ENVIRONMENTAL MONITORING METHOD:

eDNA Water Sampling with EnviroDNA Kits

Each section can be completed separately but you should read all three parts before starting. You need to complete all three parts to finish the monitoring method.

Part 1: Getting Ready

GATHER YOUR GEAR

Equipment required for this part:

- ☐ Electronic device(s) – charge ready for use and check that it has:
  - ability to take photos
  - data collection systems (app and form) (e.g. Fulcrum)
  - navigation system (e.g. Avenza) and site maps
- ☐ Laptop or computer with software for mapping (e.g. QGIS, ArcGIS, Google Earth)

Remember to check **GATHER YOUR GEAR** lists for **Out on Country** and **Back in the Office**. See the full list of equipment needed to complete this monitoring method on the last page.

KEEP IN MIND

Why?

Make sure there is a clear aim for your monitoring project and that the method you have selected will give you the answers you need.

When?

Prepare well before heading out on Country so that you have time to gather equipment or train staff, if needed.

Who?

At least one ranger/staff to plan and prepare.

Training and skills

Staff involved in planning are trained and competent in:

- ☐ Mapping software (e.g. QGIS, ArcGIS, Google Earth)
- ☐ Navigation systems (e.g. Avenza, GPS)
- ☐ Data collection systems (e.g. Fulcrum, datasheets)

eDNA Water Sampling with EnviroDNA Kits

Check permissions

Consult with Traditional Owners, landholders and relevant government agencies and authorities, to determine appropriate access and approvals for environmental monitoring:

1. Where you can go – consult with the owners/managers of the land.
2. What you can do – check if you need scientific licencing, approvals or ethics.
3. What or who can you take photos of
4. What can be done with data and photos – who owns them, where will they be stored and how will data be interpreted and communicated.

ACTIONS

Make a plan and prepare

1. Plan which dates you will collect water samples
   - Some sites may not be accessible immediately after rain.
   - Some site may not have water in them all year round
   - Sampling needs to be done when the target species has recently been in or using the water.
2. If this is the first year you are monitoring, gather records of the target species in your area and/or identify where the target species might be on Country Such as from Traditional Custodians , Atlas of Living Australia (ALA) or government databases.
3. Select your sites using mapping software and/or traditional knowledge
   - A reconnaissance trip can be helpful to check that your sites have water and are accessible.
4. Give each site a unique name, and save the location data in your data management system.
5. Prepare maps of sites/load onto navigation devices
6. Contact the EnviroDNA lab to organise eDNA analysis
   - Remember to ask what the cost and timeline is for getting samples processed.
1. Plan how you will record information on Country e.g. Fulcrum electronic data forms.
2. Plan your data management system e.g. how you will store eDNA sample data
3. Check **GATHER YOUR GEAR** lists for **Get Ready, Out on Country** and **Back in the Office** (complete list of equipment on last page) and get any equipment you don't have. See eDNA buying guide(s) for advice on which sampling kits may be suitable to buy.
4. Be clear on how many people will be involved and what everyone needs to do the work.

5. Check the training requirements for **Get Ready, Out on Country** and **Back in the Office** steps to ensure that rangers know how to use the devices, data collection apps, navigation systems etc.

Next Section – Part 2: Out on Country

Part 2: Out on Country

GATHER YOUR GEAR

One set of this equipment for each site:

- ☐ EnviroDNA eDNA sampling kit:
  - 50mL syringe, 1.2-micron PES filter, preservative syringe, ziplock bag
  - 50mL syringe, 5-micron PES filter, preservative syringe, ziplock bag

One set of this equipment for each team:

- ☐ Electronic device(s) – charged and ready to record data, take photos and navigate to sites
- ☐ Power bank – charged and ready to charge devices (optional)
- ☐ GPS device and spare batteries (recommended)
- ☐ Disposable gloves – powder free and stored in a clean ziplock bag
- ☐ Esky with ice or portable car freezer
- ☐ Pencil
- ☐ Permanent marker
- ☐ 50ml falcon tubes
- ☐ Extension pole
- ☐ Zipties

KEEP IN MIND

When?

Has recent rain made your sampling site inaccessible?

Who?

At least two people per team

Training and skills

Make sure everyone knows the plan.

Field staff are trained and competent in:

- ☐ Navigation systems (e.g. Avenza, GPS)
- ☐ Data collection systems (e.g. Fulcrum, paper datasheets)
- ☐ Collecting eDNA water samples with EnviroDNA kits

ACTIONS

Collect eDNA water samples

Once you have arrived at the site, avoid disturbing or touching the water until you have finished the eDNA sampling.

1. Take a photo of the site.
   - Photos can help to describe the site features.
2. Record **site data**
3. Put on a fresh pair of gloves
4. Using a fresh syringe, pull 50ml of water into the syringe from 5-15 cm below the water surface.
   - If the waterbody is not within arm's reach or it is dangerous to approach the water, collect the water in a falcon tube first by attach it to an extension pole and scooping water up.
5. Screw the filter onto the syringe
   - Use 1.2-micron PES filters for surface samples and 5-micron PES filters for benthic samples
6. Push the water from the syringe through the filter
7. Continue collecting and filtering water from the waterbody until no more water can be pushed through the filter.
8. Remove the filter from the syringe before collecting more water, and then screw the same filter back on
9. Keep track of how much total water is pushed through the syringe, it will be about 100-300 mL total.
10. Unscrew the filter.
11. Uncap the preservative syringe, screw it onto the filter and push the preservative in. Leave the preservative syringe attached and put the preservative cap onto the other end of the filter.
12. Place the filter into a ziplock bag, label the bag and put it in an esky on ice/in a portable car freezer.
13. Labels need to include site code, type of water source, coordinates, date, volume of water filtered.
14. Using a stick, gently disturb the sediment layer at the bottom of the waterbody.
15. Put on a fresh pair of gloves
16. Using a fresh syringe, pull 50ml of water into the syringe from the bottom of the pool (benthic zone)

17. If the water is too deep, collect the water in a falcon tube first by attaching the falcon tube onto an extendable pole and scooping water up from the bottom of the pool.
18. Repeat steps 8-13 but using a 5-micron PES filter
19. Record **eDNA sampling data**

RECORD DATA

Data to record when at the site

What to record	Required?	Notes
Information to record about each site		
Project name	Yes	Make it clear which project this data belongs to and its purpose
Date	Yes	Record the date the site (waterbody) was visiting for water sampling
Personnel	Yes	Record the name of the people who did the sampling - this is helpful if any questions come up about the data
Site name/number	Yes	Name of the site (waterbody)
Location coordinates	Yes	Record an accurate location (using a handheld GPS if possible) (latitude and longitude or eastings and northings)
Photo of site	Yes	Which camera/tablet/phone the photo was taken on, and the filename of the photo (usually ends in .JPG)
Water flow	Yes	Stagnant, slow, moderate or fast
Habitat type	Yes	Describe the habitat type (e.g. mangrove, shrubland) and type of waterbody (e.g. waterhole, creek, dam)
Presence of target species	Optional	Did you see or find signs of your target species?
Signs of disturbance	Optional	Types and causes of disturbance you can see at the site

Data to record when collecting water samples

What to record	Required?	Notes
Information to record about water samples		
Site name/number	Yes	Name of the site (waterbody) and a description of where the samples were collected from in that waterbody (e.g. if there is a specific gnamma or edge of the lake that was used for sampling)
eDNA samples taken?	Yes	Record whether you did or didn't take the three eDNA sample types (surface sample, filter membrane sample, benthic sample) and if you didn't, why
Start and finish times	Yes	Time from when the water sampling began and ended
eDNA sampling method	Yes	Record the method used to collect the water samples – in this case it is the EnviroDNA Kit

Total water filtered for surface sample	Yes	Record how much water was filtered for the eDNA water sample collected from near the surface of the water
Total water filtered for benthic sample	Yes	Record how much water was filtered for the eDNA water sample collected from near the bottom of the gnamma
Water sampling issues	Yes	Describe any problems encountered during water sampling
Stories and notes	Optional	Record information or stories from Elders, and anything else worth noting about the area or animals.
Video	Optional	Record videos of information or stories from Elders, and rangers performing or describing the work they are doing.

Next section – Part 3: Back in the Office

Part 3: Back in the Office

GATHER YOUR GEAR

Equipment required for this part:

- ☐ Electronic device(s) that you used to record your data
- ☐ Data management system, e.g. cloud storage
- ☐ Laptop or computer with software for spreadsheets (e.g. Microsoft Excel) and mapping (e.g. QGIS, ArcGIS, Google Earth)
- ☐ Freezer (less than -20˚C)
- ☐ Postage materials

KEEP IN MIND

When?

Always try to complete this work as soon as you can after returning from your time on Country so that you can send the samples off for analysis so that the DNA doesn't have time to degrade and so that you get your data back as quickly as possible.

Who?

At least one person to manage the data and send samples

Training and skills

Staff managing data are trained and competent in:

- ☐ Mapping software (e.g. QGIS, ArcGIS, Google Earth)
- ☐ Spreadsheet software (e.g. Microsoft Excel)
- ☐ Data collection systems (e.g. Fulcrum, datasheets)
- ☐ Data management systems (e.g. databases, cloud storage, external hard drives)

ACTIONS

Store and send samples

1. On return to the office, transfer the eDNA samples from the esky/car freezer into a -20˚C freezer so that they stay frozen.
2. As soon as possible, send the eDNA samples via courier to the eDNA lab for analysis
3. The eDNA lab will be able to give you instructions on packaging and sending samples.
4. Wait for the results

5. Update the data in your data management system with the eDNA results

Data entry, analysis and reporting

1. Record a summary of what you did and why, any observations (e.g. weather conditions, fire history, site condition), anything that went wrong or didn't work and things that worked well.
2. Upload the **site and water sampling data** to your data management system.
   - Recommended: get someone else to proof the data to check for mistakes.
3. Upload any photos or videos taken during the survey to your data management system.
4. Your data analysis will depend on the project purpose and the data collected. It could include:
   a. Use GIS software to map where species were detected to see if there are patterns in their distribution across the landscape.
   b. Calculate species richness – count how many species you detected. Consider splitting it up into different taxonomic groups (e.g. mammals, reptiles, birds, invertebrates, plants).
   c. Create bar graphs in excel to compare species richness across different sites.
   d. Create line or bar graphs in excel to track changes over time. Add reference lines to show things like baseline data.
   e. Create before/after graphs to show the impact of a management action.
5. Discuss with the ranger team or community the results of the monitoring, any reasons for the species being detected or not, and if there have been any changes to previous years.
   - Consider whether trends might be related to your management (e.g. fencing waterbodies) to check how well management is working, or if you need to make adjustments.
6. Share the data according to any data sharing or funding agreements you have made

Next section – Full Equipment List

Gather Your Gear – Complete List

The complete **GATHER YOUR GEAR** lists for **Get Ready**, **Out on Country** and **Back in the Office**.

Gear List	Required?	Get Ready	On Country	In Office
Electronic device(s): • Charged • Ability to take photos • App for data collection (e.g. Fulcrum) • App for navigation (e.g. Avenza)	✓	✓	✓	✓
Power bank • Charged	**Recommended**		✓	
Laptop or computer with software for: • Mapping (e.g. QGIS, ArcGIS, Google Earth) • Spreadsheets (e.g. Microsoft Excel)	✓	✓	✓	✓
GPS (e.g. Garmin handheld device) & spare batteries	**Recommended**	✓	✓	
EnviroDNA eDNA sampling kit: • 50mL syringe, 1.2-micron PES filter, preservative syringe, ziplock bag • 50mL syringe, 5-micron PES filter, preservative syringe, ziplock bag	✓		✓	✓
Disposable gloves: • Powder free • Stored in clean ziplock bag	✓		✓	
Esky with ice or portable car fridge/freezer	✓		✓	✓
Stationery: pencil, permanent marker	✓		✓	
50ml falcon tubes	**For deep waterbodies**		✓	
Extension pole	**For deep waterbodies**		✓	
Zipties	**For deep waterbodies**		✓	
Postage materials	✓		✓	✓
Freezer • -20˚C	✓			✓
Data management system (e.g. cloud storage)	✓			✓