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Abarenicola pacifica Burrowing Behavior and Its Implications for *Zostera marina* Seed Burial, Restoration, and Expansion¹

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Abstract: Seed dispersal and burial are important processes in the expansion and restoration of *Zostera marina* (eelgrass) meadows. The depth at which seeds are buried is a significant factor contributing to the success of seedling survival. If seeds are buried below 6 cm, it is unlikely that viable seedlings will develop, while shallow burials protect seeds from predation on the sediment surface. Burrowing behavior of infaunal organisms is one factor that contributes to seed burial with a potentially positive or negative influence on seedling survival. In this study, we designed a laboratory experiment to determine the relationship between lugworm (*Abarenicola pacifica*) density and eelgrass seed burial. Three treatments (no worms, low-density, and high-density of worms) with three replicates each were used to quantify seed burial. Each replicate was seeded with a blend of seed mimics and real seeds. After 25 days, three cores were extracted from each replicate and the depths of the seeds recorded. In the high-density worm treatments, most of the seeds and mimics were buried below the 6 cm critical depth, while in the low-density treatments most seeds were found shallower than 3 cm. These results agree with previous work on the burying capacity of infaunal organisms, and strongly suggest that the presence and activity of infauna can determine the success of *Z. marina* meadow expansion and restoration.

Keywords: *Abarenicola pacifica*, *Zostera marina*, lugworm, seed burial, False Bay

MANY PLANTS RELY ON BIOTIC as well as abiotic factors for seed dispersal and burial (Chambers and MacMahon 1994, McConkey et al. 2012). These factors are well documented for terrestrial plants; however, less is known about the dispersal and burial mechanisms of aquatic angiosperm seeds, notably seagrasses (Orth et al. 2006, Kendrick et al. 2012).

Zostera marina (eelgrass) is a seagrass found throughout the Northern Hemisphere, from subarctic to subtropical regions (Den Hartog 1970, Green and Short 2003). *Zostera marina* reproduces through vegetative clonal growth as well as sexually through seed production (Luckenbach and Orth 1997, Moore and Short 2006). Seeds are a critical mechanism for dispersal and patch expansion as they can travel far from the parent plant and colonize new areas (Chambers and MacMahon 1994). Seeds can disperse a variety of distances, from a few meters (Ruckelshaus 1996), up to 200 m via an attached air bubble (Churchill et al. 1985), or up to 34 km within floating reproductive shoots (Harwell and Orth 2002). Although seagrasses can expand clonally, recruitment via seed dispersal increases genetic diversity and is important for eelgrass evolution and resilience (Reynolds et al. 2012).

Zostera marina seeds are often characterized as being negatively buoyant, meaning

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they sink to the sediment surface after the fruit is dehiscence and the seed is released (De Cock 1980, Orth et al. 1994). Once the seed reaches the sediment surface, it can either be carried away as bedload (Luckenbach and Orth 1997) or incorporated into the sediment (Delefosse and Kristensen 2011, Blackburn and Orth 2013). Eelgrass seeds can be buried through the resuspension of sediment from waves and currents, as well as by organisms living in the sediment (Delefosse and Kristensen 2011, Blackburn and Orth 2013). Infauna has the capability of reworking sediment and consequently cause seeds to be buried (Delefosse and Kristensen 2011, Blackburn and Orth 2013). The burial may affect survival depending on how deep the seed is buried. Shallow burials can protect seeds from consumers and predators (Delefosse and Kristensen 2011, Blackburn and Orth 2013). However, if buried to a depth of 6 cm or more, the hypocotyl may not reach the surface and the seedling fails to fully develop and survive (Churchill 1983, 1992, Valdemarsen et al. 2011).

Abarenicola pacifica is a lugworm species found in the Pacific Northwest that inhabits the upper intertidal zone down to about +0.8 m MLLW (Healy and Wells 1959, Krager and Woodin 1993). Sediment characteristics control worm distribution more than tidal exposure, as they prefer firm, heterogeneous sediment (Hobson 1967, Crane and Merz 2017). Lugworms are ecosystem engineers due to their burrowing behavior and large capability for sediment reworking (Berke et al. 2010, Crane and Merz 2017). *Abarenicola pacifica* found in False Bay, San Juan Island, WA inhabit muddy sand near the head of the Bay, with a patchy distribution (Crane and Merz 2017). Their presence is marked by the fecal coils that accumulate on the sediment surface from the tail shafts (Krager and Woodin 1993, Crane and Merz 2017). *Abarenicola pacifica* creates “J-shaped” burrows in the sediment which they line with mucus (Healy and Wells 1959, Crane and Merz 2017). They live head-down and consume sediment from the surface via the head shaft and then, using the tail shaft, deposit their waste back onto the surface (Hobson 1967). The subsidence of sediment

into the head shaft creates a funnel shaped hole in the sediment surface; however, this funnel cannot always be seen as it can lie below the sediment surface (Healy and Wells 1959). *Abarenicola pacifica* burrows are not permanent as the worms remain in one burrow for an average of only 3 days (Crane and Merz 2017).

There have been no studies on the impact of bioturbation by *A. pacifica* on *Z. marina* seed burial, however there is evidence that a similar lugworm species, *Arenicola marina*, buries *Z. marina* seeds deep enough to prevent successful seedling development (Valdemarsen et al. 2011). In systems inhabited by both *Z. marina* and *A. marina* there are clear boundaries between their beds, suggesting an exclusionary relationship (Valdemarsen et al. 2011, Onken et al. 2022). Valdemarsen et al. (2011) found that *A. marina* can bury *Z. marina* seeds greater than 6 cm below the sediment surface and thus block successful seedling establishment. A similar study by Delefosse and Kristensen (2011) reported that the density of *A. marina* significantly impacts the rate and depth of burial of seagrass seeds. At high worm densities, the seeds not only get buried deeper but also faster when compared to low densities. The main method of burial by *A. marina* is from the accumulation of fecal coils on the surface (Delefosse and Kristensen 2011). Seed and seedling burial by *A. marina* is hypothesized to limit *Z. marina* recovery and meadow expansion (Valdemarsen et al. 2011).

In this study, we examined the effects of *A. pacifica* density on *Z. marina* seed burial using both field and lab studies. Due to the similarity of burrow shape and behavior between *A. marina* and *A. pacifica*, we hypothesized that *A. pacifica* will bury *Z. marina* seeds in a manner similar to its congener.

METHODS

Collections and observations of *Abarenicola pacifica* took place in False Bay, San Juan Island, WA, during June 2020 (Figure 1). False Bay is a circular tidal flat owned by Friday Harbor Laboratories (FHL) where two

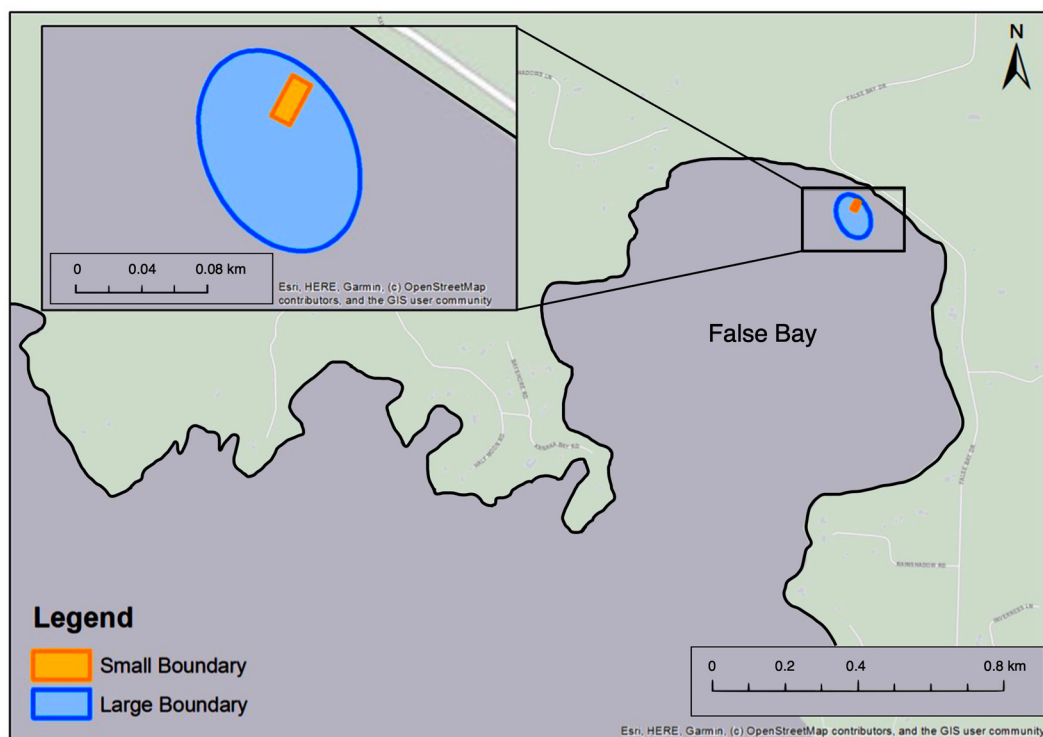


FIGURE 1. False Bay, San Juan Island, Washington. Blue area represents where many *A. pacifica* burrows were found. The orange area represents an area inhabited by *A. pacifica* with no large patches lacking fecal coils and where the density calculations were established.

species of lugworms (*A. pacifica* and *Abarenicola clapedi*) and *Zostera marina* beds have been found historically (Hobson 1967, Ruckelshaus 1994).

The natural density of *A. pacifica* in False Bay was determined from the observed distribution of fecal coils on the sediment surface. PVC posts were placed into the sediment every 20 m surrounding the *A. pacifica* beds. A smaller area inside this large plot was a defined bed where there were no large (1 m²) areas lacking fecal coils. Inside this smaller plot a 0.25 m² quadrat was haphazardly tossed 50 times and the number of fecal coils was recorded. Data from the smaller plot were then used to quantify and identify the natural variation in *A. pacifica* densities.

Sediment was collected in June 2020 at False Bay, San Juan Island, WA, in areas where *A. pacifica* presence was confirmed by fecal

coils. Sediment was brought back to FHL and sieved through a 2 mm sieve to remove unwanted materials and infauna not under investigation. Sieved sediment was then placed into buckets (i.d. 28.5 cm, 40.6 cm tall, and capacity of 18.9 L) with screened holes in the sides allowing water circulation through the porewater in the sediment. The depth of sediment in each bucket was 18 cm. Buckets were placed into flow-through seawater tanks, with approximately 3 cm of overlying water to allow the lugworms to ventilate their burrows. Sediment was left to settle until the start of the experiment.

Abarenicola pacifica were collected in June 2020 within the large boundary encompassing their habitat (Figure 1). Worms were excavated using a shovel and then placed into a plastic bag with a handful of sediment and cold seawater. To maintain the low temperature found in the burrows the bags were

placed into a cooler with ice. Worms and ice were separated by a thick layer of paper. The cooler was immediately transported to FHL after collection. Before placement into the experimental treatments, the volume of each worm was recorded using displacement of water in a graduated cylinder. Worms that did not bury within 20 min after placement on the sediment surface in experimental buckets were replaced.

Burial of *Z. marina* seeds by *A. pacifica* was tracked using both real seeds (collected in 2019 and stored in seawater at 5 °C) (Dooley et al. 2013) as well as seed mimics. To ensure sufficient statistical power for our experiment and due to limited access to enough real seeds, we employed seed mimics as a substitute for actual seeds in our study. Mimics were created following protocols described by Valdermarsen et al. (2011) and Delefosse and Kristensen (2011). Blue nylon cord was

cut into approximately 3 mm long pieces to match the length of naturally occurring seeds (Wyllie-Echeverria et al. 2003). Delefosse and Kristensen (2011) found these cord pieces to be a valid mimic that were buried to depths comparable to actual seeds. Real seeds were spray-painted for easy recovery at the end of the experiment. However, because we did not test seed viability, we do not know the germination potential of real seeds used in the study.

A total of nine buckets were set up for the experiment with three replicates for each treatment: control ($n = \text{zero worms}$), low-density of worms ($n = \text{two worms per bucket}$), and high-density of worms ($n = \text{eight worms per bucket}$). The low-density treatment corresponded to about 32 worms m^{-2} , whereas the high-density treatment corresponded to about 128 worms m^{-2} . The number of worms in the low-density treatment was based on the natural density

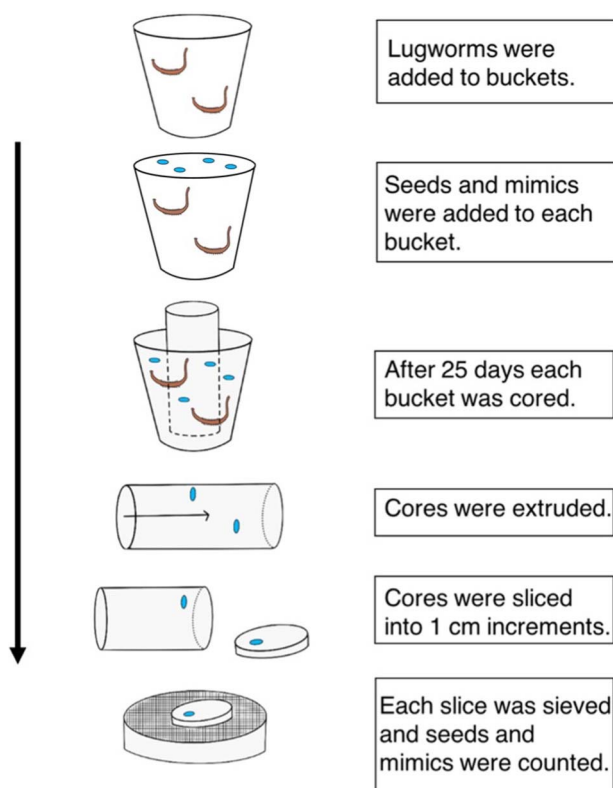


FIGURE 2. Experimental setup for lugworm burial experiment.

observed in False Bay. The high-density treatment was used to determine if there was an effect on seed burial dynamics under an extreme scenario. To avoid pseudo-replication, each seawater tank contained one bucket of each treatment (Hurlbert 1984). There were 450 seed mimics and 10 real seeds added to each bucket 12 hours after all worms had buried. Real seeds and mimics were carefully pushed approximately 1 mm into the sediment surface to keep them submerged throughout the experiment. Buckets were left undisturbed except for cleaning the seawater tanks to remove algae and diatoms.

After 25 days, all treatments were sampled using a ~10 cm diameter, 25 cm long sediment core. Each bucket was cored three times to the bottom (approx. 18 cm). Cores were removed one at a time and the sediment was extruded into a core holder where the core was then pushed out of the holder and sliced into 1 cm increments. These slices were sieved through a 1 mm sieve and the number of real seeds or mimics recorded (Figure 2).

From the field, lugworm densities were calculated as the mean ($\pm$ s.d.) number of fecal coils. Seed counts from the core samples were summed at the treatment level for hypothesis testing. A Welch ANOVA test (factor: worm density) was used to test whether the burial depths of real seeds and mimics were significantly ($p < 0.05$) different depending on worm density. The depths were transformed using a variance stabilizing transformation to conform to the normality assumption of the test, but had differing variances, meaning the Welch ANOVA test was more appropriate than other canonical tests.

RESULTS AND DISCUSSION

To understand the density and distribution of the natural population of *Abarenicola pacifica*, fecal coils on the surface of the sediment in False Bay were quantified. At this site, the distribution of *A. pacifica* tends to be patchy. Within the large boundary (Figure 1) the mean number of fecal coils was $7.2 \pm 10.8 \text{ m}^{-2}$ ($n = 50$). There were large ($>1 \text{ m}^2$) areas with no fecal piles on the

surface of the sediment, indicating a patchy distribution. Within the smaller boundary, encompassing a denser worm population, the mean number of fecal coils was $39.2 \pm 15.2 \text{ m}^{-2}$ ($n = 50$), and a maximum number of 56 coils m^{-2} . Counting fecal coils to calculate the density of lugworms tends to provide an underestimate of 10%–15% because of worms that are inactive at the time of sampling (Valdermarsen et al. 2011).

To ensure that each treatment had similarly sized worms, the volume of each captured worm was recorded. The mean per-worm volume of *A. pacifica* used for each treatment was $2.83 \pm 0.29 \text{ mL}$ ($n = 6$) for the low-density treatments, and $2.35 \pm 0.65 \text{ mL}$ ($n = 24$) for the high-density treatment. Thus, each treatment contained worms of a similar size however the low-density treatments had larger worms on average which could potentially cause increased burial capacity.

In a previous study with a congeneric lugworm, it was found that *Arenicola marina* was capable of burying *Zostera marina* seeds to a depth that blocked successful seedling development (Valdermarsen et al. 2011). In our study with *A. pacifica*, the treatments where seeds and seed mimics were exposed to worm activities resulted in the seeds being buried to significantly ($p < 0.05$) greater depths than in the treatment lacking worms (Figure 3). Using the Welch-ANOVA test, the depth at which the seeds and mimics were buried was significantly greater with increasing numbers of worms ($p < 0.05$). This depth was not significantly affected by the type of seed; mimics and real seeds were buried to similar depths and the burial patterns observed for real *Z. marina* seeds in our study were similar to those of the mimics (Figure 3). The maximum seed burial depths of the treatments were 2, 15, and 17 cm, under the Control, low-density, and high-density worms, respectively. It is possible that the seed found at 2 cm in the Control treatment may have been displaced due to sediment settling from the flow through system or inadvertently pushed deeper during the coring process. The percentage of seed mimics and actual seeds recovered was 35.8% and

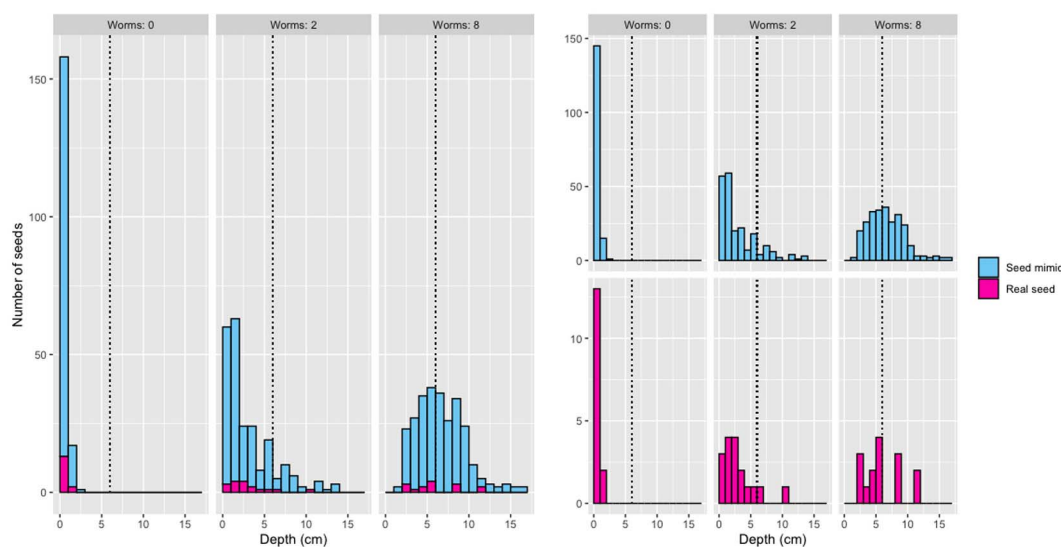


FIGURE 3. Histogram of depths of burial of *Z. marina* seeds and mimics under different *A. pacifica* densities. Values represent the summed values among all nine cores (three cores in each of the three buckets) per treatment. Blue represents nylon seed mimics (450 per treatment) and pink represents real *Z. marina* seeds (10 per treatment). Dotted line represents 6 cm depth.

50% for the Control group, 47.3% and 56.7% for the low-density treatment, and 56.7% and 50% for the high-density treatment, respectively. The less than 100% recovery is due to the coring protocol which did not allow for the excavation of the entire bucket.

All seeds in the high-density treatments were found at least 1 cm below the surface. Depths were further analyzed with respect to the 6 cm cutoff, as this depth is potentially too deep for successful seedling establishment (Valdemarsen et al. 2011). The number of seeds buried below 6 cm varied between the treatments, with no seeds found at this deep in the treatment lacking worms, 13% with low-density worm populations, and 53% with high-density worm populations (Figure 3). The mean depth at which seeds were buried was 0.11 cm for Control (zero worms), 2.53 cm for low-density (two worms), and 6.04 cm for high-density (eight worms).

Our results suggest that *A. pacifica* behaves similarly to *A. marina* in the burial of *Z. marina* seeds (Valdemarsen et al. 2011, Delefosse and Kristensen 2011). The main mechanism of burial by *A. pacifica* was by the deposition of fecal casts onto the surface of the

sediment, and *A. pacifica* is capable of depositing 15 g of feces in a single day (Hylleberg 1975). However, it is hypothesized that the seeds could also be buried by falling into the worms' burrows either during the formation of the tail or head shaft. This burial method could account for seeds found extremely deep, such as the seed found at 17 cm below the surface in the high-density treatment. As *A. pacifica* cannot ingest *Z. marina* seeds due to their size, buried seeds are expected to continue moving downward unless other infauna are present to move the seeds back up towards the sediment surface (Valdemarsen et al. 2011).

Seed burial by *A. pacifica* is a potentially important phenomenon for eelgrass restoration efforts that depend on seeding techniques. Eelgrass restoration projects at other sites in North America such as the Chesapeake Bay and Peconic Bay, New York, rely on the use of seeds, as this method provides a low-cost solution for large-scale projects compared to planting adult eelgrass shoots (Pickerell et al. 2005, Marion and Orth 2010). In 2020 a restoration effort was conducted in the Salish Sea by dispersing *Z. marina* seeds to

restore lost or declining eelgrass populations. Preliminary findings from this work demonstrated that dispersed seeds followed by clonal expansion results in eelgrass patch formation within the restoration site (Wyllie-Echeverria pers. comm.). While no evidence of *A. pacifica* has been found at the current eelgrass restoration site (Wyllie-Echeverria pers. comm.), our assessment highlights the importance of evaluating the presence and population density of *A. pacifica* in planning restoration projects in the Salish Sea region. This is necessary as we demonstrated that *A. pacifica* has the potential to bury *Z. marina* seeds below 6 cm, and burial at this depth is likely to result in the failure of the hypocotyl of sprouted seeds to reach the sediment surface thereby preventing the growth of a viable seedlings. However, in areas with a relatively low density of *A. pacifica*, these worms could potentially help in the success of a restoration effort, as the burial depth under these conditions is sufficient to protect seeds from consumers (>1 cm), but shallow enough for successful seedling production (<6 cm). It should be noted that the observed relationship between seed burial and *A. pacifica* burrowing behavior in our 25-day study may not necessarily hold true over longer time frames, meaning that even a low density of worms could potentially bury the majority of seeds below 6 cm and hinder successful restoration efforts. Beyond restoration, *A. pacifica*'s capacity to bury *Z. marina* seeds could limit areas available for colonization and disrupt the natural expansion of extant *Z. marina* meadows in the Salish Sea.

Our study provides valuable insights into the potential role of *A. pacifica* in the burial of *Z. marina* seeds and underscores the need for continued monitoring and evaluation of the benthos in eelgrass restoration projects and natural expansion.

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