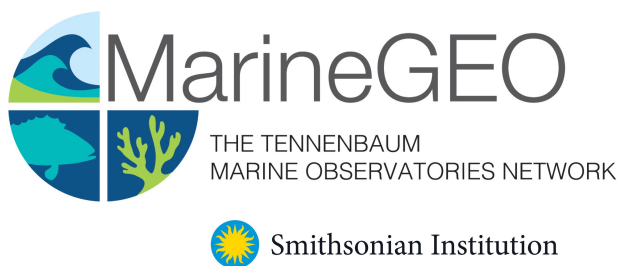


Protocol: Tea Bag Decomposition



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Introduction

This is the Tea Bag Decomposition 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 minimum, 3-4 preferable

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: 1-2 people x 2 days
- Data processing: 1 person x 1 day

*Estimated times will vary by site and conditions

Replication: Break down of the experimental replication per location.

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. If any materials are missing from the kit, please contact Isis Guibert (iguibert@hku.hk).

Survey Design:

Materials included in the KIT:

- ☐ 8 frames (20cm x 20cm) filled with Fertilizer with red buoyant chain, label and tea bags
- ☐ 8 frames (20cm x 20cm) not filled with yellow buoyant chain, label and tea bags
- ☐ Small shovel

Materials required from the partner:

- ☐ 40 pieces of metal bars (40 cm long)
- ☐ 2 pieces of metal bars (50 cm long)
- ☐ Hammer
- ☐ Ziptie (> 40)
- ☐ 4 Hobo logger (HOBO Pendant temp/light)
- ☐ 1 or 2 Transect tape(s)

Fieldwork:

Materials required from the partner:

- ☐ Tape
- ☐ Pruning shears
- ☐ Zip ties and large Ziplock bags (1-Gallon)
- ☐ 1-2 large box(es) or totes to transport the frames

Post-Processing:

Materials required from the partner:

- ☐ Weight
 - ☐ Dessicator (if tea is unlikely to air-dry)
 - ☐ Ziplock bags
-

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:

Choosing a site: For the partners that are planning to use SCUBA, we recommend working in a shallow area such that the water level at low tide never goes below 1m in depth. By doing so, participants can have 1-2 divers deploying/retrieving the frames while 1-2 persons prepare, handle, and pass the materials above water. A good option is to have all the materials close by on a float or in a small boat or a kayak for example. If you are not planning on using SCUBA, we recommend deploying the frames in an area such that the water level at low tide never goes below 0.5m in depth so the work can be completed while snorkeling.

Hobo logger:

1. Program the Hobo logger to record the temperature every hour. Do not use the light recording function for loggers that record irradiance because it uses too much battery power.
2. Attach the Hobo logger to the frames named: siteX-C1, siteX-C8, siteX-F1, siteX-F8

Fieldwork:**Tea Bag Deployment:**

1. Use the shovel to dig a 10cm deep, 20cm x 20cm wide, square hole.
2. Place the frame in the hole with the tea bags facing upward.
3. Secure the frame with two 40cm metal bars and zip ties.
4. Cover the frame with 10cm of displaced sediment, DO NOT bury the buoyant chain. If the frame has a Hobo logger attached, it should be buried along with the frame. *Note: If visibility is poor, you can place and secure all the frames before burying them. Each frame should be spaced at least 0.5m apart, and a minimum of 5m should be left between the area with control frames and the area with fertilizer frames. Use the transect tape to place the frame at the required distance. The frames should be positioned as referred in the illustration below.*

Tea Bag Retrieval

We recommend carrying out the retrievals during the morning low tide if possible. After the retrieval, the tea bags need to be processed. Those steps can take a few hours.

1. Remove the frames one by one. For each frame make sure that the label and tea bags are present. *If the label of the frame is missing, place the frame in the Ziplock bag and label it. You should be able*

to determine the label of the frame by its position in the area. If a tea bag is detached from the frame, attach it with a zip tie or place the frame together with the tea bags in a large Ziplock bag. It is important to know which tea bags go with each frame in case the tea bags' labels are missing. Use the pruning shears to detach the frame from the metal bars.

Post-Processing:

1. Remove the tea bags of the frame. Make sure that the tea bags have their label, if not add a new one. *Refer to the illustration to determine the label of the tea bag.*
2. Place the tea bags for 30min in RO/DI water
3. Carefully clean each tea bag for 20s in a bath. Change the water after washing 5-10 bags.
4. Dry the tea bags for 48h at 70°C. *If you can't process the samples after 48h, place them in a desiccator.*
5. Remove the tea from the bag. Weigh the oven-dried tea (0.001g). *If you are working in a humid area, the best way to weigh the tea is to pour it in a small Ziplock bag and remove the air before closing it (do not forget to tare the Ziplock bag first).*
6. Upload the data in the sheet

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