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PECO eDNA field sampling protocol V.1

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We use this protocol and it is working.

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Abstract

This protocol summarizes field sampling techniques used by a network of partners that contribute to the Pacific eDNA Coastal Observatory. It includes information on water sample collection, filtration, preservation, and material cleaning between samples. Samples are shipped to a central facility for further processing, and bench methods and data post-processing will be provided elsewhere. The [Pacific eDNA Coastal Observatory](#) is part of the UN Ocean Decade and the Ocean Biomolecular Observation Network.

Guidelines

- Two or more people are required to do this protocol.
- There are four parts to sampling: collection, filtration, preservation, and bleach cleaning.
- The processing of 5 samples per site should take about 1- 3 hours once you are good at it, but the first time might take longer (e.g. plan for 2-4 hours the first time).
- Samples should be filtered within 2 hours of seawater collection, and stored in the dark on ice during any storage time (unless immediately filtering). Note: Some groups have required longer storage time, and this is noted.
- At all stages, please be aware of the possibility of sample contamination. Our PCR metabarcoding amplifies even the tiniest concentration of any fish DNA. Wear gloves at all times throughout the process, change gloves any time they come into contact with anything outside of the provided kit e.g. your face or body, the ground etc.

Materials

Materials you will need to supply

- Pole for pole sampler (6' of 1" PVC preferred, or a broom handle with duck tape)
- 5L of clean water for every site visited. For every site, 1L needed for negative control, plus 4L for cleaning. If 5 sites are visited, 25L are needed.
- 750ml of regular household bleach (Clorox brand regular strength or equivalent)
- Cooler
- Ice in cooler if storing bottles for more than 20 minutes
- possibly some extra clean gloves and paper towels if kit supplies run low

Rinse and Control water

Ideally this would be deionized water from a research lab, poured directly into the Control sample Nalgene bottle. However, this could also be distilled water from a grocery store. If transporting water in any of your own containers, the container itself needs to be bleached and thoroughly rinsed before use. The project will still work if the water isn't fully sterile or salt-free, but the key is: water that does not have fish DNA in it. Consider this order of priority depending on what is available to you:

1. Deionized, distilled water directly from lab source into the control Nalgene bottle prior to each field visit, and used to rinse materials after bleaching at a lab sink
2. Distilled water from grocery store, avoiding water that contains additional minerals, transported in the original bottle (eg. 7 x 4L bottles)
3. Deionized, distilled water from a lab, transported in a container that has been bleached (10% bleach for 20 minutes) and thoroughly rinsed 3 times

Materials provided inside the PECO sampling kit (N = number of sites for your kit)

- Pole sampler end-piece in large Ziploc bag
- 5 x 1.5 L Nalgene Bottles (sterile, bagged)
- 5N Sterivex filters with end caps in individual Whirl-paks (bagged)
- 5N syringes (3ml) with Longmire's buffer + end cap in individual Whirl-paks (bagged)
- 2N Acrodisc filters (bagged with buffer syringes)
- 2L (1/2 gal) pressure sprayer/pump with tubing and HEPA air filter
- N Bulkhead Caps with tubing and fittings (new in 2024)
- Clipboard and pencil
- N large syringes (60ml, sterile, packaged)
- Bag/box of nitrile gloves (small, medium or large)
- 1 L plastic graduated beaker/jug
- Foam stand for 1.5 L bottle
- 5 cable ties
- Bag of blue wipe towels
- 2N bench coats in a Ziplock bags

Safety warnings

⚠ WHMIS guidelines for bleach disposal is to check with local municipal regulations. In Vancouver, for example, it is okay to put down the sink, but recommended that it first be used for other cleaning first, which helps to denature it. Some septic systems and natural environments are sensitive to bleach. If you are unsure, please pack out the bleach until you are in a sewage system that can handle the addition, and/or use the bleach for cleaning.

Before start

- Check Materials for list of materials you need to have ready, including control water, a pole, and a cooler.
- Most of the protocol is illustrated in [this video](#), except for the bleach cleaning and rinsing part.
- Read guidelines as a reminder of site selection and tide characteristics for timing. If samples will not be filtered immediately, prepare a cooler of ice for cold & dark storage
- We ask that you collect eDNA from field sites before any beach seining or other activities that involve adding gear or people into the water.
- Plan your collection dates for a tide in which the habitat will be accessible and water surface will be 1-3 m above bottom at the collection sites.
- If your transit time between sites and your home base is >2hrs, plan to bring the filtration kit to the field so that you can filter within 2 hours of seawater collection.
- Avoid 'fishy' field clothes, boots, coolers, or tables, and always use the pole sampler to reach away from your body.
- If possible please bring a device for assaying temperature and salinity at your site.

Day-of preparation

- 1 Print and bring datasheets. [!\[\]\(2824aab9645d9fab95bae27ff6828dab_img.jpg\) PECO - field datasheet_2024.pdf 91KB](#)
- 2 Without removing them from their bags, find a bag of 5 unused sterivex filters labeled "PECOe-xxx" or "PECOe-XXXX". These will be the 5 sample names for your next site.
- 3 Label your 5 Nalgene bottles, from 1-4 and C for control.
- 4 With clean gloves and in a clean space, fill the control bottle with "control water" (see description in Materials), label this as the control, and place it back into its clean ZipLoc bag. Bring the control bottle into the field with you as the negative control.

Seawater Collection Stage

- 5 Exact locations of water samples, as well as precise transect lines, are expected to vary from year to year. Each sample should be taken at least 10 m apart (aka 10-13 large steps) within the habitat of interest; water can be accessed by wading from shore or by boat. The sample location surface should be 1-3m above the bottom at the time of sampling.

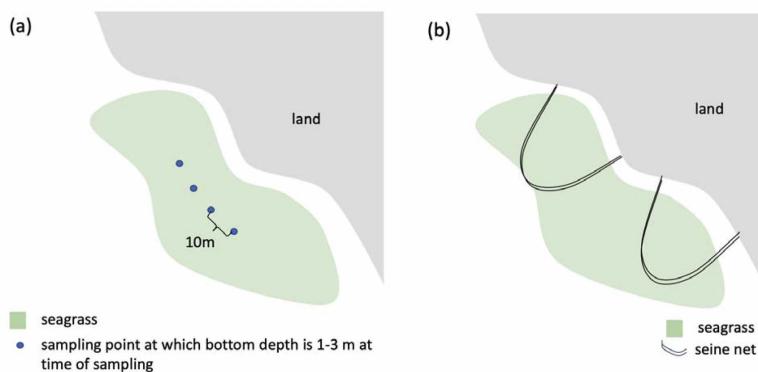


Fig. 1. Example of sampling scheme at a single site, showing spatial orientation of eDNA samples relative to land and seagrass bed (a). Schematic for optional paired beach seines shown in (b).

- 6 Advance to collection location and visually inspect the habitat and check that water depth is between 1-3m.
- 7 Have one person hold the pole while the other person, wearing a clean set of gloves, remove a bottle from the sterile Ziploc bag, mount the bottle, tighten the hose clamp around the bottle, and remove the lid.



Fig. 2 Attaching the Nalgene for a wading-access sample.

- 8 Have the person holding the pole plunge the Nalgene bottle in the water upside-down away from the boat or body, and turn it right-side-up when it is one foot below the surface. Have same person bring filled bottle to the surface; and gently decant field water over the lid and back into field. This is a rinse.
- 9 Repeat for three total rinses of the bottle with collection-site water.
- 10 For seawater collection, again have the person with the pole plunge the Nalgene bottle into the water upside-down away from the boat or body, and turn it right-side-up when it is one foot (30cm) below the surface and bring the sample to the surface.



Fig. 3. Sampling seawater from a boat. Bottle has just been rotated to release bubbles.

- 11 At the surface, shake out a small amount of water to allow for some air headspace at the top.
- 12 Have person holding the lid cap the bottle immediately after retrieval, tighten the lid, remove it from the pole, and place it in a Ziploc in the cooler with the lid closed.
- 13 Note the time and location from which the samples were collected on the eDNA Sampling Data Sheet.
- 14 Repeat for a total of 4 water samples at the collection site, separated by ~10m.
- 15 Remove bottle with control water from Ziplock bag, open the lid, hold the control bottle open for ~5 seconds at your sampling site, gently moving the bottle in the air, cap the lid and replace to Ziploc in the cooler.

- 16 Before leaving the sampled site, fill in site-level characteristics on the data sheet. Note seagrass characteristics, estimate the size of the seagrass bed, canopy height, the patchiness, cover and density of the seagrass. Note the weather, tide status, and take a decimal latitude and longitude of your location.
- 17 If possible, take a salinity and temperature reading at 30cm below surface.

Filtration Stage

- 18 This can be done on the shore near the sample site or in the lab. Be sure to move away from any possible sources of contamination. Indoors is preferred.
- 19 Wearing clean, dry gloves, remove the control water from the sterile bag.
- 20 Remove the cap and replace it with a sterile bulkhead cap and hose assembly. CAUTION: Do NOT touch the hose that will go into the sample bottle. Be careful to keep the hoses from contacting any surfaces outside of the sterile bag and bottle.

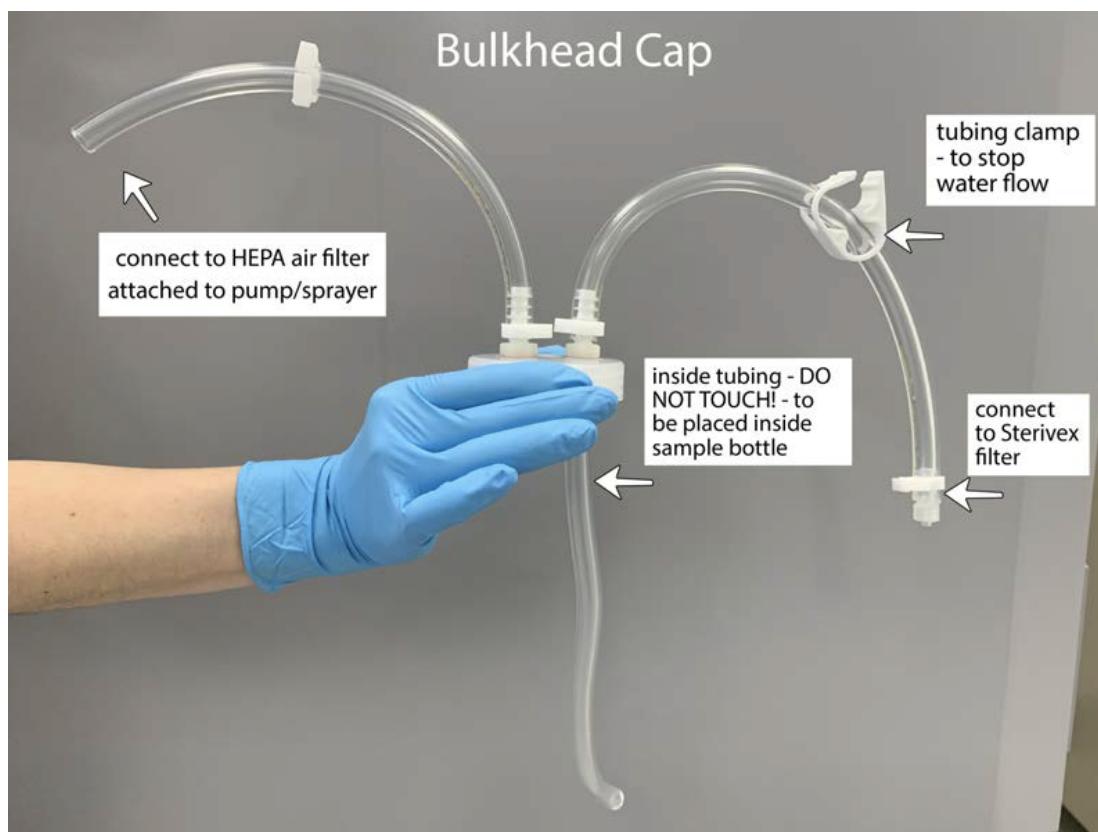


Fig. 4. This is the bulkhead cap. There will be one of these for every site, so they only need to be used once, labelled as used, and returned for sterilization at Hakai.

- 21 Once the bulkhead cap is screwed on, place the bottle in the pink foam stand for stability.
- 22 Connect the hose on the bulkhead cap to the HEPA air filter attached to the pump/water sprayer hose and clamp/cinch in place using the small white hose clamp. It is possible to set up to filter inside the kit tote, or you can set this up on a clean bench top.

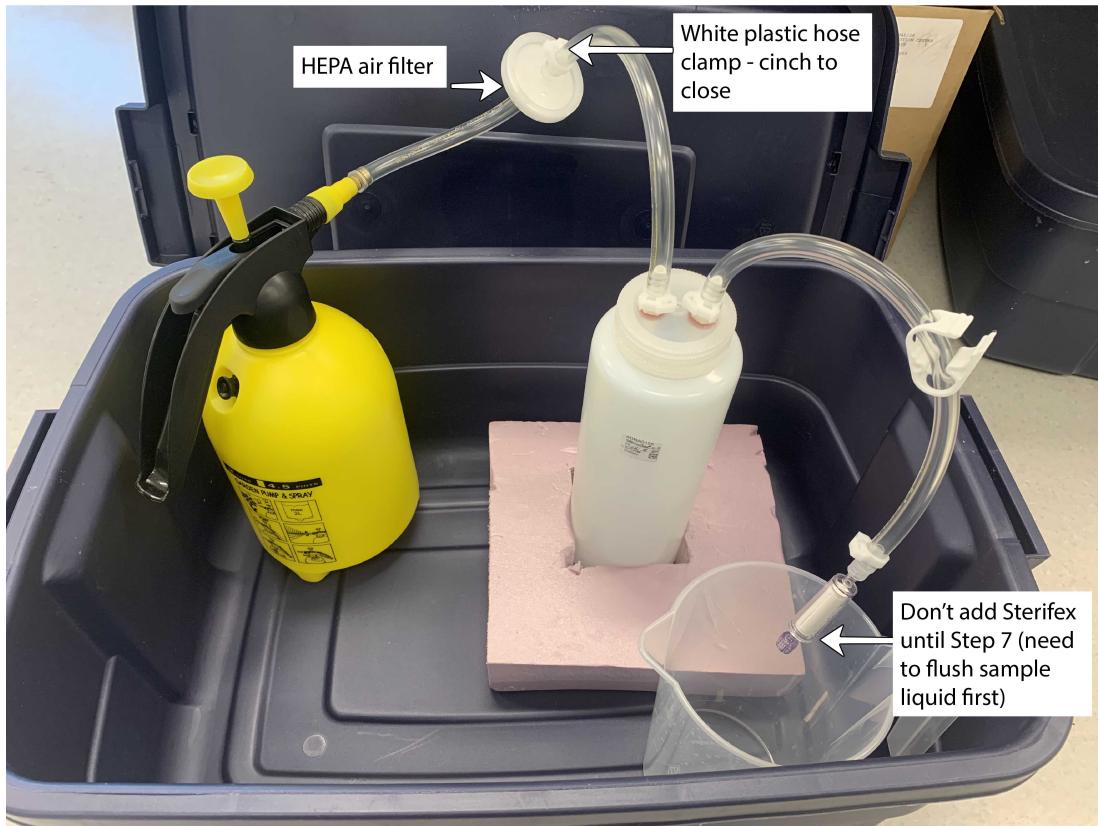


Fig. 5. Filtration set-up in tote, with control water sample attached. But: don't add the Sterivex filter until you have pumped out ~100ml of sample a few steps below.

- 23 If there is no locking mechanism on the handle of the water/pressure sprayer, use the black spring clamp to hold down the handle lever.

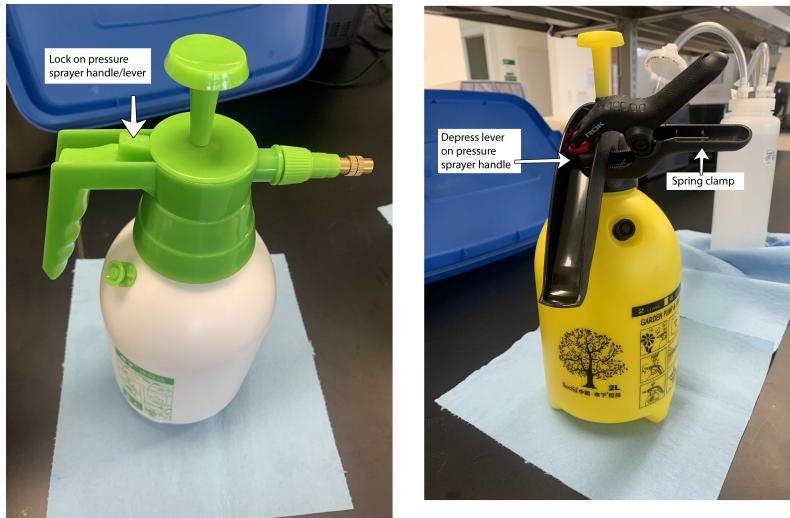


Fig. 6. Example pumps/water sprayers. RHS shows spring clamp holding down spray lever.

- 24 Before adding the Sterivex: pump the plunger on the sprayer a few times to allow water to pass through the hose into the plastic beaker. Allow about 100 ml to flow through and then press the white plastic tubing clamp on the hose to stop the flow of water.



Fig. 7. Water sample moving through filter. Hand pinches tubing clamp to stop water flow.

- 25 Discard water from the beaker. While the hose is still clamped, identify the correct labeled Sterivex filter inside its Whirl-pak bag.

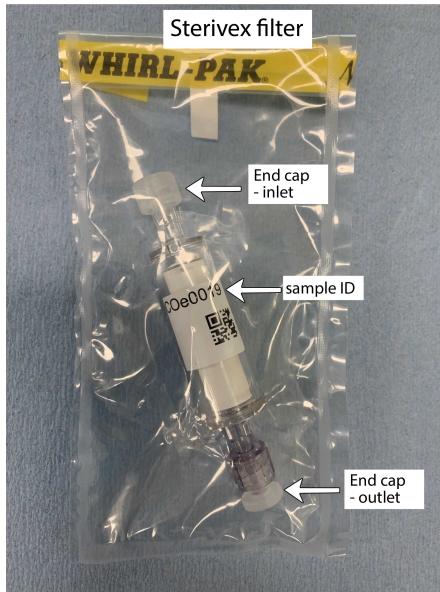


Fig. 8 Components of Sterivex filter in Whirl-Pak

- 26 Record the Sample ID and time of day on the data sheet. Be sure to indicate if it is a "Control" sample (YES/NO).
- 27 Remove the end pieces from the Sterivex filter and place them back in the Whirl-pak. New in 2024: one end piece will be a push-cap rather than a twist-on Luer lock lid.



Fig. 9. (New in 2024) Push-cap lid on outflow end of Sterivex

- 28 Screw the inlet end of the filter to the hose outlet, and place the filter over the graduated beaker so that water will flow through the filter and into beaker.
- 29 Filter 500- 1000 ml of water and close the white plastic clamp on the hose to stop the flow of water. Stopping point: If you feel strong resistance, and the water is just slowly coming out drop by drop, that is a sign to stop. We are aiming for 1000 ml of filtered water, but anything above 500ml we can still use in our analysis, and estuarine sites are known to have higher turbidity so many teams have been stopping at 500ml. Note the volume filtered on the data sheet.

- 30 Remove the filter and attach to a sterile 60 ml syringe full of air (Fig. 11). Point the syringe down and depress the plunger slowly over the beaker until all the water is removed from the filter. Detach the syringe and screw the push-cap from the whirl-pak bag on the male-end (outlet end) of the filter.

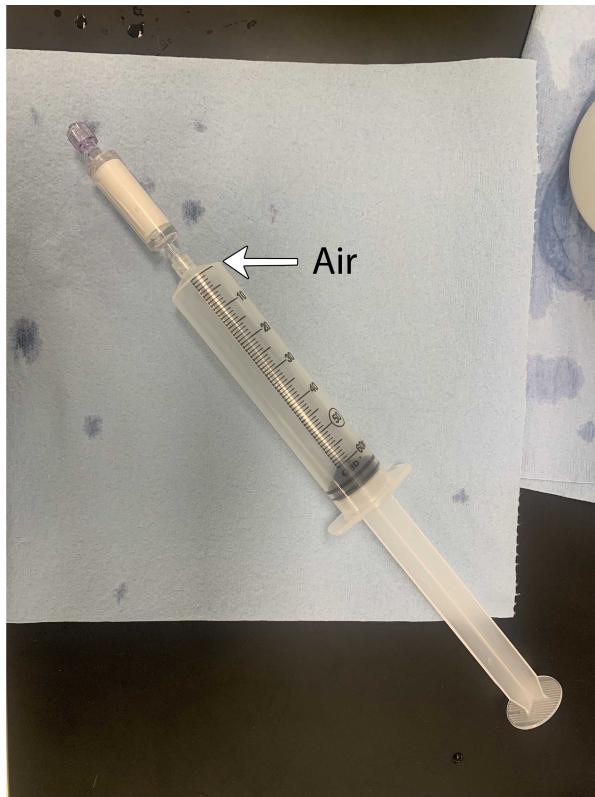


Fig 10. Sterivex and syringe (attached)

Preservation Stage

- 31 While one person is holding the Sterivex filter with the bottom end cap on, the other can remove the 3 ml syringe of buffer solution from the Whirl-pak bag labeled 'buffer'. PLEASE NOTE that if the buffer has come out of solution (ie. resembles liquid soap; opaque), warm the syringe in your hand until the buffer solution turns clear.
- 32 While holding the buffer syringe upright, remove the end cap from the syringe, peel back the top of the Acrodisc filter packet and attach/screw the filter on to the end of the syringe.

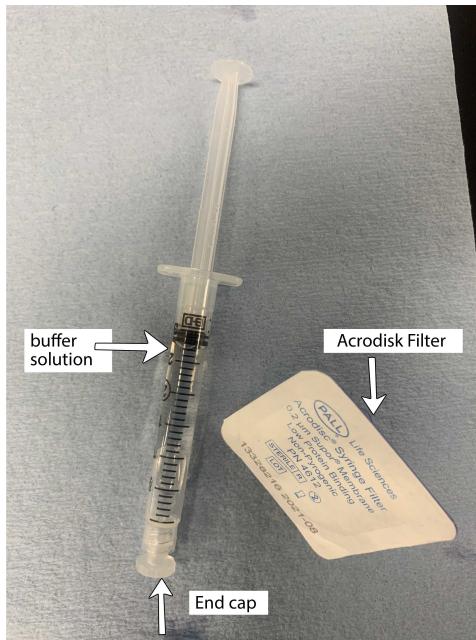


Fig. 11. Buffer solution in pre-filled syringe with Acrodisc filter.

- 33 Immediately after taking the Acrodisc out of the packet and while holding the Sterivex filter upright, push down the plunger on the buffer syringe until the white porous material inside the filter housing is covered. Note: in 2024 a different end cap will be used, so plunge slowly and we might need to hold end cap in place.

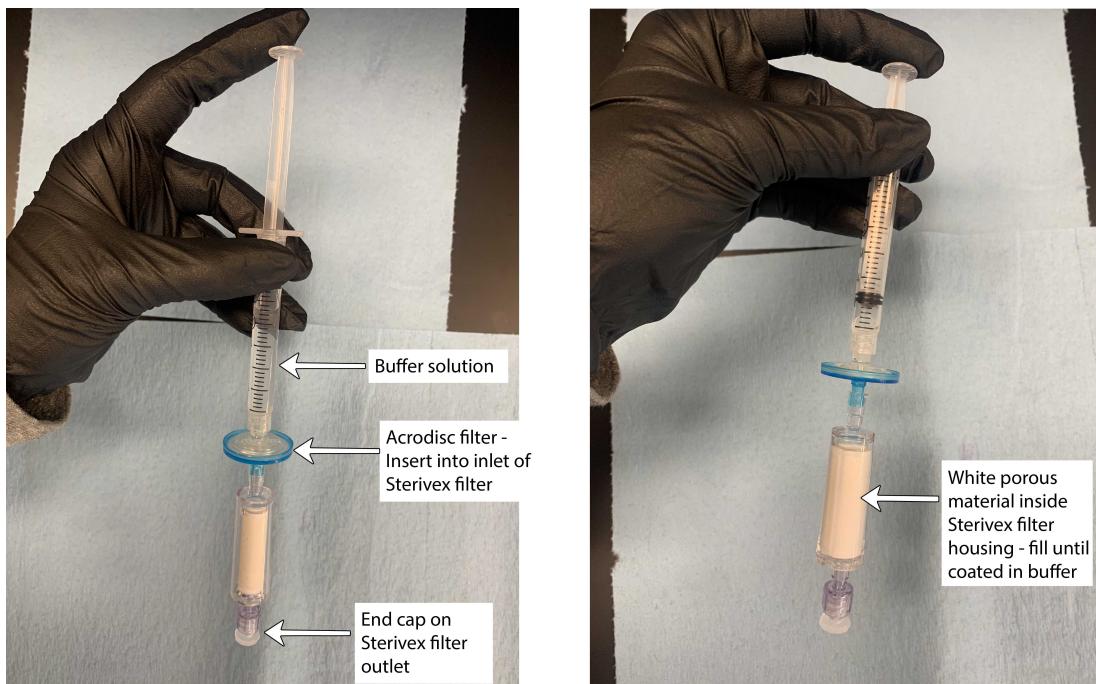


Fig. 12. Filling Sterivex with filtered buffer solution. LHS: Sterivex attached to Acrodisc and buffer solution syringe ready for depression. RHS: Depressed plunger on buffer solution into Sterivex filter.

- 34 Screw the remaining cap on to the female end (inlet end) of the Sterivex filter and gently shake to ensure the entire filter surface (white) has been coated in buffer solution. Place the filter back in its original small Whirl-pak bag. Please be sure to only screw caps on FINGER TIGHT (don't tighten with force).

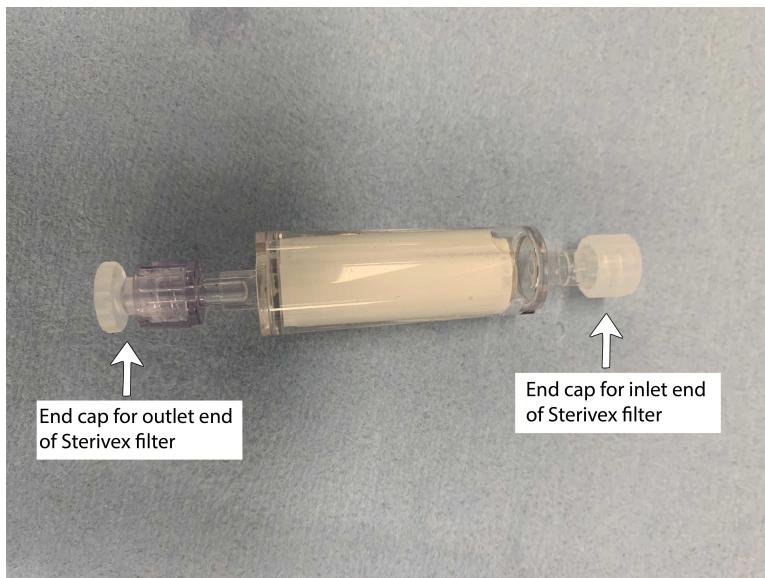


Fig. 13. Sterivex filter with buffer and both end caps. Note: in 2024 a different end cap will be used at outlet end.

Next Sample Set Up

- 35 To set up filtration for the next sample, first release the pressure in the hosing by opening the valve on the pump/sprayer. In case all of the pressure has not been removed from the tubing, unscrew the bulkhead cap slowly.
- 36 While one person slowly unscrews the bulkhead cap, the other person should uncap the next sample.
- 37 When ready to sample bottles, carefully remove the bulkhead cap and slowly pull it upwards until the inner tubing clears the bottle (be careful not to touch other surfaces with the tubing). Place the next sample bottle under the bulkhead cap while being careful not to touch the inner tubing, and screw on the cap.
- 38 Place the bottle in the pink foam stand. Repeat the Filtration, Preservation, and Next Sample Set Up steps for all four of the seawater samples.

Take down

- 39 Place all 5 samples (in individual Whirlpak bags) in a larger Ziploc bag and store in the fridge/dark until you are ready to ship.
- 40 To lightly rinse the pump, connect the Control water bottle back to the bulkhead cap and pump all remaining water through with no filter.
- 41 Remove the bulkhead cap, and place the bulkhead cap and the large syringe in a used ziplock for return to Hakai for bleaching and reuse.
- 42 Discard all remaining sample and control water from the Nalgene bottles, discard used buffer syringes and Acrodisc filters in garbage.

Bleach Cleaning - new in 2024: just bottles and beaker

- 43 Wear gloves and protective equipment and conduct bleaching in a space with good ventilation. Prepare a clean working environment or bench top where lids and parts can be put down between rinses, plan to use one bench coat for this. If available (optional), rinse all bottles with tap water before starting.
- 44 Prepare 1 L of 10% bleach solution by adding 100ml of regular-strength Clorox bleach to the Control Nalgene bottle and top up with rinse water (see notes in Materials), filling the 1.5L Nalgene about 2/3 in height. This will be a 10:90 mixture of regular strength Clorox bleach (10%) and water (90%). Close with lid and invert 5 times.
- 45 Remove cap of the next bottle (first sample bottle) and place lid on bench coat. Decant bleach solution to the new bottle, replace lid to empty bottle, and set aside in a designated spot. Cap lid of bleach-filled bottle and invert 5 times.
- 46 Repeat until all bottles are bleached, and decant bleach solution into plastic beaker.
- 47 Remove all 5 lids and place them in the beaker with bleach solution. One by one dip the open end of each bottle into the beaker. Remove lids, shake off access liquid, and return to bottles. Dispose of bleach (see below).
- 48 Fill the first Nalgene to 2/3 full with rinse water, cap and invert 5 times. Decant into the next bottle.
Repeat until the 5th Nalgene bottle is done and decant to the beaker.
- 49 Remove all 5 lids and place them in the beaker with the rinse water. One by one dip the open end of each bottle into the beaker. Carefully drain access water, remove lids, shake off access liquid, and return to bottles. Dispose of rest of the rinse water.

- 50 Repeat this rinse cycle for 3 full rinse water rinses.
- 51 If the next field sampling is more than 1 day away: shake out excess water, and allow the bottles, lids, and beaker to dry with a sterile bench pad lain over top (provided in kit). Once dry, place lids on Nalgenes and place each item in a clean Ziploc bag for the next field day.
- 52 If the next collection day is the following day, put wet-but-sterile lids on wet-but-sterile Nalgenes and place in clean Ziploc bags.

Protocol references

Much of this protocol was developed based on the Integrated Coastal Observatory (ICO) eDNA protocol.