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

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

40

41 Keywords:

42 Behaviour – Gastropods – *Littorina littorea* – Geotaxis – Scototaxis – Thigmotaxis

43

44 1. Introduction

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

59 A considerable amount of attention has been devoted to understand the role of
60 chemoreception in driving the trail-following behaviour involved in the homing and
61 aggregation behaviour of intertidal gastropods, and the localization of sexual partners, preys
62 and predators, and preferential habitats (Croll, 1983; Chapperon and Seuront, 2009, 2011a, b;
63 Ng et al., 2013). There is still, in turn, a limited amount of information related to their sensory
64 ecology and their subsequent ability to navigate through topographically complex landscapes,
65 despite the documented role of substrate spatial structure in the movement rates and
66 directionality of gastropods (Evans, 1961; Emson and Faller-Fritsch, 1976; Underwood, 1977;
67 Petraitis, 1982; Underwood and Chapman, 1985; Jones and Boulding, 1999; Fraser et al., 2014,
68 2015). Responses to gravity and visual cues have nevertheless been documented in a range of

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

82 These contradictory observations suggest, however, the presence of both positive phototaxis
83 (*i.e.* the orientation and movement toward a source of light) and scototaxis (*i.e.* a natural
84 preference for dark areas) in intertidal snails. Despite the acknowledged role of topographic
85 features, such as boulders and pebbles, crevices, cavities and pits in the ecology of intertidal
86 ecosystems (Little et al., 1998) and early suggestions on the existence of a kinaesthetic memory
87 in limpets, which determine their location based on previous movements and local topography
88 (Pieron, 1909), the role of topographic complexity and the related alternation of shaded and
89 unshaded areas on the motion behaviour of intertidal gastropod is still unknown. In particular,
90 no evidence exists for thigmotaxis (*i.e.* movement towards or away from the stimulus induced
91 by a physical contact) in intertidal gastropods.

92 In the present study, we chose the intertidal herbivorous gastropod, *Littorina littorea*, as a
93 model species due to its abundance on most Western and Northern European coasts where it

94 can reach densities up to hundreds of individuals per metre square (Petraitis, 1982; Bertness,
95 1999; Seuront et al., 2007), and its role in controlling algal growth (Stafford and Davies, 2005),
96 sediment dynamics (Kamimura and Tsuchiya, 2006) and the recruitment of both algae and
97 invertebrates (Buschbaum, 2000; Lotze and Worm, 2002). The net distance travelled by *L.*
98 *littorea* between two low tides in its rocky shore habitats ranged from 1 cm to 10-15 metres
99 (Seuront et al., 2007). Over this range of scales, *L. littorea* is typically exposed to a broad range
100 of topographic features, suggesting that it may have adapted its senses to navigate in such a
101 complex environments. In this regard, we aimed to elucidate the behavioural response of *L.*
102 *littorea* to substrate slope, topographic discontinuities and light using purpose-designed
103 experimental set-ups under laboratory-controlled conditions. Specifically, we assessed the
104 presence of negative geotaxis in *L. littorea*, and we further tested the alternative hypothesis that
105 geotaxis may either be positive or negative following the encounter with a topographic
106 discontinuity. We also assessed the behavioural response of *L. littorea* to light using two distinct
107 though complementary approaches based on the ability to actively (i) choose between a dark
108 and a light area and (ii) orient towards a dark shape. If *L. littorea* demonstrate such behavioural
109 adaptations to structurally complex environments, the behavioural repertoire and resilience of
110 this keystone species may respectively be much wider and stronger than previously thought.

111

112 **2. Methods**

113 *2.1 Collection and Acclimation of L. littorea*

114 *Littorina littorea* individuals were collected from the Fort de Croy (Wimereux, France;
115 50°45'48"N, 1°35'59"E) an intertidal reef typical of the rocky habitats found along the French
116 coasts of the eastern English Channel (Chappon and Seuront, 2009; Seuront and Spilmont,
117 2015; Spilmont et al., 2016). Before any experiment took place, *L. littorea* individuals (10 to
118 15 mm in length) were acclimatized for 24 h in the laboratory in acrylic glass (*i.e.* polymethyl

119 methacrylate, PMMA) cylinders (50 cm tall and 20 cm in inner diameter, riddle with holes 5
120 mm in diameter) held in 120-l (90 × 50 × 30 cm) tanks of running natural seawater, aerated at
121 temperatures representative of *in situ* conditions. These perforated ‘acclimation towers’
122 (Seuront and Spilmont, 2015) allow both seawater to be continuously renewed and captive
123 snails to move freely in and out of the water without being able to escape. No food was available
124 during the acclimation period. Each individual was only use once. Between each trial the
125 behavioural set-up was rinsed with 70% ethanol and seawater to remove mucus cues
126 (Erlandsson and Kostylev, 1995). In each of the behavioural assays described hereafter, the
127 motion behaviour of *L. littorea* individuals was recorded every 5 seconds during 120 minutes
128 using a Raspberry Pi NOiR camera overlooking the experimental set-up and operated through
129 a Raspberry computer under homogenous dim light conditions (*i.e.* 168 lux) measured with a
130 digital lightmeter (Extech Instruments, 403125). The resulting 1480 images where subsequently
131 assembled using Time Lapse Tool (©AI Devs) before behavioural analyses took place.

132

133 2.2 Geotaxis Assay

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

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

151

152 2.3 Thigmotaxis Assay

153 The response of *Littorina littorea* to topographic discontinuities was studied at a scale
154 pertinent to individual snails through the development of a LEGO-based behavioural apparatus.
155 More fundamentally, the experimental set-up was designed to assess if the previously reported
156 observations of snail moving up upon encountering the wall of an aquarium (Kanda, 1916;
157 Petraitis, 1982) were actually an evidence of negative geotaxis, and not an idiosyncratic artefact
158 due to the impossibility for the snails to move downward. Our apparatus was specifically
159 designed to associate the encounter of a discontinuity (*i.e.* a surface-to-wall transition or a
160 surface-to-drop-off transition) with the possibility of moving upward or downward. Note that
161 the height of the walls is identical to the height of the drop-offs, *i.e.* 3 cm. The apparatus was
162 uniformly made of light grey LEGO ® Bricks (Fig. 1A), and included four replicate cubic
163 structures (length: 11 cm, height: 6 cm), constituted of vertical walls where snail can move up
164 or down, that were built on a LEGO plaque (25.5×25.5 cm) glued on the bottom of a cubic
165 glass aquarium and immersed in 10 cm of seawater. This design was specifically chosen to offer
166 the same amount of discontinuities leading to upward and downward surfaces to *L. littorea*
167 individuals.

168 One snail was used in each replicate structure and 10 replicates were done ($N = 40$). *L.*
169 *littorea* behaviour was dichotomized in four behavioural displays following the encounter with
170 a discontinuity: (i) discontinuity avoidance (*i.e.* the behaviour by which a snail actively avoids
171 a discontinuity upon contact, through a drastic change in direction, without following it; Fig.
172 3A), (ii) discontinuity following (*i.e.* the behaviour by which a snail follows a discontinuity at
173 a distance less than one bodylength; Fig. 3B), (iii) moving upward, and (iv) moving downward.
174 The time allocated to each behaviour was noted for each individual and the frequency of
175 occurrence of the four behaviours was subsequently calculated. The total time spent in our
176 LEGO apparatus (t_{total}) and the time of activity (t_{active}) were recorded for each snail as well as
177 the time of initial scanning behaviour (t_{scan} ; *i.e.* non-oriented movements of a snail spinning
178 around its vertical axis before heading into a specific direction), the time to resume movement
179 and the activity index. A behavioural complexity index I was also calculated for each snail as I
180 $= N_i \times f_{active}$, where N_i is the total number of observed behaviours and f_{active} the percentage of
181 activity.

182

183 2.4 Scototaxis Assay

184 Two distinct but complementary approaches were used to infer the presence of scototaxis in
185 *Littorina littorea*. First, we used a binary choice assay conducted in a PVC container (length:
186 51 cm, width: 11 cm, depth: 20 cm) half black and half white with two removable vertical
187 panels used to isolate a central area from the rest of the apparatus. Five *Littorina littorea* were
188 placed in circle at the centre of the container filled with 10 cm of seawater. To enhance the
189 visibility of individuals when they crawled on the black section, they were marked with white
190 nail polish. After marking the snails, the nail polish was allowed to dry for 30 minutes and the
191 snails were kept in flowing seawater aquaria until the next day (Seuront et al., 2018a). The
192 experiment was repeated four times ($N = 20$). Control experiments were run in totally black and

193 totally white version of our experimental container to ensure no bias intrinsic to the
194 experimental set-up was present. The parameters considered for the behavioural analysis were
195 the position of each snail after two hours of experimentation, the proportion of individuals that
196 left the aquaria and the total time (t_{total}) spend in each section. This time was decomposed into
197 activity (t_{active}) and inactivity ($t_{inactive}$) times in each section of the aquaria.

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

213

214 2.5 Statistical Analyses

215 As the distribution of measured parameters was non-normally distributed (Shapiro-Wilk test,
216 $p = 0.17$), non-parametric statistics were used throughout this work. In the geotaxis assay, the
217 potential differences between inclination angles and the time taken by *Littorina littorea* to

218 resume movement (t_{move}), their time of activity (t_{active}), and their movement speed were assessed
219 using a Kruskal-Wallis test (hereafter referred to as KW test) and when necessary a subsequent
220 post-hoc test was performed using a Dunn test (Zar, 2010). The Rayleigh test was further used
221 to assess the circular uniformity of the snail dispersal for each inclination angle and control
222 (Zar, 2010). The initial orientation of *L. littorea* in the thigmotaxis assay (*i.e.* toward upward or
223 downward surfaces) was compared to a theoretical equi-repartition of the individuals using a
224 χ^2 test (Zar, 2010). The presence of a causal link between the time of activity and (i) the number
225 of behaviour and (ii) the activity index (*i.e.* percentage of activity) was further tested using the
226 Pearson correlation coefficient.

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

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

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

241

242

243 3. Results

244 3.1 Geotaxis Assay

245 The time taken by *Littorina littorea* to resume movement (t_{move}) did not significantly differ
246 (KW test, $p = 0.1295$) between the different treatments (Fig. 2A). The time of activity (t_{active} ,
247 *i.e.* the time between the first displacement and the moment snails reached a maximum net
248 distance $d_{max} = W/2$, where W is the width of the aquarium) was significantly different between
249 treatments (KW test, $p = 0.00329$). More specifically, t_{active} was significantly shorter for
250 individuals in aquarium tilted by 25° angles than for all the others treatments (Fig. 2B).

251 The distribution of the net orientation of *Littorina littorea* did not significantly differ from a
252 uniform distribution (Rayleigh test, $p = 0.2146$) under all experimental treatments (Fig. 2D). In
253 contrast, *L. littorea* speed significantly differed between treatments (KW test, $p < 0.0001$).
254 Specifically, *L. littorea* moved significantly faster on surface tilted by 25° (Dunn test, $p <$
255 0.0001) than for the surfaces tilted to 6 and 13° and the control, where speed did not
256 significantly differ (Fig. 2C). Finally, all the snails that encountered the wall systematically
257 followed it, before 78 to 100% of them climbed it.

258

259 3.2 Thigmotaxis Assay

260 Respectively 18 and 22 individuals moved toward the vertical walls and the sections of our
261 LEGO-based structure without wall. There was no significant difference between this
262 distribution and a theoretical equi-repartition of the individuals (χ^2 , $p = 0.6578$). The most
263 frequent behaviour after the first encounter with either a surface-to-wall or a surface-to-drop-
264 off discontinuity was discontinuity-following (67.5%), in contrast to discontinuity-avoidance
265 which was only marginally observed (2.5%). Climbing directly downward and upward after
266 encountering a topographical discontinuity (*i.e.* a surface-to-drop-off discontinuity and a
267 surface-to-wall discontinuity, respectively) represented respectively 10% and 20%. After

268 following a discontinuity, 100% of the individuals moved up after following a surface-to-wall
269 discontinuity or down after following a surface-to-drop-off discontinuity.

270

271 3.3 Scototaxis Assay

272 Control experiments run in either uniformly black or uniformly white versions of both the
273 binary container and target-based LEGO structure respectively showed that the proportion of
274 individuals found in each half of the aquaria did not significantly differ from a theoretical equi-
275 repartition (χ^2 test, $p = 0.177$), and that the net directions followed by *Littorina littorea* were
276 uniform ($p = 0.295$), hence that no intrinsic directionality related to the experimental set-up was
277 present.

278 In the binary choice assay, any group effects or inter-individual interactions such as trail-
279 following between snails in a group trial have never been observed. All individuals can then be
280 considered as being independent. The time spent in the black section was more than twice as
281 high as the time spent in the white section (Wilcoxon test, $p = 0.00195$). In contrast, there is no
282 significant difference in the percentages of activity and inactivity in the two different sections
283 (Wilcoxon test, $p = 0.51$).

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

289

290 4. Discussion

291 The ability to navigate through structurally complex environments is a key determinant of
292 the ecological success of a species. Intertidal gastropods are no exception, especially given the

293 highly complex nature of their environment, and the heavy constraints exerted by a range of
294 stressors such as thermal and desiccation stresses, wave exposure and the related risk of
295 dislodgement by wave action (Denny and Gaines 2007). However, despite their critical role in
296 the structure and function of intertidal ecosystems, little is still known — with the noticeable
297 exception of trail-following, see Ng et al., (2013) for a review — on the role of sensory
298 mechanisms and the related navigational capabilities of intertidal gastropods in general, and
299 *Littorina littorea* in particular. Based on the existing and often contradictory literature, this
300 work has inferred the presence of geotaxis, thigmotaxis and scototaxis in *L. littorea* under
301 laboratory-controlled conditions.

302

303 4.1 Geotaxis as a by-product of thigmotaxis

304 Despite the presence of significant differences between treatments in the time taken by *L.*
305 *littorea* to resume movement (t_{move}), their time of activity (t_{active}) and their speed, no significant
306 relationship was found between any of these variables and the slope of their substrate. These
307 results suggest that *Littorina littorea* may behave similarly on substrates of different inclination.
308 Specifically, the lack of dependence of *L. littorea* movement direction to substrate slope further
309 suggests an absence of geotactic behaviour in *L. littorea* investigated on substrates inclined
310 from 6 to 25°. *L. littorea* was also observed to move consistently significantly faster on surface
311 tilted by 25°. These results suggest that despite the absence of geotactic response, *L. littorea*
312 exhibited a kinetic response triggered by the conditions and the angles of the substratum. In
313 addition, the high proportion of *L. littorea* that climbed the wall of the experimental aquarium
314 after his encounter (78 to 100%) may have indicated a high expression of geotaxis. However,
315 in all instances, this behaviour consistently occurred after the snails followed the discontinuity
316 between the bottom and the wall of the aquarium. These observations further suggest that the

317 observed seemingly negative geotactic responses may instead be triggered by a discontinuity
318 encounter, *i.e.* a thigmotactically-induced negative geotactic response.

319

320 *4.2 Thigmotaxis as a way to navigate*

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

332 Though the following may be considered as highly speculative, the fact that discontinuity-
333 following was the predominant behaviour following an encounter with a vertical discontinuity
334 and was consistently followed by a geotactic response may also be thought as an obstacle
335 response of individual *L. littorea* which were attempting to go around an obstacle before
336 actually choosing to moving up or down. Additionally, the mechanical constraints of vertical
337 crawling (up or down) due to e.g. gravity and the shape of *L. littorina* shell may be such that it
338 may be easier to begin by turning and follow a discontinuity rather than crawling directly
339 downward or upward after encountering a surface-to-drop-off discontinuity and a surface-to-
340 wall discontinuity, respectively. In particular, the hypothesis of a mechanical constraint related
341 to the shape of *L. littorina* shell is consistent with the observed 2-fold difference between the

342 frequency of climbing downward after encountering a surface-to-drop-off discontinuity (i.e.
343 20%) and the frequency of climbing upward after encountering a surface-to-wall discontinuity
344 (i.e. 10%). This observation may indicate a higher mechanical constraint associated to a surface-
345 to-wall.

346 Despite the fundamental importance of substrate topography in the ecology of intertidal
347 organisms (Denny and Gaines, 2007), only a limited amount of work has been devoted to the
348 understanding of the mechanisms relating substrate topography and the navigational abilities
349 of intertidal gastropods. For instance, the complexity of surface topography has been shown to
350 affect different aspects of the ecology of intertidal snails such as distance travelled, population
351 density and structure (Underwood and Chapman, 1989; Chapman and Underwood, 1994). In
352 addition, the presence of species such as mussels or barnacles further creates biogenic habitats
353 that contribute to increase the complexity of surface topography and in turn affect different
354 aspects of mobile fauna biology and ecology. For instance, in gastropods the pattern of limpet
355 orientation was affect by this complex biogenic habitat (Fraser et al., 2014). In the early 20th
356 century, Pieron, (1909) introduced the idea of a kinaesthetic memory, which allowed homing
357 limpets to determine their location based on previous movement and local topography. In
358 contrast to limpets, littorinids do not exhibit homing behaviour, though evidence exist for
359 navigation back to a preferred shore height or resting habitat following dislodgment or
360 transplantation (Evans, 1961; Bock and Johnson, 1967; Gendron, 1977; Thain et al., 1985;
361 Rolán-Alvarez et al., 1997; Miller et al., 2007; Chapperon and Seuront, 2009; Seuront et al.,
362 2018a). As such, the consideration of thigmotaxis, and in particular both the prevalent
363 discontinuity-following behaviour and the thigmotactically-induced negative geotaxis observed
364 in the present work, may be a first step into furthering our understanding of how these organisms
365 may navigate through complex landscapes and is a plausible candidate to explain how the
366 aforementioned putative kinaesthetic memory may work.

367

368 *4.3 Scototaxis assay as an adaptation to a contrasted environment*

369 *Littorina littorea* orient toward black areas, hence exhibit a strong positive scototaxis (Fig.
370 4). This behaviour is consistent with early suggestions that eyes of *Littorina* could be used for
371 monitoring light intensity and for orientation (Stoll, 1973), and with littorinids visual abilities
372 as *L. littorea* have simple eyes which are optimum for under water vision during the day (Seyer,
373 1992). Our results are also consistent with previous evidence that a range of gastropod species
374 orient towards black forms, with the strength of the response increasing with their dimensions
375 (Thain et al., 1985; Hamilton and Winter, 1982, 1984). This behaviour may reflect the fact that
376 objects with ecological importance essentially appear as vertical for benthic organisms
377 (Hamilton and Winter, 1984). Though further work is needed to untangle the details of the
378 mechanisms involved, the capacity to distinguish and orient toward black areas could be
379 important for snails to allow them to find their way back after feeding excursions or
380 dislodgement as recently demonstrated for *L. littorea* (Seuront et al., 2018a).

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

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

392

393 *4.4 Conclusion*

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

410

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421

422 **References**

423 Bertness, M.D., 1999. The Ecology of Atlantic Shorelines, Sinauer As. ed. Sunderland.

424 Bock, C.E., Johnson, R.E., 1967. The role of behavior in determining the intertidal zonation
425 of *Littorina planaxis* Philippi, 1847, and *Littorina scutalata* Gould, 1849. The Veliger
426 10, 42–54.

427 Buschbaum, C., 2000. Direct and indirect effects of *Littorina littorea* (L.) on barnacles
428 growing on mussel beds in the Wadden Sea. Hydrobiologia 440, 119–128.
429 <https://doi.org/10.1023/A:1004142306396>

430 Chapman, M.G., 2000. Poor design of behavioural experiments gets poor results: examples
431 from intertidal habitats. J. Exp. Mar. Bio. Ecol. 250, 77–95.
432 [https://doi.org/10.1016/S0022-0981\(00\)00180-5](https://doi.org/10.1016/S0022-0981(00)00180-5)

433 Chapman, M.G., Underwood, A.J., 1994. Dispersal of the intertidal snail, *Nodilittorina*
434 *pyramidalis*, in response to the topographic complexity of the substratum. J. Exp. Mar.
435 Bio. Ecol. 179, 145–169. [https://doi.org/10.1016/0022-0981\(94\)90111-2](https://doi.org/10.1016/0022-0981(94)90111-2)

436 Chapperon, C., Seuront, L., 2011a. Space-time variability in environmental thermal properties
437 and snail thermoregulatory behaviour. Funct. Ecol. 25, 1040–1050.
438 <https://doi.org/10.1111/j.1365-2435.2011.01859.x>

439 Chapperon, C., Seuront, L., 2011b. Behavioral thermoregulation in a tropical gastropod: links

- 440 to climate change scenarios. *Glob. Chang. Biol.* 17, 1740–1749.
441 <https://doi.org/10.1111/j.1365-2486.2010.02356.x>
- 442 Chapperon, C., Seuront, L., 2009. Cue synergy in *Littorina littorea* navigation following
443 wave dislodgement. *J. Mar. Biol. Assoc. United Kingdom* 89, 1133–1136.
444 <https://doi.org/10.1017/S0025315409000150>
- 445 Chelazzi, G., Vannini, M., 1976. Researches on the coast of Somalia. The shore and the dune
446 of Sar Uanle. 9. Coastward orientation in *Nerita textilis* Dillwyn (Gastropoda
447 Prosobranchia). *Monit. Zool. Ital.* 8, 161–178.
448 <https://doi.org/10.1080/03749444.1976.10736839>
- 449 Croll, R.P., 1983. Gastropod chemoreception. *Biol. Rev.* 58, 293–319.
450 <https://doi.org/10.1111/j.1469-185X.1983.tb00391.x>
- 451 Denny, M.W., Gaines, S.D., 2007. *Encyclopedia of tidepools and rocky shores*, University.
452 ed.
- 453 Emson, R.H., Faller-Fritsch, R.J., 1976. An experimental investigation into the effect of
454 crevice availability on abundance and size-structure in a population of *Littorina rudis*
455 (Maton): Gastropoda: Prosobranchia. *J. Exp. Mar. Bio. Ecol.* 23, 285–297.
456 [https://doi.org/10.1016/0022-0981\(76\)90026-5](https://doi.org/10.1016/0022-0981(76)90026-5)
- 457 Erlandsson, J., Kostylev, V., 1995. Trail following, speed and fractal dimension of movement
458 in a marine prosobranch, *Littorina littorea*, during a mating and a non-mating season.
459 *Mar. Biol.* 122, 87–94. <https://doi.org/10.1007/BF00349281>
- 460 Evans, F., 1961. Responses to disturbance of the periwinkle *Littorina punctata* (Gmelin) on a
461 shore in Ghana. *Proc. Zool. Soc.* 137, 393–402.

- 462 Fraser, C.M.L., Coleman, R.A., Seebacher, F., 2015. Orientation in a keystone grazer:
463 interactions between habitat and individual identity drive patterns of resting behaviour.
464 Mar. Ecol. Prog. Ser. 522, 145–156. <https://doi.org/10.3354/meps11132>
- 465 Fraser, C.M.L., Coleman, R.A., Seebacher, F., 2014. Trying to fit in: are patterns of
466 orientation of a keystone grazer set by behavioural responses to ecosystem engineers or
467 wave action? *Oecologia* 174, 67–75. <https://doi.org/10.1007/s00442-013-2743-3>
- 468 Fratini, S., Cannicci, S., Vannini, M., 2001. Feeding clusters and olfaction in the mangrove
469 snail *Terebralia palustris* (Linnaeus) (Potamididae : Gastropoda). *J. Exp. Mar. Bio. Ecol.*
470 261, 173–183.
- 471 Gendron, R.P., 1977. Habitat selection and migratory behaviour of the intertidal gastropod
472 *Littorina littorea* (L .). *J. Anim. Ecol.* 46, 79–92.
- 473 Hamilton, P.V., 1978. The role of vision in adaptive oriented movements of *Littorina irrorata*
474 (Mollusca: Gastropoda) when displaced from their natural habitat. *Mar. Behav. Physiol.*
475 5, 255–271.
- 476 Hamilton, P.V., 1977. Daily movements and visual relocation of plant stems by *Littorina*
477 *irrorata* (Mollusca: Gastropoda). *Mar. Behav. Physiol.* 4, 293–304.
- 478 Hamilton, P. V., Russell, B.J., 1982. Celestial orientation by surface-swimming *Aplysia*
479 *brasiliensis* Rang (Mollusca: Gastropoda). *J. Exp. Mar. Bio. Ecol.* 56, 145–152.
- 480 Hamilton, P. V., Winter, M.A., 1984. Behavioural responses to visual stimuli by the snails
481 *Tectarius muricatus*, *Turbo castanea*, and *Helix aspersa*. *Anim. Behav.* 32, 51–57.
482 [https://doi.org/10.1016/S0003-3472\(84\)80323-1](https://doi.org/10.1016/S0003-3472(84)80323-1)
- 483 Hayes, F.R., 1926. The negative geotropism of the periwinkle: a study in littoral ecology.

- 484 Proc. Nov. Scotian Inst. Sci. 16, 155–173.
- 485 Haynes, K.J., Cronin, J.T., de Roos, A., 2006. Interpatch movement and edge effects : the role
486 of behavioral responses to the landscape matrix. *Oikos* 113, 43–54.
- 487 Jackson, A.C., 2010. Effects of topography on the environment. *J. Mar. Biol. Assoc. United*
488 *Kingdom* 90, 169. <https://doi.org/10.1017/S0025315409991123>
- 489 Jones, K.M.M., Boulding, E.G., 1999. State-dependent habitat selection by an intertidal snail:
490 the costs of selecting a physically stressful microhabitat. *J. Exp. Mar. Bio. Ecol.* 242,
491 149–177. [https://doi.org/10.1016/S0022-0981\(99\)00090-8](https://doi.org/10.1016/S0022-0981(99)00090-8)
- 492 Kamimura, S., Tsuchiya, M., 2006. Effects of opportunistic feeding by the intertidal
493 gastropods *Batillaria zonalis* and *B. flectosiphonata* on material flux on a tidal flat. *Mar.*
494 *Ecol. Prog. Ser.* 318, 203–211. <https://doi.org/10.3354/meps318203>
- 495 Kanda, S., 1916. Studies on the geotropism of the marine snail, *Littorina littorea*. *Biol. Bull.*
496 30, 57–84.
- 497 Keppel, E., Scrosati, R., 2004. Chemically mediated avoidance of *Hemigrapsus nudus*
498 (Crustacea) by *Littorina scutulata* (Gastropoda): effects of species coexistence and
499 variable cues. *Anim. Behav.* 68, 915–920. <https://doi.org/10.1016/j.anbehav.2003.11.020>
- 500 Levin, S.A., Whitfield, M., 1994. Patchiness in marine and terrestrial systems: from
501 individuals to populations. *Philos. Trans. R. Soc. B Biol. Sci.* 343, 99–103.
- 502 Little, C., Williams, G.A., Trowbridge, C.D., 2009. *The biology of rocky shores*, Oxford Uni.
503 ed.
- 504 Lotze, H.K., Worm, B., 2002. Complex interactions of climatic and ecological controls on
505 macroalgal recruitment. *Limnol. Oceanogr.* 47, 1734–1741.

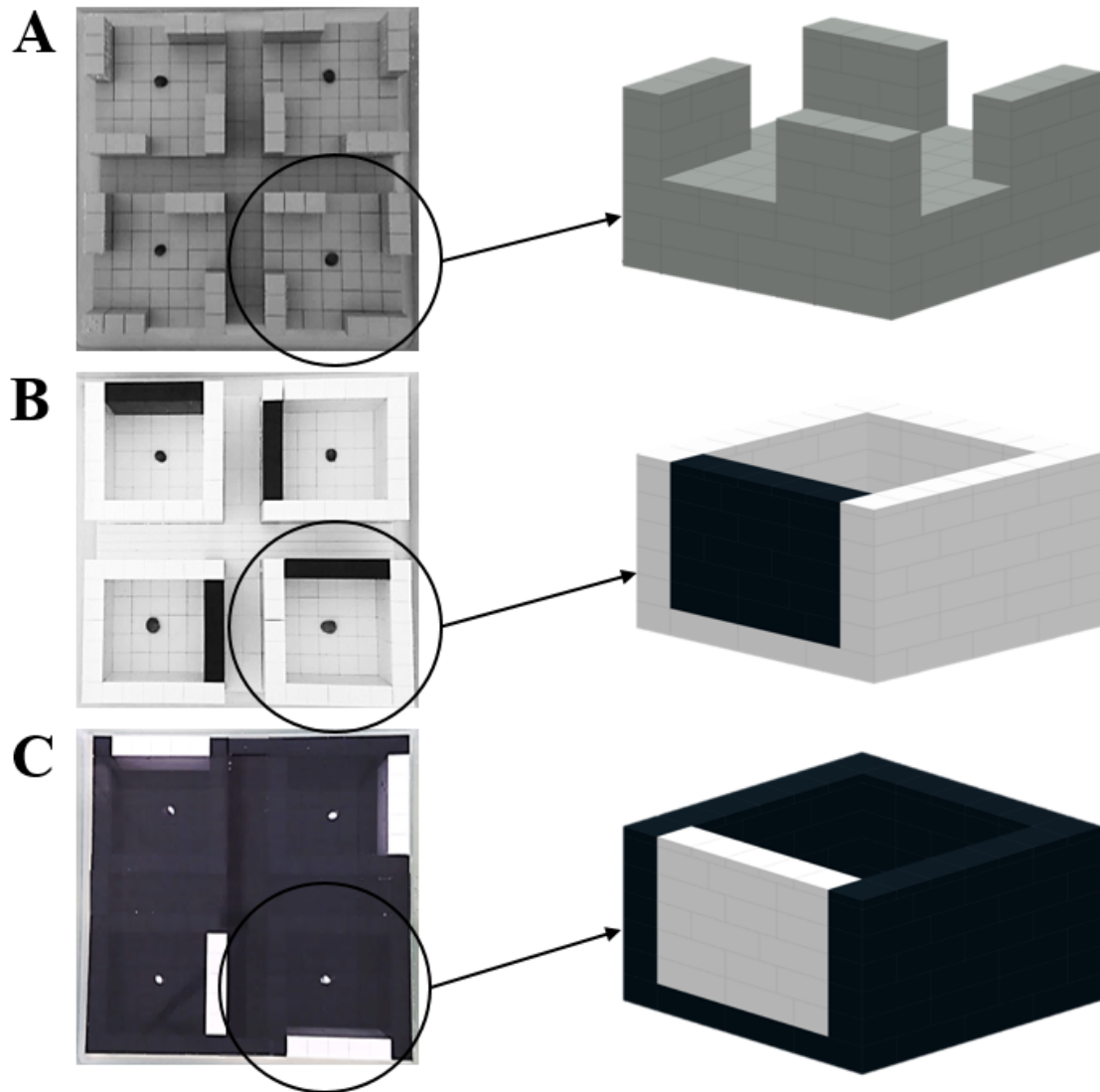
- 506 <https://doi.org/10.4319/lo.2002.47.6.1734>
- 507 Miller, L.P., O'Donnell, M.J., Mach, K.J., 2007. Dislodged but not dead: survivorship of a
508 high intertidal snail following wave dislodgement. *J. Mar. Biol. Assoc. UK* 87, 735–739.
509 <https://doi.org/10.1017/S0025315407055221>
- 510 Morales, J.M., Ellner, 2002. Scaling up animal movements in heterogeneous landscapes : the
511 importance of behavior. *Ecology* 83, 2240–2247.
- 512 Newell, G.E., 1958a. The behaviour of *Littorina littorea* (L.) under natural conditions and its
513 relation to position on the shore. *J. Mar. Biol. Assoc. United Kingdom* 37, 229–239.
- 514 Newell, G.E., 1958b. An experimental analysis of the behaviour of *Littorina littorea* (L.)
515 under natural conditions and in laboratory. *J. Mar. Biol. Assoc. United Kingdom* 37,
516 241–266.
- 517 Ng, T.P.T., Saltin, S.H., Davies, M.S., Johannesson, K., Stafford, R., Williams, G.A., 2013.
518 Snails and their trails: the multiple functions of trail-following in gastropods. *Biol. Rev.*
519 88, 683–700. <https://doi.org/10.1111/brv.12023>
- 520 Petraitis, P.S., 1982. Occurrence of random and directional movements in the periwinkle,
521 *Littorina littorea* (L.). *J. Exp. Mar. Bio. Ecol.* 59, 207–217.
522 [https://doi.org/10.1016/0022-0981\(82\)90116-2](https://doi.org/10.1016/0022-0981(82)90116-2)
- 523 Pieron, H., 1909. Sens de l'orientation et mémoire topographique de la Patella. *Comptes*
524 *Rendus l'Académie des Sci.* 148, 530–532.
- 525 Rolán-Alvarez, E., Johannesson, K., Erlandsson, J., 1997. The maintenance of a cline in the
526 marine snail *Littorina saxatilis*: the role of home site advantage and hybrid fitness.
527 *Evolution* (N. Y). 51, 1838–1847.

- 528 Seuront, L., Duponchel, A.C., Chapperon, C., 2007. Heavy-tailed distributions in the
529 intermittent motion behaviour of the intertidal gastropod *Littorina littorea*. Phys. A Stat.
530 Mech. its Appl. 385, 573–582. <https://doi.org/10.1016/j.physa.2007.07.029>
- 531 Seuront, L., Moisez, E., Hennion, C., Seuront-Scheffbuch, D., Seuront, L.M.Y., 2018a.
532 *Littorina littorea* show small-scale persistent tidal height and habitat partitioning that is
533 resilient to dislodgement through specific movement rates. J. Exp. Mar. Bio. Ecol. 509,
534 24–35. <https://doi.org/10.1016/j.jembe.2018.08.011>
- 535 Seuront, L., Ng, T.P.T., Lathlean, J.A., 2018b. A review of the thermal biology and ecology
536 of molluscs, and of the use of infrared thermography in molluscan research. J. Molluscan
537 Stud. 203–232. <https://doi.org/10.1093/mollus/eyy023>
- 538 Seuront, L., Spilmont, N., 2015. The smell of sex: water-borne and air-borne sex pheromones
539 in the intertidal gastropod *Littorina littorea*. J. Molluscan Stud. 81, 96–103.
540 <https://doi.org/10.1093/mollus/eyu060>
- 541 Seyer, J.-O., 1992. Resolution and sensitivity in the eye of the winkle *Littorina littorea*. J.
542 Exp. Biol. 170, 57–69.
- 543 Spilmont, N., Hachet, A., Faasse, M.A., Jourde, J., Luczak, C., Seuront, L., Rolet, C., 2016.
544 First records of *Ptilohyale littoralis* (Amphipoda : Hyalidae) and *Boccardia*
545 *proboscidea* (Polychaeta : Spionidae) from the coast of the English Channel : habitat
546 use and coexistence with other species. Mar. Biodivers. 1–11.
547 <https://doi.org/10.1007/s12526-016-0557-3>
- 548 Stafford, R., Davies, M.S., 2005. Spatial patchiness of epilithic biofilm caused by refuge-
549 inhabiting high shore gastropods. Hydrobiologia 545, 279–287.
550 <https://doi.org/10.1007/s10750-005-3320-5>

- 551 Stoll, C.J., 1973. On the role of the eyes and non-ocular light receptors in orientational
552 behavior of *Lymnaea stagnalis* (L). Proc. K. Nederlandse Akad. van Wet. Ser. C 76,
553 203–214.
- 554 Thain, V.M., Thain, J.F., Kitching, J.A., 1985. Return of the prosobranch *Gibbula umbilicus*
555 (Da Costa) to the littoral region after displacement to the shallow sublittoral. J.
556 Molluscan Stud. 51, 205–210.
- 557 Tilman, D., 1994. Competition and biodiversity in spatially structured habitats. Ecology 75,
558 2–16. <https://doi.org/10.2307/1939377>
- 559 Underwood, A.J., 1977. Movements of intertidal gastropods. Adv. Mar. Biol. 16, 111–210.
560 [https://doi.org/10.1016/S0065-2881\(08\)60293-X](https://doi.org/10.1016/S0065-2881(08)60293-X)
- 561 Underwood, A.J., Chapman, M.G., 1989. Experimental analyses of the influences of
562 topography of the substratum on movements and density of an intertidal snail, *Littorina*
563 *unifasciata*. J. Exp. Mar. Bio. Ecol. 134, 175–196. [https://doi.org/10.1016/0022-](https://doi.org/10.1016/0022-0981(89)90068-3)
564 [0981\(89\)90068-3](https://doi.org/10.1016/0022-0981(89)90068-3)
- 565 Underwood, A.J., Chapman, M.G., 1985. Multifactorial analyses of directions of movement
566 of animals. J. Exp. Mar. Bio. Ecol. 91, 17–43. [https://doi.org/10.1016/0022-](https://doi.org/10.1016/0022-0981(85)90218-7)
567 [0981\(85\)90218-7](https://doi.org/10.1016/0022-0981(85)90218-7)
- 568 Warburton, K., 1973. Solar orientation in the snail *Nerita plicata* (Prosobranchia: Neritacea)
569 on a beach near Watamu, Kenya. Mar. Biol. 23, 93–100.
570 <https://doi.org/10.1007/BF00389165>
- 571 Wyeth, R.C., Woodward, O.M., Willows, A.O.D., 2006. Orientation and navigation relative
572 to water flow, prey, conspecifics, and predators by the nudibranch mollusc *Tritonia*
573 *diomedea*. Biol. Bull. 210, 97–108. <https://doi.org/10.2307/4134599>

574 Zar, J.H., 2010. Biostatistical analysis, Fifth edit. ed.

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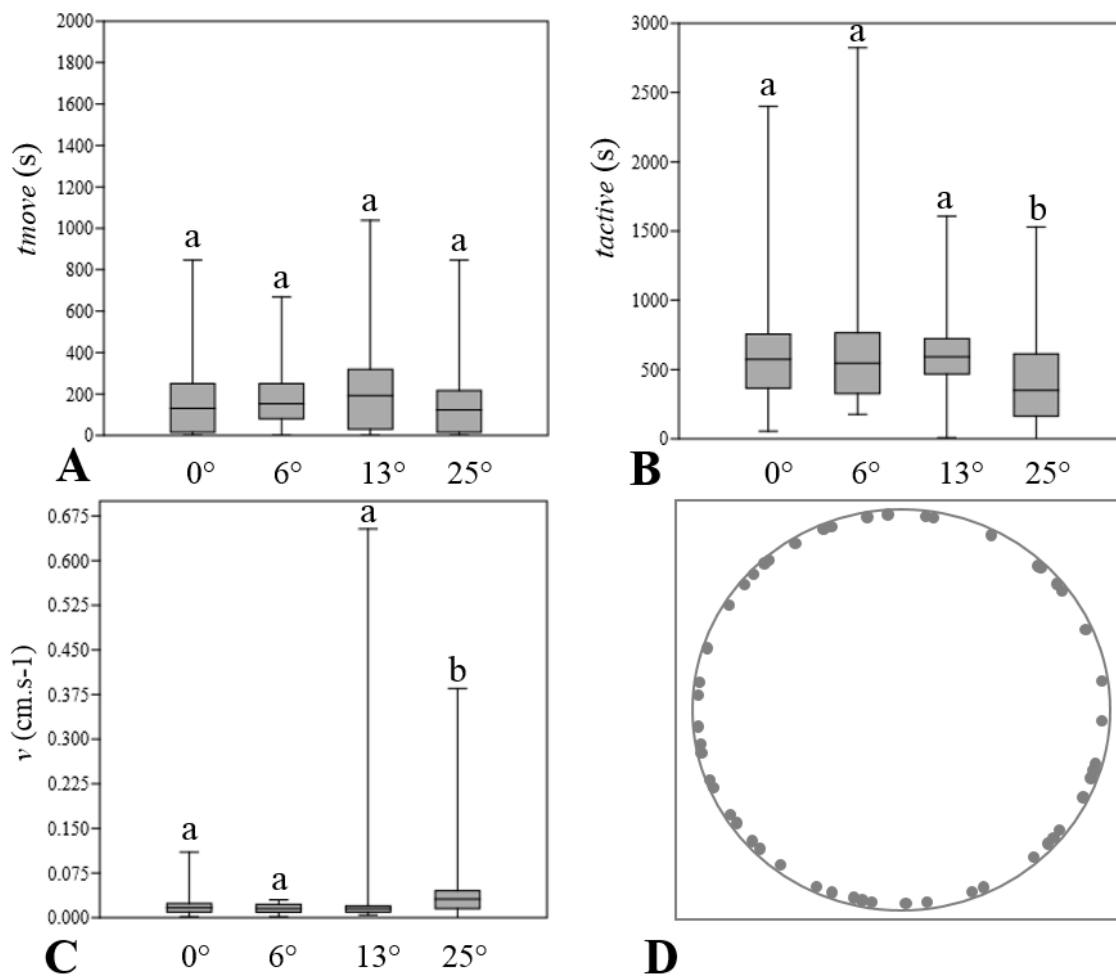
577 Fig. 1. Illustration of the LEGO-based structures designed to assess (A) the presence of geotaxis and thigmotaxis
 578 in *Littorina littorea* and the presence of scototaxis in *L. littorea* based on experiments run on four subsections
 579 made of (B) 3 white walls and 1 target black wall (W₃B₁) or (C) 3 black walls and 1 target white wall (B₃W₁). The
 580 white points from the panel C correspond to the *Littorina littorea* individuals marked with white nail polish.

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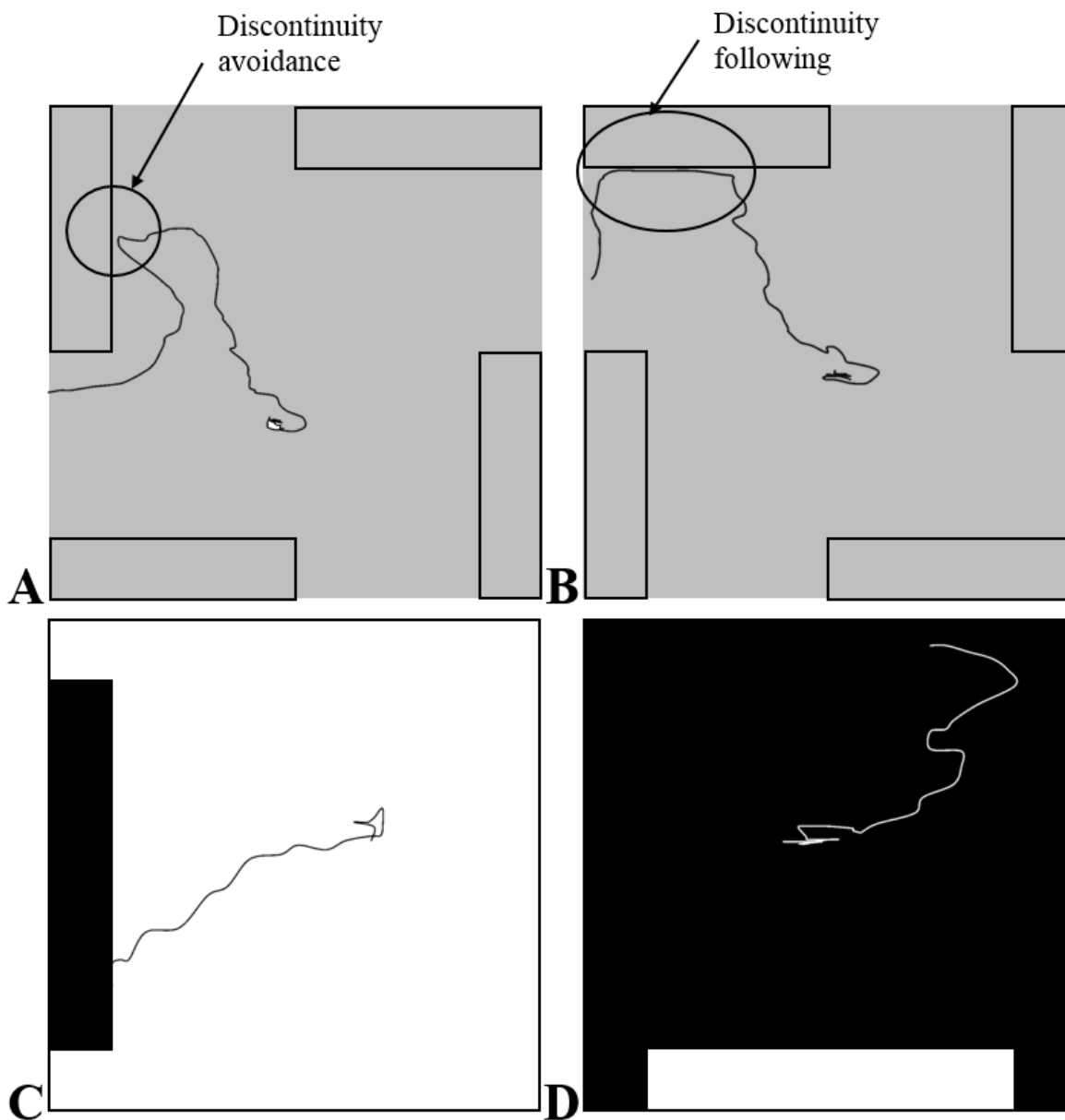


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586 Fig. 2. Behavioural measurement of *Littorina littorea* for the different treatments in the geotactic experiment, (A)
 587 time to resume movement (t_{move}), (B) time of activity (t_{active}), (C) movement speed (v) and (D) the net orientation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

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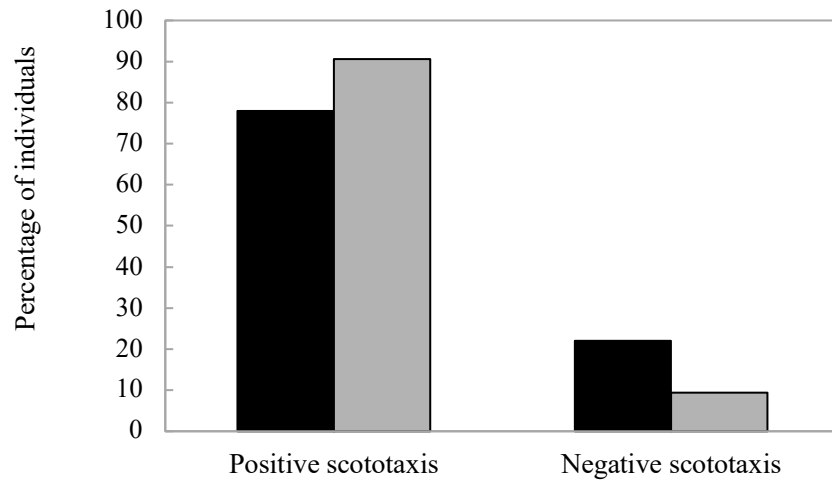
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594 Fig. 3. Illustrations of representative tracks from our thigmotactic assay (A) representing the discontinuity
 595 avoidance behaviour, (B) the discontinuity following behaviour, and from ours scototaxis assay (C) W₃B₁ assay
 596 and (D) B₃W₁ assay.

597



598

599 Fig. 4. Frequency of occurrence of target-based positive and negative scototaxis in *Littorina littorea*. The black
600 and grey bars correspond to the version of the experimental set-up respectively including 3 white walls and a black
601 one (W₃B₁) and 3 black walls and a white one (B₃W₁).

602