Ecological Engineering 92 (2016) 1–9

Contents lists available at ScienceDirect

Ecological Engineering

journal homepage: www.elsevier.com/locate/ecoleng

Assessment of the establishment success of *Zostera marina* (eelgrass) from seeds in natural waters: Implications for large-scale restoration

Chao-Jie Yang[a], Yan-Shan Liu[a], Jie Liu[b], Qiang Xu[a], Wen-Tao Li[a], Pei-Dong Zhang[a,*]

[a] Fisheries College, Ocean University of China, Qingdao 266003, PR China
[b] Marine Biology Institute of Shandong Province, Qingdao 266002, PR China

ARTICLE INFO

Article history:
Received 11 August 2015
Received in revised form 25 February 2016
Accepted 21 March 2016
Available online 30 March 2016

Keywords:
Culture
Restoration
Seed germination
Seedling establishment
New plants
Zostera marina

ABSTRACT

We described and evaluated an aquaculture system that can grow *Zostera marina* (eelgrass) from seeds in natural waters for restoration projects through a two-year field experiment. The system included protective seed bags filled with high-silted sediments, flowerpots, and culture beds. To determine the suitable conditions for seedling establishment, we subjected *Z. marina* seeds to treatments including different durations of low temperature (4 °C) prior to seed planting, seed planting densities, and numbers of holes on the side of each flowerpot. In the spring following seed distribution, the seedling establishment of *Z. marina* was significantly promoted within 30 days by low-temperature storage prior to seed planting and seven holes per flowerpot, but the seed planting density showed no effects. The average shoot density of the plants large enough for transplanting was 614 shoots m^{-2} in the optimal eelgrass transplant season, which was 10 months after planting. New plants from the seeds were fully developed and well maintained 2 years after distribution with the maximum shoot density of 2661 shoots m^{-2}. Results demonstrate that growing *Z. marina* from seeds is an alternative approach to harvest plants from donor beds. The presented aquaculture system should be considered for future large-scale restoration projects of *Z. marina*.

1. Introduction

Seagrass meadows are highly valuable ecologically and economically, but these areas have been reduced worldwide because of natural and anthropogenic causes (Short and Wyllie-Echeverria, 1996; Costanza et al., 1997; Ruiz and Romero, 2003; Tomasko et al., 2005; Burkholder et al., 2007; Montefalcone et al., 2010). Seagrass seeds contribute to genetic and population structure, bed maintenance, plant demographics, development of new beds, and improved recovery of disturbed stands compared with vegetative propagation alone (Orth et al., 2000; Whitfield et al., 2004; Greve et al., 2005). Thus, recent efforts have been attempted to restore seagrass habitats by using seeds instead of plants or sods as planting stock.

Some seed-planting field experiments have been developed focusing on *Zostera marina* (eelgrass) by applying mechanical seed planters (Orth et al., 2009; Marion and Orth, 2010), hand broadcast techniques (Busch et al., 2010; Golden et al., 2010), buoy-deployed seeding techniques (Pickerell et al., 2005), and seed protection techniques (Harwell and Orth, 1999). Restoration efforts varied with seedling establishment, which generally use less than 10% of seeds (Orth et al., 2009). Low seedling establishment rates remain a bottleneck for seed-based seagrass restoration (Orth et al., 2009). Among the efforts that did report "success" (i.e., at least some planting units survived), many projects were based on <1 year of monitoring, but long-term success remains unclear (Tanner, 2015).

Unlike emergent wetland plants and other freshwater plant species that are commercially propagated and available in sufficient quantities for restoration, seagrasses and other estuarine aquatic plants are generally not available as commercial nursery stock (Shafer and Bergstrom, 2010). Tissue culture has been used to propagate some species of submersed aquatic vegetation for restoration; however, tissue culture methods are only beginning to be developed for *Z. marina* (Tanner and Parham, 2010). An approach that has been used for large-scale restoration of *Z. marina* involves collecting seeds, germinating them, and growing seedlings in culture systems until they can be out-planted to restoration sites (Tanner and Parham, 2010; Niu et al., 2012). This approach can reduce damage to donor beds, increase survival rates of plantings, and maintain high genetic diversity in the restored *Z. marina* bed (Tanner and Parham, 2010).

Tanner and Parham (2010) reported that *Z. marina* plants large enough for transplanting can be grown from seeds within 90 days in land-based culture systems. However, growing *Z. marina* in culture

* Corresponding author.
E-mail address: zhangpdsg@ouc.edu.cn (P.-D. Zhang).

http://dx.doi.org/10.1016/j.ecoleng.2016.03.039
0925-8574/

systems is expensive because of electrical and labor costs, which may limit the general application of this method. In addition, long-term monitoring (>1 year) of the growth and expansion of field-planted *Z. marina* is lacking.

In seagrass systems, the period between seed germination and seedling establishment is one of the most vulnerable phases for successful sexual reproduction (Alagna et al., 2013; Jarvis and Moore, 2015). This period is closely linked to environmental factors, including low temperature, seed density, and pore water exchange. Generally, *Z. marina* seed germination is facilitated by low temperature (Hootsmans et al., 1987; Probert and Brenchley, 1999; Tanner and Parham, 2010). However, a previous 45-day observation showed that prolonged low-temperature treatments (cold storage) increased the premature germination of *Z. marina* seeds, which is detrimental to seed germination and seedling establishment in field restoration (Zhang unpublished data). To date, the optimal duration of low temperature for seeds prior to planting is not completely understood. Conflicting results concerning the effects of seed density on the seed germination and seedling establishment have been reported. Orth et al. (2003) found that seed germination and seedling establishment of *Z. marina* were not significantly affected by initial seed densities in a seed-broadcast field experiment. However, their results were in contrast with those of Zhang et al. (2015a), who reported significant density-dependent effects through a seed-protecting field experiment. Hence, further research is needed to evaluate the relative role of seed density in germination and early seedling survival. Several studies have reported that the exchange of pore water may affect the biological and chemical properties, as well as functions of the sediment, but no data on its effect on seed germination and seedling establishment in seagrasses have been reported (Goodman et al., 1995; Terrados et al., 1999; Koch, 2001).

In the present study, we described a cost-effective culture system that can grow *Z. marina* from seeds in natural waters for potential use in restoration projects. The system included protective seed bags made of sewn burlap and filled with high-silted sediments, ceramic flowerpots as planting plots, and culture beds welded by painted iron bar. A preliminary germination experiment (45 days) was conducted using seeds without low-temperature treatment and under controlled laboratory conditions (salinity: 15–20; temperature: 14 °C–15 °C) to determine whether burlap bags and flowerpots increased or prevented seed germination. The germination rate increased when seeds were planted 2 cm–3 cm deep in the burlap bags filled with high-silted sediments and placed in the flowerpots compared with those directly planted 2 cm–3 cm below the surface of the same high-silted sediments.

On the basis of the preliminary study results, a field experiment was conducted using seeds exposed to different treatments including duration of low temperature (4 °C) prior to planting, seed planting density, and the number of holes on the side wall of each flowerpot (representative of the exchange of pore water) from 2012 to 2014. The primary aims of this research were to (1) determine the suitable conditions required by the seeds of *Z. marina* and (2) assess the effectiveness of this system designed for increasing the establishment success of new plants from seeds. Furthermore, this study aimed to develop an efficient and cost-effective method for *Z. marina* culture to provide donor plants for large-scale restoration projects.

2. Materials and methods

2.1. Experimental site

The study was conducted in Swan Lake (Yuehu) on the eastern coast of Shandong Peninsula, China (Fig. 1). This lake is a tidal lagoon with an area of 4.94 km^2 and is separated from the open sea (i.e., Rongcheng Bay and Yellow Sea) by a 2.5 km-long sand spit, which lies to the east of the lagoon (Wei and Zhuang, 1997). The entrance channel connecting the lagoon to the open sea is 132 m wide at its narrowest part. The mean water depth was about 1.0 m relative to the mean sea level. The floor of the lagoon is generally dominated by fine-grained material, with mud and sandy mud covering approximately 40% of the lagoon area (Jia et al., 2003). Anecdotal reports, fishery practices, and local historical knowledge indicate that *Z. marina* was abundant in Swan Lake in the early 1970s. However, the eelgrass beds were almost completely eliminated by the end of 1982 because of the poor water exchange induced by the artificial closure of the entrance to the lagoon for aquaculture purposes in 1979 (Gao et al., 1998). The upper part of the artificial dam was removed in 1986, and the ecosystem of the lagoon gradually recovered.

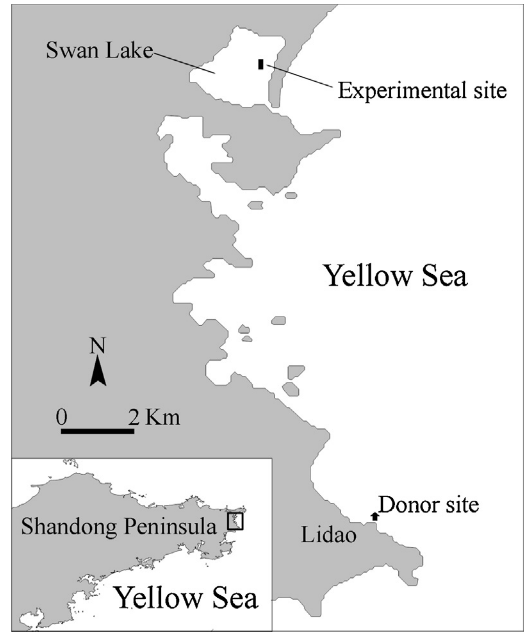

Fig. 1. Map of Shandong Peninsula showing donor and experimental sites.

2.2. Seed collection and storage

Reproductive shoots of eelgrass with inflorescences containing developed or developing seeds were collected by hand on July 2012 at the Gaojia Inlet in Rongcheng, Shandong Peninsula (Fig. 1). Reproductive shoots were broken from their root systems and loaded into nylon mesh bags (mesh size <1 mm, 84.5 L). These bags were placed in the donor site and submerged in ambient water until the shoots degenerated and seeds were released (up to 5 weeks after collection). Seeds were then sieved and placed in 2 L polythene cups with seawater for transport to the laboratory. In the laboratory, the seeds were stored in mesh bags contained in a tank with flowing oxygenated seawater at air temperature until the plants were prepared for planting.

2.3. Plant culture systems

The plant culture system included protective seed bags, filling sediments, planting pots, and culture beds. Protective seed bags were made of approximately 1 mm mesh-sized sewn burlap, which was converted into 15 cm × 20 cm (diameter × height) packets (Fig. 2a). Filling sediments were collected at the beach just outside the experimental site. Fine sand (sediment particle size