How-to guide

eDNA sampling for native, threatened and invasive species

Project 3.2

Threatened Species Recovery Hub

National Environmental Science Programme

Background

All animals leave traces of their genetic material as they move through their environment. This material is known as "environmental DNA", and it can originate from skin cells, mucous, faeces or even individual hairs. Environmental DNA (eDNA) sampling is the detection of species' DNA from a sample that could come from water, soil or even air. So far, water sampling has been the most common application of this emerging technology in biodiversity monitoring.

Traditional monitoring methods may be unable to efficiently detect cryptic species and species that occur at low population densities. These may include rare or threatened species, as well as exotic species that are in the initial stages of an invasion. eDNA sampling can be a suitable option for the detection of any of these types of species. Other ideal candidates for eDNA sampling are cryptic species that are similar in appearance to one or more other species but genetically distinct from them.

These species can also be difficult to monitor using traditional methods.

Environmental DNA sampling can be divided into two broad categories, single- or multi-species detection methods. The field sampling for these two methods can be the same, but sample processing in the laboratory differs significantly. Multi-species detection methods are also known as eDNA metabarcoding.

Before you get started

For single-species detection methods, primers/probes first need to be designed (e.g., by sequencing tissue samples from the target species in the study area, or from sequences on public repositories such as GenBank, https://www.ncbi.nlm.nih.gov) to only amplify the DNA of a target species.

If, however, you are using a multi-species detection method, and targeting an entire biological group, such as fish, frogs or crustaceans, the process is more complex. This approach requires primers that target not just a single species, but are able to target an entire group based on regions of DNA (primer sequences) that are common to the whole group, but that other groups do not possess. A reference database is also needed, so that collected DNA sequences can be matched to known species sequences. Once this library is established, however, it can be re-used for different projects or for long-term monitoring. The reference DNA databases should ideally contain sequences from species groups in the area where you will be sampling, so that intraspecific variation in DNA sequences can be accounted for.

You will also need sampling equipment such as syringes, sterile bottles and/or peristaltic pumps, also known as hose pumps or tube pumps. Other essential field equipment includes single-use gloves, buckets, and a sturdy pair of boots. A sampling pole may be necessary in some cases. You should also have a >10% commercial bleach solution for decontaminating field equipment so that you can reuse it.

There are various field- and laboratory-based preservation methods for filters, including drying, freezing, ethanol, or the use of a buffer (e.g., Longmire's solution). Choice of preservation method may depend on availability of field resources, such as a freezer.

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Researcher Lucy Howell taking samples at Avon River. Image: Emily McColl-Gausden

Research aims determine methods

It is critical that you be clear about the aims of the study, as this will determine whether you choose a single-species eDNA detection method or a multi-species one. Single-species detection methods can successfully detect native species, rare and/or threatened species, and invasive species in aquatic and terrestrial environments. Multi-species detection methods provide information about more species, but are also more complex.

Your study aims will also help you decide whether you would prefer the greater sensitivity of single-species detection or the additional data that multi-species detection can provide about community composition. If you wish to determine whether a threatened species is present at a site – for example, for an environmental impact assessment – then you might prefer the sensitivity of single-species detection methods. If, however, you wish to gather information about the presence of competitors, prey and predators to inform management decisions about food webs, multi-species methods may be more suitable. Similarly, if biodiversity measures are your goal.

If your research goals require sensitivity for a target species but you also need additional data on community composition, you can combine the methods. This will have some cost advantages, as sampling and DNA extraction performed for one method will significantly reduce the cost for the second one. There is, however, a cost difference between the two methods, with multi-species detection methods being more expensive. The cost efficiency of the two methods therefore needs to be considered in the context of your research goals.

If you are interested in species abundance at a site, some single-species assays have shown that there is a relationship between the DNA concentration in a sample and abundance. You can also estimate DNA copy number using the multi-species detection method; however, the relationship with abundance is much more complex and is generally considered a relatively weak indictor of species abundance at a site.

Finally, if you are trying to determine rapid management interventions, the faster implementation of single-species detection methods makes them a more attractive option. This could be in response, for example, to biosecurity concerns such as incursions by invasive species or pathogens causing disease outbreaks, or to make environmental impact assessments for threatened species. When a species assay (validated primer/probes) is already available, the turnaround times in the laboratory can be as short as 24 hours. There are also technologies that allow for in-field processing of samples, where results can be available in as little as two hours. This contrasts to turnaround times for multi-species methods, which can be several weeks.

Comparison table: Single-species versus multi-species eDNA sampling

	Complexity	Sensitivity	Estimating abundance	Time and cost
Single-species eDNA detection methods	Primers/probes amplify the DNA of one target species (assays available for some species)	More likely to detect target species	Some studies show a relationship between eDNA concentration and species abundance	Can be implemented in the field in <2 hours; 24-hour turnaround in the lab
Multi-species eDNA detection methods	General primers based on a DNA region common to the whole taxonomic group Reference DNA databases contain sequences from multiple species in the sample area	Potentially lower detection probability can be offset by additional data gained (e.g., about presence of prey, competitors, and predators)	Weak/inaccurate indication of site abundance for individual species	Currently lab implementation only; process can take weeks

Choose sampling locations in good quality habitat. Image: Emily McColl-Gausden

So how do you do it?

eDNA sampling is conducted by taking water from a site, using either sterile bottles, syringes, or peristaltic pumps. This water sample is then filtered to trap the DNA and other particulate matter. You can do this filtering on-site or later, in a laboratory. Next, you must extract the DNA from the filter and process it, choosing your eDNA detection method: single-species or multiple-species, according to the focus of your research.

As for other survey methods, you will need to carefully consider the timing and location of your eDNA sampling. Optimal timing will depend on your species' life cycle, as well as the likelihood of DNA being transported to or retained at a site.

If your primary objective is to maximise the chance of detecting a particular target species, sampling at a time of year when that species is most active (e.g., during the breeding season) or in highest abundance at a site may maximise the amount of DNA present in the system. This assumes that you have this information for the target species, and that DNA shedding rates are equivalent across its life stages. You should also choose a sampling location in good quality habitat.

Be aware that flowing freshwater systems can transport eDNA downstream to an unoccupied site or dilute it to undetectable levels at the point of origin. However, the impact of these processes on the interpretation of survey data may be minimal in some cases, especially when you consider the typical size of management sites and how far many aquatic organisms can travel within aquatic systems. For example, while estimates of eDNA transport vary from around 100 m to 9 km downstream, if the only information you need to make a management decision is verification of the presence of a species within a catchment area, then it may be irrelevant how far upstream the eDNA has originated. eDNA transport is a growing area of research.

If you are seeking data about recent or current occupancy, you should also take care to select eDNA samples from the water column rather than from substrate, such as sediment. eDNA can be trapped in sediment for much longer after a species has left an area than it would stay present in the water column.

Applications

eDNA sampling is an efficient detection method for cryptic species and species at low abundances because it is sensitive.

The efficiency of eDNA sampling makes it suitable for baseline data collection and long-term monitoring of sites. Because you can collect data quickly and accurately, the results can be readily compared between years.

Researcher Emily Gregg taking eDNA samples by the Murray River at Jingellic. Image: Emily McColl-Gausden

eDNA sampling: Pros and cons

Compared to traditional sampling methods, eDNA has been shown to be more effective at detecting species and communities in many aquatic (and some terrestrial) systems.

eDNA sampling is also particularly efficient for large study areas – many sites can be visited in a relatively short period of time, as sampling only takes around 10 minutes. Given the sensitivity of the method, repeat visits are not usually necessary to determine occupancy of a target species. This sets it apart from some other aquatic sampling methods, such as trapping, which can involve many trapping sessions at a given site to return a high probability of detection.

Where more detailed data is sought about population or individual health (e.g., reproductive output, juvenile recruitment, sex, genetic variation, abundance), eDNA sampling can pinpoint sampling locations for more traditional, time-intensive capture methods, thereby reducing survey efforts and costs.

Reference

McColl-Gausden, E.F., Weeks, A., and Tingley, R. 2020. A field ecologist's guide to environmental DNA sampling in freshwater environments. *Australian Zoologist*. 40(4), 641-651.

Further information

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eDNA sampling: Pros and cons (continued)

eDNA sampling has a further advantage of largely eliminating concerns around animal ethics, as animals do not need to be captured or even sighted. eDNA sampling also reduces high-risk activities, such as entering waterways to check fyke nets for fish or platypuses, or conducting backpack or boat electrofishing surveys for fish.

A final benefit of eDNA sampling is that it can be easily adopted by citizen scientists after a small amount of training. Indeed, there are already examples of successful eDNA surveys conducted by citizen scientists in Australia and overseas.

Despite these benefits, using eDNA sampling in biodiversity surveys is currently more complex than most traditional sampling methods. It is still a relatively new monitoring technique, and standardised approaches have not yet been developed.

Sampling at Goulburn River. eDNA sampling can be easily adopted by citizen scientists. Image: Sarah McColl-Gausden

Tips and recommendations

Like other monitoring methods, eDNA can result in false positive detections. False positives can occur for different reasons. Two of the most common are:

1. **eDNA is transported via flows or non-target species to a second location**. For example, if a bird of prey ate the carcass of a target species at one site and then flew to a second site, it could excrete eDNA of the target species at the second site.
2. **The sample or site is contaminated during the sampling process.** For example, field staff could accidentally carry eDNA samples between sites on their boots, or could accidentally contaminate samples with materials from other sites.

To minimise false positives, it is important to strictly follow protocols to avoid contamination between sites. Such protocols should involve, at a minimum:

- utilising single-use gloves
- sterilising field equipment such as syringes
- decontaminating field equipment for re-use with a >10% commercial bleach solution (boots, buckets, trays, sampling poles, bottles, etc.)
- avoiding entering the water.

False positives can also occur in the laboratory, although there are steps you can take to determine laboratory contamination. To minimise this and to ensure robust results, collaborate with a fully equipped genetics laboratory that implements quality control practices.

eDNA sampling is also susceptible to false negative errors. False negatives can originate from qPCR inhibition (due to factors that prevent DNA amplification), or can arise because eDNA is not captured in a given water sample, despite eDNA being present in a water body. qPCR inhibition can be identified using internal positive controls, whereas the latter issue can be partially remedied by taking multiple water samples at a site.

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This research project is being funded by the Threatened Species Recovery Hub of the Australian Government's National Environmental Science Program.