Delivering Zostera marina (eelgrass) seeds to a Restoration Site

The primary objective of this pilot study was to determine the feasibility of restoring *Zostera marina* (eelgrass) using seeds in the San Juan Archipelago. To do this, we chose two methods to deliver seeds to a restoration site: Buoy Deployed Seeding (BuDS) and broadcast seeding. BuDS[1,2] involved harvesting flowering shoots containing between 1 and 51 seeds per shoot[3] (results from this study; Figure 1), transporting them to the restoration site on the same day, and deploying them in a BuDS array at targeted areas at the restoration site. Broadcast seeding [4, 5, 2, 6] was carried out by collecting eelgrass flowering shoots, transferring them to a culture system on the same day, culturing these shoots until seeds were released, and broadcasting released seeds by hand at the restoration site.

BuDS and seed broadcasting locations were selected by reviewing benthic surveys of the restoration site (Figure 2). General locations were chosen based on a history of previous eelgrass cover or at depth similar to upper limit of eelgrass growth in the San Juan Archipelago (WDNR). Locations were marked on a field map and evaluated before BuDS were deployed or seeds broadcasted. GPS coordinates were recorded for each seeding treatment and added as a layer in the 2020 base maps (Figure 2). Individual steps required for both seeding treatments are detailed below.

Figure 1:

Figure 2: (courtesy of H. Gary Greene and Norman Maher)

Buoy Deployed Seeding

On 20 July 2020, we harvested eelgrass flowering shoots at Griffin Bay, San Juan Island (48.469456 N; -123.005024 W). On these dates, maximum low tide was -0.67 m MLLW. Flowering shoots were pulled gently from the sediment with approximately four cm of rhizome. On each day, we collected 100 flowering shoots and placed them in mesh bags. Following collection, mesh bags were placed in a cooler and transported to the public boat launch at Jackson Beach, San Juan Island (48. 31101N; -123.00475 W).

After arrival at Jackson Beach, flowering shoots were pooled in a tub with seawater. While in the tub, attached macroalgae was removed from the seed-bearing branches of the shoot and sediment was rinsed from the shoot and attached rhizomes. Shoots were returned to the cooler and covered with

seawater. The cooler and BuDS arrays were loaded on the boat and transported to Bell Point in Westcott Bay for deployment. Each pearl net within the BuDS assembly (Figure 3) was stocked with 30 flowering shoots[2] and the array was placed in the identified location.

During the transfer of shoots to the cooler, a subsample of 10 shoots was randomly selected, placed in a tub, and covered with seawater. These shoots were transported to the lab and analyzed to determine the number of spathes and stage of flower development (see [7]) within the same day of collection. From the 10 subsampled shoots, we counted 156 individual spathes in which developing seeds were forming. 44% of these spathes were in stages four or five[7] (see flowering collecting guide (Wyllie-Echeverria unpublished)).

Figure 3:

Broadcast Seeding

We collected eelgrass flowering shoots at Griffin Bay, San Juan Island (48.469456 N, -123.005024) on 22 and 23 June 2020 (collecting period one) and 6 and 7 July 2020 (collecting period two) during maximum low tides (-0.67 m MLLW) on each day. Shoots were gently extracted from the sediment with at least a four cm section of the rhizome and placed in a mesh collecting bag. During each collecting period, we collected 275 eelgrass flowering shoots for a total of 550 shoots. After collection, shoots were transferred to a culture system at the Friday Harbor Laboratories. The culture system consisted of five-gallon buckets serviced by flowing seawater (water temperature 11; salinity 29 PSU). Seawater flow was adjusted to a moderate velocity so each bucket had a similar flow rate. Each bucket was also covered with fine mesh to prevent released seeds from escaping.

Our culture array consisted of five five-gallon buckets for each collecting period. Each bucket was stocked with 55 flowering shoots. After nine weeks in culture and following a seed maturation schedule (adapted from [7]; Seed collecting guide (Wyllie-Echeverria unpublished)), we recorded ripening characteristics of seeds in haphazardly selected spathes in the separate treatments. We extracted approximately five ml of the bottom water from each bucket to estimate seed presence on the bottom. Individual buckets were determined ready to collect ripe seeds when all of the randomly selected spathes were in stage five[7], the flowering shoots were wilted and brown, and the number of seeds found in the bottom water was greater than or equal to 10.

When a treatment was ready for seed collection, we removed the wilting and brown flowering shoots and sieved bucket contents through a three-tiered system of

sieves ranging from a mesh size of 5.6 mm to 1.0 mm. We processed flowering shoots and counted seeds from 8 September until 21 September. Seeds were retained in the one mm sieve and counted. After counting all seeds from each collecting period, they were combined in a bulk sample for that collecting period. We collected a total of 1,255 seeds from collecting period one and 3,675 seeds from collecting period two.

When batches of flowering shoots were processed, leaf residue from each treatment was combined in a five-gallon bucket and set aside. On 24 September, we sieved this residue and counted seeds retained in the one mm sieve. We counted a total of 110 and 144 seeds from collecting period one and collecting period two, respectively. These seeds were added to the bulk sample for each collecting period. When summing seed yield from both collecting periods, we estimate a total of 5,184 seeds were collected.

On 17 October, we hand broadcasted seeds into Bell Point at the restoration site. To do this, we deployed a 65 m transect at the same depth as Seed Buoy treatments and recorded GPS end points (Figure 2)). We hand broadcasted seeds along the transect according to seed yield numbers for each collection (Figures 4, 5, and 6). In spring 2021 (date to be determined), we will also broadcast seeds along a 65 m transect at the same depth, but in a different location. Our objective is to compare results from fall and spring broadcast seeding events.

Figure 4:

Figure 5:

Figure 6:

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REFERENCES

1 Pickerell, C. H., S. Schott and S. Wyllie-Echeverria. 2005. Buoy deployed seeding: A new approach to restoring seagrass. Ecological Engineering 25(2):127-136.

2 Boyer, K.E. and Wyllie-Echeverria, S., 2010. Eelgrass conservation and restoration in San Francisco Bay: opportunities and constraints. San Francisco Bay Subtidal Habitat Goals Project, 83 pp Folk, R.L., 1968. Petrology of sedimentary rocks: Hemphill's. Austin, Texas, 170, p.85.

3 Wyllie-Echeverria, S., Cox, P.A., Churchill, A.C., Brotherson, J.D. and Wyllie-Echeverria, T., 2003. Seed size variation within *Zostera marina* L. (Zosteraceae). Botanical Journal of the Linnean Society, 142(3), pp.281-288.

4 Marion, S.R. and Orth, R.J., 2010. Innovative Techniques for large-scale seagrass restoration using *Zostera marina* (eelgrass) seeds. Restoration Ecology, 18(4), pp.514-526.

5 Busch, K.E., Golden, R.R., Parham, T.A., Karrh, L.P., Lewandowski, M.J. and Naylor, M.D., 2010. Large-scale *Zostera marina* (eelgrass) restoration in Chesapeake Bay, Maryland, USA. Part I: a comparison of techniques and associated costs. Restoration Ecology, 18(4), pp.490-500.

6 Orth, R.J., Lefcheck, J.S., McGlathery, K.S., Aoki, L., Luckenbach, M.W., Moore, K.A., Oreska, M.P., Snyder, R., Wilcox, D.J. and Lusk, B., 2020. Restoration of seagrass habitat leads to rapid recovery of coastal ecosystem services. Science advances, 6(41), p.eabc6434.

7 De Cock, A.W.A.M., 1980. Flowering, pollination and fruiting in Zostera marina L. *Aquatic Botany*, *9*, pp.201-220.