Timing Flower Collection is Key to Maximum Seed Yield:

A study in restoring *Zostera marina* with seeds in the San Juan Archipelago

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Introduction

- *Zostera marina*, eelgrass, is a keystone species that increases biodiversity and productivity in the nearshore as this meadow forming, flowering plant engineers and provides valuable habitat (1,2).
- The decline and fragmentation of eelgrass meadows is a concern and projects are underway to restore the Salish Sea region of Washington State (3).
- Transplanting adult shoots into a damaged site is the most common method of restoration in the Salish Sea yet this technique is expensive, and may not ensure genetic variation at the restoration site.
- Each year eelgrass naturally disperses seeds, these can be collected and used to restore eelgrass where damage or disappearance is observed (4,5,6).
- In a pilot project, we plan to use seeds to restore two locations in the San Juan Archipelago region of the Salish Sea.
- As a necessary first step, we launched an investigation to determine the appropriate time of harvest to obtain maximum seed yield.

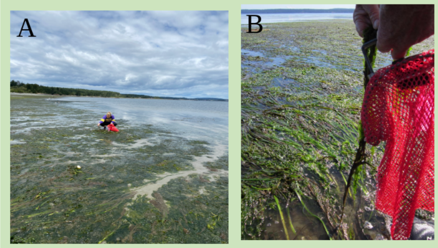

Figure 1. shoot collection
Collection at 4th of July Beach, July 6, 2020.
Photo credit: A: House B: O'Connell

Figure 2. Culture System Configuration
Photo credit: Crow

Figure 3. Seeds in Spathe
Photo credit: Coogan

Methods

- Collected eelgrass flowering shoots at 4th of July Beach, San Juan Island (48.469456 N, -123.005024) during maximum low tides (-2.2 ft MLLW) during time period 1: 22-23 June 2020, and time period 2: 6-7 July 2020 (Figure 1).
- Flowering shoots were visually identified, gently extracted from the sediment taking care to include at least a 4 cm section of the rhizome, and placed in a mesh collecting bag.
- Collected 275 flowering shoots, used a cooler to keep the shoots cool and moist, then transferred shoots to our culture system at the Friday Harbor Laboratories within 2 hrs of collection.
- Assembled a culturing system (Figure 2) and placed individual flowering shoots in 5 gal buckets serviced by flowing seawater separated by collection period (water temp 11 degrees C; salinity 29 PSU). Adjusted seawater flow to a moderate velocity so that each bucket had a similar flow rate. Buckets were covered with a fine mesh to prevent seeds from escaping.
- Each time period collection was assigned 5 buckets, and each bucket held 55 shoots.
- After 4 weeks in culture and following a seed maturation schedule (7), we recorded ripening characteristics of seeds in haphazardly selected spathes in the separate treatments and extracted a volumetric sample from the bottom water to estimate seed presence (Figure 3).
- Treatments were determined ready to extract seeds when all of the haphazardly selected spathes were in stage 5 (7), the flowering shoots were wilted and brown, and the number of seeds found at the bottom of the treatment was ≥10.
- When treatment was determined as ready for seed collection, we removed the wilting and brown flowering shoots and sieved bucket contents through a three-tier system of sieves ranging from 5.6mm to 1mm to count seed yield from each separate treatment.
- Once counted, seeds were combined into a bulk sample for each collection period and then volumetrically separated into batches for dispersal at the two restoration sites.
- Viability of a subsample of 10 seeds from each bulk sample was tested using a 1% solution of Tetrazolium Chloride (8).

Results and Discussion

- Seed yield varied significantly between the two collection dates. **Seed yield was 2.9 fold higher** (independent two-tailed t8 = -2.63, p=0.030178) **in flowering shoots collected on July 6 & 7** when **compared** to those collected on **June 22 & 23** (Figure 4).
- Seed viability varied between collection times. **80%** of the seeds from the field collection of flowering shoots **on June 22 & 23 tested viable** while **50%** of the seeds from field collections of flowering shoots on **6 & 7 July tested viable.**
- Combining total seed yield from each time period and results from viability tests, could indicate that **June 22 & 23** collections could yield approximately **1004 seedlings** while **July 6 & 7 collections** could yield approximately **1,837 seedlings**.
- Discerning peak collection time based on optimal seed yield and viability is essential for the efficiency of future restoration efforts.

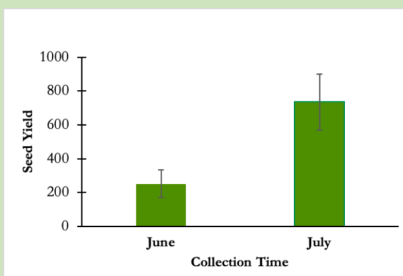

Figure 4. Average Seed Yeild by Collection Time
June collection dates are 22-6-20 & 23-6-20, July collection dates are 6-7-20 & 7-7-20, N=5, error bars represent standard error of the mean.

References

1. Green, E. P., Short, F. T., & Frederick, T. (2003). *World atlas of seagrasses*. Univ of California Press.

2. Wyllie-Echeverria, S., & Ackerman, J. D. (2003). THE PACIFIC COAST OF NORTH AMERICA. *World atlas of seagrasses*, 199-206.

3.Thom, R., Gaeckle, J., Buenau, K., Borde, A., Vavrinec, J., Aston, L., ... & Kaldy, J. (2018). Eelgrass (Zostera marina L.) restoration in Puget Sound: development of a site suitability assessment process. *Restoration ecology*, *26*(6), 1066-1074.

4.Pickerell, C. H., Schott, S., & Wyllie-Echeverria, S. (2005). Buoy-deployed seeding: demonstration of a new eelgrass (Zostera marina L.) planting method. *Ecological Engineering*, *25*(2), 127-136.

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

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

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

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

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