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Behavioral 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 (BTs) of the tropical snail Littoraria scabra at the niche level. A correlation between BT and substrate temperature (ST) was detected from field observations which suggests a possible causal relationship between both substrate and BTs. In contrast, there was no correlation between BT 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. As L. scabra individuals were free to choose their substrata, the observed distribution pattern at the niche level is related to L. scabra's behavior. Additionally, substrate surface temperatures were very heterogeneous at centimeter scale (i.e. from 22.5 to 53.1 °C) and L. scabra was shown to select specific STs (i.e. between 22.5 and 33.4 °C) rather than microhabitat type. Therefore, L. scabra did not seem to behaviorally thermoregulate through microhabitat selection nor aggregation. In contrast, behavioral experiments showed that L. scabra has the ability to actively select a thermally favorable site over short temporal scale (i.e. individual average speed of $1.26 \,\mathrm{cm}\,\mathrm{min}^{-1}$) following exposure to high temperatures above 33.4 °C. Hence, this study supports the crucial need to integrate intertidal invertebrate behavioral responses to thermal constraints in climate change studies.

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

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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 favorable 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, 2010) in their behavioral 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 slowmoving ectotherms are, respectively, attached to the

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substrates permanently and during the low tide. Contrarily to mobile invertebrates, sessile individuals cannot choose to 'take flight' to a more thermally suitable microhabitat (Muñoz et al., 2005) when the thermal conditions are not favorable. 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 (Muñoz et al., 2005). Mobile organisms have the potential to flee to nonfavorable 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 (Hutchinson, 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 (BTs) through a range of physiological (Somero, 2002), behavioral (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 nonseasonal environments have limited physiological acclimation abilities to thermal stress (Tewksbury et al., 2008) as 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 behavioral flexibility to compensate for their reduced physiological adaptive abilities, hence to adjust their BTs over short temporal scales. This implies a good understanding of the factors involved in the determinism of BT changes and an assessment of the impact of behavior on BT patterns.

BTs of intertidal ectotherms during aerial exposure are determined by the heat fluxes 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 (AT) (Broitman *et al.*, 2009). In addition, intrinsic properties of ectotherms such as thermal history (Meng *et al.*, 2009) and morphology (Jost & Helmuth, 2007) influence BTs. Finally, some ectothermic invertebrates can behaviorally thermoregulate (Pincebourde *et al.*, 2009). Specifically, gastropods may actively flee extreme temperatures, hence reduce their BTs, by selecting and moving towards a thermally favorable microhabitat (Garrity, 1984).

In this framework, we investigated the BT 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 behaviorally 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 BTs, (ii) if microhabitat selection or aggregation behavior affect individual BTs, 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 (17°35.471'S-178°29.505'E). The mangrove was characterized by a high abundance (ca. $40 \text{ ind } \text{m}^{-2}$) of the generalist herbivorous gastropod L. scabra (Linnaeus, 1758). L. 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 characterized 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 (C. Chapperon & L. Seuront, unpublished results). 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 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 behavior. More specifically, the study was undertaken at midday low tides over four different days in September 2009, and AT reported to influence the BT (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 BT

Thermal imaging was used as a noncontact and noninvasive temperature measurement. Infrared thermography is commonly used in a wide range of fields that includes animal physiology and behavior (Bulanon et al., 2009; Montanholi et al., 2010). However, thermal imaging has rarely been used 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, Everett, WA, USA; Fig. 1a.). The thermal sensitivity of the thermal camera is < 0.02 °C at 30 °C and the temperature measurement accuracy is 2%. Images of 466 L. scabra adult individuals $(15.9 \pm 0.2 \text{ cm} \text{ long and } 8.9 \pm 0.1 \text{ cm high; mean} \pm \text{SE})$ were captured on randomly chosen mangrove roots. For each thermal picture, a digital picture (Olympus μ Tough-6000, Olympus Imaging Corporation, Center Valley, PA, USA) was jointly taken to analyze the individual BT in regards to behavioral 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 min. Rhizophora sp. mangrove trees are characterized by stilt roots that emerge from the lower trunk and move downward to the ground (Menezes de, 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



Fig. 1 Thermal (a) and digital (b) pictures of eight *Littoraria scabra* individuals (30.1 ± 0.1 °C, mean BT \pm SE) situated under a *Rhizophora* sp. root (30.0 ± 0.1 °C, mean \pm SE). The white numbers indicate the position of each snail. BT, body temperature.

classified into three 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. 45° 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, 45° 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, 45° 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, USA, 2006). For each picture, mean snail BT and mean temperature of the surrounding substrate (ST) were assessed.

Microhabitat selection and aggregation behavior

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 six adult snails (14.3 ± 0.3 cm long and 8.3 ± 0.3 cm high; mean \pm 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 eight times with a new set of six individuals. All individuals involved in this experiment were immobile for at least 15 min 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 (C. Chapperon & L. Seuront, personal observations). 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 2 consecutive days have shown that both motion and aggregation behaviors 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 min to assess the frequency of trail following behavior 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 (ST) constitutes a good proxy for BT. More specifically, BST >1 means that the BT is higher than the ST. In contrast, when BST < 1, the BT is lower than the ST. 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's 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., Chicago, IL, USA, 2009).

Results

L. 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 \pm SE), 27.34 ± 0.11 °C and 0.99. Overall, BT varied from 22.8 to 32.6 °C and no individual was found on a substrate warmer than 33.4 °C (Fig. 2a). STs, 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



Fig. 2 (a) *Littoraria scabra* individual body temperatures (BT; N = 466) vs. substrate temperatures (ST), as a function of the different *Rhizophora* sp. root microhabitats (b; top, grey squares; side, open dots; under, black triangles). The grey area in (a) corresponds to the range of STs where *L. scabra* individuals were never observed, i.e. between 33.4 and 53.1 °C. The black line represents the first bissectrix, i.e. BT = ST.

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 (r = 0.80, P = 0.20, n = 4) was found between mean BT, ST and mean AT. On average, BT and ST were about 1.2-fold warmer than AT. More specifically, BT and ST were on average 4.23 and 4.52 °C warmer than AT over the 4 days of field observations.

L. scabra thermal properties and behavior

Of the *L. scabra* individuals studied here, 57% were aggregated on *Rhizophora* sp. roots. Aggregation size varied from two to 26 individuals per aggregate (Fig. 4). However, the majority of aggregates were formed by two to five individuals (Fig. 4). The temperatures of



Fig. 3 Thermal (a) and digital (b) pictures of the *Rhizophora* sp. root shown in Fig. 1. Black lines in (a) and (b) corresponds 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.

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: $\chi^2 = 2.247$, df = 2, *P* = 0.325; ST: $\chi^2 = 2.693$, df = 2, *P* = 0.260; BST-ratio: $\chi^2 = 5.464$, df = 2, *P* = 0.065; Fig. 2b). On average, BT was cooler than the substrate under (BST = 0.99) and on the side of roots (BST = 1.02 ± 0.01). Furthermore, 23%, 40% and 48% of BST-ratio were significantly >1 on the side (*Z* = -8.125, *P* = 0.01),



Fig. 4 Frequency of occurrence of *Littoraria scabra* aggregation size (N = 263).

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 \pm 0.07, 0.47 \pm 0.02 and 0.47 \pm 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 and 27.04 ± 0.28 °C, respectively. Maximum temperatures observed on the tops and bottoms of roots were, respectively, 53.1 and 33.0 °C. All the translocated individuals (N = 48) to the top of the roots returned to their previous microhabitat in 17 ± 2 min. The average speed of individuals returning the root bottom was 1.26 ± 0.06 cm min⁻¹.

In contrast, no individual returned to its previous microhabitat during the control experiment conducted during the night on a thermally homogeneous substrate after 30 min. In total, 18 individuals over the 27 recorded individuals displayed trail following behavior (Fig. 5). Snails involved in trail following behavior 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

ST as a proxy for L. scabra BTs

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



Fig. 5 Sequence of *Littoraria scabra* trail following behaviour recorded on a *Rhizophora* sp. root. Successive images (a–d) were taken at 1 min intervals. All recorded individuals (N = 6) ended up in a patch under the root (d).

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 STs 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 BTs were neither similar nor correlated to AT which demonstrates that climate variables and individual BTs are decoupled. More specifically, individuals were on average 4.23 °C warmer than AT. This result suggests that climate change models using AT as a proxy for BTs may underestimate the effect of global warming on BT patterns of tropical invertebrate species. Likewise, correlated models may also overestimate ectotherms' physiological tolerance, which is already reduced compared with 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's BTs were positively correlated to and did not significantly differ from their STs (Fig. 2a). This result suggests that ST may be used as a good proxy for L. scabra's BT. However, it also points out that the variables explaining BT 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 BT 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, nonclimatic variables such as STs 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 STs could potentially be estimated from average ATs; i.e. substrate was $4.52 \,^{\circ}$ C warmer than AT. However, the relationship between ATs and STs should be investigated at fine spatial scale and site by site. This is particularly necessary since STs were heterogeneous at small spatial scale (0.47–0.67 to 6.8 $^{\circ}$ C at the mm and cm scale, respectively) which entails the existence of a thermal mosaic of BT patterns at the niche level (Helmuth *et al.*, 2006).

Micro-scale selection of STs 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 ST 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, STs 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. <0.7 °C) surpassed those observed between microhabitats (i.e. >6 °C). 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, nonexposed 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 favorable 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 (C. Chapperon & L. Seuront, personal observations), 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, as 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 speciesspecific and environment-specific.

Here, on substrates of temperatures ranging from 22.5 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 (C. Chapperon & L. Seuront, personal observations). However, the selection of a specific range of STs (Fig. 2a) out of an available range reaching 53.1 °C suggests a behavior to keep BTs 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, ST could be used as a good proxy for BT of organisms that attach substrates, but only up to a critical temperature threshold, here 33.4 °C. Based on STs estimated from ATs 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 behavior as a response to thermal stress

This study provides insight into the behavioral 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 STs 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 $(0.6 \text{ cm min}^{-1}; \text{ Alfaro, 2007})$. This reinforces the advantage of mobile ectotherms that have the ability to take flight from STs beyond their critical upper limit temperature (Muñoz et al., 2005) At the opposite, in such a situation, sessile or slow moving organisms must sit tight and fight the thermal conditions (Muñoz 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 behavior 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 behavior 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 behaviors (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 STs. 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 behavior 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 behavior 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. AT) and physiology alone cannot accurately predict the future of organisms in the warming world. Here, we highlight the need to focus on the nonclimatic variables (i.e. ST, solar exposure) that directly interact with mobile individuals at the niche level. This is particularly relevant since behavioral adaptations to nonclimatic thermal sources could enhance the tolerance levels of intertidal ectotherms to climatic variables (Marshall et al., 2010). In this context, thermoregulatory behaviors developed by both sessile and mobile ectotherms under high thermal stress, e.g. mushrooming behaviors in limpets (Williams & Morritt, 1995; Williams et al., 2008), raised posture and shell orientation in snails (Lang et al., 1998; Muñoz et al., 2005), should be integrated in climate change models. Here, the behavioral thermoregulation of L. scabra was an escape strategy to STs >33.4 °C. This flight behavior (Muñoz *et al.*, 2005) coupled with the existence of ST 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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