Plasticity in egg placement in response to predator cues in the mud snail, Ilyanassa obsoleta

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Plasticity in egg placement in response to predator cues in the mud snail, *Ilyanassa obsoleta*

A thesis submitted in partial fulfillment of the requirement for the degree of Bachelor of Science in Biology from The College of William and Mary

by

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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 other 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 abundant mud snail, *Ilyanassa obsoleta*. In intertidal and shallow subtidal habitats in Maine, the mud snail preferentially lays egg capsules on blades of eelgrass (*Zostera marina*) that stand upright in the water column. In a field and lab study, I examined deposition of *Ilyanassa obsoleta* eggs 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 such as hermit crabs and periwinkles, mud snails increase the average lowest height of laying by 1-3 cm. In the presence of hermit crabs, capsules placed just 5 cm up a blade have survivorship as much as 4 times higher than capsules placed directly on the benthos. Placement of egg capsules off of the benthos may be an adaptive plastic response allowing mud snails to protect their embryos from benthic predators.
**Introduction**

Most marine invertebrates exhibit complex life cycles, composed of a benthic adult stage and a free-living planktonic larval stage (Thorson, 1950). Many marine invertebrates are broadcast spawners (Giese, 1959). For broadcast spawners, eggs are fertilized in open water where the embryo develops through larval stages for several weeks or months before settling onto the benthos as a juvenile, where it will spend the rest of its life. Microscopic embryonic and larval stages face high mortality (Rumrill, 1990; Vaughn and Allen, 2010); one argument for the evolution and persistence of this complex life history is that developing invertebrates face lower levels of predation in planktonic habitats than on the benthos (Rumrill, 1990; Morgan, 1995; Strathmann, 2007).

The development of planktonic marine larval stages likely arose in the late Cambrian (Signor and Vermeij, 1994). At this time, few organisms were pelagic and benthic organisms were rapidly diversifying. Escaping benthic predation could have been an important driving force for the development of planktonic larvae (Peterson, 2005). Though many organisms are now pelagic, the persistence of the complex marine invertebrate life history is still hypothesized to be due to a release from predation (Strathmann, 1985, 1993). Predation is much greater (by up to 28 times) for the same organism placed on the benthos versus in the water column (Allen and McAlister, 2007).

Many studies of the evolution of marine invertebrate life histories include estimates of planktonic predation, but are forced to make simplifying assumptions about levels of larval planktonic mortality in the absence of good evidence about natural levels of mortality (Vance, 1973; Christiansen and Fenchel, 1979; Levitan, 2000). Many fewer studies take into account benthic mortality of larvae or juveniles (Pechenik, 1999), even
though developing forms of marine invertebrates face much higher levels of predation from benthic predators than from planktonic predators (Pechenik, 1979; Allen and McAlister, 2007).

Benthic development exposes vulnerable eggs, embryos, and larvae to higher rates of predation and thus we might expect it to be selected against over evolutionary time (Strathmann, 2007; Allen and McAlister, 2007). However, polychaetes, nemerteans, turbellarians, and gastropods all deposit egg capsules on the benthos for several weeks or months as the embryos develop, suggesting that benthic development can be a successful life history strategy (Pechenik, 1999). 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 nemerteans, polychaetes, and flatworms; Christiansen and Fenchel, 1979; Crothers, 1985; Martel and Chia, 1991; Ruiz-Trillo *et al.*, 1999) while others develop in egg capsules and then hatch as swimming larvae that have an obligate planktonic period (the gastropod *Ilyanassa obsoleta* and many others; Grassle and Grassle, 1974; Pechenik, 1979; Caswell, 1981; Strathmann, 1985). The presence of both benthic encapsulation and free-living planktonic larvae in the same life history is called mixed development (Pechenik, 1979). Mixed development seems counterintuitive since it exposes developing young to threats of both the benthic and planktonic habitats (Caswell, 1981; Vaughn and Allen, 2010). However, encapsulation can increase the fitness of organisms by equipping embryos with structural and chemical defenses while allowing mothers to strategically place capsules in ways that minimize environmental stress (Pechenik, 1979; Caswell, 1981; White, 2011).
Encapsulation likely helps protect developing embryos from the constant threat of predation, and at least 65% of gastropods exhibit encapsulated development (Pechenik 1979; Pechenik 1999). Still, gastropod egg capsules can experience benthic predation rates as high as 35% prior to hatching (White, 2011). While encapsulation alone provides some protection to developing embryos (Pechenik, 1979; Pechenik, 1982; Rawlings, 1994; Rawlings, 1999; Przeslawski, 2004; Schwab and Allen, 2014), maternal reproductive plasticity has the potential to further increase the survival of young in the benthic environment. For example, the dogwhelk *Nucella lapillus* creates thicker capsules to surround their embryos when predatory isopods share their habitat (Rawlings, 1990). *N. lapillus* also places capsules in aggregations, which can be more effective in reducing mortality from both predation and desiccation than placing capsules separately (White, 2011).

These types of phenotypic plasticity, or the ability of a single genotype to produce multiple phenotypes under different environmental conditions, are common in many biological systems (Pigliucci, 2001) and allow organisms to adjust their phenotype in the face of environmental stresses (Agrawal, 2001; Ghalambor et al., 2007). Environmental stressors that induce plasticity can take many forms including, but not limited to, abiotic stressors such as temperature (e.g., butterflies display different markings if they emerge during the wet season than during the dry season; Brakefield and Reitsma, 1991) and biotic stressors like competition (e.g., the intensity of competition determines whether toads produce tadpoles that are omnivorous or carnivorous; Pfennig and Murphy, 2002) or predation (e.g., elk change their foraging preferences in the presence of wolves; Creel et al., 2005). Predator-induced plasticity is often triggered in response to predator kairomones,
or interspecific chemical cues (Ruther et al., 2002), and can induce changes in the prey’s foraging behavior, morphology, or even the timing of life history events (Gilliam and Fraser, 1987; Crowl and Covich, 1990; Peckarsky et al., 1993, Relyea, 2001). The presence of predators can lead to the formation of morphological defenses such as spines (Harvell, 1990) or thicker shells (Trussell, 1996) that deter predation. Prey may also change life-history traits in the presence of predators. For example, the freshwater snail *Helisoma trivolvis* is particularly susceptible to predators such as crayfish and water bugs when it is smallest (Hoverman et al., 2005). In the presence of predators, young snails allocate more energy into growing to a larger, less vulnerable size rather than allocating energy towards reproduction (Hoverman et al., 2005).

In multiple studies, marine gastropods exhibit plastic responses to predators (Vermeij, 1974, 1978, 1982a, 1982b, Oyarzun and Strathmann, 2011). For example, adult mud snails exposed to a waterborne chemical cue from green crabs lay egg capsules with longer protective spines, and small snails exposed to cues from green urchins lay fewer eggs (Schwab and Allen, 2014). The longer spines can be effective at reducing predation by hermit crabs, suggesting an adaptive value to the plasticity in this trait (Schwab and Allen, 2014). Many marine snails also grow thicker shells, or change shell shape or aperture size to deter nearby predators (Bourdeau, 2009b; Bourdeau, 2012; Moody and Aronson, 2012; Santoni and Allen, in preparation).

In general, gastropods respond to predator cues by adjusting both their morphology and their behavior (Trussell, 1996; Bourdeau, 2009a). Additionally, snails can balance threats from predators possessing different feeding tactics by prioritizing plastic responses to the more dangerous predator, particularly by adjusting behavior (Bourdeau, 2009b).
These risks are quickly assessed, and snails in the same habitat may choose whether to flee or hide based upon the cue, or combination of cues received (Mach and Bourdeau, 2011).

The eastern mud snail, *Ilyanassa obsoleta*, is a common gastropod and a useful model organism for studies of phenotypic plasticity because of its high abundance (up to 8000 m$^{-2}$ in roving groups of adults) on intertidal mudflats along the East Coast ranging from the Gulf of St. Lawrence to the Gulf of Mexico (Cranford, 1988; Harmon and Allen, unpublished data). *I. obsoleta* is also an invasive species on the West Coast of the United States and is now the most common gastropod in San Francisco Bay, displacing native species (Carlton, 1979). Its presence on mudflats across the Atlantic coast of North America make it a key species in the nutrient cycling (Edwards and Welsh, 1981; Giannotti and McGlathery, 2001; Kelaher *et al.*, 2003) and ecology of these habitats, yet its basic ecology remains understudied. Reproductive adults vary in size from about 14 to 26 mm in length (Scheltema, 1962; Schwab & Allen, unpublished data). During the reproductive season, an individual adult *I. obsoleta* deposits eggs in about 100 capsules, each containing 30-300 eggs (Pechenik, 1978; Brenchley, 1982; Rittschof *et al.*, 2002). *I. obsoleta* lays 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 and Maugel, 1984). Embryos develop within the capsules for two weeks before hatching as pelagic veliger larvae (Sullivan and Maugel, 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 lay their egg capsules may have substantial implications for the survival of their offspring.
Off the coast of Maine, mud snails tend to lay many of their egg capsules upon blades of eelgrass (*Zostera marina*; Allen, unpublished observation). In this habitat, egg capsules are preyed upon by benthic predators including green crabs (*Carcinus maenas*), hermit crabs (*Pagurus longicarpus*), and periwinkles (*Littorina littorea*; Brenchley, 1982). Though periwinkles more often displace egg capsules while foraging than directly consume eggs, mud snails are known to evacuate tide pools once periwinkles are introduced (Brenchley, 1983). Adult mud snails are also wary of crab predators (Tagatz, 1968; Brenchley, 1982), to which they demonstrate various plastic responses that can reduce the threat of predation throughout their life cycle (e.g. changing aperture size and capsule morphology; Hazlett, 1988; Schwab and Allen, 2014, Santoni and Allen, in preparation).

Because mud snails are highly plastic in response to predators and because the benthic environment where they deposit their eggs can be dangerous for developing offspring, I used the mud snail *I. obsoleta* to test basic hypotheses related to the evolution of complex life cycles and phenotypic plasticity. In particular, I hypothesized that 1) *Ilyanassa obsoleta* lays egg capsules predominately on eelgrass; 2) egg capsules on eelgrass are deposited off of the benthos; 3) *I. obsoleta* increases the height of egg capsule deposition in the presence of predators; 4) egg capsules which are deposited farther off of the benthos are less likely to be consumed by predators.
Methods

Experiment Sites

For most experiments, organisms including mud snails (*Ilyanassa obsoleta*), periwinkles (*Littorina littorea*), hermit crabs (*Pagurus longicarpus*), eelgrass (*Zostera marina*), and sand collars (egg masses of *Euspira heros*) were collected from the mudflat adjacent to the Bowdoin College Coastal Studies Center (CSC) on Orr’s Island, Maine (43° 79’ N, 69° 95’ W). Tide pools in this mudflat were the site of field surveys. Laboratory experiments were conducted in the flow-through seawater lab of the Bowdoin CSC. Coarsely filtered seawater pumped directly from Harpswell Sound was used for all laboratory experiments.

In a follow up experiment, mud snails, *Laminaria* spp., *Z. marina*, and *Chondrus crispus* were obtained from Massachusetts, and *Fucus distichus* and *Ascophyllum nodosum* were obtained from Maine. The work was conducted at the Allen laboratory at the College of William and Mary, VA.

Field observations

To assess spatial distribution of mud snail egg capsules, 56 plots were surveyed along three transects in a large tide pool of the CSC mudflat. For each plot, I used a 1 m² quadrat to count the number of mud snails, eelgrass plants, sand collars, the proportion of sand collars bearing mud snail egg capsules, and the proportion of blades of eelgrass bearing mud snail egg capsules. If there was a large number of mud snails in a plot (>100 snails), the quadrat was randomly subsampled in five 10 cm² divisions, and the number of mud snails extrapolated to the whole plot. The density and vertical distribution (on
eelgrass) of egg capsule predators, namely hermit crabs and periwinkles, was also recorded. I used the curve estimation procedure in SPSS (version 22; used for all analyses) to determine whether these data were significantly correlated with one another and, where there were significant correlations, what type of curve best fit the data. The curve estimation fit 11 models to my data: linear, logarithmic, inverse, quadratic, cubic, compound, power, S, growth, exponential, and logistic. Curves were determined to be the best fit by selecting those with the highest adjusted $R^2$ value.

_Eelgrass Transplant_

To determine whether mud snails and eelgrass inhabit similar areas in the field or whether snails flock to eelgrass as a preferred laying substrate during the reproductive season, I conducted a transplant experiment in a section of the CSC mudflat in which eelgrass was absent. I marked out nine plots in the mud using a 1 m$^2$ quadrat. The 1 m$^2$ plots were set 2 m apart in a 3 x 3 grid (Figure 1). The number of snails in these plots was recorded, as well as in nine haphazardly sampled background plots on the same section of mudflat. I then planted 10 eelgrass plants in each of the nine marked plots. I found that pushing the roots of the plant approximately a finger’s depth into the mud and then packing around the base of the plant was an effective method of securely planting the eelgrass in the mudflat. Five days later, I returned and once again counted the number of snails in the marked plots and in nine haphazardly sampled background plots. I conducted a square-root transformation of these data to obtain normally distributed residuals for a univariate analysis of variance (ANOVA). Normality was confirmed by Kolmogorov–Smirnov and Shapiro-Wilk tests. I then performed a 2-way ANOVA on the number of snails present with time (before and after transplant), location (plot with transplants or
background plots), and the interaction term between time and location as fixed effects. Where significant effects were found, I ran a Bonferroni post-hoc test to test for pair-wise differences among treatments.

**Substrate Preference**

To determine whether eelgrass was the preferred laying substrate for mud snails, I conducted a preference experiment in the lab. Large adult mud snails (20+ mm shell length) were collected from the field and randomly assigned into 10 replicate Sterilite® containers (15 x 21 x 7.5 cm) at a density of 18 snails per container, well within the density of snails I observed in the field. Water from the CSC flow-through system was directed into a bucket with 30 holes drilled into the bottom. Lengths of clear vinyl tubing, 5/16” in outer diameter and 3/16” in inner diameter, were fit tightly into these holes and directed into the Sterilite containers through a hole in the lid (Figure 2). Excess water escaped around the edges of the lid. This design allowed us to directly deliver flowing water to many replicates within the same sea water table. Each replicate held a sample of hard substrates which were prevalent on the CSC mudflat: an oyster shell (*Ostrea edulis*), a mussel shell (*Mytilus edulis*), a hard-shell clam shell (*Mercenaria mercenaria*), a soft-shell clam shell (*Mya arenaria*), an eelgrass plant (*Z. marina*), and a sand collar (*E. heros*; Figure 3). As the experiment progressed, snails also started to lay egg capsules upon the walls of the Sterilite container, and so the container was added as a substrate for counting purposes. The experiment ran for 1 week, and the number of egg capsules laid upon each substrate was recorded each day.

A follow-up experiment was performed in March 2016 at the College of William & Mary to evaluate snail substrate preference among algae and other marine vegetation. The
given substrates were *Laminaria* spp., *Z. marina, C. crispus, F. distichus* and *A. nodosum*. A section of each of these five substrates with similar surface area was attached to the bottom of 5 replicate 43 x 36 x 25 cm containers with hot glue and Krazy Glue ®. Placement was randomized among the 4 corners of a 6” x 6” square and the central point. Each container was filled with artificial sea water and equipped with an air pump. Ten mud snails were randomly placed in each replicate, and the number of egg capsules on each substrate, including the container walls, was recorded after 3 and 6 days.

*Egg capsule deposition on sand collars*

Since mud snail egg capsules were frequently found on sand collars in the field, I performed an additional experiment to determine whether mud snails would lay capsules on sand collars in the absence of eelgrass. Mud snails were either given one eelgrass plant or one sand collar to lay egg capsules upon. I used the laboratory set-up using Sterilite containers (15 x 21 x 7.5 cm), a bucket, and tubing as described above. Twenty containers were filled with 2-3 cm of mud from the mudflat; eelgrass was planted in ten containers, and a sand collar placed on top of the mud in an additional ten containers. I randomly assigned 18 snails to each of the 20 total containers. The experiment ran for 11 days, and the number and position of egg capsules laid upon each substrate was recorded each day. In addition to the sand collar and eelgrass, mud snails also began laying egg capsules on the walls of the Sterilite containers in which the experiments were held. Therefore, counts of egg capsules laid on containers were also collected.

I recorded the number of days until the first capsules were laid for sand collar and eelgrass treatment to determine if there is a delay of laying when the preferred substrate, eelgrass, was not available. I performed Kolmogorov-Smirnov and Shapiro-Wilk tests on
the data to confirm residuals met the assumption of normality for univariate ANOVA. Data for laying on the four substrates were square-root transformed to meet this assumption. Where significant effects were found, I ran a Bonferroni post-hoc test to test for pair-wise differences among treatments.

*Predation of mud snail egg capsules on sand collars*

The widespread presence of mud snail egg capsules under sand collars but not on top of sand collars in the field suggests either that 1) snails prefer to deposit egg capsules on the underside of a sand collar or 2) snails deposit egg capsules haphazardly on substrates, but those that are laid under a sand collar are more likely to survive than capsules laid elsewhere on the sand collar. To determine if the latter was the case, I glued 10 egg capsules in an evenly spaced ring on the top and underside of sand collars (Figure 4). I used Krazy glue gel® as an adhesive, which can be effective for attaching developing stages of marine invertebrates without deterring predators (Allen and McAlister, 2007). Manipulated sand collars were placed in a Sterilite container holding either 3 hermit crabs or 10 periwinkles, the numbers of which were estimated to possess similar foraging levels. Each treatment consisted of 15 replicate containers. The survival of egg capsules was monitored over 48 hours, and a binomial logistic regression was run on the results using the variables of predator, side of sand collar, day, and interaction terms as predictors. The inclusion of all interaction terms resulted in the lowest log-likelihood score. Following the regression analysis, I performed a Hosmer and Lemeshow test to determine the goodness of fit of the model (Kramer and Zimmerman, 2007).
**Egg capsules placement**

To determine where mud snails lay egg capsules on eelgrass, I quantified the position of egg capsules upon eelgrass in the field. Twenty-eight eelgrass plants bearing egg capsules were haphazardly collected from the CSC mudflat and brought into the lab. 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 (Figure 5).

For every eelgrass blade, I measured the placement of egg capsules. All measurements were taken from the node as 0 cm (Figure 6). I 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. If necessary for plants with high densities of capsules, layers of egg capsules were carefully scraped off with forceps to count via the aid of a tally counter. These measurements allowed me to assess the top and bottom height at which egg capsules were found along blades of eelgrass from the field. 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.

Laboratory measurements of egg capsule placement on eelgrass were taken during the experiment entitled “egg capsule deposition on sand collars”. I tracked where capsules were deposited on eelgrass over 11 days. Day 1 was recorded as the first day that at least five egg capsules were laid in a container. Egg capsule position was tracked for only five days after laying began in the first container. Therefore, containers in which snails started laying after a few days were not able to be recorded for a full 4 days of laying, and so not
every container is included for every day. These data were used to determine whether the position of mud snail egg capsules in the field is due to where snails deposit the capsules or where capsules remain after predation. The bottom and top height data were square-root transformed to fit the univariate ANOVA assumption of normality, as confirmed by Kolmogorov-Smirnov and Shapiro-Wilk tests. I ran a one-way ANOVA on the transformed data, with day as the fixed effect.

**Effect of predators on egg capsules placement**

I wanted to determine whether mud snails change their laying substrate preference or the height at which they place egg capsules in the presence of predators. Hermit crabs and periwinkles were chosen as the egg-capsule predators since hermit crabs were observed to be voracious predators of egg capsules, and periwinkles bulldoze mud snail egg capsules and are extremely abundant on the CSC mudflat (Brenchley, 1982). To deliver a chemical cue from the predator to adult mud snails without allowing the predators direct contact with egg capsules, predators were kept in separate Sterilite containers. A hole was drilled in the side of the containers (15 x 21 x 7.5 cm) so that tubing (5/16” od, 3/16” id) could run out. The lid was fit so that the incoming water from the bucket would enter this container on one side and flow over the predators, exiting through tubing on the other side. This tubing then ran through a hole in the lid of the experimental chambers, which held 18 randomly assigned adult mud snails, an eelgrass plant, and a sand collar (Figure 7).

There were 10 replicates each of three predator cue treatments: hermit crab, periwinkle, and control (ambient sea water). Each treatment container held 10 predators of similar size so that each experimental chamber received a similar strength of chemical cue from the predators. A sheet of egg-crate ceiling tile separated predator containers
(above) from laying containers (below), with tubing running through the ceiling tile to connect the containers (Figure 7).

Every four days, I recorded the number and position of egg capsules on the eelgrass and sand collar. Blades of eelgrass that broke off from the plant were discarded if I could not determine the bottom height of egg capsules, and hermit crabs were replaced after infrequent mortality. The experiment ran for 18 days. Afterwards, the snails were sexed to calculate the number of egg capsules laid per female. Sex was determined by viewing the gonads (Figure 8) after using a C-clamp to remove the shell. Many of the snails were castrated by parasites, and the sex was, therefore, indeterminate.

Data on the lowest height at which capsules were laid were square-root transformed, and data for the highest height at which capsules were laid were ln transformed to fit the univariate ANOVA assumption of normality, as confirmed by Kolmogorov-Smirnov and Shapiro-Wilk tests. I ran an ANOVA on the transformed data, with predator as the fixed effect. Where significant effects were found, I ran a Bonferroni post-hoc test to test for differences between the three treatments. To account for laying amount per female, I ran a one-way ANOVA on the per-female data with predator as the fixed effect.

**Survival of egg capsules on eelgrass**

To assess how predation varied with height along an eelgrass plant, I manipulated the position of egg capsules along blades of eelgrass. Each blade was 17 cm long, and a 2 x 5 clump of egg capsules (approximately 1 cm in height) was glued at 0 cm, 5 cm, 10 cm, and 15 cm from the base (Figure 9). One-cm sections 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®. One cm of blade was left at the bottom to attach the blade to its container. To evaluate any differences in predation by height caused by tidal height, I attached the manipulated eelgrass blades in two separate types of containers: the 7.5 cm deep Sterilite containers, as well as 20 cm deep containers. These different heights mimic low and high tide respectively and differ in the degree to which the tip of the eelgrass can float above the benthos. Fourteen replicates of each type of container were supplied flow-through water with tubes through the lid and filled with 3 hermit crabs.

Every day for 8 days, the number of egg capsules surviving at each height in each container was recorded. Any blades or sections of egg capsules that came unglued (1-2 detachments per day) were reattached. The survival of egg capsules was monitored over the course of 48 hours and a binomial logistic regression was run on the results 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, I performed a Hosmer and Lemeshow test to determine the goodness of fit of the model (Kramer and Zimmerman, 2007).
Results

Field Observations

Observational field data included number and position of mud snails, egg capsule predators, eelgrass, sand collars, and egg capsules. The correlation between the number of mud snails and the number of blades of eelgrass in the field (Figure 10) was positive, significant (p < 0.001) and best fit by a cubic function ($y = -0.0005x^3 + .118x^2 - 3.262x + 26.002$, Adjusted $R^2 = 0.486$). High densities of snails (>200 m$^{-2}$) were only seen in plots with 75 or more blades of eelgrass m$^{-2}$ (Figure 10), supporting the hypothesis that mud snail density is higher in areas with more eelgrass. There does not appear to be any significant relationship between the presence of sand collars and mud snails, despite my observation that the underside of every sand collar encountered bore mud snail egg capsules (Figure 11).

The proportion of eelgrass blades on which mud snail egg capsules were laid was positively and significantly (p < 0.001) correlated with the number of eelgrass blades in a plot (Figure 12) and the relationship was best fit by a power function ($y = 0.054x^{0.622}$, Adjusted $R^2 = 0.780$). With one exception, plots with high densities of eelgrass (>50 blades m$^{-2}$) had 70% or more eelgrass blades with egg capsules (Figure 12). Areas with sparser eelgrass, with fewer than 50 eelgrass blades m$^{-2}$, had 0-100% of eelgrass blades bearing egg capsules. In addition, the proportion of eelgrass blades bearing egg capsules was positively and significantly (p < 0.001) correlated with the number of snails in a plot (Figure 13) and this relationship was best fit by a logarithmic function ($y = 0.059\ln(x) + 0.390$, Adjusted $R^2 = 0.379$). In all plots with 200 or more mud snails m$^{-2}$, egg capsules were present on 90% or more of the eelgrass blades.
Egg capsule predators, namely hermit crabs and periwinkles, were predominately observed directly on the benthos. Most periwinkles were found along the bottom of eelgrass plants, with fewer periwinkles found the higher the position up a blade of eelgrass. In a survey of submerged eelgrass plants, there were 70 periwinkles on the bottom third of eelgrass plants, 32 periwinkles in the middle third, and 6 on the top third. Similarly, all hermit crabs observed in the field over the course of the summer were on the benthos. I observed average predator densities on the mudflat of 4.9 hermit crabs m\(^{-2}\) (SE = 0.84) and 0.26 periwinkles m\(^{-2}\) (SE = 0.10).

**Eelgrass Transplant**

The experimental addition of eelgrass significantly increased the mean number of snails in each plot (Figure 14). The number of snails varied by time (before versus after transplantation) and location (inside versus outside transplant zones), but there was an interaction precluding conclusions about the main effects (Table 1). Snail numbers increased inside plots with eelgrass over time, but did not increase in plots without eelgrass additions (Figure 14).

**Substrate Preference**

In the lab experiment to determine mud snail preferences for laying substrate, over the course of one week, 98.56% of the egg capsules laid were deposited on eelgrass. 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 even the walls of the container which held the experiment (0.28%), were laid upon to a much lesser extent, totaling only 1.44% of egg capsules laid.
When given the choice of *Laminaria* spp., *Z. marina*, *C. crispus*, *F. distichus*, or *A. nodosum* as a laying substrate, mud snails initially tended to prefer to place egg capsules on eelgrass (Figure 15A). However, this preference was not statistically significant (univariate ANOVA, df = 4, F = 2.428, p = 0.090). After six days, the preference diminished until snails laid predominately on the container, an unnatural substrate (Figure 15B; univariate ANOVA, df = 5, F = 11.812, p < 0.001).

*Egg capsule deposition on sand collars*

Mud snails preferred to lay egg capsules on eelgrass and even the container that experiments were held in, over sand collars (Figure 16). While the total amount of laying after 11 days in containers that held eelgrass was statistically indistinguishable from the amount of laying in containers that held sand collars (Figure 16; univariate ANOVA, df = 1, F = 0.179, p = 0.678), there was a statistically significant, one-day delay of laying in containers with a sand collar (Figure 16; univariate ANOVA, df = 1, F = 7.480, p = 0.015).

When broken down into four substrates, including the container of each treatment as a unique substrate (eelgrass, Sterilite container of eelgrass treatment, sand collar, and Sterilite container of sand collar treatment), there was a significant difference among substrates (Figure 16; univariate ANOVA, df = 3, F = 27.416, p < 0.001). A Bonferroni post-hoc test revealed that the number of capsules laid on eelgrass and the container walls of the sand collar treatment were not significantly different, and that the number of capsules laid on sand collars and the container walls of the eelgrass treatment were not significantly different (Figure 16). There was, however, a statistically significant difference between these two sets, as approximately 92% of egg capsules were laid upon the eelgrass rather
than on the container of the eelgrass treatment, but only 3% of egg capsules were laid upon the sand collar rather than the container of the sand collar treatment.

**Predation of mud snail egg capsules on sand collars**

Hermit crabs preferentially fed upon egg capsules that were on the top side of sand collars (Figure 17). The survival of snail egg capsules upon a sand collar depended both upon which side of the sand collar the capsules were placed (binomial logistic regression, $\text{Exp}(\beta) = 6.081, p < 0.001$) as well as whether the sand collars are exposed to periwinkles or hermit crabs ($\text{Exp}(\beta) = 0.001, p < 0.001$; Table 2). A Hosmer and Lemeshow test of this binomial logistic regression model was nonsignificant ($p = 1.00$), suggesting that the model fit the data well. The model correctly predicted survival 89.5% of the time. On the top side of sand collars, hermit crabs removed 124 times more capsules than did periwinkles, and on the underside of sand collars hermit crabs removed 5 times more capsules than periwinkles. Overall, egg capsules on the underside of a sand collar had a 38% greater chance of survival than capsules on the top side.

**Egg capsules placement**

On average, mud snail egg capsules were not present on the bottom 6 cm of eelgrass blades in the field (Figure 18). This average lowest height of capsules is not significantly different for eelgrass plants with either high or low densities of egg capsules (independent sample t-test, $df = 185, t = 1.470, p = 0.143$). For eelgrass plants with low densities of egg capsules, the average height of the highest capsules are about 12 cm off the bottom, whereas the average top height is significantly higher, at 17 cm, for eelgrass plants with high densities of egg capsules (Figure 18; independent samples t-test, $df = 186, t = -3.564, p$
Thus, when the density of egg capsules increases in the field, the top height, but not the bottom height of capsules, increases.

When laying egg capsules in the lab, mud snails exhibit a pattern of capsule placement similar to that in the field. I plotted the highest and lowest position at which egg capsules were laid over four days on blades of eelgrass (Figure 19). Up to 4 days after laying began, there was a significant effect of day on the bottom height at which egg capsules were laid (Univariate ANOVA, df = 3, F = 3.697, p = 0.013). The top height did not significantly vary by day (Univariate ANOVA, df = 3, F = 0.547, p = 0.651). In contrast to my field observations, top height remained constant over the course of up to four days of laying, while bottom height decreased as laying progressed. Snails may lay fewer than 6 cm off the benthos in the field, as they did in this experiment (by day four, capsules were an average of 5 cm off the benthos), but the pattern could be obscured by predation upon lower capsules in the field.

**Effect of predators on egg capsules placement**

In the field, the bottom height of egg capsules along a blade of eelgrass, or “bottom height”, was positively correlated to the total length of the eelgrass blade (Figure 20). I found the bottom height of capsules versus total length of an eelgrass blade for snails in the presence of 3 different predators could be significantly fit by a linear regression (Hermit crab: Adjusted $R^2 = 0.250$, $p < 0.001$; Periwinkle: Adjusted $R^2 = 0.386$, $p < 0.001$; Control: Adjusted $R^2 = 0.454$, $p < 0.001$). While these were not necessarily the best fit regressions (the hermit crab treatment was best fit by a cubic model, adjusted $R^2 = 0.374$, and the periwinkle treatment by a power model, adjusted $R^2 = 0.431$) there does not seem to be any
biological reason for these models to be more accurate than the linear model, which I therefore continued to use for my analysis.

Total length of an eelgrass blade, but not predator cue, significantly affected the bottom height of capsule placement for 9 days of the experiment (Mixed model ANOVA; Factor: Predator, Numerator df = 2, Denominator df = 18, F = 0.843, p = 0.447; Factor: Eelgrass Length, Numerator df = 1, Denominator df = 161, F = 95.259, p < 0.001). Despite the relationship that longer blades of eelgrass have a higher bottom height of egg capsules (Figure 20), heights are reported as raw heights off the benthos, rather than a height adjusted for the length of the eelgrass, as the height off of the benthos is the biologically relevant value for my study.

Sand collars and eelgrass were presented to mud snails in the presence of predator cues. Though mud snails did not previously lay capsules on sand collars in the lab, I wanted to determine if this preference would change in the presence of predator cues. Over the course of 18 days, mud snails did not deposit any egg capsules upon sand collars when exposed to ambient sea water, hermit crabs, or periwinkles. All egg capsules were laid either upon the eelgrass or walls of the container in which the experiment was enclosed.

The placement of egg capsules on eelgrass was separately analyzed for the first and last day of egg capsule deposition to determine if placement changed over time in the presence of predator cues. When egg capsules were first deposited in the presence of hermit crab cues, they were placed an average of 1 cm higher off the benthos than for controls (Figure 21). In the presence of periwinkles, snails deposited their egg capsules an average of 1.8 cm higher than controls (Figure 21). However, there was no significant difference among treatments in either the top (univariate ANOVA, df = 2, f = 1.556, p =
0.216) or bottom height (univariate ANOVA, df = 2, f = 1.513, p = 0.225) of egg capsules after one day in the presence of predator cues.

After nine days, in the presence of hermit crab cues, snails deposited their egg capsules an average of 0.4 cm higher off the benthos than control (Figure 22). In the presence of periwinkles, snails deposited their egg capsules an average of 2.6 cm higher than control (Figure 22). Bonferroni post-hoc tests were used to distinguish significance among treatments. The bottom height of capsules for periwinkle cues was significantly greater than for hermit crab cue (p = 0.014) and control (p = 0.007; univariate ANOVA, df = 2, F = 6.114, p = 0.003). The top height of laying followed the same pattern; the top height of capsules when exposed to periwinkle was significantly higher than for control or hermit crab treatments (univariate ANOVA, df = 2, F = 4.427, p = 0.013).

Since fewer egg capsules were laid in the control treatment than in either predator treatment, snails were sexed to obtain a count of capsules laid per female. Snails ranged in size from 10 – 29 mm, averaging 21 mm in length. When exposed to egg capsule predator cues (hermit crabs and periwinkles), snails tended to lay more eggs than when they were exposed to ambient seawater alone (Figure 23). However, this trend was not statistically significant (univariate ANOVA, df = 2, F = 2.806, p = 0.078).

**Survival of egg capsules on eelgrass**

Hermit crabs preferentially fed upon egg capsules deposited lower upon a blade of eelgrass (Figure 24). The survival of egg capsules upon eelgrass in the presence of hermit crabs decreased with time exposed (day 1-8), and increased with height along the blade of eelgrass (1, 5, 10, or 15 cm; binomial logistic regression, p < 0.001, Table 3). However, interactions with tide phase precludes conclusions about the main effects (Table 3). 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 7,840 capsules scored for survival) and since the model accurately predicted survival in 89.5% of cases, I continued to use this model for analysis (Kramer and Zimmerman, 2007).

Survivorship decreased each day, but capsules placed just 5 cm higher on a blade had survivorship that was four times higher than capsules placed directly on or 1 cm off the benthos. Additionally, the interaction term between tide and height or tide and day may indicate that high tide conditions played a role in increasing survival of egg capsules over low tide conditions (Figure 24). 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.
Discussion

My study tested whether a gastropod (the mud snail *Ilyanassa obsoleta*) with mixed development was plastic in terms of egg deposition in response to environmental conditions, particularly predator cues. Mixed development exposes the developing offspring of marine invertebrates to the risks of both the benthic and planktonic environments (Caswell, 1981), and benthic encapsulation confines embryos in an area with high exposure to predators (Allen and McAlister, 2007). In Maine, mud snails preferred to lay their egg capsules on eelgrass, at a height approximately 5 cm off the benthos or higher. Also, the mud snail responded to predator cues by increasing the height at which egg capsules were laid off the benthos. Egg capsules that were placed higher on eelgrass experienced lower rates of predation, suggesting that this response is adaptive and enhances offspring survival. This finding supports the hypothesis that adult organisms with mixed development can exhibit plasticity in their reproductive behaviors to increase survival of their offspring in the face of the dangerous benthic environment.

Substrate Preference

On the coast of Maine, mud snails deposit their egg capsules off the benthos by laying them on blades of eelgrass. Eelgrass was the preferred substrate for deposition of egg capsules both in the field and in the lab. In areas of my study site with abundant eelgrass, I observed large groups of mud snails in the act of laying thousands of egg capsules. These roving herds of adult mud snails would swarm a group of eelgrass one day and then move on to another area by the following day, mirroring existing reports of mud snail behavior in other areas of New England (Brenchley and Carlton, 1983).
The preference for eelgrass as a laying substrate may foretell a future decline in mud snail populations. Eelgrass has been in decline along the coast of Maine, and in Casco Bay, over half of the eelgrass has been decimated due to disturbance by the green crab, *Carcinus maenas* (Neckles, 2015). The reproductive output of mud snails is limited by the amount of hard substrate in their habitat, and lack of suitable substrates can result in lower hatching success (Pechenik, 1978; Brenchley, 1981). Therefore, the decline of eelgrass may be detrimental to mud snail populations in Casco Bay. Eelgrass is in decline not only in Casco Bay, but globally (Waycott *et al.*, 2009), and may dampen mud snail populations in the Chesapeake Bay as well (Orth *et al.*, 2006). However, in our experiments and observations, the mud snail preference for eelgrass becomes less pronounced as the season progresses, and later in the season, mud snails will deposit capsules on a greater variety of substrates. Additionally, areas with dwindling eelgrass populations still have abundant mud snail populations in Maine (Neckles, 2015). In fact, there is some concern that mud snail abundance will make eelgrass restoration difficult, since new shoots will be covered and weighed down by egg capsules (HA Neckles, personal communication).

It is not entirely clear why mud snails prefer eelgrass as a laying substrate. In our study area, the mudflat in Harpswell Sound off Orr’s Island, eelgrass was the most common hard substrate that was elevated off the benthos. Eelgrass could be preferable to mud snails since it allows placement of capsules out of the reach of benthic predators (Ambrose and Irlandi, 1992), as I discuss further below. However, when I provided snails with a variety of plants and algae, which are also native to the Maine coast (eelgrass, *Chondrus crispus*, *Ascophyllum nodosum*, *Fucus distichus*, *Laminaria* spp.), eelgrass was not significantly preferred to *C. crispus* (although eelgrass tended to be the top choice for egg
deposition). *Chondrus crispus* does not often reach more than 5-8 cm above the benthos, while less preferred substrates, such as *A. nodosum, F. distichus, and Laminaria spp.* can reach many centimeters or even meters above the benthos. Thus, it is not clear that increasing height above the benthos (beyond the first 5 cm) is attractive to adult mud snails for their deposition site. However, it should be considered that the mud snails used in this follow-up choice experiment were from a habitat in Massachusetts rather than Maine, and thus may not exhibit the same responses as the snail population in Maine.

While I have established the oviposition substrate preference for the Harpswell Sound mud snail population, other populations of mud snails prefer to lay on other available substrates. In Narragansett Bay, RI, eelgrass is not abundant and therefore was not offered as a substrate in recent substrate preference experiments (Guidone et al., 2014). In this habitat, 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). Similarly, off Beaufort, NC, mud snail egg capsules were primarily placed on shells; living oyster shells were found to be the preferred substrate (Rittschof et al., 2002). Future work should be done to determine the egg capsule position preference of other populations of mud snails, particularly if preference changes when a population of snails from an area with little eelgrass is provided with eelgrass as a laying substrate. For example, snails in NC and RI historically have had access to eelgrass as a laying substrate (Penhale, 1977; Nixon et al., 2009) and it may be possible to determine how quickly, if at all, preferences for laying substrate can change over time.

In Maine, preference for eelgrass is strong enough that mud snails flocked to transplanted eelgrass within a week. It is known 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 will 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 other reproductive snails are attractive (Rittschof et al., 2002). It is quite possible that the Harpswell Sound mud snail aggregations occur as snails follow the odor 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. My experiments were unable to distinguish between these two hypotheses, but further studies could test whether chemical cues from eelgrass are attractive to adult mud snails.

**Plasticity of Laying Height**

Mud snails exhibit plastic responses to chemical cues, or kairomones, of predators (Ruther et al., 2002; Schwab and Allen, 2014). In the face of odors or extracts of predators such as green crabs and periwinkles, mud snails may burrow or flee (Brenchley and Carlton, 1983; Rahman et al., 2000). There is an even stronger, chemically-triggered, alarm response to crushed conspecifics (Atema and Burd, 1975; Atema and Stenzler, 1977). Beyond these 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 a smaller aperture for a given shell size, reducing the success of predator attacks through the shell opening (Santoni and Allen, in preparation). Mud snails also will vary their reproductive output, both in laying quantity and capsule spine length, in the presence of predator cues (Schwab and Allen, 2014).
Increasing the length of spines on egg capsules is likely adaptive, as it reduces predation by hermit crabs on the capsules (Schwab and Allen, 2014). Given these examples, it seems very plausible that laying egg capsules off the benthos could be a plastic response in mud snails triggered by predator cues.

While mud snails laid egg capsules 4-8 cm off the benthos even in the absence of predators, they placed egg capsules 1-3 cm higher off the benthos in the presence of periwinkles. In the presence of hermit crabs, capsules were placed at a height that did not significantly differ from the control. Egg capsule predators were found primarily on the benthos and did not crawl higher than a few cm up a blade of eelgrass. Therefore, it is plausible that laying egg capsules off the benthos is a plastic response that allows mud snails to increase the survival of their young. Though hermit crabs were observed to be the more dangerous predator, they primarily stay on the benthos, while periwinkles can crawl up eelgrass plants. Thus, in the presence of periwinkles, mud snails tend to lay their capsules farther off the benthos, possibly to prevent bulldozing. Though periwinkles rarely eat egg capsules, bulldozing occurs as periwinkles displace and destroy egg capsules while grazing on the epiphytes of eelgrass (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 prioritized response (Bourdeau, 2009b; Mach and Bourdeau, 2011). Based on my observations, I 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. Therefore, in the field or when receiving control sea water with ambient cues from a mixture of predators, the mud snail
may prioritize to lay capsules at a height that protects from hermit crabs. This may also be the default response for snails in this high risk population, explaining why there was no significant difference between the heights at which capsules were placed in the presence of hermit crabs and control (Bourdeau, 2012). The response to lay even farther off the benthos may be reserved only for an instance in which there is a strong periwinkle cue.

As noted earlier, as the summer progressed, mud snails became less particular in their selection of laying substrate. In late June and July, I observed egg capsules on a greater variety of substrates in the field, especially *Fucus* spp. In my laboratory experiments, mud snails switched their preference from natural substrates to the container walls after only a few days. I hypothesize that there is a seasonally driven response (possibly triggered by temperature) for mud snails to lay their remaining egg capsules even in non-desirable locations as it becomes late in the season. Many non-feeding larvae of marine invertebrates will become less particular about where they settle onto the benthos when their energy reserves run low; this is known as the desperate larva hypothesis (Marshall and Keough, 2003). A “desperate” larva may settle on a worse quality surface as it runs out of time in the search for a better quality surface. The desperate larva hypothesis may be analogous to the shifts in substrate preferences seen in mud snail egg capsule placement over the reproductive season. I propose three hypotheses, which are not mutually exclusive, which could explain this pattern of substrate preference shifts. First, as the summer progresses, water temperatures rise. Within tolerable temperatures, the encapsulation period decreases with increasing water temperature in many gastropods including mud snails (Scheltema, 1967; Kress, 1975). Embryos will hatch out more quickly and have less time of exposure to predators, potentially rendering the height of attachment of capsules less
significant in determining embryo survival. Second, high summer temperatures that can be tolerated by gastropod adults may be deadly to embryos (Dehnel and Kong, 1979). Warming temperatures may cue mother mud snails that it is the last chance to deposit surviving young before it becomes too warm for successful development: a “desperate mother” hypothesis. Laboratory trials to determine the temperature tolerance of developing mud snail embryos would allow a determination of whether environmental temperatures ever reach this hypothetical threshold. Third, adult snails may need to lay egg capsules early in the season before they succumb to parasitic infections that can result in permanent sterilization (Curtis, 1983). Mud snails are common hosts for trematode parasites (approximately 60% of a population may be parasitized; Curtis, 1983), though larger and older snails are more likely to be parasitized than smaller snails. Additionally, the transmission of trematodes to their gastropod hosts may be dependent upon suitable temperatures and thus have a seasonal cycle (Esch et al., 2001). Mud snails may be in a race to lay their egg capsules before their gonads are destroyed by castrating parasites, many of which I observed in adult snails sacrificed at the end of my field season in Maine.

**Consequences of Deposition Strategy**

If adult mud snails deposited egg capsules on the first portion of eelgrass they encountered (i.e. the part in contact with the benthos), lower reproductive success would result. In my work, I have shown that egg capsules that were placed directly on the benthos experienced up to 4 times greater rates of predation than those placed just 5 cm higher. This pattern has also been demonstrated in juvenile scallops; young scallops that attached higher on blades of eelgrass experienced significantly less predation than those closer to the benthos (Pohle et al., 1991; Ambrose and Irlandi, 1992). Planktonic larvae of
Argopecten irradians will settle and attach primarily to eelgrass with byssal threads, but byssal attachment to eelgrass is transient and reversible (Garcia-Esquivel and Bricelj, 1993). Most juvenile scallops will first crawl into the heights of the eelgrass canopy by extending their foot and pulling up the body (Belding 1910, Pohle et al., 1991). Smaller juveniles will swim above the eelgrass and attempt to attach to a plant by extending the foot as they fall, but this method often fails, and they resort to crawling up the blade (Pohle et al., 1991). While the juvenile scallop or mother mud snail may expend more energy crawling up a blade of eelgrass, this trades off against the benefit of increased survival of young for both species.

However, there is likely an additional tradeoff between the benefits of placing capsules in locations that are protected from predators and costs from other environmental factors; otherwise one would expect mud snails to always place capsules far above the benthos. As with other plastic responses, phenotypic plasticity allows organisms to adapt to environmental variability, but it can be costly and require trade-offs with growth, fecundity, or other genetic or energetic costs (DeWitt, 1998, Relyea, 2002). I 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 are poorly adapted to 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, exposing egg capsules laid at the tip to benthic
predation. There might therefore be an ideal vertical zone for the placement of mud snail egg capsules on eelgrass that strikes a balance between abiotic and biotic stressors.

Attaching egg capsules off the benthos allows animals with mixed development to place vulnerable young up in the water column and out of the predator-dense benthos (Allen and McAlister, 2007). For marine invertebrates that lay eggs in capsules or masses, it would be evolutionarily advantageous to be plastic in response to environmental conditions. Egg capsules are attached to some surface on the benthos, a dangerous and variable zone, to develop for weeks to months without parental care. While encapsulated young may be more protected from predation than benthic larvae (of which there are very few examples), mixed development seems to expose developing young to dangers of both the benthic and planktonic habitats (Pechenik, 1979, 1999). Any opportunity for the mother to equip her young to survive in the face of environmental variability has great potential to increase her fitness. A further test of this hypothesis would be to examine whether other groups of animals exhibiting mixed development similarly adjust their laying behavior in the presence of predators.

The measures displayed by mud snails and other organisms that develop on the benthos to protect embryos from predation is further evidence that benthic predation is a factor in maintaining marine invertebrate complex life cycles (Strathmann, 1985). The planktonic larvae featured in the life histories of many marine invertebrates may have originated as a means to escape benthic predation during the late Cambrian (Signor and Vermeij, 1994; Peterson, 2005). Though many gastropods, nemerteans, polychaetes, and turbellarians place embryos on the benthos instead of immediately sending embryos into the plankton, they exhibit many plastic responses to protect their young from benthic
predators. The efforts made by mothers to adjust the site of egg capsule deposition in response to predator cues support the hypothesis that benthic predation plays a large role in maintaining planktonic larvae as a part of marine invertebrate life histories.
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References


Figures

Figure 1: Nine 1 m$^2$ plots were laid out in the field. Ten eelgrass plants, each represented by a green circle, were transplanted inside of each plot.
**Figure 2:** Water from the lab flow-through system runs into a bucket. Tubes run from holes in the bottom of the bucket into Sterilite containers to carry water through the lid of each replicate chamber. This system was used for the majority of laboratory experiments.
Figure 3: Substrate preference experiment. Mud snails were given eelgrass, oyster shell, hard-shell clam, sand collar, soft-shell clam, or mussel shell to lay capsules upon.
Figure 4: Ten mud snail egg capsules were glued to the top side of the sand collar, and ten capsules were glued on the underside of the sand collar. These sand collars were then exposed to periwinkles and hermit crabs, and the survival of egg capsules recorded.

Figure 5: Laying quantity was designated as low (A) or high (B) density, depending upon whether one or many layers of egg capsules were on eelgrass.
**Figure 6:** Morphology of eelgrass. The height of egg capsules along a leaf blade was measured from the node as 0 cm. (Catherine Collier, Integration and Application Network, University of Maryland Center for Environmental Science).
Figure 7: Schematic of the experiment for laying position in the presence of predators. The flow-through water system delivered water into treatment containers that were empty (control), held hermit crabs (brown and yellow), or held periwinkles (brown and gray). Water from these containers then flowed through a tube into the experimental chambers holding mud snails (black and gray), eelgrass (green), and sand collars (brown). The blue arrow indicates direction of water flow. Ceiling tile separated the two layers of containers to facilitate water flow downwards.
**Figure 8**: Sex determination for *Ilyanassa obsoleta*. (A) The female gonad is cream to orange in color at the apex of the body, and granular in appearance. (B) The male gonad is dark orange or red at the apex of the body and has enlarged seminal vesicles during the reproductive season (indicated by the arrow). Pictures from dissection scope at 0.8x magnification (Schwab and Allen, 2014).

**Figure 9**: Egg capsules were glued in groups of 10 capsules at 0, 5, 10, and 15 cm along a blade of eelgrass. The blades were attached in containers holding three hermit crabs.
Figure 10: Number of adult mud snails in a 1 m$^2$ plot in relation to the number of blades of eelgrass present. A cubic regression best fit the data, showing a significant effect of amount of eelgrass on presence of snails ($y = -0.0005x^3 + 0.118x^2 - 3.262x + 26.002$, Adjusted $R^2 = 0.486$, $p < 0.001$). Each data point represents one field plot.
Figure 11: Number of adult mud snails in a 1 m$^2$ plot in relation to the number of sand collars present. No tested function sufficiently fit the data, showing that the number of sand collars in a plot is not a reliable indicator of snail presence. Each data point represents one field plot.
Figure 12: Proportion of blades of eelgrass holding mud snail egg capsules in a 1 m$^2$ plot in relation to the total number of eelgrass blades. A power function best fit the data, showing that areas with more eelgrass are more covered in egg capsules ($y = 0.054x^{0.622}$, Adjusted $R^2 = 0.780$, $p < 0.001$). Each data point represents one field plot.
Figure 13: Proportion of blades of eelgrass holding mud snail egg capsules in a 1 m$^2$ plot in relation to the number of snails present. A logarithmic regression best fit the data, showing there are large aggregations of snails in areas with many egg capsules ($y = 0.059\ln(x) + 0.390$, Adjusted $R^2 = 0.379$, $p < 0.001$). Each data point represents one field plot.
Figure 14: Number of snails present in 1 m$^2$ plots before and five days after the experimental addition of eelgrass plants. “Background” represents quadrats taken from outside of the eelgrass zones, and “plots” represents the nine plots into which eelgrass was transplanted. Each bar represents the average number of snails in nine plots ± 1 SE. The interaction between time and plot location had a significant effect on the number of snails present in the plots ($p < 0.001$).
Figure 15: Number of capsules deposited on each of laying substrate after 3 days (A) and after 6 days (B). There was no significant difference among substrates other than the preference to lay egg capsules on the container after 6 days ($p < 0.001$). Each bar represents the average number of capsules laid on the substrate per replicate ± 1 SE ($n = 5$).
Figure 16: Rate of total egg capsules laid for snails given only eelgrass or only a sand collar as a laying substrate. There was a significant delay of laying in the sand collar treatment ($p = 0.015$). Each data point represents the mean total amount of laying $\pm 1$ SE for 10 containers of each treatment. Data were broken into the amount of laying on four substrates: not only the eelgrass and sand collar, but also the containers in which these treatments were held. There is a significant difference among substrates ($p < 0.001$) and a Bonferroni post-hoc test showed there was a significant difference between the top two points (eelgrass and sand collar container) versus the bottom two points (sand collar and eelgrass container) ($p < 0.001$).
Figure 17: Survivorship of snail egg capsules placed either on the top or underside of a sand collar and then exposed to either hermit crabs or periwinkles for 48 hours. Ten egg capsules were attached to either side of the sand collar. Each bar represents the mean proportion ± 1 SE of surviving egg capsules in fifteen containers for each treatment. The survival of egg capsules depended both upon the predator and side of the sand collar (p < 0.001).
Figure 18: Average position of highest and lowest mud snail egg capsules along blades of eelgrass from the field. The bottom point indicates the average height of the lowest egg capsule ± 1 SE, and the top point indicates the average height of the highest egg capsule ± 1 SE. Eelgrass plants are characterized as bearing a low density of egg capsules (n=148 blades) or high density of egg capsules (n=93 blades). The top height of capsules is significantly different between low- and high-density plants (p < 0.001). The bottom height is not significantly different between the two groups (p = 0.143).
Figure 19: The top and bottom height of egg capsules upon blades of eelgrass. Each point represents the average height per container. Day 1 was recorded as the first day at least 5 egg capsules were laid in a container. Laying was tracked for 5 days after this point, thus containers in which snails started laying after a few days were not able to be recorded for a full 4 days of laying. Top height remains constant over the course of 4 days (p = 0.651), while bottom height decreases (p = 0.013).
Figure 20: There was a positive linear correlation between the total length of a blade of eelgrass and the bottom height of egg capsules upon this blade (Hermit crab $y = 0.311x + 2.928$, Adjusted $R^2 = 0.250$, $p < 0.001$; Periwinkle $y = 0.394x + 1.638$, Adjusted $R^2 = 0.386$, $p < 0.001$; Control $y = 0.389x + 0.816$, Adjusted $R^2 = 0.454$, $p < 0.001$). This correlation was not affected by predator cue (Factor: Predator; numerator df = 2, denominator df = 18, $F = 0.843$, $p = 0.447$).
Figure 21: Bottom and top height of egg capsules for snails exposed to predator cue after one day. Each point represents the mean ± 1 SE for each treatment. For the first day of laying, hermit crab n = 7, periwinkle n = 8, and control n = 2. There was no significant difference among the treatments for the top height (p = 0.216) or bottom height (p = 0.225).
Figure 22: Bottom and top height of egg capsules for snails exposed to predator cue after nine days. Each point represents the mean ± 1 SE for each treatment. For this last day of laying, hermit crab n = 9, periwinkle n = 10, and control n = 7. The bottom height of capsules for periwinkle cue was significantly higher than for both the control (p = 0.007) and hermit crab (p = 0.014) treatment. The top height of capsules for both periwinkle cues was significantly higher than for control (p = 0.023). Different letters represent values that significantly differ.
Figure 23: Number of egg capsules laid per female snail when exposed to predator cues from hermit crabs or periwinkles or to ambient seawater. Female snails tended to lay more egg capsules when exposed to predator cues; however, this was not statistically significant (univariate ANOVA, df = 2, F = 2.806, p = 0.078). Each bar represents the mean number of capsules laid per female in 10 containers over 9 days ± 1 SE.
Figure 24: Hermit crab predation upon egg capsules at listed heights on eelgrass for high tide (A) and low tide (B). Survivorship increased with height above the benthos, both on eelgrass and via tide. Each point represents the mean number of egg capsules at the given height in 14 containers ± 1 SE. Day, height off bottom, and interactions of day and height with tide height are predictors of egg capsule survival ($p < 0.001$).
Tables

Table 1: Univariate ANOVA for eelgrass transplants. The factor “Time” indicates data from either before or after the transplant, and the factor “Location” indicates whether the data were from an experimental or background plot. Significant effects are in **bold**.

<table>
<thead>
<tr>
<th>Factor</th>
<th>df</th>
<th>F</th>
<th>P</th>
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<tbody>
<tr>
<td>Time</td>
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<td><strong>0.002</strong></td>
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<tr>
<td>Location</td>
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<td>20.595</td>
<td>&gt;<strong>0.001</strong></td>
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<tr>
<td>Time*Location</td>
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<td>18.321</td>
<td>&gt;<strong>0.001</strong></td>
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</table>

Table 2: Binomial logistic regression for predation upon egg capsules attached to sand collars. These data suggest that egg capsule survivorship on sand collars can be significantly predicted by both side of sand collar and predator. All interaction terms are included as the best model as determined by lowest log likelihood. Significant effects are listed in **bold**.

<table>
<thead>
<tr>
<th>Variable</th>
<th>β</th>
<th>P-value</th>
<th>Exp(β)</th>
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</thead>
<tbody>
<tr>
<td>Predator</td>
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<td>0.001</td>
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<tr>
<td>Side</td>
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<td><strong>0.001</strong></td>
<td>6.081</td>
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<tr>
<td>Day</td>
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<td>0.338</td>
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<tr>
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<tr>
<td>Day<em>Side</em>Predator</td>
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<td>0.449</td>
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<tr>
<td>β₀</td>
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<td>0.000</td>
<td>4.769</td>
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Table 3: Binomial logistic regression for predation upon egg capsules at heights above the benthos. These data suggest that survivorship of egg capsules on eelgrass in the presence of hermit crabs can be significantly predicted by length of exposure, height along eelgrass, and interaction factors between day, height, and tide. All interaction terms are included as the best model as determined by lowest log likelihood. Significant effects are listed in bold.

<table>
<thead>
<tr>
<th>Variable</th>
<th>β</th>
<th>P-value</th>
<th>Exp(β)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
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<td>0.562</td>
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<tr>
<td>Tide</td>
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<tr>
<td>Height</td>
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<td>Day*Height</td>
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<td>1.004</td>
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<td>Day*Tide</td>
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<td>Height*Tide</td>
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<td>&gt;0.001</td>
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<td>Day<em>Height</em>Tide</td>
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<td>β₀</td>
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<td>0.825</td>
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