Carbon and Water Program Protocols for Neutrally Buoyant Sediment Traps – Twilight Zone Explorer at BATS, June 2007-December 2009

MODIFIED ON OCTOBER 30, 2008 (S.OWENS); IN EFFECT NOVEMBER 2008

- Wear gloves and at all times during processing
- Strive to be as clean as possible – if in doubt, bag or rinse with DI
- Label all samples with sample ID, date (month), and depth (or blank) & document on data sheets
- Keep all water, samples, and solutions in cool, dark places (see below for details)

On Shore – Before Cruise

- Inspect the trap tubes for leaks, drain storage solution, and rinse with DI
- Inspect the bungee, baffle, lid, and fishing wire and replace as needed
- Load 350µm screens into Teflon filter holders in laminar flow hood
- Load equipment onto ship:
    - 5 tubes per trap + blank tube(s) + back-up spares
    - Carboys for filtered seawater
    - Mounting board, filter cassette and holder for filtering seawater
    - Flojet pump
    - Tubing (CTD to Flojet to filter holder to carboy)
    - Small carboys of brine
    - Volumetric flasks for brine and funnels, tubes, and valves for brine delivery
    - Red, temporary caps
    - Bunged 4 L bottles and 500 mL bottles for BIO ID tubes
    - Loaded Teflon filter holders
    - Log sheets

During Cruise (Standard 2-NBST month – adapt as necessary for additional traps)

Before Deployment:

- Collect ~90 L of 150 m water (enough for 2 traps) at least 2 hours before deployment – Hydrostation S is appropriate
- Rinse containers used to collect water 3 times before filling
- COLLECT WATER FOR BRINE TOO, IF NECESSARY (see below)
- Filter the water from the CTD through the high volume system into carboys
- A few hours before deployment, fill tubes to baffle with new filtered seawater
- Save ~1-2 L of filtered 150 m seawater for rinsing purposes – store in fridge
- Pour 500 mL of buffered brine/formalin solution down fill tube into bottom of sediment trap tubes

    - Measure out 500 mL of brine with volumetric flask
    - Insert tubing into tube with the valve open
    - Close the valve and fill funnel with solution
    - Slowly open valve so that the flow is highest without bubble going all the way down the tubing
    - Once complete, remove tubing slowly
- Repeat for all tubes

Deployment (at least 2 people):

- Mount and use PVC deck plate to help stabilize the NBSTs while on deck
- Mount full tubes with titanium hardware to NBST wings
- Once the tubes are mounted on NBST, immediately prior to deployment (check with bridge), pull back the tube lids and secure asymmetrically around center pin
- If any delay is necessary, use red, temporary caps to protect tubes (i.e. from smoke stack particulates which can contaminate for trace metals)

Post-Deployment

- Prepare the blank tube(s) as above for sample tubes
- Keep racked in lap for duration of deployment

Recovery:

- If any tube lids are open upon recovery, close manually and note which tube(s)
- Once NBST is securely back on board, remove tubes from wings, and rack in lab

On-board processing:

- Make sure to keep track of which tubes came from which trap in the lab and note on cast/data sheets which tubes were open/closed, any large, visible swimmers, missing/broken parts, etc.
- Allow the tubes to sit for at least 1 hour (no more than 3) before continuing processing
- Remove most of the overlying water, down to the second bottom ring, with the Flojet and clean tubing for *all* tubes
- Attach a pre-loaded Teflon filter holder to gray PVC bottle adapter and screw onto a bunged 4 L fluorinated PE bottle
- Attach to spigot of a tube and decant particle/water mixture into 4 L bottle through the screen
- Rinse the inside of each tube, from above, with filtered seawater, still passing the material through the screen. Repeat as necessary.
- Repeat for 3 more tubes from the same NBST (all through the same screen)
- Once complete, with filter holder still attached to bottle, rinse the screened material with a small amount of filtered seawater to remove any particles that may be adhered to large swimmers
- Store filter holder in bag in refrigerator – make sure to label it!
- Repeat for blank tube
- Drain brine/particle mixture from fifth tube into a well-labeled 500 mL bottle for BIO ID

- Cap the 4 L bottle and store in fridge or cooler until shore
- Rinse trap tubes with copious amounts of DI water

After Cruise – On Shore (Blue text refers to @ WHOI processing)

- Still keep all samples cool and transfer to lab on shore
- Add 1 mL of extra formalin to BIO ID samples -make sure these samples are well-labeled, tightly sealed, and wrap the outside of the lid with parafilm. Store in dark, cool place until shipping.
- For samples being shipped to WHOI for processing, add 1.2 mL to each bunged 4 L bottle, including the blank, to aid in preservation during shipping
- Rinse the screens from the tube processing on the ship onto Ag filters and dry – ship both the *dry* Ag filters and the rinsed screens to WHOI with other materials

Splitting

- Rinse the cone, top-plate, and fill-tube of the splitter with DI water
- Label n-traps x 8 clean 500 mL bottles – load first 8 into splitter
- Replace the top-plate, the spinning cone, and the fill tube
- Attach the PVC bottle top adapter to the first bunged 4 L bottle
- With the cone spinning and the valve open, gently swirl the 4 L bottle, invert it, place in shaker stand, and start shaker (or bottle can be swirled manually – be gentle to minimize any particle disaggregation)
- Unscrew bung to aid in flow
- When the bottle has drained, keep the splitter spinning, take the bottle of the stand, unscrew the PVC adapter, and rinse the bottle with small amounts of filtered seawater, pouring the rinses through the adapter
- Rinse the tube leading to the splitter, then turn the splitter off
- Remove the splitter parts; cap and store the splits in the fridge until filtration
- Rinse cone, fill-tube, and top plate with DI before the next set of samples
- Repeat for all samples, including blanks
- Rinse splitter components with DI, allow to dry, then pack and store

Filtering (as of this version, we will no longer pick the d split for small swimmers)

- Set up the filtering manifold, pump, and waste carboy
- Filter the splits onto their respective filter types (3 Ag, 3 Nuclepore, 2 QMA)
- Handle all filters with tweezers and rinse the bottles with a small amount of filtered seawater once they are empty
- Rinse the sides of the filter funnels with filtered seawater – perform a final rinse of the Nuclepore filters with a small amount of DI water
- After filtering, place the filters in labeled, clean Petri dishes – the b, e, and h splits will come in their own dishes, return to same dish from which they came

- Dry a, d, and g splits in drying oven; dry b, e, and h splits in laminar flow hood. At WHOI, dry b, e, and h splits further in dessicator.
- Cut the c fraction in half with ceramic scissors and freeze each half and the f split in separate cryovials in liquid nitrogen or a -40°C freezer

Making Brine

- Fill 4 L bottles or carboys with filtered seawater collected at sea from 400 m from a single cast and freeze in large freezer (at least minus 20 is usually required)
- Allow the bottles to thaw at room temperature in the clean bench
- Collect melt-water periodically in a clean 4L bottle – measure volume and salinity of these periodic collections to mix and produce brine with a final salinity of S=70
- Store brine in refrigerator until needed. Brine must be re-filtered through acid clean 1 µm filter prior to use
- Immediately before a cruise, buffer and add formalin to the brine and store cold
    - About 5.5 L of brine is required for a two-trap cruise
    - Add 3 mL of 37% formalin to each 5L of brine
    - Add 100 mL of pH=8 150 mM borate buffer to 5L
        - To make 500 mL of 150 mM borate:
        - Add about 200 mL of MQ to 500 mL bottle
        - Add about 4.6 g boric acid to MQ
        - Add NaOH to adjust to pH=8 (about 0.78 g) – check with pH paper
        - Fill to 500 mL with MQ

Cleaning @ BIOS

- Acid-wash (HCl):
    - Bunged 4 L bottles
    - Splitting 500 mL bottles

Rinse bottles with Milli-Q, then soak overnight in 5% HCl. Rinse 3x with Milli-Q and allow to dry on counter. For trace metal clean bottles, rinse with Milli-Q and fill with 10% HCl and sit in hood overnight. Rinse 3x with Milli-Q and allow to dry in hood.

- Rinse in Milli-Q (allow to dry in flow bench and place in plastic bags):
    - Filter-cartridge holder and tubing for filtering seawater
    - Brine-delivery materials – funnels, tubing, volumetric flasks
    - Tubes (including lids and baffles)
    - Tubing used to siphon off overlying water
    - Teflon filter holders
    - Splitter and all parts
    - Filter rig
    - Tweezers

- At least twice a year, or as needed, perform a more thorough tube (and parts) cleaning by rinsing with a weak acid solution followed by a DI rinse

Shipping

Keep samples cool and Parafilm all lids for extra protection.

Ship traps samples (in 4 L bottles) or dry filters, filtered SW as needed (in small bottles), and screen samples (on dry Ag and original screen) to:

Stephanie Owens/Steve Pike

Woods Hole Oceanographic Institution

MS#25, Clark 447

266 Woods Hole Road

Woods Hole, MA 02540, USA

Ship BIO ID samples (in 500 mL bottles) to:

Joe Cope/Debbie Steinberg

Virginia Institute of Marine Science: Biological Sciences

Rt. 1208 Greate Road

Gloucester Pt., VA 23062, USA

Contact info:

	Email	Office	Cell	
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Stephanie Owens	sowens@whoi.edu	x3843	(770) 313-2019	Grad student – contact about sample processing, data, etc.
Andrew McDonnell	drewmcd@whoi.edu	x2553	(951) 265-5224	Grad student – contact about auxiliary projects (RESPIRE, VPR)
Jim Valdes	jvaldes@whoi.edu	x2263	(508) 498-0715	Engineer – NBST, CLAP, etc. contact
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*Michael Lomas at BIOS and Dave Siegel at UCSB are additional co-PIs

Bunged 4 L bottles and small bottles for filtered SW (occasionally) will be shipped back to BIOS.