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Periwinkle climbing response to water- and airborne predator chemical cues may depend on home-marsh geography

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The salt marsh periwinkle, *Littorina irrorata*, exhibits a spatial refuge from predation by climbing the stems of *Spartina alterniflora* in order to avoid benthic predators. Salt marsh periwinkles have a broad geographic distribution, and for many species, responses to predators also varies with biogeography. This study sought to determine if the geographical location of the home marsh influenced the response of periwinkles (climbing height) to blue crab predator cues both via air and water. Snails from Louisiana (LA) climbed higher in general than those from North Carolina (NC), regardless of chemical cue. However, LA snails climbed 11cm higher in the presence of waterborne predators than control snails with no cue, while NC snails only climbed 5cm higher in the same comparisons. Airborne chemical cue tended to have snails climbing at intermediate heights. These responses were significantly enhanced when both populations of snails were housed together. Periwinkle response to predator cues was stronger in LA than NC, and so it is possible that the behavioral response of these snails to predators varies with biogeography of the home marsh. Also interestingly, the results of this study also suggest that cue delivery is probably occurring via mechanisms other than water, and potentially via airborne cues. Therefore, salt marsh periwinkles likely respond to numerous cues that initiate behavioral responses, including airborne cues, and these responses may vary by home-marsh geography.

16 **ABSTRACT** - The salt marsh periwinkle, *Littorina irrorata*, exhibits a spatial refuge from
17 predation by climbing the stems of *Spartina alterniflora* in order to avoid benthic predators. Salt
18 marsh periwinkles have a broad geographic distribution, and for many species, responses to
19 predators also varies with biogeography. This study sought to determine if the geographical
20 location of the home marsh influenced the response of periwinkles (climbing height) to blue crab
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26 populations of snails were housed together. Periwinkle response to predator cues was stronger in
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31 responses, including airborne cues, and these responses may vary by home-marsh geography.

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34 INTRODUCTION

35 Predation is one of the most important interactions affecting marine populations (Connell
36 1975, Behrens Yamada, Navarrete & Needham 1998). Predators can directly affect the
37 distribution, abundance, size structure and genetic make-up of prey populations (Menge 1983,
38 Yoshida et al., 2003). As a result of intense predation pressure, prey have evolved various means
39 to reduce predation risk that vary on ecological and evolutionary timescales (Vermeij 1982,
40 Trussell and Smith 2000). For example, natural selection is thought to drive changes in prey
41 morphology over evolutionary timescales, with prey growing thicker, more ornate exoskeletons
42 in response to high or increasing predation pressure (Vermeij 1982, 1983, 1987). However, prey
43 can respond to predators at ecological (within lifetime) timescales (Lima and Dill 1990). In
44 particular, predators have increasingly been demonstrated to rapidly induce prey defenses, which
45 act to reduce prey vulnerability (Trussell and Smith 2000). These inducible defenses occur
46 across diverse taxa and include fast growth, chemical defenses, skeleton thickening, changes in
47 behavior, and using refugia (Harvell 1990, Berenbaun and Zangerl 1999).

48 A number of gastropods have demonstrated inducible defenses as a result of predation
49 pressure in experimental settings (Behrens Yamada, Navarrete & Needham 1998, Brandwood
50 1985, Duncan and Szelistowski 1998, Large and Smee 2010, 2013). A common defense is
51 changing behavior, including predator avoidance and/or fleeing (Legault and Himmelman 1993).
52 However, these defenses typically vary across broad geographic scales. Both predator diversity
53 and predation pressure generally vary inversely with latitude, so prey organisms have responded
54 by increasing defenses with decreasing latitude (Laurila, Lindgren & Laugen 2008, Freestone et
55 al. 2011), which includes latitudinal differences in behavioral responses (Aschaffenburg 2008,
56 Donahue et al. 2009, Duval, Calzetta & Rittschof 1994, Large and Smee 2013). Induced

57 defenses are affected at broad biogeographic scales by differences in environmental conditions
58 and stimuli (Trussell and Smith 2000). Further, there are costs associated with induced defenses
59 (Trussell and Nicklin 2002), so geographic patterns in prey response likely reflect the greater
60 predictability of predation risk at certain locations (Trussell and Smith 2000).

61 For intertidal snails, predator avoidance includes using spatial refugia to avoid capture
62 which has been demonstrated in both rocky-intertidal (Menge and Lubchenco 1981) and salt
63 marsh habitats (Warren 1985). The salt marsh periwinkle, *Littorina irrorata* Say, is an important
64 resident of tidal marshes along the US Atlantic and Gulf coasts, which exhibit spatially variable
65 distribution dependent upon the tidal stage (Hovel, Bartholomew & Lipcius 2001). Historically,
66 the distribution of periwinkles in the salt marsh was initially considered to be the result of
67 environmental variables (Bingham 1972). However, considerable evidence suggests this vertical
68 distribution is to avoid predators when the tide returns, such as the blue crab, *Callinectes sapidus*
69 Rathbun, and the crown conch, *Melongena corona* Gmelin (Hamilton 1976, Warren 1985), and
70 periwinkles tend to migrate higher and/or faster in the presence of predators (Dix and Hamilton
71 1993, Duval, Calzetta & Rittschof 1994, Kimbro 2012, Wollerman, Duva & Ferrier 2003),
72 although these activities are constrained by environmental stressors, such as temperature
73 (Iacarella and Helmuth 2012). Thus, periwinkles exhibit a spatial refuge from predation by
74 climbing the stems of salt marsh cordgrass, *Spartina alterniflora* Loisel, in order to avoid benthic
75 predators (Dix and Hamilton 1993, Vaughn and Fisher 1988).

76 *L. irrorata* has an extensive geographic range (Bingham 1972) and climbing behavior has
77 been noted at the local scale in Virginia (Stanhope, Banta & Temkin 1982), North Carolina
78 (Duval, Calzetta & Rittschof 1994, Lewis and Eby 2002), Georgia (Silliman and Bertness 2002),
79 Florida (Hamilton 1976, Warren 1985), Alabama (Henry, McBride & Williams 1993), Louisiana

80 (*pers. obs.*) and Texas (Vaughn and Fisher 1988). Since predator diversity and predation
81 pressure vary with latitude (Bertness, Garrity & Levings 1981, Freestone et al. 2011), and salt
82 marsh periwinkles inhabit this broad geographic range, they are useful model organisms to
83 explore biogeographic variation in behavioral responses. Regional comparisons in climbing
84 height and response to predators in marsh periwinkles have not been made previously, although
85 a number of other similar species have exhibited differential responses to predators across
86 geographic ranges (Fawcett 1984, Large & Smee 2013). Therefore, periwinkles might exhibit
87 similar differences in induced avoidance responses according to home-marsh geography.

88 The mechanism thought to be driving climbing behavior is response to waterborne
89 chemical cues from either predators or injured conspecifics (Duval, Calzetta & Rittschof 1994,
90 Jacobsen and Stabell 1999), although periwinkles often migrate in advance of the tide. For other
91 intertidal snails, such as *Cerithidea decollata*, it has been suggested that there is an internal clock
92 that might drive migrations (Lazzeri et al. 2014), however *L. irrorata* has been demonstrated to
93 rapidly respond to out of phase tidal cycles in the lab (Hovel, Bartholomew & Lipcius 2001). It
94 is possible that some cues might become aerosolized prior to the arrival of the incoming tide,
95 forewarning snails and cueing them to start climbing (Lazzeri 2017). A number of terrestrial
96 gastropods respond to airborne cues for homing (Chelazzi, Le Vovi & Parpagnoli 1988), feeding
97 (Davis 2004), mating (Ng et al. 2013), and avoiding predators (Bursztyka et al. 2013).
98 Interestingly, it has been suggested that at least two species of intertidal snails may respond to
99 airborne cues from either food (Fratini, Cannicci & Vannini 2001) or the environment (Lazzeri
100 2017). Given the responses to other airborne chemical cues, it is possible that intertidal marine
101 gastropods would also react to airborne cues from predators, particularly snails such as *L.*

102 *irrorata*, which spend much of their time emersed. Yet, responses to potential airborne chemical
103 cues from predators has not been investigated in Littorinids.

104 Relatively little is known about how species might respond to different chemical cues
105 across geographical locations. Due to its geographic range and behavior, the marsh periwinkle is
106 a useful model organism to explore whether geographic location and the presence of airborne
107 cues affects anti-predator behavioral responses. Further, periwinkles could also be a useful model
108 organism to see whether behavioral responses change in the presence of individuals from
109 different populations, which may come into contact due to human activities. Field observations
110 in Louisiana demonstrated that periwinkles responded to crabs by climbing up *Spartina*
111 *alterniflora*, however, similar field observations in North Carolina suggested a lesser response.
112 Thus, we designed a controlled lab experiment to investigate the difference in behavioral
113 response (climbing) of two periwinkle populations to cues from a common predator, the blue
114 crab *Callinectes sapidus*. Specifically, we tested whether the presence of both waterborne and
115 airborne blue crab cues would cause snails to migrate up *Spartina* mimics, and whether the two
116 populations would climb to different heights. Since behavioral responses to predation are likely
117 to vary at different geographic locations and predation pressure often increases with decreasing
118 latitude, we hypothesized the Louisiana population of periwinkles would show a greater response
119 to the predator than the North Carolina population by migrating higher on the mimics.
120 Additionally, since intertidal salt marsh periwinkles spend the majority of their time out of the
121 water, we hypothesized that airborne cues would elicit a behavioral response, although given the
122 marine origin of this species, we expected the airborne response to be intermediate.

123 **METHODS AND MATERIALS**

124 Louisiana snails were collected from *Spartina alterniflora* marsh adjacent to the
125 Louisiana Universities Marine Consortium (29°15'20.65"N, 90°39'42.93"W) and transported to
126 North Carolina. North Carolina snails were collected from the salt marsh adjacent to the Center
127 for Marine Sciences (34°08'26.26"N, 077°51'47.81"W). These locations were chosen because
128 they are within the range of *L. irrorata* and they share an abundant common predator, blue crabs.
129 In order to acclimate snails to laboratory conditions, individuals were held in the lab for 2 days
130 prior to the start of the experiment since the species has been shown to rapidly (~1 d) respond to
131 changes in tidal conditions in the lab (Hovel, Bartholomew & Lipcius 2001). Blue crabs were
132 purchased from a local supplier. All animals were kept in flowing filtered seawater holding
133 tanks. Crabs were fed crushed snails *ad libitum* for 48 hours prior to use in experiments.

134 Lab assays were conducted to investigate regional differences in climbing and to test for
135 chemical cue responses. The experimental unit was a box-in-box mesocosm set-up (Figure 1A).
136 Briefly, we placed a small plastic tub (27 x 41 x 18.5cm), used to house the periwinkles during
137 the experiment, within a larger plastic tub (39 x 54 x 16cm). *Spartina*-mimics were used to
138 simulate marsh grass in lab assays (Hovel, Bartholomew & Lipcius 2001). Eight 75cm tall x
139 1.5cm wide PVC pipes were used in each replicate. The PVC mimics were preferable to natural
140 grass because they are inert (Sueiro, Bortlous & Schwindt 2012), can be easily washed between
141 trials, and are not likely to give off chemical cues like wooden dowel rods or *Spartina* stems. In
142 crab water cue treatments, the small, inner plastic tubs were drilled with small holes to allow
143 water to mix between the inner and outer tubs when filled (Figure 1A). For airborne cues and no
144 cue treatments, the inner boxes were not drilled in order to isolate the water in the small tub.
145 However, the airborne cue treatments held a crab in the outer box, whereas no cue treatments did
146 not receive a crab. Plastic mesh was used to cover the space between the small and large tubs to

147 prevent movement of animals between the tubs (Figure 1B). We used six box-in-box set-ups per
148 trial for the experiments. Air stones, modified to reduce splashing, were placed in the outer tub
149 for all treatments. Each experimental unit was surrounded on four sides by a 45 x 64 x 90cm
150 open top cage constructed of PVC pipe and a thick black plastic curtain to isolate the replicates
151 from each other, preventing potential transfer of airborne cues between units and reducing the
152 visual disturbance on both snails and crabs. Fluorescent work lights were provided directly
153 above each experimental unit. Temperature was maintained at 28° C in holding tanks and
154 experimental units.

155 Three different treatments were established – a no crab control, a crab present with
156 chemical cues mixing via water exchange between tubs, and a crab present with no water
157 exchange. For the no crab control, ~26 L of clean, filtered and sterilized seawater was placed
158 into the two tubs, for a water depth of 12.5cm. For the crab present treatments, crabs were
159 placed in the large outer tub and allowed to move freely throughout the space between the tubs.
160 For the waterborne cue, the small, inner tubs drilled to allow water exchange were used,
161 circulating the water between tubs and allowing snails to contact water exposed to the blue crab.
162 For the non-waterborne cue, tubs that were not drilled were used, restricting both the crab and
163 crab-cue water to the outer tub, and thus the snails could not directly sample water with crab
164 cues. These tubs were covered with a mesh top which allowed airborne cues to escape. Our
165 mesocosms were undisturbed during the experiment to minimize stimulation of test subjects. We
166 did not notice surface splashing by crabs, they were either stationary or remained submerged for
167 the duration of the trials. Thus any response in these treatments is indicative of an airborne cue.

168 Two sets of experiments were conducted. The first set used either LA or NC snails alone,
169 and two trials were used for each of the NC and LA snails. Sets of 30 snails were placed directly

170 on the PVC approximately 5cm above the water line (~17 cm above the bottom of the tubs) in
171 each mesocosm set-up (n = 180 total snails per trial) and exposed to the different treatments for 6
172 hours. Observations of snails demonstrated that many initially approached and entered the water
173 at the start of the experiment. Since the airborne cue was the target of the investigation, each trial
174 had airborne cue treatments. However, due to space limitations for the experimental set-ups,
175 waterborne cue and no cue treatment replicates were only used in a single trial.

176 To eliminate any perceived differences between populations that might have been due to
177 running separate experiments, we conducted a second common garden experiment where we
178 combined snails from the two populations. For the second experiment, we also used 2 trials,
179 although to keep density per mesocosm the same, we only used 15 snails per home marsh. This
180 allowed us to directly examine the two populations in the same experimental conditions. At the
181 end of each trial, the height of each individual snail was measured.

182 For the single population experiments, North Carolina and Louisiana trials were
183 combined and analyzed. A two-factor generalized linear mixed model (GLMM) was used to
184 determine the effect of site and cue treatment on average climbing height of snails. Site (NC or
185 LA) and cue treatment (no cue, airborne cue, waterborne cue) were modeled as a fixed effect.
186 Since 30 snails were placed into each tub for the experiment, snails within each tubs could not be
187 considered independent of each other (Supplement 1). To account for potential effects of snails
188 located within the same tub, a tub identifier was included as a random effect in the model.
189 GLMMs were also used to determine the effect of home marsh and cue type in the mixed
190 population experiment, with treatment (no cue, airborne cue, waterborne cue) and location (NC
191 and LA) as fixed effects, and tub modeled as a random effect. Models were fit with the function
192 ‘glmer’ and a gamma distribution using the ‘lme4’ package (Bates et al. 2015) in R (R Core

193 Team 2015). When significant effects were found, post-hoc general linear hypothesis
194 comparisons were performed using 'ghlt' function 'multcomp' package in R (Hothorn, Bretz &
195 Westfall. 2008).

196 RESULTS

197 Snails actively moved up and down the PVC mimics during the experimental period. LA
198 snails climbed significantly higher than NC snails ($p < 0.001$), although there was a significant
199 interaction between the two fixed factors ($p < 0.001$), suggesting the response in the different
200 snail populations varied dependent upon the cue treatment (Figure 2). There was a trend in the
201 LA snails to climb higher when exposed to predator cues, although there was high variability
202 within each treatment, and these trends were not significant (Figure 2). The minimum height for
203 waterborne cue boxes was 16.4cm, whereas it was 19.3 for airborne cue and 22.5 for control
204 boxes. The maximum was similar for all (74.8, 76.5 and 76.4cm for waterborne, airborne and
205 control boxes. No individuals were below the water line at the end of the experiment. The within
206 trial variability in mean snail height among boxes of the same treatments was 5.4, 12.6, and 2.9
207 cm for waterborne, airborne and control treatments, respectively.

208 For NC snails, there was also a trend for those exposed to waterborne crab cues to climb
209 higher than those with no cue (Figure 2), although like in the LA snail population, there was
210 considerable variability among individuals. Interestingly, the minimum height for NC snails was
211 4.8, 0, and 0cm for the waterborne, airborne and control treatments, respectively. The maximum
212 height climbed in the waterborne cue was 66.3cm, whereas the maximum height in the airborne
213 cue was 68.5cm. The maximum height climbed in the control boxes was lower (51.6cm).
214 Across all control boxes, 27% of the individuals were submerged at the end of the trial, while
215 36% of snails in the airborne boxes and only 11% of snails in the waterborne boxes were

216 submerged. As with LA snails, there was also within trial variability in mean height across
217 boxes of the same treatments. Mean height varied by 5.3, 10.9 and 5.2cm across boxes in the
218 waterborne, airborne and control treatments, respectively.

219 When snail populations were placed together, there were significant treatment and
220 location effects ($p < 0.001$ for both), but no significant interaction ($p = 0.585$; Table 1). LA snails
221 climbed significantly higher (41.4 ± 1.2 cm, mean \pm SE) than NC snails (22.8 ± 1.2 , $p < 0.001$)
222 across all treatments (Figure 3A). In addition, across both sites, snails in the airborne (38.3 ± 1.6
223 cm) and waterborne (36.7 ± 1.8 cm) cues climbed significantly higher than those in no cue
224 treatments (21.8 ± 1.3 ; $p < 0.001$ for both). Climbing height in the presence of airborne or
225 waterborne cues were not different from each other ($p = 0.648$; Figure 3B). Within the mixed
226 treatment, LA snails in the presence of crab cues climbed between 19 and 24cm higher on
227 *Spartina* mimics than those not exposed to crab cues, whereas NC snails climbed between 9 and
228 10cm higher when crab cues were present versus absent.

229 **DISCUSSION**

230 Marsh periwinkles have an inducible defense that they exhibit over their broad
231 geographic range, making them an ideal model species of examining geographic variation in
232 predator avoidance behavior. In this study, salt marsh periwinkles from both populations
233 responded to the presence of blue crabs by migrating higher up *Spartina* mimics than those in
234 control treatments, a result consistent with earlier findings (Warren 1985), although here the
235 differences were more apparent when the snails were housed in mixed population treatments.
236 However, it was previously unknown whether the behavioral response, in this case migration
237 distance, might be greater in the lower latitudes. Snails from Louisiana demonstrated a stronger
238 response by migrating farther up the mimics when kept in both the single population and mixed

239 assemblages than their North Carolina counterparts. This mimicked our field observations at
240 both LA and NC, where LA periwinkles consistently and reliably climbed up *Spartina*
241 *alterniflora* stems in response to crab odors, including airborne cues, while those in NC were less
242 consistent in their response. It is therefore possible that the behavioral response of *L. irrorata* to
243 crab presence varies geographically.

244 Several environmental factors may have influenced marsh periwinkle vertical migration
245 (Bingham 1972, Henry, McBride & Williams 1993), including tidal regime (Kimbrow 2012).
246 Louisiana snails in our experiment consistently climbed higher than North Carolina snails, even
247 in the absence of predator cues. The snails should have become entrained with their new
248 conditions in the lab, since periwinkles have been demonstrated to rapidly respond to changing
249 tidal cycles and constant water depth (Hovel, Bartholomew & Lipcius 2001). Further, the tidal
250 amplitude in NC (2m) is greater than in LA (<0.5m; Wang, Lu & Sikora 1993), so we might
251 expect snails from NC to climb higher if amplitude was engrained in their behavioral response.
252 Thus, our observations of snail climbing was opposite the home marsh tidal amplitudes. The
253 different heights between populations in the no predator treatments is unclear. Perhaps the
254 smaller, diurnal tidal range which results in more prolonged periods of marsh flooding
255 experienced in Gulf Coast marshes like LA (Eleuterius & Eleuterius 1979) entrains local snail
256 populations to remain higher when there is water present, since their ecological history suggests
257 some predictability in predation risk. This would suggest some localized adaptation in the
258 induced behavioral response (Trussell & Smith 2000), and further support that different climbing
259 heights is representative of a predator response, even if it is only a site-effect.

260 There are a number of mechanisms that might influence prey response to predators,
261 including both physical and biotic, and unfortunately, these are difficult to elucidate without

262 further experimentation and more sample sites along the geographic range of periwinkle snails.
263 However, in our controlled setting, snails from LA consistently climbed higher than the NC
264 snails. Biotic history and predator differences between the home marshes is a possible
265 explanation for the differential behavioral responses. Although we did not measure crab
266 abundance at the two collection sites, it is possible that there are differences between sites due to
267 geographic locality (Figure 4). Predation pressure varies biogeographically, with predation
268 increasing as latitude decreases (Bertness, Garrity & Levings 1981, Peterson et al. 2001), and
269 numerous prey have responded by increasing defenses along this predation pressure gradient
270 (Bertness, Garrity & Levings 1981, Freestone et al. 2011, Vermeij 1982). Further, predator
271 identity and species composition, which can vary biogeographically, also lead to differential
272 responses in prey species (Large and Smee 2013). Multiple gastropods exhibited different
273 avoidance behaviors across a broad temperate to tropical latitudinal gradient as a result of
274 increased predator diversity (Bertness, Garrity & Levings 1981). It is therefore possible that the
275 observed differences in climbing height between the Louisiana and North Carolina populations
276 of *L. irrorata* in our experiment might reflect differences in predation pressure experienced by
277 the snails at their home marshes.

278 Unfortunately, it is difficult to make broad conclusions about geographic differences
279 using only two study sites, and intraspecific trait variation could be due to a number of other
280 factors that may vary independent of geographic location. For example, parasite load could
281 reduce snail behavioral responses to predators (Belgrad & Smith 2014), and it is unclear whether
282 snails from either site had a higher parasite load which was beyond the scope of this experiment.
283 Additionally, there could be other factors beyond predation pressure that could result in different
284 climbing responses. While our periwinkles were offered food prior to the experiment, biotic

285 history and tissue condition could play a role in response, as hungrier individuals may be more
286 risky (Gilliam & Fraser 1987), and other metrics of condition can alter activity (Pardo & Johnson
287 2004). Although we controlled for hunger by feeding the snails while they were in captivity, the
288 history prior to capture for this experiment could have played a role in site differences.
289 Regrettably, we did not measure condition of the snails after experiments. Additionally, size and
290 shell morphology (i.e., thickness, aperture size, spire length, etc.) might vary across locations for
291 snails (Sepulveda & Ibanez 2012, Ramajo et al. 2013, Kosloski, Dietl & Handley 2016), and
292 could alter their escape responses to predators. Although we sought to use similarly-sized
293 periwinkles from both locations, we did not measure morphometric variables. Finally, it is
294 possible that even though blue crabs are common at both locations, we only used blue crabs
295 collected locally in NC for our experiments, potentially leading to LA snails that were more
296 alarmed by water- and airborne cues from a less familiar population of blue crabs, resulting in an
297 exaggerated response. Regardless of the mechanism driving the differential responses, the snails
298 collected in LA snails exhibited stronger responses to predators in our study system.

299 In addition, the results of this study demonstrate that periwinkles are likely responding to
300 airborne predator cues, a novel observation for an intertidal, marine snail. When the crab was
301 present but water was not allowed to exchange, there appeared to be an intermediate response in
302 single population assemblages with LA snails, resulting in snails climbing ~5cm higher than the
303 no cue treatment snails. While this was not a statistically significant difference, it is the same
304 size of the response in the NC snails with the waterborne cue. It is possible that a volatile
305 compound given off by the crab can become aerosolized and perceived by the periwinkles. This
306 has not been previously documented for marine organisms, however, chemosensory cues are
307 common in terrestrial fauna, including gastropods (Chase et al., 1978, Croll 1983), and a variety

308 of aqueous compounds can be transported via the air, including HAB toxins (Fleming, Backer &
309 Baden 2005), as well as pyrazines, trimethylamine and dimethyl sulfide DMS (Nevitt 2000).
310 Terrestrial gastropods use airborne cues for homing, mating, and finding food (Croll 1983,
311 Chelazzi, Le Voci & Parpagnoli 1988), as well as to avoid predators (Bursztyka et al., 2013,
312 Lefcort, Ben-Ami & Heller 2006). The response to airborne cues from predators has not been
313 identified for other marine, intertidal snails, although, there is some indication that intertidal
314 snails respond to airborne food (Fratini, Cannicci & Vannini 2001) and habitat cues (Lazzeri
315 2017). Since marsh periwinkles spend much of their time out of the water, it is possible they
316 could also be sensitive to airborne cues, and our experimental design was such that all of the
317 snails could have been responding to airborne cues. It is beyond the scope of this experiment to
318 determine which compound is becoming aerosolized and stimulating a response in periwinkles,
319 but that such a chemical might exist warrants further attention.

320 We also note that snail response to cues only became significantly different when the two
321 populations were mixed; that is regardless of origin, snails in mixed assemblages climbed at least
322 twice as high as snails in single population assemblages in response to predator presence. This
323 result is particularly interesting, because it suggests that some alteration in behavior might occur
324 if distinct populations of the same species come into contact. While the probability of LA snails
325 encountering NC snails in the field is low, rafted plant material can transport and disperse fauna
326 great distances (Theil & Fraser 2016), tens to hundreds of kilometers (Dame 1982, Thiel &
327 Gutow 2005, Thiel and Fraser 2016). Further, climate change related distribution shifts (Barry et
328 al. 1995, Zacherl, Gaines & Lonhart 2003, Mieszkowska et al. 2007, Poloczanska et al. 2013)
329 might lead to populations of snails with different predator responses to interact with each other.
330 Thus, it is increasingly likely that distinct populations with different predator responses can come

331 into contact with each other. In the predator cue treatments, it is possible that there was some
332 avoidance between the populations, although the pattern of higher climbing was not observed in
333 the mixed assemblage no cue control treatments. More likely, mixing the two populations
334 together may have led to amplified alarm cues and signaling to other snails. Alarm cues are
335 common, and while typically emitted from injured conspecifics (Jacobsen and Stabell 1999),
336 they could also come from disturbed, but undamaged, conspecifics (Jacobsen and Stabell 2004).
337 Alternatively, the periwinkles could have been responding to mucus trails of the other
338 populations. Conspecific mucus trails have been shown to aid in navigation, homing,
339 aggregation, and mating (Ng et al. 2013), and trails may also be a source of nutrition (Davies and
340 Beckwith 1999). Further, periwinkles may respond to both predator and alarm cues in mucus
341 trails (Duval, Calzetta & Rittschof 1994, Ng et al. 2013). The mechanism for the heightened
342 response in mixed assemblages is unclear, and this experiment was unfortunately not designed to
343 examine this. However, why this response might change in single population vs. mixed
344 assemblages should be pursued in the future.

345 CONCLUSIONS

346 This study demonstrates that geographic origin likely influence the behavioral response
347 to a common predator for periwinkles. It is possible that the differential response to the common
348 predator might be due to different abundance/distribution of blue crabs at the Louisiana/North
349 Carolina home marshes, or just general trends of higher predator density/diversity with
350 decreasing latitude. While further studies are required across more sites to ensure our
351 observations are not just a site effect, these results are promising. In addition, this study is the
352 first to demonstrate that these intertidal snails may also respond to airborne cues from predators.
353 Although the chemical is unknown, that some volatile compound might become aerosolized and

354 illicit a behavioral response in littorinid snails should be explored further. The broad geographic
355 range and behavioral responses of *Littorina irrorata* make it a useful model organism to explore
356 responses to waterborne, airborne, and even mucus-bound predator and alarm cues. Future work
357 should investigate responses of snails from multiple locations along their biogeographic
358 distribution, across multiple predator species which might also vary in abundance throughout the
359 periwinkles' range, and identify compounds from predators and injured conspecifics that might
360 become aerosolized.

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544

545

Figure 1

Conceptual diagram and photograph of experimental box-within-a-box design

Conceptual diagram (A) of the box-within-a-box experimental design. Snails and *Spartina* mimics were placed in the inner plastic box, and when a crab was present, it was placed in the outer box. Inner boxes were either perforated to allow water exchange (as shown in A) or kept solid to prevent water exchange. Photo (B) of the experimental set-up showing the mesh screen to prevent the crab from entering snail tubs and the PVC frames and black plastic curtains that surround each experimental unit to minimize mixing of cues. Symbols courtesy of the Integration and Application Network, University of Maryland Center for Environmental Science (ian.umces.edu/symbols/). Photo by CM Finelli.

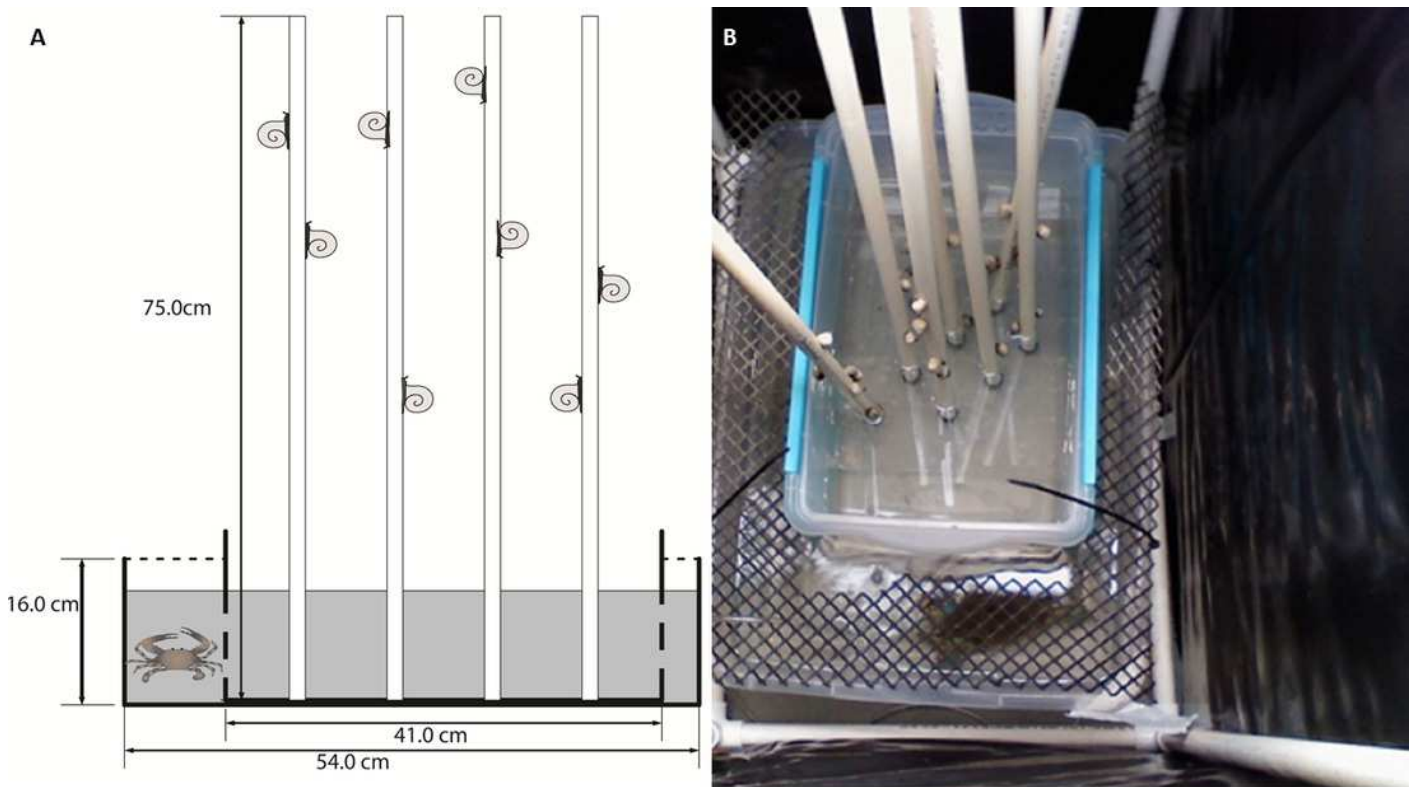


Figure 2

Climbing height of different snail populations across different cue treatments.

Climbing height in single population assemblages for Louisiana (light gray boxes) and North Carolina snails (dark gray boxes) in the presence of no cue, an airborne cue, and a waterborne cue. The boundaries of the box represent the 25th and 75th percentiles, the solid line represents the median, the whiskers are the 10th and 90th percentiles, and the dots represent outliers. Letters above the boxes indicate significant differences in post-hoc analysis.

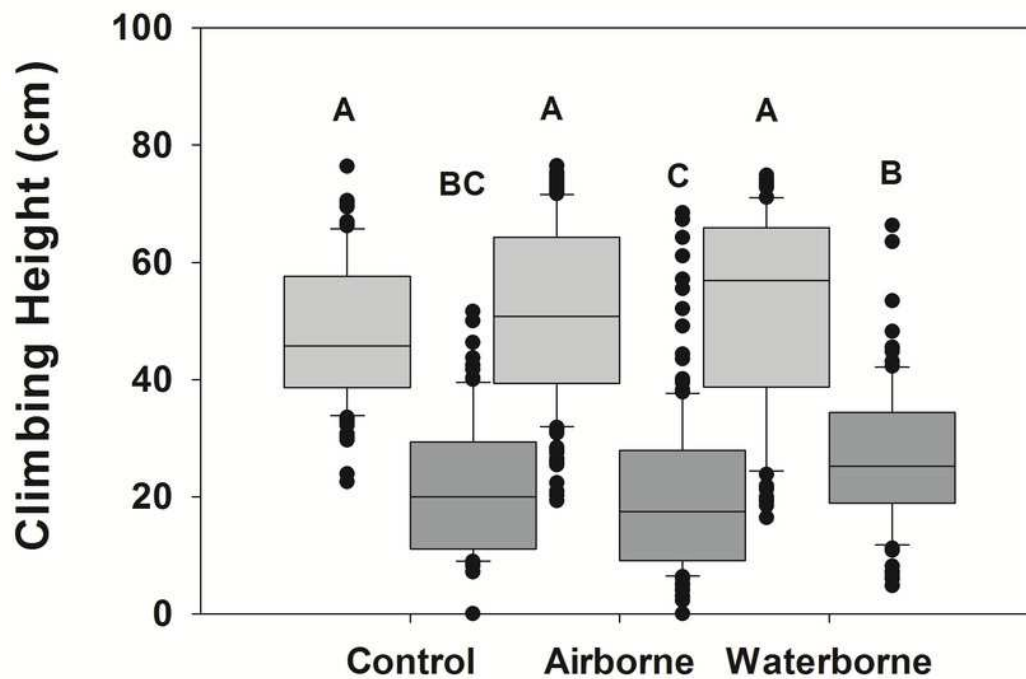


Figure 3

Climbing height for each population and cue treatment in the mixed-population assemblages

Differences in climbing height between the two populations (A) and across all three cue treatments (B) in the mixed-population assemblage experiment. The boundaries of the box represent the 25th and 75th percentiles, the solid line represents the median, the whiskers are the 10th and 90th percentiles, and the dots represent outliers.

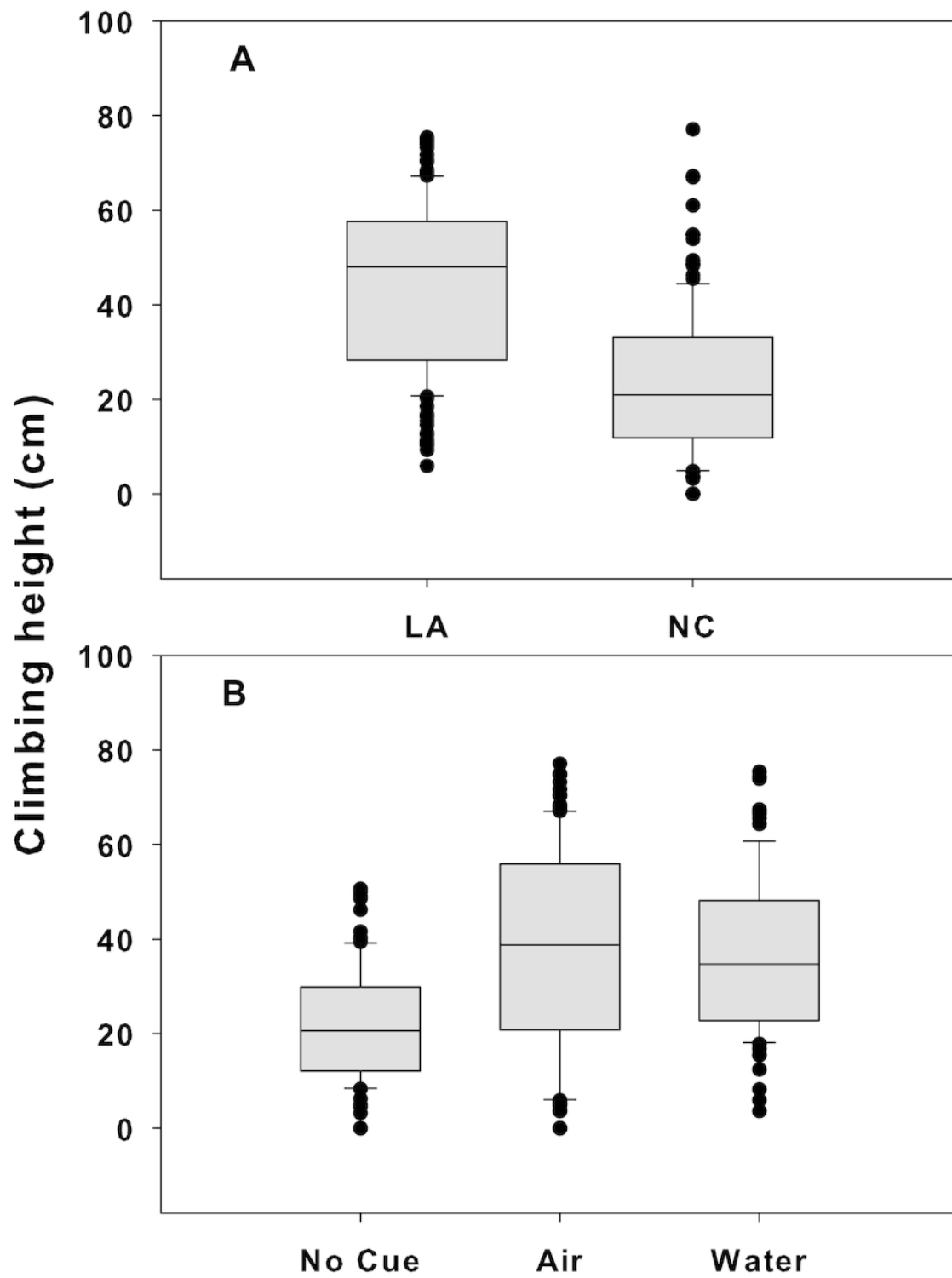


Figure 4

Landings of blue crabs in Louisiana and North Carolina

Blue crab landings from NOAA landings data for blue crabs in LA (solid line) and NC (dotted line) from 1950-2016.

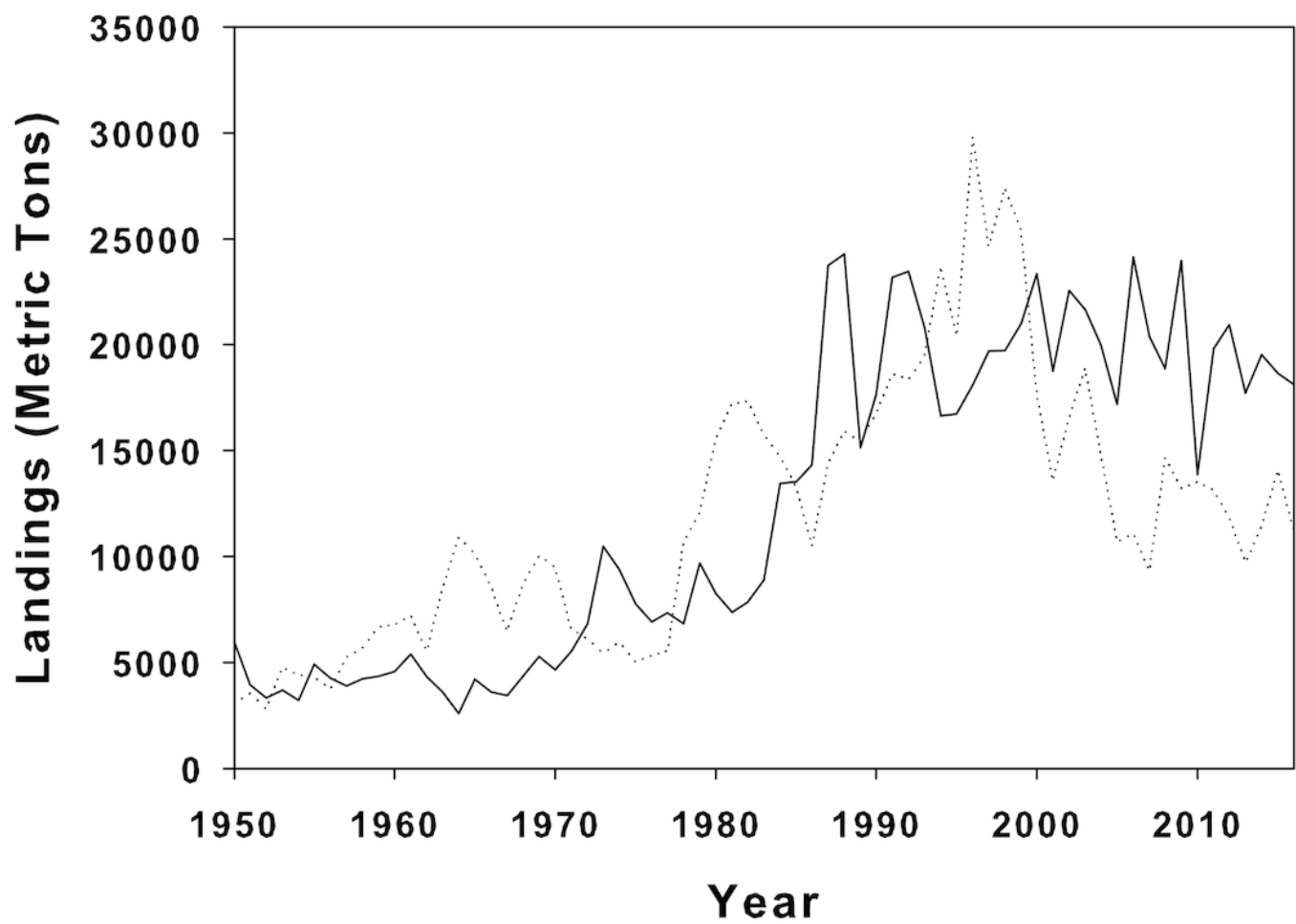


Table 1 (on next page)

Analysis of deviance table for mixed population experiments

Analysis of deviance table for differences in climbing height by site (LA and NC) and cue treatment (no cue, airborne cue, waterborne cue) in the mixed population assemblage using GLMM with site and cue treatment as the fixed factors and tub as the random effect.

1 Response: Climbing Height

	χ^2	Df	p
<i>Treatment</i>	30.9221	2	1.929e-07 ***
<i>Site</i>	63.6062	1	1.520e-15 ***
<i>Treatment x Site</i>	1.0741	2	0.5845

2