A unique meadow of the marine angiosperm *Zostera japonica*, covering a large area in the turbid intertidal Yellow River Delta, China

Xiaomei Zhang\textsuperscript{a,b,c,1}, Haiying Lin\textsuperscript{d,1}, Xiaoyue Song\textsuperscript{a,b,c,1}, Shaochun Xu\textsuperscript{a,b,c,1}, Shidong Yue\textsuperscript{a,b,c}, Ruiting Gu\textsuperscript{a,b,c}, Shuai Xu\textsuperscript{a,b,c}, Shuyu Zhu\textsuperscript{e}, Yajie Zhao\textsuperscript{e}, Shuyan Zhang\textsuperscript{e}, Guangxuan Han\textsuperscript{f}, Andong Wang\textsuperscript{e}, Tao Sun\textsuperscript{d}, Yi Zhou\textsuperscript{a,b,g,}\textsuperscript{⁎}

\textsuperscript{a} CAS Key Laboratory of Marine Ecology and Environmental Sciences, Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China
\textsuperscript{b} Laboratory for Marine Ecology and Environmental Science, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266071, China
\textsuperscript{c} University of Chinese Academy of Sciences, Beijing 100049, China
\textsuperscript{d} State Key Laboratory of Water Environment Simulation, School of Environment, Beijing Normal University, Beijing 100875, China
\textsuperscript{e} Yellow River Delta National Nature Reserve Management Bureau, Dongying 257200, China
\textsuperscript{f} Key Laboratory of Coastal Zone Environmental Processes and Ecological Remediation, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai, Shandong 264003, China
\textsuperscript{g} Center for Ocean Mega-Science, Chinese Academy of Sciences, Qingdao 266071, China

HIGHLIGHTS

- A large *Z. japonica* meadow was discovered in the turbid intertidal Yellow River Delta.
- The meadow showed highest coverage and biomass in August.
- The seed bank contributed greatly to population recruitment.
- A high genetic exchange occurred between the two sides of the estuary.
- This meadow is in good condition, which is attributed to the reserve establishment.

GRAPHICAL ABSTRACT

ABSTRACT

Marine submerged aquatic angiosperms (seagrasses) are declining globally. The species *Zostera japonica* Asch. & Graebn. is endangered in its native range in Asia, but has been successfully introduced to North America. A large area (1031.8 ha) of *Z. japonica* meadow has recently been discovered in the intertidal zone of Yellow River Delta, China. This seagrass occurs along both sides of the river mouth, forming dense meadows in turbid water conditions. Seasonal investigations over two years were conducted to examine the distribution, biomass, seed reproduction, seed bank, and population recruitment of the seagrass meadows at three sites in the intertidal zone. The meadows generally showed relatively high coverage, biomass, reproductive effort, and seed production in August. The seed bank was found to be large and contributed to population recruitment. There were significant inter-annual variations overall, and at individual sites. These variations are likely due to winter temperatures, which determine the abundance of overwintering shoots and seedling success. Differences in microtopography may also play a role in producing variations in seedling success between sites. Microsatellite analysis...
1. Introduction

Seagrasses are a group of aquatic angiosperms which are adapted to living fully submersed in the sea (Green and Short, 2003). Seagrasses occur in the intertidal and subtidal zones along temperate and tropical coastlines (Short et al., 2007). Despite its global distribution, this group has a relatively low diversity, with 72 species known worldwide, compared with approximately 250,000 terrestrial angiosperms (Orth et al., 2006). Seagrasses form the foundation of one of the most important coastal marine ecosystems. They can influence physical, chemical, and biological conditions in coastal waters, and act as ecological engineers (sensu Wright and Jones, 2006). Seagrass beds act as nursery grounds for juvenile and larval stages of many commercially important fishery species (Watson et al., 1993; Beck et al., 2001; Heck et al., 2003; Liu et al., 2013; Taylor et al., 2017; Unsworth et al., 2018a). Seagrasses also produce large quantities of organic carbon and seagrass meadows represent a significant carbon sink (Fourqurean et al., 2012; Macreadie et al., 2014; Thorhaug et al., 2017).

Seagrasses are declining globally (Green and Short, 2003; Orth et al., 2006; Waycott et al., 2009; Unsworth et al., 2018b) due to anthropogenic pressures (Short et al., 2011; Unsworth et al., 2017). A review of 215 studies showed that seagrasses have declined at a rate of 110 km² yr⁻¹ since 1980. This rate of decline is accelerating, and 29% of the known extent of seagrass has disappeared since 1879 (Waycott et al., 2009). This decline is believed to be far greater in China (Zhou et al., 2014), although a precise assessment is not possible due to a lack of historical data. Based on information from hundreds of seagrass specimens (Biological Museum, Chinese Academy of Sciences, Qingdao, China) and published studies (e.g., Yang, 1979; den Hartog and Yang, 1990), there appear to be 22 seagrass species distributed along the coasts of nine provinces and regions of China. A large number of seagrass meadows have contracted sharply or disappeared within the last 30 years, according to the national seagrass resource survey, which was initiated in 2015.

Zostera japonica Asch. & Graebn., the most widely distributed seagrass species in China, has undergone a severe decline, and some populations have almost, if not entirely, disappeared (Lin et al., 2016, 2018a, 2018b). This species is also declining and recognized as endangered species in other parts of Asia, including Japan (Abe et al., 2009; Hodoki et al., 2013) and Korea (Lee et al., 2004). Z. japonica is native to east Asia and is distributed from the temperate area of Sakhalin, Russia to subtropical southern Vietnam (Green and Short, 2003). This species has been introduced to the coastlines of British Columbia (Canada), Washington, Oregon, and North California (USA), where it has become established (Baldwin and Lovvorn, 1994; Shafer et al., 2014). The decline of Z. japonica in its native range has been attributed to anthropogenic disturbances such as coastal development, river channel improvements, aquaculture and harvesting activities (Lee, 1997; Lee et al., 2004; Huang et al., 2006; Abe et al., 2009).

In order to provide comprehensive baseline data on the seagrass resource in China, which will aid conservation, restoration and management, a national project called “A survey of seagrass populations and habitats on Chinese coasts” was initiated in 2015. During the course of this project, an unusually large area of Z. japonica, covering over 1000 ha, was discovered in the Yellow River Delta shallow waters. The water is very turbid in this location, and this area of seagrass had not previously been recorded. The Yellow River Delta National Nature Reserve covers an area of 1530.0 km². The reserve was established in 1992 to protect the wetland ecosystem and rare and endangered bird species present in the Yellow River Delta. This reserve covers 827.0 km² of terrestrial habitat, 382.5 km² of the intertidal zone and 320.5 km² of shallow water. Z. japonica was found widely distributed in the intertidal zone.

Given the lack of information on this newly discovered seagrass population, the aims of the current study were to conduct seasonal ecological investigations over two years on Z. japonica in the Yellow River Delta shallow waters, in order to describe its distribution, examine the seasonal dynamics of population recruitment, and analyze the effect of different environmental factors on population dynamics. The data collected during this study will fill the knowledge gap of seagrass meadows in this unique geographic environment and inform strategies for future conservation and management.

2. Materials and methods

2.1. Study sites

The Yellow River Delta, Dongying city, Shandong province, adjoins Laizhou Bay to the east and Bohai Sea to the north (Fig. 1). The Yellow River is the second longest river in China and carries an average of 1.0 billion tons of sediment to the sea annually (Hu et al., 1998). The Yellow River Delta is weakly tidal, with a lower tidal range of <1 m (Wang et al., 2001). There are multiple tidal patterns within the Yellow River Delta. Most of the delta experiences irregular semi-diurnal tides, but irregular diurnal tides occur in a small area of the delta (Hu et al., 1998). The highest average concentrations of the nutrients NO₃⁻, NO₂⁻, NH₄⁺, DON, DIP, and DOP in the adjacent surface waters of the Yellow River Delta during April to September were 3.12 ± 1.37 μM (July), 34.7 ± 10.2 μM (July), 4.22 ± 1.54 μM (April), 65.7 ± 33.1 μM (July), 0.12 ± 0.11 μM (April), and 0.40 ± 0.08 μM (July), respectively (Wang et al., 2017). The largest newly created wetland ecosystem in coastal China has developed in the Yellow River Delta. In the Yellow River Delta National Nature Reserve, average vegetation cover is 55.1%, with Sueda salsa L. Pall. (1803), Tamarix chinensis Lour., and Apocynum venetum L. commonly occurring. Fishery resources in the Yellow River Delta shallow waters including seagrass meadows are much abundant, and Z. japonica has been observed to be the food source for wetland herbivorous birds, such as the whooper swan Cygnus Cygnus (1758) and the tundra swan Cygnus columbianus Ord. (1815) (Zhang et al., unpublished data).

Three study sites (DY-1, DY-2, and DY-3; Fig. 1), located on both sides of the river mouth were selected. These choices were chosen in order to compare seagrass meadows on both sides of the river mouth and also due to limited road access to the meadows. DY-1 was located at 37°48′9″N, 119°8′49″E, DY-2 was located at 37°51′7″N, 119°5′47″E, and DY-3 was located at 37°43′45″, 119°14′29″E.

Three parallel transect lines for sampling were marked at DY-1, with a 100 m interval between each transect; only one single transect line was marked at both DY-2 and DY-3 (Fig. 2). The transects were perpendicular to the coastline at DY-1 and DY-2 and parallel to the coastline at DY-3. Based on the distance between the upper and lower limits of the meadows, 7–10 sampling points at an interval of 50 m, were marked along the transect lines in three study sites.
2.2. Environmental parameters

The air temperature in the intertidal zone (37°47′20″N, 119°10′23″) was measured with a HMP45C temperature logger (Vaisala, Helsinki, Finland), which is part of the micro-meteorological monitoring system built by the Yellow River Delta ecology coastal wetland research station, Chinese Academy of Sciences. The light intensity at the canopy height was measured using an ECO-PAR sensor deployed on the top of the canopy within the seagrass bed at DY1. Instantaneous photosynthetic photon flux densities (PPFD; mol photons m⁻² s⁻¹) were measured every 10 min and the daily PPFD (mol photons m⁻² d⁻¹) calculated as the sum of the quantum flux within a 24-h period. Salinity was measured during each survey at water surface using a portable conductivity, salinity instrument (YSI Pro30, USA). A multiparameter water quality monitor (YSI 6600, USA) was placed in shallow water adjacent to DY-1 during August 17th–18th 2015 and December 29th–30th 2016. Parameters such as temperature (°C), salinity, turbidity (NTU), dissolved oxygen (DO; mg L⁻¹) and pH were measured every 10 min. The content

![Fig. 1. Yellow River Delta. Left panel: study sites (DY-1, DY-2, and DY-3) and distribution (green areas) of Zostera japonica. Right panel: geometry of the sampling transects at the three study sites.](image)

![Fig. 2. Daily mean air temperature, light intensity, salinity and sediment grain size measured in this study. (A) Daily mean air temperatures in the intertidal zone in the Yellow River Delta. (B) Light intensity on the top of the canopy of Z. japonica in DY-2. (C) Surface water salinity at the three study sites (DY-1, DY-2, and DY-3). (D) Sediment grain size at the three study sites (DY-1, DY-2, and DY-3).](image)
(mg L⁻¹) of suspended particle matter (SPM) in different months was measured from samples of seawater (3 × 500 ml) randomly collected during the investigation. The water was filtered through glass microfiber filters (25 mm diameter). Three sediment cores (diameter 10.6 cm, height 12 cm) were randomly collected in the center of the meadow at each of the three study sites in June 2016. The cores were used to determine the grain size distribution within the sediment, based on laser diffraction analysis, sieve analysis, and comprehensive analysis.

2.3. Meadow area and coverage

In August 2015, during peak biomass, the extent of *Z. japonica* meadows was examined. GPS was used to record accessible boundary points by walking during low tide or from boat during mid to high tide. A drone was used to assist field observations and confirm meadow boundaries which were inaccessible. Meadow extent was mapped using GIS software based on available geographic coordinates, field observations and video.

Percentage cover of seagrass meadows was measured based on three 0.25 m² quadrats at each sampling point along one transect line at each study sites. At DY-1 the middle transect was chosen for cover estimation. Seagrass cover is represented by the area covered by seagrass as a proportion of the total quadrat area.

2.4. Shoot height, density, biomass, and seed production

Seasonal investigations were conducted once every 2–3 months at three study sites from May 2015 to June 2017. In order to understand the seedling recruitment process, investigations were conducted monthly from March to June 2016 and 2017. At the three transects in DY-1, two sediment cores (d = 10.6 cm, h = 12 cm) containing *Z. japonica* plants were haphazardly collected within 5 m of each sampling point along each transect. At the single transect in DY-2 and DY-3, two to three cores were taken at each site along the transect.

The sediment cores were carefully sieved and washed in the field using aluminum sifters (aperture = 0.07 mm). The *Z. japonica* plants in each core were taken back to the laboratory for processing. Plant material was washed using tap water and divided into above-ground (shoots: sheath and leaves) and below-ground (rhizomes and roots) parts. The numbers of vegetative and flowering shoots were counted and shoot height (cm) was measured. Any excess tap water was dried off the plant material and the wet weight (g m⁻²) was measured. During each survey, nine to twelve cores were randomly selected and subsampled for above- and below-ground plant material. These were dried to a constant weight at 60 °C, and the ratio of wet to dry weight was calculated for the above and below ground biomass. The aboveground, belowground, and total wet weight of other samples were converted into dry weight based on the coefficients calculated.

Seeds were first observed in August, and thus seed production of flowering shoots was investigated in August 2015 and 2016. The numbers of spathes per flowering shoot, pollinated spathes per flowering shoot, and seeds per spathe were counted. The proportion of pollinated spathes to total spathes was calculated. Seed production per flowering shoot was calculated by multiplying the number of seeds per spathe by the number of spathes per flowering shoot. The seed production per unit area (seeds m⁻²) was determined by multiplying the number of seeds per flowering shoot by flowering shoot density.

2.5. Sediment seed bank

Investigations of the sediment seed bank were conducted in the autumn, winter and spring from 2015 to 2017. Sediment cores containing seeds were sampled and sieved following the same methods as used for investigations of the biomass of *Z. japonica* plants. Larger plant material and shells were picked out, and the remaining mixture of seeds, smaller sand, shell and plant debris was taken back to the laboratory for processing. The mixture of seeds and other materials was then sieved again using tap water and placed in a flat layer in a white tray. Seeds and seed coats were picked out using a tweezers, and counted. A shallow layer of water was often kept in the tray to facilitate the identification of seeds from other material.

2.6. Population recruitment

Sexual reproduction via seeds and clonal growth has been observed for *Z. japonica* populations in temperate areas (Harrison, 1979, 1982; Henderson and Hacker, 2015). Investigations on overwintering shoots and seedlings were conducted in the spring (March to May) of 2016 and 2017. Sediment cores with overwintering shoots or seedlings inside were collected following the same methods used when investigating the biomass of *Z. japonica* plants; however the sediment cores were sieved more carefully to minimize the damage to seedlings. The number of overwintering shoots and seedling shoots in each sample were counted, allowing the proportion of seedling shoots from total shoots to be calculated.

2.7. Genetic diversity and gene flow

Ten microsatellite loci (Zj008, 011, 018, 025, 026, 028, 029, 030, 036, 042 (Zhang et al., 2015b)) were used to assess the genetic diversity and gene exchange of *Z. japonica* from both sides of the Yellow River mouth. In May 2015, 32 individuals of *Z. japonica* 10–20 m apart, were randomly collected in each of the three study sites. Shoots from the same rhizome were genetically identical and regarded as one individual. The fresh sheath and leaves were cleaned with deionized water and stored at −80 °C. The DNA extraction and PCR amplification procedures followed are described by Zhang et al. (2015b).

2.8. Data analysis

Results are presented as mean ± SD. Seasonal differences in biological and environmental variables were tested using repeated analysis of variance (ANOVA). Differences in biological and environmental variables between locations were analyzed using paired t-tests. One-way ANOVA, paired t-tests, and Duncan’s multiple comparisons were conducted using SPSS 20.0. Before the analyses, the data were examined to confirm the homogeneity of variance (Levene’s test). Differences were considered significant at p < 0.05.

Measures of genetic diversity including observed heterozygosity, expected heterozygosity, polymorphic information content, and mean number of alleles per locus were calculated using the Excel Microsatellite Toolkit (Park, 2001). Pairwise FST estimates were calculated to explore patterns of differentiation among each pair of sampled sites. Pairwise FST values were calculated using Arlequin 3.5 and the significance of each pairwise FST value was assessed using 10,000 bootstrap permutations (Excoffier and Lischer, 2010). The significance threshold (α = 0.05) of pairwise FST values was adjusted using a modified false discovery rate (FDR) correction for multiple comparisons (Benjamini and Yekutieli, 2001). Recent migration rates among populations were estimated using the Bayesian method implemented in BAYESASS 3.0.4 (Wilson and Rannala, 2003).

3. Results

3.1. Environmental parameters

There was no significant difference (p > 0.05) in annual mean temperature between 2015 (14.00 ± 10.20 °C) and 2016 (13.42 ± 10.40 °C) (Fig. 2A). However, the winter of 2015–2016 was much colder than that of 2016–2017. In January 2016, the temperature dropped dramatically to −12.57 °C, producing a monthly average of −5.29 ± 2.67
°C, which was significantly lower than that of 2017 \((−0.64 ± 2.44 °C, p < 0.01)\). The mean temperature in February 2016 was also lower than that in February 2017 \((−0.35 ± 2.88 °C vs. 1.32 ± 2.84 °C, p < 0.05)\). The daily PPFD were relatively higher in spring and early summer than those in autumn and winter (Fig. 2B). The salinity at the three sites did not show a clear seasonal pattern. Salinity levels ranged from 19.8 to 35.3, with an averaged salinity of approximately 28 (Fig. 2C).

The general water column characteristics are showed in Tables 1 and 2. The turbidity (NTU) was higher in August than in December (Table 1). The concentrations of SPM at DY-2, which was the farthest site from the river mouth, were much lower than the other two sites. The concentrations of SPM at DY-1 and DY-3 (Table 2) exhibited higher values in summer and autumn (May to November) with an average of 163.19 ± 29.36 mg L−1 and lower values in winter and spring (December to April) with an average of 63.02 ± 78.02 mg L−1.

There was no significant difference (\(p > 0.05\)) in sediment grain size between the three study sites (Fig. 2D). The content of clay and silt in sediment collected in June were relatively high, ranging from 30.09 ± 1.14% (DY-2) - 38.23 ± 4.72% (DY-1) and 27.85 ± 2.20% (DY-1) - 34.12 ± 0.31% (DY-3), respectively. The proportions of sand were lower, ranging from 13.02 ± 3.71% (DY-3) - 20.12 ± 11.44% (DY-2). The largest particles in the sediment were shells of Potamocorbula laevis Hinds. (1843), although living P. laevis were scarcely seen in the seagrass meadows.

### 3.2. Extent and coverage of Z. japonica

The extent of the \(Z. \) japonica meadows was greatest in August (Fig. 1). Meadows in DY-1 and DY-2 were found to be connected and continuously extending towards the river mouth. In DY-3, \(Z. \) japonica was also recorded to extend towards the river mouth. The distribution of \(Z. \) japonica on both sides of the mouth of the Yellow River was estimated to be 1031.8 ha, with 563.2 ha on the north side and 468.6 ha on the south side.

The peak growing season for \(Z. \) japonica in the intertidal Yellow River Delta was the summer. The meadows became denser in July to August, with almost 100% cover recorded at the three study sites (Appendix 1A). At sites near the river mouth, the seagrass became sparse (Appendix 1B), and was completely absent in areas of low salinity. The majority of shoots decayed in winter (Appendix 1C).

The wide mud flat of the Yellow River Delta displays distinct vegetation zones, producing a unique wetland landscape extending from sea to land (Appendix 2). \(Z. \) japonica meadows occur in the sea, with Spartina alterniflora Loisel, S. salsa, T. chinensis, and reed marsh occurring as you move landward.

### 3.3. Temporal changes of \(Z. \) japonica biomass in the meadow centers

There were significant seasonal changes in shoot height, shoot density and biomass at all three study sites (\(p < 0.01\)) (Fig. 3). Growth increased in June and July, peaked in August and had dramatically declined by October. Maximum shoot height, shoot density and biomass were recorded in DY-3 with values of 34.3 ± 9.2 cm, 58.0 cm in DY-3, 52.7 cm in DY-1, and 42.0 cm in DY-2. Total density increased from June to August in DY-2 and DY-3, but decreased in DY-1 over this time period. The flowering shoots were not uniformly distributed along the transects. Total biomass increased two to three times from June to August, mainly attributed to the increase in aboveground biomass.

### 3.4. Spatial changes of \(Z. \) japonica biomass along the transect lines

The shoot height, density and biomass changed along the transects following a seasonal trend (Appendices 3, 4, 5). From June to August, the seagrass rapidly propagated through clonal growth and shoot heights doubled. The observed maximum shoot height in August was 58.0 cm in DY-3, 52.7 cm in DY-1, and 42.0 cm in DY-2. Total density increased from June to August in DY-2 and DY-3, but decreased in DY-1 over this time period. The flowering shoots were not uniformly distributed along the transects. Total biomass increased two to three times from June to August, mainly attributed to the increase in aboveground biomass.

### 3.5. Seed production

There were significant inter-annual variations in seed production at each of the three study sites (Appendix 6). The number of spathes per flowering shoot, number of pollinated spathes per flowering shoot, and number of seeds per spathe at DY-2 were significantly higher in August 2015 than 2016 (\(p < 0.01\), \(p < 0.05\), respectively). The number of seed per spathe at each of the three study sites were all significantly higher in 2015 compared to 2016. The proportions of pollinated spathes at DY-1 were 49.84 ± 17.08% in 2015, much higher than that of 2016 (22.10 ± 18.96%). Howev-er, the indicators of seed production showed no significant differences among sites (\(p > 0.05\)). There was no variation in the number of seeds per spathe between years at this site (\(p > 0.05\)). The proportions of pollinated spathes among total spathes at the three sites were all significantly higher in 2015 compared to 2016. The proportions of pollinated spathes at DY-1 were 49.84 ± 17.08% in 2015, much higher than that of 2016 (22.10 ± 18.96%). However, the indicators of seed production showed no significant differences among sites (\(p > 0.05\)).

Seed production per flowering shoot was 14.5 ± 6.1 seeds at DY-1 in 2015, but was significantly lower in 2016 with an average of 6.3 ± 3.1 seeds (\(p < 0.001\)). At DY-3, seed production per flowering shoot was 14.2 ± 6.3 seeds in 2015, which was also significantly higher than 2016 (8.1 ± 2.1 seeds). There was no significant difference in seed

Table 1

| Temp (°C) | Salinity | DOsat (%) | DO (mg L−1) | pH | Turbidity+ (NTU) | Chl a (µg L−1) |
|----------|----------|-----------|-------------|----|----------------|----------------|
| 16-18Aug 2015 | 30.54 ± 0.64 | 26.37 ± 1.85 | 104.53 ± 32.70 | 6.77 ± 2.11 | 7.76 ± 0.31 | 63.22 ± 148.33 | 4.72 ± 6.24 |
| 29-30Dec 2016 | 0.26 ± 0.83 | 33.45 ± 0.80 | 18.63 ± 0.72 | 2.14 ± 0.11 | 7.25 ± 0.05 | 17.62 ± 0.70 | 121.38 ± 191.43 |

Temp, temperature; DO, dissolved oxygen; DOsat, dissolved oxygen saturation; Chl a, chlorophyll a.
Fig. 3. Seasonal changes in shoot density (A1, A2, A3), biomass (B1, B2, B3), and shoot height (C) in the central areas of the three study sites (DY-1, DY-2, and DY-3).
production per flowering shoot at DY-2 between 2015 (10.8 ± 6.4 seeds) and 2016 (6.5 ± 2.4 seeds) (p > 0.05). Based on the average flowering shoot density, seed production per unit area was 13,137 seeds m⁻² in at DY-1 in August 2015, 30,784 seeds m⁻² at DY-3 and were 29,160 seeds m⁻² at DY-2, respectively.

3.6. Seed bank

The distribution of seeds from samples collected in the center of the meadows was extremely heterogeneous (Fig. 4). There were significant differences in seed density between seasons (p < 0.01). Seed density was higher in autumn and winter, and decreased gradually during the spring until none were present. The highest recorded density was 2382 ± 1606 seeds m⁻² in October 2016 at DY-1. The seed density in the winter of 2016 was significantly lower than that of 2015 (p < 0.01). There was no consistent trend observed for seed density along transect lines (Fig. 5).

3.7. Population recruitment

Population recruitment from seedlings and overwintering shoots at DY-1 are showed in Fig. 6. Overwintering shoot density was low on the 18th of March 2016 (29.8 ± 30.4 shoots m⁻²), at which time clonal growth and seed germination had not yet begun. Seed germination generally occurred during April and May and seedling density along transects was not homogeneous. In April 2016, average seedling density was 847 ± 711 shoots m⁻², ranging from 0 to 2948 shoots m⁻², and seedling density increased to 3084 ± 716 shoots m⁻² with a range of 0–3855 shoots m⁻² in May 2016. Seedling density was significantly higher than overwintering shoot density (p < 0.01). The ratio of seedling shoots to total shoots was 96.51 ± 5.51%, ranging from 85.71 to 100%.

There were significant inter-annual variations in overwintering shoot and seedling shoot density (Fig. 6). Overwintering shoot density was significantly higher in March 2017 (162.0 ± 205.5 shoots m⁻²) than March 2016 (p < 0.05). On the contrary, seedling density in 2017 was significantly lower than those of 2016 (p < 0.001), with an average of 224 ± 264 shoots m⁻² and 343 ± 395 shoots m⁻² in April and May 2017. And during April to May 2017, overwintering shoot density increased from 343 ± 395 shoots m⁻² to 1609 ± 1408 shoots m⁻², which were much higher than seedling density. Seedling shoot ratio in 2017 was 35.46 ± 34.36%, ranging from 0 to 100%, significantly lower than that of 2016 (p < 0.001).

The seedling and overwintering shoot density of DY-2 and DY-3 are shown in Fig. 7. There were significant differences in seedling shoot density between study sites (p < 0.05). The seedling shoot density in April and May 2017 at DY-2 was 59 ± 63 shoots m⁻² and 164 ± 283 shoots m⁻², respectively. Seedling shoot density in DY-3 was significantly higher than DY-2 with 624 ± 317 shoots m⁻² in April 2017 and 605 ± 513 shoots m⁻² in May 2017 (p < 0.01). The seedling shoot ratio was 4.35 ± 5.82% and 50.78 ± 46.35% in May 2017 at DY-1 and DY-3, respectively. Field observations during 2017 revealed that overwintering shoots were more abundant at the end of the transects at DY-1 and DY-2, while they were more abundant at the beginning of the transect in DY-3.

Comparisons of the morphology of seedling shoots and overwintering shoots (Table 3), revealed that the number of shoots per seedling was significantly lower than those of overwintering individuals (clone) at DY-2 and DY-1 (p < 0.05). However, there were no significant differences in shoot height between seedlings and overwintering clones (p > 0.05).

Genetic diversity and gene flow of Z. japonica.

There were no significant differences in the expected and observed heterozygosity among three sites (p > 0.05) (Table 4). None of the pairwise FST values were statistically significant. Results of the BAYESASS analysis showed a high self-assignment rate for each site, ranging from 0.687 to 0.961. Migration rates from DY-2 to DY-1 and DY-3 were high (0.288 and 0.299, respectively).

4. Discussion

Seagrass meadows are at risk globally (Orth et al., 2006; Short et al., 2011; Waycott et al., 2009), and are particularly threatened in developing countries. This study describes an extensive intertidal population of the seagrass Z. japonica, which appears to be both stable and resilient, in an intertidal zone with a high amount of suspended sediments. This unusually large area of Z. japonica meadow is regarded as the most extensive area (>1000 ha) of this species in China. It is located in the intertidal zone of the Yellow River Delta and is most extensive in the summer, covering almost 100% of the central areas at the three study sites. The extensive wetlands in the Yellow River Delta comprise a mosaic of riparian boundaries, intertidal marshes, and seagrass meadows, supporting a continuum of different vegetation zones from land to sea. National seagrass surveys have recorded high rates of habitat loss in recent years, increasing the importance of this newly discovered area of Z. japonica.

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**Fig. 4.** Temporal changes in sediment seed banks of Zostera japonica in DY-1, DY-2, and DY-3.
Fig. 5. Spatial changes in sediment seed banks of *Zostera japonica* along transects in DY-1.
The Yellow River carries large amounts of freshwater and SPM into the sea every year, which inevitably led to a decrease in salinity and increase in turbidity. *Z. japonica* is usually considered to be a euryhaline species, tolerating long-term exposure to estuarine salinities ranging from 5 to 35. This species can occasionally tolerate short-term exposure to zero salinity in situ, but is best adapted to intermediate to high salinities (20–35) (Shafer et al., 2011, Kaldy and Shafer, 2013). The salinity levels recorded during this study ranged from 19.8 to 35 and are suitable for the growth of *Z. japonica*, revealing the limited attenuation effects of the water of the Yellow River on salinity in the study area. However, based on our observations the salinity levels close to the river mouth were very low (near to 0) and have limited the range of the *Z. japonica* meadow.

Though the average sediment load delivered from the Yellow River to the sea has drastically decreased from $>1 \times 10^9$ t a$^{-1}$ before 1970 to $0.13 \times 10^9$ t a$^{-1}$ from 2006 to 2013 (Hu et al., 1998; Gao et al., 2015), the SPM contents at the three study sites are at high levels compared with most other seagrass meadows. Based on the results of this
study, SPM content is highly variable, with higher values in summer and autumn (163.19 ± 90.73 mg L\(^{-1}\)), nearly three times as high as that in winter and spring (63.02 ± 78.02 mg L\(^{-1}\)). Similarly, Gao et al. (2015) found that SPM content in a river plume showed a significant seasonal pattern with a very high mean of 1880 mg L\(^{-1}\) in flood season (August) and a lower mean of 755 mg L\(^{-1}\) in dry season (April). In addition, suspended sediments from the Yellow River mouth primarily extend in a southerly and southeasterly direction during the flood season and appear to be concentrated within 35 km of river mouth (Qiao et al., 2010). Thus, the three seagrass sites in this study were all affected, but at different levels, by the turbid plume from the river mouth.

Many large-scale field surveys have suggested that high levels of turbidity can limit the cover of aquatic vegetation (Iverson and Bittaker, 1986; Duarte, 1991; Sand-Jensen et al., 2008; Krause-jensen et al., 2011). To understand the existence of a huge and thriving seagrass meadow distributed under highly turbid conditions, the intertidal nature of this species must be considered. Intertidal seagrass species such as \textit{Z. japonica} undergo several hours of exposure to the air during low tide (Leuschner et al., 1998; Ruesink et al., 2010), and it was observed that the three study sites can be emersed for 4–8 h during low tides in the growing season (May to August). Thus, it is expected that \textit{Z. japonica} only “suffers” from turbidity (by being almost in the dark) when it is immersed. This species can photosynthesize actively in the air during the emersion phase, which may provide sufficient energy to sustain growth, and could explain its occurrence in very turbid waters.

The life history strategy of \textit{Z. japonica} in the intertidal Yellow River Delta is relatively successful. The shoot height, density and biomass increased rapidly from spring to summer and were high in comparison with other geographic populations (Zhang et al., 2015). The flowering period, from the appearance of the first flowering shoot to the last seed release, mainly occurred over three months from July to September, though there were slight annual differences in the timing of these reproductive events. Reproductive efforts (flowering shoot ratio) were consistently high in different years. The maximum reproductive effort was 57.35 ± 12.04% (DY-3), which is higher than most \textit{Z. japonica} populations (Zhang et al., 2015). Seagrasses in disturbed environments are often observed to invest higher amounts of energy into sexual reproduction (see Cabaço and Santos, 2012), which is likely the reason for the high flowering shoot ratios in this study. The seed production (13137–30,784 seeds m\(^{-2}\)) recorded in this study was comparable to another mixed annual \textit{Z. japonica} population in a marine lagoon called Swan Lake (Zhang et al. unpublished). The seed bank (2382 ± 1606 seeds m\(^{-2}\)) in the current study was much higher than that in Swan Lake, but similar to that in another estuarine population (Henderson and Hacker, 2015). This may be due to the limited dispersal of seeds under a relatively low tidal range and hydrodynamics (Hu et al.,

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**Table 3**

Morphological comparisons of the shoots from seedlings and overwintering shoots.

|            | DY-1          | DY-2          | DY-3          |
|------------|---------------|---------------|---------------|
|            | 10-May-15     | 20-Apr-17     | 18-May-17     | 10-May-15     | 20-Apr-17     | 18-May-17     | 10-May-15     | 20-Apr-17     | 18-May-17     |
| No. shoots per seedling | 1.70 ± 0.40  | 1.00 ± 0.00  | 1.79 ± 1.12  | 1            | 0.61 ± 0.48  | 1.37 ± 0.66  | 1.27 ± 0.10  | 0.00 ± 0.00  | 2.66 ± 1.62  |
| No. shoots per overwintering clone | - 2.07 ± 0.72 | 3.64 ± 1.74  | -            | 2.23 ± 0.93  | 4.38 ± 2.63  | -            | 2.23 ± 0.93  | 5.05 ± 1.05  | 6.86 ± 2.49  |
| Seedling shoot height (cm) | 7.14 ± 1.28  | 4.69 ± 1.69  | 5.59 ± 2.18  | 4.16 ± 0.66  | -            | 5.81 ± 2.36  | 5.77 ± 1.02  | 5.05 ± 1.05  | 6.86 ± 2.49  |
| Overwintering shoot height (cm) | 9.08 ± 1.91  | 7.33 ± 4.17  | 7.27 ± 2.69  | 8.67 ± 2.40  | 4.02 ± 3.47  | 7.71 ± 2.66  | 7.83 ± 2.90  | 8.28 ± 3.47  | 8.09 ± 2.97  |

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**Fig. 7.** Seedling (shoot) density, overwintering shoot density, and seedling ratio of Zostera japonica along transects in DY-2 and DY-3 during the spring of 2017.
The large seed bank in this study also indicates high potential recruitment success. Although the *Z. japonica* meadow is almost bare in winter, it can rapidly recover from seasonal declines via sexual (seeds) and asexual reproduction (overwintering shoots) within two months (April to May) in the following year. Some authors have estimated the contribution of seedling recruitment for *Z. japonica* populations, ranging from a minor role in subtropical or warm temperate areas, to >90% (major role) in cool temperate zones (Zhang et al. unpublished data; Ruesink et al., 2010; Henderson and Hacker, 2015; Harrison, 1979; Harrison, 1982). Harrison (1979) observed that seedling success fluctuated from year to year, apparently in response to variations in weather in early spring when germination and seedling establishment occurred. The contribution of seed in this study was highest in 2016 with a value of 96.51 ± 5.51% and intermediate in 2017 at 35.46 ± 33.6%. The reason for this annual variation is likely due to the extremely cold weather experienced in January 2016, during which almost all provinces of China were subject to severely cold conditions. While the unusually low temperatures froze the overwintering shoots/rhizomes, leading to significant loss of overwintering shoots in March 2016 in comparison to 2017. It is possible that the rarity of overwintering shoots has improved seedling success, as seedlings under a perennial canopy may be negatively affected by competition with adult shoots (Olesen, 1999; Rivers et al., 2011).

The genetic structure of seagrass meadows is greatly influenced by the balance between seedling recruitment and clonal growth via rhizomes (Diekmann et al., 2005). Responding to the frequent seedling recruitment events in the field, *Z. japonica* meadows at the three study sites of the Yellow River Delta are all genetically diverse with consistently high values of observed heterozygosity ($H_o$), expected heterozygosity ($H_e$), and number of alleles observed ($N_a$). Moreover, the meadows on both sides of Yellow River mouth are connected by gene flow, suggesting frequent local transport of reproductive propagules. Though the dispersal capacity is limited, seagrass still can disperse via seeds attached to gas bubbles or fragments of reproductive shoot (den Hartog, 1970; Churchill et al., 1985; Harwell and Orth, 2002a), likely aided by the rotating counterclockwise tides of the Bohai Sea (Hu et al., 1998).

In conclusion, the limited inter-annual changes in seagrass abundance and seed banks during this study confirm that this seagrass population is stable and resilient to extremely turbulent conditions. Although the status of *Z. japonica* in the intertidal Yellow River Delta is strikingly good, this may be a unique situation in China. In the surrounding areas adjacent to the Yellow River Delta, most natural coastlines have been replaced by coastal highways and culture ponds, which occupy intertidal zones and have altered the hydrodynamics of this environment. In addition, excessive clamming activities directly damage the persistence of seagrass meadows. In fact, land or sea reclamation, destructive fishing and aquaculture were the most common reasons for seagrass loss in the north and south of China (Lee, 1997; Huang et al., 2008; Zheng et al.) and in Korea (Lee et al., 2004). Though the seagrass *Z. japonica* was not one of the target species for conservation at the establishment of the national nature reserve, the *Z. japonica* meadows are in the core zone of the reserve. Land or sea reclamation, destructive fishing and aquaculture are completely forbidden in the reserve. Tourists are not allowed to enter the core zone of the reserve, and only researchers can enter this area upon approval. These restrictions have protected the seagrass from direct human activities. The role of protected areas in seagrass conservation has been highlighted in previous studies, given the reduced pressure from human activities within reserves (Bulthuis, 1995; Zhou et al., 2015; Lopez-calderon et al., 2016).

Protected areas can often contain the last populations of one seagrass species in a region, in the broader context of widespread severe seagrass declines. To date, efforts to conserve seagrass habitats in China have been limited and no reserve specifically aimed at protecting seagrasses has been established. Therefore, increased attention and efforts should be devoted to establishing reserves to protect extant areas of seagrasses. Given its rich genetic diversity and potential to act as a transplant and seed resource, *Z. japonica* in the intertidal Yellow River Delta could be a suitable donor population for seagrass conservation and restoration projects in the future.

### Table 4

| Sites          | Genetic diversity | Pairwise $F_{ST}$ | Migration rate |
|---------------|------------------|------------------|---------------|
|               | $H_o$ | $H_e$ | $P_{EC}$ | $N_a$ | $F_{DY-1}$ | $F_{DY-2}$ | $F_{DY-3}$ | $F_{DY-1}$ | $F_{DY-2}$ | $F_{DY-3}$ | $F_{DY-1}$ | $F_{DY-2}$ | $F_{DY-3}$ |
| DY-1          | 0.675 | 0.715 | 0.655 | 6.80 | 0.000 | 0.692 | 0.288 | 0.021 |
| DY-2          | 0.619 | 0.705 | 0.650 | 6.30 | 0.011 | 0.000 | 0.015 | 0.061 | 0.025 |
| DY-3          | 0.641 | 0.710 | 0.658 | 6.40 | -0.004 | 0.008 | 0.000 | 0.014 | 0.289 | 0.687 |

Measures of genetic variations are: $N_a$ = number of alleles observed; $H_o$ = observed heterozygosity; $H_e$ = expected heterozygosity; $P_{EC}$ = polymorphic information; recent migration rate: the first column represents the destination site, while the first row represents origin site.

### CRediT authorship contribution statement

**Xiaomei Zhang:** Writing - original draft, Conceptualization, Methodology, Investigation, Funding acquisition. **Haiyling Lin:** Data curation, Formal analysis, Investigation, Validation, Writing - review & editing. **Xiaoyue Song:** Data curation, Formal analysis, Investigation, Writing - review & editing. **Shaochun Xu:** Data curation, Formal analysis, Investigation, Writing - review & editing. **Shidong Yue:** Investigation, Data curation. **Ruiting Gu:** Investigation, **Shuai Xu:** Investigation, **Shuyu Zhu:** Resources. **Yajie Zhao:** Resources. **Shuyan Zhang:** Resources. **Guangxuan Han:** Resources. **Andong Wang:** Resources. **Tao Sun:** Investigation. **Yi Zhou:** Funding acquisition, Supervision, Methodology, Investigation, Writing - review & editing.

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