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Reproductive properties of *Zostera marina* and effects of sediment type and burial depth on seed germination and seedling establishment

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ABSTRACT

The dynamics of reproductive properties of *Zostera marina* in the Shandong Peninsula was recorded based on field investigations. Influences of sediment type and burial depth on seed germination and seedling establishment were studied in controlled laboratory conditions. In the field, the reproductive stage occurred from late March to late August. The reproductive properties showed obvious seasonal variation and had a significant impact on the cumulative seed production. Seed release began in early June, and the cumulative seed production was 10451 ± 371.0 seeds m^{-2}, while the maximum production in a single week was 2907 ± 258.1 seeds m^{-2}. The seed germination experiment showed significant effects of seed burial depth as well as the interaction between sediment type and burial depth on both seed germination and seedling establishment percentages ($p < 0.001$). Seeds on the sediment surface or buried to 1 cm could maintain high germination percentages ($76 \pm 11.5\%$ and $90 \pm 1.5\%$, respectively) regardless of sediment type. With increasing burial depth, germination percentages decreased gradually and were lower than 40% in all sediment types. Only seeds in sand:silt mixtures 2:1 could germinate when the burial depth increased to 5 cm; the earliest germination appeared in the fifth week and the cumulative germination percentage was lower than 3%. Seedling establishment showed a similar trend to seed germination; seeds buried in shallow depths showed a higher seedling establishment percentage, and the maximum value was recorded in seeds that were buried to 1 cm in sand:silt mixtures 2:1 (30.67%).

1. Introduction

Seagrasses form productive and species-rich habitats in coastal areas throughout the world (Personnic et al., 2014) and contribute to human well-being by stabilizing sediments (Orth et al., 2006), improving water quality, enhancing fishery resources (Duffy, 2006; Koch et al., 2006), promoting nutrient cycling (McGlathery et al., 2007) and sequestering carbon (Fourqurean et al., 2012; Duarte et al., 2013). Unfortunately, seagrass populations have been declining globally in recent years as a result of human perturbations (Waycott et al., 2009), and nearly 25% of all seagrass species are considered threatened or near threatened (Short et al., 2011). Increasing concerns are focusing on global climate change, which is suggested to accelerate the loss of seagrass meadows (Waycott et al., 2009; Valle et al., 2014).

Seagrasses mainly take advantage of asexual growth to maintain extant meadows (Uhrin et al., 2011). The natural recovery of reduced seagrass populations is very slow and insufficient for recolonization of large areas (Walker et al., 2006; Uhrin et al., 2011). Naturally recruited meadows are less diverse and exhibit signs of genetic drift (Reynolds et al., 2013). In recent years, various transplantation methods using shoots or seeds have been developed in restoration practices, with varying degrees of success (Short et al., 2002; Park and Lee, 2010). However, adult seagrass transplantation is often criticized, not only because of damage to donor meadows (Balestri et al., 2011) but also because of the lack of consideration for the potential roles of sexual reproduction and seed production in the establishment of new seagrass meadows and the maintenance of population genetic structures (Harwell and Orth, 2002; Jarvis and Moore, 2010). Furthermore, transplanted seagrass often exhibits high mortality during the initial period because of poor adaptation to new environments (Li et al., 2013). Delivering seagrass seeds to the seafloor could successfully avoid the shortcomings of adult transplantation and is suggested to be a beneficial restoration practice (Reynolds et al., 2012; Terrados et al., 2013). The practices

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of seed collection and processing, short-term storage and sowing of seeds from only a single species are now widely accepted and implemented (Marion and Orth, 2010; Tanner and Parham, 2010). Although using seeds can be much more efficient than transplanting adult plants (Orth et al., 2006), the low percentage of germination in the field, typically 1–10% of seeds distributed, still represents a major bottleneck for seed-based restoration projects (Marion and Orth, 2010).

As a seagrass widely distributed in the Northern Hemisphere, *Zostera marina* L. (eelgrass) has been a key species in seagrass studies and marine ecosystem restoration (Jarvis et al., 2014; Olesen et al., 2015). The development of effective techniques for seed collection and storage has provided a basis for the ecological restoration of *Z. marina* populations (Dooley et al., 2013; Pan et al., 2014). Different burial thresholds for successful seed germination have been proposed, from 1 to 3.7 cm (Churchill, 1992; Moore et al., 1993; Granger et al., 2000) to 8–9 cm (Kawasaki, 1993; Morita et al., 2007). There are also contrasting evidences about the effect of burial depth on seed germination (Marion and Orth, 2011; Jarvis and Moore, 2015). Tanner and Parham (2010) found greater germination of seeds in fine sediments with high organic matter content compared to coarse sediments. Hypoxia in muddy sediment has been documented to increase seed germination of *Z. marina* and *Zostera capricorni* (Moore et al., 1993; Brenchley and Probert, 1998). Actually, the combined effects of sediment type and seed burial depth are complex and may be related to dissolved oxygen and organic matter content as well as to other factors. Furthermore, although seedling establishment is a crucial stage for seagrass, there are few studies focused on the influences of environmental conditions on this issue. So we hypothesized that sediment type and burial depth have significant influence on both eelgrass seed germination and seedling establishment in the Shandong Peninsula, and sediment features could be a major concern for site selection in seagrass restoration. Different sediment types were prepared by mixing in different proportions natural sediment collected in sandy and muddy beaches. The primary aims of this research were to (1) describe the reproductive properties of natural *Z. marina* populations in the Shandong Peninsula based on a continuous field investigation and (2) explore the effects of sediment type and burial depth on eelgrass seed germination and seedling establishment.

2. Materials and methods

2.1. Field monitoring of reproductive properties

The reproductive properties of natural *Z. marina* populations were investigated every two weeks from April to August in 2013 in Lidao Bay (122.586°E, 37.246°N), a shallow (1.5–3.5 m depth) tidal basin in the northeast of Shandong Peninsula (Shandong Province, North China). The floor of Lidao Bay is generally dominated by fine-grained material, with mud and sandy mud covering approximately 40% of the area. Local historical data show that the annual average air temperature is 12 °C, and the highest and lowest air temperatures are attained in August and February, respectively. In Lidao Bay, *Zostera marina* is patchy in distribution, the average distribution density was 1650 shoots m^{-2} and the average biomass was 808.995 gDW m^{-2}. Monitored parameters were vegetative and reproductive shoot density, spathe numbers per reproductive shoot and seed production. The first three parameters were investigated using a 30 × 30 cm quadrat with three replicates, the distance between quadrats was 100 m, and the percentage of reproductive shoots was calculated. During the reproductive stage, all shoots bearing spathes and seeds in each quadrat (n = 3) were collected in sieve bags with a mesh diameter of 0.5 mm, which were then tied to a floating raft. All seeds released from mesh bags were collected and counted weekly to calculate seed production (seed m^{-2} $week^{-1}$), while the cumulative seed production was calculated as the total seed yield during the corresponding investigation period.

2.2. Germination experiments under controlled lab conditions

To study the effects of sediment type and burial depth on seed germination and seedling establishment, a germination experiment was designed and conducted as a factorial experiment in which two factors, sediment type and burial depth, were assessed. Sediment types tested were 100% sand, sand:silt mixtures 1:1 (v/v), sand:silt mixtures 2:1 (v/v), sand:silt mixtures 1:2 (v/v) and 100% silt, and seed burial depths were set at 0, 1, 3 and 5 cm in each sediment type. Sediment used in the experiment was excavated from sandy and muddy coastal areas in Lidao Bay. Sand (sediment particle size between 63 and 250 μm) and silt (sediment particle size <63 μm) were obtained using a standard series of sieves and mixed in different proportions after removal of shell clasts and plant fragments. Sediment collected from the *Zostera marina* meadows without any treatment (but screened to remove shell clasts and plant fragments and mixed) and natural seawater (PSU ∼31.0) were used as controls. Seeds used in this experiment were those released by the reproductive shoots collected in early July in the mesh bags and transported back to lab in Mid-August. For each treatment, three replicates of 100 seeds were cultured in 2000 mL beakers filled with a 5 cm sediment layer and a ∼15 cm water layer; the water used was sterile seawater collected from Lidao Bay (PSU ∼31.0). To ensure that all seeds were buried at the same depth and were easily observed, seeds were placed at the bottom of the beakers, which meant that burial depth was equivalent to sediment thickness. For the 0 cm burial depth, seeds were placed on the surface of the corresponding sediment types; the sediment layer thickness was ∼5 cm with a ∼15 cm water layer. Seeds were germinated in an illuminated incubator, the photoperiod used in all treatments was 12/12 h, and temperature and light intensity were set at 14 °C and 150 μmol m^{-2} s^{-1} PAR, respectively. Seeds imbibited and with visible cotyledons were considered to have germinated. Seedlings that reached sediment surface with new leaf and were visible in the beakers were considered to be established. The number of germinated seeds and emerged seedlings with new leaves were recorded every day, and the duration of the experiment was seven weeks. The percentages of seed germination and seedling establishment were calculated as the number of germinated seeds and emerged seedlings out of total seeds, respectively.

2.3. Statistical analysis

Two-way ANOVA was performed with the data obtained in the final sampling time (week 7) to examine the effects of sediment type and burial depth on seed germination and seedling establishment. The Student-Newman-Keuls post-hoc multiple comparison test and Duncan post-hoc test were used if ANOVA indicated a significant effect. Prior to analysis, data were initially examined for normality using the Shapiro-Wilk test and for homogeneity of variances using Levene's test. The results were presented as the mean ± SE and significance level was set at $\alpha = 0.05$. Data were analyzed using IBM SPSS Statistics 22.0 (SPSS Inc., U.S.).

3. Results

3.1. The processes of flowering and seed production of Z. marina

The results of field investigations showed that the reproductive stage of natural *Z. marina* populations occurred from late March to late August in the Shandong peninsula with dynamic changes in a