

SOP 4

Water eDNA



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PROTOCOL REFERENCE

California Molecular Methods Workgroup SOPs for DNA sampling best practices and water eDNA collection: <https://github.com/stheroux/MMWG/>

OBJECTIVE

Environmental DNA methods provide an opportunity to survey multiple taxa at one time from an environmental sample, including microbes, fish, and mammals. In conjunction with the other biological indicators, water eDNA samples will be collected and analyzed to profile species biodiversity. This SOP describes the collection and preservation of water eDNA samples using gravity filtration.

Instructional video: https://youtu.be/sQ_zVaddKt8

MATERIALS

Field:

1. Kangaroo gravity feeding bags
2. MasterFlex tubing, cut into 2 inch pieces
3. Adapter for connecting Sterivex to tubing
4. 0.22um Sterivex filters
5. Coffee filters and filter holder
6. Sterivex labels with sample site code, date, and replicate number
7. Sterivex blue caps
8. 2ml screw cap tube pre-loaded with preservation solution
9. Sample collection bottle (500ml or 1L) *
10. 3 mL syringe*
11. DNAway
12. Kimwipes
13. MilliQ water
14. Nitrile gloves
15. Duct tape or binder clip for securing Sterivex filter (ie to bucket)
16. Whirl Pak or Ziploc bags labeled with sample site code, date, and replicate number

* Items should be sterilized before use and between sampling sites to prevent cross-contamination. To sterilize, soak in acid wash (1% solution of hydrochloric or nitric acid) or bleach (final concentration 1-5%), rinse 3x in DI H₂O, and autoclave. Alternatively, soak in bleach solution (final concentration 1-5%) and rinse 3x with DI H₂O.

Field methods: Water column

Instructional video: https://youtu.be/sQ_zVaddKt8

Sample Collection:

One kangaroo bag will be used **per sampling station** – filtering 1 field blank and 3 replicates. The field blank should be filtered first before filtering the replicates. **Only 1 field blank is needed per estuary.**



If field crews are short on time, water samples can be collected following the instructions below and then **brought back to the lab for filtering**. Samples can be collected using either 1L Nalgene bottles or 1L whirlpaks. Samples should be kept **cold on ice** until filtering (samples should be filtered within 24 hours).

Preparation:

1. Prior to removing eDNA supplies or collecting the sample, put on **nitrile gloves**.
2. Open eDNA supplies on a flat, dry, **clean** stable surface (lab bench, folding table, small storage container, upside down bucket, etc.).
 - a. **Sterilize the surface** before unpacking the eDNA supplies with a Clorox or bleach wipe or DNAway.

Collection of field blank:

3. **Before sample collection**, one field blank should be processed per estuary.
 - a. Filter ~100ml of MilliQ water through a clean Kangaroo pouch and Sterivex filter following the instructions below.

Collection of water eDNA sample:

4. Sample water should be collected at high tide using a 1L sample bottle. Surveyors can wade into the water, so that samples are collected in the intertidal zone at high tide. There should be at least 1 m of water at time of collection.
5. Stand in the water until the **sediment settles**, and then begin collecting the samples.
6. Rinse sample bottle 3x with ~10ml of sample (estuarine) water; discard rinse water offshore or away from sampling site. Fill bottle with 1L of sample water. If bottle numbers are limited, large WhirlPaks can be used for water collection.
7. Carry sample water back to shore.

8. After this stage, **samples could be brought back to lab for filtering**. Samples should be kept **cold on ice** until filtering and filtered within 24 hours.

Filtering with coffee filter, Sterivex filter, and kangaroo bag:

9. Ensure that Kangaroo bag tubing switch is in “off” position.
 - a. Load a coffee filter onto the filter holder and place in opening of kangaroo pouch; hold filter holder and kangaroo pouch steady with 2 hands (**Figure 1**).
 - b. Pour sample water into coffee filter, allowing volume to filter through before adding more water. This should take ~5-10 minutes.
 - c. After all water has passed through the coffee filter, remove filter squeezing out remaining liquid. Avoid touching the inside of the filter. Store coffee filter in labeled whirlpak. Place on ice immediately.
10. Hang Kangaroo bag out of direct sunlight, climate control preferred, about 4-6 feet above the floor. Place container/bucket underneath bags to collect outflow (or let drain on floor if boat or outdoor site filtering allows).
 - a. The best and fastest filtering will occur when the bag is high enough so that the filter line is straight and extended.
11. Open the Sterivex filter and label with site name, station number, sample replicate, and date using the provided labels. Attach the Masterflex tubing to the purple end of the Kangaroo bag tubing. Insert the white adapter to the other end of the Masterflex tubing. Attach (twist) the Sterivex filter to the white adapter (**Figure 2**).
12. Use a binder clip or duct tape to secure the Sterivex filter pointing into the bucket. Avoid the Sterivex filter touching the mud or sediment.
13. Open Kangaroo tubing switch to allow water to begin flowing.
14. Stop filtering after **40-50 minutes** even if less than 1L has been filtered.
 - a. **Record the volume filtered on the sample sheet.**
15. When done filtering, use a 3 mL syringe to expel the remaining water out of the Sterivex filter. Expel the remaining water 3x until the filter is dry. Cap the larger end of the Sterivex with a blue cap.
16. Proceed to **Sample Preservation** (below). Between stations, rinse filter holder with DI water 3x and wipe down with DNAway.



Figure 1. Coffee filter and kangaroo pouch.

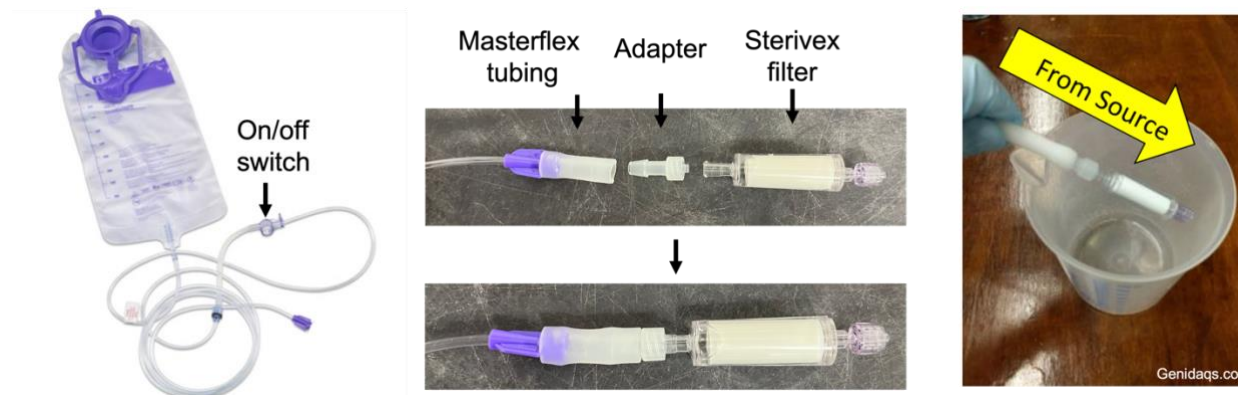


Figure 2. Plan A. Left: Kangaroo feeding bag. Center: Assembling the Masterflex tubing, adapter, and Sterivex filter. Right: Sterivex draining into bucket.

Sample Preservation:

17. Load ~1.5 ml of preservation solution using a disposable pipette. Insert the pipette into the open end of the Sterivex and flood the membrane with the preservation solution. When complete, cap the remaining end of the Sterivex with another blue cap (**Figure 3**).
18. Soak the Sterivex membrane by inverting the Sterivex 3x.
19. Place the labelled Sterivex into a labeled whirlpak
 - a. All three replicates can go into the same whirlpak or bag.
20. Keep tubes with filters on ice until able to be transported to -20°C or -80°C freezer. Samples at -20°C are stable for six months.
21. Repeat all steps for as many replicates as requested (default = 3 replicates). A single kangaroo bag can be reused for three replicates within a sampling station. Each replicate requires its own Sterivex filter.

****Filtering before storage will help preserve the integrity of the eDNA. The freeze-thaw cycle is known to degrade DNA, especially extracellular DNA which we are targeting in the water samples. Additionally, thawing large volumes of water will take a long time and could result in more DNA decay.**

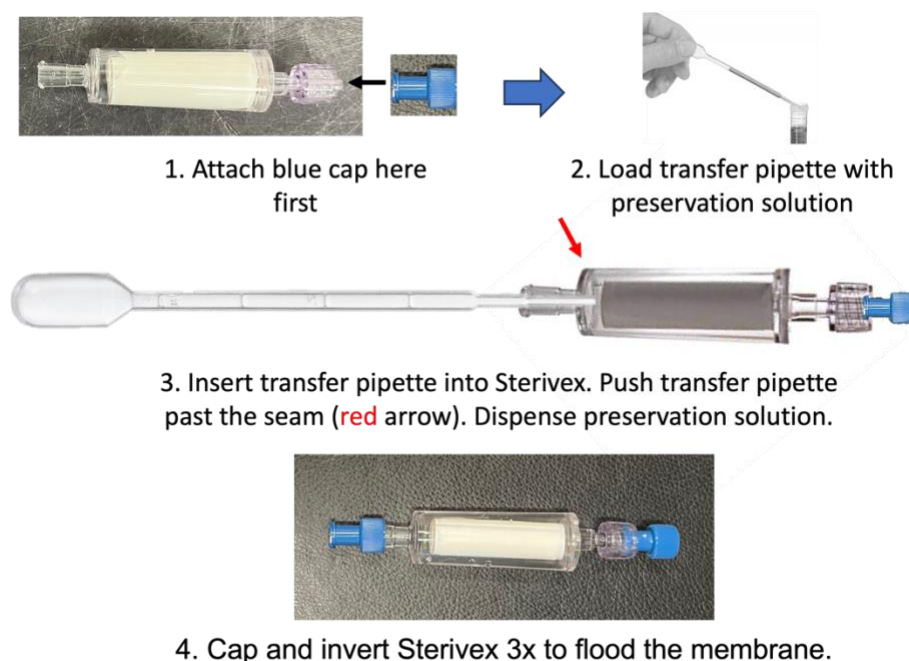


Figure 3. Steps for loading Sterivex filter with preservation solution.

QUALITY ASSURANCE/ QUALITY CONTROL (QA/QC)

Field personnel must be thoroughly trained in the proper use of sample collection gear and must be able to distinguish acceptable versus unacceptable water grab samples in accordance with pre-established criteria.

Field personnel must be thoroughly trained to recognize and avoid potential sources of sample contamination (e.g., non-sterile sampling supplies, poor sterile technique).

All data collected on the eDNA data sheet should be entered clearly. Team Leaders should double check that all eDNA samples have been collected and properly filtered and that the Data sheets have been filled out before leaving a transect site. eDNA Filters relinquished to SCCWRP need to have the following eDNA COC documentation: [SCCWRP eDNA CoC template_forBight.xlsx](#)