EMO BON Handbook Condensation, Protocol for Metadata and WaSOP 1 (Basic) sampling

This protocol originates from: https://www.embrc.eu/documents/2024.04_EMOBON-Handbook_FINAL.pdf , and is modified for sampling in the Sub-Arctic Tromsø area. Responsible for the modifications and revisions:

Kristel Berg, Julie Bitz-Thorsen, Simon N. Schmidt, and Kim Præbel
Research Group for Genetics, UiT The Arctic University of Norway.
Contact person: kim.praebel@uit.no

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1) MATERIALS

Table 1: Overview of materials needed to perform proper sampling

Materials	Quantity
Bleach	2 liters
Purified water (Milli-Q or Nanopure)	20 liters
Nitrile gloves, various sizes	1 box per size
50 ml falcon tubes	2
Wash bottle	1
Forceps	2
Scissors	1
Plastic bag for samples	10 per size
Peristaltic pump(s) and related equipment	1
Electric tape roll	1
Gaffa-tape roll	2
Filtration tripods, 142mm	5
PC membranes 3 µm, ø=142 mm	5
PC membranes 0.2 µm, ø=142 mm	5
5 ml cryotubes + caps	10
Permanent markers	1
Transparent tape roll	1
6 l Niskin bottle	1
200 µm mesh net	2
Sampling containers	1
Funnel	1
DNA/RNA Shield bottle	1
Sterivex filters	10
140mm petri dishes	10
Dry paper	2
70 % ethanol	1
Bench cover/plastic bags	1 pk
Rack for tubes (50 ml and 5 ml)	1
Box of dry ice	1
CTD	1
Hoses	
Rubberband Niskin	1
Foil	1

2) PRIOR TO SAMPLING

When preparing the work surface and cleaning, always wear gloves and adequate protection.

- Clean table with 10% bleach solution followed by Milli-Q, and 70% EtOH wash.
- Take a piece of bench-cover (thick plastic bags) and cover the worktable.
- Find the peristaltic pump, three cassettes and matching tubes and set up for filtration. Clean the pump and tubes with 10% bleach followed by milli-Q water.
- Prepare two 50 ml Falcon tubes, one with ~40 ml 10% bleach solution and one who will contain filtered seawater – label these accordingly on sides and on caps.

3) SAMPLING

Sampling consists of metadata-logging using the CTD and collecting seawater using the Niskin bottle.

- Take the following equipment with you to the sample site:
    - CTD and rope (rope is found in the tub with the equipment for the Niskin bottle)
    - Tub containing the 6 liter Niskin bottle and line
    - 20 liter Nalgene sampling container
    - Funnel and filter (found on top the sampling containers)
    - Gaffa tape (if alone, to attach the filter to funnel and funnel to sampling container if windy)
    - One spray bottle with 10% bleach solution and one with milli-Q water
    - Mobile phone (for accurate time and date logging for CTD data retrieval)
    - Other safety equipment
    - Nitrile gloves
- Attach the rope securely to the top of the CTD and make sure it is long enough to reach the sampling depth. Attach the other end of the rope securely to the pier/boat.
- Remove the “flushing syringe” from the bottom of the CTD and store it securely.
- Note date and time (as specific as possible, use screenshot of https://time.is for example) when you turn on the CTD and when it is below the water surface.
- Keep the CTD just below the surface for at least one minute to calibrate the equipment, now lower the CTD to the sampling depth (~2 meters) **Important: Note time very precisely** stay at the sampling depth for two full minutes.
- Pull the CTD out of the water and turn it off, flush the bottom of the CTD using the “flushing syringe” filled with milli-Q water at least two times. The CTD also needs a proper shower with tab water, this can with advantage be done during the long filtering steps to save time.
- Wash funnel and filter with 10% bleach and rinse with milli-Q.
- Sample containers were cleaned properly prior to storage so no need to clean those with bleach if the lids are attached securely. If lids are open follow the cleaning procedure for Niskin bottle.
- Clean the 6 liter Niskin bottle with 10% bleach, wait > 5minutes, and rinse in the sea.

- Sample 2x6 liter using the Niskin bottle at the sample depth at the chosen location.
- Empty the Niskin bottle into the funnel through the filter and into the sample container, if the filters gets clogged up, rinse and clean between samples. Clean Niskin bottle with 10% bleach and shower with tab water. This can with advantage be done during the long filtering steps.

4) FILTERING

Bring all equipment back from the sampling site.

- Swirl the seawater container to homogenize the water, fill a small aliquot container (e.g. a clean 1 l jar) with seawater, attack a sterivex filter to each of the tube ends and place the filter on the edge of the three 1-liter jars taped together. Start the pump on 60 rpm and pump until the water level in all three jars have reached the black line (each sterivex filter has now filtered minimum 500 ml water), the initial jar must be refilled accordingly, but waste as little seawater as possible. Remember to fill one of the previously labeled falcon tubes with ~40 ml filtered seawater (run-through from one of these sterivex filters).
- Remove the sterivex filters from the tubes and put them into falcon tubes labeled accordingly.
- Clean tubes with 10% bleach solution and milli-Q water before disassembling the construction. Return tubes and cassettes to their dedicated zip-lock bags, but leave one cassette out for the next filtration setup.
- Find three new, wider, tubes in a bag labeled GO-sampling and attach them to the cassette and the peristaltic pump like **figure 1** below (do not add PC filters yet):

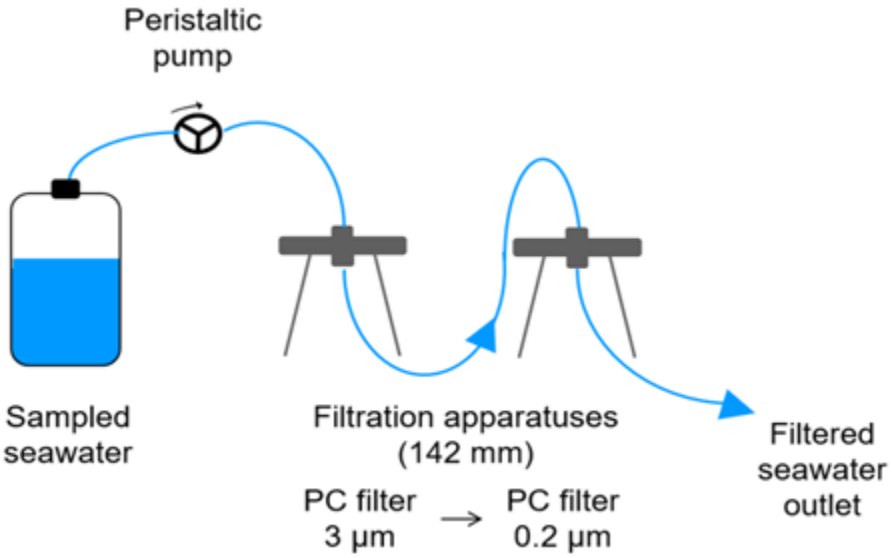

Figure 1: Simplification of filtering setup.

- Clean the filtration apparatuses and tubes using 10% bleach solution and flush thoroughly with milli-Q, and ca. 500ml sample seawater, before applying the filters.
- Clean forceps in the falcon tubes containing 10% bleach and rinse in the filtered seawater falcon tube. Store in bleach after use.
- Apply filters using the clean forceps in the order shown in **figure 1.**
- Properly mix the seawater sampling container. Use the 10 liter Nalgene sampling bottle as waste bottle and to measure out 5 liters.
- Add electronic tape to the threads of the ventilation valves and secure them using a wrench (do not disassemble valves after filtration – leave on until next sampling). Tighten knobs and secure tube endings. Leave the ventilation valves open (leaver straight up in the air).
- Start filtration of the first 5 l seawater at 60 rpm, however, the speed may be adjusted accordingly depending on the amount of particles in the water (higher speed in winter, slower in spring). When water flows regularly through the ventilation valves, close them.
- Cover the mouth of the sampling container during filtration (e.g parafilm, tin foil).
- When the filtration step is finished, guide the remaining water through the tubes and allow air to run through the system (with the valves closed) for a while before releasing the valves and the pressure in the system.
- Clean forceps and scissors in 10% bleach and rinse in filtered seawater.
- Open tripod holders and remove filter (one at a time!) using the forceps. Put the first filter in a 140mm petri dish and cut it in two equally sized pieces using the scissors. Place each half (or “replicate”) in separate labeled 5 ml cryotubes according to **Figure 2.** It is recommended to roll the filters like cigarette paper and folding while avoiding to mash it down the tube. Repeat for the second filter. Replicates 1 and 2 are placed in DNA/RNA shield fully covering the filter while replicate 3 and 4 are not. Place the cryotubes on dry ice as soon as possible.
- Run 2 liters of milli-Q water through the system without any filters.
- Repeat the above mentioned steps for a second run using another 5 liters of seawater for replicated 3 and 4. Do not add DNA/RNA shield on these samples. Place on dry ice as soon as possible.
- Run 2 liters of milli-Q water through the system without any filters.
- Run 5 liters of milli-Q water through the system with filters (these serve as negative controls). Negative controls will be placed in DNA/RNA shield like replicates 1 and 2, but without being cut in two. Place on dry ice as soon as possible.
- Disassemble tripods and pumps, clean setup and workspace accordingly (rinse all parts with 70 % ethanol and allow to dry, possibly overnight) and store properly.
- **Note down CTD sampling times in the small yellow book and take a photo of the page.**
- **Update the materials list for the next visit!**

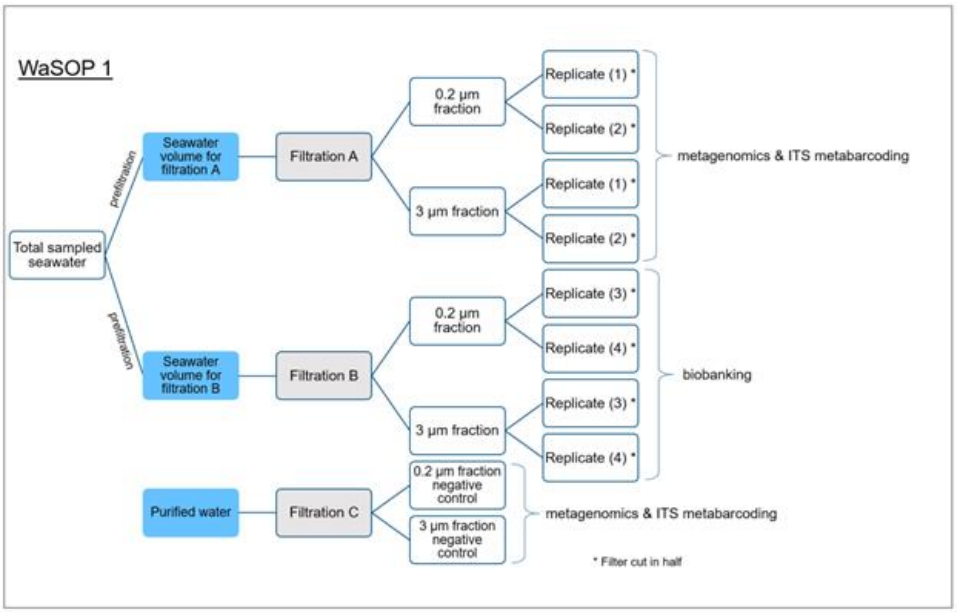

Figure 2: Summary of the sampling procedure. Look over the samples here, this is important.