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Predator-induced plasticity in egg capsule deposition in the mud snail,
Tritia obsoleta

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Running Page Head: Plasticity in mud snail egg deposition

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Abstract:

Most marine invertebrates develop in the plankton, where microscopic offspring can avoid abundant benthic predators until settlement. However, at least four phyla of marine invertebrates (Annelida, Mollusca, Nemertea, and Platyhelminthes) deposit benthic egg capsules or masses. Often, these animals possess additional means to protect their young, including chemical or morphological defenses or nonrandom selection of deposition sites. Egg capsule deposition is the dominant reproductive strategy among gastropod molluscs, including the mud snail, *Tritia obsoleta*. In intertidal and shallow subtidal habitats in New England, the mud snail preferentially deposits egg capsules on blades of eelgrass (*Zostera marina*), a substrate that stands upright in the water column. In a field and lab study, we examined deposition of *T. obsoleta* egg capsules and found that mud snails lay their egg capsules on eelgrass at 6-8 cm off the benthos or higher. When exposed to egg capsule predators, hermit crabs and periwinkles, mud snails increase the average height of deposition off the benthos by 1-3 cm. In the presence of hermit crabs, capsules deposited on a blade of eelgrass 5 cm above the benthos have survivorship as much as 4 times higher than capsules deposited directly on the benthos. We suggest that deposition of egg capsules off of the benthos is an adaptive response allowing mud snails to protect their embryos from benthic predators. We also provide evidence that snails use characteristics of the eelgrass itself to ensure capsules are laid well above the benthos.

Keywords: Plasticity, Predator-prey interactions, Gastropods, Eggs, Eelgrass, Mixed Development

1 **Introduction**

2 The vast majority of marine invertebrates exhibit complex life cycles with embryonic and
3 larval development occurring independent of the mother, either as freely spawned eggs, embryos
4 and larvae, or as encapsulated embryos (Thorson 1950, Strathmann 1985). Thorson (1950)
5 estimated that 55-85% of all benthic marine invertebrate species exhibit extended (weeks or
6 months) planktonic development through a series of embryonic and larval stages. Despite the
7 prevalence of planktonic development, many species spend part or all of their developmental
8 period on the benthos (Strathmann 1987, Pechenik 1999, Strathmann 2007). Among those
9 species that develop in benthic egg capsules, some hatch directly as crawl-away juveniles (e.g.,
10 the gastropods *Nucella lapillus*, and *Urosalpinx cinerea*, and some nemerteans, polychaetes, and
11 flatworms; Christiansen & Fenchel 1979, Crothers 1985, Martel & Chia 1991, Ruiz-Trillo et al.
12 1999) while others exhibit mixed development (Pechenik, 1979), starting from benthic egg
13 capsules from which planktonic larvae hatch (Grassle & Grassle 1974, Pechenik 1979, Caswell
14 1981, Strathmann 1985). Compared to planktonic development, benthic development exposes
15 vulnerable eggs, embryos, and larvae to higher rates of predation (Allen & McAlister 2007) and
16 thus we might expect unprotected benthic development to be selected against over evolutionary
17 time (Strathmann 2007). However, protected (i.e. encapsulated or brooded) benthic development
18 may be favored in areas with many predators, high risk of desiccation or other environmental
19 stressors (Pechenik 1999). As in other animals with complex life cycles, the timing of transitions
20 from one habitat to another during development is likely governed by a tradeoff between growth
21 and risk of mortality (Werner 1986). In mixed development, an initial period of benthic
22 encapsulation may decrease the probability of planktonic mortality before metamorphosis by
23 reducing the amount of development time spent in the plankton (Pechenik 1979). In general,
24 pelagic habitats have been found to be a lower mortality environment than the benthos for

25 unprotected embryos (Allen & McAlister 2007, Vaughn & Allen 2010). However there is also
26 evidence that later developmental stages of invertebrates are less vulnerable to planktonic
27 predators (Pennington & Chia 1984, Rumrill et al. 1985, Allen 2008). Benthic encapsulation may
28 thus be favored when it allows embryos to be protected until they are later stage larvae that are
29 better able to resist planktonic threats (Pechenik 1979).

30 In addition to switching habitats during development to optimize mortality and growth
31 rates, phenotypic plasticity can be another response to development under stressful conditions.
32 For example, larval gastropods change shell morphology during planktonic development in the
33 presence of predatory zooplankton, resulting in increased survival in the presence of predators
34 (Vaughn 2007). On the benthos, the nudibranch *Phestilla sibogae* lays eggs that may hatch early
35 if they are disturbed by a potential predator (Strathmann et al. 2010, Oyarzun & Strathmann
36 2011), while the embryos of another gastropod, *Nucella lamellosa*, delay hatching in the
37 presence of crab predators (Miner et al. 2010). In addition to the direct responses by embryos and
38 larvae described above, adults can also modulate their investment in protective structures in
39 response to predator cues. The dogwhelk, *Nucella emarginata*, produces egg capsules with
40 thicker walls in habitats with higher concentrations of the predatory isopod, *Idotea wosnesenskii*,
41 which feeds by chewing through the capsule wall (Rawlings 1990). Similarly, Schwab & Allen
42 (2014) found that the mud snail, *Tritia obsoleta*, when exposed to predatory crab cues, lays egg
43 capsules with longer protective spines. In both *N. emarginata* and *T. obsoleta*, these changes in
44 maternal investment resulted in reduced susceptibility to crustacean predators.

45 The eastern mud snail, *T. obsoleta*, is a common gastropod and a useful model organism
46 for studying plasticity because of its high abundance (up to 8000 snails m⁻², personal
47 observation) on intertidal mudflats across a wide geographic range along the East Coast of North

48 America from the Gulf of St. Lawrence to the Gulf of Mexico (Cranford 1988). During the
49 reproductive season, an individual adult *T. obsoleta* deposits about 100 capsules, each containing
50 30-300 eggs (Pechenik 1978, Brenchley 1982, Rittschof et al. 2002). *T. obsoleta* deposits benthic
51 egg capsules on solid objects (shells, worm tubes, algae, vegetation, etc.) which are frequently
52 limiting in the soft sediment systems where mud snails are most common (Scheltema 1967,
53 Sullivan & Mangel 1984). Embryos develop within the capsules for two weeks before hatching
54 as planktonic veliger larvae (Sullivan & Mangel 1984). With no parental care beyond the
55 investment in the protective capsule, the eggs are at high risk from benthic predators (Brenchley
56 1982). Thus, where adult snails deposit their egg capsules may have substantial implications for
57 the survival of their offspring. In New England, mud snails tend to lay their egg capsules on
58 blades of eelgrass (*Zostera marina*) and egg capsules are preyed upon by benthic predators
59 including green crabs (*Carcinus maenas*) and hermit crabs (*Pagurus longicarpus*), and displaced
60 by the foraging behavior of periwinkles (*Littorina littorea*; Brenchley 1982).

61 The goal of this project was to examine the deposition of egg capsules of the mud snail,
62 *T. obsoleta*, on the coast of Maine and to determine if and how snails may exhibit plasticity in
63 capsule placement in response to egg capsule predators. In particular, we hypothesized that (1) *T.*
64 *obsoleta* lays egg capsules preferentially on eelgrass over other available substrates, (2) egg
65 capsules on eelgrass are preferentially deposited above the benthos, (3) *T. obsoleta* increases the
66 height of egg capsule deposition in the presence of predators, and (4) egg capsules which are
67 deposited farther off of the benthos are less likely to be consumed by predators.

68 **Methods**

69 **Field observations of mud snails, egg capsules and eelgrass**

70 Our field site was a mudflat adjacent to the Bowdoin College Coastal Studies Center
71 (CSC) on Orr's Island, Maine (43° 79' N, 69° 95' W). To assess the distribution of mud snail
72 (*Tritia obsoleta*) egg capsules, 56 plots were surveyed along three equidistant and parallel
73 transects in a large tide pool on the CSC mudflat. We used a 1 m² quadrat to count the number of
74 mud snails, eelgrass plants (*Zostera marina*), and the proportion of blades of eelgrass bearing
75 mud snail egg capsules in each plot. If there were >100 mud snails in a plot, the number of mud
76 snails was extrapolated from five randomly subsampled 10 cm² divisions. We used the curve
77 estimation procedure in SPSS version 22 to determine significant correlations and relationships
78 between the number of mud snails, eelgrass plants, and the proportion of eelgrass blades bearing
79 egg capsules.

80 **Eelgrass transplant experiment**

81 Initial observations indicated that the vast majority of egg capsules were laid on eelgrass.
82 To determine whether this was due to convenience or preference, we conducted a transplant
83 experiment in a section of the CSC mudflat in which eelgrass was absent. Nine 1 m² plots were
84 marked in the mud set 2m apart in a 3 x 3 grid. The initial number of snails in each plot was
85 recorded, as well as in nine haphazardly sampled background plots. We then planted a ring of 10
86 bare eelgrass plants in each plot by pushing the roots of the plants approximately a finger's depth
87 into the mud and then packing around the base of the plants. After five days, we once again
88 counted the number of snails in the marked plots and in nine haphazardly sampled background
89 plots.

90 We conducted a 2-way analysis of variance (ANOVA) for the number of snails as the
91 dependent variable. Time (before and after the transplant), location (plot with transplants or
92 background plots), and the interaction between time and location were modeled as fixed effects.
93 Data were square-root transformed to obtain normally distributed residuals, as confirmed by
94 Kolmogorov–Smirnov and Shapiro-Wilk tests.

95 **Substrate Preference**

96 We conducted a preference experiment in the CSC laboratory to determine the preferred
97 laying substrate for mud snails. Coarsely filtered (250 μm) seawater pumped directly from
98 Harpswell Sound was used in the flow-through seawater lab of the CSC. Any background levels
99 of chemical cues in the water from Harpswell Sound were therefore provided to all treatments,
100 including controls. Seawater was directed into a bucket with 30 holes drilled into the bottom.
101 Lengths of clear vinyl tubing, 5/16" in outer diameter, were fit tightly into these holes and then
102 into replicate plastic containers through holes in the lids. Excess water escaped around the edges
103 of the lids.

104 Large adult mud snails (≥ 20 mm shell length) were collected from the field and randomly
105 assigned into each of 10 replicate plastic containers (15 x 21 x 7.5 cm) at a density of 18 snails
106 per container, well within the range of natural densities of snails observed in the field. Each
107 replicate container held a sample of hard substrates which were prevalent on the CSC mudflat:
108 an oyster shell (*Ostrea edulis*), a mussel shell (*Mytilus edulis*), a hard-shell clam shell
109 (*Mercenaria mercenaria*), a soft-shell clam shell (*Mya arenaria*), an eelgrass plant (*Z. marina*),
110 and a sand collar (*Euspira heros* eggs). The container was added as a substrate, though not of
111 experimental interest, once snails laid egg capsules upon the container walls. The experiment ran
112 for 1 week, and the number of egg capsules laid upon each substrate was recorded each day.

113 **Egg capsule placement on eelgrass**

114 To determine at what height mud snails lay egg capsules on eelgrass, we quantified the
115 position of egg capsules upon eelgrass from the field. Twenty-eight eelgrass plants bearing egg
116 capsules were haphazardly collected from the CSC mudflat. The plants were categorized as
117 exhibiting either low or high densities of egg capsules. Low-density plants had patchy coverage
118 of a single layer of egg capsules, while high-density plants had many (thousands) more egg
119 capsules in multiple layers covering the blades of eelgrass.

120 We measured the placement of egg capsules along the length of each eelgrass blade,
121 designating the plant's node as 0 cm. We recorded the total length of the blade and the bottom
122 and top height of each group of capsules as well as the number of capsules per group. Heights
123 are reported as measurements from the node rather than as percentage of height along the blade
124 of eelgrass since the raw height off the benthos is more relevant for this study. If necessary, for
125 plants with high densities of capsules, layers of egg capsules were removed and counted. We
126 performed independent samples t-tests to compare the bottom and top heights of egg capsules
127 between the two density levels. The bottom height data were ln transformed, and the top height
128 square-root transformed to fit the independent t-test assumption of normality, as confirmed by
129 Kolmogorov-Smirnov and Shapiro-Wilk tests.

130 We also monitored egg capsule placement on eelgrass in the laboratory. Ten containers
131 (15 x 21 x 7.5 cm) were filled with 2-3 cm of mud, and one eelgrass plant was planted in each
132 container. We randomly assigned 18 large adult snails to each container and tracked where
133 capsules were deposited on eelgrass over 11 days. Since not all snails started laying eggs at the
134 same time, not all containers are represented past two or three days of laying. These data were
135 used to determine whether the position of mud snail egg capsules in the field indicates preference

136 or is an artifact of predation (i.e. snails could deposit egg capsules all along a blade of eelgrass,
137 but the lowest capsules deposited could be preferentially consumed post-deposition). We
138 conducted one-way ANOVAs for the dependent variables of top height and bottom height, with
139 day as the fixed effect. The bottom and top height data were square-root transformed to fit the 1-
140 way ANOVA assumption of normality, as confirmed by Kolmogorov-Smirnov and Shapiro-
141 Wilk tests.

142 We next performed an experiment to determine if snails were selecting deposition heights
143 based on height *per se* or based on plant morphology (blade vs. sheath). To do so, eelgrass plant
144 blades and sheaths were separated and attached to the bottom of 5 replicate plastic containers.
145 Two blades and two sheaths were randomly assigned to positions in each container along with 18
146 large adult snails. After 24 hours, we recorded the number of egg capsules on each surface and
147 the bottom height of capsules. We ran t-tests with substrate as the grouping variable. The bottom
148 height was ln transformed to fit the t-test's assumption of normality, as confirmed by
149 Kolmogorov-Smirnov and Shapiro-Wilk tests.

150 **Effect of predators on egg capsules placement**

151 We hypothesized that mud snails would change the height at which they deposit egg
152 capsules in the presence of predators. Hermit crabs and periwinkles were used as egg-capsule
153 predators since hermit crabs were observed to be voracious predators of egg capsules, and
154 periwinkles bulldoze mud snail egg capsules (Brenchley 1982) and are extremely abundant on
155 the CSC mudflat. There were 10 replicates each of three predator cue treatments: hermit crab,
156 periwinkle, and control (ambient sea water only). Treatment containers (15 x 21 x 7.5 cm) held
157 10 predators of similar size so that each experimental chamber received a similar strength of
158 chemical cue from the predators. Predators fasted during the duration of the experiment. Holes

159 were drilled in the sides of treatment containers so that tubing (5/16" outer diameter) could
160 deliver cued water from treatment containers to experimental containers (Figure 1). Experimental
161 containers each held 18 randomly assigned large adult mud snails and an eelgrass plant. Excess
162 water escaped from around the lid. Once laying began, we recorded the number and position of
163 egg capsules on the eelgrass every four days. Blades of eelgrass that broke off from the plant
164 were discarded if we could not determine the bottom height of egg capsules, and approximately 6
165 hermit crabs were replaced after mortality. The experiment ran for 10 days before the majority of
166 eelgrass blades broke.

167 In order to test for predator effects on capsule deposition, we ran 1-way ANOVAs on the
168 transformed data with predator as the fixed effect. Data on the lowest height at which capsules
169 were laid were square-root transformed, and data for the highest height at which capsules were
170 laid were ln transformed to fit the 1-way ANOVA assumption of normality, as confirmed by
171 Kolmogorov-Smirnov and Shapiro-Wilk tests. Where significant effects of predator were found,
172 we ran a Bonferroni post-hoc test to test for differences between the three treatments.

173 **Survival of egg capsules on eelgrass**

174 To assess how predation varied with height along an eelgrass plant, we manipulated the
175 position of egg capsules along blades of eelgrass. Each blade was 17 cm long, and the first 1 cm
176 of eelgrass was glued to the bottom of the container. A 2 x 5 clump of egg capsules
177 (approximately 1 cm in height) was then glued at 0 cm, 5 cm, 10 cm, and 15 cm from the base,
178 for a total of 40 capsules added to each blade. One-cm sections of eelgrass blades bearing egg
179 capsules were cut from other plants using a razor blade and then the eelgrass/capsule assembly
180 was glued to the intact 17 cm length of eelgrass blade using Krazy glue ®. Two container heights
181 were used to mimic low and high tide conditions: the 7.5 cm deep plastic containers used in prior

182 experiments, as well as 20 cm deep containers. These containers differed in the degree to which
183 the tip of the eelgrass could float above the benthos. Fourteen replicates of each type of container
184 were supplied with flow-through water and 3 hermit crabs were randomly assigned to each
185 container.

186 Every day for 8 days, the number of egg capsules surviving at each height in each
187 container was recorded. Any blades or sections of egg capsules that came unglued (1-2
188 detachments per day) were reattached. A binomial logistic regression was run using the variables
189 of capsule height, container depth, day, and interaction terms as predictors. The inclusion of all
190 interaction terms resulted in the lowest log-likelihood score. Following the regression analysis,
191 we performed a Hosmer and Lemeshow test to determine the goodness of fit of the model
192 (Hosmer & Lemeshow 1980).

193 **Results**

194 **Field Observations**

195 In the field, the correlation between the number of mud snails and the number of blades
196 of eelgrass m^{-2} was positive, significant and best fit by a cubic function (Figure 2A). High
197 densities of snails ($>200 m^{-2}$) were only seen in plots with ≥ 75 blades of eelgrass m^{-2} (Figure
198 2A), suggesting that mud snail density is higher in areas with more eelgrass.

199 The proportion of eelgrass blades on which mud snail egg capsules were laid was also
200 positively and significantly correlated with the number of eelgrass blades m^{-2} (Figure 2B) and the
201 relationship was best fit by a power function. In areas with sparser eelgrass (<75 blades m^{-2}) egg
202 capsule deposition was maximally variable, with anywhere from 0-100% of eelgrass blades
203 bearing egg capsules. In plots with high densities of eelgrass (>75 blades m^{-2}), an average of

204 90% of eelgrass blades bore egg capsules, whereas in plots with low densities of eelgrass (<75
205 blades m⁻²), an average of 40% of eelgrass blades bore egg capsules (Figure 2B).

206 **Eelgrass Transplant**

207 The experimental addition of eelgrass yielded significant increases in the mean number of
208 snails in each plot (Figure 3). The number of snails varied with time (before versus after
209 transplantation; 2-way ANOVA, $F_{1,34} = 10.717$, $p = 0.002$) and location (inside versus outside
210 transplant zones; 2-way ANOVA, $F_{1,34} = 20.595$, $p < 0.001$), and there was a statistically
211 significant interaction between time and location (2-way ANOVA, $F_{1,34} = 18.321$, $p < 0.001$)
212 such that snails increased by an order of magnitude in the plots into which eelgrass was
213 transplanted while slightly declining in plots without eelgrass addition (Figure 3).

214 **Substrate Preference**

215 In the lab experiment to determine mud snail preferences for laying substrate, 98.56% of
216 the egg capsules laid were deposited on eelgrass (N = 4968 capsules). Other substrates, including
217 oyster shell (0.15%), mussel shell (0.12%), hard-shell clam (0.25%), soft-shell clam (0.22%),
218 sand collar (0.42%), and the container walls (0.28%), were laid upon to a much lesser extent,
219 totaling only 1.44% of egg capsules laid.

220 **Egg capsule placement**

221 In the field, the mean bottom heights of mud snail egg capsules were not significantly
222 different between blades of eelgrass with a low or high density of egg capsules, 6.74 cm and 5.95
223 cm respectively (Figure 4A; independent samples t-test, $df = 185$, $t = 1.470$, $p = 0.143$). For
224 eelgrass plants with low densities of egg capsules, the mean height of the highest capsules was
225 11.93 cm off the bottom, whereas the mean top height was significantly higher, at 16.77 cm, for

226 eelgrass plants with high densities of egg capsules (Figure 4A; independent samples t-test, $df =$
227 186, $t = -3.564$, $p < 0.001$).

228 In the lab, mud snails exhibited a pattern of capsule placement similar to that in the field.
229 We plotted the highest and lowest position at which egg capsules were laid over four days on
230 blades of eelgrass (Figure 4B). Four days after laying began, there was a significant effect of day
231 on the bottom height at which egg capsules were laid (1-way ANOVA, $F_{3,153} = 3.697$, $p = 0.013$),
232 but not on the top height (1-way ANOVA, $F_{3,153} = 0.547$, $p = 0.651$). Top height remained
233 constant over the course of up to four days of laying while bottom height decreased as laying
234 progressed, suggesting that prior egg capsule deposition may cause mud snails to place their egg
235 capsules closer to the benthos. Driven by the changes in bottom height, the mean height of egg
236 capsules also decreased over time.

237 When given the choice to lay egg capsules on either an eelgrass blade or sheath directly
238 in contact with the benthos, snails preferred the blade. After 24 h, mud snails deposited 78.0% of
239 egg capsules on eelgrass blades, 15.4% on eelgrass sheaths, and 6.6% on the experimental
240 containers ($N = 836$ capsules). Capsules were laid on all 10 of the provided blades and 5 of the
241 10 provided sheaths. Capsules that were deposited on the blades were primarily deposited
242 directly on the bottom (mean bottom height of 0.33 ± 0.14 cm) but some were laid higher up the
243 blade as well (Figure 5). In contrast, those few capsules which were deposited on the sheath had
244 a mean bottom height of 4.68 ± 2.83 cm; significantly higher than the mean bottom height for the
245 blades (t-test, $df = 13$, $t = -2.681$, $p = 0.019$).

246 **Effect of predators on egg capsule placement**

247 For the experiment where adult snails were exposed to cues from potential egg capsule
248 predators, the placement of egg capsules on eelgrass was separately analyzed for the first and last

249 day of egg capsule deposition to determine if placement changed over time in the presence of
250 predator cues. There was no significant difference among predator-cue treatments in either the
251 initial top (Figure 6A; 1-way ANOVA, $F_{2,114} = 1.545$, $p = 0.218$) or bottom height (1-way
252 ANOVA, $F_{2,114} = 1.513$, $p = 0.225$) of egg capsules. Similar results were found when mean
253 capsule height was examined.

254 After ten days, in the presence of hermit crab cues, snails deposited their egg capsules an
255 average of 0.4 cm higher off the benthos than control (Figure 6B). In the presence of
256 periwinkles, snails deposited their egg capsules an average of 2.6 cm higher than control (Figure
257 6B). Predator cue treatment had a significant effect on both bottom height (1-way ANOVA,
258 $F_{2,205} = 6.114$, $p = 0.003$) and top height of egg capsules (1-way ANOVA, $F_{2,204} = 4.427$, $p =$
259 0.013). Final top heights were lower for some treatments than on the first day of laying because
260 snails began laying on shorter blades of the eelgrass plant as time progressed. The bottom height
261 of capsules for periwinkle cues was significantly greater than for hermit crab cue ($p = 0.014$) and
262 the control ($p = 0.007$), which were not significantly different from each other ($p > 0.99$). In
263 addition, the top height of capsules when exposed to periwinkle cue was significantly higher than
264 the control ($p = 0.023$), while the height for hermit crab treatments was not significantly different
265 from either periwinkle or control treatments ($p > 0.05$).

266 **Survival of egg capsules on eelgrass**

267 Hermit crabs preferentially fed upon egg capsules deposited lower on blades of eelgrass
268 (Figure 7). The survival of egg capsules on eelgrass in the presence of hermit crabs decreased
269 with time exposed (day 1-8), and increased with height along the blade of eelgrass (1, 5, 10, or
270 15 cm; binomial logistic regression, Table 1). A Hosmer and Lemeshow test of this binomial
271 logistic regression model revealed a significant lack of fit to the data ($p < 0.001$). However, this

272 statistic can yield significant lack of fit for data with large sample sizes (as in this case with
273 7,840 capsules scored for survival) and since the model accurately predicted survival in 89.5% of
274 cases, we continued to use this model for our analysis (Kramer & Zimmerman 2007).

275 Survivorship decreased each day at all heights, but capsules deposited just 5 cm higher on
276 a blade had survivorship that was four times higher than capsules deposited directly on the
277 benthos. Additionally, the interaction term between tide and height or day may indicate that high
278 tide conditions play a role in increasing survival of egg capsules over low tide conditions (Figure
279 7). Egg capsule survivorship is likely a function of total height off the benthos, which is
280 determined by a combination of height along the blade of eelgrass and tidal period (eel grass
281 blades lay flat on the surface at low tide and rise to a more vertical position at high tide).

282 **Discussion**

283 The gastropod *Tritia obsoleta* exhibits mixed development (sensu Pechenik 1979) and
284 responds in a plastic fashion to predators throughout its life cycle (e.g. Schwab & Allen 2014,
285 Santoni et al. unpubl. data). Marine invertebrates exhibiting mixed development must navigate
286 both the benthic and planktonic environments as embryos and larvae (Caswell 1981), and the
287 period of benthic encapsulation may expose embryos to high levels of predation (Allen &
288 McAlister 2007). In Maine, mud snails strongly preferred to lay their egg capsules on eelgrass, at
289 a height approximately 5 cm off the benthos or higher. Introduction of periwinkle cues induced
290 the mud snails to increase the height at which egg capsules were deposited and egg capsules that
291 were deposited higher on eelgrass experienced lower rates of predation, suggesting that this
292 response may be an adaptation to enhance offspring survival.

293 **Substrate Preference**

294 Eelgrass was the preferred substrate for deposition of egg capsules both in the field and in
295 the lab. In areas of our study site with abundant eelgrass, we observed large groups ($>8000\text{ m}^{-2}$)
296 of adult mud snails in the act of laying hundreds of thousands of egg capsules. These dense
297 aggregations of mud snails would swarm a patch of eelgrass one day and then move on to
298 another area by the following day, mirroring existing reports of mud snail aggregative behavior
299 during the reproductive season in other areas of New England (Brenchley & Carlton 1983).

300 In Maine, preference for eelgrass is strong enough that mud snails were attracted to and
301 began laying on transplanted eelgrass within a week. The mechanism by which snails locate
302 eelgrass is not known. It is known, however, that a southern population of mud snails will follow
303 the odor of living bivalves (their preferred laying substrate in North Carolina) as well as track a
304 chemical contained within egg capsules (Rittschof et al. 2002). Snails aggregate to the source of
305 these cues for copulation and egg deposition (Rittschof et al. 2002). While the snails may also
306 respond to Egg Laying Hormone (Painter et al. 1991), there is no evidence that other
307 reproductive snails are attractive (Rittschof et al. 2002). It is possible that the Harpswell Sound
308 mud snail aggregations occur as a byproduct of snails following cues produced by patches of
309 eelgrass. It is equally possible that if one or a small number of snails were to find a patch of
310 eelgrass at random and begin to deposit capsules on it due to tactile cues, chemical cues from egg
311 capsule deposition could draw other reproductive snails to the same patch. Our experiments were
312 unable to distinguish between these two hypotheses, but further studies could test whether
313 chemical cues from eelgrass are attractive to adult mud snails.

314 While the results described here establish the substrate preference for oviposition in
315 Harpswell Sound mud snail populations, populations of mud snails in other locations prefer to

316 lay on other available substrates. For example, in Narragansett Bay, RI, eelgrass is not abundant
317 and therefore was not offered as a substrate in recent substrate preference experiments (Guidone
318 et al. 2014). Instead, the local alga *Ceramium virgatum* and invasive alga *Gracilaria*
319 *vermiculophylla* were found to be the preferred substrates for mud snail egg deposition (Guidone
320 et al. 2014). Further south, in Beaufort, North Carolina, mud snail egg capsules were primarily
321 deposited on shells, and living oyster shells were found to be the preferred substrate (Rittschof et
322 al. 2002). Based on these results, future work investigating the regional preferences of snails for
323 depositional substrates is warranted, particularly to determine whether a preference for vertical
324 substrates exists across this regional gradient. For example, in the results reported here, we found
325 that snails deposit preferentially on the unfamiliar walls of an experimental chamber rather than a
326 number of familiar substrates that lack substantial vertical definition.

327 It is unclear how snails might respond to a lack of suitable laying substrate in the field.
328 For comparison, the bubble-shell snail, *Haminaea vesicula*, also preferentially deposits on
329 eelgrass and when artificial eelgrass was provided in a field experiment, laying dramatically
330 increased both in sum and on a per-capita basis (von Dassow & Strathmann 2005). We did not
331 collect data on whether the per-capita abundance of egg capsules increased in *T. obsoleta*, but
332 did find that snails were attracted to and began laying on transplanted eelgrass in areas where it
333 was absent or low in abundance. Qualitative observations made during our field season suggest
334 that snails may deposit later in the summer when eelgrass is absent, but this remains to be
335 formally tested.

336 **Laying Height Preference**

337 In our study area, a mudflat in Harpswell Sound off Orr's Island, eelgrass was the most
338 common solid substrate that was elevated off the benthos. Eelgrass may be preferable to mud

339 snails because it allows placement of capsules out of the reach of benthic predators. A similar
340 preference has been demonstrated in juvenile scallops; young scallops attached higher on blades
341 of eelgrass experienced significantly less predation than those closer to the benthos (Pohle et al.
342 1991, Ambrose & Irlandi 1992).

343 There are likely to be additional tradeoffs between the benefits of placing capsules in
344 locations that are protected from predators and costs from other environmental factors; otherwise
345 mud snails might always be expected to deposit capsules far above the benthos. As with other
346 plastic responses, phenotypic plasticity allows organisms to respond to environmental variability,
347 but it can be costly and require trade-offs with growth, fecundity, or other genetic or energetic
348 costs (e.g. DeWitt 1998, Relyea 2002). We have demonstrated that it is advantageous to lay
349 capsules higher, out of the reach of benthic predators. However, gastropod egg capsules are also
350 vulnerable to high temperatures, poor oxygen availability, and reduced salinity, and do not
351 always protect developing embryos from environmental stresses associated with periodic
352 exposure to air (Pechenik 1978, Rawlings 1990, Przeslawski 2004). Egg capsules laid on the top
353 portions of an eelgrass plant may be more susceptible to these abiotic stresses as the tide goes
354 out. Similarly, the tips of eelgrass may bend down to the benthos, especially at low tide and
355 when weighted with egg capsules (personal observation), counterintuitively exposing egg
356 capsules laid at the tip to benthic predation while those in the middle of the blade remain
357 suspended above the surface. Our data and observations support the hypothesis that there might
358 be an ideal vertical zone for the placement of mud snail egg capsules on eelgrass that strikes a
359 balance between abiotic and biotic stressors.

360 **Plasticity of Laying Height**

361 Mud snails are known to exhibit plastic responses to chemical cues, or kairomones
362 (Ruther et al. 2002), of predators (e.g. Schwab & Allen 2014). When exposed to cues from
363 predators such as green crabs and periwinkles, mud snails may burrow or flee (Brenchley &
364 Carlton 1983, Rahman et al. 2000). There is an even stronger, chemically-triggered, alarm
365 response to crushed conspecifics (Atema & Burd 1975, Atema & Stenzler 1977). Beyond
366 behavioral responses, mud snails also demonstrate morphological plasticity in the presence of
367 predator cues. For example, when given waterborne cues from the blue crab, *Callinectes sapidus*,
368 mud snails developed apertures that were smaller relative to shell size, reducing the success of
369 predator attacks through the shell opening (Santoni et al. unpubl. data). In the presence of
370 predator cues, mud snails also vary their reproductive output both in terms of laying quantity and
371 capsule morphology, creating longer protective spines surrounding the capsule opening (Schwab
372 & Allen 2014). Given these prior examples of predator-induced plasticity, it seems reasonable
373 that deposition of egg capsules off the benthos could be an additional adaptive plastic response
374 of mud snails to predator cues.

375 Mud snails typically deposited egg capsules 4-8 cm off the benthos and elevated their
376 deposition by a further 1-3 cm in the presence of periwinkles. In the presence of hermit crabs,
377 capsules were deposited at a height that did not significantly differ from the control. Periwinkles
378 and hermit crabs are both known egg capsule predators (Brenchley 1982) that are abundant on
379 the benthos but were not observed to crawl higher than a few centimeters up a blade of eelgrass
380 in the field. Therefore, we hypothesize that depositing egg capsules several cm above the benthos
381 is a plastic response that allows mud snails to increase the survival of their young in the presence
382 of egg capsule predators. Though hermit crabs have been observed to be the more voracious

383 predator (Brenchley 1982), they primarily inhabit the benthos, while periwinkles tended to crawl
384 up eelgrass plants while grazing (personal observation). In the presence of periwinkles, mud
385 snail deposition of capsules farther off the benthos may prevent bulldozing, which occurs as
386 periwinkles displace and destroy egg capsules while grazing on the epiphytes of eelgrass and
387 occasionally directly consuming capsules and eggs (Brenchley 1982).

388 In the future, it would be beneficial to perform an experiment with combined cues from
389 periwinkles and hermit crabs. In other species of snails, combined predator cues can either lead
390 to an intermediate or a prioritized response (Bourdeau 2009; Mach & Bourdeau 2011). Based on
391 our observations, we would predict that hermit crabs are the more dangerous predator; when egg
392 capsules were attached to sand collars, hermit crabs removed 124 times more egg capsules than
393 did periwinkles (unpubl. data), yet changes in height were more dramatic in response to
394 periwinkle cues. If, as seems likely, hermit crab cues are always present then there may be a
395 default laying height for snails in this high risk population, explaining why there was no
396 significant difference between the heights at which capsules were deposited in the presence of
397 hermit crabs and control (Bourdeau 2012). Alternatively, our 'control' cue water may have been
398 carrying cues from hermit crabs in the sound, where water came into the marine lab and thus
399 contaminated with hermit crab cue. Or snails may respond more strongly to crab cues if the crabs
400 are actively consuming egg capsules, which they were prevented from doing in our experimental
401 design. The response to lay even farther off the benthos may also be reserved only for an
402 instance in which there is a strong periwinkle cue, to which mud snails are known to be sensitive
403 and to which they adjust their behavior in response (Brenchley and Carlton 1983).

404 We also found that mud snails use the morphology of eelgrass plants rather than height
405 *per se* to determine where to deposit egg capsules. Mud snails strongly prefer to deposit capsules

406 on the blades of eelgrass rather than the sheath, thus the change from sheath to blade appears to
407 provide a tactile clue for the snail as it climbs the plant as to where to deposit egg capsules.
408 When given blades without the attached sheath as a laying substrate, mud snails deposit capsules
409 directly at the interface of the blade and the benthos. Very few capsules were deposited on the
410 sheath, and those that were tended to be deposited several cm off the benthos. It is unclear
411 whether height above the benthos is the only benefit to placing egg capsules on eelgrass blades
412 rather than the sheath, but height seems likely to be related to reduced capsule predation.

413 There are few studies demonstrating that selection of deposition site has strong effects on
414 offspring survival in marine invertebrates. One example is the report that Pribilof whelks
415 preferentially deposit their egg capsules near large sea anemones (*Urticina crassicornis*) that
416 deter urchin predation by consuming urchins that approach the egg capsules (Shimek 1981). We
417 are also aware of one unpublished report of preferential deposition of egg masses on the upper
418 portion of eelgrass blades by the gastropod *Lacuna vincta*. Similar to our report, adult *L. vincta*
419 migrate up the blades of eelgrass to deposit their egg masses away from the benthos and a
420 manipulative field experiment found that when masses were placed within 2 cm of the benthos,
421 damage from predatory crabs was significantly greater than masses located at the upper end of
422 the blade (Martel and Friedman 1986).

423 **Consequences of Deposition Strategy**

424 If adult mud snails deposited egg capsules on the first portion of eelgrass they
425 encountered (i.e. the sheath in contact with the benthos), lower reproductive success would
426 result. In our work, we have shown that egg capsules that were deposited directly on the benthos
427 experienced up to 4 times greater rates of predation than those deposited just 5 cm higher. The
428 ideal zone for egg capsule placement is likely determined by a combination of benthic predators

429 and abiotic stresses. As the number of previously laid capsules increases, we observed that mud
430 snails will deposit capsules both higher and lower on eelgrass blades, presumably outside of the
431 preferable zone. Survival of these egg capsules is likely reduced compared to capsules laid
432 earlier and in a more optimal part of the eelgrass blade.

433 Attaching egg capsules above the benthos allows animals with mixed development to
434 place vulnerable young above the predator-dense benthos, and where their survival is likely to
435 increase (Allen & McAlister 2007). Marine invertebrates with an obligate period of benthic
436 development prior to planktonic dispersal (i.e. mixed development) may frequently exhibit
437 plasticity in their reproductive behaviors to increase survival of their offspring in the dangerous
438 benthic environment. Egg capsules are generally attached to benthic surfaces to develop for
439 weeks to months without parental care. While encapsulated young may be more protected from
440 predation than benthic/demersal larvae (of which there are few examples), mixed development
441 potentially exposes developing young to dangers of both the benthic and planktonic habitats
442 (Pechenik 1979, 1999). Thus, any opportunity for mothers to equip their young to better survive
443 in the face of environmental variability has great potential to increase her fitness.

444

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Figures

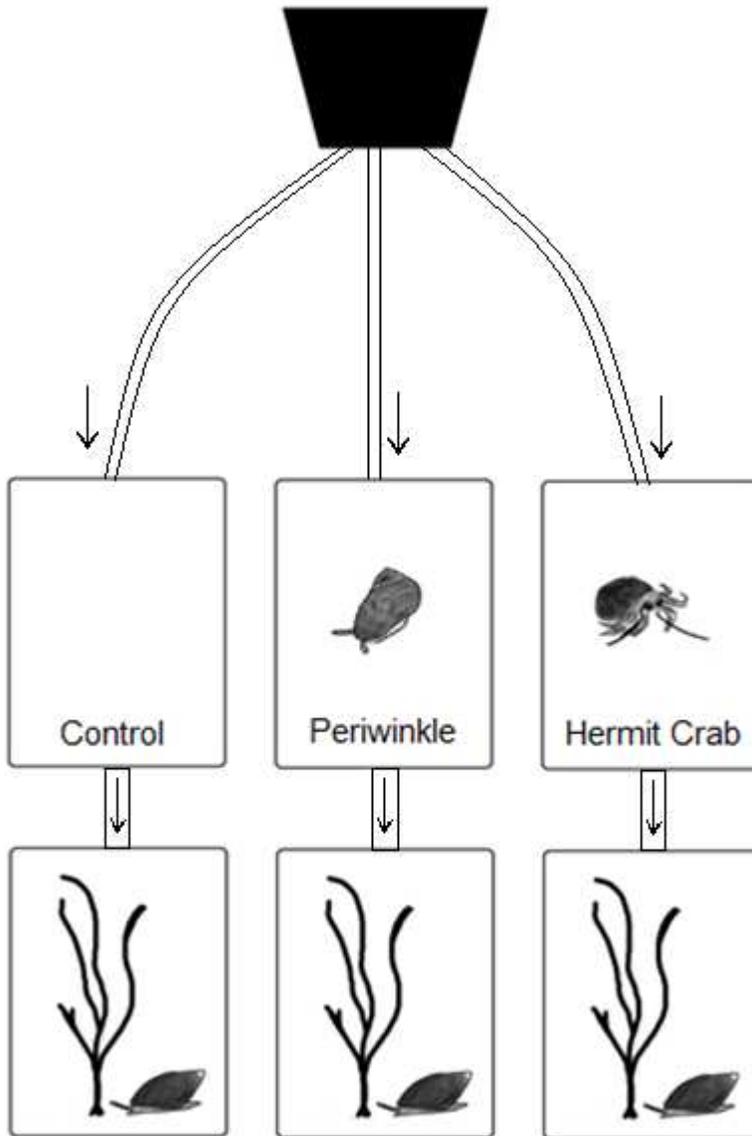


Figure 1: Schematic of the experiment to determine if predator cues induce laying height plasticity. Water from the flow-through water system gathered in a bucket and was then gravity-fed through tubes into treatment containers that were empty (control), held 10 periwinkles or 10 hermit crabs. Water from these containers then flowed through a tube into the experimental chambers holding 18 mud snails and an eelgrass plant. The arrows indicate direction of water flow.

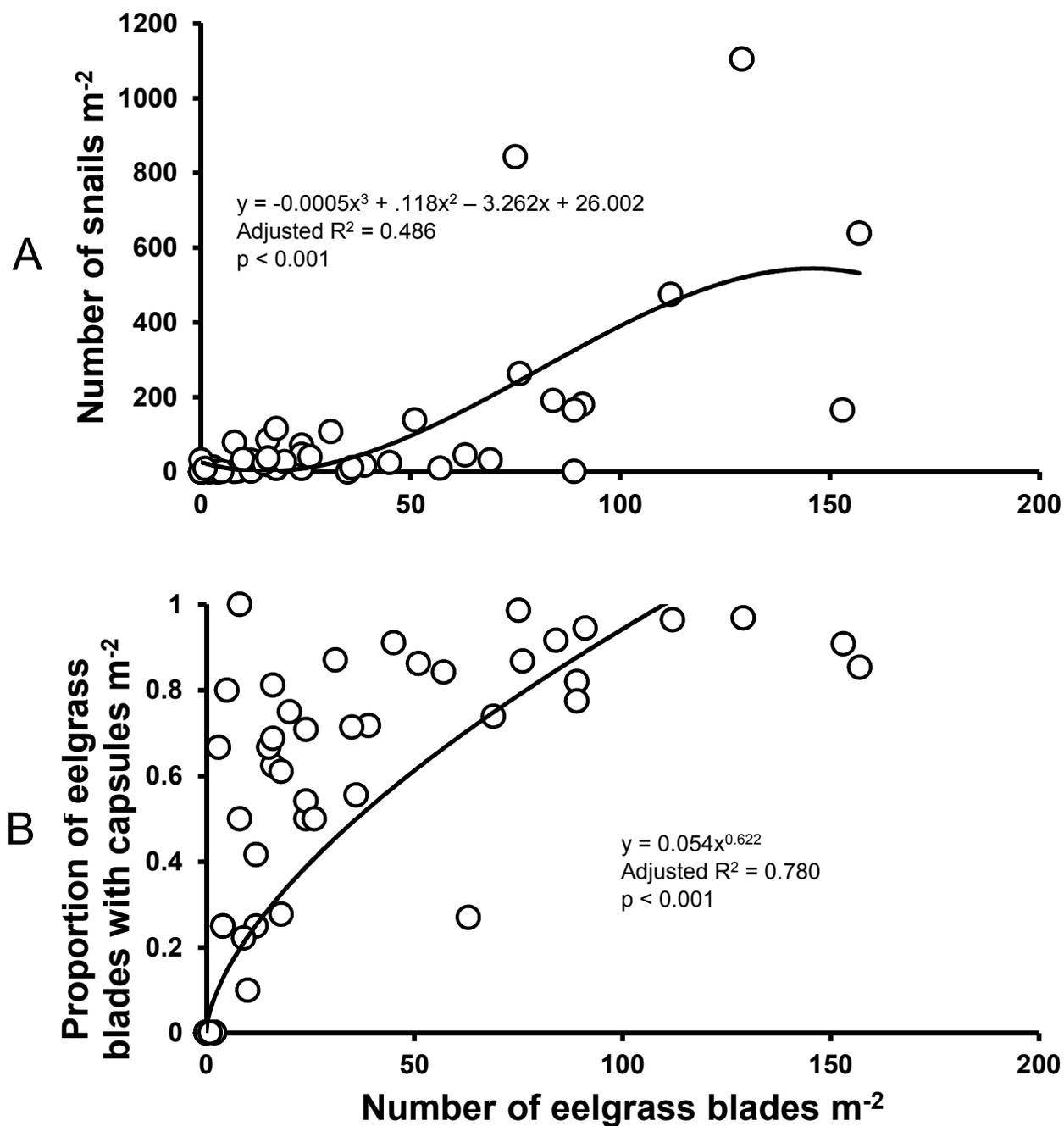


Figure 2: (A) Number of adult mud snails m^{-2} in relation to the number of blades of eelgrass present. (B) Proportion of blades of eelgrass bearing mud snail egg capsules m^{-2} in relation to the total number of eelgrass blades. Each data point represents a $1 m^2$ plot.

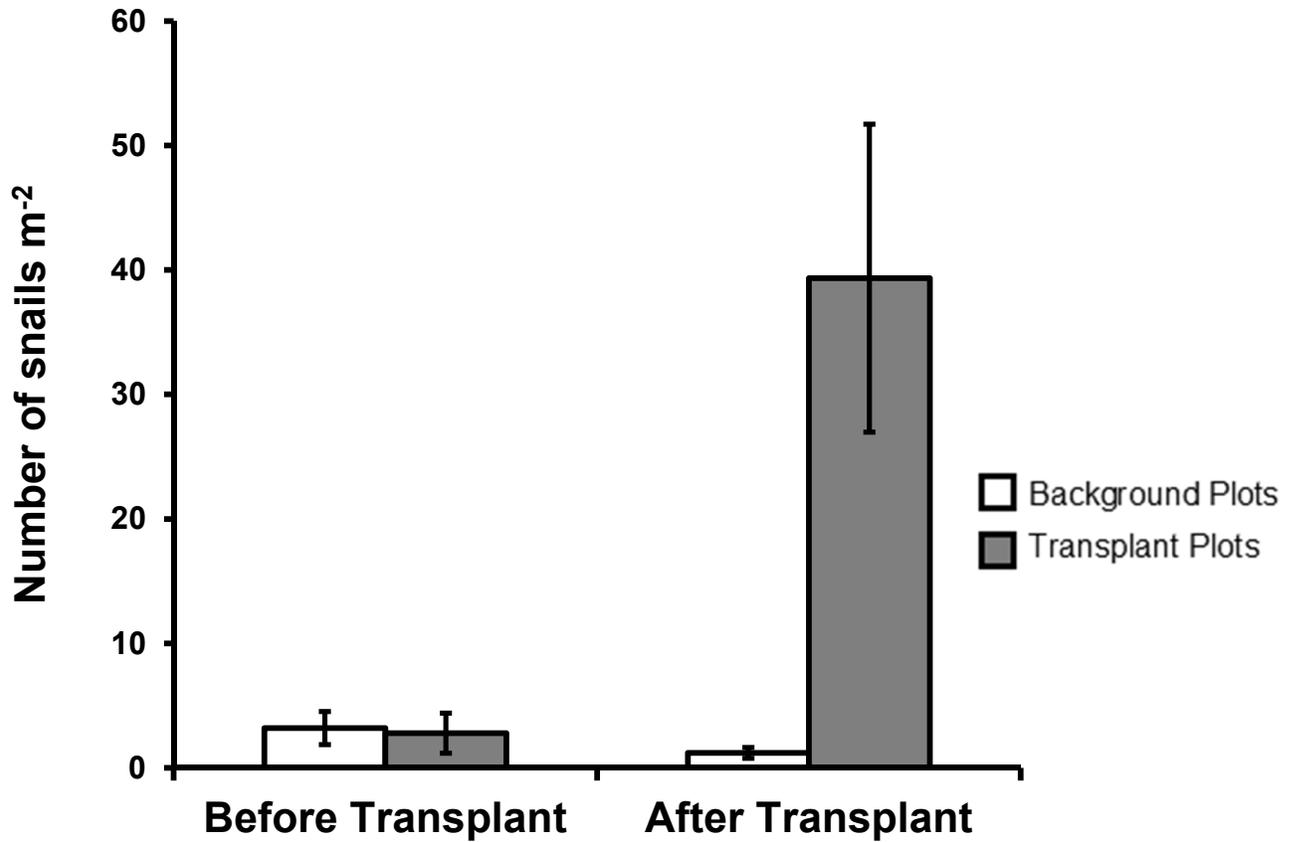


Figure 3: Number of snails present in 1 m² plots before and five days after the experimental addition of eelgrass plants. Gray bars represent the plots into which eelgrass was transplanted, and white bars represent background plots in which there was no eelgrass. Each bar represents mean \pm SE for N = 9 plots.

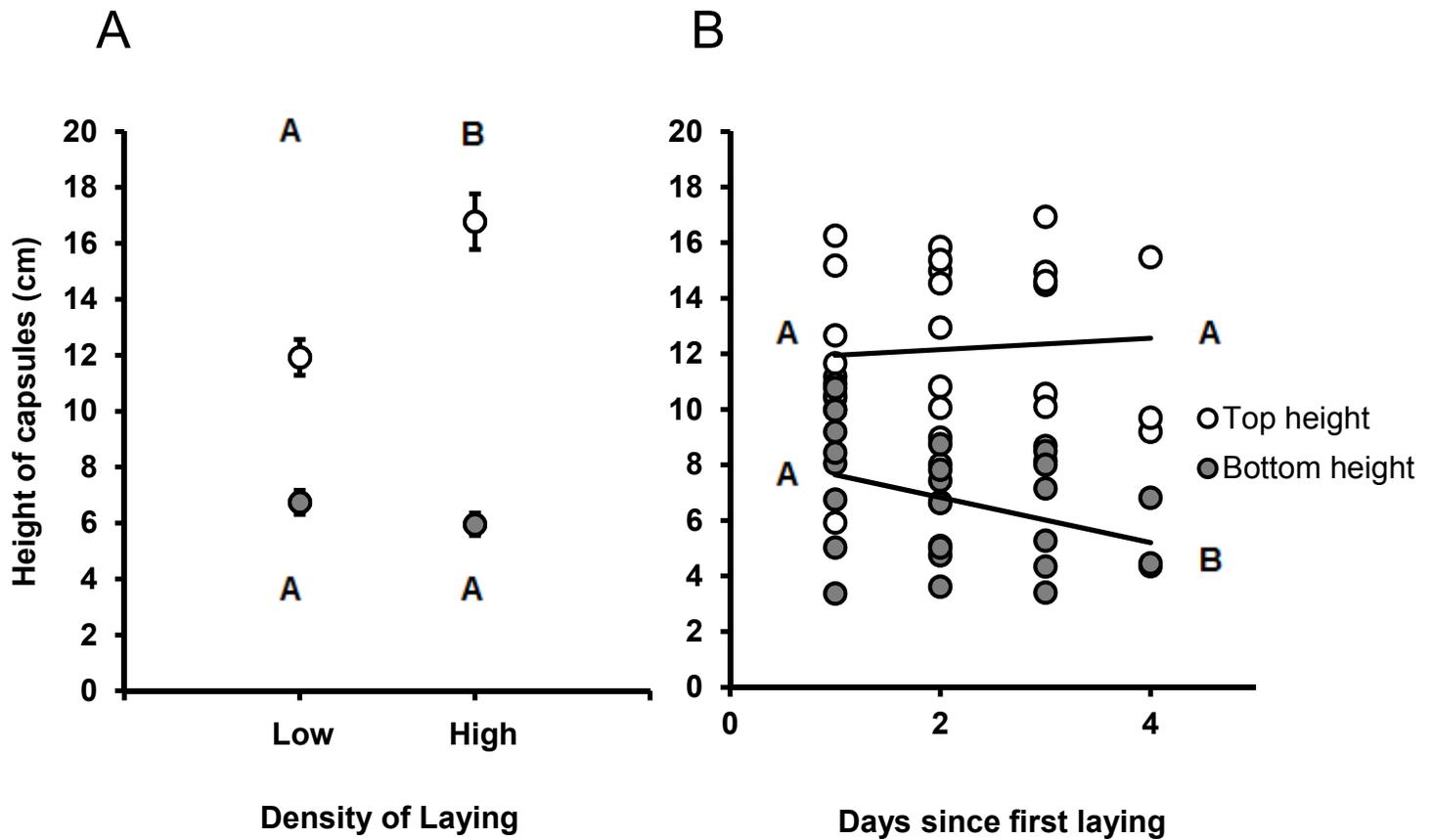


Figure 4: (A) Mean position of highest and lowest mud snail egg capsules along blades of eelgrass collected from the field. Gray and white circles indicate the mean (\pm SE) of lowest and highest egg capsules, respectively. (B) The top and bottom height of egg capsules laid upon blades of eelgrass in the laboratory. Each point represents the mean height per container. Gray and white circles indicate the mean (\pm SE) of lowest and highest egg capsules, respectively. Day 1 was recorded as the first day that at least 5 egg capsules were laid in a container. The number of containers sampled declined over time because 4 days of laying could not be recorded for containers in which snails started laying later. Different letters indicate significant differences among treatments within each height (top or bottom).

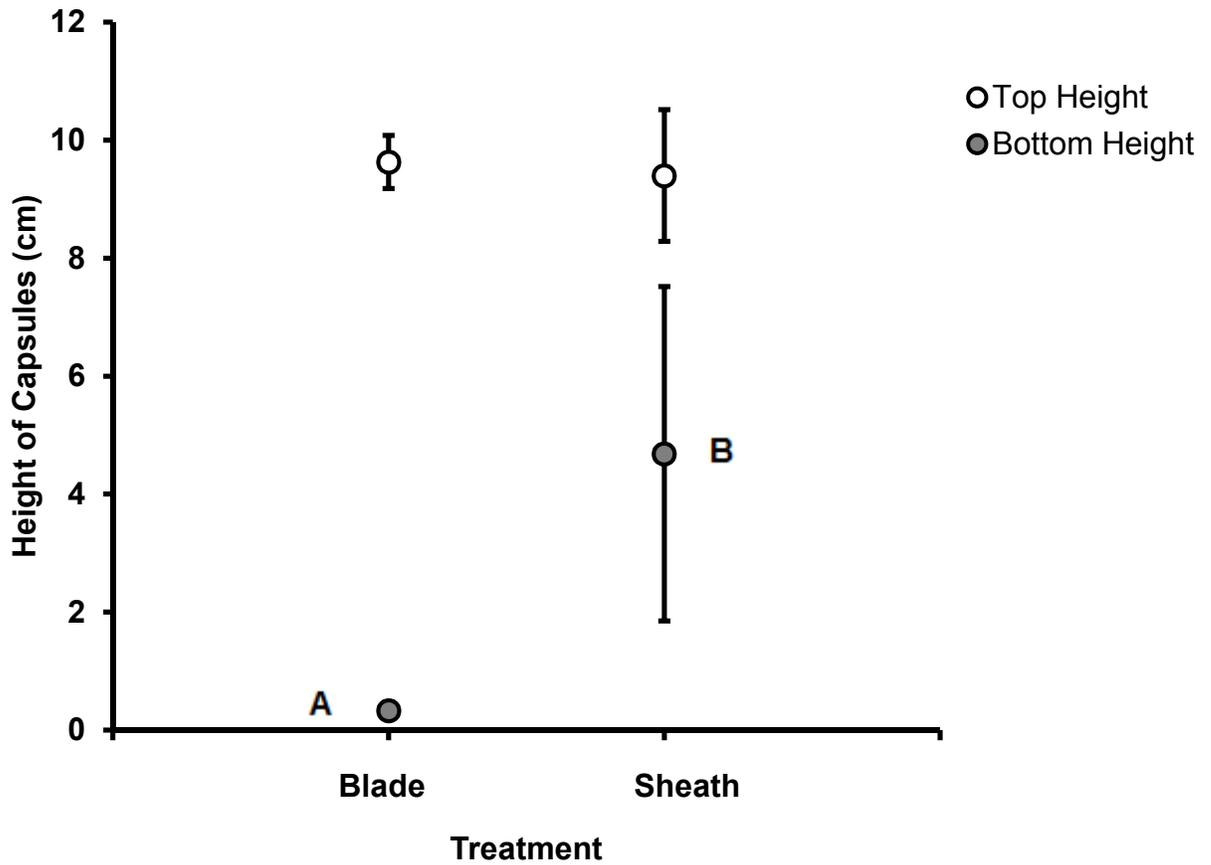


Figure 5: Mean position of highest and lowest mud snail egg capsules on either blades (n = 10) or sheaths (n = 5) of eelgrass. Mud snails deposited eggs on 10/10 provided eelgrass blades but only 5/10 provided eelgrass sheaths. Gray and white circles indicate the mean (\pm SE) of lowest and highest egg capsules, respectively. Different letters indicate significant differences among treatments.

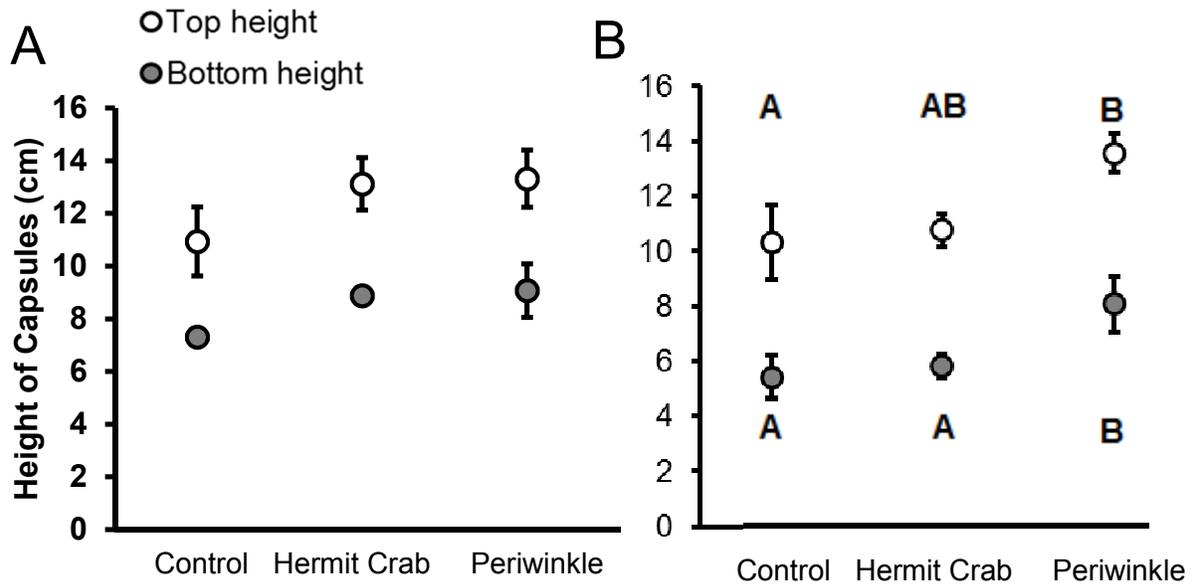


Figure 6: (A) Bottom and top height of egg capsules for snails exposed to predator cues after one day. Gray circles indicate the mean \pm SE height of the lowest egg capsule, and white circles indicate the mean \pm SE height of the highest egg capsule (Control N = 2, Hermit Crab N = 7, Periwinkle N = 8). (B) Bottom (gray circles) and top (white circles) height of egg capsules for snails exposed to predator cues after ten days (Control N = 7, Hermit Crab N = 9, Periwinkle N = 10). Different letters above each point indicate significant differences among treatments within each height (top or bottom).

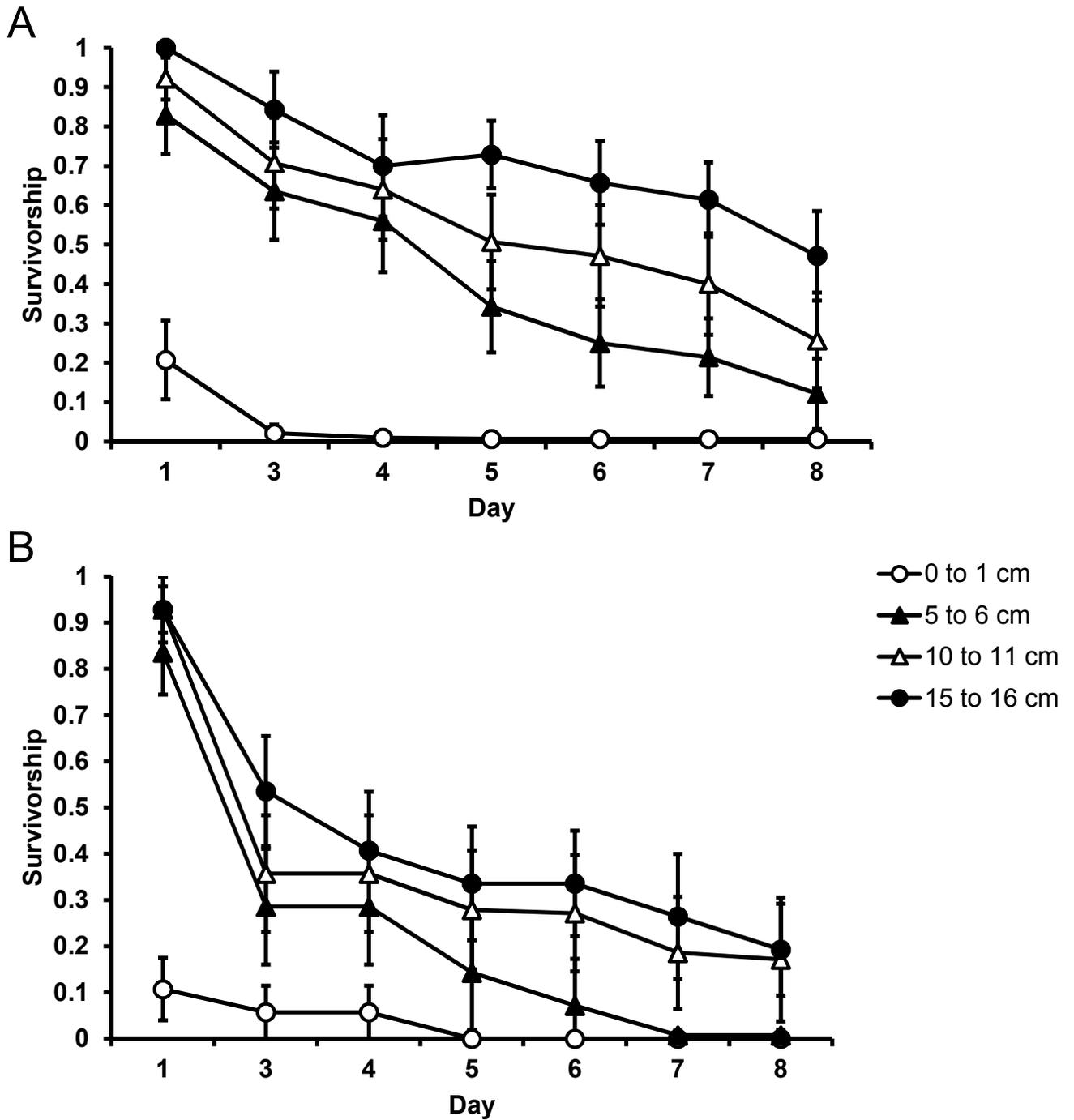


Figure 7: Hermit crab predation upon egg capsules placed at different heights above the benthos on eelgrass for simulated high tide (**A**) and low tide (**B**). Each point represents the mean \pm SE proportion of surviving egg capsules at the given height in N = 14 containers.

Table 1: Binomial logistic regression for predation upon egg capsules at heights above the benthos. All interaction terms are included as the best model as determined by lower log likelihood. Significant effects are listed in **bold**.

Variable	β	P-value	Exp(β)
Day	-0.576	<0.001	0.562
Tide	-0.230	0.324	0.794
Height	0.172	<0.001	1.188
Day*Height	0.004	0.308	1.004
Day*Tide	0.212	<0.001	1.236
Height*Tide	0.099	<0.001	1.104
Day*Height*Tide	-0.011	0.040	0.989
β_0	-0.193	0.257	0.825