Periwinkle climbing response to predator chemical cues depends 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.

1   Periwinkle climbing response to predator chemical cues depends on home-marsh geography

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15 **ABSTRACT** - The salt marsh periwinkle, *Littorina irrorata*, exhibits a spatial refuge from

16 predation by climbing the stems of *Spartina alternaflora* in order to avoid benthic predators.

17 Salt marsh periwinkles have a broad geographic distribution, and for many species, responses to

18 predators also varies with biogeography. This study sought to determine if the geographical

19 location of the home marsh influenced the response of periwinkles (climbing height) to blue crab

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25 populations of snails were housed together. Periwinkle response to predator cues was stronger in

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29 airborne cues. Therefore, salt marsh periwinkles likely respond to numerous cues that initiate

30 behavioral responses, including airborne cues, and these responses may vary by home-marsh

31 geography.

INTRODUCTION

34

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 georgraphic scales. Both predator diversity

53   and predation pressure vary inversely with latitude, so prey organisms have responded by

54   increasing defenses with decreasing latitude (Laurila, Lindgren & Laugen 2008, Freestone et al.

55   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-interidal (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   perwinkles 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   Thus, periwinkles exhibit a spatial refuge from predation by climbing the stems of salt marsh

73   cordgrass, *Spartina alternaflora* Loisel, in order to avoid benthic predators (Dix and Hamilton

74   1993, Vaughn and Fisher 1988).

75   *L. irrorata* has an extensive geographic range (Bingham 1972) and climbing behavior has

76   been noted at the local scale in Virginia (Stanhope, Banta & Temkin 1982), North Carolina

77   (Duval, Calzetta & Rittschof 1994, Lewis and Eby 2002), Georgia (Silliman and Bertness 2002),

78   Florida (Hamilton 1976, Warren 1985), Alabama (Henry, McBride & Williams 1993), Louisiana

79   (*pers. obs.*) and Texas (Vaughn and Fisher 1988). Since predator diversity and predation

80   pressure vary with latitude (Bertness, Garrity & Levings 1981, Freestone et al. 2011), and salt

81   marsh periwinkles exhibit this broad geographic range, they are useful model organisms to

82   explore biogeographic variation in behavioral responses. However, regional comparisons in

83   climbing height and response to predators in marsh periwinkles have not been made previously.

84   A number of other similar species have exhibited differential responses to predators across

85   geographic ranges (Fawcett 1984, Large & Smee 2013). Therefore, periwinkles might exhibit

86   similar differences in induced avoidance responses according to home-marsh geography.

87   The mechanism thought to be driving climbing behavior is response to waterborne

88   chemical cues from either predators or injured conspecifics (Duval, Calzetta & Rittschof 1994,

89   Jacobsen and Stabell 1999), although periwinkles often migrate in advance of the tide. For other

90   intertidal snails, such as *Cerithidea decollata*, it has been suggested that there is an internal clock

91   that might drive migrations (Lazzeri et al. 2014), however *L. irrorata* has been demonstrated to

92   rapidly respond to out of phase tidal cycles in the lab (Hovel, Bartholomew & Lipcius 2001). It

93   is possible that some cues might become aerosolized prior to the arrival of the incoming tide,

94   forewarning snails and cueing them to start climbing (Lazzeri 2017). A number of terrestrial

95   gastropods respond to airborne cues for homing (Chelazzi, Le Vovi & Parpagnoli 1988), feeding

96   (Davis 2004), mating (Ng et al. 2013), and avoiding predators (Bursztyka et al. 2013).

97   Interestingly, it has been suggested that at least two species of intertidal snails may respond to

98   airborne cues from either food (Fratini, Cannicci & Vannini 2001) or the environment (Lazzeri

99   2017). Given the responses to other airborne chemical cues, it is possible that intertidal marine

100   gastropods would also react to airborne cues from predators, particularly snails such as *L.*

101   *irrorata*, which spend much of their time emersed. Yet, responses to potential airborne chemical

102   cues from predators has not been investigated in Littorinids.

103   Relatively little is known about how species might respond to different chemical cues

104   across geographical locations. Since the marsh periwinkle has a broad range and spends much of

105   its time out of the water, it is a useful model organism to explore whether geographic location

106   and the presence of airborne cues affects anti-predator behavioral responses. Further, since

107   climate change could lead to increasing interactions between populations of the same species,

108   periwinkles could also be a useful model organism to see whether behavioral responses change

109   in the presence of individuals from different populations. Field observations in Louisiana

110   demonstrated that periwinkles responded to crabs by climbing up *Spartina alterniflora*, however,

111   similar field observations in North Carolina suggested a lesser response. Thus, we designed a

112   controlled lab experiment to investigate the difference in behavioral response (climbing) of two

113   periwinkle populations to cues from a common predator, the blue crab *Callinectes sapidus*.

114   Specifically, we tested whether the presence of both waterborne and airborne blue crab cues

115   would cause snails to migrate up *Spartina* mimics, and whether the two populations would climb

116   to different heights. Since behavioral responses to predation are likely to vary at different

117   geographic locations and predation pressure increases with decreasing latitude, we hypothesized

118   the Louisiana population of periwinkles would show a greater response to the predator than the

119   North Carolina population by migrating higher on the mimics. Additionally, since intertidal salt

120   marsh periwinkles spend the majority of their time out of the water, we hypothesized that

121   airborne cues would elicit an intermediate behavioral response.

122   **METHODS AND MATERIALS**

123   Louisiana snails were collected from *Spartina alterniflora* marsh adjacent to the

124   Louisiana Universities Marine Consortium (29°15′20.65″N, 90°39′42.93″W) and transported to

125   North Carolina. North Carolina snails were collected from the salt marsh adjacent to the Center

126 for Marine Sciences (34°08'26.26"N, 077°51'47.81"W). These locations were chosen because

127 they are within the range of *L. irrorata* and they share an abundant common predator, blue crabs.

128 However, snails were held in the lab for 2 days prior to the start of the experiment since the

129 snails have been shown to rapidly (~1 d) respond to changes in tidal conditions in the lab (Hovel,

130 Bartholomew & Lipcius 2001). Blue crabs were purchased from a local supplier. All animals

131 were kept in flowing filtered seawater holding tanks. Crabs were fed crushed snails *ad libitum*

132 for 48 hours prior to use in experiments.

133   Lab assays were conducted to investigate regional differences in climbing and to test for

134 chemical cue responses. The experimental unit was a box-in-box mesocosm set-up (Figure 1A).

135 Briefly, we placed a small plastic tub (27 x 41 x 18.5cm), used to house the periwinkles during

136 the experiment, within a larger plastic tub (39 x 54 x 16cm). *Spartina*-mimics were used to

137 simulate marsh grass in lab assays (Hovel, Bartholomew & Lipcius 2001). Eight 75cm tall x

138 1.5cm wide PVC pipes were used in each replicate. The PVC mimics were preferable to natural

139 grass because they are inert (Sueiro, Bortlous & Schwindt 2012), can be easily washed between

140 trials, and are not likely to give off chemical cues like wooden dowel rods or *Spartina* stems. In

141 crab water cue treatments, the small, inner plastic tubs were drilled with small holes to allow

142 water to mix between the inner and outer tubs when filled (Figure 1A). For airborne cues and no

143 cue treatments, the inner boxes were not drilled in order to isolate the water in the small tub.

144 Plastic mesh was used to cover the space between the small and large tubs to prevent movement

145 of animals between the tubs (Figure 1B). We used six box-in-box set-ups per trial for the

146 experiments. Air stones, modified to reduce splashing, were placed in the outer tub for all

147 treatments. Each experimental unit was surrounded on four sides by a 45 x 64 x 90cm open top

148 cage constructed of PVC pipe and a thick black plastic curtain to isolate the replicates from each

149   other, preventing potential transfer of airborne cues between units and reducing the visual

150   disturbance on both snails and crabs. Fluorescent work lights were provided directly above each

151   experimental unit. Temperature was maintained at 28° C in holding tanks and experimental

152   units.

153   Three different treatments were established – a no crab control, a crab present with

154   chemical cues mixing via water exchange between tubs, and a crab present with no water

155   exchange. For the no crab control, ~26 L of clean, filtered and sterilized seawater was placed

156   into the two tubs, for a water depth of 12.5cm. For the crab present treatments, crabs were

157   placed in the large outer tub and allowed to move freely throughout the space between the tubs.

158   For the waterborne cue, the small, inner tubs drilled to allow water exchange were used,

159   circulating the water between tubs and allowing snails to contact water exposed to the blue crab.

160   For the non-waterborne cue, tubs that were not drilled were used, restricting both the crab and

161   crab-cue water to the outer tub, and thus the snails could not directly sample water with crab

162   cues. These tubs were covered with a mesh top which allowed airborne cues to escape. Our

163   mesocosms were undisturbed during the experiment to minimize stimulation of test subjects. We

164   did not notice surface splashing by crabs, they were either stationary or remained submerged for

165   the duration of the trials. Thus any response in these treatments is indicative of an airborne cue.

166   Two sets of experiments were conducted. The first set used either LA or NC snails alone,

167   and two trials were used for each of the NC and LA snails. Sets of 30 snails were placed directly

168   on the PVC approximately 5cm above the water line (~17 cm above the bottom of the tubs) in

169   each mesocosm set-up (n = 180 per trial) and exposed to the different treatments for 6 hours.

170   Observations of snails demonstrated that many initially approached the water at the start of the

171   experiment.

172   Since rafted marsh macrodetritus and debris is common and can travel for tens to

173   hundreds of kilometers and disperse fauna (Dame 1982, Thiel & Gutow 2005, Thiel and Fraser

174   2016) and climate change related distribution shifts (Zacherl, Gaines & Lonhart 2003,

175   Mieszkowska et al. 2007, Poloczanska et al. 2013) might lead to populations of snails with

176   different predator responses to interact with each other, we conducted a second experiment

177   where we combined snails from the two populations. For the second experiment, we also used 2

178   trials, although to keep density per mesocosm the same, we only used 15 snails per home marsh.

179   At the end of each trial, the height of each individual snail was measured.

180   For the single population experiments, North Carolina and Louisiana trials were analyzed

181   separately. Generalized linear mixed models (GLMMs) were used to determing the effect of cue

182   treatment on average climbing height of snails. Cue treatment (no cue, airborne cue, waterborne

183   cue) was modeled as a fixed effect. Since 30 snails were placed into each tub for the experiment,

184   snails within each tubs could not be considered independent of each other (Supplement 1). To

185   account for potential effects of snails located within the same tub, a tub identifier was included

186   as a random effect in the model. GLMMs were also used to determine the effect of home marsh

187   and cue type in the mixed population experiment, with treatment (no cue, airborne cue,

188   waterborne cue) and location (NC and LA) as fixed effects, and tub modeled as a random effect.

189   Models were fit with the function 'glmer' and a gamma distribution using the 'lme4' package

190   (Bates et al. 2015) in R (R Core Team 2015). When significant effects were found, post-hoc

191   general linear hypothesis comparisons were performed using 'ghlt' function 'multcomp' package

192   in R (Hothorn, Bretz & Westfall. 2008).

193   **RESULTS**

194   Snails actively moved up and down the PVC mimics during the experimental period.

195   Within each population, snail height depended on crab presence. The LA snails in the waterborne

196   cue treatment tended to climb the highest, while those with no cue tended to climb the least, and

197   those with airborne cues had an intermediate median height (Figure 2A). LA snails climbed

198   >11cm higher when exposed to a waterborne cue vs. no cue, although there was high variability

199   within each treatment, and these trends were not significant (p = 0.134). For NC snails, those

200   exposed to waterbone crab cues tended to climb >5cm higher than those with no cue present

201   (Figure 2B), although like in the LA snail population, there was considerable variability among

202   individuals. These differences were also not significant (p = 0.062).

203   When snail populations were placed together, there were significant treatment and

204   location effects (p<0.001 for both), but no significant interaction (p = 0.585; Table 1). LA snails

205   climbed significantly higher (41.4 ± 1.2cm, mean ± SE) than NC snails (22.8 ± 1.2, p < 0.001)

206   across all treatments (Figure 3A). In addition, across both sites, snails in the airborne (38.3 ± 1.6

207   cm) and waterborne (36.7 ± 1.8 cm) cues climbed significantly higher than those in no cue

208   treatments (21.8 ± 1.3; p < 0.001 for both), which were not different from each other (p = 0.648;

209   Figure 3B). Within each population in the mixed treatment, LA snails in the presence of crab

210   cues climbed between 19 and 24cm higher on *Spartina* mimics than those not exposed to crab

211   cues, whereas NC snails climbed between 9 and 10cm higher when crab cues were present

212   versus absent.

213   **DISCUSSION**

214   Marsh periwinkles have an inducible defense that they exhibit over their broad

215   geographic range, making them an ideal model species of examining geographic variation in

216   predator avoidance behavior. In this study, salt marsh periwinkles from both populations

217   responded to the presence of blue crabs by migrating higher up *Spartina* mimics than those in

218   control treatments, a result consistent with earlier findings (Warren 1985). However, it was

219   previously unknown whether the behavioral response, in this case migration distance, might be

220   greater in the lower latitudes. Snails from Louisiana demonstrated a stronger response by

221   migrating farther up the mimics when kept in both the single population and mixed assemblages

222   than their North Carolina counterparts. This mimicked our field observations at both LA and NC,

223   where LA periwinkles consistently and reliably climbed up *Spartina alterniflora* stems in

224   response to crab odors, including airborne cues, while those in NC were less consistent in their

225   response. It is therefore possible that the behavioral response of *L. irrorata* to crab presence

226   varies geographically.

227   Environmental factors might influence marsh periwinkle vertical migration (Bingham

228   1972, Henry, McBride & Williams 1993), including tidal regime (Kimbro 2012). Louisiana

229   snails in our experiment consistently climbed higher than North Carolina snails, even in the

230   absence of predator cues. The snails should have become entrained with their new conditions in

231   the lab, since periwinkles have been demonstrated to rapidly respond to changing tidal cycles

232   and constant water depth (Hovel, Bartholomew & Lipcius 2001). Further, the tidal amplitude in

233   NC (2m) is greater than in LA (<0.5m; Wang, Lu & Sikora 1993), so we might expect snails

234   from NC to climb higher if amplitude was engrained in their behavioral response. Thus, our

235   observations of snail climbing was opposite the home marsh tidal amplitudes. The different

236   heights between populations in the no predator treatments is unclear. Perhaps the smaller, diurnal

237   tidal range which results in more prolonged periods of marsh flooding experienced in Gulf Coast

238   marshes like LA (Eleuterius & Eleuterius 1979) entrains local snail populations to remain higher

239   when there is water present, since their ecological history suggests some predictability in

240 predation risk. This would suggest some localized adaptation in the induced behavioral response

241 (Trussell & Smith 2000), and further support that different climbing heights is representative of a

242 predator response, even if it is only a site-effect.

243   There are a number of mechanisms that might influence prey response to predators,

244 including both physical and biotic, and unfortunately, these are difficult to elucidate without

245 further experimentation and more sample sites along the geographic range of periwinkle snails.

246 However, in our controlled setting, snails from LA consistently climbed higher than the NC

247 snails. Biotic history and predator differences between the home marshes is a possible

248 explanation for the differential behavioral responses. Although we did not measure crab

249 abundance at the two collection sites, it is possible that there are differences between sites due to

250 geographic locality (Figure 4). Predation pressure varies biogeographically, with predation

251 increasing as latitude decreases (Bertness, Garrity & Levings 1981, Peterson et al. 2001), and

252 numerous prey have responded by increasing defenses along this predation pressure gradient

253 (Bertness, Garrity & Levings 1981, Freestone et al. 2011, Vermeij 1982). Further, predator

254 identity and species composition, which can vary biogeographically, also lead to differential

255 responses in prey species (Large and Smee 2013). Similarly, multiple gastropods exhibited

256 different avoidance behaviors across a broad temperate to tropical latitudinal gradient as a result

257 of increased predator diversity (Bertness, Garrity & Levings 1981). It is therefore possible that

258 the observed differences in climbing height between the Louisiana and North Carolina

259 populations of *L. irrorata* in our experiment might reflect differences in predation pressure

260 experienced by the snails at their home marshes.

261   Unfortunately, it is difficult to make broad conclusions about geographic differences

262 using only two study sites. Other factors could lead to site differences in predation pressure

263   independent of geographic location. For example, parasite load could reduce snail behavioral

264   responses to predators (Belgrad & Smith 2014), and it is unclear whether snails from either site

265   had a higher parasite load which was beyond the scope of this experiment. Additionally, there

266   could be other factors beyond predation pressure that could result in different climbing

267   responses. While our periwinkles were offered food prior to the experiment, biotic history and

268   tissue condition could play a role in response, as hungrier individuals may be more risky

269   (Gilliam & Fraser 1987), and other metrics of condition can alter activity (Pardo & Johnson

270   2004). Additionally, size and shell morphology (i.e., thickness, aperture size, spire length, etc.)

271   might vary across locations for snails (Sepulveda & Ibanez 2012, Ramajo et al. 2013, Kosloski,

272   Dietl & Handley 2016), and could alter their escape responses to predators. Although we sought

273   to use similarly-sized periwinkles from both locations, we did not measure other morphometric

274   variables. Finally, it is possible that even though blue crabs are common at both locations, we

275   only used blue crabs collected locally in NC for our experiments, potentially leading to LA snails

276   that were more alarmed by water- and airborne cues from a less familiar population of blue

277   crabs, resulting in an exaggerated response. Regardless of the mechanism driving the differential

278   responses, the snails collected in LA snails exhibited stronger responses to predators in our study

279   system.

280   The results of this study also provide a novel observation for an intertidal, marine snail –

281   they are likely responding to airborne predator cues. When the crab was present but water was

282   not allowed to exchange, there appeared to be an intermediate response in single population

283   assemblages with LA snails, resulting in snails climbing ~5cm higher than the no cue treatment

284   snails. While this was not a statistically significant difference, it is the same size of the response

285   in the NC snails with the waterborne cue. It is possible that a volatile compound given off by the

286   crab can become aerosolized and perceived by the periwinkles. This has not been previously

287   documented for marine organisms, however, chemosensory cues are common in terrestrial fauna,

288   including gastropods (Chase et al., 1978, Croll 1983), and a variety of aqueous compounds can

289   be transported via the air, including HAB toxins (Fleming, Backer & Baden 2005), as well as

290   pyrazines, trimethylamine and dimethyl sulfide DMS (Nevitt 2000). Terrestrial gastropods use

291   airborne cues for homing, mating, and finding food (Croll 1983, Chelazzi, Le Voci & Parpagnoli

292   1988), as well as to avoid predators (Bursztyka et al., 2013, Lefcort, Ben-Ami & Heller 2006).

293   The response to airborne cues from predators has not been identified for other marine, intertidal

294   snails, although, there is some indication that intertidal snails respond to airborne food (Fratini,

295   Cannicci & Vannini 2001) and habitat cues (Lazzeri 2017). Since marsh periwinkles spend

296   much of their time out of the water, it is possible they could also be sensitive to airbone cues, and

297   our experimental design was such that all of the snails could have been responding to airborne

298   cues. It is beyond the scope of this experiment to determine which compound is becoming

299   aerosolized and stimulating a response in periwinkles, but that such a chemical might exists

300   warrants further attention.

301   We also note that snails in mixed assemblages, regardless of origin, also climbed at least

302   twice as high as snails in single population assemblages in response to predator presence. This

303   result is particularly interesting, because it suggests that some alteration in behavior might occur

304   if distinct populations of the same species come into contact. While the probability of LA snails

305   encountering NC snails in the field is low, rafted plant material can transport and disperse fauna

306   great distances (Theil & Fraser 2016). Combined with distribution shifts due to climate change

307   (Barry et al. 1995), it is increasingly likely that distinct populations with different predator

308   responses can come into contact with each other.   In the predator cue treatments, it is possible

309   that there was some avoidance between the populations, although the pattern of higher climbing

310   was not observed in the mixed assemblage no cue control treatments. More likely, mixing the

311   two populations together may have led to amplified alarm cues and signaling to other snails.

312   Alarm cues are common, and while typically emitted from injured conspecifics (Jacobsen and

313   Stabell 1999), they could also come from disturbed, but undamaged, conspecifics (Jacobsen and

314   Stabell 2004).   Alternatively, the periwinkles could have been responding to mucus trails of the

315   other populations. Conspecific mucus trails have been shown to aid in navigation, homing,

316   aggregation, and mating (Ng et al. 2013), and trails may also be a source of nutrition (Davies and

317   Beckwith 1999). Further, periwinkles may respond to both predator and alarm cues in mucus

318   trails (Duval, Calzetta & Rittschof 1994, Ng et al. 2013).   The mechanism for the heightened

319   response in mixed assemblages is unclear, and this experiment was unfortunately not designed to

320   examine this. However, why this response might change in single population vs. mixed

321   assemblages should be pursued in the future.

322   **CONCLUSIONS**

323   This study demonstrates that geographic origin might influence the behavioral response

324   to a common predator for periwinkles. It is possible that the differential response to the common

325   predator might be due to different abundance/distribution of blue crabs at the Louisiana/North

326   Carolina home marshes, or just general trends of higher predator density/diversity with

327   decreasing latitude.   While further studies are required across more sites to ensure our

328   observations are not just a site effect, these results are promising. In addition, this study is the

329   first to demonstrate that these intertidal snails may also respond to airborne cues from predators.

330   Although the chemical is unknown, that some volatile compound might become aerosolized and

331   illicit a behavioral response in littorinid snails should be explored further.   The broad geographic

332 range and behavioral responses of *Littorina irrorata* make it a useful model organism to explore

333 responses to waterborne, airborne, and even mucus-bound predator and alarm cues. Future work

334 should investigate responses of snails from multiple locations along their biogeographic

335 distribution, across multiple predator species which might also vary in abundance throughout the

336 periwinkles' range, and identify compounds from predators and injured conspecifics that might

337 become aerosolized.

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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 places 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/)

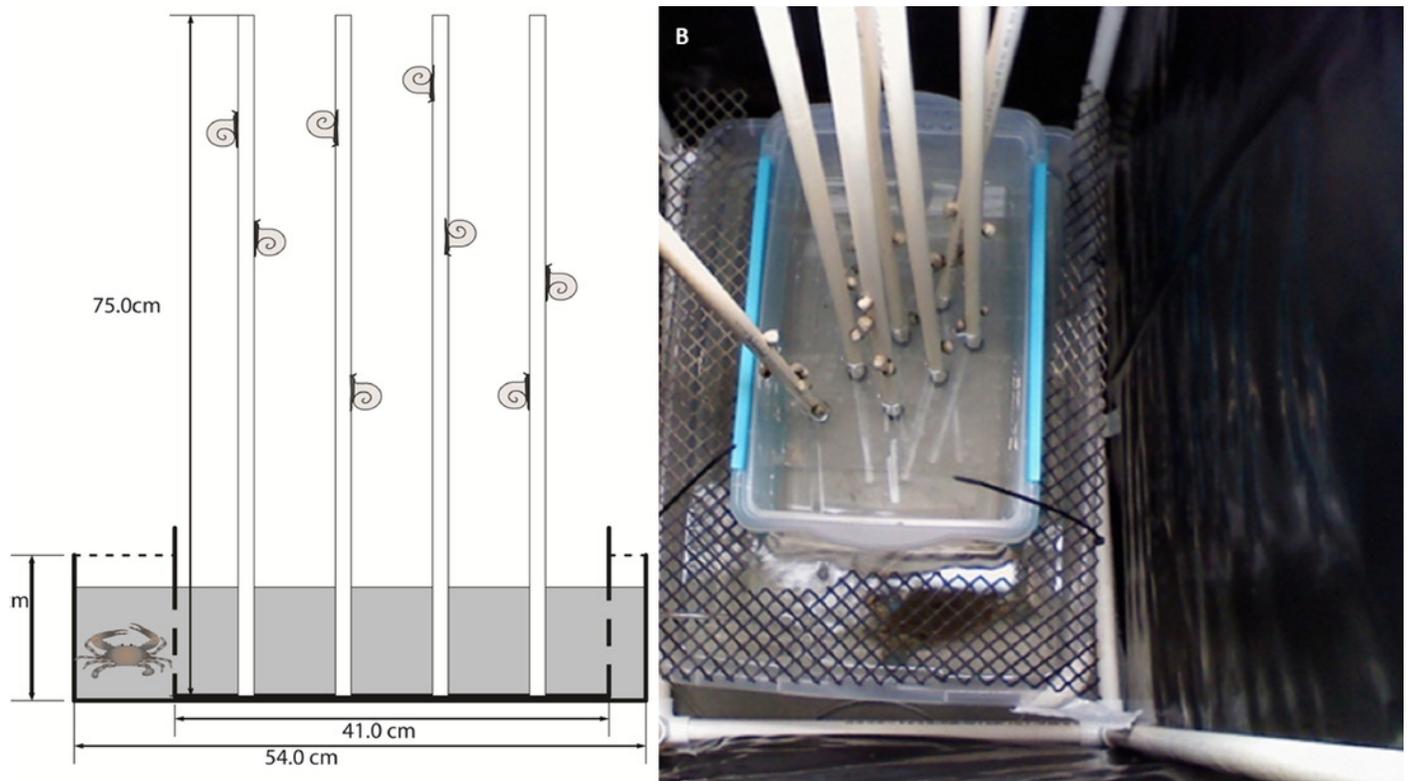



Figure 2

Climbing height of different snail populations across different cue treatments.

Climbing height in single population assemblages for Louisiana (A) and North Carolina snails (B) 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.

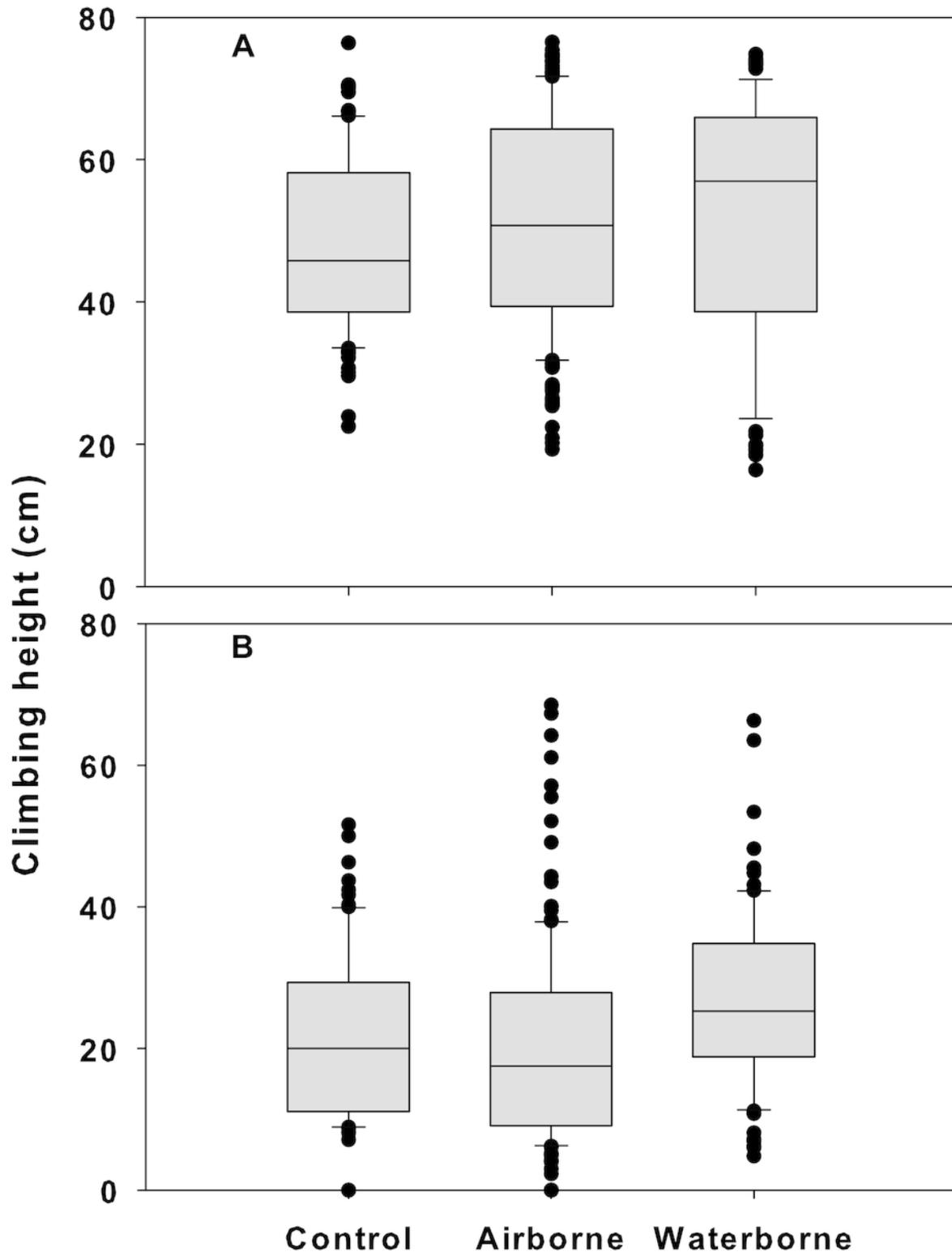



Figure 3

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.

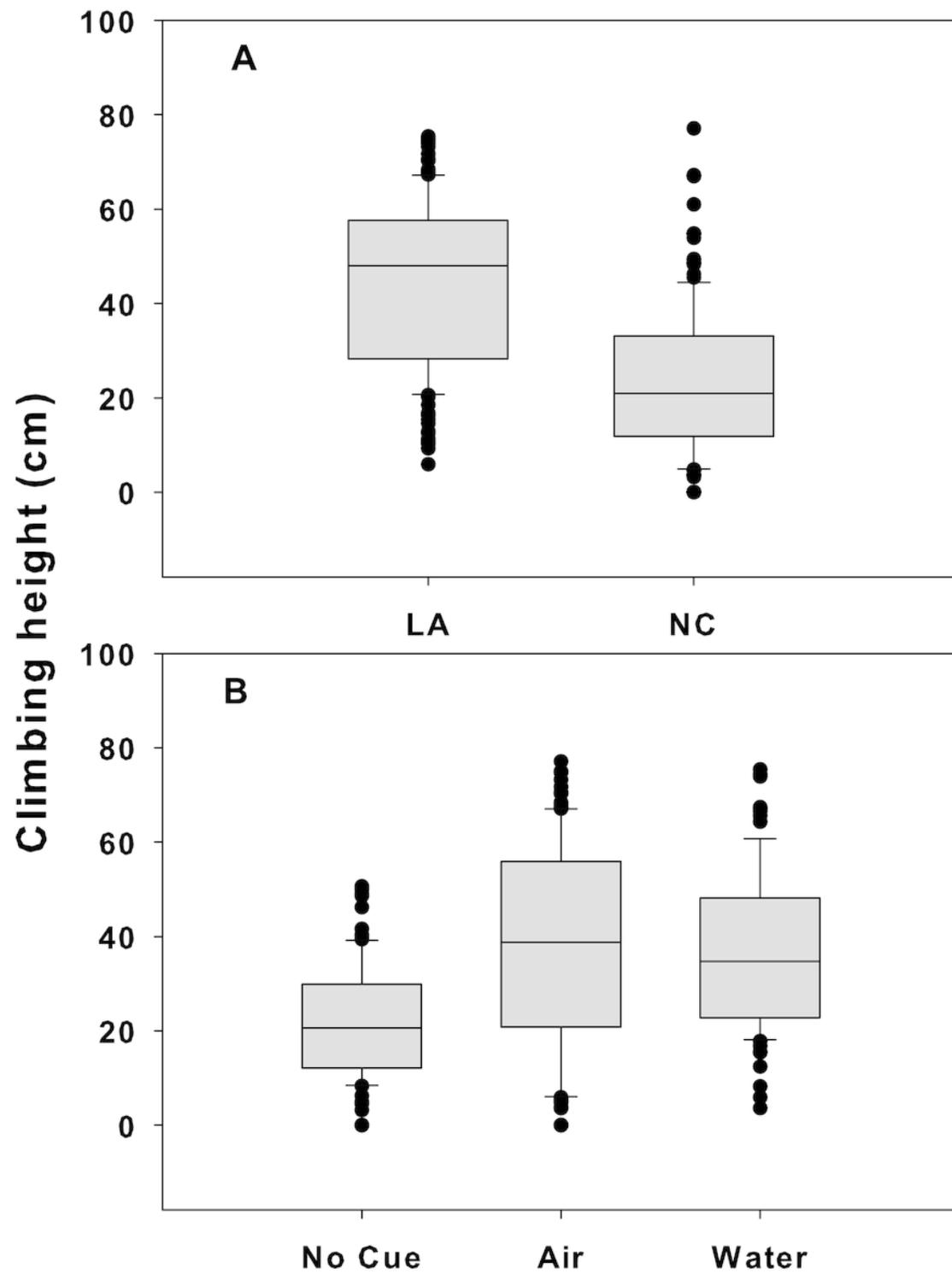
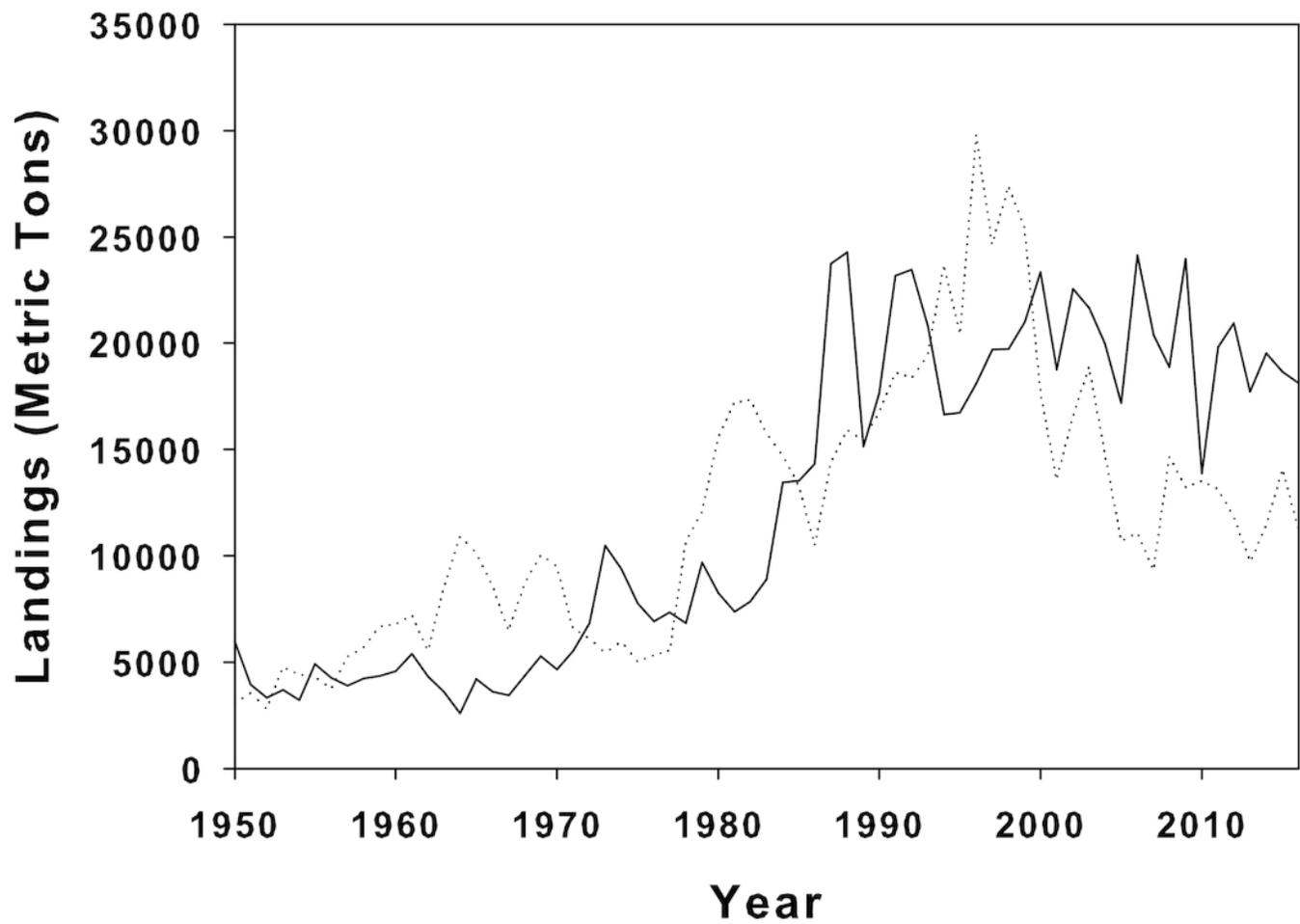



Figure 4

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.

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
Treatment	30.9221	2	1.929e-07 ***
Site	63.6062	1	1.520e-15 ***
Treatment x Site	1.0741	2	0.5845