SAMPLING PROTOCOL
STANDARD AQUATIC eDNA KIT

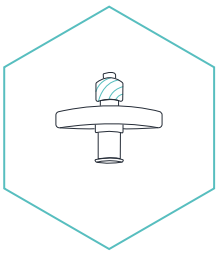
USING THE KIT

KIT CONTENTS

Kit contents are based on one person collecting an eDNA sample. If two or more people will be handling kit contents and collecting an eDNA sample, please contact us about inclusion of extra gloves in kits. Extra gloves are not provided as standard to minimise waste.

1 x pair nitrile gloves

1 x 3.5 L sampling bag
1 x specimen bag



1 x enclosed filter (0.8 µm pore size, polyether-sulfone)

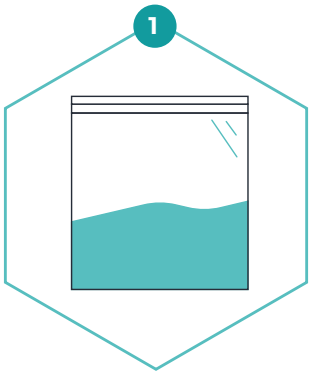
1 x 100 mL Luer Lock plastic syringe

1 x sampling datasheet

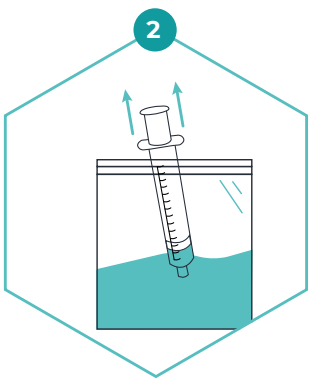
1 x resealable bag containing: 1 x small syringe filled with 1.5 mL of DNA preservative solution (sealed with reusable Luer Lock cap) and 1 x separate Luer Lock cap

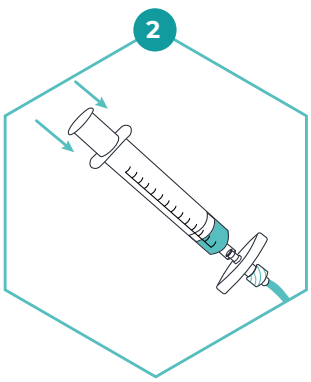
EXPERIENCED USER PROTOCOL

1. Put on the provided gloves. Use the provided sampling bag or a new mineral water bottle (with the water discarded) to collect your (sub)samples from the waterbody (see **Appendix**). Minimise contact with the water during collection. Deposit the (sub)sample in the sampling bag, seal, and make sure the water is well mixed by shaking for 20-30 seconds. You should use a new bag/bottle for each sample (i.e. each kit) and for each waterbody to avoid cross-contamination.



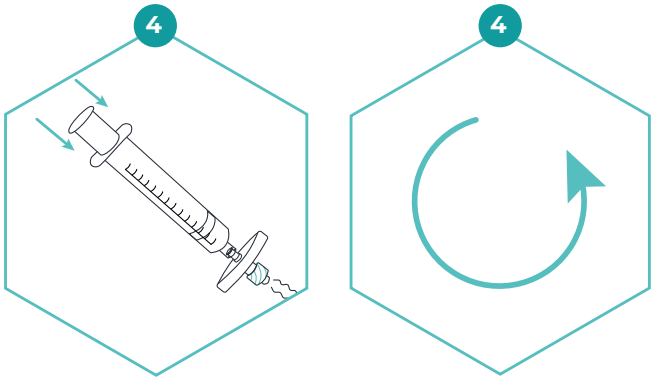
3. Draw up 100 mL of water from the sampling bag into the large syringe. Attach the syringe to the filter inlet (narrow end). Press the plunger to push the water through the filter.





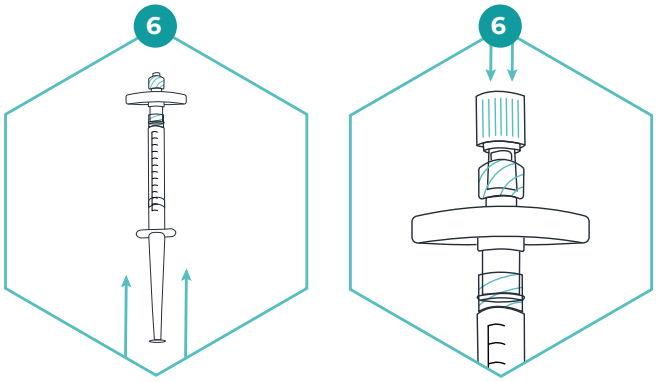
3. **Repeat steps 1 and 2 until all water has been filtered or the filter clogs**. Make a note on the sampling datasheet of the total volume processed.

4. Detach the syringe from the filter, hold the filter in one hand, and pull back the plunger to fill the syringe with air. Reattach the filter and push the air through to expel any water trapped inside the filter. **Repeat several times** to remove as much water as possible.

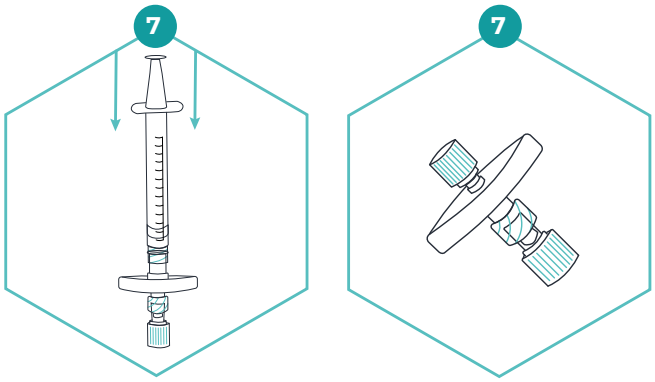


5. Uncap the small syringe (already filled with preservative solution) and twist it onto the filter inlet. **Do not discard the Luer Lock cap** - hold the cap in one hand as it will be needed in step 6.

6. Hold the filter so that the outlet (wide end) points upwards. **Carefully and slowly** press the plunger to push the preservative solution into the filter. Stop when the first drop can be seen emerging from the filter outlet, but **do not remove the small syringe** with preservative solution. Cap the filter outlet using the Luer Lock cap that was on the small syringe in step 5.



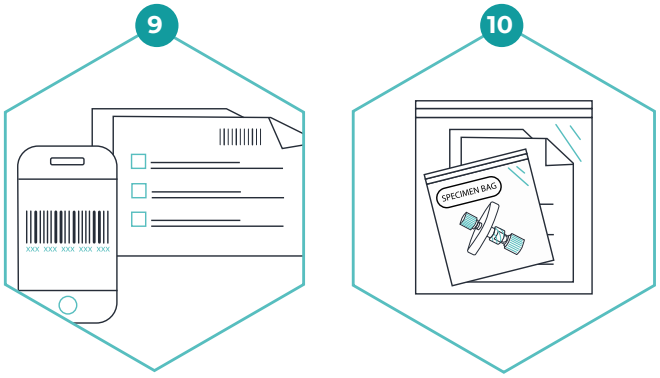
7. Invert the filter so that the filter outlet points down, and **slowly** press the plunger to expel the rest of the preservative solution. The entire volume of preservative solution should be added to the filter and the **small syringe should be empty**. Detach the small syringe whilst keeping the plunger depressed and cap the filter inlet with the separate Luer Lock cap.



8. Place the filter inside the specimen bag and seal. If storing samples at ambient temperatures, samples should be received by the lab **within 2 weeks of sampling**. If this is not possible, we recommend that samples are **frozen as soon as possible after sampling** to enhance DNA preservation until samples are sent back to us. Cold shipping is not necessary for frozen samples. **Please contact NatureMetrics if return within 2 weeks or long-term freezing is not possible**.

9. Complete the sampling datasheet and use the NatureMetrics sampling app to scan the barcode on the sampling datasheet to complete your online submission.

10. Place the bagged filter and sampling datasheet in the bag that the kit contents arrived in. For UK sample returns, simply use the standard postal service with recorded delivery. Follow the packaging notes and send to: **Nature Metrics, 1 Occam Court, Surrey Research Park, Guildford, GU2 7HJ, United Kingdom**. For international sample returns, contact your NatureMetrics Business Development Manager or our Logistics team (**logistics@naturemetrics.co.uk**) for further information. **We are only able to analyse samples that are returned using the correct NatureMetrics logistics procedure**.



DETAILED PROTOCOL

PART 1: COLLECTING THE WATER

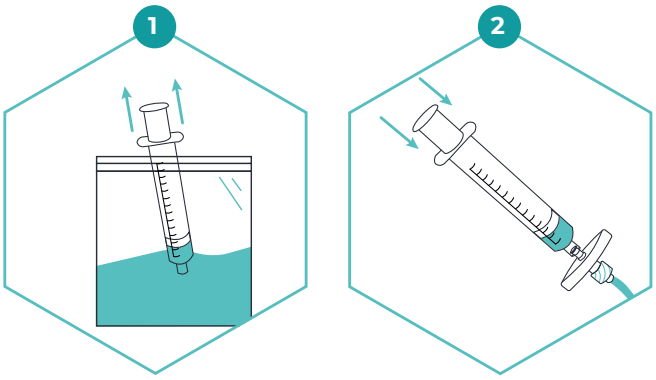
Ideal sampling strategies will vary among habitats and monitoring contexts. We recommend that you speak to a member of our team to plan your survey. We also recommend the inclusion of **field negative controls (blanks)** to detect possible contamination introduced during sampling. NatureMetrics provide a free kit to be used as a field blank if 20 or more Standard Aquatic eDNA Kits are purchased.

Download and log into the NatureMetrics sampling app before arriving in the field. Avoid introducing DNA (from yourself, soil or other waterbodies) into the water and prevent spread of disease or invasive species by wearing the provided gloves and minimising contact with the water.

PART 2: FILTERING THE WATER

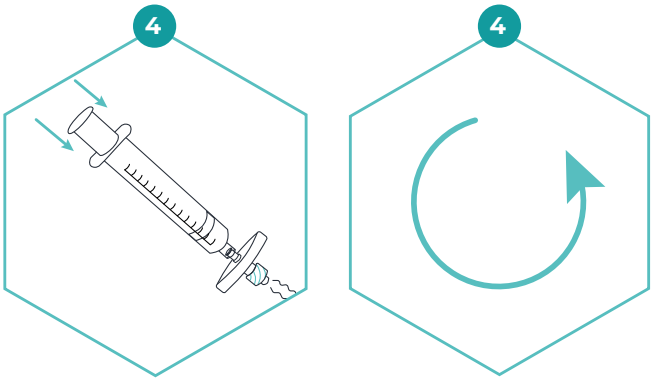
1. Draw up 100 mL of water from the sampling bag into the large syringe. **If there is a high sediment load, then leave the sample for a minute to allow the sediment to settle before drawing water up into the syringe. This should increase the amount of water that you can filter.**

2. Attach the syringe to the filter inlet (narrow end). **The syringe should easily twist onto the inlet side of the filter.** Press the plunger to push the water through the filter.



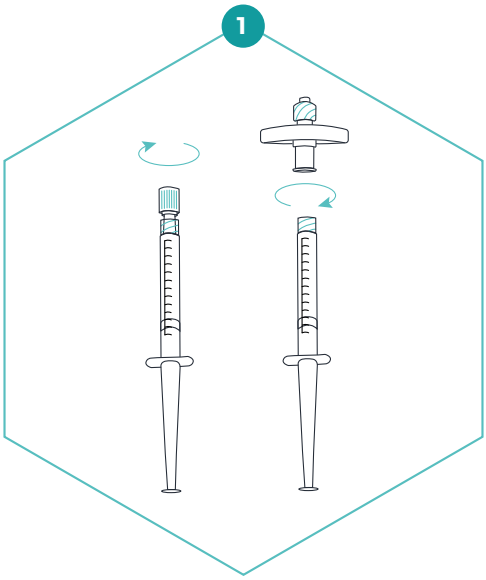
3. **Repeat steps 1 and 2** (holding the filter in one hand while it is not attached to the syringe) until all water has been filtered or the filter clogs. **Keep track of the number of times the syringe is refilled**. Make a note on the sampling datasheet of the total volume processed.

4. Detach the syringe from the filter, hold the filter in one hand, and pull back the plunger to fill the syringe with air. Reattach the filter and push the air through to expel any water trapped inside the filter. **Repeat several times** to remove as much water as possible. **It helps to gently shake the filter as you do this**.

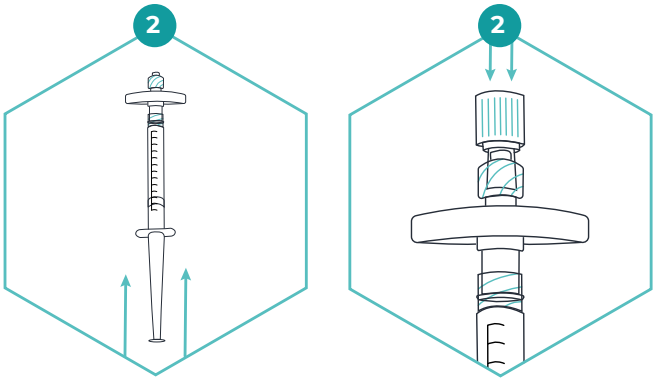


PART 3: PRESERVING THE DNA

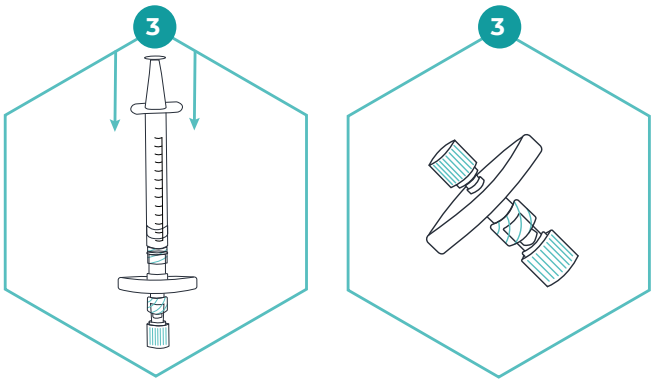
1. Uncap the small syringe (already filled with preservative solution) and twist it on to the filter inlet. **Do not discard the Luer Lock cap** – hold the cap in one hand as it will be needed in step 2. **The solution is non-hazardous to aquatic life but release into the environment should be avoided**. **We advise performing this step away from the water's edge and avoiding spills**.



2. Hold the filter so that the outlet (wide end) points upwards. **We advise you to do this step away from anyone's eyes**. **Carefully and slowly** press the plunger to push the preservative solution into the filter. This must be done slowly to allow the preservative solution to spread out over the filter surface. Stop when the first drop can be seen emerging from the filter outlet, but **do not remove the small syringe** with preservative solution. Cap the filter outlet using the Luer Lock cap that was on the small syringe in step 1.



3. Invert the filter so that the filter outlet points down, and **slowly** press the plunger to expel the rest of the preservative solution. The entire volume of preservative solution should be added to the filter and **the small syringe should be empty**. Detach the small syringe whilst keeping the plunger depressed and cap the filter inlet with the separate Luer Lock cap. **The preservative solution contains a detergent – don't be alarmed if it foams a little!**



4. Place the filter inside the specimen bag and seal. If storing samples at ambient temperatures, samples should be received by the lab **within 2 weeks of sampling**. If this is not possible, we recommend that samples are **frozen as soon as possible after sampling** to enhance DNA preservation until samples are sent back to us. Cold shipping is not necessary for frozen samples. It should be noted that although freezing is recommended as best practice for optimal storage, we understand that it is not always feasible. Storing at ambient temperatures is a tried and tested method that yields excellent data. The addition of freezing is to further safeguard the samples against potential DNA degradation, which can affect species detection when samples are stored at high temperatures for long periods. **Please contact NatureMetrics if return within 2 weeks or long-term freezing is not possible**.

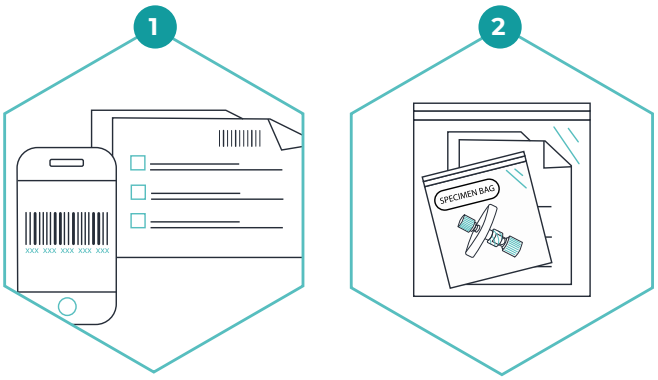
You can view a video demonstration of filtration with the Standard Aquatic eDNA Kit at: **https://www.naturemetrics.co.uk/sampling-protocol-video-guides/#aquaticednavideo**.

PART 4: RETURNING YOUR SAMPLES

Once you have collected and filtered your sample, follow our guidelines below to safely transport your sample to the NatureMetrics laboratory.

1. Complete the sampling datasheet and use the NatureMetrics sampling app to scan the barcode on the sampling datasheet to complete your online submission. **Please record your sampling data on paper and through the NatureMetrics sampling app so there is a physical and electronic copy of your data**.

2. Place the bagged filter and sampling datasheet inside the bag that the kit contents arrived in.



3. For UK sample returns, simply use the standard postal service with recorded delivery. Follow the packaging notes and send to: **Nature Metrics, 1 Occam Court, Surrey Research Park, Guildford, GU2 7HJ, United Kingdom**.

4. For international sample returns, contact your NatureMetrics Business Development Manager or our Logistics team (**logistics@naturemetrics.co.uk**) for further information. NatureMetrics is fully compliant with current legislation on the transport of biological material and our operations team are on hand to ensure that all return shipments meet the required specification. **We are only able to analyse samples that are returned using the correct NatureMetrics logistics procedure**.

PACKAGING NOTES AND QUICK LINKS

- Return your samples using the box that kits were sent in, where possible.
- If using an alternative box, ensure that it is in good condition and remove any old labels.
- Avoid excess spacing by adding cushioning material (e.g. bubble wrap, newspaper) to avoid the product shifting inside the box.
- Use good quality sealing tape and seal along box edges in a H pattern.
- The only essential thing to send back to us is the specimen bag containing the filter and the sampling datasheet. However, all kit contents can be sent back to NatureMetrics for recycling.
- Register for your my.naturemetrics account **here**.
- Please find our **Terms and Conditions here**.

CLIENT RESPONSIBILITIES

Important considerations for your sampling

- The absence of a detection does not necessarily imply the absence of a taxon from a location.
- Samples from some locations might have compounds that inhibit analyses resulting in reduced data generation – while we routinely test for inhibition, it is not always possible to overcome.
- It is the responsibility of the client to ensure that all efforts have been made to avoid contamination from external sources and between samples.
- Handling samples without gloves can increase the content of human DNA, reducing data generation.
- After sampling, movement of samples across borders without permission is not allowed as they are classed as biological samples.

Important considerations for interpreting your results

It should be noted that DNA can enter an ecosystem via many routes (e.g. wastewater from commercial and domestic sources). While DNA from a given taxon may be present and detectable, it is not possible to discern the source of the DNA. Results from common food items, domestic species and livestock species need to be interpreted with caution.

Disclaimer: Safe sample collection is the responsibility of the Client. NatureMetrics accepts no liability associated with the use of the kits and sample collection. The Client is solely responsible for the quality of the samples and the representativeness of the samples received by NatureMetrics. The information contained within the Final Report provided by NatureMetrics to the Client is not intended to be advisory, it is informational. Interpretation and decisions are the sole responsibility of the Client. NatureMetrics does not accept any liability whatsoever for any reliance placed on any information contained within, or any use that may be made of, the Final Report by the Client. Please read the full limitation of liability statement in the Terms and Conditions.

APPENDIX
SAMPLING STRATEGIES FOR AQUATIC ENVIRONMENTS

Ideal sampling strategies will vary among habitats and monitoring contexts. We recommend that you speak to a member of our team to plan your survey. However, below is some guidance synthesised from eDNA research to date.

PONDS

In a pond, eDNA does not mix well due to absence of flow or wave action, so multiple water samples are key to capture the eDNA present. A pond can be sampled for eDNA in two ways: **1) independent samples**, each comprised of subsamples (e.g. 2 L comprised of 5 x 400 mL subsamples), can be taken at multiple locations around the pond perimeter, or **2) subsamples** can be taken at multiple locations and pooled into a single composite sample (e.g. 2 L comprised of 20 x 100 mL subsamples) representing the entire pond.

Independent samples each passed through a separate filter will likely have higher detection rates as eDNA is more likely to be captured in at least one of the samples. Composite samples will reduce cost and allow more ponds to be sampled, but DNA from rare or low-density species may not be detected. Several composite samples may be needed for adequate eDNA representation from larger ponds.

When sampling ponds:

- Surface water (sub)samples should be collected from the shoreline at roughly equidistant intervals or targeting preferred habitat (if detection of a particular species is a priority) around the pond perimeter.
- Use the provided sampling bag or a clean bottle (we recommend a small mineral water bottle with the water discarded) to collect water. Deposit the collected (sub)sample in the provided sampling bag. Repeat for each subsample if applicable. Seal the bag and make sure the water is well mixed by shaking for 20-30 seconds. The bag is not self-standing, but can be propped against a log, tree stump or rock to stabilise it for filtration.
- You should use a new bag/bottle for independent samples and for each pond to avoid cross-contamination.
- Depending on water clarity, it may be possible to pass up to 2 L of pond water through each filter used, but smaller volumes (e.g. 150-250 mL) are more typical for turbid ponds or ponds with dense vegetation.

LAKES

In a lake, eDNA can still be localised, so multiple water samples remain key to capture the eDNA present. We recommend **at least 10 independent samples**, each comprised of subsamples (e.g. 2 L comprised of 5 x 400 mL subsamples), are taken from multiple locations around the lake perimeter. You should consider collecting one independent sample (i.e. using 1 kit) along 400 m of shoreline, but contact NatureMetrics to discuss alternative sampling strategies to suit all budgets.

When sampling lakes:

- Surface water samples should be collected from the shoreline at roughly equidistant intervals around the lake perimeter.
- Use the provided sampling bag or a clean bottle (we recommend a 2 L mineral water bottle with the water discarded) to scoop up water every 20-50 m along a 400 m stretch of shoreline. Deposit the collected subsample in the provided sampling bag. Repeat for each subsample, seal the bag, and make sure the water is well mixed by shaking the bag for 20-30 seconds. The bag is not self-standing, but can be propped against a log, tree stump or rock to stabilise it for filtration.

- Depending on water clarity, it may be possible to pass up to 5 L of lake water through each filter used, but smaller volumes (e.g. 2 L) are more typical.
- Sampling when the lake is not thermally stratified is ideal as more mixing of the water will occur. This means there is a higher chance of detecting eDNA from shallow and deep-water aquatic species. However, detection of invertebrates and some other taxonomic groups is generally lower in colder temperatures. In some cases, it is advisable to sample by boat and at varying depths to maximise detection rates. Please contact NatureMetrics to discuss your sampling plans.

RIVERS OR STREAMS

In a river or stream, eDNA can be well-mixed depending on local environmental conditions, but flow means that eDNA can be transported hundreds to thousands of metres from its source. As such, multiple sampling locations along the length of the stream/river are recommended. In small streams or rivers, **at least three sampling locations** (i.e. upstream, mid-section, downstream) should be identified. In larger rivers, **20-60 sampling locations** should be identified for comprehensive survey.

A **minimum of three independent samples** (e.g. 1 L), spanning the width of the stream/river section (e.g. left bank, centre, right bank) should be collected **from each sampling location**. More water samples at each sampling location are recommended for wider rivers, and possibly at downstream sampling locations if the river has a large catchment and/or is fast-flowing. If your budget does not allow for independent samples, subsamples (e.g. 3 x 1 L) can be taken at each sampling location, mixed, and as much of the pooled sample filtered as possible, but detection of rare or low-density species may be impeded.

When sampling rivers or streams:

- Start at the most downstream sampling location and work your way upstream.
- Use of a sampling vessel/device and sampling from the shoreline is recommended. If shoreline sampling is not possible, surveyors should enter the water downstream of where they will collect the sample and be careful not to disturb sediment as they move to the collection point. If the water is too deep to enter, a boat or similar should be used for sampling.
- Use the provided sampling bag or a clean bottle (we recommend a 2 L mineral water bottle with the water discarded) to collect water by holding it with the opening pointing upstream at the water surface. Stand downstream of the sampling bag/bottle to avoid collecting your own DNA. Deposit the collected sample in the provided sampling bag. Repeat for each sample, seal the bag, and make sure the water is well mixed by shaking for 20-30 seconds. The bag is not self-standing, but can be propped against a log, tree stump or rock to stabilise it for filtration.
- Depending on water clarity, it may be possible to pass up to 5 L of stream/river water through each filter used, but smaller volumes (e.g. 2 L) are more typical.

ESTUARIES, SEAS, OR OCEANS

In marine waterbodies, less is currently known about how hydrological systems affect eDNA transportation and distribution. However, it has been shown that communities obtained from marine eDNA metabarcoding are highly representative of the immediate local habitat where the sample was collected, both on horizontal and vertical planes. This means that when samples are collected in a transect going from shore to offshore, different communities will be detected, sometimes even within the range of tens of meters. As in lakes, vertical stratification of the water (as a result of thermoclines) restricts mixing of eDNA, meaning that water samples should be collected from each depth zone of interest to fully characterise the marine communities at the sampling location.

eDNA in marine systems is generally much more dilute compared to that in freshwater systems. This is in part dependent on the target group or species but is particularly pertinent for larger vertebrates (extra-organismal vertebrate eDNA). Planktonic or microbial taxa usually require smaller volumes. Therefore, sample volume should

be maximized to be representative of the environment and the taxa that are targeted. **Each sample should be at least 2 L volume**, and the volume of water filtered should be in the range of 2-5 L **(more is always better)**. Turbidity is usually less of a problem in marine water, although inshore areas (e.g. mangrove forests, marinas, areas with high population density) can become turbid due to coastal run-off and wave action disturbing the sea floor. In this case, filter as much water as possible from each sample until the filter completely clogs.

Sample number will depend on the spatial scale of the study or monitoring project. In order to characterize a community or compare sites, **a minimum of 20 samples**, even for relatively small areas, is strongly advised. This usually involves collecting independent samples (rather than subsamples) spread out across the sampling area.

Sampling design will equally be dependent on the size of the sampling area, and also on the type of ecosystem (coral reef, mangrove forest, pelagic, etc.) that is targeted. Season (and even time of day) may need to be taken into consideration, as many fish species move to (in)shore areas for mating and spawning and move to deeper or warmer waters in winter, while other species may prefer cold, deep water during summer. Thus, it is important to consider migration patterns as well as mating and spawning sites.

When sampling estuaries, seas, or oceans:

- Sampling is most often done from a boat, but samples may also be collected from the shoreline.
- Use the provided sampling bag or a Kemmerer/Niskin sampler to collect a minimum of 2 L of sea water per sample. Contact NatureMetrics if you do not have access to a Kemmerer/Niskin sampler.
- When using the Kemmerer/Niskin sampler to collect water near the bottom, be careful not to disturb the sediment as sediment present in water samples can clog filters and may contain 'old' eDNA that can skew inferences of species' presence.
- You can use a manual syringe to filter the water, but vacuum pumps are more commonly used for larger volume samples. Depending on water clarity, it may be possible to pass up to 5 L of lake water through each filter used, but smaller volumes (e.g. 2 L) are more typical.
- Sampling when the lake is not thermally stratified is ideal as more mixing of the water will occur. This means there is a higher chance of detecting eDNA from shallow and deep-water aquatic species. However, detection of invertebrates and some other taxonomic groups is generally lower in colder temperatures. In some cases, it is advisable to sample by boat and at varying depths to maximise detection rates. Please contact NatureMetrics to discuss your sampling plans.

CONTROLS

NatureMetrics recommend the inclusion of **field negative controls (blanks)** to detect possible contamination introduced during sampling. NatureMetrics Standard, MAXI and Pump Aquatic eDNA Kits contain only sterile, single-use components so no contamination is present before the kit is opened. However, contamination can be introduced by the surveyor as water samples are collected and filtered.

We recommend including **at least one field blank** at the end of each day that water sampling occurs, but ideally a field blank should be included for each waterbody sampled. NatureMetrics provide a free kit to be used as a field blank if 20 or more Standard, MAXI or Pump Aquatic eDNA Kits are purchased.

If you will be entering the water to collect samples, boots or waders should be decontaminated with bleach (containing sodium hypochlorite) in between waterbodies. Please contact NatureMetrics for advice on appropriate decontamination procedures.