Northern Australia Environmental Resources Hub

National Environmental Science Programme

Standard operating procedure for environmental DNA field sample collection

Report

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Cover photographs: Left: eDNA sampling in the Burdekin River catchment (photo: Department of Natural Resources, Mines and Energy Queensland). Right: eDNA sampling in the Barron River catchment (photo: Biosecurity Queensland).

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Acronyms & abbreviations

eDNA..... environmental DNA

NESP..... National Environmental Science Program

qPCR..... quantitative real-time polymerase chain reaction

TropWATER ... Centre for Tropical Water and Aquatic Ecosystem Research

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Executive summary

Northern Australia is a large and often remote area, yet faces numerous development and environmental pressures that may have destabilising effects on natural communities. In the face of these threats, there is an urgent need to better understand and monitor the distribution of key plant and animal species.

The environmental DNA (eDNA) technique represents a sensitive tool that allows for cost-effective monitoring of plant and animal species across large geographical areas. It is commonly used for species that occur at low abundance, such as threatened species or invasive species at an invasion front. Northern Australia is remote and sparsely populated, making it logistically difficult for scientists to conduct extensive fieldwork, which results in many species being under-monitored. Engaging with non-scientists (community members, Indigenous ranger groups and natural resource management organisations) to use eDNA methods can increase monitoring capability in remote areas by orders of magnitude.

Conventional eDNA sampling involves filtering large volumes of water, using lengthy procedures that can result in higher contamination risk and reluctance from non-scientists to carry out field sampling. It is therefore crucial to develop user-friendly field methods that need minimal equipment or training and allow for successful engagement with the community. This standard operating procedure provides a step-by-step guide to a simple eDNA water sample collection method developed for use by non-scientists.

1. Standard operating procedure

1.1 Purpose

The following protocol was developed for collecting and preserving whole water samples for environmental DNA (eDNA) detection of aquatic species.

1.2 Overview of water sampling for eDNA analysis

Environmental DNA sampling can detect animals or plants in soil or water, even when they occur in very low numbers (such as rare/threatened species or newly arrived invasive species). The protocol described here is focused on eDNA water samples. Contamination of samples with eDNA between sites or from other locations (false positives) is a real risk and can compromise sampling efforts. In order to prevent this, some general considerations need to be taken.

- Read the instructions and familiarise yourself with them prior to going to the field.
- Follow instructions and use the data sheets provided. A new datasheet should be used for each site visited.
- Use a new pair of gloves at each site.
- Make sure tubes/jars remain upright at all times.
- Do not handle any of the kit materials without wearing gloves.

Two sampling procedures are used for eDNA analysis. The first one involves small water volume collection in a Falcon tube of 50 mL capacity (Figure 1.1a) and it is used when sampling small creeks or waterholes. The second procedure involves large water volume collection in a jar of 450 mL capacity (Figure 1.1b) and it is recommended when sampling rivers or other large waterbodies (lakes, dams, estuaries, etc). Both sampling strategies have shown to provide reliable results for detection of invasive and threatened species (Villacorta-Rath et al., 2020; Villacorta-Rath, et al., 2021).

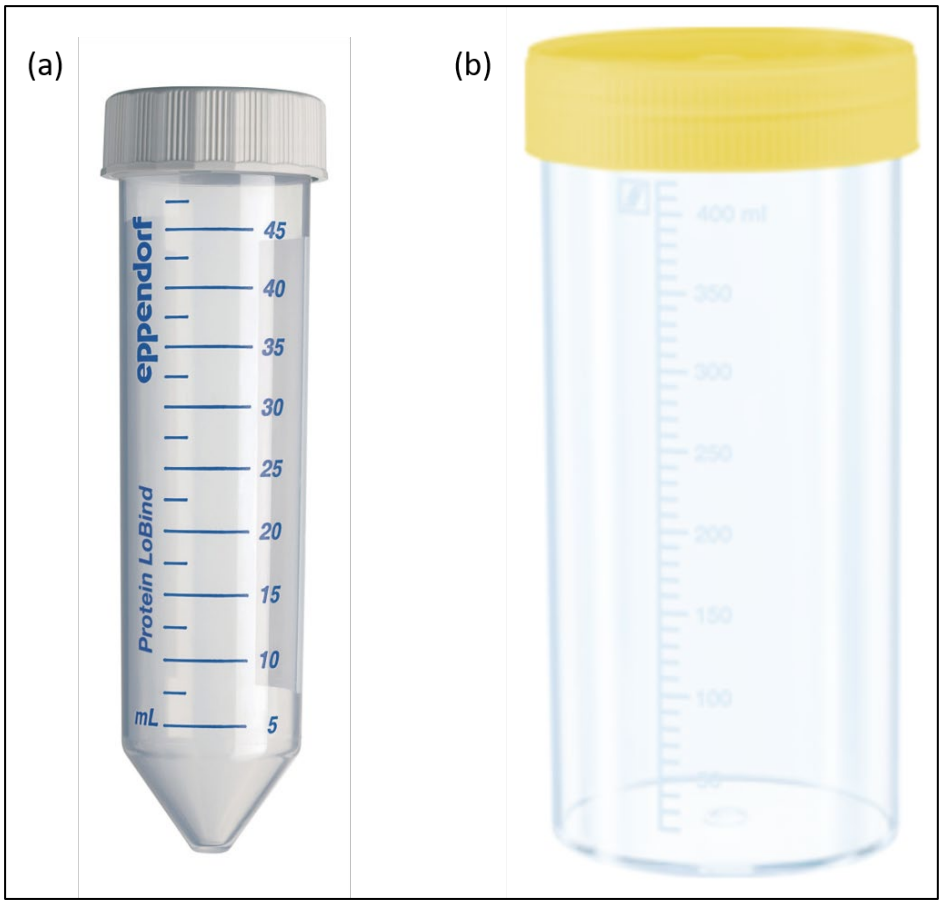

Figure 1.1. Falcon tube of 50 mL capacity (a) for sample collection from small waterbodies, and jar of 450 mL capacity (b) for sample collection from large waterbodies.

Water samples are mixed with Longmire's preservative buffer (Longmire et al, 1997) after collection. This buffer is non-hazardous and does not contain alcohol, allowing for shipping samples without any special permits. Additionally, Longmire's buffer can maintain eDNA intact at high temperatures (up to 50°C) for up to six weeks (Edmunds & Burrows, 2020) and at room temperature (average 25°C) for up to three months (Cooper et al., *in review*) so collected samples can be kept at room temperature if refridgeration is not available.

1.3 Quality control

Environmental DNA is not evenly distributed in water (Goldberg, et al., 2018). In order to maximise capture of eDNA present in water, five field samples need to be collected across a representative area of each site. Additionally, due to the highly sensitive nature of the technique, multiple safeguards are necessary to prevent cross-contamination during field collection (Goldberg et al., 2016). A control sample (field blank) needs to be collected at each site in order to prove that no contamination was introduced during field sampling procedure. Finally, changing gloves between sites is required to avoid cross-contamination between sites (Barnes et al., 2014).

1.4 Site selection for eDNA sampling

Water sample collection needs to be carried out at sites that exhibit suitable habitat for the target species. For example, if a species occurs in deep pools along a river, water samples need to be collected along the extension of the pool, or immediately downstream from it, if there is no access to the pool.

When sampling closed or semi-closed waterbodies (i.e. waterholes, lakes, dams), the rule of thumb is to try to collect samples from the majority of the accessible perimeter of a site. If your site is a large waterbody or a running stream, collect each of the samples at intervals of similar length over a maximum stretch of 200–400 m (see Figure 1.2). In the case of very large waterbodies, it is better to split them into several sites located at accessible areas.

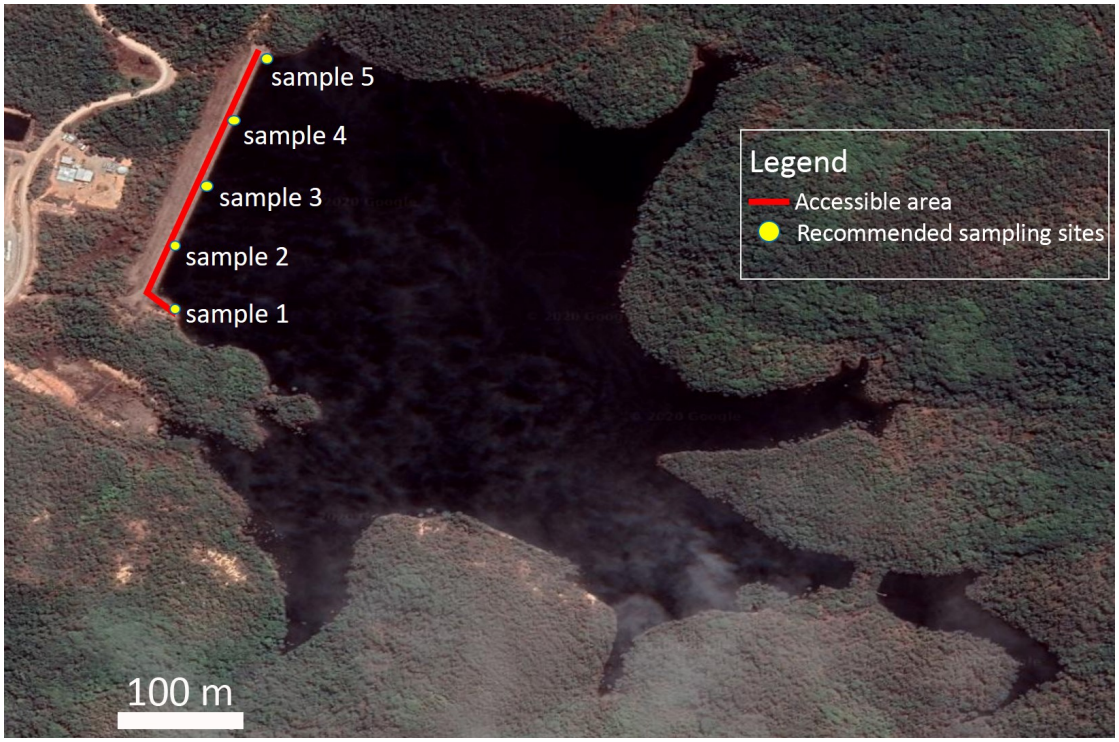

Figure 1.2. Example of a large waterbody (dam). The only accessible area is delineated with a red line. In this case it would be recommended to collect water samples that are spaced approximately 90 m apart (indicated by the yellow dots) to cover the full length of the accessible area.

1.5 Materials for eDNA sampling

One set of the following materials per sampling site.

- 5 Falcon tubes (5 x 50 mL) with grey cap*, not labelled, containing 10 mL Longmire's buffer
- 1 Falcon tube (50 mL) with grey cap*, labelled 'CONTROL', containing 10 mL Longmire's buffer

- 1 Falcon tube (50 mL) with blue cap*, labelled 'DISTILLED WATER FOR CONTROL', containing 30 mL distilled water
- 1 Falcon tube (50 mL) with blue cap*, not labelled and empty, stored in a Ziploc bag
- 8 parafilm strips
- 1 Ziploc bag to be used for rubbish disposal
- 1 datasheet
- 1 box of latex gloves (one box for all sampling sites)

*** Note:** Falcon tubes are appropriate when sampling from waterholes and small creeks. When sampling from larger waterbodies (i.e. dams, rivers, lakes), collection jars of 450 mL capacity pre-filled with 100 mL Longmire's buffer need to be used.

1.6 Procedure for eDNA sampling

1. Take a general photo of the area and at least one photo of each site (this helps to better understand the site features).
2. Fill out the datasheet with the information needed (Table 1.1).

1.6.1 Collecting the control sample (also called a field blank)

Do this step once for each visited site at the beginning of the sampling.

1. Wearing new gloves, open the 'CONTROL' tube* with the preservative solution (grey cap).
2. Pour the distilled water from the tube labeled 'DISTILLED WATER FOR CONTROL' (blue cap) into the open tube.
3. Label tube following the labelling instructions at the end of this section.
4. Peel off one parafilm strip and place it around the tube's cap in order to seal it and prevent the tube from leaking.
5. Close the control tube and place it in the rack. Make sure the tube cap is screwed in properly and that tube remains upright and in the rack at all times after sample collection (especially during transport). If using jars for sample collection, make sure cap is screwed in properly (not askew, Figure 1.3). Place the jar in plastic box.
6. Place the tube with the blue cap in the rubbish bag.
7. Place used gloves in the rubbish bag.

***Note:** For large waterbodies, the larger jars should be used instead for sample collection and preservation

1.6.2 Collecting water samples

Do this step five times for each visited site at the beginning of the sampling.

1. If the waterbody is not within arm's reach, or it is dangerous to approach the water, use an extension pole. Whilst wearing new, clean gloves, affix a new tube* with blue cap from the Ziploc bag using cable ties. The cap sould remain on the tube until right before sampling the water. After finishing affixing tube to the extension pole, discard gloves.
2. Put on new, clean gloves.
3. Collect a water sample either using the extension pole or directly from the waterbody.

4. Slowly pour the collected water into a tube containing the preservative solution until the final volume reaches the black line (40 mL). Be careful not to pour the water too fast, otherwise the tube might overflow. Do not fill tubes past the black line.
5. Close the tube with the grey cap. Make sure cap is screwed in properly. If using jars for sample collection, make sure cap is screwed in properly (not askew, Figure 1.3). Place the jar in plastic box.
6. Peel off one parafilm strip and place it around the tube's cap in order to seal it and prevent the sample from leaking.
7. Place tube in the rack. Make sure tubes remain upright and in the rack at all times after sample collection (especially during transport).
8. Repeat steps 3–7 until a total of five tubes have been filled with water samples.
9. Place the tube with the blue cap in the rubbish bag.
10. Place used gloves in the rubbish bag.
11. With new gloves, label tube with grey cap.

***Note:** For large waterbodies, the larger jars should be used instead for sample collection and preservation

Table 1.1. Environmental DNA sampling datasheet.

Personnel	
Date	
Location	
Site – include GPS coordinates	
Starting time	
Finishing time	

Sample number	Water flow (stagnant/slow/moderate/fast)	Habitat type (i.e. mangrove, sand, mud, etc.)	Photo taken (yes/no). If yes, photo number	Observations (write down any problems that you encountered during water collection)
1				
2				
3				
4				
5				
CONTROL	N/A	N/A	N/A	

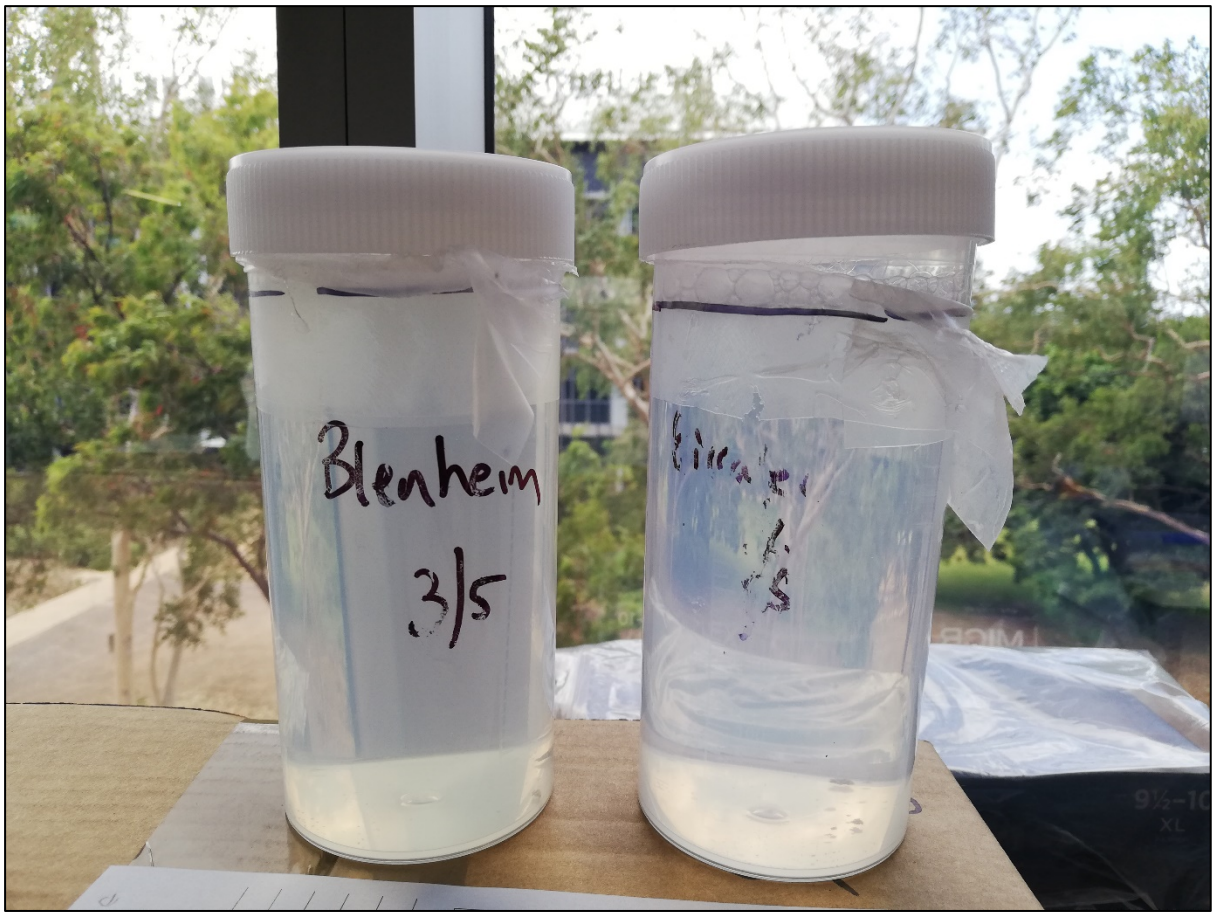

Figure 1.3. Lid properly closed (left jar) and lid askew (right jar).

1.6.3 Labeling tubes

1. Using a permanent marker pen, write down the name of the location, site, sample number and date. A location is the main area (i.e. island or river) where the samples are collected from, whereas a site is where each lot of five samples is being collected (i.e. junction, bridge, etc).
2. For the field equipment control, write down the name of the location, site, and “Control” instead of sample number.
3. Information needs to be written down on the side of the tube/jar as well as on the top of the lid, in case one of them rubs off during shipping.

1.7 Shipping water samples to the laboratory

1. Make sure all tubes/jars with samples are labelled and in the tube racks.
2. Wrap tube racks with cling wrap (to prevent tubes from jumping out of rack during transport).
3. Place all tube racks in upright position inside the box they came in. In the case of jars, make sure they are in upright position inside the box they came in.
4. Prior to sending samples to the laboratory, fill any empty gaps on the sides and top of tube racks inside the box they came in with scrunched paper.
5. Send rubbish bag back to the laboratory – we can recycle used gloves and re-use tubes.

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