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Ecological Engineering 25 (2005) 127–136

ECOLOGICAL ENGINEERING

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Buoy-deployed seeding: Demonstration of a new eelgrass (*Zostera marina* L.) planting method

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Received 19 June 2004; received in revised form 21 January 2005; accepted 28 January 2005

Abstract

We describe an innovative method of dispersing *Zostera marina* L. (eelgrass) seed that has the potential to facilitate large-scale, citizen-based restoration programs. Mature reproductive shoots of eelgrass were collected during the second week of seed release and stocked into mesh pearl nets suspended from buoys set in 0.04 ha plots. As the seeds ripened, they were naturally released from the nets, fell to the bottom and germinated to form a distinct arc-shaped meadow under each buoy. A survey of seedling survival indicated that recruitment was at least 6.9% based on estimated seed abundance within each net. The advantages of this method are that (1) harvest and deployment of reproductive shoots takes place on the same day, eliminating the need to store reproductive shoots in an on-shore storage facility, to obtain a sufficient number of seeds for large-scale restoration programs and (2) once trained, citizens can participate in both the collection and seeding phases, thereby, increasing awareness and value of *Z. marina* restoration programs. The technique presented allows for a low-cost, efficient, and simple method for successfully dispersing seed, which consequently has a significant impact on establishment of plants from seed. These attributes can also influence restoration programs for other species of aquatic plants for which the seeding of sites, historically degraded but now habitable, is possible.

Keywords: *Zostera marina*; Eelgrass; Restoration; Seeds; Citizen volunteers

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1. Introduction

"The critical role that seagrasses play in many coastal environments, coupled with their extensive losses, have created widespread support for their conservation and restoration" (Fonseca et al., 1998). Globally, resource managers have taken steps to protect seagrass habitat and support the study and testing of suitable restoration methods (Fonseca et al., 1998; Butler and Jernakoff, 1999; Balestri et al., 1998; Coles and Fortes, 2001; Campbell, 2002). A review of international restoration programs (Gordon, 1996) and guidelines for work in the United States and adjacent waters (Fonseca et al., 1998) indicate that although there has

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0925-8574/$ – see front matter © 2005 Published by Elsevier B.V.
doi:10.1016/j.ecoleng.2005.03.005

been some success, it is clear that seagrass restoration is an "evolving technology that remains difficult and challenging" (Lord et al., 1999).

In the North Atlantic and Northeast Pacific regions of the United States, programs to protect and conserve the seagrass *Zostera marina* L. (eelgrass) are structured around efforts to restore areas where historical losses have occurred and mitigate through whole plant transplantation, for populations that are damaged by development (Thom, 1990; Fonseca et al., 1998; Orth et al., 2002). For example, the Chesapeake Bay Program has targeted a submersed aquatic vegetation restoration goal of 74,900 ha by 2010, a portion of which is expected to involve direct restoration of *Z. marina* (Naylor, Maryland Department of Natural Resources, personal communication); losses of 83% of *Z. marina* meadows since 1930 (Schott, unpublished data) prompt large-scale restoration efforts in the Peconic Estuary, Long Island, NY, and water dependent projects such as ferry terminals, submarine cables and shipyard construction that can not avoid the destruction of extant populations of *Z. marina* in Puget Sound, Washington, require a mitigation plan that includes transplantation (Stamey, 2004; Austin et al., 2004).

The most common method of transplanting *Z. marina* and other seagrasses to date has been the manual removal and relocation of adult shoots (Fonseca et al., 1998). Recent attempts at mechanization of this process to accommodate the requirements of a large-scale program, using a specially designed vessel, have met with limited success (Fishman et al., 2004a,b). Moreover, while human activities that originally destroyed *Z. marina* meadows, have ceased (e.g., improvement in water quality; Orth et al., 2002), source populations are far enough removed that recolonization, via seed dispersal, may not occur.

In light of these limitations, and within the last decade, the harvest and sowing of seeds has been rigorously investigated (Orth et al., 1994, 2003; Harwell and Orth, 1999; Granger et al., 2002). Earlier studies document the potential of seeds to restore *Z. marina* populations, but this work did not proceed beyond initial trials (Addy, 1947a,b; Lamounette, 1977; Churchill and Riner, 1978). Whereas this work demonstrated that seeds held potential for restoration programs, recent studies more fully describing methods to collect, store and sow seeds have launched the technique as a viable alternative to whole plant removal and relocation (Orth et al., 1994, 2003; Ifuku and Hayashi, 1998; Harwell and Orth, 1999; Granger et al., 1996, 2002). However, despite the evidence that sowing *Z. marina* seeds may be an efficient and effective method, large-scale programs may be constrained by the labor required to collect reproductive shoots and the infrastructure necessary to store and process seed-bearing reproductive shoots from the time of collection until broadcast (i.e., 3–5 months).

Clues to the development of a system, whereby detached reproductive shoots can be deployed immediately upon harvest, eliminating the need to store and process this material, are readily apparent in the reproductive ecology of *Z. marina*. Early work (McRoy, 1968) suggested and recent work (Harwell and Orth, 2002) has demonstrated that detached, floating reproductive shoots can transport viable seeds greater than 100 km from the donor meadows. As they drift these shoots release mature seeds over a period of approximately 4 weeks, corresponding to the time it takes for an ovule to develop after successful pollination (Churchill and Riner, 1978; De Cock, 1980; Harwell and Orth, 2002).

The initiation of flowering in *Z. marina* is dependent on the water temperature increase associated with the onset of spring conditions (Setchell, 1929; Tutin, 1938; Phillips et al., 1983). As the reproductive shoot develops, branching occurs and within individual branches spathes, containing stamens and pistils, form and pollination occurs (Churchill and Riner, 1978; Cox et al., 1992). Spathes adjacent to the distil portion of the reproductive shoot develop earlier than those in the basal portion which influences the release sequence of mature seeds (Churchill and Riner, 1978); however, once pollination occurs, mature seeds are dehisced in approximately 24–34 days (De Cock, 1980). After release, most seeds sink relatively quickly to the sea floor (Orth et al., 1994). A portion of the seed rain can be transported by gas bubbles formed during release from the ovary to a distance greater than 200 m (Tutin, 1938; Churchill et al., 1985), but studies show that most of the seed rain is concentrated adjacent to the parent plant (Ruckelshaus, 1996). Because seeds continue to mature in detached reproductive shoots, these shoots can also disperse batches of seed over a great distance (Harwell and Orth, 2002). Therefore, a system capable of holding mature reproductive shoots underwater in the same location, during seed release, could be a viable seeding technique. Moreover, an efficient design for seed dispersal would mimic the natural position and movements of a single reproductive shoot in the water column.

The development of a system that links harvest and deployment has the following advantages over current seeding methods: (1) it eliminates the need to construct and staff a storage facility to hold the reproductive shoots and (2) it increases the potential for volunteers to be involved in restoration activities (e.g., seed harvest and sowing), a practice that has enhanced programs involving whole plant transplanting (e.g., Orth et al., 2002). In our study, we tested: (1) the potential of buoy deployed seeding to disperse seeds within a defined area over time and (2) the practicality that a number of these buoys could be deployed to seed a larger area.

2. Methods

2.1. Seed harvest

We collected reproductive shoots in the second week of seed release, following a protocol developed by Churchill and Riner (1978). The second week of release was confirmed through regular SCUBA reconnaissance of the harvest site and an estimate of mean viable seed yield per reproductive shoot was calculated based on collections at several sites in the Peconic Estuary during June 2002. These shoots ($n = 142$) were transported to the laboratory and all ripening fruits (i.e., Stage IV seeds, *sensu* De Cock, 1980) were counted. On June 28, 2002, mature reproductive shoots were harvested by hand using SCUBA (Granger et al., 2002) at a meadow in Sag Harbor, NY (72.2900311W; 41.0108084N).

2.2. Buoy deployed system

Our system was designed using readily available, off-the-shelf components. Fig. 1 shows the components of a single buoy dispersal unit. After experimenting with various mesh cages, commercial aquaculture pearl nets (9 mm) were selected to hold the reproductive shoots. This mesh size was selected to maximize release of a range of seed sizes (Wyllie-Echeverria et al., 2003), while preventing loss of entire reproductive shoots or detached spathes. Other components include: a 1/2 cement block (39.4 cm × 19 cm × 8.9 cm) for anchorage (full blocks can be used at more exposed sites), a 12.7 cm × 28 cm lobster pot buoy (Carlon

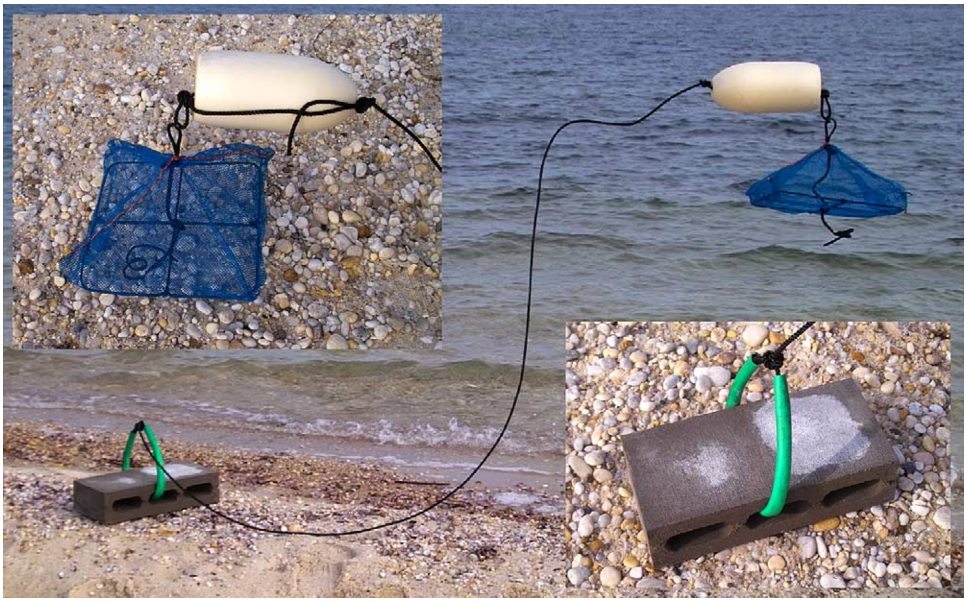

Fig. 1. A single seed buoy line showing detail of net and block attachment.

Rubber Products Co., Derby, CT), 5 m of 6.4 mm floating polypropylene line to secure the net and buoy to the anchor (total length of 3.3 m from the center of the block to the loop at the outer end of the buoy), a 35.6 cm section of recycled garden hose to protect the line from chafing where it runs through the block and a 22.7 kg capacity wire tie to attach the net to the buoy. Use of floating line is critical in keeping the line off the bottom where it could otherwise disturb recently settled seeds or rip out seedlings. The blocks and buoys are pre-assembled prior to deployment such that the total length from the center of the block to the loop at the far end of the buoy was 3.3 m.

2.3. Buoy deployment

We selected a planting site in Sag Harbor Cove, NY (72.3210912W; 40.9989839N), in an area deemed appropriate for restoration based on historic occurrence of *Z. marina* prior to a nuisance “brown tide” bloom in 1993 and 1994 and the success of previous broadcast seeding trials (Pickerell, unpublished). Average salinity at the site is 28 ppt, depth at mean low water is 1.3 m and average tidal range is 0.75 m. Bottom type at this site had been previously characterized using stainless steel sieves and the pipette method (Folk, 1974) with triplicate 10 cm deep cores as 0% gravel, 95.3% (S.E. ±0.55) sand, 4.7% (S.E. ±0.56) clay and 5.2% (S.E. ±1.77) organic matter. In order to provide for a precise grid facilitating monitoring during this first trial, buoy grids without nets were set out a week prior to the harvest of reproductive shoots. Each grid covered an area of 0.04 ha and consisted of three parallel rows of five buoys; spacing between buoys was 4.5 m on center (OC), providing approximately 1.5 m of overlap between adjacent buoy arcs. Buoy lines circled an area of approximately 30 m^2.

The transfer of reproductive shoots to pearl nets immediately followed collection from the meadow at Sag Harbor on June 28, 2002. Nets were stocked using a 1.9 L plastic pot (Poly-Tainer-Can, No. 1, Nursery Suppliers Inc., Orange, CA) filled with fresh reproductive shoots ($n \cong 100$), sewn closed using the polyethylene thread and transported to the planting site in covered 73 L fish totes partially filled with seawater to keep the shoots moist and cool. Deployment involved attaching one net to each buoy in the grid using wire ties.

2.4. Buoy observations, tides and meteorological conditions

Following deployment, the site was visited weekly to observe the progress of seed release and determine if any of the buoys had been moved from the grid or otherwise disturbed.

Mean wind speed and direction data for the month of July 2002 (the month in which seed release occurred) were obtained from the nearest National Weather Service Station located at Gabreski airport in West Hampton, NY, to determine the effect of wind on buoy movement and seed distribution. A windrose was generated by multiplying the mean wind speed and frequency for the entire month and plotting the data according to direction. Buoy movements, tidal stage and wind direction were also recorded at 30 min intervals, over a full ebb and flood cycle for several of the buoys in the grid during two separate days following deployment.

2.5. Seedling survival

Seedling abundance and distribution in the plots were determined a year later during the week of June 20–26, 2003, by counting the number of shoots in a 3-m^2 sampling grid subdivided into 12 0.25-m^2 units (Orth et al., 2003). Counts were completed by SCUBA and consisted of working along longitudinal transects running the entire length of the plot. The area adjacent to the plots was observed for the presence of seedlings. An estimate of lateral shoot formation was determined by randomly excavating 10 groups of shoots in each of the plots, counting the number of shoots per genet and returning the plants to the bottom. This number was used later to transform the raw seedling density numbers to seedling recruitment values. Raw seedling distribution data were converted into a color contour plot using Sigma Plot 2000 Software (SPSS Inc., Chicago, IL) showing observed (i.e., uncorrected for lateral shoot production) seedling density 0.25 m^{-2}.

3. Results

3.1. Harvest

On June 28, 2002, approximately 3000 reproductive shoots were collected by four divers working for approximately 2 h. The mean seed yield per reproductive shoot was calculated to be 35.6 (S.E. ±1.95) based on the sample ($n = 142$) collected and counted on June 11, 2002. This stocking rate was expected to yield 3560 seeds per net resulting in an overall planting density of 227 seeds per square metre.

3.2. Buoy observations, tides and meteorological conditions

Follow-up observations of the buoys at weekly intervals indicated that none of the buoys became dislodged or otherwise moved from the original grid. Observations of individual nets, though not quantitative, indicated that seeds were released throughout the entire 4 weeks of deployment. Most of the seeds appear to have been released within the first 3 weeks. During this time, the fresh material in the nets changed color from green, in fresh collected material, to brown, indicating that much of the fresh tissue had senesced. With this change in color, there was also a gradual reduction in overall volume, indicating that material was either breaking down or being released from the nets. Fig. 2 shows the distribution of seedlings for the southwestern corner of the buoy grid with an overlay of wind data for the month of deployment. The influence of the direction and frequency of wind on seedling distribution during the 5 weeks of deployment is clearly seen. The highest density of seedlings is directly opposite (i.e., down wind from) the strongest wind vector. Observations of buoy movements relative to tide stage and wind direction indicated that wind was an important factor in determining the position of the buoys at any point in time. Given the shape of the basin and location of the inlet to the open bay, tidal currents either work in concert with the wind at this direction (ebb) or in opposite directions (flood). Despite this, winds less than 10 mph were able to alter movement of the buoys such that they were against the direction of the incoming tide.

3.3. Seedling recruitment

An estimate of seedling recruitment was calculated based on counting all the shoots within one of the buoy plots. In this plot, a total of 10,330 seedlings were observed and after correcting for the presence of laterals (i.e., 2.8 per shoot), total seed yield was estimated at 3689. Based on this number and a predicted yield of 53,400 (i.e., 3560 seeds per net multiplied by 15 nets) seedling recruitment was calculated to be 6.9%. In both plots, seedling distribution closely corresponded to the arc of each buoy, appearing to indicate that there was minimal lateral transport following release (Fig. 2). Few seedlings were observed outside the plots; in all cases, they were found within 2 m of the plot boundaries. Focusing on a single buoy arc with the lowest seedling density (bottom left corner of Fig. 3) along the shoreward side of the grid shows that seed rain could be described as a band representing the maximum swing radius (3.3 m) of the buoy. Few seedlings were observed within the center of the radius except those that appear to have resulted from the overlap of an adjacent buoy arc. Seedling density was greatest in the northeast quadrant of the arc, followed by the south and west quadrants. Few seedlings were found in the southeast quadrant of the arc. A similar non-uniform distribution can be observed in the overall plot (Fig. 2).

4. Discussion

We have demonstrated that the natural ability of detached reproductive shoots to release viable seeds can be an efficient and effective means of restoring *Z. marina* over a defined area. Our observations indicate that the seeds are released over a 4-week period. This is in keeping with observations in Virginia (Harwell and Orth, 2002) and Great South Bay, Long Island, NY (Gates, 1984).

Seedling recruitment resulting from the seeds falling from each buoy was predictable, but not evenly distributed. Mapping of seedlings clearly indicates that the majority of seeds released fit a distribution tied to oscillations of the buoy around the central anchor. Despite the fact that the buoy can move freely within the circular area defined by the maximum swing radius of the buoy, seedling distribution indicated that buoy movements were not random. We discount the idea that seeds or seedlings could have been dislodged from the area immediately surrounding the block, given that we used floating line and did not observe the line coming in contact with the bottom during observations conducted over the 4 weeks following deployment. In addition, it is unlikely that seeds were

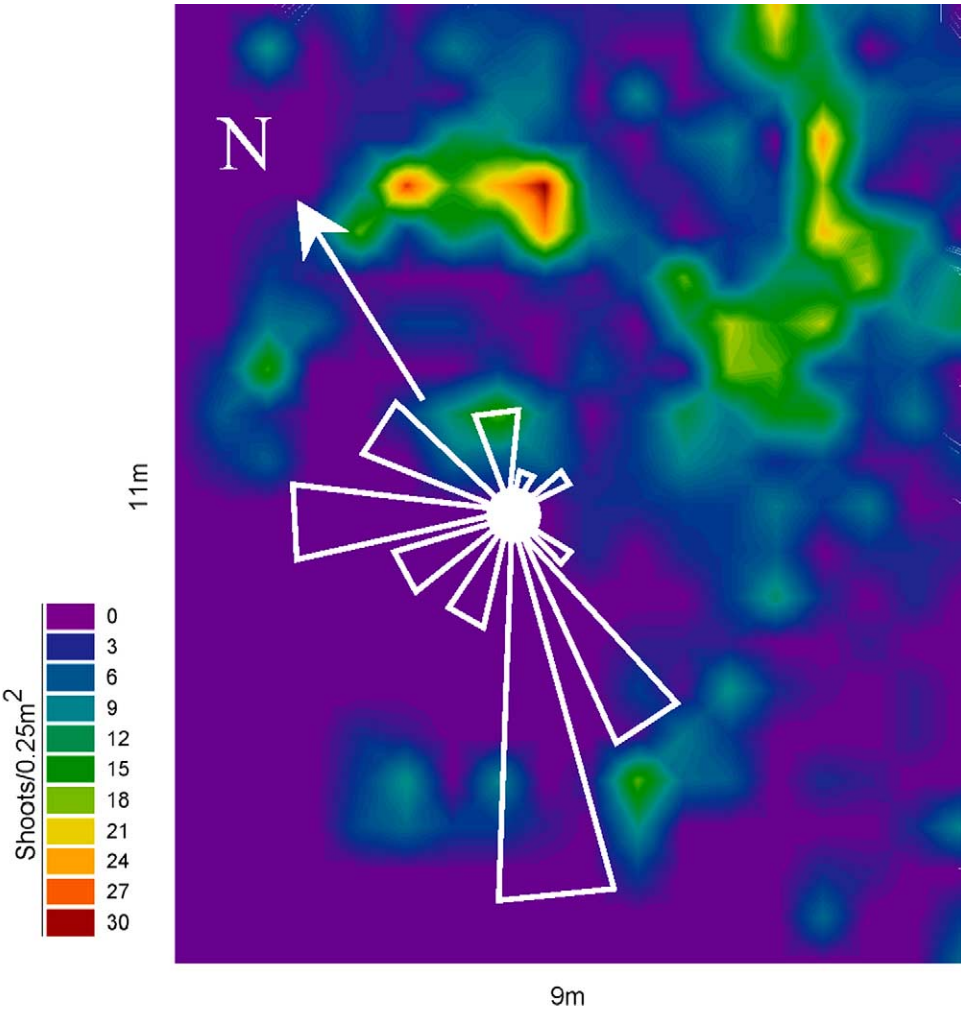

Fig. 2. Detail of contour plot showing distribution from a single buoy. Note that presence of seedlings in the top right corner and center of the plot are the result of overlap from the adjacent buoy. Windrose vectors (July 2002) represent the product of frequency and mean velocity and indicate the direction from which the wind was blowing.

transported far from where they came in contact with the bottom, based on observations of previous broadcast work at this site (Pickerell, personal observation).

Our recruitment rate of 6.9% for this trial was fairly low, but was within the range of 0.6–39.8% observed for broadcast work in the Chesapeake Bay region (Orth et al., 1994, 2003) and 5–15% observed for field plantings in Rhode Island (Granger et al., 2002). Higher germination rates (23–68%) have been demonstrated in trials using aquaria, but these rates have not been observed in field plantings (Granger et al., 2000). Estimating the actual seed yield potential from stocked nets is a difficult task, given the variability in the size of reproductive shoots and uncertainty in determining the percent of Stage IV (ripening fruit; De Cock, 1980) seeds that develop to maturity and release (Stage V; De Cock, 1980). In addition, there was a variability of stocking rate inherent in the use of a volumetric estimate. We have no reason to think that conditions in our nets are inhospitable to seed development (e.g., anaerobic, *sensu* Hootsmans et al., 1987) any more than holding shoots in tanks, as is typically the practice for broadcast seeding.

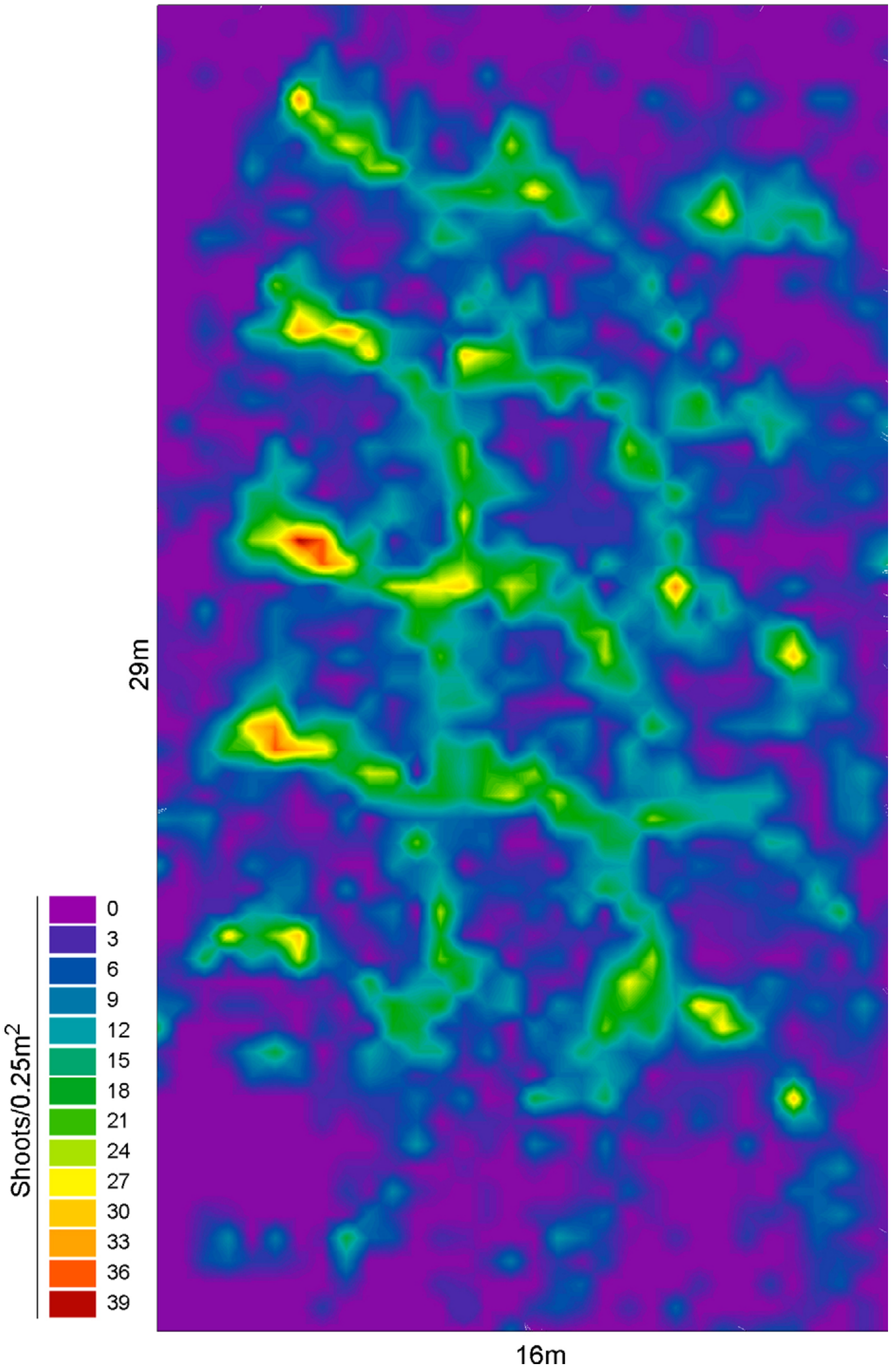

Fig. 3. Contour plot of a single (0.04 ha; 16 m × 29 m) 15-buoy grid showing distribution and density of seedlings as they appeared in June 2003, approximately 1 year after initial deployment. Each ring of seedlings corresponds to 1 of the 15 buoys (3 rows of 5) in the grid. Color bar indicates density of seedlings (shoots per 0.25 m^2).

One possible benefit of the buoy-deployed system is that re-establishment of the natural phenological timing of seed maturation and dispersal in situ may allow more time for incorporation into the sediment, more even distribution (i.e., seed rain) and lower predation due to lower initial densities, but this remains to be tested. Density dependent seed predation has been identified for numerous species of terrestrial plants (Crawley, 2000) and shellfish "seeding" is subject to predation by crabs (Boulding and Hay, 1984; Eggleston et al., 1992). Even though post dispersal predation has been identified as an important factor in loss of *Z. marina* seeds (Wigand and Churchill, 1988; Fishman and Orth, 1996; Luckenbach and Orth, 1999; Dumbauld and Wyllie-Echeverria, 2003), specific studies of density dependent predation are lacking. Given the similarities between the practice of shellfish seeding and broadcast seeding of *Z. marina* and the fact that some of the same predators (e.g., blue crabs) are at work, it would seem that reductions in density (e.g., one seed clam per square metre) that serve to protect young shellfish from predation (Peterson et al., 1995) may also hold true for *Z. marina*. However, the potential benefits of decreased predation must be balanced with the need to disperse sufficient seeds to establish a self-sustaining meadow.

Our method can be modified to accommodate a range of environmental conditions. Line length can be adjusted according to depth at low water and tidal amplitude, as well as the desired coverage for each buoy. Additional reproductive shoots can be stocked into each net, based on predicted yield or additional nets can be attached to single buoys if required. Buoy spacing and arrangement does not have to be as precise as used in our initial trial. In fact, deployment can be achieved by deploying buoy and block assemblies with attached nets, from a moving vessel passing over the restoration site, much like the TERFS method for adult shoots (Fonseca et al., 1998; Burdick and Short, 2002). Depending on the coverage desired, buoy spacing can be increased so that adjacent radii do not overlap; however, given the recent evidence that distances of 3 m between flowering shoots can lead to pollen limitation and reduced reproductive output (Reusch, 2003), a close spacing may be warranted. Conversely, a test planting consisting of a single buoy can be used to assess the potential effectiveness of seeding at candidate restoration sites before large-scale efforts are undertaken.

The simplicity of our method, as well as the fact that a storage and handling facility is not required, allows for a larger pool of practitioners to become involved in the process of seagrass restoration than was previously possible. Although we did not set out to utilize volunteer labor during this first trial, the highly repetitive nature of set-up and deployment is conducive to such labor.

Our system design can also be adapted to other species of submersed aquatic vegetation with similar flowering ecologies, including freshwater species such as those in the Potamogetonaceae. For example, Muenscher (1936) found that the seeds from several species in this family were viable up to a year after collection with appropriate storage. Because our technique does not demand storage, we suspect that restoration programs involving species in this family (e.g., *Potamogeton pectinatus*, *Potamogeton pusillus* and *Potamogeton perfoliatus*) could be successful using a seed dispensing buoy system. Also, preliminary work with other seagrass species—*Thalassia testudinum* (Lewis and Phillips, 1980) and *Posidonia oceanica* (Balestri et al., 1998) suggests that the buoy method of seed release could be effective to restore these plants. However, trial experiments are needed to determine appropriate collection methods, net mesh size and buoy spacing.

Acknowledgements

This work was funded in part by grants from the New York State Department of State, Environmental Protection Fund, the Long Island Community Foundation and the National Fish and Wildlife Foundation. The Peconic Estuary Program sponsored our early eelgrass restoration efforts. The authors would like to acknowledge the contributions of the following individuals: R.J. Orth provided critical guidance on the harvest, processing and broadcast of seeds. S. Granger shared his experience in the collection and processing of reproductive shoots in Rhode Island. A.C. Churchill's knowledge of local seed and seedling ecology was crucial to the successful completion of this work. J. Semlear provided critical information regarding site selection and facilitated coordination with the Southampton Town Trustees. K. Petersen, M. Delany and M. Parsons provided field assistance. K. Peterson created the contour plots. R. MacNish took the photographs of the buoy line. L. Brousseau, M. Sclafani, and Annette Deerr provided helpful reviews of drafts of this paper.

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