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eDNA water sampling in remote rocky shore environments

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We use this protocol and it's working

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Abstract

This is a protocol to collect marine environmental DNA samples from remote rocky intertidal sites by filtering on-site using Sterivex filtering units and a syringe/sealant gun approach. By the end of this protocol, the user will have self-contained and preserved samples in stervix filters ready for DNA extraction. This protocol has been developed using multiple sources (see references for details).

Image Attribution

All photos were taken and edited by Dina-Leigh Simons.

Safety warnings

 This protocol uses EtOH as a preservation solution and concentrated bleach as a cleaning reagent.

Before start

Please follow the cleaning regime in a clean lab before sampling. If a clean lab is not available, wipe down all possible surfaces with 10% bleach solution to create a sterile work area.



Cleaning and packing equipment (pre-sampling)

1h 10m

- 1 Put on lab coat and clean non-powdered gloves. Wipe down work area with 10% bleach. If excess dirt is present, scrub surfaces with soap and water before bleaching. 5m
- 2 Submerge 1L Nalgene bottles in 20% bleach solution (see solution preparation below) for at least 10-minutes. Be sure to completely expose all surfaces to bleach. If excessive dirt present, scrub with soap and water before submerging in bleach. If previous labelling is present on bottles, use ethanol to wipe off. 15m

Note

Bleach solution preparation: If large amounts of deionised water is not available, which is often the case in remote locations, diluting bleach with tap water is suitable as long as it is used immediately. For a 0.5% active chlorine solution, use 25x dilution of 13% active chlorine (i.e. 🧴 5 L solution = 🧴 200 mL 13% active chlorine bleach + 🧴 4800 mL tap water; 🧴 10 L solution = 🧴 400 mL 13% active chlorine bleach + 🧴 9600 mL tap water).

- 3 Remove 1L Nalgene bottles from bleach and rinse (inside and outside) with deionised water (fill 1/3 of bottle, cap lid, shake, discard water on inside, rinse; repeat 3 times). 20m

Note

Deionised water must be transported with other equipment prior to arrival at sampling location when a source is not available at site. Approximately 🧴 2 L of deionised water is required per wash of 20x Nalgene bottles.

- 4 Thoroughly dry 1L Nalgene bottles with clean blue roll or air dry on blue roll. Place into separate plastic bag/sterile box for transport to site. 10m
- 5 For all other field equipment (e.g. boxes, sealant guns, consumables), wipe down all surfaces with 10% bleach using blue roll. 15m
- 6 Count and pack consumables into field box, accounting for the number of desired samples and one negative field control per site. Add one extra set of all consumables to account for breakages, contamination, or unexpected sampling opportunities. 5m



Sensitive consumables (sterivex filters, syringes, combistoppers, and ethanol) should be stored in a secondary smaller box. For ethanol, use a fresh aliquot for each site to minimise contamination.

Note

One field sample requires the following consumables:

- 1x sterivex filter
- 1x 60mL syringe
- 1x 3mL syringe
-  2 mL ethanol
- 1x small plastic sample bag
- 2x combistoppers

Equipment required per site:

- 2x sealant gun (with optional adaptors)
- 1x large plastic site bag
- 1x box of non-powdered gloves
- 1x blue roll
- 1x marker pen
- Sample carrier bag (with separators; wine bottle bag works well)
- One-meter ruler (optional)
- pH/temperature/salinity sensor (optional)
- Camera (optional)

- 7 Transport all equipment in clean plastic bags within field bags (e.g. heavy duty holdall or similar) or sterile boxes to reduce contamination in transit. Avoid supplies from touching the transport bag directly. Use plastic bags to secure waste later.
- 8 Purchase sealed bottled drinking water on route to the site for a negative field control.

Note

If accessible, use deionised water instead for the negative field control. If you are interested in microorganisms, bottled drinking water will not be sufficiently sterile as a control.

Sample collection

1h

- 9 Find an accessible and safe route onto the shore, carrying the bag with 1L Nalgene bottles and field box containing consumables. Find a suitable location to set-up a work station either on the shore or near the car.
- 10 Record field metadata (most can be prepared beforehand):
 - Site name and grid reference
 - County/Area

5m

2m



- Date
- Recorder/s
- Latitude/longitude of access point (e.g., car park)
- Latitude/longitude of centre of survey area (e.g., midshore)
- Exposure scale of the shore
- Weather at the time of the survey, especially the visibility
- Mark site on virtual or physical map
- Tide (incoming or outgoing)

Site name:		Replicate ID code:	03.....	Replicate ID code:	04.....
Field ID:		Time:		Time:	
Coordinates:		Preservation method:	EtOH.....	Preservation method:	EtOH.....
Date:		Shore position:		Shore position:	
Recorder:		Depth:		Depth:	
Lat long of access point:		Temperature:		Temperature:	
Lat long of centre:		pH:		pH:	
Exposure:		Notes (terrain/%algae):		Notes (terrain/%algae):	
Weather and visibility:					
Tide (incoming or outgoing):					
Position of tidal cycle (+/- m):					
Notes:					

Replicate ID code:	01.....	Replicate ID code:	02.....	Replicate ID code:	05.....	Replicate ID code:	06.....
Time:		Time:		Time:		Time:	
Preservation method:	EtOH.....	Preservation method:	EtOH.....	Preservation method:	EtOH.....	Preservation method:	EtOH.....
Shore position:		Shore position:		Shore position:		Shore position:	
Depth:		Depth:		Depth:		Depth:	
Temperature:		Temperature:		Temperature:		Temperature:	
pH:		pH:		pH:		pH:	
Notes (terrain/%algae):		Notes (terrain/%algae):		Notes (terrain/%algae):		Notes (terrain/%algae):	

Notes:		Notes:	
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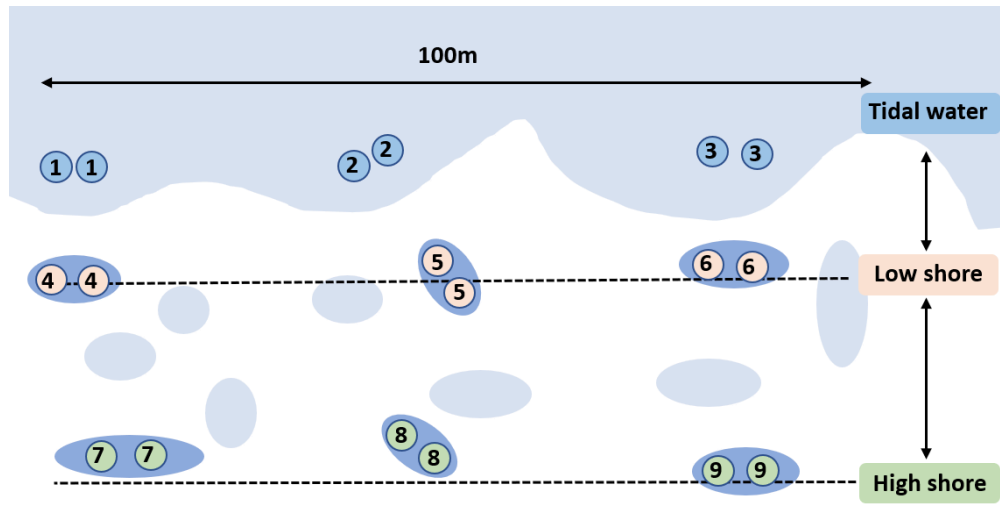
Example of field recording sheet.

- 11 Locate three rockpools at the same vertical level. Start with lowshore rockpools if tide incoming and highshore rockpools if tide outgoing.

2m

Note

This step might vary depending on what the user's specific research question is.



Example of sampling set-up.

- 12 Put on non-powdered gloves and remove a 1L Nalgene from the transport bag. Rinse the bottle in the sample water at least three times, ensuring discarded water does not re-enter the sample area.

1m

Note

Avoid entering the water when collecting samples. This will prevent disturbance of any substrate, limit contamination from other sources, and reduce health & safety risk.

If gloves touch contaminated surfaces, replace them with clean gloves or rinse gloves with bleach. Same applies throughout the whole protocol.

- 13 Fill the bottle halfway ( 500 mL). Proceed to other side of the rockpool and repeat (the bottle should now be full). Secure the lid.

1m

Note

If the rockpool is not deep enough to fully submerge the collection bottle, the bottle lid can be used to scoop the water into the bottle instead.



Collecting water sample in rockpool.

- 14 Label sample with ID on top of lid and on the side of bottle using a permanent marker pen.

1m



Labelling sample.

- 15 Shake grab bottle to homogenise DNA in water sample. Place the sample in the carrier bag with separators.

1m



Placing sample in carrier bag.



Nine samples placed in carrying bag with separators.

- 16 **Optional:** Take an image of the rockpool sampled with one-metre measuring ruler for scale. Record depth, temperature, pH and salinity using a two-point measurement system (average when entering the data). 2m
- 17 Change gloves. 1m
- 18 Repeat steps 12-17 for two more rockpools at same shore height (i.e. lowshore), three more rockpools at different shore height (i.e. highshore), and three open water samples (image and depth measurement not required for open water samples). 45m

Sample filtration

1h 45m

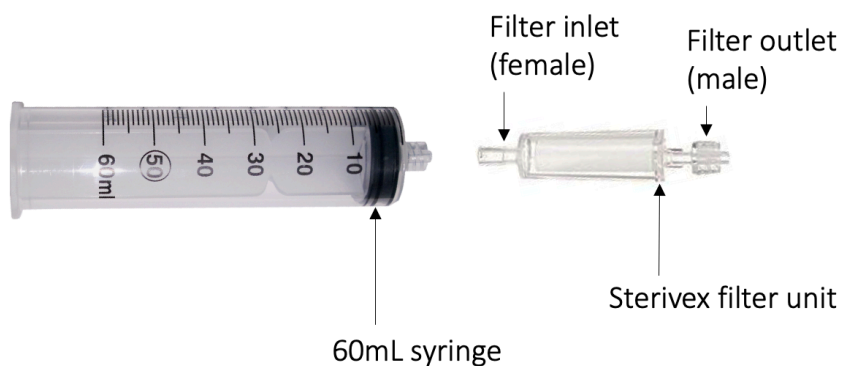
- 19 Prepare a stable work area using the lid from the storage box, and lay out all consumables needed. 1m



Example of field box containing all consumables, gloves and sealant guns. Note the secondary box (black lid) for sensitive consumables.

- 20 Partially tear open the sterivex filter packaging to expose the filter inlet (female), as instructed on the packaging. Tear open the 60mL syringe packaging and remove the plunger. Twist the Sterivex inlet into the syringe.

1m



Sterivex filter and syringe with labelled inlet and outlet.

Note

This technique is to prevent touching either end of the filter housing with external environment. Avoid touching the ends of the housing throughout the protocol.

- 21 Fill the syringe with  60 mL of water sample and place the plunger back into the end.

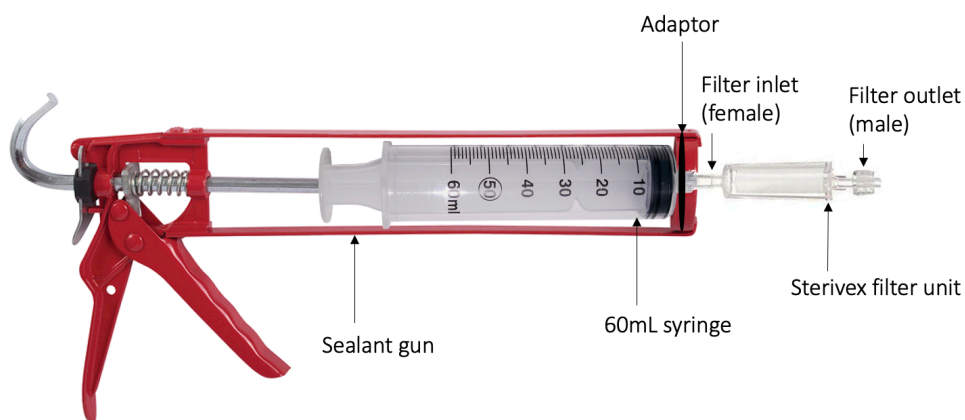
1m

Note

The filter only connects to the Luer extension in one way, ensuring proper connection. If the filter is installed backward, water will simply flow through the filter assembly and not come into contact with the filter material (i.e. no DNA will be captured).

- 22 Insert the syringe and filter into the sealant gun and fire the gun until the liquid has gone through, making sure to pump waste water away from equipment. Once all the water has passed through, detach the filter, pull the syringe plunger out, and reattach filter.

2m



Equipment set-up for filtering using a sealant gun and 60mL syringe.



Firing the sealant gun to filter sample water.

Note

Do not to push too rapidly to prevent breaking the filter. Let the liquid pass through the filter and out the end before firing again to prevent tearing the paper inside or bursting the plastic housing.

- 23 Repeat steps 21-22 until you have filtered all the water in the grab bottle.

10m

Note

All water filtration should occur directly at each site if possible. If this is not possible, keep samples cool, out of direct sunlight, and filter as soon as possible (no later than 12 hours).

- 24 Once the desired volume has been reached, detach the filter. Then, remove and reinsert the plunger to fill the syringe with air, and reattached the filter. Press the syringe down while attached to the filter to drain remaining water from inside the filter.

1m

Note

It is very important to remove water from inside filter as it can reduce the effectiveness of the preservation solution. In turbid water samples, sediment may accumulate inside filter which is normal.

- 25 Cap the filter outlet (male) with a combistopper. Remove the syringe from the filter inlet (female) and throw in waste bag. 1m



Capping filter outlet with combistopper.

- 26 Draw up  2 mL preservation solution from the ethanol aliquot using 3mL syringe. 1m

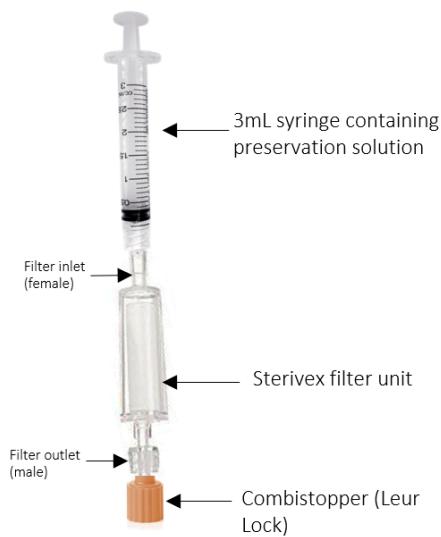
- 27 Attach 3mL syringe filled with  2 mL preservation solution to the inlet (female) and add to filter. 1m

Note

Be careful to firmly insert the tip into the Sterivex to ensure the buffer does not overflow the cartridge.



Adding preservation solution to sample.



Set-up for adding preservation solution into capped filter after water filtration.

- 28 Dispose of 3 mL syringe. Cap the filter inlet (female) with a combistopper. Gently invert to mix the solution over the filter. Label the outside of the filter with corresponding sample number. Filter is considered self-contained at this point.

1m

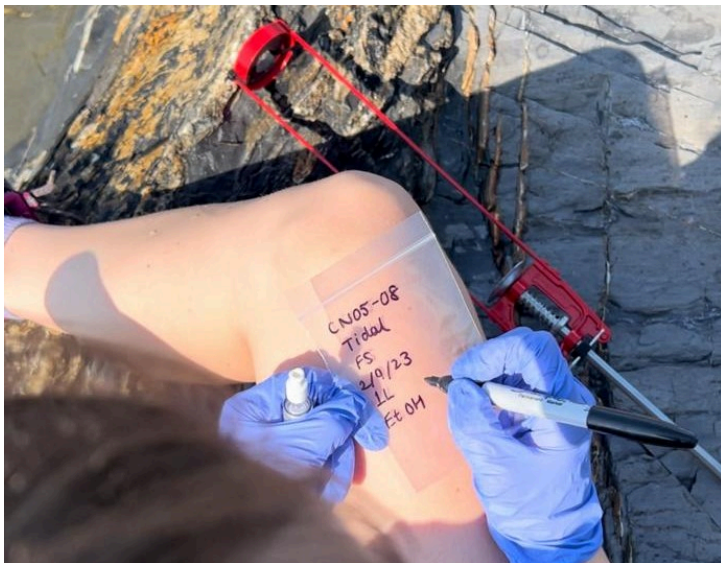


Self-contained filtered sample.

29 Place filter into a small plastic bag and label with the following information:

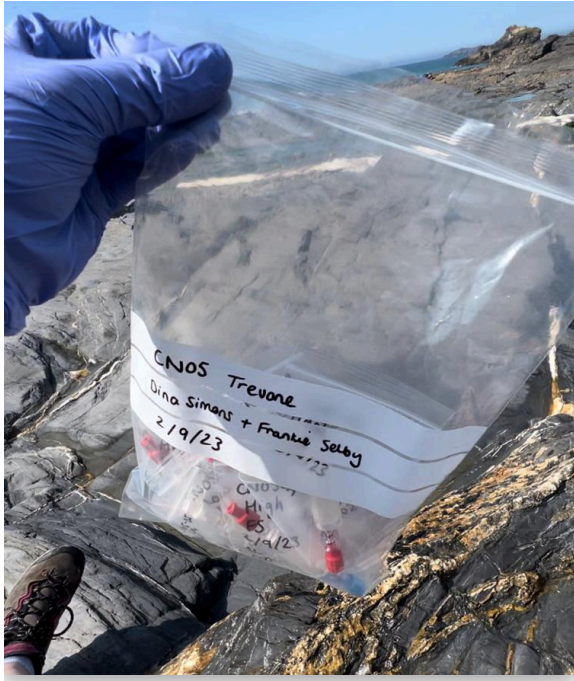
- Field ID code
- Date
- Preservation solution
- Volume filtered
- Recorder
- Shore position

1m



Example of sample bag labelled with metadata.

30 Place sample into a large plastic 'site' bag labelled with site ID, date, and recorders.



Example of site bag containing all samples.

- 31 Repeat steps 20-30 for all collected water samples.

1h 20m

Note

Each sample will take between 15-20 minutes to filter. Time on shore is significantly reduced if two people filter at the same time (approx 01:20:00 with two people, or 02:40:00 with one person).

- 32 For the negative control, rinse a clean grab bottle at least three times using the sealed bottled water. Pour 200 mL of the bottled water into the grab bottle and secure the lid. Repeat steps 20-30. The control should be filtered and preserved using the same equipment and procedures as the samples.

5m

Post-sampling checks

2m

- 33 Before leaving the site, have one last walk around the sample site to confirm metadata details. Check that all equipment and waste has removed from the shore.
- 34 All filters should be transferred to a -20 °C spark proof laboratory freezer as soon as possible. Filters can be stored unfrozen for up to one month, in a fridge or on ice packs if possible.

2m



Note

Ethanol may slightly leak out of filter housing during storage. It is good practice to wipe down housing with bleach before further processing. This bleaching step breaks the connection between field and lab environments.

- 35 All equipment must be cleaned and packed away before next use (following the cleaning and packing instructions above). It is important to clean thoroughly between sites.

Protocol references

This protocol is adapted from multiple sources, listed below, as well as direct from advice and support from NEOF (University of Sheffield, UK) and Dr Matthew Spencer (University of Liverpool, UK).

Blankenship, S. M. & Schumer, G. (2017), Environmental DNA Protocols Field Collection Procedure for Aquatic Environmental DNA sample collection and analysis, Technical report, Cramer Fish Sciences – GENIDAQS.

Bruce, K., Blackman, R. C., Bourlat, S. J., Hellström, M., Bakker, J., Bista, I., Bohmann, K., Bouchez, A., Brys, R., Clark, K., Elbrecht, V., Fazi, S., Fonseca, V. G., Hänfling, B., Leese, F., Mächler, E., Mahon, A. R., Meissner, K., Panksep, K., Pawlowski, J., Luis, P., Yáñez, S., Seymour, M., Thalinger, B., Valentini, A., Woodcock, P., Traugott, M., Vasselon, V. & Deiner, K. (2021), A practical guide to DNA-based methods for biodiversity assessment, Vol. 1, Pensoft Publishers. Publication Title: Advanced Books. URL: <https://ab.pensoft.net/article/68634/>

Goldberg, C. & Strickler, K. (2017), eDNA PROTOCOL SAMPLE COLLECTION, Technical report, Washington State University.

Laramie, M.B., Pilliod, D.S., Goldberg, C.S., and Strickler, K.M., 2015, Environmental DNA sampling protocol- Filtering water to capture DNA from aquatic organisms: U.S. Geological Survey Techniques and Methods, book 2, chap. A13, 15 p., <http://dx.doi.org/10.3133/tm2A13>.