MarineGEO

Seagrass Shoots

V 0.1.1

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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.

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 quantifies physical characteristics of seagrass blades and the associated 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:

- ☐ 18 plastic bags with external and internal labels
- ☐ 1 cooler (with ice)

Post-processing

- ☐ Sorting tray
- ☐ 72+ Pre-weighed foil tins
- ☐ Pencil/pen
- ☐ Microscope slide
- ☐ Ruler (mm)
- ☐ Drying oven
- ☐ *RECOMMENDED* – Combustion furnace

Methods

Preparation:

1. Identify sampling scheme. If following the MarineGEO survey design, review the materials here (6 replicates *x* 3 transects = 18 replicates total). Alternately, samples can be taken haphazardly within the bed (if done, record GPS coordinates of each sample)
2. Label 18 disposable plastic bags with the sampling location, transect, and replicate number
3. Place 18 internal labels with the same metadata written on waterproof paper inside the corresponding plastic bag
4. Fill a cooler with ice immediately before departing for the field

Fieldwork:

1. At each sampling location, identify a vegetated patch at the corresponding point the transect (if following the MarineGEO survey design) and select the corresponding labeled plastic bag
2. 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 and store in the same labeled plastic bag
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 2-5 at the at the remaining 5 sampling locations along the transect
7. Repeat steps 2-6 for the remaining two transects
8. Transport cooler with samples back to the lab for immediate processin

Post-processing:

Samples are best processed within 24 hours upon returning from the field. Samples can be stored for longer in the freezer but risks decay

1. Print lab data sheets
2. Weigh foil tins and record the weight of the tin directly on the foil using a pen. Tins can be either pre-made, or constructed by folding an aluminum foil square over on itself and sealing the sides
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 seagrasses by species (if more than one) and into above- and belowground components by gently pinching at the meristem (the intersection of the shoots and rhizomes) until they separate. Discard any belowground material
6. For each seagrass species, select a pre-weighed tin and label with the sample metadata (replicate number, date, location) and species name (to lowest taxonomic group), and contents (fouling material)
7. Use a microscope slide to lightly scrape the fouling material from the surface of the blades into one of the pre-weighed tins
    - *Be careful that no animals are transferred with the macrophytes*. This may require picking animals one-by-one out of more complex substrates
8. Next, for each shoot of each species, measure and record:
    - The length and width of the single longest leaf
    - The sheath length – from the top of the sheath surrounding the leaf bundle to the meristem (the visible constriction at the shoot base) (Fig 1)
    - The length (mm) of the largest disease lesion, if present (Fig. 2)
9. Examine the blades for any evidence of grazing scars and record the number on the lab data sheet. If possible, take photos of representative grazing scars

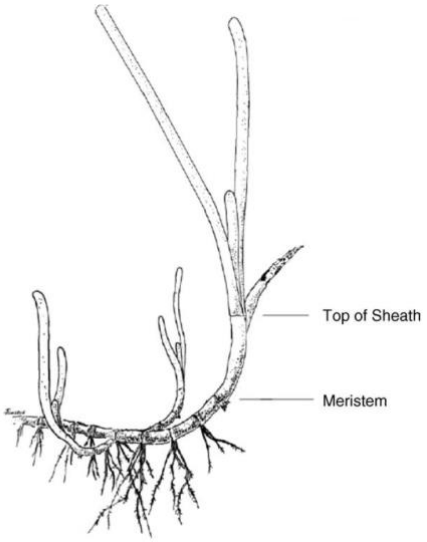

Figure 1. Morphology of an eelgrass (*Zostera marina*) shoot, showing leaves and sheath. Adapted from: Gaeckle et al. (2006) *Aquatic Botany* 843:226-232.

Figure 2. Example of diseased lesions on the blades of eelgrass (*Zostera marina*). From: Ralph & Short (2002) *Marine Ecology Progress Series* 226: 265-271.

10. Transfer the scraped blades into a pre-weighed tin labeled with the sample metadata (replicate number, date, location) and species name (to lowest taxonomic group), and contents (blades). If the sample contains more than one species of seagrass, weigh each species in a separate tin.
11. Place all the tins (fouling material and blades) 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)
    - 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 at least two weights per sample: fouling dry-mass, and blade dry-mass of the dominant species
12. *RECOMMENDED* – 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

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Data Submission

1. Enter data into provided data entry templates
2. Scan the completed lab data sheets and save both paper and electronic versions
3. E-mail data entry file, any photos, and scanned lab data sheets to: marinegeo-data@si.edu