Standard Operating Procedure (SOP) for *Zostera marina* (Eelgrass) Field Trials and Monitoring

About this resource:
This document is a detailed standard operating procedure for implementing and monitoring *Zostera marina* (eelgrass) restoration field trials. It provides comprehensive, step-by-step guidance on seed collection, processing, transport, and field deployment, with a focus on best practices for maintaining seed viability and ensuring consistent data collection. This resource supports the development of replicable, science-based restoration projects across estuarine systems.

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About the project:
This resource was developed through a 2022-2025 Collaborative Research project titled *Evaluating and Enhancing Eelgrass Resiliency and Restoration Potential in a Changing Climate*.

In the lower Chesapeake Bay, Virginia, warmer water temperatures in recent years have resulted in large scale diebacks of eelgrass meadows (Zostera marina). In contrast, many eelgrass populations in Back Sound, North Carolina appear to be more resilient to warming water temperatures. Understanding the drivers of these regional differences in eelgrass resilience could help more effectively restore eelgrass meadows in a changing climate.

With a network of the intended users from reserves, state agencies, and Chesapeake Bay nonprofits, this project compared resiliency traits of eelgrass populations in Virginia and North Carolina by conducting reciprocal restoration trials and genomic sequencing. The project results indicate the importance of seed sources in potential future eelgrass restoration, in addition to site selection.

This webpage provides more information about the project.

Standard Operating Procedure (SOP) for Seagrass Field Trials and Monitoring

1. Seed Collection, Storage, and Transport for Field Trials

1.1 Identifying Field Trial Sites and Donor Populations

1. Utilize habitat suitability models incorporating existing ecological data to identify the most appropriate field trial locations.
2. Select seagrass meadows that have previously been genotyped and found to comprise populations exhibiting strong adaptive capacity and resilience to climate stressors as donor sites.
3. Use genomic analyses and additional environmental assessments to refine seed sourcing and field trial locations. If genomic analysis is not available, select meadows that produce a high number of viable seeds.
4. Deploy continuous temperature and light loggers at each donor site to monitor temperature throughout the year.

1.2 Reproductive Shoot Collection

1. Conduct reproductive shoot collection at donor meadows during peak reproductive output.
2. Harvest reproductive shoots from each donor population to generate seeds for both local field trials and trials at other sites. Seeds should be stored in site water under ambient conditions until transported to the nearest harvesting center.

1.3 Seed Storage and Processing

1. Transport harvested reproductive shoots to the nearest seed storage center.
2. Store flowering shoots in separate flow-through or recirculating tanks under ambient light and temperature conditions until seeds are released. To prevent mixing of seeds from different populations, all flowering shoots should be stored in separate tanks designated for each population or physically separated within tanks using dividers or mesh barriers.
3. Store seeds under ambient temperatures with aeration provided by bubblers to minimize sulfide accumulation. Stir the seeds at least once daily to ensure even oxygenation and prevent sediment buildup.
4. Flowering shoot material should be maintained for a minimum of three weeks or up until all mature seeds are released from the flowering shoots. Following seed release, floating reproductive material will be removed from the tank.
5. Seeds will then be collected by draining the tanks into a 1-mm mesh screen. Any remaining debris will be rinsed from the seeds which will then be stored in filtered seawater (salinity 25), at 22°C, under aerated conditions.
6. Initial seed viability from each donor population will be tested using tetrazolium chloride methods, and store according to best practices (see Tetrazolium Chloride SOP).

7. One week prior to setting up restoration plots, soak seeds in 0.2 ppm copper sulfate to eliminate potential pathogens (see Copper Sulfate for seed treatment SOP).
8. Immediately prior to transportation, place 400 seeds in 20 ml scintillation vials filled with ambient salinity seawater that has been treated with copper sulfate. To reduce counting time, count out 5 reps of 400 seeds per population and measure their volume in a graduated cylinder. Record the volumes for each rep, use the greatest volume to estimate 400 seeds for all future reps. Place a waterproof label with the seed source and rep number in each vial prior to closure. Store in a water bath to maintain ambient temperatures.
9. Transport copper treated seeds to the nearest seed storage facility in the location where restoration plots will be established approximately two weeks before peak in situ germination (late summer). All seeds should be shipped in aerated conditions at ambient temperatures.

2. Field Trials, Monitoring, and Evaluation

2.1 Seed Dispersion in Field Trial Sites

1. Disperse seeds from both local and outsourced donor populations at three identified field trial sites in each state.
2. Establish triplicate seed-specific subplots within each site. Mark the opposite corner of each plot with a PVC pole with an attached labeled float, so that the plot measures 3 X 3m. Record GPS coordinates in middle of plot along with plot location, rep number, and seed source.
3. Ensure a minimum of 3 meters of spacing between each plot to prevent genetic exchange.
4. Deploy seeds at a density of 400 seeds/m^2 in the middle 1m^2 of the subplot to maximize germination rates and seedling survival. To ensure that seeds do not move from their intended plot, keep a lid on the scintillation vial until seeds are near the soil surface. Once at bottom of the middle of the plot, remove the cap and gently disperse seeds in a small area.
5. Deploy continuous photosynthetically active radiation (PAR) sensor arrays at each field trial site for a one-year duration.
6. Download all GPS coordinate data and scan all datasheets immediately upon return to the lab.

2.2 Monitoring and Data Collection

1. Conduct monthly monitoring from early spring through fall following seed planting.
2. In each subplot, use a 1m^2 quadrat to assess percent cover using the Braun-Blanquet scale.
3. In same quadrat, determine seedling shoot counts by counting a sub-set of seedings in a density ring of known diameter. Place the ring in the center of the quadrat and count all eelgrass shoots inside of the ring. Record the data.

4. Collect two shoots per subplot for DNA extraction and genetic analysis seasonally, once prior to heat stress, and once following heat stress.
5. At local seed-donor meadows, conduct percent cover assessments using $1m^2$ quadrats at least once during late spring, summer, and fall to establish a baseline for comparison. For each assessment, haphazardly collect percent cover and density data from 12 $1m^2$ quadrats.
6. Download PAR and temperature data from all loggers. Clean loggers as necessary and redeploy.

3. Data Management and Analysis

3.1 Data Collection and Quality Control

1. Ensure all data are collected and processed to maintain high quality.
2. Clean, check for drift, and re-calibrate sensors per manufacturer specifications.
3. Store sensor data in data loggers and download every time the meadow is sampled.
4. Perform quality control checks using protocols recommended by the Integrated Ocean Observing System Program.
5. Record all biotic/eelgrass field data on standardized physical datasheets.
6. Inspect datasheets for completeness, reasonableness, and absence of entry errors.
7. Transfer data daily into digital spreadsheets using Microsoft Excel, an open-source equivalent, or RStudio.
8. Retain original paper copies for a minimum of three years post-project completion.