

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

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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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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 (Chappon 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 were subsequently assembled using Time Lapse Tool (©AI 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 $d_{\text{max}} = W/2$, where W is the width of the aquarium. Movement speed and direction were respectively calculated as $v = d_{\text{max}}/t_{\text{active}}$. 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 $d_{\text{max}} = W/2$ ($x_{W/2}, y_{W/2}$). 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

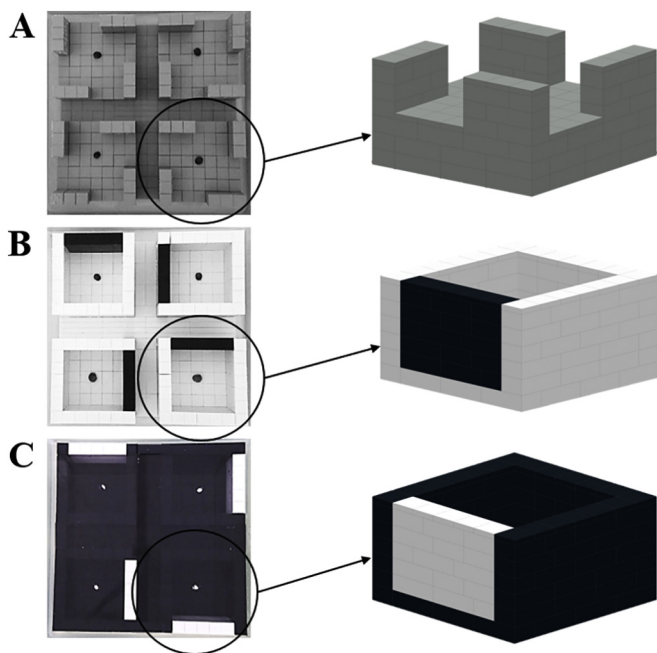


Fig. 1. Illustration of the LEGO-based structures designed to assess (A) the presence of geotaxis and thigmotaxis in *Littorina littorea* and the presence of scototaxis in *L. littorea* based on experiments run on four subsections made of (B) 3 white walls and 1 target black wall (W_3B_1) or (C) 3 black 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-repartition 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).

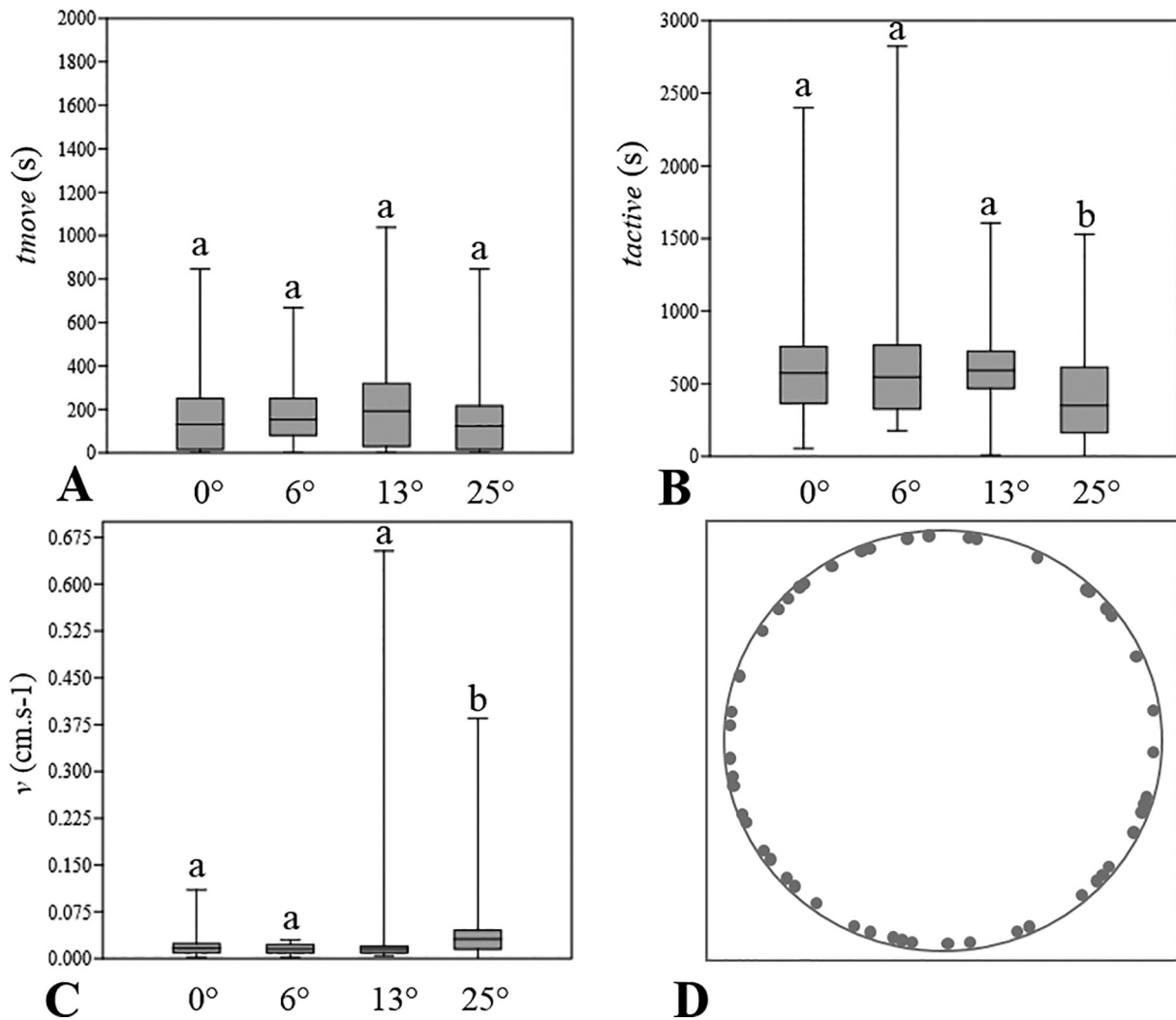


Fig. 2. Behavioural measurement of *Littorina littorea* for the different treatments in the geotactic experiment, (A) time to resume movement (t_{move}), (B) time of activity (t_{active}), (C) movement speed (v) and (D) the net orientation travelled by *L. littorea* for the experiment tilted to 6°. The 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

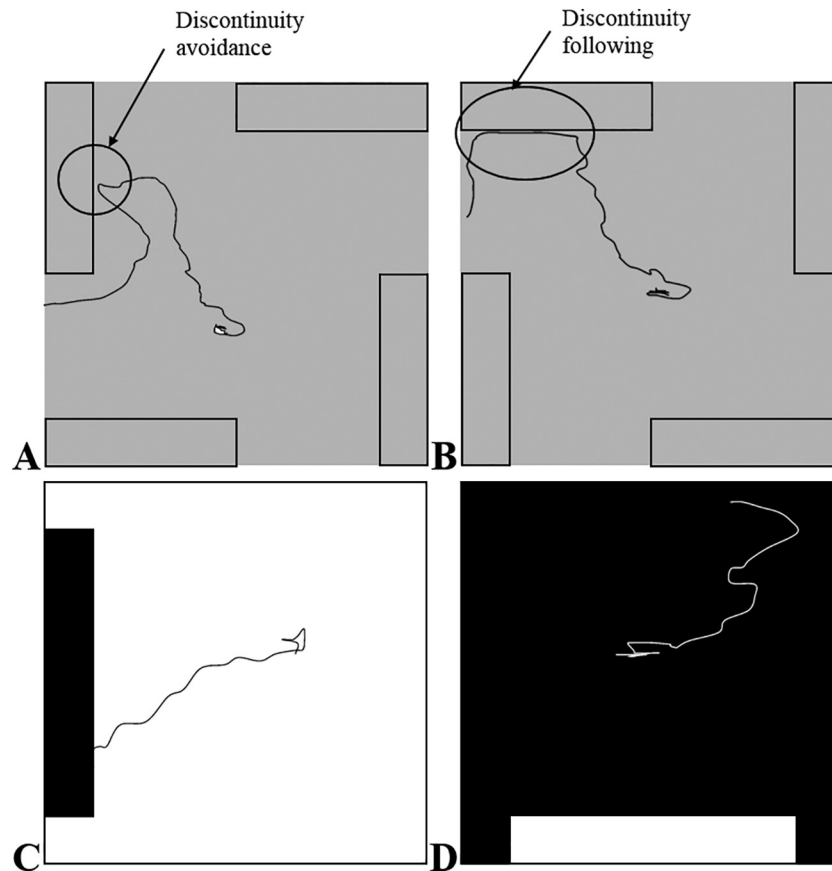


Fig. 3. Illustrations of representative tracks from our thigmotactic assay (A) representing the discontinuity avoidance behaviour, (B) the discontinuity following behaviour, and from our scototaxis assay (C) W_3B_1 assay and (D) B_3W_1 assay.

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).

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

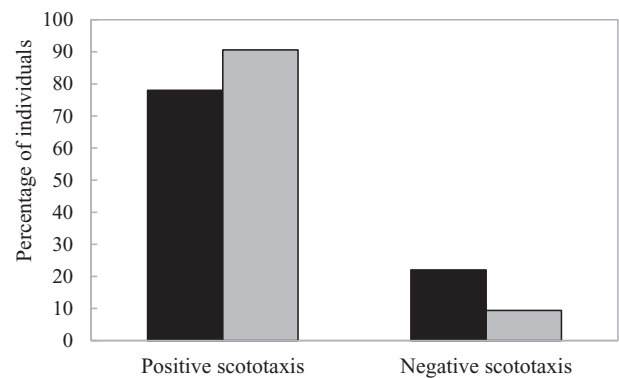


Fig. 4. Frequency of occurrence of target-based positive and negative scototaxis in *Littorina littorea*. The black and grey bars correspond to the version of the experimental set-up respectively including 3 white walls and a black one (W_3B_1) and 3 black walls and a white one (B_3W_1).

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 (Moizez, 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 thought 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 affected 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. Moizez conducted the experiments, ran the statistical analyses under the supervision of L. Seuront. E. Moizez and L. Seuront wrote the manuscript.

Declaration of Competing Interest

The authors have no conflict of interest.

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