MarineGEO

Seagrass Shoot Collections

V 0.1

Introduction

This protocol provides standardized data on characteristics of the seagrass canopy and sessile (attached) organisms on the seagrass blades from **shoot collections**. The density and lengths of seagrass blades provide information on the quality and quantity of seagrass habitat for animals, and measurement of sheath length provides a proxy estimate of leaf growth rate (primary production). The protocol also measures the amount of fouling material on the seagrass blades, which can inhibit photosynthesis and provide food for animals.

> Three (3) shoots of each of the dominant seagrass species are collected from twelve (12) locations along three (3) transects (total $N = 108$). Shoots are post-processed to measure leaf characteristics, fouling mass, shoot dry mass.

Additional copies of this protocol, field datasheets, data entry templates, instructional videos, literature, and more can be found on the Seagrass section of the MarineGEO protocol website: https://marinegeo.github.io/seagrass-habitat.

Measured Parameters

This assay determines physical characteristics of seagrasses and the fouling community, measured as:

- Blade length (mm)
- Blade width (mm)
- Sheath length (mm)
- Disease lesions (number and length in mm)
- Grazing scars (number)
- Fouling biomass (mg)

Requirements

Personnel: 2 persons

Time: Preparation: 2 persons x 1 hour
Field work: 2 persons x 0.5 days

Post processing: 1 persons x 3 days
Data processing: 1 persons x 1 hour

Replication: 3 shoots x ≥1 species x 12 locations x 3 transects = ≥108 samples

Materials Checklist:

Field:

- ☐ 36 plastic bags per seagrass species with external and internal labels
- ☐ 1 cooler (with ice)

Post-processing

- ☐ Sorting tray
- ☐ Pre-weighed foil tins (≥ 72)
- ☐ Pencil/pen
- ☐ Microscope slide
- ☐ Ruler (mm)
- ☐ Drying oven
- ☐ OPTIONAL: Combustion furnace

Methods

Preparation

1. Label 36 disposable plastic bags with the sampling location, transect, and replicate number.
2. Place 36 internal labels with the same metadata written on waterproof paper inside the corresponding plastic bag.
3. Fill a cooler with ice immediately before departing for the field.

Fieldwork

1. Sampling locations should align with the 36 quadrat locations (12 replicates x 3 transects) as determined in the Seagrass Quadrats Protocol. Alternatively, the 36 sampling locations can be determined haphazardly within the full area of the bed.
2. At each location, use your fingers to gently break off 3 seagrass shoots of the dominant seagrass species at the sediment surface. Be careful not to disturb attached material.
3. If the sampling location contains equal cover of >1 seagrass species, repeat this procedure for each dominant seagrass species.

4. Gently place the shoots and attached material into the corresponding labeled plastic bag.
5. Place the bag and contents on ice in the cooler.
6. Repeat steps 2-4 at the at the remaining 35 sampling locations.
7. Transport cooler with samples back to the lab for immediate processing.

Sample Processing: Samples are best processed immediately upon returning from the field. Samples can be stored for up to 24 hours in the refrigerator; any longer risks decay.

1. Print lab data sheets
2. Making and weighing foil tins:
    a. Use a balance to pre-weigh foil tins (either manufactured, or made by folding an aluminum foil square over on itself and sealing the sides)
    b. Record the weight of the tin directly on the foil using a pen.
    c. At least 36 tins are required, although more may be necessary depending on how many species were sampled in the bed

Figure 1. Example of diseased lesions on the blades of eelgrass (*Zostera marina*). From Ralph & Short (2002, Marine Ecology Progress Series 226: 265-271).

3. Select a labeled bag and record the metadata on the lab data sheet
4. Gently transfer the shoot from the bag into a shallow sorting tray without any water
5. Separate seagrass' above-ground biomass from below-ground biomass by cutting the shoot where color changes from green (above-ground) to white/clear (below-ground). Discard any below-ground material
6. Use a microscope slide to lightly scrape the fouling material from the surface of the blade into one of the pre-weighed tins
7. Record the tin's empty/dry weight on the datasheet and label the tin with the sample number
8. From each shoot of each species, measure and record:
    a. The length and width of the single longest leaf
    b. The sheath length – from the top of the sheath surrounding the leaf bundle to the meristem (the visible constriction at the shoot base) to (Figure 1)
    c. The length (mm) of the largest disease lesion, if present (Figure 2)
9. Examine the blades for any evidence of grazing scars and record the number on the lab data sheet. If possible, record photos of representative grazing scars

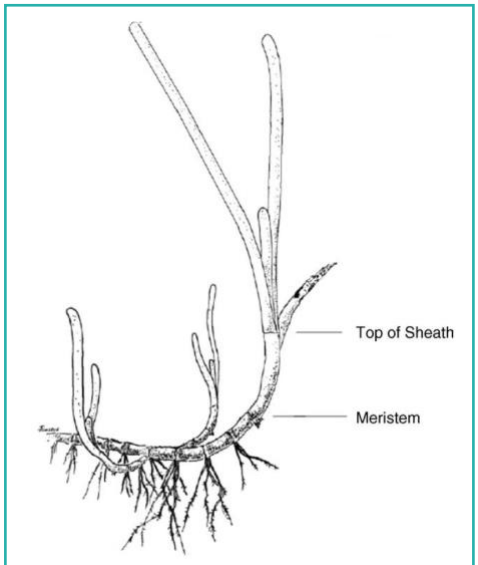

Figure 2. Morphology of an eelgrass (*Zostera marina*) shoot, showing leaves and sheath. Adapted from Gaeckle et al. (2006, Aquatic botany 843:226-232).

10. Transfer the scraped blades into a labeled pre-weighed tin and record the tin's empty/dry weight on the lab data sheet. If the sample contains more than one species of seagrass, weigh each species in a separate tin.
11. Place all the tins (blades and fouling material) in a drying oven at 60°C. Dry samples until they register a constant weight (usually 1-3 days, depending on the volume of material).
12. Remove tins from the oven and weigh each to the nearest mg. Record this dry mass (including foil) on the lab data sheet. Note: you will have two weights per sample
    a. Epibiont/fouling dry-mass
    b. Blade dry-mass
13. OPTIONAL: If a combustion furnace is available, combust the samples at 450°C for 4 hours. Allow the samples to cool in the drying oven. Weigh the sample and record the weight (including foil) to the nearest mg. Ash-free dry mass (an estimate of organic matter in the sample) can be calculated by subtracting the ash mass from the dry mass.

Data Submission

1. Enter data into provided excel data entry template.
2. Scan the completed lab data sheets as PDFs and save both paper and electronic versions
3. Place all documents (excel datasheet, scanned lab datasheets, and any photos in a new file and rename
4. Email data entry file, scanned lab data sheets, and any phot to: marinegeo-data@si.edu