MarineGEO

Seagrass Epifauna (Mesh Bags)

v 0.1

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Introduction

This protocol provides standardized data on seagrass mobile epifauna including taxonomic composition, diversity, and estimates of abundance/biomass and/or secondary production. These variables provide information on the prey resources available to fishes using seagrass habitats, and other effects of seagrass structural habitat on higher trophic levels.

Samples are post-processed to quantify seagrass biomass and estimate the biomass/secondary production of mobile epifauna. In post-processing, we recommend using a series of stacked sieves of sequentially smaller mesh sizes to sort animals by size class (Edgar. G.J. 1990. JEMBE 137:195-214). The resultant size fractionation can be combined with empirical equations relating size class to biomass and production of different taxonomic groups, providing non-destructive estimates of invertebrate biomass and production. Alternatively, you can identity, count, and measure (using photographs) each individual of each species. Both sets of instructions are provided in this guide.

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.

Measured Parameters

This assay determines characteristics of seagrass' mobile epifaunal communities, measured as:

- Macrophyte biomass (mg)
- Epifaunal abundance and taxonomic composition (individuals)
- Epifaunal biomass (mg dry or ash-free dry mass)

Requirements

Personnel: 2 persons

Time: Preparation: 2 persons x 0.5 hours
Field work: 2 persons x 0.5 days
Post processing: 1 persons x 30 days
Data processing: 1 persons x 10 hours

Replication: Six (6) grab samples of seagrass and associated invertebrates are taken using mesh bags along three (3) transects (total N = 18).

Materials Checklist:

Field:

- ☐ 18 draw-string mesh bags (500 µm mesh size, approximate dimensions: 75 cm x 20 cm, with 20 cm opening)
- ☐ Waterproof paper for internal labels
- ☐ 1 plastic cooler (with ice)

Post-processing:

- ☐ 20+ pre-weighed foil tins
- ☐ 500 µm mesh sieve
- ☐ Sorting tray
- ☐ 20+ scintillation vials (20-mL) with lids
- ☐ 70% ethanol (0.5-1.0 L)
- ☐ Petri dishes
- ☐ Forceps (fine-tip)
- ☐ Pen/pencil
- ☐ Drying oven
- ☐ OPTIONAL: nested sieve set with appropriate mesh sizes (8.0, 5.6, 4.0, 2.8, 2.0, 1.4, 1.0, 0.71 and 0.5 mm)

Methods

Preparation

1. Write 18 internal labels on waterproof paper with the sampling location, transect, and replicate number, and place inside 18 mesh bags (500 µm mesh size).
2. Fill a cooler with ice immediately before departing for the field.

Fieldwork

1. Sampling locations should align with half of the 36 quadrat locations as determined in the Seagrass Quadrats Protocol. The epifaunal samples will be taken at every other point along the transect (e.g., replicates 1, 3, 5, etc.). Alternatively, the 16 sampling locations can be determined haphazardly within the full area of the bed.
2. At each sampling location, first select the corresponding labeled mesh bag.
3. Position the mesh bag over the canopy and gently lower it over the seagrass, being careful to avoid disturbing or dislodging any organisms or macroalgae. It may be necessary to move the bag from side to side, or gently guide the seagrass blades, to aid in passing the shoots through the opening.

4. Once the opening of the bag is just above the surface of the sediment, close the bag by pulling the drawstring and either cut or tear the exposed shoots at the sediment surface to enclose them within the bag.
5. Invert the closed bag and bring it up to the surface, flushing the contents fully toward the bottom of the bag, then tie the mesh into a knot to prevent the drawstring from opening.
6. Place the bag with contents on ice in the cooler.
7. Repeat steps 2-6 at the next location along the first transect until all 6 replicates are taken.
8. Repeat steps 2-7 for the remaining two transects for a total of 18 samples.
9. Transport cooler with samples back to the lab for processing.

Sample Processing

1. Samples are best processed within 24 hours upon returning from the field. Samples can be stored for longer in the freezer but frozen organisms often become damaged and difficult to identify.
2. 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). Record the weight of the tin directly on the foil using a pen.
3. Open a mesh bag and record the metadata from the internal label on the lab data sheet.
4. Gently rinse the contents of the bag into a shallow sorting tray with water (white is best).
5. Separate macrophytes (e.g., seagrass, macroalgae) by species, place into separate pre-weighed tins, and record the tin weight(s) on the lab data sheet for that sample. Be very careful that no animals are transferred with the macrophytes! This may require picking animals one-by-one out of more complex substrates.
6. When all seagrass and algal material has been removed from the tray, pass the contents through the 500 µm mesh sieve. Gently rinse any loose material through the sieve, allow residual water to drain briefly, then transfer the remaining contents (including epifauna) to a 20-mL vial filled with 70% ethanol. The vial should be labeled externally (on the lid is best) and with an internal label of waterproof paper written in pencil (ethanol will dissolve most inks). Make sure that the sample fills no more than half of the vial volume, and that there is at least equal volume of sample and overlying ethanol, otherwise samples can decompose. Set the vial aside for processing at a later date.
7. Place the labeled foil tins containing the macrophytes in a drying oven at 60°C and dry to constant weight (usually 1-3 days, depending on the volume of material).
8. When the samples have dried thoroughly, remove the foil container from the oven, weigh to nearest mg, and record the dry mass (including foil) on the lab data sheet.

For the following steps. if you have a sieve tower, conduct Sieve Processing; if not, conduct Photographic Sizing. Conduct only one of the two, not both.

Sieve Processing

9a. Print a Mesh Bag Sieve Lab Data Sheet
10a. Stack the sieves from smallest mesh on the bottom to largest mesh on top (0.5, 0.71, 1.0, 1.4, 2.0, 2.8, 4.0, 5.6, 8.0 mm)
11a. Select a sample vial and gently invert it over the top sieve, allowing the contents to pass onto the sieve tower
12a. Use a hose or squirt bottle to gently rinse out the vial with tap or distilled water and empty its contents into the sieve tower until nothing remains in the vial.
13a. With the same water source, gently rinse the animals through the sieve tower. Take care not to use too much pressure to avoid damage fragile specimens. The goal is for animals to pass through larger sieves until they reach and are retained by the sieve mesh appropriate to their body size.
14a. Transfer the contents of each sieve into a separate labeled Petri dish, filled with 70% ethanol and labeled by sample ID and sieve size. A squirt bottle of 70% ethanol can be used to gently rinse the animals into the dish. Forceps will be useful in extracting some of the smaller or more articulated organisms.
15a. Identity and count the animals in each sieve size class, and record this information (including sieve size) on the lab data sheet provided.
16a. Record photographs of unidentified species at the highest magnification possible. Label these images with the sample information and taxonomic identification you assigned on the data sheet. These images can be used later to clarify the species' identity.
17a. Return all specimens to the labeled 20-mL vial, fill with 70% ethanol, and seal for long-term storage.

Note: Because certain invertebrates (especially amphipods and worms) often become fragmented during processing, only count specimens with heads.

Note: Accuracy is more important than specificity in identifying organisms. That is, identify the species to the lowest (most specific) taxonomic level you feel confident of, but not lower. If you think an animal might be *Gammarus mucronatus*, but are pretty certain it is *Gammarus* sp., then enter *Gammarus* sp. You can write '*Gammarus mucronatus*?' in the notes section.

Photographic Sizing

9b. Transfer the contents of a 20-mL epifauna sample into a 10-cm Petri dish or similar vessel for sorting. If the sample is large, begin with a portion of the sample, and repeat until the sample is completed.
10b. Prepare the sample for photographic measurement of epifaunal body sizes: remove as much debris as possible and spread the animals out so they are separated on the bottom of the dish. If there are too many animals to position so that none are touching, return some to the vial to clear space.

11b. Place a centimeter rule under or adjacent to the dish to provide a scale for measurement.
12b. Take one or more digital photographs to capture all animals in the dish at sufficient resolution that their body sizes can later be measured from the image using the included scale. Photographs may be captured with a camera attached to the microscope or (if the animals are large enough) with a cell phone fitted with a macro lens.
13b. After photographing the organisms, identity and count all animals and record this information on the lab data sheet provided.
14b. Place any unidentified species in a separate vessel and photograph at the highest magnification possible. Label these images with the sample information and provisional species name you assigned on the data sheet. These images can be used to later clarify the species' identity.
15b. Return all specimens to the labeled 20-mL vial and seal for long-term storage.

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Data Submission

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