

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

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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 4 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 6 to 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 to 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 the benthos is an adaptive response allowing mud snails to protect their embryos from benthic predators. We also provide evidence that snails use the characteristics of the eelgrass itself to ensure capsules are laid well above the benthos.

KEY WORDS: Plasticity · Predator–prey interactions · Gastropods · Eggs · Eelgrass · Mixed development

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INTRODUCTION

The vast majority of marine invertebrates exhibit complex life cycles with embryonic and larval development occurring independent of the mother, either as freely spawned eggs, embryos and larvae, or as encapsulated embryos (Thorson 1950, Strathmann 1985). Thorson (1950) estimated that 55 to 85% of all benthic marine invertebrate species exhibit extended (weeks or months) planktonic development through a series of embryonic and larval stages. Despite the prevalence of planktonic development, many species spend part or all of their developmental period on the benthos (Strathmann 1987, Pechenik 1999, Strathmann 2007). Among those species that

develop in benthic egg capsules, some hatch directly as crawl-away juveniles (e.g. the gastropods *Nucella lapillus* and *Urosalpinx cinerea*, and some nemertean, polychaetes, and flatworms; Christiansen & Fenchel 1979, Crothers 1985, Martel & Chia 1991, Ruiz-Trillo et al. 1999); others exhibit mixed development (Pechenik 1979), starting from benthic egg capsules from which planktonic larvae hatch (Grassle & Grassle 1974, Pechenik 1979, Caswell 1981, Strathmann 1985). Compared to planktonic development, benthic development exposes vulnerable eggs, embryos, and larvae to higher rates of predation (Allen & McAlister 2007), and thus we might expect unprotected benthic development to be selected against over evolutionary time (Strathmann 2007). However,

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protected (i.e. encapsulated or brooded) benthic development may be favored in areas with many predators, high risk of desiccation, or other environmental stressors (Pechenik 1999). As in other animals with complex life cycles, the timing of transitions from one habitat to another during development is likely governed by a tradeoff between growth and risk of mortality (Werner 1986). In mixed development, an initial period of benthic encapsulation may decrease the probability of planktonic mortality before metamorphosis by reducing the amount of development time spent in the plankton (Pechenik 1979). In general, pelagic habitats have been found to be a lower mortality environment than the benthos for unprotected embryos (Allen & McAlister 2007, Vaughn & Allen 2010). However, there is also evidence that later developmental stages of invertebrates are less vulnerable to planktonic predators (Pennington & Chia 1984, Rumrill et al. 1985, Allen 2008). Benthic encapsulation may thus be favored when it allows embryos to be protected until they are later-stage larvae that are better able to resist planktonic threats (Pechenik 1979).

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

The eastern mud snail *T. obsoleta* is a common gastropod and a useful model organism for studying

plasticity because of its high abundance (up to 8000 snails m⁻²; authors' pers. obs.) on intertidal mudflats across a wide geographic range along the east coast of North America, from the Gulf of St. Lawrence to the Gulf of Mexico (Cranford 1988). During the reproductive season, an individual adult *T. obsoleta* deposits about 100 capsules, each containing 30 to 300 eggs (Pechenik 1978, Brenchley 1982, Rittschof et al. 2002). *T. obsoleta* deposits benthic egg capsules on solid objects (shells, worm tubes, algae, vegetation, etc.) which are frequently limiting in the soft sediment systems where mud snails are most common (Scheltema 1967, Sullivan & Mangel 1984). Embryos develop within the capsules for 2 wk before hatching as planktonic veliger larvae (Sullivan & Mangel 1984). With no parental care beyond the investment in the protective capsule, the eggs are at high risk from benthic predators (Brenchley 1982). Thus, where adult snails deposit their egg capsules may have substantial implications for the survival of their offspring. In New England, mud snails tend to lay their egg capsules on blades of eelgrass *Zostera marina*, and egg capsules are preyed upon by benthic predators including green crabs *Carcinus maenas* and hermit crabs *Pagurus longicarpus*, and displaced by the foraging behavior of periwinkles *Littorina littorea* (Brenchley 1982).

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

MATERIALS AND METHODS

Field observations of mud snails, egg capsules and eelgrass

Our field site was a mudflat adjacent to the Bowdoin College Coastal Studies Center (CSC) on Orr's Island, Maine (43° 79' N, 69° 95' W). To assess the distribution of mud snail *Tritia obsoleta* egg capsules, 56 plots were surveyed along 3 equidistant and parallel transects in a large tide pool on the CSC mud-

flat. We used a 1 m² quadrat to count the number of mud snails, eelgrass plants *Zostera marina*, and the proportion of blades of eelgrass bearing mud snail egg capsules in each plot. If there were >100 mud snails in a plot, the number of mud snails was extrapolated from 5 randomly subsampled 10 cm² divisions. We used the curve estimation procedure in SPSS version 22 to determine significant correlations and relationships between the number of mud snails, eelgrass plants, and the proportion of eelgrass blades bearing egg capsules.

Eelgrass transplant experiment

Initial observations indicated that the vast majority of egg capsules were laid on eelgrass. To determine whether this was due to convenience or preference, we conducted a transplant experiment in a section of the CSC mudflat in which eelgrass was absent. Nine 1 m² plots were marked in the mud, set 2 m apart in a 3 × 3 grid. The initial number of snails in each plot was recorded, as well as in 9 haphazardly sampled background plots. We then planted a ring of 10 bare eelgrass plants in each plot by pushing the roots of the plants approximately a finger's depth into the mud and then packing around the base of the plants. After 5 d, we once again counted the number of snails in the marked plots and in 9 haphazardly sampled background plots.

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

Substrate preference

We conducted a preference experiment in the CSC laboratory to determine the preferred laying substrate for mud snails. Coarsely filtered (250 μm) seawater pumped directly from Harpswell Sound was used in the flow-through seawater lab of the CSC. Any background levels of chemical cues in the water from Harpswell Sound were therefore provided to all treatments, including controls. Seawater was directed into a bucket with 30 holes drilled into the bottom. Lengths of clear vinyl tubing, 5/16" (7.9 mm) in outer

diameter, were fit tightly into these holes and then into replicate plastic containers through holes in the lids. Excess water escaped around the edges of the lids.

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

Egg capsule placement on eelgrass

To determine at what height mud snails lay egg capsules on eelgrass, we quantified the position of egg capsules upon eelgrass from the field. A total of 28 eelgrass plants bearing egg capsules were haphazardly collected from the CSC mudflat. The plants were categorized as exhibiting either low or high densities of egg capsules. Low-density plants had patchy coverage of a single layer of egg capsules, while high-density plants had many (thousands) more egg capsules in multiple layers covering the blades of eelgrass.

We measured the placement of egg capsules along the length of each eelgrass blade, designating the plant's node as 0 cm. We recorded the total length of the blade and the bottom and top height of each group of capsules as well as the number of capsules per group. Heights are reported as measurements from the node rather than as percentage of height along the blade of eelgrass since the raw height off the benthos is more relevant for this study. If necessary, for plants with high densities of capsules, layers of egg capsules were removed and counted. We performed independent samples *t*-tests to compare the bottom and top heights of egg capsules between the 2 density levels. The bottom height data were ln-transformed, and the top height square-root transformed to fit the independent *t*-test assumption of normality, as confirmed by Kolmogorov-Smirnov and Shapiro-Wilk tests.

We also monitored egg capsule placement on eelgrass in the laboratory. Ten containers ($15 \times 21 \times 7.5$ cm) were filled with 2 to 3 cm of mud, and 1 eelgrass plant was planted in each container. We randomly assigned 18 large adult snails to each container and tracked where capsules were deposited on eelgrass over 11 d. Within these 11 d, capsules were counted from the first day of laying. Therefore, fewer days of data are reported for containers in which snails delayed egg laying. These data were used to determine whether the position of mud snail egg capsules in the field indicates preference or is an artefact of predation (i.e. snails could deposit egg capsules all along a blade of eelgrass, but the lowest capsules deposited could be preferentially consumed post-deposition). We conducted 1-way ANOVAs for the dependent variables of top height and bottom height, with day as the fixed effect. The bottom and top height data were square-root transformed to fit the 1-way ANOVA assumption of normality, as confirmed by Kolmogorov-Smirnov and Shapiro-Wilk tests.

We next performed an experiment to determine if snails were selecting deposition heights based on height per se or based on plant morphology (blade vs. sheath). To do so, eelgrass plant blades and sheaths were separated and attached to the bottom of 5 replicate plastic containers. Two blades and 2 sheaths were randomly assigned to positions in each container along with 18 large adult snails. After 24 h, we recorded the number of egg capsules on each surface and the bottom height of capsules. We ran *t*-tests with substrate as the grouping variable. The bottom height was ln-transformed to fit the *t*-test's assumption of normality, as confirmed by Kolmogorov-Smirnov and Shapiro-Wilk tests.

Effect of predators on egg capsules placement

We hypothesized that mud snails would change the height at which they deposit egg capsules in the presence of predators. Hermit crabs and periwinkles were used as egg-capsule predators since hermit crabs were observed to be voracious predators of egg capsules, and periwinkles bulldoze mud snail egg capsules (Brenchley 1982) and are extremely abundant on the CSC mudflat. There were 10 replicates each of 3 predator cue treatments: hermit crab, periwinkle, and control (ambient sea water only). Treatment containers ($15 \times 21 \times 7.5$ cm) held 10 predators of similar size so that each experimental chamber received a similar strength of chemical cue from the predators. Predators fasted over the duration of the

experiment. Holes were drilled in the sides of treatment containers so that tubing ($5/16''$ [7.9 mm] outer diameter) could deliver cued water from treatment containers to experimental containers (Fig. 1). Experimental containers each held 18 randomly assigned large adult mud snails and an eelgrass plant. Excess water escaped from around the lid. Once laying began, we recorded the number and position of egg capsules on the eelgrass every 4 d. Blades of eelgrass that broke off from the plant were discarded if we could not determine the bottom height of egg capsules, and approximately 6 hermit crabs were replaced after mortality. The experiment ran for 10 d before the majority of eelgrass blades broke.

In order to test for predator effects on capsule deposition, we ran 1-way ANOVAs on the transformed data with predator as the fixed effect. Data on the lowest height at which capsules were laid were square-root transformed, and data for the highest

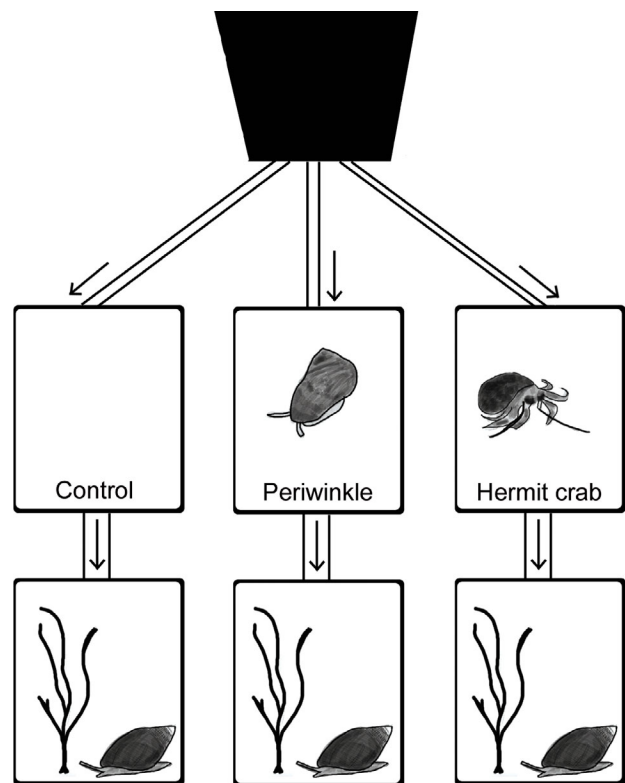


Fig. 1. Schematic of the experiment to determine if predator cues induce laying height plasticity in the mud snail *Tritia obsoleta*. Water from the flow-through system gathered in a bucket and was then gravity-fed through tubes into treatment containers that were empty (control), held 10 periwinkles 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. Arrows indicate direction of water flow

height at which capsules were laid were ln-transformed to fit the 1-way ANOVA assumption of normality, as confirmed by Kolmogorov-Smirnov and Shapiro-Wilk tests. Where significant effects of predator were found, we ran a Bonferroni post hoc test to test for differences between the 3 treatments.

Survival of egg capsules on eelgrass

To assess how predation varied with height along an eelgrass plant, we manipulated the position of egg capsules along blades of eelgrass. Each blade was 17 cm long, and the first 1 cm of eelgrass was glued to the bottom of the container. A 2 × 5 clump of egg capsules (approximately 1 cm in height) was then glued at 0, 5, 10, and 15 cm from the base, for a total of 40 capsules added to each blade. Sections (1 cm) of eelgrass blades bearing egg capsules were cut from other plants using a razor blade and then the eelgrass/capsule assembly was glued to the intact 17 cm length of eelgrass blade using Krazy glue®. Two container heights were used to mimic low and high tide conditions: the 7.5 cm deep plastic containers used in prior experiments, as well as 20 cm deep containers. These containers differed in the degree to which the tip of the eelgrass could float above the benthos. In total, 14 replicates of each type of container were supplied with flow-through water and 3 hermit crabs were randomly assigned to each container.

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

RESULTS

Field observations

In the field, the correlation between number of mud snails and number of blades of eelgrass m⁻² was positive, significant, and best fit by a cubic function (Fig. 2A). High densities of snails (>200 m⁻²) were only seen in plots with ≥75 blades of eelgrass m⁻²

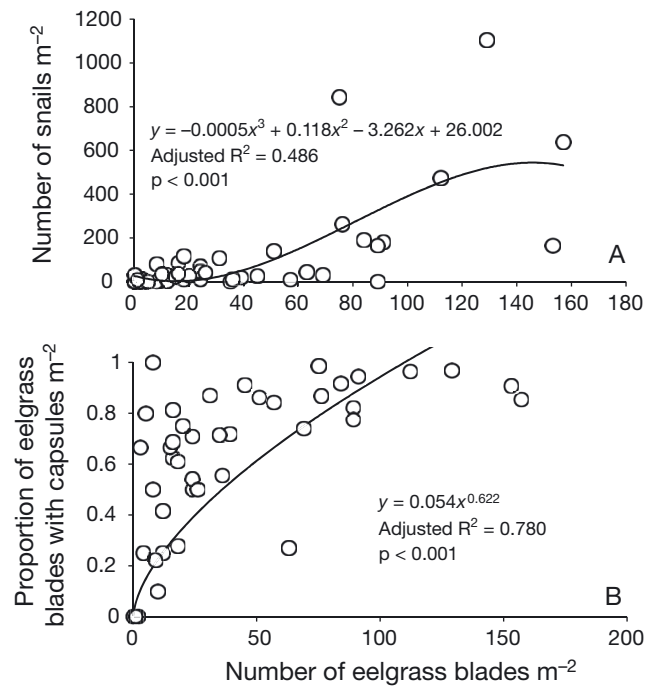


Fig. 2. (A) Number of adult mud snails m⁻² in relation to the number of blades of eelgrass present. (B) Proportion of blades of eelgrass bearing mud snail egg capsules m⁻² in relation to the total number of eelgrass blades. Each data point represents a 1 m² plot

(Fig. 2A), suggesting that mud snail density is higher in areas with more eelgrass.

The proportion of eelgrass blades on which mud snail egg capsules were laid was also positively and significantly correlated with the number of eelgrass blades m⁻² (Fig. 2B) and the relationship was best fit by a power function. In areas with sparser eelgrass (<75 blades m⁻²) egg capsule deposition was maximally variable, with anywhere from 0 to 100% of eelgrass blades bearing egg capsules. In plots with high densities of eelgrass (>75 blades m⁻²), an average of 90% of eelgrass blades bore egg capsules, whereas in plots with low densities of eelgrass (<75 blades m⁻²), an average of 40% of eelgrass blades bore egg capsules (Fig. 2B).

Eelgrass transplant

The experimental addition of eelgrass yielded significant increases in the mean number of snails in each plot (Fig. 3). The number of snails varied with time (before versus after transplantation; 2-way ANOVA, $F_{1,34} = 10.717$, $p = 0.002$) and location (inside versus outside transplant zones; 2-way ANOVA,

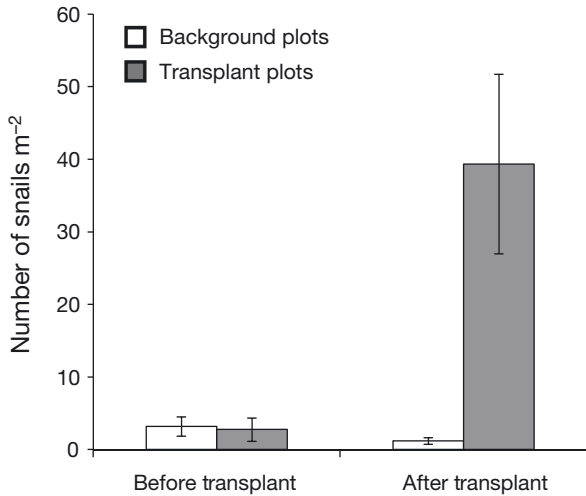


Fig. 3. Number of mud snails present in 1 m² plots before and 5 d after the experimental addition of eelgrass plants. Gray bars: plots into which eelgrass was transplanted; white bars: background plots in which there was no eelgrass. Each bar represents mean ± SE for n = 9 plots

$F_{1,34} = 20.595$, $p < 0.001$), and there was a statistically significant interaction between time and location (2-way ANOVA, $F_{1,34} = 18.321$, $p < 0.001$) such that snails increased by an order of magnitude in the plots into which eelgrass was transplanted while slightly declining in plots without eelgrass addition (Fig. 3).

Substrate preference

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

Egg capsule placement

In the field, the mean bottom heights of mud snail egg capsules were not significantly different between blades of eelgrass with a low or high density of egg capsules: 6.74 and 5.95 cm, respectively (Fig. 4A; independent samples *t*-test, $t = 1.470$, $df = 185$, $p = 0.143$). For eelgrass plants with low densities of egg capsules, the mean height of the highest capsules was 11.93 cm off the bottom, whereas the mean top height was significantly higher, at 16.77 cm, for eelgrass plants with high densities of egg capsules (Fig. 4A; independent samples *t*-test, $t = -3.564$, $df = 186$, $p < 0.001$).

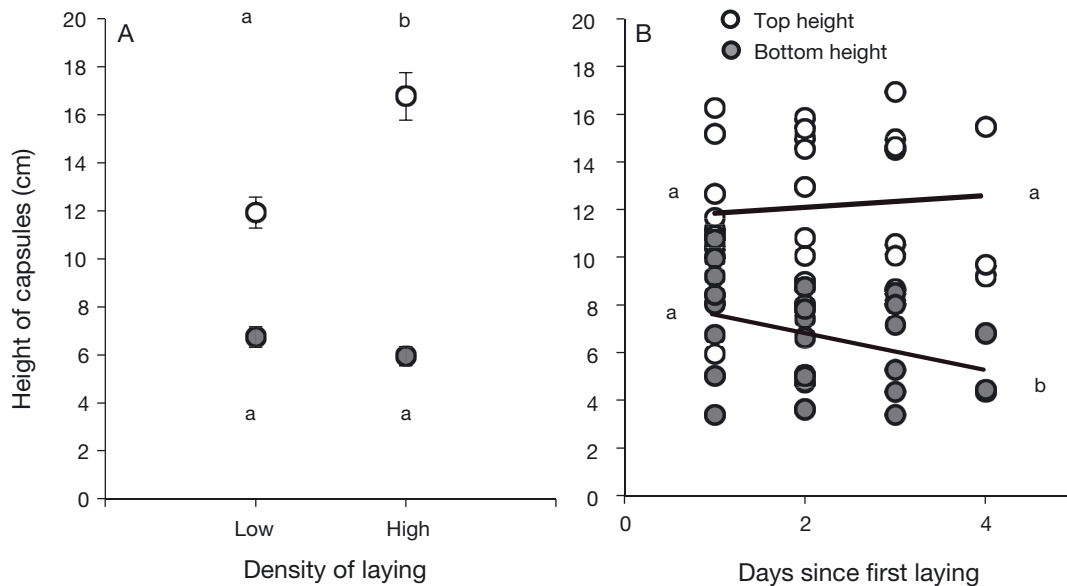


Fig. 4. (A) Mean position of highest and lowest mud snail egg capsules along blades of eelgrass collected from the field. Plants with a low density of laying had patchy coverage of a single layer of egg capsules; plants with a high density of laying had many egg capsules in multiple layers covering the eelgrass. Gray and white circles indicate the mean (±SE) of lowest and highest egg capsules, respectively. (B) Top and bottom height of egg capsules laid upon blades of eelgrass in the laboratory over 4 d of laying. Each point represents the mean height per container. Gray and white circles indicate the mean (±SE) of lowest and highest egg capsules, respectively. Day 1 was recorded as the first day on which at least 5 egg capsules were laid in a container. The number of containers sampled declined over time because 4 d of laying could not be recorded for containers in which snails started laying later. Black lines indicate the linear trend line for top or bottom height of laying over time. Different letters indicate significant differences among treatments within each height (top or bottom)

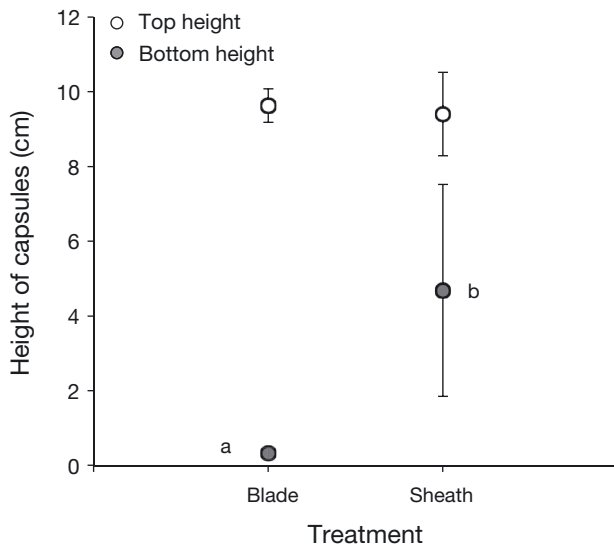


Fig. 5. Mean position of highest and lowest mud snail egg capsules on either blades ($n = 10$) or sheaths ($n = 5$) of eelgrass. Mud snails deposited eggs on 10/10 provided eelgrass blades but only 5/10 provided eelgrass sheaths. Gray and white circles indicate the mean (\pm SE) of lowest and highest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

In the lab, mud snails exhibited a pattern of capsule placement similar to that in the field. We plotted the highest and lowest position at which egg capsules were laid over 4 d on blades of eelgrass (Fig. 4B); 4 d after laying began, there was a significant effect of day on the bottom height at which egg capsules were laid (1-way ANOVA, $F_{3,153} = 3.697$, $p = 0.013$), but not

on the top height (1-way ANOVA, $F_{3,153} = 0.547$, $p = 0.651$). Top height remained constant over the course of up to 4 d of laying while bottom height decreased as laying progressed, suggesting that prior egg capsule deposition may cause mud snails to place their egg capsules closer to the benthos. Driven by the changes in bottom height, the mean height of egg capsules also decreased over time.

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

Effect of predators on egg capsule placement

For the experiment where adult snails were exposed to cues from potential egg capsule predators, the placement of egg capsules on eelgrass was sepa-

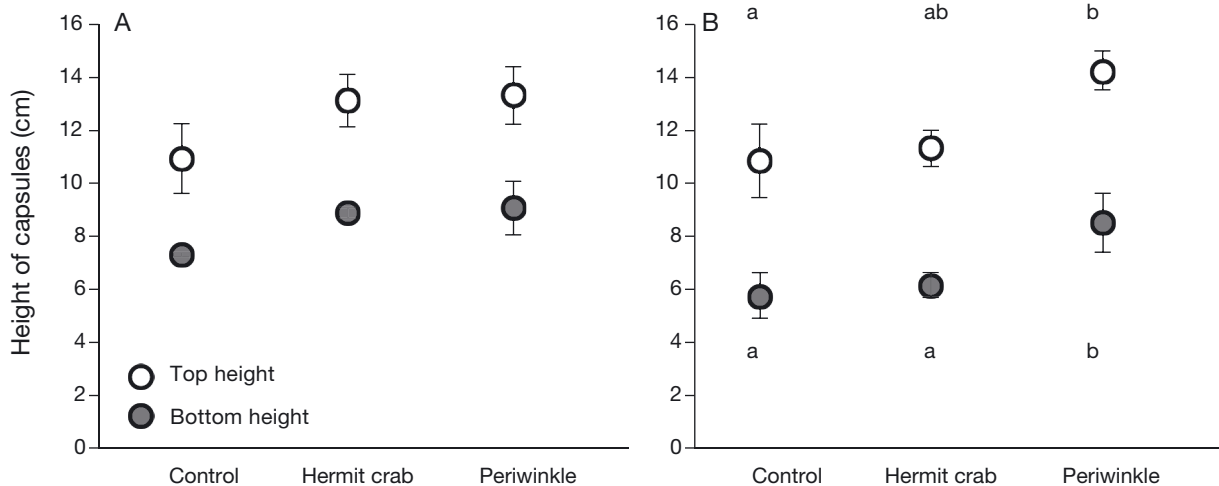


Fig. 6. (A) Bottom and top height of egg capsules for snails exposed to predator cues after 1 d. Gray circles indicate the mean (\pm SE) height of the lowest egg capsule, and white circles indicate the mean (\pm SE) height of the highest egg capsule (control $n = 2$, hermit crab $n = 7$, periwinkle $n = 8$). (B) Bottom (gray circles) and top (white circles) height of egg capsules for snails exposed to predator cues after 10 d (control $n = 7$, hermit crab $n = 9$, periwinkle $n = 10$). Different letters indicate significant differences among treatments within each height (top or bottom)

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

After 10 d, in the presence of hermit crab cues, snails deposited their egg capsules an average of 0.4 cm higher off the benthos than control (Fig. 6B). In the presence of periwinkles, snails deposited their egg capsules an average of 2.6 cm higher than control (Fig. 6B). Predator cue treatment had a significant effect on both bottom height (1-way ANOVA, $F_{2,205} = 6.114$, $p = 0.003$) and top height of egg capsules (1-way ANOVA, $F_{2,204} = 4.427$, $p = 0.013$). Final top heights were lower for some treatments than on the first day of laying because snails began laying on shorter blades of the eelgrass plant as time progressed. The bottom height of capsules for periwinkle cue was significantly greater than for hermit crab cue ($p = 0.014$) and the control ($p = 0.007$), which were not significantly different from each other ($p > 0.99$). In addition, the top height of capsules when exposed to periwinkle cue was significantly higher than the control ($p = 0.023$), while the height for hermit crab treatments was not significantly different from either periwinkle or control treatments ($p > 0.05$).

Survival of egg capsules on eelgrass

Hermit crabs preferentially fed upon egg capsules deposited lower on blades of eelgrass (Fig. 7). The survival of egg capsules on eelgrass in the presence of hermit crabs decreased with time exposed (Day 1 to 8), and increased with height along the blade of eelgrass (1, 5, 10, or 15 cm; binomial logistic regression; Table 1). A Hosmer and Lemeshow test of this binomial logistic regression model revealed a significant lack of fit to the data ($p < 0.001$). However, this statistic can yield significant lack of fit for data with large sample sizes (as in this case with 7840 capsules scored for survival), and since the model accurately predicted survival in 89.5% of cases, we continued to use this model for our analysis (Kramer & Zimmerman 2007).

Survivorship decreased each day at all heights, but capsules deposited just 5 cm higher on a blade had survivorship that was 4 times higher than capsules deposited directly on the benthos. Additionally, the

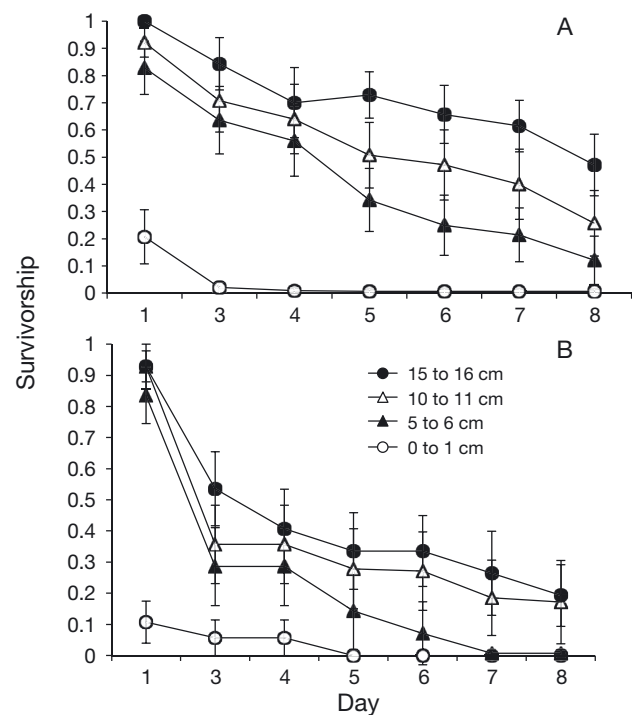


Fig. 7. Hermit crab predation upon mud snail *Tritia obsoleta* egg capsules placed at different heights above the benthos on eelgrass for simulated (A) high tide and (B) low tide. Each point represents the mean (\pm SE) proportion of surviving egg capsules at the given height in $n = 14$ containers

Table 1. Binomial logistic regression for predation upon egg capsules at heights above the benthos. β is the coefficient of the regression equation for each term. 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 \times Height	0.004	0.308	1.004
Day \times Tide	0.212	<0.001	1.236
Height \times Tide	0.099	<0.001	1.104
Day \times Height \times Tide	-0.011	0.040	0.989
β_0	-0.193	0.257	0.825

interaction term between tide and height or day may indicate that high tide conditions play a role in increasing survival of egg capsules over low tide conditions (Fig. 7). Egg capsule survivorship is likely a function of total height off the benthos, which is determined by a combination of height along the blade of eelgrass and tidal period (eelgrass blades lay flat on the surface at low tide and rise to a more vertical position at high tide).

DISCUSSION

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

Substrate preference

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

In Maine, preference for eelgrass is strong enough that mud snails were attracted to and began laying on transplanted eelgrass within 1 wk. The mechanism by which snails locate eelgrass is not known. It is known, however, that a southern population of mud snails will follow the odor of living bivalves (their preferred laying substrate in North Carolina) as well as track a chemical contained within egg capsules (Rittschof et al. 2002). Snails aggregate to the source of these cues for copulation and egg deposition (Rittschof et al. 2002). While the snails may also respond to egg laying hormone (Painter et al. 1991), there is no evidence that snails are attracted to other reproductive snails (Rittschof et al. 2002). It is possible that the Harpswell Sound mud snail aggregations occur as a byproduct of snails following cues produced by patches of eelgrass. It is equally possible that if one or a small number of snails were to find a

patch of eelgrass at random and begin to deposit capsules on it due to tactile cues, chemical cues from egg capsule deposition could draw other reproductive snails to the same patch. Our experiments were unable to distinguish between these 2 hypotheses, but further studies could test whether chemical cues from eelgrass are attractive to adult mud snails.

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

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

Laying height preference

In our study area, a mudflat in Harpswell Sound off Orr's Island, eelgrass was the most common solid substrate that was elevated off the benthos. Eelgrass

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

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

Plasticity of laying height

Mud snails are known to exhibit plastic responses to chemical cues, or kairomones (Ruther et al. 2002), of predators (e.g. Schwab & Allen 2014). When exposed to cues from predators such as green crabs and periwinkles, mud snails may burrow or flee (Brenchley & Carlton 1983, Rahman et al. 2000). There is an even stronger, chemically triggered alarm response to crushed conspecifics (Atema & Burd 1975, Atema & Stenzler 1977). Beyond behavioral responses, mud snails also demonstrate morphological plasticity in the presence of predator cues.

For example, when given waterborne cues from the blue crab *Callinectes sapidus*, mud snails developed apertures that were smaller relative to shell size, reducing the success of predator attacks through the shell opening (Santoni et al. unpubl. data). In the presence of predator cues, mud snails also vary their reproductive output both in terms of laying quantity and capsule morphology, creating longer protective spines surrounding the capsule opening (Schwab & Allen 2014). Given these prior examples of predator-induced plasticity, it seems reasonable that deposition of egg capsules off the benthos could be an additional adaptive plastic response of mud snails to predator cues.

Mud snails typically deposited egg capsules 4 to 8 cm off the benthos and elevated their deposition by a further 1 to 3 cm in the presence of periwinkles. In the presence of hermit crabs, capsules were deposited at a height that did not significantly differ from the control. Periwinkles and hermit crabs are both known egg capsule predators (Brenchley 1982) that are abundant on the benthos but were not observed to crawl higher than a few cm up a blade of eelgrass in the field. Therefore, we hypothesize that depositing egg capsules several cm above the benthos is a plastic response that allows mud snails to increase the survival of their young in the presence of egg capsule predators. Though hermit crabs have been observed to be the more voracious predator (Brenchley 1982), they primarily inhabit the benthos, while periwinkles tended to crawl up eelgrass plants while grazing (E. A. Harmon pers. obs.). In the presence of periwinkles, mud snail deposition of capsules farther off the benthos may prevent bulldozing, which occurs as periwinkles displace and destroy egg capsules while grazing on the epiphytes of eelgrass and occasionally directly consuming capsules and eggs (Brenchley 1982).

In the future, it would be beneficial to perform an experiment with combined cues from periwinkles and hermit crabs. In other species of snails, combined predator cues can either lead to an intermediate or a prioritized response (Bourdeau 2009, Mach & Bourdeau 2011). Based on our observations, we would predict that hermit crabs are the more dangerous predator; when egg capsules were attached to sand collars, hermit crabs removed 124 times more egg capsules than did periwinkles (authors' unpubl. data), yet changes in height were more dramatic in response to periwinkle cues. If, as seems likely, hermit crab cues are always present then there may be a default laying height for snails in this high-risk population, explaining why there was no significant difference between the heights at which capsules were deposited in the

presence of hermit crabs and control (Bourdeau 2012). Alternatively, our 'control' cue water may have been carrying cues from hermit crabs in the sound, where water came into the marine lab and thus contaminated with hermit crab cue. Or snails may respond more strongly to crab cues if the crabs are actively consuming egg capsules, which they were prevented from doing in our experimental design. The response to lay even farther off the benthos may also be reserved only for an instance in which there is a strong periwinkle cue, to which mud snails are known to be sensitive and to which they adjust their behavioral response (Brenchley & Carlton 1983).

We also found that mud snails use the morphology of eelgrass plants rather than height per se to determine where to deposit egg capsules. Mud snails strongly prefer to deposit capsules on the blades of eelgrass rather than the sheath; thus, the change from sheath to blade appears to provide a tactile clue for the snail as it climbs the plant as to where to deposit egg capsules. When given blades without the attached sheath as a laying substrate, mud snails deposit capsules directly at the interface of the blade and the benthos. Very few capsules were deposited on the sheath, and those that were tended to be deposited several cm off the benthos. It is unclear whether height above the benthos is the only benefit to placing egg capsules on eelgrass blades rather than the sheath, but height seems likely to be related to reduced capsule predation.

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

Consequences of deposition strategy

If adult mud snails deposited egg capsules on the first portion of eelgrass they encountered (i.e. the

sheath in contact with the benthos), lower reproductive success would result. In our work, we have shown that egg capsules that were deposited directly on the benthos experienced up to 4 times greater rates of predation than those deposited just 5 cm higher. The ideal zone for egg capsule placement is likely determined by a combination of benthic predators and abiotic stresses. As the number of previously laid capsules increases, we observed that mud snails will deposit capsules both higher and lower on eelgrass blades, presumably outside of the preferable zone. Survival of these egg capsules is likely reduced compared to capsules laid earlier and in a more optimal part of the eelgrass blade.

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

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