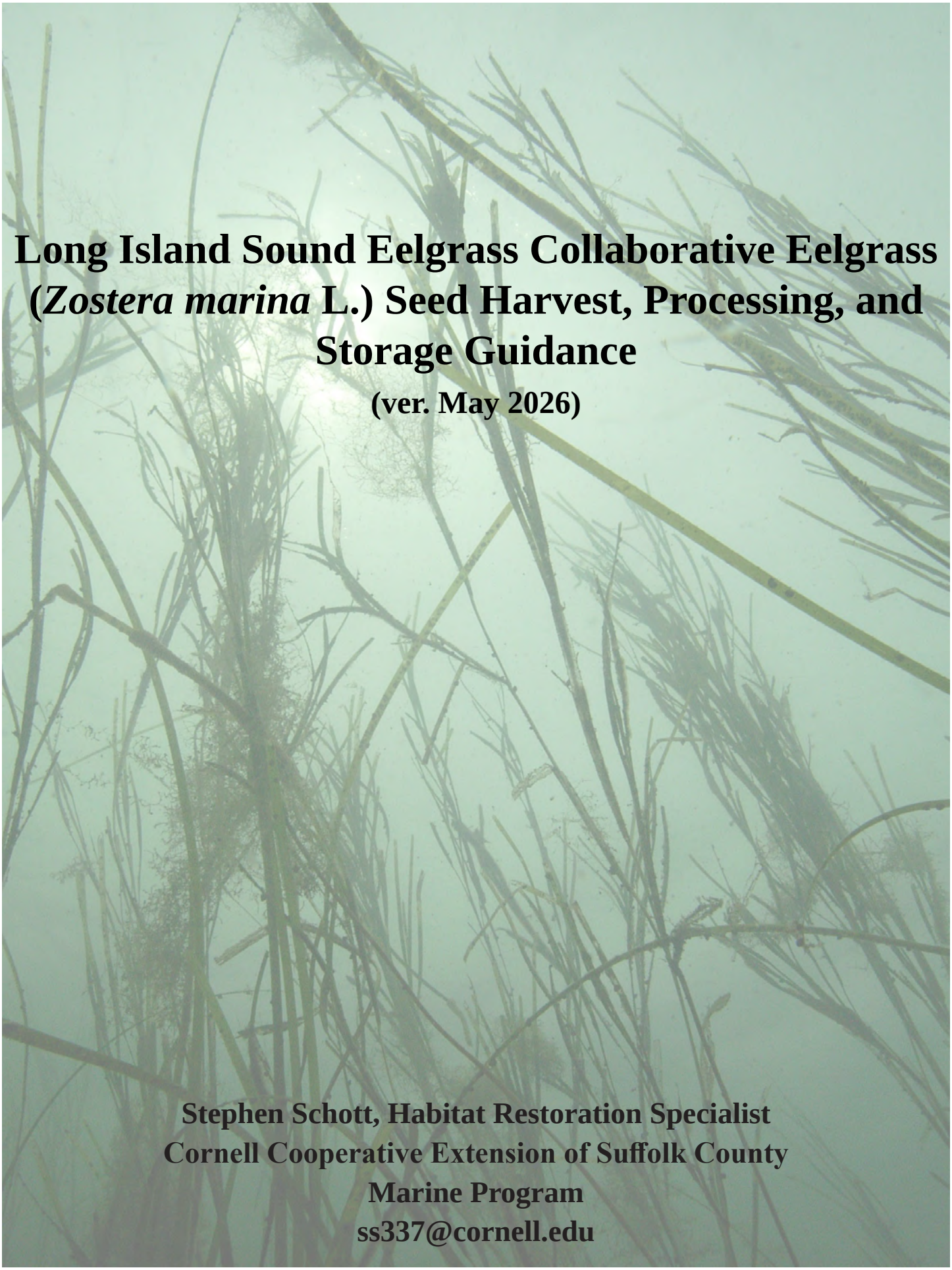

Long Island Sound Eelgrass Collaborative Eelgrass (*Zostera marina* L.) Seed Harvest, Processing, and Storage Guidance

(ver. May 2026)

Stephen Schott, Habitat Restoration Specialist
Cornell Cooperative Extension of Suffolk County
Marine Program
ss337@cornell.edu

INTRODUCTION

This guidance document includes methods and practices developed by eelgrass (Zostera marina L.) restoration practitioners along the east coast of the United States for restoring, or bolstering, declining eelgrass populations in the western North Atlantic using eelgrass seeds that have been adapted for restoration on Long Island. While many methods have been developed for restoring eelgrass since Addy's "Eel grass planting guide" (1947), most eelgrass activities have focused on the use of adult eelgrass shoots harvested from donor meadows. Adult shoot restoration has resulted in varying degrees of success in the decades that it has been utilized, however, it has not been employed at scales that offset the current rates of decline that eelgrass populations currently face. The emerging consensus in the seagrass restoration community is that seed-based restoration is the best option for undertaking large-scale eelgrass restoration. The potential for successful, large-scale eelgrass restoration is best illustrated by the work conducted in the Chesapeake Bay and its outer coastal bays, however, seed-based restoration is more infrastructure and labor intensive than the commonly utilized adult shoot restoration techniques, making it difficult for small organizations to employ.

The purpose of this document is to provide protocols that have been used for more than 25 years on Long Island for harvesting, processing, and storing eelgrass seeds for restoration, with the hope that the information presented will provide useful guidance to practitioners in the northeastern United States as they develop their own eelgrass restoration programs. Where appropriate, this guidance will also present emerging methodologies and techniques that are being developed across Zostera's native range. It is the goal of this guidance to be a "living" document that will incorporate new information as it is developed and promote eelgrass restoration and collaboration across the region.

The Cornell Cooperative Extension of Suffolk County eelgrass restoration program benefited from the generous mentorship of several incredible seagrass researchers who provided their knowledge and experience that has supported the program for almost three decades. Our sincerest appreciation goes to Drs. Jerry Churchill, Sandy Wyllie-Echeverria, J.J. Orth and Fred Short for their guidance over the years.

EELGRASS FLOWER SHOOT/SEED HARVESTING

Eelgrass seed harvesting has a finite window, unlike collection of adult, vegetative eelgrass shoots which can be harvested most of the year in the Northwest Atlantic. In the northeast region, the eelgrass seed harvest window starts in June for New Jersey and Long Island and can extend into late September-early October in the Gulf of Maine. With this in mind, it is important that goals have been set to meet the current year's need for eelgrass seeds in advance of the local harvest to address the following considerations.

Donor Meadow Selection

The process of harvesting eelgrass propagules has an impact on the donor population. With this in mind, choosing an appropriate donor meadow may not only influence harvest yields, but can determine whether harvesting has a negative impact on the donor meadow. This is especially important to consider if seeds are harvested from the donor meadow over consecutive years. Ideally, donor meadows would be identified and evaluated for their potential seed yield in advance of their selection as a donor site. Evaluation of a meadow's potential seed yield requires some scale of survey to collect flower shoot and seed density data within the meadow. The survey would collect flower shoot and seed density data from quadrats randomly sampled throughout the donor meadow. Geographic information software (GIS) is useful in creating a random sample map within a defined area (i.e., eelgrass meadow) by providing a total number of sample points and minimum distance between sample points. Existing meadow delineations can be used when available, but delineations should be as current as possible to accurately reflect the size of the meadow. The effective collection area of the meadow presented in Figure 1 is approximately 110 acres. Using ArcGIS Pro software, a random sample map was created for 30 sample points with a minimum distance of 50 meters between points using the "Create Spatial Sampling Locations" tool. Using the data collected from this type of survey, a spatial correlation map (e.g., IDW interpolation) of the meadow can be generated, highlighting areas of the meadow with higher densities of flower shoots (and seeds) (Figure 2). Identifying areas of the meadow with higher flower shoot densities increases the efficiency of divers and reduces the harvest impact on lower seed-producing areas of the meadow. The survey results also provide an estimate of total meadow seed yield which would aid in calculating a harvest threshold (e.g. 10% total seed yield) for the individual meadow, providing for the responsible management of the donor meadow.

Figure 1. An example of an eelgrass flower and seed density survey map with points randomly selected within the target eelgrass meadow using GIS software for an eelgrass meadow in Shinnecock Bay, NY.

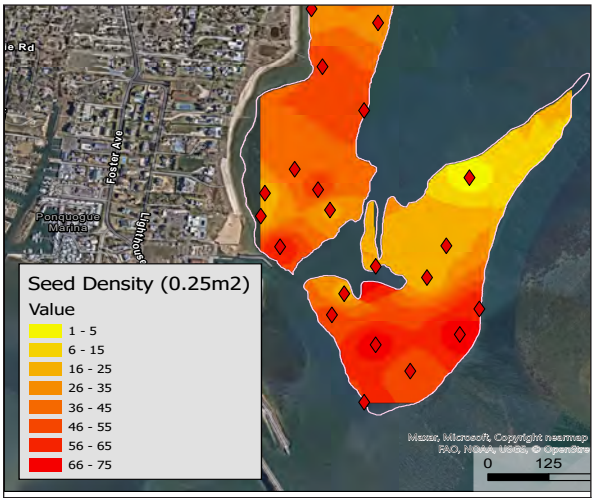

Figure 2. A spatial interpolation map (IDW interpolation) indicating the relative flower shoot density (hypothetical data) across an eelgrass meadow in Shinnecock Bay, NY.

As GIS is not a resource everyone has access to, there are general "rules" that can be used to choose donor meadows for responsible and sustainable harvest. Large, healthy and continuous eelgrass meadow should be able to sustain harvest of 10% or less of its total potential seed yield, year after year, with minimal overall impact on the health of the meadow. These large, continuous meadows typically produce more seeds than have the potential to successfully recruit into the population, especially in

the denser interior sections of the meadow where the adult shoots outcompete seedlings. In contrast, small and/or patchy meadows may be highly reliant on seedling recruitment to maintain the integrity of, or increase the size of, the meadow, and harvest would more likely have negative impacts on that population. With few exceptions, seed harvest should be avoided in these vulnerable meadows.

Harvest Timing

As mentioned above, the harvest season starts in mid-June around Long Island and can extent into the early fall for Maine. The flower shoot harvest window can be highly variable throughout the region, but at its southern extent, the season typically extends 4-weeks from start of seed release, although this window can be shortened by extreme weather events (e.g. storms or sudden swings in water temperature). The maturation rate of eelgrass seeds is correlated to water temperature, so it is important to monitor local water temperatures to identify changes that may slow or speed up seed maturation. Monitoring of flower and seed development is important in identifying the optimal harvest window.

Regionally, organizations are using the "Standard Operating Procedure: Assessing Eelgrass Flowering Density and Seed Maturity," (Carr and Colarusso, 2023; see Appendix 1) to establish monitoring transects within donor meadows to determine flower density and track seed development. These transects are established in May of each year and monitored until seed release is complete. A comprehensive guide for determining stages of eelgrass maturity was created by the Rhode Island Department of Marine Fisheries and is provided in Appendix 2.

As seed release is influenced by water temperature, seed release will occur earlier in the season at warmer locations, progressing later into the season in cooler regions. The window for seed release varies across the eastern seaboard, but the Long Island Sound region typically has a 4-week seed release window. Within this 4-week window, weeks 2 and 3 are optimal for flower shoot harvest and yield of the highest number of ripe seeds per flower shoot. Weather events (e.g. sudden changes in temperature or storms) can prematurely end the harvest window.

Flower Shoot Harvesting

Traditionally, eelgrass flower shoot harvest requires individuals to snorkel or SCUBA dive, depending on the depth of the meadow. In northern New England, there may be meadows that are accessible in shallow water at low tide, allowing for harvest using waders. Mechanical harvesting sleds have been developed in Virginia, but they have not had extensive use in the northeast. While harvesting sleds reduce or eliminate the need for individuals to enter the water, they are not selective in harvesting only flower shoots, resulting in excessive vegetative material that will need to be processed after harvest. Also, the efficacy of harvesting sleds in deeper meadows (>10ft) and rocky bottoms has not been established. Generally, hand-harvesting by a snorkeler or diver is the preferred method and results in minimal harvest of vegetative material, facilitating seed processing. Eelgrass flower shoots are readily discernible from vegetative shoots by the stature (they usually extend above the canopy) and their branching morphology, versus the strap-like blades of a vegetative shoot (Figure 3). Flower shoots have a rounded stem and are light yellow in color, making

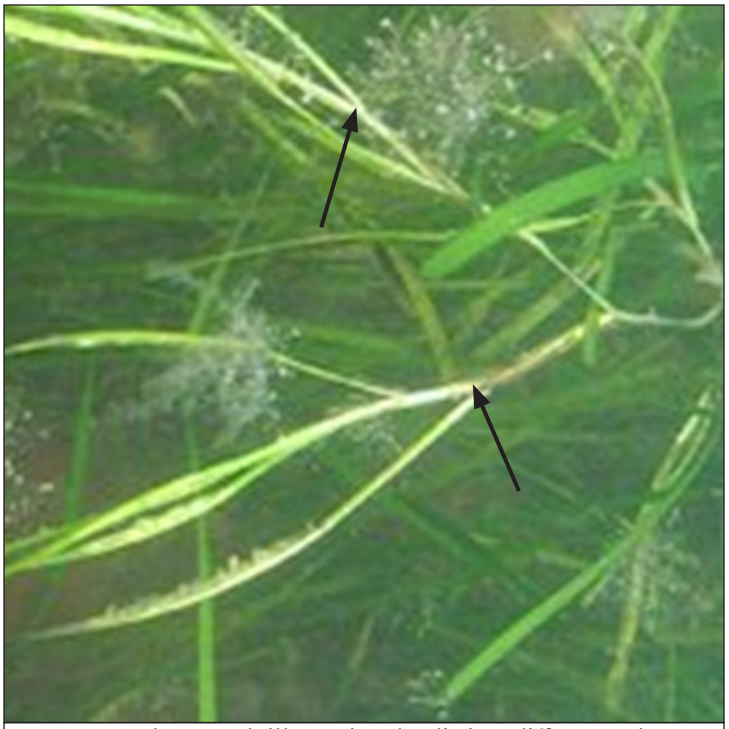

Figure 3. A photograph illustrating the distinct differences in morphology between an eelgrass flower shoot and the vegetative shoots. The flower shoot (arrows) is identified by its branching habit and light-colored stems, compared to the wider, strap-like blades of the perennial vegetative shoots.

Figure 4. Diver-harvested eelgrass flowers are placed in mesh bags, then returned to the surface where they are clipped to lines attached to a boat.

them stand out from vegetative shoots. Experienced harvesters can identify between the two shoot types by feel in low visibility or working in dense canopy. Harvested flower shoots are placed in mesh, spat bags (mesh size ≤ 1mm to minimize loss of released seeds) and once filled, can be attached to a line and floated (Figure 4) until they can be transferred to a cooler or fish tote, then transported back to the holding facility. Eelgrass seeds are intolerant to desiccation, so efforts must be made to minimize the drying of harvested material during transport, with flower shoots transferred to seawater tanks at the holding facility as soon as possible.

FLOWER SHOOT AND SEED PROCESSING

The holding and processing facility for eelgrass flower shoots and seeds typically requires flowing seawater during all of its stages. For practitioners that do not have access to flowing seawater facilities, a method was developed by A.C. Churchill (1983) whereby eelgrass flower shoots were placed in small, plastic wading pools (Figure 5) and allowed to release seeds for 48 hours. This method was later amended to include covering the wading pool with a plastic tarp and holding the flower shoots for 1-2 weeks until all seeds had been released (Churchill, pers. communication). For small volumes of flower shoots, this method is low-cost, and low effort, but it is not efficient for large-scale restoration.

Figure 5. The "Churchill Method" of processing harvested eelgrass flower shoots utilizes small, plastic wading pools into which flower shoots were placed. Enough water is added to the pools to cover the material, then a tarp cover is placed over the pools. Flowers are mixed daily for up to 2 weeks until the completion of seed release.

If flowing seawater facilities are available, flower shoots can be transferred into tanks or raceways for holding until the seeds have completely released (3-4 weeks) (Figure 6). Tanks should not be stocked too densely to avoid developing anoxic/hypoxic conditions that could slow seed development or degrade viability. If tanks need to be densely stocked, or if flow is low, supplemental aeration may be required to prevent anoxic/hypoxic conditions. Mixing the flower shoot material within each tank by hand or with a paddle daily will also minimize low oxygen conditions and facilitate the release of seeds from their spathes. By the third week of holding the seeds, the flower shoots can be evaluated to determine the progress of seed release. A thorough inspection of the flower shoots will determine when a majority of the seeds have been released and seed processing can begin.

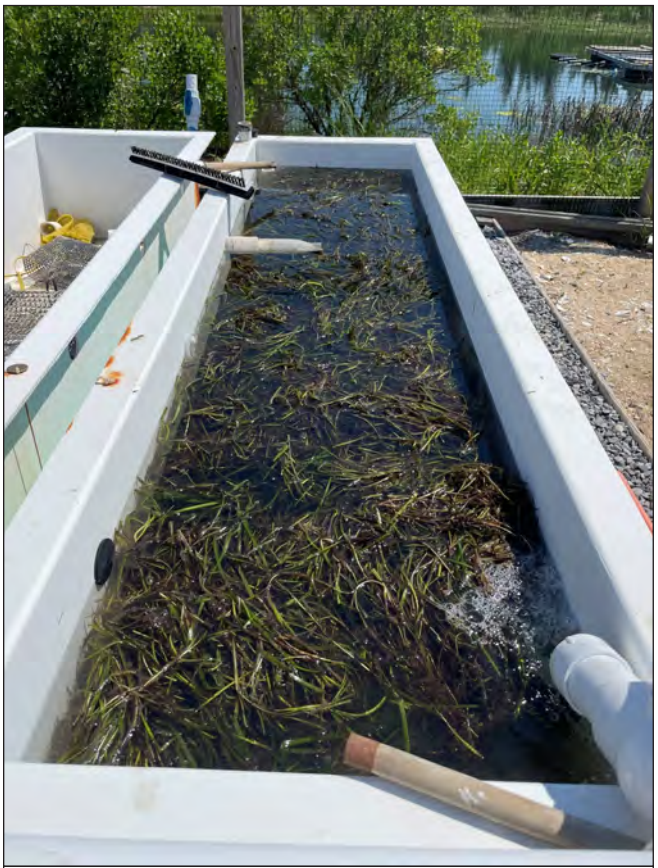

Figure 6. A raceway used to hold harvested eelgrass flower shoots in flowing seawater.

Once the majority of seeds have been released, the degraded flower shoot material can be removed from the tank. As seeds could be caught in the floating mat of flower shoots, the material should be removed a handful at a time, taking care to shake out each handful into an area of open water in the tank or into a seawater-filled tote or bucket. This process will release seeds that were caught in the flower shoots and allow the seeds to sink to the bottom of the tank/container. Once all the flower shoots and large detritus have been removed, the remaining material, seeds and smaller organic detritus, will need to be passed through a series of sieves to separate the seeds from detritus and shells. At no point in the seed cleaning process should freshwater be used on or near the seeds. The sudden osmotic change can and will result in seeds splitting their seed coat and prematurely germinating.

Seed Cleaning (Traditional Sieving Method)

The seed cleaning process, traditionally, requires a series of

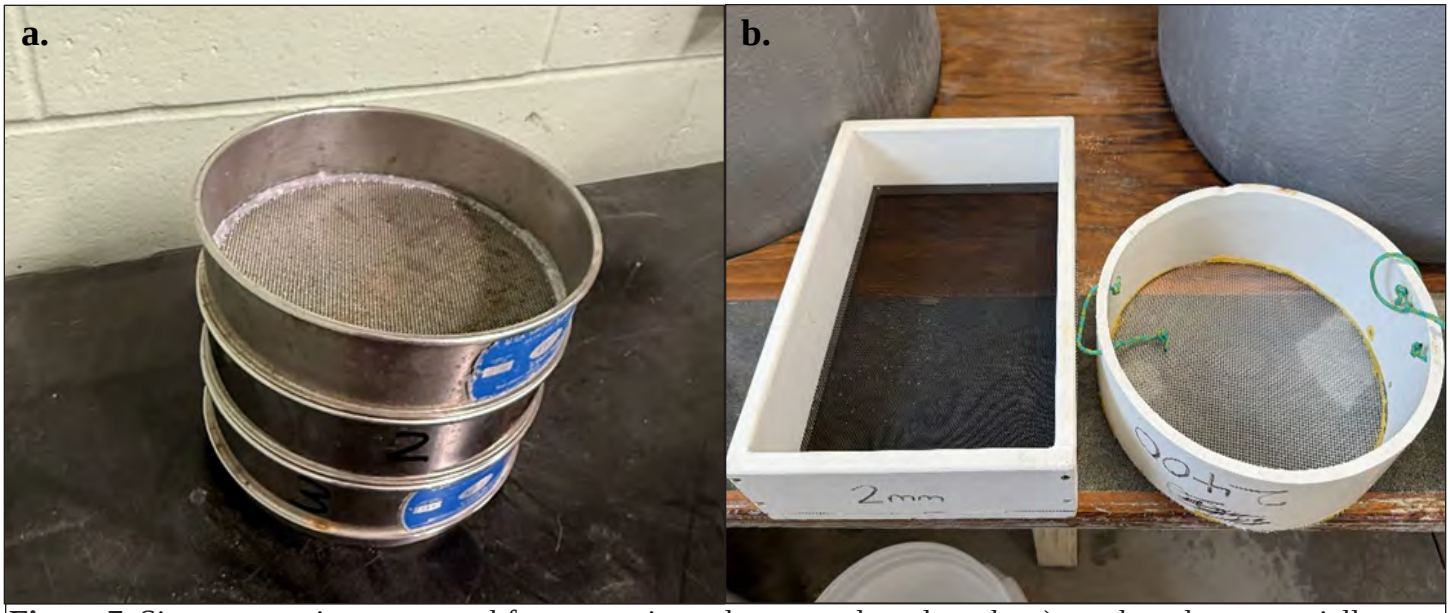

Figure 7. Sieves are an important tool for processing eelgrass seeds and can be a) purchased commercially individually or in sets, or b) constructed from available materials (sieve construction is covered in Appendix 3).

progressively smaller sieves, ranging from 13mm down to 1mm, with the recommendation of at least 3 different-sized sieves within this range culminating with 1mm. A commercial set of sediment sieves can be purchased from a laboratory supply, but they can be costly and may be limited in size (Figure 7a). Alternatively, sieves can be constructed out wood/plastic lumber or large diameter PVC duct pipe with a mesh screen secured to the bottom of the frame (Figure 7b). Homemade sieves are low-cost and highly customizable, with plastic/nylon mesh readily available from aquaculture or manufacturing suppliers.

Proceeding with the cleaning of the seeds can take two routes. If the tank has a bottom drain, a fine-mesh bag (spat bags, ≤ 1mm mesh) or large, fine-mesh sieve (mesh <1mm) can be attached to or placed under the drain outflow to catch the remaining seeds and detritus left on the bottom. The draining process should be monitored as the fine organic-seed mix from the tank can clog the mesh and cause a sieve to overflow or "blow-off" the mesh bag leading to loss of seeds. Depending on the amount of material, there may be a need to switch out a new bag or sieve to accommodate all of the material in the tank. Once the seeds and detritus have been collected, they should be consolidated into a smaller tank or placed into high-walled, mesh-bottomed containers (<1mm mesh), then placed back into seawater (eelgrass seeds are desiccation intolerant). Using aquaculture up-

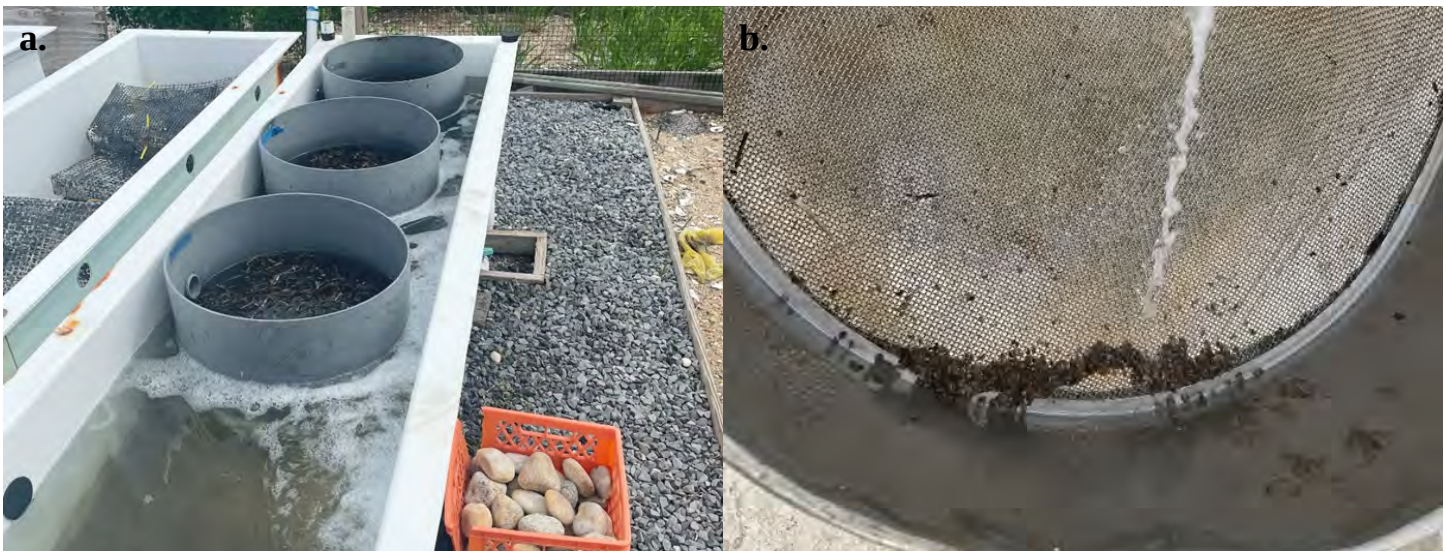

Figure 8. a) Upwelling silos holding unprocessed eelgrass seeds prior to cleaning and b) eelgrass seeds that have completed the cleaning process.

welling silos allows for the retention of seeds while providing a continuous flow of water up through the material (Figure 8a), or, at this stage, the seeds-detritus material can be held submerged with no flow for several days while processing. Aeration can be added to silos if there is concern with flow rate or concern of development of hypoxic/anoxic conditions. An alternative to draining down the tank with the seeds and detritus is to lower the water level in the tank to a manageable level and siphon the material directly from the tank into a sieve, or series of sieves, positioned over a large container (e.g. fish tote) (Figure 8b). This method allows the seeds to remain within their original tank with flowing seawater while awaiting sieving through the siphon. Seeds processed in this manner can be moved to a holding tank or container once the cleaning process has been completed down to the 1mm mesh sieve. The 1mm sieve should retain eelgrass seeds and detritus (shell and organics) that cannot pass through the mesh. While small crabs and polychaete worms are usually separated from the seeds prior to washing over the 1mm sieve, very small individuals/segments can make it into the final sieving and should be removed from the processed seeds if they are observed. The retained organics will continue to break down over time and can be rinsed from the system, however, snail shells (particularly *Lacuna vincta*) are of a similar diameter to eelgrass seeds and require additional methods of separation (e.g. manual extraction or use of flumes) to produce relatively pure seeds. (Note: Depending on the eelgrass meadow, *Lacuna* shell may constitute as much as a 1:1 ration with eelgrass seeds in processed material.) Clean seeds can be transferred to storage at this stage until their deployment (see Storage Section below). A video documenting this process will be assembled during the summer of 2026 and made available to the Collaborative.

Seed Cleaning (Flumes, Vortex Separators, and Sluices)

There have been some advances in seed cleaning technology that make the process more efficient, reducing the labor of sieving materials multiple times to produce clean seed. Some of these methods may also effectively deal with separating small snail shells from the seed which sieving alone cannot accomplish.

The first group can be classified as flumes and includes vertical (Infantes) or horizontal (Virginia Institute of Marine Sciences, VIMS). Flumes use water velocity to separate materials by density, with dense objects sinking closest to the current source, while less dense being carried further "downstream" from the current source. Infantes and Moksnes (2018) developed a 70cm long by 9.5cm diameter vertical flume (Figure 9) that can separate viable eelgrass seeds from non-viable seeds and organic material, after traditional sieving was completed. Viable seeds were found to have a sinking velocity of greater than 5.2cm s-1, while non-viable seeds had sinking velocities lower than 4cm s-1. They calculated that a flume velocity of 0.45L s-1 would push the non-viable seeds and remaining organic material out of the top of the flume, leaving viable seeds. While separating eelgrass seeds from snail shells was not discussed in their work, determining the sinking velocity difference between shell and seeds could solve the separation problem and result in truly clean eelgrass seeds. Marion and Orth (2010), in the Chesapeake Bay, took a slightly different approach. Their flume was oriented horizontally with seed-laden material being introduced at the inflow of the flume and sank to the bottom of the flume before reaching the outflow drain on the far end. Material would settle to the bottom of the flume based on their sinking velocities. The band(s) of seeds are siphoned out of the flume and run through three stacked sieves 2mm, 1.4mm, and 1mm. Overall, either model of flume is not difficult to construct, however, calibration and control of flow rates

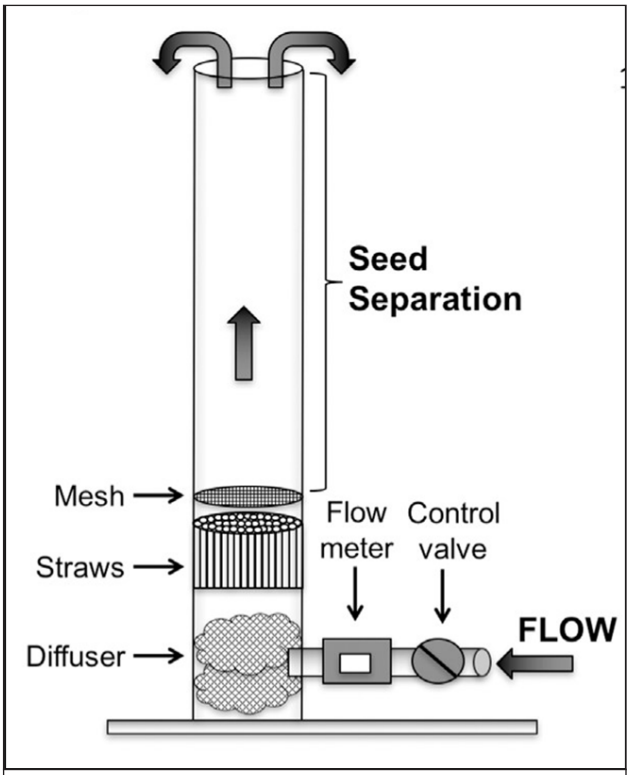

Figure 9. A diagram of the vertical flume designed by Infantes (2018) for sorting viable seeds from low-quality seeds and organic debris.

Figure 10. A vortex separator developed by University of Delft students to process eelgrass seeds for restoration.

would require testing and practice to run. Flumes may also address the need to separate large volumes of Lacuna shell from eelgrass seed in an efficient manner.

A newer technique to be applied to eelgrass seed cleaning is the use of a vortex separator (Figure 10). Vortex separators have been used in agriculture to separate grains and other seeds from chaff, but a group of engineering students from the University of Delft was asked by the Hampshire and Isle of Wight Wildlife Trust to evaluate current methods for seed separation and try to develop an efficient method to process large amounts of seeds. The result is the vortex separator detailed on the Wildlife Trusts website and demonstrated in a YouTube video. The method shows some promise, but the results appear to still require some level of sieving to produce clean seed. The ability of the vortex separator to address Lacuna shell contamination is less impressive than the flumes, but further work with a vortex system may prove successful at producing clean eelgrass seed.

A final method for producing clean eelgrass seeds efficiently may be a sluice system. A sluice is a more extensive, shallow flume that separates seeds over distance by density and, theoretically, could separate eelgrass seeds from both organic material and Lacuna snail shells. Additionally, the sinking rate of eelgrass seeds is directly related to the density of the seed which may allow a sluice system to, not only clean eelgrass seeds, but sort seeds in relation to their potential viability. No published reports were found that have explored this method for use with eelgrass or other seagrasses, but sluicing of eelgrass seeds will be investigated as by CCE and UConn to evaluate the method's ability to produce clean seed stock and classify seed viability. Figure 11 represents first version of the eelgrass sluice that built by CCE to test in 2026.

Figure 11. A prototype eelgrass sluice that will be tested by CCE and UConn in 2026.

SEED STORAGE

Once the eelgrass seed cleaning process has been completed, seeds need to be stored until they will be deployed to the field for restoration or used for experimentation. Seeds can be stored in silos or shallow raceways in a flow-through system for as long as 4 months before suffering significant seed stock shrinkage to increased germination resulting from declining water temperatures. For longer term storage needs, seeds should be refrigerated in containers with sterilized seawater.

Flowing-Seawater Storage (Short-term)

For many eelgrass restoration practitioners, seed-based restoration efforts, specifically broad-cast seeding, occurs in the fall between the end of September and into late-November. As seed harvest and processing is not complete until mid- to late-August, for Long Island, maintaining cleaned seeds in a flow-through system, instead of transferring to containers and placing them in refrigeration, is a more efficient and lower-cost method when outplanting in the fall. Clean seed can be held in upwelling silos nested in raceways with flowing seawater and held for the few months until they are deployed (Figure 13). To minimize maintenance of the seed stock due to fouling organisms, simple steps can be taken. First, filtering the incoming water can significantly reduce the introduction of fouling organisms. Sea squirts (*Molgula,* in particular) can become problematic in seed stock as they adhere to seeds and are more likely to float out of a silo or raceway taking the seeds with them. Adding a bag filter, or sand filter for larger systems, would decrease labor and seed loss. Macroalgae growth can also become problematic. Filamentous, green algae (*Chaetomorpha* or *Cladophora*) can quickly overgrow seed silos or raceways. Fortunately, eelgrass seeds do not require light and seed stocks can be deprived of light without impacting viability, while preventing macroalgae from establishing. One drawback of holding seeds in a flow-through, ambient water system is the inability to control the environmental cues that the seeds are receiving, specifically declining water temperatures. As seeds are held longer into the fall, germination rates in seed stock increases significantly, resulting in an expedited need to deploy the seeds by late-November in the Long Island region. Flowing seawater systems, even employing basic filtration, are still susceptible to infection by pathogens. For eelgrass, *Phytophthora* infection of seeds stock has garnered increased scrutiny as it's widespread prevalence in eelgrass populations has been realized. Flowing seawater systems cannot be treated, chemically, to inhibit pathogen growth, leaving the seed stock vulnerable to infection and decline in viability.

Figure 13. Upwelling silos can be used to hold clean eelgrass seeds in flowing seawater until they are outplanted.

Static, Refrigerated Storage (Long-term)

When the need arises to hold eelgrass seeds past 4 months, containerized refrigeration has had the most success in preserving seed viability. Published reports on the long-term storage of eelgrass seeds suggest that seeds held at cool temperatures, 0-7ºC (39-45°F), and salinities greater than 30 PSU (up to 70 PSU) resulted in the highest germination rates after long-term (>6 months) storage (Marion and Orth, 2010; Pan et al., 2014; Xu et al., 2020; Thomson, 2023). Also for consideration is whether seeds should be aerated or left stagnant during storage. Findings by Marion and Orth (2010) and Probert and Brenchly (1999) suggest that low levels of aeration benefit eelgrass seed storage by maintaining dormancy and resulting in higher germination rates after storage. Moore et al. (1993) concluded that eelgrass seeds were adapted to anoxic conditions and showed elevated rates of germination in storage when not oxygenated, suggesting that aeration of seeds could prolong dormancy.

Long-term storage of eelgrass seeds can lead to infection with bacterial and fungal pathogens, even after seeds have been surface-sterilized with accepted methods, such as rinsing with 70% ethanol followed by soaking in 1% sodium hypochlorite-sterilized seawater solution (5 minutes, followed by 3 rinses with sterilized seawater). Pathogens, like *Phytophthora* and *Halophytophthora*, are ubiquitous in seawater and can easily contaminate seeds stock. Chemical treatment of seed stock solutions with antimicrobial and antifungal agents including copper sulfate, nano-silver, and tannic acid have been found to provide levels of pathogen control without significantly impacting seed viability, when used at appropriate concentrations (Govers et al., 2017; Xu et al., 2019; Alagna et al., 2024). For copper sulfate, 0.2ppm was found to inhibit *Phytophthora* with no significant effects on seed viability (Alagna et al., 2024). Nano-silver (4nm diameter particles) was tested by Xu et al. (2019) and found to be a highly effective antimicrobial treatment for long-term storage of seeds at 20 ppm concentration. Tannins were found to be half as effective as copper sulfate treatment (Alagna et al., 2024) when treating *Halophytophthora* at a 1% volume/volume concentration with significant phytotoxic effects on germination rates and seedling development, but the tannin sources used were derived from trees. If tannins, and phenolic compounds, could be isolated from eelgrass wrack, potential phytotoxic effects may be reduced/eliminated. Eelgrass seeds dropping to a meadow floor would have contact with these compounds from decomposing wrack, which suggests that there may lower, or no, effects from Zostera-generated tannins and phenolics on seeds.

Regulation of Germination in Storage

While low temperature and high salinity have been shown to inhibit germination in stored seed stock, the addition of germination inhibiting hormones could also be employed to reduce germination rates in storage if water temperature and salinity are not proving to be adequate controls. Abscisic Acid (ABA) and Karrikins (KAR) have been used to inhibit germination in seeds of other plant species and may provide a viable method to maintain dormancy in eelgrass seeds for long-term storage. Karrikins have recently been found to have a strong inhibitory effect on eelgrass seeds (Pieraccini et al., 2025). ABA was found to be ineffective in inducing dormancy in Posidonia oceanica seeds (Sutera et al., 2025), but no work has been published on eelgrass seeds. The potential of Karrikins and ABA to prolong dormancy of eelgrass seeds needs to investigated further.

Table 1. A summary of the optimal parameters for the "closed" container storage of eelgrass seeds and treatments/additives that may maintain viability and dormancy.

Parameter	Optimal Range	References
Salinity	30-70 PSU	Marion and Orth (2010); Pan et al. (2014); Xu et al. (2020); Thomson (2023)
Water Temperature	0-7°C (32-45°F)	Marion and Orth (2010); Pan et al. (2014); Xu et al. (2020); Thomson (2023)
Dissolved Oxygen	≥2-3 mg/L	Moore et al. (1993)
Treatment (antifungal)		
Copper Sulfate	0.2 ppm	Govers et al. (2017); Xu et al. (2019)
Nano-silver	20 ppm	Xu et al. (2019)
Tannins	1% (v/v)	Alagna et al. (2024)
Treatment (dormancy)		
Karrikins	≥1:100 v:v	Pieraccini et al. (2025)

EELGRASS SEED VIABILITY TESTING

Determining eelgrass seed viability in collected seed stock is an important step in planning restoration plantings. Calculating viability for a given supply of seeds allows practitioners to estimate the number of seeds needed for outplanting to achieve a target seedling density. Viability testing can be used to determine baseline viability of a seed source, then track changes over time. There are several methods that have been used to test viability. Methods like the "squeeze" test and Tetrazolium staining can be subjective as determination of seed firmness or intensity of staining can vary considerable between investigators. More reliable viability tests include the seed sinking velocity and germination trials. The seed sinking velocity for viable seeds was first determined by Marion and Orth (2010) to be above 5cm s^{-1}, which was corroborated by Infantes and Moksnes (2018). Eelgrass seed germination testing can be conducted at different scales, from petri dishes to sediment filled trays in shallow tanks. Germination of eelgrass seeds can take up to 14 days with no priming, but exposure to freshwater or certain hormones before placing into the petri dish or tray may speed the germination process.

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APPENDIX 1.
Assessing Eelgrass Flowering Shoot and Seed Maturity

Carr and Colarusso (2023)

Standard Operating Procedure: Assessing Eelgrass Flowering Density and Seed Maturity

Version 1, 5/30/23
Contact: jillian.carr@umb.edu, colarusso.phil@epa.gov

Purpose: *There is great interest in using eelgrass (Zostera marina) seeds for restoration efforts, but little is known about the optimal location and timing of harvest activities. This field protocol was developed to address a regional data gap and provide a standardized approach to data collection across several National Estuary Programs and NGO organizations located in New England. The protocol can be implemented from shore or boat, and via snorkel, wading or scuba, by professional or trained volunteer scientists.*

Rationale/background

Traditionally, eelgrass restoration in New England has been predominantly done by adult shoot transplants. The actual method of deploying the uprooted shoots at the restoration site may vary (e.g., horizontal rhizome method, TERFs, tortilla method), but these just represent a minor variation on a theme. Success rates have for the most part been low and unpredictable. Adult shoot transplanting is labor intensive and as a result expensive. Due to the labor and costs involved, most practitioners are attempting to restore areas of < 1 acre over a period of 1-3 years, often not long enough to result in success. This track record has led to some funders no longer supporting eelgrass restoration projects.

In the Chesapeake Bay region, eelgrass restoration is no longer attempted by adult shoot transplants, and all restoration efforts are carried out via seeding. In the coastal bays of Virginia, close to 10,000 acres of eelgrass have been restored after a persistent large scale seeding effort, involving the deployment of over a million seeds a year for a decade. From year to year, they had highly variable rates of success. After a decade, they had accumulated enough success that the surviving restoration areas become seed sources spurring natural expansion.

Using a seeding approach for restoration has some benefits and some challenges. The challenges include having sufficient infrastructure to hold the reproductive shoots and an efficient way of separating seeds from the rest of the plant material. Benefits include easy transport and deployment of seeds to restoration sites and a relatively easy way to increase genetic diversity by using seeds from multiple meadows. In order to initiate seed-based restoration at the scale needed to combat regional declines in eelgrass, key data gaps must be filled to inform restoration planning.

This protocol was developed to fill knowledge gaps while accommodating programs with varying resources and capacity for field work. Programs may elect to conduct one, two, or all of three assessments described herein.

Site Selection

Many states have online-accessible eelgrass maps derived from aerial surveys. These maps are a good initial step to determine the current distribution of eelgrass in your geographic area of interest. From the mapped meadows in your area, consider these factors to select target sampling meadow(s):

> *Logistics:* Does the site have easy public access? Is there parking? Can you swim to the meadow from the shore (if needed)? Is a boat required? Does water depth dictate a sampling method (i.e. scuba, snorkeling, wading) available to you? Is the site close enough to allow for every-other week visits?

Safety: Is the site far removed from substantial of boat traffic or sewage outfalls? Are the tidal currents excessively strong?

Data Collection

Beginning May 1 of any year and continuing until seed release has ended, visit each site and conduct the following assessments:

(**A**) Phase of seed maturation (seed scoring), *at least every-other week*, and/or
(**B**) Flowering shoot density, *every-other week, or at least once per year when at least 50% of spathes reach stage 4*, and/or
(**C**) Seed density, *at least once per year when at least 50% of spathes reach stage 4*.

If sampling every-other week, approximately 8-10 visits are anticipated per site. Weekly records are useful if capacity allows, especially as seeds reach the dehiscing stage. Once on site, the assessments are expected to take 0.5 to 2 hours.

Assessment A: Seed maturity field sampling *(every-other week)*

Reproductive shoots are morphologically very distinctive. They tend to grow taller than the rest of the meadow canopy and are often a lighter green, almost yellowish in color, with a spindle-like stem (Figure 1).

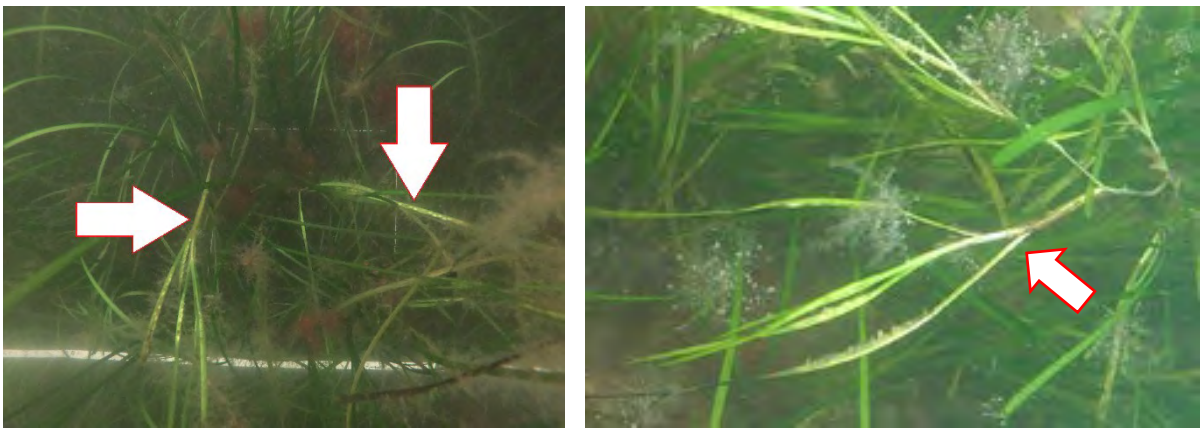

Fig 1 : Examples of reproductive shoots in the field. Source: MA DMF (left), SeagrassLI.org (right)

The seeds on a reproductive shoot are contained within spathes, which protect the developing seeds until they dehisce or separate from the plant. Spathes are clustered in branches called rhipidia (Fig 2). Immature seeds are green in color, and mature seeds tend to be dark brown or almost black in color. The timing of seed maturation can extend over a number weeks in one meadow, and is a critical piece of information to gather for restoration planning purposes. We would like to know the earliest date when seeds reach maturity and when most seeds have dropped.

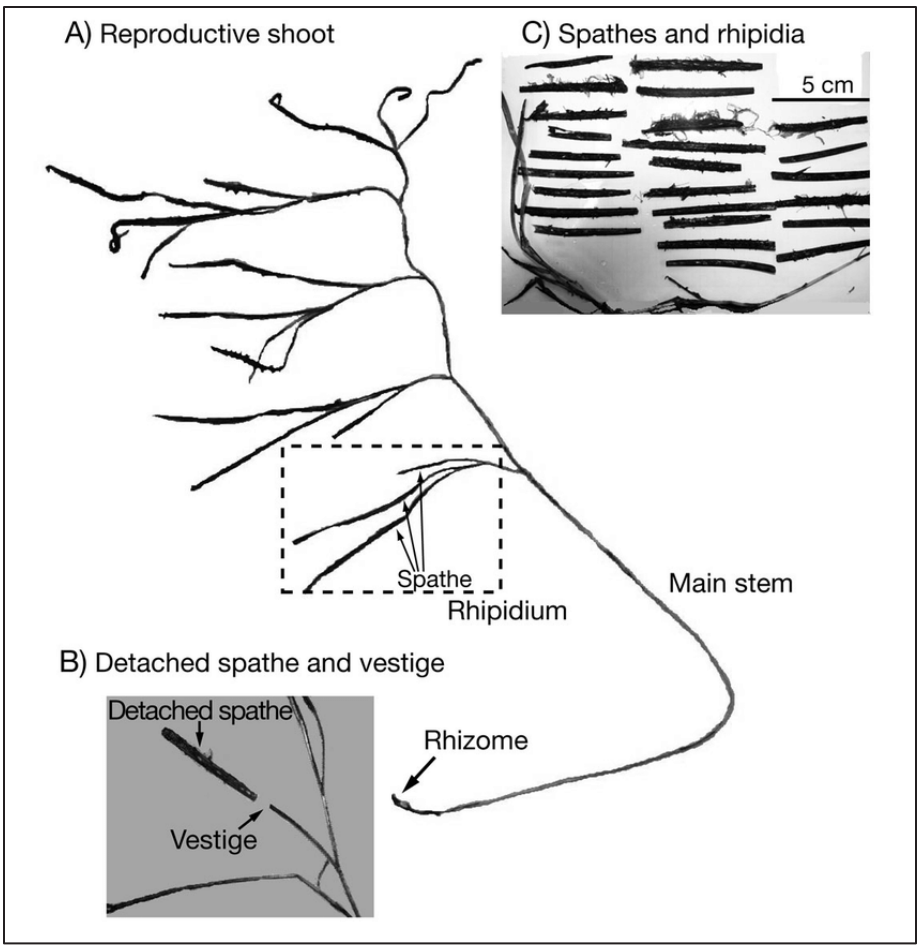

Fig 2. Eelgrass reproductive shoot morphology. Source: Hosokawa et al 2015

It is important to note that seeds on the same flowering plant do not mature uniformly. Spathes lower on the plant, within older rhipidia, tend to contain mature seeds sooner than those higher on the plant (De Cock 1980, Kuo and McComb 1998). Thus, sampling will include multiple parts of the plant, which will be scored using a key to describe the stage of seed maturity.

Field Protocol

1. Record site details on the Site Information Datasheet.
2. From each site, collect **five flowering shoots** from locations spread across the sampling area, by reaching to the bottom of the plant and pinching / snapping the stem where it meets the sediment, and give a gentle pull. Collect shoots at least 1 m apart, ideally spacing samples out over 10-20 m sampling area. Avoid sample collection within quadrats used for density sampling (Assessment B), if applicable.
3. Combine all samples from the site into one zip-close bag and keep in a cool and dark place until you can score the plants, ideally within 24 hours. Scoring at the site is acceptable.

Plant Scoring

1. Identify the reproductive components of the plant (Fig 2).
2. Find the first (lowest and oldest) rhipidium. Record this as rhipidium #1 in your datasheet. For each spathe on that rhipidium, in any order, identify the maturity stage (0-6) using Figure 3. Enter UNS if unsure. Consider taking a photo if unsure and ask for a second opinion.

3. Repeat step 2 for the next rhipidium moving up the plant, which will be #2. Continue working upward to the youngest, uppermost rhipidium.
4. Complete for each of the five shoot samples. Record stages on the field sheet.
5. For each sample, take a representative photo of the stages observed. This will help QA/QC data later.
6. Collect additional seed data (Assessment C) once per year when *at least 50% of the spathes* are in stage 4. Otherwise, discard samples.

Flowering stages of *Z. marina*. Stage 0: Spathes have developed, but styles have not yet erected; stage 1: Styles erect out of spadix; stage 2: Styles bend back into spathe after pollination; stage 3: Half-anthers release pollen; stage 4: Half-anthers have been released, seeds maturing; stage 5: Seeds are starting to release; and stage 6: Post-seed release and the flowering shoot begins to wither. Stages 1–6 are described in more detail in De Cock (1980)

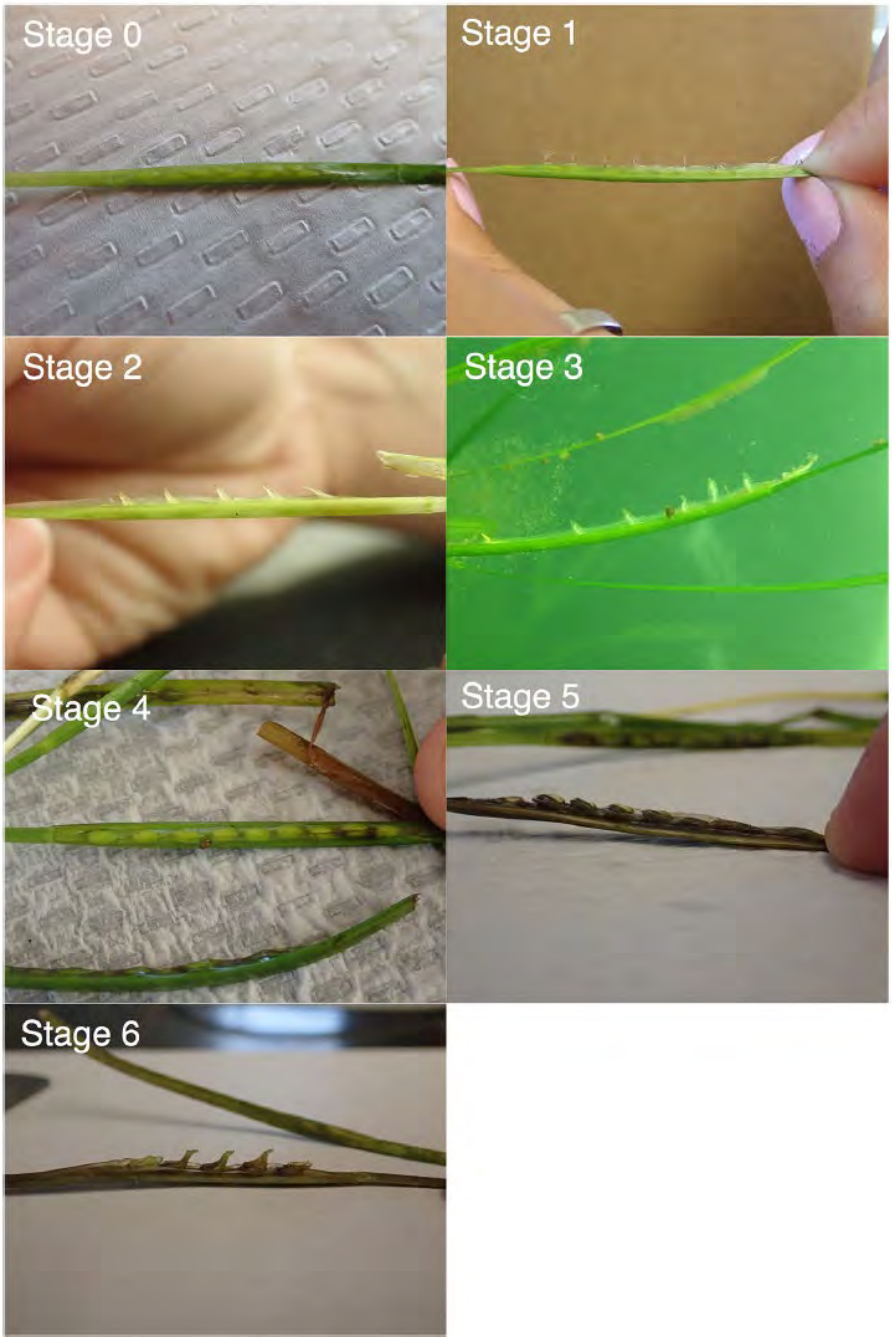

Fig 3A. Stages of eelgrass seed development (von Staats et al. 2021).

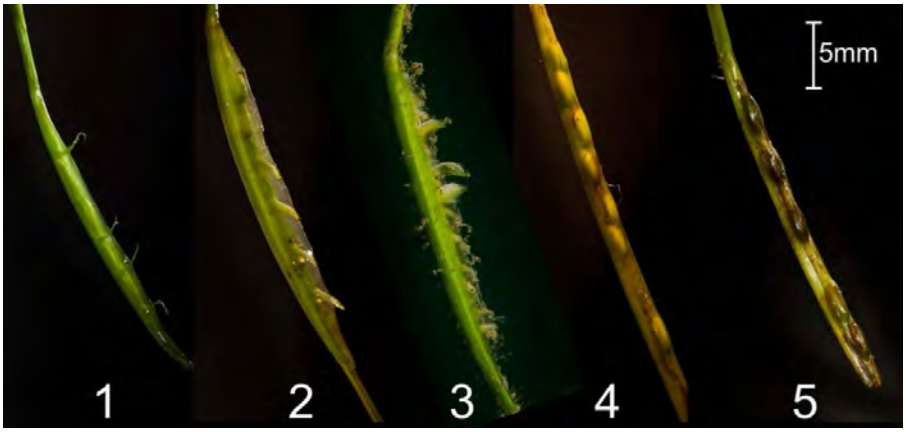

Fig 3B. Stages of eelgrass seed development (Infantes and Moksnes 2018).

Assessment B: Flowering shoot density

(every-other week, or at least once annually when at least 50% of spathes are in stage 4)

Establish sampling design & equipment

To determine flowering density, the number of reproductive shoots are counted in a fixed area as defined by a square shaped quadrat. Quadrats come in many sizes, designs, and materials. The largest quadrats use for seagrass assessments are generally 1 m^2, with other common quadrat sizes being 0.25 m^2 ($1/4^{th}$) or 0.0625 m^2 ($1/16^{th}$). *The 0.25 m^2 size is preferred* for ease of maneuvering and efficiency when performing shoot counts, though any size may be used as long as quadrat size is recorded in the data. If you do not own a quadrat, they can be inexpensively built with PVC pipes and PVC elbows. Most home improvement stores will cut the pipe to size for you (e.g., into four 1 m, 0.5 m or 0.25 m segments), and then you must glue the segments to the elbows to form a square. It is recommended that you drill several holes in each pipe segment to allow for water flow and reduce buoyancy of the quadrat. PVC of diameter 1" to 2" works well.

Aim to sample at least 3 square meters of eelgrass per site (e.g., 12 x 0.25 m^2 samples (*preferred)*; but if needed, can sample 3 x 1 m^2 samples or 36 x 0.0625 m^2 samples).

There is flexibility in approaches to spacing of the quadrat samples, depending on site conditions and access. Attempts should be made to sample quadrats separated at least 1 m from each other.

1. Completely random sampling: Throw the quadrat in completely random distance and direction. The advantage of this approach is it can save time. The disadvantage is you might miss areas of specific interest and you can't define the exact locations sampled.
2. Directed sampling: After doing some initial reconnaissance, one can target areas of a meadow that may appear to have higher flowering rates. Timing and quantity of flowering will vary spatially within individual meadows. This approach will ensure flowering shoots are captured. The disadvantage is this might result in an overestimate of the actual flowering rate throughout the entire meadow, however, literature has already documented that different parts of the meadow flower at different rates, a phenomenon that is largely depth-driven.
3. Transect sampling (*preferred*): A transect is simply a measured line laid out and quadrat samples are taken at regular *predetermined* intervals (e.g., every two meters). By taking GPS coordinates at the

beginning and end points of the transect, fairly precise sample locations can be revisited over time. Resampling sections of the meadow through time is a valuable approach. If one is trying to define the time of maximum flowering and seed ripening, it is best done by resampling the same area through time. This approach does take more time to complete. To expedite subsequent sampling visits, one can deploy semi-permanent markers (e.g., metal screw anchors, wooden stakes) at the beginning and end points of the transect.

Field protocol: Quadrat data collection

1. Record site details on the Site Information Datasheet.
2. Access the meadow by snorkel, scuba or wading. If wading, be mindful of impacts caused by footsteps.
3. If possible, collect a GPS point of the sampling location. You can get coordinates using phone apps like Google Maps. Otherwise, interpolate the location as accurately as possible from a map (e.g., Google Earth, ArcGIS).
4. Place the first quadrat per the sampling design chosen, above. Count the number of reproductive shoots that are rooted within the quadrat. It is best practice to go around the outside edge of the quadrat and ensure the shoots rooted outside the quadrat are not laying down and included incorrectly in the count.
5. *Optional:* if time and capacity allow, also count vegetative (non-reproductive) shoots in each quadrat.
6. Carefully lift the quadrat and move on to the next, until all are completed. Complete the field data sheet as you work.

Assessment C: Seed density *(once annually when at least 50% of spathes are in stage 4)*

Once per year, collect data on the number of rhipidium, spathes, and seeds per spathe using a 5-shoot sample from each site. This is best done when at least 50% of the spathes are in stage 4 (Fig 3) for accuracy and ease of observation. The timing of this is likely mid- to late-summer but will vary by location. Information about seed density per plant is useful for restoration planning and a helpful tool in donor bed prioritization. The more sites you can assess, the better for your local restoration planning. This assessment can take place while at the site or in the lab.

Field/Lab Protocol:

1. Record site details on the Site Information Datasheet.
2. Use a sample from A above (e.g., 5 flowering shoots from one site).
3. Starting with rhipidia #1 (lowest), count and record the number of spathes.
4. For each spathe, count and record the number of seeds, which can be directly seen and felt through the spathe. Stage 4 seeds are still maturing and are mostly green in color. Use a magnifying glass and a pointing tool or probe if needed to assist counting.
5. Continue for ALL rhipidia on the plant (there may be 4 or more).
6. Note qualitative variations in seed size, condition or color within the sheath in the Notes column.
7. Record using the datasheet, discard samples.

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Site information datasheet

Site Name:_____
Site Address:_____
Lat (dd.dddd°):_____
Long (dd.dddd°):_____
Organization:_____

Access Notes:_____
_____
_____

Site Location Type
Tidal River ____ Embayment ____ Open Ocean____
Other _____

Bottom Type (*select all that apply*)
Mud_____Sand____Silt_____Gravel_____Shell hash____
Other _____

Meadow Characteristics
Sparse___Dense___Patchy___Mixed___ Other:_____
Stressed___Healthy___Other:_____

Describe meadow size, shape, stressors present, etc.:
_____
_____
_____

Sketch of meadow and sampling sites

Assessment A: Seed maturity data sheet

Site Name:_____Sample **Collection** Date/Time:_____
Sample Scorer _____Sample **Scoring** Date/Time:_____
Names: Org Name & _____
Contact:

Sample (shoot) *Values: 1-5*	**Rhipidium** *Values: 1 - x* (1 is lowest on plant)	**Spathe Stage** *Values: 0-6, UNK* (Separate by comma, include as many spathes as present)	**Notes**

Numbers shown as example.

Assessment B: Quadrat sampling datasheet

Site Name:_____ Date:_____
Sampler Names:_____ Time:_____
Org Name:_____ Water Temp:_____

Quadrat size used: 1 m^2_____ 0.25 m^2_____ 0.0625 m^2_____ Other:_____

Quadrat placement strategy: Random_____ Directed_____ Transect_____
Other:_____

Quadrat Number	Repro Shoot Count	Vegetative Shoot Count *optional*		Quadrat Number	Repro Shoot Count	Vegetative Shoot Count *optional*
1	*8*					
2	*3*					
3	*0*					
4	*3*					

Notes:_____
_____
_____

Numbers shown as example.

Assessment C: Seed density data sheet (once per year)

Site Name:_____Sample **Collection** Date/Time:_____
Sampler Names:_____Sample **Processing** Date/Time:_____
Org Name & Contact:_____

Sample (shoot) *Values: 1-5*	**Rhipidium** *Values: 1-x* (1 is lowest on plant)	**# Seeds per Spathe** *Values: 0-x* (Separate by comma, include as many spathes as present)	**Notes**
1	*1*	*22, 20, 18*	
1	*2*	*14, 10, 19, 20*	
1	*3*	*23, 20, 18*	
1	*4*	*13, 18, 18*	
1	*5*	*22, 21*	

Numbers shown as example.

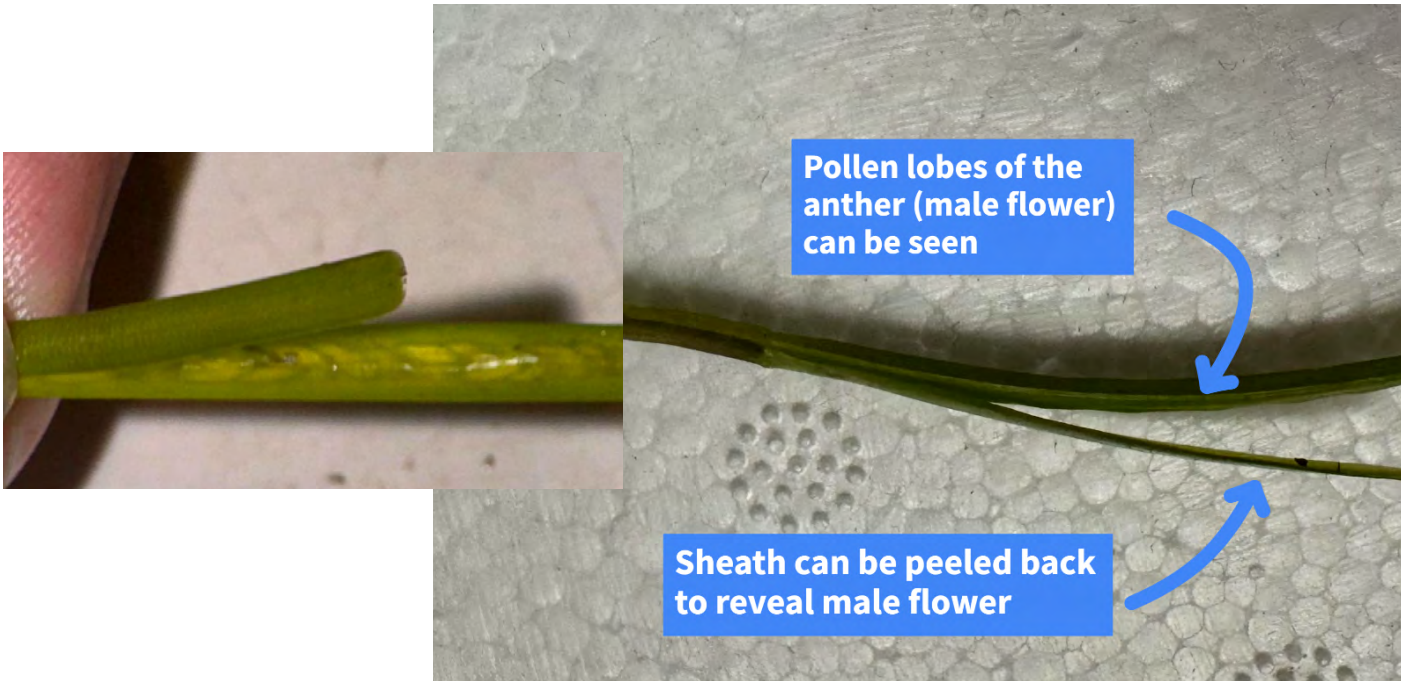
APPENDIX 2.

Eelgrass Seed Maturity Stage Guide

Rhode Island Department of Marine Fisheries (2025)

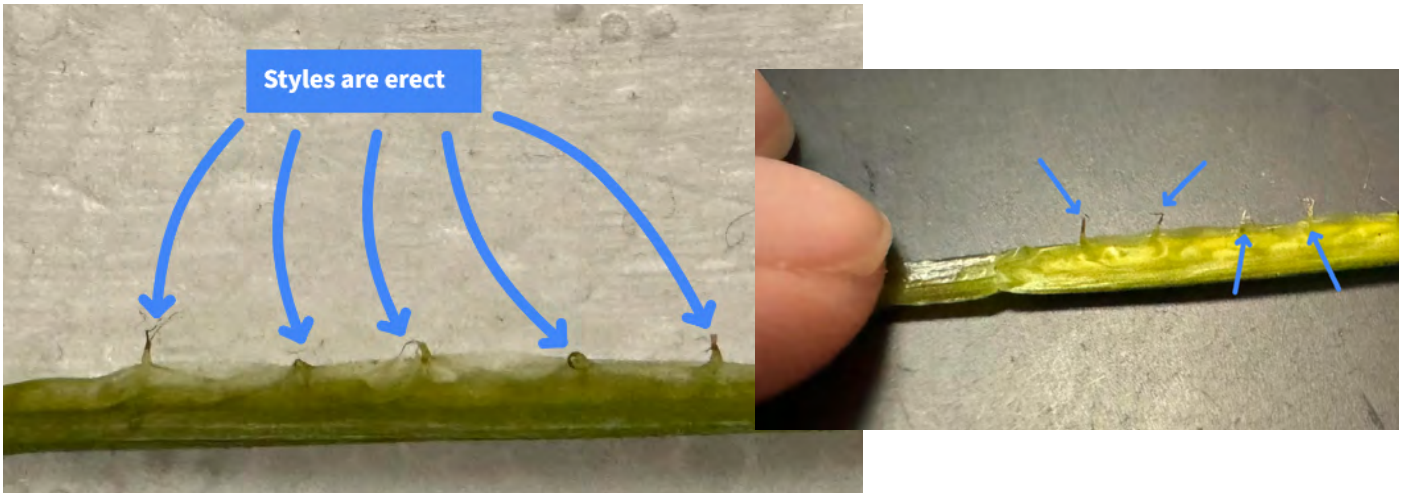
Eelgrass Staging

Stage PS (Pre-spathe):
In this stage, a sheath will be connected to the rest of the blade. The sheath can be peeled back to reveal multiple male flowers (the pollen lobes of the anther). Underneath this, the female flower (ovule) is still developing.

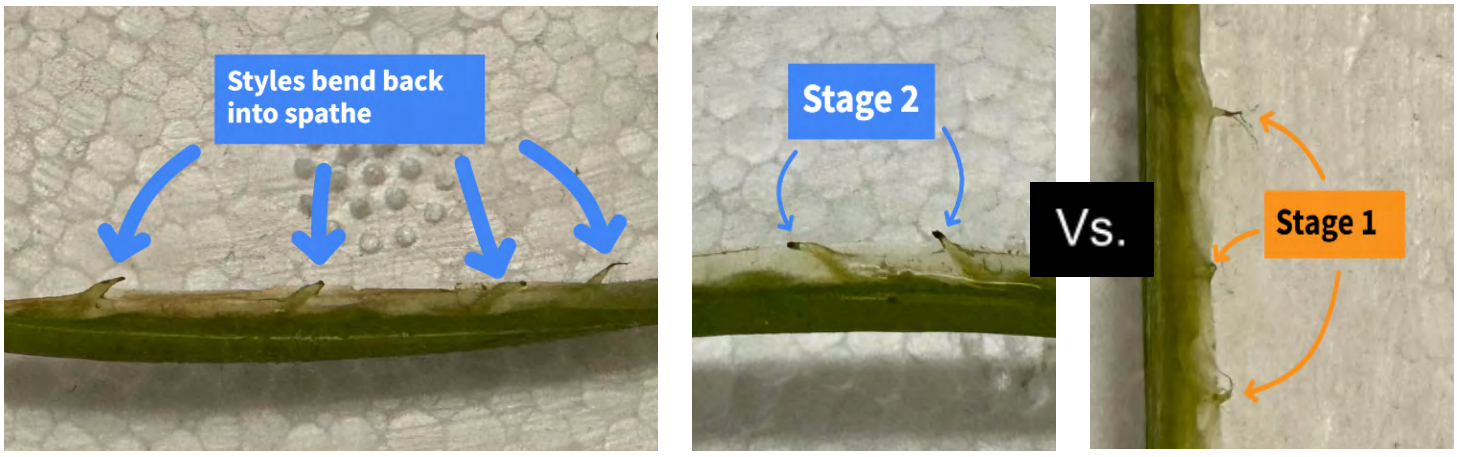


Stage 0:
Spathes have developed but the styles have not yet been erected and the sheath cover has yet to split open, differentiating it from stages 1 and 2 respectively. Anthers are arranged in a chevron-like pattern.

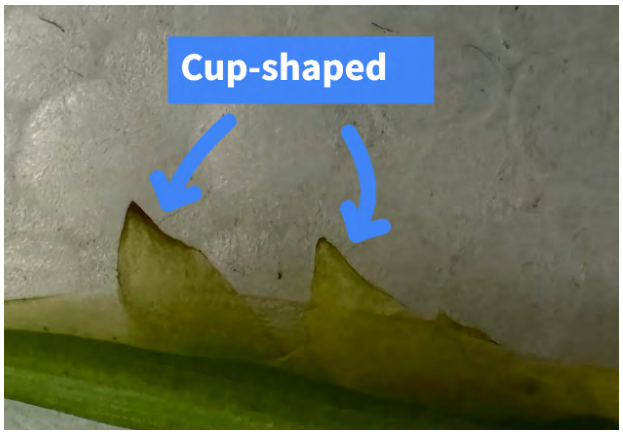
Stage 1:
Styles erect out of the spadix and protrude in a line down the middle of the spathe. The stigmas appear thin and hairlike in pairs at the tip of each style. Pollen lobes and anthers are still present.



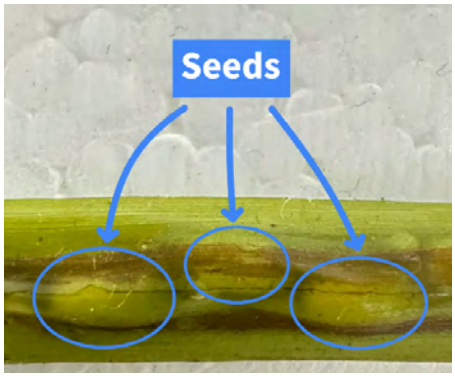
Stage 2:
Styles bend back into the spathe after pollination has occurred and pollen has not been released – anthers are still present. In stage 2, styles will be noticeably thicker than in stage 1. If pollen has been released, anthers will be absent and the spathe is considered stage 4.

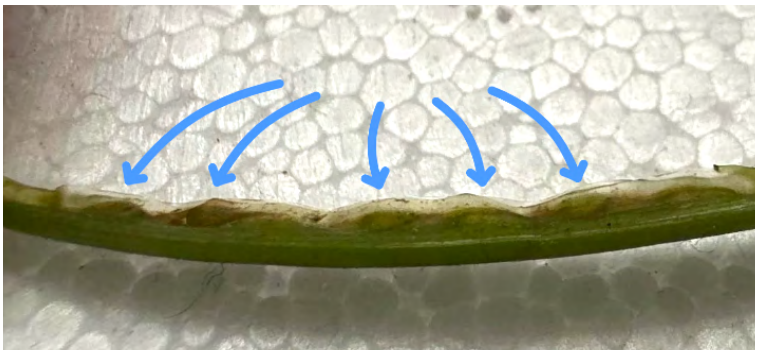


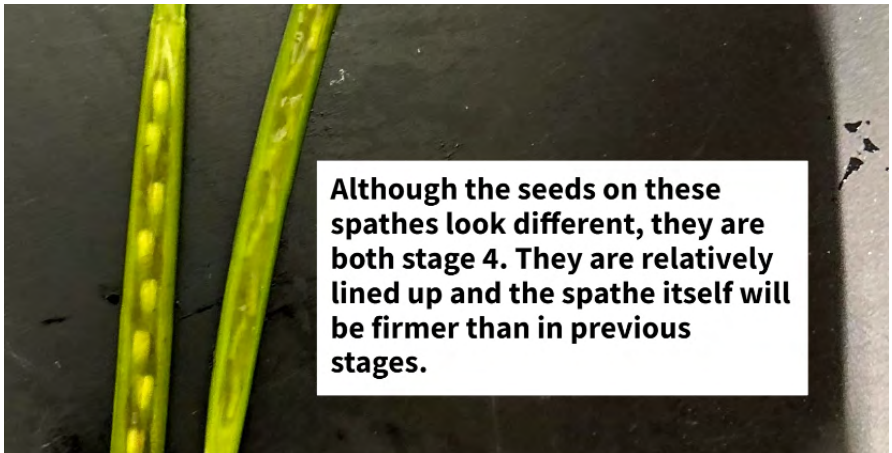
Stage 3:
Half-anthers release pollen. This stage is much easier to identify underwater, but can be identified after collection by looking for the presence of translucent, jelly filled “cups” where styles are found in earlier stages. The longer the shoots are out of the water, the harder this stage becomes to identify.



Stage 4:
Half-anthers have been released and pistils have begun to develop into seeds. Pistils will be lined up underneath the spathe in a loose line rather than in a snug chevron (*e.g.*, stage 0). Some pistils may retain styles and stigma, while others may lose these structures. The presence or loss of stigma or styles is not necessarily indicative of pollination. Ovaries vary in shape – from thin to rounded, and color – green to white or grey or brown, as they develop into seeds.







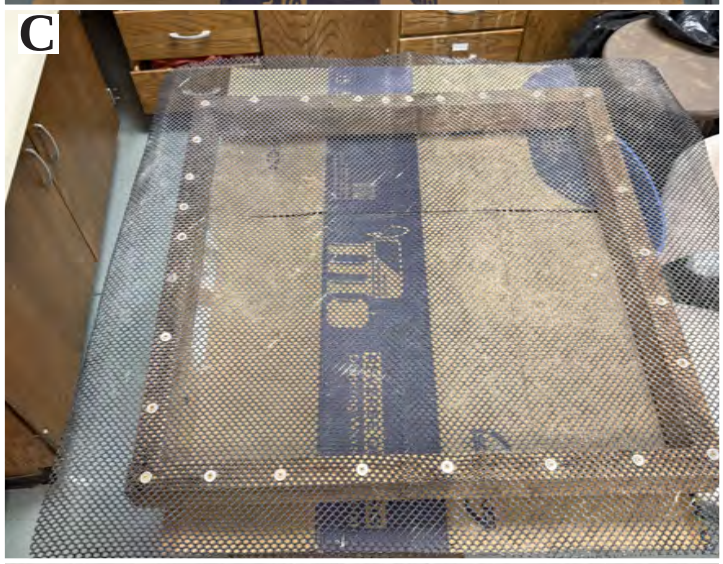
Stage 5:
Seeds are beginning to be released. The spathe will be mostly split down the middle and some seeds will be missing while others may be protruding from the spathe. The shoot will begin to brown. There may be cups present, but they will be hollow.

Stage 6:
All seeds have been released. The spathe is often brown/black and withered. This stage may be hard to identify due to decay and the presence of algae and tunicates.

APPENDIX 3.
Sieve Construction

Sieve Construction (Plastic Lumber)

The design described below was adapted from shellfish aquaculture screens used to hold post-set shellfish less that 0.5mm in diameter. The design has been adapted to be used to replace commercially-produced soil sieves and are readily customizable. The design used plastic lumber (plastic decking or trim boards) that is available at lumber and home improvement stores.



Instructions

A) Cut the plastic lumber to the desire dimension for your sieve box (not pictured). Corner clamps or a jig can be used to assist with the assembly and the box pieces are fastened with stainless-steel screws. It is highly recommended that screw holes be pre-drilled. A cross-brace can be added to larger boxes to reduce bulging and act as a handle, but it is optional. Spline pieces (plastic strips with screws pictured) to fasten the mesh/screen to the sieve box are cut to fit the box from additional plastic lumber.

B) After the sieve box is assembled, a screen jig is assembled that is 1/2-1" larger, on all sides, than the box. The screen jig will have mesh attached to it by staples or screws (see step C) and allow even tensioning of the mesh across the sieve box, creating a tight screen surface in the completed jig.

C) As mentioned above, the mesh is affixed to the screen jig using staples (for fine mesh screens) or screws with washers (for the coarse, 13mm screen, as pictured).

D) The screen jig is placed over the sieve box and pushed down to tension the mesh/screen. Two people will be needed to push down on the screen jig while a third person crews screws down the spline to lock the screen in place.

E) After all the spline has been secured, the excess mesh/screen material can be cut away from the sieve using the outside edge of the sieve box as a guide.

Sieve/Upwelling Silo Construction (PVC Duct Pipe)

The following sieve design is a modification of the method for constructing upwelling silos from PVC duct pipe. PVC duct pipe is a more expensive option and the materials are not as readily available as plastic lumber, and sieve size is limited to available pipe diamters. Circular sieve and silos are used in shellfish aquaculture due to the absence of corners, reducing dead-zones in corners, which is not a significant problem for eelgrass seeds.

Instructions

A) Cutting duct pipe (not pictured). Duct pipe is cut to desired length. Sieve heights are typically short (6-8”) while upwellers are taller (≥18”). The easiest method for cutting duct pipe is to use a table saw. This will require two people for safe implementation. The table saw fence is set to the desired height and, for safety, the saw blade height is set to just higher than the pipe wall’s thickness. With one person on each end of the pipe, it is slowly lowered onto the blade with the end butted up against the fence. Once situated on the blade the pipe can be slowly turned into the blade, yielding sections as pictured above. This method produces clean, square ends when properly completed.

B) The end of the pipe section that will have the mesh attached should be roughened prior to attaching the mesh for gluing. This will increase the surface area for the glue to adhere to the pipe.

C) The sieve mesh is cut large enough to allow for the mesh to extend at least 3” from the edge of the pipe to allow for a hose clamp to fix the mesh in place. The hose clamp is tighten enough to hold the mesh in place, but loose enough to tension the mesh over the pipe opening. Working around the circumference of the pipe and gently pulling the mesh to remove any creases should yield a very taunt sieve surface with no sag. The hose clamp is tighten to maintain tension.

D) PVC primer is applied over the mesh and pipe between the hose clamp and the edge of the pipe. Primer should also be applied along the pipe edge. PVC cement is applied over the primed areas, making sure that there is adequate coverage over the areas of mesh covered pipe. A good seal between the mesh and the edge of the pipe will prevent seeds and debris from getting caught between the pipe and the mesh. Let the cement dry, as specified on the can, then add a second coat of cement over the same area and let cure for at least 24 hours before use.

E) After the cement has cured, the excess mesh is cut along the glued edge of the hose clamp, then the clamp can be removed. The result is relatively durable and should last several seasons if stored out of the sun.

F) To make an upwelling silo for seed storage, follow the above process to create a taller version of a sieve. “Feet” are added from scrap PVC and can be attached with PVC cement to the silo (light-load use) or cemented and riveted/bolted (heavier-load use). A hole will need to be drilled near the upper end of the silo to allow for attachement to a drain which produces the “upwelling” effect. Alternatively, an “air-lift”

When the mesh eventually fails, the old mesh can be scraped/sanded from the pipe and replaced with new mesh.