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RESEARCH ARTICLE

Innovative Techniques for Large-scale Seagrass Restoration Using *Zostera marina* (eelgrass) Seeds

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Abstract

The use of *Zostera marina* (eelgrass) seeds for seagrass restoration is increasingly recognized as an alternative to transplanting shoots as losses of seagrass habitat generate interest in large-scale restoration. We explored new techniques for efficient large-scale restoration of *Z. marina* using seeds by addressing the factors limiting seed collection, processing, survival, and distribution. We tested an existing mechanical harvesting system for expanding the scale of seed collections, and developed and evaluated two new experimental systems. A seeding technique using buoys holding reproductive shoots at restoration sites to eliminate seed storage was tested along with new techniques for reducing seed-processing labor. A series of experiments evaluated storage conditions that maintain viability of seeds during summer storage for fall planting. Finally, a new mechanical seed-planting technique appropriate for large scales was developed and tested. Mechanical harvesting was an effective approach for collecting seeds, and impacts on donor beds were low. Deploying seed-bearing shoots in buoys produced fewer seedlings and required more effort than isolating, storing, and hand-broadcasting seeds in the fall. We show that viable seeds can be separated from grass wrack based on seed fall velocity and that seed survival during storage can be high (92–95% survival over 3 months). Mechanical seed-planting did not enhance seedling establishment at our sites, but may be a useful tool for evaluating restoration sites. Our work demonstrates the potential for expanding the scale of seed-based *Z. marina* restoration but the limiting factor remains the low rate of initial seedling establishment from broadcast seeds.

Key words: eelgrass, seed harvest, seed planting, seagrass restoration, seed viability, *Zostera marina*.

Introduction

Seagrasses and the numerous ecosystem services they provide are being lost at an alarming rate (Short & Wyllie-Echeverria 1996; Green & Short 2003; Orth et al. 2006*a*; Waycott et al. 2009). This has led to an increase in the number of policies, laws, and regulations to protect and conserve seagrasses in the developed countries where much of the research on seagrasses has been conducted (Duarte 1999, 2002; Kenworthy et al. 2006; Duarte et al. 2008). These conservation measures have in turn led to increased efforts to restore seagrass (Fonseca et al. 1998; Treat & Lewis 2006) with the goal of restoring lost ecosystem services.

In Chesapeake Bay, seagrasses have declined precipitously from historic levels (Orth & Moore 1983; Orth et al. 2010). In 2003, a major policy placed priority on restoring more than 400 ha (1000 acres) of submersed aquatic vegetation (SAV) by 2008 (Chesapeake Executive Council 2003). However, achieving this goal would have required dramatic expansion of the previously attempted scale of restoration, and substantially lower-cost techniques given the available funding.

Early attempts at transplanting seagrass came in the 1940s, shortly after the pandemic *Zostera marina* decline (Cottam & Munro 1954; Rasmussen 1977). Though Addy (1947) published a transplanting guide in 1947 for adult *Z. marina* plants and seeds, it was not until the 1970s and 1980s that interest in seagrass transplanting became more widespread (Fonseca et al. 1998). Since then, a wide variety of transplant techniques using primarily adult plants have been attempted in North America, Europe, and Australia with some limited success (Fonseca et al. 1998). Typically targeted at small (<0.5 ha) areas, many of these methods are labor intensive and costly. Some mechanized efforts have been attempted but were costly to develop and implement, and have shown limited success relative to manual methods (Paling et al. 2001*a*, 2001*b*; Fishman et al. 2004).

Initial attempts at *Z. marina* restoration in Chesapeake Bay began in the late 1970s, with increased efforts in the 1980s and 1990s using primarily adult plants (Orth et al. 2006*c*; Orth et al. 2010). Until recently, seeds were not widely used in seagrass restoration projects (Pickerell et al. 2005; Orth et al. 2006*c*). However, some species produce large quantities of seeds (Inglis 2000; Orth et al. 2006*c*) that are released

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over a period of weeks, allowing for targeted seed collection. Seeds can be an important mechanism for seagrass recovery following disturbances and in new patch creation (Harwell & Orth 2002; Plus et al. 2003; Greve et al. 2005; Moore & Jarvis 2008), and in one location, seeds have been used to successfully reestablish large areas of seagrass coverage (Orth et al. 2006*d*).

Limitations on the scale of seed-based *Z. marina* restoration attempts have come from three sources: acquisition and processing of seeds, maintenance of viable seed supplies, and low initial seedling establishment rates (Orth et al. 2003, 2009, 2010). The goal of the work reported here was to explore new techniques for efficient large-scale restoration of *Z. marina* populations using seeds by addressing each of these limitations. We tested several mechanical harvesting systems for expanding the scale of seed collections, and paired this with an immediate seed distribution technique (after Pickerell et al. 2005) to attempt to circumvent the infrastructure requirements for processing and holding large numbers of seeds. New techniques were developed for reducing seed-processing labor, and a series of experiments were conducted to maximize seed survival during storage. We use the terms "seed survival" to denote maintenance of viability, and "seed mortality" to denote loss of viability. Finally, a new mechanical seed-planting technique appropriate for large scales was developed and tested.

Methods

Mechanized Seed Harvest

Mechanized harvesting systems were used to acquire seeds in 2004, 2005, and 2007. In 2004, in partnership with the Maryland Department of Natural Resources, we contracted a commercial mechanical harvester for large-scale collection of *Z. marina* reproductive shoots. The barge-mounted harvester, originally designed for removing exotic species, uses a pair of horizontal, toothed cutting bars driven in opposition to cut the upper part of the canopy containing the seed-bearing spathes at an adjustable depth below the surface (Fig. 1). Paddle-wheels propel the harvester over the shallow collecting sites. Cut material is collected by a moving conveyor belt and transferred to a separate transport boat. A differential global positioning system (GPS) unit with submeter post-processed accuracy was used to record the track of the harvester, allowing identification of the total harvested area. Harvesting was conducted in two large donor beds in Mobjack Bay (lat 37.341°N, long 76.406°W) (bed area >40 ha) and the Lower York River (lat 37.262°N, long 76.399°W) (bed area >50 ha) over three days in mid-May. To assess the harvester's impact, evaluation sites were randomly chosen along the GPS track, and also at intersections of two harvested tracks. At each site, divers measured maximum leaf height (mean of four subsamples) and percent cover in one 1-m^2 plot within the harvested track and one unharvested 1-m^2 plot nearby.

In 2005, a new harvesting system was designed and custom-built at a smaller scale, allowing easy deployment and relocation. The cutting mechanism, a Lake Mower (Jensen Technologies Development Corporation, San Marcos, TX, U.S.A.), is mounted on a benthic sled pulled alongside a small boat by a beam, and is deployed by a davit and winch (Fig. 2). The height of the cutting bar can be adjusted to target taller reproductive shoots while minimizing removal of leaves. Cut material is pumped through a 7.6-cm hose from the collecting cage on the sled via a Venturi nozzle attached to a gas-powered pump, and collected in a mesh bag.

Figure 1. Commercial SAV harvester contracted in 2004. *Zostera marina* shoots are cut and collected on a conveyor belt (arrow a) for offloading to a separate transport boat (arrow b).

Aerial photographs of the donor beds were acquired on June 14, 2005, approximately two weeks after harvesting, and were inspected for visible evidence of harvesting impacts. Field assessments of the harvested sites were planned for September, four months after harvesting, but could not be completed due to a bay-wide *Z. marina* die-off related to extremely high water temperatures (Moore & Jarvis 2008).

In 2007, the harvester was reconfigured to better target small areas identified during pre-season surveys as particularly rich in reproductive shoots. The sled was relocated to the front of a shallow draft boat, and the collection apparatus was replaced with a net to passively catch the cut shoots (Fig. 3). A system of ropes holds the net open at the bottom just behind the cutting mechanism, allowing the net to be periodically manually retrieved to empty the collected shoots.

For all collections, harvested grass volume was estimated volumetrically by loosely piling damp cut shoots in a 121-L container. The seed yield of the harvested material was estimated by counting the number of spathes in 10 replicate 5-L subsamples, and the number of seeds per spathe in 100 randomly selected spathes.

Seed Separation

Harvested shoots were stored in outdoor 3,500-L tanks with flow-through seawater for approximately three weeks until all mature seeds were released from flowering shoots. Water flow was adjusted to produce a full exchange of water in approximately 2 hours, and air lines along the bottom vigorously aerated tank contents. Mesh covers shaded tank contents but did not exclude rain. Each tank held 1,000–1,300 L of harvested shoots. When shoot collections exceeded expectations, a 14,000-L plastic swimming pool was also filled, and grass

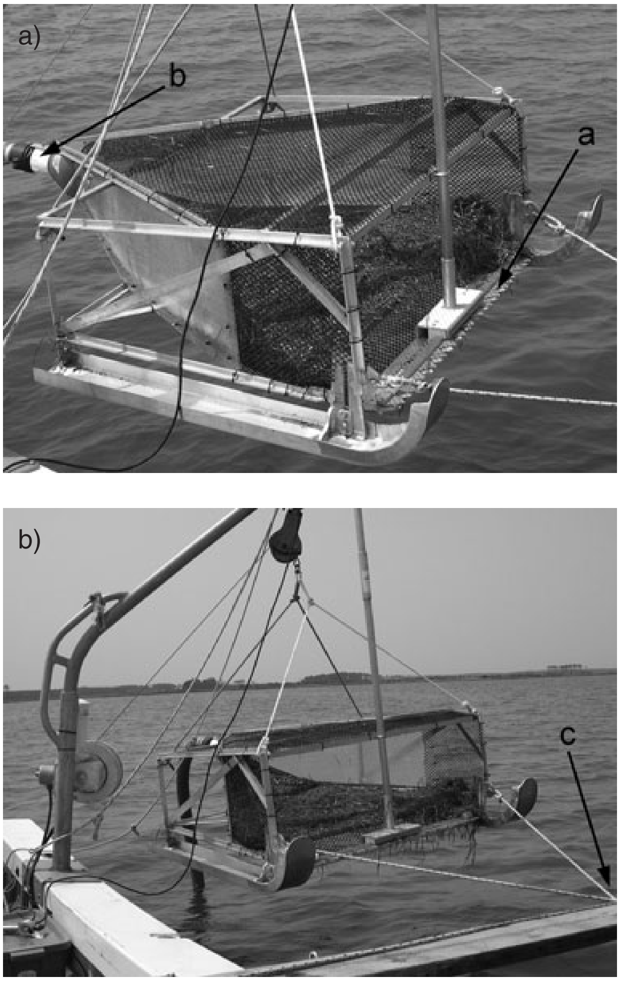

Figure 2. Small-scale harvester built in 2005. (a) *Zostera marina* shoots cut by the Lake Mower (arrow a) are directed to a vacuum hose (arrow b). A pump on the boat creates the suction using a Venturi nozzle, and the shoots are delivered to a mesh collection bag on the boat. (b) The sled is positioned for deployment by a davit from a small boat, and is pulled alongside the boat via a pulley system from a beam (arrow c).

volume, water flow, and aeration were established proportionately to mimic conditions in the smaller tanks. Tanks were stirred daily to facilitate flushing of decomposing material, and to prevent establishment of anoxic zones on the tank bottom due to accumulation of fine particles.

We used a multi-stage process to isolate seeds from the large volume of decomposing plant matter present after seed release, relying on the rapid sinking rate of viable, mature seeds (see below) to achieve separation without sieving. After three to four weeks many reproductive shoots had released their seeds, allowing a portion of the floating grass wrack to be removed. To remove the remaining submerged leaves, the following two alternative methods were developed, both suitable only for round, flat-bottomed tanks:

(1) Tanks were vigorously stirred, and after allowing seeds to fall to the bottom for at least 10 seconds, vegetative fragments were removed by dipping 1-cm mesh screens in the surface layer. This cycle was repeated until little material appeared on the screens. Then we created a strong rotational flow in the tank, using a canoe paddle or other means. The resulting vortex deposited the heaviest material (including seeds) near the center, with lighter fragments deposited closer to the tank walls. Seeds were removed from tanks by siphoning, pumping, or draining the tank onto a 1-mm mesh screen.

(2) The 5.5-m-diameter circular pool was filled with water, and the water inflow spigot was pointed along the perimeter to create a slow, circular flow, approximately 25 seconds/revolution. A mixture of grass and seeds from a nearby holding tank was slowly added near the inflow using a diaphragm pump, which can pass seeds without damaging them. The water inflow rate was adjusted until almost all grass fragments settled to the bottom before circulating back to the point of entry. Finally, the water level was lowered and the material that had settled nearest the introduction point, including viable seeds, was siphoned out.

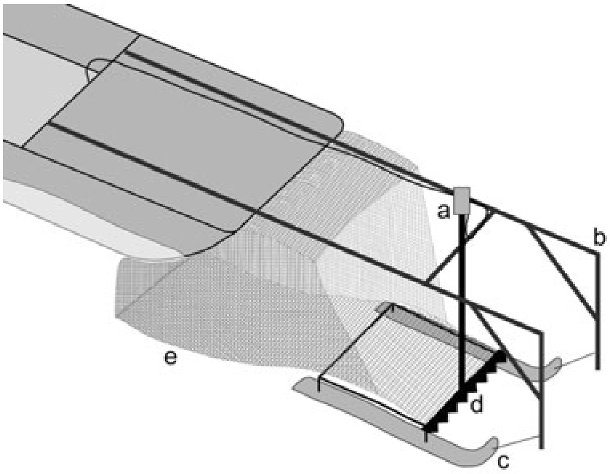

Figure 3. Schematic diagram of 2007 *Zostera marina* seed harvester. (a) Electric motor powering cutting mechanism; (b) cantilevered strut extending from bow of boat to pull sled; (c) aluminum sled; (d) cutting mechanism; (e) grass collection net.

For the final separation stage, the isolated product of the primary separation was introduced to a small flow-through seawater flume. The bottom of the flume was 15 cm below the level of the entering water, such that seeds falling to the bottom entered a low-flow zone and were not resuspended. Water column height and flow speed were carefully calibrated to insure that seeds settled to the bottom before reaching the drain, while the lighter detritus was flushed out the drain. Seeds were siphoned from the bottom of the flume, and then sieved through stacked 2.0-, 1.4-, and 1.0-mm sieves to remove most remaining spathe fragments, shells, worms, and sand.

Seed Quality Determination

In the absence of any rapid method to determine viability of large numbers of seeds without destroying the seeds (e.g. tetrazolium staining), we have traditionally assessed seed quality by individually examining seeds in subsamples and categorizing each seed as "good" (firm seeds resisting compression when squeezed lightly with forceps, having an intact seed coat, and sinking rapidly in seawater), or "bad" (soft, damaged, or slow-sinking). Fall velocity was subjectively assessed by dropping each seed in a watch glass filled with seawater. The assumption that "good" seeds are viable is supported by observations of >90% germination of these seeds planted in sediment in lab conditions (Orth, unpublished data). In order to assess the utility of fall velocity for identifying viable seeds (as well for separating viable seeds using the seed isolation techniques described above), we tested the relationship between fall velocity and seed viability. In October 2006, we measured the fall rate of 316 individual seeds in an aquarium with 22 cm of water at salinity 20, then planted the seeds and tracked the germination success of each seed. Seeds were individually planted 5–7-mm deep in sieved, sandy (>95% sand) sediments in 10-L containers within temperature-regulated recirculating seawater tanks that mimicked ambient river temperature changes. Seed outcomes were determined three months after planting in February, after the emergence of new seedlings was complete.

Seed Storage

A series of experiments were conducted to identify conditions minimizing loss of seed viability during storage. Initial hypotheses focused on seed mortality in anoxic conditions, and transitioned to examining roles of salinity and temperature. For each experiment, the number of "good" seeds was counted in five 2-mL subsamples from a single seed source. Seed batches with the desired number of "good" seeds for each treatment were created volumetrically. At the end of the experiment, the volume of each seed batch was again measured, the condition of all seeds in 5 2-mL subsamples was assessed, and seed mortality (percentage of seeds in the batch transitioning from "good" to "bad" condition) was calculated.

Results were analyzed with R statistical software (R Development Core Team 2008) by logistic regression. Logistic regression is appropriate for binary response variables, and we used survival versus mortality of each seed as the binary response. The data for the 2004 experiment exhibited overdispersion (more variability than expected under a strict binomial distribution); so we employed a quasibinomial distribution rather than a standard logistic regression (McCullagh & Nelder 1989). This procedure allows the software to adjust the modeled distribution to account for overdispersion, producing a more appropriate assessment of treatment differences. Analyses for the 2005 and 2006 experiments employed logistic regression without overdispersion correction.

2004 Aeration and Layering Experiment. Immediately following seed isolation in July 2004, five seed treatments were established by nesting 10-L tubs in a single outdoor, flow-through seawater tank. Three treatments manipulated air delivery for batches of seeds in flow-through, raw seawater: (1) no added air and no seed disturbance ("no air" treatment); (2) air delivered at approximately 0.8 L/min through two airstones placed among the seeds, with weekly mixing to winnow any accumulated fine sediments ("low air"); and (3) continuous turnover of seeds in a cone-bottom container with air introduced at approximately 1.5 L/min at the bottom ("high air"). Two additional treatments placed seeds under a substrate: (1) seeds were covered with 2–3 cm of clean, sieved sand ("sand"); and (2) seeds were covered with 2–3 cm of *Z. marina* leaf wrack ("grass"). Three replicates of all five treatments were created, each initially receiving 500 mL of seeds (approximately 53,000 viable seeds). The experiment was terminated in late September and the number of surviving seeds calculated as described above. The conventional "low air" treatment was used as the reference level in analysis for comparison with the four alternative treatments.

2005 Aeration Experiment. Three treatments manipulating air delivery, slightly modified from the 2004 treatments, were nested within three different water supply systems. Modifications to the air treatments involved increasing the frequency of stirring in the "low air" treatment to daily, and establishing the "high air" treatments in circulating airlift chambers made of 7.6-cm-diameter PVC tubing. The chambers insured a continuously oxygenated seed environment using a vertical stream of air bubbles to induce a constant flow, preventing seeds and sediments from settling at the bottom. The three water supply systems consisted of the following: (1) an outdoor tank with flow-through raw seawater; (2) an outdoor tank with flow-through filtered seawater; and (3) an indoor tank with recirculating, filtered, UV-sterilized, temperature-controlled seawater, in a greenhouse with ambient lighting. Four replicates of each treatment combination were created using 150 mL of seeds (approximately 17,000 viable seeds), for a total of 36 seed batches and 612,000 seeds. Separate logistic regressions compared air treatments within each of the three water supply treatments.

2005 Minimal-maintenance Experiment. To investigate alternative seed storage strategies that would minimize water treatment needs (i.e. recirculating pump, filter, UV sterilizer, and chiller), seeds were held in an air-conditioned room (20–24°C) in a thin layer just a few millimeters deep on the bottom of 1.85-L tubs containing artificial seawater with two airstones. To test whether unwanted growth of microbes could be reduced by initial bleach treatment (e.g. Moore et al. 1993), three replicate batches of 16,000 seeds each were briefly rinsed in a 5% bleach solution, and then repeatedly rinsed in deionized (DI) water before being placed in the tubs at a salinity of 20. Three other replicates were rinsed in only DI water. Salinity was adjusted periodically to compensate for evaporation, but seeds were otherwise left undisturbed between July and October.

2006 Salinity and Temperature Experiment. A final seed storage experiment examined the interactive effects of salinity and temperature on seed survival during July–October storage. Each treatment consisted of three 1.85-L containers holding 35,000 seeds, placed in a single 60-L aquarium. Each aquarium was filled with artificial seawater at a salinity of 12, 20, or 30. Three aquaria (one per salinity treatment) were held indoors at 21–24°C or in a temperature-controlled water bath in a shaded greenhouse at 23–28°C. Similar salinity treatments were created at 4°C in a refrigerator, but due to space constraints, nine independent containers (three for each salinity) were used without a surrounding water bath. These containers were loosely covered to reduce evaporation. Salinity in all treatments was monitored twice weekly using a YSI 85 sonde (YSI, Inc., Yellow Springs, OH, U.S.A.) and adjusted as necessary, and oxygen saturation was maintained in each aquarium with two airstones. Separate logistic regressions compared salinity treatments within each of the three temperature regimes.

Seed Dispersal

Buoy-based Seeding. In 2004, a new technique adapted from Pickerell et al. (2005) for distributing *Z. marina* seeds immediately after harvest in May was compared with the traditional broadcast of seeds in October. Mechanically harvested *Z. marina* was transported in bulk to restoration sites and used to fill 0.5-m-long 1-cm mesh tubes (Fig. 4). The seed yield of the harvested material was assessed initially by counting the number of spathes in 10 replicate 5-L subsamples, and the number of seeds per spathe in 100 randomly selected spathes. Only moderately developed seeds were counted, so estimates of the number of viable seeds distributed were conservative. The estimated volume of grass holding 5,000 seeds (approximately 3.7 L) was placed in each tube along with two small floats. Each mesh bag was tied by 5 m of polypropylene rope to a cinder block or to two bricks. Completed buoy assemblies were deployed in 0.8–2-ha plots at 12-m spacing to achieve a 370,000 seeds/ha seeding density. Our approach departed from the method of Pickerell et al. (2005) in these primary ways: (1) mechanically harvested reproductive shoots were used instead of shoots collected from shoreline wrack; (2) the mechanically harvested material contained many partial reproductive shoots and vegetative shoots; and (3) buoy construction was simplified by using mesh bags without any frame.

Figure 4. Seed distribution buoy containing seed-bearing *Zostera marina* reproductive shoots.

In a separate experiment we quantified individual buoy seedling yield. For six buoys we counted seed-bearing spathes to insure 5,000 seeds per buoy, and deployed the buoys at 50-m spacing to insure that all emerging seedlings could be attributed to a single buoy. In addition, we individually deployed 12 buoys haphazardly selected from among those filled volumetrically for restoration plots. All buoy assemblies were retrieved approximately one month after deployment and any material remaining in the bags was released on the spot. The following April, divers counted all seedlings within 15 m of the buoy anchoring point.

Hand Broadcasting. For comparison with the buoy distribution method, batches of seeds from the same collection were stored through the summer and measured volumetrically in October of 2004. The number of viable seeds was estimated in five 2-mL subsamples and used to allocate batches of 75,000 and 150,000 seeds. These batches were then broadcast by hand into 0.2- or 0.4-ha plots to achieve a density of 370,000 seeds/ha.

Seed Planting. Based on preliminary investigations showing increased seedling establishment rates at some sites through direct seed burial (Orth et al. 2009; unpublished data), we developed a system capable of rapidly injecting seeds directly into sediments over large areas. While the previous system developed by Traber et al. (2003) actively pumped seeds suspended in a gel matrix through distribution tubes, this new system uses a gravity-driven flow of water to deliver seeds through supply tubes to a planting sled pulled behind a boat. Seeds are measured by hand into a central hopper mounted 2 m above the water surface and are rinsed down by a pumped water supply. The water flow exiting the hopper is diverted into eight secondary funnels arrayed in a circle by a rotating arm driven by a centrally mounted motor, so that each secondary funnel receives a pulse of water and seeds approximately once per second. Seeds flow out of the secondary funnels into 7-mm inner diameter plastic tubes leading to the planting sled, with a separate water supply into each funnel providing a constant flow. At the planting sled, eight angled stainless-steel planting tines allow the seeds and water to exit just below the sediment surface as the sled is dragged forward. The sled has no moving parts, and simply holds the planting tines at the target depth. Field trials with divers observing the planting showed that in the sandy sediments at our restoration sites, seeds were trapped under the sediment surface without any additional burial mechanism as long as the forward speed of the sled exceeded the flow rate of water out of the planting tines.

The planter was evaluated in October 2007 by creating a single 3 × 40-m test plot of 10,000 seeds at each of three potential restoration sites in the Piankatank River (lat 37.504°N, long

Table 1. *Zostera marina* seed harvesting yields.

Harvest Technique	*Year*	*L/hour*	*Seeds/L*	*Seeds/labor-hour*
Mechanical	2004	1,080	1,340*	132,000*
	2005	390	430	84,000
	2007	480	230	55,000
Manual	2001	ND	NA	25,000
	2002	ND	NA	10,000
	2003	ND	NA	11,000
	2006	43	NA	19,000
	2007	100	NA	62,000
	2008	45	NA	13,000

Liters refer to estimates of grass volume collected (see *Methods*).
ND: no data; NA: not applicable.
* Seed estimates derived from counts of seed-bearing spathes. Seed counts for other years are direct volumetric measurements of isolated seeds.

76.330°W), York River (lat 37.268°N, long 76.515°W), and Spider Crab Bay (lat 37.357°N, long 75.802°W), a shallow coastal bay on Virginia's Eastern Shore. At each site a similarly sized plot was created 100 m away by hand-broadcasting 10,000 seeds. In April 2008, divers counted all seedlings within a series of 2-m-wide transects running perpendicular to the axis of the long, rectangular plots. One-third of each plot's total area was evaluated.

Results

Mechanized Seed Harvest

The commercial harvester used in 2004 (Fig. 1) accumulated *Zostera marina* at a mean rate of 1,080 L/hour of active cutting, with the accumulation rate dependent on the height and density of the seagrass canopy. The largest one-day harvest totaled approximately 5,000 L of grass yielding 6.7 million seeds. The average collection rate was 132,000 seeds/person-hour (Table 1), calculated by dividing the estimated seed yield by the labor expended during active harvesting operations by many individuals involved in operating the harvester and handling the grass. This does not include labor related to setup, travel to the site, breaks, or post-collection processing. In comparison, the mean seed accumulation rate for manual reproductive shoot collections over five "typical" years (2001, 2002, 2003, 2006, and 2008) was approximately 16,000 seeds/person-hour (Table 1), excluding the extraordinary yields achieved in 2007 resulting from an anomalously high seed output in one donor bed. GIS analysis determined that the total harvested area was less than 10% of the total bed area at each site. Diver measurements showed no significant difference in canopy cover between harvested and nearby unharvested plots in June in either donor bed (paired t-tests, $p = 0.24$ and $p = 0.13$, Fig. 5a). Maximum leaf height was lower in harvested plots by an average of approximately 8% (paired t-tests, $p < 0.001$ and $p = 0.014$, Fig. 5b).

The beam-pulled harvester used in 2005 (Fig. 2) was also effective at collecting reproductive shoots without removing excessive vegetative cover. Divers observing the operation documented the most effective collections during the early part of the seed release period (after May 17, 2005) when reproductive shoots stood most erect, though collections extended through June 7. The easily relocated cutting machine permitted moving the harvester to seed-rich sites that would have been inaccessible by the larger machine. A total of 11,397 L of shoot material was harvested and processed at VIMS, producing an initial seed yield of 2.5 million seeds. The mean collection rate was 390 L/hour, resulting in a yield of 84,000 seeds/person-hour. Less than 5% of the total bed area at each of the two harvesting sites was traversed by the harvester. GIS overlay of the harvester track on aerial photography from June 14 revealed no visible trace of the harvesting (Orth & Marion 2007).

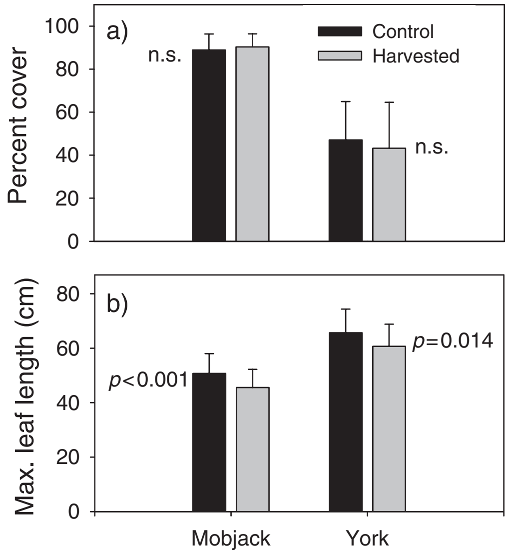

Figure 5. 2004 mechanized harvesting impact study. (a) Mean (+SD) percent cover in harvested and control plots. (b) Mean (+SD) height of tallest *Zostera marina* leaf. p-values indicate results of paired t-tests at each site. Mobjack $n = 31$, York $n = 17$.

The revised, bow-mounted harvester used in 2007 (Fig. 3) accumulated shoots at approximately 480 L/hour. The net captured the cut shoots effectively, but required substantial effort to repeatedly retrieve and empty. With only moderate seed production at the harvesting site used for the machine in 2007 (which was different from the site used for hand collections that year), the harvester yielded 55,000 seeds/person-hour.

Seed Separation

Both the rotational water flow in the swimming pool and the linear water flow in the flume were extremely effective at separating high-quality, dense seeds from grass wrack. In the pool, high-quality seeds fell several feet downflow from the entry point, whereas most grass wrack settled on the far side of the pool, with fine particles carried in eddies and eventually settling near the center of the pool. With increasing distance from the point of entry in each apparatus, a gradual reduction in seed quality was observed beyond the initial concentrated pile of very high-quality seeds. Once constructed, the flume was more efficient than the pool due to the time required to repeatedly raise and lower water levels and to remove waste material in the latter.

Seed Quality Determination

One hundred and ninety-three of the 316 seeds (61%) representing the full range of fall rates germinated (Fig. 6). Few seeds falling slower than 5 cm/second produced seedlings, and the 5.0–5.5 cm/second range was a critical transition zone, with the proportion of seeds producing seedlings rising sharply for seeds falling faster than 5.5 cm/second (Fig. 6d). Only 14% of seeds falling slower than 5.0 cm/second produced seedlings (18 of 133), whereas 89% of seeds falling 5.5 cm/second or faster did so (133 of 150). Among the recovered non-germinating seeds, 95% of those falling slower than 5.0 cm/second (82 of 86) were assessed as poor quality seeds (soft or structurally degraded), compared to 38% of those falling faster than 5.5 cm/second (5 of 13).

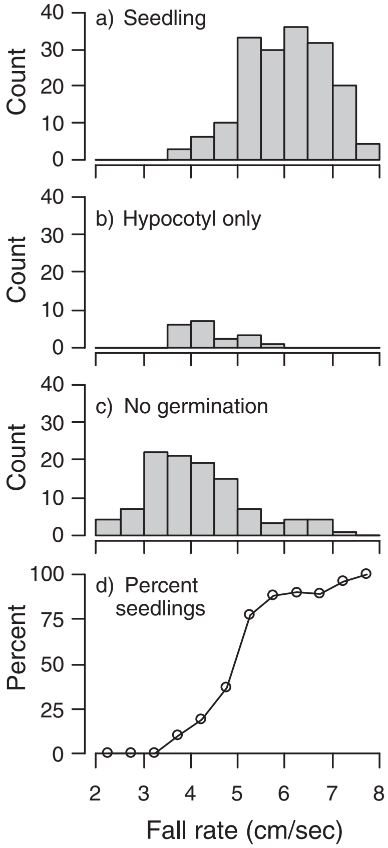

Figure 6. Histograms of *Zostera marina* seed fall rates for seeds that later produced one of the three germination outcomes (a–c), and the percent of seeds in each 0.5 cm/second range that produced seedlings (d). "Seedling" refers to seeds that produced a leaf, regardless of whether it emerged from the sediment. "Hypocotyl only" refers to seeds that initiated germination but failed to produce a leaf before the experiment's termination.

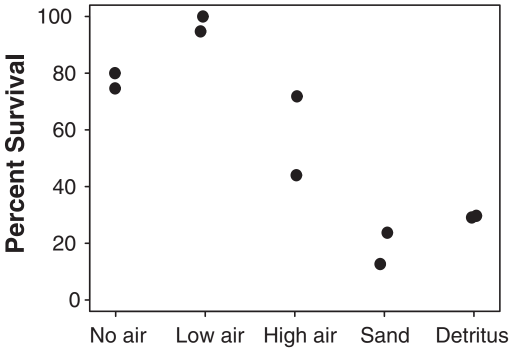

Figure 7. Seed survival rates for the 2004 seed aeration and layering experiment ($n = 2$). Each replicate held either 53,000 isolated seeds (the three air treatments) or the same number of seeds layered under sand or leaf wrack (see *Methods*).

Table 2. Logistic regression results for 2004 summer seed storage experiment comparing four alternative storage conditions with the conventional "low air" treatment ($n = 2$).

		95% Confidence Interval				
Treatment	*Coefficient*	*Lower*	*Upper*	*SE*	*T*	*p*
No air	2.38	0.26	4.50	1.08	2.20	0.079
High air	−0.91	−1.90	0.09	0.51	−1.79	0.133
Sand	−2.73	−3.85	−1.61	0.57	−4.78	0.005
Detritus	−2.10	−3.13	−1.07	0.53	−4.00	0.010

Seed Storage

2004 Aeration and Layering Experiment. Seeds held in outdoor tanks survived best during summertime storage in lightly aerated containers (Fig. 7). Data were compromised for one replicate by methodological error, so analyses were conducted with only two replicates. Logistic regression showed significantly reduced likelihood of survival for seeds buried under either seagrass detritus or sand relative to seeds in the low-air treatment (Table 2). Although the two alternative aeration treatments were not statistically different from the Low-air treatment, this is not a robust comparison result given the low replication.

2005 Aeration Experiment. Holding seeds indoors in clean, recirculating, temperature-controlled water without aeration or disturbance produced the highest mean seed survival (Fig. 8). In comparison, logistic regression showed a slight but significant reduction in survival for seeds receiving light aeration and mixing, whereas highly aerated seeds survived at similar rates (Table 3). Seed survival was generally lower and more variable in flow-through treatments receiving either raw or filtered water.

2005 Minimal-maintenance Experiment. The overall seed survival rate of the thin layer of seeds held in individual, untreated tubs was 47% (±9% SD). The initial bleach rinse had no effect; survival was 48% (±9% SD) in bleach-rinsed batches and 46% (±11% SD) in DI-rinsed batches.

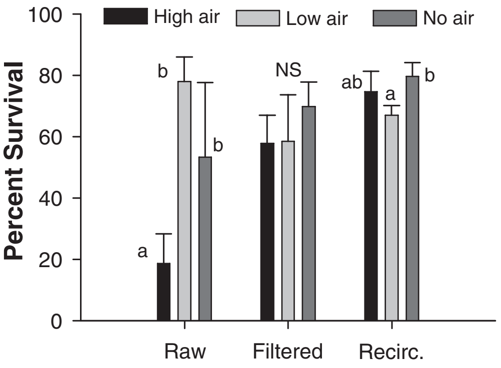

Figure 8. 2005 summer seed storage experiment comparing three aeration treatments within each of three water sources ($n = 4$). Differing lowercase letters within each water treatment indicate significantly different means. NS = non-significant.

2006 Salinity and Temperature Experiment. High salinity maximized seed survival across all three temperature regimes tested (Fig. 9a). Logistic regression showed significantly higher survival at salinity 30 than at salinity 12 at all temperatures (Table 4). Survival at salinity 30 was also higher than at salinity 20 in cold (4°C) and hot (23–28°C) treatments. Low temperatures and low salinity triggered substantial seed germination during storage, rendering the germinated seeds unusable for field distribution (Fig. 9b).

Seed Dispersal

Buoy-based Seeding. Plots seeded with reproductive shoot-bearing buoys had substantially lower seedling establishment rates (approximately 1% of seeds deployed) than plots seeded by hand-broadcasting seeds isolated from the same collections (approximately 5%) (Table 5). Test buoys containing only reproductive shoots with a known number of seed-bearing spathes, intended to provide a precise assessment of seedling yields, appeared much less successful (0.1%) than the buoys used for large-scale restoration plots (1%). This may have been a result of the physical differences between the buoy types, because the test buoys contained no vegetative shoots, perhaps allowing reproductive shoots to more easily escape the mesh netting and float away from the evaluated test plot.

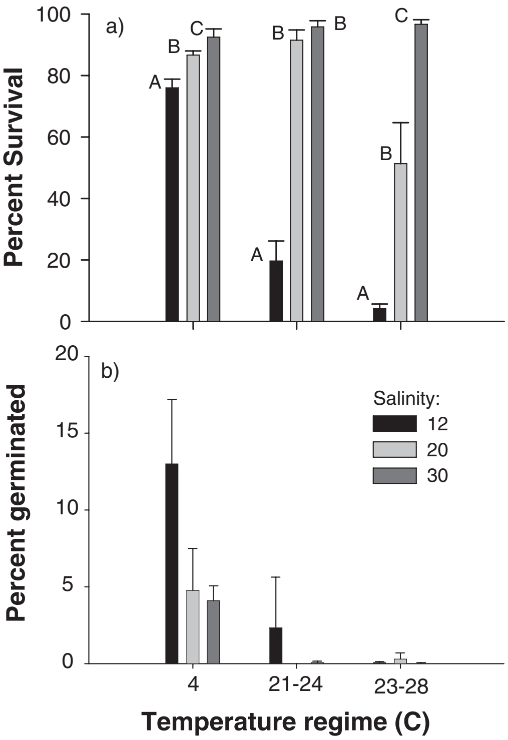

Figure 9. 2006 summer seed storage experiment comparing three salinity treatments within each of three temperature regimes (mean +SD, $n = 3$). (a) Seed survival. Differing lowercase letters within each temperature regime indicate significantly different means (logistic regression, Table 4). (b) Percent of seeds germinating during storage. Germinated seeds are not subsequently available for restoration, so they are not considered "surviving" in panel a.

Many buoys in Spider Crab Bay accumulated a coating of drifting macroalgae, which may have reduced seed release or influenced seed development. Buoys were typically heavily populated with amphipods and juvenile crabs.

Seed Planting. Seedling establishment rates (the proportion of seeds generating a seedling surviving until April) at the Piankatank River site were 28% in both planted and broadcast test plots, dramatically higher than previously observed (typically 1–5%) at that site. At the York River site, seedling

Table 3. Logistic regression results for 2005 summer seed storage experiment comparing three aeration treatments' conditions within each of three water sources ($n = 4$).

				95% Confidence Interval				
Water source	*Reference Treatment*	*Test Treatment*	*Coefficient*	*Lower*	*Upper*	*SE*	*T*	*p*
Raw	Low air	No air	1.13	0.11	2.16	0.52	2.17	0.059
	High air	No air	−1.60	−2.67	−0.54	0.54	−2.95	0.016
	High air	Low air	−2.74	−3.89	−1.59	0.59	−4.66	0.001
Filtered (Non-significant model)								
Recirculating	Low air	No air	−0.66	−1.04	−0.29	0.19	−3.50	0.007
	High air	No air	−0.28	−0.67	0.10	0.20	−1.45	0.182
	High air	Low air	0.38	0.02	0.74	0.18	2.09	0.066

Table 4. Logistic regression results for 2006 summer seed storage experiment comparing three salinity treatments within each of three temperature regimes ($n = 3$).

				95% Confidence Interval				
Temperature (°C)	*Test Salinity*	*Reference Salinity*	*Coefficient*	*Lower*	*Upper*	*SE*	*T*	*p*
1–4	12	30	−1.35	−1.72	−0.97	0.19	−7.04	<0.001
	20	30	−0.63	−1.03	−0.22	0.21	−3.04	0.023
	20	12	0.72	0.40	1.04	0.16	4.44	0.004
21–24	12	30	−4.53	−5.34	−3.71	0.42	−10.87	<0.001
	20	30	−0.74	−1.64	0.16	0.46	−1.61	0.160
	20	12	3.79	3.15	4.43	0.33	11.55	<0.001
23–28	12	30	−6.49	−7.90	−5.08	0.72	−9.01	<0.001
	20	30	−3.32	−4.44	−2.20	0.57	−5.81	0.001
	20	12	3.17	2.16	4.18	0.52	6.15	0.001

Table 5. Initial success (seedlings established as a percent of seeds distributed) measured by direct diver counts in April 2005 after seeding in either May 2004 (for buoys) or October 2004 (for broadcasts) in Spider Crab Bay.

				% Initial Success		
Method	*Plot Type*[a]	*Distribution Scale*	*Month Seeded*	*Mean*	*SD*	*n*
Buoy	Test plot	Single 5,000-seed buoy[b]	May	0.09	0.05	6
Buoy	Test plot	Single “sample” buoy[c]	May	1.00	0.72	12
Buoy	Restoration plot	144-buoy array	May	1.11	1.01	3
Broadcast	Test plot	5,000-seed broadcast	October	5.50	1.40	5
Broadcast	Restoration plot	150,000-seed broadcast	October	3.98	2.90	4

[a] All seedlings emerging in test plots were individually counted, whereas restoration plots were evaluated with two sampled transects.

[b] Reproductive shoots containing a known number of spathes were used to load each test buoy with approximately 5,000 seeds.

[c] Sample buoys were haphazardly selected from among those being deployed in large-scale restoration plots.

establishment in the planted plot was 8%, similar to previous years’ typical rates for seed broadcasting, whereas the broadcast plot had 14% establishment. Only four seedlings were observed in both plots at the coastal bay site, which may have been impacted by an overgrowth of algae. In both rivers, many seedlings emerging in the machine-planted test plots were visibly aligned in rows produced by each planting tine, and over 90% were found within 3 m to either side of the central axis of the planted plots. In the broadcast plot at the Piankatank River site, where tidal currents ran perpendicular to the long axis of the test plot, only 62% of seedlings were found within 3 m of the plot’s center. At the York River site, where tidal currents ran along the plot’s axis, however, 92% of seedlings were within that distance. Divers observed few seeds on the sediment surface immediately after planting, confirming that most seeds were retained under the sand. Together with the previous observations of seed transport, these data suggest that at our sites, currents are the primary mechanism for locally redistributing seeds short distances on unvegetated shoals, and that minimal redistribution takes place once seeds are covered with sediment.

The planting system appeared to reduce clumping of seedlings. In broadcast plots, 29 and 19% of seedlings were found in high-density clusters in the York and Piankatank Rivers, respectively, whereas in the planted plots only 9 and 13% of seedlings were found in clusters. Seedlings growing in high-density clusters are expected to experience higher mortality as a result of competition among closely packed shoots (Orth et al. 2009).

Discussion

Mechanized Seed Collection

Three years of investigations with three different mechanical harvesting systems demonstrate a strong potential for collecting large numbers of *Zostera marina* seeds if a number of conditions are satisfied. First, large donor beds with high densities of reproductive shoots must be available if collection, storage, and processing of the harvested material are to surpass manual shoot collection in efficiency. Reproductive shoot densities were typically 100–200/m^2 at our donor sites, with a wide range (approximately 20–100) of seeds per shoot. Second, seed-rich locations within the donor beds must be identified, and the timing of harvesting must be adjusted to take place within a narrow (1–2 weeks) window. Third, infrastructure must be in place to either store large quantities of harvested material, or rapidly deploy harvested shoots to restoration sites.

The bow-mounted harvesting system used in 2007 was the most cost-effective and adaptable of the mechanized harvesting systems we tested. Although it could not match the collection scale of the commercial harvester and required substantial effort to regularly empty the collection net, the system allowed precise targeting of seed-rich areas and easy transport to multiple remote harvesting locations. The commercial harvester used in 2004, while capable of rapid collections, was expensive to contract, difficult to transport to distant collection sites, and limited in its depth range and cutting height precision. The harvesting system deployed alongside the boat in 2005 proved difficult to maneuver as the drag of the sled imposed a constantly curving boat trajectory, and the suction system was susceptible to clogs in the delivery hose. Redesigning the bow-mounted system to accommodate a larger-diameter collection hose rather than a collection net might be an optimal solution, theoretically capable of delivering over 200,000 seeds/hour with a crew of two in an average year (i.e. 480 L/hour × 430 seeds/L).

Seed production in donor beds can vary dramatically from year to year, as exemplified by the extraordinary effectiveness of manual collections in 2007 compared to 2008. Although most years' collections targeted multiple donor beds, the 2007 and 2008 collections took place in the same rapidly expanding, restored coastal bay *Z. marina* bed (Orth et al. 2006*d*), yet collection efficiency differed dramatically. As the three harvesting systems were used in different years and would likely have produced similar seed yields per liter of grass if tested simultaneously, the best direct comparison of their effectiveness is the rate of grass accumulation.

The 2004 impact study results showing only slight reduction of leaf height suggest that most leaves were oriented at a low angle in the canopy during cutting, allowing much of the canopy to escape cutting, because the cutting blades were closer to the bottom than the leaf heights observed. A subsequent use of the 2007 harvester in 2009 at a different site with a taller (0.6 m), erect canopy, and little wave action resulted in a much more systematic removal of the canopy to approximately 18 cm height within the harvested track. Divers surveying multiple tracks 60 days after the 2009 cutting found no visible indications of the harvested tracks in terms of canopy height or algal accumulation, reflecting the very rapid leaf production for *Z. marina* in our region during this time period (Orth & Moore 1986).

We consider the cumulative evidence from the field-based and photographic aerial observations of harvesting impacts to suggest that large-scale mechanized harvesting, if carefully directed to appropriate donor beds and limited in both cutting height and proportion of the bed area harvested, can effectively collect *Z. marina* seeds with minimal impact on the health of the donor bed. While temporary reduction of ecosystem services provided by the canopy would need to be carefully considered if intensive harvesting were planned, the lack of damage to the plants' rhizomes, and the rapid leaf regrowth suggest less donor bed impact than would be caused by propeller scarring or use as an adult-plant transplanting source. Longer-term and broader-scale studies are needed to evaluate what proportion of total seed production can be safely removed from a donor bed without adversely affecting its ability to recover from natural perturbations.

Seed Processing and Storage

Direct comparison of average seed quality in different batches of seeds has largely been an unproductive exercise, because seed separation techniques determine how many low-density, poor quality seeds are included in the initial isolate. We use the term seed quality rather than seed viability to reflect our separation of firm, rapidly falling seeds, which are very likely to be viable, from soft, slow-falling seeds, which are very unlikely to be viable. Once seeds are isolated, however, repeated assessments of seed quality in a given seed batch can be used to track changes in quality for each batch (as in the seed storage experiments described here). For the seed separation techniques we describe, the initial concentration of viable seeds is largely dependent on how much of the pile of low-density seeds is included during final extraction. This is not an easily quantified process due to slight variations in water height and flow speed. We used an inclusive strategy, preferring to retain all potentially viable seeds, because inclusion of poor quality seeds is thought to incur a very low incremental cost during seed storage. Seed quality tests typically indicated 85–90% viable seeds (assessed as described above) at the time of initial extraction.

Three years of seed storage experiments provide a much clearer picture of the conditions allowing *Z. marina* seed survival. High salinity (20–30) and controlled temperatures (21–24°C) in recirculating water systems (preventing accumulation of organic matter) emerged as the most important factors. Direct aeration and regular mixing of the seed pile, once thought necessary to prevent anoxic conditions from possibly triggering seed germination (Kawasaki 1993; Moore et al. 1993; Brenchley & Probert 1998), were likely beneficial primarily in limiting the development of anaerobic zones which may have generated toxic sulfides. We now know that seeds can be left without disturbance in recirculating systems if almost all excess organic materials are removed during seed separation. Oxygenating the surrounding water with airstones is necessary for creating water flow and preventing growth of anaerobic bacteria but the seed pile itself may be left undisturbed. Further qualitative observations from two subsequent years of storing seeds in the bottom of 10-L tubs within recirculating tanks (salinity 25, temperature 18–20°C) suggest that the depth of the seed pile in storage containers should be restricted to less than 3–4 cm. Combined with the results of the 2005 minimal-maintenance experiment showing that very thin layers of undisturbed seeds in well-oxygenated, clean water exhibited poor survival (47%), these observations suggest that there may be an optimal redox level for seed survival, with poorer survival under both highly reducing conditions (e.g. Hootsmans et al. 1987) and fully oxygenated conditions.

Fall velocity was confirmed to be a useful tool for identifying seeds with high potential for producing seedlings, with a rapid reduction of seed viability below 5 cm/second in water at salinity 20. While we had not precisely measured the velocities of seeds considered to be viable in each years' experiments and restoration efforts, our threshold in subjectively estimating fall velocity for categorizing "good" seeds (firm, intact seeds presumed to be viable) after individual squeezing was at least 5 cm/second, suggesting a conservative identification of viable seeds (i.e. few bad seeds misidentified as good). Variation in seed size (Wyllie-Echeverria et al. 2003) likely influences fall velocity and may be partly responsible for the mixture of germinating and non-germinating seeds observed even at higher fall velocities. Separate investigation of fall velocity for viable seeds may be necessary for *Z. marina* populations with seeds larger or smaller than those in our area, generally 3.0 ± 0.49 (SD) mm long, 1.3 ± 0.23-mm wide (Orth et al. 1994).

Seed Dispersal

While the buoy-based seed distribution method circumvented the need for grass storage infrastructure and processing labor, other constraints were imposed by the material-handling requirements of the buoy-based method. Given a fixed level of effort and narrow collection window, potential harvesting effort had to be transferred to grass transport, buoy construction and distribution. Buoy distribution immediately following the harvest also presented logistical challenges due to weather and tide conditions. Flexibility to adaptively target optimal seed collections was lost due to the coordination of collection and distribution efforts. A large on-site crew was required to construct, deploy, and retrieve buoys, possibly exceeding the total effort required to maintain and process reproductive shoots by traditional methods. Although the method used by Pickerell et al. (2005) is well suited for its originally intended context capitalizing on volunteer-based effort and small-scale collection of *Z. marina* wrack, the modifications we made were necessary to adapt their method to our potential seed sources (mechanically harvested shoots), sites (e.g. high-flow, heavily fouled sites where a wire frame in each buoy would produce excess drag), and desired scale. The combination of low seedling establishment at our sites, high labor requirements, and logistical constraints do not recommend our modification of the Pickerell et al. (2005) method for large-scale application in our region given the available alternatives. While seed maturation in the buoys may potentially have been reduced due to fouling by macroalgae, overpacking with shoots, or perhaps seed predation, and this may have contributed to low seedling establishment rates, other observations of very low success rates for seeds broadcast at the same sites in summer (Marion & Orth 2010) suggest that our results may be explained by processes occurring after seeds are released from the buoys. Seed predation or mortality may limit seedling establishment for any seeds present in summer, regardless of the method by which they were deployed.

The seed-planting machine demonstrated that seeds can be rapidly and successfully planted over large scales using inexpensive materials. Seedling establishment was not enhanced by seed planting, relative to manual seed broadcasting, in this preliminary assessment. However, the uncharacteristically high seedling success for hand-broadcast seeds at the two riverine sites suggests that unidentified factors typically impacting developing seedlings, perhaps winter storms and bioturbation, were diminished during the period following our test planting. The usefulness of seed planting for large-scale restoration efforts remains unresolved, but our preliminary evidence suggests that it may be most useful at high-energy sites with unconsolidated sandy sediments where planting seeds deeply might confer a benefit in reducing physical removal of early-stage seedlings from the sediment.

Conclusions

Our work has demonstrated the feasibility of a variety of seed collection, storage, and dispersal techniques for large-scale *Zostera marina* restoration. Several of these techniques are now being used in the successful reintroduction of *Z. marina* to Virginia's coastal lagoons (Orth et al. 2006*d*).

While most seagrass restoration efforts have relied principally on adult plants (Paling et al. 2009), seed-based efforts may be possible for other species given that some seagrass species produce seeds in large quantities on an annual basis (Inglis 2000; Orth et al. 2006*b*). Some of our techniques have applicability for seagrass species producing abundant, easy-to-harvest seeds that have some degree of dormancy (e.g. all species in the family Zosteracea) (Orth et al. 2000). For species that produce abundant large fleshy fruits with seeds with no dormancy (e.g. species in the family Posidoniacea), manual techniques might be more appropriate. For example, Kirkman (1998) collected thousands of *Posidonia australis* fruits, although seedling performance was poor. Mechanical harvesting techniques would not be efficient for species (or *Z. marina* populations) with low densities of flowering shoots, and would not be appropriate for species with slow leaf growth that would compromise rapid recovery. Seed isolation and planting techniques would also need to be adapted to other species' seed size and seed density, and optimal storage conditions examined for each species. At deeper restoration sites, seed distribution techniques (especially seed planting) might need substantial modification.

Although efficient techniques for large-scale seagrass restoration have the potential to dramatically reduce the cost per hectare of initiating restoration attempts, they do not substitute for identification and correction of the factors that caused and maintained seagrass loss at a proposed restoration site. Substantial project costs are incurred by the need for careful site selection and monitoring long after seeding (e.g. Fonseca et al. 1998). Increasing the number of seeds available for restoration through the adoption of more efficient techniques does, however, present opportunities for expanding the scale of exploratory site assessment. For example, the additional available seeds can be used to create seed test plots at more potential sites, repeatedly test seedling establishment rates over multiple years, and investigate restoration sites where seed planting might help overcome physical limitations to seedling development.

Implications for Practice

- Mechanical seed collection is a feasible, efficient strategy if large donor *Zostera marina* beds with high densities of seed-bearing reproductive shoots are available.
- Passive methods for seed separation based on fall velocity through seawater can be used to separate high-quality seeds from grass wrack and poor seeds.
- In the Chesapeake Bay region, *Z. marina* seed stocks are most efficiently used by maintaining them through the summer at high salinity and cool temperatures, and broadcasting them in the fall.
- Seed injection into the sediment can be achieved rapidly over large scales, although evidence for beneficial effects of planting seeds is currently lacking.

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