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Keeping warm in the cold: On the thermal benefits of aggregation behaviour in an intertidal ectotherm

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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 behavior 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 temperature. *N. atramentosa* aggregates and solitary individuals were significantly warmer than their surrounding substratum. The temperature deviation between aggregates and substratum was, however, ca. 2 °C 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 centers are significantly warmer than their edges, hence snails experience greater thermal advantages in the aggregate center. 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 behavior 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.

1. 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 behavior 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 and 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 20 °C 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

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to the fast warming climate. Most recent studies have therefore focused upon the thermoregulatory abilities of intertidal ectotherms to adapt to 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 behavioral adaptation to extreme heat stress (Chapperon and Seuront, 2011a,b; Miller and Denny, 2011). Behavioral adaptations to thermal stress 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 and Seuront, 2011a,b) and solar heat (Marshall et al., 2010; Helmuth et al., 2011). Such behavioral responses to heat stress include resting posture (Lang et al., 1998), body orientation (Munoz et al., 2005; Miller and Denny, 2011), mushrooming behavior (Williams et al., 2005), aggregation behavior (Chapperon and Seuront, 2011a; Stafford et al., 2011, 2012) and habitat selection (Jones and Boulding, 1999; Chapperon and 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), localized 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 behavioral adaptations to cold conditions (e.g. Lencioni, 2004 such as habitat selection through migration to thermally-favorable microhabitats, basking behavior, shuttling heliothermy and aggregation. Nevertheless, the thermal benefits of aggregation behavior (also referred to as huddling behavior, crèching behavior, grouping behavior and clustering behavior) 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 and 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 behavior 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 favorable position such as the center of the huddle, but rather share it by continuously moving within the huddle (e.g. Zitterbart et al., 2011). Aggregation such as huddling behavior has also been shown to provide thermal benefits over winter in ectothermic organisms such as lizards (Shah et al., 2003) and frogs (Espinoza and Quinteros, 2008). In the intertidal, aggregation behavior is, however, typically considered as an adaptation to heat and desiccation stresses (Garrity, 1984; Chapman and Underwood, 1992; Raffaelli and Hawkins, 1996), and to our knowledge the potential thermoregulatory role of aggregation behavior under winter conditions is still to be quantitatively investigated in intertidal snails.

Aggregation behavior in the intertidal gastropod *Nerita atramentosa*, a dominant competitor for microalgae on Australian rocky shores, reduces body temperatures (Chapperon and Seuront, 2011a) and increases water content (Chapperon and Seuront, non-published data) on boulder fields under conditions of high thermal and desiccation stresses. However, the factors underlying the aggregation behavior observed at others times (e.g. winter) and places (e.g. under rocks on rock platform; Chapperon and Seuront, 2011a) are still not clearly understood. In this context, the present study investigated the potential thermoregulation role played by *N. atramentosa* aggregation behavior 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.

2. Materials and methods

2.1. 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 behavior 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 characterized 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).

2.2. Thermal properties of solitary and aggregated snails, and surrounding substratum

To determine the potential role played by the aggregation behavior on *N. atramentosa* thermoregulation, we examined the potential temperature difference between solitary individuals and aggregates during different morning low tides (i.e. 9–12 am) in the austral winter 2009. In July 2009, air temperatures in the studied area ranged between 5 °C and 21.3 °C, which are typical of the South Australian winter (Bureau of Meteorology, Australia). An aggregate was defined as the grouping of at least two individuals in direct shell contact (Chapperon and 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

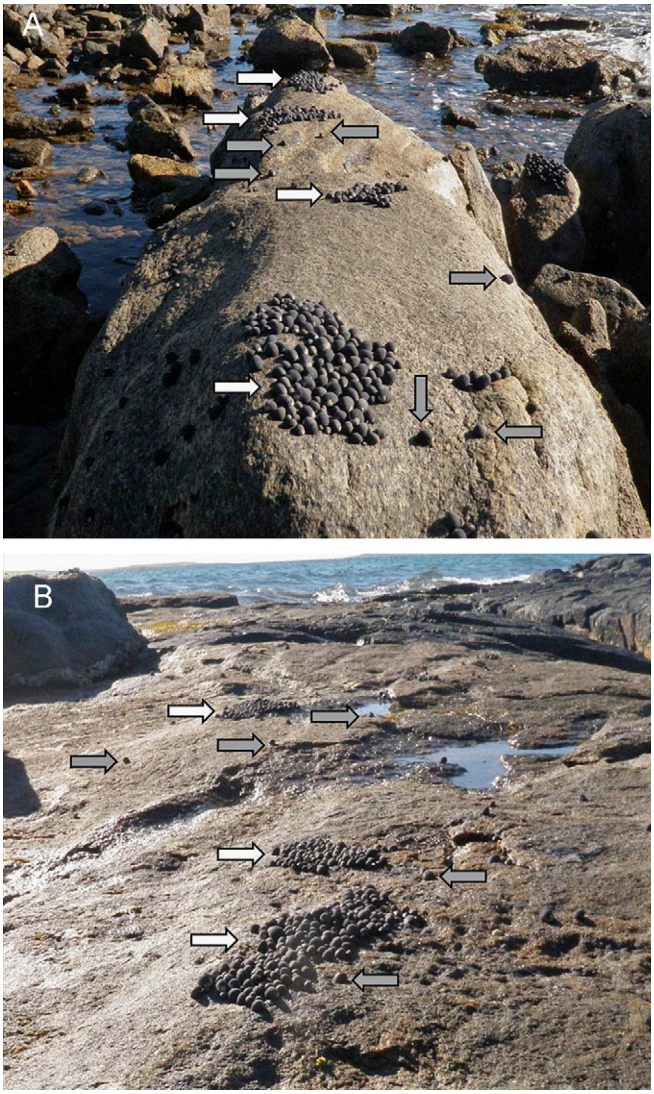

Fig. 1. *Nerita atramentosa* aggregates observed at the top of boulders (A) and on a rock platform (B) at Billy Light's point (Port Lincoln, South Australia). White and gray arrows respectively indicate examples of aggregates and solitary individuals.

35 aggregates (i.e. 3681 individuals) that were haphazardly chosen on flat exposed surfaces situated on rock platforms and boulders (Fig. 1). The sensitivity of the thermal camera (Fluke® Ti20, Fluke Corporation, Everett, Washington, USA) is ≤ 0.2 °C at 30 °C and the temperature measurement accuracy is 2% or 2 °C, whichever is greater. Emissivity values of both shell snails and substrata were set at 0.95; see Chapperon and Seuront (2011a) for further details. The body temperatures of solitary individuals, the mean temperatures of aggregates and the temperatures of surrounding substratum were subsequently calculated after analyzing the thermal pictures with the InsideIR 4.0 software (Fluke Corporation, 2006, USA). 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 bodies and aggregates (BT_s and BT_a, respectively; Chapperon and Seuront, 2011a). Here, an aggregate was considered as a single entity, hence the mean temperature of a given aggregate corresponds to the average of temperature of all individuals present in this 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 and 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 and 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 and Seuront, 2011a,b).

2.3. *Thermal heterogeneity within aggregates*

2.3.1. *Absolute thermal heterogeneity*

To determine the potential existence of a thermally favorable 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 center, inner center (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 1/4 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)

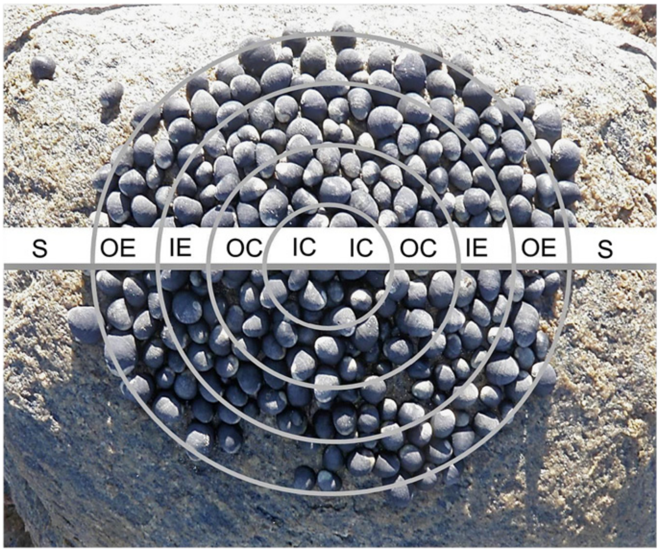

Fig. 2. *Nerita atramentosa* aggregate ($N=268$) and surrounding substratum. The black line represents the thermal profile that was drawn through the corresponding thermal picture. Five categories were defined along the thermal profile on the surrounding substratum (S) and within the aggregate i.e. outer edge (OE), inner edge (IE), outer center (OC), inner center (IC).

in the different spatial categories of each aggregate was subsequently calculated.

2.3.2. Relative thermal heterogeneity

The mantle temperature *MT* at the inner center, outer center, inner edge and outer edge of each aggregate (i.e. MT_{IC}, MT_{OC}, MT_{IE} and MT_{OE}) was estimated from $BT_{\mathrm{a(IC)}}$, $BT_{\mathrm{a(OC)}}$, $BT_{\mathrm{a(IE)}}$ and $BT_{\mathrm{a(OE)}}$ (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.

2.4. 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 Imaging Corporation, Center 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 $MT_{\mathrm{IC}}/MT_{\mathrm{OC}}$, $MT_{\mathrm{IC}}/MT_{\mathrm{OC}}$ and $MT_{\mathrm{IC}}/MT_{\mathrm{OC}}$ in order to investigate how *MT* varies within aggregates with aggregate size.

2.5. 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 (χ^2) analysis of contingency tables was run to determine the potential independence between *MT* and the spatial position within aggregates (Zar, 2010).

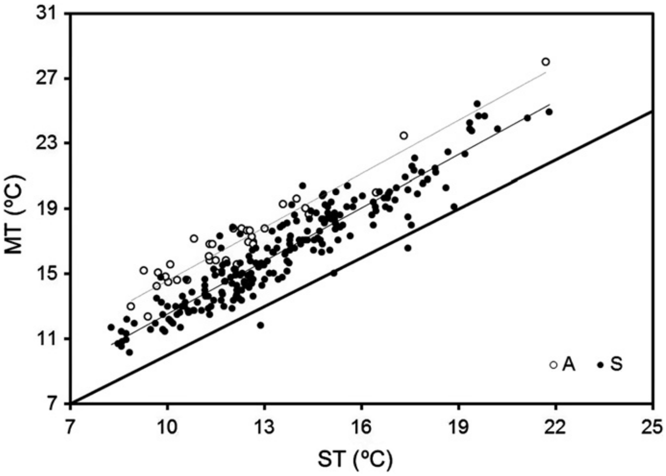

Fig. 3. Mantle temperature (*MT*) of *Nerita atramentosa* aggregates (A, white dots, $N=35$) and solitary individuals (S, black dots, $N=226$) to the surrounding substratum temperature (*ST*). The large black line represents the first bissectrix $MT=ST$. The gray and black lines are the significant linear regressions between *MT* and *ST* for aggregates ($MT=1.093ST+3.667$) and solitary individuals ($MT=1.0886ST+1.6127$), respectively.

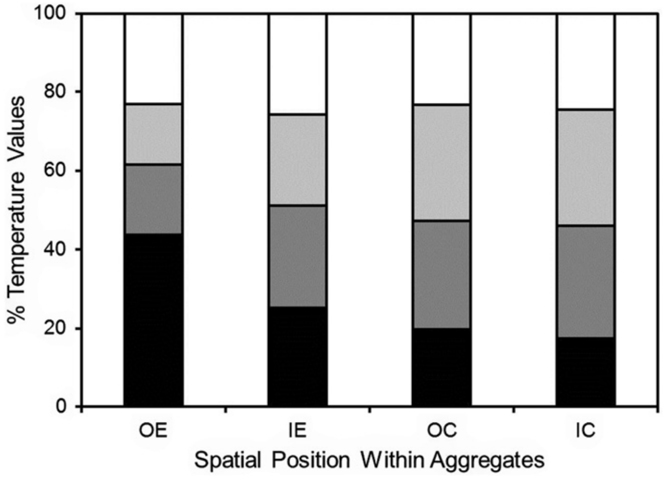

Fig. 4. Proportions of values for each temperature category i.e. the coldest C_1 (black bars), cold C_2 (dark gray bars), warm C_3 (light gray bars) and the warmest C_4 (white bars), calculated in each spatial category within aggregates i.e. outer edge (OE), inner edge (IE), outer center (OC), inner center (IC).

3. Results

3.1. 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 ($MT_{\mathrm{s}}=16.41 \pm 0.21$ °C, mean ± standard error) and aggregates ($MT_{\mathrm{a}}=16.96 \pm 0.49$ °C). MT_{s} and MT_{a} respectively ranged from 10.16 °C to 25.42 °C and 12.38 °C to 28 °C. In contrast, solitary individuals were consistently found on substratum significantly warmer ($ST=13.59 \pm 0.18$ °C) than aggregates ($ST=12.17 \pm 0.43$ °C; $t=-2.891$, df=259, $p=0.004$). The temperature of solitary individuals and aggregates were significantly and positively correlated with the surrounding substratum temperature ($r_{\mathrm{s}}=0.937$, $p < 0.001$; $r_{\mathrm{a}}=0.959$, $p < 0.001$). The temperature deviation between aggregates and the adjacent substratum ($MST_{\mathrm{ratio}}=1.41 \pm 0.02$ °C) was significantly greater than the one estimated for solitary individuals ($MST_{\mathrm{ratio}}=1.21 \pm 0.01$ °C, $t=12.099$, df=259, $p < 0.001$). More specifically, aggregates and solitary individuals were on average 4.78 ± 0.14 °C and 2.82 ± 0.08 °C warmer than rocks, respectively.

3.2. Thermal heterogeneity within aggregates

3.2.1. 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 depended on the spatial position within a patch ($\chi^2=715.747$, df=9, $p < 0.001$). In particular, the temperature categories C_1–C_3 were not uniformly distributed within aggregates ($\chi_1^2=527.047$, $\chi_2^2=62.996$, $\chi_3^2=140.326$, df=3, $p < 0.001$). The coldest temperatures were more frequent at the outer edge of aggregates (42%) and less frequent at the inner center (16%). Warm temperatures were instead more frequent at the inner center (30%) and less frequent at the outer edge (16%). In contrast, the warmest temperatures (thermal category C_4) were uniformly distributed in aggregates ($\chi^2=4.532$, df=3, $p > 0.05$). Overall, the inner and outer edges of aggregates were mainly characterized by cool temperatures

(55.64%), while their inner and outer centers were mainly formed by hot temperatures (54.22%).

3.2.2. *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 ± 0.18 °C. 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 center ($p=0.013$). In addition, MST_{ratio} estimated for solitary individuals was consistently significantly lower than the MST_{ratio} estimated from the aggregate spatial categories ($p<0.001$).

3.3. *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 center ($r=0.125$, $p=0.257$) and inner center ($r=0.087$, $p=0.430$). 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 center ($r=-0.298$, $p=0.006$) and inner center ($r=-0.320$, $p=0.003$). The ratio, MT_{ratio}, between the mantle temperatures of the inner center of aggregates, and their outer edge and the inner center significantly decreased with increasing aggregate size ($MT_{\text{IC}}/MT_{\text{OE}}$: $r=-0.361$, $p<0.0001$; $MT_{\text{IC}}/MT_{\text{IE}}$: $r=-0.224$, $p<0.05$). 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 $MT_{\text{IC}}/MT_{\text{OE}}$ and $MT_{\text{IC}}/MT_{\text{IE}}$and, aggregate size (i.e.$MT_{\text{IC}}/MT_{\text{OE}}=-0.0003N+1.064$; $MT_{\text{IC}}/\ MT_{\text{IE}}=-0.0001N+1.0216$) implies that $MT_{\text{IC}}/MT_{\text{OE}}=1$ and $MT_{\text{IC}}/MT_{\text{IE}}=1$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, vanished beyond a threshold aggregate size of 216 individuals.

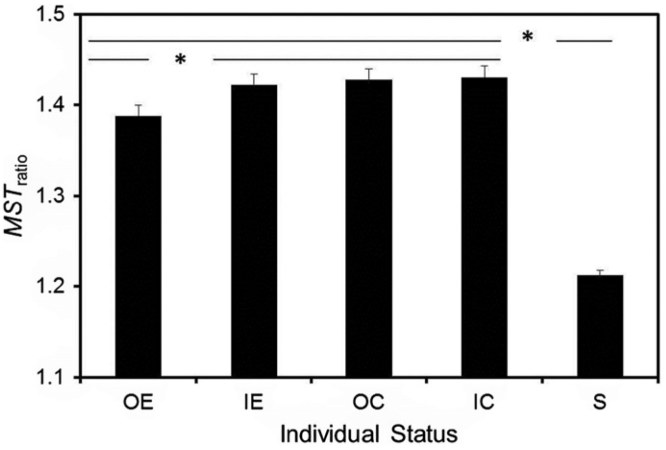

Fig. 5. Mean mantle to substratum temperature calculated between the different spatial categories within aggregates i.e. outer edge (OE), inner edge (IE), outer center (OC), inner center (IC) and surrounding substratum, and between solitary individuals (S) and surrounding substratum. $^{*}p<0.05$.

4. Discussion

4.1. *Winter aggregation in the intertidal: a behavioral adaptation to the cold*

Many invertebrates behaviorally 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), predation (Coleman et al., 2004), and hydrodynamism (Moran, 1985). Most attention has, however, been given to the advantages of aggregation behavior under desiccation stress (Chapman and Underwood, 1996; Soto and Bozinovic, 1998; Rojas et al., 2000; Stafford et al., 2011, 2012) and heat stress (Garrity, 1984; Munoz et al., 2008; Chapperon and Seuront, 2011a). Aggregation is, however, a complex, species-specific behavior 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 behavior also appears to vary spatially (between habitats and shore height) and temporally on both diel and seasonal scales 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 and 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 behavior 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 and Toboev, 2011). For instance, sun-exposed toads in knots have been shown to be on average 2.3 °C warmer than solitary ones (Espinoza and Quinteros, 2008), which is consistent with our findings. This thermoregulatory behavior 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 and Murray, 2004), growth (Klok and Chown, 1999; Espinoza and 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 behavior 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 behavior 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.

4.2. 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, color and behavior (Helmuth, 1998; Marshall et al., 2010; Chapperon and Seuront, 2011a,b; Miller and Denny, 2011); see also Denny and 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 maximized, 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 and 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 and 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* individuals 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 behavior observed in vertebrate ectotherms, e.g. lizards and turtles (Díaz and Cabezas-Díaz, 2004, Dubois et al., 2009). Likewise, the absorption and retention of solar radiation is probably enhanced by *N. atramentosa* black shell (Mc Mahon, 1990). The need to minimize energy losses during winter might explain why this species has maintained a dark pigmented color 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 behaviors, such as microhabitat selection and aggregation behaviors, in order to face heat stress during summer (Chapperon and Seuront, 2011a).

4.3. 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 center 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 (Morel and 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 and Ruxton, 2002). For instance, predation risk is greater on peripheral positions, and referred to as marginal predation (Hamilton, 1971; Okamura, 1986; Stankowich, 2003; Morel and 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 (Morel and 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 (Morel and 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 optimize the exploitation of microalgal resources that are abundant in winter (Morel and 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 (Morel and Romey, 2008), which is consistent with our observations. Besides, larger groups can reduce predation attacks (Krause and 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. Minimizing predation. Further investigations are, however, required to validate this hypothesis and also to identify others parameters, e.g. feeding behavior, which might participate in the determinism of this aggregation size threshold and/or the maintenance of aggregation size below 216 individuals.

5. Conclusion

Aggregations in intertidal snails have been identified as self-organized 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 behavioral 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 and Seuront, 2011b). Since many others factors could have initiated *N. atramentosa* aggregations in the present study (e.g. food distribution, Chapperon and Seuront, in press; pre-breeding, Feare, 1971; chemical trails released by conspecifics and/or micrologal resources, Chapperon and 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 behavior as in Littorinids (Stafford et al., 2012) which provide thermoregulatory advantages under both cold conditions (this study) and heat stress (Chapperon and 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 behavior 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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