



# Phase 2 Summary Brief

## Developing Habitat Scale DNA Monitoring in Support of Post 2020 Biodiversity Reporting Requirements

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A project set up to inform and unlock the potential for Scotland to implement environmental DNA (eDNA) methods for biodiversity monitoring and reporting purposes. This Summary Brief highlights the project's key findings, learning opportunities, remaining knowledge gaps, and future perspectives to consider when using eDNA-based approaches for biodiversity assessment at the habitat-scale in the future.

**eDNA is genetic material present in the environment, such as in water, soil, or air, that comes from organisms living in that environment. For example, eDNA can be traces from shed skin cells and bodily fluids (saliva, blood, urine, faeces, etc) which animals leave behind when they enter, inhabit, or pass through the environment. By analysing eDNA, we can detect and identify species without directly observing or capturing them, offering a powerful tool for biodiversity monitoring and reporting purposes at the habitat scale.**

## Overview

- Conventional habitat monitoring requires a wide variety of survey techniques, expertise, and resources for assessing different species, communities, and habitat types.
- eDNA-based monitoring can survey a vast range of species across the tree of life, using simple field sampling methods that can be applied to any habitat type. We tested its use for biodiversity assessment across four habitat types in Scotland: marine lochs, freshwater lochs, woodland, and peatland.
- eDNA-based monitoring enables efficient detection and distribution mapping of many priority species, such as threatened, invasive, or indicator species. The data can feed directly into some established ecological indices, but there are also opportunities for novel indices to be introduced.
- In many cases there was a strong eDNA-based signal across habitat gradients, and we demonstrate the potential for new tools to classify sites by habitat condition. Development of these tools for use at national level will require investment in relevant expertise and representative training datasets at sufficient scale.
- While there are still some standardisation challenges to overcome, the potential for using eDNA-based methods in future monitoring programmes is highly promising.

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# Background

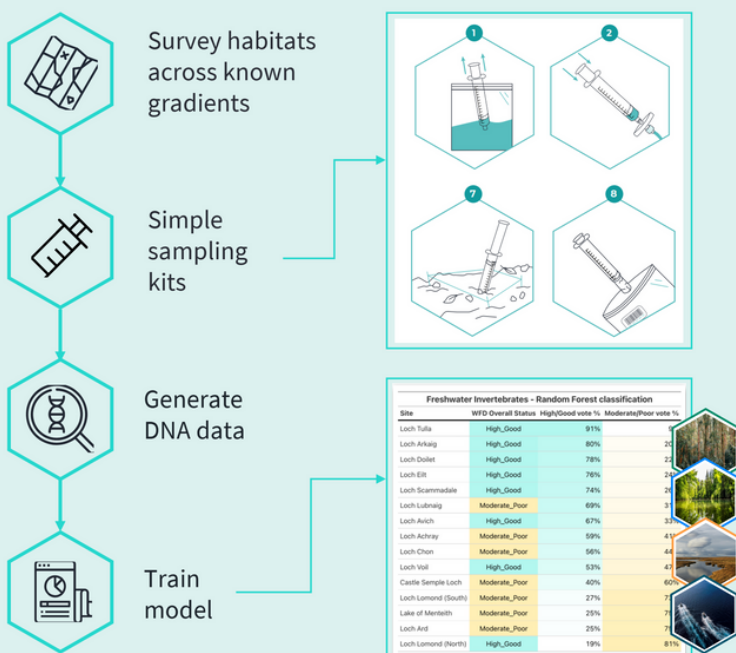
The Scottish Biodiversity Strategy sets out a clear ambition to be Nature Positive by 2030, and to have restored and regenerated biodiversity across the country by 2045. To do this, we need to be able to accurately describe and quantify ecological change. Biodiversity monitoring and reporting through eDNA-based methods is increasingly being used for tracking species diversity and community composition in ecosystems as it is scalable and can be used to detect hundreds or even thousands of species in one sample, using simple sample collection kits.

The primary project research questions were: *Can eDNA-based community data be used in habitat-scale monitoring programmes to i) apply indicators of biodiversity, and ii) be used to classify between habitat conditions<sup>3</sup> and detect condition gradients across habitats in Scotland?* The overall answer was: *Yes, eDNA-based data can be used to apply biodiversity indicators and to classify/detect habitat conditions, although investment is required to operationalise the approaches at national scale.*

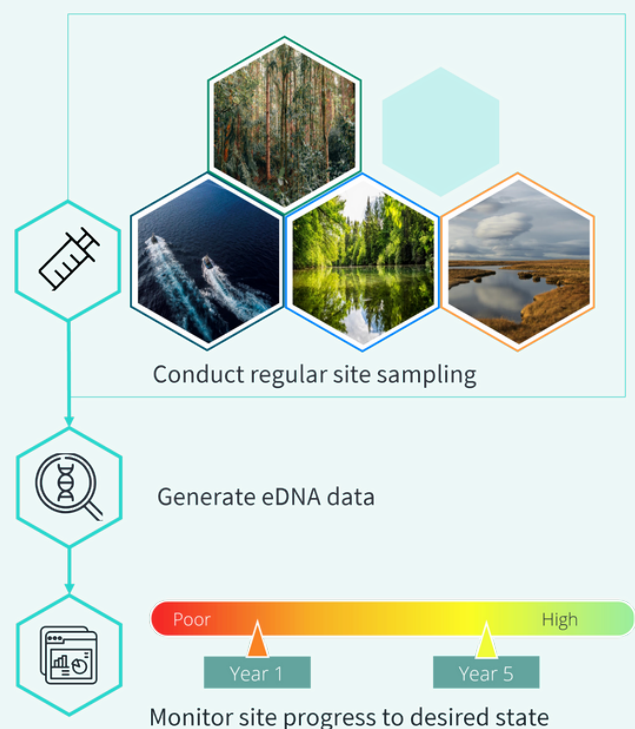
Samples were collected across four habitat types: marine lochs, freshwater lochs, woodland, and peatland. The survey sites were mostly situated in and around Loch Lomond and the Trossachs National Park but eDNA-based sampling included other parts of Scotland such as the Cairngorms National Park. Sites within each habitat were chosen based on predefined habitat conditions derived from conventional monitoring approaches.

## eDNA-based data can be used to build models to monitor habitat condition

### Build Habitat Condition Model



### Use Model to Monitor Habitats



<sup>3</sup> The state of a habitat with regards to its appearance, quality, health, and ecosystem functioning.

# Key Findings

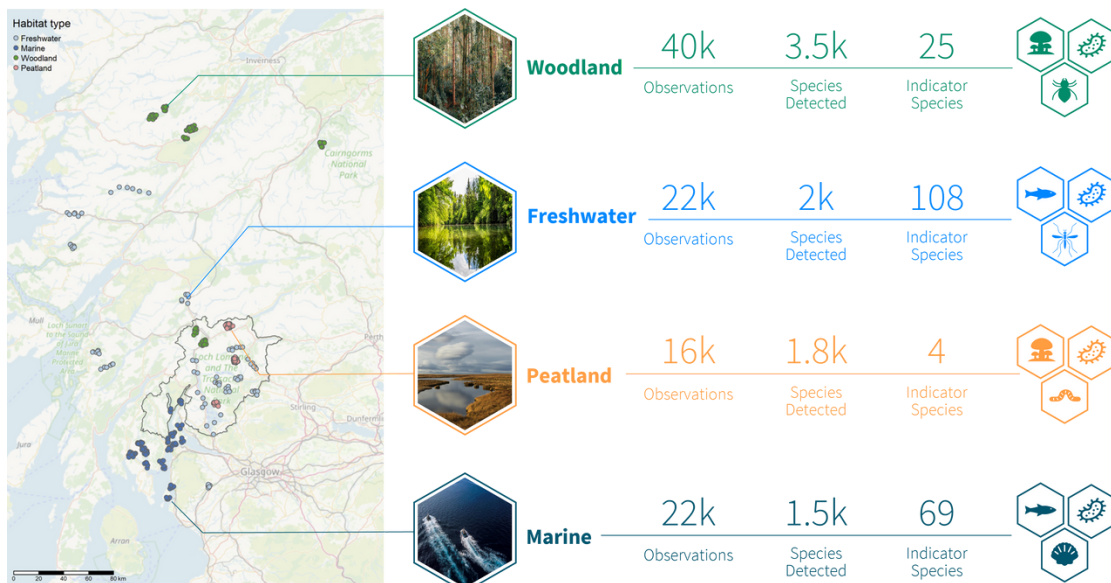
358 eDNA samples were collected, during August-September 2022, resulting in the detection of nearly 9,000 different species<sup>4</sup> with over 100,000 observations<sup>5</sup>. Among these, we found threatened species (species facing a high risk of extinction in the wild), invasive species (species whose introduction or spread threatens biological diversity), indicator species (species sensitive to environmental changes or have dependence on particular habitat conditions), Priority Marine Feature species (species with high conservation value in marine environments), and species used to designate Sites of Special Scientific Interest<sup>6</sup>.

In some cases, the data obtained through eDNA-based methods can be directly used in existing methods that assess the health of habitats. For example, evaluating marine sediment health was similar for both conventional and eDNA-based surveys. Similarly, for freshwater habitats, we found that eDNA-based data produced comparable results to data obtained through conventional methods for monitoring nutrient enrichment pressure in lochs. However, it is important to note that the communities detected by eDNA-based approaches were often different from those identified using conventional methods. Further validation is necessary to fully integrate eDNA-based approaches into existing assessment methods.

We were able to identify differences in species communities that were associated with distinct habitat conditions. By using these differences, we could then classify sites based on their condition. This classification showed promising results for freshwater and woodland habitats. However, for marine and peatland habitats, we could not test as many suitable sites, but we still observed differences between the communities. This indicates that eDNA-based monitoring has the potential to classify conditions in these habitats as well, but we need more data to refine the classification tools.

This project has resulted in new eDNA insights, informing both high-level decision making and practical end use, as well as producing extensive datasets and physical eDNA samples that will be available for future reuses.

eDNA-based monitoring can be used to survey a vast range of species across the tree of life<sup>7</sup>, enabling efficient detection and distribution mapping, which can feed into established ecological indices



<sup>4</sup> Species refers to Operational Taxonomic Units (OTUs) – DNA sequences found in a sample that, in most cases, are equivalent to species. However, not all Operational Taxonomic Units can be named to species level.

<sup>5</sup> Number of times we detected species in samples.

<sup>6</sup> Areas of special interest by reason of any of its flora, fauna, or geological or physiographical features (Wildlife and Countryside Act 1981).

<sup>7</sup> Plants, animals, and all other forms of life.

# Key Recommendations

There is a potential strategic opportunity for coordinating governance of eDNA-based priorities, and strengthening partnerships, to build capacity for Scotland going forward.



## Freshwater lochs

Create eDNA-based tools (sampling and data analysis methods) to predict the condition of freshwater lochs at a regional or national level. These tools will help us understand impacts of pressures<sup>8</sup> on ecosystem health and monitor the quality of the lochs. We can use existing classifications of loch health to develop these tools. To make them more accurate, we need to collect samples from multiple lochs across a wider geographic area and capture eDNA-based signals from a wide range of organisms which may be present. This approach could be expanded to assess the status of other freshwater habitats such as rivers, streams, and ponds.



## Marine lochs

Develop guidelines for how to collect samples of marine species in a consistent way, enabling eDNA-based faunal monitoring programmes in marine lochs. Further validate a method of scoring the health of marine sediment by using sites that experience contrasting impacts of pollution. Additionally, research the best lab techniques for identifying Priority Marine Feature species using eDNA-based methods, which can help us better characterise marine habitats at a regional or national level.



## Woodland

To monitor the progress of woodland restoration projects, we recommend focusing on individual sites from the beginning and tracking ecological responses through time. In the longer term, it would be possible to conduct a national survey using eDNA-based methods to rank woodlands based on their conservation value. This will allow prioritisation of restoration efforts for the most valuable woodlands. We also recommend validating the use of eDNA-based monitoring to detect specific fungal species that are listed as important for conservation.



## Peatland

We found that degraded and restored peatlands host different communities. However, our current model was unable to accurately predict the status of peatlands due to the limited data available. To develop a reliable model, we need a large eDNA-based dataset and clear definitions of peatland condition.

eDNA-based methods are not the only emerging technologies for routine and investigative national monitoring purposes. It should be combined with existing monitoring tools, as well as considering other emerging technologies such as earth observation (satellite images), lidar (light scanning technology that can create 3D habitat models), and bioacoustics (recording and analysing sounds to monitor species).

<sup>8</sup> Five pressures that the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES) have identified as driving the biodiversity crisis are: Changes in Land and Sea Use, Pollution, Species Exploitation, Climate change, and Invasive Non-native Species (INNS).

## Key Knowledge Gaps & Barriers

**1. eDNA benchmarks:** Using eDNA-based methods for monitoring the biodiversity of a country, such as Scotland, in a regulatory context requires a framework based on national benchmarks. These benchmarks are established by studying undisturbed environments and using them as references to assess the health and integrity of other ecosystems. Currently, there are only a few frameworks that incorporate eDNA-based community data, such as the Lake Fish Classification Index.<sup>9</sup>

**2. Sampling design:** Determining the number of samples needed for monitoring using eDNA-based methods is still largely unknown. This is because the potential applications of these methods are vast, covering different groups of organisms, habitats, and monitoring objectives. It is important to identify the number of samples required for each specific monitoring objective.

**3. Standardisation:** Standardised, open, and transparent methods and processes for eDNA-based analysis are crucial, especially when the results are used for reporting and regulation purposes.

Nationally and globally, the data from different eDNA-based projects are currently not being collected in a systematic and unified manner. Establishing standardised guidelines for formatting and storing eDNA-based data in publicly accessible databases would accelerate progress in this field.

## Future Perspectives

Adopting eDNA-based approaches holds incredible promise for enhancing existing frameworks, surveying priority species, and assessing sites along ecological gradients. To fully unlock their potential, we need to build extensive datasets and train robust models. Furthermore, the ongoing advancements in eDNA assays and cost reduction in sample processing are pivotal for widespread adoption in national reporting frameworks.

While we await the development of larger eDNA-based biomonitoring frameworks, it is important to recognise that using eDNA-based methods can already make a positive impact on biodiversity monitoring at local scales. Through thoughtful study design, we can gather valuable insights and contribute to the understanding of our local ecosystems.

The true power of eDNA-based approaches lies in their ability to generate vast datasets using standardised sampling kits. These datasets serve as tools for characterising habitat conditions, ensuring consistent monitoring, and facilitating accurate reporting. By harnessing them, we can gain deeper insights into our environment in order to work towards its preservation, restoration, and regeneration in the future.

<sup>9</sup> Nigel Willby, Alan Law, Colin Bull, Bernd Hänfling, Lori Lawson Handley, Ian Winfield (2020) A tool for classifying the ecological status of lake fish in Britain based on eDNA metabarcoding. Report to the Scottish Environment Protection Agency (SEPA).