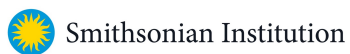


Protocol: Sediment and Porewater Sampling



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Introduction

This is the Sediment and Porewater Sampling protocol for the MarineGEO network research project.

Additional copies of this protocol, field datasheets, data entry templates, literature, and more can be found at: [website link to the network project information](#).

Measured Parameters

This assay quantifies:

Measured as:

Requirements

Personnel: 2 people

Estimated Total Time Per Location (n = number of replicates per location):

Preparation: 1 person x 1 day

Field work: 2 people x 1 day

Post processing: 1 person x 2-4 hours

Data processing: 1 person x 1 day

*Estimated times will vary by site and conditions

Replication: Break down of the experimental replication per location. (i.e. porewater (3) mini-cores (4) across control and treatment areas, total of 6 porewater and 8 mini-cores per site)

Materials:

Make sure that you have all the materials required: some materials need to be provided or procured by the partners as they cannot be sent through post.

Survey Design:

Suggestions: Transect to sample along and a handheld GPS.

Fieldwork:

Materials included in the KIT:

- ☐ 6 Rhizon samplers
- ☐ 6 retainers
- ☐ 6 x 10ml syringes
- ☐ 6 x 15ml falcon tubes
- ☐ 8 mini-cores

Materials required from the partner:

- ☐ Tape
- ☐ Cooler filled with ice
- ☐ Sterile gloves

Post-Processing:

Materials included in the KIT:

- ☐ 16 x 50ml pre-filled falcon tubes with RNAlater
- ☐ 4 empty 50ml falcon tubes
- ☐ 4 x 50ml falcon tubes

Materials required from the partner:

- ☐ 8 sterilized (autoclaved), sealable storage containers with lids that hold at least 50mL of material (prepare extra just in case)
- ☐ 8 sterilized (autoclaved) spatulas (prepare extra just in case)
- ☐ 500ml Beaker

Methods

Fully review this and any additional protocols necessary for the sampling excursion and watch the videos provided. We encourage our partner to take pictures of the experiment and share them with us. Address any questions or concerns to marinegeo@si.edu before beginning this protocol.

Preparation:Fieldwork:

We recommend carrying out the sediment and porewater sampling during the morning low tide if possible. After sampling, the mini-core samples need to be processed. Those steps can take a few hours.

1. Put your gloves on in the control area.
2. Sample the porewater from the sediment for each replicate
 - a. Remove the protection cap that is screwed on the female luer lock
 - b. Screw the syringe directly onto the luer
 - c. Underwater, insert the Rhizon sampler vertically into the sediment (in the middle of the frame)
 - d. Pull out the piston of the syringe and place the retainer along the piston (the vacuum in the syringe is the driving force for extraction of pore water).
3. On the boat or the beach, carefully transfer the seawater to the 15ml falcon tubes
4. Sample the sediment for each replicate
 - a. Remove the lids of the mini-core just before sampling
 - b. Put the core in the sediment and pull the piston out slowly while pushing the mini-core deeper. Be careful to avoid sampling seawater as much as possible.
 - c. Remove the mini-core and close it immediately
5. On the boat or the beach, secure the piston with tape and place the syringe on ice in the cooler. Position the syringe vertically so that the lid is at the bottom and the piston upward. This should help you to process the sample more easily.
6. Repeat steps 2-5 for the treatment area if one is present.

Post-Processing:

1. Wipe down surfaces with 10% bleach and 70% Etoh
2. Turn on a UV light for 15 minutes
3. Without shaking the sample, remove the tape from the mini-core syringe
4. Carefully and slowly, remove the piston as to not agitate the sediment within
5. Pour the water into the Beaker
6. Replace the piston and pour the sediment into the sterilized container
7. Mix the sediment with the spatula

8. Pour 30ml of sediment into Falcon tube #1 and the rest into Falcon tube #2. For each sample you should have received 2 x 50ml pre-filled with RNAlater falcon tubes to aliquot the sediment
 9. Repeat steps 3-8 for the first four replicate sediment samples for both control and treatment.
 10. For the last two replicate samples of the control and treatment, remove the water and pour the sediments into Falcon tube #1
 11. Secure all Falcon tubes with parafilm
 12. Place the first four samples for control and treatment at -4°C overnight before storing them at -20°C
 13. Store the rest of the samples at -20°C
 14. Send all the sediment and porewater samples on dry ice to the Network Team
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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 provided data entry template. Each template is an Excel spreadsheet. Please provide as much protocol and sample metadata as possible, such as the protocol version and contact information. Use the “notes” columns to provide additional information or context if a relevant column doesn’t already exist, rather than renaming or creating columns.
3. Use our online submission portal to upload the Excel Spreadsheet: <https://marinegeo.github.io/data-submission>
4. Contact us if you have any questions: marinegeo@si.edu