Restoration by seeding in *Zostera marina* - a progress report

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Restoration of eelgrass beds in Frenchman Bay by transplanting vegetative shoots has proven successful, and gives rise to beds that propagate by seeding. We explored the use of deliberate seeding to enhance recolonization, and have developed techniques to this end. The widespread die-back of eelgrass in the winter and spring of 2013 rendered our collected-seeding tests moot, and the efficiency of these techniques remains to be evaluated.

In Frenchman Bay, only 34% of the eelgrass acreage that was documented in 1996 remained in 2008 and the bay has faced additional losses of this ecologically important plant since this time. We had made great progress within our restoration areas, using a range of transplanting techniques[3,6,7]. Eelgrass has an unusual underwater pollination system. The seeds are retained on the fruiting stalk and mature *in situ*. The stalk eventually breaks off and can float long distances before further disintegration releases the seeds that fall to the bottom. We noticed that after partial restoration of some areas, eelgrass had spread by currents[8] to remote parts of the bay, implying propagation by seeding from our restoration sites. Further, molecular genetic analysis of the site used as a transplant source showed great genetic diversity and low level of asexual reproduction[2], also implying seeding as a major reproductive strategy. We therefore investigated methods for harvesting and planting seeds that might be appropriate to our waters.

We conducted the following studies. 1) We tied fruiting eelgrass stalks to poles, allowed them to disintegrate and drop their seeds, and examined the surrounding substrate for seedlings. 2) We collected seeding plants, allowed them to shed their seeds in a seed separator, and planted these seeds using a new method. 3) We tested seed germination and the effects of various pretreatments in the laboratory under controlled conditions. 4) We measured seed sizes by time-lapse photography, and developed techniques for observation of seed coat cracking.

Figure 1. Left: Tying plants to seed-test poles that were later inserted in the mud in a subtidal area. Right: Underwater photo of fruiting eelgrass stalks on a pole.

1) Twenty 1.5-meter poles were driven into the mud in a 5 m x 4 m plot in the subtidal area near Hadley Point. On 7/24/2009 volunteers tied 20 fruiting plants to each pole, which at the low estimate of 5 seeds per stalk would release about 2000 seeds. All stalks had disintegrated by the following spring. The surrounding substrate was examined for seedlings in May 2010. A total of 6 seedlings were found in the vicinity of the poles, and there is no assurance that these were seeded from the poles themselves or from other beds in the vicinity.

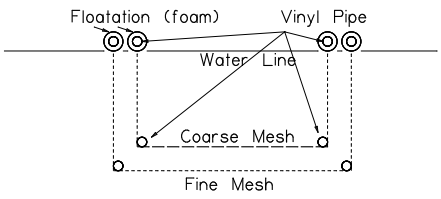

Figure 2. Diagram of seed separator. Dashed lines are plastic mesh; the coarse mesh was about 1/2", while the fine mesh was 1/16"

Figure 3. Left: Seed separator floating in the seawater tank. Right: The bottom screen with collected seeds and some stalk debris. The white dots are seeds.

2) To obtain quantities of seed, we harvested seeding plants from Hadley Point and near Bar Island in Bar Harbor in late July 2012. We used a modification of the seed separators described by Granger *et al*.[5] and Ailstock *et al*.[1] Our separators were constructed as shown in Figures 2 and 3. They were placed in a 6-ft diameter tank of flowing seawater and vigorously aerated for two weeks, during which the seeds completed maturation and fell off. Most of the seed and some of the vegetative debris passed through the coarse mesh and were retained by the fine mesh, from which they could be harvested.

3) Efficient planting of eelgrass seeds requires a method for depositing the seeds on or into the substrate that could be performed from a boat at other than low tide. Therefore, we encapsulated 10 seeds in each of 50 gelatin blocks (Fig 4) by casting them in small ice-cube trays along with a quantity of sand sufficient to ensure the blocks were denser than seawater. We investigated use of a plunger mechanism to deliver seeds, but found that while it was satisfactory for blocks without sand, the presence of sand jammed the plunger. We eventually settled on a simpler method by which the blocks were dropped down a 5' piece of ¾" PCV tubing that was inserted a few inches into the mud.

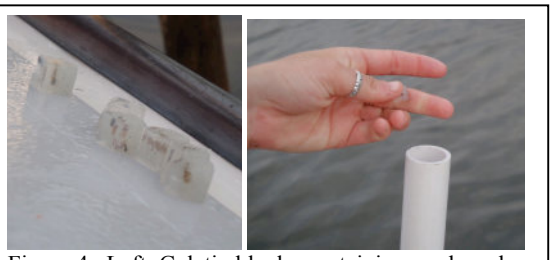

Figure 4. Left: Gelatin blocks containing seeds and sand, on the boat deck, waiting for planting. Blocks were transported on ice to avoid melting of the gelatin. Right: A block is inserted into the planting pipe.

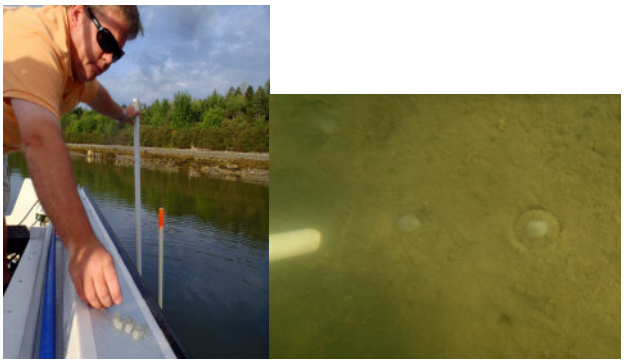

Figure 5. Left: Seed blocks being planted; the red-topped pole is a site marker. Right: Underwater view of the bottom just after the planting pipe was removed. In this photograph, three of the 10 blocks at this site are shown.

We marked 5 sites in the Berry Cove restoration area (Lamoine shore) with ½" PVC pipes, and planted 10 seed blocks for a total of 100 seeds in the vicinity of each pole, as shown in Figures 4 and 5. We could not detect evidence of seed germination in Spring 2013. Upper Frenchman Bay, including areas of Berry Cove, suffered an extensive eelgrass die-off at the end of the 2012 summer season. Green crabs are thought to be responsible for the eelgrass loss[4], which could explain why we observed no plants where we had planted seeds.

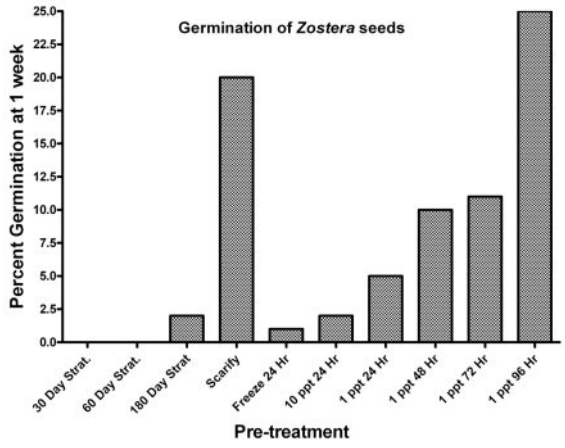

Figure 6. Percent germination of groups of 10 seeds at 18˚C in full seawater following the treatment indicated.

4) Using seeds that were stored in seawater at 4˚C, laboratory experiments were conducted to assess the optimal conditions for seed germination. After several types of pretreatment, we tested germination of seeds after one week at 18˚C in 32 ppt artificial seawater (ASW). As shown in Figure 6, percent germination is low under most conditions, with the exception of seeds that were scarified by rubbing between rough sandpaper sheets and seeds given a hypo-osmotic shock in 1 ppt diluted seawater. While scarified seeds seem to develop normally after this treatment, the seeds from hypo-osmotic treatment died shortly after germination, even if returned to 30 ppt seawater.

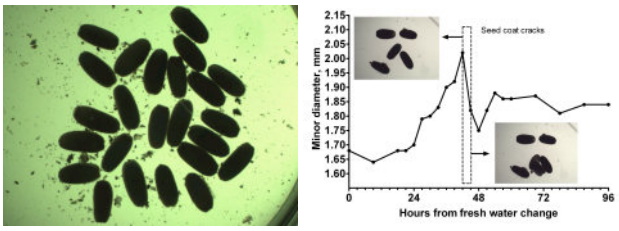

Figure 7. Left: Image of 20 seeds, ready for measurement. Right: Time course of the width (minor diameter) of a seed after changing from ASW to distilled water. The upper image shows the central seed had burst between 42 and 45 h after hypo-osmotic shock.

To record and characterize seeds, 20 seeds from each of the two harvest sites were imaged with an inverted dissecting microscope in ASW at room temperature, producing an image such as that shown in Figure 7. The length of each seed was measured; the results are shown in Table I. The 11% difference is seed size is highly significant; it is not known whether the difference is genetic, environmental or both.

The barrier to germination might be the integrity of the seed coat. Therefore, an experiment was conducted in which 5 seeds were photographed by a time-lapse camera (one frame every 3 hours) to observe any swelling and/or cracking. Of those 5 seeds, the width of the central seed increased by nearly 25% before bursting. This established that the method was appropriate for observing these events in a controlled and reproducible manner with significant numbers of seeds in a batch.

TABLE I

Seed Source	Mean Length (mm)	Standard Error of the Mean	Difference	Probability (t test)
Bar Harbor Bar	3.37	0.043	0.42	7.94×10^{-8}
Stave Island	3.79	0.039		

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