Environmental DNA (eDNA) is an effective and affordable way to survey species and biodiversity. This report provides an introduction to eDNA: how it works, the science and why it is valuable.

# eDNA: a powerful tool for conservation and biosecurity.

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To make decisions about an environment, we need to know what lives there. Unfortunately, biological surveys can be difficult, time-consuming, expensive and invasive. Environmental DNA (eDNA) is a new tool that overcomes these problems. The eDNA in just a small sample of water, soil or scat can reveal which native species, pests and even diseases are found there. Here, we outline how eDNA works, the advantages of using eDNA, and the ways in which eDNA is revolutionising environmental management.

A key challenge for conservation and biosecurity is detecting species. To protect native species, we need to know where they live. To avoid the spread of introduced species, pests and diseases, we must detect them early. To assess whether conservation programs are working, we need to monitor biodiversity over time. Unfortunately, these seemingly basic tasks can take up huge amounts of time and resources.

The problem is that not all species are easy to see, catch or identify. Confirming the presence or absence of even just a single species can be extremely labour-intensive. Sometimes, the species of greatest interest are also the rarest and most difficult to find – including threatened native species, and newly introduced species that could pose a threat. Some surveying techniques, such as trapping, can also cause stress and other risks for wildlife. These challenges tend to restrict the scale and frequency of biological surveys, ultimately limiting the information available to environmental managers.

Analysis of environmental DNA (eDNA) is a relatively new, cheap, quick and non-invasive method for detecting species. eDNA, as its name suggests, is the DNA that an organism leaves behind in its environment. eDNA was first used in the 1980s to study communities of bacteria in marine sediments[1]. In 2008, scientists published the first paper on using eDNA from a water sample to detect vertebrate species[2]. Today, eDNA is used to detect threatened and invasive species, monitor biodiversity, study population genetics and even learn about long-extinct flora and fauna. While new applications for eDNA continue to be developed, this highly adaptable tool is already an essential addition to any environmental manager's toolkit.

# How does eDNA work?

eDNA is like a fingerprint. All organisms – including animals, plants, fungi and bacteria – leave fragments of their DNA in the environment. These DNA fragments can come from shed skin, hair, saliva, faeces or other secretions. By extracting eDNA from an environmental sample, such as water or soil, we can find out what has been there without having to actually observe or capture the species.

There are two main ways that eDNA can be used. eDNA can either be used to target one species at a time (a single species approach) or to detect many species at once (e.g. DNA metabarcoding). The most useful approach depends on the question (see Box 1: Single species approach vs. DNA metabarcoding). A single species approach optimises the detection of one species, whereas a multispecies approach such as DNA metabarcoding, is more efficient for assessing biodiversity. Both approaches can be better at detecting species than traditional surveying methods[3-5].

## There are 5 steps required[6]

**STEP ONE**

**Sample collection** Samples (such as water, soil or scat) are collected from the environment.

**STEP TWO**

**DNA extraction** DNA is extracted from environmental samples. This is usually done in the laboratory, but future developments might allow DNA extraction in the field.

**STEP THREE**

**Amplification** Polymerase chain reaction (PCR) is used to make many copies of a targeted region of DNA. The target region might be unique to one species (single species approach). Or, the amplified target region might be conserved across a group of species (e.g. fish), but contain a DNA sequence that differs between species that can be determined through DNA metabarcoding.

**STEP FOUR**

**Sequencing (DNA metabarcoding only)** The amplified DNA is rapidly sequenced using next generation sequencing.

**STEP FIVE**

**Processing results** For a single species approach, results require relatively little processing – PCR amplification indicates whether a targeted species is present. For DNA metabarcoding, the raw DNA sequences are carefully processed against a reference database of DNA sequences, to identify which species are present. Raw datafiles can be large, and bioinformatic tools are used to assist with processing.

## Single species approach vs. DNA metabarcoding

There are two main ways that eDNA can be analysed: the single species approach and DNA metabarcoding. There are advantages and disadvantages of each method, and the method you choose will depend on the question being asked.

When used to target a limited number of species, the single species approach is quicker and cheaper than DNA metabarcoding. The single species approach can also be more sensitive than DNA metabarcoding, meaning that if a species is present, you might be more likely to detect it[7]. In addition, the single species approach can be used to estimate the relative abundance of different species, based on the concentration of DNA in a sample[8,9].

In comparison, the DNA metabarcoding approach is quicker and more cost-efficient for detecting many species at once. This makes DNA metabarcoding ideal for assessing biodiversity. Processing the results for DNA metabarcoding can take longer, but this method is still more efficient than a single species approach at detecting all species within a taxonomic group (e.g. fish, amphibians). However, some species at very low abundance might be missed, and relating eDNA to species abundance can be more challenging[7]. Still, these methods are rapidly evolving, and some limitations may be overcome with future developments.

# What can eDNA tell us?

If the eDNA of a species is detected, how recently was the species there? The answer depends on the environment. In aquatic environments, eDNA breaks down in a matter of days or weeks[4,10]. Consequently, water samples provide up-to-date information about the presence of species, which is critical for conservation and biosecurity. In contrast, eDNA can remain in soil for decades or centuries after an organism is gone[11,12]. In frozen sediments, traces of eDNA can even persist for hundreds of thousands of years[13]. So unlike water samples, soil and sediment may not always provide recent information – but in such cases they can still provide important historical insights.

The distance over which eDNA can be detected also depends on the environment. In a river or stream, eDNA can disperse small distances or even kilometres away from its original source depending on flow and DNA degradation rates. These environments, rather than just providing local information, are like conveyer belts of information for the broader landscape[14] allowing species to be detected over larger distances. The movement of eDNA also makes it easier to collect more concentrated samples, using filters that capture DNA[15]. In other types of environments, such as in soils and sediments, an organism often needs to have been physically present at a precise location for its eDNA to be detected[12]. This can make eDNA sampling in terrestrial environments more challenging, but also more locally precise, compared with aquatic environments.

Importantly, there is some information that eDNA cannot currently provide. Currently, eDNA is most useful for determining the presence or absence of a species. In some circumstances, eDNA can also indicate the relative abundance of different species (see Single species approach vs. DNA metabarcoding). However, eDNA cannot necessarily tell us the sex, size, developmental stage or health of individuals. To learn more about the individuals within a population, other monitoring techniques – such as trapping – are still needed. When deciding whether to utilise eDNA, environmental managers therefore need to carefully consider the questions that need answering.

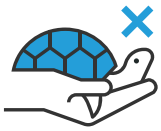
Still, new opportunities for eDNA continue to be developed. For example, changes in the relative amounts of nuclear and mitochondrial eDNA have even been used to monitor spawning of endangered fish[17]. With further research and advances in technology, other valuable applications for eDNA will likely be discovered.

## Examples of EnviroDNA's wildlife detection tests

# Why use eDNA?

## To find rare or elusive species

eDNA is an efficient way to detect species that are rare or otherwise difficult to find. For example, you only need two water samples to have a 95% probability of detecting a resident platypus – compared with up to 10 nights of trapping with fyke nets[18]. Using eDNA in this way is especially useful for conservation and for environmental impact assessments.



## To survey species non-invasively

Collecting eDNA samples is completely non-invasive. Even if eDNA cannot provide all the information you need, it can complement other tools to reduce impacts on wildlife, as well as reduce health and safety concerns for field staff. For example, eDNA can efficiently reveal where species are at a broad scale, before using other more resource intensive and invasive tools – such as trapping – to learn more about the size and breeding status of individuals[3].

## For early detection of invasive species and pests

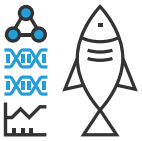
Because analysing eDNA is relatively quick, cost-effective and sensitive, it is ideal for monitoring environments for introduced species and pests. Some species are also more likely to be detected using eDNA than using traditional surveying methods[19-21], which is critical for effective eradication of a new invasive species.

## To assess and monitor biodiversity

eDNA (with DNA metabarcoding) is currently the most efficient tool for assessing biodiversity in aquatic ecosystems. Compared with other surveying methods, eDNA sampling allows detection of an equal or greater number of species, for much lower effort[3,4]. In river networks, where eDNA may travel further, eDNA can also be used to measure total biodiversity across the riverscape[14].

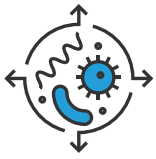
## For large-scale, long-term projects and citizen science

Besides being relatively quick and inexpensive, eDNA sampling is also easy to standardise[19]. This makes eDNA a useful tool for large-scale and long-term projects. For those who engage with the community in environmental programs, eDNA is also proving to be an innovative and successful citizen science tool. For example, eDNA samples collected by volunteers in the U.K. have been successfully used to monitor the distribution of endangered newts[22].



## To study population genetics

A more recent application of eDNA has been for studying population genetics. For example, eDNA from seawater has provided useful information about the genetic diversity of whale sharks that lead to better estimations of whale shark school size[23]. Measuring genetic diversity is particularly important for conserving threatened species, since low diversity can cause further population declines.



## To detect and avoid the spread of diseases

Disease-causing organisms, like any other organism, leave traces of DNA in their environment. eDNA can therefore help detect and track diseases. For example, eDNA can be used for early detection of chytrid fungus, a disease-causing fungus that can wipe out entire populations of amphibians[24]. Also, because eDNA sampling is non-invasive, there is less risk of spreading these diseases when searching for them.

## Using eDNA today

When making decisions about an environment – for conservation, biosecurity or proposals for development – two questions are often critical: "Which species are here?" and "Is this species here?". eDNA is a highly sensitive, efficient and non-invasive tool for answering these questions. By using eDNA to complement other surveying tools, environmental managers can not only save time and resources, but also obtain more environmental data than ever before.

## Find out more

This white paper was created by EnviroDNA, a company at the forefront of applying eDNA research and technology to industry. Along with undertaking eDNA research, we are the first company in Australia dedicated to providing eDNA species detection services to clients. You can learn more about EnviroDNA at **www.envirodna.com**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