APPENDIX A

Outreach Materials

School to Sea Boat Trip Eelgrass Lesson

Salem Sound Coastwatch

Abstract

This interactive lesson is meant to explain what eelgrass is, what threats it faces, and why it's important to the health of Salem Sound. It introduces how scientists collect data to monitor the abundance of eelgrass. Students will learn to estimate the percent coverage of vegetation within a quadrat. This lesson is intended for an audience of roughly 20-25 students and designed for a boat equipped with an underwater camera in Salem Harbor. It's flexible, so please tailor it to your needs; feel free to include as much or as little of the information below as you'd like, based on the age of the students.

Materials

- ☐ SplashCam underwater camera
- ☐ PVC 0.25 m^2 quadrat camera frame (with 30 ft. line)
- ☐ Laminated data sheets with reference charts (x2)
- ☐ Clipboards for data sheets (x2)
- ☐ Dry-erase markers for data sheets
- ☐ Laminated map of eelgrass beds in Salem Sound

Lesson Outline

What is Eelgrass?

- First, move to a location with fairly shallow water (roughly 15 to 25 ft.) before lowering the camera, which should be attached to the quadrat frame facing down toward the seafloor. The camera should project onto a monitor.
- Gently lower the drop-frame and camera to the bottom and wait 10 seconds for the sediment to settle. Make sure the quadrat lands upright and there is some amount of eelgrass within the quadrat.
- Ask the students what they see in the square: Do they know what's growing on the bottom?
- **Explain what they're seeing:** This is an eelgrass bed! Eelgrass (*Zostera marina*) is a native sea grass that lives in shallow waters with plenty of sunlight. It roots in the substrate and sprouts from rhizomes. It's a kind of flowering plant (unlike seaweed and other algae), which means it produces seeds.
    - Eelgrass is unique because it's one of very few plants that can survive entirely underwater! Its leaves have small air pockets to keep them buoyant, and its roots can grow in substrates where oxygen is unavailable.
- **Explain why it's important:** Eelgrass provides food and shelter to marine life and protects our coastlines from erosion.
    - Eelgrass is a nursery habitat for animals like young lobsters, flounder, mussels, and scallops, all of which are important to our fisheries.

- The leaves (which grow up to 3 ft. tall) reduce incoming wave energy, and their roots secure the soil and trap sediment, which builds the shoreline and prevents coastal erosion.
- It's a food source for sea turtles, ducks, geese, sea urchins, and snails.
- It filters out pollutants / runoff and sequesters carbon dioxide as it grows, which keeps our water clean and reduces the impacts of climate change.
    - It's known as a "blue carbon" habitat because it combats ocean acidification and global warming by storing CO_2 underwater.

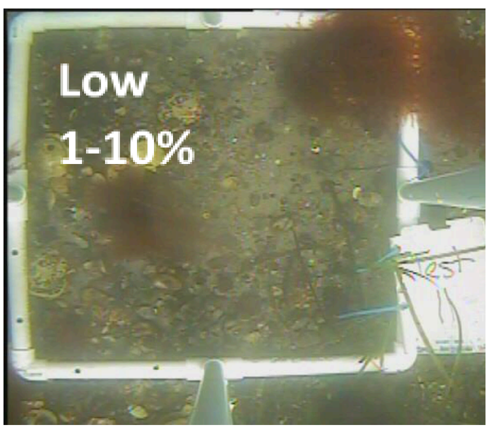
Quadrat Exercise

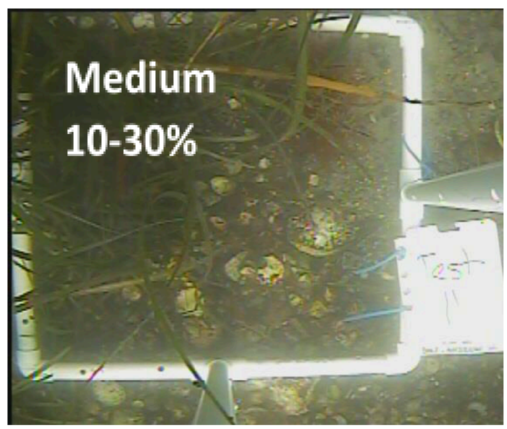
- Next, the students get to make their own observations. Move the frame and camera to a slightly different spot so the camera is looking at a new location on the seafloor.
- Split the students into two teams, and provide each team with a data sheet.
    - One team will observe the sediment and decide what types of substrate are present in the quadrat (sand, gravel, and shell hash).
    - One team will observe the eelgrass and estimate the percent coverage of grass in the quadrat using the chart below.
        - Older students can estimate the percent coverage numerically using four bins (1-10%, 10-30%, 30-75%, 75-100%).
        - Younger students can estimate the coverage qualitatively using three categories (low, medium, high).

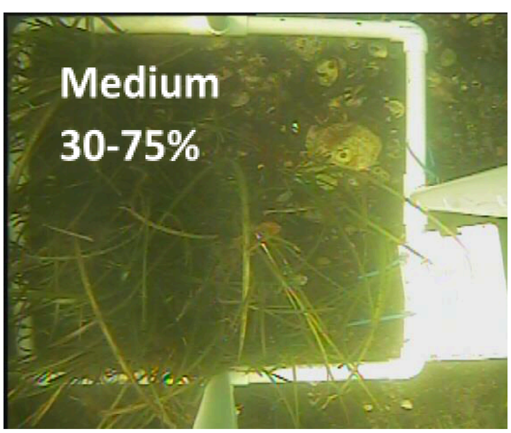
Sea Grass Field Sampling (Winter Island)				
	Coverage	**Sediment**		
	Eelgrass	Sand	Gravel	Shell Hash
Team A		Y / N	Y / N	Y / N
Team B		Y / N	Y / N	Y / N

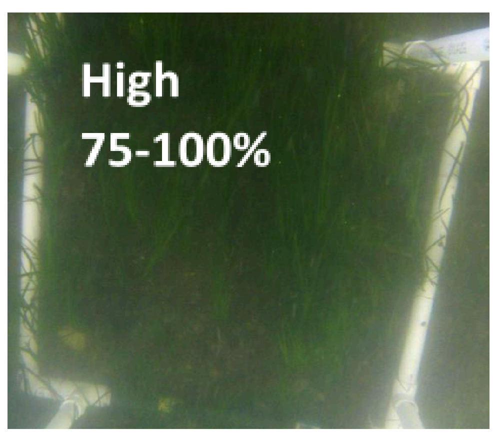
Substrates:

Percent Cover:









- Allow the teams to discuss and work on their data sheets for about 3-5 minutes, then change roles and repeat so that each team observes both the eelgrass and substrate.
    - If multiple chaperones are present, it may be helpful to have one adult act as a scribe for each team.
- After both teams have finished, reconvene the students as a group. Ask them to **share what they noticed**: were there any differences between the two teams' results? What might their observations mean about where eelgrass grows?
    - For instance, if there was dense eelgrass coverage and lots of sand, does eelgrass prefer to grow in sandy substrates?
- Finally, collect the data sheets and erase them to be reused next time.

Conclusion & Takeaways

- Use this exercise to **explain scientific monitoring efforts.** The students just used a quadrat to take a random sample of the harbor; scientists do the same thing to monitor the health of eelgrass beds. By doing lots of quadrat surveys in different places, we can get an idea of where eelgrass is growing and how its distribution is changing over time!
    - Salem Sound Coastwatch is doing exactly this – we're using an underwater camera to map changes in eelgrass habitat all along the coast of Massachusetts.
    - While explaining this, feel free to pass around a map of eelgrass beds in Salem Sound.
- **Explain what scientists have discovered this way:** it turns out, eelgrass has been disappearing from the harbor for a number of reasons.
    - Sediment runoff increases the turbidity (murkiness) of the water, as do algal blooms caused by excess nutrients. This reduces the amount of sunlight that

eelgrass receives, causing it to die off. Even shade from small docks can have the same effect!

  - Boating activities can harm eelgrass in a number of ways. Propellers and anchors tear up the leaves, and moorings can rip through the roots and rhizomes as they're dragged by the current, known as "scarring."
  - Severe storms and dredging projects can directly uproot eelgrass beds.
  - Higher temperatures, disease, and predation from invasive species also damage eelgrass populations.

- **Explain what people are doing to protect eelgrass:** Lots of people are trying to help eelgrass by improving our water quality and replanting eelgrass beds in Salem, Beverly, and Marblehead.
  - Salem Sound Coastwatch is currently working on a project funded by WHOI SeaGrant with MassBays and MA DMF to study the use of eelgrass seeds in eelgrass restoration at Winter Island. This is unusual in Massachusetts, because restoration projects usually involve transplanting entire eelgrass shoots (or "plugs"). If it proves to be successful, a seed-based approach could allow restoration projects to cover a much greater area.
  - Scientists are also investigating whether heat-tolerant eelgrass from southern regions could be brought further north in order to protect eelgrass beds from global warming in the future.
- Don't forget to pull up the camera and frame again before leaving!

Oceans & Climate
Fall Research

Name: ____________________
Partner(s): ________________

Eelgrass Seed Viability Testing

Eelgrass (*Zostera marina*) is a critical marine habitat that provides a myriad of ecosystem services, yet its extent continues to decline across the Massachusetts coastline and beyond. Restoration efforts to date have largely focused on the transplantation of adult shoots, the effectiveness of which is limited by the high implementation cost and low potential for scaling up to the extent needed to reverse recent declines. Seed-based restoration offers an alternative that can be implemented at greater scales and at a lower per-area cost. The Massachusetts Bays National Estuary Partnership (MassBays), the Massachusetts Division of Marine Fisheries (MA DMF), and Salem Sound Coastwatch (SSCW) were funded by the Woods Hole Oceanographic Institution (WHOI) SeaGrant to determine if seed-based restoration can be an effective method for eelgrass restoration.

Viable seeds are the foundation of this work. We'll be contributing to this study by testing the viability of seeds from a local and a non-local source to measure their fall velocity, shell hardness, and color. Marion and Orth (2010) found that viable ("good") seeds had an intact coat, resisted compression, and had a fall velocity of 5.5cm/sec or higher, with 89% of those seeds producing seedlings.

"Good" seed checklist:
☑ Intact coat
☑ Firm seed
☑ Fast fall velocity

Materials:

- 2 white ice cube trays (drop test)
- 1 gray ice cube tray (tetrazolium)
- Forceps
- Drop tank
- Ruler
- Seawater (20ppt)
- Stopwatch
- Tetrazolium solution (1%)
- Scalpel
- Dissecting microscope

Drop-test Procedure:
Each group will be testing a subset of seeds from either the LOCAL (West Beach, Beverly) or the NONLOCAL (Provincetown) seed source.

1. Fill your tank with 22cm of seawater (20 ppt). Be sure to measure the water level from the inside of the tank.
2. Obtain a sample of seeds from either the Local or the Nonlocal study site.
3. With forceps, randomly select a single seed from your sample. Inspect the seed. Note its color (light, medium, dark), hardness (soft/firm), and whether or not the seed coat is intact or damaged. Record this on the data sheet.

4. While one partner waits ready with the stopwatch, the other partner should hold the seed just below the water's surface with the forceps. Count down (3, 2, 1) and then simultaneously release the seed and start the timer. Stop the timer when the seed hits the bottom of the tank. Record the drop time on your data sheet.
5. Repeat until you have tested a total of 32 seeds.

DROP TEST DATA SHEET

Circle Seed Location: LOCAL // NONLOCAL

Seed #	Color	Hardness	Seed Coat	Drop Time (sec)
	Light / Medium / Dark	*Soft / Firm*	*Intact / Damaged*	

Seed #	Color	Hardness	Seed Coat	Drop Time (sec)

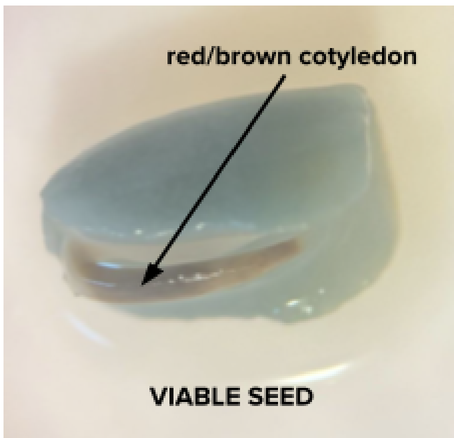
Tetrazolium Staining Procedure:

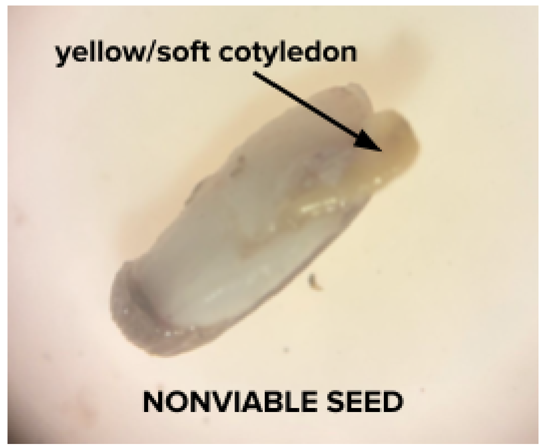
Viability is determined via tetrazolium staining. A seed's cotyledon will be stained brown/red if viable and will remain yellow if nonviable. Each group will test half of the seeds from the drop test to determine if there is a correlation between drop velocity and viability.

1. Put on gloves and safety goggles. Obtain the gray tetrazolium ice cube tray, forceps, and a scalpel (CAUTION: SCALPEL BLADES ARE EXTREMELY SHARP).
2. Using a pipette, fill each ice cube tray compartment with enough tetrazolium solution to cover the bottom (about 1ml).
3. Select 16 seeds to test in the following categories:
    a. 4 of the fastest seeds.
    b. 4 of the slowest seeds.
    c. 4 medium-fast seeds.
    d. 4 medium-slow seeds.
4. One at a time, remove a selected seed from the drop test ice cube tray and, using the scalpel, CAREFULLY cut away the seed coat, ensuring the inside embryo is not damaged.
5. Place the unsheathed seed in the tetrazolium solution, ensuring you've noted which seed # corresponds to the tetrazolium bin #.
6. Repeat these steps for the remaining seeds. Seeds will be inspected after 24 hours.

Day 2 Tetrazolium Procedure:

1. Put on gloves and goggles. Obtain forceps, a class petri dish, and dissecting microscope.
2. Remove the first seed from the tetrazolium solution with forceps and place in the glass dish.
3. Observe the seed through the dissecting microscope. Specifically, you're looking for the cotyledon (the first shoot during germination) is stained. If it is stained red/brown, consider the seed viable. If yellow or soft/mushy, consider the seed nonviable. See photos below.
4. Record your findings for all 16 seeds on the data sheet provided.





TETRAZOLIUM VIABILITY DATA SHEET

Seed #	**Viability** (*viable / nonviable*)	**Observation Notes**

GRAPHS

Calculate the seeds' velocity (cm/sec) in Google sheets. Compile the results of the class data on a Google sheet. Calculate the average seed velocity per site. Calculate the average number of viable seeds per site. Make two bar graphs to visualize these data.

CONCLUSION

Write a conclusion summarizing your results.

- Brief overview of eelgrass restoration, our study, and why this is important.
- An analysis of the results of our (the class') study
- A comparison of our results to Marion and Orth's (2010)
- Concluding thoughts about the big picture of eelgrass restoration.

Seagrass Field Sampling (Winter Island)

	Coverage	Sediment		
	Eelgrass	Sand	Gravel	Shell Hash
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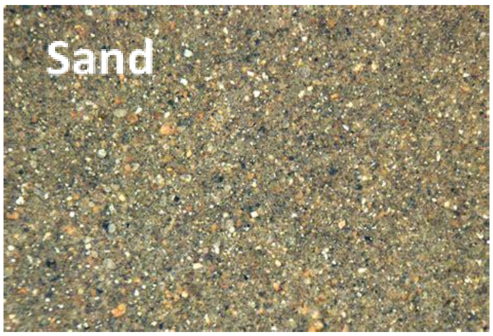
Team B		Y / N	Y / N	Y / N

Substrates:



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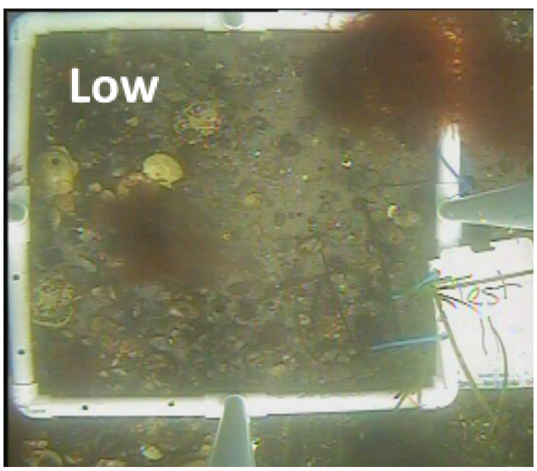
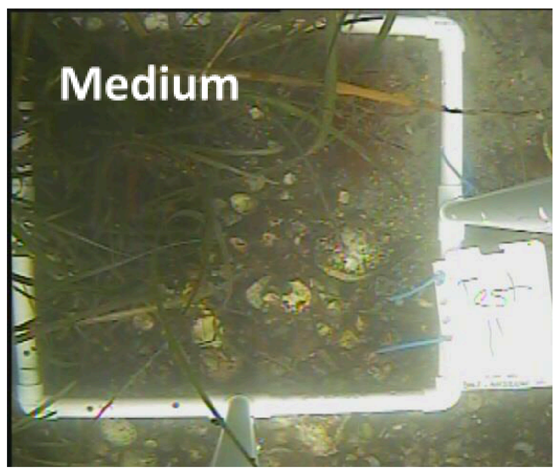
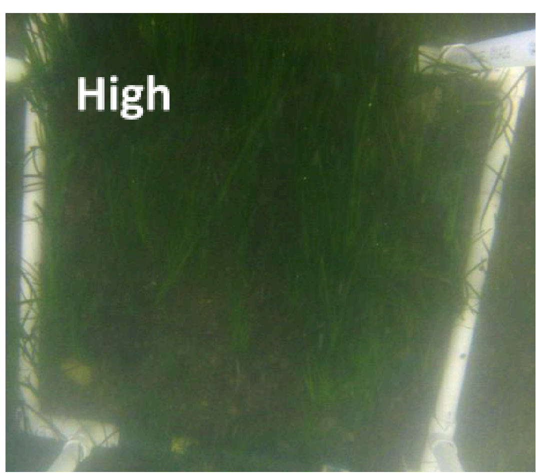






Moving toward seed-based approaches to eelgrass restoration and resiliency

MassBays
NATIONAL ESTUARY PARTNERSHIP

Jill Carr
NEP Tech Transfer 2024

Continued losses & attempts to restore

- Losing 2 football fields per hour globally
- Historic declines across most of Massachusetts, ~50% loss since 90's
- Traditional "adult shoot" restoration methods not scalable

Working Regionally

Working Regionally

- 2022: started convening New England NEPs to assess needs and resources

Intent: This spreadsheet will be shared with New England NEPs to assess resources and needs related to seed-based eelgrass restoration and monitoring; with the ultimate goal of developing region-wide project ideas that fill knowledge gaps and result in eelgrass restoration.

Instructions: Review the Components listed in columns A-C. In two blank columns to the right, work down the rows listing your resources and needs for each Component. Columns D and E can be used as an example (MassBays entry). Resources you list need not be in-house: in fact, it will be helpful to know who might be potential partners with resources (e.g., aquarium has seawater tanks, agency has boats, etc)

Data, Infrastructure, and Process Components to Support a Regional Large Scale Eelgrass Seeding Restorations

	Component	Description	*NEP (State)*: MassBays (Massachusetts) *Completed by*: Jill Carr — Resources Available	Needs
Data & Modeling	Data: eelgrass meadow locations and extents	Maps showing where eelgrass currently and/or historically exists. These data will inform all other modeling and groundtruthing efforts. Routine mapping programs may also be able to document restoration success or failure post-seeding.	MassDEP has run a nearly statewide mapping program since 1995. They fly and groundtruth one region per year on a 4 year cycle	An eelgrass condition assessment program
	Data: eelgrass meadow flowering shoots	We don't have a great sense for which meadows have the highest proportion of flowering shoots. Collecting from meadows with a greater proportion of flowering shoots will result in more	We have limited flowering shoot density data from three SeaGrassNet sites in MA, monitored for 10+	Need data from a larger sample of meadows. Included some of this work in WHOI pre-proposal for
	Data: optimal seed collection time	To optimize the viability of the seeds collected, collection timing should target ripened seeds that are beginning to dehisce (about to fall off). The timing of seed maturity will vary between states	No real dataset in MA, but could gather anecdotes from folks currently doing seed experiments (BU,	MassBays wrote this monitoring into a WHOI pre-proposal for 2024-2025 for field sampling. However,
	Data: traits and genetics of eelgrass populations	Eventually, a regional collaboration might include intentional selection of donor plants based on favorable genetic traits to acheive climate change resilience, e.g. moving plants from south to north	Limited genetics analysis performed on north shore by DMF and NEU as part of a restoration project (adult	Better coastwide coverage of genetics info; better understanding about what genetic similarities mean in
	Model: Donor bed site selection	Combine information about eelgrass extent, meadow persistence over time, density of flowering shoots, and access/safety to determine which meadows could be donors. Eventually,	No model in MA. We do have GIS and GIS skills in house.	MassBays wrote this monitoring in WHOI pre-proposal for 2024-2025.
	Model: Restoration site selection	To assess restoration suitability, assess historic and current eelgrass distribution, sediment type and potential human use conflicts, water temperature, water clarity/light, exposure/fetch	DMF has a restoration site selection model	DMF model needs enhancement; DMF and MassBays plan to work together in summer 2023 to
People	Staff personnel for project management, plan	A region-wide program would require a point-person in each state to lead the charge there. This person would directly manage that state's efforts (grant writing, reporting, planning,	I could take this on for MassBays' portion, and/or co-lead with agency partners.	...
	Staff or partner scientist for experimental design e.g. common gardens, genetics assessments	Ideally, each state would have an eelgrass practioner, either on staff or in a partner/subcontractor role (academic or NGO partner?) to develop research plans,	I could take this on and/or co-lead with agency (DMF) or academic partners (NEU? UMB? BU?)	Would need to lean on partners for experimental design assistance to ensure statistical strength.
	Field Personnel to collect reproductive shoots and distribute seeds	Depending on the location and tidal range, reproductive shoots can be collected via scuba, snorkeling or wading. They are easily recognizable in the field. Brad Peterson's lab	I could do some field work, and could train and manage field team. There would be a great deal of interest in	Funds to pay for external field team / partners.
	Lab Personnel to manage intake, storage and processing workflow	After collection, seed shoots must be promptly transported to seawater tanks. There is a spectrum of processing activities - but excess plant material should be removed to reduce tank	MassBays using BIL funds (2023-2025) to set up tanks and hire a university work-study student for tank	need funds after 2025 to sustain.
Infrastructure	Boats and/or shore-based access for harvest activities	Small skiffs are the ideal platform but shore entry can work if safety, swim distance, and beach access permission are addressed. Due to the high cost and skill required for boat ownership,	MassBays securing boat MOU with state university. Will have access to small center console and pontoon	need funds to sustain.
	Flow-through seawater tanks to hold the shoots until the seeds drop	Flow-through sea water systems are ideal (e.g. StonyBrook, VIMS) and there are a variety of setups and specs (e.g. aeration bubblers, drainable tanks for collecting seeds off bottom).	MassBays using BIL funds (2023-2025) to set up seed storage, processing, and experimental tanks at	need funds after 2025 to sustain.
	Materials to monitor germination success and other experiments (field equipment, lab	The exact materials you need to monitor a seeding effort really depend on the specific	DMF has all sorts of field monitoring and lab	

Component	*NEP Casco Bay Estuary Partnership (Maine)*: *Completed by*: Curtis Bohlen, Matt Craig — Resources Available	Needs	*NEP (State)*: Long Island Sound Study *Completed by*: Cayla Sullivan — Resources Available	Needs
Data: eelgrass meadow locations and extents	Maine DEP collected data in Casco Bay last year. Will be flying whole coast of Maine on 5 year schedule.	Finer-resolution data and surveys looking for seedlings may be needed. Daat outside of Casco BAy will help define regional	Since 2002, eelgrass have been intermittently monitored through USFWS aerial surveys. The most recent survey was	A defined monitoring approach to produce maps annually to better understand interannual and seasonal variability.
Data: eelgrass meadow flowering shoots	Limited diver surveys, expanding interest from volunteers, but I don't think this is an explicit focus of	Start collecting data. Consider ways to mobilize volunteers.	No information available. CT NERR, in collaboration with EPA, will be setting up SeagrassNet sites so there	Start collecting data.
Data: optimal seed collection time	I'm unaware of any relevant data.	Need protocols and methods., especially ones that can be implemented in part by volunteers.	No information available. If this information can be pulled from satellite imagery, we are looking to	Start collecting data in meadows with HOBO loggers
Data: traits and genetics of eelgrass populations	Very limited genetic data	Gather info that already exists from Maine, assess gaps & needs.	One study has conducted genetic work in LIS (Short et al. 2012).	Conduct another study to identify populations in Long Island Sound specifically resilient to temperature
Model: Donor bed site selection	I'll bet some of our colleagues have recommendations. No formal data or model	Need more info on criteria. Suspect we can find donor beds relatively easily once criteria are defined.	Working on a proposal to update the Eelgrass Habitat Suitability Index Model (originally developed by	Data collection on all parameters noted.
Model: Restoration site selection	Data is available, but no validated local model, little prior analysis. Analysis complicated by highly	Determine primary eelgrass stressors in Maine waters. Test regional habitat models in Maine and/or develop	We have a model, but we need to update it (working on developing a proposal now that will be competed).	Update to our current model
Staff personnel for project management, plan	Maine DEP, Maine TNC and CBEP all have staff with related responsibilities. Not yet clear who has capacity	Once project scope is better defined, reconvene Maine organizations and evaluate available capacity.	TBD - NYS is hiring a seagrass coordinator. Additionally, CT NERR is looking to lead eelgrass	
Staff or partner scientist for experimental design e.g. common gardens, genetics assessments	Few active eelgrass scientists in Maine. Some CBEP capacity to assist. May have to lean on		TBD - LISS has academics that could potentially play a role here	
Field Personnel to collect reproductive shoots and distribute seeds	Limited staff capacity. Very likely that we could recruit volunteers.	We'll need a lot more information on tasks and time involved to discuss who can contribute capacity	I can help out with this (pending joining EPA's dive team/management approval). Could potentially	
Lab Personnel to manage intake, storage and processing workflow	Limited capacity, given competing priorities. Prior (2015) eelgrass transplant collaboration with So. Maine	Initial outreach to existing labs (SMCC, GMRI, Bowdoin) to explore interest & fit. We don't have many	Could partner with UConn or Stony Brook. We have funding available to do this through Research Grant	
Boats and/or shore-based access for harvest activities	Several CBEP partners have boats. Boat time is in short supply. MUltiple suitable launch sites near	Check with Partners to determine available boat time, and/or raise funds to purchase boat time and	USFWS has offered up boat and captain in the past to LISS. Potentially NRCS?	
Flow-through seawater tanks to hold the shoots until the seeds drop	So. Maine Community College and other institutions have limited capacity in Casco Bay.	We need more details on requirements so we can begin conversations with Bowdoin, SMCC.	Could partner with UConn or Stony Brook. We have funding available to do this through Research Grant	
Materials to monitor germination success and other experiments (field equipment, lab			Depending who would lead this effort for LISS, we have	

Component	*NEP (State)*: Piscataqua Region Estuaries Partnership (NH/Southern ME) *Completed by*: Trevor Mattera — Resources Available	Needs	*NEP (State)*: Buzzards Bay NEP (MA) *Completed by*: Joe Costa — Resources Available	Needs
Data: eelgrass meadow locations and extents	PREP runs an annual "tier-1" aerial eelgrass monitoring program in the Great Bay Estuary that continues assessments		Same as MassBays; DEP now conducting assessments about every 5 years. The Buzzards Bay NEP may conduct	Worthwhile to bring in survey information from Chapter 91 licenses and NOIs for historic assessments. Baywide
Data: eelgrass meadow flowering shoots	NH has spatially limited flowering shoot density data from two SeagrassNet sites (one in Great Bay with	More monitoring. We don't have a good idea of when the flowering happens - it varies quite a bit spatially	Perhaps a few old site-specific assessments can be mined, but information on flowering	Characterizations of flowering may be variable from year to year among beds, but timing likely to be
Data: optimal seed collection time	No formal dataset currently exists in NH.	Protocols & methods to build this into current monitoring programs	Nil.	Seed collection will generally focus on shallow beds, so a seasonal collection time range for
Data: traits and genetics of eelgrass populations	Limited genetic data available, which includes an ongoing study by Cynthia Hays from Keene State and	Additional studies as well as knowing how specific traits affect resilience	?	
Model: Donor bed site selection	This type of model does not currently exist for Great Bay.	While we have a few donor beds commonly used for transplanting, this specific analysis for seed donors	Nil.	Because local permitting is also a factor, there will be a tendency for municipalities to want harvested seeds to
Model: Restoration site selection	NH has a model - Alyssa Novak (BU) and other updated the model originally developed in Short et al.	Additional data collection and possibly enhancing the 2021 updated model with additional parameters and	Site selection models have been developed.	Site selection models require logic model validation of common-sense principals to avoid GIGO.
Staff personnel for project management, plan	I could head this for PREP with the assistance of UNH JEL		None at the BBNEP; Mass DEP (Tay Evans?) should be heavily involved.	Agency staff can guide state0wide goals and help direct funding. for getting things done, I tend to favor
Staff or partner scientist for experimental design e.g. common gardens, genetics assessments	I can take this on. PREP is currently leading a multi-org/agency eelgrass restoration group that has	Coodination with other programs here to align experimental design	potential grad students, WHOI and MBL, possible Buzzards Bay Coalition participation	best driven by partner group or research lab that are the lead for site-specific projects
Field Personnel to collect reproductive shoots and distribute seeds	I can lead most annual fieldwork for a short window like this. A volunteer program is certainly not out	Potentially funding after 2025 depending on harvesting program that is established	potential grad students, WHOI and MBL, possible Buzzards Bay Coalition participation	best driven by partner group or research lab that are the lead for site-specific projects
Lab Personnel to manage intake, storage and processing workflow	PREP would likely use BIL finding through 2025 to pay for equipment and student/intern time for	Funding after 2025	potential grad students, WHOI and MBL, possible Buzzards Bay Coalition participation	best driven by partner group or research lab that are the lead for site-specific projects
Boats and/or shore-based access for harvest activities	PREP has access to boats and the boat launch at UNH JEL. Costs could be funded w/ BIL.	Funding after 2025	WHOI and MBL facilities	
Flow-through seawater tanks to hold the shoots until the seeds drop	UNH JEL has some flow-through tanks available, depending on the scale required.	Funding after 2025	WHOI and MBL facilities	
Materials to monitor germination success and other experiments (field equipment, lab	PREP has access to the majority of the field supplies		best driven by partner group or research lab that are the	

Component	*NEP (State)*: Peconic Estuary Partnership (NY) *Completed by*: Joyce Novak — Resources Available	Needs
Data: eelgrass meadow locations and extents	We have an existing aerial map of eelgrass from 2016. We are fully funded for a 2023 aerial survey and groundtruthing. We pay a	Need increased funds for better annual monitoring which may include high tech satellite imagery and trained staff to groundtruth. Funds
Data: eelgrass meadow flowering shoots	We have shoot density but not flowering shoots	
Data: optimal seed collection time	Seed collection is carried out by partners. WIth warming waters, we are looking to characterize	research funding
Data: traits and genetics of eelgrass populations	We are looking to begint his work this year with a CWG grant and target resistent seeds/plants	additional funding for targeting and transplant of seeds from resistent individuals
Model: Donor bed site selection	n/a	all
Model: Restoration site selection	We had a bio-optical model / site selection tool completed in 2019. We excluded embayments	Thermal dynamics in all embayments
Staff personnel for project management, plan	We need additional project management as this initiative grows - it has started to take up too much	Eelgrass coordinator
Staff or partner scientist for experimental design e.g. common gardens, genetics assessments	Dr Brad Peterson and the Peterson Lab at Stony Brook University has been leding this effort here.	His lab can handle growing work but will likley require funding for even expanding work.
Field Personnel to collect reproductive shoots and distribute seeds	The Cornell COoperative Extension can be used for this	funding
Lab Personnel to manage intake, storage and processing workflow	Peterson Lab	funding
Boats and/or shore-based access for harvest activities	access to these at additonal cost	funding
Flow-through seawater tanks to hold the shoots until the seeds drop	unsure	
Materials to monitor germination success and other experiments (field equipment, lab		

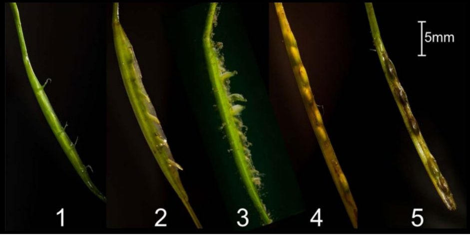
Working Regionally

- 2022: started convening New England NEPs to assess [needs and resources]
- 2023: Create and distribute flowering phenology protocol, based on NEP request

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 col... Programs and NGO organizations located in New England. The proto... boat, and via snorkel, wading or scuba, by professional or trained vol...*



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

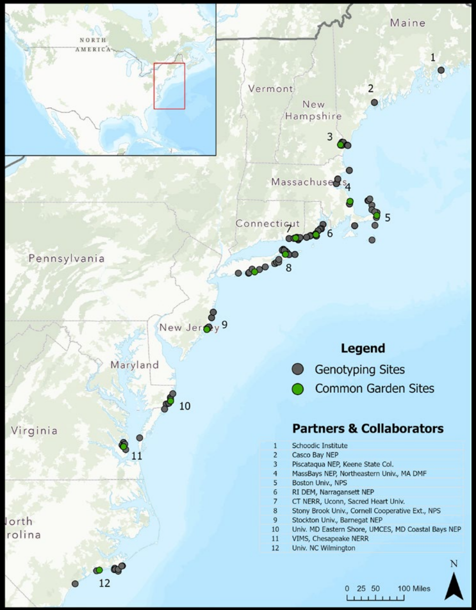
(B) Flowering shoot density, *every-other week, or at least once per year when at least 50% of spathes reach stage 4*, and/or

(C) Seed density, *at least once per year when at least 50% of spathes reach stage 4*.

Hoping to convene all partners this winter!

Working Regionally

- 2022: started convening New England NEPs to assess needs and resources
- 2023: Create and distribute flowering phenology protocol, based on NEP request
- 2023: connect with National Parks Service, with parallel interests
    - Giant $18M "HEAT" Proposal to NOAA → ☹ (still looking for $$!)
    - NPS able to continue with internal funding for subset of sites



- 2023 - ? : MassBays uses BIL funding to build and staff shared tank infrastructure

(Cat Cove Marine Lab, Salem MA)

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  (Cat Cove Marine Lab, Salem MA)
- 2024: WHOI SG funds for seeding study across MassBays area,

  MADMF-RIDMF get funding for sister study in MA-RI

WHOI SeaGrant Study:

Fill data gaps for seed-based restoration

1) when and where reproductive eelgrass shoots should be harvested

2) the quantity, quality and germination rate of MA origin seeds

3) potential impacts of seed harvest on a donor meadow

4) the regulatory processes needed to permit large-scale routine harvest and seeding

Develop Best Practice Guide

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Photo: Forest Schenck

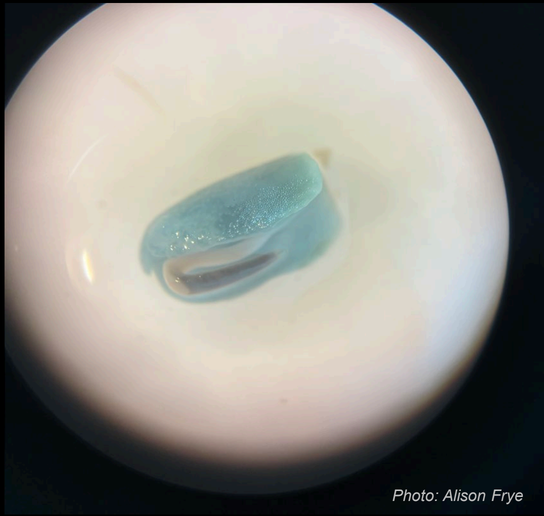

Photo: Alison Frye

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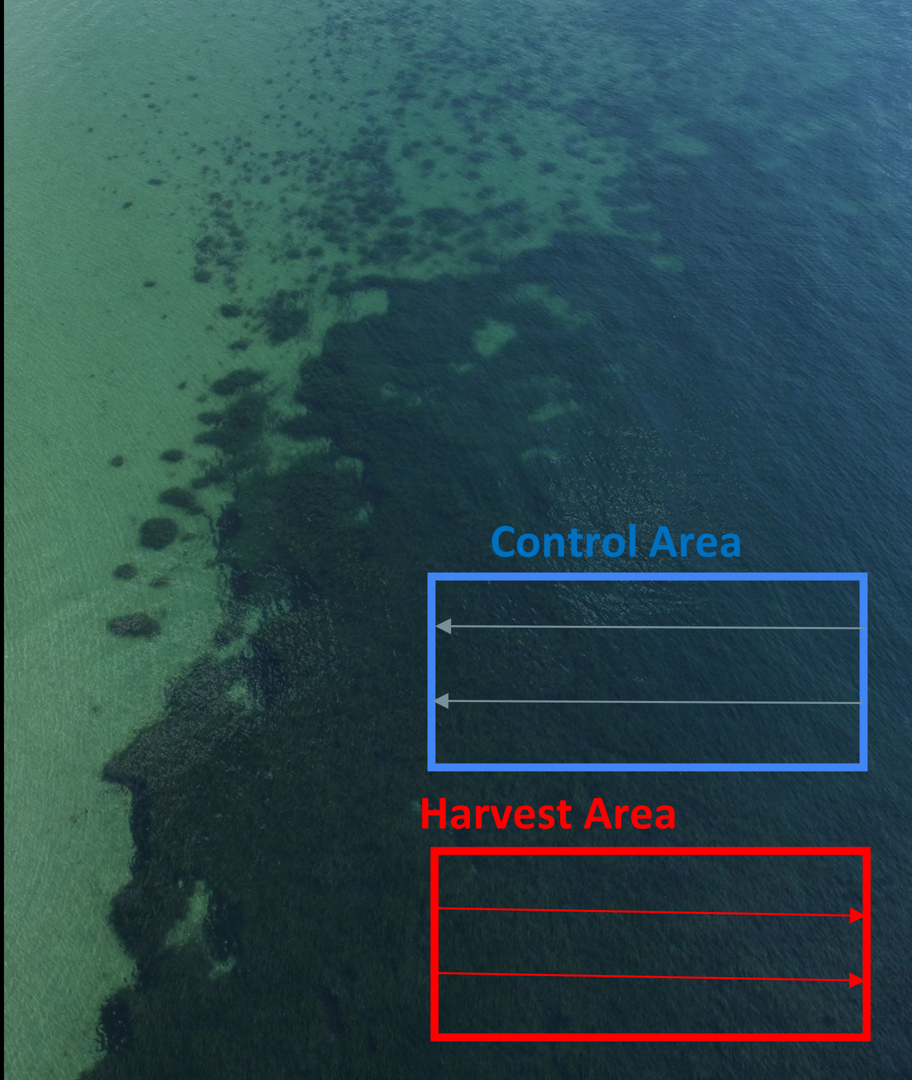

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Develop Best Practice Guide

EPA

US Army Corps of Engineers®

MassDEP
Commonwealth of Massachusetts
Department of Environmental Protection

Massachusetts Environmental Policy Act Office (MEPA)

Massachusetts Office of Coastal Zone Management

Commonwealth of Massachusetts DIVISION of MARINE FISHERIES

CONSERVATION COMMISSION

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Develop Best Practice Guide


thank you!

APPENDIX B

Protocols

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).

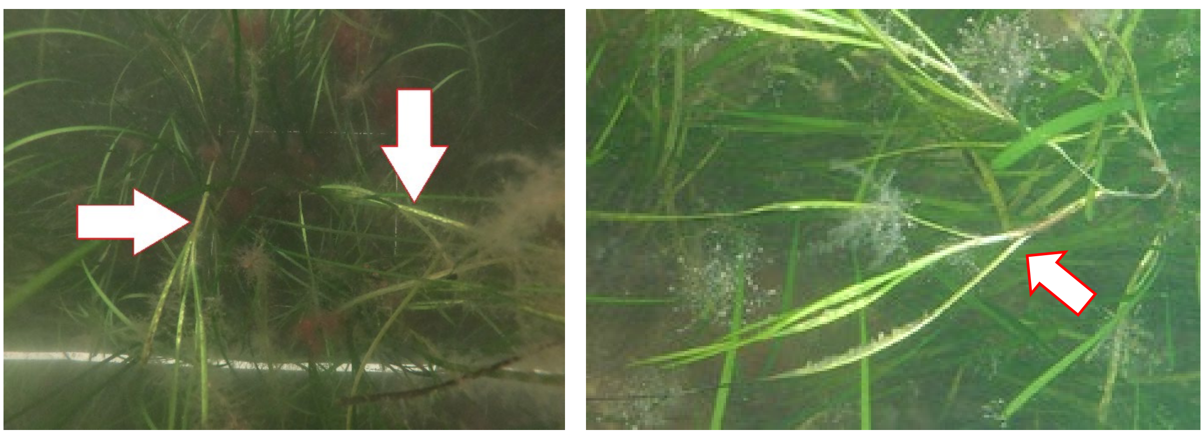


Fig 1 : Examples of reproductive shoots in the field. Source: MA DMF (left), SeagrassLI.org (right)

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.

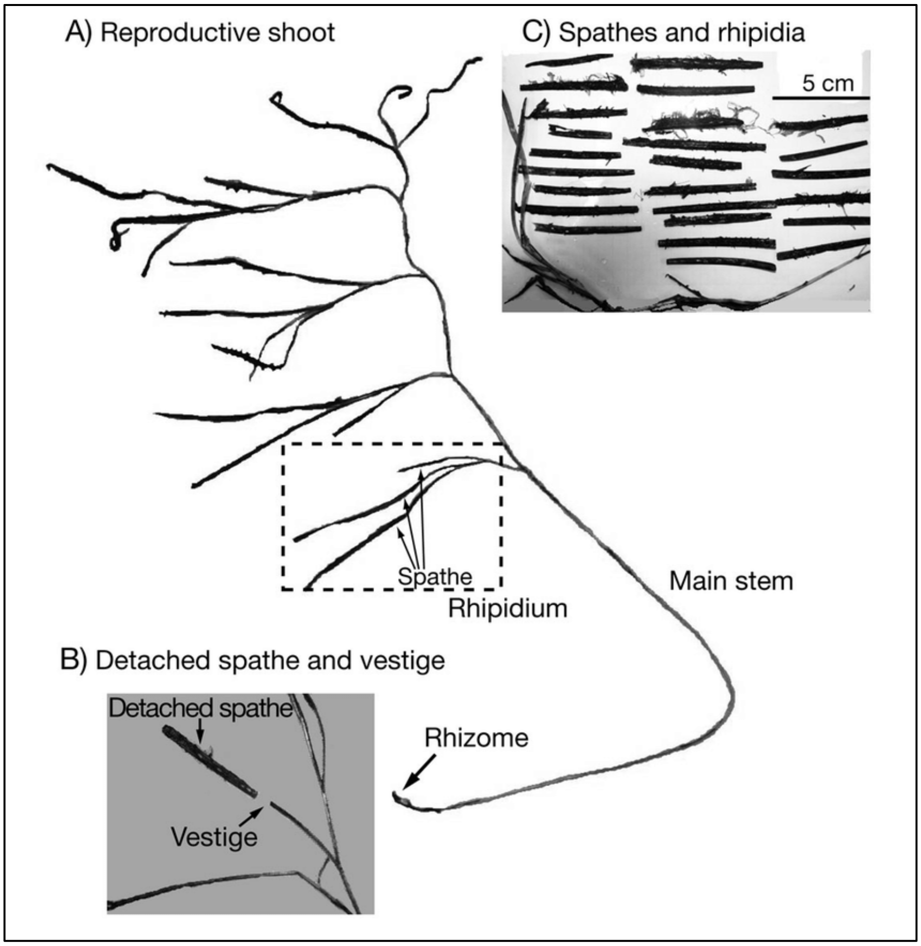


Fig 2. Eelgrass reproductive shoot morphology. Source: Hosokawa et al 2015

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)

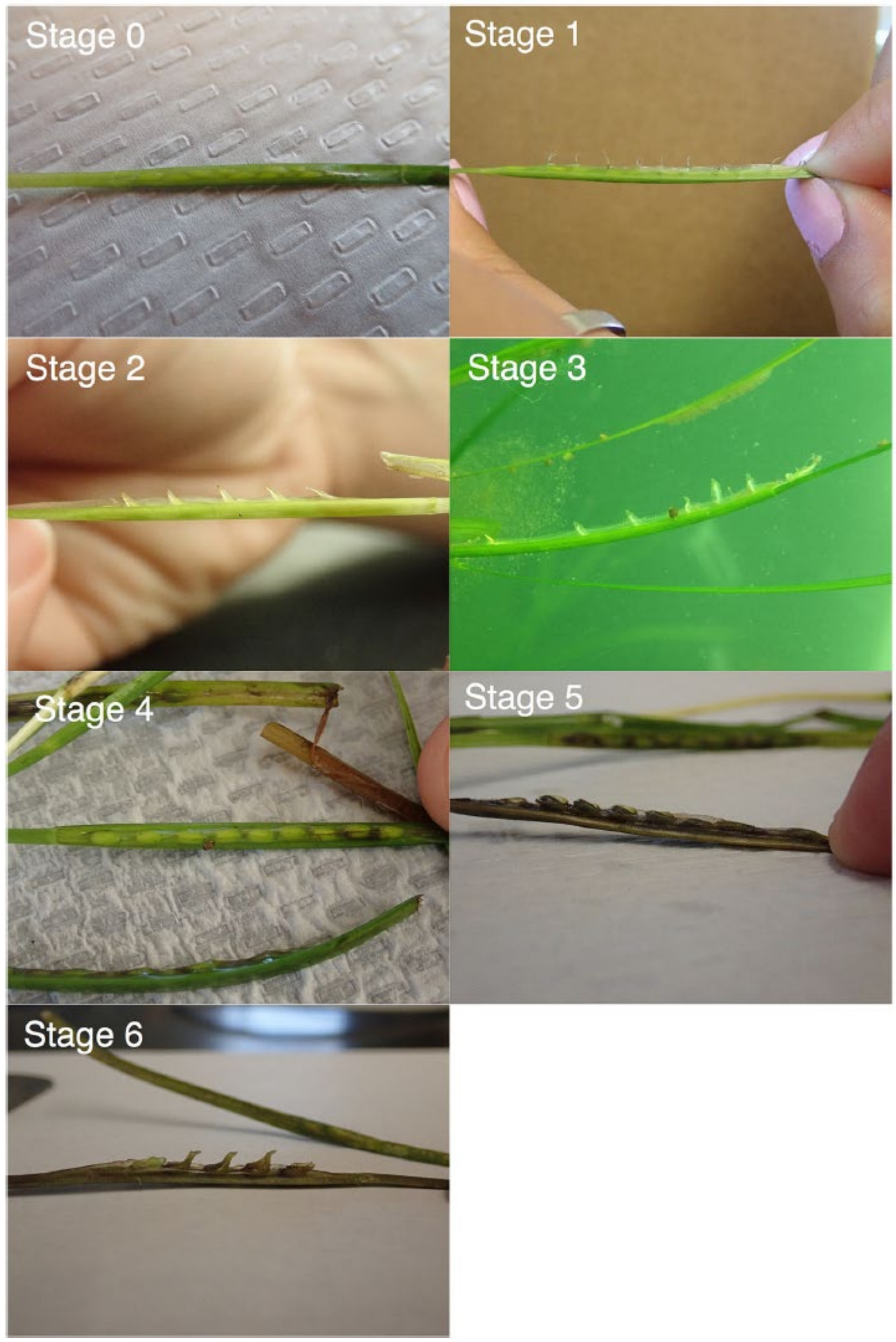


Fig 3A. Stages of eelgrass seed development (von Staats et al. 2021).

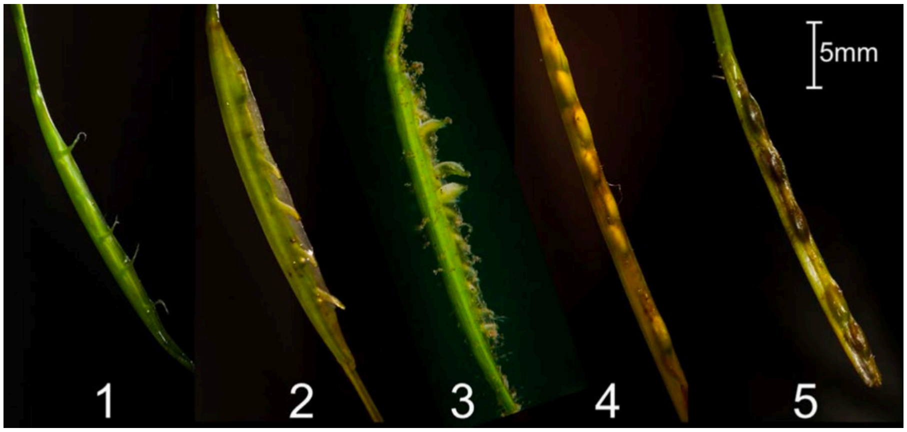


Fig 3B. Stages of eelgrass seed development (Infantes and Moksnes 2018).

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 (1/4th) or 0.0625 m^2 (1/16th). *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.

References

De Cock, A.W.A.M., 1980. Flowering, pollination and fruiting in Zostera marina L. Aquat. Bot. 9, 201–220

Hosokawa, Shinya & Nakaoka, Masahiro & Miyoshi, Eiichi & Kuwae, Tomohiro. 2015. Seed dispersal in the seagrass Zostera marina is mostly within the parent bed in a protected bay. Marine Ecology Progress Series. 523. 41-56. 10.3354/meps11146.

Infantes, E. and Moksnes, P.O., 2018. Eelgrass seed harvesting: Flowering shoots development and restoration on the Swedish west coast. Aquatic botany, 144, pp.9-19.

Kuo, J., McComb, A.J., 1998. Zosteraceae. In: Kubitzki, K. (eds) Flowering Plants · Monocotyledons. The Families and Genera of Vascular Plants, vol 4. Springer, Berlin, Heidelberg.

von Staats, D.A., Hanley, T.C., Hays, C.G., Madden, S.R., Sotka, E.E. and Hughes, A.R., 2021. Intra-meadow variation in seagrass flowering phenology across depths. Estuaries and Coasts, 44, pp.325-338.

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 Names:___________________Sample **Scoring** Date/Time:_____________________
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**
1	*1*	*3, 3, 4, 4*	
1	*2*	*3, 3, 4, 5*	
1	*3*	*4, 5, 4*	
1	*4*	*2, 2, 2*	
2	*1*	*4, 5*	
2	*2*	*3, 5, 3*	

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 C

Advisory Committee Meeting Materials

WHOI SeaGrant - Advisory Committee Meeting

2/26/24

In attendance: Forest, Alison, Jill
Phil, Randall, Alyssa, Jessie, Brad, Holly
Note: Action Items added to each section in a 3/11/24 team meeting following the AC meeting

Field Methods:
Task 4 (Slide 5)

- Brad: will all 8 sites have light/temp sensors? - Yes
- OK to reduce density counts at less than 12 quadrats? Jessie - use smaller quadrat (coffee can!) for high density areas; Brad - use ¼ for flowers and 1/16th for vegetative instead; Phil and Randall agree. Keep all 12 quadrats.
- Keep sensors out thru winters? Yes, agreement
- Harvest efficiency: harvest by time or target count? Phil - should disperse impact over area, so target count may not be as representative as target time. Brad - divers track to reach specific shoot count target rather than harvest as much as possible in fixed time. Jessie - how long to fill a big trash can? Lets you average out for variability among individuals, worked great for harvest at scale. Brad - order of mag difference between divers and snorkelers; 1 diver probably = 3 snorkelers. Brad and Jessie - would be nice to be able to gauge differences between novice and seasoned harvesters, would better inform best practices

Action Items:
Keep 12 quadrats, use 1/4m2 for repro counts and 1/16 for vegetative counts. Always put 1/16 in the same spot.
Keep timed harvest as time-based rather than shoot count based

Sites (Slide 6)

- Phil- value in revisiting Von Staats sites. Value in sampling stressed meadows since some data indicating higher flowering rates. However if harvest for restoration is the goal these may not be sites we'd want to target.
- Randall- harvesting stressed beds feels like a separate question.
- Holly - would want to see representation of all types of beds: estuaries, open coast, sparse, dense, healthy, stressed
- Phil interested in helping collect on north shore (Niles Beach Gloucester and Pirates Cove Nahant)

Action items:
Final result is 10 DMF stations; confirmed a good mix of estuarine/open water, dense/patchy.
We can't confidently assess healthy vs stressed at this time, out of scope.
Phil committed to sampling Nahant/Dorothy and Niles; CCS/NPS committed to outer cape. FS will connect with CSCR
Forest removed BH; assigned some sites to partners in new GIS layer.

MBL - any chance they can add a site, or have a site mid cape?

C-I Experiment (Slide 7)

- Is the experimental area (800m2, 0.2 ac) big enough? Too big? Alyssa - this is a big area and a lot of work. Has done it with grad students over a 10x10m area and they've not been able to finish after several hours. Experienced divers can be more efficient. Maybe plan on a couple days.
- Jessie - since they develop over time, you might not get every flowering shoot. Consider a pre or post check to see if you got them all.
- Brad - how do you know when? - Pair with the temporal maturity sampling and use one of those sites.
- Phil - for this experiment the exact timing is less important because flowering shoots are not generally produced over the course of the season?
- Jessie - can you look into seed bank? - we're considering a coring component
- Brad - likely to miss some. - Maybe we aim for a certain number of shoots within the area instead?
- Using the West Beach SGN site?
    - Holly - only overlap control site with SGN, no harvesting along the SGN transects. 100 m away seems far enough at first thought.
    - Brad in favor of control site overlapping with SGN site
- Brad and Jessie - Separate treatments based on seed dispersal distances from literature

Action Items:
Keep 0.2ac for now, but may adjust after getting maturity/density data in 2024 to the appropriate size to collect x # of seeds. Based more in reality of restoration
Time the project as we would for restoration (seeds stage4/5)
Keep coring / seed bank component at each monitoring along diver transects
Use SGN site; keep SGN shallow transect in the control site; ensure impact >100 m away

Seed Quality (Slide 8)

- Jessie - based on marion and orth paper, NONE of her seeds would be considered viable. Consider adding additional metrics; and staining the cotyledon with tetrazolium and see if they still are viable. Fall velocity may be very region specific
    - FS has methods papers for the tetrazolium technique
- Brad - Infantes reduced fall velocity threshold; that's what they use

Action Items:
Keep 5cm/s threshold; can include staining of 40 seeds per site, only sample from the 5 DMF sites during timed collections, spaced out geographically (Duxbury, Scituate, Lynn, Salem Sound, Cape Ann).
Forest will put historic drop test data in the shared folder for reference.

Seed Germination / predator exclusion (Slide 9-11)

- Should we also include a cage control? Jessie - always. Big regrets when they are left out - Alison - may be able to do 16 pouches per quadrat instead of 9. No objections.
- Holly- do you have capacity to test nonlocal sites? Should do it if possible! -Yes

- Holly to group - is 10 seeds per pouch enough? Jessie - worry that germ rate will be too low, as it usually is in the field, to have successful experiment. Brad- may have a bunch of 0’s and a bunch of 1’s and 2’s for results. MAy be difficult on back end to analyze these low numbers.
- Phil - predator exclusion - any attempt to quantify green crab pressure might be? Perhaps trapping?
- Brad - crabs want to burrow under the pouches. Consider putting a predator exclusion net/cage around the pouches to prevent things from digging in and under. Alyssa agreed. Consider adjusting exp. Design to incorporate this comment.

Action Items:
Collections from local (Salem SOund) and non-local site (Duxbury) in germination experiment (from timed harvest experiments)
Will now place 16 replicates within each quadrat; 20 quadrats (2 rows of 10). 160seeds/m2 (1,600 seeds from each site)
Include sediment in the pouch for predator exclusion with cage
Reduce burrowing: use 1mx1m burlap squares, and stitch 16 treatments to it for pouch treatments. For control, keep bare with stakes if 4 quadrat corners to ensure collection within the right area. Cage control - nix this plan; no great way to cage the treatments
Place a baited green crab trap, mark crabs, note recapture (AF will source some gear): trap out for 24 hours, mark/release, 1 week later another 24 hour catch to find recaptures. Sex/measure upon capture. Will look into trap mesh sizes, ask alyssa what she uses

Seed harvest model (slides 12-14)

- Phil - water depth impact on flowering rates. Highest rates tend to be shallow.
- Jessie - use observation station data; at least use to overlay onto grid
- Phil - weekly should be the goal; monthly not helpful. Jessie - weekly good, good to get a measure of how much that target week changes year to year
- Brad - may be able to use SST instead after correlating with air temperature
- Holly - USGS Joel Carr did that correlation already for pleasant bay using local airport air temp aired with SST satellite and in situ water temp data; generated relationship and created a temp curve and recreated a 30 yr temp record. Its in progress
- Alyssa - could project out in time, major benefit
- Jessie - understanding how long the viable window lasts and how sharply the window of prime seed collection closes would be good to try and include (in NC the seed collection window closes quickly!).
- Forest/Jill (afterwards) - consider producing model after field data collection

Action Items:
Create regression of day of maturity against air temp from point stations. Use regression relationship to generate model using air temp raster (highest resolution possible - Jill to look for more data sources since modern NOAA rasters are 32km). Incorporate SNEP data.

Regulatory (slide 15)

- Phil - **do involve some local representative ConComs** (sites most likely to receive resto projects); target communities with some knowledge of the topic (gloucester, nahant, salem?).
    - Forest: Maybe could use a case study approach to permitting?
    - Forest: Identify towns with greatest difference between existing eelgrass habitat and suitable eelgrass habitat / historic eelgrass habitat to target for case study?
- Phil- Permitting guide: helpful to describe the process but with the understanding that different reviewers will provide different answers. Layout process, but goal is not to provide answers.
- Discussion around regulators needs to happen. Regulators need to be prepared for these types of applications. Either react individually or develop regulations / guide.

Action items:
Select regulatory participants, incl a couple ConComs
Develop a risk of components of large-scale seed-based eelgrass restoration that are/could be subject to regulation (Summer 2024)
Identify 2-3 'key' components of large-scale seed-based eelgrass restoration to highlight with case studies (Summer/Fall 2024)
Develop case studies tailored to these focused questions/components, but all around concepts of large scale restoration and bring these through permitting with the resource agencies (Winter-Summer 2025)
Write up summary of how the process went (Fall 2025)
Keep regulatory meeting scheduled for fall 2024

    -General description/background of large-scale seed-based eelgrass restoration
    -What's involved, what are the outcomes, fielding questions
    -Where has it been used
    -Has it been successful

Modeling & field studies to prepare for large-scale eelgrass restoration

Advisory Committee Meeting

February 26, 2024

OVERVIEW

- WHOI Sea Grant funding, 2024-2026
- Fill data gaps for seed-based restoration

  1) when and where reproductive eelgrass shoots should be harvested,

  2) the quantity, quality and germination rate of MA origin seeds,

  3) potential impacts of seed harvest on a donor meadow, and

  4) the regulatory processes needed to permit large-scale

  routine harvest and seeding.

- Develop Best Practice Guide

OUR ASK OF YOU

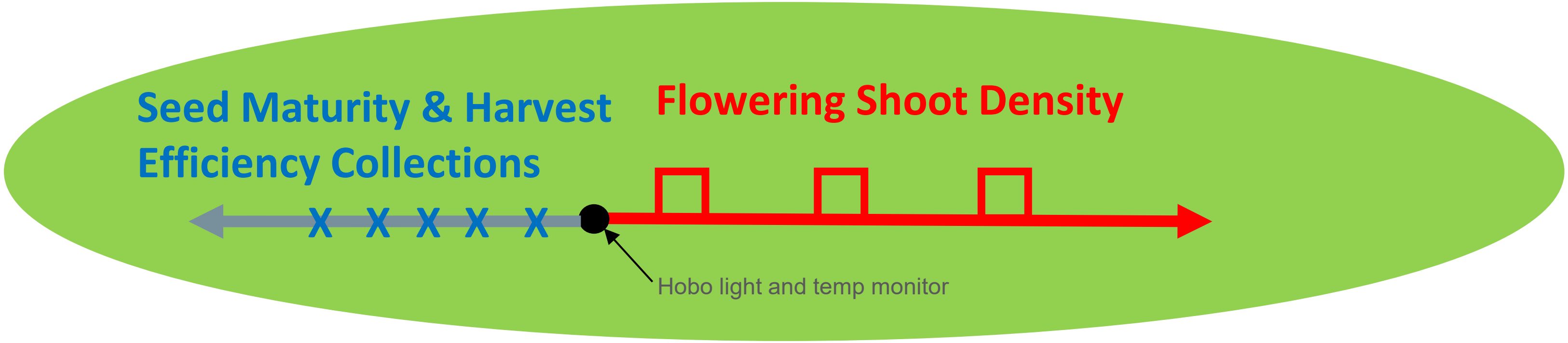
- Weigh in on project concept, scientific design, site selection
- Review QAPP and/or Best Practice Guide as able
- Provide expert guidance for permitting tasks

Field Methods

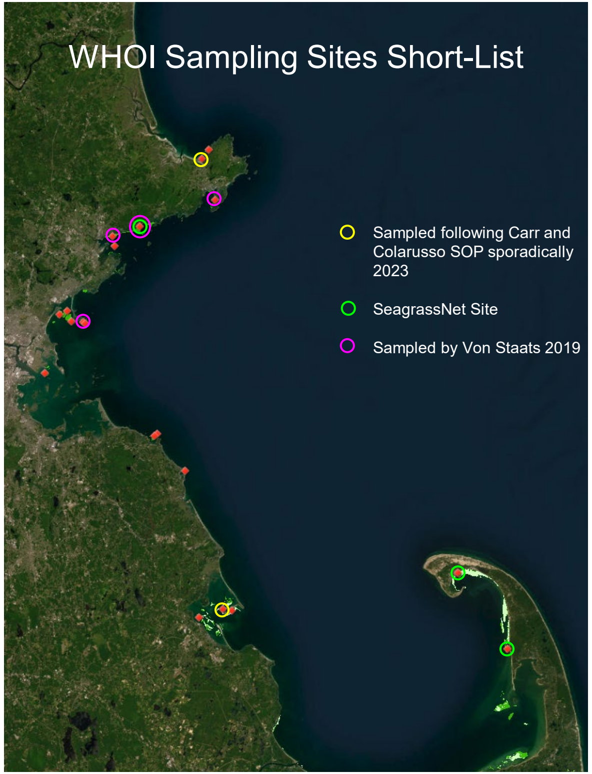
Seed Development and Timing Field Sampling (Task 4; 2024 & 2025*)

*If funds allow; budgeted for 1 year

- Follow Carr and Colarusso 'Assessing Eelgrass Flowering Density and Seed Maturity' SOP
  - Sampling 8 sites every 2-weeks from early May through early August (targeting depth of 2 m MLLW)
  - Score 5 flowering shoots following staging protocol from von Staats et al. 2021 within 24 hrs of collection
  - Count flowering and vegetative shoot density in 12 ¼ m^2 quadrats spaced at 2 m intervals along a transect
- Harvest efficiency
  - 1 sampling event per site (when at least 50% of spathes reach stage 4- seeds present)
  - Timed collection (surface to surface) by 2-3 divers of 100 shoots each



Sampling Locations

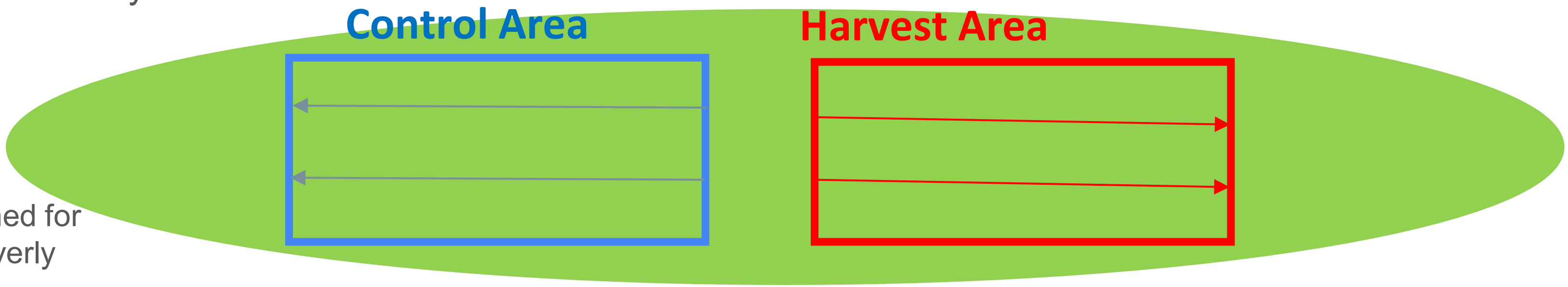


Notes:

- Goal is to sample 8 sites
- Center for Coastal Studies has agreed to monitor 2 sites on Outer Cape
- Coordinating with Cohasset Center for Student Coastal Research regarding sampling in Cohasset

Flowering Shoot Harvest Control-Impact Experiment (2025)

- All reproductive shoots will be harvested from a 40mx20m area
  - Collections by hand or mechanical harvester
- A second 40mx20m ‘control’ area will not be harvested
- Areas will be surveyed by divers and side scan sonar 1-week prior, within 1-week of harvest, 1-month and 3-months following harvest
  - Divers will record eelgrass density, % cover, canopy height, and note signs of damage at 20 points along 2 transects in each treatment area and possibly core to estimate seed density?).
  - The presence of eelgrass will be estimated from the side scan sonar imagery at 100 points randomly distributed in each treatment area.



Tentatively planned for
West Beach, Beverly

Seed Quality Determination (2024 & 2025)

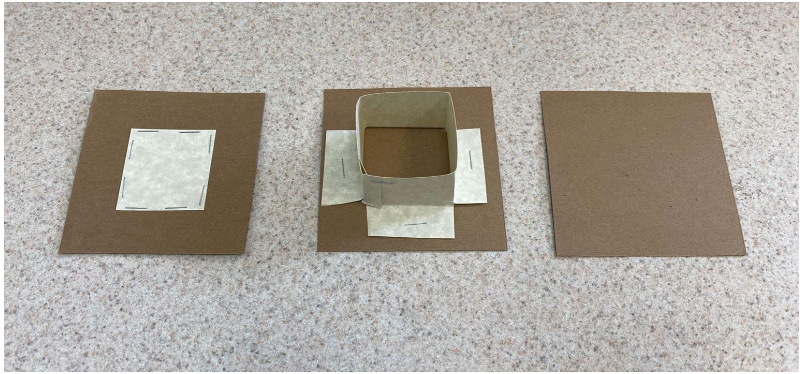
- Seeds from timed collections and maturity sampling? (Task 4)
  - July-August 2024 & 2025
- Marion & Orth (2010) seed quality protocols:
  - Seed hardness (firm vs. soft)
  - Seed coat (intact vs. damaged)
  - Fall velocity (rapid vs. slow)
    - Tested in an aquarium with 22cm of seawater at 20ppt
    - 5.5cm/s fall velocity (89% germinated)
- Outreach opportunities

Seed Field Germination Study (2024 & 2025)

- “Good” quality seeds will be used in a predator-exclusion experiment to investigate germination rate in the field
- Shallow, subtidal water off Winter Island, Salem, MA
- Install Sept. 2024, retrieve by June 2025 and assess germination rate

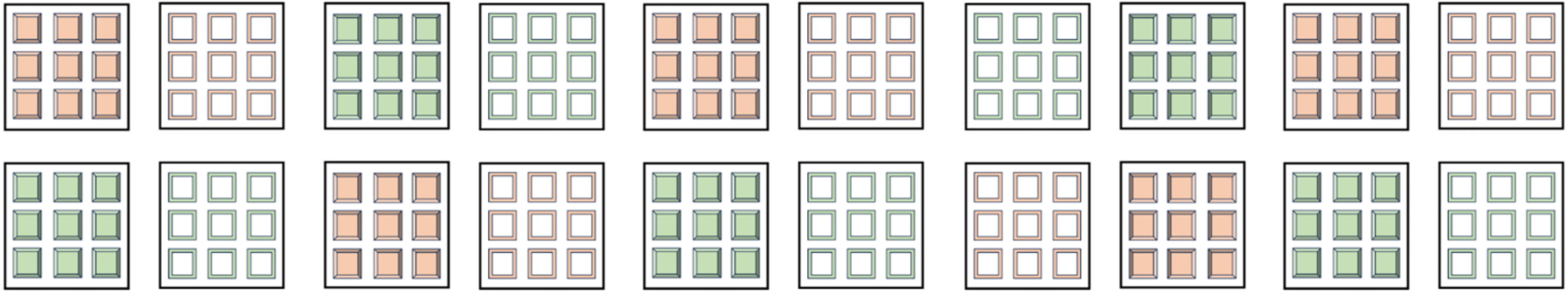
Seed Field Germination Study (2024-2025)

- Harwell & Orth (1999) and Morro Bay NEP method
- Burlap pouches (predator-exclusion)
    - 10 seeds enclosed in 2” x 2” pouch with 1” border
    - Covered with 2-3cm of sediment
- Cage control?
    - Harwell & Orth: pouch did not increase seed mortality (50% germination rate in greenhouse bag vs. no bag)



Seed Field Germination Study (2024-2025)

- **Research focus:** germination rate of “good” seeds from Salem Sound
  - Is there value in investigating local vs. nonlocal seeds?
- Assuming two locations:
  - Twenty $1m^2$ quadrats, each with 9 pouch treatments
  - Per treatment: 5 quadrats = 45 pouch replicates



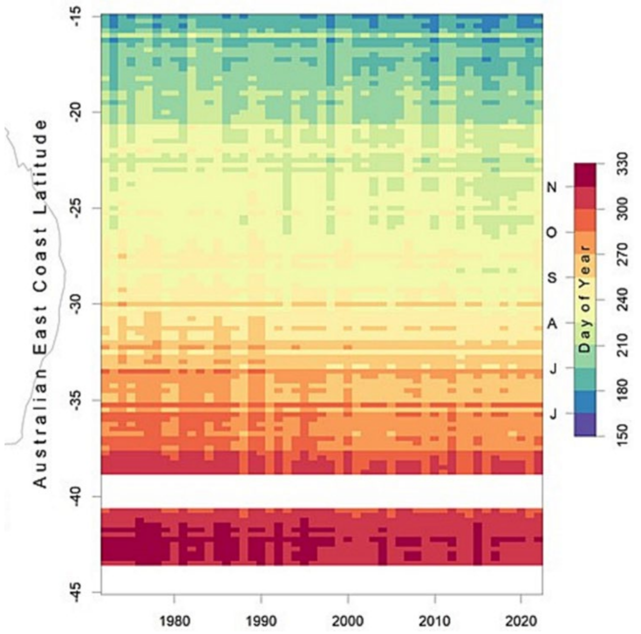
Seed Harvest Model

- Optimal timing for efficiency and viability: just before majority of seeds start to dehisce (but when is that?)
- Blok et al. (2018) found:
  - peak maturation of seeds when avg monthly air temp = 20.9°C
  - timing of peak seed maturity increased 9.8 days per 1° increase in mean annual air temperature and by 5.7 days per 1° decrease in latitude
- Lekammudiyanse (2024) found:
  - timing of peak seed maturity increased ~8 days per 1° increase in mean annual air temperature
  - Also looked at solar radiation, water temp, tidal variation, biotics

Seed Harvest Model

Proposed method, Step 1:

- Predict peak maturity
  - Mean air temperatures over 20+ years (?)
  - Sources (any advice?):
    - WorldClim, 1970-2000, monthly (~1 km resolution)
    - USDA, 1975-2005, monthly (~4 km)
    - NOAA, 1979-2024, daily, weekly (preferred), monthly (~32 km)
  - Model when avg temp reaches 20.9 C



from Lekammudiyanse (2024)

Seed Harvest Model

Proposed method, Step 2:

- Ground truth the model
  - Assess plant maturity over several weeks at 8+ sites
  - Determine peak maturation (stage 5) day of year
  - Compare against modern air temp data (local stations?)
  - Regression analysis
  - Adjust model
  - Make pretty for GIS!

Adapted from Von Staats et al. 2021

Regulatory: How to best permit seed-based restorations?

- Host workshop with DEP, ACOE, MEPA (others?)
- Pose hypothetical scenarios of:
  - Harvesting with varying methods, frequency and scale
  - Restoring at different scales
  - Restoring with non-local seeds
- Discuss submission of 1-2 real projects for permitting:
  - What regulatory language exists currently?
  - What additional science is needed from regulators?
- Is a “permitting guide” too ambitious for our project? Pros/Cons prescriptive regs vs more discretionary process?

Discussion