Seed Burial in the Seagrass Zostera marina: The Role of Infauna

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Seed Burial in the Seagrass *Zostera marina*:

The Role of Infauna

A Thesis
Presented to

The Faculty of the School of Marine Science
The College of William and Mary in Virginia

In Partial Fulfillment
of the Requirements for the Degree of
Master of Science

by
Natalia J. Blackburn
2012
APPROVAL SHEET

This thesis is submitted in partial fulfillment of the requirements for the degree of

Master of Science

Natalia J. Blackburn

Approved by the Committee, April 2012

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ABSTRACT

In terrestrial systems, seed burial is widely recognized as a vital process that influences small- and large-scale plant population patterns. Despite its demonstrated importance in terrestrial literature, very little is known about seed burial in seagrasses. *Zostera marina* is a perennial seagrass found in northern temperate oceans worldwide, and is the dominant seagrass found in the Chesapeake Bay. In terrestrial systems, seed burial is frequently mediated by soil-dwelling invertebrates. The goal of this work was to determine the role that benthic infauna play in the burial of *Z. marina* seeds by addressing the following questions: 1. Are seeds on sediments containing infauna buried more quickly than sediments without infauna, and 2. Does the infaunal feeding mode (e.g. head-up vs. head-down feeder, sessile vs. errant, or deposit feeder vs. omnivore) affect seed burial? Three mesocosm studies were conducted in sediment cores (80cm^2 x 11cm) collected from the Chesapeake Bay, defaunated, and populated with single specimens of infauna of different feeding modes: *Amphitrite ornata* (sessile head-up deposit feeder), *Neanthes succinea* (errant omnivore), or *Clymenella torquata* (sessile head down deposit feeder), or *Pectinaria gouldii* (errant head down deposit feeder). Control cores had no specimen added. Ten particles (either *Z. marina* seeds or colored beads) were added to the surface of each core, and the depth of the particles was determined at different time scales up to 14 days. Seeds in all animal cores were significantly more likely to be buried than seeds in control cores (p<1.03x10^-6), although burial rates varied by species. *N. succinea* and *P. gouldii* showed the most dramatic burial: ~55% of seeds buried after 3 days and seeds buried below 2.5cm and 4.0cm, respectively, after 2 weeks. *N. succinea* also showed evidence for actively burying seeds. *A. ornata* and *C. torquata* had 12% and 24% of seeds buried, respectively, after 3 days and both had seeds buried below 1.0cm after 2 weeks. The results of this study indicate that *Z. marina* seed burial is facilitated by infaunal activity, and that burial patterns are species specific. In addition, burial is rapid and occurs on a time scale of days. While abiotic processes may be initially important in seed burial, the direct (active movement of sediment), and indirect (formation of mounds and holes) consequences of biotic processes by infauna may prove to be dominant and relevant to seed escape from predation, retention in suitable settlement sites, and movement to a sediment depth suitable for germination.
Seed Burial in the Seagrass *Zostera marina*:

The Role of Infauna
Introduction

In terrestrial systems, seed burial has been shown to influence both small- and large-scale population patterns of adult plants (Warr et al, 1993), as well as species invasion dynamics and the intensity of interspecific competition (Reigner et al, 2008). There are three major categories of burial processes: burial by abiotic processes, such as wind or rain, (Benvenuti, 2007), seed-mediated burial (Chambers et al, 1991), and burial by other organisms (Chambers et al, 1994). In the terrestrial literature, seed burial by other organisms has been shown to be the most universally important seed burial process (Chambers et al, 1994).

While vertebrates have a clear role in seed burial, e.g. birds and rodents caching large seeds (Beck and Vander Wall, 2010, Haugaasen et al, 2010), invertebrates influence the burial of a much wider variety of seed shapes and sizes. Many plants take advantage of myrmecochory, or dispersal by ants, to move and bury their seeds. Myrmecochorous seeds generally have an eliasome, or lipid rich appendage, to encourage ants to collect them and bring them back to the nest (Lengyel, 2010) where they are protected from seed predators (Renard, 2010). Earthworms (terrestrial oligochaetes) represent a very important group (Darwin, 1837) responsible for burying seeds. *Lumbricus terrestris* L. has been shown to bury *Ambrosia trifida* L. seeds eight times more quickly than abiotic processes (Regnier, 2008). Anecic worms pull plant litter into their burrows for later consumption, and have been shown to increase both the burial and germination rates of seeds (Eisenhauer, 2008). Worms can also bury seeds simply by moving through the soil and building and maintaining burrows (Vanderreest and Rogaar, 1988; Willems and Huijsmans, 1994).

While the terrestrial literature contains numerous references on all aspects of seed ecology, including seed burial processes and mechanisms, very little is known about seed burial in aquatic plants in general (Koch, 2010), and even less in marine angiosperms, or seagrasses.

Seagrasses diverged from their terrestrial ancestors 100 million years ago (Orth et al, 2006), and, like other angiosperms, they flower and produce seeds. These seeds, which are dispersed primarily by abiotic mechanisms (Kendrick et al., 2012), are negatively buoyant and settle rapidly to the sediment surface where they become incorporated into the sediment matrix and germinate (Orth et al., 1994).

*Zostera marina* (eelgrass) is a seagrass found in northern temperate oceans throughout the world (den Hartog, 1970; Green and Short, 2003). In the Chesapeake Bay
region, *Z. marina* flowers and produces seeds in May and June. Seedling establishment patterns in the field indicate that the seeds do not move far once they settle to the sediment surface (Orth et al., 1994), which implies that they are quickly buried. Burial is important for successful seedling establishment because it reduces seed predation by blue crabs (Fishman and Orth, 1996), provides the hypoxic conditions necessary for germination (Moore et al., 1993), and reduces the chance that the seedling will get washed away by winter storms (Marion and Orth, 2012).

Despite the clear importance of seed burial to the germination and establishment of *Z. marina*, very little work has been done to determine how its seeds are buried. It is likely that burial is mediated in part by abiotic processes, such as sedimentation and sand ripple migration, but, as in terrestrial systems, burial is probably influenced or augmented by the animals living in the sediment. A recent study by Valdemarsen et al. (2011) concluded that bioturbation by *Arenicola marina* inhibits *Z. marina* recovery by burying seeds too deeply for successful germination. Luckenbach and Orth (1999) showed that *Z. marina* seeds moving through a flume are retained near the burial mounds of the maldanid polychaete, *Clymenella torquata*, and this retention increases with worm density. Harwell and Orth (2001) found that an onuphid polychaete, *Diopatra cuprea*, can influence seed dispersal by attaching flowering shoots to its tube cap, which results in the seeds being deposited near the tube. Some work has been done on seed retention in marsh species and freshwater submersed aquatic vegetation (see Chang et al., 2008 and Koch, 2010), but neither study addressed seed burial.

The goal of this study was to better understand what role infauna play in *Z. marina* seed burial. The following questions were addressed: 1. Are seeds deposited on sediments containing infauna buried more quickly and deeply than sediments without infauna, and 2. Does the infaunal feeding mode such as head-up vs. head-down feeder, sessile vs. errant, or deposit feeder vs. omnivore affect seed burial depth?
Methods

Seed collection, storage, and viability assessment

*Zostera marina* seeds were harvested by mechanical harvester in early June 2011 from a bed on the Atlantic side of the Delmarva Peninsula. Reproductive shoots bearing nearly-ripe seeds were returned to Gloucester Point, VA, and placed in well-aerated 3,500L flow-through seawater tanks. After the seeds ripened, the plant material was separated from the seeds by straining out shoot fragments, placing the remaining, seed-bearing, material in a separation flume, and then passing the flumed material through a 1mm sieve. Seeds were stored in re-circulating tanks held at 25 PSU and 18-20°C. For comprehensive methods, see Marion and Orth, 2010.

Seed viability was determined first by closely examining and gently squeezing the seed with forceps, then by dropping the seed through a still column of water. Firm, intact seeds exhibiting a fall velocity greater than 4 cm/second were considered viable (Marion and Orth, 2010) and used in the following experiments. Seed viability at the end of each experiment was determined by gently squeezing the seed and examining the seed coat for damage.

Core collection and preparation

Sediment cores were collected from a shallow, unvegetated site using 10cm diameter PVC sleeves inserted to a depth of approximately 11 cm, capped at the bottom, and transported back to the laboratory. Cores were frozen for a minimum of one week in order to eliminate macroinvertebrates without disrupting the sediment matrix.

Animal collection

The animals used in these experiments were locally common representatives from four major functional groups of bioturbators.

*Clymenella torquata* is a sessile head down deposit feeder which builds a 10-20cm long tube perpendicular to the sediment surface. Acting as a conveyer belt species (*sensu* Rhoads, 1974), *C. torquata* ingests medium-sized sediment at depth and defecates on the sediment surface, creating a defecation mound around the tube opening. *Pectinaria gouldi* is an errant head down deposit feeder which builds a 4-6cm long tube oblique to the sediment surface. Not only does *P. gouldi* act as a conveyer belt species, it moves laterally through the sediment, dragging its tube with it. *Amphitrite ornata* is a sessile head up deposit feeder which constructs a U-shaped tube up to 60cm in length and protruding from the sediment at either end. *A. ornata* uses tentacles to selectively transport sediment to the mouth. Manipulated but non-ingested material forms a conical
deposit around the anterior end of the tube, and defecated material forms a conical deposit around the posterior end of the tube. *Neanthes succinea* is an errant omnivore which creates a gallery of mucus-lined burrows. *N. succinea* moves through and over the sediment, searching for specific large organic particles for ingestion.

The *C. torquata* used in these experiments ranged in length from 1.0cm to 4.5cm and in wet mass from 0.036g to 0.167g, with a mean length of 2.7cm and a mean wet mass of 0.2g. The *P. gouldi* ranged in length from 1.0cm (2.0cm with tube) to 2.5cm (5.5cm with tube) and in wet mass from 0.087g to 0.578g, with a mean length of 1.6cm (3cm with tube) and a mean wet mass of 0.3g. The *A. ornata* ranged from 0.5cm to 5cm and from 0.052g to 1.265g, with a mean length of 2.7cm and a mean wet mass of 0.4g. The *N. succinea* ranged from 2.0cm to 8.5cm and 0.095g to 0.787g, with a mean length of 4.7cm and a mean wet mass of 0.4g.

Infauna were harvested by sieving sediment collected by shovel or suction dredge through 1.0mm mesh sieves or bags (Orth and van Montfrans, 1987). Whole, uninjured animals were carefully selected from detritus, removed from their tubes (except *P. gouldi*), and placed in aerated water bowls without sediment until the initiation of the experiment. At the end of each experiment, animals were generally recovered uninjured, and lengths and wet weights were recorded.

**Sediment analysis**

Sediment samples were taken from control cores at the end of the seed burial depth experiment and the seed burial rate experiment. Sediment was analyzed for percent gravel, sand, silt, clay, nitrogen, and total organic carbon. Gravel (>2000 microns) and sand (2000 to 62.5 microns) fractions were determined by dry and wet sieving, respectively. Silt (4 to 62.5 microns) and clay (<4 microns) fractions were determined by the pipette method (Folk, 1980). Nitrogen and total organic carbon fractions were analyzed on an Exeter CHN Model 440 CE analyzer using the methods from the Exeter Analytical, Inc. Model 440 CHN/O/S Elemental Analyzer Manual, Part no. 150-000.

**Experimental set-up**

All experiments were run in a shaded recirculating tank held at 21-23°C and 20 PSU. The tank was housed in a greenhouse located at Gloucester Point, VA. The experiments were conducted from June-August, 2011.

**Seed burial depth experiment**

The objective of this experiment was to determine the seed burial potential of infaunal species in these distinct functional groups.
Frozen cores were placed in the experiment tank and allowed to thaw completely before a single infaunal individual was added to each non-control core. Twenty cores were assigned to each of five treatments: *N. succinea, C. torquata, P. gouldi, A. ornata,* and no-animal control. Animals were allowed to acclimate overnight and establish themselves in a natural manner so their subsequent interactions with seeds were realistic. Animals that did not rebury were replaced with new animals following the same protocol. Direct observation showed that animals rapidly reburied and were actively bioturbating within 12 hours. Following this acclimation, ten viable *Z. marina* seeds were scattered on the surface of each core. Cores containing *N. succinea* were covered with fine mesh netting in order to retain the worm in the core.

After two weeks, the depth of each seed in each core was determined by sieving. The rubber cap on the bottom of the PVC sleeve was removed and replaced with a 10cm diameter disk of rigid plastic. This disk was pushed up into the sleeve to extrude the sediment in 0.5cm increments. Each increment was sliced off the main core and rinsed through a 1mm sieve, and the number of seeds in each increment was recorded.

Seed burial rate experiments

The objective of this experiment was to determine the daily rate at which seeds were buried by several infaunal species. Two separate experiments were conducted. The first incorporated *N. succinea, C. torquata, A. ornata,* and a no-animal control, with twenty cores assigned to each treatment. The experiment was conducted as in the seed burial depth experiment, except that five cores from each treatment were sieved one day, three days, seven days, and thirteen days after the addition of the seeds.

The second experiment incorporated only *P. gouldi* because only four *P. gouldi* were available. The experiment was conducted to be as comparable as possible to the seed burial rate experiment with the other infaunal species. Each of the four *P. gouldi* were added to a core and allowed to bury. Ten *Z. marina* seeds were added to the surface of those four cores as well as four control cores. All eight cores were sieved after one day. The *P. gouldi* were then added to four new cores and allowed to bury. Ten *Z. marina* seeds were added to the surface of those four cores as well as four control cores. All eight cores were sieved after three days. This was repeated twice more, with sieving occurring seven days and thirteen days after the addition of the seeds.

Sediment movement experiment

The objective of this experiment was to more thoroughly explore how different infaunal species distribute large particles in the sediment on a time scale of days. Eighteen cores were assigned to each of four treatments: *N. succinea, C. torquata, A. ornata,* or no-animal control. On each of seven consecutive days ten small colored glass
beads (size 15/0, ~1mm long and 2mm in diameter) were placed on the surface of each core, with a different color of bead added on each day. On day eight one half of the cores from each treatment were sieved as in the seed burial experiment, and on day fifteen the remaining cores were sieved. The number and color of beads in each 0.5cm increment were recorded.

Data analysis
All data analysis was conducted in the R statistical package, version 2.11.1
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Seed burial depth experiment
For each core, the mean, median, and maximum seed burial depths were calculated and averaged within treatments. The proportion of seeds found below the sediment surface was calculated for each core and used in a logistic regression (Peng et al, 2002). Using the regression outputs, ratios of the probability of seed burial in each of the animal treatments vs. the probability of seed burial in the control treatment were calculated. A Kruskal-Wallis test (Chan et al., 1997) (p=0.05) was conducted on mean seed depth per core, and two-tailed post-hoc testing was used to make all possible pairwise comparisons of seed burial between treatments.

Seed burial rate experiment
For each core the mean, median, and maximum seed burial depths at day 14, as well as the proportion of seeds buried at each sampling day, were calculated and averaged within treatments. A Kruskal-Wallis test (Chan et al., 1997) (p=0.05) was conducted on the proportion of seeds buried at days 1, 3, 7, and 13, and one-tailed post-hoc testing was used to compare proportion of seeds buried in each animal treatment with the proportion of seeds buried in the control treatment.

Sediment movement experiment
For each core, the mean, median, and maximum bead burial depths at day 15, as well as the proportion of beads buried at each sampling day, were calculated for each bead color and averaged by color within treatments. A logistic regression was used to analyze the difference in proportion of beads buried between beads representing 1 day and 14 days of burial. A non-parametric Mann-Whitney Rank Sum test (Frick and Rahlfs, 1998) was used to compare the mean bead depth and mean seed depth after 13 days of burial for each treatment (seed data was from the seed burial rate experiment). A Kruskal-Wallis test (Chan et al., 1997) (p=0.05) was conducted on the proportion of beads buried at days 1, 3, 7, and 13, and one-tailed post-hoc testing was used to compare proportion of beads buried in each animal treatment with the proportion of beads buried in the control treatment.
Results

Sediment analysis
The sediment used in these experiments was found to be on average 0.54% gravel, 97.20% sand, 0.65% silt, and 1.62% clay. Total organic carbon was below the detection limit (0.170%), and total nitrogen was 0.015%.

Seed burial depth experiment
Seed burial was recorded in all treatments after two weeks, and at least some seeds remained on the sediment surface at the end of the experiment in all treatments. All animal treatments yielded deeper seed burial than the control treatment.

Median seed depth was in the 0-0.5cm sediment layer for all animal treatments, and the mean and maximum seed depths ranged from 0.4-1.0cm and 1.0-4.5cm, respectively. Control cores had a median seed depth of 0.0cm, a mean seed depth of 0.1±0.2cm, and a maximum seed depth in the 0.5-1.0cm layer (Table 1).

The depth distribution of seeds varied between species, but generally the largest fraction of seeds was found in the 0-0.5cm layer (Figure 1). Burial was most dramatic in both *N. succinea* and *P. gouldi* cores. In *N. succinea* cores, 61.5, 20.5, and 12.8% of seeds were found in the 0-0.5cm, 0.5-1.0cm, and 1.0-1.5cm depth intervals, respectively, with 2.6% found in the 2.5-3.0cm interval. In *P. gouldi* cores, seeds were found in all depth intervals with the deepest buried seeds (3.5%) in the 4.0-4.5cm interval. In *N. succinea* cores, seeds were frequently found inside the worm’s burrow, rather than in the surrounding sediment. In all other animal cores the seeds were never found inside the worm’s tube. In control cores, 80% of seeds were found on the surface.

Logistic regression revealed that seeds in all animal cores had a significantly higher chance of being buried than the seeds in the control cores ($p \leq 1.03 \times 10^{-6}$), with the odds of being buried depending on the species, but increasing by a factor of more than 6 over the control cores in all cases (Table 2).

A Kruskal-Wallis test showed the burial depth to be significantly different between all animal treatments and the control treatment. The burial depth in *P. gouldi* cores was found to be significantly different than the burial depth in *C. torquata* or *A. ornata* cores.

There was no change in seed viability over the course of the experiment.

Seed burial rate experiment
The proportion of seeds buried after each sampling period varied widely in the different animal cores (Figure 2). However, *N. succinea* and *P. gouldi* cores consistently
showed the greatest proportion of seeds buried followed by *A. ornata* and *C. torquata* cores, with control cores showing the lowest proportion of seeds buried. After one day, *A. ornata* and *C. torquata* cores had an average of 3% and 5% of seeds buried, respectively, while *N. succinea* cores had an average of 45% of seeds buried. After 3 days, *A. ornata* and *C. torquata* cores had an average of 12% and 24% of seeds buried, respectively, while *N. succinea* cores had an average of 56% of seeds buried. After 7 days, *A. ornata* and *C. torquata* cores had an average of 24% and 38% of seeds buried, respectively, while *N. succinea* cores had an average of 87% of seeds buried. After 13 days, *A. ornata* and *C. torquata* cores had an average of 51% and 41% of seeds buried, respectively, and *N. succinea* cores had an average of 77% of seeds buried. Control cores had no seeds buried until after 13 days, when an average of 3% of seeds was buried.

After 13 days, the mean seed depth was 0.0±0.1cm in the control cores, 0.3±0.4cm in the *A. ornata* cores, 0.2±0.2cm in the *C. torquata* cores, and 0.8±1.0cm in the *N. succinea* cores. The median seed depth was 0.0cm for control and *C. torquata* cores, and in the 0-0.5cm sediment layer for *A. ornata* and *N. succinea* cores. The maximum seed depth was in the 0-0.5cm sediment layer for the control and *C. torquata* cores, in the 1.5-2.0cm layer for the *A. ornata* cores, and in the 4.0-4.5cm layer for the *N. succinea* cores.

The pattern of seed burial in *P. gouidi* cores was similar to that of *N. succinea* cores (Figure 2). After 1, 3, and 7 days, an average of 25, 55, and 78% of seeds were buried, respectively. The proportion of seeds buried reached an asymptote between 3 and 7 days, so after 13 days, the proportion of seeds buried had remained at 78%. Control cores had no seeds buried after 1 day or 3 days, and an average of 3 and 5% of seeds buried after 7 and 13 days, respectively.

After 13 days, the mean seed depth was 0.0±0.1cm in the control cores and 1.2±1.6cm in the *P. gouidi* cores. The median seed depth was 0.0cm for the control cores and in the 0-0.5cm sediment layer for the *P. gouidi* cores. The maximum seed depth was in the 0-0.5 cm sediment layer for the control cores and in the 6.0-6.5cm sediment layer for the *P. gouidi* cores.

A Kruskal-Wallis test found the proportion of seeds buried in *N. succinea* and *P. gouidi* cores to be significantly higher than the proportion of seeds buried in control cores after 1 day of burial. After 3 days of burial, *N. succinea, P. gouidi*, and *C. torquata* cores had a significantly higher proportion of seeds buried than control cores. After both 7 and 13 days, all animal cores had a significantly higher proportion of beads buried than control cores.

There was no change in seed viability over the course of the experiment.
Sediment movement experiment

All treatments showed an increase in the proportion of beads buried over the two week experiment (Figure 3). The proportion of beads buried in the animal cores increased throughout the experiment, while the proportion of beads buried in the control cores stayed near 0, but varied by up to about 10% per day. At the first sampling, on day 8, the data represented burial of beads after 1 day, 2 days, etc, through 7 days. After 1 day, *A. ornata*, *C. torquata*, and *N. succinea* cores had an average of 4, 18, and 40% of beads buried, respectively. There was an average of 1% of beads buried in the control cores. At the second sampling, on day 15, the data represented burial of beads after 8 days, 9 days, etc, through 14 days. After 14 days, *A. ornata*, *C. torquata*, and *N. succinea* cores had an average of 34, 40, and 50% of beads buried, respectively. Control cores had an average of 6% of beads buried.

In *N. succinea* cores, beads were occasionally found inside the worm’s burrow, rather than in the surrounding sediment. In all other animal cores the beads were never found inside the worm’s tube.

Logistic regression revealed a significant difference between the proportion of beads buried after 1 day and 14 days in *A. ornata* cores (p=4.1 x 10^-5) and *C. torquata* cores (p=0.03). There was no significant difference in *N. succinea* cores (p=0.47) and control cores (p=0.14).

At the sampling on day 15, the beads that had been placed on day 2 represented burial after 13 days, which was comparable to the data collected after 13 days in the seed burial rate experiment. After 13 days, the mean bead depth was 0.0±0.1cm in the control cores, 0.2±0.2cm in the *A. ornata* cores, 0.2±0.3cm in the *C. torquata* cores, and 0.4±0.4cm in the *N. succinea* cores. A non-parametric Mann-Whitney rank sum test showed that the mean bead depth after 13 days was not significantly different than the mean seed depth after 13 days for control cores (p=0.626), *A. ornata* cores (p=0.13), or *C. torquata* cores (p=0.52), but was significantly different for *N. succinea* cores (p=0.0058). The median bead depth was 0.0cm for control, *A. ornata*, and *C. torquata* cores and in the 0-0.5cm sediment layer for *N. succinea* cores. The maximum bead depth was in the 0-0.5cm sediment layer in the *C. torquata* and control cores and in the 0.5-1.0cm sediment layer for *A. ornata* and *N. succinea* cores.

A Kruskal-Wallis test found the proportion of beads buried in *N. succinea* and *C. torquata* cores to be significantly higher than the proportion of beads buried in control cores after 1 day of burial. There were no significant differences found after 3 days of burial, but after 7 and 13 days of burial, all animal treatments had a significantly higher proportion of beads buried than the control treatment.
Discussion

The results of this study indicate that *Zostera marina* seed burial in low organic sandy sediments is facilitated by infaunal activity, and that burial patterns relate at least in part to feeding mode. In addition, burial rates are rapid and occur on a time scale of days. This provides direct support for a mechanism of rapid seed burial and a partial explanation for observed patterns in previous seed dispersal studies, where it was found that seeds do not move far from where they settle on the sediment surface (Orth et al. 1994).

Seed burial depths for *A. ornata*, *C. torquata*, and *P. gouldi* related well to individual bioturbation rates for those species, indicating that seeds were being passively buried by these animals and acting as any other large particles in the sediment (de Brouwer et al., 2000). *P. gouldi*, which buried seeds the most deeply, has the highest bioturbation rate, 6 grams of sediment per individual per day (Gordon, 1966). The seeds buried most deeply were found near the animal’s head, where its feeding activity creates small, continually collapsing caverns called feeding voids. Seeds in other areas of the core were much closer to the surface and likely covered by defecated material. *A. ornata* and *C. torquata* had similar burial depths, despite representing different feeding modes. This was likely due to their similar bioturbation rates, 3.8 and 4.1 grams of sediment per worm per day, respectively (Rhodes, 1967). In these cores there was no significant difference between the mean burial depth of seeds and that of beads, which were both generally buried by being covered with defecated material, though some evidence of *P. gouldi*-like feeding voids was observed in *C. torquata* cores. This similarity between beads and seeds indicates that both were treated as passive particles. Seeds being buried passively will result in net downward movement, as they are too large for the conveyor belt feeders such as *P. gouldi* or *C. torquata* to ingest (Rhodes, 1967) and bring back to the surface.

The high proportion of seeds buried in *N. succinea* cores, and the depths to which those seeds were buried, related poorly to the species’ comparably low individual bioturbation rate observed in this experiment. The low proportion of seeds left on the core surface and the number of seeds found in the animals’ burrows suggest that *N. succinea* actively take seeds into their burrows. This is supported by the significant difference between mean burial depths of seeds and beads—they were not being passively buried and the worms had different preferences for seeds and beads. This active burial results in rapid seed burial, but could also result in deeply buried seeds being
discovered and brought toward the surface, rather than the net downward movement seen with passively buried seeds. In terrestrial literature, animals that actively bury seeds, such as ants and squirrels, have been shown to bring previously buried seeds to the soil surface (Hughes and Westoby, 1992, Hulme and Borelli, 1999). This leads to much more complicated burial dynamics than seen with animals that passively bury seeds.

The rapid burial of *Z. marina* seeds is biologically important for several reasons. First, it allows seeds to escape predation by animals foraging on the sediment surface. Second, burial provides a way for a seed to settle into and remain at a site suitable for germination and seedling establishment. Third, burial is required for the seed to reach a region of the sediment that has low oxygen levels, which have been found to be important in initiating germination.

Predation is a major risk to survival that all seeds face (Janzen, 1971). In the Chesapeake Bay, one of the major *Z. marina* seed predators is the blue crab, *Callinectes sapidus*. Fishman and Orth (1996) found that 65% of seeds in field manipulative experiments could be lost to predation by *C. sapidus* in 7 days, and that burial protected seeds from predation. In this study ~50% of seeds were buried after just 3 days, and ~80% after 7 days, by *N. succinea* and *P. gouldi*. 10-20% of seeds were buried after 3 days, and ~40% after 7-9 days, by *A. ornata* and *C. torquata*. This burial, even by the slower species, is fast enough to reduce seed predation by *C. sapidus*.

For seeds undergoing dispersal, settling into and remaining in a suitable germination site (Harper, 1977) requires rapid burial. Sediments with infauna provide a mechanism for this burial. All four species of infauna were able to bury a large proportion of seeds below the sediment surface in a matter of a few days. This study found that *P. gouldi* can bury 10% of nearby seeds in 0.5 hours and 33% in 3.5 hours with 6.7% of seeds to below 0.5cm (data not shown). This rapid biotic burial is likely to rival burial due to abiotic processes, especially as seeds are released from reproductive shoots in the late spring when sediment transport is lessened by the lack of energetic waves and currents and bioturbation rates are increasing with increased temperatures (Dellapenna et al., 1998).

Burial is also critical for *Z. marina* seedling establishment because the seeds require hypoxic conditions to germinate (Moore et al., 1993; Probert and Brenchley, 1999) and must be buried to at least 0.5cm to achieve low enough oxygen levels to cue germination (Probert and Brenchley, 1999). In addition, the seedlings produced from seeds buried to the depths found here are more likely to survive winter storms which can erode superficial sediments (Marion and Orth, 2012). Over the course of the summer, seeds must be buried below 0.5cm in order to germinate and establish as successful
seedlings. All infauna treatments were able to get at least some seeds below this threshold over a two week period, and it is expected that over an entire summer more seeds would be buried to these deeper depths.

Seed burial could also have negative consequences for seedling establishment if the seed is buried too deeply. Valdermarsen et al (2011) found that bioturbation by *Arenicola marina* had a strong negative effect on the successful establishment of *Z. marina* seedlings. However, *A. marina* was found to rework the top 18-20cm of sediment, while the infauna used in this study only rework the top ~10cm (Rhodes, 1967). It is therefore unlikely that the species in this study have the same negative effect on seedling establishment as *A. marina*. *Z. marina* seeds buried below ~5-6cm are generally not considered able to successfully germinate (Jarvis and Moore, 2010), but this lower threshold for successful germination depends on sediment type and whether the seed is in an erosional or depositional zone. In sandier sediments and in areas of sediment erosion, deeper burial as recorded with *P. gouldi* and *N. succinea* may be favorable or even necessary for successful seedling establishment.

In the field, infauna are patchily distributed, but the infaunal density used in this study, which corresponds with 127 individuals per square meter, is not unrealistic for these species [Dauer et al., 1989; Schaffner, 1990], with the exception of *A. ornata*, which is generally found at tens of individuals per square meter (Blackburn, personal observation). The burial estimates presented here are likely to be underestimates of the potential for these animals to bury seeds when they are at the high end of their natural densities, especially for the non-mobile *C. torquata* and *A. ornata*. Many times there was an area in the core clearly under the influence of the animal, and seeds outside of this area were completely unaffected by the presence of the animal. At higher infaunal densities like those seen in the field, more seeds would fall under the influence of an animal and potentially become buried. The estimated number of seeds buried by *N. succinea* in this study is also likely to be low, since individuals usually range more widely than the core allowed and would naturally encounter more seeds.

While the experiments presented here evaluated the influence of just one individual, natural infaunal communities consist of individuals of many different species and many feeding modes. Total infaunal densities in the field far exceed the density used in this study, generally in the range of three to four thousand individuals per square meter in unvegetated sediments [Schaffner, 1990], and even higher in vegetated sediments (Orth et al., 1984). This corresponds to approximately 30 infaunal individuals or more in each of the cores used in this experiment. Even if many of the species have little individual burial effect, the combined community effect is likely to be much larger than the effect of the single animals used in this study. An initial 2 week experiment with
infaunal cores from a natural sand community showed all seeds buried below the sediment surface, 50% of the seeds in the 1.5-2.0cm sediment layer, and the deepest seed in the 4.0-4.5cm sediment layer (Blackburn, unpublished data). With a rich and complex infaunal community, seeds are expected to be buried quickly and placed below the 0.5cm threshold. This community, with a combination of animals that would bury seeds actively and passively, will likely lead to much more complex seed burial, with seeds being moved up, down, and laterally through the sediment.

A diverse community will also facilitate burial by increasing the topographic complexity of the sediment surface, allowing seeds to be retained near a mound or depression. For infauna that passively bury seeds, like *C. torquata*, seed proximity was very important in determining the likelihood of burial (personal observation). Luckenbach and Orth (1999) reported that seeds entrapped near a *C. torquata* tube frequently became covered by the worm’s biodeposits, and that sediment microtopography observed in the field was not only likely caused by deposit-feeding worms but also sufficient to both retain seeds and promote shallow seed burial. For infauna that actively bury seeds, increased seed retention by small-scale sediment features can increase the chance that a seed will be encountered and collected by an individual. Seed entrapment and retention, and the time-scale over which it takes seeds to become entrapped, may prove to be important in determining the overall effectiveness of an infaunal community in burying seeds, and will be driven by the microtopography created in part by that community.

The microtopography created by an infaunal community, as well as that community’s effectiveness in burying seeds, is also likely to be affected by the strength of currents and waves in that area. Currents that are too weak to move seeds along a flat bottom (<0.7m/s) (Orth et al., 1994) will still be strong enough to transport the unconsolidated sediment ejected into the water column by defecating infauna, which will spread the influence of an individual beyond its still-water defecation mound, but reduce the rate at which it could bury a nearby seed. However, these low-shear currents are not likely affect the efficiency of active burial, as animals will still encounter and collect seeds. Currents or waves that produce enough shear stress to cause seed transport via bedload will have more of an effect on burial. Under those conditions, it is the entrapment of seeds by microtopography that will allow seeds to remain in one place long enough to be buried passively or encountered and buried actively.

The seed burial processes found here are analogous to processes described in the terrestrial literature dating back to Darwin (1837), who described the burial of cinders and pieces of lime and marl in pastureland and agricultural fields, and ascribed this burial to the activities of earthworms in the soil. In many forested areas and grasslands, the top
several inches of soil can consist entirely of earthworm castings, with earthworms normally depositing castings at a rate of 1.2-25.5 tons per acre per year (Thorpe, 1949, Wilkinson et al., 2009). While impressive, this is only a few percent of the rate that sediment is processed by the infauna used in this study, which can be calculated from individual bioturbation rates to be around 1600 tons of sediment per acre per year. With such a large volume of sediment being processed in marine systems, it is not surprising that this study found burial happening much more quickly than terrestrial estimates (Westerman et al., 2009, Willems, 1994). Despite the difference in magnitude between terrestrial and marine seed burial, there was evidence for both active and passive seed burial, both of which occur in terrestrial systems. In the terrestrial literature the implications of active burial range from influencing seedling distributions on small spatial scales (Warr et al., 1993) to allowing for interspecific seed competition based on an animal’s seed preferences (Regnier et al., 2008). Activity by *N. succinea* and other animals that bury seeds actively may be an important consideration when examining seedling distributions, and the strength of competition between *Z. marina* and other seagrasses, in the field.

*Z. marina* seed burial is complex, with both biotic and abiotic processes acting in concert to produce the seedling establishment patterns observed in the field. While abiotic processes may be initially important in seed burial, the direct (active movement of sediment) and indirect (formation of mounds and holes) consequences of biotic processes by infauna may prove to be dominant and need to be considered in understanding seedling establishment rates and patterns.
Literature Cited


Jarvis J.C., Moore K.A. 2010 The role of seedlings and seed bank viability in the recovery of Chesapeake Bay, USA, Zostera marina populations following a large-scale decline. Hydrobiologia 649:55-68.


Table 1. Seed burial depth experiment: summary of seed burial.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Median Seed Sediment Layer (cm)</th>
<th>Mean Seed Depth (cm)</th>
<th>Maximum Seed Sediment Layer (cm)</th>
<th>Percent Seeds Buried</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.0</td>
<td>0.1±0.2</td>
<td>0.5-1.0</td>
<td>20</td>
</tr>
<tr>
<td><em>A. ornata</em></td>
<td>0.0-0.5</td>
<td>0.4±0.3</td>
<td>1.5-2.0</td>
<td>67</td>
</tr>
<tr>
<td><em>C. torquata</em></td>
<td>0.0-0.5</td>
<td>0.4±0.4</td>
<td>1.0-1.5</td>
<td>60</td>
</tr>
<tr>
<td><em>N. succinea</em></td>
<td>0.0-0.5</td>
<td>0.8±0.5</td>
<td>2.5-3.0</td>
<td>97</td>
</tr>
<tr>
<td><em>P. gouldi</em></td>
<td>0.0-0.5</td>
<td>1.0±1.2</td>
<td>4.0-4.5</td>
<td>68</td>
</tr>
</tbody>
</table>

Table 2. Seed burial depth experiment: summary of logistic regression output.

For example, an odds ratio of 8.3 means that a seed is 8.3 times more likely to be buried in an *A. ornata* core than in a control core.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Log(odds)</th>
<th>Odds Ratio</th>
<th>95% CI of Odds Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. ornata</em></td>
<td>+2.1, p &lt; 2x10^-16</td>
<td>8.3</td>
<td>5.1-13.7</td>
</tr>
<tr>
<td><em>C. torquata</em></td>
<td>+1.8, p = 4.2x10^-13</td>
<td>6.1</td>
<td>3.8-10.1</td>
</tr>
<tr>
<td><em>N. succinea</em></td>
<td>+5.0, p = 1.03x10^-6</td>
<td>153.0</td>
<td>31.6-2760.4</td>
</tr>
<tr>
<td><em>P. gouldi</em></td>
<td>+2.1, p &lt; 2x10^-16</td>
<td>8.5</td>
<td>5.3-13.9</td>
</tr>
</tbody>
</table>
Figure 1. Seed burial depth experiment: seed burial distributions after 2 weeks. Bars give the median number of seeds found in each core at that depth, whiskers give the range. All animal treatment burial depths are significantly (p<0.05) different from control. *P. gouldii* burial depth is significantly different from *A. ornata* and *C. torquata* burial depth.
Figure 2. Seed burial rate experiment: percent of seeds buried at each sampling. Note that day corresponds with how many days the seeds were in the core. Standard Error shown. * indicates significant difference from control on that day (p<0.05).
Figure 3. Sediment movement experiment: percent of beads buried. Note that day corresponds to how many days the beads were in the cores. Sampling occurred on day 8 and day 15 (after 7 and 14 days, respectively). Darker lines show the data as if the beads had been sampled as the seeds in the seed burial rate experiment. Standard Error shown. * indicates significant difference from control on that day (p<0.05).
VITA

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