**Protocol for Extracting Meiofauna from Sediment**

(Frozen samples for downstream DNA sequencing)

**Decanting**

Equipment:

* 2 Liter glass graduated cylinder with stopper
* 45μm stainless steel sieve
* New, sterile wash bottles
* Sodium Metabisulfite
* Instant Ocean (or other commercial salt solution for marine aquariums)
* Funnels (one large, one small)
* Falcon tubes or Small plastic pots to store extracted meiofauna and sediment archives
* 5-gallon bucket (or large plastic tub)
* 20-Liter plastic carboy with spigot

Protocol:

1. Locate a reliable supply of ultrapurified water (either distilled water or tap water passed through a Millipore or Reverse Osmosis filtration system). **Do not use tap water for processing samples that will be used for DNA sequencing**; tap water often contains traces of DNA from protists, fungi, and meiofauna (Buse et al. 2012 *Envi Sci Pollut Res*. doi:10.1007/s11356-013-1646-5)
2. In a 5-gallon plastic bucket, prepare a 10% sodium metabisulfite solution for sterilizing equipment (mix 2.5g per gallon of distilled water). Sodium metabisulfite is used to ensure that there is no DNA crossover contamination between samples.
3. In a 20-liter plastic carboy with spigot, prepare a batch of Instant Ocean according to manufacturer’s instructions (dissolve salt mix into distilled water). Instant Ocean is used to decant samples in an isotonic solution, and to prevent cell lysis in marine organisms.
4. Soak all sample processing equipment (graduated cylinder, funnels, sieve) in sodium metabisulfite solution for 1-2 hours. Do not leave longer than this, or solution may start to corrode the metal sieve.
5. After soaking, rinse all equipment thoroughly with distilled water. This step is necessary in order to remove any remaining traces of sodium metabisulfite before sample processing
6. Fill one or more plastic wash bottles with distilled water, and another wash bottle with Instant Ocean.
7. Prepare two sterile sample containers: one for the extracted meiofuanal fraction, and another for archiving the sediment that remains after decantation. **Before starting, ensure that all sample containers are labeled with sample name, extraction date, and any other appropriate metadata.**
8. Using the equipment that has been sterilized, place a 2-liter glass graduated cylinder into a deep sink (or another area with appropriate drainage in case of spillage), and place large plastic funnel into the mouth of the cylinder.
9. Remove sediment sample from the freezer. Before beginning the decanting procedure, a subsample of raw, unprocessed sediment should be retained and put directly back into the freezer (~10g of raw sediment, at minimum if possible, although more sediment can be subsampled depending on study design – e.g. if more sediment is needed for parallel bacterial 16S sequencing or shotgun metagenomics). **Confirm that the bag/tube containing the sediment subsample is appropriately labeled before returning it the freezer.**
10. Empty the remaining portion of the sediment sample into the funnel (the fraction that will be used for meiofauna extraction). Use the wash bottle filled with Instant Ocean to break up frozen chunks of sediment and wash the sample into the glass cylinder. (Note: Muddy or extremely fine-grained sediment samples can be poured directly onto the sterile 45μm sieve and pre-washed with Instant Ocean before the sample is transferred to the graduated cylinder. Pre-washing may be useful to remove excess sediment before decanting, especially for large sample volumes.)
11. Once the sample is fully washed into the cylinder, remove funnel and wash it down with Instant Ocean over the mouth of the graduated cylinder (including the bottom opening of the funnel; this is to ensure that no meiofauna have become “stuck” to the plastic edges).
12. With the sample now in the graduated cylinder, add Instant Ocean to the cylinder (if needed) until the liquid level reaches the 2-liter mark.
13. Seal the graduated cylinder using the glass/plastic stopper.
14. To fully suspend the sediment into the water column, invert the graduated cylinder 10 times. For muddy or fine-grained sediment samples, it may be necessary to slightly shake cylinder on the first inversion, to break up any sediment plug that may have formed at the bottom of the cylinder.
15. After the 10th inversion, place the cylinder back into the sink and let the suspension settle for 30 seconds. Immediately after placing the cylinder down, remove the stopper; use Instant Ocean to wash down the stopper and the sides of the cylinder remaining above the water line. This washing step ensures that all sediment particles and meiofauna are returned to the water column while the sample settles. This settling step separates the sediment from the meiofauna; heavier sediment particles settle to the bottom of the gradated cylinder, while lighter meiofaunal organisms remain suspended in the water column.
16. After 30 seconds has passed, lift the cylinder out of the sink and gently pour the water out onto the 45μm stainless steel sieve (the sieve can be held over a large beaker or plastic bucket to save the Instant Ocean for reuse in the next decantation; for fine-grained sediments, it is advisable to use fresh Instant Ocean for each decantation until the supernatant does not look dirty/cloudy). **DO NOT pour out all the water in the cylinder; stop pouring once the decanting is about to reach the sediment plug that has now settled on the bottom of the cylinder.** During this step, meiofauna in the water column are concentrated onto the 45μm sieve.
    1. Note: Handling the cylinder and pouring off the water can require some getting used to. The glass cylinder will initially be heavy when full of water, and typically is held with one hand while the your other hand holds the 45μm sieve. When first picking up the cylinder, carefully rest it in the crook of your arm with your hand near the neck of the cylinder while you start pouring the water through the sieve. It may also be helpful to rest the cylinder on your hip for support. To prevent spash-back and facilitate water passing through the sieve, it may be useful to hold the sieve at a slight angle while pouring.
17. On the 45μm sieve, use Instant Ocean to wash down the meiofauna and concentrate them on one side of the sieve. Wash this concentrated fraction into the new, sterile sample container that was labeled in step 7 (plastic pot or falcon tube; a small sterilized funnel may be necessary to avoid spillage if the sample container has a narrow opening. If a funnel is used, ensure that all meiofauna are washed off the funnel into sample pot, as in step 11).
18. Repeat steps 12-17 nine times (decanting the sample a total of 10 times).
19. Following the last decantation, take the sample pot containing the decanted meiofauna fraction (step 17) and pour it back onto the sieve. Wash out the sample pot onto the sieve using a wash bottle containing **ultrapure or distilled water** (NOT Instant Ocean). Wash the meiofauna on the sieve using ultrapure/distilled water, and then wash the meiofauna fraction back into the sample pot using as little water as possible. This step is necessary to remove any salts/minerals from the sample (since salt may interfere with downstream DNA extraction). Minimizing water used to wash the sample will help to concentrate the meiofauna community and facilitate downstream DNA extraction and sequencing.
20. The sample pot containing the decanted meiofaunal fraction washed with distilled water is now ready for use in downstream applications (DNA extraction, taxonomic identifications, etc.). Decanted samples should be stored at -80°C if not needed immediately for downstream applications; **confirm that the sample pot is appropriately labeled before storing it the freezer.**
21. All sediment remaining in the graduated cylinder should be washed onto the 45μm sieve using Instant Ocean, and archived into the second sample container (labeled in step 7). Sediment archives should be labeled with “DECANT ARCHIVE, DO NOT USE” and stored at -80°C. **Confirm that the sample pot is appropriately labeled before storing it the freezer.** Most meiofauna are extracted from sediment samples after 10 decantations, however some organisms remain in the sediment archive; as standard practice, sediment archives are usually retained during the while a project is ongoing (and sometimes permanently).
22. After completing sample processing, wash all equipment thoroughly with distilled/ultrapure water. If further samples will be processed for DNA work, repeat protocol starting at step 4.

**Protocol Notes:**

Batches of sodium metabisulphite can be reused between samples (usually for a couple weeks, depending on how many samples are being processed); it is not necessary to mix a new batch of sodium metabisulphite every time equipment needs to be soaked. New batches can be made if the sodium metabisulphite starts looking dirty, or there will be a long lag time in between sample processing.

Sterilized sample processing equipment can be stored in the lab after being treated with sodium metabisulphite and rinsed off with distilled water. Use sealed, sterilized containers (large Ziploc bags, sterile buckets with lids) to prevent DNA contamination during storage.

The amount of sediment processed for meiofauna work varies according to sample collection methods and study design. 100ml to 500ml of sediment is an approximate range of sample volumes that are appropriate for characterizing the microbial eukaryote community.

For fine-grained or muddy sediments, additional sample processing could be applied to remove excess sediment. Typically this is carried out by centrifugation with Ludox (using the extracted meiofauna fraction as a starting point). However, this step is generally not recommended unless absolutely necessary, since it can remove some taxa and thus introduce bias in interpreting meiofauna communities (see discussion in Escobar-Briones et al. (2008). *Deep Sea Research Part II: Topical Studies in Oceanography*, 55(24-26), 2627–2633).

Instruments for Picking:

Micro Dissecting Needles: <http://www.roboz.com/micro_dissecting_needles.asp>

Other Nematode Guides:

Sampling and Extracting Nematodes: <http://xyala.cap.ed.ac.uk/research/nematodes/fgn/worm/extrafix.html>