



# MarineGEO Seagrass Epifauna Protocol



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## 1. Introduction

### About the MarineGEO seagrass epifauna protocol

Seagrass habitats support a diverse community of animals from tiny invertebrates to sea turtles and dugongs. Among the most abundant animals in seagrass habitats are the small, mobile invertebrates that live on or among seagrass leaves, referred to as mobile epifauna. The mobile epifauna of seagrass habitats are important to ecosystem processes because these animals are often highly productive and provide key food resources coastal fishes, both juveniles and adults. This document describes methods for sampling and characterizing seagrass mobile epifauna, specifically the abundance, taxonomic composition, and body size distributions of mobile epifaunal invertebrates associated with seagrass.

Epifaunal sampling and characterization are time-consuming, so MarineGEO requires only that seagrass mobile epifauna be sampled at least once from each site. Sampling should be done around the time of highest seagrass productivity at the site, usually early summer in temperate areas, coincident with annual sampling of other seagrass measurements<sup>1</sup>. Most of these activities can be completed by two experienced field workers, but additional team members improve sampling quality and efficiency. This protocol provides guidelines on how to collect seagrass epifaunal data that are comparable to other sites and how to manage the data.

Following the protocol described below produces data on the following metrics of seagrass mobile epifauna assemblages:

- Mobile epifaunal abundance (g seagrass<sup>-1</sup>)
- Mobile epifaunal biomass (g seagrass<sup>-1</sup>)
- Mobile epifaunal taxonomic composition and diversity
- Mobile epifaunal body size distribution

Combining the data produced by this protocol with co-located data from the MarineGEO Seagrass Habitat Monitoring Protocol<sup>2</sup> yields the following metrics:

- Mobile epifaunal abundance and biomass (m<sup>-2</sup>)

This MarineGEO protocol is designed to work across a broad range of seagrass species, environments, and geographic regions. As seagrasses and their environments differ widely across the globe, these protocols may need modification for particular situations. Please contact us at [marinegeo@si.edu](mailto:marinegeo@si.edu) to discuss how to adapt this protocol to ensure data from your specific site are comparable with data from other seagrass habitats worldwide.

This and other MarineGEO protocols are open-access, for anyone who wishes to use them. Electronic copies, with associated field and laboratory sheets, can be downloaded at the MarineGEO website<sup>3</sup>.

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<sup>1</sup> See MarineGEO toolkit, seagrass habitats: <https://marinegeo.si.edu/protocols/seagrass-habitats>

<sup>2</sup> <https://doi.org/10.25573/serc.14925114>

<sup>3</sup> Smithsonian MarineGEO: <https://marinegeo.si.edu/research/marinegeo-toolkit>

### *Responsible science*

It is your responsibility to comply with all legal and institutional regulations that apply in your area and to obtain any permits necessary for your work. Participants in Smithsonian MarineGEO research and users of our protocols are also expected to act in safe and ethically responsible ways; we invite you to review MarineGEO's code of conduct<sup>4</sup>. Please contact us with questions or to discuss your approach.

### *Acknowledgments*

We are grateful to the many colleagues in and outside the Smithsonian's MarineGEO network who contributed to development of this protocol over several years, including from SeagrassNet, Seagrass-Watch, the GOOS Biology and Ecosystems panel, and Scientific Committee for Oceanic Research working group 158, Coordinated Global Research Assessment of Seagrass Systems (C-GRASS).

## 2. Field collection of seagrass epifauna

### Measured Parameters and Requirements

- Mobile epifaunal abundance, taxonomic composition, and body size distribution
  - 3 transects x 4 epifaunal bag samples = 12 per site

### Materials needed

- 1 x 50m metric transect; this may be a tape, rope marked at intervals, or similar measuring aid.
- Hand-held GPS unit
- 2 x marker poles, if desired
- 12 x draw-string mesh bags (500 micron mesh) or similar sampling apparatus
- 12 x internal sample labels on waterproof paper
- 1 cooler with ice (optional)

## Methods

### *Preparation*

Fully review this and any additional protocols necessary for the sampling excursion. Address any questions or concerns to [marinegeo@si.edu](mailto:marinegeo@si.edu) before beginning this protocol.

1. Review the [MarineGEO Seagrass Habitat Monitoring Protocol Survey design](#).
2. Print out or write 12 internal labels on waterproof paper. Each label should be legible and include the sampling location, transect number, and replicate number. Place each label inside a separate mesh bag (500 um mesh) or similar sample container.

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<sup>4</sup> MarineGEO Guiding Documents: <https://marinegeo.si.edu/about-us/guiding-documents>

3. In warm weather and/or when the return trip from field to lab is long, it is best to bring a suitably sized container filled with ice to keep samples fresh and undamaged until they can be processed.

### *Field collection*

1. Relocate permanent transect coordinates, if established at the site, and lay out the 50 m transect. For the full protocol on site selection and transect layouts, review the [MarineGEO Seagrass Monitoring Protocol](#).
2. At each point along the transect where an epifaunal sample is to be collected, randomly select a seagrass patch ~1 m to one side of the transect. Do not sample within the quadrat used for quantifying seagrass cover and density, as this may affect surveys in subsequent years.
3. Position the mesh bag over the canopy and gently lower it over the seagrass, being careful to disturb or dislodge organisms as little as possible. It may be necessary to move the bag from side to side and gently guide the seagrass blades through the bag opening. For intertidal sites, blades can be gently lifted and inserted into the bag.
4. When the bag opening is just above the sediment surface, close the bag by pulling the drawstring and cut the exposed shoots at the sediment surface to release them into the bag. Avoid bagging sediment along with the sample, which can result in accidental collection of sediment and infauna, and increase processing time.
5. Invert the closed bag, bring it to the water surface, and flush the contents into the bottom of the bag. Close the drawstring and knot it to secure the contents during travel.
6. Repeat steps 1-5 at the next location along the first transect until all 4 replicates are taken.
7. Place the bags with contents on ice in container.
8. Repeat steps 1-6 for the remaining two transects for a total of 12 epifaunal samples.
9. Transport container with samples back to the lab for processing.

## 3. Post-field processing of seagrass epifauna

Samples collected in this protocol are post-processed to quantify aboveground seagrass biomass and estimate the biomass/secondary production of mobile epifauna. For post-processing, we recommend using a series of stacked sieves of sequentially smaller mesh sizes to sort animals into size classes (Fig. 2) as in Edgar, G.J. (1990) *Journal of Experimental Marine Biology and Ecology* 137:195-214. The abundance of each species in the size class can be combined with [empirical equations](#) relating abundance to biomass and production of different taxonomic groups, providing non-destructive estimates of epifaunal biomass and production.

The invertebrates are also sorted through a set of sieves to estimate body size distribution, which can in turn be used to estimate epifaunal biomass and secondary production. These metrics are standardized per mass of macrophytes, which also requires weighing the macrophytes in the sample from which epifauna were extracted.

## Methods

### *Measured Parameters and Requirements*

Same as above (2. *Field collection of seagrass epifauna*):

- Mobile epifaunal abundance, taxonomic composition, and body size distribution
  - 3 transects x 4 epifaunal bag samples = 12 per site

### *Materials needed*

- 20 + pre-weighted aluminum weighing tins<sup>5</sup>
- Shallow sorting tray, preferably light color, one per person
- 20+ scintillation vials (20-mL), or similarly secure containers, with lids
- 70 Petri dishes
- Forceps (fine-tip)
- Pen/pencil
- Drying oven
- Nested sieve set<sup>6</sup> with the following sizes: 8.0, 5.6, 4.0, 2.8, 2.0, 1.4, 1.0, 0.71 and 0.5 mm (5/16-in, #3.5, #5, #7, #10, #14, #18, #25, #35 mesh sizes respectively)

### *Measure Macrophyte Dry Mass*

1. Print lab data sheets (“datasheet\_macrophyte\_weight.pdf”, download at <https://doi.org/10.25573/serc.28842893>).
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. 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. Be sure to check the seams and folds of the bag for organisms clinging to the mesh.
5. Separate all seagrasses and macroalgae by species.
6. For each seagrass and macroalgal species, select a pre-weighed tin, and write the sample metadata (replicate number, date, location) and name of the species or lowest taxonomic level you are confident identifying it. For each seagrass species, gently scrape epibiota (algae, detritus, sessile organisms) into one labeled tin, and place the seagrass blades in a separate labeled tin. If the

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<sup>5</sup> Example: <https://www.thomassci.com/p/aluminum-weighing-boats?q=Weigh+Boat>

<sup>6</sup> Example: <https://www.thomassci.com/p/3-diameter-astm-brass-stainless-round-test-sieves?q=Sieves>

sample contains any (unrooted) macroalgae, place each by taxon into a separate labeled tin. *Avoid transferring animals with the macrophytes.* This may require picking animals one-by-one out of more complex substrates. Gently shaking the blades in the shallow tray may also help.

7. For each taxon placed in a tin above: record the sample metadata, species name, and the empty tin weight on the lab data sheet.
8. Once all macrophyte material has been removed, gently wash the contents of the sorting tray through a 500-um mesh sieve. Gently rinse any loose material through the sieve, and then transfer the animals and other material remaining on each sieve to a separate 20-mL vial, with both an internal and external label. Fill the vial to ~80% with 70% ethanol (or 95% ethanol if desired to preserve the organisms for DNA sequencing). Multiple vials may be required for larger samples.
9. Place the well-labeled vial in a secure place for storage until epifaunal processing.
10. Repeat steps 3-9 for each epifaunal sample.
11. Place all tins containing macrophytes into a drying oven. Dry at 60°C to constant weight (usually 1-3 days, depending on the volume of material).
12. Once dried, remove all tins from the oven and weigh to nearest mg. Record this weight (including tin weight) on the same lab data sheet where initial weights were previously recorded.

### *Measure epifaunal body size*

Once the epifaunal samples are preserved they can be stored until there is time to process them.

1. Print lab data sheets (“datasheet\_sieved\_counts.pdf”, download at <https://doi.org/10.25573/serc.28842893>).
2. Stack the sieves from the smallest mesh size on the bottom to the largest mesh on top (Fig. 2).
3. Select a 20-mL vial with epifauna, open the top, and gently pour the contents onto the top sieve.
4. Use a squirt bottle filled with water to gently rinse any remaining contents of the vial into the sieve tower.
5. Gently rinse the animals through the sieve tower. Avoid using too much water pressure as it can damage fragile specimens. The goal is for specimens to pass through larger sieves until they reach and are retained by the sieve mesh appropriate to their body size. This may be aided by removing the top sieve once smaller animals have washed through, and rinsing the second sieve down the tower, then removing that second sieve, and repeating until the last sieve.
6. Label a separate 10-cm Petri dish, or similar vessel, for contents of each sieve. Record sample



**Figure 2. Nested tower of sieves.** These sieves are made for measuring sediment size distribution and are available from scientific suppliers. See text.



metadata and sieve size on the dish. Transfer the contents of each sieve into its corresponding dish for sorting and counting.

7. Identify each mobile epifaunal specimen in the dish to species, if possible, and record the species name on the lab datasheet. If you cannot reliably identify a specimen to species, record the lowest taxonomic group that you feel confident, and assign it a provisional name (e.g., Nereid polychaete A). Photograph unidentified species if possible and name the image files with the sample information and provisional name you assigned on the data sheet. These images can be used to clarify the species' identity later. *Strive to maintain the same naming scheme for all future samples* (especially if samples are processed by different people).
8. Only count *mobile metazoans*. That is, do not count protozoans (e.g. foraminera), meiofauna that would normally pass through a 500- $\mu$ m mesh (e.g., nematodes, copepods), or sessile invertebrates such as bivalves, bryozoans, and sponges.
9. Do not count animals that were clearly dead when collected, e.g., empty shells and exoskeletons.
10. Only count heads: this prevents counting the same individual twice. Discard disembodied limbs, fragments of polychaetes, crustacean bodies with missing heads, etc.
11. Count and record the number of individuals for each species on the provided lab sheet.
12. Return all specimens to the labeled 20-mL vial, refill with 70% ethanol, and seal for storage

## 4. Data submission

1. Scan the completed field data sheets and save both paper and electronic versions locally. We do not require you to submit the scanned forms.
2. Enter data into the custom data entry spreadsheet for your site provided by MarineGEO (contact [marinegeo@si.edu](mailto:marinegeo@si.edu) if you don't have this yet). Provide as complete metadata as possible for protocol, samples, and any modifications you used. Use the "notes" column to provide additional information or context if a relevant column doesn't already exist, rather than renaming or creating columns.
3. The data entry spreadsheet has been pre-filled with your site information and IDs for species seen in previous surveys. If you observe additional species, enter a species code (abbreviation) of your choice and the full species name (or closest taxonomic identification) in the "SPECIES" sheet. Note that any new codes must be unique to your pre-existing codes.
4. Email spreadsheets as an attachment to [marinegeo-data@si.edu](mailto:marinegeo-data@si.edu).