Université de Lille

Ecole Doctorale n°104 – Sciences de la Matière, du Rayonnement et de l'Environnement

THÈSE

Pour obtenir le grade de docteur délivré par

l'Université de Lille

Spécialité doctorale Géosciences, Ecologie, Paléontologie, Océanographie

présentée et soutenue publiquement par

Emilie Moisez

Le 17 février 2021

A step forward in the understanding of behavioural and thermal ecology of intertidal gastropods

-

Contribution à la compréhension de l'écologie comportementale et thermique des gastéropodes intertidaux

Directeur de thèse : Laurent Seuront

Co-directeur de thèse : Nicolas Spilmont

Jury

PINCEBOURDE Sylvain	CNRS	Rapporteur
ZARDI Gerardo	Rhodes University	Rapporteur
ELIAS Florence	Université Paris Diderot	Examinateur
ELLIEN Céline	Sorbonne Université	Examinateur
GAUDRON Sylvie	Sorbonne Université	Examinateur
MEZIANE Tarik	MNHN	Président du jury
SEURONT Laurent	CNRS	Directeur de thèse
SPILMONT Nicolas	Université de Lille	Co-directeur de thèse

Laboratoire d'Océanologie et de Géosciences (LOG) – UMR 8187

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On peut toujours plus que ce que l'on croit pourvoir.

Joseph Kessel

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SUMMARY

Intertidal organisms inhabit highly variable and complex environments and are submitted to a large range of stimuli and stresses. Recently, new insights into the sensory abilities of intertidal gastropods and their subsequent ability to navigate through topographically complex landscapes demonstrated that the behavioural repertoire of intertidal gastropods is much wider than previously thought. In this context, the present thesis first aimed to ameliorate our knowledge in the thigmotactic behaviour of *Littorina littorea*. Specifically, thigmotactic behaviour was tested in experimental containers of different sizes and shapes. This approach allowed individuals to encounter two-dimensional and three-dimensional discontinuities in order to understand how the topographical complexity of their environment can modify this behaviour. Thigmotaxis was further tested under decreasing salinity concentrations to understand how salinity can modify gastropods behaviour, since they are typically submitted to large salinity variations during emersion periods. During emersion, intertidal organisms are also submitted to large temperature variations (up to 20°C). Thus, this work also investigated the thermal behaviour of intertidal gastropods by studying body temperature of *L. littorea* and *Patella vulgata* and their microhabitat temperature on a rocky platform on the French coasts of the eastern English Channel. Temperatures were investigated on four sunny days from June to December to test the microhabitat choice of these two intertidal gastropods species under temperature conditions typically experienced through a seasonal cycle on the Opal Coast. Aggregation behaviour in *L. littorea* and the associated thermal benefits previously found in gastropods literature were also investigated, (i) *in situ* under a moderate heat wave and (ii) *ex situ* under an extreme heat wave simulated under laboratory-controlled conditions. Specifically, the body temperature of individuals inside and at the edge of aggregates were investigated to assess the importance of the position within aggregates.

Taken together, the results of this thesis suggests that the thigmotactic behaviour can be one of the major taxes involved in intertidal gastropods orientation. Indeed, even under unfavourable conditions (*e.g.* low salinity conditions), the thigmotactic behaviour is still observed in *L. littorea*. This work also highlighted macrohabitat preference in *L. littorea* and *P. vulgata* for biogenic structures which allow to reduce both desiccation and thermal stresses. Within a macrohabitat, intertidal organisms are able to take advantage of the thermal mosaic encountered in intertidal environment and select microhabitat. This microhabitat selection in gastropods, is species- temperature- and habitat-dependent and allows individuals to maintain their body into their optimal range of temperatures. Finally, this work stressed the absence of thermal benefits of being aggregated in *L. littorea* under heat stress conditions. Specifically, under extreme heat wave, body temperature of individuals inside the aggregate is higher than for individuals at the edge of the aggregate. This result raised the rather unexpected and still unresolved question to understand the mechanisms driving aggregation behaviour in *L. littorea*. These results nevertheless contribute to the growing evidence that studying behaviour in intertidal gastropods is critical to understand how they move, how they perceive their environment and how they cope with raising temperature under global warming. This work also highlighted the importance of a better understanding of the thermoregulatory behaviour in intertidal organisms, for a better estimation of the impact of global warming in their ecology.

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Chapter I. General Introduction

Behavioural ecology is a key component to understand how animals interact with their conspecifics how they perceive adapt to their environment. A lot of attention has been devoted to the behavioural ecology of large mammals such as great apes, elephants, whales and dolphins. For examples, Goodall, (1986) described the rich repertoire of communicative signals of chimpanzees and the social organization of their societies. In invertebrates, the behaviour of social insects such as bees and ants (*i.e.* brood care, nest building, division of labour) has been largely studied. In intertidal invertebrates in general, and gastropods in particular, the most studied behaviour is trail-following (*i.e.* when individuals follow the tracks or paths of other individuals; see Ng et al., 2013 for a review). Trail-following in gastropods have multiple roles such as mate location (*i.e.* locating the right species, the right sex, hence facilitating sexual selection), nutrition (predatory snails follow the trails of other snails, or snails can feed on particles on mucus trails) and energy conservation (by re-using previously laid trails), and both aggregation and homing behaviour (Ng et al., 2013). However, many homing species do not necessary return to their resting site *via* the same path as they left and thus do not only used trail-following. Pieron, (1909) introduced the idea of kinaesthetic memory, which allowed homing limpets to determine their location based on previous movements and local topography. Non-homing species, such as littorinids snails are known to find their way back after dislodgement and return to their preferred habitats (Evans, 1961; Bock and Johnson, 1967; Gendron, 1977; Thain et al., 1985; Miller et al., 2007; Chapperon and Seuront, 2009; Seuront et al., 2018a). Specifically, Seuront et al. (2018a) suggested that intertidal snails are likely using a variety of directional cues to accomplish such directed movements. Numerous directional cues have been observed in intertidal snails such as chemotaxis[1] (see Croll (1983) and Ng et al. (2013) for reviews), rheotaxis[2] (Overholser, 1964; Neale, 1965; Crisp, 1969; Duch, 1971;

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[1] A movement either towards or away of an organism in response to chemical stimulus.

[2] A movement either towards or away from the stimulus induced by a flow.

Gendron, 1977), phototaxis[3] (Charles, 1961; Evans, 1961; Warburton, 1973; Petraitis, 1982; Thain et al., 1985), scototaxis[4] (Moisez and Seuront, 2020) or geotaxis[5] (Kanda, 1916; Newell, 1958a; Evans, 1961; Petraitis, 1982; Thain et al., 1985). However, a recent work highlighted the fact that, when *Littorina littorea* individuals encountered a topographical discontinuity in their environment, individuals consistently followed it before moving to a geotactic response. It suggested that the aforementioned geotaxis is essentially a by-product of thigmotaxis (Moisez and Seuront, 2020). As rocky intertidal shores are highly variable in term of topographical features, with the presence of pits, crevices, boulders and pools, thigmotaxis can be one of the major behaviours that can drive intertidal gastropods movements.

Intertidal habitats are one of the most complex and variable ecosystem and are characterized by four different gradients: the vertical gradient, the horizontal gradient, the particle size gradient and the salinity gradient (Raffaelli and Hawkins, 1996; Thompson et al., 2002). These habitats are fundamentally submitted to extreme physical conditions such as immersion time, wave exposure or microclimatic variations (*i.e.* salinity, temperature, oxygen concentration; Raffaelli and Hawkins, 1996). For example, intertidal species can typically face temperature variations as large as 20°C or more during a tidal cycle (Helmuth, 1999, 2002). Most intertidal invertebrates live close to the upper limit of their thermal tolerance window (Somero, 2002); intertidal invertebrates are thus highly vulnerable to the warming climate. Extreme events such as heat waves, droughts, storms and floods increase in frequency and duration and may be the norm under future climate scenarios (Meehl and Tebaldi, 2004; Rahmstorf and Coumou, 2011), and heat waves would become more intense, longer and more frequent (Meehl and Tebaldi, 2004). Mass mortality consequential to heat waves have already been reported in juvenile barnacles, limpets and mussels (Harley, 2008; Seuront et al., 2019). These mass mortality

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[3] A movement either towards or away from the stimulus induced by light.

[4] The natural preference for dark areas.

[5] A movement of an organism either or toward the stimulus of gravity.

caused changes in species abundance and diversity (Harley, 2011), but also impact trophic interactions, community structure and ecosystem functioning (Petchey et al., 1999; Dossena et al., 2012). Intertidal invertebrates and in particular molluscs contribute to the functioning, structure and productivity of intertidal habitats. They occupy key positions in the trophic webs as grazers that transfer the matter and energy from the primary producers to the higher consumers (Mcquaid and Branch, 1985; Peduzzi and Herndl, 1991). They also control algal growth (Stafford and Davies, 2005a) and the patterns of distribution and dispersion of others species (Chapman, 2000). Thus, their potential diminution in number due to heat waves could disrupt the ecological balance of intertidal shores. Intertidal gastropods developed a range of physiological, morphological and behavioural adaptations to adjust their body temperature in this changing environment. For examples, the production of heat shock proteins or antioxidant enzymes that protect biomolecules against thermal and oxidative stresses (Somero, 2002; Somero et al., 2017) or the shift to an anaerobic metabolism are typical examples of physiological adaptations to heat stress (McMahon and Russel-Hunter, 1977). Morphological adaptations such as shell colour, shell shapes or shell sculpture affect heating by absorption of shortwave radiation from the sun and thus can modify body temperature of intertidal snails and thus limit the impact of heat stress (Etter, 1988; Chapman, 1995; Harley et al., 2009). To limit the amount of solar radiation, gastropods change their resting posture and adjust their shell orientation towards the sun (Miller and Denny, 2011). To limit heat flux by conduction from the substratum, gastropods retract their foot in their shell and close their operculum (Miller and Denny, 2011; Rojas et al., 2013). Shell-posturing also allow the minimisation of foot surface in contact with the substratum and thus, limit heat conduction. For examples, mushrooming in limpets allow to reduce body temperature (Garrity, 1984; Williams et al., 2005) by ~2°C compared to individuals that are not able to mushroom (Williams et al., 2005). Shell lifting is widely observed in littorinids (Garrity, 1984; Miller and Denny, 2011), however this role on thermoregulatory behaviour is still unclear. Shell standing and towering are more efficient as shell standing can reduce body temperature by up to 6°C on hot and sunny in tropical species (Marshall and Chua, 2012). Towering can reduce body temperature by up to 10°C in sub-tropical snails (Seuront and Ng, 2016) and the upper individuals have significantly lower body temperature than the basal individual of the tower (~3-6°C; Marshall et al., 2010; Seuront and Ng, 2016). Mobile organisms can also select thermally favourable microhabitat such as shaded refuges, crevices or biogenic habitats created by mussels or barnacles (Jones and Boulding, 1999; Cartwright and Williams, 2012). Littorinids, by selecting thermal refuges, can maintain their body temperature below their thermal limits by up to 11°C for some species (Marshall et al., 2013). Generally, behavioural adaptations are more efficient than morphological ones (Miller and Denny, 2001). For examples, shell colour or shell sculpture can reduce body temperature about 0.2-2°C, whereas behavioural adaptations as removing the foot from the substrate or reorienting the shell can lower body temperature by 2-4°C on average (Miller and Denny, 2011). However, behavioural adaptations are still excluded from climate change models. Climate envelope models also neglect the variability in thermal stress over a wide range of spatio-temporal scales (Helmuth et al., 2005). Chapperon and Seuront, (2011a) showed that, between two different microhabitats, the temperature can vary by than 6°C. The small-scale variations in temperature between microhabitats is sometimes higher than temperature variations at larger scales. Climate change models argued that body temperature of intertidal ectotherms have the same thermal properties than their surrounding environment, however, body temperature of littorinid species is not correlated to air temperature but closely to the substrate temperature (Chapperon and Seuront, 2011a, 2011b). Similar observations were found on the blue mussel *Mytilus edulis*, for which body temperatures were consistently significantly higher than air temperature and there is no significant correlation between body temperature and air temperature (Seuront et al., 2019). Thus, using the air temperature as a proxy for body temperature of intertidal ectotherms may underestimate the impact of global warming on intertidal species (Chapperon and Seuront, 2011a; Seuront et al., 2019). In summer 2018, heat waves cause mussels mass mortality, however these successive heat waves were minor when studied based on air temperature, but drastic when considered in terms of thermal properties of both substrate and mussels (Seuront et al., 2019). Understanding how intertidal species can use behaviour to thermoregulate is then a key component to understand how they can face global warming.

Aims of this thesis.

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

(i) to investigate the thigmotactic behaviour of *Littorina littorea* under various topographical stimuli, and also under various conditions of salinity at scales pertinent for individual organisms;

(ii) to assess the space-time variability in environmental temperatures and to determine the consequence of this variability on body temperature and behaviour of *Littorina littorea* and *Patella vulgata*;

(iii) to investigate the behavioural adaptations of intertidal snails under heat stress and the importance of behaviour to compensate global climate change.

This thesis has been prepared as a suite of manuscripts accepted, submitted or to be submitted to scientific journals. The same methods (*i.e.* thermal imaging) was used to measure both the body temperature and surrounding substrate temperature of intertidal gastropods, implying some redundancies in the Methods of Chapters III and IV. The studies conducted during this thesis focused on the intertidal gastropods *Littorina littorea* and *Patella vulgata* along the French coast of the eastern English Channel. In Chapter II, I examined *Littorina littorea* behavioural response (*i.e.* thigmotactic response) to topographic discontinuities varying in forms and sizes and also to salinity variations. In Chapter III, I assessed temperature variations in four different habitats, during four sampling dates which are really different in term of temperature conditions. Hence, I assessed the effect of the temperature variations between and within the four habitats on the body temperature of *L. littorea* and *P. vulgata*. I also assessed the microhabitat selection relative to temperature of these two species and their aggregation behaviour which is typically considered to reduce desiccation and thermal stresses. Finally, in Chapter IV, I investigated the effect of aggregation behaviour on *L. littorea* individuals inside and at the edge of the aggregates under a moderate heat wave on the Opal Coast. I also tested, under laboratory conditions, the effect of a more drastic heat wave on *L. littorea* shells located inside and at the edge of aggregates.

Chapter II. Thigmotactic behaviour plays an understated poorly known, though major, role in *Littorina littorea* displacements under variable topographical complexity and various salinity scenarii

ABSTRACT

Intertidal rocky shores are characterized by a highly complex and variable topography, with the presence of pits, crevices or boulders. Intertidal rocky shores are also characterized by salinity short-term variations related to both evaporation and precipitation occurring at low tide. Intertidal species, such as gastropods, are de facto constrained by the interplay between topographic complexity and salinity variations. Intertidal gastropods are able to orient themselves through the topographical complexity of their environment using thigmotaxis (i.e. movement of an organism either towards or away from the stimulus induced by a physical contact). However, less is known about how the variability in the topographical discontinuities encounter may affect gastropods displacement. Here, we investigated the thigmotactic behaviour of Littorina littorea, *a common grazer typically found over the whole intertidal zone of temperate rocky shores, towards different topographic discontinuities and salinity variations. Specifically, we assessed its thigmotactic behaviour in cylindric and cubic experimental containers of three different sizes that were specifically chosen to mimic the presence of (i) an uninterrupted two-dimensional discontinuity (i.e. a surface-to-wall transition) and (ii) three-dimensional discontinuities characterized by both horizontal broken surface-to-wall transitions and vertical wall-to-wall-transitions. We further inferred the effect of decreasing salinity in the activity of* L. littorea *and its thigmotactic behaviour. We observed that the movement of* L. littorea *were not impacted by the size of the nature of the discontinuities. Moreover, the size of the discontinuities encountered by* L. littorea *had no impact on its thigmotactic response. However, we observed that the higher the complexity, the higher the discontinuity following. We observed that in cylindric containers, individuals consistently followed the discontinuity and subsequently switched to a negative geotactic behaviour by climbing the walls. In the cubic container, individuals also followed the horizontal discontinuities, but only climbed the wall once they reached one of the cube corner (i.e. at the point of contact between two surface-to-wall transitions and a wall-to-wall transition). These* *results suggest that what may be thought as a geotactic behaviour is actually a thigmotactic response to a vertical discontinuity. We also observed, in both cylindric and cubic containers, a general decrease of* L. littorea *activity with decreasing salinity. The occurrence of thigmotaxis was not affected by salinity, though the time individual snails spent following discontinuities decreased with decreasing salinity. Specifically, in the cubic containers, snails consistently climbed the walls mostly following wall-to-wall discontinuities. Taken together, our results suggest that thigmotaxis is one of the most important behaviour in gastropods species to navigate through topographically complex environment. They also suggest that movement behaviour of intertidal gastropods are only marginally affected by salinity changes.*

Key words: Gastropods – behaviour – thigmotaxis – topographical complexity – salinity

1. INTRODUCTION

Thigmotaxis is the movement of an organism either towards or away from the stimulus induced by a physical contact (Kalueff et al., 2013). This behaviour is known to contribute to the movement orientation of numerous species. For examples, some evidence for thigmotaxis behaviour were found in some terrestrial invertebrate species such as the meal worm *Tenebrio molitor* (Street, 1968), the earthworm *Lumbricus terrestris* (Doolittle, 1972) and the cockroaches *Periplaneta americana* (Okada and Toh, 2006). In aquatic species, thigmotaxis has been observed in the zebrafish *Dario rerio* (Kalueff et al., 2013) and the freshwater gastropods *Lymnaea stagnalis* (De Vlieger, 1968). Specifically, the intensity of the stimulus changes the thigmotactic response of *L. stagnalis*; positive thigmotaxis is caused by the repetition of weak tactile stimuli and negative thigmotaxis is the result of one strong stimulus (De Vlieger, 1968).

Marine species and in particular rocky intertidal species typically move in a highly complex environment (Denny and Gaines, 2007) characterized by a highly variable topography, and thus face variable stimuli. Rocky shores are characterized by the presence of pits, crevices or boulders (Raffaelli and Hawkins, 1996) and the presence of biogenic habitats typically created by species such as mussels and barnacles further contributes to increase the topographical complexity of rocky shores (Underwood and Chapman, 1989). Substrate topography affects different aspect of the ecology of intertidal gastropods such as distance travelled, population density and structure (Underwood and Chapman, 1989; Chapman and Underwood, 1994). In intertidal species, and in particular in gastropods, the ability to orientate across different habitats rely on essentially non-visual senses in environments characterized by topographically complex substrate and resource heterogeneity (Fratini et al., 2001; Keppel and Scrosati, 2004; Wyeth et al., 2006). Movements of intertidal gastropods are also triggered by different taxies such as chemotaxis (Croll, 1983; Chapperon and Seuront, 2009; Ng et al., 2013; Seuront and Spilmont, 2015), phototaxis (Newell, 1958a, 1958b; Evans, 1961; Warburton, 1973), scototaxis

(Hamilton and Russell, 1982; Thain et al., 1985; Moisez and Seuront, 2020) or geotaxis (Kanda, 1916; Hayes, 1926; Newell, 1958a, 1958b; Petraitis, 1982; Moisez and Seuront, 2020). A recent study conducted on *Littorina littorea* suggested that the directed movements consistently observed after a dislodgement toward their original habitat resulted from a combination of geotaxis, chemotaxis and rheotaxis (Seuront et al., 2018a).

Recently, evidence for a thigmotactic behaviour was found in the intertidal gastropod *Littorina littorea* (Moisez and Seuront, 2020). Specifically, individuals followed the topographical discontinuities of their environment and their thigmotactic behaviour induced a geotactic response. These observations suggest that previously reported positive or negative geotactic response (Kanda, 1916; Hayes, 1926; Newell, 1958a, 1958b; Petraitis, 1982) may actually have been essentially triggered by a thigmotactic response (Moisez and Seuront, 2020). Abiotic factors such as light (Newell, 1958a, 1958b; Charles, 1961; Evans, 1961), temperature, (Garrity, 1984) or salinity (Perez, 1969; Moser et al., 1989; Jury et al., 1994, 1995) can modify behaviour and in particular movements in intertidal gastropods. For example, to escape salinity variations, motile organisms can move in areas with optimal conditions (Perez, 1969; Moser et al., 1989; Jury et al., 1994, 1995). However, salinity stress can also reduce motility; for example, in the gastropods species, *Batillaria attramentaria,* a diminution in salinity lead to a diminution of the distance travelled by the individuals (Ho et al., 2019). The impact of salinity variations on taxies in intertidal gastropods is still unknown. Specifically, how diminution in salinity can affects thigmotaxis behaviour in intertidal gastropods?

In the present study, we choose the intertidal gastropod *Littorina littorea* as a model species due to its abundance on Western and Northern European coasts and its role in controlling algal growth (Stafford and Davies, 2005a), sediments dynamics (Kamimura and Tsuchiya, 2006) and the recruitment of both algae and invertebrates (Buschbaum, 2000; Lotze and Worm, 2002). *L. littorea* is found on rocky shore from the upper shore into the sublittoral and thus is submitted to large salinity variations. *L. littorea* is exposed to a broad range of topographic features, suggesting that it may also have adapted its senses to navigate in such a complex environment. In this context, we aimed to elucidate the behavioural response of *L. littorea* to different topographic discontinuities and salinity variations. Specifically, we assessed the thigmotactic response of *L. littorea* in cylindric and cubic experimental containers that were specifically chosen to mimic the presence of (i) an uninterrupted two-dimensional discontinuity (*i.e.* a surface-to-wall transition) and (ii) three-dimensional discontinuities characterized by both horizontal broken surface-to-wall transitions and vertical wall-to-wall-transitions. We also tested the thigmotactic response of *L. littorea* in the two experimental containers previously described of three different sizes. The behavioural activity and thigmotactic response of *L. littorea* were also considered under five conditions of salinity to assess the effect of this major intertidal stressor on their behavioural and navigational abilities.

2. METHODS

2.1 Collection and acclimation of Littorina littorea

Littorina littorea individuals were collected from the Fort de Croy (Wimereux, France; 50°45’48”N, 1°35’59”E) an intertidal reef typical of the rocky habitats found along the French coasts of the eastern English Chanel (Chapperon and Seuront, 2009; Seuront and Spilmont, 2015; Spilmont et al., 2018). Before any experiment took place, *L. littorea* individuals (10 to 15 mm in length) were acclimatized for 24 h in the laboratory in acrylic glass (*i.e.* polymethyl methacrylate, PMMA) cylinders (50 cm tall and 20 cm in inner diameter, riddle with holes 5mm in diameter) held in 120-l (90×50×30 cm) tanks of running natural seawater, aerated at temperatures representative of in situ conditions at the time of collection. These perforated ‘acclimation towers' (Seuront and Spilmont, 2015) allow both seawater to be continuously renewed and captive snails to move freely in and out of the water without being able to escape.

No food was available during the acclimation period. During the experiments, each individual was only used once.

2.2 Experimental conditions

The thigmotactic response of *L. littorea* was studied at scales pertinent to individual snails in six types of experimental containers. Specifically, we considered circular containers with three different diameters (*i.e.* 5, 8 and 11 cm, hereafter referred as D1, D2 and D3) and three cubic containers with three different sizes (*i.e.* 5, 8 and 11cm in side length, hereafter referred as C1, C2 and C3). The circular experimental containers were glass beakers (Fisherbrand) which were separated by light grey LEGO® Bricks walls to homogenize the visual field of each individuals as *L. littorea* used scototaxis to orient themselves (Moisez & Seuront, 2020). Cubic experimental containers were uniformly made of light grey LEGO® Bricks (Fig. 1). These experimental containers were built on a LEGO® plate (25.5 × 25.5 cm) glued on the bottom of a cubic glass aquarium and immersed in 10 cm of seawater.

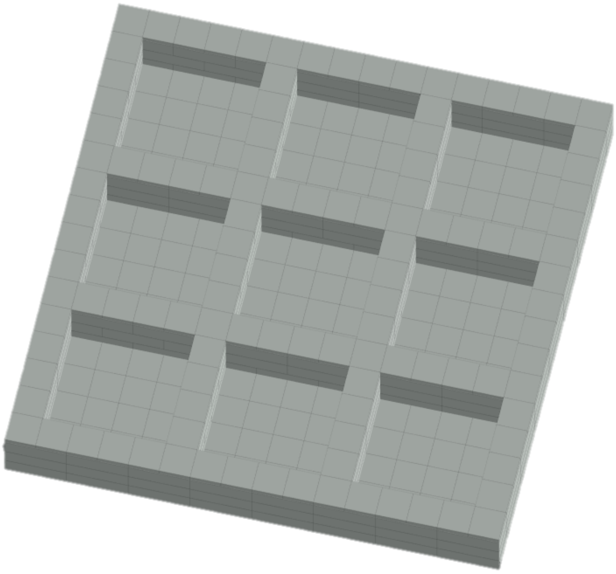

Fig. 1. Illustration of our Lego-based experimental container. In the cube experimental container, one *L. littorea* was put in each sub-section and in the circle experimental beakers were put in the cubic subsections and *L. littorea* individuals were put in the beakers.

More fundamentally, these different experimental containers were specifically used to allow *L. littorea* to face two distinct forms of topographic discontinuities of three different sizes, that is (i) an uninterrupted two-dimensional discontinuity, *i.e.* a surface-to-wall transition in circular containers (Fig. 2A) and (ii) three-dimensional discontinuities characterized by both horizontal broken surface-to-wall transitions and vertical wall-to-wall-transitions in cubic containers (Fig. 2B). One snail was used in each replicate structure; for the cubic containers, $N = 32$ for C1, C2 and C3 and for the cylindric containers, $N = 12$ for D1 and D2 and $N = 32$ for D3.

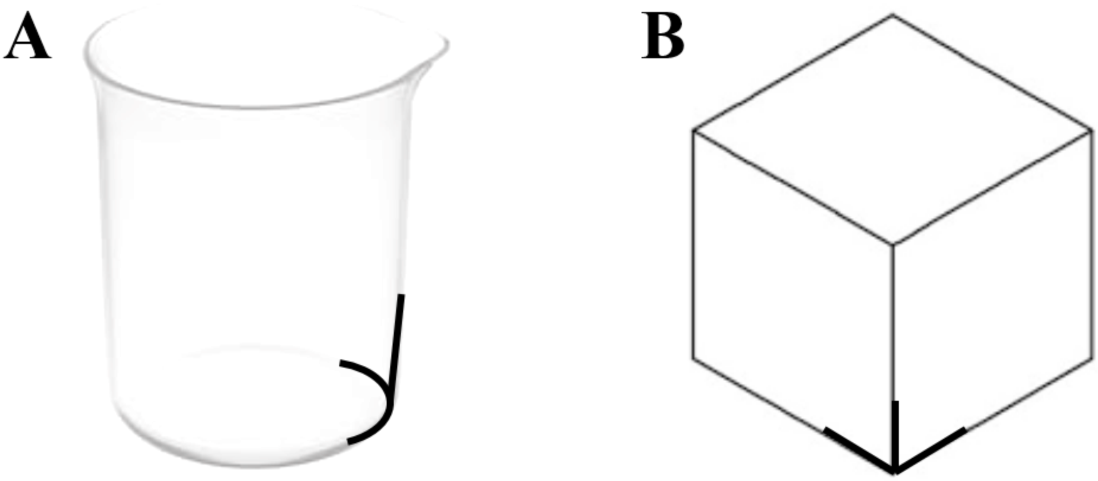

Fig. 2. Illustration of ours two different experimental containers were specifically used to allow *Littorina littorea* individuals to face two distinct forms of topographic discontinuities, that is (A) an uninterrupted two-dimensional discontinuity and (B) three-dimensional discontinuities characterized by both horizontal broken surface-to-wall transitions and vertical wall-to-wall-transitions.

The thigmotactic response of *L. littorea* was also studied under different salinity conditions in the two types of experimental containers previously described (5 cm diameter for the cylindric ones and 5 cm of size for the cubic ones). Specifically, four conditions of salinity and a control (hereafter referred as *C*) were tested in each experimental container. The control was natural seawater collected on Wimereux (France) with a salinity equal to 33.4 PSU. Natural seawater was diluted at 20% (26.7 PSU), 30% (23.4 PSU), 40% (20 PSU) and 50% (16.7 PSU). Control and dilution at 20% and 30% are realistic salinity find in the intertidal environment, whereas dilutions at 40 and 50% are extreme decreasing salinity conditions to have a pronounced answer of *L. littorea*. One snail was used in each replicate structure, which were further triplicated (N = 24 for the cylindric container and N = 27 for the cubic one) for each salinity condition.

The motion behaviour of *L. littorea* individuals was recorded every 5 s during 60 min using a Raspberry Pi NOiR camera overlooking the experimental set-up and operated through a Raspberry computer under homogenous dim light conditions (*i.e.* 168 lx) measured with a digital lightmeter (Extech Instruments, 403,125; Moisez and Seuront, 2020). The resulting 720 images where subsequently assembled using Time Lapse Tool (©Al Devs) before behavioural analyses took place. We considered the end of the experiment when the individuals exit the cylinder or the cube. Between each trial, the behavioural set-up was rinsed with 70% ethanol and seawater to remove mucus cues (Erlandsson and Kostylev, 1995).

2.3 Behavioural analysis

For each snail we measured the time spent actively moving (*i.e.* activity time, T_{act}) and being inactive (T_{inact}). The activity time included three distinct behavioural activities: (i) the time spent displacing on the bottom of the apparatus (T_b), (ii) the time spent in discontinuity-following (T_f) and (iii) the time spent displacing on the wall of the apparatus (T_w). The intensity of discontinuity-following was assessed by the number N of revolutions that snails continuously made on the discontinuity, through the following classification: $N = 0$, $N < 1$ and $N \geq 1$. The percentage of individuals that followed the discontinuity in the two experimental containers was recorded. The percentage of individuals that climbed the wall in the two experimental containers was recorded, and specifically we also recorded the percentage of individuals that climbed on the corner of the wall in the cubic experimental container.

2.4 Statistical analysis

As the distribution of measured parameters was non-normally distributed (Shapiro-Wilk test, $p > 0.05$), non-parametric statistics were used throughout this work. The inactivity time (T_{inact}), activity time (T_{act}) and the durations which composed the activity time (T_b, T_f, T_w) were compared using a Kruskal-Wallis (hereafter K-W test) test between each size condition for each experimental container and also between each salinity conditions for each experimental containers; when necessary a subsequent post-hoc test was performed using a Dunn test (Zar, 2010). These different times were also compared between circular and cubic experimental container of the same size or of same salinity conditions using a Mann-Whitney (hereafter M-W test) pairwise test.

3. RESULTS

3.1. Behavioural activity of Littorina littorea

3.1.1 Thigmotactic experiment

In the cubic containers, there was significant difference in the activity and inactivity times, time spent on the bottom and on the wall between the three different sizes of experimental containers (K-W test, $p < 0.05$). Specifically, for T_{act}, a subsequent Dunn test identified significantly distinct groups of measurements as C3 > C1 = C2 ($p < 0.05$; Fig. 3A). T_{inact} in C1 was significantly lower than in C3 (Dunn test, $p < 0.01$) and there was no significant difference between C2 and C3 and between C1 and C2 (Dunn test, $p > 0.05$; Fig. 3A). In T_b distinct groups of measurements were significantly identified as C1 < C2 = C3 (Dunn test, $p < 0.001$; Fig. 3A). In T_w the groups of measurements ranked as C1 = C2 < C3 (Dunn test, $p < 0.001$; Fig. 3A).

There was no significant difference in the time of discontinuity following between C1, C2 and C3 (K-W test, $p > 0.05$; Fig. 3A).

In the cylindric containers there was a significant difference in the time spent on the bottom and on the walls (K-W test, $p < 0.01$) between the three different sizes of the experimental containers. Specifically, for T_b a subsequent Dunn test identified significantly distinct groups of measurements as D3 > D1 = D2 ($p < 0.001$; Fig. 3B). In T_w, the groups of measurements ranked as D2 > D1 = D3 (Dunn test, $p < 0.01$; Fig. 3B). There was no significant difference in the activity and inactivity times and the duration of discontinuity following between the three sizes of cylindric containers (K-W test, $p > 0.05$; Fig. 3A, B).

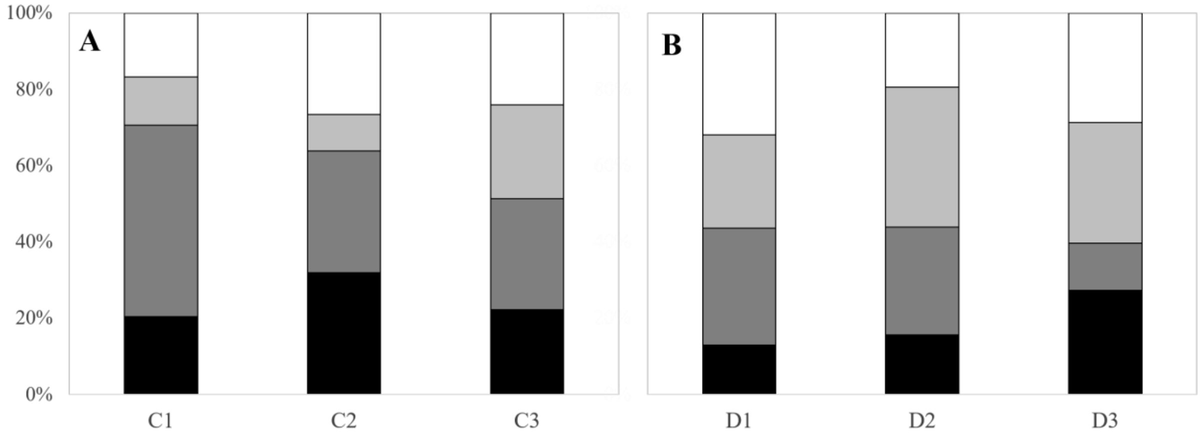

Fig. 3. Percentage of the different studied times. In black, the percentage of time of displacement on the bottom of the apparatus; in dark grey the percentage of time of discontinuity-following; in grey the percentage of time of displacement on the walls of the apparatus and in white the percentage of time of inactivity in (A) the cubic experimental containers and in (B) the cylindric ones for the three sizes of experimental containers. Noted that, the time of displacement on the bottom, the time of displacement on the walls and the time of discontinuity-following formed the time of activity.

The inactivity time and the time spent on the wall were significantly higher on D1 than on C1 (M-W test, $p < 0.01$). The activity time, time spent on the bottom and following discontinuity were not significantly different between C1 and D1 (M-W test, $p > 0.05$). The time spent on the bottom and on the walls significantly differed between C3 and D3. Specifically, T_b was higher on C3 than on D3 and T_w was higher on D3 than on C3 (M-W test, $p < 0.01$). There was no significant difference on the activity and inactivity times and the duration of discontinuity following between C3 and D3 (M-W test, $p > 0.05$). The activity time of C5 was significantly higher than in D5 (M-W test, $p < 0.05$). The duration of discontinuity following was significantly higher in C5 than in D5 (M-W test, $p < 0.05$). There was no significant difference in the inactivity time, durations on the bottom and the walls between C5 and D5 (M-W test, $p > 0.05$).

3.1.2 Thigmotactic and salinity experiment

The time of inactivity was significantly higher for high dilutions (20 PSU and 16.7 PSU) than for the control and low dilutions (26.7 PSU and 23.4 PSU) for both experimental containers (K-W test, $p < 0.01$ and Dunn post-hoc test, $p < 0.05$, Fig. 4, Table. 1).

The time of activity differed between salinity conditions only in the cylindric experimental container, being significantly lower for low salinities (K-W test, $p < 0.0001$ and Dunn post-hoc test, $p < 0.01$, Fig. 4B, Table. 1), whereas, in the cubic container, no distinct pattern was found (Dunn post-hoc test, $p < 0.01$, Fig. 4A, Table. 1).

Table. 1. Results of the Dunn post-hoc test comparing the total time (T_t), the activity time (T_{act}), the inactivity time (T_{inact}), the time on the bottom (T_b), the time of discontinuity-following (T_f) and the time on the wall (T_w) between the five salinity conditions. The *p*-value for the cylindric containers are overhead the grey cells and the *p*-value for the cubic containers are below the grey cells. The significant difference are represented in bold. For T_b on the cubic container there is no significant difference in the Kruskal-Wallis test.

Tt

	33.4 PSU	**26.7 PSU**	**23.4 PSU**	**20 PSU**	**16.7 PSU**
33.4 PSU		0,3884	0,1707	**0,0003306**	**0,008398**
26.7 PSU	0,3839		0,6118	**6,38E-03**	0,07621
23.4 PSU	0,6445	0,6722		**2,64E-02**	0,2057
20 PSU	**0,01006**	**0,0005719**	**0,002516**		0,3399
16.7 PSU	0,1038	**0,01252**	**0,03807**	0,3434	

Tact

	33.4 PSU	**26.7 PSU**	**23.4 PSU**	**20 PSU**	**16.7 PSU**
33.4 PSU		0,3371	0,1692	**0,002578**	**0,02269**
26.7 PSU	0,1273		0,6781	**7,73E-05**	**0,001245**
23.4 PSU	0,2002	0,8075		**1,31E-05**	**0,0002739**
20 PSU	**0,01487**	0,4723	0,3358		0,4512
16.7 PSU	**0,003286**	0,1571	0,09718	0,4863	

Tinact

	33.4 PSU	**26.7 PSU**	**23.4 PSU**	**20 PSU**	**16.7 PSU**
33.4 PSU		0,4705	0,5955	**0,0005628**	**6,67E-05**
26.7 PSU	0,9763		0,8632	**5,52E-05**	**5,78E-05**
23.4 PSU	0,8902	0,9178		**1,54E-04**	**1,80E-05**
20 PSU	**7,89E-05**	**0,0001126**	**2,25E-05**		0,4976
16.7 PSU	**0,01756**	**0,01961**	**0,009053**	0,09703	

Tb

	33.4 PSU	**26.7 PSU**	**23.4 PSU**	**20 PSU**	**16.7 PSU**
33.4 PSU		0,05545	**0,006632**	**0,002273**	**0,001298**
26.7 PSU	--		0,424	0,2557	0,1932
23.4 PSU	--	--		0,7359	0,6159
20 PSU	--	--	--		0,8694
16.7 PSU	--	--	--	--	

Tf

	33.4 PSU	**26.7 PSU**	**23.4 PSU**	**20 PSU**	**16.7 PSU**
33.4 PSU		0,7303	**0,02559**	**0,04**	**0,006441**
26.7 PSU	0,08833		0,05908	**0,01647**	**0,002147**
23.4 PSU	0,1786	0,7193		**1,82E-05**	**7,17E-07**
20 PSU	**0,00171**	0,1521	0,07321		0,5024
16.7 PSU	**0,0001441**	**0,03601**	**0,01404**	0,5063	

Tw

	33.4 PSU	**26.7 PSU**	**23.4 PSU**	**20 PSU**	**16.7 PSU**
33.4 PSU		0,2963	0,05796	**0,0004099**	0,1353
26.7 PSU	0,1932		**0,003278**	**4,69E-06**	**0,01115**
23.4 PSU	0,341	0,7843		1,02E-01	6,87E-01
20 PSU	**7,85E-04**	**3,97E-02**	**1,98E-02**		**0,04134**
16.7 PSU	**0,02935**	0,3802	0,2497	0,2382	

In the cylindric and cubic experimental containers, there were significant differences in T_f and T_w between the five salinity conditions (K-W test, $p < 0.001$). However, no clear pattern was found (Dunn post-hoc test, $p < 0.005$, Fig. 4, Table. 1). In the cylindric experimental container, T_b significantly differ between salinity conditions (K-W test, $p < 0.01$) and no distinct patter was found (Dunn post-hoc test, Table. 1). In contrast, there was no significant difference (K-W test, $p > 0.05$) in T_b between salinity conditions in the cubic experimental container.

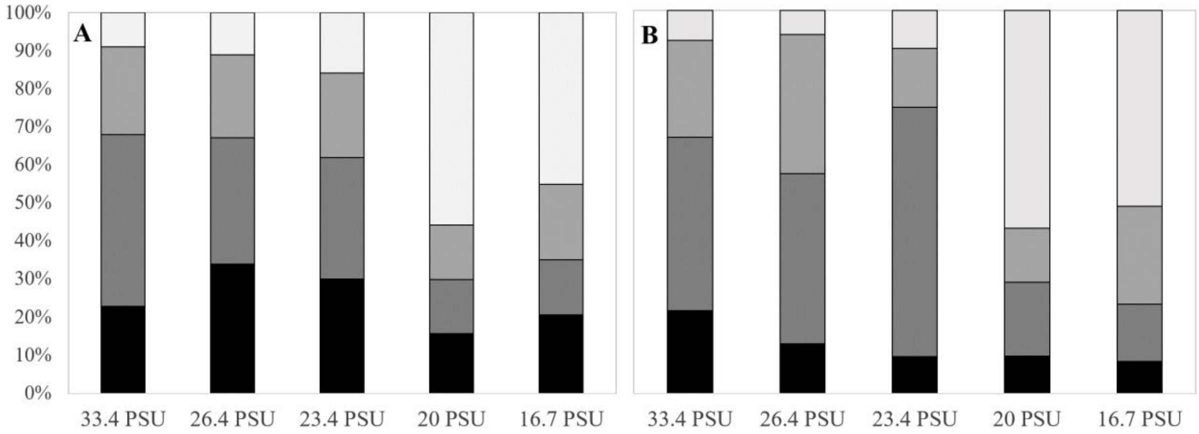

Fig. 4. Percentage of the different studied times. In black, the percentage of time of displacement on the bottom of the apparatus; in dark grey the percentage of time of discontinuity-following; in grey the percentage of time of displacement on the walls of the apparatus and in white the percentage of time of inactivity in (A) the cubic experimental container and in (B) the cylindric ones for the five salinity conditions. Noted that, the time displacement on the bottom, the time of displacement on the walls and the time of discontinuity-following formed the time of activity.

There was no significant difference in T_{inact} and T_b between the cylindric and cubic experimental devices for the five salinity conditions (M-W test, $p > 0.05$; Fig. 4). The activity time T_{act} between the circle and cube experimental containers was significantly different for *C*, 26.7, 23.4 and 16.7 PSU conditions (M-W test, $p < 0.01$; Fig. 4). T_f in the cylindric container was significantly higher than T_f in the cubic container for the 26.7 and 23.4 PSU conditions (M-

W test, $p < 0.0001$, Fig. 4A, B). T_w in the cylindric container was significantly higher than T_w in the cubic one for C and 26.7 PSU conditions (M-W test, $p < 0.001$, Fig. 4A, B).

3.2 Discontinuity-following behaviour

3.2.1 Thigmotactic experiment

In the cubic container C1, 100% of *L. littorea* individuals followed the discontinuity, in C2 96.7% and in C3 96.8% of individuals followed it. In the cylindric containers, the percentage of individuals following the discontinuity in D1, D2 and D3 was respectively 75%, 40% and 50%. The individuals that did not follow the discontinuity did not avoid it, but instead either directly climbed the wall without discontinuity-following. In the cubic containers, geotaxis without discontinuity-following (*i.e.* thigmotaxis) occurred only for 3.3% of *L. littorea* individuals in C2 and C3 conditions and did not occur in C1. In the cylindric containers, geotaxis without thigmotaxis occurred for 25%, 60% and 50% of *L. littorea* individuals in respectively D1, D2 and D3 conditions.

In the cubic containers, the percentage of *L. littorea* individuals with number of revolutions, $N = 0$ was lower than 4% in C1, C2 and C3. The percentage of individuals for $N < 1$ increased with the increasing size of the cubic containers and ranged from 86.7% to 93.5%. Finally, the percentage of individuals for $N \geq 1$ decreased with the increasing size of the cubic containers and ranged from 3.2 to 13.1% (Fig. 5A). In the cylindric containers, the percentage of individuals for $N = 0$ was 25%, 60% and 50% for D1, D2 and D3 respectively. The percentage of *L. littorea* individuals for $N < 1$ was 50%, 20% and 50% for D1, D2 and D3 respectively. Finally, the percentage of individuals for $N \geq 1$ decreased with the increasing diameter of the cylindric containers, and ranged from 0 to 25% (Fig. 5B).

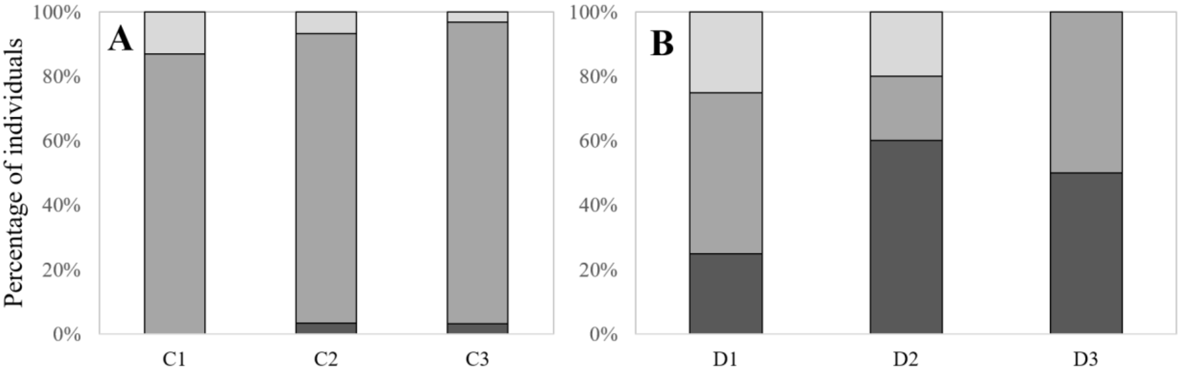

Fig. 5. Percentage of *Littorina littorea* individuals doing $N = 0$ revolutions on the discontinuity in dark grey, $N < 1$ revolutions in grey and $N \geq 1$ in light grey for the three size of the cubic (A) and the cylindric (B) experimental containers.

3.2.2 Thigmotactic and salinity experiment

In *C*, 26.7 and 23.4 PSU conditions, 100% of *L. littorea* individuals followed the discontinuity in the cylindric container. In 20 and 16.7 PSU conditions, 75 and 70.8% of individuals followed the discontinuity, respectively. In the cubic container, 100% of the individuals followed the discontinuity in *C* and 26.7 PSU conditions. In 23.4, 20 and 16.7 PSU conditions, 92.6, 77.8 and 66.7% of *L. littorea* individuals respectively followed at the discontinuity. There was no significant difference in the number of individuals that followed the discontinuity between the cylindric and the cubic containers (Chi², $p > 0.05$). The individuals that did not follow the discontinuity, did not avoid it, but instead either directly climbed the wall without discontinuity-following or did not move or only on the bottom of the container. In the cubic container, geotaxis without discontinuity-following (*i.e.* thigmotaxis) occurred only for 7.4 and 3.7% of *L. littorea* individuals respectively in 23.4 and 16.7 PSU conditions. In the cylindric container, geotaxis without thigmotaxis only occurred in the 20 and 16.7 PSU conditions for respectively 4.16 and 16.7% of the individuals.

In the cylindric container, the percentage of *L. littorea* individuals with number of revolutions, $N = 0$ was inferior to 5% in C, 26.7 and 23.4 PSU conditions whereas for the 20 and 16.7 PSU conditions was equal to 33.3 and 29.1% respectively. The percentage of individuals for $N < 1$ ranged from 16.7 to 33.3% for C, 26.7 and 23.4 PSU conditions and was equal to 41.7% in 20 and 16.7 PSU conditions. Finally, the percentage of snails for $N \geq 1$ ranged from 62.5 to 83.3% for C, 26.7 and 23.4 PSU conditions and dropped to 25 and 29.2% for 20 and 16.7 PSU conditions respectively (Fig. 6A). In the cubic container, the percentage of individuals for $N = 0$ increased with the increasing salinity; this percentage ranged from 11.1 to 14.8% for C, 26.7 and 23.4 PSU conditions and was equal to 40.7 and 29.6% for 20 and 16.7 PSU conditions, respectively. The percentage of individuals for $N < 1$ ranged from 44.4% to 70.3% for the five salinity conditions. Finally, the percentage of individuals for $N \geq 1$ decreased with the decreasing salinity, and ranged from 0 to 40.7% in the five salinity conditions (Fig. 6B).

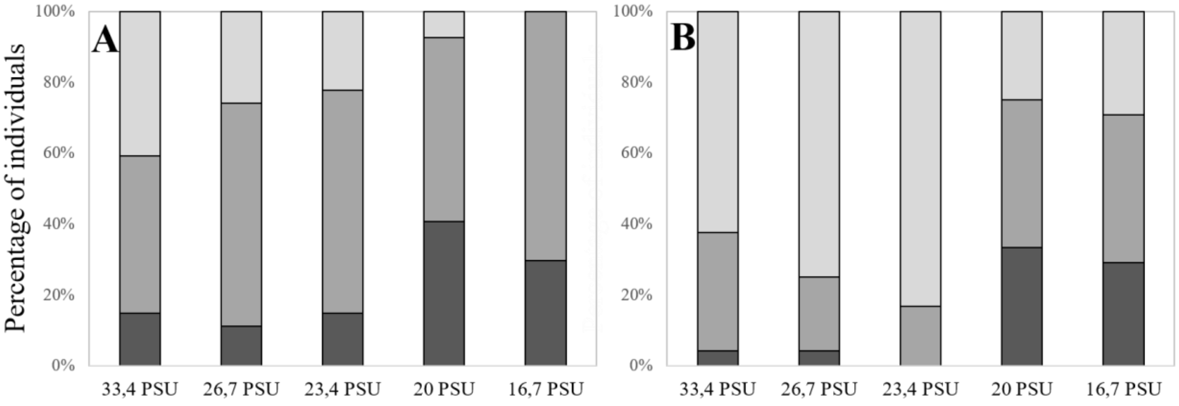

Fig. 6. Percentage of *Littorina littorea* individuals doing $N = 0$ revolutions on the discontinuity in dark grey, $N < 1$ revolutions in grey and $N \geq 1$ in light grey for the five salinity conditions in the cylindric container (A) and the cubic one (B).

3.3. Frequency of occurrence of climbing behaviour

3.3.1 Thigmotactic experiment

In the three cubic and three cylindric containers, 100% of *L. littorea* individuals climbed the walls. In the cubic containers, the vast majority (*i.e.* 87%) of the individuals climbed the wall in the corner (*i.e.* following a wall-to-wall discontinuity) in C1, 70% in C2 and 61.3% in C3.

3.3.2 Thigmotactic and salinity experiment

In the cylindric container, in *C* and 26.7 PSU conditions, 95.8 and 91.7% of *L. littorea* individuals climbed on the wall. The percentage of individuals climbed on the wall dropped to 58.3, 33.3 and 45.8% in 23.4, 20 and 16.7 PSU conditions, respectively. In the cubic experimental container, more than 90% of *L. littorea* individuals climbed on the wall (*i.e.* in the corner or not) in C, 26.7 and 23.4 PSU conditions. In 20 and 16.7 PSU conditions, this percentage dropped to 40.7 and 51.9% respectively. Specifically, the percentage of *L. littorea* individuals climbing the wall in the corner (*i.e.* following a wall-to-wall discontinuity) was 88.9, 72, 84, 81.8 and 78.6% in *C,* 26.7, 23.4, 20 and 16.7 PSU conditions, respectively.

4. DISCUSSION

4.1 Increase in topographical complexity increase Littorina littorea *thigmotactic behaviour*

Littorina littorea thigmotactic behaviour was assessed through its capacity to follow the topographical discontinuities in both cylindric and cubic experimental containers. This behaviour was assessed using purposed-designed two- and three-dimensional discontinuities. The inactivity time and the different durations that composed the activity time were not impacted by the size and by the nature of the discontinuities (*i.e.* 2D or 3D discontinuities) encountered in the experimental containers. These results suggest that the nature of the discontinuities and their size did not impact movement in *L. littorea*. *L. littorea* is found on most temperate rocky shores and is the most abundant herbivore found on the Western and the Northern European coasts. Individuals are found from the upper shore to the sublittoral (Seuront et al., 2007) and thus can encounter a large diversity of substrate such as pits, crevices or boulders (Raffaelli and Hawkins, 1996). The topography of rocky shores was also increase by the presence of biogenic structures such as mussels and barnacles beds (Underwood and Chapman, 1989). *L. littorea* is, thus, able to use the topography of its environment to orientate itself through such a complex environment. The complexity of the substrate also allows intertidal snails to find their way back to preferred habitat after dislodgement or transplantation (Evans, 1961; Bock and Johnson, 1967; Gendron, 1977; Thain et al., 1985; Rolán-Alvarez et al., 1997; Miller et al., 2007; Chapperon and Seuront, 2009; Seuront et al., 2018a).

No matter the size of the discontinuity encountered, *Littorina littorea* individuals kept following it, and thus maintained their thigmotactic behaviour. However, the nature of the discontinuities appeared to have an impact on the thigmotactic behaviour of *L. littorea*. The higher the complexity of the discontinuities, the higher the proportion of individuals that followed them. Furthermore, the speed of displacement of *L. littorea* was higher in the cubic containers than in the cylindric ones. The proportion of *L. littorea* individuals climbing on the walls of the experimental container without discontinuity-following was higher in the cylindric containers than in the cubic ones. In the three cubic containers, more than 60% of the individuals climbed the wall in the corner, and thus kept following a discontinuity. These results suggested that two-dimensional discontinuity essentially induced thigmotaxis and then a geotactic response, previously identified as a thigmotactically-induced negative geotactic response (Moisez and Seuront, 2020). In turn, when the discontinuity is three-dimensional, individuals essentially keep following it, and as such the seemingly geotactic behaviour is actually a manifestation of thigmotaxis. Geotaxis has already been reported as a by-product of thigmotactic behaviour and was consistently observed after that *L. littorea* followed topographic discontinuities (Moisez and Seuront, 2020). These results provide further insights into our understanding of how intertidal gastropods may navigate through complex landscape. In fact, topography of intertidal rocky shores is usually highly variable with presence of boulders, pits, crevices and rock pools (Raffaelli and Hawkins, 1996). The presence of biogenic habitats typically created by barnacles and mussels also contributes to increase the complexity of surface topography on rocky shores (Underwood and Chapman, 1989). The topography of intertidal rocky shore is really important for intertidal snails and affects different aspect of their ecology such as distance travelled, population density and structure (Underwood and Chapman, 1989; Chapman and Underwood, 1994). Features of the substratum such as rock pools (Underwood, 1977), crevices (Underwood and Chapman, 1985), or slope (Evans, 1961; Petraitis, 1982) affected movements of intertidal gastropods. The complexity of surface topography also allows littorinids to find their way back to a preferred habitat following dislodgment or transplantation (Evans, 1961; Bock and Johnson, 1967; Gendron, 1977; Thain et al., 1985; Rolán-Alvarez et al., 1997; Miller et al., 2007; Chapperon and Seuront, 2009; Seuront et al., 2018a). The idea of a kinaesthetic memory based on previous movement and local topography allows homing behaviour on limpets (Pieron, 1909). Our results suggested that what may be considered as geotaxis in intertidal gastropods is actually a manifestation of thigmotaxis. This can be relevant for our understanding of their way to move, their ability to found their way back or their repartition on the shore. Despite the highly complex intertidal environment, less is still known about the role of substrate topography on navigational abilities of intertidal gastropods.

4.2 Decreasing salinity decrease activity and intensity of thigmotactic behaviour on Littorina littorea

In both cylindric and cubic experimental devices, we observed a general decrease in the activity with decreasing salinity. These results are consistent with previous evidence that *L.*

littorea and *L. saxatilis* decrease their activity for salinities down to 18 PSU and 15 PSU respectively (Arnold, 1972). Similar results were found in the mud intertidal snail *Batillaria attramentaria*, when exposed to large range of decreasing salinity, displacement and distance travelled were significantly reduced (Ho et al., 2019). The activity and inactivity times of our control *C* (33.4 PSU), dilutions at 20 and 30% (26.7 and 23.4 PSU) were not significantly different and were significantly different from the 20 and 16.7 PSU conditions. These results may indicate the presence of a threshold for low salinity tolerance between 23.4 PSU and 20 PSU. This hypothesis is consistent with the threshold identified for *B. attramentaria* (between 23 and 13 PSU; Ho et al., 2019). At 23 PSU, locomotion of individuals were not impacted by the decrease in salinity, whereas at 13 PSU individuals were less active than at higher salinity (Ho et al., 2019). This threshold suggested that *L. littorea* and gastropods species in general were able to cope with short-term (*i.e.* tidal cycle scale) decrease in salinity.

Our results also showed that the time of discontinuity-following (T_f) significantly decreased in low salinity conditions. Moreover, the intensity of discontinuity-following decreased with salinity. However, the percentage of individuals climbing in the corner did not decrease with salinity. These results suggested that salinity affected not only movement of *L. littorea* but also modified the intensity of thigmotactic behaviour. *L. littorea* thigmotactic behaviour was shorter and less intense with the diminution of salinity but was consistently observed. On rocky intertidal shores, decrease in salinity occurred at low tide and is accentuated in tide pools, which are really common (Raffaelli and Hawkins, 1996; Little et al., 2009). Rock pools act as refuges against stresses linked to the emersion and are generally more beneficial habitats than nearby open rock (Raffaelli and Hawkins, 1996). However, these pools are submitted to physico-chemical variations with changes in O_2 and CO_2 concentrations, pH and salinity (Morris and Taylor, 1983). Evaporation, due to solar radiations, and dilution, due to precipitations, lead to salinity variations in rock pools (Raffaelli and Hawkins, 1996; Little et al., 2009). Salinity variations are known to affect physiology with changes in oxygen consumption (Cheung, 1997) and in cell volume regulation (Deaton, 2009) or diminution in metabolism and cardiac activity (Liu et al., 1990); and behaviour with closure of the operculum (Davenport, 1981) or movement into less hostile zone (Perez, 1969; Moser et al., 1989; Jury et al., 1994, 1995) on intertidal gastropods. Thigmotaxis behaviour are still observed with salinity diminution even if it is less intense. This mean that, even at low tide, when the conditions of salinity became more variable *L. littorea* and gastropods in general are able to use the topographical complexity of their environment to orient themselves and to find their way back to preferred shore levels or resting habitat after dislodgement. However, much work is still needed to understand how some biotic and abiotic factors, as food availability, presence of conspecifics or predators, presence of pollutant or temperature variations, can interact with the thigmotactic behaviour in *L. littorea*. For example, the presence of predators' cues is already known to increase the vigilance and antipredator responses in *L. littorea* (Seuront, 2018a) and to reduce its grazing time (Curtin, 2017); how the presence of predators can impact thigmotactic behaviour? Furthermore, an increase in seawater temperature by up to 10°C (from 10°C to 20°C) can reduce the activity time in *L. littorea* (Curtin, 2017); what about the impact of temperature variations on the thigmotactic behaviour in *L. littorea*?

CONCLUSION

This study suggests that the more complex the topographical discontinuity (*i.e.* three-dimensional discontinuity), the more intense the thigmotactic behaviour in *Littorina littorea.* It also suggested that no matter the size of the discontinuity, individuals keep following it. These results suggested that thigmotactic behaviour had a key role in the orientation and displacement of *L. littorea* in rocky shores. We also stressed that what was once thought a geotaxis is actually a thigmotactic. Geotaxis is still encountered when topographical discontinuities are less complex and is generally preceded by thigmotaxis, hence it should be referred to as a thigmotactically-induced negative geotactic response *sensu* Moisez and Seuront, (2020). Moreover, decrease in salinity decreases the activity level of *L. littorea*, and has a negative impact on the time of discontinuity-following though thigmotaxis remained by far the dominant behaviour even when salinity was reduced by half. These results suggested that thigmotaxis is a key behaviour in *L. littorea* in particular, and eventually in intertidal gastropods in general. Much work is, however, still needed to understand how others parameters that also varied during emersion time as pH, temperatures or increasing salinity, and also how biotic factors can modify thigmotactic behaviour on intertidal gastropods.

Chapter III. Microhabitats choice in intertidal gastropods is species-, temperature- and habitat-specific

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ABSTRACT

Understanding how behavioural adaptations can limit thermal stress for intertidal gastropods will be crucial for climate models. Some behavioural adaptations are already known to limit desiccation and thermal stresses as shell-lifting, shell-standing, towering, aggregation of conspecifics or habitat selection. Here we used the IRT (i.e. infrared thermography) to investigate the thermal heterogeneity of a rocky platform at different spatial scales, with four different macrohabitats (i.e. bare rock, rock with barnacles, mussels and mussels incrusted by barnacles) during four seasons. We investigated the body temperature of Littorina littorea *and Patella vulgata found on this platform and the temperature of their microhabitat (i.e. the substratum within one body length around of each individual). We also considered the aggregation behaviour of each species and assessed the percentage of thermal microhabitat choice (i.e. choice for a microhabitat with a temperature different than the surrounding substrate). We did not find any aggregation of* L. littorea *on the rocky platform during the four studied months. In contrast,* P. vulgata *were found in aggregates in all the studied periods and within each habitat, but there was no difference in body temperature between aggregated and solitary individuals. We noted that these two gastropods species were preferentially found on rock covered by barnacles in the four studied months. The presence of a thermal microhabitat choice in* L. littorea *and* P. vulgata *is habitat-dependent and also season-dependent. In June, July and November the choice was for a microhabitat with temperatures lower than the temperatures of the surrounding substrate whereas in December, individuals choose microhabitats with higher temperatures than the temperatures of their substratum. All together, these results suggest that gastropods species are able to explore their environment to find sustainable thermal macrohabitats and microhabitats and adapt this behaviour in function of the conditions of temperatures.*

Key words: Gastropods – behaviour – temperature – temperature – microhabitat choice – aggregation

1. INTRODUCTION

For both terrestrial and aquatic animals, habitat selection is a way to attract mates, raise offspring, avoid both predation and extreme climatic conditions, and may be influenced by biotic (competition, predation) and abiotic (temperature, relative humidity) factors. Among marine systems, rocky intertidal shores are considered as one of the most thermally variable and stressful environments (Harley, 2008). In these environments, intertidal species have to cope with physical and chemical stresses which intensity depends on the interaction of four major environmental gradients: the vertical gradient (*i.e.* "tidal" gradient), the horizontal gradient (*i.e.* exposure to waves), the sediment particle size gradient, and the marine-freshwater gradient of salinity (Raffaelli and Hawkins, 1996). These gradients, together with species interactions, lead to a zonation in the distribution of intertidal rocky shores species (Raffaelli and Hawkins, 1996; Little et al., 2009). Within this meso-scale zonation, mobile species can select different microhabitats, such as boulders, crevices or pools. The thermal stress has been shown to be triggering this microhabitat choice. Indeed, intertidal species can typically face temperatures variations up to ca. 20°C during a tidal cycle (Helmuth, 2002) and most intertidal invertebrates live close to the upper limit of their thermal tolerance window (Somero, 2002) that lead them to choose the most favourable environment for their survival. The body temperature of intertidal invertebrates can vary by 8-12°C depending on the microhabitat they occupy (Seabra et al., 2011); for instance, the gastropod *Nerita atramentosa* is found on rock platforms and boulder fields with different topographic complexity and thus different substratum temperatures (*i.e.* up to 4.18 °C of mean temperature difference in autumn; Chapperon and Seuront, 2011a). On the other hand, it has been shown that other littorinid snails, such as *Cenchritis muricatus*, rarely inhabit open areas and are preferentially found in crevices or in depressions (Judge et al., 2011). The orientation of the microhabitat towards sunlight can also influence the distribution of intertidal species; for example, the snail *Littoraria scabra*, is consistently found on the shaded part of its habitat and thus actively chose a microhabitat with lower temperatures than microhabitats directly exposed to solar radiations (Chapperon and Seuront, 2011b).

In a context of global climate change, rare events such as storms, floods, droughts or heat waves are likely to become the norm (Meehl and Tebaldi, 2004; Rahmstorf and Coumou, 2011; Oswald and Rood, 2014). In particular, heat waves may have dramatic consequences on intertidal species as massive mortality in juvenile barnacles, limpets and mussels (Harley, 2008; Seuront et al., 2019). Intertidal ectotherms developed some physiological adaptations as heat-shock protein expression, modifications in heart function or in mitochondrial respiration (Somero, 2002) and behavioural adaptations as habitat selection, aggregation, retraction of the foot into the shell or orientation of the shell (Chapperon and Seuront, 2011a, 2011b; Miller and Denny, 2011) to face these heat waves. These adaptations may become the norm under future climatic scenarios and their understanding in thus critical to assess the diversity and functioning of intertidal communities for the next decades.

Temperatures of rocky intertidal substrates are highly variable between shaded and exposed areas (Bertness and Leonard, 1997), vertical and horizontal surfaces (Helmuth, 1998; Miller et al., 2009), north-and south-facing substrates (Denny et al., 2006; Harley, 2008; Seabra et al., 2011), and between crevices and emergent rock (Jackson, 2010). The presence of ecosystem engineers also contributes to the natural variability in temperatures (Bertness and Leonard, 1997) and modify the biotic and abiotic properties of the rocky shores (Jones et al., 1994). Mussels are the dominant ecosystem engineers in the intertidal zone of temperate rocky shores through the formation of multi-layered beds that create microhabitats. Particularly, mussel beds generate interstitial space where water is trapped and thus reduce the effect of desiccation and heat stress (Suchanek, 1985; Gutiérrez et al., 2003; Sousa et al., 2009). Their presence also offers protection against wave action during high tides (Lewis and Bowman, 1975; Choat, 1977). Mussel beds also increase the reflection of solar radiation, which leads to a reduction of substratum temperature (Little et al., 2009). Other ecosystem engineers, such as barnacles, have also been shown to increase the fine-scale topographic complexity of the substratum, reduce the temperature (by up to 8°C compared to bare rock or to rock with low cover of barnacles) of the surrounding substratum by shading and increase interstitial humidity within patches (Lathlean et al., 2012). Barnacles have also been shown to reduce interspecific competition between the limpets *Cellana tramoserica* and *Patelloida latistrigata*, reduce heat stress and wave exposure that facilitated settlement of *P. latistrigata* (Lathlean, 2014).

In these environments, the use of thermal imaging has drastically increased over the last 10 years, in particular to investigate the role of thermal stress at scale pertinent for individual organisms (Lathlean and Seuront, 2014; Lathlean et al., 2017; Seuront et al., 2018b). Infrared thermography (IRT) is a non-invasive tool for simultaneously measuring the temperature of multiple organisms and their environment. IRT thus allows assessing the thermal heterogeneity of complex environments such as intertidal rocky shores (Lathlean and Seuront, 2014; Seuront et al., 2018b). This methodology was previously applied on mussel beds, in which observed peak temperatures were 10-15°C lower than on the adjacent bedrock (Jurgens and Gaylord, 2016), and on barnacle-covered bedrocks, an increasing barnacle density reducing the heat stress for inhabiting gastropods (the limpet *Patelloida latistrigata*; Lathlean, 2014). For intertidal molluscs, the body temperature is significantly correlated with the substrate temperature but not with the air temperature (Chapperon et al, 2013; Seuront et al., 2019) and the aggregation behaviour of some gastropod species is suspected to favour a decrease in individual body temperature (e.g. *Nerita atramentosa*; Chapperon et al., 2013). Overall, these results stress the fact that climate change models should integrate both abiotic variables, such as substratum temperature (rather than air temperature: Seuront et al., 2019), and biotic parameters, such as behavioural adaptations (Ng et al., 2017).

Noticeably, the previous studies that dealt with both habitat and microhabitat choice in intertidal organisms, in particular gastropods (Chapperon and Seuront, 2011a, 2011b; Chapperon et al., 2013, 2017; Lathlean, 2014), essentially focused on one season, one species and/or one habitat. In this context, the present study aimed at assessing microhabitat choice in two common temperate gastropod species, *Littorina littorea* and *Patella vulgata*, within four distinct habitats (*i.e.* bare rock, rock covered by barnacles or mussels, and rock covered by mussels encrusted by barnacles) occurring on a topographically homogenous intertidal rock platform extending over the whole intertidal zone during four distinct months. Specifically, we first assessed where these habitats occur on the rocky platform and in which habitat *L. littorea* and *P. vulgata* occur on the platform under different meteorological conditions (*i.e.* different months). We also assessed the presence and intensity of aggregation behaviour of these two species often reported as gregarious (Little et al., 2009). We subsequently inferred the presence of a habitat preference for *P. vulgata* and *L. littorea*, and if this choice was species-specific/month-specific. Finally, we described the thermal properties of the four habitats, assessed potential differences between the body temperature of each species and substratum temperature, and inferred the presence of a choice for a thermal microhabitat.

2. METHODS

2.1 Study site

This work was conducted on a temperate intertidal rocky platform, typical of the rocky habitats found along the French coast of the Eastern English Channel, located in Wimereux, France (50°45’09.94”N, 1°35’44.01”E). This platform ranges over the whole intertidal zone and is directly exposed to the dominant South-South West swell direction and submitted to a semi diurnal megatidal regime with a tidal range ranging between 3 and 9 m. We specifically chose this study site for its topographic homogeneity and the presence of four distinct macrohabitats from the supralittoral zone to the low intertidal zone. These habitats were (i) bare rock (Fig. 1A), (ii) rock covered by barnacles *Semibalanus balanoides* and *Austrominus modestus*, respectively native and introduced (Fig. 1B), (iii) rock covered by the blue mussels *Mytilus edulis* (Fig. 1C) and (iv) rock covered by mussels with barnacles (Fig. 1D).

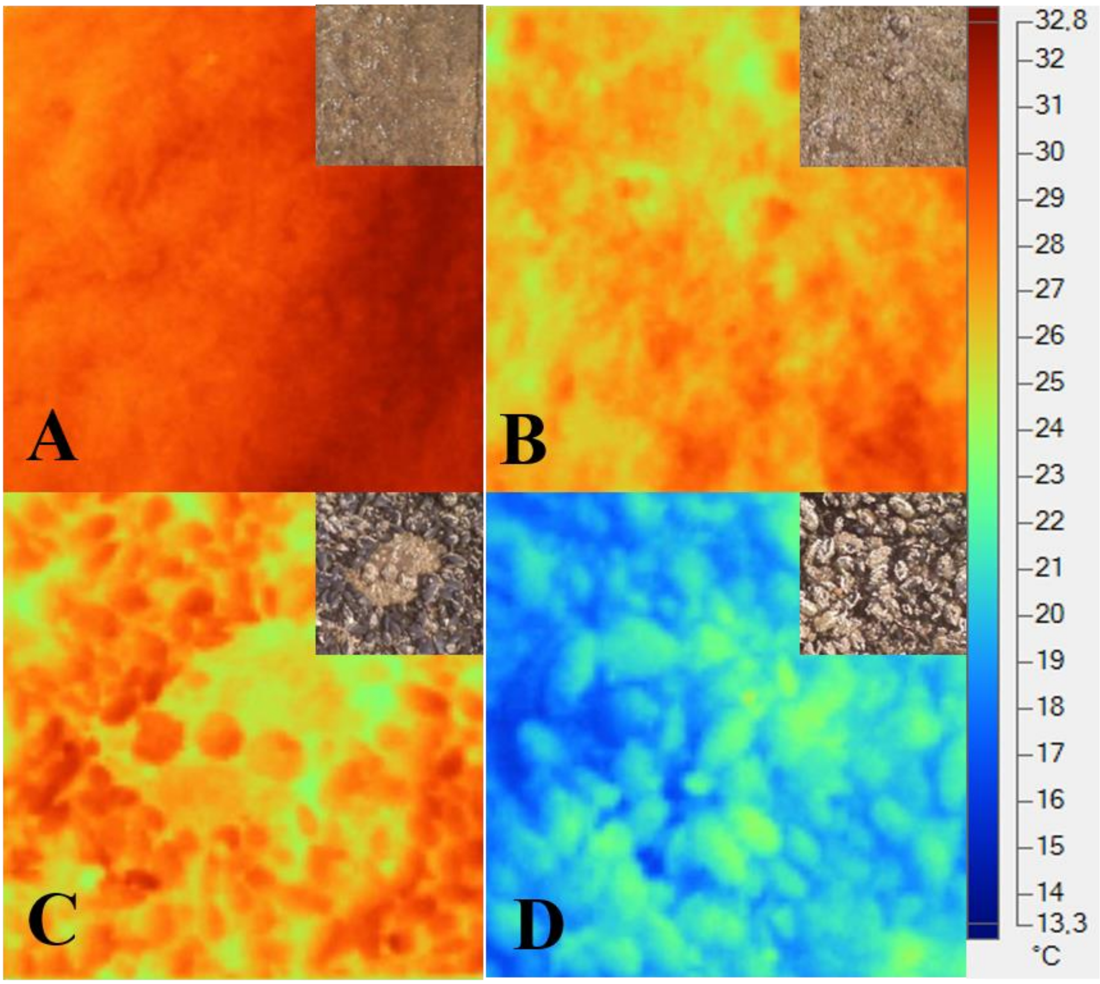

Fig. 1. Thermal images and associated digital pictures of the four types of habitats found on the rocky platform (A) bare rock, (B) rock covered by barnacles, (C) rock covered by mussels and (D) rock covered by mussels with barnacles. These images were taken in June.

The location is submitted to a large range of temperatures over a seasonal cycle (*i.e.* from -2°C to 32°C in 2019). Thus, four periods of sampling were chosen for the present study, to encompass typical temperature variations of the location: June, November and December 2018 and in July 2019. The common periwinkle *Littorina littorea* and the common limpet *Patella vulgata* were observed on this platform and were chosen as target species for the study. These two species can face large variations of temperatures during a year; typically, *L. littorea* can be exposed to air temperature as low as -15°C (Murphy, 1979) and its lethal temperature is 46°C (Evans, 1948), whereas the lethal temperature of *P. vulgata* is 42.8°C (Evans, 1948).

2.2 Thermal imaging

In order to characterize the thermal properties of these four different habitats, thermal imaging was used as a non-invasive temperature measurement (Lathlean and Seuront, 2014; Seuront et al., 2018b). We used a Fluke Ti25 camera (Fluke Corporation, Everett, Washington, USA), which sensitivity is ≤ 0.1°C at 30°C and the temperature measurement accuracy is ±2°C for the temperature range measured in the present study (*i.e.* temperatures <100°C; Fluke Corporation). The emissivity (*i.e.* ability of an object to emit thermal radiation; Lathlean and Seuront, 2014) value of substrata was set at 0.95 as previous studies show that emissivity values of rocky shores typically vary between 0.95 and 1 (Helmuth, 1998; Denny and Harley, 2006; Miller et al., 2009; Chapperon and Seuront, 2011a; Cox and Smith, 2011). All thermal imagines were analysed using the software SmartView 4.1 (Fluke Corporation). For each thermal picture, a closed curve marker was drawn around each photographed individual to calculate the mean value of body temperature (*BT*) and line were drawn to determine the surrounding substrate temperature.

2.2.1 Thermal properties of the four habitats

The experiments were conducted on one day per studied month. Three replicate IR images of 25 cm × 25 cm quadrats were haphazardly taken every 10 meters along a transect on the platform from the low intertidal zone to the supralittoral zone. The photo-quadrats were not fixed but varied in their relative position along the shore for each month. Specifically, 26 stations were sampled in June (n = 78), 27 in July (n = 81), 19 in November (n = 57) and 30 in December (n = 90). The difference in the number of stations sampled is due to differences in the tidal range at the different sampling occasions. We recorded the presence of each habitat along each transect and we calculated the percentage of occurrence of each habitat on the rocky platform as N_{hab} / n, where N_{hab} is the number of sampling stations where the habitat was observed and n is the total number of sampling stations for each month. This allowed the estimation of the vertical distribution of the different habitats at each sampling date. The abundance of *L. littorea* and *P. vulgata* on each habitat were based on the photo-quadrats; specifically, we choose the photo-quadrats where only one habitat was found to calculate the maximal abundance of each species.

To minimise the potential sources of bias in the measurements of intertidal habitat and species temperatures, IR images were consistently taken on days when low tide fell between 11 am and 1 pm (*i.e.* midday ± 1h) and cloud cover was minimal or absent. Air and water temperature and wind speed were considered as environmental variables that were most likely to affect substrate temperature and the behaviour and distribution of both *L. littorea* and *P. vulgata* (Seuront et al., 2018b). Hourly air temperature and wind speed were obtained from Meteo France (www.donneespubliques.meteofrance.fr) weather station of Boulogne-Sur-Mer (50°43′54N, 1°35′53E). Water temperature was measured using temperature loggers (DSL1922L iButtons; resolution 0.1 °C) that were installed throughout the rocky platform. iButtons were wrapped in parafilm, epoxied into shallow depressions chiseled into the rock, and covered by a 1–2 mm layer of epoxy, which was flush with the rock surface.

To determine the temperature of each habitat found on each quadrat sampled, 3 horizontal or vertical lines were haphazardly drawn in SmartView 4.1 to measure the range of temperature of the substratum (*ST*). The temperature of each pixel found on each line was subsequently extracted with SmartView 4.1.

2.2.2 Body temperatures (*BT*) and microhabitat substrate temperatures ($ST_{\mu hab}$)

To determine the presence of a thermal microhabitat choice (*i.e.* choice for a microhabitat with a temperature significantly different from the temperature of the habitat *ST*) in the intertidal gastropods *Littorina littorea* and *Patella vulgata*, we measured the body temperature (*BT*) of individual organisms and the temperature of their microhabitat ($ST_{\mu hab}$), *i.e.* the substratum within one body length of each individual, from infrared images with SmartView 4.1 (Chapperon and Seuront, 2011a, 2012). A thermal microhabitat choice can either be defined as positive (*i.e.* choice for a microhabitat with a temperature significantly warmer than the temperature of the habitat, *i.e.* $ST_{\mu hab}>ST$) or negative (*i.e.* choice for a microhabitat with temperature significantly cooler than the temperature of the habitat, *i.e.* $ST_{\mu hab}<ST$). The percentage of positive choice was defined as n_1/N, where *N* is the total number of observations, n_1 the number of observations with $ST_{\mu hab} > ST$, and the percentage of negative choice was defined as n_2/N where n_2 is the number of observations with $ST_{\mu hab} < ST$.

2.3 Aggregation status

Patella vulgata and *Littorina littorea* individuals were considered as being aggregated when there was a direct shell contact with the shell of at least one conspecific (Chapperon and Seuront, 2011a, 2011b; Chapperon et al., 2013).

2.4 Statistical analysis

Depending on the normality of the data, body temperature of solitary and aggregated individuals were compared using either a Mann-Whitney test or a t-test, within each habitat for each month. *ST* were compared between habitats within each month using a Kruskall-Wallis test (hereafter referred to as K-W test) and when necessary a subsequent post-hoc test was performed using a Dunn test (Zar, 2010). *BT* and $ST_{\mu hab}$ were compared using a Mann-Whitney test for all habitats and months for both species. The Spearman's coefficient of correlation ρ between BT and $ST_{\mu hab}$ was calculated for the two species. The mean temperature of the microhabitat ($ST_{\mu hab}$) of *L. littorea* and *P. vulgata* individuals was compared with the habitat temperature (ST) for each habitat at each tidal level using a Mann-Whitney test.

3. RESULTS

3.1 Environmental conditions

Recorded air temperature (Meteo France data) ranged between 15 and 16°C in June, 23 and 27°C in July, 10 and 13°C in November and 2 and 3°C in December. These temperatures were characteristic of the studied months except in July, where the experiment was performed during what could be considered as a heat wave period (www.donneespubliques.meteofrance.fr). Wind speed (Meteo France data) was 5.1 ± 1 m.s^{-1} in June, November and December, but decreased to 2.1 ± 0.5 m.s^{-1} in July. Mean sea surface temperature (iButtons recordings) during the periods of experiment was 16.4°C ± 0.4°C in June, 19.1°C ± 0.7°C in July, 11.9°C ± 0.5 °C in November and 7.5°C ± 0.8°C in December.

3.2 Habitat distribution

The four habitats were encountered at all tidal levels along the rocky platform, except on the very high shore where only bare rock was found. However, there were variations in the habitats' distribution and overlap during the four months (Fig. 2). Specifically, in June, November and December, all four habitats were present on the platform (Fig. 2A, C, D). In contrast, in July mussels covered by barnacles were absent (Fig. 2B). In June, the habitats the most represented were the rock covered by barnacles found on 80.8% of the platform and the rock covered by mussels with barnacles found on 69.2% of the platform. In contrast, in July, November and December, the habitats the most represented were the rock covered by barnacles, respectively found on 88.9%, 84.2% and 73.3% of the platform, and the bare rock, respectively found on 85.2%, 57.9% and 70% of the platform.

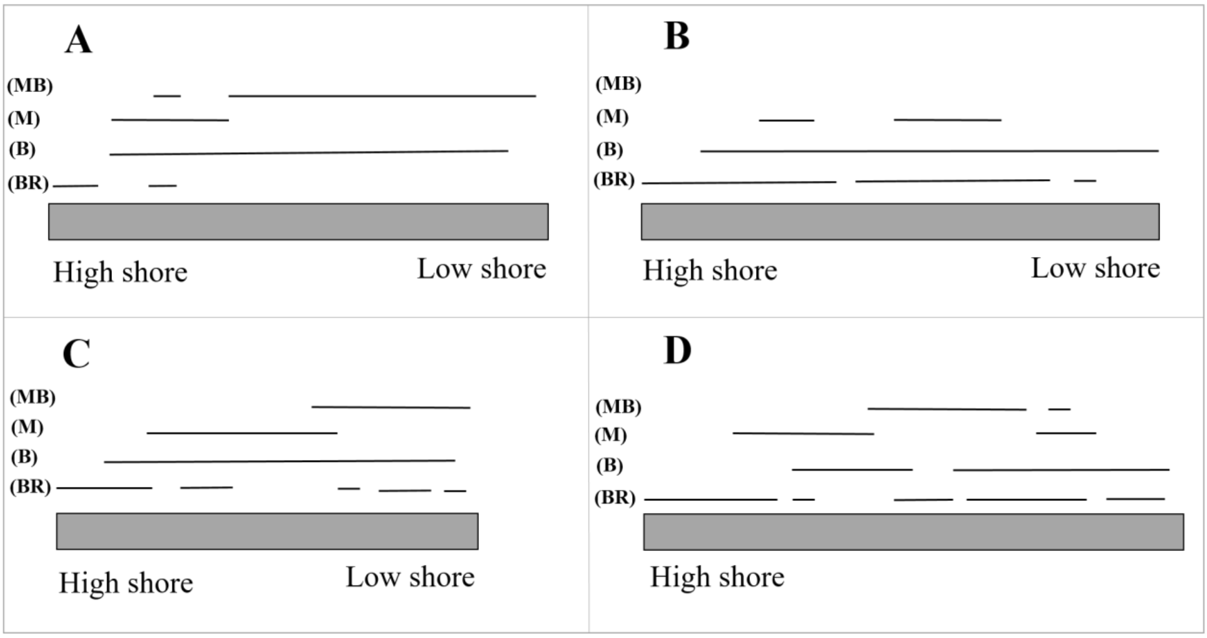

Fig. 2. Distribution of the four habitats found along the rocky platform (in grey) (BR) bare rock, (B) rock covered by barnacles, (M) mussels, (MB) mussels encrusted by barnacles at the different months (A) June, (B) July, (C) November and (D) December. The black line represents the presence of the habitat.

3.3 Abundance and occurrence of Patella vulgata and Littorina littorea

Patella vulgata was totally absent from mussel beds during the four studied months and also from mussel beds encrusted by barnacles in November and December. The maximal abundances of *Patella vulgata* and *Littorina littorea* were found on the rock incrusted by barnacles for the four studied months excepted in July for *P. vulgata* which was more abundant on bare rock (Table. 1).

Aggregation was never observed in *L. littorea* in any habitat, at any sampling date. On the contrary, for *P. vulgata,* some patches were found in all habitats at each sampling occasion (Table. 1). The size of the patches varied between 2 and 5 individuals, except in June on mussels with barnacles where a patch of 8 individuals was observed.

Table 1. Maximal abundance (ind.m^{-2}) of *Patella vulgata* and *Littorina littorea* on the different studied months in the four habitats. The number of patches of *P. vulgata* are given within brackets. *L. littorea* was never found on aggregate in this study.

		Bare rock	Rock with barnacles	Mussels	Mussels with barnacles
Patella vulgata	**June**	240 (8)	336 (8)	ABS	176 (1)
	July	256 (6)	208 (5)	ABS	ABS
	November	272 (4)	320 (7)	ABS	ABS
	December	288 (10)	384 (14)	ABS	ABS
Littorina littorea	**June**	80	700	416	320
	July	272	944	288	ABS
	November	96	352	160	64
	December	400	416	320	144

3.4 Substrate temperature (ST)

The substrate temperature of the four habitats exhibited a clear seasonality (Fig. 3), with significant differences consistently observed between months (K-W test, $p < 0.001$). For each habitat, *ST* ranked from high to low temperatures as July > June > November > December (Dunn test, $p < 0.001$). Within each month, there was significant difference in *ST* between the different habitats (K-W test, $p < 0.001$). In July, November and December, *ST* of the four habitats were significantly different (Dunn test, $p < 0.001$). In June, *ST* on bare rock was significantly hotter than the three others habitats (Dunn test, $p < 0.001$). *ST* was not significantly different between the habitats mussels with barnacles and mussels (Dunn test, $p > 0.05$). There was no significant difference in *ST* between the habitats rock covered by barnacles, mussels and mussels with barnacles (Dunn test, $p > 0.05$).

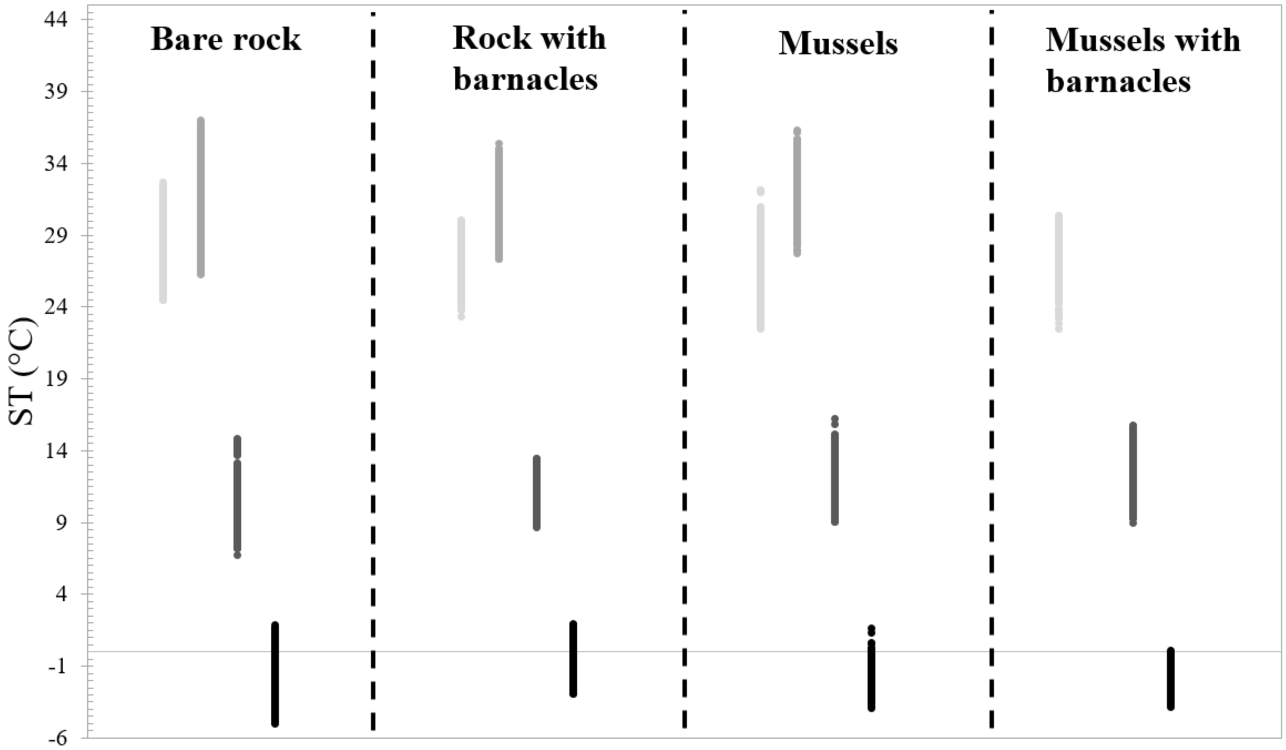

Fig. 3. Substrate temperature (*ST*) found on the different habitats in June (light grey), in July (grey), in November (dark grey) and in December (black). The habitat mussels with barnacles was absent in July.

3.5 *Body temperatures (BT) and microhabitat temperatures* ($ST_{\mu hab}$)

For *P. vulgata,* a significant positive correlation ($\rho > 0.805$, $p < 0.001$) was consistently found between *BT* and $ST_{\mu hab}$ for the three habitats where the species was found, *i.e.* bare rock, substratum with barnacles and mussels with barnacles (Fig. 4). *BT* was significantly higher than $ST_{\mu hab}$ in July and November for all the habitats, and only for bare rock and rock with barnacles in June (Mann-Whitney test, $p < 0.05$). No significant difference was found between *BT* and $ST_{\mu hab}$ in mussels encrusted with barnacles in June, and in all habitats in December (Mann-Whitney test, $p > 0.05$). Finally, no significant difference ($p > 0.05$) was found between the body temperature of solitary and aggregated individuals.

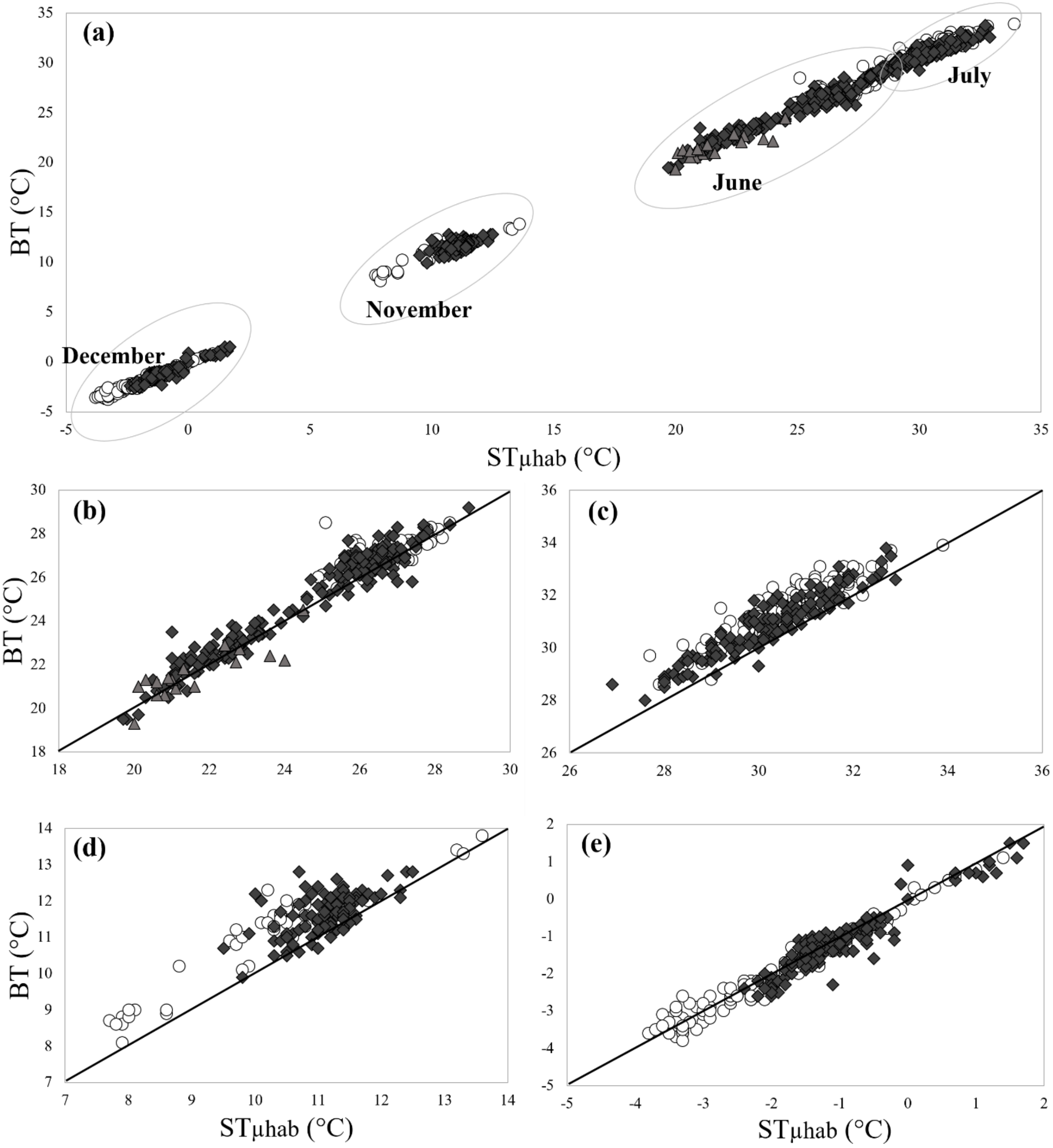

Fig. 4. Body temperature (*BT*) as a function microhabitat substrate temperature (*STµhab*) for *Patella vulgata* for the four months sutdied (a) and in particular for June (b), July (c), November (d) and December (e). The open dots represented the bare rock habitat, the diamond ones rock with barnacles, the square markers mussels and the triangle markers represented mussels with barnacles. The black line represents the first bissectrix, i.e. *BT* = *STµhab*.

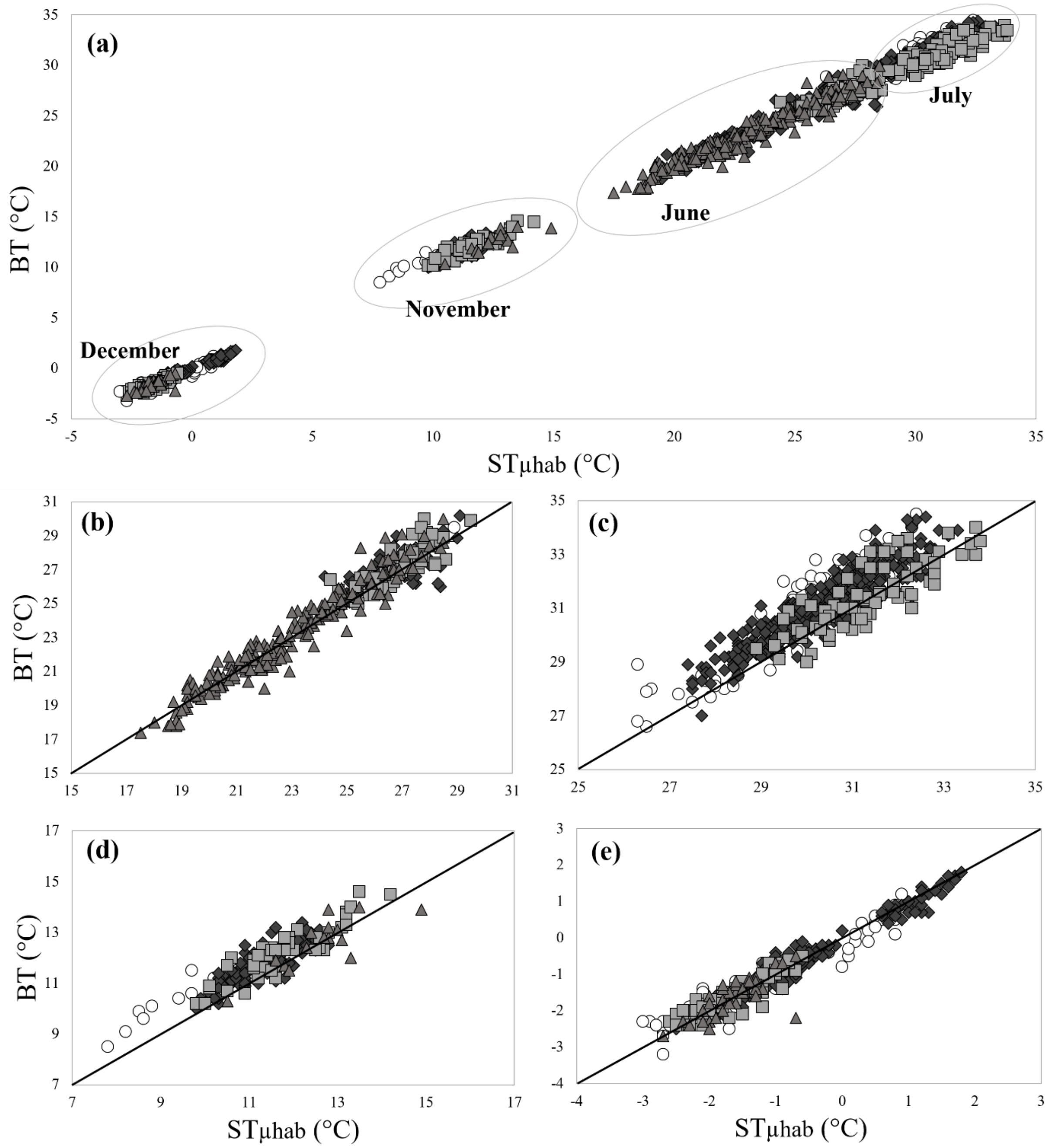

Fig. 5. Body temperature (*BT*) as function of microhabitat substrate temperature (STµhab) for *Littorina littorea* for the four months sutdied (a) and in particular for June (b), July (c), November (d) and December (e). The open dots represented the bare rock habitat, the diamond ones rock with barnacles, the square markers mussels and the triangle markers represented mussels with barnacles. The black line represents the first bissectrix, i.e. *BT* = *STµhab*.

For L. littorea, a significant positive correlation was found between *BT* and $ST_{\mu hab}$ in the four habitats ($\rho > 0.978$, $p < 0.001$; Fig. 5). In July and November on bare rock and on rock with barnacles, *BT* was significantly higher than $ST_{\mu hab}$ (Mann-Whitney test, $p < 0.05$). For the others months and habitats there was no significant difference between *BT* and $ST_{\mu hab}$ (Mann-Whitney test, $p > 0.05$).

3.6 Frequency of thermal microhabitat choice

Thermal microhabitat choice was observed for *Patella vulgata* in all the habitats and for all the studied months. Specifically, the proportion of individuals performing choice ranged from 16.7% (on rock incrusted by barnacles in November and on mussels incrusted by barnacles in June) to 57.1% (on bare rock in June; Fig. 6A). Both positive and negative choice were observed; on bare rock and on rock with barnacles in November and on mussels with barnacles in June, there was only negative choice, whereas on bare rock in December, only positive choice was detected. For the other habitats and seasons there was a co-occurrence of both negative and positive choice (Fig. 6A) with a predominance of negative choice in June, July and November and a predominance of positive choice in December.

For *Littorina littorea*, there was no thermal microhabitat choice in June and November on bare rock, and in November on mussels incrusted by barnacles. The maximum proportion of individuals displaying a choice was observed in June on rock incrusted by barnacles (47.4%; Fig. 6B). As for *P. vulgata*, both positive and negative were observed: there was only negative choice in July on the three habitats were the species was found, as well as on mussels with barnacles in June whereas in December, we only found positive choice on rock with barnacles, mussels and mussels with barnacles. For the other habitats and months, both negative and positive choice were found (Fig. 6B) with a predominance of negative choice in June, July and November and a predominance of positive choice in December, as for *P. vulgata*.

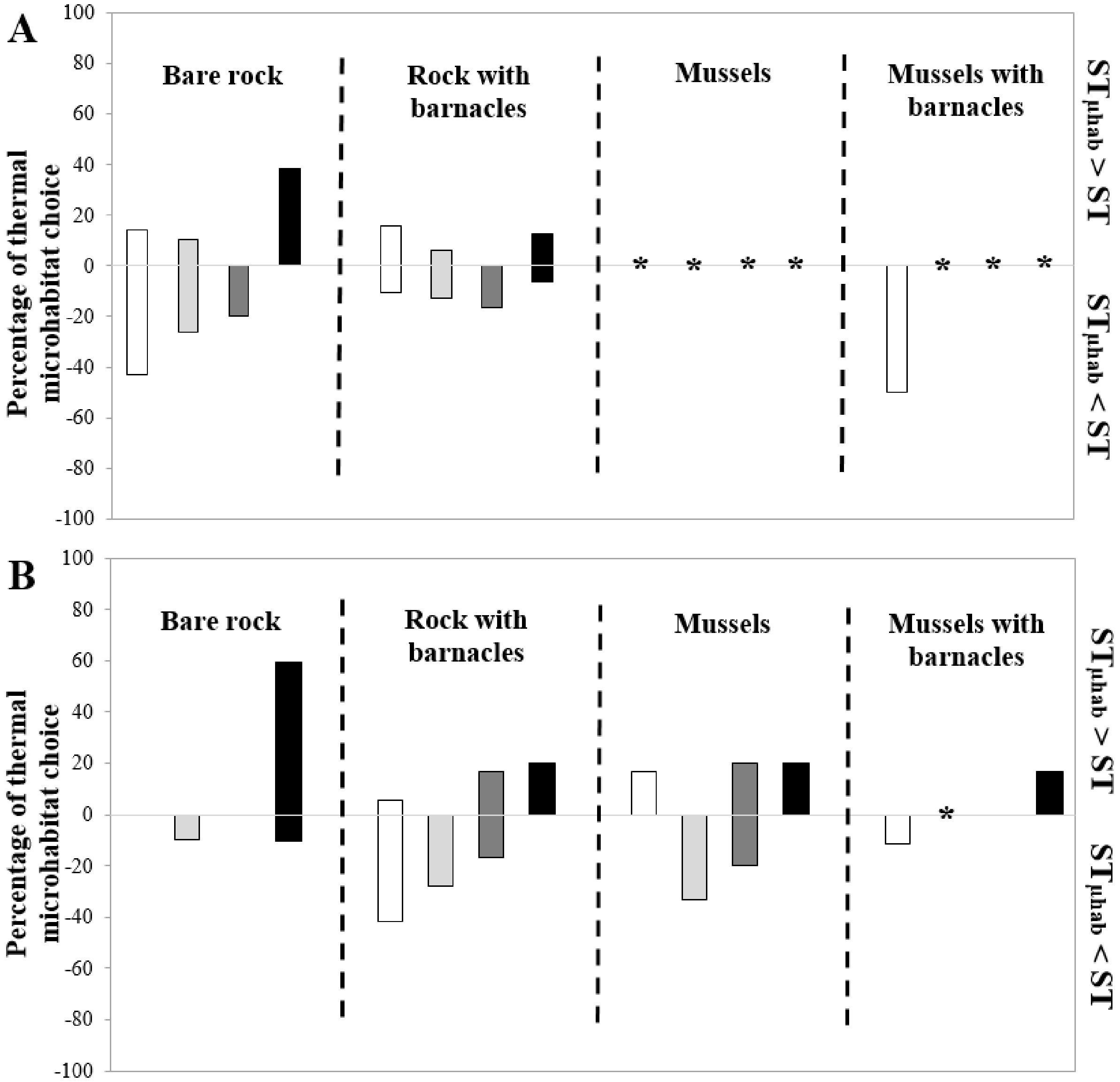

Fig. 6. Percentage of thermal microhabitat choice for *Patella vulgata* (A) and for *Littorina littorea* (B) in June in white, in July in light grey, in November in dark grey and in December in black, in the different four habitats found on the rocky reef. The asterisk means that *P. vulgata* and *L. Littorina* were absent from the habitat.

4. DISCUSSION

4.1 Aggregation

The aggregation behaviour of intertidal snails was reported in numerous studies (Chapman and Underwood, 1996; Chapperon and Seuront, 2009, 2012; Chapperon et al., 2013). These studies suggested that aggregation behaviour was controlled by local climatic conditions

(Bertness, 1989; Chapman and Underwood, 1996; Rojas et al., 2000; Chapperon and Seuront, 2012; Chapperon et al., 2013;) or by substrate topography (Stafford et al., 2008; Rickards and Boulding, 2015). However, in the present study, aggregation of *Littorina littorea* was not observed on the topographically homogeneous rocky reef. On nearby rocky platform with a highly complex topography, many *L. littorea* aggregates were previously observed (Chapperon and Seuront, 2009). These results suggest that, in the study area, the aggregation behaviour of *L. littorea* was not governed by climatic conditions (*i.e.* temperature) but mainly by substrate topography. Our observations are also consistent with the hypothesis that aggregation behaviour is selected to cope with thermal and desiccation stresses only on shores with a high crevice density (Stafford et al., 2008).

Some aggregates of *Patella vulgata* were however found. Benefit from living in aggregation for *P. vulgata* is likely to be a reduction in physical or biological stresses (Coleman et al., 2004). Living in group may allow to retain moisture on rock surface and modify air flow over the individuals and thus reduce evaporation. However, there is only relatively weak evidence of the effect of aggregation on desiccation stress in limpets (Coleman et al., 2004). Here, we found no difference on body temperature between solitary and aggregated individuals. This result suggests that aggregation behaviour was not used as a response against thermal and desiccation stresses but was driven by others factors. As suggested by Coleman (2010), aggregation in limpets may more likely be a behavioural response against predation risks than a way to reduce desiccation stress (Coleman, 2010).

4.2 The selection of a macrohabitat as refuge

The four habitats described here were encountered at all tidal levels during the study and thus often overlapped (Fig. 2). Both species were consistently observed in these habitats, whatever the tidal level, except for the limpet *P. vulgata* which was absent from mussel beds whatever the distribution of this habitat along the shore. These two results testify for a meso-habitat choice rather than a distribution of the species related to tidal height of the shore.

The four habitats previously described on the rocky platform (*i.e.* bare rock, rock covered by barnacles, rock covered by mussels and rock covered by mussels with barnacles) were found in June, November and December. In contrast, in July the habitat "mussels covered by barnacles" was absent and there were only a few mussels present. This absence of mussels is most probably related to the massive mortality of the blue mussel *Mytilus edulis* observed on the Opal Coast in summer 2018 (Seuront et al., 2019). Limpets are usually absent from mussel beds, unless boulders are present, as described for this habitat in the EUNIS classification (Connor et al., 2004) and previously observed on temperate intertidal mussel beds (Saier, 2002; Norling and Kautsky, 2007). On our study site, *Littorina littorea* and *Patella vulgata* were mostly found on substrate covered by barnacles at all sampling months. This result suggests that, at the scale of the platform, the rock covered by barnacles is the most favourable habitat for *P. vulgata* and *L. littorea*. This is in accordance with the results of Lathlean, (2014), who showed that the abundance of the limpet *Patelloida latistrigata* decreased with the diminution of barnacles cover associated to an increase of heat stress which increases with the free space due to the diminution of barnacle cover (Lathlean, 2014). Barnacles create biogenic structures and provide refuges from desiccation and predation, increase the surface for attachment for mobile gastropods species (Barnes, 2000) and also create topographically complex microhabitats which greatly reduce flow velocity and allow snails to persist against wave-swept (Denny and Wethey, 2001; Silva et al., 2015). Thus, the biogenic structures formed by barnacles can act as thermal refuges for gastropod species. Regarding littorinids, the body temperature of the temperate snail *Littorina saxatilis* in summer is on average 1°C cooler than their surrounding substrate when found on substrate with barnacles than on bare rock (Chapperon et al., 2017). Furthermore, Cartwright and Williams, (2012) showed that *Echinolittorina malaccana* and *E.*

vidua used the barnacles as refuges during times of the year where the environmental conditions, and in particular temperature, were stressful. They also showed that these two species did not used barnacles as refuges all along the year; when environmental conditions became cooler *E. malaccana* and *E. vidua* were found on other areas without the need of thermal refuges.

4.3 Microhabitat selection: a way to escape from unfavourable temperatures?

The habitat selection is a consistent behavioural feature in molluscs. This behavioural adaptation allows molluscs to reduce or avoid desiccation stress or predators. For examples *Littorina saxatilis* hide in crevices to reduce water loss (Little et al., 2009) or the high shore snails *Cenchritis muricatus* climb on terrestrial vegetation to avoid extremes temperature of the rocks surface (Emson et al., 2002). Limpets usually select their habitat through their homing behaviour, thus reducing desiccation stress and also predation risks (Little et al., 2009). In the present study, food availability was not estimated in the different habitats and we assume that all resting positions in air recorded are based on temperature. Controlled laboratory experiments or controlled field observations would be needed to tease out this potential effect. The rationale behind the thermal microhabitat choice behaviour relies on the fact that gastropods body temperature is primarily determined by the substrate temperature, due to conductance between the foot and the substratum (Garrity, 1984; Chapperon and Seuront, 2011a, 2011b); in a thermally heterogeneous environment, gastropods would then be able to actively chose the most favourable thermal habitat, to either favour or limit heat exchange with the substrate. Our results showed that the body temperatures (*BT*) of *Littorina littorea* and *Patella vulgata* were always significantly and positively correlated with the microhabitat temperatures ($ST_{\mu hab}$) in each habitat for each season which supports the thermal microhabitat choice hypothesis. However, for both species, this choice was both habitat-dependent and season-dependent. In December, individuals predominantly choose microhabitats with higher temperatures than the temperatures of their substratum (*i.e.* positive thermal microhabitat choice). Under cold conditions of temperature, *L. littorea* individuals drastically reduced their metabolic rate (down to 20% of the regular metabolic rate; Jackson, 2008); the choice for a microhabitat with higher temperature during cold seasons allows individuals to maintain their metabolic rate or at least to limit the reduction of metabolic rate. On the contrary, in June, July and November the choice was principally for a microhabitat with temperatures lower than the temperatures of the surrounding substrate (*i.e.* negative thermal microhabitat choice) which suggests a selection of a cool substrate to reduce the increase of body temperature by conduction between gastropod's foot and the substratum. Individuals, were thus able to navigate in their thermally heterogeneous environment to find the most sustainable temperatures, in particular during the heat wave of July when this choice was predominantly positive for *P. vulgata* and always positive for *L. littorea*. *P. vulgata* and *L. littorea* cessed their spontaneous movement around 32°C and their lethal temperature are 42°C and 46°C, respectively (Evans, 1948). Thus, the choice of microhabitat with lower temperature, allows individuals to maintain their body temperature under lethal or sub-lethal temperature as previously observed in tropical littorinids (*Littoraria scabra*; Chapperon and Seuront, 2011b) and limpets (*Cellana tramoserica*; Sinclair et al., 2006; *Cellana grata*; Williams and Morritt, 1995). In addition to microhabitat choice, others behavioural adaptations have previously been described, such as shell-lifting, shell-standing and towering that reduce conduction from the substrate in littorinids (Seuront and Ng, 2016; Ng et al., 2017) and "mushrooming" in limpets which raise their shell to allow evaporative cooling (Garrity, 1984). These behaviours were, however, never observed in the present study, not even, to our knowledge, in the study area. This suggests that microhabitat choice is the predominant behaviour and that, under particularly harsh thermal conditions, other behavioural adaptations may be involved. With the expected rise in both mean temperatures and frequency of extreme high temperature events (IPCC, 2018), behavioural adaptations remain highly important for intertidal gastropods to maintain their body temperature under thermal stress and to avoid lethal temperature, and we may observe in the near future in temperate areas (such as the present study site) behaviours that are usually seen in tropical environments (e.g. shell-towering) where the thermal stress level is already high. The critical importance of the survival of herbivores such as limpets and littorinids goes beyond the individual and population interest, since their presence has been shown to dampen the impact of warming on the overall intertidal community composition and functioning (Kordas et al., 2017).

CONCLUSION

This study suggests that the two intertidal gastropods species, *Littorina littorea* and *Patella vulgata* showed a clear preference for the thermally favourable biogenic habitat created by barnacles. Moreover, these two gastropods species, at the individual scale, also showed a choice for thermally favourable microhabitats. In winter, both species showed a clear behavioural preference for microhabitats with temperatures higher than the temperature of the substratum, whereas during other seasons, they showed a preference for microhabitats with temperatures lower than the ones measured on the substratum. These differences between temperature of their microhabitats and their substrate may lead to a difference in the way they face thermal stresses such as frost or heat wave. It also means that, temperature variations at very small scales are really important to understand how intertidal gastropods, and intertidal ectotherms in general, can survive in a context of global change. Specially, climate change scenarios do not integrate variations in temperature at pertinent scales for the individuals. Furthermore, these scenarios do not considerate that the body temperature of gastropods is strongly correlated to the temperature of the substrate and never to the air temperature. These results showed that climate models probably underestimate the effect of climate change in the body temperature of intertidal gastropods. This study also reveals that gastropods species are able to explore their environment to find thermal refuge and thus may find refuges under new environmental conditions and possibly adopt locally new adaptive behaviours.

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Chapter IV. Aggregation behaviour in *Littorina littorea* has limited thermal benefits under conditions of thermal stress

ABSTRACT

Aggregation is a widely reported behaviour in intertidal organisms. Specifically, in intertidal gastropods, it is known to limit predation risk and wave action and also to provide benefits under thermal and desiccation stresses. However, less is known about benefit differences for individuals inside and at the edge of an aggregate. In this context, we investigated the importance of the location in aggregates in Littorina littorea *under in situ and laboratory conditions. Specifically, we measured body temperature, using thermal imaging, of solitary* L. littorea *individuals and of individuals inside and at the edge of aggregates under a moderate heat wave on the French coast of the eastern English Channel (Opal coast). We also measured body temperature of individuals inside and at the edge of experimental aggregates under simulated heat wave conditions and in two different artificial substrates (i.e. a black and a white substrate). In the in situ experiment, there was no significant difference between the body temperature of individuals inside and at the edge of the aggregates. Furthermore, solitary individuals were cooler than aggregated ones. In the laboratory experiment, temperature reached higher values than in the in situ experiment. When laboratory temperatures were higher than those observed in the field, body temperature of individuals inside the aggregates were higher than body temperature of individuals at the edge of the aggregates. On the contrary, when temperatures were similar to those in the in situ experiment, there was no significant difference between individuals inside and at the edge of the aggregates. Body temperature of individuals on the white substrate was lower than on the black substrate. All together, these results suggested that, in* L. littorea *individuals aggregation behaviour is not driven by temperature and thermal stress. Others factors, such as desiccation, predation, reproduction or feeding can drive aggregation behaviour. It also suggested that the colour of the substrate is really important to limit the heating of body temperature of intertidal gastropods.*

Key words: aggregation – behaviour – *Littorina littorea* – temperature – thermal stress

1. INTRODUCTION

Intertidal species frequently face temperature variations greater than 20°C during a tidal cycle (Helmuth, 2002). Furthermore, intertidal invertebrates live at, or near, the upper limit of their thermal tolerance window (Somero, 2002) and are thus likely to be highly vulnerable to a warming climate. In a context of global change, rare events such as heat waves, droughts, storms and floods increased in frequency and are likely to become the norm (Meehl and Tebaldi, 2004; Rahmstorf and Coumou, 2011; Oswald and Rood, 2014). Heat waves can have dramatic consequences on intertidal species and are responsible for the mass mortality events documented in a range of ectothermic organisms such as juvenile barnacles (Wethey, 1984), limpets (Kohn, 1993) and mussels (Suchanek, 1978; Petes et al., 2007; Harley, 2008; Seuront et al., 2019). To face heat waves, intertidal ectotherms evolved physiological, morphological and behavioural adaptations. Some defence mechanisms protect biomolecules for thermal and oxidative stresses such as the production of heat shock proteins or antioxidant enzymes (Somero et al., 2017). Shell morphology and colour also play a potentially important role in gastropods species to face heat stress. For instance, limpets with the highest spired shell were predicted to be the coolest individuals (Harley et al., 2009), whereas in the dogwelk *Nucella lapillus*, brown morphs heated faster and attained higher temperatures than white morphs (Etter, 1988). Behavioural adaptations in gastropods consist in decreasing the body surface area in contact with the substratum (Chapperon and Seuront, 2011a, 2011b) by retracting their foot in their shell and close their opercula (Miller and Denny, 2011). Shell posturing as shell-lifting, shell-standing, towering and mushrooming are also known to decrease body temperature of intertidal gastropods (Garrity, 1984; Seuront and Ng, 2016). Habitat selection is used by intertidal gastropods to find microhabitat with sustainable temperatures (Levings and Garrity, 1984; Williams and Morritt, 1995; Emson et al., 2002; Gray and Hodgson, 2004; Little et al., 2009). Aggregation behaviour has also been considered to reduce both thermal and desiccation stresses

(Garrity, 1984; Raffaelli and Hawkins, 1996; Stafford and Davies, 2005a; Stafford et al., 2011, 2012). For example, *Nodilittorina peruviana* maintain higher water content and lower temperature when aggregated than solitary individuals (Rojas et al., 2000) and, in winter, the temperature of aggregated individuals of the temperate snail *Nerita atramentosa* is higher than the temperature of solitary individuals (Chapperon and Seuront, 2012). However, in *Littorina unifasciata*, aggregation behaviour does not decrease temperature of the aggregated snails but aggregated snails have more water reserves than solitary ones (Chapman and Underwood, 1996). In July, during a heat wave that hit the French coasts of the eastern English Channel, *L. littorea* individuals reached temperatures as high as 34°C and, however, were never observed in aggregate (Moisez et al., 2020). Aggregation behaviour is also controlled by local climatic conditions (Bertness, 1989; Chapman and Underwood, 1996; Rojas et al., 2000; Chapperon and Seuront, 2012) or by substrate topography (Stafford et al., 2008; Rickards and Boulding, 2015). For example, aggregation behaviour reduces both desiccation and heat stresses on *N. atramentosa*, but only in boulder fields (Chapperon et al., 2013). Thus, the advantages of being aggregated in terms of desiccation and thermal stresses and the associated mechanisms remain unclear.

The common periwinkle *Littorina littorea* is one of the most abundant species of gastropods on the intertidal rocky shores of North-western Europe (Chapman et al., 2007) and is commonly found in large aggregates of up to 700 to 800 individuals (Chapperon and Seuront, 2009). The behavioural ecology of *L. littorea* has been well studied (Kanda, 1916; Hayes, 1926; Newell, 1958b; Petraitis, 1982; Davies and Beckwith, 1999; Chapperon and Seuront, 2009; Seuront and Spilmont, 2015; Moisez and Seuront, 2020), however, little is still known about their aggregation behaviour. A combination of cues from a given substrate and conspecifics seem to play a role in *L. littorea* navigation following a dislodgment, and the subsequent aggregation formation (Chapperon and Seuront, 2009). Aggregation can also be driven by water-borne chemicals in the mating season (Croll, 1983) and by the detection of food resources (Lauzon-Guay and Robert, 2009). However, little is still known on the effect of aggregation on the body temperature of *Littorina littorea*. In this context, we measured the temperature of *L. littorea* individuals both at the centre and at the edge of aggregates observed during a moderate heat wave that occurred along the French coasts of the eastern English Channel to infer if being aggregated induce any thermal benefit. We further assessed, under laboratory conditions, the effect of a simulated extreme heat waves on *L. littorea* temperature inside and outside aggregates that were standardised in terms of both the number and the organization of individuals relative to each other within an aggregate. We also measured the body temperature of solitary individuals in both field and laboratory experiments. Finally, we also tested the impact of two different substrate colours on the temperature of *L. littorea*.

2. METHODS

Throughout this work we used a Fluke Ti25 camera (Fluke Corporation, Everett, Washington, USA) for thermal imaging, which sensitivity is ≤ 0.1°C at 30°C and the temperature measurement accuracy is 2°C or 2%, whichever is greater. The emissivity value of substrata was set at 0.95 as previous studies show that emissivity values of rocky shores typically vary between 0.95 and 1 (Helmuth, 1998; Denny and Harley, 2006; Miller et al., 2009; Chapperon and Seuront, 2011b; Cox and Smith, 2011). All thermal images were analysed using the software SmartView 4.1 (Fluke Corporation).

2.1 Field survey

Thermal implications of aggregation behaviour in *Littorina littorea* were investigated on one day of moderate heat wave in June 2020 (www.donneespubliques.meteofrance.fr) on a rocky intertidal platform located in Wimereux (Opal Coast, France, 50°45’20.45”N, 1°35’53.08”E).

This platform was topographically heterogeneous and was characterized by the presence of crevices and boulders. However, we selected patches on relatively flat surfaces, with similar inclinations, to avoid shades and temperature variations due to shades. The sampling was performed at solar midday (*i.e.* between 11 am and 13 pm). We measured the body temperature of 236 *L. littorea* individuals distributed in 13 aggregates formed of 6 to 32 individuals. We also measured the body temperature of 20 solitary *L. littorea* individuals to compared body temperature of aggregated and solitary snails.

2.2 Laboratory experiments

Littorina littorea individuals (23.8 ± 1.6 mm; mean ± standard deviation) were boiled during 10 minutes, their soft tissue removed from the shells and shells were cleaned. After drying, shells were filled with silicone. Silicone was chosen as this polymer has similar thermal properties than mantle of gastropods (Helmuth and Hofmann, 2001; Seuront et al., 2019). *L. littorea* shells were assembled in aggregates of 10 individuals with 2 individuals within the aggregate and 8 individuals at the edge of the aggregate (Fig. 1). Solitary shells of *L. littorea* were also tested. Aggregates and solitary shells were placed under infrared lamps at 125 cm (Philips PAR38 IR Red 175W 230V E27 ES) during 3 hours and temperature were recorded every 15 min using thermal imaging. The infrared lamps allowed to reach temperatures higher than temperatures found on the field site, and to simulate a heat wave in the context of global warming. The first image was taken at t_0 (*i.e.* the start of the 3 hours of experiment) and the last one at t_{180} (*i.e.* 180 minutes corresponding at the end of the 3 hours of experiment). The infrared lamps were lighted 15 minutes before the beginning of the experiment to homogenize the temperature before experiment. Two substrates were used, white and black ceramic tiles (15 × 15 × 0.4 cm) laying on granite tiles. The colour of the substratum has critical implications for its temperature as the colour defined the way it absorbed and reflected visible light (Lathlean and Seuront, 2014; Seuront et al., 2018b). Specifically, a black substrate absorbs most of the light in the visual spectrum, in contrast with a white substrate that reflects most of the light. Consequently, black substrates absorb more heat than white ones and exhibit higher temperatures (Seuront et al., 2018b).

Fig. 1. Representation of our laboratory aggregates of 10 *Littorina littorea* individuals. The individuals surrounding of a white line are the 2 individuals considered as inside of the aggregate. The others 8 individuals are considered as at the edge of the aggregate.

2.3 Thermal analysis

To determine the presence of a thermal advantage of being aggregated in the intertidal gastropods *Littorina littorea* in both field and laboratory experiments, we measured the body temperature of individuals located (i) within the aggregate and (ii) at the edge of the aggregate.

An individual was considered inside an aggregate when it was totally surrounded by conspecifics. More specifically, a close curve marker was drawn around each individual in order to calculate the mean temperature of inner and outside individuals (BT_i and BT_o respectively; Chapperon and Seuront, 2011b, 2012). Similarly, we measured the body temperature of solitary individuals (BT_s) in both field and laboratory experiments. The temperature of the substrate (*ST*) was averaged from a close curve marker drawn on the substratum directly surrounding the aggregate.

2.4 Statistical analysis

The body temperature of individuals inside and outside the aggregate were compared using a *t*-test or a Mann-Whitney test (hereafter referred as M-W test) if the data were respectively normally and non-normally distributed on both field and laboratory experiment at each time. We also compared the body temperature of solitary individuals to the body temperature of the individuals inside and outside the patch using a Mann-Whitney test on both field and laboratory experiment. We compared *ST* from the two substrates for the three hours of experiment using a Mann-Whitney or a *t*-test following the normality of the data. We also compared BT_i and BT_o between the two different substrates using a M-W test or *t*-test.

3. RESULTS

3.1 Field survey

Air temperature was 29°C ± 0.5°C, the wind speed 11km h^{-1} (www.donneespubliques.meteofrance.fr) and the cloud cover minimal during the solar midday when the sampling took place. Over the 13 aggregates considered, 127 individuals were considered at the edge of the aggregates and 52 inside them. The body temperature of individuals inside (BT_i) and at the edge (BT_o) of aggregates respectively ranged from 26.9°C to

33.2°C and from 26.6°C to 33.1°C (Fig. 2). There was no significant difference between BT_i and BT_o (M-W test, $p > 0.05$). The body temperature of solitary individuals, BT_s, ranged from 24.5°C to 30.2°C (Fig. 2). There was significant difference between BT_s and BT_o and also between BT_s and BT_i (M-W test, $p < 0.01$). Specifically, BT_s was significantly lower than BT_o and BT_i.

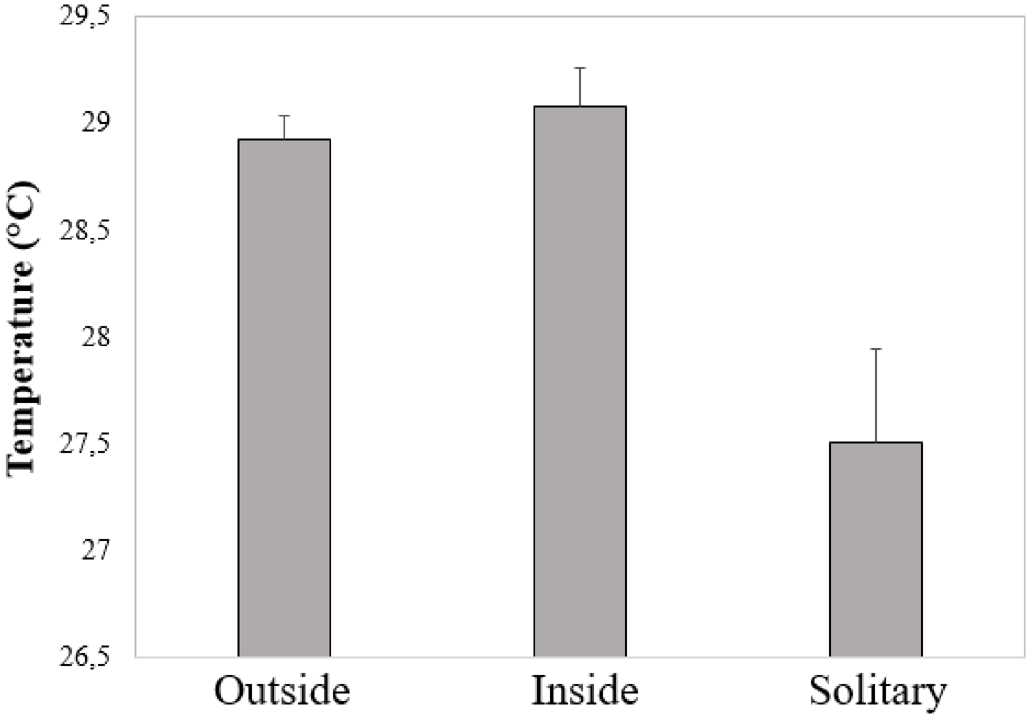

Fig. 2. Mean body temperature of *Littorina littorea* individuals outside and inside the aggregates and for solitary individuals in the field experiment. The bare represented the standard error.

3.2 Laboratory experiments

3.2.1 Substrate temperatures

The temperature of the white substrates ranged from 19.2°C to 22.9°C at t_0 and from 38°C to 43.9°C at t_{180}. The temperature of the black substrates ranged from 24.8°C to 27.4°C at t_0 and from 42.4°C to 47.2°C at t_{180}. Throughout the 3 hours of experiment, *ST* of white substrate was consistently significantly lower than *ST* of black substrate (M-W test for t_0 until t_{45}, $p < 0.001$; t-test for t_{60} until t_{180}, $p < 0.001$; Fig. 3).

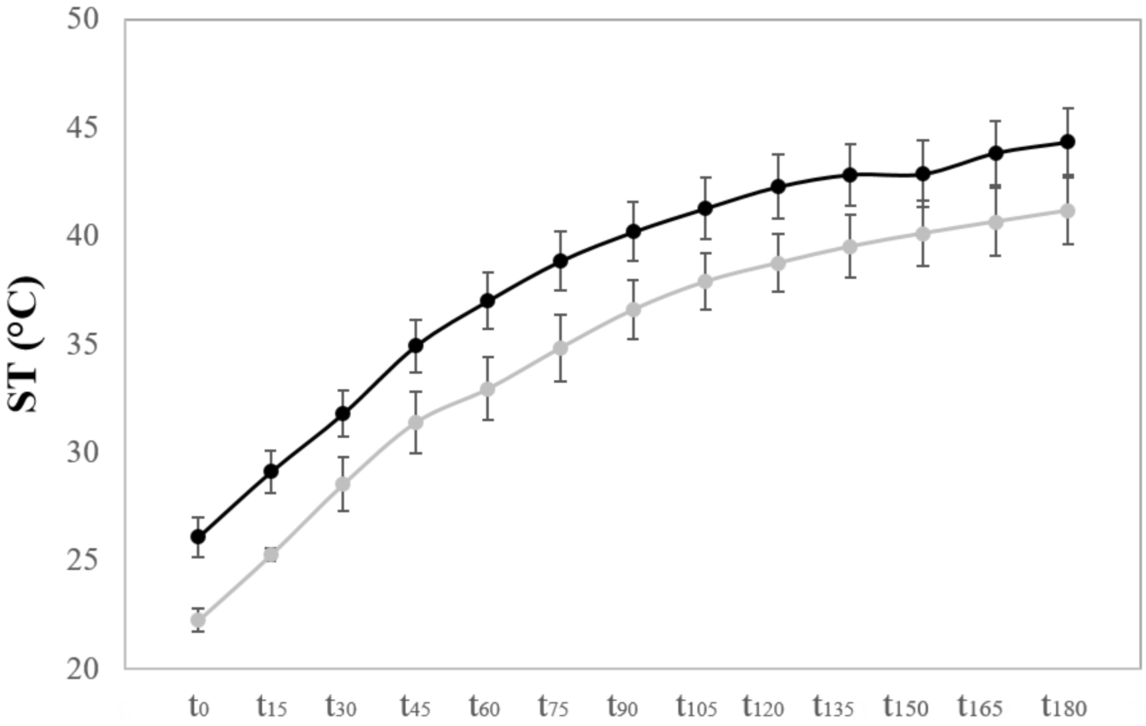

Fig. 3. Temperature of the two different substrates in function of the time of experiment. In black, *ST* of the black substrate, and in light grey *ST* of the white substrate. The bars represented the standard deviation.

3.2.2 Body temperatures

On the black substrate, BT_i ranged from 24.3°C to 26.8°C at t_0 and from 40.8°C to 45.4°C at t_{180}. BT_o ranged from 23.8°C to 27.1°C at t_0 and from 38.3°C to 44.3°C at t_{180}. There was no significant difference between BT_i and BT_o at t_0 and t_{15} for the black substrate ($p > 0.05$; Fig. 3A). From t_{30}, BT_i was consistently significantly higher than BT_o ($p < 0.05$; Fig. 4A). BT_s ranged from 24.1°C to 26.1°C at t_0 and from 34.6°C to 39.8°C at t_{180}. From t_{30}, BT_s is significantly lower than BT_o and BT_i ($p < 0.05$).

On the white substrate, BT_i ranged from 19.4°C to 23.7°C at t_0 and from 39.5°C to 46°C at t_{180}. Mean BT_o ranged from 19.3°C to 23.7°C at t_0 and from 36.8°C to 45.5°C at t_{180}. For the white substrate, there was no significant difference between BT_i and BT_o at t_0 and BT_i was significantly higher than BT_o for t_{15} until t_{180} ($p < 0.05$; Fig. 4B). BT_s ranged from 20.1°C to 26°C at t_0 and from 35°C to 42°C at t_{180}. From t_{45}, BT_s is significantly lower than BT_i and BT_o ($p < 0.05$).

BT_o of individuals on black substrate was significantly higher than BT_o of individuals on white substrate (M-W test, $p < 0.05$). BT_i of individuals on black substrate was significantly higher than on white substrates only for t0, t60 and t75 ($p < 0.05$). For all the other times of experiment, there was no significant difference in BT_i between black and white substrates ($p > 0.05$).

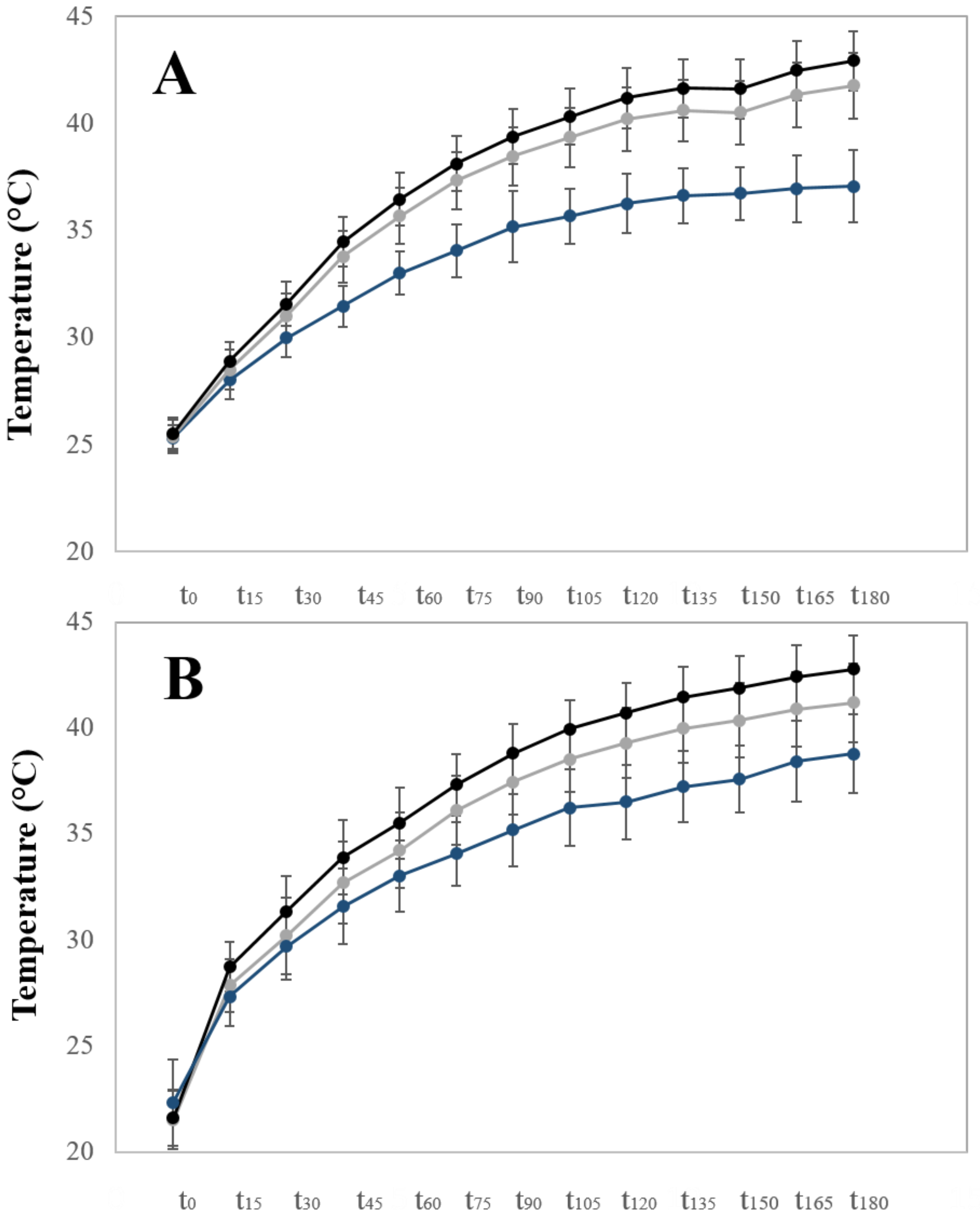

Fig. 4. Body temperature of *Littorina littorea* individuals in function of the time of experiment. In grey, the individuals outside the aggregate, in black the individuals inside the aggregate and in blue the solitary individuals on the (A) black substrate and (B) white substrate. The bars represented the standard deviation.

4. DISCUSSION

4.1 Aggregation behaviour does not lead to a thermal benefit in Littorina littorea

Aggregation behaviour has long been considered to provide benefits in terms of desiccation and thermal stresses compared to solitary individuals (Helmuth, 1998; Rojas et al., 2000; Chapperon and Seuront, 2012). However, little is still known about the impact of the position within an aggregate, especially in *L. littorea* despite the considerable amount of work devoted to the behavioural ecology of this species (Kanda, 1916; Hayes, 1926; Newell, 1958; Petraitis, 1982; Davies and Beckwith, 1999; Chapperon and Seuront, 2009; Seuront and Spilmont, 2015; Seuront et al., 2017; Moisez and Seuront, 2020).

Body temperature of solitary individuals are significantly lower than body temperature of aggregated individuals under high temperature conditions in both field and laboratory experiments. Furthermore, the lack of significant difference between the body temperature of *L. littorea* individuals inside and at the edge of aggregates observed in the field experiment implies a lack of thermal benefit of this behaviour. In contrast, the body temperature of *Nerita atramentosa* individuals inside aggregates are higher than body temperature for individuals at the edge of aggregates (Chapperon and Seuront, 2012). *N. atramentosa* is a temperate species on the extreme side of the temperature spectrum contrary to the temperate snail *L. littorea.* However, these two species have similar heat coma temperature, 38.9°C for *N. atramentosa* (McMahon, 1990) and 39°C for *L. littorea* (Evans, 1948). It may suggest that aggregation behaviour is not a way to provide any thermal benefit. Furthermore, our experiment took place in the end of spring whereas in Chapperon and Seuront (2012), experiment was conducted in winter. The difference of results may also be explained by the difference in the size of the studied aggregates. The aggregates of *N. atramentosa* contained from 37 to 268 individuals whereas ours aggregates only contained from 6 to 32 individuals. Furthermore, Chapperon and Seuront, (2012) found that in aggregates smaller than 216 individuals, all snails would have the same thermal benefit no matter their spatial positions on the aggregate. However, aggregated snails still have benefit under winter thermal conditions, as they are warmer than solitary ones (Chapperon and Seuront, 2012). Similarly, aggregated *Nodilittorina peruviana* individuals thermoregulated better than solitary ones (Rojas et al., 2000), contrary to our study, where solitary *L. littorea* individuals were cooler than aggregated ones. Similarly, aggregated *Littorina unifasciata* are hotter than solitary individuals and showing that aggregation did not reduce internal temperatures (Chapman and Underwood, 1996). Aggregation is thus not always a behavioural response to thermal stress. For example, during a heat wave on the Opal Coast, where body temperature of *L. littorea* can reach 34 °C, individuals were never found in aggregate in four different habitats (Moisez et al., 2020). To face high temperatures and reduce their body temperatures, individuals choose microhabitat with lower temperature than the surrounding substrate (Moisez et al., 2020). Similarly, aggregation was not observed to provide any thermoregulatory benefit in the temperate snail *Littorina saxatilis* under summer conditions where body temperature can reach up to 40.5°C (Chapperon et al., 2017). In this study, shell standing and habitat selection are more efficient than aggregation behaviour to reduce body temperature of *L. saxatilis* individuals (Chapperon et al., 2017). Moreover, the proportion of *L. saxatilis* individuals that forming aggregates do not change with the increase in temperature (Stafford and Davies, 2004).

Few studies suggested that aggregation is more advantageous under desiccation stress rather than thermal stress (Moulton, 1962; Chapman and Underwood, 1996). Aggregation retains moisture and allows individuals to can keep their opercula open for longer time than isolated individuals in response to desiccation stress and hence could prolong gaseous exchange (Rojas et al., 2013). Being wet during emersion and heat stress is important as water at the surface of individuals evaporated and their temperature cooled down (Seuront et al., 2018). Specifically, convection (*i.e.* the energy needed to convert water to gas) allows individuals to lose heat

(Seuront et al., 2018). However, many others factors can drive aggregation behaviour in intertidal gastropods such as substrate topography (Stafford et al., 2008; Rickards and Boulding, 2015), the following of conspecifics mucous trails (Stafford and Davies, 2005b; Chapperon and Seuront, 2011a), or the availability of food resource (Lauzon-Guay and Robert, 2009). Aggregation can also be a way to reduce predation risks (Côté and Jelnikar, 1999; Coleman et al., 2004; Nicastro et al., 2007) or to find a mate (Cudney-Bueno et al., 2008). The observation of aggregations may also be simply the natural consequence of a common preference of snails to avoid dry substrata and to congregate where substrata topography creates a humid microclimate (Stafford and Davies, 2005a; Jackson, 2010; Chapperon and Seuront, 2011a, 2011b; Stafford et al., 2012).

4.2 Aggregation behaviour of Littorina littorea*: to an obsolete behaviour under warming climate?*

Our laboratory experiment allows to increase the body temperature of *Littorina littorea* higher than temperatures experience on the field survey. Specifically, these very high temperatures for the region can simulate future conditions of temperature that intertidal gastropods can face under global warming.

The body temperature of individuals inside the aggregate was consistently significantly higher than the body temperature of the individuals located at the edge of aggregates for the two substrates considered in our experiments. Furthermore, solitary individuals exhibited lower body temperature than aggregated ones. However, this result may somehow sound counterintuitive as aggregation has widely been described as beneficial under thermal and desiccation stresses, though counterexamples exist (Chapman and Underwood, 1996; Chapperon et al., 2017). For examples, aggregated mussels (Helmuth, 1998) or aggregated *N. peruviana* (Rojas et al., 2000) exhibit lower body temperatures than solitary individuals. Thus, we expected, under high temperatures, that individuals inside the aggregate will be cooler than individuals at the edge of the aggregate. Besides, similar results were found on *N. atramentosa* in winter, where body temperature can reach 28°C; cooler body temperatures were preferentially observed at the edge of the aggregate (Chapperon and Seuront, 2012). This result can be explained by the fact that, at the edge of an aggregate, the surface-to-volume ratio in contact with air increases that increase the convective and evaporative rates (Denny et al., 2011). Moreover, air currents can be blocked by individuals at the edge of the aggregate, and thus the effect of convective cooling produce by air currents is reduced for the individuals inside the aggregate (Seuront et al., 2018).

The difference between BT_i and BT_o seems contradictory compared to our previous results in the field survey. However, this difference is probably due to the high difference in temperature between the field survey (temperature around 28°C) and the laboratory experiment (temperature can reach up to 40°C). At t_0 and t_{15}, where the temperature was comparable to temperature found in the field survey, there was no significant difference between BT_i and BT_o and the difference between the individuals inside and outside the aggregate appears with the increase of temperature. These results may indicate that beyond a temperature threshold, the thermal benefit related to aggregation behaviour might vanish. In a future warmer climate with increased mean temperatures, it seems that heat waves would become more intense, longer and more frequent (IPCC, 2018). In this context, aggregation and the related thermal benefit (Helmuth, 1998; Rojas et al., 2000; Chapperon and Seuront, 2012; Chapperon et al., 2013; Rojas et al., 2013) may become less advantageous than other behavioural adaptations such as shell-standing, shell-towering in intertidal gastropods.

The impact of high temperatures on body temperature of *L. littorea* is higher on black substrates than on white ones. Indeed, temperature of black substrate is significantly higher than temperature of white substrate. Similar results were found on an *in situ* experiment, black substrates deployed on the Opal Coast over a year had always higher temperature than white substrates (Moisez, unpublished data). Similarly, in British Columbia, the average maximum on black substrates was approximately 4 °C warmer than white ones (Kordas et al., 2015). As body temperature of intertidal gastropods is strongly correlated to the temperature of their surrounding substrate (Bertness, 1989; Chapperon and Seuront, 2011a, 2011b), reducing the heating of the substrate can be crucial for thermoregulation of intertidal species. Anthropic structures, such as port facilities, sea walls or pontoon are principally built in grey or dark concrete. Building these structures in white or in light colours could limit the heating of these anthropic substrates and thus, the body temperature of intertidal organisms that colonized these substrates.

CONCLUSION

The present work illustrates the fact that aggregation behaviour in *Littorina littorea* may only be marginally advantageous as a thermoregulatory behaviour, especially under high temperature conditions. The present study demonstrated that under moderate heat wave conditions solitary individuals were consistently cooler than aggregated ones and individuals inside an aggregate had the same body temperatures than individuals at the edge. Furthermore, under simulated and more pronounced heat wave, individuals inside the aggregate had higher temperature than individuals at the edge of the aggregate. Being in the centre of an aggregate is, thus, unfavourable for intertidal gastropods. However, being in an aggregate allowed to maintain higher water content, thus gastropods faced a trade-off between being hotter and wetter or being cooler and dryer. Under global climate change, with the expected increase of temperatures, other behaviours can be more advantageous than aggregation, as the shell posturing or the habitat selection. As behavioural adaptations considerably modified body temperature of intertidal gastropods, their integration in climate models is primordial.

Chapter V. General Discussion and Perspectives

Orientation behaviour in intertidal gastropods: the case of thigmotaxis

Recently, Seuront et al. (2018a) suggested that orientation in intertidal gastropods, and in *Littorina littorea* in particular, is controlled by the interplay between a large range of taxes, including rheotaxis, phototaxis, chemotaxis and geotaxis. These taxes allow snails to return to their original habitat after dislodgement, thus exhibit both habitat selection and tidal height partitioning (Seuront et al., 2018a; see Annexe II). Other taxes have been studied on *L. littorea* such as scototaxis, thigmotaxis and geotaxis (Moisez and Seuront, 2020; see Annexe I). Particularly, *L. littorea* showed a clear preference for dark areas and darks forms (*i.e.* positive scototaxis) with individuals typically following topographical discontinuities and thus exhibiting a thigmotactic behaviour. The results of the present work further indicate that the geotactic behaviour previously described in the literature seems to be essentially a by-product of thigmotaxis rather than an actual geotactic behaviour (Moisez and Seuront, 2020). As rocky shores are highly topographically complex and heterogeneous, intertidal gastropods are submitted to a broad range of tactile stimuli. Thus, thigmotaxis can be one of the major behaviours involved in snails orientation. Under decreasing salinity conditions, thigmotactic behaviour is less intense and shorter but was still observed (Chapter II). Even when conditions are unfavourable (*i.e.* salinity down to 16.7 PSU, Chapter II), individuals keep following the topographical discontinuities of their environment. For example, in rocky pools, salinity can drastically decrease due to heavy rains or drastically increase due to evaporation during a tidal cycle (Raffaelli and Hawkins, 1996; Little et al., 2009); the ability of intertidal snails to follow the discontinuities of their environment under low salinity conditions may help them to find a less stressful environment, hence to maintain their physiological functions (*i.e.* oxygen consumption, cell volume or cardiac activity). Noticeably, the size of the discontinuities encountered had no impact on the thigmotactic behaviour of *L. littorea* (Chapter II). This result suggests that, even small discontinuities can be followed by *L. littorea* to orient themselves. This observation further implies that, even on shores with small topographical discontinuities, intertidal gastropods can use thigmotaxis for their displacement. However, the nature of the discontinuities (*i.e.* 2D or 3D discontinuities) fundamentally changed thigmotaxis. On discontinuities with a low complexity, thigmotaxis induced a geotactic behaviour whereas in more complex ones, individuals kept following topographical discontinuities (Chapter II). These changes in thigmotactic behaviour may help intertidal gastropods to "memorise" the complexity of their environment. Though it may still be speculative, this hypothesis is consistent with the early proposal of the presence of a kinaesthetic memory (*i.e.* topographical memory) in limpets, which is involve in their homing behaviour (Pieron, 1909; Cook et al., 1969). The same kind of mechanisms may occur in littorinid snails and help them to navigate in intertidal shores and to find their way back to their resting habitat after feeding excursion or dislodgement. This hypothesis is consistent with recent results showing that dislodged *L. littorina* have the ability to return to both their original habitat and tidal height after a dislodgment (Seuront et al. 2018a). Topography of intertidal rocky shores is highly variable and characterized by the presence of boulders, crevices and pits. Barnacles, as well as mussels, create biogenic habitat and also contribute to increase the topographical complexity of rocky shores (Underwood and Chapman, 1989; Lathlean et al., 2012). The laboratory approach used to characterize thigmotaxis in *L. littorea* is really simple compared to the actual topographic complexity inherent to natural rocky shores. For a better understanding of thigmotactic behaviour in intertidal gastropods much work is still needed. New experimental designs can be build using Lego® Bricks of different sizes to create discontinuities of different thickness at scales pertinent for the organisms, and the topographical complexity can be gradually increased. As natural substrate is not smooth like Lego® Bricks, concrete can be used to mould topographical discontinuities and, thus, get closer to the complexity of natural rocky shores. Empty mussel shells can also be added to the experimental designs to artificially create mussel beds, of different layers, and thus observe the impact of a biogenic structure on gastropod's thigmotactic behaviour. As many other stimulus can modify behaviour of intertidal gastropods such as the presence of food, congener or predator cues, changes in pH and in temperature or the presence of pollutant such as microplastic leachates (Seuront, (2018a) already showed that microplastics disturbed behavioural response of *L. littorea* to the presence of predator chemical cues), it would be interesting to test if these factors can impact thigmotactic behaviour in intertidal gastropods. In this work, thigmotactic behaviour was only test on solitary individuals and thus neglected the impact of the presence of conspecifics. However, as shown in *Lasius niger*, an ant species with a trail-following behaviour as in *L. littorea*, the presence of congeners can increase the thigmotactic behaviour (Dussutour et al., 2005).

Thermal heterogeneity of intertidal shores

At large spatial scales, the temperatures of intertidal habitats are highly heterogeneous and variable. At the scale of an intertidal rocky platform on the French coasts of the eastern English Channel, substrate temperature can typically vary by up to 10°C in June between bare rock and rock covered by mussels encrusted with barnacles (Chapter III). Within a specific macrohabitat such as substrate covered by mussels, substrate temperature can also vary by up to 10°C (Chapter III). Similarly, difference in substrate temperature between rock platform and boulder field can reach 4°C in autumn (Chapperon and Seuront, 2011a) and more than 30°C between the top and the bottom of the mangrove tree *Rhizophora* sp. roots where the littorinid *Littoraria scabra* abound (Chapperon and Seuront, 2011b). These variations of substrate temperature can be explained by the presence of biogenic structures (Chapter IV), the topography of the substrate (Chapperon and Seuront, 2011a; 2011b), the inclination (Williams and Morritt, 1995; Helmuth, 1998; Miller et al., 2009), the orientation (north- and south-facing; Denny et al., 2006; Harley, 2008; Seabra et al., 2011; Chapperon et al., 2016) or the amount of incoming solar radiations (Chapperon and Seuront, 2011b; Chapperon et al., 2016). These thermal mosaics create macro- and micro-thermal refuges. For example, *Patella vulgata* individuals from sun exposed microhabitats (*i.e.* flat or vertical south-exposed rocks) can face variations in body temperature of up to 20°C (Chapperon et al., 2016). In contrast, crevices and north faced surfaces appear to be more stable microhabitats with lower temperature variations (Chapperon et al., 2016). Variations in temperature due to sun exposition have been demonstrated to be greater between sun exposed and shaded microhabitat than between shore levels or between sites (Seabra et al., 2011). Individuals which select vertical north- and vertical west- exposed rocky walls seems to be less vulnerable to extreme heat events than individuals found on south-exposed rocks (Chapperon et al., 2016). The macro- and microhabitat selection is, thus, crucial for settlement and survival of intertidal gastropods. At the scale of a population, macrohabitat selection is more important than microhabitat selection as population will select macrohabitat with sustainable conditions (*i.e.* less thermal and desiccation stress, sheltered habitat, more food resources…). In contrast, an individual within a population will be able to select microhabitat with more sustainable conditions (*i.e.* substrate temperature of the microhabitat different than the temperature of the surrounding substrate, Chapter III) within a favourable macrohabitat. The selection of thermal refuges is also species-specific, as each species have different ecophysiological tolerances. For instance, substrate covered by barnacles seems to act as thermal refuges for *L. littorea* and *P. vulgata* (Chapter III). At the scale of a macrohabitat, barnacles are known to provide refuges from predation, desiccation (Barnes, 2000) and thermal stresses for intertidal gastropods (Chapperon et al., 2017). Barnacles and mussels also create interstices where water is trapped (Suchanek, 1985; Gutiérrez et al., 2003; Sousa et al., 2009) and thus create favourable microhabitats inside beds. To find these favourable habitats characterized by a highly complex topography, *L. littorea* and intertidal snails in general, may use, among others, the abovementioned thigmotactic behaviour to orient themselves towards these habitats (Chapter II). To better understand how intertidal gastropods selected thermal refuges, thermal mosaic can create by using substrate of different colours (*i.e.* white and black, as their heating is significantly different, Chapter IV) and be place under infrared lamps to heat the substrate. By modifying the size, the number and/or the position of thermal refuges created by white substrates, we can observed how mobile gastropods, such as *L. littorea*, are able to use it to limit the increase of their body temperature.

Microhabitat selection: a strategy to compensate temperature variations

Intertidal species can experience extreme temperature variations during a tidal cycle (more than 20°C; Helmuth, 1999, 2002). Temperatures can reach lethal limits for some intertidal organisms during summer low tides (Newell, 1979; Garrity and Levings, 1984). Even if temperatures are below lethal limits, there might be a long-term cost for organisms which experience extreme metabolic stress from water loss and temperature (Jones and Boulding, 1999). Intertidal organisms and gastropods in particular select particular microhabitat to reduce thermal and desiccation stresses. For example, *Littoraria scabra* is preferentially found on the bottom of *Rhizophora* sp. roots than on the top which is directly exposed to solar radiations and thus which is a more thermally stressful microhabitat (Chapperon and Seuront, 2011b). The microhabitat selection can be species-dependent (Chapter III), but can also dependent on the size of the individuals (Jones and Boulding, 1999). Specifically, larger individuals of *Littorina sitkana* preferentially choose barnacles and algae microhabitats whereas small individuals selected less complex microhabitats as crevices and bare rock (Jones and Boulding, 1999). Jones and Boulding (1999) suggested that small individuals cannot selected a complex microhabitat on warm days because they stopped moving very early and thus were unable to find protective microhabitats. This selection is also temperature-specific. Specifically, when temperatures are below 2°C (*i.e.* in December on the French coasts of the eastern English Channel), *L. littorea* and *P. vulgata* individuals preferentially selected microhabitats with temperature higher than the temperature of the surrounding substrate. In contrast, in June, July and November, individuals preferentially selected microhabitat with temperature lower than the temperature of surrounding substrate (Chapter III). This suggests that selection of microhabitat allow gastropods to maintain their body temperature inside their optimum range by preventing, or at least minimizing, thermal stress. These results also suggested that within a favourable macrohabitat (*i.e.* barnacles or mussels beds) gastropods are able to find a microhabitats with optimal temperature (Chapter III). In a context of global change, rocky shores will become more hostile and inhospitable for intertidal organisms which are submitted to terrestrial conditions few hours a day. The most pessimistic scenario of global warming predicts an increase of about 3.7°C of global temperatures (GIEC, 2013), and heat waves will become longer, more frequent and more extreme (Meehl and Tebaldi, 2004; Rahmstorf and Coumou, 2011). As many intertidal species already lived close to their upper thermal limits (Somero, 2002), increase in temperature by up to 3.7°C would have dramatic consequences on intertidal organisms. Body temperatures of intertidal molluscs are not correlated to air temperature but to substrate temperature (Chapperon and Seuront, 2011a, 2011b; Seuront et al., 2019), this suggest that climate change models based on air temperature as a proxy of body temperature may underestimate the impact of global warming on intertidal species. Mass mortality of intertidal organisms due to heat waves have already been observed in juvenile barnacles (Wethey, 1984), limpets (Kohn, 1993) and mussels (Suchanek, 1978; Petes et al., 2007; Harley, 2008; Seuront et al., 2019). Mortality of organisms such as mussels and barnacles, which create biogenic habitats can have dramatic consequences for numerous species that live in these habitats (up to 62 taxa of epifauna lived in mussels beds in the eastern English Channel; Seuront et al., 2017) and for gastropods species that used both barnacles and mussels bed as thermal refuges (Chapter III). As temperature will increase, distribution and abundance of organisms will change. The intertidal gastropods *Osilinus lineatus* and *Gibulla umbilicalis* have already extended their northern distribution in Europe and increased their abundance (Mieszkowska et al., 2006, 2007). Similar observations were found for zooplankton (Beaugrand et al., 2002; Beaugrand and Ibanez, 2004), kelp forest and the associated communities (Beas-Luna et al., 2020), and fishes (Bañón et al., 2020; Schickele et al., 2020) and Hastings et al. (2020) made similar observations on 304 marine species across a range of taxa. Kordas et al. (2015) manipulated substrate temperatures for studying the thermal stress at the community level. For instance, barnacle abundance decreased with the increasing temperature, herbivores were absent from the warmest zone and ephemeral green algae exhibited a delay in phenology under warm conditions. Thus, the species richness on the warmer zone decreased and the invertebrate assemblage structure was altered (Kordas et al., 2015). Global warming will change the distribution of numerous species and thus disrupt ecological balance of numerous environments. Thus, understanding how behavioural adaptations of intertidal gastropods can buffer temperature is challenging under global warming.

Aggregation behaviour: a non-adaptive response to thermal stress

In gastropods, aggregation behaviour is considered to provide benefits in terms of desiccation and thermal stresses compared to solitary individuals (Helmuth, 1998; Rojas et al., 2000; Chapperon and Seuront, 2012). However, the body temperature of aggregated *L. littorea* were higher than the temperature of solitary individuals (Chapter IV). At 28°C, whatever the location within the aggregate (*i.e.* inside or at the edge) individuals exhibited the same body temperature. When temperature was higher than 28°C, individuals inside the aggregate had higher body temperature than individuals at the edge of the aggregate (Chapter IV). These results suggested that aggregation behaviour in *L. littorea* is not an advantageous behaviour in terms of thermal stress, especially under conditions of high thermal stress. *L. littorea* individuals were never observed in aggregate even though their body temperature can reach up to 35°C (Chapter III). Thus, aggregation behaviour cannot be consider as a way to reduce body temperature in the intertidal gastropods *L. littorea*. Stafford and Davies, (2004) also showed that for high shore littorinids in north-east England, the sheltering and aggregation behaviours were not related to the increase of temperatures. On the intertidal mussel species *Perna perna* and *Mytillus galloprovincialis*, position within beds is, however, important. Mussel's body temperature is lower for individuals in the centre of beds than at the edges for both species (Nicastro et al., 2012). Contrary to sedentary organisms such as mussels, mobile organisms may use, rather than aggregation behaviour, habitat selection to decrease their body temperature under heat stress (Chapter III). Aggregation behaviour in littorinids seems to be driven by other factors than thermal benefit, such as prevention of dislodgement, reduction in the risk of predation, reduction in desiccation stress or mating (Stafford and Davies, 2004). In the gastropod species *Strombus gigas*, aggregation is a way to reduce the risk of predation by the "gain safety in numbers" effect (*i.e.* the probability of being preyed upon is inversely related to the number of alternative prey available; Ray and Stoner, 1994). However, this effect remains to be tested in *L. littorea*. Similar results were found in the limpets *Patella vulgata* suggesting that aggregation also allowed to reduce predation susceptibility (Coleman et al., 2004). Potential prey in aggregates can detect predator vibrations during an attack on a group member. As a reaction, limpets clamp down and thus become harder to remove by predators (Coleman et al., 2004). In Chapter IV, we only tested the potential thermal benefit of being aggregated. However, the absence of thermal benefits provided by aggregation (Chapman and Underwood, 1996; Chapter IV) compared to studies where aggregation behaviour provides thermal benefits (Helmuth, 1998; Rojas et al., 2000; Chapperon and Seuront, 2012) showed that temperature may not be the first driver of aggregation in intertidal gastropods. However, some complementary experiments could be tested, as for example a continuous record of the body temperature of solitary and aggregated individuals all along a tidal cycle to a better understanding of body temperature variations during emersion period. In the laboratory, we can also record body temperature of living individuals in aggregate or solitary and compared these results with body temperature of the same empty shells in the same experimental conditions. In this way, we can better understand the part of physiological processes in the body temperature of intertidal snails and the potential benefits of aggregation on snails' body temperature.

The results of the Chapter IV raise the following question: which factors drive aggregation behaviour in intertidal snails? Aggregation against predation can be tested under laboratory conditions; for example, *L. littorea* individuals can be put in contact with predator cues (*i.e.* water infused with the crab *Carcinus maenas*) and their aggregation behaviour will be compared between experiments with and without predator cues. Different concentrations of predator cues can also be used and the size of the aggregates can be compared. Aggregation as a reproductive behaviour can be compared during mating and non-mating seasons. As both males and females of *L. littorea* are attracted by conspecifics of the opposite sex only during mating season (Seuront and Spilmont, 2015); if aggregation is continuously observed, no matter the mating or non-mating seasons, reproduction will not be the first driver of aggregation behaviour. Sex-ratio in natural aggregates can also be a way to understand if reproduction is a driver for aggregation behaviour.

Conclusion

The present work provides new insights in our knowledge of *Littorina littorea* behaviour and, in particular, about its thigmotactic behaviour, considered here as a major taxis involved in its displacement and orientation. This work also provides a better understanding of the ability of *L. littorea* and *P. vulgata* to take advantage of the thermal mosaic of their environment. Indeed, individuals, at the scale of both macro- and microhabitats are able to find sustainable substrate temperature to maintain their body temperature within their optimal range and to adapt this thermal selection in function of the surrounding temperatures. This work also suggested that *L. littorea* had no thermal advantage of being aggregated and suggested that individuals found others benefits from aggregation. This work stresses the importance of behavioural adaptations in intertidal gastropods, to orientate and to tolerate variations in temperature. Integration of behavioural adaptations to predictions of increasing temperature seems crucial as behavioural adaptations such as habitat selection allow reduction in body temperature of intertidal gastropods. However, much work is still needed to incorporate the role of behavioural adaptations to the prediction of geographical shifts in the distribution of intertidal species and to understand the impact of changes in species distribution on the ecology and functioning of intertidal ecosystems.

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Annexe I. Deciphering the known unknowns in the behavioural ecology of the intertidal gastropod *Littorina littorea*

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Deciphering the known unknowns in the behavioural ecology of the intertidal gastropod *Littorina littorea*

Emilie Moisez[a], Laurent Seuront[b,c,d,*]

[a] Univ. Lille, CNRS, Univ. Littoral Côte d'Opale, UMR 8187, LOG, Laboratoire d'Océanologie et de Géosciences, F 62930 Wimereux, France
[b] CNRS, Univ. Lille, Univ. Littoral Côte d'Opale, UMR 8187, LOG, Laboratoire d'Océanologie et de Géosciences, F 62930 Wimereux, France
[c] Department of Marine Energy and Resource, Tokyo University of Marine Science and Technology, 4-5-7 Konan, Minato-ku, Tokyo 108-8477, Japan
[d] Department of Zoology and Entomology, Rhodes University, Grahamstown 6140, South Africa

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ABSTRACT

A considerable amount of attention has been devoted to understand the role of chemoreception in the behavioural ecology of gastropods. There is still, however, a limited amount of information related to their sensory ecology and their subsequent ability to navigate through topographically complex landscapes, such as intertidal rocky shores, despite the documented role of substrate spatial structure in the movement rates and directionality of gastropods. Responses to gravity and visual cues have been documented in a range of intertidal gastropods, but often led to contradictory conclusions. We examined the response of the gastropod *Littorina littorea*, a keystone grazer on most temperate intertidal ecosystems of the northern hemisphere, to substrate slope, topographic discontinuities and light using purpose-designed experimental set-ups under laboratory-controlled conditions. In contrast to previous results, we showed that *L. littorea* did not exhibit a strict geotactic behaviour. Instead, they were predominantly thigmotactic following an encounter with a topographic discontinuity, and we suggest that the observed seemingly negative geotactic responses are only a by-product of thigmotaxis, *i.e.* a thigmotactically-induced negative geotactic response. *L. littorea* also showed a strong preference for dark areas and shapes. Taken together, our results suggest that thigmotaxis and scototaxis are evolutionary advantageous to navigate through topographically complex and thermally stressful environments, and that the behavioural repertoire of gastropod may be much wider than previously thought.

1. Introduction

The understanding of animal movements is a key determinant of population viability and species dynamics (Tilman, 1994; Chapman, 2000; Morales and Ellner, 2002). Typically, animal search for food, mates and hosts, while avoiding predators in spatially and temporally complex, structured environment (Levin and Whitfield, 1994; Tilman, 1994; Morales and Ellner, 2002; Haynes et al., 2006). This issue is particularly relevant in tidepools and rocky shores that are exemplary complex environments (Denny and Gaines, 2007). Habitat selection and the ability of invertebrates, in particular gastropods, to make decisions about moving across different habitats rely on essentially non-visual senses in environments characterized by topographically complex substrate and resource heterogeneity (Fratini et al., 2001; Keppel and Scrosati, 2004; Wyeth et al., 2006). Noticeably, the presence of biogenic habitats typically created by species such as mussels and barnacles further contributes to the complex topography of rocky shores (Underwood and Chapman, 1989). Understanding the determinism of gastropod navigation is hence an absolute prerequisite to explain the dispersion, distribution and local biodiversity of other sessile and mobile species (Chapman, 2000).

A considerable amount of attention has been devoted to understand the role of chemoreception in driving the trail-following behaviour involved in the homing and aggregation behaviour of intertidal gastropods, and the localization of sexual partners, preys and predators, and preferential habitats (Croll, 1983; Chapperon and Seuront, 2009, 2011a, 2011b; Ng et al., 2013). There is still, in turn, a limited amount of information related to their sensory ecology and their subsequent ability to navigate through topographically complex landscapes, despite the documented role of substrate spatial structure in the movement rates and directionality of gastropods (Evans, 1961; Emson and Faller-Fritsch, 1976; Underwood, 1977; Petraitis, 1982; Underwood and

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[*] Corresponding author at: CNRS, Univ. Lille, Univ. Littoral Côte d'Opale, UMR 8187, LOG, Laboratoire d'Océanologie et de Géosciences, F 62930 Wimereux, France.

E-mail address: laurent.seuront@cnrs.fr (L. Seuront).

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Chapman, 1985; Jones and Boulding, 1999; Fraser et al., 2014, 2015). Responses to gravity and visual cues have nevertheless been documented in a range of intertidal gastropods. For instance, negative geotaxis (*i.e.* orientation away from a gravitational force) has been observed in *Littorina littorea* (Kanda, 1916). This response has further been shown to increase with the slope of the substrate (Kanda, 1916), to be more pronounced in high-shore than in low-shore snails (Hayes, 1926), and to be habitat-related (Newell, 1958a, 1958b). Snails originating from horizontal and vertical surfaces respectively showed positive and negative geotaxis (Newell, 1958b), and this response was more pronounced in featureless environments (Newell, 1958b) — though contradictory results exist (Petraitis, 1982). Intertidal gastropods exhibit oriented responses to the light of the sun in *L.littorea* (Newell, 1958a, 1958b), *L. punctata* (Evans, 1961) and *Nerita plicata* (Warburton, 1973) and unidentified celestial objects in *Aplysia brasiliana* (Hamilton and Russell, 1981), and to a range of visual cues such as black walls (Thain et al., 1985), shore-line topographic features and nocturnal silhouettes of vegetation (Evans, 1961; Chelazzi and Vannini, 1976), and to large areas of vegetation and individuals plant stems (Hamilton, 1977, 1978).

These contradictory observations suggest, however, the presence of both positive phototaxis (*i.e.* the orientation and movement towards a source of light) and scototaxis (*i.e.* a natural preference for dark areas) in intertidal snails. Despite the acknowledged role of topographic features, such as boulders and pebbles, crevices, cavities and pits in the ecology of intertidal ecosystems (Little et al., 2009) and early suggestions on the existence of a kinaesthetic memory in limpets, which determine their location based on previous movements and local topography (Pieron, 1909), the role of topographic complexity and the related alternation of shaded and unshaded areas on the motion behaviour of intertidal gastropod is still unknown. In particular, no evidence exists for thigmotaxis (*i.e.* movement towards or away from the stimulus induced by a physical contact) in intertidal gastropods.

In the present study, we chose the intertidal herbivorous gastropod, *Littorina littorea*, as a model species due to its abundance on most Western and Northern European coasts where it can reach densities up to hundreds of individuals per metre square (Petraitis, 1982; Bertness, 1999; Seuront et al., 2007), and its role in controlling algal growth (Stafford and Davies, 2005), sediment dynamics (Kamimura and Tsuchiya, 2006) and the recruitment of both algae and invertebrates (Buschbaum, 2000; Lotze and Worm, 2002). The net distance travelled by *L. littorea* between two low tides in its rocky shore habitats ranged from 1 cm to 10–15 m (Seuront et al., 2007). Over this range of scales, *L. littorea* is typically exposed to a broad range of topographic features, suggesting that it may have adapted its senses to navigate in such a complex environment. In this regard, we aimed to elucidate the behavioural response of *L. littorea* to substrate slope, topographic discontinuities and light using purpose-designed experimental set-ups under laboratory-controlled conditions. Specifically, we assessed the presence of negative geotaxis in *L. littorea*, and we further tested the alternative hypothesis that geotaxis may either be positive or negative following the encounter with a topographic discontinuity. We also assessed the behavioural response of *L. littorea* to light using two distinct though complementary approaches based on the ability to actively (i) choose between a dark and a light area and (ii) orient towards a dark shape. If *L. littorea* demonstrate such behavioural adaptations to structurally complex environments, the behavioural repertoire and resilience of this keystone species may respectively be much wider and stronger than previously thought.

2. Methods

2.1. Collection and acclimation of L. littorea

Littorina littorea individuals were collected from the Fort de Croy (Wimereux, France; 50°45′48″N, 1°35′59″E) an intertidal reef typical of the rocky habitats found along the French coasts of the eastern English Channel (Chapperon and Seuront, 2009; Seuront and Spilmont, 2015; Spilmont et al., 2016). Before any experiment took place, *L. littorea* individuals (10 to 15 mm in length) were acclimatized for 24 h in the laboratory in acrylic glass (*i.e.* polymethyl methacrylate, PMMA) cylinders (50 cm tall and 20 cm in inner diameter, riddle with holes 5 mm in diameter) held in 120-l (90 × 50 × 30 cm) tanks of running natural seawater, aerated at temperatures representative of *in situ* conditions. These perforated 'acclimation towers' (Seuront and Spilmont, 2015) allow both seawater to be continuously renewed and captive snails to move freely in and out of the water without being able to escape. No food was available during the acclimation period. Each individual was only use once. Between each trial the behavioural set-up was rinsed with 70% ethanol and seawater to remove mucus cues (Erlandsson and Kostylev, 1995). In each of the behavioural assays described hereafter, the motion behaviour of *L. littorea* individuals was recorded every 5 s during 120 min using a Raspberry Pi NOiR camera overlooking the experimental set-up and operated through a Raspberry computer under homogenous dim light conditions (*i.e.* 168 lx) measured with a digital lightmeter (Extech Instruments, 403,125). The resulting 1480 images where subsequently assembled using Time Lapse Tool (©Al Devs) before behavioural analyses took place.

2.2. Geotaxis assay

Geotactic experiments were conducted in glass aquaria (length: 34.5 cm, width: 25 cm, depth: 18 cm) where *Littorina littorea* were immersed under 10 cm of seawater. To assess the potential geotactic response of *L. littorea*, behavioural assays were conducted in aquaria tilted with three different angles, *i.e.* 6, 13 and 25°. These angles were specifically chosen to cover the range of slopes reported to induce a geotactic response in this species (Kanda, 1916). Control experiments were conducted in aquaria lying horizontally. One individual was placed in the middle of 5 separate aquaria, with their head initially oriented randomly, and each experimental condition replicated 10 times ($N = 50$). Treatments and control experiments were randomized.

The time taken by *L. littorea* to resume movement (t_{move}) was estimated for each snail. The time of activity, t_{active}, was defined as the time between the first displacement at location (x_0, y_0) and the moment snails reached a maximum net distance $d_{max} = W/2$, where W is the width of the aquarium. Movement speed and direction were respectively calculated as $v = d_{max}/t_{\text{active}}$. Direction was the angle of the vector between a snail starting point (x_0, y_0) and the point where snails reached a maximum net distance $d_{max} = W/2$ ($x_{W/2}$, $y_{W/2}$). The behaviour of snails that encountered the wall was also recorded, and the percentage of individuals that followed and/or climbed the wall of the aquarium was also recorded.

2.3. Thigmotaxis assay

The response of *Littorina littorea* to topographic discontinuities was studied at a scale pertinent to individual snails through the development of a LEGO-based behavioural apparatus. More fundamentally, the experimental set-up was designed to assess if the previously reported observations of snail moving up upon encountering the wall of an aquarium (Kanda, 1916; Petraitis, 1982) were actually an evidence of negative geotaxis, and not an idiosyncratic artefact due to the impossibility for the snails to move downward. Our apparatus was specifically designed to associate the encounter of a discontinuity (*i.e.* a surface-to-wall transition or a surface-to-drop-off transition) with the possibility of moving upward or downward. Note that the height of the walls is identical to the height of the drop-offs, *i.e.* 3 cm. The apparatus was uniformly made of light grey LEGO ® Bricks (Fig. 1A), and included four replicate cubic structures (length: 11 cm, height: 6 cm), constituted of vertical walls where snail can move up or down, that were built on a LEGO plaque (25.5 × 25.5 cm) glued on the bottom of a cubic glass

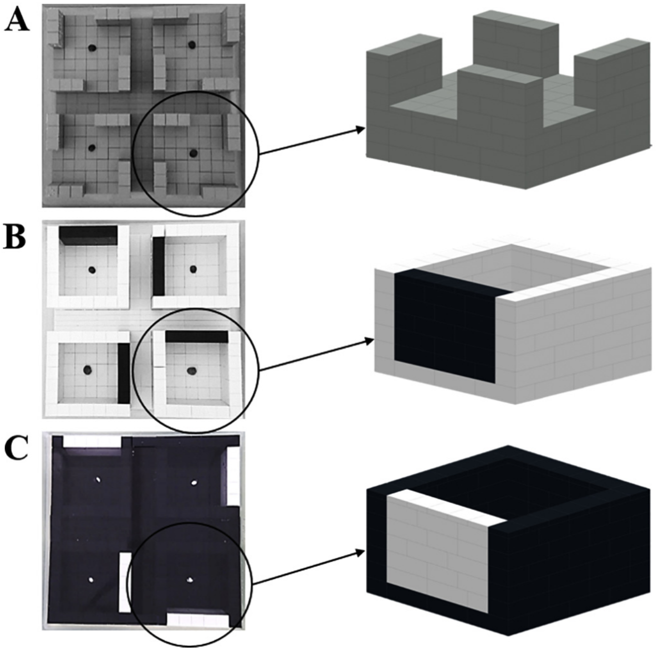

Fig. 1. Illustration of the LEGO-based structures designed to assess (A) the presence of geotaxis and thigmotaxis in *Littorina littorea* and the presence of scototaxis in *L. littorea* based on experiments run on four subsections made of (B) 3 white walls and 1 target black wall (W_3B_1) or (C) 3 black walls and 1 target white wall (B_3W_1). The white points from the panel C correspond to the *Littorina littorea* individuals marked with white nail polish.

aquarium and immersed in 10 cm of seawater. This design was specifically chosen to offer the same amount of discontinuities leading to upward and downward surfaces to *L. littorea* individuals.

One snail was used in each replicate structure and 10 replicates were done ($N = 40$). *L. littorea* behaviour was dichotomized in four behavioural displays following the encounter with a discontinuity: (i) discontinuity avoidance (*i.e.* the behaviour by which a snail actively avoids a discontinuity upon contact, through a drastic change in direction, without following it; Fig. 3A), (ii) discontinuity following (*i.e.* the behaviour by which a snail follows a discontinuity at a distance less than one bodylength; Fig. 3B), (iii) moving upward, and (iv) moving downward. The time allocated to each behaviour was noted for each individual and the frequency of occurrence of the four behaviours was subsequently calculated. The total time spent in our LEGO apparatus (t_{total}) and the time of activity (t_{active}) were recorded for each snail as well as the time of initial scanning behaviour (t_{scan}; *i.e.* non-oriented movements of a snail spinning around its vertical axis before heading into a specific direction), the time to resume movement and the activity index. A behavioural complexity index I was also calculated for each snail as $I = N_i \times f_{active}$, where N_i is the total number of observed behaviours and f_{active} the percentage of activity.

2.4. Scototaxis assay

Two distinct but complementary approaches were used to infer the presence of scototaxis in *Littorina littorea*. First, we used a binary choice assay conducted in a PVC container (length: 51 cm, width: 11 cm, depth: 20 cm) half black and half white with two removable vertical panels used to isolate a central area from the rest of the apparatus. Five *Littorina littorea* were placed in circle at the centre of the container filled with 10 cm of seawater. To enhance the visibility of individuals when they crawled on the black section, they were marked with white nail polish. After marking the snails, the nail polish was allowed to dry for 30 min and the snails were kept in flowing seawater aquaria until the next day (Seuront et al., 2018a). The experiment was repeated four times ($N = 20$). Control experiments were run in totally black and totally white version of our experimental container to ensure no bias intrinsic to the experimental set-up was present. The parameters considered for the behavioural analysis were the position of each snail after two hours of experimentation, the proportion of individuals that left the aquaria and the total time (t_{total}) spend in each section. This time was decomposed into activity (t_{active}) and inactivity ($t_{inactive}$) times in each section of the aquaria.

Second, we used a target-based assay that was conducted using a modification of the aforementioned LEGO thigmotaxis assay. Specifically, four replicate cubes (length: 11 cm, height: 6 cm) were built on a LEGO plaque (25.5 × 25.5 cm), and each cube was either made of three white walls and a target black wall (W_3B_1 assay) with a white bottom or three black walls and a target white wall (B_3W_1 assay) with a black bottom (Fig. 1B, C). We used black and white LEGO bricks to maximise the contrast between the target wall and the other walls. The position of the black and white walls was assigned randomly and the supporting LEGO plaque was glued at the bottom of a cubic glass aquarium and immersed in 10 cm of seawater. One snail was put in the middle of each replicate cube with the head oriented randomly and each assay replicated 8 times ($N = 32$). *L. littorea* behaviour was recorded as described for the geotactic and thigmotactic experiments. In the B_3W_1 assay, the 32 individuals were marked with white nail polish to be able to see them on the black background. Control experiments were run in both uniformly black and white LEGO structures. The behavioural analysis was based on the scanning behaviour (*i.e.* non-oriented movements of a snail spinning around its vertical axis before heading into a specific direction) and the net direction.

2.5. Statistical analyses

As the distribution of measured parameters was non-normally distributed (Shapiro-Wilk test, $p = .17$), non-parametric statistics were used throughout this work. In the geotaxis assay, the potential differences between inclination angles and the time taken by *Littorina littorea* to resume movement (t_{move}), their time of activity (t_{active}), and their movement speed were assessed using a Kruskal-Wallis test (hereafter referred to as KW test) and when necessary a subsequent post-hoc test was performed using a Dunn test (Zar, 2010). The Rayleigh test was further used to assess the circular uniformity of the snail dispersal for each inclination angle and control (Zar, 2010). The initial orientation of *L. littorea* in the thigmotaxis assay (*i.e.* towards upward or downward surfaces) was compared to a theoretical equi-repartition of the individuals using a χ^2 test (Zar, 2010). The presence of a causal link between the time of activity and (i) the number of behaviour and (ii) the activity index (*i.e.* percentage of activity) was further tested using the Pearson correlation coefficient.

In the control experiments of the binary scototaxis assay, the proportion of individuals found on each half of the uniformly coloured containers (*i.e.* black or white) was compared to a theoretical equi-repartiton using a χ^2 test.

A Wilcoxon test for paired samples was performed to compare the proportion of time spend in the black and the white section. The percentage of activity and inactivity times were also compared for the two sides and between the wall and the bottom using a Wilcoxon test for paired samples. The proportion of activity and inactivity on the walls and on the bottom were compared for each side using a Friedman test. A Mann-Whitney pairwise test for paired samples was performed when necessary.

The uniformity of the net direction followed by *Littorina littorea* in the control target-based scototaxis assay run in uniformly black and white LEGO set-up were compared using a Rayleigh test. The net direction of the 32 snails was recorded (*i.e.* towards black or white wall) and the time of displacement between the initial position and the first wall touched (*i.e.* black or white) was compared using a Wilcoxon-Mann-Whitney U test (hereafter called U test).

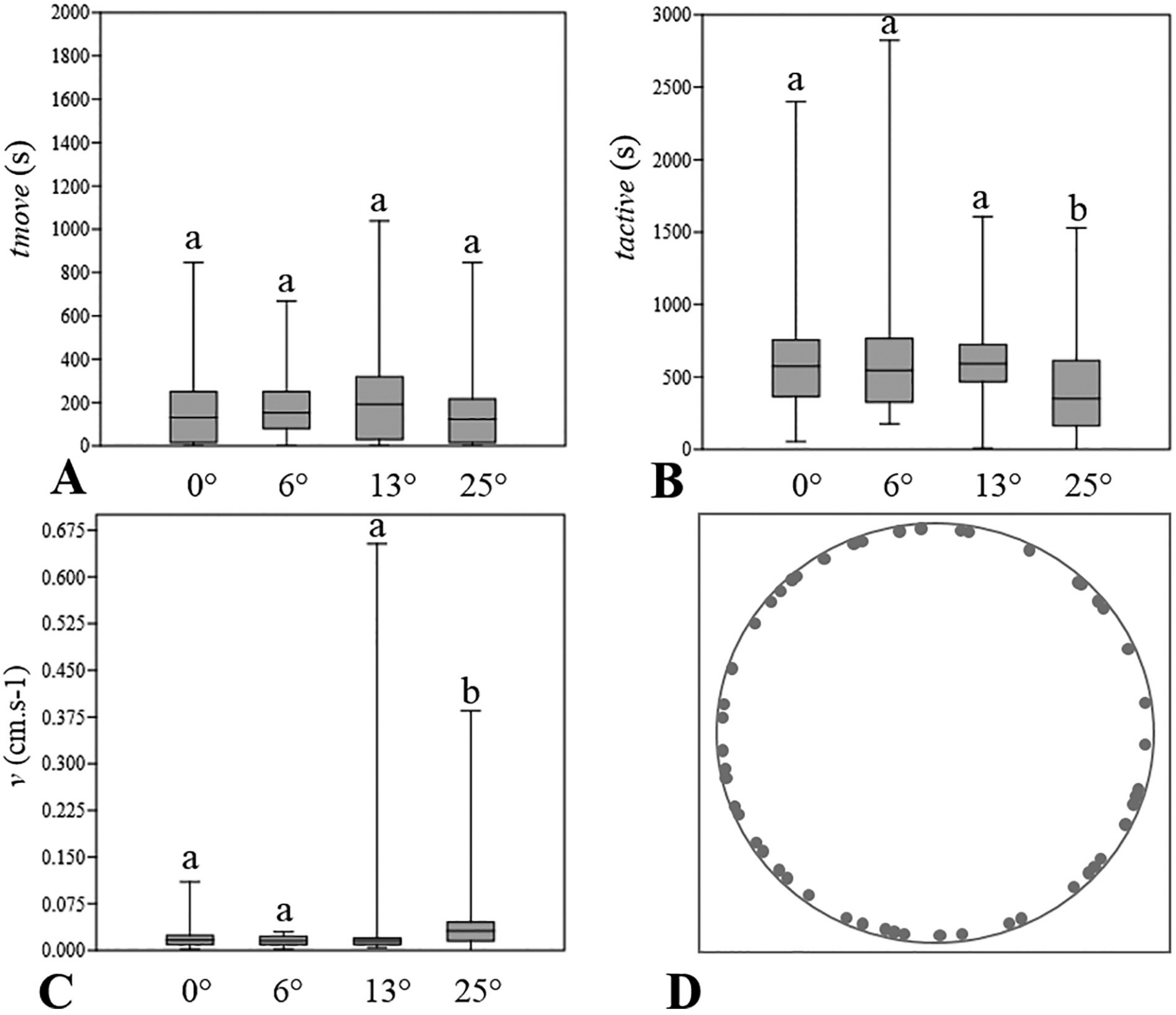

Fig. 2. Behavioural measurement of *Littorina littorea* for the different treatments in the geotactic experiment, (A) time to resume movement (t_{move}), (B) time of activity (t_{active}), (C) movement speed (v) and (D) the net orientation travelled by *L. littorea* for the experiment tilted to 6°. The limits of the box are the 25 and 75% quartiles. The median is shown with a horizontal line inside the box and the minimal and maximal values are shown with short horizontal lines.

3. Results

3.1. Geotaxis assay

The time taken by *Littorina littorea* to resume movement (t_{move}) did not significantly differ (KW test, $p = .1295$) between the different treatments (Fig. 2A). The time of activity (t_{active}, *i.e.* the time between the first displacement and the moment snails reached a maximum net distance d_{max} = W/2, where W is the width of the aquarium) was significantly different between treatments (KW test, $p = .00329$). More specifically, t_{active} was significantly shorter for individuals in aquarium tilted by 25° angles than for all the others treatments (Fig. 2B).

The distribution of the net orientation of *Littorina littorea* did not significantly differ from a uniform distribution (Rayleigh test, $p = .2146$) under all experimental treatments (Fig. 2D). In contrast, *L. littorea* speed significantly differed between treatments (KW test, $p < .0001$). Specifically, *L. littorea* moved significantly faster on surface tilted by 25° (Dunn test, $p < .0001$) than for the surfaces tilted to 6 and 13° and the control, where speed did not significantly differ (Fig. 2C). Finally, all the snails that encountered the wall systematically followed it, before 78 to 100% of them climbed it.

3.2. Thigmotaxis assay

Respectively 18 and 22 individuals moved towards the vertical walls and the sections of our LEGO-based structure without wall. There was no significant difference between this distribution and a theoretical equi-repartition of the individuals (χ^2, $p = .6578$). The most frequent behaviour after the first encounter with either a surface-to-wall or a surface-to-drop-off discontinuity was discontinuity-following (67.5%), in contrast to discontinuity-avoidance which was only marginally observed (2.5%). Climbing directly downward and upward after encountering a topographical discontinuity (*i.e.* a surface-to-drop-off discontinuity and a surface-to-wall discontinuity, respectively) represented respectively 10% and 20%. After following a discontinuity, 100% of the individuals moved up after following a surface-to-wall discontinuity or down after following a surface-to-drop-off discontinuity.

3.3. Scototaxis assay

Control experiments run in either uniformly black or uniformly white versions of both the binary container and target-based LEGO structure respectively showed that the proportion of individuals found in each half of the aquaria did not significantly differ from a theoretical

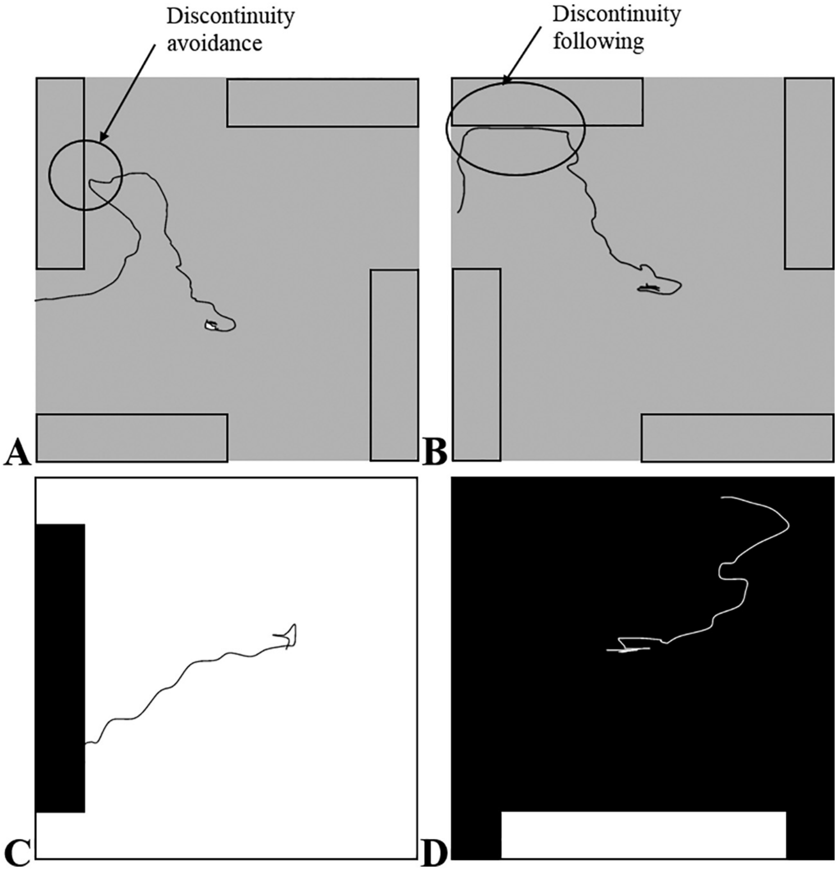

Fig. 3. Illustrations of representative tracks from our thigmotactic assay (A) representing the discontinuity avoidance behaviour, (B) the discontinuity following behaviour, and from ours scototaxis assay (C) W_3B_1 assay and (D) B_3W_1 assay.

equi-repartition (χ^2 test, $p = .177$), and that the net directions followed by *Littorina littorea* were uniform ($p = .295$), hence that no intrinsic directionality related to the experimental set-up was present.

In the binary choice assay, any group effects or inter-individual interactions such as trail-following between snails in a group trial have never been observed. All individuals can then be considered as being independent. The time spent in the black section was more than twice as high as the time spent in the white section (Wilcoxon test, $p = .00195$). In contrast, there is no significant difference in the percentages of activity and inactivity in the two different sections (Wilcoxon test, $p = .51$).

In the W_3B_1 assay (*i.e.* experimental set-up with three white wall, a black wall and a white bottom), 78% of the individuals oriented towards the black wall, whereas only 22% oriented towards a white wall (Fig. 3C, D, Fig. 4). Similarly, in the B_3W_1 assay (*i.e.* experimental set-up with three black wall, a white wall and a black bottom) a vast majority of the snails (90.6%) moved towards a black wall, while only 9.4% moved towards the white wall (Fig. 4).

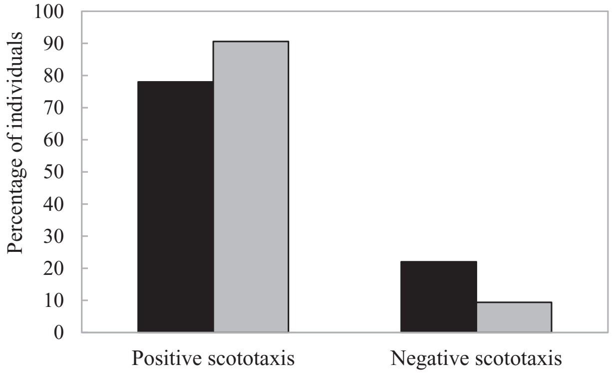

Fig. 4. Frequency of occurrence of target-based positive and negative scototaxis in *Littorina littorea*. The black and grey bars correspond to the version of the experimental set-up respectively including 3 white walls and a black one (W_3B_1) and 3 black walls and a white one (B_3W_1).

4. Discussion

The ability to navigate through structurally complex environments is a key determinant of the ecological success of a species. Intertidal gastropods are no exception, especially given the highly complex nature of their environment, and the heavy constraints exerted by a range of stressors such as thermal and desiccation stresses, wave exposure and the related risk of dislodgement by wave action (Denny and Gaines, 2007). However, despite their critical role in the structure and function of intertidal ecosystems, little is still known — with the noticeable exception of trail-following, see Ng et al. (2013) for a review — on the role of sensory mechanisms and the related navigational capabilities of intertidal gastropods in general, and *Littorina littorea* in particular. Based on the existing and often contradictory literature, this work has inferred the presence of geotaxis, thigmotaxis and scototaxis in *L. littorea* under laboratory-controlled conditions.

4.1. Geotaxis as a by-product of thigmotaxis

Despite the presence of significant differences between treatments in the time taken by *L. littorea* to resume movement (t_{move}), their time of activity (t_{active}) and their speed, no significant relationship was found between any of these variables and the slope of their substrate. These results suggest that *Littorina littorea* may behave similarly on substrates of different inclination.

Specifically, the lack of dependence of *L. littorea* movement direction to substrate slope further suggests an absence of geotactic behaviour in *L. littorea* investigated on substrates inclined from 6 to 25°. *L. littorea* was also observed to move consistently significantly faster on surface tilted by 25°. These results suggest that despite the absence of geotactic response, *L. littorea* exhibited a kinetic response triggered by the conditions and the angles of the substratum. In addition, the high proportion of *L. littorea* that climbed the wall of the experimental aquarium after his encounter (78 to 100%) may have indicated a high expression of geotaxis. However, in all instances, this behaviour consistently occurred after the snails followed the discontinuity between the bottom and the wall of the aquarium. These observations further suggest that the observed seemingly negative geotactic responses may instead be triggered by a discontinuity encounter, *i.e.* a thigmotactically-induced negative geotactic response.

4.2. Thigmotaxis as a way to navigate

The predominant behaviour of the thigmotactic assay was by far discontinuity-following, before 100% of *Littorina littorea* individuals actually moved up or down, hence switched to a geotactic behaviour. This switch from thigmotaxis to geotaxis consistently happened along both surface-to-drop-off and surface-to-wall discontinuities. However, though these results have not been included in the present work, *L. littorea* have also been observed to switch from following a surface-to-wall discontinuity to following a surface-to-drop-off discontinuity and *vice versa*, and these transitions were consistently followed by a geotactic response (Moisez, personal observations). Even though geotaxis has been observed in the absence of thigmotaxis in 30% of our observations, overall our observations suggest that previously reported positive or negative geotactic response may actually have been essentially triggered by a thigmotactic response.

Though the following may be considered as highly speculative, the fact that discontinuity-following was the predominant behaviour following an encounter with a vertical discontinuity and was consistently followed by a geotactic response may also be though as an obstacle response of individual *L. littorea* which were attempting to go around an obstacle before actually choosing to moving up or down. Additionally, the mechanical constraints of vertical crawling (up or down) due to *e.g.* gravity and the shape of *L. littorea* shell may be such that it may be easier to begin by turning and follow a discontinuity rather than crawling directly downward or upward after encountering a surface-to-drop-off discontinuity and a surface-to-wall discontinuity, respectively. In particular, the hypothesis of a mechanical constraint related to the shape of *L. littorea* shell is consistent with the observed 2-fold difference between the frequency of climbing downward after encountering a surface-to-drop-off discontinuity (*i.e.* 20%) and the frequency of climbing upward after encountering a surface-to-wall discontinuity (*i.e.* 10%). This observation may indicate a higher mechanical constraint associated to moving across a surface-to-wall discontinuity than across a surface-to-drop-off discontinuity.

Despite the fundamental importance of substrate topography in the ecology of intertidal organisms (Denny and Gaines, 2007), only a limited amount of work has been devoted to the understanding of the mechanisms relating substrate topography and the navigational abilities of intertidal gastropods. For instance, the complexity of surface topography has been shown to affect different aspects of the ecology of intertidal snails such as distance travelled, population density and structure (Underwood and Chapman, 1989; Chapman and Underwood, 1994). In addition, the presence of species such as mussels or barnacles further creates biogenic habitats that contribute to increase the complexity of surface topography and in turn affect different aspects of mobile fauna biology and ecology. For instance, in gastropods the pattern of limpet orientation was affect by this complex biogenic habitat (Fraser et al., 2014). In the early 20th century, Pieron (1909) introduced the idea of a kinaesthetic memory, which allowed homing limpets to determine their location based on previous movement and local topography. In contrast to limpets, littorinids do not exhibit homing behaviour, though evidence exist for navigation back to a preferred shore height or resting habitat following dislodgment or transplantation (Evans, 1961; Bock and Johnson, 1967; Gendron, 1977; Thain et al., 1985; Rolán-Alvarez et al., 1997; Miller et al., 2007; Chapperon and Seuront, 2009; Seuront et al., 2018a). As such, the consideration of thigmotaxis, and in particular both the prevalent discontinuity-following behaviour and the thigmotactically-induced negative geotaxis observed in the present work, may be a first step into furthering our understanding of how these organisms may navigate through complex landscapes and is a plausible candidate to explain how the aforementioned putative kinaesthetic memory may work.

4.3. Scototaxis assay as an adaptation to a contrasted environment

Littorina littorea orient towards black areas, hence exhibit a strong positive scototaxis (Fig. 4). This behaviour is consistent with early suggestions that eyes of *Littorina* could be used for monitoring light intensity and for orientation (Stoll, 1973), and with littorinids visual abilities as *L. littorea* have simple eyes which are optimum for under water vision during the day (Seyer, 1992). Our results are also consistent with previous evidence that a range of gastropod species orient towards black forms, with the strength of the response increasing with their dimensions (Thain et al., 1985; Hamilton and Winter, 1984, Hamilton and Russell, 1981). This behaviour may reflect the fact that objects with ecological importance essentially appear as vertical for benthic organisms (Hamilton and Winter, 1984). Though further work is needed to untangle the details of the mechanisms involved, the capacity to distinguish and orient towards black areas could be important for snails to allow them to find their way back after feeding excursions or dislodgement as recently demonstrated for *L. littorea* (Seuront et al., 2018a).

Note that the orientation towards dark areas is also likely to be relevant to gastropods thermal ecology and predation avoidance. Indeed, crevices, pits and the lower part of boulders typically appear as darker areas in comparison to the more exposed flat substrates, hence they can exploit topographical complexity of the rocky shore to find darker microhabitats which provide shelter from desiccation and thermal stresses (Jackson, 2010; Chapperon and Seuront, 2011b; Seuront et al., 2018b) This hypothesis is also consistent with the reported decreased activity of *L. littorea* in dark environments.

It is finally stressed that because the presence of other individuals in the scototaxis assays may implicitly generate a time-dependent dark cue on a light background depending on the relative position of individual snails respective to each other and to the dark and black walls, it is likely that the snails considered in each scototaxis assay.

4.4. Conclusion

This study suggests that the geotactic behaviour of *Littorina littorea*, previously reported in the literature (Kanda, 1916; Hayes, 1926; Newell, 1958a, 1958b; Petraitis, 1982) is more likely to be a by-product of the thigmotactic response induced by a contact with topographical discontinuities. The new evidence of the existence of the prevalence of thigmotactic response in *L. littorea* presented in the present work as well as demonstration that this species actively chooses dark areas where they consistently decreased their level of activity provide new insights into our understanding of intertidal gastropods may navigate through complex landscapes. Much work is, however, still needed to entangle the complex relationship that exist between the structure and functions at play in their environment, the cognitive abilities of intertidal snails and the adaptive value of using different taxes as a response to environmental cues. In particular, increasing the complexity of our LEGO-based structure designed to assess the presence of thigmotaxis, to be more coherent with the complex landscape of the rocky shore would contribute to generalise our results. In addition, because our experiments were consistently undertaken under laboratory conditions, underwater and on substrates devoid of food, further work is needed to entangle the role of *e.g.* food availability, temperature or humidity gradient, as well as different heat and air exposure stress may play in the observed behavioural patterns.

Author statement

L. Seuront designed and funded the research. E. Moisez conducted the experiments, ran the statistical analyses under the supervision of L. Seuront. E. Moisez and L. Seuront wrote the manuscript.

Declaration of Competing Interest

The authors have no conflict of interest.

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Annexe II. *Littorina littorea* show small-scale persistent tidal height and habitat partitioning that is resilient to dislodgement through specific movement rates

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Littorina littorea show small-scale persistent tidal height and habitat partitioning that is resilient to dislodgement through specific movement rates

Laurent Seuront[a,*], Emilie Moisez[b], Camille Hennion[b], Dorine Seuront-Scheffbuch[c], Leena M.Y. Seuront[d]

[a] CNRS, Univ. Lille, Univ. Littoral Côte d'Opale, UMR 8187, LOG, Laboratoire d'Océanologie et de Géosciences, F 62930 Wimereux, France
[b] Univ. Lille, CNRS, UMR 8187, LOG, Laboratoire d'Océanologie et de Géosciences, F 62930 Wimereux, France
[c] Agence Régionale de Santé Hauts-de-France, 556 avenue Willy Brandt, 59777 Euralille, France
[d] Ecole de la Scierie, 62720 Wierre-Effroy, France

ABSTRACT

Dislodgment, in particular by breaking waves, is a widely acknowledged source of stress, if not mortality, in rocky shores intertidal gastropods. This raises the question of understanding if snails dislodged from a specific habitat have the ability to navigate back to their original substrate, and how this is achieved. In this context, we investigated the consequences of dislodgement on a population of *Littorina littorea* inhabiting two proximate habitats (a seawall ranging from the low to the high intertidal zone and the related low intertidal rocky platform) located on an isolated offshore reef over a three-month period. Using tagged undisturbed, disturbed, translocated and transplanted *L. littorea*, we assessed a potential tidal height preference and found that *L. littorea* showed a clear tendency to remain on their original tidal height, with recapture rates ranging between 100% and 88%, respectively 1 and 78 days after dislodgment. We subsequently measured the recapture rates of individuals dislodged from three distinct tidal levels on the seawall and from a horizontal platform. Dislodged *L. littorea* showed a clear tendency to return to their original tidal level, with return rates ranging between 32 and 48% 78 days after dislodgement; snails from the horizontal platform were never found on the proximate seawall. In addition, high frequency observations conducted on individually tagged dislodged *L. littorea* during the first 8 successive low tides of our survey indicate that individuals originating from the seawall consistently showed higher movement rates and directionality towards the seawall, while individuals originating from the platform showed no directionality and reduced migration rate. These results show that dislodged *L. littorea* navigate back to their original substrate and tidal height. Putative processes include a combination of geotaxis, chemotaxis and rheotaxis. At relatively short time scales (i.e. days to months), *L. littorea* show both tidal and habitat partitioning that is distinct from the movement patterns previously reported in littorinids over a range of temporal scales.

1. Introduction

Life in the intertidal zone is heavily constrained by a range of stressors including thermal and desiccation stresses, wave exposure and the related risk of dislodgement by wave action (Denny and Gaines, 2007). Understanding the direct and indirect effects of wave exposure, force and activity on the structure and function of intertidal populations and communities has been a prolific area of research over four decades (Dayton, 1971; Wolcott, 1973; Menge and Sutherland, 1976, 1987; Sousa, 1979, 1984; Pain and Levin, 1981; Helmuth and Hofmann, 2001; Harley and Helmuth, 2003; Finke et al., 2007; Scrosati et al., 2010). Specifically, waves can dislodge intertidal organisms by imparting lift, acceleration reaction, and drag forces (Denny et al., 1985). Measuring or predicting these forces makes it possible to estimate the risk of dislodgement from the substratum under various wave conditions for a range of organisms such as macroalgae (Carrington, 1990; Gaylord et al., 1994; Blanchette, 1997), crustaceans (Martinez, 2001; Lau and Martinez, 2003), and various molluscs (Denny, 1985, 1987, 1995; Bell and Gosline, 1997; Denny and Blanchette, 2000; Carrington, 2002; Wyeth and Willows, 2006; Brenner and Buck, 2010), including littorinids (Trussell et al., 1993).

Snails on exposed shores tend to express traits that increase dislodgement resistance such as longer, broader feet and greater tenacity (Miller, 1974; Etter, 1988; Trussell et al., 1993; Trussell, 1997a, 1997b; Parsons, 1997a, 1997b), though some exceptions exist (Hohenlohe, 2003; Prowse and Pile, 2005). The ability of intertidal gastropods to survive on wave-swept environments is hence partially determined by their ability to resist dislodgement by breaking waves, which are also likely to reduce foraging efficiencies and reproductive success (Denny et al., 1985; Etter, 1989; Trussell et al., 1993). The consequences of dislodgement have, however, been far less studied (Miller et al., 2007; Chapperon and Seuront, 2009), and there is still seldom information on

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* Corresponding author.
E-mail address: laurent.seuront@cnrs.fr (L. Seuront).

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how dislodged intertidal gastropods may navigate back to their original (or more generally suitable) habitat or tidal height despite decades of investigations of littorinid sensory abilities (Evans, 1951, 1961; Charles, 1961; Newell, 1965; Warburton, 1973; Hamilton, 1976; Petraitis, 1982; Hamilton and Winter, 1982; Thain et al., 1985; Seyer, 1992; Land and Nilsson, 2002; Chapperon and Seuront, 2009; Seuront and Spilmont, 2015).

In this context, the aim of this study is to improve our understanding of littorinids behavioural ecology and navigational capabilities following a dislodgment from a preferred resting site or tidal height. Specifically, the present work uniquely focuses on a population of *L. littorea* inhabiting two proximate habitats (*i.e.* a seawall ranging from the low to high intertidal zone and the proximate low intertidal rocky platform). We first assessed whether *L. littorea* exhibit both tidal height and habitat partitioning through a temporal survey of the location of undisturbed, disturbed and translocated individuals from three distinct tidal levels on the seawall, and the horizontal platform. Specifically, if *L. littorea* exhibit tidal height and habitat partitioning, we would expect to consistently observe the same individuals on distinct tidal levels and on the horizontal platform. We further investigate the robustness of this partitioning following simulated wave dislodgement through the ability of dislodged *L. littorea* to navigate back to their original habitat whether they were dislodged from the horizontal platform or the seawall for time scales ranging from 1 to 90 days. In an attempt to assess whether the movement of dislodged individuals is directed or random, we also monitored the successive positions of individually tagged individuals sampled from both the horizontal platform and the three distinct tidal levels considered on the seawall during 8 consecutive low tides.

2. Materials and methods

2.1. Study site

Littorina littorea experiments were carried out on the landward side of the Fort de l'Heurt (50°42′29″N, 1°33′35″E; Fig. 1), where their abundance typically ranges from 15 to 250 individuals m^{-2} (Seuront, unpublished data). Note that *L. littorea* are consistently absent from the seaward wave-exposed side of the Fort (Seuront, personal observation), which is directly exposed to the main SSW swell direction that characterizes this area. The landward side of the Fort, though less exposed to the dominant swell regime, is nevertheless heavily wave-swept under stormy conditions. This consistently results in massive *L. littorea* dislodgment with 2- to 5-fold decrease in abundance observed during the low tide immediately following a storm (L. Seuront, personal observation). The Fort de l'Heurt is located 650 m away from the Mean High Water Spring (MHWS) line, on an intertidal rocky reef south of Boulogne-sur-Mer on the French side of the eastern English Channel (Fig. 1A–D). This coastal area is characterised by the amplitude of its tidal range (between 3 and 9 m), and both the strength of the tidal circulation with strong (1 to 2 m s^{-1}) tidal currents parallel to the coast, and the shallow water generate extremely high levels of mixing, with turbulence intensities ranging from 10^{-7} to 10^{-4} m^2 s^{-3} (Seuront, 2005). Once a massive structure covering nearly 1800 m^2 (50 m × 35 m), the Fort de l'Heurt has been heavily damaged by wave impacts and its original walls made of large stones (190 cm × 130 cm × 40 cm) have been destroyed and scattered around the remaining inner core, which is about 25 m long, 10 m wide and 10 m tall. Nowadays, each side of the Fort similarly consists of vertical walls made of stones of various sizes and shapes bounded with a mortar of cement or lime (Fig. 1E). The landward wall of the Fort de l'Heurt ranges from a height of 3 metres above the Mean Lower Low Water (MLLW) to the supratidal zone. *L. littorea* were sampled from 3 tidal levels on the seawall, *i.e.* 3 to 5 metres, 5 to 7 metres and 7 to 9 metres above MLLW that are hereafter referred to as low, intermediate and high tidal levels. *L. littorea* individuals were also sampled from the proximate horizontal platform (20 m long, and 10 m wide) located 2 meters above MLLW, and dominated by small-scale topographic features such as crevices, pits and small boulders, and hosting a bed of the blue mussel *Mytilus edulis*. Note that *L. littorina* were never observed in aggregations or trail-following on this site, in sharp contrast with nearby rocky platforms devoid of topographic features (L. Seuront, unpublished data).

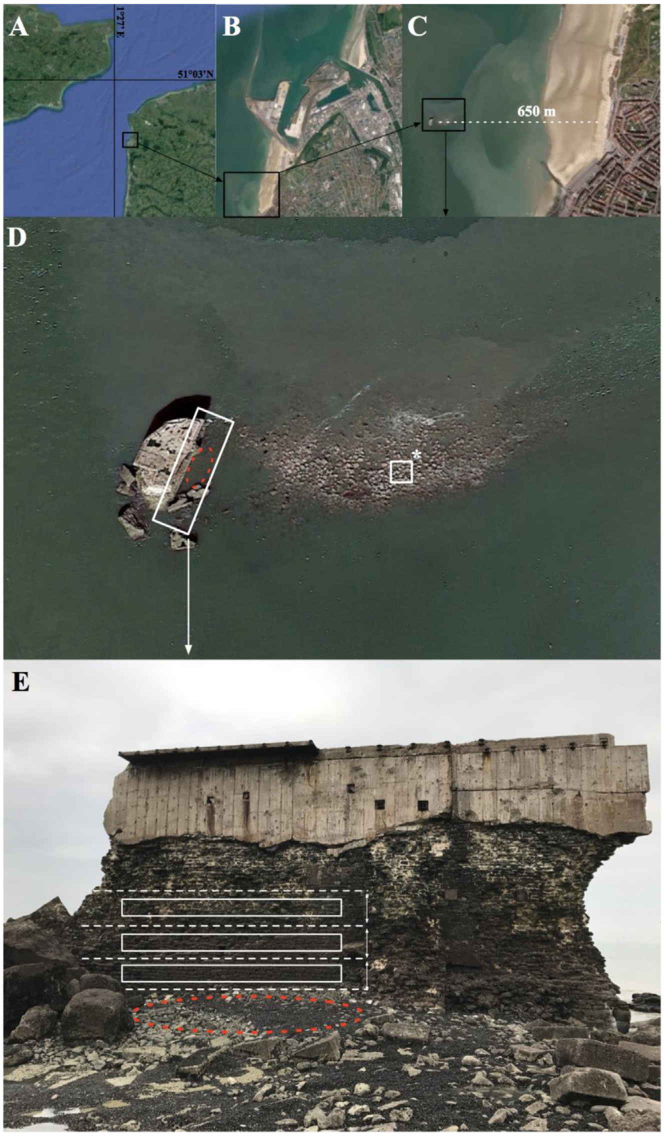

Fig. 1. The study area is located on the French coast of the eastern English Channel (a), south of Boulogne-sur-Mer (b). The study site, the Fort de l'Heurt, is situated 650 m away from the Mean High Water Spring on an intertidal rocky reef (c, d). Experiments were conducted on the landward wall of the Fort and the proximate horizontal reef (e). The dashed lines separate the three 2-m high tidal levels used in this work, the rectangles identify the sampling bands where *Littorina littorea* were sampled, and the double pointed arrows represent 2 m. The red dashed ovals identify the studied horizontal platform, and the asterisk the flat section of the reef where the transplanted control snails where sampled form.

2.2. Experimental design

The existence of tidal height and habitat partitioning in *L. littorea* was investigated using a combination of experiments involving (i) undislodged marked snails, (ii) snails that were dislodged, marked and replaced in the location they were taken from, (iii) translocated snails that were dislodged, marked and moved to another location in their habitat, and (iv) transplanted snails that were dislodged, marked and transplanted to a different habitat. These experiments have been specifically chosen to ensure that the effect of transplantation in a new habitat (here following a manual dislodgement) can be distinguished from the effects of disturbance and translocation; see Chapman (1986); Chapman and Underwood (1994a, b) and Chapman and Underwood (1992). The corresponding experimental procedures are detailed hereafter.

2.2.1. Control experiment

L. littorina tidal height and habitat preference was investigated through experiments conducted by marking in situ during the same low tide 4 groups of 40 undisturbed snails (i.e. '*control snails*') — one from the horizontal platform and 3 from each tidal level along 1.5-m wide sampling bands on the landward wall (Fig. 1E) — with acrylic nail polish of distinct bright colours to facilitate subsequent location in the field.

2.2.2. Disturbance experiment

During the same low tide, we also manually dislodged individual snails (i.e. '*disturbed snails*') from the horizontal platform ($N = 35$) and each tidal levels ($N = 40$ per level), subsequently marked them as done with the control snails, and replaced them in the exact position they were taken from.

2.2.3. Translocation and transplantation experiments

Four control groups of 100 snails were haphazardly collected from the horizontal platform and each tidal level, and returned to the laboratory for tagging. The shells of these individuals were coated with acrylic nail polish of distinct bright colours. After marking the snails, we allowed the nail polish to dry for three hours and then kept the snails in flowing seawater overnight before returning them to the field. Snails were judged unaffected by the handling, painting and drying process since they attached to the sides of the aquaria and crawled out of the seawater, as unmanipulated *L. littorea* do (L. Seuront, personal observation). This criterion is consistent with previous evidence that tagging does not affect the motion behaviour of gastropods (*e.g.*Chapman, 1986, 2000a, 2000b; Henry and Jarne, 2007; Seuront et al., 2007; Chapperon and Seuront, 2012).

During the next daytime low tide, 50 individuals of each group (i.e. '*translocated snails*') were transported in distinct aerated plastic containers to the Fort de l'Heurt, haphazardly placed back in their initial habitat (i.e. horizontal platform and tidal level on the seawall) and gently sprayed with *in situ* seawater to allow them to reattach with their foot on the substratum. During the subsequent high tide, the remaining 4 groups of 50 individuals (hereafter referred to as '*transplanted snails*') were mixed together in aerated plastic containers, brought back to the Fort de l'Heurt using a Stand-Up Paddle (Lokahi Aka Entry 10'6), and haphazardly dropped into the water over a 10 m × 10 m area facing the seawall and located directly above the horizontal platform. This procedure was used (i) to simulate snails being dislodged from their vertical substratum, with no opportunity for quick reattachment before falling into lower intertidal levels, and (ii) to guarantee that all snails were released in the same habitat, hence will experience similar stressors and cues.

Finally, because the horizontal platform is located below the low tidal level considered on the seawall (i.e. 2 metres and 3 to 5 metres above MLLW, respectively), we conducted an additional control experiment to ensure that the return rates observed on the seawall were driven by an actual preference for the seawall rather than by a tidal level preference. We sampled 50 *L. littorea* individuals (i.e. '*control transplanted snails*') from a nearby flat section of the reef surrounding the Fort de l'Heurt that is located 3.5 meters above MLLW (Fig. 1D). These snails were subsequently manipulated as described above before being released at high tide above the horizontal platform.

2.3. Behavioural experiments

Four groups of 50 snails were sampled from the horizontal platform and the three tidal levels, coated with acrylic nail polish of distinct bright colours and individually tagged with glue-on shellfish tags (4 × 2 mm, Hallprint, Hallprint Pty Ltd, Hindmarsh, South Australia). Each group of 50 individuals was subsequently randomly placed within a 25 × 25 cm area surrounding a release point haphazardly situated on the horizontal platform, sprayed with *in situ* seawater to allow them to reattach with their foot on the substratum, and left undisturbed from the release day. The position of marked snails was subsequently monitored during 8 consecutive low tides to assess whether the movement of dislodged individuals is directed or random.

2.4. Morphologic traits and dislodgement resistance

Because resistance to dislodgment may be habitat-related and driven by specific differences in shell size and morphology (Miller, 1974; Etter, 1988; Trussell et al., 1993; Trussell, 1997a, 1997b; Parsons, 1997a, 1997b), the height, width and length of all snail shells considered in the present work were systematically measured. We additionally measured the foot surface area of *L. littorea* for individuals ($n = 10$) sampled from the horizontal platform and the three tidal levels of the seawall. In the laboratory, snails were allowed to crawl over a glass plate in air (T = 22°C) until they became stationary and attached (typically after 60 to 75 s). The foot was then photographed (Nikon V1 mounted with a Nikkor 10–30 mm lens) from below, the foot area A (cm^2) measured using the software Image J (Schneider et al., 2012; Schindelin et al., 2015), and expressed as a function of shell length L (cm) following $A_i = a_i L_i^{b_i}$, where a_i and b_i are empirical parameters related to the snail habitat i (i.e. horizontal platform and the three tidal levels on the seawall).

2.5. Environmental variables

Air and water temperature, and wind speed were considered as environmental variables that are most likely to affect *L. littorea* navigation and tenacity as (i) *L. littorea* speed has been shown to significantly covary with temperature (Newell, 1958a, 1958b; Erlandsson and Kostylev, 1995) and (ii) the risk of dislodgment of intertidal organisms, including gastropods, increases with wave force, that is essentially driven by wind speed in coastal seas (Wright et al., 1999). Air and water temperatures were measured every 5 minutes using HOBO® Tidbit v2 Temperature Data Logger (Onset Computer Corporation, Bourne, MA, USA), that were glued on the horizontal platform and each tidal level on the seawall. Hourly wind speed recorded at the Boulogne-sur-Mer weather station were obtained from Météo-France.

2.6. Census

The three seawall tidal levels and the horizontal platform were surveyed at low tide 1, 2, 8, 17, 27, 37, 45, 57, 67, 78 and 90 days after the control, disturbed, translocated and transplanted snails were tagged. The censuses of each seawall level and the horizontal platform were implemented through a total of twenty person-minutes of searching per site per day (Miller et al., 2007). To avoid potential biases related to additional disturbances, snails were never manipulated during counts.

2.7. Behavioural analysis

To determine the nature of the daily individual displacements of *L. littorea*, we monitored the orientation angle α_t and the distance displaced d_t by each individual from the release point to the monitored snail position during 8 successive low tide, i.e. $t = 1$ to 8. The orientation angle α_t was estimated from the release point using the

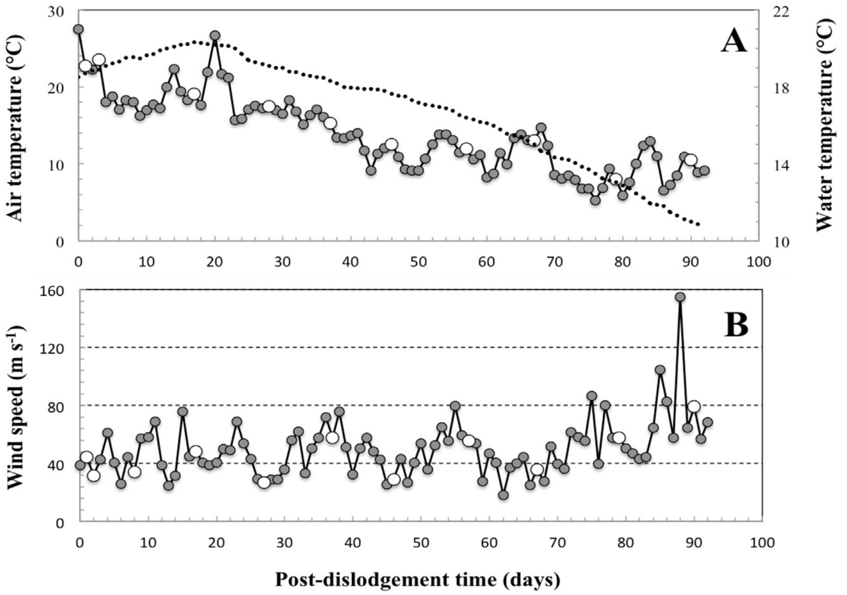

Fig. 2. Temporal patterns of (a) daily averaged air temperature (grey dots) and seawater temperature (•••), and (b) daily maximum wind speed observed over the course of our survey. The open symbols in (a) and (b) indicate the sampling days.

direction of the landward wall of the Fort de l'Heurt as a reference. The distance displaced d_t was measured by a plastic tape measure (resolution 0.1 cm) laid down the substratum topography from the release point to the snail's position. The (x_t,y_t) coordinates were subsequently estimated from the distance displaced d_t and orientation angle α_t as $x_t = d_t \cos\alpha_t$ and $y_t = d_t \sin\alpha_t$. The daily orientation angles α_i were subsequently defined as the angle taken by an individual between two successive low tides and calculated as $\alpha_i = 180 - \alpha_e(180/\pi)$, where $\alpha_e = \arccos(D_1^2 + D_2^2 + D_3^2)/(2D_1D_2)$ D_1, D_2 and D_3 are the distances between positions $(x_{t=1},y_{t=1})$ and $(x_{t=2},y_{t=2})$, $(x_{t=2},y_{t=2})$ and $(x_{t=3},y_{t=3})$, and $(x_{t=1},y_{t=1})$ and $(x_{t=3},y_{t=3})$ (Jerde and Visscher, 2005). The daily distance displaced d_i was estimated as the distance displaced by an individual between two successive low tides as $d_i = [(x_{t+1} - x_t)^2 + (y_{t+1} - y_t)^2]^{1/2}$. The net orientation α_{net} and distance d_{net} were finally estimated from the final position of the tagged snails, i.e. $\alpha_{\text{net}} = \alpha_{t=8}$ and $d_{\text{net}} = d_{t=8}$. We also quantified the level of activity of dislodged *L. littorea* individuals through using the ratio N_i/N, where N_i is the total number of mobile individuals between two successive low tides (i.e. the number of individuals that changed position between two successive low tides) and N is the total number of individuals.

2.8. Data analysis

Because *L. littorea* abundance, size and successive displacements were non-normally distributed (Kolmogorov-Smirnov test, $p < 0.05$), nonparametric statistics were used throughout. Multiple comparisons between sampling sites (i.e. three tidal levels on the seawall and the horizontal platform) and both net and daily displacements were conducted using the Kruskal-Wallis test (KW test hereafter), and a subsequent multiple comparison procedure based on the Tukey test was used to identify distinct groups of measurements (Zar, 2010). The Mardia's extension of the non-parametric Wheeler and Watson test (WW test hereafter; Zar (2010)) was used to infer (i) differences in the net orientation angles α_n for snails originating from different habitats, and (ii) temporal differences in the daily orientation angles α_i for snails originating from the same habitat. The distributions of daily orientation angles and net orientation angles were further compared to a uniform distribution using the Watson one-sample U^2 test (Zar, 2010). 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). Differences in the empirical parameters a_i and b_i of the allometric relationships $A_i = a_i L_i^{b_i}$ were respectively assessed on log-transformed data with a F test and an analysis of covariance which were respectively used to infer regression intercepts and slopes (Zar, 2010). It is finally stressed that because *L. littorina* individuals have never been observed forming aggregations or trail-following on our study site, we rule out the possibility of trail-following to occur subsequently to our transplantation experiment, hence we consider that *L. littorea* individuals were independent in their movements and each recapture has been considered an independent event.

3. Results

3.1. Littorina littorea abundance on the study site

At the beginning of our study, significant differences were found in the abundance of *L. littorea* between the seawall tidal levels and the horizontal platform (Kruskal-Wallis test, $p < 0.05$). No significant differences were, however, found in the abundance of *L. littorea* between the three tidal levels (Tukey test, $p > 0.05$). Seawall abundance, which ranged between 10 and 134 ind m^{-2} were significantly lower than on the horizontal platform (Tukey test, $p < 0.05$), where they reached up to 200 ind m^{-2} (69-202 ind m^{-2}).

3.2. Environmental conditions

Over the course of our 90 days experiment, air temperature ranged between 26.6°C and 8.9°C, and significantly decreased (Pearson test, $p < 0.01$) from August 24 to November 24. No significant difference in temperature where observed between the horizontal platform and the three tidal levels of the seawall (KW test, $p > 0.05$). Seawater temperature increased from 18.7 to 20.3°C over the first 16 days of our survey, and subsequently steadily decreased down to 11°C at the end of the survey (Fig. 2A). Daily averaged wind speed ranged from 1.0 to 7.2 m s^{-1}, with daily maximum ranging from 5 to 43 m s^{-1} (Fig. 2B). Note that the maximum averaged and instantaneous wind speeds were observed two days before the end of our experiments.

3.3. Morphologic traits

No significant differences were found in snail size between the

Table 1
Best nonlinear fits describing the temporal evolution of the recapture rates of control (R_c), disturbed (R_c), translocated ($R_{translocated}$) and transplanted ($R_{transplanted}$) *Littorina littorea* depending on their initial habitat, i.e. a horizontal platform 2 metres above the Mean Lower Low Water, a flat reef 3.5 metres above MLLW and the proximate vertical wall at tidal levels ranging from 3 to 5 metres (Level 1), 5 to 7 metres (Level 2) and 7 to 9 metres (Level 3) above MLLW. To assess whether the relationship between the recapture rates and time *t* was best fitted by a linear, power-law, logarithmic, or an exponential function, we used the theory of model selection based on Akaike's information criterion (AIC; Edwards, 2008).

Recapture rate	Habitat	Model	r^2
R_c	Platform	$Rc = -11.6\ln(t)+99.0$	0.99
	Level 1	$Rc = -2.2\ln(t)+99.9$	0.96
	Level 2	$Rc = -2.1\ln(t)+98.5$	0.96
	Level 3	$Rc = -2.4\ln(t)+99.2$	0.95
R_d	Platform	$Rc = -11.9\ln(t)+98.6$	0.99
	Level 1	$Rc = -2.5\ln(t)+98.7$	0.98
	Level 2	$Rc = -2.4\ln(t)+99.2$	0.96
	Level 3	$Rc = -2.6\ln(t)+97.7$	0.95
$R_{translocated}$	Platform	$Rc = -12.1\ln(t)+97.8$	0.98
	Level 1	$R_{translocated} = -2.7\ln(t)+100.7$	0.87
	Level 2	$R_{2translocated} = -2.5\ln(t)+97.9$	0.96
	Level 3	$R_{translocated} = -2.5\ln(t)+99.9$	0.95
$R_{transplanted}$	Platform	$R_{transplanted} = -12.8\ln(t)+98.0$	0.99
	Flat reef	$R_{transplanted} = -13.1\ln(t)+97.8$	0.98
	Level 1	$R_{transplanted} = 78.9e^{-0.02t}$	0.99
	Level 2	$R_{transplanted} = 81.1e^{-0.027t}$	0.99
	Level 3	$R_{transplanted} = 92.4e^{-0.027t}$	0.99

seawall tidal levels and the horizontal platform (KW test, $p > 0.05$), with *L. littorea* consistently ranging in length between 11.2 and 17.8 mm. *L. littorea* foot area A_i did not significantly differ between habitats (KW test, $p > 0.05$), with A ranging from 0.9 to 1.3 cm^2. More specifically, A_i consistently significantly ($p < 0.05$) scaled as a power function of shell length L_i, *i.e.* $A_i = a_i L_i^{b_i}$, irrespective of the sample location. No significant differences were found between the empirical parameters a_i (*F*-test, $p > 0.05$) or b_i (analysis of covariance, $p > 0.05$) for snails originating from the horizontal platform and from the seawall three tidal heights; overall, foot area scales as a function of shell length as $A = 0.12L^{0.84}$.

3.4. Control, disturbance and translocation experiments

The recapture rates of control snails (i.e. undisturbed snails marked in situ), disturbed snails (i.e. snails manually dislodged, marked in situ and replaced to their initial position) and translocated snails (i.e. snails manually dislodged, marked and moved to another location in their habitat) on the horizontal platform significantly ($p < 0.01$) decayed logarithmically with time *t* (Table 1). The decay typically occurred from 98% to 42% respectively 1 and 78 days after snails were marked, and an additional drop to 25-21% consistently occurred after 90 days (Fig. 3A). These rates were non-significantly different from each other (*F*-test, $p > 0.05$). On the seawall, the recapture rates of control, disturbed and translocated snails also decayed logarithmically with time *t* (Table 1), from 100 to 90%, 98 to 88% and 99 to 88% at tidal levels 1, 2 and 3 respectively over the first 78 days of the experiment (Fig. 3B). These rates did not significantly differ between tidal levels (*F*-test, $p > 0.05$), but were significantly slower than on the horizontal platform (*F*-test, $p < 0.01$). After 90 days, recapture rates drastically dropped to 44, 48 and 46% at tidal levels 1, 2 and 3, respectively (Fig. 3B). Note that the decrease in recapture rates observed both on the platform and the seawall is likely to be related to the massive storm that hit the French shores of the eastern English Channel from November 17 till November 20 (Fig. 2B). Noticeably, snails originating from different tidal levels were never observed outside of their original tidal level over the course of the experiment.

3.5. Transplantation experiments

The recapture rates of snails transplanted from the horizontal platform (i.e. snails sampled from the horizontal platform, tagged and released on the horizontal platform at high tide) and control transplanted snails (i.e. snails sampled from an elevated flat section of the reef, tagged and released on the horizontal platform at high tide) both significantly decayed logarithmically with time *t* (Table 1) from 96% to 42% respectively 1 and 78 days after being released over the horizontal platform, and an additional drop to 20% occurred after 90 days (Fig. 3C). These rates did not significantly differ from each other (*F*-test, $p > 0.05$), and were not significantly different from the recapture rates observed for control, disturbed and translocated snails (Fig. 3A, Table 1).

The recapture rate of snails transplanted from the seawall to the horizontal platform decayed exponentially, irrespective of the tidal level they were sampled from (Fig. 3D, Table 1). More specifically, these rates decayed from 82 to 12%, 84 to 7%, and 88 to 7% over the course of the 90 days survey for snails originating from tidal levels 1, 2 and 3, respectively. No significant differences were found between the 3 groups of snails in the decay of their recapture rates (*F*-test, $p > 0.05$). These rates decayed highly significantly faster (*F*-test, $p < 0.01$) than the recapture rate of snails transplanted from the horizontal platform and the flat section of the reef (Table 1).

Experimentally dislodged *L. littorea* concomitantly returned to the seawall at rates increasing from 8 to 54%, 6 to 58% and 4 to 54% respectively for snails originating from tidal levels 1, 2 and 3 (Fig. 4); these rates were best described by a logarithmic increase (Table 1), which did not significantly differ with the origin of the snails (*F*-test, $p > 0.05$). Note the decrease in return rates from 54 to 40%, 58 to 42% and 54 to 43% observed 90 days after dislodgment (Fig. 4). Noticeably, transplanted snails originating from the horizontal platform and the flat section of the reef were only rarely (0 to 6%) found on the seawall, and when present they were typically observed over the first few centimetres of the lowest limit of the tidal level 1. These observations suggests that the return rates observed on the seawall were habitat-driven (i.e. snails originating from the seawall tend to return to the seawall), and not driven by a tidal height preference. In the latter case, the snails originating from the flat section of the reef located 3.5 metres above MLLW and transplanted on a horizontal platform located 2 metres above MLLW should have exhibited a tendency to return to a similar tidal height on the seawall. Though the temporal patterns of the overall return rates of *L. littorea* on the seawall did not significantly differ between the experimental groups of snails (Fig. 4), these rates were clearly dependent from the tidal level they were dislodged from (Fig. 5).

Snails dislodged from the low tidal level (Fig. 5C) essentially returned to their tidal level, with a maximum return rate (48%) reached 45 days after dislodgement, which remained stable, until it dropped down to 26% at the end of the survey. This pattern indicates that these snails progressively come back to the tidal level they originate from. The plateau observed from 45 to 78 days after dislodgment further suggests that the return rates of these snails compensate the natural losses occurring over this period, which typically range from 0.1 to 0.3 snails per day (see Fig. 3A). Some of these snails marginally (2 to 6%) reached the intermediate tidal level 17 days after dislodgement.

L. littorea dislodged from the intermediate tidal level first returned to the low tidal level until they reached a maximum (36%) 27 days after dislodgement, and subsequently decayed down to 8% at the end of the survey. The proportion of snails reaching the intermediate tidal level consistently increased from 4% eight days after dislodgement to 40% seventy-eight days after dislodgement (Fig. 5B).

Finally, the return rate of snails dislodged from the high tidal level exhibited a more complex temporal pattern (Fig. 5A), that indicates a continuous upward movement of these snails though low and intermediate tidal levels toward the high tidal level. Specifically, dislodged

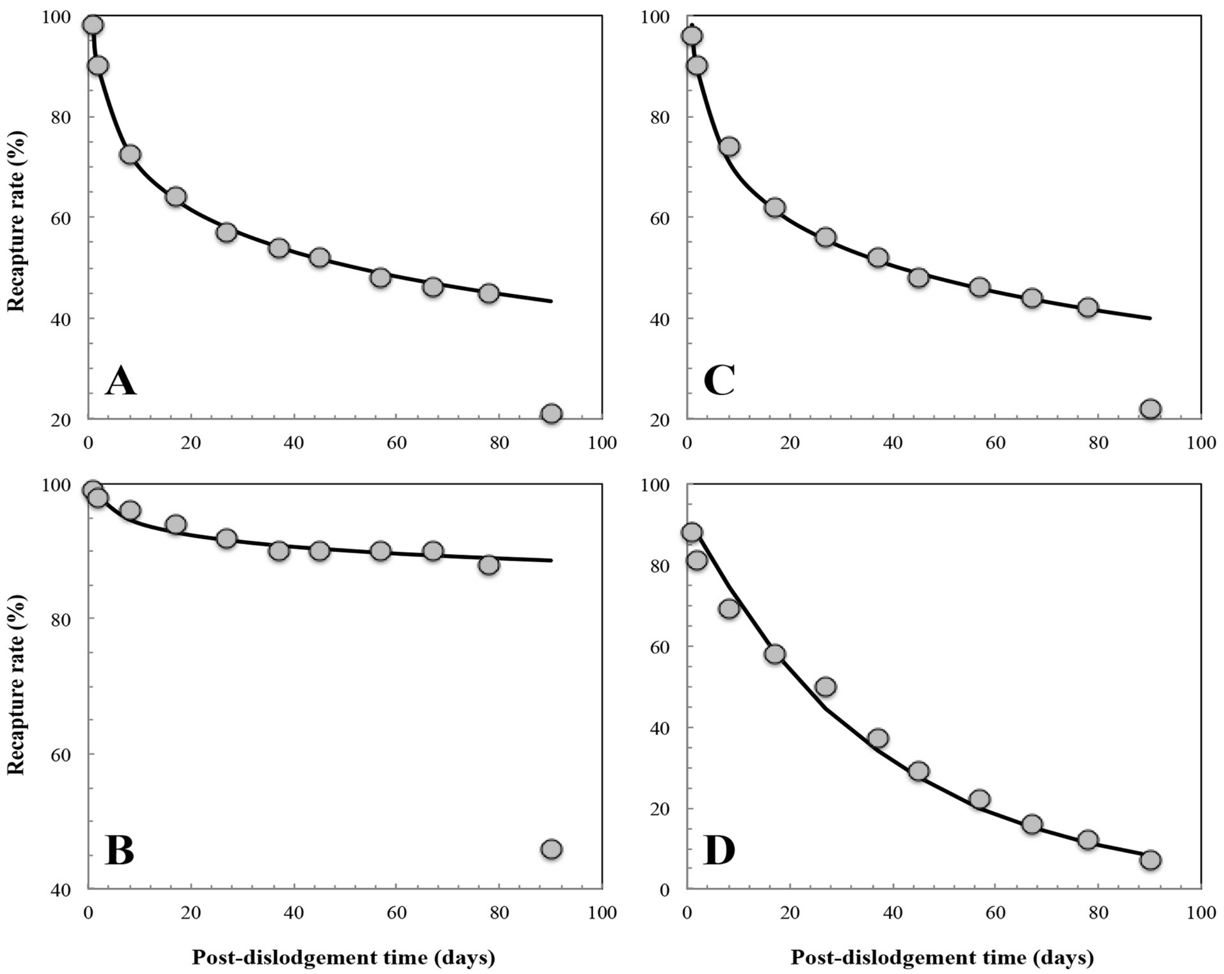

Fig. 3. Recapture rate (%) of *Littorina littorea* on the horizontal platform of the Fort de l'Heurt after being (a, b) translocated (i.e. manually dislodged, marked and moved to another location in their habitat) and (c, d) transplanted (i.e. manually dislodged, marked and moved to a different habitat), shown for snails sampled from the horizontal rocky platform (a, c) and the (b, d) the high tidal level on the Fort de l'Heurt.

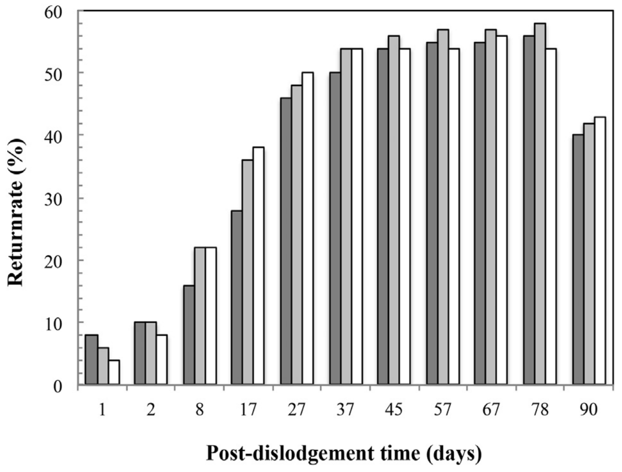

Fig. 4. Return rates of *Littorina littorea* dislodged from low (dark grey), intermediate (light grey) and high (white) tidal levels of a vertical wall that returned to the vertical wall, irrespective of original tidal height.

L. littorea reached a maximum of 26% twenty-seven days after dislodgement, and consistently decreased afterwards. In turn, these snails reached the intermediate and high tidal levels respectively 8 and 17 days after dislodgement. Their return rate reached a maximum on the intermediate tidal level (16%) 32 days after dislodgement and steadily decreased afterward down to 6% at the end of the experiment. The return rate observed on the high tidal level kept increasing up to 32% seventy-eight days after dislodgement. Note that the sharp decrease in return rates observed 90 days after dislodgement similarly affects the three experimental groups of dislodged snails, with a 2- to 3-fold decay in return rates (Fig. 5).

3.6. Motion behaviour of dislodged L. littorea

The number n_i of individually tagged *L. littorea* that were recaptured on the horizontal platform over our 8 successive low tide surveys was consistently high (i.e. 86 to 96%) for individuals originating from the low, intermediate and high tidal levels of the seawall, with n_1, n_2 and n_3 ranging respectively between 47 and 44, 48 and 45, and 48 and 43. In contrast, the number N of individuals originating from the horizontal platform significantly decreased with time t ($r^2 = 0.99$, $p < 0.01$) from 50 to 38 as $N = 51.5 - 1.7t$. Note that in contrast to previous reports (Chapperon and Seuront, 2012), no individual was ever observed at the exact same location between two successive low tides, nor between any low tides over our 8-days survey. This resulted in an activity index of 1 for all the *L. littorea* considered in this work, irrespective of their habitat (platform or seawall) or tidal height.

The daily orientation angles α_i followed by *L. littorea* originating from the horizontal platform did not significantly differ between low tides (WW test, $p > 0.05$), and were consistently best fitted by a uniform distribution (U^2 test, $p > 0.05$; Fig. 6). In contrast, α_i followed by

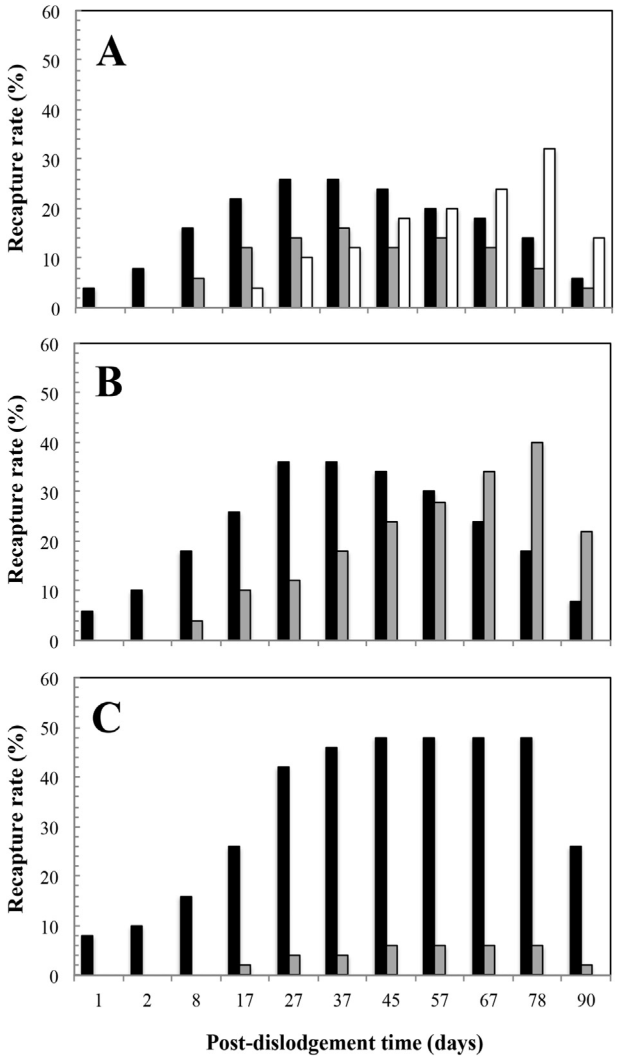

Fig. 5. Percentage of *Littorina littorea* dislodged from (a) high, (b) intermediate and (c) low tidal levels of the Fort de l'Heurt that returned to low (black bars), intermediate (dark grey bars) and high (light grey bars) tidal levels.

L. littorea originating from the seawall were highly directional (Fig. 7) and consistently significantly diverged from a uniform distribution (U^2 test, $p < 0.01$), irrespective of the tidal height considered. The daily distances d_i did not significantly differ between successive low tides (KW test, $p < 0.05$), for both *L. littorea* originating from the horizontal platform (Fig. 6) and the seawall (Fig. 7). These distances ranged from 23 to 85 cm for *L. littorea* originating from the horizontal platform, and from 16 to 212 cm for snails originating from the seawall. These figures resulted in daily displacement rates of 49 cm and 75 cm for *L. littorea* originating from the horizontal platform and the seawall, respectively. The net orientation angles α_{net} were uniformly distributed (U^2 test, $p > 0.05$) for *L. littorea* originating from the horizontal platform (Fig. 6H). In contrast, *L. littorea* individuals originating from the vertical wall were highly directional, and consistently travelled preferentially towards the seawall (Fig. 7H).

Significant differences were found in the daily distances d_i between the four groups of *L. littorea* (KW test, $p < 0.05$). The distances d_i did not significantly differ between the three groups of snails originating from the seawall (Tukey test, $p > 0.05$), and ranged between 0.6 and 2.1 metres. These distances were significantly greater (Tukey test, $p < 0.05$) than those travelled by snails originating from the horizontal platform (d_i ranged between 0.3 and 0.9 metres). No significant autocorrelation in d_i was found between successive low tides ($p > 0.05$).

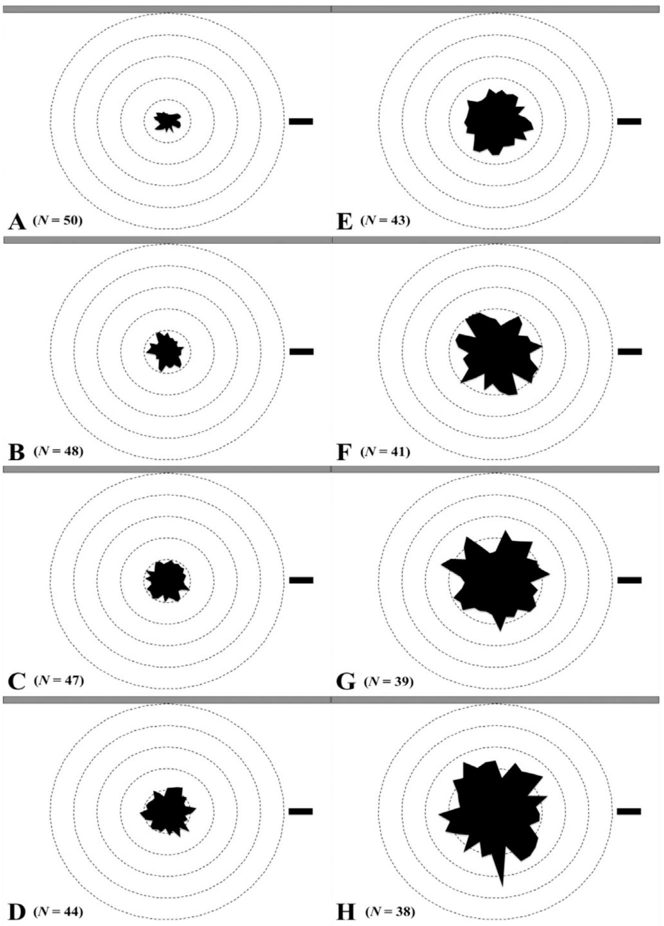

Fig. 6. Locations of *L. littorea* originating from the horizontal platform, subsequently individually tagged ($n = 50$), released on the platform and resampled (n) daily during 8 successive low tides (a to h). At each date, the polygon joins the N snails that were found on the platform. The black scale bar represents 2 metres, and the grey bars the landward seawall of the Fort de l'Heurt. Note that the distribution of angles and distances shown in the final distribution (h) are the net orientation α_{net} and distance d_{net}.

Finally, the net distance d_{net} significantly differed between the four groups of snails (KW test, $p < 0.05$). Specifically, d_{net} did not significantly differ between individuals from different tidal heights (Tukey test, $p > 0.05$), and ranged from 7.1 to 11.8 metres. These distances were highly significantly greater (Tukey test, $p < 0.01$) than those observed for individuals originating from the horizontal platform, which ranged between 3 to 7 metres.

4. Discussion

This work investigated the return rates of *L. littorea* individuals over a 90-day period after they were manually dislodged from proximate horizontal and vertical habitats. Specifically, our results show that *L. littorea* exhibit both habitat selection and tidal height partitioning, as undisturbed, disturbed, translocated and transplanted individuals show a clear preference for both the habitat (*i.e.* horizontal or vertical surfaces) and the tidal level they originate from. In addition, the high frequency resampling of individually tagged dislodged *L. littorea* during the first 8 successive low tides of our survey indicates that individuals originating from the seawall consistently showed higher movement rates and directionality towards the seawall, while individuals originating from the platform showed no directionality and reduced migration rate. It is acknowledged that this study suffers from what may be seen as a complete lack of spatial replication. Our conclusions would

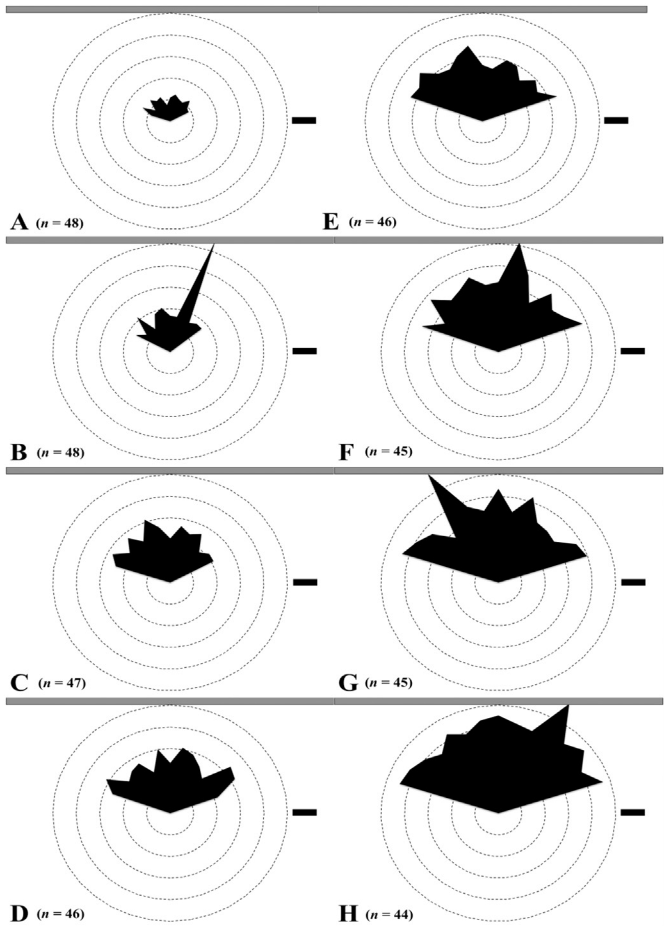

Fig. 7. Locations of *L. littorea* originating from the high tidal level of the seawall, subsequently individually tagged ($n = 50$), released on the horizontal platform and resampled (N) daily during 8 successive low tides (a to h). At each date, the polygon joins the N snails that were found on the platform. The black scale bar represents 2 metres, and the grey bars the landward seawall of the Fort de l'Heurt. Note that the distribution of angles and distances shown in the final distribution (h) are the net orientation α_{net} and distance d_{net}.

have undeniably been stronger through site replication (i.e. different locations with rock platforms and seawalls) and using 'site' as a random factor in subsequent analyses. This limitation is, however, intrinsically driven by the unique nature of our study site, i.e. a seawall located on an isolated intertidal reef, and the absence of similar structures in the region that could have been used to replicate our experiments and assessed *e.g.* how the observed differences among tidal levels compare to differences among sites, regardless they are (or not) at different levels.

4.1. Tidal height and habitat partitioning in L. littorea

A significant fraction (88-90%) of *L. littorea* individuals manually dislodged from three distinct tidal heights on a seawall, tagged and subsequently placed back to their original location remained in their tidal height over our 3-month survey (Fig. 3B). These results suggest a vertical partitioning of a *L. littorea* population as a function of tidal height. This hypothesis is further supported by (i) the low capture rate (0-6%) of control snails originating from the horizontal platform on the seawall, (ii) the relatively high return rates (54 to 58%) of experimental individuals dislodged from the seawall to their vertical substratum within 45 days (Fig. 4), and (iii) the fact that snails dislodged from a given tidal level on the seawall showed a clear tendency to return to that specific level (Fig. 5). The absence of mixing between undisturbed, disturbed and translocated snails of distinct tidal height over a 3-month period, further suggests the temporal persistence of the observed pattern. This observation is consistent with previous work showing that *L. saxatilis* did not migrate out of their microhabitat, but if moved to another habitat they showed a tendency of returning to their intertidal zone (Erlandsson et al., 1998). Our results are also consistent with — and generalised both to several tidal levels and to longer time scales — previous work conducted on the high shore littorinid *Littorina keenae* that showed a high retention rate (92-100%) of marked control snails within 1m^2 high-shore sites over a 3-week period (Miller et al., 2007). More generally, our results are consistent with previous studies showing (i) movement up the shore for a range of intertidal gastropod species transplanted lower on the shore during low tide periods (Evans, 1961; Bock and Johnson, 1967; Gendron, 1977; Thain et al., 1985; Erlandsson et al., 1998), (ii) the ability of *L. littorea* to navigate back towards their original habitat following dislodgment from a localised rocky substrate to the surrounding tidal flat (Chapperon and Seuront, 2009), and (iii) more directionality and higher movement rates in *L. saxatilis* released at an alien shore level (Erlandsson et al., 1998).

The distinct temporal decays observed in the recapture rates of undisturbed, disturbed and translocated *L. littorina* originating from the horizontal platform and the seawall (Table 1, Fig. 3) may be an indication of the different processes at play in the behaviour of *L. littorea* in distinct habitats.

The slower decay of recapture rates on the seawall of undisturbed, disturbed and translocated snails may indicate a stronger attachment of *L. littorea* to vertical surfaces, hence a lower risk of dislodgment. While this hypothesis is not consistent with the lack of significant differences observed in shell morphology and foot surfaces for snails sampled on the horizontal platform and the seawall three tidal heights, it is supported by preliminary results suggesting a stronger tenacity of *L. littorea* on vertical than horizontal surfaces (L. Seuront, unpublished data). This observation is consistent with previous work showing that periwinkles out of water for longer periods use a stronger mucus attachment, which may result in greater recapture rates for snails, which are out of the water for longer periods (Smith and Morin, 2002). This observation also indicates that beyond the expression of traits that increase dislodgement resistance on exposed shores (e.g. longer, broader feet and greater tenacity; see Miller, 1974; Etter, 1988; Trussell et al., 1993; Trussell, 1997a, 1997b; Parsons, 1997a, 1997b), intertidal snails may also tend to express habitat-specific traits that modulate attachment strength depending on the horizontal or vertical nature of their substratum, but also on their tidal height.

Alternatively, the tidal height partitioning observed in *L. littorea* on the seawall suggests that snails attached to the seawall are likely to be spatially constrained in their movement by the limited size of their habitat. This hypothesis is consistent with both the high recapture rates over our 3-month survey and the slow decrease in the recapture rate of undisturbed, disturbed and translocated snails with time (typically between 100 and 88%), which indicates a persistent presence of *L. littorea* on the seawall. This is not the case, however, on the horizontal platform where movements are not limited spatially (i.e. horizontally). It is then likely that the stronger logarithmic decay of the recapture rate of undisturbed, disturbed and translocated snails on the horizontal platform (Fig. 3A) reflects faster movement rates of *L. littorea* on the horizontal platform than on the seawall. The exponential decay observed in the recapture rate of seawall snails transplanted on the horizontal platform (Fig. 3D) indicate, in turn, a faster diffusion *via* a directed movement towards the seawall. This observation is consistent with the behavioural observations conducted during 8 successive low tides on individually tagged individuals originating from the horizontal platform (Fig. 6) and the seawall (Fig. 7), which showed higher movement rates and directionality towards the seawall in the latter, and no directionality and lower movement rates in the former. Note that these behavioural observations are also consistent with both theoretical and experimental studies showing faster movement rates in directed diffusion than in pure diffusion; see *e.g.*Berg (1993) and Viswanathan et al. (2011).

Finally, it is stressed that one may argue that because there is no horizontal habitat on the mid- to high-shore levels, the nature of the study does not allow disentangling whether the snails are selecting a shore-level or a habitat. However, this contradicts (i) the lack of difference between the recapture rates of control transplanted snails (i.e. snails sampled from a flat section of the reef located 1.5 meters above the horizontal platform, tagged and released on the horizontal platform at high tide) and the return rates of control, disturbed, transplanted and translocated snails (Fig. 3A, Table 1) and (ii) the fact that control transplanted snails did not show any tendency to climb the seawall. These results indicate that the return rates observed on the seawall were driven by an actual preference for the seawall rather than by a tidal level preference. This more generally suggests that dislodged *L. littorea* may be more prone to navigate back to their initial habitat (i.e. a vertical or a horizontal substrate) than their initial tidal height in an alien habitat. Tidal height partitioning may also be habitat-specific and/or occur on shorter time scales on vertical substrates that on horizontal ones as suggested by the widely reported ontogenetic and seasonal preferences in shore height (Underwood, 1977, 1979; Gendron, 1977; Hamilton, 1978; Petraitis, 1982; Takada, 1996; Warner, 2001; Bishop et al., 2007). Further work is, however, needed to disentangle what may be a complex relationship between habitat and tidal level in the ecology of intertidal gastropods through e.g. the translocation of snails from different tidal levels of a vertical wall to a ‘horizontal’ reef offering the same range of tidal height.

4.2. On the horizontal navigational abilities of *L. littorea*

More fundamentally, our results indicate that *L. littorea* dislodged from a seawall have the ability to navigate back to that surface, and subsequently to return to their original vertical position. These observations are consistent with previous work on *L. littorea* short-term migratory behaviour (Gendron, 1977) and imply that these snails can determine their relative position on the shore, and orient in the direction of their preferred habitat. This implies that *L. littorea* use a variety of directional cues to accomplish such directed movements. Numerous directional cues have been found in intertidal snails, including phototaxis (Charles, 1961; Evans, 1961; Warburton, 1973; Petraitis, 1982; Thain et al., 1985), geotaxis (Evans, 1961; Petraitis, 1982; Thain et al., 1985), chemotaxis towards both water-borne and air-born infochemicals from food sources and conspecifics (Fratini et al., 2001; Shearer and Atkinson, 2001; Fink et al., 2006; Chapperon and Seuront, 2009; Seuront and Spilmont, 2015), and rheotaxis, *i.e.* a movement either towards or away from the stimulus induced by a flow (Overholser, 1964; Neale, 1965; Crisp, 1969; Duch, 1971; Gendron, 1977).

Specifically, the horizontal platform where dislodged snails were released does not exhibit a significant slope towards the seawall, suggesting that navigation towards the seawall cannot be driven by geotaxis. Navigation towards the seawall may, however, be driven by phototaxis as littorinids have image-forming eyes with relatively greater resolving capabilities than many other molluscs (Newell, 1965; Seyer, 1992; Land and Nilsson, 2002), which may allow them to pick out the silhouette of the 10 metre high landward side of the Fort de l'Heurt rising above them. This hypothesis is consistent with empirical results conducted on a range of intertidal gastropods (including littorinids) showing oriented responses to plastic black walls in *Gibbula umbilicalis* (Thain et al., 1985), to shore-line topographic features and nocturnal silhouettes of vegetation in *Littorina punctata* (Evans, 1961), *Nerita textilis* (Chelazzi and Vannini, 1976) and *N. atramentosa* (L. Seuront, unpublished data) and to large areas of vegetation and individual plant stems in *Littorina irrorata* (Hamilton, 1977, 1978) and *Littoraria scabra* (Seuront, unpublished data). Note that these responses contrast with the oriented responses to the light (i.e. positive phototaxis) of the sun in *L. littorea* (Newell, 1958a, 1958b), *L. punctata* (Evans, 1961) and *Nerita plicata* (Warburton, 1973) and unidentified celestial objects in *Aplysia brasiliana* (Hamilton and Russell, 1982). As such if the navigation of *L. littorina* towards the Fort de l'Heurt relies on visual cues, the processes involved are *sensu stricto* more likely to be related to positive scototaxis (i.e. a movement towards dark targets; Alverdes, 1930) than negative phototaxis (i.e. a movement in the direction of decreasing light intensity). Though little is still know on the role of scototaxis in the development of complex oriented behaviour in intertidal gastropods, scototaxis has been shown to involve several derived behaviour in arthropod orientation behaviour (Lehrer, 1997) including perigrammotaxis (orientation towards contrasting edges), photohorotaxis (a tendendy to follow continuous contrasting edges) and hypsotaxis (attraction to the highest outline at the horizon) that may all be relevant to gastropods.

The infochemicals present on the seawall may also differ from those on the horizontal platform due to clear faunistic differences between the two habitats, hence generate a concentration gradient that snails can navigate to. In particular, the abundance of the blue mussel *Mytilus edulis* was significantly higher on the horizontal platform (6,225 ± 1,050 ind m^{-2}) than on the seawall (4,050 ± 725 ind m^{-2}), and both annelids and nematods reached densities that are up to 1 to 2 orders of magnitude greater on the platform than on the seawall (C. Hennion, unpublished data). Note that the infochemicals related to the presence of conspecific and epilithic biofilm can act synergistically in *L. littorea* horizontal migration (Chapperon and Seuront, 2009). The resolution of this specific issue warrants further work, as we did not attempt to assess the nature of the distribution (i.e. uniform vs. patchy), the concentration nor the specific composition of the epilithic biofilm present on the horizontal platform and the seawall.

The landward side of the Fort de l'Heurt lies in the wake of the main SSW swell direction that characterizes this area. As such, infochemicals are likely spread away from the rock through odour plumes (Zimmer and Butman, 2000) heading landward during incoming tide, hence creating a gradient of concentration towards the seawall. The potential turbulence dampening effect of the Fort de l'Heurt may also induce a rheotatic response in *L. littorea* as several species of gastropod have long been known to respond to unidirectional currents (*e.g.*Overholser, 1964; Neale, 1965; Crisp, 1969; Duch, 1971; Rochette et al., 1997). In addition, the presence of chemical cues have been shown to trigger and enhance positive rheotaxis (Seuffert and Martin, 2012), suggesting that odour cues from the seawall and the current pattern characterizing our study site may act synergistically in *L. littorea* navigation. Even in high Reynolds number flow, where diffusive odour gradients are eroded if not destroyed, a behavioural strategy referred to as odour-gated rheotaxis (Vickers, 2000; Weissburg, 2000) still allows an organism to find attractive odour sources. This strategy is particularly efficient for slow moving organisms, hence *L. littorea*, that follow relatively straight paths towards an odour source even in fast turbulent flows (Webster and Weissburg, 2009; Ferner and Weissburg, 2005). Specifically, a slower pace allows a temporally integrated chemosensation (typically through the klinotactic behaviour — i.e. a movement either towards or away from the stimulus induced by a physical contact — exhibited by gastropod in response to a source of stimulation; Audesirk and Audesirk, 1985; Chelazzi, 1990) that in turn might lead to some measurement of average odour concentrations, possibly creating circumstances when the animals could follow the time-averaged chemical gradients found in odour plumes (Ferner and Weissburg, 2005; Wilson and Weissburg, 2012; Wyeth and Willows, 2006; Wyeth et al., 2006). Further work is, however, needed to quantify the nature of the flow at our study site under various tide and swell conditions to infer the potential role of flow in *L. littorea* navigation.

4.3. On the vertical navigational abilities of *L. littorea*

Once they reached the seawall, *L. littorea* showed a clear preference for their original tidal level (Fig. 5). While this observation may suggest a negative geotactic response of *L. littorea* on vertical surfaces, it contradicts the results of our control transplantation experiments where snails from a flat section of the reef located 1.5 meters above the horizontal platform did not show any tendency to climb the seawall. The observed vertical partitioning (Fig. 3B) instead suggests that individuals migrating upward know when to stop once they reached their initial habitat. Each tidal level may then be characterized by specific airborne and/or waterborne infochemicals that *L. littorea* use in their navigation (Seuront and Spilmont, 2015). The three tidal levels considered in this work are also intrinsically characterized by different hydrostatic pressures at high tide. *L. littorea* may then use changes in hydrostatic pressure to adjust their vertical position on the seawall. This ability has been suggested to help *L. littorea* to navigate up and down the shore using the maximum and minimum pressures associated with shoreward and seaward currents, respectively (Gendron, 1977). However, to our knowledge, no evidence exists for this behaviour in either *L. littorea*, littorinids, or more generally in prosobranchs. Finally, as littorinids typically show some seasonality in their preferred shore height (Gendron, 1977; Hamilton, 1978; Petraitis, 1982; Warner, 2001), further work is needed to generalised the observed tidal height preference through the consideration of (i) the potential seasonal vertical migration of *L. littorea* on the studied seawall, and (ii) the seasonal fluctuations in the abundance and diversity of the fauna and flora characterizing each tidal level as they are likely to drive the nature and quantity of infochemicals available to navigation.

5. Conclusion

These results suggests that at relatively short time scales (i.e. days to months), *L. littorea* show both tidal height and habitat partitioning that is distinct from the range of movement patterns previously reported in littorinids over a range of temporal scales such as (i) ontogenetic migration (Underwood, 1977, 1979; Takada, 1996; Bishop et al., 2007), (ii) seasonality in preferred shore height (Gendron, 1977; Hamilton, 1978; Petraitis, 1982; Warner, 2001), (iii) migration to preferred shore height following dislodgment or transplantation (Evans, 1961; Bock and Johnson, 1967; Gendron, 1977; Thain et al., 1985; Miller et al., 2007), eventually in relation to different ecotypes (Rolán-Alvarez et al., 1997; Erlandsson et al., 1998), (iv) navigation back to a preferred resting site (Chapperon and Seuront, 2009), and (v) daily displacement rates related to tidal height, surface topography and food distribution of microhabitats, prey availability and shelter, and trail-following (Fairweather, 1988; Underwood and Chapman, 1989, 1992; Chapman, 1998, 2000a, 2000b; Byers, 2000; Chapman and Underwood, 1994a, 1994b; Seuront et al., 2007; Chapperon and Seuront, 2012). Additional factors such as parasitism are also known to affect the short-term movements of littorinids through either movements higher on the shore depending on the parasite present (Curtis, 1987; McCarthy et al., 2000) or reduced movement (Williams and Ellis, 1975; Miller and Poulin, 2001; O'Dwyer et al., 2014). Note, however, that this is unlikely to have affected our results as preliminary work conducted on our study site showed that *L. littorea* did not exhibit either patent or non-patent parasitic infections, as no swimming cercariae were found in 100-ml seawater jars that held individual snails overnight and no parasites were microscopically observed in dissected visceral mass tissue (Seuront and Spilmont, 2015). The potential effect of parasitism on *L. littorea* movements nevertheless remains an issue that needs to be considered with caution in any behavioural study of this species.

More fundamentally, our results unambiguously show that *L. littorea* has a strong ability to navigate back to their original habitat and tidal height following dislodgment. It is nevertheless stressed that despite decades of investigations of littorinid sensory abilities little is still known on the actual mechanisms involved, which is in deep contrast with the state of knowledge related to the sensory ecology of other invertebrates, especially crustaceans (Vickers, 2000; Webster and Weissburg 2001; Ferner and Weissburg, 2005; Wyeth and Willows, 2006; Wyeth et al., 2006; Wilson and Weissburg, 2012). The relative paucity of information available on the mechanisms driving littorinid navigation in the literature and the strong evidence provided in this work of the ability of *L. litorrea* to navigate back to a preferred habitat and/or tidal height warrants further work to decipher the relative contribution of various cues and the related taxes to the field of littorinid sensory ecology. Our journey to understand intertidal ecosystems through the lens of gastropod chemical ecology may hence still be in its infancy.

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