Using Environmental DNA to manage biodiversity risks

- An eDNA approach complements traditional survey methods and is particularly useful for aquatic species.
- eDNA has multiple potential applications, from understanding ecological communities at a landscape scale to confirming the presence of rare and elusive species at a project site or demonstrating the effectiveness of mitigation measures.
- Collecting eDNA samples requires no expert skills and while limitations exist, eDNA approaches can reduce the cost of data collection and provide answers to clearly defined questions and objectives.

Introduction

The use of environmental DNA (eDNA) as a tool for biodiversity surveys is a fast-developing field. Despite its relative infancy, eDNA is proving itself useful for surveying a wide-range of macro-organisms (animals, plants, fungi) – particularly for aquatic and riparian species where traditional capture-based methods may not detect rare species and can be time-consuming and expensive.

While eDNA approaches hold the potential to advance the scale and cost-effectiveness of biodiversity surveys, limitations still exist. Challenges include incomplete genetic reference databases, limited differentiation of recently evolved species clusters, and movement of eDNA within the environment. Projects therefore need to be carefully scoped out with specialists to ensure the approach can meet monitoring objectives, and it remains important to integrate ecological and environmental expertise relevant to the specific project site.

Two important concepts underpin the eDNA approach:

eDNA: the genetic material that higher organisms deposit in the environment through biological processes such as excretion, shedding, secretion, and saliva. In water bodies, this DNA remains viable for species identification for several days and can be captured by passing the water through a filter to gather an eDNA sample. Genetic material from terrestrial species as well as aquatic species can be captured in an eDNA water sample.

eDNA Metabarcoding: the analysis used to identify the taxa and species present within an eDNA sample. DNA sequences are extracted from eDNA samples, sequenced and compared to a

At a glance

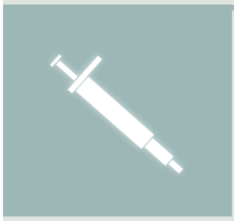
- eDNA analysis uses a non-invasive genetic technique to monitor species presence/absence and distribution. eDNA consists of small fragments of genetic material left in the environment by organisms.
- eDNA present in a water, sediment or soil sample is sequenced and compared with DNA sequences available in open global genetic databases, e.g. GenBank or Bold, through a process called metabarcoding.
- During early project scoping, eDNA can quickly confirm if species of interest are present, helping to focus the design of biodiversity mitigation and management.
- During construction and operations, eDNA can complement traditional monitoring techniques, enabling fast, regular monitoring of species presence and distribution and changes in habitat quality.

library of species' DNA sequences from previously collected specimens. This is then used to identify species present at the collection point. For aquatic species, using an eDNA approach can be an efficient way of conducting biodiversity surveys. For assessing species presence/absence it is much more effective than conventional methods of surveying aquatic biodiversity[1] and can detect a larger number of species[2,3]. In still water, eDNA can also be used to infer the relative abundance of aquatic species[4]. This makes eDNA useful for developing species lists early in project planning, and for long-term monitoring of aquatic species.

Since eDNA can identify multiple taxa and species in a single sample, composite indicators can be selected as proxies for habitat quality or condition. These can be species-based (e.g., fish species diversity) or function-based (e.g., relative abundance of predatory vs herbivorous species). This can enable cost-effective monitoring of positive and negative changes in habitat quality over time, whilst expanding the scope of monitoring to ecosystem functioning. In the terrestrial environment, academic research and commercial case studies[5] are also now demonstrating the value of eDNA for detecting species presence, complementing more conventional data collection techniques.

Simplified eDNA process

Samples are collected in the field that contain eDNA. Whether a species' eDNA is present depends on multiple factors including the species ecology (e.g. amount of secretion made by the species which may vary temporally, size of the species, migration) environmental factors (e.g. water flow rate/ circulation, salinity, decay rate, temperature, sediment levels).



At the laboratory, DNA is extracted from the sample and amplified using a specialised method. Primers are used to target a particular taxonomic group; they can be designed to capture very broad groups (e.g. Eukaryotes) or narrower ones (e.g. fish, or particular families of molluscs) with trade-offs in breadth and sensitivity to detect rare species. Amplified DNA is sequenced on a high-throughput sequencing platform.

Results are cross-checked with genetic databases to determine the suite of species present. Only species registered on the database will be identified to species level but a barcoding campaign can be added on to any project to improve the reference database for the species currently missing. Unidentified species are named at a higher taxonomic level (family or genus).

Reporting provides the species identified within each sample as well as key findings and summary graphics.

Figure 1: Simplified eDNA process

[1] Handley *et al.* 2019. Temporal and spatial variation in distribution of fish environmental DNA in England's largest lake. Environmental DNA. 1(1), 26-39. https://doi.org/10.1002/edn3.5

[2] Hinlo *et al.* 2018. Performance of eDNA assays to detect and quantify an elusive benthic fish in upland streams. Biol Invasions. 20, 3079–3093. https://10.1007/s10530-018-1760-x

[3] Coble *et al.* 2019. eDNA as a tool for identifying freshwater species in sustainable forestry: A critical review and potential future applications. Science of the Total Environment. 649, 1157-1170. https://10.1016/j.scitotenv.2018.08.370

[4] Di Muri *et al.* 2020. Read counts from environmental DNA (eDNA) metabarcoding reflect fish abundance and biomass in drained ponds. Metabarcoding and Metagenomics. 4, 97-112. https://doi.org/10.3897/mbmg.4.56959

[5] Sales *et al.* 2020. Fishing for mammals: Landscape-level monitoring of terrestrial and semi-aquatic communities using eDNA from riverine systems. Journal of Applied Ecology, 57(4), 707-716. https://doi.org/10.1111/1365-2664.13592

Benefits, opportunities and limitations of using eDNA

The eDNA approach is a relatively new and developing field. To become increasingly useful for projects, there is a need for more species' genetic information to be obtained and shared in open global databases such as GenBank. As gene sequences may not currently be available for some species, projects that undertake gene sequencing as part of the application of an eDNA approach are encouraged to publish and share gene sequence information to support the development of global databases and align with international good practice (e.g. Equator Principles guidance on data sharing).

Table 1 below summarises the specific benefits and limitations of an eDNA approach. Like any scientific method, eDNA should be applied to answer carefully framed questions, and consideration of factors like seasonality (e.g., of migratory species) remains essential.

Benefits	Limitations
• Sampling methodology is simple, non-invasive, rapid and amenable to collection by non-specialists. • As a non-invasive technique no impacts occur to individuals or their habitats compared to some traditional approaches. • Can efficiently detect a wide range of taxa, including rare and elusive species often not detected by conventional methods (if reference database is available). • Rapid degradation of eDNA in freshwater samples (days to weeks) and marine samples (hours to days) means that a positive detection is likely to be associated with current presence of the species[6]. • Easy to store samples for long periods for future analysis. • Archived samples allow auditors to commission their own surveys using the exact same methods, eliminating any observer bias. • Cost effective before-and-after assessment of aquatic habitats and species to track project impacts[7].	• When target species' DNA sequences are not available in genetic databases, capture and ID verification is needed to obtain a tissue sample for sequencing and future reference. This has a cost implication[8] and may require permits to collect the samples and send them to specialised laboratories for analysis. • For running water, identifying DNA from a species at a particular point shows the species is present either at the survey point or upstream of it. Stream flow and networks need careful consideration when interpreting findings. • eDNA has limitations in distinguishing among species in recently-evolved species clusters, where the genetic codes of commonly sequenced genes are very similar - e.g. some Haplochromine fish. Such species can be differentiated through additional research and analysis, at an additional cost. • In soil, eDNA can persist for decades and this needs to be taken into account when designing survey and analysis methods to target contemporary DNA.

Table 1: Benefits and limitations of an eDNA approach.

When is eDNA most likely to be useful?

eDNA methodologies are now sufficiently mature and cost effective that they are worth considering for virtually any project wishing to understand and mitigate impacts on biodiversity. However, they are likely to be of particular interest for projects:

- which are concerned about impacts on freshwater species and habitats, or
- where it is important to understand impacts on rare or elusive species, or difficult to access habitats, which are difficult to survey through other means, or
- where assessment of broad measures of ecosystem health and function is important, or
- where traditional survey methods are impracticable (e.g, for logistical, cost or health and safety reasons).

As with any scientific data collection method, the value of eDNA depends on clear and precise framing of the survey objectives, careful planning of sampling, and appropriate analysis and interpretation of the data.

[6] Thomsen, P. and Willersley, E. 2015. Environmental DNA – An emerging tool in conservation for monitoring past and present biodiversity. Biological Conservation. 183, 4–18. https://doi.org/10.1016/j.biocon.2014.11.019

[7] eDNA surveys have been found to more than halve the survey cost relative to equivalent surveys using traditional sampling. In a remote EIA survey, the field effort was reduced by 90 % for some groups using an eDNA survey that also detected more species than conventional methods

[8] Apart from field survey costs, there will be a small cost for bar coding fin clips. If the species is new to science then publication of a description of the species is recommended which may have researcher costs and take several months.

eDNA for biodiversity risk

Integrating eDNA into project surveys

Biodiversity surveys enable the evaluation of risks and impacts, support the design and implementation of effective mitigation actions, and provide long-term monitoring data to demonstrate project outcomes. Projects generally conduct three types of surveys (detailed in the biodiversity surveys IBN - TBC, 2018) and an eDNA approach can be applied to strengthen the outcomes and reduce uncertainty during each:

Scoping	Design	Construction & Operation	

Purpose

Risk Surveys	Impact & Mitigation Surveys	Monitoring Baseline	Monitoring
• Identify biodiversity at risk from project impact • Scope detailed assessments	• Enable impact assessment and quantification • Develop mitigation measures	• Track effectiveness of mitigation measures, project progress towards biodiversity goals or lender requirements	

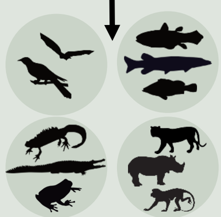
Role / potential value-add of eDNA

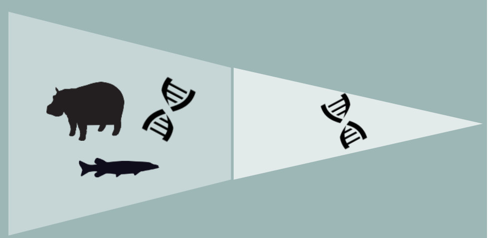
• Confirm presence/ absence of desktop-screened species • Provide understanding of species spatial distribution • Highlight species/ families that should be assessed in detail in field studies	• Confirm presence and distribution of high-risk species in relation to project infrastructure • Obtain high-resolution data on species of conservation concern	• Indicate baseline presence, absence, inferred abundance and distribution of priority species in relation to the project • Inference of trends in loss and gain of priority species • Repeatable and auditable monitoring methods and results

How?

High risk species likely/present in project area

Natural Habitats in protected areas





Rapid, **low intensity eDNA** sampling across key habitats	**Targeted eDNA** at species' preferred habitats	**Broad eDNA** sampling at regular intervals	**eDNA & traditional sampling** with tissue sampling for project database building	**Increasing role of eDNA** as measures validated / calibrated against traditional sampling

\+ Traditional sampling for **tissue collection** and confirmation

Table 2: Use of eDNA during different phases of project development

Case study: PERU[9]

An eDNA approach was used during a landscape-level survey in the Northern Peruvian Amazon focusing on the Pink River Dolphin (Endangered) and the Amazon Manatee (Vulnerable). Four replicate water samples were collected at 40 locations, separated by 10 to 15 km. The results from the survey gave a clear picture of the distribution and frequency of occurrence of the key target species based on the number of times they were detected during the replicates at each location (Figure 1).

Metabarcoding analysis of the samples identified a further 675 vertebrate species: 155 mammals (most of them terrestrial), 375 fish, 74 amphibians, 65 birds and six reptiles. Large numbers of identified fish species enabled the different ecosystems and their connectivity to be mapped in the vast landscape, highlighting habitats that were ecologically distinct and supported unique communities, and identifying where natural and man-made barriers prevent easy movement within the landscape.

The case study highlights the advantages of using an eDNA approach for landscape-level surveys and the effectiveness of eDNA as a first screening tool to assess presence/absence of species, understand presence and occupancy of target species (if included in the reference database), and understand ecological specificities of habitats within a landscape.

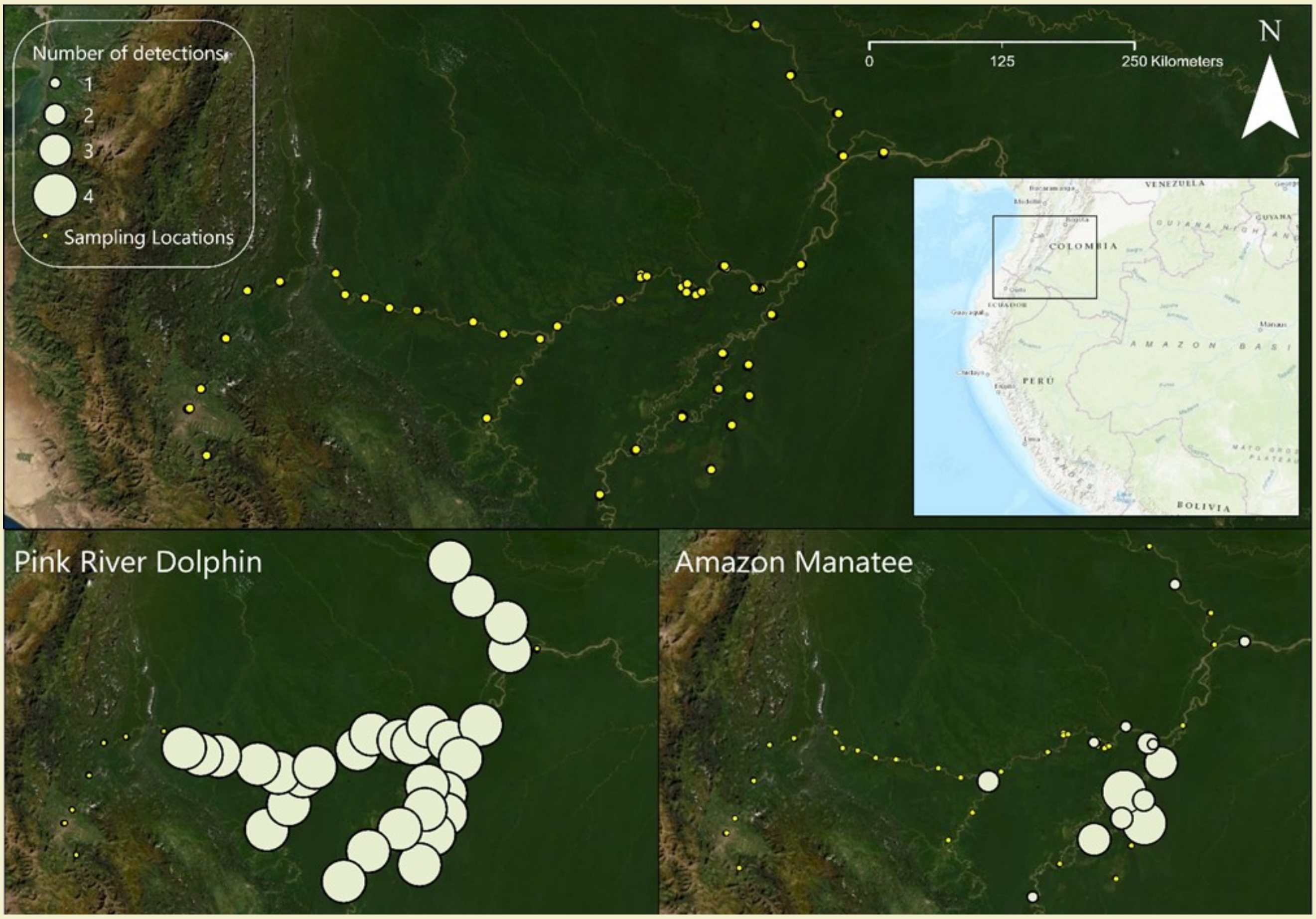


Figure 2: Sampling locations on the Northern Peruvian Amazon alongside locations and number of detections for each replicate using the eDNA approach for the Pink River Dolphin and the Amazon Manatee. Results show that the Pink River Dolphin is present in the entire landscape except upstream sites; the high detection rate across replicates suggests that the species regularly uses the entire landscape and is probably present in a relatively high density. The Amazon Manatee has a patchy distribution, suggesting they are mainly found in one tributary (flowing by a Protected Area). The lower level of replicates in the main river suggests they are either present at low density or infrequently use that part of the river.

[9] WWF (Healthy basins for the future programme) and NatureMetrics

Selected project examples

Project type	Ecosystem	Use of eDNA	Advantage of eDNA
• Oil and gas	• Marine	• Water and sediment scoping surveys to determine meiofauna, bacteria and vertebrates	• Provided information on fish diversity and other vertebrates and far lower cost than traditional surveys. Data gathered can be used to create biotic indicators that can track changes to the ecosystem over time.
• Oil and gas	• Freshwater	• Scoping survey	• Captured five times the number of fish species in large rivers compared to traditional netting techniques and identified 12 IUCN threatened species missed by conventional surveys.
• Hydropower	• Freshwater	• Targeted species survey	• Rapid confirmation of species presence in multiple sections of river compared to traditional survey approach.
• Mine	• Freshwater	• Scoping survey	• Identified additional species compared to traditional sampling techniques including an endangered aquatic mammal.
• Infrastructure	• Woodland	• Establishment of a habitat monitoring baseline for woodland restoration based on soil fauna	• Provided a habitat quality index based on soil data of fauna, fungi and bacteria. The baseline is established prior to restoration to track future restoration progress.

Table 3: Project uses and advantages of eDNA across different ecosystem types

The future of eDNA

eDNA is a rapidly evolving field of research and practice. Advances are underway that will further enhance the value of eDNA approaches for development projects, including:

- Pilot projects testing the use of an eDNA approach in different geographies and ecosystems e.g. snow, soil, deep sea and marine biomes;
- In-situ analysis such as the use of hand-held genetic sequencing devices[10] and hand-held biosensor devices using CRISPR technology[11] to rapidly detect invasive species, pests and species of conservation concern;
- Enhanced analysis techniques to provide species-specific population data (e.g. abundance) for aquatic species.

[10]Parker, S. 2019. Counting on eDNA for a faster, easier way to count coral. Mongabay Environmental News. https://news.mongabay.com/2019/05/counting–on–edna–for–a–faster–easier–way–to–count–coral/

[11]Williams, M. et al. 2019. The application of CRISPR-Cas for single species identification from environmental DNA. Molecular Ecology Resources. 19, 5 1106-1114. https://doi.org/10.1111/1755-0998.13045

eDNA for biodiversity risk

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