Vol. 678: 51–61, 2021
https://doi.org/10.3354/meps13888

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published November 11

Responses of eelgrass seed germination and seedling establishment to water depth, sediment type, and burial depth: implications for restoration

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ABSTRACT: When seagrass beds are restored by seeding, many factors affect the transition of seeds to seedlings. In this study, the effects of water depth (light availability), sediment type, and burial depth on seed germination and seedling establishment of *Zostera marina* L. were tested through 2 *in situ* suspended culture experiments in Ailian Bay, China. In the first experiment, water depth (1, 3, and 6 m) had no significant effect on seed germination and seedling establishment, but did affect seedling survival. In the second experiment, seed burial depth (2, 5, and 10 cm) had significant effects on seed germination and seedling establishment. Seeds buried at shallow depths exhibited higher percentages of seed germination and seedling establishment, and maximum values (mean ±SD = 21.33 ± 9.30%) were recorded in seeds buried at 2 cm in 100% sand. There was no significant effect of sediment type on seed germination and seedling establishment at 2 cm ($p > 0.05$), but seeds buried at 5 cm were affected by sediment type, with seeds cultured in 100% sand exhibiting the highest percentages of seed germination (12.33 ± 4.51%) and seedling establishment (12.33 ± 4.51%). No seedlings or germinated seeds were found at 10 cm, regardless of sediment type. Once plants are established, sediment type and seed burial depth do not play a decisive role in plant growth, with plants performing equally in terms of shoot density (33–90 shoots per pot) and shoot height (20 ± 6 cm) in different sediment types and at different burial depths 1 yr after seedling establishment. Our findings help clarify the complex combined effects of sediment type and burial depth on seed germination and seedling establishment.

KEY WORDS: *Zostera marina* L. · Seagrass restoration · Methodology · *In situ* suspended culture experiments

1. INTRODUCTION

Seagrass habitats contribute to many ecological functions, including sediment stabilization (Orth et al. 2006), nutrient transformation (McGlathery et al. 2007), carbon sequestration (Fourqurean et al. 2012), and fishery resource enhancement (Unsworth et al. 2018). However, anthropogenic degradation of water quality has led to a global decline in seagrass beds (Short & Wyllie-Echeverria 1996), with losses of 7% per year worldwide since 1990 (Waycott et al. 2009). These declines have led to the conservation and

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restoration of these vulnerable ecosystems in some areas of the world (Unsworth et al. 2019, Orth et al. 2020). However, most seagrass restoration has focused on adult plants, and more research into seed-based restoration is required (Orth et al. 2000).

Seagrasses occur in coastal waters and reproduce through both asexual and sexual means, allowing them to recover from disturbance (Phillips & Meñez 1988, Xu et al. 2018, 2020a). Although asexual reproduction is the principal mode of seagrass meadow maintenance (Duarte & Sandjensen 1990, Xu et al. 2018), sexual reproduction plays a vital role in colonizing new habitats and areas of large-scale decline or complete destruction (Lee et al. 2007). Moreover, sexual reproduction may enhance long-term resilience to stress by increasing genetic diversity (Reynolds et al. 2012). However, successful sexual reproduction is constrained by many potential bottlenecks along the reproductive cycle (e.g. seed germination and seedling establishment; Orth et al. 2000, Marion & Orth 2010). As seed-based restoration initiatives become more common (Orth et al. 2020), a fundamental knowledge of the processes influencing the transition from seed to seedling is essential, as this may affect seagrass resilience to and recovery from disturbances.

Successful seed germination and seedling establishment are affected by many factors, often in combination. Seed germination is influenced by the interaction between physiological, genetic, and environmental factors (e.g. temperature, sediment, and light; Orth et al. 2000, Xu et al. 2016). Sediment oxygen levels, related to sediment characteristics (e.g. sediment type), may be the most important factor influencing germination processes for some species (Moore et al. 1993, Orth et al. 2000), and low dissolved oxygen conditions have been shown to increase the successful germination of *Zostera marina* L. seeds (Moore et al. 1993).

Rather than germination failure, low initial seedling establishment has been identified as the limitation of sexual reproduction in seagrass (Moore et al. 1993, Marion & Orth 2012). Waves and currents can remove ungerminated and germinated seeds on the sediment surface (Koch et al. 2006, 2010). Seeds can be buried in sediment to reduce these risks, and thus enhance seedling establishment and survival (Jørgensen et al. 2019). However, seeds buried below a certain threshold may not have sufficient energy reserves for the cotyledons to reach the sediment surface (Jørgensen et al. 2019), leading to failure of seedling establishment. Identifying the factors (e.g. sediment type and seed burial depth) limiting seed germination and seedling establishment is critical to understanding seagrass sexual processes and developing seed-based restoration techniques.

The eelgrass *Z. marina* is widely distributed in the temperate northern hemisphere (Green & Short 2003) and is also a dominant seagrass in the Bohai and Yellow Seas in northern China (Zheng et al. 2013, Xu et al. 2018, 2020a,b, Liu et al. 2019). *Z. marina* shoots are found in habitats where substrates range from sand to silty clay (Bradley & Stolt 2006), and distribution depths range from intertidal mud flats (van Katwijk & Wijgergangs 2004) to waters 20 m deep (Boutahar et al. 2020). Eelgrass beds have been drastically reduced in China. For example, there has been a marked decline in eelgrass meadows in the coastal waters of Shandong Province, with losses of >80% since the 1980s (Ye & Zhao 2002, Xu et al. 2020b).

Eelgrass seed germination and seedling establishment are affected by many factors (e.g. sediment type, burial depth, and temperature; van Katwijk & Wijgergangs 2004, Wang et al. 2016, Xu et al. 2016, 2018). van Katwijk & Wijgergangs (2004) found that muddy sites were more suitable than sandy sites for eelgrass seed germination in the field. The interaction between sediment type and burial depth has been found to have significant effects on eelgrass seed germination and seedling establishment in controlled laboratory conditions (Wang et al. 2016). However, Jarvis & Moore (2015) found that sediment type (>90% sand vs. <50% sand) did not affect mean time to germination in the field. Therefore, additional research may be necessary to further examine seed germination and seedling establishment responses across a wider range of sediment compositions (Jarvis & Moore 2015). Additionally, laboratory experimental systems remove important contextual factors of natural ecosystems and make studies less ecologically meaningful (Orth et al. 2000), a problem that can be resolved through *in situ* or field-based experiments. Water depth is a proxy for light availability (Xu et al. 2018), and the impact of light availability on seed germination and seedling establishment in the field is unknown, which limits our current understanding of limitations for seed-based restoration.

In this study, 2 *in situ* suspended culture experiments were conducted in Ailian Bay, China, to test the effects of water depth, sediment type, and seed burial depth on *Z. marina* seed germination and seedling establishment. It was hypothesized that light availability would not impact seed germination and seedling establishment in eelgrass, but that both sediment type and burial depth would have significant effects on seed germination and seedling establishment. The results of this study provide important information for future management and seed-based restoration of eelgrass beds. In addition, the *in situ* suspended seagrass culture methods described in this study may provide a useful methodology for future field simulation experiments.

2. MATERIALS AND METHODS

2.1. Study sites

Seeds were collected from a donor site located in Swan Lake, north China (Fig. 1). Swan Lake, with an average water depth of ~2 m, is a 4.8 km^2 marine lagoon containing a seagrass meadow with an area of ~2.3 km^2 (Xu et al. 2020b). The seagrass meadow is a mix of *Zostera marina* and *Z. japonica*. Seed germination of *Z. marina* mainly occurred from the middle of March to the end of May (Xu et al. 2018). The range of photosynthetic photon flux densities at the seagrass plant canopy was 10–30 µmol photons m^{-2} d^{-1} (Xu et al. 2018).

The experimental site was located in Ailian Bay, ~20 km from Swan Lake (Fig. 1). More than 60% of the bay is used for floating raft culture of kelp or shellfish. There are 2 seagrass species present: *Z. marina* and *Phyllospadix iwatensis*. With increasing coastal water turbidity, *Z. marina*, formerly distributed between 2 and 6 m depth, has disappeared and is now mainly found in sea cucumber ponds in the bay. In Ailian Bay, almost no light penetrates to 6 m, with <4% of surface irradiance at 6 m (Zhang et al. 2020).

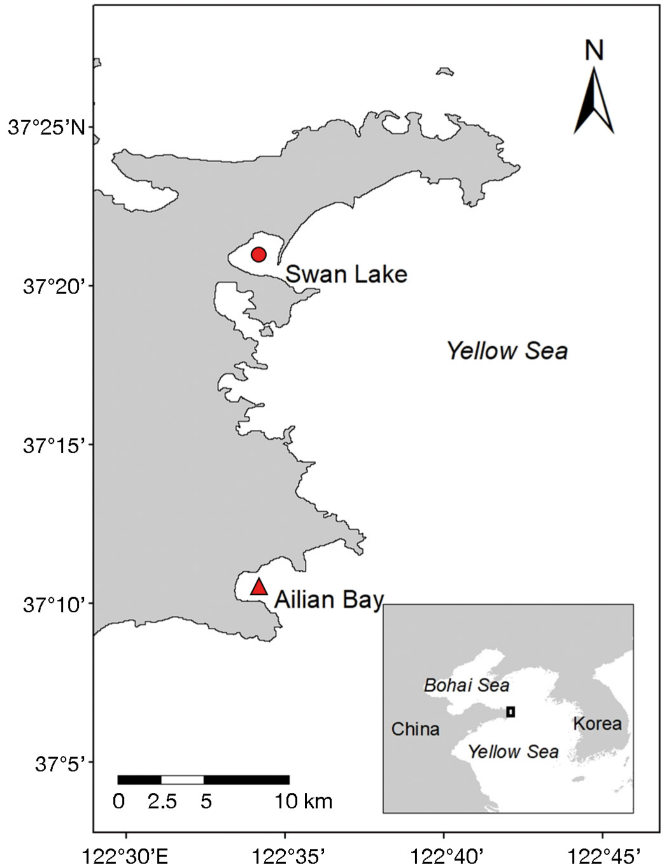

Fig. 1. Location of the donor site in Swan Lake (●) and the experimental site in Ailian Bay (▲), north China

2.2. Seed collection

Eelgrass seeds from reproductive shoots were collected from Swan Lake in mid-July 2014 and 2015, and stored in 600 µm mesh bags. These bags were held in a circular, aerated flow-through tank (1.0 × 1.2 × 1.5 m) until the initiation of experiments.

2.3. Suspended seed experiment

Given that 6 m was previously the depth limit of natural eelgrass in the system, to study the effects of water depth on seed germination and seedling establishment, a seed experiment was initiated in 2014 and conducted at depths of 1, 3, and 6 m (Table 1). On October 8, 2014, 9 pots (diameter = 20 cm, height = 12 cm; Fig. 2) were filled with dry sediment collected from the banks of Swan Lake, and the sediment was saturated with natural seawater from the lagoon. The sediment was mainly composed of sands (particle size 0.063–2.0 mm), which made up 80.68 ± 1.45% (weight) of the total sediment (Xu et al. 2018). Intact *Z. marina* seeds, with a viable seed percentage of >95% (tetrazolium chloride test; Sawma & Mohler 2002), were selected, and groups of 10 seeds were planted in each pot at a burial depth of 2–3 cm. The pots were then transported to Ailian Bay and tied to rafts with polyethylene ropes (Fig. 2), with 3 replicates at each depth (Table 1). To balance the experimental pots in the water, a 1 kg plumb ball was tied to the bottom of each pot (Xu et al. 2020b).

To study the effects of sediment type and burial depth on seed germination and seedling establishment, another seed experiment was initiated in 2015 (Table 1). Sediment types were set at 100% silt, 3:1 silt:sand mixture (v/v), 1:3 silt:sand mixture (v/v), and

Table 1. Details of the 2 suspended *Zostera marina* seed experiments

Seed planting time	Experiment period	Factors and factor levels	Seeds per pot	Number of duplicates per treatment	Monitoring dates	Monitoring index
Oct 8, 2014	Oct 8, 2014, to Aug 31, 2015	Depth (1, 3, and 6 m)	10	3	Dec 1, 2014 May 5, 2015 May 21, 2015 Jun 2, 2015 Jun 14, 2015 Aug 31, 2015	Seedling number, seedling shoot number, and seedling shoot height
Oct 1, 2015	Oct 1, 2015, to Feb 17, 2017	Sediment type: 100% silt, silt:sand mixture 3:1 (v/v), silt:sand mixture 1:3 (v/v), 100% sand; burial depth: 2, 5, and 10 cm	100	3	Mar 23, 2016 Apr 8, 2016 Apr 25, 2016 May 26, 2016 Feb 17, 2017	Germinated seed number, seedling number, seedling shoot number, and seedling shoot height

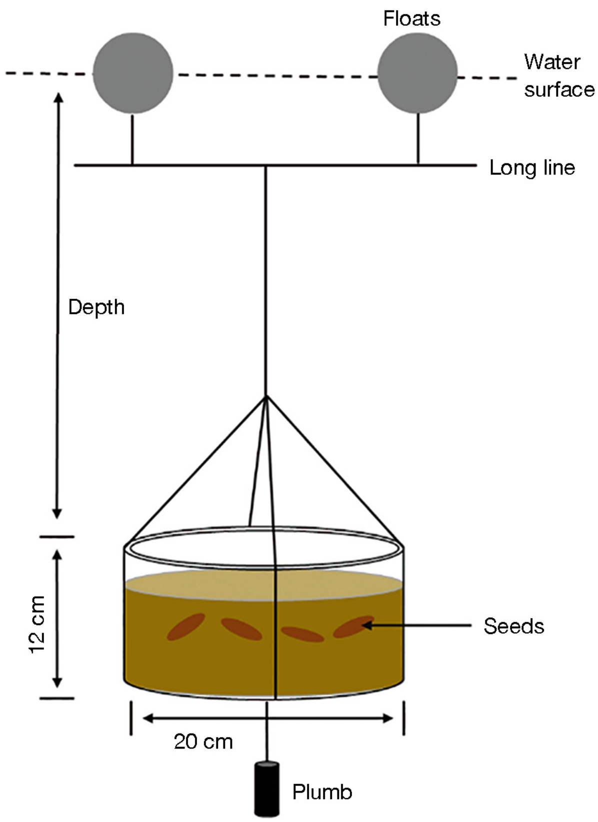

Fig. 2. Set up of PVC pots used for suspended *Zostera marina* seed experiments

100% sand. Seed burial depths were set at 2, 5, and 10 cm in each sediment type. The sediments used in the experiment were excavated from the banks of Swan Lake. The particle size of sand ranged from 0.063 to 2.0 mm, and that of silt was <0.063 mm. Given the low percentages of seed germination and seedling establishment in the first experiment, we used more seeds in the second experiment. Intact *Z. marina* seeds were selected, and groups of 100 seeds were planted in each pot, with 3 replicates for each treatment. On October 1, 2015, 36 pots (diameter = 20 cm, height = 12 cm; Fig. 2) with seeds were transported to Ailian Bay and tied to rafts with polyethylene ropes (Fig. 2). The second experiment was conducted at water depth of 2 m.

2.4. Monitoring method

In the present study, germinated seeds were defined as those with visible cotyledons that reached the sediment surface. Seedlings were considered established when germinated seeds formed visible new leaves at the sediment surface. Percent seed germination and seedling establishment were calculated as the number of germinated seeds and established seedlings divided by total seeds, respectively. Percent seedling establishment was only evaluated until early June, since clonal growth of eelgrass seedlings, which would lead to overestimating the percentage seedling establishment, mainly occurred from June onwards in Swan Lake (Xu et al. 2018).

In the first experiment, 6 monitoring visits were conducted, in December 2014, May, June, and August 2015 (Table 1). In the second experiment, to observe the germination process of seeds, 4 monitoring visits were conducted between March and May 2016, which is the main period of seed germination, and another monitoring visit was conducted in February 2017. The second experiment was conducted over a longer time period of 2 winters.

Given that only the number of established seedlings and the number of shoots produced by seedlings (hereafter called seedling shoots) were counted in the first experiment, the highest number of established seedlings was used to calculate percent seed germination and seedling establishment. To calculate percent seed germination and seedling establishment in the second experiment, the number of germinated seeds, the number of established seedlings, and the number of seedling shoots per pot were counted. Seedling height was also recorded in both experiments. During monitoring visits, the macroalgae and ascidians attached to suspended pots and polyethylene ropes were also cleaned.

2.5. Environmental parameters

To study the effect of water depth on eelgrass seed germination and seedling establishment, light availability (μmol photons m^{-2} s^{-1}) was measured at different depths (0, 1, 2, 3, and 6 m) in May and August 2015, using an ECO-PARSB sensor (Sea-Bird Scientific). In addition, other water parameters including temperature (°C), salinity (ppt), dissolved oxygen content (DO, mg l^{-1}), pH, and chlorophyll content (μg l^{-1}) were measured at different depths (1, 3, and 6 m) using a multi-parameter water quality sonde (YSI 6600). Water parameters were measured over a 2–15 min period at each depth, 3 times in total in May, June, and August 2015. For the second experiment, initiated in 2015, water temperature and salinity were measured at the water's surface (YSI Pro 30).

2.6. Data analysis

Values are presented as mean ± SD. Effects of water depth and date on seedling shoot density and height in the first experiment, and sediment type and burial depth on percent seed germination and seedling establishment in the second experiment, were analyzed using the Scheirer-Ray-Hare test. Two-sided *t*-tests were used to identify specific treatment differences. Differences were considered significant at $p < 0.05$. R v. 3.6.2 for Windows 10 was used for all data analyses (R Core Team 2016).

The spatial correlation of water turbidity and depth was assessed (see Zhang et al. 2020) using nonlinear regression to fit each of the 2 distributions, based on the measurements in May and August 2015, for the dependence of light intensity on depth to Beer's Law (Kirk 1985):

$$I = I_0 \exp(-K_d^* z) \quad (1)$$

where I represents the light availability at water depth z (m), I_0 represents the surface light, and K_d^* represents the light attenuation coefficient (m^{-1}), which was not corrected for the sun's location in the sky.

3. RESULTS

3.1. 2014 suspended seed experiment

The Scheirer-Ray-Hare test showed no significant effect of the interaction between water depth and date on seedling shoot density (shoots per pot) (Fig. 3A). In addition, there were no significant differences in seedling shoot density and height across different depths (Fig. 3; $p > 0.05$). Just one seedling was observed at 6 m in December 2014, but seedlings were observed at all depths in May 2015. The seedlings at 6 m had all died by August 31, 2015; however, there were still some surviving seedlings at shallower depths (Fig. 3A).

Light intensity changed significantly as water depth increased, and light versus depth distributions on May 21 and August 31, 2015, adhered well to Beer's Law ($R^2 \geq 0.945$; Fig. 4). Between 41.09 and 54.90% of surface irradiance was available at 1 m, and 12.78–18.75% was available at 3 m. However, there was <4% of surface irradiance at 6 m, a value lower than the minimum light requirement of seagrass (4–36% of surface irradiance; Ralph et al. 2007).

The ranges of water parameters at different depths (1, 3, and 6 m) are shown in Table 2. Salinity and pH varied slightly with water depth (Table 2). Range of DO was 4.42–11.35 mg l^{-1}. Variation in temperature with water depth was generally less than 1°C m^{-1}. The chlorophyll content was relatively low (1.55–2.70 μg l^{-1}) at all depths in May and June, but in August 2015, the chlorophyll content was much higher in shallow water than deep water.

3.2. 2015 suspended seed experiment

The Scheirer-Ray-Hare test showed no significant effect of the interaction between sediment type and seed burial depth on seed germination and seedling establishment ($p > 0.05$). However, there were significant effects of burial depth ($p < 0.05$), with higher percentages of seed germination and seedling establishment at shallower burial depths. No seedlings or

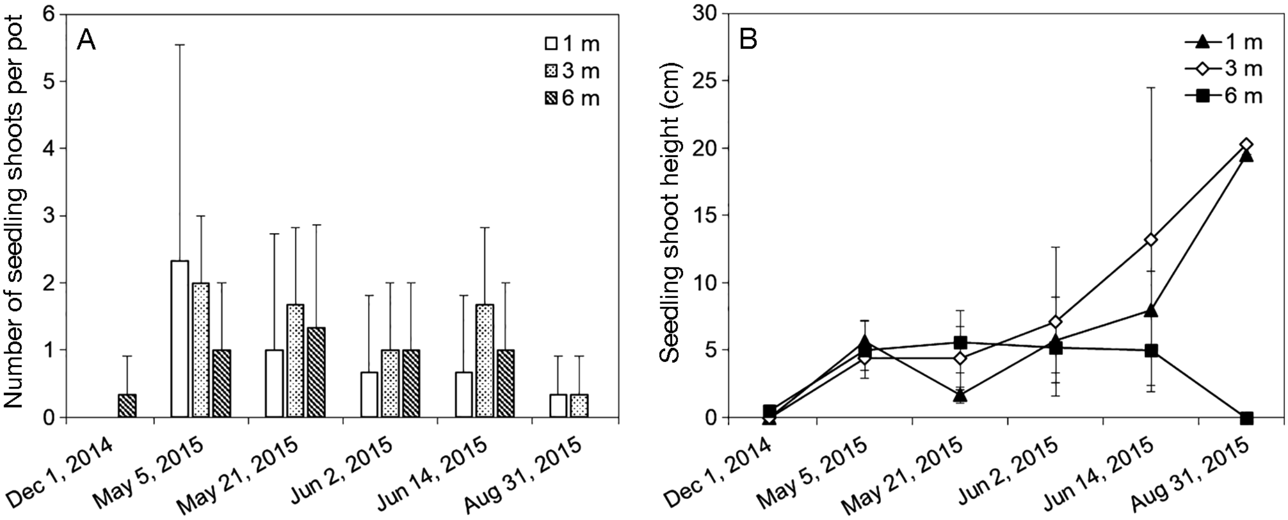

Fig. 3. Temporal changes in (A) shoot density and (B) shoot height of *Z. marina* seedlings along a gradient of water depth in Ailian Bay. Values shown are mean ± SD

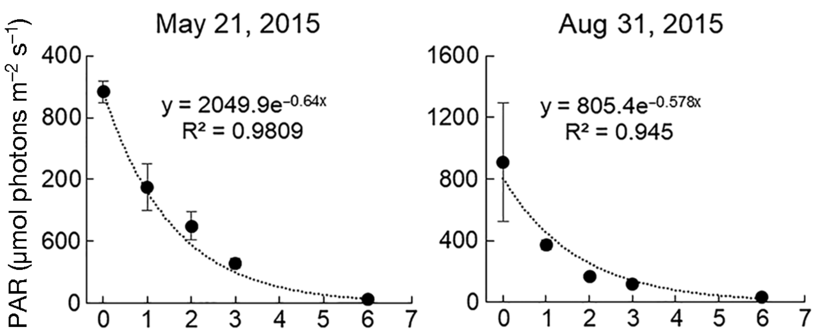

Fig. 4. Variation in light availability with water depth in Ailian Bay in 2015. The fitted line is an exponential curve representing the fit of Beer's law (see Eq. 1) to the data, adapted from Zhang et al. (2020). Values shown are mean ± SD

Table 2. Variation in water parameters with water depth (1, 3, and 6 m) in Ailian Bay in 2015; modified from Zhang et al. (2020)

Date	Depth (m)	Temperature (°C)	Salinity (ppt)	DO (mg l^{-1})	pH	Chlorophyll (μg l^{-1})
May 21, 2015	1	12.76	32.48	5.14	7.96	2.53
	3	11.44	32.62	5.61	7.96	2.70
	6	10.70	32.64	5.10	7.94	1.55
Jun 14, 2015	1	18.01	32.58	4.42	7.76	2.37
	3	15.80	32.82	4.71	7.82	1.80
	6	14.24	32.88	4.82	7.86	2.07
Aug 31, 2015	1	24.64	31.36	11.35	8.25	13.40
	3	23.77	31.57	8.47	8.10	7.02
	6	23.24	31.62	5.98	7.94	2.90

germinated seeds were found at burial depths of 10 cm, regardless of sediment type. When seeds were buried at 2 cm, there were no significant differences in seed germination and seedling establishment among sediment types ($p > 0.05$) on all observation dates except May 2016, when significant differences among sediment types were detected in germination ($p < 0.05$). However, when seeds were buried at 5 cm, there were significant differences in germination and establishment among sediment types ($p < 0.05$) on all observation dates except March 2016, when no significant differences among sediment types were detected in establishment. In general, there were higher percentages of seed germination and seedling establishment in sandy sediments. At burial depths of 2 and 5 cm, seeds cultured in 100% sand showed the highest percentage germination and establishment, with cumulative germination percentages of 21.33 ± 9.30% and 12.33 ± 4.51%, respectively (Fig. 5). Overall, seed germination and seedling establishment were mainly determined by seed burial depth, but seeds buried at 5 cm were affected by sediment type. In addition, seedling shoot height varied significantly with date ($p < 0.05$) but not seed burial depth ($p > 0.05$) (Fig. 6).

After 505 d of culture, there were no significant differences in seedling shoot density among sediment types at a burial depth of 2 cm, with density ranging from 37 to 60 shoots per pot. At a burial

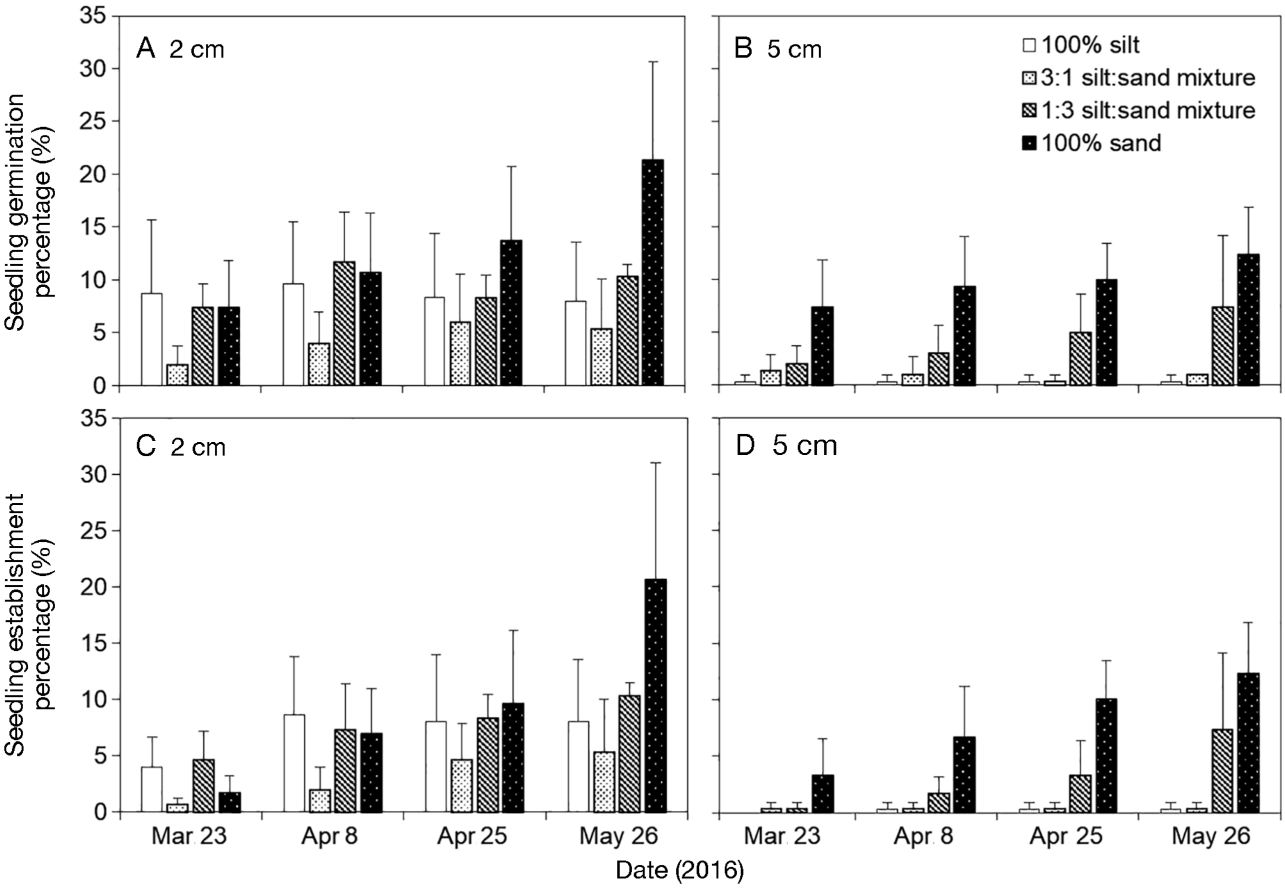

Fig. 5. Percent (A,B) seed germination and (C,D) seeding establishment of *Z. marina* under different combinations of sediment type and seed burial depth in Ailian Bay. Data for 10 cm burial depth are not shown as no seed germination or seedling establishment were observed at this depth. Values shown are mean ± SD

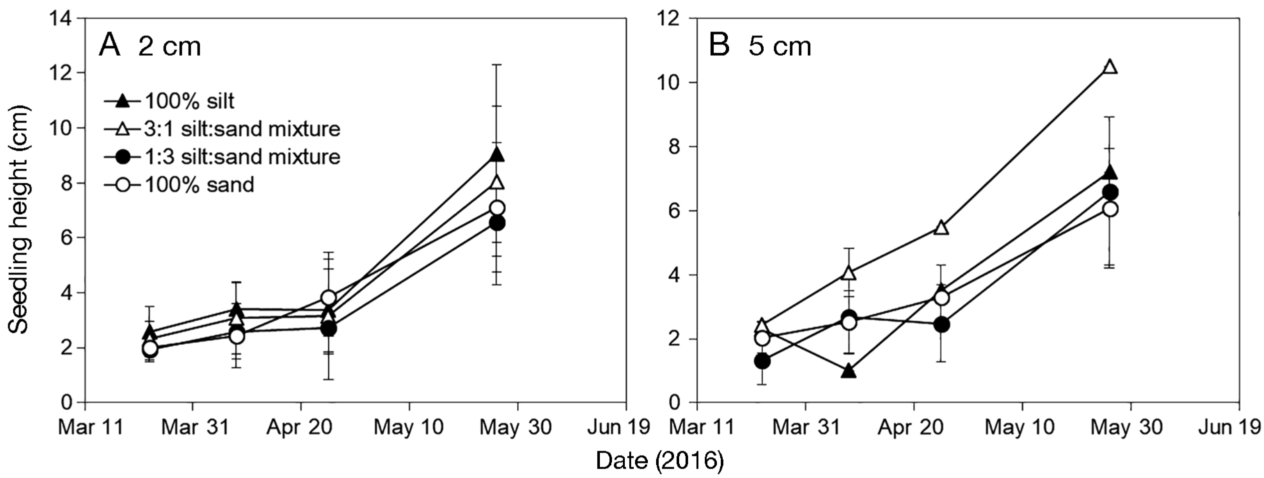

Fig. 6. Temporal changes in *Z. marina* seedling height under different combinations of sediment type and seed burial depth in Ailian Bay. Values shown are mean ± SD

depth of 5 cm, 90 ± 60 shoots were found in 1:3 silt:sand mixture, indicating that shoot density increased from 7 ± 7 shoots in May 2016 by a factor of about 13 times in less than 1 yr. No shoots were found in 100% silt or 3:1 silt:sand mixture at a burial depth of 5 cm (Table 3), which could be explained by low seed germination and seedling establishment (Fig. 5). Additionally, 37 ± 12 shoots

Table 3. Shoot density and height of *Z. marina* seedlings after 505 d of culture initiated in October 2015 in Ailian Bay. Values shown are mean ± SD

Parameters	Burial depth	100% silt (cm)	3:1 silt:sand mixture	1:3 silt:sand mixture	100% sand
No. seedling shoots per pot	2	52 ± 27	60 ± 15	51 ± 16	37 ± 12
	5	0	0	90 ± 60	33 ± 14
	10	0	0	0	0
Shoot height (cm)	2	19.51 ± 5.39	20.84 ± 4.83	21.00 ± 5.40	19.93 ± 5.09
	5	0	0	20.64 ± 7.85	20.60 ± 8.07
	10	0	0	0	0

were found in 100% sand at a burial depth of 5 cm. There were no significant differences in shoot height of all surviving seedlings across sediment types and burial depths, with a seedling height of 20 ± 6 cm (Table 3). Overall, although the effects of sediment type and burial depth are important for the initial seed germination/seedling establishment period, if the plants can establish, they may be able to perform equally well in all sediment types and at all burial depths.

Water temperature showed a seasonal pattern (Table 4). It was 6.2°C in March 2016 and increased to 15.6°C by May 2016. Salinity ranged from 28.3 to 33.9 in Ailian Bay during the period of the second experiment (Table 4).

4. DISCUSSION AND CONCLUSION

Our 2 *in situ* suspended experiments tracking seed germination and seedling establishment of *Zostera marina* revealed that seeds buried at shallow depths exhibited higher levels of germination and establishment regardless of sediment type, and no seedlings were found when seeds were buried at depth of 10 cm. Sediment type did not have a detectable effect on germination and establishment at a burial depth of 2 cm ($p > 0.05$), but sandy sediment clearly enhanced percent seed germination and seedling establishment at a burial depth of 5 cm. Our findings imply that sandy sediment and a burial depth of 2 cm would maximize successful seedling establishment in *Z. marina* seed-based restoration efforts. In addition, water depth had no significant effect on seed germination and seedling establishment, but can significantly affect seedling survival, and thus water depth of restored areas will determine success for seed-based restoration projects.

Seed burial can be favorable to sexual recruitment, and seed burial at the optimal depth is important for maximizing seedling establishment (Marion & Orth 2010, Blackburn & Orth 2013). However, burial below the optimal depth can negatively affect seedling emergence (Jørgensen et al. 2019). In the present study, deeply buried seeds (5 and 10 cm) exhibited lower levels of germination and seedling establishment than those buried at 2 cm, similar to the results of Jarvis & Moore (2015), who concluded that *Z. marina* seeds buried at 5 cm showed significantly lower emergence than seeds buried at 1 cm. There are 2 possible explanations for this pattern. Firstly, seeds may contain insufficient energy reserves to reach the sediment surface when buried deeply (Jarvis & Moore 2015). The axial hypocotyl of deeply buried seeds must elongate further before the cotyledon can reach the sediment surface. Deeply buried seeds need more energy to complete this process, and may run out of energy before the cotyledon reaches the sediment surface, directly negatively affecting the development of true leaves (Taylor 1957, Sugiura et al. 2009). Secondly, the mortality rate of seeds buried below the optimal depth increases with increasing burial depth (Jørgensen et al. 2019). Churchill (1992) found that deeper (>3.7 cm) seeds were mostly rotten, and Jørgensen et al. (2019) found that the major source of seed loss was mortality, with the fraction of dead seeds more than doubling from 2 to 8 cm depth. Deeper seeds may be subject to lethal

Table 4. Temporal changes in temperature and salinity in Ailian Bay from October 1, 2015 to February 17, 2017

Date	Temperature (°C)	Salinity (ppt)
Oct 1, 2015	24.2	31.6
Mar 23, 2016	6.2	28.3
Apr 8, 2016	11.6	28.3
Apr 25, 2016	13.1	32.7
May 26, 2016	15.6	33.7
Feb 17, 2017	3.1	33.9

effects of reduced metabolites (organically enriched sediments and low redox conditions), leading to seed mortality (Hootsmans et al. 1987, Tanner & Parham 2010, Jørgensen et al. 2019). While deeper seeds are unlikely to be washed away, they may not be able to reach the surface, leading to restoration failure; 2 cm is the optimal burial depth to maximize successful seedling establishment in *Z. marina* seed-based restoration efforts.

Several studies have reported greater germination of seeds in muddy sediments than in sandy sediments (Ostenfeld 1908, Tutin 1938, Short 1987, van Katwijk et al. 1997). However, in the present study, sediment type did not have a significant effect on *Z. marina* seed germination at a burial depth of 2 cm ($p > 0.05$). These results are similar to the findings of Jarvis & Moore (2015). Moreover, we found higher levels of seed germination in sandy sediments than in muddy sediments at a burial depth of 5 cm ($p < 0.05$).

The effects of sediment characteristics on seed germination are very complex, and sediment oxygen levels may be the most important factor influencing germination processes for some species in the field (Moore et al. 1993, Orth et al. 2000). Low dissolved oxygen conditions have been shown to facilitate seed germination over a shorter time period (Moore et al. 1993), which might explain reports of higher levels of seed germination in muddy sediments, which have lower dissolved oxygen content than sandy sediments, which generally have a deeper oxygenated layer (van Katwijk & Wijgergangs 2004). However, while seeds held in oxygenated conditions in the field germinated later than those held in anoxic conditions, both groups of seeds eventually reached similar levels of germination (Moore et al. 1993). These results indicate that dissolved oxygen may have no impact on eventual germination success in some circumstances, which could explain our results showing no effect of sediment type on maximum germination of seeds at a burial depth of 2 cm.

However, for deeply buried seeds (5 cm), sediment type did have significant influence on seed germination. As sandy sediments generally have a deeper oxygenated layer than muddy sediments (van Katwijk & Wijgergangs 2004), muddy sediments at 5 cm depth have lower dissolved oxygen levels (van Katwijk & Wijgergangs 2004). Lethal effects of low redox conditions have been described for *Z. marina* seeds (Hootsmans et al. 1987, Tanner & Parham 2010). Similarly, seeds buried at a depth of 10 cm did not successfully germinate or establish as seedlings, possibly due to hypoxic conditions. Overall, more attention should be paid to seed burial depth in muddy sediments than in sandy sediments for future *Z. marina* seed-based restoration efforts.

There may be no consistent effects of water depth on seed germination and seedling establishment (Moore et al. 1993). In our study, percent seed germination and seedling establishment did not vary significantly with water depth, which agrees with others' findings (Moore et al. 1993). However, the relatively low replication in our first experiment (only 10 seeds per pot), leading to few seedlings, may be one possible reason we found no significant effects. Although water depth had little effect on seed germination and seedling establishment in our study, the survival of seedlings after establishment varied significantly with water depth, similar to the findings of Bintz & Nixon (2001), who found that reduction of light decreased survival rate of *Z. marina* seedlings. In our study, there was <4% of surface irradiance at 6 m, below the minimum light requirement of seagrass (4–36% of surface irradiance; Ralph et al. 2007), resulting in the death of all seedlings at 6 m by August 31, 2015.

It may be important to continue monitoring for long periods (>1 yr) in seagrass restoration efforts. In our study, although the effects of different sediment types/burial depths were important for the initial seed germination/seedling establishment period, it seems these effects do not play a decisive role in plant growth after 1 yr, implying that restored areas need to be monitored for a longer period to determine if seeding was successful. In our study, the maximum rate of seedling shoot density increase is about 13 times in less than 1 yr, which could be explained by rapid clonal growth of seedlings rather than germination of seeds that did not germinate the previous year. The seed bank of Swan Lake lasted less than 1 yr (Xu et al. 2018) and even if reserve seeds germinated in the second year, new seedlings would not reach 20 cm in height by February. In a previous study, we found that adult shoots of eelgrass transplanted in pots can increase about 10 times, from 10 to about 100 shoots per pot, in less than 1 yr (Xu et al. 2020b). Many seagrass restoration projects are monitored for less than a year (van Katwijk et al. 2016), but our results show that it may be important to continue monitoring for longer periods, in view of rapid clone growth after successful seedling establishment.

In the current study, the effects of water depth, sediment type, and burial depth on seed germination and seedling establishment of *Z. marina* were tested through 2 *in situ* suspended culture experiments. Our findings provide a better understanding of the potential resilience of this species and a theoretical basis for seed-based restoration practices in this location.

Acknowledgements. This research was supported by the National Key R&D Program of China (2019YFD0901300), the National Science and Technology Basic Work Program (2015FY110600), the Key Research Project of Frontier Sciences of CAS (QYZDB-SSW-DQC041-1), the International Partners Program of the Chinese Academy of Sciences (133137KYSB20180069), and the Taishan Scholars Program (Distinguished Taishan Scholars).

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Editorial responsibility: Morten Pedersen, Roskilde, Denmark
Reviewed by: 3 anonymous referees

Submitted: January 4, 2021
Accepted: September 3, 2021
Proofs received from author(s): November 7, 2021