreover, individuals were found to be more aggregated within crevices and under rocks in both seasons (Fig. 2B).

Space-time dynamics of environment and snail body thermal properties

Significant positive linear relationships were found between *MT* and *ST* in both habitats and seasons (Fig. 3A, B). More specifically, in autumn, *MT*, *ST*, MST_{ratio} and MST_{diff} were significantly higher on the rock platform than on boulders ($Z_{MT} = -12.183$, $Z_{ST} = -11.432$, $Z_{MST_{ratio}} = -11.915$, $Z_{MST_{diff}} = -12.028$, $P < 0.001$; Table 1, Fig. 3A, B). In summer, no significant differences in *MT*, *ST*, MST_{ratio} and MST_{diff} were found between the two habitats ($Z_{MT} = -0.005$, P = 0.996; $Z_{ST} = -1.619$, P = 0.105; $Z_{MST_{ratio}} = -1.866$, P = 0.062; $Z_{MST_{diff}} = -1.607$, P = 0.108; Table 1, Fig. 3A, B).

Boulder field

In autumn, mantle and surrounding substratum temperatures were significantly higher at low shore level than at high shore level ($Z_{MT} = -6.849$, $Z_{ST} = -7.663$, $P < 0.001$; Fig. 3C). However, no significant differences in MST_{ratio} and MST_{diff} were found between shore levels ($Z_{MST_{ratio}} = -0.846$, P = 0.398; $Z_{MST_{diff}} = -0.830$, P = 0.407). In summer, *MT*, MST_{ratio} and MST_{diff} were significantly greater at low shore level than at high shore level ($Z_{MT} = -4.409$, $Z_{MST_{ratio}} = -8.413$, $Z_{MST_{diff}} = -7.626$, $P < 0.001$; Table 1, Fig. 3C). *ST*, however, was not significantly different between shore levels ($Z_{ST} = -0.453$, P = 0.651).

Rock platform

In autumn, *MT*, *ST*, MST_{ratio} and MST_{diff} were significantly greater at low shore level than at high shore level ($Z_{MT} = -6.665$, $Z_{ST} = -6.808$, $Z_{MST_{ratio}} = -5.086$,

$Z_{MST_{diff}} = -5.606$, P < 0.001; Table 1, Fig. 3D). In summer, *MT*, *ST*, MST_{ratio} and MST_{diff} were significantly higher at high shore level than at low shore level ($Z_{MT} = -5.031$, $Z_{ST} = -4.332$, $Z_{MST_{ratio}} = -3.170$, $Z_{MST_{diff}} = -3.363$, P < 0.001; Table 1, Fig. 3D).

Microhabitat occupation and thermal properties

Boulder field

All results are summarized in Table 2. In autumn, *MT, ST,* MST_{ratio} and MST_{diff} were significantly higher on flat rocks than in crevices (Fig. 4A). At high shore level, no significant difference in *MT, ST,* MST_{ratio} and MST_{diff} was observed between microhabitats. At low shore level, MST_{ratio} and MST_{diff} were significantly greater on flat rocks than in crevices. *MT* and *ST* did not significantly differ between microhabitats. In summer, *ST* was significantly cooler on flat rocks than within crevices (Fig. 4C). However, *MT* did not significantly differ between microhabitats (Fig. 4C). MST_{ratio} and MST_{diff} values were significantly greater on flat rocks than within crevices. At high shore level, *MT* and *ST* were significantly warmer in crevices than on flat rocks. MST_{ratio} and MST_{diff} were not significantly different between microhabitats. At low shore level, no significant difference was found in *MT, ST,* MST_{ratio} and MST_{diff} between microhabitats.

Rock platform

All results are summarized in Table 3. In autumn, *MT, ST,* MST_{ratio} and MST_{diff} were significantly greater on flat rocks than under rocks than in crevices (Fig. 4B). At the high shore level, *MT*, *ST*, MST_{ratio} and MST_{diff} were significantly higher on flat rocks and under rocks than within crevices. No significant difference in *MT*, *ST*, MST_{ratio} and MST_{diff} were found between flat rocks and under rocks. At low shore level, *N. atramentosa* was only found on flat rocks with the exception of one observation under rock. In summer, *MT*, *ST* and MST_{diff} were significantly warmer in crevices and on flat rocks than under rocks. No significant difference in *MT*, *ST* and MST_{diff} was found between crevices and flat rocks. MST_{ratio} was warmer on flat rocks than under rocks. No significant difference in MST_{ratio} was found between crevices and under rocks, and flat rocks. At the high shore level, *MT* and *ST* did not significantly differ between microhabitats. $\mathrm{MST}_{\mathrm{ratio}}$ and MST_{diff} were significantly higher on flat rocks than under rocks. At the opposite, at low shore level, *MT* and *ST* were significantly warmer on flat rocks than under rocks. *MT* within crevices was not significantly different from *MT* measured in the two others microhabitats. Similarly, *ST* in crevices was not significantly different from *ST* on flat rocks but was significantly higher than *ST* under rocks. MST_{ratio} and MST_{diff} were not significantly different between microhabitats.

Aggregation behaviour and thermal properties

Boulder field

All results are summarized in Table 4. In autumn, no significant difference was found in *MT*, *ST*, MST_{ratio} and MST_{diff} between aggregated and solitary individuals. Similar results were found at high shore level. At low shore level, aggregated individuals were warmer and rested on warmer substrata than solitary individuals. MST_{ratio} and MST_{diff} did not significantly differ between aggregated and solitary snails. At the opposite, in summer, aggregated individuals displayed significant cooler *MT* and were observed on cooler substrata than solitary individuals. MST_{ratio} and MST_{diff} were significantly lower among aggregated individuals. At high shore level, *MT*, *ST*, MST_{ratio} and MST_{diff} were significantly greater for solitary individuals. At low shore level, MT, ST, MST_{ratio} and MST_{diff} did not significantly differ between aggregated than solitary individuals.

Rock platform

All results are summarized in Table 4. In autumn, aggregated individuals exhibited significantly cooler *MT* and occupied significantly cooler substrata than solitary individuals. MST_{ratio} and MST_{diff} were significantly greater for solitary than aggregated individuals. At high shore level, mantles of aggregated snails were significantly cooler than those of solitary snails. No significant differences in ST, MST_{ratio} and MST_{diff} were observed between aggregated and solitary individuals. At low shore level, all individuals were solitary. In summer, no significant differences in *MT*, *ST*, MST_{ratio} and MST_{diff} were found between aggregated and solitary individuals. Similar results were found at both shore levels.

DISCUSSION

Substratum temperature as a primary determinant of *N. atramentosa* mantle temperature

Substratum temperature appears to be the primary factor that determines *N. atramentosa* body temperature as demonstrated in others organisms that attach the substratum (*e.g.* barnacles, snails; Bertness, 1989; Chapperon & Seuront, 2011a). *N. atramentosa* mantle temperature was significantly and positively correlated to that of the surrounding substratum at each season and in both habitats. This temperature correlation may result from the high thermal conductance between mantle and substratum in organisms that exhibit a direct foot contact to the substratum (Wethey, 2002). This also emphasizes the need to integrate in climate change studies organismal adaptations to heat sources such as substratum that contribute more importantly to the determinism of body temperature patterns than air or water temperatures (Marshall *et al.*, 2010; Fig. 3C-D). These adaptations seem critical since they are likely to increase the thermal tolerance of organisms in the warming climate (Marshall *et al.*, 2010; Chapperon & Seuront, 2011a).

N. atramentosa mantle temperature did not perfectly match that of the surrounding substratum (as expressed by MST_{ratio} and MST_{diff}; see Table 1), as previously observed in others mollusc species (Williams & Morritt, 1995). This deviation between snail mantle and substratum temperatures likely resulted from the interaction between a range of abiotic factors such as air temperature (Helmuth, 1998) and solar exposure (Schneider & Helmuth, 2007) and biotic factors (*e.g.* body size, Helmuth, 1998).

Mosaics of substrata and body temperature patterns at different spatial scales

N. atramentosa experienced a heterogeneous thermal stress conveyed by substratum temperature that was highly variable between two habitats of different topographic complexity (a rock platform and a boulder field), especially in autumn (*i.e.* 4.18ºC of mean temperature difference) and reached extreme temperatures in summer (*e.g.* up to 47.93ºC). The variability in thermal properties between the two habitats could be related to the difference in the topographic complexity of the substratum to which an individual is attached (Bertness, 1989). For instance, the lower substratum temperatures observed on the boulder field in autumn can be related to the potential greater buffering effect of large boulders to extreme temperatures than cobbles, present on the rock platform (Bertness, 1989). Besides the environmental temperature variability between habitats, *N. atramentosa* also faced a thermal stress that was variable within a habitat, *i.e.* at the two different shore levels.

The observed differences in thermal stress between shore heights did not always follow the intuitive pattern that high shore is the most stressful intertidal location due to prolonged emersion and the related high mortality rate (Bertness *et al.,* 1999). The seasonal variability in thermal stress observed on the rock platform between shore levels may be explained by the intensity of solar radiations received by organisms and the substratum (*i.e.* more intense in summer), the duration of sun exposure related to the emersion time (*i.e.* prolonged immersion at low shore level) and the water temperature during preceding high tide (Wethey, 2002). On the boulder field, the temperature deviation between snails and substrata between shore levels may be related to differences in the physiological adaptations of individuals to thermal stress *i.e.* high shore snails have likely developed greater physiological adaptations than low shore individuals since they inhabit a more thermally stressful environment (McMahon, 1990; Somero, 2002). This last hypothesis was however far beyond the scope of the present study and thus requires further investigations.

Substratum temperatures were highly variable at the niche level. For instance, the maximum substratum temperature range observed on the boulder field at high shore level in summer (*i.e.* 28.54ºC) is consistent with the existence of mosaic patterns of thermal properties at the niche level in intertidal areas (Helmuth *et al.*, 2006). Similarly, thermal microhabitat mosaics have been observed in terrestrial habitats such as mountain terrains of complex topography (Scherrer & Körner, 2010). In addition, substratum temperature difference at the niche level was far greater than that observed between the two habitats (*i.e.* 4.18ºC in autumn and 0.44ºC in summer). This is consistent with recent studies suggesting that microhabitat variations in thermal properties may surpass large scale variations in both marine and terrestrial environments (Jost & Helmuth, 2007; Scherrer & Körner, 2010).

Variability in thermoregulatory behaviour: a gap in climate change studies

Motile ectotherms such as reptiles, insects and invertebrates are not passively subject to environmental conditions (Kearney & Porter, 2009). Indeed, they can exploit the environment thermal heterogeneity to seek favourable microclimatic conditions in order to avert low or high environmental temperatures (Kearney *et al.*, 2009). For instance, lacertid lizards and wood turtles move back and forth between sunlit and sun-shade patches to bask or avoid the sun (*e.g.* Días & Cabezas- Días, 2004; Dubois *et al.*, 2009). In the intertidal, microhabitats such as crevices are commonly assumed to provide shelter from thermal and desiccation stresses (Jackson, 2010). The present study, however, stresses the space-time variability (*i.e.* season, habitat and shore height) in the potential role (*e.g.* thermal haven) played by different microhabitats on body temperature patterns. For instance, in autumn in both habitats, crevices were the cooler microhabitats, hence the most efficient in reducing the temperature deviation between individuals and substrata. On the other hand, in summer in both habitats, crevices (and flat rocks on the rock platform) constituted the most thermally stressful microhabitat. This result highlights that microhabitats usually considered as shelters (*i.e.* crevices) in the literature do not always follow this intuitive pattern but rather vary from it in both space and time.

On the rock platform in summer, the higher frequency of individuals and lower MT, ST, MST_{ratio} and MST_{diff} values observed under rocks suggest that individuals actively selected this microhabitat. The bottoms of rocks likely created a local microclimate and thermally stable conditions that protected individuals from summer thermal conditions as shown for the limpet *Cellana tramoserica* (Sinclair *et al.*, 2006). Similarly, the limpet *Cellana grata* took refuge in the cooler microhabitats available on the rocky shore (*e.g.* vertical surfaces non-exposed to full heat of the sun, Williams & Morritt, 1995). This result demonstrates that *N. atramentosa* was able to behaviourally thermoregulate through the selection of thermally advantageous microhabitats (*i.e.* under rocks) available within the mosaics of substratum temperatures.

On the other hand, microhabitat occupation did not play a role in *N. atramentosa* thermoregulation on the boulder field in summer. Individuals occupied more frequently crevices that were thermally more stressful than flat rocks. The high frequency of crevice occupation may thus result from the potential benefits of this microhabitat in terms of desiccation (Jones & Boulding, 1999) or feeding (Underwood & Murphy, 2008). In contrast to the rock platform, aggregated individuals at the high shore level maintained cooler mantle temperatures and smaller temperature deviations with the substratum than solitary individuals. Aggregation in *N. atramentosa* may hence be considered as a thermoregulatory behaviour. Aggregation behaviour has previously been demonstrated to be variable in space and time (Chapman, 1995). *N. atramentosa* aggregates found at other time or location could therefore be related to the diverse benefits of grouping evocated in the literature, such as reduction of predation risk (Coleman, 2010) and resource exploitation (Lauzon-Guay & Scheibling, 2009). Aggregation may also result from conspecific trail following behaviour (Chapperon & Seuront, 2011a) or may be simply caused by the tendency of *N. atramentosa* to group in depressions, as shown in other gastropod species (Stafford & Davies, 2004).

CONCLUSION

Substratum temperature appeared to be the main determinant of *N. atramentosa* mantle temperature. Variables such as substratum heat (at least for species with large areas of contact with the substratum) and solar irradiance critically need to be integrated in climate change models to improve our predictions of future body temperature patterns. The present study demonstrated space-time variability in substratum and snail thermal properties. Mosaics of substratum temperatures at the niche level were characterised by a range of temperatures greater than that observed between habitats separated by 250 m. *N. atramentosa* have developed a thermoregulatory behaviour specific to each habitat. The behavioural flexibility in *N. atramentosa* (Chapperon & Seuront, 2011b, present study) may be particularly advantageous in the warming climate and increase the survival of organisms locally. In particular, the behavioural ability to explore environment thermal heterogeneity implies that individuals are likely to find a refuge under new environmental conditions. The behaviour of ectotherms challenges the predicted shifts in species distribution patterns and hence critically needs to be integrated in climate change models.

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Table 1.

Mean (standard error), minimum and maximum values in *MT* (mantle temperature), *ST* (substratum temperature), MST_{ratio} (body-to-substratum temperature ratio) and MST_{diff} (difference of temperature between the mantle of individuals and the surrounding substratum) on RP (rock platform) and B (boulders) in A (autumn) and S (summer).

		MT (ºC)	*ST* (ºC)	MST_{ratio}	MST_{diff} (ºC)
RP-A (N=142)	Mean (SE)	22.13 (0.44)	19.64 (0.29)	1.12 (0.01)	2.49 (0.18)
	Min	15.84	15.44	0.89	-1.81
	Max	36.19	32.34	1.44	9.31
B-A (N=91)	Mean (SE)	15.48 (0.13)	15.46 (0.13)	1.00 (0.93)	0.01 (0.04)
	Min	11.44	11.42	0.93	-1.08
	Max	18.74	18.24	1.06	1.14
RP-S (N=61)	Mean (SE)	29.30 (0.43)	26.15 (0.32)	1.12 (0.01)	3.15 (0.22)
	Min	24.33	22.43	1.01	0.40
	Max	37.55	34.29	1.26	7.05
B-S (N=216)	Mean (SE)	29.55 (0.32)	25.71 (0.22)	1.15 (0.01)	3.84 (0.17)
	Min	21.30	19.40	0.79	-5.98
	Max	54,34	47.93	1.36	8.88

Table 2.

Mann-Whitney *U*-Test to investigate the variation in *MT*, *ST*, MST_{ratio} and MST_{diff} between the two microhabitats *i.e.* crevice (C) and flat rock (FR) on the boulder field in autumn (A) and summer (S) in total (all), and specifically at the low and high shore levels (LSL and HSL, respectively). Results of the tests are indicated in the last column (NS means non-significant).

Boulders			Z	P	
A	All	*MT*	-2.72	0.007	FR > C
		ST	-2.85	0.004	
		MST_{ratio}	-2.94	0.003	
		MST_{diff}	-2.92	0.003	
	LSL	*MT*	-1.43	0.154	NS
		ST	-0.92	0.358	
		MST_{ratio}	-3.30	<0.001	FR > C
		MST_{diff}	-3.36	<0.001	
	HSL	*MT*	0.46	0.470	NS
		ST	0.58	0.581	
		MST_{ratio}	0.51	0.524	
		MST_{diff}	0.55	0.562	
S	All	*MT*	-1.03	0.303	NS
		ST	-3.09	0.002	FR < C
		MST_{ratio}	-5.95	<0.001	FR > C
		MST_{diff}	-5.14	<0.001	
	LSL	*MT*	0.59	0.600	NS
		ST	0.59	0.600	
		MST_{ratio}	0.16	0.162	
		MST_{diff}	0.28	0.286	
	HSL	*MT*	-3.33	<0.001	FR < C
		ST	-4.21	<0.001	
		MST_{ratio}	-0.55	0.585	NS
		MST_{diff}	-0.17	0.864	

Table 3.

Kruskall-Wallis Test and and subsequent multiple comparisons (post hoc based on the Tukey test) to investigate the variation in MT, ST, MST_{ratio} and MST_{diff} between the three microhabitats *i.e.* crevice (C), flat rock (FR) and under rock (UR) on the rock platform in autumn (Au) and summer (Su) in total (all), and specifically at the low and high shore levels (LSL and HSL, respectively). Results of the tests are indicated in the last column (NS means non-significant).

Rock platform			df	χ^2	P	Post-Hoc
A	All	MT	2	31.74	<0.001	FR > UR > C
		ST	2	26.57		
		MST_{ratio}	2	23.65		
		MST_{diff}	2	25.94		
	HSL	MT	2	41.07	<0.001	FR = UR > C
		ST	2	33.58		
		MST_{ratio}	2	31.15		
		MST_{diff}	2	34.30		
S	All	MT	2	19.89	<0.001	C = FR > UR
		ST	2	12.98	0.002	
		MST_{ratio}	2	12.96	0.002	FR > UR = C
		MST_{diff}	2	17.43	<0.001	C = FR > UR
	HSL	MT	2	3.78	0.151	NS
		ST	2	4.04	0.133	
		MST_{ratio}	2	6.29	0.043	FR > UR = C
		MST_{diff}	2	6.43	0.040	
	LSL	MT	2	7.71	0.021	FR > UR; FR = C; UR = C
		ST	2	10.824	0.004	FR = C > UR
		MST_{ratio}	2	0.070	0.966	NS
		MST_{diff}	2	0.682	0.711	

Table 4.

Mann-Whitney *U*-Test to investigate the variation in MT, ST, MST_{ratio} and MST_{diff} between aggregated and solitary individuals at both seasons and in both habitats, in total (all), and specifically at the low and high shore levels (LSL and HSL, respectively). All individuals were solitary at the low shore level on the rock platform in autumn. Results of the tests are indicated in the last column (NS means non-significant).

			Boulders			Rock platform		
			Z	P		Z	P	
Au	All	MT	-0.728	0.466	NS	-4.052	0.001	A < S
		ST	-1.392	0.164		-3.781	0.001	
		MST_{ratio}	-1.550	0.121		-2.969	0.003	
		MST_{diff}	-1.507	0.132		-3.211	0.001	
	HSL	MT	-1.019	0.308	NS	-2.068	0.039	A < S
		ST	-1.161	0.246		-1.569	0.117	NS
		MST_{ratio}	-0.659	0.510		-1.205	0.228	
		MST_{diff}	-0.659	0.510		1.331	0.183	
	LSL	MT	-2.654	0.008	A > S			
		ST	-2.700	0.007				
		MST_{ratio}	0.572	0.567	NS			
		MST_{diff}	-0.572	0.567				
Su	All	MT	-4.533	0.001	A < S	-0.056	0.955	NS
		ST	2.889	0.004		-0.702	0.483	
		MST_{ratio}	-4.290	0.001		-1.565	0.118	
		MST_{diff}	-4.553	0.001		-1.468	0.142	
	HSL	MT	-4.319	0.001	A < S	-0.452	0.651	NS
		ST	-3.656	0.001		-1.684	0.092	
		MST_{ratio}	-3.638	0.001		-1.937	0.053	
		MST_{diff}	-4.025	0.001		-1.679	0.093	
	LSL	MT	0	1	NS	0.225	0.822	NS
		ST	-0.426	0.670		-0.644	0.520	
		MST_{ratio}	-0.426	0.670		-0.435	0.664	
		MST_{diff}	-0.213	0.831		0.306	0.760	

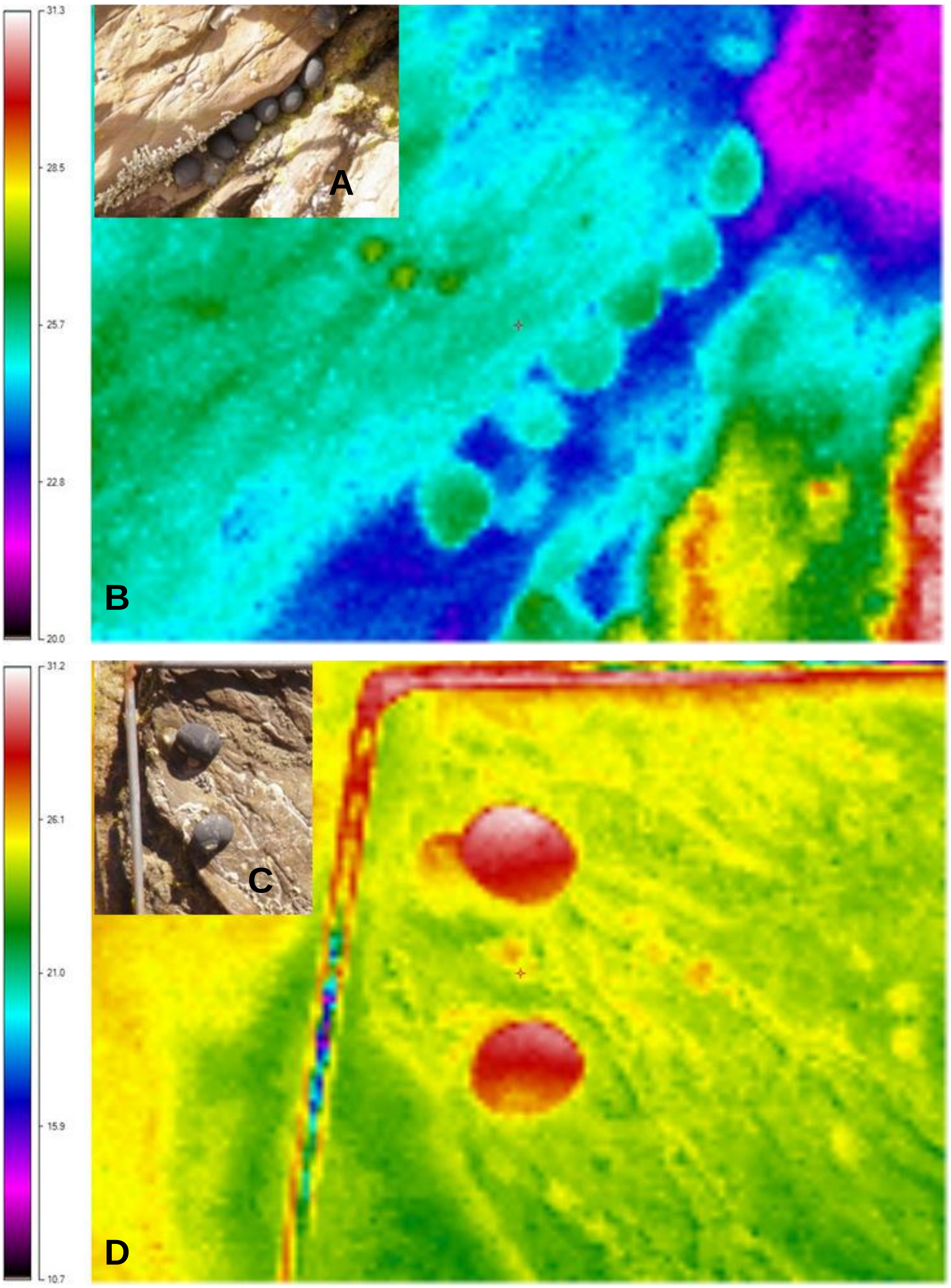

Figure 1.

Thermal images (B, D) and associated digital pictures (A, C) of *Nerita atramentosa* collected in summer on boulders, (A, B) at the high shore level within a crevice, and (C, D) at the low shore level on a flat rock. Average body temperature of snails and surrounding substratum surface within the crevice (A, B) were respectively 25.27 ± 0.19°C (N = 7; mean ± standard error) and 23.4 ± 0.12°C. On the flat rock, snails exhibited an average body temperature of 28.2 ± 0.40°C (N = 2) and the surrounding substratum surface was 24.81 ± 0.09°C.

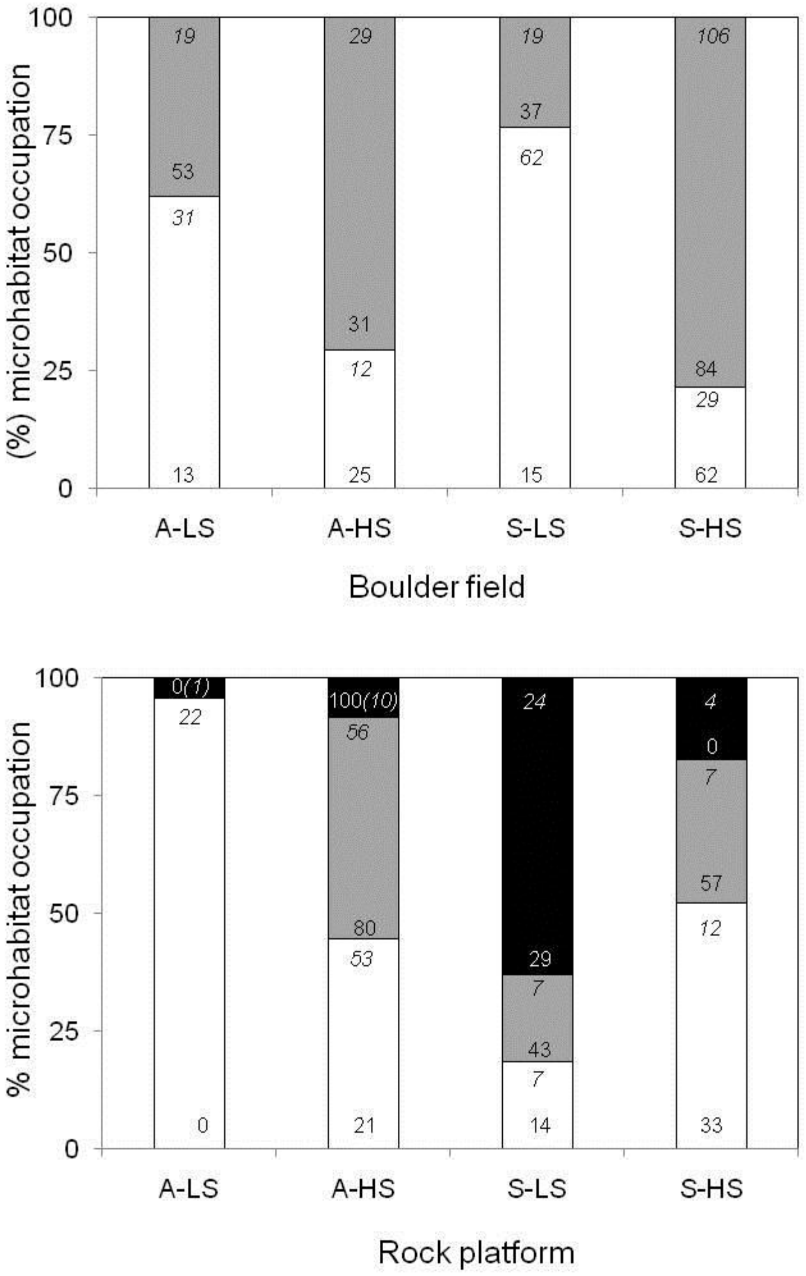

Figure 2.

Nerita atramentosa microhabitat occupation and aggregation frequency in both seasons and shore levels on boulder field (top**)** and rock platform (bottom**).** A-LS: autumn low shore, A-HS: autumn high shore, S-LS: summer low shore, S-HS: summer high shore. The white, grey and black bars respectively correspond to flat rock, crevice and under rock microhabitats. The numbers at the bottom of each bar indicate the proportion of aggregation observed within each microhabitat. The italic numbers at the top of each bar represent the total number of individuals observed within each microhabitat.

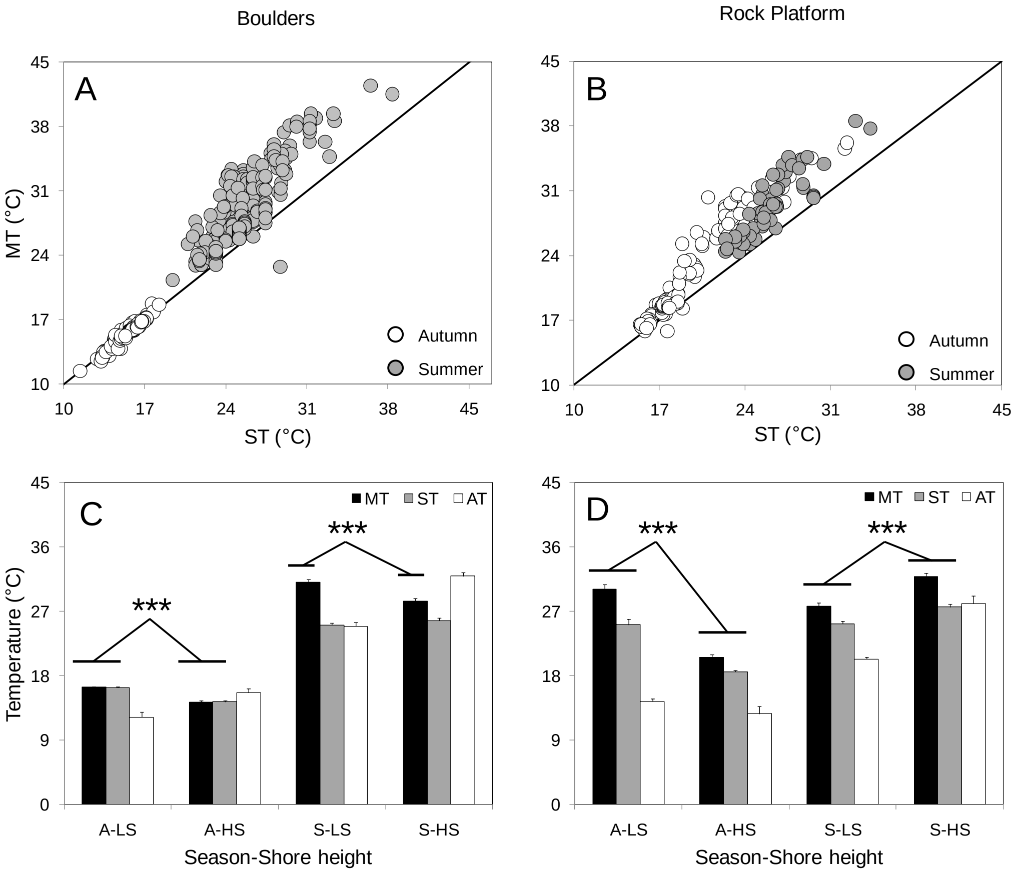

Figure 3.

Nerita atramentosa individual mantle temperatures (*MT*) and substratum temperatures (*ST*) and air temperature (*AT*; only on C and D) on the boulder field (A, C) and the rock platform (B, D) in both seasons. A-B: autumn and summer temperature values are respectively represented by white and grey circles (N = 510). The black line represents the first bissectrix, *i.e.* *MT* = *ST*. To improve the clarity of the graph, a value (47.93, 54.34) recorded in summer on the boulder field was removed. Positive and significant correlations were found between *MT* and *ST* in both habitats and at both seasons (Autumn: $\rho_B = 0.967$, P < 0.001, n = 91; $\rho_{RP} = 0.954$, P < 0.001, n = 142. Summer: $\rho_B = 0.827$, P < 0.001, n = 216; $\rho_{RP} = 0.883$, P < 0.001, n = 61). C-D: Mean values of *MT* (black bars) and *ST* (grey bar) observed in both season and shore levels. A-HS: autumn high shore level, A-LS: autumn low shore level, S-HS: summer high shore level, S-LS. Errors bars are standard errors. Mean *AT*s were calculated from the data collected in Port Stanvac (*i.e.* closest meteorological station from Marino Rocks, ca. 10 km apart) during the studied low tides (source: Bureau of Meteorology of Australia). ***: < 0.001 (Mann-Whitney U-Test).

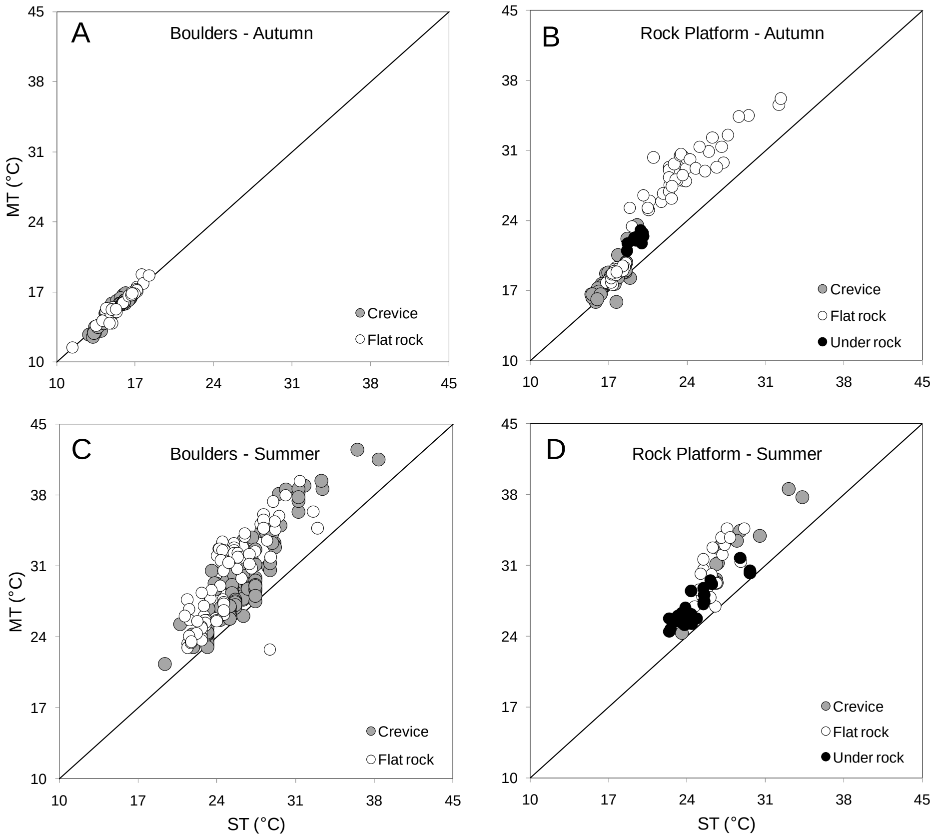

Figure 4.

Nerita atramentosa individual mantle temperatures (*MT*) versus substratum temperatures (*ST*) on the boulder field (A, C; N = 307) and the rock platform (B, D; N = 203) in autumn (A, B) and summer (C, D) in different microhabitats (white: flat rock, grey: crevice, black: under rocks). The black lines represent the first bissectrix, *i.e. MT* = *ST*. To improve the clarity of the graph, a value (47.93, 54.34) recorded in summer on the boulder field was removed.

Chapter V. Thermally-Mediated Body Temperature, Water Content and Aggregation Behaviour in the Intertidal Gastropod *Nerita atramentosa*

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CHAPTER V. TEMPERATURE, WATER CONTENT, AGGREGATION IN AN INTERTIDAL SNAIL

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 (i) 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 nighttimes, (ii) the potential relationship between environment temperature (air and substrata) and snail water content and body temperature, and (iii) 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 cm to a few m) than between habitats. This reinforces the evidence that mobile intertidal ectotherms could locally survive under warmer conditions if they can locate and behaviourally move 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.

CHAPTER V. TEMPERATURE, WATER CONTENT, AGGREGATION IN AN INTERTIDAL SNAIL

INTRODUCTION

Understanding how ectotherms physiologically adapt to heat stress and thermoregulate in response to climate warming is essential to determine the limits of their thermal tolerance window (Hofmann & Togdham, 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 & Berrigan, 2001), and ultimately sets species biogeographic ranges (Hofmann & Togdham, 2010). 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.

Ectothermic species can maintain body temperatures within their thermal tolerance window by responding to heat stress over a range of (i) temporal scales spanning from minutes to generations, hence involving processes such as behavioural thermoregulation (Dubois *et al.*, 2009; Munoz *et al.*, 2005), acclimatization (Sinclair *et al.*, 2006) and evolutionary adaptation (Somero, 2010), and (ii) spatial scales ranging from small-scale habitats (Chapperon & Seuront, 2011a; Denny *et al.*, 2011) to large-scale geographic distances (Osovitz & Hofmann, 2007). In particular, the survival of ectothermic species in the warming climate is strongly linked 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 & 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 & Underwood, 1996) has typically been considered to reduce both thermal and desiccation stresses (Garrity, 1984; Chapman & Underwood, 1992; Raffaelli & Hawkins, 1996; Stafford *et al.*, 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 *Nodilitorrina peruviana* maintain water content and thermoregulate better than solitary individuals (Rojas *et al.*, 2000). Similarly, aggregated mussels exhibit lower body temperatures (4 to 5ºC) 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 (i) the heterogeneity in snail temperature, water content and environment temperature, (ii) the relationship between snail water content, body temperature and environment temperature, and (iii) 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.

MATERIAL 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 (Chapperon & Seuront, pers. obs), 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 (i) geographic position, (ii) shell colour and (iii) 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* 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 & Seuront, 2011a, b). However, all aggregated individuals were randomly collected from different aggregations containing at least 5 individuals. Individuals were only collected from the centre of aggregations. Overall, body temperature and water content were measured from 90 to 100 adult individuals (17.3 ± 2.71 mm, $\overline{x} \pm \mathrm{SD}$) during each sampling session (*i.e.* daytime and nighttime low tides). All individuals were randomly chosen 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 significantly differ 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 mainly found under rocks within aggregations at daytime low tides (Chapperon & 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 cm up to 80 cm deep). *N. atramentosa* individuals mainly occupied crevices within aggregations at daytime low tides (Chapperon & 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 nighttimes. Daytime field work was conducted at midday low tide (*i.e.* 12 am to 2 pm) during four 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 hours 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 temperature 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 rocks (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, USA). 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 recently been used 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 & Seuront, 2011a; *Littoraria scabra* in mangroves, Chapperon & Seuront, 2011b). The thermal sensitivity of the thermal camera is ≤0.2ºC at 30ºC 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 & 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 (Fluke Corporation, 2006, USA) to assess snail body and substratum temperatures (Chapperon & Seuront, 2011a). For each thermal picture, a digital picture (Olympus J1 Tough-60, Olympus lmaging Corporation, Centre Valley, PA, USA) was jointly taken 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 ($N_{\text{total}} = 570$), and 304 solitary and 261 aggregated individuals on the rock platform ($N_{\text{total}} = 565$). 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 significantly differ between these two microhabitats (Chapperon & Seuront, 2011a). On the rock platform, studied individuals were found under rocks where they consistently aggregate and shelter from thermal stress (Chapperon & Seuront, 2011a).

Snail water content

Individual water content (N = 1135) 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 hours 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 which moves for 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 (Chapperon & Seuront, unpublished data).

Statistical analysis

Because the distributions of the variables *WC*, *MT*, *ST* and MST_{ratio} significantly diverged 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 Inc., 2009, USA).

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 nighttime, 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 nighttimes. On the rock platform, the air temperature measured under rocks (AT_{UR}) varied between 24ºC and 39ºC averaging at 30.1 ± 3.1ºC at daytime, and between 20ºC and 28ºC averaging at 23.1 ± 1.6ºC 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 significantly correlated 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 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 significantly differ 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 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 significantly increase the water content of snails (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 & 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 ºC on the boulder field on a single day) was greater than the temperature variability (*i.e.* 1.5ºC) 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 & 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 emphasize 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 ºC, 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 & Seuront, 2011a).

In contrast, the water content of *N. atramentosa* was not significantly correlated to any measured environmental temperature nor to snail temperature in both habitats during both day- and nighttimes. 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), mass (Gedan *et al.*, 2011), that create niche-specific micro-climatic conditions which vary over time. In the present study, individuals were directly exposed 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 greater substratum and individual body temperatures (*i.e.* up to 1.5ºC, 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 other abiotic factors (*e.g.* wind speed that was not measured in this study) determining of *N. atramentosa* body temperature and water content. Nevertheless, the boulder field did not seem to be as thermally stressful as anticipated since MST_{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 & 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 & 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 (Sainclair *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 & 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 generalize 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 & Seuront, 2011a).

N. atramentosa aggregations observed under rocks on the rock platform were not induced 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 *et al.*, 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 & Bull, 1984) and/or to breed as observed in others invertebrates (Cudney-Bueno *et al.*, 2008). Aggregations may also result from the following of conspecific mucous trails (*e.g.* Chelazzi *et al.*, 1985; Stafford & Davies, 2005; Chapperon & Seuront, 2011b) and food, home or conspecific source odour trails (Chelazzi *et al.*, 1985; Chapperon & Seuront, 2009). Since individuals preferentially select rock bottoms in summer to thermoregulate (Chapperon & 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 (i) the heterogeneity in environment thermal properties identified at the centimetre scale (*e.g.* this study; Chapperon & Seuront, 2011a, b; Denny *et al.*, 2011) that creates potential thermal refuges under heat stress (*e.g.* rock bottoms in this study), (ii) 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), (iii) 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 & Seuront, 2011a, b; Miller & Denny, 2011) and (iv) 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 & 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 & Seuront, 2011a, b; Miller & 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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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	$\bar{x}$ (SD	0.112 (0.022)	0.115 (0.016)	0.115 (0.019)	0.130 (0.012)
(g. cm^{-3})	Min	0.031	0.089	0.049	0.083
	Max	0.314	0.160	0.187	0.161
S-WC	$\bar{x}$ (SD	0.119 (0.048)	0.113 (0.014)	0.109 (0.020)	0.133 (0.011)
(g. cm^{-3})	Min	0.057	0.079	0.041	0.103
	Max	0.443	0.162	0.188	0.161
A-MT	$\bar{x}$ (SD	29.096 (3.123)	18.827 (1.727)	30.898 (3.251)	17.977 (1.357)
(°C)	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
$A\text{-}MST_{ratio}$	$\bar{x}$ (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
$S\text{-}MST_{ratio}$	$\bar{x}$ (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

Table 2.

Mann-Whitney *U* test to investigate differences in *WC*, *MT*, *ST*, MST_{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). NS: non significant.

Individual Status	RP. vs. B	Day			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
	MST_{ratio}	-1.540	0.124	NS	-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
	MST_{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
	MST_{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
	MST_{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
	MST_{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
	MST_{ratio}	-17.206	<0.001	D > N	-14.222	<0.001	D > N

Table 3.

Mann-Whitney *U* test to investigate differences in *WC*, *MT*, *ST*, MST_{ratio} between aggregated (A) and solitary individuals (S) at both times (day time D and night time N) in both habitats (boulders B and rock platform RP). NS: non significant.

Boulder Field	Day			Night		
S vs. A	Z	P	Sig.	Z	P	Sig.
WC	-3.254	.001	S < A	-1.833	.067	NS
MT	-1.188	.235	NS	-1.478	.139	NS
ST	-2.996	.003	S < A	-.176	.860	NS
MST_{ratio}	-3.283	.001	S > A	-2.025	.043	S > A
Rock Platform	Day			Night		
S vs. A	Z	P	Sig.	Z	P	Sig.
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
MST_{ratio}	-0.933	0.351	NS	-0.743	0.458	NS

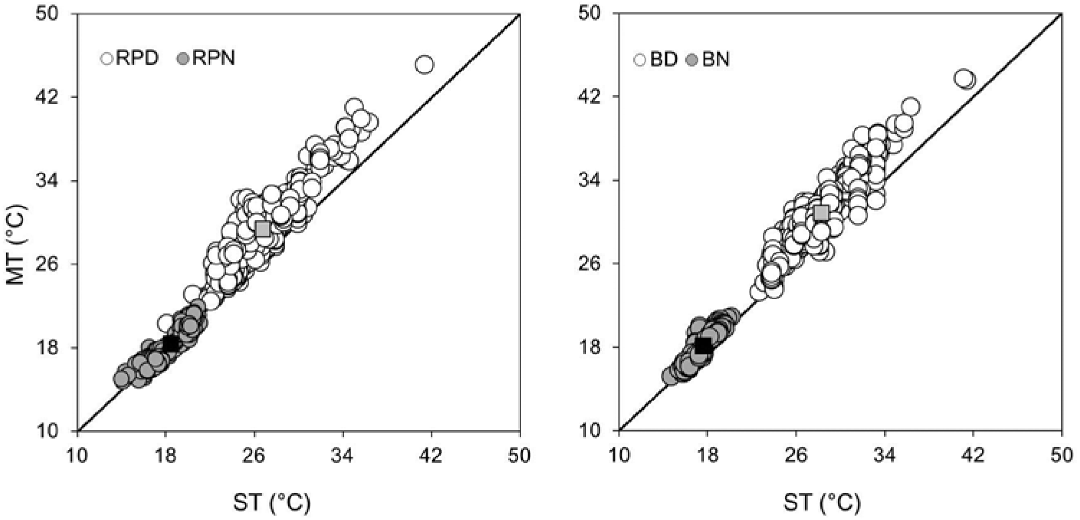

Figure 1.

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 bisectrix, *i.e.* *MT* = *ST*. RP-Day: N = 378, RP-Night: N = 187, B-Day: N = 380, B-Night: N = 190.

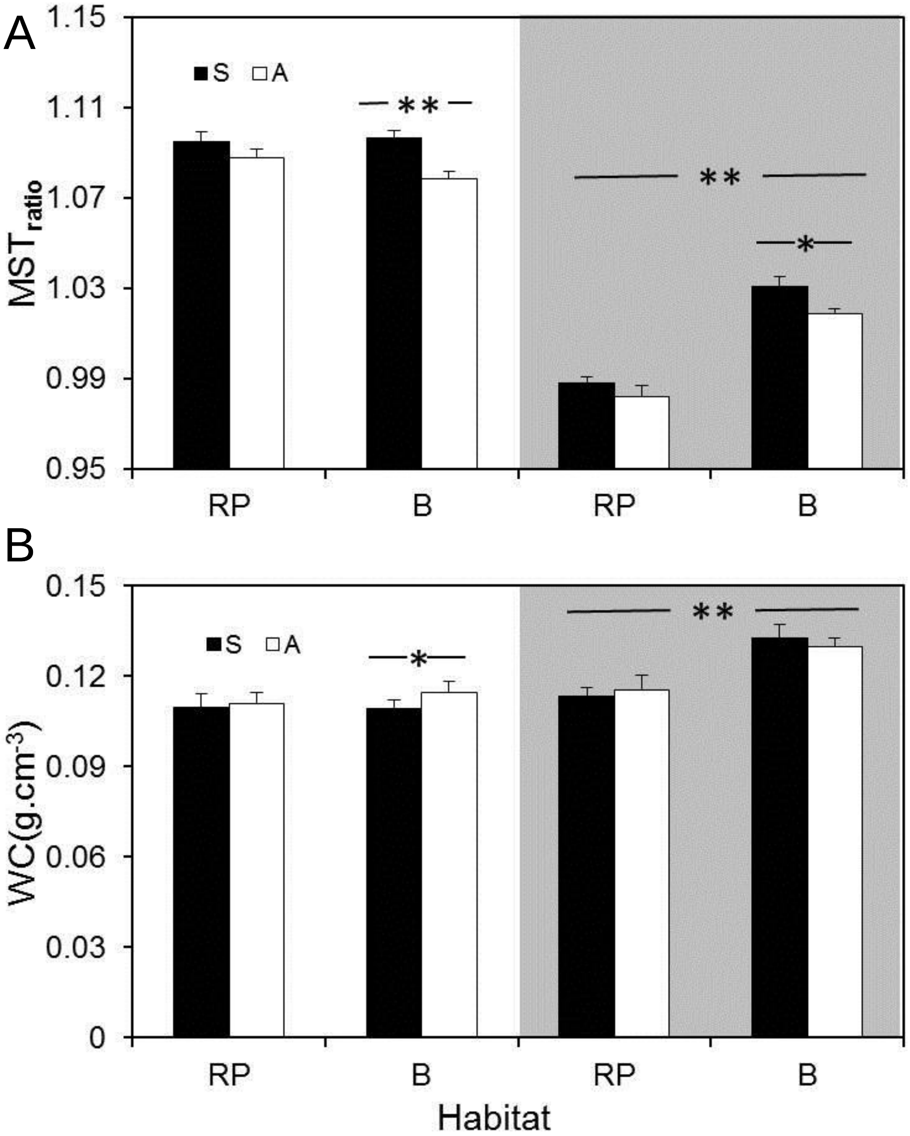

Figure 2.

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 were 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.

Chapter VI. Keeping warm in the cold: on the thermal benefits of aggregation behaviour in an intertidal ectotherm

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CHAPTER VI. AGGREGATION IN INTERTIDAL SNAIL: THERMAL BENEFITS IN THE COLD

ABSTRACT

Temperature is a primary determinant for species geographic ranges. In the context of global warming, most attention focuses upon the potential effects of heat stress on the future distribution of ectothermic species. Much less attention has, however, been given to cold thermal stress although it also sets species thermal window limits, hence distribution ranges. This study was conducted in winter on a south-Australian rocky shore in order to investigate the potential thermal benefits of the aggregation behaviour observed in the dominant gastropod Nerita atramentosa*. Thermal imaging was used to measure the body temperatures of 3681 aggregated individuals and 226 solitary individuals, and surrounding substratum.* N. atramentosa *aggregates and solitary individuals were significantly warmer than their surrounding substratum. The temperature deviation between aggregates and substratum was, however, ca. 2 ºC warmer than the one observed between solitary individuals and substratum. This result is critical since a body temperature increase of only a few degrees might enhance individual performance, hence organismal fitness, and could potentially drive changes in interspecific relationships. Besides, the potential higher thermal inertia of aggregates might increase the snail adaptive ability to abrupt environmental changes. We further investigate the potential thermal heterogeneity within an aggregate in order to identify any thermally advantageous position. Patch centres are significantly warmer than their edges, hence snails experience greater thermal advantages in the aggregate centre. Finally, we examined the potential effect of aggregate size on snail temperature and thermal spatial heterogeneity. We identified an aggregate size threshold (216 individuals) beyond which all snails had equal thermal benefits, regardless of* *their spatial positions within an aggregate. While the determinism of this aggregate size threshold requires further investigations, the present work uniquely identified the thermal benefits of aggregation behaviour for intertidal ectotherms under cold weather conditions. The implications of the present finding are discussed in the general framework of the ability of ectothermic populations to face environmental changes.*

Keywords: thermoregulatory behaviour; aggregation; ectotherm; intertidal; cold adaptation; *Nerita* atramentosa; thermal imaging

CHAPTER VI. AGGREGATION IN INTERTIDAL SNAIL: THERMAL BENEFITS IN THE COLD

INTRODUCTION

In the changing climate, the scientific community faces the challenge to determine the future distribution ranges of ectothermic species. Temperature is indeed one of the main factors that set both the patterns of activity and the limits of biogeographic distribution of marine invertebrates (*e.g.* Jones *et al.*, 2010). Temperature impacts the physiology and the behaviour of intertidal organisms (Somero, 2002; Angilletta, 2009; Munoz *et al.*, 2008), hence has a cascading effect on all ecological processes that impact populations and communities structure and dynamics of intertidal ecosystems (Morelissen & Harley, 2007; Kordas *et al.*, 2011). More specifically, temperature impacts the performance of organisms (*e.g.* locomotion, growth, reproduction, feeding activities), which appears to be optimal at intermediate body temperatures (Angilletta *et al.*, 2002). Beyond minimal and maximal limits of thermal tolerance, exposures to extremely high or low temperatures disrupt rates of metabolical and physiological processes (Pörtner, 2002), hence affect organismal fitness.

Intertidal ectotherms experience extreme thermal stress and frequently face variations of temperatures greater than 20ºC over a tidal cycle (Helmuth, 2002). As a consequence, they live at, or close to, the upper edge of their thermal tolerance window (Somero, 2002) and as such are likely to be particularly vulnerable to the fast warming climate. Most recent studies have therefore focused upon the thermoregulatory abilities of intertidal ectotherms to adapt the predicted increase in environmental temperatures and the frequency and intensity of extreme events, *e.g.* heat waves and droughts (IPCC, 2007). In particular under heat stress, individuals may either shift to an anaerobic metabolism (Pörtner, 2002) or exhibit physiological and biochemical responses at the genomic, molecular and cellular levels to maintain the homeostasis of the whole organism physiological system (*e.g.* Meng *et al.*, 2009). Besides, ectothermic organisms may maintain body temperatures within the thermal tolerance window through behavioural adaptation to extreme heat stress (Chapperon & Seuront, 2011a, b; Miller & Denny, 2011). Behavioural adaptations to thermal stresses mainly consist in modifying the heat fluxes from and toward organisms by respectively enhancing or decreasing the body surface area in contact with the environmental variables that drive body temperature, *e.g.* substratum (Chapperon & Seuront, 2011a, b) and solar heat (Marshall *et al.*, 2010; Helmuth *et al.*, 2011). Such behavioural responses to heat stress include resting posture (Lang *et al.*, 1998), body orientation (Munoz *et al.*, 2005; Miller & Denny, 2011), mushrooming behaviour (Williams *et al.*, 2005), aggregation behaviour (Chapperon & Seuront, 2011a; Stafford *et al.*, 2011, 2012) and habitat selection (Jones & Boulding, 1999; Chapperon & Seuront, 2011b). Understanding how ectotherms may adapt to heat stress is critical since heat-waves have recently caused local invertebrate mass mortalities (Garrabou *et al.*, 2009), that are in turn likely to impact the balance of the whole ecosystem. Although long-term climate change predictions suggest a global decrease in the number of cold days/nights (*e.g.* Planton *et al.*, 2008), localised extremely cold winters still occur and may have significant impacts on the structure and functioning of the intertidal ecosystem by affecting both recruitment, survival and hence distribution of invertebrates (Wethey *et al.*, 2011; Firth *et al.*, 2011). Despite recent evidence that the geographic range of ectothermic species is also set by their ability to adapt, tolerate or resist temperatures below the lower limit of their thermal tolerance window (Wethey *et* *al.*, 2011), much less attention has been given to the thermoregulatory abilities of intertidal ecotherms under extremely cold conditions.

Under freezing conditions, ectotherms exhibit two types of cold hardiness strategies: freezing tolerance and freezing avoidance, which are respectively driven by the production of cryoprotectant substances and antifreeze proteins (Voituron *et al.*, 2002). However, under non-freezing conditions, ectotherms have developed a range of behavioural adaptations to cold conditions (*e.g.* Lencioni, 2004) such as habitat selection through migration to thermally-favourable microhabitats, basking behaviour, shuttling heliothermy and aggregation. Nevertheless, the thermal benefits of aggregation behaviour (also referred to as huddling behaviour, crèching behaviour, grouping behaviour and clustering behaviour) in cold conditions has mainly been studied among birds and mammals; see Gilbert *et al.* (2010) for a review. Typically "huddles" (*i.e.* group in which animals keep close body contacts in order to save energy; Canals & Bozinovic, 2011), reduce metabolic rates and maintain all individuals (up to hundreds of organisms) warmer than the surrounding environment (Gilbert *et al.*, 2010). In endotherms, huddling behaviour is considered as a social and cooperative thermoregulation (Haig, 2008; Gilbert *et al.*, 2010). In particular, individuals within a huddle do not appear to compete for a thermally favourable position such as the centre of the huddle, but rather share it by continuously moving within the huddle (*e.g.* Zitterbart *et al.*, 2011). Aggregation such as huddling behaviour has also been shown to provide thermal benefits over winter in ectothermic organisms such as lizards (Shah *et al.*, 2003) and frogs (Espinoza & Quinteros, 2008). In the intertidal, aggregation behaviour is, however, typically considered as an adaptation to heat and desiccation stresses (Garrity, 1984;

Chapman & Underwood, 1992; Raffaelli & Hawkins, 1996), and to our knowledge the potential thermoregulatory role of aggregation behaviour under winter conditions is still to be quantitatively investigated in intertidal snails.

Aggregation behaviour in the intertidal gastropod *Nerita atramentosa*, a dominant competitor for microalgae on Australian rocky shores, reduces body temperatures (Chapperon & Seuront, 2011a) and increases water content (Chapperon & Seuront, non-published data) on boulder fields under conditions of high thermal and desiccation stresses. However, the factors underlying the aggregation behaviour observed at others times (*e.g.* winter) and places (*e.g.* under rocks on rock platform; Chapperon & Seuront, 2011a) are still not clearly understood. In this context, the present study investigates the potential thermoregulation role played by *N. atramentosa* aggregation behaviour in winter under cool conditions. More specifically, the aims of this work were (i) to compare the body temperatures of aggregated and solitary individuals, (ii) to investigate the potential thermal heterogeneity within an aggregate in order to identify any potential thermally advantageous position within an aggregate and (iii) to assess the potential effect of aggregate size on its temperature and thermal heterogeneity.

MATERIALS AND METHODS

Study species and site

The intertidal gastropod *Nerita atramentosa* (Reeve, 1855) plays a strategic role in intertidal trophic web; it is a superior competitor for microalgae on intertidal rocky shores (Underwood, 1984) and facilitates the growth of microalgal food resources (Murphy *et al.*, 2008). This species grazes while immerged and tends to form aggregates at low tide throughout the year (Underwood, 1976).

We investigated *N. atramentosa* aggregation behaviour in winter 2009 on a temperate South Australian rocky shore at Billy Light's point (34° 43' 74" S, 135° 24' 17" E), which is situated on Boston Bay at the southern extremity of the Eyre Peninsula. This site is characterised by a variety of habitats of different topographic complexity (*e.g.* rock platform to boulders) and inhabitants (*e.g.* the snail *Nodilittorina* sp. and the brown algae *Hormosira banksii*), and especially abounds with *N. atramentosa*. During the study period (July 2009), most *N. atramentosa* individuals distributed within large aggregates (up to several hundreds of individuals), consistently situated on exposed flat substratum either on top of boulders (Fig. 1A) or on rock platform (Fig. 1B).

Thermal properties of solitary and aggregated snails, and surrounding substratum

To determine the potential role played by the aggregation behaviour on *N. atramentosa* thermoregulation, we examined the potential temperature difference between solitary individuals and aggregates during different morning low tides (*i.e.* 9 to 12 am) in the austral winter 2009. In July 2009, air temperatures in the studied area ranged between 5 ºC and 21.3ºC, which are temperatures characteristic of a typical South Australian winter (Bureau of Meteorology, Australia). An aggregate was defined as the grouping of at least two individuals in direct shell contact (Chapperon & Seuront, 2011a, b). In this study, however, the minimum aggregate size among all randomly chosen aggregates was 37 individuals. Thermal imaging was used to measure the individual body temperature of 226 solitary snails and the mean temperature of 35 aggregates (*i.e.* 3681 individuals) that were haphazardly chosen on flat exposed surfaces situated on rock platforms and boulders (Fig. 1). The thermal sensitivity of the thermal camera (Fluke® Ti20, Fluke Corporation, Everett, Washington, USA) is ≤0.2ºC at 30ºC 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 & Seuront (2011a) for further details. The body temperatures of solitary individuals, the mean temperatures of aggregates and the temperatures of surrounding substratum were subsequently calculated after analysing the thermal pictures with the InsideIR 4.0 software (Fluke Corporation, 2006, USA). More specifically, a closed curve marker was drawn around each photographed solitary snail and each aggregate in order to calculate the mean temperature value of solitary body and aggregates (BT_s and BT_a, respectively; Chapperon & Seuront, 2011a). Here, an aggregate was considered as a single entity, hence the mean temperature of an aggregate corresponds to the average of temperature of all individuals present in an aggregate. In addition, the substratum temperature (*ST)* was averaged from 4 linear markers drawn on the substratum directly surrounding the individual shell or the aggregate (Chapperon & Seuront, 2011a). The mantle temperature (*MT*) of solitary individuals and aggregates were subsequently estimated from BT_s and BT_a following the empiric relationship (Caddy-Retalic *et al.*, 2011; Chapperon & Seuront, 2011a):

$$BT = 0.8875MT + 2.7044 \tag{1}$$

The ratio between *MT* and *ST* (MST_{ratio} hereafter) was calculated for each individual and each aggregate to identify the temperature deviation between organism, aggregate and surrounding substratum (Chapperon & Seuront, 2011a, b).

Thermal heterogeneity within aggregates

Absolute thermal heterogeneity

To determine the potential existence of a thermally favourable position within an aggregate, five thermal profiles were haphazardly drawn on each thermal picture of aggregates and surrounding substratum using the InsideIR 4.0 software (Fluke Corporation, 2006, USA). Each thermal profile was drawn through the core of an aggregate and extended outside each edge of the aggregate on the surrounding substratum (Fig. 2). Each thermal profile was divided into five spatial categories, *i.e.* substratum, outer edge, inner edge, outer centre, inner centre (Fig. 2). More specifically, the spatial category "substratum" corresponds to the substratum temperature surrounding the aggregate along the thermal profile, while the width of the four remaining spatial categories each corresponds to ¼ of the thermal profile length. Note that for consistency, the substratum category has the same width as the categories within a given patch. The same number of temperature values was considered for each spatial category within an aggregate. In order to determine the potential heterogeneity in the thermal properties of the different spatial categories within an aggregate, the body temperatures measured along a thermal profile were sorted from the coolest to the warmest temperatures. Four thermal categories were subsequently defined as C_1 (the coldest), C_2 (cold), C_3 (warm), and C_4 (the warmest), based on the number of values in the first, second, third and fourth quartiles of the total number of temperature values obtained along the thermal profile within an aggregate. The frequency of occurrence of each thermal category (C_1, C_2, C_3, and C_4) in the different spatial categories of each aggregate was subsequently calculated.

Relative thermal heterogeneity

The mantle temperature MT at the inner centre, outer centre, inner edge and outer edge of each aggregate *i.e.* MT_{IC}, MT_{OC}, MT_{IE} and MT_{OE} was estimated from $BT_{a(IC)}$, $BT_{a(OC)}$, $BT_{a(IE)}$ and $BT_{a(OE)}$ (see Eq. 1). The ratio, MST_{ratio}, between MT and ST was subsequently calculated for each aggregate spatial category to identify the temperature deviation between aggregated individuals occupying different position in an aggregate and the surrounding substratum.

Aggregate size and temperature

The number of individuals N within each aggregate was determined from digital pictures taken in the field (Olympus J1 Tough-60, Olympus lmaging Corporation, Centre Valley, PA, USA) in order to investigate the potential effect of aggregate size on both the MST_{ratio} estimated for a whole aggregate and each aggregate spatial category. The ratio between the mantle temperatures estimated for the four aggregate spatial categories were subsequently estimated as MT_{IC}/MT_{OC}, MT_{IC}/MT_{OC} and MT_{IC}/MT_{OC} in order to investigate how MT varies within aggregates with aggregate size.

Statistical analyses

Parametric tests were used throughout the manuscript since all studied parameters (MT, ST, MST_{ratio}) were normally distributed (Kolmogorov-Smirnoff test, $p > 0.05$). Comparisons of MT, ST and MST_{ratio} between aggregates and solitary individuals were run with the independent *t*-test. Comparisons of MST_{ratio} between the different spatial categories within aggregates were conducted with an ANOVA test and a subsequent Tukey test. Correlations between (i) *MT* of aggregates and solitary individuals, and *ST*, (ii) MST_{ratio} of aggregates and of the different aggregate spatial categories and aggregate size and (iii) MT_{ratio} between the different aggregate spatial categories were investigated with the Pearson correlation coefficient. All statistical analysis was run in PASW STATISTICS 18 (SPSS Inc., Chicago, IL, USA, 2009). Chi-square analysis of contingency tables was run to determine the potential independence between *MT* and the spatial position within aggregates (Zar, 2010).

RESULTS

Solitary individuals vs. aggregates (Fig. 3)

No significant difference ($t = 1.048$, $df = 47.923$, $p = 0.300$) was observed between the mantle temperature of solitary individuals (MT_s = 16.41 ± 0.21ºC, mean ± standard error) and aggregates (MT_a = 16.96 ± 0.49ºC). MT_s and MT_a respectively ranged from 10.16 ºC to 25.42ºC and 12.38ºC to 28ºC. In contrast, solitary individuals were consistently found on substratum significantly warmer (*ST* = 13.59 ± 0.18ºC) than aggregates (*ST* = 12.17 ± 0.43ºC; $t = -2.891$, $df = 259$, $p = 0.004$). The temperature of solitary individuals and aggregates were significantly and positively correlated with the surrounding substratum temperature ($r_s = 0.937$, $p < 0.001$; $r_a = 0.959$, $p < 0.001$). The temperature deviation between aggregates and the adjacent substratum (MST_{ratio} = 1.41 ± 0.02ºC) was significantly greater than the one estimated for solitary individuals (MST_{ratio} = 1.21 ± 0.01ºC, $t = 12.099$, $df = 259$, $p < 0.001$). More specifically, aggregates and solitary individuals were on average 4.78 ± 0.14 ºC and 2.82 ± 0.08 ºC warmer than rocks, respectively.

Thermal heterogeneity within aggregates

Absolute thermal heterogeneity (Fig. 4)

The frequency of occurrence of the four aggregate thermal categories (C_1, C_2, C_3, and C_4) significantly depends on the spatial position within a patch (χ^2 = 715.747, df = 9, p < 0.001). In particular, the temperature categories C_1 to C_3 were not uniformly distributed within aggregates ($\chi^2_1 = 527.047$, $\chi^2_2 = 62.996$, χ^2_3 = 140.326, df = 3, p < 0.001). The coldest temperatures were more frequent at the outer edge of aggregates (42 %) and less frequent at the inner centre (16 %). Warm temperatures were instead more frequent at the inner centre (30 %) and less frequent at the outer edge (16 %). In contrast, the warmest temperatures (thermal category C_4) were uniformly distributed in aggregates (χ^2 = 4.532, df = 3, p > 0.05). Overall, the inner and outer edges of aggregates were mainly characterised by cool temperatures (55.64%), while their inner and outer centres were mainly formed by hot temperatures (54.22%).

Relative thermal heterogeneity. (Fig. 5)

Mean *MT* did not significantly differ between the aggregate spatial categories (F = 0.694, df = 3, p = 0.556). On average, the range in *MT* within aggregates was 3.85 ± 0.18ºC. Mean MST_{ratio} were, however, significantly different between the aggregate spatial categories (F = 2.718, df = 3, p = 0.045). The outer edge MST_{ratio} was significantly lower than at the inner edge (p = 0.039), outer center (p = 0.018) and inner centre (p = 0.013). In addition, MST_{ratio} estimated for solitary individuals was consistently significantly lower than the MST_{ratio} estimated from the aggregate spatial categories ($p < 0.001$).

Aggregate size and temperature

Aggregates contained from 37 to 268 individuals. MST_{ratio} averaged over each aggregate was not significantly correlated to the size of aggregates (r = 0.192, p = 0.081). Similar results were obtained at the inner edge (r = 0.192, p = 0.080), outer centre (r = 0.125, p = 0.257) and inner centre (r = 0.087, p = 0.430). In contrast, at the outer edge, MST_{ratio} was significantly positively correlated to aggregate size (r = 0.325, p = 0.003).

Besides, MT averaged over each aggregate was significantly negatively correlated to aggregate size (r =-0.277, p = 0.011). This was also found at the outer edge (r = -0.215, p = 0.050), inner edge (r = -0.266, p = 0.014), outer centre (r = -0.298, p = 0.006) and inner centre (r = -0.320, p = 0.003). The ratio, MT_{ratio}, between the mantle temperatures of the inner centre of aggregates, and their outer edge and the inner centre significantly decreased with increasing aggregate size (MT_{IC}/MT_{OE}: r = -0.361, p < 0.0001; MT_{IC}/MT_{IE}: r = -0.224, p < 0.05). MT_{ratio} calculated between the aggregate inner and outer center did not correlate with the aggregate size (r = -0.124, p > 0.05). More specifically, the significant linear regression between MT_{IC}/MT_{OE} and MT_{IC}/MT_{IE} and, aggregate size (*i.e.* $MT_{IC}/MT_{OE} = -0.0003N + 1.064$; $MT_{IC}/MT_{IE} = -0.0001N + 1.0216$) implies that $MT_{IC}/MT_{OE} = 1$ and $MT_{IC}/MT_{IE} = 1$ when N = 213 and N = 216, respectively. This suggests that over winter snails placed at the core of aggregates thermally benefit from their position in comparison to snails occupying the edge of aggregates. This advantage, however, vanishes beyond a threshold aggregate size of 216 individuals.

DISCUSSION

Winter aggregation in the intertidal: a behavioural adaptation to the cold

Many invertebrates behaviourally aggregate in intertidal habitats. The formation of groups has been reported to provide benefits and/or protection in regards to a range of biological and physical factors such as reproduction (Cudney-Bueno *et al.*, 2008) and predation (Coleman *et al.*, 2004), and hydrodynamism (Moran, 1985). Most attention has, however, been given to the advantages of aggregation behaviour under desiccation stress (Chapman & Underwood, 1996; Soto & Bozinovic, 1998; Rojas *et al.*, 2000; Stafford *et al.,* 2011, 2012) and heat stress (Garrity, 1984; Munoz *et al.*, 2008; Chapperon & Seuront, 2011a). Aggregation is, however, a complex, species-specific behaviour that varies over time and space, and most likely results from the interaction or the succession of multiple factors (Munoz *et al.*, 2008). In *N. atramentosa,* aggregation behaviour also appears to vary spatially (between habitats and shore height) and temporally on both diel and seasonal bases, and although the factors beyond grouping are likely to be numerous, only a few have been clearly identified for this species (*e.g.* heat/desiccation stresses, Chapperon & Seuront, 2011a).

Here, we show that *N. atramentosa* aggregates observed in winter on the top of boulders and flat rocks were consistently warmer than solitary individuals on similar microhabitats. To our knowledge this is the first evidence of wintering thermal benefits of aggregation behaviour under non-freezing conditions among intertidal invertebrates. On average, the temperature between aggregated snails and their substratum was 2°C warmer than that identified between solitary individuals and substratum. Winter thermal aggregations have been identified in others ectotherms such as reptiles (Shah *et al.*, 2003) and insects (Eskov & Toboev, 2011). For instance, sun-exposed toads in knots have been shown to be on average 2.3°C warmer than solitary ones (Espinoza & Quinteros, 2008), which is consistent with our findings. This thermoregulatory behaviour in winter appears critically important since a body temperature increase of only a few degrees might confer advantages in terms of performance such as activity and feeding rates (Yee & Murray, 2004), growth (Klok & Chown, 1999; Espinoza & Quinteros, 2008) and energy saving that has extensively been shown in endotherms, especially penguins (Lawless *et al.*, 2001). An increase in ectotherms' body temperatures in cold conditions could therefore drive changes in interspecific relationships (Kordas *et al.*, 2011). This is critical since *N. atramentosa* is a dominant competitor for microalgae that are particularly abundant in winter. The subsequent energy saving during winter low tides may hence boost *N. atramentosa* feeding activities during the high tide, which are likely to be reduced in winter due to cold water temperature (Seuffert *et al.*, 2010). This increase in *N. atramentosa* grazing behaviour likely represents a competitive advantage towards other species such as *Austrocochlea constricta* and *A. concamerata* that co-exist in the study area with *N. atramentosa*. Competition for microalgae might therefore be intensified which could subsequently modify microalgal resource abundance. Further investigations are, however, needed to identify the potential consequences of the thermal benefits of aggregation behaviour in winter on intertidal communities.

Besides, aggregates increase thermal inertia in a range of ectotherms, *e.g.* mussels (Helmuth, 1998), geckos (Shah *et al.*, 2003), and snakes (Reiserer *et al.*, 2008), hence reduce rates of thermal exchanges better than solitary organisms. This potential higher thermal inertia in *N. atramentosa* implies that aggregated snails cool down more slowly than solitary individuals, hence buffers the effects of rapid cooling in winter. Aggregated individuals are thus better protected from environmental thermal fluctuations experienced in winter (Shah *et al.*,2003; Reiserer *et al.*, 2008) than solitary individuals.

Winter snail aggregates: why are they warmer than solitary snails?

The mechanisms conferring thermal benefits to aggregated snails in the cold are inherent to the factors that control heat transfers from and towards an individual, *e.g.* solar radiation, wind speed, air/substratum temperature, animal physiology, morphology, size, colour and behaviour (Helmuth, 1998; Marshall *et al.*, 2010; Chapperon & Seuront 2011a, b; Miller & Denny, 2011); see also Denny & Harley (2006) for a detailed description of heat-budget models. Main heat transfers occur through (i) convection (transfer of heat from snails to the environment due to air motion), (ii) conduction (transfer of energy between immobile snails, and snails and substratum in physical contact), (iii) radiation (transfer of energy from and out snails due to emission and absorption of electromagnetic radiations), and (iv) condensation (heat released during evaporative cooling). In order to increase body temperatures, outgoing and incoming heat fluxes should then be minimized and maximised, respectively.

In this context, aggregated *N. atramentosa* individuals were probably warmer than solitary individuals since grouping minimizes evaporation and convection rates, through a decrease in surface-to-volume ratio in contact with the surrounding microclimate and a thicker boundary layer over organism surfaces (Monteith & Unsworth, 1990). Aggregates thus might, to some extent, be considered as a meta-organism which allows solitary individuals to increase their relative size, hence to experience the thermal benefits related to larger animals. Indeed, large ectotherms can be warmer than small body size organisms when evaporative cooling is limited (Helmuth, 1998) which is likely be the case in winter when heat stress is inexistent or rare. Besides, large aggregates have been shown to optimize incoming heat fluxes through an increase in solar radiation absorption compared to small aggregates and solitary organisms (Klok & Chown, 1999). This is in agreement with the observed significant increase in MST_{ratio} observed at the outer edge of aggregates with increasing aggregation size in *N. atramentosa*.

In addition, the solar energy inflow might have further been facilitated by the microhabitat occupation of *N. atramentosa* that were consistently observed on top of boulders or flat rock directly exposed to solar heating (see Fig. 1). This is in accordance with the basking behaviour observed in vertebrate ectotherms, *e.g.* lizards and turtles (Díaz & Cabezas- Díaz, 2004; Dubois *et al.*, 2009). Likewise, the absorption and retention of solar radiation is probably enhanced by *N. atramentosa* black shell (McMahon, 1990). The need to minimize energy losses during winter might explain why this species has maintained a dark pigmented colour despite its potential disadvantage under the thermally extreme conditions frequently experienced in South Australia during summer. Note, however, that *N. atramentosa* has also developed some thermoregulatory behaviours, such as microhabitat selection and aggregation behaviours, in order to face heat stress during summer (Chapperon & Seuront, 2011a).

Winter snail aggregates: when spatial position and size matter

The temperature deviation between the substratum and the snails located at the edge of aggregates was lower than the one between the substratum and the centre of the aggregate. However, it was still significantly greater than the one observed for solitary snails. This indicates that it is thermally beneficial for *N. atramentosa* to group in winter regardless of the position within an aggregate. The greater frequency of cool temperatures at the edge of aggregates, however, implies that thermal benefits are more important for snails situated in central positions. This is consistent with the increase in convective and evaporative rates occurring at the edge of aggregates where the surface-to-volume ratio in contact with the air increases (Denny *et al.*, 2011). Others ectotherms such as snakes homogenize thermal benefits between different group members through individual movements within an aggregate (Reiserer *et al.*, 2008). This is, however, not applicable to *N. atramentosa*, as individuals were consistently found to be immobile on dry substratum during emersion.

Fitness has been shown to vary with the spatial position occupied within a group (Morrel & Romey, 2008). Indeed, disparities exist in performance, predation, desiccation and dislodgement risks, feeding and reproductive gains between individuals occupying different spatial position within groups (Krause & Ruxton, 2002). For instance, predation risk is greater on peripheral positions, and referred to as marginal predation (Hamilton, 1971; Okamura, 1986; Stankowich, 2003; Morrel & Romey, 2008). Feeding rates are also generally greater at the edge on dispersed food resources since individuals start to forage earlier than those situated in the middle (Morrel & Romey, 2008; but see Coleman *et al.*, 2004). Different trade-offs between fitness components hence exist in relation to the different spatial positions within aggregates. An optimal spatial position that maximizes survival, performance and reproduction thus appears unlikely and the spatial position preference within aggregates depends on a range of factors, *e.g.* individual physiological state, food abundance and availability, environmental temperature (Morrel & Romey, 2008). In this study, however, central positions within aggregates seem to be the best to occupy in order (i) to reduce the intrinsically high predation risk related to snails living on exposed flat substratum that makes them more conspicuous for crabs and birds, (ii) to optimise the exploitation of microalgal resources that are abundant in winter (Morrel & Romey, 2008) and (iii) to increase thermal benefits (this study). This implies that *N. atramentosa* aggregation in winter should be compact and increase in size as individuals would prefer to occupy central positions (Morrel & Romey, 2008), which is consistent with our observations. Besides, larger groups can reduce predation attacks (Krause & Ruxton, 2002), and they are expected to have a greater thermal inertia than smaller ones (Helmuth, 1998). This is consistent with the great proportion of *N. atramentosa* aggregates that contained more than 100 individuals (ca. 70%) in this study. It is stressed, however, that 90% of aggregates were smaller than 216 individuals, which correspond to the aggregation size at which all snails regardless of their spatial positions would have equal thermal benefits. This critical aggregation size therefore likely optimizes both individual thermal benefits and group thermal inertia, hence bigger aggregates may not be as thermally efficient, but are still likely to be beneficial in terms of *e.g.* minimising predation. Further investigations are, however, required to validate this hypothesis and also to identify others parameters, *e.g.* feeding behaviour, which might participate in the determinism of this aggregation size threshold and/or the maintenance of aggregation size below 216 individuals.

Conclusion

Aggregations in intertidal snails have been identified as self-organised systems which provide benefits to the whole aggregate under hot and dehydrating conditions (Stafford *et al.*, 2007). The present study quantitatively demonstrated the thermal benefits of snail aggregations under cold conditions. Simple behavioural rules have previously been used to explain the formation of snail aggregations on intertidal rocky shores i.e. aggregations are formed when individual movements stop on the encounter of crevices, conspecifics, mucous trails (Stafford *et al.*, 2007, 2012). Here, aggregations were found on flat surfaces which exclude the presence of crevices as a trigger of the formation of aggregations. It is however probable that *N. atramentosa* individuals either followed conspecifics and/or conspecific trails to form aggregations as demonstrated in others gastropod species (e.g. *Littoraria scabra*, Chapperon & Seuront, 2011b). Since many others factors could have initiated *N. atramentosa* aggregations in the present study (e.g. food distribution, Chapperon & Seuront, 2012; pre-breeding, Feare, 1971; chemical trails released by conspecifics and/or microlagal resources, Chapperon & Seuront, 2009), further investigation is required to identify the cause(s) of *N. atramentosa* aggregations. Regardless of what factor(s) stimulate the formation of aggregations, *N. atramentosa* aggregations can be seen as a cooperative behaviour as in Littorinids (Stafford *et* *al.*, 2012) which provide thermoregulatory advantages under both cold conditions (this study) and heat stress (Chapperon & Seuront, 2011a). In particular, snails in aggregations seem to control thermal exchange rates under cold conditions like e.g. lizards (Shah *et al.*, 2003) and to respond to the thermal fluctuations of their surrounding environment. More investigations are nevertheless required to determine whether the aggregation behaviour of *N. atramentosa*, and ectotherms in general, could increase individual fitness by buffering the more frequent thermal extremes (both cold and heat) expected in the context of global climate change, and hence be seen as an adaptive and competitive advantage.

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Figure 1.

Nerita atramentosa aggregates observed at the top of boulders (A) and on a rock platform (B) at Billy Light's point (Port Lincoln, South Australia). White and grey arrows respectively indicate examples of aggregates and solitary individuals.

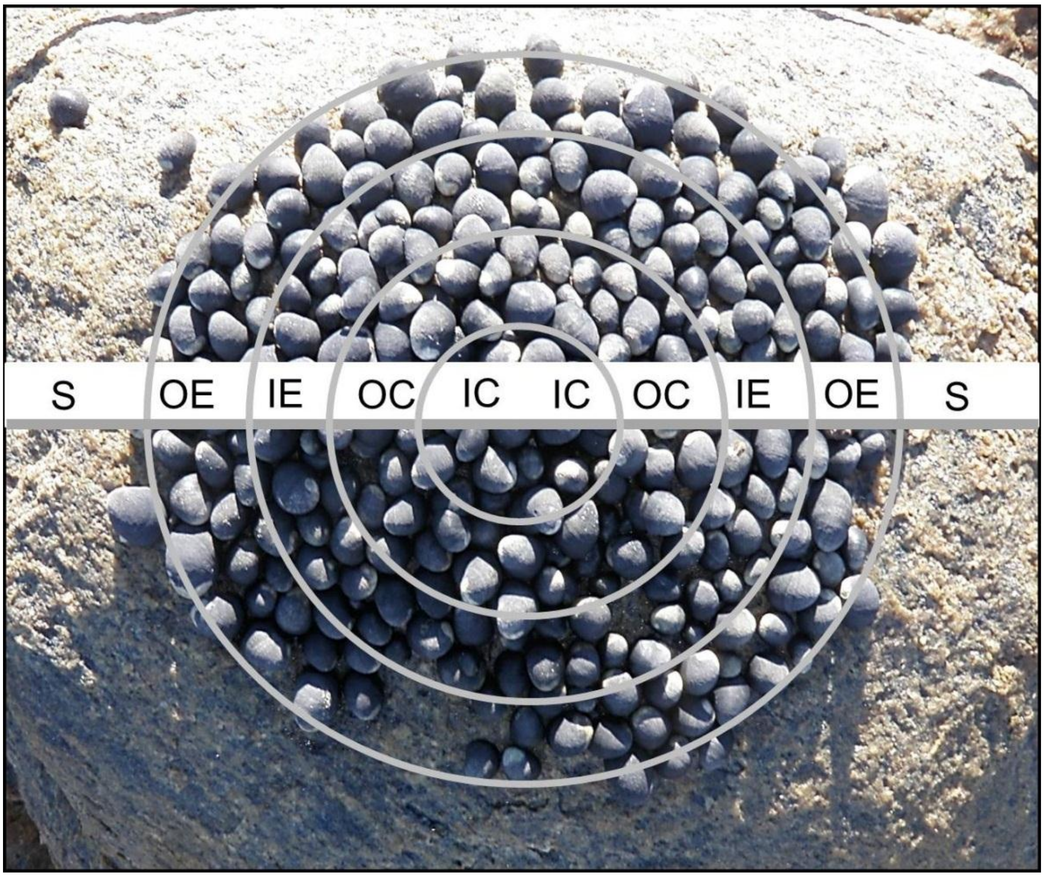

Figure 2.

Nerita atramentosa aggregate (N = 268) and surrounding substratum. The black line represents the thermal profile that was drawn through the corresponding thermal picture. Five categories were defined along the thermal profile on the surrounding substratum (S) and withing the aggregate *i.e.* outer edge (OE), inner edge (IE), outer centre (OC), inner centre (IC).

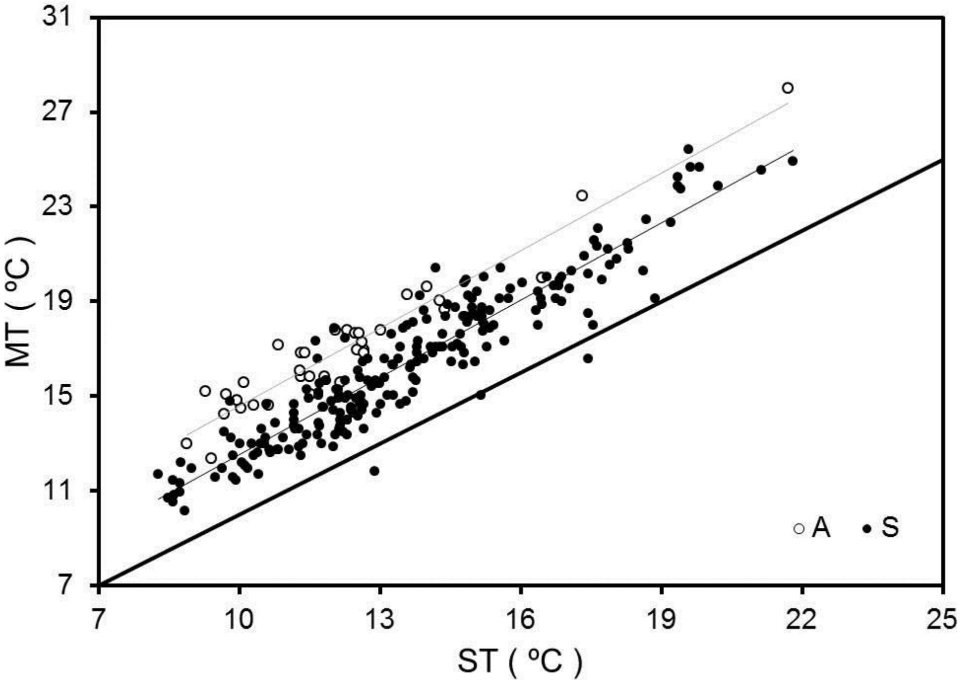

Figure 3.

Mantle temperature (*MT*) of *Nerita atramentosa* aggregates (A, white dots, N = 35) and solitary individuals (S, black dots, N = 226) to the surrounding substratum temperature (*ST*). The large black line represents the first bissectrix *MT* = *ST*. The grey and black lines are the significant linear regressions between *MT* and *ST* for aggregates (*MT* = 1.093*ST* + 3.667) and solitary individuals (*MT* = 1.0886*ST* + 1.6127), respectively.

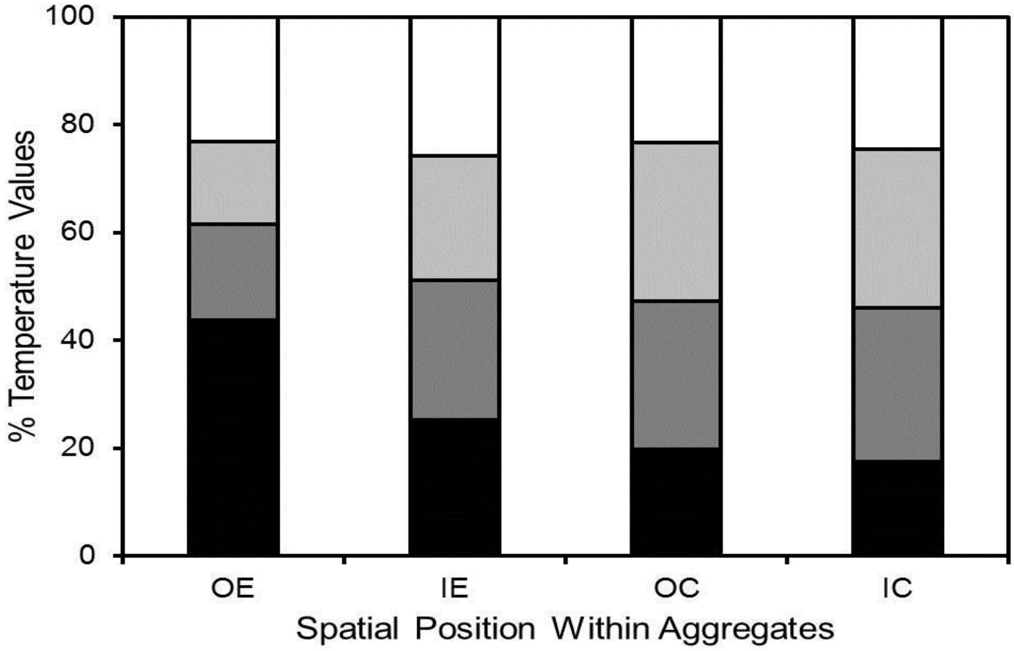

Figure 4.

Proportions of values for each temperature category *i.e.* the coldest C_1 (black bars), cold C_2 (dark grey bars), warm C_3 (light grey bars) and the warmest C_4 (white bars), calculated in each spatial category within aggregates *i.e.* outer edge (OE), inner edge (IE), outer centre (OC), inner centre (IC).

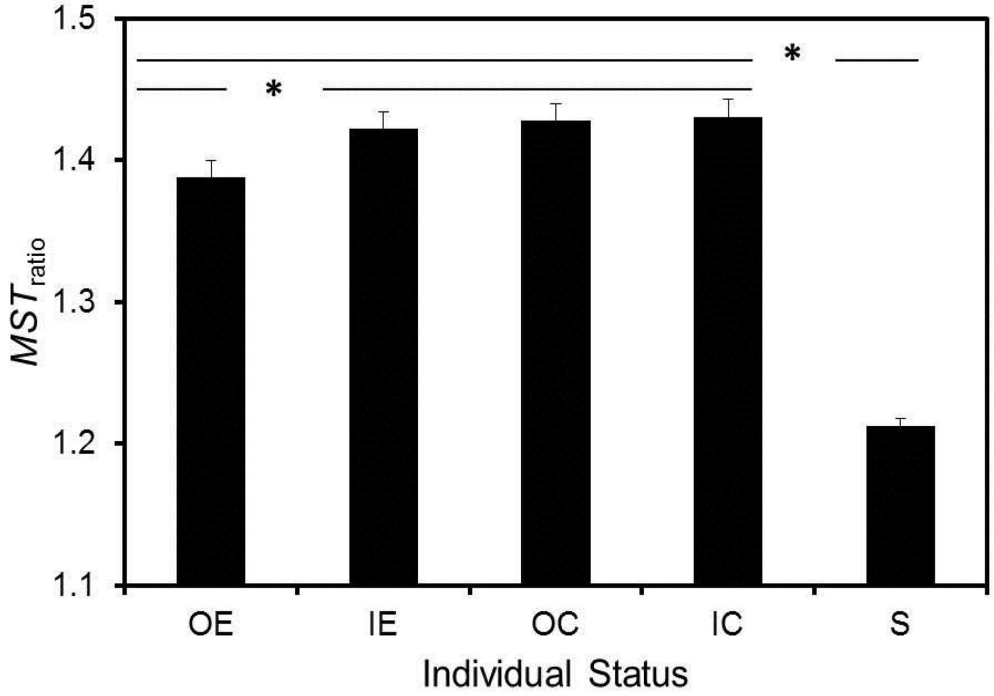

Figure 5.

Mean mantle to substratum temperature calculated between the different spatial categories within aggregates *i.e.* outer edge (OE), inner edge (IE), outer centre (OC), inner centre (IC) and surrounding substratum, and between solitary individuals (S) and surrounding substratum. *: $p < 0.05$.

Chapter VII. General Discussion

The problem of the scale: towards an organism perspective

In the warming climate, the scientific community faces the challenge of predicting the impacts of the changes in environmental conditions upon fundamental structural and functional properties of ecosystems, such as habitat structure, biodiversity, resilience, trophic interactions and competition for space and resources. It, thus, is essential to determine whether ectotherms will be able to adapt the new environmental conditions to predict future species biogeographic ranges. A major issue in climate change studies is the scale of approach, as most investigations focus on the ecological response of ecosystems or populations to averaged values of climatic variables such as air temperature, which characterize broad scale environmental conditions and implicitly assume that organisms and habitats have similar thermal properties (Helmuth, 2009).

In this context, this thesis emphasizes the need to investigate ectotherms' adaptations, especially behavioural adaptations, to new environmental conditions at a scale relevant to individual organisms, and to consider the space-time heterogeneity in (i) fluctuating environmental conditions, including extremes, and in (ii) organism responses to environmental conditions. In particular, Chapters III to V have highlighted that substratum temperature may be the primary determinant for body temperature of snails that attach to the substratum, rather than air or water temperature, and hence should be included in climate change models. Others local microclimatic and oceanographic variables such as solar radiations, wind, timing of the low tide, wave splash (Marshall *et al.*, 2010; Helmuth *et al.*, 2011), shore height (Chapter IV; Davenport & Davenport, 2005), topography and habitat stability (Chapter IV, V; Sinclair *et al.*, 2006) impact the level of thermal stress experienced by organisms, and hence their body temperature. As such, they may create counterintuitive distribution patterns broader scale in the warming climate (Helmuth *et al.*, 2002). The variability in local environmental conditions and others external pressures, *e.g.* pollution, may hence either amplify or buffer changes in climatic variables such as air temperature and create local spots where organisms are either more or less vulnerable to the warming climate. Similarly, changes in biotic properties such as food availability (Chapter VI) in the vicinity of an organism in conjunction with new environmental conditions are likely to increase its level of stress, hence its ability to maintain its fitness in the warming climate. As a consequence of this decreased fitness, complex maps of species distribution are expected in the future climate rather than simple latitudinal changes (Helmuth *et al.*, 2002). This change of individual species fitness makes it essential to understand how local environmental, biotic, anthropogenic factors and climatic variables interact at the organism scale to determine ectotherms' body temperatures, and hence determine the local dispersion and distribution patterns. The integration of local context is a fundamental prerequisite to make predictions about future species distribution patterns at large spatial scales (Russell & Connell, 2012).

Thermal mosaics: on the identification of thermal refuges

The centimetre-scale temperature variability identified in chapters III to V consolidates the evidence that thermal heterogeneity at the niche level can overwhelm that observed at larger scales in marine and terrestrial environments (Jost & Helmuth, 2007; Scherrer & Körner, 2010). In particular, the variability in substratum temperature in the temperate rocky shore of Marino Rocks, SA, Australia was up to 28 °C greater during summer at the niche level within a boulder field during the summer than the 4 °C in autumn between the distinct habitats of boulders and rock platforms separated by hundreds of meters (Chapters IV). Likewise, the small-scale heterogeneity observed over *ca*. 10 cm in substratum temperature from the top to the bottom of tropical mangrove roots could be greater than 20 °C. This thermal mosaic at the niche level in marine ecosystems can be explained by substratum inclination (Williams & Morritt, 1995; Helmuth & Hofmann, 2001), complex topography (Chapters IV & V), habitat stability (Chapters IV & V), degree of exposure to solar radiation (Chapters III to V; Schneider & Helmuth, 2007), compass orientation position (north/east-facing vertical surfaces, Williams & Morritt, 1995) and vegetation cover (*e.g.* macroalgae; Bertness *et al.*, 1999; Jost & Helmuth, 2007). Therefore, organisms inhabiting a thermally heterogeneous environment are surrounded by hot and cool environments that at the ecosystem level would only be seen if they were travelling geographic distances.

This small-scale thermal heterogeneity shows that there are thermal refuges at the local scale. For instance, boulder fields typified by their thermal stability and beach rocks not exposed to solar radiation buffer thermal stress and as such can be considered as thermal refuges (Chapters IV & V; Gedan *et al.*, 2011). The presence of thermal refuges in intertidal habitats seems essential as they could locally increase population survival as temperature increases and determines fine-scale distribution patterns (Bonnet *et al.*, 2009). This seems particularly critical for South Australian ectothermic species. Indeed, South Australia is the driest state in Australia and is predicted to become drier and warmer with an increase in the frequency of extreme maximum temperature and a further decline in precipitation over the next few decades (CSIRO, 2010). However, South

Australian ectothermic species cannot flee unfavourable environmental conditions since a poleward shift in their distribution range is limited due to an absence of land south of Australia (Wernberg *et al.*, 2011). Therefore, their survival in the warming climate is highly dependent upon their abilities to adapt local environmental conditions, hence the importance of thermal refuges.

This thesis also highlights that microhabitats intuitively anticipated to be thermal refuges such as crevices do not necessarily decrease thermal stress (Chapter III; Jackson, 2010). The identification of thermal refuges should hence be (i) species-specific, with each species having different ecophysiological properties and thermoregulatory needs, and (ii) conducted at scales ranging from microhabitats to habitats to integrate abiotic and biotic constraints. Once identified, the protection of thermal refuges through local management planning (Gedan *et al.*, 2011) combined with the setting up of artificial refuge structures to increase the number of refuges available are likely to enhance the survival and the conservation of intertidal invertebrates (Martins *et al.*, 2010), in a manner similar to that suggested for vertebrate species (Bonnet *et al.*, 2009). Conservation management plans that are implemented to increase ectotherm adaptive ability in the warming climate require a better understanding of the ecophysiology of targeted vulnerable species which underlines the need to conduct studies such as that presented in Chapter II.

Ectotherm behaviour as a buffer of the changing climate

The spatial heterogeneity in environmental temperature at small spatial scales and the resulting thermal refuges can only be advantageous in the warming climate if mobile ectotherms are able to behaviourally exploit thermal mosaics.

Results from terrestrial and marine environments show that mobile ectotherms can behaviourally change their spatial position in order to adjust their body temperatures (Chapters III to V; Kearney *et al.*, 2009; Huey & Tewsbury, 2009; Huey *et al.*, 2012). For instance, some terrestrial ectotherms move towards shaded microhabitats at midday to cool down while they lie around in the sun at others times of the day (*e.g.* Días & Cabezas-Días, 2004). A range of intertidal ectotherms also have the ability to seek less thermally stressful microhabitats. In general, they preferentially choose to settle in complex microhabitats characterised by the presence of fissures, crevices, macroalgae or to aggregate with others organisms rather than resting on exposed areas (Atkinson & Newbury, 1984; Chapman, 1995; Chapman & Underwood, 1996; Jones & Boulding, 1999; Sinclair *et al.*, 2006). In this thesis, the gastropod *L. scabra* actively selected specific substratum temperature on tropical mangrove roots under heat stress rather than a microhabitat type (Chapter III). In addition, *N. atramentosa* adopted different behavioural strategies in the two distinct habitats of boulder fields and rock platforms along the same rocky shore at Marino Rocks, SA in summer; individuals aggregated in the boulder field where crevices were not significantly cooler than flat rocks and hid under rocks on the rock platform to cool down and increase water conservation (Chapters IV & V). Note that *N. atramentosa* aggregations, as with many others terrestrial invertebrates and vertebrates (Gilbert *et al.*, 2006; Khan *et al.*, 2010), also provide individuals with thermal benefits in the cold (Chapter VI). Since evidence shows that behaviour can compensate for climate change at least over short-temporal scale (Huey *et al.*, 2012), the integration of the behaviour in climate change models is essential, yet challenging. Thermoregulatory behaviours include position adjustment, (Miller &

Denny, 2011), aggregation (Chapter IV, V), microsite selection (Chapter III), mucous holdfasts (McMahon, 1990) and as emphasized and shown in this thesis (Chapters III to VI), they are different in each species, between habitats and between seasons in temperate habitats (Chapter IV). Nonetheless, the identification of thermoregulatory behaviours could lead to the implementation of regulations within local conservation and management plans to protect ectotherms from being disturbed and stressed through removal of rocks, which could significantly decrease their vulnerability under new environmental conditions.

Remaining gaps in climate change models

Predictions of the effects of temperature changes on distribution patterns of ectothermic invertebrates are so far based on physiological responses that occur over short-term periods. However, the physiological performance of organisms, hence their fitness can be altered if the thermal stress persists over time through long-term sublethal stressors rather than extreme, short duration stressors (Helmuth *et al.*, 2010). For instance, acute physiological responses such as heat shock protein induction, damaged cells or tissues repair and replacement are high-energy-consumption processes that impact the bioenergetic budget (Somero, 2002). Thus, the energy spent to maintain homeostasis through physiological responses at the cellular and metabolic levels induces trade-offs between fitness components. Individuals survive but physiological performance and endurance such as body growth, reproduction, foraging ability can be negatively affected (Ji *et al.*, 2008; Pörtner *et al.*, 2006). Similarly, if an individual spends most of its time withdrew into its shell or takes refuge into thermally favourable microhabitat, its time spent to mate, feed is reduced and its performance, hence fitness is likely to be impacted. Therefore, acute physiological responses to thermal stress and thermoregulatory behaviours, which also impact the physiology of organisms, *e.g.* heart rates (Williams *et al.*, 2005) may not be the best adaptive strategies over time, but rather lead to a population's eventual extinction. This stresses the need to further examine the ability of a species for coping with long-term environmental changes stresses which implies a better understanding of (i) the thermal trade-offs and mechanistic links between physiology, behaviour and fitness, (ii) the limits of physiological and behavioural thermoregulation, (iii) the potential ability to genetically compensate when these limits are reached (Huey *et al.*, 2012) and (iv) the potential for evolutionary adaptations (Somero, 2010).

More specifically, (i) the natural selection of genotypes and/or phenotypes that perform well under new environmental conditions over a few generations and (ii) the potential adaptive evolution of physiological and morphological traits could ensure the persistence of a species inhabiting heterogeneous environments (Somero, 2010; Huey *et al.*, 2012). This, however, implies a good understanding of the extent genetic variability, phenotypic plasticity, and the heritability of thermal sensitivity (Huey *et al.*, 2012). Likewise, information about the body size, ornamentation, shell thickness, shape, and colour of ectotherms needs to be integrated into climate change models since it may impact ectotherms' body temperatures (Miller & Denny, 2011). This is critical since inter- and intraspecific differences in invertebrate body temperatures have been found to be primarily linked to morphological differences (Harley *et al.*, 2009) which highlights the need to conduct studies at the individual level . Besides, species persistence in the warming climate is also highly dependent on their dispersal ability in order to migrate to more favourable habitats (Bowler & Benton, 2005). More specifically, gastropod dispersal ability is a function of direct versus indirect development and the duration of larval stage; some species, for example *Bembicium vittatum*, have a direct development of their larvae into benthic eggs and some others species, for example *Austrocochlea constricta*, have mobile planktonic larvae (Pearson, 1998). Specifically, facing unfavourable environmental changes, the best strategy for planktonic developers is to displace and colonize more suitable habitats during their larval phase within the species thermal window. In contrast, species with direct development have to adapt to the local microclimate, which typically leads to a mosaic of genotypes that are genetically adapted to the local environment (Pearson, 1998). This stresses the need to incorporate in climate change models species dispersal and recruitment abilities (Helmuth, 2009), and also the responses of larval versus adult stages life stages to new environmental conditions (Du *et al.*, 2011; Huey *et al.*, 2012) since a failure to adapt new conditions at any stage of life may result in death.

Temperature is a main factor that drives patterns of activity, abundance and distributions. However, others biotic factors such as trophic interactions, competition for space and resource, local natural and anthropogenic environmental factors, and others climatic factors such as CO_2 levels also contribute to the observed patterns. Global change has already modified community dynamics of marine ecosystems (Sotka & Giddens, 2009) and has been shown to affect producers and consumers differently (Morelissen & Harley, 2007) as well as prey and predators (Pincebourde *et al.*, 2009). However, we still do not clearly understand whether trophic interactions would enhance, counteract or alter the effect of global change (Harley *et al.*, 2006). For example, the simulated removal of *Fucus* spp. under climate change induced an increase in the mortality of *Patella vulgata* that aggregates beneath *Fucus* spp. but no response from *P. depressa* that does not aggregate beneath *Fucus* spp. (Moore *et al.*, 2007). This suggests that the loss of *Fucus* spp. would affect the relative abundance of both limpet species. Different aggregation tendencies in both limpet species will likely cause changes in the spatial distribution of grazing in the intertidal, which would impact community dynamics (Moore *et al.*, 2007). In contrast, Morelissen & Harley (2007) showed that thermal effects perceptible at one trophic level for microalgae do not necessarily affect limpets that are at another trophic level. There is also a concern about the synergistic effects of climatic variables such as ocean acidification and temperature, which could amplify the consequences of global warming on ecosystems. For instance, hypercapnia has been showed to increase the sensitivity of the crab *Cancer pagurus* to heat stress, hence reducing its thermal tolerance window (Metzger *et al.*, 2007). Similarly, the sea urchin *Strongylocentrotus franciscanus* decreased its heat shock response induction under elevated CO_2 levels (O'Donnell *et al.*, 2009). These stresses need to be investigated from an organism perspective. The synergy between pollution at the local scale and acidification at the large scales, local and regional heterogeneous environmental factors (*e.g.* hydrodynamism), individual biotic factors (*e.g.* life-stage, thermal history), and trophic interactions together with temperature changes should be incorporated in climate change models. This will allow a better understanding of the consequences of climate global change from the individual scaling up to ecosystem level. This is a fundamental prerequisite to help policy makers and managers to develop tools that increase ectotherms' adaptive responses to changing environment.

Conclusion

The present work contributes to the growing evidence that small spatial scale variability in environmental conditions and the behaviour of ectotherms critically needs to be integrated in climate change models to improve predictions about future species distribution changes. However, more research needs to be conducted at the species- and habitat levels to examine further whether the individual behaviour of ectotherms could compensate for climate change. Nevertheless, critical gaps continue to persist in climate change models. In particular, since the ectotherms' adaptive behaviour can only postpone the extirpations of populations over long term warming, genetic selection and evolutionary adaptations, poorly known at best, might be the only chance of survival especially for species that cannot migrate. Trophic interactions and ocean acidification might enhance, counteract or decrease the effects of warming and/or impact upon the ability to adapt new environmental conditions. Challenges are thus still numerous; an integrative approach of the many factors which interact at a scale pertinent for organisms is required to improve climate change predictive modelling in marine studies. Ultimately, this new approach is critical to provide insight into how conservation management plans should be implemented at the local and regional scales to (i) increase the resilience of the intertidal ecosystem, (ii) protect intertidal habitats and individuals at risk, (iii) increase the chance of survival of intertidal invertebrates such as gastropods that occupy key roles in the intertidal ecosystem and (iv) maintain local biodiversity.

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