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Short communication

Evaluation of a mechanical seed planter for transplanting *Zostera marina* (eelgrass) seeds

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ABSTRACT

Few seagrass transplant projects worldwide have relied on seeds, and those projects using *Zostera marina* (eelgrass) seeds have generally found low rates of seedling establishment (<10%). We compared seedling establishment achieved by a mechanical seed planter with seeds broadcast on the sediment surface by hand. The planter injected seeds into the sediment by pumping the seeds, suspended in a gelatin-based matrix, to a benthic sled with eight planting nozzles. As a control for the gel, seeds were also injected into the sediment without gel using a hand-held pipette. Seeds were planted at a density of 300 m^{-2} into six replicate plots at each of three sites in the Chesapeake Bay region in September 2005, with seedling establishment measured in April 2006. Burying seeds, either with or without gel, had an overall positive effect on seedling establishment, but the effectiveness and the best method varied among sites. Mean seedling establishment for machine-planted seeds was significantly greater than for broadcast seeds at the Piankatank River site (4% vs. 1%), but not at the York (1.2% vs. 1.4%) or Spider Crab Bay (10.1% vs. 7.4%) sites. The effect of the gel was inconsistent among sites, with the highest seedling establishment (18.8%), resulting from seeds injected by pipette without gel at the Spider Crab site. Seed burial shows promise for increasing seedling establishment relative to seed broadcasting in the Chesapeake region, but further investigation of seed–sediment interactions at specific restoration sites is necessary. Low seedling establishment rates remain a bottleneck for seed-based eelgrass restoration.

1. Introduction

Seagrass transplanting projects worldwide have traditionally relied on adult plants (Fonseca et al., 1998) using a variety of manual and mechanical techniques (Fonseca et al., 1998; Fishman et al., 2004; Treat and Lewis, 2006). However, most techniques using adult plants are generally labor intensive and time consuming, requiring physical excavation of the donor material, which could be deleterious to the donor bed's survival, especially if annual growth rates are slow. In addition, transporting adult plants can present logistical constraints if the transplant site is located at significant distance from the donor site, or if the methodology requires moving sediment along with the plants. Transplant projects incorporating seeds have been relatively rare despite the fact that all species produce seeds, ranging up to tens of thousands m^{-2} (Orth et al., 2006a). However, seed production can be temporally and spatially variable and may require expensive seawater systems to maintain seeds until needed (Granger et al., 2002; Orth et al., 2006a). Recently, seeds have been shown to be important in creation of new patches, recovery of beds lost due to disturbance, and providing genetic diversity (Plus et al., 2003; Orth et al., 2006a), suggesting seagrass seeds could play an important role in seagrass restoration efforts (Orth et al., 2006a).

In the Chesapeake Bay region, *Zostera marina* L. (eelgrass) seeds have been used to elucidate dispersal patterns and processes (Orth et al., 1994; Luckenbach and Orth, 1999; Orth et al., 2003) and have been successfully used to initiate recovery of *Z. marina* in the coastal lagoons which lost *Z. marina* in the 1930s 'wasting disease' pandemic (Orth et al., 2006b). A bottleneck in these studies has been the low rate of seedling establishment, generally less than 10% of seeds used in each study (Orth et al., 2003, 2007). The recent development of an underwater seed planter (Traber et al., 2003) suggested an alternative method that could improve seedling success compared to techniques used in previous Chesapeake Bay studies.

The objective of this study was to assess the effectiveness of a mechanical seed planter for increasing *Z. marina* seedling establishment rates, relative to seeds broadcast on the sediment surface.

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Table 1
Sediment grain size distribution and organic content (mean ± S.D., $n = 3$) at the planting sites

Site	% Sand	% Silt/clay	% Organics
York River (Mumfort Island)	96.6 (0.6)	3.4 (0.6)	0.62 (0.00)
Piankatank River (Burtons Point)	98.6 (0.5)	1.4 (0.5)	0.73 (0.07)
Spider Crab Bay	86.2 (4.6)	13.8 (4.1)	1.41 (0.36)

2. Methods

2.1. Study sites

Three sites were chosen for the comparison test based on results of previous seed experiments and ongoing seagrass restoration efforts (Harwell and Orth, 1999; Orth et al., 2003). Two sites were located in Chesapeake Bay: Mumfort Island (37°16.0′N, 76°30.9′W) in the York River, and Burton Point (37°30.2′N, 76°19.7′W) in the Piankatank River. The third site was located in Spider Crab Bay (37°21.4′N, 75°48.2′W), one of the coastal bays of the lower Delmarva Peninsula. All sites featured unvegetated sand with low organic content (Table 1) but had historically supported dense stands of *Z. marina* (Orth and Moore, 1984). Water depths were between 0.5 and 1.0 m (mean low water).

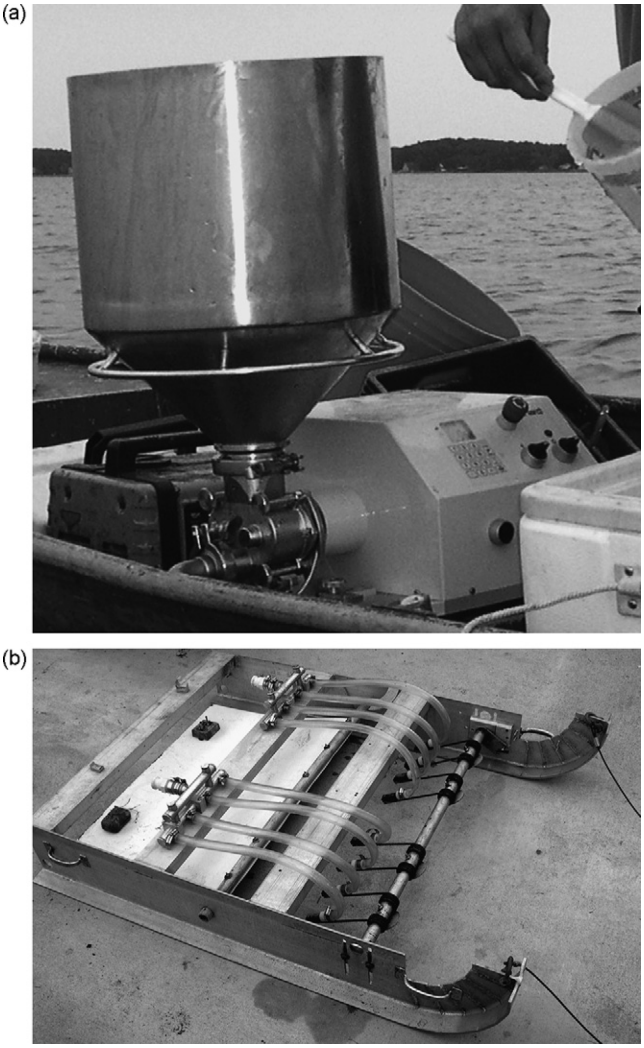

Fig. 1. *Zostera marina* seed planting machine.

2.2. Design and procedure

The mechanical seed planter (Fig. 1) (Traber et al., 2003) consists of (1) a benthic sled that creates furrows into which a seed–gel mixture is extruded and buried by a weighted pad, and (2) a pump that supplies a mixture of *Z. marina* seeds and suspension gel through flexible tubing to a manifold (distribution) system located on the sled. The gel (we used Knox® gelatin) is required to provide a viscous matrix for pumping, and keeps seeds in suspension in the supply chamber, allowing a predictable rate of seed delivery controlled directly by the pump speed. We prepared the gelatin just prior to the experiment and kept it chilled on ice until mixing with each batch of seeds. This cooling is essential, as the viscosity of the gelatin varies with temperature. The sled buries seeds to a depth of 1–2 cm below the sediment surface through eight injectors distributed along its 1 m width. To test for any potential direct effects of the gel on seedling establishment, a control treatment was established using hand-buried seeds. For this treatment, a diver used a hand-held pipette to inject seeds without any gel below the sediment surface at a similar depth to that of the mechanical planter. The two burial treatments were compared with seeds that were hand broadcast into plots on the sediment surface by a diver. Seeds on the sediment surface do not move far from where they settle (Orth et al., 1994) and this method has been successfully used in previous seed experiments (Orth et al., 2003) and restoration efforts in the Chesapeake region (Orth et al., 2006b).

At each site, six replicates of the three treatments were created, each consisting of a 1-m wide by 10-m long strip (designed to match the planting pattern created by the planting sled) receiving 3000 seeds, for a total of 18 strips per site. Strips were separated by 10 m. To establish the machine test strips, the pump was placed in a small aluminum boat and pulled along the 10 m strip by a winch on an anchored boat, with the sled towed directly behind the aluminum boat. For broadcast strips, seeds were scattered at the sediment surface by a diver along the entire strip. In actual large-scale restoration attempts using broadcast seeds, the seeds would be cast from a boat onto the water's surface, resulting in some slight (0–4 m) horizontal transport by any currents present at the time of distribution. We consider this difference inconsequential to the results. For the hand-buried seeds, small batches of seeds were pipetted into multiple linear rows along the 10 m plot, roughly simulating the pattern created by the machine.

Harvesting of seed-bearing flowering shoots, and storing of ripe seeds once they were released from the reproductive shoots followed methodologies described in Orth et al. (1994) and Harwell and Orth (1999, 2002). Seed planting occurred in September 2005, prior to the initiation of seed germination in November (Moore et al., 1993). Seed viability was assessed by planting replicate batches of 10 seeds 5–7 mm deep in sieved natural sediment. Sediment containers were held inside a greenhouse in flow-through seawater at ambient water temperatures. Germination was assessed in January (when germination was complete) by sieving the sediment and retrieving all planted seeds. The batch of seeds used in this experiment exhibited 80–90% germination.

Seedling assessment was conducted in April 2006, when all seeds had germinated and when seedlings could be most accurately counted by divers. A 4 m^2 quadrat divided into 16–0.25 m^2 cells was placed at the beginning of each line and all seedlings were recorded in each 0.25 m^2 cell. The quadrat was moved along the line to cover the entire 10-m line plus 2 m at each end of the line. In addition, a 2-m area to either side of the line was surveyed, for a total of 84 m^2 evaluated. Where necessary, seedlings were destructively sampled to count all seedlings.

Where seedlings occurred in clumps, sediment was gently removed to insure an accurate count of seedlings. Large clumps were excavated and returned to the laboratory for counting. The number of seedlings occurring individually and within clumps was recorded for each treatment.

2.3. Statistical analysis

The influence of site and seeding method on initial seedling establishment was assessed by Poisson regression due to the binary response variable, the large number of observations (54,000 seeds/site), and the preponderance of observations at very low frequencies (<10% of seeds germinating). Analyses were conducted using the GENMOD procedure in SAS version 9 software (SAS Institute, Cary, NC), with the scale factor set to log(n) to adjust for overdispersion. A significant model term ($p < 0.05$) indicates that the odds of seedling establishment for the specified treatment condition (e.g. the Machine method) relative to the reference condition (in this case, the Broadcast method) are significantly different from 1:1. Significance is also reflected in a 95% confidence interval that does not include 1.

3. Results

Burying seeds, either with gel (by the machine) or without gel (by the pipette), had an overall positive effect on seedling establishment, but the effectiveness and the best method varied among sites (Fig. 2). Poisson regression showed that across all sites the planting machine increased the odds of successful seedling establishment by a factor of 1.6 relative to hand-broadcast seeds, and injecting the seeds directly, without gel, increased the odds by a factor of 2.1 (Table 2). However, individual regression models fit for each site showed no effect of method at the York River site, a 4.0-fold increase by the planting machine at the Piankatank (but no increase for hand-injected seeds), and a 2.5-fold increase for hand-injected seeds in Spider Crab Bay (but no increase for machine-planted seeds) (Table 3).

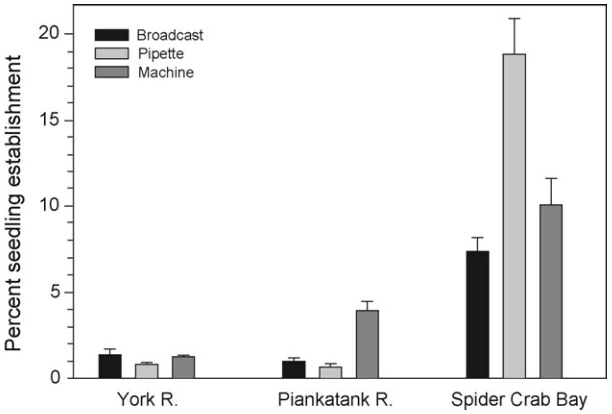

Fig. 2. Mean (+S.E.) initial seedling establishment of seeds distributed by three methods ($n = 6$).

Clumping of seedlings occurred almost exclusively at the Spider Crab Bay site, where we also had the highest seedling establishment rates for all treatments (Fig. 3). At that site, clumping was prevalent in the hand-injected plots (49% of seedlings in that treatment), rare in the broadcast plots (3%), and absent from machine-planted plots. In the 31 clumps found, the mean number of seedlings per clump was 51 ± 32.4 (S.D.), and the range was 8–142. While clumped seedlings were not measured, divers noted that most clumped seedlings were fine, single shoots, approximately 3–6 cm long, while most solitary seedlings were much larger, with 2–5 shoots each 5–10 cm long at the time of assessment.

Table 2
Poisson regression model for all sites

Variable	Parameter	d.f.	Estimate	S.E.	Chi-square	*p*	Odds	Wald 95% conf. limits on odds	
								Lower	Upper
	Intercept	1	−2.55	0.15	294.61	<0.0001			
Method	Machine	1	0.45	0.19	5.94	0.015	1.57	1.09	2.26
	Pipette	1	0.74	0.18	17.47	<0.0001	2.09	1.48	2.95
Site	York	1	−2.37	0.26	85.33	<0.0001	0.09	0.06	0.15
	Piankatank	1	−1.88	0.21	83.07	<0.0001	0.15	0.10	0.23

Parameter estimates and confidence limits were exponentiated to derive the odds of successful seedling establishment for the two seed burial methods relative to hand-broadcast seeds, and for two sites relative to the Spider Crab site.

Table 3
Poisson regression models applied to each site

Site	Parameter	d.f.	Estimate	S.E.	Chi-square	*p*	Odds	Wald 95% conf. limits on odds	
								Lower	Upper
York	Intercept	1	−4.30	0.17	672.55	<0.0001			
	Machine	1	−0.09	0.24	0.14	0.708	0.91	0.57	1.46
	Pipette	1	−0.53	0.27	3.75	0.053	0.59	0.35	1.01
Piankatank	Intercept	1	−4.63	0.24	371.06	<0.0001			
	Machine	1	1.39	0.27	26.76	<0.0001	4.01	2.37	6.79
	Pipette	1	−0.41	0.38	1.15	0.283	0.66	0.32	1.40
Spider Crab	Intercept	1	−2.61	0.16	270.83	<0.0001			
	Machine	1	0.31	0.21	2.26	0.133	1.37	0.91	2.06
	Pipette	1	0.94	0.19	25.17	<0.0001	2.56	1.77	3.69

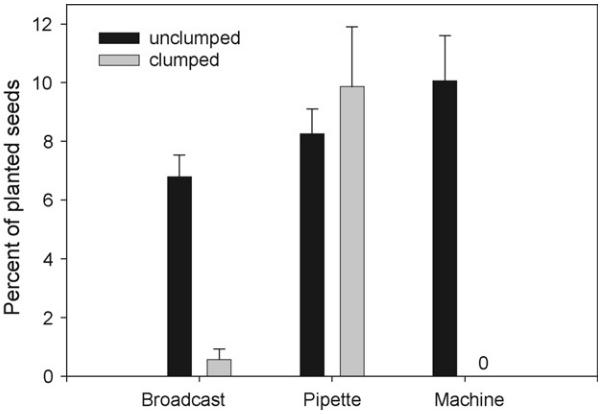

Fig. 3. Mean (+S.E.) percentage of planted seeds emerging as seedlings at low density (unclumped) or in high-density clusters (clumped) at the Spider Crab Bay site ($n = 6, 5, 6$ for broadcast, pipette, and machine, respectively).

4. Discussion

The effectiveness of the seed planting machine varied among the three restoration sites tested, with only one site showing significantly higher seedling establishing rates in the machine-planted plots than in plots established by the traditional broadcast method. The maximum rates achieved by the machine were similar to those obtained in previous seed broadcast experiments, typically in the range of 5–10% at the most successful sites. Differences in sediment characteristics and energetic regimes at the sites may explain the patterns among sites. The two sites with generally low seedling success (York and Piankatank) have sediments that contain a higher percentage of sand, have relatively little biogenic structure, and are more exposed to high-energy storm conditions in the late fall and winter during the early stages of seedling development.

At the Piankatank site, the seeds injected into the sediment with a pipette (without any gel) had similar success to those broadcast on the surface, while the machine-planted seeds achieved roughly four times that rate of establishment, implying that the presence of the gel, rather than simply the burial of the seeds, may have played an important role at that site. The gelatin is soluble in seawater, and should have dissipated quickly under field conditions, so the mechanism of any potential gel impact is unclear. Our preliminary work with seeds planted with Knox gelatin in the lab showed germination rates of 52–58%, and no difference in germination among sediments with organic contents of 0.75%, 1.5%, and 7% (unpublished data).

In the York River, by contrast, there was no difference among methods, and a substantially lower overall seedling establishment rate. We believe that the generally low seedling success at these two sites was primarily a result of high physical disturbance of both seeds and developing seedlings. The two sites differ in exposure (N/NE at the Piankatank, W/SW at the York), so storm systems would be expected to impact the sites differently.

At the Spider Crab Bay site, the machine-planted seeds underperformed those injected without gel (10% vs. 19% seedling establishment), and were marginally better than broadcast seeds (8%). That site features softer (Table 1), more cohesive sediments, much lower wave energy, rich biogenic structures (worm tubes, burrows, and clams), and presumably a shallow oxygen penetration depth due to higher organic content. Loss of seeds and developing seedlings due to physical disturbance and sediment re-suspension is thought to be a much less important process at this site.

One advantage of the machine planter appears to be the evenness in which seeds are dispersed. We noted no clumping of seedlings in the machine treatments at the Spider Crab Bay site, the site with the highest number of established seedlings (Fig. 3). This lack of clumping is related to how seeds are delivered and maintained in the bottom once injected by the machine planter. The planter's gel kept the seeds evenly spaced, whereas seeds injected by hand were not embedded in gel and were subject to the divers' ability to push seeds out of the pipette at a constant rate. Since seeds settle rapidly in seawater (Orth et al., 1994), the seeds inside the pipette tended to slide to the bottom, and apparently came out in clumps. Seedling clumps were found only at the Spider Crab Bay site, suggesting either greater clump formation at Spider Crab Bay, or greater loss at the other two sites. The occasional seedling clumps found in the broadcast treatments at Spider Crab Bay were likely due to seeds settling together in surface features. There could be both positive consequences (e.g. local stabilization of sediments, increase in O_2 availability in the rhizosphere) and negative consequences (e.g., competition for nutrients) of seedling clumps but there is currently little evidence regarding the eventual fate of clumped seedlings. However, we infer from the dramatic difference in seedling size at 6 months that those seedlings had already experienced strong negative competitive pressure relative to isolated seedlings, and that clumped seedlings would be unlikely contribute as much to restoration success as the same number of isolated seedlings, similar to the finding of Granger et al. (2000).

Each of the methods used here has requirements that need to be considered in a seed based restoration program. The planter requires a pre-made gel matrix for seed delivery, which for our experiment required 5 gallons for two 10 m lines. The gel must be kept cool during the entire process. Submerged objects such as rocks, tree stumps or old pilings, as well as high wind conditions, can compromise the efficient operation of the planter. The broadcast method requires only one individual to disperse the seeds, in addition to a boat driver for large-scale boat-based broadcasts, and can be conducted under more compromising wind conditions. Broadcast seeds settle rapidly and are generally retained near their settlement location by topographic complexities of the sediment surface which facilitate the trapping and subsequent burial of seeds (Orth et al., 1994). Seeds placed into the sediment by the planter are not dependent on sediment features for entrapment and burial, and should be minimally re-distributed from the planting site compared to broadcast seeds. In fact, distinct rows of seedlings from the machine's planting tines were obvious in the machine-planted plots. Both methods require an efficient method of storing seeds from the collection period until dispersal. We did not specifically calculate labor and time requirements for the machine planter, since our investigation imposed different constraints than large-scale restoration efforts. However, broadcasting seeds by hand can be accomplished much faster than machine planting, and labor costs would have to be considered in large-scale seed based restoration projects using a planting machine, especially in relation to seedling establishment rates. Manual injection of seeds would be impractical for large-scale restoration projects.

The pattern of low seedling establishment rates (generally less than 10%, and commonly 1–5%) has been consistent across years and with different tests of seed dispersal timing and dispersal mechanisms (Orth et al., 2003, unpublished data). While the machine planter significantly increased seedling establishment at the Piankatank site to 4%, where we have recorded seedling establishment rates of only 1% or less in previous years' experiments (Harwell and Orth, 1999; unpublished data), and across all sites increased the odds of establishment by a factor of

1.6 over broadcast seeds, this low rate remains the major bottleneck in seed-based restoration projects, especially where seed supplies are limited.

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References

Fishman, J.R., Orth, R.J., Marion, S., Bieri, J., 2004. A comparative test of mechanized and manual transplanting of eelgrass, *Zostera marina*, in Chesapeake Bay. Rest. Ecol. 12, 214–219.

Fonseca, M.S., Kenworthy, W.J., Thayer, G.W., 1998. Guidelines for the conservation and restoration of seagrasses in the United States and adjacent waters. NOAA Coastal Ocean Program Decision Analysis Series No. 12. NOAA Coastal Ocean Office, Silver Spring, MD, 222 pp.

Granger, S.L., Traber, M.S., Nixon, S.W., 2000. The influence of planting depth and density on germination and development of *Zostera marina* L. seeds. Biol. Mar. Mediterr. 7, 55–58.

Granger, S.L., Traber, M.S., Nixon, S.W., Keyes, R., 2002. A practical guide for the use of seeds in eelgrass (*Zostera marina* L.) restoration. Part 1. In: Schwartz, M. (Ed.), Collection, Processing, and Storage. Rhode Island Sea Grant, Narragansett, RI.

Harwell, M.C., Orth, R.J., 1999. Eelgrass (*Zostera marina* L.) seed protection for field experiments and implications for large scale restoration. Aquat. Bot. 64, 51–61.

Harwell, M.C., Orth, R.J., 2002. Long distance dispersal potential in a marine macrophyte. Ecology 83, 3319–3330.

Luckenbach, M.L., Orth, R.J., 1999. Effects of a deposit-feeding invertebrate on the entrapment of *Zostera marina* L. seeds. Aquat. Bot. 62, 235–247.

Moore, K.A., Orth, R.J., Nowak, J.F., 1993. Environmental regulation of seed germination in *Zostera marina* L. (eelgrass) in Chesapeake Bay: effects of light, oxygen, and sediment burial depth. Aquat. Bot. 45, 79–89.

Orth, R.J., Moore, K.A., 1984. Distribution and abundance of submerged aquatic vegetation in Chesapeake Bay: an historical perspective. Estuaries 7, 531–540.

Orth, R.J., Luckenbach, M.W., Moore, K.A., 1994. Seed dispersal in a marine macrophyte: implications for colonization and restoration. Ecology 75, 1927–1939.

Orth, R.J., Fishman, J.R., Harwell, M.C., Marion, S.R., 2003. Seed density effects on germination and initial seedling establishment in eelgrass *Zostera marina* in the Chesapeake Bay region, USA. Mar. Ecol. Prog. Ser. 250, 71–79.

Orth, R.J., Harwell, M.C., Inglis, G.J., 2006a. Ecology of seagrass seeds and dispersal strategies. In: Larkum, A.W.D., Orth, R.J., Duarte, C.M. (Eds.), Seagrasses: Biology, Ecology and Conservation. Springer, The Netherlands, pp. 111–133 (691 pp).

Orth, R.J., Luckenbach, M.L., Marion, S.R., Moore, K.A., Wilcox, D.J., 2006b. Seagrass recovery of in the Delmarva Coastal Bays, USA. Aquat. Bot. 84, 26–36.

Orth, R.J., Marion, S.R., Moore, K.A., 2007. A summary of eelgrass (*Zostera marina*) reproductive biology with an emphasis on seed biology and ecology from the Chesapeake Bay region. In: SAV Technical Notes Collection (ERDC/TN SAV-07-1), U.S. Army Engineer Research and Development Center, Vicksburg, MS.

Plus, M., Deslous-Paoli, J.-M., Dagault, F., 2003. Seagrass (*Zostera marina* L.) bed recolonization after anoxia-induced full mortality. Aquat. Bot. 77, 121–134.

Traber, M., Granger, S., Nixon, S., 2003. Mechanical seeder provides alternative method for restoring eelgrass habitat (Rhode Island). Ecol. Rest. 21, 213–214.

Treat, S.F., Lewis, R.R., 2006. Seagrass restoration: success, failure, and the costs of both. In: Proceedings of the Conference Seagrass restoration: success, failure, and the costs of both, Sarasota, Florida, March 11, 2003, p. 175.