APR 01, 2024

Protocol Citation: Anya Kardailsky, Ryan East 2024. eDNA sample collection method comparison. **protocols.io** https://protocols.io/view/edna-sample-collection-method-comparison-dbib2kan

Protocol status: In development
We are still developing and optimizing this protocol

Created: Mar 30, 2024

Last Modified: Apr 01, 2024

PROTOCOL integer ID: 97571

eDNA sample collection method comparison

Anya Kardailsky[1], Ryan East[1]

[1]Minderoo Foundation

Anya Kardailsky
Minderoo Foundation

ABSTRACT

There is an urgent global need for novel approaches to monitor our freshwater ecosystems. Current biomonitoring tools are time-consuming, costly, and dependent on taxonomic specialists. Therefore, traditional methods are falling short of fulfilling the growing public and policy obligations to monitor these vital, and fragile, ecosystems. Molecular approaches have the potential to revolutionize freshwater biomonitoring but are limited by incomplete reference databases. Furthermore, method development and testing has been lacking for benthic macro-invertebrate and fish environmental DNA (eDNA) surveys. A comparison of eDNA collection and extraction methods is initiated.

PROTOCOL REFERENCES

Elbrecht, V., & Leese, F. (2017). Validation and Development of COI Metabarcoding Primers for Freshwater Macroinvertebrate Bioassessment. *Frontiers in Environmental Science*, *5*(APR), 11. https://doi.org/10.3389/fenvs.2017.00011

Jeunen G-J, Knapp M, Spencer HG, et al. Species-level biodiversity assessment using marine environmental DNA metabarcoding requires protocol optimization and standardization. *Ecol Evol*. 2019; 9: 1323–1335. https://doi.org/10.1002/ece3.4843

Jeunen, G. J., von Ammon, U., Cross, H., Ferreira, S., Lamare, M., Day, R., Treece, J., Pochon, X., Zaiko, A., Gemmell, N. J., & Stanton, J. A. L. (2022). Moving environmental DNA (eDNA) technologies from benchtop to the field using passive sampling and PDQeX extraction. *Environmental DNA*, *4*(6), 1420–1433. https://doi.org/10.1002/EDN3.356

Leray, M., Yang, J.Y., Meyer, C.P. *et al.* A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: application for characterizing coral reef fish gut contents. *Front Zool* **10**, 34 (2013). https://doi.org/10.1186/1742-9994-10-34

Olmedo-Rojas P, Jeunen G-J, Lamare M, et al. Soil environmental DNA metabarcoding in low-biomass regions requires protocol optimization: a case study in Antarctica. *Antarctic Science*. 2023;35(1):15-30. doi:10.1017/S0954102022000384

Spens, J., Evans, A.R., Halfmaerten, D., Knudsen, S.W., Sengupta, M.E., Mak, S.S.T., Sigsgaard, E.E. and Hellström, M. (2017), Comparison of capture and storage methods for aqueous macrobial eDNA using an optimized extraction protocol: advantage of enclosed filter. Methods Ecol Evol, 8: 635-645. https://doi.org/10.1111/2041-210X.12683

Sample collection and preservation

1 Prior to sample collection, all equipment possible was sterilized using a 10-min exposure to 10% bleach solution and then rinsed with ultrapure water (UltraPure™ DNase/RNase-Free Distilled Water, Invitrogen™) twice to remove any DNA contamination.
Making sure to not handle any equipment involved with sampling without gloves.
At each site for each sampling method collect 5 samples at each site. For water it is prefereable that all the water is collected at once in one bucket.

1.1 Water samples must be collected first to minimise sediment in the water column which will clog the filters

1.2 We then added negative filtration controls by filtering 500 mL ultrapure water and extracted two extraction blanks consisting of 500 µL ultrapure water. We filtered and extracted all negative controls alongside the samples. All laboratory work prior to amplification was performed in a PCR-free designated clean room.

2 Wilderlab sampling kits with 0.45µm cellulose nitrate filter columns https://www.wilderlab.co.nz/order

2.1 Push one litre of water from the bucket through the filter using the provided collection tubes and record if the pores become too clogged to filter an entire litre.
If there are not enough collection tubes, 2ml sterivex collection tubes are also fine.

2.2 Once one litre of water is filtered reset the collection tube with no water inside and push air through the filter.

2.3 Add the provided storage buffer to the filter and cap both ends of the filter casing. Immediately label the tubes and place into a chilly bin for travel before storage in -4°C

2.4 Repeat steps 4 times to have 5 replicates at each site

3 Sterivex-GP capsule 0.22µm filters (Sterivex)

3.1 Push one litre of water from the bucket through the filter using the provided collection tubes and record if the pores become too clogged to filter an entire litre.

3.2 Once one litre of water is filtered reset the collection tube with no water inside and push air through the filter.

3.3 Add Longmire buffer to preserve the DNA in the filter and cap and parafilm the ends of the filter casing. Immediately label the tubes and place into a chilly bin for travel before storage in -4°C

3.4 Repeat steps 4 times to have 5 replicates at each site

4 Vacuum filtration 1.0µm cellulose nitrate filters by a vacuum pump

4.1 Pump one litre of water from the bucket through the filter using an active filtration pump and record if the pores become too clogged to filter an entire litre.

4.2 Once one litre of water is filtered, filters are rolled up using decontaminated tweezers, cut into ~1 mm slices and placed in 2 mL Eppendorf tubes.
Immediately label the tubes and place into a chilly bin for travel before storage in -4°C

4.3 Repeat steps 4 times to have 5 replicates at each site

5 Only once all water samples have been collected can sediment be then collected to avoid clogging of the filters due to sediment in the water column.

6 Sediment samples were collected at the same sites as water samples

6.1 Use 50ml falcon tubes to collect 3 samples of sediment at each site.

6.2 Immediately label the tubes and place into a chilly bin for travel before storage in -4°C.

7 Whirl-Pak® Speci-Sponge® were deployed at the same sampling sites and left overnight

7.1 Using decontaminated chicken wire mesh the sponges were attached using zip ties and deployed at the sites so they were completely submerged and held down with rocks overnight.

7.2 These were then collected, and all samples were kept at -4°C until extraction.

8 Physico-chemical water tests were done using YSI Pro2030 Dissolved Oxygen and Conductivity Meter and collecting 50ml falcon tubes for nitrate/nitrite analysis.

DNA extractions water samples all done using the Qiagen DNeasy Blood &…

9 Wilderlab samples and negative controls extracted as in commerial supplier's instructions

9.1 Use syringe to take out storage buffer from the inlet (don’t push the liquid through the outlet!).

9.2 Transfer buffer to a 1.5 mL Eppendorf tube.

9.3 Add 180 µL ATL buffer and 20 µL Proteinase K.

9.4 Vortex at maximum speed for 5 seconds.

9.5 Pulse-spin to bring down all liquid to bottom of tube.

9.6 Incubate samples at 56°C overnight.

9.7 Vortex at maximum speed for 5 seconds.

9.8 Pulse-spin to bring down all liquid to bottom of tube.

9.9 Add 200 µL AL buffer.

9.10 Vortex at maximum speed for 15 seconds

9.11 Pulse-spin to bring down all liquid to bottom of tube.

9.12 Add 200 µL 100% ethanol (best to use ethanol stored at -20°C).

9.13 Vortex at maximum speed for 15 seconds.

9.14 Pipet the mixture into a DNeasy Mini spin column placed in a 2 mL collection tube.

9.15 Centrifuge at 6,000 x g for 1 minute.

9.16 Discard the flow-through and collection tube.

9.17 Place the spin column in a new 2 mL collection tube.

9.18 Add 500 µL Buffer AW1.

9.19 Centrifuge samples for 1 minute at 6,000 x g.

9.20 Discard the flow-through and collection tube.

9.21 Place the spin column in a new 2 mL collection tube.

9.22 Add 500 µL Buffer AW2.

9.23 Centrifuge samples for 3 minutes at 20,000 x g.

9.24 Discard the flow-through and collection tube.

9.25 Place the spin column in a new 2 mL collection tube.

9.26 Centrifuge samples for 1 minute at 20,000 x g.

9.27 Discard the flow-through and collection tube.

9.28 Transfer the spin column in a 1.5 mL Eppendorf tube with caps removed.

9.29 Add 200 µL AE buffer to the center of the membrane (preheat AE buffer to 56°C).

9.30 Incubate for 1 minute at room temperature.

9.31 Centrifuge samples for 1 minute at 6,000 x g.

9.32 Transfer eluate to a new 1.5 mL Eppendorf tube.

9.33 Store DNA at -20°C.

10 Sterivex samples and negative controls extracted using the protocol developed by Spens et al. (2017)

10.1 Use 5 mL syringe to take out Longmire's buffer from the inlet (don't push the liquid through the outlet!).

10.2 Transfer buffer to a 1.5 mL or 2 mL Eppendorf tube.

10.3 Spin buffer at 6,000 x g for 30-45 minutes.

10.4 Discard supernatant (be careful not to disturb the pellet!)

10.5 Add 180 µL ATL buffer and 20 µL Proteinase K.

10.6 Vortex at maximum speed for 5 seconds.

10.7 Pulse-spin to bring down all liquid to bottom of tube.

10.8 Incubate samples at 56°C overnight.

10.9 Vortex at maximum speed for 5 seconds.

10.10 Pulse-spin to bring down all liquid to bottom of tube.

10.11 Add 200 µL AL buffer.

10.12 Vortex at maximum speed for 15 seconds

10.13 Pulse-spin to bring down all liquid to bottom of tube.

10.14 Add 200 µL 100% ethanol (best to use ethanol stored at -20°C).

10.15 Vortex at maximum speed for 15 seconds.

10.16 Pipet the mixture into a DNeasy Mini spin column placed in a 2 mL collection tube.

10.17 Centrifuge at 6,000 x g for 1 minute.

10.18 Discard the flow-through and collection tube.

10.19 Place the spin column in a new 2 mL collection tube.

10.20 Add 500 µL Buffer AW1.

10.21 Centrifuge samples for 1 minute at 6,000 x g.

10.22 Discard the flow-through and collection tube.

10.23 Place the spin column in a new 2 mL collection tube.

10.24 Add 500 µL Buffer AW2.

10.25 Centrifuge samples for 3 minutes at 20,000 x g.

10.26 Discard the flow-through and collection tube.

10.27 Place the spin column in a new 2 mL collection tube.

10.28 Centrifuge samples for 1 minute at 20,000 x g.

10.29 Discard the flow-through and collection tube.

10.30 Transfer the spin column in a 1.5 mL Eppendorf tube with caps removed.

10.31 Add 200 µL AE buffer to the center of the membrane (preheat AE buffer to 56°C).

10.32 Incubate for 1 minute at room temperature.

10.33 Centrifuge samples for 1 minute at 6,000 x g.

10.34 Transfer eluate to a new 1.5 mL Eppendorf tube.

10.35 Store DNA at -20°C.

11 Vacuum filtration samples and negative controls were extracted as in Jeunen et al. (2019)

11.1 Add 0.5 g of 0.5 mm Zirconia/Silica Beads to each 2ml tube from collection.

11.2 Add 720 µL ATL buffer and 80 µL Proteinase K.

11.3 Vortex at maximum speed for 10 minutes.

11.4 Incubate samples at 56°C overnight.

11.5 Vortex at maximum speed for 1 minute.

11.6 Centrifuge samples at 6,000 x g for 1 minute.

11.7 Transfer supernatant (600 µL) into a new 2 mL Eppendorf tube.

11.8 Add 600 µL AL buffer.

11.9 Add 600 µL 100% ethanol.

11.10 Vortex at maximum speed for 1 minute.

11.11 Pipet the mixture (620 µL) into a DNeasy Mini spin column placed in a 2 mL collection tube.

11.12 Centrifuge at 6,000 x g for 1 minute.

11.13 Discard the flow-through and collection tube.

11.14 Place the spin column in a new 2 mL collection tube.

11.15 Add 500 µL Buffer AW1.

11.16 Centrifuge samples for 1 minute at 6,000 x g.

11.17 Discard the flow-through and collection tube.

11.18 Place the spin column in a new 2 mL collection tube.

11.19 Add 500 µL Buffer AW2.

11.20 Centrifuge samples for 3 minutes at 20,000 x g.

11.21 Discard the flow-through and collection tube.

11.22 Place the spin column in a new 2 mL collection tube.

11.23 Centrifuge samples for 1 minute at 20,000 x g.

11.24 Discard the flow-through and collection tube.

11.25 Transfer the spin column in a 1.5 mL Eppendorf tube with caps removed.

11.26 Add 200 µL AE buffer to the center of the membrane (preheat AE buffer to 56°C).

11.27 Incubate for 1 minute at room temperature.

11.28 Centrifuge samples for 1 minute at 6,000 x g.

11.29 Transfer eluate to a new 1.5 mL Eppendorf tube.

11.30 Store DNA at -20°C.

DNA extractions sediment samples done using the PowerSoil® DNA Isolat…

12 The sediment samples were extracted using a modified extraction protocol without saturated phosphate buffer developed by Olmedo-Rojas et al. (2023)

12.1 Add 0.25 grams of soil sample to a PowerBead Tube

12.2 Vortex for 2 sec

12.3 Add 60 µL of solution C1 (if solution C1 is precipitated, heat solution to 60°C until dissolved before use)

12.4 Vortex in a horizontal position at maximum speed for 10 mins

12.5 Centrifuge at 10,000 x g for 30 s at ambient temperature

12.6 Transfer the supernatant to a clean 2 ml Collection tube

12.7 Add 250 µL of solution C2 and vortex for 5 s

12.8 Incubate at 4°C for 5 mins

12.9 Centrifuge at room temperature for 1 min at 10,000 x g

12.10 Transfer up to, but no more than, 600 µL of supernatant to a 2 ml Collection tube

12.11 Add 200 µL of Solution C3

12.12 Vortex

12.13 Incubate at 4°C for 5 mins

12.14 Centrifuge at room temperature for 1 minute at 10,000 x g

12.15 Transfer up to, but no more than, 750 µL of supernatant to a 2 ml Collection tube

12.16 Add 1200 µL of Solution C4 to the supernatant (shake Solution C4 before use)

12.17 Vortex for 5 mins

12.18 Load approximately 675 µL onto a Spin Filter

12.19 Centrifuge at 10,000 x g for 1 min at room temperature

12.20 Discard the flow-through

12.21 Add 675 µL of supernatant to the Spin Filter

12.22 Centrifuge at 10,000 x g for 1 min at room temperature

12.23 Load the remaining supernatant onto the Spin Filter

12.24 Centrifuge at 10,000 x g for 1 min at room temperature

12.25 Add 500 µL of Solution C5

12.26 Centrifuge at room temperature for 30 s at 10,000 x g

12.27 Discard the flow-through

12.28 Centrifuge at room temperature for 1 min at 10,000 x g

12.29 Place the Spin Filter in a 2 ml Collection Tube (avoid splashing Solution C5 onto the Spin Filter)

12.30 Add 100 µL of Solution C6 to the centre of the white filter membrane of the Spin Filter tube

12.31 Centrifuge at room temperature for 30 s at 10,000 x g

12.32 Discard the Spin Filter

12.33 Store DNA at -20° to -80°C

DNA extractions sponge samples

13 The extraction protocol for sponges has not been optimised and so two different extraction techniques were investigated. For each of the extractions the sponges were first cut into ten pieces that would each fit into 2ml Eppendorf tubes and then further cut into 3 pieces while in the tube.

14 For the first extraction protocol the tubes were filled with longmire buffer and left to defrost and sit for at least 3 hours.

14.1 The longmire buffer was then transferred to a new tube, squeezing out the liquid as much as possible

14.2 Transfer buffer to a 1.5 mL or 2 mL Eppendorf tube.

14.3 Spin buffer at 6,000 x g for 30-45 minutes.

14.4 Discard supernatant (be careful not to disturb the pellet!)

14.5 Add 180 µL ATL buffer and 20 µL Proteinase K.

14.6 Vortex at maximum speed for 5 seconds.

14.7 Pulse-spin to bring down all liquid to bottom of tube.

14.8 Incubate samples at 56°C overnight.

14.9 Vortex at maximum speed for 5 seconds.

14.10 Pulse-spin to bring down all liquid to bottom of tube.

14.11 Add 200 µL AL buffer.

14.12 Vortex at maximum speed for 15 seconds

14.13 Pulse-spin to bring down all liquid to bottom of tube.

14.14 Add 200 µL 100% ethanol (best to use ethanol stored at -20°C).

14.15 Vortex at maximum speed for 15 seconds.

14.16 Pipet the mixture into a DNeasy Mini spin column placed in a 2 mL collection tube.

14.17 Centrifuge at 6,000 x g for 1 minute.

14.18 Discard the flow-through and collection tube.

14.19 Place the spin column in a new 2 mL collection tube.

14.20 Add 500 µL Buffer AW1.

14.21 Centrifuge samples for 1 minute at 6,000 x g.

14.22 Discard the flow-through and collection tube.

14.23 Place the spin column in a new 2 mL collection tube.

14.24 Add 500 µL Buffer AW2.

14.25 Centrifuge samples for 3 minutes at 20,000 x g.

14.26 Discard the flow-through and collection tube.

14.27 Place the spin column in a new 2 mL collection tube.

14.28 Centrifuge samples for 1 minute at 20,000 x g.

14.29 Discard the flow-through and collection tube.

14.30 Transfer the spin column in a 1.5 mL Eppendorf tube with caps removed.

14.31 Add 200 µL AE buffer to the center of the membrane (preheat AE buffer to 56°C).

14.32 Incubate for 1 minute at room temperature.

14.33 Centrifuge samples for 1 minute at 6,000 x g.

14.34 Transfer eluate to a new 1.5 mL Eppendorf tube.

14.35 Store DNA at -20°C.

15 For the second extraction protocol the tubes were left to defrost and the water was removed from the tubes using pipettes leaving the sponge in the tube before continuing with the extract protocol

15.1 Add 0.5 g of 0.5 mm Zirconia/Silica Beads.

15.2 Add 720 µL ATL buffer and 80 µL Proteinase K.

15.3 Vortex at maximum speed for 10 minutes.

15.4 Incubate samples at 56°C overnight.

15.5 Vortex at maximum speed for 1 minute.

15.6 Centrifuge samples at 6,000 x g for 1 minute.

15.7 Transfer supernatant (600 µL) into a new 2 mL Eppendorf tube.

15.8 Add 600 µL AL buffer.

15.9 Add 600 µL 100% ethanol.

15.10 Vortex at maximum speed for 1 minute.

15.11 Pipet the mixture (620 µL) into a DNeasy Mini spin column placed in a 2 mL collection tube.

15.12 Centrifuge at 6,000 x g for 1 minute.

15.13 Discard the flow-through and collection tube.

15.14 Place the spin column in a new 2 mL collection tube.

15.15 Add 500 µL Buffer AW1.

15.16 Centrifuge samples for 1 minute at 6,000 x g.

15.17 Discard the flow-through and collection tube.

15.18 Place the spin column in a new 2 mL collection tube.

15.19 Add 500 µL Buffer AW2.

15.20 Centrifuge samples for 3 minutes at 20,000 x g.

15.21 Discard the flow-through and collection tube.

15.22 Place the spin column in a new 2 mL collection tube.

15.23 Centrifuge samples for 1 minute at 20,000 x g.

15.24 Discard the flow-through and collection tube.

15.25 Transfer the spin column in a 1.5 mL Eppendorf tube with caps removed.

15.26 Add 200 µL AE buffer to the center of the membrane (preheat AE buffer to 56°C).

15.27 Incubate for 1 minute at room temperature.

15.28 Centrifuge samples for 1 minute at 6,000 x g.

15.29 Transfer eluate to a new 1.5 mL Eppendorf tube.

15.30 Store DNA at -20°C.

qPCR

16 Metabarcoding library preparation followed the protocol described in Jeunen et al. (2022). Using universal primers developed by Leray et al. (2013) a qPCR of a dilution series of neat, 10-fold and 100-fold dilution of the input DNA in duplicate must be carried out to ensure adequate amplification and correct for potential PCR inhibitors.

	A	B
	Component	**Vol/15µl reaction**
	Buffer	12.5 mL mastermix (SensiMix SYBR Lo-ROX Kit – Meridian

A	B
	Bioscience)
Forward primer	1 mL
Reverse primer	1 mL
Water	8.5 mL
DNA	2 mL

A
PCR programme
1. *95ºC – 10 mins* 2. *95ºC – 30 secs* 3. *50ºC – 30 secs50ºC – 30 secs* 4. *72ºC – 150 secs* 5. *65ºC – 5 mins 45 secs* 6. *Back to step 2 x50 cycles in total*

Invertebrate metabarcoding sequencing

17 Once optimal DNA concentrations are found. Samples can be sequenced using invertebrate specific primers the BF1 and BR2 primers developed by Elbrecht et al. (2017).
Similar to the protocol detailed in Jeunen et al. (2022), a one-step amplification protocol was conducted to generate the amplicons for library preparation using fusion primers containing a modified Illumina sequencing adapter, a barcode tag (6 bp in length), and the template-specific primer.

Barcode sequences were selected with at least three base pair differences to minimize issues with barcode hopping. Each sample was amplified in duplicate and assigned a unique barcode combination to allow for pooling of samples.

17.1 Pool samples into minipools of similar concentrations with the negative controls in a separate minipool.
Measure the DNA concentration of each pool with a high sensitivity qubit assay.
Pool the minipools into one pool at an equimolar concentration. Except the negatve controls which just had 1ul added to the final pool.

17.2 Measure the DNA concentration of the final library pool with a high sensitivity qubit assay.
Dilute the final library to sequencing specifications.

17.3 Sequence!!!

Fish metabarcoding sequencing

18